Exploiting wheat ancestral introgression for increased photosynthetic productivity under contrasting environmental conditions

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Dedicated to my Parents

Late Mr.T. Chinnathambi and Mrs. Anjali Chinnathambi

My wife Neha, my family and friends

Abstract

Global population is expected to rise to 9 billion in another 40 years and changing climatic conditions coupled with various other abiotic and biotic stress factor have posed challenges for crop cultivars globally. Cereals like wheat, rice and maize have a central place in the human diet and require immediate attention in terms of improving yield in order to satiate the global food demand. This demand can be fulfilled by improving crop yield by exploiting natural variation in modern wheat by conventional breeding method like wheat ancestral introgression. One of the key traits that can be exploited such hybrids would be photosynthesis, ongoing debate and researcher have suggested that improving photosynthesis would be an attempt towards enhancing biomass and yield in crops like wheat and rice. In this project two different approaches were used to create interspecific hybrid, these hybrid were amphidiploids, which were created by chromosome doubling of haploid chromosome from wheat and wild relatives of wheat. First, a set of wild relatives were crossed bread wheat such as Highbury, Paragon, Chinese spring mutant, Chinese spring mutant and Pavon 76) and the amphidiploids created through these crosses where exploited for photosynthetic traits and other related physiological traits under glasshouse conditions. Second, amphidiploids created by cross using a wild relative *Thinopyrum bessarabicum* with durum wheat and tested in field conditions in India. Techniques like infra-red gas exchange, chlorophyll were used to assess photosynthetic performance in the glasshouse in optimal conditions and in the field under challenging environmental conditions. Along with the amphidiploids in the field conditions, a panel of 30 Indian genotypes were tested for natural variation in photosynthesis in field conditions. Almost similar set Indian genotypes were tested in glasshouse conditions in UK, to exploit the natural

variation in photosynthesis. Initially, these Indian genotypes and amphidiploids grown in glasshouse as well as field conditions were screened for variation in photosynthesis. Promising lines derived from these instantaneous measurements were investigated for detailed photosynthetic measurements to understand the underlying biochemical mechanism that regulates photosynthesis and also were investigated leaf morphological and anatomical features for increased photosynthetic capacity. Here we show natural variation in photosynthesis in the amphidiploid population in both field and glasshouse conditions and range parameters that regulates photosynthetic rate in introgressed lines.

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Abbreviations

A	Net CO_2 assimilation rate per unit leaf area
A/Ci	Net CO_2 assimilation rate (A) as a function of [CO_2]
A_{max}	Maximum rate of carbon assimilation/ photosynthesis
ABA	Abscisic acid
ADP	Adenine disphosphate
A_{max}	Maximum rate of photosynthesis
ANOVA	Analysis of Variance
ATP	Adenine triphosphate
C_i	Intercellular CO2 concentration
CO_2	Carbon dioxide
Cv	Cultivar
CSE	Chinese spring Euploid
CSM	Chinese spring mutant
CTD or CT:	Canopy temperature depression
ETR	Electron transport rate
Fq'/Fm'	PSII operating efficiency
Fq'/Fv'	PSII efficiency factor
FT	FLOWERING LOCUS T
Fv/Fm	Dark adapted
Fv'/Fm'	Light adapted
g_m	Mesophyll conductance
g_s	Stomatal conductance
H^+	Hydrogen ion
H_2O	Water
IRGA	Infra-red gas analysis
J	Joules
J_{max}	Maximum rate of electron transport
kJ	Kilojoules
kPa	Kilopascal
LAI	Leaf area index
LCP	Light compensation point
LHC	Light harvesting complex
LHCI	Light harvesting complex one
LHCII	Light harvesting complex two
LRC	Light response curves
LT	Leaf thickness
LVD	Leaf density
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NDVI	Normalised difference vegetative index
NPQ	Non-photochemical quenching
O_2	Oxygen
PAR	Photosynthetically active radiation
PFD	Photon flux density
PGA	Phosphoglycerate/ phosphoglyceric acid
PPFD	Photosynthetic photon flux density
PSI	Photosystem I

PSII	Photosystem II
P_{max}	Maximum rate of photosynthetic capacity
Q	Light intensity
qP	Photochemical quenching
Rd	Dark respiration/ rate of mitochondrial respiration at darkness
Rday	Day respiration Rdiurnal
RH	Relative humidity
RHmax	Relative humidity maximum
RHmin	Relative humidity minimum
ROS	Reactive oxygen species
Rubisco	Ribulose - 1,5 - biphosphate carboxylase enzyme
RuBP	Ribulose - 1,5 - biphosphate
RUE	Radiation use efficiency
SEM	Standard error of means
TPU	Triose phosphate utilisation
Trnmol	Transpiration rate
V_{cmax}	Maximum velocity of Rubisco carboxylation
VPD	Vapor pressure deficit
WHP	Wheat parent
WTP	Wild type
WUEi	Intrinsic water-use efficiency
Φ PSII	Photochemical yield/ efficiency of photosystem II

Chapter 1: Introduction and Literature Review

1.1 Introduction

The father of green revolution Dr Norman E Borlaug once quoted "Food is the moral right of who all are born into this world", not surprisingly the present situation of global food availability is at record low, diminishing arable land mainly due to urban expansion and the ever-expanding human populations have posed a major problem to global food security (Tscharntke et al., 2012). Global food demand has attained more serious concerns, which ultimately suggests that the current yields from agricultural land need to be improved for the future (Tilman et al., 2011). The Intergovernmental Panel on climate change (IPCC) has stated that the crop productivity in different geographical regions of the world has been hampered or reduced as changing climatic conditions have created negative effects on agricultural growth (Lipper et al., 2014). To meet this challenge it is necessary to improve and invest in plant and crop science research in order to transform the past and present agriculture practices (Murchie et al., 2009). Global human population has reached ~7 billion (Figure 1.1) and the projected human population is set to reach ~10 billion by 2050 (World prospectus 2015).

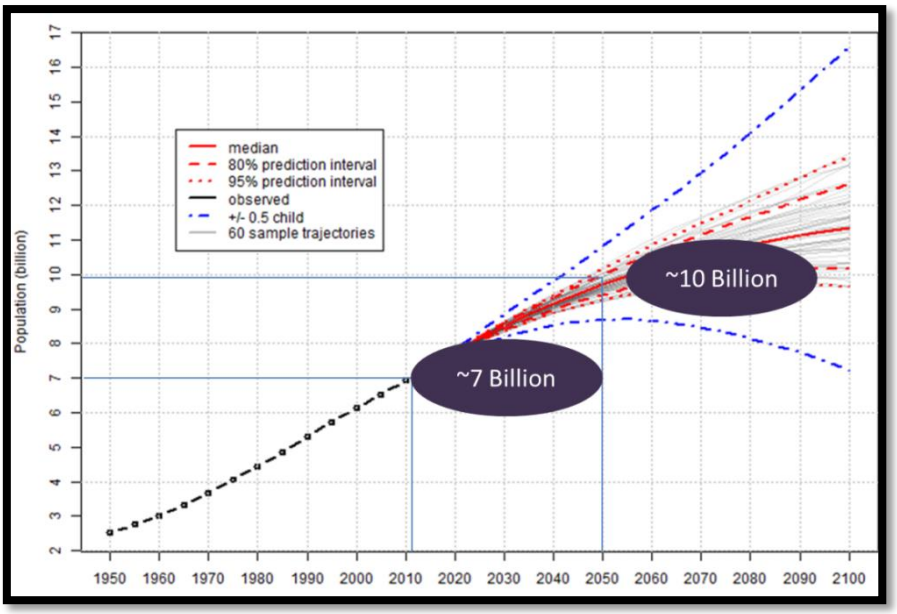


Figure 1.1: Current (~7 billion) and projected year 2050 (~10 billion) human population Source: World prospectus 2015 Revision, New York, United Nations

1.2 Wheat Evolution

The Triticeae tribe consists of 400-500 species including diploids and polyploids, which also includeseveral crop species such as a *Hordeum vulgare* (Barley), *Secale cereale* (Rye) and *Triticum aestivum*(Wheat). During the process of domestication, these species have undergone a lot of changes in their genetic diversity as compared to their wild ancestors. Triticeae consists of many polyploid species, for example Bread wheat, *T. aestivum* comprises of 3 genomes (AA, BB and DD), with ~17 Gb size (Middleton et al., 2014). The complete genome complement of *T. aestivum* was formed from the hybridisation of three diploid ancestors (Figure 1.2). For a genetic gain in wheat yields it is important to gain a better understanding of the evolution of the Triticeae. This is particularly the case for the divergence times of *Triticum urartu*, *Aegilops speltoides* and *Aegilopstauschii*, which have received little attention, as focus on

these species has generally been in their contribution to the *T. aestivum* genome.

10,000 years ago humans began to select cereals based on their traits such as non-shattering, determinate growth, increased seed number, size, and carbohydrate content, and reduced dormancy (Paterson et al., 2003). Wheat is one of the most cultivated crops globally, which provides 20% of calories to the consumers of the world. At present, total global wheat growth of yield is at a rate of 1.1% and in some places it is almost stagnant and the predicted global demand is likely to be 1.7% per annum for another few decades. It is therefore necessary to meet this demand by applyingnew technologies and approaches to avoid any shortfall in growing severity (Driever et al., 2014). The question is how to increase the existing yield per hectare. Wheat (*T. aestivum*) and Rice (*O. sativa*) ultimately originated from the same ancestor (Paterson et al., 2003). Following their divergence, both species were domesticated along with other cereal crops some 10 millennia ago, since when it has been observed that there is a decline in genetic diversity within these species (Gustafson et al., 2009).

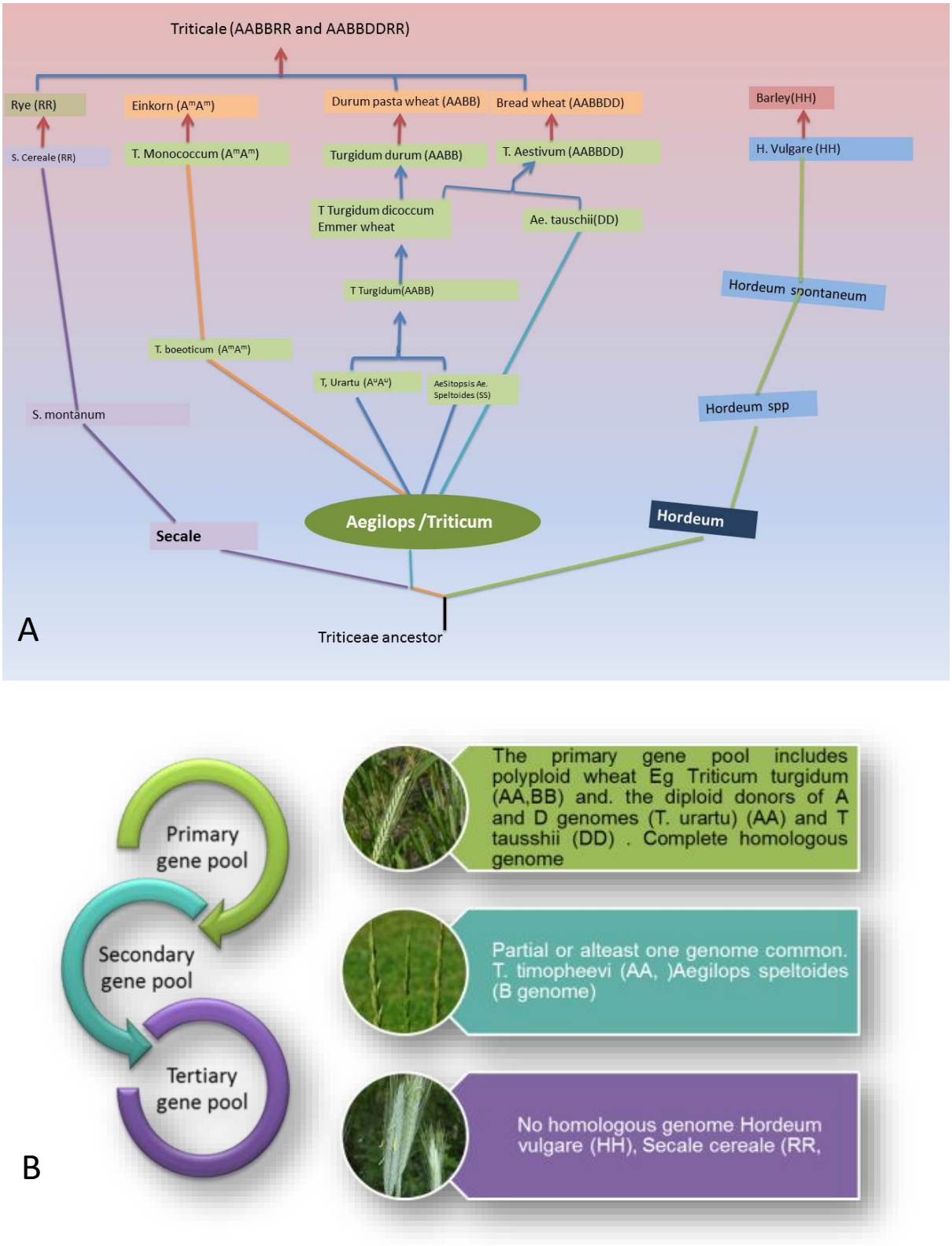


Figure 1.2 A & B: (A) Evolution of wheat and (B) Wheat gene pool. Adapted from (Feuillet et al 2007)

The first evolutionary event in wheat leading to polyploidy originated by hybridization between *Triticum urartu* and *Aegilops speltoides*, which gave rise to *T. diccocoides,* a new a variety of amphi-tetraploid species carrying 14 pairs of chromosomes (Figure 1.2). The second event of polyploidisation occurred when domesticated Emmer wheat *(T. turgidumdicoccum)*washybridized with *T. tauschii*, a wild diploid species resulting in 21 paired chromosomes forming *T.aestivum* or the common wheat, which is also now called as bread wheat, the most widely grown wheat species (Feuillet et al., 2008). *T. urartu* contributed to the bread wheat genome by donating genome A, *Aegilops speltoides* B (still unclear)and*Ae. Tauschii*D genome, all these three were constituted to form modern wheat genome *(AABBDD) Triticum aestivum* (Feuillet et al., 2008)

1.3 Wheat ancestral introgression

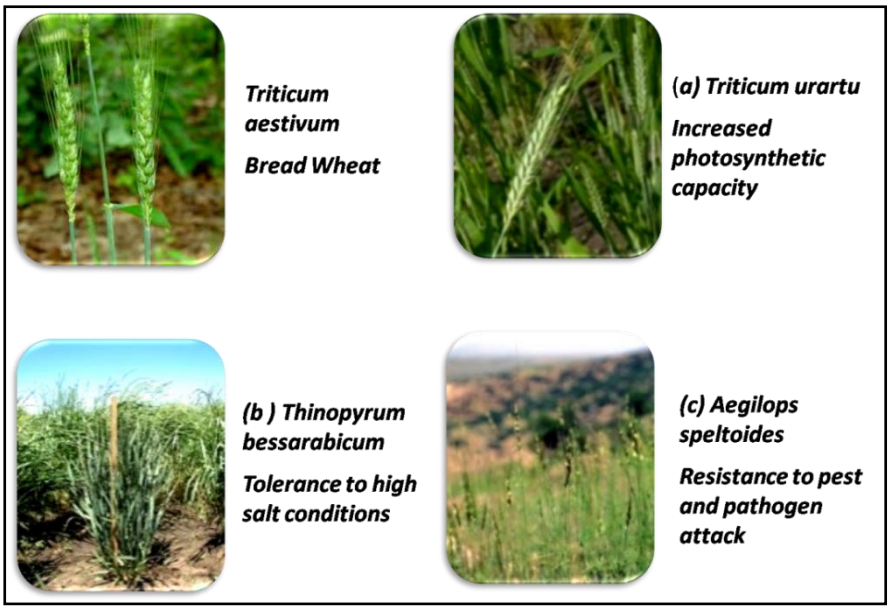


Figure 1.3: Common wheat and some of its wild relatives. The wild relatives of wheat (a) *T. urartu* (b) *T. bessarabicum* (c) *A. speltoides* that are being used as a genetic source in the wheat introgression program. Photograph source: http://www.missouriplants.com/Grasses/Triticum_aestivum_page.html,https://es.wikipedia.org/wiki/Thinopyrum,http://pfaf.org/user/Plant.aspx?LatinName=Aegilops+speltoides

The success associated with domestication of the Triticeae tribe has caused reduction in genetic diversity to a fraction of what it was previously. The possible loss of this genetic diversity could be due to elimination of undesired or even deleterious alleles, but these alleles could also prove valuable for crop improvement and future demands of food production and consumption (Reif et al., 2005). Wild relatives of wheat evolved in diverse environments and possess an array of adaptations not seen in modern wheat. The wild relatives of wheat are therefore potentially vast untapped gene pools and unlocking these resources of genetic diversity to provide key agronomic traits and quality traits could prove fruitful to present introgression programs, which are strategic priorities for wheat improvement (Feuillet et al., 2008).

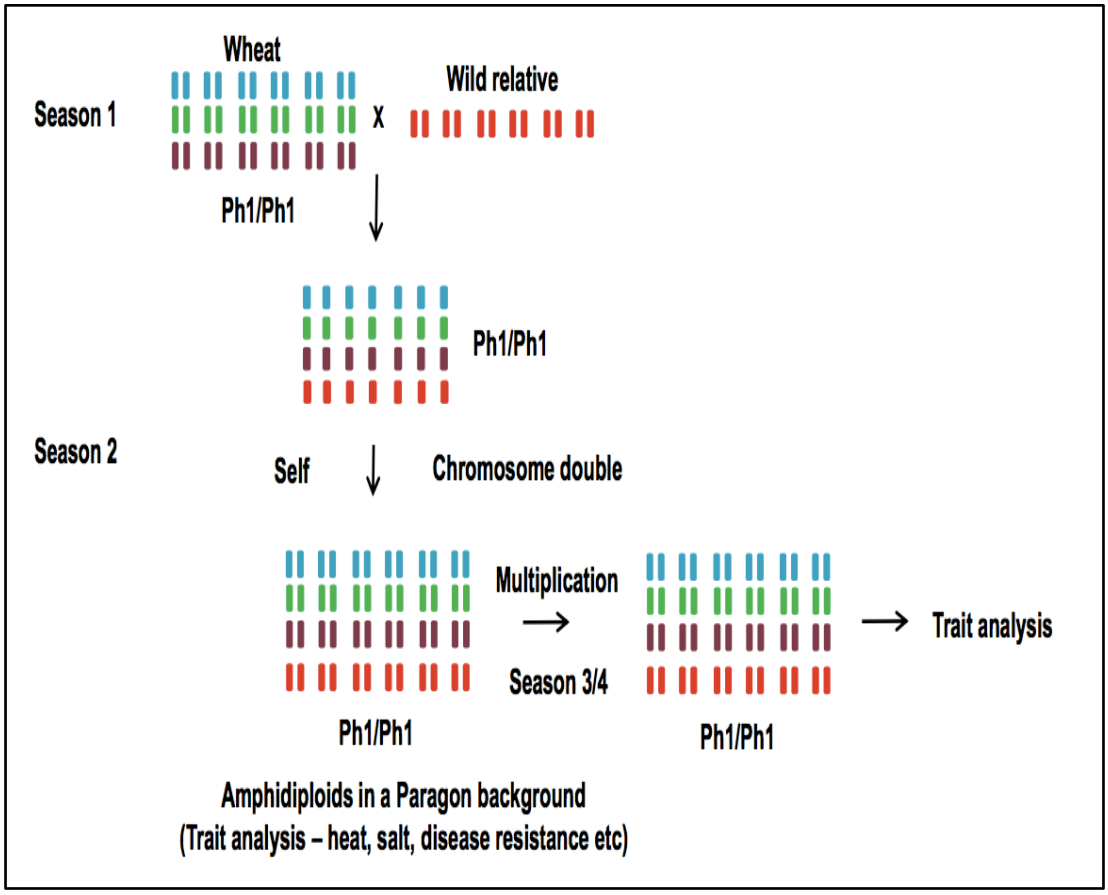


Figure 1.4: Amphidiploids derived through introgression between elite wheat variety (eg: Paragon) and wild relatives of wheat.

Hybridization is defined as crossing between individuals of different species or varieties, and introgression refers to transfer of genes through continuous backcrossing between species or varieties. The genomic composition of putative hybrids and the frequency of introgression can be estimated by genotyping the populations with molecular markers (Tywford and Ennos 2012).

Introduction of alien/ancestral chromosomes from near and distant relatives into key crop species through introgression is termed as Alien/ancestral introgression. It is the simplest form of sexual hybridization of different species to form an interspecific F1 hybrid (King et al., 2013). Introgression is usually made into a cultivated elite background (Charmet et al., 2007). Though hexaploid wheat contains three sets of seven chromosomes from A, B and D genomes, both behave as diploids at meiosis and this is because the *ph1*locus restricts the pairing of the homoeologous (i.e. those believed to be homologous in ancestral form) chromosomes which is controlled by pairing homoeologous (*ph*) genes. Homoeologous chromosomes exhibit different degrees of ancestral homology, therefore in polypoid wheat, homoeologous chromosomes can pair in the absence of *ph*1 gene (Charmet et al., 2007). Therefore, the *ph*1 gene and the use of mutants where it is absent have been essential in allowing recombination between wheat and wild relatives. Introduction of genetic material from wild relatives into wheat has to be a careful process, as they may have highly segregated homoeologous chromosomes (Qi et al., 2007).

1.4: Wild relatives of wheat

Triticum urartu is the diploid wild einkorn wheat, a progenitor species of the A genome (Ling et al., 2013). Austin et al (1982) investigated this species through gas exchange analysis and showed that *T. urartu* exhibits higher photosynthetic

rates and this trait could be very useful if incorporated into bread wheat through classical breeding. Gorham (1984) demonstrated experimentally that *T. bessarabicum* showed an ability to regulate osmosis thereby exhibiting salt tolerant properties. This is a particularly salt tolerant wild relative of wheat that could be a source of genetic material for breeders (King et al., 1997). Hsam et al (2003) showed that *A. speltoides* contains a disease resistancegene providing resistance to powdery mildew *(Pm)* which infests bread wheatand is an effective approach to reduce the use of fungicides. 'Remixing' of genetic diversity from wheat's wild relatives by successful introgression has resulted in wheat containing natural variation for breeders and farmers (Warburton et al., 2006). Many useful traits have now been incorporated into wheat cultivars from its wild relatives through introgression; examples include many high yielding cultivars that incorporated some segment of chromosome from Rye (*Secale cereale*) (Nemeth et al., 2015). *Aegilops umbellulata* was the source of leaf resistance gene *LR9* which was incorporated into some of North American wheat cultivars.Some Australian cultivars derived stem rust resistance gene *Sr26* from *Thinopyrum ponticum* (Schwarzacher et al., 1992). In the light of population-food balance, it is a prerequisite for genetic improvement programs to involve selection of desirable traits. These include higher yield potential, adaptability in diverse environments and resistance to biotic and abiotic stress.

Conventional breeding requires a screening of genotypes carrying desirable traits to inform hybridization between elite genotypes and sometimes with wild species. Genotypes generated by conventional breeding can be clearly shown in the improved cultivars of rice, wheat (Evenson and Golin, 2003) and maize (Duvick, 2001). Agronomical traits include plant height, lodging resistance, flowering time and many more (Acquaah 2012). This way, traditional breeding

has generated a resource of genetic variability to meet global requirements and adapt in a changing environment. As opposed to conventional breeding, modern methods such as marker assisted selection and genetic transformation have promised to accelerate genotype improvement but it is still too early to predict to whether they can meet the growingdemands of human food, animal feed and fossil fuels. Modern techniques have successfully integrated biotechnology tools for creation and manipulation of traits; nonetheless, contemporary methods have their roots attached to traditional knowledge of breeding and if used in concert can speed up the improvement of 21st century crops.

The close relationship between the chromosomes of these wild relatives with cultivated wheat varieties has enabled wheat breeders to generate fertile amphidiploids. Amphidiploids are a type of allopolyploid that contain a full set of chromosomes from each parent (wild grass and common wheat), which is doubled (by colchicine treatment) after the initial F1 hybrid was formed (Nemeth et al., 2015). Colchicine works as mitotic inhibitor that inhibits spindle formation during mitosis, so that the cell cannot split into daughter cells resulting into chromosome doubling (Dhooge et al., 2011). The eventual goal of such approaches is to identify a range of target traits through phenotyping, pinpoint these with molecular markers and exploit them in future breeding programs for the development of superior high yielding wheat varieties that are adapted to climate change and environment friendly agricultural practises.

1.5 The physiology of wheat yield

Importantly, productivity of wheat in the near future will have significant effect on global food security issues, being a widely cultivated crop on planet earth, and has the ability to grow in environmentally challenged conditions. To address

the problem of reduced yield, a sustainable and effective solution is required with an aim of improving yield potential. Reynolds et al (2012) defines yield potential (YP) as a function of light interception (LI), Radiation use efficiency (RUE) and Harvest Index (HI) i.e. YP= LI × HI × RUE. Net primary production has often been found to be linearly correlated to photosynthetically active radiation (PAR) that is absorbed or intercepted by plants or crops, and this is termed as **Radiation use efficiency** (Reif et al,. 2005). RUE is dependent on light, temperature, vapour deficit and factors inherent to crop species (Marsical et al., 1998). Reynolds et al (2012) states that the fundamental bottleneck to raising productivity, namely utilization of solar radiation per unit of dry matter production [radiation use efficiency (RUE)], has barely changed in the modern era of wheat breeding. Increasing dry matter of grain crops would influence **harvest index** (HI) of the crop, HI determines the reproductive efficiency of grain crop which is the ratio of biomass of crop to the harvested grain (Unkovich et al., 2010**). Light interception** from canopy closure under favourable conditions is nearly 100 % until the onset of senescence, hence light interception can only be increased by increasingrate of canopy closure or prolonging green canopy till grain filling stage (Reynolds et al., 2005). The process of photosynthesis converts solar energy into stored chemical energy i.e. biomass (Beadle et al., 1985). Thus any increase in photosynthetic efficiency should translate into biomass production. The prime determining factor of improved crop biomass is the cumulative rate of photosynthesis over the growing season (Parry et al., 2011). In the past half century, photosynthetic efficiency remained little explored despite its promising link with increasing in yield. However, to enhance yield for the future, a significant increase in the photosynthetic capacity (e.g. rate of CO_2 assimilation under saturating light) and/or efficiency (e.g.

quantum efficiency in terms of CO_2 gained per unit light absorbed) is necessary (Zhu et al., 2010).

There is strong evidence that wheat yield potential (i.e. yield under optimal or good conditions for growth) is associated with substantial improvements in photosynthesis. To accelerate gains in yield potential, many physiological traits that are complex in nature must be incorporated and combined with a strategic approach towards increasing photosynthesis. That an increase in photosynthesis translates into an increase in crop yieldhas been demonstrated by CO_2 enrichment. CO_2 enrichment in plants implies growing them under elevated CO_2 which in the field is called free air CO_2 enrichment (FACE). FACE experiments suggests that elevated CO_2 is directly linked with increased rate of photosynthesis, RUE, improved water use efficiency (WUE) including a reduction in stomatal conductance, photorespiration and transpiration (Ainsworth et al., 2005). RUE increases with an increase in leaf photosynthetic capacity, which can be monitored using gas exchange combined with growth analysis. It is imperative to introduce novel allelic diversity by exploring non-conventional sources. Reynolds (2012) discussed the potential of wild relatives of wheat for improved photosynthetic efficiency and capacity, which has not yet been explored in wheat. Employing breeding technologies in wheat to achieve vital physiological or agronomical traits from its wild relatives presents a promising method to improve yield in wheat (Warburton et al., 2005) which is one of the prime area of focus of the present wheat ancestral introgression project at the University of Nottingham.

1.6 Photosynthesis

Light, from solar energy, and carbon from CO_2enters into the biosphere via plants and algae through the process called photosynthesis. In this set of reactions, light is absorbed by the chlorophyll present in the light harvesting complexes (LHC) and photosystems (PS) in the thylakoid membrane of the chloroplast. Upon light interception, chlorophyll reaches an excited state, ultimately transducing this energy to photosystem II (PSII) and photosystem I (PSI) reaction centres, where (in PSII) a water molecule is split into protons and electrons initiating electron transport system. Electrons derived from this reaction are utilized for the reduction of NADP+ to NAPDH and the protons used by ATP synthase present in the chloroplast for the production of ATP. NADPH and ATP produced during the photosynthetic reaction are then utilized in the carbon assimilation reactions that take place in the Calvin-Benson cycle (Murchie et al., 2011). In C3 photosynthesis, (Raines et al., 2011), the first step of Calvin-Benson cycle is carboxylation where atmospheric inorganic carbon dioxide is fixed by the enzyme Rubisco (Ribulose bisphosphate carboxylase oxygenase) and the substrate RuBP (Ribulose 1, 5-biphosphate). The Calvin-Benson cycle operates with the help of ATP and NADPH generated during the light reactions. Rubisco is a dual catalytic enzyme found abundantly in all photosynthetic organisms and catalyzes carboxylation which fixes atmospheric carbon dioxide into organic carbon and oxygenation which is a net energy consuming reaction and initiates the process of photorespiration (Taiz and Zeiger 2015). The Calvin-Benson cycle works in three phase's viz. Carboxylation, reduction and regeneration. **Carboxylation** in C3 plants is catalysed by the enzyme rubisco to generate two molecules of a 3 carbon intermediate (3-phoshogylcerate) (3-PGA), followed by the next step which is **reduction,** here the generated 3-PGA

is then converted into triose phosphates by enzymatic reactions driven by ATP and NADPH generated from the light reactions. And, then the final step is **regeneration**of Rubisco's substrate RuBP, which is also termed as the Regenerative phase. RuBP is regenerated in this step through a series of enzyme catalysed reactions requiring ATP and NADPH. Rubisco thus governs the carbon flux into the plant system through of reactions. Further, a large part of the triose phosphates produced during Calvin-Benson cycle are utilized for the rubisco regeneration and the rest are used for the biosynthesis of various organic compounds such as starch in the chloroplast or transported to the cytosol for the formation of sucrose.

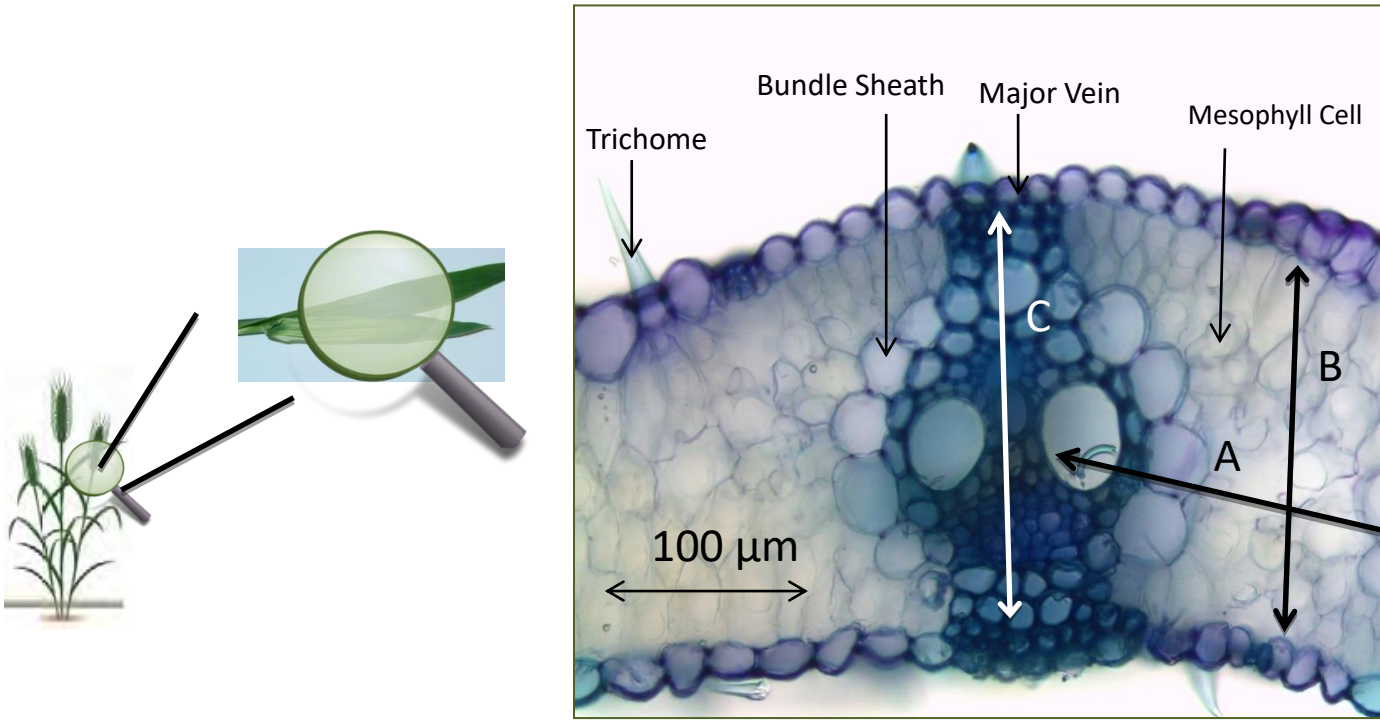


Figure 1.5: Transverse section of wheat leaf showing anatomical features under light microscope. The distance between major and minor vein (A), (B) mesophyll spongy layer between the major-minor vein, (C) the thickness major vein and (D) is the minor vein thickness. Source: Photograph by author

1.6.1 Leaf anatomy and photosynthesis

When light (full spectrum) from the sun strikes the earth less than 5 % of that energy is converted into carbohydrates through photosynthesis. The anatomical features of leaves are organized in a manner where it can efficiently absorb incident light. The top layer of the leaf is the epidermis which is usually transparent to visible light and below the epidermis are the palisade cells which are photosynthetic cells.Below the palisade cells are the spongy mesophyll cells, also photosynthetic and slightly irregular usually enveloped by air spaces (Taiz & Zeiger 2015). It was observed that the changes in the ploidy level of wheat can bring changes in the leaf anatomical features (Austin et al.,1982). Leaf morphology carries many photosynthetic traits that can develop resistance to gas exchange (Smilie et al., 2012). However, the rate of photosynthesis can be affected by various environmental factors like the intensity of light or increase in temperature as these factors, affects the leaf and cell dimensions. During the different developmental stages of plants, modifications of the leaf anatomical and morphological features are governed by the intensity of light among other factors (Murchie et al 2005, Wilson and Cooper1969). Associations between photosynthesis and mesophyll cells have undergone much analysis taking several anatomical features into consideration for example number of cells, cell size, cell surface area, chloroplast number and surface area and leaf thickness with respect to light intensities and CO_2 concentration. It is known that leaf thickness and cell size have independent action on photosynthetic rate per unit area. Early work in wheat by Wilson and Cooper (1967) suggested a negative correlation between net assimilation rate and mesophyll cell size during high light intensities. However, in non-limiting light conditions, changes in CO_2 concentration play important role in creating differences between photosynthetic

rate and mesophyll anatomical characters, against negative correlation between cell size and photosynthetic rate, cell number shows a positive correlation under light limiting conditions, it was found revealed that no significant correlation can be made between photosynthesis and any of the mesophyll parameter (Wilson and Cooper 1969). Similarly, photosynthetic activity is influenced by change in temperature when plants are subjected to lower or higher temperature. Thickness and size of the mesophyll cells varies with variation in temperature with thinner and smaller cells at higher temperature than those from lower temperature (15 or 9 ^{o}C). Analysis on single genotype by Wilson and Cooper (1969) showed that mesophyll cells developed at lower temperatures were efficient. However, studies on different genotypes exhibited faster rate of photosynthesis with smaller leaves which can be explained by possible increase in cell size. Thus that mesophyll cell size may play a crucial role in determining differences in photosynthetic rate in non-limiting light, optimal temperature and CO_2 concentration. Such modifications may be focused towards improving photosynthetic efficiency thus enhancing crop productivity. Identifying species or varieties that demonstrate such relationship between leaf anatomy and photosynthesis would be a crucial step towards improving crop productivity (Giuliani et al., 2013).

1.7 Some limiting factors of Photosynthesis

1.7.1 Rubisco

Rubisco is the most abundant enzyme that is found at high level in photosynthesizing cells (Feller et al., 2008). The activation of Rubisco (ribulose-1,5-biphosphate (RuBP) carboxylase/oxygenase), in the stroma is regulated by the enzyme rubisco activase which is essential to maintain the activation state of

rubisco under normal light conditions, which is enough to carry out photosynthesis. In C3 crops like wheat, fixing of the majority of atmospheric carbon dioxide is carried out by Rubisco Parry et al (2012), however Rubisco has two catalytic sites that carries out carboxylation (fixing of atmospheric carbon dioxide) and a wasteful energy consuming reaction called oxygenation (fixes oxygen) that leads to photorespiration. The affinity of Rubisco towards these substrates depends upon the environmental conditions. Followingthe oxygenation reaction, fixed CO_2, ammonia and energy is lost in photorespiration. Photosynthetic carbon assimilation is often limited due to Rubisco catalytic activity, however in nature there is a certain amount of variation in rubisco's catalytic properties(Parry et al., 2012), which can be used for improving photosynthesis. Under high light intensity, there can be insufficient activation of Rubisco or insufficient amount of Rubisco which results in reduction of photosynthetic rate. Similarly, at elevated temperature the oxygenation reaction becomes more prevalent and Rubisco activase can be inactivated, as was demonstrated by exposure of a leaf for 5 min at 30-35 ^{o}C and display of rapid and a readily reversible effect on light activation Rubisco inhibiting photosynthesis(Feller et al., 2007). Transferring novel variants of Rubisco through conventional breeding would allow improving existing photosynthesis in bread wheat. Photosynthesis is a light driven process, yet plants needs protection from high light intensity which induces accumulation of highly reactive intermediates and by-products such H_2O_2 that can cause oxidative damage to the photosynthetic machinery. This photo-oxidative damage if not repaired, can reduce photosynthetic efficiency resulting into photoinhibition (Niyogi et al., 1999, Murchie and Niyogi 2011).

1.7.2 Temperature:

Heat can impair the growth of wheat at any developmental stages, and photosynthesis is one of the plant's most sensitive physiological processes to be impaired at higher temperature. Inhibition of photosynthesis at moderate temperature is reversible but it does affect the activation state of Rubisco which is one of the limiting factors of photosynthesis under moderate heat stress. Parry et al (2012)explains that Rubisco is fully activated at steady state conditions and high light conditions, with an exception to high temperature, where activation state of Rubisco decreases along with the decrease in CO_2 assimilation rate.

There is a severe damage to the photosynthesis upon prolonged heat stress, where photosystem II (PSII) can be the first photosynthetic component in the 'machinery' to be affected adversely (Salvucci et al., 2004). It has been considered, that the primary cause of inhibition of photosynthesis at very high temperatures is due to heat sensitive PSII which results in the reduction of photosynthetic electron transport (Kurek et al., 2007).

1.7.3 Photoinhibition

Photoinhibition is the reduction in photosynthetic quantum efficiency and/or capacity induced upon exposure to high intensity light. It is frequently associated with damage to PSII itself but it should be noted that sustained down regulation of PSII activity has the same properties of photoinhibition but is not associated with damage (Murchie and Niyogi 2011). The extent of photoinhibition is dependent upon the photodamage occurred and the relative rate of repair of damaged PSII centres. The rate of photodamage to PSII is usually proportional to the intensity of incident light (Murata et al., 2007). Under unfavourable environmental conditions, excess light energy suppresses the repair mechanism of the photodamaged PSII and thereby increases the extent of photoinhibition.

To avoid such damage; plants perform various photoprotective mechanisms e.g. the movement of leaves or the movement of chloroplasts, and or accumulation of photoprotective compounds (Takashi et al., 2008).Photosynthetic reactions often result in the formation of singlet oxygen and other reactive oxygen species which have the potential to cause damage. These are kept under control by mechanisms such as the Mehler reaction and the water-water cycle (e.g. Murchie and Niyogi 2011).

An important photoprotective process is termed non-photochemical quenching. During photosynthesis chlorophyll attains the excited state and must immediately be de-excited by 3 mechanisms: photochemistry, the emission of light (chlorophyll fluorescence) or emission of heat (non-photochemical quenching) before reaching to the ground state. Both these processes are harmless but non photochemical quenching is a major mechanism that reduces th likelihood of over excitation of PSII and photoinhibition (Murchie and Niyogi 2011, Ruban and Murchie, 2012, Kromdjik et al 2016).

1.7.4 Photorespiration:

Another major limiting factor for many plants is **photorespiration**, which is an energetically expensive process, where the enzyme Rubisco switches to oxygenation reaction instead of carboxylation leading to loss of fixed carbon and metabolic energy. Adapting the C4 photosynthetic pathway in C3 plants would enable to avoid the photorespiration process, and is an attractive target for improved photosynthetic efficiency in C3 plants (Ort et al., 2015).

1.8 Other factors affecting photosynthesis

Crops often experience variety of stresses such as heat and drought that affects the rate of photosynthesis. The combined effect of various environmental stresses such as increased photon flux, high temperatures, pest and pathogen

attack, nutrient deficiency all that account as rate limiting factors for photosynthesis can lead to the production of reactive oxygen species in crop plants, which is detrimental to the plant. Increased production of oxidative species scavenging enzymes such as superoxide dismutase, peroxidases, catalases and other antioxidants in the plants are among the protective mechanisms induced by the plants in response to various abiotic and biotic stress factors. Furthermore, induction of various plant secondary metabolites demonstrates a strong relationship between photosynthetic carbon metabolisms for example chlorogenic acid biosynthesis, which is an area to be explored (Grace et al., 2000).These avenues of photosynthetic mechanismmay have already been partially explored but there is need for detail investigation in this aspect in order to transfer discoveries in model plants such as Arabidopsis and achieve improved photosynthetic performance in crop plants to curb the factors that limits the rate of photosynthesis.

1.9 Exploring wheat (introgressed) lines and Indian genotypes for variationin photosynthesis

As mentioned in above sections, it is apparent that improvement in photosynthetic efficiency would translate into improved or sustainable yield, thus contributing towards global food security (Taylor and Long 2017). Therefore, an attempt to improve avenues of photosynthetic mechanism which have already been explored, needs to be studied in detail in order achieve improved photosynthetic efficiency in crop plants like wheat and curb the factors that limits the rate of photosynthesis.

The present study investigates wheat introgressed lines (amphidiploids) derived from wide cross between wild relatives of wheat and bread wheat (see chapter

2) for improved and increased photosynthesis using IRGA gas exchange methods. Along with photosynthetic traits and other relevant physiological traits will also be investigated. A population of Indian genotypes derived from different agro-ecological zones of India will be tested for variation in photosynthesis under glasshouse conditions at University of Nottingham.

In the chapters to follow, description of methods and results of each experiment conducted in glasshouse and field conditions will be explained.

1.10 Aims and objective

Aims of the present study are

- Discover heritable and relevant superior photosynthetic traits in wheat and its wild relatives that can be used in breeding programs.
- Understand the basis for the above traits by analysing Leaf architecture, photo inhibition, Rubisco activity, Φ PSII in the crop plant.
- Understand how such traits benefits photosynthesis in high temperature Indian environments
- Exploring natural variation in photosynthesis in the Indian genotypes derived from different agro-ecological zones of India.

Objectives

- To identify relevant heritable photosynthetic traits in wheat and its wild relatives that can be used in breeding programs.
- To demonstrate heritable variation in photosynthetic traits populations derived from wheat alien introgression and show that these are stable across contrasting environments.

- To analyse the selected interesting individual through biochemical assays and leaf based assays to identify underlying mechanism
- To demonstrate natural variation in photosynthesis in the Indian genotypes in both environmental conditions.

The overall aim of this project to investigate natural variation in photosynthesis in the amphidiploid population derived from wheat alien introgression and in the Indian genotypes in both the environmental conditions, in the glasshouse (UK) and in the field (India)

CHAPTER 2 Materials and Methods

2.1 Introduction

This chapter includes experimental layouts and generic methods and materials. Minor variations in method are explained in the experimental chapters. Two environments were used for plant growth: a field site in India and glasshouses in the UK. This project was a collaboration between University of Nottingham and Agharkar research institute, Pune, India. Four field experiments were conducted at Hol farm, Pune, India for the years 2013-2014, 2014-2015, 2015-2016 and 2016-2017. Along with this, experiments were carried out in glasshouse conditions at the Sutton Bonington (SB) campus, University of Nottingham, UK for the years 2013-2014, 2014-2015, and 2015-2016. These experiments were conducted to examine phenotypic variation in two different sets of genetically diverse plants: first a population of amphidiploid lines derived from the interspecific hybridization between bread wheat with wheat wild relatives. Second, a selection of Indian cultivars derived from contrasting agro-ecological regions.

The origin of seed used for each experiment is described in each case and was often derived from the harvest of seed in the previous experiment.

2.2 Experimental sites

2.2.1 Glasshouse experiment

The glasshouse experiments were carried out at University Of Nottingham, Sutton Bonington campus, United Kingdom (52°49’59”N, 1°14’50”W) (Table 2.1). The following experiments were carried out in glasshouses:

1. Amphidiploids with wild relatives and wheat parents

2. Indian genotypes

2.2.1.1 Amphidiploids, wheat parents and wild relatives

The amphidiploids used in the glasshouse experiments were generated by creating interspecific hybrids between selected wild relatives of wheat (Table2b) and bread wheat (Highbury, Paragon, Pavon76 and Chinese spring). Different glasshouses were used for the amphidiploid experiments in each year: F1 for 2014, H2 for 2015 and 2016. ‘H’ glasshouses were south facing whilst F1 was west-facing and had slightly lower light levels. A complete randomization design was used for the years 2014, 2015 and 2016 in the amphidiploid experiments using 4 replicates for each line. The wild relatives (parents) were included in the experiment only for the years 2015 and 2016. Seeds were sown in module trays with 8 seeds per variety. After germination, seedlings (2-3cm long)were transferred to vernalisation conditions for 6 weeks below 8^{o}C, while the wild relatives of wheat were kept for 8 weeks in vernalisation. After vernalisation, the plants were transferred to the glasshouse and potted in low nitrogen peat compost(Klasmann medium Peat 818). Drip irrigation was used to supply most nutrients and water with the exception of nitrogen which was given manually to an equivalent of 200 kg ha^{-1} nitrogen for the complete life cycle. The dosages

were split into different growth stages and as soon as the plants were transferred to pots in glasshouse, a dosage of 60 Kg ha^{-1} was given, 60 kg ha^{-1} at stem extension, and the third dosage of 80 kg ha^{-1} was given at anthesis.

All these experiments that were carried out for the years 2014, 2015 and 2016 the soil type was the same, however there were differences between years in terms of how the nutrients were supplied. For the year 2014 all nutrients were supplied continuously through the feed that was pumped through the drippers twice daily. For the 2015 and 2016 experiments, the feed contained all nutrients except nitrogen which was supplied manually as explained above.

2.2.1.2 Indian genotypes in the glasshouse

The growth conditions for the Indian genotypes were same as described for amphidiploids in the glasshouse except for the soil conditions. John Innes No.2 soil was used for the Indian genotypes for the year 2015 and 2016. A panel of 36 Indian cultivars were grown in the glasshouse conditions in complete randomized pots in 5 replicates under regular nitrogen, potassium and phosphorus dosages (i.e. N-P-K 15-7-30 kg $ha^{-1)}$ respectively. All the 36 Indian genotypes were selected as being adapted to contrasting wheat agro-ecological zones of India. Seeds of these 36 genotypes were made available by Indian Institute of Wheat and Barley Research, Karnal, India. The first year i.e 2015, all 36 modern Indian cultivars were grown in glasshouse conditions in glasshouse H2 and tested for photosynthetic traits. From these, 16cultivars showing contrasting responses were again grown in the year 2016 and examinedagain. The glasshouse conditions, soil and nutrient conditions were same as previous year.

Table 2.1: Vernalisation period and transfer to glasshouse for amphidiploids and wild relatives of wheat for the glasshouse experiment

Experiment year	**Date of sowing**	**Vernalisation period**	**Experimental period in Glasshouse**
2014	15th March	6 weeks	May-October 2014
2015	11th August	6 weeks	October-December 2015
2016	25th March	6 weeks	June-August 2016
2016 repeat	24th July	6 weeks	September-December 2016

Table 2.2 A and 2.2 B: List of wheat parents and wild relatives

A.

Code/shorthand	**Wheat parent**
CS Eup 94	Chinese Spring
Pavon 76	Pavon 76
Paragon	Paragon
Highbury	Highbury
CSM	Chinese spring mutant

B.

Line number	**Wild grass parent**
1	*Aegilops mutica* 2130004
2	*Aegilops mutica* 2130008
3	*Aegilops mutica* 2130012
4	*Aegilops speltoides* 2140008
5	*Aegilops speltoides* 449340
6	*Aegilops umbellulata* 542377
7	*Aegilops umbellulata* 554410
8	*Aegilops comosa var. comosa* 276970
9	*Thynopyrum turcicum* P208/201
10	*Secale anatolicum* P208/142
11	*Secale anatolicum* P208/141
12	*Thinopyrum bessarabicum* 531712

2.3: Plant materials used in the glasshouse experiment.

Table 2.3: List of20 Amphidiploids generated at University of Nottingham, UK and used in the glasshouse experiment for the year 2014-2015, 2015-2016.

Line number	**Wild grass parent (Male donor)**	**Bread Wheat parent 2N=6x= 42, (AA,BB,DD)(Female)**
1	*Aegilops mutica* 2130004	CS Eup 94
2	*Aegilops mutica* 2130008	CS Eup 94
3	*Aegilops mutica* 2130012	Paragon
4	*Aegilops mutica* 2130012	Highbury
5	*Aegilops mutica* 2130012	CS Eup 94
6	*Aegilops speltoides* 2140008	CS Eup 94
7	*Aegilops speltoides* 2140008	Pavon 76
8	*Aegilops speltoides* 449340	Pavon 76
9	*Aegilops umbellulata* 542377	Pavon 76
10	*Aegilops umbellulata* 554410	CS Eup 94
11	*Aegilops umbellulata* 554410	CS Eup 94
12	*Aegilops comosa var. comosa* 276970	Paragon
13	*Thynopyrum turcicum* P208/201	CS Eup 94
14	*Secale anatolicum* P208/142	Highbury
15	*Secale anatolicum* P208/142	CS Eup 94
16	*Secale anatolicum* P208/141	CS Eup 94
17	*Secale anatolicum* P208/142	CS Eup 94
18	*Secale anatolicum* P208/142	CS Eup 94
19	*Thinopyrum bessarabicum* 531712	CS Mut P208/535
20	*Thinopyrum bessarabicum* Abers.	Chinese Spring

Table no 2.4: List of elite Indian cultivars and their physiological traits grown in glasshouse conditions at SB campus, University of Nottingham during 2014-2015, 2015-2016 and in field conditions in India at Hol farm, Pune for the year 2015-2016, 2016-2017. These lines were included in the experiment to test their photosynthetic efficiency and other related traits in field and glasshouse conditions.

