Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

SCIENZE BIOTECNOLOGICHE E FARMACEUTICHE

Ciclo 30°

Settore Concorsuale: 07/E1

Settore Scientifico Disciplinare: AGR/07

Molecular and phenotypic evaluation of durum wheat germplasm for hybrid production

Presentata da: dott.ssa Priyanka Gupta

Coordinatore Dottorato

Chi.mo Prof. Santi Spampinato

Supervisore

Chiar.mo Prof. Roberto Tuberosa

Correlatori: Dott. M. Maccaferri
Dott.ssa E. Frascaroli
Dott. F.M. Bassi

Esame finale anno 2018

Table of Contents

Dedication

To the Almighty God for immeasurable blessings upon my life, divine protection and daily guidance.

To my Maa, Premlata Gupta for her prayer, and unconditional love and care.

Abstract

Global food security is faced with serious challenges including population growth and changing climate. To cope with these challenges a paradigm shift is required to ensure sufficient and sustainable crop production. Hybrid technology is one of the strategic solutions for crops including durum wheat. To translate hybrid technology in durum wheat, an understanding of heterotic behavior is required. The present study aims at generating the basic knowledge of hybrid technology and examines the magnitude of heterosis for yield and yield components through evaluation of eight parents along with 28 F1 hybrids (half diallel) from University of Bologna and 25 F1 and 10 parents (NC II design) from ICARDA, Morocco. The hybrids along with their parents were evaluated in the field-experiment carried out in Bologna, precision phenotyping platform (Lemnatec) at different levels of water stress in Metaponto and in near-field condition via a basket method in Rabat to assess agronomic, physiological and root traits. The F1 hybrids were evaluated based on mid and best parent values as well as general and specific combining ability effects and water stress tolerance index (WSTI). The results showed >20% of mid parent heterosis, indicating the scope for exploitation of heterosis in durum wheat. Valnova x Miki was the best hybrid combination in both experiments followed by Karim x Valnova and Karim x Morocco in field while, Karim x Svevo and Iride x Miki in glasshouse for hybrid production. Based on the WSTI, three F1 hybrids (Valnova x Miki, Iride x Miki and Svevo x Miki) and one parent (Svevo) were identified as drought tolerant. In general, grain yield varied appreciably among crosses in each environment. These results suggest that hybrids in general do better under water stress environment but specific hybrid combinations need to be developed to realize the stably higher performance under drought prone environments. Another aspect of hybrid technology is to ensure adequate pollination between heterotic parents which require overlapping flowering time. To understand the genetic control of flowering time, a genome wide association study (GWAS) was conducted to identify genomic regions associated with the control of flowering time in durum wheat. A total of 384 landraces and modern germplasm were assessed in 13 environments to determine five pheno-environments based on temperatures, day length and other climatic variables. Genotyping was conducted with 35K Axiom array to generate 7,740 polymorphic SNPs. In total, 20 significant QTLs for landraces and 27 QTLs for modern germplasm were identified for flowering time consistently across the environments. The candidate gene search indicated seven novel genes, namely PRR7, GRF, SVP, RRP6L1, Hd6, TCP1, and COP1/RGA in addition to a number of already known regulatory photoperiodic genes, PPD-A and PPD-B and vernalization genes VRN-A1, VRN-B2 and VRN3 which have major impact in the genetic make-up of flowering time in landraces and elite germplasm. In summary, the results obtained from these experiments indicated sufficient heterosis in durum wheat and helped in physiological and molecular characterization of the best heterotic combinations and flowering time.

General introduction

Area, production, consumption and genetics of durum wheat

Wheat is the most widely grown cereal, providing 21% of the food calories and 20% of the protein to more than 4.5 billion people in 94 developing countries (Gomez et al., 2014) (www.wheatworld.org). Although durum wheat (*Triticum turgidum* ssp. *durum* Desf.) accounts for only 5-8% of global wheat production, it is an economically important crop because of its unique characteristics such as hardness, high protein content, intense yellow color of the semolina and cooking qualities. It offers many business opportunities as its grains are used to make pasta, couscous, bulgur, freekeh and semolina products. Durum wheat is adapted to more diverse environments than bread wheat, and it performs well in semiarid regions even under limited water availability. The latest global estimate indicates that durum wheat is grown on 13 million ha area producing 39.9 million tonnes grains with the European Union, Canada, Turkey, India, Mexico, United States, Algeria, Morocco and Kazakhstan being major producers (http://www.world-grain.com/articles/news_home/World_Grain_News/2017/10/Global_durum_wheat_use_trendin.aspx?ID=%7B04F7D478-8010-49E7-A30E-60F63024D10D%7D). The European Union is the largest durum wheat producer, averaging 8 million tonnes with major contributions from Italy, Spain and Greece. Italy, known as the 'home of pasta'. Canada produces 4.6 million tonnes of durum followed by India and Turkey (4 million tonnes each), the USA and Mexico (3.5 million tonnes each) whereas total durum wheat production of North African countries (mainly Algeria, Morocco and Tunisia) is 4.6 million tonnes. Pakistan accounts for 3.4% of global durum production (1.2 million tonnes). In summary, the Mediterranean basin is the largest producer, importer and consumer of durum wheat products in the world (FAOSTAT, 2017).

Durum wheat, first domesticated in the Fertile Crescent (7,000 BP), became a dominant tetraploid wheat in the Levant and in the Mediterranean basin ~2500 years ago (Feldman & Levy, 2015). Past studies (Feldman & Levy, 2015; Akhunov et al., 2010) have established that durum wheat is genetically very close to wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides* (2n = 4x

= 28, Genome BBAA). It is an allotetraploid species (2n = 4x = 28 chromosomes) with A and B genomes (*Triticum dicoccoides*, *T. dicoccum* and *T. turgidum* ssp. *durum* Desf.; 12 Gb genome size). The A genome is considered as the pivot genome common to all wheat species and derives from an ancestor of the wild wheat *Triticum urartu* (Dvorak et al., 2006). The origin of the B genome is more complex to be traced (Talbert et al., 1995). It has been suggested that the B genome originates from the SS genome of an *Aegilops* species belonging to the Sitopsis section (Seberg, 1999) and similar to the present *Aegilops speltoides* (Sarkar & Stebbins, 1956).

During the past 50 years, wheat production has experienced spectacular growth, due to widespread adoption of semi-dwarf varieties, use of chemical fertilizers and expansion of area under irrigation. This success has been termed as the 'Green Revolution' in South Asia which was later replicated in many other developing countries. Wheat yield has more than doubled to reach a global average of 3.3 tonnes per ha at present. However, the growth in wheat productivity has slowed down in the recent past, jeopardizing the global food security of growing population under changing climate (Hedden, 2003; Whitford et al., 2013). The demand for wheat is growing fast not only in the traditional wheat growing regions of Central Asia (5.6%), Australia (2.2%) and North Africa (2.2%) but also in new wheat growing regions such as Eastern and Southern Africa (5.8%), West and Central Africa (4.7%) and South Asia and Pacific (4.3%) (Shiferaw et al., 2013). The present growth rate of global wheat production is not sufficient to meet the demand of these regions, resulting in demand-supply gaps. To bridge the demand-supply gaps of wheat consumption, there is a need for a paradigm shift in research strategies to enhance the genetic gain in breeding improved varieties. Hybrid technology offers a viable option to step up productivity in wheat.

History of hybrid breeding

Ever since the elucidation of the phenomenon of heterosis by Shull in 1908, breeding procedures in cross-pollinated crops have evolved around the exploitation of hybrid vigour in developing open pollinated synthetics, composites or hybrid varieties. Sinha & Khanna (1975) and Srivastava (1981) have reviewed various theories proposed for understanding the complex phenomenon of heterosis at genetic, molecular, biochemical, physiological, developmental and gene regulation

levels. They concluded that the complementary intergenic and non-allelic interactions operating at different structural and functional levels are responsible for the expression of hybrid vigour at the gene product level and the observed heterosis at the phenotypic level. Srivastava (1981) also speculated that besides intragenomic interactions, the intergenomic (genome-plasmon) interactions also play an important role in the manifestation of hybrid vigour. The differences observed for hybrid vigour in reciprocal crosses in various crops also emphasize the importance of cytoplasmic nuclear interactions in the expression of heterosis. Since dominance genes in the population have evolutionary advantage (Fisher, 1930), the heterosis was initially considered a discernible phenomenon of cross-pollinated crops but later the commercial exploitation of hybrid vigour in rice and vegetable crops established its utility in self-pollinating crops as well. Falconer et al. (1996) described quantitative heterosis as mid parent heterosis (MPH) = [(F_1- MP)/ MP] x 100 and the best parent heterosis (BPH) = [(F_1- BP)/ BP] x 100].

Although heterosis has been widely utilized in crop production for the past century, its genetic and molecular basis remain poorly understood. Accordingly, although hundreds of papers have been published and many conferences have been held since the conception of heterosis, the underlying genetic and molecular basis is still being debated. In many of these forums, it appears to be a fascination with the idea that standard genetic models are not sufficient to explain heterosis while the key models of dominance, overdominance and epistasis are still in use for describing multigenic heterosis. The dominance hypothesis accounts for heterosis by the cumulative effect of favorable alleles exhibiting either partial or complete dominance while the overdominance hypothesis assumes overdominant gene action at many loci and the epistasis hypothesis attributes heterosis to epistatic interactions between non-allelic genes. The relevance of these three hypotheses has been investigated intensively using phenotypic data (Reif et al., 2006) and molecular marker-aided quantitative trait loci (QTL) mapping. Recent advances in functional genomics, epigenetics and systems level approaches have provided a new perspective to understand the complex trait of heterosis. Some pathways such as the circadian clock and energy model have shown wide influence across traits associated with heterosis. However, heterosis or hybrid vigor still remains an unsolved puzzle and an almost ‘miraculous’ agricultural phenomenon.

The exploitation of heterosis through hybrid breeding technology is one of the major breakthroughs in plant breeding (Duvick, 2001). Reliable prediction of single-cross performance is very important in hybrid breeding, because it is difficult to evaluate inbred lines in every possible cross combinations. Several prediction approaches have been suggested using phenotypic data with co-ancestry coefficients calculated from pedigree records or marker data (Schrag et al., 2009). Genomic selection based on dense molecular marker profiles has the potential to assist breeders in the selection of the most promising hybrid combinations for field evaluation (Piepho, 2009).

Hybrid breeding in cereals

In the long history of heterosis in major cereals, hybrid corn is one of the greatest success stories of all time. Maize yield has consistently increased which in part can be explained by the advances associated with hybrid technology, genetic improvement of inbred parents and higher level of investment in research from the private sector (Whitford et al., 2013). It has been estimated that 65% of the global maize area was planted with hybrid seeds, resulting in six-fold increase in production since the introduction of hybrids in the 1930s (Duvick, 2001). Another success story is hybrid rice in China, first studied in 1964. Hybrids have shown >20% yield advantage over traditional varieties in rice and now accounts for 50% of the total rice area in rice-producing countries including China, India, Vietnam and Indonesia (Cheng et al., 2007). Similarly, hybrid breeding gained the interest of scientists following the description of the first male sterile in barley in 1940 (Ramage, 1983) and triticale (× Triticosecale Wittmack) in 1980s. It was speculated that heterosis in barley might be considerably higher than in wheat and triticale, since barley is diploid whereas wheat and triticale are hexaploid. (Oettler et al., 2005) speculated that lower heterosis in triticale and wheat compared to diploid rye might be a reason of the “fixed” heterosis in allopolyploid inbred lines due to epistatic interaction between genes of the different genomes.

Contrasting results about the magnitude of heterosis for grain yield in triticale were reported. For CHA based triticale hybrids, heterosis was ~10% higher than the results in wheat (Oettler et al., 2005; Fischer et al., 2010). But the CMS-based triticale hybrids investigated by Manje Gowda et al. (2013) showed only around 2% heterosis. The contrasting findings in triticale require further research to obtain reliable estimates of heterosis and detect the reasons for the large differences.

Despite intensive research work over several decades, neither hybrid barley nor hybrid triticale could be established for a commercial use comparable to hybrid maize and hybrid rice (Longin et al., 2012).

Heterosis in hexaploid and tetraploid wheat

Heterosis was first reported in wheat by Freeman (1919) for plant height. Since then, there have been several reports on heterosis in wheat. The whole subject of hybrid wheat was reviewed by Pickett (1993), Singh et al. (2010) and more recently by van Ginkel & Ortiz (2017). A systematic hybrid wheat program was launched in the United States as early as in 1930s without much progress for 30 years. Since some of the major issues could not be solved, several attempts failed (Pickett, 1993). After the discovery of chemical hybridization agents (CHA), both public and private research programs have got a fresh boost (http://www.hybridwheat.net) especially in Europe with several private and public initiatives (Longin et al., 2012, 2013; Whitford et al., 2013; Zhao et al., 2013). Currently, Europe is the major hybrid wheat growing region with ~160,000 ha area in France and ~25,000 ha in Germany (de Castelbajac, 2010). In Europe, two hybridization systems are in use. The first system relies on CHA while the second one relies on cytoplasmic male sterility (CMS) (Whitford et al., 2013). There were no significant differences in the amount of heterosis achieved with CMS-based and CHA-based hybrids. However, the development of the CMS-based hybrids required much more time (Gowda et al., 2010).

A few successful hybrid wheats have been developed by the private sector in Europe and India (Saaten, 2013; Mahyco, 2013). Large public and commercial projects have been launched to establish hybrid wheat breeding programs in Mexico and Australia with main focus on grain yield. However, hybrids currently available offer the best economic advantage only under less than optimum growing conditions. This has been observed in several studies (Sharma & Tandon, 1995; Solomon et al., 2007; Sharma, 2013). A number of reasons have been suggested for the lack of a commercial hybrid in wheat. Pickett (1993) and Song et al. (2009) concluded that the most serious technological barriers to the development of a successful commercial wheat hybrid are the absence of adequate parental combinations, multiple genomes, stringent autogamous nature of wheat and lack of a low-cost hybridization system. The basic requirement for any commercial heterosis is the presence of genetic differences in the two parental lines (East, 1908,

1936).This notwithstanding, diverse parents will not always yield heterosis (Solomon et al., 2007). Choosing the right parental combination is central to achieving heterosis.

Based on CMS and CHA, wheat hybrids were developed and evaluated with their parents in reliable yield trial (Barbosa-Neto et al., 1996; Corbellini et at., 2002; Oury et al., 2000). Heterosis was on average around 10% in wheat. Investigations on the expected selection gain of hybrid versus line breeding were also conducted (Longin et al., 2012), but reliable predictions were difficult due to multiple influencing factors. Although lower for wheat as compared to other allogamous species, an average superiority of 10.7% with maximum of 23.8% for grain yield of hybrids compared to the mean of their parents was reported in the first large scale performance tests (Longin et al., 2013). In addition, advanced breeding technologies and a more intense screening for lines with high 'general combining ability' (GCA) have led to an increased hybrid vigor in recent time (Longin et al., 2014). It is assumed that hybrids as compared to line varieties were confirmed to have a significantly higher yield stability and lower susceptibility to abiotic and biotic stress (Longin et al., 2013; Mühleisen et al., 2014).

In durum wheat, a two-gene system has been identified that controls male sterility/fertility in specific alien cytoplasm (Maan, 1992; Simons et al., 2003). Nevertheless, flowering biology of durum wheat with limited supply of pollen hampers the controlled crossing of parental genotypes for large-scale seed production. Previous studies in durum wheat reported an average mid-parent heterosis from 12.8 to 25% for grain yield (Amaya et al., 1972; Sayar et al., 2007; Widner & Lebsock, 1973) . However, these studies were based on either single plant or small plots with low seed density, which potentially results in an overestimation of heterosis (Oettler et al., 2005). In addition, Gowda et al. (2010) investigated the association between mid-parent value and hybrid performance and reported 10% higher yield than the mid-parent performance with 22% maximum superiority. In summary, the findings in durum wheat were concurrent and further research might only be necessary to verify if the earlier findings agree with the results of present germplasm and specific growing regions.

Yield stability is another parameter in favour of hybrids. The average yield advantage of hybrids, compared to their parental inbred lines, was relatively low (~10%) in experimental studies. Compared with the better parent and/or outstanding line varieties, the yield advantage becomes

irrelevant (Oettler et al., 2005; Gowda et al., 2010). At the same time, the production of certified seed for hybrids is expensive and the development of hybrid requires higher investments. Therefore, additional benefits such as higher yield stability justifies higher investments in breeding hybrid.

A major limitation for hybrid wheat is the lack of a cost-effective hybrid seed production system as well as best heterotic groups (Gowda et al., 2010). Thus, a prerequisite for hybrid wheat breeding is the redesign of flowering and floral traits to ensure sufficient cross-fertilization in this self-pollinating species. Many traits contribute to pollination capability such as flowering time, flowering duration, plant height, extrusion of anthers and stigma, number of pollen grains per anther, adequate pollen dispersal outside the florets, opening of the glumes and longevity of pollen grains (De Vries, 1971; Lelley, 1966; Whitford et al., 2013). The male ideal form, or 'ideotype', should have long extruded anthers that shed large amount of pollen outside the florets over an extended period of time. The pollen should be viable, long-lived and have good aerodynamic qualities (De Vries, 1971 and 1974; Whitford et al., 2013). The female ideotype flowers ought to have open glumes and extrude stigmatic hair during male flowering for increased receptivity. In addition, the flowering time for both parents should be synchronized and males should be taller than females in order to facilitate cross-fertilization (De Vries, 1972; Longin et al., 2013). For most of these traits, phenotyping is difficult and time consuming. The availability of suitable high-throughput phenotyping and genotyping methods for floral traits are of utmost importance in order to facilitate the design of the male ideotype and hence increase the outcrossing rates of wheat for hybrid seed production.

Heterotic groups and combining ability

The basic requirement for a successful hybrid breeding program is a sufficient magnitude of heterosis for economically important traits to make hybrids commercially viable (Duvick, 1999). This requires efficient identification of distinct heterotic groups as manifestation of heterosis depends on the degree of heterozygosity in the genome, i.e., the presence of different alleles at several loci in the homologous chromosomes. Heterozygosity can be increased by crossing genetically distinct parental materials belonging to distinct heterotic groups. Heterotic groups

are germplasm groups that are genetically distinct from each other and that produce superior hybrids because they carry different sets of complementary and heterotic genes. (Melchinger & Gumber, 1998) defined a heterotic group as "a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from genetically distinct germplasm groups". By comparison, the term heterotic pattern refers to a specific pair of two heterotic groups, which express high hybrid performance and heterosis in their cross.

Development of divergent heterotic groups maximizes the expression of heterosis and hybrid performance (Falconer et al., 1996). Similarly, combining ability helps to know the potential of a parental line to produce a superior hybrid. General Combining Ability (GCA) refers the average performance of a genotype in hybrid combination with other genotypes. GCA measures additive gene action but if epistasis is present, then it measures additive x additive type of non-allelic gene interaction also (Falconer et al., 1996). The performance of a particular cross can deviate from the average general combining ability of two parents involved in the cross. This deviation is defined as the specific combining ability (SCA). SCA defines those cases in which certain combinations do relatively better/worse than what is expected on the basis of average performance of lines involved. It measures non-additive gene action which would also include additive x dominance and dominance x dominance kinds of non-allelic interactions. However, combining ability should be carefully examined when the objective is to develop superior hybrids for quantitative traits such as yield (Foote, 1964; Bhatt, 1971)). The ratio of the variance due to SCA and GCA is of central importance for predicting hybrid performance based on GCA effects (Fischer et al., 2008).

In autogamous crops with complex population structure such as wheat, rice, barley and triticale, heterotic groups have not been established and pose the foremost challenge. In these crops, the magnitude of heterosis is low, pollination control is difficult, and SCA for grain yield appears of greater importance than GCA (Oury et al., 2000; Oettler et al., 2005) . However, in most studies, only weak correlation was observed between inbreds belonging to divergent heterotic groups (Melchinger & Gumber, 1998). One approach to increase genetic diversity is making hybrids between adapted and non-adapted lines, e.g., winter by spring types (Koekemoer et al., 2011).

This leads to high mid-parent heterosis because of the low performance of the non-adapted parent and in some cases also to positive value of better-parent heterosis but problem occur regarding vernalization and photoperiodic reaction. New model-based clustering methods, which are implemented with the software STRUCTURE, are powerful tools to unravel the genetic structure and identify diverse groups of genotypes, and have been successfully applied in maize (Liu et al., 2003; Stich et al., 2005). Many criteria have been suggested to choose promising heterotic groups: (i) high mean performance and large genetic variance in the hybrid population in the target region(s), (ii) high *per se* performance and good adaptation of parent populations, and (iii) a higher ratio of the variance due to GCA versus SCA (Gowda et al., 2010), (iv) low inbreeding depression of inbred (Melchinger & Gumber, 1998; Fischer et al., 2009). Importantly, the magnitude of heterosis is expected to be even higher in stress environments (Oettler et al., 2005).

Key traits involved in heterosis

1. Flowering time

Flowering induction plays a pivotal role in the plant life cycle, ensuring reproductive success when the "best timing" is achieved. In durum wheat, heading and flowering time are important stages in crop development for their role in adaptation, yield potential and grain quality (Maccaferri et al., 2008). In addition, climatic stress during anthesis negatively affects pollen production (Pickett, 1993). However, in order to ensure adequate pollination between heterotic parents, their flowering time must overlap with clear understanding of the phenotypic and molecular variation (Turner et al., 2005; Wilczek et al., 2010). Therefore, plant breeders need effective tools to predict flowering time in order to transfer promising genotypes into different climatic regions.

An acquaintance of the nature of gene action controlling flowering and maturity times of spring wheat may help to assign good breeding strategies to modify these traits according to the needs of a given environment. Several studies have demonstrated the role of additive, dominance or epistatic gene actions in the inheritance of heading time of field-grown wheat. Klaimi & Qualset (1974) reported the involvement of additive, dominance and epistatic gene action in controlling

heading time of spring wheat. Nanda et al. (1981), Bhatt (1972) and Sameena et al. (2000) concluded that additive gene action was more important than dominance gene action in the inheritance of heading time in spring wheat. The importance of both additive and dominance effects in controlling heading time has been reported in winter (Edwards et al., 1976) and spring (Singh et al., 2003) wheat.

Flowering time in wheat is controlled mainly by three groups of loci, two of which interact with environmental factors, namely photoperiod sensitivity genes (*PPD*) and vernalization requirement genes (*VRN*) (Distelfeld et al., 2009). The third group of loci, controlling 'narrow-sense earliness' or 'earliness *per se*' (*EPS*), acts on the developmental rate independent of vernalization and photoperiod (Scarth & Law, 1984). Differences in the *PPD* genes divide the temperate cereals into photoperiod-sensitive and photoperiod-insensitive classes, whereas differences in the *VRN* genes divide them into winter and spring classes.

Natural variation in vernalization requirement in the temperate cereals is mainly associated with allelic differences in the *VRN1*, *VRN2*, and *VRN3* genes. Vernalization sensitivity/insensitivity in hexaploid wheat is controlled by alleles at the major vernalization loci, *VRN-A1, VRN-B1, VRN-D1* and *Vrn-D5* (Pugsley, 1972). Winter wheat possesses recessive alleles at all these loci while spring wheat have dominant alleles at one or more of them. The dominant allele of *VRN-A1* confers complete insensitivity to vernalization and is epistatic to dominant alleles of *VRN-B1, VRN-D1* and *VRN-D5*, which confer low sensitivity to vernalization (Pugsley, 1971, 1972). Conversely, durum wheat contains a homologous copy of *VRN-1*, designated *VRN-A1* and *VRN-B1* and located on the long arms of chromosomes 5A and 5B (Fu et al., 2005; Yan et al., 2004). Recent advances in wheat genomics have allowed for the cloning of *VRN-A1, VRN-B1* and *VRN-D1* genes (Yan et al., 2003).

Photoperiod response is another important factor influencing the initiation and length of flowering period. Natural variation in the response to photoperiod is mainly determined by allelic differences in the *PPD1* gene, a member of the pseudo-response regulator (PRR) gene family (Turner et al., 2005). Photoperiod-sensitive wheat is stimulated to flower only on exposure to long-days, provided that any requirement for vernalization is met, and flowering is delayed under short days. Mutation at *PPD-1* loci, however, enables the wheat plant to flower irrespective of the day length called photoperiod insensitive alleles. Photoperiod-insensitive wheat flowers

independently of day length and can be grown successfully in long- and short-day environments. These mutations have been selected in the past by plant breeders to enhance yield in certain climatic conditions by avoiding high summer temperatures through early flowering. In durum wheat, photoperiod sensitivity is determined by *PPD-A1* and *PPD-B1* loci, located on chromosomes 2AS and 2BS (Laurie, 1997). Photoperiod insensitivity in durum wheat results from mutations in *PPD-1* genes on the A or B genomes. By convention, alleles conferring photoperiod insensitivity are assigned by an 'a' suffix (e.g. *PPD*-A1a) (McIntosh et al., 2005). In durum wheat, Wilhelm et al., 2009)found two large deletions within the *PPD-A1* gene (1027 and 1117 base pair (bp) deletions designated as alleles 'GS-100' and 'GS-105, respectively), which remove a common region from the wild-type sequence. The presence of either deletion accelerated flowering, which led to the conclusion that these deletions are the likely causal basis of photoperiod insensitivity in tetraploid wheat (Wilhelm et al., 2009). Additionally, in durum wheat germplasm, the effect of functional homologs remains largely unexplored. Genetic studies showed that the most effective photoperiod insensitivity gene is the *PPD-A1* gene, followed by *PPD-B1*, which also proved that the alleles with a higher copy number of *PPD-B1* confers early flowering (Díaz et al., 2012).

Flowering control in durum wheat provides a rough classification of germplasm with maturity classes. To date, accurate knowledge about effective alleles and their estimation in elite durum wheat breeding germplasm is still lacking. However, studies of Maccaferri et al. (unpublished, 2018) reported distribution of *Ppd-A1, Ppd-B1, VRN-3* (FT) allelic variants and Copy Number Variation (CNV) of *PPD-B1* loci in the durum elite germplasm. The aim of this study was to estimate the allelic effects of *PPD* and *VRN* loci at different latitudes and temperature regimes, to find additional QTLs for heading date, and compare the frequency distribution of wild type and mutated alleles in the diversity panel subdivided into seven main gene pools according to their origin. The study showed that ICARDA and Italian local landraces maintained the wild type (photoperiod sensitive) allele whereas CIMMYT germplasm gradually replaced the wild type by the two early-flowering mutations and expressed allelic variation.

2. **Plant height**

Another key trait that revolutionized wheat production is plant height (Worland, 2001). The fine-tuning of plant height plays an important role to implement an effective hybrid wheat production system. Since it is desirable to use a taller plant as the pollen parent in order to maximize the chances of cross fertilization (Whitford et al., 2013), it is equally important that the flowering time of the pollen donor and male sterile parents must be compatible. The genetic control of plant height is very complex, determined by many major and minor genes (Worland, 2001). Thus, identification of alleles that reduce height (*Rht* gene) without negatively affecting yield *per se* is a priority for many breeders. The *Rht-1* homoeo loci, located on group 4 chromosomes is the major source of semi-dwarfism, predominantly alleles of the *Rht-B1* genes. In durum elite germplasm very few *Rht* genes have been reported on chromosomes 4Bs and 7A. This notwithstanding, the effects of key candidate loci for the adjustment of plant height remain less clear (Peng et al., 2011).

3. **Anther extrusion**

A variety of floral characteristics, like timing of floret gaping, the length of the anthers and filaments, the size of the glumes, the separation between adjacent florets and the angle subtended between opposite florets have been documented to maximize hybrid seed production (Longin et al., 2012). Similarly, higher anther extrusion stimulates cross fertilization for more effective hybrid seed production. To obtain a reasonable yield of hybrid seeds, the female parent needs to be not just male sterile, but its flowers must open sufficiently while the stigma remains receptive in order to allow access for incoming pollen; meanwhile, the male parent, rather than shedding its pollen within the closed floret, must extrude its anthers prior to anthesis. Consequently, the greater the extent of anther extrusion and pollen mass the higher will be the rate of cross fertilization for better hybrid seed production (Langer et al., 2014; Muqaddasi et al., 2016).

Genome Wide Association Study

From a couple of decades, genome wide association study (GWAS) has emerged as a powerful tool for mapping complex traits in crop plants. It can be used to identify genes responsible for

natural phenotypic variation through screening large, diverse collections of accessions with high-density genetic markers to find causal genes as a result of historical recombination (Waugh et al., 2009). Flowering time has been the subject of an intensive quantitative trait locus (QTL) mapping effort by the research community in *Arabidopsis thaliana*, with numerous QTL mapping studies published in the last 15 years (Clarke et al., 1995; El-Assal et al., 2001; Jansen et al., 1995; Kuittinen et al., 1997; Stratton, 1998; Maloof et al., 2001; Ungerer et al., 2002; Bandaranayake et al., 2004; Weinig et al., 2002, 2003; El-Lithy et al., 2004; Juenger et al., 2005; Werner et al., 2005). To date, more than 180 genes involved in flowering time control have been identified in Arabidopsis (Fornara et al., 2010; Johansson & Staiger, 2014). Furthermore, Ehrenreich et al. (2009) have found 27 quantitative trait genes (QTGs) out of 51 flowering time loci tested in Arabidopsis, through candidate gene association that have shown significant associations in various trait/environment combinations.

In barely, several novel QTLs with medium to high effects, including new QTL having major effects on developmental stages/sub-phases were found to be associated to heading time, which were added to the major genes known to regulate heading time under field conditions (Alqudah et al. 2014). Later on, Maurer et al. (2015) have identified eight major QTLs as main determinants to control flowering time in barley, upon the cultivation of 1,420 lines in multi-field trials.

Newly bioinformatics approach was used to predict flowering-related genes in wheat and barley from 190 known Arabidopsis flowering genes (Peng et al., 2015). They could identify up to 900 and 275 putative orthologues in wheat and barley, respectively. These Genome-Wide Comparative Analysis of Flowering-Related Genes in Arabidopsis, wheat, and barley showed that orthologous gene pairs in three critical flowering gene families (PEBP, MADS, and BBX) exhibited similar expression patterns among 13 developmental stages in wheat and barley, suggesting similar functions among the orthologous genes with sequence and expression similarities (Peng et al., 2015).

In Durum wheat, similarly to bread wheat, major loci with allelic variation affecting heading and flowering date are known to be associated with *PPD* and *VRN* genes. A QTL associated with *PPD*-A1a significantly reducing heading date was detected by (Maccaferri et al., 2008) in a

recombinant inbred lines population derived from the cross 'Kofa' ('GS-100' allele) × 'Svevo' ('GS-105' allele), suggesting that these alleles decrease photoperiod sensitivity to different degrees.

Subsequently, in 2014, Maccaferri et al. mapped strong molecular differentiations among sub-populations to 87 chromosome regions, in six core recombinant inbred lines populations of durum wheat. A genome-wide association scan for heading date from 27 field trials in the Mediterranean Basin and in Mexico yielded 50 chromosomal regions with evidence of association in multiple environments. GWAS analysis showed strong experiment-wise significant associations at the two chromosomal regions corresponding to the location of *PPD*-A1 and *PPD*-B1 loci and numerous chromosomal regions with highly significant ($P \leq 0.01$) marker-wise associations across environments (Maccaferri et al., 2014).

Past studies in wheat did not succeed in translating hybrid vigour into reality. There are many reasons behind the failure of heterosis in wheat, for example, the mechanism controlling heterosis in polyploids is not well understood and the number of experimental studies on the genetic basis of heterosis is low. Furthermore, the effect of inbreeding depression in a self-pollinated crop is not accurately studied and the identification of possible heterotic groups has never taken the advantage of a solid genetic study. Similarly, sufficient knowledge on novel and stable QTLs and candidate genes for heading date in durum wheat is lacking especially for different growing zones. These issues are particularly true for durum wheat and this PhD project aims at filling some of these gaps.

Objectives and structure of the study

The major goal of this research was to understand the basic knowledge of hybrid technology and examine the magnitude of heterosis for yield and yield components in durum wheat. The specific objectives were as follows:

1. Identification of genomic regions associated with flowering time in durum core collection in order to:
    a. Use them for targeted modification of flowering time for hybrid production
    b. Devise a more efficient selection of parents with synchronous flowering in major pheno-environments representing Long days (LD) and Short days (SD) conditions.

2. Estimation of heterosis for yield performance in durum wheat.
    a. Identification of superior parents based on mid- and best parent heterosis and find out the contribution of GCA and SCA.
    b. Correlation among mean values, SCA and mid- and best parent heterosis (MPH) effects.

3. Determining yield performance of hybrids under water stress conditions using
    a. High-throughput phenotyping platform (HTPP, Lemnatec method)
    b. Field evaluation and analysis of above and below ground traits heterosis.

Chapter 1: Identification of genomic regions associated with the control of flowering time in durum wheat

Objective

Identification of genomic regions based on genome-wide association study (GWAS) provides higher mapping resolution and power for detecting QTLs associated with trait of interest as compared to biparental mapping. A number of major genes for heading date have been reported in bread and durum wheat (Sukumaran et al., 2016). Most of the minor genes/QTLs have yet to be identified and characterized. Therefore, association mapping becomes more useful for identification of flowering genes/QTLs in order to synchronise flowering time of parents involved in heterotic combinations because some flowering genes such as *PPD* and *VRN* have opposing effects on heading time under Short Day (SD) and Long Day (LD) environments (Han et al., 2016). Sufficient knowledge on novel and stable QTLs and candidate genes for heading date in durum wheat is still lacking especially for different growing zones. Keeping this in mind, GWAS was conducted to identify genomic regions associated with the control of flowering time in a diverse durum wheat core set phenotyped in the Mediterranean, Senegal and Mauritanian regions representing different latitude and temperature regimes.

Materials and Methods

Plant material

A durum wheat core collection comprising of 96 landraces from 24 countries and 288 cultivars and elite breeding lines from eight countries, International Center for Agricultural Research in the Dry Areas (ICARDA), and International Maize and Wheat Improvement Center (CIMMYT) was used for this study. Detailed information regarding plant material is described in earlier study (Kabbaj et al., 2017). A total of 13 field experiments were carried out at eight locations during two crop seasons in 2014-15 and 2015-16. These locations situated at different latitudes were Marchouche, Melk Zhar and Tassaout in Morocco, Terbol main season, Kfardan and Terbol offseason from Lebanon, Fanaye in Senegal, and Kaedi in Mauritania (Fig 1.1). The experiments were conducted in augmented design with 19 blocks and four repeated checks. Days to heading

(DTH) was recorded as the number of days elapsed from the date of sowing to the onset of flowering determined at 50% of the plot heading. Average daily minimum and maximum temperatures were recorded at each environment. Out of 13 environments, two were rainfed and remaining were irrigated.

