IDENTIFYING PHYSIOLOGICAL PROCESSES LIMITING GENETIC IMPROVEMENT OF EAR FERTILITY IN WHEAT

BY OORBESSY GAJU

Thesis submitted to The University of Nottingham for the degree of Doctor of Philosophy

University of Nottingham

School of Biosciences

Sutton Bonington Campus

Leicestershire

LE12 5RD

March 2007

ACKNOWLEDGEMENTS

I would like to express my sincere thanks and gratitude to my supervisors, Dr John Foulkes, Dr. Matthew Reynolds and Dr. Debbie Sparkes, for their guidance, support and constructive criticism throughout this research study.

Many thanks go to Eugenio, Israel, Araceli, Reyna and other person who were involved in the field experiments in Cd. Obregon, Mexico. Without their invaluable help it would have been impossible to collect the necessary data in time. My deepest appreciation also goes to Mateo Vargas from CIMMYT and Jim Craigon at University of Nottingham for providing statistical advice.

Special thanks to David Hodson (Hoddy) for always being there to help out with all sorts of computer problems at the drop of a hat. I am grateful for the invaluable help provided by other technical and academic staff at Sutton Bonington. My deepest appreciation goes to all my friends who has in one way or the other contributed to this work especially Jools, Rakhi, Heike, Eunice, Bhavisha, Stella, George and Manojit.

My thanks also go to the University of Nottingham and CIMMYT for funding the research project.

Last, but not least, I would like to thank my parents, Nitish and Sindy for their love and unwavering support. I could not have done it without their encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABRREVIATIONS

Kilogram	kg
Nanometer	nm
Tonnes per hectare	$t\ ha^{-1}$
Square metre	m^2
Degrees Celcius	^oC
Photosynthetically active radiation	PAR
Radiation-use efficiency	RUE
Extinction coefficient	k
Growth stages	GS
Green area index	GAI
Leaf area index	LAI
Canopy temperature depression	CTD
Stomatal conductance	g_s
Normalized difference vegetative index	NDVI
Water soluble carbohydrates	WSC
Nitrogen	N
Specific leaf weight	SLW
Abscisic acid	ABA
Above ground dry matter	AGDM
Dry matter	DM
Harvest index	HI
Potential grain weight	PGW
Double haploid	DH
Quantitative trait loci	QTL
Degrees of freedom	DF
Standard error of means	SED
International centre for improvement of maize and wheat	CIMMYT

ABSTRACT

Wheat (*Triticum aestivum* L.) is one of the three most important cereal crop grown globally and there is a gap between yield production and world demand. Previous studies on wheat have generally shown traits influencing the capacity of the grains to store assimilate (sink) to be better correlated with yield than traits influencing potential assimilate production (source). Therefore, strategies to improve ear fertility, defined as the number of grains per ear, are one of the most relevant features in the development of new cultivars and genetic improvement of yield potential. In the present study, novel large-ear phenotype (long rachis) is investigated as a trait to increase the number of grains per ear, thus grains m^{-2}, with the aim of identifying physiological processes limiting genetic improvement of ear fertility in wheat. Two advanced lines (NL1 and NL2) developed by the International Centre for Maize and Wheat Improvement (CIMMYT) with novel ear morphology and one CIMMYT cultivar with conventional ear morphology Bacanora referred to as the parental spring wheat genotypes were characterized. In addition, 69 doubled-haploid (DH) lines from a cross between NL2 x Rialto (UK-bred cultivar) were used to investigate the physiological basis of improved ear fertility and yield potential in the novel material.

Three field experiments were carried out at the CIMMYT experimental station in Cd. Obregon (Mexico) on the parental genotypes (2003/04, 2004/05 and 2005/06) while two field experiments were carried out for the NL2 x Rialto population (2004/05 and 2005/06). An additional experiment consisting of a subset of 15 lines of the NL2 x Rialto population was carried out in 2005/06. Two controlled-environment experiments were carried out at Sutton Bonington, University of Nottingham in 2004 and 2005 to investigate the developmental basis of the large-ear phenotype on the three parental genotypes. A post-anthesis (GS 61+ 14d) degraining treatment was imposed in field experiments examining the parental genotypes in 2004, 2005 and 2006 while a similar degraining treatment was carried out for the subset of 15 DH lines of the NL2 x Rialto population in 2006. A range of physiological traits related to ear fertility were measured on the parental genotypes and the DH lines including rachis length, spikelets ear^{-1}, developmental stages, green area, radiation interception, radiation use-efficiency (RUE), dry matter production and partitioning, stem water

soluble carbohydrate reserves, potential grain weight, grain weight and combine yield. In the growth-room experiments, the rate and duration of spikelet primordia production of the main shoots were measured.

Present results showed that in the novel genotypes, longer rachis increased spikelets ear^{-1} and also grains ear^{-1}. Thus, the novel genotypes showed greater ear fertility by having more grains ear^{-1} than the benchmark cultivar Bacanora. Grains m^{-2} was not actually increased in novel genotypes; indeed lower grains m^{-2} was found in NL2 compared to Bacanora. Heavier grains were found in the novel genotypes which had greater potential and final grain weight compared to Bacanora. The NL2 genotype possesses a tiller inhibition gene (*tin*) on chromosome 1A and this genotype had fewer ears m^{-2} than the other genotypes, and this trait also contributed to the lower grains m^{-2} of NL2. The grains-to-ear DM ratio at GS 61 in NL2 was markedly lower compared to other genotypes. Results of the growth-room experiments showed that there was a developmental basis for the higher spikelets ear^{-1} observed in NL1 and NL2 than Bacanora. A longer thermal duration from floral initiation to terminal spikelet was associated with a higher spikelet number in NL1 (27) and NL2 (29) compared to Bacanora (23). Since the growth-room experiments were carried out under long photoperiod (16 hrs) results also suggested that the large-ear phenotype may have been associated with the effects of 'earliness *per se*' genes.

Grain weight of the parental genotypes did not respond differently to degraining. Averaging across parental genotypes and years, responses to degraining in the field experiments showed that although assimilate supply per grain was potentially increased by 100%, average grain weight was only increased by 15%. These findings indicated that grain yield was mainly limited by post-anthesis sink size in these experiments. In the subset of 15 DH lines experiment, there were different responses of the lines to degraining and the lines which showed larger responses of individual grain weight to degraining had lower grain weight in control intact ears.

Results of the DH experiments showed that rachis length was positively correlated with spikelets ear^{-1}, grains ear^{-1} and grains m^{-2} among the lines. There was also a positive phenotypic correlation between the grains-to-ear DM ratio at GS 61 and grains m^{-2} amongst the lines. However, the large-ear phenotype (long rachis, high

spikelets ear^{-1}) was not associated with greater grain yield due to a trade-off between grains m^{-2} and individual grain weight. The physiological mechanisms potentially explaining this trade off are analyzed. Harvest biomass was positively correlated with grain yield amongst the DH lines. So traits to improve biomass whilst maintaining harvest index may be important for future breeding. Present results showed a positive correlation between pre-anthesis RUE and harvest biomass amongst the subset of 15 lines of the NL2 x Rialto DH population. It is suggested that breeders might select for higher RUE (via high specific leaf weight) to improve grains m^{-2} and yield potential in future years.

CHAPTER 1 INTRODUCTION

1.1 WHEAT PRODUCTION AND GLOBAL DEMAND

Wheat is grown on approximately 215 million ha worldwide (FAOSTAT, 2005), the staple food of nearly 35 % of the world's population. Current global wheat production and average yield is about 627 million tonnes and 2.91 tonnes ha^{-1}, respectively (FAOSTAT, 2005).World population is increasing at a rapid pace and by 2010 the population is predicted to reach 6.8 billion at an average annual growth rate of 1.06 % (U.S. Bureau of the Census, International Database, 2003). It seems likely that the demand for food will increase at the same, if not a faster rate. According Rosengrant *et al.* (1995) demand for wheat is expected to grow by approximately 1.3% p.a. worldwide compared to a 1.8% p.a. increase in developing countries for the next twenty years. Kronstad (1998) forecasted global demand of wheat to be 1,050 million tonnes in the year 2020. Therefore, there is a requirement to increase wheat yields per unit land area, since arable land area is restricted.

Wheat was one of the first domesticated food crops and for 8,000 years has been the basic staple food of the major civilizations of Europe, West Asia and North Africa (Curtis, 2002). It is an annual grass with several 'tillers' (lateral stems) arising from the stem base each of which could terminate in an inflorescence. There are three types of wheat based on cytogenetics (i.e. chromosome number) namely:

- Einkorn group, diploid, 2n = 14
- Emmer group, tetraploid, 4n = 28
- Vulgare group, hexaploid, 6n = 42

The origin of polyploid wheats is from natural hybridizations between different species or genera of wild grasses (Figure 1.1).

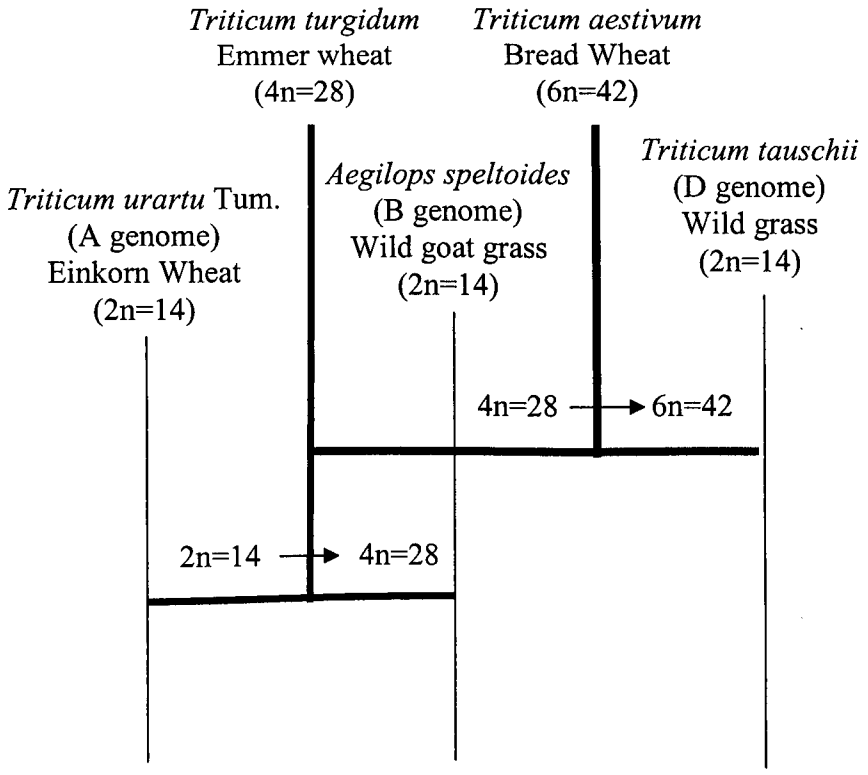


Figure 1.1. Hybridisation of different species of wheat

Bread wheat is an allohexaploid (2n=6x=42) with a genome size of 16 billion base pairs of DNA organized into 21 pairs of chromosomes, seven pairs belonging to each of the genomes A, B and D (Sears, 1954). Wheat possesses one of largest and most complex genomes, but because of its hexaploid nature and economic importance as a food source, bread wheat is the most cytogenetically studied of the crop species. Meeting expected demands by continued expansion of agricultural production into remaining natural resources is environmentally unacceptable, and the economic costs of increasing yields by intensification of agronomic infrastructure are high. Hence, a cost-effective and environmentally sound means of meeting global demand appears to be through genetic improvement of wheat yield potential (Skovmand *et al.*, 2001). The trends of global wheat production and average yield for the last decade are illustrated in Figures 1.2 and 1.3, respectively.

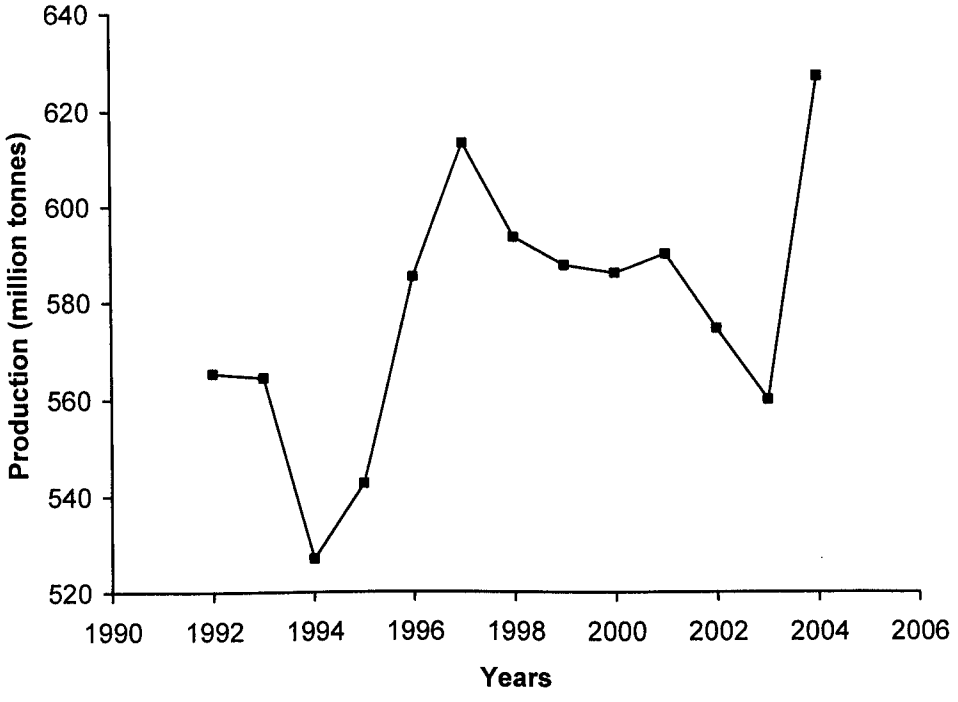


Figure 1.2. Trend in wheat global grain production

Source: FAOSTAT database, 2006

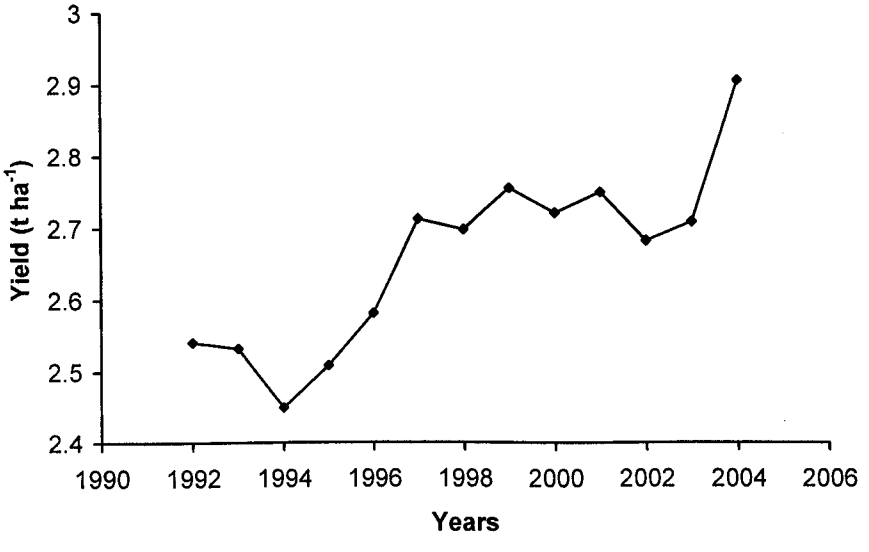


Figure 1.3. Trend in global average grain yield

Source: FAOSTAT database, 2006

The dramatic increases in cereal production between the 1940s and 1960s in developing countries was principally due to the 'green revolution' whereby plant breeders reduced the height of wheat plants by crossing traditional tall varieties with semi-dwarf varieties. The resulting high yielding semi-dwarf varieties significantly outperformed tall varieties in the presence of adequate irrigation, pesticides, and fertilizers. Cereal production in developing countries has more than doubled between the years 1961 – 1985 (Conway, 1997). Furthermore, translocations of chromosomes from rye (Villareal *et al.*, 1995), *Agropyron spp.*, and disease resistance genes have contributed to maintaining the rate of genetic gain in yield potential in the 1990s (Singh *et al.*, 1988; Reynolds *et al.*, 2001).

Until the late 1980s, there has been an almost 50:50 contribution to yield progress from better management practices and plant breeding in the UK (Silvey, 1986, Austin *et al.*, 1989) and almost the same ratio of contributions in other parts of the world (Slafer and Andrade, 1991). Future increases in wheat production will continue to be strongly dependent on the ability to achieve higher on-farm yields rather than increasing the harvested area (Slafer *et al.*, 1996; Slafer and Araus, 1998) and further increases in grain yield will likely rely more on genetic gain than on agronomic gain (Slafer and Andrade, 1991).

However, the global demand for wheat is presently increasing at a much faster rate than progress in genetic yield improvement, a little less than 1% per year in most high output regions (Reynolds *et al.*, 1999). In high output environments such as in Mexico (Sayre *et al.*, 1997), the UK (Shearman *et al.*, 2005) and France (Brancourt-Hulmel *et al.*, 2003), the genetic gain has been about 1% yr^{-1} in the previous two decades whereas in low input environments, for instance in the Great Plains, it has been only about 0.5 % (Donmez *et al.*, 2001). The focus is therefore towards genetic improvement to accelerate present rates in genetic gain in yield potential, i.e. the yield produced when a cultivar is grown under optimal conditions, in the absence of nutrient and water limitations as well as disease, pest and lodging constraints (Evans and Fischer, 1999; Fischer, 2001).

1.2 THE CONTRIBUTION OF BREEDING TO GLOBAL WHEAT PRODUCTION

Breeding success from the 1960s to 1990s in increasing yield potential has been largely due to the introduction of the semi-dwarfing genes (*Rht-D1b* and *Rht-B1b*) on chromosome 4D and 4B, respectively, reducing plant height by about 20 cm and the consequent increase in harvest index (HI), i.e. ratio of grain yield to above-ground dry matter (Austin *et al.*, 1989; Gale and Youssefian, 1985; Sayre *et al.*, 1997). However, there is a theoretical upper limit to HI of about 0.62 as estimated by Austin *et al.* (1980). This upper limit is already being approached by some cultivars in the UK, e.g. Consort for which a HI of 0.61 has been reported (Spink *et al.*, 2000). There have been relatively few reports of genetic gains in biomass production over the years and if grain yield is to be increased in the future, breeders should aim at achieving increases in biomass production whilst maintaining HI.

Future genetic improvements will have to be achieved with a crop that has already received an intense breeding effort to produce large increases in yield (Slafer *et al.*, 1999). The relatively high yield of current cultivars is likely to be responsible, at least in part, for genetic increases in wheat yields becoming harder to achieve (Reynolds *et al.*, 1996). Self-pollinated crops like wheat have a genetic structure that has implications in the choice of methods for their improvement. The pedigree method is the traditional breeding method where numerous generations of self pollination (inbreeding) within the population occurs after cross pollination (by hand) of two parent lines at the start of the breeding scheme. Wheat breeders usually use a combination of several methods for handling segregating populations in one breeding programme.

At the International Maize and Wheat Improvement Centre (the acronym in Spanish being CIMMYT), breeding is carried out by a shuttle system involving strong selection pressure on segregating wheat populations at two contrasting locations (Ciudad Obregon and Toluca) in regard to latitude, altitude and rainfall (Reynolds and Borlaug, 2006). Initially, this system was used to speed up the breeding process by allowing two generations per calendar year and screen for disease resistance genes, however, by this process it has also been possible to introduce and select

genes for photoperiod insensitivity (Ravi Singh, personal communication). At CIMMYT the methodology of handling segregating populations was changed from a pedigree system to a modified pedigree-bulk selection system in the 1990s. The new method allows one breeder to evaluate all segregating populations, in a timely fashion, except for the F_2 generation (Rajaram and Van Ginkel, 1995).

In the UK, the pedigree method has been used widely to raise the yield potential with grain yield as the main selection criterion. Selection in this way has been successful but it is a slow process as yield is also influenced by the environment. Other methods are being used to modify the pedigree method and to accelerate the breeding process, such as single seed descent so that the rate of production of early generations is faster. Similarly, the doubled-haploid (DH) technique is used whereby F1-derived homozygous lines are produced in two generations by haploidization followed by chromosome doubling with colchicine of early generation material. In the present study, this DH technique has been used to generate the populations of lines investigated and more details about the generation of the DH populations are found in Chapter 6.

During the last decades wide crosses and hybridisation between tetraploids (e.g. *Triticum turgidum*) and wild diploid relatives (e.g. *Triticum tauschii*) have been used to introduce new genes to the hexaploid wheat gene pool. For instance, synthetic lines are being used at CIMMYT to bring in novel alleles and traits from the D genome, e.g. resistance genes to stripe rust from *Triticum tauschii* in synthetic hexaploids (Ma *et al.*, 1995a). Furthermore, synthetic hexaploid wheats may be a new source of genes for breeding programmes (Narasimhamoorthy *et al.*, 2006) not only for pest resistance but for traits with complex inheritance (Mujeeb-Kazi *et al.*, 2001) and they have been reported to improve agronomic traits (del Blanco *et al.* 2001). Over the last 10 years the proportion of wheat lines that CIMMYT has distributed to rainfed wheat improvement programs around the world using its international nursery systems that are synthetic wheat derivatives has increased from approximately 10% to 50%. These synthetic wheat derivatives provide a wide range of variability for biotic and abiotic stresses, e.g. drought resistance (Reynolds *et al.* 2007). In addition, evidence is building from CIMMYT's global wheat yield trials that synthetic derivatives can also contribute to yield potential in well watered,

highly productive environments (Ogbonnaya *et al.*, 2006). In a comparison of synthetic-derived wheat with recurrent parents, final biomass and kernel weight were larger under both irrigated and moisture-stressed conditions (Reynolds *et al.*, 2007).

1.3 THE ROLE OF PHYSIOLOGY IN WHEAT BREEDING

Cereal breeding has, to date, been based principally on empirical selection for yield *per se* which is the most common selection criteria in most breeding programmes. However, yield being a genetically complex trait shows a high genotype x environment (G x E) interaction and therefore its heritability is relatively low (Jackson *et al.*, 1994). There is now a strong argument that an indirect or analytical approach, based on the understanding of the crop at the physiological and molecular levels, may help target the key traits and genes that are currently limiting yield. One of the pioneers of a more strategic approach for plant breeding was Donald (1968) who proposed the use of traits comprising a wheat ideotype as selection criteria. It was suggested that this represented an improvement over empirical breeding because individual traits have higher heritability than yield. The indirect or analytical approach may therefore complement conventional breeding programmes and accelerates yield improvement (Araus, 1996; Slafer and Araus, 1998) by identifying synergies between yield-forming traits in new ideotypes and developing new selection criteria and screening tools. A survey of wheat breeders carried out by Jackson *et al.* (1996) suggested that research in plant physiology has had a limited impact on wheat improvement. However, according to Slafer *et al.* (1994) and Slafer (2003) improved understanding of the physiological bases of past yield increases could provide better approaches for the use of candidate physiological traits as selection criteria. Therefore, the most plausible approach may be to continue to integrate our physiological and genetic understanding with established empirical approaches.

Physiology is the science of biological processes and functions. Physiological tools, for example, canopy temperature depression as measured with an infra-red thermometer (Reynolds *et al.*, 1994) can be used to provide indices for high-throughput phenotyping which serve as selection criteria in plant breeding, thereby enhancing the breeding progress (Reynolds *et al.*, 1998). However, physiological

understanding alone would not succeed in effecting major changes unless there is a concerted effort from breeders, physiologists and biotechnologists to (i) establish the correlation of the trait with yield, (ii) identify available sources of genetic diversity for the trait, (iii) generate segregating populations to quantify the trait's heritability, and (iv) develop high-throughput phenotyping methodologies so that the trait can be precisely measured, especially on early generation material (Fischer, 2001).

1.4 ROLE OF NOVEL EAR-MORPHOLOGY TRAITS IN RAISING YIELD POTENTIAL

Previous studies have shown that grain yield is mainly sink limited worldwide (Borghi *et al.*, 1986; Savin and Slafer, 1991; Shearman *et al.*, 2005; Reynolds et *al.*, 2005). Thus, studies on the physiological basis of genetic gains in grain yield in sets of historic wheat varieties globally have generally shown traits influencing the capacity of the grains to store assimilate (grain sink) to be better correlated with yield than traits influencing potential assimilate production during grain filling (grain source). Positive correlations between grains m^{-2} and yield identified in these investigations indicate that future gains in yield potential will likely depend on achieving increases in grains m^{-2} to increase grain sink size. There has been some evidence suggesting that the post-anthesis source, for example, radiation-use efficiency (RUE) can be increased by increasing the grain sink strength via an increase in grain number, i.e. ear size (Reynolds *et al.*, 2005). Therefore, strategies to improve ear fertility are one of the most relevant features in the development of new cultivars in high output environments. In this study, novel large-ear phenotype morphology is investigated as a trait to increase the number of grains per ear, thus grains m^{-2}. While particular elements of ear dry matter productivity are of a multigenic character of inheritance, ear morphological traits are more easily measured and, at the same time, controlled by fewer genes (Martinek and Bednar, 2004).

The number of grains per unit land area has contributed mostly to genetic gains in yield potential in modern UK wheat cultivars in recent years (Shearman *et al.*, 2005). Similar findings showing grains m^{-2} to be positively correlated with yield amongst sets of historic cultivars are reported elsewhere, e.g. France (Brancourt-Hulmel *et al.*, 2003), the Great Plains (Donmez *et al.*, 2001) and Mexico (Fischer *et al.*, 1998). Restructured plant types with novel ear morphology in wheat and with long ear rachis have recently been developed by CIMMYT, based on genetic combinations of including *Agro-triticum* germplasm, *Triticum polonicum* and semi-dwarf spring wheats (Rajaram and Reynolds, 2001). These plants have ears with a long rachis (up to 20 cm), high spikelet number (up to 30 spikelets per ear), high fertility (up to 200 grains ear^{-1}) and intermediate tillering capacity (up to 10 tillers). More generally, novel sources of ear anatomy for increasing ear fertility at CIMMTY are Agro-triticum (long rachis, high spikelet number), *Triticum polonicum* (long glume), tetrastichon (2 spikelets per rachis node) and multi-ovary traits.

University of Nottingham and CIMMYT have jointly generated two DH populations of lines derived from crosses between CIMMYT novel large-ear phenotype (long rachis, high spikelets ear^{-1}) (NL1 and NL2) and the productive (high radiation-use efficiency, i.e. ratio of dry matter production to radiation interception; RUE) UK adapted wheat, Rialto. In the current study, the physiological processes controlling improved ear fertility, defined as the number of grains per ear, in the novel 'large ear' CIMMYT material were investigated through analysis of the CIMMYT parents and the DH lines in field experiments at Cd. Obregon, NW Mexico and in growth-room experiments at University of Nottingham.

1.5 OBJECTIVES

The overall objectives of the present study in field experiments at Cd. Obregon, Mexico and growth-room experiments of University of Nottingham were therefore:

1. To understand the developmental and physiological basis of the large-ear phenotype in the novel CIMMYT material and quantify effects on ear fertility (grains/ear) to improve understanding of yield limiting factors in wheat
2. To investigate the source-sink balance in the large-ear phenotype material by carrying out degraining treatments
3. To investigate potential synergies between large-ear phenotype and source type traits (e.g. stem carbohydrates, pre-anthesis radiation use efficiency) by examining associations between traits in lines of the NL2 x Rialto DH population
4. To quantify and elucidate relationships between expression of ear-fertility traits in novel large ear phenotype material and other yield components, i.e. ears per m^2 and individual grain weight in the NL2 x Rialto DH population

CHAPTER 2 LITERATURE REVIEW

This chapter reviews critically past studies and investigations to determine critical stages as well as the physiological traits that have most influence on the yield potential of wheat. It starts with a few basic definitions that will be used extensively in this review followed by a description of models used previously to analyse yield potential, then the physiological determinants of yield. A brief outline of the physiological genetic gains in yield comes later together with some physiological tools used as indirect selection criteria for yield to enhance breeding. The chapter ends with an assessment of priority candidate traits for future genetic gain in yield potential.

2.1 SOURCE AND SINK LIMITATIONS TO YIELD

2.1.1 Basic definitions

The photosynthetically active tissues are designated as sources as they are the net exporters of fixed carbon dioxide whereas photosynthetically inactive parts are referred to as sinks, as they are net importers of photoassimilate. Sometimes the same organ can be both a source and a sink depending on the stage of development. For instance, newly emerged leaves are sinks but as they grow they become sources (Hay and Walker, 1989). All production of biomass relies on photosynthesis. However, the actual contribution of carbon assimilation to grain yield depends on distribution of the photoassimilate to various organs and the source and sink relationship prevailing. A large grain sink will normally be an inherent characteristic of a high yielding genotype; however, the maintenance of this large sink exerts a demand from the source. The capacity of the source to fill the sink is an essential component of high yield potential (Blum, 1996). In this study, the grain source size is defined as the potential supply of assimilates during grain filling as determined by the stem carbohydrates reserves, the green canopy area, the extinction coefficient and the radiation-use efficiency (above ground biomass production per unit radiation intercepted) whilst the grain sink size is determined by grain number, which is a function of ears m^{-2} and grains ear^{-1}, and the potential grain size.

2.1.2 Source limitation

It would be of little use to genetically improve the potential grain sink size if assimilate production of existing varieties cannot fulfil grain sink demand in favourable seasons. Source limitation during grain filling can result in shrivelled or poorly filled grains, which have smaller endosperm cells containing less starch than corresponding cells in plump grains (Brocklehurst *et al.*, 1978). If varieties are source limited, further increases in grain sites may result in a reduction in individual grain weight. The contribution of stem soluble carbohydrate reserves, which are maximal shortly after flowering, is crucial when post-anthesis photosynthates do not meet the requirements of grain growth, as they may be translocated to the ears to supplement grain demand for assimilates (Bonnett and Incoll, 1993; Schnyder, 1993). Thus, if both post-anthesis photosynthesis and stem reserves are limited, then grain growth may be severely restricted by assimilate supply (Cook and Evans, 1978).

Source limitation could theoretically be overcome by breeding to:

(i) Improve canopy architecture to increase fractional radiation interception per unit green canopy area

(ii) Increase canopy green area and /or longevity

(iii) Increase radiation-use efficiency (RUE, i.e. amount of dry matter produced per MJ m^{-2} d)

(iv) Increase stem carbohydrate reserve accumulation and efficiency of remobilisation

2.1.3 Sink limitation

Under suitable growing conditions and sufficient assimilate supply, grains can be fully developed. Potential grain sink size, however, may not be large enough to use all available assimilates efficiently, especially during early grain growth (Ma, 1994). The store of soluble carbohydrates in stem tissues may increase until 2-3 weeks after anthesis due to high assimilate production with limited grain demand (Gifford and Evans, 1981). As grains are filled with assimilates, they may reach their maximum potential size before maturing, and limitation of sink capacity will then prevent further grain growth (Martinez-Carrasco and Throne, 1979; Koshkin and Tararina,

1989). However, if the grain sink size is limiting yield, further assimilate production would be wasteful.

Sink limitation could be overcome by breeding to:

(i) Increase ear number m^{-2} through increased tiller production or tiller survival

(ii) Increase spikelets ear^{-1}

(iii) Improve floret survival $spikelet^{-1}$ at anthesis with the aim of increasing grain number ear^{-1}

(iv) Increase potential grain size

2.1.4 Relationship between grain number and grain weight

Worldwide progress in grain yield is mostly related to grains m^{-2} being increased through breeding while individual grain weight, the other major yield component, has undergone little change and in some cases even a decline (Slafer and Andrade, 1989; Acreche and Slafer, 2006). The way of analyzing the physiological basis of grain yield in terms of yield components, i.e. grains m^{-2} and individual grain weight, is quite traditional among physiologists, but this approach is sometimes considered ineffective because of the negative relationship that usually prevails between these two components (Slafer *et al.*, 1996). The latter put forward four hypotheses to explain the negative relationship, as set out below.

When grain number is increased, assimilates available during the post-anthesis period have to be shared and distributed between more grains. Reduced availability per grain would logically result in reduced weight.

1. There is a source limitation for grain growth during post-anthesis
2. There is simultaneous limitation for grain growth during post-anthesis imposed by the strengths of the source and sink

Alternatively, the reduction of grain weight is independent of the level of competition, i.e. yield is limited by the strength of the sink during the post-anthesis period and the availability of assimilates would not change the negative relationship between these components.

3. The increased number of grains may lead to a greater portion of grains being placed in grain positions (i.e. distal florets in spikelets and/or apical and basal spikelets) within the ear with reduced grain weight potential
4. There is an associated effect of 'yield increasing genes' that reduces potential grain size, for instance through an effect of grain coat, producing lower individual size for all grains in any floret position within the canopy

2.1.5 Manipulation of source and sink sizes

Genetic progress in yield potential, as stated previously, is strongly associated with increases in grain number. Nonetheless, Calderini *et al.* (1995) found that grain weight has contributed to improved yield potential in irrigated Argentinean spring wheat. In order to understand the underlying physiological mechanisms by which the relationship of source and sink is regulated, various studies incorporating source and sink manipulations have been performed. Grain growth differed among genotypes in response to enhanced assimilate supply per grain generated by spikelet removal (Ma *et al.*, 1995b). Partial reduction of grain number ear^{-1} reduces total sink size and increases the ratio of source to reproductive sink. An increase in grain mass due to spikelet removal implies that grains from intact control ears fail to reach potential grain weight due to insufficient assimilate (Fischer and HilleRisLambers, 1978; Koshkin and Tararina, 1989).

Slafer and Savin (1994) found that during the post-anthesis period, yield was either entirely sink limited or co-limited by both source and sink but never only source limited. This was because by degraining or shading treatments, the relative change in grain weight in relation to the change in assimilate supply was always less than a 1:1 ratio. According to Ma *et al.* (1995b) removal of 50% spikelets at anthesis from the main shoot in soft red wheat considerably decreased grain number and induced a range of compensatory growth responses for grains. Calderini and Reynolds's (2000)

study on degraining at heading showed a significant increase in grain weight from synthetic hexaploid lines of wheat in Mexico with the largest response occurring in grain positions with lowest grain mass. The data confirmed the physiological potential for increasing grain weight at distal spikelet positions by as much as 16%, strongly endorsing the objectives of breeders to raise yields through increasing individual grain weight potential. By contrast, Borghi *et al.* (1986) reported that at heading, partial defoliation of lamina (flag leaf or lower leaves) did not cause any significant variation in yield components. Kruk *et al.* (1997) reported that post-anthesis defoliation did not result in significant changes in grain weight in old cultivars, but showed significant reduction in individual grain weight for several positions within the ear in new cultivars, although reductions were small and many grains were unaffected.

It is very difficult to state with certainty whether source or sink is more limiting to yield potential. Post-anthesis application of source and sink manipulation did not give consistent results so that effects of modified source–sink ratios could be ascribed to environment and genotype interaction with the availability of assimilates during post anthesis for grain growth (Slafer *et al.*, 1996). Furthermore, Calderini *et al.* (2006) found that genotype and locations have a marked effect on grain yield and its components. Wheat has been reported to be a sink- limited crop (Fischer *et al.*, 1998; Austin, 1999) and grain yield is normally sink limited during the grain filling period (Borghi *et al.*, 1986; Savin and Slafer, 1991). Borrás *et al.* (2004) also reported that yield in wheat is mainly sink limited as grains grow mostly at saturated assimilate availability and that relative change in grain weight was about 0.12 of change in assimilate relative to grain thereby supporting sink limitation. However, some source limitation has been suggested (Grabau *et al.*, 1990; Ma *et al.*, 1990). Nevertheless, sink limitation appears to be the main barrier to yield progress and current modern varieties in UK (Shearman *et al.*, 2005) and at CIMMYT (Reynolds *et al.*, 2005) still seem to be more sink than source limited.

2.2 PHYSIOLOGICAL AND NUMERICAL MODELS FOR YIELD POTENTIAL ANALYSIS

Grain yield (GY) can be divided into either physiological or numerical components. Physiologically, it can be expressed as:

GY= AGDM * HI

Where AGDM and HI stand for above-ground biomass and harvest index (ratio of grain to total above ground biomass), respectively. When expressed as numerical components, yield can be considered as the product of plants per unit land area, ears plant^{-1}, spikelets ear^{-1}, grains spikelet^{-1} and average individual grain weight (Figure 2.1).

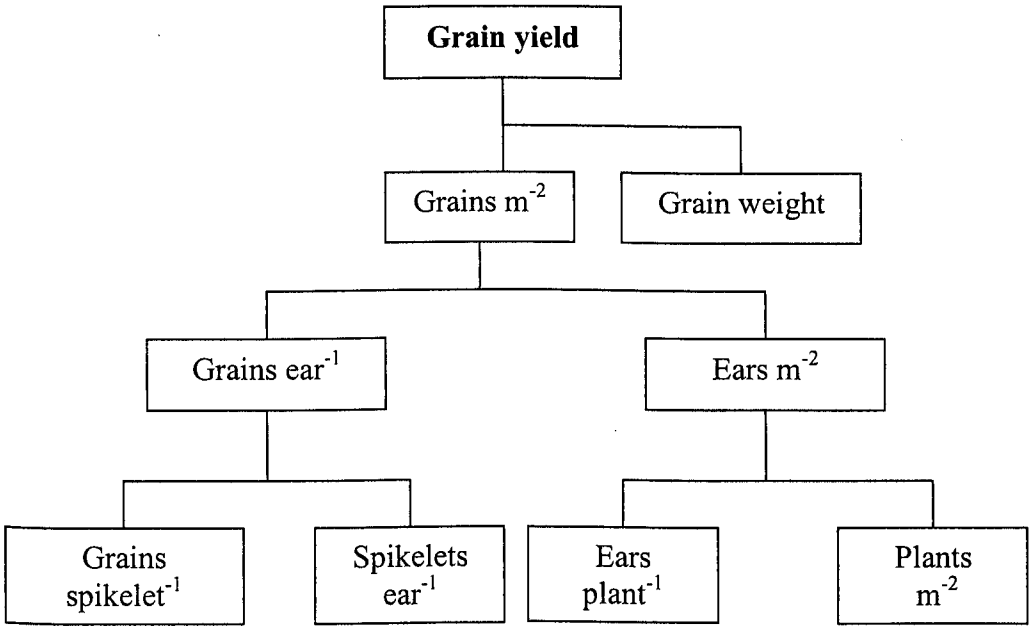


Figure 2.1. Main components into which grain yield can be numerically divided

Adapted from Slafer et al. (1996)

Since yield is a very complex trait, deconvoluting it into physiological or numerical components can help identify promising traits which can enhance yield potential. A negative relationship between grains m^{-2} and grain weight has been observed (Waddington *et al.*, 1986; Siddique *et al.*, 1989) though some exceptions can be found (Cox *et al.*, 1988; Calderini *et al.*, 1995). The number of grains m^{-2} is more associated with grain yield than individual grain weight under different environmental conditions (Fischer, 1985; Savin and Slafer, 1991) and cultivars (Slafer and Andrade, 1989). Thus, grains per unit land area has been effective in increasing yield in the past and is still the principal target trait among breeders to increase yield potential.

It is essential to understand the physiological basis of grain number variation in order to understand yield potential. However, due to compensation effects, it is difficult to isolate the effect of a given numerical yield component on grain number. Attempting to study the basis of yield through yield components is not completely satisfactory because of the difficulty in accounting for all these counteracting effects. When considering yield from the numerical components perspective the yield components are often negatively related to each other. That is, if breeding improves one component, others may be affected negatively. By contrast, the resource capture model (Equation 2.1) as suggested by Reynolds *et al.* (2005) is more informative where yield potential is expressed as the product of radiation interception (RI), radiation-use efficiency (RUE) and partitioning of above-ground biomass to grain yield (HI).

Yield potential = RI x RUE x HI **Equation 2.1**

This model may allow a more informative analysis of the physiological basis of yield if dry matter supply is what is ultimately limiting grain number.

2.3 PHYSIOLOGICAL DETERMINANTS OF YIELD POTENTIAL

2.3.1 Developmental stages

Wheat undergoes several developmental stages with each stage leading to morphological changes. Several scales have been developed for the recognition and definition of wheat developmental stages in the field. The three most popular scales used are the Feekes (1954), Haun (1973) and Zadoks (1974) scales, the latter being the most commonly used. The different developmental stages in wheat are summarized in Figure 2.2 with an arbitrary time-scale as the actual length of each phase can be affected by genetic and environmental factors, and the use of easily recognizable features of the apex as delimiters of the phases (Slafer and Rawson, 1994). The importance of these developmental stages in the determination of yield components is set out in the following sections. Vernalization, photoperiod and earliness *per se* genes affect thermal time to floral initiation and flowering and these genetic effects are discussed in detail further in Chapter 5.

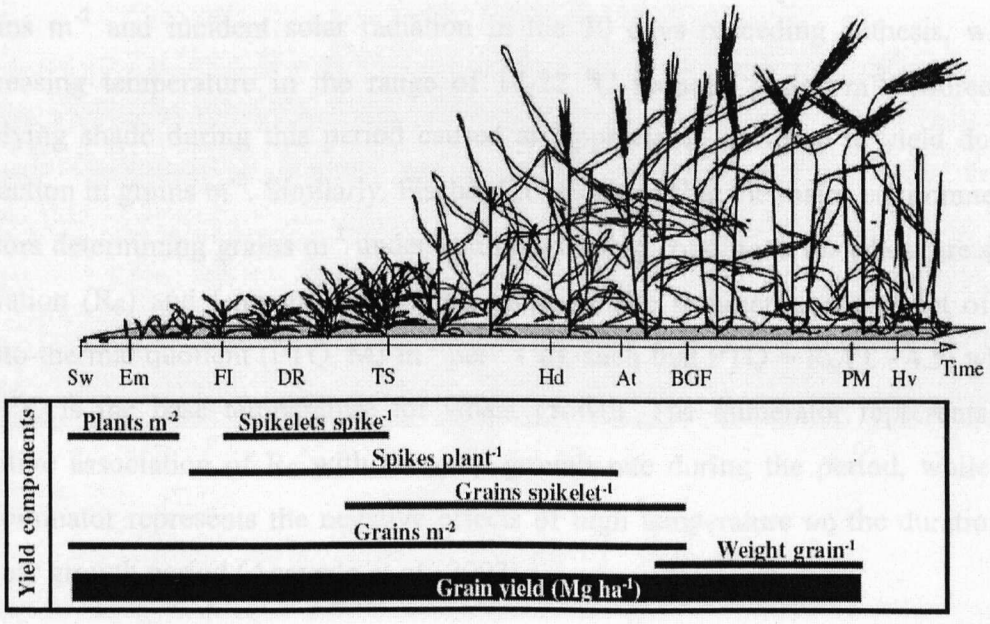


Figure 2.2. Schematic diagram of wheat growth and development adapted from Slafer and Rawson (1994) showing the stage of sowing (Sw), emergence (Em), initiation of the first double ridge (DR), terminal spikelet initiation (TS), heading (Hd), anthesis (At), beginning of the grain filling period (BGF), physiological maturity (PM) and harvest (Hv). Bars underneath the scheme show timing when different components of grain yield are produced.

2.3.2 Determination of grain sink size

2.3.2.1 Developmental stages

Particular developmental phases are very important in determining yield by influencing one or more of the components. Fischer (1985) has shown that the period between terminal spikelet initiation and anthesis is of paramount importance in determining yield usually between 20 and 30 days before flowering. The potential number of grains ear^{-1} is determined during stem elongation when a relatively small proportion of the floret primordia survive to produce fertile florets at anthesis, most of which set grains thereafter (Kirby, 1988). This short period coinciding with tiller mortality, floret death and rapid ear growth has been regarded as crucial for determination of grains m^{-2} (Fischer, 1985; Thorne and Wood, 1987; Savin and Slafer, 1991).

The role of solar radiation and temperature in determining grains m^{-2} in spring wheat was explained by Fischer (1985) who reported a positive linear relationship between grains m^{-2} and incident solar radiation in the 30 days preceding anthesis, whilst increasing temperature in the range of 14-22 °C reduced grains m^{-2}. Moreover, applying shade during this period caused an appreciable decrease in yield due to reduction in grains m^{-2}. Similarly, Fischer (2001) stated that the major environmental factors determining grains m^{-2} under optimal growing conditions for wheat are solar radiation (R_S) and temperature (T) and proposed the summarizing concept of the photo-thermal quotient (PTQ, MJ m^{-2} per °Cd), such that $PTQ = R_S/(T - 4.5)$ where 4.5 °C is the base temperature for wheat growth. The numerator represents the positive association of R_S with the crop growth rate during the period, while the denominator represents the negative effects of high temperature on the duration of the ear growth period (Acevedo *et al.*, 2002).

Indeed, Dhillon and Ortiz-Monasterio (1993) found a close positive association between the PTQ calculated for the rapid ear growth period (from 20 days prior to heading to ten days after heading) and grains m^{-2} when studying three spring-type wheat genotypes grown at ten dates of planting under optimum management. They concluded that genotypes maximized their yield when the PTQ value was highest between 20 days before and ten days after heading and suggested that all genotypes

should maximize their yield by flowering during the highest PTQ in the growing season.

Rapid ear growth normally occurs from the appearance of the penultimate leaf up to ten days past anthesis. In spring wheat, the duration of the rapid ear growth phase largely determines yield potential as shading during this period (20-30 days prior to anthesis) significantly reduced grains m^{-2} (Fischer, 1985). Furthermore, Reynolds *et al.* (2005) showed that when radiation is increased 15 days before anthesis by bending the outer rows plots to increase radiation interception in the inner rows, there was a significant increase in biomass, ears m^{-2} and grains ear^{-1}. Duration of ear growth showed genetic variability due to the presence of different alleles for *Ppd* and *Vrn* genes for photoperiod and vernalization sensitivity respectively as well as earliness *per se* genes (Slafer and Rawson, 1994). Final grain number and yield potential may be improved by manipulating these genes to increase the relative duration of the ear growth phase in the pre-anthesis period and hence fertile floret number per ear (Slafer *et al.*, 1996; Miralles and Richards, 1999; Gonzalez *et al.*, 2003).

Slafer and Savin (1994) reported that a greater ear index (ratio of ear to above-ground dry matter at anthesis) and a greater partitioning of assimilate to ear formation during the three weeks prior to anthesis resulted in more grains m^{-2}. Moreover, Slafer *et al.* (1990) reported that both ear dry weight at anthesis and ear index were closely related to grains m^{-2}. In addition, grain number was found to be closely correlated with assimilatory capacity during ear growth (Willey and Holliday, 1971). In summary, the period from early booting to anthesis occurring approximately 20 to 30 days before anthesis is considered very important in determining the two yield components (number of ears $plant^{-1}$ and grains $spikelet^{-1}$) which make up grains m^{-2}. Any factors affecting these components will therefore markedly influence grains m^{-2}.

2.3.2.2 Shoot production and death

Tillering allows plants to compensate for low plant populations or take advantage of good growing conditions. Tiller production usually ceases at around the onset of stem elongation (Hay and Walker, 1989). Tiller appearance is closely co-ordinated with the appearance of leaves on the main shoot. Thus, the rate of tiller production is related to the phyllochron which is the number of degree-days between successive emerging leaves (base temperature, 0°C). The end of tillering and the beginning of tiller death frequently coincide with terminal spikelet initiation and the start of stem elongation thereby causing a shift in the pattern of assimilate distribution. At the end of the tiller survival phase, which usually occurs at around anthesis, the final number of ears plant^{-1} and thus ears m^{-2} is usually established. The importance of tillering as a determinant of yield potential of wheat was recognised very early in the work of Engledow and Wadham (1923) who reported that at this stage the number of ears plant^{-1} was determined. Sparkes *et al.* (2006) suggested that the end of tiller production is triggered by a critical decline in light quality (R:FR). This was supported by Evers *et al.* (2006).

Tillers can form in the axils of the coleoptile and the lower leaves on the main shoot. The number of tillers formed depends on the variety and growing conditions. However, because of internal competition for assimilates between tillers and ear growth, many tillers which are produced do not survive which represents a loss of invested resources. Indeed, non-surviving shoots will reduce yield potential in the majority of situations by competing for assimilate with the developing ears in fertile shoots. Although the nitrogen content in aborted shoots can be re-translocated to surviving shoots, 90% of the dry matter cannot (Berry *et al.*, 2003). Richards *et al.* (1988) reported a tiller inhibition gene (*tin*) located on chromosome 1A contributing to the phenotype of 'gigas' wheat. The presence of the *tin* gene altered the pattern of axillary bud formation, so much so that low-tillering lines with this gene occasionally developed tillers from the first and second leaf axils whereas axillary buds failed to initiate and were absent in later leaf axils (Spielmeyer and Richards, 2004). Therefore, the number of tillers produced by a plant depends on the phyllochron, soil nutrients availability as well as other genetic and environmental factors.

2.3.2.3 Spikelet production and death

Wheat has a determinate inflorescence and the number of spikelets spike^{-1} is determined in a very narrow window from the initiation of the first to the terminal spikelet. A number of physiological and ecological factors affecting wheat plant primordia have been studied. These include vernalization requirement, photoperiod sensitivity and responses to nutrition (Midmore *et al.*, 1982; Holmes, 1973) and temperature and light intensity (Friend *et al.*, 1962). An extended review of the genetic control on the determination of spikelet number can be found in Chapter 5. Usually there is very little spikelet death in wheat.

2.3.2.4 Floret production and death and grain set

Initiation of floret primordia within the spikelet starts in the central spikelets well before terminal spikelet initiation, by which time many florets have already been initiated. Usually 7-11 florets are initiated per spikelet. However, during the period of rapid ear growth and tiller mortality, a significant portion of florets degenerates and by anthesis only 3-4 or even fewer fertile florets spikelet^{-1} can be found (Kirby, 1988). Bingham (1969) suggested that, to increase the survival rate of florets, there should be a more favourable partitioning of assimilates to the growing ear at this stage. In addition, manipulation of the photosynthetic environment can influence the number of potential grain sites by affecting fertile floret number per spikelet (Gifford, 1977). Extending the duration of pre-anthesis ear growth by manipulating the alleles involved with photoperiod sensitivity and vernalization can enhance floret survival by increasing assimilate availability to the ear (Reynolds *et al.*, 1999). Likewise, Miralles *et al.* (2000) reported the effect of photoperiod sensitivity on floret fertility through altering the duration of the stem elongation phase. Furthermore, Gonzalez *et al.* (2005) suggested a direct effect of photoperiod on floret number in addition to the effect through lengthening the ear-growth phase.

Several studies have demonstrated the role of plant hormones in determining grain set in wheat (Waters *et al.*, 1984; Zeng *et al.*, 1985, Rawson and Evans, 1970). Two growth regulators namely abscisic acid (ABA) and cytokinins have a marked effect on floret fertility. ABA has been found to promote grain abortion in wheat by reducing sugar supply to developing grains (Lee *et al*, 1988; Waters *et al.*, 1984).

Furthermore, Youssefian *et al.* (1992) showed that ABA induced floret infertility when applied over the 3 to 6 day period during the stage of pollen mother cell formation. Similar findings were reported by Zeng *et al.* (1985) with a 20% loss of florets due to ABA application during the same period.

Wang *et al.* (2001) found that ABA inhibited floret development, and decreased the number of fertile florets and grain set at almost all development stages, except at anther-lobe formation. In contrast, Zeatin (cytokinin) promoted floret development and significantly increased the number of fertile florets as well as grain set, especially at the stage of anther-lobe formation. Zeatin also increased the sugar concentrations in ears at anthesis. Lejeune *et al.* (1998) found that applied cytokinins stimulated sucrose transport into cultured ears of wheat and this resulted in more grain set. More recently, a gene from qualitative trait loci (QTL) analysis and fine mapping that increased grain number by approximately 21% was identified as *Gn1a* in rice. This gene codes for expression of a cytokinin oxidase/dehydrogenase enzyme (OsCKX2) that degrades cytokinin (Ashikari *et al.*, 2005). The latter reported that reduced expression of OsCKX2 caused cytokinin accumulation in inflorescence meristem thereby increasing the number of reproductive organs, resulting in enhanced grain yield.

2.3.2.5 Manipulation of pheno-phases to increase yield potential

To investigate the role that photoperiod sensitivity plays during the stem-elongation process in determining fertility of florets and grains m^{-2}, Gonzalez *et al.* (2005) used a factorial combination of different photoperiod (natural and 6 h extended photoperiod) and shading (unshaded and 67 ± 3% incident radiation) treatments during stem elongation of Buck Manantial, a photoperiod sensitive cultivar. The findings were that shortening duration of stem elongation through exposure to long photoperiod decreased the number of fertile florets and grains, which were positively associated with ear dry weight at anthesis. The number of fertile florets and grains m^{-2} was mostly explained by ear dry weight achieved at anthesis, independent of the treatment used to modify ear dry weight, reinforcing the hypothesis that breeding for increased photoperiod sensitivity during the stem elongation phase would improve grain number and yield due to extending the rapid ear growth phase.

Miralles and Richards (1999) manipulating the duration of the ear-growth phase by applying a short-day photoperiod to spring wheat and barley also showed a highly significant relationship between the duration of this phase and number of fertile florets per ear. By maintaining plants at a relatively short photoperiod during the growth phase, the number of days from terminal spikelet to heading was increased from 50 to 70 with 13 and 9 hour photoperiods, respectively, while the number of fertile florets per ear increased from 77 to 108. Several studies on the manipulation of the stem-elongation phase through different photoperiod regimes have shown that there was an increase in fertile florets due to a longer phase from terminal spikelet to anthesis (Slafer *et al.*, 2001; Miralles *et al.*, 2000; Gonzalez *et al.*, 2003). Furthermore, Gonzalez *et al.* (2003) reported higher ear dry weight at anthesis in addition to more fertile florets. Normally, the greater the dry weight of the ears, the smaller the floret mortality and hence the greater the number of fertile florets at anthesis, increasing ear fertility.

Calderini and Reynolds (2000) working on synthetic-derived hexaploid wheat at CIMMYT proposed that not only does extending the duration of ear growth phase increase yield potential by increasing potential grain number, but it can also increase grain weight potential. However, partitioning of biomass to favour the ear during stem elongation does not necessarily result in higher grain number as other factor may influence the determination of grain number. For example, Abbate *et al.* (1998) characterised a set of Argentinean cultivars released between 1980s and 1990s and demonstrated variation in the grain number to ear dry matter ratio at anthesis. It seems likely therefore that several factors during this critical growth stage finally determine grain number. Nevertheless, increasing the relative duration of ear growth by manipulating different environment conditions could be an avenue to increase grain yield.

2.3.2.6 Effects of large-ear phenotype on ear fertility

Various novel ear morphologies have been reported which have been the result of a recent breeding programme in Czech Republic. The greatest part of the original genetic materials was provided by research institutes in Zagreb, Novi Sad (Yugoslavia), St Petersburg (Russia) and CIMMYT (Mexico). These materials were mostly derived from wide crosses of *T. aestivum* L. with tetraploid species. The

different ear morphologies are summarised below as investigated by Martinek and Bednar (2004) in Figure 2.3.

A. **Normal spike structure** (NS), a single spikelet is situated in one node of ear rachis.
B. **Vertical sessile spikelets** (VSS), sometimes designated "banana twin spikelets", when two or three spikelets grow up vertically in an ear rachis node.
C. **Tetrastichon** (TSS – tetrastichon sessile spikelets) when three or mostly only two spikelets are sessile close to each other in a horizontal position in a ear rachis node.
D. **Floribunda** – a high number of spikelets grow up in a common ear rachis node close to each other and, at the same time, above each other.
E. **Spike branching** (TFS – transitional forms spikelets). Branchiness of the turgidum type was incorporated in *T. aestivum* L. using wide crosses between *T. aestivum* L. and branching forms of *T. turgidum* L.
F. **Larger size of glumes and lemmas** (LG). There are some wheat forms (*T. aestivum* L.) in which a larger size of glumes was transferred by wide crosses with *T. polonicum* L.
G. **Screwedness of spike rachis** (SCR – screwed spike). There are usually both directions of screwedness.

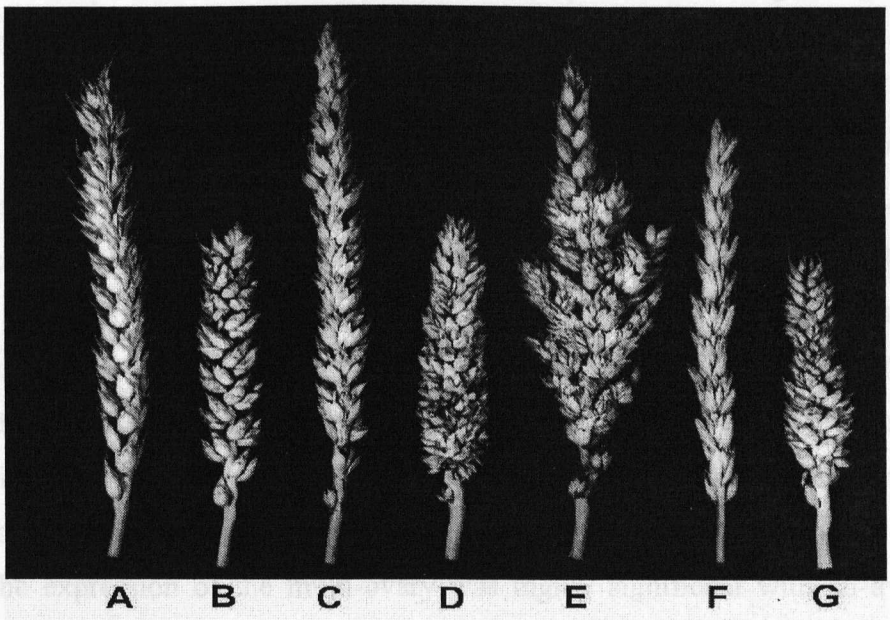


Figure 2.3. Ear morphological structure of selected gene resources

Source: (http://wheat.pw.usda.gov/ggpages/DEM/9IWGS/gene_resource.html)

All the above-described ear morphological changes however do not necessarily result in increases in grains m^{-2}. A limited number of genetic resources may be important for breeding such as those which have a higher grain number per ear rachis node (i.e. multifloret forms of NS and SCR or forms with a higher spikelet number VSS, TSS, and MRS). However, these gene resources are not widely used in breeding programmes worldwide because of some adverse characters (shrivelled grains) transferred from other less cultivated wheat species (Martinek and Bednar, 2004).

Trying to modify ear morphology is not a new concept; it has been attempted for sometime. For instance, Atsmon and Jacobs (1977) made crosses with large ears resulting in progeny having a maximum of 30 spikelets, individual grain weight of 63 mg and nine grains per spikelet. The resulting genotypes were commonly referred to as 'gigas'. Gigas wheat have restricted tiller number, large leaves, large ears and high grain number per ear when contrasted with conventional wheats (Richards, 1988), thus approaching the Donald ideotype of sparse tillering. Tiller inhibition in gigas phenotypes was attributed to a single recessive gene *tin* located on chromosome 1AS (Richards, 1998; Spielmeyer and Richards, 2004). In Australia, the *tin* gene has been reported to be advantageous in well watered conditions and also in dry environments (Richards, 1988; Duggan *et al.*, 2005). In addition, Duggan *et al.* (2005) showed that yield components were altered with lines possessing *tin* gene as fewer ears were produced at maturity but there is compensation via higher number of grains ear^{-1} and grain weight.

Dencic (1994) made crosses with branched tetrastichon lines to produce genotypes to induce changes promoting larger sink capacity. Through exhaustive recombination and selection, the following traits were improved: ear length (16%), number of spikelets ear^{-1} (10%), grains $spikelet^{-1}$ (9%) and grains m^{-2} (18%). Four lines were developed with 13% higher yields than the standards. Similarly, Chen *et al.* (1998) suggested the multi-ovary trait as another novel ear morphology trait to increase ear fertility. The parental source of the multi-ovary trait is originally from China and has been crossed with five elite spring wheat cultivars at CIMMYT (Reynolds *et al.*, 2005). The expression of the multi-ovary was highly significant with an average number of grains $floret^{-1}$ varying from 1.3 to 2.8 depending on genetic background. The trait was best associated with increased grains ear^{-1}. Ears m^{-2} was not affected

but grains m^{-2} was significantly increased by as much as 5500 grain m^{-2} in one of the cultivars (Hartog) (Reynolds *et al.*, 2005). However, there was trade-off of increasing grain m^{-2} in the lines with either tetrastichon or multi-ovary traits resulting in low grain weight (Dencic, 1994; Reynolds *et al.*, 2005).

Ear fertility is one of the most relevant strategies to increase genetically yield potential and novel large-ear phenotypes can provide a new source of variation in the hexaploids wheat gene pool. Yield is a complex trait and usually complex traits are governed by a number of genes known as quantitative trait loci (QTL) derived from natural variations (Yano, 2001). Many studies have demonstrated the use of QTL analysis as a robust approach in identifying useful genes of importance for breeding (Doebley *et al.*, 1997; Yano *et al.*, 2000; Kojima *et al.*, 2002). Suenaga *et al.* (2005) working on 'gigas' doubled-haploids (intervarietal cross between a Japanese cultivar 'Fukuhokomugi' and Israeli wheat line 'Oligoculm') reported a major QTL on chromosome 1AS that controls ears $plant^{-1}$, whereas eight QTLs were found for ear length, the largest detected on 2DS.

Jantasuriyarat *et al.* (2004) found a QTL for ear morphology on the long arm of 4A which explained 11%, 14% and 12% of the variation in ear length, spike compactness and spikelet number respectively. In addition, Sourdille *et al.* (2000) reported 4-6 QTLs for ear length, number of spikelets per ear and ear compactness in a DH population, whilst Narsimhamoorty *et al.* (2006) found ten putative QTL of which 3 QTL were derived from a synthetic parent for grain hardness, grains ear^{-1} and tiller number. QTL analysis is also being extensively used in other cereals, especially rice, whereby important agronomic traits are being localised, e.g. the gene (*Gn a1*) as previously mentioned enhancing grain number in rice (Ashikari *et al.*, 2005). Likewise, Tian *et al.* (2006) reported the presence of a QTL *gpa7* located on short arm of chromosome 7 in rice that regulates grain number per panicle. Thus, the use of QTL in locating useful genes can enhance breeding programmes by providing markers for identifying parents for use in crosses and in marker assisted selection. In addition, genes in rice can provide candidate genes in wheat for ear fertility.

2.3.2.7 Potential grain weight

Endosperm cell number represents the potential of the grain to accumulate starch, and hence the potential final grain size (Brocklehurst, 1977; Singh and Jenner, 1984) apparently by allowing a higher rate of grain growth as more sites are available for starch deposition. The total number of endosperm cells per grain is in the region of 100,000 (Briarty and Hughes, 1979; Jenner *et al.*, 1991). The regulation of endosperm cell number by assimilate supply allows the source and sink to be maintained in a balance, with the potential grain weight geared to the likely subsequent assimilate supply (Singh and Jenner, 1984). Thus, either source or sink may limit grain growth. However, source and sink are not independent. The source pre-anthesis determines the later grain sink capacity, while grain sink strength can feedback to moderate the photosynthetic source (Evans, 1993). Though assimilate availability markedly affects grain growth, storing carbohydrates in the grain is related to other factors such as floret volume (Millet, 1986), the number of endosperm cells (Brocklehurst, 1977) and A-type starch granules (Chojecki *et al.*, 1986).

Cell division ceases between 14-20 d post-anthesis (Briarty and Hughes, 1979; Nicolas *et al.*, 1985), coinciding with termination of rapid net water deposition in the grain (Schnyder and Baum, 1992) at which time potential grain size is largely determined. Grains reach their maximum volume much earlier than their maximum dry weight because endosperm cells are filled mainly with water first to establish maximum volume, and then water is replaced by assimilates (Martinez-Carrasco and Throne, 1979). It is this rapid increase in cell water content which determines cell and hence grain volume. However, maximum grain volume might be physically restricted by glumes (Millet, 1986). Therefore, potential grain size is ultimately the result of interactions between endosperm cell number and floret cavity volume and it is larger in central spikelets.

The period immediately before anthesis is important for the determination of potential grain weight as it is during this period that the structures of the ovary are formed, which are potentially important in determining grain development (Rijven and Banbury, 1960). This was supported by the observation in barley (Scott *et al.*,

1983) that there is a positive relationship between carpel weight at anthesis and final grain weight, suggesting that the carpel growth period may be important in the determination of potential grain weight. Calderini and Reynolds (2000) similarly found carpel weight at anthesis to be partially associated with the regulation of grain weight potential in synthetic hexaploids lines of wheat. Calderini *et al.* (2001) reported that temperature between booting and anthesis was closely related to grain weight differences due to differences in carpel weight at anthesis.

It has been demonstrated that high pre-anthesis temperatures can detrimentally affect the determination of carpel size (Calderini *et al.*, 1999). In addition, Weigand and Cuellar (1981) reported a decrease of an average of 1.5 mg for each degree Celsius increase during grain filling. Wardlaw (1994) demonstrated that high temperatures, for instance 22-27 °C compared with 15-18 °C, from the four leaf stage to anthesis may negatively influence final grain weight. This could be due to a shortening of the pre-anthesis period, or a smaller canopy size, reducing the pre-anthesis assimilate production and thus carpel size.

2.3.4 Traits to improve anthesis biomass

2.3.4.1 Traits to increase radiation interception

Higher biomass may be achieved by increased interception of radiation by the green canopy area, higher radiation-use efficiency and improved source and sink balance (Reynolds *et al.*, 2001). Increase in green canopy area should lead to increased radiation capture as the canopy is the light receptor. However, relatively little extra radiation is intercepted as GAI is increased above about 5; the extinction coefficient (k) is calculated from green area index (GAI) and fractional absorbance, using a modified version of Beer's Law (Equation 2.2), where Io is the incident radiation and I is the amount of radiation transmitted below a GAI value of L (Monsi and Saeki, 1953).

$$k = -\ln(I/Io)/L \qquad \textbf{Equation 2.2}$$

Calderini *et al.* (1999) and Shearman *et al.* (2005) reported leaf area index and light extinction coefficient to be poorly correlated with genetic yield progress in wheat. It appears that most modern varieties have already achieved a green area maximum above 5 and therefore there is little scope to increase radiation capture by increasing GAI. However there might be some scope to increase radiation at the start of the stem-elongation phase and also during the end of grain filling period. This could be achieved through plants possessing 'stay-green' traits.

2.3.4.2 Traits to increase radiation-use efficiency

The interception of solar radiation by plants and the utilization of radiant energy for plant biomass production represent the fundamental processes governing crop growth and yield (Monteith, 1977). Radiation-use efficiency can be defined as the amount of carbon assimilated per unit intercepted radiation (g MJ^{-1}). RUE can be improved if incoming radiation is shared amongst several leaf layers in the canopy resulting in less light saturation of upper leaves and higher photosynthetic conversion efficiency (Monteith, 1965). In addition, higher RUE is associated with smaller, thicker flag leaves (Shearman *et al.*, 2005). A wheat crop absorbs up to 90% incident PAR and RUE tends to decline as the extinction coefficient (k) increases (Green, 1989). Modern varieties have shown an increase in RUE when compared to older varieties in Australia (Siddique *et al.*, 1989b; Yanusa *et al.*, 1993), Israel (Blum, 1990) and in UK (Shearman *et al.*, 2005). However, studies on Argentinean wheat varieties over the years showed no changes in pre-anthesis RUE (Calderini *et al.*, 1997). Miralles and Slafer (1997) found that there was lower RUE in the presence of semi-dwarf alleles probably due to reduced height resulting in poorer canopy architecture and radiation distribution.

If genetic gains in wheat yield potential are to be accelerated through greater ear biomass at anthesis, a key approach would be to increase crop radiation-use efficiency (Reynolds *et al.*, 2000). Physiological avenues suggested by Reynolds *et al.* (2000) to achieve this include: photosynthetic metabolism, canopy photosynthesis, determination of grain number and size (sink strength), vascular transport of water, nutrients and assimilates, respiratory costs and buffering of these processes to environmental fluxes. In theory, RUE of a crop could be improved by genetic

manipulation at any of these levels, since their interaction determines net assimilation.

2.3.4.2a Canopy architecture

Canopy architecture could be modified so that there is more uniform distribution of the light within the canopy. Photosynthetic efficiency of a crop with GAI above *ca.* 4 is increased if the angle of the leaves is more erect so that there is reduced light saturation of the upper leaves (Innes and Blackwell, 1983; Araus *et al.*, 1993). Thus, more radiation would be available to the lower leaves to contribute to total crop biomass (Hay and Walker, 1989). An erectophile leaf has been suggested as a trait that could increase crop yield potential by improving RUE in high radiation environments. Innes and Blackwell (1983) showed a 4% yield advantage in winter wheat isolines having erect leaves in the UK. Furthermore, Araus *et al.* (1993) reported that although wheat genotypes with erect leaves were not conclusively superior to genotypes with prostrate leaves there was a slight increase in yield as the lines had 5 to 16% more grains m^{-2} and higher stomatal conductance. In fact, the majority of CIMMYT's highest yielding durum and bread wheat lines possess this trait (Fischer, 1996) and in the UK most modern varieties already have erect leaves (Shearman *et al.*, 2005). Hence, there may be relatively little scope to optimise further on canopy architecture traits in modern wheat germplasm.

Other possible ways to increase pre-flowering radiation use efficiency at the leaf and the biochemical level are to:

- optimize leaf N distribution through the canopy
- Changes in chlorophyll a:b ratio to increase adaptation of lower canopy leaves to low light intensity
- increase Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) specificity for CO_2

These physiological avenues are considered further below.

2.3.4.2b Leaf nitrogen distribution

Further avenue to increase RUE is to increase net photosynthesis rate through altering nitrogen distribution within the leaf layers of the canopy. The photosynthetic capacity of leaves is related to their nitrogen content, primarily because the proteins of the Calvin cycle and thylakoids represent the majority of leaf nitrogen (Evans, 1989). In C_3 plants, approximately 60-80% of leaf N is invested in the chloroplast (Evans and Seemann, 1989). Rubisco accounts for 25-30% of leaf N in leaves (Evans and Seemann, 1989) in C3 and about 12% of total N in C3 plants during vegetative growth (Osaki *et al.*, 1993). The allocation pattern of leaf nitrogen throughout a crop canopy can theoretically affect crop photosynthetic performance and RUE.

Leaf nitrogen concentrations decline with depth in closed canopies which is generally believed to be related to a changing radiation environment and it has been suggested by some researchers that plants allocate nitrogen in order to optimize total canopy photosynthesis (Evans, 1989). It is largely recognised that the light-saturated photosynthetic rate is positively correlated with leaf nitrogen content (Osman and Milthorpe, 1971; Yoshida and Coronel, 1976; Peng *et al.*, 1995; Takano and Tsunoda, 1971; Sadras *et al.*, 1993). Evans (1983) reported both linear and asymptotic relationships in wheat.

The findings of Dreccer *et al.* (2000) showed that wheat canopy, leaf N distribution closely followed the light gradient and thus maximized canopy photosynthesis. The association between light absorption by leaves and their N content was more stable under high than under low N supply. At low N supply, restricted canopy expansion and N remobilization had an effect on the vertical distribution of leaf N. This indicates that although the local light environment plays a major role in dictating leaf N distribution (Drouet and Bonhomme, 1999), the ultimate regulation of resource allocation is coordinated at the plant level. However, Shiratsuchi *et al.* (2006), using a simulation model, showed that daily canopy photosynthesis could be increased in rice by optimization of leaf N distribution, especially in high density canopy. Using a light model, Hirose and Wenger (1987) found that specific leaf nitrogen content (SLN) of shaded leaves at the bottom of the canopy of *Solidago altissima* stand tended to be greater than the optimum predicted from their N photosynthesis/canopy

light distribution model. Critchley (2001) suggested that nitrogen distribution in UK wheats e.g. Soissons and Spark, may already be close to optimal in comparison with predictions by Hirose and Wenger (1987). Therefore, optimising leaf nitrogen distribution through the canopy to increase the photosynthetic efficiency of the crop might not be successful in achieving this aim.

2.3.4.2c Chlorophyll a:b ratio to increase adaptation of lower canopy

Most plants have two forms of chlorophyll (Chl), designated as Chl a and Chl b. Early studies as reviewed by Thornber (1975) established that Chl b is not essential for the primary photochemical events of photosynthesis but plays a purely light harvesting role. It was further established that the photosynthetic reaction centres bind primarily Chl a while the light-harvesting antenna complexes bind both Chl a and Chl b. Chl b and its binding properties, particularly those associated with the major light-harvesting complex of PSII (mobile or peripheral LHCII), have been implicated in the regulation of energy distribution between the two photosystems (Kyle *at al.*, 1983). In addition, changes in content of Chl b and its binding properties have been shown to play a major role in adaptation to varying light intensities (Boardman, 1977; Anderson, 1986).

Decreasing the chlorophyll a:b ratio, to increase adaptation of lower canopy leaves to low light intensity, might help increase photosynthesis. Kleima *et al.* (1999) suggested that it is possible to replace most of the Chl a molecules in the light harvesting complex II (LHCII) with Chl b without destroying the structure of the complex but a decrease in the Chl a/b ratio strongly affects singlet and triplet energy transfer efficiencies within LHCII. Thus, proper functioning of the light-harvesting complex seems to require the correct Chl a/Chl b stoichiometry. In addition, Gutiérrez-Rodríguez *et al.* (2000) found that the chlorophyll content of spring wheats in a warm, irrigated environment was linked to increased net carbon dioxide fixation rate. Similarly, Gutiérrez-Rodríguez *et al.* (2004) in Mexico found positive correlations between chlorophyll content and yield and biomass amongst spring wheat genotypes. Therefore, changing the chlorophyll ratio can potentially enhance biomass production and ultimately yield.

2.3.4.2d Rubisco specificity factor

Increasing the affinity of Rubisco for CO_2 and decreasing its oxygenase activity could increase photosynthesis and hence RUE due to a reduction in photorespiration. If the catalytic properties of Rubisco could be engineered to reduce or eliminate its oxygenase activity, photosynthesis should be increased at all light intensities with increases in light-saturated leaf photosynthetic rate reaching up to 20% at 20 °C at ambient carbon dioxide (Austin, 1999). Uemura *et al.* (1997) found that the thermophilic red algae *Galdieria partita* and *Cyanidium caldarium* possessed a rubisco specificity 2.4 to 2.5 times higher than of wheat plants, offering the possibility of genetically transformed wheat Rubisco to enhance its carboxylase activity. Furthermore, Drake and Gonzalez-Meler (1997) reported that plants acclimate to elevated CO_2 with a reduction in Rubisco concentration and the subsequent reduced investment in Rubisco and associated enzymes may lead to a decline in dark respiration rate which could further improve RUE. Although this seems very promising, it is relevant more as a long-term prospect for increasing RUE in wheat.

2.3.5 Determinants of grain source size

Grain growth (dry matter) is mainly associated with current assimilate production transferred directly to the grain during the post-anthesis phase (Rawson and Evans, 1971; Slafer and Savin, 1994). The flag leaf is the principal source of photoassimilates imported by grains during grain filling (Rawson *et al.*, 1976). Evans and Rawson (1970) showed that the flag leaf can provide more than 50% of the photosynthetic requirements of the ear even in awned varieties. Moreover, up to 80% of carbon dioxide fixed by the flag leaf is translocated to the ears during rapid grain filling period (Thorne, 1982). Studies with labelled carbon dioxide also showed that most of the photosynthate of the developing grain is supplied by the flag leaf and ear (Wardlaw, 1968) and that the post-anthesis size and duration of green area would influence the rate and duration of dry matter accumulation, thus affecting yield (Gebeyehou *et al.*, 1982; Siddique *et al.*, 1989a)

2.3.5.1 Stem carbohydrate reserves

The wheat stem can function both as a source and a sink. Borrell *et al.* (1989) showed that the stem functioned as a sink until 19 days after anthesis, then became an exporter of assimilates. There is a vast body of literature which supports the role of stem reserves in providing carbohydrates to grains during grain filling in wheat (Schnyder, 1993; Blum *et al.*, 1994; Seidel, 1996; Ruuska, 2006). The accumulation of non-structural carbohydrates can reach more than 40% of the stem dry weight under favourable conditions (McCaig and Clarke, 1982; Blacklow *et al.*, 1984), yet the pre-anthesis reserves may account for only 5-15% of the final grain yield (Austin *et al.*, 1977; Wardlaw 1990; Borrell *et al.*, 1993). When photosynthetic activity is inhibited by stress conditions after anthesis, however, grain filling becomes more dependent on mobilized stem reserves, which then may represent 40-60% of the dry matter that accumulates in the grain (Bidinger *et al.*, 1977; Bell and Incoll, 1990; Blum *et al.*, 1994). Stem reserves from pre-anthesis are being increasingly recognised as an important source of carbon for grain filling when current photosynthesis is inhibited by drought, heat or disease stress during this stage (Blum, 1998; Yang *et al.*, 2000).

