Alma Mater Studiorum - Università di Bologna

DOTTORATO DI RICERCA IN

SCIENZE E TECNOLOGIE AGRARIE, AMBIENTALI E ALIMENTARI

Ciclo 35

Settore Concorsuale: 07/E1 - CHIMICA AGRARIA, GENETICA AGRARIA E PEDOLOGIA

Settore Scientifico Disciplinare: AGR/07 - GENETICA AGRARIA

EXPLORING MEDITERRANEAN DURUM WHEAT WILD RELATIVES AND ELITE DIVERSITY PANEL FOR FUTURE BREEDING

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Esame finale anno 2023

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Acknowledgments

First and foremost, I would like to express my gratitude to my esteemed supervisors Prof. Elisabetta Frascaroli and Prof. Marco Maccaferri for their invaluable advice, continuous support, and patience during my PhD degree. My gratitude extends to Prof. Roberto Tuberosa for giving me this huge opportunity to work in his lab. I am also thankful to the coordinator of this Ph.D. research program Professor Massimiliano Petracci for his direction during all the research process and evaluation courses.

Thanks go also to all members of the Agricultural genetics group of DISTAL, at the University of Bologna. I would like to offer my special thanks to my lab colleagues Giuseppe Sciara, Matteo Bozzoli, Martina Bruschi and Jad Novi for their significant contributions in my PhD thesis. I would also like to thank Sandra Stefanelli, Michele Prestera and Luca Bolognini for their technical contribution.

In addition, I would like to mention Dr. Amor Yahyaoui, Dr. Susanne Dreisigacker, Sarrah ben M'barek for the opportunity to be a part of this project and for hosting me in the CIMMYT - Septoria Precision Phenotyping Platform based in Tunisia. I also thank Dalma Castillo, Fernanda Maria Gamba Fuica, Hajer, Marwa Laribi and Chayma Araar for their valuable contribution and support.

I am also thankful to Brande Wulff and associates from the JIC (John Innes Center) and NIAB (National Institute of Agricultural Botany) for their collaboration in the *Aegilops tauschii* project.

And my biggest thanks to my family and partner for all the mental support you have shown me through this PhD journey and during the most challenging moments faced throughout the pandemic.

General abstract

Durum wheat (*Triticum durum*) is an important crop that has been used for millennia for human consumption, and modern breeding can take advantage of the wide variability useful for the adaptation to new challenges. Novel beneficial alleles can be found in wild relatives and landraces thus enhancing crop adaptation to many biotic and abiotic stresses. This dissertation considers the source of variability from both before and after wheat domestication, that caused a loss of potentially useful alleles. In particular, a panel of accessions of the progenitor specie *Aegilops tauschii* was investigated for the identification of favorable alleles for improving key agronomic and root traits, while a panel of landraces was investigated for tolerance to the septoria tritici blotch (STB) disease, determined by *Zymoseptoria tritici*.

Chapter 1. is the thesis introduction, which outlines the importance of wheat in the world, providing an historical overview of the domestication, the evolution mechanisms that led to the current forms of durum wheat and the use of wild relatives as a source of germplasm for future breeding programs is crucial. Moreover, the emergence of *Z. tritici* has been considered as the main pathogen of wheat since it contains extremely high levels of genetic variability and is thus difficult to control.

Chapter 2. Considers the contribution of the phenotypic diversity of 242 accessions of *Aegilops tauschii* from the Open Wild Wheat Consortium, involved in wheat domestication, provided with whole-genome resequencing. The accessions were phenotyped both in the field and in controlled conditions and A k-mer-based GWAS was performed to identify genomic regions involved in useful traits.

Chapter 3. Describes the genetic basis of resistance to *Z. tritici* in a durum wheat elite diversity panel representative of the germplasm bred in Mediterranean countries (Italy, Morocco, Spain, Syria, and Tunisia), Southwestern USA and Mexico. Quantitative trait loci (QTL) analysis results revealed several loci involved in the STB response that were found in several chromosome regions with a high infection rate. The genomic regions associated with STB resistance identified in this study could be of interest for marker assisted selection (MAS) in durum wheat breeding programs.

Chapter 1. General introduction

1.1. Taxonomy, etymology, and evolution of wheat

Wheat (*Triticum L.*) is an annual plant that belongs to the grassfamily *Poaceae*, tribe *Triticeae* and subtribe *Triticineae*. Sakamura (1918), Sax & Sax (1924) and Kihara (1924) used cytogenetic methods and recognized that wheat species fall into three groups based upon their ploidy level:
(i) diploids 2n = 14 = einkorn wheat (*Triticum monococcum*, genomes A^mA^m and *T. urartu*, genomes AA); (ii) tetraploids 4n = 28 = emmer wheats (*T. turgidum*, genomes BBAA and *T. timopheevii*, genomes GGAA); (iii) hexaploids 6n = 42 = bread wheats (*T. aestivum*, genomes BBAADD and *T. zhukovskyi*, $GGAAA^mA^m$).

Genome relationships show that *T. monococcum*, *T. timopheevii*, and *T. zhukovskyi* form a separate lineage irrelevant to the evolution of the principal wheat lineage, which is formed by *T. urartu*, *T. turgidum*, and *T. aestivum*. *Triticum turgidum* evolved by hybridization of *T. urartu* with a close relative of *Aegilops speltoides* (genomes SS) (Sarkar & Stebbins, 1956; Nishikawa, 1983; Dvorák & Zhang, 1990; Dvořák et al., 1993). This species was then domesticated and evolved as *T. turgidum* ssp *dicoccum* Schübl. (or *T. dicoccum*), which is the progenitor of durum wheat. The domesticated tetraploid *T. dicoccum* and the wild diploid *T. tauschii* underwent a second polyploidization event approximately 10,000 BP, resulting in the hexaploid species *Triticum aestivum* L., or bread wheat, with 21 chromosome pairs (Charmet, 2011). **Figure 1** shows the origin of cultivated bread wheat. It has long been known that *Aegilops* L. and *Triticum* are closely related, but they have usually been treated as separate genera since Linnaeus time. (Linné et al., 1753 cited by Goncharov, 2011)

According to Goncharov (2011), the morphological differences were the foundation for the earliest classification of *Triticum*. The first classification of wheat was that of Linnaeus that was based on phenology and glume morphology. Later, Dumortier (1823) added another important trait to the classification of wheat that is related to spike fragility. Since the 1950s, opposition to the morphological classifications has been based on cytogenetic (Bowden, 1959; Morris & Sears, 1967) and genetic (MacKey, 1966, 1968, 1975, 1989, 2005) principles and, more recently, on data derived from molecular-genetic investigations (Goncharov, 2002); Goncharov et al., 2009). Consequently, the classification of wheat species underwent constant revisions. Dorofeev and his collaborators

published one of the earliest nomenclature classifications of *Triticum* (Dorofeev et al., 1979). A new classification system was later introduced by MacKey (1966, 1988) and (Van Slageren, 1994), with minor modifications.

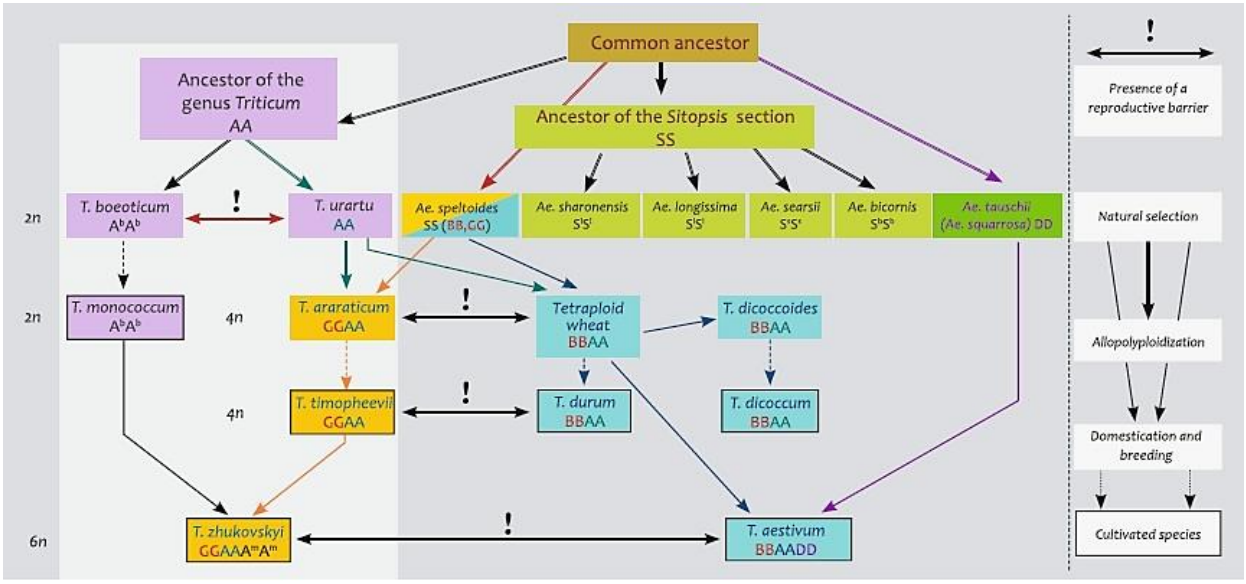


Figure 1. Diagram showing the phylogeny and origin of the genus *triticum* (Source: Goncharov et al., 2008 cited by Goncharov, 2011)

1.2. Wheat domestication

Domestication is the process of genetic selection that turns wild forms into domesticated varieties of crops by changing important features. (Salamini et al., 2002). A significant turning point in the development and evolution of human civilization was the domestication of wheat, which took place around 10,000 years ago. It enabled people to transition from a hunter-gatherer and nomadic pastoral lifestyle to a more sedentary agrarian one (Eckardt, 2010). According to Tadesse et al. (2016), tetraploid wheat (*Triticum turgidum* ssp.) was certainly one of the first cereal grains that were domesticated during the Neolithic period. Moreover, archaeological studies showed that tetraploid emmer (*T. turgidum* ssp. *dicoccum*) was one of the first wheat varieties domesticated from wild emmer, *T. dicoccoides* (2n = 2x = 28, AuAuBB). (Heun et al., 1997; Newton et al., 2010).

The transition from wild to domesticated forms of crop mostly involved changes to three principal morphological features that make the crop easier to harvest including an increased seed size, ear rachis stiffness and the ease with which the seed is released from the glumes. These modifications can be morphological, physiological, and genetic which can be termed as “domestication syndrome.” In cultivated fields, grain size has been linked to successful germination, whereas a non-shattering

seed, a hallmark of domestication, prevents natural seed dispersal and enables humans to harvest and collect the seed at the right time. (Reviewed in Fuller et al., 2007; Purugganan & Fuller, 2009 cited by (Eckardt, 2010). Wild emmer wheat underwent the first and most fundamental morphological divergence; the non-brittle rachis prevented the natural seed scattering mechanisms, thus allowing the harvest of entire heads, which must have contributed to higher grain yields (Faris et al., 2014; Peleg et al., 2011). Many studies confirmed that these alterations are primarily caused by mutations in the key genes that have marked wheat evolution. These three major genes are represented as Br, Tg and Q loci conferring brittle rachis, tenacious glumes, and a hulled seed respectively (Avni et al., 2017; Dubcovsky & Dvorak, 2007; Faris et al., 2014; Peng et al., 2011). **Figure 2** shows the spike evolution from brittle rachis to non-brittle rachis, from hulled to non-shattering kernel ("free threshing") during the shift from wild to domesticated wheat.

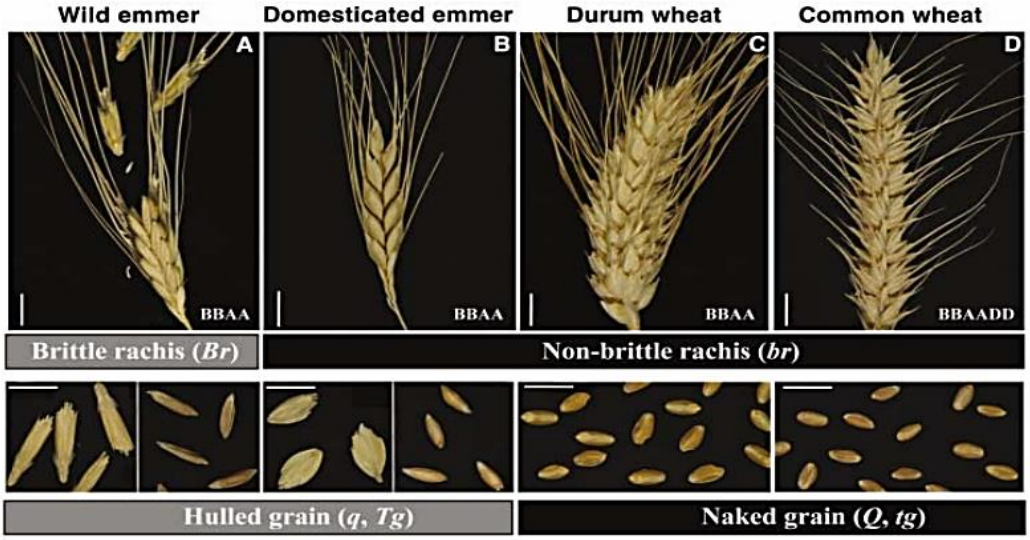


Figure 2. Wheat spikes showing a brittle rachis, (b to d) non-brittle rachis, (a and b) hulled grain, and (c and d) naked grain. (A) Wild emmer wheat (*T. dicoccoides*), (B) domesticated emmer (*T. dicoccum*), (C) durum wheat (*T. durum*), and (D) common wheat (*T. aestivum*). White scale bars represent 1 cm. The genome formula of each type of wheat is indicated at the lower right corner. (Adopted from (Dubcovsky & Dvorak, 2007)

The distribution of wild forms occurred in a ''core area'' in Southeast Turkey where the Fertile Crescent agriculture originated (Lev-Yadun et al., 2000; Abbo et al., 2006). From that point, cultivation spread throughout Europe, Asia, and Africa (Ammerman & Cavalli-Sforza, 1984; Nesbitt,

2002) making this process lasting up to one millennium in the region (Tanno & Willcox, 2006). **Figure 3** shows the origin and dispersal pathways of domesticated emmer and durum wheat.

Triticum dicoccoides was among the first cereals domesticated in the Fertile Crescent; its domesticated form is known as *T. dicoccon* (2n = 4x = 28, BBAA) (Sharma et al., 2021). This domestication step was the key to the subsequent evolution of durum and bread wheat a *turgidum* ssp. *durum*) appeared in the Fertile Crescent because of further selection and domestication of naked emmer (Zohary et al., 2012).

Kilian et al. (2007) revealed in one of their studies that einkorn underwent no reduction of diversity during domestication which raises the question of whether the earlier inference of "domestication bottlenecks" in other cereals may actually be breeding bottlenecks. Kilian et al. (2009) stated that the keys for a deep understanding of plant domestication are:

- a comprehensive germplasm collection covering the whole distribution area for each species
- the examination of numerous wild and domesticated accessions for every species
- the identification of the wild progenitor's gene pool and comparison with domesticate descendants
- the application of novel methods as molecular fingerprinting at numerous loci and the availability of novel high throughput sequencing technologies (Goldberg et al., 2006; Wicker et al., 2006).
- improvement of analytical methods addressing domestication issues based on mathematical and statistical models (Haudry et al., 2007; Pluzhnikov & Donnelly, 1996; Thuillet et al., 2005).

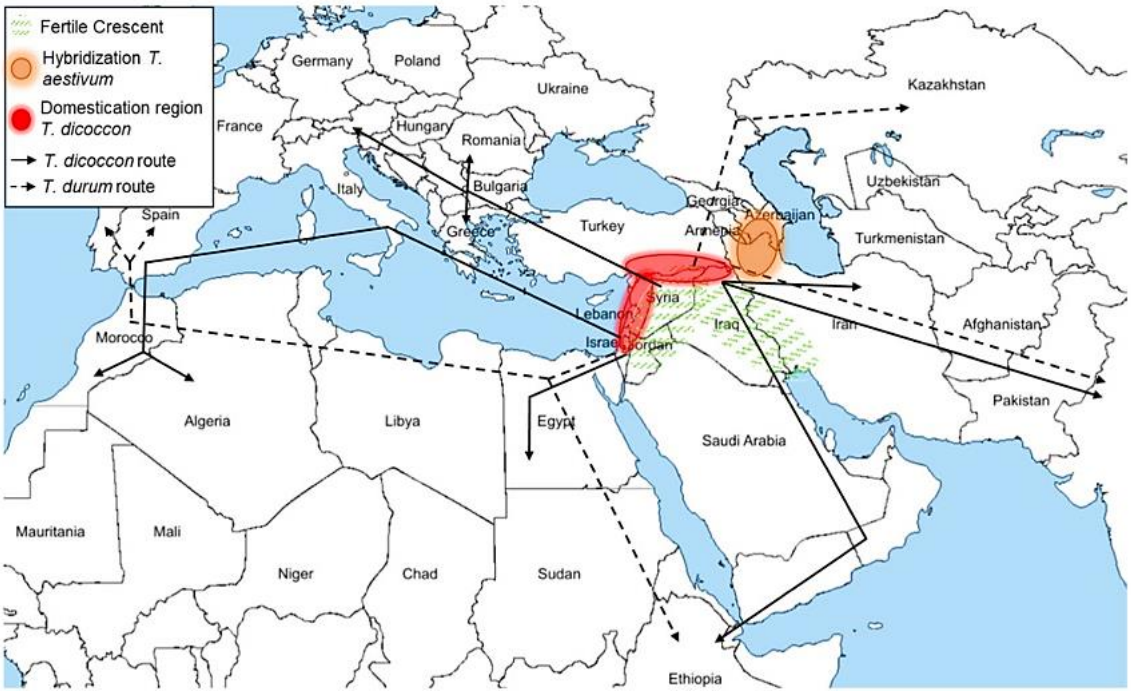


Figure 3. Center of origin of wheat and dispersion of domesticated emmer and durum wheat. Green lines show the Fertile Crescent; red circles are the domestication regions of *T. dicoccon* (Oliveira et al., 2020); solid arrows indicate dispersal routes of *T. dicoccon* (Martínez-Moreno et al., 2020; Badaeva et al., 2015); dashed arrows indicate dispersal route of *T. durum* (Moragues et al., 2007; Kabbaj et al., 2017; Martínez-Moreno et al., 2020); orange circle is the potential hybridization site of *T. aestivum* (Petersen et al., 2006; Salamini et al., 2002; Huang et al., 2002; Pont et al., 2019) cited by (Zeibig et al., 2022).

1.3. Global importance of durum wheat

Durum wheat is one of the most essential cereals that are cultivated worldwide on almost 17 million hectares (ha), with a global production of 38.1 million tonnes in 2019. The largest producer is the European Union, with 9 million tonnes in 2018, followed by Canada, Turkey, United States, Algeria, Mexico, Kazakhstan, Syria, and India (Eurostat, 2019; Tedone et al., 2018; Xynias et al., 2020). Italy is regarded as the leading producer of durum wheat in the European Union (EU), producing an average of 4.26 million tonnes per decade (on 1.28 million ha of growing area), followed by France (with 1.89 million tonnes per decade), Greece (with 1.07 million tonnes per decade), and Spain (with 0.98 million tonnes per decade) (Eurostat, 2019 cited by Xynias et al., 2020). However,

durum wheat is still grown in the Mediterranean basin and North African countries such as Algeria, Morocco, Tunisia, and Libya mainly for its culinary final products, such as semolina, pasta, couscous, frike, and bourghul. North Africa produces 18.7 million tons (MT) of durum wheat, of which 1.5 MT is in Tunisia (USDA, 2019).

Figure 4 shows the durum wheat historical percentage for the leading DW-producing countries.

According to (Moreno et al., 2022), almost all countries saw a slight decrease in the DW percentage to total wheat, except for Italy. Russia had about 20% of DW acreage to all wheat until the 1940s, when it steadily declined to 3% in 1970, a percentage that has remained constant to this day. Between 1950 and 1970, the percentage in Turkey fell from 60% to 15%. It then settled at around 15%. On the other hand, Italy is the only country where the DW percentage has increased over the study period, from 20% in the nineteenth century to 70% today. Additionally, Algeria had a high DW percentage of nearly 95% until the 1960s, when it fell to 67%. The percentage then increased again to 90%. DW was 15% of all wheat in India until 1940, when it dropped to 3% in 1950. In Morocco a rate of 70% was maintained until 1980, when it began to fall to 37% in 2000 and is currently at 30%. The United States of America had a rate of 4-5% in most of the studied period, with the exception of the peak periods of the 1920s, 1930s, and 1980s, when it reached 7%. With the exception of the years 2005 to 2015, Tunisia maintained a high DW percentage between 80% and 90% while Syria maintained a percentage between 70% and 80%.

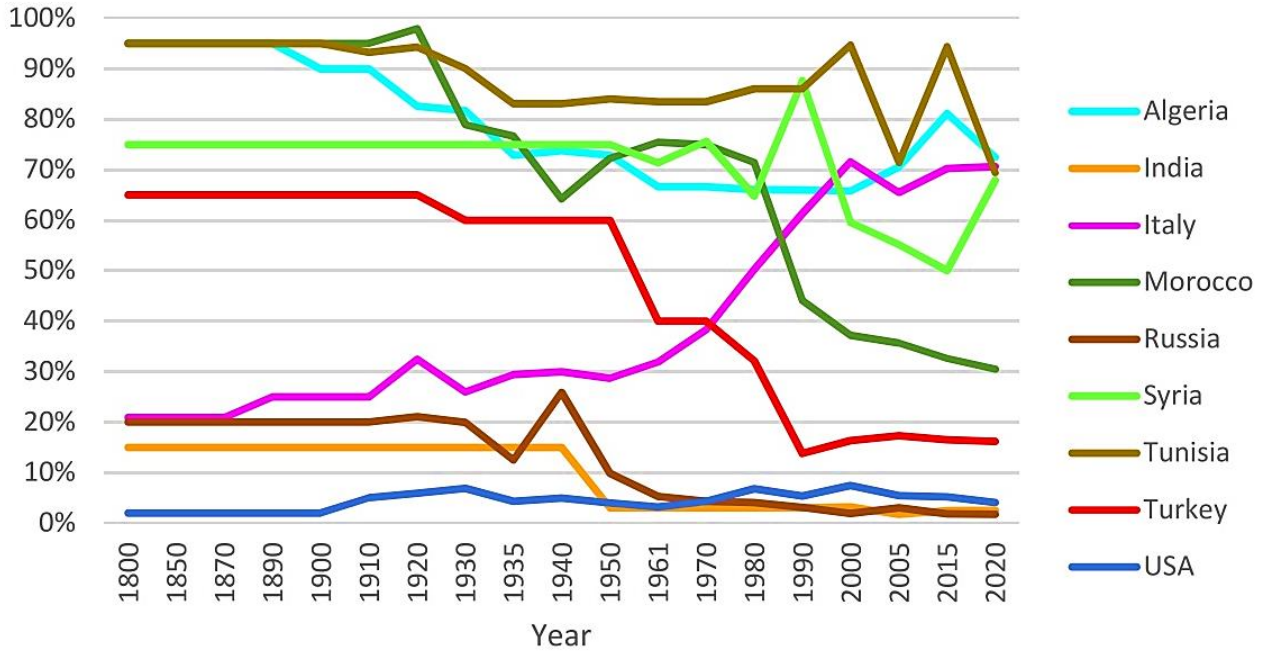


Figure 4. Percentage of the durum wheat area in the nine main durum wheat-growing countries in 1800-2020. (Source: Moreno et al., 2022)

1.4. Wild relatives and landraces: a potential source of favorable alleles

Crop wild relatives can contribute as genetic material useful for plant breeding initiatives for increased disease resistance, fertility, crop yield, and other desirable traits critical to meet the challenge of global food security through enhanced agricultural production (Maxted et al., 2012; Dempewolf et al., 2017; Warschefsky et al., 2014). *Aegilops tauschii* Coss. is the donor of the D subgenome of hexaploid bread thus a valuable genetic reservoir for wheat breeding (Kihara, 1944). This was a pioneering work that aimed to utilize wild species genome through chromosome manipulation thus helping in the improvement of various important crops. Thereafter, much focus has been given to intergeneric and interspecific hybridization to develop cultivars with increased pest tolerance, yields, and various other agronomic traits (Qandeel-e-Arsh et al., 2021).

The genetic evolution of durum wheat in the Mediterranean region, as well as natural and human selection, led to the establishment of landraces, with key quality traits including agronomic, quality characteristics, and adaptation to the region's contrasting environment (Lopes et al., 2015;

Nazco et al., 2012). Villa et al. (2005) defined a landrace as being a dynamic population of a cultivated plant that has historical origin, distinct identity, and lacks formal crop improvement, as well as often being genetically diverse, locally adapted, and associated with traditional farming systems. This shift to modern production methods encourages some varieties to be grown at the expense of more traditional and local crops (De Luca et al., 2018) and consequently leads to a significant loss of intraspecific genetic diversity (Perrings, 2018; Wallace et al., 2019). In addition, this gradual decline of genetic diversity contributes to the evolution of pests and forces us to adopt management practices such as pesticide use that harms the agroecosystem (Conversa et al., 2020).

One of the most threatened components of agricultural plant genetic resources are traditional varieties commonly referred to as landraces, which constitute the bulk of genetic diversity in domesticated species (Conversa et al., 2020; Poudel & Johnsen, 2009; Villa et al., 2007).

Mediterranean durum wheat landraces represent a particularly important group of genetic resources because of their extensive genetic variability and their documented tolerance to drought (Kyzeridis et al., 1995), resilience to pests, resistance to diseases (Talas et al., 2011), and adaptability to low-input farming systems (Srivastava & Damania, 1989). Therefore, wheat landraces are valuable sources for broadening the genetic base of cultivated wheat. Several studies using morphological, physiological, and agronomic traits have shown that genetically diverse local germplasm well adapted to a wide range of environmental conditions can be considered an important reservoir of useful genes for use in wheat breeding programs. (Lopes et al., 2015; Mangini et al., 2017; Pignone et al., 2015).

1.5. Germplasm collections

The adoption of modern, genetically uniform varieties has resulted in a reduction of performance stability under unfavorable conditions (Porceddu et al., 1988) and introduced a greater risk of vulnerability to pests and diseases in more favorable environments (Simmonds, 1979). Therefore, landraces and old cultivars have been extensively collected during the last decades to preserve these genetic resources from extinction and utilize them as a source of variation for desirable traits.

The genetic potential of the germplasm presently available to breeders can be fully exploited only if systematic evaluation for various important traits is undertaken. As accessions in gene banks are classified in function of their country of origin, the information concerning collection and evaluation data has usually been provided in terms of country gene pools (Macher et al., 2019).

The International Center for Agricultural Research in the Dry Areas (ICARDA) has a joint mandate with the Center Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) for wheat improvement in the West Asia and North Africa (WANA) region. The utilization of genetic resources such as landraces and wild progenitors of cultivated wheat to improve and stabilize crop production in the face of the biotic and abiotic stresses of the region is a key component in the development of adapted germplasm. Cavanagh et al. (2013) highlighted the considerable germplasm exchange that has occurred within the wheat-breeding community. The increased spectrum of international agricultural research from the Consultative Group on International Agricultural Research (CGIAR) centers, particularly by CIMMYT, has enhanced the flow of germplasm worldwide, which in turn has favored the use of beneficial alleles across environments.

1.6. The wheat pathogen *Zymoseptoria tritici* - *Mycosphaerella graminicola*

1.6.1. Impact on the world

Septoria tritici blotch (STB), caused by the foliar fungal pathogen *Zymoseptoria tritici* (formerly *Mycosphaerella graminicola*), is no exception and the same case scenario has been noticed and reported for the durum wheat *Z. tritici* interaction (Brown et al., 2015). Septoria tritici blotch (STB) caused by the ascomycete *Mycosphaerella graminicola*, is one of the most devastating foliar diseases of wheat. The pathogen has a bipolar, heterothallic mating system consisting of an asexual stage (anamorph) designated for Septoria tritici and a sexual stage (teleomorph) for *Mycosphaerella graminicola*. This disease impacts wheat production in Europe, the Mediterranean area, Africa, the Americas, and Australia (Dean et al., 2012; Kosina et al., 2007; Fones & Gurr, 2015) where, under favorable environmental conditions, can cause relevant yield losses (Duveiller et al., 2007; Eyal, 1999) cited by (Kidane et al., 2017). Susceptibility to *Zymoseptoria tritici*, the causal agent of Septoria tritici blotch disease, has been well documented (Chedli et al., 2018; Yahyaoui et al., 2000) as one of the most important diseases in the Mediterranean basin and it has become a serious inherent problem to Tunisian durum wheat production where the currently cultivated high yielding commercial varieties have become very susceptible to Septoria. In addition to other factors such as high seeding rate, early sowing, excessive use of fertilizers, and limited control of fungicide applications. The estimated grain yield losses in durum wheat caused by STB disease exceed 40% under conducive conditions (Berraies et al., 2014). In the United States, *Z. tritici* is among the top three pathogens causing the greatest economic damage to wheat (Ponomarenko et al., 2011) while in Australia production losses can reach 30% (Bhathal et al., 2003).

1.6.2. Epidemiology and disease cycle

The disease cycle is initiated by wind-dispersed ascospores from neighboring infected wheat debris (**Figure 5**). The ascospores are typically spread in autumn and are the primary inoculum for infection (Shaw & royle, 1989; Suffert & Sache, 2011). The biotrophic growth stage starts shortly after the emergence of seedlings in the fall when the fungi encounter the leaf. Following 24-48 hours of development on the leaf surface, the long filamentous structures of the fungus, hyphae, will enter the leaf through the stomata. The fungus obtains nutrients from the plant and hyphae will extend within the mesophyll tissue. Approximately 12-14 days after initial contact, the fungus switches from the biotrophic stage to the necrotrophic stage. In the latter, symptoms appear as necrotic lesions (**Figure 6**) on the leaves and black fruiting bodies begin to develop inside the lesions. Sexual ascospores and asexual pycnidiospores are produced inside the sexual fruiting bodies, pseudothecia, and the asexual fruiting bodies, pycnidia, respectively (Cunfer & Ueng, 1999; Sanderson, 1972). The pycnidiospores and ascospores are released after a period of high moisture in a so-called cirrhi. High levels of humidity are therefore important in order to obtain successful infection (Ponomarenko et al. 2011; Raman & Milgate, 2012). Pycnidiospores will spread to other plants as a result of physical contact and rain splash and subsequently new lesions will arise. Many cycles of asexual reproduction typically take place during the growing season but only a few cycles of sexual recombination occur. Eriksen and Munk (2003) outlined that pycnidia contain significantly higher amounts of spores compared to pseudothecia. Therefore, pycnidiospores are responsible for most of the infection in the growing season.

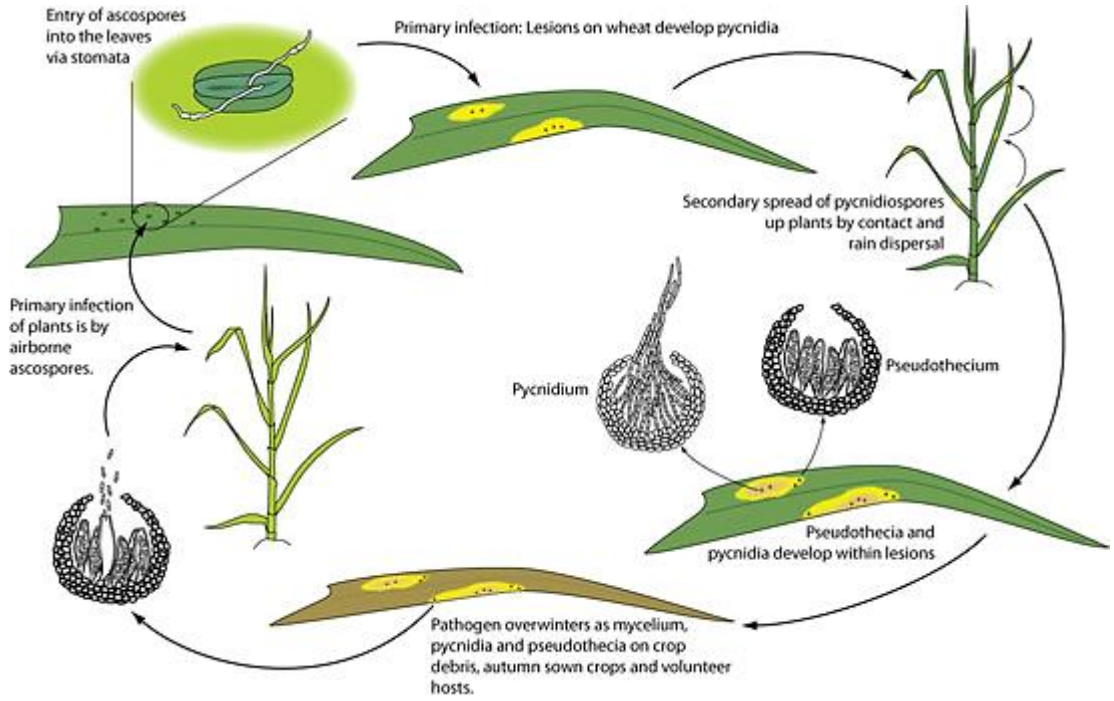


Figure 5. Disease cycle of Septoria tritici blotch. (Ponomarenko et al. 2011)

Figure 6. Necrotic lesions on a leaf showing the dark fruiting bodies (Photo provided by Meriam Nefzaoui

1.6.3. Genetics of *Zymoseptoria tritici*

The genome sequencing of *Z. tritici* was published in 2011 by (Goodwin et al., 2011). When studying host-pathogen interactions, it is critical to understand the pathogen's genetic structure. The latter can provide insight into the evolution of virulence and fungicide resistance (Linde et al., 2002).

