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Phenotyping of durum wheat (*Triticum turgidum* L. subsp. *durum)*development in a recombinant inbred line population

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Abstract

A good phenotyping of a key trait for adaptability and productivity like development is an essential pre-requisite for an integrate approach to breeding embracing physiology, molecular biology and modeling. A RIL population was grown in pots under three treatments: vernalized plants developing under long days, vernalized plants developing under short days and unvernalized plants developing under long days. Measured traits included: time to terminal spikelet, flag leaf and anthesis, final leaf number and phyllochron, number of spikelets per spike, rate and duration of tillering, maximum tiller number. The results were organized in three chapters. The first two chapters analyzed the genotypic variability in pre-anthesis phases, tillering capacity and spikelet number of durum wheat as affected by vernalization, photoperiod and earliness per se. In the third chapter the development of a sub-set of RILs was modeled by SiriusQuality2. The experimental approach utilized resulted in an accurate phenotyping and allowed to clearly distinguish between the effects of earliness per se, vernalization and photoperiod on the analyzed traits, and also to associate to each RIL a quantitative index describing its earliness per se, photoperiodic sensitivity and cold requirement. These indexes were strongly associated with the varietal parameters of the model, which was able to reproduce the phenotypic variability observed between RILs within environments.

Introduction

Importance of development

The major food crops of the world today were first domesticated in various centers of origin to which their wild ancestors were adapted. Crop production might have remained a very localized activity had not some of those species shown a remarkable ability to adapt to new environments, as expanding human populations and trade carried seeds to new areas (Hay and Porter, 2006). Thus wheat, originally adapted to grow in short Mediterranean winters at around 32 °N, produces its highest grain yields from crops grown for 10 months a 55°N (Hay and Porter, 2006).

The developmental response of a crop to the environment primarily determines its adaptability, and to a marked extent, also governs its productivity (Richards, 1991; Shorter et al., 1991; Horie, 1994). Adaptability and productivity result from a response to the environment able to generate a life-cycle that: fits the available growing season favoring an effective capture and use of resources; partition this available growing season creating an appropriate balance between the generation of 'sources of resources' (leaves and roots) and the filling of 'sinks of resources' (grains); allows the most critical phases to occur when environmental conditions guarantee an adequate availability of resources and abiotic stresses are not likely.

Development can be described as the duration of different growth phases (phasic development), or as the sequential production, differentiation, expansion and loss (for example, by orderly senescence) of the plant's organs (morphological development) (Ritchie, 1991; Hay and Porter, 2006). It determines what grows, when it starts growing and how long it keeps on growing. Plant morphological development is somewhat independent of phasic development, although it is closely coupled with phasic development and plant growth (Ritchie, 1991).

Development is primarily influenced by environmental factors which communicate the time of the year and/or growth conditions favorable for sexual reproduction and seed maturation. These factors include temperature, photoperiod (i.e. day length), light quality (i.e. spectral composition), and vernalization (i.e. exposure to a long period of cold).

Flowering is the most critical stage of wheat development (Iwaki et al., 2001; Law and Worland, 1997; Goldringer et al., 2006) and is therefore the most important developmental event for crop productivity and adaptation (Lawn et al., 1995). It partitions the whole growth season between a pre-anthesis period when the number of kernels per square meter is set, and a post anthesis period when kernels grow. The great sensitivity of this phase to environmental stresses establishes an optimal time-window available for flowering in each environment. In Mediterranean environments, the optimal flowering time for rainfed wheat is bounded by spring frosts and by late drought and temperature stress.

Genetic control of development

Flowering time is a complex trait that shows almost continuous variation in cereals (Cockram et al., 2007). The extensive studies on the genetic control of flowering time in winter cereals including wheat (Worland, 1996; Law and Worland, 1997; Laurie et al., 2004) have demonstrated that it is under the control of more than 20 loci (Snape et al., 1996; Koornneef et al., 1998) involving chromosomes of nearly all homeologous groups, often grouped as vernalization sensitive, photoperiod sensitive and earliness per se genes (Worland et al., 1987; Law et al., 1991).

Vernalization genes determine the sensitivity of wheat to cold temperatures. They differentiate vernalization insensitive or 'spring' wheats from vernalization sensitive or 'winter' wheats that require an extended period of cool (below 10 °C) temperatures before floral primordia are initiated (Worland, 1996). The vast majority of European bread wheats are vernalization sensitive (Lupton, 1992), an essential traits for autumn sown crops grown in areas where winter conditions are harsh, but not for durum wheat grown in Mediterranean environments. The mild winters of this climate makes vernalization sensitivity not indispensable to avoid flowering to occur before the end of the period with frost risks, or even detrimental when it moves flowering and grain filling to a period more prone to drought and temperature stress. Likely due to this fact, it has been traditionally assumed that Mediterranean durum wheats are not, or are only marginally, sensitive to vernalization (Gonzales et al., 2002), although Motzo and Giunta, (2007) showed the

persistence of some cold requirements also in modern durum wheat cultivars grown in Italy.

The lack of vernalization sensitivity is generally dominant and controlled by major genes for insensitivity to vernalization (*Vrn-A1*, *Vrn-B1* and *Vrn-D1* on the long arms of chromosome group 5 and *Vrn-B4* on the short arm of chromosome 7B) (Law et al., 1976; Worland et al., 1987; Snape et al., 1985).

Genes for sensitivity to photoperiod play an important role in accelerating or delaying flowering time in the spring after vernalization requirements has been satisfied. A homeologous series of major *Ppd* loci affecting the photoperiod response has been mapped to the short arm of the group 2 chromosome in wheat and are ranked *Ppd-D1>Ppd-B1>Ppd-A1* in terms of their potency (Worland et al., 1998). Dominant *Ppd* alleles greatly reduce sensitivity to photoperiod and confer an early flowering phenotype under both short and long day conditions, resulting in yield benefits under certain agro-environments (Worland et al., 1998). Presently, most European varieties of bread wheat that are day-length insensitive probably carry the *Ppd*-D1 allele deriving from the Japanese variety Akakomugi (Worland, 1996). Consistent pleiotropic effects of the *Ppd*-1 genes result in shorter plants, fewer spikelets per spike due to an earlier terminal spikelet stage but increased spike fertility (Snape et al., 2001).

Apart from these two groups of genes, many analyses of cereals have detected genes with subtle effects on flowering that have no obvious dependence on day length or low temperature (Laurie, 1997). These genes are generally referred to as 'earliness *per se*' (*eps*) genes and are generally located as QTL effects rather than as major genes (Snape et al., 2001; Griffiths et al., 2009). Also genes controlling earliness *per se* have been identified (*Eps*-2B on 2BS (Scarth and Law, 1983; Shindo et al., 2003); *Eps*-Am on 1AL sensitive to temperature (Bullrich et al., 2002; Appendino and Slafer, 2003) and other genes on 5AL (Kato et al., 2002). *Eps* genes seems relatively common in comparison to vernalization or photoperiod effects and consequently they may provide breeders with useful variation for fine-tuning flowering time (Laurie, 1997).

Breeding and development – the importance of phenotyping

Flowering time has been and actually is one of the major target of all wheat breeding programs (Snape et al., 2001). The transition from landraces to modern cultivars in durum wheat was characterized by a steady advance in anthesis date (Blum et al., 1989; Motzo et al., 2004) contributing to an increase in yield potential, at least in the Italian durum wheat cultivars (Giunta et al., 2007). A study on the effects of breeding on the phenology of Italian durum wheats (Motzo and Giunta, 2007) has demonstrated that the first substantive effect of breeding on phenology was achieved with introgression from *syriacum* germplasm, which increased precocity both by an increase in earliness *per se* and a reduction in photoperiod sensitivity. The next step, characterized by the introduction of the semi-dwarfing gene *Rht1*, had a specific effect of reducing photoperiod sensitivity, although the modern group of varieties has a relatively low level of earliness *per se*, which is fundamental for preserving and increasing the length of the terminal spikelet-anthesis period. Some quantitative cold requirement still persists in Italian germplasm, although all the cultivars are usually classified as spring types.

The importance of flowering time for yield is evidenced by the strong association often detected between yield and *Ppd* and *Eps* loci identified by QTL analysis (Reynolds et al., 2009). Although flowering time of available wheat cultivars covers the optimal range individuated for the environments where wheat is grown, a finer tuning of phenology can further improve adaptation and potential yield, i.e. by maximizing spike fertility and grain number via a change in the duration of the different pre-flowering phenological phases under the hypothesis their independent genetic control (Foulkes et al., 2011; Fischer, 2011) (Chapter I). Variation in the duration of pre-flowering phases is also related with yield potential and adaptability via the effects on the number of spikelets per spike and on tillering (Chapter II). The number of spikelets per spike influences grain yield when sowing date is changed (Arduini et al., 2009). Generally, with high soil fertility and wide spacing, a high tillering capacity can be a useful trait because the relative contribution of the main stem declines in parallel with a greater production and survival of tillers (Rawson, 1971).

Breeding science has greatly evolved in the last decades in response to the increasing knowledge and tools made available by physiology, molecular biology and modeling,

which have stimulated the integration of these new technologies into breeding and the development of a comprehensive and multidisciplinary approach to crop improvement.

The advent of various molecular markers has enabled the dissection of a phenotype into the effects of individual quantitative trait loci (QTL) (Peterson et al., 1989) and has created the opportunities for a more efficient plant breeding by the use of marker-assisted selection. On the other hand, QTLs for a given traits usually explain only a low proportion of the observed trait variation and are strongly dependent on the environment and on the genetic background (Borner et al., 1993; Blanco et al., 2002; Dudley et al., 2007). This usually results in strong genotype-by-environment or genotype-by-management interaction which render complex the application of genetic research to selection (Bertin et al., 2010) unless extensive experiments over several years at different sites are performed with numerous genotypes.

In other words, the enhanced ability to undertake genome-scale molecular biology (genotyping), have not been matched by the development of enhanced capability in phenotyping, i.e. capability to link genotype and phenotype (Campos et al., 2004) and to connect information at gene level to the expressed phenotype in a way that can be useful for selection (Miflin, 2000).

The use of ecophysiological modeling have been proposed to overcome these difficulties and give an insight into how genotype-by-environment interaction comes about (Tardieu, 2003) but models cannot account for the genetic basis of differences in response to the environment unless model parameters are linked with easily measurable physiological traits and known QTLs or genes (Yin et al., 2004; Struik et al., 2007). Many crop simulation models incorporate physiological knowledge but, to the present, few incorporate knowledge derived from genetic studies and are therefore very limited in their capacity to simulate genotypic differences. Many models make use of the so-called 'genetic' of 'varietal' parameters, intended to summarize different aspects of the genetic make-up of individuals and to represent in a quantitative manner either the presence or the absence of a group of genes that operate together as an interconnecting network, or the presence or absence of specific genes (White and Hoogenboom, 1996). These parameters, being parameters and being genetic, should be stable across environments, should represent the genetic variability available for a given species, and should have significant influences on model outputs (Boote et al., 2001). Rarely this characteristics are satisfied,

and most model genetic parameters are strongly environment-dependent. One of the most recent fields of research explore the possibility of identifying QTLs for the model genetic parameters using a genetic QTL approach and subsequently develop a QTL-based model whereby the original values of genetic parameters are replaced by QTL-based inputs (Yin et al., 2004, 2005).

Combining ecophysiological modeling and genetic mapping into a QTL-based crop models would help to resolve complex environment-dependent traits on a genetic basis. It would allow to predict performance of individuals in a RIL population under various environmental conditions (Yin et al., 2004, 2005). When QTL information is incorporated into crop models via the genetic parameters, the models should narrow genotype-phenotype gaps and become better tools for analyzing the genotype-by-environment interaction. Robust model structures and adequate input parameters are required to achieve this goal (Yin et al., 2004).

The experimental approach

The phenotyping of durum wheat development performed during my doctorate research would represent a contribution to the described evolution of breeding science.

A good phenotyping is an essential pre-requisite for the mapping of QTLs for both for the key traits controlling development, and the genetic parameters of a robust model.

Phenotyping is a very difficult work for complex growth and development traits as they are associated with genes interacting in networks with organism-environment system at a higher level of biological organization where the difference in genetic makeup express its functional consequence. The multiplicity of genes controlling development and the strong dependence of their expression on the environmental conditions create a series of gene x gene and gene x environment interactions which provide a major limitation for phenotyping.

From a methodologically point of view, a good phenotyping requires the choice of:

- the developmental traits to phenotype
- the genetic material to phenotype
- the environmental conditions/treatments

Developmental traits

The physiological knowledge of wheat development has resulted in the formulation of various frameworks which dissect time to flowering into few component traits able to explain genetic and environmental variation in development (Weir et al., 1984; Porter, 1993; Ritchie and Otter, 1985; Jamieson et al.,1998). The traditional phenological frame used to modeling phenology is to divide the whole interval from emergence to anthesis into several phenophases, assuming that vegetative and reproductive events are independent and that time to flowering can be simply thought as a progress from the vegetative to the reproductive phase. This is the base of the phenological descriptions in several simulation models (ARCWHEAT1, Weir et al., 1984; AFRCWHEAT2, Porter, 1993; CERES-Wheat, Ritchie and Otter, 1985).

Another framework was formulated by Jamieson et al. (1998) in accordance with many works demonstrating the interdependence between vegetative and reproductive events (Kirby, 1990; Hay and Kirby, 1991). In this phenology model the variation associated with vernalization requirement and daylength sensitivity is described in terms of primordia initiation, leaf production, and final main stem leaf number. The model simulates the duration of three development phase: the first is the pre-emergence phase (sowing to emergence), the second is the leaf production phase from crop emergence to flag leaf appearance and the third is the duration of the flag leaf ligule appearance – anthesis phase. The pre-emergence phase is simulated as a fixed duration in thermal time which may differ between cultivars (Weir et al., 1984). The leaf production phase integrates the effects of vernalization and photoperiod, whereas the duration of the third phase is proportional to the phyllochron (Brooking et al., 1995). This robust phenological frame is the basis for development simulation in SiriusQuality2 model (Martre et al., 2006,2008) and has been used for this work in Chapter III with the aim of verifying to which extent Sirius model is able to simulate genotypic differences in phenological traits anthesis date, final leaf number and phyllochron.

Genetic material

Traditionally, physiologists cannot afford to carry out detailed experiments on complex traits with many genotypes, and therefore one of the main limits of the existing studies analyzing development is the limited number of genotypes studied (Jackson et al., 1996).

This impeded to adequately explore the existent genetic variability for the many loci involved in the control of development, and was not useful for QTL detection. Enlarging the number of genotypes studied was made feasible in this experiment by the *a priori* individuation of a limited number of phenological traits representing the key responses of genotypes to the main environmental stimuli, namely temperature and photoperiod.

One hundred lines from a bi-parental RIL population were analyzed, deriving from two parent cultivars already characterized for their different developmental rate due to both different vernalization and different photoperiodic sensitivity (Motzo et al., 2007). This choice was a premise to the existence in the population of genetic variation for the main groups of genes controlling development and at the same time resulted in the possibility of using collected data for a future QTL mapping.

Treatments

Treatments were planned with the idea of distinguishing to what degree the variability identified was dependent on differences in sensitivity to photoperiod, vernalization requirements or earliness per se.

Numerous preceding studies have characterized vernalization requirements, photoperiod sensitivity and earliness per se in a semi-quantitative manner (Davidson et al., 1985; Hoogendoorn, 1985; Miura and Worland, 1994; Ortiz-Ferrara et al., 1998) by comparing a limited number of cultivars under different vernalization or photoperiod regimes often obtained using controlled environments. In this experiment field testing was chosen, following an alternative approach already tested for its validity by Motzo and Giunta (2007) and by Herndl et al. (2008). This approach consisted in the evaluation of genetic differences between plants subjected or not subjected to pre-planting vernalization, tested under two planting dates differing in photoperiod (December, short-day conditions and May, long-day conditions). The three combinations realized were: vernalization + long-day, no-vernalization + long-day and vernalization + short-day. These treatments resulted in the 'activation' of different groups of 'flowering genes' in the different treatments: only earliness *per se* genes in 'vernalization + long-day' conditions, earliness *per se* plus vernalization sensitivity genes in 'no-vernalization + long-day' conditions, and earliness *per s*e plus photoperiodic sensitivity genes in 'vernalization + short-day' conditions. A relative response to vernalization and photoperiod was then calculated as deviation from

the rate of development resulting from earliness per se alone by the computation of the same indexes proposed by Herndl et al. (2008).

The greatest advantages of this experimental approach were to avoid the confounding effect of the simultaneous action of all the flowering genes in the characterization of the germplasm analyzed, and to greatly simplify the calibration of varietal parameters for Chapter III.

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CHAPTER I

Genotypic variability in pre-anthesis phases of durum wheat as affected by vernalization, photoperiod and earliness per se

Introduction

Plant phenology defines the timing and duration of organ formation, and its optimization is one of the most important factors determining both adaptation to a particular environment and yield potential (Richards, 1991; Evans, 1993;Worland, 1996; Snape et al., 2001; Cockram et al., 2007). Wheat development from seedling emergence to flowering can be divided in two main phases in relation to the yield determination: a phase from emergence to terminal spikelet (TS) when all leaf and spikelet primordia on the main shoot apex are initiated and tillering occurs at the crop level; a 'reproductive phase' from TS to flowering when stem elongation and spike growth occur (Slafer and Rawson, 1994; Slafer et al., 2009).

Current understanding of wheat phenology has shown that most developmental events until anthesis can be related to the appearance of mainstem leaves (Kirby, 1990; Hay and Kirby, 1991; Miglietta, 1991). The determinate main stem of wheat develops by the sequential accumulation of primordia on the apex and their later differentiation into leaves and spikelets and this provides the mechanism by which the duration of phenophases vary (Jamieson et al., 1998). The rate of primordia production or plastochron is linearly coordinated with the rate of leaf appearance or phyllochron, as the phyllochron is two times the plastochron (Kirby, 1990). Therefore the duration of primordia initiation (sowing-TS) varies according to plastochron and number of primordia, and the duration of leaf emergence (until the appearance of the flag leaf ligula) varies according with phyllochron and final leaf number. The subsequent thermal time interval between flag leaf ligula appearance and anthesis is much more constant among genotypes and environments than the preceding period, although may vary among cultivars (Amir and Sinclair, 1991). A robust relationship has been found between Haun stage at TS and final leaf number, such

that the Haun stage at TS represents a good predictor of the final leaf number (Jamieson et al., 2007). This relationship partly derives from the small range in the number of leaves left to emerge after TS, which, in the dataset discussed by Jamieson et al. (2007), varied from a little less than 3 to about 4.5. Time to flowering and the length of the main phenophases are controlled by genotype, environment (mainly temperature and photoperiod) and their interaction (Garcia De Moral et al., 2002).