In wheat, carbohydrates available for mobilization at the stage of maximum concentration of water-soluble carbohydrates consist of 85% fructans and 10% sucrose (Blacklow *et al.*, 1984). Wardlaw and Willenbrink (1994) reported that there are differences among internodes in the amounts of carbohydrates that are stored and mobilized. Most stem sugars are accumulated in the upper internodes (Brooking and Kirby, 1981) and longer term carbohydrate reserve, i.e. fructans, are stored in the lower portions of the internodes enclosed by the leaf sheaths (Pollock and Cairns, 1991).

Cruz-Aguado *et al.* (2000) showed that the second internode from the top supplied a larger amount of non-structural carbohydrates to the grains than the other two internodes and that source-sink status does have a role in the mobilization of stored carbohydrates. In addition, Borrell *et al.* (1993) reported that mobilisation of stem reserves was positively related to final height although the presence of *Rht* alleles does not confer increased mobilisation, the efficiency of use was higher in *Rht-Blb*

than *Rht-D1b* in near isogenic lines. Also, plant height had an effect on stem storage reserves as *Rht-B1b* and *Rht-D1b* genes have been found to reduce reserve storage in a spring wheat background in Australia by 35 and 39% respectively due to a reduction of 21% in stem length (Borrell *et al.*, 1993). Ehdaie *et al.* (2006) demonstrated genotypic variation for stem reserve accumulation and mobilisation and reported that 50% of the stem dry matter was, on average, stored in the lower internodes. Likewise, Ruuska *et al.* (2006) found that examining a large and diverse group of 22 genotypes, variation in WSC concentration, when measured over multiple environments of the southern Australian wheat belt had a high genotypic component together with relatively small interaction of genotype and environment. Those authors showed that WSC is a heritable trait supporting the view that stem reserves is one important physiological trait to consider for raising yield potential by breeding.

2.3.5.2 Senescence and 'stay green'

Although the flag leaf is largely recognised as being the main photosynthetic apparatus during the post-anthesis period, all green tissues of the wheat plant are capable of photosynthesis (Evans and Rawson, 1970). Light interception decreases during the post-anthesis period with the onset of senescence. Exactly when senescence starts in a variety depends upon nitrogen and water supply and incidence of leaf diseases (Hay and Walker, 1989) but the rate of senescence is dependent mainly on accumulated temperature (Biscoe and Gallagher, 1977) with higher temperatures increasing the rate due to more rapid re-mobilisation of nitrogen (Noodén and Guiamét, 1989).

Senescence is viewed as an internally programmed degeneration leading to death but is an important developmental process occurring in many tissues and serving different purposes (Noodén *et al.* 1997). The senescent leaves become a source of nitrogen and minerals for developing grain (Turgeon, 1989). The process of senescence will be triggered even though the environmental conditions may be favourable for continued growth once the plant reaches a certain age (Buchanan-Wollaston *et al.*, 2003; Yoshida, 2003).

The specific mechanisms by which senescence is regulated are not fully understood. However, there is clear evidence that it is driven by many specific genes which may be either highly expressed (Senescence Associated Genes or SAG) or downregulated (Senescence Down-regulated Genes or SDG) during the senescence process (Gepstein, 2004; Lim *et al.*, 2003; Nam, 1997). Among the SDG are genes coding for the ribulose bisphosphate carboxylase/oxygenase or Rubisco, small subunit (*rbcS*) and the chlorophyll a/b binding protein (*cab*) (Rampino *et al.*, 2006). The latter working on a durum mutant 504 which has delayed leaf senescence, reported an altered expression profile of genes, i.e. an extended expression of photosynthesis-related genes (*rbcS* and *cab*) and a higher expression of Rubisco activase to account for the longer stay-green period. On the other hand, Uauy *et al.* (2006) found that the ancestral wild wheat allele encodes a NAC transcription factor (*NAM-B1*) that accelerates senescence and increases nutrient remobilization, whereas modern wheat varieties carry a non-functional NAM-B1 allele. Moreover, these authors showed that a reduction in RNA levels of the multiple NAM homologs by RNA interference delayed senescence by more than three weeks.

The degradation of the above-mentioned proteins, especially Rubisco, and chlorophyll loss with concomitant yellowing of leaves, slowly reduces the leaves photosynthetic capacity of leaves. Furthermore, radiation interception is reduced as the plant ages. Varieties with lower extinction coefficients tend to senesce more slowly as light penetrates further down the canopy (Green, 1989). Genotypes with erect leaves are therefore more likely to have an advantage over prostrate ones in capturing more light and delaying senescence.

Thomas and Howarth (2000) further classified the stay-green phenotype as 'functional stay green' and 'non-functional stay green/cosmetic stay green', the former continuing to photosynthesize for a longer period and the latter remaining green due to retention of chlorophyll but lacking photosynthetic competence. In addition, Spano *et al.* (2003) found that 'stay green' mutants of durum wheat were functionally 'stay green' and Borrell *et al.* (2000, 2001) showed that the stay-green trait is crucial to maintain high yield in sorghum especially during drought during grain filling.

Jenner and Rathjen (1975) reported that the onset of the declining phase of accumulation of starch was due to a fall in the capacity of the grains to synthesize starch and was not attributable to the reduction in the supply of assimilate. Prolonging the photosynthetic capacity of leaves has been suggested as being a means to improve yield (Thorne, 1971). The incorporation of 'stay green' trait to improve yield potential in a breeding programme is probably desirable as it allows a longer period of active photosynthesis during grain filling. However, in sink limited crop, stay-green may not necessarily increase yield in favourable seasons. Moreover, Reynolds *et al.* (2005) stated that there is genetic variation for stay-green trait which can be easily selected either visually or using spectral reflectance techniques.

2.3.5.3 Post anthesis RUE

Sinclair and Muchow (1999) reported that RUE during post-anthesis was lower than pre-anthesis, probably due to steady decline in rate of photosynthesis once maximum leaf area has been reached (Calderini *et al.*, 1997). There is genotypic variation in the ability of the canopy to intercept radiation and convert it into biomass (Green, 1989). The presence of dwarfing genes has an effect on post anthesis RUE whereby the lines with *Rht* alleles showed higher RUE values compared to tall lines (Miralles and Slafer, 1997). Similar findings were reported by Fischer and Stockman (1986).

There is some evidence that varieties introduced in recent decades have higher post-anthesis RUE than older varieties. Calderini *et al.* (1997) working with seven Argentinean cultivars released from 1920 to 1990 found similar pre-anthesis RUE and crop growth rates in all cultivars. However, after anthesis, the two oldest cultivars accumulated the least biomass and their radiation-use efficiencies and crop growth rates were smaller compared to the modern cultivars. Miralles and Slafer (1997) reported that post-anthesis RUE was closely and positively associated with the number of grains set per unit biomass at anthesis. They suggested that there was a regulatory effect of the sink size on the efficiency of the crop to convert radiation into biomass during this period. Similarly, Reynolds *et al.* (2005) reported that increasing the sink strength due to more grain m^{-2} increased the demand for photosynthesis during grain filling thus leading to a significant rise in post-anthesis RUE in spring wheat genotypes in Mexico.

2.4 PHYSIOLOGICAL BASIS OF GENETIC GAINS IN YIELD WORLDWIDE TO DATE

Wheat breeding in recent decades has increased grain yield in most countries with genetic gains *ca.* 1% per year (Sayre *et al.*, 1997; Shearman *et al.*, 2005; Brancourt-Hulmel *et al.*, 2003). The changes in grain yield were mainly associated with changes in number of grains m^{-2} rather than with changes in individual grain weight. From a physiological point of view, grain yield is determined by two components: amount of biomass and harvest index. The increase in grain yield during the 1960s to 1990s has been ascribed mainly to increases in harvest index rather than biomass production (Austin *et al.*, 1980; Nelson, 1988; Slafer and Andrade, 1991; Reynolds *et al.*, 1999).

Studies of sets of historic wheat cultivars worldwide have generally shown grains m^{-2} (usually due to grains ear^{-1}) and HI to be positively correlated with yield progress, but no similar positive correlation between biomass at harvest and yield progress (Slafer *et al.*, 1994; Waddington *et al.*, 1986; Austin *et al.*, 1989). Much of the increase in grain yield during recent decades has been due to the partitioning of more assimilate to the harvestable component due to the presence of dwarfing genes which were introduced during the 1970s and 1980s. This altered partitioning of assimilate to the developing ear has resulted in higher grain yields, probably due to more fertile florets $spikelet^{-1}$. Moreover, increased partitioning of biomass to ear growth in the pre-anthesis period was linked as a pleiotropic effect of dwarfing genes (Gale and Youssefian, 1985). A recent review by Flintham *et al.* (1997) confirmed the pleiotropic effects of *Rht* alleles on yield component resulting in increases in grain number.

A few more recent investigations, however, have shown harvest above-ground biomass to be positively associated with yield progress (Siddique *et al.*, 1989; Donmez *et al.*, 2001; Shearman *et al.*, 2005). Furthermore, during the 1990s there have been several reports of biomass increases attributed to introduction of alien chromatin into wheat germplasm: namely, the 1BL.1RS wheat–rye translocation in spring wheat in Mexico (Villareal *et al.*, 1995) and winter wheat in the Great Plains (Carver and Rayburn, 1994), and the 7DL.7Ag wheat-*Agropyron elongatum*

translocation in spring wheat in Mexico (Singh *et al.*, 1998; Reynolds *et al.*, 2001). In addition, synthetic hexaploid wheat, obtained by crossing tetraploid wheat and *Aegilops tauschii*, has been found to be a source of genetic diversity for important physiological traits such as enhanced photosynthetic rate (del Blanco *et al.*, 2000). Reynolds *et al.* (2007) found that synthetic derived wheat had higher biomass than recurrent parents.

Yield improvement in wheat has historically been largely due to increased partitioning of above-ground dry matter to grain, not only because of the introgression of *Rht* genes (Gale and Youssefian, 1985; Calderini *et al.*, 1995) but also as a result of continued selection for yield in the post-green revolution period (Waddington *et al.*, 1986; Calderini *et al.*, 1995 and Sayre *et al.*, 1997).With the HI of modern cultivars approaching the estimated theoretical limit of 0.62 (Austin *et al.*, 1980) there is increasing concern that HI still appears to account for the majority of improvements in genetic yield potential in wheat. Future improvements in biomass whilst maintaining HI will be an important objective of breeding programmes in the years ahead. Further reduction in plant height to improve partitioning is unlikely to be profitable as optimal plant heights have already likely been achieved (Miralles and Slafer, 1995; Fischer and Quail, 1990; Flintham *et al.*, 1997). Although, small improvements in HI are still possible, they may be increasingly difficult to achieve. Therefore, more focus on biomass production is likely to be required by wheat breeders in future.

Enhanced partitioning of assimilates from the stem to the ear has been ascribed to the *Rht* dwarfing genes; mainly *Rht-B1b* and *Rht-D1b* located on chromosome 4A and 4B, respectively. In UK germplasm the semi-dwarf allele *Rht-D1b* was first incorporated, originally from Japanese cultivar Norin 10 in the 1970's (Austin *et al.*, 1980). The majority of varieties released since then contain this allele (Austin *et al.*, 1989). Youssefian *et al.* (1992) found that the *Rht* allele reduced stem length through insensitivity to gibberrellic acid which results in smaller cell dimensions. Austin *et al.* (1980) reported that the new varieties with the *Rht* allele had greater grains spikelet^{-1} in the central spikelet than their predecessors. Furthermore, Fischer and Stockman (1986) found a positive association between *Rht-D1b* and *Rht-B1b* and grains m^{-2} in spring wheat compared to tall checks. The use of the semi dwarf genes was primarily

to reduce lodging risks and greater partitioning of assimilates to ear was a pleiotropic effect resulting in higher HI (Gale and Youssefian, 1985). The most studied gibberrellic acid sensitive dwarfing genes are *Rht-B1b (Rht 1)* and *Rht-D1b (Rht 2)* and *Rht* 3; however, several other major dwarfing genes have been reported (Gale and Youssefian, 1985).

Considering the partitioning of assimilate to favour the ear and increase grains m^{-2} it is not surprising that ear dry matter at anthesis has generally increased with breeding worldwide. For example, modern spring wheat cultivars released in 1980s compared to old cultivars from 1860s in Australia showed higher ear dry matter at anthesis than older cultivars although differences in above ground biomass were not significant (Siddique *et al.*, 1989a). The authors further demonstrated that heavier ears of modern cultivars at anthesis compared to old cultivars was due to faster rates of dry matter accumulation immediately before anthesis, exclusively due to more favourable assimilate partitioning towards ears.

Many authors have reported that the genetic gain in yield from semi-dwarf varieties is attributed to greater partitioning of dry weight to ears at anthesis. However, Abbate *et al.* (1998) observed that in Argentinean wheat cultivars the genetic gains in yield were through an increase in grains per unit ear dry weight at anthesis (more grains per gram of ears) rather than ear index. The worldwide trends for higher grains m^{-2} through breeding has been attributed mostly to increases in grains ear^{-1}(Abbate *et al.*, 1998; Siddique *et al.*, 1989b; Slafer and Andrade, 1989; Waddington *et al.*, 1986), rather than ears m^{-2}.

2.5 PHYSIOLOGICAL TOOLS TO ENHANCE BREEDING

Laborious and time-consuming measurements in the field have very often discouraged breeders from using physiological traits as selection criteria. However, with modern instrumentation, physiological indices are relatively quick and simple to measure. For instance, stomatal conductance (g_s) using a hand-held viscous-flow porometer has been found to be a robust predictor of leaf diffusive conductance when compared to the steady flow porometer. Its enhanced speed in evaluating variation for leaf conductance should enable wheat breeders to screen large breeding populations for leaf conductance more efficiently (Rebetzke *et al.*, 2000). Stomatal conductance is an indicator of leaf photosynthetic activity in irrigated environments.

Likewise, Reynolds *et al.* (1994) reported that canopy temperature depression (CTD), measured with infra-red thermometer in yield trials under warm irrigated conditions in spring wheat lines in Mexico, was significantly associated with yield variation *in situ*, as well as with the same lines grown at a number of international breeding sites. Both g_s and CTD have been found to be closely and positively correlated with yield in spring wheats in Mexico (Fischer *et al.*, 1988); and because they are associated through feedback mechanisms, measuring for CTD can give a rapid indirect determination of g_s. Pinter *et al.* (1996) reported that in irrigated trials CTD increased when g_s increased given that other variables are constant. In addition, these traits may give an indication of a line's physiological adaptation to a given environment, a factor difficult to assess visually (Reynolds and Pfeiffer, 2000).

The use of spectral reflectance is another promising technology to estimate a range of physiological measurements such as plant biomass, total leaf area, water status, and assessments of photosynthesis (Araus, 1996). Furthermore, Babar *et al.* (2006) demonstrated the potential of using spectral reflectance indices as a breeding tool to select for increased genetic gains in biomass, chlorophyll content, cooler canopies and yield. Their findings for near infra-red radiation-based indices showed a strong association with biomass and canopy temperature at heading and grain filling, while chlorophyll content was more closely related to biomass during the vegetative phase under warm irrigated conditions in Mexico.

2.6 TRAIT PRIORITISATION FOR FUTURE GENETIC GAINS IN YIELD POTENTIAL

Whether source or sink is more limiting to yield is an ongoing debate but if yield gains are to be achieved through increasing RUE, one route may be through simultaneously increasing the capacity for both assimilate production as well as sink strength (Slafer *et al.*, 1996; Richards, 1996; Kruk *et al.*, 1997). One way to achieve this would be to focus on improving assimilate supply during ear growth thereby increasing sink capacity, which itself may drive higher assimilation rates during grain filling. Most experiments indicate that yield, as determined by grain number, is limited by growth factors during the period of juvenile ear development, i.e. during floret survival prior to anthesis. Ear growth at anthesis is linked to grains m^{-2} via assimilate supply, so improved canopy photosynthesis and/or ear partitioning pre-anthesis might simultaneously increase grain source, i.e. stem WSC and possibly post anthesis RUE via sink strength, and grain sink (grains m^{-2}).

The reproductive stages of development, from initiation of floral development to anthesis, are pivotal in determining yield potential, especially the rapid spike growth phase (Fischer, 1985) which has a duration of approximately 25 days in irrigated spring wheat in Mexico (Fischer, 1985) and Argentina (Abbate *et al.*, 1997). During this period final grain number is determined, a major factor affecting the subsequent partitioning of assimilates to yield, as well as heavily influencing the assimilation rate of the photosynthetic apparatus during grain filling. The duration of the rapid ear growth phase relative to other phenological phases shows genetic variation (Slafer and Rawson, 1994) which is associated with sensitivities to photoperiod, vernalization and earliness *per se* genes. Slafer *et al.* (1996) suggested the possibility of improving final grain number and yield potential by manipulating the genes associated with sensitivity to photoperiod, vernalization and earliness *per se*. The hypothesis is again based on the idea that by increasing the partitioning of assimilates to ear growth and therefore ear biomass, potential floret survival will be increased and hence yield potential raised (Bingham, 1969; Slafer *et al.*, 2005). The large ear-phenotype traits are also of importance as they have the potential to increase grains m^{-2} by increasing grain ear^{-1}.

It is clear that understanding the physiological processes limiting yield potential can accelerate breeding programmes. While past physiological findings have contributed much to our existing fund of knowledge on yield, there is still more to discover and understand from this complex trait. It is evident that sink limitation is the main barrier to yield progress and increasing the sink strength and post-anthesis RUE has been proposed by several authors to increase yield potential. Grains m^{-2} has to date been the target component but if the physiological basis of grain weight, the other major yield component, can be more clearly understood it could open a new window of opportunity for breeders. Furthermore, physiological tools such as CTD and spectral reflectance might have a greater role in the future together with traditional physiological measurements to improve our understanding of yield potential. Hence, it can be deduced that physiological attributes determining yield can complement traditional breeding so that there could be successful genetic improvement in wheat yield potential to meet projected demands of wheat production in the future.

2.7 HYPOTHESES

The characterisation of crosses involving novel parental material is being undertaken in this work so as to examine new genetic sources of ear morphology variation to increase grain number. The use of markers would contribute immensely in the selection of complex traits in breeding programmes. This information would be useful especially if the target gene i.e. large ear phenotype is recessive and there is a need for backcrossing. Field experiments in Obregon were carried out on the parental genotypes and the two large ear-phenotype DH populations, mainly on NL2 x Rialto population than NL1 x Rialto population.

An attempt is being made in this study to understand the mechanisms limiting ear fertility and hence yield potential in large-ear phenotype material and the specific hypotheses examined are:

- There is restricted tillering in the large-ear phenotypes
- Rachis length is positively correlated with spikelets ear^{-1} and grains ear^{-1} amongst the DH lines
- Spikelet ear^{-1} is positively correlated with grains m^{-2} amongst the DH lines

- Ear index (ratio of ear dry weight to above ground dry weight) at anthesis is positively correlated with rachis length amongst the DH lines
- There is a positive correlation between ear biomass at anthesis and grains ear^{-1} amongst the DH lines
- There are genotypic differences in pre-anthesis RUE among the DH lines
- RUE is positively correlated with above-ground biomass at anthesis amongst the DH lines
- Rachis length is positively correlated with individual grain weight amongst the DH lines
- Grain filling is source limited when tested in degraining experiments
- A part of the physiological basis of large-ear phenotype in novel material is associated with variation in developmental phases i.e. the timing and duration of the spikelet primordia initiation phase

The hypotheses are tested by using the CIMMYT parental genotypes (Bacanora, NL1 and NL2) and the NL2 x Rialto doubled-haploid population.

CHAPTER 3 GENERAL MATERIALS AND METHODS

In this chapter, the methodology used in field and growth-room experiments conducted in the research study is described. The research undertaken was divided between Mexico and the UK whereby field experiments were carried out in Mexico in the crop cycles of 2003/04, 2004/05 and 2005/06 (henceforth referred as 2004, 2005 and 2006) and growth-room experiments conducted at Sutton Bonington in 2004 and 2005. The experimental design and the crop and environmental measurements for the field experiments are outlined below. The experimental design of the growth-room experiments is described later in this chapter but the methodology relating to the plant measurements in the growth-room experiments is described in detail in Chapter 5.

3.1 FIELD EXPERIMENTAL SITE

Field experiments were carried out at CIMMYT experimental station near Ciudad Obregon, North West Mexico located at 27° 20' N, 109° 54' W, 38 m above sea level in the state of Sonora. It is a dry, irrigated, low-altitude site, located in a desert climate. Mean rainfall during the wheat crop cycle from November to May is approximately 50 mm. The site is a temperate high radiation environment and irrigated wheat yields in the region are high, in the order of 8 to 11 t ha^{-1} in experimental plots and 5 to 8 t ha^{-1} in farmers' fields. The spring wheat season starts in late November with sowing and ends in late April to mid May at harvest. The soil type at the experimental station is a coarse sandy clay, mixed montmorillonitic typic calciorthid, low in organic matter (<1%) and slightly alkaline (pH 7.7) (Limon-Ortega *et al.*, 2002).

3.2 PLANT MATERIAL

Three Mexican genotypes bred at CIMMYT namely L3 also known as Bacanora (conventional ear morphology), and the novel lines 1 and 2; NL1 and NL2 (large-ear phenotype) were each previously crossed with the UK cultivar Rialto to generate three doubled-haploid (DH) populations, Bacanora x Rialto, NL1 x Rialto and NL2 x Rialto using the maize pollination technique. The three CIMMYT genotypes will be referred to hereafter as the parental genotypes. A total of 189 lines were developed

for the Bacanora x Rialto DH population, 88 lines for the NL1 x Rialto DH population and 138 lines for NL2 x Rialto DH population, but not all lines were used in the experiments in Obregon. Sixty three lines from the Bacanora x Rialto DH population were sown in 2005 and 2006; 33 lines from the NL1 x Rialto DH population in 2005 and 44 in 2006 and 59 lines from the NL2 x Rialto DH population in 2005 and 69 lines in 2006. These lines were chosen mostly based on their spring type (nil or low requirement vernalization) and photoperiod insensitivity characteristics. The progeny showed both winter and spring vernalization characteristics and photoperiod sensitivity and insensitivity since Rialto is a winter type (*vrn*) wheat which is photoperiod sensitive and the CIMMYT parents are spring types (*Vrn*) which are photoperiod insensitive. The DH populations were grown as ear rows in a greenhouse at El Batan, Mexico City in 2001/2 and 2002/3 and lines were selected on the basis of acceptable flowering dates. The parental genotypes and DH populations have provided a unique set of materials to conduct an integrated developmental and physiological evaluation to begin to understand which physiological mechanisms are responsible for enhanced ear fertility in these large-ear phenotypes genotypes as well as any trade offs between grain number per ear and other yield components at the phenotypic level.

3.3 EXPERIMENTAL TREATMENTS AND DESIGN

3.3.1 Parental characterisation field experiments

The three CIMMYT parental genotypes (Bacanora, NL1 and NL2) were sown in five replicates in 2004, six replicates in 2005 and four replicates in 2006 using a randomised block design. The lines were sown on 17 November 2003, 24 November 2004 and 22 November 2005 for 2004, 2005 and 2006, respectively. That is, experimental seasons are referred to hereafter according to harvest year. Due to uneven moisture in the field at sowing in the 2005 experiment, a proportion of seeds suffered from anoxia resulting in poor establishment. Thus, measurements were taken from the three best established replicates in this season. Seeds were sown on 5 x 1.6 m plots on raised beds (2 beds per plot; 2 rows per bed, 32 cm row spacing) at a seed rate of 80 g per plot (approximately 300 seeds m^{-2}).

In order to investigate the source and sink balance of the parental genotypes, degraining of ears was performed 14 days after anthesis for the three parental genotypes in 2004, 2005 and 2006. In 2004, a total of 30 ears per plot were tagged to carry out the degraining treatment. The number of tagged ears was reduced to 24 in 2005 and 2006. In 2004, four degraining treatments were applied as shown in Figure 3.1, but in 2005 and 2006 only two were carried out, i.e. removal of all the spikelets from one side of the spike (Treatment C- Figure 3.1) and the control (Treatment A- Figure 3.1).

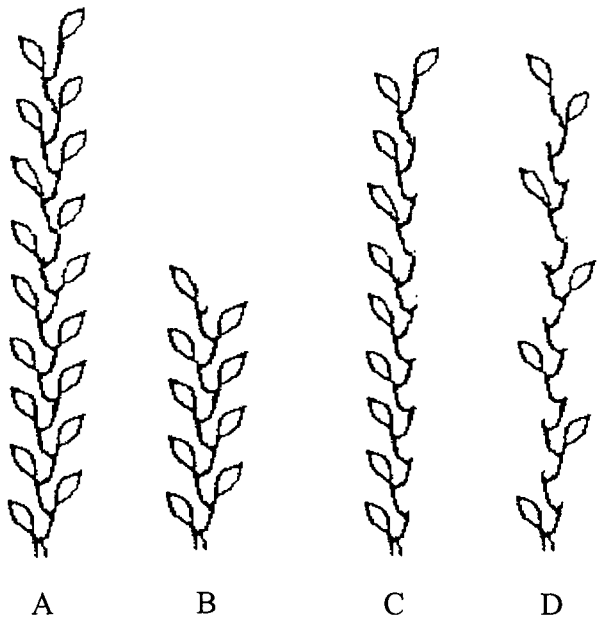


Figure 3.1. Types of degraining treatments applied at GS 61+14 d (A: control, B: top half of ear removed, C: all spikelets from one side of ear removed and D: alternate pairs of spikelets removed from the ear)

3.3.2 Doubled-haploid population field experiments

The three DH populations were sown using an alpha lattice design with 2 replicates in both 2005 and 2006. In addition, in 2006, a subset of 15 lines from NL2 x Rialto DH population was sown in 4 replicates using a randomised block design. Seeds were sown on 5 x 1.6 m plots on raised beds (2 beds per plot; 2 rows per bed, 32 cm row spacing) at a seed rate of 80 g per plot (approximately 300 seeds m^{-2}). The lines were sown on 24 November 2004 and 22 November 2005 for 2005 and 2006, respectively. Sixty three lines from the Bacanora x Rialto DH population were sown in 2005 and 2006; 33 lines from the NL1 x Rialto DH population in 2005 and 44 in

2006 and 59 lines from the NL2 x Rialto DH population in 2005 and 69 lines in 2006. The subset of lines was selected based on data collected in 2005 to represent maximum contrast in rachis length, number of spikelets, flowering date, thousand grain weight, plant height, ear index, ears m^{-2} and yield.. Two degraining treatments (treatments A and C) were carried out in the subset of 15 lines from the NL2 x Rialto DH population on 24 tagged ears in each plot.

3.3.3 Growth-room experiments at Sutton Bonington

3.3.3.1 Experimental design and treatments

The layout of pots in the growth-room experiment is shown below. To reduce any variation due to artificial light, the pots denoted by letter (x) were arranged as illustrated in Figure 3.2 as light distribution was not completely uniform throughout the room.

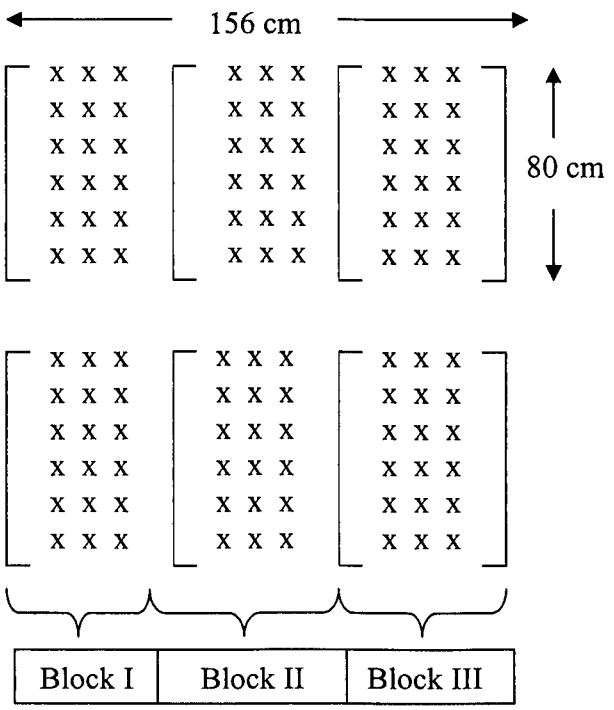


Figure 3.2. Schematic layout of pots (x) in growth room

Pots (13 x 11 cm) were laid out in three replicates in a randomised block design in one growth room. The four genotypes (Bacanora, NL1, NL2 nad UK-bred spring cultivar Tybalt) constituted the experimental treatment. In each pot one seed was sown on 13 July 2004 due to low seed availability for the parental genotypes. In each pot, two seeds were sown on 16 September 2005 and then thinned down to one plant per pot 14 days after sowing (DAS). The plants were arranged randomly in three blocks. In 2004, each block consisted of 36 pots (9 pots per genotype for destructive sampling) and in 2005 there were 48 pots (12 pots per genotype). The potting media used was John Innes Compost No. 2. The plants were watered on a regular basis and 60 days after sowing, growth solution of Sangral soluble fertiliser [3:1:3 (21:7:24 + 2MgO + TE)] was applied to ensure that the plants do not suffer from any macro- or micro-nutrient deficiency. Plant measurements are described in Chapter 5.

3.3.3.2 Radiation and temperature conditions

The growth-room experiment was conducted in 2004 and 2005 with the following environmental conditions:

- Light regime: 16 hours photoperiod with eight daylight bulbs of 400 watts providing a light intensity of 400 μ mol m^{-2} s^{-1} PAR (recorded at pot height)
- Temperature: 20 °C day temperature and 10 °C night temperature

3.4 PLOT MANAGEMENT IN FIELD EXPERIMENTS

The field experiment was undertaken under a high output environment. Appropriate cultural and management practices such as fertilisation, pests, diseases and weed control were implemented to ensure that the crops were free from any stress as outlined below.

3.4.1 Irrigation regime

Plots were irrigated when the soil started drying out by using a gravity based system where water was allowed to flow into the field until soil was saturated. The aim was to maintain the crop free from water stress from emergence to harvest. During the crop cycle, the plants were irrigated four times in 2004 (11 January, 6 February, 5 and 24 March) and 2005 (18 January, 4 February, 1 and 17 March) and five times in 2006 (5 January, 3 February, 24 February, 17 March and 7 April).

3.4.2 Fertilizer application

In the three years of study, nitrogen and phosphorus fertilizer application was the same in all field experiments. In each year, 75 kg ha^{-1} of N was applied as urea during land preparation. This was followed by 40 kg ha^{-1} of P as Triple Super Phosphate at sowing and a second application of 75 kg ha^{-1} of N as urea at the time of the first irrigation, i.e. the first week of January, close to the onset of stem elongation.

3.4.3 Herbicide, fungicide and pesticide application

Chemicals were applied using either a tractor or airplane. In the early vegetative stage, herbicides were used to control broad and narrow leaved weeds in every crop cycle. At later growth stages, fungicide Folicur 250 EW (25% tebuconazole) was applied twice in every crop cycle, usually in February (GS 45) and March (GS 65) at a dosage of 0.5 l ha^{-1} to protect crops from leaf rust. Insecticides were applied as necessary to minimise the effects of insects. Full details about the products applied and application rate, as well as application dates from 2004 to 2006 are given in Appendix I.

3.5 CROP MEASUREMENTS

All the crop measurements taken in the field experiments are described in this section.

3.5.1 Plant establishment

Plant counts were carried out in 2006 in the parental genotype experiment at GS 31(Tottman, 1987) by sampling and counting all plants in a 0.5m by 0.8 m quadrat area per plot. The same procedure was applied in the experiment examining the subset of 15 lines of Nl2 x Rialto DH population in 2006.

3.5.2 Developmental stages

Growth stages, based on the Zadoks *et al.* (1974) decimal code (GS) and (Tottman, 1987) were assessed every 3-4 days by visual inspection and the stage was taken as when more than 50% of shoots were at that stage, e.g. anthesis (GS 61) date was recorded when 50% of the plants reached the stage. In 2004 and 2005, growth stage assessments were carried out on each plot in the parental genotype experiment at GS 41 and GS 61. In 2006, GS 31, GS 41 and GS 61 were assessed in each plot of the

parental genotype experiment and the experiment examining the subset of 15 lines of the NL2 x Rialto population. In the experiments examining the wider range of lines of the NL1 x Rialto and NL2 x Rialto DH populations in 2005 and 2006 only date of GS 61 was recorded. In addition, in 2006, three randomly selected plants were dug up every 2 days in the parental genotype experiment and the 15-line subset experiment in each plot to determine the date of terminal spikelet formation. Using a microscope, the plants from each plot were dissected to reveal the stage of shoot apex development and when two or three plants had reached terminal spikelet this was taken as the date the plot reached that stage. In each plot of each field experiment, physiological maturity stage was also assessed visually as when 50% of the flag leaf and 100% of the ear had senesced and less than 10% of the stem remained green.

3.5.3 Growth analysis

3.5.3.1 Measurements in parental genotype experiments at GS 31, GS 41 and GS 61

3.5.3.1a Sampling dates and sample size

In each experiment in 2004 and 2005, samples were taken at booting (GS 41) and anthesis (GS 61). Samples consisted of the above-ground tissue from rows of a 50 cm length of the bed, situated at least 50 cm from the end of the plot to avoid border effects. From one bed per plot an area of 0.4 m^2 was sampled, plants were cut at ground level and brought to the lab. In 2006, samplings were at GS 31, GS 41 and GS 61. Sampling was carried out on the actual date of reaching the stage, therefore different lines were sampled on different calendar dates.

3.5.3.1b Measurements at GS 31 and GS 41

For the samples at GS 31 in 2006 and GS 41 in 2004 and 2005, fresh weights of the plant material were recorded and the number of fertile shoots counted. The weight of the material was recorded after placing in the oven for drying at 75 °C for 48 hours. In 2006, at GS 41, green leaf area and stem area of shoots and chlorophyll content of leaves were measured on a 25% sub-sample (SS1) by fresh weight. The remainder of the original sample was weighed after placing in the oven for 48 hrs at 75 °C. Fertile shoots were separated from infertile shoots (a fertile shoot was a healthy green shoot while an infertile was a yellow or senescent shoot) and their number was recorded

before the infertile shoots were dried to constant weight. Green leaf lamina area of the fertile shoots was measured after being separated from the dead lamina and stems by using a leaf area meter (LICOR, LI3050A/4, USA). The green area of the stem plus attached leaf sheath of the fertile shoots was calculated by assuming the shape of the organ to be a cylinder and applying the following formula: π*diameter*height+ π *r^2 (where r is the radius of the stem). Using a calliper the diameter of the stem was measured and the length was measured using a ruler. Relative chlorophyll concentration was estimated using a hand-held portable SPAD meter (SPAD-502, Minolta, Japan) on three newest fully emerged leaves and the average reading from three leaf positions of each leaf measured was recorded on 12 shoots. The measurements are given as relative units. The dead lamina, green lamina and the stem plus attached leaf sheath were then dried at 75 °C for 48 hours.

3.5.3.1c Measurements at GS 61

Samples were cut at ground level from one 0.4 m^2 area per plot as described in section 3.5.3.1b. The total fresh weight was recorded. Detailed measurements were carried out on a 25% sub-sample by fresh weight, and the weight was recorded of a 50% sub-sample by fresh weight after drying for 48 hrs at 75 °C. The remainder of original sample was discarded. The 25% sub-sample was divided into fertile shoots, potentially fertile shoots and senescent shoots and their numbers recorded. A fertile shoot was one which had an emerged ear. A potentially fertile shoot was one at the booting stage. A senescent shoot was one with no green area or one in which the newest expanding leaf was yellow. Fertile shoots were further separated into: (i) ears, (ii) dead leaf lamina, (iii) green leaf lamina and (iv) stem plus attached leaf sheath. The fresh and dry weight of each component was recorded after drying for 48 hours at 75 °C. The same procedure was carried out for the potentially fertile ears. The dry weight for senescent shoots was recorded.

Before the green leaf lamina, stem and ear fractions were placed in the oven for drying their areas were measured. Leaf lamina area was measured as described above using a leaf area meter. Stem area was calculated using the same formula as described in *3.5.3.1b*. The ear area was calculated as the product of the length and the width of the ear. In addition, rachis length was measured and total spikelets per ear counted on 12 randomly selected fertile ears in the 25% sub-sample. Finally, relative

chlorophyll concentration was measured using the SPAD meter (SPAD-502, Minolta, Japan) on the flag leaf and the two leaves below it, and the average reading from three leaf positions was recorded for 12 shoots.

3.5.3.2 Measurements in parental genotype experiments at harvest

After physiological maturity was reached, grain yield was measured by machine harvesting a plot area of 4.8 m^2 in 2004 and 2006. In 2005, the sampled area was reduced to 2.4 m^2 to reduce variability due to poor establishment. The moisture content was measured on a subsample from combine harvest. In each year, prior to harvest, a random sub-sample of 100 ear-bearing culms was removed from each plot by cutting at ground level. The shoots were dried at 75 °C for 48 hours, weighed (SDW) and the ears then threshed. Dry weight of grains (DWG) from the 100 ears was recorded. From this sample, 200 grains were randomly sampled and counted and weighed to determine thousand grain weight. Using these data, estimates of individual grain weight, all yield components and final above-ground biomass were calculated as shown below.

- Harvest index = DWG/SDW
- Grain yield (m^{-2}) = {(Plot grain weight * DW grain sub-sample /FW grain sub-sample) + DWG}/Area of plot
- Biomass (g m^{-2}) = Grain yield/Harvest Index
- TGW (g) = DW 200 grains x 5
- Grain m^{-2} = Yield/TGW x 1000
- Culm DW (g) = SDW/ No. of shoots sampled
- Ears m^{-2} = Biomass/ Shoot DW
- Grains $spike^{-1}$ = Grain m^{-2}/Ears m^{-2}

3.5.3.3 Stem water soluble carbohydrates

In the parental genotype experiment in 2004, water soluble carbohydrate content in stems plus attached leaf sheaths was estimated at 5 day intervals from GS 61+5d to GS 61+25d and at maturity. On each occasion, ten randomly selected fertile shoots were sampled per plot and their fresh weight was recorded. After the lamina and the ears were removed the stems with their attached leaf sheaths were weighed. The stems were then placed in placed in a gauze-bottomed drying tray and 'flash dried' in

a previously heated oven for exactly 2 hours at 100 °C. When handling samples care was taken not to cut but instead bend the stems to avoid sap loss. Dry weight of samples was recorded. Using a grinder (Cyclone Sample Mill Model 3010-030), dried samples were milled and put into sealed envelopes before being sent to El Batan (CIMMYT headquarters), Mexico City for laboratory analysis. Using a standard procedure, soluble carbohydrates were first extracted with water, followed by addition of anthrone and sulphuric acid mixture to the filtrate. The absorbance of the resulting coloured liquid was then read against a calibrated spectrophotometer at 630 nm to determine the concentration of water soluble carbohydrate in the samples. Laboratory results (% WSC) were multiplied by dry weight of the stem plus attached leaf sheath per m^2 to convert into the amount of stem WSC. The same procedure was repeated in the parental genotype experiments in 2005 and 2006, but with sampling at GS61 + 5 days and at maturity only.

3.5.3.4 Measurements in the doubled-haploid population experiments (examining from 33 to 69 lines) in 2005 and 2006

3.5.3.4a Measurements at GS 61

No biomass assessments were carried out in the Bacanora x Rialto and NL1 x Rialto DH population experiments. For these experiments, rachis length, spikelets ear^{-1} and chlorophyll content of the flag leaf were measured *in situ* in each plot in both 2005 and 2006 on 12 randomly selected shoots one week after GS 61. For the NL2 x Rialto experiments, in 2005 biomass samplings were taken at GS 61 following the same sampling procedure as described for parental genotype experiment. However, because of the large number of lines, a simpler and faster processing method was used. The fresh weight of the harvested plant material was recorded. Fifty shoots were randomly selected from the sample and the fresh weight and dry weight (after drying in oven for 48 hours at 75 °C) were recorded. Based on these dry weights, the above-ground biomass and the number of shoots per m^2 were calculated. Twenty shoots with ears were then taken randomly from the remaining sampled material and ears were removed from the shoots at the ear collar. Both the ears and straw were weighed after drying for 48 hours at 75 °C. From these dry weights, the ratio of ears to above-ground dry matter, i.e. the ear index of the fertile shoot was calculated. This growth analysis in NL2 x Rialto DH population was done only in 2005. Rachis

length, spikelets ear^{-1} and chlorophyll content of the flag leaf were recorded on 12 of the twenty randomly selected shoots before they were placed in the oven.

In 2006, 12 randomly selected shoots were cut at ground level from each plot. The ears were separated from the straw and the dry weight of each component recorded after drying for 48 hours at 75 °C. In addition, before the material was placed in the oven the rachis length and spikelets ear^{-1} was recorded on 12 randomly selected ears. The chlorophyll content on the 12 flag leaves was determined using the SPAD meter as described above.

3.5.3.4b Measurements at harvest

The harvest measurements in the DH experiments in 2005 and 2006 were as described in the parental genotypes in section 3.5.3.2.

3.5.3.5 Measurements in the NL2x Rialto subset (15 lines) experiment in 2006

In each plot, three biomass samplings were carried out, the first at 40 days after emergence corresponding approximately to GS 31, the second at booting (GS 41) and the third at anthesis (GS 61) plus 7 days. Sampling procedure was the same as in the other experiments. For the samplings of GS 31 and GS 41, fresh weight was recorded and the dry weight recorded after drying at 75 °C for 48 hours. At GS 61, the sample processing to measure biomass, shoot number, green areas, rachis length, spikelet ear^{-1} and flag leaf chlorophyll content was as described for the parental genotype experiments. Measurements on the tagged ears for degraining treatments A and C were carried out as described for the parental genotype experiment. The tagged ears were counted, hand threshed and grain number and grain weight was recorded at harvest. Harvest growth analysis was as described for the parental genotype experiments in section 3.5.3.2. Stem WSC was assessed at GS 61 +7 days and maturity as described for parental experiments.

3.5.4 Plant height

Plant height was measured from ground level to the tip of the ear using a wooden ruler on 12 randomly selected shoots per plot. Measurements were carried out 2 to 3 weeks after GS 61 in all field experiments.

3.6 CANOPY TEMPERATURE, STOMATAL CONDUCTANCE AND CANOPY SPECTRAL REFLECTANCE

3.6.1 Canopy temperature

Canopy temperature was measured from the early stem-extension stage to the mid grain filling stage in all experiments, except the experiment examining the NL1 x Rialto DH lines in 2005, as described in Table 3.1. Canopy temperature was measured using a handheld infrared thermometer (Model AG-42, Tela-Temperautre Crop, Fullerton, CA, Figure 3.3). To avoid incorporating soil temperature in the measurement, the thermometer was pointed at an angle to the rows, and at 50 cm above the canopy, at an angle of 30° to the horizontal. Concurrently, air temperature is also recorded to calculate the canopy temperature depression. Measurements were taken weekly from 1300 to 1500 on bright sunny days. Two readings were taken per plot on each assessment date.

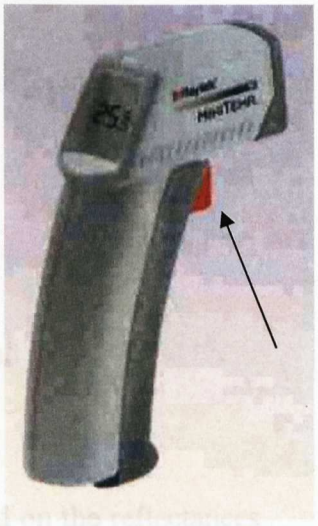


Figure 3.3. IR thermometer

3.6.2 Leaf stomatal conductance

Stomatal conductance (cm s^{-1}) was measured in the parental genotype experiments at approximately 7 day intervals from early stem extension to mid grain filling in each year; and at mid grain filling in the 15 line subset experiment (NL2 x Rialto) in 2006 as described in Table 3.2. Measurements were taken on 10 randomly selected flag leaves per plot at around 1300 on bright sunny days. In 2004, a viscous flow porometer (VFP, Thermoline and CSIRO, Australia) was used to measure leaf stomatal conductance whilst in 2005 and 2006, an AP4 dynamic diffusion porometer (Delta-T devices, Cambridge, UK) was used. An equation based on a calibration derived in Obregon using data for the VFP and Delta-T porometer collected in one experiment was used for converting "viscous flow counts" to "diffusive conductance" formally set out in Equation 3.1

Stomatal conductance = (100* 1/counts*316) + 368 **Equation 3.1**

Where counts are the values obtained from the viscous flow porometer and units are in counts (Tony Condon, personal communication).

3.6.3 Normalized difference vegetative index (NDVI)

A spectral reflectance measurement in the form of Normalized Difference Vegetative Index (NDVI) was undertaken in this study. It is calculated as:

$NDVI = (R_{NIR} - R_{RED}) / (R_{NIR} + R_{RED})$, with a range of -1 to 1 **Equation 3.2**

Where R_{NIR} is the reflectance at Near Infra Red (710-980 nm) and
R_{RED} is the reflectance at red (550-670 nm)

This measurement provides an estimate of the canopy size based on the reflectances from the canopy surface in the red and near- infrared regions. NDVI was measured using the Green Seeker (NTech Industries Inc, Ukiah, CA) and spectral radiometer readings were taken in each field experiment and in all plots at approximately 7 day intervals from early stem extension to mid grain filling on the assessment dates described in Table 3.3.

Table 3.1. Dates of canopy temperature measurements in field experiments (expts) at Cd. Obregon in 2004, 2005 and 2006

Date	Parental genotype expt (1300-1400)	Bacanora x Rialto DH expt (1300-1500)	NL1 x Rialto DH expt (1300-1500)	NL2 x Rialto DH expt (1300-1500)	NL2 x Rialto subset DH expt (1300-1400)
Feb 2004	16, 20, 25, 27				
Mar 2004	1, 10, 17, 24, 30				
Feb 2005	3, 14, 24	3, 14, 24		3, 14, 24	
Mar 2005	3, 10, 17, 21, 31	3, 10, 17, 21, 31		3, 10, 17, 21, 31	
Jan 2006	11, 18, 25	30	23		11, 18, 25
Feb 2006	1, 8, 15, 22	2, 13, 17	1, 13, 17	3, 10,15, 23	1, 10, 15, 23
Mar 2006	2, 9, 15, 23, 30	2, 16, 22, 30	2,16, 22, 29	2, 9, 16, 22, 29	2, 9, 16, 23, 30
Apr 2006	6	4, 17, 24	4, 17, 24	6, 12, 17, 24	6, 12, 24

Table 3.2. Stomatal conductance measurement dates in parental genotype experiments and NL2 x Rialto (15 lines) subset expt in 2004, 2005 and 2006

Date	Parental genotype expt (1100-1400)	NL2 x Rialto subset DH expt(1100-1400)
Feb 2004	16, 20, 26	
Mar 2004	4, 11, 17, 30	
Feb 2005	3, 14, 25	
Mar 2005	4, 10, 21	
Feb 2006	13, 16	
Mar 2006	2, 7, 16, 24,30	
Apr 2006	6	10

Table 3.3. NDVI measurement dates in field experiments at Cd. Obregon in 2004, 2005 and 2006

Date	Parental genotype expt (1300-1500)	Bacanora x Rialto DH population expt (1300-1500)	NL1 x Rialto DH population expt (1300-1500)	NL2 x Rialto DH population expt (1300-1500)	NL2 x Rialto subset DH expt (1300-1500)
Jan 2005	25			25	
Feb 2005	2, 8, 15, 22			1, 8, 15, 22	
Mar 2005	2, 8, 15, 22, 29			2, 8, 15, 22, 29	
Apr 2005	5			5, 12	
Dec 2005	13, 19				13, 19
Jan 2006	12, 17, 25				12, 17, 25
Feb 2006	1, 8, 14, 21	14, 21	14, 21	14, 21	1, 8, 14, 21
Mar 2006	1, 7, 15, 24, 29	1, 8, 15, 24, 29	1, 8, 15, 24, 29	1, 8, 15, 24, 29	1, 7, 15, 24, 29
Apr 2006	6, 19	5, 10, 21, 26	5, 10, 21, 26	6, 10, 21, 26	6, 19, 26

3.7 ENVIRONMENTAL MEASUREMENTS

3.7.1 Fractional interception of photosynthetically active radiation

Fractional interception (f) of photosynthetically active radiation (400-700 nm) (PAR) was calculated by using the equation:

$$f = 1 - (\text{Transmitted} + \text{Reflected})/\text{Incident} \qquad \textbf{Equation 3.3}$$

Where transmitted radiation is the radiation that has been not been captured and is transmitted through the canopy to ground level. Incident radiation is incident radiation above the canopy and reflected radiation is that reflected from the top of the canopy.

Measurements were made in parental genotype experiments and in the 15 line subset experiment (NL2 x Rialto) using the Sunfleck Ceptometer (Delta-T, Cambridge, UK). Readings were taken on cloudless sunny days between 11 00 and 15 00 pm. Incident PAR was obtained by placing the ceptometer above the canopy and 10 readings were taken per plot. Ten readings for transmitted PAR were obtained from below the canopy at ground level and further 10 readings for reflected PAR by inverting the ceptometer about 5 cm above the crop. A modified version of Beer's law was used to determine, the extinction coefficient, k_{PAR} using Equation 3.4.

$$k = -\ln (I/I_0)/L \qquad \textbf{Equation 3.4}$$

Where Io is incident PAR and I is transmitted PAR below a GAI value of L (Monsi and Saeki, 1953).

Fractional interception was extrapolated between readings and above-ground dry matter (AGDM) from sequential samplings was then plotted against accumulated intercepted PAR which, was calculated as a function of fractional interception and daily solar radiation. It was assumed that PAR was equal to 50% solar radiation. A linear regression of accumulated PAR interception on accumulated AGDM was fitted setting the intercept at zero and the slope of the line gave an estimate of pre-

anthesis RUE $_{PAR}$. This is a common way of calculating RUE (Sinclair and Muchow, 1999). In the experiment examining the NL2 x Rialto subset of 15 lines in 2006, RUE $_{PAR}$ was calculated from GS 31 to GS 61. Additionally, in the parental genotype in 2005 and 2006, RUE_{PAR} was calculated from GS 41 to GS 61. The RUE_{PAR} value was calculated for each plot in this way and these values were subjected to analysis of variance.

3.7.2 Meteorological data

Meteorological data were obtained from the closest meteorological station in Cd. Obregon (approximately 1 km from the research station) and a summary of weather conditions (temperature, relative humidity, rainfall and solar radiation) prevailing during the crop cycles from 2004 to 2006 is presented in Table 3.5.

3.8 DATA ANALYSIS

Data collected were subjected to analysis of variance (ANOVA) using GENSTAT 8 (Lawes Agricultural Trust, Rothamsted Experimental Station, 2006). Standard ANOVA procedures were used to calculate treatment means, standard errors and significant differences between treatments. Standard linear regression analysis using GENSTAT 8 was performed to determine relationships between crop and plant variables. Correlations between traits and between respective traits and grain yield were calculated using GENSTAT 8 and was carried out on genotype means. The statistical analysis of data collected in growth room experiments in 2004 and 2005 is described in Chapter 5.

Table 3.4. Fractional interception measurement dates in parental genotype experiments and the NL2 x Rialto subset experiment at Cd. Obregon

Date	Parental genotype expt (1100-1400)	NL2 x Rialto DH subset expt (1100-1400)
Jan 2005	17, 24	
Feb 2005	2, 7, 14, 21, 28	
Mar 2005	7	
Jan 2006	12, 18, 25	12, 18, 25
Feb 2006	1, 11, 15, 23	1, 11, 15, 23
Mar 2006	1, 9	1, 9, 16, 22, 30
Apr 2006		4

Table 3.5. Meteorological data (monthly means) from date of sowing till harvest for the Obregon cycle in 2004, 2005 and 2006

Variables	Cd. Obregon cycle 2004					Cd. Obregon cycle 2005					Cd. Obregon cycle 2006				
	Dec	Jan	Feb	Mar	Apr	Dec	Jan	Feb	Mar	Apr	Dec	Jan	Feb	Mar	Apr
Temp °C (Max)	25.8	23.2	24.1	30.1	29.6	24.0	24.6	23.8	27.1	31.1	26.3	25.6	26.5	27.1	32.2
Temp °C (Min)	4.5	8.3	6.5	10.3	11.8	8.3	9.5	9.3	8.9	10.5	7.6	5.5	8.5	8.6	10.9
Temp °C (Average)	14.5	14.9	14.7	19.5	20.8	15.5	16.4	16.0	17.7	20.8	16.1	14.5	16.5	17.2	21.1
Relative Humidity (%)	55.8	81.4	71.4	69.6	64.1	62.2	72.3	75.0	61.1	47.3	58.2	57.4	66.1	62.3	47.2
Rainfall (mm)	0.0	4.9	1.3	0.3	0.2	0.1	1.7	1.7	0.0	0.0	0.0	0.7	0.0	0.0	0.0
Solar radiation (MJ m^{-2})	13.2	14.9	18.5	24.5	26.4	13.9	14.8	17.4	25.5	27.6	15.8	16.1	19.3	22.7	25.6

CHAPTER 4 PHYSIOLOGICAL CHARACTERISATION OF THE PARENTAL GENOTYPES (BACANORA, NL1 AND NL2)

4.1 INTRODUCTION

The physiological basis of genetic gains in yield potential in wheat in recent decades has been shown to be related principally to the alleviation of grain sink size limitation. This in turn has been achieved in part by improvements in source-type traits in the pre-anthesis period. Yield is a very complex trait made up of several yield components. Variation in ears m^{-2}, spikelets ear^{-1} and grain weight form the basis to this approach related to a yield components model (yield = ears m^{-2} x grains ear^{-1} x grain weight). However, selection for any one yield component often leads to compensation in the others. Therefore, analysis of limitations to yield potential according to a physiological yield components model rather than a numerical yield components model may be more useful. In this study, an attempt is being made to understand the contribution of novel ear morphology, i.e. large-ear phenotype (long rachis, high grains ear^{-1}) to yield potential by examining the physiological basis of large ear-phenotype. Spring wheat lines possessing these characteristics have been bred at CIMMYT to attempt to raise yield potential by increasing grains m^{-2}. Prior to the present study, three CIMMYT spring wheat genotypes (one with conventional and two with novel ear phenotype) were each crossed with Rialto (a UK winter wheat) to develop three doubled-haploid populations. The selection history of the three CIMMYT parental genotypes is as follows:

- Bacanora T 88 (CM67458-4Y-1M-5Y-OB-OMEX)- conventional ear phenotype
- CMH8OA.763 (CMH8OA.763-1B-1Y-2B-3Y-OY)- large-ear phenotype
- CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC(CMH84.499-2Y-1B-1Y-5B-1Y-1B-OY)- large-ear phenotype

These parental genotypes will be henceforth referred to in the thesis as conventional Bacanora and novel lines 1 and 2 (NL1 and NL2) for CMH8OA.763 and CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC, respectively.

4.1.1 Genetic background of parental genotypes

4.1.1.1 Bacanora

This is a CIMMYT variety released in Mexico in 1988. The variety has small grains; the ears are morphologically small, but there are many grains m^{-2}. Yield is high in part due to the 1BL/1RS translocated segment from rye but the loss of the wheat loci Glu-3B (encoding low molecular weight glutenins) and Glu-1B (encoding gliadins) on the short arm of chromosome 1B resulted in poor bread-making quality (Amiour *et al.*, 2002). The variety is an excellent combiner in crosses and possesses the *Rht-B1b* semi-dwarf gene, formerly *Rht1*, on chromosome 4B. It is a spring wheat and is photoperiod insensitive, though the photoperiod sensitivity genes are not known (Maarten Van Ginkel, personal communication). The vernalization gene present in Bacanora is *Vrn-D1* (Van Beem *et al.*, 2005). This conventional line was selected for investigation in this present study due to its high grains m^{-2}.

4.1.1.2 NL1 and NL2

These two lines (cycle F7+) were developed by Ricardo Rodriguez at CIMMYT in the 1990s. The pedigrees of these materials were not rigorously recorded. However, he used the tetraploid *Triticum polonicum* L. in his crosses, and also *Triticum aestivum L.* variety Morocco, durum wheats (*Triticum turgidum*) and other wheat relatives (Van Ginkel, M., personal communication). These genotypes were selected for investigation as they have a longer rachis and more spikelets ear^{-1} than recent CIMMYT releases including Bacanora, and because out of a range of large-ear phenotype lines they were the least plastic, i.e. they retained the large-ear phenotype best when sown at commercial plant densities. However, these genotypes have been observed to have reduced tillering counteracting to some extent more grains ear^{-1}. Although this chapter focuses mostly on the three CIMMYT genotypes a brief description of Rialto is also included which is the UK-bred winter wheat parent of the three DH populations.

4.1.1.3 Rialto

Rialto was released in the UK in 1991 and bred at Plant Breeding International Cambridge, Ltd. It has a semi-dwarf stature containing the *Rht-D1b* (formerly *Rht2)* gene on chromosome 4D and the wheat-rye alien chromosome translocation 1BL/1RS. Its end use is mainly for feed but it also has some bread-making potential. Rialto was selected as it has high expression of source-sink type traits, namely high stem

carbohydrate reserves and high pre-anthesis RUE (Shearman *et al.*, 2005). It was hypothesised that these may be synergized with improved grain sink strength of the novel CIMMYT segregating lines in the relevant DH populations.

4.1.2 Traits to increase yield potential

Much of the genetic gains in grain yield potential in recent decades has been due to the partitioning of more assimilates to grain rather than to straw at harvest due to the introduction of semi-dwarfing genes in the 1960s and 1970s. Increased partitioning of assimilates to the developing ear during the pre-anthesis period has resulted in increased grain yields probably due to more fertile florets spikelet^{-1} (Fischer, 1985). It is well established that historically yield improvement has been mainly due to increases in harvest index rather than biomass. Before the 1990s, for example, the study of Austin *et al.* (1989) showed that in UK cultivars harvest index was positively associated with yield progress with non-significant increases in biomass production.

A few more recent studies, however, have shown above-ground biomass at harvest to be positively associated with genetic yield progress (Siddique *et al.*, 1989; Donmez *et al.*, 2001; Shearman *et al*, 2005). In addition, biomass increases have been reported by several authors to be associated with recent introductions of alien chromatin into the hexaploid wheat genome: namely, the 1BL.1RS from rye (*Secale cereale* L.) (Villareal, 1995) and the *Lr 19* translocation from *Agropyron elongatum* (Singh *et al.*, 1988; Reynolds *et al.*, 2001). However, there is increasing concern that if rates of genetic progress in yield are to be maintained, and ideally accelerated, more progress should be made towards increasing biomass, as in many wheat growing areas the harvest indices of modern cultivars are already close to the maximum theoretical value of 0.62 as estimated by Austin *et al.* (1980). There is evidence for sink limitation in modern wheat cultivars in the UK under optimal conditions (Shearman *et al.*, 2005) and in Mexico at CIMMYT under irrigation (Reynolds *et al.*, 2005) and increasing grains m^{-2} remains a principal target for breeders. The novel genotypes investigated in this study have sink-type traits which could increase grains m^{-2} such as long rachis associated with more spikelets ear^{-1}.

Different physiological models have been suggested as conceptual frameworks to explain the physiological basis of yield in wheat. For instance, Reynolds *et al.* (2005) used the product of radiation interception, radiation-use efficiency and harvest index to express yield potential while Shearman *et al.* (2005) explained yield advances through a combination of improved growth rate in the pre-anthesis period (higher RUE) leading to more grains m^{-2} and a larger source (higher stem sugars) for grain filling. The physiological model currently proposed to explain the physiological basis of grains m^{-2} in the novel genotypes comprises the components of pre-anthesis radiation interception (RI, MJ m^{-2}), pre-anthesis radiation-use efficiency (RUE g MJ^{-1}), ear index (i.e. the proportion of ear dry matter to above-ground dry matter at anthesis; EI), and the ratio of grains to ear dry matter (i.e. grains per gram of ear DM at anthesis; GER) as set out formally in Equation 4.1.

Grains m^{-2} = RI x RUE x EI x GER **Equation 4.1**

A numerical model will also be considered as set out in Equation 4.2 where grains m^{-2} is defined as the product of fertile shoots m^{-2} at anthesis (SM2), spikelets ear^{-1}(SE) and grains $spikelet^{-1}$ (GS).

Grains m^{-2} = SM2 x SE x GS **Equation 4.2**

The specific hypotheses examined are:

1. Novel genotypes (NL1 and NL2) have higher ear index than the conventional genotype Bacanora and therefore greater ear biomass at GS 61
2. NL1 and NL2 have more spikelets ear^{-1} and more grains $spikelet^{-1}$ hence more grains ear^{-1} than conventional genotype Bacanora
3. Novel genotypes (NL1 and NL2) have more grains m^{-2} than Bacanora
4. NL1 and NL2 have greater potential grain weight than the conventional genotype Bacanora

4.2 METHODOLOGY

The parental genotype experiments and measurements taken are described in detail in Chapter 3. Specific methodologies related to the characterization of the parental genotypes include the estimate of RUE from GS 41 to GS 61, measurements of grain weight on degrained ears, and calculation of potential grain weight (PGW) and grain sink size, and are described below.

4.2.1 RUE estimate from booting to anthesis in 2004

In the experiments in 2005 and 2006, RUE_{PAR} was calculated for pre-anthesis period in the three years as a linear regression of accumulated above-ground biomass against accumulated PAR interception at sequential samplings from GS 31 to GS 61 as explained in section 3.7.1 of Chapter 3. In 2004, fractional interception was not measured and was assumed 0.95.

4.2.2 Grain weight of different spikelet positions in degrained lines

In each experiment in the degraining treatment and the control, at harvest, the spikelets were divided into three categories namely apical, central and basal, each comprising about 1/3 of the total number of spikelets on the ear (Figure 4.1). This was done to investigate the effect of spikelet position on response of spikelet weight, grain number $spikelet^{-1}$ and individual grain weight to degraining. In each degraining treatment, the number of ears was counted. The dry weight of the separate ear sections was recorded, the grains were counted after threshing and the dry weight of the grains recorded.

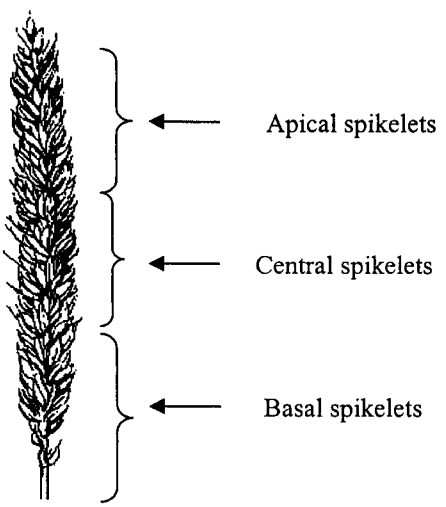


Figure 4.1. Ear showing different spikelet positions

4.2.3 Determination of potential grain weight (PGW)

Potential grain weight was determined for defined grain positions in the ear 28 days after GS61 (Macbeth, 1996) in each plot in the experiments in 2004, 2005 and 2006. For each of ten randomly selected ears per plot the central spikelet was identified by counting upwards from the ear collar, halving the total number of spikelets and rounding up to next whole number (Figure 4.2: central spikelet is 12). The grains from floret 1 and 2, i.e. proximal 2 florets of spikelet (Figure 4.2) were removed. Two additional spikelets, one above and one below the central spikelet, were also removed and again the two proximal grains were excised so that there was in total 6 central grains sampled per ear. The number and fresh weight to the nearest 0.01 g of the grains (60 grains in all for 10 ears) were recorded. Both sampled grains and the remaining ear were dried at 102 °C and the dry weight recorded after 48 hours. The results were used to calculate potential grain weight as set out below in Equation 4.3, developed by Macbeth (1996).

PGW = 44.02 + (0.51x GWC) - (0.24 x GNO)-(0.01x Sm^{-2}) **Equation 4.3**

Where PGW= potential grain weight (mg)

GWC= grain water content (mg)

GNO= grain number per ear

S m^{-2}= shoots m^{-2}

The PGW data were used to estimate sink size which is a function of PGW and grains m^{-2} at harvest (GN), as set out in Equation 4.4.

Sink size = PGW x GN **Equation 4.4**

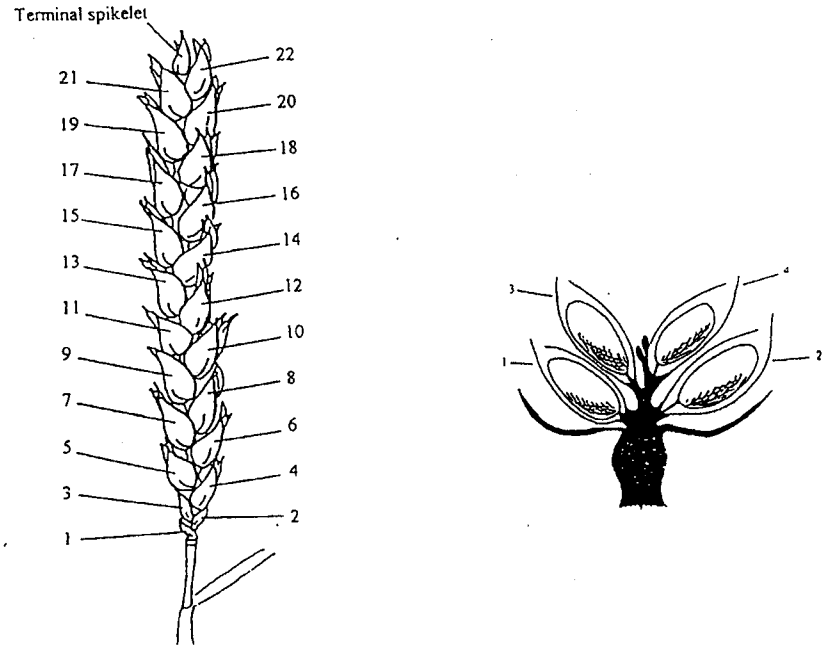


Figure 4.2. Wheat ear showing central spikelet (12) and florets number 1 and 2 (adapted from *Gay et al.*, 1998)

4.3 RESULTS

This results section will focus on data describing source and sink traits and grain yield measured in one experiment in each of 2003/04 (2004), 2004/05 (2005) and 2005/06 (2006) in Cd. Obregon, Mexico. The first part will focus on yield and its physiological and numerical components at harvest with direct comparisons between the conventional and large-ear genotypes. The second part will focus on assessments during the pre-anthesis period and attempt to identify the physiological mechanisms determining grains m^{-2} during that period. The third part will describe assessments during the post-anthesis period including an analysis of the inter-relationship between grains m^{-2} and grain size. For some crop variables, data are averaged across the three years. Some data were available from a previous experiment on the same genotypes in 2002/03 (2003) and will be referred to in some instances; these data from the 2003 experiment can be found in Appendix II.

4.3.1 Combine grain yield and yield components

The three genotypes did not differ in grain yield in 2004, but differences were observed in 2005 and 2006 ($P < 0.05$). The lowest yielding genotype in each year was NL2, and NL1 had higher yield than Bacanora in 2005 and 2006. Averaging across the years, NL2 had lower yield (630 g m^{-2}) than the other two genotypes (Table 4.1a); yields of NL1 (704 g m^{-2}) and Bacanora (688 g m^{-2}) did not differ significantly. Year had an effect on yield with highest yield recorded in 2006. There was an interaction between the genotypes and years ($P < 0.01$). NL2 had relatively lower yield compared to other genotypes in 2005 than in 2004 and 2006.

The three genotypes differed in harvest index in 2006 ($P < 0.001$), with higher HI in NL2 than in other genotypes. However, averaging over years, there were no significant differences among the genotypes. Higher HI was found in 2004 ($P < 0.001$) than in 2005 and 2006. A significant interaction between genotypes and year indicated relatively higher HI in NL2 in 2006 ($P < 0.01$) than in other years compared to other genotypes. With regard to harvest biomass, the genotypes differed just significantly in 2004 ($P = 0.05$) with higher biomass accumulated in NL1 compared to Bacanora. A similar trend was observed in 2005, and NL1 again had higher biomass at maturity compared to the other genotypes in 2006 ($P < 0.05$). Averaging across the years, NL1 accumulated more biomass than NL2 ($P < 0.001$) with a trend also for more biomass than Bacanora. There was an interaction between genotypes and year ($P < 0.01$) with Bacanora having relatively higher biomass than NL2 in 2006 but not in 2004. Overall differences amongst genotypes were explained by effects of biomass rather than HI.

Table 4.1a. Combine yield, HI and biomass in 2004, 2005 and 2006 in the three parental genotypes

Lines	2004			2005			2006			Mean		
	Yield (g m^{-2})	HI	Biomass (g m^{-2})	Yield (g m^{-2})	HI	Biomass (g m^{-2})	Yield (g m^{-2})	HI	Biomass (g m^{-2})	Yield (g m^{-2})	HI	Biomass (g m^{-2})
Bacanora	661	0.47	1398	652	0.41	1593	749	0.33	2298	688	0.41	1747
NL1	655	0.44	1511	708	0.37	1912	763	0.33	2301	704	0.38	1874
NL2	636	0.45	1424	509	0.33	1576	712	0.37	1909	630	0.39	1624
Mean	651	0.45	1444	623	0.37	1694	741	0.34	2169	674	0.40	1748
SED (DF) Genotype	20.2 (8)	0.01 (8)	40.0 (8)	42.2 (4)	0.02 (4)	180 (4)	14.0 (6)	0.006 (6)	66.1 (6)	14.0 (18)	0.009 (18)	50.9 (18)
Year										32.3 (18)	0.01 (9)	89.6 (9)
Interaction										38.3 (16.4)	0.01 (26.1)	116 (20.7)

Table 4.1b. Ear m^{-2}, grains ear $^{-1}$ and grains spikelet^{-1} in 2004, 2005 and 2006 in the three parental genotypes

Lines	2004			2005			2006			Mean		
	Ears m^{-2}	Grains ear^{-1}	Grains spikelet^{-1}	Ears m^{-2}	Grains ear^{-1}	Grains spikelet^{-1}	Ears m^{-2}	Grains ear^{-1}	Grains spikelet^{-1}	Ears m^{-2}	Grains ear^{-1}	Grains spikelet^{-1}
Bacanora	471	46.0	2.25	404	45.1	2.08	492	42.4	2.02	461	44.6	2.13
NL1	377	51.2	2.25	436	40.6	1.94	413	45.8	2.03	404	46.7	2.10
NL2	376	48.1	2.22	317	37.2	1.66	318	51.5	2.41	342	46.5	2.14
Mean	408	48.4	2.24	386	41.0	1.89	408	46.6	2.15	402	45.9	2.12
SED (DF) Genotype	18.4 (8)	2.24 (8)	0.12(8)	28.3 (4)	0.41 (4)	0.06 (4)	11.6 (6)	1.25 (6)	0.07 (6)	11.1 (18)	1.05 (18)	0.05(18)
Year										24.2 (9)	1.04 (9)	0.08 (9)
Interaction										29.1 (17.1)	1.86 (26.9)	0.11 (21.6)

Table 4.1c. Thousand grain weight (TGW) and grains m^{-2} in 2004, 2005 and 2006 in the three parental genotypes

Lines	2004		2005		2006		Mean	
	TGW (g)	Grains m^{-2}	TGW (g)	Grains m^{-2}	TGW (g)	Grains m^{-2}	TGW (g)	Grains m^{-2}
Bacanora	30.7	21 644	35.8	18 208	35.9	20 887	33.7	20 533
NL1	33.9	19 266	40.1	17 668	40.4	18 877	37.7	18 737
NL2	35.5	17 945	43.2	11 805	43.5	16 373	40.1	15 886
Mean	33.4	19 618	39.7	15 894	39.9	18 712	37.1	18 385
SED (DF) Genotype	0.58 (8)	655 (8)	0.58 (4)	1057 (4)	0.61 (6)	423 (6)	0.35 (18)	402 (18)
Year							0.44 (9)	989 (9)
Interaction							0.68 (25.9)	1150 (15.5)

Significant differences were observed among the lines in ears m^{-2} with Bacanora having more ears m^{-2} than NL1 in 2004 and 2006 ($P < 0.001$) whilst NL2 had fewer ears m^{-2} than NL1 in 2005 and 2006 ($P < 0.05$). Averaging across the years, Bacanora (461) had more ears m^{-2} ($P < 0.001$) than NL1 (404) which had more ears m^{-2} than NL2 (342). There was an interaction between genotypes and years ($P < 0.001$), where NL2 had lowest ears m^{-2} irrespective of years but by a relatively greater margin in 2005; NL1 had more ears m^{-2} compared to Bacanora in 2005 but not in 2004 and 2006.

Turning to consider grains ear^{-1}, more grains ear^{-1} were obtained in the novel genotypes than Bacanora in 2004 and 2006 ($P < 0.05$) but not in 2005. Averaging across the years, the genotypes did not differ in grains ear^{-1}; however, there was a trend for two more grains ear^{-1} in the novel genotypes. Year had an effect with most grains ear^{-1} obtained in 2004. There was also an interaction between the genotype and year ($P < 0.05$), whereby NL2 had 8 fewer grains ear^{-1} than Bacanora in 2005 but 2-9 more in 2004 and 2006. In 2005, there was a lower plant establishment than in other years which could explain the genotype x year interaction. The genotypes differed in grains $spikelet^{-1}$ in 2005 and 2006 but not in 2004 ($P < 0.01$). Averaging across years, the genotypes were not statistically significant, but more grains $spikelet^{-1}$ were obtained in 2004 (2.24) and 2006 (2.15) than in 2005 (1.89) ($P < 0.05$). There was an interaction between genotypes and years whereby novel genotypes had relatively fewer grains $spikelet^{-1}$ compared to Bacanora in 2005 than other years.

The genotypes differed in TGW in each of the three years ($P < 0.001$) with novel genotypes (37.7-40.1 g) on average having heavier grains than Bacanora (33.7 g). Averaging across the years, NL2 also had heavier grains compared to NL1 ($P < 0.001$). Lighter grains were obtained in 2004 ($P < 0.01$) compared to 2005 and 2006. The genotypes also differed in grains m^{-2} in all three years ($P < 0.01$). On average, Bacanora (20 533) had more grains m^{-2} ($P < 0.001$) than NL1 (18 737) and NL2 (15 886). Higher grains m^{-2} was observed in 2004 than other years. There was an interaction between the genotypes and years ($P < 0.01$) where NL2 had relatively fewer grains m^{-2} in 2005 compared to other genotypes than in other years.

4.3.2 Development, growth and partitioning in the pre-anthesis period

4.3.2.1 Crop development

Dissecting the shoot apex in 2006 to determine terminal spikelet date of the genotypes revealed that Bacanora and NL1 reached terminal spikelet on 9 January whereas NL2 was at this stage 4 days earlier. Dates of GS 31, 39 and 61 are summarised in Table 4.2. In each season, NL2 had a faster developmental rate than Bacanora and NL1.

Table 4.2. Dates of GS 31, 39 and 61 of the three parental genotypes in 2004, 2005 and 2006

	2004		2005			2006		
Lines	GS 39	GS 61	GS 31	GS 39	GS 61	GS 31	GS 39	GS 61
Bacanora	7 Feb	27 Feb	18 Jan	2 Feb	18 Feb	20 Jan	8 Feb	28 Feb
NL1	12 Feb	28 Feb	20 Jan	7 Feb	19 Feb	23 Jan	11 Feb	1 Mar
NL2	5 Feb	26 Feb	15 Jan	28 Jan	15 Feb	18 Jan	3 Feb	20 Feb

The time interval from GS 31 to GS 61 was 31 days for all three genotypes in 2005. In 2006, Bacanora had a longer duration of 39 days compared to 37 and 33 days for NL1 and NL2, respectively. In 2006, the time interval from terminal spikelet to anthesis was 50, 51 and 47 days for Bacanora, NL1 and NL2, respectively. NL2 reached GS 61 earlier compared to NL1 and Bacanora.

4.3.2.2 Plant number

Plants m^{-2} was not counted in 2004 and 2005, but results in 2006 at GS 31 showed that genotypes just differed (P=0.05) with NL2 (156) having fewer plants m^{-2} compared to NL1 (171) and Bacanora (179). Plants m^{-2} were assessed in 2003 and showed no differences among the genotypes, though NL2 (167) again had slightly fewer established plants compared to NL1 (173) and Bacanora (180) (Appendix II, Table 4A).

