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Cytogenetic, physiological and agronomic characterization of wheat-*Thinopyrum ponticum* recombinant lines carrying relevant breeding traits

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Coordinatore: Prof.ssa Stefania Masci

Firma .....

Tutor: Prof.ssa Carla Ceoloni

Firma .....

Dottoranda: LjiljanaKuzmanović

Firma .....

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1. INTRODUCTION

1.1. The significance of wheat for world sustainable food production

Nowadays, with an ever increasing human population (160 newborns per minute, Hoisington et al., 1999), important challenges for sufficient food production have to be faced. It is expected that in developing countries, where the number of people is increasing at a faster rate, cereals will constitute more than 60% of the world consumption, and, among them, wheat and maize will account for about 80% of the cereal needs (Hoisington et al., 1999; Ortiz et al., 2008). Currently, wheat is the most cultivated cereal crop worldwide and feeds about 40% of total human population, providing about one fifth of total calories and protein requirements (FAO, 2010). At the same time, in the view of actual global climatic changes, the best adaptability of wheat to semi-arid conditions among the three most important staple crops (rice and maize included) is expected to further favour the spread of its cultivation worldwide (Hoisington et al., 1999; Dixon et al., 2009; Dodig, 2010). Consequently, wheat as a crop occupies a central place in international strategic plans aiming at assuring food supply to mankind. Yield, adaptability to biotic and abiotic stresses, as well as characterization and widening of genetic variability are fundamental aspects to systematically focus on in the process of breeding, in order to satisfy the need of increasing the crop productivity by 2% per year, and to produce enough food for expected 9 billion people to live on Earth by 2050 (Braun et al., 1998; Hoisington et al., 1999). In numbers, this means that the production should pass from 613 million tonnes in the 2005-2007 period to more than 900 million tonnes in 2050 (Rosegrant et al., 2007). In this view, different challenges, including global climate changes, emerging pathogen species and narrowing of the wheat genetic base must be faced and overcome.

1.2. Historical milestones of wheat cultivation and breeding

Wheat belongs to the genus *Triticum* and it was one of the first domesticated crops. This occurred 10000 years ago in the Fertile Crescent, an area in the Middle East including Israel, Lebanon, Syria, Iraq, Iran and south Turkey (Cavalli-Sforza, 1996). Around this area of in South-West Asia, the main centre of diversity of wheat is situated, comprising the region from the Mediterranean cost to the West to the fertile plain of rivers Tigris and

Euphrates to the East (Hoisington et al., 1999). Here many mixed native populations of different diploid species belonging to the *Triticum* genus are still found to grow, which in the course of evolution gave origin to related species with higher ploidy level, including the cultivated polyploidy wheats. From this region wheat cultivation was extended in most Europe, North Africa, and West Asia. As a result of the adaptation to new environmental conditions in different geographical zones and due to human selection based on favourable phenotypic traits, heterogeneous landraces of wheat emerged and constituted until the 18th century the base of the crop cultivation. Deciphering of the laws of genetic inheritance by Gregor Mendel in the second half of 18th century boosted the breeding of all crops and directed it towards the creation of more productive and more homogeneous varieties, promising a better and safer future.

The importance of the wheat as a crop and the efforts put in its improvement have followed a positive trend throughout the 20th century. These were primarily driven by the so-called "Green revolutions", beginning with the one led by the Italian breeder Nazareno Strampelli in the first decades of the century (Worland, 1999), followed by the one conducted in 1960's by Nobel prize winner Norman Borlaug and his CIMMYT collaborators (Borlaug, 1968). Several outstanding results were thus achieved, a major one being introgression of dwarfing genes (*Rht1* and *Rht2*) from Japanese cultivars into varieties cultivated worldwide, which had a highly beneficial effect in reducing plant height and hence eliminating lodging problems. An increase of nutrient use efficiency (source/sink ratio) and of tillering capacity was also obtained, as well as a consistent increase of harvest index. This allowed farmers to apply high doses of fertilizers without risk of crop lodging. At the world level, the average wheat yield was increasing by 1.5% per year before the Green Revolution, while afterwards (during 1966-79) it boosted up to 3.6% per year in developing countries, mainly in South Asia (Dixon et al., 2009).

Even though the overall performance of many cultivated varieties significantly improved, this was prevalently correlated with application of high inputs (pesticides, fertilizers, irrigation). Moreover, a relatively restricted number of traits was exploited in crop selection (yield + response to high inputs) and this resulted in a small range of genotypes selected in various national programs in developed and developing countries. The increase of the average yield dropped to 1.1% per year during 1995-2005 (lower than in the preceding 30-years period) (Dixon et al., 2009), thus not meeting the trend of population growth, and this marked the end of an important era in wheat cropping. This decline in productivity of both bread (*Ttiticum aestivum*) and durum wheat (*T. durum*) has

raised the need to develop new breeding programs, also by leading research institutions like CIMMYT (Ortiz et a., 2007; Dixon et al., 2009, Reynolds et al., 2011).

Current wheat breeding strategies are animated by an increasing concern on maintenance and exploitation of natural genetic resources, including native gene pools or wild relatives, also as a means of compensating for the genetic erosion which has affected several crop species, including wheat. It is now well established that the search for new genetic variability for wheat improvement is a primary requisite for potentially overcoming constant as well as newly arisen problems and for attaining significant breeding goals.

1.3. Genetic variability for wheat breeding

1.3.1. The origin of wheat genomes - genomic organisation and interspecific relationships

Both common (= bread) wheat (*Triticum aestivum* L.) and durum wheat (*T. durum*) belong to the *Triticum* genus, sub-tribe *Triticinae*, tribe *Triticeae*, family Poaceae (Graminae). This very species-rich family includes as well other important staple crops such as rice, barley, maize, rye, and their wild relatives (e.g. *Aegilops spp*. for the *Triticinae*).

Alredy in first decades of the 20th century, Kihara (1919) and Sax (1922) showed that all members of the *Triticinae* sub-tribe belong to a vast group of species sharing the same basic chromosome number x=7. The origin of the basic chromosome set has probably to be found in a common and unknown ancestor of the *Poaceae* family which had a genome with n=5 chromosomes. Recents works of grass comparative genomics showed that this genome underwent a duplication (2n=10) and structural rearrangements (translocations and fusions) to give rise to an intermediate ancestor with n=12 chromosomes (Bolot et al., 2009; Devos, 2010). Additionally, as a result of 5 fusion events, a basic genome of x=7 chromosomes present in wheat and barley genomes arose.

After the divergence from the ancestor, some 50-70 million years ago (Kellogg, 2001; Gaut, 2002) (Fig 1), the evolution driven by polyploidization and segmental duplication events gave rise to three groups of *Triticinae* species, characterized by different ploidy levels, that can still be found nowadays: diploids (2n=2x=14), tetraploids (2n=4x=28) and hexaploids (2n=6x=42) (Gill and Friebe, 2002). Hexaploid bread wheat originated 6000-8000 years ago, as a result of spontaneous hybridization of two diploid

species, the donors of the present A and B genomes of polyploidy wheats, i.e. *Triticum urartu* (2n=2x=14, genome AA) and an *Aegilops* species of the Sitopsis section, probably extinct, but closely related to *Aegilops speltoides* (2n=2x=14, genome SS) (Miller, 1987; Feldman et al., 1995). Chromosome doubling of such probably highly infertile AS hybrid resulted in a stable, allotetraploid amphiploid (*Triticum turgidum* L. var. *dicoccoides*), corresponding to the wild progenitor of durum wheat (*T. turgidum* L. var. *durum* (2n=4x=28, genome AABB). It was probably from hybridization of the domesticated allotetraploid *T. turgidum* L. var. *dicoccum* with a diploid donor of the D genome, *Aegilops tauschii* (2n=2x=14, genome DD) that the allohexaploid progenitor of today's bread wheat (2n=6x=AABBDD) originated (Fig 2).

Figure 1 Phylogenetic relationship between cereals (figure from Bolot et al., 2009)

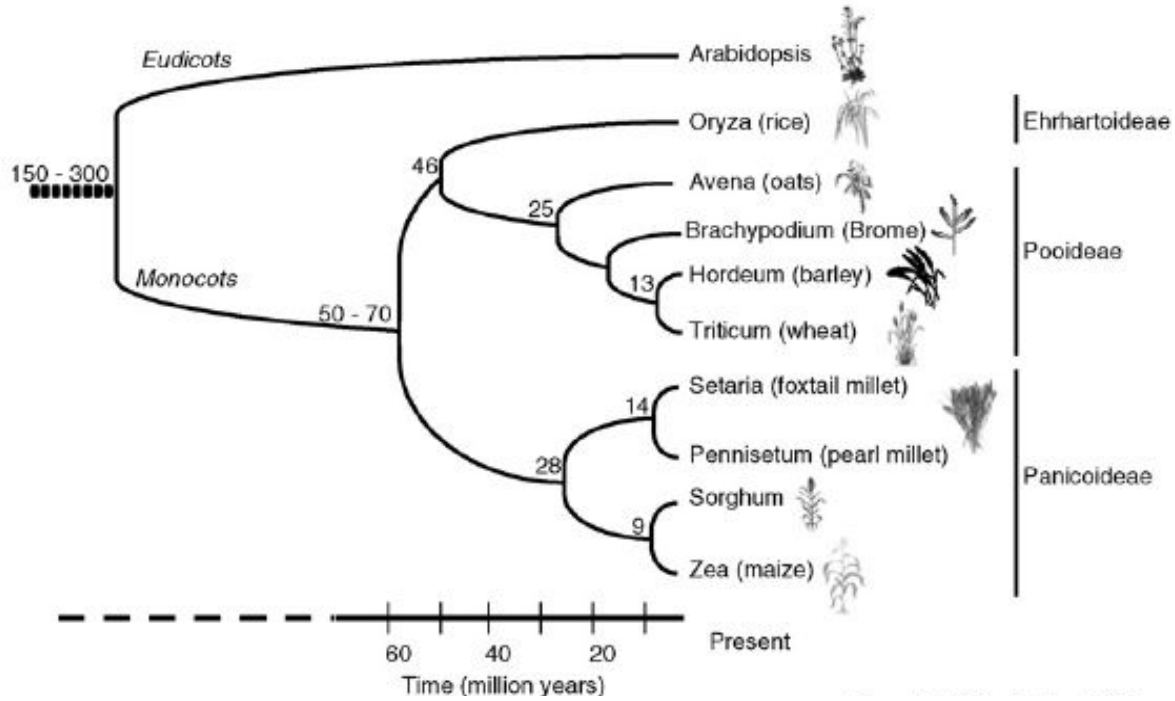
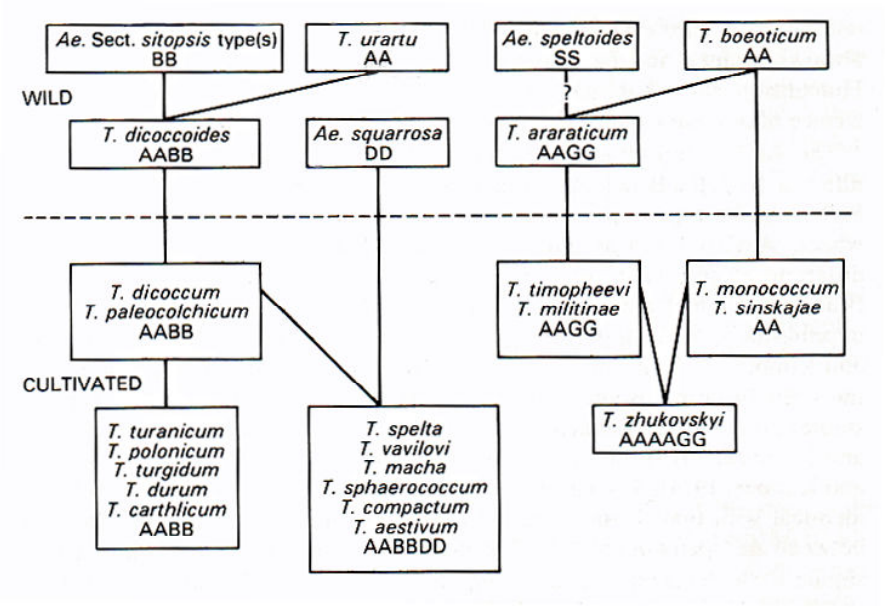
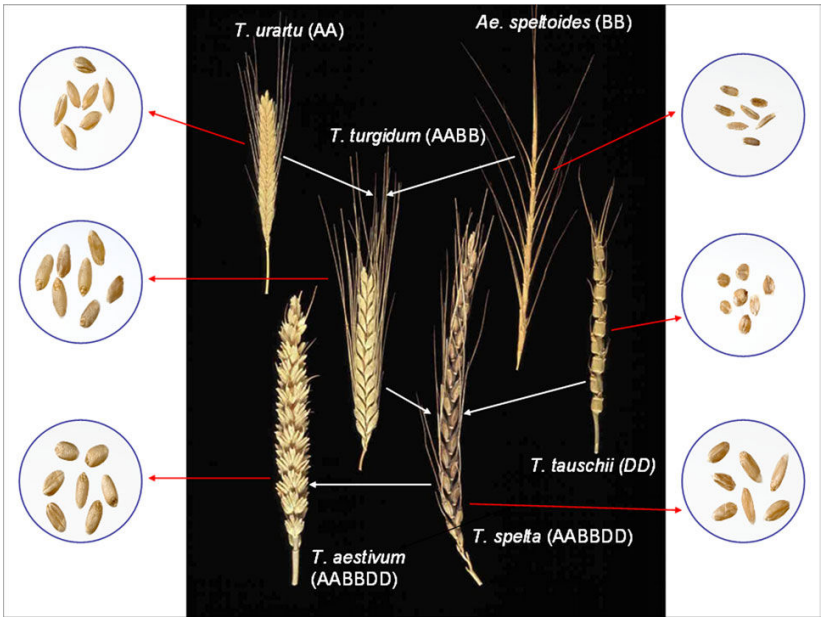


Figure 2 Evolution of polyploid wheats (a) and spikes and kernels of the main species involved (b) (figures from Miller, 1987; Shewry, 2009)



(a)



(b)

In allopolyploid wheats, each chromosome of a specific genome (e.g. A) has its corresponding chromosome in the other genomes (BD), defined "homoeologous" (partially homologous). Given the basic chromosome number of 7, there are 7 groups of homoeology[1] i.e. 7 groups of homoeologous chromosomes. The allopolyploid nature of

[1] **Homoeologous** refers to the relationships of chromosomes that share only partial homology and are brought together after inter-species hybridization or allopolyploidization.

wheats represents *per se* an important source of variability and, indeed, was a strong driving force during their evolution, allowing them to tolerate extensive cytogenetic, genetic and epigenetic changes that shaped their genomes and made them more adaptable and competitive than the diploid progenitors (Feldman and Levy, 2009). Of paramount importance was establishment of meiotic behaviour similar to that of diploids, which guaranteed regularity in disomic transmission of traits (Feldman et al., 1995). In fact, although chromosomes of two or three genomes are similar, there are always 14 or 21 bivalents formed at meiosis. This and other relevant modifications leading to genome stabilization and full fertility were stably fixed owing to the self-pollination type of reproduction.

The polyploidy condition confers to wheat genomes considerable buffering capacity toward even conspicuous alterations. This permitted an extensive cytogenetic characterisation of such species, probably the deepest ever carried out in plant species. After the initial work of Kihara (1919) and Sax (1922) on chromosome pairing behaviour, E.R. Sears started his work on the creation and selection of aneuploid lines (Sears 1952, 1954, 1966). Among a wide array of aneuploid types, stable wheat lines lacking a single chromosome pair, functionally compensated with an extra dose of another pair of the same group (the so called nulli-tetrasomic lines), were isolated, which have been essential tools to establish the homoeologous relationships among wheat chromosomes. Moreover, addition and substitution lines carrying single chromosomes or chromosome arms originating from genomes of alien species were also produced, and these showed that homoeology exists even between chromosomes of the cultivated allopolyploids and their more or less close relatives (Feldman and Sears, 1981).

In addition to the existing homoeology between *Triticeae* genomes, a remarkable aspect of their genome relationships is conservation of gene synteny[2] and also colinerity, which is also of relevance for the exploitation of alien variability (see ahead, § 1.3.2.). During the 70 million years of evolution, genomes of all members of the *Poaceae* family underwent important structural and functional changes that resulted in species with very different DNA contents, ranging from 320 Mb in *Brachypodium* genus to 2500 Mb in maize (Devos, 2010). Wheat genomes evolved at different rates: for example the D genome

[2] **Synteny** represents the preserved order of genes between related species originating from the same ancestor. In a wider way it represents only the co-localization of genes on the same chromosome that are not obligatory linked, but as to related species, the shared synteny is one of the most important criteria for establishment of orthologous genomic regions.

"suffered" from a sizable reduction in DNA amount compared to genomes A and B (Feldman and Levy, 2009), but, on the other hand, gene number remained more or less similar, as well as colinearity between them, especially for most adaptive genes (Devos and Gale, 2000). As a whole, complementing cytogenetic studies, recent advances in development of genetic and physical maps, DNA sequence availability of the most important grass species and comparative genomic analysis, confirmed a high level of orthology and colinearity of homoeologous chromosomes of *Triticum* and even Poaceace genomes (Devos and Gale, 2000; Akhunov et al., 2009; Devos, 2010).

1.3.2. **Alien variability**

Although the domestication of crops had an enormous importance for mankind in social and economic terms, for what species variability is concerned it inevitably provoked a bottleneck that became more pronounced with modern breeding (see § 1.2.).

The concept of the "smart breeding" (McCouch, 2004), however, is currently inspiring novel breeding approaches. In essence, it supports an intelligent use of natural resources, taking into account present needs and efficiently employing new technologies. Genetic erosion could thus be counteracted and variability greatly increased by resorting to wild and cultivated relatives as donors of useful traits (e.g. yield components, Reynolds et al., 2001), drought (Dodig, 2010), other stress tolerance (Takeda and Matsuka, 2008). Wheat belongs to a taxonomic family that is very rich of species with more or less related genomes, from which traits of interest can be transferred into cultivated wheat varieties by recombination. Indeed, in wheat breeding the "smart breeding" approach has already proved to be a good way to follow (Friebe et al., 1996; Hoisington et al., 1999; Gennaro et al., 2007).

As it will be discussed in details ahead (§ 1.4.1.), the first necessary step to be done is the identification and characterization of alien donor genomes and individual chromosomes of them, and establishment of their cytogenetic relationships with those of the recipient species. In fact, the success in interspecific gene transfer depends on the evolutionary distance between species. As reported in Friebe et al. (1996), the available genetic resources for wheat breeding can be divided into three gene pools (primary, secondary and tertiary) according to their homology/homoeology relationships and the ease to recombine with wheat chromosomes, in line with the classification given by Feldman (1979) (Tab 1).

Table 1 Triticinae species grouped according to their phylogenetic relationship with bread and durum wheat genomes (Feldman, 1979)

Type of intergenomic affinity	Species	Genome
I. Species sharing homologous genomes		
1. the closest progenitors	*T. turgidum var. dicoccoides*	AB
2. diploid donors of A genome	*T. monococcum var. boeticum*	A
	T. monococcum var. urartu	A
	T. tauschii (Ae. tauschii, Ae squarrosa)	D
3. polyploids sharing one genome		AG, AAG
a. A genome	*T. timopheevii*	DM^{cr}
b. D genome	*T. crassum (=Ae. crassa)*	DM^{v}
	T. ventricosum (=Ae. ventricosa)	CD
	T. cylindricum (=Ae. cylindrica)	$DM^{cr}C^{u}$
	T. juvenale (=Ae. juvenalis)	$DM^{cr}S^{l}$
II. Species sharing homoeologous genomes		
1. Species closely related	*T. searsii (=Ae. searsii)*	S^{s}
	T. longissimum (=Ae. longissima)	S^{l}
	T. bicorne (=Ae. bicornis)	S^{b}
	T. speltoides (=Ae. speltoides)	S
	T. variabilis (=Ae. . variabilis)	$C^{u}S^{v}$
	T. kotschyi (=Ae. kotschyi)	$C^{u}S^{v}$
	T. tripsacoides (=Ae. mutica)	M^{t}
2. Species less closely related	*T. dichasians (=Ae. caudata, Ae. markgrafii)*	C M
	T. comosum (=Ae. comosa)	M^{u}
	T. uniaristatum (=Ae. uniaristata)	C^{u}
	T. umbellulatum (=Ae. umbellulata)	$C^{u}M^{o}$
	T. ovatum (=Ae. ovata)	$C^{u}M^{t}$,
	T. triaristatum (=Ae. triaristata)	$C^{u}M^{t}M^{t2}$
	T. macrochaetum (=Ae. biuncialis)	$C^{u}M^{b}$
	T. columnare (=Ae. columnaris)	$C^{u}M^{c}$
	T. triunciale (=Ae. triuncialis)	$C^{u}C$
	Some *Agropyron (Thinopyrum)* species	
3. Species not closely related	Spp. belonging to genera *Secale, Haynaldia*, some *Agropyron (Thinopyrum, Elymus)* species and other *Triticinae* genera	

Species belonging to the primary gene pools share full homology with wheat genomes and have high frequency recombination rate (all hexaploid and tetraploid wheat accessions in gene banks, land races, and the donor species of the A and D genomes).

The secondary genetic pool consists of mostly polyploid species that have one or more shared genomes but overall reduced recombination rates with wheat. Gene transfer in fact is possible only between homologous chromosomes. Together with closely related *Triticum/Aegylops* species, diploid S-genome species from the *Sitopsis* section (S≈B genome donor) are included as well in the secondary gene pool.

The tertiary gene pool includes diploid and polyploid species that do not share any homologous genome with wheat and the gene transfer cannot occur by homologous recombination with wheat; in such cases, the tools of "chromosome engineering" (see later in the text) must be applied. Some of the most important genera for wheat-alien transfer from this group are *Secale* and *Agropyron.* This gene pool has been so far mainly exploited for transfer of disease resistance genes, such as the case of the famous 1BL.1RS translocation (Villareal et al., 1991).

1.4. Methods and tools for wheat-alien introgression

Genetic variability of wheat relatives started to be rather extensively explored and used for the transfer of useful genes since the mid 20th century (Friebe et. al., 1996; Ceoloni et al., 2005b); however, effective exploitation of such a rich gene reservoir can be still considered at its onset, coinciding with efficient use of recent molecular genetic and cytogenetic tools (Ceoloni et al., 1998; Varshney et al., 2006; Jiang and Gill, 2006; Qi et al., 2007).

In principle, the choice of the most suitable procedure for interspecific and intergeneric gene transfer depends primarily on whether the alien chromosome(s) carrying the desirable gene(s) is completely or only partially homologous (= homoeologous) to the corresponding chromosome(s) of the recipient species. Using this criterion, different methods for the exploitation of alien variability can be explored for incorporation of as much as the entire alien genome, particularly in the form of chromosome-doubled hybrids, i.e., amphidiploids, down to a single chromosome or chromosome-arm pair (either added or substituted), or just a small chromosomal segment (Feldman 1983; Gale and Miller 1987; Ceoloni 1987; Ceoloni and Jauhar, 2007; Qi et al., 2007). In practice, however, only rarely have sizable wheat-alien chromosome manipulations resulted in valuable materials for breeding, particularly when durum wheat is the recipient (Ceoloni and Jauhar 2006).

Synthetic amphiploids have generally proved inferior to established crops. In this respect, the man-made cereal, triticale – the amphiploid derivative from the cross between tetraploid *T. durum* and rye, *Secale cereale* L. – probably represents the only exception. In fact, in spite of a greatly stabilized pairing behavior and hence largely recovered fertility with respect to the corresponding F_1 hybrids, the excessive content of undesirable genes from the alien parent makes synthetic amphiploids unable to compete with well adapted wheat cultivars. However, they have been usefully employed as bridging material for the transfer of specific alien genes into wheat. They can also serve as valuable starting points for the production of alien chromosome addition and substitution lines in a wheat background (Feldman and Sears, 1981), which, in turn, serve as ideal materials for the introduction of alien sub-chromosomal segments. Such a sequence of steps, leading to a progressive reduction of unwanted genetic contribution from the alien donor, and allowing early verification of the alien gene expression in wheat, indeed represents the most elegant layout for targeted chromosome engineering (see ahead).

However, additions or substitutions of alien chromosomes or even chromosome arms to wheat, particularly tetraploid durum wheat (Ceoloni et al. 2005b), generally cause detrimental effects, including lower cytological stability and plant fertility. Consequently, a genome-sustainable and breeder-friendly approach for taking advantage of genetic resources present in alien Triticeae consists of aiming at the introduction of alien chromosomal segments of as much reduced entity as possible.

1.4.1. Chromosome engineering

The main cytogenetic procedures that permit to engineer the wheat genome with small alien chromosomal introductions were originally delineated and first applied already several decades ago. It was in 1972 that E.R. Sears, the "father" of such methodologies, coined the term "chromosome engineering" as illustrative of *"the transfer of segments of alien* chromosomes *carrying particular desired genes to wheat chromosomes"* (Sears 1972). Although in its widest sense, the term can be applied to any wheat-alien transfer, irrespective of the degree of relatedness between alien donor and wheat recipient chromosomes, it is for transfers between homoeologous chromosomes that the need for inducing exchanges between such chromosomes arises, given the virtual absence of pairing and recombination between them, as in the case of homoeologous chromosomes of the wheat genomes recalled above (see § 1.3.2.).

In its milestone paper of 1972, Sears reviewed the various approaches for wheat-alien transfer applicable when donor species are more distantly related to wheat (species belonging to the tertiary gene pool). In principle, the more distant the alien species from the cultivated crop, the greater the possibility of introducing totally new genetic material into the cultivated background, and, indeed, in many instances important traits from such species have been targeted for wheat improvement (see, e.g., Fedak, 1984; Friebe et al. 1996; see also next paragraph). The different strategies included use of irradiation treatment (Sears, 1956; Knott, 1961; Sharma and Knott, 1966), spontaneous breaking and fusion of different chromosomal arms (Mettin et al., 1973; Zeller, 1973), and induced wheat-alien homoeologous pairing and recombination. As to the latter, in addition to use of high-pairing accessions of of some wild species, such as *Ae. speltoides*, to induce recombination with wheat (Riley et al., 1968), genetic manipulation of the wheat pairing control system has been indicated, and indeed consistently proved to be, as the most effective way to obtain successful wheat-alien transfers.

Since the early 1960s, several studies (e.g. Riley, 1960; Sears, 1976; Feldman, 1993) showed suppression of homoeologous pairing in wheat to be due to the action of a complex genetic system, with the *Ph1* gene, inherited dominantly and located on the long arm of chromosome 5 of genome B, exerting the strongest effect (=5BL, Riley and Chapman,1958; Sears and Okamoto, 1958). Fixation of this gene in the primitive tetraploid wheat (and later of additional, though less potent ones at the hexaploid level) represented an essential step in the cytological diploidization of the newly arisen polyploids. However, other mechanisms, consisting of rapid genetic and epigenetic changes brought about concurrently with or immediately after the formation of the polyploids, accentuated the physical divergence among homoeologues, hence probably providing the physical basis for the diploid-like meiotic behavior of polyploid wheats, later reinforced by the *Ph1* system (Feldman et al. 1997; Liu et al., 1998a; Ozkan et al., 2001; Shaked et al., 2001; Kashkush et al., 2002)). The mode of action of *Ph1* is not yet fully understood, but there are evidences that it regulates the recognition between homologous chromosomes at the level of individual heteroduplexes (Luo et al., 1996). In fact, in the absence of *Ph1*, homoeologus chromosomes, both of wheat and of its hybrids with alien Triticeae, do recognize their partial homologous (homoeologous) regions, and thus pair and recombine, even though at lower rate compared to completely homologous chromosomes. The development of chromosome engineering was boosted by isolation of mutant lines for the *Ph1* gene in

bread (Sears, 1977) and durum wheat (Giorgi, 1983), which allow the access to homoeologous genomes of alien species from the *Triticeae* tribe.

Being the result of recombination, the products of *ph1*-based chromosome engineering are generally balanced from the cytogenetic and functional viewpoints. Another advantage of this approach is that wheat-alien primary recombinant chromosomes can be further manipulated by successive cycles of induced homoeologus recombination, aiming at reducing the amount of introgressed chromatin. This consequently leads to the decrease of possibly unwanted linkage drag, particularly when the donor species is a wild species. The way in which this can be done depends on the plant material available and the localization of the gene(s) of interest. If the gene is localized in a distal position, where the gene density and frequency of recombination are higher, a single interchange between donor and recipient chromosomes could be sufficient to produce wheat chromosomes containing short alien segments. Proximal localization of the target gene(s) may not allow the creation of the desired recombination by a single event, since the proximal chromosomal regions have lower recombination frequencies. A single event could lead to a significant portion of the recipient chromosome arm being replaced by the alien chromatin, probably giving a recombinant product of low practical value. As an alternative to repeated cycles of induced recombination, which would involve the majority of wheat homoeologues and cause overall genome instability, homologous recombination between two primary recombinant chromosomes, having a "complementary' wheat and alien chromatin distribution would work to achieve the desired result. In practice, if both primary recombinants are the result of a single recombination event, one of them having the wheat-alien exchange site proximal and the other distal to the gene of interest, an interstitial alien segment harboring the desired gene can be obtained by crossing-over in the shared, alien homologous region (Sears, 1983; Ceoloni et al., 2005a). The result obtained would be equal to the one produced by a double interchange between homoeologous chromosomes, a very rare event even under *ph1* permissive conditions (Lukaszewski, 1995).

There are numerous examples of successful use of chromosome engineering for the transfer of useful traits from alien relatives to cultivated bread and durum wheat (see e.g. Ceoloni, 1987; Gale and Miller, 1987; Friebe et al., 1996; Ceoloni et al., 2005a). In some cases, introgressions have been firstly obtained in bread wheat and then a selected subset of them transferred into durum wheat. Tetraploid durum wheat is in fact less tolerant to chromosome alterations and makes more difficult the transfer of desired genes if relatively large amounts of alien chromatin remain associated with them.

1.5. *Thinopyrum ponticum* – valid source of useful genes for wheat improvement

So far, different wild species belonging to the tertiary gene pool of Triticinae have been used for breeding, mainly for the transfer of genes determining disease resistance and abiotic stress tolerance (reviewed in Fedak, 1984; Friebe et al., 1996, Ceoloni et al., 2005b). One such species is the wild wheat relative *Thinopyrum ponticum*.

1.5.1. The genus Thinopyrum

Thinopyrum is a genus of *Triticeae* tribe that has significantly contributed to both bread and durum wheat improvement. The exploitation of different *Thinopyrum* species started in 1920's and since then several genes (mainly for resistance to biotic and abiotic stresses) were introduced into different wheat germplasm. Since that time Dr. N.V. Tsitsin from the former USSR (Union of Soviet Socialist Republics) demonstrated that *Thinopyrum* species could hybridize with relative ease with *Triticum* species (Chen, 2005; Li and Wang, 2009).

In the 1980's, about 20 species, formerly classified into different genera (e.g. *Agropyron, Elytrigia*) were grouped into a separate genus, i.e. *Thinopyrum. Thinopyrum* species possess different amounts of the J genome, which originates from *Lophopyrum* and partly *Elytrigia* genera (reviewed in Chen, 2005). There are five levels of ploidy present in genus *Thinopyrum* (Tab 2), from diploidy to decaploidy, and species with different ploidy have been exploited for creation of numerous wheat-*Thinopyrum* amphidiploids. The decaploid *Th. ponticum* represents the target species of the work presented in this thesis and will be the main subject of the following paragraphs.

Table 2 Ploidy levels of main *Thinopyrum* species [Dewey, 1984; Chen (2005))

Level of ploidy	Species
1. diploid, 2n=14	*Th. bessarabicum*, [Savul. and Rayss] A. Love
	Th. elongatum [Host] D. Dewey
2. segmental tetraploid, 2n=28	*Th. junceforme* [Love and Love] Love
	Th. distichum [Thunb.] Love
3. segmental allohexaploid, 2n=42	*Th.intermedium* [Host]Barkwarth and D. Dewey
	Th. junceum [L.] Love
4. segmental octoploid, 2n=56	*Th. runemarkii* Love
5. segmental decaploid, 2n=70	*Th. ponticum* [Podp.] Barkwarth and D. Dewey

1.5.2. Genomic constitution of *Thinopyrum ponticum*

Tall wheatgrass, *Thinopyrum ponticum*, 2n=10x=70 (syn. *Agropyron elongatum* [Host] Beauvois, *Lophopyron ponticum* [Podp.] A. Love, *Elytrigia elongata* [Host] Nevski, *E. pontica* [Podp.] Holub.) is a perennial wild relative of wheat of a particular interest for bread and durum wheat breeding. Thanks to an extensive work in the last 70 years, diversified pool of addition, substitution, translocation and recombinant wheat-*Th. ponticum* lines have allowed rather good cytogenetic, molecular and physiological characterization of the species and useful traits harboured in its genome. To identify the component genomes of the whole decaploid chromosome set, in addition to chromosome pairing studies and use of banding techniques, the development of fluorescence in situ hybridization (FISH) and, particularly, genomic in situ hybridization (GISH) techniques (§ 1.7.) has been of fundamental importance. In particular, by use of the S (=St) genome from the closely related genus *Pseudoroegneria* as probe in GISH led to the proposal, now largely accepted, of a haploid constitution of *Th. ponticum* as $JJJJ^sJ^s$ (Chen et al. 1998a; Chen, 2005). As described by this genome formula, *Th. ponticum* is likely to be a segmental autoallo-decaploid, with three sets of the J genome, originating from *Th. bessarabicum* (also designated as E^b), and two sets of the J^s genome, a modified J genome with partial homology (in the centromeric chromosome regions) with the S genome (Zhang et al., 1996; Chen et al., 1998a; Fedak et al., 2000). Genomes J and S are closely related and S-genome chromosomes readily pair with J and J^s chromosomes, (Chen, 2005). The exact origin of the J^s genome is unknown; even if it originated from the J genome of *Th. bessarabicum* or the E genome of *Th. elongatum*, important genome modifications must have occurred during speciation. In any case the J^s genome is a very valuable source of useful genes, such as resistance genes to viral and fungal diseases (Chen et al., 1998b; Chen, 2005). As to relative affinity with wheat ABD genomes, both the S and J genomes are genetically mostly similar to the D genome, less to the A genome and even less to the B genome (Liu et al., 2007).

Prior to the described, recent classification, chromatin of the whole *Thinopyrum* genus was designated in literature as “Ag” or “el”, referring to previous genus nomenclature (*Agropyron, Elytrigia*). In any case, also at present such designations are frequently used (and it will be so in the present thesis), particularly when affiliation of a particular chromosome to a specific genome (J or J^s) is not established.

1.6. *Th. ponticum* traits of interest for wheat improvement

1.6.1. Perennial habit

Two *Thinopyrum* species extensively used in wide crosses with wheat, are the hexaploid intermediate wheatgrass *Th. intermedium* and the decaploid tall wheatgrass *Th. ponticum* (see, e.g., Li and Wang, 2009). These are both perennial species and in fact the perennial habit of wheatgrasses was the first trait that brought to attention the genus *Thinopyrum.* The first interspecific hybridization in 1920's by N.V. Tsitsin was intended to confer perennial habit to wheat cultivars. Creation of perennial wheat appears to have today a relevant potential in many depleted environments, affected by drought, soil degradation, erosion and exhaustive cropping systems (Bell et al., 2010; Cox et al., 2010). The possibility of introducing this character from *Th. ponticum* and *Th. imtermedium* into wheat would have many positive effects on environment preservation, reduction in use of fuels, pesticides, better water and nutrient use from deeper layers in depleted soil, and in pest management, as these species harbor useful resistance genes. *Th. ponticum* is already being grown as a forage crop and it is found to be persistent in drought-prone environments in Australia (Bell et al., 2010). Characterization of wheat-*Thinopyrum* perennial hybrids has been quite extensive, particularly in the USA and Australia (Cai et al., 2001; Cox et al. 2002; Cox et al., 2005; Bell et al., 2010), but few useful hybrids have been isolated so far.

1.6.2. Resistance genes

Certainly the most numerous and effective gene transfers already made from *Thinopyrum* species into wheat lines are those conferring resistance to wheat diseases, including leaf, stem and stripe rust (caused by *Puccinia triticina, P. gramminis* f. sp. *tritici* and *P. striiformis*, respectively), powdery mildew (*Blumeria graminis*), barley yellow dwarf virus (BDYW), wheat streak mosaic virus (WSMV) and its vector, the wheat curl mite (WCM) (Tab 3.; Friebe et al., 1996; Li and Wang, 2009). In addition, some accessions of *Th. ponticum* and translocation lines derivated from them, as well as a number of interspecific hybrids harbor resistance to Fusarium Head Blight (FHB) (*Fusarium graminearum* Schwabe and other *Fusarium spp.*) (Kibirige-Sebunya and Knott, 1983; Olivier et al., 2005). Recently, to the list of resistance genes present in *Th. ponticum*, resistance to eyespot (*Oculimacula yallundae*), to common root rot (*Bipolaris sorokiniana*),

Cephalosporium stripe (*Cephalosporium gramineum*), to tan spot (*Phrenophora tritici-repentis*) and to Stagonospora nodorum blotch (*Phaeospaeria nodorum*) have been added (Li and Wang, 2009).

Table 3 Genes transferred from *Thinopyrum ponticum* (genome symbols "Ae" or "Ag") and *Th. intermedium* (genome symbol "Ai" or "E") into wheat (adapted from Li and Wang, 2009) (*Lr* – leaf rust, *Sr* – stem rust, *Pm* – powdery mildew, *Bdv* – BYDV virus, *Wsm* – WSMV virus, *Cmc* – WCM virus).

Gene	Source	Chromosomes involved (wheat-*Thynopirum*)	Cultivar or line
Lr19	*Th. ponticum*	7DL-7eL	Agata
		7D-7Ag#1	Sears transfer 7Ag No.1
Lr24	*Th. ponticum*	3DL-3AeL	Agent
		3D-3Ag1	Sears transfer
		T1BL.1BS-3AeL	Amigo
Lr29	*Th. ponticum*	CS 7D/Ag#11	Sears transfer 7Ag No.11
		7DL-7AeL.7AeS	RL6080
Lr39	*Th. intermedium*	T1DS.1DL-7AiL	T25
		T2AS.2AL-7AiL	W49 (=T33)
		T3DL.3DS-7AiL	T4
		T5AL.5AS-7AiL	T24
		T6DS.6DL-7AiL	RL6097
Sr24	*Th. ponticum*	3DL-3AeL	Agent
		3D-3Ag#1	Sears transfer
		T1BL.1BS-3AeL	Amigo
Sr25	*Th. ponticum*	7DL-7AeL	Agatha
		7D-7Ag No.1	Sears transfer 7Ag# No.1
		7A/7AeL No.12	Sears transfer 7Ag# No.12
		T7DS.7DL-7AeL	
Sr26	*Th. ponticum*	T6AS.6AL-6AeL	
		6A-6AeL	
Sr43	*Th. intermedium*	7DL-7AeL.7AeS	
		7D.7DL-7AeL	
		7DS-7AeL	
Sr44	*Th. intermedium*	T7DS-7AiL.7AiS	
Pm40	*Th. intermedium*	7BS	GRY19
Pm43	*Th. intermedium*	2DL	CH5025
Bdv2	*Th. intermedium*	T7DS.7DL-7AiL	TC14
		7DS-7AiS.AiL	TC5, TC6, TC8, TC9, TC7
		T1BS-7AiS.AiL	
Bdv3	*Th. intermedium*	7DS.7DL-7EL	P961341 (PI 635118)
Wsm1	*Th. intermedium*	T4DL.4AiS	CI 17884
		T4DL.4AiS	KS93WGRC27
		T4AL.AiS	CI 17766
		A29-1-13-2	
Cmc2	*Th. ponticum*	T6DL.6AeS	
		T5BL.6AeS	
		T6AL.6AeS	

1.6.2.1. The transfer of an important *Th. ponticum* resistance gene to bread and durum wheat: the case of *Lr19* and the associated *Yp* gene

Up to date more than 45 resistance genes have been identified toward leaf rust (*Lr* genes), one of the most damaging wheat diseases, caused by the fungus *Puccinia recondita* f. sp. *tritici*. However, the pathogen has developed virulence against most of them. From the analysis of about 30 near-isogenic lines (NILs) available in the background of the susceptible bread wheat variety Thatcher, each one harbouring a different *Lr* resistance gene, tested every year in different environments to assess the efficacy of the genes, it appears that only the lines carrying the *Lr9* and *Lr19* genes have been durably resistant over the years against this pathogen in many countries worldwide (Masterhazy et al., 2000; Pasquini et al., 2001; Singh et al., 2004; Ordoñez and Kolmer, 2008).

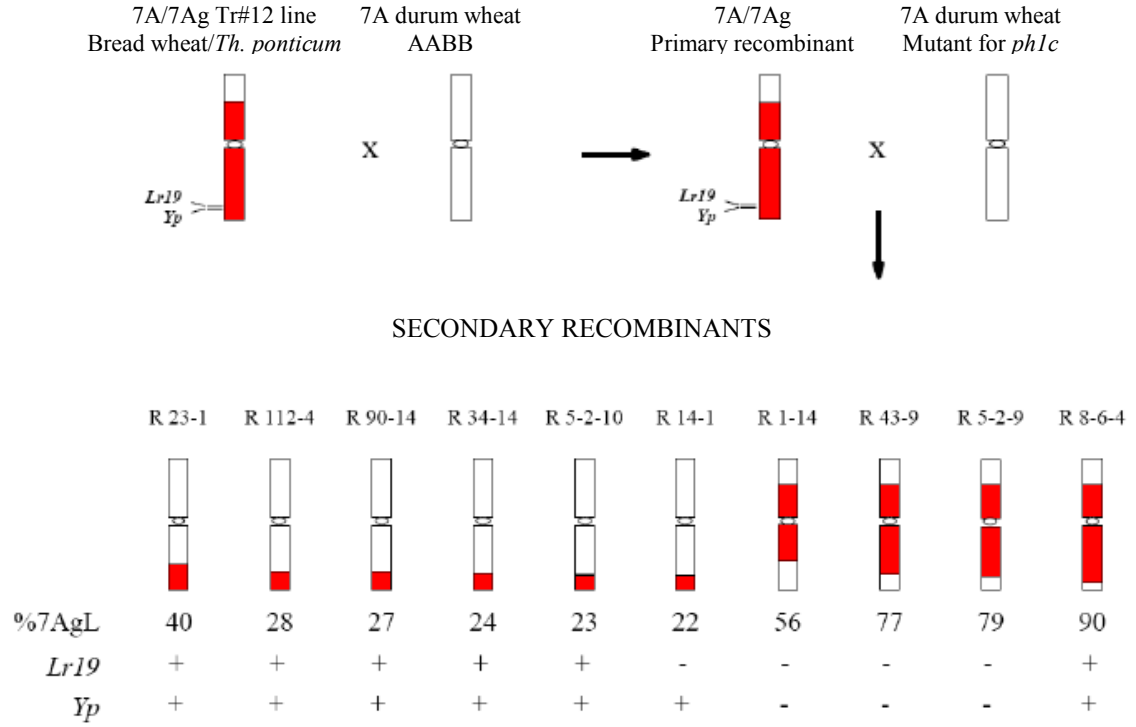
Both of the genes originate from wild *Triticinae* species, *Lr9* from *Aegilops umbellulata* (2n=2x=14) and *Lr19* from *Th. ponticum* (Friebe et al., 1996). The transfer of the *Lr19* gene from *Th. ponticum* into wheat started in 1960's. It was initially obtained in bread wheat by irradiation treatment, and a translocation line named T4 (=Agatha) was isolated, containing about 70% of the alien 7Ag (=$7el_1$) chromosome on the long arm of wheat 7DL (Sharma and Knott, 1966; Friebe et al., 1996; Kim et al., 1993). The line was characterized by a resistant phenotype to leaf rust, determined by the *Lr19* dominant gene, by the presence of a linked gene for stem rust resistance (*Sr25*), and, also in tight association, of a gene increasing yellow endosperm color (denominated *Yp* = Yellow pigment) (Knott, 1968). In subsequent genetic analysis, the *Yp* gene was localized more distal with respect to *Lr19* (Bournival et al., 1994). The yellow flour trait that the *Yp* gene introgression into wheat determined was not desirable for bread wheat breeding, and, in fact, Knott (1980) tried to separate *Lr19* from *Yp* by application of mutant agents; however, the attempt was not satisfactory. Sears (1973), starting from the same source, i.e. a disomin 7Ag(7D) substitution line named Agrus, used the absence of the *Ph1* gene to induce pairing between chromosome 7Ag and its wheat homoeologues. A series of recombinant lines were obtained, all but one involving wheat chromosome 7D. The remaining line, called Transfer N^{o}12, had recombination on wheat chromosome 7A (Eizenga, 1987). However, all recombinant lines showed the undesirable yellow flour trait (Sears, 1978).

On the other hand, the rather tight association between *Lr19* and *Yp* looked useful for durum wheat improvement, since the yellow color is a much appreciated trait for semolina and pasta products. The successful transfer of the *Lr19+Yp* genes into durum has been achieved by the Laboratory for Plant Cytogenetics at University of Tuscia.

For the combined transfer into tetraploid durum wheat, Sears' line Transfer N°12 (=Tr#12) looked as a particularly suitable donor, carrying already the *Lr19+Yp* genes on a 7A chromosome, from which they could be, in principle, homologously into chromosome 7A of durum wheat. However, from the selfed tetraploid line obtained in this way (called "primary" recombinant), which was initially was heterozygous for the 7AgL-7AL transfer, none of the plants resulted homozygous for the the recombinant chromosome (Ceoloni et al., 1996). Subsequent GISH analysis showed that the introgressed *Th. ponticum* chromatin was much bigger than what was estimated previously (Sears, 1973, 1978), replacing the whole long are and almost the half of the short arm of the 7A chromosome. As a result of the excessive length of the alien segment, male transmission of the recombinant chromosome was compromised, approaching zero. This confirmed that tetraploid durum wheat genome is less tolerant to conspicuous chromosomal alterations compared to hexaploid bread wheat. Thus, the 7AS.7AL-7AgL chromosome (from Tr#12) had to be engineered to reduce the alien portion, yet retaining the desired genes (Ceoloni et al., 2005a).

This was carried out by induced homeologous pairing. Thus, after the cross and backcross between the "primary" recombinant and the durum wheat variety Creso carrying the *Ph1* gene mutation (*ph1c/ph1c*), a set of ten "secondary" recombinant lines was obtained with a 7AL-7AgL recombination frequency of about 2.5% (Ceoloni et al., 2005a). Characterization by GISH revealed the different recombinant chromosomes to contain from 22% to 90% of the original Tr#12 *Th. ponticum* chromatin on 7AL (Fig 3). Comparison between the various recombinants, phenotypically different with respect to resistance/susceptibility to leaf rust, allowed localization of the *Lr19* gene in the 25% most distal region of the long arm of the recombinant 7A, more precisely in the portion differentiating recombinant lines R5-2-10 (23% distal 7AgL, resistant) and R14-1 (22% distal 7AgL, susceptible). The *Yp* gene was apparently located in the most distal 22%, being present in both lines.

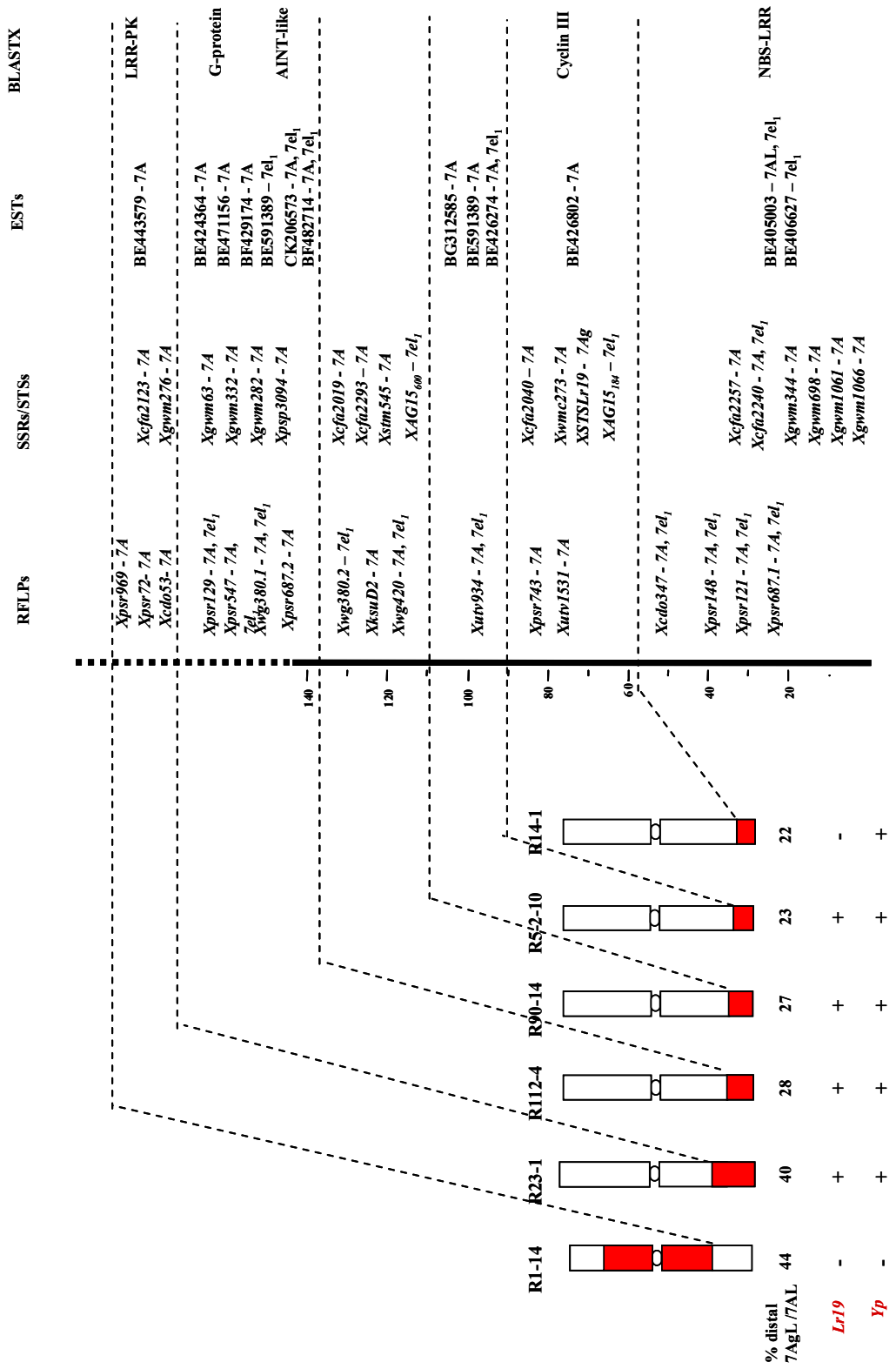
Figure 3 Production of recombinant durum wheat lines carrying different amounts of *Th. ponticum* 7Ag chromosome on wheat 7A chromosome. The approximate location of *Lr19* (leaf rust resistance) and *Yp* (Yellow pigment) genes is indicated along 7AgL. Physical maps were obtained by means of GISH (Ceoloni et al., 2005a).



