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Variation in Photosynthetic Efficiency of Spring Barley (*Hordeum vulgare* ssp. *vulgare*) Landraces

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Declaration

I hereby declare that this thesis has been composed by me and that the work is my own, except as acknowledged by means of references. This thesis has not been submitted for any other degree or professional qualification except as specified.

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Table of Contents

Table of Figures

Table of Tables

List of Abbreviations

A_{max} – Maximum rate of photosynthesis

ANOVA – Analysis of variance

ε_i – Radiation interception efficiency

ε_c – Conversion efficiency of intercepted radiation into biomass

ε_p – Partitioning efficiency of biomass into harvestable product

F_o – Minimal chlorophyll fluorescence

F_m – Maximal chlorophyll fluorescence

F_v/F_m – Ratio of chlorophyll fluorescence

GAI – Green Area Index

GLA – Green Leaf Area

GS – Growth stage

g_s – Stomatal conductance

HI – Harvest index

LAI – Leaf area index

NUE – Nitrogen use efficiency

PAR – Photosynthetically active radiation

PC – Principle component

PSII – Photosystem II

QTL – Quantitative trait loci

RUE – Radiation use efficiency

S_t – Amount of incident solar radiation

SSR – Simple sequence repeats

SLA – Specific leaf area

WUE – Water use efficiency

Y – Yield

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Abstract

Crop yields are coming under pressure to continue to grow in the face of climate change, competition, disease and pressure to reduce inputs. Photosynthetic efficiency is being targeted for improvement to increase yields. This study examined the variation in parameters of photosynthetic efficiency including canopy structure (leaf length, canopy angle, and chlorophyll content and growth rate) and gas exchange (photosynthetic rate, stomatal density and chlorophyll fluorescence) in Spring Barley (*Hordeum vulgare ssp vulgare*). These were first established for modern cultivars representing the most widely grown lines in the last 60 years. As cultivars are developed from a small pool of parents they may have limited genetic variation available for breeding. Landraces have been suggested as sources of variation. Using field and growth cabinet based studies the photosynthetic efficiencies of canopy structure and gas exchange were established for a range of European landraces under high and low nutrient inputs.

This study demonstrated that in modern cultivars the leaf length increased with year of release from 23.2 to 29.6 cm and the chlorophyll content decreased from 46.9 to 34.8 SPAD units. Once the ear had emerged no difference was seen in canopy structure or photosynthetic rate. There was variation in landrace canopy establishment rate, leaf angle and number of leaves present within the canopy.

The landraces from Northern European latitudes pushed though booting and reached full canopy establishment up to 8 days sooner than those from Southern Europe. This may be a response to a shorter growth season at Northern latitudes requiring the canopy to be established quickly. The landraces held the leaves within their canopy in a more horizontal position than the Southern European lines with leaf angle ranging from 18-45 degrees at GS39 and 31-84 degrees at GS59. This regressed negatively with temperature so it may be that a vertical canopy structure is beneficial in areas with higher temperatures. The photosynthetic rate of the landraces showed no variation but when chlorophyll fluorescence examined the efficiency of photosystem II (PSII) there was a positive regression of F_v/F_m ratio with latitude. This suggested that lines from Southern Europe were experiencing a greater stress

with a ratio of up to 0.822 compared to those lines from the North with ratios from 0.767. The stomatal density of the landraces showed a large difference in ranges from 22-41 stomata between the lines.

When high and low nutrient inputs were compared reductions from a ratio of 0.48 to 0.47 in Harvest Index and from 55g to 52g in 1000 grain weight were seen. The chlorophyll content of the lines was also reduced from 41.7 to 39.2 SPAD units at GS39 and from 44.9 to 39.8 SPAD units at GS59 by the reduction in nutrient inputs which may be a result of less N available for the production of chlorophyll.

In conclusion there is variation present in canopy structure in European landraces that may be useful for future breeding or in identifying landrace collections which could be targeted for traits of interest in photosynthetic efficiency. These landraces may provide traits which could be used to develop cultivars which are locally adapted to climate and environmental conditions.

Lay Summary

Crop yields are coming under pressure grow to meet increasing demands. In the past yield improvements have been the result of selective breeding for short height and responsiveness to fertilisers. The efficiency of photosynthesis to capture light and convert it into grain is now being targeted for improvement to lead to increased yields. This study looked at the structure of the canopy and the gas exchange efficiency of spring barley (*Hordeum vulgare* ssp. *vulgare*).

The canopy structure of modern varieties was assessed and it was seen that there was an increase in leaf length and a decrease in chlorophyll content with year of release over the last 60 years. There were no differences in rates of gas exchange between the lines. As there may not be sufficient variation present in elite breeding programs to allow improvement in photosynthetic efficiency landraces (barley cultivars typically grown before the turn of the 20th century before elite breeding) were examined to see if there was variation in traits of interest that could be introduced into modern breeding programs.

There was variation in landrace canopy establishment rate, the angle the leaves are held in regards to the stem and number of leaves present within the canopy. The landraces from Scandinavia reached full canopy establishment before the rest of the landraces meaning they could capture light over a longer time period than those from Southern Europe. The Northern European landraces held their leaves in a more horizontally structure than those from the South which means that only the top layer of leaves was involved in light interception. There was a difference in the efficiency of photosystem II (part of the system that captures light energy) with the landraces from Southern Europe being more inefficient than those from the north suggesting that they were experiencing greater levels of stress when grown in a Scottish environment. The density of the stomata (pores through which gases can pass into the leaves) showed a large range between the lines between 22 and 41 stomata per mm^2. Low fertiliser inputs caused a reduction in chlorophyll content and aspects of yield such as the 1000 grain weight.

In conclusion there is variation present in canopy structure and stomatal density in spring barley landraces that may be useful for future breeding. The results shown may also assist in identifying landrace collections from geographical and climatic locations which could possess useful traits for breeding new cultivars which are locally adapted to environmental conditions.

1. General Introduction

The demand for world crop production is forecast to increase over the next three decades with world population projected to reach 9.7 billion by the year 2050 (United Nations, 2015). This large population projection coupled with factors such as loss of arable land area and changing world climate is putting increasing pressure on crop yields to continue to increase avoiding a potential shortage (Evans, 1997). The drive for yield increase of non-food crops such as fodder crops, crops for bio energy and crops for industrial use such as malting is also under greater demand and the land use conflict between food and non-food crops is increasing (Fischer & Edmeades, 2010). Crop yields have increased significantly since the 'Green Revolution' of the 1960's with 'Harvest Index' (HI) (the proportion of a plants biomass that forms harvestable product) and high nutrient inputs as the driving factors. It has been suggested that yield improvement caused by HI and nutrient inputs is reaching a limit and other crop characteristics must be improved for crop yields to continue to increase (Reynolds *et al.*, 2009a; Fischer & Edmeades, 2010) requiring more effort in crop trait research and variety development. Crop yields must be increased without increased land areas, relying on increased inputs of water or fertilisers. This study looks at photosynthesis as a means to increase crop yields in Scottish spring barley (*Hordeum vulgare ssp. vulgare*) with a focus on landraces as a source of novel traits and the potential to provide greater local adaptation to the environment.

1.1 Pressures on Crop Yields

The most widely grown crops globally are maize (1038Mt), wheat (729Mt) and paddy rice (740Mt) (FaoStat, 2014). In Scotland, where the research in this study has been carried out, barley (*H. vulgare* L.) is the most widely grown crop in terms of land use and with a significant importance to the Scottish economy. Spring barley production in Scotland amounted to 1.52 million tonnes in 2015 along with 406,000 tonnes of winter barley (The Scottish Government, 2015).

Current pressures on crop yields include decreasing available growing area, pests and disease, climate change, and reduction or imbalance of fertiliser inputs (Evans, 1997;

Reynolds *et al*, 2009a, 2011; Hossard *et al*, 2014). To meet future population demands for crops at the rate of current population increases we would need to double crop production by 2050 (Reynolds *et al.*, 2009a; Foulkes *et al.*, 2011). Increasing arable area available for crop growth has been one of the main avenues for increasing crop yields alongside fertilisers and the development of dwarf varieties (which brought about the improvement in HI) in the past century. This is no longer going to be an option in increasing crop yields as alongside increased competition for land between crops there is also competition with other land uses such as forestry, tourism and conservation (protected areas). On top of this greater urbanisation for housing and business and increased demand for livestock production from meat based diets means that the only viable way to grow crop yields is to drive the increase of yield per plant (Parry & Hawkesford, 2010). The Scottish climate is challenging for crop production due to its high variability and unpredictability which can result in disease pressures and delayed sowing and harvesting which provide additional pressures on crop yields.

1.2 Determinants of Yield

Cereal yields are defined in many ways but at a basic level is the amount of grain, and grain weight (or harvestable product) produced by the plant which can be utilised for the end use of industry. In Scotland, the crop types which are commonly grown tend to be elite varieties which have been specially bred through pedigree breeding to be high yielding, high quality, responsive to high fertiliser inputs, uniform for ease of harvest and grown over large areas.

There are differences between the yield attainable in trials and on farm. The attainable yield (the best yield a farmer can achieve balancing management and economic risk (Fischer & Edmeades, 2010)) achievable by the farmer is normally lower than the potential yield calculated from the trials and may be due to a range of environmental or management factors. However on farm yield may be lower than the attainable yield and this is known as the yield gap (Murchie *et al.*, 2009; Fischer & Edmeades, 2010; Foulkes *et al.*, 2011). This yield gap is explained by many factors such as pests and diseases, soil quality and nutrient balances (Richards, 2000). Closing this yield gap has been an important part of research, policy and farmer

engagement work in western agriculture and this has the potential to increase yields in developing nations although increased yields have mainly come about from new cultivars having higher potential yield which is mirrored by higher attainable and farm yields (Richards, 2000; Foulkes *et al.*, 2011).

Yield production per plant can be described by a simple equation (Equation 1) in what has been come to known as the Monteith equation (Monteith & Moss, 1977) and has been remodelled multiple times (Farquhar *et al.*, 1980, 2001).

Equation 1, The Monteith Equation

$$Y = S_t \times 0.487 \times \varepsilon_i \times \varepsilon_c \times \varepsilon_p$$

Where yield potential (Y) is dependent on the amount of incident solar radiation received (S_t), the proportion of St which is photosynthetically active (0.487), the radiation interception efficiency of the plant (ε_i), the conversion efficiency of the intercepted radiation into biomass (ε_c) and the efficiency of partitioning of this biomass into harvestable product (ε_p). Only certain parameters of this equation are left open to improvement as some are dependent on environmental factors and some have already been maximised. The components of this equation which have already been maximised whether through breeding or other means will be discussed below.

The amount of S_t present for use is mainly pre-determined by the latitude, altitude, season length and weather conditions at the specific geographic growing location (Long *et al.*, 2006). Weather conditions are becoming more erratic and unpredictable as a consequence of global warming which may affect crop yields (Zhu *et al.*, 2010; Savolainen *et al.*, 2013; Henry & Nevo, 2014; Lobell *et al.*, 2014). Developing crops which are resilient to these erratic conditions will be important. Breeding to adapt crops to the amount of solar radiation present and season lengths has been achieved with adaptation of flowering time to different photoperiod lengths.

Flowering time is a photoperiod linked trait that in cereals has been shown to be locally adapted on a north/south latitudinal gradient (Jones *et al.*, 2008). Control of flowering time is significant as it ensures that switching from vegetative to reproductive growth occurs at the optimum time for reproductive success. Flowering time can be triggered by day-length but there is a large variation in daylight hours

throughout many crops range with almost continuous daylight in summer in the north. Variation in the responsiveness of barley to day-length in landraces has been observed and two major loci determining the photoperiod response have been identified as Photoperiod-H2 (Ppd-H2) and Photoperiod-H1 (Ppd-H1) which control flowering under short and long days respectively (Laurie *et al.*, 1995). A polymorphism in the Ppd-H1 locus gives a day-length non-responsive phenotype (Turner *et al.*, 2005) by down regulation of the photoperiod gene CONSTANS and it downstream flowering regulator FLOWERING TIME. It has been shown that this non-responsive form shows latitude dependent distribution and predominates in Scandinavia where there is almost constant daylight in the summer months (Jones *et al.*, 2008). This polymorphism has allowed barley to be cultivated far from its origin of domestication and will have contributed to the spread of agriculture out of the Fertile Crescent (Richards, 2000). The polymorphism has not been recorded in wild barley (*H. spontaneum)* which suggests that this occurred after the domestication event (Cockram *et al.*, 2007). Photoperiod sensitivity as a reason for local adaptation needs further study in crop species as it may affect traits such as shoot numbers and spikelets in ears (Cockram *et al.*, 2007) and it has recently been found that *PpD-H1* is involved in the control of leaf size in barley (Digel *et al.*, 2016).

Partitioning efficiency (ε_p) has been a target for crop breeders and scientists since the 'Green revolution' and has been largely maximised by the improvement of HI (Evans, 1997; Richards, 2000; Morinaka *et al.*, 2006). Harvest index is defined as the proportion of plant biomass which is partitioned between reproductive and the vegetative tissues (Chono *et al.*, 2003; Morinaka *et al.*, 2006). The improvement of HI has largely maximised ε_p in crops and there is little scope left for improvement. Dwarf cereal varieties are widely grown and crop height could not be shortened much more as there is a trade-off with lodging from top heavy plants (Berry *et al.*, 2003; Foulkes *et al.*, 2011).

Increases in yield have also come about with raising the use of fertiliser, pesticides and herbicides. There is increasing pressure to reduce the use of fertilisers as their production uses high inputs of finite resources and their application onto farmland can cause serious environmental problems such as leaching of nitrogen into water

courses. Pesticides and herbicides are coming under increasing scrutiny for their effect on the environment, human health and beneficial insects and there is increasing resistance developing in insects and weeds (Di Prisco *et al.*, 2013; Rundlof *et al.*, 2015). Increasing numbers of chemicals are being banned for use on crops which adds to the pressure to maintain high yields as losses from pests and diseases increase (Hossard *et al.*, 2014).

The lack of available yield increases from the improvement in ε_p and increased chemical inputs has led breeders and researchers to look at photosynthesis as a possible means to increase yield. Photosynthesis has not been the focus of pedigree crop breeding there may not be sufficient variation present in the current elite cultivars to improve photosynthetic c efficiency.

Breeders develop new cultivars with the aim of increasing yield or improving disease resistance and it requires a large investment of time and resources to develop a new cultivar. Phenotypic selection for yield characters (or other trait of interest such as disease resistance) informs the choice of parent lines and progeny to take through to the next generation. The development of technologies such as genomic selection, molecular markers, robotics and remote sensing are and will continue to aid not only more target selection of parents for breeding but make the process faster, cheaper and more efficient (Fischer & Edmeades, 2010).

1.3 Limits to Selection

Modern pedigree crop breeding has been intensively selective utilising only a small pool of parents which exhibit favourable traits (e.g. dwarf stature, fertiliser responsiveness) but as a consequence reducing the genetic base available for use in breeding with genetic material being recycled and no new material entering the pedigree (Russell *et al.*, 2000; Brozynska *et al.*, 2015). As current crop varieties have been developed from a small pool of parental genotypes there may be little scope for further crop improvement from these parents leading to a yield plateau where further gains will be difficult to achieve. A study by Russell et al (2000) assessed the genetic diversity of barley from wild relatives through landraces to intensively bred modern cultivars using SSR (simple sequence repeats) markers and they found that there was

a reduction in genetic variability which they concluded has arisen from selective breeding.

New avenues for crop improvement are being proposed and photosynthetic efficiency may provide prospects to improve yield. Photosynthetic efficiency has not been the focus of selection in crop breeding to date (Long *et al.*, 2006; Murchie *et al.*, 2009) and it is possible that as there is little genetic variation present in potential parents to be utilised in developing new cultivars with increased efficiencies. Photosynthetic traits may have become less efficient as a side effect of directed breeding for other traits such as yield or disease resistance so older material such as landraces may need to be assessed for higher rates of photosynthetic efficiency.

1.4 Landraces for Use in Breeding

Landraces may be a source of genetic variation that could be used in future breeding of crops to combat the yield plateau and introduce traits that may be useful which have been overlooked in modern crop breeding. Up until the introduction of modern cultivars at the turn of the 20th century the majority of cultivated barley in Europe was grown as local landraces (Villa *et al.*, 2005). A landrace is defined as a 'heterogeneous (genetically and phenotypically variable) plant variety that is reproduced by farmers as populations that are subject to both artificial and natural selection' (Bellucci *et al.*, 2013). A landrace population will be highly variable both phenotypically and genetically with higher genetic diversity than modern elite cultivars. The diversity in a landrace variety is structured between and within populations where a population is defined at the field/farmer level. Landraces are traditionally adapted to local conditions in contrast to modern varieties which have been bred to be adapted to an environment which has been created by modern agricultural practices. Landraces are still grown today in less developed countries worldwide and areas that have larger climatic variability or stress such as the Western Mediterranean as they are better at coping with the stressful climatic conditions than the modern elite cultivars (Yahiaoui *et al.*, 2014; Dwivedi *et al.*, 2016).

The genetic diversity of barley landraces from Spain was assessed (Yahiaoui *et al.*, 2008) and polymorphisms in microsatellite markers showed that the landraces

clustered into four main groups. These groups initially split into two- and six-rowed forms but thereafter segregated by climatic region which followed a north/south division. Other studies have seen a reduction in genetic variation from wild relatives through landraces to elite cultivars (Russell *et al.*, 2000, 2011). The population structure gives clues about evolutionary relationships between landrace and there is evidence that the European population is split by geographic distribution (Jones *et al.*, 2011) into nine populations. Some of these divisions are explained by two/six row or winter/spring growth type but it was also found that there were strong correlations between population structure and temperature and precipitation although it could not be seen whether this local adaptation was associated with population origin or a result of convergent evolution.

As landraces were grown over small geographic areas and developed 'on farm' by farmers there is likely to be a high degree of local adaptation to abiotic and biotic conditions such as climate, soil type, management techniques and disease. This adaptation has come about from farmers choosing the next generation of seed from those which grow the best and produce the highest yields. There has not been highly selective breeding such as has been seen with the development of the modern cultivars which has resulted in the loss of diversity (Villa *et al.*, 2005).

Local adaptation is defined as the possession of traits conferring higher fitness to an organism at its home site than at environmentally different sites (Savolainen *et al.*, 2013). Local adaptation has been recorded for natural populations of many plant species from *Arabidopsis thaliana* (Stenøien *et al.*, 2002) to Scots pine (*Pinus sylvestris*) (Salmela *et al.*, 2011, 2013) to wild barley (*H. spontaneum*) (Nevo & Beharav, 2005; Verhoeven *et al.*, 2008). Studies found that in both wild and crop species there is a correlation between photoperiod responses and latitudinal gradients (Jones *et al.*, 2008; Salmela *et al.*, 2011, 2013). It was observed in *A. thaliana* that there was a correlation between variation in hypocotyl growth in response to light and latitude with the more northerly populations (in Norway) being more responsive to red and far-red light (Stenøien *et al.*, 2002). In Eucalyptus trees (*Eucalyptus camaldulensis*), temperature and evaporation are important drivers of adaptation and that this has led to local adaptation in the populations of Australia (Dillon *et al.*,

2014). In wild cereal relatives local adaptation has been found with geographic and environmental factors correlating with a large amount of the genetic population structure in wild emmer wheat (Ren *et al.*, 2013) and wild barley (Baek *et al.*, 2003; Nevo & Beharav, 2005) using SSRs. There is a correlation between landrace latitude of origin and genetic make-up with landraces from similar latitudes being more similar than those from different latitudes as observed in Sardinian barley landraces where latitudinal distance was more important than geographical distance in determining genetic similarity (Bellucci *et al.*, 2013). This study used SSRs to establish the relatedness of different landraces.

Understanding the variation and the local adaptation in landraces will be important in advising future breeding programs as landraces have been suggested as a possible candidate for expanding the pool of parents utilised in breeding which could increase useful genetic variation (Rodriguez *et al.*, 2008). The former study found that landraces had high levels of genotype-by-environment interactions and this resulted in better yields under non-optimal growth conditions compared to modern elite varieties. Historic relatives of wheat have been suggested as sources of superior Rubisco (Prins *et al.*, 2016) and landrace germplasm has been suggested as a source of adaptation to stress factors such as water or nutrient limitation (Dwivedi *et al.*, 2016). Landrace traits related to photosynthetic efficiency and understanding how these are locally adapted to environmental conditions will help to broaden our knowledge and improve advice on possible useful sources of germplasm (Dwivedi *et al.*, 2016). Using phenotyping approaches to screen germplasm collections for physiological traits of interest would be a way of identifying possible parents for future cultivar development (Reynolds et al. 2009b).

1.5 Photosynthetic Efficiency

Photosynthetic efficiency is an area that has not been looked at in much depth in breeding for increased yield although it is one of the primary drivers of yield production (Reynolds *et al.*, 2011). Photosynthetic efficiency is defined as the amount of energy from the sun a plant is able to capture and convert into chemical energy. This contributes towards yield as described in Equation 1 and will be contributed to by the ability of the plant to capture light through its structure, the rate

of photosynthesis and the efficiency of the physiological processes and enzymes involved in energy conversion. Although certain parameters of Equation 1 are not available for improvement there are aspects of this equation which relate to photosynthetic efficiency which could be good candidates for future breeding to bring about improvements in yield (Zhu *et al.*, 2010; Parry *et al.*, 2011; Long *et al.*, 2015). There have been suggestions that radiation-use-efficiency (RUE) is higher in early cultivars and wild relatives compared to modern varieties (Muurinen & Peltonen-Sainio, 2006; Hubbart *et al.*, 2007; Gaju *et al.*, 2016). In rice (*Oryza sativa*), photosynthetic rates have decreased with year of release between 1966 and 1980 along with chlorophyll and Rubisco content (Hubbart *et al.*, 2007). Pre-heading RUE of barley was greater in older cultivars than in more recently released lines (Muurinen & Peltonen-Sainio, 2006). Landraces are one source of potential germplasm for introducing new traits of interest or improving traits (Hammer & Teklu, 2008).

S_t is largely predetermined by the latitude, altitude and time of year. However, increasing the duration of canopy maintenance by delaying leaf senescence will increase S_t (Richards, 2000) by increasing the amount of time the plant is utilising available light resources. The leaves of many crop species senesce during grain development because not all of the nutrients required to fill the grains come directly from photosynthesis and there is a remobilisation of nutrients stored in the leaves into the developing grain. The grain carbon content is contributed to by 75% from photosynthesis and 25% of remobilised carbohydrates (Emebiri, 2013). Delaying the senescence period can lead to increased yield with more of the nutrients in the developing grains coming from primary productivity (Richards, 2000; Zheng *et al.*, 2009). Scientist are beginning to understand the mechanisms behind senescence in crops with enzymes associated with senescence such as cytosolic glutamine synthetases and asparagine synthetases (Avila-Ospina *et al.*, 2015) and QTLs (quantitative trait loci) being identified. Nine QTLs involved in the loss of green colour in barley were identified with a single major locus on the short arm of chromosome 5H (Emebiri, 2013) which was independent of flowering time and 14 QTLs in maize associated with stay-green traits (Zheng *et al.*, 2009). More studies are required to understand how loss of green-colour is linked with environmental

cues. An alternative to delaying the start of senescence would be to develop cultivars which reach full canopy opening earlier and have canopy structures which maximise the volume of light captured (Richards, 2000).

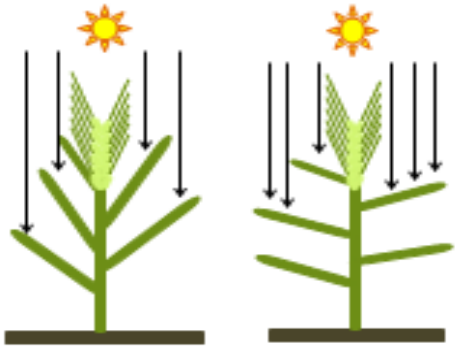

Figure 1.1 Canopy architecture. On the left is an erectophile canopy structure with light penetrating through to the lower leaf layers. On the right is a planophile canopy structure with the light only reaching the upper canopy layer. Light capture levels can be comparable between different canopy architectures but the utilisation in photosynthesis can differ.

Improvement in plants radiation interception efficiency can come from changes in plant architecture, amount of chlorophyll in the leaves, and the duration of leaf chlorophyll maintenance. Breeding for canopy structure has so far mainly focussed on structural and developmental uniformity and shorter varieties for improved HI and ease of harvest. Plant architecture (the structure of the canopy and leaves) is one of the main factors influencing ε_i and it can be maximised by designing crop canopies where light is distributed between leaves in a way where there is a reduction in the number of leaves receiving high wasteful light levels and light is allowed to penetrate though to lower canopy levels (Reynolds et al. 2009a) (Figure 1.1). In a horizontal canopy the uppermost leaf layer intercepts most of the light but will quickly become saturated at around a photosynthetic photon flux (the amount of photosynthetically active radiation falling on a leaf) density of one quarter full sunlight 500 µmol m^{-2} s^{-1} with the rest being lost for interception. Only around 10% of the light will penetrate down to the next leaf layer (Long *et al.*, 2006). A more efficient leaf arrangement would be a more vertical structure where the upper layer intercepts less light allowing a greater fraction to be transmitted to the lower leaf layers (Long *et al.*, 2006). Increasing the surface area of the leaves without altering the canopy structure will only increase self-shading. Canopy structure can also affect water-use-efficiency

(WUE) and is important in drought tolerance so altering the canopy structure must be done carefully to avoid stress due to water loss and increased photorespiration due to higher leaf temperatures (Beadle & Long, 1985; Goyne *et al.*, 1993). The WUE of a plant is defined as the amount of CO_2 taken up by a plant through photosynthesis in relation to the amount of water lost by transpiration (McAusland *et al.*, 2016). Adapting canopy structure to local climatic conditions may be a way to balance the conflict between light interception and water loss.

The chlorophyll content of the leaves and its distribution between leaf layers are also important factors in ε_i. Genes underlying the chlorophyll content are beginning to be identified in crop species (Flood *et al.*, 2011) and four QTLs for chlorophyll content in barley have been identified on chromosomes 2H, 3H and 6H (Xue *et al.*, 2008). As chlorophyll content can be highly correlated with photosynthetic capacity, it may be possible that increasing the chlorophyll content of leaves especially those in lower leaf layers could increase the amount of light energy intercepted by the leaves (Gaju *et al.*, 2016). This may require changing the shape of leaves to provide enough surface area but to minimise self-shading. It is also important to optimise the leaf surface layout in terms of stomatal spacing as stomatal size and clustering can affect water loss but maximising this can allow for an efficient spread of photosynthetic activity (Lehmann & Or, 2015; Lawson & McElwain, 2016).

The second target for potential barley (*H. vulgare*) photosynthetic efficiency improvement is in conversion efficiency ε_c. The conversion efficiency is determined by the photosynthetic rate, the efficiency of enzymes such as Rubisco and the nitrogen-use-efficiency (NUE). The main area of loss in the ε_c of cereals lies in the conversion of photosynthetically active radiation (PAR) into biomass by the enzyme Rubisco (Farquhar *et al.*, 2001; Raines, 2011).

Rubisco is the most abundant enzyme in C_3 plants such as barley and can account for up to 25% of the leaf nitrogen content. Rubisco catalyses the reaction which assimilates CO_2 but it also carries out a competing photorespiration reaction with O_2 at conditions when the CO_2 concentration in the leaf is low leading to a loss of fixed CO_2 (Keys, 1986). Up to 60% of potential assimilated carbon can be lost in photorespiration.

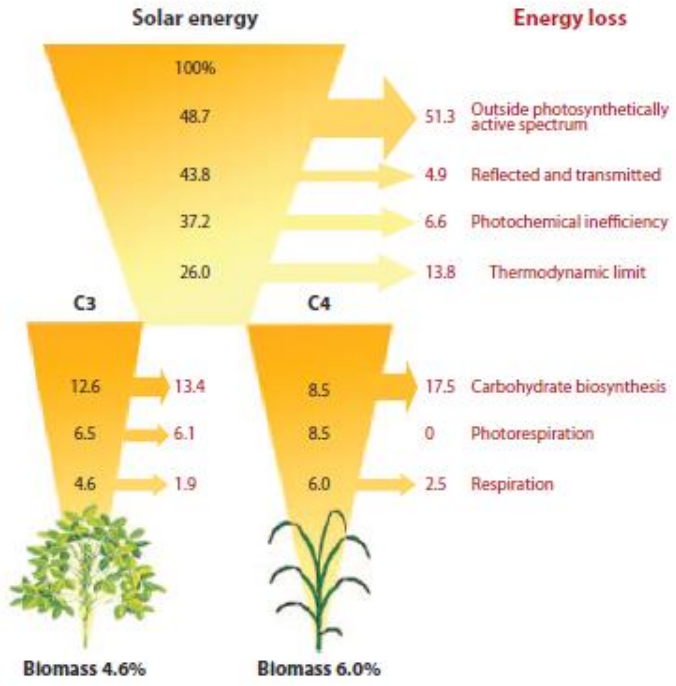

Figure 1.2 Diagram from (Zhu *et al.*, 2010). Comparison of energy losses between C3 and C4 photosynthesis from 100% solar energy at 30°C and [CO_2] of 387ppm

The activity of Rubisco is only one of the inefficient steps of photosynthesis with only around 4.6% of the solar energy available converted into biomass in C_3 crops (Figure 1.2) (Zhu *et al.*, 2010). There is a large amount of radiation from the sun outside the photosynthetically active spectrum and not all of the photosynthetically active radiation is absorbed and there are inefficiencies in the photochemistry of the reaction centres. In C_3 species Rubisco is the next main cause of loss of energy but in C_4 species such as maize CO_2 is transferred from the mesophyll cells to the bundle sheath cells increasing the concentration of CO_2 available thus reducing levels of photorespiration.

There is currently a large multinational project (IRRI, 2014) underway which is attempting to introduce the C_4 pathway into rice although so far there had been little success due to the complexity of coordinating the introduction of multiple genes required to switch from a system where the main reactions are carried out in the mesophyll cells to a system where the reactions are partitioned between the mesophyll and bundle sheath cells. Another possible approach could be to introduce

a system similar to that of certain algae and cyanobacteria termed the 'CO_2 concentrating mechanism' which increases the concentration of CO_2 around Rubisco along with the partitioning of CO_2 into compartments known as carboxysomes (Price *et al.*, 2008; Raines, 2011; Reynolds *et al.*, 2011). Approaches to consider in this would be the expression of the HCO_3^- transporter such as BicA and SbtA into the chloroplast of C_3 plants from the cyanobacteria to increase the level of CO_2 in the chloroplast.

There is also substantial variation present across species in the specificity of Rubisco to CO_2 (Murchie *et al.*, 2009) with Rubisco from the red algae found to be 2.5 time higher specificity than in higher plants (Uemura *et al.*, 1997), if this could be introduced into crop species it is predicted that the daily canopy carbon gain could increase by 27% (Zhu *et al.*, 2010). It must be taken into account that higher rates of photosynthesis may not be beneficial to the plants if they are growing in stressful environments due to the higher demands they will place on the plant in terms of nutrients (Flood *et al.*, 2011). It has been discovered that in some C_3 species such as wheat and rice there is a mechanism for the reassimilation of photorespired CO_2 to minimise losses by arranging chloroplasts to cover the areas of the mesophyll cells visible to the intercellular spaces thus capturing the photorespired CO_2 (Busch *et al.*, 2013). This suggests that although Rubisco is inefficient plants have adapted to deal with this and there is still much to be discovered in relation to photosynthetic efficiency.

One other aspect to ε_c that is still being looked at is the source to sink ratio. There have been differing opinions about whether yield production is constrained by the source i.e. photosynthetic efficiency, or the sink i.e. grain number and grain size. This may vary between crop species (Borrás *et al.*, 2004) but there is increasing evidence that for cereals yield is at least partially constrained by grain sink strength (Zhang *et al.*, 2010; Foulkes *et al.*, 2011) and that improvements in resource-use-efficiency and photosynthesis will not result in yield increases without the improvement in sink size. Studies in cereals have found that they are not limited by resource amounts during the grain filling period unless subject to high levels of stress (Wardlaw, 1994; Borrás *et al.*, 2004; Zhang *et al.*, 2010; Serrago *et al.*, 2013). Whilst

sink strength is an important factor it conversely must be true that grain yield will not increase if the ability to capture more source is not maximised and this is challenging as the size of the sink is predetermined before grain filling begins (Murchie *et al.*, 2009).

It is beyond the scope of this project to re-engineer Rubisco or attempt to introduce C_4 photosynthesis into barley however this study will look to see if there is any natural variation present in photosynthetic efficiency using spring barley (*H. vulgare*) as a study organism. Some studies have indicated that there are levels of natural variation present in different aspects of photosynthesis which may be of interest for use in future breeding (Kemanian *et al.*, 2004; Flood *et al.*, 2011; van Rooijen *et al.*, 2015). A study in wheat found substantial levels of natural variation in photosynthetic capacity in 64 elite varieties in rates of photosynthesis (Driever *et al.*, 2014).