4.3.3 Fertile shoots m^{-2}

The genotypes differed in fertile shoots m^{-2} with NL2 overall having fewer shoots m^{-2} compared to NL1 and Bacanora ($P < 0.001$) at GS 31 and booting (GS41). There was a consistent trend for NL1 to produce more shoots than NL2. Year had an effect on shoots m^{-2} with more shoots being produced in 2006 than in other years ($P < 0.05$). However, there was no significant interaction between the genotypes and years.

At anthesis, there were no significant differences among the genotypes in 2004. Significant differences were found among the genotypes in 2005 and 2006 with NL2 having fewer shoots m^{-2} compared to Bacanora ($P < 0.05$). Averaging across the years, NL2 (304) had fewer shoots m^{-2} compared to the other genotypes ($P < 0.001$), and there was a trend for Bacanora (457) to have more shoots m^{-2} than NL1 (418). Fewer shoots m^{-2} were found in 2005 than the other years ($P < 0.05$). However, there was no significant interaction between the genotypes and years at GS 61.

Table 4.3. Fertile shoots m^{-2} for the three parental genotypes in 2004, 2005 and 2006

Lines	2004	2005		2006			Mean	Mean
	GS 61	GS 41	GS 61	GS 31	GS 41	GS 61	GS 41	GS 61
Bacanora	485	428	391	545	618	480	523	457
NL1	411	435	376	665	557	455	496	418
NL2	341	282	232	318	358	322	320	304
Mean	412	382	333	509	511	419	446	393
SED (DF)	52.2	34.4	43.5	19.3	68.5	37.1	38.3	26.2
Genotype	(6)	(6)	(4)	(6)	(6)	(6)	(12)	(16)
Year							35.3 (6)	29.6 (8)
Interaction							56.6 (17.76)	48.5 (23.83)

4.3.4 Shoot production and death in 2005

Plants were tagged in 2005 to follow the temporal pattern of shoot production. The results showed that novel genotypes had fewer shoots than Bacanora from the third assessment date (27 January) at around start of stem extension (GS 31) onwards (Figure 4.3). The genotypes differed ($P < 0.05$) in shoots $plant^{-1}$ at all sampling times with NL2 having fewer shoots compared to Bacanora and NL1. As expected,

time also had a significant effect on shoot number ($P < 0.05$) whereby numbers decreased in all three genotypes levelling off shortly before anthesis. Both NL1 and Bacanora had one more shoot per plant than NL2 at maximum shoot production and the differences in shoots m^{-2} amongst the genotypes at GS 61 was due to tiller production rather than tiller survival.

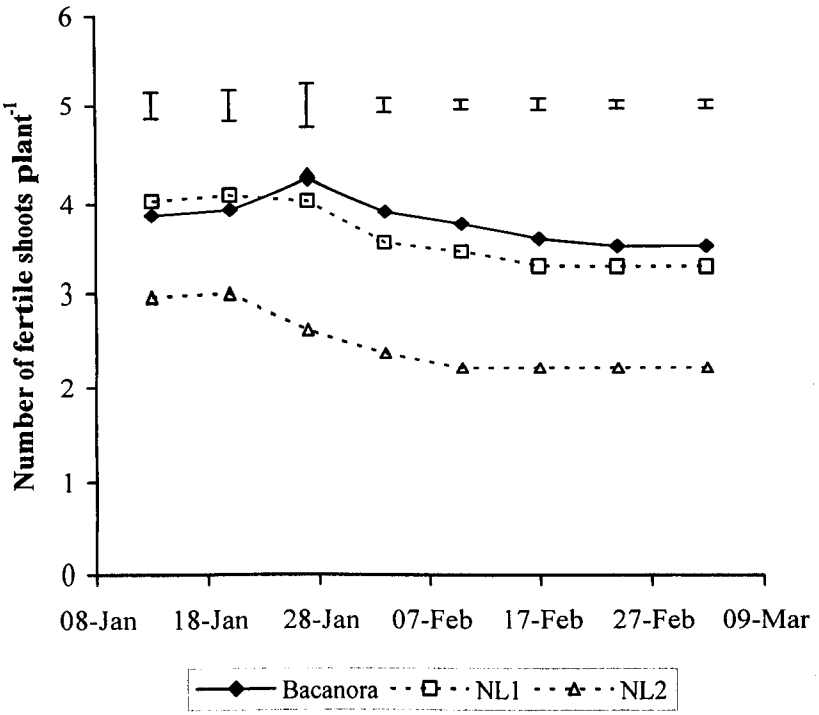


Figure 4.3. Number of shoots plant^{-1} from onset of stem extension to anthesis in 2005 (Error bars represent SED, DF =4)

4.3.5 Green area index (GAI) and leaf area index (LAI)

NL1 had more green area compared to Bacanora and NL2 at GS 31 in 2006 ($P < 0.001$). The genotypes also differed in GAI at GS 41 with NL1 having higher GAI compared to Bacanora and NL2 in 2005 and 2006 ($P < 0.05$). Averaging across 2005 and 2006, at GS 41 NL1 (7.0) had greater GAI than other genotypes ($P < 0.05$), with a trend for Bacanora (5.3) to have larger green area than NL2 (4.7). There was an interaction between genotypes and years ($P < 0.05$). In 2005, NL2 had relatively smaller green area than Bacanora compared to 2006. Similar findings were obtained for LAI at GS 31 and GS 41.

At anthesis, NL1 produced more green area than the other genotypes in 2004, 2005 and 2006, although significant differences were observed only in 2006 ($P < 0.01$).

Averaging across the years, NL1 (8.5) had greater green area than Bacanora (7.5) which in turn had greater green area than NL2 (6.7) ($P < 0.01$). Green areas were larger in 2006 compared to 2004 and 2005 ($P < 0.01$). There was no significant interaction between the genotypes and years at anthesis. The genotypes differed in LAI in each of the three years ($P < 0.05$) with NL1 having the greatest LAI in each case (Table 4.4b). Cross-year ANOVA showed that NL1 (4.8) had larger LAI than other genotypes (3.6-3.9). Greater LAI was observed in 2006 than in other years ($P < 0.01$). No significant interaction was detected between genotypes and years.

Table 4.4a. Green area index in 2004, 2005 and 2006 for the three parental genotypes

Lines	2004	2005			2006		Mean	Mean
	GS 61	GS 41	GS 61	GS 31	GS 41	GS 61	GS 41	GS 61
Bacanora	8.22	4.52	6.13	3.10	6.06	7.72	5.29	7.47
NL1	8.74	6.83	6.74	4.73	7.18	9.65	7.01	8.53
NL2	6.92	5.17	5.45	2.36	4.20	7.39	4.68	6.69
Mean	7.96	5.51	6.11	3.40	5.81	8.25	5.66	7.56
SED (DF)	0.94	0.36	0.51	0.31	0.69	0.44	0.39	0.41
Genotype	(6)	(6)	(4)	(6)	(6)	(6)	(12)	(16)
Year							0.38 (6)	0.49 (8)
Interaction							0.59 (17.4)	0.77 (23.4)

Table 4.4b. Leaf area index in 2004, 2005 and 2006 for the three parental genotypes

Lines	2004	2005			2006		Mean	Mean
	GS 61	GS 41	GS 61	GS 31	GS 41	GS 61	GS 41	GS 61
Bacanora	4.06	3.97	3.15	2.91	5.14	4.30	4.55	3.90
NL1	4.61	6.01	3.77	4.35	6.14	5.67	6.07	4.76
NL2	2.88	4.56	3.14	2.19	3.53	4.78	4.04	3.64
Mean	3.85	4.85	3.35	3.15	4.94	4.92	4.89	4.10
SED (DF)	0.56	0.39	0.16	0.28	0.63	0.33	0.37	0.24
Genotype	(6)	(6)	(4)	(6)	(6)	(6)	(12)	(16)
Year							0.36 (6)	0.29 (8)
Interaction							0.56 (17.5)	0.46 (23.4)

4.3.6 Chlorophyll content of flag leaf

The genotypes differed in chlorophyll content of flag leaves with NL1 (51.1) having a higher content than Bacanora (48.3) and NL2 (47.3) ($P < 0.001$). Higher chlorophyll content was found in 2006 compared to 2004 and 2005 ($P < 0.001$) (Table 4.5).An interaction was observed between genotypes and years ($P < 0.05$). Bacanora had lower chlorophyll than NL2 in 2004 but the reverse was the case in 2005 and 2006. Furthermore, flag leaf nitrogen (%) measured at anthesis in 2004 showed that novel genotypes had a marginally higher leaf N concentration (NL1:4.36%; NL2:4.25%) compared to Bacanora (4%) (P =0.01).

Table 4.5. Chlorophyll content of flag leaf for 3 parental genotypes in 2004, 2005 and 2006

Lines	2004 SPAD units	2005 SPAD units	2006 SPAD units	Mean SPAD units
Bacanora	45.3	49.2	50.6	48.3
NL1	49.5	49.7	53.9	51.1
NL2	45.8	46.0	49.9	47.3
Mean	46.9	48.3	51.5	48.9
SED (DF) Genotype	0.48(4)	0.13 (4)	0.33 (6)	0.21 (14)
Year				0.32 (7)
Interaction				0.44 (17.7)

4.3.7 Normalized difference vegetative index (NDVI)

NDVI is an indicator of canopy greenness and hence GAI. No pre-anthesis measurements were taken in 2004. In 2005, no significant differences were detected among the genotypes for NDVI. In 2006, the genotypes differed from 12 January till 8 February during the stem-extension period, with NL1 having higher NDVI compared to Bacanora and NL2 (Figure 4.4 (b)). In 2003, no significant differences were observed amongst the genotypes in the pre-anthesis period (Appendix II, Figure A1).

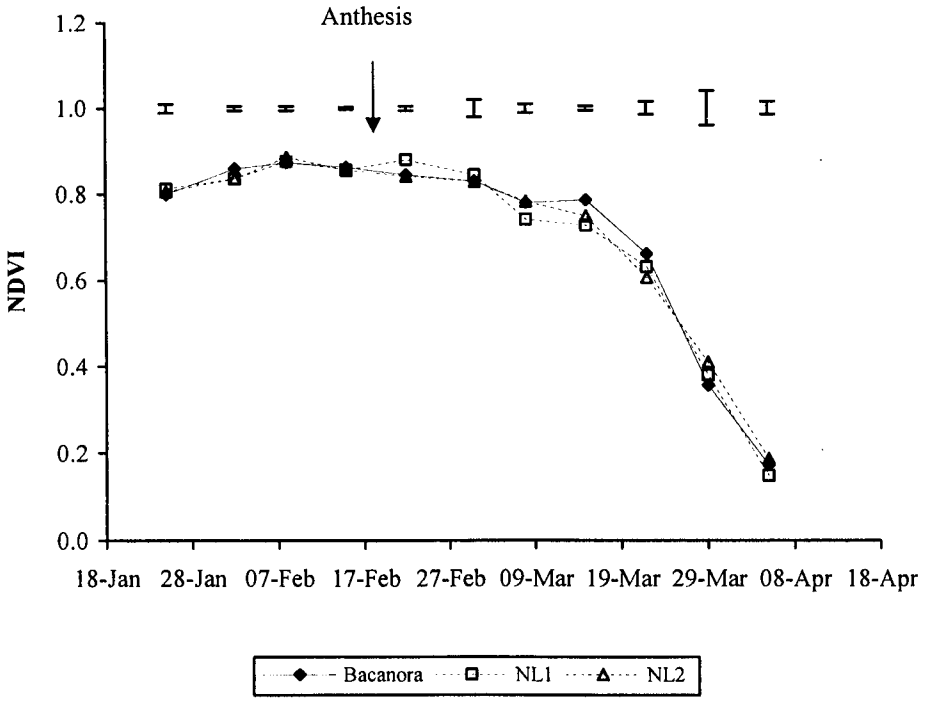


Figure 4.4(a). NDVI in 2005 for the three parental genotypes (Error bars represent SED, DF=4 and arrow indicates mean date at GS 61)

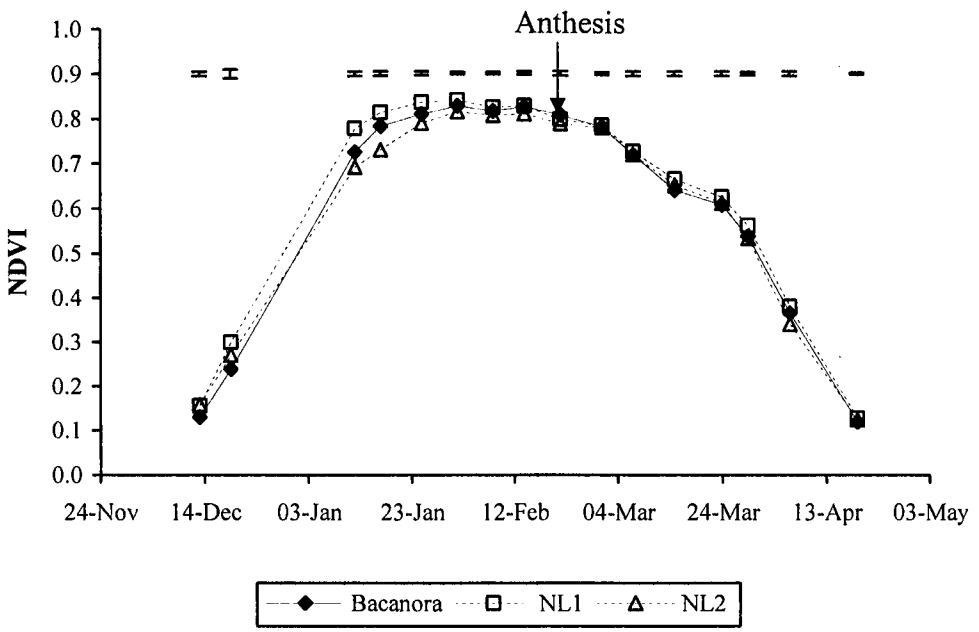


Figure 4.4(b). NDVI in 2006 for the three parental genotypes (Error bars represent SED, DF=6 and arrow indicates mean date at GS 61)

4.3.8 Fractional PAR interception (f)

No measurements of f using hand-held ceptometers were taken in 2004. There were differences ($P < 0.05$) amongst the genotypes in 2005 at the last two readings (pre-anthesis) where NL1 had slightly higher f compared to Bacanora and NL2 (Figure 4.5(a)). A similar effect was found in 2006 with NL1 intercepting a higher proportion of PAR compared to Bacanora and NL2 during early stem-elongation phase (Figure 4.5 (b)).

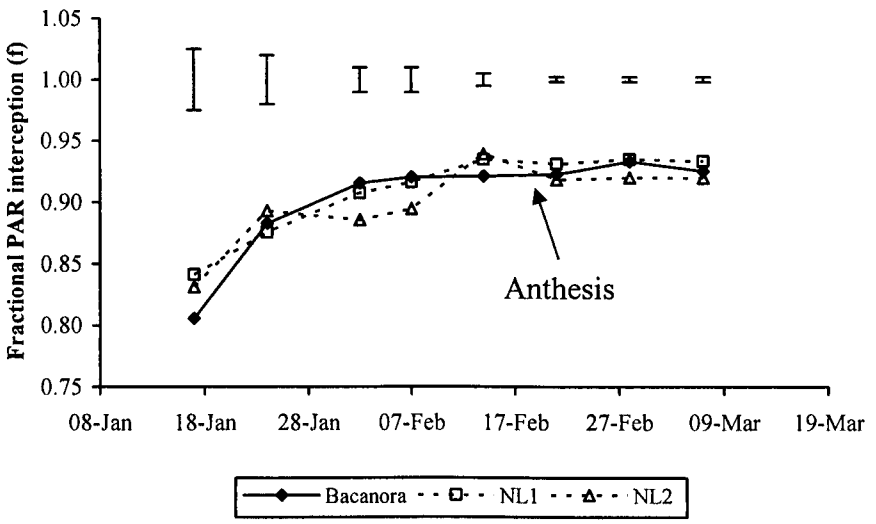


Figure 4.5(a). Fractional PAR interception in 2005 (Error bars show SED, DF=4). Arrow indicates the mean date of three genotypes at GS 61.

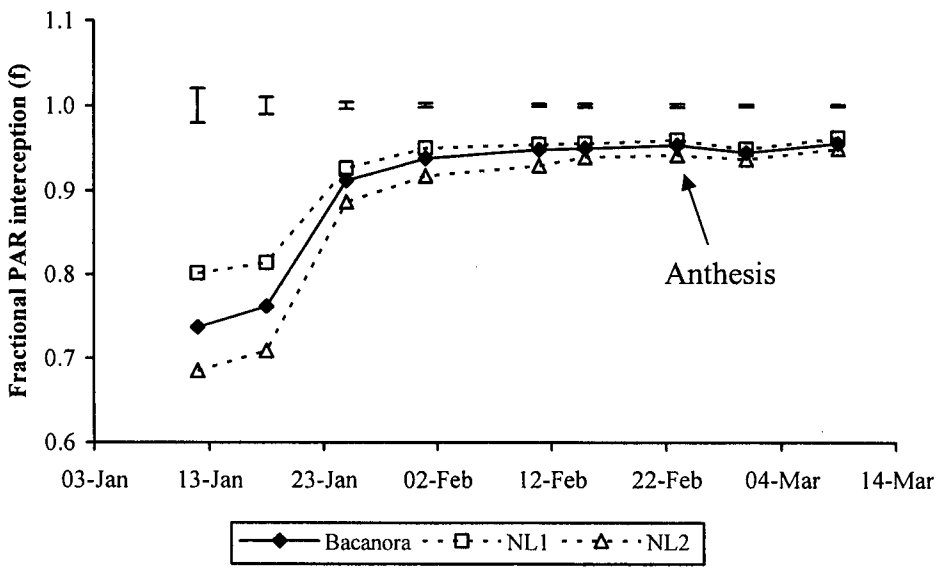


Figure 4.5(b). Fractional PAR interception in 2006 (Error bars show SED, DF=6). Arrow indicates the mean date of three genotypes at GS 61.

4.3.9 Biomass accumulation

In 2006, at GS 31 there was lower biomass accumulation in NL2 compared to the other genotypes ($P < 0.01$). In each of 2005 and 2006 at booting, NL1 had the largest biomass amongst the genotypes ($P < 0.05$). Averaging across the two years, NL1 (471 g m^{-2}) accumulated more biomass compared to Bacanora (405 g m^{-2}) and NL2 (344 g m^{-2}) and Bacanora had more biomass than NL2 ($P < 0.05$) (Table 4.6). Neither the year effect nor the year x genotype interaction was statistically significant. Biomass accumulated at anthesis did not differ significantly among the genotypes in the three years. Averaging across years, novel genotypes produced *ca.* 50 g m^{-2} less biomass than Bacanora, but these differences were not statistically significant. However, year had an effect on biomass accumulation with less biomass accumulated in 2005 than in other years ($P < 0.01$). There was no significant interaction between genotypes and years.

Table 4.6. Pre-anthesis biomass production (g m^{-2}) of the three parental genotypes in 2004, 2005 and 2006

Lines	2004	2005		2006			Mean	
	GS 61	GS 41	GS 61	GS 31	GS 41	GS 61	GS 41	GS 61
Bacanora	850	385	737	178	424	909	405	841
NL1	799	478	679	241	464	861	471	789
NL2	856	365	632	141	324	833	344	787
Mean	835	409	683	187	404	868	407	806
SED (DF)	74.2	34.6	74.6	24.8	38.7	47.5	25.9	37.9
Genotype	(6)	(6)	(4)	(6)	(6)	(6)	(12)	(16)
Year							19.1 (6)	39.7 (8)
Interaction							35.5 (17.85)	68.2 (24)

4.3.10 Pre-anthesis accumulated PAR interception and radiation-use efficiency

Accumulated PAR interception from GS 31 to GS 61 in 2006 differed ($P < 0.001$) amongst the genotypes with NL2 intercepting less radiation than other genotypes (Table 4.7). Differences were observed among the genotypes in radiation interception from GS 41 to GS 61 in 2005 and 2006 ($P < 0.001$) with novel genotypes intercepting less radiation than Bacanora in both years. Averaging across 2005 and 2006, Bacanora (450 MJ m^{-2}) intercepted more radiation than the novel genotypes, and NL1 (406 MJ m^{-2}) slightly more than NL2 (392 MJ m^{-2}) ($P < 0.001$). Year had an effect on PAR interception with more radiation intercepted in 2006 than

in 2005 ($P < 0.001$). There was an interaction between genotypes and years ($P < 0.001$), with NL2 intercepting relatively less radiation compared to other genotypes in 2006 than in 2005. The genotypes did not differ in extinction coefficient (k) in the three years. Averaging across years, Bacanora had a k_{PAR} value of 0.53 compared to 0.51 and 0.53 for NL1 and NL2, respectively. There were also no significant differences between years and no interaction between genotypes and years for k_{PAR}.

Significant differences were found among the genotypes in RUE_{PAR} from GS 31 to GS 61 in 2006 with NL2 using radiation more efficiently compared to NL1 and Bacanora ($P < 0.01$; Table 4.7). However, from GS 41 to GS 61, no significant differences were found among the genotypes in each of 2005 and 2006 although NL2 tended to have greater RUE_{PAR} (Table 4.7) than other genotypes. Averaging over the two years, the genotypes did not differ in RUE_{PAR} although NL2 tended to have slightly greater RUE_{PAR} compared to Bacanora and NL1. Higher RUE_{PAR} was observed in 2006 than in 2005 ($P < 0.05$). There was no significant interaction between genotypes and years.

Table 4.7. Accumulated PAR (MJ m^{-2}) and RUE_{PAR} (g MJ^{-1}) from GS 31 to GS 61 and from GS 41 to GS 61 in 2005 and 2006 for the three parental genotypes

Lines	2005	2006		Mean
	GS 41-61	GS 31-61	GS 41-61	GS 41-61
Accumulated PAR (MJ m^{-2})				
Bacanora	327	958	542	450
NL1	268	931	509	406
NL2	323	765	443	392
Mean	306	885	498	416
SED (DF) Genotype	5.14 (4)	3.47 (6)	1.26 (6)	2.25 (10)
Year				1.82 (5)
Interaction				3.19 (14.9)
RUE_{PAR} (g MJ^{-1})				
Bacanora	2.28	1.63	2.51	2.41
NL1	1.84	1.49	2.21	2.05
NL2	1.71	1.87	3.23	2.58
Mean	1.94	1.66	2.65	2.35
SED (DF) Genotype	0.59 (4)	0.07 (6)	1.01 (6)	0.34 (10)
Year				0.17 (5)
Interaction				0.43 (13.3)

4.3.11 Leaf activity traits

4.3.11.1 Canopy temperature

The overall summary for canopy temperature is that there were no consistent differences amongst the three genotypes observed across the years. In 2004, NL2 had higher canopy temperature compared with Bacanora and NL1 ($P < 0.05$) during the rapid ear-growth phase (Figure 4.6 (a)). The genotypes differed ($P < 0.05$) in 2005, with novel genotypes having slightly higher canopy temperature than Bacanora (Figure 4.6(b)). In 2006, NL1 had slightly higher canopy temperature ($P < 0.05$) compared to NL2 and Bacanora during early vegetative growth, i.e. at around stem extension (GS 31).

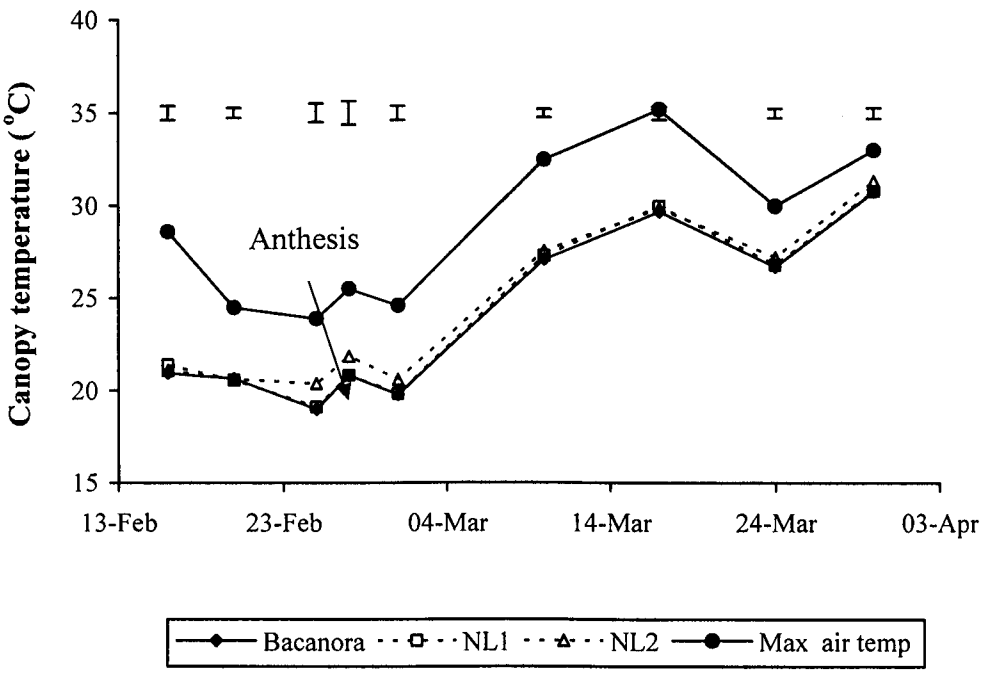


Figure 4.6(a). Canopy temperature for the three parental genotypes in 2004 (Error bars show SED, DF= 8). Arrow indicates the mean date at GS 61.

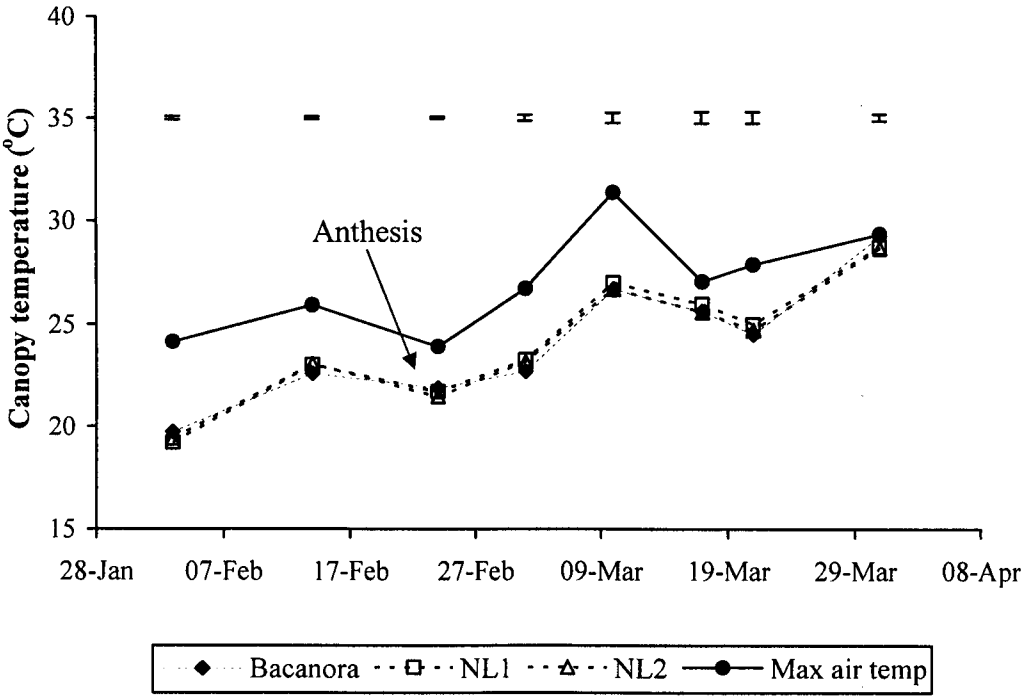


Figure 4.6 (b). Canopy temperature for the three parental genotypes in 2005 (Error bars show SED, DF=4). Arrow indicates the mean date at GS 61.

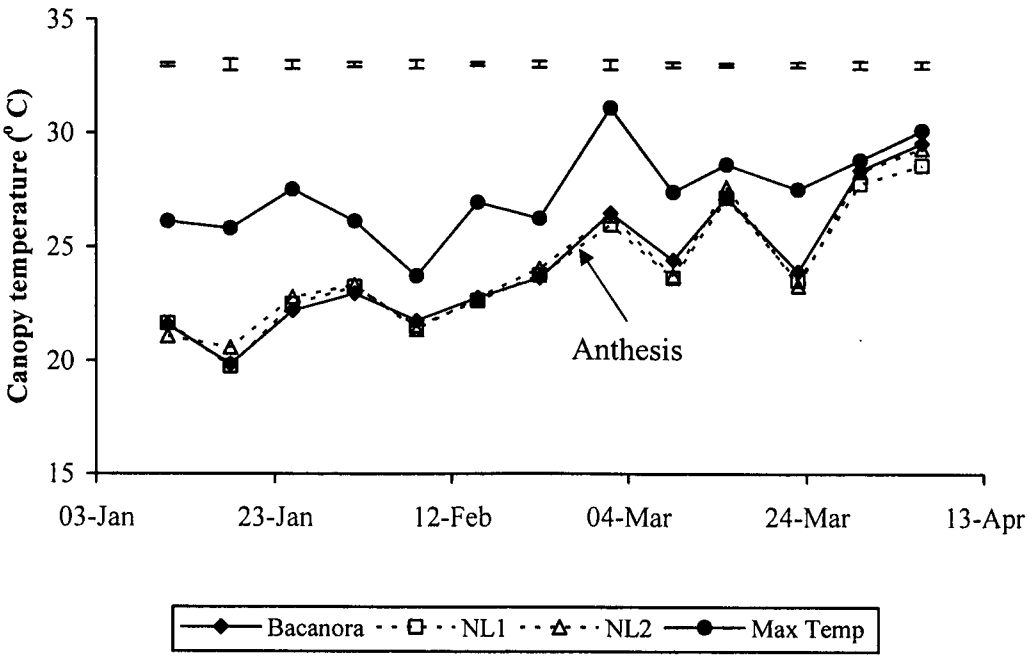


Figure 4.6(c). Canopy temperature for the three parental genotypes in 2006 (Error bars show SED, DF=6). Arrow indicates the mean date at GS 61.

4.3.11.2 Stomatal conductance

A viscous flow porometer was used in 2004 for the assessment of stomatal conductance while in 2005 and 2006 a steady flow porometer was used. In 2004 there were no significant differences amongst the genotypes, associated with large variability as indicated by the standard errors (Figure 4.7 (a)). Overall, there was a tendency for NL1 to have higher stomatal conductance than other genotypes around booting stage in 2005 and 2006. In 2005, differences ($P < 0.05$) among the genotypes were evident in the pre-anthesis period (first two readings) with NL1 having higher stomatal conductance than NL2. In 2006, no significant differences were observed amongst the genotypes.

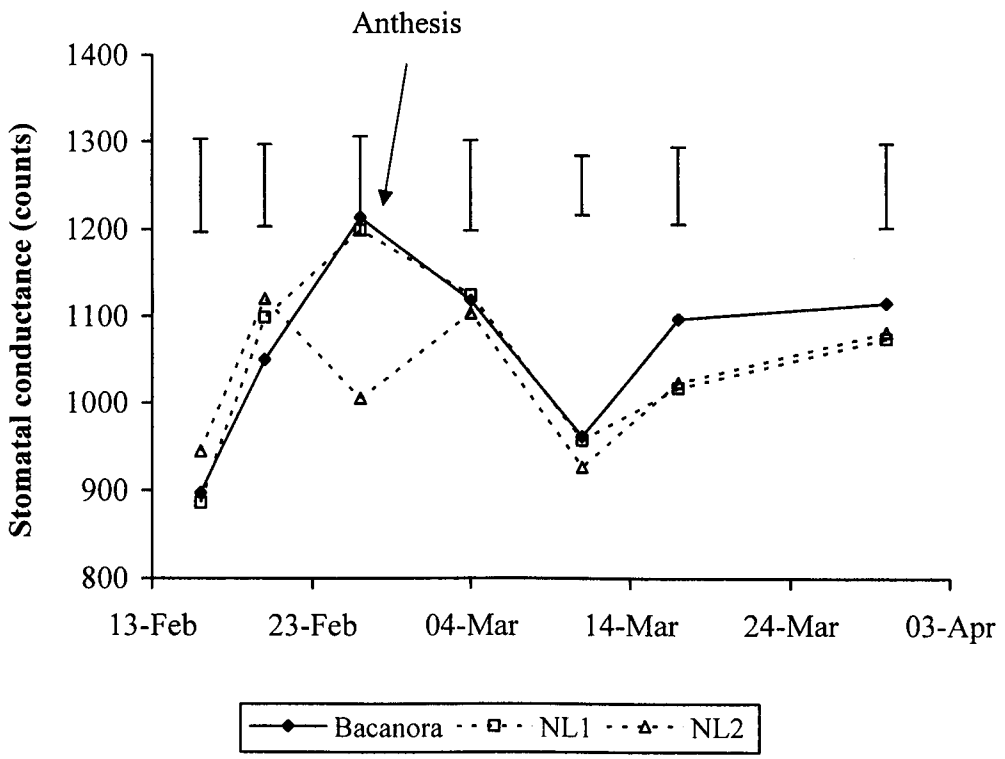


Figure 4.7(a). Stomatal conductance in 2004 for the three parental genotypes (Error bars show SED, DF=8). Arrow indicates the mean date at GS 61.

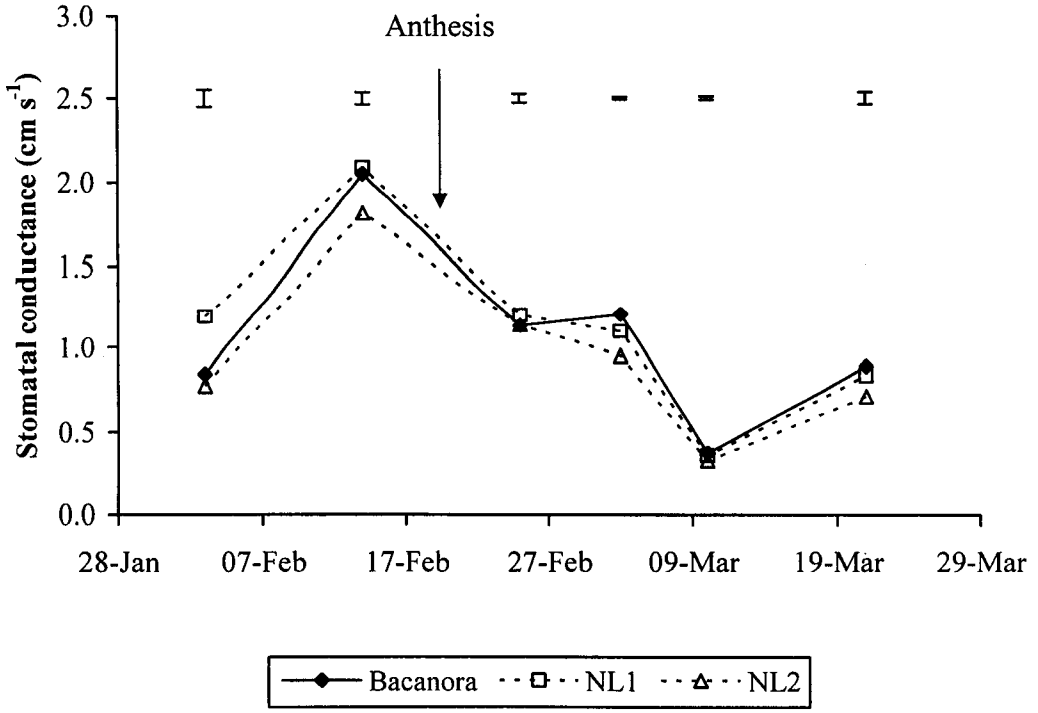


Figure 4.7(b). Stomatal conductance in 2005 for the three parental genotypes (Error bars show SED, DF=4). Arrow indicates the mean date at GS 61.

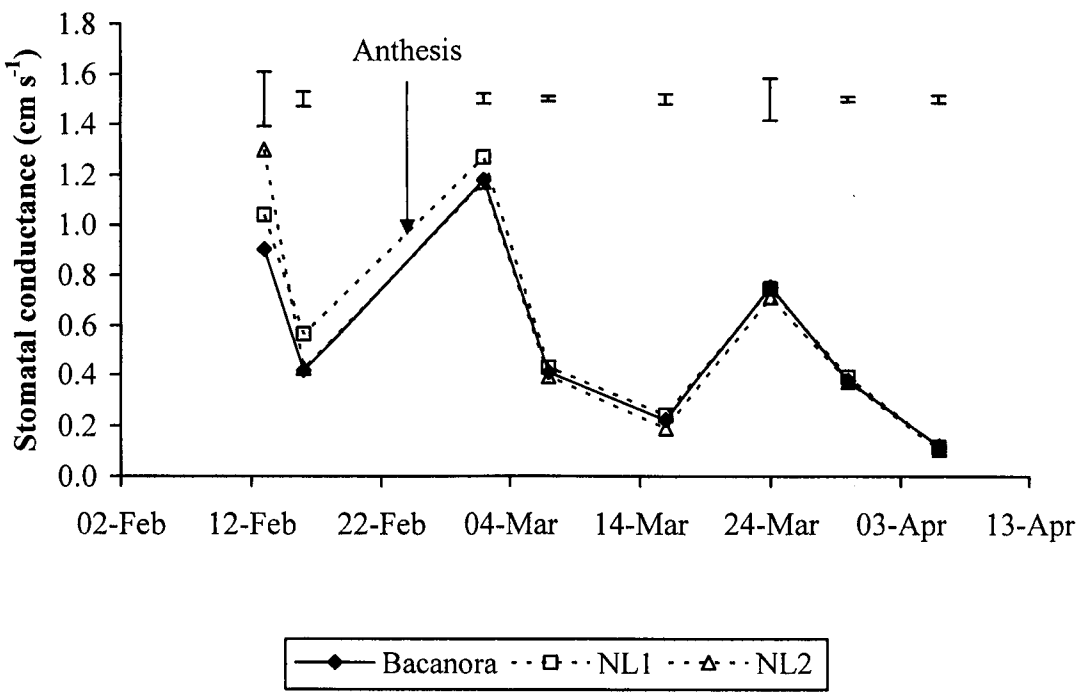


Figure 4.7(c). Stomatal conductance in 2006 for the three parental genotypes (Error bars show SED, DF=6). Arrow indicates the mean date at GS61.

4.3.12 Ear index, ear biomass, DM per ear and ratio of grains to ear DM at GS61

The genotypes differed in ear index in 2004 with NL2 having greater ear index compared to NL1 and Bacanora ($P < 0.01$; Table 4.8). No significant differences were observed in 2005 and 2006 (Table 4.8). Averaging across years, the genotypes differed in ear index; with NL2 having greater ear index compared to Bacanora and NL1. There was an interaction between the genotypes and years. In 2005, NL2 had a slightly lower ear index compared to the other genotypes, whereas in other years it was the highest ranking genotype.

The genotypes differed in ear biomass in 2004 and 2006 with NL2 having more ear biomass compared to NL1 and Bacanora ($P < 0.05$). Averaging across years, genotypes differed in ear biomass ($P < 0.001$) with NL2 (217 g m^{-2}) having more ear biomass compared to Bacanora (192 g m^{-2}) and NL1 (171 g m^{-2}). There was a significant interaction between genotypes and years ($P < 0.001$) whereby NL2 had relatively lower ear biomass in 2005 than in 2004 and 2006 compared to other genotypes.

There were differences amongst the genotypes in DM per ear in 2004 with novel genotypes having heavier ears than Bacanora, and NL2 having heavier ears than NL1 ($P = 0.001$). A similar trend was found in 2005 and 2006. Averaging across years, NL2 (0.74 g) had more dry matter ear^{-1} compared to NL1 (0.55 g), which in turn had more dry matter ear^{-1} than Bacanora (0.49 g) ($P < 0.001$). Ears were heavier in 2005 compared to 2004 and 2006 ($P < 0.05$). The genotype x and year interaction was not significant.

The genotypes differed in the ratio of grains-to-ear DM (i.e. grains per gram ear DM at GS 61) in 2004 and 2006 with NL1 having a greater ratio compared to Bacanora and NL2. No significant differences were observed in 2005, although the same trend was observed. Averaging across years, the genotypes differed in the grains-to-ear DM ratio ($P < 0.001$) with NL2 (71 grains g^{-1}) having a smaller ratio compared to the other genotypes (106-111 grains g^{-1}). There were significant differences between the years with the largest ratio in 2004, but no interaction between genotype and year.

4.3.13 Ear traits

4.2.13.1 Spikelets ear^{-1}

The genotypes differed in spikelet number per ear in 2004 and 2006 ($P < 0.05$) but not in 2005, with novel genotypes having more spikelets ear^{-1} than Bacanora (Table 4.9). Averaging across years, genotypes differed in spikelets ear^{-1} with NL1 and NL2 having two and one more spikelets ear^{-1} than Bacanora, respectively ($P < 0.05$). Year did not have an effect on spikelet number. A significant interaction was observed between the genotypes and years whereby NL1 had relatively fewer spikelets ear^{-1} than other genotypes in 2005 compared to 2004 and 2006.

4.2.13.2 Rachis length

Rachis length differed among the genotypes with novel genotypes (12.2-14.0 cm) as expected having a longer rachis than Bacanora (10.9 cm) ($P < 0.01$) averaging over years (Table 4.9). Cross-year ANOVA showed that NL1 had longer rachis than NL2 ($P < 0.001$). Rachis length was shorter in 2004 compared to 2005 and 2006 ($P < 0.05$). There was a significant interaction between genotypes and years. Rachis length was similar between NL1 and NL2 in 2005 but in other years rachis length was greater for NL1 than NL2.

Table 4.8. Ear index, ear biomass, DM per ear and grains-to-ear DM ratio in 2004, 2005 and 2006 for the three parental genotypes

Lines	2004				2005				2006				Mean			
	Ear index	Ear DM ($g\ m^{-2}$)	DM ear^{-1} (g)	Grain: Ear DM	Ear index	Ear DM ($g\ m^{-2}$)	DM ear^{-1} (g)	Grain: Ear DM	Ear index	Ear DM ($g\ m^{-2}$)	DM ear^{-1} (g)	Grain: Ear DM	Ear index	Ear DM ($g\ m^{-2}$)	DM ear^{-1} (g)	Grain: Ear DM
Bacanora	0.21	175	0.45	120	0.27	197	0.57	94	0.23	205	0.46	102	0.23	192	0.49	106
NL1	0.20	155	0.56	127	0.27	181	0.62	98	0.21	180	0.49	105	0.22	171	0.55	111
NL2	0.30	259	0.77	67	0.25	160	0.76	74	0.27	219	0.68	75	0.27	217	0.74	71
Mean	0.24	196	0.59	105	0.26	179	0.65	87	024	201	0.54	94	0.24	193	0.59	96
SED (DF)	0.02	16.8	0.04	11.6	0.01	20.4	0.03	13.8	0.02	9.87	0.04	4.00	0.01	8.91	0.02	5.80
Genotype	(6)	(6)	(6)	(6)	(4)	(4)	(4)	(4)	(6)	(6)	(6)	(6)	(16)	(16)	(16)	(16)
Year													0.01 (8)	8.35 (8)	0.03 (8)	4.30 (8)
Interaction													0.02 (23.9)	15.5 (23.8)	0.05 (22.9)	9.51 (22.3)

Table 4.9. Spikelets ear^{-1} and rachis length (cm) in 2004, 2005 and 2006 for the three parental genotypes

Lines	2004		2005		2006		Mean	
	Spikelets ear^{-1}	Rachis length (cm)	Spikelets ear^{-1}	Rachis length (cm)	Spikelets ear^{-1}	Rachis length (cm)	Spikelets ear^{-1}	Rachis length (cm)
Bacanora	21.0	10.1	21.7	11.1	20.6	11.4	21.1	10.9
NL1	22.6	14.5	20.9	13.1	23.0	14.2	22.3	14.0
NL2	21.4	11.2	22.6	12.7	21.9	12.8	21.9	12.2
Mean	21.7	11.9	21.7	12.3	21.8	12.8	21.8	12.4
SED (DF) Genotype	0.50 (6)	0.53 (6)	0.76 (4)	0.36 (4)	0.55 (6)	0.56 (6)	0.34 (16)	0.29 (16)
Year							0.51 (8)	0.23 (8)
Interaction							0.71 (21.0)	0.49 (22.8)

4.3.14 Post-anthesis measurements

4.3.14.1 Water soluble carbohydrates at GS 61+5d and physiological maturity

In 2004, water soluble carbohydrates in stems and attached leaf sheaths were determined 5 days after anthesis and then every five days until 25 days after anthesis and again at physiological maturity. The genotypes differed ($P < 0.05$) in stem WSC from 5 days after anthesis until maturity (Figure 4.8), with higher amounts accumulated by Bacanora and NL2 than by NL1. Stem WSC started to decline steadily in Bacanora and NL2 from GS 61+15d but stem WSC declined later for NL1 from GS 61+25d. The genotypes differed in stem WSC five days after GS 61 in each year ($P < 0.01$) with NL1 (0.37 t ha^{-1}) having less WSC than Bacanora (0.59 t ha^{-1}) and NL2 (0.54 t ha^{-1}) (Table 4.10). The differences in maximum amounts accumulated were probably greater than these values as stem WSC increased between GS 61+5d and 15 days for Bacanora and NL2. Cross-year ANOVA showed that NL1 had lower stem WSC compared to Bacanora and NL2 ($P < 0.001$). Year had an effect on the amount of stem WSC with highest amounts accumulated in 2005 ($P < 0.05$). There was an interaction between the genotypes and years. In 2004, NL2 accumulated more WSC in stems than Bacanora at GS 61+ 5 days whereas in 2005 and 2006 the reverse was observed.

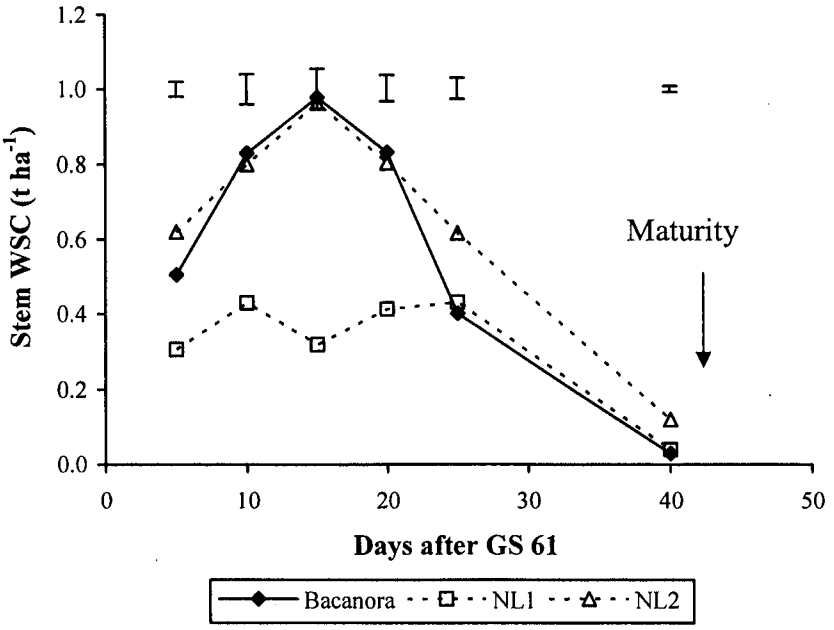


Figure 4.8. Water soluble carbohydrates in stems and leaf sheaths of parental lines in 2004 (Error bar showing SED, DF=6)

Table 4.10. Water Soluble Carbohydrates (WSC) in stems and leaf sheaths (t ha^{-1}) at GS 61 plus 5 days and maturity for the three parental genotypes in 2004, 2005 and 2006

Lines	2004	2005	2006	Mean
GS 61+5d				
Bacanora	0.51	0.67	0.60	0.59
NL1	0.31	0.47	0.35	0.37
NL2	0.62	0.61	0.40	0.54
Mean	0.48	0.58	0.45	0.50
SED (DF)Genotype	0.04 (6)	0.04 (4)	0.04 (6)	0.021 (16)
Year				0.032 (8)
Interaction				0.054 (20.9)
Maturity				
Bacanora	0.03	0.04	0.03	0.04
NL1	0.04	0.04	0.09	0.06
NL2	0.12	0.05	0.04	0.07
Mean	0.06	0.04	0.05	0.06
SED (DF) Genotype	0.01 (6)	0.008 (4)	0.005(6)	0.006 (16)
Year				0.005 (8)
Interaction				0.012 (22.4)

Significant differences were observed at maturity amongst the genotypes in 2004 and 2006 ($P < 0.01$) with NL2 having highest values in 2004 and NL1 in 2006. However, absolute differences were small. Averaging across the years, NL2 had more stem WSC at maturity than other genotypes ($P < 0.01$). The amount of stem WSC accumulated was greater in 2004 than in other years ($P < 0.001$). A significant interaction was observed between genotypes and years ($P < 0.001$) whereby in 2004 and 2005 NL2 ranked highest of the three genotypes while in 2006 Bacanora ranked highest (Table 4.10). Moreover, averaging over years, Bacanora mobilised more stem reserves (93%) compared to NL1 (83%) and NL2 (87%).

4.3.14.2 Leaf activity traits during grain filling

The lines did not differ in canopy temperature during grain filling in 2004 and 2005 (Figures 4.6a-b). However, in 2006, Bacanora had higher canopy temperature than novel genotypes (Figure 4.6 c) ($P < 0.05$). In 2004 and 2005, Bacanora had higher stomatal conductance than novel lines during grain filling ($P < 0.05$) but no significant differences were detected amongst the lines in 2006 (Figures 4.7 a-c).

4.3.14.3 Potential grain weight and grain sink size

The genotypes differed in potential grain weight with novel genotypes having greater PGW than Bacanora in each year ($P < 0.01$). Cross-year ANOVA showed that NL2 (50.9 mg) had greater PGW compared to NL1 (47.7 mg), which in turn had greater PGW than Bacanora (43.8 mg) ($P < 0.05$). PGW was greater in 2005 than in other years ($P < 0.001$). There was an interaction between genotypes and years ($P < 0.05$) with NL1 and Bacanora having similar PGW in 2004, but in other years NL1 having greater PGW than Bacanora.

Estimated grain sink size differed among the genotypes in 2004 and 2005 ($P < 0.01$) but not in 2006. In 2004, greatest sink size was found for Bacanora while in 2005 it was for NL1 (Table 4.11). In all three years, NL2 had the lowest estimated sink size. Averaging over the years, NL2 (801 g m^{-2}) had smaller sink size than the other two genotypes (891-898 gm^{-2}) ($P < 0.001$). Larger sink sizes were produced in 2004 and 2006 than in 2005 ($P = 0.05$). A significant interaction between genotypes and years indicated that in 2005 NL2 had a relatively lower sink size compared to other genotypes than in 2004 and 2006.

4.3.14.4 Crop height

The genotypes differed in height with novel genotypes being taller than Bacanora in each of the three years ($P < 0.01$). Averaging across the years, NL1 was 6.6 cm and 14 cm taller than NL2 and Bacanora, respectively (Table 4.12) ($P < 0.001$). Year had an effect on plant height with taller plants observed in 2006 than in other years ($P < 0.001$). There was no significant interaction between genotypes and years.

Table 4.11. Potential grain weight (PGW) in mg and estimated sink size (g m^{-2}) from 2004 to 2006

Lines	2004		2005		2006		Mean	
	PGW (mg)	Sink size (g m^{-2})	PGW (mg)	Sink size (g m^{-2})	PGW (mg)	Sink size (g m^{-2})	PGW (mg)	Sink size (g m^{-2})
Bacanora	44.5	962	45.2	823	41.9	872	43.8	898
NL1	45.6	877	50.9	899	47.8	903	47.7	891
NL2	47.6	853	55.1	649	51.9	849	50.9	801
Mean	45.9	897	50.4	790	47.2	875	47.5	863
SED (DF) Genotype	0.57 (8)	25 (8)	0.75 (4)	52 (4)	1.67 (6)	27.5 (6)	0.63 (18)	18.7 (18)
Year							0.52 (9)	37.5 (9)
Interaction							1.06 (25.9)	46.4 (18.5)

Table 4.12. Crop height (cm) from 2004 to 2006 of three parental genotypes

Lines	2004	2005	2006	Mean
Bacanora	80.8	78.0	85.9	81.8
NL1	93.9	90.9	102	95.8
NL2	88.1	86.6	92.4	89.2
Mean	87.6	85.2	93.4	88.9
SED (DF) Genotype	2.22 (8)	1.76 (4)	1.31 (6)	1.13 (18)
Year				0.95 (9)
Interaction				1.90 (26.2)

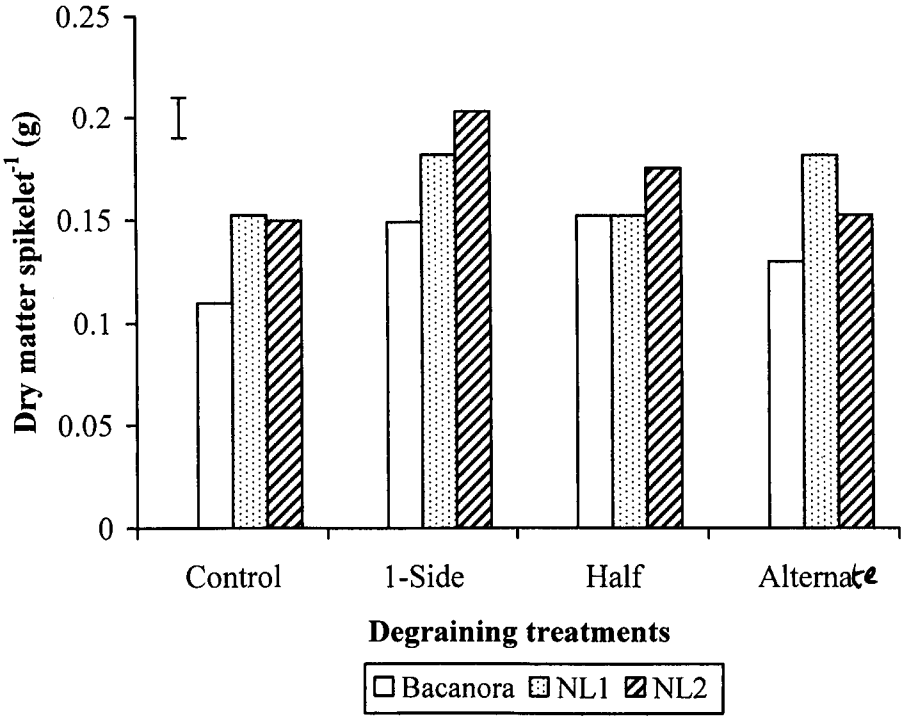
4.3.15 Response to degraining

4.3.15.1 Effects of different types of degraining in 2004

In 2004, the shoots were subjected to three types of degraining at GS 61 +14 days, namely: (i) removal of all spikelets from 1-side of the ear (ii) cutting the top half of the ear off and (iii) removing alternate spikelets from either side of the ear. Only one degraining treatment was carried out in 2005 and 2006 and the following section will compare response of 1-sided degraining treatment in different seasons.

There were significant differences among the genotypes in DM spikelet^{-1} in control shoots with novel genotypes having greater values than Bacanora ($P < 0.01$). Degraining of the ear by each of the three types of degraining resulted in more DM spikelet^{-1} compared to the control treatment ($P < 0.05$). No significant interaction was found between genotypes and treatments. The genotypes differed in individual grain weight at harvest in control shoots with novel genotypes having heavier grains compared to Bacanora ($P < 0.001$). Degraining resulted in heavier grains ($P < 0.001$) with the response obtained from the 1-sided treatment being similar to the half and the alternate treatments. A significant interaction was observed between the genotypes and treatments with NL2 showing a greater response to the 1-sided degraining treatment compared to the other genotypes ($P < 0.05$).

(a)



(b)

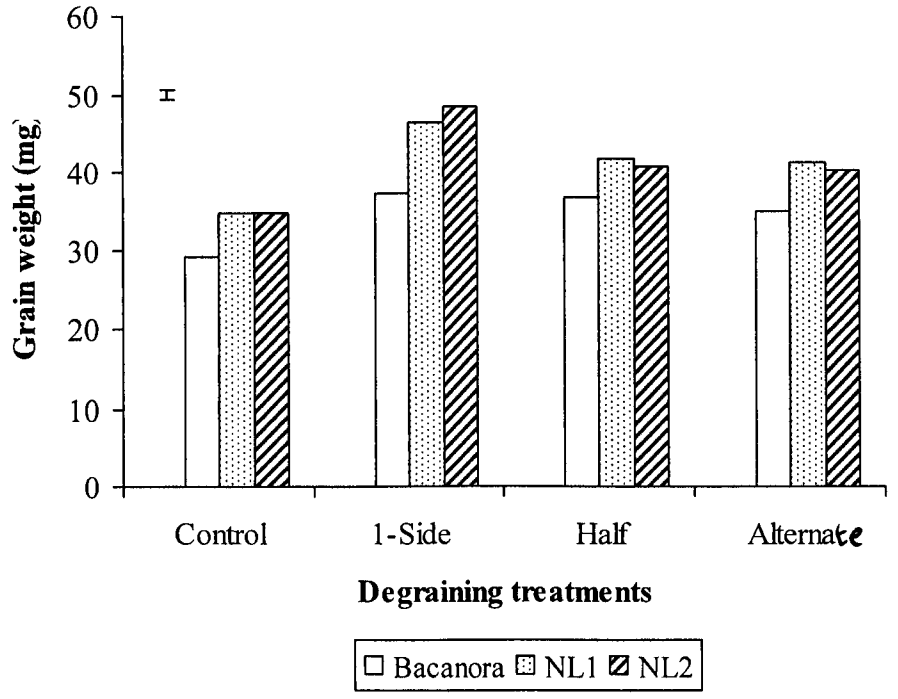


Figure 4.9. Effect of degraining treatments on (a) dry matter spikelet^{-1} and (b) individual grain weight at harvest in 2004 (Error bar represents SED)

4.3.15.2 Effect of the 1-sided degraining treatment on dry weight spikelet^{-1} in 2004, 2005 and 2006

In 2004, the genotypes differed in DM spikelet^{-1} at harvest with novel genotypes accumulating more dry matter than Bacanora in the control treatment (P <0.001). Removal of spikelets from one side of the ear had a significant positive effect on DM spikelet^{-1} at harvest (P < 0.001). An interaction between genotypes and treatment occurred with NL2 having a greater response to degraining compared to NL1 and Bacanora (P < 0.05). In 2005, differences were found among the genotypes with novel genotypes accumulating more DM spikelet^{-1} in the control treatment (P < 0.001). Degraining resulted in more DM spikelet^{-1} (P < 0.01). However, no significant interaction was found between genotypes and treatments. Similar findings were found in 2006 to those in 2005. Cross-year ANOVA showed that there were differences among the genotypes (P < 0.001) in control ears with novel genotypes having heavier spikelets than Bacanora (Table 4.13). Degraining resulted in greater DM spikelet^{-1} (P < 0.001). The response was greater in 2005 and 2006 than in 2004. Averaged across years, the genotypes responded similarly to degraining.

4.3.15.3 Effect of 1-sided degraining treatment on individual grain weight in 2004, 2005 and 2006

In 2004, the genotypes differed in individual grain weight at harvest (P < 0.001) in the control shoots with novel genotypes having heavier grains than Bacanora (Table 4.14). Degraining resulted in heavier grains compared to intact ears (P < 0.001). However, all genotypes responded in a similar way to degraining treatment. Similar findings were observed in 2005 and 2006. Averaging across degraining treatments, year had an effect on grain weight with heavier grains found in 2005 and 2006 than in 2004 (P < 0.001). A significant interaction was observed between degraining treatment and year whereby in 2004 there was a greater response to degraining than in 2005 and 2006 (P < 0.001).

Table 4.13. Effects of degraining (1-sided) on DM spikelet^{-1} (g) at harvest for the three parental genotypes in 2004, 2005 and 2006

Lines		2004		2005		2006		Mean	
		Control	Degrain	Control	Degrain	Control	Degrain	Control	Degrain
Bacanora		0.10	0.15	0.17	0.19	0.15	0.18	0.14	0.17
NL1		0.14	0.18	0.22	0.25	0.20	0.24	0.19	0.22
NL2		0.14	0.20	0.24	0.26	0.24	0.28	0.20	0.25
Mean		0.13	0.18	0.21	0.23	0.20	0.23	0.18	0.21
SED (DF)	Genotype	0.004 (10)		0.007 (10)		0.005 (10)		0.003 (24)	
	Treatment		0.003 (10)		0.006 (10)		0.004 (10)		0.002 (12)
	Year							0.002 (12)	
	Interaction							0.004 (34.8)	

Table 4.14. Effects of degraining (1-sided) on individual grain weight at harvest for the three parental genotypes in 2004, 2005 and 2006

Lines		2004		2005		2006		Mean	
		Control (mg)	Degrain (mg)	Control (mg)	Degrain (mg)	Control (mg)	Degrain (mg)	Control (mg)	Degrain (mg)
Bacanora		26.7	37.3	39.2	41.9	38.2	41.6	34.7	40.2
NL1		33.2	46.3	50.1	53.3	49.6	53.5	44.3	51.0
NL2		32.9	48.3	49.5	51.7	47.8	50.0	43.4	50.0
Mean		30.9	44.0	46.3	49.0	45.2	48.4	40.8	47.1
SED (DF)	Genotype	1.13 (10)		0.67 (10)		1.28 (10)		0.64 (24)	
	Treatment		0.92 (10)		0.54 (10)		1.04 (10)		0.43 (12)
	Year							0.52 (12)	
	Interaction							0.85 (35)	

4.3.15.4 Effect of degraining on individual grain weight in apical, basal and central spikelets

4.3.15.4a Apical spikelets

Generally, greater responses to degraining were obtained from the grains in apical spikelets than those in other spikelet positions. In 2004, novel genotypes had heavier grain weight than Bacanora ($P < 0.01$). Degraining resulted in heavier grains compared to the control shoots ($P < 0.01$). No significant interaction was found between genotypes and degraining treatments. Similar findings were observed in 2005 and 2006 (Table 4.16a). Averaging across degraining treatments, lighter grains were found in 2004 than in 2005 and 2006 ($P < 0.001$). There was an interaction between degraining treatment and year ($P < 0.05$), whereby a greater response of grain weight to degraining was found in 2004 than in 2005 and 2006.

4.3.15.4b Basal spikelets

The genotypes differed in grain weight in basal spikelets ($P < 0.001$) with novel genotypes having heavier grains than Bacanora in 2004. Degraining resulted in heavier grains compared to the control ($P < 0.001$). The genotype x degraining treatment interaction was not significant. Similar findings were observed in 2005. In 2006, the genotypes differed in grain weight with novel lines having heavier grains than Bacanora. Neither the year effect nor the year x degraining treatment interaction was significant. Averaging across the years, novel genotypes had heavier grains in basal spikelets than Bacanora ($P < 0.001$). Degraining resulted in heavier grains than the control ($P < 0.001$). Averaging across degraining treatments, lighter grains were found in 2004 than in 2005 and 2006 ($P < 0.001$). The genotype x degraining treatment interaction was not statistically significant. An interaction between year and treatment was found whereby a greater response to degraining was observed in 2004 than in other years.

4.3.15.4c Central spikelets

The genotypes differed in grain weight in the central spikelets ($P < 0.001$) with novel genotypes having heavier grains than Bacanora in 2004 (Table 4.16c). Degraining resulted in heavier grains compared to the control shoots ($P < 0.001$). The genotype x degraining treatment interaction, however, was not significant. Similar results were obtained in 2005 and 2006. Averaging across the years, novel genotypes had heavier grains in central spikelets than Bacanora ($P < 0.001$). Grains were heavier in the degrained ears than the control ears by 4.9 mg ($P < 0.001$). Lighter grains were observed in 2004 than in 2005 and 2006 ($P < 0.001$). There was an interaction between genotypes and year whereby novel genotypes had relatively heavier grains than Bacanora in 2005 and 2006 than in 2004 ($P < 0.001$). Furthermore, a year x treatment interaction was observed with a greater response to degraining observed in 2004 than in 2005 and 2006 ($P < 0.001$).

Table 4.15a: Effects of degraining (1-sided) on individual grain weight (mg) in 2004, 2005 and 2006 for apical spikelets

Lines		2004		2005		2006		Mean	
		Control	Degrain	Control	Degrain	Control	Degrain	Control	Degrain
Bacanora		25.0	32.3	32.2	33.4	33.0	35.0	30.1	33.6
NL1		31.8	42.7	44.9	48.1	45.6	49.4	40.8	46.7
NL2		30.4	38.8	44.7	46.6	44.3	45.7	39.8	43.7
Mean		29.1	37.9	40.6	42.7	41.0	43.4	36.9	41.3
SED (DF)	Genotype	2.39 (10)		0.54 (10)		1.13 (10)		0.86(24)	
	Treatment		1.95 (10)		0.44 (10)		0.92 (10)		0.86 (12)
	Year							1.05 (12)	
	Interaction							2.28 (34.7)	

Table 4.15b: Effects of degraining (1-sided) on individual grain weight (mg) in 2004, 2005 and 2006 for basal spikelets

Lines		2004		2005		2006		Mean	
		Control	Degrain	Control	Degrain	Control	Degrain	Control	Degrain
Bacanora		26.3	38.0	42.2	45.6	39.8	44.5	30.7	42.7
NL1		32.6	43.1	52.1	55.6	50.5	53.4	48.4	50.7
NL2		33.3	41.4	50.9	53.5	48.6	50.9	46.3	48.6
Mean		30.7	40.8	48.4	51.6	46.3	49.6	41.8	47.3
SED (DF)	Genotype	0.48 (10)		0.76 (10)		2.29 (10)		0.91 (24)	
	Treatment		0.39 (10)		0.62 (10)		1.87(10)		0.33(12)
	Year							0.41 (12)	
	Interaction							1.91 (28.5)	

Table 4.15c: Effects of degraining (1-sided) on individual grain weight (mg) in 2004, 2005 and 2006 for central spikelets

Lines		2004		2005		2006		Mean	
		Control	Degrain	Control	Degrain	Control	Degrain	Control	Degrain
Bacanora		28.2	37.7	40.6	43.2	40.1	43.3	32.4	40.7
NL1		34.6	41.7	51.6	54.9	51.8	56.5	47.9	50.6
NL2		34.4	42.6	51.4	53.7	49.5	52.7	47.1	50.8
Mean		32.4	40.7	47.9	50.6	47.1	50.8	42.5	47.4
SED (DF)	Genotype	0.45 (10)		0.76(10)		0.96 (10)		0.44 (24)	
	Treatment		0.37 (10)		0.62 (10)		0.78(10)		0.38 (12)
	Year							0.47(12)	
	Interaction							1.10 (35.9)	

4.4 DISCUSSION

Grain yield is determined not only during the grain filling stage but also during the pre-anthesis period. The discussion will focus firstly on the yield and its components before analysing the pre-anthesis physiological factors that determine grains m^{-2} and secondly to the post-anthesis factors that determine grain weight in the parental genotypes. The source and sink balance in relation to degraining responses will also be considered.

4.4.1 Yield components

Overall the grain yield of NL2 (6.30 t ha^{-1}) was lower than that of NL1 (7.04 t ha^{-1}) and Bacanora (6.88 t ha^{-1}). Grain yield in wheat comprises two major components, namely: the number of grains per unit area and the individual grain mass. Grain yield amongst the genotypes was more similar than would be predicted by differences amongst the genotypes in grains m^{-2}. This was because both potential grain weight and final grain weight were higher in novel genotypes compared to Bacanora. The two grain yield components, however, have been reported to be negatively associated amongst genotypes (Siddique *et al.*, 1989; Slafer and Andrade, 1993). This trend was also observed in this study too as the novel genotypes had fewer grains m^{-2} but heavier grains than Bacanora. The latter with more grains m^{-2} had lower grain weight possibly due to an increase in competition for assimilates during grain filling or alternatively due to lower average potential grain weight with probably more grains in distal floret and/or spikelet positions. Grains m^{-2} was lower in NL2 (15 886) compared to NL1 (18 737) and Bacanora (20 533); the latter two genotypes did not differ significantly. Aggarwal *et al.* (1986) stated that post-anthesis assimilate availability does not always influence grain weight in relation to grain number per unit area and reported that compensation between grain number per unit area and grain weight occurred mostly when the former exceeded 11,000 grain m^{-2} which agrees with the current findings.

Higher above-ground biomass was produced by NL1 compared to Bacanora and NL2 at physiological maturity and higher yield in NL1 compared to NL2 was attributed to biomass rather than HI. NL2, on the other hand, had the lowest biomass at maturity, a 7.5% and 15% reduction in biomass compared to Bacanora and NL1, respectively

and 'gigas' lines possessing the tiller inhibition *tin* gene can have reduction in biomass up to 7% according to Duggan *et al.* (2005a).

More grains ear^{-1} in the novel genotypes compared to Bacanora was associated with a longer rachis length of novel genotypes and 1-2 more spikelets per ear. Spikelet number was likely affected by developmental responses affecting spikelet primordia production and this will be discussed further in Chapter 5. NL2 showed attributes associated with the *tin* gene on chromosome 1A (Richards, 1988) as expected. Duggan *et al.* (2005a), averaging effects across four pairs of near-isogenic lines under irrigated conditions where 50 kg N ha^{-1} was applied in Australia, reported the *tin* gene to reduce ears m^{-2} by 21% and increase grains ear^{-1} by 15%. The presently observed difference in ears m^{-2} between NL2 and Bacanora of 26% was comparable to this. However, the increase in grains ear^{-1} in NL2 compared to Bacanora was relatively modest, only 4%. The expression of grains ear^{-1} in the present study in the *tin* genotype may have been reduced compared to that in the investigation of Duggan *et al.* (2005a), since in that investigation plant establishment was 84-89 plants m^{-2}, lower than in the current investigation at *ca.* 160 plants m^{-2}. However, the genotypes did not differ in the number of grains per spikelet; it was rather spikelets ear^{-1} that accounted for higher grains per ear^{-1} in the novel genotypes. It is possible that plant hormones may play an important role in floret fertility, cytokinins and abscisic acid are the hormones most related to floret fertility (Waters *et al.*, 1984; Zeng *et al.*, 1985, Rawson and Evans, 1970). A lower concentration of ABA at booting stage was associated with increased number of grains ear^{-1} (Reynolds, unpublished) and novel genotypes may have had a lower level of ABA and higher level of cytokinin compared to Bacanora.

The higher yield of NL1compared to NL2 was associated with more biomass produced at maturity, more grains m^{-2} and more ears m^{-2}. Similarly, lower yield of NL2 compared to Bacanora could be ascribed to NL2 producing less biomass, having fewer grains m^{-2} and fewer ears m^{-2}. The presence of *tin* gene in NL2 (Sean Mayes, personal communication) could account for its fewer ears m^{-2}, more grains ear^{-1} and heavier grains, observations which are consistent with the findings of Duggan *et al.* (2005a). When ears m^{-2} was reduced the genotype was able to compensate by increasing the grains ear^{-1} and grain weight components, probably due to the negative

relationship between ears m^{-2} and grains ear^{-1} in relation to pre-anthesis assimilate supply. However, the grain weight compensation was larger than via grains ear^{-1}.

Despite, shorter rachis length and fewer spikelets ear^{-1}, grains m^{-2} (main target trait for breeders) was higher in all three years for Bacanora while novel genotypes counteracted this to some extent through higher grain weight. What would be interesting to know is to what extent increases in grains ear^{-1} and grain weight in novel genotypes may be partially independent of decreases in ears m^{-2}. This will be examined further in Chapter 6. Although genetic progress in yield potential is strongly associated in grain number (Slafer *et al.*, 1994), Calderini *et al.* (1995) showed that increased grain weight has contributed to improved yield potential in Argentinean wheat. Heavier grains were associated with higher potential grain weight of the novel lines in the present study and the presence of *tin* gene that is in NL2 is known to contribute to heavier grains (Atsmon and Jacobs, 1977; Richards, 1988; Duggan *et al*, 2005a).

Both potential grain weight and final grain weight were higher in novel genotypes than Bacanora. Present results indicated that the higher PGW in NL2 compared to Bacanora was associated with a lower grains to ear DM ratio as expected. However, higher PGW in NL1 was achieved without at a similar reduction in this ratio. This is encouraging in that it suggests it may be possible to uncouple these two parameters genetically as a route to increasing yield potential. The mechanisms underlying the maintenance of high PGW at a high grains to ear DM ratio in NL1 are not known. It is possible that genes derived from *Triticum polonicum* have caused changes in glume characteristics facilitating larger PGW through alleviation of physical restrictions. Maximum grain weight may be physically restricted by glumes (Millet, 1986). A second possibility is that an increase in rachis length per spikelet in NL1 compared to other genotypes could have facilitated increased spikelet photosynthesis, hence enhanced assimilate supply to the florets in the periods immediately preceding and following anthesis. Potential grain weight may be influenced by assimilate supply in the week before anthesis (Calderini and Reynolds, 2000); a linear and curvilinear relationship between PGW and carpel weight at anthesis was reported in barley (Scott *et al.*, 1983) and wheat (Calderini *et al.*, 1999). Calderini *et al.* (1999) suggested the existence of a carpel weight threshold of 1 mg at anthesis for achieving

maximum grain weight. In addition, assimilate supply during the first 14 days of the post-anthesis period can affect the rate of endosperm cell division, hence the number of endosperm cell per grain in wheat (Brocklehurst, 1977). Potential grain weight can be restricted by the number of endosperm cells as each cell has limited capacity for starch storage. In summary, increases in PGW could be related to reduced competition for assimilates, both pre-anthesis and post-anthesis, associated with increased ear photosynthesis. Furthermore, present results showed a greater sink size for Bacanora and NL1 compared to NL2 by *ca.* 100 g m^{-2}. The importance of sink size post-anthesis in being the main factor limiting grain yield (Richards, 1996) appears to hold true in the present study since both Bacanora and NL1 with greater sink size had higher yield than NL2 mainly due to their higher grains m^{-2}.

The general experience around the world is that grain weight has changed little with breeding. Therefore, present findings for increased PGW in novel genotypes are of interest to CIMMYT and UK breeders since grain weight can also be a target trait for increasing yield potential. There is a trade off for the novel genotypes in reduction in grains m^{-2} with higher grain weight but there was no yield penalty in NL1 compared to Bacanora. Lower grains m^{-2} in NL2 compared to Bacanora and NL1 was associated primarily to its lower tillering ability and to a lesser extent to fewer plants m^{-2}. One important target for breeders in exploiting these novel genotypes in their programmes would be to aim to increase grain weight whilst simultaneously maintaining grains m^{-2}. If glume length could be increased by the novel material potentially alleviating physical restriction on the floret volume cavity, and simultaneously sufficient assimilate supply could be maintained to minimize limitation to carpel weight and number of endosperm cells, a balance could be achieved to increase grain weight and maintain grains m^{-2}. Moreover, if a marker could be identified that can be linked to potential grain weight this would facilitate the breeders task of selecting for higher grain weight. In addition, there is a need to identify other traits to counteract restricted tillering in novel genotypes.

4.4.2 Physiological basis of grains m^{-2} in the pre-anthesis period

4.4.2.1 Radiation interception and RUE

It is well established that radiation has a profound effect on crop growth. The earlier canopy closure is reached the more radiation is intercepted by the crop. Bacanora accumulated more PAR (MJ m^{-2}) compared to the novel genotypes from booting to anthesis, the 2- to 3-week when the ears rapidly accumulate dry matter. Fischer (1985) conducting shading experiments, found that for a given genotype radiation interception during this period principally determined grains m^{-2}. Bacanora had more grains m^{-2} in all three years compared to the novel genotypes. However, differences in grains m^{-2} were not associated with differences in anthesis biomass. More light intercepted by NL1 in the early vegetative phase from GS 31 to GS 41 could explain the higher biomass produced by the genotype at GS 41. However, by anthesis no significant differences in biomass were observed among the genotypes, although Bacanora had *ca.* 50 g m^{-2} more biomass than the novel genotypes. When averaged over years, the increment in biomass from booting to anthesis was greater in NL2, probably due to an increased investment in non-reproductive sinks of the ear structures. Light interception was largely maintained in NL2 despite smaller GAI due to the Beer's law relationship and lower interception by NL2 compared to Bacanora was due to smaller canopy size associated with the *tin* gene. However, NL2 showed higher RUE compared to the other genotypes. Overall, the trend for higher biomass accumulation at anthesis for Bacanora could be ascribed more to higher accumulated PAR rather than differences in RUE. The differences in grains m^{-2} amongst the genotypes, however, were not explained by anthesis biomass.