The genetic control of flowering time has been extensively studied in wheat and is due to three groups of genes that regulate the response to photoperiod (*Ppd* genes), the response to vernalization (*Vrn* genes), and genotypic differences in developmental rates independent of these two environmental factors, which define the so-called 'intrinsic earliness' or 'earliness per se' (*Eps* genes). Most of the variation in developmental rates is explained by vernalization and photoperiod response genes, with smaller effects of *Eps* alleles (Worland, 1996; Snape et al., 2001).

Vernalization and photoperiod influence the time to flowering by their effect on the final leaf number of the main stem as they do not affect rate of leaf primordia initiation (Miglietta, 1989, 1991; Kirby, 1990; Hay and Kirby, 1991; Brooking et al., 2002). According to Brooking and Jamieson (2002), vernalization and photoperiod have no direct effects on phyllochron when expressed in thermal time and any observed variation within a genotype can be explained through changes in final leaf number as the rate of leaf appearance usually declines after the Haun stage 6-8. On the other hand, if the number of leaves yet to appear is fixed at TS, then genotypic differences in the photoperiodic sensitivity of reproductive development after TS could lead to differences in leaf appearance rates (Pararajasingham et al., 1996). Miralles and Richards,(2000) demonstrated that the overall effect on heading date of changes in the rate of leaf emergence is small, as in their experiment it ranged between -1 and 3 days with respect to the control.

Compared to the extensive knowledge of the genetic control of flowering, less is known about the genetic control of pre-flowering phases (Borràs-Gelonch et al., 2011).

Vrn genes have major effects on the length of the emergence-TS period (Hasle and Weir, 1970; Rawson, 1970; Griffiths et al., 1985; Flood and Halloran, 1986 a and b; Roberts et al., 1988; Ritchie, 1991; Robertson et al., 1996; Snape et al., 2001; Motzo et al., 2007). This is also the period when the final leaf number is set, either by vernalization and

photoperiodic conditions, as a consequence of the transition to the reproductive phase and the appearance of the first spikelet primordium. In spite of the common assumption that all mainstem primordia at the completion of vernalization will become leaves (Jamieson et al., 2007), Brooking and Jamieson (2002) proved that final leaf number could be set after the appearance of the last primordium because, at least when vernalizing temperatures were high (11 °C), there were many more primordia on the mainstem when vernalization was saturated than eventually there were leaves.

On the other hand, the evidence of Hutley-Bull and Schwabe (1980) and Griffiths et al., (1985) suggests that also the sensitivity to photoperiod of final leaf number can continue after the initiation of the first spikelet primordium, until terminal spikelet formation, and that the final leaf number could therefore be set some time after the last foliar primordium has appeared. *Ppd* genes seems to affect also the subsequent TS-flowering phase, as a response to photoperiod after TS has been evidenced by Miralles and Richards (2000), independent of photoperiod during previous phases. In their experiment, plants with the same final leaf number had quite different durations of reproductive development, from TS to heading, in response to photoperiod treatments imposed during that phase only, demonstrating that the photoperiodic sensitivity of the sowing-TS and TS-heading phases were independent. The response to photoperiod after TS can be affected by the level of satisfaction of the vernalization requirement (Gonzales et al., 2002).

Although wheat yield components are being formed at all the time from sowing to maturity, there are particular phases that are more relevant for yield and that can be manipulated by breeding to increase crops adaptability and productivity (Slafer et al., 2009). Grain yield improvements in wheat, both bread and durum, have been highly associated with grain number per unit area (Foulkes et al, 2011; Giunta et al., 2007). The most recent reviews on the avenues to future increase in wheat yield potential highlight the possibility of increasing grain number via a greater spike dry weight around anthesis by optimizing wheat developmental pattern (Foulkes et al., 2011; Fischer, 2011; Reynolds et al., 2009). Based on the fact that spike growth mainly occurs in a rather short period within pre-anthesis development mostly coincident with the stem elongation phase, lengthening the stem elongation phase could result in improvements in spike dry matter at anthesis and in the number of grains (Slafer et al., 1996; 2001). The response of wheat grain number to duration of stem elongation mainly relates to the fate of floret primordia, and less floret

primordia are able to survive to become grains in parallel with reduction in spike dry matter when the stem elongation phase is shortened (Gonzales et al., 2003, 2005; Serrago et al., 2008; Bancal, 2008). In fact, Fischer (1984) defined a narrower spike growth period within TS-anthesis covering the time from 5 to 95% of final spike dry weight and comprised between the emergence of the penultimate leaf and anthesis. The influence of development on the length of this actual spike growth period in wheat can be assumed by its shorter duration under longer photoperiods (Fischer, 2011; Serrago et al., 2008).

Increasing the stem elongation phase to increase spike fertility would rise yield potential only if it will not cause a delayed anthesis, as anthesis date has already been optimized and several cultivars are available allowing the wheat crops to flower within an optimal window for the varying environments of wheat cultivations and sowing dates. Lengthening the stem elongation phase without producing major changes in the total period from sowing to anthesis therefore depends on the existence of genetic variability in the length of the stem elongation phase independent of any variability in the duration of the phases from sowing to the onset of stem elongation (Reynolds et al., 2009). A clear link between photoperiodic sensitivity, duration of spike growth and spike fertility has already been demonstrated in bread wheat (Slafer et al., 2005; Miralles and Slafer, 2007 and papers cited therein) but information on the extent of variability available is scarce (Whitechurch et al., 2007; Borras-Gelonch et al., 2011). Foulkes et al. (2011) suggest the manipulation of both photoperiodic sensitivity and earliness *per se* genes to obtain the optimal phenological pattern.

Most of the research in this area has involved bread wheat and its transferability to durum wheat has to take into account the absence of the D genome in durum wheat and hence of all the 'flowering genes' located there. In particular, *Ppd*-D1, the main source of photoperiod insensitivity in bread wheat (Worland and Law, 1985; Worland, 1999), seems to be more powerful than the other relevant source of photoperiod insensitivity *Ppd*-B1 (Snape et al., 2001), and *Vrn*-D1 more than *Vrn*-B1 (Eagles et al., 2010, Fischer 2011).

From an experimentally point of view, most research on wheat phenology has characterized vernalization requirements, photoperiod response and earliness *per se* of few cultivars in a semi-quantitative manner (Wall and Cartwright, 1974; Midmore, 1976; Davidson et al., 1985; Hoogendoorn, 1985; Miura and Worland, 1994; Ortiz-Ferrara et al., 1998) with different vernalization and photoperiod regimes in controlled environments,

which may reduce their quantitative reliability and applicability under field conditions (Herndl et al., 2008). An alternative approach could be field testing combining pre-planting vernalization treatments with two or more planting dates or locations that differ in photoperiod. In the absence of photoperiod and vernalization limitations, variation in development would be driven by earliness *per se*. Photoperiod sensitivity could be evidenced and quantified by deviations from the response in earliness per se, showing slower development in the environment with shorter photoperiod. In the same way, vernalization requirements can be assessed by comparing vernalized and unvernalized treatments under no limiting photoperiodic conditions (Motzo et al., 2007; Herndl et al., 2008).

This experiment was aimed at:

- characterizing the variability in duration in pre-flowering developmental phases, final leaf number and phyllochron in a recombinant inbred line population of durum wheat,
- discriminate to what degree the variability is due to differences in earliness per se, sensitivity to photoperiod or cold requirements,
- evidence whether the genotypic relationships between pre-flowering phases, final leaf number, phyllochron and anthesis date are differentially affected by earliness *per se*, sensitivity to photoperiod or sensitivity to vernalization.

Materials and methods

Plant material

A population of 100 recombinant inbred lines (RILs) randomly chosen within a population of 161 RILs obtained from a cross between durum wheat cultivars Ofanto (a semi-dwarf wheat released in 1990 and originated from a cross between Appulo and Valnova) and Cappelli (an old Italian tall cultivar derived from a selection of exotic landraces released in Italy in 1915), was used in the present study.

Treatments, experimental design and crop management

The experiments were carried out during 2010 and 2011 at Ottava, Sardinia (Italy) (41°N; 8°E; 225 m asl). In 2010 vernalized and not vernalized seedlings of each line were transplanted in pots on 24 May. A second sowing of vernalized seedlings was performed on 23 December 2010 in pots. Vernalization was achieved by imbibing grains at room temperature for 24 h and growing the seedlings in the dark at 4°C in a growth chamber for 40 d. Six-eight weeks below 5 °C are assumed to be sufficient for the full vernalization of most wheat cultivars (Davidison et al., 1985; Griffiths et al., 1985). Unvernalized material was germinated at room temperature, and seedlings of similar size were transplanted. Six seedlings were transplanted per pot, subsequently thinned to three, and two pots were assigned to each RIL/vernalization combination. In both sowing dates pots were ordered in a completely randomized design with two replication, maintained outdoors under non-controlled conditions in the May sowing and placed in a greenhouse in the December sowing.

The combination of vernalization treatment and sowing date resulted in three treatments: MAYV, where vernalized plants grew under long-day, MAYNV with unvernalized plants growing in long-days and DECV with vernalized plants growing in short-days. Under the Mediterranean conditions where durum wheat is usually grown, natural daylength will not exceed 15 h and the prevailing daylength at transplanting in the May sowing was 14.8 h.

Irrigation and fertilization were provided regularly to avoid any water or nutritional stress.

Phenotyping

The distance from the soil surface to the ligule of the youngest emerged leaf of each plant was measured twice weekly. Inspection of the pots continued on a twice a week basis, to record the stages of penultimate leaf and flag leaf emergence (FLA), booting and anthesis (ANTH) (anthers exerted from the spikelets, DC 61).

Daily values for minimum and maximum temperatures were recorded at the experimental site. Thermal time was calculated on a 0 °C basis as the sum of eight contributions each day of a cosinuosoidal variation between the observed maximum and minimum temperatures (Weir et al., 1984). Contributions for 1/8-day temperatures above 26°C (optimal temperature) were reduced falling to zero at 37 °C. Photoperiod was calculated according to Weir et al. (1984), in which photoperiod-effective radiation for each day

begins and ends when the sun is 6° below the horizon.

The number and length of leaves that had emerged on the main stem of each plant were recorded twice weekly until the flag leaf was fully extended, following Haun (1973). Rates of leaf emergence were calculated for each plant as the slope of the regression between the Haun stage and the thermal time from planting. Two separate regressions were performed for each plant: one including all the leaves and another limited to the leaves from 2 to 8. For all the plants, the linear regression was highly significant and explained more than 90% of the observed variation. An average phyllochron (PHY) was calculated as the reciprocal of the rate of leaf emergence from the regression including all the leaves, and another phyllochron was calculated from the subset of leaves (PHY28).

From the same data the total number of leaves on the main stem (FLN) and of the leaves already emerged at terminal spikelet (TS) was obtained. The latter were calculated by substituting the thermal time to TS in the regressions of Haun stage *vs* thermal time. The number of leaves emerged after TS was obtained as a difference between FLN and leaves at TS.

Derived data and statistical analysis

The terminal spikelet stage of development (TS) was estimated from data on the distance of the ligule from the soil surface. The point at which the internode began elongating (stem elongation phase) is characterized by a sharp increase in the distance of the last leaf ligule from the soil and occurs approximately simultaneously with TS (Hay, 1978). The relationship between the distance of the last leaf ligule from the soil and the thermal time from planting was therefore a bilinear one, with two linear segments of different slope: a lower slope before stem elongation, and a higher one after stem elongation. The thermal time corresponding to the change in slope was estimated by a 'segmented regression' implemented through the Splitline Regression procedure (GENSTAT, 2008).

According to Herndl et al. (2008) and White and Laing (1989), relative response to vernalization (RRV) and photoperiod (RRP) were computed using rates of development (R), defined as :

$$R= 1/F$$

where F is cumulated thermal time (°Cd) from transplanting to each stage of development. The relative response to vernalization was estimated as:

$$RRV = 1 - \frac{R_{MAYNV}}{R_{MAYV}}$$

where R_{MAYNV} was defined for plants grown in MAYNV and R_{MAYV} is the rate for plants grown in MAYV.

Similarly, the photoperiod response RRP was calculated as:

$$RRP = 1 - \frac{R_{DECV}}{R_{MAYV}}$$

The magnitude and statistical significance of the treatment, genotype and treatment x genotype interaction effects were obtained by a mixed model ANOVA (lines and treatment x line random; treatment, block within treatment and plants within block fixed), analysed using the REML procedure (GENSTAT 2008). The same type of analysis were also performed for each treatment separately. Variance components and BLUPs (Best Linear Unbiased Predictors) relative to each line and trait were then calculated and heritabilities were estimated from the resulting variance components. BLUPs (which represent the main genotypic effects) were used to broadly estimate genetic correlations among traits (Borràs-Gelonch et al. 2011).

Results

Daylength and temperature

Daylength at transplanting was equal to 14.8 h in May and to 9.4 h in December (**Fig.1**). In both MAYV and MAYNV it reached a maximum of 15.2 h in correspondence of the average TS date of the RIL. At anthesis the daylength was still 15.1 h in MAYV, 14.6 in MAYNV. In DECV daylength was always increasing, reaching 10.3 h at TS, 12.4 at FLA and 13.1 h at anthesis.

Average air temperatures were about 20.3 °C in the period before TS in the May treatments, 13.9 °C in the same period of the December treatment. They rose to 24.2 °C in MAYV during TS-ANTH, when they reached 18.1 °C in DECV.

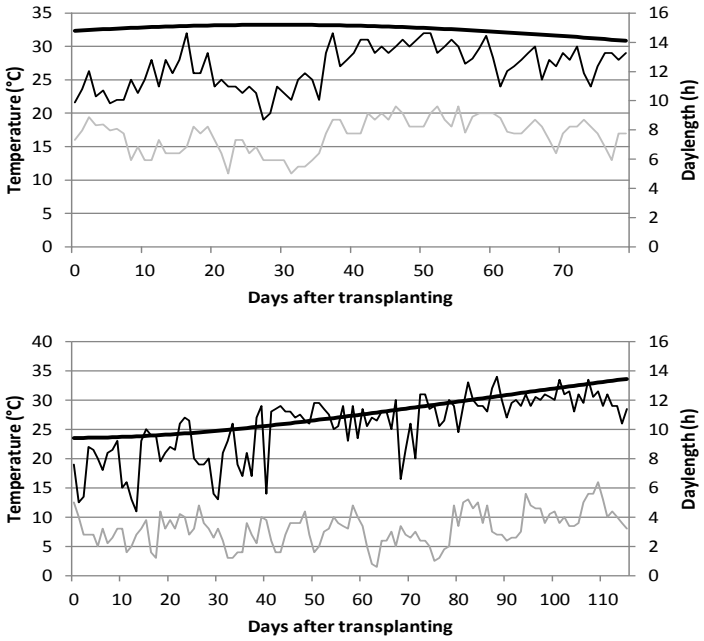

Figure 1. Maximum and minimum air temperatures and daylength of the period from transplanting to anthesis for the MAY (upper panel) and DECV treatments

Anthesis date

All plants flowered in the MAYNV treatment despite the lack of vernalizing temperatures, indicating the absence of obligate responses to vernalization.

The components of variance calculated through a mixed-model analysis of variance on the data from the three treatments/environments revealed a lack of genetic variation for anthesis date but a very relevant variation due to GxE interaction (**Tab. 1**). On the other hand, genetic variance was extremely high within each treatment.

Table 1. Variance components resulting from the mixed-model analysis of variance performed separately for each treatment and combining the three treatments (combined ANOVA) and corresponding heritability on a genotype-mean basis

	Combined ANOVA	MAYV	MAYNV	DECV
σ^2_G	147 ± 579	1138 ± 179	22951 ± 3610	3567 ± 578
σ^2_{GE}	8865 ± 966			
Residual	1639 ± 78	438 ± 32	4118 ± 439	1490 ± 117
$h^2_{Genotype\text{-}mean}$	4.4	92.6	94.2	91.4

As expected, the earliest anthesis was observed in MAYV, when earliness *per se* alone was driving development and only 819 °Cd (42 days) were sufficient on average to the

population to flower (**Tab. 2**). Parents did not differ in anthesis date in this treatment and the variability expressed by the RILs was scarce both in in days – it ranged from 40 to 48 days – and in thermal time (**Fig.2**).

The greatest delay in anthesis was induced by the photoperiodic limitation of DECV, which moved forward average population anthesis date by 796 °Cd compared to MAYV, exerting similar effects on both parents.

Table 2. Parent mean values (± standard error of the mean) and population mean and coefficient of variation for anthesis date expressed in cumulated thermal time from transplanting and in days after transplanting.

Trait		**Parents**		**Population**	
		Cappelli	Ofanto	Mean	*CV (%)*
ANTH (°Cd)	MAYV	857 ± 10	832 ± 0	819	3.9
	MAYNV	1566 ± 24	1090 ± 16	1277	11.4
	DECV	1717 ± 18	1564 ± 36	1616	3.5
ANTH (das)	MAYV	44 ± 1	43 ± 0	42	3.5
	MAYNV	80 ± 1	56 ± 1	64	10.8
	DECV	113 ± 1	107 ± 2	108	2.1

The lack of vernalization of MAYNV delayed anthesis by 457 °Cd (22 days) on average compared to MAYV, but its greatest effect was expressed on the variability amongst RILs (29 days and 600 °Cd, **Fig. 2**).

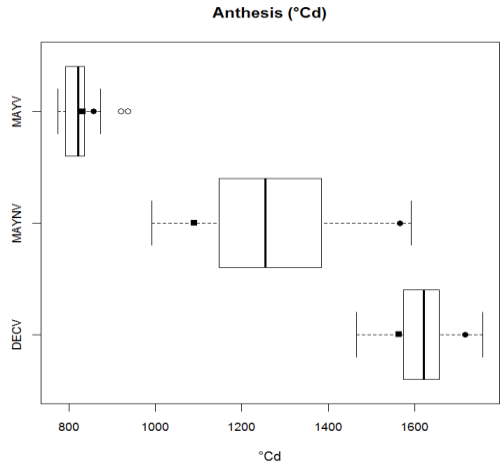

Figure 2. Boxplots of anthesis date expressed in cumulated thermal time from transplanting in the three treatments. Parents are indicated with a solid square ('Ofanto') and a solid circle ('Cappelli').

Cultivar Cappelli was always later than cultivar Ofanto and differences were greater in MAYNV (476 °Cd, 24 d) than in DECV (153 °Cd, 6 d).

Pre-flowering phases

As already reported for anthesis date, the components of variance calculated for the pre-flowering phases showed a genotypic variance close to zero or zero in the combined analysis cause most of the variance was captured by GxE interaction (**Tab. 3**).