1.6 Spring Barley (*Hordeum vulgare* L.)

This study has chosen to look at the photosynthetic efficiency of Spring Barley (*Hordeum vulgare* L.). Barley is the most widely grown arable crop in Scotland (The Scottish Government, 2015) with 1.5 million tonnes of spring barley being grown over an area of 256,000 hectares of land in 2015. Whilst this was down on the area grown in 2014 by 4% due to diversification rules associated with the EU Common Agricultural Policy, it is still the crop of the greatest economic importance to Scotland. Scottish barley is used both for the malting and the animal feed industries. High quality spring barley is bought by maltsters who then feed into the brewing and distilling industries which are a major contributor to the Scottish economy and also make up a large amount of Scottish product exports at £3950million in 2014 (The Scottish Government, 2014). Barley of a lower quality commands a lower price and is generally sold for animal feed and although this is a less well known use it can make up a large part of the annual Scottish barley crop (The Scottish Government, 2015). Barley is not just an important crop today but is also used in research as a model crop for temperate regions and is also of historic importance as one of the original crops to be domesticated. Barley (*H. vulgare*) is one of the founding crops of modern agriculture and originated in the Fertile

Crescent around 10,000 years ago (Harlan & Zohary, 1966). The wild ancestor of the modern crop is wild barley (*Hordeum vulgare ssp. spontaneum* C. Koch) which still grows naturally in its original habitat in the Fertile Crescent. It is currently thought that there was one centre of domestication in the Israeli-Jordan area of the Fertile Crescent with subsequent spread and diversification happening in a wider geographical area (Badr *et al*, 2000; Kilian *et al*, 2006; Russell *et al*, 2011).

The domestication of wild barley into crops was accompanied by a series of genetic changes which are commonly termed as the 'Domestication Syndrome' involving changes to crop phenotypes such as seed retention, seed dormancy, seed size and increased uniformity (Doebley *et al*, 2006; Jones *et al*, 2008). Some of the major genetic changes that have accompanied domestication include the photoperiod response genes (Laurie *et al.*, 1995; Cockram *et al.*, 2007; Jones *et al.*, 2008), the loss of the vernalisation requirement leading to spring varieties (Cockram *et al.*, 2007; Trevaskis *et al.*, 2007) and the development of the six-rowed ear allowing the production of triple the usual grain numbers occurring from the *vrs1* mutation which allowed the lateral spikelets to convert into fully fertile spikelets (Komatsuda *et al.*, 2007). More recently intensive selective breeding has introduced new traits which resulted in the uniform, high yielding crops that we are familiar with today such as dwarf varieties, high responsiveness to fertilisers and resistance to certain diseases (i.e. powdery mildew resistance with the *Mlo* gene in barley (Buschges *et al.*, 1997)). Photosynthetic efficiency and its associated traits have not been a target of breeding for improved efficiency in spring barley so there may be scope to increase yields through greater and more efficient resource capture through photosynthesis.

This study will begin by looking at photosynthetic efficiency in elite varieties under controlled conditions and then progress to examine in greater detail whether landraces may have variation in traits associated with photosynthetic efficiency which have room for improvement such as canopy structure and gas exchange rates. It may be that radiation-use-efficiency is greater in older cultivars and wild relatives (Muurinen & Peltonen-Sainio, 2006; Hubbart *et al.*, 2007; Gaju *et al.*, 2016).

1.7 Project Aims

The overall aim of this study is to assess whether spring barley landraces contain useful variation in traits related to photosynthetic efficiency that could be exploited to increase yields of cultivated barley. Each chapter will address a specific set of questions set around the following themes.

1) Is there variation in leaf size and shape, SPAD and photosynthetic rates in selected modern elite cultivars of spring barley released over the last 60 years and has this changed over year of release since the Green revolution?
2) Is there variation in European spring barley landraces in their ability to intercept radiation (ε_i) through canopy structure and chlorophyll content and are these traits adapted to local environmental conditions in the landraces location of origin?
3) Is there variation in the conversion efficiency (ε_c) in terms of photosynthetic rate, transpiration rate, stomatal conductance and stomatal density of spring barley landraces and are these traits adapted to local environmental conditions in the landraces location of origin?
4) How stable are the traits associated with photosynthetic efficiency such as chlorophyll content and photosynthetic rate in the landraces under low nutrient inputs? How resilient are components associated with yield such as 1000 grain weight and number of grains per ear along with the Harvest Index as an indicator of biomass production under low nutrient inputs?

1.8 Tackling the Project Aims

The proposed research questions will each be assessed in a separate chapter where the first chapter is an evaluation certain traits contributing to photosynthetic efficiency of modern cultivars of spring barley that have been released over the last 60 years. The canopy structure including leaf size and area was assessed on early and modern barley varieties under controlled conditions. The chlorophyll content was measured using a SPAD meter (Minolta Corp, NJ) which is a well cited proxy for directly measuring chlorophyll content (Giunta *et al.*, 2002; Debaeke *et al.*, 2006; Sadras *et al.*, 2012; Monostori *et al.*, 2016). The gas exchange rates were also established using a LiCor 6400 Gas Exchange System (LiCor Inc., Lincoln, NE)

which directly measures the rates of gas exchange under controlled conditions. Once the variation present in the current elite varieties of spring barley had been assessed and trends established with year of release. Spring barley landraces were then assessed for their potential to provide novel variation in traits of interest.

The ε_c and ε_i of landraces were then examined in detail to assess the variation present and any patterns of variation with environmental factors. The landraces were chosen to represent a wide latitudinal European spread in order to see whether there was evidence of local adaptation of photosynthetic efficiency to climatic and latitudinal conditions.

In order to study the ε_i of the landraces, a field trial was run over two seasons (2014, 2015). The landraces were grown in a fully randomised and blocked design in tussocks in order to minimise the effects of spatial variation in environmental conditions in the results. Direct measures of canopy structure such as leaf length, area and angle of upper leaves of the canopy were measured together with more indirect measures of canopy structure such as chlorophyll content (using SPAD as a proxy), the canopy establishment rate and the rate of senescence. This allowed the canopy structure to be assessed both in terms of green area maintenance but also at an individual plant level.

The ε_c of the landraces was assessed using a growth cabinet study under highly controlled conditions. This was not assessed in the field trial as it utilised a LiCor 6400 (LiCor Inc., Lincoln, NE) which although takes very detailed measurements of gas exchange and stomatal functioning does not lend itself to large scale remote studies such as field trials. This is due to the length of time required to take measurements which restricts the ability to compare measures taken over a number of days. The measurement window is also constrained by the time of day at which the measures can be taken as there are often circadian rhythms in photosynthesis over the day with rates rising to a peak in the morning followed by a decline in rates over the course of the day or a depression of photosynthetic rate at midday (Garcia *et al.*, 1998; Hirasawa & Hsiao, 1999; Srivastava *et al.*, 2002). In order to minimise external environmental influences, photosynthetic rates, stomatal conductance and transpiration rate of the landraces was assessed in a controlled growth cabinet

environment. These data were then coupled with stomatal density and chlorophyll fluorescence studies on the field trial in order to assess the efficiency of the gas exchange of the landraces.

Finally the stability of the photosynthetic efficiency and components of yield was assessed under nutrient stress by running a replication of the field trial under low fertiliser inputs. The components of yield assessed included 1000 grain weight and number of grains number of grain per ear and HI. The chlorophyll content under low nutrient inputs was examined to see if the reduction of nitrogen supply to the plant resulted in a reduction of chlorophyll, and carbon isotope and elemental analysis was undertaken to see how efficiently the photosynthetic apparatus is functioning and whether it is being affected by stress.

The final chapter (General Discussion) of this study attempted to answer whether there is useful variation in photosynthetic efficiency in landraces to increase yields in spring barley. Traits with potential were identified and an assessment of the likelihood that these traits will be of interest to breeders and their inclusion into future breeding programs undertaken.

2. Trends in Photosynthetic Traits in Modern Cultivars

2.1 Introduction

Spring barley (*Hordeum vulgare* ssp. *vulgare*.) is an economically important crop not just in the UK but worldwide. A large focussed breeding effort has gone into the development and improvement of spring barley to increase yield and adapt the plant to our current management practises as previously discussed in Chapter 1. The focus for future crop improvement for increased yields is turning to the traits associated with photosynthetic efficiency such as canopy structure and rates of gas exchange (Richards, 2000; Long *et al.*, 2006, 2015; Murchie *et al.*, 2009; Zhu *et al.*, 2010; Nunes-Nesi *et al.*, 2016). In order for this to be achieved the trends in barley photosynthetic efficiency since the Green Revolution and the introduction of modern breeding practises need to be assessed in order to identify characters that can mostly benefit selection to improve yield.

2.1.1 Development of Modern Crop Varieties

Intensive breeding has led to a 'Domestication Syndrome' as described in Chapter 1 of this thesis (Jones et al. 2008; Hammer 1984 (as cited in Doebley et al. 2006); Doebley et al. 2006). Early farmers would only be using a small pool of individuals to select from for the next generation resulting in a genetic bottleneck reducing the genetic diversity in the breeding population (Doebley *et al.*, 2006; Haudry *et al.*, 2007; Koenig *et al.*, 2013). Evidence of such bottlenecks have been seen in tomatoes and this was found to be the result of selection for environmental responsiveness and stress tolerance genes (Koenig *et al.*, 2013).

Around the turn of the 20th century plant breeding companies began to make a concerted effort to begin directed breeding of crop pedigrees (Evans, 1997). This began to accelerate after the end of World War II (Bellucci *et al.*, 2013). The local landraces that farmers had previously grown were then replaced with what we would recognise today as a modern elite cultivar using a small pool of these landraces to set up breeding programs. In the 1960's agriculture, including breeding, underwent a Green Revolution which resulted in the form of crops that we are familiar with today.

The main improvements in crop yields and the accompanying resource-use-efficiencies that came about during the Green Revolution were increases in Harvest Index (HI) (the proportion of a plants biomass that forms harvestable product) by the introduction of dwarf varieties and increased responsiveness to fertiliser application. In spring barley the introduction of the dwarfing gene *uzu* in Asian barley varieties has led to an increase in HI (Chono *et al.*, 2003).

After the introduction of modern breeding programs, crop selection has further intensified leading to pedigree breeding which uses a small group of parents which exhibit favourable traits but as a consequence reducing the genetic base available with genetic material being recycled and no new material entering the pedigree (Brozynska *et al.*, 2015). It has been suggested that as current crops tend to be developed from a small pool of parental genotypes there is little scope for further crop improvement leading to a yield plateau in some crops where further gains will be difficult to achieve. Barley is a highly inbreeding crop and modern varieties lack genetic variation within breeding lines; as genetic variation in barley is mostly found between varieties. New avenues for crop improvement are being proposed and photosynthetic efficiency as a means to improve yield has been suggested as an area with exciting prospects to improve yield.

2.1.2 Photosynthetic Efficiency of Modern Cultivars

Photosynthetic efficiency is one of the key factors that influence the final yield of a crop plant. Its importance in contributing to yield formation has been made clear in what has been come to known as the Monteith equation as described in Chapter 1 (Equation 1)(Monteith & Moss, 1977; Farquhar *et al.*, 1980, 2001). Factors of this equation involved in photosynthesis such as canopy structure or photosynthetic rate may possess variation that could be utilised in future breeding.

The ε_i (See Equation 1) is affected by the structure of the canopy and the chlorophyll content of the photosynthesising tissues. A study of Argentinian barley varieties (Abeledo *et al.*, 2003) found that yield increases per m^2 were related to increased ability to capture more radiation through more canopy coverage and had increased with year of cultivar release. The ε_c (See Equation 1) of barley is defined as the ability of the plant to convert the photosynthetically active radiation (PAR) to

biomass and is contributed to by the photosynthetic rate, the efficiency of enzymes such as Rubisco and the nitrogen-use-efficiency of plants. It has been suggested that there may be levels of natural variation in photosynthetic rates (Kemanian *et al.*, 2004; Flood *et al.*, 2011; van Rooijen *et al.*, 2015) amongst cultivars of cereals which could be utilised to improve efficiency. A study in wheat (*Triticum* a*estivum*) found substantial levels of natural variation in photosynthetic capacity in 64 elite varieties mainly in rates of photosynthesis (Driever *et al.*, 2014). As photosynthetic rate is not a factor that has been consciously selected for in modern breeding, it is unclear how much it has changed through development of cultivars of spring barley since the Green Revolution.

2.1.3 Variability of Photosynthetic Efficiency since the Green Revolution of the 1960s

Parts of Equation 1 that contribute to yield may have been directly selected for since the Green Revolution, such as the partitioning efficiency of the plant. A study of the history of Nordic barley breeding found that HI has improved significantly with year of introduction (Bertholdsson & Kolodinska Brantestam, 2009). Although this study spanned from the year 1880 the trend for improved HI from the 1960s was strong. Other factors of the Equation including aspects of photosynthetic efficiency such as chlorophyll content and photosynthetic rate have not improved however and indeed some seem to have declined as a consequence of modern breeding for other characters.

Studies looking into photosynthetic efficiency have suggested that factors involved in radiation-use-efficiency (RUE) (which encompass ε_i, ε_c, and ε_p) have not improved with all other developments in crop breeding since the Green Revolution and may have deteriorated (Reynolds *et al.*, 2011). This may be an unintended side effect of selection for other traits where alleles for high RUE have been lost. There have been suggestions that RUE is greater in older cultivars and wild relatives (Muurinen & Peltonen-Sainio, 2006; Hubbart *et al.*, 2007; Gaju *et al.*, 2016). A study of rice (*Oryza sativa*) varieties released since 1966 found that photosynthetic rate and chlorophyll content decreased over the time period to the 1980s (Hubbart *et al.*, 2007). Photosynthetic rate and chlorophyll content increased after the 1980s in rice

which coincided with the introduction of high levels of fertilisers. There was then a recovery of these factors but this was attributed to the application of high levels of fertilisers. As there is a push to decrease fertiliser inputs this could cause an unintended decrease in photosynthetic efficiencies. Another study which looked at wheat, barley and oats found that old (dates not defined) wheat and barley cultivars had a higher RUE than new cultivars both before and after heading (Muurinen & Peltonen-Sainio, 2006).

Evidence discussed previously showed large potential in other crops for improving ε_i and ε_c. This initial study looks to see if there is the same potential in spring barley (*H.vulgare*) by seeing whether over the years these two aspects of photosynthetic efficiency have declined as a consequence of selection in modern cultivars for other traits. This provides the basis for investigating variation in physiological traits among more diverse barley genotypes.

2.1.4 Aims

The aim of this study was to assess the change in two aspects of photosynthetic efficiency in spring barley bred over the course of the last 60 years. It is expected that a decrease in chlorophyll content and photosynthetic rate will be seen with year of release. The size of the leaves is likely to have become more uniform with year of release but is it not known whether this will have involved an increase or decrease in length or area. The main questions to be addressed are:

1) Has leaf structure including size and area, and chlorophyll content (ε_i) changed over the last 60 years of spring barley breeding?
2) Have the ε_c parameters of photosynthetic rate, stomatal conductance and transpiration rate changed over the last 60 years of spring barley breeding?

Varieties of spring barley that have been released over the last 60 years were therefore assessed for values of photosynthetic efficiency including leaf size and area, leaf chlorophyll content and photosynthetic rate to provide the baseline or trend of physiological change over time.

2.2 Methods

2.2.1 Material

Fifteen modern varieties of 2 row spring barley were selected (Table 2.1) which represents dominantly grown varieties released and included on the recommended lists (AHDB) from the last 70 years. These varieties were chosen as they represent the main varieties grown in Scotland for the malting industry over recent history and were varieties that were of the greatest importance to the Scottish economy.

Eight seeds of each variety were planted in a 0.5 litre pot using Levington's Pot and Bedding Compost High Nutrient with two pots of each barley variety. They were planted on 07/11/13 at SRUC Kings Buildings Edinburgh (UK). As this study was carried out in winter, supplementary heating and lighting was provided. Lights were set to create a 16 hour day and heating was provided to ensure the temperature did not drop below 15^{o}C. The light intensity was variable as only natural daylight was provided during the day and artificial lights only first thing in the morning and after sunset. The pots were watered five days a week to avoid waterlogging and the amount of water provided adjusted accordingly. Plants were supported with stakes if needed. The pots were arranged in the glasshouse as in Figure 2.1. There was no randomisation and blocking within this experiment due to lack of knowledge of experimental design and set up at the beginning of the training program that is a PhD research project.

Golden Promise A	Prisma B					
Glassel B	Prisma A	Shuffle B	Triumph B	Polygena B	Zephyr B	Lofa Abed B
Glassel A	Optic B	Shuffle A	Triumph A	Polygena A	Zephyr A	Lofa Abed A
Concerto B	Optic A	Propino B	Tankard B	Westminster B	Proctor B	Maris Mink B
Concerto A	Golden Promise B	Propino A	Tankard A	Westminster A	Proctor A	Maris Mink A

Figure 2.1 Experimental set-up of the pots on the bench with the door to the left. A and B are replicate pots.

Table 2.1 List of cultivars, year of first introduction on the Scottish Recommended List and breeding company. Information obtained from the AHDB recommended lists and personal communication from Dr Steve Hoad who is involved in the compilation of the recommended lists.

Variety	Introduced	Pedigree	Breeder
Proctor	1953	Kenia x Plumager Archer	Plant Breeding Institute Cambridge, United Kingdom
Zephyr	1966		Combined Cereal Breeders, Holland
Golden Promise	1968	Gamma ray mutant from Maythorpe	Zeneca Seeds, United Kingdom
Lofa Abed	1970	Proctor x Minerva	Abed Plant Breeding Station, Denmark
Maris Mink	1974	Deba Abed x (Emir x Swallow)	Plant Breeding Institute Cambridge, United Kingdom
Triumph	1980	DIAMANT X 14029/64/6	Deutsche Saatgut Handelsbetriebe, Germany
Prisma	1988	(Trumpf x Cambrinus) x Piccolo	BV Landbouwbureau Wiersum, Holland
Polygena	1993	46401/80 x 45465/78	Hadmersleben
Tankard	1994	(Corniche x CSBA 1096/1022) x (Heritage x Chariot)	Plant Breeding International Cambridge, United Kingdom
Optic	1995	Chad x (Corniche x Force)	Syngenta Seeds Ltd
Westminster	2005	NSL 97-5547 x Barke	Limagrain Europe
Concerto	2009	Minstrel x Westminster	Limagrain Europe
Propino	2010	Quench x NFC Tipple	Syngenta Seeds Ltd
Shuffle	2011	Troon \Quench\Adonis	Syngenta Seeds Ltd
Glassel	2013	Summit x Belgravia	Syngenta Seeds Ltd

2.2.2 Growth Stages

At Growth Stage (GS) 21 which occurred on 05/12/13 and is the start of shooting, measurements of canopy structure were recorded. The measurements taken were; leaf area (cm^2), leaf length (cm) and leaf dry weight (g). Leaf chlorophyll content (SPAD units) was also measured. At GS59 which occurred on 15/01/14 and is full emergence of the ear, leaf length and leaf chlorophyll content were measured along with measures of gas exchange including photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$), conductance (mol H_2O $m^{-2}s^{-1}$), intercellular CO_2 concentration (µmol CO_2 mol^{-1}) and transpiration rate (mmol H_2O $m^{-2}s^{-1}$). These measures were not taken at GS21 as the leaves had not reached an adequate size for measurements to taken. Leaf area was not measured at this stage due to the destructive nature of this sampling. Four plants out of eight plants were randomly chosen per pot and tagged with laboratory tape and all further measurements were taken on these plants. Measurements were carried out on one leaf of the main shoot of the four chosen plants and two replicate pots.

2.2.3 Canopy Measurements

Leaf length was measured with a standard 30cm ruler (Stephens Publishing, Sandusky, OH in cm from the tip of the leaf to the point where the leaf blade meets the sheath (Figure 2.2). Chlorophyll content was estimated by using a SPAD meter (Minolta corps, Ramsey, NJ) at the leaf midpoint of the leaf blade (Figure 2.2). Three measurements were taken for each leaf and the readings averaged to take into account any spatial variation present in the amount of chlorophyll present in the leaf.

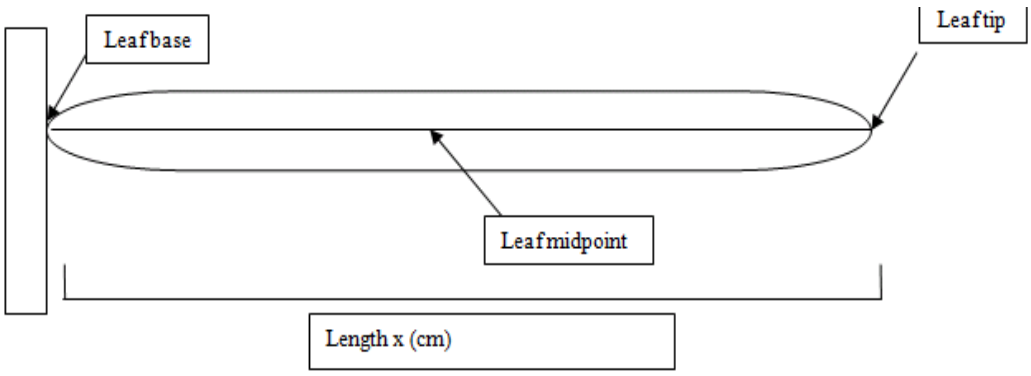

Figure 2.2 Points of measurement on the leaf blade. Length measurements were taken from leaf base to leaf tip and chlorophyll contents were taken at the leaf midpoint.

Leaf area was measured by detaching the leaves and immediately passing them through a leaf area meter (Li-3100 are meter, LiCor Inc., Lincoln, NE) to minimise wilting and breakdown which calculated leaf area in cm^2. Leaves were passed through the meter three times and the readings averaged.

The leaves that had been used for leaf area measures were then placed in individual paper bags and dried in an oven (Ecocell, MMM Medcenter, Munich, Germany) at 80^oC for 48 hours. The leaves were then weighed using a precision balance (Kern PLJ, D-72336, Kern & Sohn Gontbl, Balingen, Germany) in grams (g).

The specific leaf area (SLA) is a measure which allows the leaf thickness to be taken into account when examining the size and shape of the leaf available for light interception. It was calculated using the leaf area and leaf dry weight measures as leaf area/leaf dry weight.

2.2.4 Gas Exchange

Measures of photosynthetic rate were taken using a LiCor 6400 gas exchange system (LiCor Inc., Lincoln, NE). Chamber settings were 400ppm CO_2 concentration, 500µmol of PAR, air flow of 500µmol and block temperature of 22^0C. The light level was chosen to mimic the levels of light being received by the plants in the glasshouse which was low due the Scottish wintertime. All measures were taken between 11.00am and 1.00pm British Winter Time. The leaf was placed in the chamber and allowed to acclimatise for 15minutes prior to readings being taken. This 15minute acclimatisation period was chosen after running a number of test observing the photosynthetic rates on the Li-Cor 6400 monitor and timing how long stabilisation took. Three readings were taken per leaf and averaged to account for any variation present in the respiration of the leaf. Readings were taken on four plants per replicate pot and there were two pots of each variety. Measurements were taken on the uppermost fully expanded leaf excluding the flag leaf. Gas exchange readings were not performed for cultivars Proctor, Zephyr, Lofa Abed and Maris Mink due to the condition of the leaf deteriorating with senescence and disease burden.

2.2.5 Statistical Analysis

Using GenStat 15th Edition data was first tested for normality. A regression analysis was used to determine whether there was a significant relationship between any of the photosynthetic efficiency variables and the date of release of the cultivars.

2.3 Results

2.3.1 Canopy Structure

2.3.1.1 Growth Stage 21 – main shoot and one shoot

The leaf length (cm) of the spring barley cultivars released over the last 60 years has increased over time ($p=0.027$, $t_{1,13}=6.21$, $R^2=0.27$) (Figure 2.3) with a range of 23.2 - 29.6cm (Table 2.2). The chlorophyll content (SPAD units), measured on the 2nd leaf which at GS21 is defined as the 2nd fully expanded leaf to emerge, showed a decrease with year of release with those varieties released in the 1960s having more chlorophyll in the leaves than those released recently ($p=0.041$, $t_{1,13}=5.16$,, $R^2=0.22$) (Figure 2.3) from 46.9 - 34.8 SPAD units (Table 2.2).

Leaf surface area (cm^2), measured on the same leaf as the SPAD readings, has increased with year of release since the 1950s ($p=0.006$, $t_{1,13}=10.61$, $R^2= 0.40$) (Figure 2.4) from 14.83 - 20.24 cm^2 (Table 2.2) whereas the dry weight (g) ($p=0.194$, $t_{1,13}=1.88$, $R^2=0.05$) (Figure 2.4) and the specific leaf area (cm^2/g) ($p=0.217$, $t_{1,13}=1.68$, $R^2=0.04$) (Figure 2.4) have not changed over the course of the last 60 years. This indicates that the leaves have become thinner whilst becoming longer.

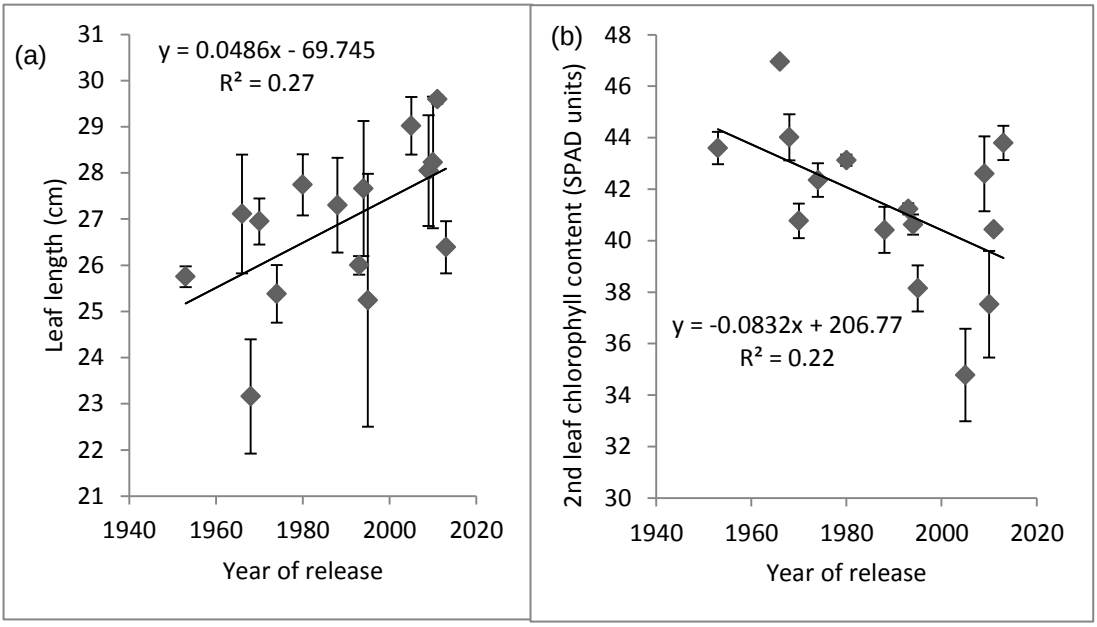

Figure 2.3 GS21 (a) Liner regression of leaf length with year of release. Line is linear regression line. (b) Linear regression of 2nd leaf chlorophyll content with year of release. Error bars are the standard error. Each point in the graphs are the means of two replicates for a different cultivar with each replicate being the mean of readings on four leaves. Measurements were taken on the second fully expanded leaf for both leaf length and SPAD readings.

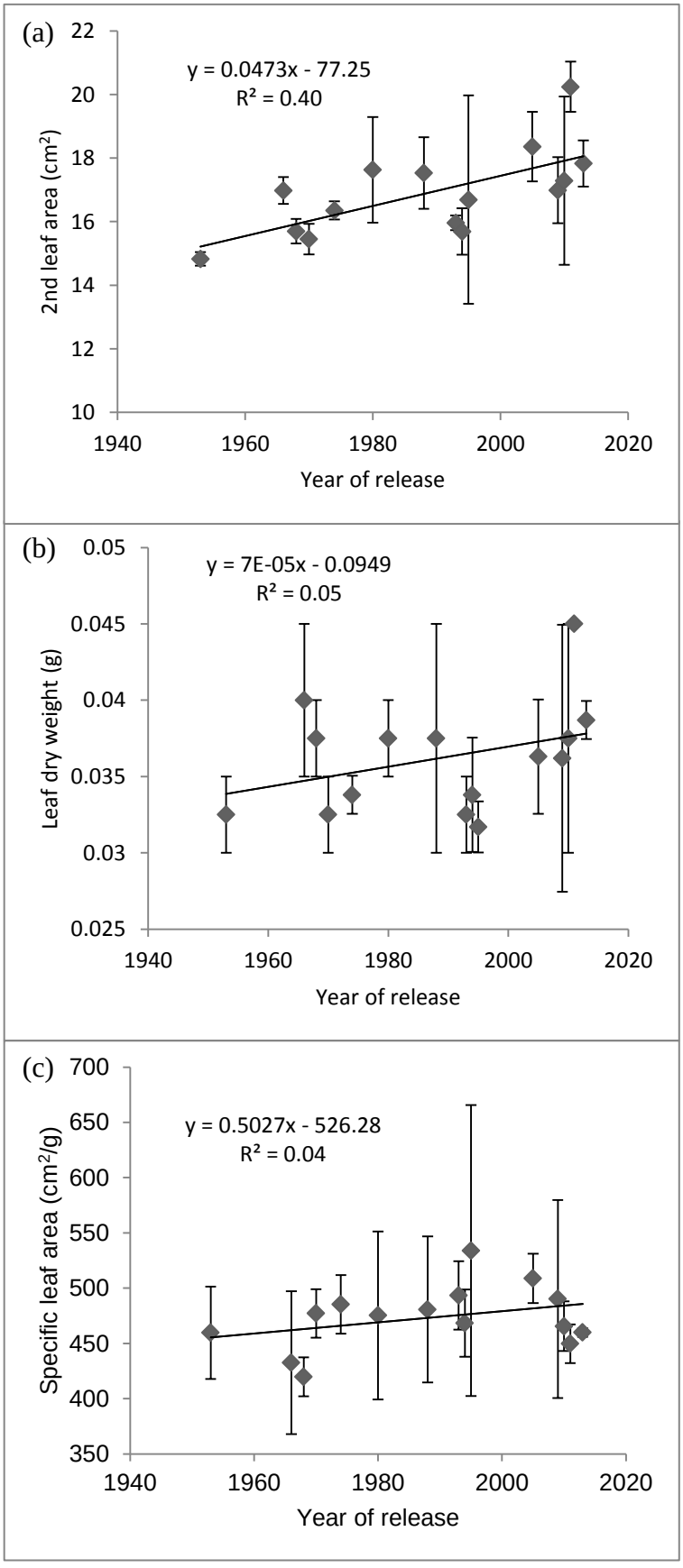

Figure 2.4 GS21 (a) Linear regression of leaf area with year of release. (b) Linear regression leaf dry weight with year of release. (c) Linear regression specific leaf area with year of release. Each point in the graphs are the means of two replicates for a different cultivar with each replicate being the mean of readings on four leaves. Measurements were taken on the second fully expanded leaf for leaf area, dry weight and specific leaf area.

Table 2.2 Canopy Structure traits at GS21. The range of means for the cultivar set and the regression with the year of release

Leaf trait	**Range**	**Regression with year of release**
Leaf Length (cm)	23.2 - 29.6	$p=0.027$, $t_{1,13}=6.21$, $R^2=0.27$
Chlorophyll content (SPAD units)	34.8 – 46.9	$p=0.041$, $t_{1,13}=5.16$, $R^2=0.22$
Leaf Area (cm^2)	14.83 – 20.24	$p=0.006$, $t_{1,13}=10.61$, $R^2=0.40$
Leaf Dry Weight (g)	0.317 – 0.045	$p=0.197$, $t_{1,13}=1.88$, $R^2=0.05$
Specific Leaf Area (cm^2g^{-1})	419.8 – 534.0	$p=0.217$, $t_{1,13}=1.68$, $R^2=0.04$

2.3.1.2 Growth Stage 59 – ear completely emerged

The leaf length (cm), measured on the uppermost fully expanded leaf excluding the flag leaf (i.e. leaf 2 where flag leaf is leaf 1), of the cultivars at this growth stage showed no changes over the course of the last 60 years ($p=0.100$, $t_{1,13}=3.13$, $R^2=0.13$) (Figure 2.5). The chlorophyll content (SPAD units), measured on the same leaf as the length, of the 2nd leaf also showed no significant relationship with year of release ($p=0.095$, $t_{1,13}=3.24$, $R^2=0.13$) (Figure 2.5).

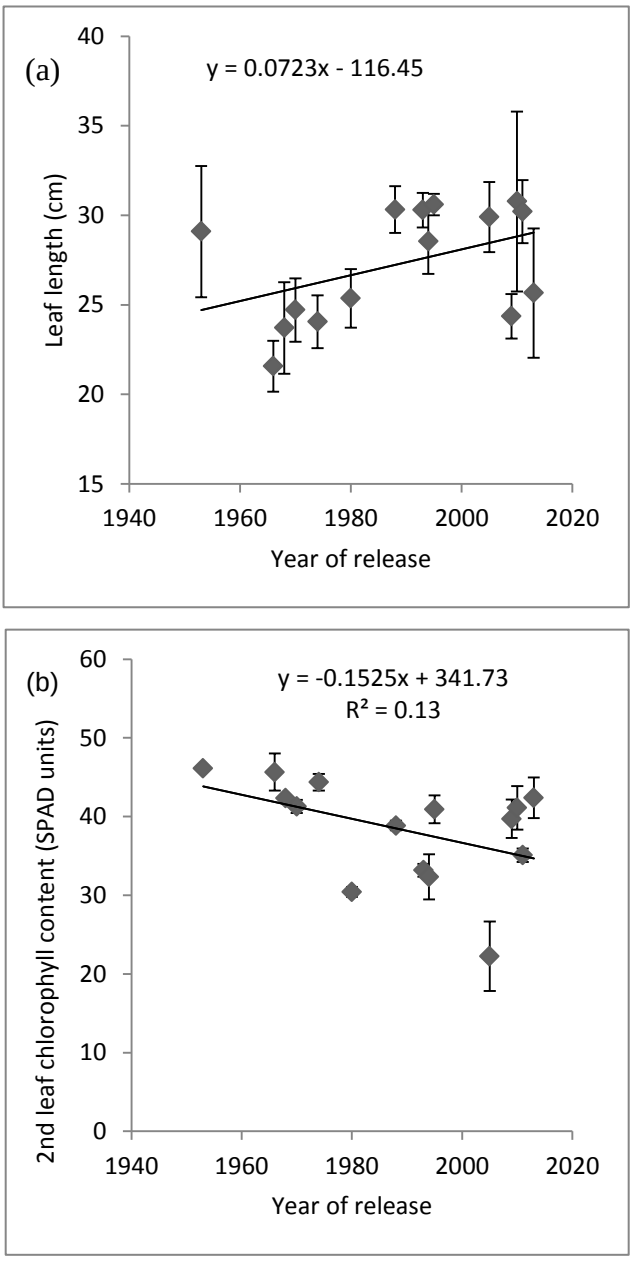

Figure 2.5 GS59 (a) Linear regression of leaf length with year of release. (b) Linear regression of 2nd leaf chlorophyll content with year of release. Each point in the graphs are the means of two replicates for a different cultivar with each replicate being the mean of readings on four leaves. Measurements were taken on the uppermost leaf excluding the flag leaf for both leaf length ad SPAD readings.