Extinction coefficient, k_{PAR} at anthesis did not differ amongst the genotypes (range of 0.51-0.52). Reported k_{PAR} for wheat until anthesis from various investigations under irrigated and high potential conditions ranged from 0.36 to 0.76 (Abbate *et al.*, 1998). Present values were intermediate in this range and typical of modern semi-dwarf varieties as demonstrated by the range of k_{PAR} in UK wheats of 0.45 to 0.70 reported by Shearman *et al.* (2005). Therefore, k_{PAR} of parental genotypes was not influencing the differences amongst genotypes in radiation capture. In addition, wheat genotypes often differ in their angle of leaf inclination (Duncan, 1971; Ledent, 1976) which may alter the pattern of light distribution in canopy (Trenbath and

Angus, 1975), photosynthetic rate (Austin *et al.*, 1976) and dry matter production (Trenbath and Angus, 1975).

Amongst the parental genotypes, NL2 had recurved leaves compared to Bacanora and NL1 having erect to semi-erect leaves. Austin *et al.* (1976) reported that in the UK canopy photosynthesis was consistently higher in erect-type than in lax-type cultivars due to greater penetration of PAR into the canopy. Similarly, Araus *et al.* (1993) showed that leaf angle played an important role in grains m^{-2} whereby erect-leaved genotypes had higher stomatal conductance than lax-leaved counterparts due to reduced light saturation of upper leaves. Hence, more irradiance could be available to lower leaves which could contribute more to total biomass (Hay and Walker, 1989). The pre-anthesis k values, however, showed that there was little difference amongst genotypes in fractional interception with increase in GAI with canopy depth in the present study.

Monteith (1977) demonstrated the linear relationship between accumulated dry matter and intercepted solar radiation in barley and suggested that for most C_3 crops under good growing conditions approximately 1.4 g of crop mass was accumulated per MJ of intercepted solar radiation. Previous studies on wheat have shown different RUE values amongst genotypes. Abbate *et al.* (1998) found the value of pre-anthesis RUE_{PAR} to be 2.7 ± 0.28 g MJ^{-1} in Argentinean cultivars whilst Shearman *et al.* (2005) reported a range of RUE_{PAR} of 2.33 to 2.64 g MJ^{-1} in eight UK winter wheats. Current findings show that pre-anthesis RUE_{PAR} was in the range of 2.05 to 2.58 g MJ^{-1} averaged over the two years with lower RUE in NL1 than in NL2 with an intermediate value for Bacanora. The current differences in RUE were not explained by differences in k_{PAR} or leaf angle. Therefore, the tendency for more biomass produced at anthesis in Bacanora than novel genotypes was due to higher radiation interception and a slightly longer duration from GS 31 to GS 61 (39 days vs. 37 and 33 days in NL1 and NL2 respectively), as observed in 2006, rather than effects of RUE. Indeed differences in RUE tended to counteract differences in radiation interception amongst the genotypes. It has been reported that a longer stem-elongation phase could provide more assimilates for the survival of tillers and fertile florets resulting in an increase in grains m^{-2} in spring wheat (Reynolds *et al.*, 2000)

4.4.2.2 Leaf activity traits

Fischer *et al.* (1998) demonstrated that stomatal conductance, photosynthetic rate and canopy temperature depression (CTD) amongst eight semi-dwarf spring wheat were closely and positively correlated with yield in North West Mexico and the present study was carried out at same site. Similar findings in sub-tropical environments were reported by Reynolds *et al.* (1994). In fact, CTD has been shown to be associated with yield differences between homozygous lines, indicating a potential for genetic gains in yield in response to selection for CTD (Reynolds *et al.*, 1998). The inconsistency among the genotypes for effects of canopy temperature and stomatal conductance, however, makes it difficult to draw any conclusion from the present data. They were not associated with genotype differences in grains m^{-2}.

4.4.2.3 Ear index and grain to ear DM ratio

NL2 had 11% and 21% more ear biomass compared to Bacanora and NL1, respectively. Results showed that NL2 had the higher ratio of ear to above ground dry matter at GS 61 compared to NL1 and Bacanora but this did not result in higher grains m^{-2}. A lower ear index of NL2 in 2005 could be due to an interaction with lower plant establishment in that year. As reported by Slafer and Savin (1994), the general observation is that the greater the ear index at anthesis the more grains m^{-2} is obtained at harvest. Therefore, present results are atypical with regard to this association between ear biomass and grain number. However, Abbate *et al.* (1998) stated than in Argentinean wheat cultivars genetic progress in grains m^{-2} occurred through the increase in the grains-to-ear DM ratio rather than an increase in ear index amongst five cultivars in the range 61-106 grains g^{-1}, and that grains m^{-2} was more closely related to this ratio than to dry weight of ears at anthesis. In the UK Shearman *et al.* (2005) reported a range of 73-129 grain g^{-1} but found that this ratio was not associated with breeding progress in grains m^{-2}.

NL2 had a lower grains-to-ear DM ratio (71 grains g^{-1}) at GS61 compared to NL1 (111 grains g^{-1}) and Bacanora (106 grains g^{-1}). This was the main physiological basis for lower grains m^{-2} in NL2. Ear index was marginally lower and grains-to-ear DM ratio in NL1 was marginally higher than for Bacanora, but these differences were not

statistically significant. Abbate *et al.* (1997) reported a value of 96 grains g^{-1} for Bacanora while present results indicated a value of 106.2 grains g^{-1} (average over 3 years). Fisher (1983) stated that cultivars with the greatest grains m^{-2} could have more grains per gram of spikes (i.e. grains-to-ear DM ratio) which again agrees with current findings. The lower value of NL2 for the ratio could be due to larger chaff dry weight in proportion to total ear dry weight or partitioning a higher proportion of ear dry weight to non-reproductive sinks. Indeed, Motzo *et al.* (2004) reported an increase in chaff weight of lines having the *tin* gene as mentioned above. Thus, this trait could potentially be of importance to assist in future selection for grain yield potential.

4.4.2.4 Tiller production

The novel genotype NL2 showed characteristics of 'gigas' wheat. Atsmon et *al.* (1986) reported that gigas characteristics appear to derive from the capacity to utilise the savings from restricted tillering for enhanced ear growth per shoot. Hence, the lower yield in NL2 could be ascribed to its restricting tillering capacity resulting in fewer ears m^{-2}.The genetically low shoot production of NL2 was due to the presence of the tiller inhibiting gene (e.g. recessive *tin* gene located on chromosome 1AS (Richards, 1988). There is evidence from recent molecular mapping work carried out at Nottingham that NL2 has the *tin* gene (Sean Mayes, personal communication).

An investigation of the *tin* gene effects using four pairs of near-isogenic lines under irrigated conditions in Australia, has been recently reported (Duggan *et al.,* 2005a). They reported that with the *tin* gene there was smaller LAI, less accumulated PAR and slightly smaller biomass production. Present findings are consistent with the effects of *tin* gene on tiller production as reported by Duggan *et al.* (2005a) who reported a 21% decrease in ears m^{-2} which is comparable to the 26% difference between nl2 and Bacanora reported here. Moreover, the lines with *tin* genes have been found to partition more of their biomass towards the ear at anthesis which could explain the high ear index for NL2.

On the other hand, NL1 was more prolific in its tiller production than NL2 and may not possess the tiller-inhibiting gene, or if it does it may be counteracted by other tiller-promoting genes. The relatively lower tiller production for NL2 in 2005 than in other years was associated with generally lower plant densities in that year, and may reflect lower intrinsic tillering potential for NL2 compared to NL1 or an effect of the *tin* gene on plant establishment. It is noteworthy that the slightly lower germination for NL2 could not fully explain effects on shoots m^{-2} in present study. This is because in 2003, when plant number was also measured, there were no differences amongst the genotypes but still similar differences in ears m^{-2} at anthesis were observed to those in other years amongst the genotypes. In fact, Duggan *et al.* (2005b) reported the inability of *tin* lines to produce tillers to compensate for gaps in the canopy in case of poor establishment consistent with effect currently reported in 2005. Greater shading of tiller buds at onset of stem extension could also lead to lower tiller production due to a high R:FR ratio (Sparkes *et al.*, 2006).

4.4.3 Relationship between post-anthesis processes and grain yield

Almost half the photosynthate fixed in stems and leaf sheaths before anthesis can be lost from wheat plants by maturity (Austin *et al.*, 1977; Bonett and Incoll, 1993). These reserves can be either re-translocated to the developing grain or lost by respiratory processes. The proportion lost to respiration has been reported to be typically 25% of accumulated reserves (Gebbing *et al.*, 1998). This is because loading of carbohydrates to the conducting phloem vessel is an active process but the amount differs between varieties. The ability of varieties to retain and efficiently remobilise photosynthate to the grain would be expected to influence their yields. Furthermore, in the novel genotypes, the generally higher stem reserves at anthesis for NL2 compared to NL1 may relate to differences in tiller production affecting partitioning of assimilate to non-structural stem DM. Duggan *et al.* (2005) reported the presence of the *tin* gene to confer higher stem WSC at anthesis.

The accumulation of stem WSC has been reported to be positively associated with genetic gains in some optimal environments (Blum, 1998; Shearman *et al.*, 2005). One component of stem WSC is the % of WSC of stem and leaf sheath biomass, the maximum level being about 30-40% as found in a range of 17 genotypes in the UK (Foulkes *et al.*, 1998).The maximum stem reserves for the three parental genotypes

in 2004 occurred at GS 61+15d and differed in the range of 0.32 to 0.98 t ha^{-1} with NL1 amassing and utilizing a smaller amount of reserves compared to NL2 and Bacanora. There was a decline in stem reserves from 15 days of anthesis in Bacanora and NL2 but slightly later for NL1. This broadly confirms previous investigations (Schnyder, 1993; Blum, 1998) that accumulation usually ceases within three weeks of anthesis, after which fructan is gradually lost from stems until grain maturity. It could have been that source was marginally limiting during grain filling in these experiments and stem reserves were important for grain filling even in the absence of stress in the present study. Greater partitioning to stem WSC DM may be associated with a lower ear index at anthesis in NL1 (0.20) compared to NL2 (0.30). Interestingly, NL1 consistently had lower stem WSC than the other two genotypes. This may have been associated with reduced competition between the ear and stem storage immediately before and after anthesis again leading to greater assimilate availability to florets of NL1 in the critical period around flowering.

Stem reserves usually make a greater contribution to performance in relatively low-yielding lines and under post-anthesis stress in most environments (Austin *et al.*, 1980). Tollenaar (1991) showed that modern hybrids usually mobilize less of their reserves during grain growth partly due to later senescence of their leaves. The contribution of stem reserves to growth of developing grains was greater in NL2 and Bacanora than NL1 but the overall contribution of reserves to yield in these experiments was quite small. Dry matter growth of grain is mainly associated with current assimilate production transferred directly to the grain during the post-anthesis phase (Rawson and Evans, 1971; Slafer and Savin, 1994). The ear itself can contribute up to 33 to 43% assimilates for grain growth (Evans and Rawson, 1970) most of which comes from the glumes (Biswas and Mandal, 1986) while the lamina will become major contributors during the later stages of grain growth (Guitman *et al.*, 1991). Ruuska *et al.* (2006) examining 22 Australian cultivars showed that stem WSC is a heritable trait and breeding for high WSC is possible in wheat. Raising RUE in the pre-anthesis period may be an important trait for increasing stem WSC whilst simultaneously increasing ear biomass (Shearman *et al.*, 2005).

4.4.4 Degraining responses

The negative relationship that exists between grains m^{-2} and grain weight makes it difficult sometimes for breeders to increase one component independently of the other. There is little information in the literature to directly test whether competition for assimilates during grain filling is responsible for reduction in grain weight. However, indirect evidence from the manipulation of source-sink ratios during grain filling and the analysis of changes in weight of grains from particular spikelet positions can be used as an indicator of source or sink limitation (Slafer *et al.*, 1996). In this study, the source-sink balance was manipulated by removal of the spikelets. Grain growth responses to degraining can indicate whether the negative correlation between the two major yield components is due to competition for assimilates between the grains (Slafer and Savin, 1994). Manipulations of the reproductive sink by degraining the parental genotypes fourteen days after anthesis showed that all the genotypes were equally sensitive to the increase in the availability of assimilates and implied that the assimilate availability was insufficient to satisfy grain growth requirements in intact ears, i.e. grain growth was source limited. Similar findings were reported by Martinez-Carasco and Thorne (1979) and Koshkin and Tararina (1989).

Apart from 2004, the positive responses to degraining were very small and there was evidence for only marginal source limitation. The act of degraining itself can decrease source activity due to an increase in leaf abscisic acid levels affecting compensatory grain growth (Blum *et al.*, 1988) or it might alter the pattern of senescence of the photosynthetic tissues (Slafer and Savin, 1994). Furthermore, even though it is assumed that cell division ceased 14 days after anthesis, it is possible that when degraining was carried out cell expansion was still taking place. The timing of degraining is very important as degraining treatments imposed during the period of endosperm cell division might increase potential grain weight of remaining grains due to an increase in assimilate availability during an increase in endosperm cell number and thus sink capacity. In addition, Calderini and Reynolds (2000) reported that within a spikelet, growth of distal grains is limited compared to proximal grains by greater resistance to assimilate translocation associated with longer vascular systems and that distal grains can be considered to be source limited.

Overall, the three genotypes showed positive responses of grain weight to degraining in the range of 5.5-6.7 mg. The novel genotypes have a higher green canopy area at GS 61 per grain than Bacanora and this may have contributed to their larger potential grain size. However, the genotypes did not differ significantly in their responses indicating that overall the source: sink balances of the genotypes were likely similar. Although assimilate supply per grain was increased by 100%, grain weight overall was only increased by 15%. Therefore, grain dry weight was only marginally responsive to changes in assimilates supply during grain filling. These findings are in general agreement with those of a recent extensive review of source:sink manipulation experiments in wheat, maize and soybean that mean grain dry weight in wheat changed by *ca.* 0.12 relative to the change in potential availability of assimilates per grain produced during seed filling and that wheat yield is mainly limited by post-anthesis sink size under optimal conditions (Borras *et al.*, 2004). Present findings would therefore suggest that yields of the three genotypes were mainly limited by grain sink size in the post-anthesis period.

Grain weight of central and basal spikelets were heavier compared to apical spikelets in novel genotypes by 7.1-7.6 mg in NL1 and by 6.5-7.3 mg in NL2. In Bacanora, central grains were slightly heavier than basal and apical spikelets. Grains at some positions accumulate starch at a faster rate (Bremner, 1972) or earlier than others (Evans *et al.*, 1972) which agrees with present findings. Jenner and Rathjen (1972) reported that central spikelets have a greater competitive advantage for limited supply of assimilates. Similarly, Bremner and Rawson (1978) revealed that grains in the central spikelets are larger than those in basal spikelets which are in turn larger than the grains in apical spikelets. Also, these authors postulated that the basal grain of a spikelet has priority for assimilate under limited assimilate supply. Moreover, Stoddard (1999) found that grains from distal florets within a spikelet were always smaller than those from proximal florets which in turn have almost the same weight. When the supply is limited, the preferential distribution of assimilates to central and basal spikelets is accentuated (Bremner, 1972).

A greater response to degraining was observed in apical spikelets (10-15% increase) than the basal (4.7-4.9% increase) and central spikelets (5.6-7.8 % increase) for the novel genotypes. The effectiveness of assimilate transportation to developing florets depends on the vascular system. There are distinct differences in the vascular system of individual florets within the same spikelet (Hanif and Langer, 1972). Bremner and Rawson (1978) suggested that the vascular system was adequate, but it was rather the maintenance of a sufficient gradient of assimilates in the rachis which is the limitation to apical spikelets. Whingwiri *et al.* (1981) also stated that the number and size of central bundles declined acropetally along the rachis. Therefore the differences in bundle size supplying the spikelets determine the resistance of assimilate movement in respective spikelets. Hergoz and Stamp (1983) on the other hand suggested differences in the sequence of morphogenesis as another mechanism that control differences in potential grain growth between spikelets along the length of the rachis. Perhaps apical spikelets were more source-limited in novel genotypes associated with longer rachis but not in Bacanora. Hence, a long rachis would not be beneficial in source-limited crops.

4.5 SUMMARY

The hypothesis that novel genotypes (NL1 and NL2) have higher ear index than the conventional genotype Bacanora was confirmed in the case of NL2 but not for NL1. However, for NL2 this did not relate to more grains m^{-2}. Greater biomass at GS 61 in novel lines were not observed as Bacanora had *ca.* 50 g m^{-2} more than novel genotypes though these differences were not statistically significant. The genotypes took different resource-capture routes to produce the same amount of dry matter. The second hypothesis that NL1 and NL2 have more spikelets ear^{-1} hence more grains ear^{-1} was supported by the results. However, the genotypes did not differ in grains $spikelet^{-1}$; more grains ear^{-1} was due to more spikelets ear^{-1}. Hence, these novel genotypes showed greater fertility of individual ear compared to Bacanora. The hypothesis that novel genotypes have more grains m^{-2} was not supported in present study. The physiological basis for grains m^{-2} in NL2 was the lower grains to ear DM ratio (71 grains g^{-1}) compared to NL1 (111 grains g^{-1}), counteracting larger ear index and more ear biomass at anthesis in NL2. In addition, both potential grain weight and final grain weight were higher in novel genotypes compared to

Bacanora. Higher PGW in NL2 was associated with a much lower value of grains to ear DM ratio compared to Bacanora. However, higher PGW in NL1 was achieved without a similar reduction in grains to ear DM ratio. This is encouraging in that it suggests it may be possible to uncouple these two parameters genetically as a route to increasing yield potential.

NL2 possesses the tiller inhibition *tin* gene whereas NL1 probably does not have this gene. Hence, there were fewer ears m^{-2} in NL2 but the genotype maintained a reasonable yield due to heavier grains through greater PGW and final grain weight. Fewer grains m^{-2} was compensated for by heavier grains, which is of interest to breeders. This study showed that potential grain weight can be a potential trait to consider for augmenting yield in the future as it is an important source of variation for grain sink size. It is suggested that the phenotype represented by NL1 (high grains-to-ear Dm ratio, high PGW) may be of value to breeders in future years in programmes aimed at increasing grains m^{-2} and yield potential in high output environments whereas with NL2 there is the need to complement this phenotype with other traits that can counteract effects of restricted tillering and a low grains-to- ear DM ratio. These traits combinations will be discussed further in Chapter 5.

CHAPTER 5 COMPARISION OF SHOOT APEX DEVELOPMENT, LEAF EMERGENCE AND TILLER PRODUCTION IN NOVEL (LARGE-EAR PHENOTYPE) AND CONVENTIONAL GENOTYPES

This chapter describes a controlled-environment experiment examining the shoot apex development of the three parental genotypes plus a UK-bred spring wheat cultivar, Tybalt. Spikelet primordia production was measured as it has a direct link with spikelet number but also indirect effects on the duration of the stem-elongation phase affecting tiller and floret survival. The chapter starts with a review of relevant literature, followed by the methodology, results, discussion and a summary.

5.1 Introduction

Plant development has been defined as a sequence of phenological events controlled by external factors, each event making important changes in the morphology and/or function of some organs (Landsberg, 1977). The shoot apex becomes organised in the embryo during grain growth and is well formed in the mature seed. It is made up of a smoothly rounded meristematic dome, on the flanks of which are formed the leaf and spikelet primordia (Kirby and Appleyard, 1984). In general, the cereal seed when sown already has a well-developed shoot with three or four leaf initials and an apical dome enclosed within the coleoptile (Figure 5.1). Upon germination the apex resumes activity and further leaf primordia are initiated. The plastochron is the thermal time interval between the initiation of successive leaf primordia. Of these primordia the first three to ten develop to form leaves so that the main stem at maturity bears 7 to 14 or more leaves. Each primordium unit may also later differentiate into an elongated internode and/or an axillary bud (tiller bud). The primordia subsequently initiated on the main shoot develop to form floral parts (Kirby, 1977).

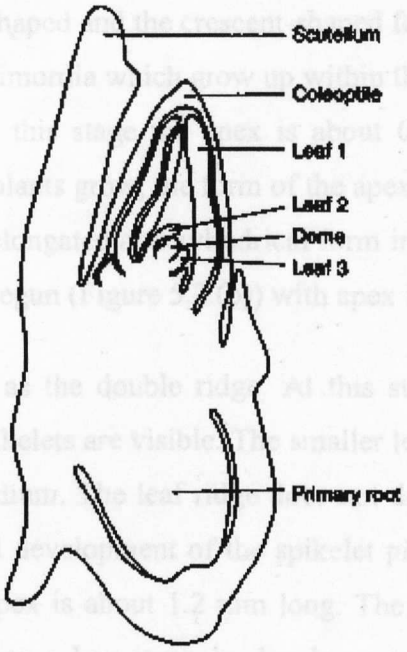


Figure 5.1. Longitudinal section of the embryo of a mature grain, with the apex and leaves of the shoot present and a tiller bud visible (Adapted from Kirby and Appleyard, 1987)

The life cycle of the cereal plant can be divided into three phases by reference to shoot apex development. In the first phase, leaves and spikelets are initiated at the shoot apex and tillers are initiated and grow from the axils of the leaves. This phase ends with the formation of the terminal spikelet. In the next phase, the stem and ear elongate rapidly. This is the stem-elongation phase that ends at anthesis: the main features are rapid ear growth involving the differentiation and maturation of florets; a proportion of florets and tillers die during this phase. In the final phase, the carpels are fertilised and grain growth occurs (Kirby, 1988).

Development of the shoot apex involves a number of distinct processes, such as the formation of single and double ridges, the terminal spikelet on the main stem and the onset of anthesis. The change in the form of the apex results from two parallel processes: first, the initiation of primordia (Kirby, 1974) and second, the increasing complexity of each primordium as development proceeds.

At first the apex is dome-shaped and the crescent-shaped folds of tissue on the flanks of the shot apex are leaf primordia which grow up within the sheaths of the expanded leaves (Figure 5.2(a)). At this stage the apex is about 0.25 mm long (Kirby and Appleyard, 1984). As the plants grow, the form of the apex undergoes morphological changes adopting a more elongated and cylindrical form indicating that the initiation of spikelet primordia has begun (Figure 5.2 (b)) with apex length about 0.5 mm long.

The next stage is known as the double ridge. At this stage primordia which will differentiate to become spikelets are visible. The smaller leaf ridge subtends a bigger bulge, the spikelet primordium. The leaf ridge does not develop further and is soon obscured by the continued development of the spikelet primordium (Figure 5.2(c)). The length of the shoot apex is about 1.2 mm long. The double-ridge stage (floral initiation) has been used as a key stage in developmental analysis and has been interpreted as marking the beginning of the ear development and the end of vegetative development of the plant. Double ridge is not visible until about half the spikelet primordia have been laid down and Rawson (1970) showed that double ridge occurs when a variable proportion of the spikelets have been initiated.

Each spikelet primordia differentiates to form, first a pair of glume primordia (Figure 5.2(d)), and then the florets start to form. The lemma primordia are initiated first and then the axillary meristems differentiate to form the other floral structures. Each floret primordium initiates anther and carpel primordia (Figure 5.2 (e)). As each of these organs is initiated, the shoot apex takes on a characteristic shape which may be used to define the stage of development (Kirby and Appleyard, 1984). The wheat ear, which is a determinate inflorescence, terminates in an apical spikelet placed at right angles to the plane of the rest of the spikelets. At this stage the last few primordia initiated by the dome of the shoot apex do not develop into spikelets but become the glumes and floret primordia of a terminal spikelet (Figure 5.2(f)). The apex at this stage is 4 mm long. When the spikelets differentiate the number is fixed but adjustment to growth conditions may be made in the number of fertile flowers in a spikelet. The above stages were described according to Kirby and Appleyard (1987). Waddington *et al.* (1983) proposed another quantitative scale based on numerical score form 1.5 to 10 with a more precise description of the floral development.

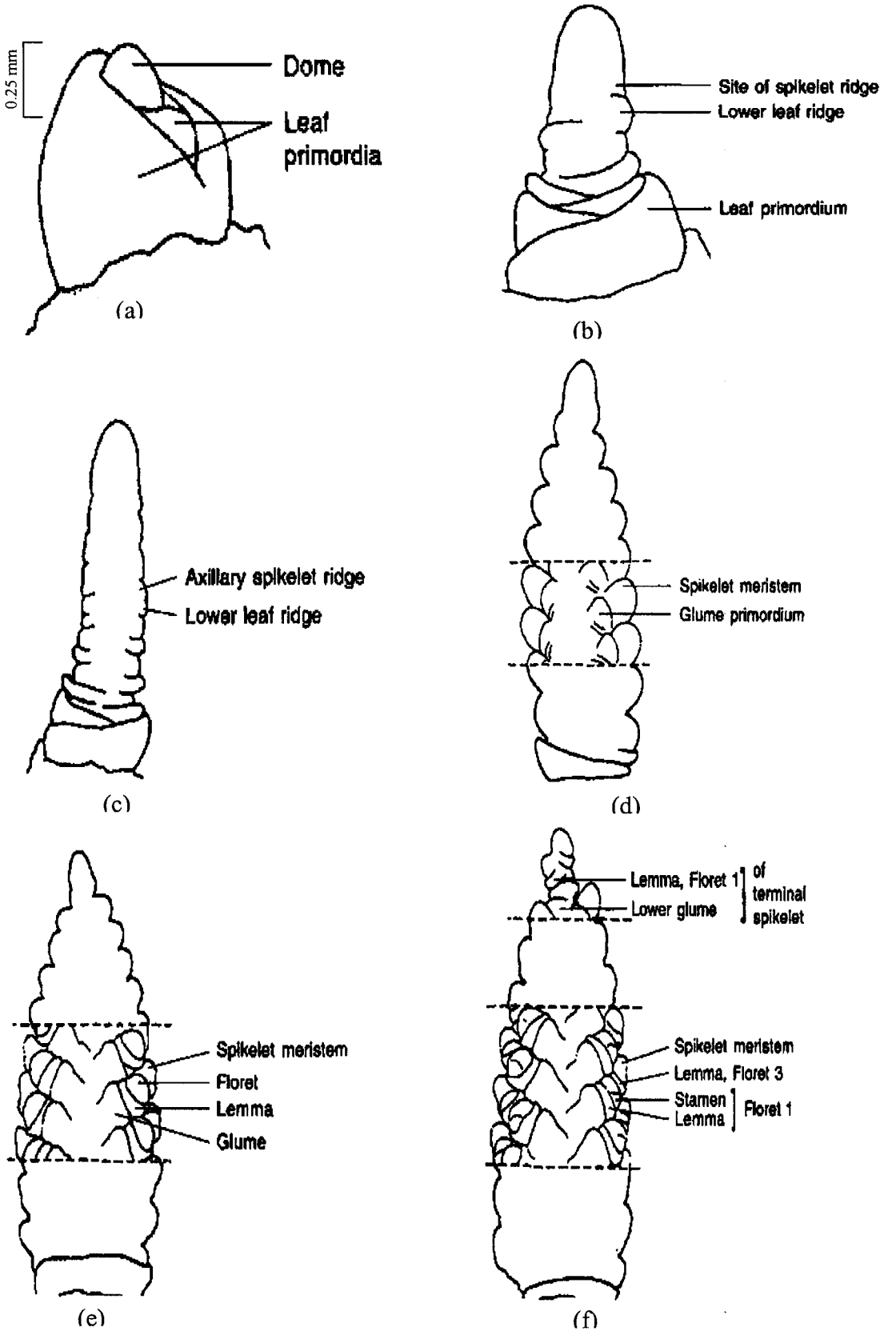


Figure 5.2. Successive stages of development of shoot apex from vegetative apex to terminal spikelet stage (Adapted from Kirby and Appleyard, 1987)

5.2 FACTORS AFFECTING RATE AND DURATION OF PRIMORDIA PRODUCTION AT THE SHOOT APEX

Morphologically, three major events occur during ear development to the terminal spikelet stage. They begin with the onset of apex elongation, followed by initiation of spikelets at the double-ridge stage and finally terminal spikelet formation. The rate and duration of each stage are under the influence of various factors. An intricate and complex series of biochemical regulatory events occurs within a relatively short time and within the small, short apex (Gardner *et al.*, 1985). Spikelet number in wheat is influenced both by the duration and rate of spikelet initiation (Rahman and Wilson, 1977a). The rate and duration of spikelet primordia production are inversely correlated (Holmes, 1973), yet they are partially independently controlled as Rahman and Wilson (1977b) found it possible to change the rate of initiation without changing the duration with phosphorus application. It has been proposed that certain relative durations of the phases of the life cycle may lead to higher numbers of spikelets or higher yields than others (Rawson, 1970) and that the differences observed in different cultivars in spikelets ear^{-1} arise from differences in the rate and duration of spikelet initiation (Rawson, 1971). Moreover, Rahman *et al.* (1978) found that under controlled photoperiod (12 h) and temperature (20°C), spikelet number was under simple genetic control with dominance for high spikelet number and that the gene that determines spikelet number does so by determining rate of spikelet initiation.

The influence of environmental stimuli on the initiation of leaves and spikelets by the stem apex has been the subject of several investigations. The time-course of primordium initiation has been found to consist of two phases, with an initial slow phase of leaf initiation being followed by a more rapid phase of spikelet initiation of the future ear (Baker and Gallagher, 1983). Spikelet primordia are initiated 2-3 times faster than leaf primordia (Baker and Gallagher, 1983; Delécolle *et al.*, 1989) but the rate varies greatly among cultivars (Allison and Daynard, 1976). A number of physiological and ecological factors affecting genetic and environmental variation in wheat primordia production have been studied. These include genetic differences in vernalization and photoperiod responses (Midmore *et al.*, 1982), earliness *per se*

responses (Flood and Halloran, 1986), and effects of nutrition (Holmes, 1973), temperature and light intensity (Friend *et al.*, 1962).

5.2.1 Effects of photoperiod

Wheat is quantitative long-day plant (Thomas and Vince-Prue, 1997) and it is well known that development is faster in long days with a reduction in the thermal time to flowering in photoperiod-sensitive varieties. The photoperiod is sensed by mature leaves and not by apical meristems (Bernier *et al.*, 1993). This advancement to flowering occurs mainly due to acceleration of development from seedling emergence to floral initiation, i.e. when the plants become reproductive (Davidson *et al.*, 1985; Rawson and Richards, 1993) and from terminal spikelet to flowering (Slafer and Rawson, 1996). Since daylength affects apical morphogenesis and the final number of leaf primordia produced, it affects also leaf production, tillering and other developmental processes in cereals (Kirby and Appleyard, 1980).

A series of major genes located on homoeologous group 2 chromosomes largely control the photoperiod response in wheat (Snape and Worland, 2001). The three genes are *Ppd-D1*, *Ppd-B1* and *Ppd-A1* (formerly known as *Ppd1, Ppd2 and Ppd3*) and are located on the short arms of chromosomes 2D, 2B and 2A, respectively (Law *et al.*, 1978). The dominant photoperiod alleles render a given genotype daylength insensitive (Pugsley, 1966; Worland, 1996). *Ppd-D1* is the most photoperiod insensitive locus followed by *Ppd-B1* and *Ppd-A1* (Worland, 1996). *Ppd-D1* was shown to reduce flowering time by 6-14 days depending on season and produced fewer spikelets ear^{-1} by bringing forward the time of terminal spikelet and thus shortening the primordia development phase (Snape *et al.*, 2001). The *Ppd* genes regulate the number of leaves to be initiated by the apex, i.e. final leaf number, which in turn marks the timing of floral initiation. Photoperiod has been shown to affect the phyllochron (thermal time between the appearance of two successive leaves) of either all leaves (Cao and Moss, 1989; Slafer and Rawson, 1997) or the last emerging leaves (Slafer and Rawson, 1997). Virtually all UK-bred varieties are photoperiod-sensitive whilst all CIMMYT varieties are photoperiod insensitive.

Accelerated development in long days is associated with reductions in final leaf and spikelet primordia number (Kirby, 1992; Rawson and Richards, 1993). Stefany (1993) reported that in photoperiod-sensitive genotypes, the shorter the day, the longer the phase from double ridge to terminal spikelet therefore increasing the period to terminal spikelet and the number of spikelets per ear. According to Kirby *et al.* (1999) photoperiod affected the duration of both the double ridge to terminal spikelet and the terminal spikelet to ear emergence phases in winter wheat. Similarly, Slafer and Rawson (1994) reported that the main factor affecting the duration of the double ridge to terminal spikelet phase is probably the prevailing photoperiod. Moreover, photoperiod appears to have a direct response on plant development from terminal spikelet to heading (Miralles and Richards, 2000) and from terminal spikelet to anthesis (Miralles *et al.*, 2000; Gonzalez *et al.*, 2002). Furthermore, Whitechurch and Slafer (2002) found that the duration of the spikelet initiation phase (floral initiation to terminal spikelet) was greatly affected by photoperiod. Whitechurch and Slafer (2001) reported that the duration of the late reproductive phase of stem elongation was directly affected by photoperiod, but no evidence was found of any specific known *Ppd* allele being responsible for major responses to photoperiod during the stem-elongation period.

With regard to the effects of environmental variation in photoperiod on rate of primordial initiation, on very limited evidence in controlled-environment experiments, it appears that the rate of leaf initiation is unaffected by day length though the rate of spikelet initiation responds strongly, increasing by up to 50% if day length is increased from 8 to 16 h (Holmes, 1973; Lucas, 1972; Rahman and Wilson, 1977; Rawson, 1971).Usually, when photoperiod is increased, rate of spikelet initiation increases but the duration of spikelet initiation decreases (Holmes, 1973; Allison and Daynard, 1976; Rahman and Wilson, 1977).Duration is relatively more affected than rate of spikelet initiation, and the number of spikelets per ear declines with increasing day length (Kirby and Appleyard, 1987).

5.2.2 Effects of vernalization

The genotypes under investigation in the present study are predominantly spring wheats although some of the NL2 x Rialto DH lines will likely contain some vernalization requirement and some days with low mean temperature (<10 °C) occur in Cd. Obregon (Mexico) in December/January to satisfy any moderate vernalization requirements. Hence, a short summary of vernalization effects on primordia production is included. Spring genotypes usually require temperatures between 7 and 18 °C for 5 to 15 days for floral initiation, while winter wheat requirements are between 0 and 7 °C for 30 to 60 days (Acevedo *et al.*, 2002). The effect of vernalization in winter genotypes is to reduce the number of leaves initiated and to promote earlier floral development (Davidson *et al.*, 1985). Spikelet initiation in fully vernalized plants is similar to that of spring wheat (Kirby and Appleyard, 1987). According to Kirby *et al.* (1999) vernalization cannot directly affect the double ridge to terminal spikelet phase whilst Weir *et al.* (1984) stated that the vernalization requirement must be saturated before the plant is competent to flower, saturation must precede the formation of double ridges. Therefore, the vernalization requirement must be satisfied first before genotypes which are sensitive to photoperiod require a certain daylength to flower. Jamieson *et al.* (1998) reported that until the vernalization response is saturated, the plant is probably insensitive to photoperiod.

Vernalization is under the influence of some major genes, which include *Vrn-A1* (formerly *Vrn1*), *Vrn-B1* (formerly *Vrn2*) and *Vrn-D1* (formerly *Vrn3*) located on homoeologous group 5 chromosomes namely, 5A, 5B and 5D respectively (Law *et al.*, 1976) and also *Vrn-A2* located on chromosome 5A (Yan *et al.*, 2004). Like photoperiod, the dominant allele is spring type. Differential effects of *Vrn* gene combinations on yield and yield components in wheat have been reported by Stelmakh (1993, 1998) and Kato *et al.* (2001). The latter found that the presence of *Vrn-D1* (spring allele) in isogenic lines reduced the number of spikelets per spike compared to those lines without this allele. There is very little evidence about the vernalization and photoperiod interactions but there are few studies carried out on the effects on photoperiod on the vernalization required in winter (*vrn*) wheat. In some photoperiod sensitive winter wheat varieties the vernalization required can be reduced by exposing the plants to short days (Dubcovsky *et al.*, 2005; Brooking and

Jamieson 2002) or photoperiod sensitivity modified by vernalization treatment such that the thermal duration to floral initiation is reduced by 36% in vernalized plants but only 12% in unvernalized ones when extreme photoperiod treatments were compared (Gonzalez *et al.*, 2002).

5.2.3 Earliness *per se (Eps)*

The main environmental factors affecting phasic development in wheat are photoperiod, vernalization and temperature (Pirasteh and Welsh, 1980; Slafer and Rawson, 1994) as covered in the previous sections. Photoperiod and vernalization are usually considered to account for the majority of the difference between cultivars in development rate. These responses have been studied in detail (e.g., Rahman and Wilson, 1977; Davidson *et al.*, 1985; Flood and Halloran, 1986 and Slafer *et al*, 1994). However, a number of studies indicate a factor(s) besides vernalization and photoperiod response influences developmental rate in wheat (Halloran and Boydell, 1967; Halloran, 1975, 1976).

Syme (1973) found that there was a basic developmental time period for all wheat cultivars and this character appears to be influenced by mean daily temperature. Ford *et al.* (1981) proposed 'earliness genes' distinct from those controlling photoperiod sensitivity to account for differences in time to ear emergence. Flood and Halloran (1984) provided further evidence for the existence of differences in the rate of development in the absence of the influences of vernalization and photoperiod and proposed the term 'basic development rate'. According to Slafer and Rawson (1995) the concepts of 'basic vegetative period,' 'intrinsic earliness' and 'basic development rate' in wheat assumed that if plants are vernalized fully and then grown at long daylength in order to remove any responses to vernalization and photoperiod, the calendar or thermal time then taken to anthesis will be a characteristic of a genotype that will be heritable. Thus, regardless of temperature, early genotypes will always be earlier than late genotypes (providing there are no vernalization and photoperiod responses). Slafer (1996) showed cross-over interactions in relation to temperature as genotypes changed their ranking for 'intrinsic earliness' depending on the temperature regime and 'intrinsic earliness' was not a static genotypic characteristic but the result of an interaction between genotype and temperature. Furthermore, the

earliness *per se* trait is likely to be related to temperature sensitivity (Slafer and Rawson, 1995; Slafer, 1996).

Because of their major influence on flowering time, vernalization and photoperiodic genes have been studied in detail, while earliness *per se* genes have not received due consideration (Kato and Wada, 1999). Earliness *per se* is determined by a minimum vegetative growth period when floral primordia are initiated, independent of external stimuli. It is quantitatively inherited and controlled by a number of minor genes whose effects can be determined only in the absence of the epistatic effects of vernalization and photoperiod (Kato and Wada, 1999).

The genes for earliness *per se* have been located on different chromosomes in various studies. The first earliness *per se* gene was reported by Scarth and Law (1983) on the long arm of chromosome 2B affecting ear emergence time in recombinant lines of Chinese Spring whilst Miura and Worland (1994) reported that chromosome 3A carried an earliness *per se* gene. In addition, Kato *et al.* (1999) identified quantitative trait loci for earliness *per se* on the proximal end of chromosome 5AL of single chromosome substitution lines. Sarma *et al.* (2000) mapped an earliness *per se* gene together with *Vrn-A1* on chromosome 5A. Moreover, intrinsic earliness could be expected to relate to final leaf number and phyllochron as well as rate of spikelet primordia production (Jamieson *et al.*, 1988).

5.2.4 Environmental effect of temperature

Temperature is probably the primary environmental factor affecting the rate of development in calendar time. It is widely recognised that development accelerates as temperature increases, and linear relationships between the rate of development (the reciprocal of the duration) and mean temperature have frequently been reported (Angus *et al.*, 1981; Slafer and Savin, 1991). Controlled-environment studies show that high temperatures shorten the leaf and spikelet initiation phases. The rate of spikelet initiation increases with increasing temperatures and is most rapid between 25 and 30 °C; above this temperature the rate declines (Kirby and Appleyard, 1987).

The duration of the spikelet initiation phase, as influenced by temperature, is inversely related to the rate of initiation according to the plastochron, and there is therefore generally little variation in the number of spikelets per ear in temperatures ranging form 10-20 °C (Rahman and Wilson, 1977). At higher temperatures (above 25 °C) the number of spikelets initiated is reduced. A field study conducted by Baker (1979) indicated that spikelet initiation ceases if the temperature drops below 3 °C. Above this, up to about 10 °C, the response to temperature is approximately linear. Rahman and Wilson (1978) found that the rate of spikelet initiation for 8 cultivars increased as the temperature was increased from 16/9 °C to 23/16 °C but the response was variable as temperature was further increased. Overall, this confirms that the rate of spikelet initiation in calendar time is temperature dependent.

5.2.5 Agronomic and other factors influencing phenological stages

Sowing date (Angus *et al.*, 1981; Kirby *et al.*, 1985; Hay, 1986) or sowing location (Bauer *et al.*, 1988) can dramatically affect the duration of different developmental phases and to differing degrees depending on the cultivar used. The major components of the environment which affect development and the association with sowing location and date are low temperature in order to satisfy vernalization responses, temperature *per se* to drive the growth associated with development, and photoperiod (Pirasteh and Welsh, 1980). There are indications that other factors such as level of nutrient, water availability, radiation and plant density (Rawson, 1993) and CO_2 (Rawson, 1992) can modify responses, but these effects are relatively small. As discussed in the previous sections, genotypes differ in their sensitivity to major factors and also appear to differ in their intrinsic rates of development (Flood and Halloran, 1984).

Low light intensity has been observed to increase the duration and reduce rate of spikelet primordia production with the outcome of fewer spikelets per spike (Fischer, 1985; McMaster *et al.*, 1987; Stockman *et al.*, 1983). In addition, Cottrell *et al.* (1981) reported that high levels of gibberellins in shoot apices under long days resulted in a higher rate of spikelet initiation. Water stress prior to heading stage does not result in spikelet death unless the whole plant dies (Morgan, 1971). Grieve *et al.* (1993) found that salinity had no effect on initiation rate but shortened the duration of spikelet primordia production of two spring wheat cultivars. Furthermore, nitrogen

fertilization has often been shown to increase the number of spikelets per ear in wheat (Whingwiri and Kemp, 1980; Frank and Bauer, 1982; Darwinkel, 1983) due to an increase in the rate of spikelet initiation (Whingwiri and Kemp, 1980).

It is unlikely that one factor will act independently from another in shoot apex development. As supported by the literature, photoperiod and temperature, including vernalization, remain the main factors that could effect changes to the rate and duration of spikelet production but other factors could also have minor effects. There are interactions between the effects of vernalization, photoperiod and earliness *per se* which makes it very difficult to determine which one has the greatest influence but recognising their importance and identifying genes controlling these responses are important for breeding. This is because breeders can take advantage of this knowledge to tailor varieties to fit particular agro-climates as well as optimise time allocated to vegetative or reproductive growth as a potential avenue for manipulating source-sink relationships in order to increase yield potential of wheat.

5.3 HYPOTHESES

The specific hypotheses tested are:

a) Novel large-ear genotypes with more spikelets per ear have a longer thermal duration and/or higher rate of spikelet primordia production than conventional genotypes

b) More spikelets per ear in novel large-ear genotypes is associated with effects of 'earliness *per se*' on the phasing of development

5.4 METHODOLOGY

A controlled-environment (CE) experiment was carried out in each of 2004 and 2005 with four spring wheat genotypes at University of Nottingham, Sutton Bonington Campus. The aim was to investigate the rate and duration of spikelet primordia production in four genotypes with contrasting large-ear phenotype and conventional ear morphology.

5.4.1 Growth-room experiments in 2004 and 2005

The design, treatments and environmental conditions of the experiment were described in Chapter 3.

5.4.1.1 Plant material

The planting materials were four spring wheat genotypes, the three parental genotypes (Bacanora, NL1 and NL2) and one UK cultivar (Tybalt-released in 2003 by Cebeco) which constitute the genotype treatment.

5.4.1.2 Plant measurements

The main stem of each plant was tagged after three leaves had fully emerged. Every 4-6 days from 21 DAS in 2004 and 15 DAS in 2005, one pot per genotype from each block was randomly selected. For each sampled pot, the plant was removed from the soil and the leaf sheaths of the main shoot were successively removed so that only one or two leaf sheaths enclosed the shoot apex. The latter was exposed using a sharp mounted needle to remove the youngest leaf sheaths under a binocular stereoscopic microscope (Wild Heerburg, Plan 1x, Switzerland) equipped with a range of fixed magnifications from x 6 to x 50. 'Blue tack' was used to hold the shoot apex in place while delicately removing the young leaves.

Three replicates per genotype per sampling time were used to assess the stage of apex development (Kirby, 1985) on the main shoot of each plant. Due to a high degree of variability between replicates, the two most advanced stages from the three replicates assessed were used to determine the number of spikelet primordia and the growth stage (Zadoks') reached. Nine assessments at 5- to 6- day intervals were made in 2004 whilst eleven assessments at 4- to 5- day intervals were carried out in

2005. The number of emerged leaves on the main shoot, number of tillers per plant, and length of shoot apex were also recorded at each sampling time on the plants in 2004 and 2005. In 2005, sequential, non-destructive measurements were carried out one set of plants (4 genotypes x 3 replicates = 12 plants) in order to quantify the phyllochron by leaf tagging on main stem and shoot production through time. No measurements of ear traits, biomass and dry matter partitioning were performed in 2004 due to a restricted number of sampling replicates. However, these measurements were carried out in 2005 on one set of plants at harvest (12 plants). The shoots were separated into main shoot, tillers 1-3 and tillers 4+ categories. Within these categories, the average rachis length, spikelets ear^{-1}, grains $spikelet^{-1}$ and individual grain weight were recorded. Grain number and dry weight were recorded after threshing the ears from each category.

5.4.2 Statistical analysis

Data were analysed using GENSTAT 8.1 (Lawes Agricultural Trust). A curve fitting program was used for estimating the rate and duration of spikelet primordia production and the final number of spikelet primordia. A two-straight-line model was fitted to the spikelet primordia data with thermal time (base temperature 0 °C) on the x-axis and spikelet primordia number on the y-axis. The slope of the first line gave the rate of spikelet production and the second line was horizontal (slope fixed =0). The break point on the y-axis accounts for the maximum spikelet number reached. Duration of spikelet primordia production was calculated by extrapolating the slope back to the x-axis and taking the duration as the difference between the intercept and the break point on the x-axis. Curve fitting was carried out for each replicate. The same programme was applied to leaf emergence data in 2005. The GENSTAT program and an example of the output from the program are given in Appendix III. Analysis of variance (ANOVA) was applied on the estimated rate, duration and final spikelet number for each genotype in each replicate to detect any significant differences among the genotypes. Linear regression was used to calculate phyllochron. ANOVA was also used to analyse data collected on ear traits in 2005.

5.5 RESULTS

5.5.1 Rate and duration of spikelet primordia production

In both years, novel genotypes produced more spikelet primordia than Bacanora and Tybalt ($P < 0.05$), with NL2 having 1-3 more spikelet primordia than NL1 ($P < 0.05$; Table 5.1). The novel genotypes had a longer thermal duration from floral initiation to reach terminal spikelet than the conventional genotypes (Figure 5.3 (a) and (b)) in both years ($P < 0.05$). The rate of production was slower in the novel genotypes compared to Bacanora and Tybalt in 2004. In 2005, Tybalt had a faster rate of production compared to the other three genotypes. The two novel genotypes had almost the same rate of primordia production in both years. Similar effects were seen in both experiments, e.g. an average of 25.2 spikelets was obtained in both 2004 and 2005.

Table 5.1. Rate and duration of spikelet primordia production and final number of spikelet primordia on the main shoot in 2004 and 2005

Lines	2004			2005		
	Rate ($^{\circ}$C d)$^{-1}$	Duration ($^{\circ}$C d)	Final number	Rate ($^{\circ}$C d)$^{-1}$	Duration ($^{\circ}$C d)	Final number
Bacanora	0.077	701	23.8	0.070	645	22.9
NL1	0.058	843	26.6	0.056	819	26.4
NL2	0.070	719	28.0	0.074	721	29.2
Tybalt	0.083	617	22.5	0.085	545	22.2
Mean	0.072	720	25.2	0.071	682	25.2
SED (DF=6)	0.01	52.9	0.87	0.004	20.5	0.45

5.5.1.1 Spikelet primordia ear^{-1} on main shoot

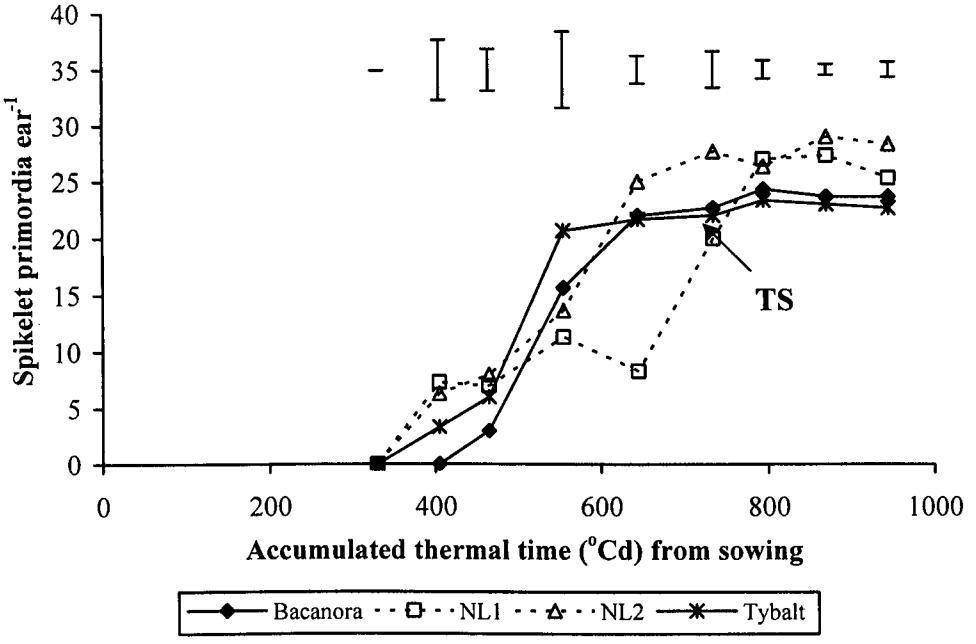


Figure 5.3a. Spikelet primordia ear^{-1} versus thermal time (base temperature 0 °C) in 2004.

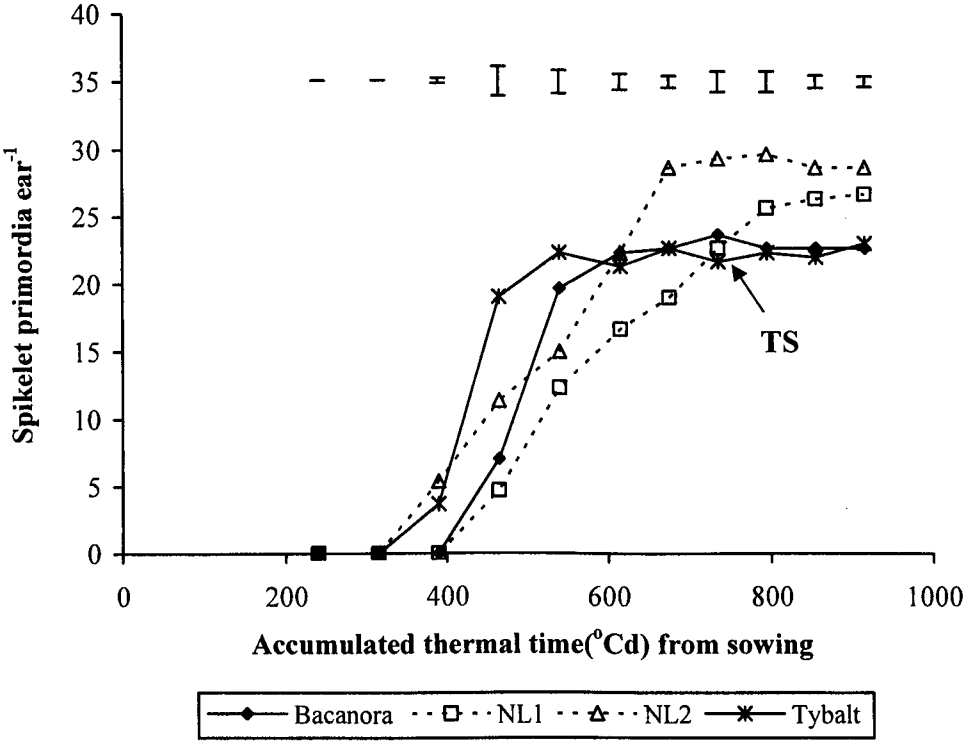


Figure 5.3b. Spikelet primordia ear^{-1} versus thermal time (base temperature 0 °C) in 2005. (Error bars indicate SED, DF=6; arrow indicates average time of terminal spikelet for 4 genotypes)

Successive changes in shoot apex development for NL2 from late vegetative to terminal spikelet in 2004.

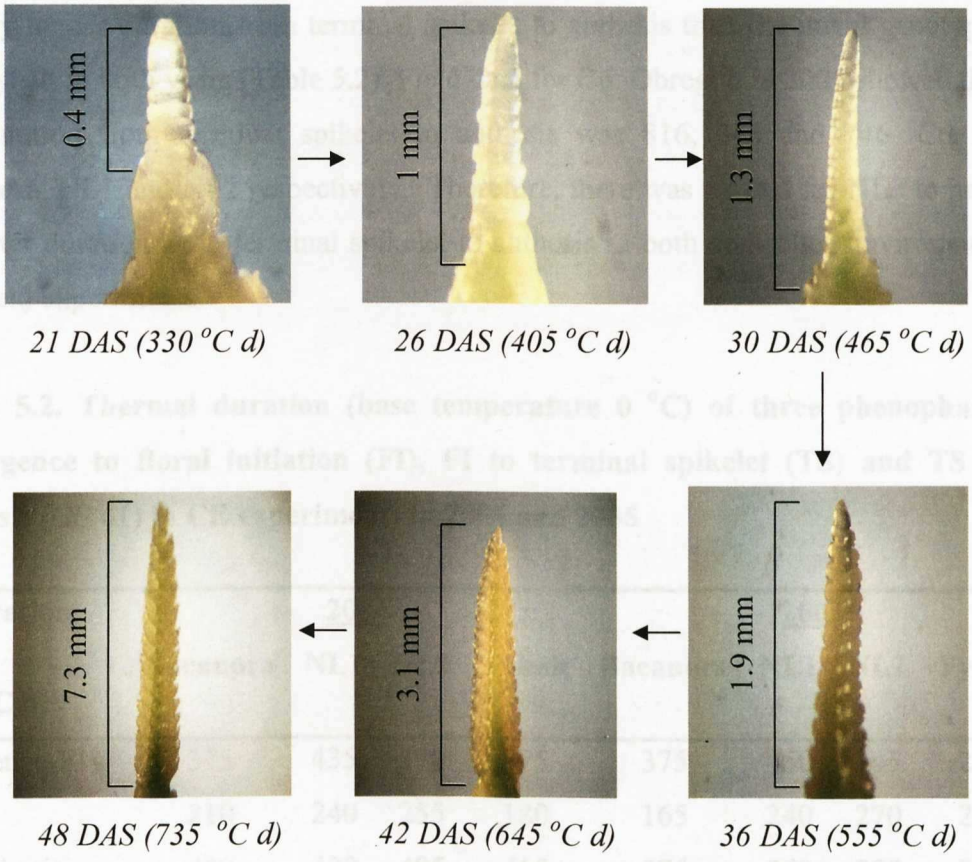


Figure 5.4. Stages of apex development for NL2

5.5.2 Duration of the different developmental phases

It should be noted that the data presented in Tables 5.2 and 5.3 on the pre-anthesis phase were based on a limited number of plants, but it did provide useful information. In 2004, date of anthesis was recorded only from one plant per genotype and it was not possible to subject the data to statistical analysis. However, the other stages (floral initiation (FI) and terminal spikelet (TS)) which were assessed on three replicates.

In both 2004 and 2005, NL1 had a longer thermal duration of the vegetative phase (Emergence-FI) than other genotypes. Novel genotypes also had a longer duration of the FI to TS phase than Bacanora and Tybalt in both years. However, Bacanora had a slightly longer duration from terminal spikelet to anthesis than the novel genotypes and Tybalt in both years (Table 5.2). Field data for Cd. Obregon in 2006 showed that the duration from terminal spikelet to anthesis was 816, 840 and 746 °Cd for Bacanora, NL1 and NL2 respectively. Therefore, there was a trend for NL2 to have a shorter duration from terminal spikelet to anthesis in both controlled environment and field experiments.

Table 5.2. Thermal duration (base temperature 0 °C) of three phenophases (emergence to floral initiation (FI), FI to terminal spikelet (TS) and TS to anthesis (GS 61) in CE experiments in 2004 and 2005

Duration	2004				2005			
(°C d)	Bacanora	NL1	NL2	Tybalt	Bacanora	NL1	NL2	Tybalt
Emergence-FI	375	435	390	375	375	450	345	285
FI-TS	210	240	255	180	165	240	270	225
TS-Anthesis	480	420	405	465	375	360	285	300

The longer duration for the novel genotypes from emergence to TS observed in the CE experiments was not, however, reflected in the parental field trial in 2006, as novel genotypes reached TS relatively earlier in the field experiments compared to the CE experiments (Table 5.3).

Table 5.3. Accumulated thermal (°Cd) from emergence to terminal spikelet in the field experiment in 2006 and the growth-room experiments in 2004 and 2005

Lines	Growth room 2004	Growth room 2005	Field cycle 2006
Bacanora	585	540	637
NL1	675	690	637
NL2	645	615	564

5.5.3 Leaf emergence and tiller production

5.5.3.1 Phyllochron of the four genotypes

Data on leaf emergence were collected only in 2005. The genotypes did not differ in phyllochron (0.01 °C d^{-1}, Table 5.4) but NL1 produced one more leaf than the other genotypes, due to a longer thermal duration from emergence to flag leaf emergence. The additional leaf in NL1 was consistent with a longer duration from emergence to FI compared to NL2 and Tybalt (Figure 5.5). Similar results for the duration of this phenophase were observed for 2004 and 2005 from the destructive samples.

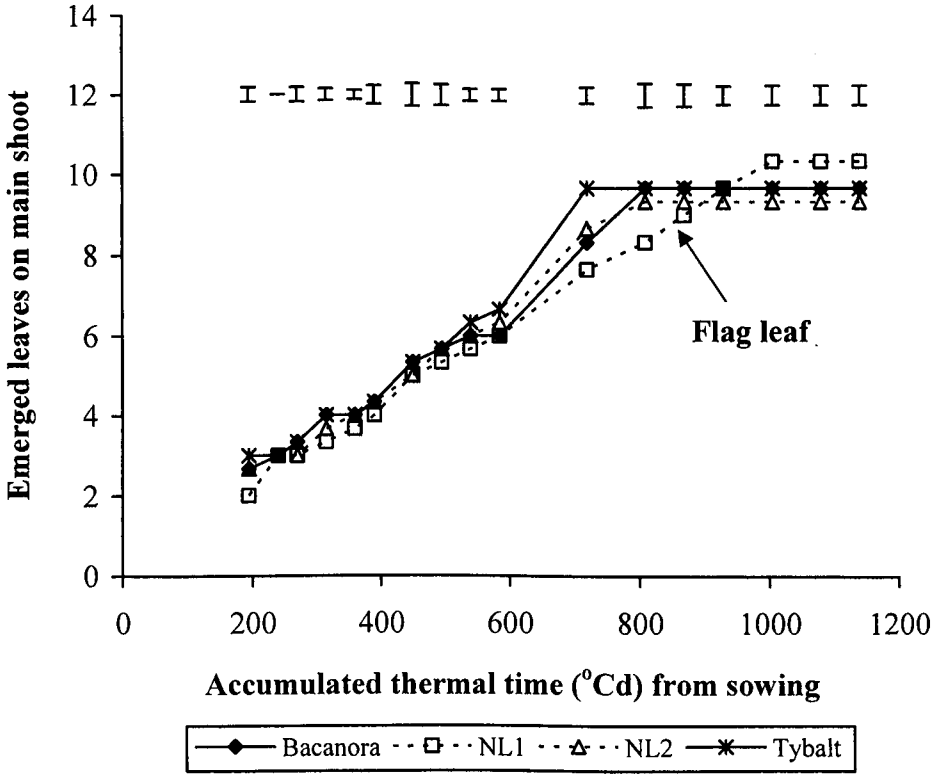


Figure 5.5. Leaf emergence on the main stem versus thermal time (base temperature, 0°C) in 2005 (Error bars indicate SED, DF=6)

Table 5.4. Rate and duration of leaf production and final leaf number on the main shoot in 2005

Lines	Leaves		
	Rate (°C d)$^{-1}$	Duration (°C d)	Final number
Bacanora	0.01	843	9.67
NL1	0.01	993	10.33
NL2	0.01	816	9.33
Tybalt	0.01	795	9.67
Mean	0.01	862	9.75
SED (DF=6)	0.0006	39.2	0.47

5.5.3.2 Tiller production of the four genotypes

The genotypes differed in tillers plant^{-1} ($P < 0.05$) with NL2 having fewer tillers plant^{-1} compared to the other genotypes at all but the first of the samplings (Figure 5.6) in 2005. Tiller survival was similar for all genotypes and differences in maximum tiller number explained the differences in final tiller number. The trend of reduced tiller production in NL2 compared to NL1 and Bacanora was consistent with the field experiments. The time to reach maximum tiller number was similar for all genotypes, but tiller death initially proceeded more slowly for NL1 than the other genotypes.

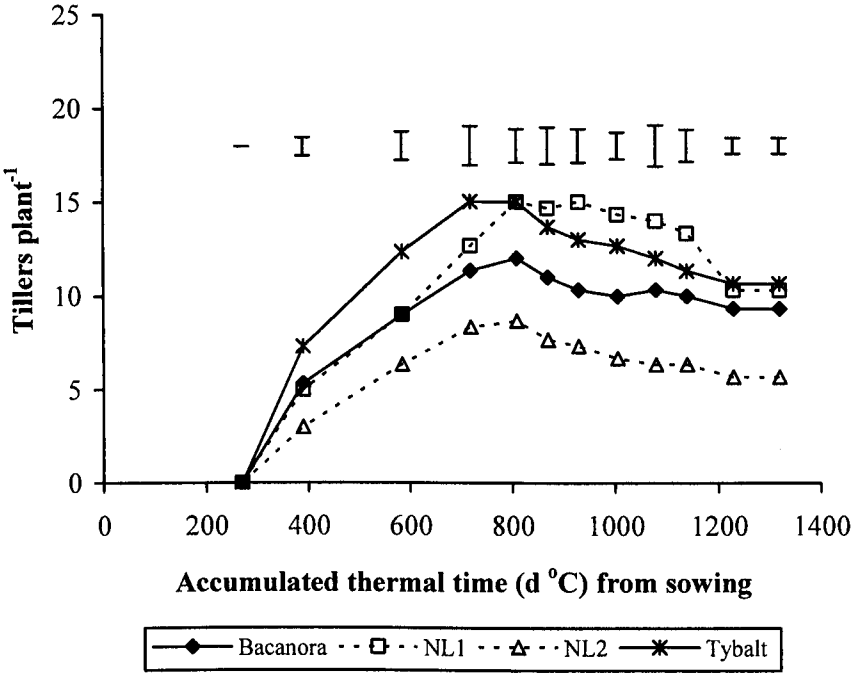


Figure 5.6. Tillers plant $^{-1}$ versus thermal time (base temperature 0 °C) in 2005

5.5.4 Relationship between leaf number and tiller number

Using a generalized linear model and a Poisson distribution, there were significant differences among the genotypes ($P < 0.001$) in tillers produced per emerged leaf. The analysis showed a linear relationship between the number of leaves on the main shoot and tillers produced by the plant. NL2 produced significantly fewer tillers per leaf than Bacanora which in turn produced fewer tillers per leaf than NL1 and Tybalt ($P < 0.05$). The parallel regression analysis can be found in Appendix III together with the output from the program.

5.5.5 Harvest data from growth-room experiment in 2005

The genotypes did not differ in biomass per shoot nor were there any statistically significant differences in HI (Table 5.5). The genotypes differed in plant height with NL1 being taller compared to the other genotypes ($P < 0.001$) which was consistent with the field results. Novel genotypes had a longer rachis and more spikelets ear^{-1} on the main shoot than Bacanora and Tybalt ($P < 0.001$). The genotypes did not differ in grains ear^{-1} or grain weight on the main shoot ears. Results for the T1-T3 category showed that the genotypes differed in rachis length ($P < 0.001$) while the other variables were not significantly different. For the T4+ category, spikelets ear^{-1} and rachis length differed among the genotypes ($P < 0.01$) but not in grains ear^{-1} and grain weight. There was a trend for NL2 (80 g) to have more grains ear^{-1} than the other genotypes (56-61 g) in main shoot ears; and also for the average of T1-T3 tillers (63 compared to 37-55, respectively). Chaff dry weight was higher in NL2 compared to NL1 and Bacanora.

Table 5.5. Harvest data in 2005 showing shoot dry weight (g), HI, chaff dry weight ear^{-1} (g), PH (plant height in cm), SN (spikelets ear^{-1}), RL (rachis length in cm), GN (grains ear^{-1}), GW (grain weight in mg) and TN (number of tillers) for the 4 genotypes

Lines	Biomass Shoot^{-1} (g)	HI	Chaff Ear^{-1} (g)	PH (cm)	Main shoot SN	Main shoot RL (cm)	Main shoot GN	Main shoot GW (mg)	T1-T3 tillers TN	T1-T3 tillers SN	T1-T3 tillers RL (cm)	T1-T3 tillers GN	T1-T3 tillers GW (mg)	T4+ tillers TN	T4+ tillers SN	T4+ tillers RL (cm)	T4+ tillers GN	T4+ tillers GW (mg)
Bacanora	1.33	0.45	0.60	49.9	21.7	9.80	60.7	37.4	3	20.5	9.05	49.2	36.3	5.33	19.5	8.35	36.8	33.4
NL1	2.04	0.31	0.61	67.2	24.0	10.3	56.3	40.5	3	23.2	9.62	37.4	43.0	6.33	19.9	9.09	28.2	43.5
NL2	1.58	0.46	1.09	49.0	28.7	13.3	80.3	43.4	3	25.2	12.3	63.2	41.1	2.11	25.7	11.7	44.3	39.2
Tybalt	2.12	0.43	0.56	65.5	22.0	10.2	61.3	47.2	3	20.8	9.39	55.2	44.6	5.67	17.6	9.06	36.7	46.5
Mean	1.77	0.41	0.72	57.9	24.1	10.9	64.7	42.1	3	22.4	10.1	51.3	41.3	4.86	20.6	9.55	36.4	40.6
SED (DF=6)	0.32	0.05	0.07	4.11	1.38	0.54	7.60	3.98	0	1.95	0.64	13.3	5.11	0.83	1.55	0.49	12.7	8.15

5.6 DISCUSSION

5.6.1 Apex development

In cereals, the changing morphology of the stem apex is the only unequivocal guide to ontogeny and observations of apical development are therefore central to studies of development in the whole plant (Baker and Gallagher, 1983). This is because the apex ultimately forms the ear on which the grains are formed and borne and patterns of apical development are implicated in controlling number and size of grains (Kirby, 1974), thereby affecting yield potential.

Significant differences among the genotypes were observed in spikelets primordia ear^{-1} in the growth-room experiments in both years. The novel genotypes NL1 and NL2 produced more spikelets than the other genotypes in the range of 26 to 29. They initiated spikelets at almost the same rate but had a longer duration of spikelet primordia production. The novel genotypes had a longer vegetative period from emergence to floral initiation than Bacanora and Tybalt in 2004, but NL2 had a shorter vegetative duration than Bacanora and NL1 in 2005. In both years, Tybalt reached FI earlier than the other genotypes. Spikelet number in wheat is influenced both by the duration and rate of spikelet initiation (Rahman and Wilson, 1977). The higher number of spikelets in the novel genotypes obtained was due to a longer duration rather than rate of spikelet initiation, for which differences were not significant in the range of 0.06 to 0.08 $(°Cd)^{-1}$ in 2004 and 2005. It has been proposed that extended durations from floral initiation to terminal spikelet may lead to higher numbers of spikelets than others (Rawson, 1971). It might be possible that differences in earliness *per se* genes could account for these differences in duration in the present study.

The parental CIMMYT genotypes are predominantly photoperiod insensitive since photoperiod in Cd. Obregon (27° 20 N, 109 54 °W) is less than 13 hours when the plant reaches terminal spikelet, while Tybalt is photoperiod sensitive. However, given the controlled conditions (16 hours photoperiod) these effects should have been negated. Usually, once spikelets have been formed, they are likely to survive (Kirby, 1977). It is generally the number of fertile florets per spikelet that has the greater impact on yield. The phase from terminal spikelet to anthesis is a crucial one

in determining grains m^{-2} as in this phase there is competition for assimilates amongst the ear, stem and leaf affecting assimilates available for floret survival.

In both 2004 and 2005, a longer duration was observed from terminal spikelet to anthesis in Bacanora and NL1 than other genotypes. NL2 had the shortest duration in both years of the four genotypes resulting in earlier flowering time. A shorter duration from terminal spikelet to anthesis could increase competition between the ear and stem and disfavour ear dry matter accumulation at anthesis. However, present results showed a strong trend for more grains ear^{-1} for NL2 than Bacanora and Tybalt. This was in contrast with reports in the literature whereby increasing the stem-elongation phase resulted in more fertile florets ear^{-1} hence more grains ear^{-1}(Gonzalez *et al.*, 2003). However, NL2 shows 'gigas' feature and therefore has fewer tillers per plant to compete for assimilates and exhibits higher ear index as discussed in Chapter 4. Thus, reduced tillering and higher ear index more than compensated for shorter duration of the TS-anthesis phase. Furthermore, in contrast to field results where NL1 had a longer rachis and therefore more spikelets ear^{-1}, in the growth room experiment NL2 had the longer rachis and hence more spikelets. This partially accounted for more grains ear^{-1} for NL2 compared to NL1 at maturity in the controlled-environment experiment. Therefore, the large-ear phenotype showed some plasticity with plant density in the present study. These findings suggest that there is evidence for a developmental basis to the large-ear phenotype probably linked to earliness *per se* genes which is new information. The fact that spikelet number can be genetically manipulated in this way offers the possibility of exploiting this trait in breeding programmes.

5.6.2 Effect of 'earliness *per se*' on development and spikelet primordia production

A wheat cultivar must have a flowering time which fits its target environment in order to maximise yield potential. The genetic control of flowering time is complex and the duration of different pheno-phases in the life cycle is under the control of three major genetic systems namely those controlling vernalization, photoperiod and 'earliness *per se*' responses. The third set of genes controls developmental rate independent of vernalization and photoperiod, so called 'earliness *per se*' genes (Snape *et al.*, 2001). Given that the four genotypes were spring wheats and that the

effect of photoperiod was negated as plants were grown under a long photoperiod, 'earliness *per se*' effects are most likely to account for the differences obtained in the duration among the genotypes. Earliness *per se* genes act to determine an intrinsic number of vegetative and floral primordia to be initiated, as quantified in plants grown with full vernalization and under long days or their rate of development after initiation. In addition, Jamieson *et al.* (1988) reported intrinsic earliness to be associated with the rate of spikelet primordia production. Slafer and Rawson (1995) found that the earliness trait is likely to be related to temperature sensitivity and that there could be an interaction between temperature and earliness *per se* (Slafer, 1996), whilst Halloran and Flood (1984) reported the genetic differences among cultivars were mostly related to temperature under the influence of the earliness *per se* trait.

An interaction between earliness *per se* and temperature could not explain the differences amongst the four genotypes in the present experiment since temperature was constant in the experiments. However, novel genotypes had longer thermal duration from floral initiation to terminal spikelet and may have had earliness *per se* alleles favouring an extended duration for this period. NL2 reached flowering earlier than Bacanora and NL1 in the field and controlled-environment conditions suggesting the possibility of the genotype carrying earliness *per se* alleles favouring a shorter thermal duration to flowering as, irrespective of environment, the genotype reached anthesis date earlier than the other parental genotypes.

5.6.3 Effect of leaf emergence rate

The number of leaves on a shoot is determined early in the life-cycle at floral initiation and varies considerably from 7 to 12 in spring wheat (Kirby *et al.*, 1985; Kirby *et al.*, 1989). The genotypes in this study did not differ in their rates of leaf appearance (0.01 $^{o}Cd^{-1}$) on the main shoot, but NL1 produced one more leaf than the others. The 16 hour photoperiod did not have any effect in enhancing the rate of leaf appearance on the main shoot, which agrees with the findings of Evans and Blundell (1994), and Pararajasingham and Hunt (1996) in spring wheat that 16 hour photoperiod does not affect the phyllochron. Based on the assumption that treatment differences in leaf primordia initiation at the shoot apex in these experiments were not affected by photoperiod, it can be deduced that the higher final number of leaves for NL1 in 2005 was associated with an earliness *per se* effect. NL1 remained in

vegetative phase for longer (450 °C d) compared to other genotypes, hence more leaf primordia were initiated due to a longer duration to floral initiation rather than a shorter plastochron resulting in higher leaf number. Therefore, earliness *per se* alleles could also be present in NL1.

5.6.4 Effect of tiller production

Results from the Obregon field cycle averaged over three years on shoots m^{-2} showed that NL2 and NL1 produced about 153 and 39 fewer shoots m^{-2} at GS 61 compared to Bacanora, respectively. NL2 has the characteristic large-ear phenotype, i.e. 'gigas' features whereby the ear has a long rachis but is quite square and compact in shape, whereas for NL1, it was mainly the rachis length which differed from the conventional genotype (Plate 1). Fewer tillers in NL2 could be ascribed to the probability of the genotype possessing the tiller inhibition *tin* gene as reported by Richards (1988), as previously mentioned. Duggan *et al.* (2002) reported that reduced tillering with the *tin* gene was due to an earlier cessation of tillering rather than a reduced tiller production rate which agrees with current findings. In the novel genotypes the tillering rate was the same (0.02 tillers °C d^{-1}), but NL1 has a maximum of 14 tillers per plant compared to 7 tillers in NL2. The lower maximum tiller number per plant observed in the field experiments at higher population density compared to single plants in the controlled-environment experiments could have been mediated by differences in the R:FR ratio. Plants at high population density will have fewer tillers due to reduced R:FR ratio associated with shading effects (Sparkes *et al.*, 2006) compared to single plants.

5.6.5 Harvest results

Spikelets ear^{-1} and rachis length were higher in novel genotypes than in conventional genotypes. While in three genotypes the spikelets ear^{-1} and rachis length decreased in later formed tillers, in Bacanora the number of spikelets ear^{-1} remained the same in late formed tillers but at high plant population density there will be fewer T4+ tillers. This could be one mechanism by which this genotype maintains its high ear biomass and grains m^{-2}. Normally, successively younger and smaller shoots reach the same phenological stage later. The difference among shoots tends to be less pronounced once the plant reaches maturity (Baker and Gallagher, 1983). There were few

differences in individual grain weight between the ear of main stem and the ears from the tillers in Bacanora.

In the novel genotypes, NL2 had the longest rachis which contrasts with the field data according to which NL1 had the longest rachis. This shows the plasticity of the novel ear morphology trait in relation to ear population density. Plant breeders would want to select those large-ear phenotypes which show the least plasticity at high ear population densities. Grain weight results were consistent with the field results whereby novel genotypes had heavier grains compared to Bacanora. With a longer rachis and more spikelets per ear, NL2 had more grains ear^{-1} compared to the other genotypes. However, the overall effect on grains $plant^{-1}$ was that conventional genotypes Bacanora (413) and Tybalt (431) produced more grains per plant than NL1 (344) and NL2 (351). Tybalt had heavier grains than novel genotypes which in turn had heavier grains than Bacanora. Moreover, NL2 had lower biomass $shoot^{-1}$ than NL1 suggesting there could have been preferential partitioning towards the ear in the pre-anthesis period as indicated by high chaff weight in NL2, which was consistent with field data. Indeed, high chaff weight has been previously reported in lines with *tin* gene (Motzo *et al.*, 2004).