Table 3. Variance components resulting from the mixed-model analysis of variance performed separately for each treatment and combining the three treatments (combined ANOVA) and corresponding heritability

Parameter	TS (°Cd)	TS-ANTH (°Cd)	PenLeaf-ANT (°Cd)	FLA-ANTH (°Cd)
Combined ANOVA				
σ^2_G	0 ± 449	46 ± 307	0 ± 81	0 ± 71
s^2_{GE}	7522 ± 848	4070 ± 518	806 ± 140	680 ± 125
Residual	2784 ± 128	3571 ± 165	2054 ± 96	2053 ± 94
$h^2_{Genotype\text{-}mean}$	0.0	2.3	0.0	0.0
MAYV				
σ^2_G	1472 ± 243	267.9 ± 65.4	196 ± 59	60.4 ± 26.1
Residual	1010 ± 72	823.9 ± 60.8	1004 ± 72	533 ± 39.5
$h^2_{Genotype\text{-}mean}$	88.2	61.1	49.4	35.1
MAYNV				
σ^2_G	17266 ± 2593	3580 ± 746	1941 ± 603	2363 ± 577
Residual	1164 ± 123	3583 ± 386	5715 ± 582	3700 ± 401
$h^2_{Genotype\text{-}mean}$	97.8	74.0	49.5	64.1
DECV				
σ^2_G	3270 ± 639	8656 ± 1411	290 ± 79	280 ± 83
Residual	4364 ± 339	3822 ± 299	1021 ± 79	1190 ± 93
$h^2_{Genotype\text{-}mean}$	77.0	91.0	55.0	51.1

Within treatment, on the contrary, genetic variance represented a significant and very high proportion of total variance, particularly for TS and TS-ANTH. A lower GxE and genetic variance was observed in the two shorter periods of 'penultimate leaf-anthesis' and FLA-Anthesis.

The lack of vernalization in MAYNV almost doubled the time needed on average by the population to reach the TS compared to MAYV (43 *vs* 22 days, 849 *vs* 450 °Cd) (**Tab. 4**). MAYNV was also the treatment in which the greatest genotypic variability was expressed (**Fig. 3)** corresponding to a range of 514 °Cd (from a minimum of 30 to a maximum of 55 d). A smaller lengthening effect compared to MAYV was induced on this phase by the photoperiod limitation of DECV. Parents showed opposite behaviors because, compared to MAYV, Cappelli showed the greatest lengthening of TS in MAYNV, and Ofanto in DECV.

Table 4. Parent mean values (± standard error of the mean) and population mean and coefficient of variation for the pre-flowering phases

Trait		**Parents**		**Population**	
		Cappelli	Ofanto	Mean	*CV (%)*
TS (°Cd)	MAYV	471 ± 3	374 ± 14	450	8.0
	MAYNV	1021 ± 25	695 ± 13	849	15.3
	DECV	536 ± 25	745 ± 36	575	8.5
TS-ANTH (°Cd)	MAYV	382 ± 10	458 ± 14	371	3.4
	MAYNV	546 ± 36	395 ± 27	430	11.6
	DECV	1182 ± 39	819 ± 64	1037	8.5
Penultimate leaf-ANTH (°Cd)	MAYV	259 ± 11	331 ± 10	246	3.5
	MAYNV	365 ± 41	291 ± 16	299	10.0
	DECV	321 ± 6	325 ± 10	314	3.9
FLA-ANTH (°Cd)	MAYV	187 ± 10	250 ± 11	168	2.7
	MAYNV	257 ± 27	217 ± 16	211	17.9
	DECV	260 ± 17	270 ± 8	258	4.5

The length of TS-ANTH period was mostly affected by the photoperiodic limitations of DECV, which induced in the population an average 2.8 fold increase in duration compared to MAYV. This was also the treatment with the greatest range among RILs, equal to 464 °Cd (30 days) on average. Cultivar Cappelli had a longer duration of this phase than cultivar Ofanto in the two vernalized treatments, and reached TS phase 800 °Cd later in DECV than in MAYV compared to the delay of only 361 °Cd observed in cultivar Ofanto.

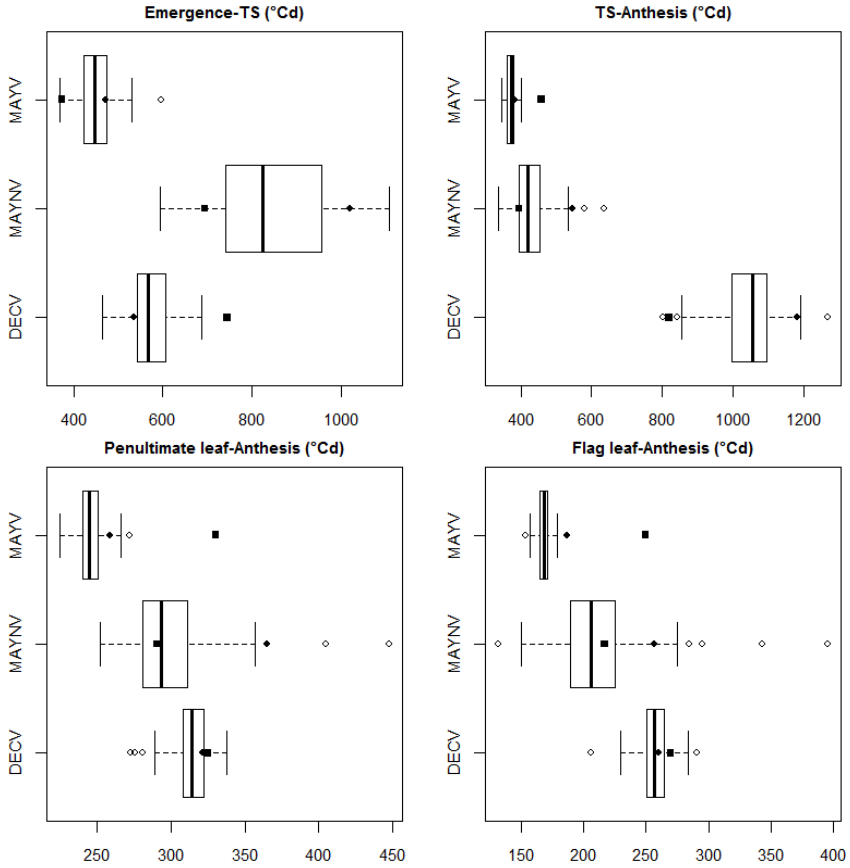

Figure 3. Boxplots of pre-flowering phases expressed in cumulated thermal time from transplanting in the three treatments. Parents are indicated with a square ('Ofanto') and a solid circle ('Cappelli').

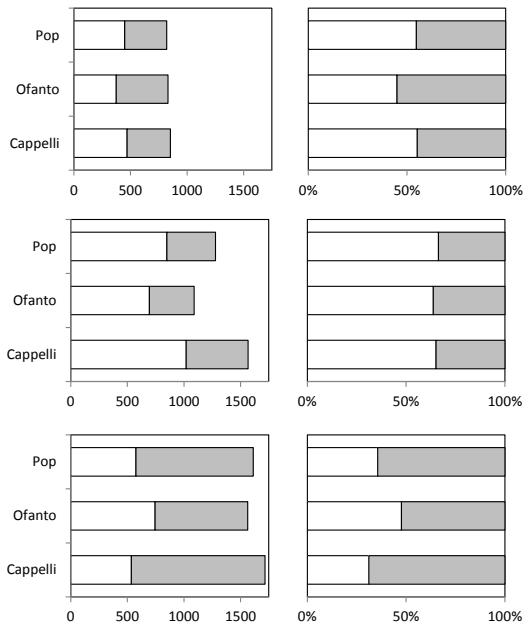

Figure 4. Proportion of the TS-anthesis phase over the whole transplanting-anthesis period in cumulated thermal time (left) and in percentage (right).

The relative contribution of the TS-ANTH to the whole pre-anthesis period varied considerably with the treatment applied (**Fig. 4**).

In MAYV it represented about half of the pre-anthesis period, but its contribution decreased to 34-36 % in MAYNV and increased in DECV, to a different extent in the population (from 45 to 64 %) and in the parents: it reached 69 % of the whole transplanting-anthesis period in cultivar Cappelli whereas it even showed a slight decrease – from 55 to 52 % - in cultivar Ofanto.

The period after the appearance of the penultimate leaf was scarcely affected by the treatments applied in absolute value (a maximum difference of 68 °Cd was observed between MAYV and DECV), but represented about 70% of the TS-ANTH period in the two MAY treatments, and only 32 % under the photoperiodic limiting conditions of DECV (**Fig. 5**). In cultivar Ofanto its proportion was higher (43 %) in this treatment only as a consequence of the shorter TS-ANTH characterizing this parent and not because of a greater absolute value.

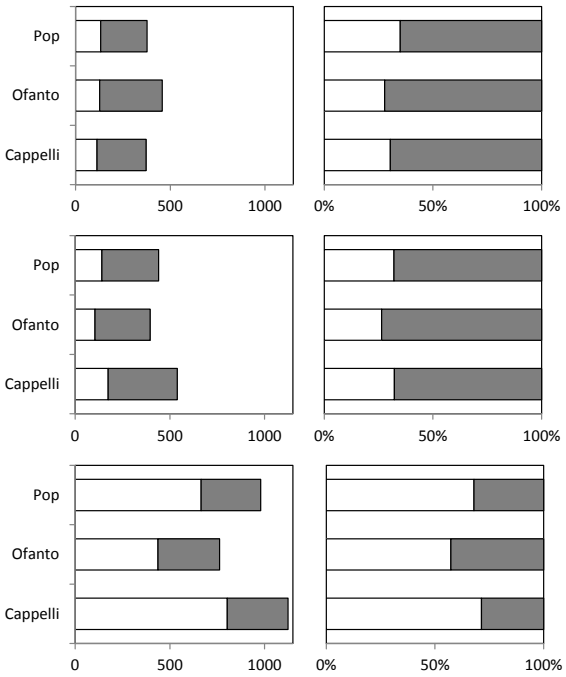

Figure 5. Proportion of the penultimate leaf-anthesis phase over the TS-anthesis period in cumulated thermal time (left) and in percentage (right).

The greatest genotypic variation in penultimate leaf-anthesis (from 252 to 365 °Cd excluding two outliers, corresponding to a range of 5 days, from 13 to 18 d in cultivar Cappelli) was observed in the MAYNV treatment (**Fig.3**).

The length of the FLA-ANTH period varied, in the three treatments, from a minimum of 168 °Cd in MAYV to a maximum of 258 °Cd in DECV. On the other hand, a three-fold variation was observed among RILs in MAYNV (from 132 to 395 °Cd, i.e. about 3 days, from 16 to 19 d). Parents only differed in the length of FLA-ANTH in the MAYV treatment, when cultivar Cappelli exhibited a shorter FLA-ANTH compared to cultivar Ofanto, whose FLA-ANTH was sensibly longer (250 °Cd, 13 d) than the maximum values observed in the population.

Number of leaves

The combined analysis of variance evidenced a significant GxE interaction but the absence of any genetic variance for any of the traits analyzed (**Tab. 5**). The genetic component of variance within each treatment/environment was higher for FLN and leaves at TS than for leaves after TS. A particularly low value was calculated for this trait in MAYV.

Table 5. Variance components of final leaf number, number of leaves emerged at terminal spikelet and after terminal spikelet, resulting from the mixed-model analysis of variance performed separately for each treatment and combining the three treatments (combined ANOVA) and corresponding heritability

Parameter	FLN (no)	Leaves at TS (no)	Leaves after TS (no)
Combined ANOVA			
σ^2_G	0.071 ± 0.06	0 ± 0.025	0.0132 ± 0.019
s^2_{GE}	0.674 ± 0.08	0.356 ± 0.044	0.1436 ± 0.029
Residual	0.422 ± 0.02	0.222 ± 0.011	0.48 ± 0.023
$h^2_{Genotype\text{-}mean}$	19.4	0	9.4
MAYV			
σ^2_G	0.201 ± 0.04	0.122 ± 0.023	0.031 ± 0.017
Residual	0.375 ± 0.03	0.161 ± 0.012	0.381 ± 0.028
$h^2_{Genotype\text{-}mean}$	73.1	78.6	28.2
MAYNV			
σ^2_G	2.119 ± 0.35	0.891 ± 0.155	0.3474 ± 0.102
Residual	0.605 ± 0.07	0.337 ± 0.039	0.77 ± 0.087
$h^2_{Genotype\text{-}mean}$	90.8	87.6	54.5
DECV			
σ^2_G	0.115 ± 0.03	0.105 ± 0.024	0.144 ± 0.037
Residual	0.363 ± 0.03	0.224 ± 0.017	0.432 ± 0.034
$h^2_{Genotype\text{-}mean}$	58.8	67.7	59.9

The minimum FLN of about 8 leaves was observed in the MAYV treatment (**Table 6**) in cultivar Ofanto. The lack of vernalization of MAYNV caused the greatest increase in FLN, with the maximum values observed in one RIL producing about 17 leaves (**Figure 6**). Cappelli was the parent cultivar with the highest number of leaves in this treatment (15 leaves).

The number and the proportion of leaves produced before and after TS changed dramatically with the treatment applied. Five-six leaves had already emerged at TS in MAYV and DECV, but this number corresponded to about 60% and 45% of FLN, respectively. The highest number (9-10) and the greatest proportion of leaves at TS (72 % of FLN) was observed in MAYNV.

Table 6. Parent mean values (± standard error of the mean) and population mean and coefficient of variation for the final leaf number, the number of leaves emerged at terminal spikelet and after terminal spikelet.

Trait		**Parents**		**Population**	
		Cappelli	Ofanto	Mean	*CV (%)*
FLN	MAYV	9.5 ± *0.3*	7.8 ± *0.3*	8.9	4.2
	MAYNV	15.0 ± *0.4*	11.6 ± *0.2*	13.6	10.1
	DECV	12.5 ± *0.3*	11.7 ± *0.2*	12.2	2.1
Leaves at TS	MAYV	5.6 ± *0.3*	4.7 ± *0.2*	5.7	5.4
	MAYNV	10.1 ± *0.6*	8.8 ± *0.4*	9.8	8.7
	DECV	4.7 ± *0.3*	6.4 ± *0.2*	5.3	4.9
Leaves after TS	MAYV	4.0 ± *0.1*	3.2 ± *0.2*	3.2	2.9
	MAYNV	4.9 ± *0.9*	2.9 ± *0.3*	3.8	11.0
	DECV	7.9 ± *0.2*	5.3 ± *0.3*	6.8	4.2

The greatest variability in FLN, and in leaves emerged at and after TS was expressed by the population in MAYNV (**Fig. 6**) when the range was equal to, respectively, 6, 4 and 2 leaves. A much narrower range was expressed in the two vernalized treatments. In DECV the two parent cultivars produced the highest (Cappelli) and the lowest (Ofanto) number of leaves after TS, with values very far from the range expressed by the population. Cultivar Ofanto, on the contrary, had the highest number of leave at TS. On the other hand, Cappelli had the highest number of leaves after TS also in MAYV.

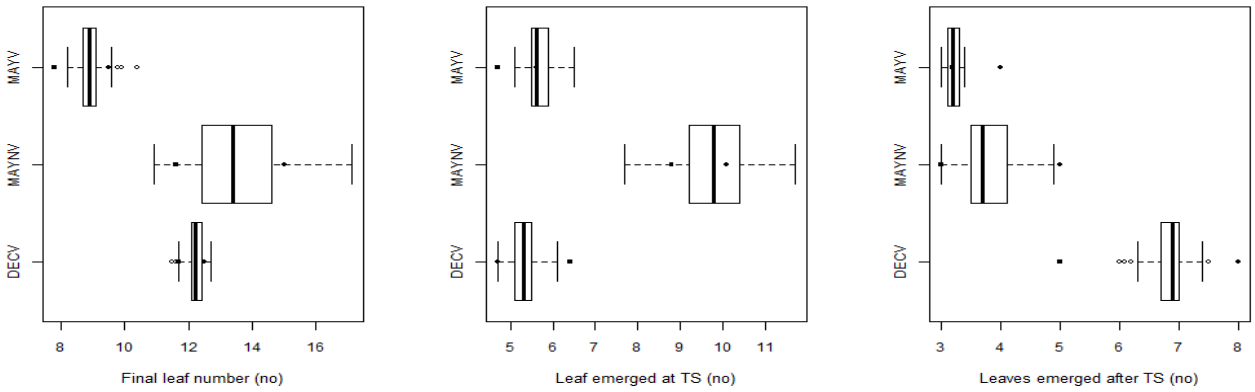

Figure 6. Boxplots of final leaf number and number of leaves emerged at and after the terminal spikelet stage in the three treatments. Parents are indicated with a solid square ('Ofanto') and a solid circle ('Cappelli').

Phyllochron

The relationship between Haun stage and cumulated thermal time was clearly linear in MAYV, when the minimum number of leaves was produced. In DECV several lines showed a slight decrease in the rate of leaf appearance after about 800 °Cd (well after TS), which was not related to any particular leaf number or stage of development or number of emerged leaves. This same pattern was observed in some plants of MAYNV. Data reported are therefore relative to both the average phyllochron obtained from the linear interpolation of Haun stage *vs* cumulated thermal time and to the phyllochron of leaves 2-8, for which a linear relationship between Haun Stage and thermal time can be assumed (Boone et al., 1990; Jamieson et al., 1995; Gonzàles et al. 2002).

Genetic variance calculated from combined analysis was irrelevant for average phyllochron, higher (it accounted for 22 % of the total variance) and equal to two times its standard error for PHY28 (**Table 7**).

Within treatments, on the contrary, more genetic variance was expressed for average PHY than for PHY28. The least genetic variance was found in the MAYV treatment for both types of phyllochron, the highest in DECV, when heritability values of 90 and 53 % were recorded for, respectively, average PHY and PHY28.

Table 7. Variance components of average phyllochron and of the phyllochron calculated for the leaves from the second to the eighth, resulting from the mixed-model analysis of variance performed separately for each treatment and combining the three treatments (combined ANOVA) and corresponding heritability

Parameter	Avg phyllochron (°Cd)	Phyllochron 2-8 (°C)
Combined ANOVA		
σ^2_G	2.07 ± 2.32	4.44 ± 2.14
s^2_{GE}	21.8 ± 3.57	7.01 ± 2.77
Residual	47.28 ± 2.19	79.5 ± 3.59
$h^2_{Genotype\text{-}mean}$	12.0	22.2
MAYV		
σ^2_G	5.74 ± 2.8	5.39 ± 3.33
Residual	66.07 ± 4.64	87.07 ± 6.02
$h^2_{Genotype\text{-}mean}$	30.4	24.7
MAYNV		
σ^2_G	21.42 ± 5.47	16.79 ± 7.52
Residual	41.22 ± 4.19	95.78 ± 9.44
$h^2_{Genotype\text{-}mean}$	61.1	34.9
DECV		
σ^2_G	53.86 ± 8.89	15.07 ± 4.29
Residual	26.54 ± 2.1	60.4 ± 4.62
$h^2_{Genotype\text{-}mean}$	89.9	52.8

A lower PHY was calculated for the late sown MAY treatments (**Tab. 8**). In fact leaves needed about 30 °Cd more to emerge in DECV compared to MAYV and MAYNV. PHY28 was very similar to the average phyllochron.