Several studies revealed that sexual recombination occurs on a regular basis and is the cause of the fungus's genetic structure as well as high levels of genetic diversity (Chen & McDonald, 1996; Eriksen & Munk, 2003). Furthermore, it was found that the Middle East is a hotspot of *Z. tritici* diversity (Zhan et al., 2003). (McDonald & Mundt, 2016) reported that wheat germplasm sampled from the Fertile Crescent including the Middle East was rich in diversity for STB resistance due to the long co-evolutionary history between *Z. tritici* and wheat. Moreover, it has been discovered that all natural-infected fields have low clonality and a high degree of genotypic diversity (Linde et al., 2002; Zhan et al., 2003). Studies have also found that several *Z. tritici* strains are present within a single lesion on the leaf adding to the high diversity (Linde et al., 2002).

Due to air dispersal, ascospores that are produced sexually can travel a great distance (Boeger et al., 1993). As a result, new strains that have adapted locally to particular conditions can quickly and easily spread to neighboring fields. STB is challenging to control due to the high level of dispersion because fungicides and host resistance are quickly overcome (McDonald & Mundt, 2016).

Resistance to Septoria tritici blotch may be qualitative, isolate-specific which depends on major genes or quantitative, isolate-nonspecific with polygenic inheritance (Goodwin, 2012). As cited by Dreisigacker et al. (2015), 18 major resistance loci from Stb1 to Stb18 (Chartrain et al., 2009; Goodwin, 2012; Ghaffary et al., 2011; Ghaffary et al., 2012) along with many quantitative genes with minor effects have been identified (Kelm et al., 2012; Kosellek et al., 2013; Risser et al., 2011; Simón et al., 2012). In wheat breeding, partial or quantitative resistance (QR) is more significant.

It is incomplete but generally effective against all genotypes of a pathogen, and is usually durable (Brown et al., 2015; Niks et al., 2015). A major gene, Stb6 on chromosome 3A (Brading et al., 2002), has been associated with QR to Septoria in field conditions (Arraiano et al., 2009), and QTLs for Septoria resistance have been detected near Stb6 in several crosses (Brown et al., 2015) cited by Arraiano & Brown (2017). Chartrain et al. (2004) used a double haploid population produced from a cross between the resistant cvs. Arina and the susceptible Riband, to identify quantitative trait loci (QTL) and determine the genetics of this partial resistance. Results showed that no QTLs could be found in Arina, suggesting that this type of resistance is probably controlled by several dispersed genes.

General objective

The main objective of this thesis is to tackle the shortage of genetic variability, impairing crop adaptations to new global agro-environmental conditions, challenging food security for the next generations. In particular, the thesis studies the variability from both before and after wheat domestication, responsible for a loss of potentially useful alleles. The molecular tools for mining of useful alleles are in both cases molecular markers already available to search genomic regions involved in the expression of the interesting traits.

The specific object of the first study was to investigate a panel of accessions of the progenitor specie *Aegilops tauschii*, providing the genetic diversity present in the wheat D-genome, to identify favorable alleles for key agronomic traits evaluated in the field and root traits investigated under controlled conditions.

The second study had the objective of analyzing a panel of widely diverse cultivars and advanced lines for tolerance to the septoria tritici blotch (STB) disease, determined by *Zymoseptoria tritici*. This study takes advantage of three years of field evaluation in a Tunisian location particularly suitable for discriminating genotypes tolerant to STB.

CHAPTER 2. Exploring trait variation in a sequenced *Aegilops tauschii* diversity panel for fast-tracking wheat breeding

2.1. Abstract

Aegilops tauschii is the diploid progenitor of the D subgenome of hexaploid wheat (*Triticum aestivum* L.) and is an important source of genetic variation for wheat breeding. As a consequence of a strong domestication bottleneck, *T. aestivum* has been strongly depleted in polymorphisms, particularly in the D subgenome. Crop wild relatives offer a wide range of novel alleles due to the broad genetic variability still present in natural populations. Our objective was to assess the phenotypic diversity of 242 accessions from the Open Wild Wheat Consortium (OWWC; http://www.openwildwheat.org/), all provided with whole-genome resequencing. Traits of agronomic interest were considered, including phenology, growth habit, tillering, spike traits, anthocyanin accumulation, shoot and root biomass as well as root system architecture (RSA). The collection includes Lineage 1 from Turkey through Afghanistan to Central Asia and Lineage 2, primarily from the southwestern coastal area of the Caspian Sea, with the latter being involved in wheat domestication. Agronomic data were gathered from two field experiments conducted in Cadriano, Italy, in 2019/2020 and 2020/2021. RSA data were obtained from growing the accessions in a growth chamber on filter paper sheets on polycarbonate plates soaked in a modified Hoagland solution. Root growth angle, root length, root diameter, root network area, lateral root density, and length were assessed on ten-day-old roots.

Field data were analyzed and corrected for outliers and spatial effects while RSA growth chamber data were analyzed including replicates and blocks. Subsequently, a *k-mer-based* GWAS was achieved with the aim of identifying novel QTLs governing the examined traits (Gaurav et al. 2021). Overall, Manhattan plots obtained from the GWAS uncovered twenty-nine peaks for all traits. Significant peaks were detected for anthocyanin presence on chromosome 1D, 5D and 7D whereas two peaks were identified on chromosome 1D and 7D for heading date. Other clear and obvious positive associations can be seen for senescence and flag leaf length on chromosomes 3D, 5D and 7D respectively. For RSA analysis, 18 most significant peaks were detected for 10 of the analyzed traits.

This study revealed the presence of high variability in the L2 collection for all the field and Root System Architecture traits, confirming that *Aegilops tauschii* possesses a large repertoire of genetic diversity. Eventually, novel beneficial RSA alleles could be introgressed into bread and durum wheat through the direct crossing and synthetic hybridization. This research is conducted under the framework of "*Rooty: A root ideotype toolbox to support improved wheat yields*" (funded through the International Wheat Yield Partnership) and "*CerealMed, Enhancing diversity in Mediterranean cereal farming systems",* a PRIMA Farming System section II 2019 project.

2.2. Introduction

The world population is projected to reach 9.7 billion by 2050, increasing pressure on the food system and challenging food security (FAO, 2014). With the combined challenge of population expansion and hotter, less favorable climates, wheat yields must be sustainably increased to ensure global food security. In fact, a 70% increase in overall food production would be necessary to feed such a growing population. The wild relatives of wheat have a rich genetic diversity reservoir that can be used to boost productivity (Dubcovsky & Dvorak, 2007; Pont et al., 2019). Indeed, breeders have been encouraged to recruit diversity from *Ae. tauschii* for a long time due to the low genetic diversity of the bread wheat D-subgenome. *Aegilops tauschii* (2n = 2x = 14, genomes DD) is a wild, self-pollinating species of goatgrass with a wide natural range that extends from China and West Pakistan to East Turkey (Van Slageren, 1994). It originated from the hybridization between diploid A and B genome progenitors (Marcussen et al., 2014), and became the diploid D-genome donor of bread wheat (*Triticum aestivum* L.). Hexaploid wheat was created through the natural hybridization of tetraploid wheat and *Ae. tauschii* between 8,000 and 10,000 years ago (Bell, 1987; Renfrew, 1973; Singh et al., 2019), with *Ae. tauschii* providing several genes that increased environmental adaptability and enhanced bread quality (Kihara, 1944; Mcfadden & Sears, 1946; Kerber & Tipples, 1969; Lagudah et al., 1991).

Gaurav et al. (2021) showed that only 25% of the genetic diversity present in the *Aegilops tauschii* species contributed to the initial geneflow into hexaploid wheat. As a matter of fact, only a small subpopulation of *Ae. tauschii* was involved in the few hybridization events that did occur between *T. turgidum* and this species. Based on population structure analysis, Ae. *tauschii* is divided into two subspecies known as *Ae. tauschii* ssp. *tauschii* (Lineage 1; L1) and ssp. *strangulata* (Lineage 2; L2). The latter is considered the major contributor to the wheat D subgenome. As cited by (Singh et al., 2019), *Ae. tauschii* genetic diversity has been exploited through synthetic hybridization of tetraploid wheat and wild Ae. tauschii (Mcfadden & Sears, 1946); Kihara & Lilienfeld, 1949), and introgressed to bread wheat via direct crossing (Gill & Raupp, 1987). Despite these results, significant amounts of untapped genetic diversity remain present in this species.

2.3. Objectives

The objectives of this study were: i) to assess the phenotypic diversity of 242 accessions from the Open Wild Wheat Consortium (OWWC; http://www.openwildwheat.org/), (*lineage 1 and 2*) all provided with whole-genome resequencing, ii) to explore the phenotypic variability of Root System Architecture (RSA) traits present within a collection of 143 accessions of *Aegilops tauschii* ssp. *Strangulata* and iii) to perform GWAS with the aim to identify loci underlying the genetic variation for RSA and harness the untapped genetic diversity present in the wheat D-genome donor *Aegilops tauschii.*

2.4. Materials and Methods

2.4.1. Plant material

Phenotypic diversity. In this study, a diverse panel of 242 *Aegilops tauschii* accessions was investigated. This collection was provided by the John Innes Centre, Norwich (UK), and displays extensive variation in flowering time, micronutrient content, and disease resistance. The panel includes Lineage 1 (L1) from Turkey through Afghanistan to Central Asia and Lineage 2 (L2), primarily from the southwestern coastal area of the Caspian Sea, with the latter being involved in wheat domestication. (**Figure 7**). The putative Lineage 3 (L3) which is considered an admixture of L1 and L2 was not included in this study.

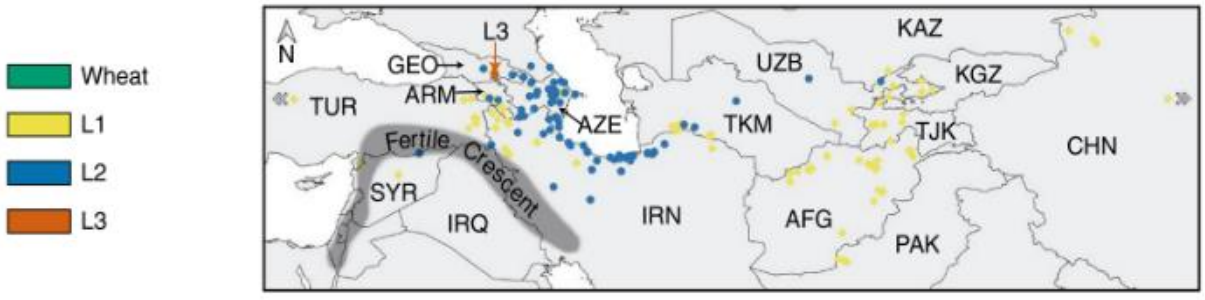


Figure 7. Geographical distribution of the *Aegilops tauschii* accessions used in this study. The accessions are color-coded according to their lineage. In yellow, Lineage 1, blue: lineage 2 and orange: lineage 3. (From Gaurav et al., 2021)

Variability of RSA. Due to the wide genetic diversity between L1 and L2 lineages and to the major representation of L2 sequenced lines in the OWWC consortium, the L2 lineages were used for further phenotypic analysis of root architecture system in collaboration with John Innes Centre and the OWWC consortium (http://www.openwildwheat.org/), and thus 143 accessions belonging to *Aegilops tauschii* ssp. *strangulata* (Lineage 2; L2) were considered.

2.4.2. Phenotypic analyses

a) **Phenotypic diversity.**

Agronomic evaluation. Phenotyping was conducted at Cadriano experimental station of Bologna university, Italy (Latitude: 44−33'03"−, Longitude: 11−24'36"−, Altitude: 33 m). The experiment was conducted in two cropping seasons (2019-2020 and 2020-2021), however, the first year only is considered in the present thesis, while the second has to be completed**.** The experiments were laid out following randomized complete block design with two replications, 1.5 m plots, and a 1-meter distance between rows. The following traits were measured in the field:

- Heading date (HD) was calculated as days from the sowing date to the date when approximately 50% of the spikes have emerged from the flag leaf sheath.
- Elevation angle or growth habit (EA). (Score per plant: 0 = completely elevated, 90 = prostrated).
- Tiller score (TS) for each plant (0= very few culms, 9 = high number of culms).
- Senescence (SEN) was estimated as the number of days from 1st June for complete senescence.
- Culms dry weight (CDW) was evaluated from a single plant harvested in the field. Blades, sheaths, and internodes were separated from each culm. Then, the tissues were weighed after being dried at 65°C.
- Root dry weight (RDW) was assessed equally to culms dry weight except on roots.
- The number of culms (NC) were counted from a single plant harvested in the field.
- Anthocyanin presence (AP) (% = percentage of the plot affected).

Six stems were harvested for every genotype and then each one was put on paper for scanning (**Figure 8**). Collected images were used to measure other traits using the open-source Java-written program ImageJ (Schneider et al., 2012). Each image was analyzed individually, and values obtained in pixels were translated to centimeters for each trait. Data were gathered from:

- Plant height (PH)
- Flag leaf length (FLL)
- Flag leaf width (FLW)
- Spike length (SL)
- Number of spikelets (NS)
- Internode length (IL)
- Node number (NN)
- Peduncle length (PL)

Figure 8. Image showing a plant of *Aegilops tauschii* scanned on paper ready to be analyzed with ImageJ software.

b) **<u>Variability for RSA.</u>**

<u>RSA evaluation.</u> The 143 accessions of lineage 2 were grown at Cadriano experimental station of Bologna university, Italy in the cropping seasons 2018-2019 in order to increase the seed lot. Eight commercial bread and durum wheat cultivars were included in the experiment as controls: Antalis, Bologna, Julius, Monastir, Neodur, Reform, Soberio and Svevo.

The accessions were characterized for different RSA traits at the seedling stage using the roots growth protocol reported in Canè et al.(2014). For each genotype, 20 seeds were collected, selecting the most developed and avoiding shriveled caryopses. After being weighted, 10 seeds of each genotype were selected for pre-germination. The seeds were placed in 50 ml Falcon tubes and sterilized with a solution of deionized water and 5-10% sodium hypochlorite solution for 5 minutes. To remove all bleach residue, seeds were rinsed three times with sterile distilled water, put in Petri dishes and placed in an incubator for 48 hours at 28 °C. Homogeneously sprouting seedlings were then grown for 10 days in moist filter paper sheets in vertical black polycarbonate screening plates (40 x 40 cm). Six pre-germinated seeds for each genotype and three seeds per replicate, were used for the experiment, selecting for homogeneity of germination.

Labor 50 x 50 cm filter paper sheets (Cordenons s.p.a.) were used for this experiment. A horizontal line was drawn with a pencil on the top of the sheet, 2 cm from the top edge. Starting 10 cm from the left edge and letting 10 cm between each other, three points were marked on the line, representing the position for the seeds to be placed. Each sheet was then soaked in the nutrient solution laid on a polycarbonate plate (42.5 x 38.5 cm, with the long side horizontal), folding the sides around the plate to anchor it. The nutrient solution was a modified version of Hoagland's solution. For 1 liter of solution, the chemical composition was the following:

- $CaNO_3$ 1M= 5 ml (f.c.: 5 mM);
- KNO_3 1M= 5 ml (f.c.: 5 mM);
- $MgSO_4$ 1 M= 2 ml (f.c.: 2 mM);
- Fe-EDTA 0.1 M= 1 ml (f.c.: 100 μM).

The experiment was conducted in two replicates, and the experimental unit consisted of three plants. For each unit, three of the ten previously pre-germinated seeds were placed on the drawn line, in correspondence of the three points marked before, with the ventral furrow in contact with the paper and with the seminal sprouting root pointing downward. The pre-germinated seeds were selected based on their size (for a higher nutrient content), uniformity and length of the seminal root that should not be very long to avoid mechanical stress. A strip of filter paper (50 x 5 cm) was marked with the name of the genotype, soaked in the nutrient solution, and placed on the plate to cover the seeds.

Each plate was covered with a black plastic bag that had been purposefully cut, leaving the top and two side edges free to allow the seedlings' leaves to emerge from the top, the nutrient solution to infiltrate from the sides, and the paper to remain moist through capillary action to better protect the seedlings from light. Clips were used to secure the plastic bags to the plates. The nutrient solution-filled tank was then filled with the prepared screening plates, which were kept in a vertical position to

prevent altering the root growth. Two additional polycarbonate plates were added, one at the beginning and one at the end of the row of plates carrying the seeds, to protect the seedlings from light and increase plate stability.

The experiment was carried out in a growth chamber with the following controlled conditions: 16 h of light and 8 h of darkness, for 10 days at 22 °C. Then, tanks were left in the growth chamber for ten days to allow root growth along the plate surface. After five days the tanks were interchanged in position and rotated at 180° to avoid possible bias due to light and humidity gradients. The level of the solution was periodically checked for evaporation and the tank was refilled accordingly using deionized water.

Image collection

After ten days images of roots grown on plates were collected. Each plate was photographed from above by means of a Nikon 5600 fixed on a vertical support at 58 cm of distance from the plate. The "DigiCamControl" app was installed on a laptop computer and connected to the camera. The app allowed seeing framed images in real time and change settings such as exposure, shooting mode, and focus mode. A ruler was included in each picture as reference for traits measurements with the software. For each plate, two pictures were taken. The first one, used for measuring the root growth angle, was taken leaving the plants and roots as they were on the plate (natural growth position) as shown in **figure 9**. The second one, used for measuring other RSA traits, was taken after “combing” the roots, this means separating the intertwined roots from each other with the help of tweezers and placing them well separated on the plate (**Figure 10**). The day after images acquisition, shoots length from the base of the hypocotyl to the tip of the longer leaf were manually measured for each seedling by means of a ruler.

Figure 9. Image of a black polycarbonate plate with uncombed roots of *Aegilops tauschii* plantlets grown on a white filter paper sheet.

Figure 10. Image of a black polycarbonate plate with combed roots of *Aegilops tauschii* seedlings grown on a white filter paper sheet.

Root Image analysis

Traits under analysis were subsequently measured on seedling images using ImageJ software combined with the SmartRoot plugin. For this type of measurement, combed roots pictures were used. The “angle tool” was used to measure Root Growth Angle (RGA). The measurement was performed for each plantlet on the “uncombed” roots picture in a rather simple way using the cursor, which allowed one to draw two lines coinciding with the initial part of the two most external roots, representing the sides of the angle, making the vertex of the angle coincide with the seed (**Figure 11**). Once the angle was traced, the software automatically measured it in degrees. For the measurement of roots length and diameter, an ImageJ plugin called “SmartRoot” was used, specifically developed for measurement of root traits. For this type of measurement, combed root pictures were used. Images opened through SmartRoot are converted to 8-bit greyscale (**Figure 12**). Once the conversion is made, it is necessary to invert the colors of the image through the ImageJ toolbar: this allows to obtain an optimal contrast between the black of the roots and the white of the panel thus allowing the software to work optimally. The program automatically converts the pixels covered by the measurement to centimeters, allowing to obtain a cm value for all subsequent measurements taken on the image. Then the measurement is carried out using the "trace" tool, starting with the roots of the first plant on the plate, from left to right. Also, in this case the measurement is done through the cursor, in manual or semi-automatic mode. In manual mode, the root is traced through the manual drop of nodes from the seed to the root apex, with a left click on the mouse. The nodes, besides representing "milestones" along the root, also are used to measure the diameter point by point, so the greater the number of nodes dropped, the greater the accuracy of the final measurement, as the program calculates an average for the values provided by the nodes along the root. Once reached the root apex, a double left-click allows to close the measurement of that specific root. In semi-automatic mode, after placing the first node with a left click, the rest of the root can be drawn semi- automatically by pressing the Alt key together with the left mouse button. This will drop a series of nodes along the root, placed at regular length intervals from each other. This mode, while faster, is not always accurate, so manual correction is needed to place nodes more precisely along the root, to avoid diameter measurements that are too small or too large, and to avoid going off root’s path.

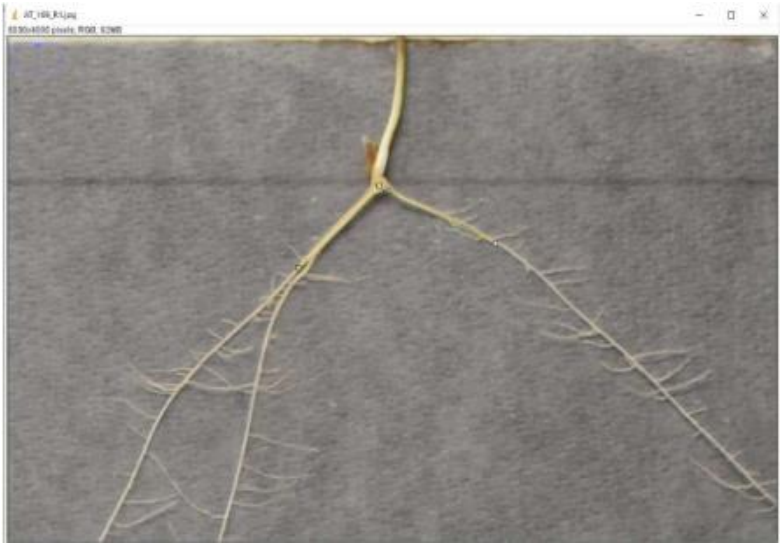


Figure 11. Example of Root Growth Angle measurement on ImageJ software using the Angle tool.

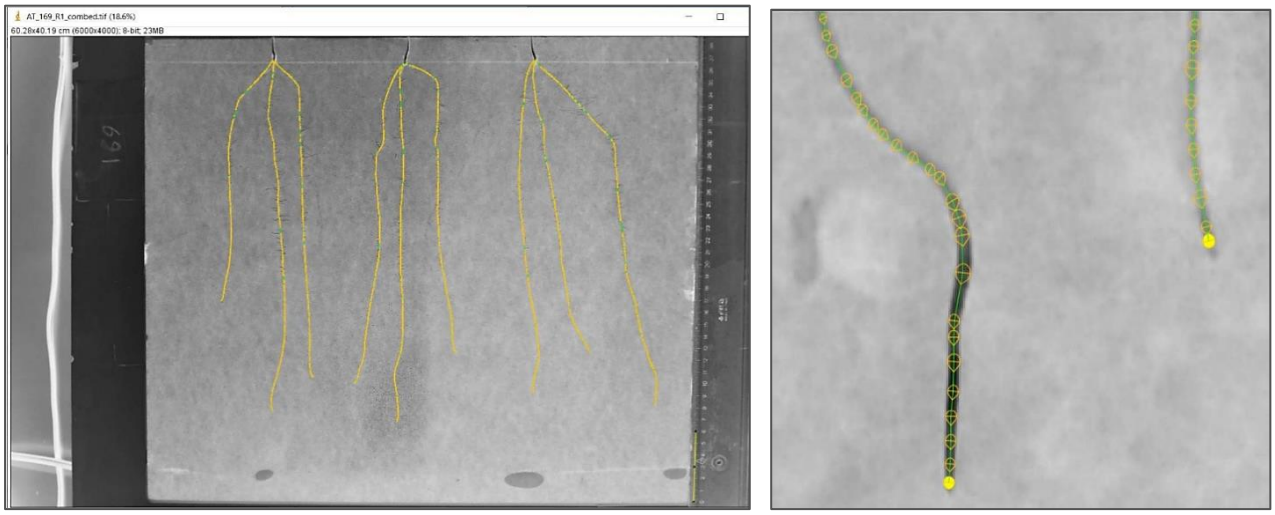


Figure 12. Photo converted to 8-bit grayscale with all the roots traced (On the left); Close up of the terminal part of two traced roots (On the right).

Root network area, lateral roots length and lateral roots density were obtained using the Gia Roots software, in collaboration with the National Institute of Agricultural Botany (NIAB), UK. This program converts the photos of the roots into binary images, in which the white pixels represent the area covered by the roots, on a background of black pixels (**Figure 13**). The network area was calculated automatically by the program, while for the traits "lateral roots length" and "lateral roots density", scoring was done visually. From manual measurements and image analysis, the following

phenotypic data were produced for each single analyzed plant: root growth angle (°), root number (no.), root total length (intended as the sum of the length of all the roots present in a plant; cm), root length average (intended as the average of the length of all the roots present in a plant; cm), primary root length (cm), root diameter average (intended as the average of the diameter of all the roots present in a plant; cm), shoot length (cm), network area (cm^2), lateral roots length (score per plant: 0 = minimum length, 9 = maximum length), lateral roots density (score per plant: 0 = minimum density, 9 = maximum density).

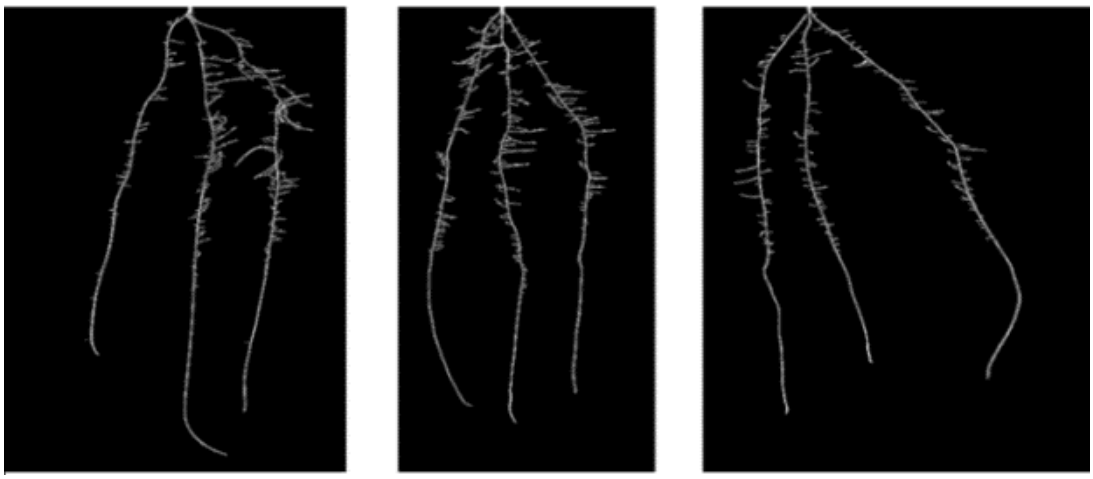


Figure 13. Binary picture output by Gia Roots. The plate image was cut into three separate plants to process them with the software and assign a score to each one.

2.4.3. Statistical analysis

Phenotypic diversity. The following descriptive statistical indices were determined for each phenotypic trait: minimum, average, maximum, standard deviation, and coefficient of variance. The broad-sense heritability was then calculated using the Smith et al. (1998) method. All data were corrected for outliers and spatial effects in the field using the Spats R package (Rodríguez-Álvarez et al., 2018). Power transform (PT) was applied to some traits with a non-normal distribution. Analysis of variance (ANOVA) was conducted following a linear mixed model in the R statistical package (R Core Team, 2021). Best linear unbiased predictions (BLUPs) were obtained for each accession and each trait.

Variability of RSA. Descriptive analysis and heritability estimates were obtained for the phenotypic data in the R statistical package (R Core Team, 2021). The normality of frequency distribution was assessed for each trait. For some traits, data distribution was normalized through quantile-quantile

(QQ) or power transform (PT) methods. ANOVA was performed following a linear mixed model. Statistical analysis produced BLUEs of each accession for each analyzed phenotypic trait.

2.4.4. Population structure analysis

Population structure was previously analyzed by (Gaurav et al., 2022) using the Bayesian approach and the model-based clustering was performed using the STRUCTURE software version 2.3.4. that was used to investigate the number of distinct lineages of *Ae. tauschii*.

STRUCTURE simulations were run using a random set of 100,000 *k*-mers with a burn-in length of 100,000 iterations followed by 150,000 Markov chain Monte Carlo iterations for five replicates each of K ranging from 1 to 6. STRUCTURE output was uploaded in STRUCTURE HARVESTER software to generate a ΔK plot for each run. The results obtained were then processed and plotted using CLUMPAK software to compare the clustering results across K.

2.4.5. Genome-wide-association study

Genotypes were filtered for redundancy based on population structure analysis (Gaurav et al., 2021a) and all the phenotypic data were filtered for outlier presence.

For association mapping, the *k*-mer *based* pipeline set by Gaurav et al. (2022) was used. *K*-mers were generated using Jellyfish (version 2.2.6) and mapped to the *Aegilops tauschii* AL8/78 reference genome. The chosen *k*-mer size was 51 bp and a (0,1) matrix for presence-absence was generated annealing each *k*-mer to the *Aegilops* accession. *K*-mers present in less than two accessions or in all but one accession were removed during the construction of the matrix. The data processing programs were written in Python and are publicly available at https://github. com/wheatgenetics/owwc.

The association mapping was performed fitting the General linear model (GLM): *k*-mer correlation (R^2) with phenotype > 0.2, MAF = 8, the GWAS was corrected for population structure performing a principal component analysis at 10 clusters (PCA=10) and -log(P) value equal to 6 representing the association threshold for each *k*-mer in a genomic position for the considered genotype. The number of *k*-mers used was between 50 and 80 million. For a significance level of 0.05, a Bonferroni-adjusted –log P value threshold equal to 7.3 was used to identify marker-trait associations. Manhattan plots were generated using Python. The plotting script is published at https:// github.com/wheatgenetics/owwc.

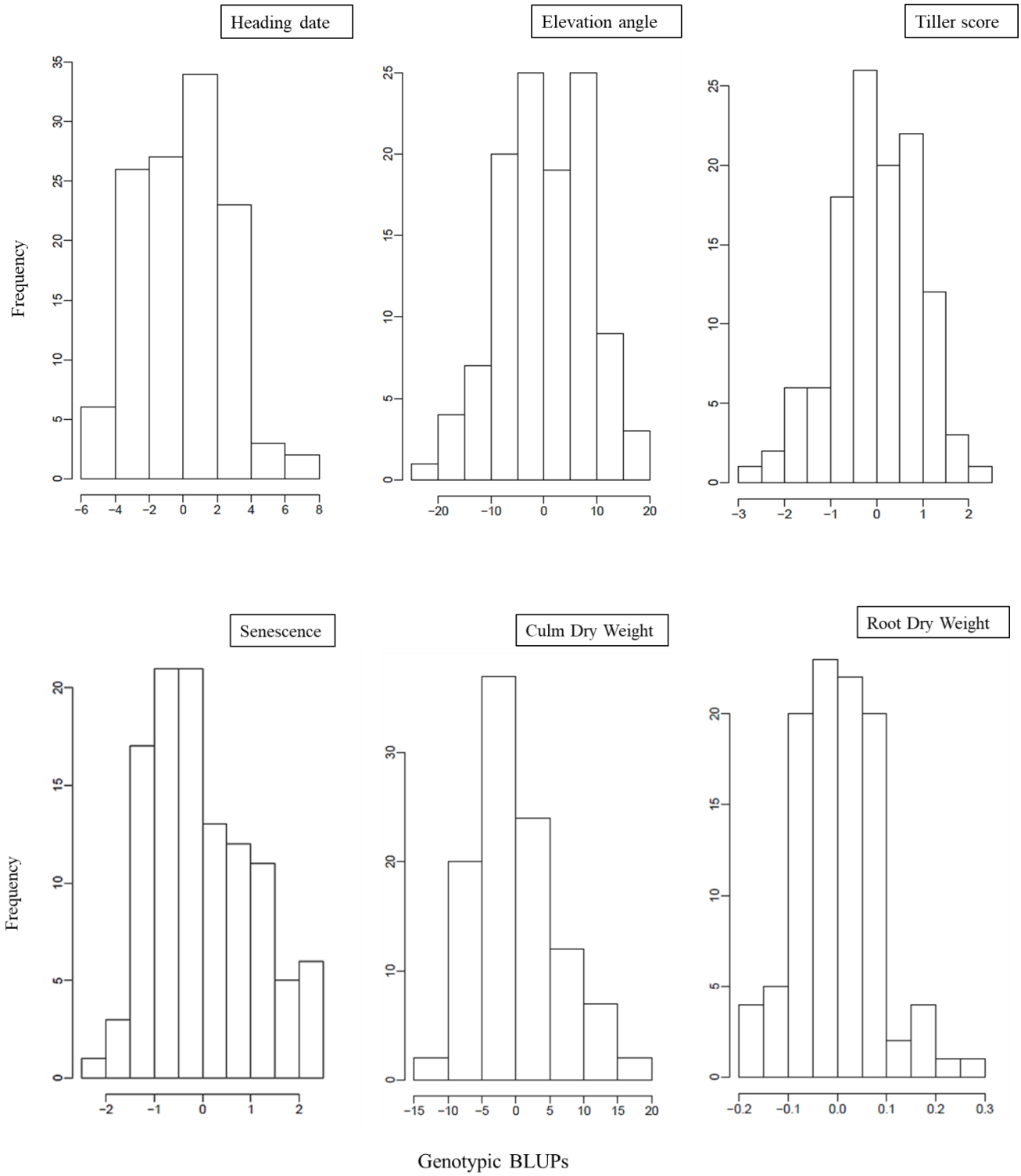
2.5. Results

Field traits

ANOVA revealed high genetic variation among the accessions, data not shown, and descriptive statistics are reported in **Table 1**. Phenotypic variation among accessions for each trait was confirmed by standard deviation and coefficient of variation (Table 1). Medium-to-high heritability estimates were obtained for all the traits. The broad-sense heritability (h^2) ranged between 0.41-0.94 and varied for the same trait among the two lineages. In L1, the highest value was observed for flag leaf length and plant height with 0.88 and 0.80 respectively. Whereas in L2, values of 0.94 and 0.82 were obtained for heading date and flag leaf length. Additionally, the coefficient of variation ranged from 5.63% to 65.34% in lineage 1, while in lineage 2 it ranged from 5.79% to 38.43%. The distributions obtained for the blups for each phenotypic trait are shown in **figure 14**, **15** and **16**. Overall, the distributions had a normal tendency.

Table 1. Mean (cm), standard deviation (Std), coefficients of variation (CV%), and heritability for the tested 14 morphological traits in the year 2020.