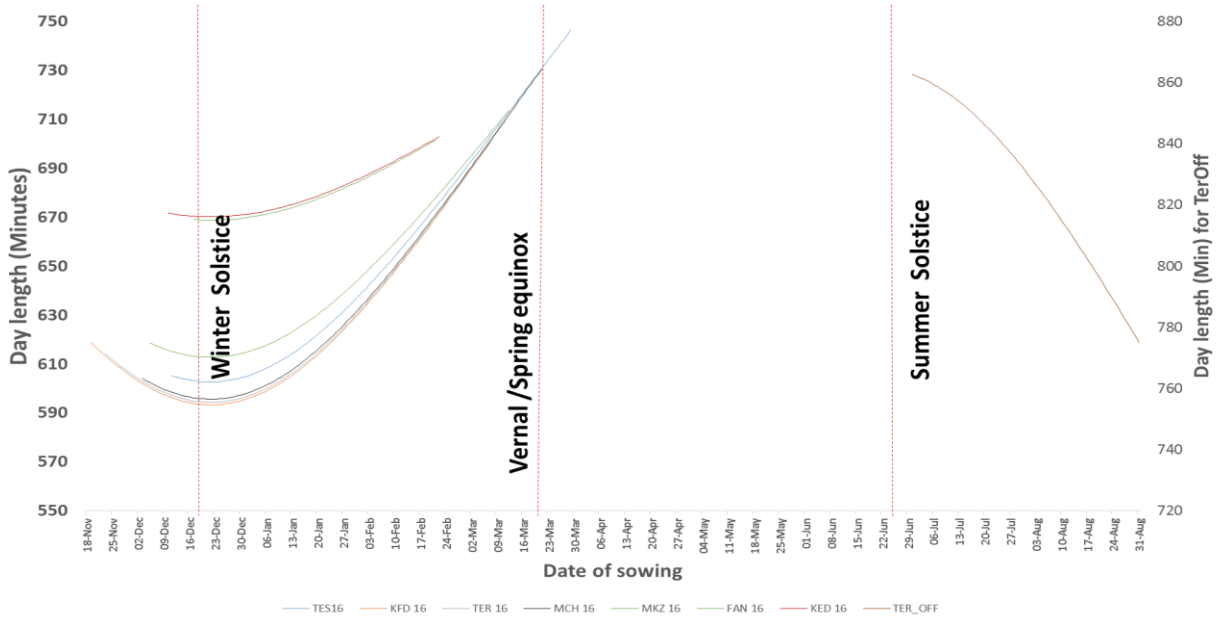


Fig 1.1. Distribution of day length at eight locations, Kaedi (Mauritania), Fanaye (Senegal), Marchouche, Melk Zhar and Tassaout (Morocco), Terbol main season, Terbol off-season, and Keardan (Lebanon) from sowing to heading.

In order to establish marker-trait association, 384 accessions were genotyped by 35K Affymetrix Axiom wheat breeders array (www.affymetrix.com) at Trait Genetics (Gatersleben, Germany) following the manufacturer instructions. A total of 10 sub-populations were identified as explained by Kabbaj et al. (2017).

Statistical analysis

Cumulative growing degree days (CGDD) and cumulative day length (CDL)

To estimate the growing degree days (GDD), the average daily temperature from planting to flowering was calculated for each site following Klepper et al. (1988). In case of wheat, a range of 0 to 32^{o}C temperature is considered optimal and, therefore, those environments depicting lower than 0^{0}C and higher than 32^{0}C were converted keeping these limits in consideration (https://ndawn.ndsu.nodak.edu). Cumulative growing degree days (CGDD) was calculated by

adding together all of the positive values from planting to the average days of heading for each genotype. Similarly, day length was obtained by using the statistical package RX64 version 3.3.2, library (maptools) and Cumulative day length (CDL, m) from emergence to flowering was calculated by summing up the daily photoperiod for each genotype in all environments.

In order to characterize environments, hierarchical cluster analysis based on Euclidean distance and average linkage method and principal component analysis (PCA) was conducted with the climatic variables measured in each environment. To determine phenological environment (PhEnv), combined analyses of variance across environments and pheno-environments were conducted for DTH, CGDD and CDL. To explain the source of variation between pheno-environments, GxE interaction was studied using the sum of squares (SS) of pheno-environments. The broad sense heritability was estimated by the following formula suggested by Falconer and Mackay (1996):

$$H = \frac{\sigma_g^2}{\sigma_p^2} * 100$$

Where;

σ_g^2 is the genotypic variance and σ_p^2 is the phenotypic variance.

The genotypic and phenotypic variance components were estimated based on the method suggested by Burton and Devane (1953).

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2 + \sigma_{ge}^2$$

$$\sigma_g^2 = \frac{MS_g - MS_e}{r}$$

$$\sigma_{ge}^2 = \frac{MS_{ge} - MS_e}{r}$$

Where;

MS_g and MS_{ge} are the mean square due to genotype and GxE interaction, MS_e is the error mean square, and r is the number of replicates.

Each year-location combination was considered as one environment. Best linear unbiased estimates of flowering time (FT-BLUEs) were derived for the individual environment, pheno-environments (PhEnv) and across environments based on the linear mixed model. All analyses were carried out with GENSTAT (version 2010) and free statistical package RX64 version 3.3.2.

Linkage disequilibrium decay (LD decay)

For determining linkage disequilibrium (LD), the genetic position of SNPs markers was retrieved by aligning marker sequences against the Svevo physical map reported by Cattivelli et al. (unpublished 2018) and then by assuming the genetic position of the nearest marker for which a genetic position was available, based on the Svevo genetic map available to ICARDA. In order to minimize the variation of LD estimates produced by considering the rare alleles, only SNP alleles with minor allele frequency (MAF) higher than 0.05 were considered for the pairwise LD calculation. LD decay rate was estimated by calculating r^2 for all pairs of SNP loci within the same chromosome (intra-chromosomal pairs) against the corresponding genetic distances. Marker-wise R^2 was calculated using the software HaploView (www.broadinstitute.org/haploview/haploview) while the nonlinear fitting was carried out using the statistical software R based on the Weir's formula relating effective population size, recombination rate and genetic distance (Rexroad & Vallejo, 2009). Data were plotted using the R package ggplot2 (Wickham, 2016). The genetic distances corresponding to LD decay to r^2= 0.5 and r^2 = 0.3 were inspected to define the average QTL confidence interval (CI is the interval within which the most associated marker contains the causal gene at 95% confidence).

The association analysis was performed with TASSEL version 5.2.38. The marker-trait association test was carried out using mixed linear model (MLM) based on the kinship value and with population structure which minimizes the false positive and increases the statistical power to conduct the analysis for across environments as well as for five pheno-environments and 13 individual environments. In each case, landraces and elite lines were measured separately. Total markers used for the association analysis were 7,740

which also comprised flowering time markers, *PPD-A1, PPD-B1*, and *VRN-A1*. Once more, all polymorphic markers were filtered for minor allele frequency (MAF >0.05) and only 5,067 and 2,824 markers were considered for GWAS analysis in landraces and elite germplasm, respectively. Thus, assuming a total genetic map length equal to 2,600 cM for durum wheat (Maccaferri et al., 2015), the marker density in this experiment was equal to 1.94 and 1.08 informative markers per cM for landraces and elite cultivars, respectively. For testing the significance of marker-trait association (MTA), the criterion of Bonferroni test (P-value/total number of independent SNP_s tested) provides a strict threshold and therefore, the LOD3 threshold was considered in the present study. It is the threshold currently adopted in many GWAS studies (Han et al., 2016). To correctly estimate and rank the QTLs based on the explained percent of phenotypic variance, a factorial regression model was adopted to determine the marker effect for five pheno-environments and across environments considering all significant markers in a global model. To reduce the effect of false-positive MTAs, population structure was included as a covariate in the regression model. The purpose to include the population structure as a covariate in the model was to remove its parallel and side-effect on DTH phenotypes of the germplasm. In addition, to take into account the effect of linked markers on the same chromosome, LD results were considered in the marker selection phase for landraces and elite germplasm, separately.

Candidate gene approach

Candidate genes were identified based on the physical linkage information for significant QTLs on each chromosomal segment. Two or more QTL peaks with less than 5 Mbp distance were considered as the same QTL. Within each QTL, marker showing stronger association (lower p-value) was chosen as tag or peak marker. Confidence interval (CI) was determined by subtracting and adding 2.5 Mbp to the position of the first and the last significantly associated markers of the QTL. Annotation of the genes present within the QTL confidence intervals was retrieved from the Svevo physical map (Cattivelli et al. unpublished 2018). Given the evolutionary similarity between durum and bread wheat, gene annotation was integrated with information from the bread wheat evidence based gene discovery tool, Knetminer (http://knetminer.rothamsted.ac.uk). Knetminer is a database where, for each gene in the bread wheat genome, evidences such as gene symbol, available literature on the gene or any of its orthologs, affected phenotypes, etc., are available.

In order to access the Knetminer database, we first searched for the ID of the bread wheat orthologs to the genes in the QTL confidence intervals and then searched for the bread wheat genes associated to the key-words “flowering time”, “heading time”, “vernalization”, and “photoperiod”. Concurrently, orthologs to the durum wheat genes were also searched within the QTL intervals. Based on the mapping position of significant markers and the average LD and LD decay rate analysis, QTL confidence intervals were inspected for their gene content along with functional annotations. In some cases, proteins could be associated with a flowering gene linked with photoperiod and vernalization response. The candidate genes for these functional proteins were retrieved from the literature and Knetminer reported for flowering time in different plant species.

Results

Phenotypic variation for flowering time

The results showed that days to heading among 384 durum lines ranged from 65 to 137 days, with an average of 97 days across the 13 environments. The best linear unbiased estimations (BLUEs) for heading time across the environments ranged from 71 to 107 days with an average of 87 days. Most of the elite lines occupied the early segment of heading day (HD) distribution while landraces flowered late with few exceptions (Fig. 1.2).

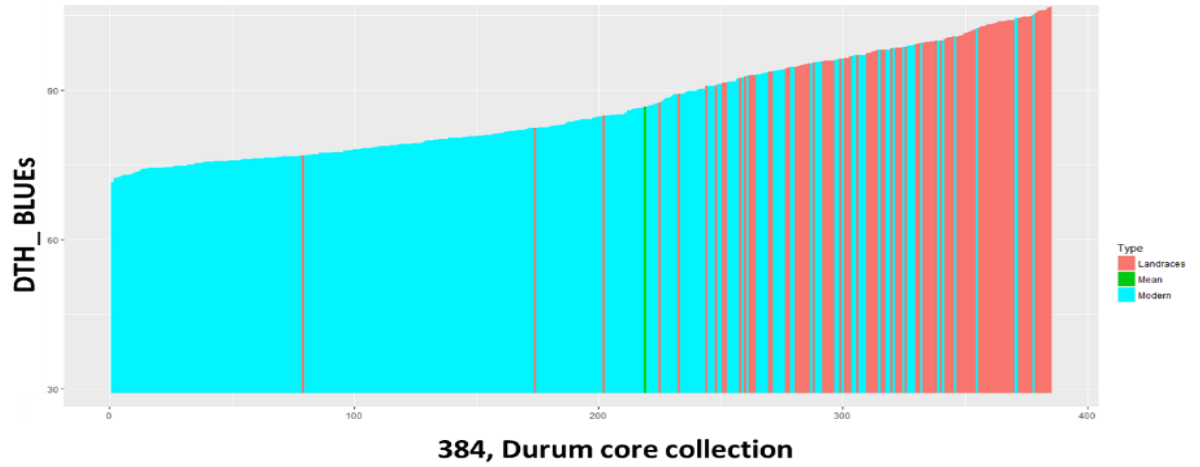


Fig 1.2: Distribution of 384 durum lines on the basis of days to heading (BLUEs) across environments

Average temperature distribution at 13 environments is presented in Fig 1.3. Temperature patterns during the crop season at 13 environments showed that the maximum temperature from sowing to flowering ranged between 23.6 ^{0}C at Kfardan and 41 ^{0}C at Kaedi whereas the minimum temperature ranged between -12.5 ^{0}C at Terbol and 15 ^{0}C at Kaedi. Meteorological data of 13 environments revealed that Kaedi is the hottest environment whereas Terbol had long periods of low temperature during winter season which is essential for meeting the strong vernalization requirements.

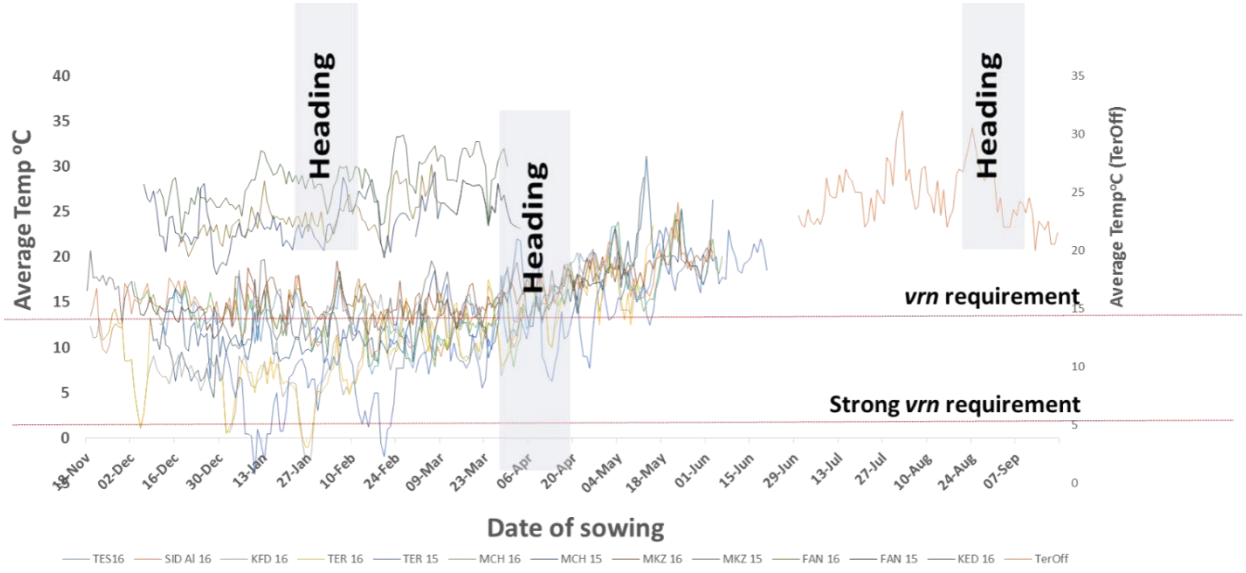


Fig 1.3: Average temperature distribution during the crop season (sowing to maturity) at 13 environments. The environments included are Kaedi (KED) 2015 and 2016 in Mauritania, Fanaye (FAN)2015 and 2016 in Senagal, Marchouche (MCH) 2015 and 2016, Melk Zhar (MKZ)2015 and 2016 and Tassaout (TES)2016 in Morocco, Terbol (TER)2015 and 2016, Kfardan (KFD) 2016 and Terbol off (TerOff) season 2016 in Lebanon.

Variation for CGDD and CDL ranged from 1049 to 2019 and 43871 to 91760, respectively (Fig. 1.4). The distribution pattern of BLUEs for DTH, CGDD, and CDL of 384 durum core collection is shown in boxplots (Fig 1.4a, 1.4b and 1.4c).

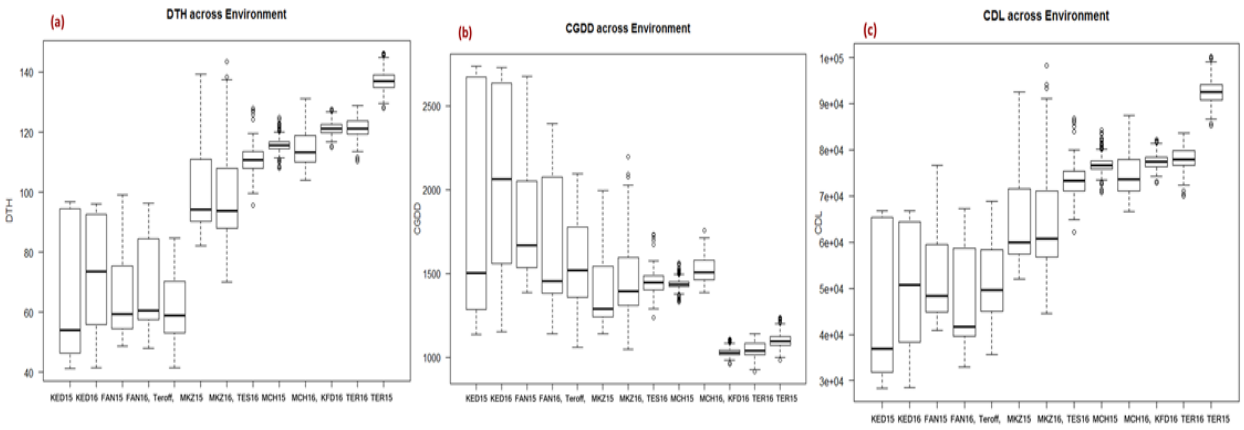
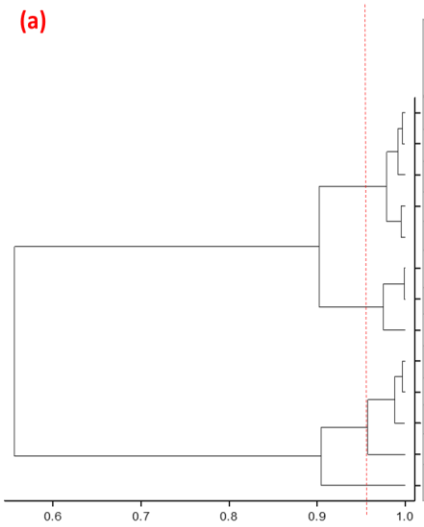


Fig 1.4. Boxplots showing the distribution pattern of DTH, CGDD, and CDL among 384 durum core collection of ICARDA evaluated at 13 environments.

Similarly, cumulative day length (CDL) at sowing (from 597 to 862 minutes) and flowering (43871 to 91760 minutes) describes the range of diversity according to latitudes at eight different locations. Short days (12h continuous light), high temperature and same photoperiod at Fanaye and Kaedi were responsible for early flowering whereas flowering was delayed at Terbol due to its high altitude and relatively low maximum temperatures in spring (Fig.1.5a). The estimated broad-sense heritability (h^2) value for DTH and CGDD across the environments was 0.93 followed by 0.89 for CDL, suggesting that DTH and its associated traits (CGDD and CDL) are highly heritable traits in durum wheat.

(a)



Environment	Acronym	Pheno Envn	Coordinates	Max temp	Avr max tmp	Min Tmp	Avr min tmp	CGDD	CDL sowing	CDL flwr	DTH
Tassaout_2016	TES 16	1	29° 49' 48" N, 8° 34' 48" W	26.6	20.9	-0.2	4.9	1420.5	605.2	72702.0	110.6
Marchouch_2016	MCH 16	1	33° 34' 3.1"N, 6° 38' 0.1"W	27.7	20.1	1.6	6.2	1499.6	604.2	74046.2	114.8
Marchouch_2015	MCH 15	1	33° 34' 3.1"N, 6° 38' 0.1"W	30.2	18.0	2.1	6.7	1431.1	597.8	76966.2	115.8
Melkzhar_2016	MKZ 16	1	30.4278° N, 9.5981° W	30.4	22.3	2	7.5	1477.9	618.6	63856.1	98.0
Melkzhar_2015	MKZ 15	1	30.4278° N, 9.5981° W	31.4	20.4	0.4	6.7	1356.4	635.1	63322.5	100.1
Kfardan_2016	KFD 16	2	33°48'29"N, 35°59'22"W	23.6	14.6	-8.5	2.2	1049.2	618.7	77263.5	121.5
Terbol_2016	TER 16	2	33°48'29"N, 35°59'22"W	25	14.9	-9	1.7	1048.5	614.1	77806.2	121.5
Terbol_2015	TER 15	2	33°48'29"N, 35°59'22"W	27	14.5	-12.5	1.4	1165.0	598.9	91764.6	137.2
Fanaye_2016	FAN 16	3	16.5259° N, 15.2224° W	37.1	32.7	9.5	14.4	1593.8	669.0	46250.4	68.0
Fanaye_2015	FAN 15	3	16.5259° N, 15.2224° W	37.9	31.1	8.3	15.0	1495.7	671.2	43870.8	65.4
Kaedi_2015	KED 15	4	16.1536° N, 13.5032° W	41	31	10	18	1885.7	673.6	45973.0	67.4
Kaedi_2016	KED 16	4	16.1536° N, 13.5032° W	39	33.4	15	21.6	2019.3	671.6	50321.6	73.2
TerbolOff_2016	TerOff 16	5	33°48'29"N, 35°59'22"W	38.3	35.5	11.4	15.3	1537.0	862.3	51248.9	61.7

Fig 1.5a. Meteorological parameters along with CGDD, CDL, and DTH for 13 environments used for phenotyping of 384 durum wheat core collection

Determination of pheno-environments

In order to group 13 individual environments into more uniform phenological-environmental groups (here called Pheno-Environment - PhEnv), clustering analysis was done based on different climatic variables resulting into four pheno-environments at 95% of amalgamation (Fig.1.5a). Within the pheno-environment, Kaedi and Fanaye were heterogeneous due to high minimum temperature at Kaedi during the crop season in 2016. To investigate the variation within pheno-environments, genotype x environment interaction (GxE) was determined according to the sum of squares (SS) of grouped environments, which indicated heterogeneity between Fanaye and Kaedi. Based on the principal component analysis (PCA) and significant percentage of GxE variance, 13 environments were finally grouped into five pheno-environments (Fig.1.5b). PhEnv1 included five environments of Morocco (TES16, MkZ15, MKZ16, MCH15, and MCH16) whereas, PhEnv2 represented three environments of Lebanon (TER15, TER16 and KFD16). PhEnv3 and PhEnv4 included two environments each of Senegal (FAN15 and FAN16) and Mauritania (KED15 and KED16) whereas, PEnv5 represented Terbol off season of Lebanon.

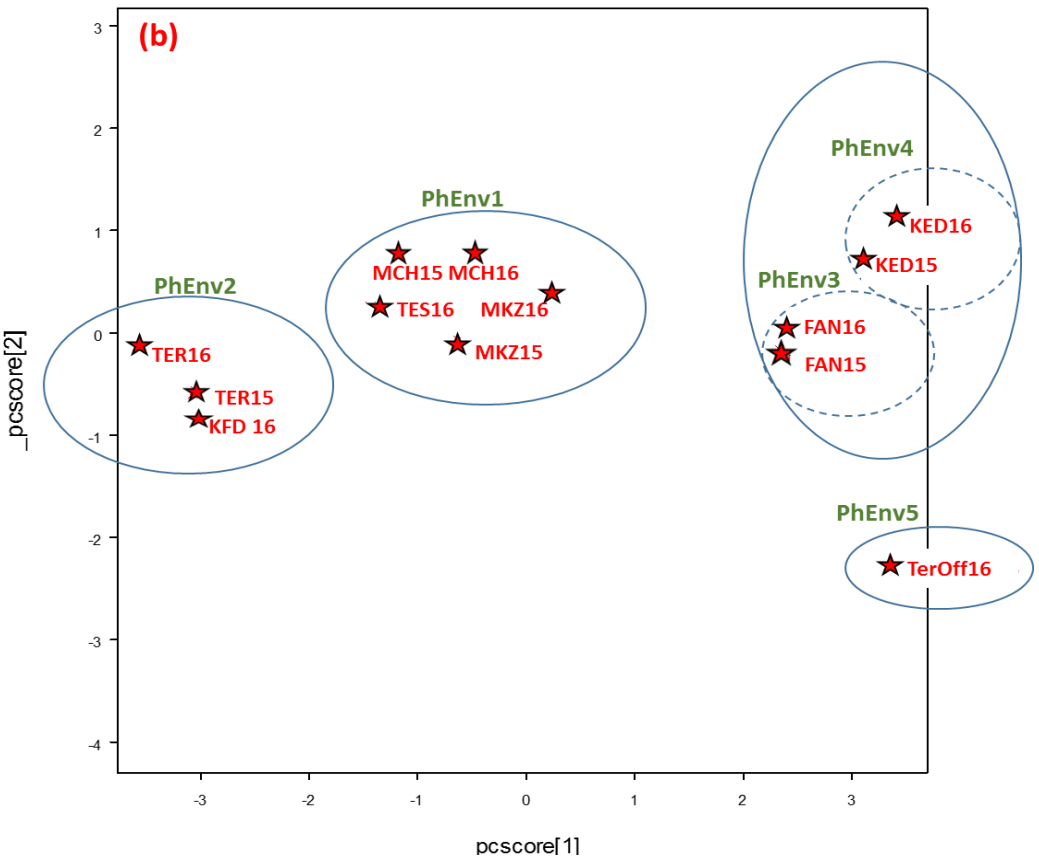


Fig 1.5b: PCA depicting the distribution of five pheno-environments based on climatic and phenotyping data.

Analysis of variance for GxE and G x PhEnv

Analysis of variance (ANOVA) was carried out over all environments as well as for the five Pheno-environments for 2015 and 2016. ANOVA revealed highly significant ($p<0.001$) variation for environment, genotype, and genotype x environment (GxE) interaction for heading date (Table 1.1). Combined ANOVA based on five pheno-environments showed that significant proportion of GxE interaction was attributed to G x PhEnv interaction. Results indicate that G x PhEnv interaction explained 68.7% of the GxE variation for DTH, 80.7% for CGDD and 66.7% for CDL (Table 1.1). These results suggest significant influence of environmental factors on flowering time in durum wheat. ANOVA for individual environment within the pheno-environment also indicated significant GxE interaction within each environment, further explaining the significant variability within pheno-environments (Table 1.2). Variance associated with PhEnv4 represented

by Kaedi (2015 and 2016) suggested that GxE interaction was small in comparison to other pheno-environments. This might be due to high temperature prevalent at Kaedi during 2016.

Table 1.1. Combined ANOVA for DTH, CGDD and CDL involving 384 durum lines at 13 environments as well as for five pheno-environments.

		Sum of square		
Source	**df**	**DTH**	**CGDD**	**CDL**
Environment	12	4.0E+06**	4.0E+08**	1.3E+12**
Pheno-Environment	4	3.8E+06**	3.8E+08**	1.1E+12**
Error	8	2.0E+05	2.2E+07	1.5E+11
Genotype	383	4.5E+05**	2.2E+08**	2.4E+11**
Genotype X Environment	4596	4.4E+05**	3.2E+08**	2.2E+11**
Genotype X Pheno-Environment	1532	3.0E+05**	2.6E+08**	1.5E+11**
Error	936	4.6E+03	1.8E+06	2.4E+09
Proportion of GxE explained by G x PhEnv		68.7%	80.7%	66.6%

**: P<0.001, df: degree of freedom, SS: sum of square, DTH: Days to heading, CGDD: cumulative growing degree days, CDL: cumulative day length.

Table 1.2. ANOVA of GxE interaction for DTH, CGDD and CDL in five pheno-environments.

Pheno-Environment	**df**	**Mean Sum of Square for GxE interaction**		
		DTH	**CGDD**	**CDL**
PhEnv1	1532	55.99	12205	2.91E+07
PhEnv2	766	08.25	1377	5.18E+06
PhEnv3	383	27.13	17912	1.36E+07
PhEnv4	383	99.03	93256	5.46E+07

Note: PhEnv5 represents only one environment and therefore, GxE interaction was not possible.

Correlations among phenological traits and correlation among pheno-environment for DTH

Since CGDD and CDL were derived from the heading days of each genotype, simple correlation coefficients among three traits namely, days to heading (DTH), cumulative growing degree days (CGDD) and cumulative day length (CDL) showed significantly positive correlation (0.99***).

Correlations among five pheno-environments for DTH suggested that PhenoEnv1 (Marchouche, Melk Zhar, and Tassaout), PhenoEnv2 (Terbol and Kfardan) and PhenoEnv3 (Fanaye) showed significantly positive correlation whereas PhenoEnv4 (Kaedi) showed the least correlations with PhenoEnv1 and phenoEnv2 (Fig 1.6). These results indicated strong but diverse climatic effect on phenology of genotypes among the five pheno-environments.

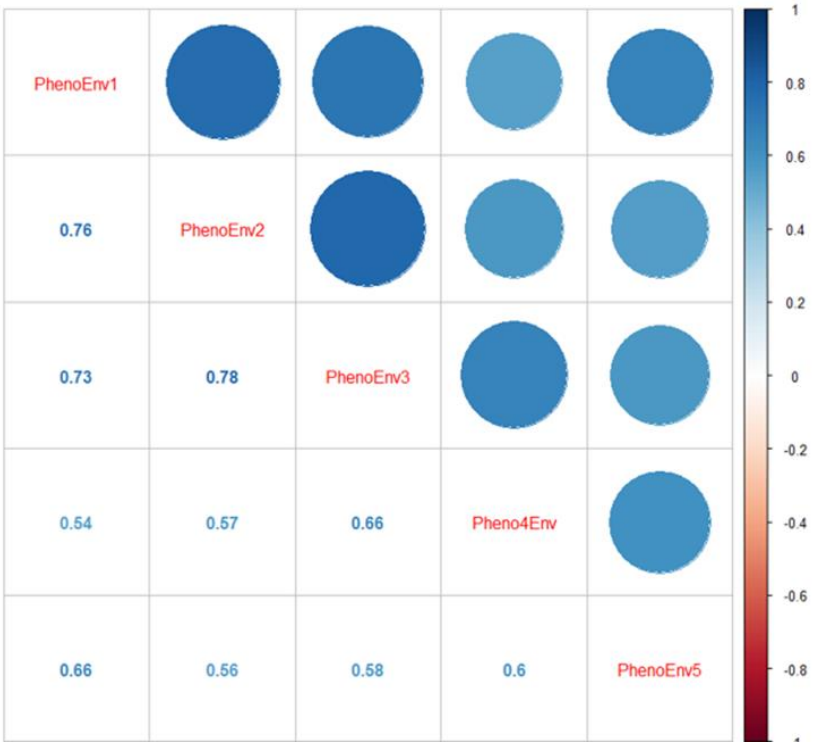


Fig 1.6: Phenotypic correlation between five phenological environments for days to heading (DTH) of 384 durum core collection of ICARDA.

Genotyping and population structure

Out of 384 durum lines genotyped by 35k Axiom Breeds Array, only 370 showed DNA quality sufficient for single nucleotide polymorphism (SNP) calls. A total of 35,143 SNPs were assessed, of these 11,642 (34%) failed to meet the minimum call rate, suggesting that these markers are located on the D genome, and therefore, not present in durum wheat. A total of 14,851 (42%) met the quality cut-off but remained monomorphic while 8,173 (36%) were found to be of high quality and polymorphic, and were finally used for GWAS study (Kabbaj et. al., 2017). Investigation of population structure is crucial for understanding marker-trait relationships and allele partitioning in the whole germplasm. The Q matrix groups the genotypes according to the

fraction of their genomes belonging to each of the sub-populations (as estimated based on the mapped markers). It is also crucial to estimate the optimal number of sub-populations based on model-based Bayesian analysis or Principal component analysis. In this study, 384 durum lines clustered into 10 groups. Four groups were composed of landraces and six groups included mostly cultivars and elite lines.

LD decay

Linkage disequilibrium extent is highly dependent on the mating system of the species and on the germplasm considered. A total of 5,076 SNPs in landraces and 2,825 SNPs in elite (MAF≥ 0.05) were used for evaluating the range of LD in durum wheat germplasm. First, r^2-value distribution of the unlinked marker-pairs (marker to marker genetic distance > 50 cM) was used to estimate the level of 'background LD' present in germplasm and mainly caused by population structure. The 95% percentile of r^2 distributions was estimated in landraces and elite germplasm (Figs 1.7a and 1.7b). The higher background-LD observed for landraces as compared to elite germplasm is indicative of stronger population structure present in the former germplasm. Subsequently, the pattern of LD decay rate was investigated in order to set appropriate confidence intervals to the QTLs identified in the GWAS. As expected in case of landraces the LD decay rate was very low and equal to 0.02 cM for r^2 values of 0.3 whereas for elite lines, LD decay rate was 0.06 cM for r^2 value of 0.5. Reimer et al. (2008) and Maccaferri et al. (2014) estimated a more relaxed LD decay rate for modern durum germplasm, in the 2-5 cM range. The elite durum lines included in the core collection herein considered are more diverse with complex pedigrees which could be one of the reasons to set low LD rate in the modern germplasm (Fig 1.7b). Based on these results, the GWAS-QTL confidence interval was set at 0.04 cM for landraces and at 0.06 cM for the elite germplasm for higher genetic resolution of this panel to determine unique position of QTLs of interest.

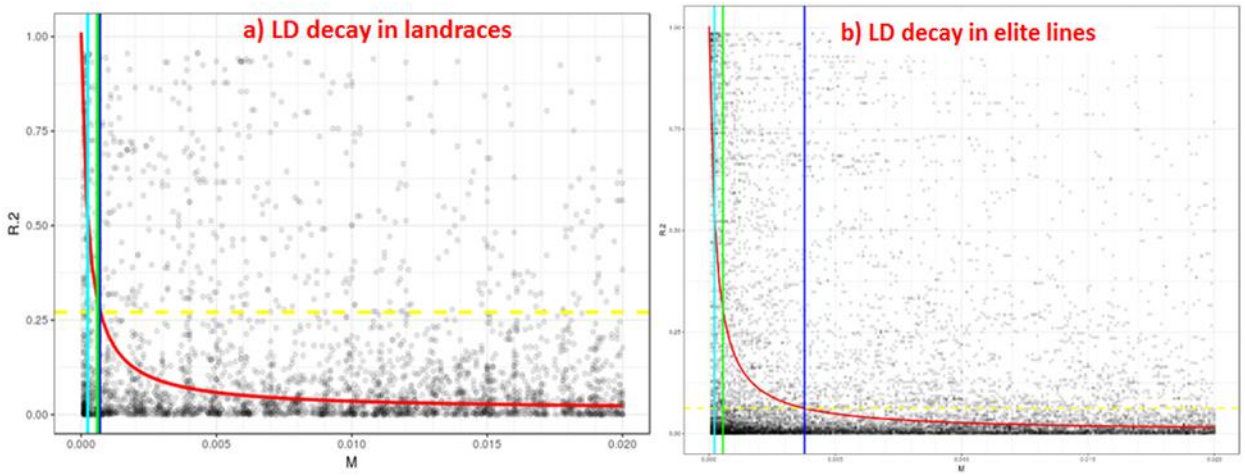


Fig 1.7. Scatter plot showing the linkage disequilibrium (LD) decay across the chromosomes. The physical distance in cM plotted against the LD estimate (R^2) for pairs of markers. The horizontal line indicates the 95% percentile of the distribution of the unlinked r^2. LD decay in a) landraces and b) elite lines of durum wheat.