Table 8. Parent mean values (± standard error of the mean) and population mean and coefficient of variation for average phyllochron and the phyllochron calculated for the leaves from the second to the eighth

Trait		**Parents**		**Population**	
		Cappelli	Ofanto	Mean	*CV (%)*
Average PHY (°Cd)	MAYV	72 ± *2*	81 ± *4*	73	1.8
	MAYNV	87 ± *2*	73 ± *1*	79	4.4
	DECV	118 ± *4*	111 ± *4*	115	5.3
PHY leaves 2-8 (°Cd)	MAYV	73 ± *3*	79 ± *3*	74	1.5
	MAYNV	82 ± *1*	81 ± *2*	81	2.9
	DECV	115 ± *2*	109 ± *4*	113	2.4

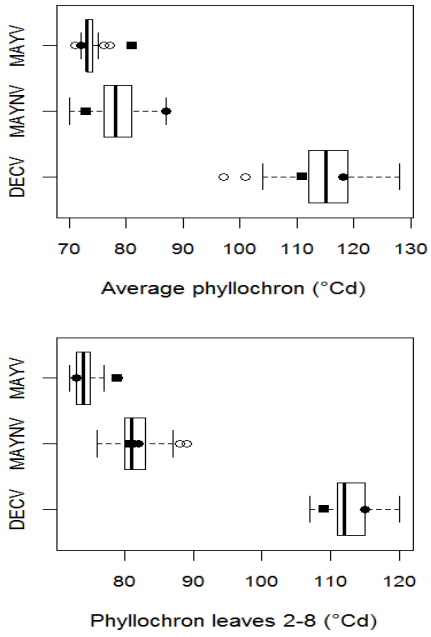

Figure 7. Boxplots of average phyllochron and the phyllochron calculated for the leaves from the second to the eighth in the three treatments. Parents are indicated with a solid square ('Ofanto') and a solid circle ('Cappelli').

Discussion

A large genotypic variability was expressed by the RIL population in anthesis date and pre-flowering phases partly as a consequence of the great difference in phenology between the two parent cultivars. Cappelli, is a tall late cultivar with both high photoperiodic sensitivity and high cold requirements (Motzo et al., 2007), whereas Ofanto is a modern semi-dwarf cultivar, earlier than Cappelli when sown in winter (De Vita et al., 2007), with presumably lower cold requirements and photoperiodic sensitivity than Cappelli as a consequence of the intense breeding work carried out by Italian breeders on durum wheat to hasten flowering. A large difference in earliness per se was also expected in this population as modern Italian cultivars are characterized by a lower earliness *per se* compared to the older constitutions (Motzo et al., 2007).

An accurate phenotyping of the terminal spikelet stage could only be made by destructive samplings followed by dissection of the apex, an inapplicable procedure when many plants have to be checked. Alternatively, TS can be estimated through the closely associated and

more easily detectable onset of stem elongation (Kirby and Appleyard, 1981; Kirby et al., 1994; McMaster, 1997). Recently, Borràs-Gelonch et al. (2011) found no substantial differences between the results using phases estimated with TS and phases estimated using the onset of stem elongation evaluated by the elongation of the first internode.

Apart from TS and TS-ANTH, two more sub-phases of the TS-ANTH period were considered: the period between the emergence of the penultimate leaf and anthesis and the FLA-Anthesis. The first has been indicated by Fischer (1984) as the period when the spikes in a crop accumulate the last 95% of final dry weight, grains excluded and is therefore more tightly linked with spike growth and fertility than TS-ANTH. The FLA-anthesis is important for anthesis date prediction and some models assume that it is only sensitive to temperature and is therefore equal to a fixed thermal time or proportional to the phyllochron (Amir and Sinlair, 1991; Brooking et al., 1995).

The extremely low contribution of genetic effects to the total variance and the very high GxE contribution found for all the phenophases, FLN and leaves at TS can be interpreted by considering that, although we compared the same lines in the three treatments, the development of each line was controlled by a different set of genes in each treatment. We can therefore assume from this result that treatments applied were effective in activating different gene groups –*Eps* in MAYV, *Eps* and *Vrn* in MAYNV, *Eps* and *Ppd* in DECV, an assumption reinforced by the concomitant high genetic variance and heritability within each treatment. Only the number of leaves after TS and the phyllochron performed differently. The number of leaves after TS showed a genetic component of variance within treatment very low compared to FLN and leaves at TS, consistently with the scarce variation in leaves left to emerge after TS found by Jamieson et al. (2007). The phyllochron of the leaves 2-8 showed a significant genetic component at the combined analysis, in contrast with the average phyllochron. Heritabilities of leaves after TS were lower of the ones calculated for the average phyllochron in those treatments where photoperiod or vernalization were limiting development, but not with plants vernalized grown under long days. The low to moderate heritability values calculated in this experiment for PHY28 are lower than those reported by Syme et al. (1974) for some Mexican and Australian bread wheats (0.68). These Authors proposed a polygenic control of phyllochron and sustained the possibility to select for this trait. Our results let us assume that the 'flowering genes' exerted only a limited control the PHY28, and that the higher

heritability calculated for the average phyllochron simply reflected 'flowering genes' effects on FLN. Whenever FLN is large cause photoperiodic or vernalization limitations, the slower rate of emergence of later emerged leaves establishes a relationship between FLN and average phyllochron (Gonzales et al., 2002; Brooking and Jamieson (2002).

Earliness per se

The MAYV treatment was utilized to analyze earliness per se because it was characterized by the least limiting conditions in both cold and daylength, as confirmed by the short transplanting-TS period – 450 °Cd the average of the RIL population – comparable to the value of 400 °Cd proposed by Ritchie (1991) for fully vernalized wheat plants grown under long days. The minimum FLN was also observed in this treatment – 7.8 in cultivar Ofanto, 8.9 on the average of the RIL population - although it was higher than the minimum values of 6-8 leaves reported by Levy and Peterson (1972) and Rahman (1980) for spring wheats under more than 16 h of light. On the other hand, Brooking and Jamieson (2002) observed that the minimum FLN in vernalized treatments varied in response to vernalizing temperature, from a minimum of 8 to higher values. We can therefore hypothesize that in our experiment some leaf primordia were initiated prior to saturation during the 40 days of vernalization.

Compared to vernalization and photoperiod sensitivity, earliness per se generated the least genotypic variability in the anthesis date of the population, although still comparable with the variability detected by van Beem et al. (2005) across 51 cultivars, largely from CIMMYT. Consistently with this limited range in anthesis date, a narrow variability was also observed in FLN and in phyllochron, although still sufficient to detect a negative association between anthesis date and FLN ($r = -0.59$, $P<0.001$) and anthesis date and PHY28 ($r = -0.25$, $P<0.05$).

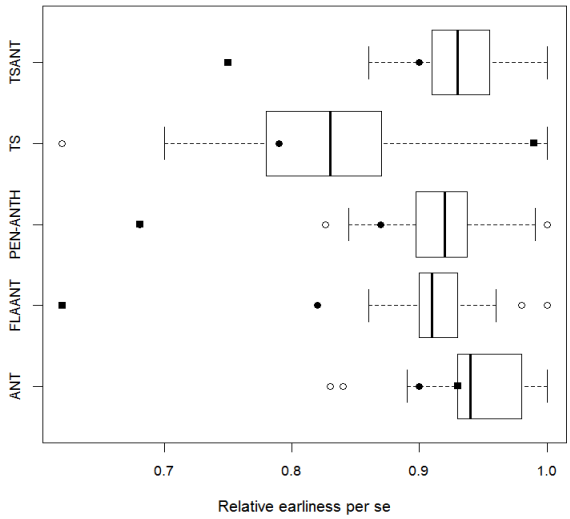

Figure 8. Boxplots showing the relative earliness per se of the RILs and of the parents for anthesis date and for the pre-flowering phases considered. Parents are indicated with a solid square ('Ofanto') and a solid circle ('Cappelli').

Slafer and Rawson (1995) suggested that basic developmental rate can vary both between genotypes and for a same genotype with stage of development. A relative earliness per se index was calculated with respect to the earliest genotype in the MAYV treatment to compare the variability in earliness *per se* of the different pre-flowering phases (**Fig.8** , the higher the index, the greater the earliness *per se*). Among the four pre-flowering phases considered, TS showed the greatest genotypic variability in earliness per se and FLA-ANTH the smallest. This scarce variability, combined with that expressed in phyllochron, resulted in a quite constant value of the number of phyllochrons necessary to reach anthesis (2.1 -2.6) after the appearance of the flag leaf ligula. Extreme values of earliness per se were observed in cultivar Ofanto, which showed the greatest earliness per se for TS, but the lowest earliness per se for all the phenophases after TS. A similar result was obtained by Motzo et. (2007) which showed that the earliest anthesis of modern durum wheat cultivars such as Ofanto is mainly the result of a low photoperiodic sensitivity combined with a low earliness *per se.*This combination resulted in a shorter duration of TS and a longer duration of TS-anthesis and FLA-anthesis in the modern compared to the older constitutions. This same negative association between earliness per se before and after TS was found for the whole RIL population (**Fig. 9a** , $r = -0.55$, $P<0.001$). At the same time, a high earliness *per se* in TS-ANTH is mirrored in a high earliness *per se* in the FLA-ANTH period ($R = 0.70$, $P<0.001$).

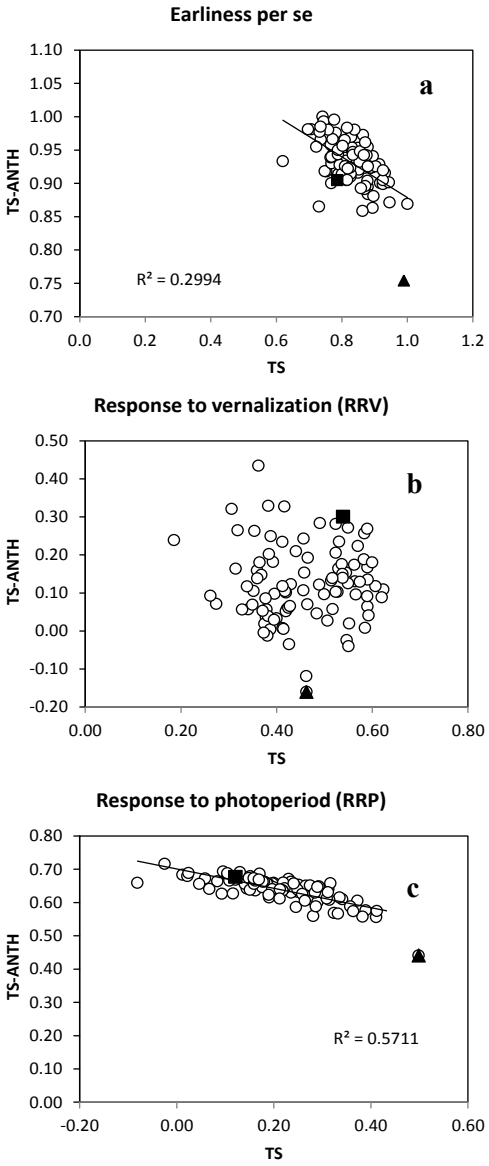

Figure 9. Relationship between the periods transplanting-terminal spikelet and terminal spikelet-anthesis based on the RILs' earliness per se (a), relative sensitivity to vernalization (b) and to photoperiod (c) calculated for the two phenophases.

Cold requirements

The RRV and RRP indexes calculated according to Herndl et al. (2008) were used to quantify, respectively, the sensitivity to vernalization and photoperiod of each line and of the parents (the higher the index, the greater the sensitivity).

RRV for anthesis ranged between 0.20 and 0.49 (**Fig. 10**) and was almost identical to the range quoted by Herndl et al. (2008) for a set of 26 bread wheat cultivars from the North Europe. Although the presence of Cappelli as one of the parents (RRV = 0.45) justifies the presence of some cold requirements in the RIL population analyzed, Motzo et al. (2007)

demonstrated that some cold requirements persists also in modern durum wheat cultivars. The traditional assumption that Mediterranean durum wheats are not – or are only marginally – sensitive to vernalization, derive from the consideration that vernalization sensitivity is not indispensable to avoid flowering to occur before the end of the period with frost risks in this type of environment. In fact the winter sowing dates commonly adopted in this environments did not favor a selection against vernalization sensitivity as any cold requirement is usually satisfied.

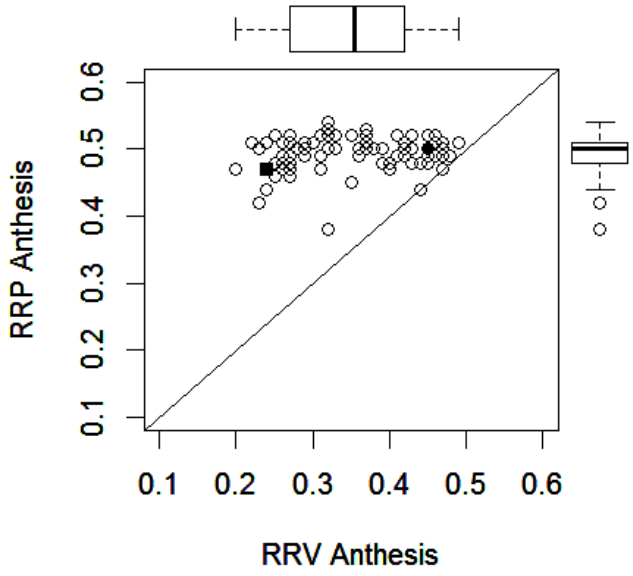

Figure 10. RILs' relative sensitivity to photoperiod (RRP) estimated from anthesis date plotted against relative sensitivity to vernalization (RRV) and corresponding boxplots. Parents are indicated with a square ('Ofanto') and a solid circle ('Cappelli').

The pre-flowering phase most affected by vernalization was TS (RRV 0.46 are average **Fig.11**), in accordance with the known remarkable effects of *Vrn* genes on the length of the emergence-TS period (Hasle and Weir, 1970; Rawson, 1970; Flood and Halloran, 1986 a and b; Griffiths et al., 1985; Roberts et al., 1988, Ritchie, 1991; Robertson et al., 1996; Snape et al., 2001; Motzo et al., 2007). The average population RRV was high and higher than the average population RRP only for this phenophase, implying a greater effect of vernalization than of photoperiod. This result could partly be the outcome of a greater limitation encountered by the non-vernalized plants in May, when no natural vernalization occurred, compared to the relatively short days of DECV (between 9.4 h at transplanting and 10.3 h at TS, on average). As expected by the lack of any direct effect of vernalization after TS (Jamieson et al., 2007), RILs did not lengthen or only marginally lengthened the

duration of the phases after TS, as shown by RRV indexes very low and close to zero for many lines.

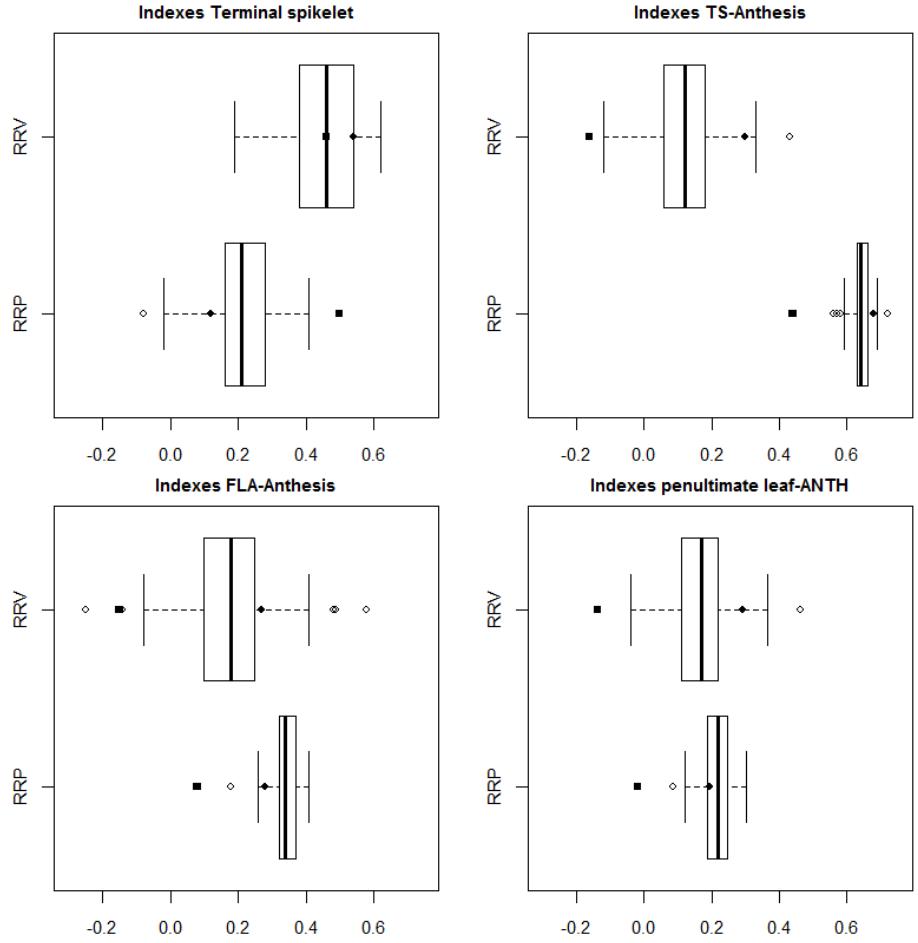

Figure 11. Boxplots showing the variability among RILs in relative photoperiodic and vernalization sensitivity for the pre-flowering phases considered. Parents are indicated with a square ('Ofanto') and a solid circle ('Cappelli').

The still large variability evident in **Fig. 11** for these phases cannot be interpreted as a carry-on effect of the variability expressed for TS because no significant relationship was calculated between RRV at TS and after TS (**Fig. 9b**), mirrored in the absence of any relationship between the number of leaves emerged at TS and after TS.

The vernalization effect on the length of TS, i.e. the phase during which leaf and spikelet primordia are produced (Kirby et al., 1999), helps to explain both the great genotypic variability in FLN, leaves at TS and after TS, and the positive association between RRV for anthesis date with FLN ($r = 0.79$, $P<0.001$) and leaves at TS ($r = 0.66$, $P<0.001$). Vernalization influence the time to flowering by its effect on the final leaf number of the

main stem as it does not affect rate of leaf primordia initiation (Miglietta, 1989, 1991; Kirby, 1990; Hay and Kirby, 1991; Brooking et al., 2002).

According to Brooking and Jamieson (2002) and Gonzales et al. (2002), vernalization had no direct effects on PHY28 and the positive association found with average phyllochron ($r = 0.43$, $P<0.001$) can be explained through the changes in final leaf number because the rate of leaf appearance usually declines after the Haun stage 6-8.