Variables	**L1**				**L2**			
	Mean	**Std**	h^2	**CV (%)**	**Mean**	**Std**	h^2	**CV (%)**
Elevation.angle	54.52	8.31	0.47	15.24	65.26	11.65	0.72	17.85
Heading.date	3.91	2.56	0.51	65.34	0.26	3.40	0.94	12.89
Senescence	8.24	1.07	0.50	13.03	10.78	1.94	0.68	17.96
Tiller.number	5.11	0.93	0.44	18.27	4.99	0.79	0.41	15.81
Culm.dry.weight	24.50	6.08	0.46	24.83	26.09	6.82	0.53	26.13
Number.culms	88.46	20.62	0.47	23.31	80.73	19.80	0.50	24.52
Root.dry.weight	0.64	0.08	0.46	12.96	0.77	0.12	0.30	15.14
Anthocyan.presence	77.05	6.86	0.46	8.90	53.07	20.40	0.79	38.43
Plant.height	49.89	5.86	0.80	11.75	46.43	3.81	0.77	8.21
Flag.leaf. length	10.13	1.73	0.88	17.04	8.36	1.61	0.82	19.27
Flag.leaf.width	0.47	0.07	0.76	15.02	0.84	0.14	0.67	16.33
Internode.length	9.29	1.37	0.77	14.74	7.36	0.86	0.74	11.75
Node.number	3.35	0.19	0.54	5.63	3.62	0.21	0.60	5.79
Peduncle.length	5.86	1.62	0.77	27.75	5.46	1.50	0.79	27.55


Heading date
Elevation angle
Tiller score
Senescence
Culm Dry Weight
Root Dry Weight
Frequency
Frequency
Genotypic BLUPs

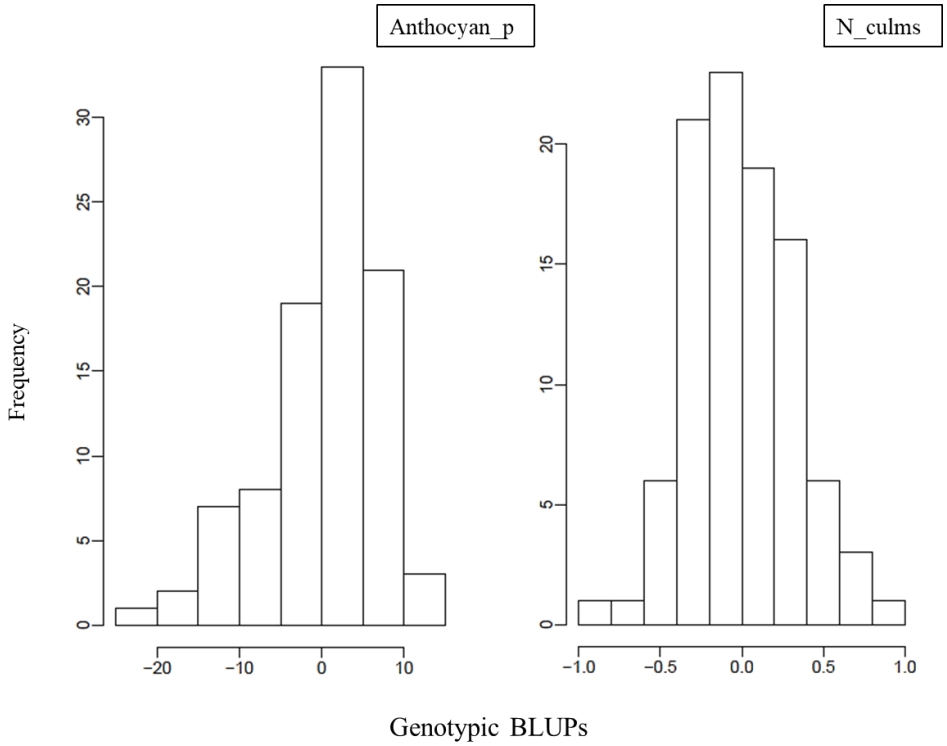
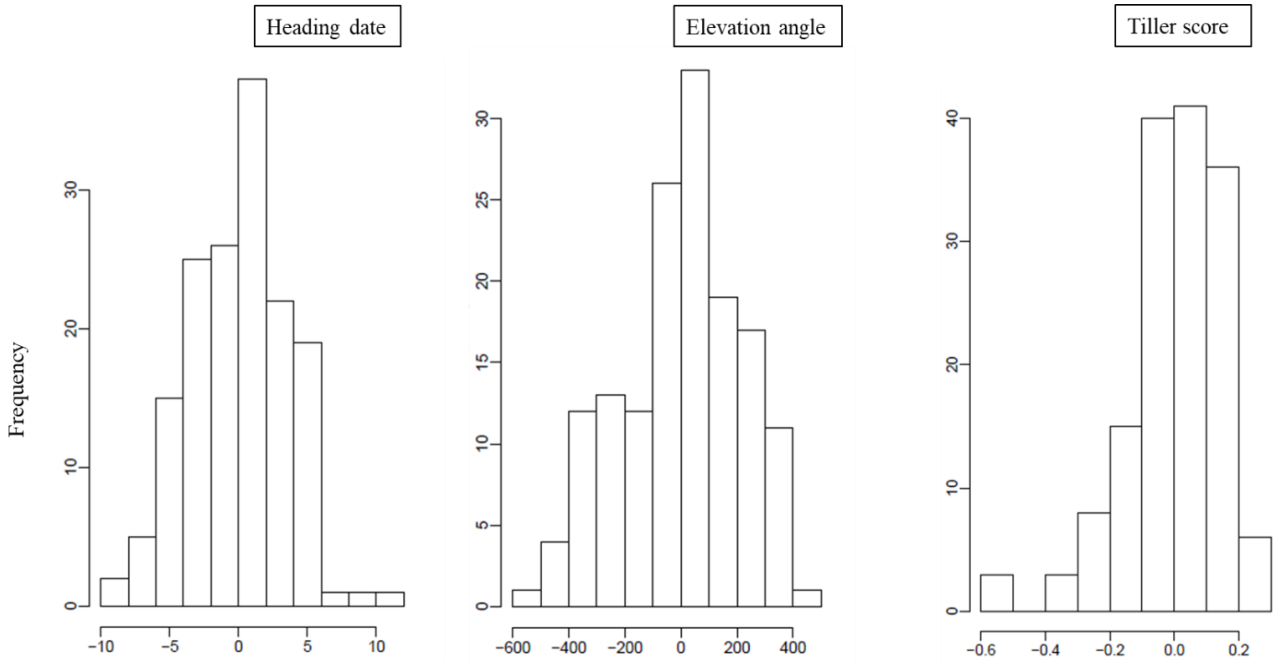


Figure 14. Frequency distribution histograms for the eight phenotypic traits measured in the field. Reported values of the BLUPS obtained from raw data (without normalization) refer to lineage 1 (*ssp. Tauschii*). Only number of culms and root dry weight were power transformed.



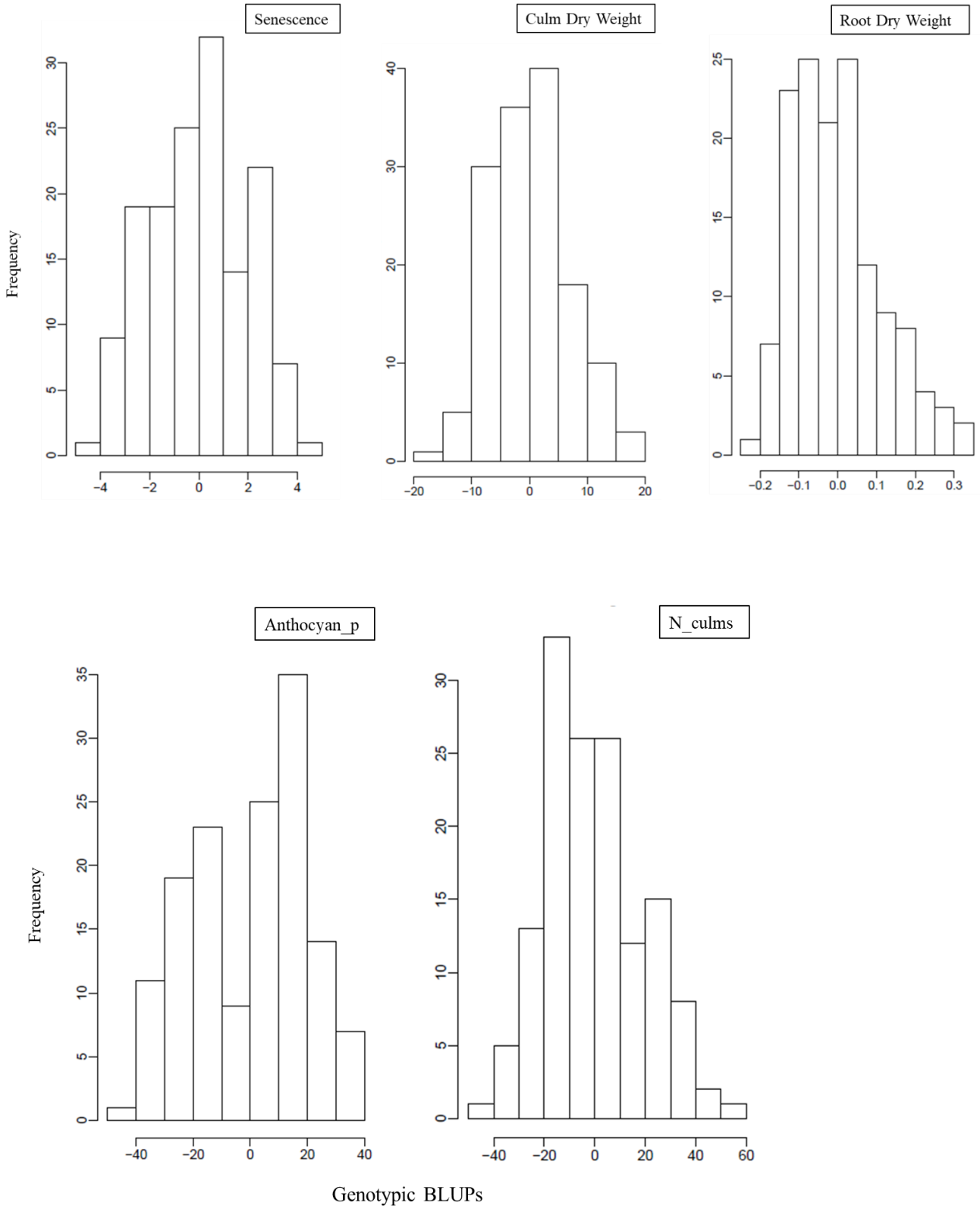
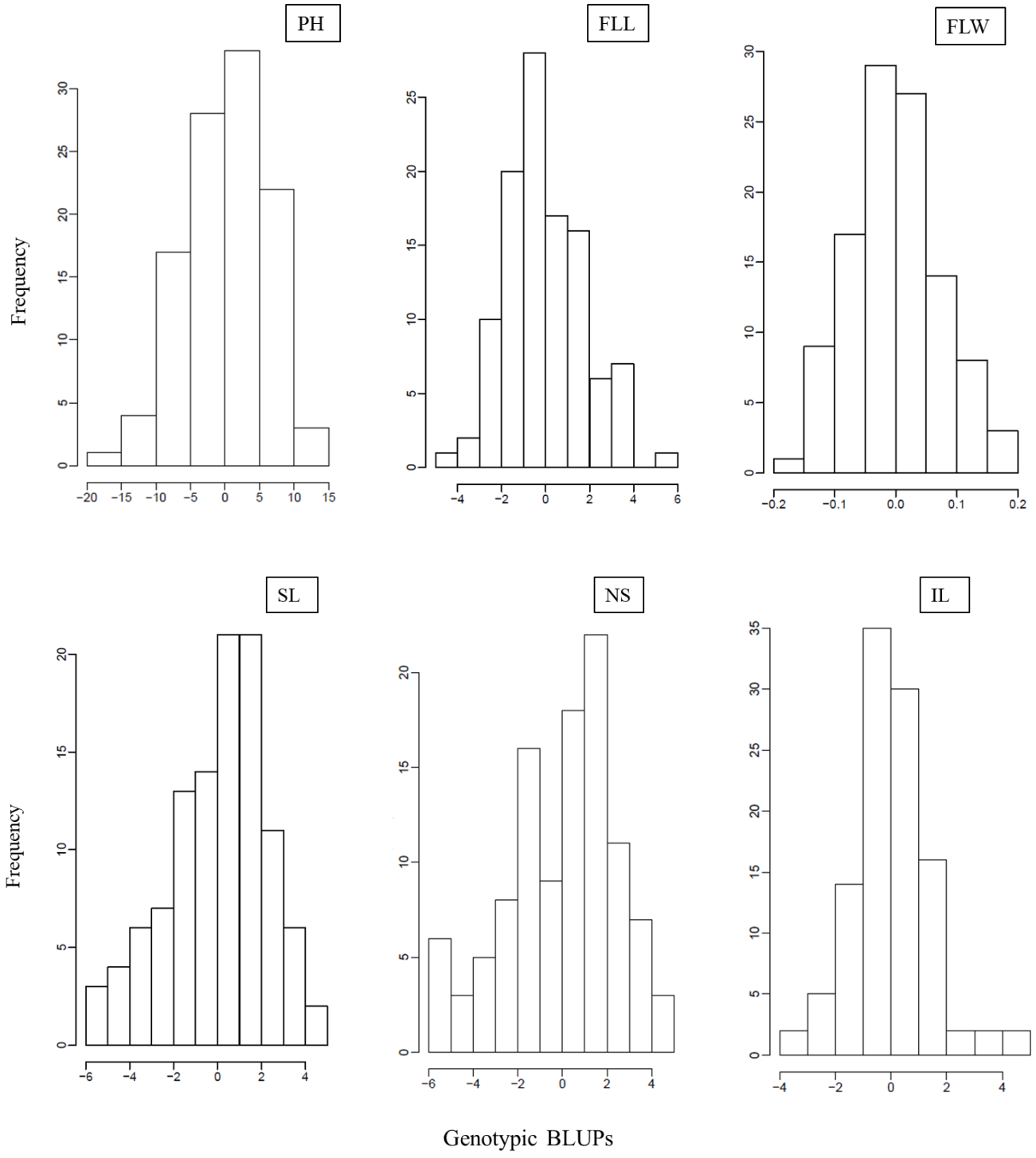


Figure 15. Frequency distribution histograms for the eight phenotypic traits measured in the field. Reported values of the BLUPS obtained from raw data (without normalization) and refer to lineage 2 (*ssp. Strangulata*). Only tiller score and elevation angle were power transformed.


PH
FLL
FLW
SL
NS
IL
Frequency
Frequency
Genotypic BLUPs

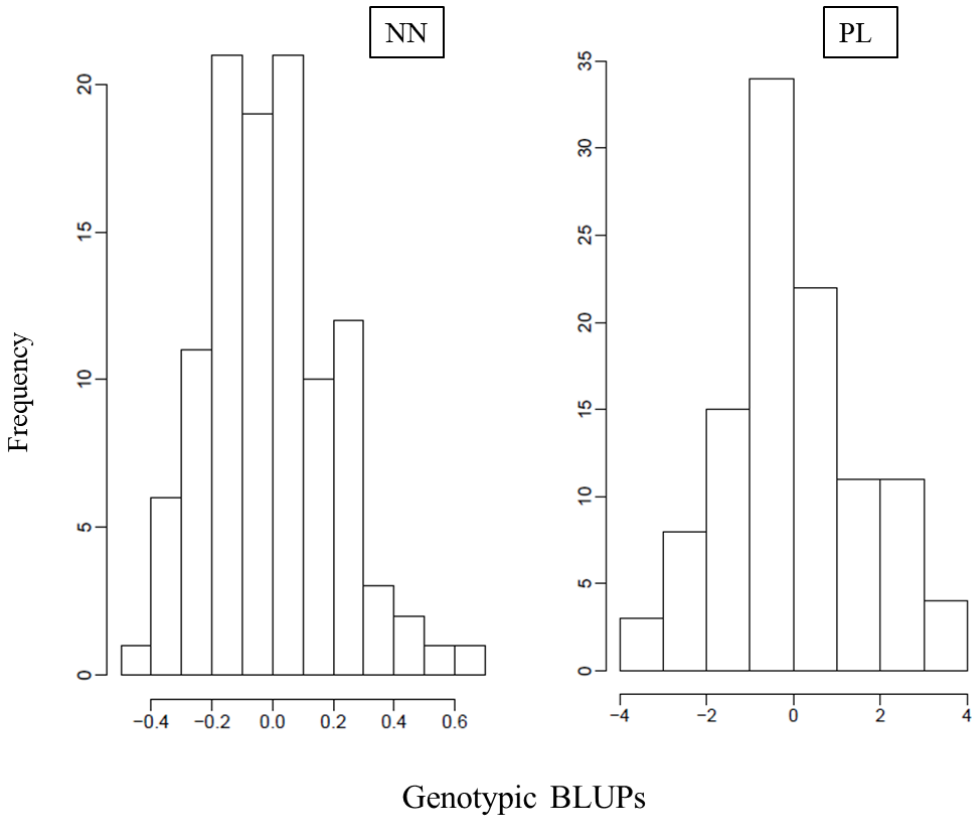
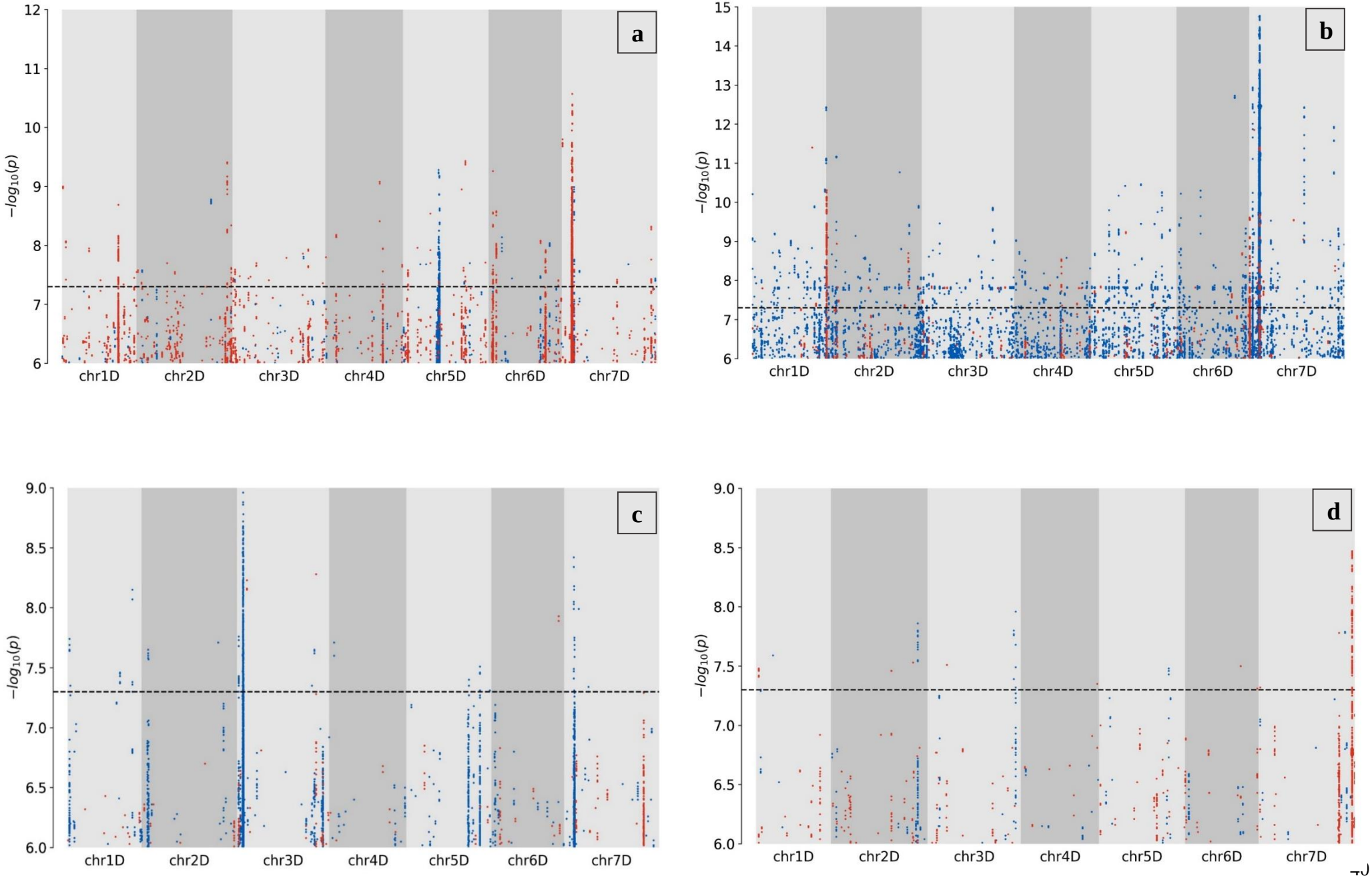


Figure 16. Frequencies distribution histograms for the eight phenotypic traits measured from the ImageJ analysis. Reported values of the BLUPS obtained from raw data (without normalization) and refer to lineage 2 (*ssp. Strangulata*). Only tiller score and elevation angle were power transformed. **PH:** Plant Height; **FLL:** Flag leaf length; **FLW:** Flag leaf width; **SL:** Spike length; **NS:** number of spikelets; **IL:** Internode length; **NN:** Node number; **PL:** Peduncle length.

GWAS results

The Genome wide association analysis for the phenotypic traits revealed 12 significant peaks associated to the eight traits investigated (**Figure 17**). For each phenotypic trait the GLM pipeline produced eight Manhattan plots for each trait (Node number was discarded because no peak was visible). The output plot presents blue and red peaks, the first are associated with a negative correlation to the phenotype and the blue dots to a positive one. Three significant peaks were detected on chromosomes 1D, 5D and 7D for Anthocyanin presence.

Additionally, two peaks were detected for heading date, a red peak was identified on chromosome 1D and a blue peak on 7D. For senescence, two peaks were detected on 3D and 7D whereas for plant height a peak can be seen on chromosome 7D. For flag leaf length, three clear and defined peaks were identified on chromosomes 1D, 4D and 7D. For peduncle length and culms wry weight, peaks were detected on chromosome 2D and 3D respectively.


a
b
c
d
$-log_{10}(p)$
chr1D
chr2D
chr3D
chr4D
chr5D
chr6D
chr7D

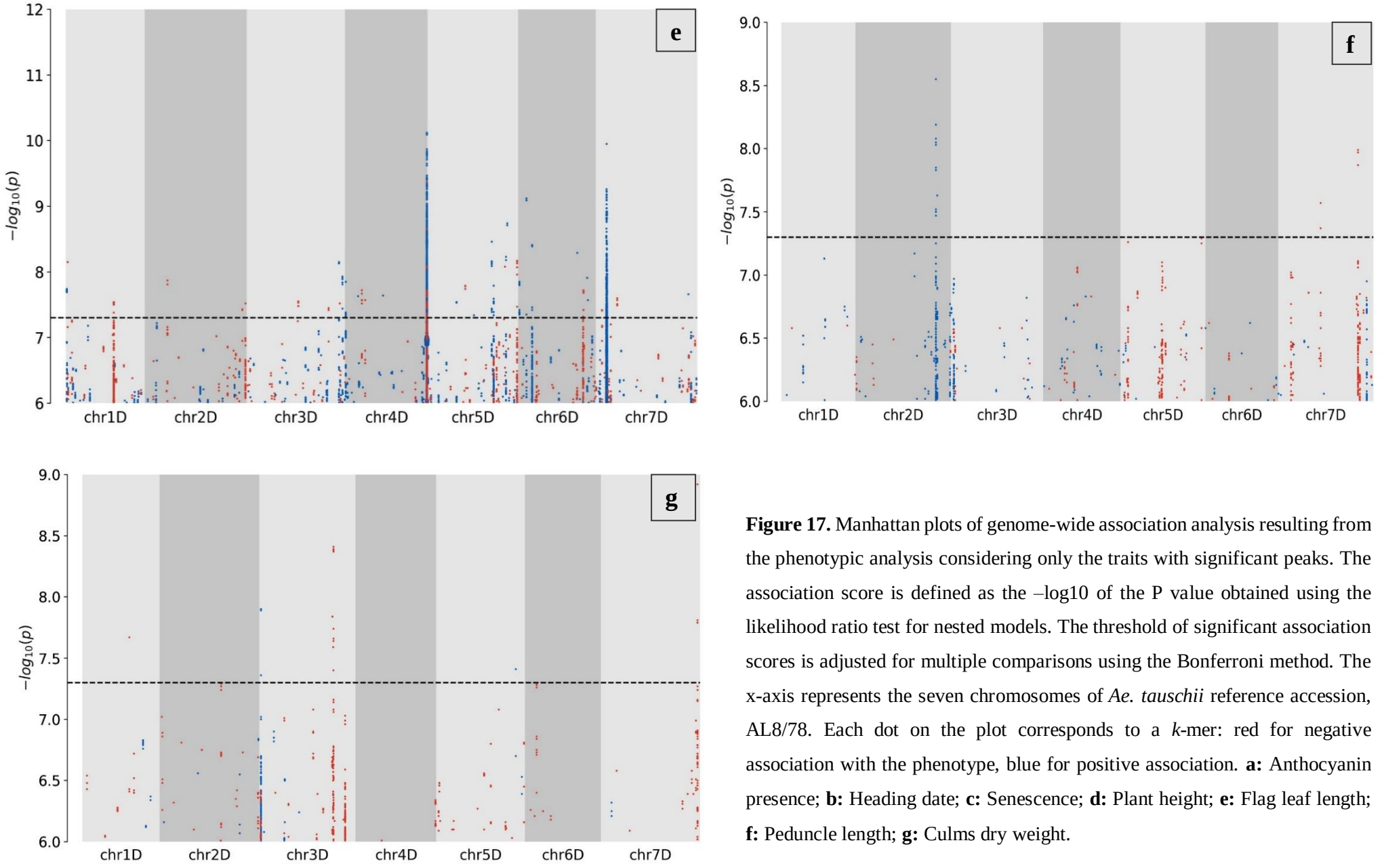


Figure 17. Manhattan plots of genome-wide association analysis resulting from the phenotypic analysis considering only the traits with significant peaks. The association score is defined as the –log10 of the P value obtained using the likelihood ratio test for nested models. The threshold of significant association scores is adjusted for multiple comparisons using the Bonferroni method. The x-axis represents the seven chromosomes of *Ae. tauschii* reference accession, AL8/78. Each dot on the plot corresponds to a *k*-mer: red for negative association with the phenotype, blue for positive association. **a:** Anthocyanin presence; **b:** Heading date; **c:** Senescence; **d:** Plant height; **e:** Flag leaf length; **f:** Peduncle length; **g:** Culms dry weight.

Table 2. Table illustrating the main association peaks resulted from the GWAS analysis of field phenotypic traits. Only peaks with a (-) log (pvalue)≥8 are listed in the table.

Chromosome	Scaffold interval (start-end in bp)	-log(pvalue)	R^2	k-mers no.	Phenotype (1)

7D	69,33-69,34	10.37	-0.59	1	**AP**
5D	24,321- 24,322	9.17	0.56	2	**AP**
1D	507-508	8.97	-0.22	2	**AP**
2D	218-219	10.31	-0.28	1	**HD**
7D	65,82-65,83	14.75	0.55	1	**HD**
3D	39,10-39,11	8.96	0.41	1	**SEN**
7D	64,25-64,26	8.42	0.43	1	**SEN**
7D	62,714-62,715	8.47	-0.5	3	**PH**
7D	66,49-66,50	9.95	0.51	1	**FLL**
4D	51,941-51,942	10.11	0.47	1	**FLL**
2D	54,653-54,654	8.55	0.49	1	**PL**
3D	48,103-48,104	8.38	-0.51	1	**CDW**

(1): AP, diameter average; HD, lateral roots density; SEN, lateral roots length; PH, network area; FLL, root growth angle; PL, root length average; CDW,

Root traits.

Descriptive statistics are reported in **Table 3**. Phenotypic variation for each trait was confirmed by standard deviation and min-max values. High heritability estimates were observed for all the root traits. The broad-sense heritability (h^2) ranged between 0.768-0.961.

Table 3. Min, Max, Average, standard deviation (Std) and heritability for the tested 11 root traits in the year 2020.

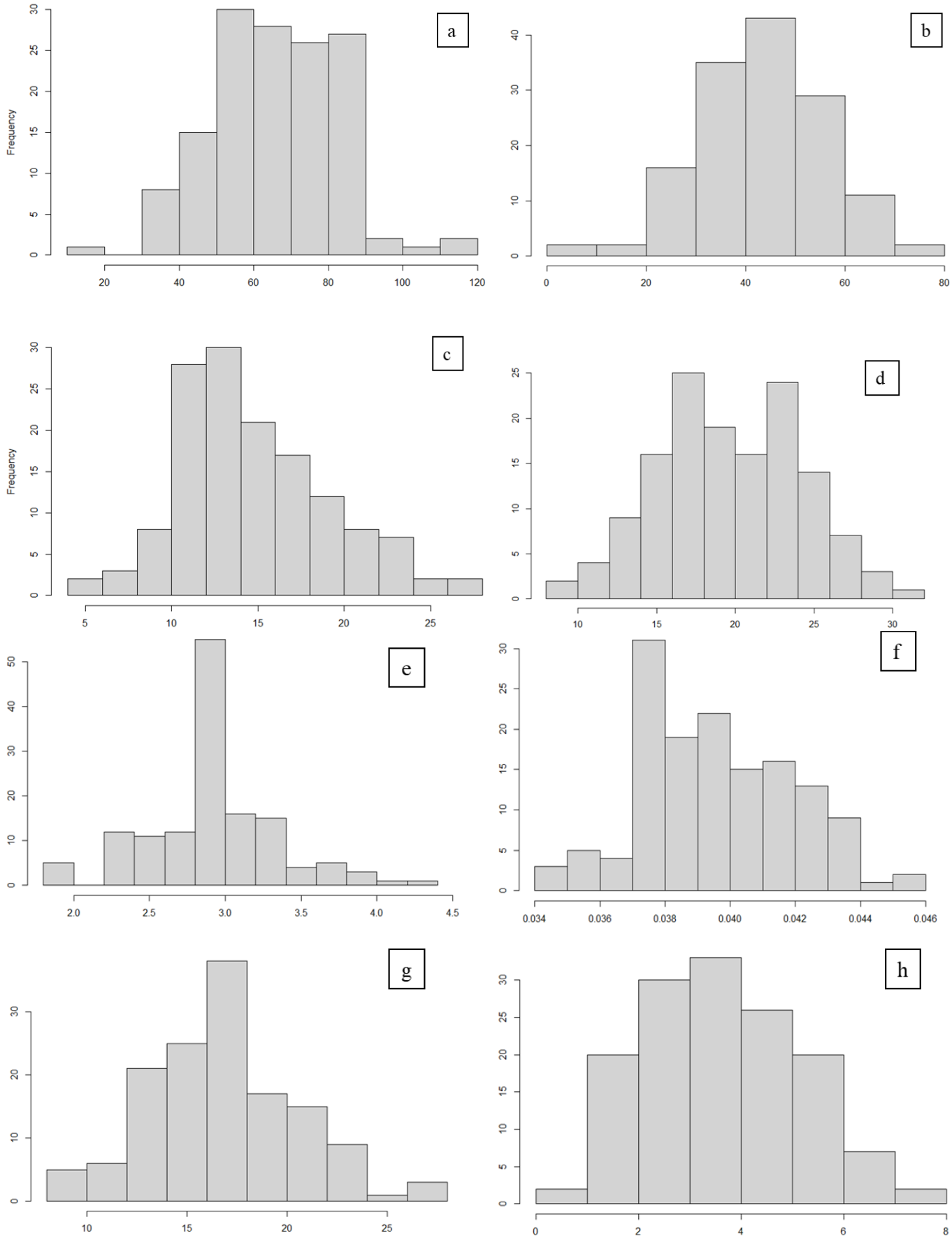
Variables	Min	Average	Max	Std	h^2
Root growth angle	14.194	65.189	117.484	16.752	0.880
Root total length	4.436	14.796	27.83	4.393	0.911
Root length average	4.436	14.796	27.83	4.393	0.910
Primary root length	8.507	19.601	30.784	4.593	0.935
Root number	1.954	2.949	4.391	0.439	0.961
Root diameter average	0.034	0.04	0.046	0.002	0.809
Shoot length	8.82	16.802	26.375	3.589	0.848
Network Area	0.319	3.647	7.348	1.492	0.950
Lateral roots length	0.159	2.553	6.778	1.247	0.768
Lateral roots density	0.455	2.316	4.848	0.864	0.805
Twenty kernel weight	0.06	0.231	0.37	0.063	0.801

The analysis was performed for the first season (2019-2020) only. According to the BLUES obtained from raw data of the root analysis, the following phenotypes showed a normal distribution of values: root total length, network area, lateral root density and twenty kernel weight. For the primary root length, raw data showed a bimodal distribution. In the other cases, data distribution deviated significantly from normal (**Figure 18**).