Field (F)/ Glasshouse (G)	Name	Year of Introduction	Physiological traits
F,G	BH-1146	NA	NA
F,G	CBW-38	2008	Potential for heat tolerance in Eastern India
F,G	DBW-14	2002	Acidic tolerant, high protein content and good grain quality. Tolerant to yellow and brown rust and leaf blight.
F,G	DBW-16	2006	High level of resistance against the prevalent pathotypes of stripe rust.
F,G	DBW-17	2007	Tolerant to terminal stress and has faster grain filling.
F,G	DBW-39	2010	Shows resistance to leaf and stripe rusts and leaf blight. Also shows wide adaptability and stability.
F,G	DBW-46	N/A	NA
F,G	DBW-51	N/A	NA
F,G	DBW-71	2013	Possessing disease resistance and heat tolerance.
F,G	DPW 621-50	2011	Resistance yellow and leaf rusts.
F,G	GW 322	2002	Drought tolerant, increased WUE, good grain protein content.
F,G	HD 2733	2001	High yield wider adaptation, high tillering capacity and tolerance.
F,G	HD 2932	2008	Contains high zinc and iron. Shows wider adaptability.
F,G	HD 2967	2011	Resistance to Yellow and brown rust. Contains High Zinc, Iron and Copper.
F,G	HI 8498	1999	Resistance to stem and leaf rust resistance to leaf blight and lead rust.

2.4 Field experiment

Field station: ARI is located at Hol farm, Pune, Maharashtra, India (Peninsular region), Latitude: 18^{o} 04 N, Longitude: 74^{o} 21E, Altitude: 557.5 M. The soil type in this region is black cotton soil or vertisol, while the irrigation was flood irrigation supplied by the local canal. India has 6 different wheat agro-ecological zones, shown in figure 2.1.

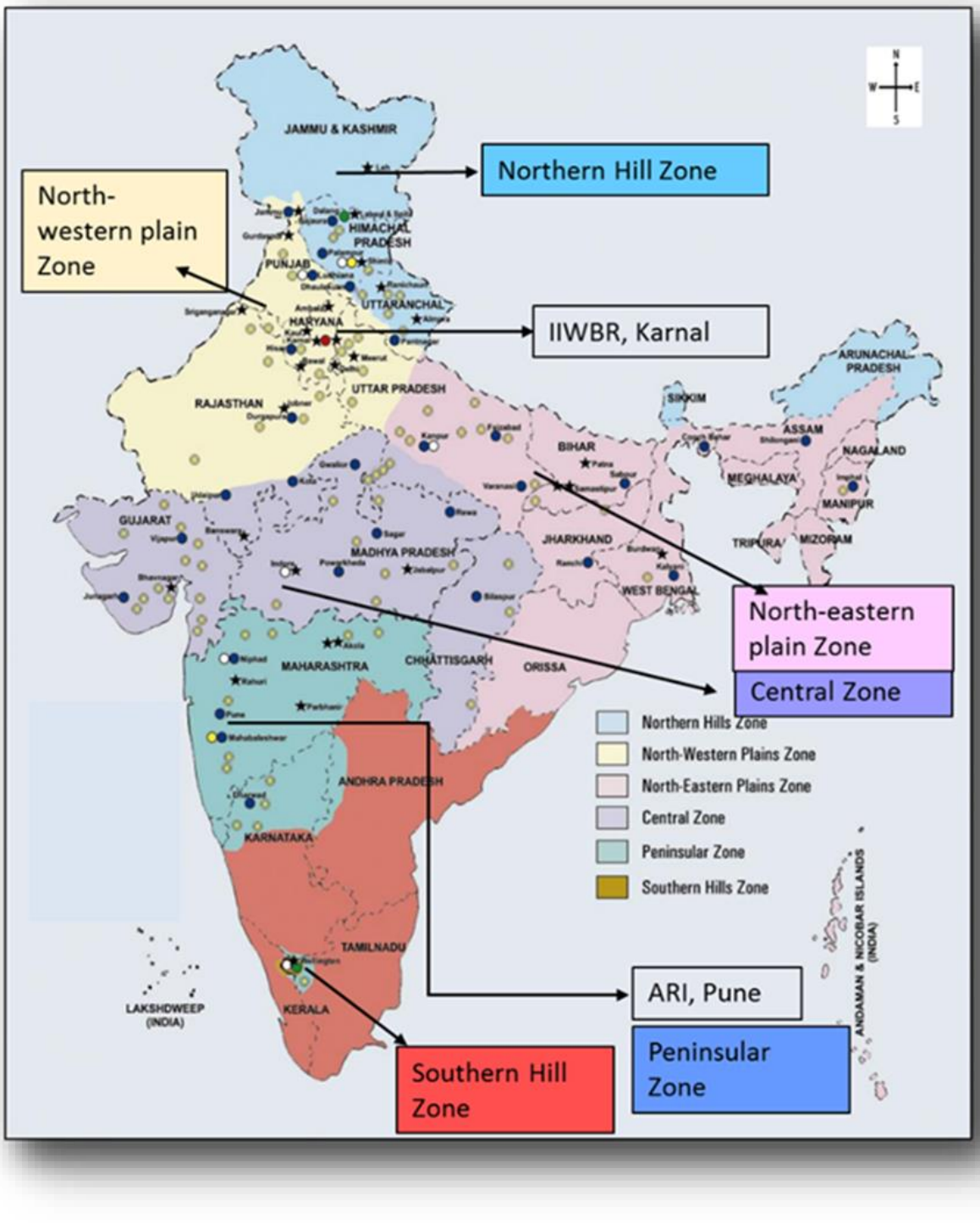


Figure 2.1: Map of agro-ecological zones (Source: JP Jaiswal 2009 http://agropedia.iitk.ac.in).

2.4.1 Amphidiploids in the field

The amphidiploids that were grown in field conditions at Pune site, India were not the same 20 amphidiploids grown in glasshouse conditions at University of Nottingham. All amphidiploids were derived by crossing between durum wheat and one of the wild relatives of wheat *Thinopyrum bessarabicum* (the amphidiploids are listed in table 2.6), only one of the amphidiploid from the population nine was a cross between Chinese spring (*Triticum aestivum)* and *Th. bessarabicum* cross. These amphidiploids were generated at the University of Nottingham, SB campus, and were then sent to the Institute in India, Karnal, IIWBR (Indian Institute for wheat and barley research) for multiplication before distribution to different wheat research centres for field trials.

The amphidiploids were photoperiod sensitive; therefore, supplementary lights were provided by installing fluorescent tubelights approx. 1m above each amphidiploid plots. As the daylight in the peninsular zones is approximately 12 hours, the lights were switched on manually every day 2 hours before the sunrise and two hours after sunset to complete 16 hours of photoperiod (sunrise 6.30±am & sunset: 6.30 pm±).



Figure 2.2: Supplementary lights for Amphidiploids at field site, Pune.

2.4.2 Indian genotypes in the field

The field experiment in India included a panel of 30 modern Indian cultures selected from contrasting wheat agro-ecological zones of India (Figure 2.1). These 30 elite varieties were selected to represent genetic and phenotypic variation in traits relating to photosynthesis and other physiological parameters.

Table 2.5: Eight amphidiploids planted for the field experiment in India during season 2013-14

Amphidiploid code	**Durum wheat parent**	**Wild parent**	**Bread wheat grown as a comparison**
EC-78007	Langdon	*T. bessarabicum*	
EC-7008	Macoun	*T. bessarabicum*	
EC-787009	Karim	*T. bessarabicum*	
EC-787010	Neodur	*T. bessarabicum*	
EC-787011	Creso	*T. bessarabicum*	
EC-787012	Azaziah	*T. bessarabicum*	
EC-787013	Stewart	*T. bessarabicum*	
EC-787014	*T. aestivum* wheat	*T. bessarabicum*	Chinese spring
NA	NA	NA	Highbury
NA	NA	NA	Paragon
NA	NA	NA	HD2967
NA	NA	NA	HI
NA	NA	NA	KRL210
NA	NA	NA	MACS6222

2.5 Field experiment and layout for the year 2014-15, 2015-2016 and 2016-17



Figure 2.3: Wheat trials grown at the field site, Hol farm.Source: Photograph by author

The field experiment for the years 2013-14, 2014-2015 and 2015-2016 were intended to be replicated, using the same lines each year. For the first year i.e. 2013-14, only the Indian genotypes were studied; however the following years 2014-15, 2015-16 and 2016-17, nine amphidiploids and 30 Indian genotypes were grown at the field site, Pune. Figure 2.5 shows the experimental layout. This experiment was part of another study into N nutrition. In this thesis only data from high N plots is shown. The plot size was 6 rows of 4 meter length with row spacing of 0.20 cm for the Indian genotypes. An important and additional measurement was the gas exchange measurements on both, the 30 Indian cultivars as well as the amphidiploids and its respective wheat parents for the year 2015-16 and 2016-17. For the year 2016-17 the number rows were

reduced to 3 for the Indian genotypes, however the amphidiploids experimental setup remained the same previous year.

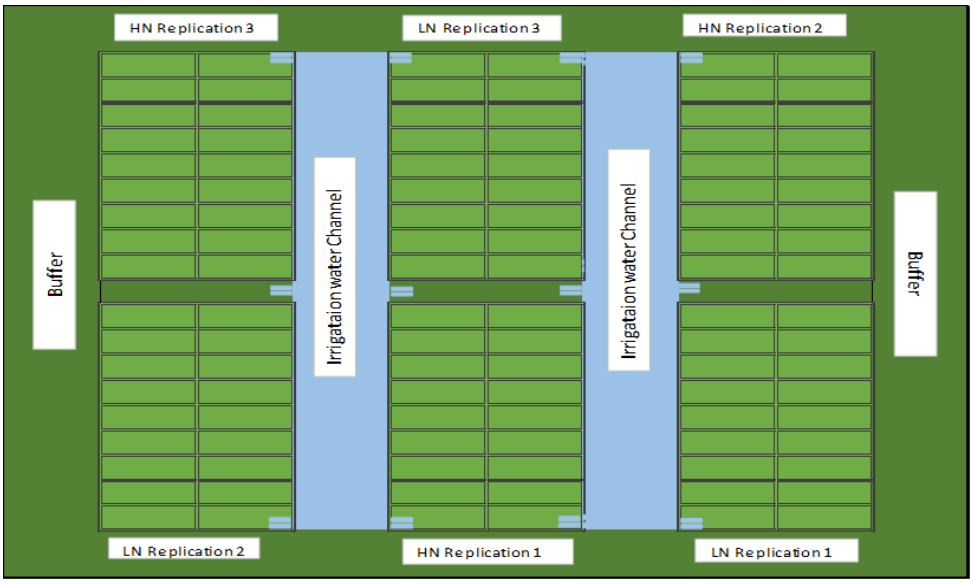


Figure 2.4: Field plan for amphidiploids for the year 2015-2016

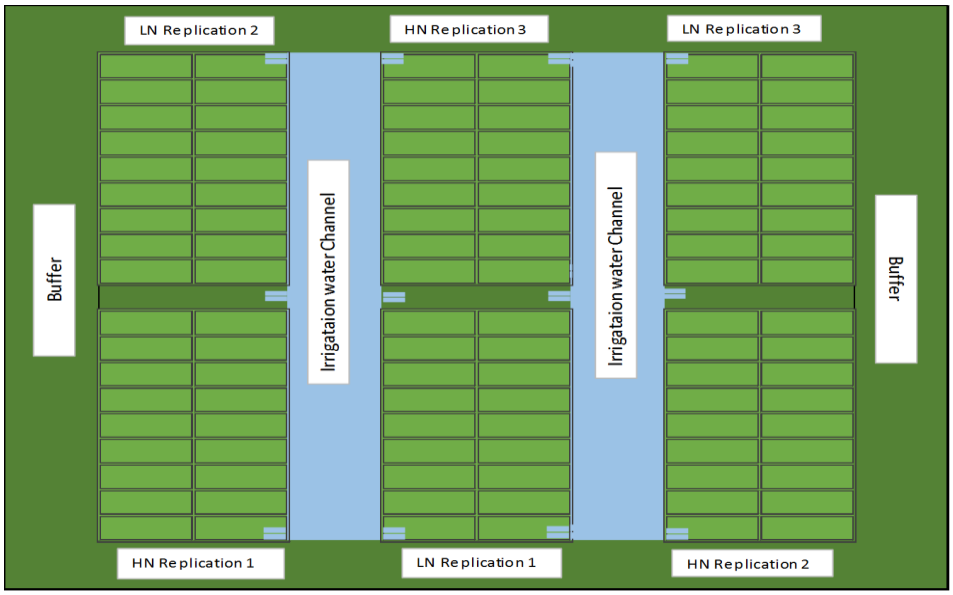


Figure 2.5: Field plan 2015-2016 for Indian genotypesthat were grown at field site, Hol farm.

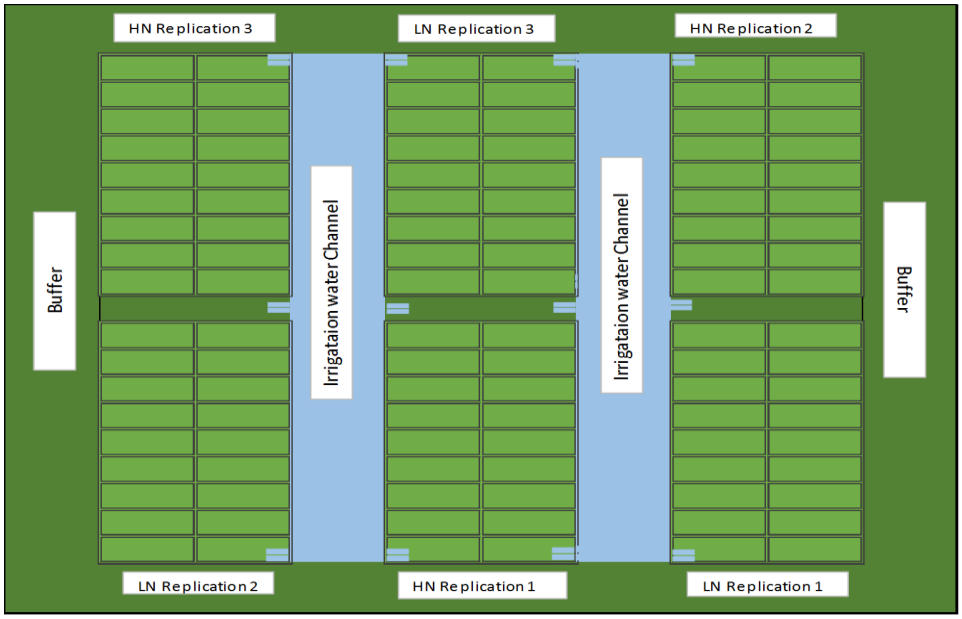


Figure 2.6: Field plan 2016-2017 for amphidiploidsthat were grown at field site, Hol farm.

2.6 Crop physiological measurements

2.6.1 A. NDVI (Normalised difference vegetative index)

B. Canopy temperature

A **B**





Figure 2.7 A and B: The NDVI meter and the hand held Infra-red gun used to measure canopy temperature, infra red thermal gun used in this experiment. Photograph source:https://spinoff.nasa.gov/Spinoff2009/er_10.htmlhttps://www.instrumart.com/products/18425/sixth-sense-lt300-infrared-thermometer

A: NDVI

The radiation reflectance of the wheat cultivars in the field conditions were measured by using NDVI (CM1000 NDVI meter, Fieldscout, spectrum technologies Inc, USA). Care was taken that the measurement was taken at the canopy level approximately for each plot 30 cm above from the canopy during mid-day or late morning and avoid cloudy days to maintain consist environmental conditions. The measurement was taken starting from growth stage GS41until maturity.

The wavelength equation is

$$NDVI= (R_{900}-R_{680})/ (R_{900}+R_{680})$$

Where R900 and R680 are the reflectance at 900 and 680nm respectively.

B: Canopy temperature

Canopy temperature was measured using infra-red thermometer (LT300, Sixth sense, USA). The infra-red gun measurement was taken at mid-day and the gun was kept at height of a foot above the whole canopy of each plot and the measurement was taken per week during sunny days.

2.6. 3 Chlorophyll fluorescence

Chlorophyll fluorescence is a non-invasive method of assessing photosynthesis efficiency and a direct measurement of photosystem II activity.

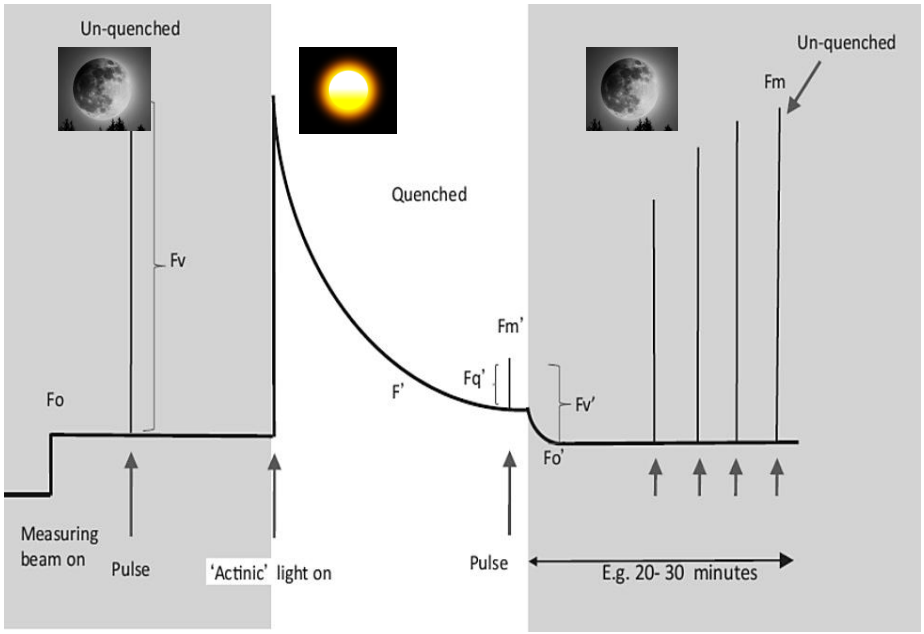


Figure2.8: Fluorescence quenching plot for dark adapted leaf (adapted from Murchie and Lawson, 2013).

A basic summary of chlorophyll florescence analysis is as follows. When light is absorbed by chlorophyll, an electron is promoted to a higher state of excitation. It must immediately return to ground state (be quenched) and it can do this by one of three processes: photochemistry or resonance transfer, emission of heat (non photochemical quenching) or emission of light (fluorescence). In the dark adapted state in optimal conditions it is possible to measure the maximum quantum yield of photosystem II. If a weak measuring light is switched on, the intensity of light is not strong enough to initiate electron transport, but it is enough to elicit the minimal fluorescence for chlorophyll fluorescence termed as Fo, (Fo' in light adapted state). Abrief saturating flash of light is applied in order to obtain the maximum value of chlorophyll fluorescence Fm (Fm' in light adapted state); the difference between Foand Fmis taken as the variable chlorophyll fluorescence in the dark adapted state Fv (Figure 2.12) (Fv' in the light adapted state). The ratio of Fv/Fm is directly proportional to the quantum

yield of photosynthesis (e.g. Maxwell and Johnson 2000, Murchie Lawson 2013) and its maximum value should be around 0.83.

As the leaf is exposed to actinic light (i.e. this light that can be used to drive photochemistry) the leaf can be described as being in quenched state (Figure 2.12); with quenching of fluorescence occurring due to both photochemical and non-photochemical processes. When a leaf is exposed to a saturating pulse at this point, a maximum value of chlorophyll fluorescence in the light adapted state Fm' is obtained. The difference between Fm' and the steady rate of fluorescence, prior to the application of the saturating pulse (F'), gives the value Fq' which reflects the degree of quenching of by Fm' PSII photochemistry. Fo' is measured immediately after the removal of actinic light, the difference between this value and Fm' is taken as the value of variable fluorescence in the light adapted state; Fv'. Therefore, in light adapted state operational yield of PSII is measured as Fq'/Fm' (also ΦPSII or ΔF/Fm') and does not require Fo. The level of non photochemical quenching (NPQ, a form of photoprotection) is usually measured using the difference between Fm and Fm' and therefore requires a period of dark adaptation ((Fm-Fm')/Fm'). Fv'/Fm' is sometimes used as a convenient proxy for NPQ because no dark adaptation is required and it correlates with measured NPQ.

2.6.4 Operational yield (Fq'/Fm', ΦPSII orΔF/Fm')



Figure 2.9: Open window option in PSI Fluorpen.

Using PSI Fluorpen (FP 100, Photon system instruments, CZ), ΦPSIIwas measured on previously tagged plants (to make sure the same plant in each plot was measured each time). The measurement was usually carried out mid-day, when the sun was overhead on flag leaf of varieties in replicates of three per plot. The Fluorpen 'open window' option was used in order that electron transport at light saturation could be measured. The device was clamped over the leaf with the open window facing upward. Sunlight was therefore shining on the light from below the leaf and fluorescence measured on the opposite side of the leaf.

2.6.5 Dark adapted (Fv/Fm)



Figure 2.10: Flag leaf of wheat dark adapted by aluminium foil

Using the same PSI Fluorpen, dark adapted Fv/Fmwas measured at two growth stages in order to understand whether photoinhibition was present. This was done by dark adapting the flag leaf with aluminium foil for minimum 20 mins on each tagged plants. The fluorpen with 'closed window' option was used in order to avoid light interference and evaluate dark adapted fluorescence. Initially, the flag leaf of the main tiller of two plants per plot was covered with aluminium foil for 20 mins. Then after 20 mins, the device was clamped over the aluminium foil and the aluminium foil was removed carefully and then after few secs a reading of Fv/Fm was made.

2.6.6 Gas exchange measurements



Figure 2.11: Gas exchange measurements in the field and glasshouse using LI-COR 6400XT. Source: Photograph by author

Gas exchange measurements were made using (LI-COR 6400XT, Lincoln Nebraska, USA), which had leaf chamber fluorescence adaptor (LCF 1810). Each leaf was clamped to the leaf chamber sealed with gaskets in order to avoid leaks.

Three type of measurement was made:

1. Instantaneous ('spot') photosynthetic measurement where light saturated rate of photosynthesis is measured (P_{max}).
2. Light response curves (LRC curves).(not shown in thesis)
3. CO_2 response curves (AC_i curves).

Instantaneous photosynthetic measurements were made at a PPFD (photosynthetic photon flux density) of 1500 µlmol $m^{-2}s^{-1}$ over a 3 minute period. The CO_2 (C_a) in the cuvette (sample) CO_2 was controlled at 400 ppm (µmol mol^{-1}), the flow rate was kept at 500 µ mol $s^{-1}$and the block temperature was maintained at 22^0C. Humidity was set as ambient(i.e. full scrub using the drierite tube). The leaf was maintained under these conditions for a minimum of two and a maximum of three minutes with the machine measuring when readings were stable.

Analysing the data from such spot measurements, promising varieties or cultivars were selected which demonstrated higher and lower photosynthetic values.These promising lines were further subjected to detailed photosynthetic measurements like AC_i curves (CO_2 response curve) and LRC curves (Light response curves) (not shown in thesis) to understand the underlying biochemical mechanism that might be causing variation in photosynthetic rate within the wheat varieties. AC_ianalysis is carried out by comparing the CO2 assimilation rate with response to different internal leaf concentrations of CO_2 (C_i)by manipulating the CO_2 supply from pure CO_2 cylinders (LI-COR Inc, Lincoln, Nebraska) fitted to CO_2 injector installed in the LI-COR console. CO_2 response curves or AC_i curves indicates the plant's response to different levels of CO_2 concentrations, which provides the information of the biochemical limitation of photosynthesis following fitting a biochemical model (Farquhar and von Caemmerer 1980, Sharkey et al 2007). Such curves allow us to assess A_{max} (maximum photosynthetic rate at saturating CO_2), V_{cmax} (maximum rate of Rubisco's carboxylation), J_{max} (maximum rate of electron transport for RuBP regeneration), Triose phosphate utilisation (TPU) and other related parameters.

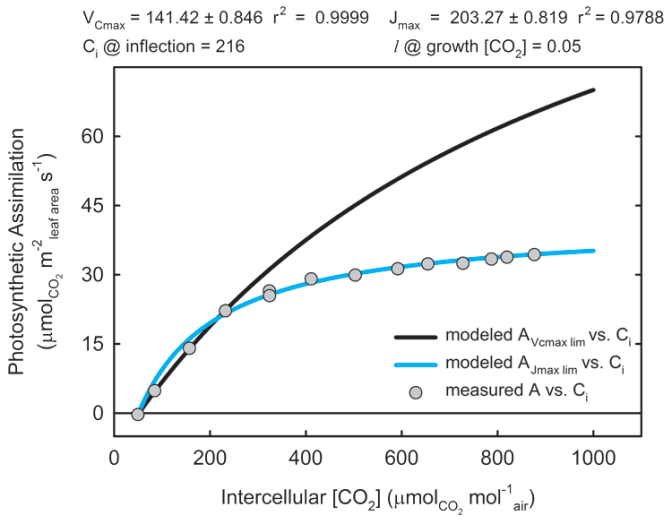


Figure 2.12: Example of CO_2 response curves (AC_i curve) and the fitting of the biochemical model

(Source: https://www.licor.com/env/products/photosynthesis)

The AC_i curve or the CO_2 response was measured at different levels of CO_2 (sequence 400, 300, 200, 100, 80, 50, 400, 400, 600, 800, 1000, 1200, 1500ppm). The CO_2 was set 400 µmol mol^{-1} initially, so that the plant could acclimatise to ambient CO_2 levels, then it was subjected to lower CO_2 levels (in order to asses Rubisco activity at lower levels of CO_2) and then again the CO_2 brought ambient levels, after this CO_2 levels gradually increase to assess RuBP regeneration capability. At each level the time was set for maximum 3 mins, of which one min is kept for stability.Derived values from the AC_i curve measurement were then analysed to assess the maximum carboxylation rate of rubisco (V_{cmax}), maximum rate of electron transport (J_{max}) using curve fitting model as explained by (Sharkey et al., 2007).

2.7: Leaf anatomy

2.7.1 Clearing and staining

Leaf anatomical features were observed by using the flag leaf of the main tiller and middlemost portion of the leaf was used for leaf sectioning. The leaf sections were hand cut into thin strips, these thin strips were placed on a microscopic slide and few drops of 85 % lactic acid saturated chloral hydrate (supplied by Sigma Aldrich, UK) were added as shown in the procedure (Lux et al., 2005), addition of this chemical was carried out in a fume hood as the chemical is toxic in nature. This slide was kept in a rectangular Petridish and was allowed to float in a water bath; the temperature of the water bath was set to 70 ^oC for approximately 1 hour. The slide with leaf section was then allowed to cool at room temperature in the fume hood. To remove the chloral hydrate from the

thin leaf strips, it was washed with distilled water 2-3 times. Once cleared, these leaf sections were stained using 1% Toluidine blue. Toluidine blue was prepared by using 1 % sodium tetraborate solution (supplied by Sigma Aldrich, UK). This method is similar to that used by Smillie et al (2012).

2.7.2 Imaging and Image analysis

The leaf sections were imaged under a light microscope (Leica) and under 20X resolution using Leica DFC 320 digital camera (Leica microsystems, Germany) and the software used was (ImageJ). These images were saved as JPEGfiles, which were used for analysis on a PC (ImageJ software).The parameters measured, are described in the previous chapter (figure 1.5).

2.8 Vein density



Figure 2.13: Handheld microscope, Source: Photograph by author

Vein density measurements were made using handheld microscope (Palm trekker, Palm microscope, UK). The leaf area that was considered for measurement was 1 cm avoiding the midrib; all these measurements were taken only for the amphidiploid field experiment on the flag leaf of each plant (9 reps, 3 each plot)and at anthesis stage (GS61-69).

2.9 Crop measurements

2.9.1 Growth stages

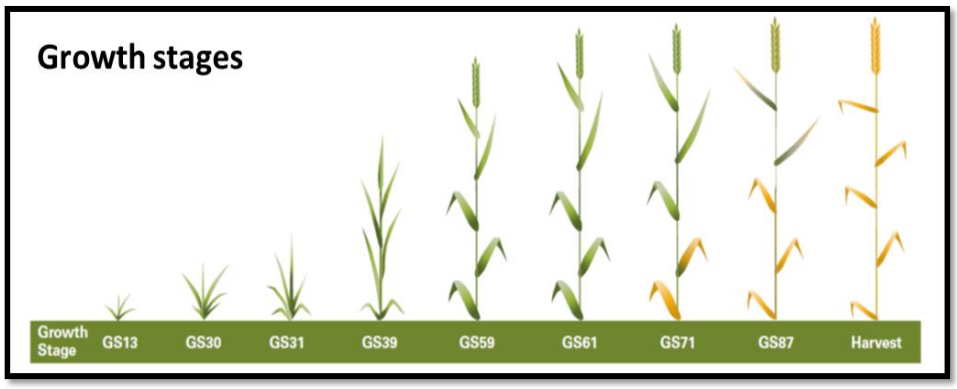


Figure 2.14: Growth stages of wheat (Source: Wheat growth guide, AHDB, cereals and oilseeds)

Growth stages were recorded for each genotype every week/day, the main tiller was tagged, once they reached GS41 and this main tiller was used throughout for all photosynthetic measurements and physiological measurements. All the AC_i photosynthetic measurements using LI-COR 6400 XT infra red gas exchange analyser were done at anthesis stage i.e. between GS61-69.

2.9.2 Plant height

Plant height was measured at growth stage (GS69-80, Zadok's nomenclature) for all the glasshouse experiments as well as in the field experiments. Plant height was measured on the main tiller using a metal ruler.

2.9.3 Leaf length and width

Length and width of leaves were measured at growth stage (GS69-80) for all the glasshouse experiments and the field experiments. Length and width of the flag leaves of the main tiller was measured at growth stages (GS69-80).

2.9.4 Tillers

Tillers were counted from the middlemost rows in the field experiment. Tillers were counted by placing a ruler of one meter length in the middlemost row of

the plot and numbers of tillers were counted along its length. For the glasshouse experiments, number of tillers were counted from each plant.

2.9.5 Biomass

To estimate the biomass of the plants in the field conditions, plants were harvested at maturity following grain filling in two separate procedures. First plants were harvested from the middlemost row of the plot by placing a ruler of one meter length and harvesting all plants along its length. Second, the total biomass was estimated by harvesting plants from entire plots, this included the one meter biomass harvest. For the glasshouse experiment, all plants were harvested at maturity.

2.9.6 Yield

For the field experiments, total plot yield was measured by harvesting plants from the entire plots after plants attained maturity. As the sun is intense in this region of India with temperature ~38°C (Pune, meteorological station data) during the harvest season (March-April), the plants after harvest were kept outside in the open for drying. Spikes of the amphidiploids were harvested manually and were collected in a cloth bag and then 5 spikes of each amphidiploids and wheat parents were used for post-harvest analysis (grains per spike) and grains for the amphidiploids were separated by hand threshing because by machine threshing, there is risk of damaging the grains. The grains harvested from the total plot of each amphidiploid and UK wheat parents (both in 3 reps) were weighed and for the thousand grain weight, grains were counted manually. For the glasshouse experiments yield in terms of weight of grain was not calculated because the seed was needed to sow subsequent experiments. Instead numbers of grains were counted.

2.10 Statistical analysis

Data collected in the field experiments and glasshouse experiments were subjected to one and two way analysis of variance (ANOVA), correlation and regression using statistical package Genstat v17.0 (VSN international) as described in each chapter.

Chapter 3

Exploring the variation in photosynthesis in wheat ancestral introgression (Amphidiploids) linesunder glasshouse conditions

3.1 Introduction

Modern wheat varieties suffer from a shortage of genetic variability post green revolution. Changing climatic conditions are compelling breeders to develop wheat varieties that can both be productive under biotic and abiotic stresses, in order to satiate global food demand (Warburton et al., 2005).Wild relatives of wheat like *Triticum urartu, Aegilops speltoides* and *Thinopyrum bessarabicum*can carry important genetic traits can be introduced into modern wheat through wheat ancestral introgression which isclassified as a conventional wheat breeding technique (Placido et al., 2013). Evidence suggests that enhanced leaf and canopy photosynthesis, above that which currently exists, is necessary to support an increase in crop yield (Murchie et al., 2009). Traits conferring superior photosynthesis may be present in wild relatives which are generally adapted to environments with fewer resources and higher levels of abiotic stress in comparison with modern wheat.

In this chapter screening of a new amphidiploid population derived from introgression of wild relatives into a wheat background (wheat parents: Paragon, Chinese spring, Pavon 76, and Highbury) were carried outusing leaf photosynthesis as the physiological parameter. Rapid screening techniques such as chlorophyll fluorescence and IRGA (infrared gas exchange analysis) were used

to assess photosynthetic performance in the glasshouse in optimal conditions and subsequently (in chapter 4) in the field under a higher level of physiological stress. To investigate the role of leaf morphological and anatomical features for increased photosynthetic capacity, leaf sections of the amphidiploid populations were also made to assess cellular structure and vein arrangement in the leaf. These were compared to P_{max} (maximum light saturated photosynthetic capacity), light response curves and AC_i curves. The overall aim is to identify the physiological and morphological basis of variation in photosynthesis in the amphidiploid population and parents.

Hypotheses tested:

1. Amphidiploid lines might have higher P_{max} than the wheat parents
2. Amphidiploids lines should exhibit P_{max} as their wild relatives.
3. Amphidiploids will demonstrate variation in key photosynthetic parameters.
4. Leaf anatomical features might influence the rate of photosynthesis in these amphidiploid lines.

3.2 Results

This chapter is a study of variation in maximum photosynthetic capacity (P_{max}) and underlying traits in a population of 20 amphidiploid wheat lines and an attempt to identify wheat with superior photosynthetic traits. The experiments were carried out in glasshouse conditions, University of Nottingham for the year 2014 and 2015. The 2014 year experiment was composed of amphidiploids, their respective wheat parents and wheat lines including Highbury, Paragon, Pavon 76, Chinese spring euploid and Chinese spring mutant. It should be noted that the dosages and method of application of nitrogen in the amphidiploid

experiment for the year 2014 were different than the year 2015, which is explained in chapter 2. The first experimental year had 20 amphidiploids genotypes in 4 replicates; initially these genotypes were tested for maximum photosynthetic capacity. The data derived from photosynthetic capacity measurements allowed selection of promising lines, which were further investigated for maximum carboxylation velocity of Rubisco (V_{cmax}), maximum electron transport rate (J_{max}), these values were derived from curve fitting of the AC_i curve. To investigate the leaf morphology, leaf sections were made on the lines that showed higher P_{max} and lower P_{max}, to try to further understand the relationship between leaf morphological characteristic and photosynthetic efficiency of these amphidiploids. For the experimental year 2015, wild wheat parents that were used in generating these 20 amphidiploids were included for the P_{max} measurements, however detailed measurement such as AC_i curves and LRC curves were not made on these wild relatives to reduce the number of detailed photosynthetic measurements due to time limitation. Leaf sections were made on the flag leaf of the lines that demonstrated high and low P_{max}. All the P_{max}measurementswere done at vegetative stage (GS39-GS45 (Zadok's nomenclature) and all the detailed photosynthetic measurements were done at anthesis stage (GS61-GS69). In the body text to follow, wheat wild type parents will be abbreviated as WTP (i.e. wild relatives of wheat).

3.2.1 Light saturated photosynthetic measurements for the year 2014

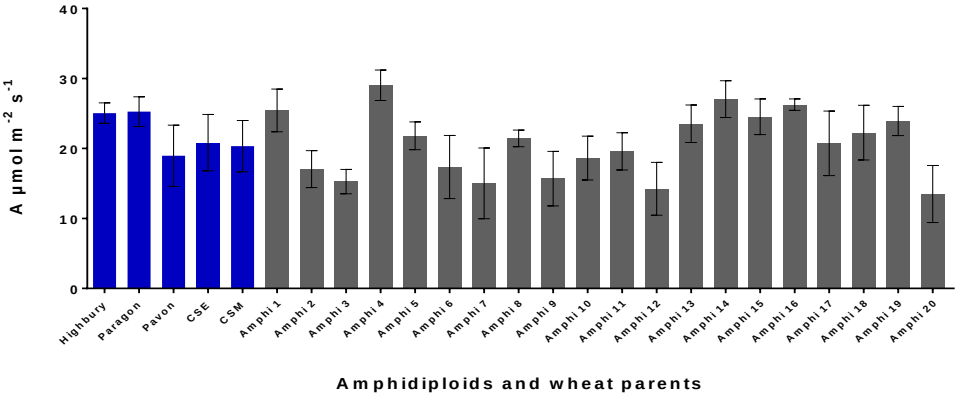
To understand the variation in photosynthesis in the amphidiploids, wheat parents and wild parents, light saturated photosynthesis measurements under ambient CO_2(P_{max}) were carried out. The amount of nitrogen supplied through the drippers for the year 2014 was higher than the year 2015, hence the experiment year was treated separately and common trends examined subsequently. Figure 3.1 shows the photosynthetic parameters of the amphidiploids and the wheat parents, P_{max} of the amphidiploids for the year 2014 and statistical analysis (ANOVA) for 2014 is presented in Table 3.1. Statistical comparisons were made between the amphidiploids and their wheat parents and among the amphidiploid lines.

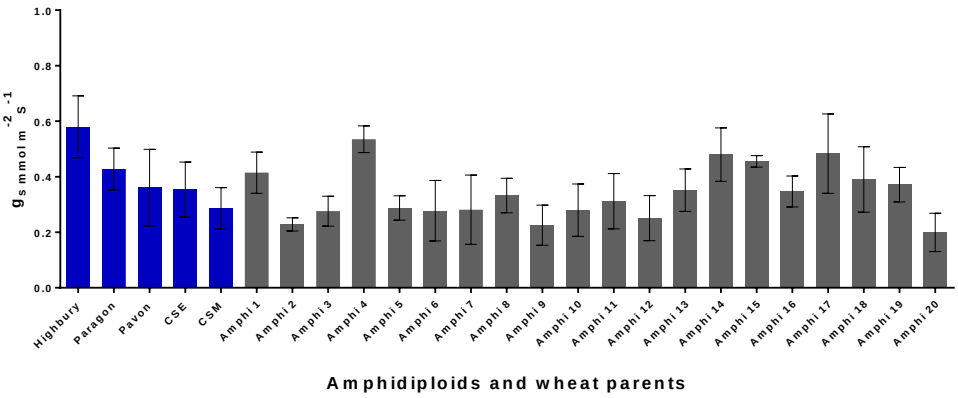
Despite significant variation in P_{max}, ΦPSII, ETR among the lines, few of the amphidiploids showed higher or equal P_{max} value than the UK wheat in the year 2014. Amphi 4 recorded highest P_{max} for the year 2014 at 29 μmol$CO_2$$m^{-2}s^{-1}$. Neither stomatal conductance nor transpiration for the year 2014 was significantly different. Intercellular CO_2 was ~280 mmol $m^{-2}s^{-1}$ in all the amphidiploids and UK wheat for 2014. No differences in Fv'/Fm' (a proxy for non-photochemical quenching or NPQ (Murchie and Lawson 2013)) or qP (indicating state of closure of PSII reaction centres) was observed. Intrinsic water use efficiency (WUE_i) is the ratio of photosynthesis to stomatal conductance and in breeding programs this is sometimes used as an index of crop water use efficiency (Gilbert et al., 2011). Stomatal closure is a common response to avoid water loss and its relationship with CO_2 conductance defines the efficiency of water use or 'crop per drop'. Although there were no

significantdifferences, Amphi 6 and 9 showed higher values of WUE_i, this could be due to stomatal closure with a lower relative reduction in leaf photosynthesis.

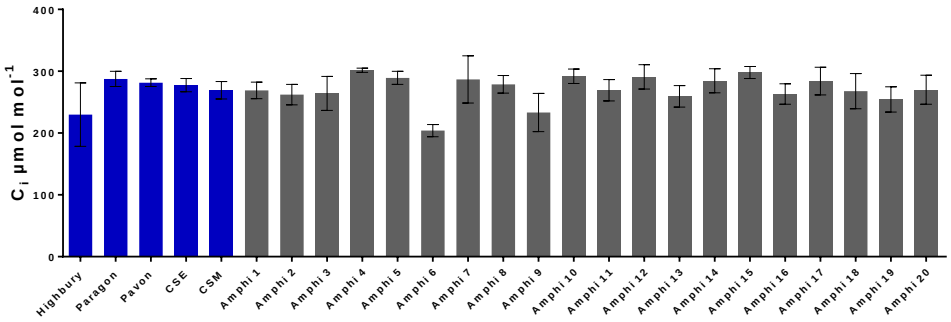
Overall these photosynthetic parameters should be good indicators for selecting individuals that exhibit high and low in photosynthesis in control conditions. However, no lines were found that were significantly better than their wheat parents in 2014.

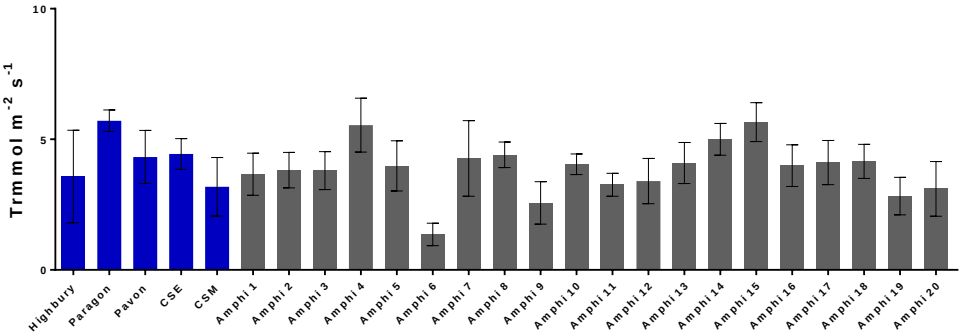
Legends of all the graphs are shown at the end.

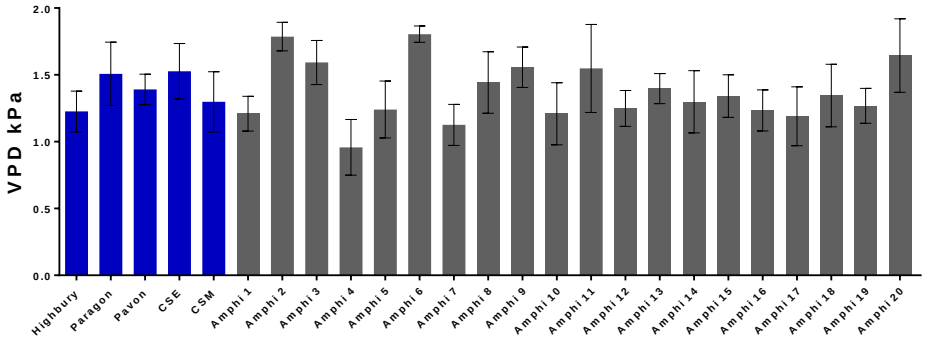


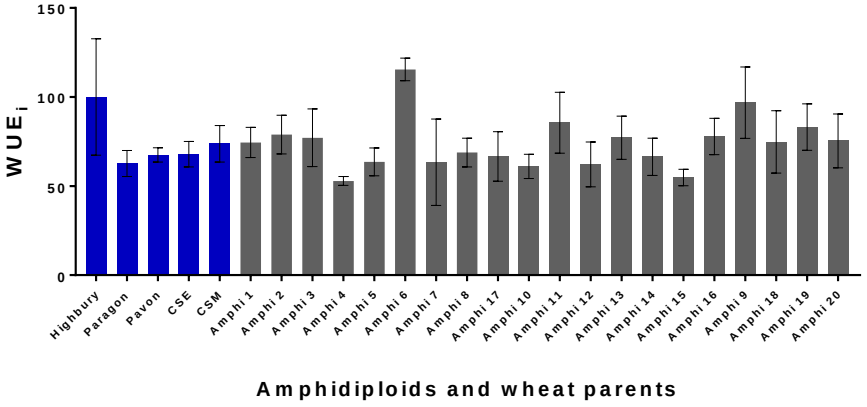


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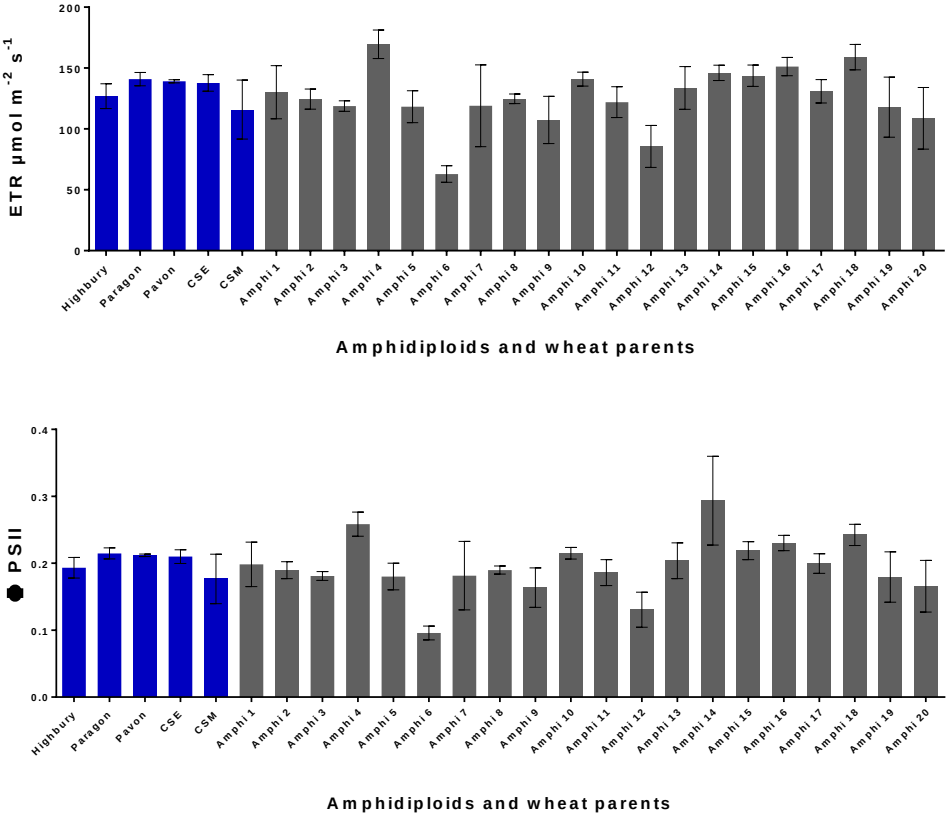


Figure 3.1: Light saturated photosynthetic capacity (P_{max}), stomatal conductance (g_s), Leaf internal CO_2 (Ci), transpiration (Trm), Fv'/Fm', photochemical quenching (qP), operating efficiency of PSII (ΦPSII), electron transport rate (ETR) and intrinsic water use efficiency (WUE_i) of the amphidiploids and wheat parents for the year 2014 measured at vegetative stage (GS39-GS45 (Zadok's nomenclature).

Amphidiploids are represented in grey bars and the UK wheat in blue bars. The cuvette conditions were 400 µmol of CO_2, block temperature was set to 22°C, 1500 PPFD $\mu molm^{-2}s^{-1}$, and ambient humidity was kept ~ 60%. Values are means, error bars are standard errors of the means (n = 4 (individual plants)).

Table 3.1: Analysis of Variance of the photosynthetic parameters (all the parameters) of the amphidiploids and wheat parents for the experimental year 2014. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05).Groups refers to a comparison between amphidiploids and the wheat parents. Amphidiploid lines refer to a comparison within the amphidiploid lines and wheat parents refer to comparison within the wheat lines.

Year 2014	**Groups (Lines vs wheat parents)**	**SEM**	**Amphi diploid Lines**	**SEM**	**Wheat parents**	**SEM**
P_{max}	0.38	±1.60	0.034	±4.53	0.193	±4.40
C_i	0.614	±10.39	0.448	±29.38	0.098	±38.3
g_s	0.157	±0.04	0.264	±0.12	0.177	±0.10
Fv'/Fm'	0.54	±0.01	0.133	±0.03	0.014	±0.02
qP	0.509	±0.066	0.163	±0.02	0.125	±0.04
ΦPSII	0.652	±0.01	0.02	±0.04	0.365	±0.02
ETR	0.651	±7.70	0.01	±21.79	0.365	±16.22
Transpiration	0.613	±0.43	0.233	±1.23	0.06	±1.30
VPD	0.659	±0.15	0.636	±0.44	0.283	±0.19
WUE_i	0.983	±6.86	0.686	±19.41	0.084	±23.70

3.2.2: Relationship between P_{max} and other photosynthetic parameters of the amphidiploids and wheat parents for the year 2014.

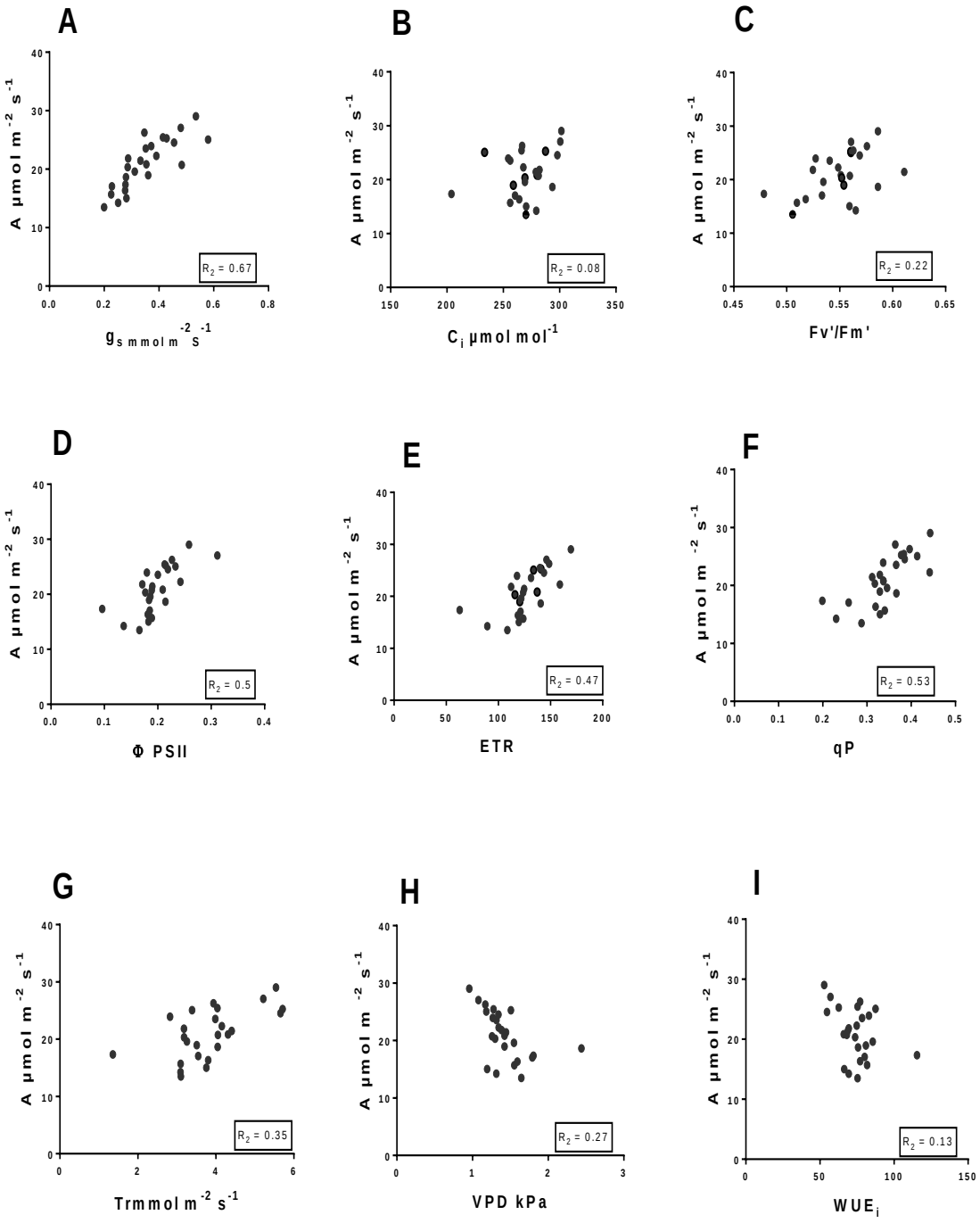


Figure 3.2: Relationshipbetween P_{max} and (A) stomatal conductance, (B) C_i (C) Fv'/Fm' (D) ΦPSII (E) ETR, (F) qP, (G) Transpiration rate(H) VPD and (I)WUE_i derived from the P_{max} measurements for the amphidiploids. The line denotes regression; values are means of each parameter.