5.7 SUMMARY

NL1 and NL2 had almost the same rate of spikelet primordia initiation but a longer duration than Bacanora and Tybalt resulting in more spikelets per ear than conventional genotypes. Therefore, at least in part, there is a developmental basis to the large-ear phenotype. This is encouraging because spikelets ear^{-1} should therefore be partially independent of any effects associated with reduced tiller production. Tiller production in NL2 remained low (7 tillers $plant^{-1}$) compared to 10-14 tillers $plant^{-1}$ in other genotypes. There was a positive relationship between main stem leaf number and tillers $plant^{-1}$ with NL2 producing fewer main stem leaves and tillers than the other genotypes. Therefore, present results also indicate part of physiological basis of large-ear phenotype may be associated with genes conferring restricted tillering, at least in the case of NL2. From two years data from the growth-room experiments, the hypothesis that a higher number of spikelets ear^{-1} has a developmental basis for the novel lines related to a longer duration of spikelet primordia production was confirmed. Also, the second hypothesis that the effects of

earliness *per se* were associated with more spikelets per ear and a longer rachis in NL2 and NL1 was supported.

In order to obtain environmental adaptability in wheat varieties it is important that they flower at appropriate times to particular environmental conditions. According to Worland (1996) earliness *per se* genes appear to be widespread in European wheats and play a significant role in determining flowering time. Therefore, present results confirm that these developmental responses are important in CIMMYT germplasm. Present results showed that in novel genotypes the period from floral initiation to terminal spikelet was crucial in determining spikelet number and hence grains ear^{-1}. Therefore, further information on allelic variation in earliness per se genes in novel large-ear genotypes may provide scope for breeders to enhance grains m^{-2} by increasing spikelet fertility in future years.

Plate 1. Novel genotypes NL1 (first picture) and NL2 (second picture)

CHAPTER 6 PHYSIOLOGICAL BASIS OF EAR FERTILITY AND YIELD POTENTIAL IN THE DOUBLED-HAPLOID POPULATIONS

This chapter focuses on an analysis of large-ear phenotype traits principally in the NL2 x Rialto doubled-haploid (DH) population. Three DH populations were generated, by crossing each of the three parental genotypes with Rialto. Some data were collected from the Bacanora x Rialto population and the NL1 x Rialto population in 2005 and 2006. However, much more extensive measurements were carried out in NL2 x Rialto population in 2005 and 2006 and in particular for a subset of 15 lines from NL2 x Rialto population in 2006. The chapter starts with a brief description of the production of the DH populations and then reports the results of experiments examining the subset of 15 lines of the NL2 x Rialto population in 2006, followed by 59 and 69 DH lines of NL2 x Rialto in 2005 and 2006, respectively. No data are presented on the Bacanora x Rialto DH population and the NL1 x Rialto DH population but relevant summaries of the data are included in Appendix IV. The chapter concludes with the discussion and summary.

6.1 INTRODUCTION

Breeding programmes in bread wheat comprise a three-step process for developing germplasm:

1. Genetic recombination for enlarging variation
2. Identification and selection of recombinant genotypes according to their phenotype
3. Fixation of genes in homozygous genotypes

Pedigree breeding is the conventional method of accumulating genetic recombination in each generation which involves an initial hand-crossing and then selfing for up to eight generations to obtain homozygosity for loci associated with agronomic traits. The heterozygosity in early generations makes the efficient identification and selection of recombinant genotypes more difficult. Pedigree breeding has been the preferred method of breeders involving repeated selection in each generation (Inagaki, 1998), but it has the disadvantage of requiring about eight generation cycles

to develop a new cultivar. Artificial production of haploid plants followed by chromosome doubling is a quick method for obtaining homozygous recombinant genotypes from heterozygous F1 genotypes, derived from parental crosses, in a single generation (Nei, 1963). This greatly reduces the time required for cultivar development. In addition, yield evaluation can start earlier and cultivar development is accelerated. This technique also facilitates the production of mapping populations for genetic studies. By this method the efficiency of selection is increased because DH lines do not express dominance variation and segregation within lines (Snape, 1989). Fixing lines to achieve homozygosity at an early-generation stage has assisted global testing of elite lines, and has helped to reduce costs of national wheat improvement programmes (Mujeeb-Kazi, personal communication). The major disadvantage of the DH technique is that there are fewer lines within a cross to select amongst and therefore less genetic and phenotypic variation. In summary, the use of DH populations with selected breeding objectives can complement conventional breeding programmes to accelerate the release of new varieties in both developed and developing countries where rapid varietal development is crucial to maintain sustainable wheat production.

6.1.1 Use of doubled-haploid techniques in wheat

Haploid plants are important in reducing the number of generations it takes to fix the homozygosity of wheat. A homozygous plant is obtained when a haploid's chromosomes are doubled. Anther culture and maize hybridization are two frequently used techniques for DH production in wheat. However, the major limitations of anther culture are the occurrence of somaclonal variation, aneuploidy and genotypic specificity (Picard, 1989), whilst the crossability of homologous group 5 loci (*Kr1, Kr2, Kr3*) is a limiting factor for the sexual crossing route. In addition, anther culture in winter wheat is very much genotype dependent and offers very low success compared to that in barley, durum and spring wheat (Laurie and Reymondie, 1991; Fedak *et al.*, 1997). Maize has been identified as an alternative sexual route for haploid production since it appears to be insensitive to the Kr crossability alleles of wheat (Laurie and Bennett, 1987) and polyhaploids can be recovered across different genotypes (Inagaki and Tahir, 1990). Rieza-Lizarazu and Mujeeb-Kazi (1990) reported an embryo recovery frequency of 29% and 81% plant regeneration using a detached-tiller culture method of wheat and maize hybridisation. Furthermore,

gametoclonal variation induced in DH lines using the maize pollination system was similar to that found in DHs obtained from wheat and *Hordeum bulbosum* L. crosses (Laurie and Snape, 1990).

6.1.1.1 Maize pollination technique

This sexual hybridisation technique as a means of producing polyhaploid wheat plants is based on preferential elimination of the parental chromosomes derived from the maize pollen in early stages of embryo development. After fertilisation between maize pollen and the ovule of the F1 wheat plant, chromosomes of the male parent are eliminated very early during embryo development (Laurie and Bennett, 1986) resulting in a haploid female embryo. Normally, the embryo aborts very early but the use of exogenous synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) enhances seed and embryo development until the latter can be excised and plated on a synthetic medium for continued growth and plantlet regeneration (Laurie *et al.*, 1990). When 2-3 small leaves are initiated in the media dish the seedling with its roots is transferred to a pot. This stage is about five weeks after embryo differentiation. When the plants are at the 6-leaf stage and have about 2-3 tillers, colchicine treatment is applied to restore chromosome number by changing the gamete cells from haploid to diploid (Figure 6.1). If the material is of winter type then about 8 to 10 weeks of low temperatures (5-8 °C) for vernalization should be given before the colchicine treatment is carried out, as was the case with lines of the NL2 x Rialto cross (Mujeeb-Kazi, personal communication).

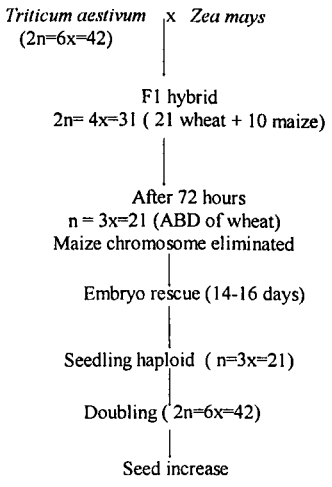


Figure 6.1. Schematic diagram of double haploid production in wheat using the maize pollination technique

6.1.2 Genetic background of the parents of the NL2 x Rialto doubled-haploid population

The reasons for selecting the NL2 and Rialto parents were described in sections 4.1.1.2 and 4.1.1.3 of Chapter 4, and relate to differences in ear morphology traits and likely differences in source-type traits (radiation-use efficiency and stem WSC). Major genes controlling agronomic traits where allelic expression is known to differ for the parents are described in Table 6.1.

Table 6.1. Major agronomic genes of NL2 and Rialto (Sean Mayes, personal communication) and Rialto

Genotypes	Photoperiod sensitivity	Vernalization requirement	Tiller inhibition	Height
NL2	*Ppd-D1a* (photoperiod insensitive)	*Vrn-A1* (winter type)	*tin* gene (1AS) (tiller inhibition)	*Rht-B1b* (semi-dwarf)
Rialto	*Ppd-D1b* (photoperiod sensitive)	*vrn-A1* (spring type)	Non *tin* gene (1AS) (no tiller inhibition)	*Rht-D1b* (semi-dwarf)

The lines of this population will allow a more rigorous analysis of the physiological basis of the large-ear phenotype and its contribution to grain number per unit area and yield than by comparing a range of cultivars and/or advanced lines. This is because it is possible to compare target trait differences against a relatively uniform genetic background in contrast to comparing cultivars. One hundred and thirty eight (138) DH lines of the NL2 x Rialto population were generated of which 69 were studied in the present experiments. These 69 lines were selected after initial screening for flowering date in the glasshouse at CIMMYT headquarters, El Batan. The 69 earliest flowering lines were selected, i.e. those presumably having a low or nil vernalization requirement and which were photoperiod insensitive.

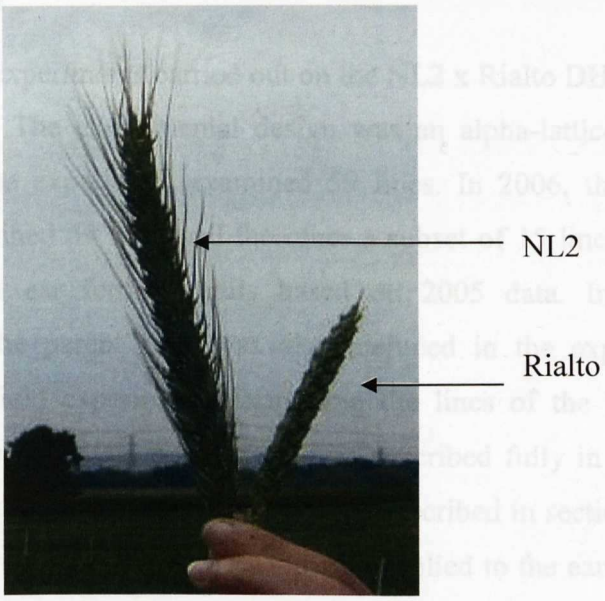


Figure 6.2. Ear phenotypes of NL2 and Rialto

6.1.3 Objectives and hypotheses

The objectives were to examine the physiological basis of grain number determination in the lines of the DH populations contrasting for large-ear phenotype and to quantify inter-relationships between ear fertility traits and yield and yield components.

The specific hypotheses examined are:

- Rachis length is positively correlated with spikelets ear^{-1} and grains ear^{-1} amongst the DH lines
- Spikelet ear^{-1} is positively correlated with grains m^{-2} amongst the DH lines
- Rachis length is positively correlated with ear index (ratio of ear dry weight to above ground dry weight) at anthesis amongst the DH lines
- There is a positive correlation between ear biomass at anthesis and grains ear^{-1} amongst the DH lines
- There are genotypic differences in pre-anthesis RUE among the DH lines, and RUE is positively correlated with above-ground biomass at anthesis
- Rachis length is positively correlated with individual grain weight amongst the DH lines

6.2 METHODOLOGY

There were three field experiments carried out on the NL2 x Rialto DH population in Cd. Obregon, Mexico. The experimental design was an alpha-lattice laid on two replicates. In 2005, one experiment examined 59 lines. In 2006, there were two experiments; one examined 69 lines and the other a subset of 15 lines representing maximum contrasts in ear fertility traits based on 2005 data. In the 69 line experiment in 2006, the parent NL2 was also included in the experiment. The methodology for the field experiments examining the lines of the NL2 x Rialto population and the two other DH populations was described fully in section 3.3.2. Growth analyses carried out on this population were described in section 3.5.3.4 and 3.5.3.5. A degraining treatment at GS 61 + 14d was applied to the ears of randomly selected shoots in the experiment examining the 15 lines of NL2 x Rialto DH population as described in section 3.3.1. In the subset experiment, fractional PAR interception using a ceptometer was measured on a weekly basis until GS 61. Canopy temperature and NDVI were measured on a weekly basis from GS31 to grain filling and booting to mid grain filling, in the subset experiment and the NL2 x Rialto DH population experiments, respectively. Further details of these measurements are described in Chapter 3.

6.3 RESULTS

In the first part of this section, results for the experiment examining the subset of 15 DH lines are presented and in the second part results of the experiments examining 59 and 69 lines of the NL2 x Rialto DH population in 2005 and 2006, respectively.

6.3.1 Results of the field experiment examining the subset of 15 DH lines in 2006

6.3.1.1 Crop development

The dates of developmental stages of the 15 lines at terminal spikelet, onset of stem extension (GS 31), booting (GS 41), anthesis (GS 61) and physiological maturity are shown in Table 6.2. There was large variation amongst the lines at GS 41 and GS 61 in the ranges 20 February-13 March and 7-27 March, respectively but the ranking of lines at the two growth stages was similar (Table 6.2). There were also differences amounting to *ca.* 20 days among the lines at terminal spikelet, GS 31 and physiological maturity. The variation of flowering date among the lines subjected the lines to different environmental conditions during grain filling particularly with

regard to temperature, as the late flowering lines incurred higher temperatures compared to earlier flowering lines.

Table 6.2. Dates of developmental stages (TS=terminal spikelet, GS 31= onset of stem extension, GS 41=booting, GS 61=anthesis, PM=physiological maturity) and GF(number of days to accumulate 700 °Cd from GS 61) for the subset of 15 DH lines of NL2 x Rialto population in 2006

Lines	TS	GS 31	GS 41	GS 61	PM	GF (days)
1	12 Jan	11 Feb	27 Feb	20 Mar	02 May	35
5	12 Jan	16 Feb	05 Mar	23 Mar	04 May	34
7	12 Jan	18 Feb	13 Mar	27 Mar	10 May	33
17	13 Jan	08 Feb	24 Feb	16 Mar	28 Apr	36
21	10 Jan	09 Feb	24 Feb	15 Mar	25 Apr	36
27	08 Jan	09 Feb	20 Feb	07 Mar	18 Apr	38
31	11 Jan	12 Feb	24 Feb	21 Mar	30 Apr	34
32	10 Jan	28 Jan	22 Feb	07 Mar	21 Apr	38
52	11 Jan	30 Jan	27 Feb	17 Mar	22 Apr	35
67	10 Jan	29 Jan	22 Feb	20 Mar	28 Apr	34
74	08 Jan	04 Feb	20 Feb	09 Mar	20 Apr	38
106	12 Jan	17 Feb	05 Mar	20 Mar	30 Apr	35
116	12 Jan	09 Feb	22 Feb	16 Mar	22 Apr	36
124	09 Jan	31 Jan	22 Feb	17 Mar	28 Apr	35
138	10 Jan	30 Jan	21 Feb	08 Mar	14 Apr	38

6.3.1.2 Grain yield, harvest index, harvest above-ground dry weight and yield components

The 15 lines differed in yield and all the yield components ($P < 0.001$). Yield ranged from 4.7 to 7.6 t ha^{-1} with lower yields observed for the later flowering lines. Above-ground dry matter (AGDM) and HI differed amongst the lines in the ranges 1443-1946 g m^{-2} and 0.34-0.45, respectively ($P < 0.001$). Number of ears per m^2 ranged from 274 to 565 amongst the lines ($P < 0.001$), whilst grains ear^{-1} ranged from 30.5 to 52.3. Differences were observed amongst the lines ($P < 0.001$) in grains m^{-2} ranging from 12 814 to 25 981 and also in grain weight in the range 24.9-52.8 mg.

Yield was positively correlated with grain weight ($P < 0.05$, r=0.46) but not with grains m^{-2} (Table 6.4). A positive association was found between each of harvest AGDM ($P < 0.001$, r=0.76) and HI ($P < 0.001$, r=0.60) and yield. Positive

correlations were observed between each of ears m^{-2} ($P < 0.05$; r=0.61) and grains ear^{-1} ($P < 0.05$, r=0.51) and grains m^{-2}. Negative trade-offs between yield components were also found amongst the 15 lines. Thus, grains m^{-2} was negatively associated with grain weight ($P < 0.001$, r= -0.84). There was also a strong negative correlation between ears m^{-2} and grain weight ($P < 0.001$, r= -0.73). Overall, grains ear^{-1} was positively associated with grains m^{-2} but was not significantly correlated with grain yield amongst the lines.

The effect of flowering date on yield and grain weight is shown in Figures 6.3 and 6.4, respectively. The late flowering lines were exposed to higher temperatures during grain filling and this may have resulted in the negative linear regression between flowering date and each of yield and grain weight. Figure 6.5 shows the mean air temperature against calendar date during the experimental season. Assuming the duration of the grain filling period to be 700°C days (above a base of 0°C) in winter wheat (Kirby *et al.*, 1998), the grain filling period was reduced for the latest flowering line (Line 3, 33 days) by 5 days compared to the earliest flowering lines (Lines 27 and 32, 38 days)

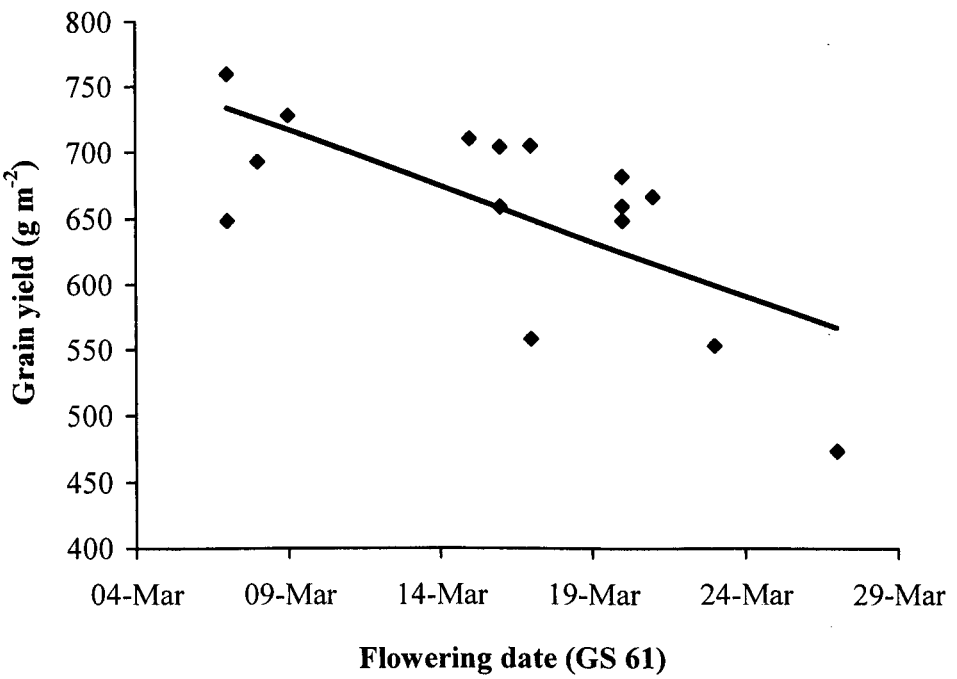


Figure 6.3. Effect of flowering date on grain yield amongst subset of 15 DH lines of the NL2 x Rialto population in 2006 (y= -8.324x + 326555; R^2=0.45, P <0.001)

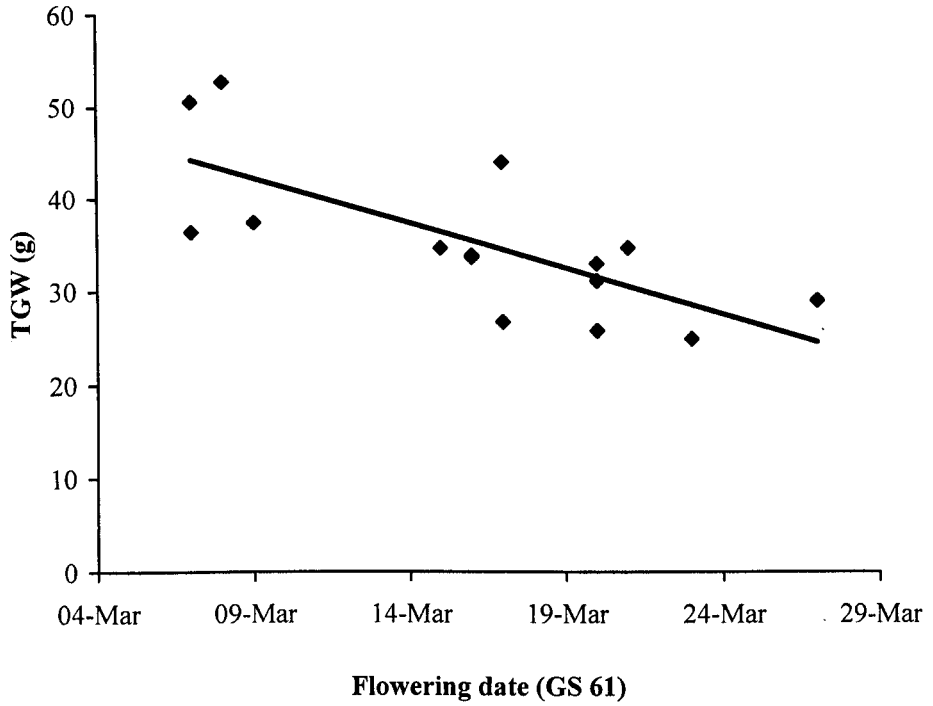


Figure 6.4. Effect of flowering date on thousand grain weight (TGW) amongst of 15 DH lines of NL2 x Rialto population in 2006 (y=-0.984x + 38563; R^2= 0.53, $P < 0.001$)

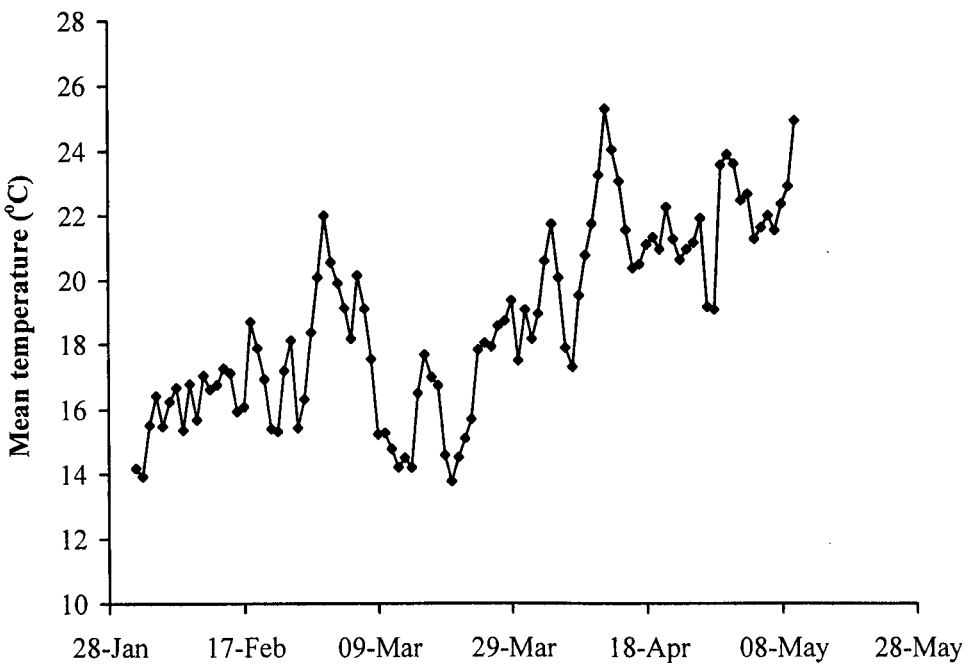


Figure 6.5. Daily mean air temperature from 1 Feb to 10 May in 2006 at CIMMYT Cd. Obregon experimental station

Table 6.3. Combine grain yield (85% DM), harvest index (HI), above-ground dry matter (AGDM), ears m^{-2}, grains ear^{-1}, thousand grain weight (TGW) and grains m^{-2} at harvest for 15 NL2 x Rialto DH lines in 2006

Lines	Yield (g m^{-2})	HI	AGDM (g m^{-2})	Ears m^{-2}	Grains ear^{-1}	TGW (g)	Grains m^{-2}
1	660	0.37	1811	498	52.3	25.8	25 981
5	553	0.36	1529	470	47.6	24.9	22 380
7	473	0.34	1403	542	30.5	29.0	16 390
17	704	0.36	1946	565	37.3	33.8	20 864
21	710	0.42	1712	454	45.5	34.6	20 596
27	648	0.42	1536	363	35.5	50.6	12 814
31	667	0.39	1718	458	42.4	34.6	19 327
32	759	0.41	1881	427	49.2	36.4	20 862
52	705	0.40	1810	407	40.1	43.9	16 056
67	649	0.41	1588	385	51.5	32.9	19 762
74	727	0.44	1653	441	44.1	37.5	19 432
106	682	0.40	1713	529	41.6	31.1	22 082
116	659	0.45	1468	410	47.9	33.6	19 604
124	558	0.39	1443	444	47.0	26.7	20 911
138	692	0.42	1645	274	47.8	52.8	13 108
Mean	657	0.40	1657	444	44.0	35.2	19345
SED (DF=42)	31.4	0.022	120.2	31.4	2.58	1.49	1270

Table 6.4. The phenotypic correlation (r) between grain yield, yield components and other physiological traits amongst the subset of 15 DH lines in 2006 (r values significant at 5% are indicated in italics and those at 1% level of significance are indicated in bold)

	PAR	RUE	k	GAI	AGDM GS 61	Ear index	Grains to ear DM ratio	Spikelet ear^{-1}	Rachis length	Grains ear^{-1}	Ears m^{-2}	Grains m^{-2}	Grain weight	Grain yield	AGDM at harvest	HI	Ears DM gm^{-2}	FD	Dur	PH
PAR at GS 31-61	1																			
RUE_{PAR} at GS 31- 61	-0.38	1																		
k_{PAR} at GS 61	-0.44	-0.11	1																	
GAI at GS 61	**0.63**	-0.12	**-0.93**	1																
AGDM at GS 61	*0.61*	0.46	*-0.50*	*0.51*	1															
Ear index at GS 61	-0.12	-0.11	**0.77**	*-0.57*	-0.13	1														
Grains: ear DM	0.08	**-0.66**	*-0.58*	*0.55*	-0.08	**-0.72**	1													
Spikelets ear^{-1}	0.44	-0.26	0.09	0.14	0.23	0.43	-0.15	1												
Rachis length	0.16	-0.33	0.46	-0.20	-0.03	**0.74**	-0.37	**0.83**	1											
Grains ear^{-1}	-0.13	-0.09	0.16	0.03	-0.12	0.28	0.16	*0.57*	*0.60*	1										
Ears m^{-2} at harvest	**0.65**	-0.21	*-0.54*	*0.60*	0.43	-0.38	*0.54*	0.04	-0.20	-0.36	1									
Grains m^{-2}	0.47	-0.28	-0.33	*0.54*	0.27	-0.07	**0.62**	*0.54*	0.33	*0.51*	*0.61*	1								
Grain weight (mg)	**-0.75**	*0.53*	0.40	**-0.63**	-0.30	0.09	*-0.49*	*-0.50*	-0.33	-0.21	**-0.73**	**-0.84**	1							
Grain yield (g m^{-2})	*-0.61*	**0.69**	0.21	-0.33	0.05	0.04	0.09	-0.15	-0.11	0.31	-0.26	0.04	0.46	1						
AGDM harvest (g m^{-2})	-0.20	**0.62**	-0.31	0.20	0.36	-0.36	0.42	-0.26	-0.44	0.13	0.20	0.29	0.13	**0.76**	1					
HI	**-0.67**	0.30	**0.70**	**-0.75**	-0.35	*0.50*	-0.37	0.13	0.40	0.35	**-0.65**	-0.27	*0.52*	*0.60*	-0.06	1				
Ears DM (g m^{-2})	0.20	0.11	0.47	-0.27	0.37	**0.87**	**-0.70**	*0.53*	**0.69**	0.24	-0.14	0.09	-0.08	0.06	-0.15	0.28	1			
Flowering date (FD)	**0.99**	-0.43	-0.44	*0.61*	-0.27	-0.17	0.07	0.41	0.14	-0.16	*0.61*	0.40	**-0.72**	**-0.68**	-0.27	**-0.69**	0.13	1		
Duration d (GS31-61)	0.29	0.12	-0.04	0.16	0.34	0.12	-0.13	0.32	0.25	0.36	-0.14	0.11	-0.21	-0.11	0.01	-0.14	-0.14	0.30	1	
Plant Height (PH)	-0.48	**0.66**	-0.26	0.06	0.09	-0.42	0.22	-0.46	-0.48	0.11	-0.27	-0.20	0.47	*0.54*	**0.68**	0.00	-0.35	-0.47	0.23	1

6.3.1.3 Shoot production, green canopy area, radiation interception and radiation-use efficiency in pre-anthesis period

The 15 lines differed in fertile shoots m^{-2} and GAI at anthesis in the ranges 291-622 and 5.50-9.42, respectively ($P < 0.001$) (Table 6.5). The lines also differed in chlorophyll content of each of the flag leaf and leaf 2 in the ranges 49.4-55.2 and 45.7-52.9 SPAD units, respectively ($P < 0.001$). PAR interception from GS 31 to GS 61 was measured from interpolation of weekly ceptometer readings of fractional PAR interception during this period and incident PAR. There were differences among the lines in PAR interception in the range of 1481 to 2121 MJ m^{-2} and in RUE_{PAR} over the same period in the range of $_{1.09}$ to 1.59 g MJ^{-1}($P < 0.001$). In addition, the lines differed in k_{PAR} values at GS 61($P < 0.01$) in the range of 0.46 to 0.73.

Considering the inter-relationships between resource-capture traits and grains m^{-2} in an attempt to understand the physiological basis of grains m^{-2}, there was a positive phenotypic correlation between PAR interception and anthesis AGDM amongst the lines ($P < 0.05$, r=0.61; Table 6.4), and GAI at GS 61 was positively correlated with intercepted PAR ($P < 0.001$, r=0.63). A strong positive association was found between PAR interception and flowering date ($P < 0.001$, r=0.99) but a weak association between the duration of the GS 31 to GS 61 phase and PAR interception. There was a trend for RUE_{PAR} to be positively correlated with above-ground biomass at GS 61 (P=0.05, r=0.46). A positive association was also observed between biomass and GAI ($P<0.05$, r=0.51). In summary, the main physiological determinants governing the amount of AGDM produced at GS 61 were canopy green area, light interception and the duration from GS 31 to GS 61.

Table 6.5. Fertile shoots m^{-2}, green area index at GS 61, SPAD chlorophyll content for the flag leaf and leaf 2 (L2), accumulated intercepted photosynthetically active radiation (PAR) (GS 31-61), radiation-use efficiency, RUE_{PAR} (GS 31-61) and k_{PAR} at GS 61 for the 15 lines of the NL2 x Rialto DH population in 2006

Lines	Fertile shoots m^{-2}	GAI (GS 61)	SPAD flag	SPAD L2	Accumulated PAR (MJ m^{-2}) GS 31-61	RUE_{PAR} (g MJ^{-1})	k_{PAR} (GS 61)
1	494	9.42	54.7	48.8	1969	1.17	0.49
5	517	8.67	52.1	48.1	1979	1.09	0.50
7	622	9.12	49.4	45.9	2121	1.12	0.46
17	584	7.88	49.6	45.7	1787	1.31	0.52
21	417	7.73	53.4	50.3	1756	1.15	0.58
27	350	5.50	55.0	51.9	1481	1.20	0.71
31	505	8.73	53.2	48.4	1968	1.36	0.48
32	413	8.21	55.2	52.9	1510	1.43	0.49
52	421	7.72	53.4	50.3	1821	1.59	0.52
67	346	6.42	55.0	52.1	1919	1.17	0.73
74	435	6.74	54.7	50.5	1623	1.41	0.66
106	552	7.86	50.9	47.7	1910	1.26	0.53
116	394	6.64	52.0	48.6	1773	1.20	0.65
124	451	8.15	52.1	48.4	1792	1.10	0.55
138	291	7.25	53.9	50.7	1487	1.33	0.57
Mean	453	7.74	53.0	49.3	1793	1.26	0.56
SED (DF=28)	40.2	0.634	0.912	1.33	27.4	0.092	0.061

6.3.1.4 Above-ground dry matter production and partitioning in the pre-anthesis period, ear traits at anthesis and crop height

The lines differed in AGDM accumulation at the three samplings up to anthesis ($P < 0.05$). At the onset of stem elongation, AGDM ranged from 101 to 178 g m^{-2}, and at booting from 252 to 445 g m^{-2} ($P < 0.05$). However, at anthesis, the range was larger from 867 to 1344 g m^{-2} ($P < 0.05$). Flowering time was positively correlated with AGDM at GS 61 ($P < 0.05$, r=0.55). There was a positive correlation between AGDM and the duration of the period from GS 31 to GS 61 (r=0.34) but it was not significant.

The lines differed in ear biomass at GS 61 ($P < 0.001$) in the range 240 to 506 g m^{-2} (Table 6.6). Similarly, there were differences amongst the lines in ear DM per shoot ($P < 0.001$) from 0.49 to 1.67, in ear index ($P < 0.001$) from 0.23 to 0.46 and the grains-to-ear DM ratio at GS 61 ($P < 0.001$) from 41 to 89 grains g^{-1}. The ranges ($P < 0.001$) in rachis length, spikelets ear^{-1} and plant height were 11.4-14.7 cm, 22.1-26.3 and 61.0-98.2 cm, respectively.

Rachis length was positively correlated with spikelets ear^{-1} ($P < 0.001$, r=0.83) and ear index ($P < 0.001$, r=0.74). A positive association was also found between rachis length and grains ear^{-1} ($P < 0.05$, r=0.57). Spikelets ear^{-1} ($P < 0.05$, r=0.54) and grains ear^{-1} ($P < 0.05$, r=0.51) was positively correlated with grains m^{-2} but not with grains $spikelet^{-1}$ (r=0.28). There was no association between AGDM at GS 61 or ear index and grains m^{-2}. However, there was a highly significant positive correlation between grains to ear DM ratio and grains m^{-2} ($P < 0.001$, r=0.62). Plant height was also positively correlated with yield ($P < 0.05$, r=0.54).

6.3.1.5 Normalized difference vegetative index (NDVI), stomatal conductance and canopy temperature

Differences were observed amongst the lines ($P < 0.001$) in NDVI measured on calendar dates at around GS 31 (71-0.83), GS 41 (0.80-0.87), GS 61 (0.75-0.86) and early grain filling stage, when the lines had begun to senesce (0.37-0.66). Stomatal conductance was measured once at the early grain filling stage (3 April) and showed no significant differences amongst the lines. The lines differed in canopy temperature at GS 31 in the range 0.71-0.83 ($P < 0.05$). No significant differences were observed at booting or at anthesis. At early grain filling there were significant differences amongst the lines in the range 0.37-0.66 ($P < 0.05$).

Canopy temperature at the early grain filling stage was positively correlated with yield ($P < 0.05$, r=0.50). There was no association between yield and NDVI. Flowering time was positively correlated with canopy temperature at grain filling ($P < 0.001$, r=0.65) but negatively at vegetative ($P < 0.001$, r=-0.61). Flowering time was positively associated with NDVI at the early grain filling stage ($P < 0.001$, r=0.69).

Table 6.6. Above-ground dry matter (g m^{-2}) (AGDM) at GS 31, GS 41, GS 61, ear DM g m^{-2}, dry matter per ear, ear index, the grains to ear DM ratio, spikelets ear^{-1}, rachis length and plant height at GS 61 for the 15 DH lines of the NL2 x Rialto population in 2006

Lines	AGDM			Ear DM	DM ear^{-1}	Ear	Grains to ear DM ratio	Spikelets	Rachis length	Plant height
	GS 31	GS 41	GS 61	g m^{-2}	(g)	Index	(grains g^{-1})	ear^{-1}	(cm)	(cm)
1	144	361	1138	351	0.74	0.31	73.3	26.3	13.4	72.1
5	141	352	1104	343	0.71	0.31	65.9	25.0	13.8	70.3
7	137	343	1159	287	0.49	0.25	59.2	22.7	11.7	61.0
17	127	318	1154	280	0.50	0.24	76.9	22.1	11.6	93.9
21	126	315	1001	311	0.74	0.31	67.7	23.7	13.2	70.6
27	119	296	867	325	0.97	0.37	40.9	22.1	12.0	63.9
31	151	377	1344	439	0.92	0.33	44.2	24.8	13.2	66.9
32	168	420	1060	241	0.60	0.23	89.3	22.2	11.4	94.6
52	129	322	1275	369	0.89	0.28	44.6	24.3	12.3	94.4
67	162	405	1115	494	1.44	0.44	39.9	25.7	14.6	66.5
74	178	445	1143	506	1.67	0.46	39.9	24.8	14.7	71.6
106	139	347	1154	334	0.62	0.29	67.9	26.2	13.2	61.2
116	129	323	1054	332	0.89	0.32	58.7	25.8	14.5	61.9
124	118	296	999	353	0.86	0.35	60.8	26.3	14.5	65.9
138	101	252	1000	296	1.04	0.30	46.6	23.7	13.0	98.2
Mean	138	345	1105	351	0.87	0.32	58.4	24.4	13.1	74.2
SED (DF=28)	18.6	46.7	85.9	32.0	0.101	0.023	8.85	1.13	0.44	1.47

Table 6.7. Normalized difference vegetative index (NDVI) and canopy temperature for the 15 NL2 x Rialto DH lines in 2006

Lines	NDVI				Canopy temperature °C			
	Vegetative (GS 31)	Booting (GS 41)	Anthesis (GS 61)	Grain filling (GS 75)	Vegetative (GS 31)	Booting (GS 41)	Anthesis (GS 61)	Grain filling (GS 75)
1	0.83	0.86	0.85	0.54	21.76	22.39	23.50	29.78
5	0.77	0.82	0.83	0.58	22.03	22.41	23.36	29.69
7	0.81	0.85	0.86	0.66	21.76	22.42	23.33	29.23
17	0.78	0.85	0.79	0.43	21.84	22.30	23.67	30.16
21	0.79	0.83	0.80	0.44	22.18	22.28	23.45	29.88
27	0.76	0.81	0.76	0.37	21.93	22.58	23.68	30.86
31	0.82	0.86	0.81	0.48	21.25	22.56	23.03	30.19
32	0.78	0.84	0.75	0.39	21.72	22.06	23.55	30.27
52	0.81	0.84	0.77	0.40	21.98	22.45	23.56	30.43
67	0.81	0.85	0.78	0.42	21.71	22.52	23.86	30.88
74	0.80	0.84	0.76	0.38	21.30	22.40	23.72	30.41
106	0.81	0.87	0.82	0.47	21.82	22.68	23.50	30.21
116	0.81	0.85	0.78	0.38	21.72	22.38	23.30	30.76
124	0.75	0.82	0.81	0.46	22.06	22.44	23.49	30.31
138	0.71	0.80	0.77	0.37	21.89	22.42	23.48	30.40
Mean	0.79	0.84	0.81	0.45	21.80	22.42	23.50	30.23
SED (DF=28)	0.023	0.012	0.015	0.024	0.33	0.36	0.52	0.40

6.3.1.6 Stem soluble carbohydrate reserves

Differences were observed among the lines ($P < 0.001$) in stem WSC at GS 61+7 days in the range 0.58-1.56 t ha^{-1}. At maturity, differences were much smaller in the range of 0.04-0.16 t ha^{-1} ($P < 0.05$). There was positive relationship between RUE_{PAR} and stem WSC at anthesis ($P<0.001$, r=0.63). Stem WSC was negatively correlated with grains m^{-2} ($P<0.05$, r=0.54) but there was a positive relationship between stem WSC and grain weight at anthesis ($P<0.05$, r=0.60). Overall, stem WSC at anthesis was not significantly correlated with yield (r=0.30). No association was found between stem WSC and ear DM. Plant height, however, was positively associated with WSC at anthesis ($P < 0.05$, r=0.57). An average of 92% (difference between stem WSC at GS 61+7d and maturity/stem WSC at GS 61+7d) of stem reserves were mobilised from the lines.

Table 6.8. Water soluble carbohydrates (WSC) in stems and attached leaf sheaths at anthesis plus 7d and at maturity in the 15 lines of the NL2 x Rialto DH population

Lines	Stem WSC at GS 61 + 7 d (t ha^{-1})	Stem WSC at maturity (t ha^{-1})	%Mobilised WSC
1	0.58	0.04	93%
5	0.60	0.05	92%
7	0.80	0.06	93%
17	1.09	0.11	90%
21	0.76	0.05	93%
27	0.69	0.11	84%
31	0.89	0.05	94%
32	0.56	0.07	88%
52	1.56	0.16	90%
67	0.94	0.06	94%
74	0.96	0.06	94%
106	0.90	0.05	94%
116	0.79	0.06	92%
124	0.70	0.04	94%
138	1.49	0.16	89%
Mean	0.89	0.07	
SED (DF=28)	0.19	0.03	

6.3.1.7 Responses to degraining at GS 61+14d

Degraining of ears was carried out 14 days after GS 61 on the 15 lines. The spikelets were removed from one side of the ear. The lines differed in dry matter per spikelet at maturity in control shoots ($P < 0.001$), with the heaviest spikelets for Lines 27 and 138 (0.22 g) and the lightest spikelets in Line 52 (0.15 g). Averaging across the years, degraining increased dry matter per spikelet in degrained ears at harvest ($P < 0.001$). However, no significant interaction was found between the lines and the degraining treatment. Significant differences were found among the lines in individual grain weight in the control ears in the range 32.3 to 60.2 mg ($P < 0.001$). Degraining resulted in heavier grains ($P < 0.001$) compared to the control ears by 2.5 mg. An interaction between the lines and the degraining treatment was observed ($P < 0.001$), where those lines with lighter grains in intact control ears (< 40 mg) tended to show a positive response to degraining and those with heavier grains (> 40 mg) in intact ears tended to show a neutral or negative response.

Table 6.9 Effect of degraining on individual spikelet weight and individual grain weight for the 15 lines of the NL2 x Rialto DH population

Lines	DM of spikelet (g)		Individual grain weight (mg)	
	Control	Degrain	Control	Degrain
1	0.12	0.16	29.4	33.9
5	0.11	0.13	29.3	32.3
7	0.10	0.14	33.7	41.2
17	0.11	0.13	32.9	39.1
21	0.14	0.16	38.8	40.2
27	0.20	0.22	62.4	60.2
31	0.14	0.17	34.7	37.3
32	0.17	0.20	39.7	44.9
52	0.09	0.15	44.4	44.5
67	0.16	0.16	34.7	34.5
74	0.17	0.20	41.3	42.6
106	0.11	0.13	31.9	35.3
116	0.12	0.13	32.7	36.9
124	0.12	0.14	29.8	33.6
138	0.20	0.22	58.1	55.9
Mean	0.14	0.16	38.3	40.8
SED (DF=58)				
Lines	0.01		0.75	
Degraining	0.003		0.28	
Interaction	0.01		1.09	

6.3.2 Field experiment examining the 59 lines of the NL2 x Rialto DH population in 2005

6.3.2.1 Effects of genotype on yield, yield components, harvest above-ground dry matter and harvest index

Yield differed amongst the 59 lines ($P < 0.001$) ranging from 1.7 to 7.3 t ha^{-1} with the lowest yield associated with the latest flowering lines. Harvest AGDM and HI differed among the lines ($P < 0.001$) in the ranges 641 to 2157 g m^{-2} and 0.21-0.49, respectively. Differences were observed among the lines ($P < 0.001$) in ears m^{-2} (196-512), grains ear^{-1} (24.5-51.5), grains m^{-2} (5694-19222) and grain weight (20.8-52.9).

6.3.2.2 Effects of flowering time on grain yield and grain weight and effect of plant height on yield for 59 lines of NL2 x Rialto DH population

Flowering time (15 February to 12 April) again had a large effect on the grain yield and grain weight (Figures 6.6 and 6.7). Later flowering lines had lower yields and lighter grains ($P < 0.05$). There were differences among the lines ($P < 0.001$) in plant height in the range 46-106 cm. Plant height was positively correlated with yield (Figure 6.8). Since flowering of the lines ranged from 15 February to 12 April, differences in temperature during the respective grain filling periods were large and probably affected yield. Daily mean temperatures during the crop cycle are shown in Figure 6.9.

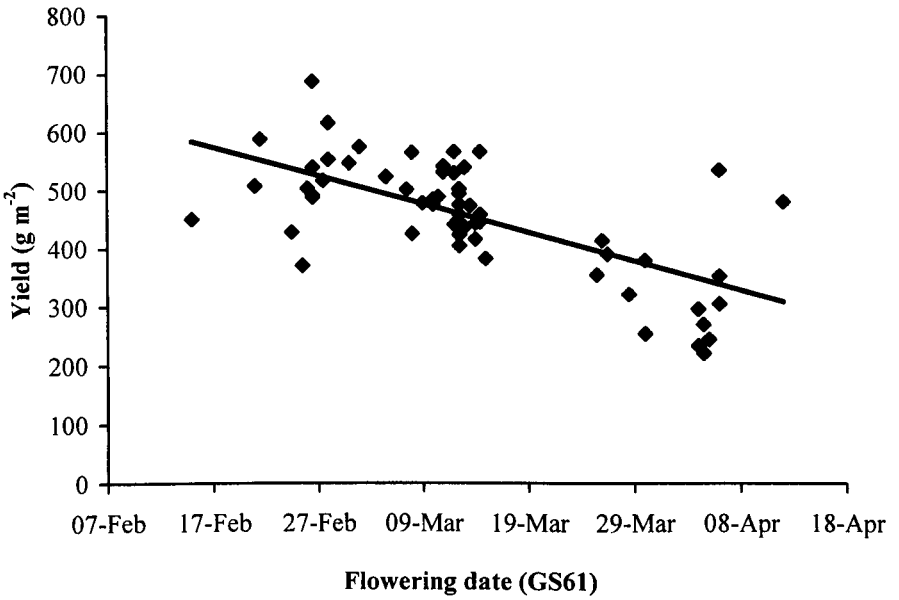


Figure 6.6. Effect of flowering time on grain yield of 59 lines of NL2 x Rialto DH population in 2005 (y= -4.92x + 189636; R^2=0.45, $P < 0.001$)

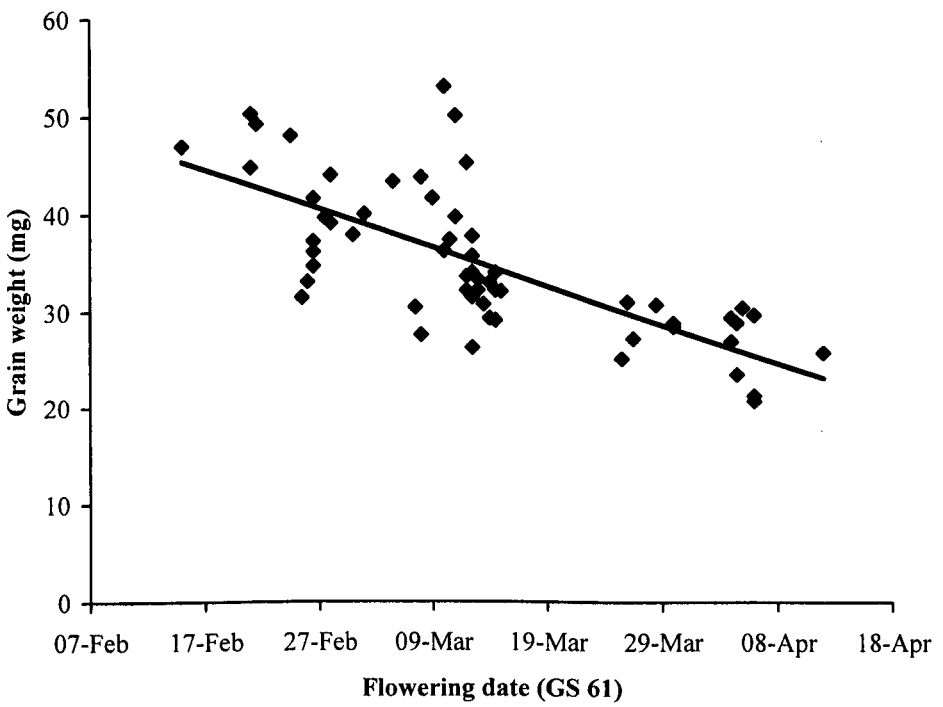


Figure 6.7. Effect of flowering time on grain weight of 59 lines of NL2 x Rialto DH population in 2005 (y = -0.395x + 15233; R^2=0.52, $P < 0.001$)

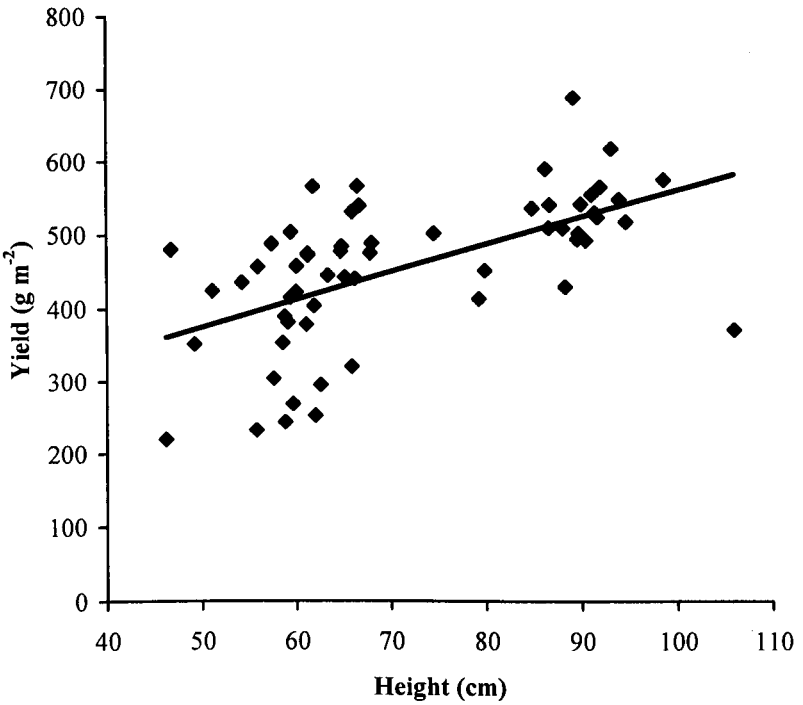


Figure 6.8. Effect of plant height on grain yield of 59 lines of NL2 x Rialto DH population in 2005 (y= 3.701x + 190; R^2=0.33, $P < 0.001$)

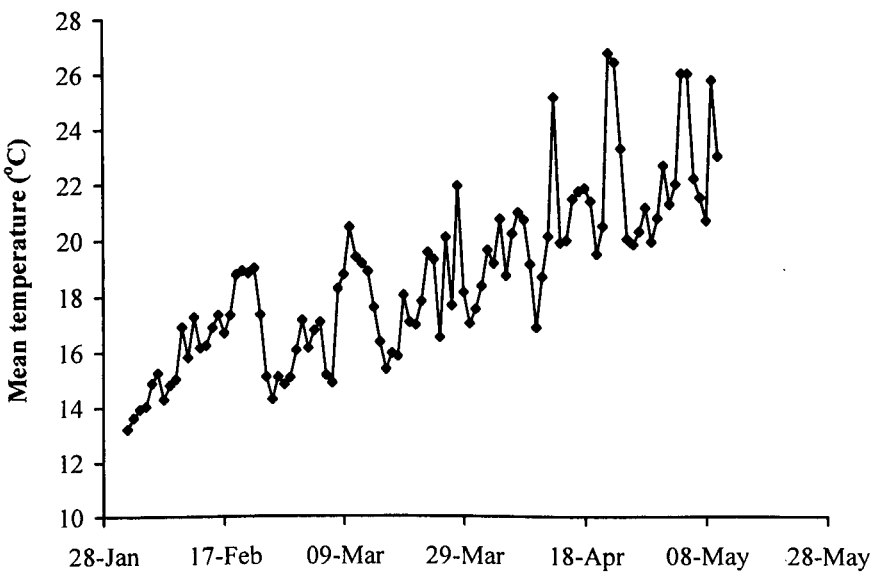


Figure 6.9. Daily mean air temperature from 1 Feb to 10 May in 2005 at CIMMYT Cd. Obregon experimental station

6.3.2.3 Effects of genotype on above- ground dry matter, dry matter partitioning and ear traits at GS 61

The 59 lines differed in fertile shoots m^{-2} and AGDM and at GS 61 in the ranges 237-583 and 537-1208 g m^{-2} respectively ($P < 0.001$). The lines varied in spikelets ear^{-1} ($P < 0.001$) from 19 to 30 and rachis length ($P < 0.001$) from 10.2 to 16.1 cm; later flowering lines had longer rachis. Relative chlorophyll content differed amongst the lines ($P < 0.001$) varying from 42 to 54.8 SPAD units. Differences were observed amongst the lines in ear biomass (176-510 g m^{-2}), ear index (0.21-0.44) and the grains to ear DM ratio at GS 61 (25.4-51.7 grains g^{-1}) ($P < 0.001$).

The large effect of flowering time and plant height on yield and physiological traits made it difficult to satisfactorily normalise the data for flowering. The inter-relationships between the traits will be first examined in the full set of 59 lines. Secondly, in order to examine the physiological basis of grains m^{-2} and yield according to the traits whilst minimising any confounding effects of flowering date and plant height on the relationship between ear fertility traits and grains m^{-2} and yield, trait correlations will be also quantified in a set of 20 'median' lines in which the extremes of flowering date and plant height have been removed. In order to select the set of 20 median lines, the full set of 59 lines was first ranked for plant height and the top ten and bottom ten ranking lines were removed. The remaining lines were then ranked for flowering time and the 20 median lines were selected as the subset for further physiological analysis. Trait interactions will be first considered as the basis for the determinants of grains m^{-2} and then for the further determinants of grain yield.

6.3.2.4 Relationships between traits amongst the 59 DH lines

Rachis length was positively correlated with spikelets ear^{-1}(P < 0 .001, r=0.77). Grains m^{-2} was positively correlated with ears m^{-2} (P <0.001, r=0.67) and grains spikelet^{-1} (P <0.001, r=0.48) but not with spikelets ear^{-1}. There was no association between ears m^{-2} and spikelets ear^{-1}. Neither ear index (r=-0.15) nor AGDM at GS 61 (r=-0.21) were significantly associated with grains m^{-2}. However, a strong positive correlation existed between grains m^{-2} and grains to ear DM ratio (P <0.001, r=0.58). Neither ear index nor the grains-to-ear DM ratio was significantly correlated with rachis length.

Yield was positively correlated with AGDM at harvest (P< 0.001, r=0.80) and HI (P <0.001, r=0.48). A positive correlation was found between yield and grains m^{-2} (P <0.001, r=0.71) and grain weight (P < 0.001, r=0.53). The expected negative relationship between grains m^{-2} and grain weight was observed (r=-0.21).

6.3.2.5 Relationships amongst traits for the subset of 20 median DH lines of the NL2 x Rialto population

Rachis length was positively correlated with spikelets ear^{-1} (P< 0.001, r=0.74) and grains ear^{-1}(P <0.001, r=0.62). Spikelets ear^{-1} (P <0.001, r=0.72) and grains spikelet^{-1} (P <0.001, r=0.57) were positively correlated with grains m^{-2}. A strong correlation was found between grains-to-ear DM ratio and grains m^{-2} (P <0.001, r=0.73). No association was found between grains m^{-2} and AGDM at GS 61. Ear index was negatively associated with grains m^{-2} (r=-0.25). There was no association between rachis length and ear index. Rachis length was positively correlated with the grains-to-ear DM ratio (P <0.001, r=0.60). Neither flowering date nor height was correlated with yield.

Yield was positively but not significantly associated with grains m^{-2} (r= 0.42). There was no association between yield and grain weight (r=0.17). A strong negative correlation was found between grains m^{-2} and grain weight (P<0.001, r=-0.79). A positive association was found between yield and AGDM at harvest (P < 0.001, r=0.63). No association was found between HI and yield.

Table 6.10. The phenotypic correlation among 59 lines of the NL2 x Rialto DH population in 2005 for yield (g m^{-2}), yield components, harvest AGDM, HI, ear traits, dry matter (g m^{-2}), dry matter partitioning, flowering date and plant height (cm) at GS 61 (r values significant at 5% level in italics and at 1% in bold and italics)

	AGDM GS 61 (g m^{-2})	Rachis length (cm)	Ear Index	Spikelets ear^{-1}	Plant height (cm)	HI	Yield (g m^{-2})	AGDM Harvest (g m^{-2})	Grain weight (mg)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}	Grains $spiklelet^{-1}$	Grains : Ear DM	Ear DM g m^{-2}	Flowering date
AGDM (GS 61)	1															
Rachis length	***0.42***	1														
Ear Index	-0.12	0.08	1													
Spikelets ear^{-1}	*0.27*	***0.77***	0.15	1												
Plant height	0.02	-0.20	***-0.68***	*-0.27*	1											
HI	***-0.47***	***-0.43***	0.12	*-0.31*	0.18	1										
Grain yield	-0.18	***-0.37***	*-0.33*	-0.25	***0.54***	***0.48***	1									
AGDM at harvest	0.15	-0.14	***-0.49***	-0.09	***0.52***	-0.10	***0.80***	1								
Grain weight	-0.06	***-0.46***	-0.21	***-0.50***	***0.62***	***0.38***	***0.53***	*0.35*	1							
Grains m^{-2}	-0.15	-0.10	-0.21	0.09	0.09	*0.27*	***0.71***	***0.62***	-0.21	1						
Ears m^{-2} at harvest	0.07	-0.04	-0.17	0.07	-0.14	*-0.28*	*0.35*	***0.63***	*-0.30*	***0.67***	1					
Grains ear^{-1}	*-0.26*	-0.06	-0.11	0.05	*0.28*	***0.68***	***0.52***	0.14	0.03	***0.58***	-0.17	1				
Grains $spikelet^{-1}$	***-0.37***	***-0.41***	-0.19	***-0.45***	***0.38***	***0.75***	***0.58***	0.16	*0.26*	***0.48***	-0.19	***0.87***	1			
Grains: Ear DM ratio	*-0.26*	-0.06	-0.09	0.06	*0.27*	***0.69***	***0.52***	0.11	0.03	***0.58***	-0.19	***0.99***	***0.86***	1		
Ear DM (GS 61)	***0.42***	*0.28*	***0.43***	*0.34*	*-0.27*	-0.01	-0.01	*0.26*	-0.14	0.11	***0.44***	*-0.28*	***-0.42***	*-0.29*	1	
Flowering date	***0.45***	***0.49***	***0.52***	*0.28*	***-0.61***	***-0.64***	***-0.68***	*-0.35*	***-0.72***	-0.20	0.18	***-0.46***	***-0.68***	***-0.46***	***0.40***	1

Table 6.11. (a)The phenotypic correlation among 20 median lines of the NL2 x Rialto DH population in 2005 for yield (g m^{-2}), yield components, harvest AGDM, HI, ear traits, dry matter (g m^{-2}), dry matter partitioning, flowering date and plant height (cm) (r values significant at 5% level in italics and at 1% in bold and italics) (b) Minimum and maximum values of traits in median lines

(a)	AGDM GS 61 (g m^{-2})	Rachis length (cm)	Ear Index	Spikelets ear^{-1}	Plant height (cm)	HI	Yield (g m^{-2})	AGDM harvest (g m^{-2})	Grain weight (mg)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}	Grains $spiklelet^{-1}$	Grains : Ear DM	Ear DM g m^{-2}	Flowering date
AGDM (GS 61)	1															
Rachis length	0.03	1														
Ear Index	-0.18	0.00	1													
Spikelets ear^{-1}	-0.01	***0.74***	-0.14	1												
Plant height	***0.52***	0.03	-0.02	-0.04	1											
HI	-0.23	0.08	0.32	0.13	-0.30	1										
Yield	*0.48*	0.22	-0.16	0.21	0.15	0.04	1									
AGDM at harvest	*0.44*	0.16	-0.38	0.13	0.32	***-0.70***	***0.63***	1								
Grain weight	0.32	*-0.47*	0.11	***-0.58***	0.45	-0.17	0.17	0.25	1							
Grains m^{-2}	0.02	*0.55*	-0.25	***0.72***	-0.32	0.12	0.42	0.17	***-0.79***	1						
Ears m^{-2}	-0.33	-0.01	-0.26	0.07	***-0.56***	-0.41	0.16	0.47	-0.31	0.37	1					
Grains ear^{-1}	0.29	***0.62***	-0.10	***0.72***	0.11	0.42	0.33	-0.11	***-0.56***	***0.73***	-0.34	1				
Grains $spikelet^{-1}$	0.38	0.41	-0.05	0.39	0.18	*0.48*	0.31	-0.23	-0.43	***0.57***	*-0.48*	***0.92***	1			
Grains: Ear DM ratio	0.27	***0.60***	-0.10	***0.70***	0.11	0.42	0.31	-0.14	***-0.57***	***0.73***	-0.35	***0.95***	***0.92***	1		
Ear DM (GS 61)	0.47	0.16	*0.46*	0.05	*0.49*	-0.28	0.25	0.43	0.39	-0.23	-0.11	-0.10	-0.16	-0.13	1	
Flowering date	-0.02	0.35	0.05	0.34	-0.22	0.30	-0.30	*-0.48*	***-0.67***	0.42	-0.15	*0.53*	*0.52*	*0.52*	-0.15	1

(b)	AGDM GS 61 (g m^{-2})	Rachis length (cm)	Ear Index	Spikelets ear^{-1}	Plant height (cm)	HI	Yield (g m^{-2})	AGDM harvest (g m^{-2})	Grain weight (mg)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}	Grains $spiklelet^{-1}$	Grains : Ear DM	Ear DM g m^{-2}	Flowering date
Minimum	644	11.4	0.34	21.4	59	0.30	385	796	26	9063	258	27	1.20	26.2	212	10 Mar
Maximum	978	14.8	0.44	27.4	90	0.50	581	1574	53	18491	455	51	1.90	51.7	433	15 Mar

6.3.3 Field experiment examining the 69 lines of the NL2 x Rialto DH population in 2006

6.3.3.1 Effects of genotype on yield, yield components, harvest above-ground dry matter and HI

The 69 lines differed in yield ranging from 1.9 to 6.5 t ha^{-1} ($P < 0.001$). Likewise, differences were obtained in harvest biomass which ranged from 457 to 1632 g m^{-2}. Harvest index differed among the lines ranging from 0.22 to 0.38 ($P < 0.001$). Differences were observed in grains m^{-2} and grain weight ranging from 8165 to 17 966 and 18.2 to 50.5 g respectively ($P < 0.001$). Ears m^{-2} ranged from 198-523 whilst grains ear $^{-1}$ ranged from 21 to 63 ($P < 0.001$). The NL2 parent response was intermediate in terms of grain yield and the different yield components.

6.3.3.2 Effects of flowering time on grain yield and grain weight and effect of plant height on yield for 69 lines of NL2 x Rialto DH population

Later flowering time in 2006 also had a negative effect on yield and grain weight (Figures 6.10 and 6.11). One of the parents of the DH population, i.e. NL2, was included in the experiment and is shown as the open symbol. Yield was also positively correlated with plant height (Figure 6.12) lines, differing in height in the range 51.7- 103.2 cm ($P < 0.001$).

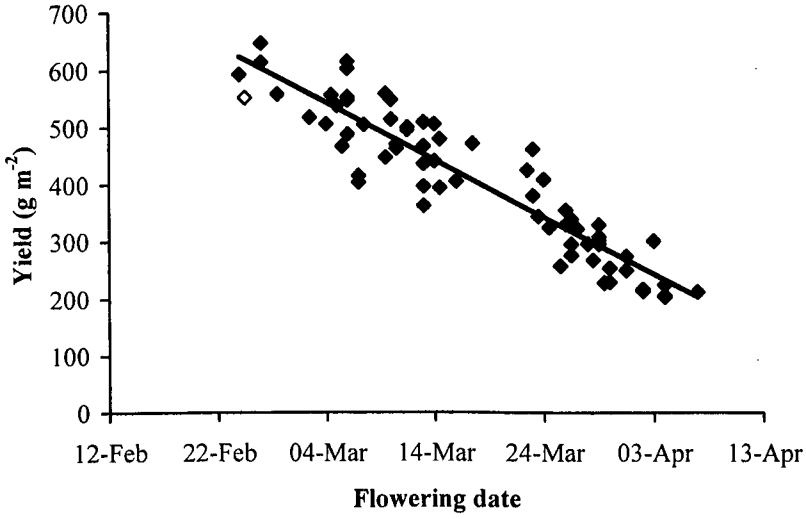


Figure 6.10. Effect of flowering time on yield for 69 lines of NL2 x Rialto DH population in 2006 (y=-9.98x + 387510; R^2=0.86, $P < 0.001$)

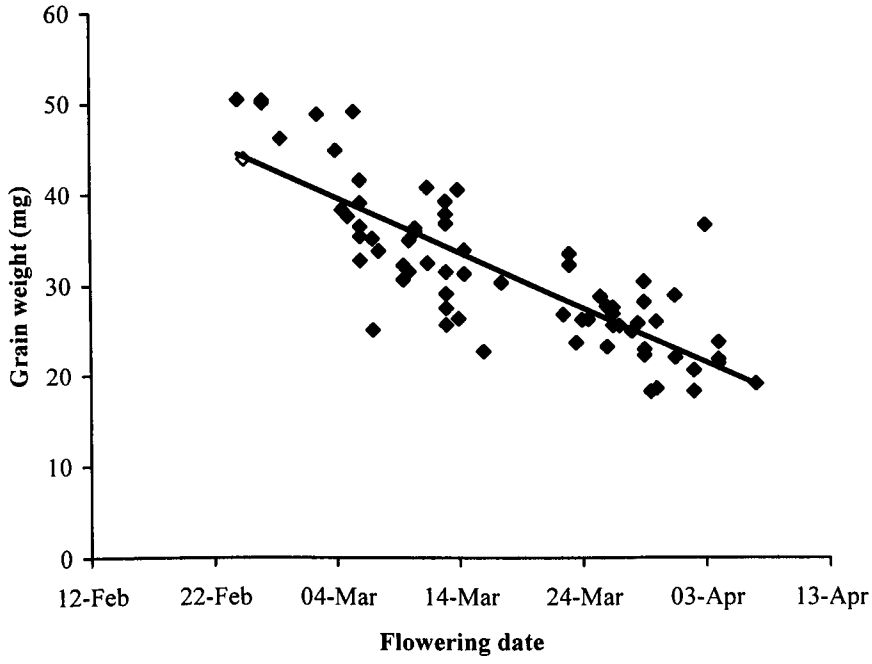


Figure 6.11. Effect of flowering time on grain weight for 69 lines of NL2 x Rialto DH population in 2006 (y=-0.61x + 23532; R^2=0.67, $P < 0.001$)

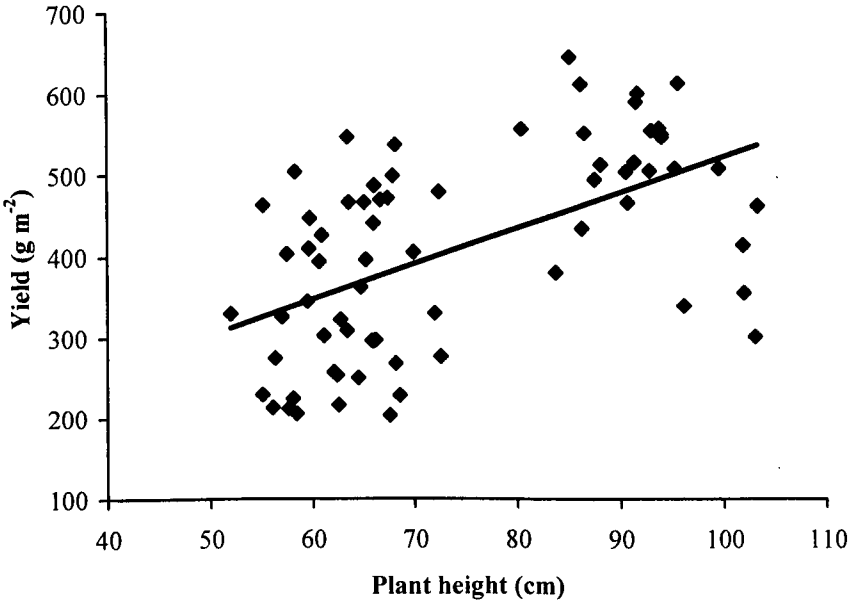


Figure 6.12. Effect of plant height on grain yield for 69 lines of NL2 x Rialto DH population in 2006 (y= 4.39x + 83.7; R^2=0.30, $P < 0.001$)

6.3.3.3 Effects of genotype on above-ground dry matter, DM partitioning and ear traits at GS 61

The lines differed in AGDM at GS 61 from 568 to 890 g m^{-2} ($P < 0.001$). Differences were also observed in spikelets ear^{-1} ($P < 0.001$) and rachis length ($P < 0.001$) ranging from 19.2 to 29.3 and 10.6 to 16.2 cm, respectively. Relative chlorophyll content ranged from 46.6 to 58.1 SPAD units ($P < 0.001$). Differences were observed amongst the lines in ear biomass (158-742 g m^{-2}), ear index (0.28-0.66) and the grains-to-ear DM ratio (14.1-93.1 grains g^{-1}) ($P < 0.001$).

The same method was applied to the data as in 2005 to identify a subset of 20 median lines and examine the inter-relationships between yield and the other traits minimising confounding effects of flowering time and plant height. The traits examined were the same as in 2005.

6.3.3.4 Relationships amongst traits for the 69 DH lines of the NL2 x Rialto population

Rachis length was positively correlated to spikelets ear^{-1} ($P< 0.001$, r=0.39). There was no association between rachis length and grains per ear. A positive correlation was found between grains $spikelet^{-1}$ and grains m^{-2} ($P <0.001$, r=0.42). Grains m^{-2} was positively associated with ears m^{-2} (r=0.17) and spikelet ear^{-1} (r=0.28) but these correlations were not statistically significant. Neither AGDM at GS 61 nor ear index was associated with grains m^{-2}. Grains m^{-2} was positively correlated with the grains-to-ear DM ratio ($P < 0.001$, r=0.50). Rachis length was not associated with either ear index (r=0.15) or the grains-to-ear DM ratio (r=-0.20). There was no association of flowering date or plant height with yield.

There was no association between grains m^{-2} and grain weight. Yield was positively correlated with AGDM ($P<0.001$, r=0.62) and HI ($P <0.001$, r=0.62). Similarly, yield was positively associated with grains m^{-2} ($P <0.001$, r=0.54) and grain weight ($P <0.001$, r=0.80). Ears m^{-2} was negatively correlated with grains ear^{-1} ($P < 0.001$, r=0.70)

6.3.3.5 Relationships amongst traits for the subset of 20 median DH lines of the NL2 x Rialto population

Rachis length was positively associated with spikelet ear^{-1} (r=0.32) but not statistically significant. No association was found between rachis length and grains ear^{-1}. Grains m^{-2} was positively correlated with spikelets ear^{-1} (P<0.001, r=0.64), grains ear^{-1} (P < 0.001, r=0.69) and grains $spikelet^{-1}$(P <0.001, r=0.59). A positive correlation was found between grains-to-ear DM ratio and grains m^{-2} (P < 0.001, r=0.53). No association was found between grains m^{-2} and ears m^{-2}. Grains m^{-2} was positively associated with AGDM at GS 61 (r=0.26) and ear index (r=0.15) but not statistically significant. A positive correlation was found between rachis length and ear index (P< 0.001, r=0.63). Ears m^{-2} was negatively associated with grains ear^{-1} (P < 0.001, r=0.74).

The negative association between grains m^{-2} and grain weight was observed among the 20 median lines (P <0.05, r=0.46). A positive correlation was found between AGDM at harvest and yield (P < 0.05, r=0.51). Positive but insignificant associations were found between each of HI (r=0.39) and grains m^{-2} (r=0.38) and yield. Grain weight was positively correlated with yield (P<0.001, r=0.64).

6.3.4 Relationships amongst traits for subset of 20 median DH lines of the NL2 x Rialto population using data for 59 lines averaged over 2005 and 2006.

Rachis length was positively associated with spikelets ear^{-1} (P < 0.05, r=0.53). Grains m^{-2} was positively correlated with spikelets ear^{-1}(P < 0.001, r=0.65) and grains ear^{-1}(P < 0.001, r=0.65), but not with ears m^{-2}. A weak positive correlation was found between the grains-to-ear DM ratio and grains m^{-2} (r=0.48). Yield was positively associated with grain weight (P < 0.001, r=0.76). A negative association was found between grain weight and grains m^{-2} (P < 0.001, r=-0.67). Ears m^{-2} was negatively associated with grains ear^{-1} (P < 0.001, r=-0.75). There was no association between grains m^{-2} and yield. The correlation matrix is presented in Appendix IV (Table 5).

Table 6.12. The phenotypic correlation among 69 lines of the NL2 x Rialto DH population in 2006 for yield (g m^{-2}),ADDM at harvest, HI, yield components, ear traits, dry matter (g m^{-2}), dry matter partitioning, flowering date and plant height (cm) at GS 61 (r values significant at 5% level in italics and at 1% in bold and italics)

	Ear index	AGDM GS 61 (g m^{-2})	Rachis length (cm)	Spikelets ear^{-1}	Plant height (cm)	HI	Yield (g m^{-2})	AGDM Harvest (g m^{-2})	Grain weight (mg)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}	Grains $spikelet^{-1}$	Grains to Ear DM ratio	Ears DM (g m^{-2})	Flowering date
Ear index	1															
AGDM GS 61	-0.13	1														
Rachis length	0.15	*0.34*	1													
Spikelets ear^{-1}	0.03	0.18	***0.39***	1												
Plant height	***-0.85***	0.22	-0.01	-0.11	1											
HI	-0.16	***-0.60***	*-0.23*	-0.13	0.11	1										
Yield	***-0.48***	-0.09	-0.20	*-0.24*	***0.54***	***0.62***	1									
AGDM at harvest	***-0.44***	***0.53***	0.01	-0.18	***0.58***	-0.20	***0.62***	1								
Grain weight	***-0.50***	-0.14	***-0.28***	***-0.49***	***0.55***	***0.50***	***0.80***	***0.47***	1							
Grains m^{-2}	-0.07	0.04	0.08	*0.28*	0.10	***0.36***	***0.54***	***0.35***	-0.05	1						
Ears m^{-2}	0.22	***0.67***	0.04	0.07	-0.20	***-0.66***	-0.23	***0.40***	***-0.40***	0.17	1					
Grains ear^{-1}	-0.22	***-0.56***	0.02	0.14	0.19	***0.81***	***0.49***	-0.16	*0.23*	***0.53***	***-0.70***	1				
Grains $spikelets^{-1}$	*-0.23*	***-0.62***	-0.14	-0.21	*0.24*	***0.85***	***0.58***	-0.09	***0.42***	***0.42***	***-0.72***	***0.94***	1			
Grains: ear DM	***-0.46***	***-0.59***	-0.20	0.06	***0.34***	***0.68***	***0.50***	-0.04	*0.24*	***0.50***	***-0.51***	***0.83***	***0.80***	1		
Ears DM (g m^{-2})	***0.43***	***0.83***	***0.42***	0.15	*-0.26*	***-0.64***	***-0.35***	*0.24*	***-0.39***	-0.03	***0.72***	***-0.62***	***-0.67***	***-0.76***	1	
Flowering date	***0.39***	0.24	0.22	***0.39***	***-0.39***	***-0.63***	***-0.85***	***-0.42***	***-0.79***	***-0.35***	*0.30*	***-0.43***	***-0.58***	***-0.46***	***0.43***	1

Table 6.13. (a) The phenotypic correlation among 20 median lines of the NL2 x Rialto DH population in 2006 for yield (g m^{-2}), harvest AGDM, HI yield components, ear traits, dry matter (g m^{-2}), dry matter partitioning, flowering date and plant height (cm) (r values significant at 5% level in italics and at 1% in bold and italics) (b) Minimum and maximum values of traits in median lines

(a)	Ear index	AGDM GS 61 (g m^{-2})	Rachis length (cm)	Spikelets ear^{-1}	Plant height (cm)	HI	Yield (g m^{-2})	AGDM harvest (g m^{-2})	Grain weight (mg)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}	Grains $spikelet^{-1}$	Grains :Ear DM	Ears DM (g m^{-2})	Flowering date
Ear index	1															
AGDM at GS 61	-0.32	1														
Rachis length	***0.63***	-0.10	1													
Spikelets ear^{-1}	-0.16	-0.04	0.32	1												
Plant height	***-0.88***	***0.45***	***-0.55***	-0.16	1											
HI	0.00	***-0.66***	-0.17	0.23	-0.20	1										
Yield g m^{-2}	-0.41	0.23	-0.25	0.27	0.39	0.39	1									
AGDM at harvest	-0.42	***0.79***	-0.10	0.08	***0.58***	***-0.58***	*0.51*	1								
Grain weight	*-0.53*	*0.43*	***-0.60***	-0.24	*0.49*	0.07	***0.64***	*0.46*	1							
Grains m^{-2}	0.15	-0.26	0.41	***0.64***	-0.13	0.39	0.38	0.01	*-0.46*	1						
Ears m^{-2}	0.01	***0.58***	0.15	-0.11	0.20	***-0.77***	-0.03	***0.70***	0.01	-0.07	1					
Grains ear^{-1}	-0.02	***-0.62***	0.08	*0.48*	-0.11	***0.80***	0.27	*-0.48*	-0.30	***0.69***	***-0.74***	1				
Grains $spikelet^{-1}$	0.01	***-0.66***	-0.01	0.27	-0.14	***0.81***	0.24	*-0.52*	-0.26	***0.59***	***-0.78***	***0.97***	1			
Grains: ear DM	-0.32	***-0.67***	-0.18	0.35	0.19	***0.66***	0.18	-0.38	-0.25	*0.53*	*-0.54*	***0.84***	***0.83***	1		
Ears DM (g m^{-2})	*0.52*	***0.64***	0.42	-0.22	-0.29	***-0.61***	-0.13	0.37	-0.05	-0.13	*0.53*	***-0.57***	***-0.57***	***-0.85***		
Flowering date	0.12	0.13	0.24	-0.02	0.07	*-0.54*	-0.42	0.18	-0.35	-0.05	0.30	-0.20	-0.21	-0.14	0.24	1

(b)	Ear index	AGDM GS 61 (g m^{-2})	Rachis length (cm)	Spikelets ear^{-1}	Plant height (cm)	HI	Yield (g m^{-2})	AGDM harvest (g m^{-2})	Grain weight (mg)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}	Grains $spikelet^{-1}$	Grains :Ear DM	Ears DM (g m^{-2})	Flowering date
Minimum	0.33	618	11.4	20.8	61	0.29	250	734	23	8640	243	26	1.21	24.2	158	17 Mar
Maximum	0.63	1013	14.7	26.3	91	0.55	507	1357	41	17921	440	66	2.87	93.1	477	4 Apr

6.4 DISCUSSION

The discussion aims at analysing the physiological basis of the processes limiting ear fertility and yield potential amongst the lines of the NL2 x Rialto DH population. It will firstly focus on the differences amongst lines in source- and sink-type traits in the pre-anthesis period determining grains m^{-2}. The discussion will then examined relationships between grains m^{-2} and grain weight influencing yield potential.