Identification of photoperiod and vernalization responsive genotypes

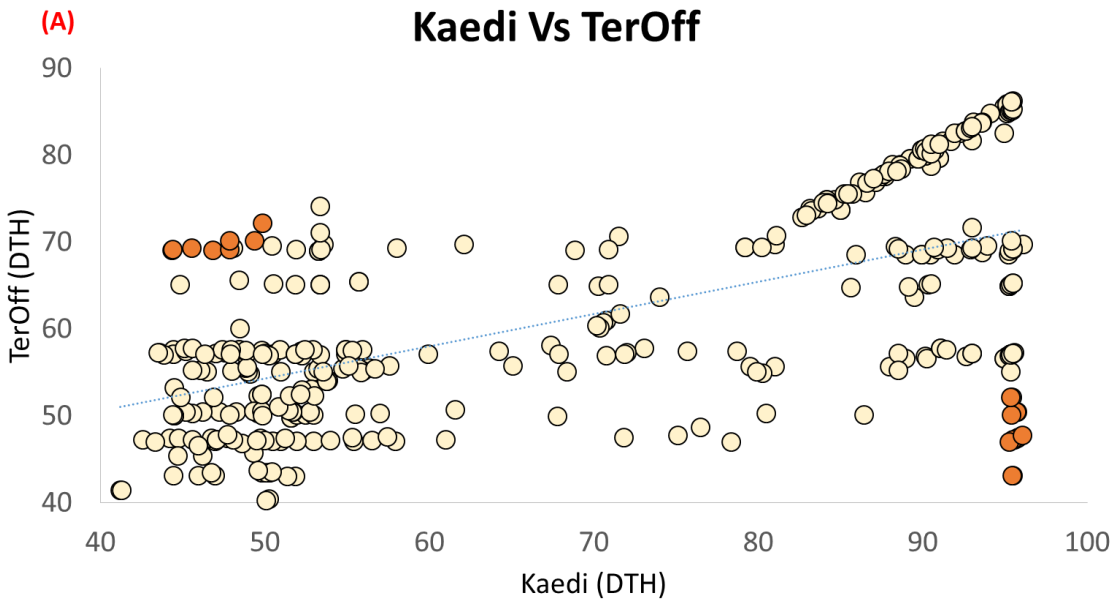
Evaluating the role of photoperiod and vernalization response is essential for better understanding of adaptation to a specific geographical location in wheat breeding. At the same time, it is also important to identify germplasm for early flowering based on different climatic features (Langer et al., 2014). For this purpose, two specific locations were selected based on its temperature and day length details to assess *PPD* and *VRN* responsive genotypes.

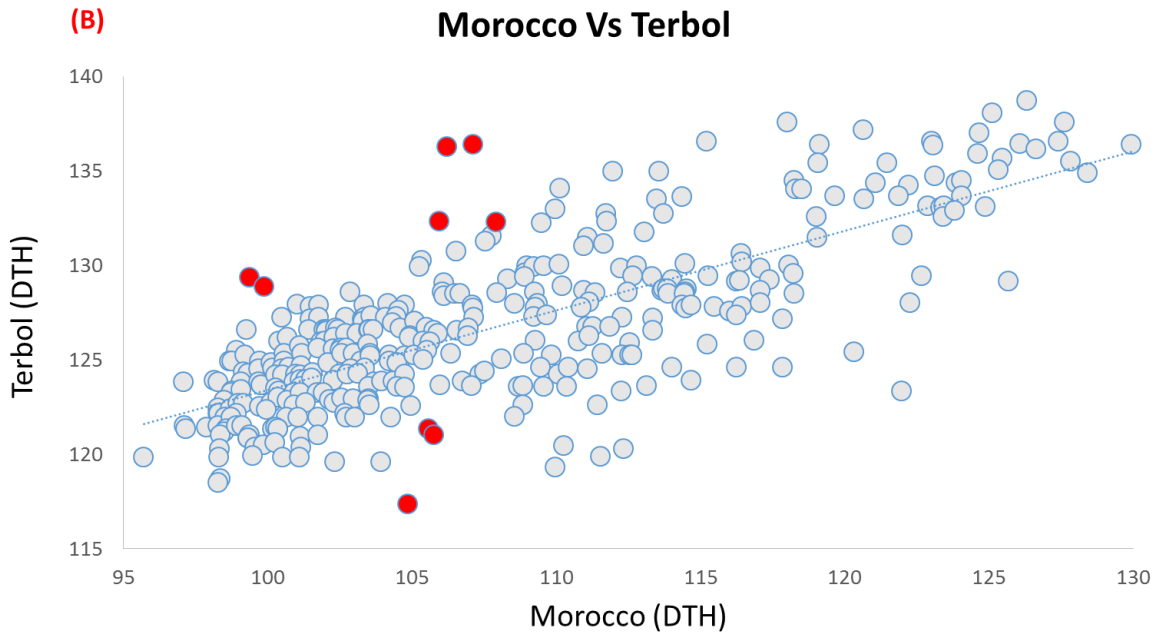
Among the 13 environments, two environments, Kaedi and Terbol off-season represent different day length with similar temperature regime (warm) and thus selected to explain the *PPD* responsive genotypes. Similarly, Morocco locations and Terbol main season with almost similar day length were compared to define *VRN* responsive genotypes as Terbol represents the coolest temperature in winter season and Morocco represents diverse temperature regimes. To understand the strong and mild vernalization requirements, Fanaye and Kaedi were observed where day length had similar effect with varied minimum temperature. Considering different climatic features, BLUEs of heading days from Kaedi versus Terbol off-season, Morocco versus Terbol and Fanaye versus Kaedi were compared to identify *VRN* and *PPD* responsive lines in durum core collection.

Based on the two-year DTH data of Kaedi and Terbol off-season, 20 lines were identified which flowered differently at these locations. Five genotypes, namely Egypt_118, Egypt_119, Egypt_181, Egypt_160, and Egypt_124 flowered early in Terbol off-season but late in Kaedi. Difference in day length was responsible for this variation in DTH which ranged from 45 to 52 days (Fig 1.8a). In contrast, four genotypes, Egypt_377, Egypt_314, Egypt_152, and Egypt_233 flowering early in Kaedi but late in Terbol off-season and difference in DTH varied from 20 to 25 days. These two locations had almost similar flowering days for many genotypes. This may be due to high temperature at both locations.

Similarly, five genotypes, Egypt_330, Egypt_216, Egypt_68, Egypt_155 and Egypt_185 flowered early in Morocco but late in Terbol main season while Egypt_51 and Egypt_261 flowered late in Morocco but early in Terbol. This variation was more prominent particularly at Tessout and Melk Zehr locations which were the warmest locations at Morocco. Lower temperature prolonged heading time difference in Terbol whereas vernalization requirements resulted in late flowering in Morocco which varied from 12 to 30 days for heading time in durum core collection (Fig 1.8b).

Fanaye and Kaedi locations had almost similar day length but due to prevalence of higher temperature at Kaedi as compared to Fanaye, genotypes desired strong vernalization to flower while temperature at Fanaye fulfilled the mild requirement of vernalization (Fig1.8c). Thus, five genotypes, Egypt_124, Egypt_160, Egypt_181, Egypt_182 and Egypt_319, flowered early in Fanaye while Egypt_344, Egypt_355 and Egypt_357 were early in Kaedi (differences ranged from 19 to 49 days) which explain the effect of mild and strong vernalization requirements in durum core collection. These results showed the strong effect of day length and temperature regime in durum wheat.


(A)
Kaedi Vs TerOff
TerOff (DTH)
Kaedi (DTH)
90
80
70
60
50
40
40
50
60
70
80
90
100


(B)
Morocco Vs Terbol
Terbol (DTH)
Morocco (DTH)
140
135
130
125
120
115
95
100
105
110
115
120
125
130

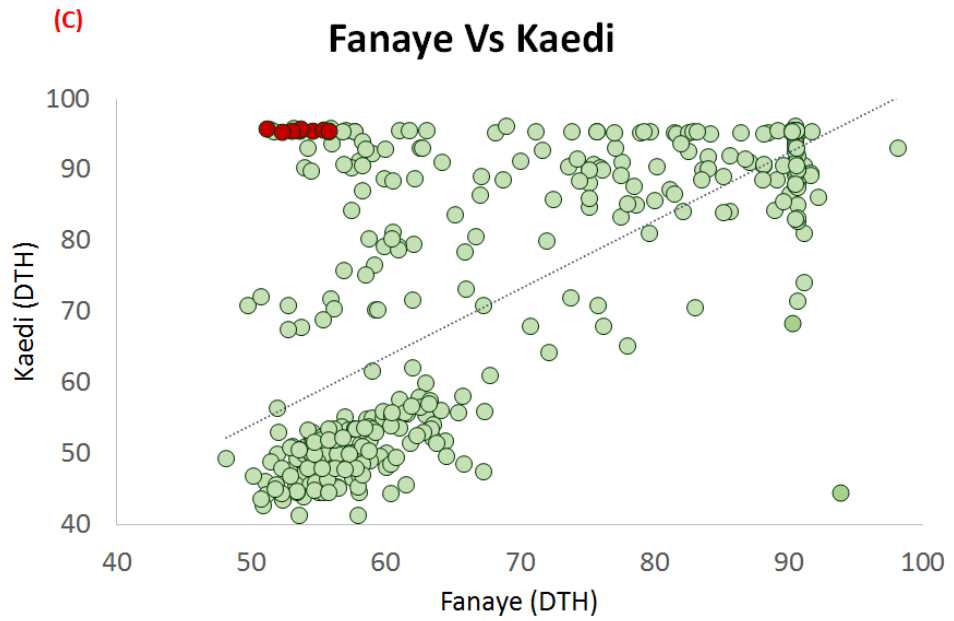


Fig 1.8: Comparison of BLUEs of DTH in a) Kaedi versus Terbol off-season, b) Morocco versus Terbol main season, and c) Fanaye versus Kaedi, illustrating the PPD and VRN responsive genotypes of durum wheat. (Red dots are describing the early flowering in one environment and late in another or vice versa)

Manhattan plot

Manhattan plots for CGDD and CDL for across environments and five pheno-environments are depicted in Fig 1.9 where the y-axis plots -log10 (P-values) against the physical position (Mb) of SNPs markers for landraces and elite germplasm. In case of landraces, most significant QTLs were located on chromosome 2A and 2B for all pheno-environments and across environment except PhEnv1 (Fig 1.9a). Additionally, PhEnv2 (Terbol) followed by PhEnv5 (Terbol off-season) had the highest number of QTLs while PhEnv4 (Kaedi) and PhEnv1 (Moroccan stations) had least number of significant QTLs. Similarly, elite germplasm showed higher number of significant QTLs in PhEnv2 and PhEnv3 followed by across environments (BLUEs) located on 1B, 2A, 2B, 3A, 4B, 5A, 6B, 7A and 7B chromosomes whereas PhEnv1 and PhEnv5 revealed least number of significant QTLs (Fig 1.9b). Among the five pheno-environments, PhEnv2 and PhEnv3 along with across environment (BLUEs) had best LOD on Chr2A. PhEnv2 had revealed 8 significant QTLs in landraces and 12 in elite lines dispersed across seven chromosomes.

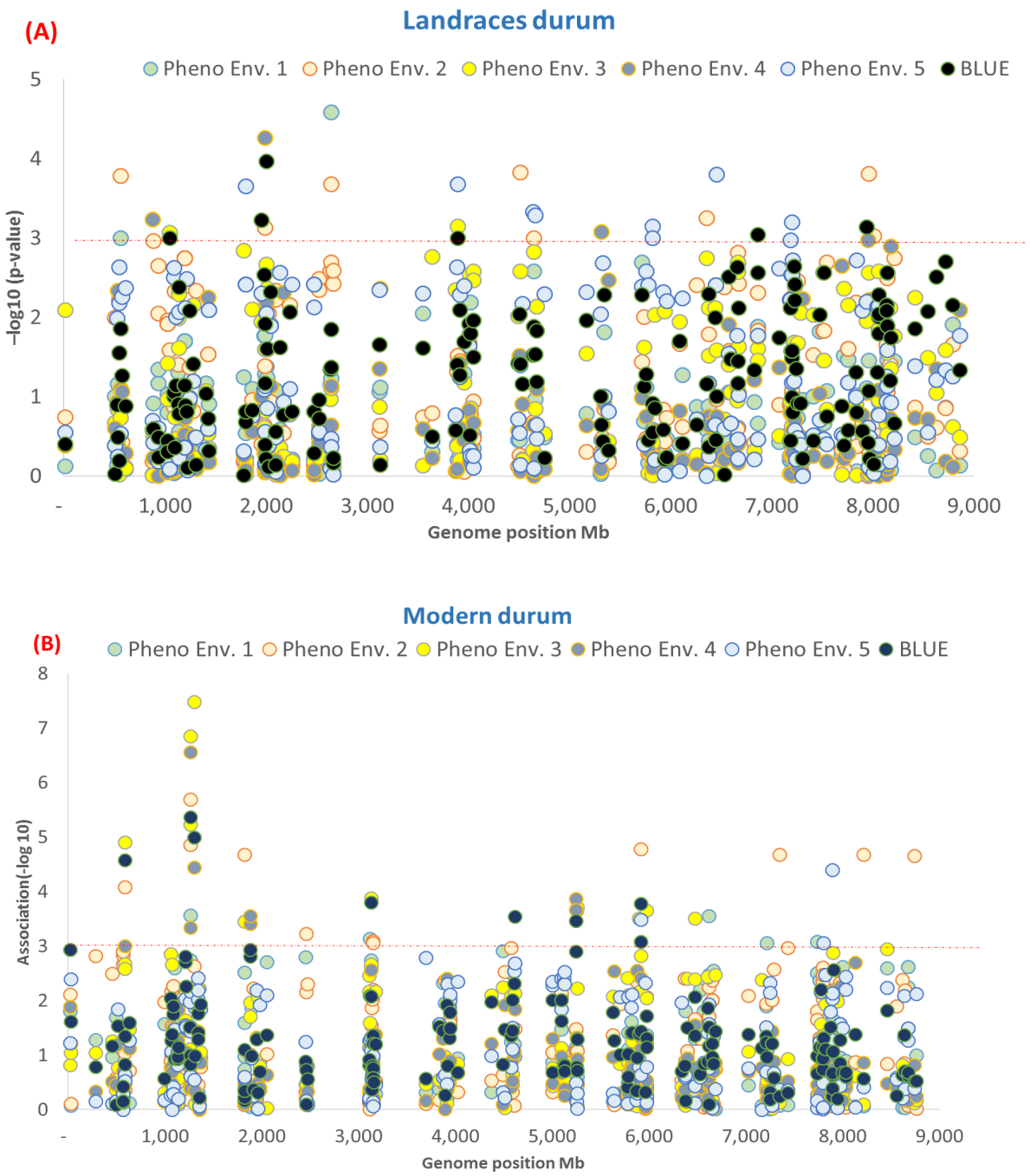


Fig 1.9: Manhattan plot for heading days in five pheno-environments and across environments (BLUEs) for A) landraces and B) Elite lines of durum wheat

Genome wide association study

BLUEs of DTH, CGDD and CDL were used to perform association analysis across environments, five pheno environments and 13 individual environments for landraces and elite lines. Results

showed that 34 and 71 markers for DTH, 34 and 63 for CGDD, and 27 and 66 markers for CDL were significant at threshold 3 among the landraces and elite lines, respectively. Since most of the markers were common among DTH, CGDD and CDL, a particular trait was assigned to a common marker based on high LOD value. Seven markers in landraces and 12 markers in elite lines were significant across the environments and pheno-environments with some exceptions. Based on the corresponding map position of significant markers, QTLs were identified in each chromosome resulting in 30 QTLs with 34 markers in landraces and 47 QTLs with 66 markers in elite lines. To sum up the significant QTLs with high percentage of variance, factorial regression model along with population structure was adopted to determine the marker effect at each of the five pheno-environments and across environments. This analysis resulted in 27 significant QTLs for landraces and 36 QTLs for elite lines. Since few markers were still closely linked on the same chromosome as a consequence of linkage disequilibrium, LD analysis was carried out to confirm the independence of linked markers in each of the five pheno-environments and across environment. Based on LD analysis, 20 and 27 highly significant QTLs for landraces and elite lines were identified at P-value <0.005 (Table 1.3a, b). This table illustrates significant QTLs along with high peak marker, LOD value, minor allele frequency (MAF), r^2, assigned trait and candidate genes for landraces (Table 1.3a) and elite germplasm (Table1.3b). Candidate genes were identified for significant QTLs based on the combined strategy that revealed interesting correspondences. Significant QTLs with its novel gene content and associated protein identified in the present study are described below:

Significant QTLs for landraces

Among 30 major QTLs derived from 34 significant markers in landraces, 20 QTLs accounted for significant part of variance, high r^2 and MAF (ranged from 0.1 to 0.5) with more than 3 –log10 (p-value, LOD). The results revealed two QTLs for PhEnv1 and PhEnv3, eight QTLs for PhEnv2, four QTLs for PhEnv4, and seven QTLs for PheEnv5, while six QTLs were significant across environments.

Table 1.3a: Significant QTLs with its MAF, r2, LOD, F value, variance, trait and candidate genes identified in durum landraces

	Landraces					PhEnv 1			PhEnv 2			PhEnv 3			PhEnv 4			PhEnv 5			Across Env				
QTL	Peak marker ID	Chr	Position	MAF	r2	LOD	F	Var%	LOD	F	Var%	LOD	F	Var%	LOD	F	Var%	LOD	F	Var%	LOD	F	Var%	Trait	Candidate genes
1	AX-94736642	1A	561710549	0.2	0.22	3.0	**	25.0	3.8	**	17.0	0.7	-	-	1.1	-	-	0.5	-	-	1.9	-	-	CGDD	
2	AX-94635647	1B	317460455	0.1	0.18	0.4	-	-	2.9	-	-	3.1	**	13.5	3.2	**	15.5	0.1	-	-	3.0	**	17.9	DTH	*HUB2*
3	AX-94963816	2A	556658473	0.2	0.17	0.5	-	-	0.1	-	-	0.2	-	-	0.1	-	-	3.7	0.0	3.6	0.7	-	-	DTH	*GRF5, VIT1 & NRPE1*
4	AX-94488406	2A	744540573	0.2	0.18	1.8	-	-	3.2	**	11.8	1.6	-	-	0.3	-	-	0.2	-	-	3.2	0.1	2.6	DTH	*SCL3,TGA7 & HO1*
5	AX-94939920	2B	8627526	0.2	0.23	1.4	-	-	0.9	-	-	2.7	-	-	2.2	-	-	2.0	-	-	4.0	0.1	1.7	DTH	*TCP1, DREB1C & PVA11*
6	*PpdB*	2B	56297789	0.5	0.28	0.5	-	-	0.1	-	-	0.8	-	-	4.3	**	81.4	1.1	-	-	1.2	-	-	DTH	
7	AX-94452589	3A	5383646	0.4	0.21	4.6	**	6.0	3.7	**	4.6	0.2	-	-	1.1	-	-	0.4	-	-	1.8	-	-	CGDD	*PIE1*
8	AX-95021774	3B	765093681	0.4	0.17	0.2	-	-	1.5	-	-	3.2	**	8.7	0.6	-	-	3.7	**	11.5	3.0	**	7.8	CGDD	*PIE1*
9	AX-94439386	4A	597712617	0.3	0.18	1.4	-	-	3.8	**	5.4	2.6	-	-	0.1	-	-	0.1	-	-	1.4	-	-	DTH	*HUB2*
10	AX-95217431	4A	725814985	0.2	0.18	0.2	-	-	3.0	**	10.0	0.3	-	-	0.6	-	-	3.4	**	22.3	1.5	-	-	DTH	*UBC4/Hd6*
11	AX-95101347	4B	12541653	0.1	0.15	1.0	-	-	0.1	-	-	0.8	-	-	0.1	-	-	3.3	**	5.8	1.5	-	-	DTH	*TaBRI1 & TaSERK*
12	AX-94920631	5A	10352444	0.1	0.17	0.5	-	-	0.1	-	-	0.1	-	-	3.2	**	15.1	0.3	-	-	0.6	-	-	DTH	
13	AX-94978708	5A	511042719	0.2	0.13	0.6	-	-	0.3	-	-	0.0	-	-	0.2	-	-	3.2	-	-	0.9	-	-	CGDD	*Vrn1, SVP, RRP6L1 & AGL18*
14	AX-94939814	5B	392721850	0.2	0.14	0.6	-	-	3.3	**	7.9	2.8	-	-	0.0	-	-	0.0	-	-	1.2	-	-	DTH	
15	AX-94653414	5B	492698137	0.1	0.18	0.2	-	-	1.0	-	-	1.6	-	-	0.7	-	-	3.8	0.1	1.5	1.0	-	-	DTH	*RF2b, PCF2 & TR*
16	AX-94930415	6A	195636135	0.1	0.17	1.9	-	-	2.3	-	-	1.6	-	-	1.8	-	-	1.8	-	-	3.1	**	13.9	CGDD	
17	AX-94761286	6A	530569927	0.2	0.17	0.5	-	-	1.1	-	-	0.4	-	-	0.3	-	-	3.2	**	31.1	1.5	-	-	CGDD	
18	AX-94451862	6B	686030300	0.3	0.18	1.1	-	-	0.4	-	-	2.0	-	-	1.1	-	-	2.2	-	-	3.1	0.0	3.9	CDL	
19	AX-94667805	7A	8306193	0.3	0.05	0.0	-	-	3.1	**	4.0	0.0	-	-	3.0	-	-	0.6	-	-	0.4	-	-	DTH	*SVP, AGL18 & SPY*
20	AX-94634646	7A	21115627	0.1	0.06	1.5	-	-	4.0	*	3.0	2.6	-	-	0.1	-	-	0.1	-	-	1.1	-	-	DTH	*SVP & AGL15*

MAF: minor allele frequency, LOD: -log10(p-value), **: P<0.001, Var%: accounted variance, PhEnv: Pheno-environment, AcrossEnv: Across environment

Table 1.3b: Significant QTLs with its MAF, r2, LOD, F value, variance, trait and candidate genes identified in durum elite lines

	Elite lines					PhEnv 1			PhEnv 2			PhEnv 3			PhEnv 4			PhEnv 5			Across Env				
QTL	Peak marker ID	Chr	Position	MAF	r2	LOD	F	Var%	LOD	F	Var%	LOD	F	Var%	LOD	F	Var%	LOD	F	Var%	LOD	F	Var%	Trait	Candidate genes
1	AX-94498055	1B	313590	0.1	0.09	0.5	-	-	4.1	**	7.5	4.9	**	6.9	3.0	*	0.5	0.6	-	-	4.6	**	25.4	DTH	
2	AX-94385320	2A	36350414	0.1	0.13	0.3	-	-	2.6	-	-	7.5	**	25.8	4.4	**	6.6	0.5	-	-	5.0	**	16.5	DTH	*PPD,PRR & SRT1*
3	*Ppd-A1*	2A	36577899	0.8	0.17	3.6	**	1.2	5.7	**	2.3	6.8	**	2.2	6.6	*	0.7	2.2	-	-	8.5	**	20.7	DTH	*PPD*
4	AX-94460586	2A	556826480	0.1	0.08	0.7	-	-	4.7	**	1.1	3.5	*	2.1	0.5	-	-	0.6	-	-	0.2	-	-	CDL	*GRF5 & VIT1*
5	AX-94956877	2B	54211755	0.5	0.06	0.1	-	-	1.0	-	-	2.0	-	-	3.6	**	3.7	0.2	-	-	3.0	-	-	DTH	*SRT1 TKL-2 & BHLH74*
6	*PPD-B*	2B	56297789	0.7	0.16	1.7	-	-	3.6	**	7.6	1.3	-	-	4.5	**	30.0	-	-	-	4.2	**	2.0	DTH	*PPD*
7	AX-94452589	3A	5383646	0.4	0.05	0.5	-	-	3.2	*	2.0	0.5	-	-	0.1	-	-	0.2	-	-	0.1	-	-	CGDD	
8	AX-94479255	3A	676783381	0.2	0.05	3.1	-	-	3.1	**	5.5	3.9	**	1.3	1.2	-	-	1.3	-	-	3.8	**	1.7	DTH	
9	AX-94973426	3B	306838	0.5	0.05	0.5	-	-	3.1	*	2.0	0.6	-	-	0.8	-	-	0.7	-	-	1.2	-	-	CGDD	*DXR*
10	AX-95630216	4A	687435178	0.1	0.04	1.3	-	-	3.0	**	2.0	2.1	-	-	0.4	-	-	0.8	-	-	1.4	-	-	CGDD	LRR-RLKs
11	AX-94554200	4B	26874224	0.1	0.06	1.0	-	-	1.5	-	-	2.1	-	-	1.8	-	-	3.5	*	1.1	3.5	**	1.2	DTH	
12	AX-95630515	4B	656495066	0.2	0.05	1.5	-	-	3.7	**	19.0	0.7	-	-	3.9	**	14.9	0.2	-	-	3.5	*	1.9	DTH	FACoR
13	AX-94577903	5A	11396690	0.1	0.07	0.5	-	-	0.5	-	-	3.7	**	5.0	0.1	-	-	0.3	-	-	0.7	-	-	DTH	*DUT, AGAL2 & RBG8*
14	*VRN-A1*	5A	549156928	0.2	0.03	0.4	-	-	1.6	-	-	3.0	*	20.0	2.6	-	-	3.9	**	10.0	0.6	-	-	DTH	*vrn*
15	AX-95213349	5A	644672371	0.1	0.06	0.9	-	-	0.4	-	-	3.5	**	3.6	0.6	-	-	0.8	-	-	1.4	-	-	DTH	CSN1, PCS1 & DREB1F
16	AX-95071189	5A	665732472	0.1	0.06	0.6	-	-	0.5	-	-	3.5	**	3.6	2.4	-	-	3.5	**	4.2	3.8	**	5.2	DTH	*RGA & SMD3A*
17	AX-95140644	5B	61484239	0.2	0.05	0.4	-	-	0.2	-	-	3.7	**	2.4	0.3	-	-	0.1	-	-	1.1	-	-	DTH	PIF4
18	AX-95259336	5B	557607202	0.1	0.06	0.3	-	-	0.8	-	-	3.5	**	8.2	0.8	-	-	0.4	-	-	1.4	-	-	DTH	*RRP6L1, SVP, AGL18 & SPY*
19	AX-94531833	5B	700885816	0.1	0.05	3.6	**	2.3	1.7	-	-	0.1	-	-	0.6	-	-	1.9	-	-	1.5	-	-	DTH	PFT, LUH & HUB2
20	AX-94707895	6B	6588578	0.1	0.05	3.1	**	5.4	1.2	-	-	0.5	-	-	0.2	-	-	1.1	-	-	1.0	-	-	DTH	PHY
21	AX-94805681	6B	136694784	0.1	0.08	0.1	-	-	4.7	**	7.0	0.1	-	-	0.5	-	-	0.4	-	-	0.2	-	-	DTH	
22	AX-94637897	6B	528609908	0.4	0.04	3.1	**	2.6	1.6	-	-	0.2	-	-	0.7	-	-	3.1	**	8.4	1.0	-	-	DTH	
23	AX-95080277	7A	3076784	0.4	0.06	1.6	-	-	0.2	-	-	0.5	-	-	0.3	-	-	4.4	**	5.9	0.7	-	-	DTH	NTL9, BAG7 & GPA1
24	*VRN3*	7A	69364420	0.1	0.03	0.5	-	-	0.2	-	-	3.2	**	30.0	2.3	-	-	0.5	-	-	2.0	-	-	DTH	*vrn*
25	AX-94905964	7A	323655493	0.5	0.08	0.1	-	-	4.7	**	2.7	0.1	-	-	0.9	-	-	0.4	-	-	0.6	-	-	DTH	*PTB*
26	AX-94701740	7B	127472771	0.4	0.01	0.2	-	-	4.7	**	6.8	0.1	-	-	0.8	-	-	0.4	-	-	0.5	-	-	DTH	*SYD, CONSTANS*
27	AX-94878591	7B	685133195	0.1	0.03	0.8	-	-	3.4	*	3.0	0.3	-	-	1.2	-	-	0.1	-	-	1.4	-	-	DTH	*NRPD3B*

MAF: minor allele frequency, LOD: -log10(p-value), **: P<0.001, Var%: accounted variance, PhEnv: Pheno-environment, AcrossEnv: Across environment

Among the significant QTLs, photoperiod specific marker, *PPD-B1* (56.3 Mbp on Chr2B) was reported in landraces with high LOD (4.6) and significant variance (81%) in PhEnv4 (Fanaye). QTL3 (peak marker AX-94963816) located on Chr2A was prominent in the warmest location PhEnv5 (Terbol off-season), explaining 12-15% of the total variance. Annotation with the Svevo physical map showed 84 genes within the confidence interval of QTL3 (556 Mbp to 558 Mbp) and 59 of them were, to a certain extent, associated with the key-word “flowering time” in the Knetminer

database. Two genes in particular were found orthologue to *Arabidopsis* genes, *GRF5* (Knetminer score 149.38) and *VIT1* (Knetminer score 35.88). QTL4 (AX-94488406 on Chr2A) accounted for 3-12% of the total variance and was found significant in PhEnv2 (Terbol) as well as across environment. Annotation of this locus with the Svevo physical map showed 176 genes within its critical interval. Of them, 124 novel genes were discovered for flowering time with 47 major publications in the knetminer database (knetminer.rothamsted.ac.uk). Four major genes (*SCL3, TGA7, OsFCA and HO1*) found orthologue to *Arabidopsis* and rice genes were reportedly involved in delayed flowering and regulation of photoperiodism pathway (Pepper et al. 1997; Lee et al., 2001; Emborg et al., 2006; Willige et al., 2007; Zhang et al., 2009). Similarly, QTL5 (AX-94939920 on Chr2B) was significant across environment with 154 genes within its confidence interval. Three candidate genes, *TCP1, DREB1C, and PVA11* with novel gene content were selected based on the knetminer score. Fig 1.10 illustrates the network view of *TCP1* gene (scored 754 based on Knetminer database as highly cited gene for "heading days") which includes three major genes, namely *FT, BRC1 and TSF* (blue triangle in dotted circle). These genes are primarily mapped in *Arabidopsis* and rice (Li et al., 2005, 2011; Reeves et al. 2001) with 679 evidences for DTH (knetminer.rothamsted.ac.uk).

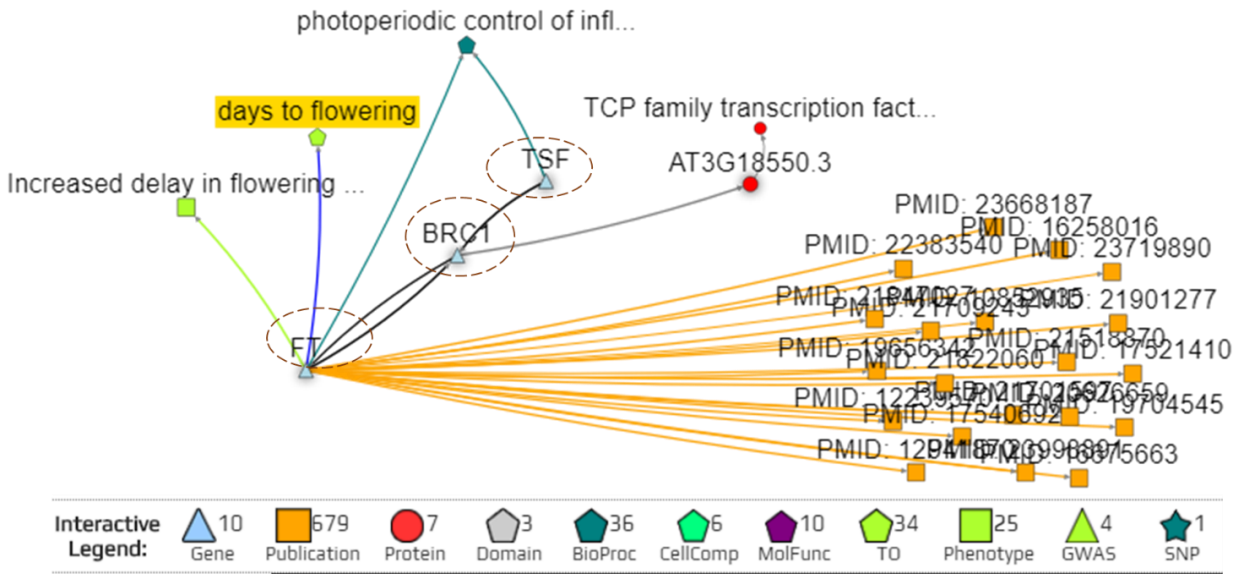


Fig 1.10. Network view of TCP1 gene displaying knowledge web of main selected genes for QTL5 on Chr2B responsible for flowering time. Gene are displayed in blue triangle with dotted circle. Orange node provides a document-centric view based on relevant search; red node shows protein; and green node explains the assigned trait.

QTL13 (peak marker AX-94978708 on Chr5A with 511Mbp) followed by QTL15 (AX-94653414 on Chr5B with 493Mbp) explained 5.8% and 1.5% of total variance at PhEnv5 (Terbol off-season). QTL13 showed strong involvement at *VRN*-A1 position (549 Mbp) with 172 genes within its confidence interval while 114 genes were reported on QTL15 according to the Svevo physical map. However, Knetminer database indicated 103615 genes for QTL13 and 72 genes for QTL15. Knetminer database indicated *SVP, RRP6L1, AGL18, AGL24, AGL15, AGL16, SPY, ELF3, FTIP1, DET1, DI19-7, HUA2, FRL5* and *MADS18* with high score at QTL13 (30.78 to 1399.82 score) (Fig. 1.11) while only three candidate genes, *RF2b*, *PCF2* and *TR* were found with QTL15 which in turn were associated with *FT*, *bZIP30* and *SVP* genes (Jang, 2009).

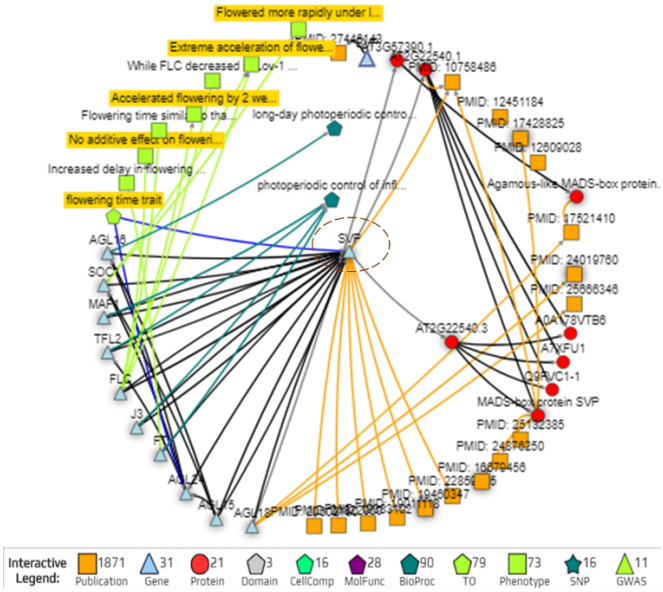


Fig 1.11: Web view of SVP gene displaying knowledge network of selected genes for QTL13 on Chr5A responsible for early flowering. Genes are displayed in blue triangle with dotted circle. Orange node provides a document-centric view based on heading days; red node shows protein; green node explains assigned phenotype.