This material has been genetically and cytogenetically characterized in details. Rather dense genetic maps of the 7A-7Ag chromosome contained in each recombinant line have been developed with a variety of molecular markers, allowing an easy follow-up of the transferred DNA segments in segregating progeny from crosses between different recombinant lines or between these lines and cultivated genotypes (Fig 4).

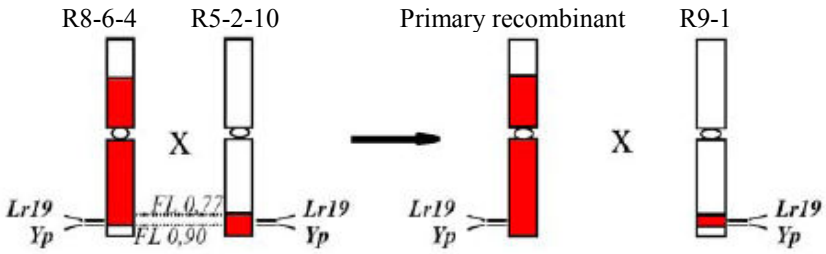
The R5-2-10 line, which contains the smallest 7AgL segment still harboring the *Lr19+Yp* genes, was selected in particular as good material for further practical use. Several analyses have shown that the presence of 7AgL segment does not have any negative effect in terms of stability, including gametic transmission of the carrier chromosome, as well as on agronomic performance. In fact the durum wheat variety Cincinnato, a derivative of R5-2-10, has been recently registered in the Italian National Variety list, hence released for cultivation.

Figure 4 Genetic and physical maps of distal 7AL/7AgL regions: a high resolution mapping



Notably, the distribution of chromatin in R5-2-10 line (77% 7AL + 23% distal 7AgL) is such that in the region of interest it overlaps to part of 7AgL present in line R8-6-4 (90% 7AgL+10% distal 7AL) (Fig 3), both recombinant chromosomes carrying *Lr19+Yp* genes. This allowed a further reduction in the quantity of 7AgL chromatin, resulting in the development of a new ("tertiary") recombinant line, R9-1, with an interstitial and shortened 7AgL segment.. In the cross between lines R8-6-4 and R5-2-10, homologous recombination occurred within the about 13% of 7AgL chromatin shared by the two recombinant chromosomes (Fig 5). As a consequence, R9-1 has the same proximal limit of the 7A-7AgL chromosome as in R5-2-10 line (FL = fractional length with respect to the centromere = 0.77) and the distal limit corresponding to the one present in R8-6-4 line (FL = 0.9). Similarly to R5-2-10, R9-1 proved to be very stable across generations and to give a remarkable performance in field conditions (Gennaro et al., 2003 and unpubl.). It is thus being used in breeding programs aimed at exploiting *Lr19* and *Yp*.

Figure 5 Creation of the "tertiary" recombinant durum wheat line R9-1, having an interstitial portion of 7AgL chromatin harboring *Lr19+Yp* genes (Ceoloni et al., 2005a)



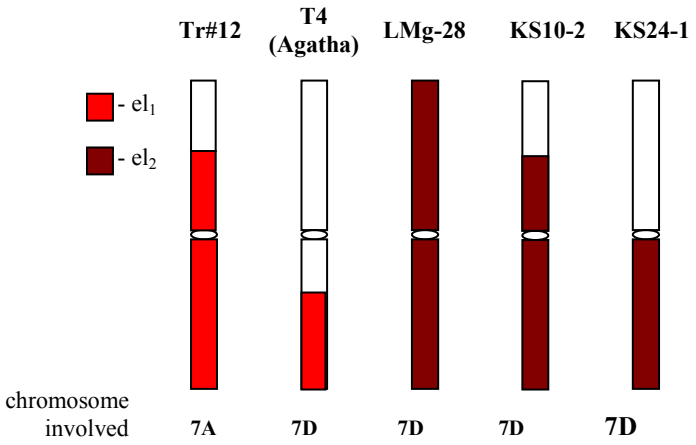
1.6.2.2. **FHB resistance**

FHB currently represents one of the most important and severe wheat diseases, causing up to 100% yield losses and deterioration of technological and nutritional grain quality. The fungus *Fusarium graminearum* can infect wheat ears from flowering time until late stages of maturity. Besides the reduction in grain size and reduced germinability, the pathogen produces also mycotoxines, such as deoxynivalenol (DON), which can cause serial health risk for humans and livestock. The expansion of the pathogen infestations beyond its traditional world areas (e.g. China humid regions) has been greatly enhanced by recent climate changes. A sustainable approach for disease control is selection of resistant

cultivars, but very few resistance sources exist within the cultivated wheat germplasm. In bread wheat, the most well-known and deployed is the Chinese variety Sumai 3, which contains several QTLs contributing to the resistant phenotype, the major one being located on the 3BS arm (Liu and Anderson, 2003). Few other genes/QTLs have been identified in Asian cultivars, such as Ning 894037, Wuhan 1, Wangshuibai, in the Brasilian cultivar Frontana and some European winter wheat varieties, such as Arina, Fundulea 201R and Renan, currently being under evaluation (Badea et al., 2008; Buerstmyer et al., 2009). In durum wheat varieties there is no important and valid source of FHB resistance identified so far. Besides the possibility of transferring the Sumai3 QTL on 3BS, resorting to wild wheat relatives may represent a valuable approach also for this disease, as very effective resistance has been detected in the diploid *Lophopyrum elongatum* (Shen and Ohm, 2006; Jahuar et al., 2009) and in *Th. ponticum* (Shen et al., 2004; Shen and Ohm, 2007). As to the latter, a different accession of *Th. ponticum* from that containing the *Lr19+Yp* genes, which is FHB susceptible, turned out to be highly resistant (Shen et al., 2004). Interestingly, the FHB gene/QTL was found to be located in a roughly coinciding region to that containing *Lr19+Yp*, i.e. the distal half of a chromosome 7. The two *Th. ponticum* accessions, and so their group 7 chromosomes carrying either resistance gene, have been given different designations, $7el_2$ indicating the one carrying FHB resistance, and $7el_1$=7Ag identifying the carrier of *Lr19+Yp*. A set of substitution and translocation lines containing $7el_2$ chromatin on chromosome 7D is available, including a substitution line in cv. Marquiz background, named LMq-28 (Knott et al, 1977), having a pair of $7el_2$ chromosomes in the place of the 7D pair, and various translocation lines obtained in both cv. Marquiz and Thatcher backgrounds (Kibirige-Sebunya and Knott, 1983) (Fig 6). Pairing analyses have demonstrated that homology between el_1 and el_2 chromatin is almost complete (Dvořák, 1975; Knott et al., 1977).

These materials are being further characterized, and the genetic map of the bread wheat $7el_2$-7D as well as $7el_1$-7D (T4) translocations (Shen and Ohm, 2007) as well as of the durum wheat $7el_1$ (=7Ag)-7A recombinants (Fig 12) is being enriched with new markers able to reveal $7el_1$ vs. $7el_2$ polymorphisms in the target region. Since the $7el_2$ *Th. ponticum* appears to carry different alleles at corresponding loci to *Lr19* and *Yp* of $7el_1$, ideal *Th. ponticum* allelic combinations of the target genes for durum wheat on one side and bread wheat on the other are likely to be selectable for the benefit of both species (see ahead § 1.7.).

Figure 6 Recombinant chromosomes of available bread wheat translocation and substitution lines carrying chromatin from two accessions of *Th. ponticum*



1.6.3. Yield QTL

The existence of a QTL for yield in wheat-*Th. ponticum* genetic stocks (tentatively called here *Yld-7AgL*) has been originally suggested on the basis of results obtained by CIMMYT, using near-isogenic lines (NILs) of the original T4 (=Agatha) translocation (Sharma and Knott, 1966) inserted into various genetic backgrounds (Singh et al., 1998; Reynolds et al., 1999, 2001, 2005; Monneveaux et al., 2003). Notably, the *Th. ponticum* 7AgL chromatin present in T4 originates from the same 7Ag chromosome from which Sears' transfer N^{o} 12 and hence the durum wheat recombinant lines produced by Ceoloni et al. (2005a) derive. Form CIMMYT work it resulted that a gene(s) on the T4 7AgL translocation contributed to an increase of >13% in grain yield and >10% in biomass (Reynolds et al., 2001). According to Singh et al. (1998), the positive effect of this QTL was particularly evident under drought stress; however, Sibikeev et al. (2000), testing derivatives of the same T4 translocation but in different varietal backgrounds, observed a yield increase associated with presence of the translocation also in non-stressed environments. Moreover, background effects make the results not always clear-cut (Monneveux et al., 2003).

As to wheat, not until recently a complex trait such as yield has been dissected in details in bread and durum wheat (see, e.g., Quarrie et al., 2005, 2006; Maccaferri et al., 2008; Zhang et al., 2010). Quarrie et al. (2005) reported the strongest yield QTL to be on wheat 7AL, mainly affecting grain number per ear, associated with two distal markers, WMC273 and PSP3094. Using the set of durum wheat-*Th. ponticum* recombinant lines

mentioned above (Ceoloni et al., 2005a), these markers turned out to be relatively distant on 7AL in physical and genetic terms, withWMC273 in the terminal quarter of the arm (included in the 23% 7AgL of line R5-2-10, see Fig 3), and PSP3094 being more proximal (comprised between the 7AgL FL 0.72 and 0.60 of lines R112-4 and R23-1, respectively, Fig 3). More recent work by Quarrie et al. (2006 and unpubl.) pointed more specifically at PSP3094 as a closer marker to the 7AL yield QTL (among four identified clusters along the 7AL arm) and suggested it might correspond to a gene controlling flag leaf width and chlorophyll content, and indirectly affecting the amount of assimilates transferred into the spike.

QTLs affecting grain number on the 7AL chromosome arm at relatively close locations to that of Quarrie et al. (2005, 2006) were identified also in two recent works by Kumar et al. (2007) and McIntyre et al. (2010). The former associates the QTL to the GWM276 marker, and the latter reports a strong QTL, stably expressed across high and low-yielding environments, associated with markers BARC121 and GWM282 (see Fig 4 and Fig 12).

Since substantial colinearity has been observed in 7AL and 7AgL genetic map comparison (Fig 4; Gennaro et al., 2010), it can be hypothesized that the location of possible *Th. ponticum* homoeoloci of the mentioned wheat 7AL QTLs corresponds to certain 7AgL regions differentiating the durum wheat 7AL-7AgL recombinant chromosomes of Ceoloni et al. (2005a). For instance, a ortholog of Quarrie's QTL, marked by PSP3094, might be comprised in the 7AgL segment present in R23-1, but lacking in R5-2-10 (Fig 3).

Similarly to what observed for such wheat yield QTL, also the NILs with the T4 7DL-7AgL translocation (70% of the 7AgL on the 7DL) used by CYMMIT showed to have increased photosynthetic capacity and better source-sink balance, mainly expressed as increase in grain number/ear, compared to control lines (Reynolds et al., 2001; Monneveux et al., 2003). There is also preliminary evidence (Reynolds et al., 2005, 2009) that the higher yielding NILs possessing the 7AgL segment on 7DL, and, in particular, their increase in grain number/ear, would be associated with reduced floral abortion, which, in turn, would be related to a lower amount of endogenous abscisic acid (ABA) in spike tissue at boot stage and higher resistance to water stress conditions (Reynolds et al., 2008). Interestingly enough, a cabinet experiment by Quarrie et al. (2007) with exogenously applied ABA showed that the 7AL QTL linked to PSP3094, affecting leaf width and yield,

is highly responsive to stress conditions (increased ABA concentration and low nutrient regime),

No doubt, analysis of various aspects of potential yield QTL(s) located along the 7AgL of both the T4 line and of the durum wheat-*Th. ponticum* recombinant lines is a very interesting area to explore. In fact, accumulation of positive alleles for different yield-related traits, including photosynthetic activity (capacity and efficiency), sink-source optimization and several others, represents a priority in current and future strategies for wheat breeding (Reynolds et al., 2011).

1.6.4. Segregation distortion genes

Segregation distortion (SD) is the deviation of observed genetic ratios from the expected Mendelian ratios of a given genotypic class within a segregating population. Distorted segregation ratios may result from gametophytic competition, resulting in preferential fertilization or abortion of the male or female gametes or zygotes, with most systems affecting the male gametes. Because in progeny from heterozygous individuals segregation distortion (*Sd*) genes frequently act by rendering non-functional gametes that do not carry them, they have been also referred to as gametocidal (*Gc*) genes. SD has been observed in a wide variety of organisms, including fungi, plants, insects, and mammals. In fact, genetic elements that cause SD may be potent evolutionary forces, particularly in terms of species differentiation and genome restructuring (Hurst, 1993; Taylor and Ingvarsson, 2003).

Genomic regions harboring markers with abnormal segregation ratios have been reported in many crop species including barley, pearl millet, tomato, rice, maize and wheat. Chromosomes carrying *Sd* genes have been also identified in several wild wheat relatives and their effect revealed upon hybridization with wheat. In most cases such alien chromosomes or chromosome segments are selectively or sometimes exclusively retained in the wheat background, by causing sterility of both male and/or female gametes lacking them. This was observed for various *Aegilops* species and for species of the *Thinopyrum* genus.

For at least some of these genes, direction and magnitude of the SD effect appear to be determined by the genetic background of the recipient wheat. This is the case for the *Sd1* gene, located on the 7AgL arm, proximal to the leaf-rust resistance *Lr19* gene. Its incorporation into different bread wheat varieties through 7AgL translocations, resulted in

either preferential transmission or self-elimination, or even normal segregation of the carrier chromosome (Zhang and Dvorak, 1990; Marais, 1990, 1992; Marais et al., 2001), apparently depending on the allelic variation at several wheat "responder" loci (Prins and Marais, 1999). Interestingly enough, shortening the long 7AgL segment of the T4 translocation chromosome turned its original preferential transmission into a generally defective one (Marais, 1992; Marais et al., 2001). Similar observations were made on 7DL homoeologous transfers of 7AgL segments of different lengths (Sears, 1978; Zhang and Dvorak, 1990). The shortened segments were interpreted as having lost either the *Sd1* gene (Zhang and Dvorak, 1990) or some additional factor(s) present on the original alien segment, able to interact with it and modify its effect (Marais et al., 2001).

As to the set of durum wheat–*Th. ponticum* recombinant lines,.during the creation of NILs through backcrosses with the cultivar Simeto (used as male), the 7A-7Ag recombinant chromosome of R23-1 line (and others with even larger amount of 7AgL chromatin) showed low transmission rate through the male germline (Ceoloni et al., 2005a). Initially, by comparing genetic maps of the R23-1 line (Gennaro et al., unpubl.) and of bread wheat recombinant chromosomes supposed to carry *Sd1* (Marais et al., 2001), the presence of *Sd1* in R23-1 was excluded. On the other hand, more recent work by Groenewald et al. (2005) indicates the existence of the second segregation distortion gene, *Sd2*, on the introgressed *Th. ponticum* chromosome and distally associated with the *Lr19* gene. Thus, the question on the exact localization of *Sd1* and *Sd2* remains open, whereas the variability of its effects in relation to the recipient varietal background seems to be confirmed also in durum wheat (Grossi, 2010).

1.7. Techniques of molecular genetics and cytogenetics aiding efficient chromosome engineering

Technological development in the fields of genetics and cytogenetics, and, in particular, the recent "genomics revolution" is offering unrivalled opportunities for understanding structure and function and hence for targeted manipulation of even complex plant genomes. No doubt, it is greatly impacting all breeding strategies, including chromosome engineering. In fact, molecular marker technology, as well as molecular cytogenetic techniques, such non-radioactive *in situ* hybridization (e.g. FISH and GISH), effectively complementing classical methods of analysis (including meiotic pairing, gene location and mapping by use of aneuploid lines, use of insufficient biochemical,

cytological and morphological markers), are making detection and characterization of wheat-alien introgression products much more efficient and accurate than ever so far (see, e.g., Ceoloni and Jauhar, 2006 for a review).

Molecular marker technology has several possibilities of application in basic and applied genetics, and a very wide array of marker types is available nowadays, several of which are definitely time- and cost-effective. The first type of widely used molecular markers were RFLPs (Restriction Fragment Length Polymorphism) (Halentjaris, 1987), which do not require knowledge of the DNA sequence for application. By 1995, more than 1100 were placed on the wheat genome map (Law, 1995). However the real revolution was achieved with the discovery of the Polymerase Chain Reaction (PCR) (Mullis, 1986), which gave the base for future development of almost unlimited molecular marker types. PCR allowed the conversion of RFLPs into a new PCR-based marker type, STS (Sequence Tagged Site) and development of a variety of markers such as RAPD (Random Amplified Polymorphic DNA) (Williams et al., 1990), AFLP (Amplified Fragment Length Polymorphism) (Vos et al., 1995), microsatellites or SSR (Simple Sequence Repeats) (Tingley and Rafalski, 1996) which for long time were considered perfect markers, and finally EST-SSR (Expressed Sequence Tag SSR). In the last decade a "third generation" of markers has been introduced, called DArT (Diversity Array Technology) (Jaccoud et al., 2001), which are based on microarray technology and are highly specific. The increase in number of ever more diversified markers was followed by construction of ever more dense genetic maps of wheat. Still, SSRs represent the markers of choice for Marker Assisted Selection (MAS), because they are often co-dominant, locus-specific and highly polymorphic even at the interspecific level (Roder et al., 1995; Plaschke et al., 1993).

On the other hand, the resolution and time-efficacy of cytogenetic analyses was greatly improved by the introduction of in situ hybridization (ISH) techniques, used on cytological material to detect DNA (or RNA) used as a probe and the chromosomal DNA fixed on the glass slide. Currently, ISH does not make use of radioactively labelled probes as it was earlier (Gall and Pardue, 1969; John et al., 1969), but probes labelled by insertion into the DNA sequence of a modified nucleotide (often dUTP=deoxyuridine triphosphate) attached to a molecule of biotin (vitamin H), or digoxygenin (a steroid naturally present in the genus *Digitalis*) or a fluorochrome (Leitch et al., 1994). In the latter case, or when the fluorochrome is involved in the detection phase by interacting with the modified nucleotide (Trask, 1991, see also § 2.2.1.4.), the technique is called FISH (Fluorescence In Situ Hybridization). This is presently the mostly used ISH method, because of its advantages in

terms of high probe signal resolution, possibility of visualisation of multiple probes (marked with different fluorochromes) on the same slide, and even the possibility of repeated hybridization of the same slide (Cuadrado et al., 1997).

For identification by ISH of chromosome segments transferred from alien species, both species-specific or genome-specific probes can be used. As to *Triticinae* species though, not many such specific sequences are available, and they are not always able to cover in an interspersed manner the entire genome or chromosome of interest. To this aim, total genomic DNA of the donor species of the alien segment can be used as the probe (Anamthawat-Jonsson et al., 1990), following procedures, collectively called GISH (Genomic In Situ Hybridization), which are otherwise similar to FISH with specific sequences (see, for details, § 2.2.1.4.).

In spite of some obstacles when working with plant material (presence of the cell wall, inherent higher chromosome condensation, difficulties in synchronization of cell cycle), in situ hybridization is today a widely used technique for identification of genomes, chromosomes or chromosome segments, at both intra- and inter-specific levels (e.g., Ceoloni et al., 1996, 1998); analysis of structural alterations of the genome (deletions, duplication, translocation or inversions); meiotic pairing studies (Cuadrado et al., 1997); analysis of physical organization of sequences and of relationship between physical and genetic maps (e.g., Biagetti et al., 1999).

1.8. OBJECTIVES

The durum wheat-*Th. ponticum* lines developed by Ceoloni et al. (2005a) represent an extremely useful tool to carry out different research activities of potential breeding value. The rationale of two such activities is described in the following.

1. On one hand, they offer the unique opportunity to further enrich their gene content with valuable alleles for additional useful traits, i.e. to perform a gene pyramiding targeting desirable allelic variants originating from different donor accession of the same alien species.

2. On the other hand, the same materials contain segments of at least part of the 70% 7AgL of the bread wheat translocation line T4 (see § 1.6.3.), thus allowing a higher resolution in the analysis of possibly multiple genetic determinants and corresponding phenotypes of the collectively called *Yld-7AgL* (see § 1.6.3.). For this, NILs (BC_{4-6} to the variety Simeto) of three of the durum wheat recombinant lines, i.e. R5-2-10, R112-4 and R23-1, having their distal 7AL replaced by 23%, 28% and 40%, and respectively, of the *Th. ponticum* 7AgL homoeologous arm, as well as NILs of the T4 line into the bread wheat variety Thatcher will be the material of choice. No other line with shorter 7AgL segments than that of T4 is in fact available, particularly as NIL, whereas durum wheat recombinant chromosomes with more than 40% 7AgL, or anyway with more proximal segments, are not well tolerated, hence not available in homozygous condition. In fact, the same R23-1 homozygous line shows some instability (see § 1.6.4.) and a substitute as carrier of the 28% (R112-4)-to-40% (R23-1) segment will be developed in the course of this thesis with hopefully reduced or no stability problems.

In pursuing both research targets, the following main actions have been performed in the course of the present work:

a. enrichment of the 7AL/7AgL genetic map with additional PCR-based markers, mainly SSRs and ESTs;
b. setting up of the pyramiding of the *Lr19+Yp* genes present in the durum wheat R5-2-10 and R23-1 lines and bread wheat T4 line with a *FHBres* gene/QTL originating from a different *Th. ponticum* accession;
c. development of a new recombinant durum wheat/*Th. ponticum* line containing an interstitial chromosomal segment spanning from about 20% to

40% of the recombinant 7AL arm, to minimize or eliminate stability problems associated with the entire 7AgL segment of R23-1 line;

d. analysis of available NILs of durum wheat R5-2-10, R112-4 and R23-1 lines, and of bread wheat NIL of line T4 for an array of yield-related parameters, recorded during growth (field conditions, see § 2.2.2.2.) or at post-harvest stages, and of their correlation with defined segments of the 7AgL arm;
e. analysis of the effect on yield traits of exogenously applied ABA, as a mimicking agent of stress conditions, on the same materials as above, and of its correlation with defined segments of the 7AgL arm;
f. assessment of endogenous ABA concentration in field and greenhouse-grown juvenile spikes (boot stage) of recombinant genotypes and control lines.
g. assessment of physiological parameters describing the plant water status of plant materials grown under field and controlled conditions.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1. Genetic and cytogenetic analyses

2.1.1.1. Plant material

To pursue the research objectives indicated in § 1.8, different durum and bread wheat recombinant and translocation lines involving the *Th. ponticum* 7AgL chromosome arm, represented the target materials.

The durum wheat-*Th. ponticum* recombinant lines used were produced by Ceoloni et al., (2005a), as described earlier (see Introduction, § 1.6.2.1.). Five lines (R23-1, R112-4, R90-14, R5-2-10 and R14-1) have mainly 7A chromatin on the recombinant chromosome, and a distal portion of the long arm replaced with 7AgL (=$7el_1L$) chromatin. All five lines posses the *Yp* gene and all except the R14-1 line, possess the *Lr19* gene as well (Figs 3 to 5). On the other hand, in three recombinant lines (R1-14, R5-2-9, R8-6-4) 7Ag chromatin is present in the proximal region of the short and long arms, while 7A chromatin occupies distal regions. For R5-2-9 and R8-6-4 (79 and 90% of the long arm, respectively) are heterozygous for the recombinant 7A/7Ag chromosome and have the other 7A chromosome replaced by a 7D. For these lines it was not possible to obtain vital homozygous lines, probably because of low capability of durum wheat to tolerate a sizable 7Ag segment in homozygous condition. Hence, they are being maintained in heterozygosity, with either a normal 7A or 7D. The 7D chromosome was transferred by a cross with the durum wheat substitution line, developed in cv. Langdon (Joppa and Williams, 1988), which has the 7A chromosomes pair substituted with a 7D pair [LDN 7D(7A)]. This chromosomal constitution allowed the use of R5-2-9 and R8-6-4 lines in mapping of dominant molecular markers in 7AL/7AgL regions, which would have not been possible in the presence of a normal 7A chromosome. Finally, R9-1 line is a "tertiary" recombinant containing an interstitial 7AgL segment harbouring both *Lr19* and *Yp* genes (Ceoloni et al., 2005a and Fig 5).

For the development of a new recombinant line, named R5-2-23, with an interstitial 7AgL segment that corresponds to the proximal portion of the R23-1 segment, recombinant lines R5-2-9 (heterozygous for a normal 7A chromosome) and R23-1 were used as parents in crosses.

Bread wheat lines used in the study for molecular marker screening and *Th. ponticum* gene pyramiding are recombinant lines carrying different amounts of *Th.*

ponticum chromatin on chromosome 7D or 7A. Alien chromatin originates from two accessions of *Th. ponticum* and to discriminate between them, such chromatin has been given a different designation (el_1 or el_2). The el_1 stocks include the wheat-*Th. ponticum* translocation line T4 (Agatha, 7DS.7DL-7el_1L), obtained by irradiation treatment by Sharma and Knott (1966) and carrying about 70% of 7DL arm replaced with 7el_1 (see § 1.6.2.1., § 1.6.3 and Fig 6), and Sears' recombinant line transfer N°12, (7AS-7el_1S.7el_1L), with nearly half of the short arm and the whole long arm of 7A replaced by 7el_1 (Ceoloni et al., 1996 and Fig 1).

The el_2 stocks consist of substitution and translocation lines carrying *Th. ponticum* chromatin on chromosome 7D, i.e. line LMq-28 (Knott et al., 1977) obtained in cv. Marquiz background, in which a pair of 7el_2 chromosomes replaces the 7D pair; and two translocation lines obtained in mixed background of cvs Marquiz and Thatcher, named KS10-2 (7el_2S.7el_2L-7DL) and KS24-1 (7DS.7el_2L) (Kibirige-Sebunya and Knott, 1983) (Fig 6)

Allelic variants of interest for the gene pyramiding work concern FHB resistance (el_1 = susceptible; el_2 = resistant) and leaf-rust resistance (el_1 = *Lr19* carrier, highly resistant; el_2 = *Lr19* non-carrier, moderately resistant at the adult plant stage, see Kim et al., 1993). The yellow pigmentation trait seems to be expressed by both sets of stocks (Sharma and Knott, 1966; Sears, 1978; Kibirige-Sebunya and Knott, 1983)

A series of other control lines were used for correct interpretation and verification of molecular markers' patterns: durum wheat cvs. Simeto, Creso and Langdon (LDN), bread wheat cvs. Chinese Spring, Marquiz and Thatcher, nulli-tetrasomic and ditelosomic lines of Chinese Spring involving arms and entire chromosomes of homoeologous group 7 (see Sears and Sears, 1978) and durum wheat substitution lines LDN 7D(7A) and LDN 7D(7B) (Joppa and Williams, 1988).

2.1.1.2. Probes used for *in situ* hybridization

For *in situ* hybridization experiments (pre-annealing-GISH (§ 2.2.1.4.) total genomic DNAs of *Th. ponticum* and durum wheat were used as probes.

2.1.1.3. Molecular markers

Microsatellite or SSR (Simple Sequence Repeats), STS (Sequence Tagged Site) and EST (Expressed Sequence Tag) markers were used.. To search for el_1-el_2 polymorphism, available markers already mapped in our laboratory along the 7A-7Ag (=el_1) recombinant chromosomes were used to screen lines carrying 7el_2 chromatin. Moreover, a bibliographic and database search was performed to identify other markers mapped on the wheat homoeologous group 7 chromosomes, aiming at increasing the marker density in the 7AL/7AgL chromosomal regions carrying the target genes.

SSRs markers were widely used, due to their very good distribution across the genome, their high polymorphism and transferability between genomes, and the vast number of SSRs already available for wheat.

Series of SSRs employed in this study are listed below (each series being developed by a different Institution):

- GWM – developed by Röder et al. (1998) and Wang et al. (2002). In particular, primer sequences for markers GWM698, GWM1061 and GWM1066 are not publicly available and were kindly provided by M.S. Röder (Institut für Pflanzengenik Kulturpflanzenforschung Gatersleben, Germany).
- PSP – only PSP3094 was used from this series and primer sequences have been kindly provided by P: Stephenson and S.A. Quarrie (John Innes Centre, Norwich, UK).
- WMC – developed within the international Wheat Microsatellite Mapping Network (IWMMN) and reported in Gupta et al. (2002).
- CFA – series developed by Sourdille et al. (2001) and genetically and physically mapped by means of wheat deletion lines (Somers et al., 2004; Sourdille et al., 2004); primer sequences are available at http://wheat.pw.usda.gov/.
- BARC – developed by Song et al. (2005) from a BAC library of the cv. Chinese Spring; primer sequences are available at http://wheat.pw.usda.gov/.
- MAG – series of EST-SSRs developed from available wheat EST sequences by Xue et al. (2008); primer sequences are available at http://wheat.pw.usda.gov/

Two STS markers employed were developed in our laboratory and are tightly linked to the *Lr19* gene (Gennaro et al., 2009) and to *Yp*. (Gennaro et al., 2010). Both are codominant, allowing discrimination between 7AL and $7el_1$ alleles.

ESTs primer pairs used consisted of two sets. One, already available in our laboratory (Gennaro, 2006), was developed starting from wheat EST sequences databases. (http://wheat.pw.usda.gov/cgi-bin/westsql/est_blast.cgi). Primers were designed exploiting the ISP (Intron Spanning Polymorphism) technique, which targets sequence polymorphism in non-coding regions by using primer pairs designed on coding regions flanking the intron of interest. The other set of ESTs primer pairs, consisted of few recently mapped ESTs markers in the very distal region of 7AL chromosome arm, in the region of FHB resistance locus (BF145935, BE637476, BE445653, BG607810). Primer sequences of these EST were published by Ayala-Navarette et al. (2007) and Shen and Ohm (2007).

2.1.2. Physiological and agronomic analysies

A second important group of analyses performed in this study consisted in: a) phenotyping the recombinant genotypes grown in field conditions for yield-related traits; b) evaluating the effect of exogenously applied ABA on yield-related traits of the treated (first) culm; c) measuring the endogenous ABA content in juvenile spikes, and d) carrying out a water relations analysis in the same materials, also under induced drought.

2.1.2.1. Plant material

For physiological and agronomic analyses the same comprehensive a set of 3 three durum and 1 one bread wheat genotypes were employed in each of different trials, differing only in number of plants included in each of the experiments (Tab 4).

Table 4 Plant material genotypes and respective corresponding number of plants sawn for physiological and agronomic analyses: 1 – yield-related traits assessment in field conditions and analysis of the effect of exogenously applied ABA on yield traits of the first culm yield traits; 2 – assessment of endogenous ABA content in juvenile spikes; 3 – analysis of water relations

Genotype	Wheat type	BC	HOM+ families	HOM- families	2009 (1)		2010 (1)		2010 (2)		2010 (3)	
					HOM+	HOM-	HOM+	HOM-	HOM+	HOM-	HOM+	HOM-
R23-1	Durum	4	2	3	48	72	48	72	12	18	10	10
R112-4	Durum	5	2	2	24	24	30	30	12	12	10	10
R5-2-10	Durum	6	2	1	24	12	30	30	12	6	10	10
T4	Bread	-	T4	-	7	-	30	-	6	-	15	-
Thatcher	Bread	-	-	Thatcher	-	7	-	30	-	6	-	15
Total	**-**	**-**	**7**	**7**	**103**	**115**	**138**	**162**	**42**	**42**	**45**	**45**

The durum wheat lines included the 7AL-7AgL (=7el$_1$L) recombinants R23-1, R112-4, R5-2-10 (Fig 3), each carrying part of the 7AgL portion present in line T4 which has been originally associated with a yield increase (Singh et al., 1998; Reynolds et al., 2001). The three durum wheat recombinant genotypes are represented in this study by homozygous carriers (=HOM+) and homozygous non-carriers (=HOM-) of the corresponding 7AgL segment, selected in F_{2-3} generations following a number of backcrosses (BC) to the recurrent variety Simeto. The number of backcrosses performed to produce the recombinant near-isogenic lines (NILs) differs among the three lines, with R5-2-10 and R112-4 being more advanced compared to R23-1 (BC_6, BC_5, and BC_4, respectively). Therefore the number of R23-1 plants analyzed for yield-related traits was larger than that of the other two lines, in order to compensate for the expected higher heterogeneity of the line. All HOM+ and HOM- variants are represented with 1, 2 or 3 families (§.2.2.2.2.) originating from sister lines.

The bread wheat genotype used is the T4 translocation line, tested together with the donor of the recurrent background of T4 NILs, i.e. cv. Thatcher. T4 was included in physiological and agronomic analyses as a sort of reference line (Singh et al., 1998; Reynolds et al., 2001; Monneveux et al., 2003), and also to assess its behaviour in a central Italy environment (Viterbo).

2.2. METHODS

2.2.1. Genetic and cytogenetic study

2.2.1.1. Genomic DNA extraction

For efficient molecular PCR-based marker analyses, availability of a time-saving protocol for DNA extraction is important, since it allows analysis of numerous plants at early developmental stages. In this study, conventional SDS (Sodium dodecyl sulfate) protocol by Dellaporta et al. (1983) was used for the extraction of total genomic DNA from young wheat leaves or half seeds, which resulted in good PCR-grade quality of DNA. Composition of extraction buffer used was: Tris 100mM pH8, EDTA 50 mM pH 8, NaCl 500 mM, SDS 1.25%. DNA obtained was dissolved in different volumes of ultra pure water (40-100µl) depending on the amount of initial plant tissue and conserved at +4°C or -20°C prior the use. In general, the quality of DNA extracted allowed its conservation at +4°C for long periods without any deterioration.

For the use of genomic DNA in GISH, the protocol by Tai and Tanksley (1990) was applied which allowed extraction of greater amount of rather pure DNA. The extraction buffer used has the following composition: TrisHCl 100 mM pH8, EDTA 50 mM pH 8, NaCl 500 mM, SDS 1.25%, NaOH 8.3 mM and Sodium-bisulfite 0,38%). DNA samples were resuspended in TE solution and conserved at -20°C prior to use.

2.2.1.2. Determination of DNA concentration

Ethidium-bromide fluorescence was used to estimate the concentration of the extracted DNA. The method is based on direct proportion between the DNA quantity present in a particular volume and the emitted fluorescence by ethidium-bromide incorporated in DNA double helix. Namely, 0.5-1 µl (DNA concentration from extraction or diluted) are charged on 1% agarose gel in 1xTBE solution (Tris 89 mM; boric acid 89 mM; EDTA 2 mM). The gel contained 0,5 µl/ml ethidium-bromide and several standards of the λ DNA of known concentration. After a short electrophoretic run (10-20'), a photo of the gel has been taken by means of the KODAK 1D software, under UV light, to later proceed to a direct comparison of the fluorescence emitted by individual DNA samples and control samples of known concentration.

2.2.1.3. Molecular analysis analyses based on Polymerase Chain Reaction (PCR)

The conventional protocol proposed by Röder et al. (1998) was initially applied for PCR, later modified for specific needs. The optimization protocol consisted of a gradient-PCR useful to set up the most suitable annealing temperature for each primer pairs. This was particularly useful for genotypes that had not been previously analyzed (e.g. el_2 chromatin-bread wheat stocks), and, in general, to maximize marker polymorphisms. The list of all markers employed, together with their optimized annealing temperatures is presented in Table 5.

For all markers, the reaction volume was 10µl and in almost all cases it consisted of 1x PCR Buffer, 1.5 mM $MgCl_2$, 200µM dNTP mix (Promega), 200 nM of each one of primers (forward and reverse), 0.35U of *Taq* Polymerase (Amersham, cat# 270799), and 20-40 ng of target DNA. It was only in the case of marker BE429174_3'Fx3'R that the two primers had different concentrations (200 nM of forward and 400 nM of reverse). When larger scale progeny screening was performed instead of *Taq* Polymerase by Amersham, GoTaq® Green Master Mix (Promega, cat#M7112) was used instead, because of a substantial time-saving. For 10µl reaction volume, 5 µl of GoTaq® Green Master Mix was added, while primer concentration remained unchanged (200nM).

PCR amplification conditions were basically similar for all markers. However, slight differences applied are reported in Tab 6.

Amplification products were separated by horizontal electrophoresis on agarose gel in 1xTBE running buffer (Tris 89mM, boric acid 89 mM, EDTA 2mM), using a gel concentration of up to 3% (depending on the size of amplified DNA fragments). Agarose used was of two types, one for routine use, and MetaPhor® (Cambrex Bio Science, Rockland Inc., USA), a more specific one used for separation of bands having a few base pair difference. Gel images were acquired by means of KODAK 1D software, by the use of ethydium-bromide staining (0.5µg/ml).

Table 5 Molecular markers employed and optimized in course of work (information on markers' map position retrieved from http://wheat.pw.usda.gov/GG2/index.shtml; T_a= annealing temperature; * from Baga et al., 2007)

Molecular marker	Type	Map position reported	T_a (°C)	Previously mapped in our laboratory
GWM63	SSR	7A	60	+
GWM146	SSR	7B, 7A*	60	-
GWM282	SSR	7A.1, 7A.2	55	+
GWM344	SSR	7A, 7B	52	+
GWM698	SSR	7AL	55	+
GWM1061	SSR	7AL	58	+
PSP3094	SSR	7A	61	+
WMC17	SSR	7A, 7B	60	-
WMC107	SSR	7A	58	-
wwWMC116	SSR	7AL	61	-
WMC273	SSR	1A, 5D, 7A, 7B, 7D	51	+
WMC488	SSR	7A, 7D	61	-
WMC607	SSR	7A	61	-
WMC633	SSR	7AL	61	-
WMC790	SSR	7AL	58	-
CFA2019	SSR	7A	60	+
CFA2040	SSR	7AL, 7B, 7D	52	+
CFA2123	SSR	7A	60	+
CFA2240	SSR	7A	56	+
CFA2257	SSR	7A, 7B, 7D*	55	+
CFA2293	SSR	7A	58	+
BARC49	SSR	7A, 5D	55	-
BARC108	SSR	7A	55	-
BARC121	SSR	7A, 6D, 7D	55	-
BARC172	SSR	7D, 5B, 7A*	58	-
BARC195	SSR	6A, 7A	61	-
BARC231	SSR	2D, 6A, 6D, 7A*	58	-
MAG2999	STS	7A	51	-
MAG4134	STS	7A	61	-
AG15_600 (*Lr19*)	STS	7Ag	57	+
AG15_184 (*Lr19*)	STS	7Ag	62	+
Psy1_A1 (*Yp*)	STS	7AL, 7Ag	60	+
BE405003	EST	7AL,7BL,7DL	55	+
BE406627	EST	7AL	62	+
BE426274_Ex3/Ex2	EST	7AL	58	+
BE426802_int8	EST	7AL	58	+
BE429174_3'Fx3'R	EST	7AL	61	+
BE445653	EST	7AL, 7BL, 7DL	52	-
BE591389	EST	7AL	62	+
BE637476	EST	7AL, 7BL, 7DL	58	-
BF145935	EST	7BL, 7DL	64	-
BG312585	EST	7AL	60	+
BG607810	EST	7AL, 7BL, 7DL	58	-

Table 6 PCR amplification conditions of various types of primers used (T_a = annealing temperature)

Marker	Type	Stage 1	Stage 2				Stage 3
		95°C	Cycle N°	94°C	T_a	72°C	72°C
SSR	all	5’	35	30”	30/40”	30/40”	8’
PSP3094	SSR	5’	30	1’	1’	1’	5’
$AG15_{600}$	STS	3’	35	45”	45”	45”	10’
$AG15_{184}$	STS	5’	35	30”	1’	1’	10’
Psy_A1	STS	5’	35	30”	1’	1’	8’
EST	all	5’	35	30”	30/40”	30/40”	10’

2.2.1.4. Genomic *In situ* hybridization (GISH)

As an alternative approach to C-banding technique and *in situ* hybridization (ISH) with specific DNA sequences, GISH is a method that allows a more homogeneous coverage of wheat and alien genomes (see § 1.7.). GISH is not substantially different from the ISH, if not in probe and hybridization mix preparation.

Because of existing partial homology between the DNAs of the donor- and recipient-species as they belong to the same Triticeae family, use as the probe of total genomic DNA of donor species (e.g. *Th. ponticum*) , causes a diffuse hybridization (cross-hybridization) of the probe across the chromosomal DNA of recipient species (e.g. wheat in a wheat-*Th. ponticum* recombinant genotype), previously fixed on microscopic slide.. There are various methods to avoid or minimize cross-hybridization. One of them is based on the addition of an excess amount of unlabelled DNA of recipient species (in our case wheat). This excess DNA, called “block”, will hybridize in rather stringent conditions of hybridization with completely homologous wheat DNA, thus preventing the labelled, alien DNA probe from hybridizing at corresponding locations; the labelled probe, instead, will hybridize with its completely homologous DNA, overall allowing a satisfactory discrimination of the target alien chromosomal region from the wheat chromosomes.

A variant of GISH was later set up, called “pre-annealing-GISH” (Anamthawat-Jonsson and Reader, 1995), which allows an even clearer interspecific/intergenomic discrimination. Following this method, the two genomic DNAs (wheat and alien) are typically labelled with different fluorochromes and used in equal quantities in a “pre-annealing” phase, in which highly similar sequences between the two DNAs associate, hence are ”subtracted” from the following hybridization. Although the success of the protocol, and of GISH in general, depends on the intergenomic relatedness (the more

distant the two genomes, the higher the possibility of their differentiation), it generally provides with an overall satisfactory identification of intergenomic transfers.

The "pre-annealing"protocol described by Anamthawat-Jonsson and Reader (1995) has been adopted in the present work. Its main steps, including specimen preparation microscopic image acquisition, are described in the following.

2.2.1.4.1. Preparation of microscopic slides for somatic chromosome analysis by GISH

Meristematic tissue of root tips is used for preparation of microscopic slides. Starting from seed the following steps were carried out:

- Germination, synchronization of cell cycle and cell fixation in metaphase – after being immersed for 4 h in water, seeds are transferred on two layers of humid filter paper inside a Petri dish. Seeds are left in this way for about 24 h at 20-22°C. When seminal roots are 0.5-1 cm long, they are cut and treated with ice-water for about 24 h, in order to accumulate cells at the metaphase stage where chromosome contraction is maximal.
- Fixation – at the end of the ice treatment, root tips are fixed in a 3:1 solution of ethanol and acetic acid (Carnoy) for at least some hours but preferentially for one week at room temperature. Plant material can be preserved for long periods at -20°C when in Carnoy.
- Enzymatic treatment – in order to obtain a microscopic slide with elevated chromosome quality as required for final high quality results in GISH, it is necessary to eliminate all possible cellular debris (cell wall, membranes, cytoplasm). This is necessary in order to make the DNA fixed on the slide easily accessible to the probe. Therefore, root tips are left in digestion buffer (0.01M citric acid, 0.01M trisodium-citrate pH 4.6) for 5', then transferred in a fresh buffer for 20', and finally in enzymatic solution (2% cellulose, 4% liquid pectinase, Sigma) for 1-2h at 37°C. At the end of digestion, root tips are washed and processed for slide preparation.

Preparation of slides – digested plant tissue is fragmented in a drop of 45% acetic acid under a stereomicroscope in order to remove the unwanted tissues, leaving only the inner, meristematic cells on the slide. After squashing and quality control at the microscope, the slide is frozen in liquid nitrogen or dry ice, the cover slip removed and the

slide, with the chromosome preaparation, fixed with alcohol and let to dry. Slides can be preserved at 4°C or -20°C (for longer periods) prior the use

2.2.1.4.2. Epifluorescence microscopy and automatic digital image acquisition of images in GISH

To prevent fluorescence decay, slides must be protected with a drop of "Vectashield" (Vector Laboratories). Hybridization sites are visualized by a proper choice of epifluorescence filters of a Nikon Microphot-FXA epifluorescence microscope, equipped with a cooled B/W Leica DFC360-FX camera. Selected cells were captured and processed with an ImageJ software (Abràmoff et al. 2004), allowing improvement of image parameters (light, contrast, etc.) and measurement of the alien segment length on wheat chromosome.

2.2.1.5. Crosses of plants and auto-pollination

In order to prevent self-pollination, ears to be crossed must be emasculated while anthers are still green and below the immature stigma. In this phase ears are normally outside of flag leaf sheath. Pollination is performed only on the three first florets of each spikelet, since the others are usually sterile or very small and therefore eliminated. Pollination is performed a couple of days after emasculation: mature anthers are removed from the male parent and wiped over the stigmas of emasculated flowers (female parent). Since maturity of florets is not synchronized, pollination is usually repeated after a few days.

2.2.1.6. Plant growing conditions

When genetic and cytogenetic analysis had to be performed, plants were grown in controlled conditions in growth chambers. This allowed completion of two generations per year. Seeds were germinated for few days in Petri dishes on a double layer of humidified filter paper and then transplanted in pots with soil and put for about 3-4 weeks in a cold growth chamber at 4°C and 12 h photoperiod for vernalisation. Afterwards, plants were maintained at 13-15°C (night) and 18-20°C (day), and 16 h photoperiod, and later at 20-25°C until ripening. Over time, plants were fertilized with different nutrient solutions depending on the plant phenological phase: until the end of tillering, a solution with N:P:K

ratio of 21:7:14 was used, while in a second phase of growth a solution with 8:10:23 ratio was applied.

2.2.1.7. Meiotic chromosome preparations

Plant tissue used for meiotic pairing analyses were typically immature anthers containing pollen mother cells (PMC) at different meiosis stages.

The culm with the immature spike positioned in the penultimate internode is cut and the spike either readily sectioned or fixed in Carnoy solution and stored at -20°C until analysis. Selected anthers are put on the microscopic slide, covered with a drop of 2% aceto- rimord and gently opened to allow PMCs to be released. The cover slip is put on the top and similarly to somatic chromosomes' preparation, a series of delicate movements performed, in order to obtain a slide with minimum overlapping and well spread chromosomes. Images of chromosome spreads were captured and processed by ImageJ software (see § 2.2.4.2.) and later analyzed to determine pairing frequency of target chromosomes and presence of possible irregularities (e.g. univalents, micronuclei etc.).

2.2.1.8. Analysis of post-meiotic pollen divisions

After meiosis, pollen grains undergo two mitotic divisions. After the first one, two unequal nuclei are formed and the bigger one starts very soon to distend its chromosomes and dissolve within the cytoplasm (=vegetative nucleus). The smaller one has a more compact chromatin and normally is positioned at the opposite side of the pollen grain pore (= generative nucleus). This nucleus will give rise to two generative nuclei, formed at the end of the second mitotic division, thus giving rise to 3n mature pollen grains.

Analysis of the first post-meiotic division is performed on the immature anthers from spikelets sampled when the spike is emerging from the flag leaf sheath. Spikelets were excised from the spike with a pair of forceps and immediately fixed in Carnoy before analysis. Sampling of anthers at the second post-meiotic division is done 5-7 days later, when the spike is completely outside the flag leaf sheath.

By the same procedure followed for meiotic analyses (see §2.2.1.7), anthers with pollen grains at various stages of mitotic divisions are analysed at the microscope, and images captured for subsequent detailed investigations.

2.2.2. Physiological and agronomic study

2.2.2.1. Assessment of the ABA effect on yield-related traits

The experiment was performed in 2009 and 2010 in controlled conditions with the main purpose to examine the response of putative *Yld-7AgL* QTL(s) to the treatment with a high concentration of abscissic acic (ABA), applied to the first emerging spike of each plant, on fertility and other yield-related traits of the treated culm. A detailed time-line of the experiments carried out in the two years is presented in (Tab 7).

Table 7 Time line followed in experiments characterized by ABA application on juvenile first spikes

Activity	2009	2010
Sawing date (growth chamber)	February, 12	January, 22
Vernalization period at 4°C	Feb, 18 – Mar, 11	Jan, 29 – Feb, 19
Transfer of plants in glasshouse	March, 12	March, 8
Experiment start – 1st ABA treatment	April, 4	March, 23
2nd ABA treatment	April, 6	March, 26
Leaf 6 growth measurement	April, 4-9	March, 23-29
Transfer of plants in the field	April, 16	March, 31
Start of heading recording	May, 4	April, 28
Flag leaf measurements	May	May-June
Yield traits measurements	June	June-July

2.2.2.2. Growing of plants

Seeds of plants representing 3 durum and 1 bread wheat recombinant/translocation lines carrying 7AgL (=el$_1$L) segments (Tab 8) were sterilized for 5' in 1% Sodium hypochlorite and then washed thoroughly in distilled water. They were put to germinate for several days in Petry dishes, on a double-layered humid filter paper, at 22°C. When seminal roots were developed, plantlets were transferred in small pots with soil and placed in a growth chamber at 4°C with 12h photoperiod for a 3 weeks vernalisation period. Afterwards, plants were placed for a few days at 16°C, then transplanted in final 1.8 l pots and transferred to a glasshouse where the treatment with ABA was performed. Temperature and light conditions were not controlled, but remained rather stable throughout the treatment. Plants were regularly watered and protected against powdery mildew by mild fungicide treatments (at 2-3 leaf stage).