Photoperiodic sensitivity

On the average of the whole population, the RRP index quantifying the photoperiodic sensitivity of each line for anthesis date was equal to 0.49, a higher value compared to the one reported by Herndl et al. (2008). This result may reflect the action of different *Ppd* genes – the *Ppd D1* was involved in bread wheat variation but not in our durum wheat variation – but also the different photoperiodic conditions due to the different latitude of cultivation and sowing date.

RRP index for anthesis date was higher than RRV index (0.35) i.e., photoperiodic limitations delayed anthesis more than the lack of satisfaction in cold requirements (**Fig. 8**). This marked effect of photoperiod on anthesis date was totally due to the notable increase in phyllochron induced by the limiting photoperiodic conditions, as FLN was 1.4 leaves lower than the one observed when vernalization was the limiting factor. A higher phyllochron is usually detected under autumn and winter than in spring sowings (Kirby et al., 1982; Baker et al., 1980, Bassu et al., 2009), and photoperiod has been hypothesized as one possible cause of this difference (Baker et al., 1980; Belford et al., 1987; Delecollé et al., 1985). According to Jamieson et al. (2008), the reason of this difference should be searched in the use of air temperature in place of soil temperature to measure phyllochron, and daylength some time after emergence should simply be considered as a predictive surrogate for seasonal variation in the air-apex temperature difference.

The range in RRP between 0.38 and 0.54 expressed by the test population for anthesis date was narrower than the one expressed in vernalization sensitivity, according to the very limited variation in FLN associated with the vernalization of imbibed seeds causing their rapid switch to floral induction once transplanted.

Figure 8 also shows that the majority of RILs and cultivar Ofanto had a greater photoperiodic sensitivity compared to cold requirement, whereas a few lines and cultivar

Cappelli were characterized by a similar impact of vernalization and photoperiod on their developmental rate. No genotypic relationship existed between cold requirement and photoperiodic sensitivity, mirroring the physiological independence of vernalization and photoperiodic responses indicated by Halloran (1975). On the other hand, an association was found between RRP indexes and the relatively low genetic variability in earliness per se (**Fig. 12**): the RILs with a higher RRP also showed a higher earliness per se. This result could be ascribed to the co-location on the homeologous chromosomes of group 2 of the principal *Eps* genes mapped in wheat (*Eps*-2B, *Eps*-2D) with *Ppd*B1 (Snape et al. 2001). This result adds to the goodness of the experimental approach utilized to phenotype wheat phenology, which allowed to discriminate the two responses and to find out an association otherwise masked the contemporary action of the two groups of genes.

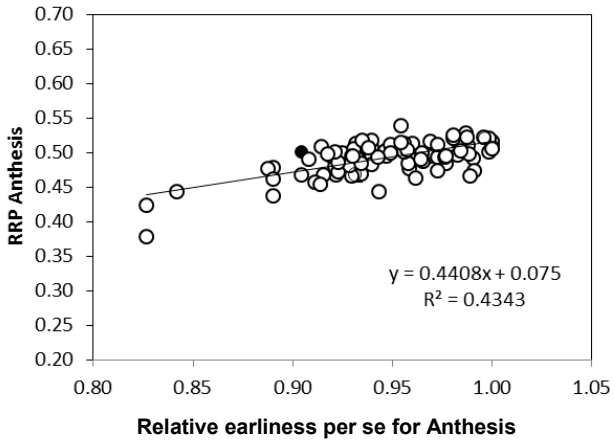

Figure 12. Relationship between RILs' relative photoperiodic sensitivity and earliness per se. Parents are indicated with a solid square ('Ofanto') and a solid circle ('Cappelli').

RRP for TS was low and close to zero for many lines (**Fig.** 11) and TS-ANTH was the pre-flowering phase most affected by photoperiodic sensitivity, confirming the prolonged sensitivity to photoperiod after TS demonstrated by Miralles and Richards (2000). The lengthening of the TS-anthesis was associated to the highest number of leaves yet to emerge after TS, which amplified the effect of the high phyllochron on this phase. A positive genotypic association was found between phyllochron and RRP for TS-ANTH ($r = 0.56$, $P<0.001$), confirming that photoperiodic conditions during this phase can directly

affect development, as demonstrated by Miralles and Richards (2000). The largest variability in PHY (37 °Cd range) was induced by photoperiodic limitations.

A negative genotypic correlation was calculated between RRP for this phase and RRP for the preceding TS phase (**Fig. 9**, R = - 0.76, P<0.001), mirrored in the negative association between the number of leaves produced before and after TS (r = - 0.63, P<0.001). It was the case of cultivar Ofanto, characterized by a very high photoperiodic sensitivity and the highest number of emerged leaves from transplanting to TS, but an apparent very low photoperiodic sensitivity accompanied by the lowest number of leaves left to emerge in the TS-anthesis period. In other words, the RILs with the lower photoperiodic sensitivity, by reaching earlier and hence with less leaves the TS stage, had more leaves yet to emerged after TS, confirming a genetic base of those negative correlations. At the same time, the RILs which reached earlier TS were subjected to more limiting photoperiodic conditions in comparison with the lines that reached TS later because daylength increased from about 10 h at TS to 13 h at anthesis. This environmental effect could have partially contributed to the above mentioned genetic correlation.

The negative relationship described between TS and TS-ANTH is mirrored in the lack of any significant effect of the duration of the TS-ANTH interval on anthesis date. This fact, coupled with the genetic variability detected in photoperiodic sensitivity, let us hypothesizes that it could be possible to increase the length of the stem elongation phase without delaying anthesis by simply selecting for a low photoperiodic sensitivity within spring genotypes. Our results are in contrast with the overall significant and positive genetic correlation found by Borràs-Gelonch et al. (2011) between TS and TS-ANTH in bread wheat, likely because in their experiment all the three groups of flowering genes were contemporary involved in the control of development. On the other hand, we did not found any positive relationship between TS and TS-ANTH also when vernalization alone was driving development.

Consistently with their minimal variation in thermal time, the sub-phases FLA-anthesis and penultimate leaf-anthesis showed very low RRP indexes, i.e. a lower sensitivity to photoperiod than the whole TS-anthesis period. This result confirm the common assumption of many models that the thermal time interval between flag leaf ligula appearance and anthesis is much more constant among genotypes and environments than the preceding period, although may vary among cultivars (Amir and Sinclair, 1991). On

the other hand, this result implies that the impact of a lengthening of the TS-ANTH on the period of maximum spike growth (penultimate leaf anthesis, Fischer, 1984) and hence on kernel number could be less than expected.

Conclusions

In spite of the impossibility to evaluate the interaction between the three groups of 'flowering genes', the experimental approach utilized allowed to clearly distinguish between the effects of the three groups of genes on both duration and variability of anthesis and pre-anthesis phases, and also to associate to each RIL a quantitative index describing its earliness per se, photoperiodic sensitivity and cold requirement.

The combination of this experimental approach with the use of an adequate phenological framework defining the key traits responsible for the genotypic differences in development resulted in an accurate phenotyping, eventually useful for a subsequent genetic analysis. The contemporary use of the length of phenophases and of the number and rate of emergence of leaves during these phenophases was useful for discriminating the responses to environment and the real genetic responses.

The relationships between pre-flowering sub-phase were strongly dependent on the group of flowering genes that was driving development and each group of genes differentially affected the developmental traits analyzed.

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CHAPTER II

Genotypic variability in durum wheat tillering capacity and spikelet number as affected by development

Introduction

Grain yield improvement in wheat has been highly associated with grain number per unit area (Foulkes et al., 2011 and paper cited therein) and grain number per unit area is the result of the number of fertile spikes per unit area by the number of kernels per spike.

The number of tillers produced by a wheat plant – a component of the number of spikes per unit area - and the number of spikelets per spike - a component of spike fertility - are determined by the wheat morphological development. According to Ritchie (1991), morphological development is closely coupled with phasic development and plant growth, although phasic and morphological development are somewhat independent of each other. Phasic development defines the duration of different growth phases and is primarily affected by genetic (*Ppd*, *Vrn* and *Eps* genes) and environmental (temperature and photoperiod) factors.

Wheat plants show variable degrees of tillering and, although the grain yield of the main stem is always superior to that of any tiller and only few tillers survive and bear a spike, the relative contribution of tillers to grain yield can vary between 30 and 50% depending on sowing density and environmental conditions (Bremner, 1969; Elhani et al., 2007). Variation among varieties in the genetic potential for tillering is normally masked by the effects of high plant-population density (Hay and Porter, 2006), although Rawson (1971) found appreciable varietal differences also under relative close spacing. On the other hand, the close spacing negatively affected tillers fertility as the better tillering cultivars had a lower percentage of ear-bearing tillers than those which produced fewer shoots. Generally, with high soil fertility and wide spacing, the relative contribution of the main stem declines due to the greater production and survival of tillers (Rawson, 1971). In those situations a high tillering capacity can be a useful trait.

The emergence of the first leaf tiller coincides with the appearance of the tip of leaf 4, i.e. three phyllochrons after the appearance of the first leaf; subsequent primary tillers emerge at regular intervals of one phyllochron (Baker and Gallagher, 1983; Kirby et al., 1985; Masle, 1985). Around the time that the main shoot apex reaches the terminal spikelet stage, the tillers begin to die in the reverse order of their emergence (Hay and Kirby, 1991; Evers and Vos, 2006 and papers cited therein), normally leaving up two, but occasionally more, primary tillers to complete the course of development and bear ears (e.g. Thorne and Wood, 1988), depending on environmental factors. In this respect, an early onset of tillering may be an advantage as yield per tiller is directly related to age (Rawson, 1971).

In both bread wheat and barley, the leaf number of the main shoot at which tillering ceases is correlated with total number of leaves on the main shoot, i.e., the rate of tillering varies with the rate of leaf production (Kirby, 1985; Syme, 1974). Accordingly, some significant genetic correlations were found in these species between duration of phenological phases, phyllochron and tillering traits (Borras et al., 2009; Borras-Gelonch et al., 2011).

The impact on yield of variation in spikelet number induced by phasic development was evidenced by Rawson (1970), which found that grain yield per ear was clearly dependent on spikelet number within each cultivar when spikelet number was varied by day-length or vernalization treatments. Spikelet number plays a critical role in the determination of the number of kernels per unit area and grain yield whenever the limitation imposed to the sowing operations by the extremely variable amount and timing of rainfall in semi-arid environments cause sowing to be delayed from the autumn to the late winter or the beginning of spring (Baker and Gallagher, 1983; Li et al., 2001; Arduini et al., 2009). The shortening of the period available for spikelet initiation is the main reason for the decrease in spikelet number and yield with delayed sowings (Arduini et al., 2009). In this sense, a strong genetic control of spikelet number via 'flowering genes' can be assumed, which makes this trait amenable of being improved by breeding to a greater extent than other traits related to kernel number determination such as number of fertile florets and kernels per spikelet or spike weight at anthesis.

The period of spikelet initiation on the apex begins when the interplay of the response of the genotype to photoperiod and vernalization determines the switch from the vegetative to the reproductive phase (Mc Master, 1997). The duration of spikelet initiation, from the formation of the first reproductive primordium or collar to the appearance of terminal

spikelet, span at least 2-3- weeks (several hundred degree-days) (Hay and Kirby, 1991) and is directly controlled by temperature and photoperiod (Kirby, 1990). The effect of vernalization on the length and rate of spikelet initiation is indirect. Brooking and Jamieson (2002) demonstrated that the level of saturation in cold requirement, not only set the switch to from leaves to spikelet initiation, but also influence the length of the spikelet initiation phase and its rate. In their experiment, the shorter the vernalization period, the longer the length and the lower the rate of spikelet initiation. This means that the rate of spikelet primordium initiation, usually equal to 2-3 times the rate of leaf primordium initiation, can coincide with the rate of leaf primordia initiation as an indirect effect of vernalization.

Spikelet number and final leaf number on the main stem are related, their number being an indication of the length of the vegetative phase (Pugsley, 1966; Rawson, 1971; Giunta et al., 2001). As a consequence, a longer development is usually associated with an increase in spikelet number per ear, whether due to limiting photoperiodic conditions or to the response to vernalization (Rawson, 1970). In cultivars with a pronounced response to vernalization, the length of the period to floral initiation is of prime importance, because during this period potential spikelet sites are established. When daylength controls development, on the other hand, it is the duration of the period from floral initiation to terminal spikelet, during which all the spikelet initials appear, which determines spikelet number. The studies of Rawson (1970) on the control of spikelet number by photoperiod manipulations demonstrated that floral initiation and terminal spikelet production may have different quantitative requirements depending on the cultivar, causing genotypic differences in the length of the period from floral initiation to terminal spikelet leading to different number of spikelets. The conclusion of Rawson (1970) that short-season wheats must pay a price in terms of spikelet number and yield did not take into account that, although the duration of each development processes in later-sown plants and late-developing organs will tend to be shorter, this does not necessarily mean that the number of structures (leaves, ears, spikelets, florets) formed will be reduced, because the rate of production of structure can also vary (Hay and Walker, 1989).

In consideration of the tight link between both tillering and spikelet number and development , any breeding work aimed at modifying these traits has to take into account their genetic and physiological interrelationships with flowering genes.

This experiment was aimed at:

- characterizing the variability in tillering traits and spikelet number in a recombinant inbred line population of durum wheat,
- discriminate to what degree the variability in tillering traits and spikelet number is associated to the variability in phasic development and, more specifically, to genotypic differences in earliness per se, sensitivity to photoperiod or cold requirements

Materials and methods

Plant material, treatments, experimental design and crop management have already been described in Chapter I.

Phenotyping

The number of tillers per plant was recorded once weekly in correspondence with Haun stage determination and continued until no more tillers were produced for three subsequent sampling dates.

Tillering data were used to calculate the thermal time and the Haun stage at the end of tillering and the maximum number of tillers. The average rate of tiller production was expressed on a leaf basis as:

$$\frac{\text{Maximum tiller number} - \text{tiller number at the first sampling}}{\text{Haun stage at the max tiller number} - \text{Haun stage at the first sampling}}$$

At maturity the spikes of the main stems were harvested separately from the spikes on tillers and the number of tillers bearing spikes for each plant was counted together with the number of fertile spikes.

On the main stem spikes the following data were recorded: number of total and fertile spikelets per spike, number of kernels per spike and per fertile spikelet.

Details on thermal time calculation and statistical analysis are reported in Chapter I.

Results

Tillering

No tillering trait showed a significant genetic variance at the combined ANOVA as most of the variance was captured by the GxE interaction (**Table 1**).

Within each treatment the contribution of genetic variance to total variance was generally above 30 % for most traits, with the highest values in DECV. No genetic variation was found for the end of tillering in MAYNV and for the Haun stage at the end of tillering in MAYV.

Table 1. Variance components resulting from the mixed-model analysis of variance performed separately for each treatment and combining the three treatments (combined ANOVA) and corresponding heritability

Parameter	End of till (°Cd)	HS end till	Max tiller no	Rate of till till per leaf	Tot spikes on tillers	Fert spikes on tillers
Combined ANOVA						
σ^2_G	569 ± 693	0.010 ± 0.05	0.007 ± 0.179	0.009 ± 0.007	0.030 ± 0.039	0.028 ± 0.0339
s^2_{GE}	5172 ± 1081	0.288 ± 0.09	2.093 ± 0.306	0.035 ± 0.010	0.360 ± 0.061	0.283 ± 0.0524
Residual	15931 ± 846	1.725 ± 0.09	2.447 ± 0.132	0.185 ± 0.010	0.846 ± 0.039	0.844 ± 0.039
$h^2_{Genotype\text{-}mean}$	11.5	2.5	0.6	18.2	10.3	10.5
MAYV						
σ^2_G	3720 ± 1471	0.048 ± 0.053	0.390 ± 0.121	0.027 ± 0.012	0.080 ± 0.030	0.072 ± 0.0293
Residual	16969 ± 1707	0.876 ± 0.086	1.108 ± 0.114	0.144 ± 0.015	0.622 ± 0.044	0.640 ± 0.0449
$h^2_{Genotype\text{-}mean}$	40.2	14.3	50.9	35.5	39.9	36.7
MAYNV						
σ^2_G	0 ± 147	0.335 ± 0.19	4.930 ± 1.054	0.054 ± 0.029	0.708 ± 0.168	0.519 ± 0.139
Residual	3663 ± 353	2.552 ± 0.26	5.842 ± 0.595	0.381 ± 0.039	1.109 ± 0.114	1.099 ± 0.113
$h^2_{Genotype\text{-}mean}$	0.0	28.1	71.9	29.8	65.0	57.9
DECV						
σ^2_G	11679 ± 2641	0.391 ± 0.12	0.628 ± 0.134	0.038 ± 0.009	0.449 ± 0.097	0.395 ± 0.0886
Residual	23929 ± 1937	1.731 ± 0.140	1.106 ± 0.089	0.079 ± 0.006	0.866 ± 0.067	0.846 ± 0.0657
$h^2_{Genotype\text{-}mean}$	67.2	48.7	70.2	66.5	69.4	67.1

The shortest duration of tillering (time to the maximum tiller number) was observed in MAYV and was of 541 °Cd on the average of the population (**Table 2**). A greater increase in duration was induced by the photoperiodic limitations of DECV – tillering phase longer by 90% - than by the absence of vernalization of MAYNV, which caused an increase by only 30% in the duration of tillering.

Ofanto responded to the treatments in the same way described for the population, whereas MAYNV exerted on cultivar Cappelli an effect as remarkable as DECV, mirrored in an even greater increase in Haun stage at the end of tillering (+4.6 leaves) than in MAYV, compared to +2.5 leaves in DECV. As a consequence tillering lasted more in cultivar Cappelli than in Ofanto only in MAYNV.

Table 2. Parent mean values (± standard error of the mean) and population mean and coefficient of variation for the tillering traits

Trait		**Parents**		**Population**	
		Cappelli	Ofanto	Mean	*CV (%)*
End of tillering (°Cd)	MAYV	440 ± *15*	463 ± *48*	541	6.7
	MAYNV	953 ± *80*	683 ± *67*	703	5.3
	DECV	1012 ± *119*	1182 ± *55*	1029	8.4
Haun stage at the end of tillering	MAYV	6.2 ± *0.3*	6.6 ± *0.5*	7.7	1.1
	MAYNV	10.8 ± *0.9*	7.9 ± *2*	8.8	3.4
	DECV	8.7 ± *0.9*	10.1 ± *0.5*	9.3	4.6
Rate of tillering (tillers per emerged leaf)	MAYV	1.3 ± *0.1*	0.6 ± *0.1*	1.0	9.1
	MAYNV	1.4 ± *0.5*	1.4 ± *0.1*	2.0	6.1
	DECV	0.6 ± *0.1*	0.8 ± *0.1*	0.6	25.0
Max n° of tillers	MAYV	4.4 ± *0.4*	3.0 ± *0.4*	5.2	8.3
	MAYNV	11.5 ± *2.5*	8.0 ± *3.0*	11.5	15.8
	DECV	3.8 ± *0.8*	4.5 ± *0.7*	3.6	17.9
Total spikes on tillers	MAYV	0.8 ± *0.2*	0.8 ± *0.2*	1.5	12.0
	MAYNV	3.3 ± *0.6*	1.0 ± *0.3*	2.2	29.9
	DECV	2.8 ± *0.6*	4.3 ± *0.8*	3.0	18.6
Fertile spikes on tillers	MAYV	0.8 ± *0.2*	0.8 ± *0.2*	1.4	11.3
	MAYNV	2.5 ± *1.0*	1.0 ± *0.3*	1.9	26.8
	DECV	2.5 ± *0.5*	4.2 ± *0.8*	2.8	17.5

The wider range in the population was recorded in DECV (about 300 °Cd between the minimum and the maximum value) which was also the treatment with the wider range in Haun stage at the end of tillering (about 2 leaves) (**Fig. 1**). A limited treatment effect was observed in the Haun stage at the end of tillering, which for the population varied between 7.7 in MAYV, to 8.8 in MAYNV and 9.3 in DECV.