The GWAS analysis for the second year was still in progress when this thesis was submitted. However, some peaks for several phenotypic traits had already been identified for first-year data. For each

phenotypic trait the GLM pipeline produced two outputs files and two Manhattan plots, the first Manhattan plot considers all the associated peaks above -log pvalue=6, whilst the second retains only those peaks with at least 30 k-mers associated. It is worth considering the non-filtered output in order to look at all the associated peaks but, in case the background noise is too strong, the filtered output highlights only the principal peaks. In addition, the output plot presents blue and red peaks, the first are associated with a negative correlation to the phenotype and the blue dots to a positive one. 18 most significant peaks were detected for 10 of the analyzed RSA traits, with both positive and negative correlation with the phenotype under investigation (**Figure 19**). Our results showed the following peaks for each analyzed RSA trait:

For Diameter average on chr5D, a red peak (negative correlation with phenotype) was detected at 410kbp and 7 were genes associated to the phenotype. For lateral root density score, a blue peak (positive correlation) can be seen at 330kbp with the presence of 6 genes. Another red peak at 580kbp with 12 genes detected and on ch7D, 4 genes were detected at 220kbp. For lateral roots length, a blue peak was revealed on chr2D at180kbp with 5 genes. Another peak with a negative correlation was detected with 19 genes. For the network area, a blue peak was detected on chr7D with 16 genes at 1.18Mbp. Moreover, for primary root length, a positive correlation appeared at 130kbp with the presence of two genes. For root growth angle, the presence of a negative association (red peak) can be seen on chr3D with 47 genes. In order to obtain a better peak, phenotypic data could be improved in this case. On the other hand, 3 peaks were detected on 3 chromosomes chr2D (2genes), chr3D (6 genes) and finally on chr7D with 23 genes negatively associated to the trait.


a
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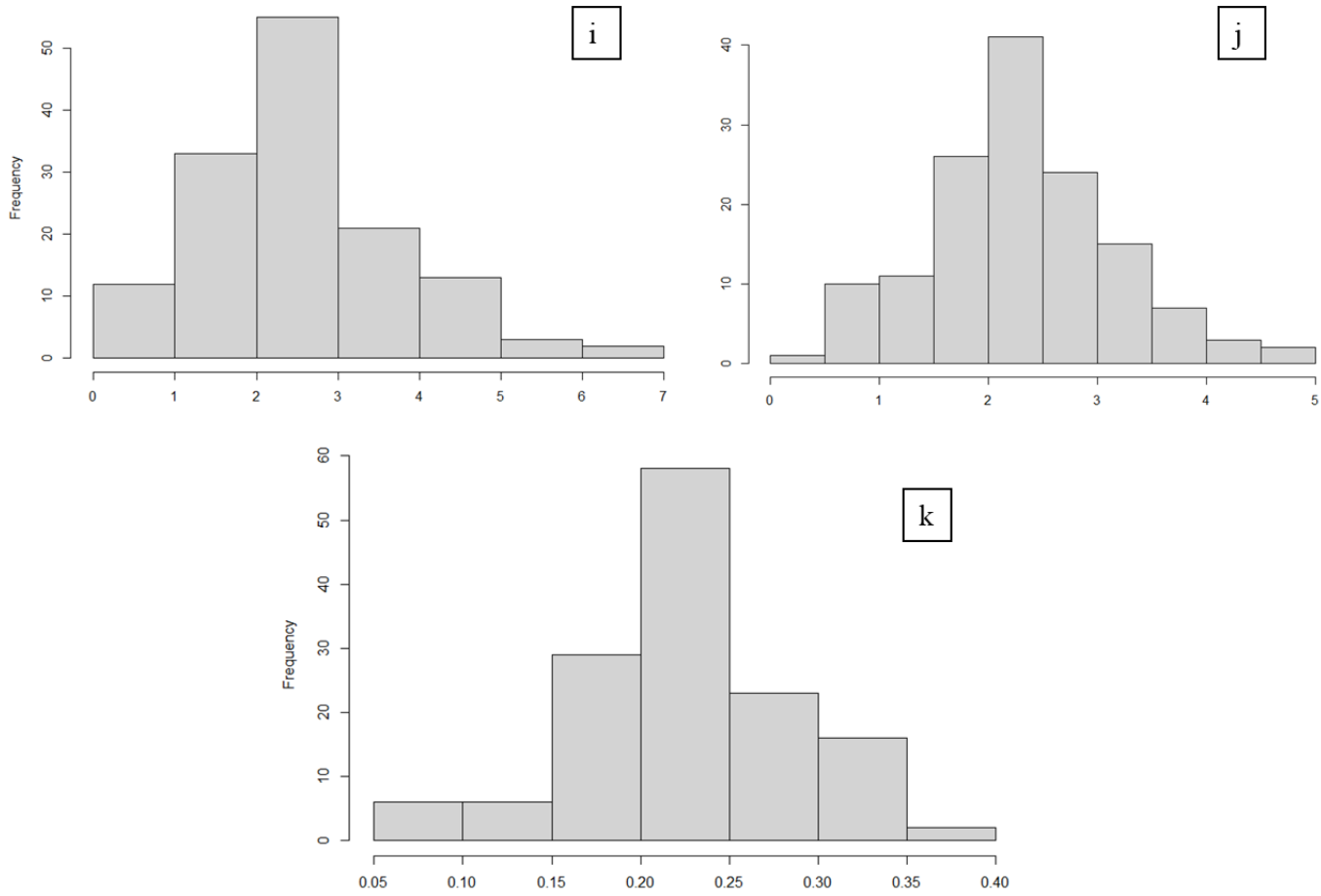
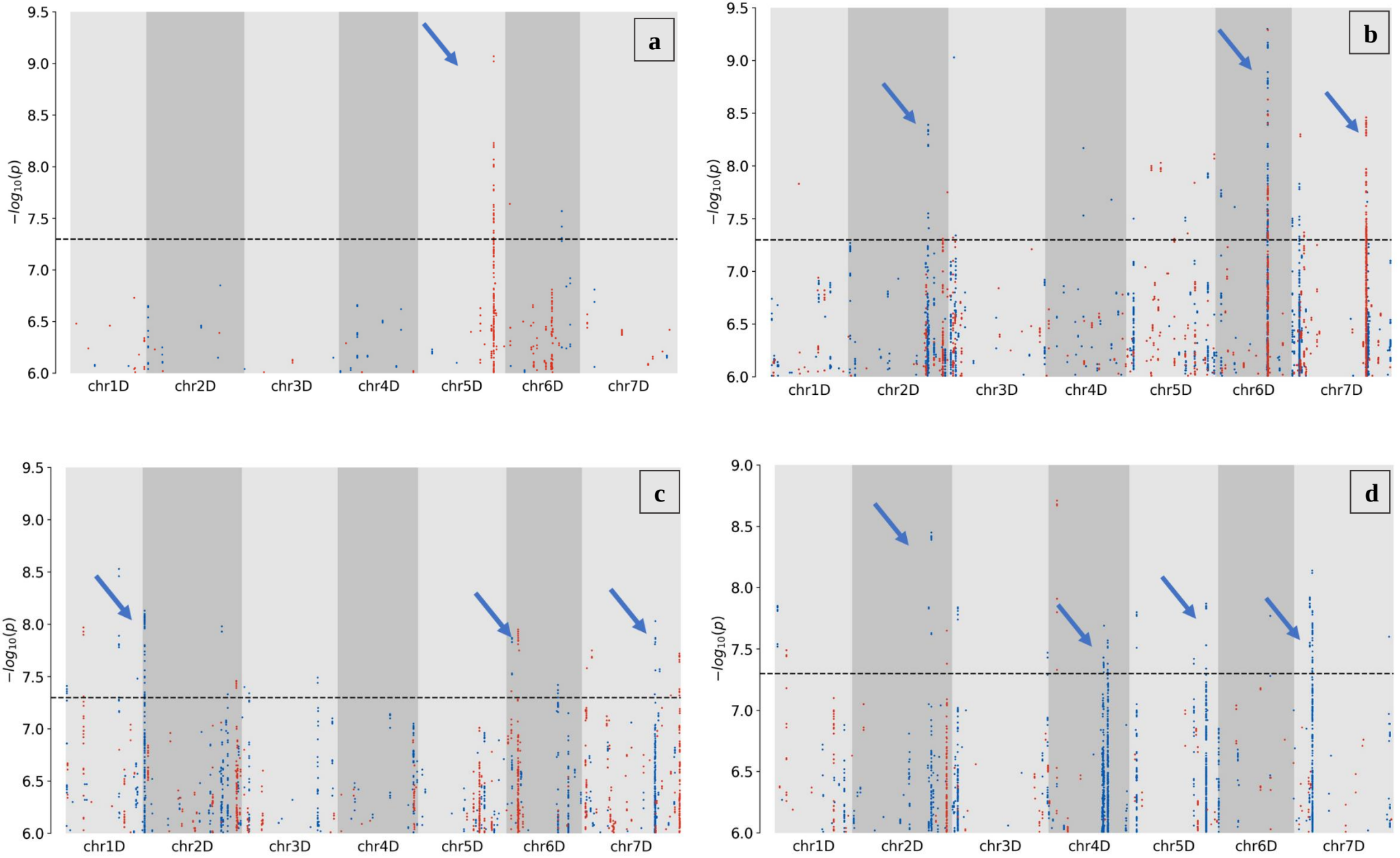


Figure 18. Frequency distribution histograms and tables with the results of the statistical analysis for each trait measured during the root phenotypic analysis. Reported values refer to BLUES obtained from raw data (without normalization) and refer to lineage 2 accessions only. **a:** Root Growth Angle (°); **b:** Root Total Length (cm); **c:** Root Length Average (cm); **d:** Primary Root Length (cm); **e:** Root Number (no.); **f:** Root Diameter Average (cm); **g:** Shoot Length (cm); **h:** Network Area (cm^2); **i:** Lateral Roots Length (score: 0=minimum length; 9 = maximum length); **j:** Lateral Roots Density (score: 0=minimum length; 9 = maximum length); **k:** Twenty Kernel Weight (g).


a
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chr7D

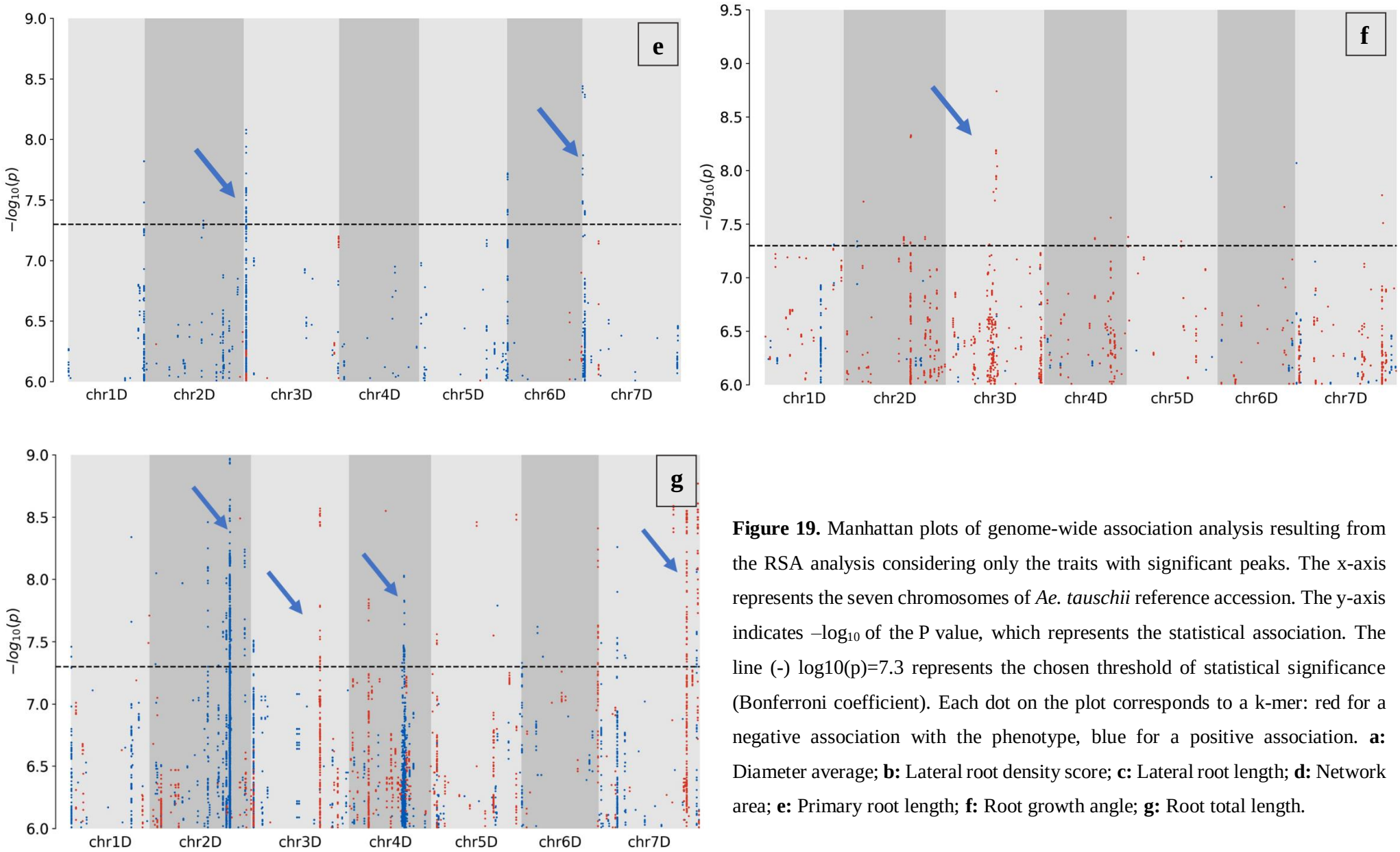

Figure 19. Manhattan plots of genome-wide association analysis resulting from the RSA analysis considering only the traits with significant peaks. The x-axis represents the seven chromosomes of *Ae. tauschii* reference accession. The y-axis indicates $-\log_{10}$ of the P value, which represents the statistical association. The line (-) log10(p)=7.3 represents the chosen threshold of statistical significance (Bonferroni coefficient). Each dot on the plot corresponds to a k-mer: red for a negative association with the phenotype, blue for a positive association. **a:** Diameter average; **b:** Lateral root density score; **c:** Lateral root length; **d:** Network area; **e:** Primary root length; **f:** Root growth angle; **g:** Root total length.

Table 4. Table illustrating the main association peaks resulted from the GWAS analysis. Only peaks with a (-) log (pvalue) ≥ 8 are listed in the table, except for the peak on chromosome 4D, which, although it did not reach this threshold value, presented a remarkable solidity.

Chromosome	Scaffold interval (start-end in bp)	- log(pvalue)	Phenotypic correlation	*k*-mers no.	RSA trait (1)
5D	499,48-499,49	9.07	-0.23	1	**DMA**
6D	336,98-336,99	9.62	0.42	1	**LRD**
6D	338,99-339	9.64	-0.4	1	**LRD**
7D	480,42-480,43	8.46	-0.25	1	**LRD**
2D	12,12-12,13	8.13	0.45	2	**LRL**
4D	383,35-383,36	7.57	0.26	2	**NWA**
7D	114,93-114-94	8.14	0.45	1	**NWA**
3D	279,54-279,55	12.71	-0.45	1	**RGA**
2D	514,89-514,90	9.33	0.5	1	**RLA**
4D	349,82-349,83	8.03	0.36	1	**RLA**

(1): DMA, diameter average; LRD, lateral roots density; LRL, lateral roots length; NWA, network area; RGA, root growth angle; RLA, root length average.

2.6. Discussion

To date, wild species have been considered more as sources of resistance to biotic and abiotic stresses than as sources of diversity, permitting the deep modification of the architecture and physiology of cultivated species. *Ae tauschii* displays a high level of genetic differentiation among local populations, and genetic marker analysis suggests that the wheat D subgenome donor was recruited from an L2 population of *Ae. tauschii* in the southwestern coastal area of the Caspian Sea. To explore this diversity, we performed association mapping and discovered new gene candidates for agro-morphological and root traits, exemplifying the potential of *Ae. tauschii* for wheat improvement. K-mer based genome wide association study allowed us to identify many quantitative trait loci

governing field agronomic traits but root architecture as well. Indeed, phenotypic analysis revealed extraordinary variability in the L2 collection for all the Field and Root System Architecture traits, confirming that *Aegilops tauschii* possesses a large repertoire of genetic variability.

Previous genetic research has uncovered many genes that affect important agronomic traits, but only a few have been practically used in plant breeding. Among the QTLs identified in our study, some were confirmed by other findings in literature. Chromosome 2DS is known to harbor the Ppd-D1 and Rht8 genes that control flowering time and PH, respectively, and also could affect the spikelet number (Muqaddasi et al., 2019; Shaw et al., 2013). Besides chromosome 5D, three chromosomes, 2D, 6D and 7D, were found to be involved in the regulation of flowering time. Gaurav et al. (2021) found in their study that flowering time is mapped to a broad peak on chromosome arm 7DS containing 35 genes.

Unfortunately, the detected loci represented minor QTLs that make them unattractive for fine mapping. One of the possible reasons for the finding of minor favorable loci alone is the long size of introgressions: beneficial alleles of *Ae. tauschii* could be still masked by many deleterious alleles located on the same chromosomal segment (Pestsova et al., 2006).

A study carried by Christopher et al. (2013) revealed 4 QTLs that were identified for the seminal root angle on chromosomes 2A, 6A, and 3D. This confirmed the significant peak on Manhattan plot detected on 3D chromosome, associated to the Root Growth Angle. In another study by Atkinson et al. (2015), 29 QTL for seedling root traits were identified in 94 DH progenies derived from a cross between two winter wheat cultivars. 22 of which were located on the D genome. Three of these 22 QTLs were all located on chromosome 3D and correlated with root angle traits. QTLs for the number of lateral roots, located on chromosomes 6D and 7D, were also reported in this thesis. For lateral root length, Atkinson et al. (2015) found several QTLs, again on chromosomes 6D and 7D. This was confirmed on the Manhattan plots reported in this thesis in which two weak peaks can be observed on chromosomes 6D and 7D. Other contributions reported by (Kabir et al., 2015) and Xie et al. (2017), reveal QTLs for root architectural characteristics in wheat as well as a large QTL for root total length on chromosome 4 which also confirms our results.

In recent years, breeding for root system traits has received increasing attention because of its importance in determining final crop yields, especially under stress conditions. In fact, in the specific case of the wheat D genome, there are several studies conducted with the aim of identifying QTLs that correspond to complex root traits, many of them performed by linkage analysis on DH (double haploids) or RIL (recombinant inbred lines) populations of wheat.

Further analysis of the 2021 data already available would be crucial to confirm these results and novel alleles could be introgressed into bread and/or durum wheat through direct crossing and synthetic hybridization.

CHAPTER 3. Resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in the elite durum wheat germplasm assessed through association mapping

3.1. Abstract

Septoria tritici blotch (STB), caused by the foliar fungal pathogen *Z. tritici*, is one of the most important threats to productivity in the Mediterranean basin, and particularly in Tunisia. Ever since its emergence in 1970 which coincided with the introduction of the commonly high-yielding durum wheat varieties that became susceptible to Septoria over a few years in Tunisia, and possibly other countries, experienced serious recurrent epidemics of STB, with yield losses reaching up to 40%. Resistance to Septoria tritici blotch may be qualitative, isolate-specific which depends on major genes or quantitative, isolate-nonspecific with polygenic inheritance. This study aimed at elucidating the genetic basis of resistance to *Z. tritici* in 160 accessions belonging to a durum wheat elite diversity panel representative of the germplasm bred in Mediterranean countries (Italy, Morocco, Spain, Syria, and Tunisia), Southwestern USA and Mexico. The STB responses of the Durum Panel accessions were assessed for Argelato (2008, 2014), Ferrara (2008, 2015) and Beja station in Tunisian for three consecutive years (2008, 2009 and 2010). The latter is located in the sub-humid bioclimatic zone, particularly known to be a hot spot for STB especially on durum wheat.

Accessions were scored for 23423 SNP markers, and genetic structure was investigated, assigning the accessions to five groups. Some of the accessions of the panel proved to be more tolerant than the best checks and differences for STB responses among the five subgroups were highly significant in all the three evaluation years. A Genome-wide association Study was performed for STB infection across the six environments using five different models (GLM, MLM, MLMM, BLINK, FarmCPU).

The preliminary GWAS highlighted numerous peaks that can be attributed to QTL of interest for resistance to Septoria tritici blotch response which could be of interest for MAS in durum wheat breeding programs, providing strategies are adopted that could effectively deal with a relatively high number of markers to accumulate and maintain these small-effect QTLs to achieve an acceptable and durable level of resistance.

3.2. Introduction

Wheat, with ca. 680 million tons global production, accounts for ca. 30 % of the global annual production obtained from the major staple food cereal crops (rice, wheat and maize). Tetraploid durum wheat (*Triticum durum* Desf.; AABB genomes) is an important crop in the Mediterranean Basin and particularly in West Asian and North African (WANA) countries where it is annually grown over more than 13 million hectares. Mediterranean countries account for approximately 75% of global worldwide durum wheat production (Belaid, 2000; Habash et al., 2009). Wheat production is affected world-wide by numerous serious biotic and abiotic threats. Estimates of potential and actual losses despite the current crop protection practices have been presented for wheat and the other major crops on a regional basis as well as globally (Oerke, 2006) the total global potential loss due to pests can spike to 30-50% for wheat.

Among the most important diseases in wheat that significantly reduce production are those caused by rusts, powdery mildew, fusarium, and the leaf blotch diseases, including Septoria leaf blotch. Septoria leaf blotch, caused by *Mycosphaerella graminicola* (asexual stage: *Septoria tritici*), is economically important in most hexaploid and tetraploid wheat growing areas of the world. Controlling the disease through fungicide treatments is economically and environmentally burdensome. Furthermore, the effectiveness of fungicides decays rapidly because of the fast evolving and unique fungal population genetics.

However, the genetic bases of the elite durum germplasm are still relatively narrow as compared to those of the corresponding elite germplasm of hexaploid wheat.

Despite its broad adaptation, durum wheat production and kernel quality under such environments are negatively affected by various fungal diseases such as Septoria tritici blotch.

Mycosphaerella graminicola has recently been reported as devastating pathogens in the sub-humid areas of the Mediterranean basin including Spain, Southern Italy, Morocco, Algeria and Tunisia, where difficulty in managing Septoria disease and yield losses ranging from 30 to 70% have been reported (Rezgui et al., 2008). Under these conditions, the early and semi-dwarf plant ideotype of the elite lines is favorable to disease development, particularly in the sub-humid regions and under irrigated farming systems. The tight quality parameters required by the international semolina and pasta market contribute to limiting the widening of genetic diversity in the elite pool.

Marker-based approaches allow breeders to identify the genes/quantitative trait loci (QTL) governing plant response to diseases. To effectively deploy new STB resistance alleles from different sources, the genetic characterization of the available germplasm is required. The standard approach for such an attempt is to construct bi-parental crosses between resistant and susceptible parents and

then phenotype and genotype (with molecular markers) progeny populations to determine the number and chromosomal location of resistance loci (Gupta et al., 1999; M. Maccaferri et al., 2008). An alternative to mapping with bi-parental crosses is association mapping (AM) or linkage disequilibrium (LD)-based mapping where genotype-phenotype correlations are searched in germplasm collections or natural populations (Flint-Garcia et al., 2003; Rafalski, 2002). The underlying principle of this approach is that LD tends to be maintained over many generations between loci that are genetically linked to one another. With AM, statistical assessments will be made for associations between genotypes based on molecular markers and phenotypes of various traits in reference germplasm sets (Buntjer et al., 2005).

Since its first use with plants a decade ago (Thornsberry et al., 2001), AM has gained wide application in many important crops due to advances in high-throughput genotyping technologies, increased interest in identifying novel alleles, and improvements in statistical methods (J. Yu et al., 2006; Zhu et al., 2008). In both tetraploid and hexaploid wheat, AM has already proven to be an effective strategy to identify marker-trait associations for agronomically valuable traits (Breseghello & Sorrells, 2006; Crossa et al., 2007; Maccaferri et al., 2011; Maccaferri et al., 2010), including also resistance to stem rust (Yu et al., 2011), leaf rust (Maccaferri et al., 2010), *stagonospora nodorum* blotch (Tommasini et al., 2007), fusarium head blight (Miedaner et al., 2011) and Soil-borne cereal mosaic virus (Maccaferri et al., 2011).

3.3. Objectives

Therefore, the objectives of this study were to use association mapping to identify genomic regions for field-based resistance to septoria tritici blotch in a durum wheat elite diversity panel.

3.4. Materials and Methods

3.4.1. Plant material

This study evaluated a durum wheat elite diversity panel ("UNIBO Durum Panel") of 160 accessions (mainly cultivars and advanced lines) representative of the germplasm bred in Mediterranean countries (Italy, Morocco, Spain, Syria, and Tunisia), Southwestern USA and Mexico (Supplementary table 1).

The accessions included in the collection were chosen from a larger pool of 330 accessions that were obtained from various sources and previously evaluated on a comparative field trial carried out in 2003 in Cadriano, near Bologna, Italy (Maccaferri et al., 2006). The accessions to be included in

this panel were chosen based on their pedigrees and morpho-physiological traits critical to adaptation, such as plant height and heading date. Accessions highly related to each other in particular siblings from the same cross and backcrossed lines with significant heading date differences, which may have influenced the phenotypic evaluation of flowering time-influenced traits (Maccaferri et al., 2010), were excluded. Most of the accessions were semi-dwarf, early to medium heading elite cultivars and advanced breeding lines released from the early '70s up to the late '90s. The collection comprises also 'founder genotypes' widely used as parents in breeding programs throughout the Mediterranean Basin and at International CGIAR Centers (CIMMYT and ICARDA). Four checks were used including Simeto (pedigree: Capeiti 8/Valnova, from the Italian germplasm) which has been the first-ranking cultivar in Italy since the early 1980s and is still widely cultivated throughout the Mediterranean Basin (Maccaferri et al., 2010). Yavaros79' was developed from 'Bittern' with pedigree Jori's'//Anhinga's'/Flamingo's' (Royo et al., 2010) and is characterized by wide adaptation. Nasr99 is known as a durum wheat variety with better resistance to Septoria (Gharbi et al., 2000). Altar89 was selected from the cross Ruff's'/Flamingo's'//Mexicali75/3/Shwa's' and is characterized by high yield potential (Royo et al., 2010).

A detailed phenotypic and molecular characterization of the panel was previously reported in Maccaferri et al. (2006 and 2010). The latter included accessions belonging to one of five main population subgroups as follows: accessions from ICARDA bred for the dryland areas (subgroup 1), from ICARDA bred for temperate areas (subgroup 2), from the Italian and early '70 CIMMYT breeding programs (subgroup 3), from CIMMYT in the late '70s-early '80s (subgroup 4), from CIMMYT in the late'80s-early '90s (subgroup 5).

3.4.2. Field phenotyping

The STB responses of the Durum Panel accessions were assessed for three consecutive years (2008, 2009 and 2010) at Beja station in Tunisia, two years for Argelato (2008 and 2014), Ferrara (2008 and 2015) in unreplicated plots including the internal checks Simeto, Yavaros_79, Altar_84, and Nasr_99. The experimental station of the CRRGC at Oued-Beja (36°44′05"N, 9°13″35"E, governorate of Beja, northwest of Tunisia) is located in the sub-humid bioclimatic zone where the average annual rainfall ranges from 500 to 850 mm and a daily mean temperature varies between 10 and 28 °C. This area is particularly known to be a hot spot for STB especially on durum wheat.

Septoria tritici blotch disease severity (DS) was visually scored plot wise as coverage of flag leaves with lesions bearing pycnidia on a scale from 1 (fully resistant) to 9 (fully susceptible). Infection types were categorized into four discrete classes: resistant (R), moderately resistant (MR), moderately

susceptible (MS) and susceptible (S). Infection responses overlapping between any two categories were recorded using a dash (e.g. MR-MS to represent overlapping between MR and MS responses). For each evaluation season, the terminal disease severity at the soft-dough stage, in coincidence with the peaks of disease severity, was considered as the most informative disease score and was therefore used to carry out the molecular-phenotype association tests. Other traits such as plant height (PH) and heading date (HD) were also taken in consideration in this study for further use as potential covariates for the genome wide studies.

3.4.3. Statistical analysis

The analysis of Variance (ANOVA), combined over years, was conducted on DS based on the mean values of the experimental units. Due to the unbalanced design, the LSMEANS (least squares means) were generated for the fixed effects. ANOVA was carried out considering seasons as replicates and using the genotype × season interaction as error variance.

The LSM values for disease severity (% of leaf area covered by pycnidia) for all isolates was calculated using the PROC MIXED procedure in SAS v9.3 (SAS Institute Inc., Cary, NC, USA) considering the genotype as a fixed effect and replication considered as random effect.

Descriptive statistics such as mean, maximum, minimum, heritability (h^2) values were calculated, the distribution frequencies were obtained. Principal component analysis and Pearson correlation coefficients were generated using R Statistical software (v4.1.3, R Core Team 2021).

3.4.4. Molecular analysis

SNP genotyping

A bulk of ca. 25 seeds from the original pure stock of each accession was germinated and grown in growth chamber at 20 ^{o}C. After 2 weeks, seedling leaves were collected, freeze-dried, grounded, and used for genomic DNA extraction. DNA extraction and other molecular procedures were carried out as previously described in Maccaferri et al. (2010).

Genotyping was processed using the Illumina iSelect 90K wheat SNP assay (S. Wang et al., 2014) and genotypes were called as described by Maccaferri et al. (2015a) , while polymorphisms were mapped based on the tetraploid wheat consensus map reported in Maccaferri et al. (2015b).

According to the SNP consensus map, up to 23,423 SNPs were informative and genetically arranged.

Population structure and Genome-wide association study

Estimating LD between markers measures whether markers segregate independently or not. The program TASSEL (http://www.maizegenetics.net) was used to calculate Linkage Disequilibrium (LD) decay among markers for the A and B genomes, and only Single Nucleotide Polymorphisms (SNPs) with minor allele frequency (MAF) > 0.05 were considered. LD decay pattern based on the consensus genetic distances was inspected considering squared allele frequency correlation (r^2) estimates from all pairwise comparisons among intra-chromosomal SNPs. If, within a chromosome region, all pairs of adjacent loci were in LD, this region was referred to as an LD block (Stich et al., 2005). Curve fit and distance at which LD decays below r^2=0.3 were used to define the confidence intervals of QTLs detected in this study using a custom script in R (The R Core Team, 2022) as described in Maccaferri et al. (2015a). SNP imputation was performed using Beagle 5 software using default parameters (Browning et al., 2018). The imputation accuracy was measured at 98.6% by running 1,000 replicates of randomly masked 1% of the called genotypes (Nothnagel et al., 2009; Hancock et al., 2012). Using the software PLINK (Chang et al., 2015), redundant markers were pruned based on genome wide linkage disequilibrium set at r2 = 0.99 and merged into one unique SNP call. Moreover, three additional pruned hapmaps were produced selecting a single SNP among those with r2 of 0.8, 0.5 and 0.3 to run the population structure analysis (Mazzucotelli et al., 2020).

Kinship based on Identity-by-State (IBS) among accessions was calculated in TASSEL5. In addition, a subset of non-redundant 12,064 SNP markers (r2 < 0.5) was used to evaluate the population structure (Q) in STRUCTURE 2.3.4. software using the corresponding tagger function in Haploview 4.2 software. Numbers of hypothetical subpopulations ranging from k = 2 to 20 were assessed using 10,000 burn-in iterations, followed by 100,000 recorded Markov-Chain iterations, in five independent runs for each k in order to estimate the sampling variance of population structure inference. The rate of change in the logarithm of the probability of likelihood [LnP(D)] value between successive k values was considered Δk statistics, (Evanno et al., 2005) together with the rate of variation (decline) in number of accessions clearly attributed to subpopulations (accessions with Q membership's coefficient ≥ 0.5). Finally, the level of differentiation among subpopulations was measured using the Fixation Index (Fst) among all possible population pairwise combinations (Condorelli et al., 2018).

Prior knowledge of the breeding populations suggested the presence of significant population structure in panel. To decrease the number of false positives, this structure was accounted for in the association test models. An optimum number of five hypothetical subgroups were chosen to obtain the

Q matrix of membership coefficients of each accession to all subgroups as reported in Maccaferri et al., 2011).

Subsequently, genome-wide scans for association mapping of loci governing STB resistance were conducted using the disease severity (DS) phenotypic data. 12,064 SNP markers with MAF > 0.05 were tested for significance of marker-trait associations using the R package GAPIT (Wang & Zhang, 2021) under two pipelines using several models. The first pipeline included the following models:

(1) the fixed general linear model (GLM), (2) the mixed linear model (MLM) plus the K kinship matrix (MLM+K), (3) multiple loci mixed model plus the kinship (K+ MLMM), (4) FarmCPU and (5) Blink.

In contrast, the second pipeline included: (1) the fixed general linear model (GLM) including the Q population structure results as covariates (Q GLM), (2) the mixed linear model (MLM) including the Q population structure results plus the K kinship matrix (Q + K MLM), (3) multiple loci mixed model including Q population structure results plus the kinship (Q + K MLMM), (4) FarmCPU + Q and (5) Blink + Q;

For a significance level of 0.05, a Bonferroni-adjusted –log P value threshold (LOD score) equal to 2.99 was used to identify marker-trait associations. An initial scan for sites highly associated to STB response showed that polymorphisms at the *PPD-A1* and *Rht-B1b* loci on chromosome 2A and 4B stood out for association. Since it is known that alleles for earliness and semi dwarfing at the *PPD* and *RHT* loci increase susceptibility because of either synchronization of phenology with disease-favorable environmental conditions or reduced vigor, the allelic states of the accessions at both *PPD-A1* and *Rht-B1b* (Achilli et al., 2022; Bentley et al., 2011; Wilhelm et al., 2013) were included in the MLM model as additional covariates.

3.5. Results

Response to septoria tritici blotch

The mean, coefficient of variation (CV), heritability (h^2) and range STB response values of the durum panel accessions for each evaluation year as well as for the averaged responses across years are reported in **Table 5**. STB infection was high across all the three evaluation years in Beja, allowing for robust phenotyping of STB response. The mean DS rating was 5.8 for B-2008, 6.3 for B-2009 and 6.7 for B-2010. The overall infection level was slightly higher for B-2010 as compared to B-2009 and B-2008. In contrast, less STB infection was observed in Italy.

Moderate to high heritability was evident across all environments, with values ranging between 0.59 and 0.96. Coefficient of variation was maximum for STB infection in Argelato (0.44) followed by Ferrara (0.37) and minimum in Beja (0.20).

Table 5. Summary statistics of septoria tritici blotch (STB) response, disease severity (DS), in a panel of 160 durum wheat elite advanced lines and cultivars evaluated across 8 environments.