QTL19 (AX-94667805 on Chr7A at 8.3Mbp) and QTL20 (AX-94634646 on Chr7A at 21Mbp) were significant in PhEnv2 (Terbol) accounting for 3 to 4% variance. Annotation with the Svevo physical map revealed 207 and 142 genes within the respective QTL confidence intervals. Knetminer database revealed that QTL19 and QTL20 encoded *SVP, AGL15, 18, 24* and *SPY* genes (score 1399) for the regulation of photoperiod with 375 citations from *Arabidopsis*, rice and wheat (Lee et al., 2010; Krishna et al., 2016). Similarly, QTL9 (AX-94439386) and QTL10 (AX-95217431) located on

Chr4A accounted for significant variance (5.4-22%), r^2 (0.22), MAF (0.2-0.3) and high LOD (3.4-3.8) for PhEnv2 and PhEnv3. QTL9 is related to *UBC4* gene involved with ubiquitination protein which regulates various biological processes including growth and development, and regulation of chromatin structure (Moon, 2004; Ramadan et al., 2015). QTL10 associated with (LRR-RK) protein encodes *TaBRI1* gene which interacts with members of *TaSERK* gene family which cause early flowering and seed yield enhancement in *Arabidopsis* and respond to changing environment (Singh et al., 2016). QTL10 was associated to a protein kinase and its alpha subunit closely related to the *Arabidopsis* genes AtCO, FLOWERING LOCUS T (AtFT) which is linked to the flowering-time QTL (Hd6) of rice involved in photoperiod sensitivity (Kane et al., 2005; Takahashi et al., 2001).

In addition, QTL2 (AX-94635647 on Chr1B) and QTL8 (AX-95021774 on Chr3B) had high values for variance (13% to 26%), r^2 (0.16 to 0.18), MAF (0.1 to 0.4) and max LOD (3.7) consistently in all pheno-environments. QTL8 encodes *HUB2* gene which indirectly connects with the epigenetic mechanism of vernalization (Zhou et al., 2017). QTL1 (AX-94736642 on Chr1A) accounted for 25% variance and significant F-value for PhEnv1 and PhEnv2 followed by QTL7 (AX-94452589 on Chr3A) with high LOD (4.6). Similarly, QTL14 (AX-94939814 on Chr5B), QTL11 (AX-95101347 on Chr4B), QTL16 (AX-94930415 on Chr6A) and QTL17 (AX-94761286 on Chr6A) were significant in PhEnv5 and across environment accounting for significant variance (5 to 30%).

Significant markers for elite germplasm

In elite lines, 27 QTLs were derived from 66 significant markers on seven chromosomes. Of the 27 QTLs, *PPD*-A1 (36.6 Mbp) and *PPD-B*1 (56.29 Mbp) were photoperiod specific, consistently significant in all pheno-environments and across environments. Among the photoperiodic specific QTLs, QTL3 (*Ppd*-A1 on Chr2A) showed the highest LOD (3.6 to 8.5), high variance (2 to 20%), 0.17 r^2 and best MAF (0.8) consistently in all pheno-environments except PhEnv5 whereas QTL7 (*PPD*-B on Chr2B) was significant for PhEnv2, PhEnv4 and across environment with high significance of variance (2 to 30 %), 0.73 MAF and 0.17 r^2. Earlier studies have reported the vital role of photoperiod-insensitive genes (*PPD-A1* and *PPD-B*) for early flowering and significance of these QTLs across the environments in the present study confirm the effect of day length on

flowering time. Similarly, two vernalization specific QTLs named *VRN*-A1 (549 Mbp) and *VRN*3-7A (69.4 Mbp) were significant for PhEnv3 (Fanaye) and PhEnv5 (TerbolOff) with 2.35% and 30% of variance, respectively.

QTL2 (AX-94385320 on Chr2A) had exactly similar position of PPD-A with high LOD (7.8) and significant variance (6 to 26%) which explained the importance of this locus for photoperiod in PhEnv3, PhEnv4 and across environment. Annotation of this locus with the Svevo physical map showed 140 genes within its chromosomal interval (36.1 to 36.6 Mbp). Of them, 98 novel genes were discovered for flowering time in orthologous species especially two genes of photoperiod, *PRR37* (scored 109) and *SRT1* (scored 52) with 97 major publications in the knetminer database (knetminer.rothamsted.ac.uk). *PRR37* gene is associated with many other flowering genes like *PHY, TOC1, LHY*, etc., which regulate photoperiod (Fig. 1-12).

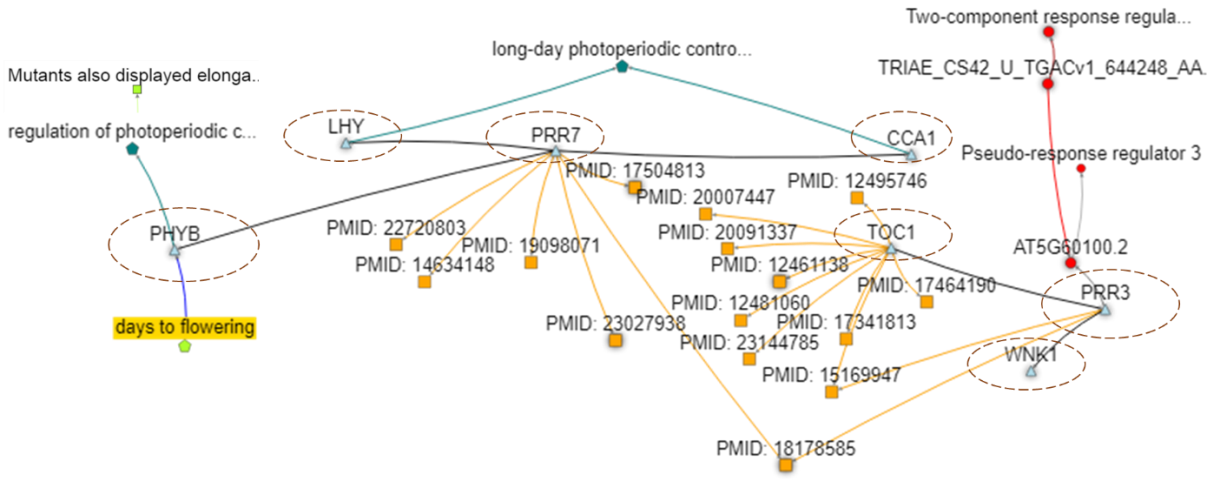


Fig 1.12: Network view of PRR37 gene displaying knowledge web of main selected genes for QTL2 on Chr2A responsible for regulation of photoperiod in long and short days. Genes are displayed in blue triangle with dotted circle. Legend direct to the other nodes e.g. orange node provides citation based on its function for flowering time. Similarly, red node shows associated protein and green node explains the phenotype express by specific gene.

Within the confidence interval (555.7 to 561.2Mbp), QTL4 (AX-94460586 on Chr2A) contained 162 genes as revealed by the Svevo map annotation. Of them, Knetminer database revealed 100 genes with two major genes, *GRF5* and *VIT1* associated with the response to flowering time. The same QTL was also identified in landraces headed by the peak marker AX-94963816 (QTL3). Annotation QTL5 (AX-94956877 on Chr2B) with the Svevo physical map showed 124 genes within the confidence interval of 53.9 to 54.5 Mbp. Of them, 88 were significant for PhEnv4 and across

environment with 3.4% variance. Three genes, *SRT1, TKL-2,* and *BHLH74* with a function of controlling flowering time in cereals under short- and long-day environments also showed strong association with PRR, TOC1, APRR, CCA1, LHY, and WNK1 in the Knetminer database.

QTL13 (AX-94577903 on Chr5A, positioned at 11.4 Mbp) accounted for significant variance (5%) at PhEnv3 (Fanaye) reported 98 genes within its confidence interval. Of them, 62 genes with three major genes, namely *DUT, AGAL2 and RBG8* were recovered with the key-word "flowering time" in the Knetminer database. *DUT* gene controlled starch metabolism in *Arabidopsis* and its mutant control flowering time (Streb et al., 2012). The critical intervals (644.6 to 665 Mbp) of QTL15 and QTL16 (AX-95213349 and AX-95071189 on Chr5A) showed 92 and 166 genes with significant variance for PhEnv3 (QTL15 and QTL16) and PhEnv5 (QTL16) and across environment (QTL16). QTL15 encoded *CSN1, PCS1* and *DREB1F* genes whereas QTL16 encoded *RGA* and *SMD3A* genes associated with the keyword "flowering time" in the Knetminer database. The database showed linkage of *CSN1* gene with *COP1, DET* and *COP1* genes. Furthermore, QTL17 (AX-95140644 on Chr5B) was significant for PhEnv3 and contained 79 genes with *PIF4* gene encoding PHY controlling photoperiodic mechanism and shade avoidance which alter flowering time. Phytochrome-E influences internode elongation and flowering time in *Arabidopsis* (Devlin et al., 1998). On the other hand, QTL18 (AX-95259336 positioned at 557Mbp adjacent to *VRN-5B* gene) revealed significant effect, accounting for 8.2% variance with high r^2 in PhEnv3 (Fanaye). In the Knetminer database, QTL18 contained 103616 genes orthologous to flowering genes in *Arabidopsis*, rice and wheat. QTL18 contained four novel genes, *RRP6L1, SVP, AGL18* and *SPY* with 832 publications with the keyword "flowering time" (Fig 1.13).

Another significant QTL19 at 700 Mbp accounted for 2.3% variation in PhEnv1 and contained 122 genes within its confidence interval. Of them, four major genes, *PFT (ERF15)*, *LUH*, *HUB2* and Histone H2B monoubiquitination in the chromatin of FLOWERING LOCUS C regulate flowering time in *Arabidopsis* (Ying et al., 2008). QTL23 (AX-95080277 on Chr7A, 3.1Mbp) was highly significant for PhEnv5 and accounted for 5.9% variability. This locus reported 184 genes including three major genes, namely *NTL9, BAG7* and *GPA1* which characterize extreme acceleration of flowering under long-day environments. This QTL is linked with Mono copper oxidase-like protein SKU5 and SKS1. It has been reported that *SKU5* and *SKS1* genes in *Arabidopsis* express in

expanding tissues and account for earliness per se phenotype. *SKU5* is expressed in all tissues like roots, hypocotyls, cotyledons, leaves, inflorescence stems, and flowers (Sedbrook et al., 2002).

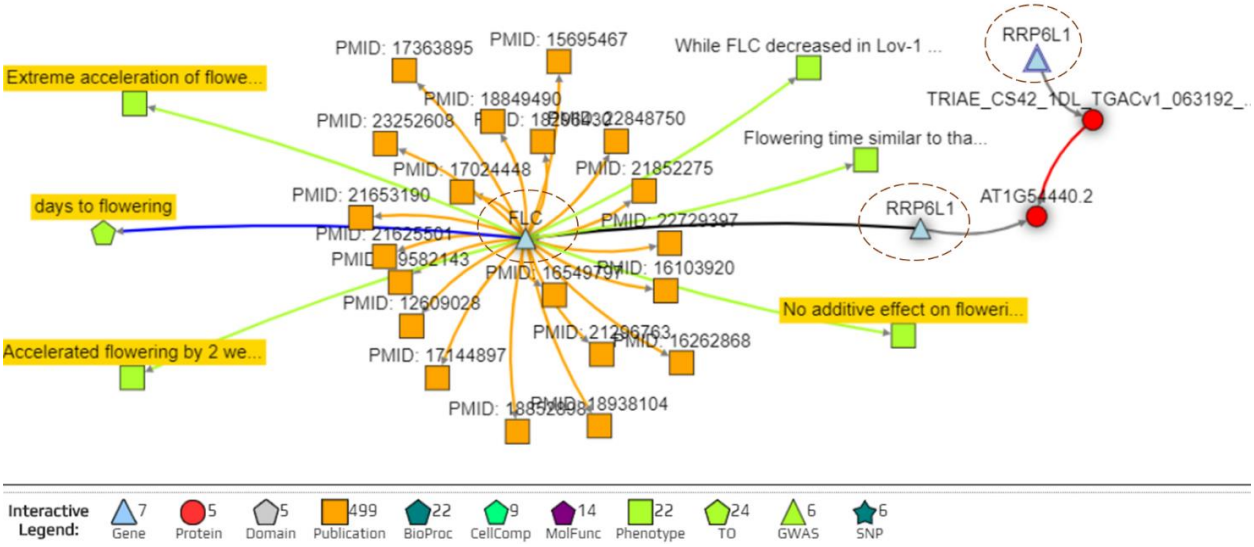


Fig 1.13. Web view of RRP6L1 gene displaying knowledge network of main selected genes for QTL19 on Chr5B for the keyword "days to flowering". Genes are displayed in blue triangle with dotted circle; Lead with legends explains other nodes e.g. orange node provides a document-centric view; red node shows protein and green node explains assigned trait.

QTL25 (AX-94905964 on Chr7A), QTL26 (AX-94701740 on Chr7B) and QTL27 (AX-94878591 on Chr7B) showed significant effect in PhEnv2 accounting for 2.5 to 6.8% variability. Annotation with the Svevo physical map discovered 36, 87, and 112 genes within the critical interval of QTL25, QTL26, and QTL27, respectively. Knetminer database identified 17 genes for QTL25 with *PTB* as major gene involved in floral transition in *Arabidopsis* (Streitner et al., 2008). QTL26 involved *SYD* gene associated with AGAMOUS-LIKE 17, a novel flowering promoter, which acts in a FT-independent photoperiod pathway (Ping et al., 2008). QTL2 contained a major gene *NRPD3B* which interacts with CONSTANS in the photoperiod pathway to directly regulate the transcription of SOC1, a major floral pathway integrator (Hou et al., 2014). QTL9 (AX-94973426 on Chr3B) and QTL10 (AX-95630216 on Chr4A) were significant in PhEnv2. Among them QTL9 belongs to DXR gene. Usually this gene targets to plastids and localizes into chloroplasts of leaf cells but it also catalyses for isoprenoid biosynthesis and some of the isoprenoid products derived from the MEP pathway in *Arabidopsis* might be essential for flower development because flower primordia of the cla1-1 mutant or dark-grown wild-type plants never mature into normal flowers (Mandel et

al., 1996; Carretero-Paulet et al., 2002). QTL10 belongs to Leucine-rich repeat receptor-like protein kinase (LRR-RLKs or TaLRRKs) which plays a critical role in plant development and involved in growth, floral development and flower abscission (Shpak et al., 2004; Shumayla et al., 2016). QTL20 (AX-94707895 on chr6B) was significant accounting for 5.4% variance in PhEnv1 and belonged to WAT1-related protein regulating auxin transporter which interrelates and precisely regulates intensity or quality of light with the help of Phytochromes (PHY). In wheat, PHYC is required for early flowering under long day environments (Pearce et al., 2016).

In this study, there were markers with no direct relationship with flowering genes. For example, QTL1 (AX-94498055-Chr1B) and QTL8 (AX-94479255 on Chr3A) were consistently significant at all pheno-environments except PhEnv5 and QTL11 (AX-94554200 on Chr4B) in PhEnv5 and across environment. Similarly, QTL21 (AX-94805681 on Chr6B) was significant in PhEnv2. These QTLs explained significant level of variance (1.2 to 19%) at different locations but no candidate genes were annotated. It could be possible that these QTLs might be associated with *EPS* genes for determining flowering response and could not be retrieved as mechanism of EPS has not been studied in depth as it is for *PPD* and *VRN* genes. QTL12 (AX-95630515 on Chr4B) for PhEnv2 was associated with Fatty acyl-CoA reductase involved in biosynthesis of the leaf blade wax in wheat (Yong et al., 2015) whereas QTL22 (AX-94637897on Chr6B) was linked with AT-rich interactive domain protein with no evidence to support relationship for flowering time.

In addition to photoperiodic (*PPD-A, PPD-B*) and vernalization (*VRN-A1, VRN-B1, VRN3-FT*) specific genes already known, the candidate gene search in landraces and elite germplasm resulted in retrieval of seven novel genes, namely *PRR7, GRF, SVP, RRP6L1, Hd6, TCP1,* and *COP1 & RGA* in response to the call for heading date in the Knetminer database (Table 1.4). These genes are not only significant but also stable across environment and pheno-environments.

Table 1.4. Description of highly significant candidate genes in landraces and elite germplasm of durum wheat retrieved from pheno-environments and across environment

Candidate gene	**PhEnv1** (Morocco)	**PhEnv2** (Lebanon)	**PhEnv3** (Fanaye)	**PhEnv4** (Kaedi)	**PhEnv5** (TerOff)	**BLUEs** (Across)	**Germplasm**
PPD-A	X	X	X	X		X	Elite
PPD-B		X		X		X	Landraces /Elite
VRN-A1			X		X		Elite
VRN-B1			X				Landraces
VRN3-FT			X				Elite
PRR7	X	X	X	X		X	Elite
RRP6L1			X		X		Landraces/Elite
GRF		X	X		X		Landraces/Elite
TCP1						X	Landraces
SVP			X		X		Landraces/Elite
Hd6		X			X		Landraces
COP1 & RGA			X		X	X	Elite
Total	2	5	9	4	6	5	

Chapter 2: Estimation of heterosis for yield performance in durum wheat

Objective

Identification of superior hybrid combinations in durum wheat is prerequisite for realizing hybrid vigour commercially. Therefore, the main objective of this study was to investigate the role of parental selection and identify the best heterotic groups based on mid- (MPH) and best (BPH) parent heterosis, and general (GCA) or specific (SCA) combining ability effects.

Materials and methods

Genetic material and development of hybrids

In a previous study, genetic diversity of a panel of 230 elite lines representing major breeding groups in durum wheat was investigated. Based on the single nucleotide polymorphism (SNP) analysis (Maccaferri et al., 2005), five genetically diverse groups were identified and characterized by their geographical origin: GP1 (Italian), GP2 (CIMMYT 70s), GP3 (CIMMYT 80s), GP4 (ICARDA 80s for temperate areas) and GP5 (ICARDA 80s for dryland areas) (Fig 2.1) by means of STRUCTURE that was used as described in Maccaferri et al. (2005).

Based on the above information, eight high-yielding elite durum lines were chosen to represent the genetic diversity of the five groups. The production of F_1 hybrid seed was carried out under greenhouse as well as field conditions. In order to coincide and extend the flowering interval of parental lines used for F_1 hybrid production, the parental lines were planted in the greenhouse at two different dates. The same set of parental lines was sown on November 2014 at the field experimental station, University of Bologna (Cadriano, Italy) in order to produce sufficient F_1 seeds. Emasculation and hand pollination were performed to produce F_1 hybrid seeds for 28 combinations following a half-diallel scheme (without reciprocals). Because the first round of crossing did not yield sufficient number of F_1 seeds, a second round of crossing was performed in field as well as in glass-house during 2015 in order to fulfill the seed requirements for further experiments (Fig 2.2). In total, 344 spikes in glasshouse and 650 spikes in field were emasculated

and pollinated, resulting in 1,270 seeds produced in glasshouse and 2,316 seeds produced in field for 28 hybrid combinations.

Experimental design

Eight parents along with 28 F_1 progenies of the half-diallel scheme were sown in a field trial at UniBO experimental station, Cadriano, Italy on November 10, 2015 to assess heterosis for various agronomic traits including yield components. The experiment was laid out in a randomized complete block design (RCBD) with three replicates. To monitor the spatial field variability, a commercial bread wheat hybrid (HyFi, commercialized by RV Venturoli, Bologna) was sown in each block in multiple plots. Each entry was sown in twin-row micro-plots consisting of 2 x 5 plants, each spaced 20 cm apart. The inter-plot distance was kept at 70 cm while the twin rows were spaced 20 cm apart in order to identify most promising combinations among all possible crosses. Three plants from both durum and bread wheat were considered for measuring the morphological traits.

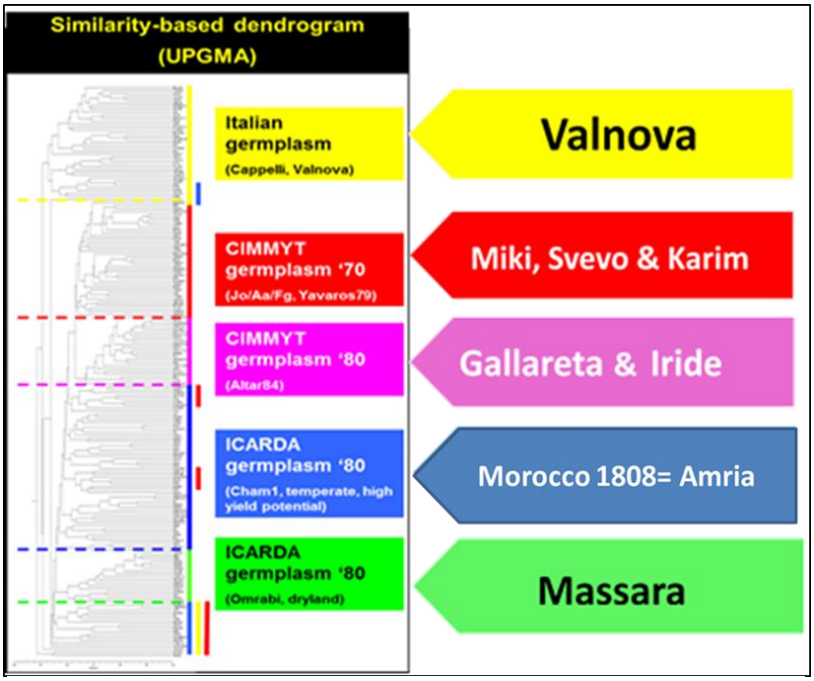


Fig 2.1: Selection of parents for performing crosses from 230 elite durum lines.

Fig 2.2. Crossing block of field and glasshouse at the University of Bologna.

Fig 2.3. Evaluation of 28 F_1 hybrids and corresponding parents in the field of University of Bologna, Italy.

Phenotyping evaluation

The following agronomic traits were measured on the F_1 plants and their parents:

1) Heading date (HD): The number of days elapsed from planting to the date when 50% of the plants in a plot showed at least one ear extruded from the flag leaf was taken as heading date.
2) Plant height (PH in cm): The average distance from the ground to the top of the terminal spikelet at maturity from three randomly selected plants/plot was taken as plant height.
3) Grain yield (GY in g/plant): All spikes of selected three plants were harvested (at maturity) and threshed. Weights were recorded in grams using an electronic balance.

4) Thousand-kernel weight (TKW in g): Weight of total number of seeds harvested from a plant divided by the number of seeds and then multiplied by 1000 was taken as 1000-kernel weight in g.

5) Harvest index (HI in %): Harvest index was calculated as the ratio of grain yield to total above-ground biomass weight.

6) Total biomass weight (TBW in g /plant): The average weight of above-ground biomass (straw and grain weight) of randomly selected three plants was taken as total biomass weight in g.

7) Chlorophyll content (SPAD unit): Flag leaf greenness was measured by SPAD meter which is the average of four readings beginning from anthesis (Zadok 60) to the end of grain filling period. At flowering, SPAD readings are generally correlated to yield potential while during grain filling, SPAD value represents a "greenness" measurement which can estimate plant senescence.

8) Leaf area index: Three flag leaves per plant were scanned to characterize the plant canopy to provide indication of biomass accumulation and plant growth.

In addition, number of spikes per plant, number of grains per spike, number of spikelets per spike and number of grains per spikelet were also recorded to assess correlations with above main traits.

Statistical analysis

The analysis of variance (ANOVA) was first performed according to a RCBD. Diallel analysis was used to estimate GCA and SCA for those traits that showed significant differences among the genotypes comprising 28 F_1 progenies and 8 parents. The procedure given by Griffing (1956) for the Method II and Method IV was followed by using a statistical program in R X 64 version 3.3.2 and Plant breeding statistical tools (IRRI, 2014). In GF-Method II, half diallel (when no reciprocal differences exist) with parents and one set of F_1s were analyzed whereas for GF-Method IV, only set of F_1s were considered (PB Tool, IRRI, 2014). Percentages of heterosis relative to mid (MPH) and better (BPH) parents were calculated using Fonseca and Patterson (1968) as follows. For a given F_1 cross,

MPH = (mean value of the F_1 cross – mean of the two parents)/mean of the two parents × 100.

BPH = (mean value of the F_1 cross – mean value of the best parent)/value of the best parent) × 100.

Parental means, heterotic means and phenotypic coefficient of variation (PCV) were estimated along with repeatability based on variance components of three replications (SAS® Institute, 2006). The following linear model was fitted to data to estimate variance components.

$$\mathbf{Y\ ijk} = \boldsymbol{\mu} + \mathbf{Gi} + \mathbf{Gj} + \mathbf{Sij} + \mathbf{eij}$$

Where,

Y ijk = trait value,

μ = overall mean,

G_i = General combining ability effect of i^{th} genotype,

G_j = General combining ability effect of j^{th} genotype,

Sij = Specific combining ability effect between i^{th} and j^{th} parents and

eij= pooled error for means over replicate.

Results

Diallel analysis

Analysis of variance showed significance among genotypes for all examined traits. For all traits, CV was acceptable (ranging from 3.2% for plant height to 21.13% for spike number) and repeatability ranged from 49.4% for SPKN to 89.9% for TKW (Table 2.1). The F_1 hybrid mean was higher than the parental mean for all the traits studied except for harvest index. Analysis showed

25.8% mid parent heterosis (MPH%) and 18% best parent heterosis (BPH%) for grain yield, indicating the scope for exploitation of heterosis in durum wheat (Table 2.1). SPKN was the trait with higher MPH (29.2 %). The diallel analysis among the 28 F_1s and their parents showed significant SCA effects for all the investigated traits namely, plant height, number of spikes per plant, total biomass per plant, grain yield per plant, 1000-kernel weight, straw weight per plant and harvest index while GCA effects were significant only for harvest index and 1000-kernel weight (Suppl. Table 1).

Table 2.1: ANOVA and combing ability analysis for seven traits in 28 F_1 and eight parents of durum wheat during a field trial in 2016.

Traits	GCA	SCA	CV	Repeatability%	Mean	MPH%	Parental mean	F_1 mean	BPH%
GY	NS	**	14.9	79.6	63.5	25.8	52.8	66.5	18.2
SBW	NS	**	20.6	59.0	55.4	20.1	47.9	57.5	14.6
TBW	NS	**	19.6	60.7	117.8	22.7	100.5	122.8	16.4
PH	NS	**	3.2	78.1	85.5	5.5	82.0	86.5	3.9
SPKN	NS	**	21.1	49.4	13.2	29.2	10.8	13.8	22.7
HI	**	**	12.1	62.9	0.5	2.5	0.5	0.5	-1.1
TKW	**	**	5.2	89.9	59.0	8.6	55.3	60.0	1.6

*: P<0.05. **: P<0.001, NS: not significant (P>0.05), GCA – General combining activity, SCA – Specific combining ability, CV - Coefficient of variation, MPH - Mid-parent heterosis, BPH - Best parent heterosis, GY - Grain yield, SBW – Straw biomass weight, TBW – Total biomass weight, PH – Plant height, SPKN – Spike number, HI – Harvest Index, TKW: 1000-kernel weight

To compare MPH, BPH and SCA for grain yield among different heterotic groups of durum elite lines, eight parents were classified into five groups but Group5 parent 'Massara' was merged with Group4 parent 'Morocco' due to low genetic distance between these two parents which also have common source (ICARDA 80s) and registered low genetic distance (Maccaferri et al., 2015). Moreover, the merging was convenient for estimation of highest number of possible combinations among groups. The analysis of these four groups provided the average of MPH, BPH and SCA with the number of combinations between each group. Similarly, the Italian group was represented by the Valnova line only, thus the combination Italian x Italian was not present. Table 2.2 shows MPH, BPH and SCA for hybrids within and between each of the four groups of

lines. The results indicated negative values for SCA for F_1 hybrids involving parents from the same group (on the diagonal) with low mid and best parent heterosis. On the contrary, F_1 hybrids between elite lines representing different groups (above the diagonal) showed high MPH, BPH and SCA. Hybrids between Valnova (the only parent representing Group1, Italian) and Group2 (CIMMYT '70s) showed the highest mid and better parent heterosis followed by F_1 hybrids between Group2 (CIMMYT '70s) and Group4 (ICARDA '80s) and Group2 (CIMMYT '70s) and Group3 (CIMMYT '70s) (Table 2.2). The higher the value of MPH and BPH, the better is the heterosis.

Table 2.2: MPH, BPH and SCA for grain yield (g) among four groups of durum elite lines

Groups	Values	Group2 **CIMMYT (70s)**	Group3 **CIMMYT (80s)**	Group4 **ICARDA (80s)**
Group1	MPH	69.48	30.09	43.33
ITALIAN	BPH	63.06	19.49	37.49
	SCA	27.55	-1.45	6.06
Group2	MPH	23.99	45.99	49.2
CIMMYT(70s)	BPH	17.99	33.48	46.26
	SCA	-11.07	16.01	19.21
Group3	MPH		7.19	30.33
CIMMYT (80s)	BPH		0.52	19.8
	SCA		-16.74	7.15
Group4	MPH			1.03
ICARDA (80s)	BPH			15.58
	SCA			-26.22

GCA – General combining activity, SCA – Specific combining ability, CV - Coefficient of variation, MPH - Mid-parent heterosis, BPH - Best parent heterosis

Table 2.3: SCA and mean grain yield for best heterotic parents among four groups of durum elite lines (the entries in shades are for the best heterotic parents).

GCA/SCA	Gl	Ir	Kr	Mk	Mr	Ms	Sv	Vl
Gl	**2.77**	-5.01	8.62	10.38	-11.63	3.02	10.44	8.81
Ir		**0.14**	6.8	2.72	2.91	14.39	-7.03	-9.8
Kr			**3.18**	-19.15	25.13	-11.91	-0.92	20.57
Mk				**-1.04**	5.99	1.9	9.1	21.95
Mr					**-2.4**	-8.54	0.9	-1.61
Ms						**-0.67**	16.17	5.72
Sv							**-2.48**	-14.97
Vl								**0.5**

Note: Gl; Gallareta, Ir; Iride, Kr; Karim, Mk; Miki, Mr; Morocco, Ms; Massara, Sv; Svevo, Vl; Valnova. Bold and underlined values on diagonal are GCA and upper diagonal are SCA values

Mean	Gl	Ir	Kr	Mk	Mr	Ms	Sv	Vl
Gl	**56.67**	61.34	78.02	75.56	52.19	68.57	74.18	75.52
Ir		**61.24**	73.56	65.26	64.09	77.3	54.07	54.28
Kr			**55.24**	46.44	89.36	54.05	63.22	87.7
Mk				**44.93**	66	63.64	69.02	84.86
Mr					**52.08**	51.84	59.46	59.93
Ms						**51.74**	76.46	69
Sv							**51.64**	46.49
Vl								**49.11**

Note: Gl; Gallareta, Ir; Iride, Kr; Karim, Mk; Miki, Mr; Morocco, Ms; Massara, Sv; Svevo, Vl; Valnova. Bold and underlined values on diagonal are parental means and upper diagonal are F_1 hybrid means

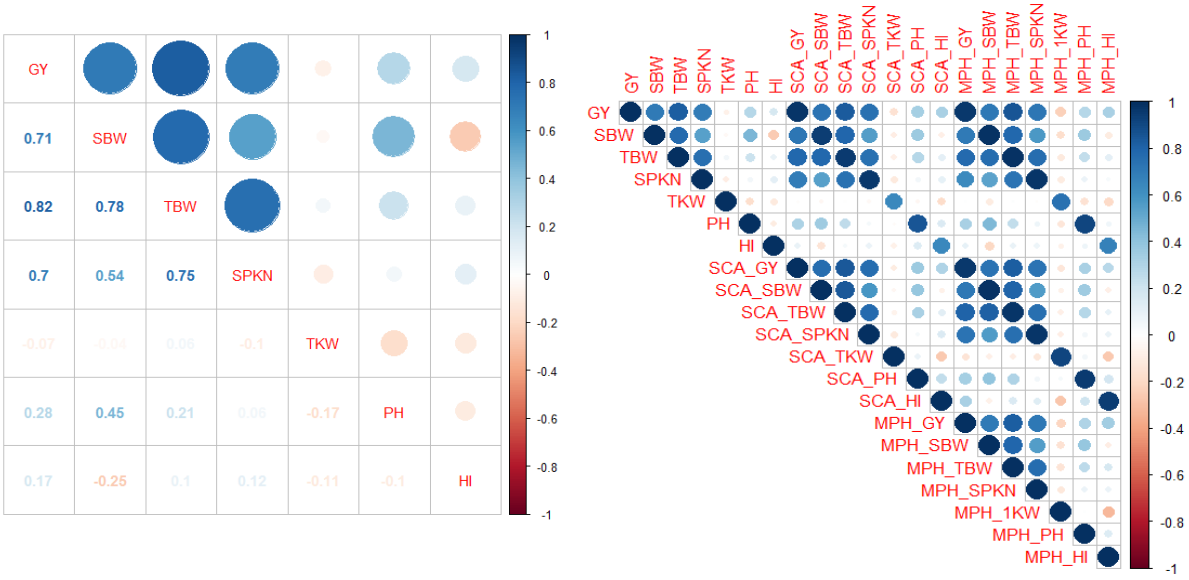
Table 2.3 provides the best parental combinations among those groups by comparing the mean performance of F_1 hybrids for grain yield along with SCA and GCA of the parents involved in the hybrid. The results revealed that the best parental combinations were Karim-Morocco, Karim-Valnova, and Miki-Valnova. Based on mean performance of F_1 hybrids for grain yield along with its MPH and BPH as well as SCA and GCA of the parents, the hybrids were grouped into three heterotic combinations: (i) high yield performance with high value of mid and better parent heterosis as well as high SCA, (ii) high performance with average value of mid and better parent heterosis and low parental GCA and (iii) average yield performance with average value of MPH and BPH, low SCA but either one or both parents have good GCA. The remaining hybrids showed negative or very low heterosis. Similar patterns of MPH, BPH and SCA were observed for heterotic

combinations for total biomass weight (TBW), straw weight (SBW), plant height (PH), spike number (SPKN), harvest index (HI) and thousand kernel weight (TKW). However, for PH and TKW, some parental groups differed between first and second category (Suppl. Table 2).

Correlation between genotype mean of the traits, their SCA effects and MPH effects

Correlation analysis showed significant and positive correlation between seven of the 21 pairs among the seven agronomic traits (Fig 2.3a). Grain yield (GY) showed significant and positive correlations with TBW (0.82**) followed by SBW (0.71**) and SPKN (0.70**). Straw biomass weight (SBW) was positively correlated with TBW (0.78**), SPKN (0.54**) and plant height (0.45**). Total biomass weight (TBW) was positively correlated with spike number per plant (0.75**). Harvest index (HI) and 1000-kernel weig6ht (TKW) showed weak and non-significant correlation with other agronomic traits.