Correlation analysis between P_{max} other photosynthetic parameters (Figure 3.2 and table 3.2) reveals that P_{max} showed high and significant relationship with all parameters shown including stomatal conductance (G_s) (figure 3.2A), Fv'/Fm' (figure 3.2C), Φ PSII (figure 3.2D), ETR (figure 3.2E) and qP (figure 3.2F), P_{max} also showed strong relationship with C_i, Transpiration rate, VPD and WUE_i (figure 3.2 B, C, H & I).

This indicates that each photosynthetic parameter may have had an influence in determination of P_{max} of the amphidiploids and the wheat parents. However linear correlation does not mean causation: for example the close and linear relationship with G_s indicates a high level of control of gas exchange via stomata. However G_s and CO_2 assimilation have a strong mechanistic link with each other and it is also possible that higher photosynthesis stimulates a high stomatal conductance which would lead to a lower level of stomatal limitation over photosynthetic rate. If that were the case it may be anticipated that C_i would be more variable (because it would not be driven down by stomatal closure). The observed weak correlation between C_i and P_{max} provides partial support for this. The commonly cited and major limiting factor at light saturation under optimal conditions for C3 species is the amount of active Rubisco in the leaf (e.g. Makino 1997, Murchie et al 1999). Recent work also indicates that the rate of RuBP regeneration and electron transport have an effect in the co-determination of photosynthetic rate (Lefebvre et al 2005, Simkin et al 2017). Thus, a co limitation of P_{max} by different components with Rubisco dominating is consistent with the data in Table 3.2

In detail, Table 3.2 shows a high level of correlation amongst most other parameters indicating a strong interdependence of the different components of

leaf photosynthesis. For example highly significant relationship could be observed between $C_i$and g_s, Fv'/Fm', ΦPSII, ETR, transpiration rate, WUE_i (P <0.001), and VPD (P value 0.025), but no correlation could be drawn between C_i and qP, VPD. Stomatal conductance showed significant relationship with, ΦPSII, Fv'/Fm', qP, ETR, transpiration rate, WUE_i (P <0.001) and VPD (0.03). Fv'/Fm' showed a highly significant relationship with ΦPSII, ETR, transpiration rate and WUE_i, no relationship could be drawn between Fv'/Fm' and qP and VPD. ΦPSII was highly significant with qP, ETR, transpiration rate, WUE_i (P <0.001) and VPD (0.02). qP was highly significant with ETR, transpiration rate (P <0.001) and with VPD &WUE_i (0.02). ETR was highly significant with transpiration rate and with WUE_i (0.03). Transpiration rate was highly significant with WUE_i (P <0.001), VPD was highly significant with WUE_i (P <0.001).

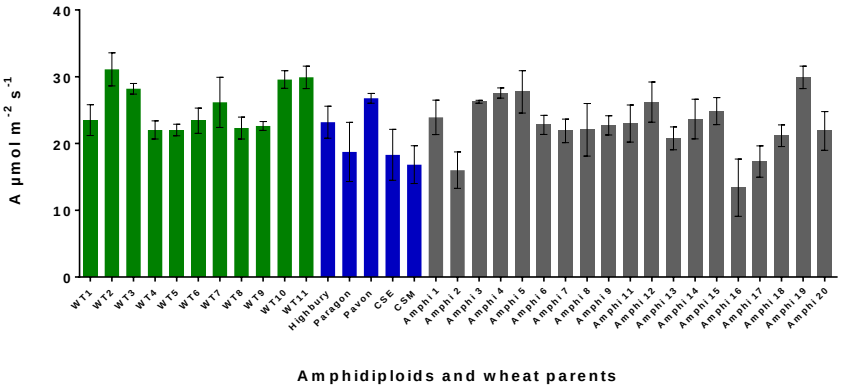
Table 3.2: Correlation analysis between P_{max} and other photosynthetic parameters of the amphidiploids and wheat parents for the experimental years 2014. Values are P values, shaded boxes indicate significant relationships (P<0.05). Here, P_{max} and other photosynthetic parameters were correlated against each other.

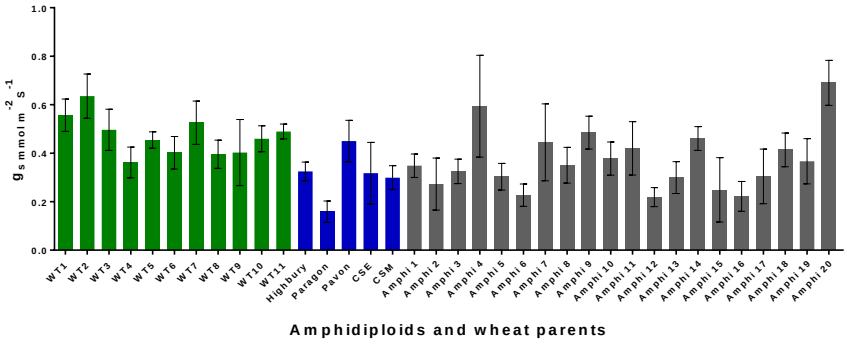
Year 2014	C_i	g_s	Fv'/Fm'	ΦPSII	qP	ETR	Transpiration	VPD	WUE_i
P_{max}	0.005	<.001	<.001	<.001	<.001	<.001	<.001	0.043	0.001
C_i		<.001	<.001	<.001	0.09	<.001	<.001	0.06	<.001
g_s			<.001	<.001	<.001	<.001	<.001	0.03	<.001
Fv'/Fm'				<.001	0.14	<.001	<.001	0.097	<.001
ΦPSII					<.001	<.001	<.001	0.02	<.001
qP						<.001	<.001	0.02	0.02
ETR							<.001	0.03	<.001
Transpiration								0.045	<.001
VPD									<.001

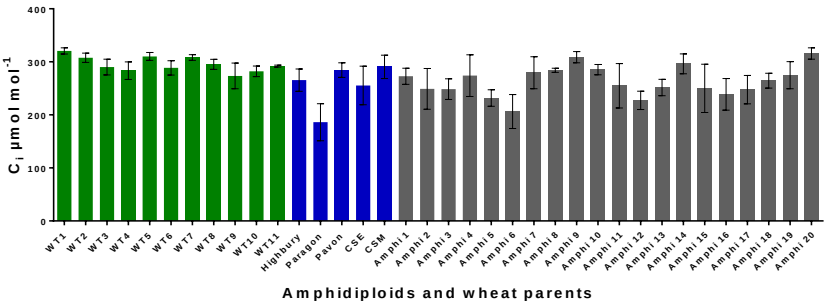
3.2.3 Light saturated photosynthesis measurements for 2015

For the experimental year 2015, the same set of amphidiploids (as year 2014) was included, maintaining similar environmental conditions as for the previous year experiment (2014). A major difference was that wild relatives of wheat were grown along with the amphidiploids and the nitrogen was added manually for year 2015 (For details see Chapter 2).

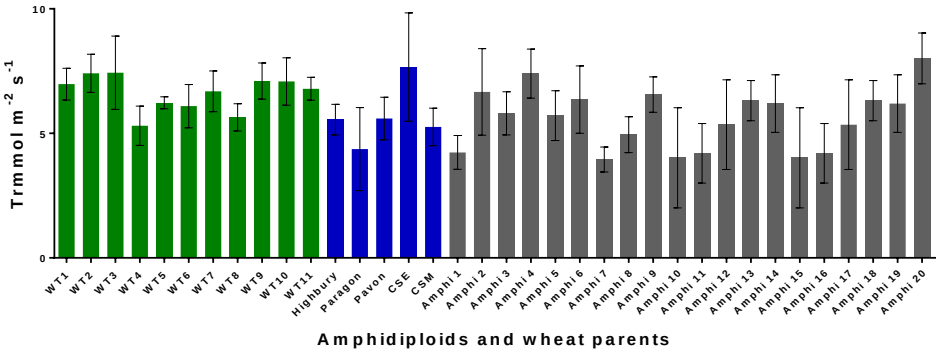
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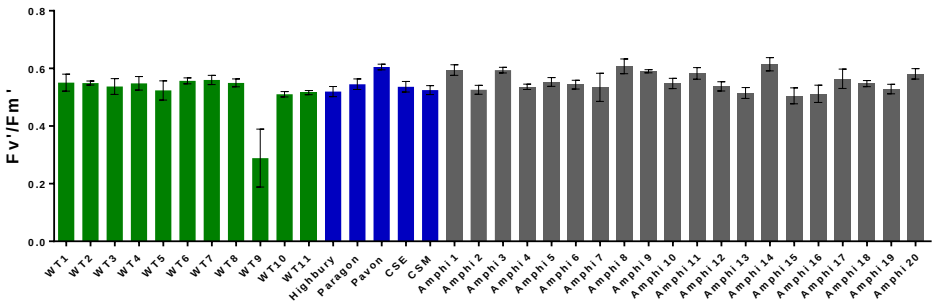


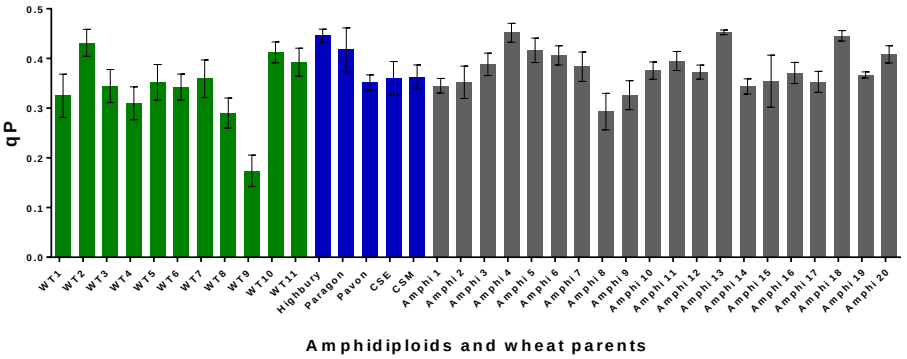


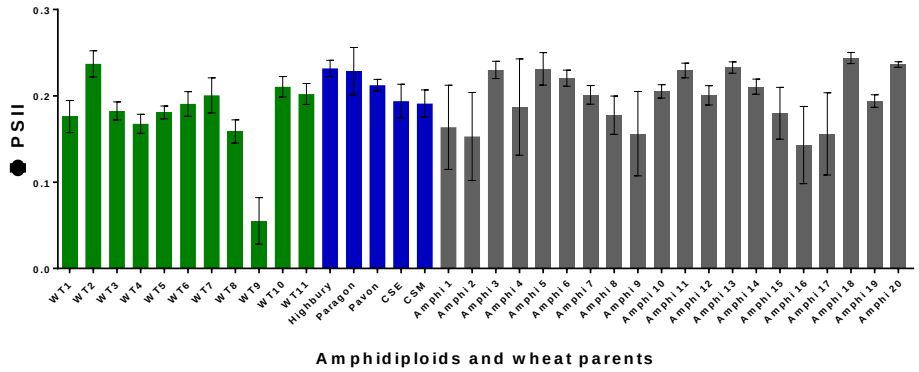


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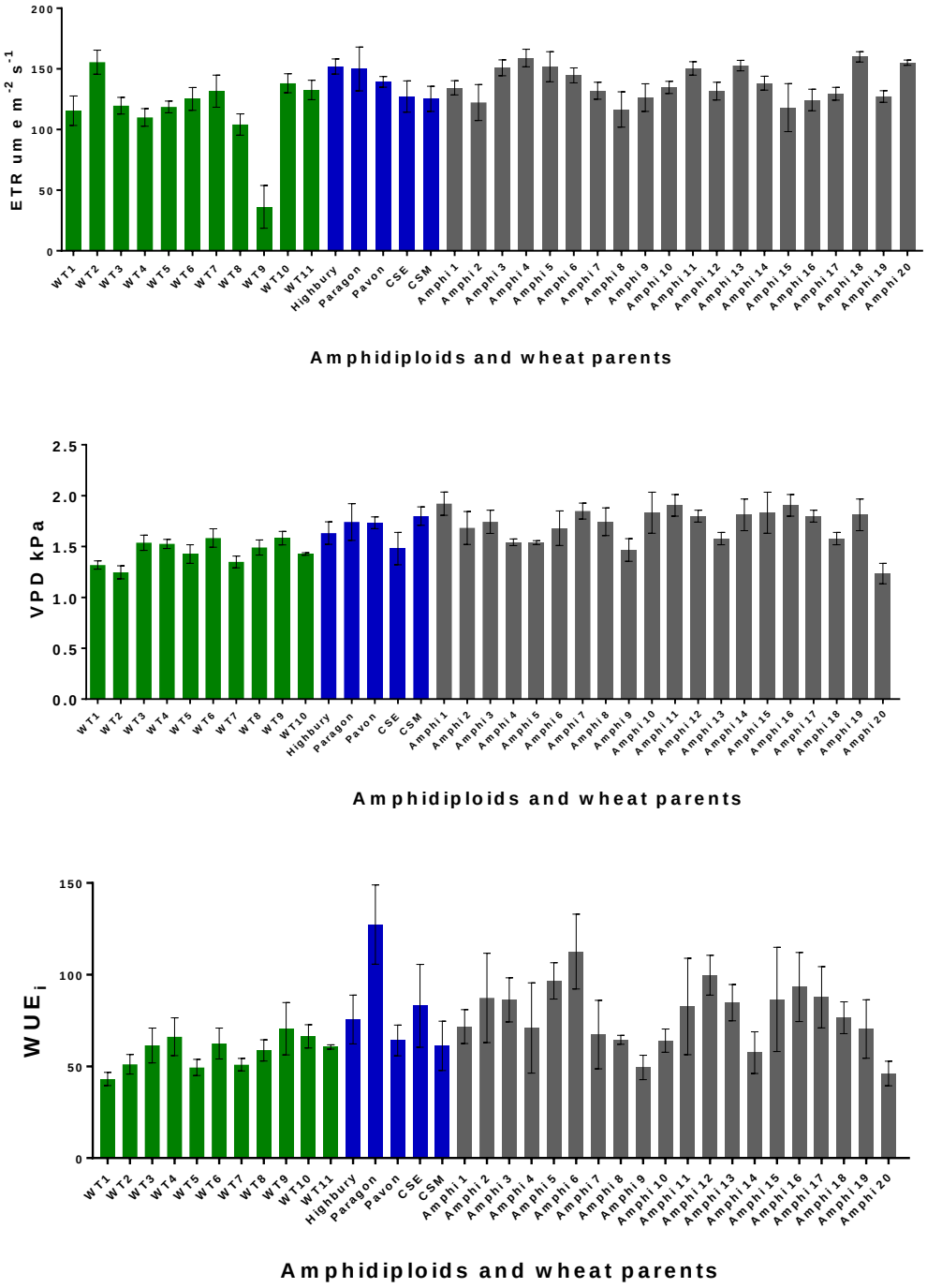


Figure 3.3: Light saturated photosynthetic capacity (P_{max}), stomatal conductance (g_s), Leaf internal CO_2 (Ci), transpiration (Trm), Fv'/Fm', photochemical quenching (qP) ,operating efficiency of PSII (ΦPSII), electron transport rate (ETR) and intrinsic water use efficiency (WUE_i)of the amphidiploids and wheat parents for the year 2014 measured at vegetative stage (GS39-GS45 (Zadok's nomenclature).

Amphidiploids are represented in grey bars, the UK wheat in blue bars and the wild type parents in green bars. The cuvette conditions were 400 µmol CO_2, block temperature was set to 22°C, 1500 PPFD µmol$m^{-2}s^{-1}$, and ambient

humidity was kept ~ 60%. Values are means, error bars are standard errors of the means (n = 4 (individual plants)).

This section describes all the photosynthetic measurements of the amphidiploids, the wheat parents (WHP) and wild parents (WTP) (Figures 3.3 and table 3.3) for 2015. As in 2015 there were significant levels of variation among the amphidiploid lines (Table 3.2). The ranges of most parameters were similar between years; however transpiration and VPD were generally lower for 2015.

The Anova used two approaches: first, comparing the amphidiploids (lines) with the wild parent (group 1), second, comparing with wheat parents (group 2) and also the comparison of the amphidiploids (lines) against each other. Significant differences ($P<0.001$) were observed between the wild parents and amphidiploids lines in some photosynthetic parameters such as C_i, g_s, Fv'/Fm', qP, ΦPSII, ETR, VPD and WUE_i (Table 3.1), P_{max}, and transpiration rate ($P <0.05$). A key observation is that both gs and P_{max} were lower in amphidiploid lines whilst WUE_i was higher. Significant differences ($P<0.001$) were observed among the amphidiploid lines in some photosynthetic parameters P_{max}, Fv'/Fm', qP, ΦPSII and ETR (column 4 is the table 3.3). While comparing the amphidiploids with the wheat parents, no significant difference could be observed in any of the photosynthetic parameters for the year 2015.

Table 3.3: Analysis of variance of the photosynthetic parameters of the amphidiploids, wild type parents and wheat parents for the experimental year 2015. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05).Groups refers to a comparison between amphidiploids and the wheat parents. Amphidiploid lines and Wheat parents refer to a comparison among the amphidiploid lines and among parent lines.

Year 2015	Wild Parents vs Amphidiploids (Group 1)	SEM	Amphidiploids Lines	SEM	Wheat Parents vs Amphidiploids (Group 2)	SEM	Wheat parents	SEM
P_{max}	0.003	±0.8724	<.001	±3.256	0.133	±1.33	0.193	±4.40
C_i	<.001	±8.47	0.551	±31.64	0.773	±13.42	0.098	±38.3
gs	<.001	±0.033	0.394	±0.12	0.373	±0.04	0.177	±0.10
Fv'/Fm'	<.001	±0.01	<.001	±0.03	0.446	±0.01	0.014	±0.02
qP	<.001	±0.01	<.001	±0.03	0.475	0.01	0.125	±0.04
ΦPSII	<.001	0	<.001	±0.03	0.264	0.01	0.365	±0.02
ETR	<.001	±3.66	<.001	±13.69	0.739	±4.94	0.365	±16.22
Transpiration	0.016	±0.41	0.792	±1.56	0.353	±0.59	0.06	±1.30
VPD	<.001	±0.03	0.09	±0.14	0.674	±0.06	0.283	±0.19
WUE_i	<.001	±5.3	0.593	±19.78	0.741	±8.38	0.084	±23.72

3.2.4: Relationships between P_{max} and other photosynthetic parameters for the experimental year 2015.

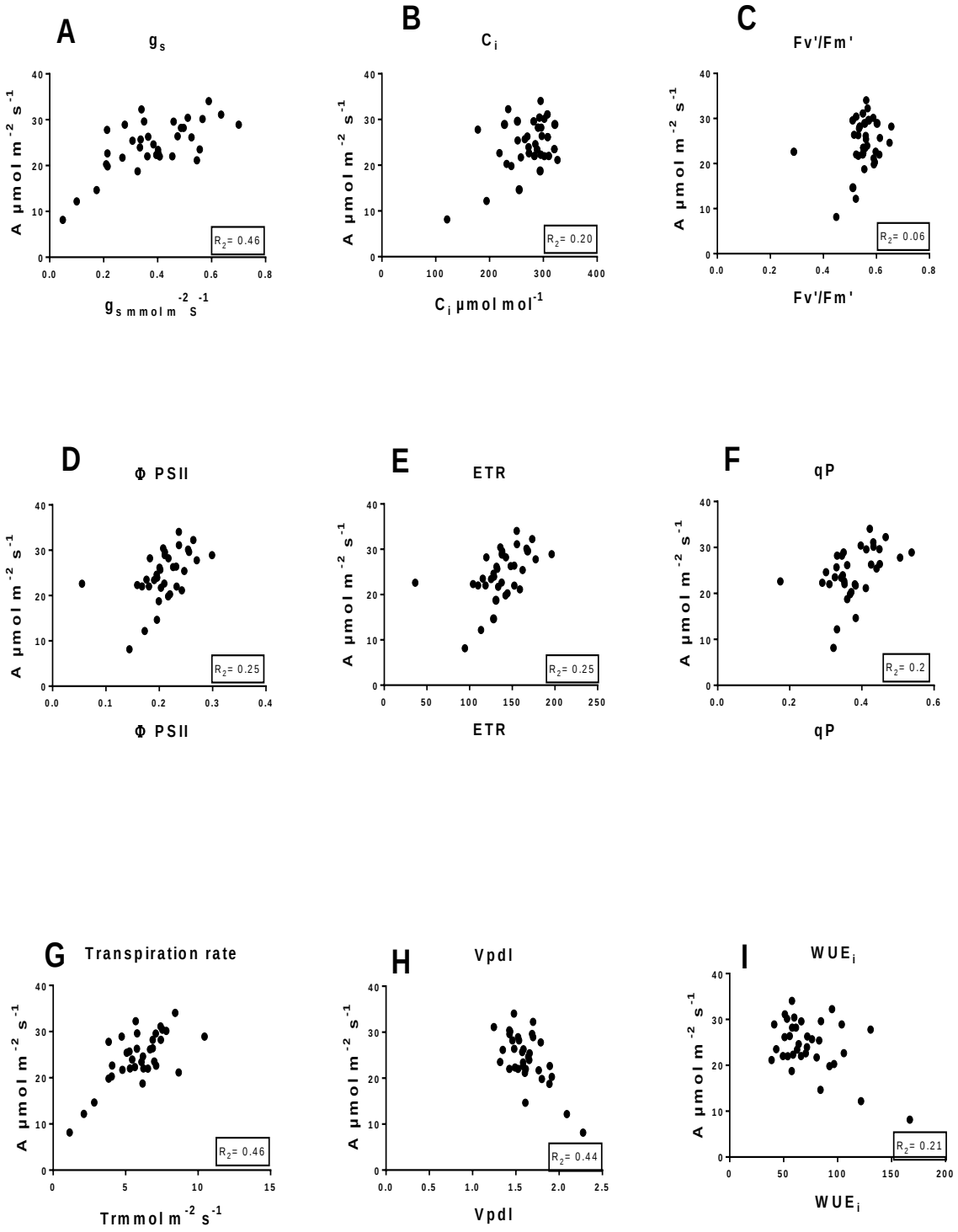


Figure 3.4: Relationshipbetween P_{max} and (A) stomatal conductance, (B) C_i (C) Fv'/Fm' (D) ΦPSII (E) ETR, (F) qP, (G) Transpiration rate(H) VPD and (I)WUE_i derived from the

P_{max} measurements for the amphidiploids for the year 2015 Values are means of each parameter, (n=4).

As in 2015, correlation analysis between P_{max} other photosynthetic parameters (Figure 3.22 and table 3.4) reveals that P_{max} showed positive and significant relationships with all other photosynthetic parameters figure 3.22 (A) g_s, (B) C_i, (C) Fv'/Fm' (D), Φ PSII, (E) ETR (F) qP and (G)Transpiration rate. P_{max} showed a negative correlation with (H) VPD and (I) WUE_i. The pattern of correlation was similar to that shown in 2014.

Table 3.4: Correlation analysis between P_{max} and other photosynthetic parameters of the amphidiploids, wheat parents and wild parents for the experimental years 2015. Values are P values, shaded boxes indicate significant relationships (P<0.05).

Year 2015	C_i	g_s	Fv'/Fm'	ΦPSII	qP	ETR	Transpiration	VPD	WUE_i
P_{max}	0.02	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.02
C_i		<.001	<.001	0.814	0.253	0.613	<.001	<.001	<.001
g_s			<.001	0.388	0.44	0.172	<.001	<.001	<.001
Fv'/Fm'				0.07	0.05	0.013	<.001	<.001	<.001
ΦPSII					<.001	<.001	0.019	0.1	0.853
qP						<.001	0.257	0.904	0.28
ETR							0.002	0.012	0.576
Transpiration								<.001	<.001
VPD									<.001

Table 3.4 shows that P_{max} was highly significant with g_s, ΦPSII, Fv'/Fm', qP, ETR, Transpiration rate and VPD ($P < 0.001$), while C_i and WUE_i were significant with ($P < 0.05$). Highly significant relationship could be observed between C_i with g_s, Fv'/Fm', transpiration rate, VPD and WUE_i ($P < 0.001$), but no correlation could be drawn between C_i and ΦPSII,ETR, qP. Stomatal conductance showed significant relationship with, Fv'/Fm', transpiration rate, VPD and WUE_i ($P < 0.001$), no correlation could be observed between gs and ΦPSII,qP, ETR. Fv'/Fm' showed highly significant relationship with transpiration rate, VPD and WUE_i ($P < 0.001$, ETR and transpiration rate (P value <0.05), no relationship could be drawn between Fv'/Fm' ΦPSII. ΦPSII was highly significant with qP and ETR, ($P < 0.001$) and transpiration rate (P value <0.05). qP was highly significant with ETR, ($P < 0.001$), ETR showed significant relationship with transpiration rate and VPD. Transpiration rate was highly significant with VPD and WUE_i ($P < 0.001$), VPD was highly significant with WUE_i ($P < 0.001$).

3.2.5: AC_i curves

Table 3.5 shows that there was a poor correspondence between the P_{max} values attained in 2015 compared with that in 2014. Noting the lack of a statistical difference if the means are ranked it can be seen that some of the lines that were classified as Low P_{max} in 2014 were classed as high in 2015.

There was a need to limit the number of lines that underwent detailed analysis (A-C_i and leaf structure). Amphidiploids that demonstrated high and low mean P_{max}at the vegetative stage of development for the year 2014 were selected and were subjected to detailed photosynthetic measurement at the anthesis stage of development. AC_i curves were performed to help understand which components were limiting photosynthesis in these leaves (or example Rubisco carboxylation efficiency) which can be analysed using curve fitting model (Sharkey et al., 2007) to calculate V_{cmax}, and J_{max}. V_{cmax} is the maximum carboxylation velocity of the enzyme Rubisco and J_{max} is the maximum rate of electron transport.

Due to time constraints, the AC_i and leaf morphology data is presented onlyfor 2014.

Table 3.5: Selected amphidiploid lines showing high and low P_{max} for the years 2014 and 2015. Note that this is based on a ranking of means and not based on a statistical test.

2014 High P_{max}	**2015 High P_{max}**	**2014 Low P_{max}**	**2015 Low P_{max}**
Amphi 1	Amphi 3	Amphi 3	Amphi 2
Amphi 4	Amphi4	Amphi 5	Amphi 13
Amphi 13	Amphi 5	Amphi 9	Amphi 16
Amphi 14	Amphi 12	Amphi 20	Amphi 17
Amphi 15	Amphi 15		
Amphi 16	Amphi 19		

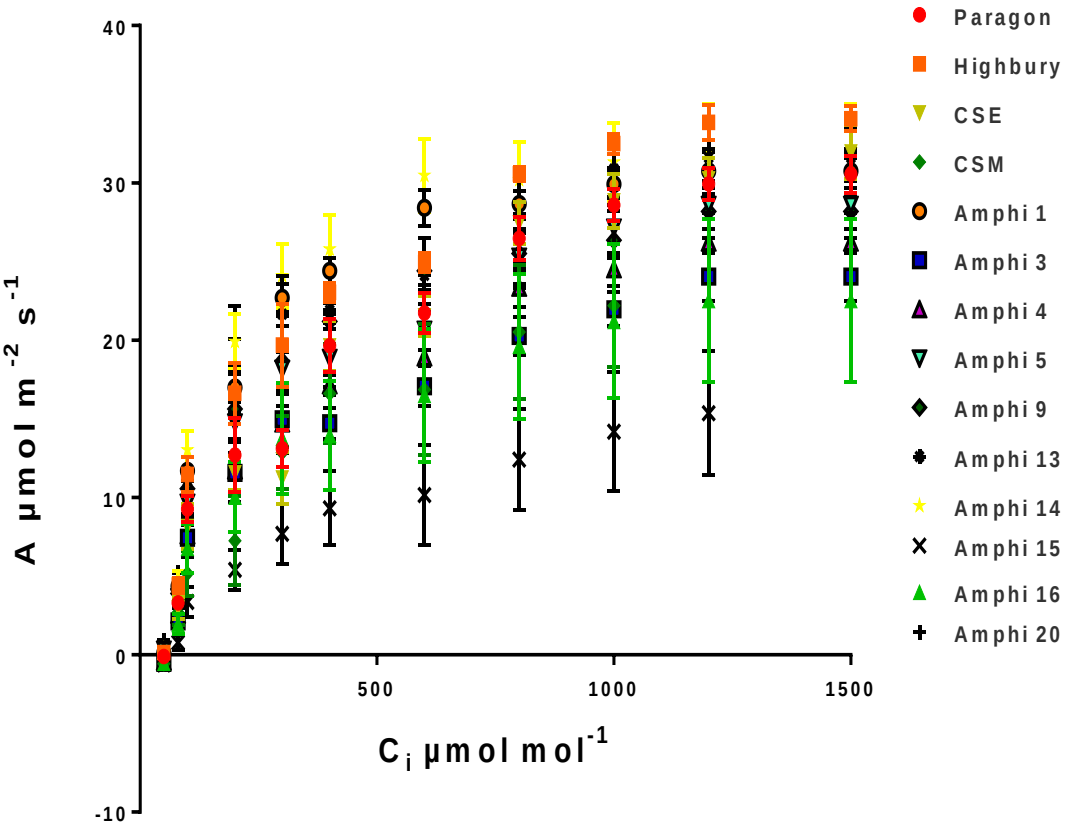


Figure 3.5: Example of AC_i curves / CO_2 response curves of the wheat parents and amphidiploids that demonstrated higher and lower P_{max} for the experimental year 2014. The data is plotted as CO_2 assimilation rate A ($\mu molm^{-2}\ s^{-1}$), to increasing CO_2 concentration, the settings for CO_2 concentration are mentioned in Chapter 2. The cuvette conditions were 1500 PPFD and the block temperature set as 22 ° C, humidity was set to ~ 60 %. Values are means, the error bars are the standard errors of the means (n=4). Curve fitting (not shown here) was carried using CO_2 level 1000 ppm as the maximum assimilation rate.

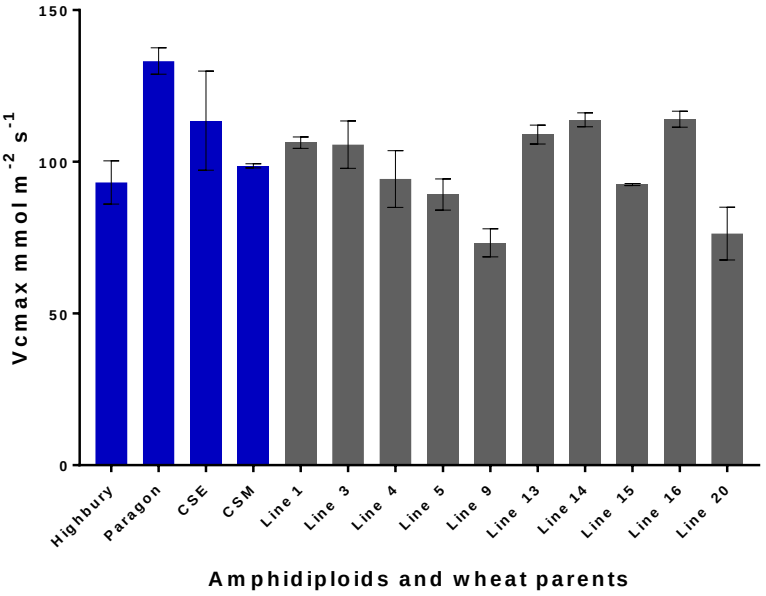


Figure 3.6: Mean of V_{cmax} derived from curve fitting of the amphidiploids with higher and lower P_{max} along with wheat parents for the experimental year 2014. Values are means, error bars are the standard errors of mean, (n=4).

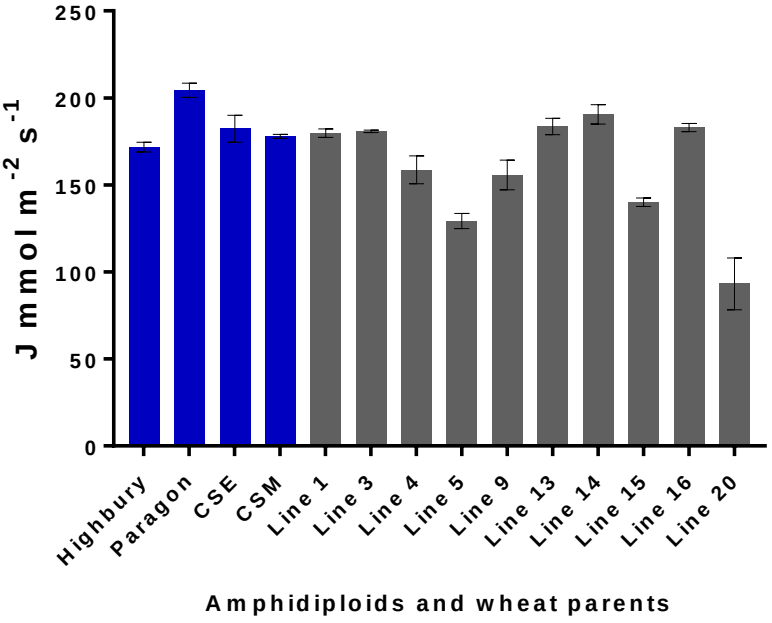


Figure 3.7: Mean of J_{max} derived from curve fitting of the amphidiploids with higher and lower P_{max} along with Wheat parents for the experimental year 2014. Values are means, error bars are the standard errors of mean, (n=4).

Table 3.6: Analysis of Variance of J_{max} and V_{cmax} of the amphidiploids and wheat parents for the experimental year 2014. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Groups refers to a comparison between amphidiploids and the wheat parents. Lines refer to amphidiploid and Wheat parents refer to a comparison within the wheat parent lines.

Year 2014	**J_{max}**	**SEM**	**V_{cmax}**	**SEM**
Groups (Amphidiploid lines vs Wheat parents)	<0.001	±8.16	<0.001	±8.11
Amphidiploid (Lines)	<0.001	± 8.17	<0.001	±8.11
Wheat parents	0.005	± 7.59	0.08	± 14.99

Figure 3.6, 3.7 and Table 3.6 show differences between wheat parents and the amphidiploid lines. Individual comparisons show that these correspond to a decrease between amphidiploids and lines that are not their parental wheat lines. For example V_{cmax} in Amphi 9 was comparatively lower than in paragon which was not the parent of this line. J_{max} in Amphi 5 was lower than Paragon which was not the wheat parent of this line. Therefore there is no evidence that these traits have been improved in the amphidiploid lines, consistent with the P_{max} data. Table 3.6 shows that J_{max} and V_{cmax} was highly significant (<0.001) when compared in groups i.e. amphidiploid vs wheat parents, in lines i.e comparison within the amphidiploids, however, comparison within the wheat parents, J_{max} was significant (P value <0.005) but no significance could be observed in V_{cmax} in the wheat parents.

3.2.6: Relationships between P_{max} and other photosynthetic parameters for the experimental year 2014.

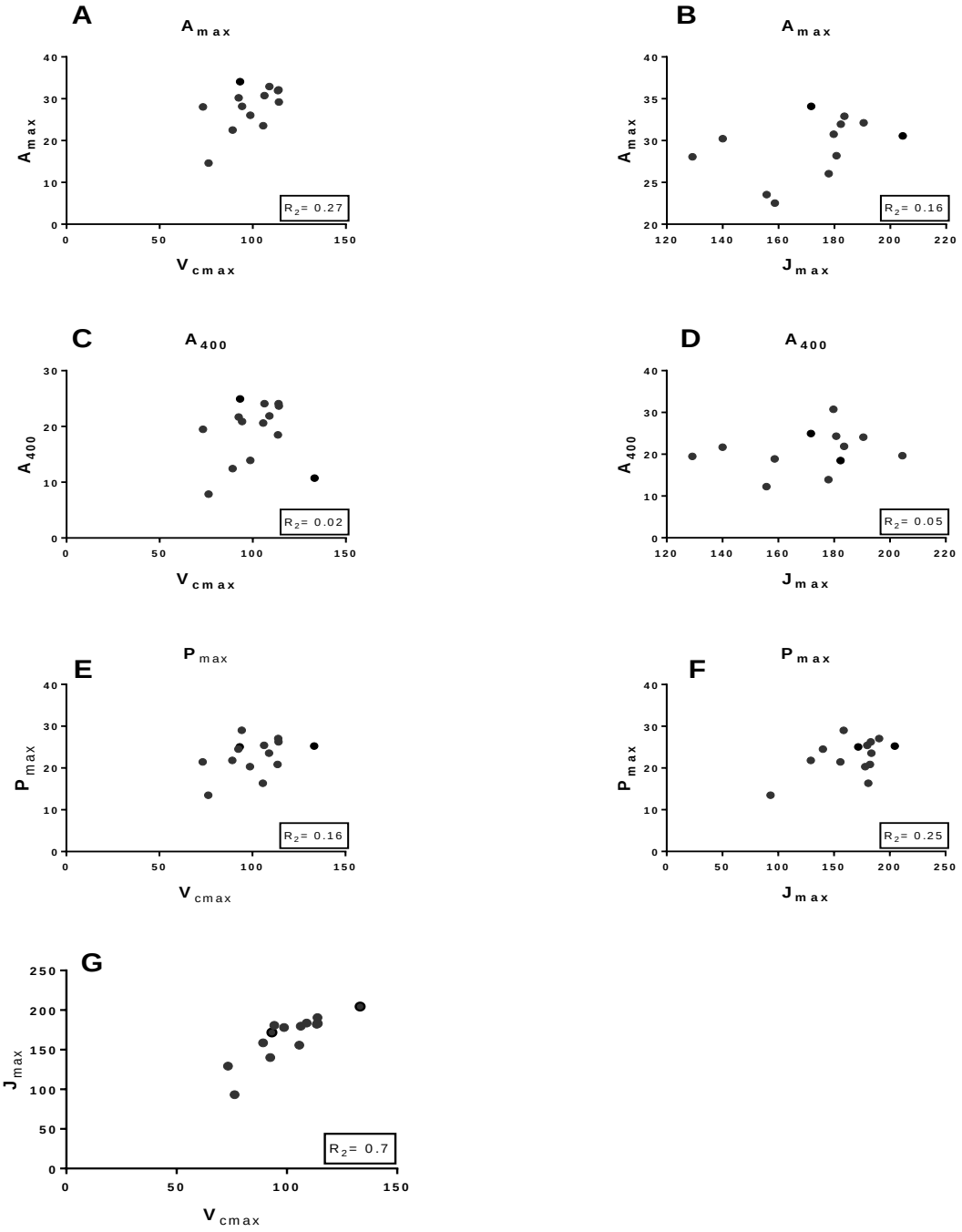


Figure3.8: Relationships between Correlation analysis of J_{max}, A_{400} (assimilation rate at 400 ppm CO_2), A_{max} (assimilation rate at saturating CO_2) and P_{max} (from the spot measurements in figure 3.1) derived from AC_i curves of the amphidiploids and the UK wheat from the experimental year 2014. Values shown are means.

Table 3.7: Correlation of theresults of curve fitting of AC_i curve measurements for the experimental year 2014. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). P_{max} is the measurement made at the vegetative stage (GS39-GS45, Zadok's nomenclature) of development.

Year 2014	J_{max}	A_{400}	A_{max}	P_{max}
V_{cmax}	<.001	0.986	0.869	0.971
J_{max}		0.573	0.966	0.67
A_{400}			<.001	0.06
A_{max}				0.504

Correlation analysis of J_{max}, A_{400}(assimilation rate at 400 ppm CO_2), A_{max} (assimilation rate at saturating CO_2) and P_{max}(from the spot measurements in figure 3.8) is shown in Table 3.7. The comparison was made between the wheat parents and amphidiploids. A high level of significance could be observed between V_{cmax} and J_{max} (P <0.001). A_{400} showed significant relationship with A_{max} (P <0.001). No correlation could be observed between V_{cmax} and A_{400}, A_{max}, P_{max}. When interaction was considered between J_{max} and A_{400}, A_{max} and P_{max}, similarly no relationship could be observed between P_{max} with A_{400} and A_{max}.

3.2.7 Leaf anatomy

The amphidiploids were screened on the basis of their photosynthetic responses however it isalso necessary to understand the underlying mechanism that is regulating photosynthesis. Early work has established (e.g. Austin *et al* 1982)that variation in leaf anatomical characteristics can establish a relationship with flag leaf photosynthesis. Contemporary work across species has shown that leaf anatomy features such as leaf thickness, stomatal density and mesophyll cell size may have a significant influence on photosynthetic capacity (Falcioni et al 2017, Fullana-Pericàs, Murchie et al., 2017,Paradiso et al., 2017).Hence, in

this section of the chapter, an attempt was made to understand the anatomical characteristics in the selected amphidiploids and the wheat parents in the 2014 experiment by performing leaf sectioning, staining and imaging on the flag of the amphidiploids and wheat parents. This section shows the results of analysis of mesophyll cell area, leaf thickness, distance between major and minor veins and the number of mesophyll cells between major and minor vein.

Leaf section example

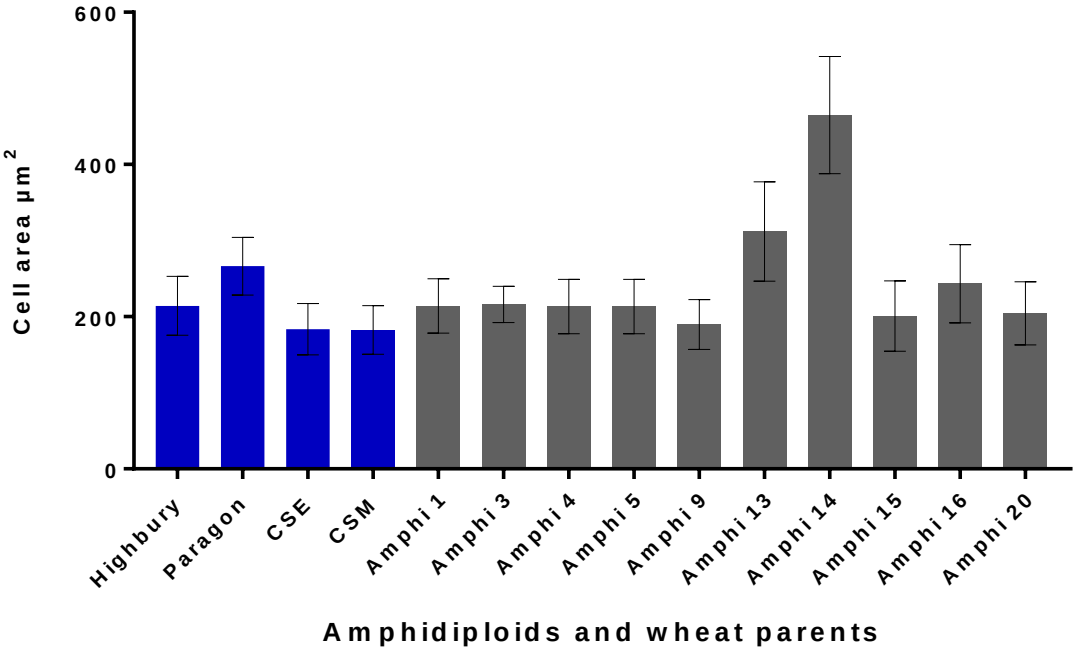


Figure 3.9: Mesophyll cell area of the amphidiploids (grey bars) and wheat parents (blue bars). Values are means, error bars are standard errors of the means (n = 4).

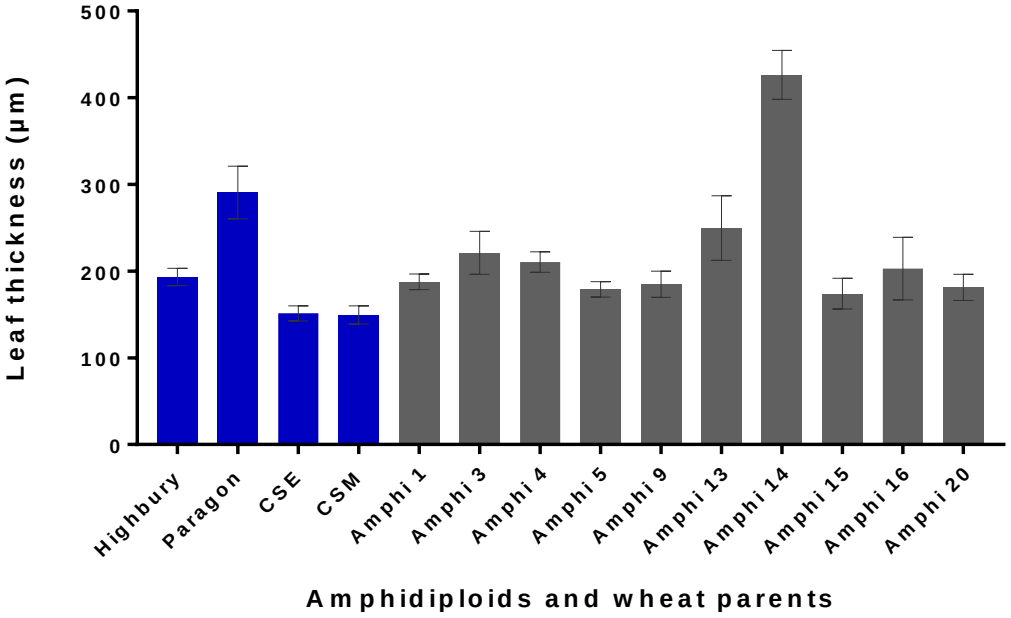


Figure 3.10: Leaf thickness of the amphidiploids (grey bars) and wheat parents (blue bars). Values are means, error bars are standard errors of the means (n = 4).

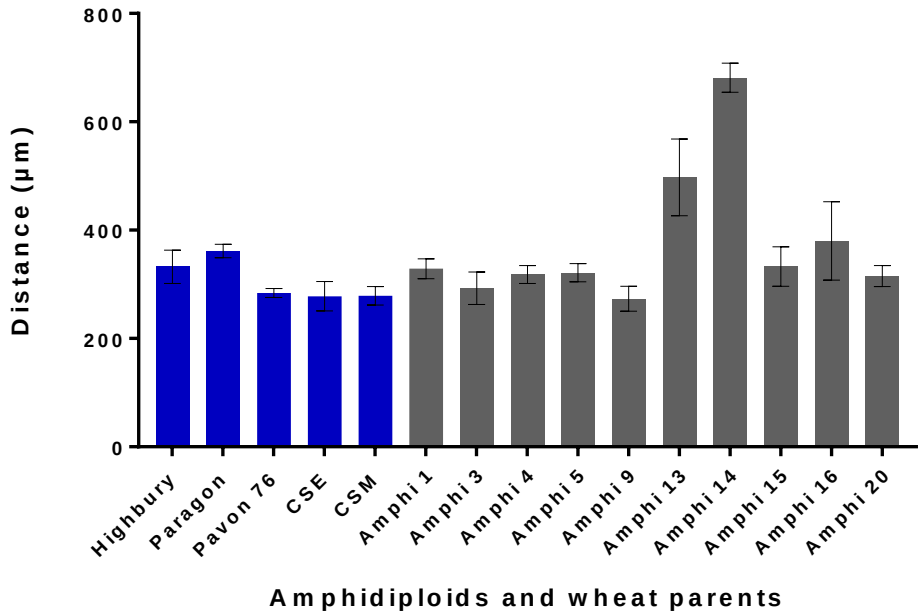


Figure 3.11: Distance between major and minor veins of the amphidiploids (grey bars) and wheat parents (blue bars). Values are means, error bars are standard errors of the means (n = 4).

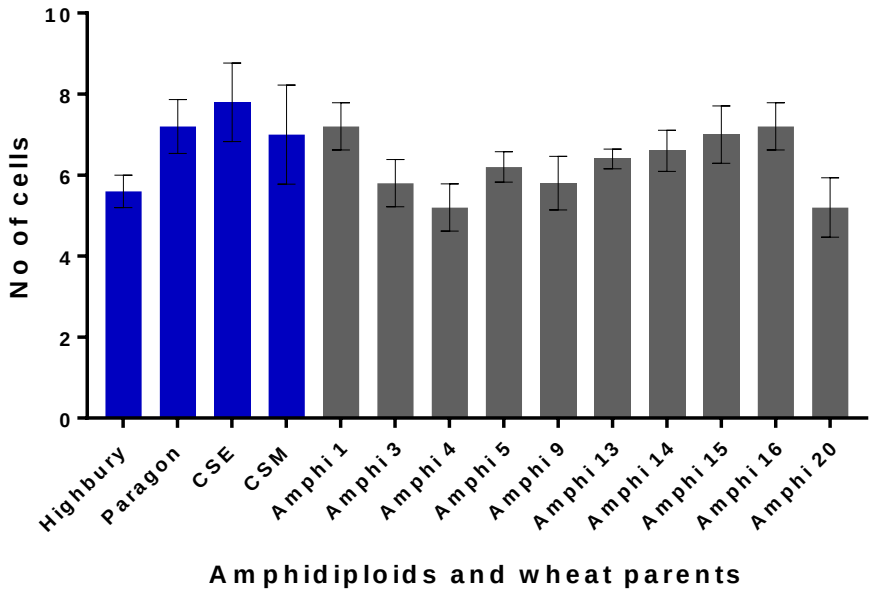


Figure 3.12: Number of mesophyll cells of the amphidiploids (grey bars) and wheat parents (blue bars). Values are means, error bars are standard errors of the means (n = 4, i.etransverse section of four leaves). Yaxis represents number of mesophyll cells.

Figures 3.9-3.12 and Tables 3.8 and 3.9 show the leaf anatomical parameters of the amphidiploids and the wheat parents for the year 2014. Mesophyll cell area was highest in amphi 14 (464 μm^2) and amphi 13 (311 μm^2) (Figure 3.9), most of the amphidiploids and wheat parents were in the range 200-250 μm^2.The distance between the major vein and minor vein were in the range 250-400 μm^2 in most of the amphidiploid and wheat parents, but in amphi 14 the distance was the maximum more than 681 μm^2 and in amphi 13 it was 497 μm^2 (Figure 3.11). The number of mesophyll cells between major and minor veins was in the range (5-8) (Figure 3.12).

Table 3.8: Two way anova of leaf anatomical parameters for the experimental year 2014. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Here, group means comparison between wheat parents and amphidiploids and lines indicate comparison within the amphidiploid lines.

Year 2014	No. of Mesophyll	SEM	Distance between veins	SEM	Leaf thickness	SEM	Mesophyll cell area	SEM
Group, Wheat parents vs amphidiploids	0.126	±0.37	0.022	±20.54	0.01	±14.25	0.391	±57.17
Lines, Amphidiploids	0.383	±0.87	<0.001	±47.44	<0.001	±32.9	0.483	±132

Table 3.9: Correlation matrix of the leaf anatomical parameters with P_{max}, V_{cmax}, J_{max}, A_{max} and A_{400} for the year 2014. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Here, P_{max} derived from the photosynthetic measurements from vegetative stage (GS39-45, Zadok's nomenclature), the AC_i curve parameters V_{cmax}, J_{max}, A_{max} and $A_{400}$are compared with the mesophyll cell area, thickness of flag leaf, distance between the veins and the number of mesophyll cells between two veins.