6.4.1 Crop development

The idea of crossing winter wheat and spring wheat parents has been around since the early 1960s at CIMMYT, and there have been some successful crossing combinations. For example, Villareal *et al.* (1991) showed that crosses between spring wheat and winter wheat containing the 1B/1R translocation can enhance yield potential in spring wheat backgrounds by 1B/1R conferring more grains through either more ears m^{-2} or through larger ears bearing more grains. Similarly, Rees *et al.* (1993) reported that the lines from a winter and spring cross kept their canopies cooler than the surrounding environment, showing higher stomatal conductance and photosynthetic efficiency. The present DH population was derived from a cross between winter wheat Rialto and the CIMMYT spring wheat advanced line NL2 in an attempt to increase grains m^{-2} and yield potential by exploiting synergies between high grains ear^{-1} in NL2 and higher expression of source-type traits, e.g. pre-anthesis RUE in Rialto. Although 138 lines were generated from this cross, only 69 were adapted to grow in short days of Mexico in the crop cycle with few vernalizing days.

Manipulation of the photosynthetic environment can influence the number of potential grain sites by affecting fertile floret number per spikelet (Gifford, 1977). The respective lines were subjected to different temperatures in the pre- and post-anthesis periods because of the range of flowering time. In this regard, some of the lines could have experienced more favourable conditions to develop maximum grain size than others. The temperature difference from booting to grain filling between the early and late flowering lines was on average 8 °C. This could have an effect on cell development, duration of cell division and expansion and activity of enzymes in the regulation of starch accumulation. Hawker and Jenner (1993) showed that the effect of heat on grain dry weight was due to a reduction in the activity of starch synthase which could explain the low grain weight of the late flowering lines. The late

flowering lines tended to have reduced grains m^{-2} associated with smaller ear biomass at GS 61 and grains ear^{-1} but the bigger effect of phenology was on grain weight which was directly related to grain yield. The main effect of later flowering was to have a shorter grain filling period. Moreover, the role of hormones is important in the determination of floret fertility. There could have been differences in reduced endogenous cytokinins amongst some of the lines according to flowering date. Abscisic acid is another hormone governing the floret fertility and given that there was potentially some heat stress to the later flowering lines, this hormone could have been produced to buffer the crop against the stress.

6.4.2 Physiological basis of genetic variation in grains m^{-2} in pre-anthesis period

Present results showed that anthesis biomass was not correlated with grains m^{-2} but there were differences amongst the lines in light interception and RUE. Yield was negatively correlated with ear index in the median lines and the full set of DH lines in both 2005 and 2006 which is in contrast to most past studies (Slafer and Savin, 1994; Gonzalez *et al.*, 2003). In 2005 and 2006, there was no association between ear index and grains m^{-2} amongst all lines of the population. Ear index was negatively correlated with anthesis biomass and also tended to be negatively correlated with the grains-to-ear DM ratio in the 20 median lines. It was assumed that with the higher ear dry mater partitioning at anthesis there will be enhanced ear fertility due to more grain setting in the spikelets. In fact, in the present DH population larger partitioning of DM to the ear at anthesis did not necessarily materialise into more grains m^{-2} but it did result in more ear biomass. Ear biomass was positively correlated with grains m^{-2} in 2006, but not in 2005. The main reason for this inconsistent association was that in the large ear-phenotype lines more assimilates were likely invested in non-reproductive tissues in the ear such as glumes, paleas and lemmas as was discussed in Chapter 4 and 5 with NL2 having greater chaff weight.

The trait which therefore overall appeared to be most positively correlated with grains m^{-2} in this population was the grains-to-ear dry matter ratio at GS 61. Data from the 15 lines of the subset showed that grains g^{-1} DM was positively associated with grains m^{-2} in the range of 40-90 grains g^{-1}. There was a positive association between this trait and grains m^{-2} in both years in the median lines and the full set of

lines with values ranging from 14-93 grains g^{-1}. Similar findings were reported by Abbate *et al.* (1998) in Argentinean wheat cultivars in the range of 61-106 grains g^{-1}, i.e. grains m^{-2} was more closely related to this ratio than ear biomass at anthesis. In UK wheats, Shearman *et al.* (2005) reported a range of 73-129 grains g^{-1} but found that this ratio was not associated with breeding progress in grain number. Lower ratios in the ranges reported in present study were likely associated with greater chaff weight and Motzo *et al.* (2004) reported an increase in chaff weight of lines having the *tin* gene which was present in NL2. The *tin* gene was most likely responsible for more variation in the ratio in this population than in most population reported worldwide. Moreover, there could have been effects of traits at the biochemical level whereby phytohormones such as cytokinins or ABA could have influenced this ratio. Ashikari *et al.* (2005) found increased level of cytokinin increased grain number in rice. Furthermore, with the exception of 15 DH lines experiment, ear biomass at anthesis was negatively associated to grains ear^{-1}. Overall, these findings showed that additional ear biomass associated with the large-ear phenotype did not translate into more grains m^{-2} principally due to a trade off with the grains-to-ear DM ratio.

As discussed in Chapter 5, there was a developmental basis accounting for the high number of spikelets ear^{-1} in the parental line NL2. In the field experiments examining the NL2 x Rialto population rachis length was positively associated with spikelets ear^{-1} and grains ear^{-1} in the 20 median lines. This supports the hypothesis that 'rachis length is positively correlated with spikelet number per ear amongst the DH lines'. It is encouraging that genetic variation of rachis length was positively associated with spikelet number per ear at commercial plant densities. The hypothesis that 'rachis length is positively correlated with ear index' was supported in the 20 median lines in 2006 but was not supported in 20 median lines in 2005. Moreover, rachis length was positively correlated with grains m^{-2} amongst the 20 median lines in both years. There was also a strong positive association in the subset of 15 DH lines between these two traits, suggesting that developmental genes influencing rachis length and spikelets ear^{-1} in this population may have contributed to more grains and could be of potential use in future breeding programmes. It was encouraging that rachis length was not negatively with the grains-to-ear DM ratio. The positive association between rachis length and grains ear^{-1} suggest that grains ear^{-1} could be increased in the future by incorporating rachis length as a selection trait in breeding programmes opening a

new avenue to enhance yield potential. However, future attempts to increase grain yield via improved grain number might be unwise if they are counteracted by reductions in grain weight. The relationship between grains m^{-2} and individual grain weight amongst the lines will now be further discussed.

6.4.3 Relationship between grains m^{-2} and grain weight and determination of grain yield

The negative relationship between grains m^{-2} and grain weight is widely reported amongst cultivars (Acreche and Slafer, 2006; Sherman *et al.*, 2005; Reynolds *et al.*, 2005; Slafer *et al.*, 1996). There is a compensation process between grain weight and grains m^{-2} and the success of breeders in increasing grains m^{-2} has often been partially counteracted by a reduction in mean grain weight due to an increase in grain number at distal positions of the spikelets with lower potential grain weight (Slafer and Andrade, 1993). Present results showed that grain weight was negatively correlated with grains m^{-2} and there was broadly full compensation. The negative relationship between grains m^{-2} and grain weight could be due, in part, to the impact of semi-dwarfing genes which were segregating in this population on yield components. Presents results in the full sets of lines showed that plant height was not correlated with grains m^{-2} and Flintham *et al.* (1997) reported that shorter lines in the approximate range 70-100 cm had more grains m^{-2}. This could have been due to the negative association between plant height and flowering date as the late flowering lines were shorter and therefore experienced higher temperatures around meiosis which could possibly have reduced ear fertility. However, plant height was positively correlated with grain weight.

It is very important to understand the causes of the negative relationship between grains m^{-2} and grain weight for future breeding progress, i.e. to identify candidate traits to break negative linkage. In this respect, a negative relationship between the grains-to-ear DM ratio and grain weight was observed amongst the median lines as has been reported by Fischer and HilleRislambers (1978) and Fischer (1983). That is, ears with high dry mass per competent floret have larger grain weight, presumably through the effects on potential grain weight. Larger PGW was observed in both NL1 and NL2 compared to Bacanora (see Chapter 4). The hypothesis that 'rachis length is

positively correlated with grain weight', however, was not supported from the results of the DH experiments. This was probably because less assimilate supply per competent floret amongst the lines resulted in a decrease in potential grain weight.

Genetic progress in yield potential is strongly associated with increases in grain number per unit area, while weight per grain has generally declined (Slafer *et al.*, 1994; Waddington *et al.*, 1986) or remained static with breeding (Shearman *et al.*, 2005). Nonetheless, Calderini *et al.* (1995) showed that grain weight has contributed to improved yield potential in irrigated wheat in Argentina. In the present study, because of the trade off, DH lines with large-ear phenotype traits were not associated with increased yield. The ear of NL1 was less compact than NL2 and it may be more favourable for higher PGW and plant hormones cytokinins might help break the linkage. Present results showed that there was also an effect of temperature on grain weight due to the large variation in flowering time amongst the lines. It is during the pre-anthesis period that the structures of the ovary are formed which is potentially important in determining grain development (Rijven and Banbury, 1960). This was supported by the observation in barley (Scott *et al.*, 1983) that there is a positive relationship between carpel weight at anthesis and final grain weight, suggesting that the carpel growth period may be important in the determination of potential grain weight. Moreover, Calderini and Reynolds (2000) showed a relationship between carpel weight at anthesis and the rate of grain growth. It has been hypothesised that high pre-anthesis temperatures can detrimentally affect the determination of carpel size (Calderini *et al.*, 1999). Similarly, Wardlaw (1994) demonstrated that high temperatures, for instance 27/22 °C compared to 15/18 °C, from the four leaf stage to anthesis may negatively influence final grain weight. However, there was no effect of flowering on grains m^{-2} in the 20 median lines, but there was still a strong negative correlation between grains m^{-2} and grain weight.

NL2 has the *tin* gene and there was distorted segregation among the lines in the population as when averaged over two years, ears m^{-2} ranged from 225 to 520 amongst the 59 lines. Duggan *et al.* (2005a) reported that lines with the *tin* gene usually have fewer than 350 ears m^{-2} at maturity at a lower seed rate. Some lines in this DH population had even fewer than 350 ears m^{-2} consistent with the presence of

this gene. There was a negative relationship between ears m^{-2} and grains ear^{-1} amongst the lines showing the compensation effect of the yield components in maintaining yield. Preliminary results from another group working on the genetic basis of NL2 x Rialto population at Sutton Bonington have genotyped the lines for a molecular marker for the presence of the *tin* gene and 49 lines out of the 138 lines possess the gene. However, this genotyping data is preliminary. Nevertheless, a general linear regression analysis was carried out on the 59 lines of NL2 x Rialto in 2005 and 2006 to investigate the effect of *tin* gene on yield and yield components. The *tin* gene had the most profound effect on grains ear^{-1}, ears m^{-2} and rachis length (Appendix IV, Table A6).

There is much interest in avenues to increase the biomass at harvest as various studies have shown that the harvest index in wheat is approaching its theoretical maximum (Austin *et al.*, 1980; Nelson, 1988) while historically there has been relatively little genetic gain in above-ground biomass (Evans, 1998). There was a very strong correlation between AGDM at harvest and yield and there were genotypic differences among the lines suggesting genetic variation in biomass independent of phenology for breeders to exploit. For the median lines in 2006, although there was no association between flowering and AGDM, still there was a positive correlation between AGDM and yield.

Among these lines, the harvest index ranged from 0.20 to 0.50 when averaged over two years, suggesting that there is still scope for improving this trait in future crosses as the upper theoretical limit of HI has been estimated to be 0.62 (Austin *et al.*, 1980). Differences in HI were expected due to segregation for the *Rht* gene. There were no obvious double-semi-dwarfs, i.e. plants less than 40 cm in this population, so there may be one or more minor height promoting genes segregating in the population linked to one of the semi-dwarf genes. Preliminary molecular work on the NL2 x Rialto population has shown that NL2 had the *Rht-B1b* gene (Sean Mayes, personal communication) while Rialto is known to have the *Rht-D1b* gene. Spikelet number is not affected by *Rht* dwarfism; the increases in grain number per ear derive rather from increased fertility of distal florets within spikelets (Flintham and Gale, 1983; Miralles and Slafer, 1995). Interestingly in this population, there were some tall lines as well as semi-dwarf lines that were high yielding and these lines taking different

routes to obtain the same yield. There were four lines (5.8-6.5 t ha^{-1}) that were higher yielding than NL2 (5.4 t ha^{-1}) out of 69 DH lines of NL2 x Rialto population showing transgressive segregation among the lines. Compared to the highest yielding line, NL2 had lower biomass at harvest and fewer ears m^{-2}.

6.4.4 Genetic variation in source and sink strength

Rawson *et al.* (1983) reported that about half of the photosynthate during grain growth comes from current photosynthesis of the flag leaf whilst Sylvester-Bradley *et al.* (1990) showed that the contribution is about 40% in UK wheat. The rate and duration of grain growth are therefore limited by the rate and duration of photosynthesis by the flag leaf and also leaf 2 and leaf 3 during grain filling. By anthesis, stem reserves of WSC mostly as fructans have accumulated and the amounts are maximal shortly after flowering (Foulkes *et al.*, 2002). Results from the experiment examining 15 lines of the NL2 x Rialto population showed that, on average, about 90 % of the stem reserves accumulated at GS 61+14d are apparently mobilised in these lines, indicating the potential importance of this trait to realised yield. This trait could have been more important for the late flowering lines if they incurred accelerated senescence associated with higher temperature; as stem WSC has been reported to be important in maintaining yield under stressful post-anthesis conditions (Blum, 1988). Recently Ruuska *et al.* (2006) showed it to be a highly heritable trait. There was a weak positive association between WSC and ear biomass at anthesis (r=0.31) indicating very little evidence of pre-anthesis competition for assimilates in present study. Stem reserves was not associated with yield, indicating no major source limitation amongst the lines. In this respect, greater accumulation of stem WSC reserves may be beneficial, providing this is not competitive with ear growth during the rapid ear-growth phase (Blum 1998; Shearman *et al.*, 2005) and stem reserves have increased with breeding in the UK (Shearman *et al.*, 2005).

Yield was positively correlated to pre-anthesis RUE_{PAR} in the 15 lines subset of NL2 x Rialto population. However, AGDM at anthesis was not correlated with yield; therefore genotype rankings for post-anthesis RUE may have been different to pre-anthesis rankings. Leaf posture is potentially an important trait to maximise light interception in these lines, the range of k_{PAR} was from 0.46-0.73 showing variation in leaf angles in these lines but k_{PAR} was not linked with RUE differences in population.

It was not possible to say from data collected what were the underlying traits for RUE_{PAR}. Miralles and Slafer (1997) found that there was lower RUE in the presence of semi-dwarf alleles, probably due to reduced height resulting in poorer canopy architecture and radiation distribution which could support current findings as RUE_{PAR} was positively associated with plant height.

Nitrogen distribution in leaf layers has been suggested as a factor which can account for genetic differences in RUE as N should be optimally distributed so that leaves receiving the maximum light also have the largest specific leaf N content (Grindlay, 1997). An increase in RUE could also occur due to an increase in the concentration of photosynthetic tissues in the leaf and an indicator of this could be an increase of specific leaf weight (SLW: DM per cm^2 leaf area) as thicker leaves may contain more chlorophyll (Morgan *et al.*, 1990). In fact, a trend for a positive association was found ($r=0.34$) between RUE and SLW in the subset of 15 lines which could partially account for the genetic differences of RUE found amongst the lines. Differences in RUE could also have been due to a smaller flag leaf area which may reduce saturating light intensities at the top of the canopy and improve distribution of light to lower leaves. However, flag leaf size was not measured in this experiment. In addition, improvements in grain sink strength have also been shown to increase post-anthesis RUE by alleviation of feedback inhibition of photosynthesis in spring wheat in Mexico (Reynolds *et al.* 2005).

The degraining results showed most of the lines were sink limited and using the green canopy area at anthesis to grains m^{-2} ratio (cm^2 green area per grain) it was found that there was a trend for a lower value of this ratio in the lines with smaller grain size which were those showing more positive grain growth responses to degraining. There was a positive correlation between this ratio and grain weight in degrained ears ($r=0.41$). Ma *et al.* (1990) reported greatest change in grain size with degraining where grain size of intact plants was smallest which agrees with present results. In summary, it seems likely that lines of the NL2 x Rialto population were broadly in close source-sink balance and that breeders may need to select increasingly for traits which are associated with more or less simultaneous increases

in source and sink size to maintain current rates of yield progress in high-output environments in future years.

6.4.5 Application of spectral reflectance in breeding

The use of spectral reflectance is another promising technology to estimate a range of physiological measurements such as plant biomass, total leaf area, water status, and assessments of photosynthesis (Araus, 1996). Furthermore, Babar *et al.* (2006) demonstrated the potential of using spectral reflectance indices as a breeding tool to select for increased genetic gains in biomass, chlorophyll content, cooler canopies and yield. Their findings on the utility of near infra red radiation-based indices showed a strong association with biomass and canopy temperature at heading and grain filling, while chlorophyll content was more closely related to biomass during the vegetative phase under warm irrigated conditions in Mexico.

Likewise, Reynolds *et al.* (1994) reported that canopy temperature depression (CTD) measured with infra-red thermometer in yield trials under warm irrigated conditions in spring wheat lines in Mexico was significantly associated with yield variation *in situ*, as well as with the same lines grown at a number of international breeding sites. Both g_s and CTD have been found to be closely and positively correlated with yield in spring wheats in Mexico (Fischer *et al.*, 1988) and because they are associated through feedback mechanisms, measuring for CTD can give a rapid indirect determination of g_s. In this study also, both canopy temperature and normalized difference vegetative index (NDVI), which gives a measure of greenness, was used. Canopy temperature and NDVI were negatively associated with yield suggesting that these physiological tools can be used in a warm climate to select for superior genotypes which can help in the selection process, cutting down on expensive and time consuming measurements.

6.5 SUMMARY

An attempt has been made to explain the physiological basis of grains m^{-2} in the progeny of NL2 x Rialto DH population despite the big effect of flowering time on the traits measured. The expected negative relationships between yield components were found. The hypothesis that 'rachis length is positively correlated with spikelets ear^{-1} and grains ear^{-1}' was supported. The hypothesis that 'spikelets ear^{-1} is positively correlated with grains m^{-2}' was confirmed. 'Ear index is positively associated with rachis length' hypothesis was supported. Ear biomass at anthesis was negatively correlated with grains ear^{-1}, this did not support the hypothesis that 'there is a positive correlation between ear biomass at anthesis and grains ear^{-1}'. Grains-to-ear DM ratio were positively correlated to grains m^{-2}. Grain yield in this population was more correlated to grain weight than grain m^{-2}. The hypothesis that 'rachis length is positively correlated with grain weight' was not proved. The hypothesis that 'there are genotypic differences amongst the lines in RUE_{PAR} and in AGDM at harvest which were both positively correlated with yield' was confirmed. There has been transgressive segregation among the lines and also distorted segregation for the *tin* gene and the latter had a significant effect on ears m^{-2}, grains ear^{-1} and rachis length.

CHAPTER 7 GENERAL DISCUSSION

This chapter starts with an introduction on the importance of crop physiology in plant breeding followed by the importance of grains m^{-2} and source-sink balance of the lines as determinants of yield. The effects of temperature in the DH experiemnts are briefly discussed. The major findings of the study are compared with reported literature. Avenues to increase grains m^{-2} and grain weight are discussed followed by overall conclusions and recommendations for future work.

7.1 INTRODUCTION

One of the principal aims of crop physiology is to understand the yield-forming processes underlying yield potential in crops and incorporate or impart this knowledge to breeders so that there is efficient selection of lines and choice of parental crosses. Many attempts to identify traits to raise yield potential have been made since Donald's ideotype (1968) for wheat. In the current study the parental genotypes were selected according to target traits (ear morphology, stem WSC and RUE) which plant breeders could potentially use to raise the genetic yield potential. However, physiological characteristics have often proved to vary in their effects on yield with both environment and genetic background. The genes controlling the desired traits may often be pleiotropic in their expression and trade-offs between their various traits may result in counter-intuitive outcomes (Evans, 1994). Knowledge of the changes in physiological traits associated with genetic gains in yield potential can help in the understanding of yield-limiting factors and therefore in planning future breeding strategies. Furthermore, yield limitations due to the source or sink capacity vary during the crop cycle and hence understanding these source-sink limitations is important not only for future breeding strategies but also for the optimisation of agronomic practices (Gambin and Borras, 2007).

The present study investigated the yield determinants of grain number per unit area and yield potential in the three CIMMYT parental genotypes and principally one DH population (NL2 x Rialto) of the three DH populations that were developed. The parental genotypes were chosen for their high expression of either source (Rialto, high pre-anthesis RUE, stem water soluble carbohydrates) or sink (NL1 and NL2, rachis length, spikelet number, potential grain weight) type traits. This study was

undertaken to investigate the central hypothesis that physiological avenues could be identified through improved ear fertility in large ear-phenotypes for future genetic gain in grains m^{-2} and yield potential. Yield potential of wheat has been primarily increased worldwide to date through breeding by direct selection for higher grain yield (Loss and Siddique, 1994) though during the green revolution a quantum step increase in grain yield was achieved by introgression of the semi-dwarf genes (Gale and Youssefian, 1985). More recently, the increased use of alien chromatin such as the 1BL/1RS chromosome translocation from rye and the 7DL.7Ag translocation from *Agropyron elongatum* have made major genetic contributions to raise yield potential in wheat (Villareal *et al.*, 1998; Reynolds *et al.*, 2001). The physiological basis of these increases will be referred to later.

7.2 THE IMPORTANCE OF GRAINS M^{-2} TO YIELD POTENTIAL

It is generally accepted among physiologists and breeders that grain number per unit area is the most important grain yield determinant (Fischer, 1985; Slafer *et al.*, 1996). However, Sinclair and Jamieson (2006) stated that grain number may be a carefully controlled consequence of more fundamental limitations to yield, rather than a determinant of yield itself. These authors postulated that yield is seldom limited by grain number *per se*, but that genetic or environmental crop conditions that cause reductions in grain number are accompanied by reductions in the ability of the crop to fill grains. Carbon and nitrogen availability in the plant are the crucial factors in determining both grain number and yield. Nitrogen accumulation is considered the critical determinate of yield by Sinclair and Jamieson (2006) because the size and longevity of the photosynthetic canopy are associated closely with nitrogen accumulation, and nitrogen availability in the developing ear is apparently correlated highly with grain number and yield. Fischer (2006) stated that grain number linked to assimilate supply in the pre-anthesis rapid ear growth phase is still probably the major determinant for grain yield. However, he did not rule out the possibility that both carbon and nitrogen are important resources that contribute to grain number determination. Therefore, there remains a general consensus that grain yield under optimal conditions is still limited by post-anthesis sink size and understanding grain number determination is useful for predicting physiological routes to higher yield. In

summary, assimilate supply is therefore the likely critical determinant of grain number not nitrogen supply and grains m^{-2} remains the main limitation to grain yield.

7.3 EFFECTS OF FLOWERING TIME ON GRAIN NUMBER AND YIELD POTENTIAL

In this study, due to segregation for vernalization and photoperiod sensitivity, the lines differed by up to 50 days in flowering time. The field experiments were conducted in Cd. Obregon (Mexico) where temperature typically rises above 30 °C in April and if the lines have reached anthesis shortly prior to the beginning of April, there will likely be a yield penalty compared to the earlier flowering lines as the grain filling period would take place in a much hotter environment. Usually, under hot grain filling conditions, the grain filling rate increases but this is more than compensated by a shorter duration, resulting in lighter grains and hence a decrease in grain yield. Such an effect with flowering date was observed in the NL2 x Rialto DH lines.

Egli (1998) reported that temperature was probably the most conspicuous environmental factor affecting grain weight and quality. Previous studies have shown that higher temperature imposed at grain filling reduced grain weight (Sofield *et al.*, 1977) and even brief periods (3-6 days) of high temperatures interrupting a rather cool grain filling period resulted in lighter grains (Stone and Nicolas, 1994; Savin and Nicolas, 1996). Pre-anthesis environmental conditions affect the potential number of grains but the conditions at anthesis and the next few days then determine the number of grains set and high temperature, low light intensity and water stress all contribute negatively to grain yield through lower grain set (Wardlaw, 1970; Fischer, 1973). In Obregon, air mean temperature rose from 18 to 28 °C from February to April.

The grain filling period of the NL2 x Rialto DH population was under the influence of increasing temperatures which probably accounts for the negative correlation between flowering date and yield in the DH lines. In the period immediately pre-anthesis, there could have been an effect on the carpel weight which has been reported to affect final grain weight (Wardlaw, 1994; Calderini *et al.*, 2001). High

temperature limits the yield of wheat by reducing grain weight (McDonald *et al.*, 1983), which is attributable to a reduction in starch synthase activity (Hawker and Jenner, 1993). Temperature hastens development of the grain but the rate of grain-filling does not increase enough to compensate for reduced duration of grain-filling (Sofield *et al.*, 1977; Caley *et al.* 1990). Wiegand and Cuellar (1981) investigating the effect of temperature at grain filling showed that there was a 3.1 day shortening of grain filling per ^{o}C increase in mean daily air temperature. Because temperature plays such a significant role in grain yield, breeders should look for genetic variability in both plant senescence and grain filling rates and durations to help stabilize grain size. There is also a need to select for those genotypes which are more heat tolerant which can minimise the effects of heat on the concentration of several of the metabolites involved in the pathway of conversion of sucrose to starch. Temperature did not affect the genotype differences in parental experiments meaningfully and by using median lines of the DH populations the effect of temperature on the DH lines was minimised.

7.4 SOURCE/SINK BALANCE IN PARENTAL GENOTYPES AND DH LINES

The timing of the degraining treatment is also crucial in determining whether grain yield is source or sink-limited during grain filling. Degraining carried out just after anthesis could be misleading as at that time cell division is still taking place and assimilate availability would be diverted to these cells. This is why degraining in present study was performed 14 days after anthesis under the assumption that cell division has ceased and that the pattern of senescence has not changed with the imposed treatment.

It has been generally reported worldwide that wheats are mostly sink-limited during grain filling (Borras *et al.*, 2004; Reynolds *et al.*, 2005; Calderini *et al.*, 2006; Bingham *et al.*, 2007; Shearman *et al.*, 2005; Brancourt-Hulmel *et al.*, 2003). Grain filling can be limited either through the supply of photosynthate, i.e. source limitation, or the capacity of the grain to accumulate available carbohydrate, i.e. sink limitation (Bingham *et al.*, 2007) whichever is lower. An improved understanding of source-sink balance in modern wheat germplasm can help to inform plant breeding

strategies. In the present study, source-sink balance was investigated by manipulating the source and sink ratio through degraining. Grain growth responses to removal of the spikelets from one side of the ear indicated that in the three parental genotypes there was marginal source limitation whereas in the subset of 15 DH lines of the NL2 x Rialto population, 10 lines were showing marginal source limitation while the remaining five were sink-limited. However, one has to be cautious in concluding definitely whether yield was sink or source limited as the extent of responses was inconsistent among the years for the parental genotypes and in the NL2 x Rialto DH population the results were based only on one year.

All three parental genotypes showed positive responses of grain weight to degraining in the range 5.5-6.8 mg. The results of the green canopy area to grain ratio showed that novel genotypes had a higher ratio compared to Bacanora and in the subset of 15 DH lines the ratio was lower for those lines which showed larger responses to degraining. These lines also had smaller grain size in intact control ears. This was similar to the findings of Ma *et al.* (1990) who reported a greater response to degraining in genotypes with smaller grain size. There was a larger increase in grain weight in response to degraining at apical spikelets compared to central and basal spikelets for the parental genotypes. This could potentially be related to poorer vascular connections to the apical spikelets. It appears that these grains may be more source-limited than at other positions in the ear. Overall, although assimilate supply per grain was potentially increased by 100%, average grain weight was only increased by 15%. Therefore, grains dry weight was only marginally responsive to changes in assimilate supply during grain filling.

These findings are in general agreement with those of a recent extensive review of source: sink manipulation experiments in wheat, maize and soyabean which concluded that mean grain dry weight in wheat changed by *ca.* 0.12 relative to the change in potential availability of assimilates per grain produced during seed filling and that wheat yield is mainly limited by post-anthesis sink size under optimal conditions (Borras *et al.*, 2004). Present findings would suggest that yields of the lines were primarily limited by grain sink size in the post-anthesis period. Results also indicated that source and sink was in quite close balance, so there is a need to improve the source and sink simultaneously in future breeding strategies. The

hypothesis 'grain filling is source limited when tested in degraining experiments' was only partially supported. If future progress is to be made in raising grain yield potential there should be a concomitant increase in both the source and sink size. Increasing both grains m^{-2} and grain weight through increased post-anthesis assimilate supply in sink limited wheats can efficiently increase yield.

7.5 PHYSIOLOGICAL BASIS OF GENETIC VARIATION IN GRAINS M^{-2}

7.5.1 Radiation-use efficiency and radiation capture

The amount of radiation intercepted by the crop is dependent on the amount of incident radiation and the crop's fractional interception according to its canopy size and architecture (Slafer *et al.*, 1999). Light interception was correlated with flowering date among the 15 DH lines in 2006. However, a major finding was that pre-anthesis RUE_{PAR} was positively associated with biomass at anthesis and was positively correlated to grain m^{-2}. The hypothesis 'RUE is positively correlated with above-ground biomass at anthesis amongst the DH lines' was supported by results in the present study. The extinction coefficient k_{PAR} was not correlated with RUE, so leaf angle may not be the explanation of the genotypic differences observed amongst the lines.

Large-ear phenotype (rachis length) was negatively correlated with RUE. Thus, there may be sub-traits underpinning the differences in RUE amongst the lines of this population which might have come from genetic traits inherited from Rialto. Abbate *et al.* (1998) found the value of pre-anthesis RUE_{PAR} to be 2.7 ± 0.28 g MJ^{-1} in Argentinean cultivars whilst Shearman *et al.* (2005) reported a range of RUE_{PAR} of 2.33 to 2.64 g MJ^{-1} in eight UK winter wheats. In the subset of 15 DH lines the highest value was 1.59 g MJ^{-1} lower than those reported. Shearman *et al.* (2005) reported that in UK wheats released from 1972 to 1995 the increase in the RUE was associated with an increase in flag leaf specific weight and present results also showed a trend for a positive correlation of SLW (culm leaves) with RUE (r=0.34). This indicates that the lines with higher RUE had thicker leaves and likely more photosynthetic apparatus per unit leaf area averaged across green culm leaves, which might have reduced light saturation of upper leaves.

RUE can also theoretically be increased by optimising the distribution of incident radiation within the canopy and increasing the proportion of incident radiation intercepted by the lower leaves and this can be potentially achieved by reducing the flag leaf area (Richards, 1996). It is possible that smaller flag leaves were associated with higher RUE in present study as was found by Foulkes *et al.* (2006) for four UK wheat cultivars. However, flag leaf size was not measured in the present study. Furthermore, optimising N distribution within canopy leaf layers could theoretically increase RUE due to higher canopy photosynthesis (Lemaire and Gastal, 1997) and preferential N allocation to illuminated leaves (Dreccer *et al.*, 1998). However, Critchley (2001) reported that N distribution in the top leaves of modern UK wheat varieties is already close to values for optimum photosynthesis as predicted by the light/photosynthesis model of Hirose and Wenger (1987).

Radiation-use efficiency can potentially be improved by manipulating traits at the biochemical, cellular, leaf or canopy levels of organization as well as optimising source and sink (Reynolds *et al.*, 2000). Selection for greater SLW would be of interest to breeders as this trait can be relatively easily phenotyped and could offer scope for increasing RUE both pre- and post-anthesis in future years. In the longer term, radiation-use efficiency could potentially be improved by doubling the specificity of the key photosynthetic enzyme Rubisco for CO_2 which could theoretically increase net photosynthetic rate at saturating light intensities (A_{MAX}) by 20 % (Austin, 1999). However, attempts to select for low rates of photorespiration in wheat have so far met with little success (Evans 1983). Nevertheless, Uemura *et al.* (1997) found that the thermophilic red algae *Galdieria partita* and *Cyanidium caldarium* possessed a Rubisco specificity 2.4 to 2.5 times greater than of higher plants, offering the possibility of genetically transformed wheat Rubisco with enhanced carboxylase activity.

Increasing pre-anthesis RUE should enhance yield potential as the period of GS 31-61 is largely recognised as being crucial in determining grains m^{-2} (Fischer, 1985). Present results and reports on grain growth of modern wheat cultivars worldwide indicate yield to be sink limited during the post-anthesis period (Shearman *et al.*, 2005; Reynolds *et al.*, 2005; Cartelle *et al.*, 2006) and some studies have shown that post-anthesis RUE was positively associated with number of grains, hence implying

a regulation of post-anthesis RUE by sink size (Miralles and Slafer, 1997; Reynolds *et al.*, 2005). Therefore, if potential grain number can be increased, RUE during grain filling may be increased in response to alleviation of feedback inhibition thereby increasing biomass and yield. Ear biomass was not correlated with grain number, therefore ear index and grains-to-ear DM ratio will be looked at in the following section to explain the basis of genetic differences in grain number in the present study.

7.5.2 Ear index, ear biomass and grains-to-ear DM ratio

There was no association between ear index and grains m^{-2} in the median 20 lines in 2005 and 2006. Neither was there any association between ear index and grains ear^{-1} in the median lines. Ear biomass at anthesis was not consistently correlated with grains m^{-2} in the 20 median lines which is in contrast to previous work whereby high ear biomass was positively associated with grains m^{-2} (Gonzalez *et al.*, 2003; Slafer and Savin, 1994; Fischer, 2006; Bindraban *et al.*, 1998). These lack of associations were mainly due to the confounding effect of large genetic variation for the grains-to-ear DM ratio which was positively associated with grains m^{-2}. Some of the DH lines had the *tin* gene and therefore there was more investment of assimilates towards non-reproductive ear structures (palea, lemma, glumes and rachis) thereby accounting for higher ear biomass at anthesis but not translating into more grains. This ratio itself might be also affected by plant hormones such as cytokinins which have recently been reported to have a direct influence in grain setting in rice increasing grain number by up to 21% (Ashikari *et al.*, 2005).

The grains-to-ear DM ratio in the NL2 parent was markedly lower (71 grains g^{-1}) than the other parental genotypes (101-116 grains g^{-1}) suggesting that other genes (perhaps from *T. polonicum* believed to be in the pedigree of NL2 and known to affect glume size) were also contributing to the low value. According to Fischer (2001) grain number determination is strongly related to ear dry weight at anthesis usually governed by events in the last 20-30 days before anthesis, although some modern cultivars were reported to show a high grains-to-ear DM ratio contributing to genetic gains in yield potential which was consistent with present findings (Abbate *et al.*, 1998). Highest ratio obtained in the DH lines was 93 grains g^{-1}, below the value of benchmark Bacanora in the parental genotype experiments (106 grains g^{-1}).

However, the large-ear phenotype lines may still have potential use in breeding programmes as the addition of other traits could be used to counteract the low value of the ratio.

The relative duration of the GS 31-61 phase as a proportion of thermal time to GS 61 is regarded as critical to determining grains m^{-2} and genetic manipulation to increase the duration of the stem-elongation phase, hence radiation receipt has been shown to enhance yield potential (Slafer *et al.*, 1996; Reynolds *et al.*, 2000). An extended duration for the rapid ear-growth phase pre-anthesis by manipulating the photoperiod sensitivity (*Ppd*) and vernalization (*Vrn*) genes has been reported to increase grains m^{-2} (Reynolds *et al.*, 2000; Gonzalez *et al.*, 2003; Miralles and Richards, 1999) due to more radiation interception during this phase, increasing assimilate supply and floret survival. This was further supported by the work of Reynolds *et al.* (2005) whereby opening the rows for more radiation capture at booting led to a significant increase in grains m^{-2}. Present findings from growth-room experiments have shown that an extended period from floral initiation to terminal spikelet phase resulted in more spikelets ear^{-1} and hence higher grain number. Presents results also indicated there was probably an effect of earliness *per se* in the novel genotypes leading to an additional 1-2 spikelets per ear. This was associated with a longer duration of the floral initiation to terminal spikelet phase as discussed in Chapter 5. Thus, identifying and manipulating these earliness *per se* genes to optimize specific phases, remains one prospective avenue for yield progress in wheat breeding.

7.5.3 The effects of large-ear phenotype in determining sink capacity

The main physiological avenue examined in this study to improve ear fertility was through genetic modification of ear morphology. Thus, the CIMMYT parent NL2 was selected due to its longer rachis and potentially higher spikelets ear^{-1}, grains ear^{-1} and ultimately grains m^{-2}. However, amongst the three CIMMYT parents, despite having more grains ear^{-1} than Bacanora, NL2 did not have higher grains m^{-2}. The *tin* gene was probably responsible for this because of a trade-off with ears m^{-2}. In the novel genotypes the extra grains were contributed by the apical and central spikelets. In contrast to NL1, NL2 possessed the typical gigas features described by Atsmon *et al.* (1986). Thus, possessing the *tin* gene, the NL2 genotype had large ear index and higher ear biomass but these effects were counteracted by a lower grains-to-ear DM

ratio linked to high chaff weight. Motzo *et al.* (2004) examining the progeny of bread wheat genotypes Kite and Janz containing the *tin* gene crossed with the durum wheat (*Triticum turgidum* subsp. *durum*) cultivars Simeto and Valbelice found the *tin* gene increased chaff weight more than proportional to the increase in ear fertility (grains/ear). Both novel genotypes produced fewer ears m^{-2} at harvest than Bacanora, although the reduction in ears m^{-2} for NL2 containing the *tin* gene (26%) was larger than for NL1 (8%). In the NL1 x Rialto DH population, only 44 lines were examined in 2006. Rachis length was positively correlated with spikelets ear-1 (r=0.21) but not associated with grains ear^{-1} and grains m^{-2}. In 2005, 33 lines were examined but the data set was not reliable due to poor plant establishment and variation in the experimental error associated with measurements.

There was genetic variation in rachis length and it was positively correlated to spikelets ear^{-1}. Ear index was positively associated with rachis length as hypothesized. In addition, rachis length was negatively associated with grain weight. Preliminary analysis of the DH lines according to *tin* and *non-tin* groups has shown to be significantly associated with grains ear^{-1}, ears m^{-2} and rachis length. However, there was still significant variation for rachis length in each group and positive correlation with spikelets ear^{-1}. Overall, rachis length was positively associated with grains m^{-2} in the median lines which was encouraging. However, grains m^{-2} in the DH lines increased above values for NL2 but it was not above that of Bacanora.

7.5.4 Post-anthesis source supply

About 70-90% of grain dry weight in wheat under optimal conditions comes from current assimilates during the grain filling period (Austin *et al.*, 1977). Higher yield potential could be achieved by increasing the post-anthesis source size concomitant with sink size if source and sink supply were in quite close balance. Some lines of the NL2 x Rialto population which flowered late were exposed to higher temperatures resulting in lower yield with reduced grain weight. An important trait to select for genotypes under post-anthesis stress is high stem WSC. Although stem WSC was only measured in 15 lines of the NL2 x Rialto population, values showed a large range from 0.56-1.56 t ha^{-1}. High expression of stem WSC was a trait probably inherited from Rialto, and around 90% of WSC were apparently mobilised. Stem WSC was positively correlated with grain weight. Stem reserves was not correlated

with yield showing evidence for sink limitation in the population. Furthermore, stem reserves was not competitive with ear dry matter. In fact, stem reserves from pre-anthesis plant assimilation are being increasingly recognised as an important source of carbon for grain filling when current photosynthesis is inhibited by stress and environmental conditions that decrease current assimilation (Blum, 1998). The results of Shearman *et al.* (2005) showed the potential importance of stem WSC for grain yield potential even under favourable post-anthesis conditions. A high heritability of stem WSC has been found by Ruuska *et al.* (2006) showing that this is a trait which can be improved through breeding but may be more important for yield potential in UK than in Mexico.

7.6 RELATIONSHIP BETWEEN GRAINS M^{-2} AND GRAIN WEIGHT

From investigations reported worldwide, progress in grain yield under optimal conditions is mostly related to grains m^{-2} being modified through breeding while individual grain weight, the other major yield component, has undergone little change and in some cases even a decline (Waddington *et al.*, 1986; Slafer *et al.*, 1994; Slafer and Andrade, 1993; Shearman *et al.*, 2005; Acreche and Slafer, 2006). A negative relationship between grains m^{-2} and grain weight was also found in present study in the 20 median lines in 2005 and 2006. Understanding the physiological basis of this negative relationship is important for future breeding progress. However, it is not that straightforward to identify possible avenues to break the negative linkage due to the many factors potentially affecting these two components. The present results will now be discussed in relation to these considerations.

7.6.1 Physiological avenues to increase grains m^{-2}

Several authors have reported that grains m^{-2} was positively associated with ear index at anthesis, i.e. a measure of investment assimilates in sink production (Fischer and Stockman, 1986; Siddique *et al.*, 1989a; Slafer *et al.*, 1990; Slafer and Savin, 1994; Gonzalez *et al.*, 2003). However, present results contrasted with these findings and rather it was the ratio of grains-to-ear DM ratio that was correlated with grains m^{-2}. Similar findings for a positive correlation in spring wheat cultivars in Argentina were reported by Abbate *et al.* (1998). Reynolds *et al.* (2001) reported an increase in

the grains-to-ear DM ratio from 108 to 118 grains g^{-1} in genotypes containing the 7Ag.7DL translocation from *Agropyron elongatum* compared to check lines. In the parental genotypes, NL2 had the lowest grains-to-ear DM ratio in the range of 71-111 grains g^{-1} and in the DH lines the range was 24-93 grains g^{-1}. Several lines of the NL2 x Rialto population had a lower grains-to-ear DM ratio than NL2 showing transgressive segregation in this population. Although longer rachis increased ear index it was negatively correlated with the ratio. The genotypic differences obtained amongst these lines could be a pleiotropic tiller inhibition effect of the *tin* gene on chromosome 1AS. In the NL2 x Rialto DH population the ratio showed positive correlation with grains m^{-2} in both 2005 and 2006. Despite showing a lower value to other reported values, there may be scope for genetic improvement for specific lines in the population by incorporating other traits to counteract the low ratio.

The assessment of spikelet primordia production in the growth-room experiments showed that in the novel genotypes more spikelets ear^{-1} was due to a longer thermal duration of the floral initiation to the terminal spikelet phase. The hypothesis that large-ear phenotype in NL1 and NL2 was partly associated with developmental effects was thus confirmed. Lengthening this period, i.e. floral initiation to terminal spikelet can increase the number of spikelets ear^{-1} and ultimately increase grains m^{-2} via more grains ear^{-1}. There was only 1-2 more spikelets ear^{-1} in novel parental genotypes in field experiments but 3-4 more spikelets ear^{-1} in single plants in the growth-room experiments. This could be due to less competition for resources between shoots for the single plants compared plants at high plant population density. Also, the plants were exposed to different temperature and photoperiod conditions in the field and growth-rooms. In the growth rooms assessments were carried out on the main shoots compared to the average of all shoots in the field. Moreover, the effects of earliness *per se* effects were associated with more spikelets per ear and a longer rachis in NL2 and NL1 in the growth-room experiments which supported the hypothesis 'More spikelets per ear in novel large-ear genotypes is associated with effects of 'earliness *per se*' on the phasing of development'.

Tillering is an important characteristic in wheat determining final ears m^{-2}. There is considerable genetic variation for tillering capacity and Atsmon and Jacobs (1977) reported the low-tillering of genotypes possessing 'gigas' features which was

consistent with present results for NL2. Wheat with a genetic disposition to produce fewer tillers is possible through the introgression of the *tin* gene (Richards, 1988). The microsatellite marker (*Xgwm 136*) was used by Spielmeyer and Richards (2004) to map the *tin* gene to the distal region of chromosome 1AS. The *tin* gene was closely linked to the marker when subjected to single marker regression analysis ($P < 0.001$). Ongoing parallel work at Nottingham at the molecular level has revealed through preliminary mapping of the NL2 x Rialto DH population, an association between tillers plant^{-1} grown as ear-rows at Sutton Bonington and *Xgwn 136*. Therefore, inheritance of the latter from NL2 seems to be conferring a reduced tillering capacity amongst lines of the NL2 x Rialto population (Sean Mayes, personal communication).

NL2 has consistently shown low shoot production both in the field experiments in Obregon and the growth-room experiments at Sutton Bonington. Thus, while selecting for large-ear phenotype, breeders at CIMMYT might have inadvertently selected for fewer tillers as well. Low-tillering lines in populations which segregated for the *tin* gene produced a greater harvest index and a larger grain size (Richards, 1988; Duggan *et al.*, 2005a). Lines possessing *tin* gene produced fewer ears at maturity but there is compensation via more grains ear^{-1} and increased grain weight (Duggan *et al.*, 2005b) which were consistent with present results. The expression of grains ear^{-1} in the present study in the *tin* genotype may have been reduced compared to that in the investigation of Duggan *et al.* (2005a), since in that investigation plant establishment was 84-89 plants m^{-2}, compared to 160 plants m^{-2} in the current experiment. Plants possessing the *tin* gene do not have the ability to compensate for poor establishment (Duggan *et al.*, 2005a) and therefore, to optimise yield, perhaps sowing at higher seed rate would be necessary to exploit this phenotype.

Grains m^{-2} is determined by the numbers of tillers, i.e. ears m^{-2} and grains ear^{-1} and many factors affect these two components, e.g. genotypes, cultural practices and growing conditions. In the present study, the reason for some lines to have fewer tillers was ascribed to the *tin* gene, as mentioned above, but there was distorted segregation of the gene as not all the DH lines had few tillers. Slafer and Andrade (1993) found that very little changes with breeding took place in ears m^{-2} while most of the yield improvement from grains m^{-2} came from grains ear^{-1} when old and

modern cultivars were compared. Newer varieties in Australia (Siddique *et al.*, 1989a) and some varieties in the UK (Austin *et al.*, 1980) have shown that there was a reduction in tiller number. Over production of tillers may reduce the ear size of remaining shoots which is a waste of resources. On the other hand, Shearman *et al.* (2005) reported increases in ears m^{-2} associated with genetic gains in grains m^{-2} in UK cultivars released form 1972 to 1995.

In the selection of wheat varieties for high yield conditions, considerable emphasis is placed on large ears with many grains. However, 'gigas' wheat, though following Donald's ideotype in many characteristics, departs from the small erect leaves by having large thick leaf blades. These leaf features to some extent were observed in the NL2 parent and some of the lines in NL2 x Rialto DH population. The advantage that gigas ears have at anthesis was somewhat diminished during grain growth. The ability to set grains in six or more florets in each spikelet in the gigas lines could be due to more distal florets within the spikelet reaching anthesis closer in time to that in the basal florets than occurs in normal cultivars (Atsmon *et al.*, 1986). This could account for more grains found in the *tin* lines. However, present results showed more grains ear^{-1} found in the novel genotypes were due to more grains located in apical as well as central spikelets. Marshall and Boyd (1985) found gigas lines to have a similar phyllochron to those of Australian cultivars, but the leaves were larger. Similar findings were reported by Atsmon *et al.* (1986) consistent with results of the controlled-environment study.

Atsmon, *et al.* (1986) found that although individual grains do not grow faster in the gigas lines, grain weight was greater but the major advantage of gigas ears was in the greater number of grains per spikelet. However, present findings do not support this as the number of grains per spikelet was the same in the parental genotypes. It was more spikelets per ear that accounted for more grains ear^{-1} in these genotypes. NL1 and NL2 had more spikelets ear^{-1} and more grains $spikelet^{-1}$ hence more grains ear^{-1} and DH lines with *tin* gene had more grains ear^{-1} as shown by the results of the *tin* gene analysis (Appendix IV, Table A6). The hypothesis that 'novel genotypes have more grains m^{-2} than Bacanora' was not supported. However, the hypothesis that 'NL1 and NL2 have more spikelets ear^{-1} and more grains $spikelet^{-1}$ hence more grains ear^{-1} than Bacanora' was confirmed. The *tin* gene did not contribute to

increased grains m^{-2} in the present study. Earliness *per se* effects associated with large-ear phenotype may be more useful than *tin* effects as a trait for breeders to exploit to improve grain number.

7.6.2 Physiological avenues to increase potential grain weight

Results showed that the potential and final grain weight of the novel parental genotypes were higher than Bacanora. Higher PGW in NL2 was associated with a much lower value of grains to ear DM ratio compared to Bacanora. However, higher PGW in NL1 was achieved without a similar reduction in the grains to ear DM ratio. This is encouraging as it suggests it may be possible to uncouple these two parameters genetically as a route to increasing yield potential. The grains- to-ear DM ratio was negatively correlated with grain weight in the median lines. However, there was no association between rachis length (cm) per spikelet and grain weight. A negative correlation was found between grain weight and post-anthesis canopy temperature at grain filling showing a link between post-anthesis leaf activity traits in part explaining the negative relationship found.

It is known that endosperm cell number per grain is closely related to final grain weight (Brocklehurst, 1977) but little is known about the genetic variation in cell number in wheat. Usually, endosperm cell number has been reported to be regulated by assimilate supply (Singh and Jenner, 1984) until 14 days after anthesis when cells stop dividing (Nicolas *et al.*, 1985). Therefore, in order to increase the cell number, it is necessary to increase assimilate availability during cell division. Selecting genotypes for a longer cell division period could also increase assimilate supply and thus increase potential grain size. However, there may be other restrictions on the maximum grain size, such as physical restrictions by glumes (Millet, 1986) or an inadequate vascular system within the ear (Rawson and Ruwali, 1972). Furthermore, Miralles and Slafer (1995) showed that semi-dwarf varieties with *Rht-B1b* and *Rht-D1b* are pre-disposed to have smaller grains compared to tall controls. These varieties are insensitive to gibberellic acid which results in smaller cell dimensions (Yousseffian *et al.*, 1992). Insufficient vascular connections within the ear (Natrova and Natra, 1993) might restrict potential grain size despite sufficient assimilate

availability during cell division and expansion phase. The potential grain size can also be affected by carpel weight (Scott *et al.*, 1983; Calderini *et al.*, 1999).

Whilst potential grain size can be increased by the above-mentioned mechanisms, the rate and duration of grain growth also have an impact on final grain weight. Egli (1998) reported that grain weight can be increased by increasing the rate and/or duration of grain filling and Gooding *et al.* (1994) found an increased rate of grain filling with fungicides application which resulted in increased grain size. The rate of grain growth could be increased genetically by reducing any physical restriction of potential grain size, improving the vascular connections within the spikelets and the ear and perhaps increasing the rate of sucrose accumulation in the grain through an enhanced activity of the starch synthase enzyme (Jiang *et al.*, 2003). The negative relationship between grains m^{-2} and grain weight among the lines showed extra grains were set in the distal positions with reduced weight potential thus overall reducing the grain weight (Slafer *et al.*, 1996; Areche and Slafer, 2006) and in NL1 and NL2 some additional grains were set at apical spikelets which had potentially lower grain weight.

7.6.3 Harvest index

The introduction of the semi-dwarf genes increased the partitioning of assimilates from the stem to the ear in the pre-anthesis period and increased yield potential through improvement of floret survival (Brooking and Kirby, 1981; Fischer, 1983). Historically, genetic gains in yield have been associated with increases in harvest index (Hay, 1995). However, some studies have suggested that wheat HI in many countries may be asymptotically approaching a theoretical maximum (Calderini and Slafer, 1998), estimated by Austin *et al.* (1980) to be 0.62. For example, already in the UK, harvest index of 0.61 has been reported by Spink *et al.* (2000) for the modern cultivar Consort (released in 1996) and further increases in HI may be increasingly harder to achieve. Plants taller than *ca.* 80 cm have an increased risk of lodging (Flintham *et al.*, 1997) whilst those with shorter height have a high risk of foliar disease (Austin, 1999). Further reduction in plant height may not yield any increase in HI as optimum height has probably already been achieved by most modern cultivars (Fischer and Quail, 1990); indeed it would probably be disadvantageous as reduced biomass production is obtained due to poor light

distribution among the canopy with lines possessing both the *Rht-B1b* and *Rht-D1b* alleles (Miralles and Slafer, 1995).

In the present study, the range of HI for the three parental genotypes was 0.44-0.47 whilst in the progeny of the NL2 x Rialto population was 0.29 to 0.55 in the median lines. HI for elite lines in most breeding programmes worldwide is around 0.45-0.55 (Slafer and Andrade, 1993) so there are still prospects for achieving small increases in HI but at the same time there is a need for greater physiological understanding of how reduced investment in stems can be most effectively converted into greater grain growth. Rachis length was not correlated with HI but spikelets ear^{-1} was positively correlated with HI in the median lines (r=0.23) and 15 DH lines subset (r=0.40). The presence of *Rht-D1b* was known in Rialto whereas NL2 possesses the *Rht-B1b* gene (Sean Mayes, personal communication). The overall yield differences in the parental genotypes were explained by biomass rather than HI, while among the NL2 x Rialto DH lines differences in yield could be explained by the differences in both HI and biomass in the median lines. Thus there might still be scope to increase HI in the large ear-phenotype by increasing spikelets ear^{-1}. Current findings showed that HI was positively related to plant height which contradicts previous work (Miralles and Slafer, 1995) and grains m^{-2} was associated with HI. The tallest lines had highest yields in this population but then plant height was negatively linked to flowering date i.e. late flowering lines were shorter which could have confounded the normal relationship between the semi-dwarf alleles and grains m^{-2} and hence grain yield.

7.6.4 Above-ground dry matter (AGDM) production

A few investigations have reported a positive association between harvest biomass to genetic gains in yield potential in spring and winter wheats (Reynolds *et al.*, 2005; Shearman *et al.*, 2005; Slafer *et al.*, 1996). In the present study there was a positive correlation between harvest biomass and grain yield. When an increase in HI is limited, an increase in biomass is required to increase yield potential, and the findings of Singh *et al.* (1998) and Reynolds *et al.* (2001) using alien chromatin associated with the *Lr*19 translocation introgressed from *Agropyron elongatum*, showed an increase of 10% in final biomass and also a 21% increase in photosynthetic rate during grain filling, suggesting scope for introgression of this translocation to increase yield potential. The direct selection for increased grain yield,

though successful, has nonetheless modified specific physiological and morphological traits linked to yield although breeders are often unaware of the processes underlying the yield advantage (Snape, 2001). Present results showed that NL1 had greater AGDM compared to Bacanora and both of these genotypes had greater biomass than NL2. The reduction in biomass in NL2 has been attributed to the presence of *tin* gene as reported by Duggan *et al.* (2005a). RUE was positively associated with harvest AGDM in the subset of 15 DH lines. A strong positive association was found between AGDM and grain yield in the NL2 x Rialto DH populations in the median lines and in the full set of lines in both 2005 and 2006, indicating the importance of biomass as a determinant of yield potential in this population. It is desirable to increase biomass production concurrently with harvest index so as to raise yield potential. Indeed, a breeding approach that selects for high biomass capacity together with high harvest index has been suggested by several authors to increase grain yield in the future (Babar *et al.*, 2006; Muurinen and Peltonen-Sainio, 2006).

7.7 APPLICATION OF PHYSIOLOGICAL TRAITS IN BREEDING

Crop physiologists are coming under increasing pressure, as the molecular techniques for identifying and transferring genes, become more reliable and applicable to the cereals, to identify individual characteristics or processes for improvement (Evans, 1994; Collard, 2005). Field evaluations are still necessary to identify superior lines which need to be tested at different locations for several years before a cultivar is released; a time consuming and laborious process which exerts a huge demand for resources. An easy, rapid, inexpensive and reliable tool that allows breeders to confidently screen large numbers of genotypes in a relatively short time can relieve the burden of intensive field phenotyping, especially if such screening tools have high heritability and good correlation with grain yield (Babar *et al.*, 2006). The use of morphological and physiological selection criteria differentiating between genotypes is an indirect breeding approach. However, Jackson *et al.* (1996) reported that physiological tools have limited contribution to breeding, most probably due to lengthy and therefore time consuming measurements plus the lack of associations with yield (Richards, 1996).

The genetic basis of yield improvement is not well established (Reynolds *et al.*, 1999) but continued progress is still made through classical breeding programmes. Though grain yield remains the main selection criteria, the high genotype x environmental interaction that sometimes prevails makes it difficult to differentiate between genotypes and good lines can be easily discarded. Genetic improvement in wheat yield can be attributed to selection for improved traits which confer higher yield potential. The exact physiological basis for the genetic gain in grain yield potential is unknown. However, it is worth noting that the physiological processes controlling yield have been altered during the course of yield improvement and that new cultural practices have changed how these processes affect wheat growth and development. It is generally believed that yield is a function and integration of processes (e.g. nutrient uptake, photosynthesis, carbon partitioning and senescence) having a significant role in growth and development and each of them are affected by the vagaries of weather during the crop cycle.

In order to narrow the apparent gap between the genotype and the phenotype with regard to yield, it is important to identify key traits related to yield and then attempt to identify and locate the genes controlling them (Slafer, 2003). Ear fertility is a complex, quantitative trait and it is therefore subjected to genotype x environment and there is a need to understand the genotype x environment to exploit this trait in breeding. The application of physiological traits is breeding depends on traits having:

(i) genetic variability
(ii) relatively high correlation with yield
(iii) relatively high heritability compared with yield
(iv) high throughput screens

Present results showed that there was genetic variability in rachis length, spikelets per ear^{-1} and the grains-to-ear DM ratio amongst the parental genotypes and the DH lines. These three traits were not highly correlated with yield; they are likely to be moderate to high heritability. In this study long rachis and high spikelet ear^{-1} were targeted as candidate traits. These two traits were positively correlated and indicated that ear morphology traits could be useful for incorporating in breeding programmes to increase grain number. Results of the parental experiments showed that the phenotype represented by NL1 could be more useful than that represented by NL2.

The latter might need other traits to maintain its potential grain weight. Other physiological candidates coming from the study for use in breeding include pre-anthesis RUE, stomatal conductance, NDVI and the grains-to-ear DM ratio. In a comparison of synthetic-derived wheat with recurrent parents, final biomass and grain weight were larger under both irrigated and moisture-stressed conditions (Reynolds *et al.* 2007). Hence, there is much scope for the use wide sources of wheat germplasm for increasing yield potential in the future.

7.8 CONCLUSIONS

- Present results have shown that in the novel genotypes, longer rachis increased spikelets ear^{-1} and also grains ear^{-1} confirming the hypothesis that rachis length was positively associated with spikelets ear^{-1} in the large-ear phenotype lines. The novel genotypes showed greater ear fertility (grains ear^{-1}) than the benchmark Bacanora. The negative relationship between the two major yield components, grains m^{-2} and grain weight, was observed in present study. Grains m^{-2} was not actually increased by novel genotypes, indeed lower grains m^{-2} was found in NL2. Heavier grains were found in the novel genotypes compared to Bacanora and it appears that NL1 may be more useful to breeders in high-output environments than NL2. This genotype maintained a high PGW at a high grains-to ear DM ratio.

- Current findings showed that there was a developmental basis for the higher number of spikelets per ear in the large-ear phenotype. A longer thermal duration from floral initiation to terminal spikelet was associated with an increase spikelet number in these novel genotypes. The large-ear phenotype was likely associated with the earliness *per se* genes. The ear growth phase from GS 31 to GS 61 has been reported as influential in determining grains m^{-2}. Present results showed that to increase spikelet fertility the floral initiation to terminal spikelet phase is also important and extending the duration of that phase can increase grains ear^{-1}. Extending the spikelet primordia production phase is therefore another potential avenue to increase grains m^{-2} via ear fertility.

- Variation in harvest biomass production independent of flowering time effects was observed amongst the DH lines. Raising biomass is regarded as an important future avenue of increasing genetic yield potential as there is concern about the theoretical upper limit of harvest index being approached. Present results showed genetic differences among the lines in harvest biomass and biomass was positively correlated with grain yield. At CIMMYT, various crossing programmes are ongoing using wide germplasm to increase biomass production. The transfer of alien gene such as 7DL.7Ag has been shown to

increase biomass by 10% (Reynolds *et al.*, 2001). Furthermore, synthetic lines are being used at CIMMYT to bring in novel alleles and traits from the D genome, from *Triticum tauschii* in synthetic hexaploids (Ma *et al.*, 1995) and synthetic derived lines have been shown to have increased biomass production in optimal environments (Reynolds *et al.*, 2007).

- Higher ear index in NL2 was associated with a lower grains-to-ear DM ratio compared to NL1. A difference of 40 grains g^{-1} was found between the two novel genotypes. The grains-to-ear DM ratio in NL2 was markedly lower compared to other genotypes and it is possible that other genes in addition to *tin*, perhaps from *T. polonicum*, were contributing to the low value. In fact the main physiological trait limiting yield in NL2 was the lower grains to ear DM ratio. NL1 had a lower ear index than NL2, but was more efficient at producing grains per unit ear biomass and may be the more useful parental genotype in high output environments for improving yield potential. Similarly, this trait explained a significant proportion of the genotypic yield differences among the DH lines in the NL2 x Rialto population and was positively correlated to grains m^{-2}. This trait has been postulated by Abbate *et al.* (1998) to increase yield potential and this study also showed that it is a very important trait to consider for future breeding strategies, and it may be possible to combine high expression for this trait with high PGW (NL1).

- NL2 has the tiller inhibition *tin* gene whereas NL1 probably does not. Gigas features were more pronounced in NL2 than in NL1. In the NL2 x Rialto population, the *tin* gene resulted in fewer ears m^{-2} which agrees with Duggan *et al.* (2005) findings. Berry *et al.* (2003) reported up to 120 g m^{-2} net loss of dry matter from non-surviving shoots and the incorporation of the *tin* gene could increase ear fertility by restricting excessive tillering. The maximum number of grains ear^{-1} reached in the NL2 x Rialto population was 63. The expected negative relationship was found between ears m^{-2} and grains ear^{-1}, but it was encouraging to find that some lines did have high ears m^{-2} and high number of grains ear^{-1}. There are feedback mechanisms between ear fertility and tillering which should be considered as indirect determinants of grains m^{-2}.

- It is probable that the novel genotypes have *Triticum polonicum* in their genetic background which could have contributed to their greater potential grain size (due to longer glumes) thereby increasing final grain weight. The physical restriction by the glumes could have been alleviated contributing to greater potential grain size. The proposal that potential grain size can be increased by manipulation of ear morphology (Reynolds *et al.*, 2000) was supported by the results on the parental genotypes. However, in the results of the DH populations there was trade-off between grain number per unit area and grain weight.

- Genotypic differences were found in stem WSC in the parental genotypes and NL2 x Rialto DH population. There was a positive association between grain weight and WSC in DH subset of NL2 x Rialto population but not between WSC and yield. WSC has been shown to increase with breeding in UK cultivars in the last decades (Shearman *et al.*, 2005) and Ruuska *et al.* (2006) showed the trait to be heritable, providing the possibility to incorporate WSC in future breeding programmes. However, present results suggested this trait may be more important to raising yield in the UK environment than in Mexico.

- No significant differences were found in RUE_{PAR} for the parental genotypes but genotypic variation was observed in the subset of 15 lines of the NL2 x Rialto population. RUE_{PAR} was positively correlated to biomass at anthesis and grain yield. The extinction coefficient, k_{PAR}, was not linked to differences in RUE. However, there was an association with SLW. So breeders could perhaps select for smaller, thicker leaves. RUE is a complex trait but the results of the presents study suggested there may be scope for manipulating it genetically in future years.

7.9 FUTURE WORK

- There are many ways by which yield potential can be potentially increased in the future. Longer rachis with greater spikelets ear^{-1} has increased grains ear^{-1} and hence potentially yield. The effects of rachis length and spikelets ear^{-1} should be examined in a wider range of novel parents and derived populations. In the NL2 x Rialto DH population, the genes that control the ear fertility are currently being mapped in the UK environment. Furthermore there is a need to map ear fertility traits in the NL1 x Rialto. Further field studies should be carried out in these DH populations in the UK environment, especially in regard to the winter type lines, with a view to introgressing traits for ear fertility into elite wheat backgrounds.

- Based on present findings, the physiological basis of the differences in RUE could be further investigated in this population. There should be development of high-throughput screens for RUE for use in CIMMYT and UK breeding programmes. NL1 appeared to be more useful than NL2 therefore it will be useful to characterise this population further particularly with regard to understanding the physiological basis of PGW and relationships with grains m^{-2}.

- It would be useful to examine if the traits of NL1 and NL2 are representative of other CIMMYT large-ear phenotype lines, or whether there are other sources of different or complementary variation.

- The large ear phenotype if controlled by recessive genes may be difficult to handle in breeding programmes. So it will be important to develop QTL-based markers for use in backcrossing.

- The controlled-environment experiments showed the importance of pheno-phases in grain yield determination. Future work should focus on identifying QTLs for earliness *per se* through mapping studies.

- There should be mapping studies and QTL analysis of RUE sub-traits and stem WSC in both the NL1 x Rialto and NL2 x Rialto DH populations.

- Exploitation of the large-ear phenotype trait in the longer term will depend on maintaining lodging resistance. Recent work in the UK on the development of a lodging-proof ideotype (Berry *et al.*, 2004) is therefore relevant in this respect. It suggests that breeders should select for wide, deep root plate spread and wide stems with increased material strength of the stem wall. A further consideration in bread-making cultivars will be the negative relationship that is usually observed between yield and grain protein concentration. For bread-making cultivars, it will be important in the new plant types to identify and select for traits associated with favourable departures from the grain yield to protein concentration relationship. Otherwise, these genotypes might be better suited for feed wheats instead of bread making. So conceptual ideotypes should be developed to incorporate lodging resistance and stable high protein content into new cultivars incorporating large-ear phenotype traits.

- Plant hormones play an important role in floret fertility, cytokinins and ABA are the hormones most related to floret fertility. Ashikari *et al.* (2005) reported the gene *Gn1a* which determines grain set in rice by regulating cytokinin levels to correspond to major QTLs for yield potential. Investigation of the effect of orthologues of this gene in wheat could offer a new avenue to the understanding of floret fertility. ABA has been found to promote grain abortion in wheat by reducing sugar supply to developing grains (Lee *et al.*, 1988; Waters *et al.*, 1984). Preliminary studies of ABA levels measured in the ear tissue level at late booting stage when grain abortion took place in the 7DL.7Ag lines showed a lower concentration of ABA associated with increased number of grains ear^{-1} in these lines, indicating a possible biochemical link (Matthew Reynolds, personal communication). The role of these hormones could become increasing important in the future in understanding the physiological basis of ear fertility and there should be a concentrated effort of both geneticists and physiologists in unravelling the underlying mechanisms.

BIBLIOGRAPHY

ABBATE, P.E., ANDRADE, F.H. AND CULOT, J.P. (1995) The effects of radiation and nitrogen on number of grains in wheat. *Journal of Agricultural Sciences (Cambridge)*, **14**, 351-360.

ABBATE, P.E., ANDRADE, F.H., CULOT, J.P. AND BINDRABAN, P.S (1997) Grain yield in wheat: Effects of radiation during spike growth. *Field Crops Research*, **5** (2), 245-257.

ABBATE, P.E., ANDRADE, F.H., LAZARO, L., BRIFFI, J.H., BERARDOCCO, V.H. AND MARTURANO, F. (1998) Grain yield increase in recent Argentine wheat cultivars. *Crop Science*, **38**, 1203-1209.

ACEVEDO, E., SILVA, P. AND SILVA, H. (2002) Wheat growth and physiology. In: *Bread Wheat Improvement and Production* (Eds. Curtis, B.C., Rajaram S and Gómez Macpherson, H.) FAO, Rome. pp 39-70.

ACRECHE, M.M. AND SLAFER, G.A. (2006) Grain weight response to increases in number of grains in wheat in a Mediterranean area. *Filed Crops Research,* **98**, 52-59.

AGGARWAL, P.K., CHATURVEDI, G.S., SINGH, A.K. AND SINHA, S.K. (1986) Performance of wheat and triticale cultivars in a variable soil–water environment. III. Source-Sink relationships. *Field Crops Research*, **13**, 317-330.

ALLISON, J.C.S. AND DAYNARD, T.B. (1976) Effect of photoperiod on development and number of spikelet of a temperate and some low-latitude wheats. *Annals of Applied Biology*, **83**, 93-102.

AMIOUR, N., JAHIER., J., TANGUY, A.M., CHIRON., H., AND BRANLARD, G. (2002) Effect of 1R (1A), 1R (1B) and 1R (1D) substitution on technological value of bread wheat. *Journal of Cereal Science*, **35**, 149-160.

ANDERSON, J.M. (1986) Photo regulation of the composition, function and structure of thylakoid membranes. *Annual Review of Plant physiology*, **37**, 93-136.

ANGUS, J.F., MACKENZIE, D.H., MORTON, R., AND SCHAFER, C.A. (1981) Phasic development in field crops. I. Thermal and photoperiodic responses of spring wheat. *Field Crops Research*, **4**, 269-283.

ARAUS, J.L. (1996) Integrative physiological criteria associated with yield potential. In *Increasing yield potential in wheat: breaking the barriers*. Edited by Reynolds M.P., Rajaram, S. and McNab, A., pp. 150-166, CIMMYT, Mexico, DF.

ARAUS, J.L., REYNOLDS, M.P AND ACEVEDO, E. (1993) Leaf posture, grain yield, growth, leaf structure, and carbon isotope discrimination in wheat. *Crop Science*, **33**, 1273-1279.

ASHIKARI, M., SAKAKIBARA, H., LIN, S., YAMAMOTO, T., TAKASHI, T, NISHIMURA, A., ANGELES, E.R., QIAN, Q., KITANO, H. AND MATSUOKA, M. (2005) Cytokinin oxidase regulates rice in grain production. *Science*, **309**, 741-745.

ATSMON, D. AND JACOBS E. (1977) A newly bred 'gigas' form of bread wheat (*Triticum aestivum* L.). Morphological features and thermo-photoperiodic responses. *Crop Science*, **17**, 31-35.

ATSMON, D., BUSCH, M.G., EVANS, L.T. (1986) Effects of environmental conditions on expression of the 'Gigas' characters in wheat. *Australian Journal of Plant Physiology*, **13** (3), 365-379.

AUSTIN, R.B. (1999) Yield of Wheat in the United Kingdom: Recent Advances and Prospects. *Crop Science*, **39,** 1604–1610.

AUSTIN, R.B., BINGHAM, J., BLACKWELL, R.D., EVANS, L.T., FORD, M.A., MORGAN, C.L. AND TAYLOR, M. (1980) Genetic improvement in winter wheat yield since 1900 and associated physiological changes. *Journal of Agricultural Science*, **94**, 675-689.

AUSTIN, R.B., EDRICH, J.A., FORD, M.A. AND BLACKWELL, R.D. (1977) The fate of dry matter, carbohydrates and 14C lost from the leaves and stems of wheat during grain filling. *Annals of Botany*, **41**, 1309-1321.

AUSTIN, R.B., FORD, M.A. AND MORGAN, C.L. (1989) Genetic improvement in the yield of winter: a further evaluation. *Journal of Agricultural Science (Cambridge)*, **112**, 295-301.

AUSTIN, R.B., FORD, M.A., EDRICH, J.A. AND HOOPER, B.E.(1976) Some effects of leaf posture on photosynthesis and yield in wheat. *Annals of Applied Biology,* **83**, 425-446.

BABAR, M.A., REYNOLDS, M.P., VAN GINKEL, M., KLATT, A.R., RAUN, W.R. AND STONE, M.L. (2006) Spectral Reflectance to Estimate Genetic Variation for In-Season Biomass, Leaf Chlorophyll, and Canopy Temperature in Wheat. *Crop Science,* **46**, 1046-1057.

BAKER, C.K. (1979) The environmental control of development in winter wheat. PhD thesis. University of Nottingham.UK.

BAKER, C.K. AND GALLAGHER, J.N. (1983) The development of winter wheat in the field. 1. Relation between apical development and plant morphology within and between seasons. *Journal of Agricultural Science*, **101**, 327-335.

BAKER, C.K., GALLAGHER, J.N. AND MONTEITH, J.L. (1980) Daylength change and leaf appearance in winter wheat. *Plant Cell and Environment*, **3**, 285-287.

BAUER, A., GARCIA, R., KANEMASU, E.T., BLAD, B.L., HATFIELD, J.L., MAJOR, D.J., REGGINATO, R.J. AND HUBBARD, K.G. (1988) Effect of latitude on phenology of 'colt' winter wheat. *Agricultural and Forest meteorology*, **44**, 131-140.

BELL, C.J. AND INCOLL, L.D. (1990) The redistribution of assimilate in field-grown winter wheat. *Journal of Experimental Botany,* **41**, 949-960.

BERNIER, G., HAVELANGE, A., HOUSA, C., PETITJEAN, A. AND LEJEUNE, P. (1993) Physiological signals that induce flowering. *Plant Cell,* **5**, 1147-1155.

BERRY, P. M., STERLING, M., SPINK, J. H., BAKER, C. J., SYLVESTER-BRADLEY, R., MOONEY, S. J., TAMS, A. R. & ENNOS, A R. (2004). Understanding and reducing lodging in cereals. *Advances in Agronomy* **84**, 217–271.

BERRY, P.M., SPINK, J.H., FOULKES, M.J. AND WADE, A. (2003) Quantifying the contributions and losses of dry matter from non-surviving shoots in four cultivars of winter wheat. *Nature*, **270**, 431-433.

BIDINGER, F.R., MUSGRAVE, R.B. AND FISCHER, R.A. (1977) Contribution of stored pre-anthesis assimilates to grain yield in wheat and barley. *Nature*, **270**, 431-433.

BINDRABAN, P.S., SAYRE, K.D. AND SOLIS-MOYA, E.(1998) identifying factors that determine kernel number in wheat. *Field Crops Research*, **58** (3) 223-234.

BINGHAM, I.J., BLAKE, J., FOULKES, M.J. AND SPINK, J. (2007) Is barley yield in the UK sink limited? I. Post-anthesis radiation interception, radiation-use efficiency and source and sink balance. *Field Crops Research*, **101**, 198-211.

BINGHAM, J. (1969) The physiological determinants of grain yield in cereals. *Agricultural Progress*, **44**, 177-189.