Correlation analysis was also carried out between mean performance of the hybrids, mid parent heterosis (MPH) and specific combining ability (SCA) for all the seven agronomic traits (Fig. 2.3b). Hybrid means were significantly positively correlated ($P < 0.05$) with corresponding MPH and SCA values for grain yield (GY), straw biomass weight (SBW), total biomass weight (TBW) and spikes number (SPKN). Mid-parent heterosis (MPH) for GY, SBW, TBW and SPKN was significantly positively correlated with their corresponding SCAs. The same, i.e. the positive and significance, was not found true for the remaining traits, namely 1000-kernel weight (TKW), plant height (PH) and harvest index (HI) as mean performance of these traits in hybrids had insignificant ($P>0.05$) correlation with MPH and SCA (Fig 2.3b). Generally, hybrid performance is estimated based on the accuracy of correlation of mean values with GCA, SCA and MPH. The present analysis illustrated strong and significant correlation ($P<0.01$) among mean value, MPH and SCA for GY, SBW, TBW and SPKN in durum F_1 hybrids but not with GCA, hence suggesting that GCA should not be the only criteria to determine performance of heterosis among these durum accessions.



(A) **(B)**

Fig. 2.3. *Correlation between A) mean value of phenotypic traits and B) among mean, SCA and MPH effect in field. *Size and color of circle represent strong correlation (blue for positive correlation and red for negative correlation).*

Chapter 3a: Yield performance of hybrids under well-watered and water stressed conditions on Lemnatec (HTPP)

Objective

The major objective of this experiment was to assess mean performance of durum F_1 hybrids along with their parents for various yield related traits along with its root description under well-watered (WW) and water stressed (WS) conditions on a high-throughput phenotyping platform (HTPP) provided by Lemnatec.

Material and methods

This experiment was conducted in greenhouse using the HTPP by Lemnatec in Metaponto, Agrobios ALSIA (from December 2015 to June 2016) to assess the performance of hybrids and its parents under full and reduced moisture (Fig. 3.1). Precise measurements of various growth parameters were recorded to complement the field observations, particularly for early vigour to estimate genotypic variation under stress conditions.

Genetic material and experimental design

The experiment involved eight parents and their 28 F_1 hybrids developed via half-diallel scheme previously described in Chapter2.

A. Glasshouse setup

Genotypes were planted in plastic pots (16 cm diameter × 20 cm tall) filled with 2 kg of peat/sandy soil. For each of the 36 genotypes, 10 pots were sown with one seed each. In total, 360 pots were arranged in a rectangular layout with 10 rows and 36 pots within each row. All the genotypes were randomly allotted to the pots within each row, independently. The 10 rows were grouped into five pairs of nearby rows. A row in a pair was randomly assigned to one of the two stress treatments, well-watered (WW) or water stress (WS). The experimental design therefore was split-plot design in greenhouse where main plots were the rows assigned to the two stress treatments, sub-plots were the pots assigned to the 36 genotypes, and pair of rows were the 5 replicates (Fig 3.1). The rows grown under well-watered (WW) conditions were maintained at

100% of soil water-holding capacity while the rows assigned to the water stress (WS) treatment were left unwatered until the late-booting stage, corresponding to stage 47 of the Zadok scale, by maintaining the soil water content at 50% of the water holding capacity. To increase the precision of target-weight watering, pre-filled pots of soil were used. The use of pre-filled pots also helped to ensure that soil density was similar between pots.

Phenotypic evaluation

Glasshouse experiment

The following agronomic traits were measured for F_1 hybrids and their parents in glasshouse experiment:

1) DTH - Days to heading (days)
2) PH - Plant height (cm)
3) GY - Grain yield (g/plant)
4) TKW -1000-kernel weight (g)
5) SPKSS - Number of spikes per plant
6) SDN - Number of grains per spike
7) SPKNN - Number of spikelets per spike
8) NGPS - number of grains per spikelet
9) TBW - Total biomass (g/plant)
10) HI - Harvest index

In addition to comparing the transpiration efficiency and canopy area in both treatments (WW and WS), stomatal conductance, relative water content (RWC) and leaf area were also measured.

B. Lemnatec setup (High Throughput Phenotyping Platform)

The level of water stress and phenotypic responses of the plants were assessed by using an automatic plant phenotyping platform equipped with image capture and processing technologies (http://www.lemnatec.com/products/hardware-solutions/scanalyzer-3d/). This automated HTPP allows for repeated non-destructive image capture for multi-parametric analysis and provides valuable information on the physiological changes of the plants over time that combine quantification of growth and phenotyping with high reproducibility while allowing for long-term

data storage for data mining. The system consists of three parts: a conveyor unit, an imaging unit with three imaging modes, RGB (visible), UV (ultra violet), NIR (near infra-red) cameras and software to analyze morphometric parameters (Fig. 3.2). Visible light imaging was used to capture and quantify morphological and physiological parameters such as plant shape, color, size and biomass. Scanalyzer UV-Fluorescence imaging module used for the analysis of photosynthetic efficiency, which used to estimate the response to plant treatment or stress, or to measure photosynthetic differences among genotypes and NIR imaging used to estimate the range of leaf water content.

Lemnatec Experiment (High throughput phenotyping)

Each of the 360 plants were monitored weekly through the Scanalyzer 3-D system to record visible, fluorescence and near-infrared images and capture top-view and two side-view images (Fig. 3.1). These data were used to assess the plant performance for the following traits.

1) Biovolume (BV) and Fresh weight (FW)
2) Green area (GA)
3) Green index (GI)
4) Yellow index (YI)
5) Dry index (DI)
6) Hue angle (HA)

Each of these traits was calculated as an integral of 0^0 and 90^0 and analyzed. Here, biovolume (BV) and fresh weight (FW) indicated plant biomass whereas green area, green index and yellow index specified the leaf chlorophyll content, senescence and health status of plant which somehow correlated with yield and yield components. Moreover, hue angle indicated the fluorescence images which segmented in red green blue color (RGB) to characterize the fluorescence emissions from red to yellow (performed in the hue channel). However, dry index indicated the water content of the plant which correlated with susceptible and tolerance status of the genotypes (Fig 3.2).

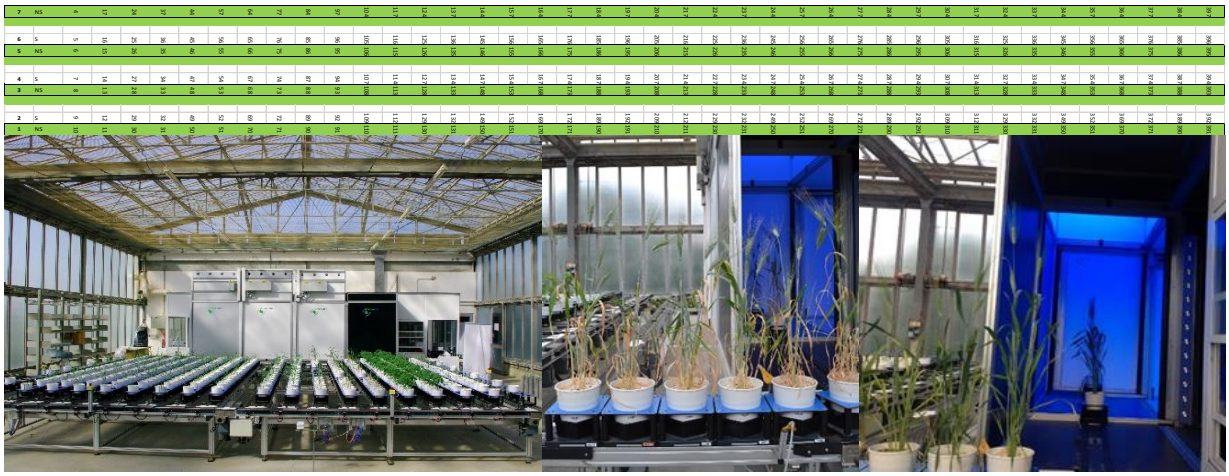


Fig 3.1. Evaluation of F_1 hybrids on Lemnatec platform at Metapontum Agrobios, Italy during April 2016.

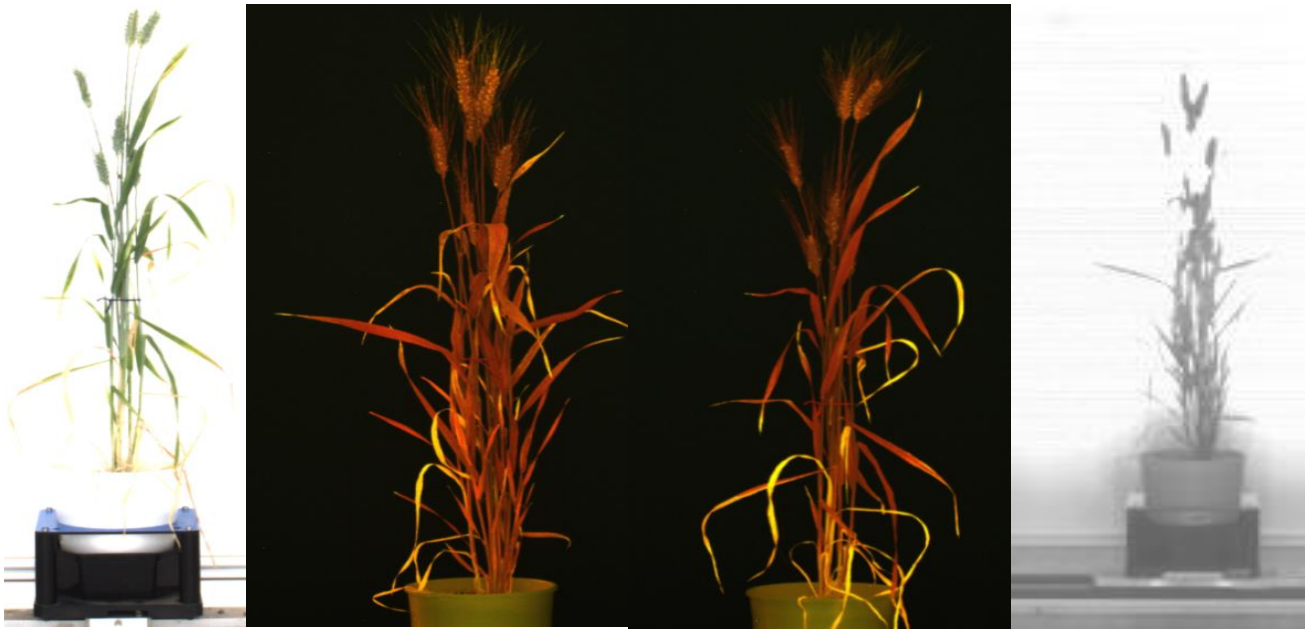


Fig 3.2. Three different images (a) RGB (visible), (b) UV (ultra violet), (c) NIR (near infra-red) of F_1 hybrid in WW and WS conditions on HTPP at Metapontum Agrobios during April to May 2016.

Statistical analysis

A. Greenhouse experiment

ANOVA was performed according to a split plot design. Diallel analysis was performed as reported in the Chapter 1 Section.

B. Lemnatec Experiment (High throughput phenotyping)

The following approaches were taken for the analysis:

1. Day-wise genotype and stress effects and interaction

An analysis of variance (ANOVA) was carried out day-wise, using the linear model accounting for main effects and interaction of stress, genotypes and their interactions and experimental errors under the split-plot design. The model, for each source of variation, was fitted following the directives under GenStat software.

BLOCKSTRUCTURE	Rep/Stress/Geno
TREATMENTSTUCTURE	Stress * Geno
ANOVA	Response

where abbreviations 'Rep', 'Stress', 'Geno' and 'Response' stand for the factors representing replications, stress levels and genotypes, and response variate, respectively.

2. Interaction with days under repeated measures

Since the same pot was observed over several weeks, the response (BV, GA, G, Y, DI and HA) values may be expected to be correlated. Genotypic and stress effect behavior of all variables over time were examined using repeated measures models. Since, no stress was applied to the pots in rows allotted to WS until 46 days after germination (47 of the Zadok scale), the analysis of repeated measures was carried out on the dataset starting from day 53 onward till day 79 i.e., for five weeks (day 53, 60, 66, 72 and 79). For the observations from these 5 weeks, Rep, Stress, Geno, and Days, denote (each of length 360 × 5 = 1800) the factors replicates, stress, genotypes and weeks, respectively. The response variables analyzed were: Bio-volume (BV), fresh weight (FW), green area (GA), green index (GI), yellow index (YI), dry index (DI) and hue angle (HA). The

mixed model for all the variables was fitted using the restricted maximum likelihood (REML) method directives of Genstat software illustrated in the following.

VCOMPONENTS [Fixed=Stress*Geno*Days] Rep/Stress/Geno/Days; constraints=positive

VSTRUCTURE [Terms=Rep.Stress.Geno.Days] Model=AR; Order=1; \ Factor=Days

3. Water Stress Tolerance Index (WSTI)

Response variables for all the traits on the last day (day 79) and average from day 60 to 79 were used to examine the water-stress tolerance as the genotype would have adjusted its internal mechanism to moisture available. An indicator of relative change in mean response due to water stress, was called Water Stress Tolerance Index (WSTI) and defined, for a genotype G_i as

WSTI % = | (mean response of G_i under no stress – mean response of Gi under water-stress)/ (mean response of Gi under no stress)| × 100, or equivalently

= 100 ×|1- (mean response of Gi under water-stress)/ (mean response of Gi under no stress)

WSTI was used to classify the genotypes into broad tolerance groups. Keeping their statistical distribution in view, the classes were defined as follows: If mean and SD stand for the mean and standard deviations of the 36 WSTI values (i.e., for 79 day and mean of 60 to 79 day), then a genotype Gi with its WSTI value as WSTIi, was defined as

Tolerant if WSTIi ≤ Mean – SD

Moderate if Mean – SD < WSTIi ≤ Mean + SD

Susceptible if WSTIi > Mean + SD

Beside these analysis, based on WSTI specific and general combining ability were estimated for Lemnatec traits and Pearson correlation coefficient was calculated with means values of each traits and among MPH and GCA or SCA effect. Moreover, correlations were also calculated between the trait values obtained in these experiments and those obtained in the field.

Results

Glasshouse (GH) experiment

ANOVA revealed a moderate effect of stress treatment on agronomic traits and significant differences among genotypes. In general, CV and repeatability estimates in glasshouse experiment were smaller in magnitude than the corresponding estimates in field experiments for all the traits. In order to evaluate the performance of the genotypes under GH conditions in relation with the performance in the field, the results of the well-watered treatment are hereafter reported. In case of WW treatment, the overall F_1 hybrid mean was higher than the parental mean, indicating superiority of hybrids over parents. Diallel analysis showed significant GCA and SCA effects ($P<0.001$) among 28 F_1s and their parents for all the traits except GCA effect for TBW and SCA effect for TBW and SPKN. Variance component for the eight parental genotypes was significant for each trait, indicating imbalance data to estimate GCA and SCA effects. Glasshouse results were in agreement with the results obtained in field experiment. The MPH ranged from 3.14% for spikes number (SPKN) to 19.70% for straw biomass weight (SBW). The mid parent heterosis was 19.6% for grain yield and total biomass weight.

Table 3.1: ANOVA and combing ability results for seven traits in 28 F_1 and eight parents of durum elite lines grown in the glasshouse.

Traits	GCA	SCA	P1/P2	CV	Repeatability %	Mean	MPH%	Parental mean	F_1 mean
GY	**	**	**	18.6	54.7	9.8	19.6	9.0	10.1
SBW	**	**	**	16.1	78.0	7.6	19.7	7.0	7.8
TBW	ns	ns	**	14.9	27.4	17.5	19.6	16.0	17.9
SPKN	**	ns	**	18.2	44.5	6.2	3.1	6.2	6.2
HI	**	**	**	16.8	79.0	0.5	6.6	0.5	0.5
TKW	**	**	**	19.5	66.9	34.6	9.3	32.7	35.2
TSDN	**	**	**	10.3	37.4	180.5	11.9	172.4	182.9

*: P<0.05. **: P<0.01, ns: not significant (P>0.05), GCA – General combining ability, SCA – Specific combining ability, P1/P2 - variation among parents, CV - coefficient of variation, MPH - mid parental heterosis

Analysis of F_1 hybrids within and among the four heterotic groups of parents exhibited higher values of mean, MPH, BPH and SCA within the group as compared to the F_1 hybrids among parents from different groups. These results differed from the results reported in the field experiment. Mean (10.50 g) and BPH (2.28 g) for grain yield were highest in F_1 hybrids among the parents within Group2 (CIMMYT '70s), which comprised best parental lines namely, Karim, Svevo and Miki. F_1 hybrids between parents representing Group1 (Italian) and Group3 (CIMMYT '80s) expressed the higher MPH (2.41) and SCA (1.50) Table 3.2.

Table 3.2: Mean, MPH, BPH and SCA for grain yield among four groups of durum elite lines in the glasshouse experiment.

Groups	Values	CIMMYT (70s)	CIMMYT (80s)	ICARDA (80s)
ITALIAN	Mean	8.58	9.31	6.19
	MPH	2.24	2.41	0.15
	BPH	0.11	-0.30	-1.70
	SCA	0.66	1.50	-0.57
CIMMYT(70s)	Mean	10.50	9.53	8.71
	MPH	2.28	0.49	0.53
	BPH	1.67	-0.08	-0.14
	SCA	0.82	-0.08	0.16
CIMMYT(80s)	Mean		9.74	7.59
	MPH		0.13	-1.17
	BPH		-0.32	-2.05
	SCA		0.24	-0.87
ICARDA (80s)	Mean			8.26
	MPH			0.37
	BPH			-0.98
	SCA			0.86

Note: MPH – Mid-parent heterosis, BPH – Best parent heterosis, SCA – Specific combining ability

The glasshouse experiment revealed that the hybrids Karim x Svevo and Karim x Miki (represent same group) followed by Iride x Valnova, Iride x Miki and Iride x Karim (represent different groups) were the best parental combinations in terms of grain yield (Table 2.3). The range of grain yield was higher among the F_1 hybrids than that in their parents. Grain yield among the parents ranged from 4.2 g in Valnova to 10.06 g in Gallareta whereas grain yield among F_1 hybrids varied from 5.72 g (Iride x Massara and Massara x Valnova) to 12.62 g (Karim x Svevo). SCA effect for grain yield showed the similar patterns among the F_1 hybrids. The SCA for grain yield was maximum in Karim x Svevo (3.097) followed by Iride x Valnova (2.827), Miki x Valnova (1.941), Iride x Miki (1.051) and Iride x Karim (0.873).

Similar analysis was performed for yield-related traits like harvest index (HI), spike number (SPKN), thousand kernel weight (TKW), straw weight (SBW) and total biomass weight (TBW). For all traits, the best combinations showed higher mean, SCA, MPH and BPH except for thousand kernel weight in some combinations. Based on all the traits studied, the best parental combination in glass house study was Svevo X Karim followed by Valnova X Miki.

Table 3.3. Estimates of GCAs and SCAs in the upper panel, and mean performance (lower panel) of grain yield for parents among four groups in glasshouse. GCAs (in diagonal) and SCAs (in upper triangle). The entries in shades are for the best heterotic parents.

GCA/SCA	**Gl**	**Ir**	**Kr**	**Mk**	Mr	Ms	**Sv**	**Vl**
Gl	**0.33**	0.24	-0.72	-0.35	1.32	-1.38	-0.81	0.17
Ir		**0.53**	0.87	1.05	-0.49	-2.90	-0.49	2.82
Kr			**0.58**	-0.23	-0.60	1.40	3.09	0.003
Mk				**0.70**	0.21	-0.19	-0.40	1.94
Mr					**-0.69**	0.85	0.14	-0.03
Ms						**-0.53**	-0.02	-1.12
Sv							**0.33**	0.05
Vl								**-1.25**

Note: Gl; Gallareta, Ir; Iride, Kr; Karim, Mk; Miki, Mr; Morocco, Ms; Massara, Sv; Svevo, Vl; Valnova. Bold and underlined values on diagonal are GCA and upper diagonal are SCA values

Mean (g)	**Gl**	**Ir**	**Kr**	**Mk**	Mr	Ms	**Sv**	**Vl**
Gl	**10.06**	9.74	8.82	9.32	9.60	7.04	8.48	7.88
Ir		**9.16**	10.62	10.92	7.98	5.72	9.00	10.74
Kr			**7.88**	9.68	7.92	10.08	12.64	7.96
Mk				**9.02**	8.86	8.60	9.26	10.02
Mr					**6.54**	8.26	8.42	6.66
Ms						**9.24**	8.40	5.72
Sv							**8.52**	7.76
Vl								**4.20**

Note: Gl; Gallareta, Ir; Iride, Kr; Karim, Mk; Miki, Mr; Morocco, Ms; Massara, Sv; Svevo, Vl; Valnova. Bold and underlined values on diagonal are parental means and upper diagonal are F_1 hybrid means

Correlations among mean values, SCA effects and MPH effects (GH experiment)

Correlation studies among agronomic traits under the glasshouse conditions depicted that grain yield had significant positive correlation with total biomass weight (0.59**), spike number (0.61**), 1000-kernel weight (0.60**) and harvest index (0.72**) and negative correlation with straw weight (-0.47**). Harvest index and straw biomass weight had inverse relationship (0.74**). Spike number was positively associated with grain yield, harvest index, 1000-kernel weight and total biomass weight. Mean value showed significant positive correlation with SCA for grain yield and 1000-kernel weight. Strong and positive correlation between F_1 hybrid mean and SCA indicates that non-additive and epistasis gene action are more important in grain yield and 1000-kernel weight. Mean performance of few traits expressed weak relationship with SCA, indicating predominance of GCA or additive gene action for heterotic performance (Fig 3.3).

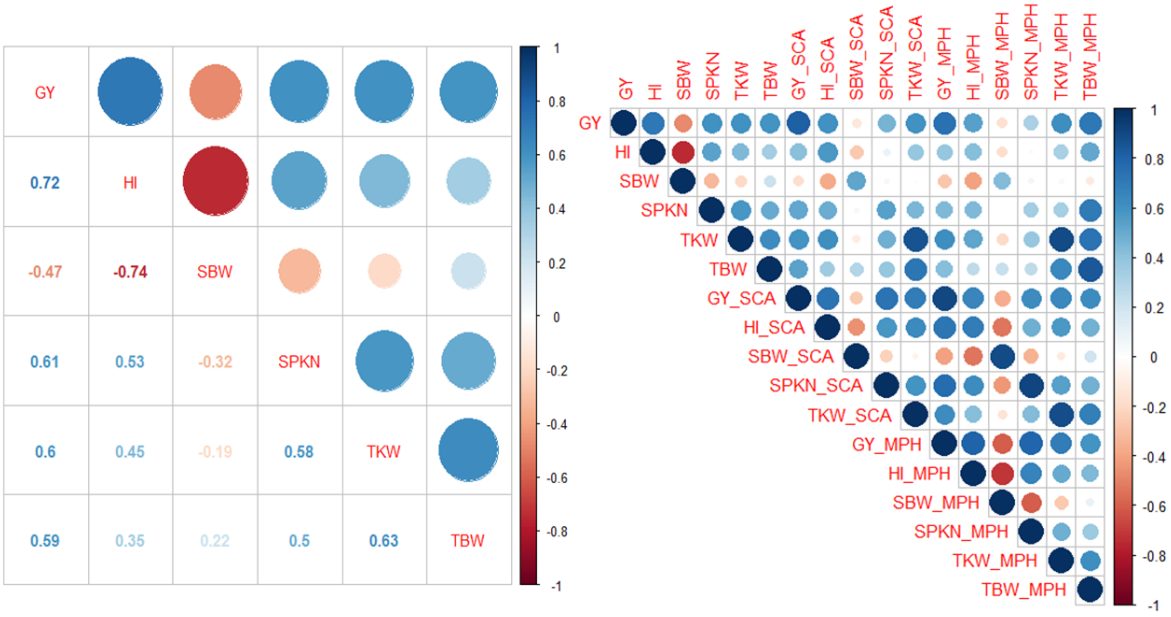


Fig 3.3. Correlation between A) phenotypic traits means and B) among mean values of traits, specific combining ability and MPH of six traits in glasshouse. Size and color of the circle represent correlation (blue for positive correlation whereas red for negative correlation).

Correlation between the results under field and glasshouse conditions

Analysis of data collected from glasshouse and field experiments revealed that the two conditions were not comparable as there was no trend of phenotypic traits among glasshouse and field experiment. Nevertheless, harvest index of field had significant positive relationship with harvest index (0.37) and spike number (0.50) followed by grain yield (0.37) of glasshouse which is clearly visible in Fig. 3.4. These results indicated that in both environments hybrids were able to produce more than its parents but in different heterotic combinations.

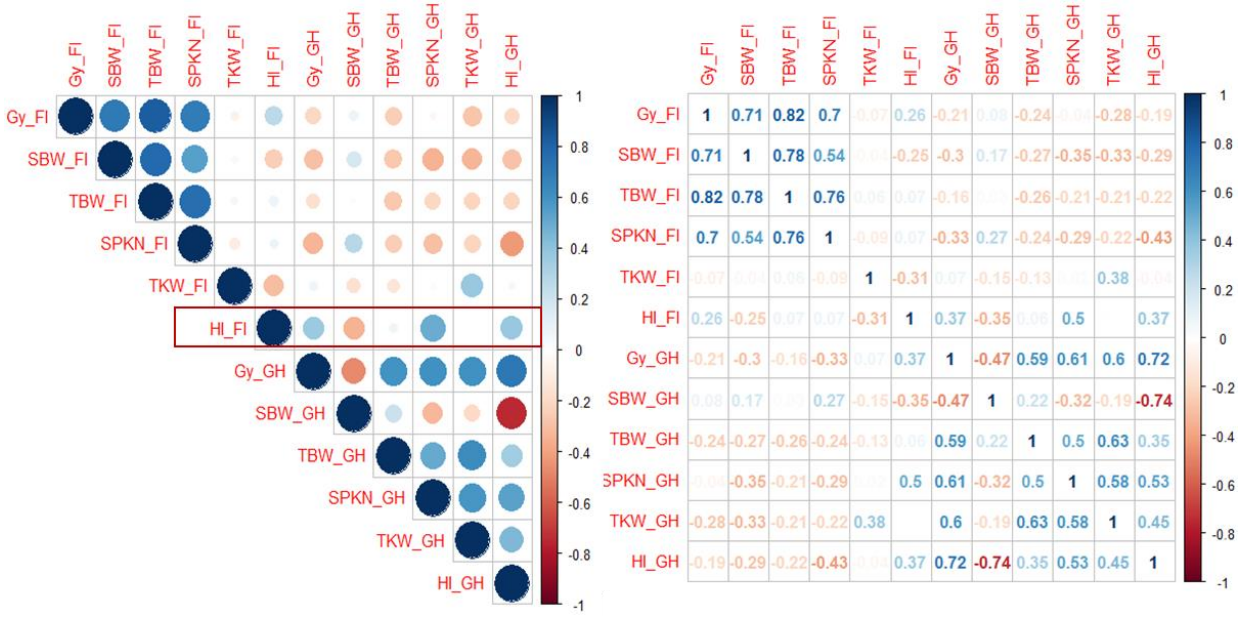


Fig 3.4. Phenotypic correlation between field and glasshouse. Big size and color of circle represent strong correlation (blue for positive correlation whereas red for negative correlation).

Lemnatec (HTPP) experiment

Part 1. Day-wise genotype and stress effects and interaction

ANOVA for six variables at different time interval under well-watered and water stressed conditions showed significant differences for all six traits between WW and WS conditions after the day 53 (Fig 3.5). These significant differences continued until the last day of observation (day 79). Genotypes exhibited significant differences under WW and WS conditions throughout the period of observations (from 5 to day 79). The result showed that Genotype x Stress interaction was significant only at the later stage from day 53 to 79. However dry index did not express

significant differences in Geno x Stress at later stage that revealed that plants were partially moisture (Table 3.4).

Table 3.4. ANOVA at each specific time point with treatment as a factor.

Source	d.f.	Day5	Day25	Day39	Day46	Day53	Day60	Day66	Day72	Day79
					Biovolume (BV)					
Stress (S)	**1**	NA	NA	NA	NA	**	**	**	**	**
Genotype(G)	**35**	**	**	**	**	**	**	**	**	**
S x G	**35**	NA	NA	NA	NA	*	**	**	**	**
					Yellow index (YI)					
Stress (S)	**1**	NA	NA	NA	*	NA	**	**	**	**
Genotype(G)	**35**	**	**	**	**	**	**	**	**	**
S x G	**35**	NA	NA	NA	NA	**	**	**	**	**
					Green area (GA)					
Stress (S)	**1**	NA	NA	NA	NA	**	**	**	**	**
Genotype(G)	**35**	NA	**	**	**	**	**	**	**	**
S x G	**35**	NA	NA	NA	NA	NA	**	**	**	**
					Green Index (GI)					
Stress (S)	**1**	NA	NA	NA	NA	*	**	**	**	**
Genotype(G)	**35**	**	**	**	**	**	**	**	**	**
S x G	**35**	NA	NA	NA	NA	NA	**	**	**	**
					Hue angle (HA)					
Stress (S)	**1**	NA	NA	NA	**	**	**	**	**	**
Genotype(G)	**35**	**	**	**	**	**	**	**	**	**
S x G	**35**	NA	NA	NA	NA	*	**	**	**	**
					Dry index(DI)					
Stress (S)	**1**	NA	NA	NA	NA	**	**	**	**	**
Genotype(G)	**35**	**	**	**	**	**	**	**	**	**
S x G	**35**	NA	NA	NA	NA	*	NA	NA	NA	NA

NA: not applicable, d.f.: degrees of freedom. P-value: Probability of observing more extreme data under the hypothesis of no effect or no interaction and based on F-distribution. An ANOVA model for split-plot design in RCBD, with stress factor in main-plots and genotype factor in sub-plots, was fitted. Blue numbers represent the significance after day53.

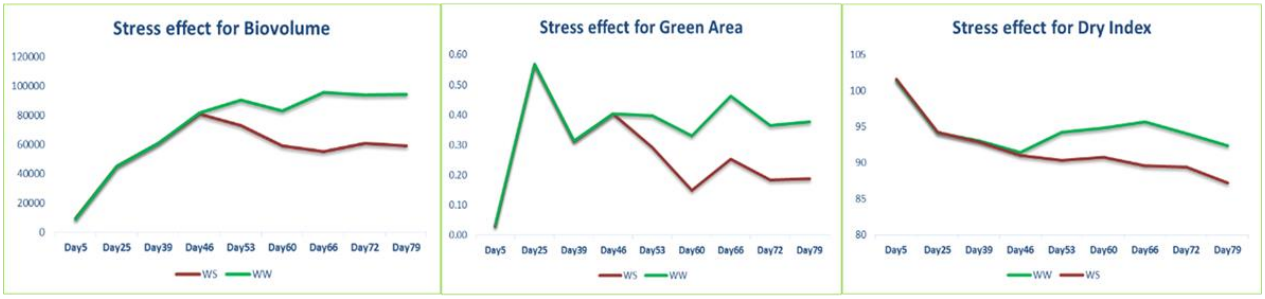


Fig 3.5. Illustrating the effect of stress imposition from day 46 in both WW and WS treatments for biovolume, green area and dry index.

Part 2: Interaction with days under repeated measures

Combined ANOVA across time points (from day 53 to 79), with time and treatments as factors was carried out for biovolume, fresh weight, green area, green index, yellow index, hue angle and dry index. Repeated measures analysis over time points from days 53 to 79 (53, 60, 66, 72, 79) showed significant differences for stress, genotypes and their interaction with time (Stress x Time, Geno x Time) for all the traits studied (Table 3.5). However, higher order interaction among Stress x Geno x Time did not show significant for any of the traits. These results indicate that genotypes differed significantly with time and stress levels for all the traits.

Table 3.5. Combined ANOVA across time points (from day 53 to 79), with time and treatments as factors.

Traits		Biovolume		Fresh weight		Green area		Green index		Yellow index		Hue angle		Dry index	
Source	d.f.	m.s.a	*p*	m.s.	*p*	m.s.	*p*	m.s.	*p*	m.s.	*p*	m.s.	*p*	m.s.	*p*
Rep	4	75.5	NS	34.2	NS	0.1	NS	0.7	NS	0.1	NS	6.6	NS	1.4	NS
Stress	1	4048.0	**	1835.8	**	11.2	**	14.2	**	5.2	**	305.0	**	102.9	**
Main plot Residual	4	1.1		0.5		0.1		0.1		0.0		0.9		0.0	
Geno	35	37.6	**	17.1	**	0.1	**	0.2	**	0.1	**	2.0	**	1.4	**
Stress.Geno	35	13.9	**	6.3	**	0.0	**	0.1	**	0.0	**	1.7	**	0.3	*
Sub-plot Residual	280	4.1		1.9		0.0		0.0		0.0		0.4		0.1	
Time	4	54.0	**	24.5	**	1.3	**	1.0	**	1.7	**	14.8	**	5.3	**
Stress.Time	4	76.8	**	34.8	**	0.3	**	0.2	**	0.2	**	9.0	**	0.7	*
Geno.Time	140	0.8	NS	0.4	NS	0.0	NS	0.0	NS	0.0	NS	0.3	NS	0.0	NS
Stress.Geno.Time	140	0.4	NS	0.2	NS	0.0	NS	0.0	NS	0.0	NS	0.2	NS	0.0	NS
Residual	1152	1.8		0.8		0.0		0.0		0.0		0.3		0.1	

**: P<0.001, m.s.: Mean square. d.f: degrees of freedom. P-value: Probability of observing more extreme data under the hypothesis of no effect or no interaction and based on F-distribution. An ANOVA model for split-plot design in RCBD, with stress factor in main-plots and genotype factor in sub-plots, was fitted. m.s.a: where a=m.s x 10-3

Part 3: Water Stress Tolerance Index (WSTI)

In order to identify genotypes for tolerance to the water stress, the average response of genotypes over time was analyzed under both treatments from day 5 to day 79 where stress was imposed from booting stage (from day 46 until day 79) for biomass, green index, yellow index, green area, and dry index (Fig 3.5). Based on Water Stress Tolerance Index (WSTI), hybrids and parents were classified for their tolerance to water stress (WS). Since stress had strong effect from the day 60 to day 79, the average of day 60 to 79 was correlated with last day (day 79) which

resulted in the same ranking for genotypes. Therefore, the last day point (day 79) was chosen to characterize for WSTI. Based on WSTI, genotypes were ranked into three categories namely, tolerant, moderately tolerant and susceptible.