Year 2014	P_{max}	V_{cmax}	J_{max}	A_{max}	A_{400}
Mesophyll cell area	0.893	0.376	0.245	0.754	0.752
Leaf thickness	0.284	0.282	0.023	0.327	0.05
Distance between veins	0.178	0.746	0.122	0.133	0.053
No. of Cell	0.344	0.953	0.708	0.895	0.691

Table 3.10: Correlation matrix of the leaf anatomical parameters for the year 2014. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Here, leaf anatomical parameters (mesophyll cell area, leaf thickness, distance between the veins and number of mesophyll cells) are compared against each other.

Year 2014	Mesophyll cell area	Leaf thickness	Distance between veins	No. of Cell
Mesophyll cell area		0.042	0.283	0.126
Leaf thickness			<0.001	0.8
Distance between Veins				0.926

The two way anova was carried out for the leaf anatomical parameters of the amphidiploids (lines) with the wheat parents (groups) and also the comparison of the amphidiploids (lines) against each other (Table 3.8) Significant differences were observed for the distance between major and minor veins between the lines (P value <0.001) and the groups (P value <0.05) and leaf thickness also showed significance between the lines (P value <0.001) and the groups (P value <0.05) , no significance was seen either in the groups or lines in the number of mesophyll cells between major and minor vein and the mesophyll cell area. Of these, amphidiploid line 14 and 13 had higher leaf thickness and interveinal distance than its wheat parent (P<0.05). However there was no significant difference in any photosynthetic trait. Overall there is weak evidence for a link between leaf anatomy and photosynthesis.

Table 3.9 shows the correlation matrix between photosynthetic parameters and parameters derived from AC_i curve measurements. Of all the leaf anatomical parameters, leaf thickness and distance between major and minor vein showed significant relationship A_{400} (P <0.05) indicating that thicker leaves may be required to support a higher photosynthetic capacity as has been observed previously (e.g. Murchie et al 2005).

Another correlation was done between the leaf anatomical parameters (Table 3.10) leaf thickness showed significant relationship with mesophyll cell area (P <0.05) and distance major and minor vein (<0.001). This suggests that the mesophyll cells are in greater number as the distance between major and minor veins increase and also when leaf thickness is greater, which can be observed in amphi 14. Again this was not associated with higher photosynthesis.

3.3 Discussion and conclusion

The goal of this work was to identify variation in leaf photosynthesis in populations of wheat amphidiploid lines ultimately revealing lines with superior photosynthesis. The most important target, and the focus of discussion here, is to outperform the wheat parents since this will form the basis for selection and breeding for higher photosynthesis. When comparing the parents with amphidiploid lines as groups, there was no statistical evidence that amphidiploids possessed a higher photosynthetic capacity for either year (2014 or 2015), or that there was any potential for higher photosynthesis (V_{cmax}, J_{max}) in the amphidiploid lines. Therefore hypothesis 1 must be rejected.

There was a high degree of similarity between the P_{max} of the wheat and amphidiploid lines showing that at least the latter were able to retain the rates of photosynthesis present in the wheat parents (AC_i results notwithstanding). Hypothesis 2 can therefore be supported. This is important because in any wide crossing programme it is crucial that any negative traits from the wild species are not carried over into the breeding or pre-breeding wheat material. There is reason to suspect this is a real possibility. In this chapter there was no difference between wild relative lines and the wheat parents (Figs 3.1,3.3) but many other wild species have conservative photosynthetic rates especially where such species are geared towards low input and multi stress environments (e.g. see Murchie et al 2009). Since the wild relatives did not generally show enhanced photosynthetic rates themselves (though see 2015 data, Figure 3.3) it is perhaps not surprising that no changes were seen in the amphidiploids. In this case there was no choice over which wild relatives are used to produce the amphidiploid lines but a future study should screen the wild species and accessions before crossing. A separate

screen of wild relatives and accessions at the University of Nottingham has revealed a large degree of variation among a selection of wild relative species of wheat (Murchie personal communication), including those used in this study. Within these species many accessions with superior photosynthetic capacity to wheat have been identified. Surprisingly this seems to depend more on the individual accession rather than species suggesting that it is not possible to generalise about species dependent photosynthetic traits and rigorous screening of accessions is necessary before a introgression programme begins (Murchie and McAusland unpublished data).

One of the reasons for the lack of difference could be growth conditions or GxE interaction. The glasshouse conditions used here were 'unchallenging' since the temperature was within optimal range; often non-saturating and the plants had sufficient water and nutrients. It's becoming clear that to improve photosynthesis in crops, we need to improve photosynthesis under naturally dynamic conditions. For example, photosynthesis in natural and agronomic environments is subjected to fluctuations in light intensity due to cloud cover and plant architecture. It has recently been demonstrated that improving the way in which quantum yield of photosynthesis responds to light fluctuations can improve yield and biomass (Kromdjik et al 2016, Murchie 2017). In that case it is not the photosynthetic capacity that is important but rather the reduction in quantum yield that occurs under temporary low light, the screens used in the current chapter would not have revealed such traits. Therefore, it will be necessary to develop dynamic screens for photosynthesis in addition to steady state measurements. This will be difficult to achieve for gas exchange but possible for chlorophyll fluorescence which can be measured remotely. Such techniques are being developed e.g. at the University of Nottingham (McAusland and Murchie, personal communication).

In addition to light, screening should be done at different temperatures, this is especially important for C3 plants which are sensitive at higher temperatures due to the oxygenation reaction of Rubisco, photorespiration, Rubisco activase and oxidative stress tolerance. In fact, many of these properties may be more relevant to wild relative introgression since these species and accessions will be subject to such conditions and more likely to exhibit tolerance. However, it should be noted that there is a difference between improving yield potential (e.g. by studying photosynthesis under optimal conditions such as a glasshouse) and improving yield under sub optimal conditions (such as high temperature).

The data indicated that there was no consistency between the years in terms of P_{max} and other associated traits. Due to the lack of statistical significance this cannot be explored in depth here, but differences could be due to differences in light level between the years which may cause differences in acclimation of photosynthesis (e.g. see Murchie and Horton 1997 and Athanasiou et al 2010). The differing methods of application of N may have had an effect. Lastly it might be expected that some segregation of the amphidiploid lines occurred. Although expected to be relatively stable, several generations are usually required. The seed in 2015 was the progeny of the plants grown in 2014.

There is growing evidence that leaf structure (functional anatomy) may have an influence on photosynthetic rate in C3 plants. Here we do not discuss Kranz anatomy which is specific to C4 species and associated CO_2 concentrating mechanisms. For example, mesophyll conductance determines the rate of transfer from the intercellular spaces of the leaf into the mesophyll itself (Flexas et al., 2012 Terashima et al., 2011 It can be related to features such as the mesophyll surface area and size of mesophyll cells. Here we did not note any

differences in mesophyll area and this was consistent with the lack of difference between wheat and amphidiploid plants in P_{max} or other photosynthesis parameters and it can be concluded that hypothesis 3 must be rejected.Murchie et al (2005) demonstrated that leaf thickness is related to accumulation of rubisco content in plants, however in this chapter, table 3.7 shows that leaf thickness had significant relationship with A_{400} (<0.05), A_{400} is the assimilation rate at 400 $\mu molCO_2m^{-2}s^{-1}$ derived from the AC_i curve measurements, which can be attributed to rubisco accumulation. It could be observed that the leaf thickness showed significant relationship with mesophyll cell area, mesophyll cell are the site of photosynthetic process, where rubisco is found. Exploring such variation in leaf anatomical features in the amphidiploid populations would be an interesting work to be carried out in future to investigate other leaf anatomical features that are not mentioned in this chapter.

Another important aspect is the link between leaf hydraulics (water movement), morphology and water. It is thought that leaf venation characteristics can influence the ability of the leaf to draw water from root to shoot. Since transpiration rate correlates with photosynthesis in many circumstances this may be important in achieving high photosynthetic rates of land plants. Leaf venation in C3 was important in terms of enhancing photosynthesis of plants at an early evolutionary stage (e.g. Brodribb et al., 2010).

It is important to consider that an increase in carbon gain may come at the cost of an increase in water use (transpiration). This may be important in areas that are water limited or where the growing season is defined by water availability (see subsequent chapters in this thesis). It is therefore interesting that in 2015 an enhanced WUE_i was noted in amphidiploid lines in comparison with wheat

parents, although no difference was observed in 2014. There was no loss in WUE in the amphidiploid lines.

Stomatal conductance deservesparticular attention especially stomatal density. Stomatal conductance in both experiments was found to be lower in most of the amphidiploids, this could be due to soil water evaporation, as the compost used did not retain water for longer period, regardless the plants received water twice a day. There are various reasons for stomatal closure, higher light intensity, lower water content, high temperature or higher CO_2 levels. However, the closure of stomata did not show its effect on intercellular CO_2, suggesting it could be partial closure of stomata, and there was no resistance for diffusion of CO_2 into the leaf air spaces. In the year 2015, the temperature was slightly higher in the summer, which might have contributed towards effect of stomatal closure. The stomatal conductance in the amphidiploids and wheat parents for the year 2014 was lower with values in the range of (0.1-4) $mmolm^{-2}s^{-1}$, similar trend could be observed in the amphidiploids, wheat parents for the year 2015; however the wild relatives of wheat showed higher stomatal conductance than the amphidiploids and wheats (Figure 3.13). WUE_i of the amphidiploids were comparatively lower in the year 2014, this could be due to lower transpiration rate in the amphidiploids. Wild relatives of wheat in the year 2015 demonstrated lower WUE_i than the wheat and amphidiploids suggesting they have traits related water use efficiency.

Conclusions

- No improved photosynthetic capacity (P_{max}) was observed in amphidiploids lines but equally there was little evidence of an introduction of detrimental traits including no reduction in intrinsic water use efficiency.
- Strong correlations between P_{max}, stomatal conductance and ΦPSII efficiency were observed.
- Whilst limited variation in leaf cellular morphology was observed there was little evidence of a link with photosynthetic capacity.

Chapter 4

The physiology and photosynthetic responses of amphidiploids derived from *Thinopyrum bessarabicum*and durum wheat cross under field conditions in India

4.1 Introduction

Wild relatives of wheat are potentially vast resources of genetic variation for various traits. To exploit these new traits by incorporation into modern wheat would be a promising method to develop advanced wheat varieties superior to modern wheat which has lost most of its genetic diversity due to domestication and breeding. Wheat introgression or generating wheat introgression lines that carry new agronomically important traits derived from wild relatives would be a great initiative (Nemeth et al., 2015).

An example of how introgression could introduce new properties is seen in salinity tolerance. Durum wheat or pasta wheat is a tetraploid (2n=14) and is sensitive to saline conditions; however it is well adapted to drought stress (Woodrow et al., 2017). Globally soil conditions have increasingly deteriorated toward higher salinity in many areas, a hostile growth environment for crop plants thus affecting agricultural productivity. Increasing salinity of arable land with changing environmental conditions and irrigation methods has made it difficult in some areas to grow cereal crops such as wheat and rice. However, some wild relatives of wheat such as *Thinopyrum bessarabicum* still thrive in saline conditions and are tolerant to high salt concentration; geographically they can be found on the coast of Crimea (Gorham et al., 1985). A solution or approach to cultivate wheat varieties to such hostile conditions would be to incorporate drought tolerant genes from *T. bessarabicum* into domesticated wheat through introgression. *T. bessarabicum* is a diploid (2n=2X=14) and can

be hybridized with a tetraploid wheat (2n=4x=28) which results in 42 set of chromosome amphidiploids and which is fertile (Kings et al., 1997).

Such integration of the *T. bessarabicum* genome into durum wheat could result in promising wheat varieties with properties that do not just include salinity tolerance but also those experienced by *T.bessarabicum* such as drought and high temperature. These will prove useful in future deteriorating environmental conditions as well as deteriorating soil conditions (IPPC 2013).

In this chapter a series of wheat ancestral amphidiploids was developed from different species and accessions (retaining the ancestral parents) that were not the same lines used in chapter 3. Amphidiploid plants contain diploid sets of chromosome from both the parents owing to chromosome doubling induced by colchicine treatment. Colchicine inhibits chromosome segregation during cell division which results in chromosome doubling (Chapter 2).

Here, diploid (2n) wild relatives of wheat were used. Tetraploid durum wheat (Table 4.1) were used with an exception to line no 9 which is a cross between Chinese spring (hexaploid=6n) X *T. bessarabicum* (diploid) (Figure 4.1). The details of the crossing method have been explained in material and method chapter, section 2.2.3.1. The durum wheat used in this program were Neodur, Azaziah, Langdon, Macoun, Karim, Creso and Stewart, EC0014 amphidiploid is the only exception within these amphidiploids, which is a cross of *T. bessarabicum* with Chinese spring (tritium species). However the durum wheat parents were not available for field analysis. Instead we compare to UK wheat Paragon, Highbury, Pavon 76, Chinese Spring and Indian check lines KRL210, HD2967, HI8468, and MACS6222.

4.2 Material and methods:

Most methods that are generic have been explained in chapter 2. This chapter includes any variation in methodology.

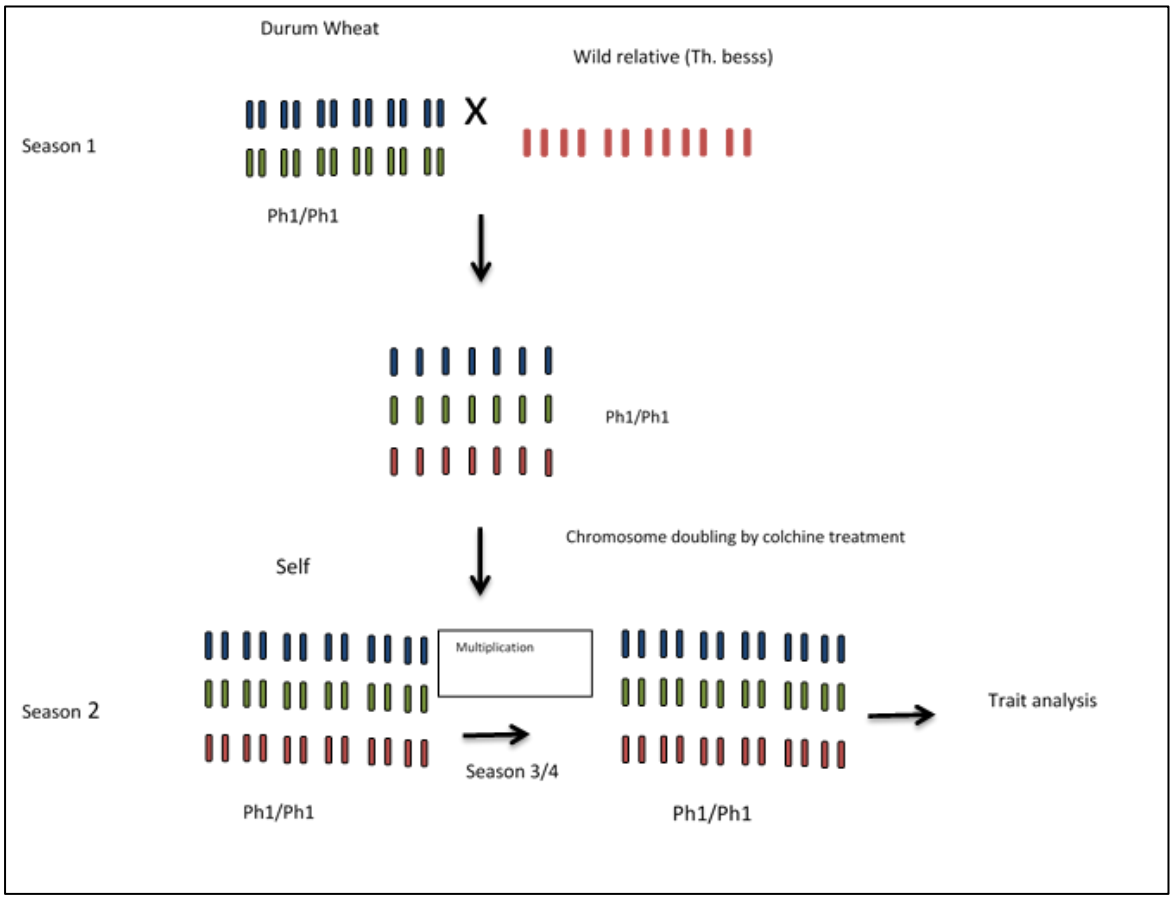


Figure 4.1: Diagram showing the procedures to generate amphidiploids by crossing tetraploid durum wheat and wild diploid (wild wheat) *T. bessarabicum.* This is a variation on the methodology already shown in chapters 1 and 2.

4.3 Specific hypotheses tested in this chapter

1. The amphidiploids exhibit higher light saturated photosynthetic capacity (P_{max}) in the field conditions with no reduction in leaf water use efficiency compared to the UK wheat lines.
2. The amphidiploids exhibit lower canopy temperature in the field compared to the UK wheat lines and the Indian check lines.
3. The amphidiploids exhibit higher biomass and related agronomic traits in the field compared to the UK wheat lines.

4.4 Results

This chapter presents results for photosynthesis, various physiological parameters and agronomy analysis of the amphidiploid lines carried out during the year 2016 and 2017. Weekly physiological measurements such as NDVI and canopy temperature were measured on all lines. Days to flowering and vein density were recorded. However, photosynthetic measurements were made only on the UK wheat lines (check lines) and amphidiploids excluding the Indian genotypes. The following strategy was adopted for photosynthesis measurements which is the same strategy as chapter 3: after initial light saturated rate at ambient CO_2 (P_{max}), amphidiploid lines with higher values were investigated for detailed photosynthetic measurements such as CO_2 response (AC_i) curves, along with the UK check lines. The P_{max} measurements were taken during the vegetative stage (GS39-GS45, Zadok's nomenclature) of all the lines, whereas the CO_2 response curves were taken at anthesis stage (GS61-GS69, Zadok's nomenclature). For statistical analysis the comparison between UK wheat lines and amphidiploids are assigned as groups, and comparison within each lines within the amphidiploids in result tables.

4.4.1 Crop phenology

Days to flowering

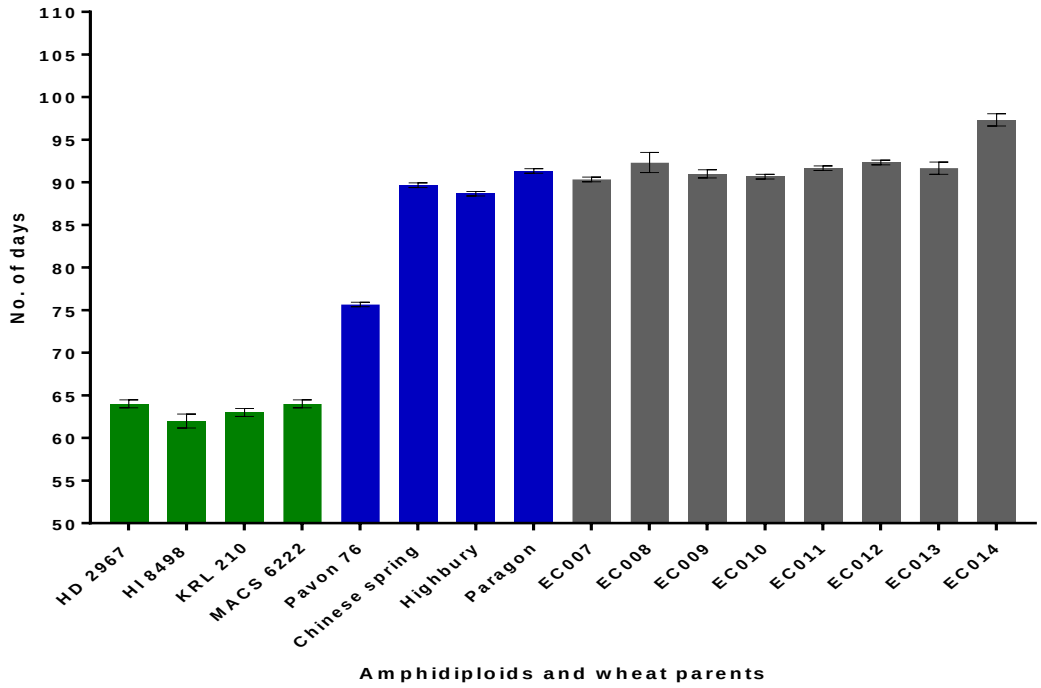


Figure 4.2: Days to flowering. The green bars show Indian genotypes, the blue bars show UK wheat lines, the grey bars show amphidiploids. Values are means, error bars are standard error of the means (n=3).

Phenology varied considerably between the groups. Indian genotypes flowered considerably earlier (62-65 days) compared to UK wheat lines (75-90 days) and amphidiploids (90-97 days) (Figure 4.2). This may be due to the adaptation to shorter seasons, high temperature and possible water deficit of the Indian genotypes. The amphidiploids are likely to have derived this phenology from their respective wheat parents or wild parent (*T. bessarabicum*) which do not possess the same adaptations as the Indian lines.

4.4.2 Environmental measurements

4.4.2.1 Temperature and growth stages

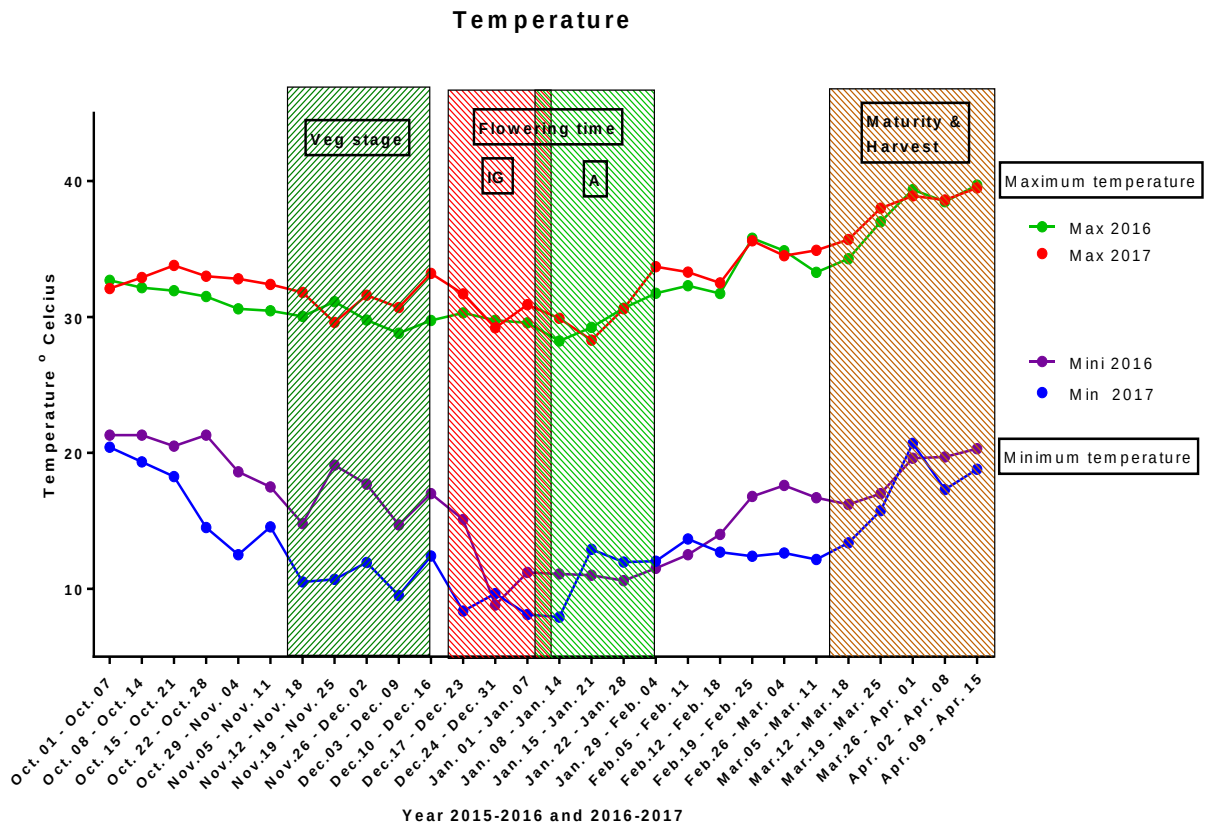


Figure 4.3: Temperature during wheat growing season, where the minimum temperature is shown in blue and purple solid circles. Lowest values were 8.8°C and 7.9°C for the year 2015-16 and 2016-17 respectively. The maximum temperature shown in red and green solid squares which were around 40°C in April for both the experimental years). The first rectangle shaded in dark green is the vegetative or pre-anthesis stage (GS31-59), while the anthesis or the flowering stage (GS61-69) is shown in red shaded rectangle for (IG) Indian genotypes and light green shaded rectangle for (A) amphidiploids. Brown shaded rectangle is the maturity stage and harvesting period of both Indian genotypes and amphidiploids including the UK wheat lines. Values are weekly means. Meteorological data are recorded from Pune field meteorological station.

Maximum temperature increased from 33°C to almost 40°C during the growing season (figure 4.3). The lowest maximum temperature was experienced during the flowering period (30 °C) for the Indian genotypes but was higher for the amphidiploids. Minimum temperature was 20 °C at sowing, reduced to 10 °C at flowering and rose to 20 °C again at harvest. The mean maximum temperature

was 32.25 °C (2016) and 33.20 °C (2017) and mean minimum temperature 16.19 °C (2016) and 13.39 °C (2017).

4.4.2.2 Relative humidity and growth stages

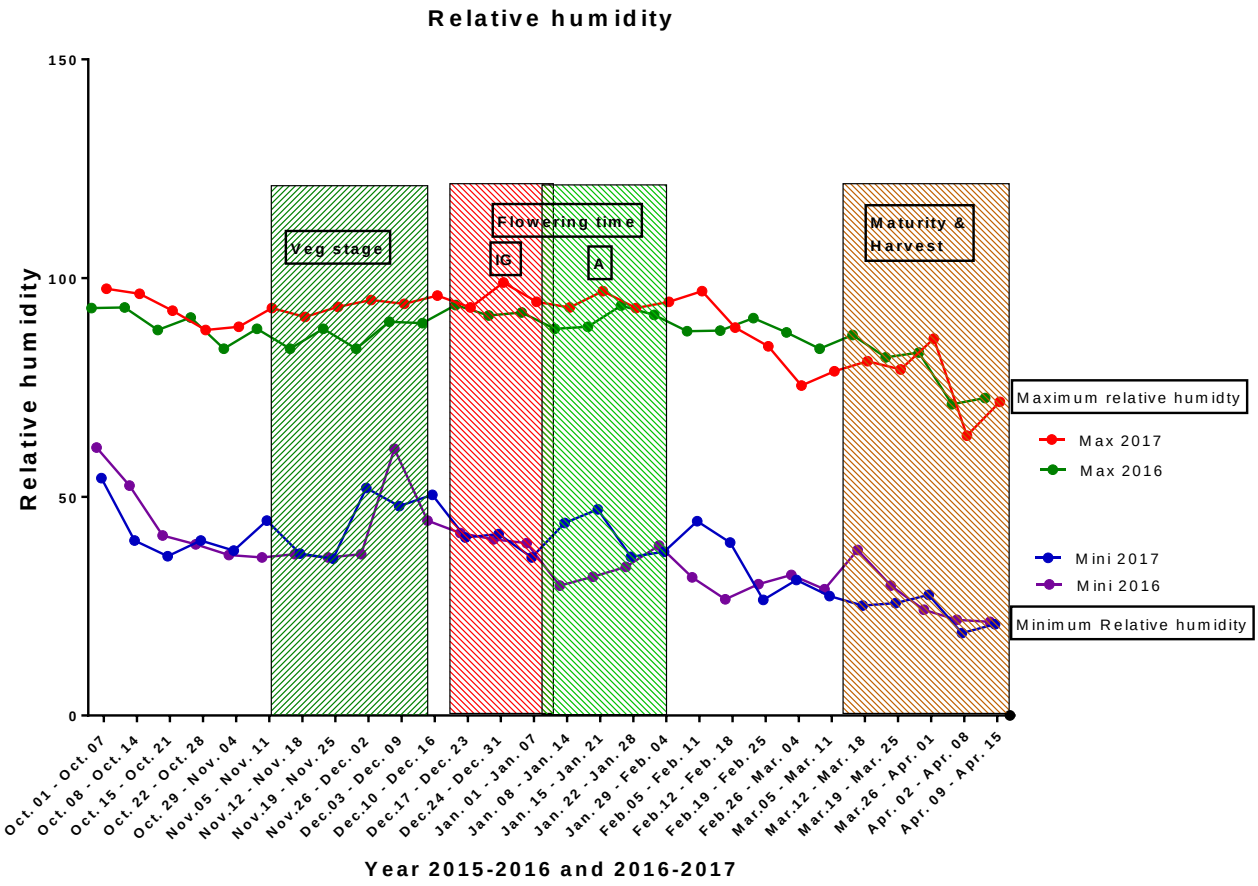


Figure 4.4: Relative humidity (RH) during wheat growing season, where RH_{min} is shown in purple and blue solid circles.(A) is amphidiploids and (IG) is the Indian genotypes, the growth stages are explained in figure 4.2. Values are weekly means.Meteorological data are recorded from Pune field meteorological station.

Lowest values were 21.85 % and 18.85 % for the year 2015-16 and 2016-17 respectively. The RH_{max} shown in green and red solid circle which ~ 93 % for both the experimental years. The mean RH_{max} was 87.40 % (2016) and 89.19 % (2017) and mean RH_{min} was 36.51 % (2016) and 37.36 % (2017). Highest RH_{max} was observed as 93 % for the both experimental year during flowering time (figure 4.3). The lowest RH_{max} was 71 % (2016) & 64 % (2017) and lowest RH_{min} was 21.85 % (2017) & 18% (2016) during the harvesting or maturity period.

4.4.2.3: Canopy temperature

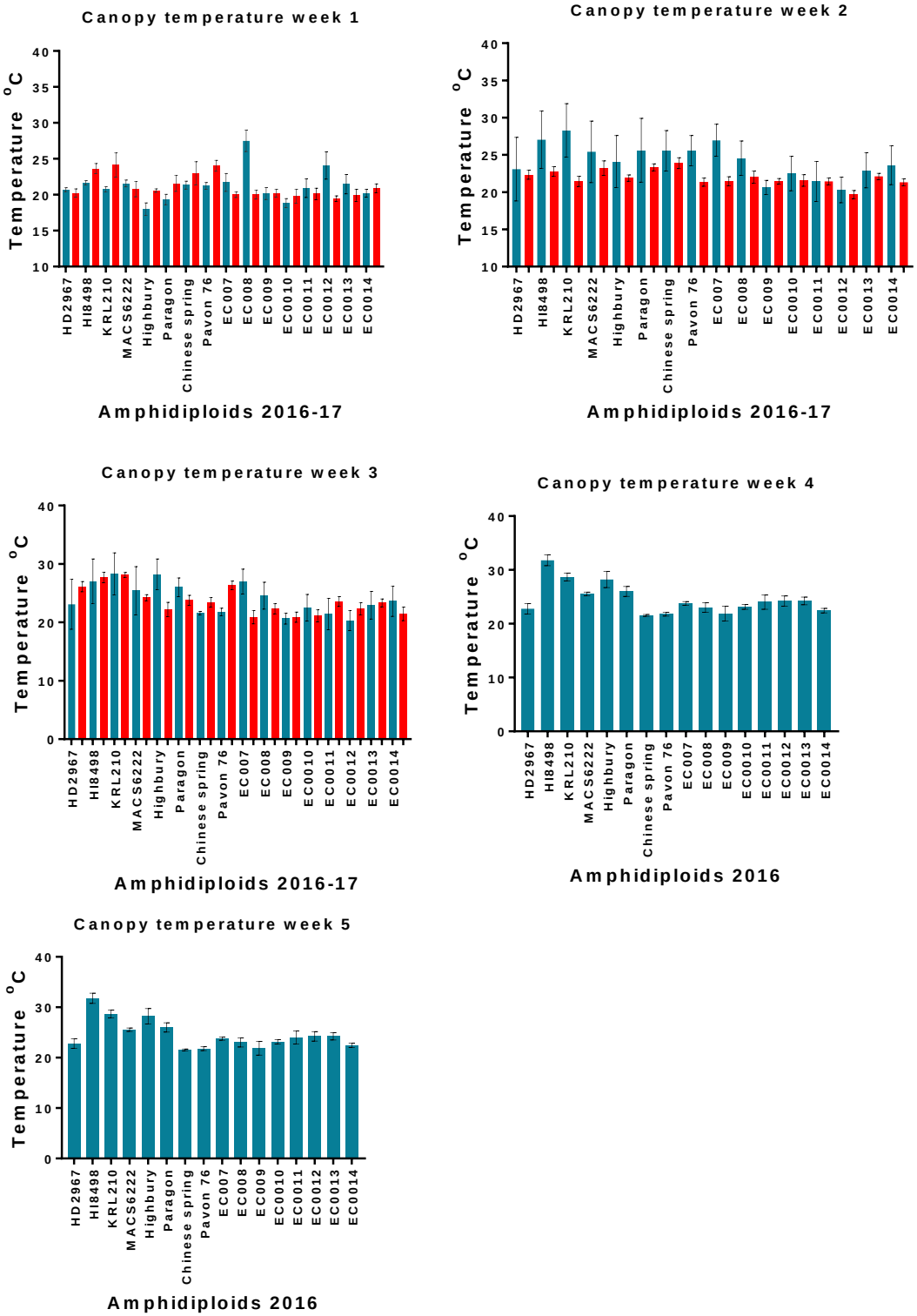


Figure 4.5: Weekly canopy temperatures, which are shown until week 5 of the amphidiploids, UK wheat parents and Indian genotypes. The canopy temperature for the experimental year 2017 is represented in light blue bars and dark blue bars for the

experimental year 2016. Values are weekly means, error bars are standard errors of the means (n = 9).Note: For the year 2017, experiment was carried out for limited period therefore the canopy temperature measurement are available only until 3 weeks.

Weekly canopy temperature was measured via Infra-red to identify any changes within the population across the growing season (figure 4.5 and table 4.1-4.3). For both the experimental years the amphidiploids, UK wheat and Indian genotypes were used. The amphidiploids had cooler canopy temperature compared to UK wheat and Indian genotypes for the both the experimental year (week 3 in 2017) however if the canopy temperature is compared between the two experimental years, then year 2017 experienced cooler climatic conditions than 2016 (see table 4.3).

A lower canopy temperature (CT) indicates stomatal closure and a restriction of transpiration cooling. Under well-watered conditions, CT depression below the ambient temperature can be used to predict photosynthetic rate. It can also be used to predict a closure of stomata in response to soil water deficit. In this case the plants were well watered so it represents important differences in photosynthetic rate.

Two way analysis of canopy temperature (table 4.3) reveals that there were significant differences between the lines and between the groups for each week for the year 2016. For the experimental year 2017, significant differences were observed in week 3, within the lines and between the groups for each week (table 4.3 B). This could be due to a rise in atmospheric temperature from weeks 1-3 or a physiological effect such as stomatal closure.

In order to understand how climatic conditions between two experimental years affected the canopy temperature, two way anova was carried out to observe any significant differences in canopy temperature between the lines, groups and the

two experimental years. Canopy temperature was significant when compared within the lines for week 1 and week 3 (table 4.3) and was significant in week 3 within the lines and groups, suggesting an increase in atmospheric temperature or stomatal closure from week 3 onwards.

Table 4.1: Anova of canopy temperature 2016 for 5 weeks. Values shown are means, shaded boxes indicate significant relationships (P<0.05). Here, CT is canopy temperature. Group refers to a comparison between amphidiploid and UK wheat and Indian genotypes and lines refers to variance among the amphidiploid lines.

CT	Group	Lines	SEM
Week 1	0.001	<.001	±0.01
Week 2	<.001	<.001	±0.01
Week 3	<.001	<.001	±0.01
Week 4	<.001	<.001	±0.02
Week 5	<.001	<.001	±0.02

Table 4.2: Anova of canopy temperature for the year 2017 for 3 weeks of measurements. Values shown are p values, shaded boxes indicate significant relationships (P<0.05). Here, Group refers to comparison between UK wheat parents, Indian genotypes and amphidiploids and lines refers to comparison within the amphidiploids.

CT	Group	SEM	Lines	SEM
Week 1	0.809	± 0.32	0.999	± 0.70
Week 2	0.399	± 0.31	0.633	± 0.69
Week 3	<.001	± 0.29	<.001	± 0.64

Table 4.3: Anova of canopy temperature (CT) for both the years for 3 weeks of measurements. Values shown are means, shaded boxes. Here, Group refers to comparison between UK wheat parents, Indian genotypes and amphidiploids and lines refers to comparison within the amphidiploids. Group.year means interaction of groups with respect to the years and lines.year refers to interaction of the amphidiploids with the years.

CT	Week 1	SEM	Week 2	SEM	Week 3	SEM
Group	0.108	±0.33	0.008	±0.58	<.001	±0.39
Group.Year	0.028	±0.47	0.013	±0.82	0.008	±0.56
Lines	<.001	± 0.58	0.018	± 1.27	<.001	± 0.69
Lines. Year	<.001	±0.83	0.095	± 1.79	<.001	± 0.98
Year	<.001	± 0.20	<.001	± 0.44	<.001	± 0.24

4.4.2.4: Normalised difference vegetative index (NDVI)

The interception of vegetation by photosynthetically active radiation is closely related to NDVI(Gamon et al., 1995), as it is one of most commonly used spectral indices, it is closely related to the green biomass, leaf area index (LAI) and chlorophyll content Hansen and Schjoerring, (2003).



Figure 4.6: Weekly NDVI measurements on the amphidiploids, UK wheat lines and Indian genotypes until week 5. Blue bars represents experimental year 2016 and red bars represent experimental year 2017. Values are means, error bars are standard errors of the means (n = 9). Note: For the year 2017, experiment was carried out for limited period therefore the NDVI measurement are available only until 3 weeks.

NDVI measurements were carried out weekly alongside canopy temperature measurements. NDVI is a reflectance measurement and indicates green leaf area (Figure 4.6). There was little consistent difference between groups in terms of NDVI with the exception of the Indian genotypes where the values dropped by week 4 and 5 for the experimental year 2016 probably due to senescence. This is consistent with the earlier flowering and shorter life cycle in this group. Amphidiploids also demonstrated early decline in NDVI in comparison to UK lines. For the experimental year 2017, the NDVI values did not show differences.

Two way anova of NDVI for the experimental year 2016 (table 4.5) reveals that there were significant differences between the lines and between the groups for each week. After two way analysis of NDVI (table 4.6) for 2017, significant differences were observed in groups across 3 weeks but within the lines it was significant in week 3. According to usual plant developmental stages, canopy cover decreases due to senescence and there is evidence here of this occurring in Indian lines but less so in the amphidiploids. Two way anova of NDVI for both experimental years were done, to compare the leaf green area between lines, groups and experimental years. NDVI values were highly significant within the lines, between groups and the experimental years for all 3 weeks, except for in week 2 for comparison between year and year to group interaction.

Table 4.4: Anova of NDVI of the amphidiploids, UK wheat lines and Indian genotypes for the experimental year 2016. Values shown are the P values. Here, Group refers to comparison between UK wheat lines, Indian genotypes and amphidiploids and lines refers to comparison within the amphidiploids.

NDVI	Group	Lines	SEM
Week 1	0.001	<.001	±0.01
Week 2	<.001	<.001	±0.01
Week 3	<.001	<.001	±0.01
Week 4	<.001	<.001	±0.02
Week 5	<.001	<.001	±0.02

Table 4.5: Anova of NDVI of the amphidiploids, UK wheat and Indian genotypes for the experimental year 2017. Values shown are the P values.Here, Group refers to comparison between UK wheat lines, Indian genotypes and amphidiploids and lines refers to comparison within the amphidiploids.

NDVI	Group	Lines	SEM
Week 1	<.001	0.019	±0.01
Week 2	<.001	0.22	±0.02
Week 3	<.001	<.001	±0.00

Table 4.6: Anova of NDVI of the amphidiploids, UK wheat and Indian genotypes for the experimental year 2016 and 2017. Values shown are the P values.Here, Group refers to comparison between UK wheat lines, Indian genotypes and amphidiploids and lines refers to comparison within the amphidiploids. Group.year means interaction of groups with respect to the years and lines.year refers to interaction of the amphidiploids with the years.

NDVI	Week 1	SEM	Week 2	SEM	Week 3	SEM
Group	<.001	±0.00	<.001	±0.00	<.001	±0.00
Year	<.001	±0.00	0.346	±0.00	<.001	±0.00
Group.Year	<.001	±0.00	0.677	±0.00	<.001	±0.00
Lines	<.001	±0.00	<.001	±0.01	<.001	±0.00
Lines.Year	<.001	±0.01	<.001	±0.01	<.001	±0.01

4.4.3 Chlorophyll fluorescence

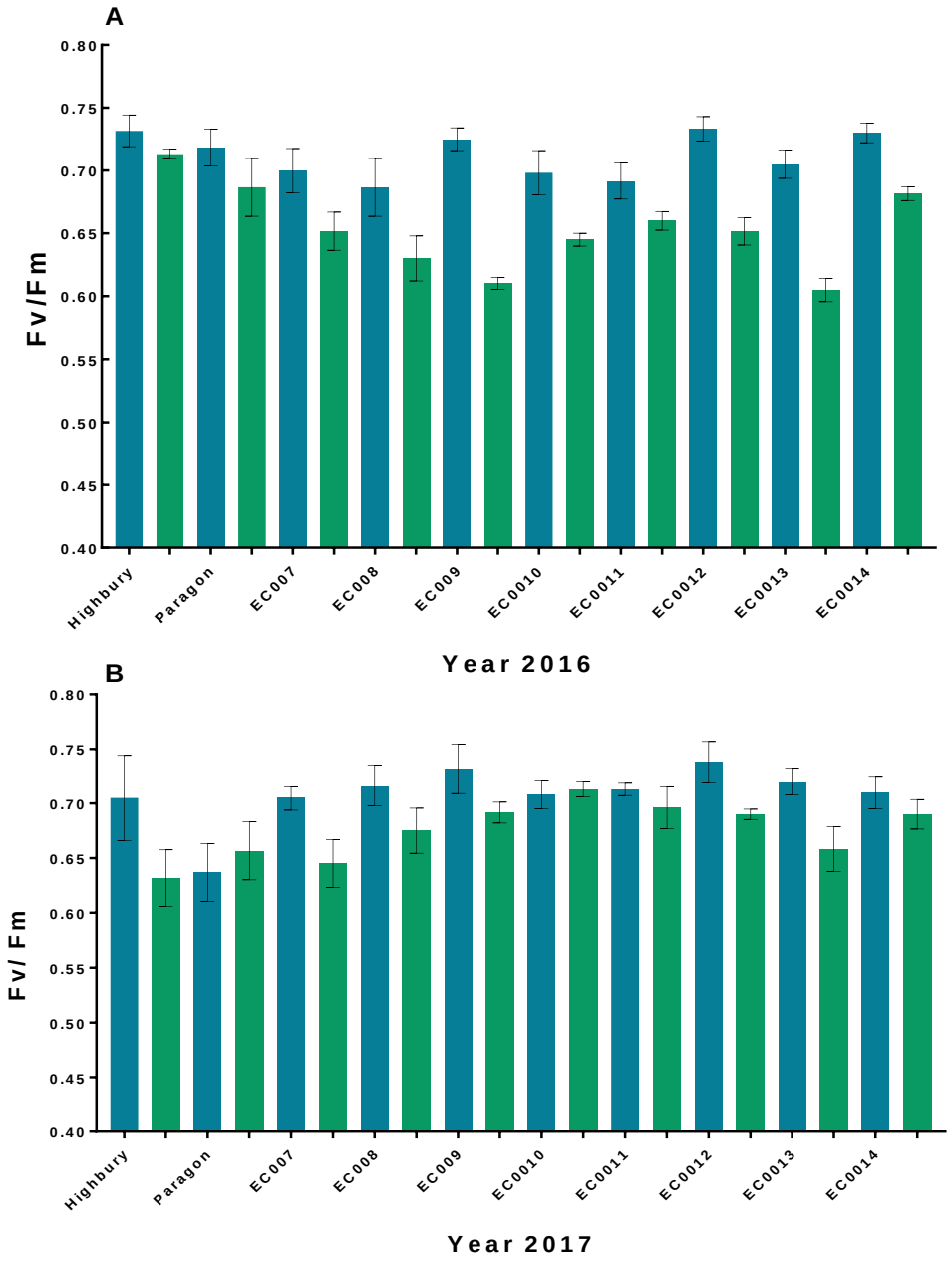


Figure4.7 Dark adapted chlorophyll Fv/Fm measurements on the amphidiploids and UK wheat lines. Blue bars are pre anthesis, green bars are post anthesis. Values are means, error bars are standard errors of the means (n = 6).

Dark adapted Fv/Fm represents sustained quenching within ΦPSII and is usually used as an indirect measurement of photoinhibition and lowered quantum yield. The measurements were made during two growth stages, pre-anthesis and post-

anthesis. Such measurement gives us an understanding of whether the plants are experiencing stressful environmental conditions.

Table 4.7: Anova of the dark adapted (DA) chlorophyll fluorescence (Fv/Fm) of the amphidiploids and UK wheat lines for the experimental year 2016 and 2017. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Here, Group refers to comparison between UK wheat lines, Indian genotypes and amphidiploids and lines refers to comparison within the amphidiploids.

Growth stage	**Pre-anthesis**		**Post-anthesis**	
Year 2016	**DA Fv/Fm**	**SEM**	**DA Fv/Fm**	**SEM**
Lines	0.009	0.01	0.161	0.03
Group	0.021	0.01	0.132	0.02
Year 2017				
Group	0.293	0.02	<0.001	0.03
Lines	0.206	0.01	<.001	0.02

The maximum Fv/Fm is around 0.83 and values here are substantially lower than this, reaching 0.65 or below, indicating substantial photoinhibition levels in these field conditions. Two way anova analysis demonstrated some significant differences in dark adapted Fv/Fm during the postanthesis stage among the lines and groups (P <0.05), the year to lines interaction was highly significant (P <0.001). In general values were higher for pre-anthesis measurements, indicating that the plants were not photoinhibited.

4.4.4 Light saturated photosynthetic measurements

To understand variation in photosynthesis in the amphidiploids, light saturated photosynthetic measurements (P_{max}) were carried out using gas exchange. (figures 4.8-4.12 and table 4.8)

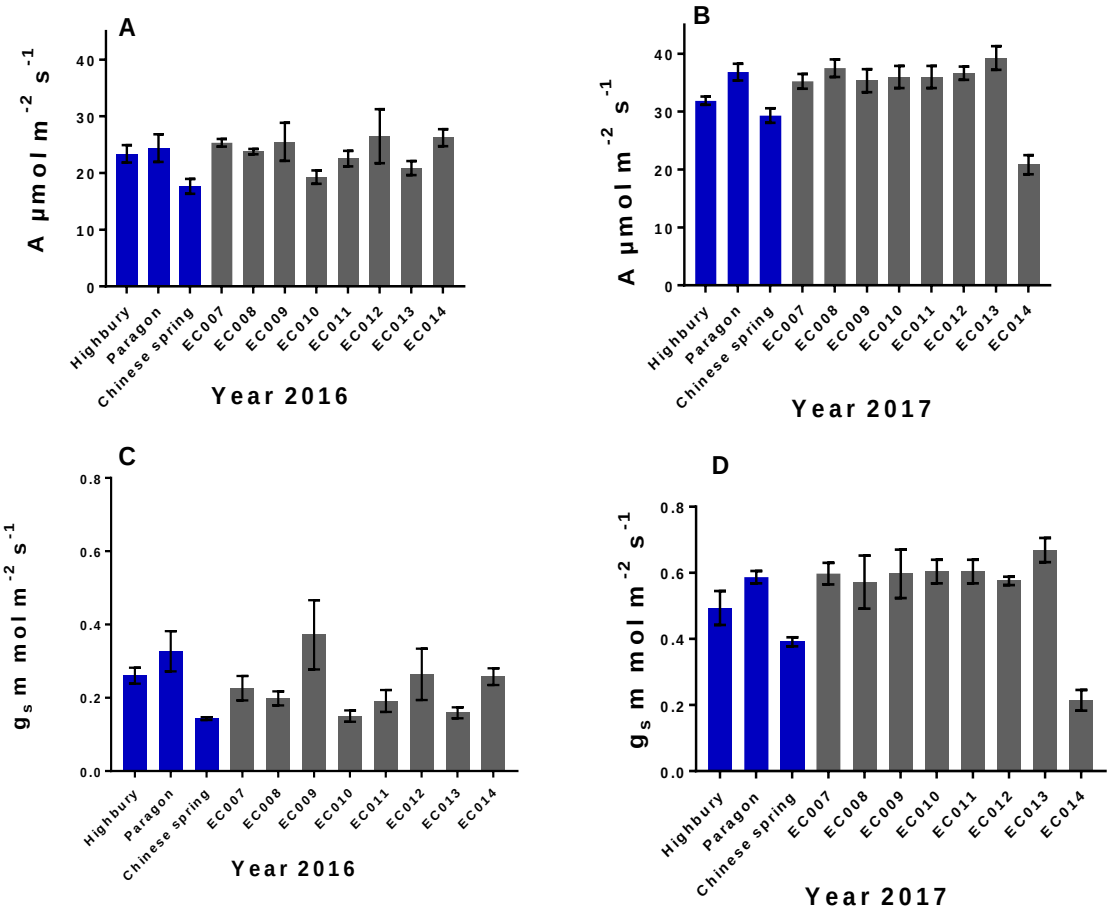


Figure 4.8: (A) & (B) P_{max} of amphidiploids and UK wheat lines for the year 2016 & 17 respectively. (C) & (D) stomatal conductance (g_s) for the year 2016 & 2017 respectively. Values are means, error bars are standard errors of the means (n = 3).