BISCOE, P. V. AND GALLAGHER, J. N. (1977) Weather, dry matter production and yield. In *Environmental Effects on Crop Physiology*, Edited by Landsberg, J.J and Cutting, C.V. pp. 75-100., Academic Press, London.

BISWAS, A.K. AND MANDAL, S.K (1986) Monocarpic senescence in wheat: Influence of sterile glumes and ear. *Physiology Plantarum*, **64**, 431-434.

BLACKLOW, W.M., DARBYSHIRE, M., PHELOUNG, P. (1984) Fructan polymerized and depolymerized in the internodes of winter wheat as grain filling progressed. *Plant Science Letters*, **36**, 213-218.

BLUM, A. (1990) Variation among wheat cultivars in the response of leaf gas exchange to light. *Journal of Agricultural Science,* **115**, 305-311.

BLUM, A. (1996) Yield potential and drought resistance. In *Increasing Yield Potential in Wheat: Breaking the Barriers,* Edited by Reynolds, M.P., Rajaram, S. and McNab, A. pp. 90-97,CIMMYT, Mexico, D.F.

BLUM, A. (1998) Improving wheat grain filling under stress by stem reserves mobilisation. *Euphytica*, **100**, 77-83.

BLUM, A., MAYER, J. AND GOLAN, G. (1988) The effect of grain number per ear (sink size) on source activity and its water relations in wheat. *Journal of Experimental Botany*, **39**, 106-114.

BLUM, A., SINMENA, B., MAYER, J., GOLAN, G., SHPILER, L. (1994) Stem reserve mobilization supports wheat-grain filling under heat stress. *Australian Journal of Plant Physiology*, **21**, 771-781.

BOARDMAN, N.K. (1977) Comparative photosynthesis of sun and shade plants. *Annual Review of Plant Physiology*, **28**, 355-377.

BONNETT G.D. AND INCOLL L.D. (1993) Effects on the stem of winter barley of manipulating the source and sink during grain-filling. II. Changes in the composition of water-soluble carbohydrates of internodes. *Journal of Experimental Botany,* **44**, 83–91.

BORGHI, B., CORBELLINI, M., CATTANEO, M., FORNASARI, M.A. AND ZUCCCHELLI, L. (1986) Modification of the source/sink relationship in bread wheat and its influence on grain yield and protein content. *Journal of Agronomy and Crop Science*, **157**, 245-254.

BORRAS, L., SLAFER, G.A AND OTEGUE, M.E (2004) Seed dry weight response to source sink manipulation in wheat, maize and soybean: a quantitative reappraisal. *Field Crop Research,* **86**, 131-146.

BORRELL, A.K., HAMMER, G.L AND HENZELL, R.G. (2000) Does maintaining green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. *Crop Science*, **40**, 1037-1048.

BORRELL, A.K., HAMMER, G.L AND VAN OOSTEROM, E. (2001) Stay-green: A consequence of the balance between supply and demand fro nitrogen during grain filling? *Annals of Applied Biology*, **138**, 91-95.

BORRELL, A.K., INCOLL, L.D. AND DALLING, M.J. (1993) The influence of the Rht_1 and Rht_2 alleles on the deposition and use of stem reserves in wheat. *Annals of Botany*, **71**, 317-326.

BORRELL, A.K., INCOLL, L.D., SIMPSON, R.J. AND DALLING, M.J (1989) Partitioning of dry matter and the deposition and use of stem reserves in a semi-dwarf wheat crop. *Annals of Botany*, **63**, 527-539.

BRANCOURT-HULMEL, M., DOUSSINAULT, G., LECOMTE, C., BÉRARD, P., LE BUANEC, B AND TROTTET, M. (2003) Genetic improvement of agronomic traits of winter wheat cultivars released in France from 1946 to 1992. *Crop Science*, **43**, 37-45.

BREMNER, P.M. 1972. Accumulation of dry matter and nitrogen by grains in different positions of the wheat ear as influenced by shading and defoliation. *Australian Journal of Biological Science*, **25**, 657-668.

BREMNER, P.M. AND RAWSON, H.M (1978) The weights of individual grains of the wheat ear in relation to their potential, the supply of assimilate and interaction between grains. *Australian Journal of Plant Physiology*, **5**, 61-72.

BRIARTY, L.G AND HUGHES, C.E. (1979) The developing endosperm of wheat-A stereological analysis. *Annals of Botany*, **44**, 641-658.

BROCKLEHURST P.A., MOSS, J.P. AND WILLIAMS W. (1978) Effects of irradiance and water supply on grain development in wheat. *Annals of Applied Biology*, **90**, 265–276.

BROCKLEHURST, P.A. (1977) Factors controlling grain weight in wheat. *Nature*, **266**, 348-349.

BROOKING, I.R. AND JAMIESON, P.D. (2002) Temperature and photoperiod response of vernalization in near-isogenic lines of wheat. *Field Crops Research*, **79**(1) 21-38.

BROOKING, I.R. AND KIRBY, E.J.M. (1981) Interrelationships between stem and ear development in winter- wheat- The effects of a Norin-10 dwarfing gene, GAI-Rht2. *Journal of Agricultural Science*, **97**, 373-381.

BUCHANAN-WOLLASTON, V., EARL, S., HARRISON, E., MATHAS, E., NAVABPOUR, S., PAGE, T. AND PINK, D. (2003) The molecular analysis of leaf senescence—a genomics approach. *Plant Biotechnology Journal*, **1**, 3–22.

CALDERINI, D. F. AND SLAFER, G. A. (1998) Changes in yield and yield stability in wheat during the 20th century. *Field Crops Research,* **57**, 335-347.

CALDERINI, D.F AND REYNOLDS, M.P. (2000) Changes in grain weight as a consequence of degraining treatments at pre- and post anthesis in synthetic hexaploid lines of wheat (*Triticum durum* x *T. tauschii*). *Australian Journal of Plant Physiology*, **27**, 183-191.

CALDERINI, D.F, DRECCER, M.F AND SLAFER, G.A. (1995) Genetic improvement in wheat yield and associated traits. A re-examination of previous results and the latest trends. *Plant Breeding*, **114**, 108-112.

CALDERINI, D.F., ABELEDO, L.G., SAVIN, R. AND SLAFER, G.A. (1999) Effect of temperature and carpel size on during pre-anthesis on potential grain weight in wheat. *Journal of Agricultural Science, Cambridge,* **132**, 453-459.

CALDERINI, D.F., DRECCER, M.F., AND SLAFER, G.A. (1997) Consequences of breeding on biomass, radiation interception and radiation use efficiency in wheat. *Field Crops Research*, **52**, 271-281.

CALDERINI, D.F., REYNOLDS, M.P. AND SALFER, G.A (2006) Source-sink effects on grain weight of bread wheat, durum wheat and triticale at different locations. *Australian Journal of Agricultural Research*, **57**, 227-233.

CALDERINI, D.F., SAVIN, R., ABELEDO, L.G., REYNOLDS, M.P. AND SALFER, G.A. (2001) The importance of the period immediately preceding anthesis for grain weight determination in wheat. *Euphytica*, **119**, 199-204.

CALEY, C,Y., DUFFUS, C.M. AND JEFFCOAT, B. (1990) Effects of elevated temperature and reduced water uptake on enzymes of starch synthesis in developing wheat grains. *Australian Journal of Plant Physiology*, **17**, 431-439.

CAO, W AND MOSS, D.N. (1989) Daylength effect on leaf emergence and phyllochron in wheat and barley. *Crop Science*, **29**, 1021-1025.

CARTELLE, J., PEDRO, A., SAVIN, R. AND SLAFER, G.A. (2006) Grain weight responses to post anthesis spikelet- trimming in an old and a modern wheat under Mediterranean conditions. *European Journal of Agronomy,* **25**, 365-371.

CARVER, B.F AND RAYBURN, A.L. (1994) Comparison of related wheat stocks possessing 1B or 1RS.1BL chromosomes: Agronomic performance. *Crop Science*, **34**, 1505-1510.

CHEN, T.Y., SKOVMAND, B.S., RAJARAM, S., REYNOLDS, M.P. (1998) Novel source of increased spike fertility in wheat multi-seeded flowers. In *Abstracts of the 90th Annual meeting of the American Society of Agronomy, 18-22 October 1998.* Baltimore, MD, American Society of Agronomy.

CHOJECKI, A.J.S., GALE, M.D. AND BAYLISS, M.W. (1986) The number and sizes of starch granules in wheat endosperm and their association with grain weight. *Annals of Botany,* **58,** 819-831.

COLLARD, B.C.Y., JAHUFER, M.Z.Z., BROUWER, J.B AND PANG, E.C.K. (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica,* **142**, 169-195.

CONWAY, G. (1997). The Doubly Green Revolution: Food for All in the 21st Century. London: Penguin books.

COOK, M.G. AND EVANS, L.T. (1978) Effect of relative size and distance of competing sinks on the distribution of photosynthetic assimilate in wheat. *Australian Journal of Plant Physiology*, **5,** 495-509.

COTTRELL, J.E., DALE, J.E. AND JEFFCOAT, B.(1981) Development of the apical dome of barley in response to treatment with gibberellic acid. *Plant Science Letters*, **22**, 161-168.

COX, T.S., SHROYER, J.P., LIU, B.H., SEARS, R.G. AND MARTIN, T.J. (1988) Genetic improvement in agronomic traits of hard red winter wheat cultivars from 1919 to 1987. *Crop Science,* **28**(5), 756-760.

CRITCHLEY, C.S (2001) A physiological explanation for the canopy nitrogen requirement of wheat. PhD thesis, University of Nottingham, p 231.

CRUZ-AGUADO, J.A., RODES, R., PEREZ, I.P. DORADO, M. (2000) Morphological characteristics and yield components associated with accumulation and loss of dry mass in the internodes of wheat. *Field Crops Research,* **66,** 129-139.

CURTIS, B.C. (2002) Wheat in the world. In *Bread Wheat: Improvement and Production*. Edited by Curtis, B.C., Rajaram, S. and Gomez Macpherson, H., pp 1-17, FAO, Rome, Italy.

DARWINKEL, A. (1983) Ear formation and grain yield of winter wheat as affected by time of nitrogen supply. *Netherlands Journal of Agricultural Science*, **31**, 211–225.

DAVIDSON, J.L., KRISTIAN. K.R., JONES, D.B. AND BREMNER, P.M. (1985) Responses of wheat to vernalization and photoperiod. *Australian Journal of Agricultural Research*, **36**, 347-359.

DEL BLANCO, I.A., RAJARAM, S. AND KRONSTAD, W. E. (2001) Agronomic potential of synthetic hexaploid wheat-derived populations. *Crop Science*, **41**, 670-676.

DELÉCOLLE, R., HAY, R.K.M., GUÉRIF, M., PLUCHARD, P. AND VARLET-GRANCHER, C. (1989) A method of describing the progress of apical development in wheat based on the time of course of organogenesis. *Field Crops Research*, **21**, 147-160.

DENCIC, S. (1994) Designing a wheat ideotype with increased sink capacity. *Plant Breeding*, **112**, 311-317.

DHILLON, S.S. AND ORTIZ-MONASTERIO, J.I. (1993) Effect of date of sowing on the yield and yield components of spring wheat and their relationship with solar radiation and temperature at Ludhiana, Punjab, India. Wheat Special Report No. 23, CIMMYT, Mexico, DF.

DOEBLEY, J., STEC, A. AND HUBBARD, L. (1997) The evolution of apical dominance in maize. *Nature*, **386**, 485-488.

DONALD, C.M. (1968) The breeding of crop ideotypes. *Euphytica*, **17** (3), 385-403.

DONMEZ, E., SEARS, R.G., SHROYER, J.P. AND PAULSEN, G.M. (2001) Genetic gain in yield attributes of winter wheat in the Great Plains. *Crop Science*, **41**, 1412-1419.

DRAKE, B.G. AND GONZALEZ-MELER, M.A. (1997) More efficient plants: a consequence of rising atmospheric CO_2? *Annual Review of Plant physiology and Plant Molecular Biology*, **48**, 609-639.

DRECCER, M.F., SLAFER, G.A. AND RABBINGE, R. (1998) Optimisation of vertical distribution of canopy nitrogen: an alternative trait to increase yield potential in winter cereals. *Journal of Crop Production*, **1**, 47-77.

DRECCER, M.F., VAN OIJEN, M., SCHAPENDONK, H.C.M., POT, C.S. AND RABBINGE, R. (2000) Dynamics of vertical leaf nitrogen distribution in a vegetative wheat canopy. Impact on canopy photosynthesis. *Annals of Botany*, **86**, 821-831.

DROUET, J.L. AND BONHOMME, R. (1999) Do variations in local leaf irradiance explain changes to leaf nitrogen within row maize canopies? *Annals of Botany*, **84**, 61-69.

DUBCOVSKY, J., CHEN, C. AND YAN, L. (2005) Molecular characterization of the allelic variation at the *VRN-H2* vernalization locus in barley. *Molecular Breeding*, **15** (4), 395-407.

DUGGAN, B.L., RICHARDS, R.A. AND TSUYUZAKI, H. (2002) Environmental effects on stunting and the expression of a tiller inhibition (tin) gene in wheat. *Functional Plant Biology*, **29**(1), 45-53.

DUGGAN, B.L., RICHARDS, R.A., VAN HEERWAARDEN, A.F. (2005a) Agronomic evaluation of a tiller inhibition gene (tin) in wheat. I. Effect of yield, yield components and grain protein. *Australian Journal of Agricultural Research*, **56**, 169-178.

DUGGAN, B.L., RICHARDS, R.A., VAN HEERWAARDEN, A.F. (2005b) Agronomic evaluation of a tiller inhibition gene (tin) in wheat. II. Growth and partition of assimilates. *Australian Journal of Agricultural Research*, **56**, 179-186.

DUNCAN, W.G. (1971) Leaf angle, leaf area, and canopy photosynthesis. *Crop Science,* **11**, 482-485.

EGLI, D.B (1988) Seed growth and development. In: *Seed biology and the yield of grain crops*. Edited by Egli, D.B., CAB International, New York.

EHDAIE, B., ALLOUSH, G. A., MADORE, M. A. AND WAINES, J. G. (2006) Genotypic Variation for Stem Reserves and Mobilization in Wheat: I. Postanthesis Changes in Internode Dry Matter. *Crop Science*, **46**, 735-746.

ENGLEDOW, F.L. AND WADHAM, S.M. (1923) Investigations on yield in cereals. Part I. *Journal of Agricultural Science, Cambridge*, **13**, 390-439.

EVANS, J.R. (1983) Nitrogen and photosynthesis in flag leaf of wheat (*Triticum aestivum*, L.). *Plant Physiology*, **72**, 297-302.

EVANS, J.R. (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia*, **78** (1), 9-19.

EVANS, J.R. AND SEEMANN, J.R. (1989) The allocation of protein nitrogen in the photosynthetic apparatus: coasts, consequences and control. In *Photosynthesis, Proceedings of C.S French Symposium on Photosynthesis held in Stanford, California,* July, *17-23, 1988. Plant Biology 8(* Ed. Briggs, W.R.), pp. 183-205. Alan R. Liss Inc, New York.

EVANS, L. T. (1983). Raising the yield potential: by selection or design? In *Genetic Engineering of Plants: an Agronomic Perspective* (Eds T. Kosuge, C. P. Meredith & A. Hollaender), pp 371–389. New York: Plenum Press.

EVANS, L.T (1994) Crop physiology: Prospects for the retrospective science. In: *Physiology and determination of Crop Yield.* Edited by Boote, K.J., Bennett, J.M., Sinclair, T.R and Paulsen, G.M. pp 19-35, Madison, Wisconsin, USA.

EVANS, L.T AND FISCHER, R.A. (1999) Yield potential: Its definition, measurements and significance. *Crop Science,* **39**, 1544-1551.

EVANS, L.T AND RAWSON, H.M. (1970) Photosynthesis and respiration by the flag leaf and components of the ear during grain development in wheat. *Australian Journal of Biological Sciences*, **23**, 245-254.

EVANS, L.T. (1993) Crop evolution, adaptation and yield. Cambridge University Press. Cambridge, pp 1-500.

EVANS, L.T. AND BLUNDELL, C. (1994) Some aspects of Photoperiodism in wheat and its wild relatives. *Australian Journal of Plant Physiology*, **21**, 551-562.

EVANS, L.T. AND RAWSON, H.M. (1970) Photosynthesis and respiration by the flag leaf and components of the ear during grain development in wheat. *Australian Journal of Biological Sciences*, **23**, 245-254.

EVANS, L.T.(1998) Feeding the ten billion: Plants and Population growth. *The challenge of increasing photosynthesis*, p 170 Cambridge University Press.

EVANS, L.T., BINGHAM, J., JACKSON, P. AND SUTHERLAND, J. (1972) Effects of awns and drought on the supply of photosynthate and its distribution within wheat ears. *Annals of Applied Biology*, **70**, 67-76.

EVERS, J.B., VOS, J., ANDRIEU, B., STRUICK, P.C. (2006) Cessation of tillering in Spring wheat in relation to light interception and red:far red ratio. *Annals of Botany*, **97**, 649-658.

FEDAK, G., BURVILL, M. AND VOLDENG, H.D. (1997) A comparison of anther culture and maize pollination for haploid production in wheat. *Journal of Applied Genetics*, **38 (4)**, 407-414.

FISCHER, F.A (2006) Understanding the physiological basis of yield potential in wheat. *International Symposium on wheat yield potential*: Challenges to international wheat breeding, p 25, Cd. Obregon, Mexico.

FISCHER, R.A (2001) Selection traits for improving yield potential. In: *Applications of physiology in wheat breeding*, p 148-160.CIMMYT, DF Mexico.

FISCHER, R.A. (1973) The effect of water stress at various stages of development on yield processes in wheat. In: *Plant Response to Climatic Factors*, pp.233-241, Paris.

FISCHER, R.A. (1983) Growth and yield of wheat. In *Potential productivity of field crops under different environments*. Edited by Smith, W.H and Bante, S.J, International Rice Research Institute, pp 129-154, Los Banõs, Phillipines.

FISCHER, R.A. (1985) Number of Kernels in wheat crops and the influence of solar radiation and temperature. *Journal of Agricultural Science*, **105**, 447-461.

FISCHER, R.A. (1996) Wheat Physiology at CIMMYT and Raising the Yield Plateau. In *Increasing Yield Potential in Wheat: Breaking the Barriers*. Edited by Reynolds, M.P., Rajaram, S. and McNab, A., pp. 195-203, CIMMYT, Mexico, D.F.

FISCHER, R.A. AND HILLERISLAMBERS, D. (1978) Effect of environment and cultivar on source limitation to grain weight in wheat. *Australian Journal of Agricultural Research*, **29**, 443-458.

FISCHER, R.A. AND QUAIL, K.J. (1990) The effect of major dwarfing genes on yield potential in spring wheats. *Euphytica*, **46**, 51-56.

FISCHER, R.A. AND STOCKMAN, Y.M. (1986) Increased kernel number in Norin-10 derived dwarf wheat: evaluation of the cause. *Australian Journal of Plant physiology*, **13**, 767-784.

FISCHER, R.A., REES, D., SAYRE, K.D., LU, Z.M., CONDON, A.G. AND LARQUE SAAVEDRA, A. (1998) Wheat yield progress associated with high stomatal conductance and photosynthetic rate, and cooler canopies. *Crop Science*, **38**, 1467-1475.

FLINTHAM, J.E AND GALE, M.D (1983) The 'Tom Thumb' dwarfing gene, *Rht3*, in wheat. 2. Effects on height, yield and grain quality. *Theoretical and Applied Genetics*, **66**, 249-256.

FLINTHAM, J.E., BORNER, A., WORLAND, A.J. AND GALE, M.D. (1997) Optimizing wheat grain yield: effects of *Rht* (gibberellin-insensitive) dwarfing genes. *Journal of Agricultural Science,* **128**, 11-25.

FLOOD, R.G. AND HALLORAN, G.M. (1984) Basic development rate in spring wheat. *Agronomy Journal*, **76**, 260-264.

FLOOD, R.G. AND HALLORAN, G.M. (1986) The influence of genes for vernalization response on development and growth in wheat. *Annals of Botany*, **58**, 505-513.

FORD, M.A., AUSTIN, R.B., ANGUS, W.J. AND SAGE, G.C.M. (1981) Relationships between responses of spring wheat genotypes to temperatures and photoperiodic treatments and their performance in the field. *Journal of Agricultural Science (Cambridge)*, **96**, 623-634.

FOULKES, M.J., PAVELEY, N.D., WORLAND, A., WELHAM, S.J., THOMAS, J AND SNAPE, J.W (2006) Major genetic changes in wheat with potential to affect disease tolerance. *Phytopathology*, **96**, 680-688.

FOULKES, M.J., SCOTT, R.K. AND SYLVESTER-BRADLEY, R. (2002) The ability of wheat cultivars to withstand drought in UK conditions: formation of grain yield. *Journal of Agricultural Sciences*, **138**, 153-169.

FOULKES. M.J, SPINK, J.H., SCOTT, R.K. AND CLARE, R.W. (1998) Varietal typing trials and NIAB additional character assessments. *Home Grown Cereals Authority Final Project Report No. 174*, Vol. V., London, HGCA.

FRANK, A.B., BAUER, A. (1982) Effect of temperature and fertiliser N on apex development in spring wheat. *Agronomy Journal*, **74**, 504-509.

FRIEND, V.A., HELSON, A. AND FISHER, J.E. (1962) Leaf growth in marquis wheat, as regulated by temperature, light intensity and day length. *Canadian Journal of Botany*, **40**, 1299-1311.

GALE, M.D. AND YOUSSEFIAN, S. (1985) Dwarfing genes in wheat. In *Progress in Plant Breeding*. Edited by Russell, G.E., pp. 1-35. Butterworth, London.

GAMBIN, B.L. AND BORRAS, L. (2007) Plasticity of sorghum kernel weight to increased assimilate availability. *Field Crops Research*, **100**, 272-284.

GAMON, J.A., FIELD, C.B., GOULDEN, M.L., GRIFFIN, K.L., HARTLEY, A.E., JOEL, G., PENUELAS, J. AND VALENTINI, R. (1995) Relationships between NDVI, canopy structure, and photosynthesis in three Californian vegetation types. *Ecological Applications*, **5**, 28-41.

GARDNER, J.S., HESS, W.M. AND TRIONE, E.J. (1985) Development of the young wheat spike: A SEM study of Chinese spring wheat. *American Journal of Botany,* **72**(4), 548-559.

GAY, A.P., STOKES, D.J., WEIGHTMAN, R.M AND SYLVESTER-BRADLEY, R. (1998). How to run a reference crop. *HGCA Research and Development,* Project report 151, p. 43.

GEBBING, T., SCHNYDER, H. AND KÜHBAUCH, W. (1988) Carbon mobilization in shoot parts and roots of wheat during grain filling: assessment by $^{13}C/^{12}C$ steady-state labelling, growth analysis and balance sheets of reserves. *Plant, Cell and Environment*, **21** (3) 301-313.

GEBEYEHOU, G., KNOTT, D.R., AND BAKER, R.J. (1982) Relationship among durations of vegetative and grain filling phases, yield components, and grain yield in durum wheat cultivars. *Crop Science,* **22**, 287-290.

GEPSTEIN, S. (2004) Leaf senescence—not just a 'wear and tear' phenomenon. *Genome Biology*, **5** (3), 212.

GIFFORD, R. M., AND EVANS, L. T. (1981) Photosynthesis, carbon partitioning, and yield. *Annual Review of Plant Physiology,* **32,** 485-509.

GIFFORD, R.M. (1977) Growth pattern, carbon dioxide exchange and dry matter distribution in wheat growing in differing photosynthetic environments. *Australian Journal of Plant Physiology*, **4**, 99-110.

GONZALEZ, F.G., SALFER, G.A. AND MIRALLES, D.J. (2005) Photoperiod during stem elongation in wheat: is its impact on fertile floret and grain number determination similar to that of radiation? *Functional Plant Biology*, **32**, 181-188.

GONZALEZ, F.G., SLAFER, G.A. AND MIRALLES, D.J (2002) Vernalization and photoperiod responses in wheat pre-flowering reproductive phases. *Field Crops Research,* **74** (2), 183-195.

GONZALEZ, F.G., SLAFER, G.A. AND MIRALLES, D.J. (2003) Grain and floret number in response to photoperiod during stem elongation in fully and slightly vernalized wheats. *Field Crops Research*, **81**, 17-23.

GOODING, M.J., SMITH, S.P., DAVIES, W.P. AND KETTLEWELL, PS. (1994) Effects of late-season applications of propiconazole and tridemorph on disease, senescence, grain development and the bread making quality of winter wheat. *Crop Protection* **13**, 362–370.

GRABAU, L.J., VAN STANFORD, D.A. AND MENG, Q.W.(1990) Reproductive characteristics of winter wheat cultivars subjected to post anthesis shading. *Crop Science,* **30**, 771-774.

GREEN, C.F. (1989) Genotypic differences in the growth of *Triticum aestivum* in relation to absorbed solar radiation. *Field Crops Research*, **19**, 285-295.

GRIEVE C.M., LESCH, S.M., MAAS, E.V., AND FRANCOIS, L.E. (1993) Leaf and spikelet primordia initiation in salt-stressed wheat. *Crop Science*, **33**, 1286-1294.

GRINDLAY, J.D.C. (1997) Towards an explanation of crop nitrogen demand based on the optimisation of leaf nitrogen per unit leaf area. *Journal of Agricultural Science, Cambridge*, **128**, 377-396.

GUITERREZ-RODRIGUEZ, M., REYNOLDS, M.P., ESCALANTE-ESTRADA, J.A. AND RODRIGUEZ-GONZALEZ, M.T. (2004) Association between canopy reflectance indices and yield and physiological traits in bread wheat under drought and well-irrigated conditions. *Australian Journal of Agricultural Research*, **55**, 1139-1147.

GUITMAN, M.R., ARNOZIS, P.A. AND BARNEIX, A.J (1991) Effect of source-sink relations and nitrogen nutrition on senescence and N remobilisation in the flag leaf of wheat. *Physiology Plantarum*, **82**, 278-284.

HALLORAN, G.M. (1975) Genotype differences in photoperiodic sensitivity and vernalization response in wheat. *Annals in Botany*, **39**, 845-851.

HALLORAN, G.M. (1976) Genetic control of photoperiodic sensitivity and maturity in spring wheat within narrow limits of adaptation. *Euphytica*, **25**, 489-498.

HALLORAN, G.M. AND BOYDELL, C.W. (1967). Wheat chromosome with genes for vernalization response. *Canadian Journal of Genetics and Cytology*, **9**, 632-639.

HANIF, M. AND LANGER, H.M. (1972) The vascular system of the spikelet in wheat (*Triticum aestivum*). *Annals of Botany*, **36**, 721-727.

HAUN, J.R. (1973) Visual qualification of wheat development. *Agronomy Journal*, **65**, 116-119.

HAWKER, J.S. AND JENNER, C.F. (1993) High temperature affects the activity of enzymes in the committed pathway of starch synthesis in developing wheat endosperm. *Australian Journal of Plant Physiology*, **20**, 197-209.

HAY, K.M. AND WALKER. A.J. (1989) Temperate cereals. In *An Introduction to the physiology of crop yield*, pp. 160-175, Longman Scientific and Technical, Essex.

HAY, R.K.M. (1986) Sowing date and the relationships between plant and apex development in winter cereals. *Field Crops Research*, **14**, 321-337.

HAY, R.K.M. (1995) Harvest index: A review of its use in plant breeding and crop physiology. *Annals of Applied Biology*, **126**, 197-216.

HERGOZ, H. AND STAMP, P. (1983) Dry matter and nitrogen accumulation in grains at different ear positions in 'gigas' semi dwarf and normal spring wheats. *Euphytica*, **32**, 511-520.

HIROSE, T AND WENGER, M.J.A (1987) Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oceologia*, 72, 520-526.

HOLMES, D.P. (1973) Inflorescence development of semi dwarf and standard height wheat cultivars in different photoperiod and nitrogen treatments. *Canadian Journal of Botany*, **51**, 941-956.

INAGAKI, M. AND TAHIR, M. (1990) Comparison of haploid production frequencies in wheat varieties crossed with *Hordeum bulbosum* L. and maize. *Japanese Journal of Breeding*, **40**, 209-216.

INAGAKI, M.N. (1998) Evaluation of doubled haploid breeding scheme in wheat improvement.http://ss.jircas.affrc.go.jp/engpage/annualreport/1998/divisions/BRDtopic.htm

INNES, P. AND BLACKWELL, R.D. (1983) Some effects of leaf posture on the yield and water economy of winter wheat. *Journal of Agricultural Science,* **101**, 367-376.

JACKSON, P., ROBERTSON, M., COOPER, M. AND HAMMER G. (1996) The role of physiological understanding in plant breeding: from a breeding perspective. *Field Crops Research*, **49**, 11-37.

JACKSON, P.A., BYTH, D.E., FISCHER, K.S. AND JOHNSTON, R.P. (1994) Genotype x environment interactions in progeny from a barley cross. II. Variation in grain yield, yield components and dry matter production among lines with similar times to anthesis. *Field Crops Research*, **37**, 11-23.

JAMIESON, P.D., BROOKING, I.R., SEMENOV, M.A. AND PORTER, J.R. (1988) Making sense of wheat development: a critique of methodology. *Field Crops Research*, **55**, 117-127.

JANTASURIYARAT, C., VALES, M.I., WATSON, J.W. AND RIERA-LIZARARU, O. (2004) identification and mapping of genetic loci affecting the free threshing habit and spike compactness in wheat (*Triticum aestivum* L.).*Theoretical and Applied Genetics,* **108**, 261-273.

JENNER, C.F AND RATHJEN A.J. (1975) Factors regulating the accumulation of starch in ripening wheat grain. *Australian Journal of Plant Physiology,* **2**, 311-322.

JENNER, C.F. AND RATHJEN, A.J. 1972. Limitations to the accumulation of starch in the developing wheat grain. *Annals of Botany*, **36**, 743-754.

JENNER, C.F., UGALDE, T.D. AND ASPINALL, D. (1991) The physiology of starch and protein deposition in the endosperm of wheat. *Australian Journal of Plant Physiology*, **18**, 211-216.

JIANG, D., CAO, W., DAI., T AND JING, Q. (2003) Activities of key enzymes for starch synthesis in relation to growth of superior and inferior grains on winter wheat (*Triticum aestivum* L.) spike. *Plant growth Regulation*, **41**, 247-251.

KATO, H, TAKETA, S, BAN, T, IRIKI, N. AND MURAI, K. (2001) The influence of a spring habit gene, *Vrn-D1* on heading time in wheat. *Plant Breeding*, **120** (2) 115-120.

KATO, K. AND WADA, T (1999) Genetic analysis and selection experiment for narrow sense earliness in wheat by using segregating hybrid progenies. *Breeding Science*, **49**, 233-238.

KATO, K., MIURA, S. AND SAWADA, S. (1999) QTL mapping of genes controlling ear emergence time and plant height on chromosome 5A of wheat. *Theoretical and Applied Genetics*, **98**, 472-477.

KIRBY, E.J.M. (1974) Ear development in spring wheat. *Journal of Agricultural Sciences*, **82**, 437-447.

KIRBY, E.J.M. (1977) The growth of the shoot apex and the apical dome of barley during ear initiation. *Annals of Botany*, **41**, 1297-1308.

KIRBY, E.J.M. (1985) Significant stages of ear development in winter wheat. In *Wheat growth and modelling*. Edited by Day, W. and Atkinson, R. K. Plenum Press, New York and London. pp. 7-24.

KIRBY, E.J.M. (1988) Analysis of leaf, stem and ear growth in wheat from terminal spikelet stage to anthesis. *Field crops Research*, **18**, 127-140.

KIRBY, E.J.M. (1992) A field study of the number of main shoot leaves in wheat in relation to vernalization and photoperiod. *Journal of Agricultural Science*, **118**, 271-278.

KIRBY, E.J.M. AND APPLEYARD, M. (1980) Effects of photoperiod on the relation between development and yield per plant of a range of spring barley varieties. *Z. Pflanzen zuchthg*, **85**, 226-239.

KIRBY, E.J.M. AND APPLEYARD, M. (1984) *Cereal Development Guide,* 2nd Edition, National Agricultural Centre, Cereal Unit, Stoneleigh, England.

KIRBY, E.J.M. AND APPLEYARD, M. (1987) Development and structure of the wheat plant. In: Wheat Breeding: Its scientific basis, Lupton, F.G.H (Ed), Chapman and Hall Ltd, p 287-311.

KIRBY, E.J.M., APPLEYARD, M. AND FELLOWES, G. (1985) Effect of sowing date and variety on main shoot leaf emergence and number of leaves of barley and wheat. *Agronomie*, **5**, 117-126.

KIRBY, E.J.M., EVANS, E.J., FROST, D.L., SYLVESTER-BRADLEY, R., CLARE, R.W., SPINK, J.H., FOULKES, M.J. AND SCOTT, R.K. (1998) Varietal responses to length of growing season. *Project Report 174,* Vol. IV, Exploitation of varieties for UK cereal production. HGCA London.

KIRBY, E.J.M., SIDDIQUE, K. H. M., PERRY, M. W., KAESEHAGEN, D. AND STERN, W. R. (1989) variation in spikelet initiation and ear development of old and modern Australian wheat varieties. *Field Crops Research,* **20**, 113-128.

KIRBY, E.J.M., SPINK, J.H., FROST, D.L., SYLVESTER-BRADLEY, R., SCOTT, R.K., FOULKES, M.J. CLARE, R.W. AND EVANS, E.J. (1999) A study of wheat development in the field: analysis by phases. *European Journal of Agronomy*, **11**, 63-82.

KLEIMA, F.J., HOBE, S., CALKOEN, F., URBANUS, M.L., PETERMAN, E.J., VAN GRONDELLE, R., PAULSEN, H. AND VAN AMERONGEN, H. (1999) Decreasing the chlorophyll a/b ratio in reconstituted LHCII: Structural and Functional consequences. *Biochemistry*, **38**, 6587-6596.

KOJIMA, S., TAKAHASHI, Y.,KOBAYASHI, Y., MONNA, L., SASAKI, T., ARAKI, T. AND YANO, M. (2002) *Hd3a*, a rice ortholog of the Arabidopsis FT gene promotes transition to flowering downstream of *Hd1* under short day conditions. *Plant and Cell Physiology*, **43** (10), 1096-1105.

KOSHKIN, E.I AND TARARINA, V.V. (1989) Yield and source/sink relations of spring wheat cultivars. *Field Crops Research*, **22**, 297-306.

KRONSTAD, W.E. (1998) Agricultural development and wheat breeding in the 20th century. In *Wheat: Prospects for Global improvement. Proceedings of the 5th International Wheat Conference, Ankara, Turkey.* Edited by Braun, H.J., Altay,

F., Kronstad, W.E., Beniwal, S.P.S. and Mc Nab, A. pp. 1-10. Developments in plant breeding, V. 6. Kluwer Academic Publishers. Dordrecht.

KRUK, B.C., CALDERINI, D.F. AND SLAFER, G.A. (1997) Grain weight in wheat cultivars released from 1920 to 1990 as affected by post-anthesis defoliation. *Journal of Agricultural Science*, **128**,273-281.

KYLE, D.J., STAEHELIN, L.A., ARNTZEN, C.J. (1983) Lateral mobility of LHCII in chloroplast membranes controls excitation energy distribution in higher plants. *Archives of Biochemistry and Biophysics*. **222**, 527-541.

LANSBERG, J.J. (1977) Effects of weather on plant development. In "Environmental effects on crop physiology" (Eds. J.J Landsberg, and C.V. Cutting) p 289-307. Academic Press, London.

LAURIE, D.A. AND BENNETT, M.D. (1986) Wheat x maize hybridisation. *Canadian Journal of Genetics and Cytology*, **28**, 313-316.

LAURIE, D.A. AND BENNETT, M.D. (1987) The effect of crossability loci Kr1 and Kr2 on fertilization frequency in hexaploid wheat x maize crosses. *Theoretical and Applied Genetics*, **73**, 403-409.

LAURIE, D.A. AND SNAPE, J.W. (1990) The agronomic performance of wheat doubled haploid lines derived form wheat x maize crosses. *Theoretical and Applied Genetics*, **79**, 813-816.

LAURIE, D.A. AND REYMONDIE, S. (1991) High frequencies of fertilization and haploid seedling production in crosses between commercial hexaploid wheat varieties and maize. *Plant breeding*, **106**, 182-189.

LAURIE, D.A., O'DONOUGHUE, L.S. AND BENNETT, M.D. (1990) Wheat x maize and other wide sexual hybrids: their potential for crop improvement and genetic manipulations. In *Gene Manipulation in Plant Improvement* II. Edited by Gustafson, J.P. *Proceedings of 19th Stadler Genetics Symposium* pp. 95-126, Plenum Press, New York.

LAW, C.N., SUTKA, J. AND WORLAND, A.J. (1978) A genetic study of daylength response in wheat. *Heredity,* 41, 575-585.

LAW, C.N., WORLAND, A.J. AND GIORGI, B. (1976) The genetic control of ear-emergence time by chromosomes 5A and 5D of wheat. *Heredity*, **36**, 49-58.

LEDENT, J.F. (1976) Anatomical aspects of leaf angle changes during growth in wheat. *Phytomorphology*, **26**, 309–314.

LEE, B.T., MARTIN, M. AND BANGERTH, F. (1988) Phytohormones levels in the floret of a single wheat spikelet during pre-anthesis development and relationships to grain set. *Journal of Experimental Botany,* **39** (204), 927-933.

LEJEUNE P., PRINSEN E., VAN ONCKELEN H. AND BERNIER G. (1998) Hormonal control of ear abortion in a stress-sensitive maize inbred. *Australian Journal of Plant Physiology*, **25**, 481–488.

LEMAIRE, G. AND GASTAL, F. (1997) N uptake and distribution in plant canopies. In: *Diagnosis of the Nitrogen Status in Crop*. pp 3-43. (Ed. Lemaire, G.). Springer-Verlag, Heidelberg, Berlin.

LIM, P.O., WOO, H.R. AND NAM, H.G. (2003) Molecular genetics of leaf senescence in Arabidopsis. *Trends in Plant Science,* **8**, 272–278.

LIMON-ORTEGA, A., SAYRE, K.D., DRIJBER, R.A. AND FRANCIS, C.A. (2002) Soil attributes in a furrow-irrigated bed planting system in northwest Mexico. *Soil and Tillage Research,* **63** (3), 123-132.

LOSS, S.P. AND SIDDIQUE, K.H.M. (1994) Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. *Advances in Agronomy*, **52**, 229-276.

LUCAS, D. (1972) The effect of day length in primordia production of the wheat apex. *Australian Journal of Biological Sciences*, **25**, 649-656.

MA, H., SINGH, R.P. AND MUJEEB-KAZI, A. (1995a) Resistance to stripe rust in *Triticum turgidum*, *T. tauschii*, and their synthetic hexaploids. *Euphytica,* **82**, 117–124

MA, Y.Z. (1994) Source and sink manipulation in wheat: effects of kernel traits and assimilates in vegetative tissues. PhD thesis. University of Kentucky.

MA, Y.Z., MACKNOWN, C.T. AND VAN SANFORD, D.A. (1990) Sink manipulation in wheat: compensatory changes in kernel size. *Crop Science*, **30**, 1099-1105.

MA, Y.Z., MACKOWN, C.T. AND VAN SANFORD, D.A. (1995b) Kernel mass and assimilate accumulation of wheat: cultivar responses to 50% spikelet removal at anthesis. *Field crops Research*, **42**, 93-99.

MACBETH, J.E. (1996) The prediction of mature grain weight in winter wheat (*Triticum aestivum*, L.) PhD Thesis. Harper Adams Agricultural College, Shropshire.

MARSHALL, C. AND BOYD, W.J.R. (1985) A comparison of the growth and development of biculm wheat lines with freely tillering cultivars. *Journal of Agricultural Science*, **104** (1), 163-171.

MARTINEK, P. AND BEDNÁR, J. (2004) Gene resources with non-standard spike morphology in wheat. http://wheat.pw.usda.gov/ggpages/DEM/9IWGS/gene_resource.html

MARTINEZ-CARRASCO, R. AND THORNE, G.N. (1979) Physiological factors limiting grain size in wheat. *Journal of Experimental Botany*, **30**, 669-679.

MCCAIG, T.N. AND CLARKE, J.M. (1982) Seasonal changes in non-structural carbohydrate levels of wheat and oats grown in a semi-arid environment. *Crop Science*, **22**, 963-970.

MCDONALD, G.K., SUTTON, B.G. AND ELLISON, F.W. (1983) The effect of time of sowing on the grain yield of irrigated wheat in the Namoi valley, New South Wales. *Australian Journal of Agricultural Research*, **34**, 229-240.

MCMASTER, G.S., MORGAN, J.A. AND WILLIS, W.O. (1987) Effects of shading on winter wheat yield, spike characteristics and carbohydrate allocation. *Crop Science*, **27**, 967-973.

MIDMORE, D.J., CARTWRIGHT, P.M. AND FISCHER, T.A. (1982) Wheat in tropical environments. I. Phasic development and spike size. *Field crops Research*, **5**,185-200.

MILLET, E. (1986) Relationship between floret size and grain weight in aneuploid lines of homogenous group 5 chromosomes of common wheat cv. Chinese Spring. *Canadian Journal of Genetics and Cytology*, **28**, 497-501.

MIRALLES, D.J. AND RICHARDS, R.A. (1999) Sensitivity to photoperiod during the reproductive phase changes grain number in wheat and barley. *Proceedings of the Australian Society of Plant physiology*, 28 September-3 October, 1998. Adelaide, Australia.

MIRALLES, D.J. AND RICHARDS, R.A.(2000) Responses of leaf and tiller emergence and primordium initiation in wheat and barley to interchanged photoperiod. *Annals of Botany*, **85**, 655-663.

MIRALLES, D.J. AND SLAFER, G.A. (1997) Radiation interception and radiation use efficiency of near- isogenic wheat lines with different height. *Euphytica*, **97**, 201-208.

MIRALLES, D.J., RICHARDS, R.A. AND SLAFER, G.A. (2000) Duration of the stem elongation period influences the number of fertile florets in wheat and barley. *Australian Journal of Plant Physiology,* **27** (10), 931-940.

MIRALLES, D.J.AND SLAFER, G.A. (1995) Yield, biomass and yield components in dwarf, semidwarf and tall isogenic lines of spring wheat under recommended and late sowing dates. *Plant Breeding*, **114**, 392-396.

MONSI, M. AND SAEKI, T. (1953) Uber der lichtfactor in den pflanzengesellschaften und seine bedeutung fur die stoffproduktion. *Japanese Journal of Botany*, **14**, 22-52.

MONTEITH, J.L. (1965) Light distribution and photosynthesis in field crops. *Annals of Botany*, **29**, 17-37.

MONTEITH, J.L. (1977) Climate and the efficiency of crop production in Britain. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **281**, 277-294.

MORGAN, J.A., LECAIN, D.R. AND WELLS R. (1990) Semidwarfing genes concenrtrate photosynthetic machinery and affect leaf gas exchange of wheat. *Crop Science*, **30**, 602-608.

MORGAN, J.M. (1971) The death of spikelets in wheat due to water deficits. *Australian Journal of Experimental Agriculture and Animal Husbandry*, **11**, 349-351.

MOTZO, R., GIUNTA, F AND DEIDDA, M. (2004) Expression of a tiller inhibitor gene in the progenies of interspecific crosses Triticum aestivum L. x T. turgidum subsp. durum. *Field Crops Research*, **85** (1), 15-20.

MUIRA, H. AND WORLAND, A.J. (1994) Genetic control of vernalization, day length response, and earliness per se by homoeologous group 3 chromosomes in wheat. *Plant Breeding*, **113**, 160-169.

MUJEEB-KAZI A, FUENTES-DAVILA G, VILLAREAL RL, CORTES A, ROSAS V, DELGADO R (2001) Registration of 10 synthetic hexaploid wheat and 6 bread wheat germplasm resistant to Karnal bunt. *Crop Science*, **41**, 274.

MUURINEN, S. AND PELTONEN-SAINIO, P. (2006) Radiation-use efficiency of modern and old spring cereal cultivars and its response to nitrogen in northern growing conditions. *Field Crops Research*, **96**, 363-373.

NAM, H.G. (1997) The molecular genetic analysis of leaf senescence. *Current Opinion in Biotechnology*, **8**, 200–207.

NARSIMHAMOORTHY, B., GILL, B.S., FRITZ, AK., NELSON, J.C. AND BROWN-GUEDIRA, G.L. (2006) Advanced backcross QTL analysis of a hard winter wheat x synthetic wheat population. *Theoretical and Applied Genetics*, **112** (5), 787-796.

NATROVA, Z. AND NATR, L. (1993) Limitation of kernel yield by the size of conducting tissue in winter wheat varieties. *Field Crops Research*, **31**, 121-130.

NEI, N. (1963) The efficiency of haploid method in plant breeding. *Heredity*, **18**, 95-100.

NELSON, J.C. (1998) Genetic associations between photosynthetic characteristics and yield: review of the evidence. *Plant Physiology and Biochemistry*, **26**, 543-554.

NICOLAS, M. E., GLEADOW, R.M. AND DALLING, M. J. (1985) Effect of post-anthesis drought on cell division and starch accumulation in developing wheat grains. *Annals of Botany*, **55**, 433-444.

NOODÉN, L.D. AND GUIAMÉT, J.J. (1989) Regulation of assimilation and senescence by the fruit in monocarpic plants. *Physiologia Plantarum*, **77**, 267-274.

NOODÉN, L.D., GUIAMÉT, J.J. AND JOHN, I (1997) Senescence mechanisms. *Physiologia Plantarum*, **101**, 746-753.

OGBONNAYA, F., DRECCER, F., YEL, G. TRETHOWAN, R., LUSH, D., SHEPPERD, J. & VAN GINKEL, M. (2006). Yield of synthetic backcross-derived lines. pp12. . Reynolds MP & Godinez D (Eds): Extended Abstracts of the International Symposium on Wheat Yield Potential "Challenges to International Wheat Breeding" March 20-24th, 2006 Cd. Obregon, Mexico. CIMMYT, Mexico, D.F.

OSAKI, M., SHINANO, T AND TADONO, T. (1993) Effect of nitrogen application on the accumulation of ribulose, 1,5 bisphosphate carboxylae oxygenase and chlorophyll in several field crops. *Soil Science and Plant Nutrition*, **39**, 427-436.

OSMAN, A.M. AND MILTHROPE, F.L. (1971) Photosynthesis of wheat leaves in relation to age, illuminance and nutrient supply. II. Results. *Photosynthetica*, **5**, 61-70.

PARARAJASINGHAM, S. AND HUNT, L.A. (1996) Effects of photoperiod on leaf appearance rate and leaf dimensions in winter and spring wheats. *Canadian Journal of Plant Science*, **76**, 43-50.

PENG, S., CASSMAN, K.G. AND KROPFF, M.J. (1995) Relationship between leaf photosynthesis and nitrogen content of field-grown rice in tropics. *Crop Science*, **35**, 1627–1630.

PICARD, E. (1989) The male gamete as a tool in the genetic improvement of cereals. *Genome,* **31**, 1005-1013.

PINTER, P.J., ZIPOLI, G., REGINATO, R.J., JACKSON, R.D., IDSO, S.B. AND HOHMAN, J.P. (1990) Canopy temperature as an indicator of differential water use and yield performance among wheat cultivars. *Agricultural Water and Management*, **18**, 35-48.

PIRASTEH, B. AND WELSH, J.R. (1980) Effect of temperature on the heading date of wheat cultivars under a lengthening photoperiod. *Crop Science*, **20**, 453-456.

POLLOCK, C.J. AND CAIRNS, A.J. (1991) Fructan metabolism in grasses and cereals. *Annual Review of Plant Physiology and Plant Molecular Biology*, **42**, 77-101.

PUGSLEY, A.T (1966) The photoperiodic sensitivity of some spring wheats with special reference to the variety Thatcher. *Australian Journal of Agricultural Research*, **17** (5) 591-599.

RAHMAN, M.S. AND WILSON, J.H. (1977a) Determination of spikelet number in wheat. I. Effect of varying photoperiod on ear development. *Australian Journal of Agricultural Research*, **28**, 537-546.

RAHMAN, M.S. AND WILSON, J.H. (1977b) Genetic control of spikelet number in hexaploid wheat. *Crop Science*, **17**, 296-299.

RAHMAN, M.S. AND WILSON, J.H. (1978) Determination of spikelet number in wheat. III. Effect of varying temperature on ear development. *Australian Journal of Agricultural Science*, **29** (3), 459-467.

RAHMAN, M.S., HALLORAN, G.M. AND WILSON, J.H. (1978) Genetic control of spikelet number per ear with particular reference to rate of spikelet initiation in hexaploid. *Triticum aestivum* L. em. Thell. *Euphytica*, **27**, 69-74.

RAJARAM, S. AND REYNOLDS, M. P. (2001). *International Wheat Breeding.* Abstracts of XVIth EUCARPIA Congress, Edinburgh, Scotland, 'Plant Breeding: Sustaining the Future' 10-14 September 2001. EUCARPIA.

RAJARAM, S. AND VAN GINKEL, M. (1995) Wheat breeding methodology: International perspectives. Proceedings 20th hard red winter wheat workers workshop. Oklahoma City, USA.

RAMPINO, P., SPANO, G., PATALEO, S., MITA, G., NAPIER, J.A., DI FONZO, N., SHEWRY , P.R. AND PERROTTA, C. (2006) Molecular analysis of a durum wheat 'stay green' mutant: Expression pattern of photosynthesis-related genes. *Journal of Cereal Science*, **43**, 160–168.

RAWSON, H.M AND RUWALI, K.N. (1972) Ear branching as a means of increasing grain uniformity in wheat. *Australian Journal of Agricultural Research*, **23**, 551-559.

RAWSON, H.M. (1971) An upper limit for spikelet number per ear in wheat as controlled by photoperiod. *Australian Journal of Agricultural Research*, **22**, 537-546.

RAWSON, H.M. (1992) Plant responses to temperature under conditions of elevated CO_2. *Australian journal of Botany,* **40** (5), 473-490.

RAWSON, H.M. (1993) Radiation effects on development rate in a spring wheat grown under different photoperiods and high and low temperatures. *Australian Journal of Plant Physiology*, **20**, 719-727.

RAWSON, H.M. AND EVANS, L.T. (1970) The pattern of grain growth within the ear of wheat. *Australian Journal of Biological Sciences*, **23**, 753-764.

RAWSON, H.M. AND EVANS, L.T. (1971) The contribution of stem reserves to grain development in a range of wheat cultivars of different height. *Australian Journal of Agricultural Research*, **22**, 851-863.

RAWSON, H.M. AND RICHARDS, R.A. (1993) Effects of high temperature and photoperiod on floral development in wheat isolines differing in vernalisation and photoperiod genes. *Field crops Research*, **32**, 181-192.

RAWSON, H.M., GIFFORD, R.M. AND BREMMER, P.M. (1976) Carbon dioxide exchange in relation to sink demand in wheat. *Planta,* **132,** 19-23.

RAWSON, H.M., HINDMARSH, J.H., FISCHER,R.A., AND STOCKMAN,Y.M. (1983) Changes in leaf photosynthesis with plant ontogeny and relationships with yield per se in wheat cultivars and 120 progeny. *Australian Journal of Plant Physiology,* **10**, 503-514.

RAWSON, H.M.(1970) Spikelet number, its control and relation to yield per ear in wheat. *Australian Journal of Biological Sciences*, **23**, 1-15.

REBETZKE, G.J., READ, J.J, BARBOUR, M.M., CONDON, A.G. AND RAWSON, H.M. (2000) A handheld porometer for rapid assessment of leaf conductance in wheat. *Crop Science,* **40**, 277-280.

REES, D., SAYRE, K.D., ACEVEDO, E., NAVA SANCHEZ, T., LU, Z., ZEIGER, E., AND LIMON, A. (1993) Canopy temperatures of wheat: Relationship with yield and potential as a technique for early generation selection. Wheat Selection Report No. 10. Mexico, D.F., CIMMYT.

REYNOLDS, M. P., CALDERINI, D.F., CONDON, A.G. AND RAJARAM, S. (2001) Physiological basis of yield gains in wheat associated with the LR19 translocation from *Agropyron elongatum. Euphytica,* **119,** 137-41.

REYNOLDS, M. P., DRECCER F. AND TRETHOWAN, R. (2007). Drought Adaptive Traits derived from Wheat Wild Relatives and Landraces. *Journal of Experimental Botany,* **58** (2), 177-186.

REYNOLDS, M.P, SINGH, R.P., IBRAHIM, A. AGEEB, O.A.A. LARQUÉ-SAAVEDRA, A. AND QUICK, J.S.(1998) Evaluating physiological traits to complement empirical selection for wheat in warm environments. *Euphytica*, **100**, 85-94.

REYNOLDS, M.P. AND BORLAUG, N.E. (2006) Impacts of breeding on international collaborative wheat improvement. *Journal of Agricultural Science*, **144**, 3-17.

REYNOLDS, M.P. AND PFEIFFER, W.H. (2000) Applying physiological strategies to improve yield potential. CIHEAM-options Mediterranean's, 95-103.

REYNOLDS, M.P. BALOTA, M., DELGADO, M.I.B., AMANI, I. AND FISCHER, R.A. (1994) Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. *Australian Journal of Plant Physiology,* **21**, 717-730.

REYNOLDS, M.P., PELLEGRINESCHI, A. AND SKOVMAND, B. (2005) Sink limitation to yield and biomass: a summary of investigations in spring wheat. Annals of Applied Biology, **146**, 39-49.

REYNOLDS, M.P., RAJARAM, S. AND SAYRE, K.D. (1999) Physiological and genetic changes of irrigated wheat in the post-green revolution period and approaches for meeting projected global demand. *Crop Science*, **39**, 1611-1621.

REYNOLDS, M.P., SKOVMAND, B., TRETHOWAN, R., SINGH, R.P. AND VAN GINKEL. M. (2001) Applications of Physiology to Wheat Breeding. In *Proceeding of the Warren Kronstad Symposium*, Edited by Reeves J., McNab A. and Rajaram S. pp 139-141, CIMMYT, Mexico, D.F

REYNOLDS, M.P., VAN BEEM, J., VAN GINKEL, M. AND HOISINGTON, D. (1996) Breaking the yield barriers in wheat: a brief summary of the outcomes of an international consultation. In *Increasing yield potential in wheat:*

breaking the barriers. Edited by Reynolds, M.P., Rajaram, S., McNab, A., pp. 1-10, CIMMYT, Mexico, DF.

REYNOLDS, M.P., VAN GINKEL, M. AND RIBAUT, J.M. (2000) Avenues for genetic modification of radiation use efficiency in wheat. *Journal of Experimental Botany*, **51**, 459-473.

RICHARDS, R.A. (1988) A tiller inhibitor gene in wheat and its effect on plant growth. *Australian Journal of Agricultural Research*, **39**, 749–757.

RICHARDS, R.A. (1996) Increasing the Yield Potential of Wheat: Manipulating Source and Sinks. In *Increasing Yield Potential in Wheat: Breaking the Barriers*.Edited by Reynolds, M.P., Rajaram, S. and McNab, A., pp.134-149. CIMMYT, Mexico, D.F.

RICHARDS, R.A., REBETZKE, G.J., CONDON, A.G. AND VAN HERWAARDEN, A.F. (2002) Breeding opportunities for increasing the efficiency of water use and crop yield in temperate crops. *Crop Science,* **42,** 111-121.

RIERA-LIZARAZU, O. AND MUJEEB-KAZI, A. (1990) Maize (*Zea mays* L.) mediated wheat (*Triticum aestivum* L.) polyhaploid production using various crossing methods. *Cereal Research Communications*, **18**, 335-346.

RIJVEN, A.H.G.C. AND BANBURY, C.A. (1960) Role of the grain coat in wheat grain development. *Nature*, **188,** 546-547.

ROSENGRANT, M.W., AGCAOILI-SOMBILLA, M. AND PEREZ, N.D. (1995) Global food projections to 2020: Implication for investment. IFPRI, Washington, DC.

RUUSKA, S.A., REBETZKE, G.J., VAN HERWAARDENE, A.F., RICHARDS, R.A., FETTELL, N.A., TABE, L. AND JENKINS, C.L.D. (2006) Genotypic variation in water-soluble carbohydrate accumulation in wheat. *Functional Plant Biology*, **33**, 799-809.

SADRAS, V.O., HALL, A.J. AND CONNOR, D.J. (1993) Light-associated nitrogen distribution profile in flowering canopies of sunflower (*Helianthus annuus* L.) altered during grain growth. *Oecologia*, **95**, 488–494.

SARMA, R.N., FISH, L., GILL, B.S. AND SNAPE, J.W. (2000) Physical characterization of the homoeologous Group 5 chromosomes of wheat in terms of rice linkage blocks, and physical mapping of some important genes. *Genome*, **43**, 191-198.

SAVIN, R AND SLAFER, G.A. (1991) Shading effects in the yield of an Argentinean wheat cultivar. *Journal of Agricultural Science (Cambridge),* **116**, 1-7.

SAVIN, R. AND NICOLAS, M. (1996) Effects of short periods of drought and high temperature on grain growth and starch accumulation of two malting barley cultivars. *Australian Journal of Plant Physiology*, **23**, 201-210.

SAYRE, K.D., RAJARAM, S. AND FISCHER, R.A. (1997) Yield potential progress in short bread wheats in northwest Mexico. *Crop Science*, **37**, 36-42.

SCARTH, R. AND LAW, C.N. (1983) The location of the photoperiod gene Ppd 2 and an additional genetic factor for ear emergence time on chromosome 2B of wheat. *Heredity*, **51**, 607-619.

SCHNYDER, H. (1993) The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling-a review. *New Phytologist,* **123**, 233–245.

SCHNYDER, H. AND BAUM, U. (1992) Growth of the grain of wheat 9 (*Triticum aestivum* L.). The relationship between water content and dry matter accumulation. *European Journal of Agronomy*, **1**, 51-57.

SCOTT, R.W, APPLEYARD, M., FELLOWES, G. AND KIRBY, E.J.M. (1983) Effect of genotype and position in the ear on carpel and grain-growth and mature grain weight of spring barley. *Journal of Agricultural Science*, **100**, 383-391.

SEARS, E.R. (1954) The aneuploids of common wheat. *Missouri Agricultural Experimental Station Research Bulletin*, **572**, 1-58.

SEIDEL, P. (1996) Tolerance responses of plants to stress. The unused reserve in plant protection. *Plant Research and Development*, **44**, 81-99.

SHEARMAN, V.J, SYLVESTER-BRADLEY, R, SCOTT, R.K. AND FOULKES, M.J. (2005) Physiological processes associated with wheat yield progress in UK. *Crop Science*, **45**, 175-185.

SHIRATSUCHI, H., YAMAGISHI, T. AND ISHII, R. (2006) Leaf nitrogen distribution to maximize the canopy photosynthesis in rice. *Field Crops Research*, **95**, 291–304

SIDDIQUE, K.H.M., KIRBY, E.J.M. AND PERRY, M.W. (1989a) Ear to stem ratio in old and modern wheats: Relationship with improvements in number of grains per ear and yield. *Field crops Research*, **21**, 59-78.

SIDDIQUE, K.H.M., R.K. BELFORD, M.W. PERRY & D. TENNANT. (1989b) Growth, development and light interception of old and modern wheat cultivars in a Mediterranean-type environment. *Australian Journal of Agricultural Research*, **40**, 473-487.

SILVEY, V. (1986) The contribution of new varieties to cereal yields in England and Wales between 1947 and 1983. *Journal of National Institute of Agricultural Botany*, **17**, 155-168.

SINCLAIR, T.R. AND JAMIESON, P.D. (2006) Grain number, wheat yield, and bottling beer: An analysis. *Field Crops Research*, **98**, 60-67.

SINCLAIR, T.R. AND MUCHOW, R.C. (1999) Radiation Use Efficiency. *Advances in Agronomy*, **65**, 215-265.

SINGH, B.K AND JENNER, C.F. (1984) Factors controlling endosperm cell number and grain dry weight in wheat: Effects of shading on intact plants and of variation and nutritional supply to detached cultured ears. *Australian Journal of Plant Physiology*, **11**, 151-163.

SINGH, R.P., HUERTA-ESPINO, J. AND CROSSA, J. (1998) Agronomic effects from chromosome translocations 7Dl.7AG and 1BL.1RS in spring wheat. *Crop Science*, **38**, 27-33.

SINHA, S.K, AGGARWAL, P.K., CHATURVEDI, G.S., KOUNDAL, K.R. AND KHANNA-CHOPRA, R. (1981) A comparison of physiological and yield characters in old and new wheat varieties. *Journal of Agricultural Sciences,* **97**, 33-236.

SKOVMAND, B., REYNOLDS, M.P. AND DELACY, I.H. (2001) Searching genetic resources for physiological traits with potential for increasing yield. In *Application of Physiology in wheat breeding.* Edited by Reynolds, M.P., Ortiz-Monasterio,J.I. and McNab, A., pp. 11-18, CIMMYT, Mexico D.F.

SLAFER, G, CALDERINI, D. AND MIRALLES, D. (1996) Yield components and compensation in wheat. In *Increasing yield potential in wheat: Breaking the barriers.* Edited by Reynolds, M.P., Rajaram, S. and McNab, A., pp. 101 – 133, CIMMYT, Mexico D.F.

SLAFER, G.A AND ANDRADE, F.H. (1989) Genetic improvement in bread wheat (*Triticum aestivum*) yield in Argentina. *Field Crops Research*, **21**, 289-296.

SLAFER, G.A AND ANDRADE, F.H. (1991) Changes in physiological attributes of the dry matter economy of bread wheat (*Triticum aestivum*, L.) through genetic improvement of grain yield potential at different regions of the world. A review. *Euphytica*, **58**, 37-49.

SLAFER, G.A. (1996) Differences in phasic development rate amongst wheat cultivars independent of responses to photoperiod and vernalization. A viewpoint of the intrinsic earliness hypothesis. *Journal of Agricultural Science,* **126**(4), 403-419.

SLAFER, G.A. (2003) Genetic basis of yield as viewed from a crop physiologist's perspective. *Annals of Applied Biology*, 142 (2), 117-128.

SLAFER, G.A. AND ANDRADE, F.H. (1993) Physiological attributes related to the generation of grain yield in bread wheat cultivars released at different eras. *Field Crops Research* **31**: 351–367.

SLAFER, G.A. AND ARAUS, J.L. (1998) Keynote address: Improving wheat responses to abiotic stresses. In: Proceedings 9th International Wheat Genetics Symposium, Vol. 1. Saskatoon: University of Saskatchewan Extension Press.

SLAFER, G.A. AND RAWSON, H.M. (1994) Sensitivity of wheat phasic development to major environmental factors: A re-examination of some assumptions made by physiologists and modellers. *Australian Journal of Plant Physiology*, **21**, 393-426.

SLAFER, G.A. AND RAWSON, H.M. (1995) Intrinsic earliness and basic development rate assessed for their response to temperature in wheat. *Euphytica*, **83**, 175-183.

SLAFER, G.A. AND RAWSON, H.M. (1996) Responses to photoperiod change with phenophase and temperature during wheat development. *Field Crops Research*, **44,** 73-83.

SLAFER, G.A. AND RAWSON, H.M. (1997) Phyllochron in wheat as affected by photoperiod under two temperature regimes. *Australian Journal of Plant Physiology,* **24** (2), 151-158.

SLAFER, G.A. AND SAVIN, R. (1994) Source-sink relationships and grain mass at different positions within the spike in wheat. *Field Crops Research*, **37**, 39-49.

SLAFER, G.A. AND SAVIN, R. (1991) Developmental base temperature in different phonological phases of wheat (*Triticum aestivum*). *Journal of experimental Botany*, **42**, 1077-1082.

SLAFER, G.A., ABELEDO, L.G., MIRALLES, D.J., GONZALEZ, F.G. AND WHITECHURCH, E.M. (2001) Photoperiod sensitivity during stem elongation as an avenue to raise potential yield in wheat. *Euphytica*, **119**, 191-197.

SLAFER, G.A., ANDRADE, F.H. AND SATORRE, E.H. (1990) Genetic improvements effects on pre-anthesis physiological attributes related to wheat grain yield. *Field Crops Research*, **23**, 255-263.

SLAFER, G.A., ARAUS, J.L. AND RICHARDS, R.A. (1999) Physiological traits that increase the tield potential of wheat. In: Wheat: Ecology and Physiology of Yield Determination, pp.379-415. (Eds. Satorre, E.H. and Slafer, G.A.) Food Products Press, New York.

SLAFER, G.A., SATORRE, E.H. AND ANDRADE, F.H. (1994) Increases in grain yield in bread wheat from breeding and associated physiological changes. In *Genetic improvement of field crops*. Edited by Slafer, G.A. pp. 1-68, Marcel Dekker, New York.

SLAFER, G.A., ARAUS, J.L., ROYO, C., GARCIA DEL MORAL, L.F. (2005) Promising eco-physiological traits for genetic improvement of cereals yields in Mediterranean environments. *Annals of Applied Biology*, **146** (1), 61-70.

SNAPE, J.W. (1989) Doubled haploid breeding: Theoretical basis and practical applications. In: Mujeeb-Kazi, A, Sitch, LA (eds) Review of advances in plant biotechnology, 1985-88. CIMMYT/IRRI, Mexico DF, Mexico, Manila, Philippines, p 19-30.

SNAPE, J.W. (2001) The influence of genetics on future crop production strategies: from traits to genes and genes to traits. *Annals of Applied Biology*, **138**, 203-206.

SNAPE, J.W. AND WORLAND, A.J. (2001) Genetic basis of worldwide wheat varietal improvement. In: *The World Wheat Book. A History of Wheat Breeding*. Edited by Angus W.J. and Bonjeau, A.P. pp 59-100. Lavoisier Publishing, Paris.

SNAPE, J.W., BUTTEROWRTH, K., WHITECHURCH, E. AND WORLAND, A.J. (2001) Waiting for fine times: Genetics of flowering time in wheat. *Euphytica*, **119**, 185-190.

SOFIELD, I., EVANS, L.T., COOK, M.G. AND WARDLAW, I.F. (1977) Factors influencing the rate and duration of grain filling in wheat. *Australian Journal of Plant Physiology*, **4**, 785-797.

SOURDILLE, P., TIXIER, M.H., CHAREMT, G., GAY, G., CADALEN, T., BERNARD, S. AND BERNARD, M. (2000) Location of genes involve din ear compactness in wheat (*Triticum aestivum*) by means of molecular markers. *Molecular Breeding*, **6**, 247-255.

SPANO, G., DI FONZO, N., PERROTTA, C., PLATANI, C., RONGA, G., LAWLOR, D.W., NAPIER, J.A. AND SHEWRY, P.R. (2003) Physiological characterization of 'stay green' mutants in durum wheat. *Journal of Experimental Botany,* **54** (386), 1415-1420.

SPARKES, D.L., HOLME, S.J AND GAJU, O. (2006) Does light quality initiate tiller death in wheat? *European Journal of Agronomy,* **24**, 212-217.

SPIELMEYER, W. AND RICHARDS, R. (2004) Comparative mapping of wheat chromosome 1AS which contains the tiller inhibition gene (*tin*) with rice chromosome 5S. *Theoretical and Applied Genetics*, **109**, 1303-1310.

SPINK, J.H., SEMERE, T., SPARKES, D.L., WHALEY, J.M., FOULKES, M.J., CLARE, R.W. AND SCOTT, R.K. (2000) Effect of sowing date on the optimum plant density of winter wheat. *Annals of Applied Biology*, **137**, 179-188.

STEFANY, P. (1993) Vernalization requirement and response to day length in guiding development in wheat. Wheat special Report No. 22, CIMMYT, Mexico, D.F.

STELMAKH, A.F (1993) Genetic effects of *Vrn* genes on heading date and agronomic traits in bread wheat. *Euphytica*, **65**, 53-60.

STELMAKH, A.F. (1998) Genetic systems regulating flowering response in wheat. *Euphytica*, **100**, 359-369.

STOCKMAN, Y.M., FISCHER, R.A. AND BRITTAIN, E.G. (1983) Assimilate supply and floret development within the spike of wheat (*Triticum aestivum* L.). *Australian Journal of Plant Physiology*, **10**, 585-594.

STODDARD, F.L (1999) Variation in grain mass, grain nitrogen, and starch B-granule content within wheat heads. *Cereal Chemistry*, **76**, 139-144.

STONE, P.J. AND NICOLAS, M. (1994) Wheat cultivars vary widely in their responses of grain yield and quality to short periods of post-anthesis heat stress. *Australian Journal of Plant Physiology*, **21**, 887-900.

SUENAGA, K., KHAIRALLAH, M., WILLIAM, H.M. AND HOISINGTON, D.A. (2005) A new inter-varietal linkage amp and its application for quantitative trait locus analysis of 'gigas' features in bread wheat. *Genome*, **48**, 65-75.

SYLVESTER-BRADLEY, R., STOKES, D.T., SCOTT, R.K. AND WILLINGTON, V.B.A. (1990) A physiological analysis of the diminishing responses of winter wheat to applied nitrogen. 2. Evidence. *Aspects of Applied Biology*, **25**,289–300.

SYME, J.R. (1973) Quantitative control of flowering time in wheat cultivars by vernalization and photoperiod sensitivities. *Australian Journal of Agricultural Research*, **24**, 657-665.

TAKANO, Y. AND TSUNODA, S. (1971) Curvilinear regression of the leaf photosynthetic rate on leaf nitrogen content among strains of *Oryza* species. *Japanese Journal of Breeding*, **21**, 69-76.

THOMAS, B. AND VINCE-PRUE, D. (1997) Photoperiodism in plants. Academic Press, San Diego, CA. pp 428.

THOMAS, H AND HOWARTH, C.J.(2000) Five ways to stay green. *Journal of Experimental Botany*, **51**, 329-337.

THORNBER, J.P. 1975. Chlorophyll pigments: light harvesting and reaction centre components in plants. *Annual Review of Plant Physiology*, **26**, 127-158.

THORNE, G. N. (1971) Physiological factors limiting the yield of arable crops. In *Potential Crop Production*. Edited by Wareing, P. F. and Cooper J. P. pp. 143-158, Heinemann, London.

THORNE, G.N. (1982) Distribution between parts of the main shoot and the tillers of photosynthate produced before and after anthesis in the top 3 leaves of main shoots of Hobbit and Maris Huntsman winter-wheat. *Annals of Applied Biology,* **101**, 553-559.

THORNE, G. N. AND WOOD, D.W. (1987) Effects of radiation and temperature on tiller survival, grain number and grain yield in winter wheat. *Annals of Botany,* **59**, 413-426.

TOTTMAN, D.R AND BROAD, H. (1987) The decimal code for the growth stages of cereals with illustrations. *Annals of Applied Biology*, **110**, 441-454.

TIAN, F., ZHU, Z., ZHANG, B., TAN, L., FU, Y., WANG, X AND SUN, C.Q. (2006) Fine mapping of a quantitative trait locus for grain number per panicle from wild rice (*Oryza rufipogon* Griff.). *Theoretical and Applied Genetics*, **113**, 619-629.

TOLLENAAR, M. (1991) Physiological basis of genetic improvement of maize hybrids in Ontario from 1959 to 1988. *Crop Science,* **31**, 119-124.

TRENBATH, B. R. AND ANGUS, J. F. (1975) Leaf inclination and crop production. *Field Crop Abstracts,* **28**, 231-244.

TURGEON, R. (1989). The sink-source transition in leaves. *Annual Revenue of Plant Physiology and Plant Molecular Biology*, **40**, 119-138.

UAUY, C., DISTELFELD, A., FAHIMA, T., BLECHL, A. AND DUBCOVSKY, J (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science*, **314**, 1298-1301.

UEMURA, K., ANWARUZZAMAN, S., MIYACHI, S. AND YAKOTA, A. (1997) Ribulose-1,5-bisphosphate carboxylase/oxygenase from thermophilic red algae with a strong specificity for CO2 fixation. *Biochemistry and Biophysics Research Communications*, **233**, 568-571.

VAN BEEM, J., MOHLER, V., LUKMAN, R., VAN GINKEL, M., WILLIAM, M., CROSSA, J. AND WORLAND, A.J. (2005) Analysis of genetic factors influencing the developmental rate of globally important CIMMYT wheat cultivars. *Crop Science,* **45**, 2113-2119.

VILLAREAL, R.L., BAÑUELOS, O., MUJEEB-KAZI, A. AND RAJARAM, S. (1998) Agronomic performance of chromosomes 1B and T1BL.1RS near-isolines in spring bread wheat Seri M82. *Euphytica*, **103**, 195–202.

VILLAREAL, R.L., DEL TORO, E., MUJEEB-KAZI, A. AND RAJARAM, S. (1995) The 1BL/1RS chromosome translocation effect on yield characteristics in a *Triticum aestivum* L. cross. *Plant Breeding*, **114**, 497-500.

VILLEREAL, R.L., RAJARAM, S., MUJEEB-KAZI, A. AND DEL TORO, E. (1991) The effect of chromosome 1B/1R translocation on the yield potential of certain spring wheats (*Triticum aestivum* L.). *Plant Breeding,* **106**, 77-81.

WADDINGTON, S.R., CARTWRIGHT, PM., WALL, P.C (1983) A quantitative scale of spike initial and pistil development in barley and wheat. *Annals of Botany,* **51**, 119-130.

WADDINGTON, S.R., RANSOM, J.K., OSMANZAI, M. AND SAAUNDERS, D.A. (1986) Improvement in the yield potential of bread wheat adapted to northwest Mexico. *Crop Science*, **26**, 698-703.

WANG, Z., CAO, W., DAI, T. AND ZHOU, Q. (2001) effects of exogenous hormones on floret development and grain set in wheat. *Plant Growth Regulation*, **35**, 225-231.

WARDALW, I.F. (1994) The effect of high temperature on kernel development in wheat: variability related to pre-heading and post-anthesis conditions. *Australian Journal of Plant Physiology*, **21**, 731-739.

WARDLAW, I.F. (1968) The control and pattern of movement of carbohydrate in plants. *Botanical Review*, **34**, 79-105.

WARDLAW, I.F. (1970) The early stages of grain development in wheat: response to light and temperature in a single variety. *Australian Journal of Biological Sciences*, **23**, 765-774.

WARDLAW, I.F. (1990) The control of carbon partitioning in plants. *New Phytologist*, **116**, 341-381.

WARDLAW, I.F. (1994) The effect of high temperature on kernel development in wheat: variability related to pre-heading and post-anthesis conditions. *Australian Journal of Plant Physiology*, **21**, 731-739.

WARDLAW, I.F. AND WILLENBRINK, J. (1994) Carbohydrate storage and mobilization by the culm of wheat between heading and grain maturity: the relation to sucrose synthase and sucrose phosphate synthase. *Australian Journal of Plant Physiology*, **21**, 255-271.

WATERS, S. P., MARTIN, P. AND LEE, B. T. (1984) The influence of sucrose and abscisic acid on the determination of grain number in wheat. *Journal of Experimental Botany,* **35,** 829-840.

WEIR, A.H., BRAGG, P.L., PORTER, J.R., RAYNER, J.H. (1984) A winter wheat crop simulation model without water or nutrient limitations. *Journal of Agricultural Sciences, Cambridge,* **102**, 371-382.

WHINGWIRI, E.E., KUO, J. AND STERN, W.R. (1981) The vascular system in the rachis of a wheat ear. *Annals of Botany*, **48**, 189-201.

WHINGWIRI, E.E. AND KEMP, D.R. (1980) Spikelet development and grain yield of the wheat ear in response to applied nitrogen. *Australian Journal of Agricultural Research*, **31**, 637–647.

WHITECHURCH, E.M AND SLAFER, G.A. (2002) Contrasting Ppd alleles in wheat: effects on sensitivity to photoperiod in different phases. *Field Crops Research*, **73**(2) 95-105.

WHITECHURCH, E.M. AND SLAFER, G.A. (2001) Responses to photoperiod before and after jointing in wheat substitution lines. *Euphytica,* **118** (1), 47-51.

WIEGAND, C.L. AND CUELLAR, J.A. (1981) Duration of grain filling and kernel weight of wheat as affected by temperature. *Crop Science*, **21**, 95-101.

WILLEY, R.W. AND HOLLIDAY, R. (1971) Plant population, shading and thinning studies in wheat. *Journal of Agricultural Science, Cambridge* 77:453-461.

WORLAND, A.J. (1996) The influence of flowering time genes on environmental adaptability in European wheats. *Euphytica*, 89 (1) 49-57.