For biovolume, WSTI showed that out of 36 genotypes, four F_1 hybrids (Svevo x Iride, Svevo x Miki, Iride x Miki, Valnova x Miki) and one parent (Svevo) were moderately tolerant. Based on the WSTI index, six F_1s were classified as susceptible and the remaining were moderately tolerant (Table 3.6). WSTI was also calculated for other traits like green area, green index, yellow index and dry index. Similar to biovolume, these traits had strong correlation with day 79 and average of day 60 to day 79. Therefore, raking was done for a last day point. Since all these traits are interdependent due to its chlorophyll and dry index characterization thus these traits were ranked collectively. Results of these traits depicted that hybrids, Svevo x Miki, Valnova x Miki, Iride x Miki, Karim x Iride and Gallareta x Morocco were tolerant and Morocco was tolerant parent whereas four crosses were susceptible for chlorophyll content and dry index. Remarkably, outcomes of lemnatec studies for all traits including biovolume confirmed that Svevo x Miki, Valnova x Miki and Iride x Miki were most tolerant hybrids whereas Massara x Miki, Karim x Valnova, and Iride x Massara were susceptible hybrid combinations. The remaining F_1 and parents were characterized as moderate among 36 genotypes in durum wheat (Table 3.6). In all this analysis helped to understand the three common robust tolerant and three worst heterotic combinations in durum wheat.

Following are the summary statistics of last day 79 for BV illustrating the classification of genotypes.

Mean =36.39, Minimum = 15.13, Maximum = 50.32, SD= 7.95

Scale points for the groups: < Mean –SD, (Mean-SD, Mean + SD), Mean+SD

Mean-SD = 36.39(Day79) – 7.95(Day79) = 28.45

Mean+SD = 36.39 (Day79) + 7.95(Day79) = 44.34

Tolerant group comprised of genotypes with BV less than 28.45

Table 3.6. Estimation of a Water Stress Index and its ranking for last time point (day 79) for biovolume, yellow index, dry Index, green index and green area.

Geno SN.	P1 x P2	Biovolume (BV)			Yellow index	Dry index	Green index	Green area	Based on YI,DI, GI and GA	
		WSTI	Group	Rank (Geno)	WSTI	WSTI	WSTI	WSTI	Group	Rank (Geno)
1	Gl x Mr	40	Moderate	25	-71	-5	24	10	**Tolerant**	3
2	Gl x Kr	36	Moderate	18	-180	-3	46	21	Moderate	19
3	Gl x Ir	30	Moderate	7	-240	-7	46	29	Moderate	28
4	Gl x Sv	38	Moderate	20	-280	-6	62	27	Moderate	31
5	Gl x Vl	36	Moderate	19	-220	-7	56	28	Moderate	32
6	Gl x Ms	41	Moderate	28	-78	-7	63	24	Moderate	24
7	Gl x Mk	36	Moderate	17	-100	-5	40	15	Moderate	16
8	Mr x Kr	34	Moderate	12	-67	-4	50	13	Moderate	11
9	Mr x Ir	45	Susceptible	31	-57	-6	43	3	Moderate	8
10	Mr x Sv	36	Moderate	16	-100	-6	55	18	Moderate	21
11	Mr x Vl	39	Moderate	24	-133	-6	43	20	Moderate	20
12	Mr x Ms	39	Moderate	23	-250	-9	77	45	Susceptible	36
13	Mr x Mk	41	Moderate	27	-42	-8	47	21	Moderate	18
14	Kr x Ir	34	Moderate	13	-57	-5	32	15	**Tolerant**	5
15	Kr x Sv	35	Moderate	15	-80	-6	76	23	Moderate	23
16	Kr x Vl	47	Susceptible	33	-200	-8	73	36	Susceptible	34
17	Kr x Ms	41	Moderate	29	-78	-7	60	33	Moderate	25
18	Kr x Mk	40	Moderate	26	-63	-7	48	13	Moderate	17
19	Ir x Sv	28	**Tolerant**	4	-100	-5	43	18	Moderate	13
20	Ir x Vl	43	Moderate	30	-180	-5	44	27	Moderate	22
21	Ir x Ms	50	Susceptible	36	-111	-8	70	38	Susceptible	33
22	Ir x Mk	25	**Tolerant**	3	-86	-3	30	20	**Tolerant**	6
23	Sv x Vl	39	Moderate	22	-86	-5	33	17	Moderate	14
24	Sv x Ms	45	Susceptible	32	-100	-8	53	32	Moderate	30
25	Sv x Mk	19	**Tolerant**	2	-17	-3	29	13	**Tolerant**	1
26	Vl x Ms	50	Susceptible	35	-180	-8	50	23	Moderate	29
27	Vl x Mk	29	**Tolerant**	5	-75	-5	26	13	**Tolerant**	4
28	Ms x Mk	48	Susceptible	34	-157	-9	65	34	Susceptible	35
29	Gl x Gl	30	Moderate	8	-120	-3	31	15	Moderate	7
30	Mr x Mr	30	Moderate	6	-43	-4	11	10	**Tolerant**	2
31	Kr x Kr	31	Moderate	9	-138	-6	62	28	Moderate	26
32	Ir x Ir	34	Moderate	11	-120	-5	32	15	Moderate	15
33	Sv x Sv	15	**Tolerant**	1	-55	-3	38	26	Moderate	9
34	Vl x Vl	38	Moderate	21	-100	-5	29	15	Moderate	10
35	Ms x Ms	33	Moderate	10	-140	-8	52	21	Moderate	27
36	Mk x Mk	34	Moderate	14	-40	-7	44	10	Moderate	12

Geno: Genotype, YI: Yellow index, DI: Dry index, GI: Green index, GA: Green area,Gl: Gallareta, Ir: Iride, Kr: Karim, Mk: Miki, Mr: Morocco, Ms: Massara, Sv: Svevo, Vl: Valnova.

Furthermore, the genotypic variation among 36 genotypes from day 53 to day 79 (after stress imposition) significant. Among them four best tolerant F_1 hybrids with their parents for biovolume and four Susceptible F_1 hybrids with their parents for dry index were obtained (Fig 3.6). The pattern differences between tolerant (increasing with time) and susceptible (decreasing with time) genotypes are depicted in Fig 3.6.

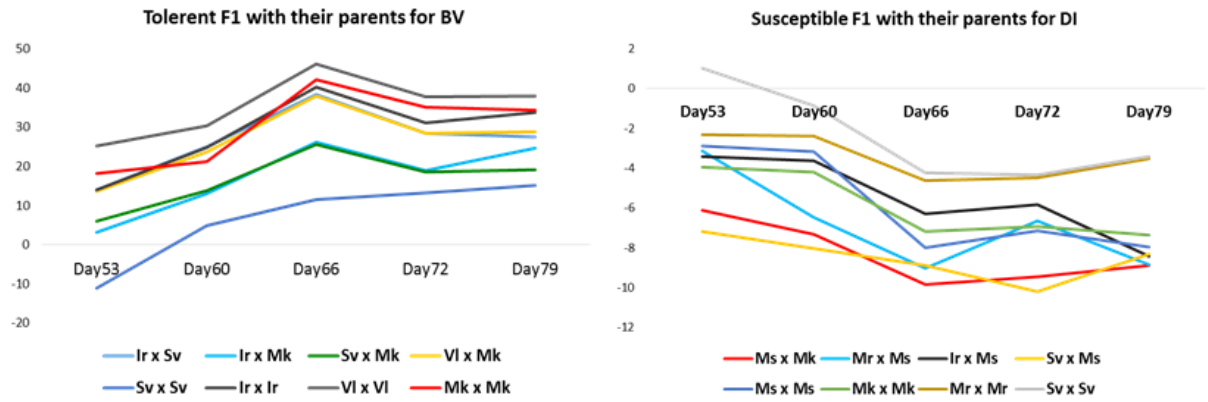


Fig 3.6. Comparing genotypic variation among four tolerant and susceptible f_1 with its parents from day 53 to day 79 (after stress imposition) for biovolume and dry index in durum wheat.

Part4. Specific and General combining ability on Lemnatec traits based on WSTI

Analysis of variance was carried out based on mean genotypic value calculated as WSTI of each trait namely, biomass, green index, yellow index, green area, and dry index. Significant differences were observed for GCA for all traits whereas SCA was significant only for yellow index and dry index. Significant SCA for yellow index and dry index suggests that non-additive gene actions were more important compared to additive gene action. Relative parent heterosis was high for all traits (Table 3.7). Parental mean was lower than F_1 hybrid mean for all traits except biovolume.

Table 3.7. ANOVA and combing ability results for seven traits in 28F_1 and eight parents of measured on Lemnatec in durum elite line.

Lemnatec	GCA	SCA	P1/P2	Mean	MPH%	Parents mean	F_1 mean
BV	**	Ns	**	34.6	7.1	36.1	34.0
Dark Green	**	Ns	**	45.8	13.0	42.9	46.6
Yellow	**	**	**	-106.4	-28.9	-92.4	-108.2
Green area	**	Ns	**	21.5	4.8	18.4	22.3
Hue angle	**	Ns	**	11.5	3.4	9.8	12.0
Dry Index	**	**	**	-5.4	1.2	-4.9	-5.6

**: P<0.01, Ns: not significant (P>0.05)

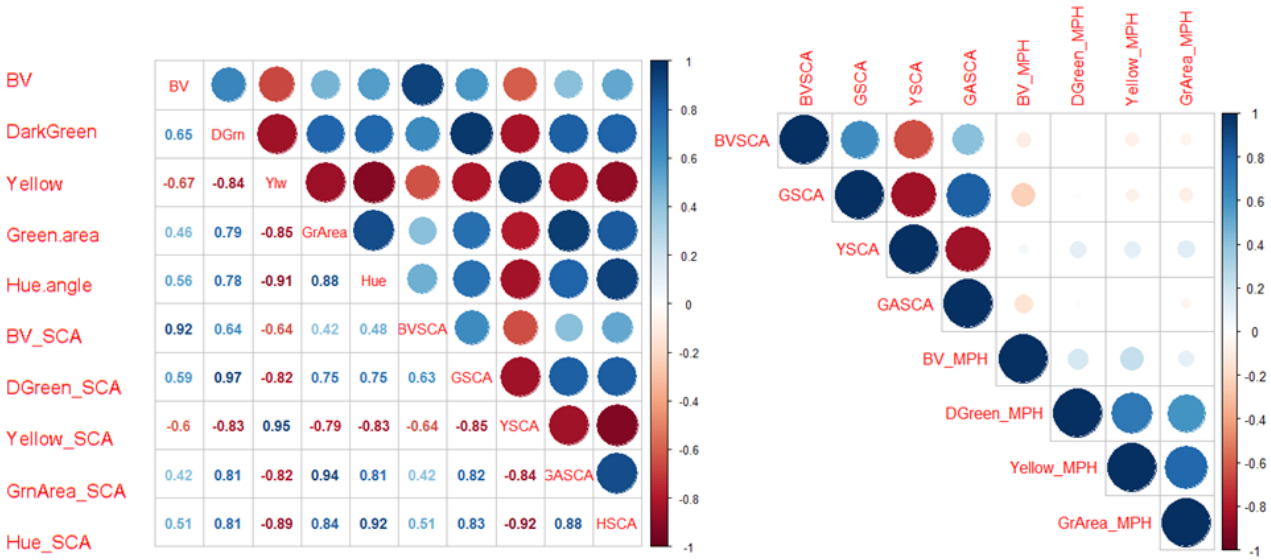
Further, analysis was done based on geographical location which clustered in four groups where comparison of the values of mean, MPH and SCA were observed. Results of these analysis described that different groups for example Group3 (CIMMYT80s) with Group4 (ICARDA80s) and Group2 (CIMMYT70s) were performed better which subsequently followed by Group1 (Italian) for green index (Table 3.8).

Table 3.8. Values of mean, MPH% and SCA among four groups of durum elite lines based on the evaluation with the Lemnatec platform for green index.

Groups	Values	CIMMYT (70s)	CIMMYT (80s)	ICARDA (80s)
ITALIAN	Mean	36.7	46.2	40.6
	MPH%	9.7	16.5	-1.5
	SCA	-5.2	5.5	-0.2
CIMMYT(70s)	Mean	41.6	49.8	54.3
	MPH%	14.5	17.3	16.1
	SCA	-7.0	2.5	6.7
CIMMYT (80s)	Mean		48.0	52.7
	MPH%		6.3	27.8
	SCA		1.8	6.3
ICARDA (80s)	Mean			13.0
	MPH%			14.2
	SCA			-33.6

Part5: Correlation analysis of Lemnatec traits

Correlation analysis of Lemnatec study depicted strong relationship between all traits either positively or negatively. For example, biovolume had positive correlation with dark green (0.65) green area (0.46) and hue (0.56) whereas negative correlation with yellow or dry index (-0.67 with BV and -0.84 with Dark green). As expected, genotypes which produced high biomass and green area had low yellow pigment and may poor water depletion. Similarly, correlation among SCA of each trait with its means indicated strong positive correlation between green area, green index and biovolume. However, among all lemnatec traits MPH had very weak correlation with SCA that showed GCA played significant role and determine the heterosis for green index and biovolume of genotypes rather than SCA (Fig. 3.7).



(A) **(B)**

Fig 3.7. Correlation analysis for Lemnatec traits A) mean value with SCA and, B) SCA with MPH.

Dark and big circle indicate strong correlation whereas blue and red show positive and negative relationship respectively **BV: biovolume, DGrn: dark green index, Ylw: yellow index, GrArea: green area, Hue: hue angle, SCA: specific combining ability and, MPH: mid parent heterosis.

Chapter 3b: Yield performance of hybrids under near-field conditions

Objective

Another experiment was carried out to determine shallow or deep rooting behavior under near field conditions using a basket method at ICARDA, Rabat, Morocco during 2016-17. The aim of this study was to identify the most heterotic combinations with adaptation to drought stress. This experiment evaluated above ground biomass, below ground root weight, and average growth angle of roots with 3D structure of roots and yield related traits.

Material and methods

Genetic material

Ten genotypes, two from each of the five structured groups were evaluated at the International Center of Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco during 2017. Among the 10 genotypes, two genotypes, namely Valnova and Karim were common with those chosen for crossing at the University of Bologna (Table 3.9). A total of 25 F_1 hybrids were performed in the ICARDA Marchouche experimental station, Morocco following North Carolina Design II (Nduwumuremyi et al., 2013), which allows for the estimation of both general and specific combining ability. For this experiment, 53 F_1 along with its 18 parents and one local check were assessed following the root basket method (Uga, 2012). This material included 28 F_1 and 8 parents (half diallel) from the University of Bologna and 25F_1 and 10 parents (NC II design) from ICARDA, Morocco.

Experimental design and phenotypic evaluation

For phenotyping root architecture, open plastic mesh baskets (top diameter of 18 cm, bottom diameter of 11 cm, height of 11 cm, and mesh size of 3 mm) were used. These baskets were filled with soil mixed evenly with fertilizer @ 15N:15P:15K before planting, and during the vegetative to flowering period, 33.5% of nitrogen (2 mg per basket) was also applied. These baskets were buried in the field maintaining a spacing of 20 x 17.5 cm between baskets. The experimental design was alpha lattice with two replications and two treatments, well-watered (WW) and water stressed (WS) (Fig 3.8).

Three seeds were sown in each basket, and plants were thinned to one per basket after seedling establishment. Observations were recorded for above ground traits like days of heading, plant height, spike number, spikelet number, spike length, seed number, 1000-kernel weight, seed weight and total biomass weight as well as SPAD at different time intervals from heading to maturity. For underground observation at maturity, the baskets were pulled out carefully and soil was removed around the basket from the field. To understand the root growth pattern, root growth angel (RGA) were recorded at three levels of basket and then washed. Further, to study the root physiology, images of root lengths were taken following WinRhizo software as well as other traits like root level per ratio and root biomass (Fig 3.9).

Table 3.9. Crossing scheme implemented in Morocco.

	Male parent	Omrabi5	Colosseo	Ofanto	Terbol 973	Stot//Altar 84/Ald
Female parent	Germplasm Group	Group1 ITALIAN	Group 2 CIMMYT(70s)	Group3 CIMMYT (80s)	Group 4 ICARDA (80s)	Group 5 ICARDA (80s II)
Awalbit	ITALIAN	Hyb1	Hyb6	Hyb11	Hyb16	Hyb21
Marouane	CIMMYT(70s)	Hyb2	Hyb7	Hyb12	Hyb17	Hyb22
Valnova	CIMMYT (80s)	Hyb3	Hyb8	Hyb13	Hyb18	Hyb23
Karim	ICARDA (80s)	Hyb4	Hyb9	Hyb14	Hyb19	Hyb24
Hessian-F2/3/Stot// Altar 84/Ald	ICARDA (80s II)	Hyb5	Hyb10	Hyb15	Hyb20	Hyb25

Statistical analysis

Data on individual traits obtained from the baskets were analyzed following a model for alpha design to estimate the genotype effects and interaction with stress treatment. The REML directives of Genstat software was used with the following:

Fixed effect: Genotype + Treatment+ Genotype x Treatment

Random effect: Replication+ Replication.Block

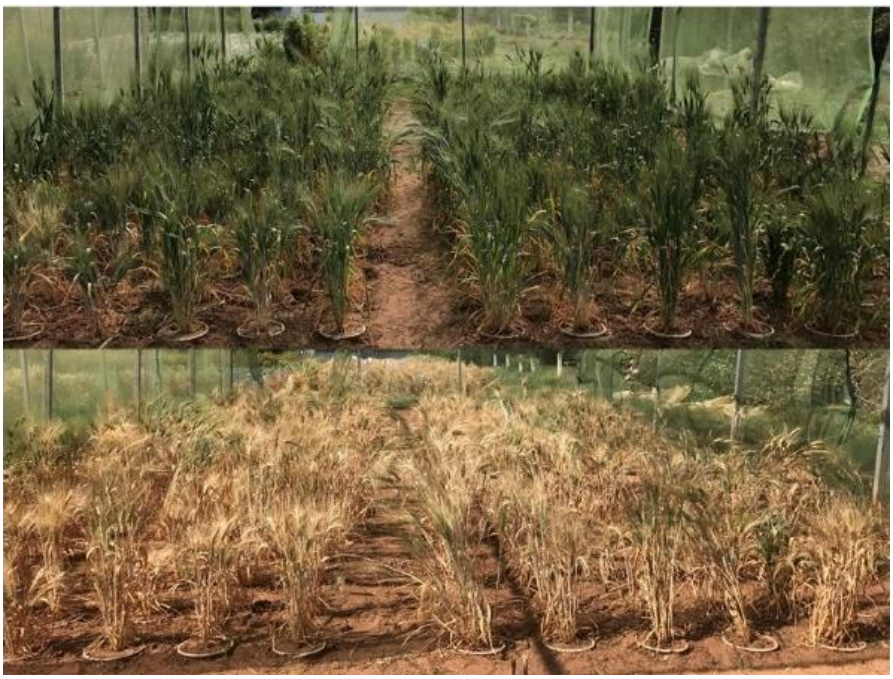


Fig 3.8: Evaluation of 53 F_1 hybrids and its parents by root basket method in ICARDA, Rabat (2016-2017)

Fig 3.9: Characterization of below ground (root) traits by WinRhizo software (scanned images).

Further, combining ability analysis for 28F_1 and eight parents (by diallel) from university of Bologna and 25 F_1 and 10 parents (by NCII design) from ICARDA was carried out. Diallel analysis was carried out by adopting the same model and software as outlined in Chapter 2. Mid-parent and better-parent heterosis (MPH and BPH) were calculated using formula proposed by Fonseca and Patterson (1968) and described in Chapter 2. For the North Carolina II analysis, following model proposed by Athanase et al. (2013) was adopted:

$yijk = \mu + mi + fj + (mf)ij + eijk$,

where,

μ= overall mean

si= General combining ability effect of male i^{th} genotype

dj= General combining ability effect of female j^{th} genotype

(*sd*)*ij*= Specific combining ability effect between male i^{th} and female j^{th} parents

eijk = Pooled error

Results

Analysis of variance (Table 3.10) indicated significant differences among genotypes for all the above-ground (SPAD, grain yield, 1000-kernel weight, harvest index, plant height, days to heading) and below-ground traits except root level-1 (root level2, root level3, total root number and root biomass). Water stress treatments (well-water versus water stress) showed significant effect on all traits except for root level1 and root level3. Analysis showed significant genotype by treatment (GxT) interaction only for the four above-ground traits (SPAD, grain yield, 1000-kernel weight and harvest index), indicating that different genotypes responded to water stress differently for these traits while for under-ground traits, no such interaction between genotypes and water regime treatments was observed.

Table 3.10: Analysis of variance for above and below ground traits in 18 parents and 53 F_1 hybrids in durum wheat evaluated during 2017

Source	SPAD	GY	TKW	HI	PH	DTH	RTL 1	RTL2	RTL3	TRTN	RTBM
Genotype (G)	**	**	**	**	**	**	NS	**	**	**	**
Treatment (T)	**	*	**	**	**	**	NS	*	NS	*	*
G x T	**	**	**	**	NS	NS	NS	NS	NS	NS	NS

*: P<0.05. **: P<0.01, GY: grain yield, TKW: 1000-kernel weight, HI: harvest index, PH: plant height, RTL1: root level1, RTL2: root level2, RTL3: root level3, TRTN: total root number and RTBM: root biomass.

Diallel analysis

In order to evaluate the performance of the parents and F_1 hybrids under water-stress conditions, 8 parents along with 28 F_1 hybrids made in half-diallel fashion were evaluated under well-water and water stress conditions following root basket methods in the net-house. Analysis of variance showed no significant differences in performance between well-water and water stress treatments. Analysis of variance showed significance among genotypes for all the traits except spike number (Table 3.11). The mean performance of F_1 hybrids was higher than the parental mean for all the traits studied except for root level1. Combining ability analysis among the 28 F_1s and their parents showed significant SCA effects for all the traits namely, grain yield (GY), straw biomass weight (SBW), total biomass weight (TBW), 1000-kernel weight (TKW), harvest index (HI), root biomass (RtBiomass), total root number (TotalRtNo), rootLevel1, rootLevel2 and rootLevel3 except spikes number (SPKN). However, GCA effects were significant only for 1000-kernel weight, root level1 and root level2. Diallel analysis showed 43.1% mid-parent heterosis (MPH) and 13.1% best parent heterosis (BPH) for grain yield, indicating the scope for exploitation of heterosis in durum wheat. Other above-ground traits with substantial BPH% was straw weight (20.4%) followed by total biomass weight (6.2%). Among the below ground traits, only rootlevel3 (9.7%), and rootlevel1 (7.5%) showed above 5% best parent heterosis. However, all the below ground traits, root biomass (RtBiomass), total root number (TotalRtNo), rootLevel1, rootLevel2 and rootLevel3 had above 17% mid parent heterosis, suggesting interplay of both additive and non-additive gene effects in the expression of root traits and predominance of non-additive gene effect in the expression of above ground traits.

Table 3.11: Combing ability analysis for above and below ground traits in 28 F_1 and eight parents of durum wheat in net house at ICARDA, Morocco during the crop season of 2017

Traits	P1/P2*	GCA	SCA	Mean	MPH%	BPH%	Parents mean	F1 mean	
GY	**	NS	**	25.6	43.1	13.1	21.8	26.8	Above ground traits
SBW	**	NS	**	59.0	21.6	20.4	51.7	61.0	
TBW	**	NS	**	84.6	4.4	6.2	73.5	87.8	
TKW	**	**	**	39.4	26.6	-0.4	38.1	39.8	
HI	**	NS	**	0.3	10.0	1.62	0.3	0.4	
SPKN	NS	NS	NS	12.2	12.3	-1.9	11.1	12.6	
RtBiomass	**	NS	**	4.6	24.1	1.5	4.2	4.8	Below ground traits
Total RtNo	**	NS	**	27.2	24.6	3.9	23.7	28.2	
RootLeve1	**	*	**	10.5	39.6	7.5	11.6	10.2	
RootLeve2	**	**	**	14.5	17.7	-5.6	9.6	15.9	
RootLeve3	**	NS	**	10.1	30.8	9.7	9.3	10.4	

*: P<0.05. **: P<0.01, ns: not significant (P>0.05), P1/P2: variation among parents, mean values, mid parental heterosis (MPH%), best parent heterosis (BPH%), total parental and F1 mean for 11 traits, GY: grain yield, SBW: straw biomass weight, TBW: total biomass, 1KW: 1000-kernel weight, HI: harvest index, SPKN: spikes number, RtBiomass: root biomass and Total RtNo: total root number.

Analysis of F_1 hybrids for root biomass within and among the four heterotic groups of parents exhibited higher values of mean, MPH, BPH and SCA (Table 3.12). For root biomass, values of MPH, BPH within each of the four groups of durum elite lines were low and SCA were negative on the diagonal which represent within the group. However, group2 (CIMMYT 70s) performed better within. The composition of the group2 showed best elite lines (Svevo, Miki and Karim). Above diagonal values showed that only few combinations between the groups had robust performance, for example, F_1 hybrids between parents representing Group3 (CIMMYT '80s) and Group4 (ICARDA 80s) expressed the higher MPH (39.6), BPH (22.4) and SCA (0.5) followed by between the parents from Group1 (Italian) and Group3 (CIMMYT '80s) [MPH (22.9), BPH (5.6) and SCA (0.2)] for root biomass. These results support the results obtained in the glasshouse experiment for grain yield.

Table 3.12: Mean, MPH, BPH and SCA for root biomass among four groups of durum elite lines in the net house experiment

Root biomass		Group2	Group3	Group4
Group1	Values	CIMMYT (70s)	CIMMYT (80s)	ICARDA (80s)
ITALIAN	Mean	5.2	5.5	4.3
	MPH%	-2.0	22.9	3.4
	BPH%	-29.2	5.6	-8.9
Group2	SCA	0.3	0.2	-0.9
CIMMYT(70s)	Mean	4.3	4.4	4.7
	MPH%	35.4	18.4	38.7
	BPH%	10.5	-4.8	11.8
Group3	SCA	0.2	-0.1	0.3
CIMMYT (80s)	Mean		4.8	5.2
	MPH%		14.9	39.6
	BPH%		-10.1	22.4
Group4	SCA		-0.1	0.5
ICARDA (80s)	Mean			5.1
	MPH%			4.6
	BPH%			-17.1
	SCA			0.5

Table 3.13 provides the best parental combinations among four groups by comparing the mean performance of F_1 hybrids for root biomass along with SCA and GCA of the parents involved in the hybrid. The results revealed that the best parental combinations were Valnova-Svevo, Iride-Morocco, followed by Gallareta-Svevo and Gallareta- Valnova for root biomass. In net house experiment, Valnova-Miki performed better for grain yield and Valnova-Svevo combination for root biomass. In case of root level1 and root level2, Gallareta-Massara combination performed better followed by Valnova –Svevo while for root level3, two combinations, Miki-Svevo, Karim-Morocco and Svevo as parent performed better. This indicates that parents, Karim, Miki and Svevo have deep roots as compared to Gallareta, Massara, and Valnova.

Table 3.13 Estimates of mean performance in the upper panel, and GCAs and SCAs (lower panel) of root biomass for F_1 and parents among four groups in the net house. GCAs (in diagonal) and SCAs (in upper triangle).

Root biomass	**Gl**	**Ir**	**Kr**	**Mk**	**Mr**	**Ms**	**Sv**	**Vl**
Gl	**5.4**	4.8	4.2	5.7	4.1	6	6.2	6.3
Ir		**3.6**	2.8	5.1	6.8	3.9	2.5	4.8
Kr			**2**	4.9	5.6	5.9	2.9	4.1
Mk				**3.1**	4.3	3.4	5.1	4.6
Mr					**3.5**	5.1	4.1	3.4
Ms						**4.4**	4.7	5.1
Sv							**5.6**	6.8
Vl								**6**

GCA/SCA	**Gl**	**Ir**	**Kr**	**Mk**	**Mr**	**Ms**	**Sv**	**Vl**
Gl	**0.6**	-0.1	-0.4	0.7	-1.1	0.6	0.7	0.5
Ir		**-0.4**	-0.7	1.1	2.7	-0.5	-1.9	0
Kr			**-0.7**	1.3	1.8	1.8	-1.2	-0.3
Mk				**-0.2**	0	-1.1	0.5	-0.3
Mr					**-0.1**	0.5	-0.6	-1.6
Ms						**0.1**	-0.2	-0.1
Sv							**0.2**	1.5
Vl								**0.5**

Gl; Gallareta, Ir; Iride, Kr; Karim, Mk; Miki, Mr; Morocco, Ms; Massara, Sv; Svevo, Vl; Valnova. The entries in shades with border are the parents (on diagonal) while upper diagonal are F_1 hybrids

North Carolina-II Analysis

In order to evaluate the performance of the parents and F_1 hybrids in the net-house, 10 parents along with 25 F_1 hybrids were evaluated under well-water and water stress conditions following root basket methods. The mean performance of F_1 hybrids was higher than the parental mean for all the traits studied except for root level1. Analysis showed significant SCA effects for straw biomass weight (SBW), total biomass weight (TBW), 1000-kernel weight (TKW), harvest index (HI), spike number (SPKN), root biomass (RtBiomass), total root number (TotalRtNo), and rootLevel2 while non-significant for grain yield and harvest index. However, GCA effects were significant only for grain yield, straw weight, total biomass, harvest index, root length (level1, 2 and3). F_1 hybrids showed positive mid-parent heterosis (MPH) and best parent heterosis (BPH) except rootlevel1 (Table 3.14).

Table 3.14 ANOVA and combing ability analysis for above and below ground traits in 25 F_1 and ten parents of durum wheat in net house 2017

Traits	P1/P2	GCA	SCA	Mean	MPH%	BPH%	Parents mean	F1 mean	
GY	**	**	NS	21.8	14.8	12.7	18.5	23.2	
SBW	**	**	**	60.0	12.6	10.4	51.8	63.3	
TBW	**	**	**	81.8	13.6	10.1	70.3	86.4	Above ground traits
TKW	**	NS	**	38.1	12.0	7.7	34.7	39.5	
HI	**	**	NS	0.4	9.9	0.2	0.3	0.4	
SPKN	**	NS	**	14.4	11.5	9.0	12.9	15.0	
RtBiomass	**	NS	**	5.4	11.1	9.7	4.8	5.7	
TotalRtNo	**	NS	**	25.9	10.8	2.2	23.4	26.8	Below ground traits
RootLevel1	**	**	NS	10.4	-16.8	-22.8	10.7	10.3	
RootLevel2	**	**	**	9.1	9.4	2.2	7.3	8.7	
RootLevel3	**	**	NS	7.3	1.7	8.6	6.2	7.8	

*: P<0.05. **: P<0.01, ns: not significant (P>0.05), P1/P2: variation among parents, mean values, mid parental heterosis (MPH%), best parent heterosis (BPH%), total parental and F1 mean for 11 traits, GY: grain yield, SBW: straw biomass weight, TBW: total biomass, 1KW: 1000-kernel weight, HI: harvest index, SPKN: spikes number, RtBiomass: root biomass and Total RtNo: total root number.

The results obtained from both the analysis, diallel along with North Carolina II in the net- house conditions confirmed the higher heterosis over their parents for all the traits studied except root level1. Root level1 represented shallow root type whereas root level3 associated with the deep rooting system and the results indicated that F_1 hybrids were higher than the parental mean for rootlevel3 rather than root level1. Hybrids are known to perform better under marginal condition and phenotyping for roots traits on root basket method at different level confirmed the presence of deep rooting type (root level3) over their parents in both the analysis. However, detail study needs to be done to find out the best heterotic groups in durum elite lines at below ground.

Discussion

From an agricultural perspective, plants are considered better adapted to a specific region when they flower at the appropriate time and yield acceptably well in certain environments (Wilczek et al., 2010). To identify genes/QTLS associated with flowering time variation, the present study was conducted to seek the flowering time loci in 384 durum core collection of ICARDA for a particular environment reflecting different climatic features. Observation on days to heading (DTH) was measured across 13-environments (eight locations over two growing seasons 2014/2015 and 2015/2016) located at different latitudes and temperature regimes (Fig 1.5a). Moreover, the germplasm in the panel was vastly diverse with two main subgroups corresponding to landraces and elite lines. This wide phenotypic database endorsed application of genome-wide association mapping approach for understanding the genetic basis of flowering variation evaluated under a wide range of environmental factors. Phenotyping results demonstrated that environmental variables such as temperature and photoperiod had significant effect in determining the phenotype of durum germplasm in five diverse pheno-environments which justified by both PCA and variance analysis of GxE and GxPhEnv interaction. It also confirmed significant genotypic variation for flowering time in five pheno-environments.

Early flowering has been an important selection criteria to avoid yield losses in wheat, particularly under terminal drought and heat stress conditions (Iqbal et al., 2007). Many studies have indicated that regulatory photoperiodic genes, such as *Ppd*-A1, *Ppd*-B1, *Ppd*-D1 and the vernalization gene *Vrn*-A have major influence on flowering time (Worland, 2001; Zanke et al., 2014). Recent studies revealed complementary interaction between *Vrn*-1 and *Ppd*-D1 imparting super/very-early flowering habit in spring wheat (Sukumaran et al., 2016). In addition to the *Vrn* and *Ppd* genes, effective flowering genes called as “earliness per se” (*Eps*) genes are also identified in wheat (Worland, 2001). *Eps* genes can speed up flowering time in any stage of development which can be used for shortening the life cycle of wheat (Kato & Wada, 1999; Dubcovsky et al., 2016). Recent study indicated that vernalization and photoperiod genes mask the effect of *Eps* genes in spring wheat (Sukumaran et al., 2016). Thus, genotypic variation for flowering time can be utilized to match crop duration with agro-climatic conditions prevailing in

the target production zones (Lewis et al., 2008) and to synchronise flowering time of heterotic parents for hybrid seed production.

Genome-wide association studies (GWAS) in bread and durum wheat have identified QTLs for flowering time, disease resistance and key agronomic traits (Barbosa-Neto et al., 1996; Crossa et al., 2007; Le Gouis et al., 2012; Maccaferri et al., 2008, 2014, & 2015; Reif et al., 2011; Zhang et al., 2014). The present study analysed QTL effect as a function of environmental variables to determine flowering genes/QTLs in different populations under wide range of environmental conditions by analysing 384 diverse durum core set of ICARDA with 7740 SNPs across 13 environments with different latitudes, longitudes and temperatures. Association analysis recognized 47 QTLs (20 in landraces and 27 in elite lines) consistently significant across five pheno-environments. Based on the annotation at the defined confidence interval, candidate genes were retrieved from the Svevo physical map (Cattivelli et al. unpublished 2018) and Knetminer database (http://knetminer.rothamsted.ac.uk). These approaches resulted in many candidate genes already identified in orthologues species like *Arabidopsis*, rice, wheat and other cereals for flowering time. Out of 20 and 27 QTLs identified in landraces and elite germplasm, only 13 and 21 QTLs contained possible candidate genes.

Among the identified QTLs, photoperiod and VRN specific markers were significant with high LOD and variance consistently across pheno-environments. In landraces, PPD-B1 was significant with very high variance (80%) while in elite lines, photoperiod dependent PPD-A and PPD-B were significant in across environment and all pheno-environments except PhEnv5 (Terbol off-season). Vernalization dependent markers, VrnA1 and Vrn3 were significant only in PhEnv5 and PhEnv3 (Fanaye) in elite lines. Both environments represent warmer climate. This confirms the key role of *PPD-A, PPD-B, VrnA1* and *Vrn3* genes already reported in many studies for regulation of flowering time (Turner et al., 2005, 2012; Laurie, 1997; McIntosh et al., 2005; Wilhelm et al., 2009; Diaz et al., 2012; Liuling et al., 2003). Recently, it is reported that *Vrn*-B1 allele was responsible for adaptation to different environmental conditions by accelerating or delaying flowering time (Shcherban et al., 2017).