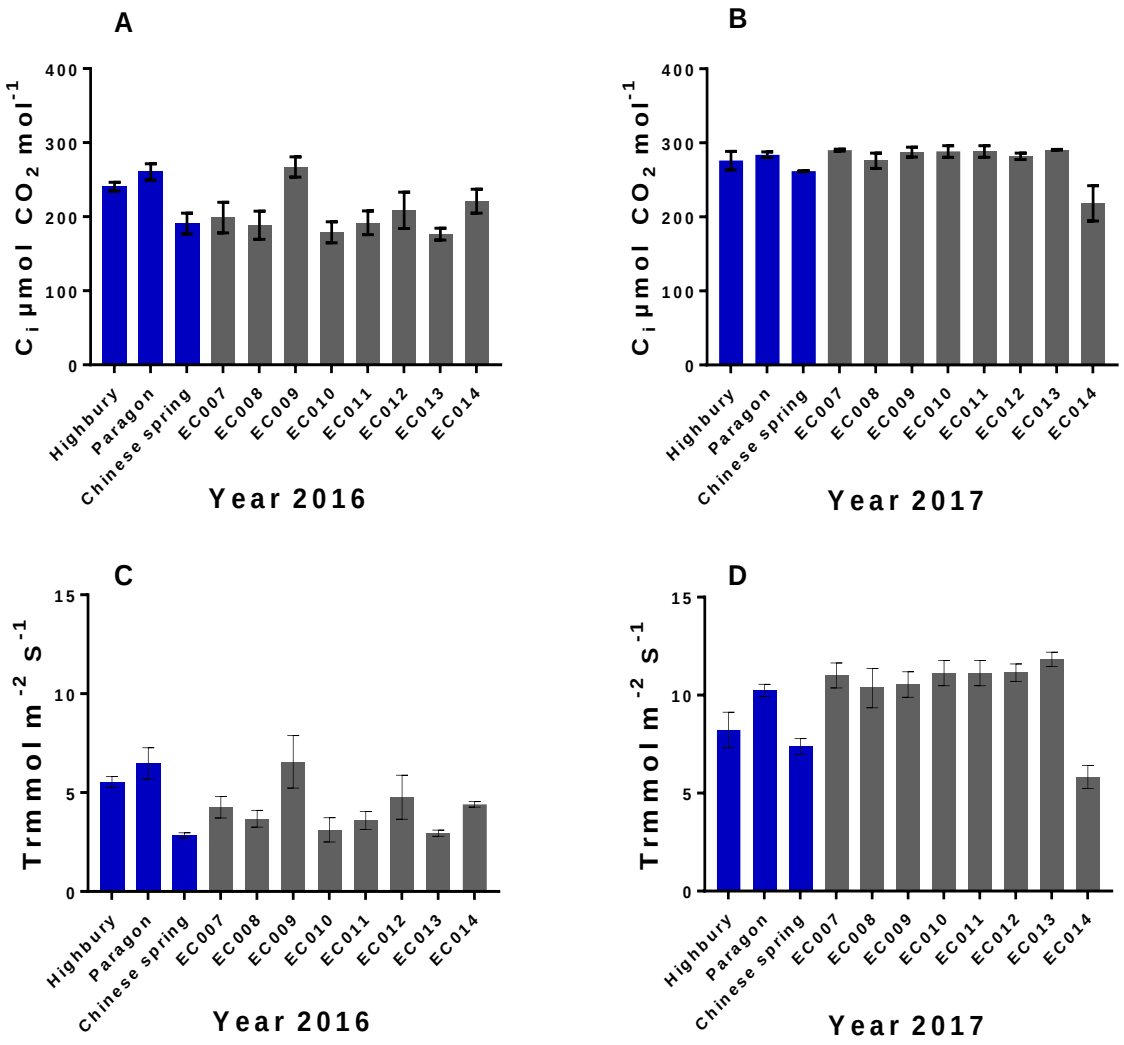


Figure 4.9: (A) & (B) intercellular CO_2 (C_i) of the amphidiploids and UK wheat lines for the year 2016 and 2017 respectively, (C) & (D) Transpiration rate of the amphidiploids and UK wheat lines for the year 2016 & 2017 respectively. Values are means, error bars are standard errors of the means (n = 3).

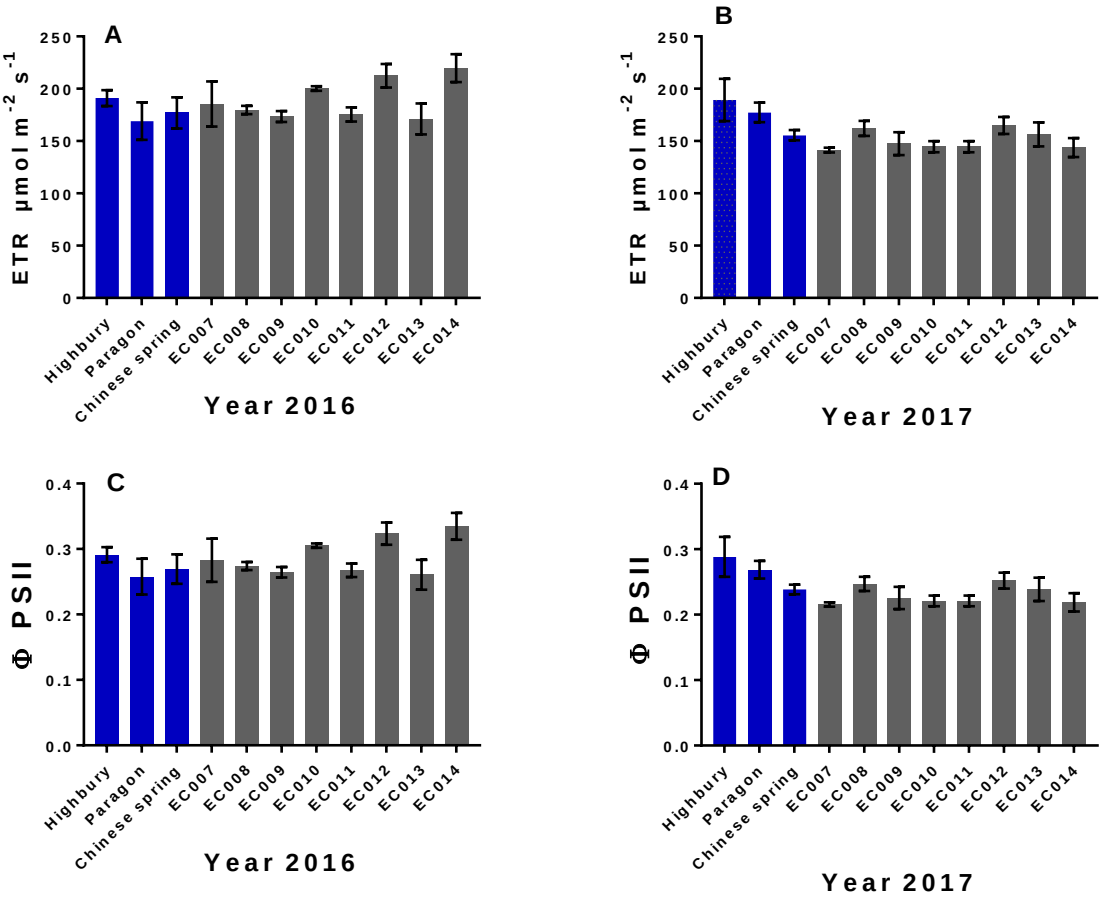


Figure 4.10: (A) & (B) Electron transport rate (ETR) of the amphidiploids and UK wheat lines for the year 2016 & 2017 respectively, (C & D) ΦPSII of the amphidiploids and UK wheat lines for the year 2016 & 2017 respectively. Values are means, error bars are standard errors of the means (n = 3).

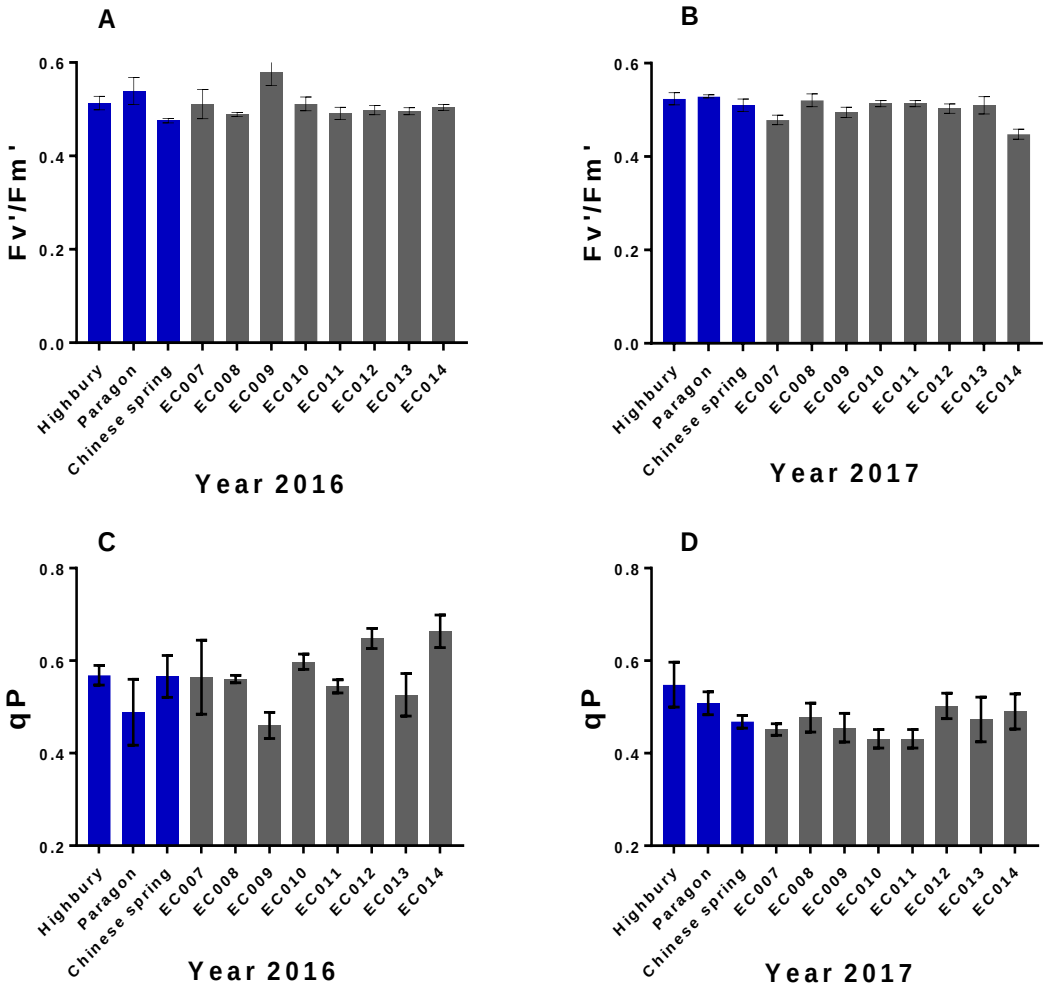


Figure 4.11: (A) & (B) Fv'/Fm'of the amphidiploids and UK wheat lines for the year 2016 & 2017, (C) &(D)qP of the amphidiploids and UK wheat lines for the year 2016 & 2017 respectively. Values are means, error bars are standard errors of the means (n = 3).

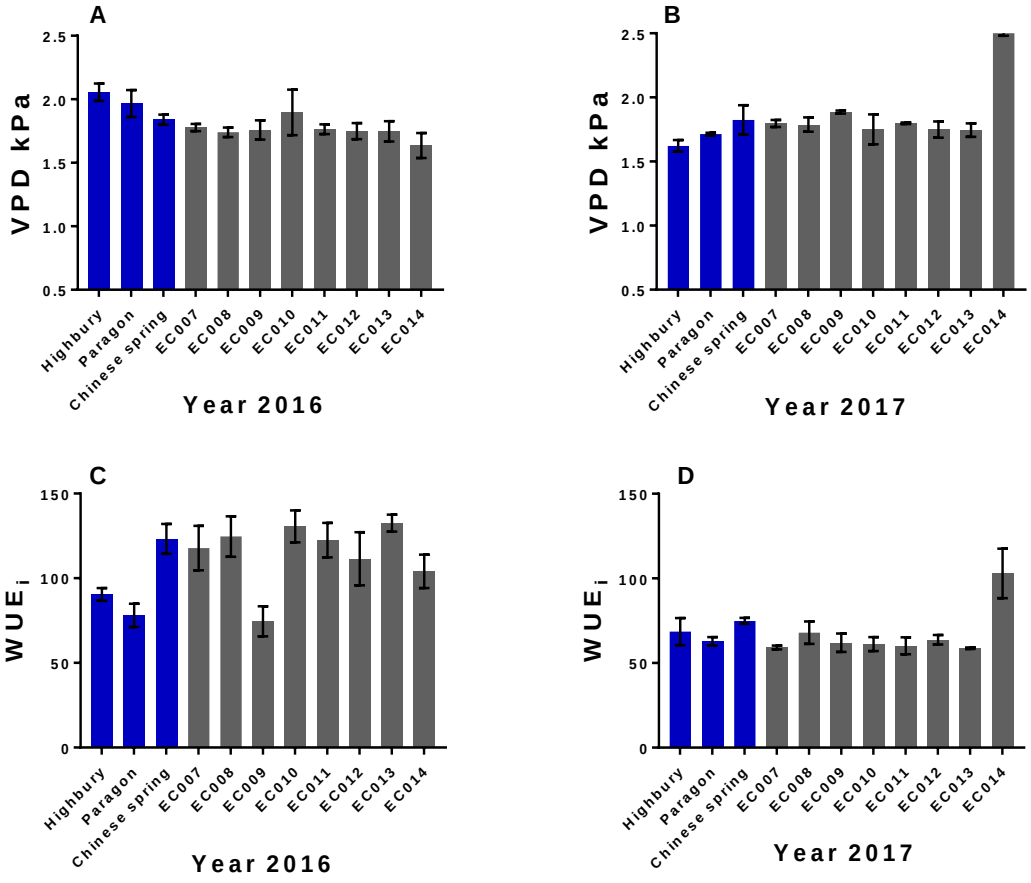


Figure 4.12: (A) & (B) VPD of the amphidiploids and UK wheat lines for the year 2016 & 2017 respectively, (C) & (D) WUE_i of the amphidiploids and UK wheat lines for the year 2016 & 2017 respectively. Values are means, error bars are standard errors of the means (n = 3).

The amphidiploids are represented in grey colour and the UK wheat lines in blue colour in all the bars graphs from figures 4.8- 4.12, Only two UK wheat lines Highbury and Paragon were used as control / check lines to keep the number of measurements limited, therefore UK wheat Chinese spring was excluded from the AC_i curve measurements. Statistical analysis is shown in table 4.5. P_{max} for the year 2017 (figure 4.8 (A & B)) was comparatively higher than the year 2016,

amphidiploids performed better than the UK wheat lines in the year 2017. Stomatal conductance for the year 2016 was significantly lower than the experimental year 2017 (figure 4.8 (C & D), this could have resulted due to closure of stomata as the year 2016 may have experienced water deficit conditions as well as higher temperature during the growing season (figure 4.3 and discussion). Intercellular CO_2 showed variation in year 2016 (figure 4.9 (A & B)) compare to experimental year 2017, lower stomatal conductance tends to decrease C_i, thus decreasing photosynthetic rate via enhanced Rubisco oxygenation and increasing photorespiration (Broeckx et al., 2014). During the 2016 growing season, closure of stomata indicates plant's response to water deficit (Lawlor and Cornic, 2002), affecting transpiration rate (figure 4.9 (C)). In the amphidiploids and the UK wheat lines especially, this could be the plant's mechanism to avoid water loss. The year 2017 (figure 4.9 (D)) showed a higher transpiration rate compared to year 2016. Electron transport in figure 4.10 (A) was observed to be higher in the year 2016 compared to year 2017 (figure 4.10 B), the UK wheat lines showed higher ETR compare to the amphidiploids, quantum yield of PSII comparatively higher in 2016 than 2017 (figure 4.10 (C & D). Figure 4.10 (A& B) is the light saturated Fv'/Fm', which is negatively correlated with total non-photochemical quenching and was comparatively higher in the year 2017 than the year 2016, suggesting a higher level of photoprotection in 2016, consistent with lower P_{max} (Figure 4.11 C, D). WUE_i showed higher values in the year 2016 in contrast to year 2017. An amphidiploid with accession number EC014 showed higher values of WUE_i (figure 4.12 D) due to particularly low g_s. The amphidiploids exhibited large variation in WUE_i in the year 2016 (figure 4.12 C) and values were higher than those in 2017, probably due to a water deficit-induced stomatal closure. If stomata partially close to

restrict water loss but there is not a proportional decline in photosynthesis (and no other factor reducing photosynthetic rate such as temperature) then WUE_i should rise. These photosynthetic parameters are good indicators for selecting individuals that respond differently to change in environmental conditions.

4.4.5: Statistical analysis

Anova of all the photosynthetic parameters is done in table 4.11, so that there is an overall comparison with the AC_i curve parameters.

4.4.6: Correlation between P_{max} and other photosynthetic parametersfor the year 2016

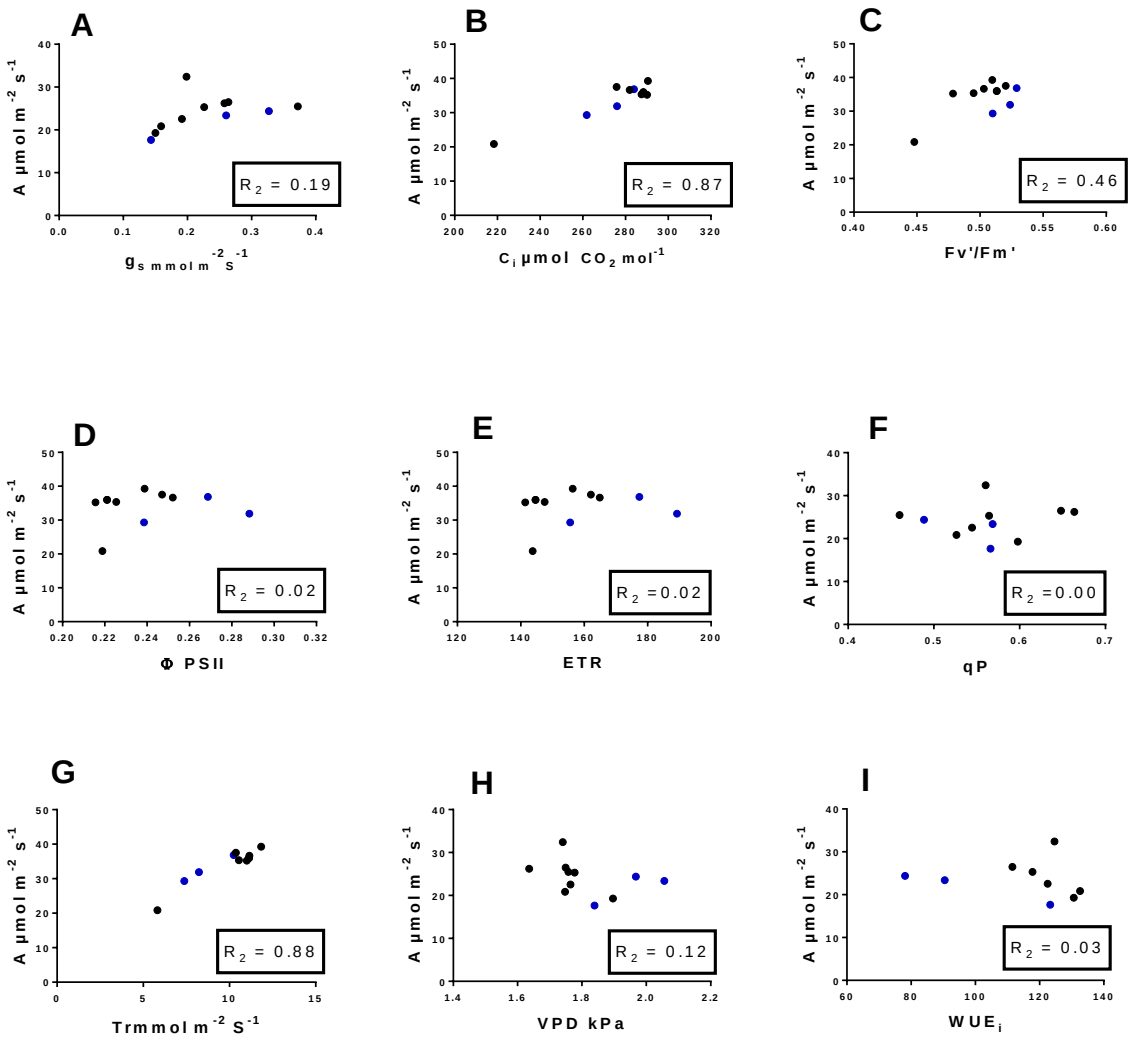


Figure 4.13: Relationship between Pmax and other parameters (A) gs, (B) C_i (C) Fv'/Fm', (D) ΦPSII, (E), ETR (F) qP (G) Transpiration rate ,(H) VPD and (I) WUE_iderived from the instantaneous photosynthetic measurements of the amphidiploids from the year 2016. The blue solid circles represent UK wheat and the black solid circles are the amphidiploids. Values are means of each parameter, (n= 3)

Figure 4.13 plots P_{max} against other photosynthetic parameters. A correlation analysis is shown in table 4.6. P_{max} showed significant and strong correlations with gs, C_i, Fv'/Fm' and transpiration rate. Negative relationship was observed between P_{max} with VPD (figure 4.13 H) and WUE_i (figure 4.13 I).No relationship could be drawn with qP.

4.4.7: Correlation between Pmax and other photosynthetic parametersfor the year 2017

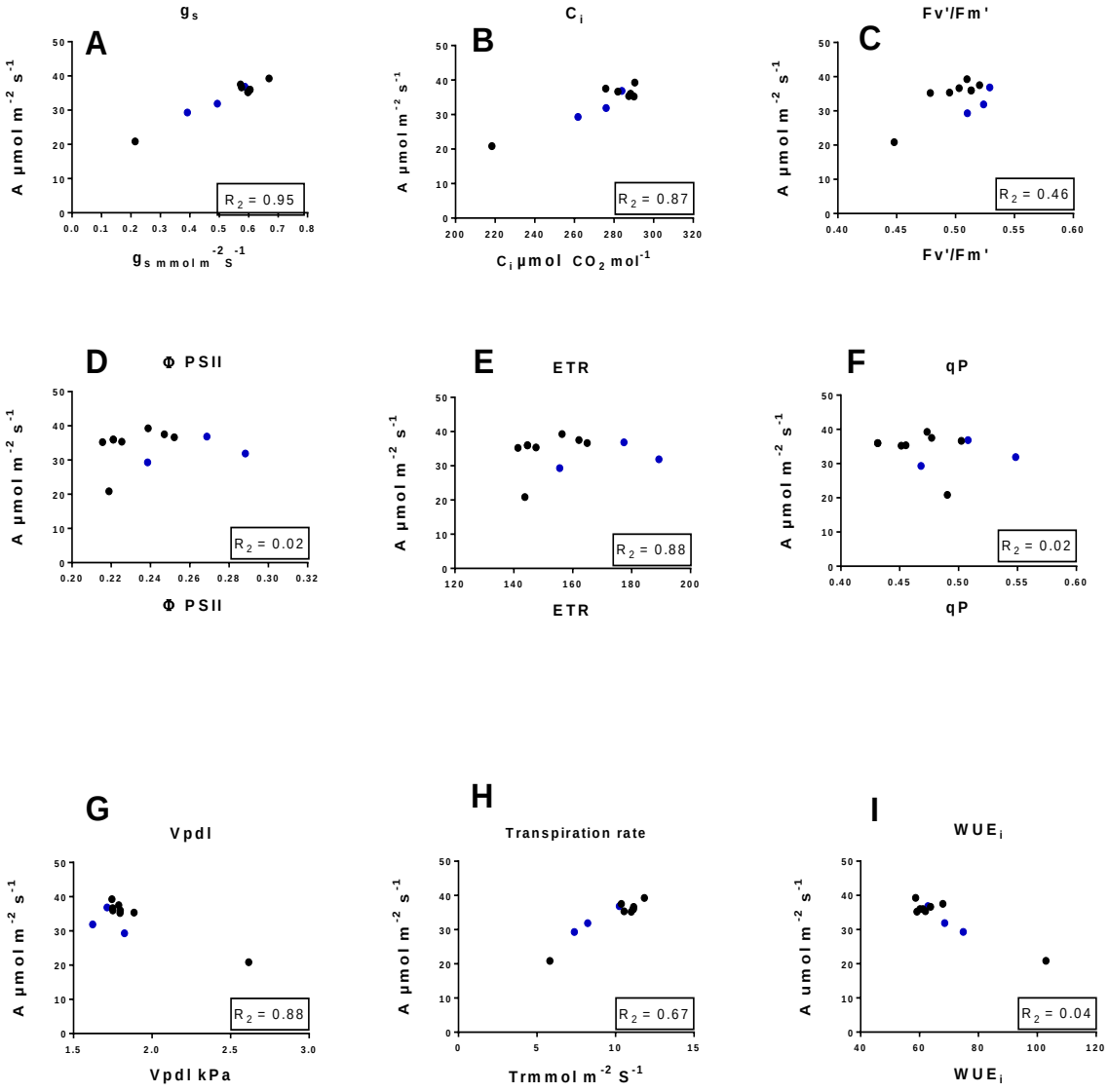


Figure 4.14: Relationship between Pmax and other parameters (A) gs, (B) C_i (C) Fv'/Fm', (D) ΦPSII, (E), ETR (F) qP (G) Transpiration rate,(H) VPD and (I) WUE_iderived from the instantaneous photosynthetic measurements of the amphidiploids from theyear 2017. The blue solid circles represent UK wheat lines and the black solid circles are the amphidiploids. Values are means of each parameter, mean (n= 3).

For the experimental year 2017, P_{max} showed strong relationship with stomatal conductance, C_i, transpiration rate, Fv'/Fm', a negative relationship could be observed P_{max} with VPD (figure 4.14 H) and negative correlation with WUE_i (figure 4.14 I), no correlation could be drawn between P_{max} and ΦPSII, ETR or qP. A detailed correlation analysis has been carried out (see table 4.10).

Table 4.8: Correlation matrix of the data derived from the photosynthetic measurements for the experimental year 2016. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Here, all the photosynthetic parameters are compared against each other for the year 2016.

Year 2016	C_i	g_s	Fv'/Fm'	ΦPSII	qP	ETR	Transpiration	VPD
P_{max}	0.002	<.001	0.047	0.0029	0.314	0.0029	<.001	0.012
C_i		<.001	<.001	0.66	0.05	0.656	<.001	0.98
g_s			<.001	0.586	0.372	0.589	<.001	0.154
Fv'/Fm'				<.001	0.732	0.334	<.001	0.252
qP					<.001	<.001	0.726	0.259
Φ PSII						<.001	0.253	0.212
ETR							0.732	0.252
Transpiration rate								0.918
VPD								

Table 4.9: Correlation matrix of the data derived from the photosynthetic measurements for the experimental year 2017. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05).Here, all the photosynthetic parameters are compared against each other for the year 2017.

Year 2017	C_i	g_s	Fv'/Fm'	ΦPSII	qP	ETR	Transpiration	VPD
P_{max}	<.001	<.001	0.008	0.079	0.477	0.082	<.001	<.001
C_i		<.001	0.012	0.266	0.013	0.265	<.001	<.001
g_s			0.01	0.774	0.144	0.771	<.001	<.001
Fv'/Fm'				0.038	0.634	0.037	0.12	<.001
qP					<.001	<.001	0.223	0.077
Φ PSII						<.001	0.045	0.975
ETR							0.22	0.079
Transpiration rate								0.003
VPD								

Correlation analysis was carried out between the photosynthetic parameters for each experimental year shown in table 4.10 and 4.11. As in chapter 3 there were high levels of correlation with stomatal conductance and transpiration but comparatively less so with electron transport, possibly due to greater stomatal limitation. For 2016, P_{max} showed significant relationship with intercellular C_i (P value: 0.002), Fv'/Fm' (P value 0.047), ETR & ΦPSII (P value: 0.029) and VPD (P value: 0.012). P_{max} was highly significant with g_s& transpiration rate (P value <0.001). For the experimental year 2017, P_{max} was significant with Fv'/Fm' and was highly significant with C_i, gs, transpiration rate, VPD and the P value for all of these parameters was (<0.001). C_i showed significant relationship with g_s and transpiration rate (P value <0.001) for both the experimental years, C_i also demonstrated significant relationship with Fv'/Fm' (P value: <0.001 for the year 2016 and 0.012 for the year 2017), qP (P value 0.05 for the year 2016), VPD (P value <0.001 for the year 2017). Stomatal conductance showed highly significant relationship with transpiration rate (P value <0.001) for both the experimental years and significant relationship with Fv'/Fm' (P value <0.001 for the year 2016, 0.01 for the year 2017).

4.4.8: AC_i curves

To understand the components which may be underlying the differences in photosynthesis in different lines it is imperative to understand the biochemical mechanism within the photosynthetic machinery, for example rubisco carboxylation efficiency and rate of photosynthetic electron transport. Therefore, promising lines were selected and were subjected to detailed photosynthetic measurement e.g. AC_i curves, to understand the biochemical limitations of photosynthesis. AC_i curves were carried out during anthesis stage on several

lines with high P_{max} and further analysed using curve fitting model (Sharkey et al., 2007) to calculate V_{cmax}, and J_{max}.

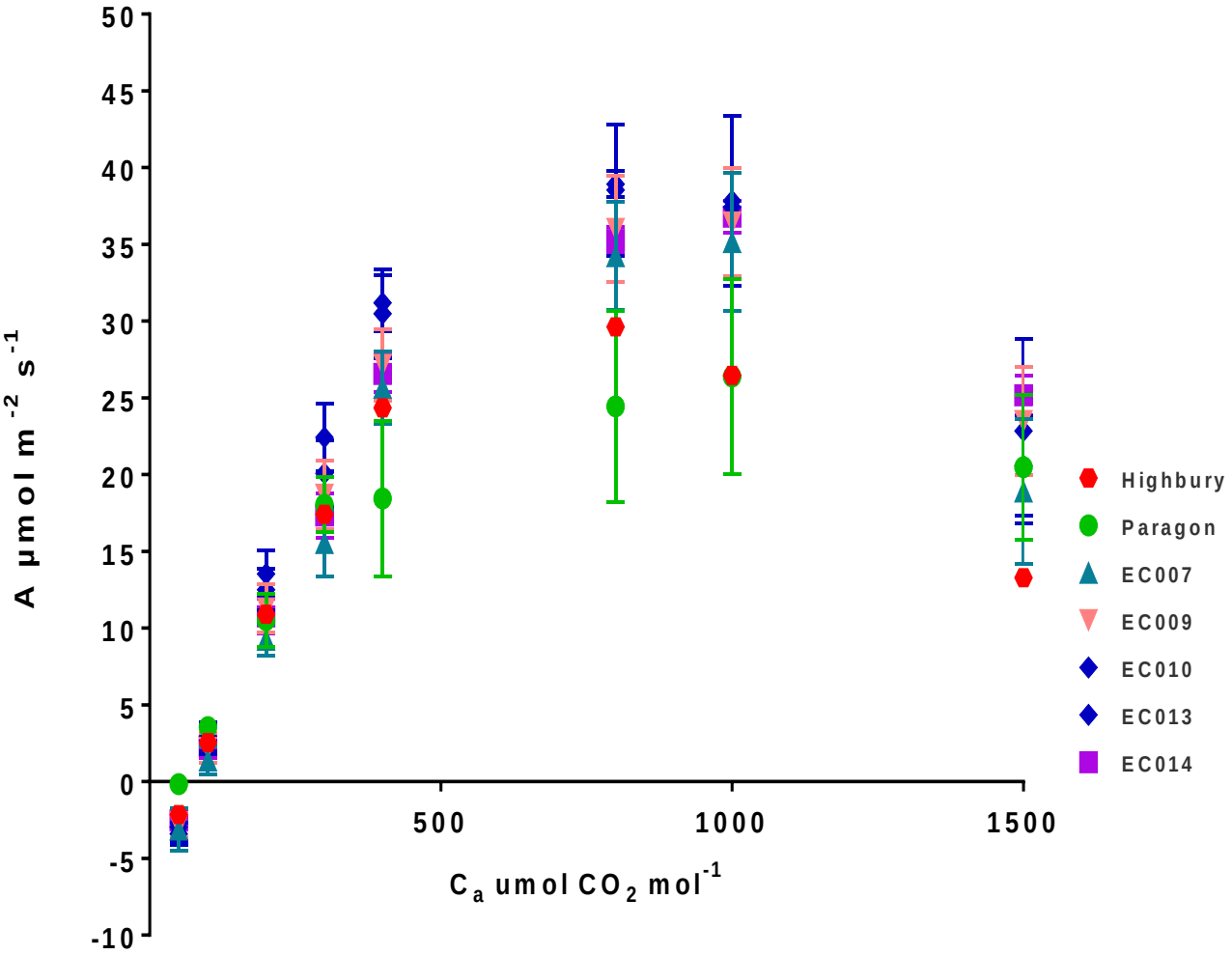


Figure 4.15: Example of variation in AC_a curves / CO_2 response curves of the amphidiploids with higher and lower P_{max}, UK wheat Paragon and Highbury for the year 2017. The data is plotted as CO_2 assimilation rate A ($\mu molm^{-2}\ s^{-1}$), to increasing CO_2 concentration, the settings for CO_2 concentration are mentioned in Chapter 2. The cuvette conditions were 1500 PPFD and the block temperature set as 25° C, humidity was set to ~ 60 %. Values are means, the error bars are the standard errors of the means (n=3). Curve fitting was carried using CO_2 level 1000 ppm as the maximum assimilation rate.

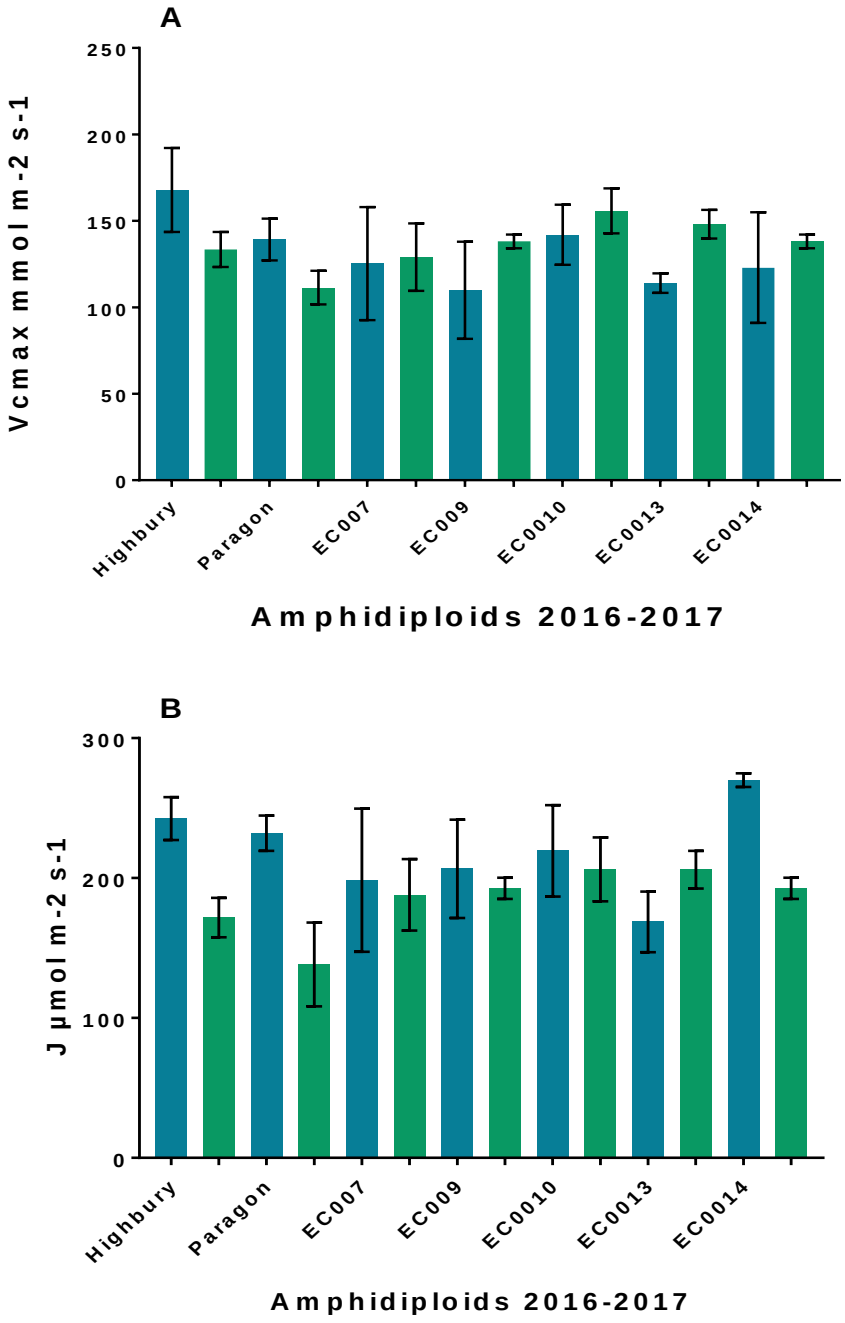


Figure 4.16: Bar graph of (A) V_{cmax} (B) J_{max} of the amphidiploids with higher and lower P_{max}, UK wheat lines derived from the AC_i curve measurement, blue and green bars represents year 2016 and 2017 respectively. Values are means, the error bars are the standard errors of the means (n=3).

V_{cmax}, and J_{max} were calculated using curve fitting model and are represent as bar graph for year 2016 (blue bars) and 2017 (green bars). The modelling only used the Ca values up to 1000 ppm due to an unexpected decline in photosynthesis above these values.

V_{cmax} was higher in the UK wheat lines for the experimental year 2016, but in the year 2017 an increase V_{cmax} in the amphidiploids could be observed (figure 4.16 A) implying that there was a decrease in rubisco carboxylation efficiency for the year 2016, probably due to water deficit and high temperature conditions.

J_{max} was higher in the year 2016 in the UK wheat lines and in the amphidiploid with accession no EC0014 (figure 4.16B) suggesting that the plants have acclimated to environmental conditions (associated with water deficit and possibly higher irradiation) in the year 2016 contrast to the year 2017 where J_{max} was lower in both UK wheat lines and the amphidiploids.

Table 4.10: Anova of photosynthetic parameters for the experimental year 2016 and 2017. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Here, Group refers to comparison between UK wheat lines and amphidiploids and lines refers to comparison within the amphidiploids and year refers to comparison between the two years.

Photosynthetic parameters	Year	SEM	Groups	SEM	Lines	SEM
P_{max}	<.001	± 1.07	0.024	± 2.01	<.001	± 2.84
g_s	<.001	± 0.02	0.005	± 0.05	<.001	± 0.07
C_i	<.001	± 8.35	0.008	± 15.63	0.002	± 22.1
Fv'/Fm'	0.04	± 0.01	0.203	± 0.02	0.116	± 0.03
ΦPSII	0.002	± 0.01	0.49	± 0.02	0.177	± 0.03
qP	0.026	± 0.02	0.45	± 0.05	0.417	± 0.07
ETR	0.002	± 8.58	0.479	± 16.04	0.169	± 22.69
Transpiration rate	<.001	± 0.42	0.004	± 0.79	<.001	± 1.11
VPD	0.419	± 0.05	0.013	± 0.09	<.001	± 0.13
WUE_i	<.001	± 5.25	0.007	± 9.83	0.002	± 13.9
V_{cmax}	0.817	± 11.78	0.752	± 22.03	0.652	± 31.16
J_{max}	0.033	± 15.81	0.783	± 29.58	0.28	± 41.84
RD	0.976	± 1.14	0.827	± 2.14	0.81	± 3.03
A_{max}	0.557	± 3.04	0.984	± 5.69	0.315	± 8.04
A_{400}	<.001	± 1.84	0.896	± 3.45	0.237	± 4.88

The two way anova was carried out in order to compare the UK wheat lines with the amphidiploids (groups) and comparison of the amphidiploids (lines) against each other. More significant differences ($P<0.05$) were shown in 2017 compared to 2016. In 2017, almost all the photosynthetic parameters except for ΦPSII, ETR and qP, showed significant differences between amphidiploid and UK wheat lines in contrast to 2016. In the year 2017, P_{max} was higher in the amphidiploids compare to UK wheat lines in the year 2017 and while comparing both the years P_{max} was overall higher in year 2017 than year 2016.

In contrast there were fewer differences ($P<0.05$) between parameters among amphidiploids: C_i for 2016 and ETR and VPD for 2017. However, there was a significant difference in the intercellular C_i in the year 2016, when the amphidiploids were compared to the UK wheat lines, Highbury and Paragon.

Two way anova analyses were carried out, the factors being the photosynthetic parameters mentioned in Table 4.10.The comparison was made between the two experimental years (2016 & 2017), interaction between year and groups (UK wheat lines and amphidiploids) and interaction within the groups. Overall, a high level of significance could be observed. In particular there was a high level of significance between experimental years, almost all the photosynthetic parameters were highly significant for the year 2017 when comparing to the year 2016. P_{max}, g_s, C_i, transpiration rate and A_{400} (P value <0.001), ΦPSII and ETR (P value 0.002), Fv'/Fm' (P value 0.04), qP (P value 0.026) and J_{max} (P value 0.033). A comparison between the groups i.e. the UK wheat lines and amphidiploids for both the experimental years revealed high significance for photosynthetic parameters such as C_i (P value: 0.008), g_s (P value: 0.005), transpiration rate (P value: 0.004), VPD (P value: 0.013) and P_{max} (P value: 0.024). When interaction was considered between experimental years and

groups, a high level significance could be observed for P_{max}, g_s, transpiration rate and VPD (P value <0.001), C_i (P value: 0.002). Significance between the experimental years suggests that the environmental conditions were contrasting in the year 2016 compared to 2017 and could be one of the major factors that limited photosynthesis in the amphidiploids and the UK wheat lines.

Temperature is one of the major factors that govern the rate of plant growth and development (Hatfield and Prueger, 2015) and most of the wheat varieties will respond differently to the fluctuation in temperature. The minimum temperature during the flowering stage in the year 2016 was higher than the year 2017, which might have affected specific aspects of plant growth and development such as flowering time, respiration and photosynthesis (Hatfield et al 2011). Knowles et al., (2006) have found that minimum air temperatures have significant effects on plant growth and phenology and with the changing climatic conditions there would be an increase in minimum air temperature in coming years.

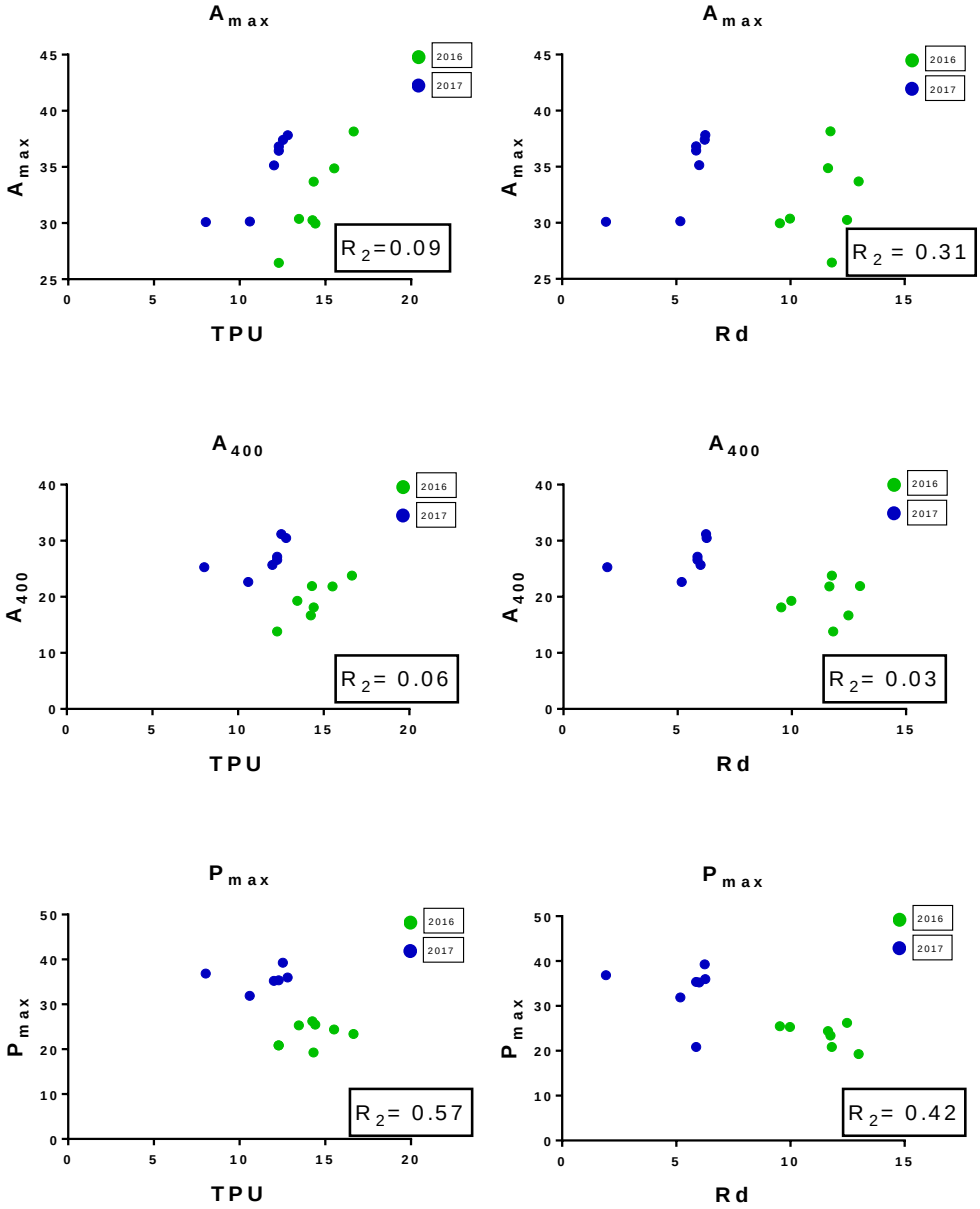


Figure4.17: Relationships between A_{400} (A_{max}) and (A) V_{cmax}, and (B) J_{max}, derived from AC_i curves of the amphidiploids and the UK wheat from the experimental year 2016 & 2017. The blue solid circles represent experimental year 2017 and green solid circles represent experimental year 2016. The statistical analysis of the correlation is described in table no. 4.9. P_{max} is the light saturated photosynthetic capacity at 400 µmol mol^{-1} CO_2 (from pre anthesis stage(GS39-GS45, Zadok's nomenclature) and A_{max} is the maximum assimilation rate at 1000 µmol mol^{-1} of CO_2 derived from the AC_i curve.

Table 4.11: Correlation analysis of the AC_i curve measurements and P_{max} of the amphidiploids and UK wheat lines for both the experimental years 2016 and 2017. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). P_{max} is the value derived from the photosynthetic measurements at vegetative stage (GS39-49, Zadok's nomenclature)

Year 2016-2017	V_{cmax}	J_{max}	RD	A_{400}	A_{max}	P_{max}
V_{cmax}		<.001	<.001	<.001	0.005	0.371
J_{max}			<.001	0.477	0.092	0.238
Rd				0.107	0.029	0.9
A_{400}					<.001	0.022
A_{max}						0.841

Correlation analysis was carried out between the photosynthetic parameters derived from AC_i curve measurements from both the experimental years and is shown in table 4.13. Here, P_{max} is the light saturated photosynthetic capacity at 400 µmol mol^{-1} CO_2 (from pre anthesis stage) and A_{max} is the maximum assimilation rate at 1000 µmol mol^{-1} of CO_2 and A_{400} is the assimilation rate at 400 µmol mol^{-1} of CO_2 derived from the AC_i curve measurements.

A_{max} showed significant relationship with V_{cmax} (P value: 0.005), TPU (P value 0.013) and RD (P value: 0.029), A_{max} was highly significant with A_{400}. A_{400} showed significant relationship with V_{cmax}, P_{max} showed significant relationship with A_{400}.

These data are important because they show a poor correlation between P_{max} and V_{cmax}, indicating that the amount of active Rubisco was not prevalent in

determining the P_{max} measured at vegetative stage and so stomatal conductance could have been more important. However, these measurements were at different leaves and different stages and possibly different environmental conditions.

4.4.9 Plant morphological and post- harvest measurements

4.4.9.1 Vein density

Plant morphological measurements such as plant height and leaf vein density were made to understand any relationship between morphological characteristics and leaf photosynthesis in these lines.

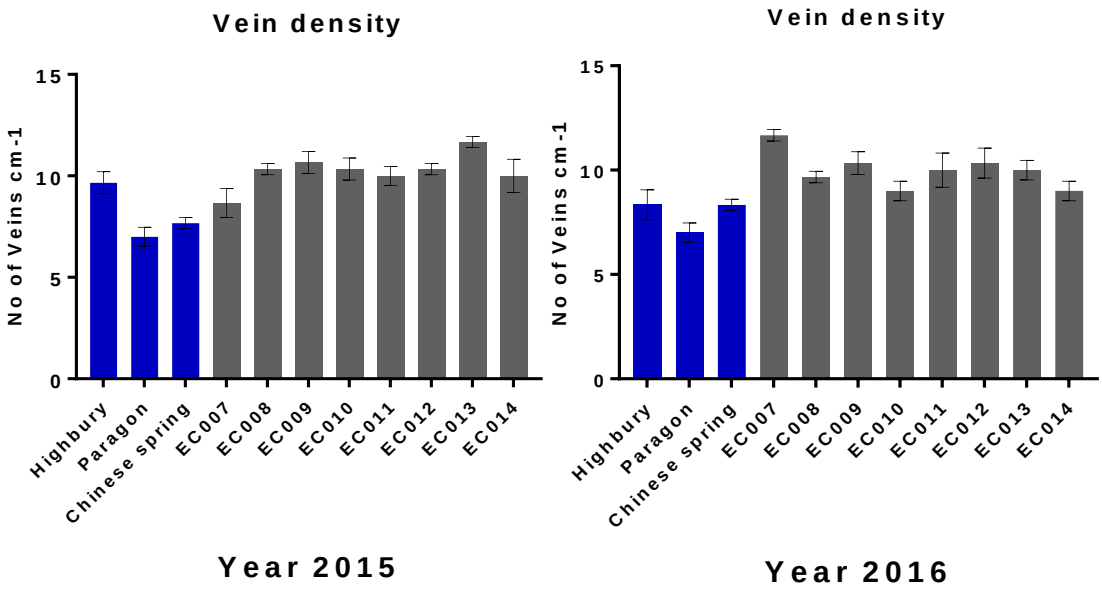


Figure4.18 Vein densities (A & B) of flag leaf of the amphidiploids and UK wheat lines for the year 2016 and 2017 respectively. The amphidiploids are shown in grey bars and the UK wheat lines in blue bars. Values are means, the error bars are the standard errors of the means (n=3)

Vein density is a trait that may be related to water transport; hydraulic conductivity and leaf gas exchange. There is still a significant gap to understand leaf venation and its relationship with the rate of photosynthesis (Brodribb et al.,

2007). It is imperative to understand the relationship between leaf venation and photosynthesis. Leaf vein density (LVD) has recently gained attention to understand the relationship of LVD to the physiology, ecological evolution of terrestrial plants (Price et al., 2014). Vein density has a direct influence on the transportation of water through the mesophyll cell space and thus affecting the physiological rates. The lesser the LVD is, longer time is taken to travel and higher LVD takes shorter time, therefore it would be beneficial to the plants to have a higher LVD than lower LVD in the leaves.

Measurements were made of UK wheat lines and amphidiploids and analysis of variance carried out (figure 4.18 and table 10) were observed to have more vein density than the UK wheat lines. No correlation could be drawn between number of veins and any photosynthetic parameters (data not shown)

Table 4.12: Anova of vein density for the year 2016 and 2017. Values shown are P values, shaded boxes indicate significant relationships (P<0.05).Here, Group refers to comparison between UK wheat lines and amphidiploids and lines refers to comparison within the amphidiploids.

Year 2016	P value	SEM
Lines	<.001	±0.85
Group	<.001	±0.44
Year 2017		
Group	0.003	±0.43
Lines	<.001	±0.95

Vein density of the amphidiploids and UK wheat lines, Highbury and Paragon were significant for both the years. Such traits could be inherited in next generation of wheat lines through introgression of amphidiploids wheat varieties.