Table 2.3 Canopy traits at GS59. The range of means for each trait and the regression of each trait value with the year of variety release

Leaf trait	Range	Regression with year of release
Leaf Length (cm)	21.6 – 30.7	$p=0.100$, $t_{1,13}=3.13$, $R^2=0.13$
Chlorophyll content (SPAD units)	30.4 – 46.1	$p=0.095$, $t_{1,13}=3.24$, $R^2=0.13$

2.3.2 Gas exchange

None of the Gas Exchange parameters of photosynthetic rate, stomatal conductance, intercellular CO_2 concentration or transpiration rate which were measured on the uppermost fully expanded leaf excluding the flag leaf showed any change either increase or decrease over the last 60 years. The photosynthetic rates (µmol CO_2 $m^{-2}s^{-1}$) of the spring barley cultivars ($p=0.267$, $t_{2,8}=1.56$, $R^2=0.10$), the stomatal conductance (mol H_2O $m^{-2}s^{-1}$) ($p=0.862$, $t_{1,19}=0.03$), the intercellular CO_2 concentration (µmol CO_2 mol^{-1}) ($p=0.762$, $t_{1,19}=0.10$) and the transpiration rate (mmol H_2O $m^{-2}s^{-1}$) ($p=0.649$, $t_{1,19}=0.22$) (Figure 2.6, Table 2.4) all showed no changes with breeding since the Green Revolution.

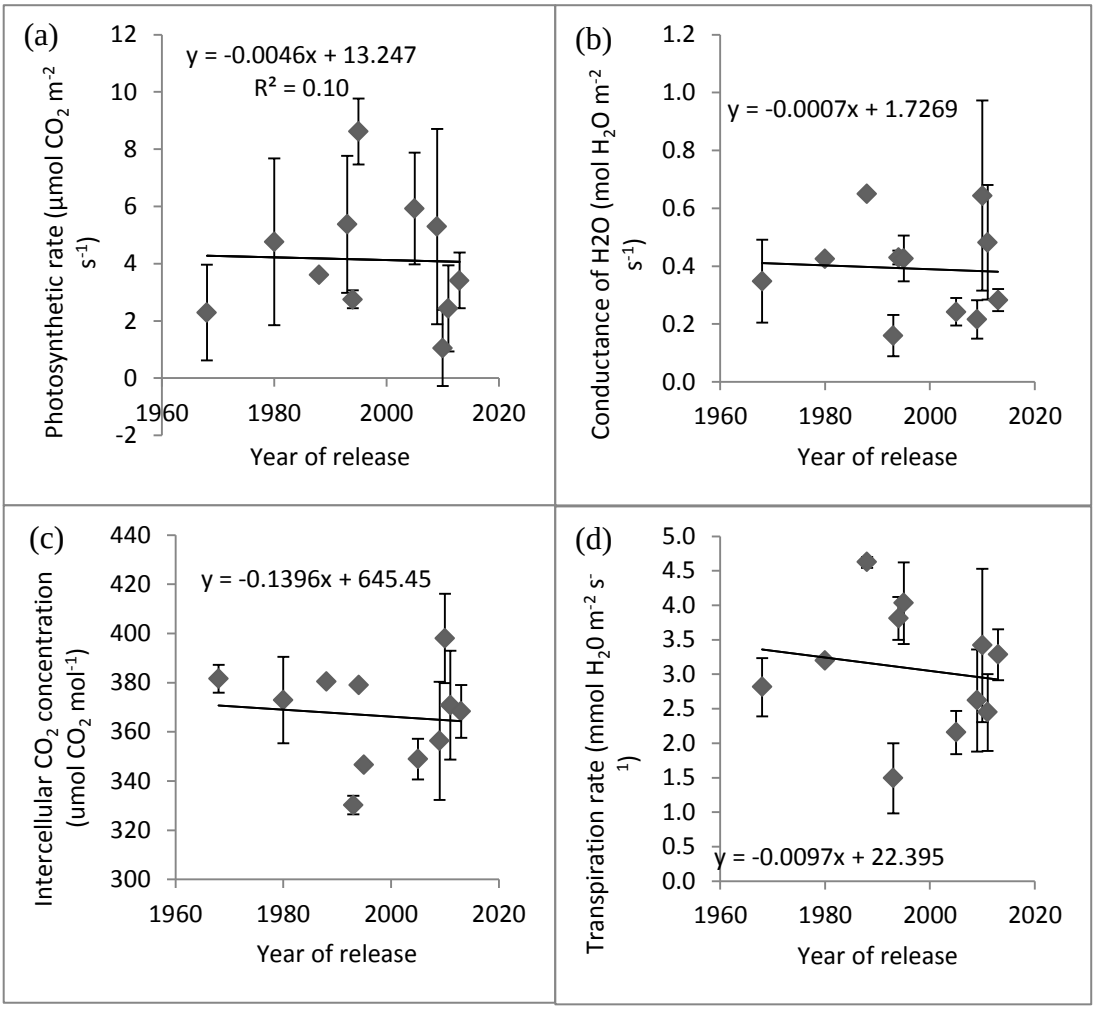

Figure 2.6 Gas exchange measures taken using a Li-Cor 6400 on the uppermost leaf excluding the flag leaf. Chamber settings were 400ppm CO_2 concentration, 500µmol of PAR, air flow of 500µmol and block temperature of 22^0C. Each point is a different cultivar with readings taken on four plants per replicate pot and two pots of each variety. (a) Quadratic regression of photosynthetic rate with year of release (b) Liner regression of conductance with year of release. (c) Linear regression of intercellular CO_2 concentration with year of release. (d) Linear regression of transpiration rate with year of release.

Table 2.4 Parameters of gas exchange of variety leaves. The range of values is given and the results of the regression with year of release.

Leaf trait	**Range**	**Regression with year of release**
Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$)	1.05 – 8.62	p=0.267, $t_{2,8}$=1.56, R^2=0.10
Stomatal conductance (mol H_2O $m^{-2}s^{-1}$)	0.16 – 0.65	p=0.862, $t_{1,19}$=0.03
Intercellular CO_2 concentration (µmol CO_2 mol^{-1})	330.2 – 398.0	p=0.762, $t_{1,19}$=0.10
Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)	1.49 – 4.62	p=0.649, $t_{1,19}$=0.22

2.4 Discussion

2.4.1 Canopy Structure

The structure and size of the leaves is an important component in photosynthetic efficiency as it determines the firsts step in light interception. The individual leaf size and shape, the chlorophyll content of these leaves, the total number of leaves and shoots and how the leaves overlap in the canopy all contribute to light interception. The selection of modern cultivars used in this study have been bred to have a uniform canopy with predominantly erect leaf habit, reduced competition between plants and a shorter stature with more biomass partitioning to grain production.

The leaf structure of the modern cultivars changed over the course of the growth season. At the early GS21, leaf shape was measured using leaf length, area, dry weight and SLA was calculated. At the later GS59 only leaf length was measured. The leaf length at the early GS has increased over the course of the last 60 years (Figure 2.3) but at the later GS, there were no difference in leaf length between the cultivars (Figure 2.5). This initial difference may be because in the more recent cultivars the leaves are growing in length quicker earlier which would allow the plants to take advantage of the early growing season. It has been shown that increasing the length of 'green time' in crops has positive results on yield (Richards, 2000; Zheng *et al.*, 2009) and in maize some QTLs (quantitative trait loci) are beginning to be identified. This could be a useful trait to keep in future cultivars especially those designed to be grown in Scotland where the light conditions can be erratic so early growth could provide more time for grain filling especially since the sowing dates have become later. The variety Shuffle had the longest leaf lengths early on, so this may be an interesting variety to use in future breeding for early leaf size.

When the leaf shape is looked at in a more comprehensive way, using SLA to account jointly for leaf area and leaf thickness (proxy by dry weight) there are no differences in the SLA between the cultivars (Figure 2.4, Table 2.2) suggesting that those varieties that have higher leaf areas may have thinner leaves. This could be an advantage meaning that the chlorophyll contained in the leaves is more able to intercept light and less shaded by the internal leaf structure. It does appear that the

leaf area has increased over the last 60 years suggesting a trend for larger leaf surface areas (Figure 2.4). Studies have shown that this increase in leaf area has led to an increase in total photosynthesis (Richards, 2000). Larger leaf surface areas may lead to a problem with self-shading (Falster & Westoby, 2003) which could limit light interception meaning that how the leaves are held within the canopy becomes an important trait. A study of Argentinian spring barley showed an increase of leaf area index (LAI), which is the green leaf area per unit ground area, with year of cultivar release (Abeledo *et al.*, 2003). This indicates that the leaf area has increased. This is in contrast to some studies which have found that leaf area and SLA have decreased with year of release. A study of durum wheat (*Triticum turgidum* L.var. *durum*) showed a decrease in these factors in cultivars released from 1900 to 2000 (Giunta *et al.*, 2008). This suggests that barley is following a different trend for leaf area than other crops.

Later on in the growth season, the leaves of all the cultivars reach the same length. Destructive sampling to calculate SLA was not possible due to the leaves having to be left intact for gas exchange measurements but it would be interesting to see if the leaf canopy at this stage was still adapted for larger thinner leaves, or whether it was an early growth advantage in these lines with the size of the leaves in the other lines reaching the same final dimension. It would be interesting to look at the spacing of stomata over the leaf surface area as this trait has been shown to be affected by domestication with the redistribution of stomata (Milla *et al.*, 2013) and the size, shape and spacing of stomata are important for gas exchange but also for minimising water loss (Lehmann & Or, 2015; de Boer *et al.*, 2016; Lawson & McElwain, 2016).

The chlorophyll content of the leaves is important in light capture although there have been conflicting arguments about whether yield is source or sink limited. At the early GS where light capture is all about vegetative growth there was a large variety of chlorophyll contents present in the different cultivars (Table 2.2). A useful cultivar for breeding may be Zephyr as it had a high chlorophyll content compared to the other varieties. There was a significant trend for chlorophyll content to decrease over the last 60 years (Figure 2.3). This may be a side effect of the chlorophyll content getting spread out over a larger leaf surface area but it may be a side effect of

selection for other traits in breeding where high chlorophyll levels have been lost. In rice chlorophyll content decreased over time between 1966 and 1980 but has recovered slightly with increased chlorophyll levels since the 1980s with improved responsiveness to fertiliser inputs causing more nitrogen to be taken up by the plant and utilised in producing chlorophyll (Hubbart *et al.*, 2007). This is in contrast to some studies in wheat where chlorophyll content has increased over time (Watanabe *et al.*, 1994; Sadras *et al.*, 2012; Gaju *et al.*, 2016) and it has also been shown that modern cultivars have higher chlorophyll contents than older landraces. One study in Australian wheat found that changes in chlorophyll content of the leaves from varieties released since 1958 varied with canopy layer with the greatest increases found in the upper canopy (Sadras *et al.*, 2012). This study did not look at canopy structure and it would be interesting to see whether there have been changes in leaf morphology to accompany this pattern. Modern barley cultivars have lower leaf chlorophyll contents than wheat so may have responded differently to breeding (Giunta *et al.*, 2002). Indeed QTLs for chlorophyll content in barley have already been identified (Xue *et al.*, 2008) which could be useful for future barley breeding.

2.4.2 Gas Exchange

The gas exchange measures taken give an idea of the conversion efficiency of the plant. This is one of the areas that is notoriously inefficient (Long *et al.*, 2006; Zhu *et al.*, 2010; Parry *et al.*, 2011). The main measure taken was photosynthetic rate alongside the stomatal conductance, intercellular CO_2 concentration and the transpiration rate.

The ε_c of the cultivars in all parameters measured has not changed with year of cultivar release (Figure 2.6, Table 2.4) and this is in contrast to what has been found in some other studies in other cereal crops. In wheat a large amount of variation was seen in photosynthetic rates and rates of CO_2 assimilation present in a range of cultivars released between 1975 and 2008 (Driever *et al.*, 2014). This study found that breeding may have resulted in photosynthetic capacity decreasing and Rubisco content certainly decreased with year of release. A contrasting study in Australian wheat under constant nitrogen conditions (Watanabe *et al.*, 1994) however found increases in photosynthetic rate over time and put this down to the introduction of the

Rht dwarfing gene allowing higher nitrogen content allocation to the leaves which allow the production of greater levels of chlorophyll and Rubisco thus increasing CO_2 assimilation rates. Studies have also been carried out in rice which shows variation in photosynthetic rate and stomatal conductance in current cultivars (Hubbart *et al.*, 2007; Gu *et al.*, 2012) and Hubbart found that between 1966 and 1980 cultivars released showed a decreased photosynthetic rate and Rubisco content.

Although no link with year of release was seen in our study, there is some evidence from other crops that shows that there may be scope to increase the conversion efficiency of the crop. It would be interesting to go further back to landraces or wild barley to establish if there were higher levels of photosynthetic capacity that may have been lost through modern breeding, as it has been suggested that RUE was higher in old cultivars than new cultivars (Muurinen & Peltonen-Sainio, 2006). It may be that this pilot study showed too small a sample size to pick up any trend in gas exchange with year of cultivar release. Alternatively, there may have already been a reduction in photosynthetic efficiency with breeding progress before the introduction of modern cultivars post-Green Revolution. It may also be that alternative measurements of gas exchange would be more informative in following photosynthetic activity through crop breeding. For example, it may have been of benefit to produce A/Ci curve, or light response curves, to assess photosynthetic capacity in more detail.

Improved photosynthetic rate and responsiveness to changing CO_2 concentrations with climatic change may be a way to improve gas exchange efficiency and there may be more exotic material which is more responsive to current CO_2 levels and less limited by Rubisco and photorespiration without having to go to the extremes of re-engineering Rubisco or introducing the C_4 pathway (Gowik & Westhoff, 2011). Increased photosynthetic rate would be accompanied by a decrease in stomatal conductance which would also be of benefit in reducing water loss.

2.4.3 Future of yield improvement through consideration of wider genotypic diversity

The main conclusions from this study are;

- The length of the leaves of the cultivars has increased with year of release at GS21.
- The chlorophyll content of the leaves has decreased with year of release of cultivar line at GS21.
- There were no changes in photosynthetic rate in the cultivars with year of release.

The reduction in chlorophyll content and the differences seen in leaf size over the last 70 years leads to the suggestion that perhaps improvement in photosynthetic efficiency through increased levels of chlorophyll or improved canopy structure for light interception could lead to improvements in yield. As most modern cultivars are bred from a small pool of parents there is not much scope for improvement without the introduction of traits from other material (Brozynska *et al.*, 2015). Landraces are an interesting suggestion for candidates for including traits of interest into the current varieties. Landraces not only contain a larger amount of variation than the current varieties but they are grown over much smaller geographical areas leading to much more local adaptation to climate conditions. This suggests that there may be large amounts of variation present geographically to be utilised in breeding new cultivars. To begin, the variation present in photosynthetic efficiency must be assessed to identify traits of interest and this must be done at a local Scottish environment with the idea that the traits must be relevant to a Scottish climate. The next two chapters look at different aspects of photosynthetic efficiency in field and controlled conditions from a range of European origins to assess the range available for use in future breeding.

3. Light Interception Efficiency of Spring Barley Landraces

3.1 Introduction

3.1.1 Yield components and limits

Crop yields are coming under pressure to feed an ever increasing world population in the face of climate change, competition for arable area, pests and diseases and an increased pressure to reduce fertiliser and chemical inputs (Evans, 1997; Reynolds *et al.*, 2009a; Foulkes *et al.*, 2011; Hossard *et al.*, 2014). There are suggestions that a 'yield ceiling' is being reached with limited yield increases from greater arable area and reduced Harvest Index (HI) and new avenues for yield increases must be explored.

Yield production per plant or crop can be described by the Monteith equation (Equation 1) (Monteith & Moss, 1977). The plants ability to intercept light (ε_i) is one of the factors that can be improved to contribute to increased yields. Landraces may be interesting to look at for possible traits of interest for use in breeding due to larger variability and the fact that they are locally adapted to climate conditions. This chapter uses spring barley landraces to examine variation in ε_i which could be utilised in breeding future varieties. Light interception is contributed to by a variety of factors from the initial establishment of the crop canopy to how long that canopy is maintained through to the structure of the canopy and the chlorophyll content.

3.1.2 Canopy Development and Maintenance

There are three distinct growth phases distinguished in the development of barley. The first involves vegetative growth and canopy establishment, the second flowering and ear development and the third grain filling and ripening. The rate at which the plant develops through each of these phases and subsequently the length of time spent in each will have an effect on the amount of time available for photosynthesising (Murchie *et al.*, 2009). The length of the growth season will vary depending on latitude and this is not a factor that can be changed (Zhu *et al.*, 2010). In Northern latitudes, the length of the growth season will be short but there will be more hours of light per day than in Southern European latitudes. If landraces are able

to optimise their growth patterns in response to these local light conditions this could be of use in developing new varieties which either pass more rapidly through canopy establishment into the phase of their life-cycle where they are capturing the maximum amount of light energy, or delay senescence and remain actively photosynthesising for longer.

Delaying senescence as a way of increasing yield and the development of 'stay-green' varieties has been a new focus in plant breeding. Slower loss of chlorophyll content through nutrient remobilisation has been associated with an increase in grain weight in barley, maize and wheat (Diaz *et al.*, 2005; Zheng *et al.*, 2009; Parry *et al.*, 2011; Emebiri, 2013). During grain filling and ripening it is usual for the plant leaves to senesce and the nutrients held within the leaves to be remobilised into the grains. This ensures no waste of nutrients and that the grains have sufficient resources to fill properly. In rice biomass increased by 0.2 tonnes per hectare for every day that the growth season was increased (Akita, 1989). In developing stay-green varieties there is a trade-off between maintaining the chlorophyll content of the leaves and ensuring sufficient supply of nutrients for grain development. More research is needed to identify the point where maintaining a green canopy is counterproductive to grain production i.e. the point at which photosynthesis is no longer providing enough resources to meet the demands of grain filling and must be supplemented by nutrient remobilisation.

Another way of increasing canopy duration would be for seedlings to push through the initial canopy establishment faster producing a full canopy of leaves earlier in the season (Richards, 2000; Murchie *et al.*, 2009; Zhu *et al.*, 2010). Early canopy establishment would allow shorter growth seasons to be taken full advantage of (Reynolds *et al.*, 2009a; Parry *et al.*, 2011). It has also been seen that early canopy cover increases the ability of the crop to deal with weed competitors (Zhang *et al.*, 2015).

An important aspect of canopy establishment and duration is the chlorophyll content of the leaves and other photosynthesising organs (stems and awns). The volume and arrangement of chlorophyll in the leaves is an important factor in efficient light interception and capture (Yin & Struik, 2015). Creating a canopy with a more even

distribution of chlorophyll throughout the leaf layers in combination with a vertical canopy structure has been suggested to aid in light distribution and reduce the number of leaves becoming saturated with light (Ort *et al.*, 2011; Yin & Struik, 2015). Some QTLs have been identified which are thought to be involved in chlorophyll production and amount. Four QTL's were identified in barley on chromosomes 2H, 3H and 6H (Xue *et al.*, 2008) whilst another study found different QTL's on chromosomes 2H and 4H (Guo *et al.*, 2008). The identification of chlorophyll production QTLs could be of use in understanding the production of chlorophyll within the plant potentially leading to breeding varieties with higher levels of chlorophyll.

3.1.3 Light Interception

Both morphological and physiological factors contribute to the ε_i efficiency of spring barley. Two attributes of the crop canopy have major effects on light interception efficiency. These attributes comprise canopy structure, determined by the dimensions of the leaves and the way they are held and spread within the canopy and the duration of canopy maintenance (Long *et al.*, 2006).

Leaf dimensions such as length and surface area are factors that could be optimised for better light capture. Although it may at first be assumed that larger leaves will be more effective in the canopy at capturing a large amount of light this is not always the case. There is a trade-off between leaf area and self-shading (Long *et al.*, 2006; Amanullah *et al.*, 2007). For the canopy to efficiently capture as much light energy as possible the way in which the leaves and leaf layers are arranged within a canopy must also be optimised as shown in figure 1.1.

For a canopy with an upper leaf angle of 75^0 from the horizontal there could be double the efficiency of energy capture of a horizontal canopy at midday (Long *et al.*, 2006). It has also been suggested that having an erectophile canopy structure may help to reduce heat stress. The number of leaf layers in a canopy will affect the photosynthetic efficiency if there is an erectophile canopy structure (Li *et al.*, 2015a) and the genetic determinants of this are beginning to be understood in maize along with its relationship with the switch from vegetative growth to flowering. The

development of an erectophile canopy has already allowed a larger leaf area per unit of ground area resulting in the increase of yield (Murchie *et al.*, 2009).

In modern elite crop cultivars leaf morphology tends to be very uniform (Russell *et al.*, 2000). There is a lack of knowledge in older material about variation in leaf structure. The leaf size and shape will affect factors such as how much leaf area is available for light capture but also how much light misses the top leaf layer and penetrates through the canopy. There has been some progress in identifying QTLs for leaf morphology traits (Xue *et al.*, 2008; Liu *et al.*, 2015) with a lot of focus on the flag leaf in barley due to its importance in carbohydrate production. In barley leaf size has recently been linked to the flowering time gene *PHOTOPERIOD-H1* (*Ppd-H1*) (Digel *et al.*, 2016) which indicates that there is a trade-off between leaf growth and utilising resources for developing ears and grain. It has been found that in modern crops leaf area is also affected by fertiliser inputs with maize showing that increasing fertiliser inputs increases the leaf area available for photosynthesis (Amanullah *et al.*, 2007). Leaf size and shape will not only affect the amount of light capture but will also influence heat stress, water loss and weed competition (Zhang *et al.*, 2015). Traits such as leaf size or canopy angle may become more important in the future as climate change increases temperatures leading to the possibility of increased heat and drought stress.

Overall, there are many factors which go into making up the ε_i of spring barley. Some of these components are involved in trade-offs with other aspects of the plant's biology and others will need to be improved with careful consideration of modern agricultural practises. Landraces offer an exciting possible source of variation in canopy structure which if identified properly, could provide a source of traits for optimising the canopy not only in the face of a changing future climate but also in refining crops for local conditions. It may be expected that there are high levels of variation in canopy establishment and maintenance and structural traits in landraces. This variation may be linked to the climate in their locations of origin which could give clues not only about the adaptation and development of these traits but also indicate likely geographical areas to explore for material which could be utilised in future breeding programs.

3.1.4 Aims

The aim of this study was to examine components of leaf canopy development and structure that are known to influence ε_i. Variation in a range of different spring barley landraces from a European latitudinal spread will be compared with a reference cultivar. The landraces were specifically chosen to represent a wide latitudinal spread as they will have become adapted to a varied range of climatic conditions. It will be assessed how diversity of growth and developmental traits manifest under a high input (nitrogen and pesticide) system, typical of UK barley growing conditions. A following chapter (5) will explore landrace performance under reduced input or stressed conditions. The specific questions addressed in this study are;

1) Is there variation in the canopy establishment, development and maintenance between the landraces in traits such as leaf size and habit (angle), leaf chlorophyll content and duration of canopy maintenance?
2) Is variation manifested under a high input system related to the diverse origins of landraces, in particular the latitude and climate of origin?
3) How might understanding of trait variation (or integration) be interpreted for improvements in plant breeding?

In order to answer these questions, measurements of canopy structure such as leaf length, area and leaf angle were taken on field grown plants. These measurements were then compared to measures of climatic conditions which were taken at the landraces location of origin. The growth rate and chlorophyll content of the leaves were measured across the season to track development and senescence.

3.2 Methods

3.2.1 Seed source

The material was collected from seed banks (Table 3.1) prior to the start of this project. The latitude and longitude of their original collection was also noted (Table 3.1) to be used for collection of climatic data. The landraces were specifically chosen to represent a wide geographical range across Europe (Figure 3.1) which encompasses a range of different climatic conditions and season lengths.

3.2.2 Field Trial

3.2.2.1 Field Trial Set-up

A field trial was carried out over two consecutive years in the spring/summer of 2014 and 2015 at Boghall farm in Midlothian, EH10 7DX. The 2014 field trial was planted in the Crofts field and the 2015 trial in the Cowloan field. The soil type at these sites is a sandy loam (Macmerry Series). The farm is situated on the South-east slope of the Pentland hills at an elevation of 200m. In 2014 the average daily maximum temperature over the season was 16^{o}C and the minimum was 7^{o}C. The average monthly rainfall was 107mm and the average monthly hours of sunlight was 119 hours. In 2015 the daily maximum temperature over the season was 14.5^{o}C and the minimum was 5.5^{o}C. The average monthly rainfall was 130.mm and the average monthly hours of sunlight was 119 hours.

This experiment was carried out over two years. In both years, a trial was planted as a split-plot design in which all plots were replicated with a normal fertiliser treatment (120kg/hectare) and a low fertiliser treatment (30kg/hectare). There were three replicate plots in 2014 and four in 2015 with each plot containing one tussock of each line and each tussock containing 25 plants (Figure 3.2). Variation among landraces grown 120kg/hectare (high input) are reported herein, whilst their response to reduced input is reported in Chapter 5.

Table 3.1 List of landraces, their site of collection with latitude and longitude coordinates, the Gene bank they were sourced from and which year of the field trial they were grown in

Landrace Number	Collection Site	Country	Latitude	Longitude	Source	Year
GER1	Pflugs Intensiv Sélection dans une orge de pays Sarroise	Germany	*49.40*	*6.96*	INRA	2014
FRA1	E71 Oisans Variété De Pays De L'oisans	France	*45.03*	*6.03*	INRA	2014/2015
GER2	Bavaria Sélection dans une variété de pays de Basse-Bavière	Germany	*48.50*	*11.5*	INRA	2014/2015
FRA2	Le Puy N12 Sélection dans une variété de pays d'Auvergne	France	45.08	3.83	INRA	2014
SWE1	Alanas parsonage, Jamtland	Sweden	64.17	15.7	NSGC	2014
NOR1	Opdal, central high-mountain region, Sor-Trondelag	Norway	62.50	9.67	NSGC	2014/2015
NOR2	Donnes, Nordland, Nordland	Norway	66.20	12.58	NSGC	2014/2015
CZE1	Libochovice, North Bohemia	Czech Republic	*50.40*	*14.03*	NSGC	2014/2015
FIN1	Sattanen EH0103 Sattanen, Sodank	Finland	67.58	26.62	NGB	2014/2015

FIN2	Järvenkylä ME0302 SEP B cereal mix Järvenkylä, Mieh	Finland	60.72	27.48	NGB	2014
FIN3	Långstrand 0102; Paavo Mix Långstrand, Kors	Finland	63.03	21.90	NGB	2014
BRI1	Chevalier selection dans une population de pays de Debenham	Britain	*52.23*	*1.18*	INRA	2014
BRI2	Hen Gymro Variété de pays du Pays de Galles	Britain	*52.50*	*-3.50*	INRA	2014/2015
SPN1	Cervecera De Burquete Variété de pays de Navarre	Spain	*43.00*	*-1.50*	INRA	2014/2015
GER3	Essleben, Bavaria	Germany	49.95	10.08	NSGC	2014/2015
IRE1	Donegal, Ireland	Ireland	54.92	-8.00	NSGC	2014
DEN1	Denmark	Denmark			IPK	2014
ITA1	a number of km SW of Castelvetere	Italy	41.47	14.97	IPK	2014/2015
CYP1		Cyprus				2014
DEN2	Binder Selection Dans Hanna	Denmark	*56.00*	*10.00*	INRA	2014/2015

SLO1	Kroviniaci, nordl, von Horna Suca, Biele Karpaty, Westhang	Slovakia	49.00	17.98	IPK	2014
SLO2	Matiaska, ONO von Presov	Slovakia	49.06	21.58	IPK	2014

Figure 3.1 Map of Europe showing locations of landrace origins, Red markers are 2014 field trial and Yellow are 2014/2015. Map produced using Google maps.

Each plot was planted as a tussock 0.5m square and contained on average 25 seeds spread evenly over the plot. In 2014 the trial was planted on 09/04/14 and in 2015 on 23/04/15. Fertiliser was applied at a rate of 120kg/hectare. The fertiliser was applied by hand. Fertiliser was applied as 60kg N per hectare for the full N treatment and 30kg per hectare for the low N treatment on 26/03/14 and 25/03/15 for respective years. A top up of 60kg N per hectare was applied to the normal fertiliser plots two weeks after sowing. An herbicide treatment was applied when the plants reached GS23 (Harmony 70g/ha + Oxytril 0.5L/ha + High load micra.m 1.0L/ha). Four replicate plants were chosen from the centre of each tussock to minimise edge effect and labelled using adhesive labels from a label printer (Brother P-touch GL200, Manchester) for all measurements to be taken on. Measurements were then averaged to represent each tussock.

Only the 120kg/hectare fertiliser treatment was used for analysis to examine interception efficiency (see chapter 5 for fertiliser treatment). Only a subset of genotypes planted in 2014 were planted in 2015 to reduce the number of individual measurements that were required to be taken. This allowed for a greater number of replicates to be planted and also additional measures (which will be discussed in chapter 5 of this thesis) to be undertaken. The lines which were excluded were all chosen as either they had had very poor growth (line 8.166) in 2014 or their location of origin was closely replicated by another line in the study (Figure 3.1). The analysis that follows will only include the lines which were present in both years to allow a balanced analysis and direct comparison to be made. The data for the additional lines can be found in Appendix 2.

3.2.3 Canopy Development

3.2.3.1 Growth Stage and Leaf Chlorophyll Content

The Growth Stage (GS) of the plants was recorded every Monday throughout the growth season and was assessed using the HGCA (AHDB) growth stage guide (HGCA (The Scottish Executive), 2006). The chlorophyll content of the uppermost leaf excluding the flag leaf on the main shoot was measured on a weekly basis. Chlorophyll content was estimated by using a SPAD meter (Minolta corps, Ramsey, NJ) at the centre point of the leaf blade.

3.2.4 Light Interception

3.2.4.1 Leaf Length and Number of Leaves

Leaf length was measured with a standard 30cm ruler (Stephens Publishing, Sandusky, OH) in cm from the tip of the leaf to the point where the leaf blade meets the sheath. The leaf length was measured at GS39 and GS59. The numbers of leaves on the main shoot were counted. The number of leaves was measured at GS24, 39 and 59.

3.2.4.2 Leaf Area and Dry Weight

Leaf area was measured by detaching the leaves and immediately passing them through a leaf area meter (Li-3100 are meter, LiCor Inc., Lincoln, NE) to minimise wilting and breakdown which calculated leaf area in cm^2. Leaves were passed through the meter three times and the readings averaged.

The leaves that had been used for leaf area measures were then placed in individual paper bags and dried in an oven (Ecocell, MMM Medcenter, Munich, Germany) at 80^oC for 48 hours. The leaves were then weighed using a precision balance (Kern PLJ, D-72336, Kern & Sohn Gontbl, Balingen, Germany) in grams.

The specific leaf area (SLA) was calculated using the leaf area and leaf dry weight measures as leaf area divided by leaf dry weight. The dried leaves were then put aside for isotope analysis. The leaf area, dry weight and SLA were all measured at GS39.

3.2.4.3 Leaf Angle

The leaf angle was measured between the second leaf and the main shoot as the angle on the upper side of the leaf. A Helix Oxford protractor (Maped Helix, West Midlands, UK) was held against the stem above the leaf and the angle noted to the nearest 5^0. Care was taken to avoid bending the leaf away from the stem and handling prior to this measure being taken was minimised by ensuring that this was always the first measure taken on the day. The leaf angle was measured at GS39 and 59.

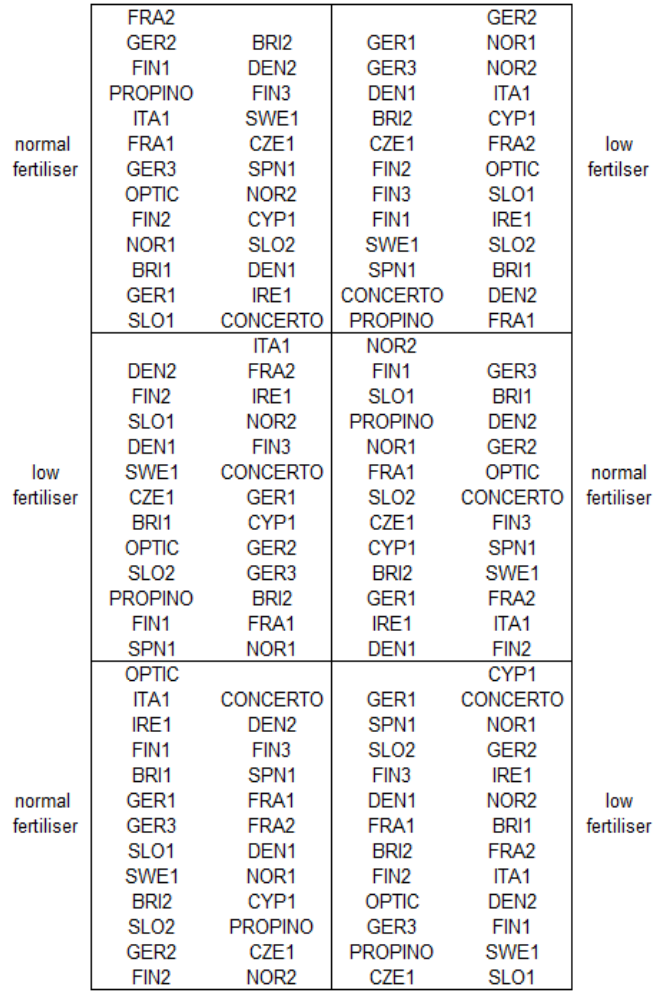

Figure 3.2 Field trial layout in (a) 2014 and (b) 2015. The trial was a split-plot with the subplot fully randomised. Variation among landraces under 120kg/hectare fertiliser plots are reported herein, whilst response to reduced fertiliser is reported in chapter 5.