In spite of the longer tillering phase in DECV, the peak tiller number was sensibly higher in MAYNV, when up to 19 tillers were counted in one RIL and the greatest genotypic variation was observed (about 11 tillers range). MAYNV was also the treatment with the highest rate of tiller produced per leaf emerged.

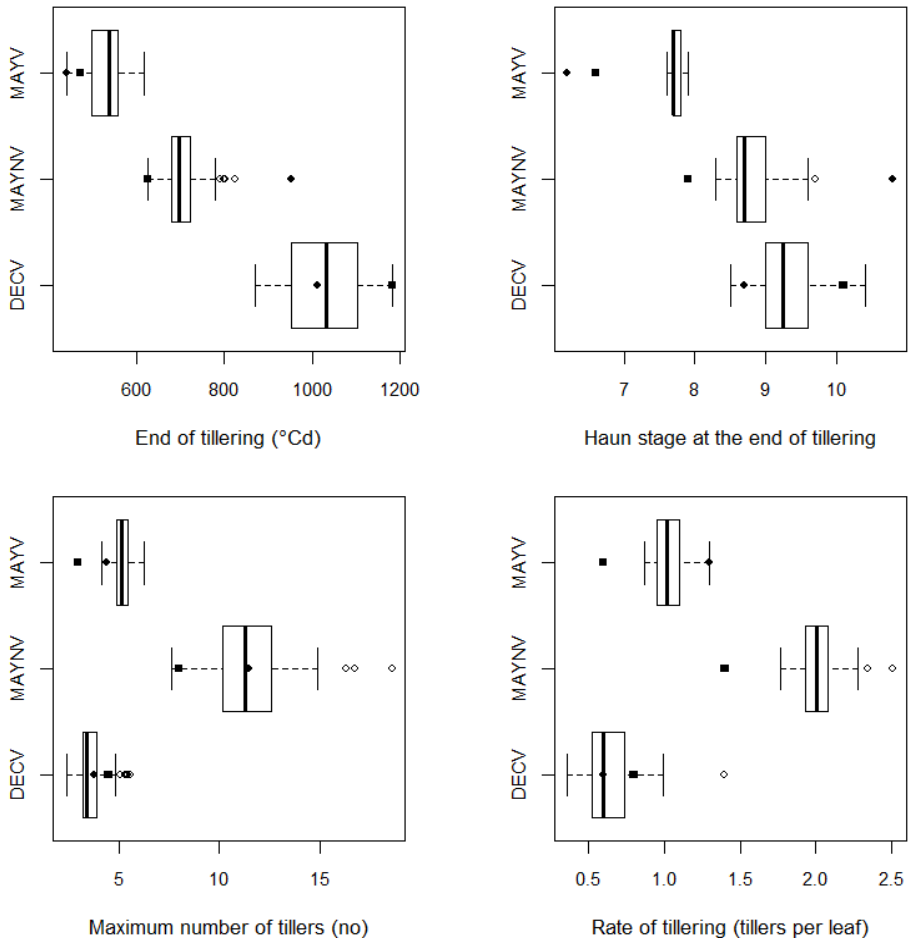

Figure 1. Boxplots of the tillering traits in the three treatments. Parents are indicated with a solid square ('Ofanto') and a solid circle ('Cappelli').

The proportion of tiller bearing spikes on the total tiller number was sensibly higher in DECV (83 %) than in MAYV (29 %) and MAYNV (19 %). DECV was the treatment with more spike-bearing tillers also in absolute value (3 tillers per plant).

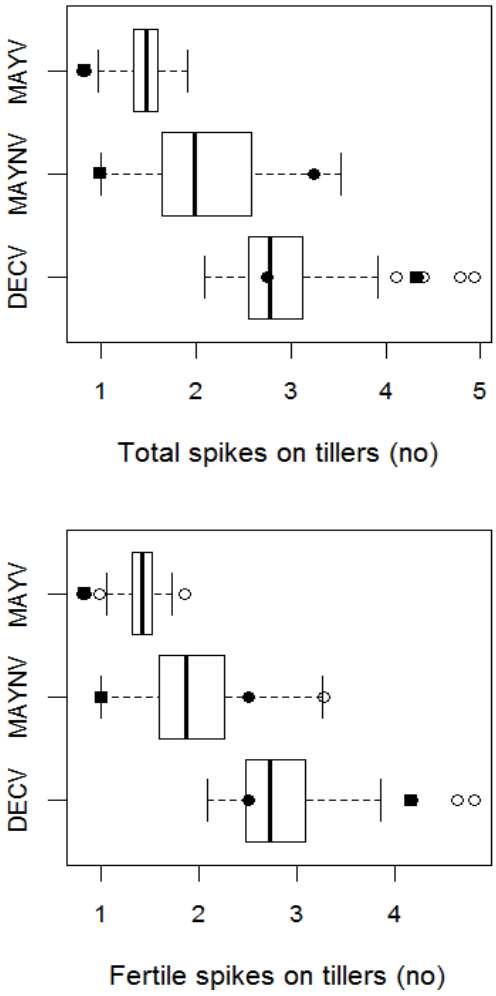

Figure 2. Boxplots of the number spikes on tillers in the three treatments. Parents are indicated with a solid square ('Ofanto') and a solid circle ('Cappelli').

Most of the spikes were fertile in all the three treatments. The lowest genotypic variability in both tillering spikes and fertile tillering spikes was observed in MAYV (**Fig. 2**).

Fertility

All the fertility traits showed a significant GxE interaction but no genetic variance at the combined ANOVA with the exception of the total number of spikelets per spike (**Table 3**). Within treatment, on the contrary, the proportion of genetic variance over the phenotypic variance was above 50% for all the traits, with slightly higher values in DECV.

Table 3. Variance components resulting from the mixed-model analysis of variance performed separately for each treatment and combining the three treatments (combined ANOVA) and corresponding heritability

Parameter	Kernels per fert.spikelet	Kernels per spike	Total spikelets per spike	Fertile spikelets per spike
Combined ANOVA				
σ^2_G	0.006 ± 0.007	1.33 ± 2.65	0.21 ± 0.10	0.00 ± 0.14
s^2_{GE}	0.055 ± 0.010	26.78 ± 4.26	0.73 ± 0.12	1.57 ± 0.25
Residual	0.159 ± 0.007	51.89 ± 2.43	1.50 ± 0.07	3.06 ± 0.14
$h^2_{Genotype\text{-}mean}$	12.0	7.0	29.8	0.0
MAYV				
σ^2_G	0.041 ± 0.012	9.88 ± 2.80	0.74 ± 0.15	0.72 ± 0.17
Residual	0.203 ± 0.014	45.97 ± 3.25	1.44 ± 0.10	2.32 ± 0.17
$h^2_{Genotype\text{-}mean}$	51.0	52.4	72.3	61.3
MAYNV				
σ^2_G	0.121 ± 0.030	40.33 ± 9.04	0.58 ± 0.17	1.93 ± 0.63
Residual	0.197 ± 0.021	50.68 ± 5.40	1.49 ± 0.16	5.91 ± 0.62
$h^2_{Genotype\text{-}mean}$	63.9	69.6	53.0	48.7
DECV				
σ^2_G	0.037 ± 0.008	38.74 ± 7.86	1.48 ± 0.28	1.86 ± 0.36
Residual	0.077 ± 0.006	57.63 ± 4.54	1.57 ± 0.12	2.22 ± 0.18
$h^2_{Genotype\text{-}mean}$	67.6	74.6	80.6	78.7

The lower spike fertility was detected in MAYNV and the highest – almost two fold the values of MAYNV in the population - in DECV, with a peak value of 71 kernels per spike in cultivar Ofanto, mainly due to its high number of kernels per spikelet. DECV was also the treatment with the highest number of spikelets per spike, both total and fertile, accompanied by a spikelet fertility comparable to MAYV.

The largest genotypic variability was observed in the number of kernels per spike in MAYNV and in DECV (**Fig. 3**). The genotypic variability was also high for the number of kernels per fertile spikelet in MAYNV – from 1.4 to 2.9 - a range almost double those observed in the other two treatments. Parents were outliers with respect to the population and showed the lowest (Cappelli) and the highest (Ofanto) value for both kernels per spike and kernels per fertile spikelet. Genotypic variation for the number of spikelet was less relevant.

Table 4. Parent mean values (± standard error of the mean) and population mean and coefficient of variation for the fertility traits

Trait		Parents		Population	
		Cappelli	Ofanto	Mean	*CV (%)*
N° of kernels per fertile spikelet	MAYV	2.5 ± *0.3*	2.8 ± *0.1*	2.4	6.1
	MAYNV	2.2 ± *0.3*	1.7 ± *0.3*	2.2	12.3
	DECV	1.7 ± *0.3*	3.2 ± *0.1*	2.4	6.3
Kernels per spike	MAYV	29.6 ± *2.7*	33.2 ± *1.9*	28.7	7.8
	MAYNV	25.5 ± *4.9*	18.0 ± *2.9*	25.2	20.3
	DECV	30.8 ± *13*	71.3 ± *4.1*	54.8	9.5
Total spikelets/spike	MAYV	14.0 ± *0.5*	12.3 ± *0.3*	13.3	5.4
	MAYNV	15.3 ± *0.9*	14.4 ± *0.2*	13.9	3.9
	DECV	24.8 ± *1.0*	23.5 ± *0.9*	24.5	4.4
Fertile spikelets/spike	MAYV	12.2 ± *0.6*	11.8 ± *0.5*	12.2	5.4
	MAYNV	11.5 ± *1.0*	11.0 ± *0.5*	11.1	8.5
	DECV	15.3 ± *5.0*	22.2 ± *1.1*	22.5	5.2

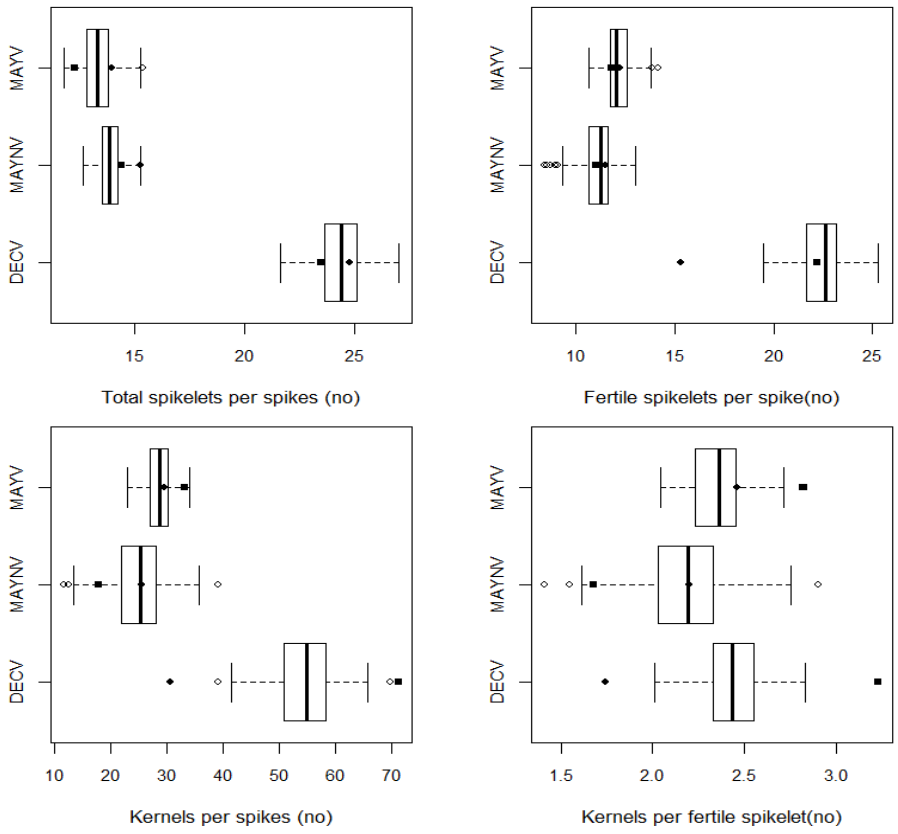

Figure 3. Boxplots of the fertility traits in the three treatments. Parents are indicated with a solid square ('Ofanto') and a solid circle ('Cappelli').

Discussion

Cultivar Cappelli belongs to the *Mediterraneum typicum* group defined by Grignac (1965) and characterized by long spikes and awns, tallness, lateness in flowering and reduced tillering. Semi-dwarf wheats, on the other hand, tillered more and had a higher rate of tiller survival compared to tall varieties in the progeny of a cross between a semi-dwarf and a tall bread wheat variety (Lupton et al, 1974). Differences between old, such as Cappelli, and modern, such as Ofanto, durum wheat Italian cultivars in spike fertility were evidenced by Giunta et al. (2007). These Authors demonstrated that the number of kernels per spike was higher in modern constitutions only in case of late sowings (February, March), whereas De Vita et al. (2007) found a greater spike fertility in Ofanto (32 kernels per spike) compared to Cappelli (29 kernels per spike) also under the common sowing time of December. As a consequence of these differences, the RIL population derived from the cross between Cappelli and Ofanto gave origin to a moderate to large genotypic variation in both tillering and fertility traits, depending on the flowering genes activated by the treatments applied.

The results of the ANOVAs performed seem to indicate an effect of flowering genes on both tillering and fertility traits, although lower compared to their effect are the traits directly involved in development discussed in Chapter I. The only exception was the total number of spikelets per spike, for which a significant genetic variance was detected at the combined ANOVA together with a high genetic variance within treatment, indicating that also genes other than the flowering ones were involved in the control of this trait.

Earliness per se

In the MAYV treatment the greatest differences among RILs was observed in the rate of tillering and in the end of tillering among the tillering traits, leading to heritability values moderate in size and of the same order of magnitude of those described by Borràs-Gelonch et al. (2011) for bread wheat. Cultivar Ofanto distinguished itself for having the lowest or one of the lowest values for all the tillering traits considered.

The relationship between tillering traits and earliness *per se* genes was established by computing the correlations of these traits with the relative earliness *per se* for anthesis date described in Chapter I.

The only trait partly under the control of earliness per se genes was the rate of tillering. The RILs with the greatest earliness per se for anthesis date (Chapter I) also showed the lowest rate of tillering (r = -0.31, P<0.0.01), although the association was too weak to result in any effect of earliness per se on the number of tillers and on the number of tiller-bearing spikes. Accordingly, neither final leaf number, nor phyllochron, influenced the maximum number of tillers, remarking the absence of any relevant effect of development on this trait when earliness per se alone was driving development.

On average, the contribution of genetic to total variance in MAYV for fertility traits was higher than that observed for tillering traits. The maximum heritability of 72 % calculated for the total number of spikelets per spike was higher compared to the low narrow-sense heritability of 9% reported by Ketata et al. (1976).

Earliness *per se* genes as quantified by the relative earliness *per se* for anthesis date only affected the number of spikelets per spike. A high earliness *per se* for anthesis date was negatively reflected on a low number of both total (r = - 0.51, P<0.001) and fertile (r = - 0.36, P<0.001) spikelets per spike on the main stem.

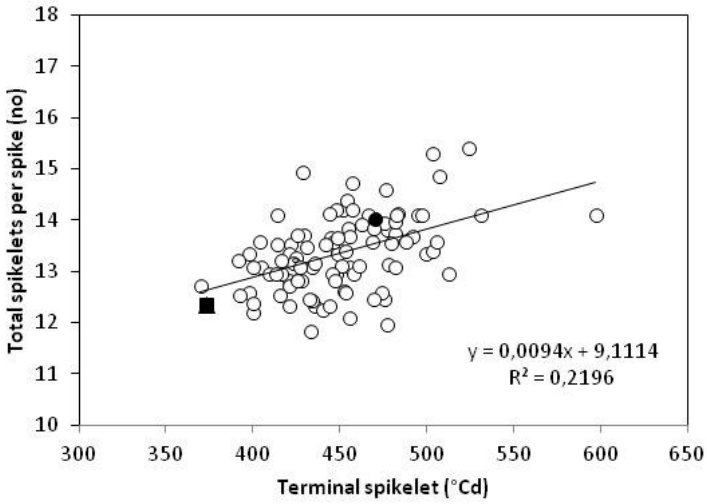

Figure 4. Relationship between the terminal spikelet period and the total number of spikelets per spike. Parents are indicated with a square ('Ofanto') and a solid circle ('Cappelli').

The relationship between development and number of spikelets was also evidenced by the positive relationship calculated between total spikelet number and TS (**Fig. 4**). Moreover, 25% of the variation in total spikelet number was explained by the final leaf number (r = 0.50, P<0.001), consistently with the negative relationship obtained between earliness per se and total spikelet number, i.e., the RILs with the greatest earliness per se and hence the lower final leaf number, also had the lower spikelet number. Earliness per se genes are

assumed to act via changes in the number of leaf or spikelet primordia being initiated (Hoogendoorn, 1985) or in the rate of primordia initiation (Gotoh, 1977), thus establishing significant correlations between time to flowering and number of spikelets produced on the main stem (Worland et al., 1994, 1996).

Cold requirements

In the no limiting photoperiodic conditions of MAYNV, the highest genetic variability, resulting in moderate to high heritability values, was expressed in rate of tillering and maximum tiller number, whereas no genetic variation was found for the end of tillering.

The tillering phase ceased on average 146 °Cd before TS. Ofanto showed the shortest duration of tillering and Cappelli the longest, both in thermal time and in Haun stage at the end of tillering, consistently with the strong genetic correlation calculated between TS and the end of the tillering phase (r = 0.68, P<0.001, **Fig. 5**), and with the remarkable difference in TS between the two parents in MAYNV evidenced in Chapter I and attributed to their different cold requirements. More generally, the genotypic variation in cold requirements (RILs' RRV for anthesis of Chapter I) was positively associated with all tillering traits, with the exception of the rate of tillering. The greater the cold requirement of a RIL, the later the end of tillering (r = 0.59, P<0.001), the higher the maximum number of tillers (r = 0.47, P< 0.001) and of tillering spikes (r = 0.58, P< 0.001).