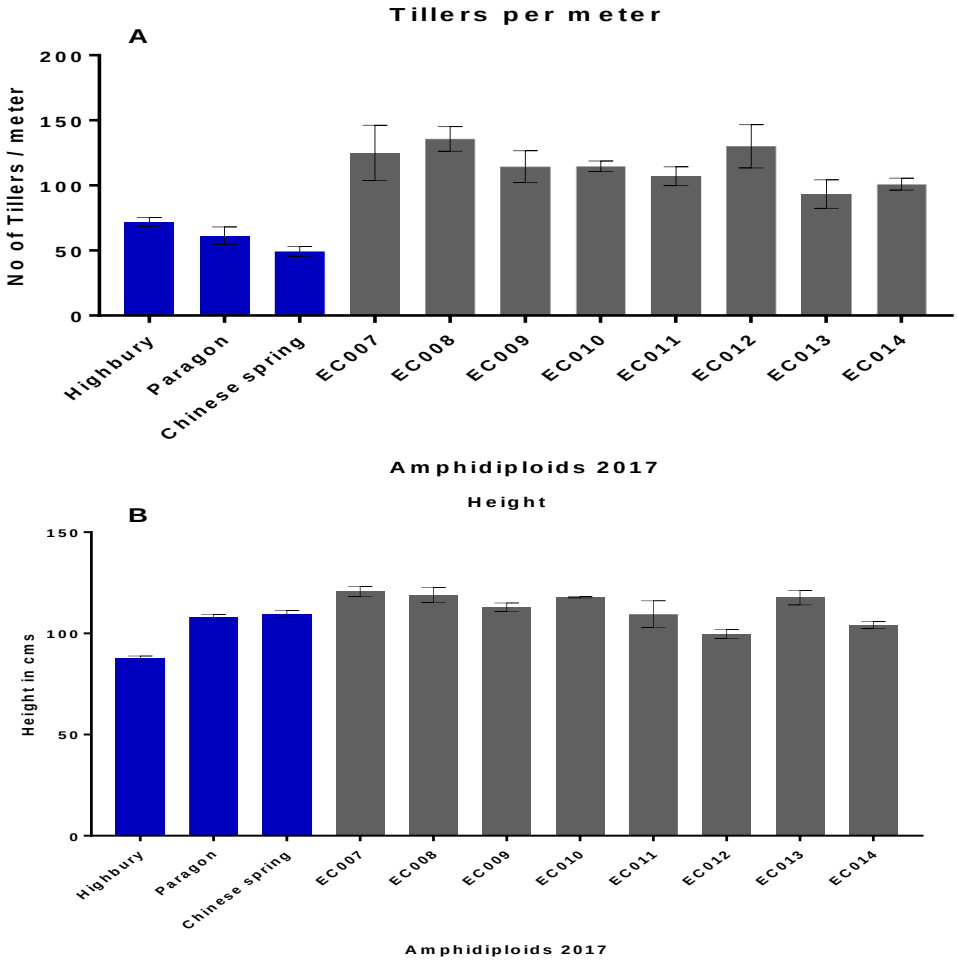


Figure4.19: (A) Tillers per meter (B) Plant height of the amphidiploids and UK Wheatlines for the year 2017. Values are means, the error bars are the standard errors of the means (n=3).

The tillers of most the amphidiploids were less in numbers compare to the UK wheat lines and the amphidiploids were taller than the UK wheat lines.

4.4.9.2 Post harvest measurements

After the completion of all other measurements, the plants were harvested and post-harvest analysis (biomass and grain weight were carried out).

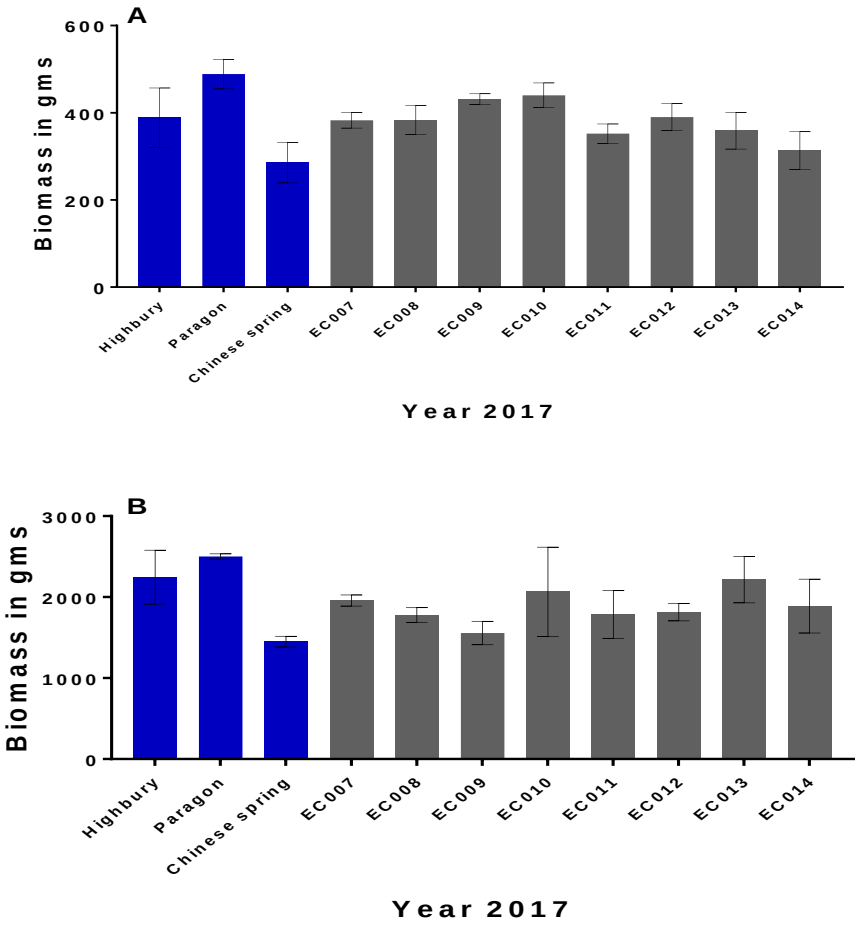


Figure4.20 (A) Biomass per meter (B) total biomass, the amphidiploids are represented in grey bars, and UK wheat lines in blue bars for the year 2017. Values are means, the error bars are the standard errors of the means (n=3)

Biomass measurements were carried out in two separate methods explained in chapter 2. Biomass was harvested from 1m lengths of the middlemost rows in each plot (4.20A), this harvest was included with the total plot harvest to calculate the total biomass (4.20 B). For the 1m biomass and total biomass, the amphidiploids biomass appeared to be higher than the UK wheat Chinese spring, which is one of the highlight of experimental year 2017.

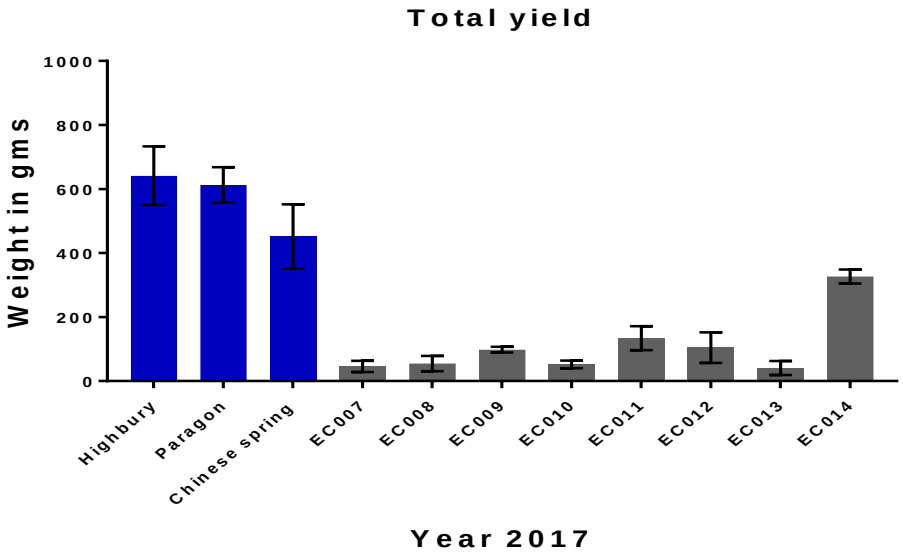


Figure4.21: Total yield (average of 3 plots per line) of the amphidiploids and UK wheat Highbury, Paragon, and Chinese spring. The amphidiploids are represented in grey bars and UK wheat in blue bars for the year 2017. Values are means, the error bars are the standard errors of the means (n=3)

There was a significantly lower yield in the amphidiploids compare to the UK wheat lines. One reason is that the amphidiploid's spike shattered which resulted in loss of spikes and ultimately grain loss.

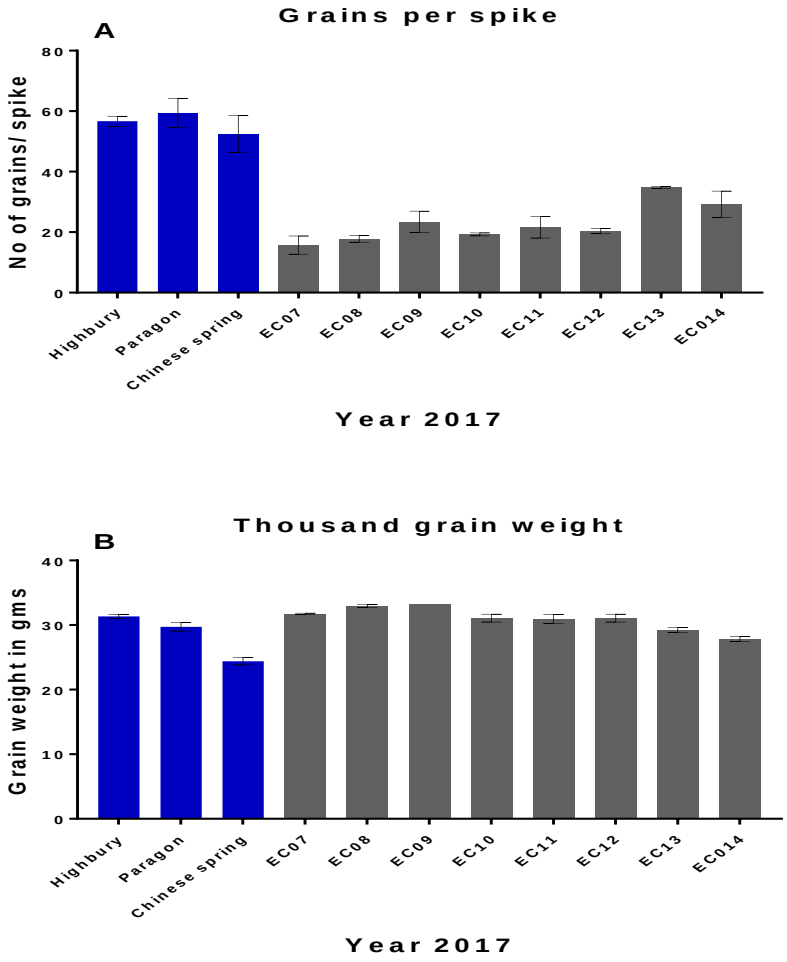


Figure4.22: (A) Grains per spike and (B) Thousand grain weight (TGW), the amphidiploids are represented in grey bars, UK wheat lines in blue bars for the year 2017. Values are means, the error bars are the standard errors of the means (n=3)

The grains per spike were lower in the amphidiploids compared to the UK wheat lines; this could again be due to spike shattering of the amphidiploids hence loss of grains. The thousand grain weight in the amphidiploids was in the same range of the UK wheat lines.

Table 4.13: Anovaof the postharvest parameters (all of the parameters) of the amphidiploids and UK wheat lines for the year 2016 and 2017. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Here, Group refers to comparison between UK wheat lines and amphidiploids and lines refers to comparison within the amphidiploids.

Year 2017	Plant Height	SED	Tillers /meter	SED	Biomass / meter	SED	Total Biomass	SED
Groups	<.001	±1.95	<.001	±7.186	0.863	±30.19	0.004	±330.3
Lines	<.001	±6.01	0.996	±22.18	0.598	±94.6	0.98	±1020

Year 2017	Total yield	SED	TGW	SED	Grains/ spike	SED
Groups	<.001	±41.82	0.121	0.38	<.001	±2.55
Lines	0.133	±129.1	<.001	1.19	0.027	±7.89

Two way anovas were done on the postharvest parameters of the amphidiploids and were compared with the UK wheat lines for grains per spike, tillers per meter, plant height and total yield were all significant with P value:<0.001 and for biomass (P <0.05), biomass per meter and thousand grain weight (TGW) were not significant. When comparison was made within the lines TGW and plant height were significant (P value < 0.001), and grains per spike (P < 0.05)

The UK wheat Highbury and Paragon were significantly higher than the amphidiploids for all the postharvest parameters; however the amphidiploids were in the same range as the other UK wheat like Chinese spring for thousand grain weights.

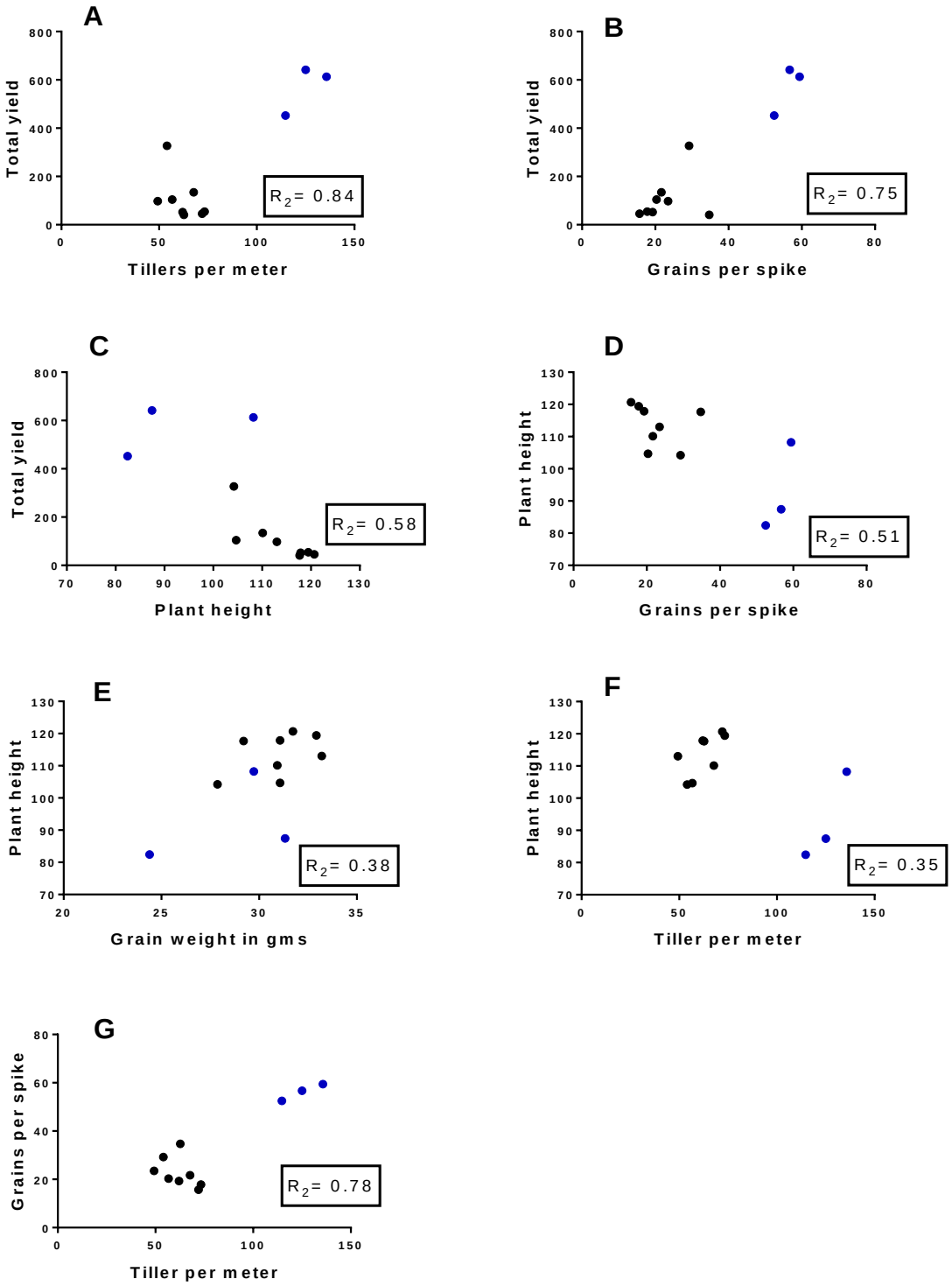


Figure4.23: Relationship between total yield and (A) tillers per meter (B) grains per spike (C) plant height, (D, E & F) plant height with grains per spike, tillers per meter & thousand grain weight and (G) tillers per meter with grains per spike for the year 2017. Values are the means of each parameter. The UK wheat lines are shown in blue solid circles and the amphidiploids in black solid circles.

The postharvest parameters were compared against each other and in this case some significant correlations were observed (Figure 4.23 and Table 4.15)Total yield of the amphidiploids and UK wheat lines showed positive correlation with tillers per meter and grains per spike (figure 23 A & B), but with plant height it showed a negative correlation (figure 23 C), suggesting the taller plants do not necessarily have high yield, however this could be confirmed if the spikes were not shattering in nature to avoid grain loss in taller plants. Plant height showed positive correlation with thousand grain weights (figure 23 E) but negative correlation with tillers per meter (figure 23 F). Grains per spike showed a positive correlation with tillers per meter (figure 23 G) and is known to be an important component in wheat grain yield.

Correlation analysis between the postharvest elements and photosynthetic parameters (all of the parameters) was carried out but no significant relationship could be drawn (data not shown), indicating that the most important factor in determining yield in these lines was tillering and grain number.

Table 4.14: Correlation of the postharvest parameters of the amphidiploids and UK wheat for the experimental year 2017. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Here, all the postharvest parameters were compared against each other.

Year 2017	Total yield	Grains per spike	Tillers per meter	Biomass per meter	Plant height	Thousand grain weight
Total Biomass	0.826	0.567	0.409	0.167	0.389	0.165
Total yield		<0.001	<0.001	0.826	<0.001	0.158
Grains per spike			<0.001	0.795	0.017	0.121
Tillers per meter				0.374	0.009	0.923
Biomass per meter					0.789	0.501
Plant height						0.02

4.6 Discussion

The discussion will highlight the phenotypic variation in photosynthesis in the amphidiploid population compared to UK wheat Highbury and Paragon. Significant differences in photosynthetic capacity were observed between amphidiploids and UK wheat lines: the possible mechanisms for these differences will be discussed along with the trade-offs that the field environment might present.

The light saturated photosynthetic capacity was higher in the amphidiploids than the UK wheat lines for the year 2017; however the amphidiploids struggled to maintain photosynthetic efficiency in the year 2016. Soil water deficit occur in drought conditions (Jones et al., 1999), in well-watered conditions when the soil water evaporation rate is high then it can pose adverse effect on plants. Perdomo (2017) revealed that under water deficit and high temperature conditions, diffusion of atmospheric CO_2 inleaves is constrained; however biochemical limitation of photosynthesis is also an effect of associated heat stress, which may have also occurred in the present experiment (temperatures of 30 – 40°C). It should also be noted that irradiance levels were high in this experiment, frequently reaching 1500 – 2000 umol m^{-2} s^{-1} and clear sunny skies common, which is consistent with the dark adapted Fv/Fm data showing values below 0.7 (figure 4.7). High irradiance will exacerbate biochemical sites of stress caused by other environmental pressures such as water deficit and high temperature. However, the AC_i data suggested that the primary cause of lower photosynthesis was stomatal closure.

In the year 2016, water scarcity and soil water evaporation rate (data not available) along with high temperature, created drought like conditions. Shah

and Paulsen (2003) have reported that drought and high temperature can adversely affect photosynthesis in wheat and yield loss in wheat especially when drought coincides with post-anthesis stage (Foulkes et al., 2002). Pour-Aboughadareh (2017) reported a decline in stomatal conductance, chlorophyll content and reduction of Fv/Fm due to drought or scarcity of water in wheat at the field station, Pune.A similar observation could be made in the year 2016 where amphidiploids and the UK wheat lines demonstrated significant reductions in stomatal conductance in contrast to year 2017 probably attributed to faster soil water evaporation rate in the year 2016.

This is corroborated by wider environmental circumstances: in 2016, World Health Organisation (WHO) declared the state of Maharashtra, where the experimental site is located, as a drought-affected region. This limited supply of water after January 2016 (The field was periodically watered as described in chapter 2), it is likely that the period of measurement coincided with a temporary water restriction that affected leaf gas exchange and another possibility could be faster evaporation rate of soil water, data for which is unavailable. However, it is unknown, if this affected grain yield or biomass because at the time of writing this data was not available for 2016.

The stomatal conductance was lower in 2016 with many values below 0.2 mmol m^{-2} s^{-1}. Canopy temperature was also higher in 2016, suggesting that this was a whole plant effect. Higher leaf temperatures combined with lower leaf C_i (Fig 4.9) may have resulted in higher rates of oxygenation vs carboxylation and hence photorespiration *in situ* which would have pushed photosynthetic rates down further at all light levels. Other values such as V_{cmax} were generally not lower in 2016 suggesting that there was no fundamental change in leaf function between the years. A significant effect of the lowered stomatal conductance

was to lower the P_{max} via restriction of stomatal aperture and hence the diffusion of CO_2 into the leaf. Another important effect was to increase the WUE_i in 2016 compared to 2017 (Fig 4.12). This is likely to be due to the fact that the 2016 reduction in transpiration and stomatal conductance was not proportionally as great as the reduction in photosynthesis. Hence a restriction of water supply will lead to a higher WUE_i but lowered biomass production through lowered. An important conclusion to this chapter is therefore that whilst high P_{max} is probably a valuable trait, within the Indian field environment used in this study it will come with the penalty of water loss. This could be a serious limitation because there are frequently limitations imposed on farmers in this region described above as a result of water supply via the network of canals that supply fields, as was the case in 2016.

An interesting addition to these observations is the higher values of qP, ΦPSII and ETR in 2016 compared to 2017, despite the lowered P_{max}. A higher ETR capacity is perhaps surprising but could occur as a result of higher 'excitation pressure' from the lowered P_{max} inducing a higher rate of flux through photorespiration and other electron sinks such as the Mehler reaction. The Fv'/Fm' is inversely correlated with NPQ (Murchie and Lawson, 2013) and here indicates that NPQ was higher in 2016, consistent with higher excitation pressure. Differences in photoinhibition between years were small or non-existent. Together these observations support the notion of a system that is flexible and able to compensate thylakoid and stromal reactions to prevent substantial photodamage during periods of water restriction caused by stomatal closure.

The wheat growing season in India is between late October to April every year, the Indian genotypes takes almost 120 days from sowing until harvest,

however the flowering of the amphidiploids and UK wheat lines ranges between 85-98 days and another 60 days to attain maturity. The number of days required for the amphidiploids to attain maturity could risk them to experience hotter climatic conditions towards the end of wheat growing season. (Blum et al., (2009) suggests that shorter time period taken by crops to reach anthesis or to attain maturity, could be an effective strategy to escape terminal drought like conditions. Therefore, it can be recommended that, an earlier sowing date for the amphidiploid could be a suitable strategy to escape higher temperature at late grain filling stage.

Chapter 5

Natural variation in photosynthesis of 36 Indian cultivars derived from different agro-ecological zones of India

5.1 Introduction

The countries from the Indian subcontinent such as India, Nepal, Pakistan and Bangladesh are some of the most important wheat producing and consuming regions of the world (Mondal et al., 2016).

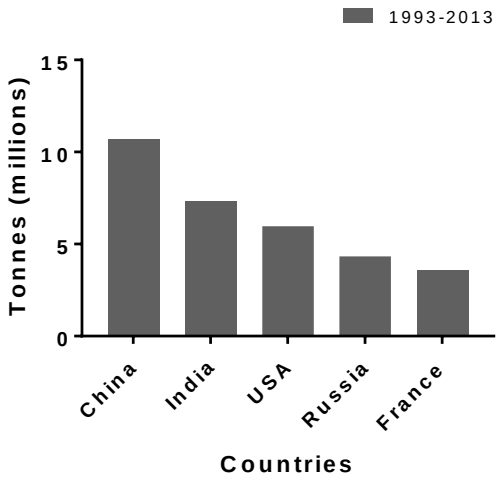


Figure 5.1: Global wheat production in tonnes from the year 1993-2013, where India ranks as the 2nd largest producer of wheat in the world.

India is the second largest producer of wheat in the world after China, with a production of 68-75 million tons for the last five years. However, this productivity will not be enough to satiate the food demand by the year 2020 which is estimated to be ~90 million tons, Rane and Nagarajan (2004). Despite

an enormous leap in the production of wheat between 1920 and 2012, the increasing population in developing countries like India means there is also a continual rise in demand for wheat (Reynolds and Borlaug, 2006). Since the new millennium India is experiencing challenging climatic and environmental conditions, which ultimately affects productivity of crops like wheat and rice (IPPC 2013). There has been a gradual increase of 0.32°C and 0.28°C in the minimum temperature in wheat growing areas per decade in India (northern parts). Warmer climatic conditions are one of the major factors slowing wheat productivity growth in South Asia and globally (Rao et al., 2015). In India, the eastern, central and southern regions of India are now warmer throughout the wheat growing season. At the vegetative stage of wheat in these regions the average temperature recorded usually ranges between 27-30°C, which gradually increases to more than 30°C during the grain filling stage (Mondal et al, 2016). Anthesis itself is directly sensitive to heat stress and can result in sterility and yield loss (Jagadish et al., 2009). However among various environmental factors, the temperature rise particularly has resulted in huge reductions of yield in wheat largely due to terminal heat stress during grain filling stage, which suggests development of heat-tolerant wheat varieties should focus on heat stress that coincides with grain filling stage (Dhyani et al., 2013). This rise in temperature during grain-filling has resulted in yield loss primarily due to decrease in photosynthetic efficiency (Hairat and Khurana 2015).Therefore, it would be productive to explore the phenotypic variation in existing Indian genotypes for improved photosynthetic efficiency.

India has different wheat agro-ecological zones; the details of the zones are mentioned in chapter 2. This chapter describes variation in photosynthesis in Indian genotypes grown in glasshouse conditions at the University of

Nottingham, Sutton Bonington campus. The Indian genotype seeds were made available from the Indian institute of Wheat and Barley research, Karnal, India. Along with the Indian genotypes, UK wheat cultivars Highbury and Paragon were also grown to compare the photosynthetic efficiency and other related physiological traits. There are limited published data on photosynthetic efficiency of Indian wheat cultivars; hence this area needs to be explored to establish the extent of variation and understand the underlying causes.

Specific hypothesis tested in this chapter:

1. 36 Indian genotypes exhibit variation in photosynthetic capacity and efficiency in glasshouse conditions (UK conditions).
2. Indian genotypes perform comparatively better than the UK wheat Paragon and Highbury in the following photosynthetic parameters as they are adapted to high light and temperature conditions
   A. Higher P_{max} (light saturated photosynthetic capacity)
   B. Higher V_{cmax} (maximum carboxylation rate of rubisco)
   C. Higher J_{max} (maximum rate of electron transport)
   D. Lower VPD (Vapour pressure deficit)

5.2 Results

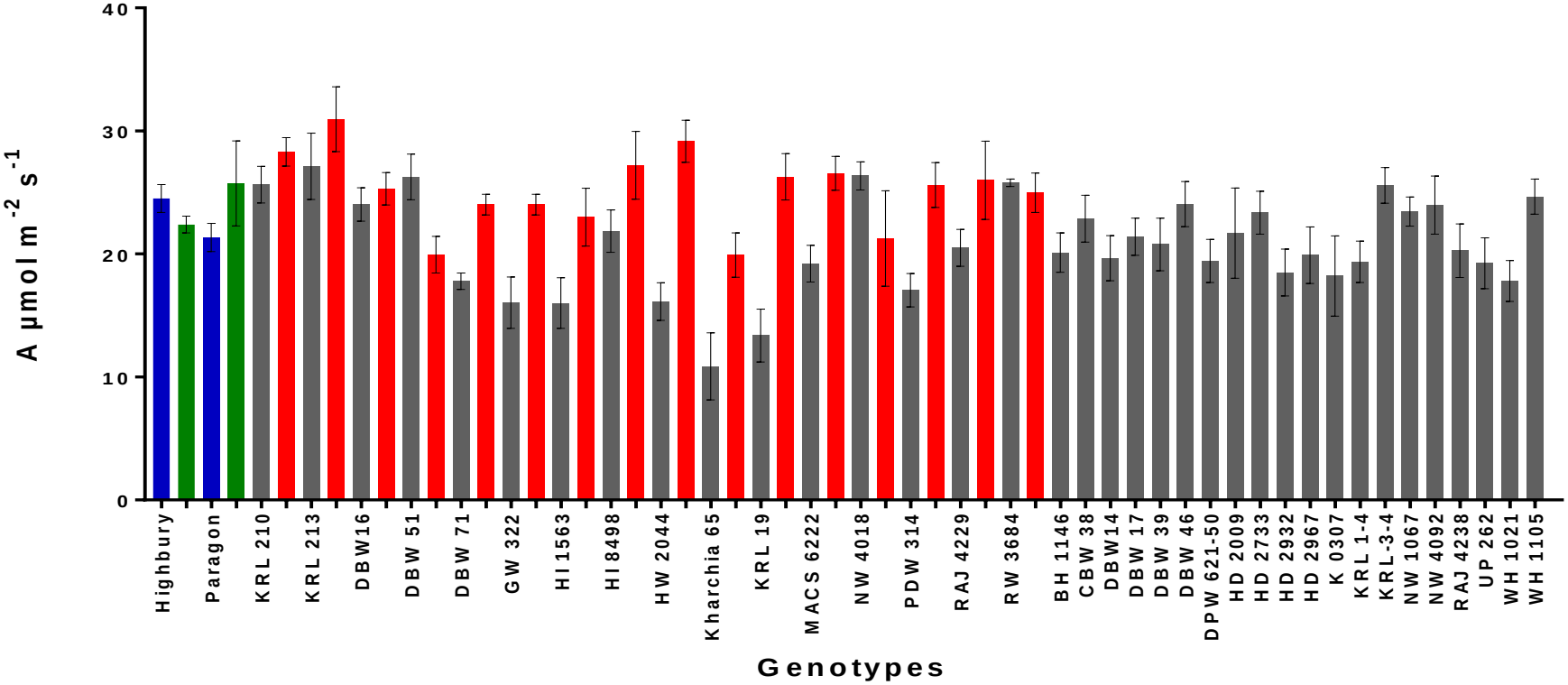
This chapter is a study of phenotypic variation in maximum photosynthetic capacity and efficiency in a panel of 36 Indian genotypes. The experiments were carried out in glasshouse conditions at University of Nottingham duplicated over the years 2015 and 2016. The first experimental year had 36 Indian genotypes and 5 replicates of each; initially these genotypes were tested for maximum light saturated photosynthetic capacity. The data derived from photosynthetic capacity measurements allowed selection of promising lines, which were further

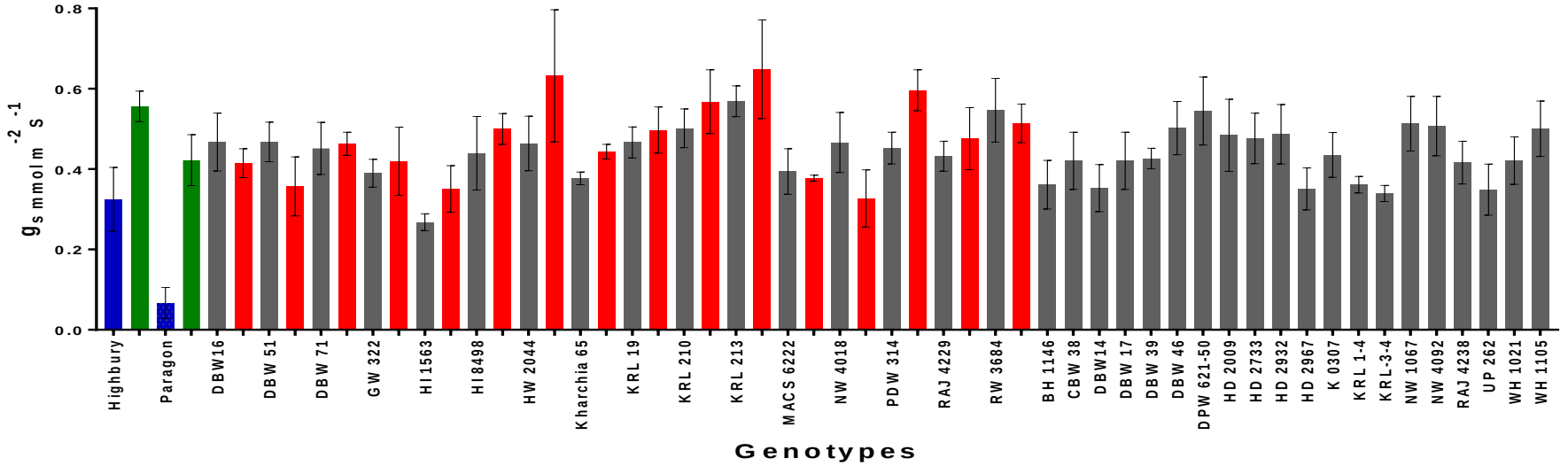
investigated for maximum carboxylation velocity of Rubisco (V_{cmax}), maximum electron transport rate (J_{max}). For the experimental year 2016, only those Indian genotypes were used which were found to be promising in the experimental year 2015. This was done for the purpose of replication. There were some differences in growing conditions between 2015 and 2016 as explained in chapter 2. All the photosynthetic measurements were done at vegetative stage (GS39-45), whereas all the detailed photosynthetic measurements were done at anthesis stage.

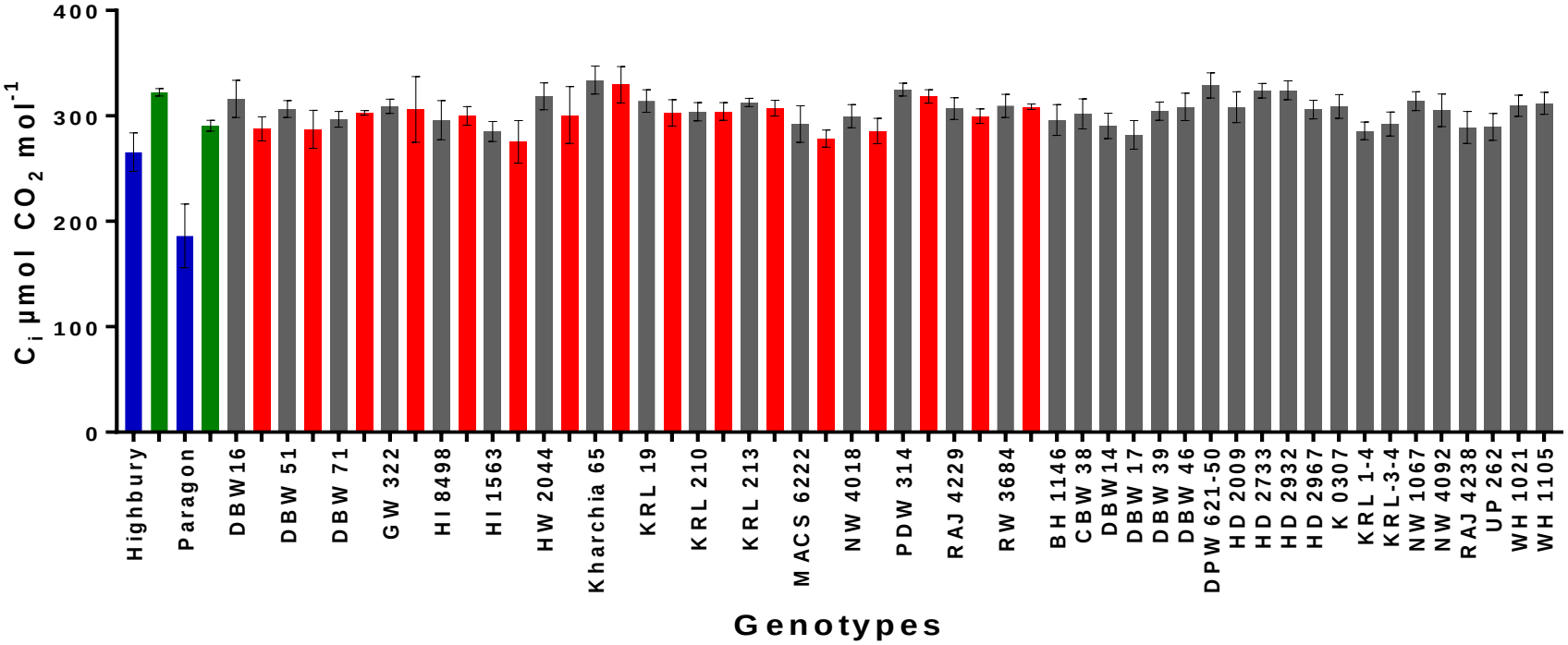
The results of the photosynthetic capacity of the Indian genotype are described for both experimental years.

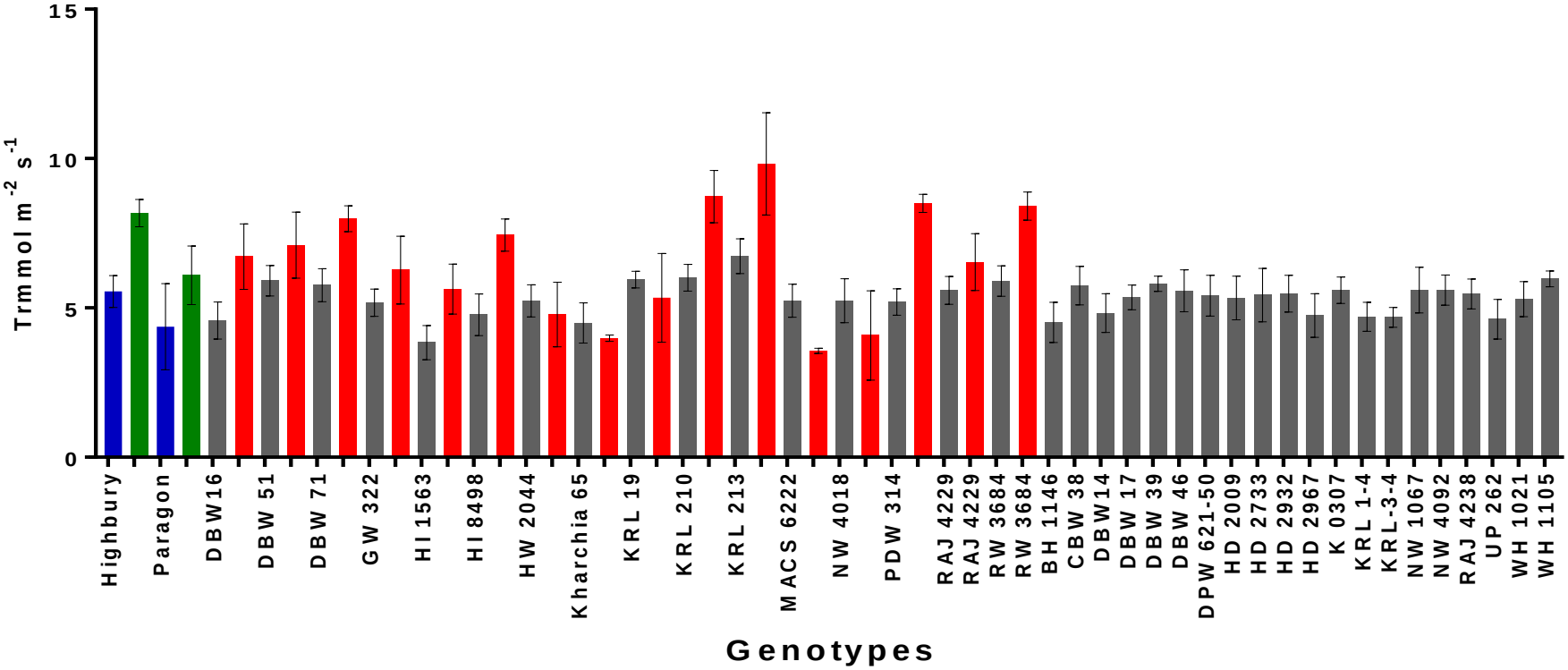
5.2.1 Light saturated photosynthetic measurements

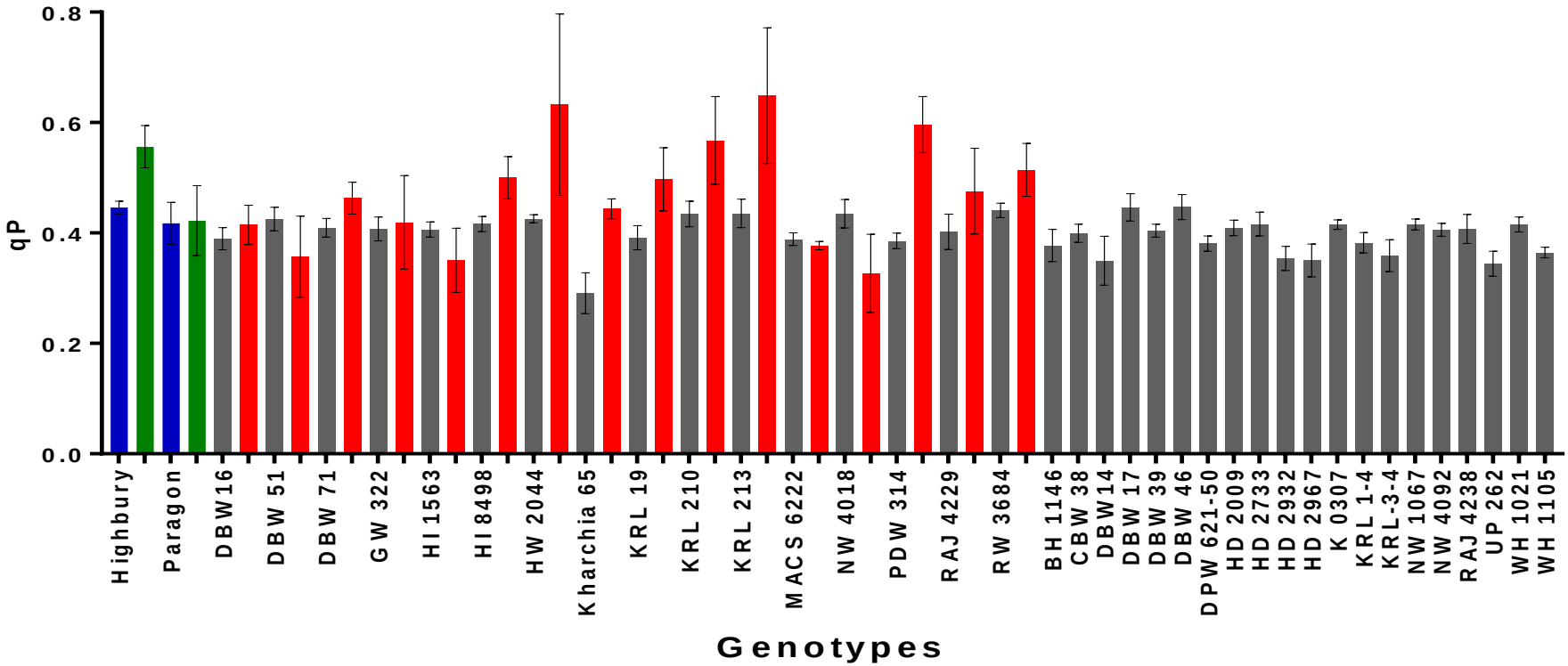
To understand genetic variation in photosynthesis in the amphidiploids, light saturated photosynthetic measurements (P_{max}) were carried out (Figures 5.2-5.11 and Table 5.1).

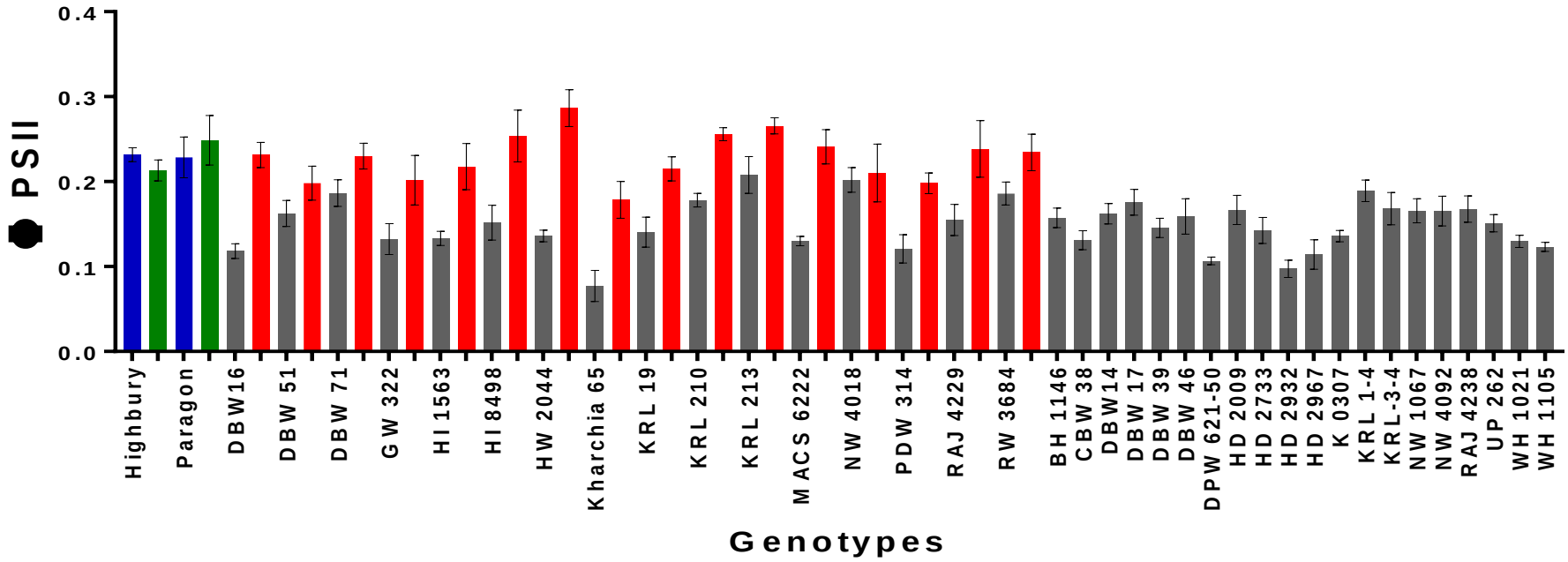

A µmol m-2 s-1
40
30
20
10
0
Highbury
Paragon
KRL 210
KRL 213
DBW16
DBW 51
DBW 71
GW 322
HI1563
HI8498
HW 2044
Kharchia 65
KRL 19
MACS 6222
NW 4018
PDW 314
RAJ 4229
RW 3684
BH 1146
CBW 38
DBW14
DBW 17
DBW 39
DBW 46
DPW 621-50
HD 2009
HD 2733
HD 2932
HD 2967
K 0307
KRL 1-4
KRL-3-4
NW 1067
NW 4092
RAJ 4238
UP 262
WH 1021
WH 1105
Genotypes

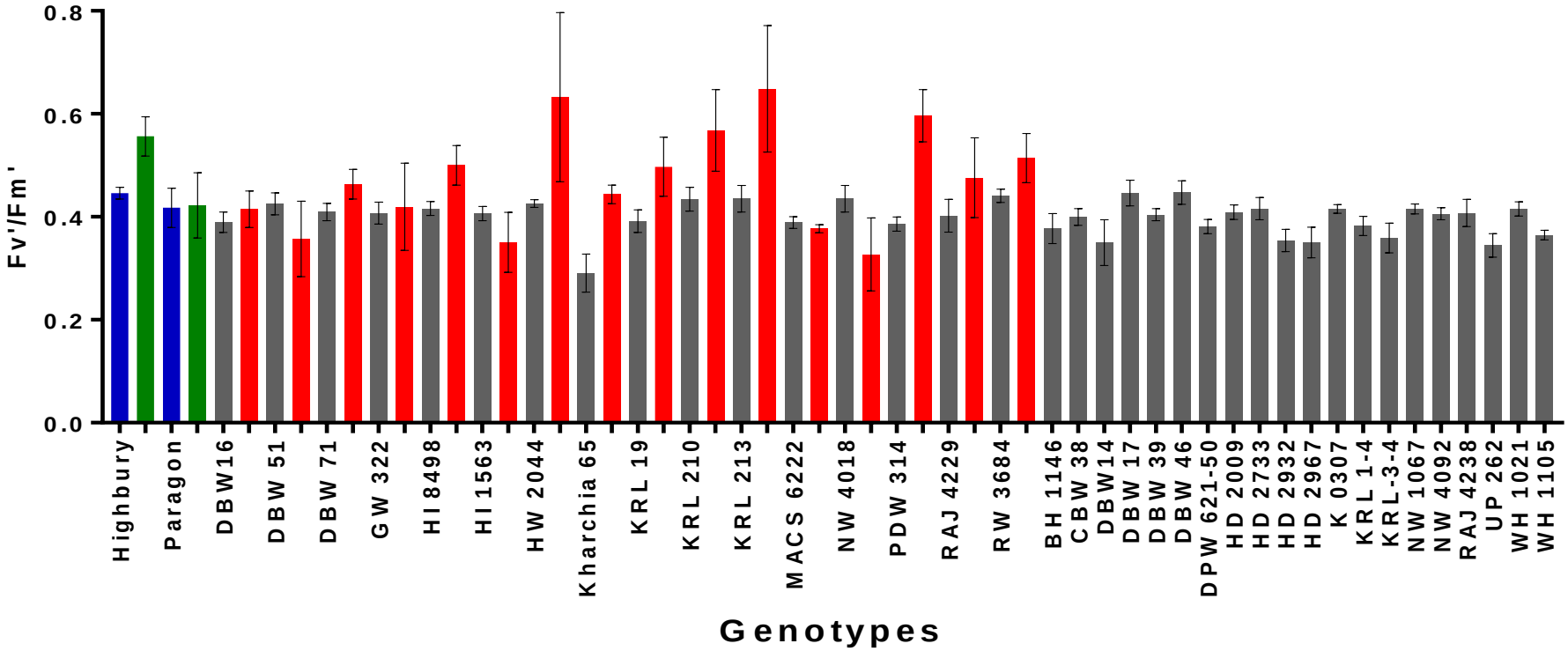

0.8
0.6
0.4
0.2
0.0
gs mmol m-2 s-1
Highbury
Paragon
DBW16
DBW 51
DBW 71
GW 322
HI1563
HI8498
HW 2044
Kharchia 65
KRL 19
KRL 210
KRL 213
MACS 6222
NW 4018
PDW 314
RAJ 4229
RW 3684
BH 1146
CBW 38
DBW14
DBW 17
DBW 39
DBW 46
DPW 621-50
HD 2009
HD 2733
HD 2932
HD 2967
K 0307
KRL 1-4
KRL-3-4
NW 1067
NW 4092
RAJ 4238
UP 262
WH 1021
WH 1105
Genotypes