3.2.4.4 Climate Study

The climate in the location for each of the landraces was looked at as a possible factor influencing the canopy structure. Climate data were obtained from the national meteorological offices in each country of origin (Table 3.2, Appendix 1). The weather data region scale varies between countries from local weather data to regional data depending on the scale of reporting. It was always taken as the closest reported point to the latitude and longitude of origin of the landraces. The climatic variables reported are the total rainfall (mm) for spring/summer, the total number of sunlight hours for spring/summer and the average daily temperature (^{o}C) for spring/summer. These are long term averages but the numbers of years the averages cover vary between countries as reporting differs in the official statistics. The data are long-term averages with FRA1, FIN1, BRI1 and SPN1 being from 1981-2010. GER2, NOR1, NOR2, CZE1 and GER3 and from 1961-1991 and ITA1 is from 1971-2000.

3.2.4.5 Statistical Analysis

Analysis of variance (ANOVA) was carried out in order to check for significant variation between the lines. Tukey's post hoc test was used to identify individual differences between the lines. Regression analysis of climate and latitude with each measure of canopy structure was carried out to see if there was a relationship with each trait and the local conditions at their place of origin. Year of field trial was included as a factor to assess if patterns seen were consistent over both years of the trial or whether environmental conditions in the field trial had an effect on the traits. All statistical analysis was carried out using GenStat 16th Edition. A principle component analysis was run on the canopy structure light interception traits using Minitab 17 to examine relationships between the traits and to examine the canopy structure as a whole.

Table 3.2 Climatic data from the location of origin of the landraces. Rainfall and sunlight are cumulative totals for one year's growth season. The average temperature is the average daily temperature over the growing season. The data are long-term averages with FRA1, FIN1, BRI1 and SPN1 being from 1981-2010. GER2, NOR1, NOR2, CZE1 and GER3 and from 1961-1991 and ITA1 is from 1971-2000.

Landrace Number	Data Location	Rainfall (mm)	Sunlight (hours)	Average Temperature (^{o}C)
FRA1	Grenoble	321	946	10
GER2	Bayern	225	890	13
NOR1	Central Norway	189	771	9
NOR2	Northern Norway	248	689	8
CZE1	Doksany	225	850	14
FIN1	North	222	2257	5
BRI2	Wales	215	800	16
SPN1	Pamplona Aeropuerto	213	991	16
GER3	Bayern	220	885	13
ITA1	Campobasso	100	1069	25
DEN2	Jutland	1612	886	13

3.3 Results

3.3.1 Canopy Development

3.3.1.1 Growth Rate

There was no significant difference in time between sowing and GS24 (growth stage) between the landraces ($p=0.387$, $F_{10,63}=1.09$) (Table 3.3). There was significant variation between the landraces in the number of days spent between GS24 and reaching GS39 ($p<0.001$, $F_{10,63}=7.43$)(Table 3.3,Figure 3.3) ranging from 12-20 days with those lines from northern latitudes taking a shorter time with NOR2, FIN1, NOR1 and GER3 being significantly different from SPN1, FRA1, GER2, CZE1 and BRI2. There was significant variation between the landraces in the number of days spent between GS39 and reaching GS59 ($p=0.001$, $F_{10,63}=3.45$)(Table 3.3,Figure 3.3) ranging from 5-14 days with NOR1 being significantly different from every line apart from GER3 and SPN1.

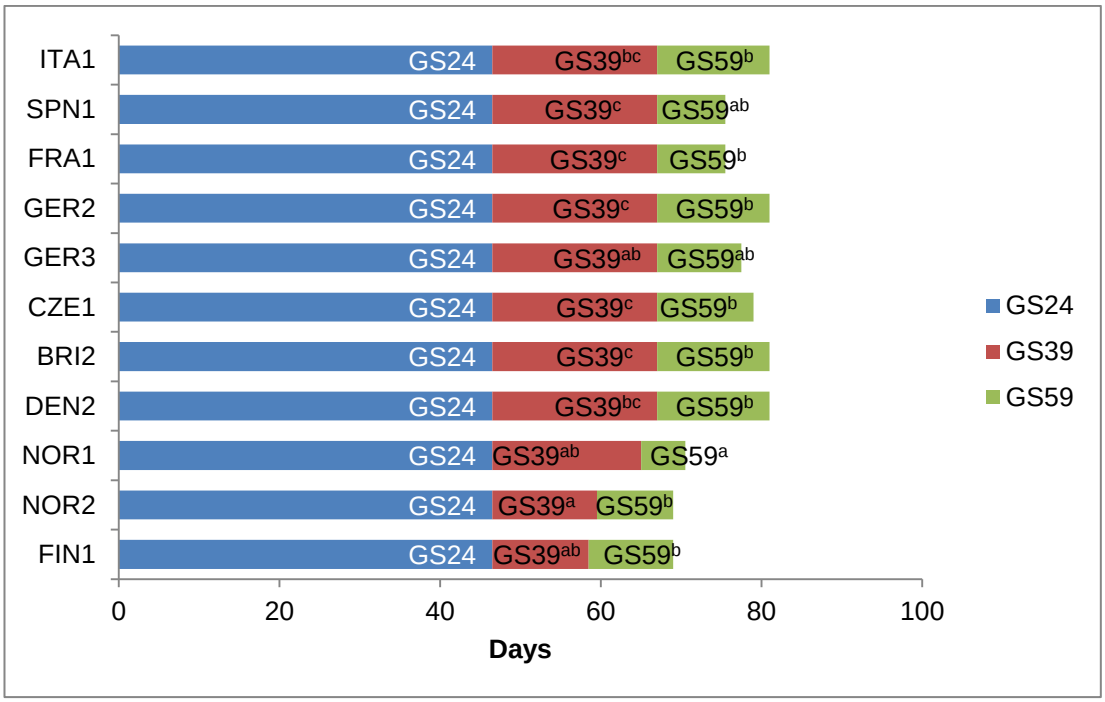

Figure 3.3. Time progression of days spent reaching each Growth Stage. Blue is emergence to GS24, red is GS24 to GS39 and green is GS39 to GS59. Landraces are listed from North to South, bottom to top respectively. Lines significantly different from each other are indicated by letters, lines which do not share a letter are different from each other. There were no significant differences between lines up until GS24. Growth Stage assessments were taken on the plants grown in the field trials at Boghall farm and the Growth Stage was assessed using the HGCA growth stage guide.

Table 3.3. (a) Time between key growth stages in days from GS24 to GS39 and GS39 to GS59. The range represents the minimum and maximum of landrace means. (b) Relationships between length of time spent in growth phases and climatic variables, indicating the overall (linear regression) and year effects.

(a)		**Range of**		**(b)**							
Growth Phase		**Means number of days**	**ANOVA**	**Latitude (degrees)**		**Precipitation (mm)**		**Hour of Sunlight**		**Temperature (^{o}C)**	
		Days		Regression	Year	Regression	Year	Regression	Year	Regression	Year
GS24	**to**	12-20	p<0.001	p<0.001, $t_{1,20}$=38.60, R^2=0.64 negative regression	p<0.001	p=0.508, $t_{1,20}$=0.45	p0.850	p=0.007, $t_{1,20}$=8.93, R^2=0.27 negative regression	p=0.822	p=0.001, $t_{1,20}$=14.71, R^2=0.39 positive regression	p=0.805
GS39	**to**	5-14	p=0.001	p=0.335, $t_{1,20}$=0.98	p=0.072	p=0.314, $t_{1,20}$=1.07	p=0.071	p=0.979, $t_{1,20}$=0.0	p=0.079	p=0.081, $t_{1,20}$=3.37, R^2=0.10 positive regression	p=0.056
GS59											

3.3.1.2 Leaf chlorophyll content

There was a significant difference in SPAD readings between the lines at GS24 ($p=0.013$, $F_{11,69}=2.51$), GS39 ($p<0.001$, $F_{11,69}=3.71$) and GS59 ($p=0.030$, $F_{11,69}=2.12$) (Table 3.4, Figure 3.4) with SPAD values ranging from 32.5-43.4, 35.2-45.4 and 41.1-48.7 respectively. At GS24 line NOR2 was significantly different from lines FIN1 and BRI1. At GS39 line ITA1 was significantly different from lines GER2, CZE1, FIN1, BRI2, NOR1, DEN2 and FRA1 and line FRA1 was also significantly different from lines GER3 and SPN1. There was no correlation between climate and latitude and leaf chlorophyll content although there was not a consistent response between the years of the field trial indicating an environmental factor. At GS24 the direction of the regression was different with latitude, precipitation and temperature. At GS39 the regression direction was different with precipitation and temperature and at GS59 there was a stronger positive regression with hours of sunlight.

3.3.2 Light Interception

3.3.2.1 Leaf Angle

The angle of the 2nd leaf in the upper canopy layer showed significant variation between the lines at both GS39 ($p<0.001$, $F_{11,69}=3.09$) and GS59 ($p<0.001$, $F_{11,69}=14.28$)(Table 3.5) with the angles from vertical ranging from 18-45 degrees and 31-84 degrees respectively. At GS39 lines CZE1 and ITA1 were significantly different from lines NOR1, FIN1 and NOR2. Line NOR2 was also significantly different from lines BRI2, GER2, DEN2, GER3 and FRA1. At GS59 lines BRI1, ITA1 and CZE1 were significantly different from all lines apart from GER2 and SPN1. Line FIN1 was significantly different from all lines apart from DEN2 and NOR2. When the angle of the leaf was regressed against average precipitation, sunlight hours, temperature and latitude there was a significant positive regression at both GS39 ($p=0.003$, $t_{1,20}=11.62$, $R^2=0.33$) and GS59 ($p<0.001$, $t_{1,20}=17.89$, $R^2=0.44$) with latitude and a significant negative regression at both GS39 ($p=0.002$, $t_{1,20}=12.31$, $R^2=0.07$) and GS59 ($p<0.001$, $t_{1,20}=28.47$, $R^2=0.56$) with temperature (Table 3.5, Figure 3.5). When year was included as a factor there was seen to be no difference in leaf angle between the years of the field trial.

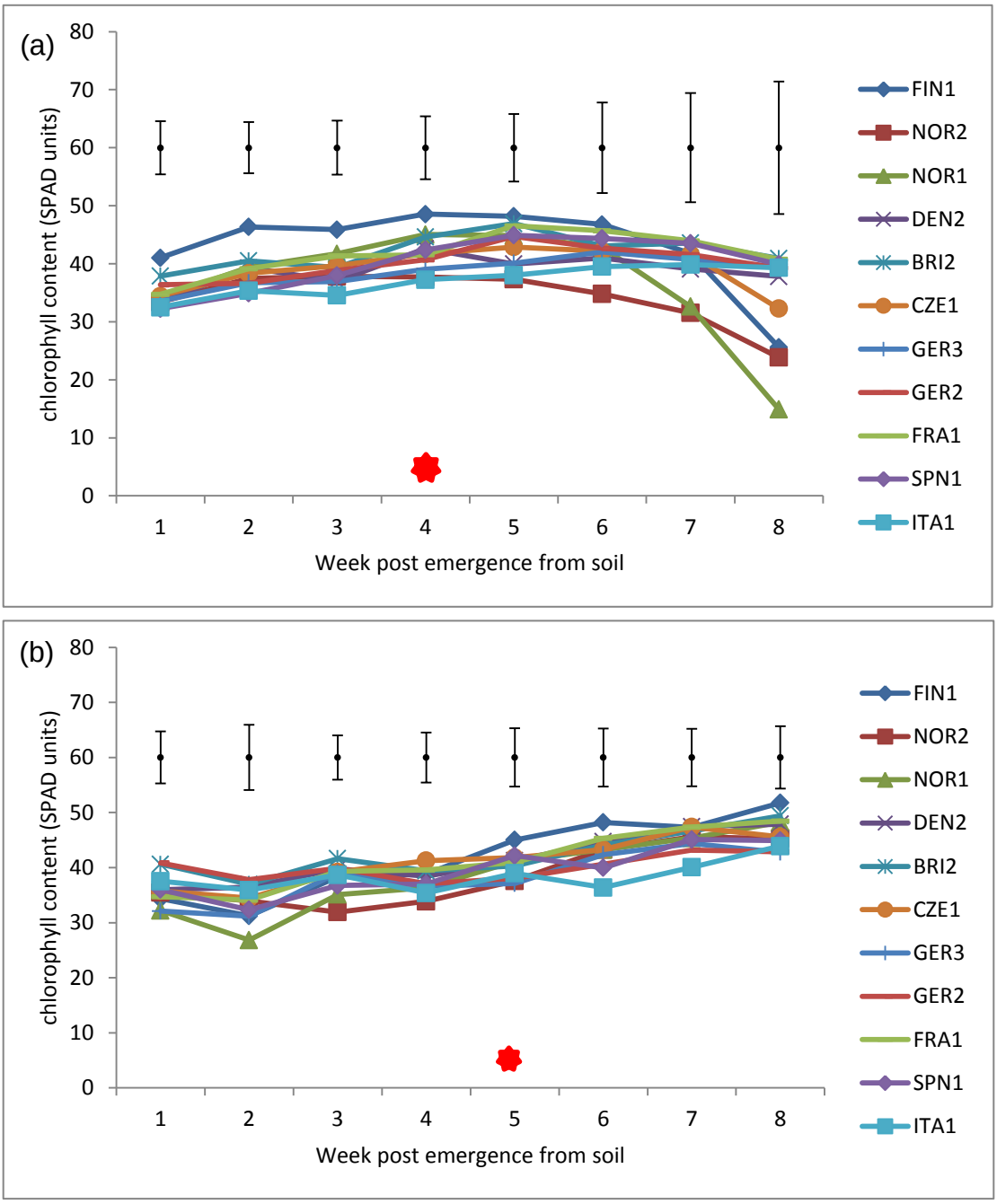

Figure 3.4 Chlorophyll content of the leaves measured each week post emergence of the plant from the soil. The chlorophyll content was measured on the uppermost leaf excluding the flag leaf throughout meaning that only after GS39 where the flag leaf was fully emerged was the same leaf tracked. This occurred as indicated on the graph by *. Each point on the figure is the average of the readings taken for one line at each time-point which was three replicates for 2014 and four replicates for 2015. The bars at the top of the graphs are the LSD bars for each time point. (a) 2014 field trial. (b) 2015 field trial. In legend the landraces are listed North to South.

Table 3.4 (a) Chlorophyll content of second leaf at key growth stages 24, 39 and 59. The range represents the minimum and maximum mean chlorophyll contents seen in the landrace lines. (b) Relationships between chlorophyll content and climatic variables indicating the overall (linear regression) and year effects.

(a)			**(b)**							
Growth Stage	**Range of Means of chlorophyll content (SPAD units)**	**ANOVA**	**Latitude (degrees)**		**Precipitation (mm)**		**Hours of Sunlight**		**Temperature (^{o}C)**	
			Regression	**Year**	**Regression**	**Year**	**Regression**	**Year**	**Regression**	**Year**
GS24	32.5 - 43.4	p=0.013 $F_{11,69}$=2.51	p=0.935, $t_{1,20}$=0.01	p=0.010	p=0.932, $t_{1,20}$=0.01	p=0.010	p=0.113, $t_{1,20}$=0.03	p=0.005	p=0.864, $t_{1,20}$=0.03	p=0.010
GS39	35.2 – 45.4	p<0.001 $F_{11,69}$=3.71	p=0.633, $t_{1,20}$=0.24	p<0.001	p=0.741, $t_{1,20}$=0.11	p<0.001	p=0.551, $t_{1,20}$=0.37	p<0.001	p=0.989, $t_{1,20}$=0.00	p<0.001
GS59	41.1 – 48.7	p=0.030 $F=_{11,69}$=2.12	p=0.519, $t_{1,20}$=0.43	p=0.064	p=0.690, $t_{1,20}$=0.16	p=0.066	p=0.066, $t_{1,20}$=3.78, R^2=0.11	p=0.044	p=0.126, $t_{1,20}$=2.55, R^2=0.06	p=0.051

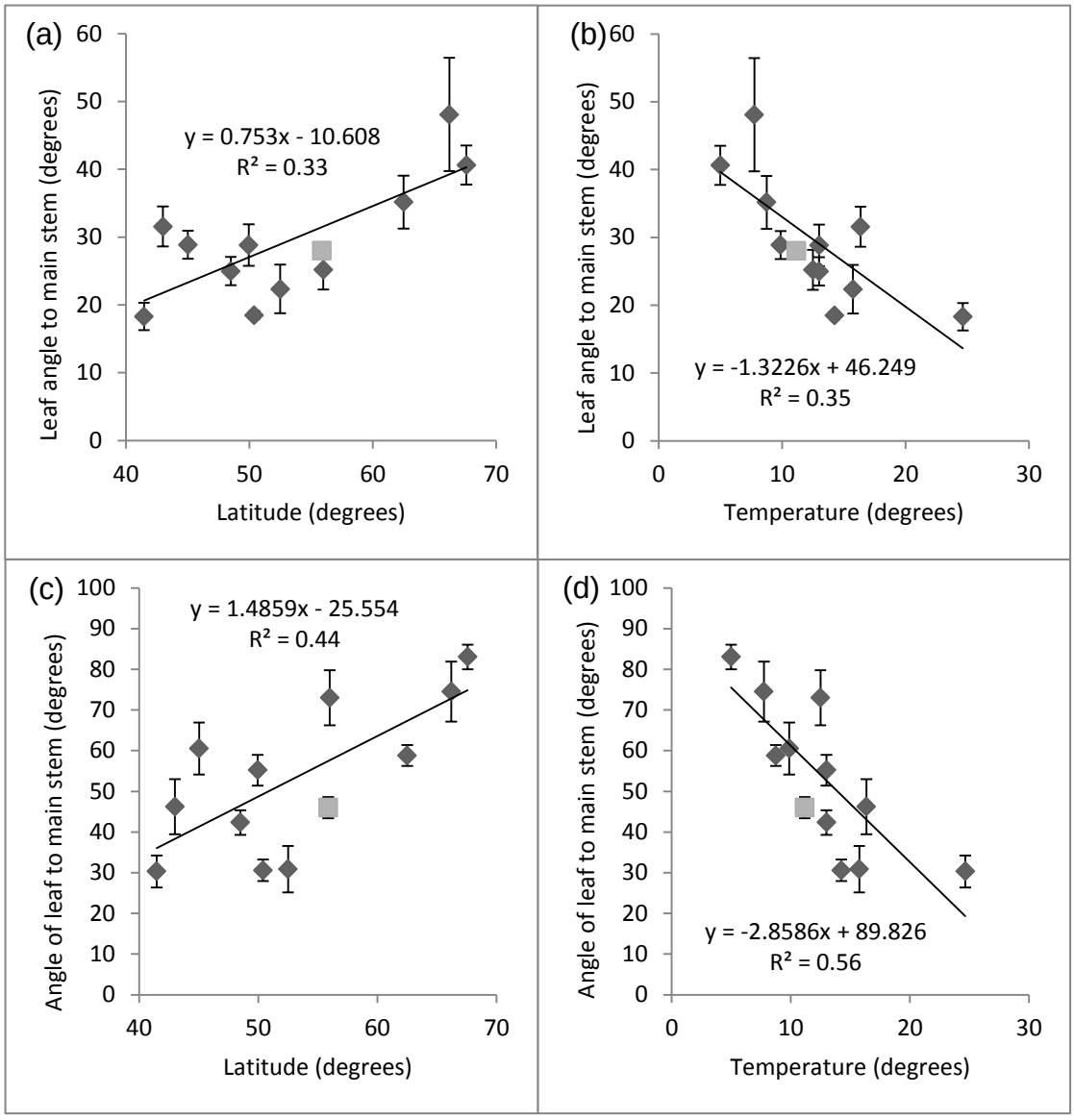

Figure 3.5 Linear regression of leaf angle of the main shoot against climate. The leaf angle was measured on the uppermost leaf excluding the flag leaf on the main shoot of each plant in the field trial at Boghall Farm. The latitude and temperature and those at the location of original collection for each of the landrace lines. Grey diamonds are the landraces and the grey square is Concerto for reference. Each point represents a different landrace. Error bars are the standard error. The solid grey line is the regression lines and the regression equation is given. (a) GS39 angle against latitude. (b) GS39 angle against temperature. (c) GS59 angle against latitude. (d) GS59 angle against temperature.

Table 3.5 (a) Canopy structure traits of the landraces at each growth stage. The range represents the minimum and maximum means of the landraces for each trait. (b) Relationships between canopy traits and climatic variables, indicating the overall (linear regression) and year effects.

(a)Canopy Trait	Growth Stage	Range Means	ANOVA	(b)Latitude (degrees)		Precipitation (mm)		Hour of Sunlight		Temperature (^oC)	
				Regression	Year	Regression	Year	Regression	Year	Regression	Year
No leaves	GS24	3	p=0.870, $F_{11,69}$=0.54	p=0.435, $t_{1,20}$=0.63	p<0.001	p=0.780, $t_{1,20}$=0.08	p<0.001	p=0.904, $t_{1,20}$=0.02	p<0.001	p=0.351, $t_{1,20}$=0.91	p<0.001
	GS39	4-5	p=0.002, $F_{11,69}$=3.09	p=0.098, $t_{1,20}$=3.02, R^2=0.08	p=0.136	p=0.808, $t_{1,20}$=0.06	p=0.166	p=0.380, $t_{1,20}$=0.80	p=0.158	p=0.013, $t_{1,20}$=7.45, R^2=0.23	p=0.102
Leaf Angle (degrees)	GS39	18-45	p<0.001, $F_{11,69}$=6.24	p=0.003, $t_{1,20}$=11.62, R^2=0.33	p=0.077	p=0.702, $t_{1,20}$=0.15	p=0.164	p=0.339, $t_{1,20}$=0.96	p=0.155	p=0.002, $t_{1,20}$=12.31, R^2=0.35	p=0.073
	GS59	31-84	p<0.001, $F_{11,69}$=14.28	p<0.001, $t_{1,20}$=17.89, R^2=0.44	p=0.980	p=0.081, $t_{1,20}$=3.38, R^2=0.10	p=0.984	p=0.059, $t_{1,20}$=4.0, R^2=0.12	p=0.984	p<0.001, $t_{1,20}$=28.47, R^2=0.56	p=0.977
Leaf Length (mm)	GS39	23.2-30.3	p<0.001, $F_{11,69}$=3.58	p=0.368, $t_{1,20}$=0.85	p<0.001	p=0.843, $t_{1,20}$=0.04	p<0.001	p=0.510, $t_{1,20}$=0.45	p<0.001	p=0.773, $t_{1,20}$=0.09	p<0.001
	GS59	20.8-29.7	p<0.001, $F_{11,69}$=5.28	p=0.851, $t_{1,20}$=0.04	p=0.004	p=0.969, $t_{1,20}$=0.00	p=0.004	p=0.134, $t_{1,20}$=2.44, R^2=0.06	p=0.002	p=0.480, $t_{1,20}$=0.52	p=0.003
SLA (cm^2/g)	GS39	180.35-210.79	p=0.718, $F_{10,69}$=0.70	p=0.973, $t_{1,20}$=0.00	p<0.001	p=0.843, $t_{1,20}$=0.04	p<0.001	p=0.670, $t_{1,20}$=0.19	p<0.001	p=0.574, $t_{1,20}$=0.33	p<0.001

3.3.2.2 Number of Leaves

The number of leaves on the main shoot showed variation between the lines at GS39 ($p=0.002$, $F_{11,69}=3.09$) with between 4 and 5 leaves and when the number of leaves was regressed against latitude and climate a positive regression with temperature was seen ($p=0.013$, $t_{1,20}=7.45$, $R^2=0.23$) (Figure 3.6,Table 3.5). Line NOR2 was significantly different from ITA1 and BRI2 and lined FRA1 and FIN1 were also different to BRI2. When year was included as a factor there was no difference between the years at GS39 however there was a different response between the years at GS24.

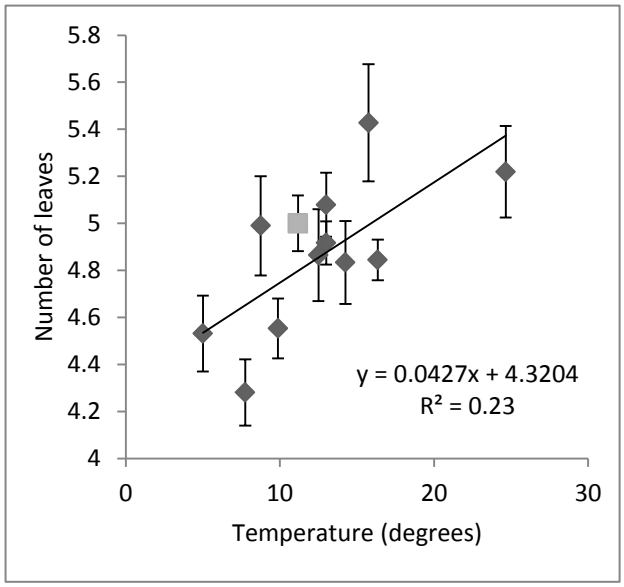

Figure 3.6 Linear regression of the number of leaves on the main stem with temperature. The number of leaves were measured on the main shoot of each plant in the field trial at Boghall Farm. The temperatures were those at the location of original collection for each of the landrace lines. Grey diamonds are the landraces and the grey square is Concerto for reference. Each point represents a different landrace. Error bars are the standard error. Grey solid line is the regression line and the regression equation is shown.

3.3.2.3 Leaf Length

The length of the second leaf showed significant differences between the landraces at both GS39 ($p<0.001$, $F_{11,69}=3.58$) and GS59 ($p<0.001$, $F_{3,11,69}=5.28$) (Table 3.5) with leaf length between 23.2-30.3 ad 20.8 ad 29.7cm respectively. At GS39 lines FIN1 and ITA1 were significantly different from line GER2. AT GS59 line ITA1 was significantly different to lines NOR1, SPN1, CZE1, BRI1, GER3 and GER2 with line GER2 also being significantly different to line FIN1.

3.3.2.4 SLA

There was no significant variation between the landraces in SLA. There was also no correlation of specific leaf area with climate or latitude.

3.3.2.5 Principle Component Analysis

The first three components of the principle component (PC) analysis described 82% of the variation. The first component accounted for 42% of the variation, the second 25% and the third 15%. The canopy traits chlorophyll content at GS39, leaf length at GS39 and number of leaves at GS24 correlated most with PC1. The number of leaves at GS39 and the leaf angle at both GS39 and 59 correlated with PC2. The chlorophyll content at GS24 and 59 and the leaf length and GS59 correlated with PC3.

Figure 3.7 shows the relationships between the variables and the components and if variables are pointing in the same direction then there may be a relationship between them. The chlorophyll content of the leaves is inversely related to the number of leaves with plants with higher chlorophyll contents having lower number of leaves (Figure 3.7, Figure 3.8, Table 3.6). The chlorophyll content is also inversely related to the leaf length at GS39 with longer leaves having lower chlorophyll contents. Figure 3.8 shows the individual lines plotted in respect to components 1 and 2 and it is seen that the lines split along component 1 into two main groups based on year of field trial. Component one is contributed towards by the leaf length and the chlorophyll content and may indicate differences in leaf size between years. Along component two the Scandinavian lines including FIN1 and NOR2 are higher than the lines from central and southern Europe and component two is contributed to by the leaf angle.

Table 3.6 Principle component analysis of canopy leaf traits at significant growth stages 24, 39 and 59. Correlations of the canopy parameters (chlorophyll content, leaf length, number of leaves and leaf angle) with the first three components.

Canopy Trait	Growth Stage	PC1	PC2	PC3
Leaf chlorophyll	24	-0.335	-0.035	0.531
content (SPAD	39	-0.441	-0.018	0.122
units)	59	-0.291	0.024	0.556
Leaf length (cm)	39	0.415	-0.200	0.377
	59	0.375	-0.117	0.440
Number of leaves	24	0.474	0.086	0.163
	39	0.126	-0.539	-0.164
Leaf angle	39	0.232	0.543	0.072
(degrees)	59	0.026	0.592	-0.012

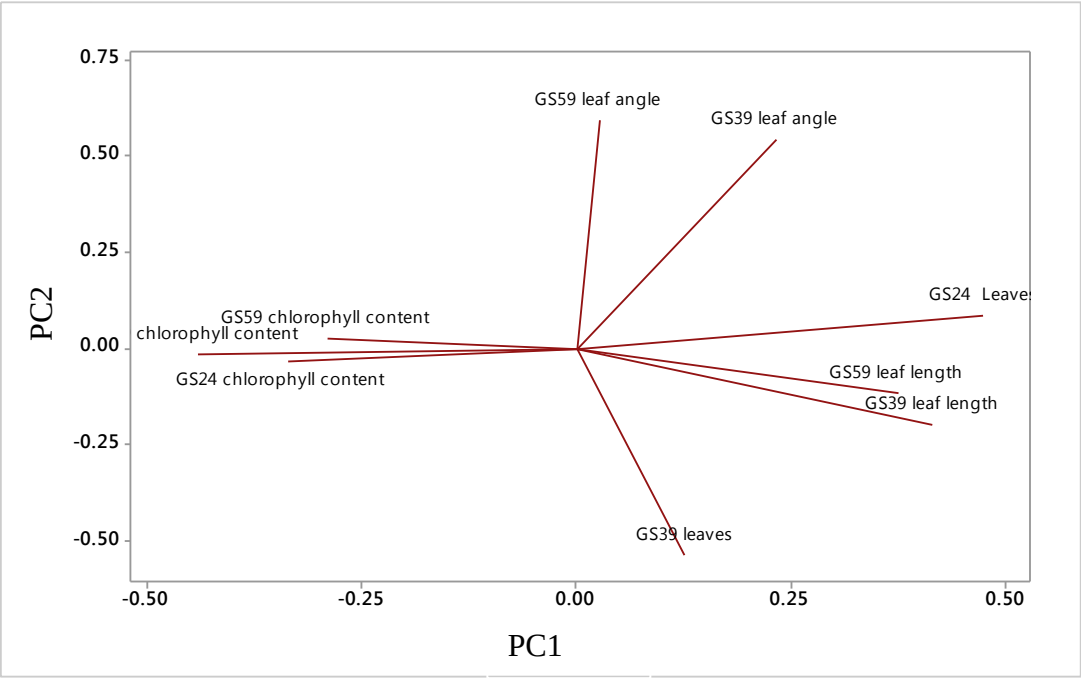

Figure 3.7 Loading plot of canopy variables plotted in regard to relationships with components 1 and 2. The canopy variables included are the chlorophyll content (SPAD units), leaf length (cm), number of leaves and the leaf angle (degrees).

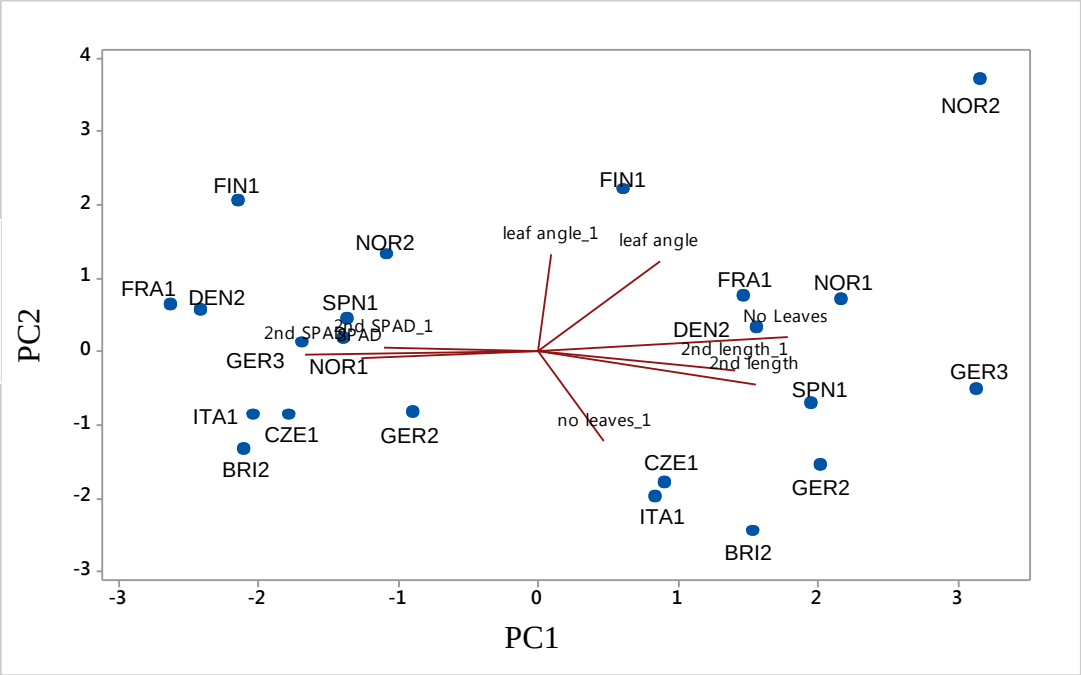

Figure 3.8 Biplot of canopy variables plotted in regard to relationships with components 1 and 2 of the principle component analysis. The canopy variables included are the chlorophyll content (SPAD units), leaf length (cm), number of leaves and the leaf angle (degrees).