	Durum Panel - DS[b]				
Environment [a]	**Mean**	**Min**	**Max**	h^2	**CV**
B-2008	5.86	1.18	8.56	0.87	0.28
B-2009	6.33	1.79	8.39	0.78	0.22
B-2010	6.72	2.21	8.90	0.96	0.29
B-mean	6.30	2.02	8.46	0.81	0.20
F-2008	3.12	1.50	7.00	0.68	0.37
F-2015	3.54	2.50	6.50	0.59	0.21
A-2008	1.88	1.00	5.00	0.66	0.44
A-2014	3.15	1.50	6.00	0.76	0.28

[a]: Field evaluation carried out in Beja (3 years), Ferrara and Argelato (2 years).

[b]: Septoria tritici blotch resistance was scored from 1 (fully resistant) to 9 (completely susceptible)

The durum panel accessions showed continuous distributions of disease severity (DS) values with minor deviations from normality (Figure 20 and 21). For each year, checks in Beja location showed STB responses in the expected ranges, with Yavaros_79 and Altar_84 characterized by a consistently susceptible response (Disease rating comprised between 6.8 and 8.9) and Nasr_99 and Simeto that showed a medium-resistant to medium susceptible response, respectively (Disease rating comprised between 3.4 and 6.0).

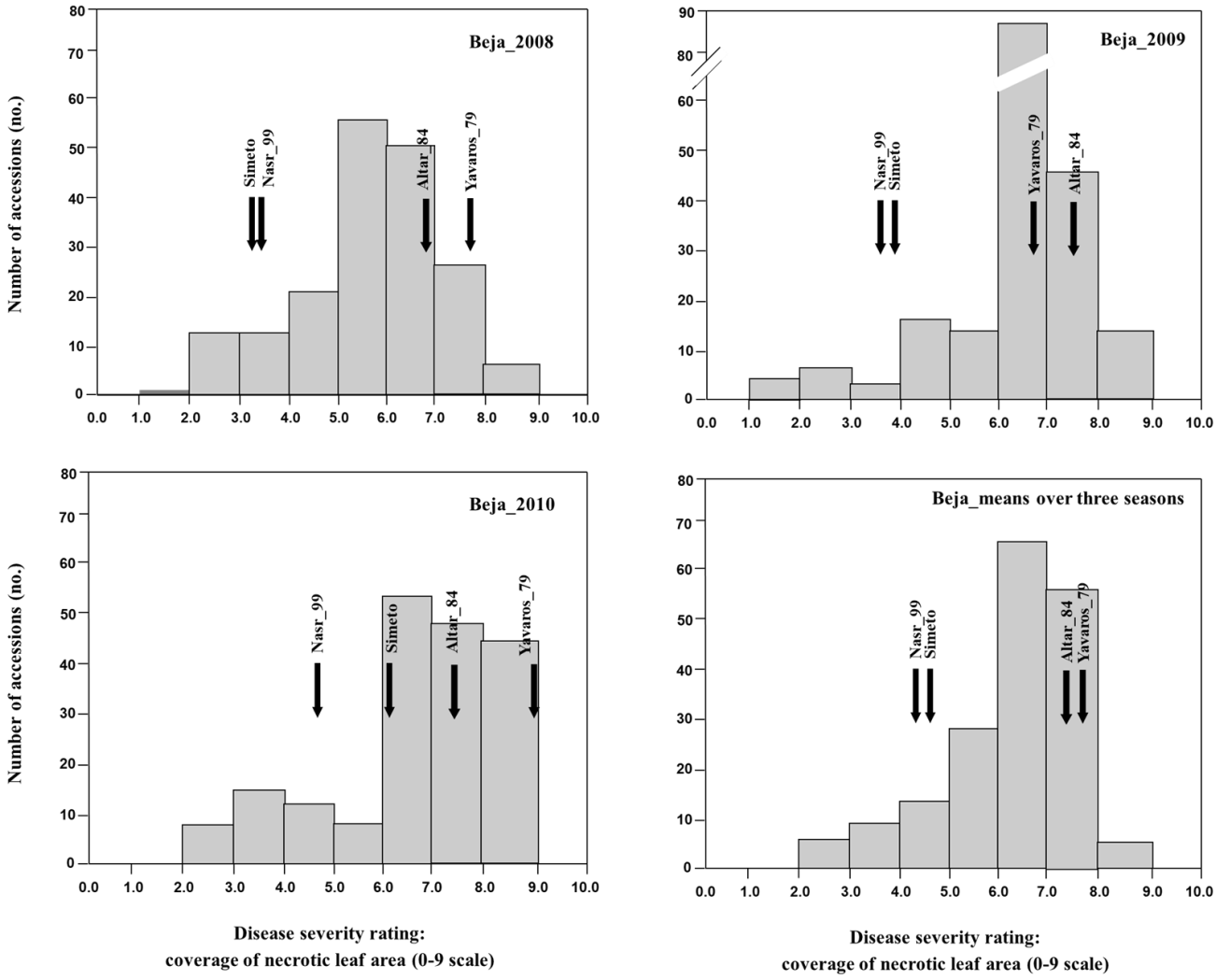

Figure 20. Distributions of disease severity values of the 160 accessions for three years and mean STB responses averaged across years in Beja, Tunisia. Black arrows indicate the four checks and their respective response to the disease.

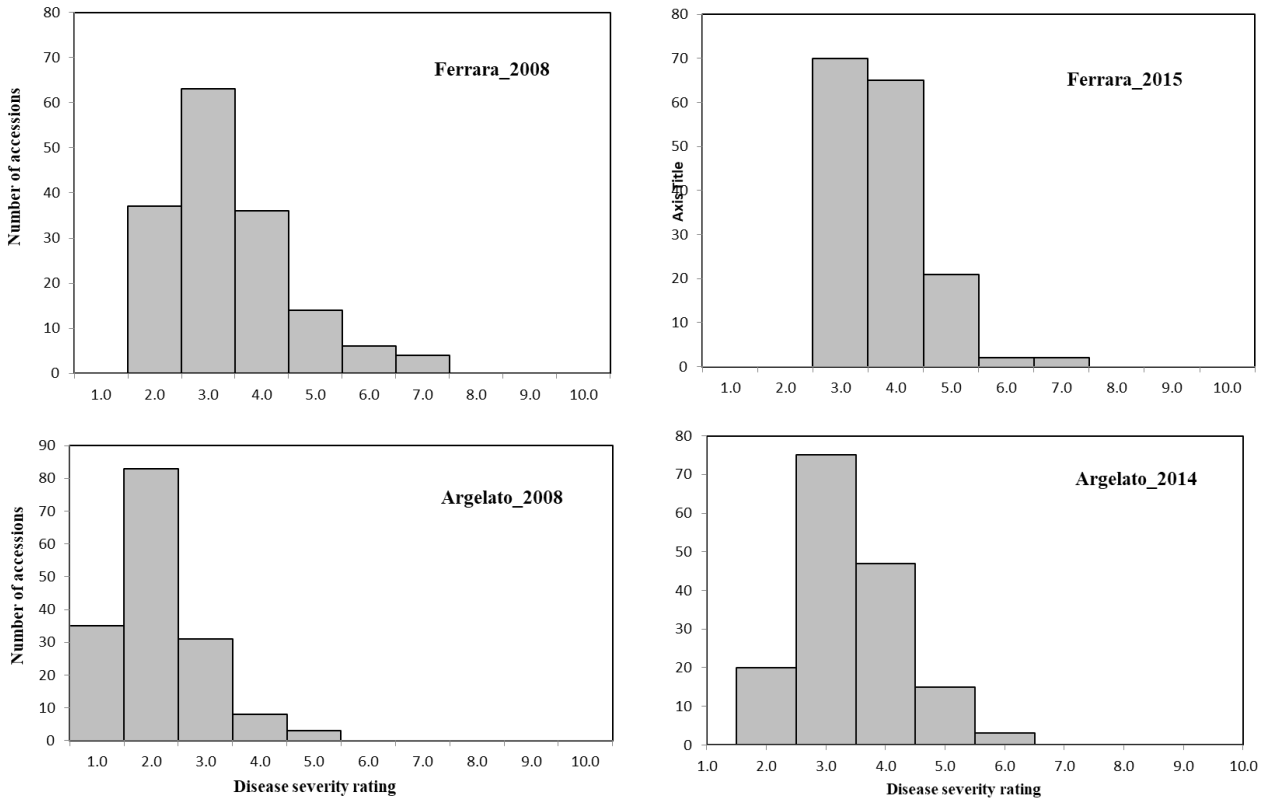

Figure 21. Distributions of disease severity values of the 160 accessions for Argelato and Ferrara across the two years. The x axis indicates disease severity scoring on a scale of 0-9 and the y-axis the number of accessions.

Principal component analysis was done to determine which of the variables more strongly contributed to the principal components. The results showed two dimensions of PCA explaining 65.3% of data variance (**Figure 22**). The first dimension accounted for 40.5% of the variances, while the second dimension accounted for 24.8% of variances. The first PC, which is the most important component, was positively correlated to SEP2008_BEJ, SEP2009_BEJ, SEP2010_BEJ and SEP_BEJALL. In contrast, the variables with the greatest weight on PC2 component were SEP2008_FER, SEP2015_FER, SEP2008_ARG and SEP2014_ARG. (**Table 6**)

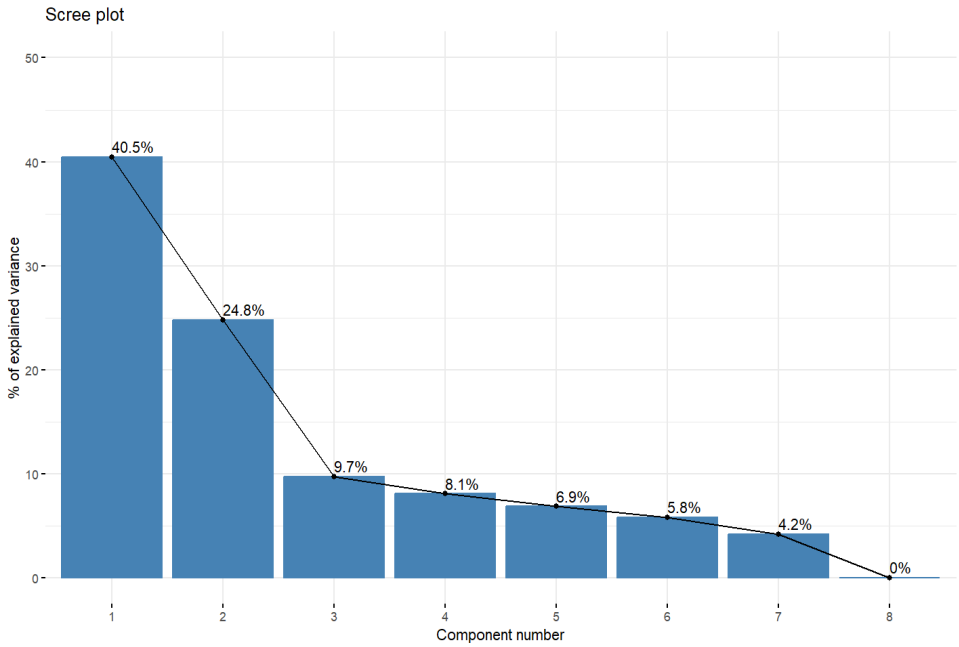

Figure 22. Screeplot of principal component analysis. The x-axis shows the principal components, which are 8 in this case. The y-axis shows the percentage of the explained variance per principal component. The elbow appears to occur at the third principal component.

Table 6. Eigenvectors of the eight principal components (PC) based on the eight variables among the durum panel.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
SEP2008_FER	-0.019	0.514	0.361	0.728	-0.071	-0.158	0.211	-0.001
SEP2015_FER	0.140	0.522	0.146	-0.396	-0.602	0.407	-0.041	-0.001
SEP2008_ARG	0.212	0.383	-0.778	0.073	-0.112	-0.396	-0.170	0.001
SEP2014_ARG	0.155	0.506	0.190	-0.353	0.740	-0.030	-0.100	0.003
SEP2008_BEJ	0.441	-0.075	-0.203	0.382	0.194	0.658	-0.188	0.329
SEP2009_BEJ	0.480	-0.092	-0.017	-0.176	-0.019	-0.160	0.781	0.306
SEP2010_BEJ	0.438	-0.165	0.400	-0.032	-0.186	-0.439	-0.519	0.351
SEP_BEJALL	0.543	-0.134	0.082	0.072	-0.005	0.015	-0.006	-0.821

Pearson correlation coefficients (at $p < 0.05$) of the phenotypic traits in the Durum panel were also evaluated (**Figure 23**). A high positive correlation was observed between Sep_Bejall and Sep_Bej2009 (0.86), same between Sep_Beja2008 and Sep_Beja2010 (0.46). For Argelato location, a positive correlation was detected between SEP2014_ARG and SEP2015_Ferrara (0.46) which shows overall that a positive correlation is present when the traits belong to the same or close environment.
On the contrary, a lower correlation coefficient can be seen between SEP2008_BEJA and SEP2008_ARG (0.26) implying that variation can differ from these two distant locations.
These wider phenotypic trait variations among the 160 accessions indicated that, the constituted association panel was suitable for association mapping.

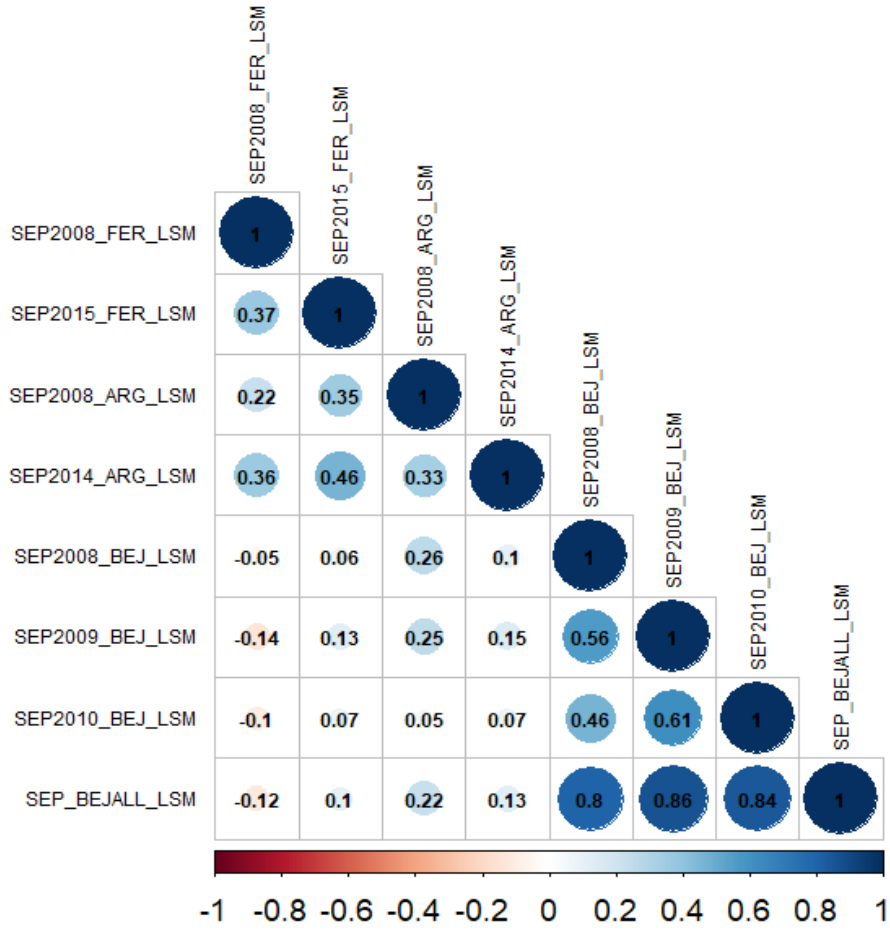

Figure 23. Correlogram showing Pearson correlation coefficients for Septoria tritici blotch response of 160 accessions of Durum wheat in 8 environments.

Population structure analysis

The population structure of all accessions included in the panel were analyzed using 12,064 SNPs that were retained after LD and MAF filtering.

The genetic structure of the durum panel was investigated by means of genetic-similarity and model-based Bayesian clustering analyses and the results have been reported elsewhere (Maccaferri et al., 2006 and 2011). It was shown that the genetic structure was accounted for by a minimum and optimum number of five distinct main subgroups, corresponding to clearly distinct breeding lineages as follows:

- The ICARDA germplasm bred for the dryland areas (subgroup S1, founder: Haurani), with founder accessions native of the West Asian countries.
- The CIMMYT60/ICARDA germplasm bred for the temperate areas (subgroup S2, founder: JoriC69)
- the late ’70s CIMMYT germplasm, widely adapted to Mediterranean conditions (subgroup S3, founders: Yavaros 79 and Karim)
- The Italian germplasm (subgroup S4, founder: Valnova)
- The late ’80s, to early ’90s CIMMYT germplasm, with increased yield potential (subgroup S5, founders: Altar 84 and Gallareta).

Based on the molecular assignment of each accession to the subgroup with the highest posterior probability, the five subgroups (from S1 to S5) included 13, 42, 60, 24 and 21 accessions, respectively. The accessions were qualitatively and quantitatively assigned to each of the five main subgroups based on the results obtained from the Bayesian analysis implemented in STRUCTURE. The membership coefficient to each of the five subgroups, averaged over all the accessions, was equal to 0.69, 0.63, 0.66, 0.73 and 0.84 from S1 to S5, respectively (**Supplementary Table 2**)

The differences for STB response among the five subgroups were highly significant in all the three evaluation years (P = 0.01 for B-2008, P = 0.001 for B-2009 and B-2010), with the among group variance component explaining 6.89, 14.01 and 15.72% of the phenotypic variation, respectively.

The effect of population structure on the STB response was also investigated by means of regression analysis. **Table 7** reports the mean and range of the accessions’ STB responses subdivided in each of the main five subgroups for each of the three evaluation years as well as for the responses averaged over years. In **Figure 24** a box-plot distribution shows the differences in STB response among subgroups for the data averaged over years. These values clearly show that all five subgroups included accessions with a wide range of response, from highly resistant (DS rating comprised between 1 and 3) to highly susceptible (DS rating higher than 7), thus indicating that all subgroups are equally informative and well-suited for AM purposes. Based on the least significant difference among

subgroups, S3, S4 and S5, which mainly included elite germplasm bred at CIMMYT, showed higher STB susceptibility than S1 and S2. S1, which included accessions with a genetic structure that mostly traced back to the native North African and West Asian germplasm, was the subgroup with the highest frequency of genotypes showing good levels of partial resistance. The complete dataset of phenotypic response and population structure membership coefficients for each of the 160 accessions included in the association panel is reported as **Supplementary Table 1**.

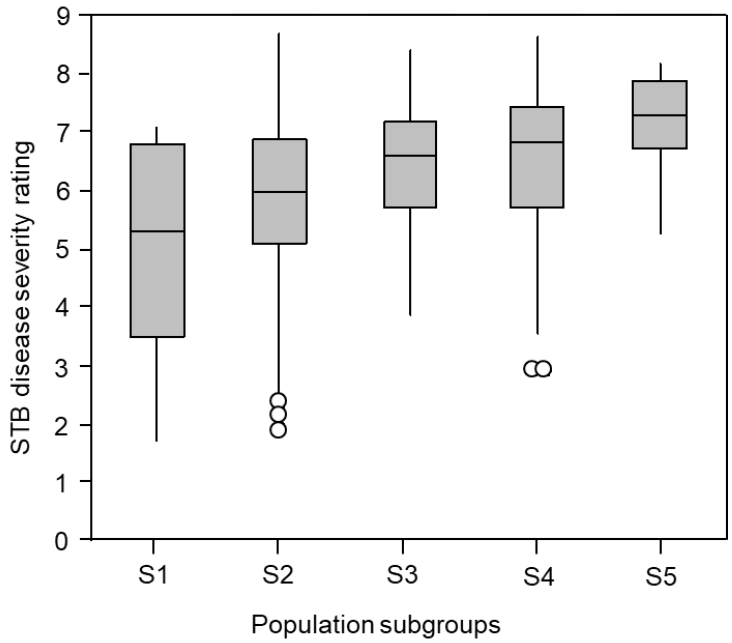

Figure 24. Boxplot distribution showing the differences in STB response among subgroups for the data averaged over years.

Table 7. Mean and range of septoria tritici blotch (STB) response (DS) in the five main germplasm subgroups of the association mapping panel.

	Subgroup 1 (S1) ICARDA drylands (13) [a]			Subgroup 2 (S2) CIMMYT60/ICARDA temperate (42)			Subgroup 3 (S3) late '70 CIMMYT (60)			Subgroup 4 (S4) Italian germplasm (24)			Subgroup 5 (S5) late '80 CIMMYT (21)		
Environment	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
B-2008	4.72	1.18	7.69	5.91	2.48	8.56	5.85	2.48	8.56	5.67	2.48	7.69	6.74	4.22	8.56
B-2009	4.87	1.79	6.84	6.56	2.96	8.39	6.39	2.96	8.39	5.92	1.79	7.62	7.06	4.51	8.39
B-2010	4.78	2.21	7.95	7.04	3.16	8.90	7.02	3.16	8.90	5.55	2.21	8.90	7.76	6.03	8.90
B-mean	4.90	2.02	6.85	6.49	3.09	8.46	6.41	3.36	8.46	5.77	2.69	7.12	7.12	5.24	7.93
F-2008	3.54	2.00	6.50	3.24	1.50	6.00	2.83	1.50	7.00	4.08	2.00	6.50	2.43	1.50	3.50
F-2015	3.62	3.00	4.50	3.58	2.50	6.50	3.42	2.50	5.00	4.10	3.00	6.50	3.12	1.50	3.50
A-2008	1.50	1.00	2.50	1.93	1.00	4.00	1.86	1.00	4.50	2.50	1.00	5.00	1.43	1.50	3.50
A-2014	2.88	2.00	4.50	3.62	2.00	5.50	2.92	1.50	6.00	3.60	2.50	5.00	2.55	1.50	3.50

[a]: number of accessions belonging to each subgroup.

Association mapping for septoria tritici blotch response

The trend of LD decay was described by a nonlinear regression of the pairwise r^2 values on the corresponding map distances based on the Illumina 9K SNP consensus map (Cavanagh et al., 2013) (**Figure 25**).

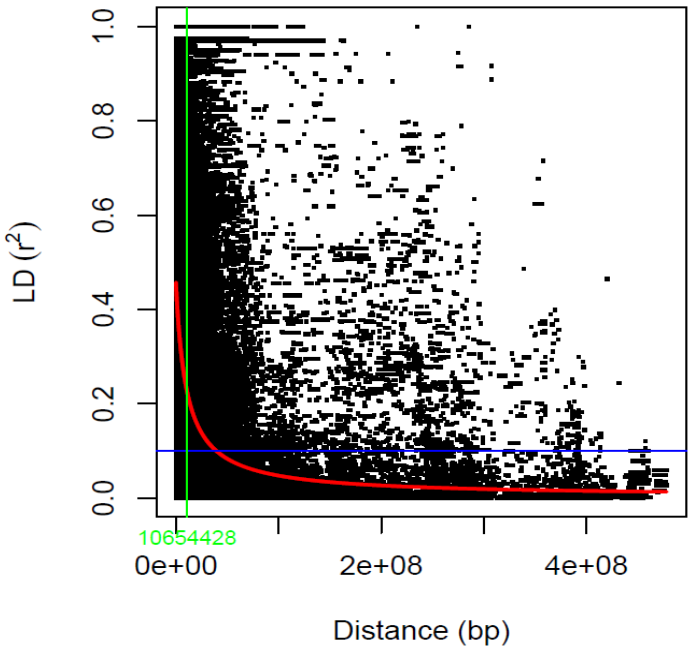

Figure 25. Genome-wide average linkage disequilibrium (LD) decay over genetic distances. (A) Plot of pair-wise single-nucleotide polymorphism LD r2 values as a function of intermarker map distance (cM) based on a reference consensus map (Cavanagh et al. 2013). The red curve represents the model fit to LD decay. The light-blue dashed line represents the confidence interval for the quantitative trait loci regions in which LD r2 = 0.3.

According to GWAS Q-Q (quantile-quantile) plot results, MLM+K+Q showed the best results to control the P-value inflation associated to population structure. Thus, all GWAS analyses were performed based on MLM+K+Q model. Additionally, relevant loci for phenology (PPD-A1, PPD-B1, FT-7A-indel, Rht-B1b and VRN-A1) were used as covariate, since these loci are strongly associated with the most

important agronomic traits influenced by wheat growth and, as such, may affect traits measured in this experiment.

The genome-wide scan with the marker-wise significance threshold set at $P = 0.05$ revealed 10 chromosome regions harboring putative QTLs for STB response. The GWAS plot depicted in **Figure 26** shows that several marker-disease response associations were detected at relatively high statistical significances (marker-wise $-\log_{10} P$ values between 3 and 6) on chromosomes 1A, 2A, 3A, 4A, 6A, 1B, 2B, 3B, 6B, 7B. Inspection of inter-marker LD between adjacent markers significantly associated to STB response showed that in several cases the multiple associated markers actually represented unique single QTL regions (based on the *P* and R^2 values and direction of the allelic effect). Such QTL regions were represented by SSR and DArT markers with inter-marker distances always comprised within 10 cM, as estimated from the durum consensus map, and LD r^2 values were higher than 0.6 in most cases. The known STB loci and QTLs for STB response previously mapped on the A- and B- genome chromosomes of *Triticum aestivum* L. have been projected, according to their published mapping intervals, on the durum consensus map used to plot the genome-wide association *P* values (**Figure 26**). Significant intervals of 20 cM have been used when such information was missing. Nine significant regions showed a tight overlap with the projected intervals of the known *Stb* and QTL loci. The nine regions included the main effect QTL regions on chr. 4A, 1B, 3B and 6B. On chr. 4AL, the two tightly associated regions tagged by wPt-1155 and wPt-0763 were located in the small interval known to harbour *Stb7*/*Stb12* in hexaploid wheat (Dreisigacker et al., 2015) as well as the *QStb.4AL-Mazurka* and *QStb.4AL-Tuareg* reported by Kelm et al., 2011. The main QTL region on chr. 1BS, tagged by *gwm762*, was coincident with the chromosome location of *Stb11* (Chartrain et al., 2005). The main QTL region is associated to *barc133* on chr. 3B, which showed the highest R^2 values in Beja, was in the same location of *Stb2* (Liu et al., 2013). Finally, the main QTL region tagged by wPt-8336 was coincident with the *QTL QStb.6BS-Tuareg* reported by Kelm et al. (2012). Among the QTL region significant at the marker-wise level, the region associated to wPt-5411 on chr. 1A overlapped with *QStb.1AS-Biscay* and *QStb.1AS-Florett* (Risser et al., 2011), one region associated to *ksum45* in the proximal region of chr. 3BS could be located close to the putative regions harboring *Stb10*/*Stb14* in hexaploid wheat (Chartrain et al., 2005; Cowling, 2006). For the markers showing significant effects at the experiment-wise level, the map locations of all the markers associated to the phenotype, together with the LD patterns (r^2 values) and the detailed association *P* and R^2 values for each season are showed in **Figure 27**.

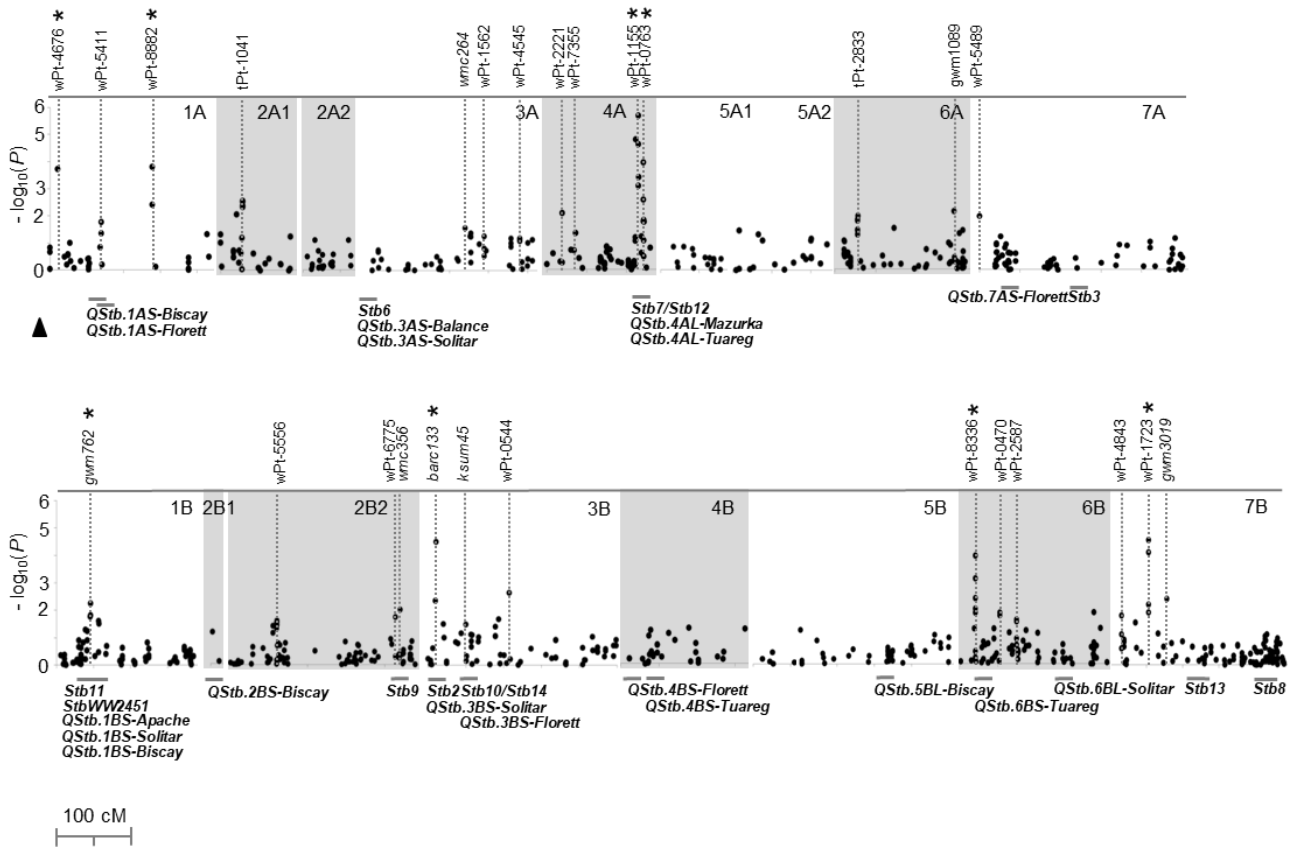

Figure 26. Association mapping probabilities, reported as -log (p), of the mapped markers tested for association to Septoria tritici blotch response of 183 elite accessions of durum wheat. Results are shown for the STB response averaged over three evaluation seasons, reported on a chromosome-by-chromosome basis. Vertical, dotted lines indicate the 27 markers tagging QTL regions with significant effects ($P < 0.05$).

The false discovery rate (FDR) multiple testing corrections was applied to identify the most valuable associations. Eight independent chromosome regions conferred putative main partial resistance effects with FDR *q* value ≤ 0.05 on the mean DS ratings averaged over years (**Table 8**) and were located on chromosomes 1AS (two QTLs), 1BS, 3BS, 4AL (two QTLs), 6BS and 7BS. Additionally, 19 unique single marker/chromosome regions that showed significant associations consistently confirmed on two to three seasons as well as on the data averaged across seasons, though at the marker-wise level only, were considered as regions potentially valuable for STB response.

Of the 27 unique associations considered as robust signals for presence of loci involved in STB response, 18 were detected as chromosome regions defined by two (or more, up to ten) adjacent marker with significant association to STB response ($P \leq 0.05$ or higher significance levels) and significant LD

($P \leq 0.05$, based on permutation test). For each of the QTLs that were identified as linkage-blocks of adjacent markers, the markers significantly associated to the phenotype were checked for consistency of their effects, the length of the chromosome region was defined in cM (QTL boundaries) and a single marker, the one most associated to STB response across seasons, was designated as the best QTL-representative marker (**Table 4**).

No single marker/QTL regions with experiment-wise significant association to STB response for one evaluation season only (season-specific association) were observed. On the contrary, numerous of these season-specific associations were observed at the marker-wise significance level ($P \leq 0.05$); however, these association signals were not considered sufficiently robust to be considered as putative STB-response QTL regions.

The QTLs with experiment-wise effects consistent across two to three seasons and on the mean data were also those with the highest R^2 values overall as well as on single seasons (**Table 8).** These QTLs showed R^2 values comprised between 4.2 and 7.8%, based on the mean data. In particular, the regions on chrs. 3BS (tagged by *barc133*), 4AL (tagged by wPt-1155) and 1BS (tagged by *gwm762*) showed the highest marker effects (R^2 values on the mean data equal to 7.8, 6.8 and 6.3%, respectively) and each of these QTLs was identified by a series of adjacent markers that supported the QTL effects. The other five exp-wise significant regions showed R^2 values (B-mean) ranging from 4.2 to 5.0%. The R^2 values of the marker-wise significant regions with effects observed over two to three seasons ranged from 1.4 to 5.7% (B-mean).

Table 8. Quantitative trait loci (QTLs) for septoria tritici blotch (STB) response identified through association mapping in a panel of 160 elite durum wheat accessions evaluated in Beja, Tunisia, with significant effects observed over at least two of the three evaluation seasons and on the mean data.