Table 8 Number of plants sawn for the experiment with exogenously applied ABA in 2009 and 2010

Genotype	Families	Replica I		No plants/r		Total plant No	
		2009	2010	2009	2010	2009	2010
R5-2-10 HOM+	R5-A	3	3	4	5	12	15
	R5-C					12	15
	R5-E					12	-
R5-2-10 HOM-	R5-D	3	3	4	10	12	30
	R14-D/1-1					12	-
	R14-D/2-1					12	-
R112-4 HOM+	V05x62-9-22	3	3	4	5	12	-
	V05x62-9-25					12	15
	V05x62-9-31					12	15
R112-4 HOM-	V05x62-9-13	3	3	4	5	12	15
	V05x62-9-17					12	15
	V05x62-9-24					12	-
R23-1 HOM+	V06x191-2-25	3	3	8	8	24	-
	V06x191-2-53					24	24
	V06x191-2-64					24	24
	V06x191-2-69					24	-
R23-1 HOM-	V06x191-2-26	3	3	8	8	24	24
	V06x191-2-31					24	24
	V06x191-2-36					24	-
	V06x191-2-60					24	24
T4	-	1	3	7	10	7	30
Thatcher	-	1	3	7	10	7	30
TOTAL						**350**	**300**

After the ABA treatment, plants remained in the glasshouse for two weeks and then were transferred in the field until harvest. In the field plants were maintained in the same pots, inserted in the soil. Genotypes were represented by 3 randomized blocks, each one containing plants derived from 3-4 families (Tab 8), arranged in 4 pots per row and a row length of 80 cm (Fig 7).

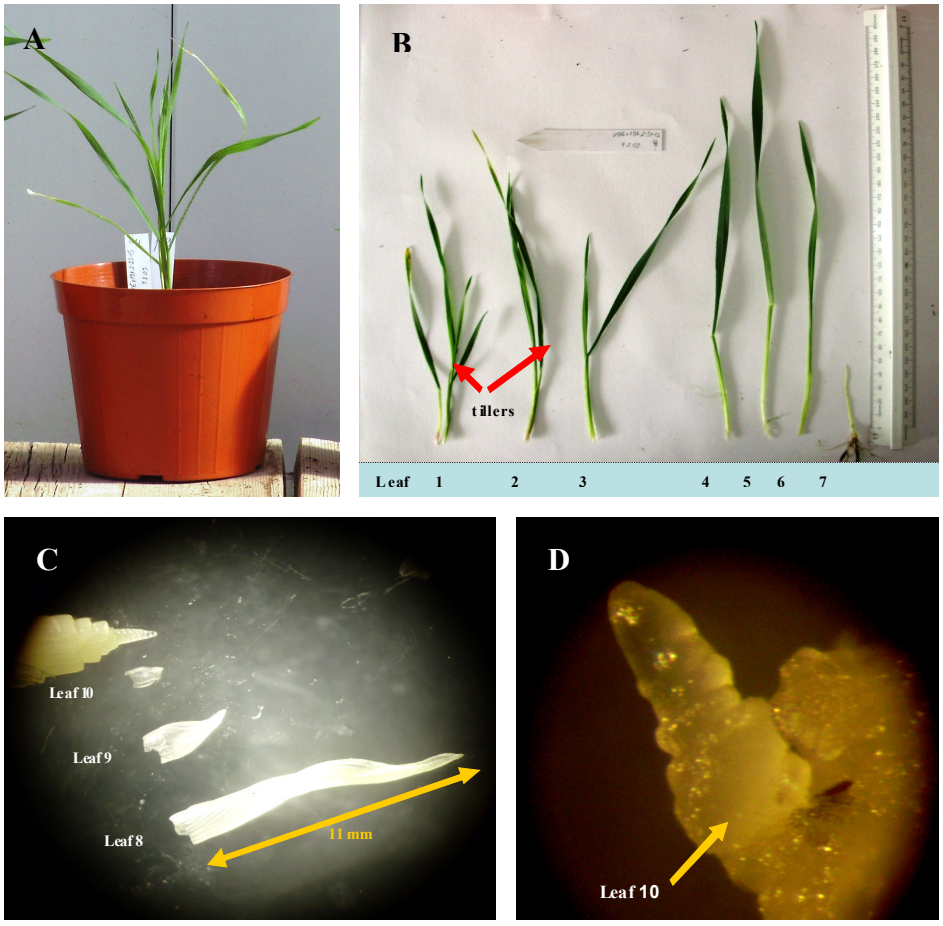
Figure 7 Plants in the field in two developmental stages

2.2.2.3. **Treatment with exogenous ABA**

ABA treatment of the first ear started at the "double ridge" stage, corresponding to initiation of floral rimordial development. In this phase, in fact, spikelets and florets begin to differentiate; ABA treatment was thus expected to affect these plant organs, representing the "sink" of assimilates, and spike traits of the treated (first) culm would have been then recorded, mainly to investigate whether the plant response would have been 7AgL genotype-dependent.

The right stage for treatment was determined at a stereoscope, by dissection of spear plants grown in the same conditions (Fig 8). The plant was extracted from the pot, the root apparatus eliminated, and leaves delicately peeled off with the help of a scalpel. This was repeated until the last leaf, enveloping the young ear, was removed. Ear's developmental phase was then checked.

Figure 8 Dissection of plant prior to ABA treatment (A–entire plant look; B-dissected leaves 1-7; C-dissected leaves 8-10 at stereoscope; D-early double ridge stage)

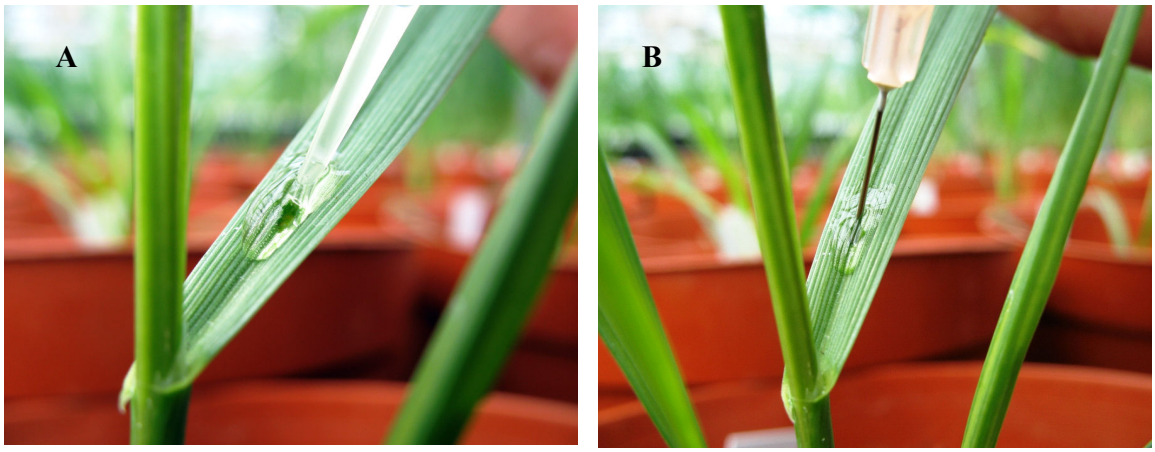


When the "double ridge" stage was detected on the dissected plant and the experiment started, the top leaf length was measured (see ahead § 2.2.2.4.). Half of the plants of each genotype were treated with ABA solution (Tab 11), and the other half, used as control, with water and TWEEN® 20 (Sigma, cat#P9416). 25µl of ABA solution or water-TWEEN was laid on each of two basal leaves (with a normal pipette), on the area just next to leaf sheath (Fig 9). A hole was than carefully made with a needle through the drop and leaf, in order to assure immediate uptake of the solution by the plant. The same treatment was repeated 3 days later

Table 9 Composition of ABA solution

Component	Concentration
Abscisic acid [a] (Sigma)	3.8×10^{-4} M
TWEEN® 20	0.1%
Water	up to the final volume

Figure 9 Steps in application of ABA onto leaves (A-laying down of a solution drop; B – hole making through leaf)



2.2.2.4. Leaf growth measurements

In order to confirm the effect of ABA treatment and to see how it affects growth rate of the top leaf, leaf length was measured on a daily basis during 7 days following the first ABA application. In this study, the "double ridge" stage was found when plants had leaf 6 emerged, and therefore, measurements were taken of this leaf. The distance from the soil surface up to leaf 2 ligule and up to the end of leaf 6 were measured, and leaf 6 length was calculated as the difference between these two values.

2.2.2.5. Measurements of the first culm yield-related traits at maturity

After the end of glasshouse experiment with exogenous ABA, plants were transferred to the field to complete their life cycle. Data recorded on the first culm (the ABA treated) were used for the assessment of the ABA effect on flag leaf dimensions, fertility and yield.

During plant growth and before maturation, while the leaves were still green and hydrated, flag leaf and flag leaf-1 dimensions, i.e. width (FLW, FL-1W, respectively) and length (FLL, FL-1L, respectively), were measured. At maturity the following traits were recorded: culm length up to the ear basis(HUPE), ear length (EL), seed number per ear (SNE), spikelet number per ear (SPNE) and grain yield (GY). From these values three other traits were calculated: total culm height (TH=HUPE+EL), seeds per spikelet (SPS=SNE/SPNE) and thousand kernel weight (TKW=GY*1000/SNE).

In 2010, the first culm dry biomass (B) was additionally recorded and this value used for harvest index (HI=GY/B) calculation.

2.2.2.6. Measurements of yield-related traits for the entire plant

Evaluation of productivity capacity of entire plant represents continuation of the experiment with ABA. In this way the same set of material was analyzed for two separated analysis. Plants were randomized in 3 blocks in the field and grown until maturity. During the entire growth period, plants were maintained watered even when there was no precipitation.

The first data collected was heading date (DTH) and total tiller number at heading (TTH) as a measure of early growth vigour. Heading date was recorded when the ear was half out of flag leaf sheath. At maturity the following traits were measured: total culm number (TCN), productive culm number (PRCN), grain yield of entire plant (GY), grain yield of productive culms (PRGY), seed number of entire plant (SEED), seed number of productive culms (SEEDPRC), and thousand kernel weight of whole plant (TKW) and of productive culms (PRCTKW). Other two parameters were derived from some of previously mentioned ones: grain yield per productive culm (GYxPRC) and seed number per productive culm (SEEDxPRC).

In 2010 dry biomass (B) of the whole above ground part of plant was additionally weighted and data used for harvest index (HI=GY/B) calculation.

2.2.2.7. Assessment of the endogenous ABA content in juvenile ear

This part of the work was made in the Laboratory of Physiology of Dr Ian Dodd, from Lancaster Environmental Centre, University of Lancaster, United Kingdom, during a 5 week stage program in June-July 2010.

The analysis can be divided in several steps:

- Growing of plants and spike sampling (Viterbo) – Plants were grown in both greenhouse and in the field. Seeds (Tab 12) were germinated as described in § 2.2.1.1. After transplantation into pots and 3 week vernalisation period at 4°C, half of seedlings were transferred to the greenhouse and the other half to the field. Plants were maintained regularly watered in both environments. In the field, plants were sawn in rows of 6 plants, at 20 cm distance between plants, each row corresponding to one genotype. When the first culms reached the "boot stage" of development (immature spike inside of the last internode, just below the flag leaf sheath; see Fig 10), spikes were detatched, wrapped in aluminium foil, frozen immediately in liquid nitrogen and stored later at -80°C prior to use.

Figure 10 Wheat spike at the "boot stage"

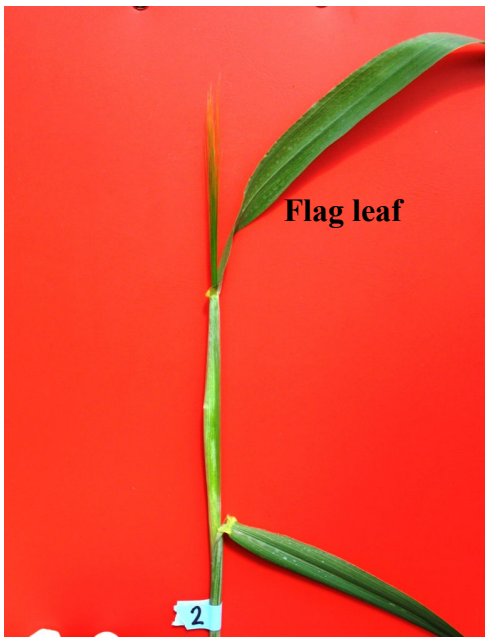


Table 10 Plant material employed for assessment of endogenous ABA content (GH – greenhouse, F – field)

Genotype	Familes	Plant No GH	Plant No F
R5-2-10 HOM+	R5-A	4	6
	R5-C	2	6
R5-2-10 HOM-	R5-D	8	6
R112-4 HOM+	V05x62-9-25	6	6
	V05x62-9-31	2	6
R112-4 HOM-	V05x62-9-13	3	6
	V05x62-9-17	8	6
R23-1 HOM+	V06x191-2-53	7	6
	V06x191-2-64	4	6
R23-1 HOM-	V06x191-2-26	6	6
	V06x191-2-31	1	6
	V06x191-2-60	4	6
T4	-	5	6
Thatcher	-	6	6
TOTAL		**66**	**84**

- Freeze-drying (Viterbo) – frozen spike tissue was lyophilized in freeze-dryer at -50°C for 48h.
- Homogenisation of tissue (Viterbo) – immediately after lyophilisation, dehydrated spikes were homogenised to powder in planetary ball mill (Retsch®, PM100); pulverized plant samples were stored in 1.5 ml tubes at room temperature and in the dark.
- ABA extraction (Lancaster) – about 0.2 g of each pulverized spike sample was diluted in deionized water in a 1:30 ratio . Tubes with diluted material were put on a shaker for overnight ABA extraction at 5°C. Samples were centrifuged and the supernatant removed for ABA assay.
- ABA radioimmunoassay (RIA) – the protocol of Quarrie et al. (1988) was applied for this purpose. The protocol is based on conditions of competition for MAC252 ABA antibodies, of radioactively H^3-labeled ABA (added during the assay) and the unlabelled ABA present in the sample. The instrument used for analysis is a scintillation counter, which detects signals from the labelled ABA and gives values reversely proportional to the amount of ABA in the sample (high readings mean that there was little ABA in the sample, and labelled ABA could efficiently bind to the antibody, and vice versa). Since aqueous extracts of barley, maize and wheat tissues are reported to have less than 10% contamination (Quarrie et al., 1988), the ABA

values recorded in this study, expressed on a dry weight (DW) basis, have not been corrected for contamination .

2.2.2.8. **Relative Water Content (RWC)**

In order to correlate endogenous ABA content with a parameter of plant water status, At the same time of juvenile spike sampling, flag leaf was sampled, due to its major contribution to the water status of the spike. Because the very same plants were used, the existence of a possible correlation between RWC and endogenous ABA content was considered worth verifying.

Flag leaf of each plant was excised, put in a 50 ml plastic tube and covered with ice to prevent evaporation of leaf tissue. Once in laboratory, ftr our leaf discs of 1 cm diameter were excised from different zones of the leaf and weighted (fresh weight = FW). Discs were then immersed in distilled water and left in dark for 2 h until the tissue had become fully turgid; disks were then re-weighted (turgid weight = TW). The final step was drying the leaf tissue at 65°C overnight. The next morning, dry leaf discs were weighted for the last time (dry weight = DW).

On the basis of 3 weighing records, RWC was calculated with the formula RWC = (FW-DW)/(TW-DW). Differences between genotypes were then evaluated with one way ANOVA. At the end, RWC data were plotted against corresponding values of endogenous ABA content and analyzed by regression analysis.

2.2.2.9. Analysis of water relations under drought stress

Also this analysis was performed at the Lancaster Environmental Centre (Lancaster, UK).

Two seeds of each plant (Tab 13) were planted in a greenhouse in a 50:50 mixture of John Innes No. 2 compost and gravel in rectangular drainpipes (6 cm x 6 cm x 30 cm) which were covered with aluminium foil until seedlings germinated and started growing (Fig 11A and B). The foil was removed when half of the plants emerged underneath. Seedlings were then thinned to 1 plant per drainpipe when leaf 2 was fully developed, which marked the beginning of 4 weeks of imposed drought by soil drying. During the experiment 12h photoperiod was maintained.

Table 11 Plant material used for analysis of water relations under drought

Genotype	Family	Plant No
R5-2-10 HOM+	R5-A	5
	R5-C	5
R5-2-10 HOM-	R5-D	10
R112-4 HOM+	V05x62-9-25	5
	V05x62-9-31	5
R112-4 HOM-	V05x62-9-13	5
	V05x62-9-17	5
R23-1 HOM+	V06x191-2-53	5
	V06x191-2-64	5
R23-1 HOM-	V06x191-2-26	5
	V06x191-2-31	5
T4	V06x191-2-60	15
Thatcher	-	15
TOTAL		90

Weekly measurements of leaf water potential (LWP; Ψ_w) and ABA content of the last fully emerged leaf were made; fresh weight (FW) and dry weight (DW) of the sampled leaf, as well as biomass at the end of experiment were also assessed.

LWP is a parameter of plant water status which measures water availability at a given time. It pertains to potential free energy of water per unit volume, and quantifies the tendency of water to move from an area to another. In the plant, water moves from areas with high to areas with low water potential. In this study, LWP was measured with a thermocoupled psychrometer (HR-33T, Wescor, Inc, Logan, USA) by dew point temperature depression (hygrometric measurements) (Neumann and Thurtell, 1972) a week, in the middle of the day, on 4 or 5 samples per each of 8 genotypes genotype. A total of 4 measurements were made, the first on the first day of soil drying experiment, the three others on a 7-day interval basis. A leaf disc (8 mm in diameter) was sampled from the last fully developed leaf and placed in a sample holder, which was then enclosed in a thermocoupled chamber and left for 2 h to allow equilibration of the psychrometer. After 2 h, the chamber was connected with a voltmeter, LWP read in μV, and consequently converted to mega Pascal (MPa).

At the same time when leaf sampling for LWP measurements was carried out, a piece of leaf was taken for assessment of endogenous ABA content. The method used for determination of ABA content was the same used for assessment of endogenous ABA in immature spikes (see § 2.2.2.7.). Leaf sample for this purpose was immediately weighted after sampling (fresh weight, FW), placed into a 1.5 ml tube with a perforated tap to allow

water evaporation during the following overnight freeze-drying process at -50°C. The dried sample was weighted again the next day (dry weight, DW) and chopped to powder. The sample was dissolved at 1:30 ratio in deionized water and ABA assay performed in the same way as described previously.

FW and DW of the sampled leaf were used for calculation of their ratio (FW/DW) as a measure of turgor[3] loss over the period of water stress.

At the end of week 4 of soil drying (Fig 11 D), final measurement of biomass produced was performed. Plants were taken out of drainpipes, the aboveground vegetative part cut off and weighted.

Figure 11 Growing of plants for analysis of water relations under drought stress; A – start of experiment; B – detail from drainpipe; C – plants after 1 week, and D – plants after 4 weeks of of water stress (end of experiment).

[3] **Turgor** is the normal rigid state of a cell, caused by pressure of the cell contents against the cell wall

2.2.2.10. Statistical analysis

In order to investigate the effects of genetic and/or environmental factors and interactions between them on variables recorded in the study, an analysis of variance (ANOVA) was conducted using SYSTAT12 software (Systat Software Incorporated, San Jose, CA, USA). A GLM was applied and each variable (i.e. traits measured) was entered as the 'dependent' factor against 'independent' factors. Independent factors common to all analysis performed were "genotype background" (G) and "7AgL segment" presence/absence (7AgL/G – nested). "Year" (Y) was included for the experiments of leaf growth and productivity assessment of the first culm and of the entire plant when the GLM was run for the 2 year data set. For the productivity experiments the "treatment" with ABA (T) and "experimental block" (= replica) I were included as well, while in the analysis of leaf growth, the factor "day" (D) was added.

For all experiments interactions of second, third and fourth order (where the number of factors was more than three) were analyzed [e.g. 7AgL/G – nested, G x Y, T x Y, R/Y – nested, Y x 7AgL(G), T x Y x 7AgL(G)]. The variations observed were considered significant at $P < 0.01$ or $P < 0.05$. All variables containing three levels (e.g. genotype) that were significantly affected with some of the fixed factors or their interactions, were further examined by selecting for pairwise mean differences (P values for pairwise comparisons using the Tukey Honestly-Significant-Difference (HSD) test).

Before performing ANOVA, obvious outliers were eliminated. For yield-related trait analysis, this was based on the whole set of data, concerning at least the majority of the assessed parameters.

Data regarding the analysis of yield-related traits were also subjected to a discriminant analysis (DA) using SYSTAT12 software (Systat Software Incorporated, San Jose, CA, USA). Only traits that were not derivatives from other traits were included in the statistical model. Data were analyzed for the two years together (2009-2010) and for separate years as well.

Classification criteria adopted were the combination of "genotype", "7AgL segment" presence/absence and "treatment" applied (ABA or control). The procedure applied for verification of discriminate power of each variable was "Complete" with 0.001 tolerance. Data were plotted on the basis of the first two scores obtained from DA with NTSYSpc 2.20N software package (Rohlf, 2008).

3. RESULTS AND DISCUSSION

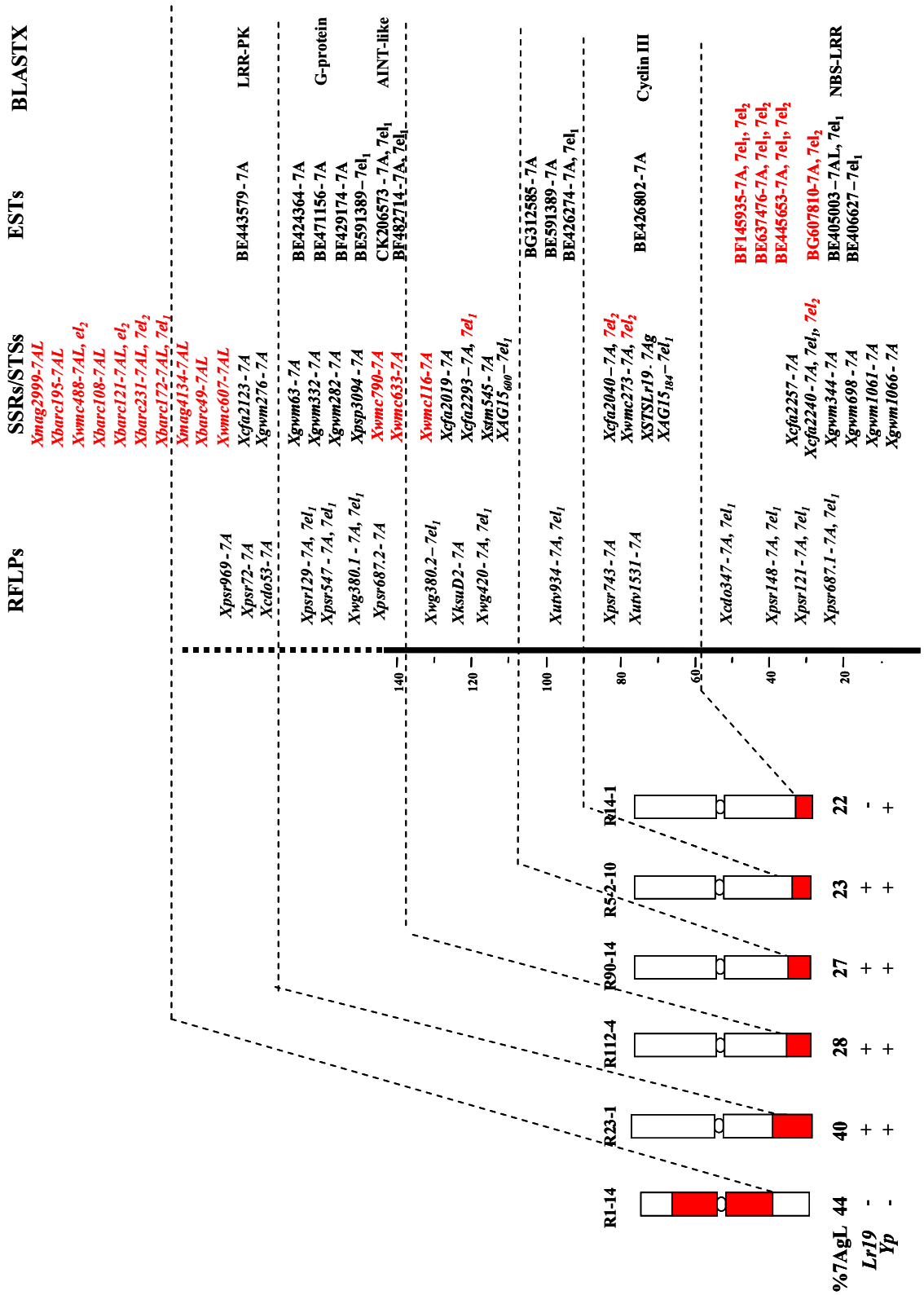
3.1. Molecular characterization of 7AL/7AgL recombinant chromosomes – development of high resolution genetic map

In order to create a useful tool for achieving the aims of the present work, a genetic map of 7AL/7AgL chromosomes developed in our laboratory (see Fig 4, § 1.6.2.1.) was enriched by assignment of new molecular markers. The existing map had a good marker density along 7AL/7AgL arm, with particular definition of distal region harbouring the *Lr19+Yp* genes, present in small *Th. ponticum* 7AgL segments of durum wheat recombinant lines R5-2-10 and R9-1 (Figs 3 and 5). As among the objectives of this study is to potentially exploit genes not only located in the vicinity of the *Lr19+Yp* genes (FHB resistance gene), but also in more proximal regions (the *Yld-7AgL* QTL), a better coverage with polymorphic markers in several 7AgL and 7AL sub-regions was obtained (Fig 12).

The availability of 8 different 7AL-7AgL breakpoints present in the durum wheat-*Th. ponticum* recombinant lines represented a very useful tool to "dissect" the genomic region of actual and potential interest in several physical/genetic sub-regions, allowing a precise marker assignment to each of them. This is of paramount importance when trying to determine the contribution of a particular segment to a given, complex phenotypic trait, such as, in this study, yield (see § 3.7.).

A total of 55 PCR-based markers were used (see §2.2.1.3.), both newly tested and already mapped in our laboratory (but tested on a limited number of materials). Forty three of them amplified 7AL and/or 7AgL bands and 12 resulted not useful (not reported here) because they amplified on regions of chromosomal groups other than 7 or gave composite band profiles difficult to interpret. Seventeen new markers, of which 13 SSRs and 4 ESTs, were assigned on the existing genetic map (Fig 12 in red). 8 gave polymorphic alleles on 7AL and on el_1 and/or el_2 *Th. ponticum* chromatin present in the genotypes assessed (see § 2.1.1.1.). For four molecular markers that had previously shown a 7AL allele only, useful polymorphism at an el_1 (CFA2293) or an el_2 (CFA2040, CFA2240 and WMC273) locus could be revealed, also thanks to the inclusion of additional genetic stocks (in particular KS stocks of bread wheat (Fig 6, § 1.6.2.2.).

Figure 12 Genetic and physical maps of distal 7AL/7AgL regions: a high resolution mapping (letters in red – newly added markers and identified alleles; el_1 = alleles from *Th. ponticum* accession susceptible to FHB and present in durum wheat material produced in our laboratory; el_2 = alleles from *Th. ponticum* accession resistant to FHB, present in available bread wheat recombinant lines included in molecular screening)



The first group of new markers was assigned in the proximal region of the 7AgL segment present in R23-1 line and even beyond the 7AL-7AgL breakpoint of this line (Fig 12). More precisely, this area spans from marker MAG2999 to GWM 276 on the proximal side and partly overlaps with the 7AgL segment present in R112-4 line on the distal side (WMC116). Assuming 7AL-7AgL colinearity and based on evidence in bread wheat (Quarrie at al., 2006), line R23-1 might harbour a gene/QTL contributing to the yield-increasing effects generally ascribed to a *Yld-7AgL* QTL (see § 1.6.3.). In addition, new molecular markers in the proximal 7AL-7AgL chromosomal region have been useful in the course of production of a new recombinant line (see § 3.3.).

A second group of markers was newly assigned in the very distal region of 7AL/7AgL arm, i.e. in the terminal 23% of the long arm of recombinant line R5-2-10 (Fig 4). Such markers are 4 ESTs, polymorphic between 7AL, $7el_1$ and $7el_2$. One of them, BE445653, and the SSR marker CFA2240, flank the region supposed to contain the FHB resistance gene/QTL on $7el_2$ in the *Th. ponticum* resistant accession (Shen and Ohm, 2007). In fact, these markers were useful in PCR selection of material produced during the work aimed at pyramiding the FHB resistance into bread and durum lines already possessing the *Lr19+Yp* genes (see § 3.2.).

3.2. Setting up of the pyramiding of *Lr19+Yp* genes present in durum wheat recombinant lines with Fusarium Head Blight (FHB) resistance from a different *Th. ponticum* accession

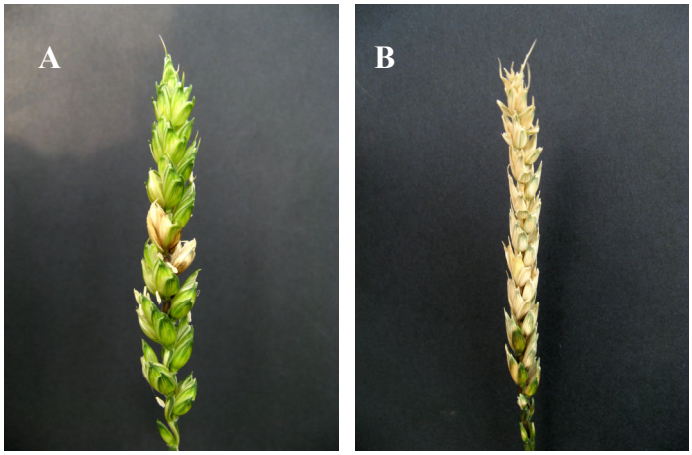
The work objective of pyramiding in a relatively short time Fusarium Head Blight resistance (*FHBres*) with the *Lr19+Yp* genes, already transferred onto the 7AL arm of durum wheat recombinants, relied on the possibility that $7el_1$ and $7el_2$ *Th. ponticum* chromatin could undergo recombination without the need of genetic promotion. In fact, bread wheat- *Th. ponticum* (el_2) FHB resistant accessions were reported to be substantially homologous with to el_1–bearing susceptible accessions, at least in their distal 7elL regions (50-80% $7el_1$-$7el_2$ pairing, Dvořák, 1975; Kibirige-Sebunya and Knott, 1983; Shen et al., 2004). In addition to this, recent genetic map data of $7el_1$ (Fig 12; Ayala-Navarette et al., 2007) and $7el_2$ (Shen and Ohm, 2007) chromosomes, confirmed the FHB resistance gene/QTL to be situated on the same distal portion of the $7el_2$L arm as are *Lr19+Yp* genes on $7el_1$L, and it is largely demonstrated that recombination frequency in these chromosomal regions is normally high in the Triticeae (Saintenac et al., 2009). Moreover, molecular markers for the tagging of all genes of interest are available (see §1.6.2.2. and § 2.1.1.3.).

Therefore, the $7el_2$ FHB resistance donor stocks (Fig 6, Tab 12) were used in crosses with bread and durum wheat translocation and recombinant lines possessing $7el_1$ chromosome segments with *Lr19+Yp*. Parental bread wheat lines (T4 and KS lines) had been previously tested for the response to inoculation with *Fusarium* spp. pathotypes spread in Italy (Gennaro, unpublished). Results obtained were in accordance with previous observations: line T4 resulted highly susceptible, while the KS genetic stocks confirmed their FHB resistance. The latter were able to stop the disease spreading soon after inoculation, and to maintain the number of infected spikelets constant throughout the testing period (Fig 13).

Table 12 Recombinant/translocation chromosomes present in bread and durum wheat lines used for FHB resistance transfer

Line	Wheat type	*Th.ponticum* chromatin introduced	Wheat chromosome arm involved	Method applied for transfer
KS24-1	bread	$7el_2$	7DL	Chrom. Eng.
KS10-2	bread	$7el_2$	7DL, 7DS	Chrom. Eng.
T4	bread	$7el_1$	7DL	Irradiation
R5-2-10	durum	$7el_1$	7AL	Chrom. Eng.
R23-1	durum	$7el_1$	7AL	Chrom. Eng

Figure 13 Phenotype of infected spikes (35 days post-inoculation) of the resistant KS lines (A) and susceptible T4 line (B) (Gennaro et al., unpublished)



3.2.1. **Molecular cytogenetic characterization of el_2 donor lines**

By means of GISH (§ 2.2.1.4.) on somatic chromosomes, the physical organisation of 7D-7el chromosomes present in the different bread wheat lines used was confirmed for lines T4 and KS 24-1 and newly defined for KS10-2 (Fig 14). Of the two KS recombinant lines, the former possesses the whole 7DL arm replaced by $7el_2L$, while the latter, contrary to earlier evidence (Kim et al., 1993) has also about half of its 7DS substituted by $7el_2$ chromatin.

GISH applied onto meiocytes of T4 x KS F_1 genotypes at metaphase I showed that homology of $7el_1$ and $7el_2$ chromosomes is nearly complete; in fact, a ring bivalent, with pairing between the two 7el long arms on one side and between the 7DS arms on the other was almost invariably observed (Fig 15), while in a few cells a rod bivalent, with pairing only between the 7DS arms, was detected (Fig 16).

Figure 14 Physical organisation of 7D-7el chromosomes in genotypes with target regions for gene pyramiding (*Th.ponticum* DNA is marked by a green fluorescent dye)

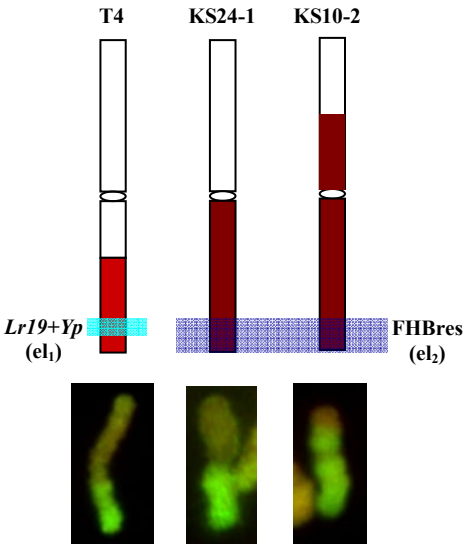


Figure 15 Metaphase I pairing in F_1 plants from the cross between KS10-2 and T4: homologous pairing between chromosomes 7DS-7el$_2$S.7el$_2$L and 7DS.7DL-7el$_1$ results almost invariably in a ring bivalent (*Th. ponticum* DNA is marked by green fluorescent dye)

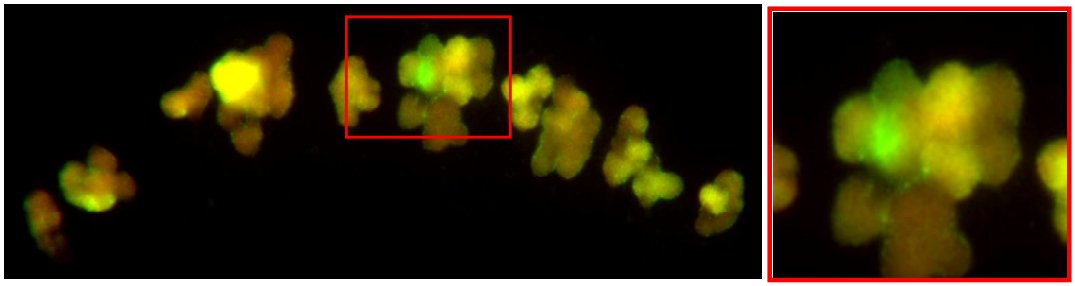
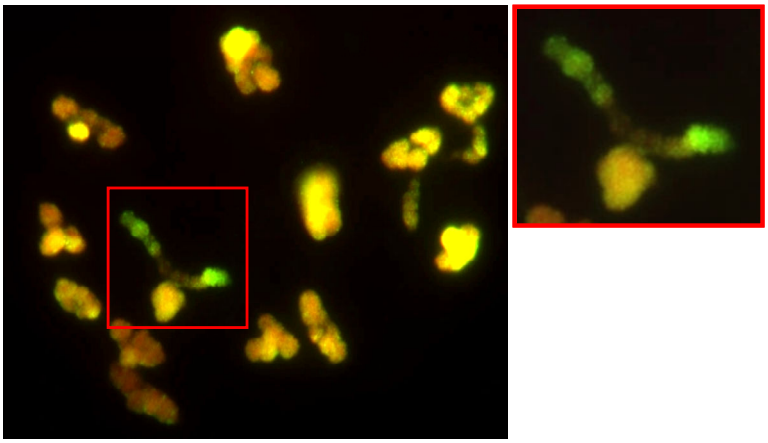


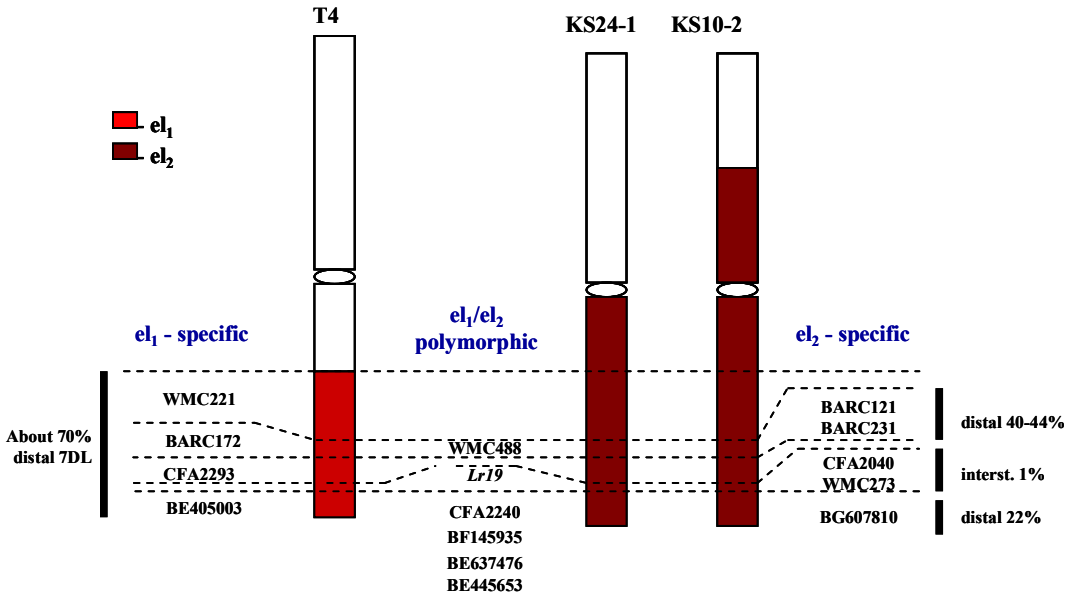
Figure 16 Homologous pairing between the two 7D short arms in KS10-2 and T4 lines results in a rod bivalent (indicated in rectangle)



3.2.2. **Marker polymorphism validation for MAS of *FHBres* + *Lr19* + *Yp***

Enrichment of the genetic map (Fig 12) consisted in fine molecular characterization of the distal 7AL/7el$_1$L region, as described in § 3.1. Marker screening of el$_1$–bearing durum and bread wheat lines (e.g. T4, Fig 6) and el$_2$-bread wheat lines KS24-1 and KS10-2 revealed useful el$_1$– el$_2$ polymorphism (Fig 17).

Figure 17 Molecular marker polymorphisms identified between el$_1$ and el$_2$ chromatin originating from two *Th. ponticum* accessions



Also the marker band profiles confirmed the physical organization of 7D recombinant chromosome of lines KS24-1 and KS10-2, as revealed by GISH (Fig 16).

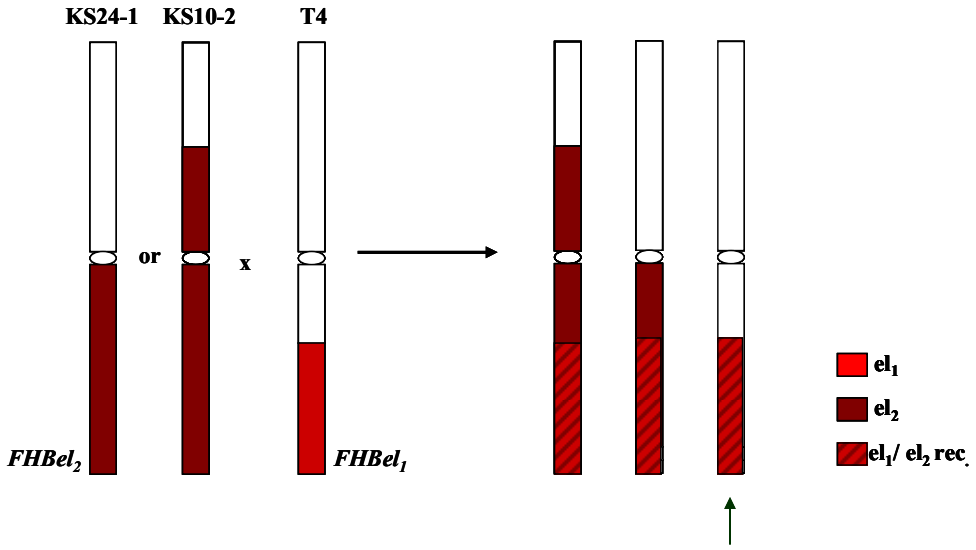
3.2.3. **The transfer procedure**

Crosses have been performed in order to obtain the desired recombination between 7el$_1$/7el$_2$ chromosomes, hence combination of different *Th. ponticum* 7e$_l$ genes, into both bread and durum wheat.

Crosses between bread wheat genotypes (T4 and KS) were not expected to have any particular obstacle, since pairing and recombination would have involved the 7D/7el chromosome of each line (Figs 15 and 16). The physical structure of the desired recombinant chromosome corresponds to that of T4, but with a mixed 7el$_1$/7el$_2$ gene content (Fig 18). In total 16 seeds have been obtained by backcrossing KS x T4 F$_1$ plants to

the Italian, FHB and leaf-rust susceptible bread wheat cv. Blasco: 4 from a cross KS24-1 x T4, and 12 from a cross KS10-2 x T4. DNA from only one plant (cross KS10-2 x T4) amplified desired alleles for FHB resistance and *Lr19+Yp,* which represent 6.25% of the gametic recombination frequency (=gam rf) and 12.5% of the total recombination frequency (=tot rf=2 x gam rf). Electrophoretic profile of three markers used for selection is reported in Fig 19. BC_1 progeny has been produced from the two selected plants, and this is currently being subjected to MAS in view of performing further BCs and selfing. On homozygous $7el_1+7el_2$ plants, as from MAS, *Lr19+Yp+FHBres* phenotypes will be validated.

Figure 18 Crosses between $7el_1/7el_2$ –bearing bread wheat genotypes and their recombinant products (the desired recombinant is marked by an arrow)



Crosses have been also performed between line KS24-1 (FHB resistance) and durum wheat-*Th. ponticum* recombinant lines R23-1 and R5-2-10, carrying the *Lr19+Yp* genes on their 7AL arm (Fig 20). Due to the fact that the yet homologous $7el_1L$ and $7el_2L$ regions reside on different wheat chromosomes (7A and 7D, respectively), an overall higher genetic instability and lower fertility was expected in the initial F_1 and BC generations. For the same reason, $7el_1L$-$7el_2L$ pairing and recombination frequency was expected to be somewhat reduced, resulting in.

The pentaploid F_1 plants have been backcrossed to durum genotypes, including the same recombinant lines R23-1 and R5-2-10 and cultivated varieties and 10 BC_1 plants in total were obtained (5 from each of crosses KS24-1 x R23-1, and KS24-1 x R5-2-10). DNA samples of BC_1 progeny were analyzed by molecular markers (Fig 17), and surprisingly

enough, four of them (2 from each type of cross) had all three desired alleles and were sawn for the following BC (estimated gam rf=40%, tot rf=80%). Determination of chromosome number in BC_1 (expected from 2n=28 to 2n=35) resulted in 30-32 chromosomes per plant. The work will continue with additional BCs of selected individuals with a range of cultivated FHB and leaf-rust susceptible varieties, also to bring the chromosome number to 2n = 28. Phenotypic verification of all the target genes will be carried out as soon as sufficient seed will be available of homozygous carriers.

Figure 19 Electrophorograms of molecular markers showing $7el_1/7el_2$ polymorphism (1– cv. Blasco; 2 – KS24-1; 3 – KS10-2; 4 – T4; 5 – F1 KS24-1 x T4; 6 – F1 KS10-2 x T4; 7 – cv. Simeto; 8 – R23-1; 9 – R5-2-10; 10 – F1 KS24-1 x R23-1; 11 – F1 KS24-1 x R5-2-10)

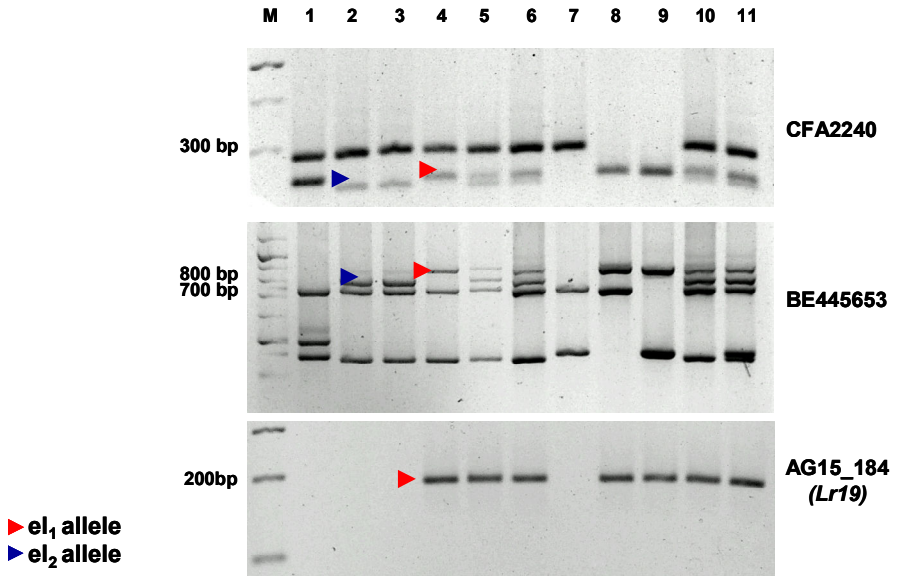
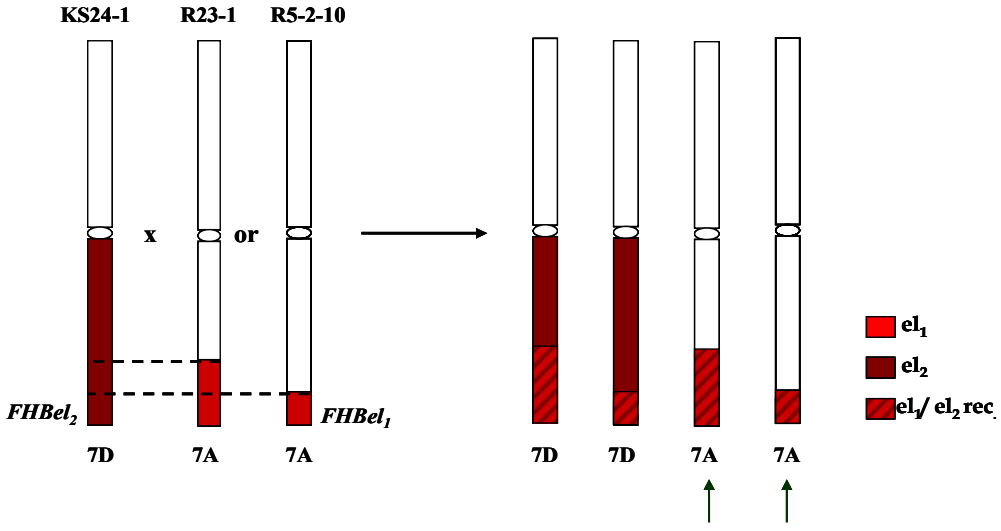


Figure 20 Interspecific crosses performed between bread and durum wheat lines for gene pyramiding in durum wheat (the desired recombinants are marked with arrows)



3.3. **Production of a new durum wheat-*Th. ponticum* recombinant line of potential value for analysis of the *Yld-7AgL* QTL.**

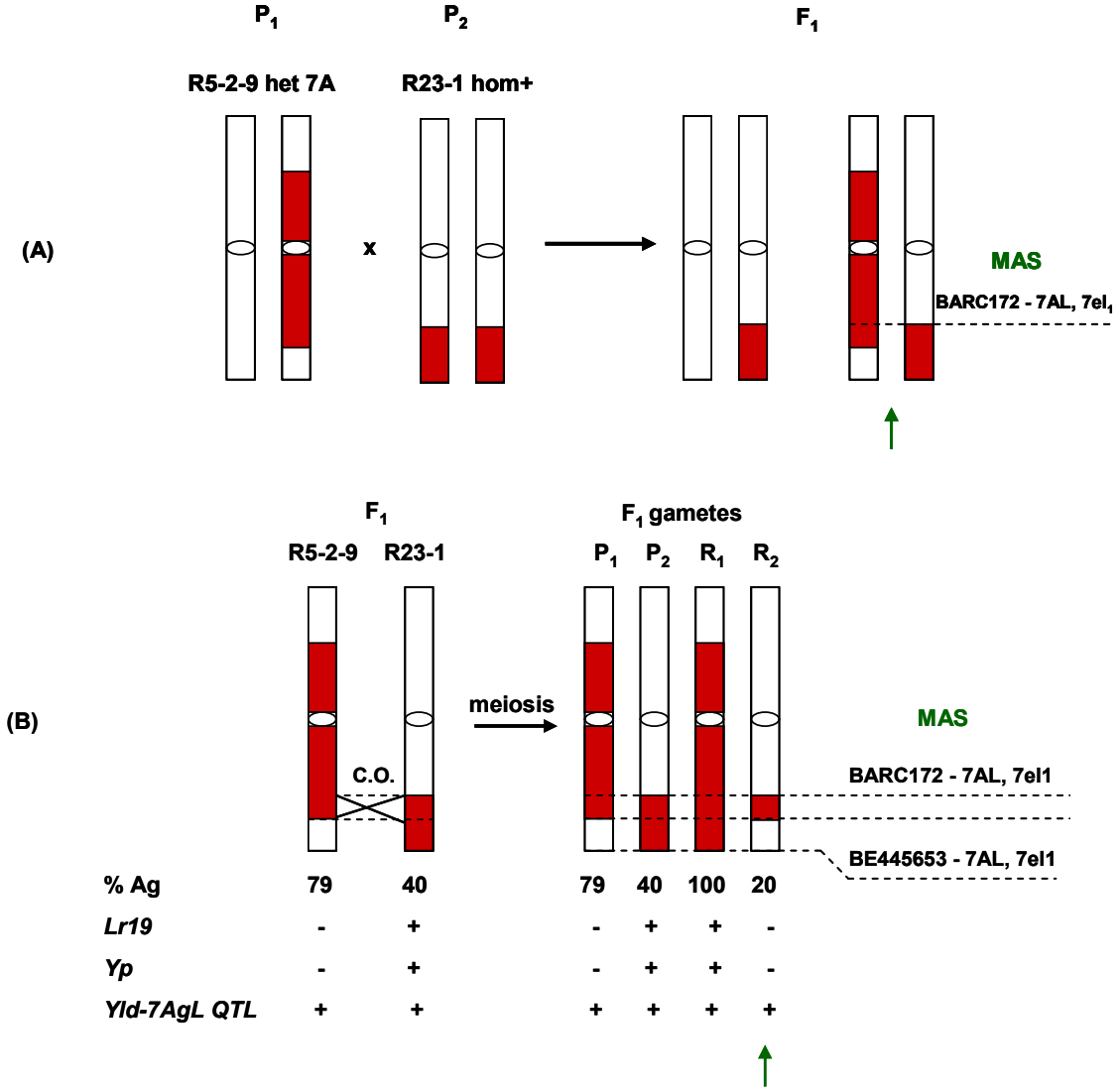
One of the objectives of the work was the shortening of 7AgL segment present in line R23-1 in order to eliminate or minimize the stability problems associated with the presence of probably two *Sd* (Segregation distortion) genes (see § 1.6.4.), thus making accessible to analysis, particularly of yield-related traits, the region comprised between the 7AL-7AgL breakpoints (BPs) of R112-4 and R23-1 (Fig 12).