3.4 Discussion

3.4.1 Canopy Development and Chlorophyll Content

The speed at which the crop canopy is fully developed and the duration of canopy greenness both have a huge impact on the light interception efficiency over the growth season allowing the maximal time for light capture.

The selection of landraces from this study showed that the duration of canopy emergence (up to GS24) was the same for all lines. Once stem extension and rapid canopy expansion began, the three Scandinavian landraces, NOR1, NOR2 and FIN1, went through the stem extension stage and reached GS39 quicker than the rest of the lines from Central and Southern Europe (Figure 3.3). The growth season in Scandinavia is shorter than in Southern latitudes so it would be advantageous for the plants to reach full canopy expansion as early as possible to take advantage of as much of the growing season as they can. It will also be an advantage in out-competing weeds and other plants to reach full canopy expansion thus shading out competitors (Sim et al. 2007; Kruk et al. 2006).

Maintaining canopy greenness could improve photosynthetic efficiency and perhaps yield by increasing the amount of time before the leaves begin to senesce. It was observed in the 2014 field trial that the landraces from Scandinavia began to decrease chlorophyll content before the lines from the rest of Europe once the same leaf was being tracked throughout. This could possibly be because the lines from Scandinavia, NOR1, NOR2, FIN1, are locally adapted to a shorter growth season. This was not seen in the 2015 field trial but it is believed that because of the late planting and unfavourable weather conditions that the growth season was compressed which means that differences would not be discernible (Figure 3.4). Compared to modern cultivars, landraces senesce more quickly once they have reached grain filling which suggests a potential negative side effect in using landraces in breeding to ensure that canopy greenness is maintained or at least established early (Gaju *et al.*, 2016). The genetic control behind senescence is beginning to be understood with QTLs identified (Emebiri, 2013) with physiological mechanisms including enzyme function understood (Avila-Ospina *et al.*, 2015) in barley which could give further

clues about the mechanisms behind senescence (Zheng *et al.*, 2009) as there could be strong correlations with stay-green traits and yield.

In terms of future breeding for an extended canopy length, the lines from Scandinavia seem promising in allowing early canopy establishment making the most of early season light. This could be especially useful with regards to the trend for sowing to be delayed until later in the season. Care would need to be taken however as the Scandinavian landraces senesce early so breeding would have to combine the early canopy establishment traits with delayed senescence from elsewhere.

The chlorophyll content of the leaves and how this is distributed over the leaf surface is important in the capture and interception of light. Greater amounts of chlorophyll combined with increased leaf surface area will allow the improved capture of light (Giunta *et al.*, 2002). If the leaves are too thick light will not be able to penetrate through to deeper cell layers so will only be captured on the surface. This study looked at the amounts of chlorophyll held within the canopy in the three different growth phases of the plant. At the early growth stage in the leaf emergence phase of development there were differences in chlorophyll content between the lines with different lines showing higher chlorophyll content in different years (Table 3.4). This may be a result of the drastically different weather conditions that were experienced between the two years of the field trial and it would be interesting to see how the chlorophyll content responded to stress in each of the lines (see chapter 5 for nutrient stress). There was no link with latitude or climate in terms of chlorophyll content. It has been seen that in modern lines there is a high level of variation in chlorophyll content between lines but that the environmental component of this is low (Giunta *et al.*, 2002). This is in contrast to what was found in this study where a lot of variation was seen between the years of the field trail. At GS39 and GS59 there were no differences between the lines in terms of chlorophyll content and there was no regression with latitude or climate in most years (Table 3.4). It must be noted that the leaf upon which SPAD readings were taken at GS24 was not the same leaf as GS39 and 59.

3.4.2 Light Interception and Canopy Structure

The angle at which plants hold their leaves is important in allowing light to penetrate through to the lower canopy layers and avoid saturation of the upper leaf layers. The angles in which upper canopy layers are held were measured as the angle of the leaf to the main stem. At both GS39 and GS59 there were significant differences between the landrace lines in terms of leaf angle (Table 3.5).When this was regressed with latitude and climate there was a positive regression of leaf angle against latitude and a negative regression with the average temperature over the growing season at the location of the landraces original collection (Table 3.5, Figure 3.5). This means that the further North that the landraces originate from the more planophile the canopy structure is with leaves following a horizontal trend relative to the stem. This may be because of the lower angle of the sun in the sky and it would be interesting to track the angle of the leaves over the course of a day to see if it changes. There were no significant regressions with rainfall or sunlight over the growing season at the location of original landrace collection against leaf angle. As the leaf angle regresses negatively with temperature it may be that in Southern European latitudes an erectophile canopy structure is beneficial in dealing with heat stress caused by higher temperatures whilst in Northern latitudes there is not so much pressure caused by heat and having a planophile canopy is an advantage in out-competing your neighbours. An old study of barley has shown that the average daily temperature had the largest effect on radiation-use-efficiency out of all the variables they measured (Goyne *et al.*, 1993). Interestingly when Concerto is plotted on the graph with the landraces (with central Scottish latitude and climate) for reference it follows the regression lines.

In terms of indications for local adaptation, temperature has been indicated as one of the major selective forces in population structure of wild barley (Nevo & Beharav, 2005; Hübner *et al.*, 2009, 2013). When genetic structure was analysed using simple sequence repeats (SSRs), temperature explained a significant amount of variation in the genetic structure of emmer wheat (Ren *et al.*, 2013). In cultivated European barley landraces it has been observed that local adaptation exists in response to climate factors, particularly temperature, along with day length adaptiveness although it is not clear whether this is a result of the origin of the landrace

populations or evolution to the environments in which they are grown (Jones *et al.*, 2011). Understanding how temperature affects the crop and how landraces have adapted to cope with local temperatures could be key to breeding varieties which are locally adapted to conditions or breeding new varieties that will be able to cope with a warming climate.

The leaf angles in this study were measured at the individual leaf level and this may be misleading if inferring how an entire plant canopy intercepts light. Leaves may behave differently when in a canopy situation with competition, shading from neighbouring plants, sowing density and management practises being additional factors. It may be better to look at how the canopy as a whole intercepts light by examining how much light is penetrating the canopy though the different leaf layers and indeed light interception at the individual leaf level is very rarely looked at. A study of maize showed that planting density had an impact on SLA and Leaf Area Index (leaf area per unit of ground surface area) (Amanullah *et al.*, 2007) and it may be that different canopy structures will lend themselves to different planting densities. It has been shown in rice that erect canopy structures improves light capture and leaf area index in dense canopies (Sinclair & Sheehy, 1999).

Another aspect of the canopy structure that is a factor in how light is intercepted is the size and shape of the leaves which is made up of the leaf length, leaf area and the SLA. In the landraces, there were differences between the lines in regard to leaf length at both GS39 and 59 but there was no relationship with latitude or climate (Table 3.5). There was a significant effect of year on the leaf length which could be explained by the different weather conditions experienced between the years of the field trail. In 2015 there was a lot more rainfall and a lower temperature than in 2014 which could have affected the growth of the leaves. It could also be an effect of the shorter growth season in 2015 due to delayed planting which may have resulted in a shorter time available for leaf growth. When the whole biomass of the leaves is taken into account using the SLA there were no differences between the lines which suggests that lines with longer leaves are thinner and *vice versa* (Table 3.5). This is supported by research in barley which showed no differences in SLA between cultivars (Giunta *et al.*, 2002) which was in contrast to their findings in wheat and

triticale which showed variation in SLA as a part of a wider study to examine the relationship of chlorophyll content with leaf parameters. Selection for larger leaves through breeding has been carried out in wheat and it was accompanied by an increase in leaf surface area and biomass (Zhang *et al.*, 2015), if a similar approach could be applied in barley then the canopy cover could be increased providing more area for light interception. The allocation of the leaf area to different cell types and the layout of stomata over the leaf surface are other important factors of leaf structure (de Boer *et al.*, 2016) and stomatal density will be looked at in chapter 4 of this thesis.

A multivariate analysis was carried out including the chlorophyll content (SPAD units), the leaf length (cm), the number of leaves and the leaf angle (degrees) across three growth stages (GS24, 39 and 59) in order to examine relationships between the different factors that make up the canopy structure. It was seen that the first three components accounted for most of the variation. There were some relationships between the different variables but the trends were not consistent across the growth stages. At GS39 the number of leaves and the leaf angle were negatively related to each other with the more horizontal the leaf angle the lower number of leaves in the canopy (Figure 3.7). This makes sense as the lower leaf layers will be shaded so will not be being utilised fully in photosynthesis so it would be wasteful to put too much energy into producing more leaves. It has been seen in wheat that there were differences in leaf area and leaf chlorophyll content between different leaf layers (Li *et al.*, 2015b) but this study saw an increase in leaf area in lower canopy layers. This makes sense as smaller upper leaves would allow the light to penetrate through to lower leaf layer. The chlorophyll content was higher in upper leaf layers.

3.4.3 Conclusions

- Significant variation was seen in the amount of time spent in the stem extension phase between GS24 and GS39 in the landraces with the Scandinavian landraces reaching ear emergence first.
- Significant variation was seen in the leaf SPAD readings between the landraces.

- There was a significant regression seen between the angle in which the uppermost leaf (excluding the flag leaf) was held in relation to the stem with latitude of origin and average growing season temperature at location of original collection at both GS39 and GS59. Landraces from Northern latitudes had a more planophile leaf arrangement than those from Southern latitudes.

4. Gas Exchange Efficiency of Spring Barley Landraces

4.1 Introduction

Crop yield increases are thought to be reaching a limit with increases in arable area and Harvest Index (HI). An example of this is rice production in China, India and Indonesia where between 1970 and 1980 yields increased by 36% but this fast growth has decreased to yield increases of 7% between 2000 and 2010 (Long & Ort, 2010). Other parameters, beside HI, of the Monteith Equation (Monteith & Moss, 1977) (see Equation 1) are now being focussed on for improvement which may lead to increased yields. This includes the ε_c which is the efficiency of the plant in converting energy captured into biomass.

The ε_c of radiation into biomass is a target for improving crop yield and this part of the study will examine how variation in ε_c might contribute to future crop improvement. There may be inherent variation in photosynthetic activity in spring barley (*Hordeum vulgare* ssp. *vulgare*) which may provide room for improvement through breeding. Landraces may contain natural variation in rates of photosynthesis that may be useful in future breeding.

4.1.1 Gas Exchange

The maximum rate of photosynthesis per unit leaf area (A_{max}) is an important measure of photosynthetic efficiency. The higher the rate of A_{max} the more CO_2 is being absorbed by the plant. This must, however be balanced with water loss though transpiration (Lawson & Blatt, 2014). As the photosynthetic rate of plants has not been targeted for improvement during crop breeding it is possible that the A_{max} of crops will have remained unchanged with little variation, there may have been a decrease in A_{max} with breeding over time or there may be high levels of variation between cultivars. Australian wheat cultivars were found to have increased photosynthetic rates over the last century (Watanabe *et al.*, 1994) which were accompanied by increased chlorophyll and leaf nitrogen contents. In contrast a study of British grown wheat cultivars introduced after 1975 showed a high level of natural variation in photosynthetic capacity but no link between high photosynthetic rate and yield (Driever *et al.*, 2014). They showed that A_{max} appeared to be lower in more

recently introduced cultivars. Another study in Australian wheat supports this showing no relationship with photosynthetic rate and year of cultivar release (Sadras *et al.*, 2012). Modelling has suggested that increasing photosynthesis could results in increases in plant biomass (Zhu *et al.*, 2007).

As most modern European cultivars are derived from a relatively small pool of parent genotypes it may be necessary to look to older material such as landraces to obtain sufficient variation to increase photosynthetic rates. A study in wheat however, found that cultivars had higher rates of A_{max} than landraces under modern high nitrogen conditions (Gaju *et al.*, 2016) but with pressure to reduce fertiliser inputs in the future landraces may be a useful source of higher photosynthetic rates under lower nitrogen inputs. Unfortunately, this study does not convey their findings under low nitrogen conditions.

Detailed measures of gas exchange and photosynthetic rate are difficult to get in field conditions where large numbers of plants are required to be looked at. It can be impractical to take the required equipment out into field conditions and as measurements can take a long time comparing readings directly can lead to problems in interpretation. Chlorophyll fluorescence is one method that is commonly used to examine the efficiency of photosystem II (PSII) and can be easily done on large numbers of plants in the field. Light energy which is absorbed into the leaf can either be used in photosynthesis, dissipated as heat or emitted as light (chlorophyll fluorescence) (Maxwell & Johnson, 2000). Information about the efficiency of the photosynthetic machinery can be gained as these responses occur in competition with each other. Fluorescence caused by the quenching of the reaction centres can be photochemical (electron transfer from the reaction centre of PSII onwards) or non-photochemical (heat dissipation).

Fluorescence can be measured on either dark or light adapted plants. The ratio of F_v'/F_m' (the ratio of variable and maximum fluorescence) from light adapted plants gives the maximum efficiency of PSII at a given photon flux density (i.e. the operating efficiency) and the F_v/F_m of dark adapted plants gives the maximum efficiency of PSII. By dark adapting the plants and then exposing them to a saturating flash of light all the reaction centres are closed which leads to an increase

in the amount of light being re-emitted. If the plants are stressed and not photosynthesising efficiently the ratio will be small as there will be less reaction centres open for use (Maxwell & Johnson, 2000). The yield of F_v/F_m may in reality be much lower than that measured during dark adaptation so it can be useful to take light-adapted readings to obtain the operating efficiency of PSII. Assumptions must be made when measuring both light and dark adapted states including the assumption that all fluorescence measured will come from PSII, whereas in reality some fluorescence will be emitted from PSI. Overestimation of the minimal fluorescence level F_o can also be a problem (Baker, 2008). Fluorescence imaging is also a technique that can be used to study the spatial heterogeneity across leaf surfaces.

This study utilised dark adapted chlorophyll fluorescence to examine the efficiency of the photosynthetic machinery in spring barley landraces in order to ascertain whether the lines are under environmental stress (an F_v/F_m ratio below 0.8). If there are differences seen in the photosynthetic capacity measured using gas exchange techniques but no difference in the efficiency of the photosynthetic machinery then this will tell us that there is variation in photosynthetic capacity that may be exploited by breeding programs.

4.1.2 Stomata and Gas Exchange

Stomata are the pores which regulate the flow of gases between the external and internal leaf environments. Stomatal behaviour is vital for the uptake of CO_2 for use in photosynthesis but also important in regulating water loss through transpiration (Lawson & Blatt, 2014; Lawson & McElwain, 2016). Stoma is an integral part of the ε_c of the plant as they function to maximise the amount of CO_2 available for use by the plant through the opening and closing of the pore through the guard cells.

The number of stomata on the surface of a leaf (and whether they are present on one or both adaxial and abaxial surfaces) is an important trait as it regulates the exchange of gases between the outside and internal leaf environment and is the main regulator of the amount CO_2 that is available for photosynthesis. It is thought that the stomatal density responds to atmospheric CO_2 concentration during the initial development of the leaf (Woodward, 1987; Gray *et al.*, 2000). The density of stomata are known to vary between species and within species depending on the environment and it has

been suggested that higher stomatal densities have the potential for increasing CO_2 uptake by optimising the gas diffusion process up until a point where there is competition for diffusion between pores (Tanaka *et al.*, 2013). A study in thale cress (*Arabidopsis thaliana*) which looked at photosynthetic rate in mutants with increasing numbers of stomata, showed a 30% higher photosynthetic rate than in wild-type plants (Tanak*a et al*, 2013).

The allocation of leaf surface area to stomata has long been studied and has resulted in the 'one cell spacing' rule which states that for efficient functioning of stomata they must be at least one cell separating them to allow gas diffusion (Serna & Fenoll, 2000). Stomata can cluster together on the leaf surface which causes CO_2 diffusion limitation, resulting in lower photosynthetic rates (Dow *et al.*, 2014). Increasing the stomatal density must be done with care to avoid stomatal clustering. The stomatal density and pore size controlled by the responsiveness of the guard cells also controls the rate of stomatal conductance (g_s) (de Boer *et al.*, 2016). The g_s is the capacity for CO_2 and H_2O to flow through the stomatal pore. Low g_s results in the limited assimilation of CO_2 into the leaf which has been shown to have an impact on crop yield. A study in wheat showed a close correlation with g_s and yield (Fischer *et al.*, 1998)..

Carbon isotope analysis can be used to examine the patterns of gas exchange over the life of the plant in response to water stress. Water loss through the stomata can be a major source of stress for the plant and there is a balance between closing the stomata under drought conditions and maintaining rates of photosynthesis. There are two isotopic forms of carbon present in the atmosphere ^{12}C and ^{13}C. Plants discriminate between the two forms of carbon preferring to take up the lighter ^{12}C when conditions are favourable (O'Leary, 1988; Farquhar *et al.*, 1989). When the plants are experiencing a stress they take up more of the ^{13}C as stomata close and they are forced to take up what carbon is available. The ratio of ^{12}C to ^{13}C ($\delta^{13}C$) gives us information on the lines intrinsic water-use-efficiency (WUE) (the ratio of carbon gain to water loss through stomatal conductance) of the plant (Dawson *et al.*, 2002) over the growing period of the tissue.

4.1.3 Aims

This study aims to examine variation in the ε_c of spring barley landraces in relation to photosynthetic and gas exchange parameters. Gas exchange will be measured using portable gas exchange technology to examine variation in photosynthetic rate and g_s. Chlorophyll fluorescence measures on field based plants will allow the maximum efficiency of PSII photochemistry to be established. The stomatal densities of selected barley landraces will be established using microscopy. The carbon isotope composition of the plants will be established to assess patterns of gas exchange. The landraces originated from a wide latitudinal spread across Europe from Scandinavia to Iberia and were specifically chosen as they will be adapted to a varied range of climatic conditions. It might be expected that there will be high levels of variation between the landraces in parameters such as photosynthetic rate and transpiration rate. As the landraces are being grown in vastly different climatic conditions to their locations of origin variation the levels of chlorophyll fluorescence will also be expected to vary due to differing levels of stress being felt by the line. The specific questions addressed in this study are:

1) Is there variation present in gas exchange parameters of a latitudinal spread of European landraces and does this regress with the climatic conditions of origin of these landraces?
2) Is there variation between stomatal densities of European barley landraces?

4.2 Methods

4.2.1 Seed Source

See Section 3.2.1 for seed source and Table 3.1 and Figure 3.1

4.2.2 Gas Exchange

4.2.2.1 Gas Exchange

One 0.5 litre pot was planted with nine seeds of each landrace for each replicate and three replicate runs of the experiment were carried out. Seeds were planted using Levington's Pot and Bedding Compost High Nutrient. Pots were arranged in a random manner (Figure 4.2) and placed inside a Sanyo Fitotron growth cabinet (Fitotron, Weiss Technik UK, Loughborough). Please note that rep 3 was carried out in a different cabinet due to sensor malfunction in the cabinet used for reps 1 and 2. Growth cabinet settings were 16/8 hours' day/night, 500µmol $m^{-2}s^{-1}$ light, 50% humidity, and 18/13^{o}C day/night temperature. Both cabinets were set to the same conditions to ensure continuity between reps. The light level was chosen as the highest light possible without incurring leaf damage through scorching by previous personal experience of use of those specific cabinets. Pots were watered as required to maintain damp compost and this was judged by touch. Plants were allowed to reach Growth Stage (GS) 24 before gas exchange measures commenced and measurements were taken at both GS24 and GS39. Three plants of each line were tagged using laboratory tape and all gas exchange measures were taken on the uppermost leaf on the main stem excluding the flag leaf of the marked plants.

Gas exchange data was gathered using a LiCor 6400 Gas Exchange System (LiCor Inc., Lincoln, NE) and the measurements taken included photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (mol H_2O $m^{-2}s^{-1}$), intercellular CO_2 concentration (µmol CO_2 mol^{-1}) and transpiration rate (mmol H_2O $m^{-2}s^{-1}$). The IRGA chamber was clamped onto the uppermost leaf excluding the flag leaf ensuring the entire area was covered with leaf (if not then the leaf area was calculated and adjustments made to readings). The conditions in the chamber were 800µmol $m^{-2}s^{-1}$ light, 400ppm CO_2, leaf temperature 18^{o}C and a fast air flow rate. The leaves were left for 15 minutes to acclimatise to the chamber conditions then a spot reading was taken for each leaf.

Gas Exchange readings were taken when the plants reached Growth Stage 24 (late tillering) and 39 (flag leaf emerged).

rep1

GER2	CZE1	GER3	FRA1
BRI2	Concerto	ITA1	SPN1
FIN1	DEN2	NOR2	NOR1

rep2

BRI2	CZE1	NOR1	FIN1
SPN1	GER2	ITA1	NOR2
GER3	Concerto	DEN2	FRA1

rep3

NOR2	Concerto	FIN1	FRA1
ITA1	SPN1	CZE1	BRI2
GER3	DEN2	NOR1	GER2

Figure 4.1 Diagram showing the layout of the landrace varieties and control Concerto within the growth cabinet. Each pot was placed in the growth cabinet in the corresponding position with the door being towards bottom row. Light was supplied from above. The growth cabinets were set at a light intensity of 500µmol, 50% humidity, 16/8 hour day/night and 18/13^{o}C. Gas Exchange readings were taken when the plants reached Growth Stage 24 and 39

4.2.2.2 Chlorophyll Fluorescence

The chlorophyll fluorescence was measured on the 2015 field trial (chapter 3 section 3.2) using a HandyPea (Hansatech Instruments, Norfolk) field fluorescence meter. The measurements were taken on the uppermost leaf excluding the flag leaf on each plant at GS39. The measurements were taken between 10.00am and 12.00pm. Four replicate measures were taken per tussock and averaged. The leaves were dark adapted by placing clips on the leaves and leaving for 20 minutes. The HandyPea was then attached to each clip individually and a reading was taken and recorded.

4.2.3 Stomata and Gas Exchange

4.2.3.1 Stomatal Density

Leaves which had been collected from the 2015 field trial (see chapter 3 for layout and conditions) were frozen at -15^{o}C (Foster, Norfolk) promptly after collection GS39 to allow storage until time was available for processing and analysis. The leaf

collected was the uppermost leaf excluding the flag leaf and two replicates per tussock and four tussocks per line were sampled.

In order to assess stomatal density imprints of the leaf surface were taken using the nail varnish method (Sekiya & Yano, 2008; Xu & Zhou, 2008). The leaves were removed from the freezer and defrosted for ten minutes. The surface of the leaves was then wiped gently with tissue paper to remove any surface dirt. A single coat of clear nail varnish (Max Factor Glossfinity) was then painted onto the lower surface of the leaf in the centre of the leaf avoiding the middle vein. This was the left to dry until the varnish was dry to touch. A piece of clear Sellotape (Sellotape, Cheshire) was then placed on top of the nail varnish sticky side down and light pressure applied using a finger to adhere the tape to the varnish. The tape was then slowly peeled off the leaf surface and the varnish which contained an imprint of the leaf surface was stuck to the tape. The tape was then placed face down on a slide.

Stomatal density was counted using an optical microscope (Leica DMRBE, Leica microsystems, Wetzlar Germany). A defined area ($1.0316mm^2$) was drawn on a coverslip which was used to define the area on which the numbers of stomata were counted. All stomata which were partially in the area were included in the stomatal count. The number of stomata was then divided by the area to give the number of stomata per mm^2.

4.2.3.2 Isotope Analysis

When the plants in the field reached GS39 a second leaf was collected from a shoot of the labelled plant and placed in an individual labelled paper bag. The leaves were then taken back into the lab and dried in an oven (Ecocell, MMM Medcenter, Munich, Germany) at 80^oC for 48 hours. The dried leaves were then ground in a ball mill (Retsch MM200, Haan Germany) at a rate of 25 1/s for 30s seconds until a fine powder was achieved. Each sample was then placed in individual 20ml scintillation vials (Fischer Scientific, Leicestershire) which was labelled with the plot number, year and line name. The samples were then sent to IsoAnalytical in Cheshire for carbon isotope analysis.

4.2.4 Climate study

The climate in the location of original collection for each of the landraces was looked at as a possible factor influencing the ε_c. Climate data was obtained from the national meteorological offices in each country of origin (Table 3.2, Appendix 1). The weather data region scale varies between countries from local weather data to regional data depending on the scale of reporting. It was always taken as the closest reported point to the latitude and longitude of origin of the landraces. The climatic variables reported are the total rainfall (mm) for spring/summer, the total number of sunlight hours for spring/summer and the average daily temperature (^oC) for spring/summer.

4.2.5 Statistical Analysis

Analysis of variance (ANOVA) was carried out in order to check for significant variation between the lines and Tukeys post hoc test was used to identify individual differences between the lines. Regression analysis of climate and latitude with each measure of gas exchange, chlorophyll fluorescence, stomatal density and isotope analysis was carried out to see if there was a relationship with each trait and the local conditions at their place of origin. All statistical analysis was carried out using GenStat 16th Edition.

4.3 Results

4.3.1 Gas Exchange

4.3.1.1 Photosynthetic Rate

The photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$) did not vary significantly between the lines at either GS and showed no regressions with latitude or climate (Table 4.2). The intercellular CO_2 concentration (µmol CO_2 mol^{-1}) did not vary significantly between the landraces at GS24 but did show significant variation between the landraces at GS39 ($p=0.0174$, $F_{10,20}=3.00$) ranging from 302.16-347.49 µmol CO_2 mol^{-1}. The landraces showed no regression with latitude or climate at GS24 but did show a significant negative regression of intercellular CO_2 concentration (µmol CO_2 mol^{-1}) with latitude ($p=0.004$, $t_{1,9}=14.65$, $R^2=0.57$) and a positive regression with temperature ($p=0.033$, $t_{1,9}=6.37$, $R^2=0.35$) at GS39 (Figure 4.2, Table 4.4). At GS39 the stomatal conductance (mol H_2O $m^{-2}s^{-1}$) showed no significant differences between the lines and no significant regression with latitude or climate. The transpiration rate (mmol H_2O $m^{-2}s^{-1}$) showed a significant variation between the lines ($p=0.024$, $F_{10,20}=2.81$) at GS39 from 1.69-3.16 mmol H_2O $m^{-2}s^{-1}$ although Tukeys Post hoc analysis did not identify any differences between individual lines. There was no significant regression with latitude or climate (Table 4.2).

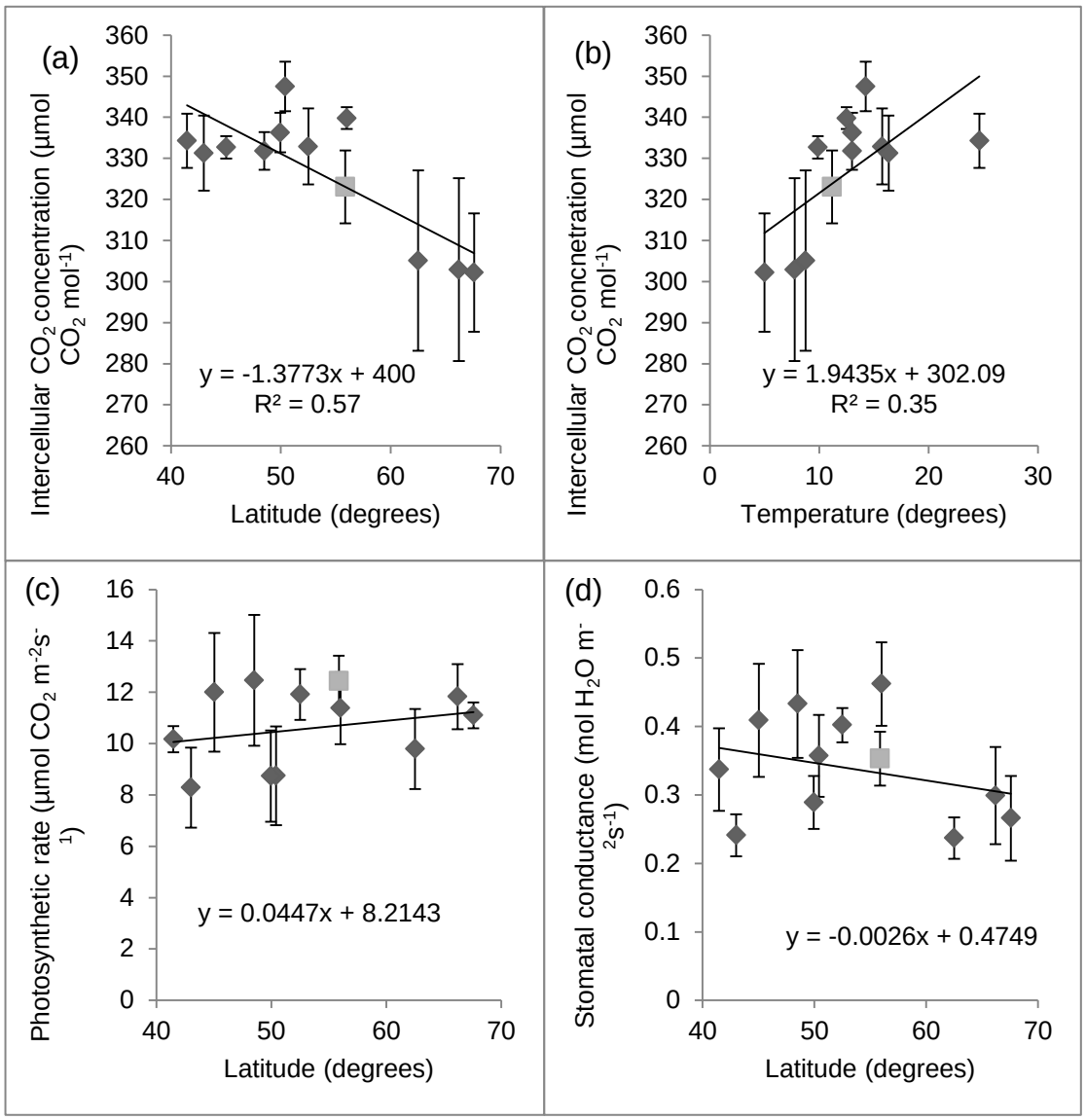

Figure 4.2 GS39 (Flag leaf blade fully visible) linear regression of landrace intercellular CO_2 concentration (µmol mol^{-1}) with (a) latitude), (b) temperature. Linear regression of (c) photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$) with latitude and (d) stomatal conductance (mol H_2O $m^{-2}s^{-1}$) with latitude. Grey diamonds are landraces and the grey square is Concerto for reference. The grey solid line is the regression line and the regression equation is provided**.** Measurements were taken at GS39 on plants grown in a growth cabinet under light intensity of 500µmol, 50% humidity, 16/8 hour day/night and 18/13^{o}C temperature. The conditions in the Li-cor chamber were 800µmol $m^{-2}s^{-1}$ light, 400ppm CO_2, leaf temperature 18^{o}C and a fast air flow rate. Each point on the figures represents one landrace line and is made up of three replicate runs of the experiment.

Table 4.1 (a) Differences between the gas exchange parameters at GS24 and 39 of the landraces. The range represents the minimum and maximum of landrace means. (b) Relationships between gas exchange variables and climatic variables, indicating the overall (linear regression).

(a) Gas exchange parameter	Growth Stage	Range of Means	ANOVA	(b) Latitude (degrees)	Precipitation (mm)	Hour of Sunlight	Temperature (^oC)
Photosynthetic rate (μmol CO_2 $m^{=2}s^{-1}$)	GS24	14.24-17.83	p=0.063, $F_{10,20}$=2.21	p=0.760, $t_{1,9}$=0.10	p=0.392, $t_{1,9}$=0.81	p=0.834, $t_{1,9}$=0.05	p=0.919, $t_{1,9}$=0.01
	GS39	8.29-12.46	p=0.278, $F_{10,20}$=1.34	p=0.424, $t_{1,9}$=0.70	p=0.332, $t_{1,9}$=1.05	p=0.897, $t_{1,9}$=0.02, R^2=0.01	p=0.379, $t_{1,9}$=0.86
Intercellular CO_2 concentration (μmol CO_2 mol^{-1})	GS24	314.18-325.46	p=0.911, $F_{10,20}$=0.44	p=0.736, $t_{1,9}$=0.12	p=0.270, $t_{1,9}$=1.38, R^2=0.03	p=0.080, $t_{1,9}$=3.88, R^2=0.22	p=0.254, $t_{1,9}$=1.49, R^2=0.04
	GS39	302.16-347.49	p=0.017, $F_{10,20}$=3.00	p=0.004, $t_{1,9}$=14.65, R^2=0.57	p=0.866, $t_{1,9}$=0.03	p=0.249, $t_{1,9}$=1.52	p=0.033, $t_{1,9}$=6.37, R^2=0.35
Stomatal Conductance (mol H_2O $m^{=2}s^{-1)}$	GS24	0.37-0.56	p=0.335, $F_{10,20}$=1.22	p=0.748, $t_{1,9}$=0.11	p=0.371, $t_{1,9}$=0.88	p=0.386, $t_{1,9}$=0.83	p=0.078, $t_{1,9}$=3.97, R^2=0.22
	GS39	0.24-0.46	p=0.104, $F_{,10,20}$=1.91	p=0.382, $t_{1,9}$=0.84	p=0.548, $t_{1,9}$=0.39	p=0.415, $t_{1,9}$=0.73	p=0.574, $t_{1,9}$=0.34
Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)	GS24	2.48-3.30	p=0.670,$F_{10,20}$ =0.75	p=0.956, $t_{1,9}$=0.00	p=0.082, $t_{1,9}$=3.84, R^2=0.22	p=0.267, $t_{1,9}$=1.40, R^2=0.03	p=0.305, $t_{1,9}$=1.18, R^2=0.01
	GS39	1.69-3.16	p=0.024, $F_{10,20}$=2.81	p=0.195, $t_{1,9}$=1.96, R^2=0.08	p=0.716, $t_{1,9}$=0.14	p=0.389, $t_{1,9}$=0.82	p=0.318, $t_{1,9}$=1.12, R^2=0.01

4.3.1.2 Chlorophyll Fluorescence

The fluorescence ratio (maximal fluorescence of photosystem II) (Fv/Fm) showed no significant variation between the lines but did show a positive regression with latitude ($p=0.036$, $t_{1,9}=6.05$, $R^2=0.33$) (Table 4.3, Figure 4.3).