Chr.	Most associ marker	Interval (cM) [b]	Significant seasons [a]	AM test *p* value	FDR *q* value	R^2 *(%)* [c]	R^2 *range* (%) [d]	Associated markers in the QTL region
1A	wPt-4676	10.0	B-08**, B-09***, B-10**, B-mean***	$1.9\ 10^{-4}$	0.013	4.2	2.5 - 4.1	-
1A	wPt-5411	67.9-69.6-69.6	B-09**, B-10***, B-mean*	0.044	0.550	1.4	2.3 – 2.6	wPt-4886, wmc469, wPt-5411
1A	wPt-8882	138.9-138.9-139.1	B-08**, B-09***, B-10**, B-mean***	$1.6\ 10^{-4}$	0.012	4.8	2.6 – 3.9	wPt-0011, wPt-8882
1B	gwm762	38.7-45.6-56.9	B-09**, B-10*, B-mean**	0.005	0.260	**6.3**	4.2 – 7.6	gwm11, barc137, wmc85, gwm759, gwm7 gwm947, wPt-8168, gwm131, wPt-3451, barc181
2A	tPt-1041	20.2-27.7-27.7	B-08**, B-09**, B-10*, B-mean**	0.003	0.157	3.0	1.5 – 3.4	wmc667, wPt-7049, gwm636, wPt-4197, tPt-1041, wPt-5647, wPt-6245
2B2	wPt-5556	55.2-60.3-60.3	Bja-08*, B-10*, B-mean*	0.024	0.494	1.7	0.8 – 3.5	gwm429, wPt-6199, wPt-2600, wPt-5672, wPt6192, wPt-7757, wPt-4125
2B2	wPt-6775	213.7	Bja-09**, B-10*, B-mean**	0.010	0.402	2.1	1.3 – 1.9	-
2B2	wmc356	220.0	Bja-09**, B-10*, B-mean**	0.010	0.360	4.1	1.6 – 6.1	-
3A	wmc264	110.5-119.6 -127.1	Bja-08*, B-09*, B-mean*	0.027	0.509	3.6	2.8 – 5.1	wmc428, wmc264, wPt-2202, wPt-8362
3A	wPt-1562	143.9	Bja-08*, B-10*, B-mean*	0.054	0.553	1.3	1.6 – 2.4	-
3A	wPt-4545	178.6-189.6-189.6	Bja-09*, B-10*, B-mean*	0.052	0.573	1.3	1.1 – 1.4	wPt-5125, wPt-7492, wPt-5133, wPt-3978, wPt-4545, wPt-8876, wPt-1888

Table 8. (continued)

Chr.	Most associated marker	Interval [b]	Significant seasons [a]	AM test *p* value	FDR *q* value	R^2 *(%)* [c]	R^2 *range* (%) [d]	Associated markers in the QTL region
3B	barc133	5.9-10.5-20.0	B-08***, B-09*, B-10**, B-mean***	$3.2\ 10^{-5}$	0.005	**7.8**	1.9 – 8.9	gwm1034, wPt-9012, barc133, CSSR7
3B	ksum45	43.4-49.7-49.7	B-09*, B-10*, B-mean*	0.033	0.536	2.4	1.8 – 2.3	wPt-4842, ksum45
3B	wPt-0544	107.3	B-08*, B-09**, B-10*, B-mean**	0.002	0.144	3.3	1.5 – 1.8	-
4A	wPt-2221	19.1	B-08*, B-10**, B-mean**	0.008	0.212	2.7	2.4 – 2.9	wPt-1355
4A	wPt-7355	35.7	B-08*, B-10*, B-mean*	0.045	0.553	1.5	1.2 – 2.8	-
4A	wPt-1155	111.0-115.1-118.6	B-08***, B-09***, B-10***, B-mean***	$2.0\ 10^{-6}$	$0.6\ 10^{-3}$	**6.8**	3.8 – 7.1	wPt-5055, wPt-0798, wPt-7354, wPt-9418, wPt-1155, wPt-4424, wmc219,
4A	wPt-0763	121.3-121.3-122.1	B-08***, B-09**, B-10**, B-mean***	$1.0\ 10^{-4}$	0.011	4.9	2.1 – 4.6	wPt-6176, wPt-0763, wPt-3729, wPt-7876, wPt-6390, wPt-1007
6A	tPt-2833	27.2-27.5-27.5	B-08*, B-09*, B-mean**	0.010	0.400	2.4	1.4 – 2.7	wPt-5652, wPt-7616, tPt-0877, wPt-6904, rPt-9065
6A	gwm1089	143.6	B-08*, B-09**, B-10*, B-mean**	0.007	0.298	5.7	4.2 – 5.5	-
6B	wPt-8336	19.4	B-08*, B-09***, B-10*, B-mean***	$1.1\ 10^{-4}$	0.011	4.6	2.1 – 6.3	wPt-2991, wPt-3304, wPt-1852, wPt-3116, wPt-8336
6B	wPt-0470	51.6-64.5-64.5	B-08*, B-09*, B-mean*	0.017	0.400	2.1	1.6 – 1.8	wPt-2297, wPt-0470, wPt-7540
6B	wPt-2587	73.9-74.2-74.2	B-08**, B-09*, B-mean*	0.025	0.500	1.9	1.2 – 2.2	wPt-9971, wPt-2479
7A	wPt-5489	0.0	B-08***, B-09*, B-mean*	0.010	0.360	2.1	1.2 – 5.5	-
7B	wPt-4843	15.6	B-09**, B-10*, B-mean*	0.016	0.400	2.1	1.4 – 2.4	wPt-0837
7B	wPt-1723	52.7-52.9-66.1	B-08***, B-09**, B-10*, B-mean***	$7.8\ 10^{-5}$	0.005	5.0	3.1 – 5.1	wPt-7064, wPt-8390, wPt-8283

7B	gwm3019	77.9	B-08*, B-09*, B-10*, B-mean**	0.004	0.194	4.2	2.4 – 2.9	-

[a]: Seasons with significant marker-trait associations

[b]: Interval width of the QTL most associated marker as from the durum consensus map used as reference.

[c]: R^2 value for the marker most associated with the QTL (averaged over the four evaluation seasons)

[d]: range of percentage of the phenotypic variation (R^2) for STB responses observed across the three seasons with significant marker-trait association.

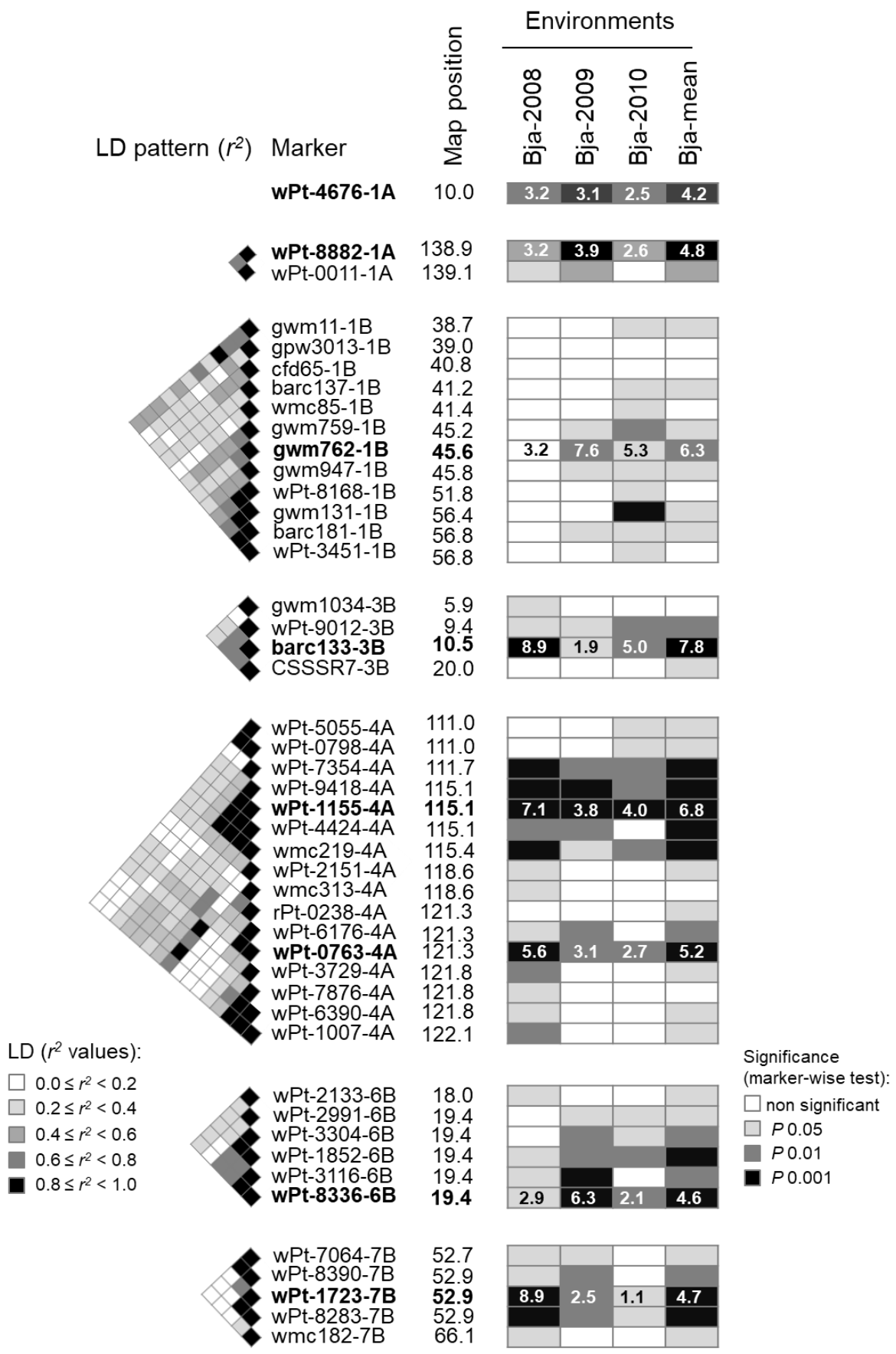

Figure 27. Map locations of all the markers associated to the phenotype, pairwise linkage disequilibrium (LD) between 9 significant markers, and the detailed association P and R^2 values for all environments. Color gradient represents LD as $r^{2.}$

3.6. Discussion

There is a growing interest in applying association mapping (AM) to a wide range of crops to identify QTLs responsible for variation of quantitative traits with agricultural and evolutionary importance (Ersoz et al., 2009; Hall et al., 2010; Stich & Melchinger, 2010). Accordingly, a better understanding of the genetic basis underlying the naturally occurring genetic diversity for STB response in durum wheat could help accelerate the breeding process for enhancing disease resistance of this crop while shedding light on the evolution of the host-pathogen relationships.

The panel of accessions evaluated in this study surveys the genetic variation present in elite germplasm pool commonly used by durum breeders, a feature that makes these results more readily transferable to and more valuable for pre-breeding activities. The few landrace selections that were included were sampled among the founders that made a strong contribution to the development of the modern germplasm. Several reasons led to this decision. First and foremost, the presence in the elite germplasm of linkage disequilibrium which extends over rather long distances as shown in Maccaferri et al. (2006 and 2011) allowed us to conduct a genome-wide scan with an average marker density matching the genotyping capacity allowed by the marker systems currently available for durum wheat, mainly SSR and DArT markers (Maccaferri et al. 2003, Mantovani et al. 2008).

The high phenological homogeneity of the elite materials and the lack of information regarding the presence of useful loci for quantitative, durable STB field resistance in durum wheat germplasm were additional reasons (Marco Maccaferri et al., 2006) as opposed to landrace accessions, which enables a more accurate and meaningful evaluation of the disease responses.

It is known that the elite durum wheat germplasm is relatively poor in sources of Septoria tritici resistance loci under field conditions as demonstrated by the very small fraction of clearly resistant materials observed in our study, which includes materials from the most important durum wheat breeding programs worldwide. Deployment of alternative sources of resistance such as minor genes with quantitative and additive effects whose beneficial alleles confer partial resistance has been advocated for improving resistance to all STB races. Given the quantitative and diverse nature of the loci conferring partial resistance, the beneficial alleles can be cumulatively selected *via* marker-assisted selection towards the release of more durable, adult-plant resistant cultivars.

As to the identification of STB resistance QTLs, most of the interest is concentrated on the genotypes consistently scored as medium resistant (MR) to medium susceptible (MS with DSS comprised between 10 and 40% of infected leaf area) across multiple seasons. The observation that MR accessions represented a rather sizeable portion of the association panel (40% of the total) and were present in all the five main subgroups, considered together with the normal distribution of phenotype frequency

suggests the validity of genome-wide association studies (GWAS) for identifying STB response QTLs in this germplasm collection.

The joint Q GLM and Q + K MLM association analyses highlighted several chromosome regions putatively harbouring QTLs with main effects on STB response in the field. Multiple-test corrections led to an excessively restrictive selection of the significant regions, particularly for an exploratory analysis like that reported in this study. Furthermore, loci conferring partial resistance could be present in the germplasm with alleles characterized by relatively small and environment- specific effects. Therefore, it was decided to report the most significant chromosome regions based on the less stringent marker-wise significance test, provided that the associations were significant over two or more seasons as well as on the averaged data. This allowed us to sort the QTLs showing the main and most stable effects, even though the experiment-wise significance was not reached.

Several QTLs identified in this study co-located at previously reported major *Stb* loci as well as to several QTLs recently identified through AM in hexaploid wheat. Moreover, QTLs uncovered on chromosome 1B, 3B, 4A and 6B were not reported elsewhere. These results highlight the effectiveness of AM to dissect the genetic basis of moderately complex traits while showing its potential to uncover the presence of previously unknown QTLs, provided that an appropriately balanced set of accessions are evaluated. The development of series of near isogenic stocks at the chromosome 1B region should be pursued to further characterize molecularly and phenotypically the favorable allele present in the elite germplasm at this region.

QTL effects estimated via AM are usually considerably lower than those estimated in biparental populations because the genetic diversity at the effector loci explored in AM panels is usually higher than that present in bi-parental mapping populations where the number of segregating effector loci is lower, and the effects are magnified by the relatively homogeneous genetic background. The identification of many QTLs with small effects is consistent with the model of complex traits that predicts an exponential decay of QTL effects with very few QTL having large effects (Robertson & Brink, 1967). Further, the rather large population size typical of AM studies typically provides estimates of QTL effects more accurate than those estimated with small population sizes of biparental linkage studies, often considerably inflated (Beavis, 2019; Melchinger et al., 1998). The elite breeding germplasm of durum wheat, in the past decades, has not been improved by means of an extensive use of wide crosses to introgress alleles with strong phenotypic effects (Maccaferri et al., 2005) as it has been the case with hexaploid wheat. Lastly, the effect of the marker is a function of the effect of the QTL and the LD between the marker and the QTL and insufficient marker density could lead to markers that are in low LD with the QTL.

The availability of high-density SNP platforms with thousands of highly multiplexed assays will soon allow researchers to carry out studies with nearly complete genome coverage and the ability to switch from single-marker to haplotype-based analyses, thus enabling to fully exploit the potential of AM in wheat. (Akhunov et al., 2009; Trebbi et al., 2011; Yu et al., 2011). Furthermore, the use of the same SNP assays in applied breeding programs will also facilitate the simultaneous selection of multiple beneficial alleles for partial resistance.

If the relatively small R^2 estimates are accurate for the QTLs herein reported, then this has important implications for the application of MAS. In contrast to large-effect QTLs that are both easier to identify and maintain in breeding populations through phenotypic selection, the small-effect QTLs identified in this study are more likely to be lost from breeding populations without the use of markers. Thus, MAS strategies that could effectively deal with a relatively high number of markers and haplotypes (use of highly multiplexable SNP assays, (Akhunov et al., 2009) may be required to accumulate and maintain these small-effect QTLs in order to achieve an acceptable and durable level of resistance for *Zymoseptoria tritici* resistance within durum breeding populations.

The results of this study showed that elite germplasm of durum wheat contained valuable genetic variation that could be used to improve adult plant resistance to Septoria tritici blotch. Across the seasons, putative QTLs involved in the STB response were consistently found in several chromosome regions with a high infection rate. The putative genomic regions associated with STB resistance identified in this study could be of interest for MAS in durum wheat breeding programs, thus making conventional breeding faster and more efficient. Comparison of the effects among different alleles at the same locus will identify the most beneficial alleles to be used in MAS. These results demonstrate that genome-wide association studies in durum wheat complement and enhance the information from linkage-based QTL studies toward the implementation of MAS.

General conclusions

In 2050, the world's population is expected to rise by nearly 25%, with the majority of that increase occurring in developing nations. This adamant growing population will continue to push the global demand for food ever higher. On the other hand, climate change, increased urbanization, conflicts and abiotic/biotic stresses may pose an additional threat to global food production and food security in the near future. In this connection, geneticists and breeders are asked to provide new varieties with improved ability to respond to the changing environment. This project showed the presence of variability in germplasm for all traits investigated. Favorable alleles have been found both in wild relatives and in diversity panels of durum wheat. Valuable information was obtained both for basic agronomic traits and for traits responsible for adaptation to climate change, such as root traits. The analyses didn't reveal major genes controlling any of the investigated traits, thus suggesting that all of them are under complex genetic control. For this reason, it is advisable that the new breeding techniques are devised to take this complexity into account. Since wheat represents a main source of food, disease control is extremely important, and the increasing difficulties in controlling *Z. tritici* require more effective wheat breeding. New genes for long-lasting host resistance are required, and the recent cloning of the first STB resistance factor is a significant step toward the understanding of STB resistance genes and their effects. Nevertheless, incorporating wild relatives alleles in durum wheat breeding for enhancing genetic diversity, improving resistance to diseases and to major abiotic stresses have proven to be promising and can significantly increase the fitness of modern cultivars against *Z. tritici* and preserve their high yield potential.

APPENDIX

Supplementary Table 1. List of the 160 accessions belonging to the elite durum wheat germplasm collection with registration details.

Code	Sample Name	Breeding program
DP002	CANNIZZO	Southern_Italy_breeding70(Valnova_Mexicali_Grazia)
DP003	CLAUDIO	Southern_Italy_breeding70(Valnova_Mexicali_Grazia)
DP004	LESINA	SouthernItaly group (Creso founders)
DP005	MERIDIANO	N/A
DP006	MONGIBELLO	Southern_Italy_breeding70(Valnova_Mexicali_Grazia)
DP007	NORBA	CIMMYT80/90_related
DP008	PIETRAFITTA	CIMMYT70_related
DP010	TORREBIANCA	Southern_Italy_breeding70(Valnova_Mexicali_Grazia)
DP011	CIMMYT-23(BISU_1/PATKA_3)	CIMMYT80/90_related
DP012	CIMMYT-36(CMH82A.1062/3/GG0VZ394//SBA81/PLC/4/AAZ…)	CentralFrance_NorthItaly
DP013	CIMMYT-41(DUKEM/3/RUFF/FGO//YAV79)	CIMMYT70_Svevo
DP015	CIMMYT-52(KULRENGI-BALIKCIL_8)	CIMMYT80(Altar84)
DP016	CIMMYT67-PLATA16	CIMMYT80(Altar84)
DP017	CIMMYT73-PORTO5	N/A
DP018	CIMMYT-78(ROK/FGO//STIL/3/BISU_1)	CIMMYT80(Altar84)
DP020	CIMMYT-108(ACUATICO/YAZI_1)	CIMMYT80(Altar84)
DP021	CIMMYT-136(FOCHA_1/5*ALAS)	CIMMYT80(Altar84)
DP023	CIMMYT-198(BUSHEN_4/TARRO_2//BUSHEN4)	CIMMYT80/90_related
DP024	CIMMYT-222(GS/CRA//SBA81/3/HO/MEXI_1/5/MEMO/6/2*…)	CIMMYT80(Altar84)
DP025	CIMMYT-247(RASCON_37/2*TARRO_2)	CIMMYT80(Altar84)
DP027	CIMMYT266	CIMMYT90_recent
DP028	ALDEANO	CentralFrance_NorthItaly
DP029	ARIESOL	CIMMYT60-earlyICARDATemperate(Cocorit71_Cham1)
DP030	ARTENA	CIMMYT60-earlyICARDATemperate(Cocorit71_Cham1)
DP031	ASTIGI	CIMMYT90_recent

DP032	BOABDIL	CIMMYT70_Svevo
DP033	BOLENGA	CentralFrance_NorthItaly
DP034	BOLIDO	CIMMYT70_Svevo
DP035	BOLO	CIMMYT80/90_related
DP036	BOMBASI	CIMMYT80(Altar84)
DP037	BORLI	CIMMYT70(YavarosC79_Karim_Duilio_ICARDAtemperate)
DP038	CANYON	ICARDA_temperate_recent
DP039	DURCAL	CIMMYT70_Svevo
DP040	DUROI	CIMMYT70_Svevo
DP041	GALLARETA	CIMMYT80(Altar84)
DP042	ILLORA	CIMMYT80/90_related
DP044	SENADUR	various_program_highAdmixture
DP045	SULA	CIMMYT80(Altar84)
DP047	1805	CIMMYT70_related
DP048	1807	various_program_highAdmixture
DP049	1808	CIMMYT60-earlyICARDATemperate(Cocorit71_Cham1)
DP050	1809	various_program_highAdmixture
DP053	JAWHAR	CIMMYT70(YavarosC79_Karim_Duilio_ICARDAtemperate)
DP054	MARJANA	CIMMYT80(Altar84)
DP055	MARZAK	CIMMYT80/90_related
DP056	OURGH	CIMMYT70(YavarosC79_Karim_Duilio_ICARDAtemperate)
DP057	TAREK	ICARDA_temperate_recent
DP060	AW12/BIT	ICARDA-Dryland(Haurani_Omrabi_Syrian_Gidara)
DP061	BIC/3/CHAM1//GRA//STK	ICARDA_temperate_recent
DP062	CHABA/DERAA	ICARDA_temperate_recent
DP063	CHACAN	CIMMYT70_related
DP064	KARIM	CIMMYT70(YavarosC79_Karim_Duilio_ICARDAtemperate)
DP065	H.MOUL(MOR)/CHABA88	N/A
DP066	KRS/HAUCAN	CIMMYT60-earlyICARDATemperate(Cocorit71_Cham1)
DP067	LAGOST3	N/A
DP069	OMBAR	ICARDA_temperate_recent
DP071	OMRABI5	ICARDA-Dryland(Haurani_Omrabi_Syrian_Gidara)
DP072	QUAD//ERP/MAL/3/UNKN	ICARDA_temperate_recent
DP073	SEBAH	CIMMYT60-earlyICARDATemperate(Cocorit71_Cham1)
DP074	STOJOCRI-3	ICARDA_temperate_recent
DP075	ZEINA1	N/A
DP076	ANTON	Southern_Italy_breeding70(Valnova_Mexicali_Grazia)
DP077	APPIO	Southern_Italy_breeding70(Valnova_Mexicali_Grazia)
DP079	ARCANGELO	SouthernItaly group (Creso founders)
DP080	ARCOBALENO	CIMMYT80(Altar84)

DP081	BRAVADUR	DesertDurum
DP082	BRONTE	CIMMYT80(Altar84)
DP083	CAPEITI8	N/A
DP085	CICCIO	SouthernItaly group (Valnova-Capeiti-Cappelli founders)
DP086	COLORADO	DesertDurum
DP087	COLOSSEO	SouthernItaly group (Creso founders)
DP088	CORTEZ	various_program_highAdmixture
DP089	CRESO	SouthernItaly group (Creso founders)
DP090	DONPEDRO	CIMMYT70_related
DP091	DUILIO	CIMMYT70(YavarosC79_Karim_Duilio_ICARDAtemperate)
DP093	FLAMINIO	CIMMYT70_related
DP094	FORTORE	SouthernItaly group (Valnova-Capeiti-Cappelli founders)
DP095	GARGANO	SouthernItaly group (Valnova-Capeiti-Cappelli founders)
DP096	GRAZIA	Southern_Italy_breeding70(Valnova_Mexicali_Grazia)
DP097	IRIDE	CIMMYT80(Altar84)
DP098	ITALO	various_program_highAdmixture
DP099	IXOS	CentralFrance_NorthItaly
DP100	KRONOS	various_program_highAdmixture
DP102	MESSAPIA	CIMMYT60-earlyICARDATemperate(Cocorit71_Cham1)
DP103	MEXICALI75	CIMMYT80/90_related
DP104	MOHAWK	various_program_highAdmixture
DP105	OFANTO	SouthernItaly group (Valnova-Capeiti-Cappelli founders)
DP106	PLATANI	SouthernItaly group (Valnova-Capeiti-Cappelli founders)
DP107	PLINIO	SouthernItaly group (Creso founders)
DP108	PRODURA	CIMMYT60-earlyICARDATemperate(Cocorit71_Cham1)
DP109	REVA	various_program_highAdmixture
DP110	ROQUENO	CIMMYT60-earlyICARDATemperate(Cocorit71_Cham1)
DP111	SVEVO	CIMMYT70_Svevo
DP112	TRINAKRIA	SouthernItaly group (Valnova-Capeiti-Cappelli founders)
DP113	VALBELICE	SouthernItaly group (Valnova-Capeiti-Cappelli founders)
DP114	VALNOVA	Southern_Italy_breeding70(Valnova_Mexicali_Grazia)
DP116	WESTBRED881	N/A
DP117	WESTBREDTURBO	N/A
DP118	AGHRASS-1	ICARDA_temperate_recent
DP119	AINZEN-1	ICARDA_temperate_recent
DP120	ANGRE	Southern_Italy_breeding70(Valnova_Mexicali_Grazia)

DP121	AMEDAKUL-1	ICARDA_temperate_recent
DP122	AMMAR-1	ICARDA_temperate_recent
DP123	ARISLAHN-5	ICARDA_temperate_recent
DP124	ATLAST-1	CIMMYT70_related
DP125	AUS-1	ICARDA_temperate_recent
DP126	AWALI-1	Southern_Italy_breeding70(Valnova_Mexicali_Grazia)
DP127	RADIOSO	SouthernItaly group (Creso founders)
DP128	AZEGHAR-2	ICARDA-Dryland(Haurani_Omrabi_Syrian_Gidara)
DP130	BICRE	ICARDA_temperate_recent
DP131	BICREDERAA-1	CIMMYT60-earlyICARDATemperate(Cocorit71_Cham1)
DP132	BIGOST-1	CIMMYT70_related
DP133	BLK2	ICARDA_temperate_recent
DP134	BRACHOUA	ICARDA_temperate_recent
DP135	CHABHA88	N/A
DP136	CHAM-1	CIMMYT60-earlyICARDATemperate(Cocorit71_Cham1)
DP137	DERAA	cimmyt60-earlyICARDATemperate(Cocorit71_Cham1)
DP139	GEROMTEL-1	ICARDA_temperate_recent
DP140	GEZIRA17	N/A
DP141	GIDARA-2	ICARDA-Dryland(Haurani_Omrabi_Syrian_Gidara)
DP142	GUEROU-1	ICARDA_temperate_recent
DP144	HAURANI	LandracesTurkishMediterranean
DP145	HEIDER	CIMMYT60-earlyICARDATemperate(Cocorit71_Cham1)
DP146	ICARDA121(Ouassel-1/4/Buc/Chrc//Prl/3/Pvn/5/Hel/3/Bit/Corm//Shwa)	N/A
DP147	SEBOU	CIMMYT60-earlyICARDATemperate(Cocorit71_Cham1)
DP149	ICARDA78(Aric31708.70/3/Bo//C.deChile/Br/4/Cit/Gta)	N/A
DP150	JORDAN	CIMMYT80_related
DP151	KABIR1	CIMMYT70_related
DP153	KHABUR-1	ICARDA_temperate_recent
DP154	KRF	Southern_Italy_breeding70(Valnova_Mexicali_Grazia)
DP155	LAGONIL-2	ICARDA_temperate_recent
DP156	LAHN	CIMMYT70(YavarosC79_Karim_Duilio_ICARDAtemperate)
DP157	LOUKOS-1	CIMMYT70(YavarosC79_Karim_Duilio_ICARDAtemperate)
DP158	MAAMOURI-1	ICARDA_temperate_recent
DP159	MARSYR-1	ICARDA_temperate_recent
DP160	MASSARA-1	ICARDA-Dryland(Haurani_Omrabi_Syrian_Gidara)
DP161	MIKI-1	ICARDA_temperate_recent
DP163	MURLAGOST-1	cimmyt60-earlyICARDATemperate (Cocorit71_Cham1)

DP164	NILE	CIMMYT70_Svevo
DP166	OMGENIL-3	ICARDA-Dryland(Haurani_Omrabi_Syrian_Gidara)
DP167	OMLAHN-3	ICARDA-Dryland(Haurani_Omrabi_Syrian_Gidara)
DP168	OMRUF-2	CIMMYT70(YavarosC79_Karim_Duilio_ICARDAtemperate)
DP169	OMSNIMA-1	ICARDA-Dryland (Haurani_Omrabi_Syrian_Gidara)
DP170	ORT-1	CIMMYT80/90_related
DP171	OTB-6	ICARDA_temperate_recent
DP172	OUASERL-1	ICARDA_temperate_recent
DP173	OUASLAHN-1	CIMMYT60-earlyICARDATemperate (Cocorit71_Cham1)
DP175	QUABRACH-1	ICARDA_temperate_recent
DP176	QUADALETE	ICARDA_temperate_recent
DP177	RAZZAK(TUN)	N/A
DP178	SAADA3/DDS//MTL1	various_program_highAdmixture
DP179	SAJUR	CIMMYT80/90_related
DP182	TELSET-5	LandracesTurkishMediterranean
DP183	TENSIFT-1	ICARDA_temperate_recent
DP184	TERBOL97-3	ICARDA_temperate_recent
DP185	TUNSYR-1	ICARDA_temperate_recent
DP186	WADALMEZ-1	ICARDA_temperate_recent
DP187	YOUNES-1	ICARDA-Dryland (Haurani_Omrabi_Syrian_Gidara)
DP188	YOUSEF-1	ICARDA_temperate_recent
DP189	KOFA	DesertDurum

Supplementary table 2. Genetic structure of the UNIBO-Durum Panel accessions determined through model-based clustering in STRUCTURE software. Subpopulation 1 (S1) included thirteen accessions from ICARDA breeding programs for dryland areas; Subpopulation 2 (S2) included forty-two accessions from CIMMYT60/ICARDA germplasm bred for the temperate areas; Subpopulation 3 (S3) included sixty accessions characterized as late 1970s CIMMYT germplasm, widely adapted to Mediterranean conditions; Subpopulation 4 (S4) included twenty-four accessions from the italian germplasm, and Subpopulation 5 (S5) included twenty-one accessions from late 1980s to early 1990s CIMMYT accessions, with high yield potential.