YAN, L., LOUKOIANOV, A., BLECHL, A., TRANQUILLI, G., RAMAKRISHNA, W., SANMIGUEL, P., BENNETZEN, J., ECHENIQUE, V. AND DUBCOVSKY, J. (2004) The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. *Science*, **303**, 1640-1644.

YANG, J., ZHANG, J., HUANG, Z., ZHU, Q., AND WANG, L. (2000) Remobilization of carbon reserves is improved by controlled soil-drying during grain filling of wheat. *Crop Science,* **40**, 1645-1655.

YANO, M. (2001) Genetic and molecular dissection of naturally occurring variation. *Current Opinion in Plant Biology*, **4** (2), 130-135

YANO, M., KAYATOSE, Y., ASHIKARI, M., YAMANOUCHI, U., MONNA, L., FUSE, T., BABA, T., YAMAMOTO, K., UMEHARA, Y., NAGAMURA, Y. AND SASAKI, T. (2000) *Hd1*, a major photoperiod sensitivity of quantitative trait locus in rice is closely related to the Arabidopsis flowering time gene CONSTANS. *The Plant Cell,* **12**, 2473-2483.

YANUSA, I.A.M., SIDDIQUE, K.H.M., BELFORD, R.K. AND KARIMI, M.M. (1993) Effect of canopy structure on efficiency of radiation interception and use in spring wheat cultivars during the pre-anthesis period in a Mediterranean-type environment. *Field Crops Research*, **35**, 113-122.

YOSHIDA, S. (2003) Molecular regulation of leaf senescence. *Current Opinion in Plant Biology*, **6**, 79–84.

YOSHIDA, S. AND CORONEL, V. (1976) Nitrogen nutrition, leaf resistance and leaf photosynthetic rate of the rice plant. *Soil Science and Plant Nutrition*, **22**, 207-211

YOUSSEFIAN, S., KIRBY, E.J.M., AND GALE, M.D. (1992) Pleiotropic effects of the GA-insensitive *Rht* dwarfing genes in wheat. I. Effects on development of the ear stem and leaves. *Field Crops Research,* **28**, 191-210.

ZADOKS, J.C., CHANG T.T. AND KONZAK, C.F. (1974) A decimal code for the growth stages in cereals. *Weed Research,* **14**, 415-421.

ZENG, Z.R., MORGAN, J.M. AND KING, R.W. (1985) Regulation of grain number in wheat: genotype difference and responses to applied abscisic acid and high temperature. *Australian Journal of Plant Physiology*, **12**, 609-619.

APPENDICES

Appendix I: Management and Cultural practices in field experiment at Cd. Obregon in 2004, 2005 and 2006

Fertilizer application

- Land preparation: 75 kg ha^{-1} of N as urea
- First irrigation: 75 kg ha^{-1} of N as urea (11 January 2004, 18 January 2005, 5 January 2006
- 40 kg ha^{-1} Triple Super Phosphate at sowing (17 November 2003, 24 November 2004, 22 November 2005)

Irrigation regime

Plots were irrigated when the soil start drying out by using a gravity-based system where water was flowed in the field until soil was saturated. During the crop cycle in 2004 the plants were irrigated four times namely on 11 January, 6 February, 5 and 24 March. In 2005, irrigation was carried out on 18 January, 4 February, 1 and 17 March. In 2006, irrigation was carried out on 5 January, 3 February, 24 February, 17 March and 7 April.

Pesticides application

Table A1: Pesticide regime throughout crop cycle 2003/04

Pesticides	Date	Dosage
Herbicides		
Broad leaves	8 January 2004	Starane (Fluroxipir) @ 750 ml/ha
		Buctril (Bromoxinil) @ 1250 ml/ha
Narrow leaves	25 January 2004	Topik (Clodinafop-Propargyl) @ 750 ml/ha
Insecticides	28 January 2004	Aflix (Dimetoato) 1L/ha (By airplane)
	24 March 2004	Lorsban (Clorpirifos) 1L/ha (By airplane)
Fungicides	3 February 2004	Folicur (Tebuconazol) @ 500 ml/ha
	15 March 2004	Folicur (Tebuconazol) @ 500 ml/ha

Table A2: Pesticide regime throughout crop cycle 2004/05

Pesticides	Date	Dosage
Herbicides		
Broad leaves	16 December 2004	Penetrator (Coadyudante)2L/ha
Narrow leaves	21 December 2004	Topik (Clodinafop-Propargyl) @ 1.7L/ha
Insecticides	28 January 2005	General
	9 February 2004	Perfekthion
	22 March 2004	Lorsban 1L/ha
Fungicides	2 October 2004	Folicur (Tebuconazole) @ 500 ml/ha
	25 February 2005	Folicur (Tebuconazole) @ 500 ml/ha
	29 March 2005	Folicur (Tebuconazole) @ 500 ml/ha

Table A3: Pesticide regime throughout crop cycle 2005/06

Pesticides	Date	Dosage
Herbicides		
Broad leaves	19 December 2005	Topik (Clodinafop-Propargyl) @ 0.85L/ha
Narrow leaves	23 December 2005	Brominal @ 0.8 L/ ha Starane @ 1.2 L/ ha
Insecticides	20 January 2006	Aflix @ 1.2 L/ha
	17 February 2006	Aflix @ 1.2 L/ha
Fungicides	21 January 2006	Folicur (Tebuconazole) @ 1.2 L/ha
	24 February 2006	Folicur (Tebuconazole) @ 1.2 L/ha
	7 March 2006	Folicur (Tebuconazole) @ 1.2 Ll/ha

Appendix II: Results form parental genotype experiment in 2002/3

Table A 4: Plants m^{-2} of parental genotypes in 2003

Genotypes	Plants m^{-2}
Bacanora	180
NL1	173
NL2	167
Mean	173
SED (df=8)	20.5

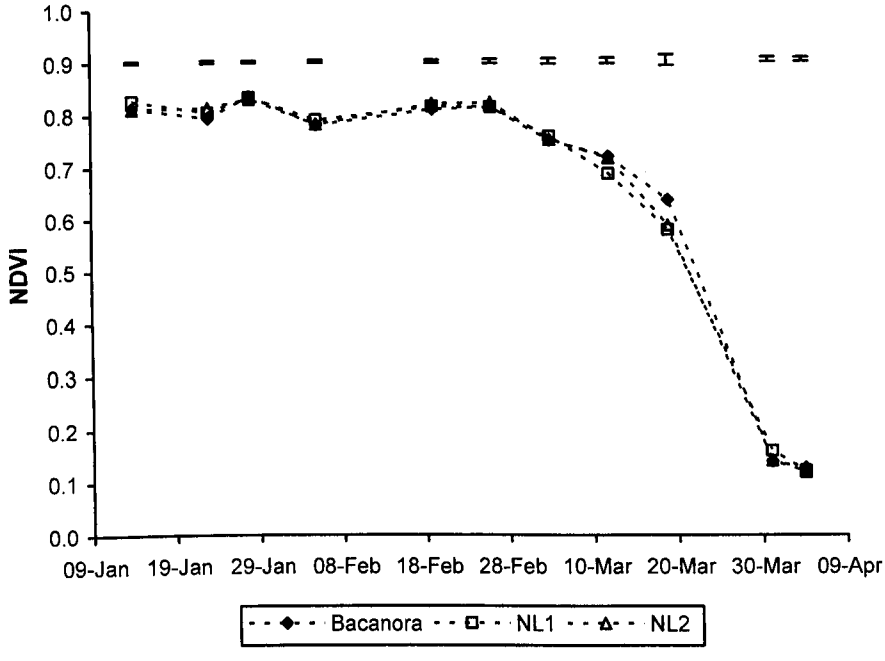


Figure A1: NDVI of parental genotypes in 2003

Appendix III

GENSTAT program and example output for (a) broken stick analysis applied to data in Chapter 5 and (b) quantifying relationship between leaf emergence and tiller production

(a) The program used in broken stick analysis to calculate rate and duration of spikelet primordia production.

Program

```
read spikelet

0 8 8 10 28 26 28 28 30
:
vari date;! ((330, 405, 465, 555, 645, 735, 795, 870,
945)1)
model spikelet
vari [9] ones
calc ones=1
expr twol [1];!e (Xmt=date-t)
&twol [2] ; !e (U=Xmt* (Xmt<=0))
&twol [3] ; !e (V=0* (Xmt>0))
calc t=750
rcycle t;step=2
fitnonl[calc=twol[1,2,3];cons=omit;selin=y;pr=mo,su,est,f
] ones,U,V
rgraph [g=h] index=date
stop
```

't' is the point where the two lines meet (giving the date, in thermal time of the duration)
'U' is te gradient of the first line (giving the rate of spikelet production)
'V' is the gradient of the second line (always 0)
'Ones' is the value of the spikelet number at the point of intersection (giving the maximum number produced)

The program selects the two lines and the point of intersection by calculating the overall fit that gives the smallest residual sum of squares. This is done iteratively within the program. An initial estimate for the point t, where the two lines meet, is put into the program to speed up the procedure. In this case; initial value is 750.

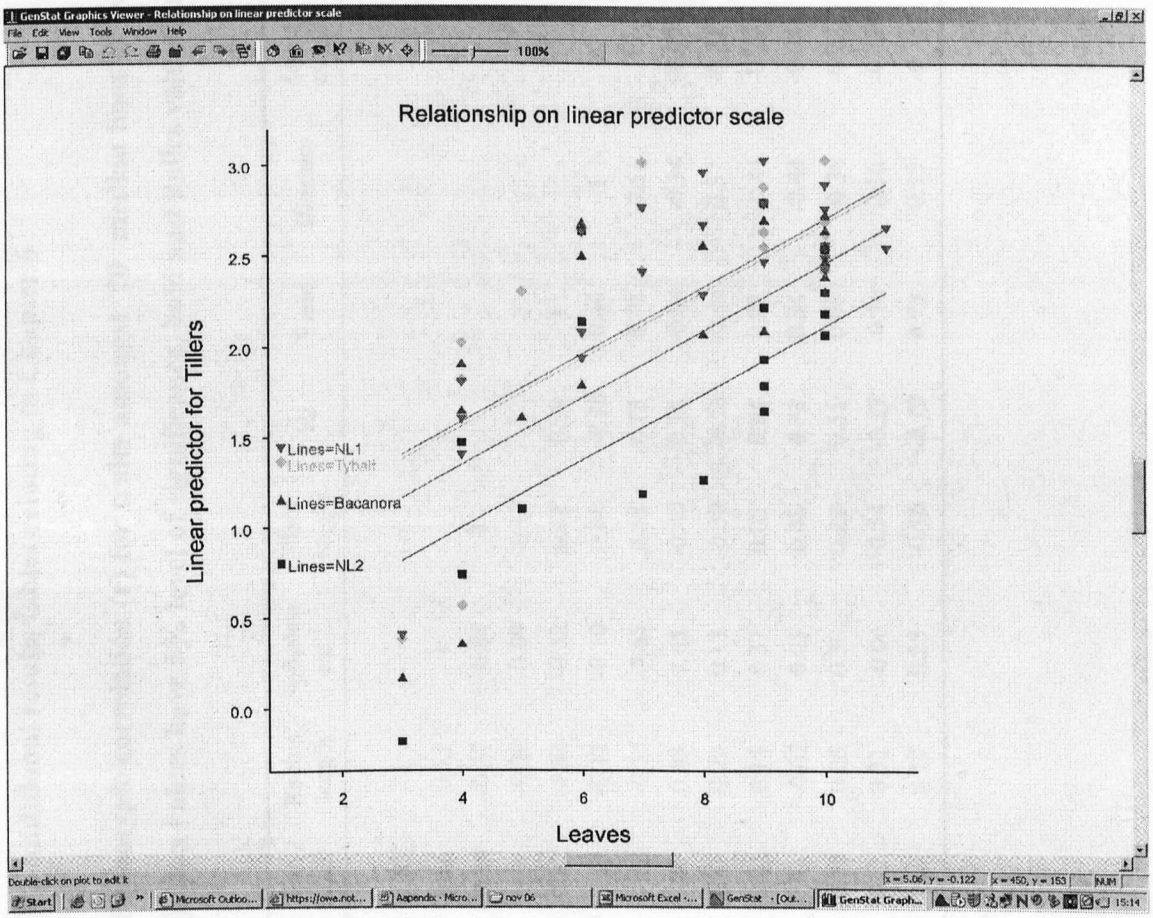
Output

```
Nonlinear regression analysis

Response variate: spikelet
Nonlinear parameters:     t
Model calculations:  twol[1], twol[2], twol[3]


Summary of analysis

Source      d.f.       s.s.       m.s.     v.r.
Regression     4    4070.14    1017.53    59.25
Residual       5      85.86      17.17
Total          9    4156.00     461.78

Percentage variance accounted for 87.4
Standard error of observations is estimated to be 4.14.


Estimates of parameters

Parameter     estimate       s.e.
t                747.0       58.7
* Linear
ones              28.67       2.39
U               0.0683     0.0122
V                    0          *

Message: some standard errors are unavailable due to
singularity.


Fitted values and residuals

                                        Standardized
Unit          Response Fitted value     residual
1                 0.00        0.19        -0.05
2                 8.00        5.31         0.65
3                 8.00        9.41        -0.34
4                10.00       15.55        -1.34
5                28.00       21.70         1.52
6                26.00       27.85        -0.45
7                28.00       28.67        -0.16
8                28.00       28.67        -0.16
9                30.00       28.67         0.32

Mean             18.44       18.44         0.00

16  rgraph [g=h] index=date
17  stop
```

(b) Relationship between leaf emergence and tiller produciton

```
Regression analysis

Response variate: Tillers
Distribution: Poisson
Link function: Log
Fitted terms: Constant + Lines + Leaves


Summary of analysis

                                        mean    deviance approx
Source          d.f.     deviance     deviance    ratiochi pr
Regression         4        238.7       59.676    59.68 <.001
Residual         115        173.6        1.510
Total            119        412.3        3.465

Dispersion parameter is fixed at 1.00.
```

GENSTAT output chart for the regression analysis



Appendix IV –Additional results tables relating to Chapter 6

Table A5. Phenotypic correlation (r) for traits amongst 20 median lines of NL2 x Rialto DH population average over two years (2005 and 2006) (italics values have 5% level of significance, bold and italics values have 1% level of significance)

	Ear index	Rachis length	Spikelets ear^{-1}	Plant height	HI	Yield	Biomass	Grain weight	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}	Grains spikelet^{-1}	Grains :Ear DM	Ears DM (g m^{-2})	Flowering date
Ear index	1														
Rachis length	0.20	1													
Spikelets ear^{-1}	-0.16	*0.53*	1												
Plant height	***-0.80***	-0.37	-0.07	1											
HI	0.23	-0.20	-0.09	-0.17	1										
Yield	-0.22	-0.34	-0.32	0.41	0.39	1									
Biomass (AGDM)	-0.53	-0.11	-0.10	*0.58*	***-0.66***	0.40	1								
Grain weight	-0.38	*-0.55*	***-0.65***	*0.58*	0.13	***0.76***	*0.51*	1							
Grains m^{-2}	0.29	0.43	***0.65***	-0.40	0.26	-0.04	-0.34	***-0.67***	1						
Ears m^{-2}	0.12	0.20	0.13	-0.30	***-0.76***	-0.44	0.37	-0.31	-0.06	1					
Grains ear^{-1}	-0.01	0.15	0.37	0.05	***0.65***	0.22	-0.44	-0.24	***0.65***	***-0.75***	1				
Grains spikelets^{-1}	0.03	-0.02	0.05	0.08	***0.73***	0.34	-0.44	-0.04	0.47	***-0.85***	***0.95***	1			
Grains: ear DM	-0.23	0.05	0.30	0.22	0.51	0.08	-0.38	-0.22	0.48	***-0.65***	***0.88***	***0.85***	1		
Ears DM (g m^{-2})	*0.52*	0.31	-0.06	-0.34	-0.37	-0.16	0.16	-0.18	0.01	*0.51*	-0.35	-0.35	*-0.58*	1	
Flowering date	0.00	0.15	0.35	-0.16	***-0.72***	***0.73***	0.15	*-0.56*	-0.02	***0.63***	-0.36	*-0.49*	-0.26	0.41	1

Table A6: Effect of *tin* gene on different traits amongst of the 59 lines of NL2x Rialto DH population in 2005 and 2006

Traits	Effect of *tin* gene
Biomass (g m^{-2})	P=0.97, + 10 g m^{-}
HI	P=0.91, 0
Ears m^{-2}	P=0.02, - 83 ears m^{-2}
Grains ear^{-1}	P=0.006, + 20.62 grains ear^{-1}
Grains m^{-2}	P=0.88, + 3643 g m^{-2}
TGW (g)	P=0.94, + 5.78 g
Yield (g m^{-2})	P=0.78, + 8.1g m^{-2}
Rachis length (cm)	P < 0.001, + 2.78 cm
Spikelets ear^{-1}	P=0.22, + 4.13 spikelets ear^{-1}
Ear index	P=0.15, 0
Grains to ear DM ratio (grains g^{-1})	P=0.17, + 3.13 grains g^{-1}

Genstat output model of regression analysis of *tin* gene effect on the different traits

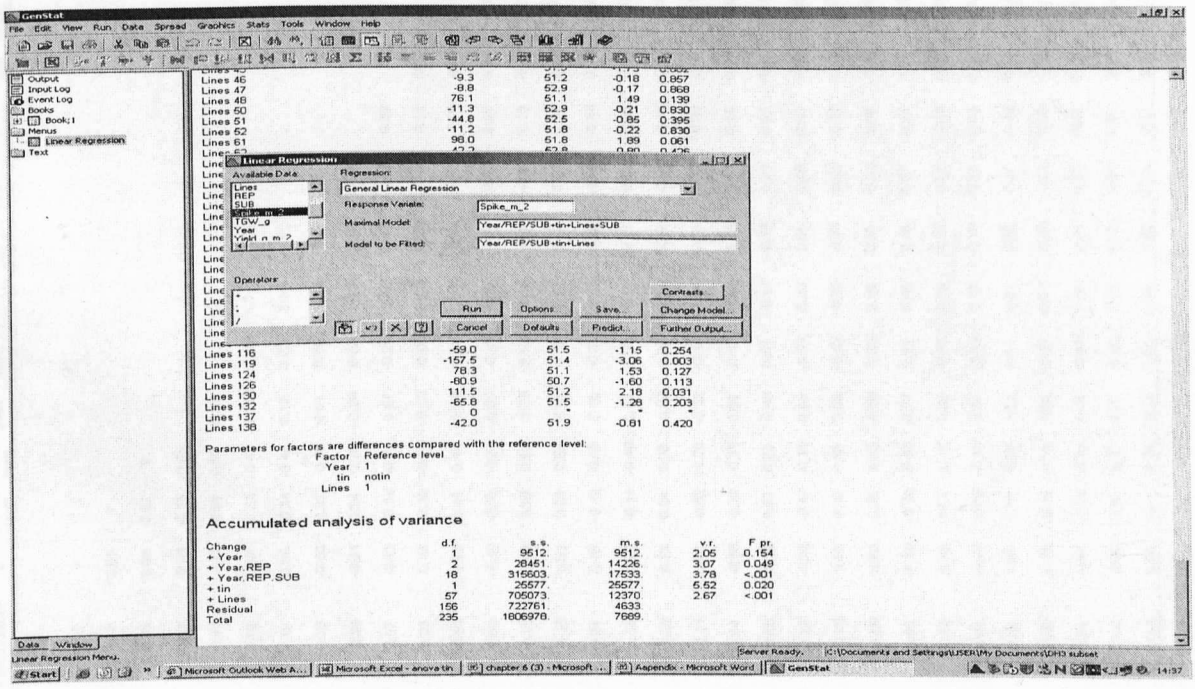


Table A7. Correlation matrix of subset of 15 DH lines of NL2 x Rialto population in 2006

	Conductance	k	WSC (ant)	WSC(mat)	PAR	RUE	AGDM (GS 31)	AGDM (GS 41)	AGDM (GS 61)	AGDM harvest	Grains/ m2	Grains/ ear	HI	Ears/ m2	TGW	Yield	Rachis	Spikelet/ ear	Ear DM	Ear index	GAI	Plant height	Grains to Ear DM	CT (veg)	CT (GF)	NDVI (veg)	NDVI (GF)	Fertile shoots	Ears DM (g m^{-2})	Grains/ spikelet	Phenology	Duration	SLW
Conductance	1																																
k	-0.52	1																															
WSC (ant)	-0.47	0.05	1																														
WSC(mat)	-0.71	0.09	0.82	1																													
PAR	0.80	-0.44	-0.18	-0.49	1																												
RUE	-0.47	-0.11	0.63	0.60	-0.38	1																											
AGDM (GS 31)	0.16	0.04	-0.33	-0.48	0.17	0.24	1																										
AGDM (GS 41)	0.16	0.05	-0.34	-0.48	0.17	0.23	1.00	1																									
AGDM (GS 61)	0.39	-0.50	0.31	-0.06	0.61	0.46	0.42	0.42	1																								
AGDM harvest	-0.24	-0.31	0.24	0.28	-0.20	0.62	0.22	0.22	0.36	1																							
Grains/m2	0.57	-0.33	-0.54	-0.72	0.47	-0.28	0.43	0.44	0.27	0.29	1																						
Grains/ear	0.02	0.16	-0.19	-0.28	-0.13	-0.09	0.23	0.23	-0.12	0.13	0.51	1																					
HI	-0.53	0.70	0.16	0.14	-0.67	0.30	0.07	0.07	-0.35	-0.06	-0.27	0.35	1																				
Ears/m2	0.60	-0.54	-0.37	-0.51	0.65	-0.21	0.26	0.27	0.43	0.20	0.61	-0.36	-0.65	1																			
TGW	-0.76	0.40	0.60	0.82	-0.75	0.53	-0.35	-0.36	-0.30	0.13	-0.84	-0.21	0.52	-0.73	1																		
Yield	-0.55	0.21	0.30	0.32	-0.61	0.69	0.23	0.23	0.05	0.76	0.04	0.31	0.60	-0.26	0.46	1																	
Rachis	0.21	0.46	-0.13	-0.46	0.16	-0.33	0.20	0.20	-0.03	-0.44	0.33	0.60	0.40	-0.20	-0.33	-0.11	1																
Spikelet/ear	0.52	0.09	-0.11	-0.49	0.44	-0.26	0.14	0.13	0.23	-0.26	0.54	0.57	0.13	0.04	-0.50	-0.15	0.83	1															
Ear DM	-0.32	0.73	0.24	0.06	-0.29	0.20	0.37	0.37	-0.04	-0.19	-0.22	0.32	0.61	-0.55	0.31	0.25	0.64	0.30	1														
Ear index	-0.11	0.77	-0.02	-0.20	-0.12	-0.11	0.34	0.34	-0.13	-0.36	-0.07	0.28	0.50	-0.38	0.09	0.04	0.74	0.43	0.92	1													
GAI	0.74	-0.93	-0.22	-0.35	0.63	-0.12	0.09	0.09	0.51	0.20	0.54	0.03	-0.75	0.60	-0.63	-0.33	-0.20	0.14	-0.60	-0.57	1												
Plant height	-0.58	-0.26	0.57	0.72	-0.48	0.66	-0.14	-0.15	0.09	0.68	-0.20	0.11	0.00	-0.27	0.47	0.54	-0.48	-0.46	-0.11	-0.42	0.06	1											
Grains to Ear DM ratio	0.22	-0.58	-0.47	-0.31	0.08	-0.16	0.05	0.05	-0.08	0.42	0.62	0.16	-0.37	0.54	-0.49	0.09	-0.37	-0.15	-0.75	-0.72	0.55	0.22	1										
CT (veg)	-0.05	0.10	0.10	0.19	0.09	-0.42	-0.70	-0.71	-0.42	-0.30	-0.16	-0.14	-0.15	-0.08	0.05	-0.36	-0.01	0.12	-0.28	-0.10	-0.13	-0.17	-0.04	1									
CT (GF)	-0.69	0.82	0.26	0.35	-0.60	0.29	0.03	0.03	-0.33	0.06	-0.32	0.24	0.71	-0.60	0.53	0.50	0.26	0.05	0.62	0.55	-0.87	0.12	-0.44	0.02	1								
NDVI (veg)	0.63	-0.30	-0.20	-0.46	0.73	0.01	0.46	0.46	0.67	0.26	0.55	-0.18	-0.38	0.75	-0.57	-0.06	-0.04	0.29	-0.23	-0.10	0.43	-0.38	0.18	-0.19	-0.32	1							
NDVI (GF)	-0.03	0.46	0.00	-0.17	0.29	-0.24	0.33	0.33	0.07	-0.15	0.08	0.31	0.00	-0.14	-0.17	-0.12	0.35	0.26	0.42	0.47	-0.24	-0.21	-0.30	0.02	0.36	0.18	1						
Fertile shoots	0.64	-0.67	-0.26	-0.40	0.70	-0.18	0.17	0.17	0.51	0.09	0.45	-0.48	-0.77	0.95	-0.66	-0.42	-0.28	-0.01	-0.59	-0.45	0.69	-0.24	0.44	-0.07	-0.72	0.67	-0.22	1					
Ears DM (g m^{-2})	0.09	0.47	0.12	-0.23	0.20	0.11	0.52	0.52	0.37	-0.15	0.09	0.24	0.28	-0.14	-0.08	0.06	0.69	0.53	0.84	0.87	-0.27	-0.35	-0.70	-0.28	0.36	0.23	0.51	-0.18	1				
Grains/spikelet	0.56	-0.73	-0.15	-0.15	0.69	-0.27	-0.05	-0.05	0.38	0.05	0.28	-0.36	-0.99	0.67	-0.53	-0.61	-0.41	-0.13	-0.63	-0.52	0.78	0.00	0.38	0.13	-0.74	0.41	-0.02	0.78	-0.30	1			
Phenology	0.76	-0.44	-0.15	-0.45	0.99	-0.43	0.06	0.06	0.55	-0.27	0.40	-0.16	-0.69	0.61	-0.72	-0.68	0.14	0.41	-0.35	-0.17	0.61	-0.47	0.07	0.18	-0.61	0.65	0.28	0.69	0.13	0.70	1		
Duration	0.04	-0.04	0.30	0.07	0.29	0.12	0.12	0.12	0.34	0.01	0.11	0.36	-0.14	-0.14	-0.21	-0.11	0.25	0.32	0.20	0.12	0.16	0.23	-0.13	-0.01	0.15	0.05	0.60	-0.15	0.31	0.16	0.30	1	
SLW	-0.13	0.35	0.11	-0.09	-0.13	0.34	0.60	0.60	0.21	0.01	0.00	-0.07	0.34	0.04	0.07	0.25	0.36	0.12	0.69	0.63	-0.30	-0.12	-0.41	-0.46	0.22	0.13	-0.02	0.00	0.66	-0.33	-0.17	0.11	1

Table A8. Analysis of variance for traits measured in 59 lines of NL2x Rialto DH population in 2005

Lines	Biomass ($g\ m^{-2}$)	Shoot m^{-2}	SPAD flag	Rachis	Spike Index	spikelet $spike^{-1}$	Height (cm)	HI	Yield ($g\ m^{-2}$)	Biomass ($g\ m^{-2}$)	TGW (g)	Grain m^{-2}	Spike m^{-2}	Grains/ spike
1	824.80	464.50	51.51	13.55	0.34	27.11	67.86	0.36	484.50	1257	26.23	18491	346.60	50.98
2	711.30	515.20	50.21	12.44	0.36	24.96	55.92	0.37	466.90	1186	32.15	14618	485.30	28.47
3	1208.30	430.50	51.15	14.56	0.25	26.30	84.96	0.22	447.90	2046	29.71	15126	518.20	28.08
5	781.00	555.00	49.44	13.48	0.38	25.55	63.34	0.41	433.60	1032	29.08	14935	335.20	43.10
7	707.50	577.30	45.96	11.75	0.34	23.66	59.42	0.34	409.60	1254	29.26	14683	445.70	33.25
10	933.50	474.70	48.72	13.93	0.32	25.00	57.59	0.27	308.10	1179	20.73	14984	484.40	30.33
11	739.90	380.40	52.23	11.19	0.24	21.76	91.16	0.41	550.90	1366	39.01	14170	307.40	46.20
12	814.80	504.10	55.62	13.15	0.33	24.56	46.75	0.31	385.70	1338	25.79	14910	541.60	28.24
13	772.90	317.80	53.05	12.61	0.42	23.51	54.28	0.44	537.30	1250	33.29	16228	377.00	43.79
14	712.50	415.80	46.27	13.78	0.23	27.16	98.67	0.41	572.50	1427	39.95	14356	322.40	44.84
17	760.20	503.20	42.27	11.08	0.24	20.56	89.77	0.42	593.10	1426	30.36	19710	419.70	45.92
18	889.50	297.50	52.63	12.83	0.29	23.31	86.32	0.36	734.70	2108	49.13	14945	412.90	37.19
19	829.90	493.70	50.13	13.88	0.35	26.00	58.8	0.39	386.20	1048	27.23	14112	333.40	44.94
21	825.00	358.00	50.13	14.38	0.38	25.61	74.56	0.39	500.20	1368	35.66	13924	346.20	42.35
23	848.60	406.80	48.71	15.50	0.35	25.11	65.83	0.26	300.00	1222	30.73	9673	371.80	28.20
24	584.10	317.70	53.94	12.28	0.42	25.30	57.38	0.40	492.20	1216	37.15	13171	304.80	43.50
27	832.80	331.30	47.55	11.48	0.37	22.01	61.21	0.39	478.00	1251	52.97	9063	349.00	26.53
31	978.30	399.80	49.14	12.88	0.39	25.01	66.51	0.43	574.30	1301	33.97	16870	351.50	47.45
32	720.20	402.00	54.74	10.96	0.26	20.51	89.19	0.33	579.60	1903	36.08	15842	503.70	34.39
33	536.70	273.30	46.94	10.35	0.27	19.05	79.95	0.46	427.40	955	46.83	8961	258.60	37.03
34	1046.30	449.00	49.41	14.23	0.21	26.85	79.32	0.31	409.20	1389	31.03	13085	378.80	36.81
36	795.90	443.10	50.22	13.01	0.36	22.48	64.69	0.40	480.90	1174	41.57	11601	305.90	37.13
38	584.80	478.40	50.08	12.83	0.39	21.33	59.41	0.44	505.40	1151	33.02	15383	332.30	47.91
42	739.80	340.60	48.10	13.40	0.28	24.58	86.69	0.38	533.20	1446	41.53	12788	333.30	40.02
45	644.90	372.70	43.25	14.01	0.23	24.55	105.92	0.37	368.90	991	31.40	11729	277.30	43.66
46	770.40	450.20	47.57	11.98	0.39	24.95	60.01	0.42	455.50	1089	31.42	14476	366.00	39.75
47	767.20	419.20	48.56	12.45	0.37	22.38	65.92	0.41	581.00	1359	39.65	14710	388.60	37.36
48	784.90	492.50	49.77	13.18	0.39	26.48	46.18	0.28	211.20	806	23.51	9117	372.60	26.05
50	742.50	480.40	48.48	11.87	0.33	22.28	59.07	0.41	385.10	923	32.05	11987	339.00	34.67
51	791.60	362.90	48.35	11.44	0.28	22.73	91.98	0.38	557.60	1512	43.66	12672	340.40	38.96
52	795.70	298.70	49.14	11.72	0.31	24.40	91.43	0.34	579.20	1674	45.17	12752	377.20	34.02
61	612.70	321.90	50.12	12.17	0.24	23.93	90.55	0.38	914.50	2328	34.63	26358	532.70	47.11
62	674.80	355.70	47.91	11.71	0.44	23.23	61.91	0.50	411.60	796	33.97	12129	320.20	36.39
65	761.30	293.60	48.77	14.21	0.30	26.65	93.21	0.36	623.60	1708	43.87	14280	355.40	38.80
66	785.90	509.20	48.61	15.09	0.35	27.23	61.08	0.33	326.40	977	28.51	11443	318.80	35.90
67	862.40	354.00	48.57	13.52	0.42	24.38	65.17	0.44	450.30	995	32.95	13860	295.80	45.73
70	918.30	444.80	49.67	12.68	0.36	23.48	59.64	0.26	169.10	637	28.91	5698	192.40	28.95
71	913.20	442.20	47.45	14.72	0.34	23.60	55.75	0.25	236.50	889	26.97	8874	307.90	27.51
74	818.30	375.60	50.78	12.93	0.36	24.13	64.85	0.30	485.90	1574	36.17	13496	455.10	28.93
75	839.30	447.80	50.84	11.43	0.34	21.48	68.04	0.41	494.30	1153	37.32	13284	346.10	37.06
77	805.50	296.00	50.49	11.78	0.32	21.40	88.36	0.43	432.80	994	47.92	9110	257.40	33.79
80	839.10	572.90	48.81	13.33	0.32	24.98	61.26	0.38	467.80	1228	30.71	15225	390.50	38.90
81	941.50	291.10	51.88	11.88	0.28	20.96	86.67	0.35	499.90	1453	50.18	9913	297.80	33.49
82	862.50	476.50	48.89	14.32	0.31	25.29	58.5	0.37	342.70	1020	25.17	13900	340.90	44.01
87	757.90	390.70	48.23	12.07	0.38	24.01	66.27	0.40	434.00	1115	33.58	12922	359.60	36.22
90	937.00	309.30	50.41	12.98	0.33	24.54	89.61	0.38	490.70	1324	37.68	12989	291.00	45.30
93	695.40	420.30	47.04	11.40	0.25	22.59	91.74	0.41	520.00	1277	43.23	12059	311.60	37.80
94	855.10	309.60	50.75	12.28	0.25	19.49	88.1	0.43	497.30	1235	44.67	11067	296.80	39.14
96	728.30	412.00	50.75	12.93	0.34	24.49	49.22	0.25	311.10	1247	21.26	14612	478.10	31.00
106	773.50	480.90	47.51	12.73	0.32	27.39	61.85	0.44	552.70	1317	32.12	17192	402.20	44.30
107	932.70	427.90	47.96	14.50	0.33	27.19	62.57	0.33	292.40	900	29.43	9932	312.50	32.38
116	780.70	387.50	49.56	13.77	0.32	26.31	60	0.49	484.30	1068	31.51	15609	320.20	49.57
119	847.80	445.40	50.32	16.12	0.33	29.66	62.01	0.31	251.90	806	28.85	8810	256.10	33.35
124	644.50	333.90	47.17	14.32	0.40	26.03	66.77	0.42	452.40	1103	32.15	14180	357.20	39.23
126	667.40	404.60	48.08	12.30	0.37	22.31	94.68	0.43	467.80	1151	39.58	11788	308.20	39.43
130	760.90	358.00	46.77	12.82	0.24	24.00	94.03	0.43	582.80	1380	37.80	15431	362.30	42.81
132	668.90	364.80	46.24	13.06	0.39	24.61	58.83	0.30	203.80	678	30.48	6806	224.40	30.13
137	536.70	495.70	48.91	10.24	0.33	22.68	51.14	0.40	418.50	1062	27.55	15261	383.80	39.44
138	881.10	249.10	48.71	11.46	0.37	23.21	90.03	0.36	451.40	1241	50.02	9112	258.00	34.24
Mean	***791.80***	***405.10***	***0.33***	***12.79***	***24.08***	***49.28***	***71.75***	***0.37***	***461***	***1251***	***35.05***	***13313.00***	***355.60***	***37.84***
P value	***< 0.001***	***< .001***	***< 0.001***	***< 0.001***	***< 0.001***	***<0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***
sed	***91.48***	***58.4***	***0.02***	***0.5***	***1.32***	***1.41***	***2.08***	***0.03***	***106***	***328.6***	***2.36***	***3142.00***	***78.49***	***5.37***

Table A9. Analysis of variance for traits measured in 69 lines of NL2x Rialto DH population in 2006

DH lines	Spike Index	Rachis length	No. of spikelets/ spike	SPAD of flag leaf	Height (cm)	HI	Yield (g m^{-2})	Biomass (g m^{-2})	TGW (g)	Grain m^{-2}	Spike m^{-2}	Grains/spike
1	0.50	13.39	26.25	52.30	66.06	0.54	403.70	756	22.58	17921	287.78	63.34
2	0.66	13.04	27.69	49.11	58.75	0.35	341.60	990	23.78	14398	474.87	30.43
3	0.49	15.05	25.42	50.24	95.30	0.24	348.40	1380	25.44	13648	340.41	40.01
5	0.60	13.48	24.17	48.56	71.07	0.29	331.90	1120	23.19	14284	419.09	33.81
7	0.59	12.09	23.45	47.58	59.83	0.26	304.40	1170	22.74	13452	503.95	27.29
10	0.54	12.54	22.35	46.95	67.83	0.22	225.50	1009	18.33	12394	457.93	26.56
11	0.31	10.99	20.36	52.74	91.83	0.33	544.60	1633	38.14	14325	435.28	32.83
12	0.56	12.73	26.04	49.66	55.40	0.27	276.30	1030	21.97	12472	498.85	25.08
13	0.65	12.07	20.28	54.32	53.91	0.50	464.60	929	35.71	13083	303.79	43.04
14	0.44	12.22	22.14	48.84	96.69	0.42	521.00	1204	37.80	13812	341.41	39.73
16	0.33	12.82	22.66	50.02	101.88	0.30	357.70	1185	28.02	12709	383.20	32.35
17	0.39	11.66	22.06	46.52	94.08	0.37	510.50	1374	31.27	16356	487.06	33.35
18	0.43	11.60	19.53	54.73	88.91	0.46	648.80	1403	50.29	12998	338.49	38.06
19	0.68	13.32	22.45	49.99	61.50	0.24	323.80	1306	25.39	12780	522.56	24.29
21	0.51	13.74	23.78	50.85	71.16	0.44	481.10	1094	33.64	14371	311.41	45.70
23	0.59	13.98	22.00	49.67	69.97	0.29	304.40	1030	26.72	11382	406.98	27.97
24	0.53	13.25	23.62	52.55	58.39	0.49	480.80	1005	33.76	14324	276.14	52.63
27	0.57	10.99	20.02	52.56	63.52	0.42	466.20	1084	48.84	9738	290.19	33.57
31	0.56	13.21	23.48	49.71	67.36	0.36	447.90	1239	29.99	15021	381.47	39.60
32	0.41	11.12	21.94	54.88	93.53	0.42	537.40	1304	34.76	15523	375.67	42.32
33	0.48	9.69	17.40	52.74	80.38	0.47	496.70	1088	46.07	10718	302.64	35.91
34	0.33	12.40	23.18	47.59	83.81	0.31	370.60	1250	32.26	11542	410.90	28.02
36	0.50	12.35	20.83	52.70	65.14	0.43	455.60	1077	36.74	12433	362.34	34.34
38	0.61	13.40	24.48	52.05	63.44	0.41	546.60	1345	32.42	16944	406.14	41.89
42	0.38	12.65	21.96	48.65	86.64	0.49	527.30	1097	39.04	13484	293.17	46.68
45	0.29	14.10	23.48	46.67	101.36	0.38	404.10	1108	34.96	11623	289.64	41.45
46	0.65	12.09	24.36	48.25	59.55	0.45	403.10	917	26.07	15535	375.81	41.59
47	0.58	12.60	21.28	51.02	63.86	0.47	470.00	1000	35.97	13225	364.97	36.37
48	0.67	11.71	22.18	48.09	54.64	0.34	222.70	676	18.27	12402	401.31	31.82
50	0.66	12.45	22.07	50.31	57.44	0.42	402.60	955	24.77	16384	409.45	40.17
51	0.33	11.85	23.91	49.84	87.58	0.39	488.00	1268	40.72	11962	325.65	37.52
52	0.42	11.92	24.31	51.16	89.25	0.44	460.20	1063	39.22	11718	341.04	35.23
56	0.50	13.15	29.04	55.93	61.96	0.37	204.10	535	20.60	9749	235.37	41.02
61	0.35	12.40	23.94	52.66	88.60	0.37	498.60	1357	31.51	15762	432.88	36.50
62	0.63	11.83	21.50	51.60	61.27	0.48	387.60	835	31.42	12329	332.66	37.74
65	0.37	12.36	20.81	47.63	92.25	0.43	551.50	1262	41.43	13403	278.95	47.90
66	0.50	12.54	21.05	49.64	72.69	0.29	274.00	941	27.48	10059	416.44	24.98
67	0.56	13.19	24.03	52.02	64.60	0.47	463.00	1007	32.46	14155	285.80	49.93
70	0.50	12.40	21.33	50.54	66.44	0.35	250.50	734	28.97	8640	335.60	25.87
71	0.54	13.73	20.65	51.81	65.00	0.28	240.20	838	28.81	8695	369.89	24.05
74	0.62	12.59	22.35	50.27	68.58	0.44	481.00	1111	35.37	13639	325.94	42.96
75	0.51	12.50	21.99	51.63	68.60	0.46	534.60	1163	37.61	14172	367.97	38.57
76	0.51	16.15	25.71	51.48	67.55	0.29	274.00	915	25.85	10547	381.98	27.21
77	0.44	12.27	21.28	56.42	92.02	0.46	583.30	1291	50.61	11400	292.67	39.21
80	0.47	13.48	26.62	51.07	64.02	0.27	300.80	1158	22.42	13598	541.44	26.09
81	0.41	11.78	20.57	53.89	86.25	0.49	606.80	1258	50.22	12025	287.57	42.77
82	0.51	12.34	20.67	48.13	58.10	0.39	327.00	843	25.95	12600	365.33	34.80
84	0.28	10.85	27.70	51.31	103.22	0.40	464.50	1157	33.80	13696	342.33	39.73
86	0.57	12.53	19.23	52.45	64.46	0.25	368.40	1474	27.60	13187	647.41	20.02
87	0.58	13.37	23.85	50.38	68.24	0.35	403.90	1151	25.28	15902	448.26	35.73
90	0.34	11.52	22.93	52.24	87.33	0.55	433.70	797	28.84	15030	243.83	65.73
93	0.34	11.44	23.06	49.56	91.34	0.38	506.80	1353	40.28	12495	445.81	28.67
94	0.35	11.87	18.70	49.18	87.18	0.48	491.10	1039	44.79	10901	307.86	35.45
96	0.57	12.14	26.46	57.26	57.18	0.29	196.30	662	17.92	10834	376.18	30.45
106	0.57	12.51	22.56	49.92	62.41	0.44	355.00	795	26.48	13480	303.83	43.59
107	0.58	12.89	21.50	49.67	72.10	0.29	280.60	973	27.82	10107	509.94	20.73
116	0.53	13.58	25.51	48.05	60.74	0.53	445.00	862	30.41	14623	330.86	45.24
117	0.62	11.47	22.60	50.80	56.94	0.23	214.10	940	19.27	11041	521.81	21.45
119	0.53	13.39	23.55	49.50	72.16	0.52	282.90	552	24.74	11463	197.85	58.82
124	0.60	14.67	23.55	50.75	66.99	0.41	408.00	1000	26.09	15592	387.11	41.20
125	0.56	10.54	22.22	56.97	58.19	0.22	205.40	880	23.75	8507	379.44	22.55
126	0.34	12.22	21.89	49.66	95.26	0.47	535.80	1152	31.98	17081	310.58	55.02
130	0.32	13.62	24.77	51.49	95.49	0.45	602.50	1327	36.10	16738	462.44	36.78
131	0.52	11.14	21.56	56.87	57.79	0.22	224.30	996	21.98	9976	450.46	21.91
132	0.62	11.92	20.33	46.59	64.02	0.53	239.60	457	25.97	9322	256.58	39.89
134	0.50	15.96	21.76	58.13	102.36	0.22	303.50	1368	36.79	8169	360.49	23.11
136	0.52	13.32	23.14	55.98	67.29	0.23	197.00	835	21.53	8897	295.01	29.77
137	0.64	11.08	23.22	51.10	51.71	0.48	335.60	685	30.60	11156	344.31	32.76
138	0.36	11.99	21.24	50.42	91.30	0.44	457.80	1035	49.15	9152	230.44	39.07
NL2	0.54	13.18	21.43	48.19	93.36	0.52	543.40	1064	44.12	12258	275.14	44.77
Mean	***0.50***	***12.58***	***22.76***	***51.03***	***74.07***	***0.38***	***400.60***	***1056***	***31.50***	***12762***	***368.55***	***36.50***
P value	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***
sed	***0.07***	***0.55***	***1.31***	***1.89***	***3.11***	***0.03***	***34.86***	***116.90***	***2.04***	***1341.00***	***57.14***	***6.11***

Table A10. Analysis of variance for traits measured in 33 line sof NL1 x Rialto DH population in 2005

Lines	Plant height (cm)	Spikelets ear^{-1}	Rachis length (cm)	HI	Yield (g m^{-2})	Biomass (g m^{-2})	TGW (g)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}
2	92.3	25.49	13.16	0.46	615.60	1342	34.69	18538	369.00	45.92
3	95.19	25.75	12.78	0.42	600.90	1448	34.19	18276	492.30	33.37
4	83.77	22.64	12.3	0.47	620.10	1351	34.91	17783	349.80	49.2
8	111.9	23.71	11.52	0.42	635.20	1579	43.02	15143	379.30	38.76
12	53.04	24.66	12.11	0.43	445.10	1028	32.47	14510	459.80	30.56
19	45.89	25.66	11.76	0.44	403.10	796	33.98	13037	244.90	35.92
20	66.87	24.91	11.17	0.45	540.90	1208	35.64	14894	375.20	42.52
22	90.1	26.36	17.94	0.39	515.40	1323	42.01	12223	356.60	34.57
23	76.09	24.36	13.91	0.47	475.00	991	37.31	13327	243.10	54.18
25	51.29	23.26	10.38	0.50	570.80	1111	29.95	20715	439.80	39.98
28	79.43	21.99	10.62	0.44	552.60	1278	34.40	16342	313.30	47.23
33	83.98	24.29	14.88	0.44	649.90	1443	31.65	21983	311.70	61.91
35	79.09	27.53	16.12	0.44	437.10	1052	42.10	10225	258.30	51.41
39	48.74	25.53	17.83	0.18	194.60	1029	27.90	6176	457.60	17.19
41	87.87	27.93	15.16	*	*	*	*	*	*	*
44	85.61	25.97	12.13	0.43	412.70	936	32.32	12884	319.40	39.16
46	102.94	22.47	16.09	0.28	377.80	1319	35.94	10944	352.50	28.47
47	115.71	25.1	16.38	0.31	389.80	1304	38.30	9001	299.40	34.74
48	80.08	23.15	13.82	0.25	265.10	1098	18.80	13086	416.10	36.1
49	65.74	25.39	12.41	0.31	483.90	1810	36.55	12109	447.60	31.13
50	84.02	18.09	11.18	0.47	551.10	1224	44.60	11407	298.70	47.41
53	79.16	26.72	17.39	0.26	361.50	1389	29.49	13277	365.10	38.38
56	85.3	23.5	10.19	0.42	544.50	1313	40.38	13225	285.60	48.13
58	84.36	29.8	14.68	0.45	665.60	1469	33.21	20786	362.40	59.63
59	101.27	24.87	11.9	0.48	519.90	1142	42.33	11281	296.40	43.24
60	76.51	25.82	12.11	0.37	460.20	1349	33.81	12915	413.20	35.77
65	93.02	24.68	11.29	0.48	657.50	1354	40.84	16312	293.90	55.2
67	53.92	24.39	12.84	0.25	506.50	2586	32.84	15506	836.50	19.05
68	49.16	21.88	10.48	0.44	370.20	935	28.99	12924	483.10	33.53
69	84.29	22.94	10.64	0.44	449.30	1101	39.43	11668	356.60	36.1
70	77.69	23.95	15.27	0.29	316.90	1132	34.33	8474	290.70	35.96
72	88.69	23.6	9.78	0.44	456.50	1069	47.38	8900	258.30	41.35
73	85.46	22.53	11	0.39	519.00	1347	39.81	12046	320.00	43.18
Mean	***79.95***	***24.51***	***13.07***	***0.40***	***486.40***	***1277.00***	***35.74***	***13747***	***367.10***	***40.29***
P value	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***ns***	***< 0.001***	***< 0.001***	***< 0.05***	***< 0.001***
sed	***2.67***	***0.63***	***0.62***	***0.05***	***90.33***	***473.20***	***2.63***	***2480***	***123.40***	***8.72***

Table A11. Analysis of variance for traits measured in 44 lines NL1x Rialto DH population in 2006

Lines	Plant height (cm)	Spikelets ear^{-1}	Rachis length (cm)	HI	Yield (g m^{-2})	Biomass (g m^{-2})	TGW (g)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}
2	97.98	20.84	11.73	0.40	469.30	1183.23	31.73	15349	412.10	38.19
3	98.98	21.50	12.97	0.38	489.70	1271.11	29.18	17398	369.20	47.03
4	87.20	19.67	10.92	0.38	390.00	1032.96	25.87	14624	355.20	42.08
7	133.76	20.25	11.62	0.14	183.70	1278.66	23.62	7760	365.80	21.74
8	106.33	18.84	10.04	0.40	436.40	1090.08	41.93	10792	267.90	39.68
10	133.83	20.92	13.59	0.17	186.80	1084.39	22.32	8236	387.40	22.03
12	50.50	20.34	10.80	0.32	243.60	760.25	28.20	9092	387.20	23.56
15	102.16	20.75	12.06	0.23	263.90	1112.97	29.72	8727	256.90	33.34
19	46.30	20.42	12.61	0.47	286.80	618.40	35.09	8382	215.80	38.27
20	71.61	22.09	11.61	0.38	424.70	1121.06	29.99	13975	340.60	41.74
21	106.69	21.83	14.43	0.19	250.90	1399.47	23.35	10370	381.30	31.03
22	98.53	21.83	13.74	0.34	419.50	1229.58	35.72	11479	298.00	38.88
22	107.44	21.42	12.18	0.30	357.50	1180.25	34.28	10156	292.70	36.86
23	81.65	19.50	19.69	0.37	394.00	1058.98	34.10	11989	321.20	36.91
25	52.44	22.25	12.20	0.50	436.20	871.57	24.91	17071	307.40	55.66
28	87.36	19.83	11.92	0.39	424.80	1095.79	31.59	13137	315.70	41.52
29	79.47	20.25	16.22	0.15	145.30	982.50	21.46	6743	312.70	22.34
30	133.44	19.34	13.54	0.17	252.50	1466.23	32.23	7712	275.40	28.40
32	57.81	21.42	10.41	0.22	172.30	773.97	17.53	9829	344.50	29.62
33	86.71	21.84	12.27	0.38	448.30	1188.06	31.02	14645	287.40	50.68
35	93.71	21.75	11.47	0.38	418.80	1099.84	35.24	12151	253.30	47.20
39	54.81	20.34	12.00	0.19	101.70	528.07	23.40	4648	283.70	15.65
41	106.31	17.84	9.00	0.33	446.00	1329.13	34.35	13388	377.40	34.84
43	83.19	22.17	14.08	0.15	161.40	1113.02	25.20	6180	353.10	18.11
44	87.08	20.34	9.74	0.35	397.50	1121.86	32.32	12744	372.90	33.39
46	136.88	20.34	13.15	0.18	208.90	1148.82	30.74	7066	265.20	25.35
47	127.71	21.67	13.48	0.17	228.80	1313.05	34.37	6835	311.30	21.09
48	86.03	19.84	11.42	0.12	130.90	1077.23	17.73	7407	506.40	14.74
49	64.51	18.50	10.94	0.42	400.00	963.80	32.73	11701	263.70	45.86
50	89.03	18.09	12.21	0.46	532.80	1167.40	44.46	12043	303.90	39.80
53	85.36	21.67	12.20	0.29	242.40	857.51	25.36	9608	228.90	42.69
56	103.34	19.25	9.53	0.39	444.20	1127.83	42.98	9869	244.60	41.68
58	82.38	23.42	11.51	0.40	411.70	1038.78	32.37	12889	262.70	49.86
59	118.46	19.25	10.16	0.37	409.60	1107.30	44.22	9325	283.00	33.51
60	75.06	23.92	11.90	0.31	311.90	1009.96	30.34	10435	322.10	33.62
65	103.64	20.92	10.02	0.40	366.40	915.61	35.24	10542	249.10	42.71
67	54.72	21.59	11.26	0.44	351.70	791.38	28.96	11406	242.10	47.89
68	51.66	20.50	11.16	0.24	296.90	1210.55	23.27	12484	417.00	30.57
69	92.01	21.17	9.85	0.44	426.90	967.72	43.60	9800	218.70	44.78
70	94.80	19.84	12.21	0.18	265.60	1448.98	27.39	9040	363.00	25.50
72	101.83	17.17	9.58	0.40	425.10	1078.10	45.95	9238	228.70	39.95
73	100.74	20.84	11.16	0.37	399.90	1073.33	37.24	10547	271.70	38.79
75	46.86	22.17	12.31	0.34	261.50	761.75	20.85	12298	290.50	42.90
83	91.22	20.84	11.55	0.13	146.30	1088.83	17.46	8387	674.00	13.04
CMH80A.763 (L8)	98.89	19.67	12.00	0.46	583.00	1250.84	41.23	14163	308.10	46.40
Mean	***90.01***	***20.62***	***11.97***	***0.32***	***334.40***	***1075.34***	***30.91***	***10704.00***	***319.80***	***35.32***
P value	***< 0.001***	***0.05***	***ns***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***
sed	***3.84***	***1.50***	***2.31***	***0.02***	***30.75***	***140.12***	***1.53***	***868.00***	***56.90***	***5.13***

Table A12. Correlation matrix of traits for 33 DH lines of NL1x Rialto population in 2005

	Floweringdate	Plant height (cm)	Spikelets ear^{-1}	Rachis length (cm)	HI	Yield (g m^{-2})	Biomass (g m^{-2})	TGW (g)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}
Flowering date	1										
Plant height (cm)	-0.55	1									
Spikelets ear^{-1}	-0.24	0.17	1								
Rachis length (cm)	0.67	-0.12	0.16	1							
HI	-0.71	0.33	0.21	-0.60	1						
Yield (g m^{-2})	-0.74	0.41	0.36	-0.39	0.75	1					
Biomass (g m^{-2})	-0.41	0.32	0.31	-0.01	0.14	0.75	1				
TGW (g)	-0.55	0.59	0.11	-0.18	0.36	0.35	0.22	1			
Grains m^{-2}	-0.51	0.13	0.29	-0.38	0.62	0.87	0.67	-0.14	1		
Ears m^{-2}	0.07	-0.53	-0.06	-0.05	-0.12	0.15	0.29	-0.48	0.38	1	
Grains ear^{-1}	-0.51	0.48	0.36	-0.28	0.69	0.70	0.38	0.23	0.65	-0.43	1

Table A13. Correlation matrix of traits for 44 DH lines of NL1x Rialto population in 2006

	Flowering date	CT (veg)	CT (GF)	NDVI (Booting)	NDVI (GF)	Plant height (cm)	Spikelets ear^{-1}	Rachis length (cm)	HI	Yield (g m^{-2})	Biomass (g m^{-2})	TGW (g)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}
Flowering date	1														
CT (veg)	0.24	1													
CT (GF)	-0.75	-0.21	1												
NDVI (Booting)	0.53	0.14	-0.23	1											
NDVI (GF)	0.85	0.17	-0.56	0.76	1										
Plant height (cm)	-0.08	0.32	-0.36	-0.24	-0.20	1									
Spikelets ear^{-1}	0.31	-0.20	-0.10	0.29	0.35	-0.23	1								
Rachis length (cm)	0.43	0.12	-0.28	0.16	0.44	0.05	0.21	1							
HI	-0.80	-0.37	0.77	-0.39	-0.60	-0.32	-0.09	-0.30	1						
Yield (g m^{-2})	-0.87	-0.34	0.64	-0.39	-0.68	0.04	-0.20	-0.26	0.86	1					
Biomass (g m^{-2})	-0.10	0.06	-0.27	-0.03	-0.10	0.70	-0.17	0.12	-0.25	0.24	1				
TGW (g)	-0.84	0.04	0.55	-0.58	-0.81	0.30	-0.40	-0.27	0.63	0.73	0.19	1			
Grains m^{-2}	-0.50	-0.53	0.46	-0.04	-0.26	-0.22	0.11	-0.14	0.69	0.78	0.15	0.16	1		
Ears m^{-2}	0.36	-0.19	-0.38	0.27	0.30	0.01	0.02	0.06	-0.49	-0.33	0.25	-0.57	0.01	1	
Grains ear^{-1}	-0.61	-0.32	0.55	-0.21	-0.38	-0.22	0.12	-0.21	0.88	0.82	-0.09	0.47	0.77	-0.56	1

Table A14. Analysis of varaince for traits of 63 DH lines of Bacanora x Rialto population in 2005

Lines	Plant height (cm)	SPAD-Flag	Rachis length (cm)	Spikelets ear^{-1}	HI	Yield (g m^{-2})	Biomass (g m^{-2})	TGW (g)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}
1	72.90	52.82	14.59	22.83	0.26	293.20	1131	27.63	10665	398.10	26.78
2	73.76	47.37	9.55	22.26	0.52	558.40	1094	37.57	14896	362.60	41.96
3	83.29	48.57	12.20	24.20	0.43	447.60	1027	39.72	11248	275.60	41.08
11	55.82	49.73	13.14	23.13	0.28	219.20	798	24.56	9049	435.50	21.96
12	89.30	47.44	12.14	25.20	0.43	599.00	1374	35.02	17087	419.20	40.90
14	100.27	50.41	13.94	24.01	0.29	313.80	1147	32.29	9671	252.50	39.98
15	45.99	54.15	12.57	24.08	0.31	151.00	551	23.74	6007	296.30	23.53
16	78.23	45.06	12.53	25.58	0.42	547.00	1281	35.32	15436	376.00	40.59
20	78.89	47.65	12.66	26.16	0.41	620.90	1501	30.79	20239	409.70	48.19
21	52.09	48.93	13.23	26.03	0.38	675.80	1865	33.68	20061	548.80	37.06
22	85.79	45.43	8.76	22.56	0.40	779.70	1964	43.50	17975	508.50	34.91
24	109.87	46.63	10.94	23.48	0.41	385.40	940	41.79	9225	256.10	35.76
27	81.33	51.26	11.29	25.54	0.43	569.70	1351	35.06	16177	400.30	40.58
28	78.23	49.72	10.55	22.71	0.37	507.00	1362	35.28	14357	400.10	36.21
30	100.02	51.19	11.87	24.21	0.35	570.50	1640	46.55	12274	308.10	39.85
31	81.91	47.44	11.09	26.78	0.46	609.70	1349	35.38	17224	400.10	42.91
32	92.96	50.80	11.63	24.36	0.42	558.30	1399	39.20	14238	359.20	40.56
35	75.00	49.03	12.27	22.99	0.23	210.00	903	27.22	7764	369.20	21.36
38	87.81	49.25	11.26	25.00	0.40	591.10	1492	39.02	15187	366.90	42.02
40	77.72	47.76	10.82	25.95	0.47	554.80	1165	35.73	15557	345.80	44.40
41	81.53	47.75	11.83	24.44	0.37	653.20	1734	37.88	17241	498.20	34.30
42	90.76	48.04	12.31	24.35	0.45	730.40	1612	35.49	20482	443.60	45.75
46	104.28	45.98	11.12	22.98	0.35	551.10	1567	39.75	13937	365.40	37.94
48	108.30	45.14	11.62	24.48	0.36	622.00	1731	46.68	13265	375.00	34.79
52	76.69	48.63	10.86	22.97	0.42	512.70	1231	33.76	15258	429.70	35.48
53	104.45	47.26	12.16	25.21	0.40	563.10	1409	42.80	13212	349.90	38.54
56	86.47	50.59	14.32	26.77	0.30	374.20	1228	30.89	12083	236.50	50.42
57	55.22	49.65	12.90	24.01	0.25	254.10	1048	29.05	8677	350.00	26.18
58	99.17	50.79	12.32	24.14	0.41	612.00	1464	41.64	14680	359.70	40.58
66	50.27	51.29	14.37	24.68	0.30	243.00	795	24.80	9874	302.90	32.47
68	75.96	51.08	10.60	22.93	0.39	908.40	2411	40.89	22345	563.30	39.97
70	46.07	52.80	14.69	23.55	0.28	193.00	680	27.55	6702	265.70	24.55
71	103.51	50.87	13.57	23.03	0.28	265.20	983	33.87	7813	268.20	30.02
75	92.32	52.28	11.45	24.56	0.34	604.70	1740	42.76	14214	361.80	39.43
76	107.51	49.93	14.67	27.13	0.32	640.70	2060	43.14	14862	389.80	39.37
78	74.85	49.50	11.58	25.28	0.45	504.20	1089	33.52	15133	307.10	47.81
80	96.89	49.13	12.68	26.95	0.36	574.10	1617	41.58	13850	340.20	39.84
87	57.52	48.77	11.13	22.27	0.47	497.50	1052	37.96	13218	333.00	38.81
91	85.94	48.53	11.28	23.30	0.43	972.10	2403	40.20	23970	577.20	41.81
93	83.59	47.05	10.95	22.44	0.42	928.50	2162	40.54	22998	560.70	40.39
105	62.44	49.23	11.23	24.62	0.49	476.60	949	33.13	14469	283.50	49.72
109	99.80	48.95	10.10	21.27	0.41	536.40	1372	45.13	11894	323.90	36.98
110	70.26	50.98	11.23	24.14	0.41	872.10	2138	34.38	25057	536.40	46.85
112	75.35	51.62	11.40	23.47	0.25	302.10	1215	26.43	11489	406.80	28.78
115	109.62	49.62	14.34	27.38	0.45	476.70	1047	41.19	11555	288.50	40.02
118	70.30	50.82	11.99	23.09	0.50	555.40	1080	39.23	14183	330.10	42.13
126	72.27	51.70	13.01	24.71	0.25	353.60	1444	24.66	14319	502.90	28.39
134	102.89	45.54	11.37	24.51	0.36	603.20	1670	45.96	13052	372.20	34.31
135	77.27	47.31	9.96	22.55	0.45	531.70	1228	35.91	14823	346.70	43.27
137	86.37	49.95	12.43	25.64	0.45	708.50	1600	38.25	18505	438.00	42.29
138	67.35	49.08	11.16	25.43	0.52	695.50	1357	33.44	20686	452.50	45.32
140	93.35	48.88	14.19	24.78	0.39	630.90	1723	39.96	15757	390.10	40.12
143	106.73	45.52	12.73	26.46	0.40	513.80	1284	38.60	13296	376.90	35.65
150	81.46	49.39	11.56	24.12	0.46	640.60	1375	37.22	17167	400.10	42.67
152	93.55	44.92	11.89	24.20	0.40	445.60	1156	34.42	12919	354.30	37.72
153	122.49	46.69	13.97	23.40	0.34	427.40	1288	43.19	9889	342.30	29.44
154	94.07	45.79	10.61	22.90	0.40	485.80	1255	39.26	12382	366.90	33.46
158	87.45	49.62	11.07	21.62	0.42	637.60	1505	43.57	14636	404.30	36.47
159	71.59	47.92	10.88	23.63	0.37	474.20	1263	34.65	13919	333.50	42.92
165	58.53	50.71	9.56	20.37	0.52	547.70	1076	33.03	16544	426.90	40.41
167	84.04	46.97	11.00	23.39	0.41	667.20	1646	42.33	15823	413.90	38.50
171	54.26	48.94	10.48	24.57	0.48	537.70	1138	30.16	17760	403.50	44.60
183	47.66	49.18	13.80	24.84	0.24	284.20	1187	24.89	11416	457.80	25.23
Mean	***82.16***	***49.13***	***11.86***	***24.08***	***0.39***	***536.30***	***1389.00***	***36.66***	***14452.00***	***383.90***	***37.78***
P value	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***NS***	***< 0.001***
sed	***3.12***	***1.22***	***0.47***	***1.08***	***0.04***	***151.20***	***434.10***	***1.63***	***3812.00***	***105.00***	***4.05***

Table A15. Analysis of varaince for traits of 63 DH lines of Bacanora x Rialto population in 2006

Lines	Plant height (cm)	Rachis length (cm)	Spikelets ear^{-1}	HI	Yield (g m^{-2})	Biomass (g m^{-2})	TGW (g)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}
1	86.32	14.29	21.51	0.25	285.30	1150	22.22	12778	377.70	33.73
2	76.93	9.69	21.35	0.47	476.20	1034	32.49	14799	549.60	34.39
3	71.70	11.33	21.94	0.42	516.50	1222	33.62	15565	381.00	40.69
11	75.92	12.02	20.08	0.13	176.90	1322	20.36	8838	603.50	16.67
12	79.69	19.64	22.91	0.44	492.10	1111	31.00	15923	293.10	53.10
14	127.80	13.87	22.74	0.31	222.00	750	30.10	7742	185.90	40.17
15	81.34	12.02	21.67	0.26	172.20	674	19.83	9165	370.00	26.04
16	79.93	12.63	24.42	0.42	464.90	1112	28.04	16855	347.80	48.31
20	83.18	10.71	20.59	0.41	427.80	1061	24.00	17693	341.60	53.17
21	81.62	12.16	23.19	0.36	321.90	899	27.22	11972	366.30	33.17
22	103.07	8.87	19.61	0.45	492.10	1083	42.17	11838	304.80	39.32
24	94.12	9.80	20.75	0.40	397.20	993	40.74	9990	337.80	30.79
27	78.39	9.67	20.00	0.34	364.30	1098	26.38	13849	379.40	36.86
28	88.17	10.00	21.08	0.24	342.40	1433	26.06	13688	528.70	26.67
30	97.29	10.42	20.76	0.42	530.50	1272	46.36	11787	306.10	40.76
31	94.54	10.73	21.59	0.48	568.00	1202	28.21	20681	371.90	56.94
32	82.05	11.39	21.53	0.47	493.70	1070	35.02	13722	340.40	39.87
35	77.24	13.97	21.28	0.23	262.30	1119	23.94	10617	366.40	28.58
38	70.69	12.19	22.54	0.42	476.40	1121	31.83	14894	293.20	49.87
40	89.98	10.63	20.76	0.45	559.60	1244	29.90	18583	366.90	51.01
41	77.08	11.47	21.27	0.41	452.40	1095	29.97	14776	371.10	39.58
42	85.03	11.76	21.59	0.36	491.90	1369	30.50	16211	361.00	44.42
46	103.07	10.42	20.70	0.38	442.60	1146	34.12	13068	279.50	47.93
48	104.32	11.09	21.36	0.36	443.00	1228	39.38	11146	479.30	25.77
52	91.21	10.51	20.73	0.31	308.90	1019	24.14	12871	362.90	35.60
53	91.49	10.79	21.40	0.36	517.00	1427	35.28	14908	366.30	40.69
56	100.51	11.49	21.24	0.25	332.00	1368	28.26	12075	371.90	31.96
57	94.65	12.93	21.55	0.12	167.70	1391	23.90	7239	630.50	11.46
58	103.26	12.74	22.63	0.36	465.00	1301	35.12	13368	330.50	40.25
66	80.87	12.11	21.37	0.16	164.50	1037	19.82	9109	472.70	19.13
68	107.49	12.40	21.23	0.46	522.40	1132	35.20	15414	308.50	50.03
70	95.99	11.67	21.06	0.25	210.70	879	27.88	8156	304.10	26.89
71	103.40	14.19	22.63	0.20	230.20	1129	28.48	7721	314.60	24.42
75	108.85	11.71	20.72	0.44	501.40	1127	40.25	12304	294.10	42.88
76	96.79	10.77	20.31	0.39	477.50	1225	35.80	13186	247.10	53.75
78	112.18	11.39	21.03	0.46	493.10	1082	33.67	14539	295.60	50.80
80	85.94	10.63	20.04	0.35	478.50	1404	33.78	13813	347.10	39.73
87	78.39	11.02	20.44	0.42	308.20	725	30.31	10063	239.60	41.87
91	74.77	10.47	20.80	0.47	534.70	1121	35.12	15293	308.70	49.77
93	84.35	9.73	19.54	0.43	516.20	1222	35.62	14444	364.80	39.96
105	89.48	11.14	22.67	0.39	392.40	1000	26.00	14746	331.20	44.75
109	70.26	9.54	18.52	0.39	432.40	1125	41.62	10345	287.90	36.29
110	94.04	10.89	22.69	0.33	370.00	1103	25.89	14271	380.30	37.46
112	101.75	10.86	20.74	0.25	275.30	1086	22.63	12066	368.10	33.43
115	117.51	12.64	23.80	0.35	483.50	1383	30.29	16192	338.20	48.87
118	133.44	11.21	20.75	0.42	427.60	1025	33.40	12632	310.90	40.61
126	80.72	10.97	19.82	0.21	259.40	1310	19.55	13726	478.00	28.66
134	128.01	10.46	21.76	0.38	440.10	1167	39.68	11290	289.40	39.24
135	96.18	9.40	19.84	0.46	526.20	1157	30.83	17067	367.30	47.15
137	82.61	12.13	23.89	0.38	520.40	1357	31.46	16292	359.50	44.80
138	96.72	11.19	22.98	0.41	393.70	960	24.70	15876	354.00	44.78
140	87.41	13.89	21.91	0.44	547.70	1237	35.03	15680	264.90	58.68
143	91.63	12.16	24.13	0.36	421.50	1172	31.09	13615	342.80	40.20
150	94.90	10.58	21.54	0.45	473.90	1057	32.78	14311	329.10	43.14
152	86.93	11.60	22.37	0.34	355.50	1056	31.84	11442	314.70	36.08
153	93.22	12.46	22.73	0.30	352.20	1184	35.99	10015	344.80	29.42
154	118.14	9.14	19.89	0.34	415.70	1262	33.82	12330	553.20	24.64
158	112.48	9.73	17.64	0.38	529.70	1375	38.71	13136	416.00	31.30
159	86.51	10.51	21.23	0.38	452.00	1179	26.40	16489	328.10	50.97
165	85.12	9.68	19.49	0.47	424.90	887	30.90	13351	322.60	41.29
167	83.17	10.62	20.04	0.51	587.20	1138	39.99	14394	313.20	46.86
171	68.19	9.87	21.96	0.42	406.60	977	21.86	17694	409.40	44.37
183	68.47	11.59	21.45	0.23	178.10	793	18.26	9670	370.70	25.96
Bacanora T 88	99.59	17.88	18.80	0.53	568.30	1087	35.67	15548	329.40	47.98
Mean	***91.66***	***11.49***	***21.29***	***0.37***	***411.50***	***1132.00***	***30.85***	***13326***	***358.80***	***39.12***
P value	***ns***	***< 0.05***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***
sed	***21.45***	***2.25***	***1.14***	***0.03***	***29.92***	***125.7***	***2.212***	***1113***	***74.26***	***5.9***

Table A16. Correlation matrix of traits for 63 DH lines of Bacanora x Rialto population in 2005

	Flowering date	CT (Veg)	CT (GF)	SPAD	Rachis length (cm)	Spikelets ear^{-1}	Plant height (cm)	HI	Yield (g m^{-2})	Biomass (g m^{-2})	TGW (g)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}
Flowering date	1													
CT (Veg)	0.07	1												
CT (GF)	-0.78	0.03	1											
SPAD	0.45	0.19	-0.29	1										
Rachis length (cm)	0.55	-0.08	-0.58	0.13	1									
Spikelets ear^{-1}	-0.01	-0.27	-0.03	-0.23	0.50	1								
Plant height (cm)	-0.41	-0.05	0.11	-0.37	0.02	0.18	1							
HI	-0.60	-0.13	0.62	-0.28	-0.48	0.08	0.00	1						
Yield (g m^{-2})	-0.71	-0.21	0.63	-0.24	-0.38	0.17	0.31	0.54	1					
Biomass (g m^{-2})	-0.39	-0.18	0.28	-0.08	-0.07	0.17	0.38	-0.09	0.78	1				
TGW (g)	-0.71	-0.08	0.52	-0.23	-0.45	-0.10	0.68	0.32	0.70	0.58	1			
Grains m^{-2}	-0.19	-0.23	0.24	-0.06	-0.03	0.35	-0.27	0.39	0.64	0.48	-0.09	1		
Ears m^{-2}	0.31	-0.24	-0.16	0.13	0.19	0.00	-0.46	-0.31	0.08	0.32	-0.39	0.52	1	
Grains ear^{-1}	-0.62	-0.05	0.53	-0.23	-0.32	0.33	0.22	0.77	0.64	0.22	0.40	0.49	-0.47	1

Table A17. Correlation matrix of traits for 63 DH lines of Bacanora x Rialto population in 2006

	Flowering date	CT (veg)	CT (GF)	NDVI (Booting)	NDVI (GF)	Plant height (cm)	Rachis length (cm)	Spikelets ear^{-1}	HI	Yield (g m^{-2})	Biomass (g m^{-2})	TGW (g)	Grains m^{-2}	Ears m^{-2}	Grains ear^{-1}
Flowering date	1														
CT (veg)	0.32	1													
CT (GF)	-0.73	-0.16	1												
NDVI (Booting)	0.54	0.02	-0.24	1											
NDVI (GF)	0.79	0.25	-0.51	0.76	1										
Plant height (cm)	-0.24	-0.27	-0.04	-0.35	-0.23	1									
Rachis length (cm)	0.34	0.22	-0.32	-0.02	0.11	-0.04	1								
Spikelets ear^{-1}	0.26	0.05	-0.26	0.21	0.13	-0.01	0.56	1							
HI	-0.81	-0.20	0.72	-0.36	-0.58	0.03	-0.25	-0.06	1						
Yield (g m^{-2})	-0.85	-0.25	0.63	-0.44	-0.65	0.09	-0.23	-0.09	0.87	1					
Biomass (g m^{-2})	-0.22	-0.15	-0.03	-0.24	-0.25	0.12	-0.04	-0.09	-0.11	0.38	1				
TGW (g)	-0.79	-0.40	0.40	-0.55	-0.65	0.35	-0.23	-0.23	0.60	0.69	0.25	1			
Grains m^{-2}	-0.46	-0.02	0.47	-0.11	-0.32	-0.17	-0.13	0.13	0.65	0.74	0.29	0.04	1		
Ears m^{-2}	0.33	0.05	-0.11	0.36	0.28	-0.18	-0.16	-0.13	-0.49	-0.31	0.37	-0.38	-0.10	1	
Grains ear^{-1}	-0.58	-0.08	0.47	-0.32	-0.44	0.02	0.03	0.15	0.80	0.76	0.01	0.31	0.78	-0.64	1

Field plan of parental genotypes in 2004, 2005 and 2006

Parental genotypes in 2004

Bacanora	**NL1**	**NL2**	**NL1**	**NL2**	**Bacanora**	**NL2**	**NL1**	**Bacanora**	**NL2**	**Bacanora**	**NL1**	**Bacanora**	**Nl1**	**NL2**
Rep 1			Rep 2			Rep 3			Rep 4			Rep 5		

Parental genotypes in 2005

NL2	**NL1**	**Bacanora**	Rep 3
Bacanora	**NL2**	**NL1**	Rep 2
Bacanora	**NL1**	**NL2**	Rep 1

Parental genotypes in 2006

NL1	**Bacanora**	**NL2**	Rep 4
NL2	**NL1**	**Bacanora**	Rep 3
Bacanora	**NL2**	**NL1**	Rep 2
Bacanora	**NL1**	**NL2**	Rep 1

Field plan of NL2 x Rialto DH population in 2005 (120 plots of 59 lines + check laid on alpha-lattice in 2 reps)

120	119	118	117	116	115	114	113	112	111
101	102	103	104	105	106	107	108	109	110
100	99	98	97	96	95	94	93	92	91
81	82	83	84	85	86	87	88	89	90
80	79	78	77	76	75	74	73	72	71
61	62	63	64	65	66	67	68	69	70
60	59	58	57	56	55	54	53	52	51
41	42	43	44	45	46	47	48	49	50
40	39	38	37	36	35	34	33	32	31
21	22	23	24	25	26	27	28	29	30
20	19	18	17	16	15	14	13	12	11
1	2	3	4	5	6	7	8	9	10

Rep 2

Rep 1

Field plan for NL2 x Rialto in 2006 (140 plots of 69 DH lines + NL2 laid on alpha-lattice design in 2 reps)

140	139	138	137	136	135	134	133	132	131	130	129	128	127
113	114	115	116	117	118	119	120	121	122	123	124	125	126
112	111	110	109	108	107	106	105	104	103	102	101	100	99
85	86	87	88	89	90	91	92	93	94	95	96	97	98
84	83	82	81	80	79	78	77	76	75	74	73	72	71
57	58	59	60	61	62	63	64	65	66	67	68	69	70
56	55	54	53	52	51	50	49	48	47	46	45	44	43
29	30	31	32	33	34	35	36	37	38	39	40	41	42
28	27	26	25	24	23	22	21	20	19	18	17	16	15
1	2	3	4	5	6	7	8	9	10	11	12	13	14

Rep 2

Rep 1