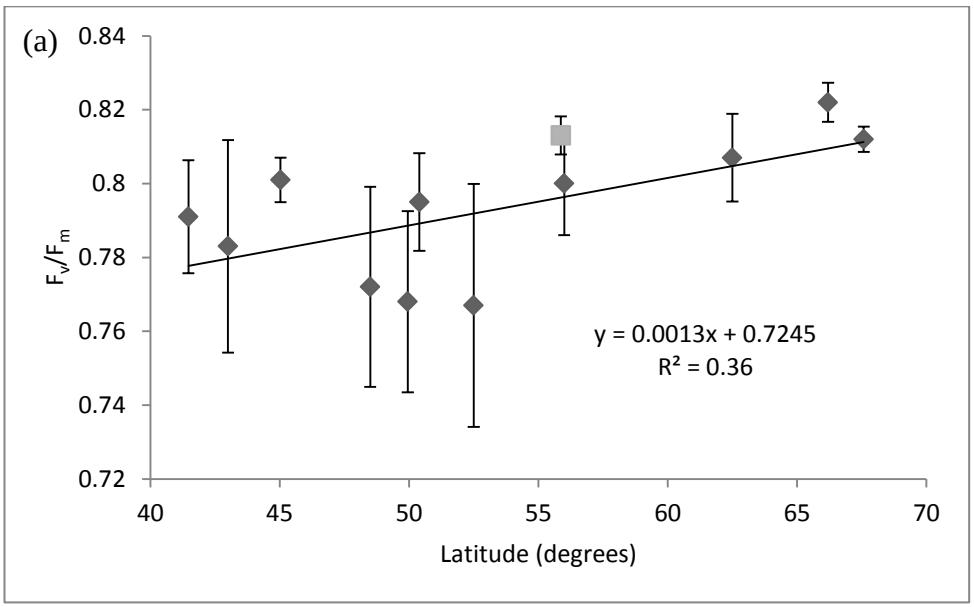

Figure 4.3 Linear regression of landrace Fv/Fm with (a) latitude. Grey diamonds are landraces and the grey square is Concerto for reference. The solid line is the regression line and the regression equation is given. Readings were taken using a Handy Pea and were taken on the uppermost leaf excluding the flag leaf at GS39. Each point represents a line and four plants per tussock and four tussocks were measured. Error bars are standard error.

Table 4.2 (a) Differences in the fluorescence ratio for the landraces. The range represents the minimum and maximum of landrace means for each trait. (b) Relationships between fluorescence trait and climatic variables, indicating the overall (linear regression).

(a)			**(b)**			
Trait	**Range of Means**	**ANOVA**	**Latitude (degrees)**	**Precipitation (mm)**	**Hours of Sunlight**	**Temperature (^{o}C)**
Fv/Fm	0.767-0.822	p=0.229, $F_{10,30}$=1.40	p=0.036, $t_{1,9}$=6.05, R^2=0.33	p=0.653, $t_{1,9}$=0.22	p=0.439, $t_{1,9}$=.66	p=0.087, $t_{1,9}$=3.70, R^2=0.21

4.3.2 Stomatal Density and Isotope Analysis

The stomatal density (stomata per mm^2) on the lower leaf surface of the landraces showed a significant variation between the lines ($p<0.001$, $F_{11,33}=5.62$) ranging from 22-41 stomata per mm^2. Lines BRI1, NOR2 and FIN1 were significantly different from lines CZE1 and DEN2. DEN 2 was also significantly different from NOR1. Stomatal density did not regress significantly with latitude or climate variables (Table 4.4). A difference in the ranges of stomatal densities between the lines was observed with some lines having large ranges (FIN1) and others being very conserved in stomatal numbers (BRI2) (Figure 4.4). There was no relationship seen in the values of carbon isotope ratio between the lines and either latitude or climatic variables (Table 4.4).

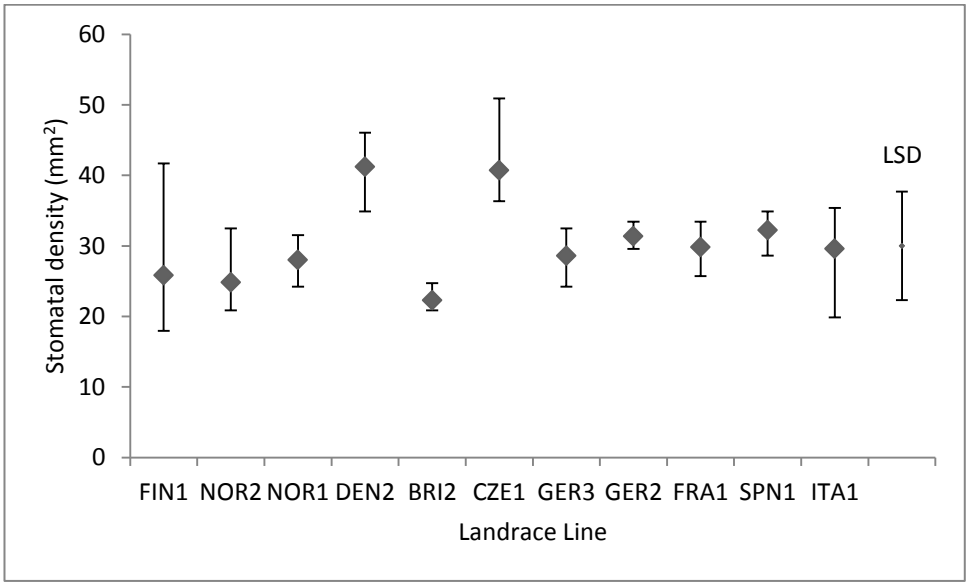

Figure 4.4 Stomatal density ranges of landraces listed from South to North by latitude (left to right). The point is the mean density for that line and the whiskers represent the maximum and minimum numbers of stomata found. The bar to the far right is the Least Significant Difference bar. Stomatal density was measured on the uppermost leaf excluding the flag leaf at GS39 in leaves collected from the 2015 field trial with each line representing four replicate tussocks.

Table 4.3 (a) Differences in stomatal density and carbon isotope composition between landraces at GS39. The range represents the minimum and maximum of landrace mean number of stomata or isotope ratio (b) Relationships between stomatal density or isotope ratio and climatic variables from the location of original collection, indicating the overall (linear regression). The stomatal density was measured on the uppermost leaf excluding the flag leaf from the 2015 field trial with two replicates per tussock and four tussocks of each line. The carbon isotope ratio was sampled on the uppermost leaf excluding the flag leaf of GS39 plants in both 2014 and 2015 field trials with four replicates per line on 2015 and three in 2014.

(a)			**(b)**			
	Range of Means	**ANOVA**	**Latitude (degrees)**	**Precipitation (mm)**	**Hour of Sunlight**	**Temperature (^{o}C)**
Stomatal Density (stomata per mm^2)	22-41	p<0.001, $F_{11,33}$=5.62	p=0.387, $t_{1,9}$=0.83	p=0.903, $t_{1,9}$=0.02	p=0.597, $t_{1,9}$=0.30	p=0.574, $t_{1,9}$=0.34
$\delta^{13}C_{V\text{-}PDB\ (‰)}$	-28.82 - -30.12	P=0.092, $F_{10,79}$=1.74	p=0.128, $t_{1,10}$=2.81, R^2=0.15	p=0.875, $t_{1,10}$=0.03	p=0.120, $t_{1,10}$=2.94, R^2=0.16	p=0.094, $t_{1,10}$=3.51, R^2=0.20

4.4 Discussion

4.4.1 Gas Exchange

The photosynthetic rate of the landraces did not vary significantly between the lines at either GS although there seemed to be more variation at GS24 than at GS39 (Table 4.2). There was no relationship between photosynthetic rate and latitude or weather data. The rate of photosynthesis was higher at GS24 than at GS39. It is possible that the higher rates of photosynthesis detected at the earlier GS were caused by the smaller size of the leaves with the stomatal pores being condensed into a smaller area thus there is higher gas exchange rates within the chamber of the detector. When the plant gets larger there is a larger surface area over which the plant can photosynthesise perhaps allowing the rates to drop. It could also be that the plants were under some sort of stress in the growth cabinets such as scorching stress from the lights causing the plant to reduce its activity at a later growth stage due to shutting down of stomatal pores to reduce water loss.

Wheat has been shown to have large variation between cultivar varieties in their photosynthetic capacity (Driever *et al.*, 2014) although no correlation with photosynthetic rate and yield was observed. They suggested that although there is no correlation with increased yields there is scope to improve the photosynthetic capacity of wheat and that previous breeding which has not been focussed on photosynthetic efficiency has resulted in loss of high levels of photosynthetic rates. This is in agreement with a study of Australian wheat which showed no relationship with photosynthetic rate and yield (Sadras *et al.*, 2012). Some studies in wheat have however found a correlation between yield and photosynthetic rate (Fischer *et al.*, 1998)

It should be noted that our study was carried out in a growth cabinet under favourable conditions. It may be that when in the field with stressful environmental factors such as fluctuating light, temperature and wind that the lines may respond differently with different rates of stomatal response affecting the photosynthetic rate (Lawson & Blatt, 2014). As increasing levels of photosynthetic rate could lead to greater water loss it may be beneficial to look at variation in photosynthetic rate

when lines are water-stressed to identify the best performing lines for maintaining water-use-efficiency (Gilbert *et al.*, 2011).

Chlorophyll fluorescence can be used to gain information about the efficiency of PSII with the ratio of F_v/F_m. In this study, there was no significant difference in the F_v/F_m ratio between the landraces although there was a significant regression with F_v/F_m and latitude with the Southern landraces having a lower ratio that those from Northern latitudes (Figure 4.3, Table 4.3). This suggests that whilst there were no excessive differences in the ability of the photosynthetic system to function there was a greater stress felt by the Southern European landraces. This may be because the conditions under which they were being grown were not similar to the conditions at their location of origin to which they may have become locally adapted and they may be experience a stress such as temperature or water supply. It may have been useful to measure the chlorophyll fluorescence in light adapted plants to gain an idea of the operational efficiency of PSII. Landraces are still grown in marginal areas of Europe where stress levels (such as drought stress) are high and have been seen to have a yield benefit of 20% compared to modern cultivars when grown in the same conditions (Ceccarelli, 1994; Dwivedi *et al.*, 2016). Spanish barley landraces have been shown to outperform modern cultivars in low productivity areas which are mainly characterised by water availability and this study suggested that this may be because traits which help cope with these conditions have been missed by modern breeders which aim to produce varieties which can be grown over much larger areas (Yahiaoui *et al.*, 2014).

4.4.2 Stomata and Gas Exchange

The density of the stomata of the landraces showed significant variation between landrace lines with the landrace with the highest density of stomata having double the number than the landrace with the lowest (Figure 4.4). This indicates that there is variation present within the landraces that perhaps could be used to boost the numbers of stomata in modern cultivar breeding. There was no regression with stomatal density and latitude or climate which is perhaps surprising as it may be expected to be a disadvantage to have high numbers of stomata in higher temperatures due to the risk of excessive water loss through transpiration.

Alternatively, higher numbers of stomata may aid in cooling the plant and avoiding heat stress. It must be noted that the line with the smallest range in stomata is the landrace which originated in the United Kingdom. It may be that the novel environment is having an effect on the other landraces which are coping with the stress by producing a wide range of stomatal densities.

Having higher numbers of stomata, it might be expected that the rate of photosynthesis might be increased due to more pores taking up a greater amount of CO_2. In this study, there was no indication of any difference in photosynthetic rates between the landraces (Table 4.2). This is a pattern that was also seen in a study on *Arabidopsis* (Schluter *et al.*, 2003) which although not measured under the same conditions as this study demonstrated that a mutant line with increased numbers of stomata showed no increased CO_2 assimilation rate when compared to the wild type and had not produced any increased biomass. This could indicate that stomatal factors are not limiting on CO_2 assimilation rates. This is in contrast to other findings also in *Arabidopsis* which showed a 30% increase in photosynthetic rates in lines with high stomatal density compared to the wild type (Tanaka *et al.*, 2013).

Although no link with climatic variables were found in this study it is possible that increased numbers of stomata are only beneficial under certain environmental circumstances. It is possible that high numbers of stomata would be detrimental under low water and high temperature conditions. It has been seen that under low water inputs the number of stomata decreases (Xu & Zhou, 2008) and the stomatal conductance rates reduced. This is possibly a response to try to limit water loss through transpiration through the stomata.

The carbon isotope ratio can be used to determine the WUE of the plants through patterns of gas exchange over the growing period. There was no difference in isotope discrimination between the lines and no relationship seen with latitude or climate of origin (Table 4.4). This indicates that there are no differences between the landraces in how they are coping with the conditions of Boghall and closing stomata. This does not follow the pattern seen in transpiration rate which showed differences between the lines. Some studies have seen links between high ^{13}C content and low transpiration rates (Roussel *et al*, 2009). It may be that there was not a large enough

sample size here to pick up any differences. It has been observed in other species of plant that there are links with climate, altitude and location of origin but this was not seen here (Huc *et al.*, 1994; Moore *et al.*, 1999).

Studies in other crops such as wheat have shown no change in the stomatal density or respiration rate with domestication and year of release (Sadras *et al.*, 2012) although other crops have varied in their response to domestication (Milla *et al.*, 2013) with increases and decreases in the number of stomata, stomatal conductance and arrangement of stomata seen. Increasing the numbers of stomata may however be of benefit in the face of increasing atmospheric CO_2 concentrations allowing the plants to take advantage of an increased abundance of CO_2. If crops are bred with higher numbers of stomata care must be taken to ensure sufficient spacing between the stomata or their function may be impaired. The 'one-cell spacing rule' ensures that there is sufficient space between the stomata that diffusion between the cells does not impair the function (de Boer *et al.*, 2016). Stomatal clustering has also been predicted to reduce stomatal functioning by impairing the aperture opening as a result of the guard cell function being limited (Lehmann & Or, 2015).

It must be noted that some of the measures (stomatal density, chlorophyll fluorescence and carbon isotope ratio) taken in this chapter were carried out of plants grown in the field trial in 2015 and others (gas exchange) were carried out on cabinet grown plants. Using material grown in the field allows a more natural setting for the plants to grow reflecting environmental influences experienced during the growth season. The downside of field grown plants is that it is impossible to control every possible environmental variable so results must always be interpreted with this in mind. Using a growth cabinet allows complete control over all environmental variables which means that interpretation of results can be more robust however growing healthy plants in growth cabinets is challenging. The growth cabinet was used in this experiment for the gas exchange work purely for practical reasons in that it was unfeasible to transport the Li-Cor 6400 to the field and maintain sufficient battery power with the resources available. This means that it is not possible to link results from the gas exchange work with the results on stomatal density and carbon isotope ratio as too many assumptions would have to be made. It would have been

useful to measure stomatal density and carbon isotope ratio on the material grown in the growth cabinet.

4.4.3 Conclusions

Improving photosynthetic efficiency has been suggested as a means to improve crop yields and one of the major targets of this is the conversion efficiency of the plants. Increasing the amount of energy and biomass produced by the plants by improving the ε_c must be accompanied by increases in sink capacity if it is to result in an increase in yield.

- There was no variation seen between the landraces in photosynthetic rates and the levels of photosynthetic rate were comparable with Concerto, a modern cultivar.
- There was a positive regression with F_v/F_m and latitude of the landraces original collection indicating differences in stress levels between the lines affecting the efficiency of photosystem II.
- Large variations in stomatal density were seen in the landraces potentially providing a source of variation for future breeding if the aim was to increase the numbers of stomata.

5. Resilience of Photosynthetic Efficiency and Yield to Nitrogen Deficiency

5.1 Introduction

Yield increases since the Green Revolution have mainly come about from the introduction of dwarf varieties of cereals (Evans, 1997; Chono *et al.*, 2003; Morinaka *et al.*, 2006) which improved the Harvest Index (HI)(the partitioning of resources between the straw and the grain) and the development of cultivars that are highly responsive to high input agricultural systems with high fertiliser rates (Ryan *et al.*, 2008). High levels of fertiliser inputs are not sustainable for the future as the materials they are produced from are a finite resource and are costly to buy (Foulkes *et al.*, 2009). They can also have a large detrimental environmental effect if not applied correctly and leaching into water systems is a problem. These issues mean that there may be a push for reduced levels of artificial fertilisation in future and new crop cultivars will have to be more efficient in their use of the nitrogen supplied and at accessing the latent nitrogen content of the soil whilst maintaining yield levels.

5.1.1 Components of Yield

Yield can be assessed by looking at its individual components namely; the number of ears per m^2, the average grain weight (usually expressed as the weight of 1000 grains) and the number of grains per ear. The 1000 grain weight is a measure commonly used by industry as it not only encapsulates the amount of biomass being produced per grain but also gives an indication of grain quality which is important if the end product is going into the malting industry. It is also a reported component of yield in a number of scientific studies (Bertholdsson & Kolodinska Brantestam, 2009; Wu *et al.*, 2012; Gaju *et al.*, 2016). When combined with the number of grains and number of ears per m^2, an idea of the productivity and partitioning of the plant can be obtained. As seen in previous chapters (chapter 3 and chapter 4) yield can also be expressed through the Monteith equation (Equation 1) (Monteith & Moss, 1977; Farquhar *et al.*, 1980, 2001).

Nitrogen supply influences canopy expansion and light interception which in turn has an effect on the rate of photosynthesis per unit of leaf area. This will directly impact on biomass production and yield.

5.1.2 Nitrogen Uptake and Stress

Nitrogen availability is a limiting factor in yield development of crop plants (Teixeira *et al.*, 2014), particularly modern cultivars which have been bred to be highly responsive to fertiliser inputs. Nitrogen is an important resource and affects not only the crops yield but also the photosynthetic efficiency (Teixeira *et al.*, 2014) and the plants biomass. At least 2% of all nitrogen taken up by plants ends up in the chlorophyll and 25% of the leaf nitrogen can be accounted for by Rubisco (Monostori *et al.*, 2016) . It has also been seen that lack of nitrogen can affect the amount of chlorophyll in the plant which has a negative effect on yield in a study of wheat (Monostori *et al.*, 2016). This study saw that when additional fertiliser was applied there was not only an increase in yield (tonnes per hectare) of the crop but there was also an increase in the SPAD reading which indicates an increase in the amount of chlorophyll within the leaves. A different study which looked at wheat, barley and oats under low and high nitrogen inputs also saw a decrease in radiation-use-efficiency (RUE) with the low nitrogen regime (Muurinen & Peltonen-Sainio, 2006) when including the leaf area and the light interception efficiency. The leaf area index has also been shown to be higher under high fertilisation in both maize and durum wheat (Amanullah *et al.*, 2007; Fois *et al.*, 2009). However, crop yields and RUE have been found to suffer when nitrogen levels get too high (Olesen et al. 2000).

The gas exchange history of the plant over its lifecycle can be assessed to see if there were differences in the stomatal functioning of the plant through carbon isotope analysis. Although it is commonly used to study drought stress it also gives an indication of the gas exchange performance over the life of the plant. See chapter 4 introduction for description of carbon isotope technique theory. The ratio of ^{12}C to ^{13}C ($\delta^{13}C$) can give information about the stomatal functioning of the line and the intrinsic water-use-efficiency (WUE) (the ratio of carbon gain to water loss through

stomatal conductance) (Dawson *et al.*, 2002) over the growth of the tissue sampled. This can give an indication of the transpiration efficiency of the crop.

In most Western farming set-ups crops are treated throughout the season with fertiliser treatments to boost the nitrogen levels in the soil. This can be expensive to source and apply and have detrimental effects to the wider environment if inappropriately used (Hirel *et al.*, 2007; Foulkes *et al.*, 2009). Nitrogen application must be carefully done in sync with the latent nitrogen content of the soil, weather conditions and crop type (Olfs *et al.*, 2005). Producing varieties which are more resilient to nitrogen deficit stress will not only be of benefit in Western farming but will also benefit areas where the costs of nitrogen fertilisers are prohibitive. Crop plants nitrogen-use-efficiency (NUE) can be divided into two components; the ability of the roots to capture nitrogen from the soil, and the conversion to grain and biomass ((Moll *et al.*, 1982) as cited in (Foulkes *et al.*, 2009; Barraclough *et al.*, 2010)). In order for improved NUE both of these parameters must be improved to ensure the maximum recovery of fertiliser inputs from the soil and then partitioning to allow the photosynthetic processes to continue unconstrained. Recovery of fertiliser nitrogen can be low and this can be caused by not applying fertiliser in response to crop demand and spatial variation in latent soil nitrogen content or weather conditions (Kirda *et al.*, 2001; Foulkes *et al.*, 2009). Improvements in the plants NUE may be brought about by increases in root biomass and rooting depth (King *et al.*, 2003), and the remobilisation of nitrogen from the roots into the shoots and then the grain. Although these aspects have not been studied directly there may be variation in the resilience of landraces to low fertiliser inputs that could be utilised in breeding programs.

5.1.3 Landraces

This study looks at the maintenance of certain components of yield (1000 grain weight and number of grains per ear), and maintenance of photosynthetic efficiency of spring barley landraces (*Hordeum vulgare* ssp. *vulgare).* The number of ears per m^2 was not measured due to space and material constraints although it is the main component of yield that is responsive to N supply. Landraces are still grown in some areas globally and in marginal areas in Europe where they are better able to cope

with the stress than modern cultivars (Yahiaoui *et al.*, 2014; Dwivedi *et al.*, 2016). This could indicate that landraces may be more resilient to nutrient stress which could provide breeders with genetic resources to develop cultivars which maintain yields at lower fertiliser inputs (Lafitte *et al.*, 1997).

Landraces were commonly grown before the invention of the Haber-Bosch process to produce nitrogenous fertilisers. Although there would have been some form of organic fertiliser commonly applied the lines would not have been bred to be highly responsive to fertiliser inputs like modern cultivars. A study in wheat, barley and oats which compared modern and old cultivars RUE under low nitrogen regimes showed older cultivars of barley had a higher resource-use-efficiency than the newer cultivars (Muurinen & Peltonen-Sainio, 2006). Yield was also maintained under a low nitrogen input better in landraces than modern cultivars when compared with the yields obtained under modern fertilisation levels in a different study of wheat (Gaju *et al.*, 2016).

5.1.4 Aims

The aim of this study was to examine variation in certain components of yield and photosynthetic efficiency among landraces when grown under a nutrient stress environment. Leaf chlorophyll content, which is an important trait related to photosynthetic efficiency will be assessed under low fertiliser inputs along with components of yield such as the 1000 grain weight and number of grains per ear. The harvest index will also be compared to see if the partitioning efficiency is affected under nutrient supply stress. The efficiency of the photosynthetic apparatus will be assessed using carbon isotope analysis and chlorophyll fluorescence. Variation in a range of different spring barley landraces from a European latitudinal spread will be compared with a reference cultivar. The landraces were specifically chosen to represent a wide latitudinal spread as they will have become adapted to a varied range of climatic conditions.

1) Is there variation in components of yield including number of grains per ear, 1000 grain weight of landraces between 120kg/hectare and 30kg/hectare input nitrogen regimes? Is the partitioning efficiency measured by HI affected by reduced nitrogen inputs?

2) Is there variation in the chlorophyll content of landraces between plants grown in a standard (120kg/hectare) nutrient input system and those under a low (30kg/hectare) input system?
3) Is there variation seen in the efficiency of the of the landraces photosynthetic systems which could be an indication that the line is experiencing stress?

5.2 Methods

5.2.1 Seed Source

See section 3.2.1 and Table 3.1 and Figure 3.2 for information on seed source.

5.2.2 Field Trial

5.2.2.1 Field Trial Set-up

See Chapter 3, section 3.2.2.1 for information on the field trial. Trial layout is given in figure 3.3. There were two levels of fertiliser inputs at 120kg N per hectare and 30 kg N per hectare in the trial design and all plots for both levels were utilised in the following measurements. Fertiliser levels were chosen in reference to the standard recommended rate for malting barley (AHDB, 2017). The field site level of nitrogen is around 63.5kg per hectare (Pappa *et al.*, 2011).

5.2.2.2 Components of Yield

Harvest took place on 12/08/14 in 2014 and 04/09/15 in 2015). The ears were individually hand threshed and the grain number, row count, grain weight recorded and the 1000grain weight calculated. Grain weight was measured on a precision balance (Kern PLJ, D-72336, Kern & Sohn Gontbl, Balingen, Germany).

5.2.2.3 Harvest Index

The ear and the straw were harvested to allow calculation of harvest index to occur. The straw was dried in an oven (Ecocell, MMM Medcenter, Munich, Germany) at 80^{o}C for 48 hours. And weighed using a precision balance (Kern PLJ, D-72336, Kern & Sohn Gontbl, Balingen, Germany). Harvest index was then calculated by dividing the grain weight by the combined weight of the grain plus the straw plus the chaff.

5.2.2.4 Chlorophyll Content and Fluorescence

See Chapter 3, section 3.2.3.1 for chlorophyll content methodology.

See Chapter 4, section 4.2.2.2 for chlorophyll fluorescence methodology.

5.2.2.5 Leaf Nitrogen Concentration and Carbon Isotope Analysis

When the plants in the field reached GS39 a second leaf (uppermost leaf excluding flag leaf) was collected from a shoot of the labelled plant and placed in an individual

labelled paper bag. The leaves were then taken back into the lab and dried in an oven (Ecocell, MMM Medcenter, Munich, Germany) at 80^{o}C for 48 hours. The dried leaves were then ground in a ball mill (Retsch MM200, Haan Germany) at a rate of 25 1/s for 30s seconds until a fine powder was achieved. Each sample was then placed in individual 20ml scintillation vials (Fischer Scientific, Leicestershire) which was labelled with the plot number, year and line name. The samples were then sent to IsoAnalytical in Cheshire for nitrogen concentration analysis.

See chapter 4, section 4.2.3.2 for carbon isotope methodology.

5.2.2.6 Climate and latitude

The climate in the location for each of the landraces was looked at as a possible factor influencing the carbon isotope ratio. Climate data was obtained from the national meteorological offices in each country of origin (Table 3.2, Appendix 1). The weather data region scale varies between countries from local weather data to regional data depending on the scale of reporting. It was always taken as the closest reported point to the latitude and longitude of origin of the landraces. The climatic variables reported are the total rainfall (mm) for spring/summer, the total number of sunlight hours for spring/summer and the average daily temperature (^{o}C) for spring/summer.

5.2.2.7 Statistical Analysis

Two-way ANOVA (analysis of variance) was carried out on the data to assess differences between the lines, differences between the fertiliser treatments and any interaction between the fertiliser treatment and the line in components of harvest, chlorophyll content, nitrogen content and carbon isotope ratio. Regression analysis was used to examine any relationship between latitude and climate from the location of origin and the carbon isotope ratio. All statistical analysis was carried out using GenStat 16th Edition.

5.3 Results

5.3.1 Harvest Index and Components of Yield

The HI showed a significant difference between the landraces in both the 2 row lines with BRI2 being significantly different from everything except GER2 and FRA1 being significantly different from CZE1 and GER3 ($p<0.001$, $F_{7,93}=27.1$) and 6 row lines with ITA1 and NOR2 being significantly different from FIN1 ($p=0.003$, $F_{3,43}=5.43$). There were differences in HI between the 120kgN/hectare and 30kgN/hectare fertiliser treatments in the 2 row lines ($p=0.037$, $F_{1,93}=4.45$) (Table 5.1, Figure 5.1) with a reduction in means of HI ratio from 0.48 to 0.47.

The 1000 grain weight showed a significant difference between the landraces in both the 2 ($p=0.044$, $F_{6,81}=2.28$) and 6 row lines ($p<0.001$, $F_{3,45}=16.28$). In the 2 row lines SPN1 was significantly different from GER2 and Concerto and in the 6 row lines NOR2, ITA1 and NOR1 were all significantly different from each other. There were differences in 1000 grain weight between the 120kg/hectare and 30kg/hectare fertiliser treatments in the 2 row lines ($p=0.014$, $F_{1,81}=6.30$) (Table 5.1, Figure 5.2) with a reduction from 55.0g to 52.0g with a reduced fertiliser input. The number of grains per ear showed a significant difference between the landraces in both the 2 ($p=0.004$, $F_{6,81}=3.53$) and 6 row lines ($p<0.001$, $F_{3,45}=6.59$) (Table 5.1). In the 2 row lines SPN1 and FRA1 were significantly difference from BRI2 and Concerto and in the 6 row lines NOR2 and FIN1 were significantly different form NOR1.

5.3.2 Chlorophyll Content and Fluorescence

5.3.2.1 Chlorophyll Content

The SPAD readings at GS39 and GS59 showed significant differences between the 120kgN/hectare and 30kgN/hectare fertiliser treatments ($p<0.001$, $F_{1,141}=15.83$),($p<0.001$, $F_{1,141}=38.64$) (Table 5.2, Figure 5.3). At GS39 there was a reduction from 41.7 to 39.2 SPAD units and at GS59 there was a reduction from 44.9 to 39.8 SPAD units with a reduction in fertiliser input.

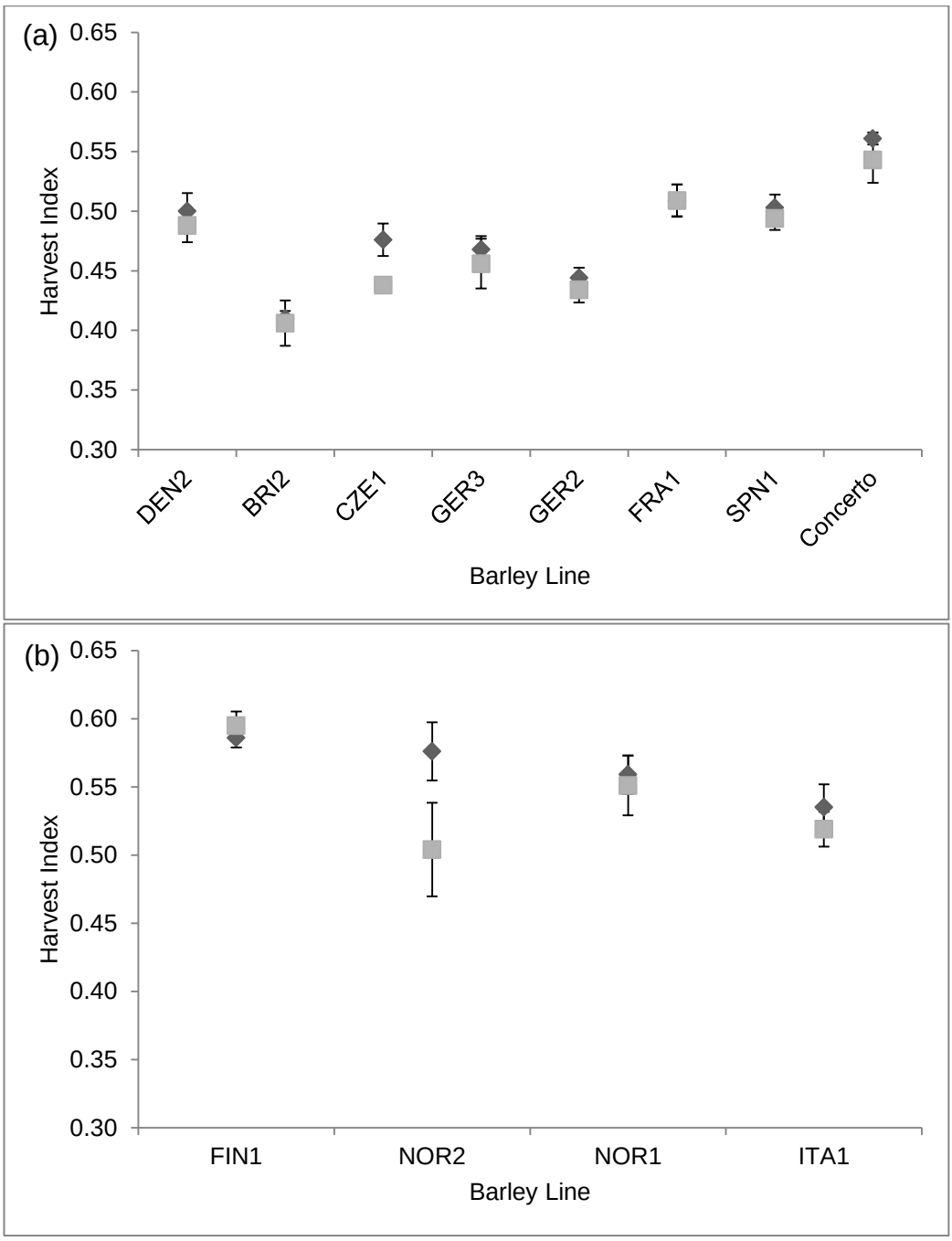

Figure 5.1 Harvest Index of the landraces at 120kg N per hectare (grey diamonds) and 30kg N per hectare (grey squares) fertiliser inputs. (a) 2 row lines, (b) 6 row lines. Error bars are the standard error. This is the average across two years of trials (2014 and 2015) and in 2014 there were three replicates of each line and in 2015 there were four replicates. Each point represents one barley line.