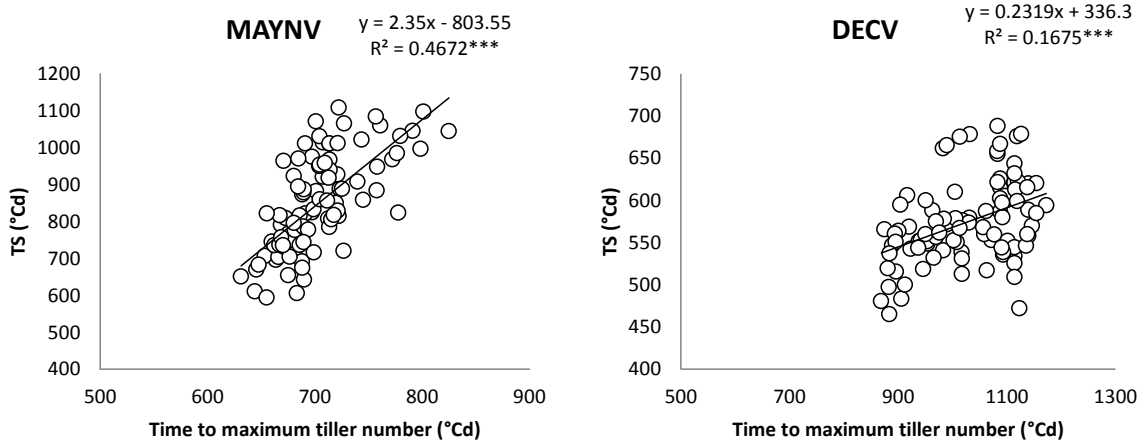

Figure 5. Relationship between the time to maximum tiller number and the terminal spikelet period in the treatments MAYNV and DECV. Parents are indicated with a square ('Ofanto') and a solid circle ('Cappelli').

In spite of the absence of genetic variability in rate of tillering, the lack of satisfaction in cold requirement was associated with the highest rate of tillering compared to MAYV, resulting in the highest tiller number. Nor temperature, neither nutrition differences could explain this difference in rate as plants were grown in the same environment without water or nutrient limitations. One reason for this large effect on rate of tillering of the lack of vernalization could be the lower apical dominance deriving from the lower developmental rate of the apical meristem evidenced by the later to TS caused by the lack of any natural vernalization. Apical dominance refers to the control exerted by the apical portion of the shoot, which include apical meristem and young leaves, on axillary bud growth following bud formation (Cline, 1991; Murphy and Briske, 1992). Most tillers were not able to survive and produce a spike, as this treatment was the one with the lowest number and proportion of tillers bearing spikes. The high rate of about 2 tillers per leaf let us suppose that the high mortality was a consequence of a high contribution of secondary or tertiary tillers to the high rate.

The lack of vernalization did not affect any fertility trait, as evidenced by the lack of differences between MAYV and MAYNV. Accordingly, no relationship was found between RILs' RRV for anthesis (Chapter I) and any fertility trait. In our experiment, the long-day conditions guaranteed a rapid differentiation of terminal spikelet in both treatments once the cold requirements were satisfied by the ageing of the plant (Wang et al., 1995) as no artificial or natural vernalization temperatures occurred. This caused the time to TS to vary only as a consequence of a longer period devoted to leaf primordia production, as demonstrated by the higher leaf number of MAYNV (Chapter I) and the lack of any relationship between time to TS and spikelet number.

The irrelevant effect of cold requirements on spikelet number is also reflected in the fact that MAYNV was the treatment characterized by the lowest genotypic variability (a range of only 2.5 spikelets between the extreme RILs) and the lowest heritability (53 %) compared to the other treatments.

Photoperiodic sensitivity

The large genetic variability expressed in terms of both genotypic variation and heritability for most tillering traits was not associated with RILs' photoperiodic sensitivity as quantified by RRP indexes for anthesis (Chapter I). The limiting photoperiodic conditions

and the temperatures characterizing the environmental conditions of DECV resulted in the longest duration of tillering both in thermal time and Haun stage at the end of tillering. Although weakly associated with TS, tillering ceased on average 444 °Cd after TS, in contrast with Miralles and Richards (2000) which, in bread wheat, reported that the peak number of tillers was reached before TS at photoperiods shorter than 19 h. Tillering usually ceases at the onset of stem elongation (Rawson, 1971; Hay and Kirby, 1991), which is what we used to estimate TS. On the other hand, tillering began at Haun stage 5 (data not shown) and hence the number of leaves already emerged at the beginning of stem elongation (5.3) was enough to guarantee the production of the 3.6 tillers produced on average in this treatment. It could be hypothesized that the slow developmental rate after TS signaled in this treatment by the lowest rate of tillering, highest phyllochron and highest number of leaves yet to emerge after TS, enlarged the time window available for tillering.

The combination of the lowest rate and the longest duration resulted in the lowest peak number of tillers, but in the highest number and proportion of tillers bearing spikes.

Together with what evidenced in MAYNV, this result indicate that tiller survival is tightly linked with rate of tillering, as high rates can only be achieved with secondary or high order tillers, which usually have less chance to survive (Anderson-Taylor & Marshall, 1983).

Our results support the possibility of manipulating the duration of pre-anthesis phases without modifying the tillering capacity already demonstrated for bread wheat by Borràs-Gelonch et al. (2011) although our data clarify that this is true only when photoperiodic sensitivity is controlling development.

The limiting photoperiodic conditions of DECV exerted a remarkable positive affect on spike fertility via a particularly high spikelet number compared with the other treatments. Rawson (1970) showed that, in cultivar with no vernalization response, spikelet number is dependent on the length of the period from floral initiation to terminal spikelet. Spikelet number plays a critical role in the determination of the number of kernels per unit area and grain yield whenever the limitation imposed to the sowing operations by the extremely variable amount and timing of rainfall in semi-arid environments cause sowing to be delayed from the autumn to the late winter or the beginning of spring (Baker and Gallagher, 1983; Li et al., 2001; Giunta et al., 2001; Arduini et al., 2009). The difference of

about 10 spikelets per spike between DECV and the two May treatments is comparable to the maximum difference in spikelet number between sowing dates observed by Arduini et al. (2009) in durum wheat sown at month intervals along a whole year.

In spite of this relevant environmental effect on spikelet number and of the large genotypic variation (a range of 5.4 spikelets per spike) and heritability for spikelet number in DECV, the genotypic variability in spikelet number in this treatment was not associated with the limited genetic variability in photoperiodic sensitivity measured by RRP indexes (Chapter I), nor was spikelet number genetically associated with leaf number or phyllochron. Based on the significant genetic variation for spikelet number and on the low to moderate heritability calculated at the combined ANOVA, we can therefore hypothesize that genes other than the flowering genes were controlling genotypic variation in spikelet number in DECV.

In other words, changing the photoperiod environment did not produce the same results as genotypic differences in photoperiod sensitivity within a similar environment, in contrast with Gonzales et al. (2005).

One consequence of this result could be that the lengthening the TS-anthesis phase via *Ppd* genes proposed as a means to increase kernel number (Foulkes et al., 2011; Fischer, 2011; Reynolds et al., 2009) would not result in any negative effect on the number of spikelets.

Conclusions

A different effect on tillering capacity and on the fertility traits analyzed was evidences for the three groups of flowering gens. Genetic variability in earliness per se influenced spikelet number but not tillering traits, which were mostly affected by the genetic differences in cold requirements. Genetic variation in photoperiodic sensitivity, on the contrary, did not affect tillering nor fertility traits. From a practical point of view, this experiment suggests that tillering capacity is a trait amenable of being manipulated by acting on *Vrn* genes, whereas spikelet fertility is more dependent on environmental manipulation of daylength via the choice of sowing time, than on genetic manipulation of *Ppd* genes, at least in the population analyzed.

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CHAPTER III

Model analysis of phenology in recombinant inbred lines of durum wheat

Introduction

Phenology, and particularly anthesis date, has a determinant role in genetic adaptation to the environment (Iwaki et al., 2001; Law and Worland, 1997; Goldringer et al., 2006). Flowering time is therefore one of the major target of all wheat breeding programs (Snape et al., 2001).

The molecular approach in plant breeding has been restricted by the inability to connect information at gene level to the expressed phenotype in a way that can be useful for selection (Miflin, 2000). The enhanced ability to undertake genome-scale molecular biology (genotyping), have not been matched by the development of enhanced capability in phenotyping, i.e. capability to link genotype and phenotype (Campos et al., 2004). Phenotyping is a very difficult work for complex growth and development traits as they are associated with genes interacting in networks with organism-environment system at a higher level of biological organization where the difference in genetic makeup expresses its functional consequence. Hence, gene x gene and gene x environment interactions create a context dependence which provide a major limitation for molecular breeding (Podlich et al., 2004). So that gene-to-phenotype relationship are not straightforward (Hammer et al., 2005).

A number of studies, in which the effects of quantitative trait loci or genes have been incorporated into existing ecophysiological models, have shown the promise of using models in analyzing genotype-phenotype relationships of some crop traits (Yin and Struik, 2010; Yin et al., 2000, 2004; Hammer et al., 2006), although this kind of utilization requires more robust crop models than do conventional agricultural applications (Yin et al., 2000) and the choice of a relevant genotypic range.

Most physiological and modeling studies have used cultivars with a limited genotypic range in a wide range of environments (Jackson et al., 1996), although, in order to interface physiological modeling with genetics, a more appropriate genetic population could be a recombinant inbred line population (Yin et al., 2000; 2005).

The robustness of a model is highly dependent on a correct simulation of development which passes on a better understanding of the physiological basis of the genetic variation by using a controlled genetic background, and on the validity with which the crop model architecture and associated coefficients capture and integrate the physiological basis of the genetic variation (Hammer et al., 2005). Yin et al. (2005) explored the ability of a phenology model to explore differences in flowering time among individual lines in a RIL population of spring barley. The model was parameterized in a greenhouse experiment in order to quantify the response of each parameter of the model to the photoperiod variation. The parameterized model was then used to predict the flowering time of the same population in independent multiple field environments. This work evidenced a poor prediction of genotypic differences in flowering time within environment which could have been the consequence of an inadequate physiological frame, as in their modeling work Yin et al. (2005) only considered photoperiodic sensitivity of the lines but didn't take into account their cold requirements.

The traditional phenological frame used to modeling phenology is to divide the whole interval from emergence to anthesis into several phenophases, assuming that vegetative and reproductive events are independent and that time to flowering can be simply thought as a progress from the vegetative to the reproductive phase. This is the base of the phenological descriptions in several simulation models (ARCWHEAT1, Weir et al., 1984; AFRCWHEAT2, Porter, 1993; CERES-Wheat, Ritchie and Otter, 1985). In fact the seminal papers by Kirby (1990) and Hay and Kirby (1991) have clearly shown that vegetative and reproductive development are coordinated and overlap in time. For example, the interval between the emergence of successive leaves (the phyllochron) is around twice the appearance of leaf primordia at the apex (the plastochron) (Frank and Bauer, 1995), but may change around the time of floral initiation (Hay and Delècolle, 1989). This change reflects the coordination of phyllochron and plastochron, since leaf initiation is controlled predominantly by temperature (Miglietta, 1989) whereas the rate of spikelet initiation is also influenced by photoperiod (Rawson, 1970). The leaf appearance

is also closely coordinate with the extension of stem internodes and with the resulting appearance of the spike (Hay and Kirby, 1991). This means that vegetative and reproductive events are not independent. Within this physiological frame, Jamieson et al. (1998) proposed a phenology model where the variation associated with vernalization requirement and daylength sensitivity are described in terms of primordia initiation, leaf production, and final main stem leaf number. The model simulates the duration of three development phase: the first is the pre-emergence phase (sowing to emergence), the second is the leaf production phase from crop emergence to flag leaf appearance, and the third is the duration of the flag leaf ligule appearance – anthesis phase. The pre-emergence phase is simulated as a fixed duration in thermal time which may differ between cultivars (Weir et al., 1984). The leaf production phase integrates the effects of vernalization and photoperiod, whereas the duration of the third phase is proportional to the phyllochron (Brooking et al., 1995). This robust phenological frame is the basis for development simulation in SiriusQuality2 model (Martre et al., 2006, 2008).

Physiological inputs are used as model parameters for characterizing genotypic differences. The model parameters thus represent coordinate genotypic responses that quantify a "meta-mechanism" at a higher level of biological organization (Hammer et al., 2005). There has been a tendency in simulation study of crop design to consider the coefficients required to drive crop models as "genetic coefficients" simply assuming that they reflects genetic variation (Hammer et al., 2002). While this can be a reasonable hypothesis, there has been little evidence connecting the action of true genetic variation with these coefficients, with the exception of some study on crop phenology (Leon et al., 2001; Yin et al., 2003). The use of models as frameworks to integrate physiology and breeding requires that the parameters of control equations of simple but physiologically robust models were linked to genetic variation (Tardieu, 2003), and also requires the understanding of the inheritance of these model parameters (Stam, 1998).

This work was aimed at:

- verify to which extent Sirius model is able to simulate genotypic differences in phenological traits (anthesis date, final leaf number, phyllochron);

- evaluate the level of association between the genetic coefficients implemented in the model and earliness *per se*, photoperiodic sensitivity and vernalization sensitivity independently estimated.

Materials and methods

Data on leaves at TS and final leaf number, phyllochron and anthesis date were obtained as described in details in Chapter I and II.

A subset of 18 RILs showing extreme values for earliness per se and photoperiodic and vernalization sensitivity was selected from the 100 RILs examined, together with the two parent cultivars Ofanto and Cappelli.

Sirius model description

Leaf production can be described by a segmented linear model of the thermal time (Boone et al., 1990; Jamieson et al., 1995; Gonzàles et al. 2002). The first two leaves appear more rapidly than the next six, and then the leaf appearance slows again for the subsequent leaves.

Table 1. Name, symbol, definition, nominal value and unit of the non-varietal and varietal parameters of Sirius phenology sub-model (modified by He et al., 2012).

Name	Symbol	Definition	Value Nominal	Unit
Non- varietal parameters				
maxDL	Dl_{sat}	Saturating photoperiod above which final leaf number is not influenced by daylength	15	h
MaxLeafSoil	$Lmax^{soil}$	Haun stage up to which thermal time is calculated based on soil temperature (0 - 2 cm deep)	4	Leaf
Ldecr	L_{decr}	Haun stage up to Phyll is decreased by $Phyll_{decr}$	2	Leaf
Lincr	L_{incr}	Haun stage up to Phyll is increased by $Phyll_{incr}$	8	Leaf
Pdecr	$Phyll_{decr}$	Factor decreasing the phyllochron for leaf number less than L_{decr}	0.75	Dimensionless
Pincr	$Phyll_{incr}$	Factor increasing the phyllochron for leaf number higher than or equal to L_{incr}	1.25	Dimensionless
Rp	R_p	Rate of decrease of the P_{SD} for winter sowing	0.003	°C d d^{-1}
PFLLAnth	$tflag^{anth}$	Phyllochronic duration of the period between flag leaf ligule appearance and anthesis	1.39	Phyllochron
SDWS	$SD_{W/S}$	Sowing date for which P_{SD} is minimum	90	Day of the year
SDSA	$SD_{S/A}$	Sowing date for which P_{SD} is maximum	200	Day of the year
IntTVern	$Tint^{vern}$	Intermadiate temperature for vernalization to occur	8	°C
MaxTvern	$Tmax^{vern}$	Maximum temperature for vernalizzation to occur	17	°C
MinTvern	$Tmin^{vern}$	Minimum temperature for vernalizzation to occur	0	°C
Varietal parameters				
D_{se}	D_{se}	Thermal time from sowing to emergence	175	°C d
MaxL	$Lmax^{abs}$	Absolute maximum leaf number	24	Leaf
MinL	$Lmin^{abs}$	Absolute minimum possible leaf number	5.5	Leaf
Phyll	**Phyll**	Phyllochron	**100**	°C d
SLDL	**SLDL**	Daylength response of leaf production	**0.15**	Leaf h^{-1} (daylength)
VAI	**VAI**	Response of vernalization rate to temperature	**0.001**	d^{-1} $°C^{-1}$
VBEE	VBEE	Vernalization rate at temperature equal to $Tmin^{ver}$	0.01	d^{-1}

The decrease factor of the phyllochron for the Haun stage less than 2 and the increase factor of the phyllochron for the Haun stage higher than 8, are, respectively 0,75 and 1,25, and their values are varietal independent (**Table 1**).

The phyllochron from Haun stage 2 to 8 is a varietal parameter (**Table 1**) but the model modifies its value according to the sowing time and to the results reported by several authors (Slafer and Rawson, 1997; McMaster et al., 2003; Bassu et al., 2009). One reason for this variation could be that the relationship between apex and air temperature change with the period of the year (i.e. sowing date) (Vinocur and Ritchie, 2001; Jamieson et al., 2008), but others have discussed putative physiological causes for this variations (Slafer and Rawson, 1997; McMaster et al., 2003). As a surrogate for the apex-air temperature correction, in the Sirius model the phyllochron decreases linearly for the winter sowings from the first day of the year to the day of the year 90 (Northern hemisphere) with a rate of 0.003 °Cd d^{-1}, and it stays minimum until mid-July (day of the year 200 for the Northern hemisphere). From mid-July to the first day of the year the phyllochron is held at the value determined by the cultivar and its varietal parameter Phyll.

The vernalization process in Sirius is modeled according to the work of Robertson et al. (1996), but using the modified vernalization rate response to temperature as proposed in Brooking (1996). The model calculates the vernalization progress toward vernalization (V_{prog}) according to five parameters. Three are non-cultivar specific parameters that relate with the range of temperature at which vernalization occurs: the minimum vernalizing temperature (minTvern, 0°C), the intermediate temperature (intermTvern, 8°C, i.e. the temperature at which vernalization rate is maximum) and the maximum vernalizing temperature (maxTvern, 17°C). One of the varietal parameter defines the vernalization rate (d^{-1}) at the minimum temperature for vernalization (VBEE, d^{-1}), the other the vernalization rate from the minimum vernalizing temperature to the maximum value at the intermediate temperature (VAI, $d^{-1}°C^{-1}$). Above this temperature the vernalization rate decreases in proportion to the difference between the maximum vernalizing temperature and the daily temperature. When the canopy or soil temperature is less than minimum vernalizing temperature or higher than maximum vernalizing temperature, the vernalization rate is 0. The progress toward the full vernalization (V_{prog}) is simulated as the time integral of the daily vernalization rate and can range from 0 to 1. This integral value is used to modulate the number of leaves that can be produced on the main stem and therefore the time to

anthesis. The number of leaves that can be produced spans from a maximum (L_{max}) and a minimum (L_{min}) values which are varietal parameters. The model assumes that plants start with the capacity to produce a number of leaves (LN_{pot}) equal to the maximum number (L_{max}) for the cultivar, but this number is daily reduced by vernalization progress:

$$LN_{pot} = L_{max} - (L_{max} - L_{min}) \times V_{prog}$$

Vernalization is complete when one of these conditions is satisfied: V_{prog} is equal at 1or the number of primordia already initiated exceeds the maximum number of leaf that the cultivar can produce. The number of primordia accumulated in the apex (PN) is determined by the temperature and by the value of phyllochron which determine the number of emerged leaves. The relationship between number of primordial and of emerged leaves has been formulated by Kirby (1990):

$$PN = 2 \times LN + 4$$

4 is the number of leaf primordia already present on apex when the plant emerge and LN is the number of emerged leaves. A third stopping rule is implemented by the model to take into account that the number of primordia already initiated can be higher than the potential leaf number (LN_{pot}). In this case the minimum leaf number (L_{min}) is chosen as the maximum between the average value between potential leaf number and number of primordia, and the value of the minimum leaf number (L_{min}):

$$L_{min} = \max ((L_{max} + PN) /2, L_{min})$$

The modeling of photoperiod response in Sirius requires the following parameters:

- one cultivar specific parameter, SLDL which is the slope of the FLN-daylength relation. The unit is in leaf hourDL^{-1}, DL stays for day length.
- one non cultivar specific parameter: Dsat, which stays for saturating daylength (15 h).