In the present study, *Ppd-A1* gene was found in the region of QTL2 (AX-94385320 on Chr2A) in elite germplasm consistently in all pheno-environments with 8.5 LOD and 20.7% of variance. This finding was further supported with retrieval of *PRR37* and *SRT1* genes at OTL2 locus. *PRR37* gene plays an important role in photoperiodism in many crops and its mechanism reviewed recently in grasses (Murphy et al., 2011; Nuñez et al., 2017). Past studies revealed that *Arabidopsis* clock-associated pseudo-response regulators *PRR9*, *PRR7* and *PRR5* positively regulate flowering time through the canonical constans-dependent photoperiodic pathway (Nakamichi et al., 2007). These studies also support the view that, not only APRR1/TOC1 but also APRR1/TOC1 quintet members are important for a better understanding of a molecular link between circadian rhythm, flowering time control and photo-morphogenesis (Masaya Murakami et al., 2004). PROTEIN 37 (PRR37) also increases expression of Hd1, Hd3 and HD6 in rice which regulate the expression of *RFT1* in short and long days (Takahashi et al., 2001; Zanke et al., 2014). The importance of the Hd6 related genes for heading days described by Zanke et al. (2014) on chromosome 5B in wheat indicated significant homology to the rice photoperiodism gene *Hd6*. Recently, Hd6 gene was cloned as a rice QTL which determined photoperiod sensitivity and involved in the plant photo transduction pathway. Similar trend was observed for *PPD-B1* gene in the confidence interval of QTL5 (AX-94956877 on Chr2B) at Kaedi location (hot environment). This QTL contained three candidate genes, *SRT1, TKL-2 and BHLH74*. Knetminer Network view revealed that these genes have strong association with *PRR, TOC1, APRR, CCA1, LHY,* and *WNK1* genes which play an important function in controlling flowering time in cereals under short and long days.

On the other hand, QTL18 (AX-95259336) in landraces contained *VRN-5B* gene (557Mbp) at Fanaye and annotation with Knetminer database revealed RRP6L1 as possible candidate gene with 832 publications. The gene *RRP6L1* explained a strong network with *FLC* gene which regulate flowering requirement for vernalization (extended winter-like temperatures), which enables rapid flowering under long days. Much of the difference in vernalization response is apparently due to variation of FRI and FLC alleles (Lempe et al., 2005). In addition, QTL18 was associated with E3 ubiquitin-protein ligase *RNF14* which is responsible for plant development in durum wheat (David et al., 2012). The gene of this protein called *TdRF1* belongs to the WNK family of kinases known to have a role in the control of flowering response (Wang et al., 2008; Chen & Ni,

2006). Recently it has been reported that E3 ligases a master regulator of the cold response, and CONSTANS, the central component of the flowering pathways which causes delay flowering in *Arabidopsis* (Dong et al., 2006; Ariizumi et al., 2011; Lazaro et al., 2012).

Our study revealed two QTLs common in landraces and elite germplasm. QTL3 (CI at 556-558 Mbp) in landraces and QTL4 (CI at 555-561.2Mbp) in elite germplasm displayed almost similar position and identified *GRF5* and *VIT1* genes and associated functional protein for the response of flowering time (Purwestri et al., 2009). The *GRF* gene encodes growing factors known to bind the florigen CO/BBx at protein level, inhibiting the downstream pathway (Purwestri et al., 2009). Since QTL3 was detected only in hot environment (Terbol offseason), it infers that the genes underlying QTL3 act in a temperature-dependent manner (Guo, H et al., 1998; Reeves et al., 2001; Sang et al. 2007; He et al., 2012; Kumar et al., 2012). However, in elite lines this QTL was significant also in Fanaye and Terbol locations, suggesting combine effect of day length and temperature on days to heading. Past studies in *Arabidopsis* and rice also reported combined effect of *Hd3a and GF14c* genes (Purwestri et al., 2009). GF14c-overexpressing plants exhibited a delay in flowering while the knockout mutants displayed early flowering relative to the wild-type plants under short-day conditions.

The present study also identified QTL7 (AX-94452589, Chr3A) accounting for significant variance in Morocco (PhEnv1) and Terbol (PhEnv2) in both groups of germplasm, landraces as well as elite lines. This marker identified homologs of SWR1C in *Arabidopsis* called PIE1 (PHOTOPERIOD-INDEPENDENT EARLY FLOWERING 1) which causes early flowering by reduction in expression of FLOWERING LOCUS C (FLC), a strong floral repressor (Choi et al., 2011). This outcome related to the *Eps* gene might have a major impact for early flowering in Terbol for landraces and elite lines whereas in Morocco stations, it was illustrated only for elites. Among the significant QTLs in landraces, QTL5 (AX-94939920 on Chr2B) was reported across environment with *TCP1* as major gene. TCP1 gene (scored 754 based on Knetminer databases proved novel genes for “heading days”) is reported to be associated with three major genes named *FT, BRC1 and TSF*. Genetic and spatial interactions between FT, TSF and SVP regulate floral transition, and its mechanisms act in the leaf and meristem to control flowering time (Jang et al., 2009).

Most important flowering gene which explained significant variation in flowering time directly or in interaction with other genes was *SVP* in both landraces and elite lines (Fig 1.11). The role of *SVP* gene in the control of flowering time is by mediating the temperature-dependent functions of FCA and FVE within the thermosensory pathway in *Arabidopsis*. It also controls flowering time by negatively regulating the expression of a floral integrator, FLOWERING LOCUS T (FT), via direct binding to the CArG motifs in the FT sequence. This is one of the molecular mechanisms that modulate flowering time under fluctuating temperature conditions (Lee et al., 2007). The *SVP* gene also encodes MYB transcription factor called EARLY FLOWERING MYB PROTEIN (EFM) which plays an important role in repressing FT mediated flowering response to environmental cues in *Arabidopsis* (Jang, 2009; Yan et al., 2014). This gene also involves in various functions like accelerating flowering by two weeks under long day and high temperature. The present study also suggests the key role of *SVP* gene in two of the hottest environments, Terbol off-season and Fanaye in landraces as well as in elite lines, in modulating flowering time under high temperature.

In landraces, QTL10 (AX-95217431 on chr4A) was significant in Fanaye and Terbol. It was found associated with a protein kinase and its alpha subunit closely relates to the *Arabidopsis* genes which are linked to the flowering-time QTL (*Hd6*) of rice involved in photoperiod sensitivity (Kane et al., 2005; Takahashi et al., 2001). Another QTL8 (AX-95021774) (Chr3B) belongs to *HUB2 gene* (Histone H2B monoubiquitination) indirectly connected with the epigenetic mechanism of vernalization (Zhou et al., 2017). In elite lines, the confidence intervals of QTL15 and QTL16 contained *CSN1* and *RGA* genes in PhEnv3 (Fanaye). The CSN1/COP1/SPA complex acts together to control photo-morphogenesis and inhibits flowering under non-inductive short-day conditions (Nixdorf et al., 2010) while the *RGA* gene encodes FRIGIDA that up-regulates expression of the floral repressor FLOWERING LOCUS C (FLC), confers vernalization requirement and delays flowering in *Arabidopsis* via a co-transcriptional mechanism (Geraldo et al., 2009).

The most significant novel genes, namely *PRR7, GRF, SVP, RRP6L1, Hd6, TCP1,* and *COP1/RGA* have been reported to affect regulation of flowering time in orthologous species, *Arabidopsis* rice, wheat, etc. The *PRR7* gene was most significant and stable as it was retrieved from all pheno-environments except PhEnv5 followed by the second most stable gene, *GRF* from PhEnv2,

PhEnv3 and PhEnv5. Among the five pheno-environments, PhEnv3 (Fanaye) was the best pheno-environment to retrieve maximum number of candidate genes for heading date (Vrn-1-5A, Vrn5B, PRR7, RRP6L1, GRF, SVP and Cop1) followed by PhEnv5 (Terbol off-season), and PhEnv2 (Terbol) and PhEnv4 (Kaedi). PhEnv1 representing Morocco locations revealed the least number of candidate genes. The function of the candidate genes revealed in different pheno-environments matches with the adaptation requirement imposed by the agro-climatic conditions prevalent in test locations. Therefore, classification of environments in diverse pheno-environments has helped to recover novel candidate genes related to specific climatic conditions like high temperature responsive genes (*SVP, RRP6L1*) in PhEnv3 (Fanaye) and PhEnv5 (Terbol off-season) in addition to *VRN1* and *VRN3* genes. Similarly, effect of photoperiodism was prominent in PhEnv2 (Lebanon) resulting in identification of PPD specific gene (*PRR7*, *SD6*) in addition to already identified genes (*PPD-A, PPD-B*). Elite germplasm and landraces had different QTLs except two QTLs, indicating that breeding efforts have led to changes in the genetic makeup of elite lines in terms of earliness, photoperiod insensitivity and vernalization requirement. Phenotyping of the same durum core germplasm for heading date in earlier study (Kabbaj et al., 2017) also indicated that landraces did either not flower or flower late as compared to elite lines.

To meet the future demand of durum wheat under climate change and variability, there is a need to accelerate productivity growth under more diverse and unpredictable agro-climatic conditions. Hybrid technology is one of the readily available options, provided there is sufficient heterosis and a cost effective seed production system. Past studies in durum wheat (Sayar et al., 2007 and M Gowda et al., 2010) suggest that exploitation of heterosis is possible if we identify superior hybrid combinations which in turn depend on the parental selection. The present study was undertaken to investigate the role of parental selection, identify the best heterotic combinations based on mid-parent value (MPH), and general (GCA) or specific (SCA) combining ability and the magnitude of heterosis. The experiments were conducted both in open field at UniBO experimental station, Cadriano, Italy, under the controlled environments using the HTPP (lemnatec with different levels of water stress) at Metaponto, Agrobios ALSIA, and in field (under net house) at the International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco in order to assess heterosis for yield and yield related traits, find the best performing

parents (based on GCA and SCA) and establish relationships among traits measured in different field and controlled environments. The experimental material included were eight parents representing different five heterotic groups based on genetic similarity, pedigree, and geographical diversity, and their 28 F_1 hybrids in half-diallel fashion.

The present field and glasshouse studies revealed that the mean performance of F_1 hybrids was higher than that of parents for all the traits studied. The results showed 25.8% and 19.6% of mid parent heterosis (MPH) in field and glasshouse experiments, respectively, indicating the scope for exploitation of heterosis in durum wheat. The average MPH observed for grain yield in the present study is in line with the findings of earlier reports of 10 to 25% MPH in durum wheat (Amaya et al., 1972) (Widner & Lebsock, 1973), (Sayar et al., 2007) and (M Gowda et al., 2010). However, above 20% average MPH for grain yield observed in the present study is higher than the MPH reported in other self-pollinated crops like bread wheat and triticale (Oury et al., 2000) (Oettler et al., 2005) (Fischer et al., 2010). This might be due to prevalence of overdominance interaction or unfavorable epistatic interactions in durum wheat. Heterosis is the variance from the mid-parent value which might result from difference in allele frequencies among the parents, the degree of dominance, and additive x additive epistatic effects (A. Melchinger, Utz, Piepho, Zeng, & Schön, 2007). In the present study, analysis of variance (ANOVA) among the 28 F_1s and their parents showed significant specific combining ability (SCA) effect for all agronomic traits in field as well as in glass house study, indicating that non-additive gene action played a predominant role in determining the heterosis in the present material. Further, significant positive correlation among F_1 hybrid mean, MPH and SCA for grain yield also indicate towards non-additive and epistasis gene actions for heterosis in field as well as under controlled environments. These results are in agreement with the results of (Goldringer, Brabant, & Gallais, 1997) who also found larger epistatic than additive variance for grain yield in wheat. In contrast, recent research showed predominance of additive and additive x additive gene actions in self-pollinated crops (Kaeppler, 2012) (Beche et al., 2013) (Huang et al., 2015). Our results of diallel and NCII analysis suggest that F_1 hybrid in durum wheat has an advantage over the inbred varieties.

Success of hybrid breeding relies on the parent selection. It is presumed that hybridization between genetically diverse parents results in better heterosis (Boeven, Longin, & Würschum, 2016). Keeping this in mind, we selected eight parents representing five genetically and geographically diverse groups of durum wheat accessions: GP1 (Italian), GP2 (CIMMYT 70s), GP3 (CIMMYT 80s), GP4 (ICARDA 80s for temperate areas) and GP5 (ICARDA 80s for dryland areas) (Maccaferri et al. 2005). The results indicated negative SCA values for F_1 hybrids involving parents from the same group with low mid and best parent heterosis. On the contrary, F_1 hybrids between elite lines representing different groups showed high MPH, BPH and SCA. Hybrids between Valnova (the only parent representing Group1, Italian) and Group2 (CIMMYT '70s) showed the highest MPH and BPH followed by F_1 hybrids between Group2 (CIMMYT '70s) and Group4 (ICARDA '80s) and Group2 (CIMMYT '70s) and Group3 (CIMMYT '70s). These results support the central dogma of hybrid breeding that higher the genetic distance between parents, the better is the heterosis (Boeven et al. 2016). However, analysis of glasshouse data suggested higher values of MPH in hybrids were affected within the group as well. This may be due to best parental lines namely, Karim, Svevo and Miki within the best group. F_1 hybrids between parents representing Group1 (Italian) and Group2 (CIMMYT '80s) expressed the higher MPH (2.42g for grain yield). Recent genomic tools have revealed that diversity alone does not consistently lead to higher heterosis (van Ginkel & Ortiz, 2017).

Correlation of field and glasshouse results revealed that the two conditions were not comparable. Nevertheless, harvest index of field experiment had significant positive relationship with grain yield, harvest index and spike number of glasshouse. These results indicated that in both environments, hybrids were able to produce more than its parents but in different heterotic combinations.

Although heterosis has been reported in many crops under normal growing conditions, there is a need to study the behaviour of hybrids under water limiting conditions. It becomes more relevant in case of durum wheat which is generally grown in drought-prone environments. To assess the performance of hybrids under water limiting conditions, precision phenotyping of hybrids along with their parents was done on a high-throughput platform. Results of this experiment described that after stress imposition plants were acted differently for biomass and

chlorophyll content but not as much for dry index as plants were partially moisture. Combined analysis of variance over time showed significant differences for stress, genotypes and their interaction with time (Stress x Time, Geno x Time) but interaction was consistent between stress, genotypes and time for all the traits studied. Significant differences occurred after the day 53 to day 79 (after stress imposition for all the traits). Based on the Water Stress Tolerance Index (WSTI), three F_1 hybrids (Svevo x Miki, Iride x Miki, Valnova x Miki) were identified as tolerant for various traits measured in terms of chlorophyll content and biomass. In general, grain yield varied appreciably among crosses in each environment. These results suggest that hybrids in general do better under water stress environment but specific hybrid combinations need to be developed to realize the stably higher performance under drought-prone environments.

Conclusion

1. Phenotyping of durum core collection for DTH, CGDD and CDL under diverse environments offered the opportunity to identify the genetic factors underlying their variations. Expression of flowering genes by environmental conditions prevalent in diverse environments provided the possibility for each group of genes associated with photoperiodic, vernalization and early per se genes to express. This was particularly useful for the photoperiod or vernalization genes, which usually have larger effects and well characterized.

2. Phenotyping results demonstrated that environmental variables such as temperature and photoperiod had significant effect in controlling the flowering time in durum wheat in five diverse pheno-environments which justified by both PCA and variance analysis of GxE and GxPhEnv interaction. It also confirmed significant genotypic variation for flowering time in five pheno-environments.

3. GWAS results suggested that 47 most significant QTLs located on chromosomes 1B, 2A, 2B, 3A, 3B, 5A, 5B, 7A and 7B had an effect on regulation of flowering time across five pheno-environments. These QTL regions potentially co-located with major *PPD, VRN* and *EPS* genes as previously described. This analysis identified 20 highly significant QTLs in landraces and 27 in elite germplasm. Some landrace specific QTLs like QTL2 on Chr1B, QTL3 on Chr2A, QTL5 on Chr2B, QTL13 on 5A and QTL19 and QTL20 on Chr7A had strong association with previously described candidate genes in orthologous species. Similarly, elite specific QTLs like QTL2 and QTL4 on Chr2A, QTL5 on Chr2B, QTL15 and QTL16 on Chr5A, and QTL18 on Chr5B contained highly significant and stable candidate genes previously identified in orthologous.

4. The candidate gene search for DTH, CGDD and CDL in durum core collection indicated seven novel genes, namely *PRR7, GRF, SVP, RRP6L1, Hd6, TCP1,* and *COP1/RGA* in addition to a number of already known regulatory photoperiodic genes, *PPD-A* and *PPD-B* and vernalization genes *VRN1, VRN2 and VRN3* which have major impact in the genetic make-up of flowering time in landraces and elite germplasm.

5. Elite germplasm and landraces had different QTLs except two QTLs, indicating that past breeding efforts have led to changes in the genetic makeup of elite lines in terms of earliness, photoperiod insensitivity and vernalization requirement.

6. The distribution of QTLs in five pheno-environments has led to the conclusion that many more genetic loci are involved in controlling flowering time in durum wheat. We were able to demonstrate the significance of novel genes, *PRR7* (DTH) and *GRF5* (CDL) on Chr2A, *RRP6L1* (CGDD) on Chr5A, and *SVP* (DTH) on Chr5A and Chr7A in the genetic control of DTH, CGDD and CDL in durum wheat.

7. The function of the candidate genes revealed in different pheno-environments matches with the adaptation requirement imposed by the agro-climatic conditions prevalent in test locations. Therefore, classification of environments in diverse pheno-environments has helped to recover novel candidate genes related to specific climatic conditions like high temperature responsive genes (*SVP, RRP6L1*) in PhEnv3 (Fanaye) and PhEnv5 (Terbol off-season) in addition to *VRN1* and *VRN3* genes.

8. We have gathered large number of candidate genes for photoperiod and vernalization within the QTL confidence interval. The sequences of the significant markers with highest LOD have been provided to LGC genomics for *in silico* design of KASP markers. These can be expected to be converted into validated markers which can be used in genomics enabled improvement in durum wheat program.

9. Combining ability analysis suggest predominance of non-additive gene action as underlying principle in the expression of heterosis for grain yield and its components in durum wheat. Mean performance of F_1 hybrids was higher than that of parents for all the traits studied. The results showed 25.8%, 19.6% and 15% of mid parent heterosis (MPH) in field, glasshouse and net-house experiments, indicating the scope for exploitation of heterosis in durum wheat. Valnova x Miki was the best hybrid combination in all the three experiments followed by Karim x Valnova and Karim x Morocco in field while, Karim x Svevo and Iride x Miki in glasshouse and Valnova x Svevo in net-house for hybrid production. Based on the Water Stress Tolerance Index (WSTI), three F_1 hybrids (Valnova x Miki, Iride x Miki and Svevo x Miki)

were identified as drought tolerant based on chlorophyll content and biomass. Whereas root study revealed that Valnova x Svevo as root biomass and Svevo x Miki as deep roots (root level3) performed better in net-house conditions.

10. Hybrids in general do better under water stress environment. For better performance, specific hybrid combinations need to be developed to realize the stably higher performance under drought prone environments. Almost all hybrids of the parents Miki, Valnova, Svevo and Karim performed better under both well-watered and water stress conditions. These heterotic combinations and parental lines could be exploited for hybrid breeding program in durum wheat.
11. To exploit the heterosis in durum wheat, there is a need to develop a robust hybrid production system including a search for cytoplasmic genic male sterility system, and standardization of CHA (chemical hybridizing agent) system. Identification of a large number of candidate genes related to flowering time, photoperiodism and vernalization in the present study offers scope to explore environment sensitive genetic male sterility system for hybrid wheat development.

References

Adamczyk, B. J., Lehti-Shiu, M. D., & Fernandez, D. E. (2007). The MADS domain factors AGL15 and AGL18 act redundantly as repressors of the floral transition in Arabidopsis. *The Plant Journal*, *50*(6), 1007-1019.

Akhunov, E. D., Akhunova, A. R., Anderson, O. D., Anderson, J. A., Blake, N., Clegg, M. T., . . . Deal, K. R. (2010). Nucleotide diversity maps reveal variation in diversity among wheat genomes and chromosomes. Bmc Genomics, 11(1), 702.

Amaya, A. A., Busch, R., & Lebsock, K. (1972). Estimates of genetic effects of heading date, plant height, and grain yield in durum wheat. Crop science, 12(4), 478-481.

Andeden, E., Yediay, F., Baloch, F., Shaaf, S., Kilian, B., Nachit, M., & Özkan, H. (2011). Distribution of vernalization and photoperiod genes (Vrn-A1, Vrn-B1, Vrn-D1, Vrn-B3, Ppd-D1) in Turkish bread wheat cultivars and landraces. Cereal Research Communications, 39(3), 352-364.

Bandaranayake, C. K., Koumproglou, R., Wang, X. Y., Wilkes, T., & Kearsey, M. J. (2004). QTL analysis of morphological and developmental traits in the Ler× Cvi population of Arabidopsis thaliana. Euphytica, 137(3), 361-371.

Barbosa-Neto, J., Sorrells, M., & Cisar, G. (1996). Prediction of heterosis in wheat using coefficient of parentage and RFLP-based estimates of genetic relationship. Genome, 39(6), 1142-1149.

Beche, E., da Silva, C. L., Pagliosa, E. S., Capelin, M. A., Franke, J., Matei, G., & Benin, G. (2013). Hybrid performance and heterosis in early segregant populations of Brazilian spring wheat. Australian Journal of Crop Science, 7(1), 51.

Bhatt, G. (1971). Heterotic performance and combining ability in a diallel cross among spring wheats (Triticum aestivum L.). Australian Journal of Agricultural Research, 22(3), 359-368.

Bhatt, G. (1972). Inheritance of heading date, plant height, and kernel weight in two spring wheat crosses. Crop science, 12(1), 95-98.

Boeven, P. H., Longin, C. F. H., & Würschum, T. (2016). A unified framework for hybrid breeding and the establishment of heterotic groups in wheat. Theoretical and applied genetics, 129(6), 1231-1245.

Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., . . . Zhang, Y. (2002). Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science, 298(5595), 1039-1043.

Carretero-Paulet, L., Ahumada, I., Cunillera, N., Rodrıguez-Concepción, M., Ferrer, A., Boronat, A., & Campos, N. (2002). Expression and Molecular Analysis of the ArabidopsisDXR Gene Encoding 1-Deoxy-d-Xylulose 5-Phosphate Reductoisomerase, the First Committed Enzyme of the 2-C-Methyl-d-Erythritol 4-Phosphate Pathway. Plant physiology, 129(4), 1581-1591.

Chen, M., & Ni, M. (2006). RFI2, a RING-domain zinc finger protein, negatively regulates CONSTANS expression and photoperiodic flowering. The Plant Journal, 46(5), 823-833.

Choi, K., Kim, J., Hwang, H.-J., Kim, S., Park, C., Kim, S. Y., & Lee, I. (2011). The FRIGIDA complex activates transcription of FLC, a strong flowering repressor in Arabidopsis, by recruiting chromatin modification factors. The Plant Cell, 23(1), 289-303.

Choi, Sang Chul, et al. "Trithorax group protein Oryza sativa Trithorax1 controls flowering time in rice via interaction with early heading date3." *Plant physiology* 164.3 (2014): 1326-1337.

Clarke, J. H., Mithen, R., Brown, J. K., & Dean, C. (1995). QTL analysis of flowering time inArabidopsis thaliana. Molecular and General Genetics MGG, 248(3), 278-286.

Corbellini, M., Perenzin, M., Accerbi, M., Vaccino, P., & Borghi, B. (2002). Genetic diversity in bread wheat, as revealed by coefficient of parentage and molecular markers, and its relationship to hybrid performance. Euphytica, 123(2), 273-285.

De Vries, A. P. (1971). Flowering biology of wheat, particularly in view of hybrid seed production—a review. Euphytica, 20(2), 152-170.

De Vries, A. P. (1972). Some aspects of cross-pollination in wheat (Triticum aestivum L.) 1. Pollen concentration in the field as influenced by variety, diurnal pattern, weather conditions and level as compared to the height of the pollen donor. Euphytica, 21(2), 185-203.

De Vries, A. P. (1974). Some aspects of cross-pollination in wheat (Triticum aestivum L.). 3. Anther length and number of pollen grains per anther. Euphytica, 23(1), 11-19.

Devlin, P. F., Patel, S. R., & Whitelam, G. C. (1998). Phytochrome E influences internode elongation and flowering time in Arabidopsis. *The Plant Cell*, *10*(9), 1479-1487.

Dhokane, D., Karre, S., Kushalappa, A. C., & McCartney, C. (2016). Integrated metabolo-transcriptomics reveals Fusarium head blight candidate resistance genes in wheat QTL-Fhb2. PLoS One, 11(5), e0155851.

Díaz, A., Zikhali, M., Turner, A. S., Isaac, P., & Laurie, D. A. (2012). Copy number variation affecting the Photoperiod-B1 and Vernalization-A1 genes is associated with altered flowering time in wheat (Triticum aestivum). PLoS One, 7(3), e33234.

Distelfeld, A., Li, C., & Dubcovsky, J. (2009). Regulation of flowering in temperate cereals. Current opinion in plant biology, 12(2), 178-184.

Dong, C.-H., Agarwal, M., Zhang, Y., Xie, Q., & Zhu, J.-K. (2006). The negative regulator of plant cold responses, HOS1, is a RING E3 ligase that mediates the ubiquitination and degradation of ICE1. Proceedings of the National Academy of Sciences, 103(21), 8281-8286.

Duvick, D. N. (1999). Heterosis: feeding people and protecting natural resources. The genetics and exploitation of heterosis in crops, 19-29.

Duvick, D. N. (2001). Biotechnology in the 1930s: the development of hybrid maize. Nature Reviews Genetics, 2(1), 69-74.

Dvorak, J., Akhunov, E. D., Akhunov, A. R., Deal, K. R., & Luo, M.-C. (2006). Molecular characterization of a diagnostic DNA marker for domesticated tetraploid wheat provides evidence for gene flow from wild tetraploid wheat to hexaploid wheat. Molecular biology and evolution, 23(7), 1386-1396.

East, E. M. (1908). Inbreeding in corn. Rep. Conn. Agric. Exp. Stn, 1907, 419-428.

East, E. M. (1936). Heterosis. Genetics, 21(4), 375.

Edwards, L., Ketata, H., & Smith, E. (1976). Gene action of heading date, plant height, and other characters in two winter wheat crosses. Crop science, 16(2), 275-277.

Ehrenreich, I. M., Hanzawa, Y., Chou, L., Roe, J. L., Kover, P. X., & Purugganan, M. D. (2009). Candidate gene association mapping of Arabidopsis flowering time. Genetics, 183(1), 325-335.

El-Assal, S. E.-D., Alonso-Blanco, C., Peeters, A. J., Raz, V., & Koornneef, M. (2001). A QTL for flowering time in Arabidopsis reveals a novel allele of CRY2. Nature genetics, 29(4), 435-440.

El-Lithy, M. E., Clerkx, E. J., Ruys, G. J., Koornneef, M., & Vreugdenhil, D. (2004). Quantitative trait locus analysis of growth-related traits in a new Arabidopsis recombinant inbred population. Plant physiology, 135(1), 444-458.

Emborg, T. J., Walker, J. M., Noh, B., & Vierstra, R. D. (2006). Multiple heme oxygenase family members contribute to the biosynthesis of the phytochrome chromophore in Arabidopsis. *Plant Physiology*, *140*(3), 856-868

Falconer, D. S., Mackay, T. F., & Frankham, R. (1996). Introduction to quantitative genetics (4th edn). TRENDS in Genetics, 12(7), 280.

Feldman, M., & Levy, A. A. (2015). Origin and evolution of wheat and related Triticeae Species Alien Introgression in Wheat (pp. 21-76): Springer.

Fischer, S., Maurer, H., Würschum, T., Möhring, J., Piepho, H.-P., Schön, C., . . . Melchinger, A. (2010). Development of heterotic groups in triticale. Crop science, 50(2), 584-590.

Fischer, S., Möhring, J., Maurer, H., Piepho, H.-P., Thiemt, E.-M., Schön, C., . . . Reif, J. (2009). Impact of genetic divergence on the ratio of variance due to specific vs. general combining ability in winter triticale. Crop science, 49(6), 2119-2122.

Fischer, S., Möhring, J., Schön, C., Piepho, H. P., Klein, D., Schipprack, W., . . . Reif, J. (2008). Trends in genetic variance components during 30 years of hybrid maize breeding at the University of Hohenheim. Plant Breeding, 127(5), 446-451.

Fornara, F., de Montaigu, A., & Coupland, G. (2010). SnapShot: control of flowering in Arabidopsis. Cell, 141(3), 550-550. e552.

Freeman, G. F. (1919). The heredity of quantitative characters in wheat. Genetics, 4(1), 1.

Fu, D., Szűcs, P., Yan, L., Helguera, M., Skinner, J. S., Von Zitzewitz, J., . . . Dubcovsky, J. (2005). Large deletions within the first intron in VRN-1 are associated with spring growth habit in barley and wheat. Molecular Genetics and Genomics, 273(1), 54-65.

Geraldo, N., Bäurle, I., Kidou, S. I., Hu, X., & Dean, C. (2009). FRIGIDA delays flowering in Arabidopsis via a cotranscriptional mechanism involving direct interaction with the nuclear cap-binding complex. *Plant Physiology*, *150*(3), 1611-1618.

Goldringer, I., Brabant, P., & Gallais, A. (1997). Estimation of additive and epistatic genetic variances for agronomic traits in a population of doubled-haploid lines of wheat. Heredity, 79(1), 60-71.

Gomez, D., Vanzetti, L., Helguera, M., Lombardo, L., Fraschina, J., & Miralles, D. J. (2014). Effect of Vrn-1, Ppd-1 genes and earliness per se on heading time in Argentinean bread wheat cultivars. Field Crops Research, 158, 73-81.

Gowda, M., Kling, C., Würschum, T., Liu, W., Maurer, H., Hahn, V., & Reif, J. (2010). Hybrid breeding in durum wheat: heterosis and combining ability. Crop science, 50(6), 2224-2230.

Gowda, M., Zhao, Y., Maurer, H. P., Weissmann, E. A., Würschum, T., & Reif, J. C. (2013). Best linear unbiased prediction of triticale hybrid performance. Euphytica, 191(2), 223-230.

Guo, H., Yang, H., Mockler, T. C., & Lin, C. (1998). Regulation of flowering time by Arabidopsis photoreceptors. *Science*, *279*(5355), 1360-1363.

Han, P., García-Ponce, B., Fonseca-Salazar, G., Alvarez-Buylla, E. R., & Yu, H. (2008). AGAMOUS-LIKE 17, a novel flowering promoter, acts in a FT-independent photoperiod pathway. *The Plant Journal*, *55*(2), 253-265.

Han, Z., Zhang, B., Zhao, H., Ayaad, M., & Xing, Y. (2016). Genome-wide association studies reveal that diverse heading date genes respond to short and long day lengths between indica and japonica rice. Frontiers in plant science, 7.

He, Yuehui. "Chromatin regulation of flowering." *Trends in plant science* 17.9 (2012): 556-562.

Hedden, P. (2003). The genes of the Green Revolution. TRENDS in Genetics, 19(1), 5-9.

Hou, X., Zhou, J., Liu, C., Liu, L., Shen, L., & Yu, H. (2014). Nuclear factor Y-mediated H3K27me3 demethylation of the SOC1 locus orchestrates flowering responses of Arabidopsis. *Nature communications*, *5*, 4601.

Huang, X., Yang, S., Gong, J., Zhao, Y., Feng, Q., Gong, H., . . . Xia, J. (2015). Genomic analysis of hybrid rice varieties reveals numerous superior alleles that contribute to heterosis. Nature communications, 6, 6258.

Iqbal, M., Navabi, A., Salmon, D. F., Yang, R.-C., Murdoch, B. M., Moore, S. S., & Spaner, D. (2007). Genetic analysis of flowering and maturity time in high latitude spring wheat. Euphytica, 154(1-2), 207-218.

Jagadish, S. V., Bahuguna, R. N., Djanaguiraman, M., Gamuyao, R., Prasad, P. V., & Craufurd, P. Q. (2016). Implications of high temperature and elevated CO2 on flowering time in plants. *Frontiers in plant science*, *7*, 913.

Jang, S., Torti, S., & Coupland, G. (2009). Genetic and spatial interactions between FT, TSF and SVP during the early stages of floral induction in Arabidopsis. *The Plant Journal*, *60*(4), 614-625.

Jansen, R., Van Ooijen, J., Stam, P., Lister, C., & Dean, C. (1995). Genotype-by-environment interaction in genetic mapping of multiple quantitative trait loci. Theoretical and applied genetics, 91(1), 33-37.

Johansson, M., & Staiger, D. (2014). Time to flower: interplay between photoperiod and the circadian clock. Journal of experimental botany, 66(3), 719-730.

Juenger, T. E., Mckay, J. K., Hausmann, N., Keurentjes, J. J., Sen, S., Stowe, K. A. Richards, J. H. (2005). Identification and characterization of QTL underlying whole-plant physiology in Arabidopsis thaliana: δ13C, stomatal conductance and transpiration efficiency. Plant, Cell & Environment, 28(6), 697-708.

Jung, J. H., Ju, Y., Seo, P. J., Lee, J. H., & Park, C. M. (2012). The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in Arabidopsis. The Plant Journal, 69(4), 577-588.

Kabbaj, H., Sall, A. T., Al-Abdallat, A., Geleta, M., Amri, A., Filali-Maltouf, A. Bassi, F. M. (2017). Genetic diversity within a global panel of durum wheat (Triticum durum) landraces and modern germplasm reveals the history of alleles exchange. Frontiers in plant science, 8, 1277.

Kaeppler, S. (2012). Heterosis: many genes, many mechanisms—end the search for an undiscovered unifying theory. ISRN Botany, 2012.

Kane, N. A., Danyluk, J., Tardif, G., Ouellet, F., Laliberté, J.-F., Limin, A. E., Sarhan, F. (2005). TaVRT-2, a member of the StMADS-11 clade of flowering repressors, is regulated by vernalization and photoperiod in wheat. Plant physiology, 138(4), 2354-2363.

Kato, K., & Wada, T. (1999). Genetic analysis and selection experiment for narrow-sense earliness in wheat by using segregating hybrid progenies. Breeding science, 49(4), 233-238.

Kim, D.-H., & Sung, S. (2014). Genetic and epigenetic mechanisms underlying vernalization. The Arabidopsis Book, e0171.

Klaimi, Y., & Qualset, C. O. (1974). Genetics of heading time in wheat (Triticum aestivum L.). II. The inheritance of vernalization response. Genetics, 76(1), 119-133.