3.3.1. Genotyping of BC_1 and F_2 populations obtained from the cross R5-2-9 x R23-1

In order to achieve this goal, the previously produced durum wheat recombinant lines R5-2-9 (heterozygous 7A) and R23-1 have been crossed (Fig 21A). Two types of F_1 progeny were obtained, and MAS with the 7AL-7AgL polymorphic marker BARC172 helped selecting the useful one (marked with arrow in Fig 21A), carrying one R5-2-9 and one R23-1 chromosomes in place of the normal 7A pair. As a result of homologous pairing in the 7AgL portions shared by the two recombinant chromosomes (Fig 21A), a new recombinant line, named R5-2-23, was obtained, carrying an interstitial 7AgL segment of about 20% of the 7AL arm, flanked by the R5-2-9 and R23-1 BPs (Fig 21B).

Selected F_1 plants were then crossed with the durum wheat cv. Hathor, a relatively low-yielding variety which might have been able to better reveal the presence of a *Yld-7AgL* QTL (if any) potentially harboured in the interval flaked by R5-2-9 (distal) and R23-1 (proximal) BPs (Fig 21B).

Figure 21 Development of the new R5-2-23 recombinant line (= R_2); (A) production of the F_1 generation from initial cross between parental recombinant lines R5-2-9 (heterozygous 7A) and R23-1; (B) recombination events at meiosis of selected F_1 plants (R5-2-9 + R23-1 chromosomes) with the resulting four gamete types (P-parental, R-recombinant; selected plants by MAS are indicated by a green arrow)



By the use of two polymorphic and co-dominant molecular markers, one (BARC172) located in the region proximal to the R23-1 BP, and the other (BE445653) in the region distal to the R5-2-9 BP (Fig 12), it was possible to identify all four gamete types expected to be produced in the F_1 meiosis, and found in different combinations with a 7A chromosome in the BC_1 progeny. A total of 105 plants were genotyped with the above markers and results are reported in Tab 13, together with the PCR profile of the two markers used (Fig 22). Plants possessing the new recombinant R5-2-23 chromosome (R_2 type) represented a 16.2% of the progeny (=gam rf, see § 3.2.3.). Considering the lower

value of R_1 frequency as the result of adverse gametic selection, total recombination frequency that occurred in the shared 7AgL segment by R5-2-9 and R23-1can be estimated as [gam rf x 2], i.e. around 32%.

Table 13 Scheme of PCR profiles of 4 expected genotypes in BC_1 progeny obtained from the cross [(R5-2-9 x R23-1) x cv. Hathor] and corresponding frequencies obtained (the genotype of interest is marked with green arrow)

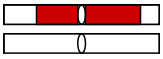
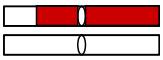
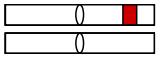
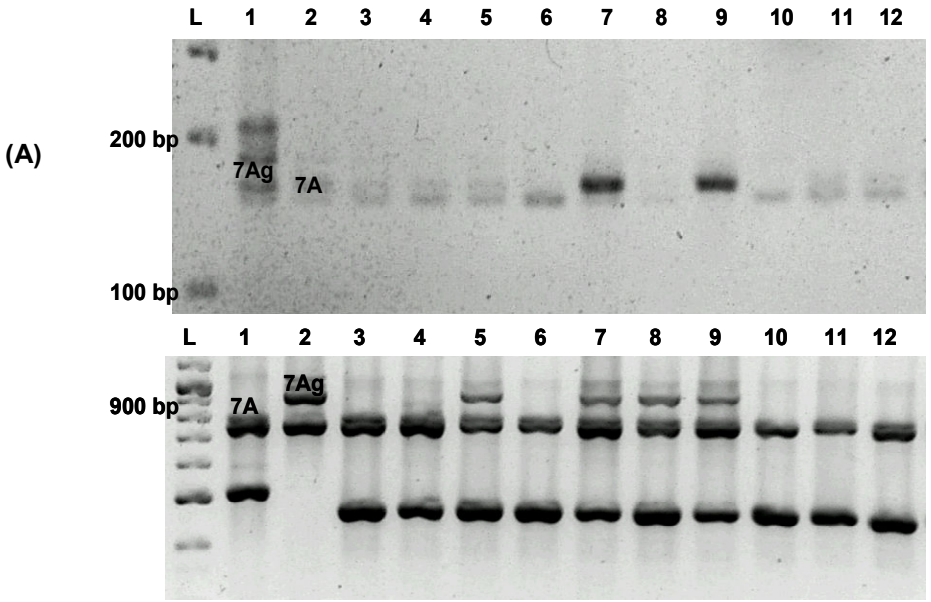
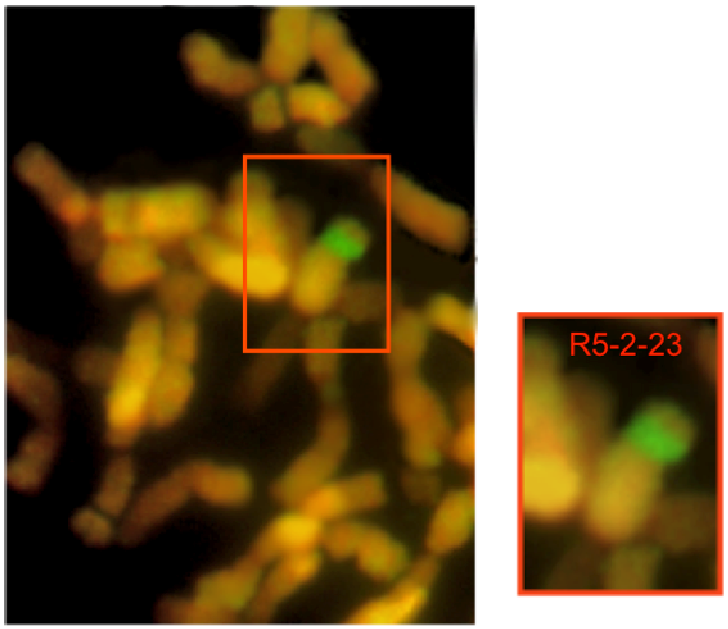
	BC_1 genotype	BARC172	BE445653	Observed plants	%	
7A P_1		7A 7A	7A 7Ag	45	42.9	
7A P_2		7A 7Ag	7A 7A	33	31.4	
7A R_1		7A 7Ag	7A 7Ag	10	9.5	
7A R_2		7A 7A	7A 7A	17	16.2	←

Figure 22 Electrophoretic profiles of the SSR marker BARC172 (A) and the EST marker BE445653 (B) used in selection of BC_1 progeny obtained from the cross [(R5-2-9 x R23-1) x cv. Hathor] (L-ladder, 1- R5-2-9, 2-R23-1, 3- cv. Hathor, 4-12 – BC_1 plants)



The structure of the R5-2-23 chromosome was clearly visualized by means of GISH (Fig 23).

Figure 23 Somatic metaphase of a BC_1 plant containing a single R5-2-23 7A-7Ag chromosome visualized by GISH: the *Th. ponticum* interstitial segment is marked by a green fluorescent dye



Once obtained in a heterozygous state with a normal 7A, transmission ability of the new recombinant chromosome was assessed in BC_1F_2 progeny from the cross with cv. Hathor, which were genotyped by MAS. In various genetic backgrounds, the R23-1 chromosome was found to be unstable (Grossi, 2010), possibly due to the presence of 7AgL segregation distortion genes *Sd1* and *Sd2* (see § 1.6.4.). As a result, transmission of R23-1 chromosome was severely reduced through the male germline. Since the new recombinant contains the proximal portion of the R23-1 7AgL segment where *Sd1*, but not *Sd2*, is probably localized (Grossi, 2010), at least part of the abnormalities associated with the R23-1 chromosome might have been retained by the R5-2-23 chromosome.

Screening of plants belonging to 8 BC_1F_2 families showed that, as a whole, male gametes carrying the R5-2-23 chromosome suffer from a competitive disadvantage compared to 7A carriers, similarly to what observed for the R23-1 chromosome. In fact, while female transmission (observed in BC progeny, not shown) was normal, only 3 out of the 8 families produced homozygous genotype for the R5-2-23 chromosome, and only 1 out of 2 families produced homozygous genotype for the R23-1 chromosome (Tab 14). The

transmissibility was still reduced in F_2 offspring with respect to the expected 25%, but comparable between R5-2-23 and R23-1 in the background of cv. Hathor.

Table 14 Observed frequencies of HOM+ plants in BC_1F_2 families from the cross R5-2-23 x Hathor

Genotype	Mother plant	Plants screened	HOM+	% HOM+
R23-1	V09x5-1	22	0	0
	V09x36-13	20	3	15.0
R5-2-23	V09x23-2	24	0	0
	V09x31-11	24	0	0
	V09x17-1	26	3	11.5
	V09x22-2	14	0	0
	V09x22-3	14	0	0
	V09x31-7	12	0	0
	V09x36-8	14	1	7.1
	V09x31-4	52	6	11.5

However, in contrast with what observed with R23-1 homozygotes (Grossi, 2010), R5-2-23 HOM+ were sawn and showed to have good seed germination ability, as well as an overall good plant development. Their phenotype seemed normal and, notably, they seem to have normal fertility. As a whole, although some effects (male gamete transmission) probably associated with presence of the *Sd1* gene still affect R5-2-23 plants, the overall effect seems to be of minor entity compared to that of R23-1.

3.3.2. **Analysis of meiotic and postmeiotic division of segregating populations of new recombinant**

Because of their low transmission ability, male gametes carrying the new recombinant chromosome have been subjected to a series of cytogenetic analyses in heterozygous from [(R5-2-9 x R23-1) x cv. Hathor] cross (Tab 13) and the following BC_1 progeny.

Meiosis showed to be regular in all phases. At metaphase I 14 bivalents were regularly observed in almost all cells. At anaphase I and telophase I, some cells with laggards[4] were observed, which probably gave origin to micronuclei detected at later stages. In 4 plants of the [(R5-2-9 x R23-1) x cv. Hathor] offspring (V09x17-1, V09x23-6,

[4] **Laggard** is a chromosome which is late in reaching one of the poles of meiotic spindle in anaphase or telophase of meiosis

V09x31-7, and V09x32-6) the frequency of such cells was around 24%, while 7 other plants were about normal (0-8%). The percentage of cells with laggards and micro-nuclei in late meiotic phases varied also in different BC_1 progeny plants, ranging to 7 up to 47% (Tab 15). This variability may be attributed to variability in the background of the different plants, still far from isogeneity.

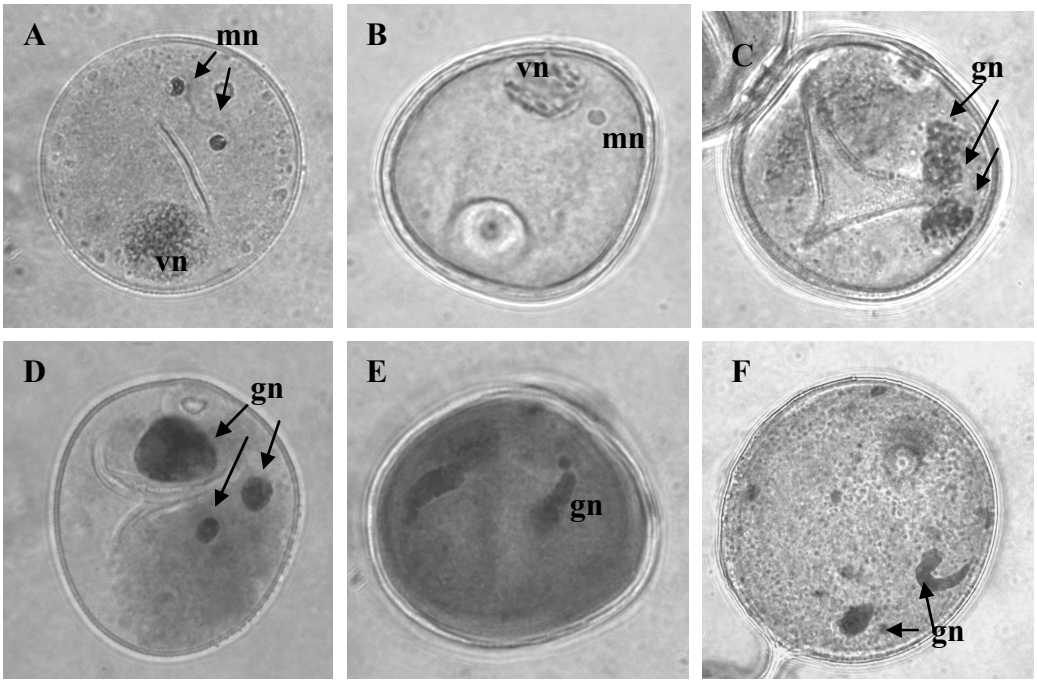
Table 15 Frequency of observed cells with irregular chromosome organisation in late phases of meiosis in heterozygous BC_1 progeny of the new recombinant R5-2-23

Line	parentals		%	%
	female	male	normal	irregular
V10x58-20	F_1	Hathor	92.9	7.1
V10x81-20	F_1	Hathor	53.0	47.0
V10x81-22	F_1	Hathor	81.6	18.5
V10x59-21	Hathor	F_1	66.7	33.3
V10x59-22	Hathor	F_1	87.9	12.2
V10x72-21	Hathor	F_1	66.4	33.6
V10x81-21	Hathor	F_1	84.0	16.0

Pollen post-meiotic mitotic divisions of the same plants were also analyzed and some irregularities (micro-nuclei, broken chromatin) were detected. Micronuclei were prevalently found at the first post-meiotic division, at a 0-17% frequency in both progeny types (Fig 24A and B). At the second post-meiotic division, cells with micro-nuclei were almost absent, but a few plants showed them with a 20-25% frequency. In these few plants some severe aberration in chromatin organization at the end of second post-meiotic division were observed: 3 instead of 2 generative nuclei (Fig 24C and D), and broken chromatin (Fig 24 E and F).

In conclusion, the work done so far provided with several vigorous R5-2-23 homozygous plants, BC_2 progeny from the cross with cv. Hathor (being currently harvested). In spite of residual irregularities still associated with the shortened 7AgL segment, selection of stable homozygous R5-2-23 plants seemed feasible; hence, this new recombinant line will be an additional tool in the progress of the research on the putative *Yld-7AgL* QTL(s).

Figure 24 Pollen grains with aberrations at post-meiotic pollen divisions: A-B micronuclei at prophase I; C-D generative nuclei of unequal size after multiple division at telophase II; E-F broken chromatin at telophase II (vn=vegetative nucleus, gn=generative nucleus, mn=micronucleus).



3.4. Evaluation of fertility and yield traits in field growing condition and in relation to simulated abiotic stress

To characterize the chromosomal region(s) harbouring the putative *Yld-7AgL* QTL(s), a series of physiological parameters and agronomic traits were evaluated in three durum wheat recombinant lines and one bread wheat translocation lines. The latter was T4, carrying on its 7DL arm the 70% long 7AgL segment to which the positive effects on yield were ascribed (§ 1.6.3.). The durum wheat lines, i.e. R5-2-10, R112-4, R23-1, contain shorter portions of the same 7AgL (Fig 3), and, although a different homoeologue is involved in their 7AgL transfers (7AL vs. 7DL), they represent a very useful tool to "dissect" the T4 segment into sub-regions (spanning more than half of its length), hence determine the contribution of different *Th. ponticum* segments to yield-related traits. Of all the lines, NILs have been used, developed in the background of durum wheat cv. Simeto and bread wheat cv. Thatcher (Tab 8). Thus, comparisons have been carried out among lines, as well as between homozygous carriers (HOM+) and non-carriers (HOM-) of each line. For the T4 line, the cv. Thatcher was used as negative control.

Since there have been reports of ABA dependence of the wheat 7AL QTL (Quarrie et al., 2007) of which the *Yld-7AgL* QTL might represent an orthologue (Quarrie et al., 2006), and of the preferential positive expression of the latter in bread wheat when grown in drought-prone environments (Singh et al., 1998), a test with exogenous application of ABA (to mimic the stress condition) was performed on the first culm of plants with the genotypes above recalled (Tab 8) at the appropriate developmental stage (§ 2.2.2.3.), and fertility parameters recorded on this culm and its spike. In addition, the overall productivity (biomass and grain yield) of the same plants was evaluated in field conditions on the basis of several parameters measured during growth, at maturity and in post-harvest period.

3.4.1. Climatic conditions in the two experimental seasons

Weather conditions in two the experimental years, 2009 and 2010, showed rather remarkable differences, which, as demonstrated in the presentation of the results, have affected the expression of some of the important yield parameters.

Meteorological parameters were retrieved from the meteorological station situated at the experimental station of University of Tuscia (Lat N 42.426418° Lon E 12.080573°), Viterbo, where greenhouse and experimental field used in the present work are located. Differences between the two years are illustrated in Fig 25 by average daily temperature and precipitation quantity during the period from the beginning of the experiment until harvest. Year 2009 was characterized by warmer spring and fewer precipitations compared to cooler and rainier 2010. Such conditions caused differences in the duration of the biological cycle of the plants (shorter in 2009), and differential expression of several other characters (see ahead in the text). Number of days to heading, for example, was on average 7-8 shorter in 2009 because of the same reason. In both years the order of heading was R5-2-10 (earliest), R112-4, R23-1 and T4-Thatcher (latest) (Fig 26, Fig 27 and Tab 17).

Figure 25 2009 and 2010 precipitation and mean daily temperatures during experimental field trials carried out in Viterbo (Italy); red boxes indicate duration of the experiment from sawing until harvesting

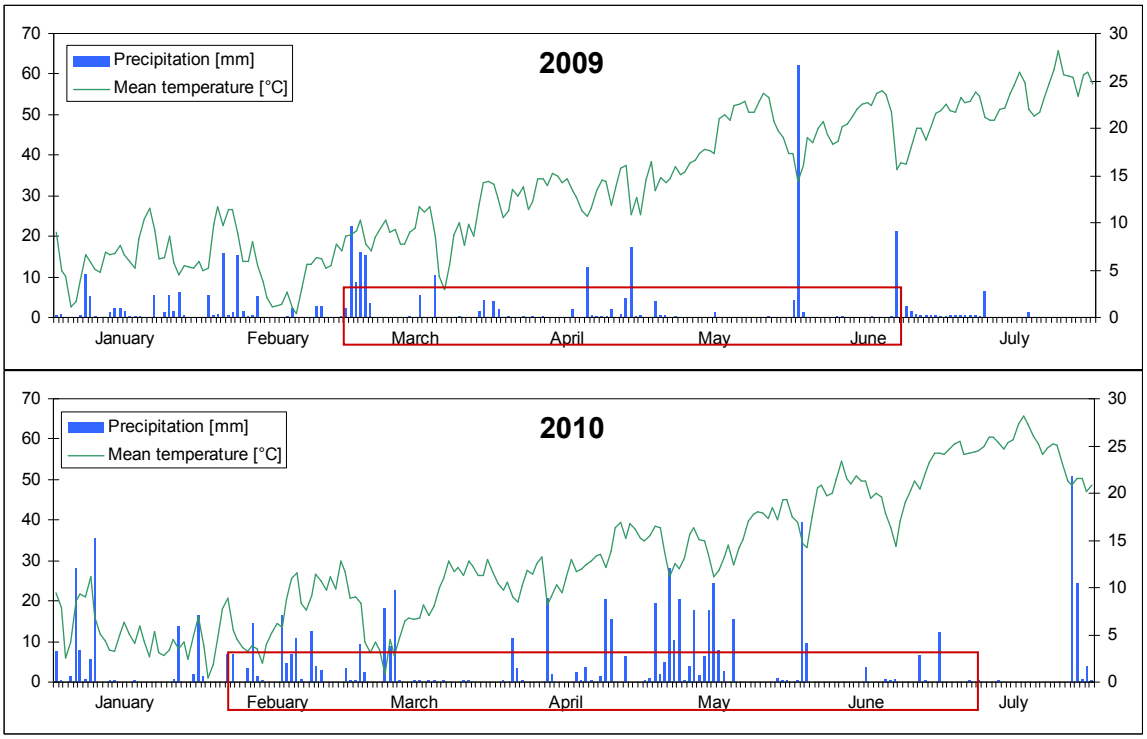


Figure 26 Distribution of heading date in durum and bread wheat lines analyzed in 2009 and 2010

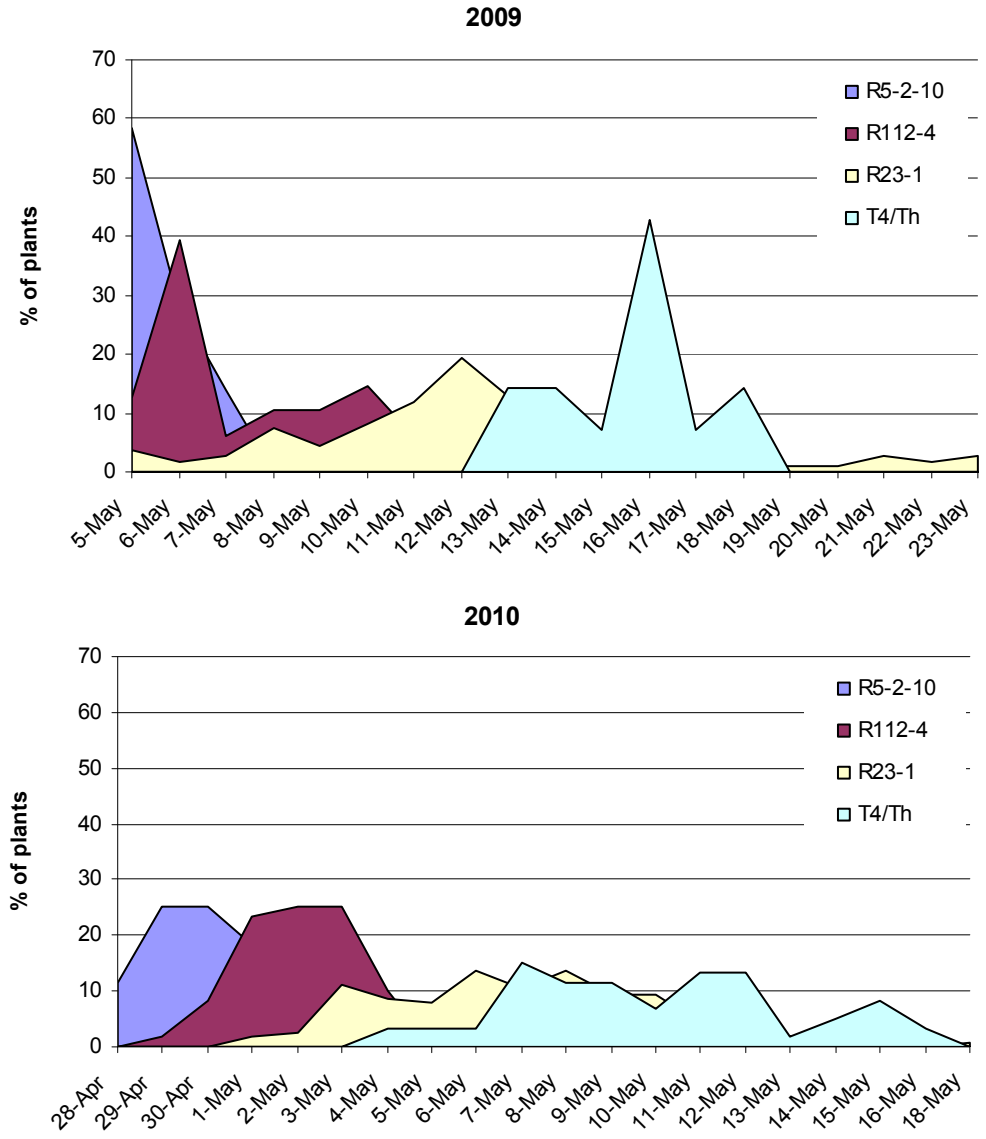
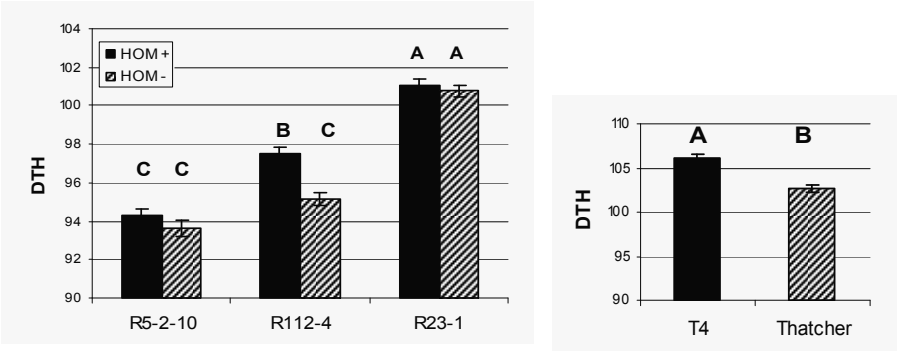


Figure 27 Days to heading (DTH) mean values in analyzed durum and bread wheat lines with Tukey HDS test ranking at 0.01 significance level



3.5. Analysis of the effect of exogenously applied ABA on yield-related traits of the 1st culm in two year experiment

Application of ABA was performed on juvenile plants at the "double ridge" developmental stage of the first spike (see § 2.2.2.3.). Since reproductive organs start differentiating at this specific period, fertility and yield-related parameters, such as spikelet number, seed number, flag leaf dimensions, could be significantly affected by an exogenous application of ABA. A possible differential response of 7AgL recombinant/translocation lines to such a simulation of stress conditions (in terms of fertility and productivity) would have indicated an ABA-dependence of 7AgL-related phenotypes.

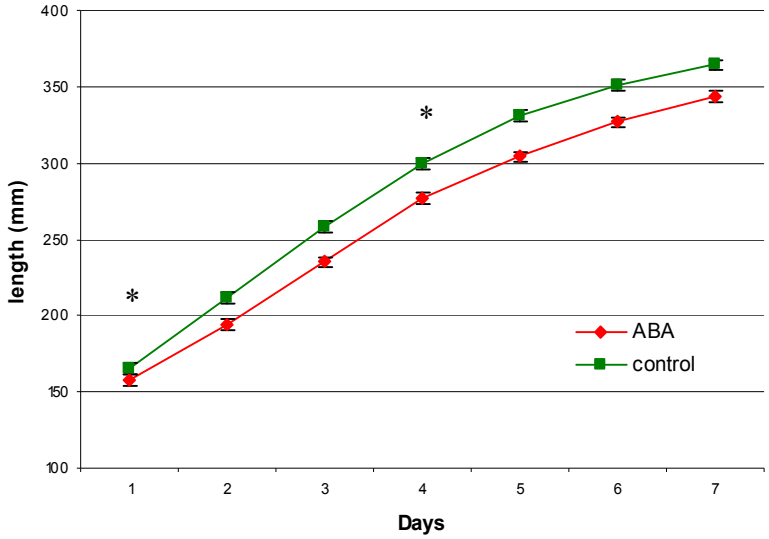
3.5.1. Leaf growth measurements

In order to confirm the efficacy of ABA treatment (see § 2.2.2.4.) at double ridge stage, top leaf growth of treated plants was measured on a daily basis in the week of treatment. In the present study, leaf 6 was actively emerging when the double ridge was detected. ANOVA analysis revealed that ABA had a negative effect on leaf 6 growth (Tab 16, Fig 28). Moreover, none of the interactions between factors analyzed in the current model was significant, indicating that the treatment had the same effect in both years and in each day during the recording period.

Table 16 Summary table of ANOVA for leaf 6 growth in the week of ABA treatment by GLM (Y-year, T-treatment, D-day)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
Y	1526747.100	1	1526747.100	606.664	0.000
T	280070.656	1	280070.656	111.288	**0.000**
D	11731288.860	6	1955214.810	776.919	0.000
T*Y	3157.789	1	3157.789	1.255	0.263
D*T	19298.956	6	3216.493	1.278	0.264
Y*D*T	18073.830	6	3012.305	1.197	0.305
Error	6621243.919	2631	2516.626		

Figure 28 Leaf 6 growth during the week of ABA treatment in 2009 and 2010 experiments. Asterisks indicates days in which ABA/water (= control) applications were performed)



ABA treatment was effective and had a depressing effect on leaf 6 growth as expected in stress conditions. It was expected that other developing tissues at the moment of treatment (developing ear in particular) would be as well affected by ABA treatment.

3.5.2. Flag leaf development and yield related traits of the first culm at maturity in durum wheat recombinant lines

Quarrie et al. (2006) correlated the identified yield QTL on 7AL with flag leaf width and proposed this QTL to be an orthologue of the *Arabidopsis* gene *AINTEGUMENTA* (*ANT*), involved in control of cell division and of leaf width. In order to investigate if a similar mechanism could be true also for the *Yld-7AgL* QTL(s), and if, this effect too was ABA-dependent as, again, in the case of the 7AL yield QTL (Quarrie et al., 2007), on the ABA–treated first culm, flag leaf dimensions and other fertility traits have been assessed and correlated with the ABA effect and presence/absence of a given 7AgL segment. Measurements of 13 traits[5] in 2009 and 15 in 2010 are reported in Appendix 2 and grouped by the treatment applied (ABA or water as control).

ANOVA[6] analysis by GLM (Tab 17) for durum wheat lines shows that treatment with exogenous ABA (T) had a highly significant (P<0.000) negative effect on FLW, FL-

[5] For the list of abbreviations used for the traits analyzed see Appendix 1
[6] For all ANOVA summary tables see Appendix 4

1W, FL-1L, EL and SPNE, but, on the other hand, the decrease in traits values was the same in all genotypes, independently of 7AgL segment presence/absence (Tx7AgL/G). Therefore, if differences in expression of the measured traits existed, they did not apparently depend on the treatment with ABA, but on some other factor. Apart from the above listed characters, ABA did not affect others traits in any manner. Thus, as a whole, the *Yld-7AgL* QTL does not seem to be ABA-dependent.

"Year" effect alone (Y) was significant for almost all traits, at $P<0.000$ for FLW, EL, TH, SPS, TKW, at $P<0.01$ for TH and GY and at $P<0.05$ for FL-1W and FL-1L. This was due to different climatic conditions in the two years, which is confirmed by rare and little significant interaction of year with other factors in the model (Yx7AgL/G) (Tab 17).

Genotype (G) (= background genetic information from the variety) was as well highly significant ($P<0.000$) in various cases, alone (e.g. EL, TH, SPNE, SPS, GY) or in interaction with year (Y x G) (FLW, FLL, FL-1L, HUPE, EL, TH, and SPNE). This was probably due to different number of backcrosses performed in development of the three durum wheat lines (lower for R23-1, see § 2.1.2.1.) which caused higher variability mainly within R23-1 HOM+ and R23-1 HOM- lines.

On the basis of the evidence that ABA treatment (T) had no significant effect associated with 7AgL segments (Tx7AgL/G), data were treated not taking into account the "T" factor but only the "7AgL/G" and "Y x 7AgL/G" interactions. Therefore the evaluation of *Yld-7AgL* QTL was made only on the basis of the alien segment size in the recombinant/translocation lines.

The most interesting independent factor included in analysis was presence/absence of 7AgL segment and its interaction with genotype (7AgL/G). Except for plant height (HUPE and TH), for all other traits measured on the first culm this was highly significant interaction ($P<0.000$) (Tab 17). Additionally, the interaction of 7AgL/G with "year"(Y x 7AgL/G) was significant for some traits but not for others, and therefore allowed the distinction of "year" dependent and "year" independent traits which will be discussed as such in the following paragraphs (Figs 29 and 31).

Tables 17, 18 and 19 Mean squares for the first culm traits of the three durum and 1 bread wheat line determined for 2 year (DTH-days to heading, FLW-flag leaf width (mm), FLL-flag leaf length (mm), FL-1W-flag leaf-1 width (mm), FL-1L-flag leaf-1 length (mm), HUPE-height up to ear (mm), EL-ear length (mm), TH-total height (mm), SNE-seed number per ear, SPNE-spikelet number per ear, SPS-seeds per spikelet, GY-grain yield (g), TKW- thousand kernel weight (g));
*, **, *** indicate significant *F* values at $P<0.05$, 0.01, and 0.001, respectively

Table 17
1st culm, 3 durum wheat lines (R5-2-10, R112-4, R23-1)

Factor	df	DTH	FLW	FLL	FL-1W	FL-1L	HUPE	EL	TH	SNE	SPNE	SPS	GY	TKW
G	2	1349.1 ***	7.3	4151.7 *	0.8	16418.6 ***	98790.0 **	4667.6 ***	145382.4 **	200.8	117.8 ***	9.2 ***	9.2 ***	2706.1 ***
Y	1	4855.4 ***	49.3 ***	1920.4	10.7 *	3527.4 *	15523.9	4250.8 ***	36021.2 **	8661.3 ***	0.0	48.9 ***	6.1 **	2189.4 ***
T	1	7.8	72.3 ***	347.0	123.1 ***	15951.4 ***	1224.5	599.7 ***	3538.1	392.3	137.2 ***	1.5	0.9	6.0
Y x G	2	0.7	39.2 ***	4057.5 *	2.6	2329.3 *	65279.4 **	214.8 ***	72516.8 **	314.9	29.1 ***	3.4 *	1.8	1419.7 ***
Y x T	1	8.7	0.0	2777.5	5.0	1649.3	38.7	182.8 *	53.3	10.8	24.5 ***	2.0	0.3	97.7
7AgL/G	3	52.0 ***	94.0 ***	12528.8 ***	38.9 ***	9105.3 ***	4440.9	333.5 ***	5859.7	400.8	31.0 ***	3.1 *	2.6 **	1812.4 ***
R/Y	4	5.3	4.0	215.7	6.9 *	1169.0	10356.7 *	42.2	11584.9 *	111.7	1.0	0.6	0.3	149.2 *
Y x 7AgL/G	3	7.9	5.8	217.1	6.7 *	1295.4	12480.7 *	54.8	13718.1 *	846.0 ***	2.3	2.9	2.1 *	104.9
T x 7AgL/G	3	4.9	1.5	106.3	4.4	718.0	462.2	11.9	542.9	61.2	1.2	0.2	0.5	123.6
Y x T x 7AgL/G	3	9.3	1.3	737.1	0.7	326.1	3016.4	11.0	2941.0	156.3	0.4	0.7	1.1	75.1
Error	338	5.4	2.7	1083.1	2.1	691.4	4167.9	30.0	4304.8	211.5	1.6	0.8	0.7	61.5

Table 18
1st culm, 2 durum wheat lines (R5-2-10, R112-4)

Factor	df	DTH	FLW	FLL	FL-1W	FL-1L	HUPE	EL	TH	SNE	SPNE	SPS	GY	TKW
G	1	225.7 ***	3.8	7023.7 **	0.0	90.3	27457.6 **	206.0 ***	32420.0 **	73.0	0.3	0.1	0.9	138.1
Y	1	2716.7 ***	63.9 ***	1807.4	6.6 *	2059.9 *	1385.3	1515.2 ***	2.9	6185.7 ***	9.2 ***	42.8 ***	2.0 *	3399.1 ***
T	1	0.0	44.1 ***	1392.3	61.6 ***	8093.4 ***	1259.4	133.7 ***	2213.9	176.3	35.5 ***	0.4	0.6	4.2
Y x G	1	0.6	46.7 ***	7120.7 ***	5.1 *	4597.7 **	52480.1 **	40.5	55434.5 **	395.3 *	9.1 ***	0.4	1.4	22.3
Y x T	1	2.0	1.4	1859.5	4.4	430.0	1587.8	19.4	1255.9	1.0	5.1 *	0.6	0.1	108.7
7AgL/G	2	67.9 ***	47.4 ***	587.0	6.2 **	435.2	6020.1	112.0 ***	6330.0	422.9 **	10.5 ***	0.8	1.4 *	57.2
R/Y	4	0.7	1.5	397.1	4.1 **	511.1	3721.0	9.2	4035.2	126.2	0.8	0.8	0.6	82.4
Y x 7AgL/G	2	10.2 **	3.7	284.2	4.1 *	1179.5	8785.9 *	4.9	8742.5 *	417.0	1.9	1.4 *	2.0 **	30.5
T x 7AgL/G	2	4.3	1.1	9.2	1.9	605.4	268.9	9.8	257.6	91.3	1.7	0.1	0.7	107.9
Y x T x 7AgL/G	2	8.0 *	2.2	1222.5	0.0	324.6	3014.7	7.2	2996.0	158.5	0.3	0.8	1.2 *	94.0
Error	163	1.8	2.1	678.9	1.2	495.3	2829.6	11.4	2839.0	84.6	0.8	0.4	0.4	46.2

Table 19
1st culm, 2 bread wheat lines (T4, Thatcher)

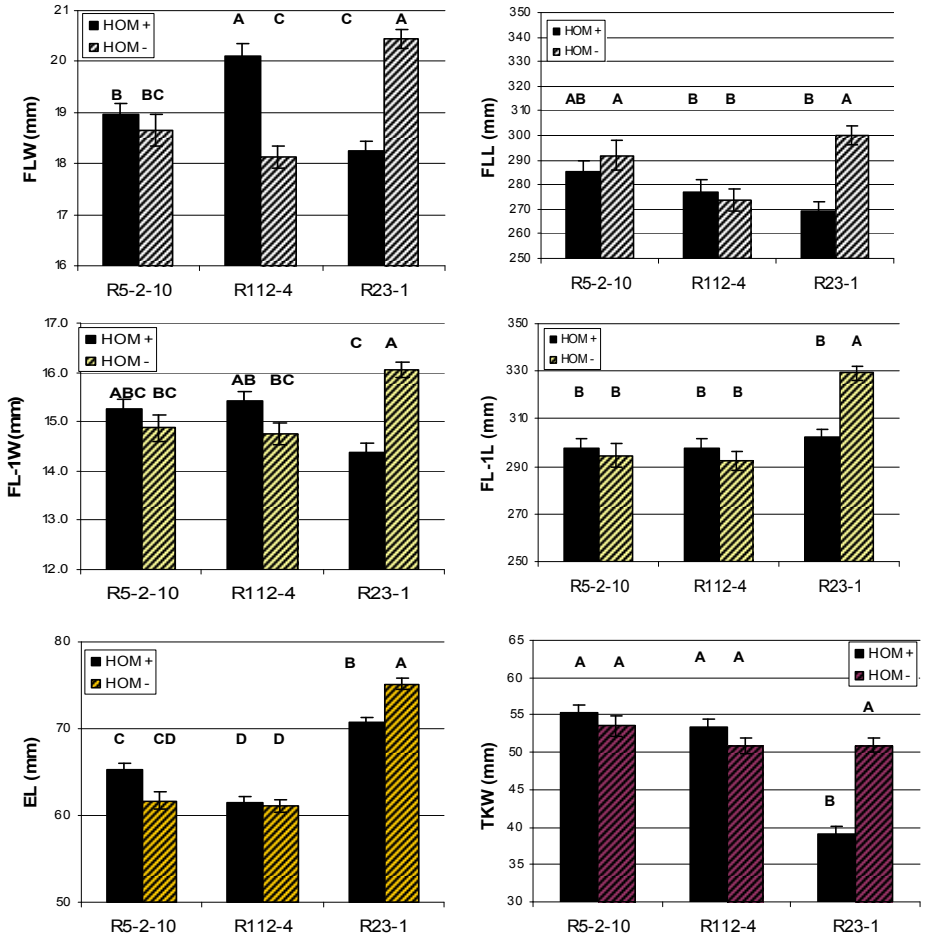
Factor	df	DTH	FLW	FLL	FL-1W	FL-1L	HUPE	EL	TH	SNE	SPNE	SPS	GY	TKW
7AgL/G	1	135.5 ***	22.2 **	13300.5 **	1.2	15618.6 ***	18993.5	127.1 *	16012.9	207.3	3.8 *	0.3	1.2 *	110.0 *
Y	1	569.8 ***	64.7 ***	957.0	75.2 ***	63.2	750320.6 **	8789.1 ***	921524.9 **	9533.8 ***	179.2 ***	10.4 ***	16.3 ***	214.8 **
T	1	3.0	2.7	2495.0	3.0	6469.8 ***	5238.7	11.3	5735.8	55.3	10.7 ***	0.0	0.0	0.4
Y x 7AgL/G	1	24.7 **	3.7	826.5	2.7	2835.0	1312.5	79.4	746.2	4.6	0.9	0.0	0.1	10.7
T x 7AgL/G	1	0.9	0.2	959.9	3.9	0.6	11405.8	14.8	12242.0	99.0	0.4	0.2	0.1	0.4
Y x T	1	0.0	0.1	7897.6 *	0.3	4559.4 *	13471.9	0.3	13600.7	3.3	0.6	0.0	0.0	2.3
Y x T x 7AgL/G	1	0.9	5.6	2313.0	0.6	3.4	3919.9	0.4	3839.0	82.6	0.0	0.3	0.2	11.0
R/Y	4	12.1 **	4.6	2952.1	0.4	256.0	23186.0	33.2	24466.5	12.0	0.8	0.1	0.0	4.1
Error	61	2.6	2.8	1432.1	1.3	750.8	11817.9	27.7	11856.3	67.2	0.9	0.2	0.2	26.1

3.5.2.1. "Year" independent traits of the first culm in durum wheat recombinant lines

Fig 29 represents traits of the first culm that were stably expressed in both experimental years, but not all of them showed to be significantly different between genotypes. Significant differences involving 7AgL segment effect were observed for FLW and EL.

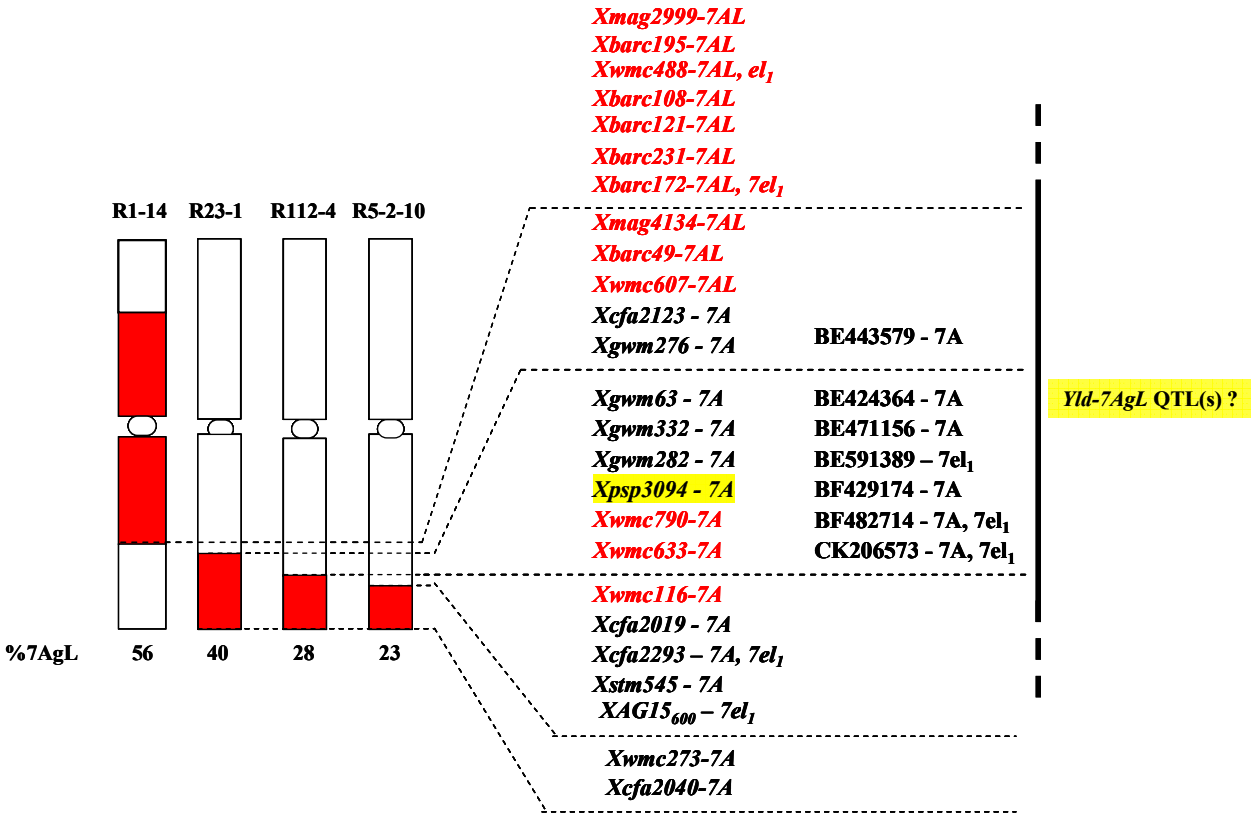
FLW was significantly increased in R112-4 HOM+ line compared to R112-4 HOM- due to the segment presence (FLW, Fig 29). The same significance was not observed in R5-2-10 HOM+ while in R23-1 HOM+ the effect of the segment on FLW was significantly depressing compared to its HOM-. Therefore, it was possible to assume that a genetic determinant (gene/QTL) contributing to the development of a larger flag leaf could be located in the 7AgL portion present in R112-4 HOM+ and absent in R5-2-10 HOM+ (=5% of alien chromatin comprised between the 7AL-7AgL breakpoints of the two lines, see Fig 12).

Figure 29 Traits of the first culm stably expressed in 2009 and 2010; letters correspond to ranking of groups after Tukey HSD test at 0.01 significance level



This observation does not correspond to what has been hypothesized to be the location of the 7AL yield QTL affecting flag leaf width (Quarrie et al., 2005, 2006). For this, the closest marker appeared to be PSP3094, hence a possible 7AgL homoeolocus should be situated in the alien segment present in line R23-1 and absent from R112-4 (region comprised between 28 and 40% of 7AL-7AgL arm, see Fig 12). However, as recalled in the Introduction (§ 1.6.3.), the 7AL yield QTL was associated with two markers, i.e. WMC273 and PSP 3094 (Quarrie et al., 2005), which turned out to be relatively distant on the 7AL-7AgL maps defined by breakpoints of the durum-*Th. ponticum* recombinant lines (Figs 12, 30). Thus, either there is no perfect colinearity between the 7AL and 7AgL in that region, or there is more than one QTL controlling FLW in that region.

Figure 30 7AgL segments present in different durum wheat recombinant lines, and corresponding molecular markers, spanning the region of putative *Yld-7AgL* QTL(s) (in red – markers developed during this study).



While there seems to be no doubt about R112-4 as carrier of a factor promoting FLW in its proximal 7AgL segment, other observations point at the more distal 7AgL portion, shared with R5-2-10 line, as the site where additional, relevant factors are probably located. In particular, a differential expression of the EL trait was observed, with line R5-2-10 HOM+ that tended to have a significantly longer ear compared to R5-2-10 HOM-

(P=0.053) and to R112-4 HOM+ (P<0.01). The overall picture was not very clear: ANOVA analysis performed with all three durum wheat recombinant lines showed doubtful results for this trait as well as for SPNE and GY (see ahead § 3.5.2.2.), probably so because of the anomalous behaviour of the R23-1 HOM+ line (phenotype "depressed" by the presence of the *Sd* genes, see above). To overcome this and to get a more correct information on trait expression, ANOVA was performed including HOM+/HOM- genotypes of R5-2-10 and R112-4 only (Tab 18).

This analysis allowed clear identification of differences between lines R5-2-10 HOM+ and R112-4 HOM+. In the case of EL, it was confirmed after Tukey HSD test, that R5-2-10 HOM+ was significantly (P<0.000) better than its HOM- control and than line R112-4 HOM+. This result suggested the presence of a genetic determinant (gene/QTL) controlling ear length in the 23% 7AgL region of R5-2-10. However, since this segment is common to all three durum recombinant lines analyzed, but it is significantly better expressed only when there is not more than 23% of 7AgL on 7AL, a possible interpretation of this result is that there might be other genes in 7AgL regions beyond the 23% (i.e., even disregarding R23-1, in line R112-4) that somehow prevents its expression.