In the model, crop responds to daylength (DL) only once vernalization is completed or at emergence for spring wheat without cold requirement for which the vernalization routine is skipped. The crop responds to daylength from the Haun stage 1.5, because at that time there will be seven primordia in the plant which corresponds to the minimum number of leaves that a plant can produce when grown without limitation in daylength, as resulting from the above mentioned equation of Kirby (1990):

The response to daylength leads to an increase in the number of the leaf primordia resulting from the vernalization routine (AmnLFNO). Brooking et al. (1995) showed that the final leaf number is determined by the daylength at the stage of two leaves after the flag leaf primordium has formed. The value of approximate final leaf number (AppFLN) at final leaf primordia initiation (FLP) plus two leaves (FLP2 in Brooking et al., 1995) is therefore considered to be a good approximation of the final leaf number (FLN). This assumption creates the need for an iterative calculation of an approximate final leaf number.

If daylength is less than saturation (Dsat), the model calculates an approximate final leaf number (AppFLN) that is the result of vernalization sub routine plus the product of variety SLDL by the difference between Dsat and the daily value of daylength:

$$AppFLN = AmnLFNO + varietySLDL * (Dsat - DAYLENGHT)$$

The calculation of the AppFLN stops when the leaf stage is equal to AppFLN / 2 because at this leaf stage we have reached the stage of FLP2. Let us consider the case of a plant with 6 leaves emerged and for which an AppFLN of 12 leaves has been calculated with the above reported equation. The model calculates first the number of primordia corresponding to 6 emerged leaves with the equation of Kirby (1990): PN = 2 * 6 + 4 = 16 primordia produced by the apex at the six leaf stage. There it adds two to this value because the last leaf primordium is committed two leaves after the production of the primordium of the last leaf (flag leaf). Therefore at the six leaf stage the model calculates 18 primordia to reach the stage of FLP2. Eighteen primordia = final leaf primordia (FLP) + 4 primordia because four more primordia are produced to reach the FLP2 stage if a constant rate is considered. Therefore the stage of FLP2 is reached when the leaf number is equal to AppFLN/2.

Varietal parameters calibration

Only three varietal parameters have been calibrated for He et al. (2012) demonstrated the possibility of correctly simulate phenology with only Phyll, VAI and SLDL.

The MAYV treatment was used to calibrate Phyll as a significant association between earliness *per se* of cultivars and their phyllochron was found by He et al. (2012) in wheat.

VAI was calibrated with data from the MAYNV treatment, and SLDL with data from DECV treatment.

Validation was only performed for cultivar Cappelli, whose varietal parameters were used to simulate anthesis date for available independent sets of data relative to six different sowing dates (September, November, January, February, March and May) (data published in an aggregate form in Giunta et al., 2007 and Motzo et al., 2007).

Results and discussion

The range in the varietal parameters Phyll and SLDL calibrated for our 20 lines (**Table 2**) were within the ones described by He et al. (2012) for 16 bread wheat cultivars from France and UK. VAI, on the contrary, spanned in our lines from 0.01 to 0.02, i.e. outside the range between 0 and 0.01 observed in the above quoted paper.

Table 2. Range of the varietal parameters Phyll, SLDL and VAI, calibrated for 20 lines.

Varietal parameters		**Min**	**Max**
Phyll	°C d	**98**	**110**
SLDL	Leaf h^{-1} (daylength)	**0.65**	**0.85**
VAI	d^{-1} $°C^{-1}$	**0.01**	**0.02**

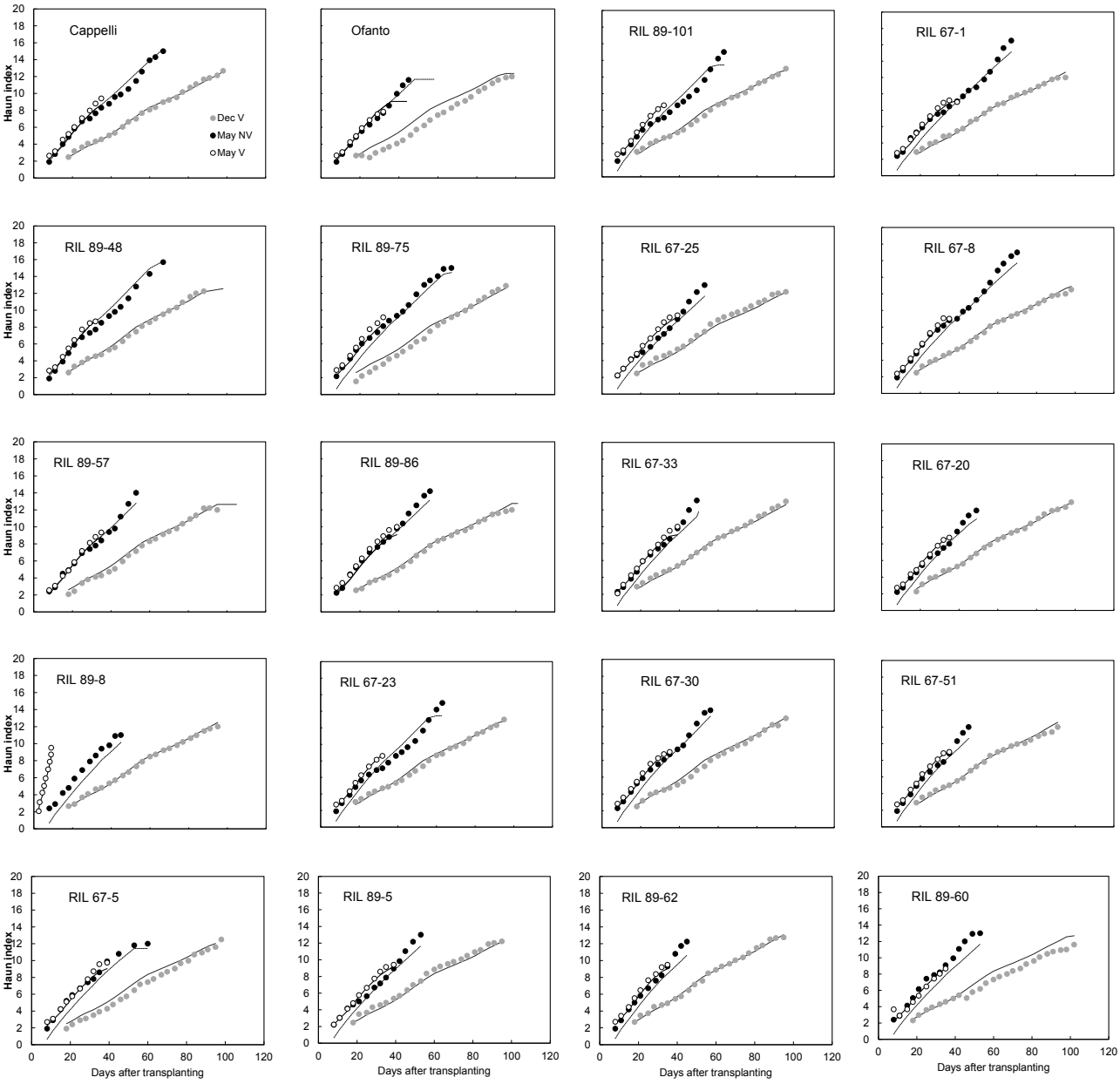

Figure 1. Haun stage vs days from transplanting simulated and observed for the 18 lines and two parents cultivar in the three treatments.

Figure 1 shows the simulated and observed Haun stage *vs* days from transplanting for the 18 lines and two parent cultivars in the three treatments. The goodness of fit apparent from the figure is summarized in **Table 3**.

Table 3 . Root Mean Square Error for the simulation of anthesis date, final leaf number.

	Anthesis (doy)	FLN (no)
Overall	0.8	0.6
DecV	0.7	0.5
May NV	1.0	0.7
May V	0.8	0.5
67-1	0.0	0.8
67-20	1.3	0.6
67-23	1.2	0.9
67-25	0.8	0.4
67-30	0.6	0.2
67-33	1.3	0.6
67-5	0.8	0.6
67-51	0.8	0.7
67-8	0.8	0.5
89-101	0.0	0.7
89-48	0.6	0.4
89-5	0.6	0.3
89-57	0.6	0.2
89-60	1.4	0.9
89-62	0.0	0.4
89-75	0.0	0.1
89-8	1.3	0.3
89-86	0.0	0.7
Cappelli	1.3	0.3
Ofanto	0.6	0.6

Anthesis date was simulated very accurately, with a minimum RMSE of 0 days in five RILs and a maximum RMSE of 1.4 days in the RIL 89-60 (**Table 3**). The treatment with the better simulation was DECV and the overall RMSE was less than 1. The good simulation of anthesis date is partly the consequence of the effectiveness with which the model simulated the final leaf number. A RMSE ranging from 0.1 to 0.9 was calculated for the single lines, with the largest mean value in MAYNV and an overall RMSE of 0.6.

The information from all the planting times was well fitted by the model. It could be argued that such a result should not be surprising, giving that each parameter was optimized in the sowing date where it was driving the phenology of the crop. However,

although the procedure allows for the selection of the best fit, it does not ensure that the error of this best fit is acceptable. The low RMSE calculated from the simulations can thus be taken as an indication that the algorithm of the model constitute a real summarization of the system. At the same time, the optimization of each phenological parameter in the sowing time where it was driving the development gave the possibility to better quantify the genotypic value of the parameter without the confounding effect of the interaction of the other environmental signals.

Calibration resulted in varietal parameters for the phyllochron ranging from 98 to 110 °Cd (**Table 4**). Starting from this varietal differences, the framework implemented in the model to take into account the effects of a variation in final leaf number and in the environment, namely sowing date, was able to cover an observed range varying from 70 to 126 °Cd.

Table 4 . Results of calibration of the varietal parameter phyllochron.

	Sirius	MAYV	MAYNV	DECV
Genotype	°Cd/leaf	°Cd/leaf	°Cd/leaf	°Cd/leaf
67-1	105	73	82	120
67-20	105	75	78	118
67-23	100	73	85	116
67-25	98	73	76	113
67-30	100	73	76	113
67-33	105	72	76	116
67-5	110	74	82	115
67-51	100	75	73	120
67-8	105	72	81	118
89-101	100	76	72	116
89-48	100	73	83	109
89-5	110	73	80	119
89-57	105	72	78	112
89-60	110	75	77	126
89-62	100	72	70	113
89-75	105	72	82	106
89-8	106	72	78	122
89-86	108	73	78	119
Cappelli	110	72	87	118
Ofanto	105	81	73	111

The three model varietal parameters considered were compared to the three indexes estimated from observed data to characterize the RILs' relative earliness *per se*,

photoperiodic sensitivity (RRP) and vernalization sensitivity (RRV) described in Chapter I. A strong relationship was calculated in all the three cases (**Figure 2**), which highlights the goodness of the Sirius varietal parameters.

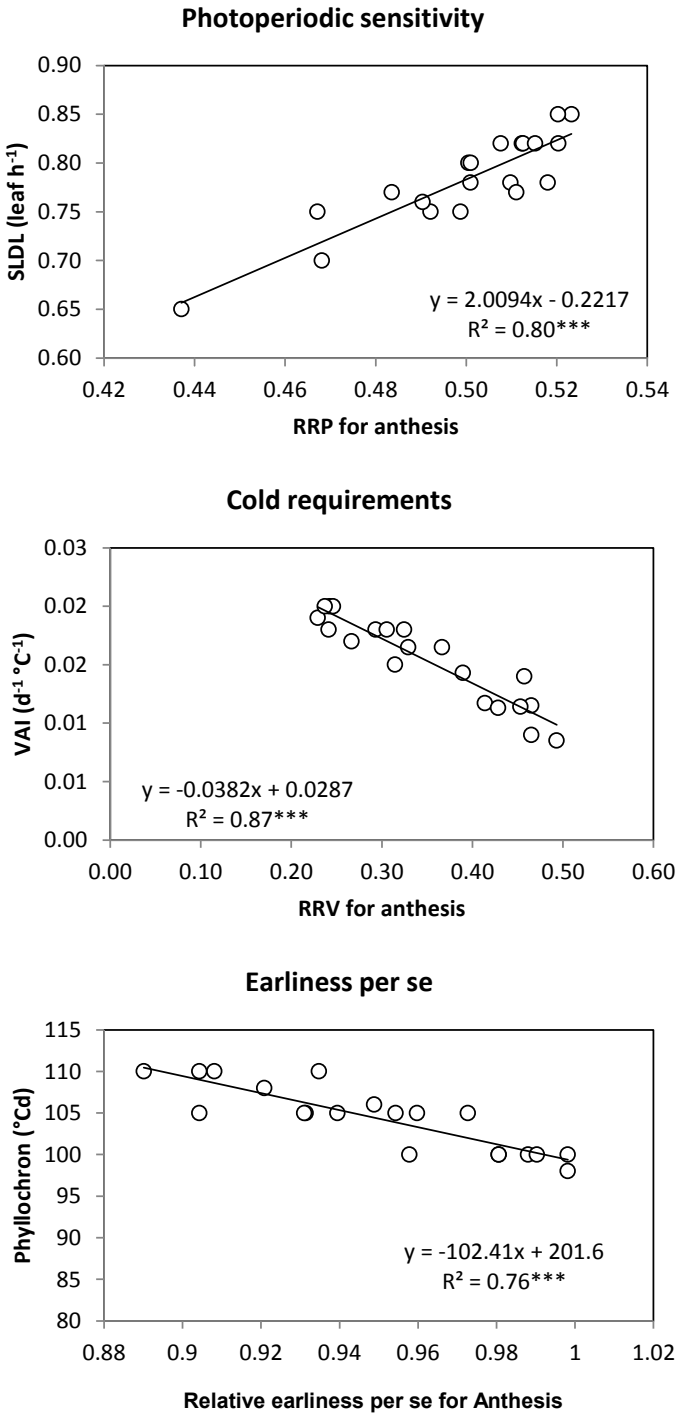

Figure 2. Relationship between three indexes RRP, RRV and RREPS vs three varietal parameters SLDL, VAI and phyllochron.

The relationship between RREPS and phyllochron mirrored the one observed by He et al. (2012) in bread wheat and could be attributed to the effect of earliness *per se* on the rate of primordia initiation (Gotoh, 1977, Worland, 1996) because of the strong coordination between rate of leaf primordia initiation and rate of leaf appearance (Kirby, 1990).

No association was found between the three varietal parameters calibrated for Sirius in spite of the one calculated by He et al. (2012). This result evidences the independent contribution of all the three of them in improving anthesis date prediction. At the same time, it confirms the goodness of the experimental approach used, which provided distinct treatments for separately calibrate the three varietal parameters. Also White et al. (2008), by estimating vernalization requirements and photoperiodic response parameters as linear effects of, respectively, *Vrn* and *Ppd* genes by means of a genetic model, implied the validity of a separate estimate of varietal parameters.

Data relative to the anthesis date of cultivar Cappelli available from preceding experiments were used to validate the model and the genetic coefficients calibrated for this cultivar in the pot experiment. These data referred to four sowing dates in the field (November, January, February and March, Giunta et al., 2007) and two sowing dates in pots (September and May, Motzo et al., 2007). Simulated anthesis date was in good agreement with observed data (**Fig. 3** , RMSE = 4 days), in spite of the wide variation in sowing time and of the different management (field and pots).

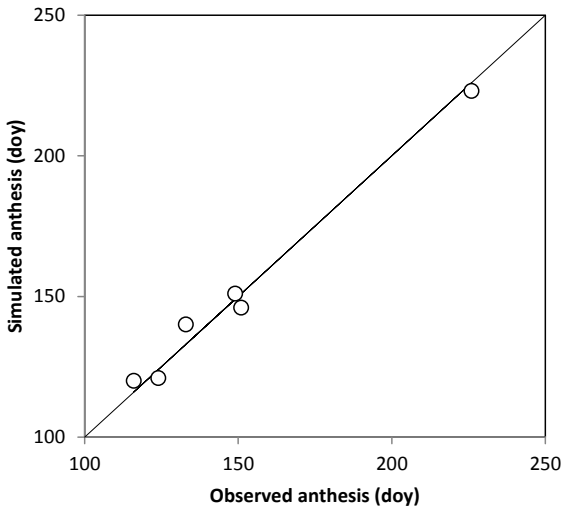

Figure 3. Observed and Simulated anthesis date of cultivar Cappelli and 1:1 line.

This is a promising result as the correct simulation of anthesis date is usually a problem when sowing dates are moved from winter to spring (Stapper, 1984). In the same

Mediterranean environment, Bassu et al. (2009) had to vary the phyllochron according to sowing date to obtain a correct simulation of anthesis with the APSIM model.

Conclusions

The sensitivity of the varietal parameters used by Sirius to simulate varietal differences in development was able to reproduce the phenotypic variability observed between RILs within environments.

The three Sirius varietal parameters considered were able to capture the phenotypic variability in development expressed by indexes independently calculated to evaluate the relative response of RILs to vernalization and photoperiod and their relative earliness per se. Although each parameter was calibrated in the sowing date where it was driving the phenology of the crop, the low RMSE calculated from the simulations indicates the validity of the physiological framework implemented in Sirius to model development. A future independent field experiment will eventually validate the calibration performed and confirm the validity of the varietal parameters optimized in defining the genetic make-up of the lines for their development.

The same procedure adopted in this experiment will be applied to calibrate the varietal parameters of the other RILs of the population in order to identify the relative QTLs and hence the genetic base of the parameters and their effective genetic independence. This information will allow the simulation of the impact of real genetic differences in development under varying environmental conditions and hence the genotype-by-environment interaction.

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