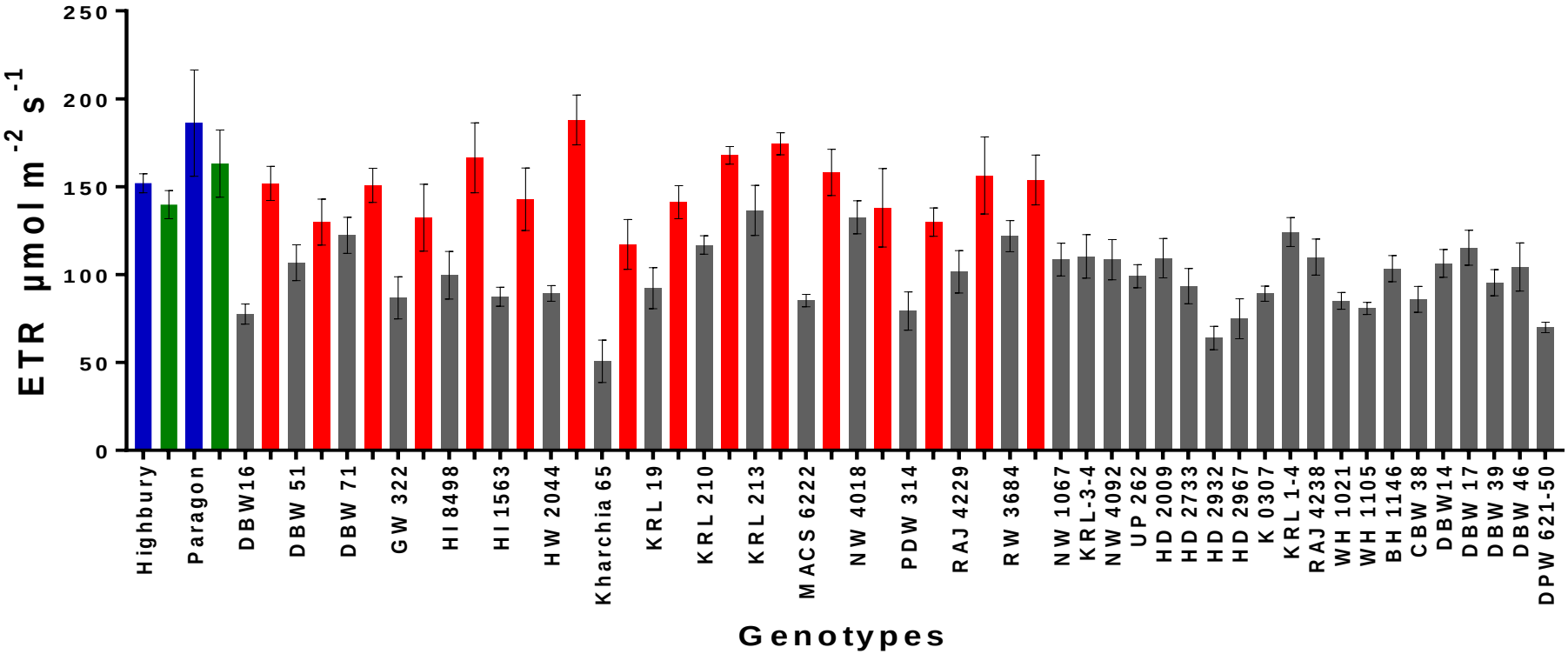

400
300
200
100
0
C_i µmol CO_2 mol^{-1}
Highbury
Paragon
DBW16
DBW 51
DBW 71
GW 322
HI 8498
HI 1563
HW 2044
Kharchia 65
KRL 19
KRL 210
KRL 213
MACS 6222
NW 4018
PDW 314
RAJ 4229
RW 3684
BH 1146
CBW 38
DBW14
DBW 17
DBW 39
DBW 46
DPW 621-50
HD 2009
HD 2733
HD 2932
HD 2967
K 0307
KRL 1-4
KRL-3-4
NW 1067
NW 4092
RAJ 4238
UP 262
WH 1021
WH 1105
Genotypes

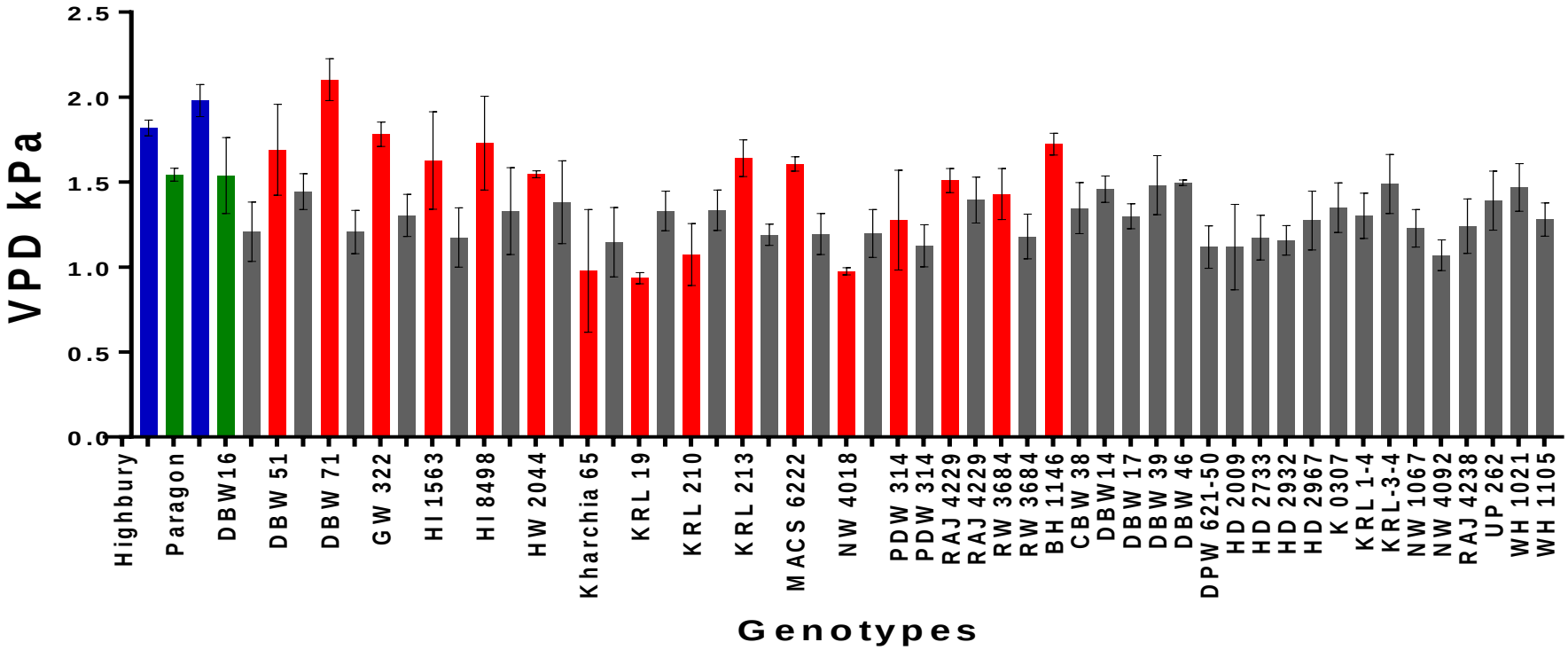

15
10
5
0
Trmmol m-2 s-1
Highbury
Paragon
DBW16
DBW 51
DBW 71
GW 322
HI 1563
HI 8498
HW 2044
Kharchia 65
KRL 19
KRL 210
KRL 213
MACS 6222
NW 4018
PDW 314
RAJ 4229
RAJ 4229
RW 3684
RW 3684
BH 1146
CBW 38
DBW14
DBW 17
DBW 39
DBW 46
DPW 621-50
HD 2009
HD 2733
HD 2932
HD 2967
K 0307
KRL 1-4
KRL-3-4
NW 1067
NW 4092
RAJ 4238
UP 262
WH 1021
WH 1105
Genotypes


qP
0.8
0.6
0.4
0.2
0.0
Highbury
Paragon
DBW16
DBW 51
DBW 71
GW 322
HI 1563
HI 8498
HW 2044
Kharchia 65
KRL 19
KRL 210
KRL 213
MACS 6222
NW 4018
PDW 314
RAJ 4229
RW 3684
BH 1146
CBW 38
DBW14
DBW 17
DBW 39
DBW 46
DPW 621-50
HD 2009
HD 2733
HD 2932
HD 2967
K 0307
KRL 1-4
KRL-3-4
NW 1067
NW 4092
RAJ 4238
UP 262
WH 1021
WH 1105
Genotypes


0.4
0.3
0.2
0.1
0.0
PSII
Highbury
Paragon
DBW16
DBW 51
DBW 71
GW 322
HI1563
HI8498
HW 2044
Kharchia 65
KRL 19
KRL 210
KRL 213
MACS 6222
NW 4018
PDW 314
RAJ 4229
RW 3684
BH 1146
CBW 38
DBW14
DBW 17
DBW 39
DBW 46
DPW 621-50
HD 2009
HD 2733
HD 2932
HD 2967
K 0307
KRL 1-4
KRL-3-4
NW 1067
NW 4092
RAJ 4238
UP 262
WH 1021
WH 1105
Genotypes


Fv'/Fm'
0.8
0.6
0.4
0.2
0.0
Highbury
Paragon
DBW16
DBW 51
DBW 71
GW 322
HI8498
HI1563
HW 2044
Kharchia 65
KRL 19
KRL 210
KRL 213
MACS 6222
NW 4018
PDW 314
RAJ 4229
RW 3684
BH 1146
CBW 38
DBW14
DBW 17
DBW 39
DBW 46
DPW 621-50
HD 2009
HD 2733
HD 2932
HD 2967
K 0307
KRL 1-4
KRL-3-4
NW 1067
NW 4092
RAJ 4238
UP 262
WH 1021
WH 1105
Genotypes


ETR µmol m-2 s-1
250
200
150
100
50
0
Highbury
Paragon
DBW16
DBW 51
DBW 71
GW 322
HI 8498
HI 1563
HW 2044
Kharchia 65
KRL 19
KRL 210
KRL 213
MACS 6222
NW 4018
PDW 314
RAJ 4229
RW 3684
NW 1067
KRL-3-4
NW 4092
UP 262
HD 2009
HD 2733
HD 2932
HD 2967
K 0307
KRL 1-4
RAJ 4238
WH 1021
WH 1105
BH 1146
CBW 38
DBW14
DBW 17
DBW 39
DBW 46
DPW 621-50
Genotypes


VPD kPa
2.5
2.0
1.5
1.0
0.5
0.0
Highbury
Paragon
DBW16
DBW 51
DBW 71
GW 322
HI 1563
HI 8498
HW 2044
Kharchia 65
KRL 19
KRL 210
KRL 213
MACS 6222
NW 4018
PDW 314
PDW 314
RAJ 4229
RAJ 4229
RW 3684
RW 3684
BH 1146
CBW 38
DBW14
DBW 17
DBW 39
DBW 46
DPW 621-50
HD 2009
HD 2733
HD 2932
HD 2967
K 0307
KRL 1-4
KRL-3-4
NW 1067
NW 4092
RAJ 4238
UP 262
WH 1021
WH 1105
Genotypes

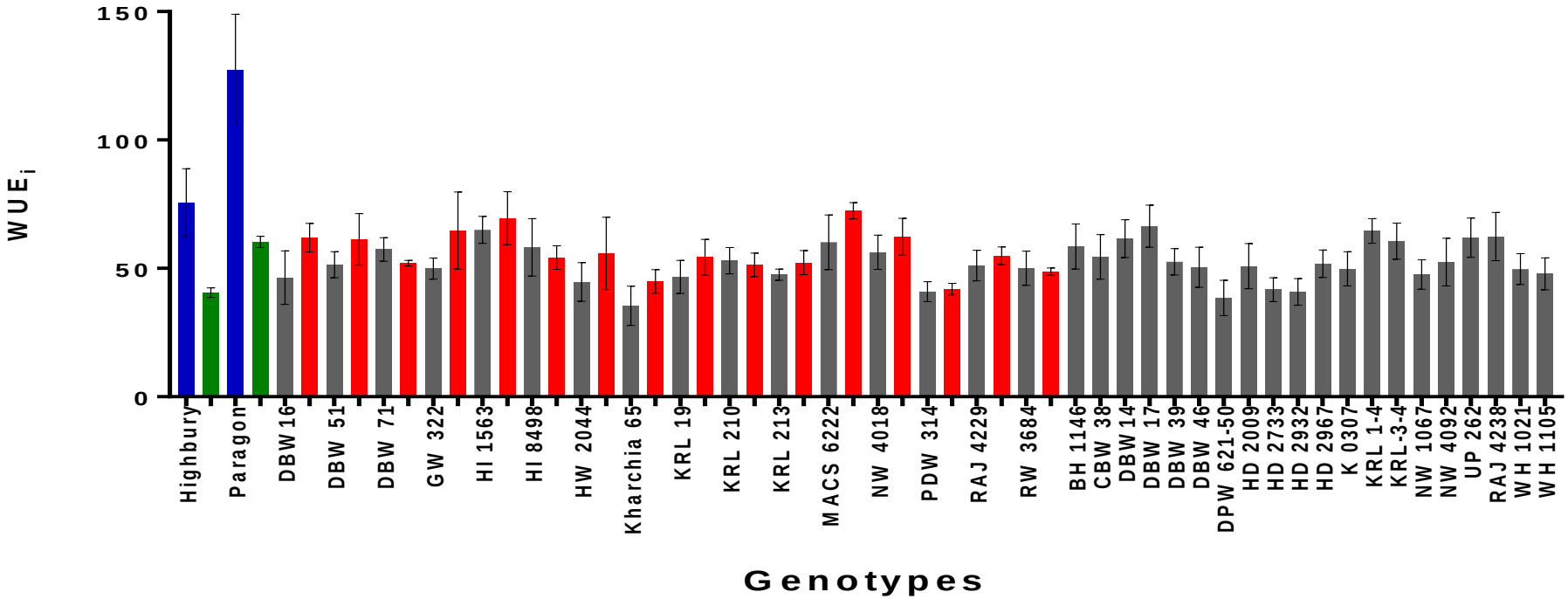


Figure 5.2:: Light saturated photosynthetic capacity (P_{max}), stomatal conductance (g_s), Leaf internal CO_2 (Ci), transpiration (Trm), VPD, intrinsic water use efficiency (WUE_i), Fv'/Fm', photochemical quenching (qP), electron transport rate (ETR) and operating efficiency of PSII (ϕPSII) of the Indian genotypes and UK wheat cultivars Highbury and Paragon for the year 2015 and 2016 measured at vegetative stage (GS39-GS45 (Zadok's nomenclature). UK wheat cultivars are represented in blue bars (2015) green bars (2016), the Indian genotypes in grey bars (2015) and red bars (2014). The cuvette conditions were 400 µmol CO_2, block temperature was set to 22°C, 1500 PPFD µmol$m^{-2}s^{-1}$, and ambient humidity was kept ~ 60%. Values are means, error bars are standard errors of the means (n = 5 (individual plants)).

The section describes all the photosynthetic measurements of the UK cultivars and the Indian genotypes for the year 2015 and 2016. The soil medium for UK wheat paragon and highbury were different than the Indian genotypes for the experimental year 2015 as explained in chapter 2. The Indian genotypes for the year 2015 demonstrated good variation in photosynthetic capacity (Figure 5.2, grey bars), genotypes with highest P_{max} were KRL213, NW4018, DBW 51 and KRL210 and among all the Indian genotypes, highest P_{max} was observed in KRL213 (30 $\mu molCO_2m^{-2}s^{-1}$) and the lowest P_{max} value in Kharchia (11 $\mu molCO_2m^{-2}s^{-1}$). There were clear differences between the years in terms of P_{max} and some notable inconsistencies for example HW2044 was one of the lowest in 2015 but one of the highest in 2016. In year 2016, KRL213, HW2044, KRL210 and HI 8498 demonstrated high photosynthesis (Figure 5.2, red bars), the P_{max} value of KRL213 was 30 $\mu molCO_2m^{-2}s^{-1}$. However, the ranking of many lines was consistent between the years e.g. KRL 213, Kharchia 65.

A large variation in stomatal conductance (g_s) could be observed in the Indian genotypes for both the years, UK wheat Highbury and Paragon showed lower g_s in the year 2015 (figure 5.2, blue bars). Paragon showed very low g_s in 2015 and it is unclear how such a low g_s could support the P_{max} observed. However in the year 2016, g_s of Highbury and Paragon were higher than 2015. The highest g_s could be observed in HW2044 (0.63 mmol $m^{-2}s^{-1}$) and KRL 213 (0.64mmol $m^{-2}s^{-1}$) for 2016. C_i for both years of the Indian genotypes were in the restricted range of 280 to 300 µmol $CO_2m^{-2}s^{-1}$ suggesting that the differences in stomatal conductance observed were compensated by changes in photosynthetic rate, or vice versa. Highbury and Paragon showed lower C_i than the Indian genotypes which could be attributed to lower stomatal conductance. Transpiration rate was comparatively higher in the year 2016 than the year 2015; this could be due to

the growing conditions which are explained in Chapter 2. The growing season for the year 2016 was during summer (July to September) and the conditions were largely warmer and brighter (Meteorological data not available), hence the response of the Indian genotypes to the rising temperature and higher light was a higher transpiration rate.

Fluorescence data was also collected during the P_{max} measurements. Photochemical quenching, qP is an indication of the state of reduction of PSII centres and overall we see limited differences with most values around 0.4 and in some lines a higher value for 2016. The exception are Indian genotypes, KRL210, KRL 213, HW2044 and PDW 314 where qP was higher (0.4-0.6) while qP was ~ 0.4 for all the genotypes including Highbury and Paragon for the year 2015. Quantum yield of PSII (ΦPSII) in the Indian genotypes for the year 2016 was consistently higher than the year 2015 whilst Fv'/Fm' was higher in HW2044, KRL19, KRL210, KRL 213, PDW314 and RW3684 for 2016. ETR responded in the same way as ΦPSII since the former is calculated from the latter and measured at the same light intensity. VPD was observed to be higher in the Indian genotypes for the year 2016 than the year 2015, consistent with the higher transpiration rate. No significant difference was observed WUE_i in the Indian genotypes for both years (table 5.1), however WUE_i was observed to be higher in the UK wheat Highbury and Paragon for the year 2015 than either the 2016 counterpart or the Indian genotypes.

5.2.2: Statistical analysis

Table 5.1: Analysis of variance of the photosynthetic parameters (all the parameters) of the Indian genotypes and UK wheat lines for the experimental year 2015 and 2016. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). This table shows analysis of the photosynthetic parameters (P_{max}, g_s, C_i etc) within the groups (i.e.UK cultivars Highbury and Paragon), within the lines (i.e. Indian genotypes), between the years 2015 and 2016 and interactions of the groups and lines between years.

Year 2016-2017	Group	SEM	Lines	SEM	Year	SEM	Lines. Year	SEM	Group. Year	SEM
P_{max}	0.763	±1.34	<.001	±0.70	<.001	±1.82	0.036	±2.11	<.001	±0.80
C_i	<.001	±7.66	<.001	±13.07	0.621	±4.59	0.003	±4.35	<.001	±10.44
g_s	0.025	±0.03	<.001	±0.05	0.016	±0.02	0.14	±0.05	0.004	±0.04
Fv'/Fm'	<.001	±0.01	<.001	±0.02	<.001	±0.01	<.001	±0.02	<.001	±0.01
qP	0.165	±0.01	<.001	±0.02	<.001	±0.02	<.001	±0.03	0.867	±0.01
ΦPSII	<.001	±0.01	<.001	±0.00	<.001	±0.00	<.001	±0.01	<.001	±0.00
ETR	<.001	±7.53	<.001	±11.34	<.001	±4.52	<.001	±11.34	0.002	±3.778
Transpiration rate	0.624	±0.49	<.001	±0.716	<.001	±0.29	<.001	±1.01	0.308	±0.67
VPD	<.001	±0.09	<.001	±0.15	0.004	±0.05	<.001	±0.05	0.004	±0.13
WUE_i	<.001	±4.56	<.001	±7.696	0.713	±2.73	<.001	±2.56	<.001	±6.22

Anova was carried out in order to compare the UK wheat lines with the Indian genotypes (groups) and also the comparison of the Indian genotypes (lines) against each other. Significant differences ($P<0.05$) were shown between the lines for all the photosynthetic parameters, which are in shaded boxes (table 5.1) While comparing the years within all groups, all the photosynthetic parameters showed significant difference but no significant difference could be observed in C_i and WUE_i when comparing both the years. P_{max} was significantly higher in the Indian genotypes for the 2016 in contrast to the year 2015. Between the lines or within the Indian genotypes there were significant differences observed in all the photosynthetic parameters.

Comparison of the UK wheat lines with the Indian genotypes for P_{max}, qP and transpiration rate showed comparatively fewer differences, however significant differences could be observed in C_i, g_s, Fv'/Fm', ΦPSII, ETR, VPD and WUE_i.

5.2.3: Correlation between P_{max} and other photosynthetic parametersfor the year 2015.

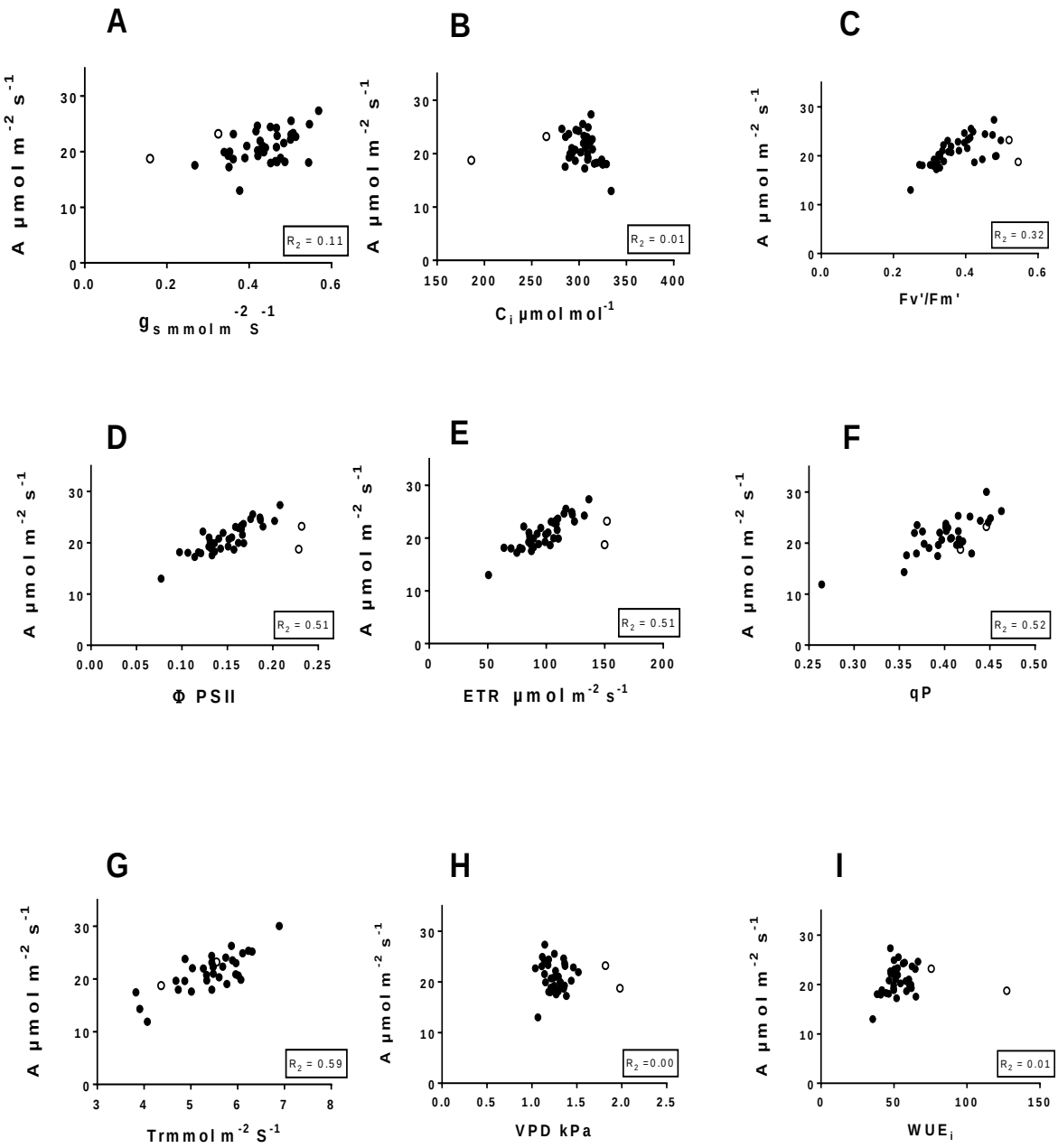


Figure 5.3: Relationshipbetween P_{max} and (A) stomatal conductance, (B) C_i (C) Fv'/Fm',(D) ΦPSII, (E) ETR, (F) qP,(G) Transpiration rate (H) VPD and (I) WUE_i, derived from the P_{max} measurements for the Indian genotypes for the year 2015. The black solid circles are Indian genotypes and the open circles are UK lines. The line denotes regression. Values are means of each parameter, (n=5).

Correlation analysis between P_{max} other photosynthetic parameters (figure 5.3 and table 5.2) reveals that P_{max} showed strong relationship with stomatal conductance (figure 5.3 A) and transpiration rate (figure 5.3 C). P_{max} significantly correlated with Fv'/Fm', ETR, qP and ΦPSII (figure 5.3 C, D, E & F). No significant relationship could be drawn between P_{max} with C_i, VPD and WUE_i.

Table 5.2: Correlation between P_{max} and other photosynthetic parameters of the Indian genotypes and UK wheat cv. for the experimental year 2015. Values are P values, shaded boxes indicate significant relationships (P<0.05). Here, the photosynthetic parameters for eg. P_{max}, C_i, g_s etc are plotted against each other to understand the relationship between them.

Year 2015	C_i	g_s	Fv'/Fm'	ΦPSII	qP	ETR	Transpiration	VPD	WUE_i
P_{max}	0.263	<.001	<.001	<.001	<.001	<.001	<.001	0.599	0.341
C_i		<.001	0.358	<.001	0.025	<.001	<.001	<.001	<.001
g_s			0.694	0.073	0.021	0.073	<.001	<.001	<.001
Fv'/Fm'				<.001	0.08	<.001	0.394	0.379	<.001
qP					<.001	<.001	0.011	0.426	<.001
Φ PSII						<.001	0.443	0.602	0.021
ETR							0.212	0.648	<.001
Transpiration rate								0.001	<.001
VPD									<.001

Correlation analysis for the year 2015 (table 5.2) shows that P_{max} was highly significant with stomatal conductance, ΦPSII, Fv'/Fm', qP, ETR, transpiration rate with a P <0.001, while C_i, VPD and WUE_i were not significant. Highly significant relationship could be observed between C_i and g_s, ΦPSII, ETR, transpiration rate, VPD,WUE_i (P <0.001) and qP (P value 0.025). Stomatal conductance showed significant relationship with transpiration rate, VPD, WUE_i(P <0.001) and qP (0.021). Fv'/Fm' showed highly significant relationship with ΦPSII, ETR and WUE_i.

5.2.4: Correlation between P_{max} and other photosynthetic parameters for the experimental years 2016

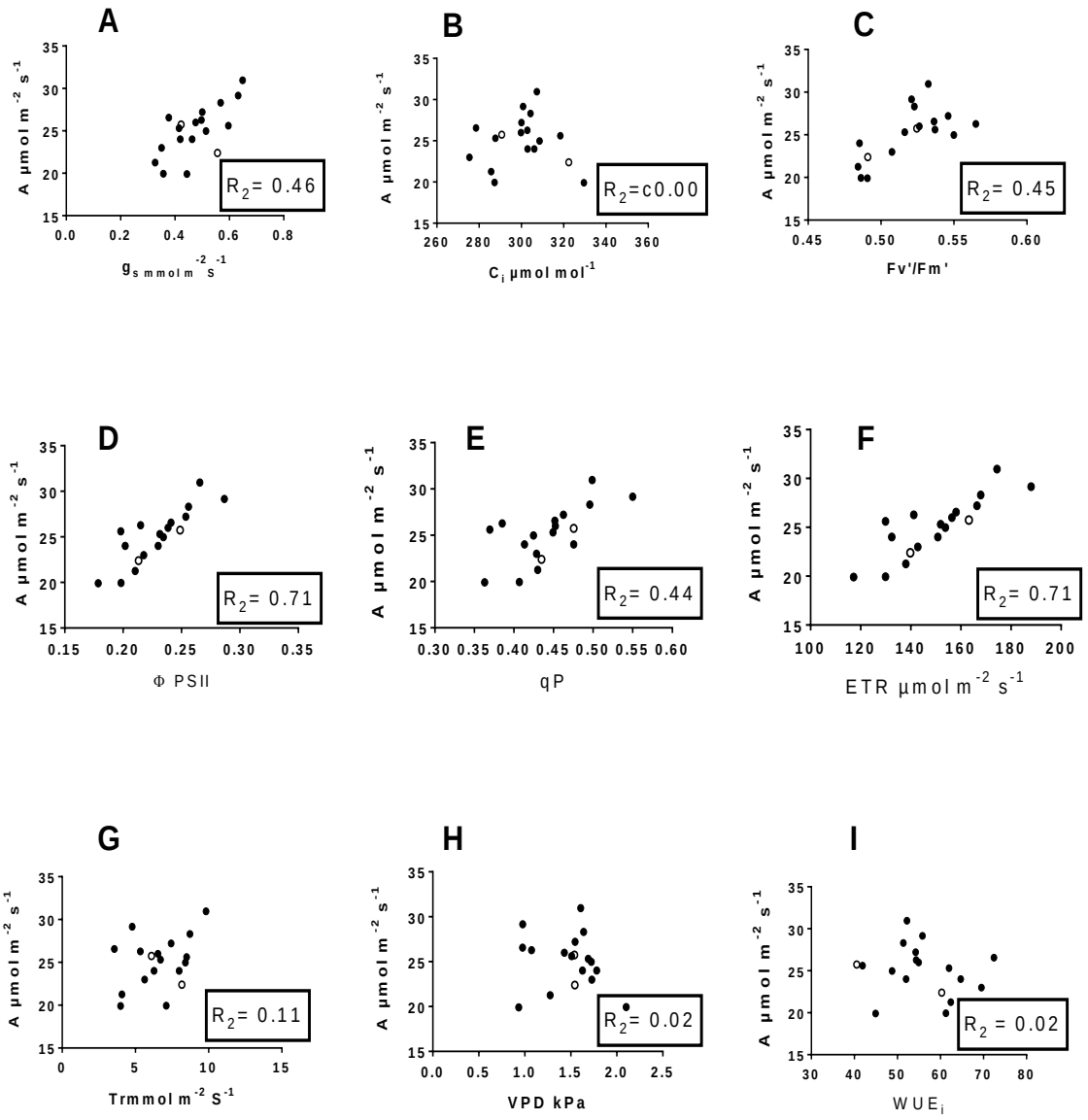


Figure 5.4: Relationship between P_{max} year 2016 (A) Stomatal conductance (B) intercellular CO_2 (C) ΦPSII (D) Fv'/Fm' (E) qP (F) ETR (G) Transpiration and (H) Vpdl and (I) WUE_i,derived from the P_{max} measurements for the Indian genotypes for the year 2016. The black solid circles are Indian genotypes and the open circles are UK lines. The line denotes regression. Values are means of each parameter, (n=5)

Correlation analysis between P_{max} other photosynthetic parameters (figure 5.4 and table 5.3) reveals that P_{max} showed a strong relationship with stomatal conductance (figure 5.4 A) and transpiration rate (figure 5.4 C). P_{max} significantly correlated with Fv'/Fm', ETR, qP and Φ PSII (figure 5.4 C, D, E & F). No significant relationship could be drawn between P_{max} with C_i, VPD and WUE_i.

Table 5.3: Correlation between P_{max} and all the photosynthetic parameters derived from the photosynthetic capacity measurements for the Indian genotypes for the experimental year 2016. Values are P values, shaded boxes indicate significant relationships ($P<0.05$). Similar relationship as mentioned in table 5.2 is observed here.

Year 2016	C_i	g_s	Fv'/Fm'	ΦPSII	qP	ETR	Transpiration	VPD	WUE_i
P_{max}	0.06	<.001	<.001	<.001	<.001	<.001	<.001	0.705	0.154
C_i		<.001	0.358	<.001	<.001	<.001	0.15	<.001	<.001
g_s			<.001	0.037	0.487	0.037	<.001	0.006	<.001
Fv'/Fm'				<.001	0.891	<.001	0.008	0.27	0.951
ΦPSII					<.001	<.001	0.011	0.426	0.001
qP						<.001	0.108	0.148	<.001
ETR							0.012	0.432	0.001
Transpiration								<.001	0.001
VPD									0.005

Correlation analysis for the year 2016 (table 5.3) shows that P_{max} was highly significant with stomatal conductance, ΦPSII, Fv'/Fm', qP, ETR, transpiration rate with a ($P<0.001$), no significant relationship could be observed in C_i, VPD and WUE_i. C_i showed highly significant relationship with g_s, ΦPSII, ETR, qP, VPD, WUE_i($P <0.001$). Stomatal conductance showed significant relationship with Fv'/Fm', transpiration rate and WUE_i ($P <0.001$) and the P values with ΦPSII (0.037), ETR (0.037) and VPD (0.006).Fv'/Fm' showed highly significant relationship with ΦPSII, ETR and transpiration rate (0.008).

5.2.5: AC_i curves

AC_i curve measurements were carried out during anthesis stage on the UK wheat cv. and Indian genotypes with high and low P_{max} and further analysed using curve fitting model (Sharkey et al., 2007) to calculate V_{cmax} and J_{max}. Here, P_{max} is the light saturated photosynthetic measurement taken at vegetative stage (GS39-GS45, Zadok's nomenclature).V_{cmax} is the maximum carboxylation velocity of Rubisco, J_{max} is the rate of maximum rate of electron transport, A_{max} is the maximum assimilation rate at 1000 μmol mol^{-1} of CO_2 derived from the AC_i curve and A_{400} is the assimilation rate at 400 μmol mol^{-1} of CO_2 derived from the AC_i curve measurements.

Table 5.4: Indian genotypes showing high and low P_{max} for the year 2015 and 2016.

2015 High P_{max}	**2016 High P_{max}**	**2015 Low P_{max}**	**2016 Low P_{max}**	**2016 Intermediate P_{max}**	**2016 Intermediate P_{max}**
KRL213	KRL213	Kharchia	Kharchia	MACS6222	GW322
NW4018	KRL210	HD2932	DBW51	RAJ4229	RW3684
DBW51	HW2044	HI1563	NW4018		
KRL210	PDW314	HW2044	Highbury		
RW3684	Raj4229	HD2967	HI1563		

Intermediate P_{max} values are the P_{max} values of those genotypes which are between high and low P_{max}. Also, considering the photosynthetic performance of these intermediate genotypes in field conditions in India (Experiment not included in the thesis due to time constraint)

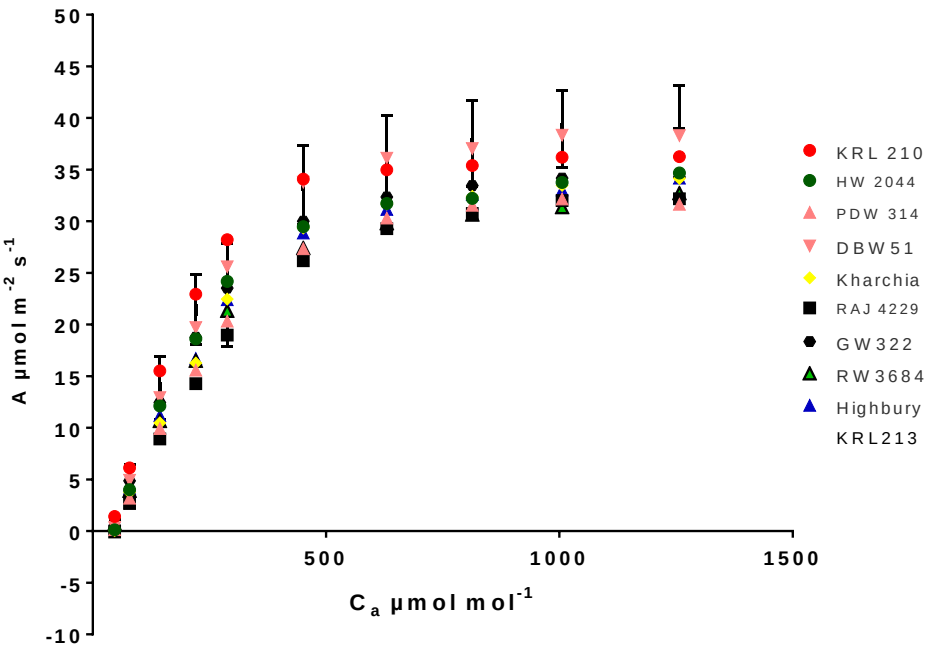


Figure 5.5: Example of variation in AC_a curves / CO_2 response curves of the Indian genotypes with higher and lower P_{max}, UK wheat cv. Paragon and Highbury for the experimental year 2016. The data is plotted as CO_2 assimilation rate A ($\mu molm^{-2}\ s^{-1}$), to increasing CO_2 concentration, the settings for CO_2 concentration are mentioned in Chapter 2. The cuvette conditions were 1500 PPFD and the block temperature set as 22 ° C, humidity was set to ~ 60 %. Values are means, the error bars are the standard errors of the means (n=3). Curve fitting was carried using CO_2 level 1000 ppm as the maximum assimilation rate.

Ranking of the Indian genotypes varied according to the experimental year for P_{max} measurements, therefore selection of the Indian genotypes demonstrating high and low P_{max} varies according to year. As a result there were more Indian genotypes for the AC_i curve measurements in the year 2016 in contrast to year 2015.

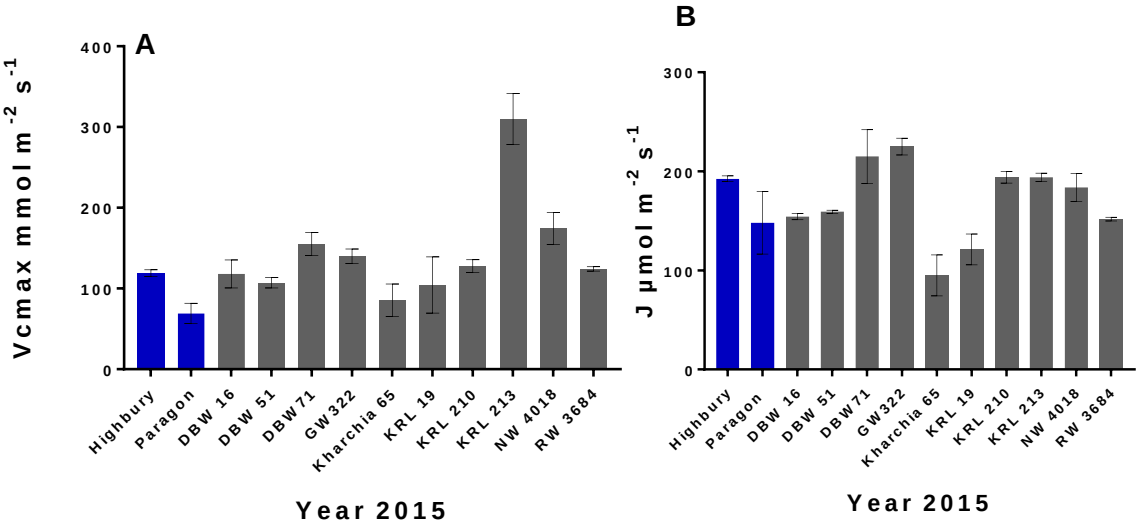


Figure 5.6: Mean of V_{cmax} and J_{max} derived from curve fitting (sharkey et al., 2009) of the Indian genotypes with higher and lower P_{max} along with UK wheat cv. Paragon and Highbury for the experimental year 2015.

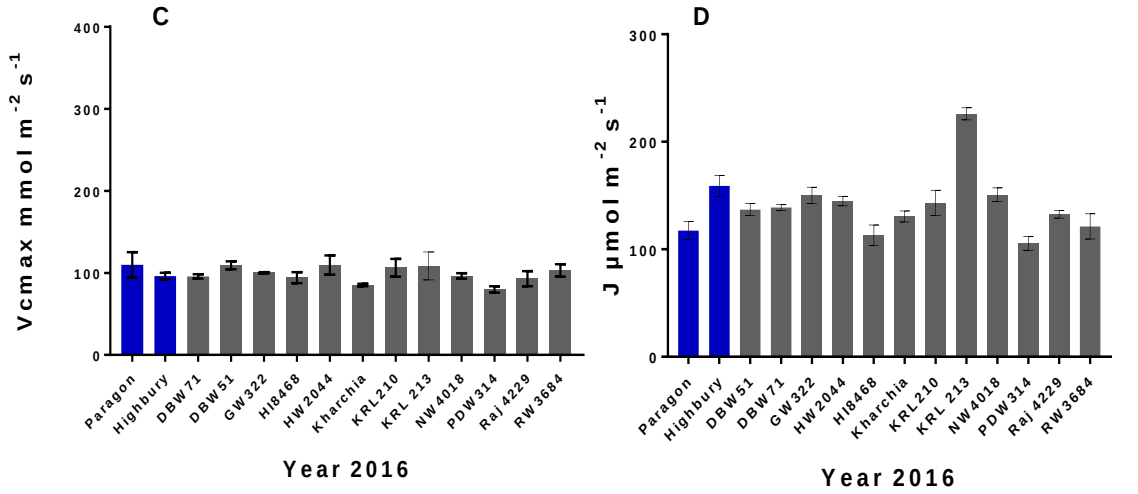


Figure 5.7: Mean of V_{cmax} and Jmaxderived from curve fitting (sharkey et al., 2009) of the Indian genotypes with high and low P_{max} along with UK wheat cv. Paragon and Highbury for the experimental year 2016.

Table 5.5 and figure 5.6-5.7 show the results of ACi analysis. $V_{cmax}$was highest in the Indian genotype KRL213 ~300 µmolm^{-2} s^{-1} and in most of the Indian genotypes V_{cmax} was higher than the UK wheat in the year 2015 (figure 5.6) and (table 5.5). J_{max} was higher in the Indian genotypes GW322 & DBW 71 ~200 µmolm^{-2} s^{-1} and lowest in Kharchia at 95 µmolm^{-2} s^{-1}.Contrastingly the V_{cmax} values for both the UK genotypes and Indian genotypes in the year 2016 (figure

5.7) were ~100 µmol m^{-2} s^{-1}. J_{max} was highest in the Indian genotype KRL213 ~226 µmol m^{-2} s^{-1} for the year 2016, other Indian genotypes and UK wheat were in the range (~100-150 µmol m^{-2} s^{-1}) for the year 2016. Therefore, there was a decrease in rubisco carboxylation efficiency and electron transport capacity for the year 2016 which seems inconsistent with P_{max} data. This could be due to developmental effects (growth stage) or environmental conditions at the time of measurement (increased temperature).

Table 5.5: Analysis of variance of AC_i curve measurements for the experimental year 2015 and 2016. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Group refers to differences between UK lines and Indian genotypes and lines refer to differences among Indian genotypes.

5.5

Year 2015	**P_{max}**	**SEM**	**J_{max}**	**SEM**	**V_{cmax}**	**SEM**	**A_{400}**	**SEM**	**A_{max}**	**SEM**
Group	0.817	±2.315	0.001	±15.43	0.497	±12.25	0.037	±31.28	<0.001	±5.035
Lines	0.036	±4.227	<0.001	±28.18	<0.001	±22.37	<0.001	±57.11	<0.001	±9.193
Year 2016										
Group	0.912	±1.94	0.497	± 7.18	0.586	±8.21	0.677	± 1.25	0.498	±1.47
Lines	0.021	±3.59	<0.001	±13.30	0.721	±15.21	0.194	±2.32	<0.001	±2.73

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Anova were carried out, the factors being the photosynthetic parameters from AC_i curve measurements in table 5.5. Here, P_{max} is the light saturated photosynthetic capacity at 400 µmol mol^{-1} CO_2 (from pre anthesis stage) and A_{max} is the maximum assimilation rate at 1000 µmol mol^{-1} of CO_2 and A_{400} is the assimilation rate at 400 µmol mol^{-1} of CO_2 derived from the AC_i curve measurements.

Significance varied according to year with a higher level of significance both between groups and lines observed for 2015 compared to 2016. In particular there was a high level of significance between the lines (Indian genotypes) for P_{max}, J_{max} and A_{max} for both years. In 2015 there was significance for V_{cmax}, J_{max}, A_{400} and A_{max}, (P value: <0.001). In terms of groups, no parameter was significantly different in 2016 but 2015 saw differences in V_{cmax}, J_{max}, and A_{max}.

5.2.6: Correlation between P_{max} and other photosynthetic parameters for the experimental year 2015.

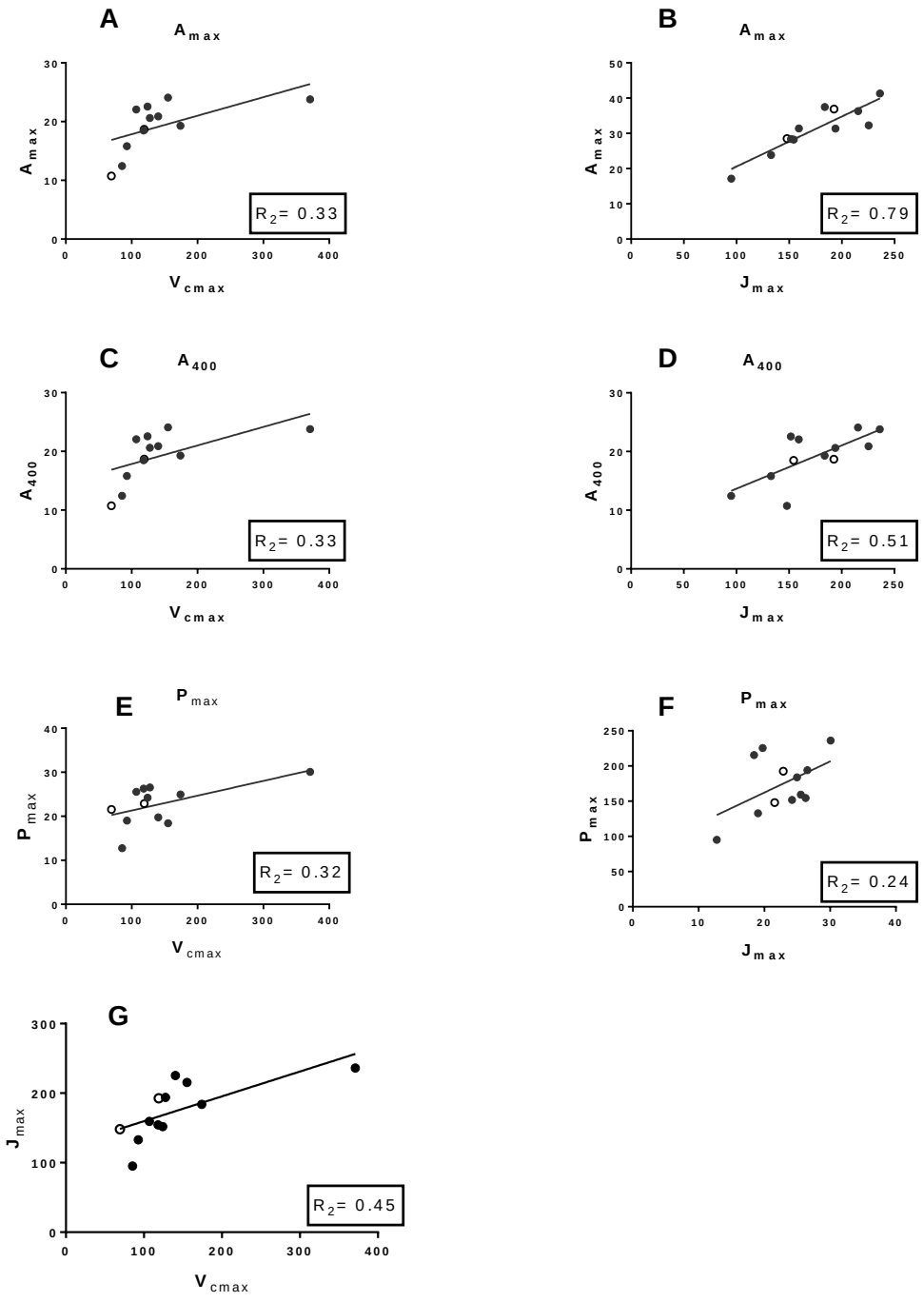


Figure5.8: Relationships between V_{cmax} with (A) A_{max} (B) A_{400} (E) P_{max}& (G) $J_{max.}$ (B) A_{max} (D) A_{400} and (F) P_{max} with J_{max}. The blue solid circles represent UK Wheat and black solid circles represent Indian genotypes. The statistical analysis of the correlation is described in table 5.5A. Values are the means of each individual genotype.

5.2.7: Correlation between P_{max} and other photosynthetic parameters for the experimental years 2016**.**

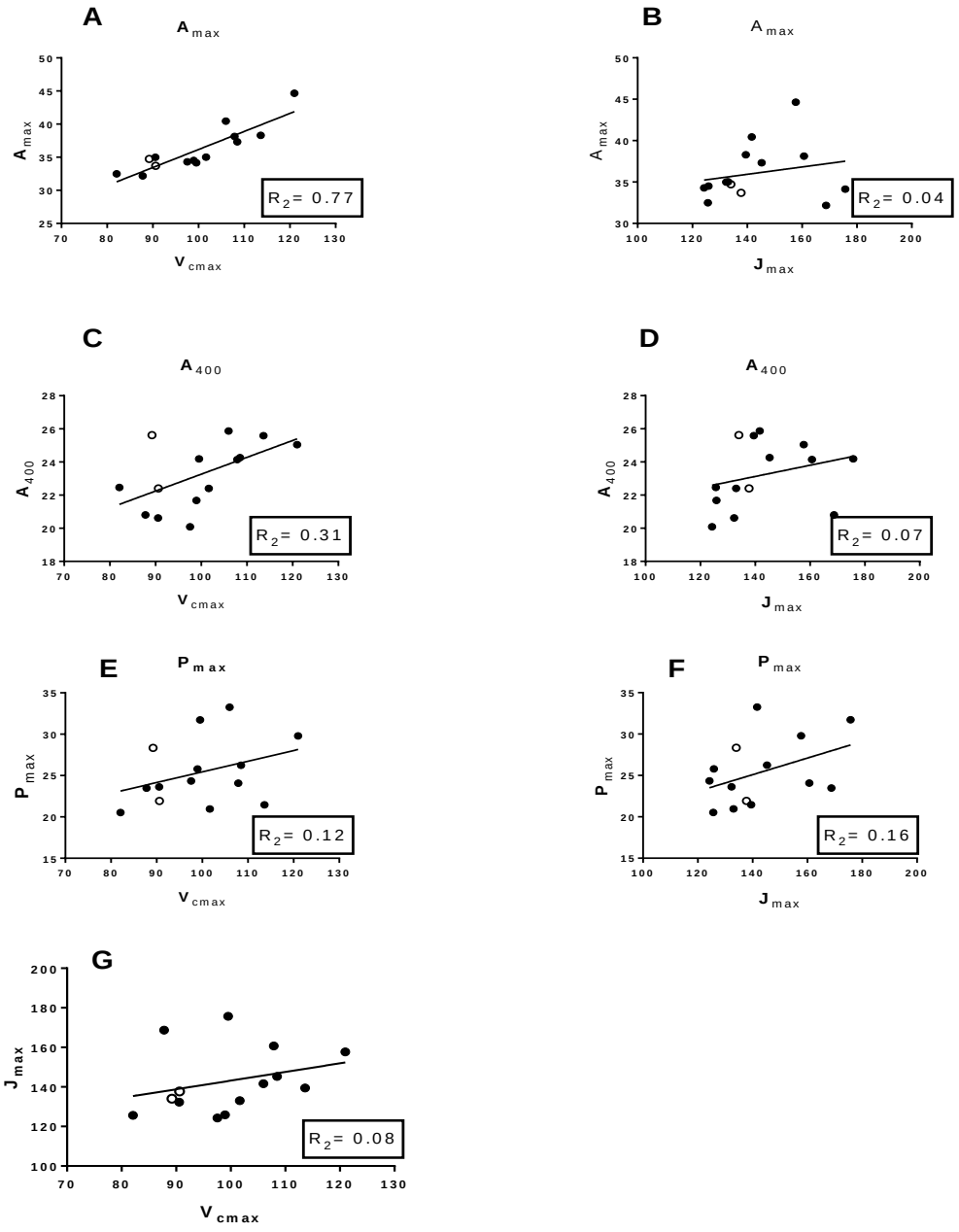


Figure5.9: Relationships between V_{cmax} with (A) A_{max} (B) A_{400} (E) Pmax & (G) $J_{max.}$ (B) A_{max} (D) A_{400} (F) P_{max} with J_{max}. The open circles represent UK Wheat lines and black solid circles represent Indian genotypes. The statistical analysis of the correlation is described in table 5.5B. Values are the means of each individual genotypes.