Accession code	**Accession name**	**S1**	**S2**	**S3**	**S4**	**S5**
		ICARDA-Dryland	ICARDA temperate	CIMMYT70	Italian	CIMMYT 80
DP060	AW12/BIT	1.000	0.000	0.000	0.000	0.000
DP071	OMRABI5	1.000	0.000	0.000	0.000	0.000
DP160	MASSARA-1	1.000	0.000	0.000	0.000	0.000
DP187	YOUNES-1	0.882	0.000	0.053	0.000	0.065
DP167	OMLAHN-3	0.773	0.000	0.007	0.000	0.220
DP182	TELSET-5	0.686	0.146	0.022	0.109	0.037
DP166	OMGENIL-3	0.593	0.000	0.399	0.000	0.007
DP083	CAPEITI8	0.585	0.000	0.000	0.415	0.000
DP169	OMSNIMA-1	0.578	0.104	0.303	0.000	0.014
DP113	VALBELICE	0.543	0.000	0.000	0.457	0.000
DP141	GIDARA-2	0.516	0.245	0.142	0.000	0.096
DP144	HAURANI	0.455	0.261	0.000	0.264	0.020
DP128	AZEGHAR-2	0.332	0.190	0.286	0.116	0.076
DP029	ARIESOL	0.000	1.000	0.000	0.000	0.000
DP147	SEBOU	0.000	1.000	0.000	0.000	0.000
DP110	ROQUENO	0.020	0.980	0.000	0.000	0.000
DP149	ICARDA78	0.000	0.972	0.028	0.000	0.000
DP030	ARTENA	0.030	0.970	0.000	0.000	0.000
DP116	WESTBRED881	0.000	0.807	0.000	0.193	0.000
DP081	BRAVADUR	0.000	0.761	0.000	0.239	0.000
DP136	CHAM-1	0.000	0.756	0.083	0.091	0.070
DP044	SENADUR	0.000	0.746	0.000	0.175	0.079
DP077	APPIO	0.000	0.719	0.000	0.243	0.037
DP120	ANGRE	0.000	0.698	0.000	0.223	0.079
DP028	ALDEANO	0.073	0.671	0.128	0.129	0.000
DP189	KOFA	0.000	0.662	0.000	0.338	0.000
DP102	MESSAPIA	0.095	0.661	0.244	0.000	0.000
DP039	DURCAL	0.000	0.657	0.327	0.000	0.016
DP086	COLORADO	0.009	0.656	0.000	0.335	0.000
DP055	MARZAK	0.147	0.655	0.199	0.000	0.000
DP109	REVA	0.008	0.652	0.197	0.116	0.027
DP034	BOLIDO	0.062	0.649	0.237	0.041	0.011
DP049	1808	0.082	0.642	0.274	0.000	0.002

DP033	BOLENGA	0.052	0.633	0.133	0.182	0.000
DP111	SVEVO	0.000	0.628	0.312	0.000	0.060
DP137	DERAA	0.000	0.625	0.247	0.071	0.057
DP145	HEIDER	0.063	0.603	0.334	0.000	0.000
DP100	KRONOS	0.000	0.600	0.059	0.282	0.059
DP104	MOHAWK	0.000	0.595	0.168	0.237	0.000
DP088	CORTEZ	0.016	0.575	0.198	0.212	0.000
DP032	BOABDIL	0.023	0.571	0.252	0.000	0.154
DP013	CIMMYT-41	0.057	0.571	0.268	0.028	0.076
DP040	DUROI	0.048	0.549	0.380	0.000	0.023
DP074	STOJOCRI-3	0.000	0.527	0.188	0.236	0.049
DP098	ITALO	0.188	0.522	0.117	0.106	0.066
DP154	KRF	0.000	0.506	0.000	0.257	0.237
DP108	PRODURA	0.000	0.475	0.376	0.149	0.000
DP103	MEXICALI75	0.000	0.406	0.000	0.351	0.242
DP179	SAJUR	0.000	0.426	0.112	0.137	0.325
DP170	ORT-1	0.000	0.445	0.046	0.114	0.395
DP012	CIMMYT-36	0.175	0.463	0.133	0.151	0.079
DP178	SAADA3/DDS	0.131	0.296	0.183	0.209	0.180
DP176	QUADALETE	0.114	0.367	0.364	0.141	0.014
DP073	SEBAH	0.100	0.453	0.376	0.071	0.000
DP050	1809	0.087	0.385	0.361	0.096	0.070
DP056	OURGH	0.000	0.000	1.000	0.000	0.000
DP064	KARIM	0.000	0.000	1.000	0.000	0.000
DP119	AINZEN-1	0.000	0.000	1.000	0.000	0.000
DP130	BICRE	0.000	0.000	1.000	0.000	0.000
DP183	TENSIFT-1	0.000	0.000	0.914	0.086	0.000
DP142	GUEROU-1	0.000	0.102	0.898	0.000	0.000
DP186	WADALMEZ-1	0.000	0.106	0.894	0.000	0.000
DP118	AGHRASS-1	0.000	0.103	0.887	0.000	0.010
DP153	KHABUR-1	0.000	0.012	0.880	0.045	0.063
DP121	AMEDAKUL-1	0.000	0.139	0.861	0.000	0.000
DP125	AUS-1	0.000	0.022	0.846	0.112	0.020
DP157	LOUKOS-1	0.000	0.000	0.836	0.000	0.164
DP171	OTB-6	0.000	0.000	0.824	0.176	0.000
DP082	BRONTE	0.006	0.000	0.812	0.182	0.000
DP135	CHABHA88	0.074	0.020	0.763	0.000	0.143
DP047	1805	0.248	0.000	0.752	0.000	0.000
DP158	MAAMOURI-1	0.000	0.142	0.752	0.030	0.077
DP061	BIC/3/CHAM1//	0.000	0.272	0.728	0.000	0.000
DP168	OMRUF-2	0.000	0.000	0.726	0.000	0.274
DP134	BRACHOUA	0.000	0.000	0.707	0.092	0.201
DP177	RAZZAK(TUN)	0.081	0.180	0.700	0.000	0.039
DP159	MARSYR-1	0.182	0.119	0.698	0.000	0.000
DP156	LAHN	0.000	0.035	0.695	0.000	0.270
DP175	QUABRACH-1	0.079	0.000	0.695	0.125	0.101
DP164	NILE	0.000	0.253	0.694	0.000	0.053
DP184	TERBOL97-3	0.114	0.000	0.680	0.000	0.206
DP188	YOUSEF-1	0.010	0.140	0.675	0.097	0.077

DP139	GEROMTEL-1	0.004	0.048	0.662	0.181	0.106
DP090	DONPEDRO	0.002	0.184	0.654	0.123	0.037
DP124	ATLAST-1	0.000	0.000	0.653	0.161	0.187
DP151	KABIR1	0.120	0.000	0.643	0.236	0.000
DP072	QUAD//ERP/MAL/3/UNKN	0.262	0.103	0.635	0.000	0.000
DP062	CHABA/DERAA	0.026	0.274	0.634	0.000	0.066
DP173	OUASLAHN-1	0.019	0.000	0.633	0.348	0.000
DP132	BIGOST-1	0.063	0.311	0.626	0.000	0.000
DP161	MIKI-1	0.062	0.280	0.621	0.037	0.000
DP155	LAGONIL-2	0.014	0.133	0.580	0.156	0.117
DP172	OUASERL-1	0.270	0.000	0.575	0.155	0.000
DP005	MERIDIANO	0.000	0.000	0.572	0.265	0.163
DP037	BORLI	0.000	0.000	0.564	0.000	0.436
DP069	OMBAR	0.350	0.000	0.554	0.000	0.096
DP063	CHACAN	0.000	0.086	0.552	0.169	0.193
DP093	FLAMINIO	0.000	0.000	0.552	0.426	0.023
DP123	ARISLAHN-5	0.051	0.193	0.547	0.114	0.096
DP053	JAWHAR	0.000	0.000	0.545	0.000	0.455
DP038	CANYON	0.000	0.425	0.544	0.000	0.031
DP131	BICREDERAA-1	0.083	0.350	0.533	0.034	0.000
DP133	BLK2	0.000	0.468	0.531	0.000	0.000
DP122	AMMAR-1	0.000	0.267	0.529	0.048	0.156
DP057	TAREK	0.159	0.254	0.523	0.000	0.064
DP066	KRS/HAUCAN	0.083	0.417	0.489	0.009	0.002
DP075	ZEINA1	0.000	0.000	0.478	0.372	0.150
DP146	ICARDA121	0.084	0.139	0.476	0.232	0.069
DP117	WESTBREDTUR	0.064	0.406	0.468	0.062	0.000
DP185	TUNSYR-1	0.000	0.000	0.467	0.314	0.220
DP163	MURLAGOST-1	0.000	0.391	0.454	0.059	0.096
DP065	H.MOUL(MOR)	0.069	0.241	0.448	0.215	0.027
DP048	1807	0.028	0.436	0.437	0.099	0.000
DP126	AWALI-1	0.000	0.326	0.366	0.189	0.119
DP067	LAGOST3	0.013	0.278	0.318	0.167	0.224
DP002	CANNIZZO	0.000	0.000	0.000	1.000	0.000
DP006	MONGIBELLO	0.000	0.000	0.000	1.000	0.000
DP095	GARGANO	0.000	0.000	0.000	1.000	0.000
DP114	VALNOVA	0.000	0.000	0.000	1.000	0.000
DP105	OFANTO	0.034	0.000	0.000	0.966	0.000
DP091	DUILIO	0.134	0.000	0.000	0.866	0.000
DP107	PLINIO	0.000	0.000	0.134	0.866	0.000
DP094	FORTORE	0.137	0.000	0.000	0.863	0.000
DP076	ANTON	0.000	0.148	0.000	0.820	0.032
DP004	LESINA	0.039	0.052	0.093	0.816	0.000
DP099	IXOS	0.000	0.196	0.000	0.804	0.000
DP085	CICCIO	0.221	0.000	0.000	0.779	0.000
DP106	PLATANI	0.283	0.000	0.000	0.717	0.000
DP140	GEZIRA17	0.092	0.193	0.000	0.715	0.000
DP079	ARCANGELO	0.157	0.000	0.152	0.691	0.000

DP010	TORREBIANCA	0.000	0.379	0.000	0.619	0.002
DP008	PIETRAFITTA	0.000	0.000	0.364	0.598	0.038
DP096	GRAZIA	0.000	0.377	0.000	0.571	0.052
DP112	TRINAKRIA	0.462	0.000	0.000	0.537	0.000
DP127	RADIOSO	0.097	0.179	0.225	0.499	0.000
DP087	COLOSSEO	0.002	0.268	0.238	0.492	0.000
DP089	CRESO	0.000	0.098	0.429	0.473	0.000
DP150	JORDAN	0.009	0.170	0.364	0.458	0.000
DP035	BOLO	0.000	0.100	0.128	0.396	0.375
DP011	CIMMYT-23	0.000	0.000	0.000	0.000	1.000
DP015	CIMMYT-52	0.000	0.000	0.000	0.000	1.000
DP016	CIMMYT67	0.000	0.000	0.000	0.000	1.000
DP017	CIMMYT73	0.000	0.000	0.000	0.000	1.000
DP021	CIMMYT-136	0.000	0.000	0.000	0.000	1.000
DP024	CIMMYT-222	0.000	0.000	0.000	0.000	1.000
DP041	GALLARETA	0.000	0.000	0.000	0.000	1.000
DP045	SULA	0.000	0.000	0.000	0.000	1.000
DP080	ARCOBALENO	0.000	0.000	0.000	0.000	1.000
DP036	BOMBASI	0.000	0.000	0.000	0.000	1.000
DP025	CIMMYT-247	0.061	0.025	0.000	0.000	0.914
DP097	IRIDE	0.000	0.000	0.138	0.000	0.862
DP020	CIMMYT-108	0.000	0.000	0.167	0.000	0.833
DP018	CIMMYT-78	0.000	0.000	0.202	0.000	0.798
DP023	CIMMYT-198	0.000	0.240	0.000	0.000	0.760
DP054	MARJANA	0.000	0.000	0.263	0.000	0.737
DP042	ILLORA	0.000	0.038	0.000	0.234	0.727
DP003	CLAUDIO	0.066	0.193	0.052	0.155	0.534
DP031	ASTIGI	0.000	0.467	0.000	0.000	0.533
DP007	NORBA	0.032	0.214	0.098	0.168	0.488
DP027	CIMMYT266	0.000	0.205	0.328	0.000	0.466

References

Abbo, S., Gopher, A., Peleg, Z., Saranga, Y., Fahima, T., Salamini, F., & Lev-Yadun, S. (2006). The ripples of "The Big (agricultural) Bang": The spread of early wheat cultivation. *Genome*, *49*(8), 861–863. https://doi.org/10.1139/G06-049

Achilli, A. L., Roncallo, P. F., Larsen, A. O., Dreisigacker, S., & Echenique, V. (2022). Population structure, allelic variation at Rht-B1 and Ppd-A1 loci and its effects on agronomic traits in Argentinian durum wheat. *Scientific Reports*, *12*(1), 1–13. https://doi.org/10.1038/s41598-022-13563-w

Akhunov, E., Nicolet, C., & Dvorak, J. (2009). Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina GoldenGate assay. *Theoretical and Applied Genetics*, *119*(3). https://doi.org/10.1007/s00122-009-1059-5

Ammerman, A. J., & Cavalli-Sforza, L. L. (1984). The Neolithic Transition and the Genetics of Populations in Europe. In *The Neolithic Transition and the Genetics of Populations in Europe*. https://doi.org/10.1515/9781400853113

Arraiano, L. S., Balaam, N., Fenwick, P. M., Chapman, C., Feuerhelm, D., Howell, P., Smith, S. J., Widdowson, J. P., & Brown, J. K. M. (2009). Contributions of disease resistance and escape to the control of septoria tritici blotch of wheat. *Plant Pathology*, *58*(5). https://doi.org/10.1111/j.1365-3059.2009.02118.x

Arraiano, Lia S., & Brown, J. K. M. (2017). Sources of resistance and susceptibility to Septoria tritici blotch of wheat. *Molecular Plant Pathology*, *18*(2), 276–292. https://doi.org/10.1111/mpp.12482

Atkinson, J. A., Wingen, L. U., Griffiths, M., Pound, M. P., Gaju, O., Foulkes, M. J., Le Gouis, J., Griffiths, S., Bennett, M. J., King, J., & Wells, D. M. (2015). Phenotyping pipeline reveals major seedling root growth QTL in hexaploid wheat. *Journal of Experimental Botany*, *66*(8), 2283–2292. https://doi.org/10.1093/jxb/erv006

Avni, R., Nave, M., Barad, O., Baruch, K., Twardziok, S. O., Gundlach, H., Hale, I., Mascher, M., Spannagl, M., Wiebe, K., Jordan, K. W., Golan, G., Deek, J., Ben-Zvi, B., Ben-Zvi, G., Himmelbach, A., Maclachlan, R. P., Sharpe, A. G., Fritz, A., … Distelfeld, A. (2017). Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. *Science*, *357*(6346), 93–97. https://doi.org/10.1126/SCIENCE.AAN0032/SUPPL_FILE/SUPPLEMENTARY_FILE7.TAB.ZIP

Badaeva, E. D., Keilwagen, J., Knüpffer, H., Waßermann, L., Dedkova, O. S., Mitrofanova, O. P., Kovaleva, O. N., Liapunova, O. A., Pukhalskiy, V. A., Özkan, H., Graner, A., Willcox, G., & Kilian, B. (2015). Chromosomal passports provide new insights into diffusion of emmer wheat. *PLoS ONE*, *10*(5), 1–25. https://doi.org/10.1371/journal.pone.0128556

Beavis, W. D. (2019). QTL Analyses: Power, Precision, and Accuracy. In *Molecular Dissection of Complex Traits*. https://doi.org/10.1201/9780429117770-10

Belaid, A. (2000). Durum wheat in WANA: production, trade, and gains from technological change. *Durum Wheat Improvement in the Mediterranean Region: New Challenges*, *40*.

Bell, G. D. H. (1987). The history of wheat cultivation. In *Wheat Breeding*. https://doi.org/10.1007/978-94-009-3131-2_2

Bentley, A. R., Turner, A. S., Gosman, N., Leigh, F. J., Maccaferri, M., Dreisigacker, S., Greenland, A., & Laurie, D. A. (2011). Frequency of photoperiod-insensitive Ppd-A1a alleles in tetraploid, hexaploid and synthetic hexaploid wheat germplasm. *Plant Breeding*, *130*(1). https://doi.org/10.1111/j.1439-0523.2010.01802.x

Berraies, S., Gharbi, M. S., Rezgui, S., & Yahyaoui, A. (2014). Estimating grain yield losses caused by septoria leaf blotch on durum wheat in Tunisia. *Chilean Journal of Agricultural Research*, *74*(4), 432–437. https://doi.org/10.4067/S0718-58392014000400009

Boeger, J. M., Chen, R. S., & McDonald, B. A. (1993). Gene flow between geographic populations of Mycosphaerella graminicola (anamorph Septoria tritici) detected with restriction fragment length polymorphism markers. *Phytopathology*, *83*(11). https://doi.org/10.1094/Phyto-83-1148

Bowden, W. M. (1959). The taxonomy and nomenclature of the wheats, barleys, and ryes and their wild relatives. *Canadian Journal of Botany*, *37*(4), 657–684.

Brading, P. A., Verstappen, E. C. P., Kema, G. H. J., & Brown, J. K. M. (2002). A gene-for-gene relationship between wheat and Mycosphaerella graminicola, the Septoria tritici blotch pathogen. *Phytopathology*, *92*(4), 439–445. https://doi.org/10.1094/PHYTO.2002.92.4.439

Breseghello, F., & Sorrells, M. E. (2006). Association analysis as a strategy for improvement of quantitative traits in plants. *Crop Science*, *46*(3). https://doi.org/10.2135/cropsci2005.09-0305

Brown, J. K. M., Chartrain, L., Lasserre-zuber, P., & Saintenac, C. (2015). Genetics of resistance to Zymoseptoria tritici and applications to wheat breeding. *Fungal Genetics and Biology*, *79*, 33–41. https://doi.org/10.1016/j.fgb.2015.04.017

Browning, B. L., Zhou, Y., & Browning, S. R. (2018). A One-Penny Imputed Genome from Next-Generation Reference Panels. *American Journal of Human Genetics*, *103*(3), 338–348. https://doi.org/10.1016/j.ajhg.2018.07.015

Buntjer, J. B., Sørensen, A. P., & Peleman, J. D. (2005). Haplotype diversity: The link between statistical and biological association. *Trends in Plant Science*, *10*(10), 466–471. https://doi.org/10.1016/j.tplants.2005.08.007

Canè, M. A., Maccaferri, M., Nazemi, G., Salvi, S., Francia, R., Colalongo, C., & Tuberosa, R. (2014). Association mapping for root architectural traits in durum wheat seedlings as related to agronomic performance. *Molecular Breeding*, *34*(4). https://doi.org/10.1007/s11032-014-0177-1

Cavanagh, C. R., Chao, S., Wang, S., Huang, B. E., Stephen, S., Kiani, S., Forrest, K., Saintenac, C., Brown-Guedira, G. L., Akhunova, A., See, D., Bai, G., Pumphrey, M., Tomar, L., Wong, D., Kong, S., Reynolds, M., Da Silva, M. L., Bockelman, H., … Akhunov, E. (2013). Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(20). https://doi.org/10.1073/pnas.1217133110

Chang, C. C., Chow, C. C., Tellier, L. C. A. M., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience*, *4*(1), 1–16. https://doi.org/10.1186/s13742-015-0047-8

Charmet, G. (2011). Wheat domestication: Lessons for the future. In *Comptes Rendus - Biologies* (Vol.

334, Issue 3). https://doi.org/10.1016/j.crvi.2010.12.013

Chartrain, L., Brading, P. A., Makepeace, J. C., & Brown, J. K. M. (2004). Sources of resistance to septoria tritici blotch and implications for wheat breeding. *Plant Pathology*, *53*(4), 454–460. https://doi.org/10.1111/J.1365-3059.2004.01052.X

Chartrain, L., Joaquim, P., Berry, S. T., Arraiano, L. S., Azanza, F., & Brown, J. K. M. (2005). Genetics of resistance to septoria tritici blotch in the Portuguese wheat breeding line TE 9111. *Theoretical and Applied Genetics*, *110*(6). https://doi.org/10.1007/s00122-005-1945-4

Chartrain, L., Sourdille, P., Bernard, M., & Brown, J. K. M. (2009). *Identification and location of Stb9 , a gene for resistance to septoria tritici blotch in wheat cultivars Courtot and Tonic*. 547–555. https://doi.org/10.1111/j.1365-3059.2008.02013.x

Chedli, R. B. H., M'barek, S. Ben, Yahyaoui, A., Kehel, Z., & Rezgui, S. (2018). Occurrence of Septoria tritici blotch (Zymoseptoria tritici) disease on durum wheat, triticale, and bread wheat in northern Tunisia. *Chilean Journal of Agricultural Research*, *78*(4). https://doi.org/10.4067/S0718-58392018000400559

Chen, R. S., & McDonald, B. A. (1996). Sexual reproduction plays a major role in the genetic structure of populations of the fungus Mycosphaerella graminicola. *Genetics*, *142*(4). https://doi.org/10.1093/genetics/142.4.1119

Christopher, J., Christopher, M., Jennings, R., Jones, S., Fletcher, S., Borrell, A., Manschadi, A. M., Jordan, D., Mace, E., & Hammer, G. (2013). QTL for root angle and number in a population developed from bread wheats (Triticum aestivum) with contrasting adaptation to water-limited environments. *Theoretical and Applied Genetics*, *126*(6), 1563–1574. https://doi.org/10.1007/s00122-013-2074-0

Condorelli, G. E., Maccaferri, M., Newcomb, M., Andrade-Sanchez, P., White, J. W., French, A. N., Sciara, G., Ward, R., & Tuberosa, R. (2018). Comparative aerial and ground based high throughput phenotyping for the genetic dissection of NDVI as a proxy for drought adaptive traits in durum wheat. *Frontiers in Plant Science*, *9*(June), 1–17. https://doi.org/10.3389/fpls.2018.00893

Conversa, G., Lazzizera, C., Bonasia, A., Cifarelli, S., Losavio, F., Sonnante, G., & Elia, A. (2020). Exploring on-farm agro-biodiversity: a study case of vegetable landraces from Puglia region (Italy). *Biodiversity and Conservation*, *29*(3). https://doi.org/10.1007/s10531-019-01908-3

Cowling, S. G. (2006). *Identification and mapping of host resistance genes to Septoria tritici blotch of wheat*. https://mspace.lib.umanitoba.ca/handle/1993/20699

Crossa, J., Burgueño, J., Dreisigacker, S., Vargas, M., Herrera-Foessel, S. A., Lillemo, M., Singh, R. P., Trethowan, R., Warburton, M., Franco, J., Reynolds, M., Crouch, J. H., & Ortiz, R. (2007). Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. *Genetics*, *177*(3). https://doi.org/10.1534/genetics.107.078659

Cunfer, B. M., & Ueng, P. P. (1999). Taxonomy and identification of Septoria and Stagonospora species on small-grain cereals. *Annual Review of Phytopathology*, *37*. https://doi.org/10.1146/annurev.phyto.37.1.267

De Luca, D., Cennamo, P., Del Guacchio, E., Di Novella, R., & Caputo, P. (2018). Conservation and genetic characterisation of common bean landraces from Cilento region (southern Italy): high differentiation in spite of low genetic diversity. *Genetica*, *146*(1). https://doi.org/10.1007/s10709-017-9994-6

Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., & Foster, G. D. (2012). The Top 10 fungal pathogens in molecular plant pathology. In *Molecular Plant Pathology* (Vol. 13, Issue 4). https://doi.org/10.1111/j.1364-3703.2011.00783.x

Dempewolf, H., Baute, G., Anderson, J., Kilian, B., Smith, C., & Guarino, L. (2017). Past and future use of wild relatives in crop breeding. In *Crop Science* (Vol. 57, Issue 3). https://doi.org/10.2135/cropsci2016.10.0885

Dorofeev VF, Filatenko AA, Migushova EF, Udaczin RA, and J. M. (1979). No Title. *Wheat. In: Flora of Cultivated Plants (Dorofeev VF and Korovina ON, Eds.) Leningrad (St. Petersburg), Russia. Kolos (in Russian).*, *1*, 346.

Dreisigacker, S., Wang, X., Martinez, B. A., Jing, R., & Singh, P. K. (2015). Adult - plant resistance to Septoria tritici blotch in hexaploid spring wheat. *Theoretical and Applied Genetics*, *128*(11), 2317–2329. https://doi.org/10.1007/s00122-015-2587-9

Dubcovsky, J., & Dvorak, J. (2007). Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science*, *316*(5833), 1862–1866. https://doi.org/10.1126/SCIENCE.1143986/SUPPL_FILE/DUBCOVSKY.SOM.PDF

Dumortier, B. C. J. (1823). Graminees de la Belgique. *Obs. Gramin. Belg*, *83*, 134.

Duveiller, E., Singh, R. P., & Nicol, J. M. (2007). The challenges of maintaining wheat productivity: Pests, diseases, and potential epidemics. *Euphytica*, *157*(3). https://doi.org/10.1007/s10681-007-9380-z

Dvořák, J., Terlizzi, P. di, Zhang, H.-B., & Resta, P. (1993). The evolution of polyploid wheats: identification of the A genome donor species. *Genome*, *36*(1), 21–31. https://doi.org/10.1139/g93-004

Dvorák, J., & Zhang, H. B. (1990). Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. *Proceedings of the National Academy of Sciences*, *87*(24), 9640–9644. https://doi.org/10.1073/pnas.87.24.9640

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

Eckardt, N. A. (2010). Evolution of domesticated bread wheat. *Plant Cell*, *22*(4), 993. https://doi.org/10.1105/tpc.110.220410

Eriksen, L., & Munk, L. (2003a). The occurrence of Mycosphaerella graminicola and its anamorph Septoria tritici in winter wheat during the growing season. *European Journal of Plant Pathology*, *109*(3). https://doi.org/10.1023/A:1022851502770

Eriksen, L., & Munk, L. (2003b). The occurrence of Mycosphaerella graminicola and its anamorph Septoria tritici in winter wheat during the growing season. *European Journal of Plant Pathology*, *109*, 253–259.

Ersoz, E. S., Yu, J., & Buckler, E. S. (2009). Applications of linkage disequilibrium and association mapping in maize. In *Biotechnology in Agriculture and Forestry* (Vol. 63). https://doi.org/10.1007/978-3-540-68922-5_13

Eurostat. (2019). Eurostat - Tables, Graphs and Maps Interface (TGM) table. In *Eurostat* (Issue 1).

EVANNO, G., REGNAUT, S., & GOUDET, J. (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/https://doi.org/10.1111/j.1365-294X.2005.02553.x

Eyal, Z. (1999). The septoria tritici and stagonospora nodorum blotch diseases of wheat. In *European Journal of Plant Pathology* (Vol. 105, Issue 7). https://doi.org/10.1023/A:1008716812259

Faris, J. D., Zhang, Z., & Chao, S. (2014). Map-based analysis of the tenacious glume gene Tg-B1 of wild emmer and its role in wheat domestication. *Gene*, *542*(2). https://doi.org/10.1016/j.gene.2014.03.034

Flint-Garcia, S. A., Thornsberry, J. M., & Buckler, E. S. (2003). STRUCTURE OF LINKAGE DISEQUILIBRIUM IN PLANTS *. *Annu. Rev. Plant Biol*, *54*, 357–374. https://doi.org/10.1146/annurev.arplant.54.031902.134907

Fones, H., & Gurr, S. (2015). The impact of Septoria tritici Blotch disease on wheat: An EU perspective. *Fungal Genetics and Biology*, *79*. https://doi.org/10.1016/j.fgb.2015.04.004

Fuller, M. P., Fuller, A. M., Kaniouras, S., Christophers, J., & Fredericks, T. (2007). The freezing characteristics of wheat at ear emergence. *European Journal of Agronomy*, *26*(4). https://doi.org/10.1016/j.eja.2007.01.001

Gaurav, K., Arora, S., Silva, P., Sánchez-martín, J., Gao, L., Brar, G. S., Widrig, V., Raupp, J., Simmonds, J., Hayta, S., Smedley, M. A., & Harwood, W. (2021a). *Evolution of the bread wheat D-subgenome and enriching it with diversity from Aegilops tauschii*.

Gaurav, K., Arora, S., Silva, P., Sánchez-martín, J., Gao, L., Brar, G. S., Widrig, V., Raupp, J., Simmonds, J., Hayta, S., Smedley, M. A., & Harwood, W. (2021b). *Evolution of the bread wheat D-subgenome and enriching it with diversity from Aegilops tauschii*. 1–63.

Gaurav, K., Arora, S., Silva, P., Sánchez-Martín, J., Horsnell, R., Gao, L., Brar, G. S., Widrig, V., John Raupp, W., Singh, N., Wu, S., Kale, S. M., Chinoy, C., Nicholson, P., Quiroz-Chávez, J., Simmonds, J., Hayta, S., Smedley, M. A., Harwood, W., … Wulff, B. B. H. (2022). Population genomic analysis of Aegilops tauschii identifies targets for bread wheat improvement. *Nature Biotechnology*, *40*(3), 422–431. https://doi.org/10.1038/s41587-021-01058-4

Gill, B. S., & Raupp, W. J. (1987). Direct Genetic Transfers from Aegilops squarrosa L. to Hexaploid Wheat 1 . *Crop Science*, *27*(3). https://doi.org/10.2135/cropsci1987.0011183x002700030004x

Goldberg, S. M. D., Johnson, J., Busam, D., Feldblyum, T., Ferriera, S., Friedman, R., Halpern, A., Khouri, H., Kravitz, S. A., Lauro, F. M., Li, K., Rogers, Y. H., Strausberg, R., Sutton, G., Tallon, L., Thomas, T., Venter, E., Frazier, M., & Venter, J. C. (2006). A Sanger/pyrosequencing hybrid approach for the generation of high-quality draft assemblies of marine microbial genomes. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(30). https://doi.org/10.1073/pnas.0604351103

Goncharov, N. P. (2002). Comparative genetics of wheats and their related species. *Siberian Un-Ty Press, Novosibirsk (Russian)*.

Goncharov, N. P. (2011). Genus Triticum L. taxonomy: The present and the future. *Plant Systematics and Evolution*, *295*(1), 1–11. https://doi.org/10.1007/s00606-011-0480-9

Goncharov, N. P., Golovnina, K. A., & Kondratenko, E. Y. (2009). Taxonomy and molecular phylogeny of natural and artificial wheat species. *Breeding Science*, *59*(5), 492–498.

Goodwin, S. B. (2012). Resistance in wheat to septoria diseases caused by mycosphaerella graminicola (septoria tritici) and phaeosphaeria (stagonospora) nodorum. In *Disease Resistance in Wheat*. https://doi.org/10.1079/9781845938185.0151

Goodwin, S. B., M'Barek, S. Ben, Dhillon, B., Wittenberg, A. H. J., Crane, C. F., Hane, J. K., Foster, A. J., van der Lee, T. A. J., Grimwood, J., Aerts, A., Antoniw, J., Bailey, A., Bluhm, B., Bowler, J., Bristow, J., van der Burgt, A., Canto-Canché, B., Churchill, A. C. L., Conde-Ferràez, L., … Kema, G. H. J. (2011). Finished genome of the fungal wheat pathogen Mycosphaerella graminicola reveals dispensome structure, chromosome plasticity, and stealth pathogenesis. *PLoS Genetics*, *7*(6). https://doi.org/10.1371/journal.pgen.1002070

Gupta, P. K., Varshney, R. K., Sharma, P. C., & Ramesh, B. (1999). Molecular markers and their applications in wheat breeding. In *Plant Breeding* (Vol. 118, Issue 5). https://doi.org/10.1046/j.1439-0523.1999.00401.x

Habash, D. Z., Kehel, Z., & Nachit, M. (2009). Genomic approaches for designing durum wheat ready for climate change with a focus on drought. *Journal of Experimental Botany*, *60*(10). https://doi.org/10.1093/jxb/erp211

Hall, M. C., Lowry, D. B., & Willis, J. H. (2010). Is local adaptation in Mimulus guttatus caused by trade-offs at individual loci? *Molecular Ecology*, *19*(13). https://doi.org/10.1111/j.1365-294X.2010.04680.x

Hancock, D. B., Levy, J. L., Gaddis, N. C., Bierut, L. J., Saccone, N. L., Page, G. P., & Johnson, E. O. (2012). Assessment of Genotype Imputation Performance Using 1000 Genomes in African American Studies. *PLoS ONE*, *7*(11). https://doi.org/10.1371/journal.pone.0050610

Haudry, A., Cenci, A., Ravel, C., Bataillon, T., Brunel, D., Poncet, C., Hochu, I., Poirier, S., Santoni, S., Glémin, S., & David, J. (2007). Grinding up wheat: A massive loss of nucleotide diversity since domestication. *Molecular Biology and Evolution*, *24*(7). https://doi.org/10.1093/molbev/msm077

Heun, M., Schafer-Pregl, R., Klawan, D., Castagna, R., Accerbi, M., Borghi, B., & Salamini, F. (1997). Site of einkorn wheat domestication identified by DNA fingerprinting. *Science*, *278*(5341), 1312–1314.

Huang, X. Q., Börner, A., Röder, M. S., & Ganal, M. W. (2002). Assessing genetic diversity of wheat (Triticum aestivum L.) germplasm using microsatellite markers. *Theoretical and Applied Genetics*, *105*(5). https://doi.org/10.1007/s00122-002-0959-4

Kabbaj, H., Sall, A. T., Al-abdallat, A., Geleta, M., & Amri, A. (2017). *Landraces and Modern Germplasm Reveals the History of Alleles Exchange Genetic Diversity within a Global Panel of Durum Wheat (Triticum durum) Landraces and Modern Germplasm Reveals the History of Alleles Exchange*. *July*. https://doi.org/10.3389/fpls.2017.01277

Kabir, M. R., Liu, G., Guan, P., Wang, F., Khan, A. A., Ni, Z., Yao, Y., Hu, Z., Xin, M., Peng, H., & Sun, Q. (2015). Mapping QTLs associated with root traits using two different populations in wheat (Triticum aestivum L.). *Euphytica*, *206*(1), 175–190. https://doi.org/10.1007/s10681-015-1495-z

Kelm, C., Ghaffary, S. M. T., Bruelheide, H., Röder, M. S., Miersch, S., Weber, W. E., Kema, G. H. J., & Saal, B. (2012). The genetic architecture of seedling resistance to Septoria tritici blotch in the winter wheat doubled-haploid population Solitär × Mazurka. *Molecular Breeding*, *29*(3), 813–830.

https://doi.org/10.1007/s11032-011-9592-8

Kerber, E. R., & Tipples, K. H. (1969). EFFECTS OF THE D GENOME ON MILLING AND BAKING PROPERTIES OF WHEAT. *Canadian Journal of Plant Science*, *49*(3). https://doi.org/10.4141/cjps69-046

Kidane, Y. G., Hailemariam, B. N., Mengistu, D. K., Fadda, C., Pè, M. E., & Dell'Acqua, M. (2017). Genome-wide association study of Septoria tritici blotch resistance in Ethiopian durum Wheat Landraces. *Frontiers in Plant Science*, *8*(September), 1–12. https://doi.org/10.3389/fpls.2017.01586

Kihara, H. (1924). Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rucksicht auf das Verhalten der Chromosomen und die Sterilitat in den Bastarden. *Mem. Coll. Sci., Kyoto Imp. Univ.*, *1*, 1–200.

Kihara, H. (1944). Discovery of the DD-analyser, one of the ancestors of Triticum vulgare. *Agric. Hort.*, *19*, 13–14.

KIHARA, H., & LILIENFELD, F. (1949). A NEW SYNTHESIZED 6x-WHEAT. *Hereditas*, *35*(1 S). https://doi.org/10.1111/j.1601-5223.1949.tb03343.x

Kilian, B, Özkan, H., Deusch, O., Effgen, S., Brandolini, A., Kohl, J., Martin, W., & Salamini, F. (2007). Independent Wheat B and G Genome Origins in Outcrossing Aegilops Progenitor Haplotypes. *Molecular Biology and Evolution*, *24*(1), 217–227. https://doi.org/10.1093/molbev/msl151

Kilian, Benjamin, Crop, G., Trust, D., Ozkan, H., & Pozzi, C. (2009). *Domestication of the Triticeae in the Fertile Crescent Chapter 3 Domestication of the Triticeae in the Fertile Crescent* (Issue May). https://doi.org/10.1007/978-0-387-77489-3

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*.