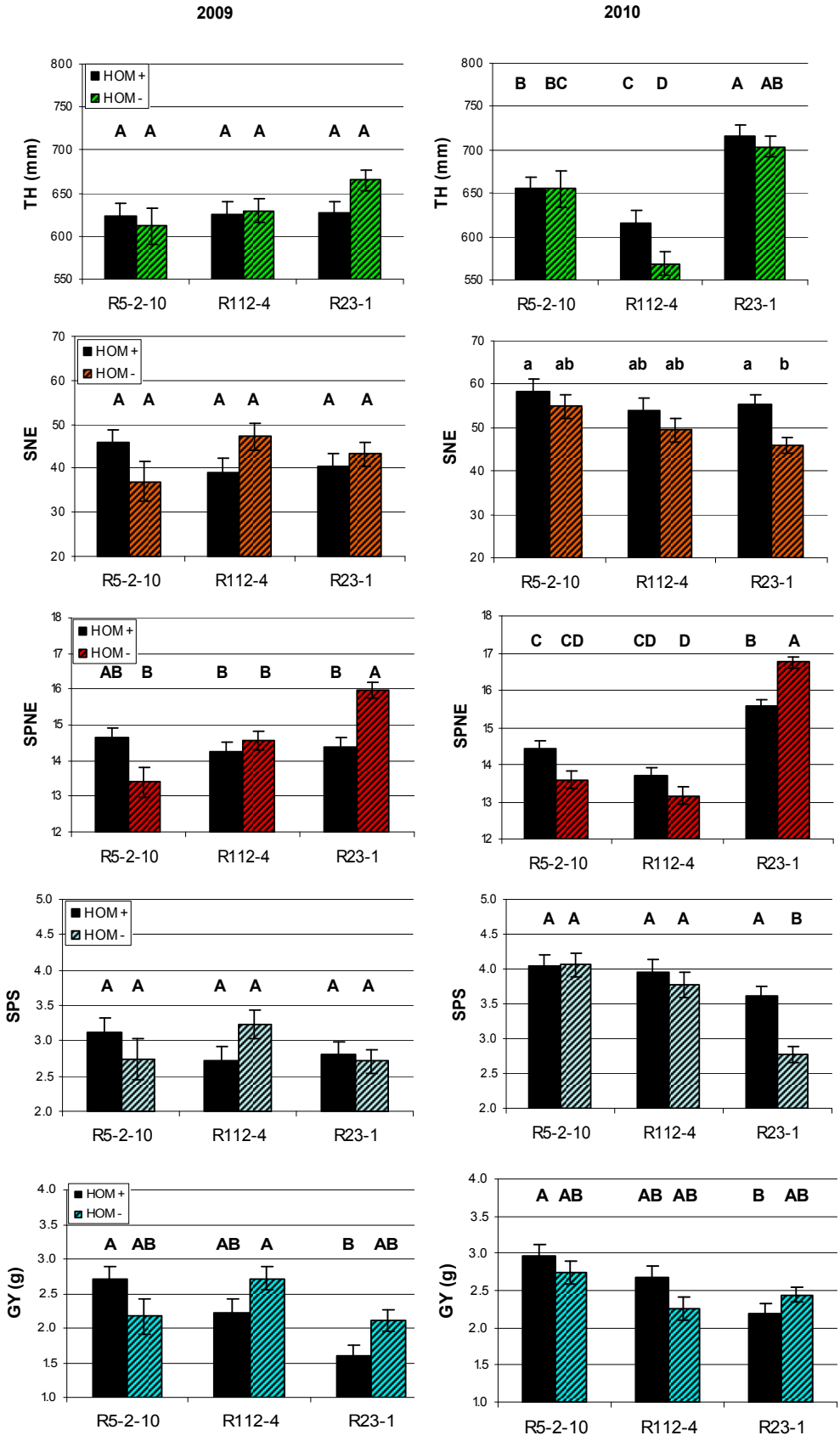
Contrary to lines R5-2-10 HOM+ and R112-4 HOM+ which showed to be boosted for some of the traits by the 7AgL segment presence, line R23-1 HOM+ had significantly reduced values of all six traits in Fig 29 due to R23-1 background or the presence of the alien segment. This could be attributed to the deleterious effects on plant growth and fertility, besides that on male gametogenesis, associated with segregation distortion gene(s) present in the 7AgL segment of this line (§ 1.6.4. and Grossi, 2010). Particularly, TKW of R23-1 HOM+ showed to be significantly (P<0.000) reduced by the 7AgL presence compared to its HOM-.

Other traits stably expressed in 2 years (FLL, FL-1W, FL-1L) were not affected by 7AgL presence, but more by the genotype background (G) (Tab 17).

3.5.2.2. **"Year" dependent traits of the first culm in durum wheat recombinant lines**

Traits that turned out to be significantly influenced by the "experimental year" (Y) variable are represented in Fig 31. In 2009, differences between lines were not as evident as in 2010 when differences were significant. Overall, in year 2010 all lines performed better, irrespectively of the 7AgL presence.

Figure 31 Traits assessed on the first culm which resulted differentially expressed in 2009 and 2010; letters correspond to the ranking of Tukey HSD test results (capital=P <0.01; lower case=P<0.05)



A particularly interesting result was observed for the fertility parameters SNE and SPS in R23-1 HOM+ line. A significant difference was detected ($P<0.05$ and $P<0.01$ respectively) between HOM+ and HOM- NILs. In the case of SPNE, there is a clear positive effect of R23-1 background (demonstrated by elevated value of HOM- control), but the presence of 7AgL segment significantly reduced the spikelet number. However, the plant maintained the ability to produce relatively high number of seeds, SNE and SPS under the 7AgL segment effect (+29.8% SPS Fig 31). Unfortunately, the overall R23-1 productivity remains relatively low, because the same line demonstrates significantly ($P<0.01$) lower GY and TKW compared to other two recombinant lines (Fig 31), due to negative effect of the segment or to interactions with some other genetic factors in the genome.

However, expression of this and other yield-related traits will be better assessed by analysis of data concerning the entire plant, since a single culm can only provide with an indication of the overall productivity potential of the various genotypes, and since the presence of 7AgL segment in the three lines analyzed did not affect significantly GY trait.

3.5.3. **Flag leaf development and yield related traits of the first culm at maturity in bread wheat line T4**

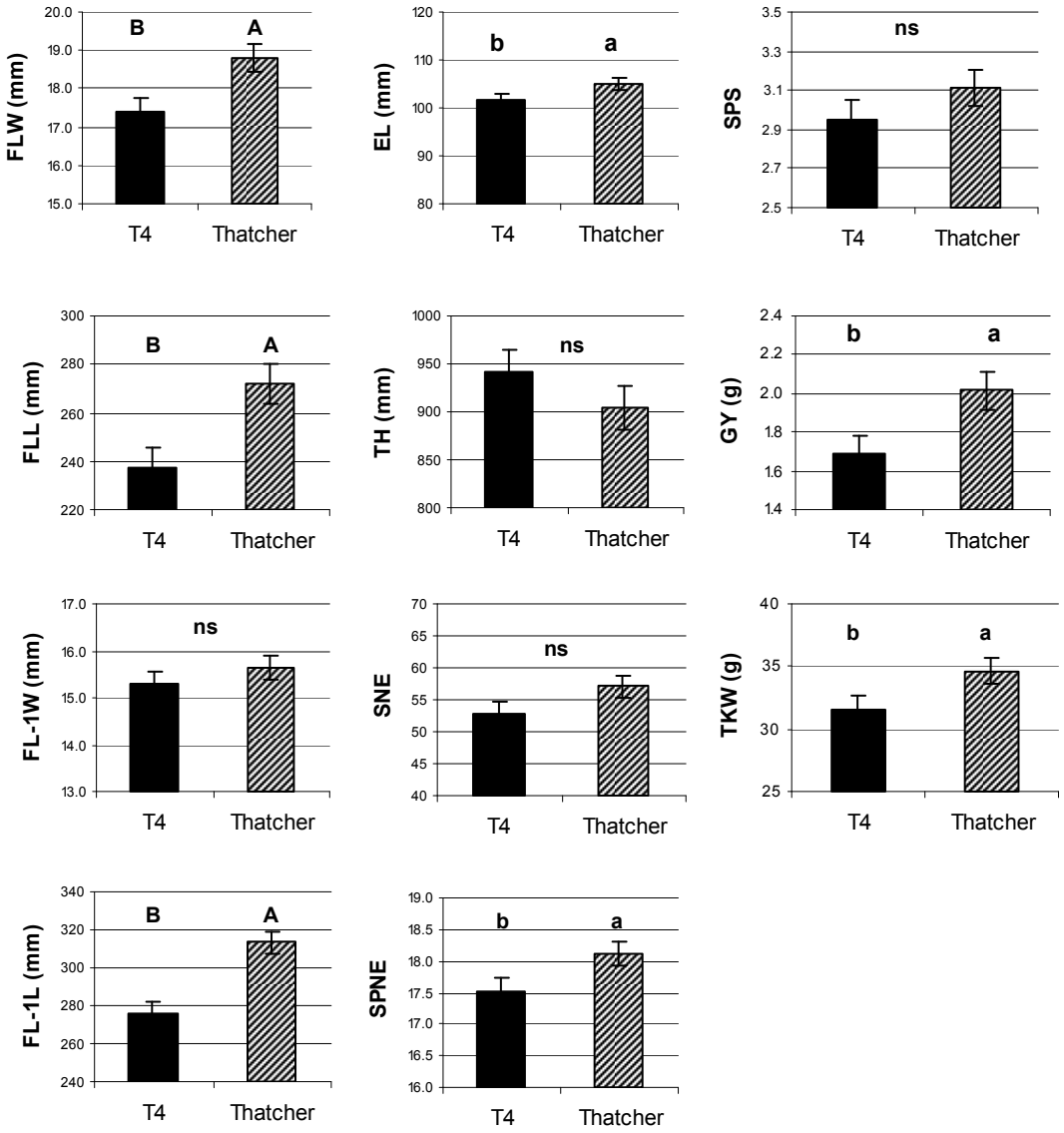
Rather unexpectedly, the bread wheat translocation line T4 showed an opposite behaviour to that previously reported by CIMMYT (e.g. Singh et al., 1998, Reynolds et al., 2001; see § 1.6.3.).

In this study, ABA had the same negative effect on FL-1L and SPNE of bread wheat lines T4 and the Thatcher control, similarly to what observed for durum lines (Tab 19). Treatment did not significantly interact with any other factor and it is noteworthy that, as in case of durum wheat recombinant lines, also in T4 the putative yield 7AgL QTL(s) does not seem to be ABA-responsive.

The 7AgL segment of line T4 did not show positive effects on the measured traits compared to Thatcher (= HOM-, T4 control) (Fig 32). Highly significant differences were observed in several cases (FLW, FL-1L, EL, SPNE, GY, TKW), but always with the Thatcher allele associated with higher values (Tab 19). At this stage of the analysis, it can only be concluded that, at least for the first culm, the *Yld-7AgL* QTL(s) of line T4 line is

not expressed during the two years and in the specific environment chosen for the present study (Viterbo, Central Italy).

Figure 32 Traits of the first culm of bread what lines (T4 vs. Thatcher as control) in 2009 and 2010; letters correspond to the ranking of Tukey HSD test results (capital=$P<0.01$; minor=$P<0.05$)



3.5.4. Biomass production and harvest index of the first culm in durum and bread wheat lines in 2010

In 2010, an additional parameter, i.e. biomass (B) was measured, and the derived Harvest Index (HI) value calculated (GY/B). Biomass was considered as the entire above ground part of the plant (in this case entire first culm). For durum wheat lines, background (G) and 7AgL segment (7AgL/G) played a significant role (Tab 20). The 7AgL segment of line R23-1 was associated with a significantly (P<0.000) reduced biomass (Fig 33), as it was for other traits (e.g. FLW, TKW) discussed in § 3.5.2.1.

HI did not show major variation due to the segment presence; in fact, while comparing R5-2-20 HOM+ to R23-1 HOM+ showed a significant difference (P<0.01), the higher values of R5-2-10 (and R112-4, though not significant) come from the background effect in durum wheat lines.

Tables 20 and 21 Mean squares for the first culm biomass and HI of the three durum and 1 bread wheat line determined in 2010 (*, **, *** indicate significant F values at P=0.05, 0.01, and 0.001, respectively)

Table 20
1st culm, 3 durum wheat lines
(R5-2-10, R112-4, R23-1)

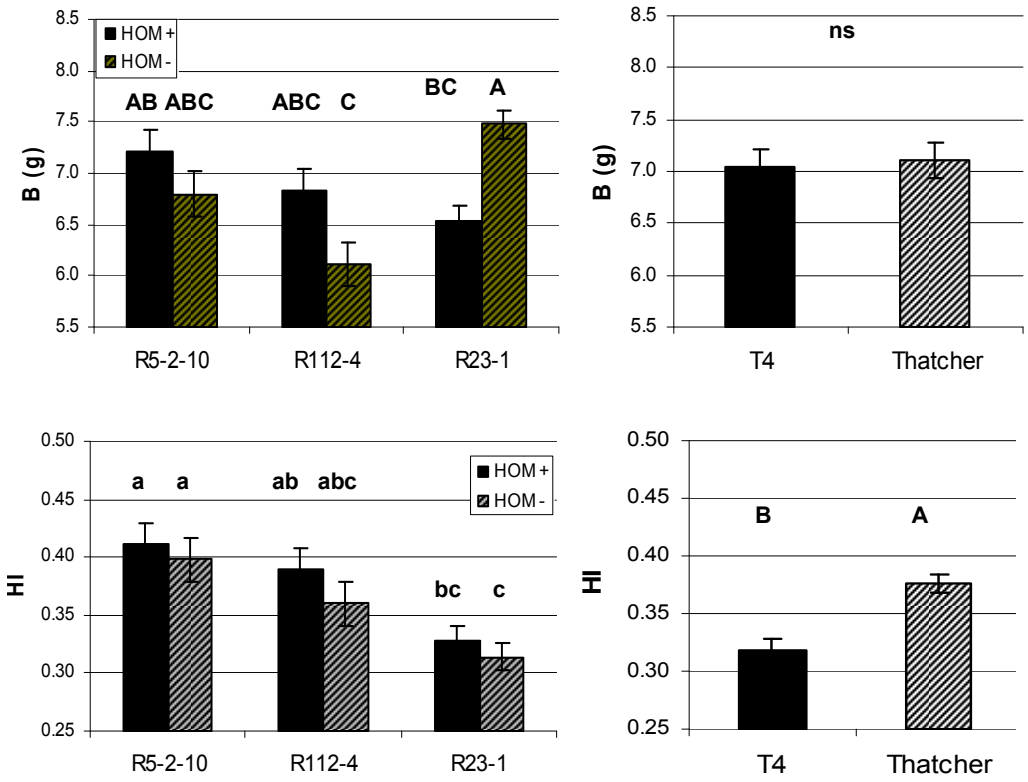
Factor	df	B	HI
G	2	6.1 **	0.1 ***
T	1	5.0	0.0
R	2	2.5	0.0
T x G	2	0.7	0.0
7AgL/G	3	11.6 ***	0.0
T x 7AgL/G	3	1.4	0.0
Error	217	1.3	0.0

Table 21
1st culm, 1 bread wheat line
(T4/Thatcher)

Factor	df	B	HI
7AgL/G	1	0.1	0.0 ***
T	1	0.5	0.0
R	2	3.7 *	0.0 *
T x 7AgL/G	1	0.4	0.0
Error	53	0.9	0.0

Bread wheat line T4 did not show differences in B due to the 7AgL segment, but was significantly different (P<0.000) in HI, with Thatcher exhibiting higher values (Tab 21, Fig 33). These results confirm that, also for the biomass trait, line T4 did not confirm in the year and environment of the present study what previously shown. However, again, an analysis of data of the whole plant, rather than its first culm only would give a more sound basis for any conclusion that will derive from.

Figure 33 Biomass and HI of the first culm of durum and bread what lines in 2010; letters correspond to the ranking of Tukey HSD test results (capital=$P<0.01$; lower case=$P<0.05$)



3.6. Analysis of yield related traits of entire plants in field growing conditions

The aim of this analysis was an assessment of grain and biomass productivity of entire plants belonging to the three durum (R5-2-10, R112-4 and R23-1) and one bread wheat (T4) genotype at maturity, in relation to presence of 7AgL segments of different size. The same plants used for analysis of fertility under simulated stress by exogenously applied ABA (§ 2.2.2.2.), were used in 2 years field experiments. While values assessed on the first culm, treated with ABA, provided information on specific traits, such as FLW, hypothesized to be correlated with grain yield (Quarrie et al., 2006), the analysis of the whole plant was expected to give a more sound basis for understanding the control of yield-related traits in relation to alien segment presence. Productivity parameters were recorded and evaluated for the entire plant, but productive culms (those that actually produced seed, excluding small, secondary tillers, only contributing to biomass) were also separately considered. Measurements taken on the whole plant are reported in § 4.2.

3.6.1. Yield related traits at maturity of durum wheat recombinant lines

ANOVA[7] by GLM was performed for the two years data regarding 11 traits[8] and separately for the two traits (B and HI) recorded in 2010 only (measurements are reported in Appendix 3). Differently from analysis of data of the first culm, this analysis did not include ABA treatment as a factor in the statistical model, because no obvious effect on productivity was expected of the treatment applied only on the first culm in early vegetative stage of development.. Since no significance of the treatment factor (T) was actually observed for any of 11 traits, data sets, initially divided per treatment, were pooled.

The "genotype" (G) (= background) factor was highly significant ($P<0.01$ or $P<0.000$) in interaction with the 7AgL segment factor (7AgL/G) for eight traits, and significant ($P<0.05$) for two (Tab 22). This indicates a relevant involvement of 7AgL segments in the control of the parameters analyzed.

[7] For all ANOVA summary tables see Appendix 4
[8] For the list of abbreviations used for the traits analyzed see Appendix 1

Given the observed significant interaction of the "year" (Y) factor with the 7AgL segment for almost all traits (Y x 7AgL/G) (Tab 22), as in the analysis of data of the first culm, traits analysis has been divided in two groups: "year–independent" and "year-dependent" traits).

Tables 22 and 23 Mean squares for whole plant traits of the three durum and one bread wheat lines determined for 2 years (TTH-total tiller number at heading, TCN-total culm number at harvest, PRCN-productive culm number, PRGY-grain yield (g) of productive culms, GYxPRC-grain yield (g) per productive culm, PRCSEED-seed number of productive culms, SEEDxPRC-seed number per productive culm, PRCTKW-thousand kernel weight (g) of productive culms, GY-grain yield (g) of entire plant, SEED-seed number of entire plant, TKW-thousand kernel weight (g) of entire plant);
*, **, *** indicate significant *F* values at *P*=0.05, 0.01, and 0.001, respectively

Table 22

Whole plant, 3 durum wheat lines (R5-2-10, R112-4, R23-1)

Factor	df	TTH	TCN	PRCN	PRGY	GYxPRC	PRCSEED	SEEDxPRC	PRCTKW	GY	SEED	TKW
G	2	79.8 **	36.9	7.9	970.6 ***	6.2 ***	28257.4	155.4 *	2258.8 ***	1638.3 ***	77333.0 *	422.7 ***
Y	1	18.8	47.6	560.3 *	0.0	18.9 ***	205562.2 ***	15389.7 ***	1402.1 ***	375.8 *	886596.8 ***	2807.0 ***
Y x G	2	68.2 **	9.3	14.6	618.5 ***	2.4 ***	95389.0 **	233.5 **	901.9 ***	878.2 ***	101387.7 *	22.1
7AgL/G	3	146.7 ***	134.0 ***	32.4 **	130.1 *	1.2 ***	97430.0 ***	89.9	1228.5 ***	174.1 *	203561.3 ***	1147.2 ***
R/Y	4	15.0	19.0	8.4	58.1	0.4 *	21472.0	53.1	88.4 *	78.3	43675.9	140.4 **
Y x 7AgL/G	3	7.0	17.7	25.4 *	237.8 **	0.4 *	102536.1 ***	64.6	53.4	280.2 **	126789.2 ***	50.7
Error		13.8	13.4	6.9	45.0	0.2	15261.5	43.8	28.9	57.1	22421.3	31.6

Table 23

Whole plant, 2 bread wheat lines (T4/Thatcher)

Factor	df	TTH	TCN	PRCN	PRGY	GYxPRC	PRCSEED	SEEDxPRC	PRCTKW	GY	SEED	TKW
G	1	19.0	63.6	2.2	10.4	0.1	1004.4	3.0	41.4	6.7	2712.0	35.7
Y	1	58.1	324.1 ***	138.6 *	1186.4 ***	8.2 ***	313529.9 **	3856.2 ***	919.0 ***	1586.3 ***	360401.0 **	866.8 ***
R	2	54.6	8.5	19.5	5.1	0.1	6699.3	259.9 *	6.3	14.8	18328.5	5.6
Y x G	1	12.5	16.6	5.8	10.9	0.0	23490.3	0.7	0.8	0.2	1108.2	0.2
Error	52	23.9	27.3	19.6	48.4	0.1	41148.0	56.0	33.2	52.0	45734.3	23.0

3.6.1.1. "Year" independent yield-related traits of the whole plant at harvest in durum wheat recombinant lines

Analysis of five "year–independent" traits, graphed in Fig. 34, gave important information on involvement of different 7AgL regions in yield determination.

TTH and TCN, i.e. total tiller number at heading and at harvest, respectively, give indication of the overall potential for productivity, in terms of both biomass and grain production, and in this study they exhibited a similar expression patterns. Presence of 7AgL segment increased these values in all three genotypes, but significantly so only in HOM+ vs. HOM- plants of the R112-4 ($P<0.000$) and R23-1 ($P<0.05$) recombinant genotypes. In particular, presence of the 7AgL segment caused a larger increase in TTH and TCN in R112-4 HOM+ vs. its HOM- control (27.8% and 25.6%, respectively), while the effect was of smaller entity but still significant in R23-1 HOM+ compared to its HOM- control (13.4% for TTH and 13% for TCN). These results indicate that a genetic factor involved in the control of the two traits is located in the 7AgL region common to R112-4 HOM+ and R23-1 HOM+ and absent from R5-2-10 HOM+ (between 23 and 28% distal 7AL/7AgL arm, see Fig 12). The 7AgL gene/QTL concerned would promote production of early vegetative mass, and, even more important, maintenance of high number of culms until harvest.

For the two additional parameters related to seed weight, TKW and PRCTKW (see § 3.6.), only the R23-1 HOM+ genotype showed a big decrease due to presence of the 7AgL segment: -20.4% of TKW and -22.5% of PRCTKW (Fig 34), as observed also for the first culm (see § 3.5.2.1. and Fig 29), whereas no significant difference was detected when the HOM+ and HOM- plants of the two other recombinant lines were compared. Thus it can be concluded that while R23-1 HOM+ plants have a compromised grain filling process, possibly caused by the deleterious genes (probably *Sd* genes, see § 1.6.4.) they carry (see Fig. 12), for R112-4, the higher number of tillers is not negatively correlated with weight of seed they carry.

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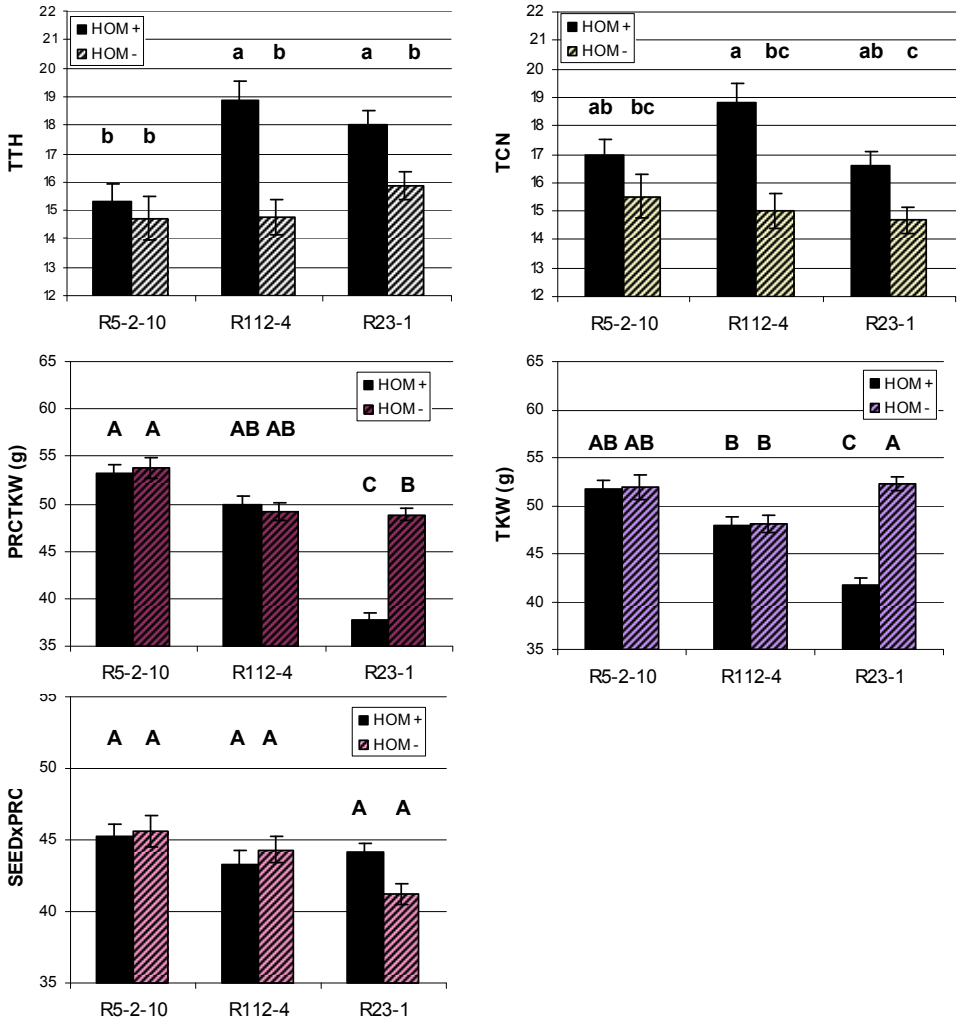
Figure 34 Yield-related traits of the whole plant in durum wheat recombinant lines, stably expressed in 2009 and 2010; letters correspond to ranking of groups after Tukey HSD test (capital – $P<0.01$, lower case – $P<0.05$ significance level)



3.6.1.2. **"Year" dependent yield-related traits of the whole plant at harvest in durum wheat recombinant lines**

Comparison between genotypes for SEED (seed number of the entire plant) proved that R23-1 HOM+ produces more seeds compared to R23-1 HOM-. This result (+34.3%) was significant ($P<0.05$, Fig 35) in 2010 rainy year only (Fig 25).

Considering productive culms only (Fig 35), the same expression pattern was observed for grain yield (PRGY) and seed number (PRCSEED). The latter was increased in all HOM+ recombinant genotypes, though significantly so ($P<0.000$) (+73%) only in R23-1 HOM+ compared to its HOM- control. PRGY was not significant in any of the three genotypes. The difference in SEED and PRCSEED increase was due to the fact that SEED

includes as well secondary culms produced by plant, which are significantly more abundant in R23-1 HOM+ than in its HOM- control (Fig 35). On the other hand, in 2010 GY resulted significantly higher in R112-4 HOM+ vs. its HOM- control (see § 3.6.4.).

Taking into consideration all traits analyzed so far, both "year-independent" (§ 3.6.1.1.) and "year–dependent" (this paragraph), it is possible to conclude that presence of the 7AgL segment confers to lines R112-4 and R23-1 high potential for vegetative biomass production (TTH in Fig. 34), for higher culm number at harvest (TCN in Figs 34), and elevated seed number (SEED in Fig 35). On the other hand, the R23-1 segment significantly decreased TKW (Fig 34), thus confirming what was hypothesised on the basis of the first culm data, i.e. that a portion of the R23-1 7AgL segment (not shared with R112-4) acts negatively on some yield-related traits, namely those involved in seed filling at ripening (SEED vs. TKW).

Figure 35 Traits of whole plant differentially expressed in 2009 and 2010; letters correspond to the ranking of Tukey HSD test results (capital=P<0.01; lower case=P<0.05)

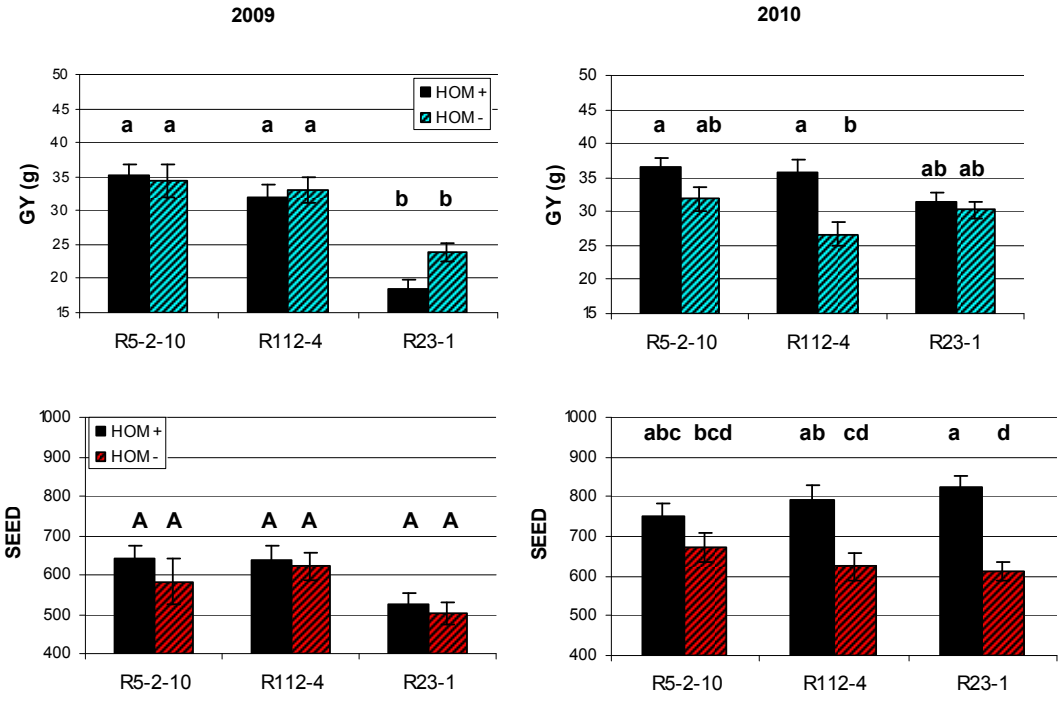
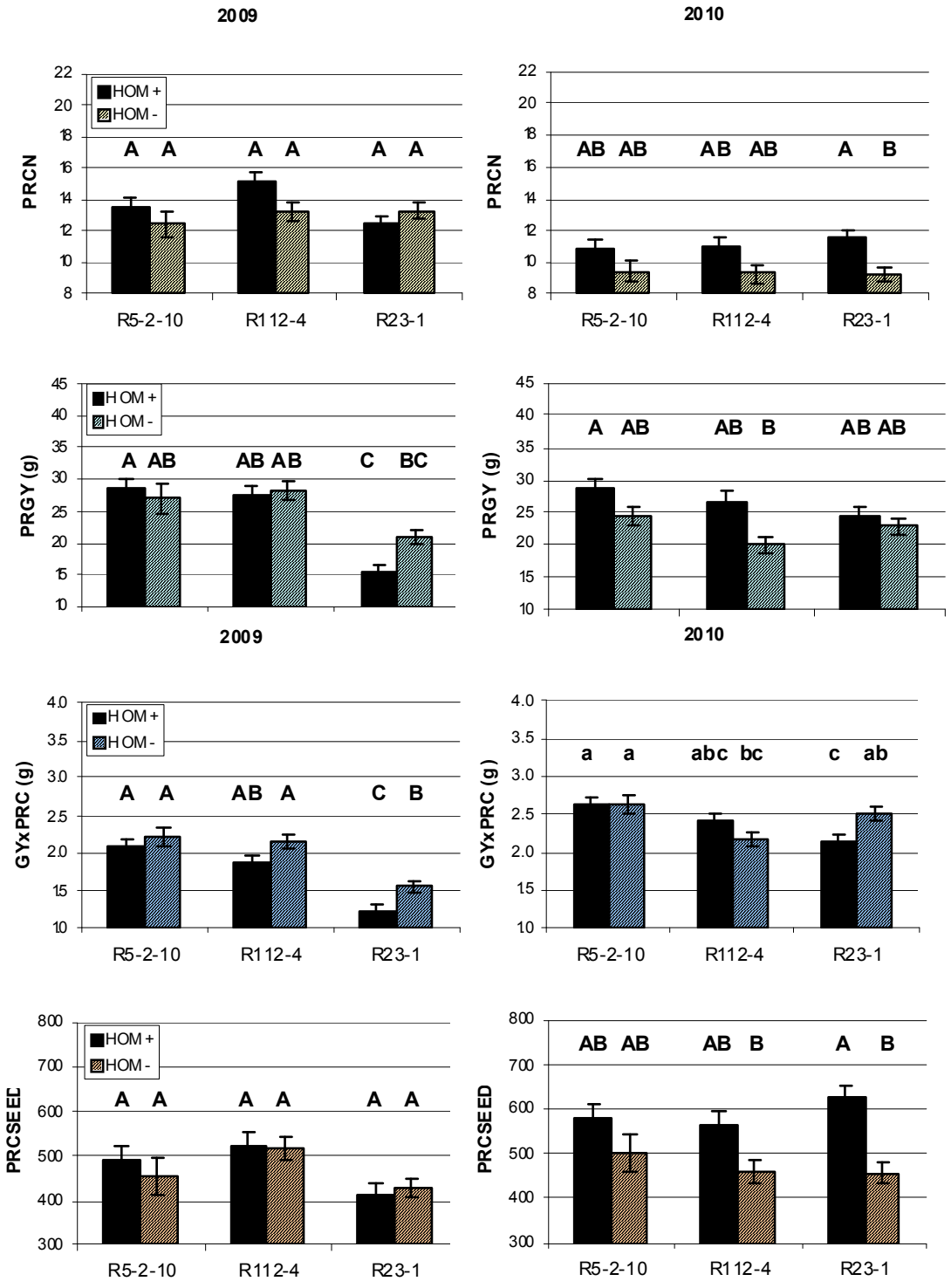


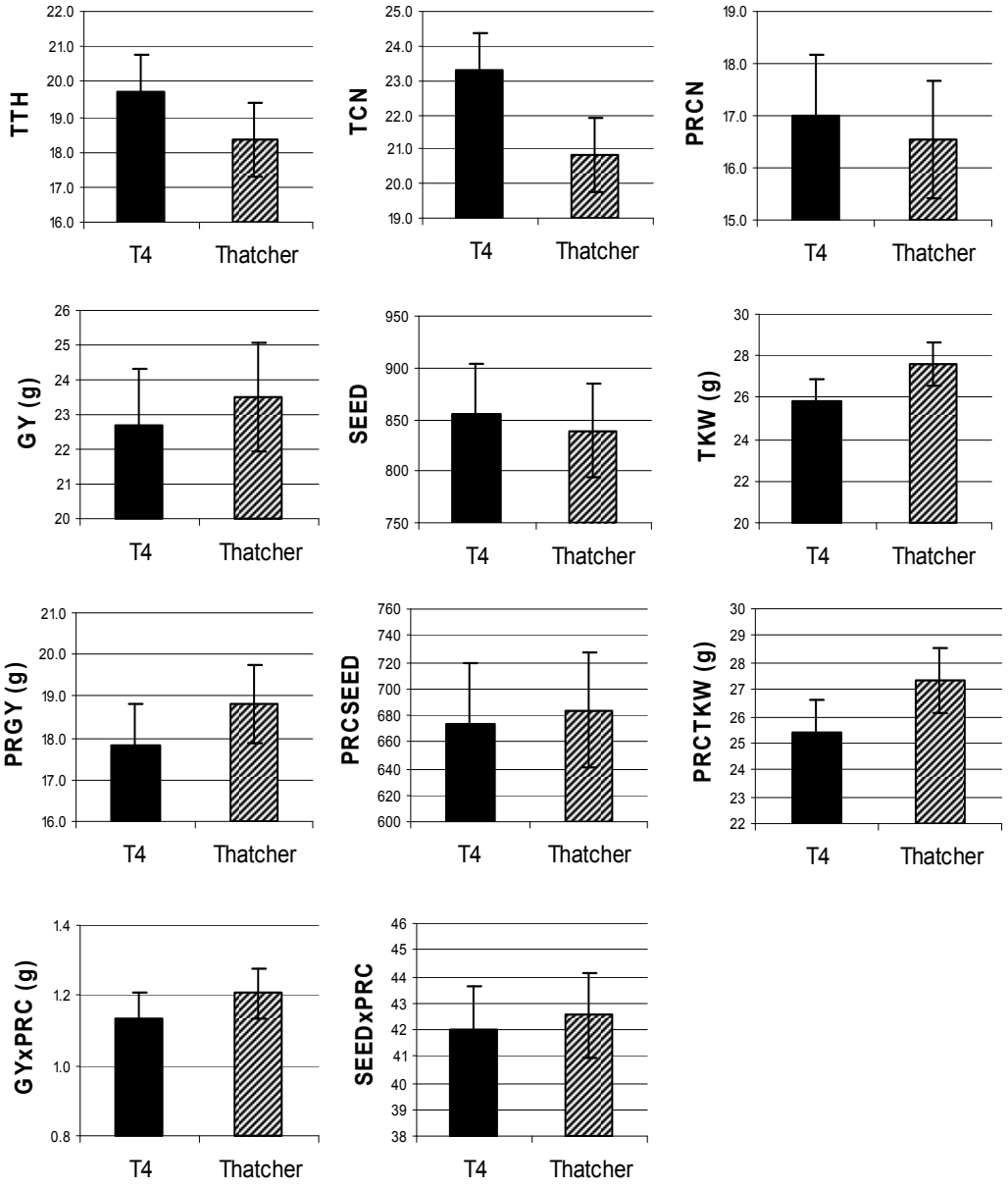
Figure 35 Continued



3.6.2. Yield-related traits at harvest of bread wheat line T4

Even less significant results compared to the first culm data, were observed for data concerning the whole plant when line T4 was compared to its control line Thatcher (Tab 23). In fact, no evidence of positive effect of the T4 7AgL segment on productivity parameters was detected in the 2 year experiments carried out in Central Italy environment (Viterbo) (Fig 36).

Figure 36 Yield-related traits of the whole plant in bread wheat line T4 in 2 year field experiment



3.6.3. Biomass production and harvest index of the whole plant in durum and bread wheat lines in 2010

Differences for biomass production (B) were observed only in R112-4 durum wheat genotype, whose 7AgL segment significantly (P<0.05) increased B with respect to its HOM- (+29.6%) (Tab 24, Fig 37). This confirms the results of production of higher culm number in HOM+ genotypes of this recombinant line (Fig 34), while for the same trait R5-2-10 HOM+ and R23-1 HOM+ showed only a positive tendency compared to their HOM- lines. The 28% 7AgL segment of R112-4 is common to that of line R23-1; however, the latter did not show a significant biomass increase. This is probably due (like for TKW, Fig 34) to the negative effects of *Sd* genes (§ 1.6.4.).

Bread wheat genotypes did not show significant differences in whole plant biomass production (Tab 25, Fig 37).

Bread wheat genotypes did not show significant differences in whole plant biomass production (Tab 25, Fig 37).

Tables 24 and 25 Mean squares for the whole plant biomass production and HI of the three durum and 1 bread wheat lines determined in 2010;
*, **, *** indicate significant F values at P=0.05, 0.01, and 0.001, respectively

Table 24
Whole plant, 3 durum wheat lines (R5-2-10, R112-4, R23-1)

Factor	df	B	HI
G	2	125.8	0.0 ***
R	2	113.9	0.0 **
7AgL/G	3	1659.4 ***	0.0
Error	135	329.8	0.0

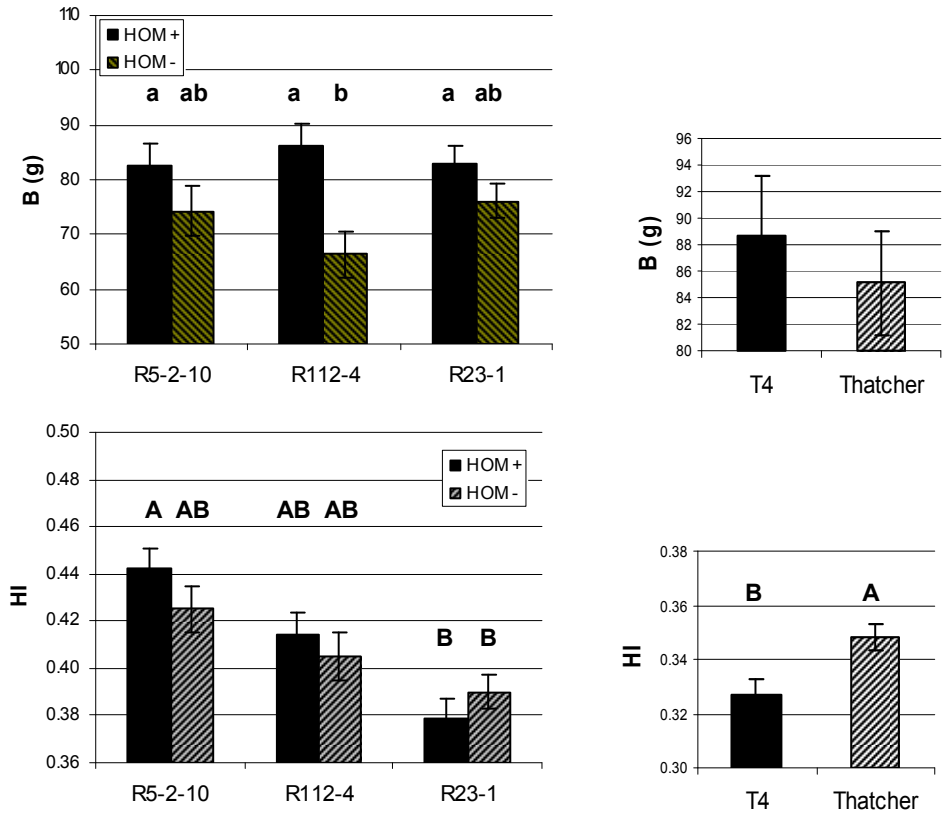
Table 25
Whole plant, 1 bread wheat line (T4/Thatcher)

Factor	df	B	HI
7AgL/G	1	139.0	0.0 ***
R	2	594.6	0.0 *
Error	40	375.0	0

As for HI, durum wheat lines exhibited almost the same pattern as that of the first culm: presence of the 7AgL segments did not affect significantly any of the genotypes (Fig. 37).

Also for bread wheat lines the HI result of the first culm was confirmed, with line Thatcher having significantly (P<0.000) higher HI (18.1%) compared to line T4 (Fig. 37). Also for this trait it can be hypothesized that the environmental conditions of Central Italy do not allow the expression of the putative *Yld-7AgL* QTL of line T4.

Figure 37 Biomass and HI of the whole plant of durum and bread what lines in 2010; letters correspond to the ranking of Tukey HSD test results (capital=$P<0.01$; minor=$P<0.05$)



3.6.4. R112-4 yield potential

An important result obtained from the present study is the strong evidence of an increased productivity ascribable to the 7AgL segment present in line R112-4. Already from the first culm data, the R112-4 7AgL segment showed a positive effect on flag leaf width (see § 3.5.2.1.). One can speculate that ability to produce a wider flag leaf (FLW, Fig 29), not at the expenses of its length (FLL, Fig 29) positively affects the size of available photosynthetic area, which could ultimately lead to higher grain yield. When whole plant data were considered, the effects seem to involve not only grain yield but biomass as well. An extraordinarily good potential for culm production (TTH and TCN Fig 34) was observed. In 2010, analysis of biomass (B) production (Fig 37) showed line R112-4 HOM+ to produce 29.6% more above-ground biomass compared to its HOM- control ($P<0.05$ significance level). In the same year, this rise in tiller number was positively correlated with grain yield increase (Fig 35): seed number (SEED) was significantly

(P<0.05) increased (+27%) and, as a result, GY (Fig 35) was also markedly increased (+34%). Since the same remarkable effects were not exhibited by the R5-2-10 line, it is plausible to think at the 5% 7AgL segment differentiating the latter from line R112-4 as the region were one or more genetic determinants of such traits are located.

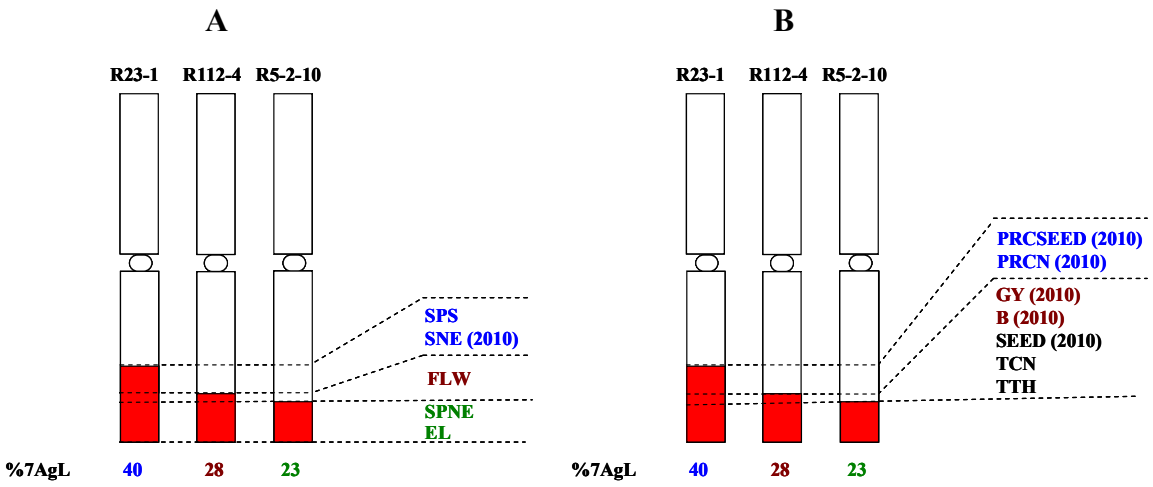
3.7. Summary result of the 1st culm and whole plant data analysis – structural/functional sub-division of 7AgL segments in durum wheat recombinant lines

The observed results provided novel and detailed information on the positive impact of portions of the 7AgL arm included in the three durum wheat recombinants. Highly significant differences between genotypes, clearly due to a given 7AgL segment presence, allow drawing an indicative structural-functional sub-division of the 40% 7AgL chromatin encompassed by the three lines, for both traits of the first culm and of the whole plant (Tab 26). On the basis of Tukey HSD significance test, the genetic control of five traits monitored on the first culm (FLW, EL, SNE, SPNE, and SPS) and seven referring to the whole plant (TTH, TCN, PRCN, PRCSEED, SEED, GY, and B) could be assigned to different 7AgL portions (Fig 38). One trait assessed on the first culm (SNE) and five on the whole plant (PRCN, PRCSEED, SEED, GY, and B) were affected by year, and significantly expressed in 2010 only.

Table 26 Significantly different expression of traits measured on the first culm (5 traits) and on the whole plant (7 traits) correlated with 7AgL segments present in one or more durum wheat-*Th. ponticum* recombinants

1st culm	**FLW**	**EL**	**SNE**	**SPNE**	**SPS**		
R5-2-10 HOM+	-	+	-	+	-		
R112-4 HOM+	+	-	-	-	-		
R23-1 HOM+	-	-	+ (2010)	-	+		
Whole plant	**TTH**	**TCN**	**PRCN**	**PRCSEED**	**SEED**	**GY**	**B**
R5-2-10 HOM+	-	-	-	-	-	-	-
R112-4 HOM+	+	+	-	-	+ (2010)	+ (2010)	+ (2010)
R23-1 HOM+	+	+	+ (2010)	+ (2010)	+ (2010)	-	-

Figure 38 Possible sub-regions of 7AgL segments within analyzed durum wheat-*Th. ponticum* recombinants controlling traits of the (A) 1st culm and (B) whole plant (in black – traits common to two recombinants, in blue – traits specific to R23-1, in brown – traits specific to R112-4, in green – traits specific to R5-2-10)



It is interesting to note that traits that are correlated in the two sets of data have been assigned to the same segment (SPS and SNE of the 1st culm with PRCSEED of the whole plant; FLW with B), which strengthen the hypothesised assignment of traits to specific 7AgL sub-regions. The information obtained represents an essential "first draft" for further confirmation (after additional trials, also in different environments) of the evidence so far accumulated and, possibly, discovery of other traits of potential interest associated with 7AgL fragments incorporated in the presently available recombinant lines and other ones under development. Combined with the information that a dense 7AL/7AgL genetic map can provide (Fig 12), the possibility that specific markers for the different traits can be isolated seems feasible.

3.8. Discriminant analysis (DA) of yield related parameters in durum wheat NILs

In order to investigate which one(s) of the traits analyzed is (are) mostly contributing to distinction between the durum wheat genotypes investigated, six DAs were performed, three on first culm data and three on the entire plant data.

Within the two sets of DAs, one was conducted with 2 years combined data set (2009-2010) and the other two with data from each year separately (2009 or 2010). Because of the specific characteristics of DA analysis, derived traits (i.e. TKW and SPS of the first culm; GYxPRC, SEEDxPRC, PRCTKW, TKW and HI of the whole plant) could not included. Since the only difference between lines is the presence/absence of the alien segment (ABA treatment resulted non significant, in the interaction with genotypes, see § 3.5.2.), the results of DA should reveal the most discriminating trait(s) among the genotypes analyzed.

Results were visualized by graphing the first two discriminant functions.

3.8.1. DA of yield related traits of the first culm related to simulated abiotic stress

In all DAs performed on the first culm, nine discriminant functions were identified, indicating that all nine traits included in analysis are to some extent distinctive between different lines. The first three discriminant functions explained 78.6-90.4% of total distribution (Tab. 27). The somewhat lower descriptive power of 2-year analysis is due to the effect of year interaction with some of the traits.

Table 27 Cumulative proportion of total dispersion of the 1st culm data obtained by three DA performed

Discriminant function	1	2	3	4	5	6	7	8	9
2009-2010	**0.453**	**0.662**	**0.786**	0.876	0.931	0.964	0.986	0.996	1.000
2009	**0.499**	**0.721**	**0.873**	0.943	0.978	0.989	0.994	0.999	1.000
2010	**0.570**	**0.799**	**0.904**	0.962	0.979	0.991	0.999	1.000	1.000

Contribution of each of the nine traits to particular discriminant functions and classification coefficients are reported in Tab. 28. In all three analyses, the first three functions were determined by SNE (seed number per ear), GY (grain yield) and/or EL (ear length). Thus, these traits were the most discriminating for the observed total distribution of

groups. The result obtained agrees with statistical significance observed by ANOVA analysis for EL (R5-2-10 HOM+, § 3.5.2.1.), and SNE and GY (R23-1 HOM+, § 3.5.2.2.).