Table 5.6 A and B: Correlation of AC_i curve measurements for the experimental year 2015 and 2016. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Here, AC_i curve parameters J_{max}, A_{max}, A_{400} and P_{max} from the spot measurements taken at vegetative stage (GS39-45) were compared against each other for the year 2015 and 2016.

Table 5.6A

Year 2015	Jmax	A_{400}	A_{max}	P_{max}
V_{cmax}	<.001	0.002	0.147	0.096
J_{max}		0.029	<.001	0.135
A_{400}			<.001	0.02
A_{max}				<.001

Table 5.6B

Year 2016	J_{max}	A_{400}	A_{max}	P_{max}
V_{cmax}	0.012	0.002	<.001	0.788
J_{max}		0.029	0.092	0.155
A_{400}			0.002	0.022
A_{max}				0.076

Correlation analysis was carried out between the photosynthetic parameters derived from AC_i curve measurements from both the experimental years and is shown in figure 5.9 and table 5.6A (year 2015) and 5.6 B (year 2016).

First it is clear that the correlations between pre-anthesis P_{max} and other parameters were weak for both years. For the year 2015, V_{cmax} showed significant relationship with J_{max} (P value :< 0.001), and A_{400} (P value: 0.002), no relationship could be drawn between V_{cmax} and A_{max}, P_{max}. J_{max} was highly significant with A_{max} (<0.001) and A_{400} (0.029). A_{400} showed significant relationship with A_{max} (0.002) and P_{max} (0.022).

5.2.8: Postharvest measurements

Plant measurements such as plant height, length and width of flag leaf were carried out before harvest. The Indian genotypes were harvested after completion of all other measurements and post-harvest analysis (biomass and grain weight) were then carried out.

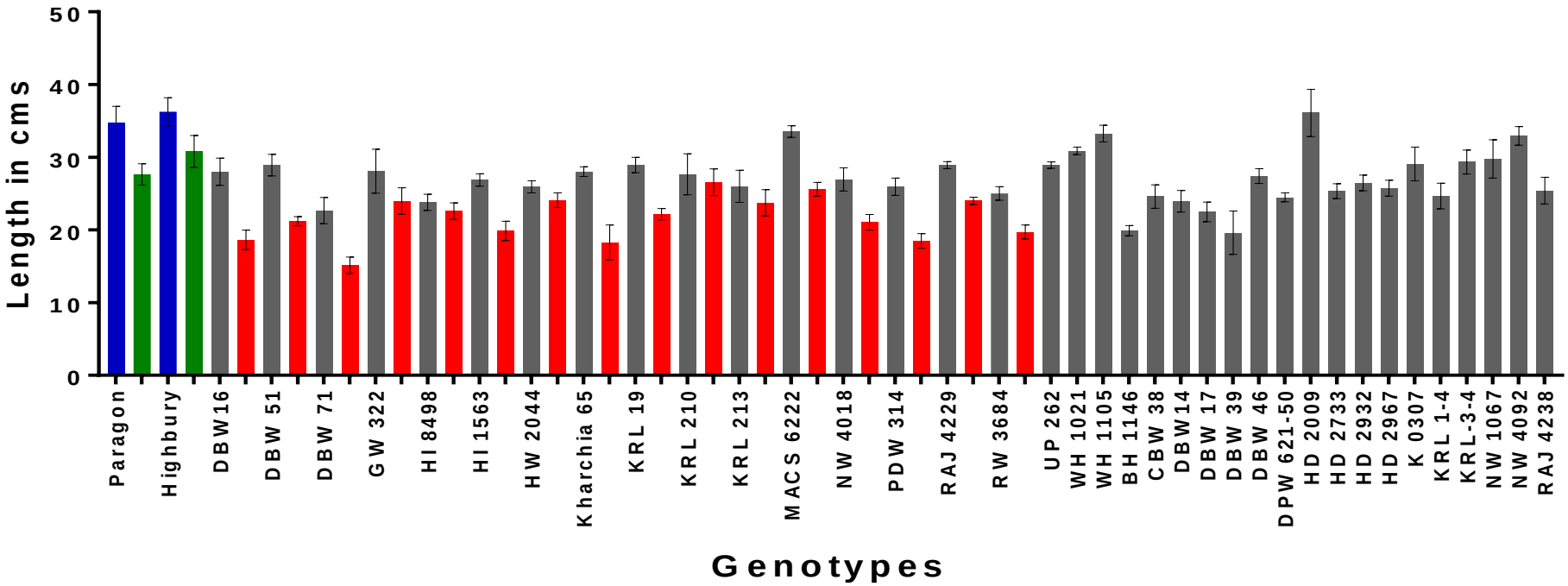


Figure 5.10: Mean leaf length for both the experimental years, of the Indian genotypes represented in grey bars (2015) and red bars (2016) and the UK wheat Highbury and paragon represented in blue bars (2015) & green bars (2016)). Values are means, error bars are the standard errors of the means, (n=5).

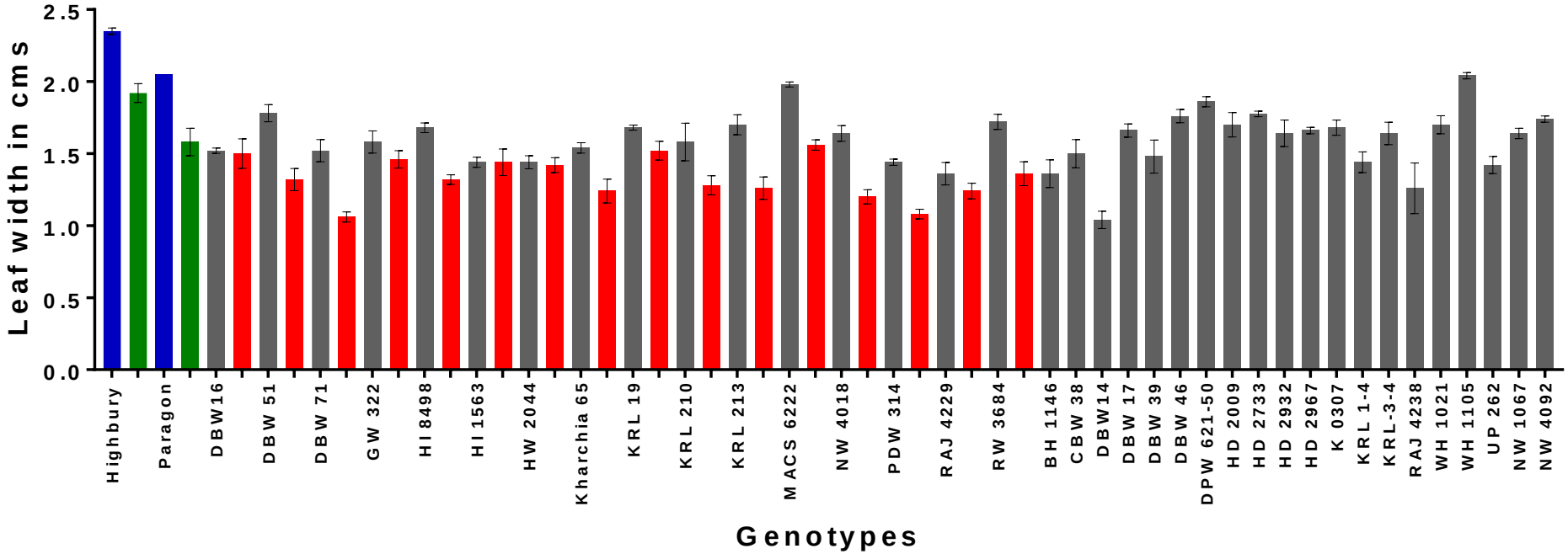


Figure 5.11: Mean leaf width for both the experimental years, of the Indian genotypes represented in grey bars (2015) and red bars (2016) and the UK wheat Highbury and paragon represented in blue bars (2015) & green bars (2016)). Values are means, error bars are the standard errors of the means, (n=5).

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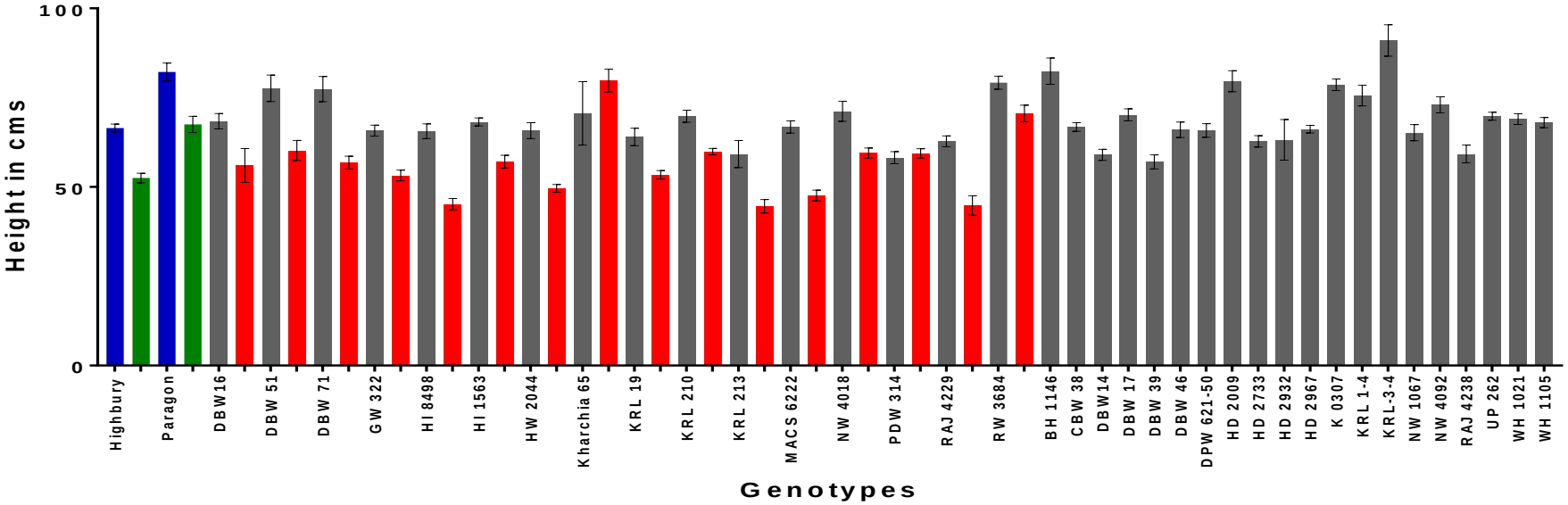


Figure 5.12: Mean plant height for both the experimental years, of the Indian genotypes represented in grey bars (2015) and red bars (2016) and the UK wheat Highbury and paragon represented in blue bars (2015) & green bars (2016)). Values are means, error bars are the standard errors of the means, (n=5).

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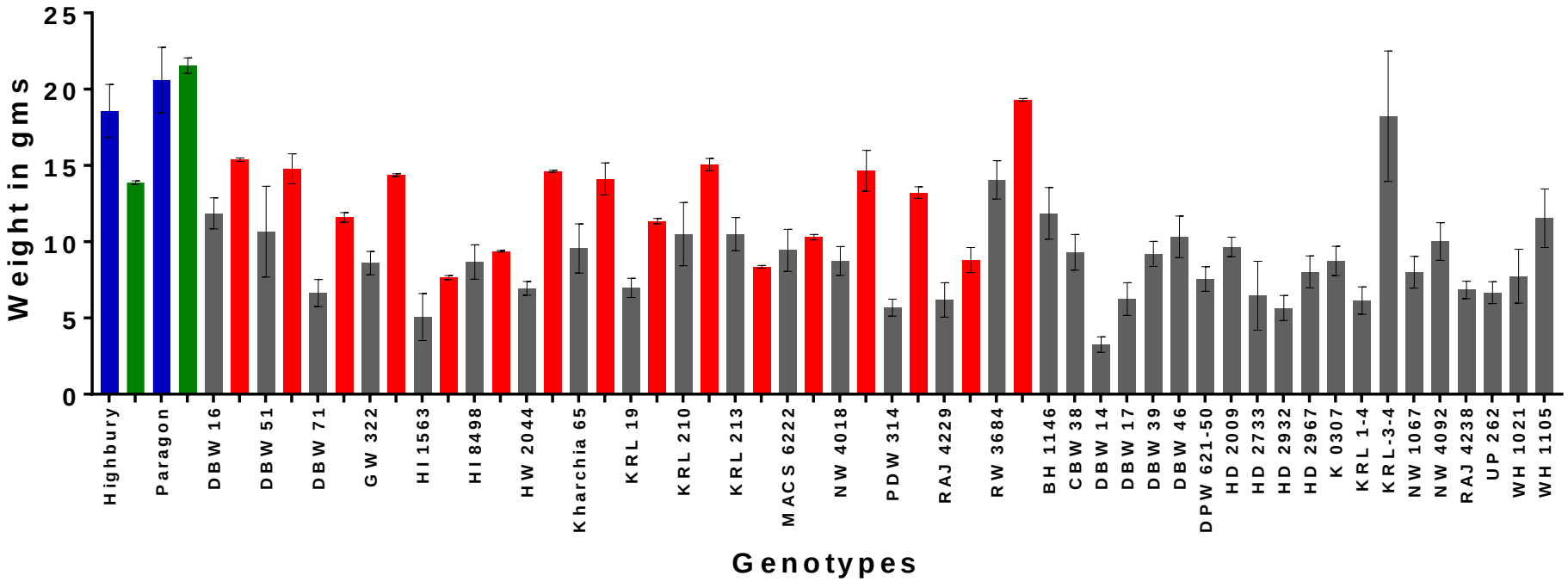


Figure 5.13: Mean of biomass (weight in gms) of the Indian genotypes represented in grey bars (2015) and red bars (2016) and the UK wheat cv. Highbury and Paragon represented in blue bars (2015) & green bars (2016). Values are means, error bars are the standard errors of the means, (n=5).

Figure 5.10 shows flag leaf length of the UK wheat cv. for the year 2015, which was longer than the Indian genotypes, a large variation could be observed in the length of leaves of the Indian genotypes for the year 2015. However, there was a decrease in the length of the leaves of the Indian genotypes for the year 2016. Figure 5.10 shows that Highbury was observed to have wider leaves (~ 2 cm) than the Indian genotypes for both the year, width of the leaves decreased in the year 2016 for the Indian genotypes. Leaf morphology can alter upon the availability of light James and Bell (1999), which could be one of the reasons for increase and decrease width of leaves. Plant height (Figure 5.11) of most of the Indian genotypes and the UK wheat cv. were in the range of 60-80 cms for the year 2015, but there was a decrease in height in most of the Indian genotype (45 cms) and UK wheat cv. (50-70 cms) for the year 2016. Kharchia was the taller variety among the Indian genotypes. Biomass (Figure 5.12) of the UK wheat cv. was significantly higher than the Indian genotype for the year 2015. There was a significant increase in the biomass of the Indian genotypes for the year 2016.

Table 5.7A & B: Analysis of variance of the postharvest elements of the Indian genotypes for the year 2015 and 2016.Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Here we observe the interaction of the groups and lines for year 2015 (5.6 A) and 2016 (5.6B). Here, for the year 2015 the number genotypes were 36 Indian genotypes and for the year 2016 the numbers of Indian genotypes were 16, therefore comparison between the years were not done.

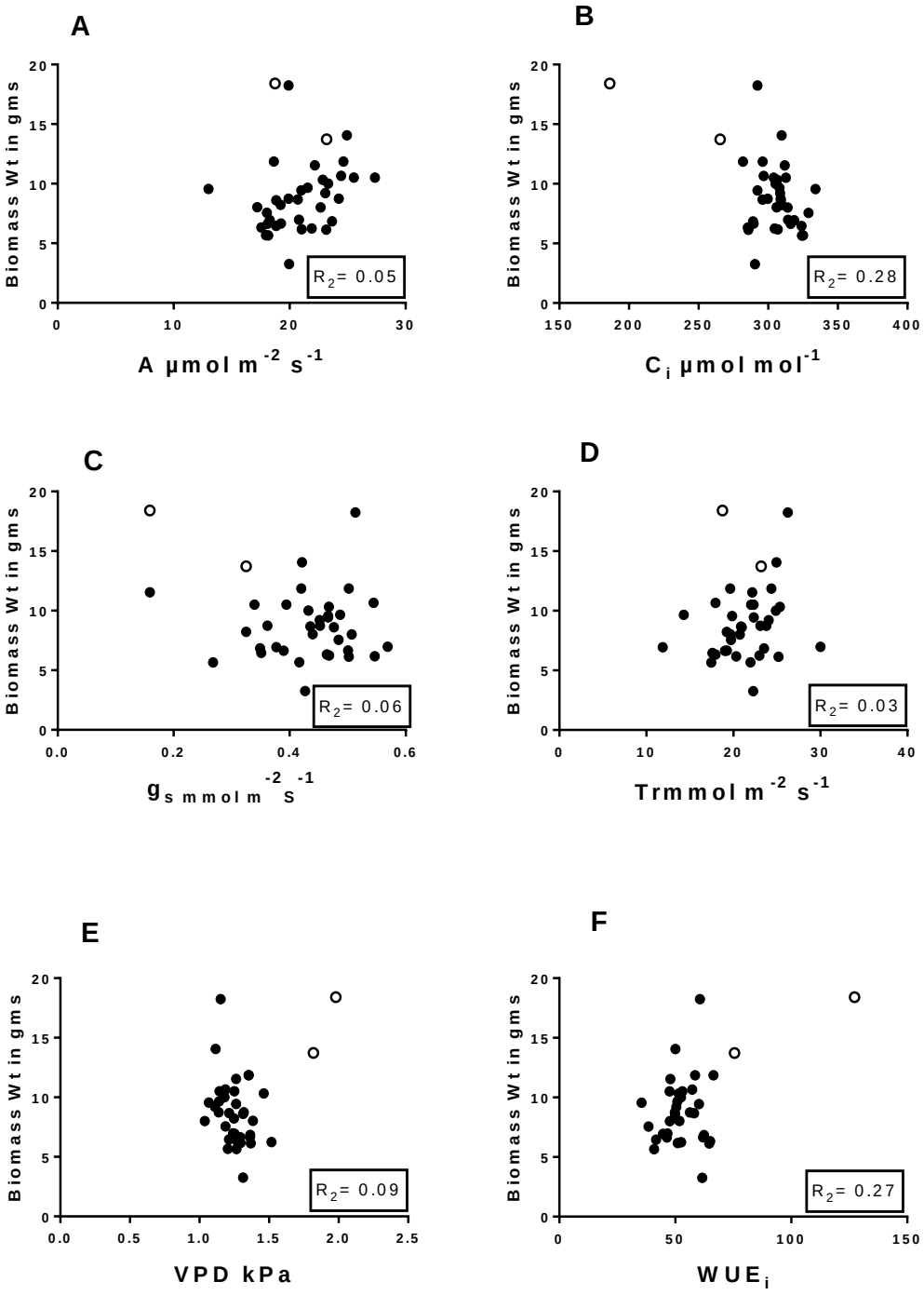
5.7A

Year 2015	Biomass	SED	Plant height	SED	Leaf length	SED	Leaf width	SED
Groups	<.001	±9.03	<.001	±68.51	<.001	±27.45	0.107	±1.60
Lines	<.001	±0.99	<.001	±3.53	<.001	±1.32	<.001	±0.06

5.7B

Year 2016	Biomass	SED	Plant height	SED	Leaf length	SED	Leaf width	SED
Groups	<.001	±0.83	0.145	±1.69	<.001	±1.17	<.001	± 0.05
Lines	<.001	±1.56	<.001	±3.19	<.001	±2.22	<.001	±0.10

ANOVA (table 5.7 A & B) was carried out for the morphology data and the post-harvest data (table 5.7A and table 5.7B). For the year 2015, when the UK wheat was compared with the Indian genotypes significant variation could be observed seen in biomass, plant height and leaf length ($P<0.001$). Within the Indian genotypes significant variation could be observed in both the years for all the postharvest elements, biomass, plant height, leaf length and leaf width ($P <0.001$). Significant variation could be observed in the year 2016 in biomass, leaf length and leaf width ($P<0.001$).


A
Biomass Wt in gms
R_2= 0.05
A µmol m^{-2} s^{-1}
B
Biomass Wt in gms
R_2= 0.28
C_i µmol mol^{-1}
C
Biomass Wt in gms
R_2= 0.06
g_s mmol m^{-2} s^{-1}
D
Biomass Wt in gms
R_2= 0.03
Tr mmol m^{-2} s^{-1}
E
Biomass Wt in gms
R_2= 0.09
VPD kPa
F
Biomass Wt in gms
R_2= 0.27
WUE_i

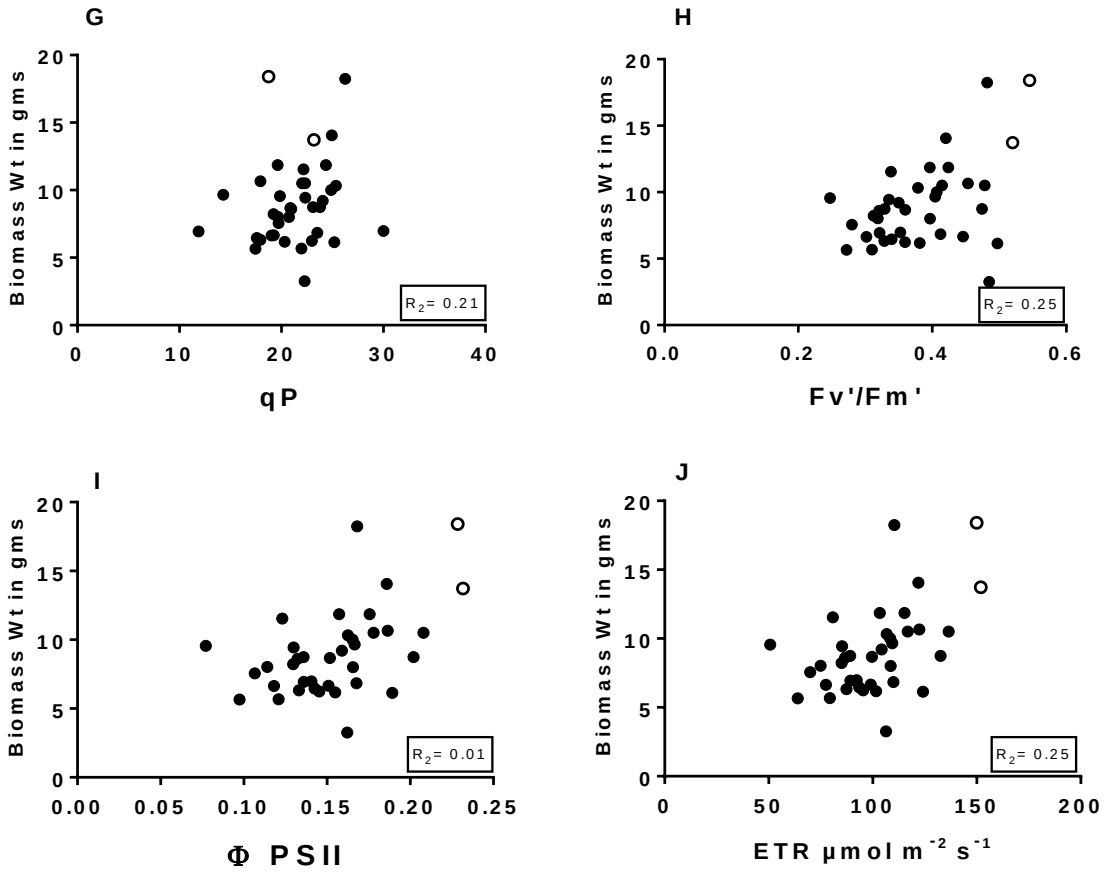


Figure 5.14: Relationship of biomass (weight in grams) with (A) P_{max} (B) C_i (C) g_s (D) Transpiration rate(E) VPD (F) WUE_i (G) qP (H)Fv'/Fm'(I)ΦPSIIand(J) ETR of the Indian genotypes for the year 2015. Indian genotypes are represented in black solid circles and UK wheat lines in open circle. Values are means of each parameter, mean (n= 5).

Figure 5.14 plots biomass against all the photosynthetic parameters. Biomass showed significant relationship with P_{max}& C_i, Fv'/Fm', ΦPSII, ETR and WUE_i ($P<0.01$).Relationship of rest of the plant morphological parameters such plant height,leaf width and leaf length are shown in table no. 5.8.

Table 5.8: Correlation of biomass, plant height, leaf length and leaf width with all the photosynthetic parameters derived from the light saturated photosynthetic measurements for the experimental year 2015. Values shown are the P values, shaded boxes indicate significant relationships (P<0.05). Here, all the photosynthetic parameters are compared with biomass and plant morphology.

Year 2015	**P_{max}**	**C_i**	**g_s**	**Fv'/Fm'**	**ΦPSII**	**qP**	**ETR**	**Trans. rate**	**VPD**	**WUE_i**
Biomass	0.01	<.001	0.439	<.001	<.001	0.038	<.001	0.616	0.699	<.001
Plant height	0.915	0.934	0.83	0.118	0.27	0.737	0.268	0.147	0.039	0.851
Leaf length	0.353	0.247	0.405	0.36	0.344	0.941	0.348	0.06	0.463	0.262
Leaf width	0.009	0.051	<.001	0.308	0.879	0.173	0.882	0.003	0.275	0.04

Biomass showed significant relationship with C_i, Fv'/Fm', PSII, ETR and WUE_i ($P < 0.001$), P_{max} ($P<0.01$), no correlation could be drawn between g_s, transpiration rate and VPD, plant height showed significant correlation with VPD ($P<0.05$). Leaf width showed significant relationship with g_s ($P<0.01$), P_{max}, C_i, transpiration rate and WUE_i ($P<0.05$).No relationship could be seen between leaf length and any of the photosynthetic parameters (biomass, leaf width, plant height).

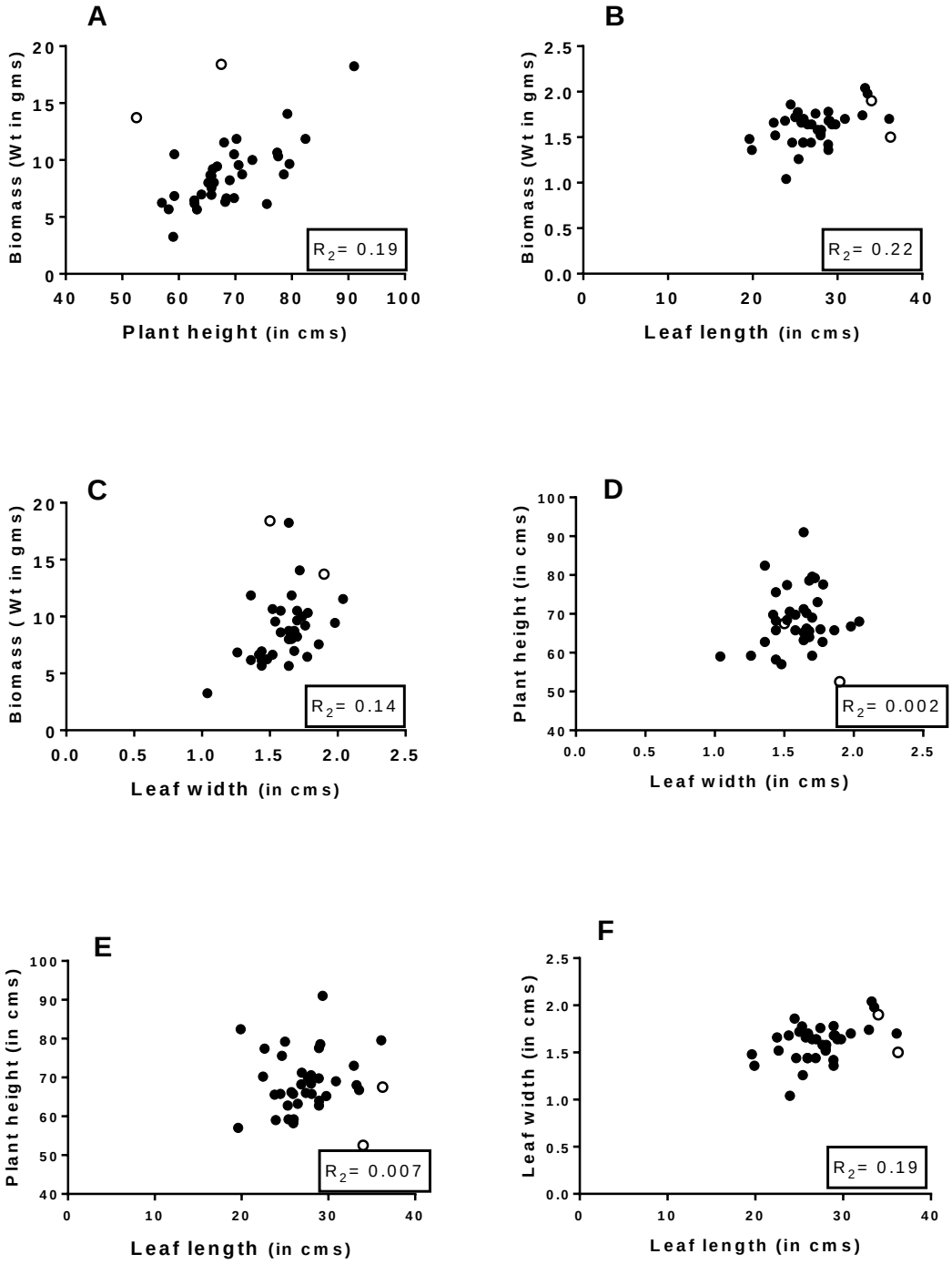


Figure 5.15: Relationship of Biomass with (A) Plant Height (B) Length of leaves (C) Leaf width, Plant height with (D) Leaf width and (E) Leaf length & (F) leaf length with leaf width of the Indian genotypes for the year 2015. Indian genotypes are represented in black solid circles and the UK wheat lines in open circle.Values are means of each parameter, mean (n= 5).

For the year 2015, Biomass was plotted against plant height, leaf length and leaf width, plant height against leaf width and leaf length and leaf width with leaf length (figure 5.15). Biomass showed strong relationship with plant height, leaf length and leaf width. Plant showed significant relationship with leaf length and leaf width, leaf length showed significant relationship with leaf width. No correlation was observed in these parameters for the year 2016 (table 5.9). Biomass for the year 2016 did not show any relationship with any of the photosynthetic parameters except for transpiration rate (data and figure not shown).

Table 5.9: Correlation of the postharvest elements of the Indian genotypes for the year 2015. Here, Biomass, plant height, leaf length and leaf width were compared against each other.

Year 2015	Plant Height	Leaf length	Leaf width
Biomass	<.001	<.001	<.001
Plant height		<.001	<.001
Leaf length			<.001

Correlation analysis for the year reveals that biomass showed significant relationship with plant height, leaf length, leaf width ($P < 0.001$) and P_{max} ($P<0.01$). Plant height showed significant correlation leaf length and leaf width ($P<0.001$). Leaf width showed significant relationship with leaf length ($P<0.001$),

5.3 Discussion and conclusion

This chapter highlights the natural variation in leaf photosynthesis in a panel 36 Indian genotypes, here the UK wheat Highbury and Paragon were used as check lines. It was hypothesised that the Indian genotypes will exhibit variation in photosynthesis under extended photoperiod in glasshouse conditions. (Figure 3.2, P_{max}) confirms the hypothesis that the Indian genotypes exhibit variation in leaf photosynthesis in glasshouse conditions though it needs to be considered that glasshouse conditions are usually favourable conditions. This could be due to acclimation of these Indian genotypes to higher irradiation and higher temperature conditions, so they are robust to various environmental conditions in India. Normally, India experiences tropical climatic conditions and during wheat growing season there is a rise in temperature, which can be detrimental to the plants. However, over a period of time, these Indian genotypes derived from different wheat growing zones of India have shown tolerance to moderately high light and temperature conditions, Rane and Nagarajan (2004).

Performance of the Indian genotypes was comparatively better than the UK wheat Highbury and Paragon. The P_{max} measurements of the Indian genotypes were higher than the UK wheat Highbury and Paragon for both the experimental years. Most the Indian genotypes for the experimental year 2016 showed higher P_{max} value than the experimental year 2015. However, Kharchia 65 had lower P_{max} values for both the years. The year of introduction of the Indian genotypes are listed in Chapter 2 (Kharchia 65 is the parent of KRL210 and KRL213), for eg: Kharchia was released or introduced in 1985, but KRL210 and KRL213 were released in 2010, a difference of ~25 years between them. Lower P_{max} value in Kharchia suggests that older varieties might have

decreasedphotosynthetic efficiency (needs to be investigated in detail). Therefore, the variation in most of the photosynthetic parameters might have some relationship with the year of introduction of the Indian genotypes.

Higher P_{max} values of the Indian genotypes were observed in the year 2016 (Figure 5.2), could be due to high light conditions as these Indian genotypes have already acclimated to high light conditions, perhaps they thrive in moderately high light conditions, as the growing period varied between both the years (2015 and 2016) (see chapter 2). Stomatal conductance of the Indian genotypes were comparatively higher in the year 2016 probably the stomata response to high light conditions, however, the UK wheat showed very low value Paragon (0.01 mmol m^{-2} s^{-1}) and Highbury (0.3 mmol m^{-2} s^{-1}) for the year 2015 but in the year 2016 Highbury showed higher stomatal conductance than Paragon. This higher value in the Indian genotype probably enhanced the diffusion of CO_2 and hence higher intercellular CO_2 (C_i) in both the year whilst the UK wheat demonstrated lower C_i values for the year 2015(Figure 5.2, C_i). Higher stomatal conductance resulted in higher transpiration rates in the Indian genotypes for the year 2016 (Figure 5.3, transpiration rate). A rise in qP could also be observed in the Indian genotypes for the year 2016, this could be due to higher light conditions as mentioned above in the discussion that this could be probably due to the growing period i.e. in Summer, resulting in higher qP in Indian genotypes such as HW2044, KRL213 and KRL 210 (Figure 5.2, qP). The quantum yield of PSII was observed to be significantly higher in the Indian genotypes for the year 2016 than year 2015; however the UK genotypes were consistent for both the years. Higher values of Fv'/Fm' in the year 2016 for the Indian genotypes HW2044, KRL213, KRL210 and PDW314 (Figure 5.2, Fv'/Fm') indicates that the plants were experiencing some kind stress conditions due to

light or any other environmental factor. Higher light conditions in the year 2016 also influenced the electron transport in the Indian genotypes as well as the UK wheat (Figure 5.2, ETR). All the Indian genotypes demonstrated low VPD compare to the UK wheat cultivars (Figure 5.3 H and 5.4 H). There is a negative or no relationship between VPD and the rate of photosynthesis and this has demonstrated by Shirkhe and Patare (2004), which suggests that wheat cultivars with low VPD are more beneficial. Lower value of WUE_i in the Indian genotypes for both the year (Figure 5.3 I, WUE_i) indicates that the plants were in well-watered conditions, however a higher WUE_i values in Paragon for the year 2015 can be understood, as the soil medium of the UK wheat were of different composition than the Indian genotypes soil type. As discussed in Chapter 3, all these Indian genotypes and UK wheat cv. were grown in glasshouse conditions which were unchallenging as compare to the field conditions in India. Therefore, if these genotypes were grown under challenging environmental conditions and then the screening them for various physiological traits would prove to be more logical. However, the glasshouse experimental results cannot be ruled out, as these experiments are to be completed within a set timeline.

Interesting or promising genotypes were investigated for detailed photosynthetic measurements such as AC_i curves, to assess the Rubisco catalytic efficiency in the genotypes that demonstrated high P_{max}, low P_{max} and genotypes with intermediate P_{max}. KRL213 demonstrated higher V_{cmax} than the Indian genotypes and UK wheat (Figure 5.6), however there was a reduction in V_{cmax} for the year 2016 the possible reason high light levels could have limited Rubisco catalytic efficiency. J_{max} for the year 2015 was higher in few other Indian genotypes (DBW71, GW322) than the UK wheat cv (Figure 5.6) and for the year 2016

KRL213 showed higher Jmax than the UK wheat cv (Figure 5.7). Nothing significant could be observed in the plant morphological characteristics, however a rise in biomass could be observed in the Indian genotypes for the year 2016, but the UK wheat had higher biomass than the Indian genotypes for both years; this could be due to their adaptation to the UK climatic conditions and greater absorption of nutrients from the soil. Thus, it can be concluded that hypothesis 2 was carefully tested and the entire hypothesis were proved to be correct. One of the highlight of this experiment was that biomass had significant relationship with most of the photosynthetic parameters derived from the 'spot' or instantaneous measurements (table 5.8) showing that it is an important trait across the diverse Indian germplasm and a fundamental trait for further improvement.

Biomass also showed strong relationship with plant height, leaf width and leaf length (Figure 5.15 and Table 5.9). Overall the Indian genotypes demonstrated a higher rate of photosynthesis than the UK and among them KRL213, KRL210 and HW2044 were the genotypes that demonstrated higher photosynthetic in the year 2016 (Figure 5.2). Such photosynthetic characteristics of these Indian genotypes could be future breeding materials which can be used to generate interspecific hybrids with UK wheat cultivars and can produce resilient wheat varieties.

With changing climatic conditions and deteriorating soil conditions globally or specifically in India, it is imperative to generate wheat cultivars that can sustain such hostile soil conditions and at the same time can exhibit greater photosynthetic efficiency. The outcome of this experiment was identifying Indian

wheat cultivars KRL213 and KRL210 which exhibit the property being salt tolerant and have also demonstrated high photosynthesis.

Conclusion

- Indian genotypes demonstrated variation in leaf photosynthesis in the glasshouse conditions coping with the extended photoperiod as opposed to the Indian climatic conditions.
- P_{max} demonstrated strong relationship with stomatal conductance, Fv'/Fm', ΦPSII, qP, ETR and Transpiration rate.
- Plant biomass showed strong relationship with P_{max}, C_i, Fv'/Fm', ΦPSII, ETR and WUE_i.

Chapter 6 General discussion and conclusion

Overall aim of this project work was to explore natural variation in leaf photosynthesis and other related physiological traits in wheat introgressed lines (amphidiploids) and a panel of 36 Indian genotypes which is discussed here to give a final outcome of this study in a broader perspective.

The primary of aim of this study was to explore variation in leaf photosynthesis in a population of amphidiploid (*T. aestivum* background) in glasshouse conditions and amphidiploids (durum wheat background) in field conditions (India). Along with this, Indian genotypes were also tested for natural variation in photosynthesis in glasshouse conditions and field conditions with minor variation in the number of genotypes between glasshouse and field conditions. In the present study it was found that the amphidiploid in the field conditions demonstrated higher photosynthetic rate than the amphidiploids in field conditions. Although, the Indian genotypes were not involved in the introgression studies, but were investigated to understand the natural variation in leaf photosynthesis and other related physiological traits.

6.1 Introduction

The present food production is not sufficient enough to satiate global food demand and with increasing world population which is expected to rise ~9 billion in the next 40 years, global food security has gained more attention (Tilman et al., 2011). And changing climatic conditions has put major concerns towards agricultural outputs (Lieper et al., 2014).Yield of crops like wheat and rice have been declining with adverse climatic conditions and deteriorating soil conditions. Therefore, there is an urgent need for crop improvement in order to meet global

food demand (Tscharntke et al., 2012). In developing countries like India where the staple food crop is rice and wheat and with increasing population there is even more food demand in coming years (Bhalla et al., 1999). Although, India is second largest producer of wheat, with deteriorating climatic conditions a decline in wheat production has been observed in many wheat growing regions of India (Rao et al., 2014) and hence crop improvement has gained a major attention.

*Is wheat ancestral introgression an effective breeding method?*At the University of Nottingham, amphidiploids were generated by wide cross between wild relatives of wheat and bread wheat. The results obtained from the amphidiploid experiment, described in Chapter 3, was not substantial enough to pinpoint lines (amphidiploids) that demonstrated higher photosynthesis or improved photosynthetic efficiency than the bread wheat or wild wheat. It was expected that the some of the amphidiploid could outperform some of the wheat parents. However, the amphidiploids that were generated with wide cross between durum wheat and a wild relative (*T. bessarabicum*) (grown in field conditions) showed comparatively higher photosynthesis than the amphidiploids with (*T. aestivum*) background (grown in glasshouse condition). Perhaps, the growing conditions for both the set of amphidiploids were different, hence the differences in the rate of photosynthesis. Whilst, comparing these two set of amphidiploids with the Indian genotypes, the Indian genotypes exhibited higher rate of photosynthesis. An improvement in photosynthetic performance plants can be achieved if they are grown in natural environmental conditions (Johnson et al., 2015, Kromdjik et al 2016, Murchie 2017). Thereforea major reason in higher rate of photosynthesis in these Indian genotypes could be due to their adaptability to challenging environmental conditions.

The present study investigates two set of wheat introgressed lines (amphidiploids) that were generated at University of Nottinghamfor variation in photosynthesis and other related physiological traits. First a set of amphidiploids lines that were generated with *T. aestivum* background and wild relatives of wheat and second a set of amphidiploids that had durum wheat background crossed with *T. bessarabicum*. Along with these two experiments, Indian genotypes were also tested in glasshouse and field conditions, but this thesis includes only the glasshouse experiment.

The first experimental chapter (Chapter 3) describes about the first set of 20 amphidiploids lines and these lines were tested in glasshouse conditions. Although the amphidiploids used in this experiment did not exhibit higher photosynthetic rates than its parents, but there were some of the amphidiploids that demonstrate photosynthetic efficiency were equal to its parents. It is also important to select appropriate accessions of the wild relatives of wheat and therefore selection of the different accessions of wild relatives suggests screening of wild relatives of wheat that exhibit higher photosynthetic rates and other related physiological traits is important and this is been carried out at the University of Nottingham at present. Not only this, it is logical to screen plants grown under fluctuating light conditions to assess plant's response as opposed to controlled conditions in glasshouse which is more favourable to the plants. This suggests that there is need in modification of methods in screening of such lines generated through wheat ancestral introgression. Although, it cannot be denied that for a larger population it would be feasible to screen them in glasshouse conditions and then the promising lines from this population can be subjected to natural growth conditions.

Chapter 4 explains about the amphidiploids that were grown in the field conditions, which seemed to be more robust than the amphidiploids with *T. aestivum* background. Highbury and Paragon that were used as a check lines in this experiment, also performed well in field condition, both the amphidiploids and UK wheat lines demonstrated higher photosynthetic rates in field conditions, especially in the year 2017. These amphidiploids responded differently to different experimental year, as explained in Chapter 4, that in the year 2016, the amphidiploids experienced drought like conditions in contrast to year 2017, which suggests that these amphidiploids were affected to the rise in temperature and water scarcity. However, the population of amphidiploid was very few in number, therefore variation in these amphidiploid was not as greater as the amphidiploids explained in Chapter 3. This experiment, allowed assessing the photosynthetic efficiency of the amphidiploids, which were promising enough. Also, these amphidiploids were crossed with some of the promising Indian genotypes (which is not included in any of this experiment), these amphidiploids could be future breeding material to generate lines that can perform better under such environmental conditions. For eg. amphidiploids that have already been acclimated to moderately high light and high temperature conditions in India could perform better in UK conditions and could be used in future research.

The final experimental chapter i.e. chapter 5 describes about the variation in leaf photosynthesis of the Indian genotypes in glasshouse conditions, here a panel 36 Indian genotypes were used in this experiment.

In the last five years, wheat production in India has been recorded to ~70 million tons and being second most populated country in the world, there is an urgent demand to increase the production of wheat from 70 million to ~90

million tons by the year 2020, Rane and Nagarajan (2014). Increasing demand for wheat production and also the changing climatic conditions has posed a tremendous challenge for breeders in India, as high temperature conditions has been one of the major factors for the decline in wheat productivity (Rao et al., 2015). Therefore, it is imperative to generate lines that can cope with future environmental conditions. Indian genotypes that were grown in glasshouse conditions described in chapter 5 exhibited higher photosynthetic rates compare to the check lines Highbury and Paragon. UK experiences 16 hour of daylight and these Indian genotypes were grown under these conditions, despite any photoperiod effect on them, these Indian genotypes demonstrated good photosynthetic performance. Some of the traits that are ideally important for crop improvement program such as having low VPD and high WUE genotypes, this was observed in the Indian genotypes (see Chapter 5). The major outcome of this chapter was identifying few lines such KRL210, KRL213 that demonstrated higher photosynthetic rates, the same lines showed high photosynthesis in field conditions as well (experiment not shown).

Overall, comparing the Indian to the amphidiploids would not yield substantial results, as the amphidiploids were new to such environmental conditions for eg. field conditions in India with moderately high light and high temperature conditions. On the contrary, the Indian lines consisted of lines that were introduced (see Table 2.4, Chapter 2) even before the amphidiploids were generated (at University of Nottingham). Suggesting that earlier introduction of these genotypes allowed them to acclimate to such conditions when comparing with the amphidiploids. But, it cannot be ignored that the Indian genotypes were grown for the first time in the glasshouse conditions in UK.

Therefore, it is difficult to derive any conclusion that whether the wheat ancestral introgression is an effective or alternative breeding strategy or not.

Considering other approaches other than breeding methodas a target of crop improvement, recent study associated with transgenic approaches to improve photosynthesis and yield in crops also needs to be considered. For eg, Simkin et al., demonstrated that over-expression of various genes and/ or enzymes involved in Calvin-Benson cycle that can have direct influence on photorespiration and electron transport and ultimately improving yield in plants. Hay et al., 2012 showed that under elevated CO_2, transgenic soya bean exhibited higher photosynthetic rate which is a result of the gene involved in HCO_3^- transportation, ictB gene, where overexpressed ictB gene allowed to fix more CO_2, which is a carbon concentrating mechanism. A suggestion that can be used in the future, is that exploring the genes that are involved in photosynthesis from the promising amphidiploids and Indian genotypes.

6.2 Summary

6.2.1Amphidiploids in glasshouse conditions

Amphidiploids that were generated through interspecific hybridization between wild relatives and UK wheat cultivars demonstrated photosynthetic rate similar to the wheat parents. As discussed in Chapter 3, the amphidiploids did not show much improved photosynthesis compared to the wheat parents and the possible reason could selection of wild relatives. In pre-breeding program, there could be a possibility of chromosomal segregation, therefore there is a need to confirm, whether the next generation (seeds derived from the first experiment) are carrying relevant genes. Also, glasshouse conditions are favourable conditions

for these wheat plants, therefore if these lines are tested under challenging environmental conditions and screened, then it would be more relevant.

Evidence suggests that there is a relation between leaf morphological traits and photosynthesis (Fullana-Pericas et al., 2017, Murchie et al., 2005). Therefore, these lines were investigated for various leaf morphological traits such as leaf thickness, distance between the veins, number of mesophyll cells and mesophyll cell area. Most of these leaf morphological traits varied in most of the lines, which suggests that some of this variation could be due to properties inherited from its respective wild parent or wheat parent. However, due to limited time period only few leaf anatomical parameters were studied. A study of stomatal density and its relationship plants was also done, however due to time constraint; it was not included in the study.

6.2.2 Amphidiploids in field

As mentioned above, the second set of amphidiploids derived from the cross between durum wheat and *T. bessarabicum* were tested in field conditions in India, in the peninsular region. Temperature and light intensity in this region of India is comparatively higher. In the year 2016, the region where the experiment was conducted was declared as drought affected area. Although, there was supply of water initially, but as the experiment progressed, water supply was limited. Therefore, a variation in leaf photosynthesis could be observed between both the years (2016 & 2017). In the peninsular region of India, where the field is located, temperature normally is 30°C; therefore the rate of photosynthesis was affected in the amphidiploids, which observed to have a close relationship with stomatal conductance. In drought conditions, grapevine roots through ABA signalling mediates stomatal closure, a water

saving strategy(Thombesi et al., 2015).Also, soil water evaporation rate could have also been one of the factors affecting photosynthesis in plants. As demonstrated by Perdomo (2017), diffusion of CO_2 in the leaves is affected in water deficit and high temperature conditions. Even in drought like conditions, the amphidiploids performed well in the year 2016 and much better in the year 2017.

6.2.3 Indian genotypes in glasshouse conditions

There are limited studies carried out Indian wheat genotypes with respect to photosynthesis. This experiment gives an opportunity to explore the variation in leaf photosynthesis in these genotypes. The Indian genotypes that were grown in the glasshouse conditions showed good variation in leaf photosynthesis and related physiological traits. One of the highlight of this experiment was that, there was good relationship between plant biomass and some of the photosynthetic parameters that were derived from the light saturated photosynthetic measurements. These Indian genotypes also demonstrated higher WUE_i, which is one of the important traits that can be used in future breeding program. Among these Indian genotypes, KRL210 and KRL213 were promising Indian cultivars, as these genotype also exhibit salt tolerant property. Genotypes with such important photosynthetic and other physiological trait could be used in future breeding program.

6.3 Conclusion

The success of wheat ancestral introgression lines cannot be judged on few experiments, also in pre-breeding program, it is difficult to derive a conclusion.

One of the highlight of this study was that the Indian genotypes exhibited higher photosynthesis compare to the amphidiploids, which could be used as breeding material in future.

As there were different introgressed lines that were used in field and glasshouse, it is recommended that in future, such breeding materials should be grown in both field and glasshouse conditions, along with their respective parents (wild and domesticated) in order to derive comparison between them. Therefore, once these introgressed lines are screened in glasshouse conditions and field conditions, it can allow us to pick the lines that demonstrate higher photosynthesis and important physiological traits. These lines can be subjected to detailed photosynthetic measurements such as ACi curves and light response curves. Along with this, leaf morphology and rubisco assays can be also be done, which will allow determining the complete trait analysis of the promising lines.

A major observation in this study was that the leaf photosynthesis in most of the experiments was governed by many of the photosynthetic parameters and one of them was stomatal conductance. In all the three experiments, it was observed that stomatal conductance had major role in affecting photosynthesis directly or indirectly. It would be an interesting work, that more focus is given towards stomatal conductance and explore the relationship with photosynthesis.

6.4 Future work

It is necessary to create more lines by crossing between wild relatives and bread wheat with different combinations and create lines that could be promising. The amphidiploid lines that were tested in in glasshouse conditions can also be tested in field conditions to understand their stability in challenging environmental conditions like in India. Similarly, the amphidiploids lines that

were tested in India could be tested in glasshouse as well as field conditions in UK. The Indian cultivars (cv) overall demonstrated good variation in photosynthesis and the promising lines that were screened could be used as breeding material in future. And interesting attempt would be to create amphidiploid lines using the Indian genotypes and test it in different environmental conditions.

Leaf anatomy was not done in any of the experiment in field conditions; this would be a useful approach to understand how leaf anatomical characteristics could influence photosynthesis in field conditions. Also, there could be few biochemical assays such as superoxide dismutase that could be done in both field and glasshouse conditions. Rubisco activation assays would also be an important approach that can be carried out in selected varieties.

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