Kosellek, C., Pillen, K., Nelson, J. C., Weber, W. E., & Saal, B. (2013). *Inheritance of field resistance to Septoria tritici blotch in the wheat doubled-haploid population ¨ r 3 Mazurka Solita*. 161–176. https://doi.org/10.1007/s10681-013-0898-y

Kosina, P., Reynolds, M., Dixon, J., & Joshi, A. (2007). Stakeholder perception of wheat production constraints, capacity building needs, and research partnerships in developing countries. *Euphytica*, *157*(3). https://doi.org/10.1007/s10681-007-9529-9

Kyzeridis, N., Biesantz, A., & Limberg, P. (1995). Vergleichende Feldversuche mit Land- und Zuchtsorten von Durumweizen (Triticum turgidum var. durum) unter verschiedenen ökologischen Bedingungen im Mittelmeerraum. *Journal of Agronomy and Crop Science*, *174*(2). https://doi.org/10.1111/j.1439-037X.1995.tb00204.x

Lagudah, E. S., Appels, R., Brown, A. H. D., & McNeil, D. (1991). The molecular-genetic analysis of Triticum tauschii, the D-genome donor to hexaploid wheat. *Genome*, *34*(3). https://doi.org/10.1139/g91-059

Lev-Yadun, S., Gopher, A., & Abbo, S. (2000). The cradle of agriculture. *Science*, *288*(5471), 1602–1603. https://doi.org/10.1126/SCIENCE.288.5471.1602/ASSET/E4F7F99D-7C16-4FA4-A43F-C031A59B5C93/ASSETS/GRAPHIC/1602-1.GIF

Linde, C. C., Zhan, J., & McDonald, B. A. (2002). Population structure of Mycosphaerella graminicola: From lesions to continents. *Phytopathology*, *92*(9). https://doi.org/10.1094/PHYTO.2002.92.9.946

Linné, C. von, Linné, C. von, & Salvius, L. (1753). Caroli Linnaei ... Species plantarum :exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas... In *Caroli Linnaei ... Species plantarum :exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas...* Impensis Laurentii Salvii. https://doi.org/10.5962/bhl.title.669

Liu, Y., Zhang, L., Thompson, I. A., Goodwin, S. B., & Ohm, H. W. (2013). Molecular mapping re-locates the Stb2 gene for resistance to Septoria tritici blotch derived from cultivar Veranopolis on wheat chromosome 1BS. *Euphytica*, *190*(1), 145–156. https://doi.org/10.1007/s10681-012-0796-8

Lopes, M. S., El-Basyoni, I., Baenziger, P. S., Singh, S., Royo, C., Ozbek, K., Aktas, H., Ozer, E., Ozdemir, F., Manickavelu, A., Ban, T., & Vikram, P. (2015). Exploiting genetic diversity from landraces in wheat breeding for adaptation to climate change. *Journal of Experimental Botany*, *66*(12). https://doi.org/10.1093/jxb/erv122

M.S., G., M., D., & F., B. A. (2000). Breeding for resistance to Septoria tritici in durum wheat. In A. J.L., D. F. N., N. M., & R. C. (Eds.), *Durum wheat improvement in the Mediterranean region: New challenges* (Vol. 40, pp. 397–401). Zaragoza : CIHEAM. http://om.ciheam.org/om/pdf/a40/00600065.pdf

Mac Key, J. (1975). The boundaries and subdivision of the genus Triticum. *Proc. 12th Int. Bot. Congr., Leningrad*, 1–23.

Mac Key, J. (1988). A plant breeder's aspect on taxonomy of cultivated plants. *Biologisches Zentralblatt*, *107*(4), 369–379.

Mac Key, J. (2005). Wheat: its concept, evolution, and taxonomy. In *Durum wheat breeding* (pp. 35–94). CRC Press.

Mac Key, J. (1968). Relationships in the Triticinae. *Proc. Third Int. Wheat Genetics Symp., Canberra, Australia. Austral. Acad. Sci*, 39–45.

Maccaferri, M., Mantovani, P., Tuberosa, R., Deambrogio, E., Giuliani, S., Demontis, A., Massi, A., & Sanguineti, M. C. (2008). A major QTL for durable leaf rust resistance widely exploited in durum wheat breeding programs maps on the distal region of chromosome arm 7BL. *Theoretical and Applied Genetics*, *117*(8), 1225–1240. https://doi.org/10.1007/s00122-008-0857-5

MacCaferri, M., Sanguineti, M. C., Demontis, A., El-Ahmed, A., Garcia Del Moral, L., Maalouf, F., Nachit, M., Nserallah, N., Ouabbou, H., Rhouma, S., Royo, C., Villegas, D., & Tuberosa, R. (2011). Association mapping in durum wheat grown across a broad range of water regimes. *Journal of Experimental Botany*, *62*(2), 409–438. https://doi.org/10.1093/jxb/erq287

Maccaferri, Marco, Ratti, C., Rubies-Autonell, C., Vallega, V., Demontis, A., Stefanelli, S., Tuberosa, R., & Sanguineti, M. C. (2011). Resistance to Soil-borne cereal mosaic virus in durum wheat is controlled by a major QTL on chromosome arm 2BS and minor loci. *Theoretical and Applied Genetics*, *123*(4). https://doi.org/10.1007/s00122-011-1605-9

Maccaferri, Marco, Ricci, A., Salvi, S., Milner, S. G., Noli, E., Martelli, P. L., Casadio, R., Akhunov, E., Scalabrin, S., Vendramin, V., Ammar, K., Blanco, A., Desiderio, F., Distelfeld, A., Dubcovsky, J., Fahima, T., Faris, J., Korol, A., Massi, A., … Tuberosa, R. (2015). A high-density, SNP-based

consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. *Plant Biotechnology Journal*, *13*(5), 648–663. https://doi.org/10.1111/pbi.12288

Maccaferri, Marco, Sanguineti, M. C., Demontis, A., El-ahmed, A., Garcia, L., Maalouf, F., Nachit, M., Nserallah, N., Ouabbou, H., Rhouma, S., & Royo, C. (2010). *Association mapping in durum wheat grown across a broad range of water regimes*. 1–30. https://doi.org/10.1093/jxb/erq287

Maccaferri, Marco, Sanguineti, M. C., Demontis, A., El-ahmed, A., Garcia, L., Maalouf, F., Nachit, M., Nserallah, N., Ouabbou, H., Rhouma, S., & Royo, C. (2011). *Association mapping in durum wheat grown across a broad range of water regimes*. *62*(2), 409–438. https://doi.org/10.1093/jxb/erq287

Maccaferri, Marco, Sanguineti, M. C., Mantovani, P., Czembor, J. H., Ezrati, S., & Tuberosa, R. (2010). *Association mapping of leaf rust response in durum wheat*. 189–228. https://doi.org/10.1007/s11032-009-9353-0

Maccaferri, Marco, Sanguineti, M. C., Natoli, V., Ortega, J. L. A., Salem, M. Ben, Bort, J., Chenenaoui, C., De Ambrogio, E., del Moral, L. G., De Montis, A., El-Ahmed, A., Maalouf, F., Machlab, H., Moragues, M., Motawaj, J., Nachit, M., Nserallah, N., Ouabbou, H., Royo, C., & Tuberosa, R. (2006). A panel of elite accessions of durum wheat (Triticum durum Desf.) suitable for association mapping studies . *Plant Genetic Resources*, *4*(1). https://doi.org/10.1079/pgr2006117

Maccaferri, Marco, Sanguineti, M. C., Noli, E., & Tuberosa, R. (2005). *Population structure and long-range linkage disequilibrium in a durum wheat elite collection*. 271–289. https://doi.org/10.1007/s11032-004-7012-z

Maccaferri, Marco, Zhang, J., Bulli, P., Abate, Z., Chao, S., Cantu, D., Bossolini, E., Chen, X., Pumphrey, M., & Dubcovsky, J. (2015). A genome-wide association study of resistance to stripe rust (Puccinia striiformis f. sp. tritici) in a worldwide collection of hexaploid spring wheat (Triticum aestivum L.). *G3: Genes, Genomes, Genetics*, *5*(3), 449–465. https://doi.org/10.1534/g3.114.014563

MacKey, J. (1966). Species relationship in Triticum. *Species Relationship in Triticum. In: MacKey J (Ed) Proceedings of the 2nd International Wheat Genetics Symposium, 1963. Hereditas Suppl 2:237–275*, 237–275.

MacKey, James. (1989). Genus Triticum and its systematics. *Genus Triticum and Its Systematics. In: Shumny VK (Ed) Vavilov's Legacy in Modern Biology (in Russian). Nauka, Moscow*, 170–185.

Mahmod, S., Ghaffary, T., Robert, O., Laurent, V., Lonnet, P., Margale, E., Visser, R. G. F., & Kema, G. H. J. (2011). *Genetic analysis of resistance to septoria tritici blotch in the French winter wheat cultivars Balance and Apache*. 741–754. https://doi.org/10.1007/s00122-011-1623-7

Mangini, G., Margiotta, B., Marcotuli, I., Signorile, M. A., Gadaleta, A., & Blanco, A. (2017). Genetic diversity and phenetic analysis in wheat (Triticum turgidum subsp. durum and Triticum aestivum subsp. aestivum) landraces based on SNP markers. *Genetic Resources and Crop Evolution*, *64*(6), 1269–1280. https://doi.org/10.1007/S10722-016-0435-7/FIGURES/1

Marcussen, T., Sandve, S. R., Heier, L., Spannagl, M., Pfeifer, M., Jakobsen, K. S., Wulff, B., Steuernagel, B., Mayer, K. F. X., Olsen, O. A., Rogers, J., Doležel, J., Pozniak, C., Eversole, K., Feuillet, C., Gill, B., Friebe, B., Lukaszewski, A. J., Sourdille, P., … Praud, S. (2014). Ancient hybridizations among the ancestral genomes of bread wheat. *Science*, *345*(6194). https://doi.org/10.1126/science.1250092

Martínez-Moreno, F., Ammar, K., & Solís, I. (2022). Global Changes in Cultivated Area and Breeding

Activities of Durum Wheat from 1800 to Date: A Historical Review. In *Agronomy* (Vol. 12, Issue 5, pp. 1–17). https://doi.org/10.3390/agronomy12051135

Martínez-Moreno, F., Solís, I., Noguero, D., Blanco, A., Özberk, İ., Nsarellah, N., Elias, E., Mylonas, I., & Soriano, J. M. (2020). Durum wheat in the Mediterranean Rim: historical evolution and genetic resources. *Genetic Resources and Crop Evolution*, *67*(6), 1415–1436. https://doi.org/10.1007/s10722-020-00913-8

Maxted, N., Kell, S., Ford-Lloyd, B., Dulloo, E., & Toledo, Á. (2012). Toward the systematic conservation of global crop wild relative diversity. *Crop Science*, *52*(2). https://doi.org/10.2135/cropsci2011.08.0415

Mazzucotelli, E., Sciara, G., Mastrangelo, A. M., Tricker, P. J., Ozkan, H., Echenique, V., Steffenson, B. J., & Kilian, B. (2020). *The Global Durum Wheat Panel (GDP): An International Platform to Identify The Global Durum Wheat Panel (GDP): An International Platform to Identify and Exchange Beneficial Alleles*. *December*. https://doi.org/10.3389/fpls.2020.569905

McDonald, B. A., & Mundt, C. C. (2016). How knowledge of pathogen population biology informs management of Septoria tritici blotch. In *Phytopathology* (Vol. 106, Issue 9). https://doi.org/10.1094/PHYTO-03-16-0131-RVW

Mcfadden, E. S., & Sears, E. R. (1946). The origin of triticum spelta and its free-threshing hexaploid relatives. *Journal of Heredity*, *37*(3). https://doi.org/10.1093/oxfordjournals.jhered.a105590

Melchinger, A. E., Friedrich Utz, H., & Schör, C. C. (1998). Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. *Genetics*, *149*(1). https://doi.org/10.1093/genetics/149.1.383

Miedaner, T., Würschum, T., Maurer, H. P., Korzun, V., Ebmeyer, E., & Reif, J. C. (2011). Association mapping for Fusarium head blight resistance in European soft winter wheat. *Molecular Breeding*, *28*(4). https://doi.org/10.1007/s11032-010-9516-z

Moragues, M., Moralejo, M., Sorrells, M. E., & Royo, C. (2007). Dispersal of durum wheat [Triticum turgidum L. ssp. turgidum convar. durum (Desf.) MacKey] landraces across the Mediterranean basin assessed by AFLPs and microsatellites. *Genetic Resources and Crop Evolution*, *54*(5). https://doi.org/10.1007/s10722-006-9005-8

Morris, R., & Sears, E. R. (1967). The cytogenetics of wheat and its relatives. *Wheat and Wheat Improvement*, *13*, 19–87.

Muqaddasi, Q. H., Brassac, J., Koppolu, R., Plieske, J., Ganal, M. W., & Röder, M. S. (2019). TaAPO-A1, an ortholog of rice ABERRANT PANICLE ORGANIZATION 1, is associated with total spikelet number per spike in elite European hexaploid winter wheat (Triticum aestivum L.) varieties. *Scientific Reports*, *9*(1), 1–12. https://doi.org/10.1038/s41598-019-50331-9

Nations, F. and A. O. of the U. (2014). State of Food Insecurity in the World. *FAO Publications*, 22–31.

Nazco, R., Villegas, D., Ammar, K., Peña, R. J., Moragues, M., & Royo, C. (2012). Can Mediterranean durum wheat landraces contribute to improved grain quality attributes in modern cultivars? *Euphytica*, *185*(1). https://doi.org/10.1007/s10681-011-0588-6

Nesbitt, M. (2002). When and where did domesticated cereals first occur in southwest Asia? *The Dawn of Farming in the Near East*, *January 2002*, 113–132.

Newton, A. C., Akar, T., Baresel, J. P., Bebeli, P. J., Bettencourt, E., Bladenopoulos, K. V., Czembor, J. H., Fasoula, D. A., Katsiotis, A., Koutis, K., Koutsika-Sotiriou, M., Kovacs, G., Larsson, H., De Carvalho, M. A. A. P., Rubiales, D., Russell, J., Dos Santos, T. M. M., & Vaz Patto, M. C. (2010). Cereal landraces for sustainable agriculture. A review. In *Agronomy for Sustainable Development* (Vol. 30, Issue 2). https://doi.org/10.1051/agro/2009032

Niks, R. E., Qi, X., & Marcel, T. C. (2015). Quantitative Resistance to Biotrophic Filamentous Plant Pathogens: Concepts, Misconceptions, and Mechanisms. *Annual Review of Phytopathology*, *53*. https://doi.org/10.1146/annurev-phyto-080614-115928

Nishikawa, K. (1983). Species relationship of wheat and its putative ancestors as viewed from isozyme variation. *Proc. 6th Int. Wheat Genet. Symp.*, 59–63.

Nothnagel, M., Ellinghaus, D., Schreiber, S., Krawczak, M., & Franke, A. (2009). A comprehensive evaluation of SNP genotype imputation. *Human Genetics*, *125*(2), 163–171. https://doi.org/10.1007/s00439-008-0606-5

Oerke, E. C. (2006). Crop losses to pests. In *Journal of Agricultural Science* (Vol. 144, Issue 1). https://doi.org/10.1017/S0021859605005708

Oliveira, H. R., Jacocks, L., Czajkowska, B. I., Kennedy, S. L., & Brown, T. A. (2020). Multiregional origins of the domesticated tetraploid wheats. *PLoS ONE*, *15*(1), 1–20. https://doi.org/10.1371/journal.pone.0227148

Peleg, Z., Fahima, T., Korol, A. B., Abbo, S., & Saranga, Y. (2011). Genetic analysis of wheat domestication and evolution under domestication. *Journal of Experimental Botany*, *62*(14). https://doi.org/10.1093/jxb/err206

Peng, J., Sun, D., & Nevo, E. (2011). Wild emmer wheat, Triticum dicoccoides, occupies a pivotal position in wheat domestication process. *Australian Journal of Crop Science*, *5*(9 SPEC. ISSUE), 1127–1143.

Perrings, C. (2018). Conservation beyond protected areas: The challenge of landraces and crop wild relatives. In *Ecology, Economy and Society: Essays in Honour of Kanchan Chopra*. https://doi.org/10.1007/978-981-10-5675-8_8

Pestsova, E. G., Börner, A., & Röder, M. S. (2006). Development and QTL assessment of Triticum aestivum-Aegilops tauschii introgression lines. *Theoretical and Applied Genetics*, *112*(4), 634–647. https://doi.org/10.1007/s00122-005-0166-1

Petersen, G., Seberg, O., Yde, M., & Berthelsen, K. (2006). Phylogenetic relationships of Triticum and Aegilops and evidence for the origin of the A, B, and D genomes of common wheat (Triticum aestivum). *Molecular Phylogenetics and Evolution*, *39*(1). https://doi.org/10.1016/j.ympev.2006.01.023

Pignone, D., De Paola, D., Rapanà, N., & Janni, M. (2015). Single seed descent: a tool to exploit durum wheat (Triticum durum Desf.) genetic resources. *Genetic Resources and Crop Evolution*, *62*(7), 1029–1035. https://doi.org/10.1007/S10722-014-0206-2/TABLES/3

Pluzhnikov, A., & Donnelly, P. (1996). Optimal sequencing strategies for surveying molecular genetic diversity. *Genetics*, *144*(3). https://doi.org/10.1093/genetics/144.3.1247

Pont, C., Leroy, T., Seidel, M., Tondelli, A., Duchemin, W., Armisen, D., Lang, D., Bustos-Korts, D., Goué, N., Balfourier, F., Molnár-Láng, M., Lage, J., Kilian, B., Özkan, H., Waite, D., Dyer, S.,

Letellier, T., Alaux, M., Russell, J., … Çakır, E. (2019). Tracing the ancestry of modern bread wheats. *Nature Genetics*, *51*(5). https://doi.org/10.1038/s41588-019-0393-z

Porceddu, E., Ceoloni, C., Lafiandra, D., Tanzarella, O. A., & Scarascia Mugnozza, G. T. (1988). Genetic resources and plant breeding: problems and prospects. *Proceedings of the Seventh International Wheat Genetics Symposium / Edited by T.E. Miller and R.M.D. Koebner*. https://doi.org/10.3/JQUERY-UI.JS

Poudel, D., & Johnsen, F. H. (2009). Valuation of crop genetic resources in Kaski, Nepal: Farmers' willingness to pay for rice landraces conservation. *Journal of Environmental Management*, *90*(1), 483–491. https://doi.org/10.1016/j.jenvman.2007.12.020

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

Purugganan, M. D., & Fuller, D. Q. (2009). The nature of selection during plant domestication. In *Nature* (Vol. 457, Issue 7231). https://doi.org/10.1038/nature07895

Qandeel-e-Arsh, Jabbar, T., Khalid, S., Atif, R. M., Rehman, H. M., & Rana, I. A. (2021). Wild Relatives and Modern Plant Breeding Technologies. In *Wild Germplasm for Genetic Improvement in Crop Plants* (pp. 343–371). Elsevier. https://doi.org/10.1016/B978-0-12-822137-2.00018-7

R Core Team. (2021). R core team (2021). In *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www. R-project. org*.

Rafalski, J. A. (2002). Novel genetic mapping tools in plants: SNPs and LD-based approaches. *Plant Science*, *162*(3), 329–333. https://doi.org/https://doi.org/10.1016/S0168-9452(01)00587-8

Raman, H., & Milgate, A. (2012). Molecular breeding for Septoria tritici blotch resistance in wheat. *Cereal Research Communications*, *40*(4), 451–466. https://doi.org/10.1556/CRC.40.2012.4.1

Renfrew, J. M. (1973). Palaeoethnobotany. The prehistoric food plants of the Near East and Europe. *Palaeoethnobotany. The Prehistoric Food Plants of the Near East and Europe.*

Rezgui, S., Fakhfakh, M. M., Boukef, S., Rhaiem, A., Chérif, M., Chérif, M., & Yahyaoui, A. H. (2008). Effect of Common Cultural Practices on Septoria Leaf Blotch Disease and Grain Yield of Irrigated Durum Wheat. *Tunisian Journal of Plant Protection*, *3*(2).

Risser, P., Ebmeyer, E., Korzun, V., Hartl, L., & Miedaner, T. (2011). Quantitative trait loci for adult-plant resistance to Mycosphaerella graminicola in two winter wheat populations. *Phytopathology*, *101*(10), 1209–1216. https://doi.org/10.1094/PHYTO-08-10-0203

Robertson, A., & Brink, R. A. (1967). Heritage from Mendel. *Madison, Wisconsin*, 265–280.

Rodríguez-Álvarez, M. X., Boer, M. P., van Eeuwijk, F. A., & Eilers, P. H. C. (2018). Correcting for spatial heterogeneity in plant breeding experiments with P-splines. *Spatial Statistics*, *23*. https://doi.org/10.1016/j.spasta.2017.10.003

Royo, C., Maccaferri, M., Álvaro, F., Moragues, M., Sanguineti, M. C., Tuberosa, R., Maalouf, F., Moral, L. F. G. del, Demontis, A., Rhouma, S., Nachit, M., Nserallah, N., & Villegas, D. (2010). Understanding the relationships between genetic and phenotypic structures of a collection of elite durum wheat accessions. *Field Crops Research*, *119*(1), 91–105. https://doi.org/10.1016/j.fcr.2010.06.020

Sakamura, T. (1918). Kurze Mitteilung über die Chromosomenzahlen und die Verwandtschaftsverhältnisse der Triticum-Arten. *Shokubutsugaku Zasshi*, *32*(379), en150–en153. https://doi.org/10.15281/jplantres1887.32.379_150

Salamini, F., Özkan, H., Brandolini, A., Schäfer-Pregl, R., & Martin, W. (2002). Genetics and geography of wild cereal domestication in the near east. In *Nature Reviews Genetics* (Vol. 3, Issue 6). https://doi.org/10.1038/nrg817

Sanderson, F. R. (1972). A Mycosphaerella species as the ascogenous state of septoria tritici rob. And Desm. *New Zealand Journal of Botany*, *10*(4). https://doi.org/10.1080/0028825X.1972.10430256

Sarkar, P., & Stebbins, G. L. (1956). Morphological Evidence Concerning the Origin of the B Genome in Wheat. *American Journal of Botany*, *43*(4), 297. https://doi.org/10.2307/2438947

Sax, K., & Sax, H. J. (1924). Chromosome Behavior in a Genus Cross. *Genetics*, *9*(5), 454. https://doi.org/10.1093/GENETICS/9.5.454

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. In *Nature Methods* (Vol. 9, Issue 7). https://doi.org/10.1038/nmeth.2089

Sharma, S., Schulthess, A. W., Bassi, F. M., Badaeva, E. D., Neumann, K., Graner, A., Özkan, H., Werner, P., Knüpffer, H., & Kilian, B. (2021). Introducing beneficial alleles from plant genetic resources into the wheat germplasm. In *Biology* (Vol. 10, Issue 10). https://doi.org/10.3390/biology10100982

Shaw, L. M., Turner, A. S., Herry, L., Griffiths, S., & Laurie, D. A. (2013). Mutant alleles of Photoperiod-1 in Wheat (Triticum aestivum L.) that confer a late flowering phenotype in long days. *PLoS ONE*, *8*(11). https://doi.org/10.1371/journal.pone.0079459

SHAW, M. W., & ROYLE, D. J. (1989). Airborne inoculum as a major source of Septoria tritici (Mycosphaerella graminicola) infections in winter wheat crops in the UK. *Plant Pathology*, *38*(1). https://doi.org/10.1111/j.1365-3059.1989.tb01425.x

Simmonds, N. W. (1979). Principles of crop improvement. *Principles of Crop Improvement.*

Simón, M. R., Cordo, C. A., Castillo, N. S., Struik, P. C., & Börner, A. (2012). Population Structure of Mycosphaerella graminicola and Location of Genes for Resistance to the Pathogen: Recent Advances in Argentina . *International Journal of Agronomy*, *2012*, 1–7. https://doi.org/10.1155/2012/680275

Singh, N., Wu, S., Tiwari, V., Sehgal, S., Raupp, J., Wilson, D., Abbasov, M., Gill, B., & Poland, J. (2019). Genomic analysis confirms population structure and identifies inter-lineage hybrids in Aegilops tauschii. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.00009

Smith, S. E., Kuehl, R. O., Ray, I. M., Hui, R., & Soleri, D. (1998). Evaluation of simple methods for estimating broad-sense heritability in stands of randomly planted genotypes. *Crop Science*, *38*(5). https://doi.org/10.2135/cropsci1998.0011183X003800050003x

Srivastava, J. P., & Damania, A. B. (1989). Use of collections in cereal improvement in semi-arid areas. *The Use of Plant Genetic Resources.*, 88–104.

Stich, B., & Melchinger, A. E. (2010). An introduction to association mapping in plants. In *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* (Vol. 5). https://doi.org/10.1079/PAVSNNR20105039

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). https://doi.org/10.1007/s00122-005-2057-x

Suffert, F., & Sache, I. (2011). Relative importance of different types of inoculum to the establishment of Mycosphaerella graminicola in wheat crops in north-west Europe. *Plant Pathology*, *60*(5). https://doi.org/10.1111/j.1365-3059.2011.02455.x

Tabib, S. M., Justin, G., Timothy, D. F., Robert, O., & Kema, G. H. J. (2012). *New broad-spectrum resistance to septoria tritici blotch derived from synthetic hexaploid wheat*. 125–142. https://doi.org/10.1007/s00122-011-1692-7

Tadesse, W., Nachit, M., Abdalla, O., & Rajaram, S. (2016). Wheat breeding at ICARDA: achievements and prospects in the CWANA region. *The World Wheat Book*, *3*, 371–401.

Talas, F., Parzies, H. K., & Miedaner, T. (2011). Diversity in genetic structure and chemotype composition of Fusarium graminearum sensu stricto populations causing wheat head blight in individual fields in Germany. *European Journal of Plant Pathology*, *131*(1). https://doi.org/10.1007/s10658-011-9785-3

Tanno, K. I., & Willcox, G. (2006). How fast was wild wheat domesticated? *Science*, *311*(5769), 1886. https://doi.org/10.1126/science.1124635

Tedone, L., Ali, S. A., & Mastro, G. De. (2018). Optimization of Nitrogen in Durum Wheat in the Mediterranean Climate: The Agronomical Aspect and Greenhouse Gas (GHG) Emissions. In *Nitrogen in Agriculture - Updates*. https://doi.org/10.5772/intechopen.70195

Thornsberry, J. M., Goodman, M. M., Doebley, J., Kresovich, S., Nielsen, D., & Buckler, E. S. (2001). Dwarf8 polymorphisms associate with variation in flowering time. *Nature Genetics*, *28*(3). https://doi.org/10.1038/90135

Thuillet, A. C., Bataillon, T., Poirier, S., Santoni, S., & David, J. L. (2005). Estimation of long-term effective population sizes through the history of durum wheat using microsatellite data. *Genetics*, *169*(3). https://doi.org/10.1534/genetics.104.029553

Tommasini, L., Schnurbusch, T., Fossati, D., Mascher, F., & Keller, B. (2007). Association mapping of Stagonospora nodorum blotch resistance in modern European winter wheat varieties. *Theoretical and Applied Genetics*, *115*(5). https://doi.org/10.1007/s00122-007-0601-6

Trebbi, D., Maccaferri, M., de Heer, P., Sørensen, A., Giuliani, S., Salvi, S., Sanguineti, M. C., Massi, A., van der Vossen, E. A. G., & Tuberosa, R. (2011). High-throughput SNP discovery and genotyping in durum wheat (Triticum durum Desf.). *Theoretical and Applied Genetics*, *123*(4). https://doi.org/10.1007/s00122-011-1607-7

USDA. (2019). *USDA*. Wheat Data. Available Online: www.ers.usda.gov/data- products/wheat-data.aspx

Van Slageren, M. W. (1994). *Wild wheats: a monograph of Aegilops L. and Amblyopyrum (Jaub. & Spach) Eig (Poaceae)*. Agricultural University Wageningen.

Villa, T. C. C., Maxted, N., Scholten, M., & Ford-Lloyd, B. (2005). Defining and identifying crop landraces. *Plant Genetic Resources*, *3*(3), 373–384. https://doi.org/10.1079/pgr200591

Wallace, M., Bonhomme, V., Russell, J., Stillman, E., George, T. S., Ramsay, L., Wishart, J., Timpany,

S., Bull, H., Booth, A., & Martin, P. (2019). Searching for the Origins of Bere Barley: a Geometric Morphometric Approach to Cereal Landrace Recognition in Archaeology. *Journal of Archaeological Method and Theory*, *26*(3). https://doi.org/10.1007/s10816-018-9402-2

Wang, J., & Zhang, Z. (2021). GAPIT Version 3: Boosting Power and Accuracy for Genomic Association and Prediction. *Genomics, Proteomics and Bioinformatics*, *19*(4), 629–640. https://doi.org/10.1016/j.gpb.2021.08.005

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Salvi, S., Milner, S. G., Cattivelli, L., Mastrangelo, A. M., Whan, A., Stephen, S., Dvorak, J., Morell, M., Dubcovsky, J., Ganal, M., Tuberosa, R., & Lawley, C. (2014). *Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array*. 787–796. https://doi.org/10.1111/pbi.12183

Warschefsky, E., Varma Penmetsa, R., Cook, D. R., & Von Wettberg, E. J. B. (2014). Back to the wilds: Tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. *American Journal of Botany*, *101*(10). https://doi.org/10.3732/ajb.1400116

Wicker, T., Schlagenhauf, E., Graner, A., Close, T. J., Keller, B., & Stein, N. (2006). 454 sequencing put to the test using the complex genome of barley. *BMC Genomics*, *7*. https://doi.org/10.1186/1471-2164-7-275

Wilhelm, E. P., Boulton, M. I., Al-Kaff, N., Balfourier, F., Bordes, J., Greenland, A. J., Powell, W., & Mackay, I. J. (2013). Rht-1 and Ppd-D1 associations with height, GA sensitivity, and days to heading in a worldwide bread wheat collection. *Theoretical and Applied Genetics*, *126*(9), 2233–2243. https://doi.org/10.1007/s00122-013-2130-9

Xie, J., Huo, N., Zhou, S., Wang, Y., Guo, G., Deal, K. R., Ouyang, S., Liang, Y., Wang, Z., Xiao, L., Zhu, T., Hu, T., Tiwari, V., Zhang, J., Li, H., Ni, Z., Yao, Y., Peng, H., Zhang, S., … Sun, Q. (2017). Sequencing and comparative analyses of Aegilops tauschii chromosome arm 3DS reveal rapid evolution of Triticeae genomes. *Journal of Genetics and Genomics*, *44*(1), 51–61. https://doi.org/10.1016/j.jgg.2016.09.005

Xynias, I. N., Mylonas, I., Korpetis, E. G., Ninou, E., Tsaballa, A., Avdikos, I. D., & Mavromatis, A. G. (2020). Durum Wheat Breeding in the Mediterranean Region: Current Status and Future Prospects. *Agronomy*, *10*(3). https://doi.org/10.3390/agronomy10030432

Yahyaoui, A., Hakim, S., Al Naimi, M., Nachit, M. M., Royo, C., Di-Fonzo, N., & Araus, J. L. (2000). Multiple disease resistance in durum wheat (Triticum turgidum L. var. durum). *Durum Wheat Improvement in the Mediterranean Region: New Challenges, Zaragoza,*(January 2000), Spain, 12-Spain, 14.

Yu, J., Pressoir, G., Briggs, W. H., Bi, I. V., Yamasaki, M., Doebley, J. F., McMullen, M. D., Gaut, B. S., Nielsen, D. M., Holland, J. B., Kresovich, S., & Buckler, E. S. (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics*, *38*(2). https://doi.org/10.1038/ng1702

Yu, L. X., Lorenz, A., Rutkoski, J., Singh, R. P., Bhavani, S., Huerta-Espino, J., & Sorrells, M. E. (2011). Association mapping and gene-gene interaction for stem rust resistance in CIMMYT spring wheat germplasm. *Theoretical and Applied Genetics*, *123*(8). https://doi.org/10.1007/s00122-011-1664-y

Zeibig, F., Kilian, B., & Frei, M. (2022). The grain quality of wheat wild relatives in the evolutionary context. *Theoretical and Applied Genetics*, *135*(11), 4029–4048. https://doi.org/10.1007/s00122-021-04013-8

Zhan, J., Pettway, R. E., & McDonald, B. A. (2003). The global genetic structure of the wheat pathogen Mycosphaerella graminicola is characterized by high nuclear diversity, low mitochondrial diversity, regular recombination, and gene flow. *Fungal Genetics and Biology*, *38*(3). https://doi.org/10.1016/S1087-1845(02)00538-8

Zhu, C., Gore, M., Buckler, E. S., & Yu, J. (2008). Status and Prospects of Association Mapping in Plants. *The Plant Genome*, *1*(1), 5–20. https://doi.org/10.3835/plantgenome2008.02.0089

Zohary, D., Hopf, M., & Weiss, E. (2012). *Domestication of Plants in the Old World: The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin*. Oxford University Press.

Abstract of the thesis

Durum wheat (*Triticum durum*) is an important crop that has been used for millennia for human consumption, and modern breeding can take advantage of the wide variability useful for the adaptation to new challenges. Novel beneficial alleles can be found in wild relatives and landraces thus enhancing crop adaptation to many biotic and abiotic stresses. This dissertation considers the source of variability from both before and after wheat domestication, that caused a loss of potentially useful alleles. In particular, a panel of accessions of the progenitor specie *Aegilops tauschii* was investigated for the identification of favorable alleles for improving key agronomic and root traits, while a panel of landraces was investigated for tolerance to the septoria tritici blotch (STB) disease, determined by *Zymoseptoria tritici*.

Chapter 1. is the thesis introduction, which outlines the importance of wheat in the world, providing an historical overview of the domestication, the evolution mechanisms that led to the current forms of durum wheat and the use of wild relatives as a source of germplasm for future breeding programs is crucial. Moreover, the emergence of *Z. tritici* has been considered as the main pathogen of wheat since it contains extremely high levels of genetic variability and is thus difficult to control.

Chapter 2. Considers the contribution of the phenotypic diversity of 242 accessions of *Aegilops tauschii* from the Open Wild Wheat Consortium, involved in wheat domestication, provided with whole-genome resequencing. The accessions were phenotyped both in the field and in controlled conditions and A k-mer-based GWAS was performed to identify genomic regions involved in useful traits.

Chapter 3. Describes the genetic basis of resistance to *Z. tritici* in a durum wheat elite diversity panel representative of the germplasm bred in Mediterranean countries (Italy, Morocco, Spain, Syria, and Tunisia), Southwestern USA and Mexico. Quantitative trait loci (QTL) analysis results revealed several loci involved in the STB response that were found in several chromosome regions with a high infection rate. The genomic regions associated with STB resistance identified in this study could be of interest for marker assisted selection (MAS) in durum wheat breeding programs.