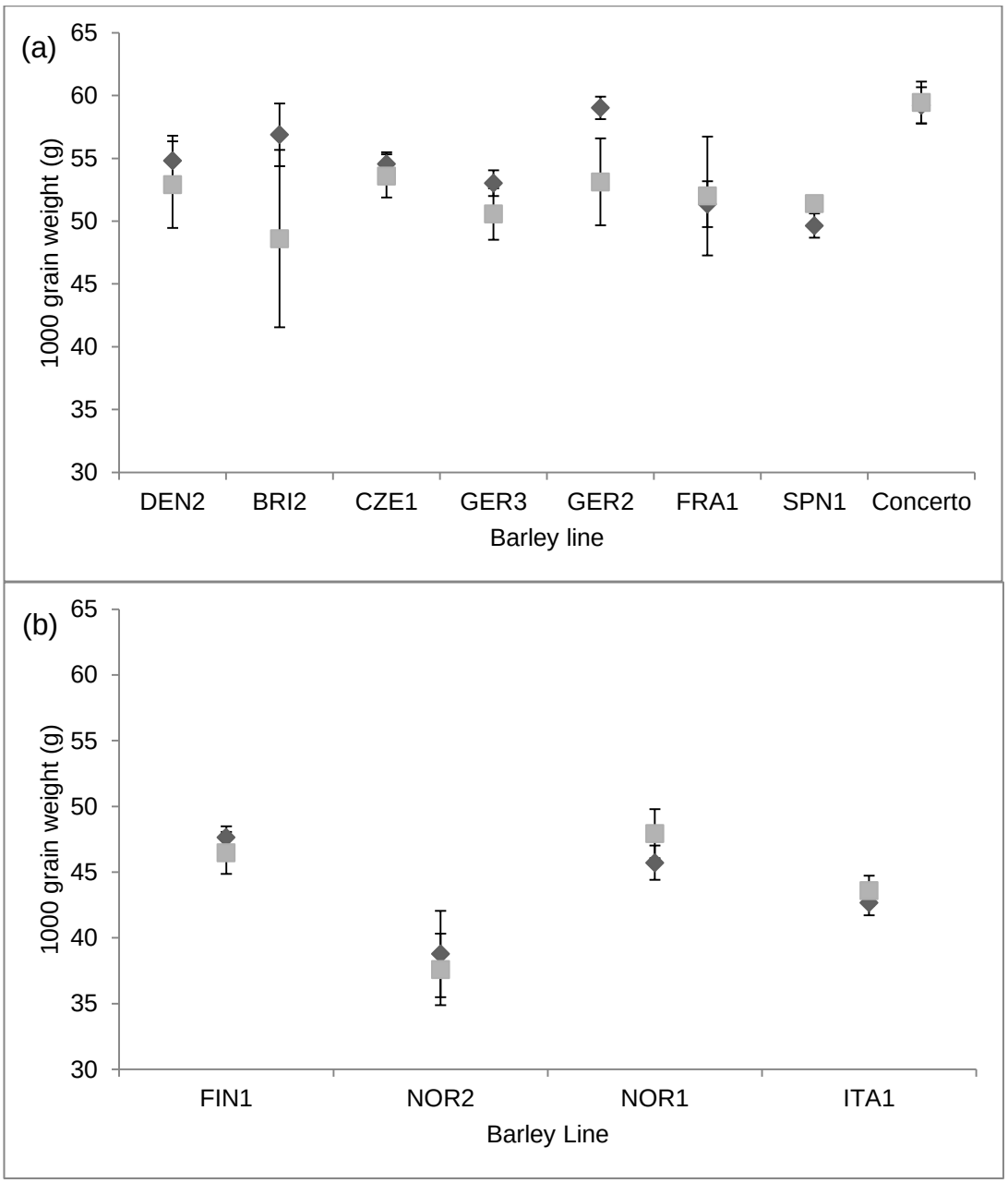

Figure 5.2 Weight of 1000 grains of landrace line under 120kg N per hectare (grey diamonds) and 30kg N per hectare (grey squares) fertiliser treatments. (a) 2 row lines (b) 6 row lines. Error bars are the standard error and each point represents the mean of one barley line and is the average of from the field trials in 2014 and 2015. There were three replicates in 2014 and four in 2015.

Table 5.1 Harvest components of the landraces. The landraces are split into 2 and 6 row lines. The mean values and the range of values are given for each of the harvest components and split between the 120kgN/hectare and 30kgN/hectare fertiliser treatments. Results of the ANOVA are given for the lines with all the data combined and then with the fertiliser treatment looked at as a factor.

Harvest Parameter	Row	Mean 120kgN/hectare	Mean 30kgN/hectare	Mean Range for 120kgN/hectare Fertiliser	Mean Range for 30kgN/hectare Fertiliser	ANOVA between lines	Fertiliser	Interaction
Harvest Index	2	0.48	0.47	0.41-0.56	0.41-0.54	$p<0.001$, $F_{7,93}=27.1$	$p=0.037$, $F_{1,93}=4.45$	$p=0.883$, $F_{7,93}=0.43$
	6	0.56	0.54	0.54-0.59	0.50-0.60	$p=0.003$, $F_{3,43}=5.43$	$p=0.067$, $F_{1,43}=3.54$	$p=0.104$, $F_{3,43}=2.18$
1000 grain weight (g)	2	55.0	52.8	49.6-59.3	50.4-54.2	$p=0.044$, $F_{6,81}=2.28$	$p=0.014$, $F_{1,81}=6.30$	$p=0.286$, $F_{6,81}=1.26$
	6	43.7	43.8	38.7-47.2	37.2-47.9	$p<0.001$, $F_{3,45}=16.28$	$p=0.895$, $F_{1,45}=0.02$	$p=0.745$, $F_{3,45}=0.41$
Number of grains per ear	2	28.0	27.1	26-29	26-29	$p=0.004$, $F_{6,81}=3.53$	$p=0.084$, $F_{1,81}=3.07$	$p=0.701$, $F_{6,81}=0.64$
	6	57.4	54.0	53-64	45-66	$p<0.001$, $F_{3,45}=6.59$	$p=0.212$, $F_{1,45}=1.60$	$p=0.562$, $F_{3,45}=0.69$

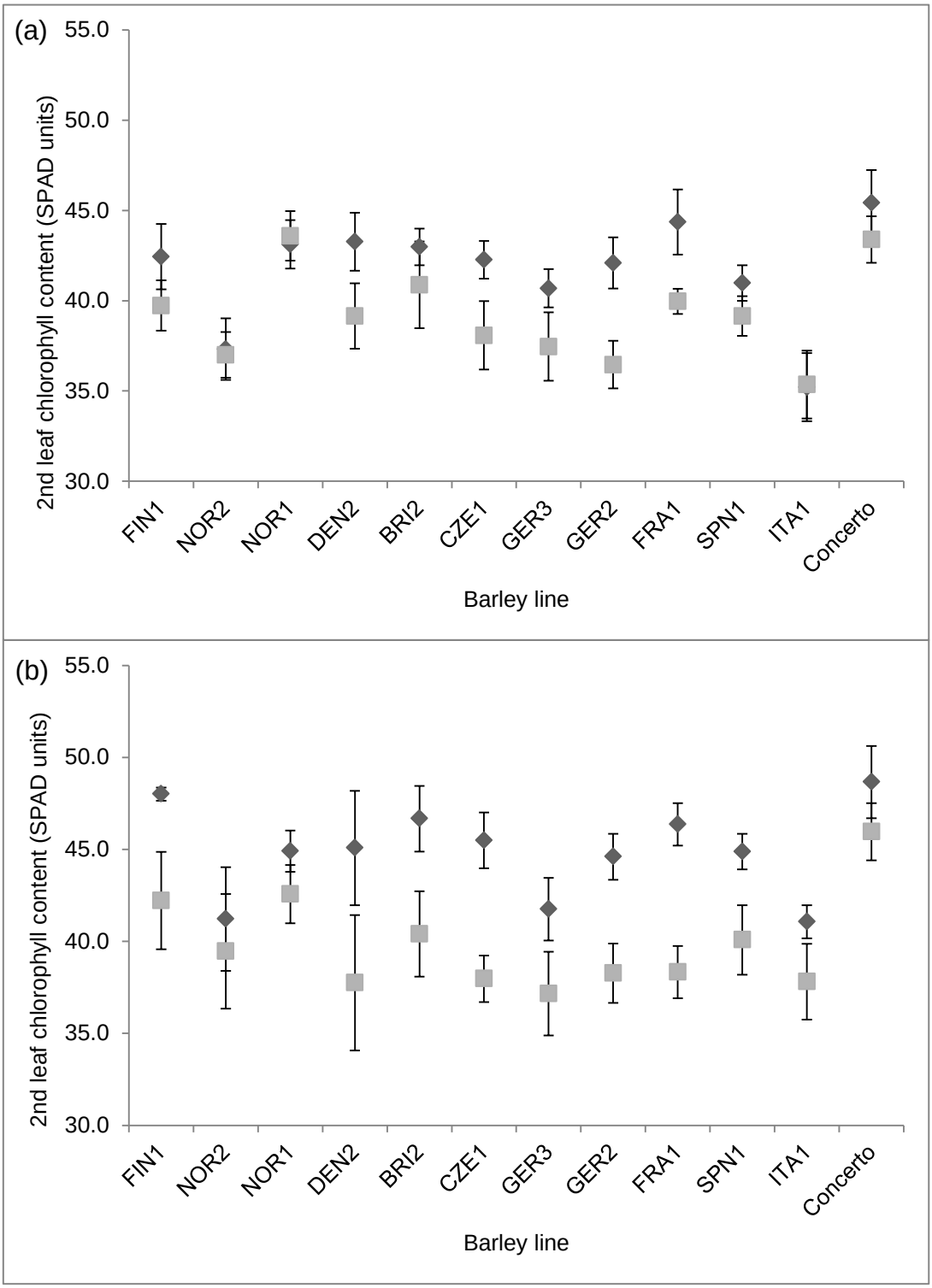

Figure 5.3 Chlorophyll content (SPAD units) of the 2nd leaf at 120kg N per hectare (grey diamonds) and 30 kg N per hectare (grey squares) fertiliser treatments. (a) at GS39 and (b) at GS59. SPAD readings were taken on the uppermost leaf excluding the flag leaf. Error bars are the standard error. Each point represents one landrace line at a fertiliser treatment. In 2014 there were three replicates for each line and in 2015 there were four replicates.

Table 5.2 Chlorophyll content of the landraces at different Growth Stages (GS). The mean and the range of values are given for each of the chlorophyll content and split between the 120kgN/hectare and 30kgN/hectare fertiliser treatments. Results of the ANOVA are given for the lines with all the data combined and then with the fertiliser treatment looked at as a factor.

Chlorophyll content (SPAD units) Growth Stage	**Mean 120kgN/hectare**	**Mean 30kgN/hectare**	**Mean Range for 120kgN/hectare Fertiliser**	**Mean Range for 30kgN/hectare Fertiliser**	**ANOVA between lines**	**Fertiliser**	**Interaction**
GS24	37.3	37.9	32.5-43.4	35.1-42.6	p=<0.001, $F_{11,141}$=4.83	p=0.277, $F_{1,141}$=1.19	p=0.429, $F_{11,141}$=1.02
GS39	41.7	39.2	35.2-45.4	35.4-43.6	p<0.001, $F_{11,141}$=5.49	p<0.001, $F_{1,141}$=15.83	p=0.654, $F_{11,141}$=0.79
GS59	44.9	39.8	41.1-48.7	37.2-46.0	p=0.004, $F_{11,141}$=2.68	p<0.001, $F_{1,141}$=38.64	p=0.839, $F_{11,141}$=0.58

5.3.2.2 Chlorophyll Fluorescence

The F_v/F_m ratio showed no difference between the lines and no differences between the fertiliser treatments ($p=0.494$, $F_{1,69}=0.47$) (Table 5.3).

5.3.3 Leaf Nitrogen Concentration and Carbon Isotope Analysis

There was a significant difference in nitrogen concentration (% dry weight) between the lines ($p<0.001$, $F_{1,124}=127.77$) between the 120kgN/hectare and 30kgN/hectare fertiliser treatments (Table 5.4, Figure 5.4) with a reduction from 5.00 to 3.91% with a reduction in fertiliser inputs. There was no significant difference seen in carbon isotope ratio between the fertiliser treatments but there was a significant difference between the landrace lines ($p=0.002$, $F_{10,124}=3.06$) (Table 5.4) with DEN2 and ITA1 being significantly different form NOR1 and FIN1. As there were significant differences seen between the lines in carbon isotope ratio which were not seen in chapter 4 regression analysis was used to look at relationships between ratio and latitude or climate. No regression was seen between latitude and climate and carbon isotope ratio (Table 5.5).

Table 5.3 Results of the analysis of the chlorophyll fluorescence data. The mean and the range of values are given for each of the measures and split between the 120kgN/hectare and 30kgN/hectare fertiliser treatments. Results of the ANOVA are given for the lines with all the data combined and then with the fertiliser treatment looked at as a factor.

	Mean 120kgN/hectare	**Mean 30kgN/hectare**	**Mean Range for 120kgN/hectare Fertiliser**	**Mean Range for 30kgN/hectare Fertiliser**	**ANOVA between lines**	**Fertiliser**	**Interaction**
F_v/F_m	0.794	0.798	0.767-0.822	0.785-0.815	p=0.086, $F_{11,69}$=1.72	p=0.494, $F_{1,69}$=0.47	p=0.803, $F_{11,69}$=0.62

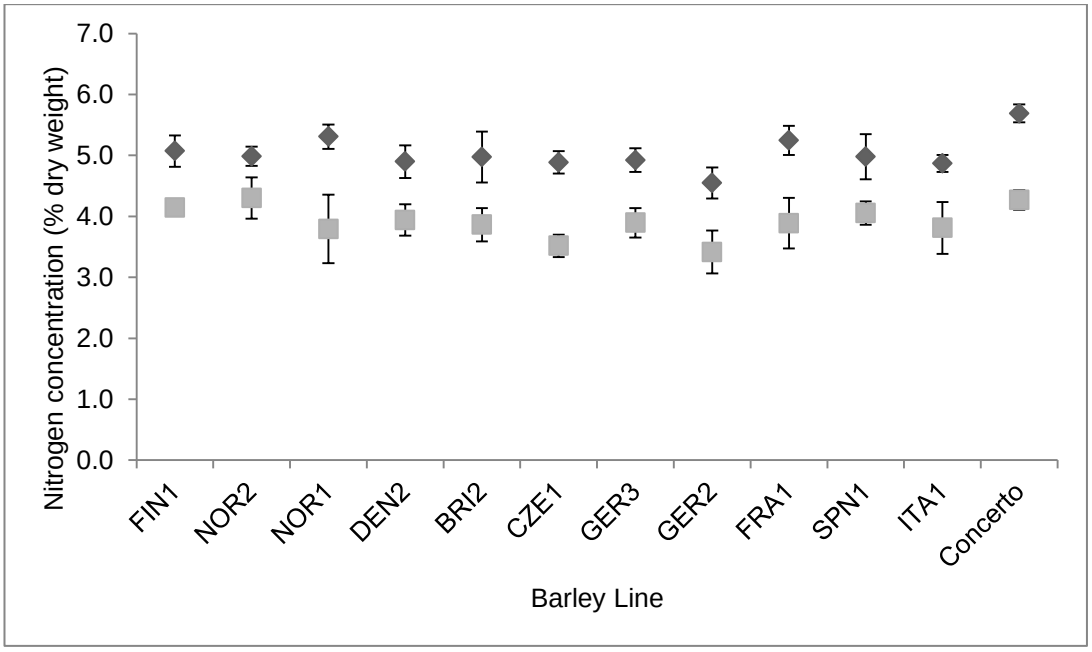

Figure 5.4 The nitrogen concentration (% dry weight) of the uppermost leaf excluding the flag leaf of the landraces at both 120kg N per hectare (grey diamonds) and 30 kg N per hectare of (grey squares) nitrogen fertiliser treatments. Error bars are standard error. Each point represents one landrace line at the stated fertiliser level averaged over two years of the field trial. In 2014 there were three replicates per line and in 2015 there were four replicates per line.

Table 5.4 The landrace isotope and elemental analysis results. The mean and the range of values are given for each of the measures and split between the 120kg N per hectare and 30kg N per hectare fertiliser treatments. Results of the ANOVA are given for the lines with all the data combined and then with the fertiliser treatment looked at as a factor.

Isotope analysis	**Mean 120kgN/hectare**	**Mean 30kgN/hectare**	**Mean Range for 120kgN/hectare Fertiliser**	**Mean Range for 30kgN/hectare Fertiliser**	**ANOVA between lines**	**Fertiliser**	**Interaction**
Nitrogen concentration (% dry weight)	5.00	3.91	4.85-5.27	3.44-4.26	p=0.239, $F_{10,124}$=1.30	p<0.001, $F_{1,124}$=127.77	p=0.833, $F_{10,124}$=0.57
$\delta^{13}C_{V\text{-}PDB\ (‰)}$	-29.67	-29.63	-28.82 - -30.12	-28.87 - -30.23	p=0.002, $F_{10,124}$=3.06	p=0.829, $F_{1,124}$=0.05	p=0.991, $F_{10,124}$=0.24

Table 5.5 Relationship between carbon isotope ratio and the latitude and climate variables in the location of origin

	Latitude (degrees)	**Precipitation (mm)**	**Hour of Sunlight**	**Temperature (^oC)**	
$\delta^{13}C_{V\text{-}PDB\ (‰)}$	p=0.258, $t_{1,10}$=1.46, R^2=0.04	p=0.908, $t_{1,10}$=0.01	p=0.166, $t_{1,10}$=2.27, R^2=0.11	p=0.206, R^2=0.07	$t_{1,10}$=1.86,

5.4 Discussion

5.4.1 Harvest Index and Components of Yield

In order to examine how well the landraces maintained their yield production and biomass partitioning under low input system two different fertiliser treatments (120kgN/hectare for a high input system and 30kgN/hectare low input system) were used and the HI, 1000 grain weight and number of grains per ear were examined. The landraces were split into 2 and 6 row lines as it was felt that there could not be a direct comparison between the two groups of lines. There was variation seen between the landrace lines in all aspects of yield and resource partitioning examined (Table 5.1). It must be noted that the number of ears per m^2 was not measured and this is perhaps the most important determinant of yield as this can be very responsive to nitrogen supply. The overall yields are not particularly of interest for this study as if the landraces were used in modern breeding it would be for the introduction of other traits such as photosynthetic efficiency. What may be of importance is the ability of the landraces to maintain yields when under stress (Richards, 2000; Dwivedi *et al.*, 2016).

The 1000 grain weight of the 2 row landraces was different between the 120kgN/hectare and 30kgN/hectare fertiliser inputs with the weight of 1000 grains grown under high inputs being heavier (Table 5.2, Figure 5.2). This was a reduction of 6% of grain weight for a 75% reduction in fertiliser. It could be that as the field site at Boghall has high residual soil nitrogen content (~63.5kgN/hectare (Pappa *et al.*, 2011)) and that at another site a larger decrease in grain weight would be observed. It may be that as there were only four 6 row lines that it may not be a large enough sample size to see an effect between the fertiliser inputs. It may also be that the 6 row lines are responding differently to the 2 row lines in terms of grain weight and that individual grain weight is more set. The number of grains per ear did not vary between the fertiliser treatments (Table 5.1) which suggest that the grain number per ear is predetermined and it is the grain filling and the grain weight which is affected in addition to the number of ears per m^2 when nutrient supply is limited. This is backed up by what was seen in the 2 row lines in terms of 1000 grain weight. Some studies have found relationships between numbers of grain and grain weight

(Calderini & Reynolds, 2000; Acreche & Slafer, 2006) with a study in wheat showing a reduction in average grain weight with increasing numbers of grain (Acreche & Slafer, 2006) and there is disagreement over whether competition between the grains for resources reduces weight when there are more grains present (Borrás *et al.*, 2004). Artificially removing grain to study source-sink limitations has shown that remaining grain become heavier when others are removed although it can depend upon the time point at which this is done (Calderini & Reynolds, 2000).

A study in wheat showed a reduction in grain yield and above ground biomass under low nitrogen inputs (Gaju *et al.*, 2016). Interestingly this study also compared landraces with modern cultivars and found that there was a relative greater reduction in yield in the modern cultivars compared to the landraces. This held true for some of the landraces studied here in the components of yield that were measured but was not a universal trend as some lines had much larger reductions in grain weight than the reference Concerto under 30kgN/hectare nitrogen conditions. Another study in wheat which looked at yield and NUE at a variety of nitrogen treatment levels found that yields increased with increasing fertiliser levels (Barraclough *et al.*, 2010). This pattern holds true for barley as well as wheat as a study which looked at both species under low and high nitrogen input environments as part of a larger study into source or sink limitations found that there were greater yields under high nitrogen treatments (Serrago *et al.*, 2013).

Reducing nitrogen inputs are going to have an impact on yields and this is not ideal as there is continued pressure for yields to remain high due to an increasing world population. When looking at landraces as possible sources for breeding a decision must be made between using lines which already have high yields such as GER2 or lines which are more resilient in terms of yield loss to lower nitrogen inputs such as CZE1. A full assessment of yield including the number of ears per m^2 should be carried out. The grain quality must also be considered depending on the industry in which the grain will feed into. Do lower nutrient inputs have a detrimental effect on grain quality?

5.4.2 Chlorophyll Content and Fluorescence

The chlorophyll content of the leaves is important in allowing effective light capture but it has the possibility to be affected by low nitrogen inputs as 2% of all nitrogen taken up ends up in the chlorophyll (Parry & Hawkesford, 2010; Monostori *et al.*, 2016). There were differences seen in leaf chlorophyll content at both GS39 and GS59 between the two fertiliser treatments (Table 5.2, Figure 5.3). At both GS's there was seen to be higher SPAD readings in the lines grown under 120kgN/hectare fertiliser inputs than in the 30kgN/hectare fertiliser treatments. This follows what was seen in the literature in other crops. A study of wheat which looked at adding increasing fertiliser treatments saw an increase in SPAD reading in the fertiliser treated lines (Monostor*i et al*, 2016). A study looking at a variety of fertiliser types on wheat showed a higher flag leaf chlorophyll content under all fertiliser treatments when compared to no fertiliser (Jiang *et al.*, 2004). Another study which looked at barley under different nitrogen regimes also saw lower readings of chlorophyll content on a chlorophyll meter than in plants grown under high nitrogen (Zhao *et al.*, 2016). The Green Area Index (GAI) of both wheat and barley was seen to be greater under higher nitrogenous fertiliser inputs (Sieling *et al.*, 2016), this means that there is a greater leaf area over which chlorophyll could be spread under high nitrogen conditions. Other aspects of photosynthetic efficiency may also be impacted in response to nitrogen deficit.

5.4.3 Leaf Nitrogen Concentration and Carbon Isotope Analysis

The biomass production and the utilisation of the nitrogen taken up by the plant may also be affected by reductions in fertiliser inputs. The nitrogen concentration (% dry weight) of the leaves were not different between the landrace lines however there was a decrease in nitrogen content under the 30kgN/hectare fertiliser treatment (Table 5.4, Figure 5.4). This is perhaps to be expected as if there is a reduced supply of nitrogen from fertilisers then this is going to be reflected in the makeup of the leaves. This is important as part of grain filling involves the remobilisation of nutrients from the leaves into the grain though senescence (Foulkes *et al.*, 2009; Parry & Hawkesford, 2010; Yang *et al.*, 2016). Fewer resources in the leaves may have an impact on the grain filling and thus the harvestable product.

The nitrogen content of the leaves follows trends seen in other studies where reduced nitrogen available for uptake is reflected in a reduced content in the tissues. The allocation of nitrogen to the different tissues has been seen to vary depending on the nutrient availability. Crops with a plentiful supply of nitrogen were seen to have higher specific leaf areas (SLA) and green leaf area (GLA) (Sieling *et al.*, 2016; Weymann *et al.*, 2017) in wheat, barley and oilseed rape. The nitrogen content of different tissues in oilseed rape was seen to respond differently with decreased nitrogen supply, the leaves nitrogen content is related to the dry matter content but the stem nitrogen content decreased (Sieling *et al.*, 2016). A study which looked at the NUE of wheat landraces and modern cultivars found that there was increased NUE when under low nitrogen inputs rather than high inputs (Gaju *et al*, 2016). This is interesting as it may be that the plants can sense a possible nitrogen deficiency and adjust their uptake to make the most of what is available. An equally possible explanation here is that the high fertiliser inputs used in this study were surplus to the requirements of the plant.

There was a difference in the carbon isotope composition between the individual lines which shows that there is variation between the landraces in the efficiency of their gas exchange over their life (Table 5.4). The isotopic composition did not change between the two fertiliser treatments indicating that there was no real change in stress level affecting gas take-up by activating the closure of the stomata, but that the plants were utilising the available resources differently in maintaining yield. A study of wheat which looked at the $\delta^{13}C$ composition of grain and flag leaves showed no relationship between leaf $\delta^{13}C$ and yield (Foulkes *et al.*, 2016) there for there is the suggestion the leaves may not give an indication of whether there will be an impact on yield, however it will give information about the transpiration efficiency. It would be interesting to look at the isotopic composition of different tissues to establish if there are differences in how the partitioning of resources between the tissues is affected by stress.

Throughout all traits measured in this study the results of the ANOVAs showed no interaction with the landrace line and the fertiliser nitrogen supply. This lack of interaction suggests that all landraces are responding equally to the change in N

supply which indicated that there will be little variation present to exploit in breeding.

5.4.4 Conclusions

In conclusion, nutrient stress affects the landraces in terms of some components of yield and biomass production. It must be noted that the intrinsic level of nitrogen in the soil at the Boghall field site is relatively large (~63.5kgN/hectare (Pappa *et al.*, 2011)) so greater differences may be observed in sites where there is a lower soil nitrogen content. It is important in fertiliser application to take the latent nitrogen content of the soil into account (Delin *et al.,* 2005; Mengel *et al.,* 2006).

- The 1000 grain weight was reduced under 30kg/hectare fertiliser input as opposed to 120kg/hectare.
- There was a reduction in SPAD readings under 30kg/hectare fertiliser input as opposed to 120kg/hectare indicting that there was a reduced chlorophyll content.
- The nitrogen concentration in the leaf was lower under 30kg/hectare fertiliser input as opposed to 120kg/hectare. This may affect the amount of nitrogen available for remobilisation into the grain during senescence.

6. General Discussion

This thesis aimed to answer the question do spring barley (*Hordeum vulgare* ssp.*vulgare*) landraces contain useful variation in components of photosynthetic efficiency that could be exploited to increase yields of cultivated barley. Initially the canopy structure and gas exchange rates of modern cultivars were measured to find out the amount of variation present in the modern germplasm and whether these traits have changed as a side effect of breeding over time (chapter 2). The canopy structure (chapter 3) and gas exchange components (chapter 4) were examined in European barley landraces in greater detail under both standard (120kgN/hectare) and low (30kgN/hectare) nutrient inputs (chapter 5) to see if there is useful variation present that may be utilised in future breeding.

6.1 Photosynthetic Efficiency of Spring Barley Cultivars Released over the Last Sixty Years

The aim of chapter 2 was to answer the question is there variation in photosynthetic efficiency in modern elite cultivars of spring barley released over the last 60 years and has this changed over year of release? Characters of canopy structure including leaf length and specific leaf area (SLA), and leaf chlorophyll content (measured using a SPAD meter) were established alongside rates of photosynthesis, stomatal conductance and transpiration.

The most interesting results from this study were that leaf length increased and chlorophyll content early in the plants establishment had decreased with cultivar release over the last 60 years (chapter 2: figure 2.3, Table 2.2). It was concluded that the increase in early growth stage leaf length may allow a greater canopy area to be established earlier in the season not only increasing the length of canopy maintenance but out competing weed establishment. Increasing the length of time of canopy maintenance or ‘green time’ through fast canopy establishment and delayed senescence has been linked to increased yields (Richards, 2000; Zheng *et al.*, 2009). The problem in this is that most modern cultivars are bred to be generally adapted to conditions over wide geographical areas so the entire length if the growth season in one location may not be utilised due to restrictions in the rest of the varieties range. It

would perhaps be of benefit to breed cultivars that are locally adapted to conditions but this will have other considerations to take into account such as economic cost and demand from further down the usage chain.

Lowering of chlorophyll content early in the plants development with year of release could be an effect of the plants initially utilising their resources in growth and biomass production (Parry & Hawkesford, 2010; Monostori *et al.*, 2016). Opposing trends in different cereals have been observed with chlorophyll content decreasing in rice yet increasing in wheat over time (Watanabe *et al.*, 1994; Hubbart *et al.*, 2007; Sadras *et al.*, 2012; Gaju *et al.*, 2016). This may be an unintended side effect of breeding for other favourable traits where in barley and rice parents with lower chlorophyll contents were selected for breeding because they contained a favourable phenotype in another area such as disease resistance. If the canopy structure could be optimised so that self-shading was not an issue then having high levels of chlorophyll spread throughout the canopy could allow more light energy to be captured. It may be that along with the decrease in chlorophyll content seen here that in order to improve the photosynthetic efficiency of barley and perhaps carry this through to yield that there is not enough variation seen in modern cultivars to be useful in breeding. This is why this study then went on to look at the photosynthetic efficiency of landraces.

6.2 Photosynthetic Efficiency of Spring Barley Landraces

Landraces are heterogeneous plant varieties which have high levels of variation and tend to be locally adapted. This study (chapters 3 and 4) looked at the canopy structure (ε_i) and the conversion efficiency (ε_c) of spring barley landraces from a wide geographical spread across Europe to assess variation and local adaptation to climatic conditions. The main questions asked in these chapters were is there variation in European spring barley landraces in aspects of their ability to intercept (ε_i) radiation and is this locally adapted to environmental conditions (chapter 3), and is there variation in the gas exchange and conversion efficiency (ε_c) of spring barley landraces (chapter 4) and does this relate to environmental conditions at the location of origin?

The main finding in canopy structure was the faster establishment of the canopy in landraces from Scandinavia compared to those from central and southern Europe (chapter 3, figure 3.3). Canopy traits at the leaf level showed variation in the angle that the leaves were held at within the canopy with northern landraces having a more planophile canopy than those from further south (chapter 3, figure 3.5). In terms of ε_c the photosynthetic rate or stomatal conductance did not show any difference between the landraces lines. It has been suggested that a high stomatal conductance sustained over a long period of time is one way in which rates of photosynthesis could be boosted but it appears that there may not be the variation present in the landrace material studied here to achieve that (Richards, 2000; Tanaka *et al.*, 2013). There were also seen to be large differences between the ranges of stomatal densities of different landrace lines (chapter 4, figure 4.5).

In order to assess whether any of this variation seen in photosynthetic efficiency of the landraces will be useful in terms of future cultivar breeding all aspects of canopy structure and photosynthetic efficiency must be taken into account. Canopy structure optimisation requires a vertical canopy structure to involve more leaf surface in light interception. This was seen in the Southern European landraces in this study but would only be beneficial if the rate of photosynthesis per unit leaf area increased rather than the existing apparatus becoming more spread out over the surface area (Parry & Hawkesford, 2010).

Ensuring that increases in photosynthetic capacity actually result in increased yields would be essential in order to ensure the use of increasing photosynthetic rates. Some studies have failed to find any correlation with yield and higher rates of photosynthesis before anthesis in wheat (Sadras *et al.*, 2012; Driever *et al.*, 2014). It may be that under non-optimal conditions (such as on farm settings) there are other factors limiting yield such as temperature, fertiliser application or stomatal limitations caused by light and water variability (Lawson & Blatt, 2014). Lines with higher rates of photosynthesis along with higher stomatal responsiveness may be a way to compensate but only if water-loss is not a limiting factor though high environmental temperatures or drought. It may also be that enzymes such as Rubisco are more limiting to yield so unless their efficiency is improved alongside rates of

photosynthesis then improvements in yield will not follow. Other studies have found a link between photosynthetic rates (Fischer *et al.*, 1998) and yields or have not drawn any definitive results (Watanabe *et al.*, 1994). Fischer (1998) found an increase of 23% in photosynthetic rate which accompanied a yield increase of 27%. It may be beneficial to monitor photosynthetic rates in field over a much longer period of time in order to assess the plant's capacity.

The variation seen in chlorophyll content, stomatal density, canopy establishment and leaf angle could be used in future breeding with the landrace utilised depending on the trait of interest. There was evidence of links between some traits and the climate in the location of origin for the landraces with could be useful in identifying collections of landraces which could be utilised in future breeding for specific traits.

6.3 Maintenance of Photosynthetic Efficiency and Yield under Low Nitrogen Inputs

In Scotland, high levels of fertilisers are applied to crops and there may be pressure in the future to reduce this due to environmental effects. When selecting landraces for inclusion in future breeding programs it is important to understand how any trait of interest responds to a low nitrogen input system. Chapter 5 aimed to answer the question are traits associated with the photosynthetic efficiency of spring barley landraces affected by low nitrogen inputs? In this study the chlorophyll content of the leaves was reduced at 30kgN/hectare of fertiliser compared to 120kgN/hectare (Chapter 5, Figure 5.3, Table 5.2) which follows what has been observed in barley and other crops. The chlorophyll content of wheat was affected by reduced fertiliser inputs (Jiang *et al.*, 2004; Monostori *et al.*, 2016) and in barley chlorophyll content readings were lower under low nitrogen regimes than under high nitrogenous fertiliser inputs (Zhao *et al.*, 2016).

Low fertiliser inputs may also be expected to have an effect on yield production and even though yield per se was not measured here it was seen here that under low nitrogen inputs components that contribute to yield such as the 1000grain weight of the 2 row landraces was reduced (Chapter 5, Table 5.1, Figure 5.1, Figure 5.2). Grain yield has been showed to decrease under low fertiliser inputs (Barraclough *et al.*, 2010; Gaju *et al.*, 2016) and it has been seen that landraces are affected less than

modern cultivars by reduced levels of nitrogenous fertilisers (Gaju *et al.*, 2016). This did not hold true for all the landraces studied here with the modern variety Concerto maintaining its 1000gw at a higher level than some of the landraces. Improved capture and utilisation of latent soil nitrogen will be an important trait to assess in conjunction with photosynthetic efficiency when selecting lines to be included in future breeding programs.