Table 28 Canonical discriminant functions standardized by within variances for the 1st culm data (values of main contributing traits to the first three functions are in bold) and parameters for classification

Variable	Discriminant function									Classification of functions	
2009-2010	1	2	3	4	5	6	7	8	9	**F-to-remove**	**Tolerance**
FLW	0.043	0.308	0.191	1.222	0.496	0.050	0.465	0.018	0.249	5.745	0.464
FLL	0.132	-0.413	0.359	-0.458	-0.170	-0.458	-0.401	-0.547	0.659	3.071	0.602
FL_1W	0.070	-0.129	0.562	-0.165	-0.864	-0.167	-0.438	-0.409	-0.674	3.121	0.512
FL_1L	-0.249	-0.046	-0.673	0.070	0.265	-0.185	-0.583	0.773	-0.261	3.308	0.613
EL	**-0.780**	0.264	0.678	-0.321	0.256	-0.172	0.166	0.392	-0.397	10.402	0.597
TH	-0.176	0.134	-0.192	0.198	0.339	0.559	-0.361	-0.705	0.010	2.324	0.848
SNE	-0.080	**1.808**	-0.746	0.227	-1.081	-0.083	-0.482	-0.063	0.776	8.687	0.170
SPNE	-0.242	-0.649	-0.721	0.043	-0.397	0.164	0.334	-0.208	0.647	6.054	0.569
GY	0.261	**-1.768**	**0.981**	-0.433	0.750	0.702	0.284	0.662	-0.357	8.973	0.165
2009	1	2	3	4	5	6	7	8	9	**F-to-remove**	**Tolerance**
FLW	0.196	0.662	**1.355**	0.362	0.300	0.061	0.188	0.129	0.437	6.764	0.360
FLL	-0.086	-0.642	-0.579	0.316	-0.341	0.516	0.188	0.448	0.350	3.794	0.627
FL_1W	-0.216	-0.602	-0.182	0.135	-0.822	-0.335	0.533	-0.087	-0.809	2.008	0.455
FL_1L	0.544	-0.093	-0.219	-0.425	-0.355	-0.415	-0.635	-0.293	0.442	2.786	0.660
EL	0.576	-0.412	-0.212	0.539	0.544	-0.541	-0.020	-0.485	-0.135	4.180	0.596
TH	0.205	0.100	0.370	-0.072	-0.424	1.009	0.176	-0.354	-0.064	1.338	0.644
SNE	0.568	**1.718**	-0.826	-0.876	-0.356	-0.057	1.213	0.333	0.436	5.593	0.151
SPNE	0.210	-0.267	-0.029	-0.697	0.388	0.474	-0.268	0.667	-0.297	1.859	0.632
GY	**-1.164**	-1.582	0.708	0.291	0.881	-0.304	-0.724	-0.579	0.088	7.778	0.162
2010	1	2	3	4	5	6	7	8	9	**F-to-remove**	**Tolerance**
FLW	0.229	0.193	0.183	0.968	0.485	0.465	0.434	0.539	0.001	2.9	0.502
FLL	0.122	0.522	-0.052	-0.498	0.219	-0.672	0.392	-0.514	0.614	2.468	0.546
FL_1W	0.141	0.305	0.571	-0.445	-0.531	-0.933	0.051	0.076	-0.382	2.703	0.515
FL_1L	-0.339	-0.304	-0.243	0.163	0.195	0.083	-1.170	0.299	0.090	2.14	0.554
EL	**-0.583**	-0.173	-0.445	-0.267	-0.418	-0.184	0.360	0.898	-0.131	4.281	0.556
TH	-0.208	-0.298	-0.130	0.706	-0.059	-0.328	0.181	-0.547	-0.175	3.476	0.892
SNE	0.342	**-1.648**	**1.424**	0.139	0.334	-0.326	0.059	0.107	0.920	9.157	0.168
SPNE	-0.467	0.322	0.518	-0.084	0.381	0.567	-0.086	-0.863	0.341	3.479	0.517
GY	-0.218	**1.624**	**-1.342**	-0.138	-1.118	0.533	-0.247	-0.178	-0.257	8.187	0.161

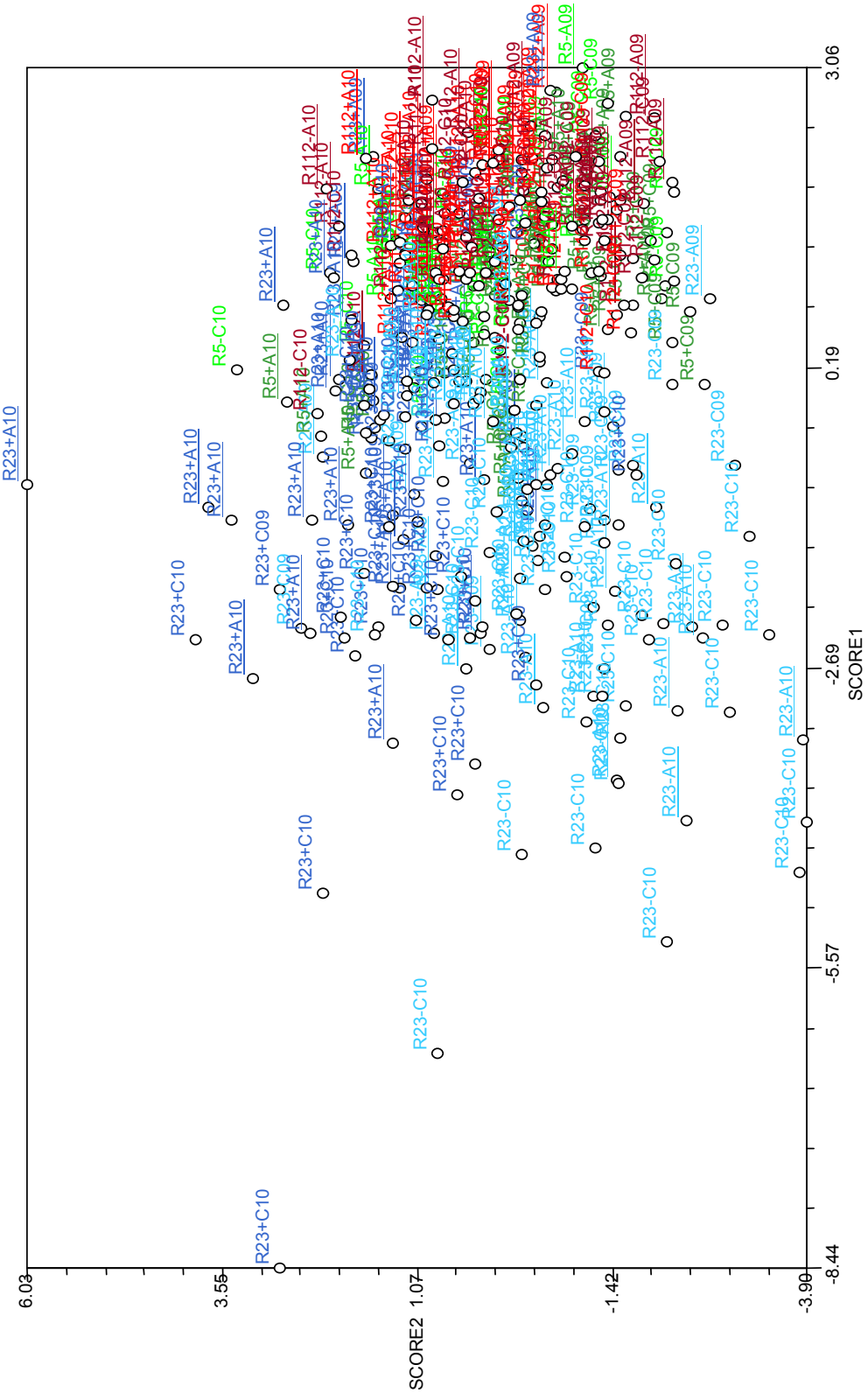
Classification matrices of analysed groups for each DA are reported in Tables 29-31. Groups present in tables and in the following Figures 39-41 are described by abbreviations of the genotype ("R5" = line R5-2-10, "R112" = line R112-4, "R23" = line R23-1), presence or absence of 7AgL segment ("+" and "-" respectively), treatment applied ("A" = ABA treated, "C"=control, water treated), and in case of 2-year analysis, the year of observation ("09" = 2009, "10" = 2010). For more obvious distinctiveness of groups, data points from ABA treated plants are also underlined.

Correct data description in DA of 2 years (Tab 29) was 52% and, as in the case of cumulative proportion of total distribution (Tab. 27), it reflected the interaction of parameters with year. Nine out of 24 groups of data had percentage of description lower than 50%, most of them representing groups treated with ABA, which somewhat contributed to dispersion of data, although the treatment itself did not result statistically significant in interaction with 7AgL segments. The other factor that may have contributed to low description of groups is that four of them refer to line R23-1, which, as described previously, has the least number of backcrosses (§ 2.1.2.1.) and *Sd* genes (§ 1.6.4.) causing higher level of variability. Two-dimension plotting of DA for 2 years is represented in Fig 39. Data were graphed by combining the first two discriminant functions determined as seen mainly by EL, GY and SNE (Tab. 28). The first discriminant function (= score 1) divided data in two major groups: one consisted of line R23-1 (in blue, mainly negative values), and the other of lines R5-2-10 and R112-4 (in red and green, positive values). The trait that contributed the most to this separation was EL (Tab 28). The second discriminant function (= score2) divided data of line R23-1 in two sub-groups, based on presence/absence of the 7AgL segment (light blue = R23-1 HOM-, dark blue = R23-1 HOM+). Traits mostly responsible for this were GY and SNE (Tab 28). In fact, these traits were negatively correlated in R23-1 HOM+ (more seeds and lower yield, § 3.5.2.2.), which coincides with the opposite sign of the coefficient for these traits (Tab. 28). The current model does not separate groups by experimental years, which indicates a rather constant expression of the variables analyzed. This is somewhat expected, since the measured traits describes only one culm of the plant. Clear classification of lines R23-1 HOM+ and R23-1 HOM- from other genotypes coincide with highly significant differences in EL, SNE and GY compared to other lines, the latter strongly depressed by the presence of the alien segment, which, on the contrary, determined a significantly high SNE.

Table 29 DA classification matrix (cases in row categories classified into columns) of 2 years data set of the1st culm

2009-2010	R112+ A09	R112+ A10	R112+ C09	R112+ C10	R112- A09	R112- A10	R112- C09	R112- C10	R23+ A09	R23+ A10	R23+ C09	R23+ C10	R23- A09	R23- A10	R23- C09	R23- C10	R5+ A09	R5+ A10	R5+ C09	R5+ C10	R5- A09	R5- A10	R5- C09	R5- C10	% correct
R112+A09	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	**80**
R112+A10	0	7	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	2	**47**
R112+C09	2	0	5	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	**50**
R112+C10	0	0	0	9	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	**64**
R112-A09	1	0	0	0	5	0	2	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2	0	**42**
R112-A10	0	2	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	**45**
R112-C09	0	0	1	0	0	0	7	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	**64**
R112-C10	0	2	0	0	0	0	0	6	1	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	**50**
R23+A09	1	0	0	0	0	1	0	3	4	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	**29**
R23+A10	0	1	0	0	0	0	0	0	1	13	1	4	2	1	0	0	0	0	0	0	0	0	0	1	**54**
R23+C09	0	0	0	0	0	0	0	0	3	1	6	1	1	0	0	0	0	0	0	0	0	0	0	1	**46**
R23+C10	0	0	0	0	0	0	0	0	0	3	0	16	1	0	0	1	0	0	0	1	0	0	1	0	**70**
R23-A09	1	0	2	0	0	0	1	0	1	0	1	0	5	2	2	0	0	0	0	0	0	0	0	0	**33**
R23-A10	0	0	0	0	0	1	1	1	0	1	1	1	1	14	0	7	0	1	0	1	0	6	0	0	**39**
R23-C09	0	0	0	1	0	0	0	0	0	0	0	1	1	1	8	1	0	0	1	0	0	0	0	0	**57**
R23-C10	0	0	0	0	0	0	0	0	0	0	0	3	1	8	2	17	0	0	0	1	0	0	0	0	**53**
R5+A09	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	7	0	2	0	1	0	0	0	**64**
R5+A10	0	0	1	1	0	0	0	1	0	2	0	0	0	1	0	0	0	5	0	0	0	1	0	1	**38**
R5+C09	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	1	0	0	0	**75**
R5+C10	0	0	0	2	0	0	0	0	0	1	1	0	0	0	1	0	0	3	0	6	0	0	0	0	**43**
R5-A09	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	**60**
R5-A10	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	10	0	2	**67**
R5-C09	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4	0	**80**
R5-C10	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	3	0	0	0	6	**50**
Total	14	13	10	15	6	8	14	13	12	23	15	26	12	27	14	26	10	14	15	13	7	20	9	17	**52**

Figure 39 Classifying durum wheat-*Th. ponticum* recombinant lines from 2009-2010 experiments using canonical discriminant analysis (1st culm data set)



DA of separate data from years 2009 and 2010 resulted in two somewhat different classifications, due do differential expression in the two years of principal discriminant variables from the model (GY, SNE and/or EL). For both years level of description of groups was higher compared to analysis of 2-years data (Tables 30 and 31) and more descriptive for 2009 compared to 2010 (67% and 56% respectively). Results below 50% per group were fewer with respect to the 2-years analysis, one in 2009 and three in 2010. From Figs. 40 and 41, it can be seen that the groups less described by the current model (R112-A in 2009; R112+A, R23-A, and R5+A in 2010) were much more variable with respect to other groups (bigger dispersion of data points in the graph), which prevented their clear separation from other groups. Again, ABA treatment did not have significant discriminating power on analyzed data. In both years the first discriminant function (= score1) divided line R23-1 from the rest of thedata, and the second function (= score2) separated HOM+ and HOM- genotypes within lines R23-1 and R112-4. Line R5-2-10 was not clearly classified on the basis of presence/absence of 7AgL, as differences caused by the segment were not as significant as in the other two genotypes. GY was the parameter mostly contributing to this result, followed by SNE and EL. In 2009, FLW (flag leaf width) showed to be contributing more than in 2010 (third discriminant function, Tab. 28), demonstrating its involvement in the first culm productivity.

In conclusion, DA of parameters assessed on the first culm indicates GY and SNE as the most discriminating between groups in all three analysis; EL and FLW also contributed to differentiation, but were year-dependent (Tab 28). Since the effect of ABA was not significant, the main criterion for the classification obtained confirmed to be the presence/absence of the 7AgL segment.

Table 30 DA classification matrix (cases in row categories classified into columns) of 2009 data set of the1st culm

2009	R112+A	R112+C	R112-A	R112-C	R23+A	R23+C	R23-A	R23-C	R5+A	R5+C	R5-A	R5-C	%correct
R112+A	9	1	0	0	0	0	0	0	0	0	0	0	90
R112+C	1	6	0	2	0	0	0	0	0	0	1	0	60
R112-A	1	0	5	1	0	0	0	0	1	1	1	2	42
R112-C	0	1	1	8	0	0	0	0	1	0	0	0	73
R23+A	0	0	0	0	11	1	2	0	0	0	0	0	79
R23+C	0	0	0	0	2	9	2	0	0	0	0	0	69
R23-A	1	0	0	0	1	1	8	2	0	1	0	1	53
R23-C	0	0	0	0	0	2	1	10	0	1	0	0	71
R5+A	0	0	0	1	0	0	0	0	6	2	2	0	55
R5+C	0	1	0	0	0	0	0	0	2	9	0	0	75
R5-A	0	0	0	1	0	0	0	0	0	0	3	1	60
R5-C	0	0	0	0	0	0	0	0	1	0	0	4	80
Total	12	9	6	13	14	13	13	12	11	14	7	8	67

Table 31 DA classification matrix (cases in row categories classified into columns) of 2010 data set of the1st culm

2010	R112+A	R112+C	R112-A	R112-C	R23+A	R23+C	R23-A	R23-C	R5+A	R5+C	R5-A	R5-C	%correct
R112+A	7	1	1	1	0	0	0	0	1	0	2	2	47
R112+C	0	9	1	1	0	0	0	0	0	1	0	2	64
R112-A	3	0	6	0	0	0	0	0	0	0	1	1	55
R112-C	1	0	1	8	0	0	0	0	2	0	0	0	67
R23+A	1	0	1	0	14	5	1	0	0	0	0	2	58
R23+C	0	0	0	1	2	18	1	0	0	1	0	0	78
R23-A	0	0	0	1	1	1	15	7	2	3	5	1	42
R23-C	0	0	0	0	0	2	10	18	0	2	0	0	56
R5+A	1	1	0	1	2	0	1	0	5	0	1	1	38
R5+C	0	2	0	0	2	0	0	0	3	7	0	0	50
R5-A	0	2	1	0	0	0	0	0	1	0	10	1	67
R5-C	0	1	1	0	0	0	0	0	0	3	0	7	58
Total	13	16	12	13	21	26	28	25	14	17	19	17	56

Figure 40 Classifying durum wheat-*Th. ponticum* recombinant lines in 2009 experiment using canonical discriminant analysis (1st culm data set)

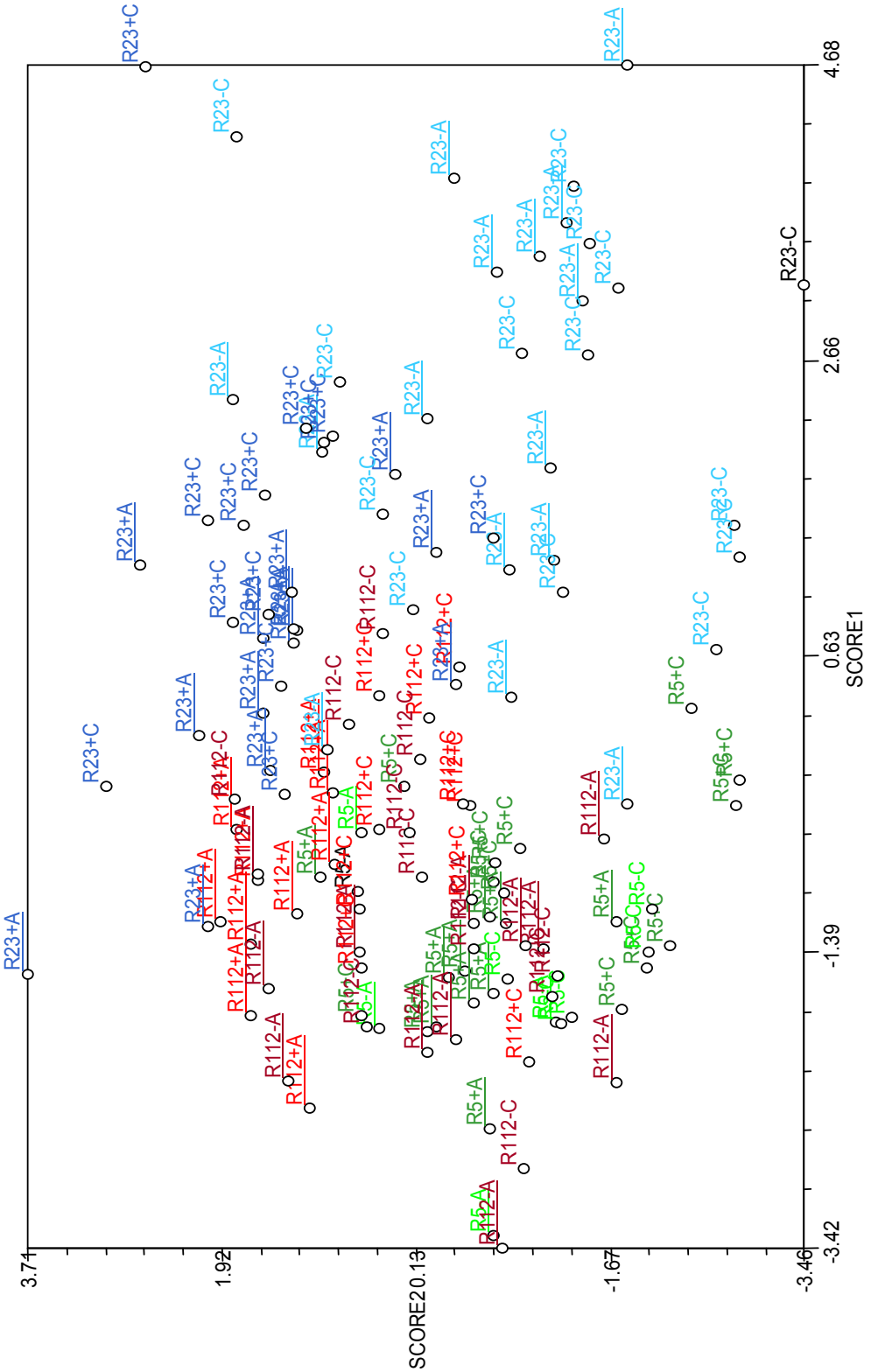
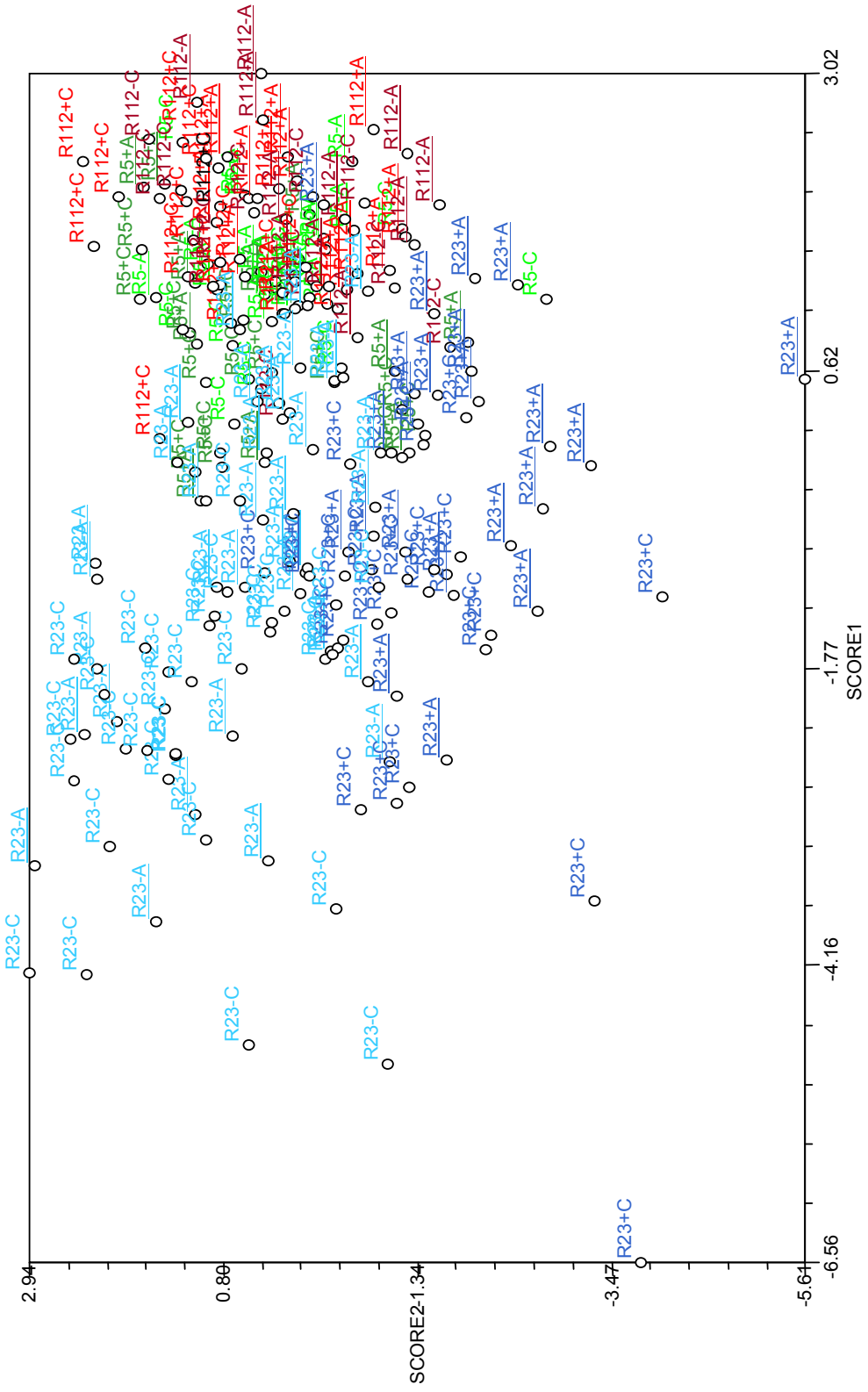


Figure 41 Classifying durum wheat-*Th. ponticum* recombinant lines in 2010 experiment using canonical discriminant analysis (1st culm data set)



3.8.2. DA of yield related traits of the whole plant at harvest

DAs on data from the entire plant were conducted including in the analyses the following traits: “TTH”, “TCN”, “PRCN”, “PRGY”, “PRCSEED”, “GY”, “SEED” number and the “B” was includes for 2010 data set.

Seven discriminant functions were identified in DA for 2-years distribution and five for the separate years. The first three functions described 93.3-94.9% of total distribution (Tab. 32).

Table 32 Cumulative proportion of total dispersion obtained by DAs of whole plant data

Discriminant function	1	2	3	4	5	6	7
2009-2010	**0.548**	**0.861**	**0.933**	0.969	0.985	0.997	1.000
2009	**0.766**	**0.888**	**0.968**	0.993	1.000	-	-
2010	**0.715**	**0.838**	**0.949**	0.992	1.000	-	-

Contributions of all variables in the model to particular functions are reported in Tab. 33. Similarly to the 1st culm analysis, for all three DAs of whole plant data, grain yield and seed number (PRGY, GY, PRCSEED, SEED) had the biggest distinctive power on the total group distribution, indicating a constant trait expression and correlation between them in both years. The first and second discriminant functions were determined by grain yield and seed number of productive culms (PRGY, PRCSEED) in all three DAs performed (Tab. 33). Grain yield and seed number of the whole plant was important in 2010, probably due to more favourable climate conditions for plant development, which can be confirmed by important contribution of TCN to second discriminant function (Tab. 33). Once more, like in case of the first culm, the most important traits determining group classification were grain yield and seed number, negatively correlated.

Classification matrices of the groups analyzed are reported in Tables 34-36. Abbreviations used in these tables and in Figs. 42-44 are the same used for Tables 29-31 and Figs 39-41.

Table 33 Canonical discriminant functions standardized by within variances for whole plant with coefficients for classification (majorly contributing traits to particular function are represented in bold)

Year(s)	Discriminant function							Classification of function	
2009-2010	1	2	3	4	5	6	7	**F-to-remove**	**Tolerance**
TTH	0.309	0.366	0.579	0.013	1.303	0.494	0.367	3.55	0.379
TCN	-0.020	-0.592	0.574	1.504	-0.682	-1.391	-2.064	2.007	0.104
PRCN	1.044	0.385	-0.711	-0.626	-0.569	1.736	1.134	4.962	0.148
PRGY	**4.786**	1.569	**-4.526**	-0.295	3.131	-6.134	4.896	6.252	0.009
PRCSEED	**-3.571**	-0.607	**3.791**	0.070	-0.492	1.272	-6.908	4.791	0.013
GY	**-4.037**	**-3.658**	**4.458**	0.345	-2.104	5.895	-4.395	7.298	0.009
SEED	1.781	**2.531**	**-4.787**	-0.521	0.179	-1.390	6.691	4.16	0.013

2009	1	2	3	4	5	**F-to-remove**	**Tolerance**
TTH	0.253	0.772	0.375	0.279	0.460	3.899	0.716
TCN	-0.152	1.072	-1.403	-1.285	-1.665	2.106	0.117
PRCN	0.293	-0.376	0.691	1.307	1.925	0.698	0.094
PRGY	-0.470	**2.738**	**5.745**	2.612	-6.292	1.286	0.007
PRCSEED	0.877	**-1.218**	-3.231	-3.540	0.356	0.86	0.011
GY	**-1.744**	-1.298	**-4.950**	-2.740	6.283	1.349	0.008
SEED	0.793	-0.843	2.970	4.539	-1.023	0.915	0.012

2010	1	2	3	4	5	**F-to-remove**	**Tolerance**
TTH	0.028	0.564	0.744	0.377	1.051	0.809	0.177
TCN	-0.882	**-1.269**	0.780	-0.969	-0.768	2.124	0.085
PRCN	0.268	0.203	-0.471	0.993	-1.194	0.857	0.186
PRGY	2.274	**-1.792**	**3.219**	-1.863	4.175	1.423	0.009
PRCSEED	-1.495	**1.290**	-1.421	-1.688	-1.310	0.748	0.014
GY	**-4.349**	0.880	**-3.362**	1.396	-3.956	3.52	0.009
SEED	**4.057**	-1.215	0.873	1.192	1.606	3.54	0.012
B	0.194	0.587	-0.619	0.832	0.737	1.663	0.303

Table 34 DA classification matrix (cases in row categories classified into columns) of 2009-2010 data set from whole plant

2009-2010	**R112+09**	**R112+10**	**R112-09**	**R112-10**	**R23+09**	**R23+10**	**R23-09**	**R23-10**	**R5+09**	**R5+10**	**R5-09**	**R5-10**	**%correct**
R112+09	9	2	2	0	0	0	1	0	1	0	1	0	**56**
R112+10	0	8	0	3	0	1	0	2	0	3	0	1	**44**
R112-09	1	0	10	0	1	0	2	1	1	0	2	0	**56**
R112-10	0	4	0	7	0	3	0	4	0	0	0	1	**37**
R23+09	0	0	0	2	20	2	2	0	1	0	0	0	**74**
R23+10	0	1	1	5	0	22	0	1	0	0	0	0	**73**
R23-09	5	1	0	0	4	0	17	0	0	0	2	1	**57**
R23-10	0	3	1	2	0	0	0	19	0	7	0	4	**53**
R5+09	1	1	2	1	0	0	0	1	9	0	4	0	**47**
R5+10	0	3	0	0	0	2	0	8	0	7	0	2	**32**
R5-09	1	0	1	0	0	0	0	0	1	0	4	0	**57**
R5-10	0	2	0	4	1	0	0	2	0	5	0	3	**18**
Total	17	25	17	24	26	30	22	38	13	22	13	12	**52**

Table 35 DA classification matrix (cases in row categories classified into columns) of 2009 data set from whole plant

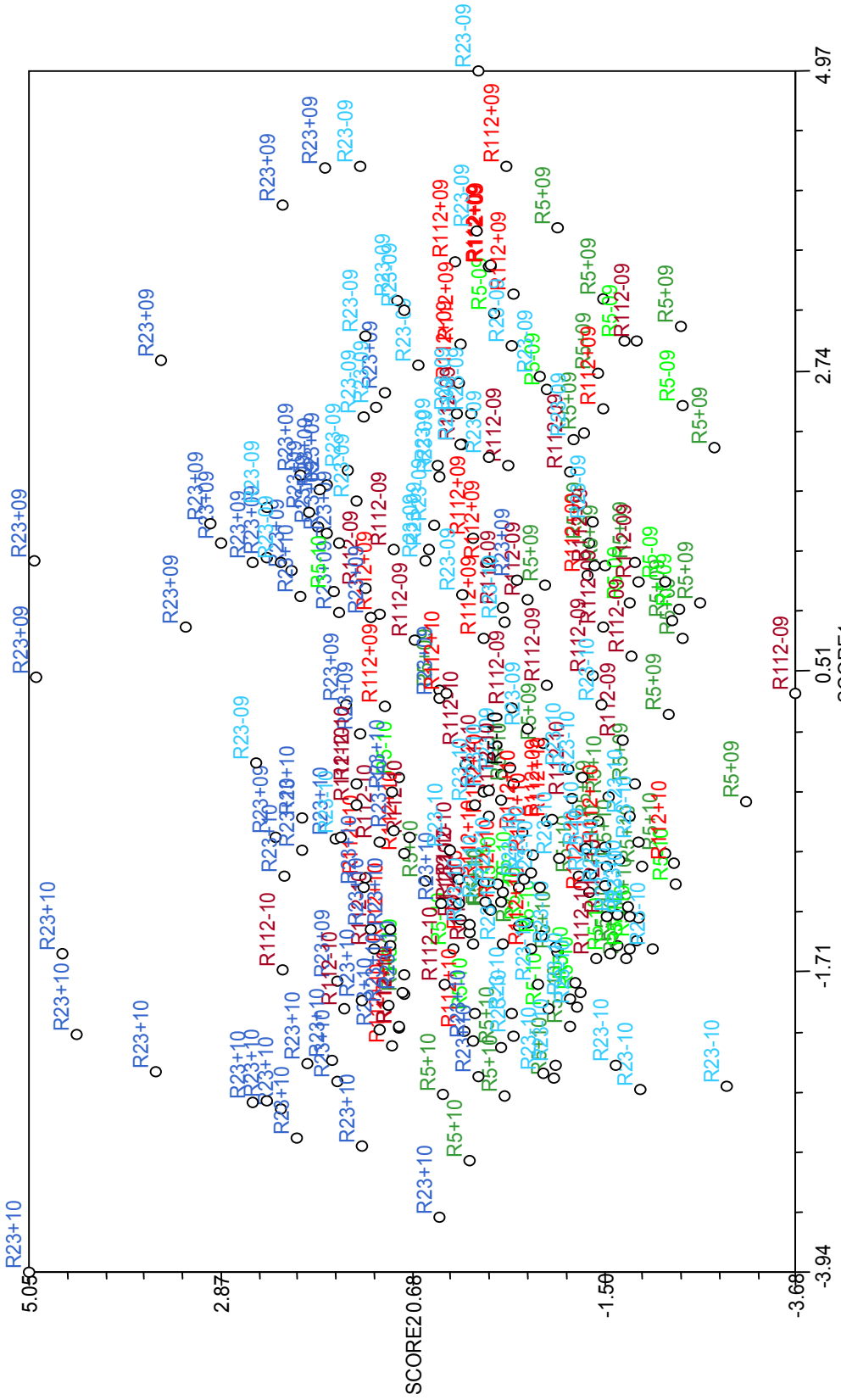
2009	R112+	R112-	R23+	R23-	R5+	R5-	%correct
R112+	11	1	0	1	3	0	**69**
R112-	0	13	1	1	2	1	**72**
R23+	2	0	21	3	1	0	**78**
R23-	8	1	4	14	1	2	**47**
R5+	1	1	1	0	11	5	**58**
R5-	1	0	0	0	2	4	**57**
Total	23	16	27	19	20	12	**63**

Table 36. DA classification matrix (cases in row categories classified into columns) of 2010 data set from whole plant

2010	R112+	R112-	R23+	R23-	R5+	R5-	%correct
R112+	5	2	1	2	5	3	**28**
R112-	4	8	3	2	0	2	**42**
R23+	1	6	23	0	0	0	**77**
R23-	2	3	0	20	5	6	**56**
R5+	2	1	2	4	13	0	**59**
R5-	2	6	0	2	3	4	**24**
Total	16	26	29	30	26	15	**51**

Overall correct description of 2-years data was 52%, obviously affected by "year" factor particularly significant on some traits included in the current model (e.g. SEED, GY, see § 3.6.1.2.). Three groups were weakly described (R5+09, R5+10, and R5-10) because of the variability within each one. Classification results of the two years data set are graphed in Fig. 42. The first discriminant function (= score1), separates clearly two groups by year of experiment due to various differentially expressed yield traits, PRGY and PRCSEED (§ 3.6.1.2., Fig. 35, Tab. 33), mainly contributing to the first function. Second discriminant function (= score 2) further separated R23-1 HOM+ from the rest of the data, which was expected since this line was significantly more variable in the two years compared to the other lines, and had a very low value of GY in 2009, due to a negative effect of 7AgL presence. Traits which mostly discriminated the groups by the second function were GY and SEED (Tab. 33).

Figure 42 Classifying durum wheat-*Th. ponticum* recombinant lines on the basis of 2009-2010data using canonical discriminant analysis (whole plant data set)



DA for individual years 2009 and 2010 showed more differentiation between groups due to the 7AgL segment. Correct description of data points was better in 2009 (63%) compared to 2010 (51%) (Tabs 35 and 36). This specifies that more stressful climate conditions, as observed in 2009, enhanced differences among genotypes. Higher variability of groups R23- in 2009 and of R112- and R5- in 2010 caused their low fitting to the model (Tab. 35). These results are graphed in Figs. 43 and 44. Classification in both years highlighted the same differences, based on the same variables, i.e. grain yield (PRGY/GY) and seed number (PRCSEED/SEED). The first discriminating function clearly separated line R23 HOM+ from the rest of groups, as a consequence of its significantly inferior yielding caused by the alien segment presence. Besides this, in 2010 line R112-4 HOM+ together with R23-1 HOM+ was separated by the first discriminant, in line with their similarities in seed number produced in comparison to the R5-2-10 genotype (Fig 35).

Second discriminant function (PRGY, PRCSEED and, TCN) divided further R112-4 HOM+ from R112-4 HOM-. The model was unable to separate HOM+ from HOM- lines of R5-2-10, which was somewhat expected since for this genotype segment presence/absence was not statistically significant. Moreover, the number of plants in 2009 of R5-2-10 HOM- was limited.

It is clearly visible form Figs. 43 and 44 that HOM+ lines are well separated from the rest of data as well as between each other, which proves that involvement of 7AgL segments of different size, introduced in the same background, has an important effect on phenotype of these three genotypes. On the other hand, HOM- lines are very similar to each other, which makes these groups unclassifiable. This is understandable, since they tend to be nearly isogenic due to the repeated backcrosses with cv. Simeto.

It is important to say that by this analysis it was confirmed that GY/PRGY and SEED/PRCSEED are the key traits determining the observed distribution of groups. The involvement of traits such as TCN, PRCN and TTH was significant in determination of third and higher discriminant functions (Tab. 33), which confirms their contribution to the overall yielding potential of the plant (grain and biomass included). The line which had significantly higher values for all the above mentioned traits (due to 7AgL segment) is line R112-4 (HOM+).

Figure 43 Classifying durum wheat-*Th. ponticum* recombinant lines from 2009 experiment using canonical discriminant analysis (whole plant data set)

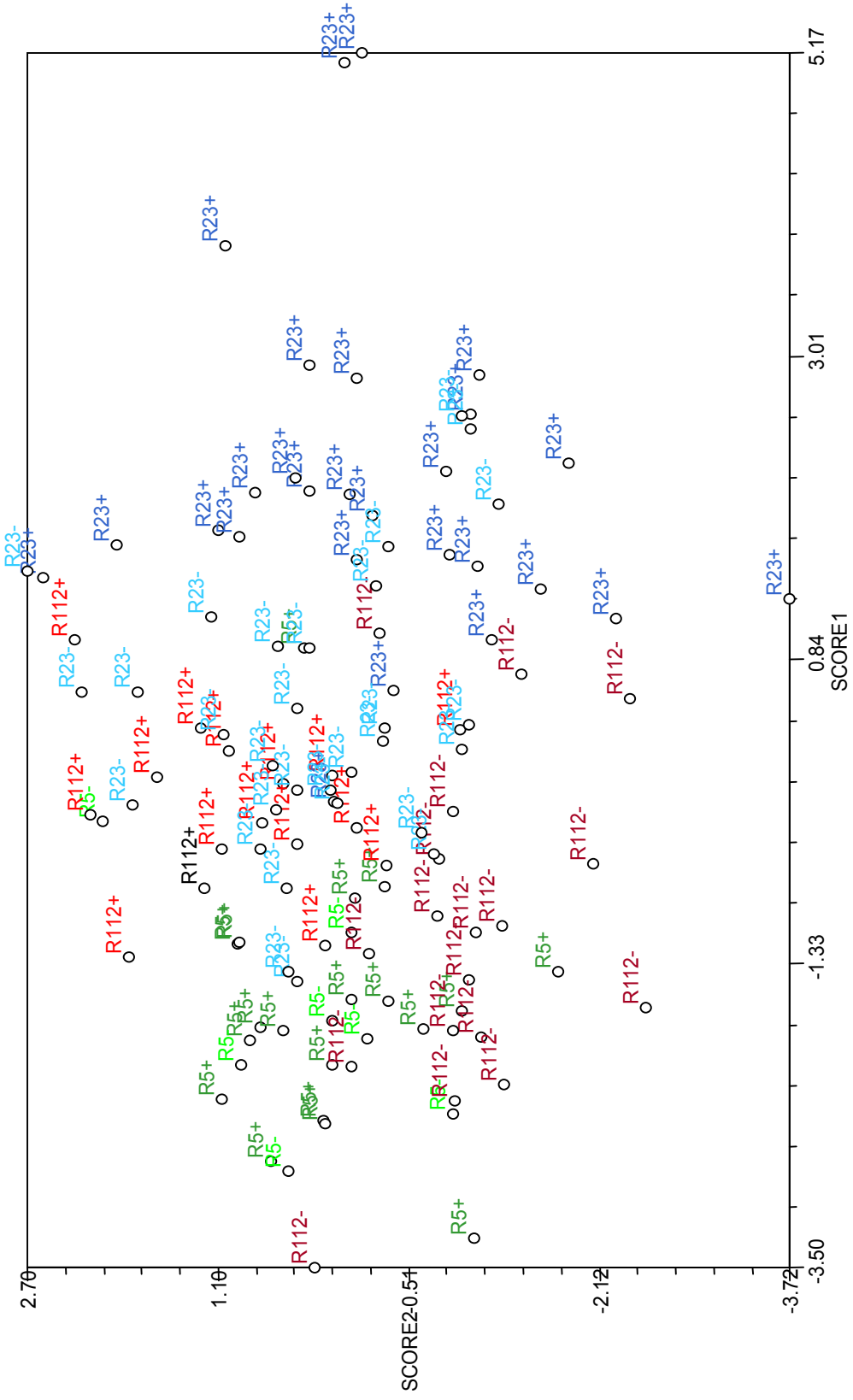
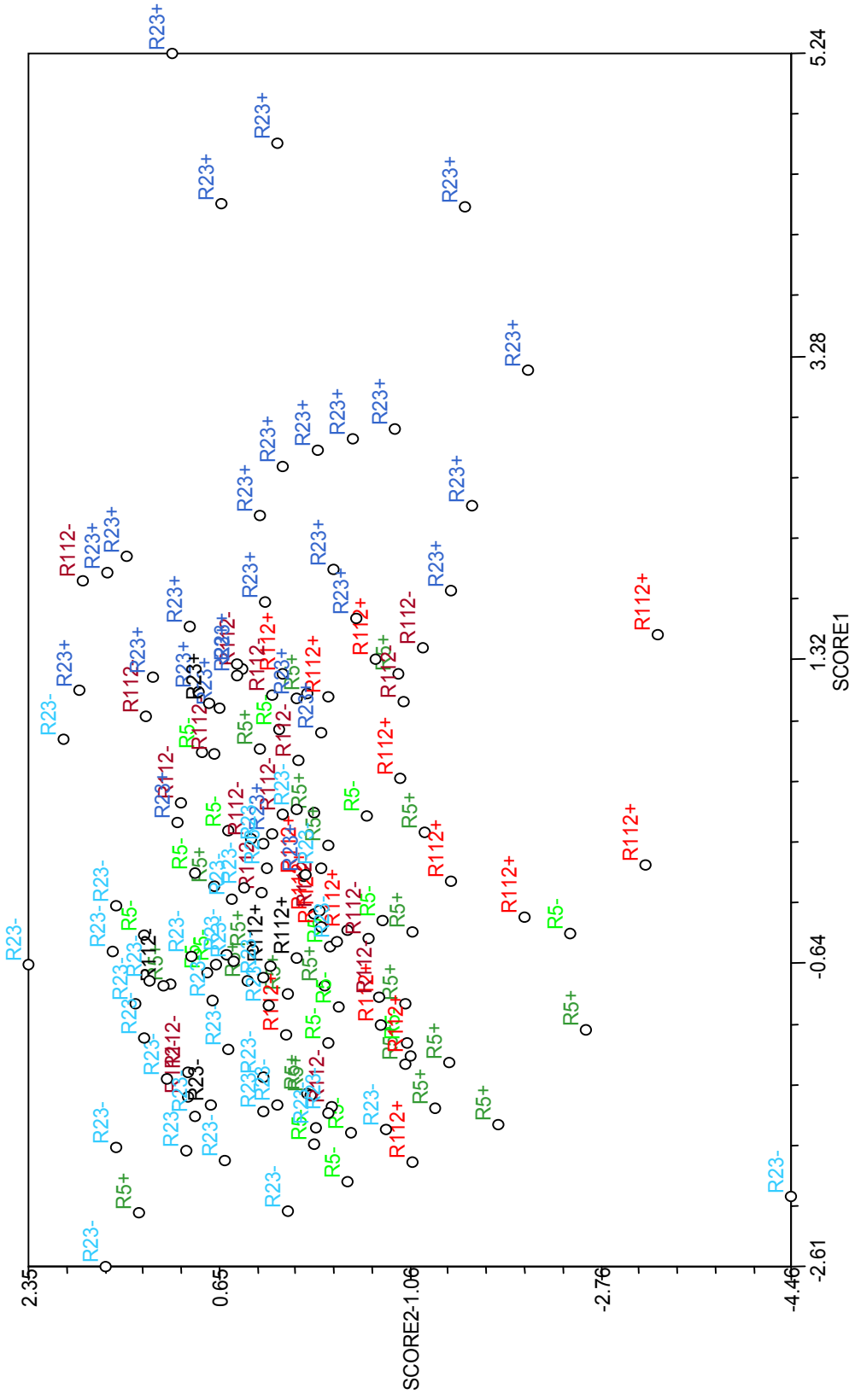


Figure 44 Classifying durum wheat-*Th. ponticum* recombinant lines from 2010 experiment using canonical discriminant analysis (whole plant data set



3.9. **Analysis of endogenous ABA content at boot stage of ear development**

There are numerous evidences that the content of endogenous ABA in wheat spike and particularly floral organs is negatively correlated with spike fertility, especially under water stress conditions (Morgan, 1980, Morgan and King, 1983, Westgate et al., 1996). Wheat ear in fact tends to act as sink of ABA from leaves, largely conditioned by leaf water status (Morgan and King, 1984). Even in the absence of water stress it was shown that fertile wheat genotypes accumulated significantly less ABA in spikes compared to male sterile lines in period around pollen meiosis (Zeng and King, 1986).

Actually, wheat spikes are characterized by changes in levels of endogenous ABA during normal development, tending to increase from floret initiation and to reach its peak just before meiosis, while rapidly decreasing afterwards (Cao et al., 2000). The pollen mother cells are the most sensitive to ABA during meiosis (Morgan and King, 1984). After meiosis, the level of endogenous ABA in the ear increases slightly, but, interestingly, the most productive and fertile genotypes maintain the lowest level of the hormone (Cao et al., 2000). This means that low endogenous ABA in developmental phases after meiosis might be an indicator of the wheat spike fertility. as considered in the present study.

As to wheat-*Th. ponticum* recombinant lines, evidence about endogenous ABA content in spikes is limited to one report by CIMMYT, indicating lower levels of ABA in T4 NILs harbouring 7AgL chromatin compared to their controls (Reynolds et al., 2008). Since the origin of the T4 7AgL segment is the same as those present in our durum wheat recombinants, the level of endogenous ABA in juvenile spikes of the latter was measured, to see if there was any difference in the ABA content of HOM+ vs. HOM- lines, which could be reflected on differential yield.

Results obtained in 2010 from the first culm of plants grown in the field and in greenhouse are reported in Tab. 37. ANOVA analysis performed by GLM did not reveal any significant difference in ABA content associated with presence of the 7AgL segment in both durum and bread wheat genotypes[9], which does not confirm (at least for bread wheat lines) what was reported by Reynolds et al., 2008. On the other hand, the observed absence of genotype-dependent differences in endogenous ABA content in juvenile spikes of our lines parallels the results obtained by the analysis discussed in § 3.5., where fertility

[9] For all ANOVA summary tables see Appendix 4

parameters of the first culm did not result as ABA-dependent. Moreover, the observed results indicate that endogenous ABA is not a distinctive physiological factor affecting fertility and productivity of the material analyzed, which does not exclude that there might be some other factors involved but were not considered in the present study.

Table 37 Measurements of endogenous ABA in juvenile spikes of plants grown in field (FIELD) and greenhouse (GH) condition, and respective relative water content (RWC) of plants grown in field in 2010 (SE-standard error).

Genotype	ABA (nd/gDW)		RWC
	FIELD	GH	FIELD
R5-2-10 hom+	108.07	176.17	0.85
SE	11.54	30.08	0.01
R5-2-10 hom-	103.83	129.97	0.87
SE	15.72	26.05	0.01
R112-4 hom+	101.66	136.68	0.84
SE	11.12	26.05	0.01
R112-4 hom-	87.13	174.02	0.86
SE	11.12	23.30	0.01
R23-1 hom+	123.03	264.58	0.89
SE	12.01	22.21	0.01
R23-1 hom-	106.95	332.95	0.87
SE	10.40	22.21	0.01
T4	128.08	295.82	0.87
SE	13.88	20.52	0.01
Thatcher	126.23	310.08	18.73
SE	13.88	310.08	18.73

Differences in endogenous ABA level were observed between plants grown in the two environments, but they were of similar entity for all genotypes (Fig. 45). Lines R5-2-10 and R112-4 accumulated about 1.5 fold ABA in greenhouse compared to field conditions, while a 3 fold increase was observed for line R23-1. The latter showed to be more sensitive to greenhouse conditions (higher temperature, lack of atmospheric precipitation); this, however, did not depend on the 7AgL segment but on background effect.

As the amount of accumulated ABA in ear directly depends on leaf water status, relative water content (RWC)[10] was assessed in plants grown in the field (Tab 37). The aim was to determine the inherent endogenous ABA production in our lines, with regard to alien segment presence and eliminating possible effects of water stress conditions. Plants were thus watered regularly (in addition to occurring precipitation) and RWC of flag leaf

[10] For ANOVA summary table see Appendix 4

measured. No significant differences were observed due to the presence of 7AgL segments (Fig. 46).

Figure 45 Observed levels of endogenous ABA in analyzed durum and bread wheat genotypes (FIELD-field conditions; GH-greenhouse conditions) in 2010.