Klepper, B., Rickman, R., Zuzel, J., & Waldman, S. (1988). Use of growing degree days to project sample dates for cereal crops. Agronomy journal, 80(5), 850-852.

Koekemoer, F., Van Eeden, E., & Bonjean, A. (2011). An overview of hybrid wheat production in South Africa and review of current worldwide wheat hybrid developments. The world wheat book—a history of wheat breeding, 2, 907-950.

Kong, D., Li, M., Dong, Z., Ji, H., & Li, X. (2015). Identification of TaWD40D, a wheat WD40 repeat-containing protein that is associated with plant tolerance to abiotic stresses. Plant cell reports, 34(3), 395-410.

Kuittinen, H., Sillanpää, M., & Savolainen, O. (1997). Genetic basis of adaptation: flowering time in Arabidopsis thaliana. Theoretical and applied genetics, 95(4), 573-583.

Kumar, S. Vinod, et al. "Transcription factor PIF4 controls the thermosensory activation of flowering." *Nature* 484.7393 (2012): 242.

Langer, S. M., Longin, C. F. H., & Würschum, T. (2014). Phenotypic evaluation of floral and flowering traits with relevance for hybrid breeding in wheat (Triticum aestivum L.). Plant Breeding, 133(4), 433-441.

Laurie, D. A. (1997). Comparative genetics of flowering time. Plant molecular biology, 35(1), 167-177.

Lazaro, A., Valverde, F., Piñeiro, M., & Jarillo, J. A. (2012). The Arabidopsis E3 ubiquitin ligase HOS1 negatively regulates CONSTANS abundance in the photoperiodic control of flowering. The Plant Cell, 24(3), 982-999.

Lee, H., Yoo, S. J., Lee, J. H., Kim, W., Yoo, S. K., Fitzgerald, H., et al. (2010). Genetic framework for flowering-time regulation by ambient temperature-responsive miRNAs in *Arabidopsis*. *Nucleic Acids Res.* 38, 3081–3093. doi: 10.1093/nar/gkp1240

Lee, J. H., Cho, Y. S., Yoon, H. S., Suh, M. C., Moon, J., Lee, I., ... & Kim, J. K. (2005). Conservation and divergence of FCA function between Arabidopsis and rice. *Plant molecular biology*, *58*(6), 823-838.

Lee, J. H., Yoo, S. J., Park, S. H., Hwang, I., Lee, J. S., & Ahn, J. H. (2007). Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. *Genes & development*, *21*(4), 397-402.

Lelley, J. (1966). Observation on the biology of fertilization with regard to seed production in hybrid wheat. Die Zuchter, 36, 314-316.

Lempe, J., Balasubramanian, S., Sureshkumar, S., Singh, A., Schmid, M., & Weigel, D. (2005). Diversity of flowering responses in wild Arabidopsis thaliana strains. *PLoS Genetics*, *1*(1), e6.

Lewis, S., Faricelli, M. E., Appendino, M. L., Valárik, M., & Dubcovsky, J. (2008). The chromosome region including the earliness per se locus Eps-Am1 affects the duration of early developmental phases and spikelet number in diploid wheat. Journal of experimental botany, 59(13), 3595-3607.

Li, W., Wang, Z., Li, J., Yang, H., Cui, S., Wang, X., & Ma, L. (2011). Overexpression of AtBMI1C, a polycomb group protein gene, accelerates flowering in Arabidopsis. *PLoS One*, *6*(6), e21364.

Liu, K., Goodman, M., Muse, S., Smith, J. S., Buckler, E., & Doebley, J. (2003). Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. Genetics, 165(4), 2117-2128.

Longin, C. F. H., Gowda, M., Mühleisen, J., Ebmeyer, E., Kazman, E., Schachschneider, R., Reif, J. C. (2013). Hybrid wheat: quantitative genetic parameters and consequences for the design of breeding programs. Theoretical and applied genetics, 126(11), 2791-2801.

Longin, C. F. H., Mühleisen, J., Maurer, H. P., Zhang, H., Gowda, M., & Reif, J. C. (2012). Hybrid breeding in autogamous cereals. Theoretical and applied genetics, 125(6), 1087-1096.

Longin, C. F. H., Reif, J. C., & Würschum, T. (2014). Long-term perspective of hybrid versus line breeding in wheat based on quantitative genetic theory. Theoretical and applied genetics, 127(7), 1635-1641.

Maan, S. S. (1992). Genetic analyses of male fertility restoration in wheat: VI. A defective-seed gene. Crop science, 32(6), 1408-1413.

Maccaferri, M., Cane, M. A., Sanguineti, M. C., Salvi, S., Colalongo, M. C., Massi, A., . . . Clarke, J. M. (2014). A consensus framework map of durum wheat (Triticum durum Desf.) suitable for linkage disequilibrium analysis and genome-wide association mapping. Bmc Genomics, 15(1), 873.

Maccaferri, M., Sanguineti, M. C., Corneti, S., Ortega, J. L. A., Salem, M. B., Bort, J., . . . El-Ahmed, A. (2008). Quantitative trait loci for grain yield and adaptation of durum wheat (Triticum durum Desf.) across a wide range of water availability. Genetics, 178(1), 489-511.

Maccaferri, M., Sanguineti, M. C., Noli, E., & Tuberosa, R. (2005). Population structure and long-range linkage disequilibrium in a durum wheat elite collection. Molecular Breeding, 15(3), 271-290.

Maloof, J. N., Borevitz, J. O., Dabi, T., Lutes, J., Nehring, R. B., Redfern, J. LBerry, C. C. (2001). Natural variation in light sensitivity of Arabidopsis. Nature genetics, 29(4).

Mandel, M. A., Feldmann, K. A., Herrera-Estrella, L., Rocha-Sosa, M., & León, P. (1996). CLA1, a novel gene required for chloroplast development, is highly conserved in evolution. The Plant Journal, 9(5), 649-658.

Maurer, A., Draba, V., Jiang, Y., Schnaithmann, F., Sharma, R., Schumann, E.Pillen, K. (2015). Modelling the genetic architecture of flowering time control in barley through nested association mapping. Bmc Genomics, 16(1), 290.

McIntosh, R., Devos, K., Dubcovsky, J., Rogers, W., Morris, C., Appels, R., & Anderson, O. (2005). Catalogue of gene symbols for wheat: 2005 supplement. Ann. Wheat Newslett., 51, 272.

Melchinger, A. E., & Gumber, R. K. (1998). Overview of heterosis and heterotic groups in agronomic crops. Concepts and breeding of heterosis in crop plants(conceptsandbree), 29-44.

Melchinger, A., Utz, H., Piepho, H.-P., Zeng, Z.-B., & Schön, C. (2007). The role of epistasis in the manifestation of heterosis: a systems-oriented approach. Genetics, 177(3), 1815-1825.

Miao, L., Mao, X., Wang, J., Liu, Z., Zhang, B., Li, WJing, R. (2017). Elite Haplotypes of a Protein Kinase Gene TaSnRK2. 3 Associated with Important Agronomic Traits in Common Wheat. Frontiers in plant science, 8.

Moon, J., Parry, G., & Estelle, M. (2004). The ubiquitin-proteasome pathway and plant development. The Plant Cell, 16(12), 3181-3195.

Mugnozza, G. S. (2005). The contribution of Italian wheat geneticists: from Nazareno Strampelli to Francesco D'Amato. Proc. Int. Congr. In the wake of the double helix: from the green devolution to the gene revolution, Bologna, Italy, 52-75.

Mühleisen, J., Piepho, H.-P., Maurer, H. P., Longin, C. F. H., & Reif, J. C. (2014). Yield stability of hybrids versus lines in wheat, barley, and triticale. Theoretical and applied genetics, 127(2), 309-316.

Muqaddasi, Q. H., Lohwasser, U., Nagel, M., Börner, A., Pillen, K., & Röder, M. S. (2016). Genome-wide association mapping of anther extrusion in hexaploid spring wheat. PLoS One, 11(5), e0155494.

Murakami, M., Yamashino, T., & Mizuno, T. (2004). Characterization of circadian-associated APRR3 pseudo-response regulator belonging to the APRR1/TOC1 quintet in Arabidopsis thaliana. *Plant and cell physiology*, *45*(5), 645-650.

Murphy, R. L., Klein, R. R., Morishige, D. T., Brady, J. A., Rooney, W. L., Miller, F. R & Mullet, J. E. (2011). Coincident light and clock regulation of pseudoresponse regulator protein 37 (PRR37) controls photoperiodic flowering in sorghum. *Proceedings of the National Academy of Sciences*, *108*(39), 16469-16474.

Nakamichi, N., Kita, M., Niinuma, K., Ito, S., Yamashino, T., Mizoguchi, T., & Mizuno, T. (2007). Arabidopsis clock-associated pseudo-response regulators PRR9, PRR7 and PRR5 coordinately and positively regulate flowering time through the canonical CONSTANS-dependent photoperiodic pathway.

Nanda, G., Hazarika, G., & Gill, K. (1981). Inheritance of heading date, plant height, ear length and spikelets per spike in an intervarietal cross of wheat. TAG Theoretical and Applied Genetics, 60(3), 167-171.

Nduwumuremyi, A., Tongoona, P., & Habimana, S. (2013). Mating designs: helpful tool for quantitative plant breeding analysis. Journal of Plant Breeding and Genetics, 1(3), 117-129.

Nixdorf, M., & Hoecker, U. (2010). SPA1 and DET1 act together to control photomorphogenesis throughout plant development. *Planta*, *231*(4), 825-833.

Nuñez, F. D., & Yamada, T. (2017). Molecular regulation of flowering time in grasses. *Agronomy*, *7*(1), 17.

Oettler, G., Tams, S., Utz, H., Bauer, E., & Melchinger, A. (2005). Prospects for hybrid breeding in winter triticale. Crop science, 45(4), 1476-1482.

Oury, F.-X., Brabant, P., Berard, P., & Pluchard, P. (2000). Predicting hybrid value in bread wheat: biometric modelling based on a" top-cross" design. TAG Theoretical and Applied Genetics, 100(1), 96-104.

Pearce, S., Kippes, N., Chen, A., Debernardi, J. M., & Dubcovsky, J. (2016). RNA-seq studies using wheat PHYTOCHROME B and PHYTOCHROME C mutants reveal shared and specific functions in the regulation of flowering and shade-avoidance pathways. BMC plant biology, 16(1), 141.

Peng, F. Y., Hu, Z., & Yang, R.-C. (2015). Genome-wide comparative analysis of flowering-related genes in Arabidopsis, wheat, and barley. International journal of plant genomics, 2015.

Peng, J. H., Sun, D., & Nevo, E. (2011). Domestication evolution, genetics and genomics in wheat. Molecular Breeding, 28(3), 281.

Pepper, A. E., & Chory, J. (1997). Extragenic suppressors of the Arabidopsis det1 mutant identify elements of flowering-time and light-response regulatory pathways. *Genetics*, *145*(4), 1125-1137.

Pickett, A. A. (1993). Hybrid wheat-results and problems. Fortschritte der Pflanzenzuechtung (Germany).

Piepho, H.-P. (2009). Ridge regression and extensions for genomewide selection in maize. Crop Science, 49(4), 1165-1176.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics, 155(2), 945-959.

Pugsley, A. (1971). A genetic analysis of the spring-winter habit of growth in wheat. Australian Journal of Agricultural Research, 22(1), 21-31.

Pugsley, A. (1972). Additional genes inhibiting winter habit in wheat. Euphytica, 21(3), 547-552.

Purwestri, Y. A., Ogaki, Y., Tamaki, S., Tsuji, H., & Shimamoto, K. (2009). The 14-3-3 protein GF14c acts as a negative regulator of flowering in rice by interacting with the florigen Hd3a. *Plant and cell physiology*, *50*(3), 429-438.

Ramadan, A., Takahashi, H., Takeda, H., Shinozaki, K., Nemoto, K., Seki, M., & Sawasaki, T. (2015). Wheat germ-based protein libraries for the functional characterisation of the Arabidopsis E2 ubiquitin conjugating enzymes and the RING-type E3 ubiquitin ligase enzymes. BMC plant biology, 15(1), 275.

Ramage, R. (1983). Heterosis and hybrid seed production in barley Heterosis (pp. 71-93): Springer.

Reif, J., Warburton, M., Xia, X., Hoisington, D., Crossa, J., Taba, S.. Melchinger, A. (2006). Grouping of accessions of Mexican races of maize revisited with SSR markers. Theoretical and applied genetics, 113(2), 177-185.

Rexroad, C. E., & Vallejo, R. L. (2009). Estimates of linkage disequilibrium and effective population size in rainbow trout. BMC genetics, 10(1), 83.

Sameena, S., Iqbal, S., & Jaivir, S. (2000). Inheritance of some quantitative traits in bread wheat (Triticum aestivum L. em. Thell). Annals of Agricultural Research, 21(1), 51-54.

Sarkar, P., & Stebbins, G. (1956). Morphological evidence concerning the origin of the B genome in wheat. American Journal of Botany, 297-304.

Sayar, R., Khemira, H., & Kharrat, M. (2007). Inheritance of deeper root length and grain yield in half-diallel durum wheat (Triticum durum) crosses. Annals of Applied Biology, 151(2), 213-220.

Scarth, R., & Law, C. (1984). The control of the day-length response in wheat by the group 2 chromosomes. Zeitschrift für Pflanzenzüchtung, 92(2), 140-150.

Schrag, T. A., Möhring, J., Maurer, H. P., Dhillon, B. S., Melchinger, A. E., Piepho, H.-P., . . . Frisch, M. (2009). Molecular marker-based prediction of hybrid performance in maize using unbalanced data from multiple experiments with factorial crosses. Theoretical and applied genetics, 118(4), 741-751.

Seberg, O. (1999). Slageren, MW van 1994. Wild Wheats: a monograph of Aegilops L. and Amblyopyrum (Jaub. & Spach) Eig (Poaceae). Botanical Journal of the Linnean Society(129), 185-186.

Sedbrook, J. C., Carroll, K. L., Hung, K. F., Masson, P. H., & Somerville, C. R. (2002). The Arabidopsis SKU5 gene encodes an extracellular glycosyl phosphatidylinositol–anchored glycoprotein involved in directional root growth. The Plant Cell, 14(7), 1635-1648.

Sharma, R. (2013). Does low yield heterosis limit commercial hybrids in wheat?

Sharma, R. K., & Tandon, J. (1995). Effect of heat stress on variation in parental lines and their F1 hybrids in wheat: a comparison. The Indian Journal of Genetics and Plant Breeding, 44(4), 455-456.

Shcherban, A. B., & Salina, E. A. (2017). Evolution of VRN-1 homoeologous loci in allopolyploids of Triticum and their diploid precursors. BMC plant biology, 17(1), 188.

Shiferaw, B., Smale, M., Braun, H.-J., Duveiller, E., Reynolds, M., & Muricho, G. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security, 5(3), 291-317.

Shpak, E. D., Berthiaume, C. T., Hill, E. J., & Torii, K. U. (2004). Synergistic interaction of three ERECTA-family receptor-like kinases controls Arabidopsis organ growth and flower development by promoting cell proliferation. Development, 131(7), 1491-1501.

Sicard, H., Faubladier, M., Noaillac-Depeyre, J., Léger-Silvestre, I., Gas, N., & Caizergues-Ferrer, M. (1998). The role of the Schizosaccharomyces pombe gar2 protein in nucleolar structure and function depends on the concerted action of its highly charged N terminus and its RNA-binding domains. Molecular biology of the cell, 9(8), 2011-2023.

Simons, K. J., Gehlhar, S. B., Maan, S. S., & Kianian, S. F. (2003). Detailed mapping of the species cytoplasm-specific (scs) gene in durum wheat. Genetics, 165(4), 2129-2136.

Singh, H., Sharma, S., Sain, R., & Singhania, D. (2003). The inheritance of production traits in bread wheat by diallel analysis. SABRAO Journal of Breeding and Genetics, 35, 1-10.

Singh, S., Chatrath, R., & Mishra, B. (2010). Perspective of hybrid wheat research: a review. Indian J Agric Sci, 80, 1013-1027.

Sinha, S. K., & Khanna, R. (1975). Physiological, biochemical, and genetic basis of heterosis. Advances in Agronomy, 27, 123-174.

Solomon, K., Labuschagne, M., & Viljoen, C. (2007). Estimates of heterosis and association of genetic distance with heterosis in durum wheat under different moisture regimes. The Journal of Agricultural Science, 145(3), 239-248.

Song, X., Ni, Z., Yao, Y., Zhang, Y., & Sun, Q. (2009). Identification of differentially expressed proteins between hybrid and parents in wheat (Triticum aestivum L.) seedling leaves. Theoretical and applied genetics, 118(2), 213.

Srivastava, H. (1981). Intergenomic interaction, heterosis, and improvement of crop yield. Advances in Agronomy, 34, 117-195.

Stich, B., Melchinger, A. E., Frisch, M., Maurer, H. P., Heckenberger, M., & Reif, J. C. (2005). Linkage disequilibrium in European elite maize germplasm investigated with SSRs. Theoretical and applied genetics, 111(4), 723-730.

Stratton, D. A. (1998). Reaction norm functions and QTL–environment interactions for flowering time in Arabidopsis thaliana. Heredity, 81(2), 144-155.

Streb, S., & Zeeman, S. C. (2012). Starch metabolism in Arabidopsis. *The Arabidopsis Book*, e0160.

Streitner, C., Danisman, S., Wehrle, F., Schöning, J. C., Alfano, J. R., & Staiger, D. (2008). The small glycine-rich RNA binding protein AtGRP7 promotes floral transition in Arabidopsis thaliana. *The Plant Journal*, *56*(2), 239-250.

Sukumaran, S., Lopes, M. S., Dreisigacker, S., Dixon, L. E., Zikhali, M., Griffiths, S., . . . Reynolds, M. P. (2016). Identification of earliness per se flowering time locus in spring wheat through a genome-wide association study. Crop Science, 56(6), 2962-2672.

Takahashi, Y., Shomura, A., Sasaki, T., & Yano, M. (2001). Hd6, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the α subunit of protein kinase CK2. Proceedings of the National Academy of Sciences, 98(14), 7922-7927.

Talbert, L., Blake, N., Storlie, E., & Lavin, M. (1995). Variability in wheat based on low-copy DNA sequence comparisons. Genome, 38(5), 951-957.

Tang, D., Wang, G., & Zhou, J.-M. (2017). Receptor kinases in plant-pathogen interactions: more than pattern recognition. The Plant Cell, 29(4), 618-637.

Turner, A., Beales, J., Faure, S., Dunford, R. P., & Laurie, D. A. (2005). The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. Science, 310(5750), 1031-1034.

Uga, Y. (2012). Quantitative measurement of root growth angle by using the basket method. *Methodologies for root drought studies in rice. The Philippines: International Rice Research Institute*, 22-26.

Ungerer, M. C., Halldorsdottir, S. S., Modliszewski, J. L., Mackay, T. F., & Purugganan, M. D. (2002). Quantitative trait loci for inflorescence development in Arabidopsis thaliana. Genetics, 160(3), 1133-1151.

van Ginkel, M., & Ortiz, R. (2017). Cross the Best with the Best, and Select the Best: HELP in Breeding Selfing Crops. Crop science.

Wang, Y., Liu, K., Liao, H., Zhuang, C., Ma, H., & Yan, X. (2008). The plant WNK gene family and regulation of flowering time in Arabidopsis. Plant Biology, 10(5), 548-562.

Waugh, R., Jannink, J.-L., Muehlbauer, G. J., & Ramsay, L. (2009). The emergence of whole genome association scans in barley. Current opinion in plant biology, 12(2), 218-222.

Weinig, C., Dorn, L. A., Kane, N. C., German, Z. M., Halldorsdottir, S. S., Ungerer, M. C., . . . Schmitt, J. (2003). Heterogeneous selection at specific loci in natural environments in Arabidopsis thaliana. Genetics, 165(1), 321-329.

Weinig, C., Ungerer, M. C., Dorn, L. A., Kane, N. C., Toyonaga, Y., Halldorsdottir, S. S., . . . Schmitt, J. (2002). Novel loci control variation in reproductive timing in Arabidopsis thaliana in natural environments. Genetics, 162(4), 1875-1884.

Werner, J. D., Borevitz, J. O., Warthmann, N., Trainer, G. T., Ecker, J. R., Chory, J., & Weigel, D. (2005). Quantitative trait locus mapping and DNA array hybridization identify an FLM deletion as a cause for natural flowering-time variation. Proceedings of the National Academy of Sciences of the United States of America, 102(7), 2460-2465.

Whitford, R., Fleury, D., Reif, J. C., Garcia, M., Okada, T., Korzun, V., & Langridge, P. (2013). Hybrid breeding in wheat: technologies to improve hybrid wheat seed production. Journal of experimental botany, 64(18), 5411-5428.

Wickham, H. (2016). ggplot2: elegant graphics for data analysis: Springer.

Widner, J., & Lebsock, K. (1973). Combining ability in durum wheat: I. Agronomic characteristics. Crop science, 13(2), 164-167.

Wilczek, A., Burghardt, L., Cobb, A., Cooper, M., Welch, S., & Schmitt, J. (2010). Genetic and physiological bases for phenological responses to current and predicted climates. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 365(1555), 3129-3147.

Wilhelm, E. P., Turner, A. S., & Laurie, D. A. (2009). Photoperiod insensitive Ppd-A1a mutations in tetraploid wheat (Triticum durum Desf.). Theoretical and applied genetics, 118(2), 285-294.

Willige, B. C., Ghosh, S., Nill, C., Zourelidou, M., Dohmann, E. M., Maier, A., & Schwechheimer, C. (2007). The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of Arabidopsis. *The Plant Cell*, *19*(4), 1209-1220.

Worland, T. (2001). Genetic basis of worldwide wheat varietal improvement. The world wheat book: A history of wheat breeding, 59-100.

Yan, L., Helguera, M., Kato, K., Fukuyama, S., Sherman, J., & Dubcovsky, J. (2004). Allelic variation at the VRN-1 promoter region in polyploid wheat. Theoretical and applied genetics, 109(8), 1677-1686.

Yan, L., Loukoianov, A., Tranquilli, G., Helguera, M., Fahima, T., & Dubcovsky, J. (2003). Positional cloning of the wheat vernalization gene VRN1. Proceedings of the National Academy of Sciences, 100(10), 6263-6268.

Yan, Y., Shen, L., Chen, Y., Bao, S., Thong, Z., & Yu, H. (2014). A MYB-domain protein EFM mediates flowering responses to environmental cues in Arabidopsis. *Developmental cell*, *30*(4), 437-448.

Zanke, C., Ling, J., Plieske, J., Kollers, S., Ebmeyer, E., Korzun, V. Beier, S. (2014). Genetic architecture of main effect QTL for heading date in European winter wheat. Frontiers in plant science, 5.

Zhang, S., Yang, C., Peng, J., Sun, S., & Wang, X. (2009). GASA5, a regulator of flowering time and stem growth in Arabidopsis thaliana. *Plant molecular biology*, *69*(6), 745-759.

Zhao, Y., Zeng, J., Fernando, R., & Reif, J. C. (2013). Genomic prediction of hybrid wheat performance. Crop science, 53(3), 802-810.

Zhou, S., Chen, Q., Sun, Y., & Li, Y. (2017). Histone H2B monoubiquitination regulates salt stress-induced microtubule depolymerization in Arabidopsis. Plant, Cell & Environment.

Zhou, S.-M., Kong, X.-Z., Kang, H.-H., Sun, X.-D., & Wang, W. (2015). The involvement of wheat F-box protein gene TaFBA1 in the oxidative stress tolerance of plants. PLoS One, 10(4), e0122117.

Zikhali, M., Wingen, L. U., & Griffiths, S. (2015). Delimitation of the Earliness per se D1 (Eps-D1) flowering gene to a subtelomeric chromosomal deletion in bread wheat (Triticum aestivum). *Journal of experimental botany*, *67*(1), 287-299.

Acknowledgment

Time marches on, the season changes and it is the time to relish what has been accomplished. This work has the patronage, encouragement, sustained interest, and help of many. This is my profound duty to begin with a '**Thank You**' note that can show at least in part many special thoughts which I keep within my heart.

With great pleasure, I express my deep sense of gratitude and devotion to Prof Roberto Tuberosa, Dipartimento di Scienze Agrarie (DiPSA), University of Bologna, and esteemed guide for his constant encouragement, valuable feedback and necessary facilities made available to me during the course of investigation.

I take this opportunity to express my deep sense of gratitude and indebtedness to Dr. Filippo M Bassi, Durum wheat breeder, ICARDA, Rabat, Morocco for his persuasion, expert guidance, constant inspiration, constructive suggestions and infinite patience that made me to accomplish the present investigation and inculcated the spirit of research. I am grateful to him forever.

I am highly grateful to Drs Marco Maccaferri and Elisabetta Frascaroli, DiPSA, University of Bologna, for their day to day guidance, valuable suggestions, statistical analysis and criticism in preparation of my thesis. I am equally grateful to Drs Murari Singh, Miguel S Garcia and Kehel Zakaria from ICARDA, for their kind support for having fruitful discussions and technical advice on statistical approaches during the course of investigation.

I have profound gratitude to Prof Ed Runge, TAMU, USA and Dr Michael Baum, Director of BIGM program of ICARDA for his patronage and encouragement during my research and to Tero International for imparting leadership skills. I extend my thanks to Dr Silvio Salvi, DiPSA, UniBo for his encouragement and support during research.

A very special thanks to Dr Angelo Petrozza and Mr Stephan Summerer from ALSIA Centro Ricerche Metapontum Agrobios for their technical and moral support during my research work at Metaponto. My sincere thanks to Mrs Sandra Stefanelli and Simona Corneti and other staff members of the DiPSA, University of Bologna, Italy and ICARDA, Rabat Morocco for their cooperation.

I avail this opportunity to thanks to all my colleagues especially Giuseppe Sciara and Meryam Zaim who helped me a lot in many ways. I also thanks to Donatella, Danara, Hafssa, Martina, Giuseppe Conderelli, Eder, Riccardo, Linda, Silvia, Francesco, Khaoula and Jitendra who were a constant source of encouragement throughout the period of investigation.

I feel the inadequacy of diction in expressing my heartfelt and affectionate regards to my family members, especially to my papa, Shiv Agrawal, my brother, Neeraj Gupta, and my Bua, Meera Seth whose unfailing love and affection have always been a source of inspiration to me.

Finally, I would like to thank Monsanto Beachell Borlaug International scholarship program for funding this research. At the last but not least, I would like to extend my thanks to all those who helped me directly and indirectly during the course of this study.

Supplementary tables

Suppl.Table1. Illustrating the mean, GCA/SCA and mid parent heterosis (MPH%) for five traits in 28 F1 and eight parents of durum wheat during a field trial in 2016.

HybN	P1	P2	**TBW**	GCA/SCATBW	MPH%TBW	**SBW**	GCA/SCASBW	MPH%SBW	**SPKN**	GCA / SCASPKN	MPH%mean	**1KW**	GCA/ SCA1KW	MPH%1KW	**PH**	GCA/SCAPH	MPH%_PH
1	Gl	Gl	100.11	4.26		46.24	0.03		11.44	0.45		48.53	-4.80		81.76	0.48	
2	Ir	Ir	108.55	2.35		50.41	0.55		11.77	0.45		53.32	-0.62		81.00	-1.21	
3	Kr	Kr	101.46	2.97		46.22	-2.12		10.73	-0.07		60.64	2.28		81.07	-1.38	
4	Mk	Mk	92.53	-4.77		47.60	0.63		9.87	-0.93		58.12	0.84		84.10	0.51	
5	Mr	Mr	94.89	-6.22		43.27	-4.79		10.62	-0.23		50.94	-0.61		78.05	-1.66	
6	Ms	Ms	97.62	-5.21		47.47	1.88		10.89	0.10		49.75	-4.08		84.14	1.95	
7	Sv	Sv	104.90	-0.32		47.38	-0.54		11.25	0.09		56.77	2.51		83.29	0.77	
8	Vl	Vl	103.83	6.95		54.72	4.36		9.79	0.16		64.36	4.48		82.34	0.53	
9	Gl	Ir	128.29	3.86	25.68	55.77	-0.19	18.61	14.11	0.08	23.90	52.39	-1.18	2.82	86.50	1.75	5.12
10	**Gl**	**Kr**	133.69	8.64	33.63	55.67	2.38	20.18	13.78	0.26	28.20	57.06	0.59	4.63	83.50	-1.08	2.08
11	**Gl**	**Mk**	133.16	15.85	37.99	57.59	1.56	22.49	15.22	2.57	42.62	54.92	-0.11	3.66	89.17	2.70	6.24
12	Gl	Mr	90.80	-25.06	-7.92	38.61	-12.01	-14.37	11.11	-2.25	-1.17	51.58	-1.99	4.50	82.33	-1.96	2.43
13	Gl	Ms	135.51	18.63	38.67	66.94	9.65	44.50	14.44	0.75	30.57	51.35	1.24	12.09	88.50	0.59	5.55
14	Gl	Sv	149.32	27.55	45.40	75.14	20.27	60.02	16.33	2.66	42.36	59.04	2.35	5.27	88.50	1.78	5.98
15	Gl	Vl	132.04	3.01	32.68	56.52	-3.25	13.98	14.89	1.14	45.20	59.48	0.82	3.19	92.05	5.56	9.98
16	Ir	Kr	137.08	13.94	30.17	63.52	9.70	31.15	14.44	0.93	28.87	63.30	2.66	11.23	83.50	0.61	2.46
17	Ir	Mk	119.22	3.81	17.73	53.96	-2.60	8.93	14.45	1.79	34.29	60.77	1.56	23.00	83.67	-1.11	1.12
18	Ir	Mr	117.79	3.84	16.58	63.37	12.22	39.65	11.33	-2.03	1.82	64.14	6.39	2.00	89.00	6.40	9.48
19	Ir	Ms	133.79	18.82	31.96	67.54	9.73	41.50	18.67	4.97	65.88	52.54	-1.75	16.45	83.99	-2.22	1.36
20	Ir	Sv	107.04	-12.82	-0.33	41.83	-13.56	-15.31	13.22	-0.46	15.89	64.09	3.21	3.35	86.67	1.64	4.52
21	Ir	Vl	123.62	-3.50	17.81	57.14	-3.15	10.02	13.00	-0.75	24.49	60.82	-2.02	9.15	81.83	-2.96	0.17
22	Kr	Mk	95.72	-20.30	-0.54	49.27	-4.62	7.17	9.22	-2.92	-10.50	63.31	1.21	13.58	83.67	-0.94	1.08
23	**Kr**	**Mr**	153.89	39.32	56.45	54.53	6.05	18.74	16.11	3.27	50.83	63.36	2.72	5.32	84.17	1.74	4.61
24	Kr	Ms	95.12	-20.47	-4.14	41.07	-14.08	-11.55	11.89	-1.28	9.20	58.09	0.91	4.20	86.17	0.12	3.56
25	Kr	Sv	114.62	-5.86	11.21	51.41	-1.32	11.22	13.44	0.29	23.16	61.16	-2.61	5.52	86.17	1.30	3.99
26	Kr	Vl	157.08	29.34	53.38	69.38	11.76	37.81	17.22	3.99	70.86	66.05	0.31	6.62	86.17	1.54	4.46
27	Mk	Mr	121.87	15.04	29.71	55.88	4.66	22.48	13.78	1.80	35.12	63.34	4.13	6.52	84.33	0.01	3.26
28	Mk	Ms	76.58	-31.27	-18.34	55.90	-1.99	12.51	10.67	-1.65	4.15	56.78	1.03	17.63	88.50	0.56	4.38
29	Mk	Sv	116.63	3.90	17.56	57.29	1.82	19.43	10.89	-1.41	3.76	61.07	-1.27	10.07	88.83	2.08	5.14
30	**Mk**	**Vl**	164.48	44.48	67.13	79.62	19.25	54.60	15.00	2.63	51.26	62.86	-1.45	16.09	88.00	1.49	4.79
31	Mr	Ms	98.33	-8.07	3.34	45.22	-7.26	0.88	13.00	-0.02	25.54	53.56	-0.73	10.59	88.33	2.57	7.24
32	Mr	Sv	126.64	15.35	27.09	56.03	5.97	23.22	15.56	2.55	43.71	63.35	2.47	4.15	84.33	-0.24	3.67
33	Mr	Vl	99.11	-19.44	-0.05	50.41	-4.54	3.73	13.89	0.81	35.16	63.50	0.65	5.39	84.00	-0.34	3.81
34	**Ms**	**Sv**	130.56	18.26	29.88	71.38	14.66	47.24	14.89	1.55	37.74	58.89	1.48	24.18	91.50	3.31	7.79
35	Ms	Vl	143.27	23.70	43.75	74.28	12.66	47.88	14.00	0.59	37.31	59.36	-0.02	6.30	93.50	5.54	10.26
36	Sv	Vl	102.66	-21.79	-2.31	45.23	-13.97	-12.70	12.33	-1.06	18.06	74.81	8.84	2.63	84.33	-2.44	1.52

**TBW: Total biomass weight, SBW: Straw weight, SPKN: Spike number, 1KW: Thousand kernel weight, PH: Plant height.

Supple Table2. Explaining the classification of best heterosis partner based on MPH, SCA, GCA (P1 and P2) and BPH in field experiment of University of Bologna.

SN	P1	P2	GY(g/3P)	MPH	SCA	GCA_P1	GCA_P2	BPH
(i) High yield performance + high value of mid and better parent heterosis + high SCA								
1	Kr	Mr	268.08	107.1	75.22	9.46	-7.03	102.35
2	Kr	Vl	263.1	106.58	61.79	9.46	1.41	97.37
3	Mk	Vl	254.59	113.54	65.95	-3.21	1.41	107.26
4	Ir	Ms	235.1	62.45	46.02	0.44	-1.8	48.36
5	Gl	Mk	226.69	74.31	31.43	8.04	-3.21	56.7
6	Ms	Sv	229.31	69.75	47.99	-1.8	-7.31	76.39
(ii) High yield performance + average value of mid and better parent heterosis + low parental GCA								
7	Gl	Vl	226.57	67.91	26.68	8.04	1.41	56.57
8	Gl	Kr	234.05	66.19	26.12	8.04	9.46	64.06
9	Gl	Sv	222.53	60.08	31.37	8.04	-7.31	52.54
10	Mk	Sv	207.01	53.28	27.09	-3.21	-7.31	56.46
11	Ms	Vl	206.99	55.72	16.94	-1.8	1.41	51.78
12	Mk	Mr	197.99	52.48	17.8	-3.21	-7.03	41.75
(iii) Average yield performance + average value of MPH and BPH + low SCA but either one or both parents have good GCA								
13	Ir	Kr	220.69	45.97	20.35	0.44	9.46	36.98
14	Gl	Ms	205.71	43.10	9.03	8.03	-1.79	35.71
15	Ir	Mk	195.78	36.54	8.11	0.44	-3.20	12.08