6.4 Utilising Landraces in Breeding Programs and Developing Locally Adapted Cultivars

As climate change begins to affect growth conditions and crops begin to experience increasing levels of stress such as drought, water-logging or extreme temperatures, breeders will need to focus on breeding for resistance or resilience to these conditions. This could be through breeding for smaller geographical areas and 'local adaptation' to climate conditions, or the development of lines which can cope with stress with as small a yield penalty as possible. This work suggested that there were traits associated with both approaches present in the material studied with variation being present in the canopy structure (chapter 3) which may show evidence of local adaptation to temperature (chapter 3, figure 3.5) although to be conclusive the trials would have to be expended to other geographical areas. This could be utilised to develop a canopy in spring barley which will allow a balance between efficient light capture and reduction of temperature stress which may lead to leaf scorching and water deficit. Developing optimal canopies for smaller geographic areas will allow the maximum amount of light energy to be captured. Alternatively, there was variation seen between the landraces in their ability to cope with stressful conditions whilst maintaining yield (chapter 5). Under nutrient stress it was seen that some of the lines are able to maintain their grain weights well such as CZE1 whereas other lines such as BRI2 are unable to continue to produce the weight of grain under stress. Tools such as chlorophyll fluorescence or isotopic analysis enable the amount of stress the lines are under to be assessed by breeders. As landraces collections contain greater genetic diversity than current pools of cultivar parents used in breeding programs and they contain adaptations to a wide range of environmental conditions they are ideal candidates for future breeding (Lopes *et al.*, 2015).

The scale of variation seen in this thesis in traits between the landraces from chapters 3 and 4 and the modern cultivars in chapter 2 varied between trait. When Concerto was plotted with the landraces it fitted in their range and indicated that although the landraces might have larger ranges of variation in some traits but were not on hugely different scales than the modern cultivars. In terms of leaf length and SLA the landraces had a larger range of leaf lengths but a smaller range of SLA's than the modern cultivars from chapter 2. The variation in leaf length could be used to breed for a canopy that maximises light capture. There were similar levels of variation seen in SPAD units between the landraces and modern cultivars but as the trend in the cultivars had been for a decrease in newer lines landraces could be used in breeding to boost the levels of chlorophyll. In photosynthetic rate the landraces and the cultivars had similar ranges but the landraces had higher rates when comparing chapters 2 and 4 but this is probably due to the difference in growth conditions between glasshouse and growth chamber as Concerto grown in the chamber had similar rates of photosynthesis to the landraces. It may be that there is not much more variation in landraces than the current pool of cultivars to use in breeding to boost photosynthetic rate.

Landraces could be utilised in breeding programs to introduce traits and some landraces are still grown in marginal areas as they outperform elite cultivars (Dwivedi *et al.*, 2016). Spanish landraces have been show to out yield modern cultivars when grown at sites which were known for their unproductivity (Yahiaoui *et al.*, 2014). It was suggested that this was because the landraces had a better capability to continue to produce well filled grains under stress. Landraces have already been used to bring important traits into elite cultivars in a number of different crops. In maize, landrace lines are incredibly diverse and work to introduce some of this allelic diversity into cultivar populations has resulted in lines which produce more grains than parents under drought stress conditions (Meseka *et al.*, 2013, 2015). Some of the lines looked at in this study contained up to 7% higher yield than their parents. Landraces have also been utilised in rice breeding for flood tolerance in which a line was identified which contained a locus called *SUBMERGENCE 1 (SUB1)* which allowed for plant submergence (Xu & Mackill, 1996). This was then identified to contain three genes of which *SUB1A'*s tolerant form was introgressed

into cultivars widely grown in Asia (Bailey-Serres *et al.*, 2010). In order to utilise possible traits from barley landraces in breeding there are a number of possible avenues that could be explored. These traits might confer better climate adaptation or may be of other beneficial traits.

6.5 Breeding with Landraces

There are large collections of landraces which are held in many different Gene banks around the world. In order for the variation held within these centres to be utilised efficiently by breeders there are a number of different approaches that could be taken, whether this is through phenotyping or genetic sequencing or both (Lopes *et al.*, 2015).

Genetic screening, alongside phenotyping, will be useful in order to assess the genetic diversity within a collection of landraces throughout the initial stages of breeding programs to form core groups of material with high genetic diversity in a specific trait of interest (i.e. nutrient deficiency tolerance) from which to utilise in breeding and to preserve variability which can be utilised later in selection (Pessoa-Filho *et al.*, 2010). As landrace collections are usually sizeable it will be of benefit to develop sub-collections around specific traits of interest, or adaptation to environmental conditions in a specific area which can be easily accessed by breeders. Identifying new allelic variation in key traits of interest in collections is also an approach to utilise landraces in breeding (Kumar *et al.*, 2010). An example of when this approach has already been utilised with breeding is the identification of new wheat powdery mildew resistance alleles of the gene *Pm3* in landraces (Bhullar *et al.*, 2009, 2010).

Phenotyping is a further way to assess landrace collections for traits that may be used for adaptation to stress or (as has been done in this study) for photosynthetic efficiency. The has been used at CIMMYT (International Maize and Wheat Improvement Centre) in three way crosses using landraces for stress adaptive traits (Reynolds *et al.*, 2009b). In Mexican wheat a landrace was used to introduce the ability to extract water from deeper in the soil and the line produced entered through into trials for release for growth. Reynolds (2009) gives a nice summary of the process of breeding for traits through phenotypic selection.

In order for the variation in photosynthetic efficiency discovered in this study to be of use in future breeding programs, the likelihood of the material being utilised must be assessed. Plant breeders, when looking to create a new variety, preferentially utilise parents from elite lines as they are familiar with the material and will have a high chance of obtaining lines which could go on to be recognised as new cultivars relatively quickly (Dwivedi *et al.*, 2016). In order for landraces to be considered as possible parents they must provide traits that will be of sufficient benefit to make the greater amount of time spent to produce a new variety worth their while economically (Sharma *et al.*, 2013; Lopes *et al.*, 2015). The development of new cultivars of spring barley is driven from a number of directions from breeders, scientists, farmers and end product users. Breeders pursue the goal to increase yield and quality and produce varieties that will pass the stringent tests to get onto the recommended lists. Scientists look for novel traits such as resistance to a disease (e.g. powdery mildew (Buschges *et al.*, 1997)), farmers look for varieties that can produce the greatest yield and highest quality and be sold for the highest possible price and end product users want grain that will be suitable for their processes. Whether a new variety is taken up by the farming community can be unaffected by whether it has better photosynthetic efficiency than other cultivars but may be dictated by the likelihood of selling their product. This could be an obstacle in the development of locally adapted cultivars of spring barley in Scotland with the maltsters dictating to farmers the varieties that they are willing to purchase. In malting it can take a lot of work to optimise the process for a specific variety and thus from a business point of view it is sensible to buy the cultivar that you are familiar with and know how to best work with it.

There must be coordinated work between scientists, breeders and end product users to ensure that including landrace material into breeding programs is done in a way that will ensure its uptake into the general market. Utilising advances in technology to make the breeding process quicker and more targeted will also be essential to keep the costs down of the longer process that will be needed in order to introduce the traits form landraces into cultivars.

6.6 Next Steps in Research

This work could be taken in a number of different directions in the future both phenotypically, physiologically, genetically or socially. It would be interesting to do a social study or survey in which breeders, farmers, maltsters and other end product users were surveyed in order to assess their views on landrace material and their willingness to utilise landraces in the future of breeding programs. This would ensure that proposing landraces as sources of variation for future breeding will be utilised by breeders and that the correct traits are targeted as being important for retention in new material.

It would also be an interesting next step to look at the genetic control behind some of the traits that exhibited greater variability within the landrace collections such as chlorophyll content, growth rate and stomatal density. There has already been work to identify QTLs (quantitative trait loci) involved in chlorophyll content in barley (Guo *et al.*, 2008; Xue *et al.*, 2008; Liu *et al.*, 2015) which could be expanded upon and could perhaps be used to develop new screens to enable large collections of landraces to be surveyed quickly for traits of interest.

Developing technology could be utilised to allow greater numbers of plants to be phenotyped in a short space of time. Measuring chlorophyll content has already benefited from advances in technology with the development of the SPAD meter which is quick, easy and practical to take into the field. Advancements of this sort in the measurements of gas exchange and perhaps the use of remote sensing such as drones to gain quick information on the canopy structure would be of benefit in identifying interesting lines rapidly.

It has been suggested that varietal mixes may be a way forward when it comes to increasing yields in environments which are variable by providing resilience to pathogen spread, lodging, and yield increases when under stress from competitive release (use of released resources by unstressed lines from stressed lines) (Kiær *et al.*, 2012; Creissen *et al.*, 2016). The mechanisms behind increased yields in varietal mixes are not entirely understood but it may be interesting to examine how the photosynthetic efficiencies of crops change when grown in varietal mixes. Are there impacts on canopy structure or light interception? Examining how the different lines

complement each other could lead to new understandings of the way different aspects of photosynthetic efficiency interact with the environment.

6.7 Final Conclusions

This thesis aimed to answer the question do spring barley (*Hordeum vulgare ssp. vulgare*) landraces contain useful variation in components of photosynthetic efficiency that could be exploited to increase yields of cultivated barley. The main take home messages from this work are:

- There have been changes in modern varieties released over time with chlorophyll content decreasing and leaf length increasing showing changes through modern pedigree breeding.
- In the landraces there was variation seen in canopy structure between the lines which could be made use of in future breeding to develop an erectophile canopy. Some novel results showed that canopy structure (the angle at which the leaves are held) varied depending on the geographical location and latitude of origin of the line reflecting possible local adaptation with lines from Northern climates possessing a more planophile canopy than the Southern lines.
- The amount of time between emergence and full canopy establishment also varied with location of origin with the Northern landraces reaching full canopy establishment faster than those from Southern European origins.
- There was large variation in the stomatal density of the landraces which could be of use in developing locally adapted crops especially in areas where there is increased unpredictability in weather and environmental conditions.

In conclusion, breeding must look to new resources, new traits and new technologies with research, breeders and product users working more closely together to allow the continuation of yield increases in the face of changing environmental and management conditions.

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8. Appendix 1

8.1 Additional Information for Landrace Climate Data Sources

Table 8.1 Sources of landrace climate information. The links lead to the national met offices for each country of landrace origin.

Landrace	Data source
FRA1	http://www.meteofrance.com/climat/france#rhone-alpes/regi82/normales
GER2	http://www.dwd.de/EN/ourservices/_functions/search/search_Formular.html?cl2Taxonomies_LSB_Zeit_1=zeit%2Fjahreszeit+zeit%2Fvergangenheit
NOR1	http://sharki.oslo.dnmi.no/pls/portal/BATCH_ORDER.PORTLET_UTIL.Download_BLob?p_BatchId=852963&p_IntervalId=1643806
NOR2	http://sharki.oslo.dnmi.no/pls/portal/BATCH_ORDER.PORTLET_UTIL.Download_BLob?p_BatchId=852963&p_IntervalId=1643806
CZE1	http://portal.chmi.cz/portal/dt?portal_lang=en&nc=1&menu=JSPTabContainer/P3_0_Informace_pro_Vas/P3_9_Historicka_data/P3_9_1_Pocasi/P3_9_1_3_Mapy_char_klim&last=false
FIN1	http://en.ilmatieteenlaitos.fi/maps-from-1961-onwards
BRI2	http://www.metoffice.gov.uk/climate/uk/regional-climates/wl
SPN1	http://www.aemet.es/en/serviciosclimaticos/datosclimatologicos/valoresclimatologicos?l=9263D&k=nav
GER3	http://www.dwd.de/EN/ourservices/_functions/search/search_Formular.html?cl2Taxonomies_LSB_Zeit_1=zeit%2Fjahreszeit+zeit%2Fvergangenheit
ITA1	http://clima.meteoam.it/atlanteClimatico.php
DEN2	http://www.dmi.dk/en/learn/in-general/dmi-publications/2013/

9. Appendix 2

9.1 Supplementary Canopy Structure Data for Additional Landrace Lines in Chapter Three

9.1.1 Chlorophyll Content

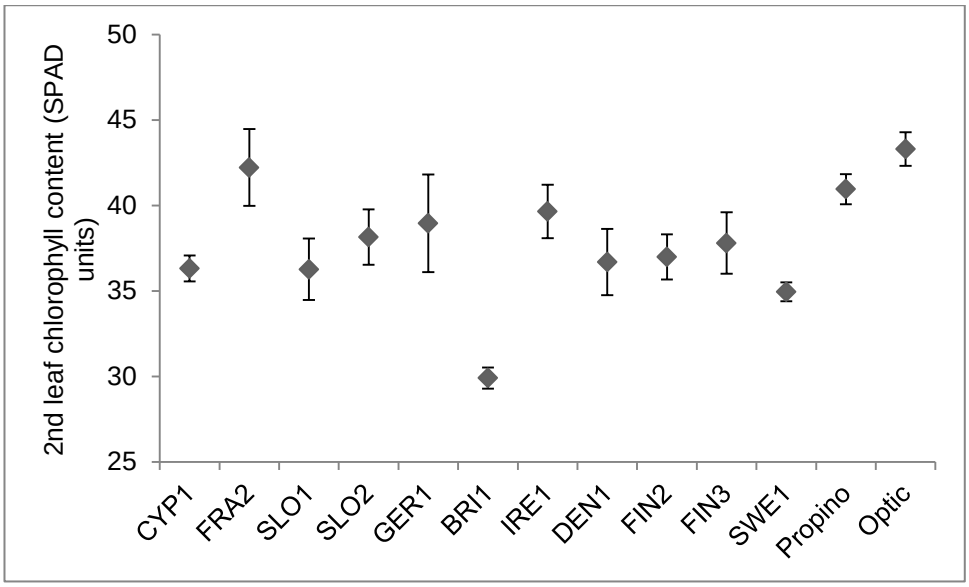

Figure 9.1 Chlorophyll content in SPAD units of the 2nd leaf at GS24. Landraces are listed from South to North left to right with modern cultivars Propino and Optic for reference. Error bars are standard error.

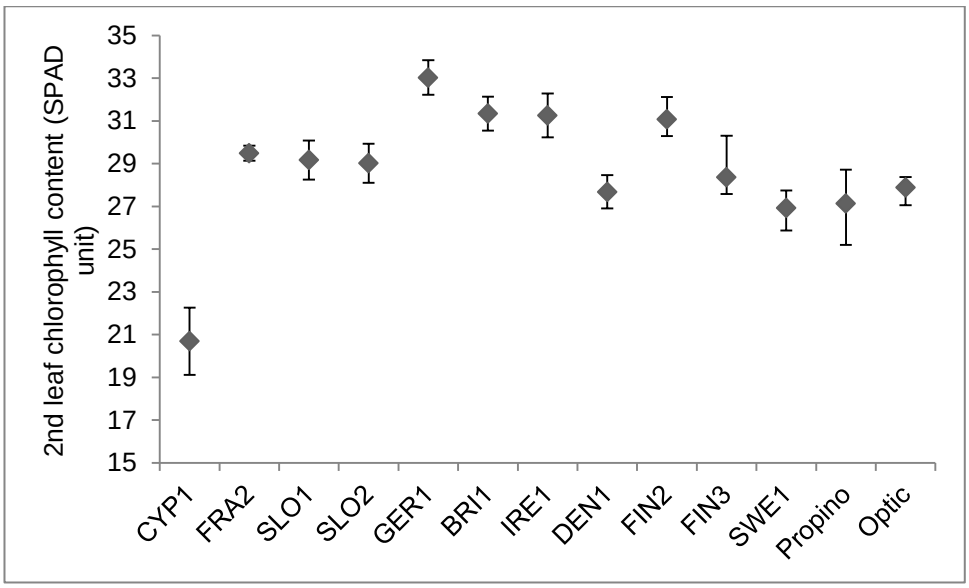

Figure 9.2 Chlorophyll content in SPAD units of the 2nd leaf at GS39. Landraces are listed from South to North left to right with modern cultivars Propino and Optic for reference. Error bars are standard error.

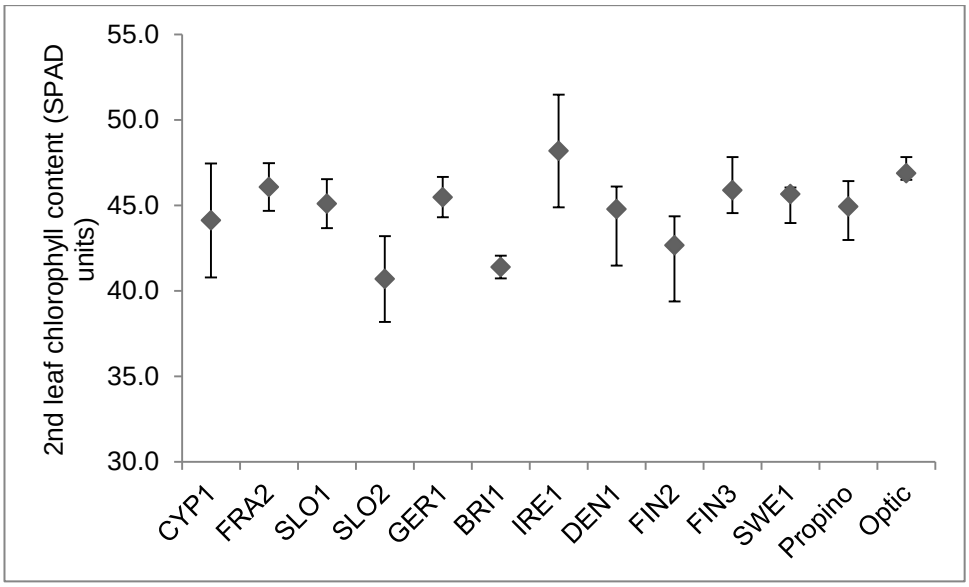

Figure 9.3 Chlorophyll content in SPAD units of the 2nd leaf at GS59. Landraces are listed from South to North left to right with modern cultivars Propino and Optic for reference. Error bars are standard error

9.1.2 Leaf Angle

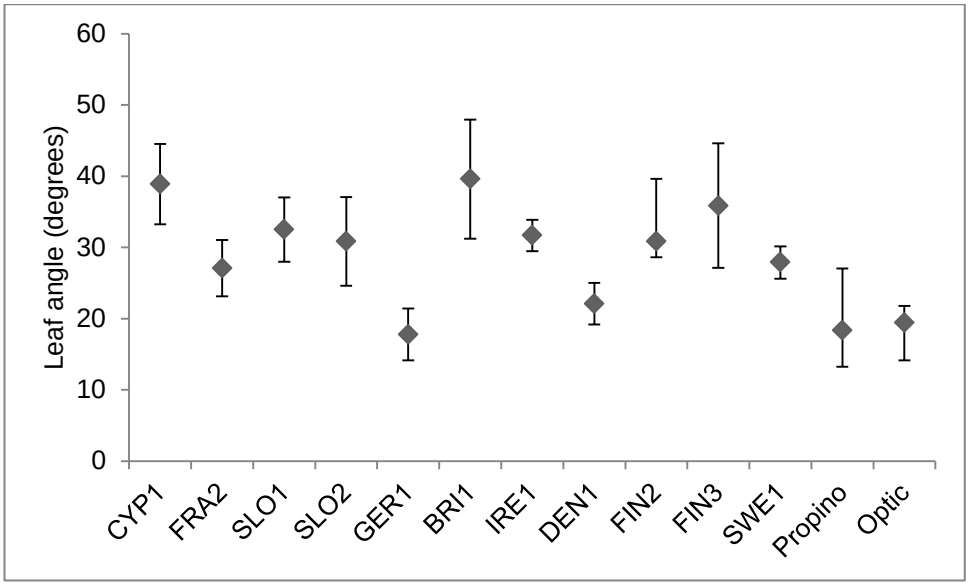

Figure 9.4 Angle of the second leaf of the main shoot to the main stem (degree) at GS39. The landraces are listed from South to North left to right with modern varieties Propino and Optic for reference. Error bar are the standard error.

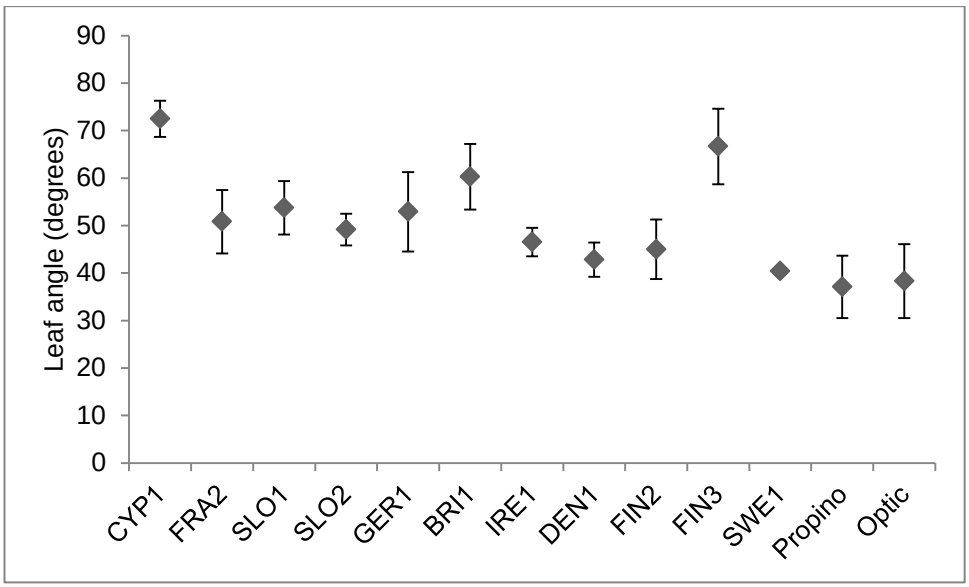

Figure 9.5 Angle of the second leaf of the main shoot to the main stem (degree) at GS59. The landraces are listed from South to North left to right with modern varieties Propino and Optic for reference. Error bar are the standard error.

9.1.3 Leaf Length

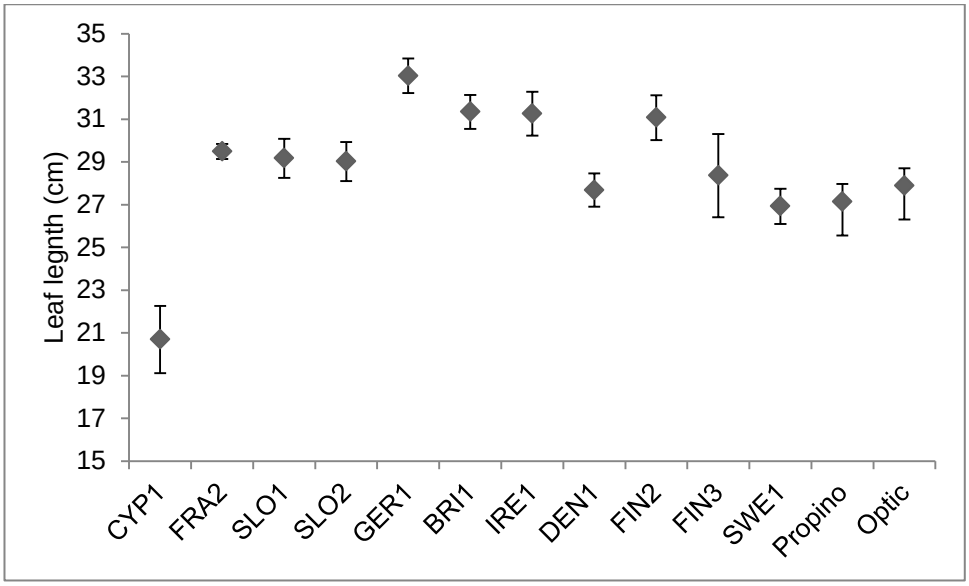

Figure 9.6 The length of the second leaf in cm on the main shoot at GS39. Landraces are listed from South to North left to right with modern varieties Propino and Optic for reference. Error bars are the standard error.

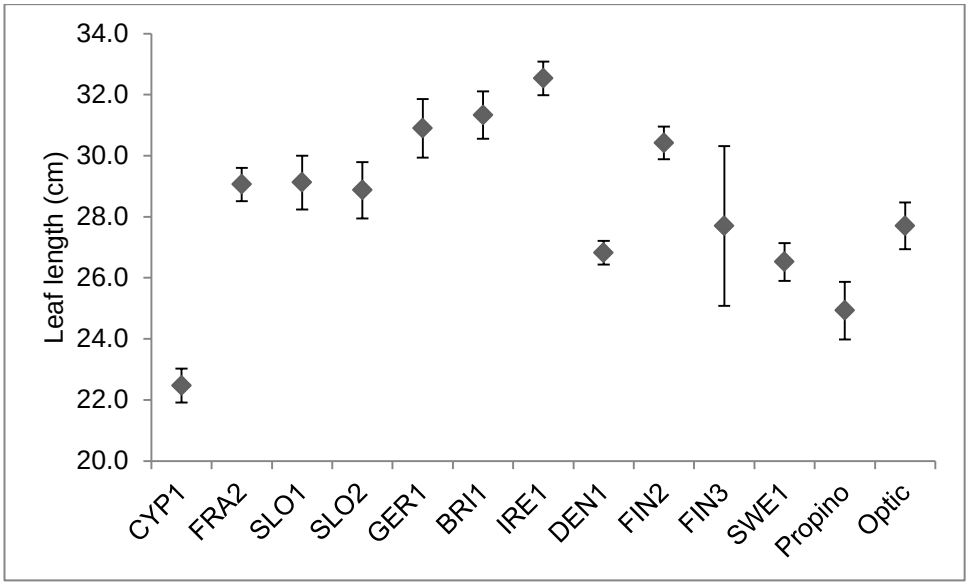

Figure 9.7 The length of the second leaf in cm on the main shoot at GS59. Landraces are listed from South to North left to right with modern varieties Propino and Optic for reference. Error bars are the standard error.

9.1.4 Specific Leaf Area

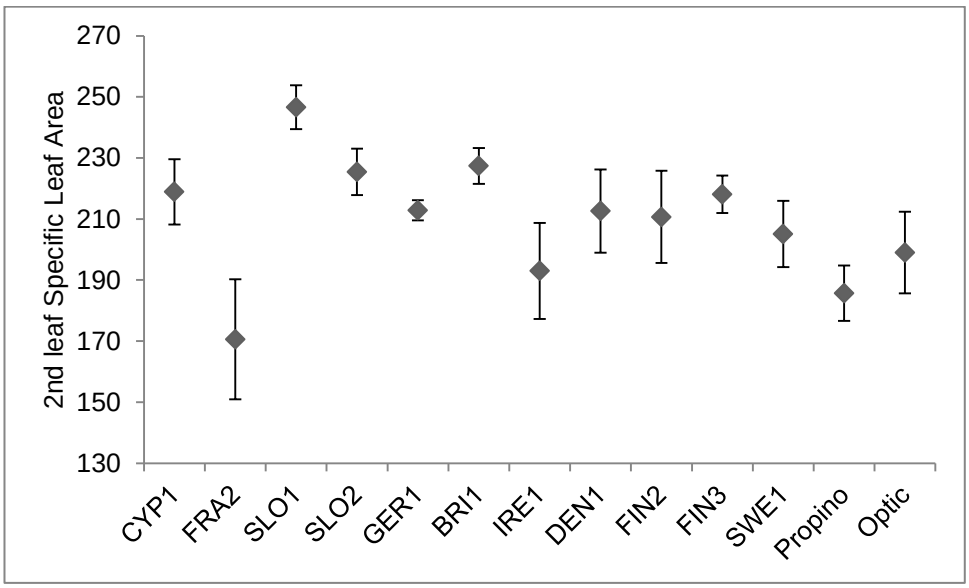

Figure 9.8 The specific leaf area of a 2nd leaf at G39. Landraces are listed from South to North, left to right with modern varieties Propino and Optic for reference. Error bars are the standard error

9.1.5 Number of Leaves

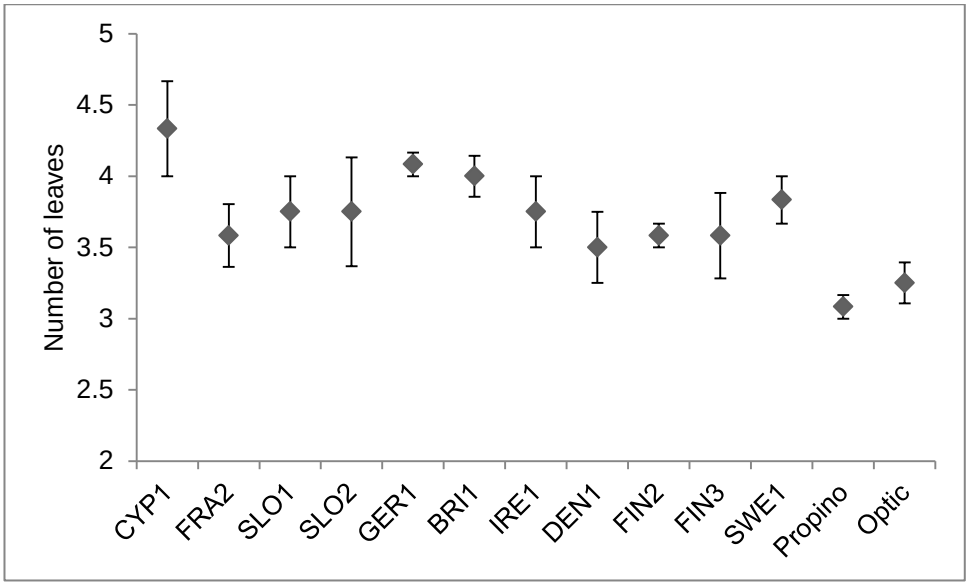

Figure 9.9 The number of leaves present at GS24. Landrace are listed from South to North left to right with the modern varieties Propino and Optic for reference. Error bars are standard error.

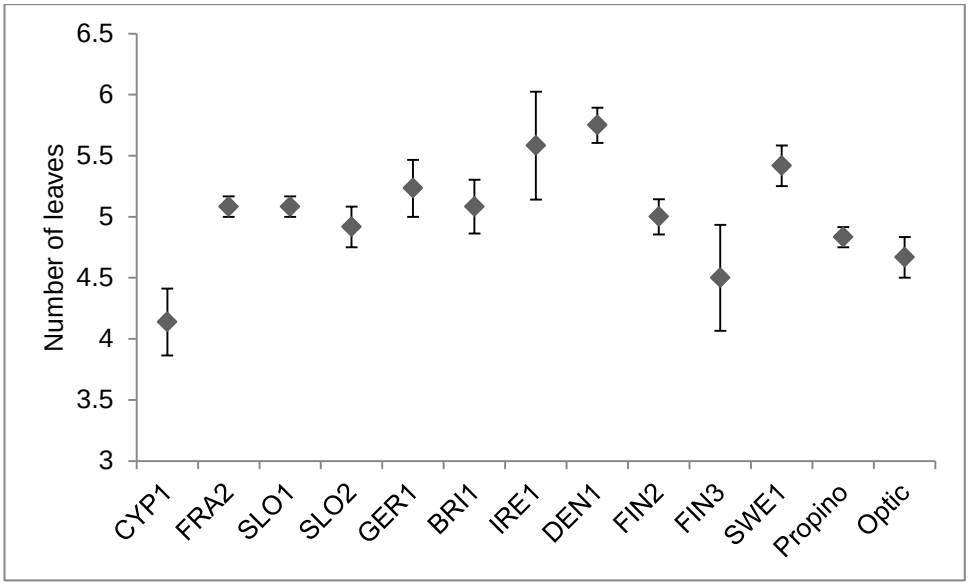

Figure 9.10 The number of leaves present on the main shoot at GS39. Landrace are listed from South to North left to right with the modern varieties Propino and Optic for reference. Error bars are standard error.

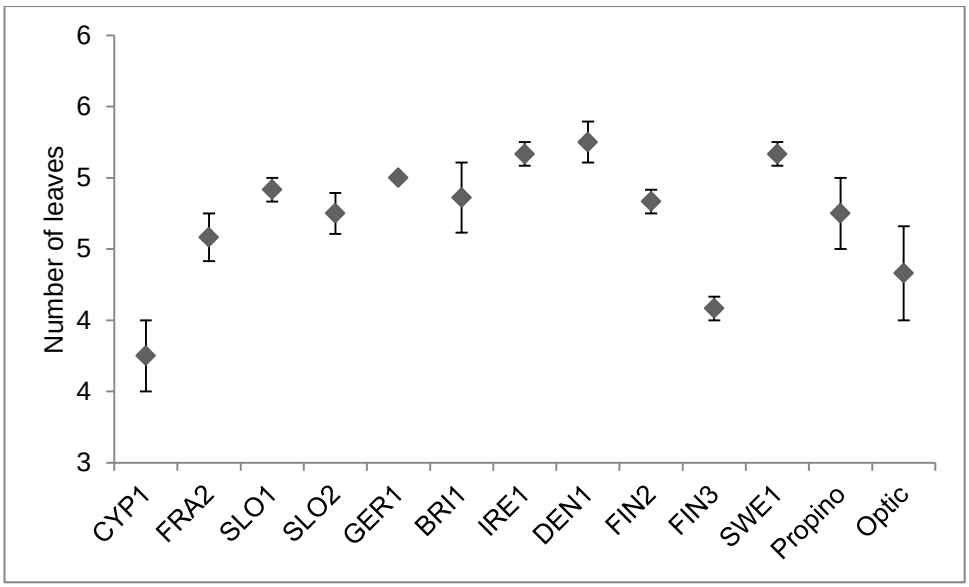

Figure 9.11 The number of leaves present on the main shoot at GS59. Landrace are listed from South to North left to right with the modern varieties Propino and Optic for reference. Error bars are standard error.