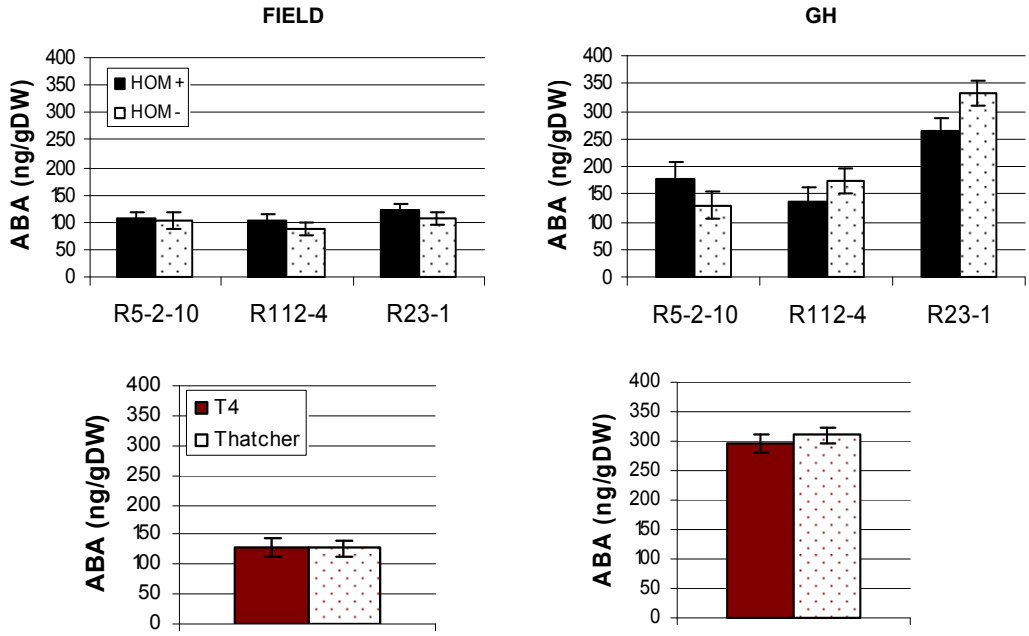
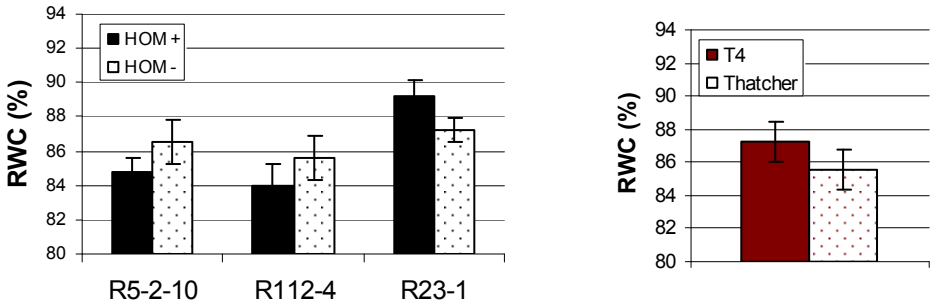


Figure 46 Observed RWC in analyzed durum and bread wheat genotypes grown in field, in 2010.



Correlation analysis was then performed for results obtained for ABA and RWC. As expected in non-stress conditions, no correlation was observed except in the case of line R5-2-10 HOM+ (Tab. 38)[11].

Table 38 Parameters of correlation analysis (Pearson statistics, Boniferroni probabilities) of endogenous ABA and RWC in analyzed durum and bread wheat lines grown in field in 2010.

ABA-RWC	Coeff.	P
R5-2-10 HOM+	-0.705	0.010
R5-2-10 HOM-	0.614	0.271
R112-4 HOM+	0.207	0.695
R112-4 HOM-	-0.430	0.470
R23-1 HOM+	0.001	0.998
R23-1 HOM-	-0.005	0.984
T4/Thatcher	-0.024	0.942

3.10. **Analysis of physiological parameters related to plant water status under water stress in controlled conditions**

The *Yld-7AgL* QTL was originally described as being better expressed under drought (Singh et al., 1998). In consideration of this, a series of experiments was performed in order to characterize the response to drought of the 7AgL recombinant genotypes used in this thesis (see § 2.2.2.9.). In particular, four useful parameters were measured during a four-week soil drying assay.

During the experiment, plants responded as expected under drought conditions, i.e. with a gradual decline of water status caused loss of turgor and slowing down of growth. At the end of 4 weeks of water withhold plants were as illustrated in Fig 47.

Measurements of three parameters concomitantly recorded on a weekly basis are reported in Tab. 39 and graphed in Figs. 48-51[leaf water potential (LWP), fresh weight/dry weight ratio(FW/DW) and the endogenous ABA content in the youngest developed leaf]. All values were in the expected ranges, and for none of them the ANOVA[12] analysis by GLM revealed significant differences correlated to the alien segment presence within a genotype (HOM+ vs. HOM- lines) or between genotypes. LWP and FW/DW decreased gradually over time (Figs 48 and 49) but was stable between genotypes, although LWP was relatively variable within all genotypes and did not show

[11] For graphs of correlation analysis performed by SYSTAT12 software see Appendix 4
[12] For all summary tables of ANOVA see Appendix 5

important changes in water available to plants until the last week of experiment Levels of measured ABA content in the youngest, fully developed leaf (Fig 50) showed that plants did not reflect the exposure to drought stress in a significant way until week 4. At that point, ABA content had 3.7-4.5 fold increase in durum and 2.3-2.4 fold increase in bread wheat lines, indicating more sensibility to drought of durum wheat seedlings. However, all lines analyzed responded in the same way, indicating that 7AgL segment presence did not confer any superior drought tolerance in early vegetative stages.

In addition, final fresh biomass weight (Fig 51) did not reveal any difference in plant growth and accumulation of fresh matter under drought at early vegetative stage. Durum wheat lines showed some tendency to grow more compared to bread wheat lines.

Figure 47 Aspect of plants used in the first and the last week of drought experiment

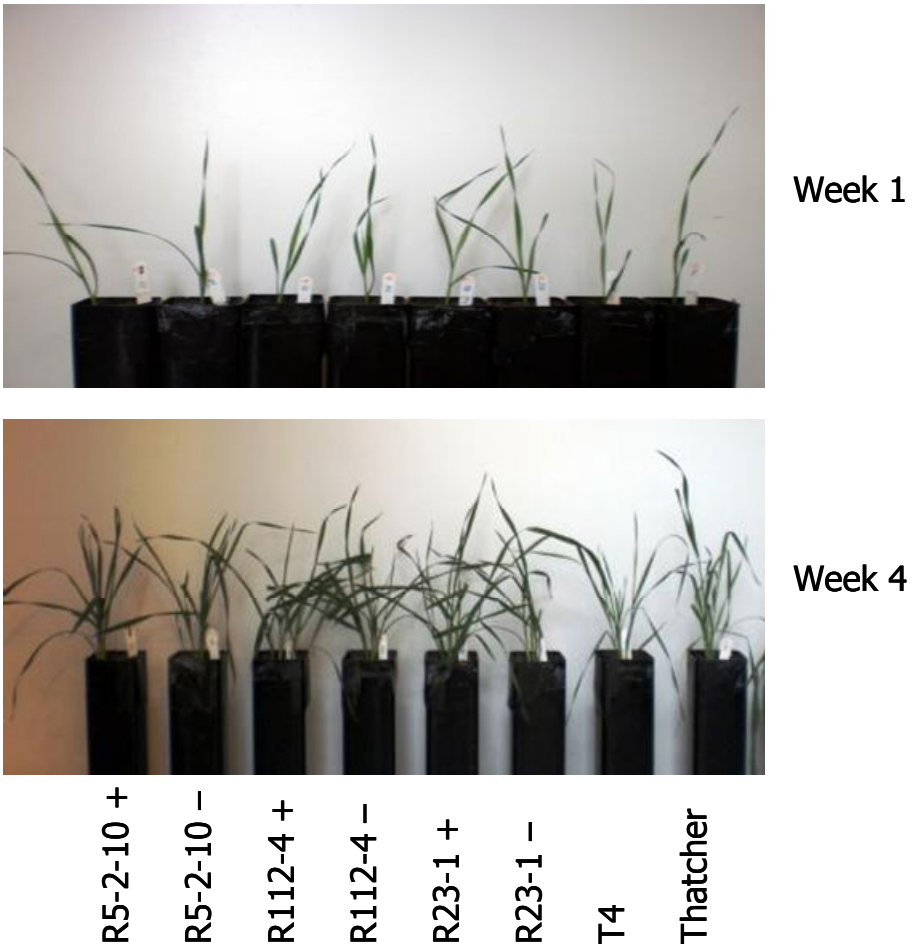


Table 39 Means of physiological parameters recorded in analyzed durum and bread wheat lines during the 4 week imposed drought stress (SE-standard error)

Genotype	FW-DW				LWP (Mpa)			ABA (ng/g DW)			
	week 1	week 2	week 3	week 4	week 2	week 3	week 4	week 1	week 2	week 3	week 4
R5-2-10 HOM+	9.09	8.12	5.76	5.32	-1.10	-1.14	-1.19	123.93	179.27	177.83	737.35
SE	0.42	0.42	0.37	0.48	0.15	0.15	0.17	27.51	29.41	27.51	31.76
R5-2-10 HOM-	8.81	9.22	5.96	5.33	-1.01	-0.99	-1.26	117.36	187.07	149.51	654.51
SE	0.37	0.39	0.37	0.48	0.17	0.15	0.15	24.60	27.51	24.60	31.76
R112-4 HOM+	9.32	7.48	5.76	5.21	-1.08	-1.06	-1.29	116.13	149.67	157.45	710.64
SE	0.39	0.37	0.37	0.48	0.12	0.17	0.15	25.93	25.93	24.60	31.76
R112-4 HOM-	8.46	7.34	6.11	5.57	-1.18	-1.13	-1.14	125.04	157.26	172.11	685.86
SE	0.39	0.42	0.37	0.48	0.17	0.17	0.17	25.93	29.41	27.51	31.76
R23-1 HOM+	8.62	7.42	5.76	4.90	-1.08	-1.06	-1.27	157.87	203.49	201.42	909.27
SE	0.42	0.42	0.42	0.48	0.21	0.17	0.15	27.51	29.41	29.41	31.76
R23-1 HOM-	8.82	7.61	5.90	5.58	-0.91	-1.11	-1.31	144.26	180.56	215.23	792.26
SE	0.37	0.45	0.39	0.48	0.21	0.13	0.13	24.60	29.41	25.93	34.79
T4	9.10	-	5.29	5.13	-0.86	-1.03	-1.09	137.11	-	379.21	856.00
SE	0.21	-	0.24	0.33	0.16	0.09	0.08	19.48	-	23.86	30.80
Thatcher	8.86	-	5.40	5.12	-1.10	-1.15	-1.33	131.81	-	360.91	848.13
SE	0.21	-	0.24	0.33	0.09	0.09	0.11	19.48	-	22.75	30.80

Figure 48 Observed FW/DW ratio during 4 week imposed drought stress in A-durum, and B-bread wheat lines

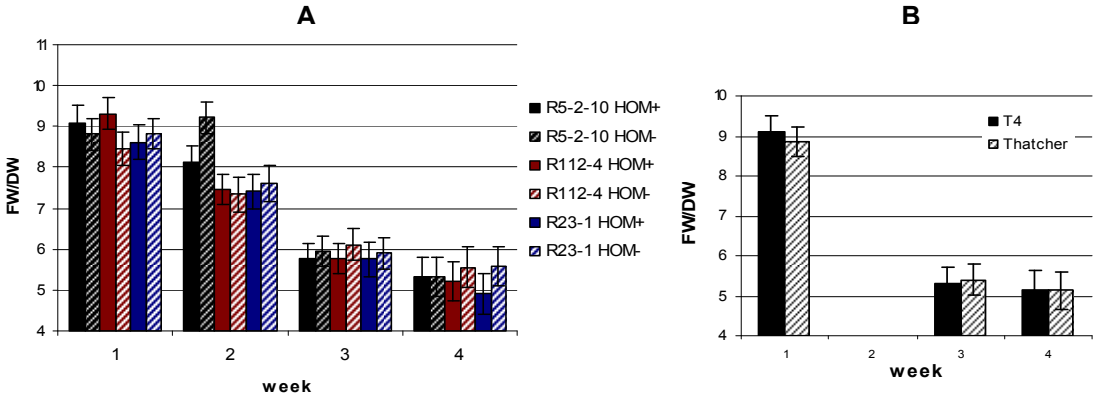


Figure 49 Observed LWP during 4 week imposed drought stress in A-durum, and B-bread wheat lines

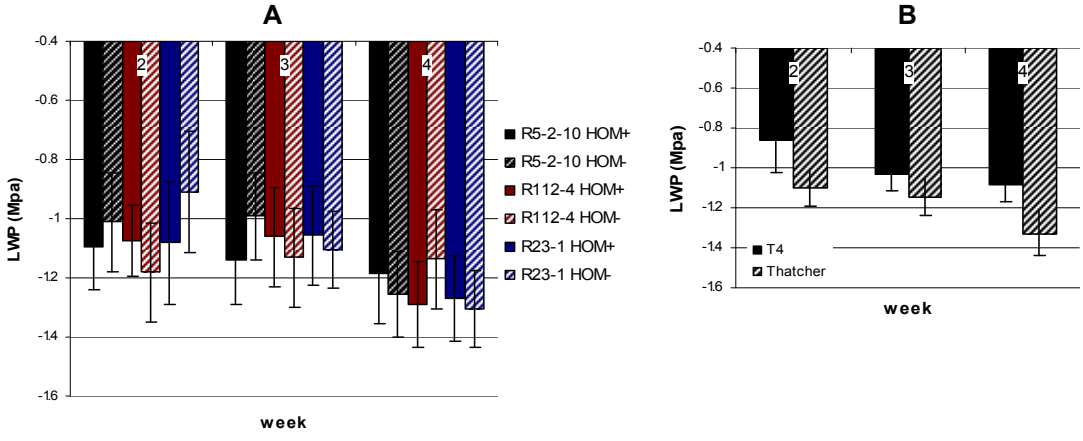


Figure 50 Observed LWP during 4 week imposed drought stress in A-durum, and B-bread wheat line

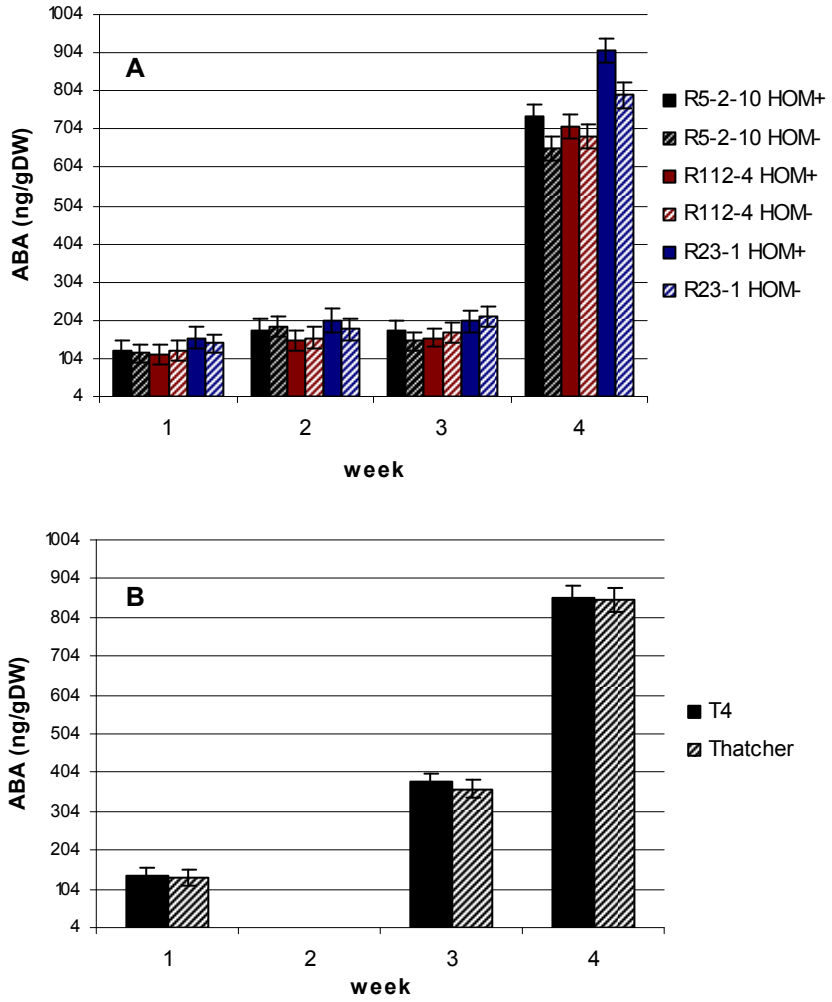
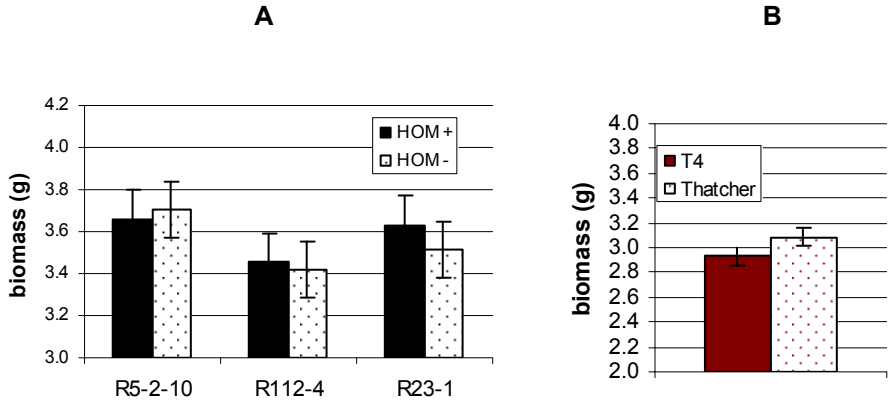


Figure 51 Observed above ground biomass at the end of experiment in A-durum, and B-bread wheat lines



3.11. CONCLUSIONS

In the view of effectively exploiting available genetic variability of wild wheat relatives for the improvement of cultivated *Triticum* species, the present study has contributed to the pyramiding of multiple useful genes of the wheatgrass species *Th. ponticum* into wheat, also developing and exploiting suitable molecular and cytogenetic tools, as well as to the identification of novel genes/QTLs of the same species that enhance a relevant trait such as yield.

The work on molecular and cytogenetic characterization of the available durum and bread wheat 7Ag recombinant lines resulted on one hand in effective selection of target regions along their 7AgL segments harbouring the *Lr19+Yp+FHBres* genes, and, on the other, allowed isolation of a new recombinant durum wheat line, R5-2-23, with about 20% interstitial 7AgL chromatin on the 7AL arm, possibly including one or few of the genetic determinants of the putative *Yld-7AgL* QTL originally ascribed to the long 7AgL segment of the bread wheat T4 line.

While this new durum wheat-*Th. ponticum* recombinant will contribute to dispose of stable lines in which fractions of at least a large portion of the T4 7AgL segment are separately present, already the use of some such lines in extensive agrononomic and physiological analyses has shed some light onto the expression of some important yield-related traits associated with 7AgL genes/QTLs. Based on the results of these analyses, the existence of multiple genetic determinants, located on different sized alien segments, and contributing to different yield parameters, can be hypothesised. Grain yield (g) and seed number showed to be the most important traits that were differentially expressed by the different recombinant genotypes, and for which the observed increased values in given genotypes could be associated to the presence of the corresponding *Th. ponticum* segment. Durum wheat lines that showed the most significant differences ascribable to the 7AgL segment were R112-4 and R23-1. With the latter being in the future replaced by the more stable R5-2-23 recombinant just isolated, they will represent a good material for further characterization of genes controlling critical yield parameters such as TTH, TCN, GY, SEED and TKW. Considering altogether the assessed yield parameters, line R112-4 has turned out to be the most promising genotype in terms of overall yield potential. In fact, compared to its control this line showed a wider flag leaf of the first culm (+10%), a higher

number of culms (+25%) and overall above-ground biomass (+30%), altogether significantly contributing to the observed higher grain yield (+25%). It is likely to hypothesize that more than one gene/QTL, located within the 28% 7AgL chromatin present in R112-4, controls the expression of these multiple traits. Several physiological assays did not show any involvement of the ABA hormone in the expression of such traits.

Overall, the results obtained confirm *Th. ponticum* as a valid source of useful genetic variability for wheat improvement and open up various possibilities for further investigations, including, in the forthcoming future: evaluation of yield potential of durum wheat recombinant lines in different years and environments; analysis of possible involvement of the root apparatus as a (further) yield-contributing factor; assessment of biochemical and physiological aspects of source-sink relations in carriers of different 7AgL segments.

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APPENDIX 1 - Abbreviations used in the evaluation of yield-related traits

The first culm analysis:

DTH	days to heading
FLW	flag leaf width
FLL	flag leaf length
FL-1W	flag leaf-1 width
FL-1L	flag leaf-1 length
HUPE	height up to ear
EL	ear length
TH	total height
SNE	seed number per ear
SPNE	spikelet number per ear
SPS	seeds per spikelet

Whole plant analysis

TTH	total tiller number at heading
TCN	total culm number at harvest
PRCN	productive culm number at harvest
PRGY	grain yield of productive culms
GYxPRC	grain yield per productive culm
PRCSEED	seed number of productive culms
SEEDxPRC	seed number per productive culm
PRCTKW	thousand kernel weight of productive culms,
SEED	seed number of entire plant

For both analysis:

GY	grain yield
TKW	thousand kernel weight
B	biomass production
HI	harvest index

APPENDIX 2 – Measurements of yield related traits of the first culm per treatment and per year

2009 ABA

Genotype	DTH	FLW	FLL	FL-1W	FL-1L	HUPE	EL	TH	SNE	SPNE	SPS	GY	TKW	N
R5-2-10 hom+	**89.63**	**18.80**	**281.63**	**14.24**	**289.22**	**568.71**	**60.87**	**629.58**	**46.95**	**14.34**	**3.27**	**2.77**	**59.35**	**12**
SE	0.60	0.44	9.29	0.41	7.43	18.23	1.55	18.53	4.11	0.36	0.26	0.23	2.22	
R5-2-10 hom-	**90.56**	**16.96**	**293.46**	**14.13**	**281.10**	**543.20**	**57.74**	**600.94**	**33.35**	**12.95**	**2.58**	**1.95**	**56.17**	**5**
SE	0.90	0.62	12.58	0.56	10.05	24.67	2.10	25.08	5.56	0.49	0.35	0.31	3.00	
R112-4 hom+	**94.80**	**19.61**	**255.42**	**14.45**	**292.99**	**564.31**	**57.60**	**621.91**	**37.23**	**13.77**	**2.69**	**2.07**	**55.76**	**10**
SE	0.67	0.47	9.44	0.42	7.55	18.53	1.57	18.83	4.17	0.37	0.26	0.23	2.25	
R112-4 hom-	**90.47**	**17.48**	**267.43**	**14.66**	**295.88**	**567.85**	**58.54**	**626.40**	**46.47**	**14.31**	**3.25**	**2.71**	**57.60**	**12**
SE	0.62	0.43	8.77	0.39	7.01	17.20	1.46	17.48	3.87	0.34	0.25	0.22	2.09	
R23-1 hom+	**96.78**	**17.60**	**267.38**	**13.78**	**294.66**	**560.27**	**63.36**	**623.63**	**41.07**	**13.97**	**2.92**	**1.66**	**39.99**	**15**
SE	0.56	0.39	7.38	0.36	6.54	15.57	1.32	15.82	3.51	0.31	0.22	0.20	1.89	
R23-1 hom-	**97.44**	**20.14**	**292.14**	**15.30**	**325.64**	**592.75**	**70.77**	**663.52**	**40.23**	**15.63**	**2.58**	**1.88**	**46.41**	**15**
SE	0.57	0.39	7.97	0.35	6.37	15.63	1.33	15.89	3.52	0.31	0.22	0.20	1.90	
T4	**101.50**	**16.57**	**237.28**	**14.83**	**261.51**	**735.71**	**88.78**	**824.48**	**39.68**	**15.04**	**2.62**	**1.19**	**30.24**	**4**
SE	0.81	0.85	19.24	0.57	13.93	55.25	2.68	55.34	4.17	0.49	0.22	0.23	2.60	
Thatcher	**99.50**	**16.57**	**239.53**	**13.48**	**281.26**	**654.46**	**88.53**	**742.98**	**38.93**	**15.79**	**2.48**	**1.21**	**31.22**	**4**
SE	0.81	0.85	19.24	0.57	13.93	55.25	2.68	55.34	4.17	0.49	0.22	0.23	2.60	
													Total	77

2010 ABA

Genotype	DTH	FLW	FLL	FL-1W	FL-1L	HUPE	EL	TH	SNE	SPNE	SPS	GY	TKW	B	HI	N
R5-2-10 hom+	**98.64**	**19.49**	**284.40**	**15.21**	**296.74**	**573.69**	**66.01**	**639.70**	**56.04**	**13.63**	**4.05**	**2.75**	**49.08**	**6.79**	**0.40**	**15**
SE	0.55	0.38	7.71	0.36	6.56	15.12	1.28	15.36	3.41	0.30	0.22	0.19	1.84	0.29	0.03	
R5-2-10 hom-	**97.68**	**19.52**	**290.23**	**14.17**	**289.88**	**599.50**	**64.01**	**663.51**	**55.46**	**12.54**	**4.35**	**2.91**	**52.30**	**6.87**	**0.42**	**15**
SE	0.55	0.38	7.71	0.34	6.20	15.12	1.28	15.37	3.41	0.30	0.22	0.19	1.84	0.29	0.03	
R112-4 hom+	**100.82**	**19.44**	**294.40**	**14.73**	**294.18**	**554.90**	**62.19**	**617.09**	**52.17**	**12.56**	**4.10**	**2.54**	**48.11**	**6.47**	**0.38**	**15**
SE	0.55	0.38	7.71	0.35	6.22	15.13	1.29	15.38	3.41	0.30	0.22	0.19	1.84	0.29	0.03	
R112-4 hom-	**99.86**	**18.06**	**279.81**	**13.98**	**268.48**	**499.15**	**61.30**	**560.45**	**49.59**	**12.44**	**3.93**	**2.35**	**46.42**	**5.99**	**0.37**	**14**
SE	0.56	0.36	7.94	0.39	7.01	15.57	1.32	15.82	3.51	0.31	0.22	0.20	1.89	0.31	0.03	
R23-1 hom+	**105.43**	**18.11**	**270.35**	**14.23**	**301.55**	**634.75**	**74.67**	**709.42**	**54.23**	**14.77**	**3.77**	**2.16**	**40.01**	**6.50**	**0.33**	**24**
SE	**0.44**	0.31	6.20	0.28	4.98	12.17	1.03	12.37	2.74	0.24	0.17	0.15	1.48	0.23	0.02	
R23-1 hom-	**104.71**	**19.55**	**303.88**	**15.07**	**313.78**	**626.33**	**77.34**	**703.67**	**45.04**	**15.71**	**2.91**	**2.43**	**53.18**	**6.50**	**0.32**	**36**
SE	0.37	0.26	5.21	0.23	4.17	10.22	0.87	10.38	2.30	0.20	0.15	0.13	1.24	0.19	0.02	
T4	**110.49**	**17.82**	**231.81**	**16.21**	**266.89**	**955.30**	**114.56**	**1069.86**	**66.65**	**19.21**	**3.48**	**2.21**	**33.17**	**6.87**	**0.32**	**14**
SE	0.43	0.45	10.13	0.30	7.33	29.09	1.41	29.14	2.19	0.26	0.12	0.12	1.37	0.25	0.01	
Thatcher	**104.93**	**20.40**	**280.13**	**16.67**	**320.73**	**889.87**	**119.27**	**1009.13**	**70.07**	**19.27**	**3.65**	**2.68**	**38.10**	**7.10**	**0.38**	**15**
SE	0.41	0.43	9.77	0.29	7.08	28.07	1.36	28.11	2.12	0.25	0.11	0.12	1.32	0.24	0.01	
															Total	148

2009 CONTROL

Genotype	DTH	FLW	FLL	FL-1W	FL-1L	HUPE	EL	TH	SNE	SPNE	SPS	GY	TKW	N
R5-2-10 hom+	**90.22**	**18.80**	**293.76**	**15.07**	**300.68**	**555.28**	**63.50**	**618.78**	**44.66**	**14.94**	**2.98**	**2.65**	**59.51**	**12**
SE	0.64	0.44	8.95	0.40	7.15	17.55	1.49	17.83	3.95	0.35	0.25	0.22	2.13	
R5-2-10 hom-	**88.68**	**17.41**	**297.10**	**15.28**	**306.97**	**565.74**	**57.54**	**623.28**	**40.73**	**13.85**	**2.91**	**2.40**	**58.75**	**5**
SE	0.97	0.62	12.59	0.56	10.06	24.70	2.10	25.10	5.56	0.49	0.35	0.31	3.00	
R112-4 hom+	**93.09**	**20.55**	**273.88**	**15.86**	**310.18**	**567.26**	**60.10**	**627.36**	**40.89**	**14.73**	**2.76**	**2.39**	**59.14**	**10**
SE	0.67	0.47	9.44	0.42	7.55	18.52	1.57	18.82	4.17	0.37	0.26	0.23	2.25	
R112-4 hom-	**90.90**	**18.42**	**264.75**	**15.24**	**316.01**	**547.01**	**58.47**	**632.48**	**47.91**	**14.84**	**3.22**	**2.73**	**56.95**	**11**
SE	0.64	0.45	9.09	0.40	7.27	17.84	1.51	18.13	4.02	0.35	0.25	0.23	2.17	
R23-1 hom+	**96.80**	**18.34**	**270.39**	**14.05**	**311.14**	**566.36**	**65.54**	**631.90**	**39.95**	**14.80**	**2.70**	**1.54**	**38.19**	**13**
SE	0.60	0.42	8.41	0.37	6.72	16.50	1.40	16.77	3.72	0.33	0.24	0.21	2.01	
R23-1 hom-	**96.14**	**21.37**	**304.90**	**17.02**	**346.49**	**595.76**	**71.02**	**666.78**	**46.45**	**16.30**	**2.84**	**2.33**	**50.94**	**15**
SE	0.58	0.40	8.14	0.36	6.51	15.97	1.36	16.23	3.60	0.32	0.23	0.21	2.00	
T4	**102.00**	**16.33**	**255.67**	**14.00**	**304.67**	**671.67**	**88.67**	**760.33**	**35.67**	**15.67**	**2.29**	**1.05**	**29.31**	**3**
SE	0.92	0.97	21.85	0.65	15.82	62.76	3.04	62.87	4.73	0.55	0.25	0.26	2.95	
Thatcher	**100.00**	**18.00**	**305.33**	**14.67**	**327.00**	**692.00**	**90.33**	**782.33**	**46.33**	**16.67**	**2.79**	**1.52**	**32.66**	**3**
SE	0.92	0.97	21.85	0.65	15.82	62.76	3.04	62.87	4.73	0.55	0.25	0.26	2.95	
													Total	72

2010 CONTROL

Genotype	DTH	FLW	FLL	FL-1W	FL-1L	HUPE	EL	TH	SNE	SPNE	SPS	GY	TKW	B	HI	N
R5-2-10 hom+	**98.81**	**20.20**	**280.02**	**16.46**	**305.26**	**599.67**	**70.83**	**670.50**	**60.85**	**15.22**	**4.04**	**3.19**	**52.89**	**7.63**	**0.42**	**15**
SE	0.55	0.38	7.70	0.35	6.37	15.10	1.28	15.35	3.40	0.30	0.22	0.19	1.84	0.29	0.03	
R5-2-10 hom-	**97.55**	**20.70**	**287.08**	**15.90**	**300.52**	**579.88**	**67.59**	**647.47**	**54.31**	**14.64**	**3.78**	**2.56**	**46.84**	**6.73**	**0.38**	**13**
SE	**0.58**	0.40	8.13	0.37	6.75	15.95	1.35	16.21	3.59	0.32	0.23	0.20	1.94	0.32	0.03	
R112-4 hom+	**101.47**	**20.83**	**284.21**	**16.57**	**293.17**	**549.74**	**65.72**	**615.46**	**55.95**	**14.82**	**3.82**	**2.82**	**50.21**	**7.18**	**0.40**	**14**
SE	0.56	0.39	7.92	0.35	6.39	15.53	1.32	15.79	3.50	0.31	0.22	0.20	1.89	0.30	0.03	
R112-4 hom-	**99.24**	**18.55**	**282.46**	**15.12**	**288.65**	**510.68**	**66.17**	**576.85**	**49.46**	**13.87**	**3.62**	**2.15**	**42.66**	**6.23**	**0.35**	**15**
SE	0.56	0.39	7.92	0.38	6.78	15.53	1.32	15.78	3.50	0.31	0.22	0.20	1.89	0.30	0.03	
R23-1 hom+	**105.36**	**18.81**	**267.54**	**15.46**	**303.22**	**644.74**	**79.04**	**723.77**	**56.36**	**16.37**	**3.45**	**2.24**	**38.22**	**6.55**	**0.32**	**23**
SE	0.46	0.31	6.31	0.28	5.07	12.39	1.05	12.59	2.70	0.25	0.18	0.16	1.51	0.24	0.02	
R23-1 hom-	**104.74**	**20.74**	**299.15**	**16.82**	**331.27**	**622.70**	**81.37**	**704.08**	**46.57**	**17.80**	**2.65**	**2.44**	**53.32**	**7.56**	**0.31**	**33**
SE	0.38	0.27	5.42	0.24	4.40	10.62	0.90	10.80	2.39	0.21	0.15	0.13	1.29	0.20	0.02	
T4	**110.47**	**18.80**	**225.13**	**16.56**	**271.13**	**999.20**	**114.40**	**1113.60**	**69.20**	**20.2**	**3.43**	**2.31**	**33.31**	**7.22**	**0.32**	**15**
SE	0.41	0.43	9.77	0.30	7.33	28.07	1.36	28.11	2.12	0.25	0.11	0.12	1.32	0.24	0.01	
Thatcher	**106.07**	**20.20**	**263.20**	**17.73**	**324.33**	**960.27**	**121.80**	**1082.07**	**73.13**	**20.73**	**3.53**	**2.66**	**36.63**	**7.12**	**0.38**	**15**
SE	0.41	0.43	9.77	0.29	7.08	28.07	1.36	28.11	2.12	0.247	0.11	0.12	1.32	0.24	0.01	
															Total	143

APPENDIX 3 – Measurements of yield related traits of the whole plant per year

2009

Genotype	TTH	TCN	PRCN	PRGY (g)	GYxPRC (g)	PRCSEED	SEED xPRC	PRCTKW (g)	GY (g)	SEED	TKW (g)	N
R5-2-10 hom+	14.93	18.15	13.58	28.52	2.10	494.24	36.76	57.23	35.07	639.75	54.57	**19**
SE	0.85	0.84	0.60	1.54	0.09	28.35	1.52	1.23	1.73	34.37	1.29	
R5-2-10 hom-	13.68	16.02	12.43	26.96	2.22	454.34	37.59	59.00	34.39	582.49	56.69	**9**
SE	1.24	1.23	0.88	2.24	0.13	41.31	2.21	1.80	2.53	57.12	2.15	
R112-4 hom+	18.05	18.60	15.16	27.42	1.85	523.67	35.46	52.55	31.96	636.47	50.69	**16**
SE	0.93	0.92	0.66	1.68	0.10	30.90	1.66	1.34	1.89	37.45	1.41	
R112-4 hom-	13.94	15.38	13.23	28.26	2.13	516.20	39.04	54.39	33.07	622.07	53.16	**18**
SE	0.88	0.87	0.62	1.58	0.09	29.16	1.56	1.27	1.78	35.35	1.33	
R23-1 hom+	18.34	16.47	12.42	15.12	1.22	413.88	33.51	36.53	18.50	523.93	44.99	**27**
SE	0.72	0.71	0.51	1.30	0.08	23.86	1.28	1.04	1.46	28.92	1.09	
R23-1 hom-	16.92	15.70	13.27	20.91	1.55	428.23	32.18	47.92	23.93	503.83	55.09	**30**
SE	0.70	0.69	0.49	1.26	0.08	23.18	1.24	1.01	1.42	28.12	1.06	
T4	19.06	26.71	18.46	12.00	0.71	563.65	32.54	20.54	16.47	757.54	21.16	**7**
SE	1.85	1.98	1.68	2.64	0.13	76.86	2.84	2.18	2.73	81.03	1.82	
Thatcher	16.64	22.99	18.74	14.01	0.74	620.58	32.81	22.80	17.41	751.75	23.16	**7**
SE	1.85	1.98	1.68	2.64	0.13	76.86	2.84	2.18	2.73	81.03	1.82	
											Total	133

2010

Genotype	TTH	TCN	PRCN	PRGY (g)	GYxPRC (g)	PRCSEED	SEEDx PRC	PRCTKW (g)	GY (g)	SEED	TKW (g)	B (g)	HI	N
R5-2-10 hom+	15.75	15.80	10.82	28.60	2.65	580.19	53.69	49.35	36.42	752.70	48.81	82.73	0.44	**23**
SE	0.78	0.77	0.55	1.40	0.08	25.82	1.38	1.12	1.58	32.00	1.20	3.80	0.01	
R5-2-10 hom-	15.75	15.02	9.42	24.49	2.63	502.57	53.69	48.38	31.83	673.22	46.96	74.32	0.43	**17**
SE	0.91	0.90	0.64	1.64	0.10	30.18	1.62	1.31	1.85	36.58	1.37	4.44	0.01	
R112-4 hom+	19.72	19.06	10.95	26.75	2.43	561.24	51.21	47.39	35.74	793.12	45.12	86.04	0.41	**18**
SE	0.88	0.87	0.62	1.59	0.10	29.26	1.57	1.27	1.79	35.47	1.33	4.30	0.01	
R112-4 hom-	15.61	14.61	9.29	20.04	2.18	460.21	49.59	43.84	26.66	624.43	43.06	66.38	0.41	**19**
SE	0.85	0.84	0.61	1.54	0.09	28.42	1.52	1.24	1.74	34.44	1.29	4.18	0.01	
R23-1 hom+	17.65	16.70	11.55	24.58	2.14	629.53	54.68	39.12	31.41	822.76	38.33	82.88	0.38	**30**
SE	0.68	0.67	0.48	1.23	0.07	22.57	1.21	0.98	1.38	27.36	1.03	3.32	0.01	
R23-1 hom-	14.82	13.65	9.23	22.77	2.51	457.18	50.26	49.77	30.28	612.47	49.57	76.08	0.39	**36**
SE	0.62	0.61	0.44	1.12	0.07	20.64	1.11	0.90	1.26	25.01	0.94	3.03	0.01	
T4	20.33	19.87	15.56	23.67	1.56	783.96	51.48	30.19	28.93	953.41	30.42	88.74	0.33	**19**
SE	1.13	1.20	1.02	1.60	0.08	46.72	1.72	1.33	1.66	49.25	1.11	4.47	0.01	
Thatcher	20.08	18.67	14.36	23.64	1.67	746.54	52.27	31.90	29.58	927.14	32.11	85.13	0.35	**25**
SE	0.98	1.07	0.89	1.40	0.07	40.76	1.50	1.16	1.45	42.97	0.96	3.90	0.01	
													Total	187

APPENDIX 4 – ANOVA summary tables of yield-related traits analysis (GLM)

The 1st culm data from two years and 3 durum wheat lines (R5-2-10, R112-4, R23-1)

Dependent Variable	DTH
N	362
Multiple R	0.915
Squared Multiple R	0.837

Analysis of Variance (DTH)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	2698.265	2	1349.133	248.367	0.000
YEAR	4855.383	1	4855.383	893.844	0.000
TREATMENT	7.843	1	7.843	1.444	0.230
YEAR*GENOTYPE$	1.412	2	0.706	0.130	0.878
TREATMENT*YEAR	8.666	1	8.666	1.595	0.207
_7AGL(GENOTYPE$)	155.852	3	51.951	9.564	0.000
REPLICA(YEAR)	21.338	4	5.334	0.982	0.417
YEAR*_7AGL(GENOTYPE$)	23.697	3	7.899	1.454	0.227
TREATMENT*_7AGL(GENOTYPE$)	14.741	3	4.914	0.905	0.439
TREATMENT*YEAR*_7AGL(GENOTYPE$)	27.951	3	9.317	1.715	0.164
Error	1836.024	338	5.432		

Dependent Variable	TTH
N	364
Multiple R	0.369
Squared Multiple R	0.137

Analysis of Variance (TTH)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	127.705	2	63.853	3.495	0.031
YEAR	0.028	1	0.028	0.002	0.969
TREATMENT	4.318	1	4.318	0.236	0.627
YEAR*GENOTYPE$	129.015	2	64.508	3.531	0.030
TREATMENT*YEAR	15.801	1	15.801	0.865	0.353
_7AGL(GENOTYPE$)	537.786	3	179.262	9.813	0.000
REPLICA(YEAR)	22.190	4	5.547	0.304	0.875
YEAR*_7AGL(GENOTYPE$)	24.628	3	8.209	0.449	0.718
TREATMENT*_7AGL(GENOTYPE$)	28.486	3	9.495	0.520	0.669
TREATMENT*YEAR*_7AGL(GENOTYPE$)	101.083	3	33.694	1.844	0.139
Error	6211.366	340	18.269		

	FLW
N	365
Multiple R	0.596
Squared Multiple R	0.355

Analysis of Variance (FLW)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	14.685	2	7.342	2.768	0.064
YEAR	49.260	1	49.260	18.570	0.000
TREATMENT	72.319	1	72.319	27.263	0.000
YEAR*GENOTYPE$	78.431	2	39.216	14.784	0.000
TREATMENT*YEAR	0.000	1	0.000	0.000	0.995
_7AGL(GENOTYPE$)	281.950	3	93.983	35.430	0.000
REPLICA(YEAR)	15.984	4	3.996	1.506	0.200
YEAR*_7AGL(GENOTYPE$)	17.443	3	5.814	2.192	0.089
TREATMENT*_7AGL(GENOTYPE$)	4.364	3	1.455	0.548	0.650
TREATMENT*YEAR*_7AGL(GENOTYPE$)	3.966	3	1.322	0.498	0.684
Error	904.552	341	2.653		

Dependent Variable	FLL
N	365
Multiple R	0.396
Squared Multiple R	0.157

Analysis of Variance (FLL)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	8303.484	2	4151.742	3.833	0.023
YEAR	1920.409	1	1920.409	1.773	0.184
TREATMENT	346.952	1	346.952	0.320	0.572
YEAR*GENOTYPE$	8115.085	2	4057.543	3.746	0.025
TREATMENT*YEAR	2777.507	1	2777.507	2.564	0.110
_7AGL(GENOTYPE$)	37586.434	3	12528.811	11.568	0.000
REPLICA(YEAR)	862.983	4	215.746	0.199	0.939
YEAR*_7AGL(GENOTYPE$)	651.237	3	217.079	0.200	0.896
TREATMENT*_7AGL(GENOTYPE$)	318.885	3	106.295	0.098	0.961
TREATMENT*YEAR*_7AGL(GENOTYPE$)	2211.270	3	737.090	0.681	0.564
Error	369325.444	341	1083.066		

Dependent Variable	FL_1W
N	354
Multiple R	0.576
Squared Multiple R	0.332

Analysis of Variance (FL-1W)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	1.698	2	0.849	0.400	0.671
YEAR	10.713	1	10.713	5.049	0.025
TREATMENT	123.106	1	123.106	58.019	0.000
YEAR*GENOTYPE$	5.119	2	2.559	1.206	0.301
TREATMENT*YEAR	4.972	1	4.972	2.343	0.127
_7AGL(GENOTYPE$)	116.764	3	38.921	18.343	0.000
REPLICA(YEAR)	27.772	4	6.943	3.272	0.012
YEAR*_7AGL(GENOTYPE$)	20.164	3	6.721	3.168	0.025
TREATMENT*_7AGL(GENOTYPE$)	13.306	3	4.435	2.090	0.101
TREATMENT*YEAR*_7AGL(GENOTYPE$)	2.094	3	0.698	0.329	0.804
Error	700.201	330	2.122		

Dependent Variable	FL_1L
N	354
Multiple R	0.556
Squared Multiple R	0.309

Analysis of Variance (FL-1L)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	32837.131	2	16418.565	23.746	0.000
YEAR	3527.401	1	3527.401	5.102	0.025
TREATMENT	15951.395	1	15951.395	23.071	0.000
YEAR*GENOTYPE$	4658.593	2	2329.296	3.369	0.036
TREATMENT*YEAR	1649.305	1	1649.305	2.385	0.123
_7AGL(GENOTYPE$)	27315.864	3	9105.288	13.169	0.000
REPLICA(YEAR)	4676.052	4	1169.013	1.691	0.152
YEAR*_7AGL(GENOTYPE$)	3886.125	3	1295.375	1.874	0.134
TREATMENT*_7AGL(GENOTYPE$)	2153.902	3	717.967	1.038	0.376
TREATMENT*YEAR*_7AGL(GENOTYPE$)	978.151	3	326.050	0.472	0.702
Error	228166.292	330	691.413		

Dependent Variable	HUPE
N	365
Multiple R	0.533
Squared Multiple R	0.284

Analysis of Variance (HUPE)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	197580.086	2	98790.043	23.703	0.000
YEAR	15523.881	1	15523.881	3.725	0.054
TREATMENT	1224.527	1	1224.527	0.294	0.588
YEAR*GENOTYPE$	130558.783	2	65279.391	15.662	0.000
TREATMENT*YEAR	38.690	1	38.690	0.009	0.923
_7AGL(GENOTYPE$)	13322.779	3	4440.926	1.066	0.364
REPLICA(YEAR)	41426.710	4	10356.677	2.485	0.043
YEAR*_7AGL(GENOTYPE$)	37442.194	3	12480.731	2.994	0.031
TREATMENT*_7AGL(GENOTYPE$)	1386.575	3	462.192	0.111	0.954
TREATMENT*YEAR*_7AGL(GENOTYPE$)	9049.133	3	3016.378	0.724	0.538
Error	1421257.334	341	4167.910		

Dependent Variable	EL
N	365
Multiple R	0.818
Squared Multiple R	0.670

Analysis of Variance (EL)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	9335.151	2	4667.576	155.386	0.000
YEAR	4250.751	1	4250.751	141.510	0.000
TREATMENT	599.675	1	599.675	19.964	0.000
YEAR*GENOTYPE$	429.601	2	214.801	7.151	0.001
TREATMENT*YEAR	182.803	1	182.803	6.086	0.014
_7AGL(GENOTYPE$)	1000.557	3	333.519	11.103	0.000
REPLICA(YEAR)	168.664	4	42.166	1.404	0.232
YEAR*_7AGL(GENOTYPE$)	164.397	3	54.799	1.824	0.142
TREATMENT*_7AGL(GENOTYPE$)	35.845	3	11.948	0.398	0.755
TREATMENT*YEAR*_7AGL(GENOTYPE$)	32.893	3	10.964	0.365	0.778
Error	10243.138	341	30.039		

Dependent Variable	TH
N	365
Multiple R	0.582
Squared Multiple R	0.339

Analysis of Variance (TH)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	290764.725	2	145382.362	33.773	0.000
YEAR	36021.249	1	36021.249	8.368	0.004
TREATMENT	3538.050	1	3538.050	0.822	0.365
YEAR*GENOTYPE$	145033.587	2	72516.794	16.846	0.000
TREATMENT*YEAR	53.294	1	53.294	0.012	0.911
_7AGL(GENOTYPE$)	17578.978	3	5859.659	1.361	0.254
REPLICA(YEAR)	46339.446	4	11584.861	2.691	0.031
YEAR*_7AGL(GENOTYPE$)	41154.183	3	13718.061	3.187	0.024
TREATMENT*_7AGL(GENOTYPE$)	1628.826	3	542.942	0.126	0.945
TREATMENT*YEAR*_7AGL(GENOTYPE$)	8822.997	3	2940.999	0.683	0.563
Error	1467921.025	341	4304.754		

Dependent Variable	SNE
N	365
Multiple R	0.416
Squared Multiple R	0.173

Analysis of Variance (SNE)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	401.557	2	200.778	0.949	0.388
YEAR	8661.294	1	8661.294	40.958	0.000
TREATMENT	392.302	1	392.302	1.855	0.174
YEAR*GENOTYPE$	629.847	2	314.924	1.489	0.227
TREATMENT*YEAR	10.772	1	10.772	0.051	0.822
_7AGL(GENOTYPE$)	1202.323	3	400.774	1.895	0.130
REPLICA(YEAR)	446.795	4	111.699	0.528	0.715
YEAR*_7AGL(GENOTYPE$)	2538.070	3	846.023	4.001	0.008
TREATMENT*_7AGL(GENOTYPE$)	183.532	3	61.177	0.289	0.833
TREATMENT*YEAR*_7AGL(GENOTYPE$)	468.836	3	156.279	0.739	0.529
Error	72109.838	341	211.466		

Dependent Variable	SPNE
N	365
Multiple R	0.757
Squared Multiple R	0.573

Analysis of Variance (SPNE)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	235.541	2	117.771	72.447	0.000
YEAR	0.002	1	0.002	0.002	0.969
TREATMENT	137.155	1	137.155	84.371	0.000
YEAR*GENOTYPE$	58.152	2	29.076	17.886	0.000
TREATMENT*YEAR	24.474	1	24.474	15.055	0.000
_7AGL(GENOTYPE$)	92.942	3	30.981	19.058	0.000
REPLICA(YEAR)	3.889	4	0.972	0.598	0.664
YEAR*_7AGL(GENOTYPE$)	6.830	3	2.277	1.401	0.242
TREATMENT*_7AGL(GENOTYPE$)	3.512	3	1.171	0.720	0.541
TREATMENT*YEAR*_7AGL(GENOTYPE$)	1.329	3	0.443	0.273	0.845
Error	554.335	341	1.626		

Dependent Variable	SPS
N	365
Multiple R	0.533
Squared Multiple R	0.284

Analysis of Variance (SPS)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	18.451	2	9.225	10.942	0.000
YEAR	48.910	1	48.910	58.011	0.000
TREATMENT	1.493	1	1.493	1.771	0.184
YEAR*GENOTYPE$	6.739	2	3.370	3.997	0.019
TREATMENT*YEAR	1.987	1	1.987	2.356	0.126
_7AGL(GENOTYPE$)	9.448	3	3.149	3.735	0.012
REPLICA(YEAR)	2.455	4	0.614	0.728	0.573
YEAR*_7AGL(GENOTYPE$)	8.632	3	2.877	3.413	0.018
TREATMENT*_7AGL(GENOTYPE$)	0.704	3	0.235	0.278	0.841
TREATMENT*YEAR*_7AGL(GENOTYPE$)	2.244	3	0.748	0.887	0.448
Error	287.499	341	0.843		

Dependent Variable	GY
N	364
Multiple R	0.431
Squared Multiple R	0.186

Analysis of Variance (GY)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	18.370	2	9.185	13.837	0.000
YEAR	6.064	1	6.064	9.136	0.003
TREATMENT	0.904	1	0.904	1.362	0.244
YEAR*GENOTYPE$	3.659	2	1.830	2.756	0.065
TREATMENT*YEAR	0.312	1	0.312	0.469	0.494
_7AGL(GENOTYPE$)	7.934	3	2.645	3.984	0.008
REPLICA(YEAR)	1.153	4	0.288	0.434	0.784
YEAR*_7AGL(GENOTYPE$)	6.177	3	2.059	3.102	0.027
TREATMENT*_7AGL(GENOTYPE$)	1.552	3	0.517	0.779	0.506
TREATMENT*YEAR*_7AGL(GENOTYPE$)	3.339	3	1.113	1.677	0.172
Error	225.686	340	0.664		

Dependent Variable	TKW
N	364
Multiple R	0.666
Squared Multiple R	0.444

Analysis of Variance (TKW)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	5412.265	2	2706.132	43.981	0.000
YEAR	2189.445	1	2189.445	35.584	0.000
TREATMENT	5.990	1	5.990	0.097	0.755
YEAR*GENOTYPE$	2839.433	2	1419.717	23.074	0.000
TREATMENT*YEAR	97.718	1	97.718	1.588	0.208
_7AGL(GENOTYPE$)	5437.073	3	1812.358	29.455	0.000
REPLICA(YEAR)	596.893	4	149.223	2.425	0.048
YEAR*_7AGL(GENOTYPE$)	314.669	3	104.890	1.705	0.166
TREATMENT*_7AGL(GENOTYPE$)	370.793	3	123.598	2.009	0.112
TREATMENT*YEAR*_7AGL(GENOTYPE$)	225.411	3	75.137	1.221	0.302
Error	20920.066	340	61.530		

Biomass and HI of the 1st culm in 2010 and 3 durum wheat lines (R5-2-10, R112-4, R23-1)

Dependent Variable	B
N	231
Multiple R	0.435
Squared Multiple R	0.189

Analysis of Variance (B)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	12.202	2	6.101	4.717	0.010
TREATMENT	5.000	1	5.000	3.866	0.051
REPLICA	4.967	2	2.484	1.920	0.149
TREATMENT*GENOTYPE$	1.448	2	0.724	0.560	0.572
_7AGL(GENOTYPE$)	34.906	3	11.635	8.996	0.000
TREATMENT*_7AGL(GENOTYPE$)	4.307	3	1.436	1.110	0.346
Error	280.655	217	1.293		

Dependent Variable	HI
N	231
Multiple R	0.384
Squared Multiple R	0.148

Analysis of Variance (HI)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.297	2	0.149	15.215	0.000
TREATMENT	0.005	1	0.005	0.526	0.469
REPLICA	0.000	2	0.000	0.016	0.984
TREATMENT*GENOTYPE$	0.002	2	0.001	0.089	0.914
_7AGL(GENOTYPE$)	0.019	3	0.006	0.652	0.582
TREATMENT*_7AGL(GENOTYPE$)	0.023	3	0.008	0.779	0.507
Error	2.121	217	0.010		

The 1st culm data for two years and 2 durum wheat lines (R5-2-10, R112-4)

Dependent Variable	DTH
N	181
Multiple R	0.959
Squared Multiple R	0.920

Analysis of Variance (DTH)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	225.659	1	225.659	126.578	0.000
YEAR	2716.656	1	2716.656	1523.843	0.000
TREATMENT	0.030	1	0.030	0.017	0.897
YEAR*GENOTYPE$	0.599	1	0.599	0.336	0.563
TREATMENT*YEAR	1.982	1	1.982	1.112	0.293
_7AGL(GENOTYPE$)	135.763	2	67.882	38.077	0.000
REPLICA(YEAR)	2.768	4	0.692	0.388	0.817
YEAR*_7AGL(GENOTYPE$)	20.425	2	10.212	5.728	0.004
TREATMENT*_7AGL(GENOTYPE$)	8.561	2	4.280	2.401	0.094
TREATMENT*YEAR*_7AGL(GENOTYPE$)	15.975	2	7.987	4.480	0.013
Error	290.591	163	1.783		

Dependent Variable	TTH
N	182
Multiple R	0.410
Squared Multiple R	0.168

Analysis of Variance (TTH)

Source	Te III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	99.648	1	99.648	5.895	0.016
YEAR	50.560	1	50.560	2.991	0.086
TREATMENT	10.309	1	10.309	0.610	0.436
YEAR*GENOTYPE$	1.745	1	1.745	0.103	0.748
TREATMENT*YEAR	10.979	1	10.979	0.649	0.421
_7AGL(GENOTYPE$)	323.327	2	161.664	9.563	0.000
REPLICA(YEAR)	27.112	4	6.778	0.401	0.808
YEAR*_7AGL(GENOTYPE$)	47.495	2	23.747	1.405	0.248
TREATMENT*_7AGL(GENOTYPE$)	10.295	2	5.148	0.305	0.738
TREATMENT*YEAR*_7AGL(GENOTYPE$)	1.026	2	0.513	0.030	0.970
Error	2772.306	164	16.904		

Dependent Variable	FLW
N	182
Multiple R	0.662
Squared Multiple R	0.438

Analysis of Variance (FLW)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	3.751	1	3.751	1.820	0.179
YEAR	63.871	1	63.871	30.995	0.000
TREATMENT	44.146	1	44.146	21.423	0.000
YEAR*GENOTYPE$	46.654	1	46.654	22.640	0.000
TREATMENT*YEAR	1.372	1	1.372	0.666	0.416
_7AGL(GENOTYPE$)	94.729	2	47.364	22.985	0.000
REPLICA(YEAR)	5.997	4	1.499	0.728	0.574
YEAR*_7AGL(GENOTYPE$)	7.307	2	3.654	1.773	0.173
TREATMENT*_7AGL(GENOTYPE$)	2.109	2	1.054	0.512	0.600
TREATMENT*YEAR*_7AGL(GENOTYPE$)	4.387	2	2.193	1.064	0.347
Error	337.953	164	2.061		

Dependent Variable	FLL
N	182
Multiple R	0.422
Squared Multiple R	0.178

Analysis of Variance (FLL)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	7023.703	1	7023.703	10.346	0.002
YEAR	1807.427	1	1807.427	2.662	0.105
TREATMENT	1392.317	1	1392.317	2.051	0.154
YEAR*GENOTYPE$	7120.684	1	7120.684	10.489	0.001
TREATMENT*YEAR	1859.528	1	1859.528	2.739	0.100
_7AGL(GENOTYPE$)	1173.945	2	586.972	0.865	0.423
REPLICA(YEAR)	1588.281	4	397.070	0.585	0.674
YEAR*_7AGL(GENOTYPE$)	568.456	2	284.228	0.419	0.659
TREATMENT*_7AGL(GENOTYPE$)	18.495	2	9.247	0.014	0.986
TREATMENT*YEAR*_7AGL(GENOTYPE$)	2445.013	2	1222.507	1.801	0.168
Error	111339.398	164	678.899		

Dependent Variable	FL_1W
N	182
Multiple R	0.639
Squared Multiple R	0.409

Analysis of Variance (FL-1W)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.004	1	0.004	0.003	0.955
YEAR	6.593	1	6.593	5.492	0.020
TREATMENT	61.598	1	61.598	51.308	0.000
YEAR*GENOTYPE$	5.067	1	5.067	4.220	0.042
TREATMENT*YEAR	4.359	1	4.359	3.631	0.058
_7AGL(GENOTYPE$)	12.404	2	6.202	5.166	0.007
REPLICA(YEAR)	16.397	4	4.099	3.414	0.010
YEAR*_7AGL(GENOTYPE$)	8.140	2	4.070	3.390	0.036
TREATMENT*_7AGL(GENOTYPE$)	3.837	2	1.918	1.598	0.205
TREATMENT*YEAR*_7AGL(GENOTYPE$)	0.075	2	0.037	0.031	0.969
Error	196.892	164	1.201		

Dependent Variable	FL_1L
N	182
Multiple R	0.455
Squared Multiple R	0.207

Analysis of Variance (FL-1L)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	90.303	1	90.303	0.182	0.670
YEAR	2059.903	1	2059.903	4.159	0.043
TREATMENT	8093.428	1	8093.428	16.341	0.000
YEAR*GENOTYPE$	4597.671	1	4597.671	9.283	0.003
TREATMENT*YEAR	430.045	1	430.045	0.868	0.353
_7AGL(GENOTYPE$)	870.321	2	435.160	0.879	0.417
REPLICA(YEAR)	2044.361	4	511.090	1.032	0.392
YEAR*_7AGL(GENOTYPE$)	2358.979	2	1179.490	2.381	0.096
TREATMENT*_7AGL(GENOTYPE$)	1210.835	2	605.418	1.222	0.297
TREATMENT*YEAR*_7AGL(GENOTYPE$)	649.112	2	324.556	0.655	0.521
Error	81226.225	164	495.282		

Dependent Variable	HUPE
N	182
Multiple R	0.496
Squared Multiple R	0.246

Analysis of Variance (HUPE)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	27457.554	1	27457.554	9.704	0.002
YEAR	1385.336	1	1385.336	0.490	0.485
TREATMENT	1259.409	1	1259.409	0.445	0.506
YEAR*GENOTYPE$	52480.050	1	52480.050	18.547	0.000
TREATMENT*YEAR	1587.816	1	1587.816	0.561	0.455
_7AGL(GENOTYPE$)	12040.282	2	6020.141	2.128	0.122
REPLICA(YEAR)	14884.138	4	3721.035	1.315	0.267
YEAR*_7AGL(GENOTYPE$)	17571.836	2	8785.918	3.105	0.047
TREATMENT*_7AGL(GENOTYPE$)	537.705	2	268.852	0.095	0.909
TREATMENT*YEAR*_7AGL(GENOTYPE$)	6029.338	2	3014.669	1.065	0.347
Error	464051.533	164	2829.583		

Dependent Variable	EL
N	182
Multiple R	0.745
Squared Multiple R	0.555

Analysis of Variance (EL)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	205.990	1	205.990	18.064	0.000
YEAR	1515.196	1	1515.196	132.876	0.000
TREATMENT	133.721	1	133.721	11.727	0.001
YEAR*GENOTYPE$	40.451	1	40.451	3.547	0.061
TREATMENT*YEAR	19.439	1	19.439	1.705	0.194
_7AGL(GENOTYPE$)	224.051	2	112.026	9.824	0.000
REPLICA(YEAR)	36.817	4	9.204	0.807	0.522
YEAR*_7AGL(GENOTYPE$)	9.748	2	4.874	0.427	0.653
TREATMENT*_7AGL(GENOTYPE$)	19.598	2	9.799	0.859	0.425
TREATMENT*YEAR*_7AGL(GENOTYPE$)	14.478	2	7.239	0.635	0.531
Error	1870.108	164	11.403		

Dependent Variable	TH
N	182
Multiple R	0.508
Squared Multiple R	0.258

Analysis of Variance (TH)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	32420.009	1	32420.009	11.420	0.001
YEAR	2.908	1	2.908	0.001	0.975
TREATMENT	2213.883	1	2213.883	0.780	0.378
YEAR*GENOTYPE$	55434.512	1	55434.512	19.526	0.000
TREATMENT*YEAR	1255.884	1	1255.884	0.442	0.507
_7AGL(GENOTYPE$)	12659.962	2	6329.981	2.230	0.111
REPLICA(YEAR)	16140.755	4	4035.189	1.421	0.229
YEAR*_7AGL(GENOTYPE$)	17485.070	2	8742.535	3.079	0.049
TREATMENT*_7AGL(GENOTYPE$)	515.274	2	257.637	0.091	0.913
TREATMENT*YEAR*_7AGL(GENOTYPE$)	5991.907	2	2995.954	1.055	0.350
Error	465593.741	164	2838.986		

Dependent Variable	SNE
N	182
Multiple R	0.633
Squared Multiple R	0.401

Analysis of Variance (SNE)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	73.037	1	73.037	0.863	0.354
YEAR	6185.732	1	6185.732	73.102	0.000
TREATMENT	176.332	1	176.332	2.084	0.151
YEAR*GENOTYPE$	395.256	1	395.256	4.671	0.032
TREATMENT*YEAR	0.971	1	0.971	0.011	0.915
_7AGL(GENOTYPE$)	845.854	2	422.927	4.998	0.008
REPLICA(YEAR)	504.798	4	126.200	1.491	0.207
YEAR*_7AGL(GENOTYPE$)	834.039	2	417.019	4.928	0.008
TREATMENT*_7AGL(GENOTYPE$)	182.537	2	91.268	1.079	0.342
TREATMENT*YEAR*_7AGL(GENOTYPE$)	316.941	2	158.471	1.873	0.157
Error	13877.234	164	84.617		

Dependent Variable	SPNE
N	182
Multiple R	0.682
Squared Multiple R	0.465

Analysis of Variance (SPNE)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.257	1	0.257	0.336	0.563
YEAR	9.157	1	9.157	11.939	0.001
TREATMENT	35.526	1	35.526	46.321	0.000
YEAR*GENOTYPE$	9.092	1	9.092	11.855	0.001
TREATMENT*YEAR	5.084	1	5.084	6.629	0.011
_7AGL(GENOTYPE$)	21.001	2	10.501	13.691	0.000
REPLICA(YEAR)	3.171	4	0.793	1.034	0.392
YEAR*_7AGL(GENOTYPE$)	3.886	2	1.943	2.534	0.082
TREATMENT*_7AGL(GENOTYPE$)	3.398	2	1.699	2.215	0.112
TREATMENT*YEAR*_7AGL(GENOTYPE$)	0.654	2	0.327	0.426	0.654
Error	125.781	164	0.767		

Dependent Variable	SPS
N	182
Multiple R	0.696
Squared Multiple R	0.485

Analysis of Variance (SPS)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.134	1	0.134	0.374	0.541
YEAR	42.846	1	42.846	119.329	0.000
TREATMENT	0.353	1	0.353	0.984	0.323
YEAR*GENOTYPE$	0.440	1	0.440	1.225	0.270
TREATMENT*YEAR	0.586	1	0.586	1.633	0.203
_7AGL(GENOTYPE$)	1.504	2	0.752	2.094	0.126
REPLICA(YEAR)	3.011	4	0.753	2.097	0.084
YEAR*_7AGL(GENOTYPE$)	2.770	2	1.385	3.857	0.023
TREATMENT*_7AGL(GENOTYPE$)	0.294	2	0.147	0.409	0.665
TREATMENT*YEAR*_7AGL(GENOTYPE$)	1.674	2	0.837	2.331	0.100
Error	58.885	164	0.359		

Dependent Variable	GY
N	182
Multiple R	0.486
Squared Multiple R	0.236

Analysis of Variance (GY)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.876	1	0.876	2.419	0.122
YEAR	2.004	1	2.004	5.530	0.020
TREATMENT	0.567	1	0.567	1.565	0.213
YEAR*GENOTYPE$	1.385	1	1.385	3.822	0.052
TREATMENT*YEAR	0.101	1	0.101	0.280	0.598
_7AGL(GENOTYPE$)	2.812	2	1.406	3.880	0.023
REPLICA(YEAR)	2.285	4	0.571	1.577	0.183
YEAR*_7AGL(GENOTYPE$)	4.069	2	2.035	5.616	0.004
TREATMENT*_7AGL(GENOTYPE$)	1.448	2	0.724	1.998	0.139
TREATMENT*YEAR*_7AGL(GENOTYPE$)	2.329	2	1.164	3.214	0.043
Error	59.420	164	0.362		

Dependent Variable	TKW
N	182
Multiple R	0.625
Squared Multiple R	0.390

Analysis of Variance (TKW)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	138.118	1	138.118	2.987	0.086
YEAR	3399.061	1	3399.061	73.521	0.000
TREATMENT	4.166	1	4.166	0.090	0.764
YEAR*GENOTYPE$	22.347	1	22.347	0.483	0.488
TREATMENT*YEAR	108.725	1	108.725	2.352	0.127
_7AGL(GENOTYPE$)	114.375	2	57.187	1.237	0.293
REPLICA(YEAR)	329.733	4	82.433	1.783	0.135
YEAR*_7AGL(GENOTYPE$)	61.084	2	30.542	0.661	0.518
TREATMENT*_7AGL(GENOTYPE$)	215.789	2	107.895	2.334	0.100
TREATMENT*YEAR*_7AGL(GENOTYPE$)	188.028	2	94.014	2.033	0.134
Error	7582.167	164	46.233		

Biomass and harvest index of the 1st culm data in 2010 and 2 durum wheat lines (R5-2-10, R112-4)

Dependent Variable	B
N	106
Multiple R	0.567
Squared Multiple R	0.322

Analysis of Variance (B)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	5.791	1	5.791	13.548	0.000
TREATMENT	2.787	1	2.787	6.522	0.012
REPLICA	0.423	2	0.212	0.495	0.611
_7AGL(GENOTYPE$)	6.473	2	3.236	7.572	0.001
TREATMENT*_7AGL(GENOTYPE$)	3.902	2	1.951	4.565	0.013
Error	41.458	97	0.427		

Dependent Variable	HI
N	106
Multiple R	0.426
Squared Multiple R	0.182

Analysis of Variance (HI)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.012	1	0.012	3.683	0.058
TREATMENT	0.006	1	0.006	1.999	0.161
REPLICA	0.010	2	0.005	1.596	0.208
_7AGL(GENOTYPE$)	0.005	2	0.003	0.820	0.443
TREATMENT*_7AGL(GENOTYPE$)	0.036	2	0.018	5.573	0.005
Error	0.312	97	0.003		

The 1st culm data for two years of bread wheat line T4

Dependent Variable	DTH
N	73
Multiple R	0.933
Squared Multiple R	0.871

Analysis of Variance (DTH)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	135.459	1	135.459	52.908	0.000
YEAR	569.832	1	569.832	222.567	0.000
TREATMENT	3.021	1	3.021	1.180	0.282
YEAR*GENOTYPE$	24.681	1	24.681	9.640	0.003
TREATMENT*GENOTYPE$	0.932	1	0.932	0.364	0.548
TREATMENT*YEAR	0.008	1	0.008	0.003	0.956
YEAR*TREATMENT*GENOTYPE$	0.932	1	0.932	0.364	0.548
REPLICA(YEAR)	48.423	4	12.106	4.728	0.002
Error	156.177	61	2.560		

Dependent Variable	FLW
N	73
Multiple R	0.699
Squared Multiple R	0.489

Analysis of Variance (FLW)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	22.151	1	22.151	7.910	0.007
YEAR	64.671	1	64.671	23.092	0.000
TREATMENT	2.658	1	2.658	0.949	0.334
YEAR*GENOTYPE$	3.714	1	3.714	1.326	0.254
TREATMENT*GENOTYPE$	0.166	1	0.166	0.059	0.808
TREATMENT*YEAR	0.121	1	0.121	0.043	0.836
YEAR*TREATMENT*GENOTYPE$	5.625	1	5.625	2.009	0.161
REPLICA(YEAR)	18.590	4	4.648	1.660	0.171
Error	170.833	61	2.801		

Dependent Variable	FLL
N	73
Multiple R	0.617
Squared Multiple R	0.380

Analysis of Variance (FLL)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	13300.543	1	13300.543	9.287	0.003
YEAR	956.974	1	956.974	0.668	0.417
TREATMENT	2494.971	1	2494.971	1.742	0.192
YEAR*GENOTYPE$	826.460	1	826.460	0.577	0.450
TREATMENT*GENOTYPE$	959.889	1	959.889	0.670	0.416
TREATMENT*YEAR	7897.616	1	7897.616	5.515	0.022
YEAR*TREATMENT*GENOTYPE$	2312.953	1	2312.953	1.615	0.209
REPLICA(YEAR)	11808.245	4	2952.061	2.061	0.097
Error	87360.134	61	1432.133		

Dependent Variable	FL_1W
N	72
Multiple R	0.762
Squared Multiple R	0.580

Analysis of Variance (FL-1W)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	1.164	1	1.164	0.929	0.339
YEAR	75.212	1	75.212	60.081	0.000
TREATMENT	3.026	1	3.026	2.417	0.125
YEAR*GENOTYPE$	2.669	1	2.669	2.132	0.149
TREATMENT*GENOTYPE$	3.945	1	3.945	3.151	0.081
TREATMENT*YEAR	0.345	1	0.345	0.276	0.602
YEAR*TREATMENT*GENOTYPE$	0.622	1	0.622	0.497	0.484
REPLICA(YEAR)	1.609	4	0.402	0.321	0.863
Error	75.110	60	1.252		

Dependent Variable	FL_1L
N	72
Multiple R	0.731
Squared Multiple R	0.534

Analysis of Variance (FL-1L)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	15618.596	1	15618.596	20.802	0.000
YEAR	63.159	1	63.159	0.084	0.773
TREATMENT	6469.797	1	6469.797	8.617	0.005
YEAR*GENOTYPE$	2834.951	1	2834.951	3.776	0.057
TREATMENT*GENOTYPE$	0.610	1	0.610	0.001	0.977
TREATMENT*YEAR	4559.384	1	4559.384	6.073	0.017
YEAR*TREATMENT*GENOTYPE$	3.441	1	3.441	0.005	0.946
REPLICA(YEAR)	1023.943	4	255.986	0.341	0.849
Error	45048.883	60	750.815		

Dependent Variable	HUPE
N	73
Multiple R	0.762
Squared Multiple R	0.581

Analysis of Variance (HUPE)

y	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	18993.467	1	18993.467	1.607	0.210
YEAR	750320.566	1	750320.566	63.490	0.000
TREATMENT	5238.684	1	5238.684	0.443	0.508
YEAR*GENOTYPE$	1312.470	1	1312.470	0.111	0.740
TREATMENT*GENOTYPE$	11405.848	1	11405.848	0.965	0.330
TREATMENT*YEAR	13471.914	1	13471.914	1.140	0.290
YEAR*TREATMENT*GENOTYPE$	3919.898	1	3919.898	0.332	0.567
REPLICA(YEAR)	92743.993	4	23185.998	1.962	0.112
Error	720893.990	61	11817.934		

Dependent Variable	EL
N	73
Multiple R	0.924
Squared Multiple R	0.854

Analysis of Variance (EL)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	127.120	1	127.120	4.584	0.036
YEAR	8789.144	1	8789.144	316.925	0.000
TREATMENT	11.265	1	11.265	0.406	0.526
YEAR*GENOTYPE$	79.428	1	79.428	2.864	0.096
TREATMENT*GENOTYPE$	14.787	1	14.787	0.533	0.468
TREATMENT*YEAR	0.307	1	0.307	0.011	0.917
YEAR*TREATMENT*GENOTYPE$	0.421	1	0.421	0.015	0.902
REPLICA(YEAR)	132.759	4	33.190	1.197	0.321
Error	1691.687	61	27.733		

Dependent Variable	TH
N	73
Multiple R	0.787
Squared Multiple R	0.619

Analysis of Variance (TH)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	16012.881	1	16012.881	1.351	0.250
YEAR	921524.926	1	921524.926	77.724	0.000
TREATMENT	5735.809	1	5735.809	0.484	0.489
YEAR*GENOTYPE$	746.152	1	746.152	0.063	0.803
TREATMENT*GENOTYPE$	12242.002	1	12242.002	1.033	0.314
TREATMENT*YEAR	13600.738	1	13600.738	1.147	0.288
YEAR*TREATMENT*GENOTYPE$	3839.046	1	3839.046	0.324	0.571
REPLICA(YEAR)	97866.173	4	24466.543	2.064	0.097
Error	723235.856	61	11856.326		

Dependent Variable	SNE
N	73
Multiple R	0.848
Squared Multiple R	0.718

Analysis of Variance (SNE)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	207.333	1	207.333	3.087	0.084
YEAR	9533.784	1	9533.784	141.965	0.000
TREATMENT	55.269	1	55.269	0.823	0.368
YEAR*GENOTYPE$	4.574	1	4.574	0.068	0.795
TREATMENT*GENOTYPE$	98.980	1	98.980	1.474	0.229
TREATMENT*YEAR	3.345	1	3.345	0.050	0.824
YEAR*TREATMENT*GENOTYPE$	82.634	1	82.634	1.230	0.272
REPLICA(YEAR)	48.073	4	12.018	0.179	0.948
Error	4096.506	61	67.156		

Dependent Variable	SPNE
N	73
Multiple R	0.896
Squared Multiple R	0.803

Analysis of Variance

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	3.816	1	3.816	4.186	0.045
YEAR	179.234	1	179.234	196.622	0.000
TREATMENT	10.652	1	10.652	11.686	0.001
YEAR*GENOTYPE$	0.931	1	0.931	1.022	0.316
TREATMENT*GENOTYPE$	0.365	1	0.365	0.400	0.530
TREATMENT*YEAR	0.625	1	0.625	0.686	0.411
YEAR*TREATMENT*GENOTYPE$	0.035	1	0.035	0.038	0.846
REPLICA(YEAR)	3.102	4	0.775	0.851	0.499
Error	55.606	61	0.912		

Dependent Variable	SPS
N	73
Multiple R	0.705
Squared Multiple R	0.497

Analysis of Variance (SPS)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.270	1	0.270	1.410	0.240
YEAR	10.396	1	10.396	54.199	0.000
TREATMENT	0.026	1	0.026	0.138	0.712
YEAR*GENOTYPE$	0.005	1	0.005	0.025	0.874
TREATMENT*GENOTYPE$	0.232	1	0.232	1.210	0.276
TREATMENT*YEAR	0.015	1	0.015	0.077	0.782
YEAR*TREATMENT*GENOTYPE$	0.345	1	0.345	1.797	0.185
REPLICA(YEAR)	0.310	4	0.077	0.404	0.805
Error	11.700	61	0.192		

Dependent Variable	GY
N	73
Multiple R	0.787
Squared Multiple R	0.620

Analysis of Variance (GY)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	1.186	1	1.186	5.908	0.018
YEAR	16.307	1	16.307	81.263	0.000
TREATMENT	0.047	1	0.047	0.234	0.631
YEAR*GENOTYPE$	0.078	1	0.078	0.389	0.535
TREATMENT*GENOTYPE$	0.070	1	0.070	0.348	0.557
TREATMENT*YEAR	0.005	1	0.005	0.025	0.875
YEAR*TREATMENT*GENOTYPE$	0.227	1	0.227	1.131	0.292
REPLICA(YEAR)	0.107	4	0.027	0.133	0.970
Error	12.241	61	0.201		

Dependent Variable	TKW
N	73
Multiple R	0.496
Squared Multiple R	0.246

Analysis of Variance (TKW)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	110.034	1	110.034	4.218	0.044
YEAR	214.769	1	214.769	8.232	0.006
TREATMENT	0.440	1	0.440	0.017	0.897
YEAR*GENOTYPE$	10.699	1	10.699	0.410	0.524
TREATMENT*GENOTYPE$	0.398	1	0.398	0.015	0.902
TREATMENT*YEAR	2.280	1	2.280	0.087	0.769
YEAR*TREATMENT*GENOTYPE$	10.992	1	10.992	0.421	0.519
REPLICA(YEAR)	16.358	4	4.090	0.157	0.959
Error	1591.445	61	26.089		

Biomass and HI of the 1st culm of bread wheat line T4 in 2010

Dependent Variable	B
N	59
Multiple R	0.392
Squared Multiple R	0.153

Analysis of Variance (B)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.053	1	0.053	0.061	0.805
TREATMENT	0.499	1	0.499	0.578	0.451
REPLICA	7.327	2	3.663	4.246	0.019
TREATMENT*GENOTYPE$	0.403	1	0.403	0.467	0.498
Error	45.731	53	0.863		

Dependent Variable	HI
N	59
Multiple R	0.637
Squared Multiple R	0.405

Analysis of Variance (HI)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.049	1	0.049	28.628	0.000
TREATMENT	0.000	1	0.000	0.023	0.881
REPLICA	0.013	2	0.006	3.676	0.032
TREATMENT*GENOTYPE$	0.000	1	0.000	0.007	0.933
Error	0.091	53	0.002		

Whole plant data for two years and three durum wheat lines (R5-2-10, R112-4, R23-1)

Dependent Variable	TTH
N	262
Multiple R	0.441
Squared Multiple R	0.194

Analysis of Variance (TTH)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	159.548	2	79.774	5.785	0.004
YEAR	18.772	1	18.772	1.361	0.244
YEAR*GENOTYPE$	136.479	2	68.240	4.948	0.008
_7AGL(GENOTYPE$)	440.030	3	146.677	10.636	0.000
REPLICA(YEAR)	59.862	4	14.965	1.085	0.364
YEAR*_7AGL(GENOTYPE$)	20.859	3	6.953	0.504	0.680
Error	3392.480	246	13.791		

Dependent Variable	TCN
N	262
Multiple R	0.430
Squared Multiple R	0.185

Analysis of Variance (TCN)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	73.897	2	36.949	2.755	0.066
YEAR	47.602	1	47.602	3.549	0.061
YEAR*GENOTYPE$	18.688	2	9.344	0.697	0.499
_7AGL(GENOTYPE$)	401.888	3	133.963	9.988	0.000
REPLICA(YEAR)	75.950	4	18.987	1.416	0.229
YEAR*_7AGL(GENOTYPE$)	53.064	3	17.688	1.319	0.269
Error	3299.588	246	13.413		

Dependent Variable	PRCN
N	262
Multiple R	0.587
Squared Multiple R	0.345

Analysis of Variance (PRCN)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	15.763	2	7.882	1.137	0.322
YEAR	560.290	1	560.290	80.850	0.000
YEAR*GENOTYPE$	29.227	2	14.614	2.109	0.124
_7AGL(GENOTYPE$)	97.122	3	32.374	4.672	0.003
REPLICA(YEAR)	33.743	4	8.436	1.217	0.304
YEAR*_7AGL(GENOTYPE$)	76.288	3	25.429	3.669	0.013
Error	1704.788	246	6.930		

Dependent Variable	PRGY
N	262
Multiple R	0.534
Squared Multiple R	0.285

Analysis of Variance (PRGY)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	1941.240	2	970.620	21.579	0.000
YEAR	0.002	1	0.002	0.000	0.994
YEAR*GENOTYPE$	1237.004	2	618.502	13.751	0.000
_7AGL(GENOTYPE$)	390.166	3	130.055	2.891	0.036
REPLICA(YEAR)	232.435	4	58.109	1.292	0.274
YEAR*_7AGL(GENOTYPE$)	713.369	3	237.790	5.287	0.002
Error	11064.903	246	44.979		

Dependent Variable	GYXPRC
N	262
Multiple R	0.753
Squared Multiple R	0.566

Analysis of Variance (GYxPRC)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	12.488	2	6.244	38.995	0.000
YEAR	18.900	1	18.900	118.034	0.000
YEAR*GENOTYPE$	4.883	2	2.441	15.246	0.000
_7AGL(GENOTYPE$)	3.677	3	1.226	7.654	0.000
REPLICA(YEAR)	1.765	4	0.441	2.756	0.029
YEAR*_7AGL(GENOTYPE$)	1.324	3	0.441	2.755	0.043
Error	39.391	246	0.160		

Dependent Variable	PRCSEED
N	262
Multiple R	0.496
Squared Multiple R	0.246

Analysis of Variance (PRCSEED)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	56514.859	2	28257.429	1.852	0.159
YEAR	205562.225	1	205562.225	13.469	0.000
YEAR*GENOTYPE$	190778.064	2	95389.032	6.250	0.002
_7AGL(GENOTYPE$)	292289.995	3	97429.998	6.384	0.000
REPLICA(YEAR)	85888.001	4	21472.000	1.407	0.232
YEAR*_7AGL(GENOTYPE$)	307608.369	3	102536.123	6.719	0.000
Error	3754330.943	246	15261.508		

Dependent Variable	SEEDXPRC
N	262
Multiple R	0.806
Squared Multiple R	0.650

Analysis of Variance (SEEDxPRC)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	310.780	2	155.390	3.544	0.030
YEAR	15389.672	1	15389.672	350.988	0.000
YEAR*GENOTYPE$	467.069	2	233.534	5.326	0.005
_7AGL(GENOTYPE$)	269.712	3	89.904	2.050	0.107
REPLICA(YEAR)	212.423	4	53.106	1.211	0.307
YEAR*_7AGL(GENOTYPE$)	193.849	3	64.616	1.474	0.222
Error	10786.304	246	43.847		

Dependent Variable	PRCTKW
N	262
Multiple R	0.772
Squared Multiple R	0.596

Analysis of Variance (PRCTKW)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	4517.617	2	2258.808	78.245	0.000
YEAR	1402.114	1	1402.114	48.569	0.000
YEAR*GENOTYPE$	1803.776	2	901.888	31.241	0.000
_7AGL(GENOTYPE$)	3685.570	3	1228.523	42.556	0.000
REPLICA(YEAR)	353.748	4	88.437	3.063	0.017
YEAR*_7AGL(GENOTYPE$)	160.073	3	53.358	1.848	0.139
Error	7101.618	246	28.868		

Dependent Variable	GY
N	262
Multiple R	0.587
Squared Multiple R	0.344

Analysis of Variance (GY)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	3276.511	2	1638.255	28.685	0.000
YEAR	375.845	1	375.845	6.581	0.011
YEAR*GENOTYPE$	1756.330	2	878.165	15.376	0.000
_7AGL(GENOTYPE$)	522.268	3	174.089	3.048	0.029
REPLICA(YEAR)	313.272	4	78.318	1.371	0.244
YEAR*_7AGL(GENOTYPE$)	840.579	3	280.193	4.906	0.002
Error	14049.618	246	57.112		

Dependent Variable	SEED
N	259
Multiple R	0.578
Squared Multiple R	0.334

Analysis of Variance (SEED)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	154665.917	2	77332.958	3.449	0.033
YEAR	886596.805	1	886596.805	39.543	0.000
YEAR*GENOTYPE$	202775.401	2	101387.701	4.522	0.012
_7AGL(GENOTYPE$)	610683.759	3	203561.253	9.079	0.000
REPLICA(YEAR)	174703.788	4	43675.947	1.948	0.103
YEAR*_7AGL(GENOTYPE$)	380367.553	3	126789.184	5.655	0.001
Error	5448371.857	243	22421.283		

Dependent Variable	TKW
N	259
Multiple R	0.713
Squared Multiple R	0.509

Analysis of Variance (TKW)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	845.410	2	422.705	13.361	0.000
YEAR	2807.047	1	2807.047	88.726	0.000
YEAR*GENOTYPE$	44.204	2	22.102	0.699	0.498
_7AGL(GENOTYPE$)	3441.482	3	1147.161	36.260	0.000
REPLICA(YEAR)	561.609	4	140.402	4.438	0.002
YEAR*_7AGL(GENOTYPE$)	152.049	3	50.683	1.602	0.190
Error	7687.888	243	31.637		

Biomass and harvest index of the whole plant and 3 durum wheat lines (R5-2-10, R112-4, R23-1) in 2010

Dependent Variable	B
N	143
Multiple R	0.333
Squared Multiple R	0.111

Analysis of Variance (B)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	251.507	2	125.754	0.381	0.684
REPLICA	227.869	2	113.934	0.346	0.708
_7AGL(GENOTYPE$)	4978.121	3	1659.374	5.032	0.002
Error	44518.092	135	329.764		

Dependent Variable	HI
N	143
Multiple R	0.509
Squared Multiple R	0.259

Analysis of Variance (HI)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.059	2	0.029	16.087	0.000
REPLICA	0.024	2	0.012	6.592	0.002
_7AGL(GENOTYPE$)	0.005	3	0.002	1.001	0.394
Error	0.246	135	0.002		

Whole plant data for two years of bread wheat line T4

Dependent Variable	TTH
N	58
Multiple R	0.377
Squared Multiple R	0.142

Analysis of Variance (TTH)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	18.986	1	18.986	0.796	0.377
YEAR	58.070	1	58.070	2.433	0.125
REPLICA	109.168	2	54.584	2.287	0.112
YEAR*GENOTYPE$	12.479	1	12.479	0.523	0.473
Error	1240.865	52	23.863		

Dependent Variable	TCN
N	57
Multiple R	0.477
Squared Multiple R	0.227

Analysis of Variance (TCN)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	63.588	1	63.588	2.333	0.133
YEAR	324.062	1	324.062	11.892	0.001
REPLICA	17.056	2	8.528	0.313	0.733
YEAR*GENOTYPE$	16.557	1	16.557	0.608	0.439
Error	1389.820	51	27.251		

Dependent Variable	PRCN
N	58
Multiple R	0.414
Squared Multiple R	0.171

Analysis of Variance (PRCN)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	2.199	1	2.199	0.112	0.739
YEAR	138.567	1	138.567	7.069	0.010
REPLICA	39.087	2	19.544	0.997	0.376
YEAR*GENOTYPE$	5.814	1	5.814	0.297	0.588
Error	1019.350	52	19.603		

Dependent Variable	PRGY
N	58
Multiple R	0.575
Squared Multiple R	0.330

Analysis of Variance (PRGY)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	10.395	1	10.395	0.215	0.645
YEAR	1186.443	1	1186.443	24.516	0.000
REPLICA	10.268	2	5.134	0.106	0.900
YEAR*GENOTYPE$	10.913	1	10.913	0.226	0.637
Error	2516.495	52	48.394		

Dependent Variable	GYXPRC
N	58
Multiple R	0.778
Squared Multiple R	0.605

Analysis of Variance (GYxPRC)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.053	1	0.053	0.472	0.495
YEAR	8.220	1	8.220	73.545	0.000
REPLICA	0.293	2	0.147	1.312	0.278
YEAR*GENOTYPE$	0.016	1	0.016	0.148	0.702
Error	5.812	52	0.112		

Dependent Variable	PRCSEED
N	58
Multiple R	0.374
Squared Multiple R	0.140

Analysis of Variance (PRCSEED)

y	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	1004.422	1	1004.422	0.024	0.876
YEAR	313529.880	1	313529.880	7.620	0.008
REPLICA	13398.692	2	6699.346	0.163	0.850
YEAR*GENOTYPE$	23490.273	1	23490.273	0.571	0.453
Error	2139698.197	52	41148.042		

Dependent Variable	SEEDXPRC
N	58
Multiple R	0.779
Squared Multiple R	0.607

Analysis of Variance (SEEDxPRC)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	2.961	1	2.961	0.053	0.819
YEAR	3856.167	1	3856.167	68.862	0.000
REPLICA	519.890	2	259.945	4.642	0.014
YEAR*GENOTYPE$	0.740	1	0.740	0.013	0.909
Error	2911.941	52	55.999		

Dependent Variable	PRCTKW
N	58
Multiple R	0.606
Squared Multiple R	0.367

Analysis of Variance (PRCTKW)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	41.407	1	41.407	1.247	0.269
YEAR	918.984	1	918.984	27.678	0.000
REPLICA	12.607	2	6.304	0.190	0.828
YEAR*GENOTYPE$	0.818	1	0.818	0.025	0.876
Error	1726.564	52	33.203		

Dependent Variable	GY
N	58
Multiple R	0.620
Squared Multiple R	0.384

Analysis of Variance (GY)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	6.733	1	6.733	0.129	0.721
YEAR	1586.312	1	1586.312	30.482	0.000
REPLICA	29.576	2	14.788	0.284	0.754
YEAR*GENOTYPE$	0.216	1	0.216	0.004	0.949
Error	2706.104	52	52.040		

Dependent Variable	SEED
N	58
Multiple R	0.384
Squared Multiple R	0.148

Analysis of Variance (SEED)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	2711.968	1	2711.968	0.059	0.809
YEAR	360401.016	1	360401.016	7.880	0.007
REPLICA	36656.955	2	18328.477	0.401	0.672
YEAR*GENOTYPE$	1108.214	1	1108.214	0.024	0.877
Error	2378185.830	52	45734.343		

Dependent Variable	TKW
N	58
Multiple R	0.663
Squared Multiple R	0.439

Analysis of Variance (TKW)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	35.749	1	35.749	1.552	0.218
YEAR	866.830	1	866.830	37.643	0.000
REPLICA	11.230	2	5.615	0.244	0.785
YEAR*GENOTYPE$	0.238	1	0.238	0.010	0.919
Error	1197.438	52	23.028		

Biomass and HI of the whole plant of bread wheat line T4 in 2010

ependent Variable	B
N	44
Multiple R	0.279
Squared Multiple R	0.078

Analysis of Variance (B)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	139.015	1	139.015	0.371	0.546
REPLICA	1189.216	2	594.608	1.586	0.217
Error	14999.465	40	374.987		

Dependent Variable	HI
N	44
Multiple R	0.554
Squared Multiple R	0.307

Analysis of Variance

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.005	1	0.005	7.518	0.009
REPLICA	0.006	2	0.003	5.195	0.010
Error	0.024	40	0.001		

APPENDIX 4 – ANOVA summary tables of endogenous ABA content in juvenile spike (GLM) and corresponding relative water content (RWC) of the flag leaf

Endogenous ABA content in field grown durum wheat genotypes (R5-2-10, R112-4, R23-1)

Dependent Variable	ABA
N	76
Multiple R	0.259
Squared Multiple R	0.067

Analysis of Variance (ENDOGENOUS ABA)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	5902.338	2	2951.169	1.706	0.189
_7AGL(GENOTYPE$)	3334.712	3	1111.571	0.642	0.590
Error	121113.868	70	1730.198		

Endogenous ABA content in field grown bread wheat genotype T4/Thatcher

Dependent Variable	ABA
N	12
Multiple R	0.030
Squared Multiple R	0.001

Analysis of Variance (ENDOGENOUS ABA)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	10.253	1	10.253	0.009	0.927
Error	11552.430	10	1155.243		

Endogenous ABA content in greenhouse grown durum wheat genotypes (R5-2-10, R112-4, R23-1)

Dependent Variable	ABA
N	54
Multiple R	0.739
Squared Multiple R	0.545

Analysis of Variance (ENDOGENOUS ABA)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	270149.560	2	135074.780	24.890	0.000
_7AGL(GENOTYPE$)	39222.315	3	13074.105	2.409	0.079
Error	260491.531	48	5426.907		

Tukey's Honestly-Significant-Difference Test

GENOTYPE$(i)	GENOTYPE$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
R112	R23	-143.418	0.000	-200.044	-86.792
R112	R5	2.282	0.996	-61.209	65.772
R23	R5	145.700	0.000	84.787	206.613

Endogenous ABA content in greenhouse grown bread wheat genotype T4/Thatcher

Dependent Variable	ABA
N	11
Multiple R	0.169
Squared Multiple R	0.028

Analysis of Variance (ENDOGENOUS ABA)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	554.156	1	554.156	0.263	0.620
Error	18949.678	9	2105.520		

RWC of field grown durum wheat genotypes (R5-2-10, R112-4, R23-1)

Dependent Variable	RWC
N	53
Multiple R	0.526
Squared Multiple R	0.276

Analysis of Variance (RWC)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.011	2	0.005	6.389	0.004
_7AGL(GENOTYPE$)	0.004	3	0.001	1.593	0.204
Error	0.039	47	0.001		

Tukey's Honestly-Significant-Difference Test

GENOTYPE$(i)	GENOTYPE$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
R112	R23	-0.034	0.007	-0.059	-0.009
R112	R5	-0.008	0.750	-0.036	0.019
R23	R5	0.026	0.032	0.004	0.048

RWC of field grown bread wheat genotype T4/Thatcher

Dependent Variable	RWC
N	12
Multiple R	0.294
Squared Multiple R	0.087

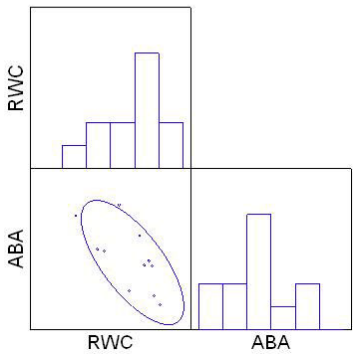
Analysis of Variance

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.001	1	0.001	0.948	0.353
Error	0.008	10	0.001		

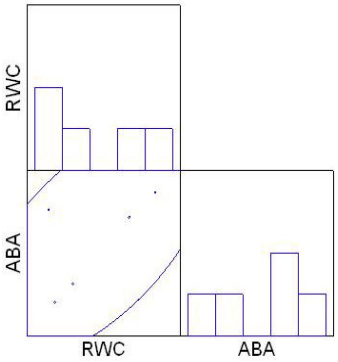
Graphs of correlation matrices between endogenous ABA content and RWC at boot stage of plant development

Durum wheat genotypes (R5-2-10, R112-4, R23-1)

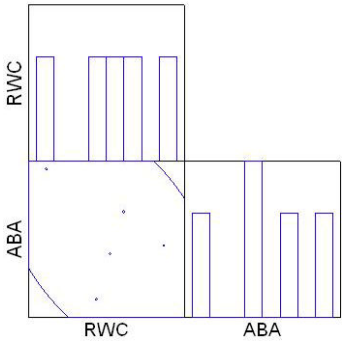
R5-2-10 HOM+



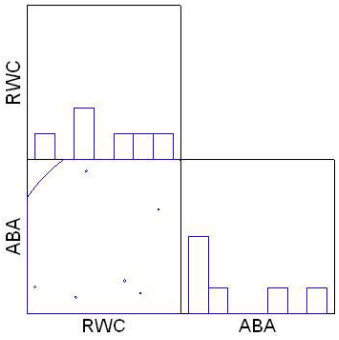
R5-2-10 HOM-



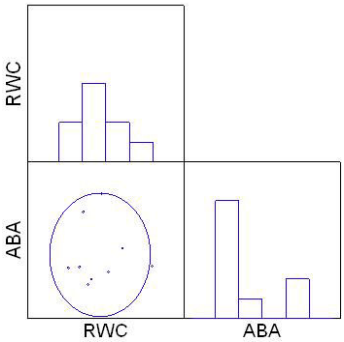
R112-4 HOM+



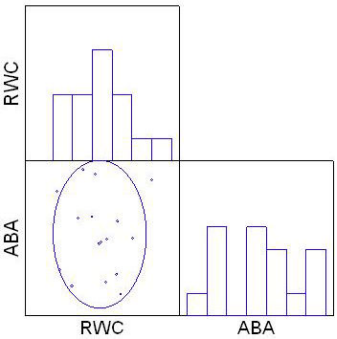
R112-4 HOM-



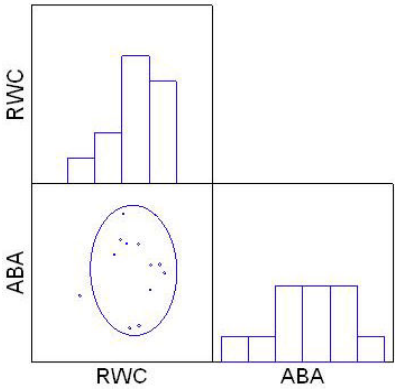
R23-1 HOM+



R23-1 HOM-



Bread wheat genotype T4/Thatcher



APPENDIX 5 – ANOVA summary tables of physiological parameters measured under induced drought stress (GLM)

Fresh weight/Dry weight ratio (FW/DW)

Durum wheat genotypes (R5-2-10, R112-4, R23-1)

Dependent Variable	FW_DW
N	197
Multiple R	0.798
Squared Multiple R	0.638

Analysis of Variance (FW/DW)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	4.970	2	2.485	1.776	0.172
WEEK	392.946	3	130.982	93.607	0.000
WEEK*GENOTYPE$	11.919	6	1.987	1.420	0.210
_7AGL(GENOTYPE$)	2.537	3	0.846	0.604	0.613
WEEK*_7AGL(GENOTYPE$)	9.407	9	1.045	0.747	0.665
Error	242.076	173	1.399		

Bread wheat genotype T4/Thatcher

Dependent Variable	FW_DW
N	64
Multiple R	0.925
Squared Multiple R	0.856

Analysis of Variance (FW/DW)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.030	1	0.030	0.047	0.829
WEEK	219.621	2	109.811	172.321	0.000
WEEK*GENOTYPE$	0.408	2	0.204	0.320	0.728
Error	36.960	58	0.637		

Leaf Water Potential (LWP)

Durum wheat genotypes (R5-2-10, R112-4, R23-1)

Dependent Variable	LWP
N	65
Multiple R	0.383
Squared Multiple R	0.147

Analysis of Variance (LWP)

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.012	2	0.006	0.070	0.932
WEEK	0.408	2	0.204	2.399	0.102
WEEK*GENOTYPE$	0.069	4	0.017	0.203	0.935
_7AGL(GENOTYPE$)	0.020	3	0.007	0.080	0.971
WEEK*_7AGL(GENOTYPE$)	0.153	6	0.025	0.299	0.934
Error	4.000	47	0.085		

Bread wheat genotype T4/Thatcher

Dependent Variable	LWP
N	16
Multiple R	0.653
Squared Multiple R	0.427

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.131	1	0.131	5.298	0.044
WEEK	0.000	2	0.000	0.007	0.993
WEEK*GENOTYPE$	0.098	2	0.049	1.997	0.186
Error	0.247	10	0.025		

Final fresh biomass

Durum wheat genotypes (R5-2-10, R112-4, R23-1)

Dependent Variable	BIOMASS
N	59
Multiple R	0.259
Squared Multiple R	0.067

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.615	2	0.307	1.690	0.194
_7AGL(GENOTYPE$)	0.081	3	0.027	0.148	0.931
Error	9.637	53	0.182		

Bread wheat genotype T4/Thatcher

Dependent Variable	BIOMASS
N	30
Multiple R	0.286
Squared Multiple R	0.082

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	0.181	1	0.181	2.489	0.126
Error	2.036	28	0.073		

Endogenous ABA content in leaf under drought – analysis of variance (GLM)

Durum wheat genotypes (R5-2-10, R112-4, R23-1)

Dependent Variable	ABA
N	186
Multiple R	0.955
Squared Multiple R	0.912

Analysis of Variance

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	155066.381	2	77533.190	12.810	0.000
WEEK	9824345.202	3	3274781.734	541.074	0.000
WEEK*GENOTYPE$	96178.884	6	16029.814	2.649	0.018
_7AGL(GENOTYPE$)	28792.301	3	9597.434	1.586	0.195
WEEK*_7AGL(GENOTYPE$)	48246.610	9	5360.734	0.886	0.539
Error	980485.025	162	6052.377		

Bread wheat genotype (T4/Thatcher)

Dependent Variable	ABA
N	63
Multiple R	0.965
Squared Multiple R	0.932

Source	Type III SS	df	Mean Squares	F-ratio	p-value
GENOTYPE$	1506.095	1	1506.095	0.265	0.609
WEEK	4426972.088	2	2213486.044	388.817	0.000
WEEK*GENOTYPE$	538.919	2	269.459	0.047	0.954
Error	324493.855	57	5692.875		