CORDOBA UNIVERSITY
ETSIAM, GENETIC DEPARTMENT
&
CIMMYT/ICARDA DURUM JOINT PROGRAM

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Ph.D. Thesis

Quantitative Trait Loci (QTL) determination of grain quality traits in durum wheat (*Triticum turgidum* L. var. *durum*)

Presented by:
Ismahane El Ouafi

Supervisors:
Dr. Miloudi M. Nachit
Dr. Antonio Martín Muñoz
Dr. Luis Miguel Martín Martín

CORDOBA (SPAIN), SEPTEMBER 2001

Cordoba University, High Technical School of Agronomy and Forestall Engineering,
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INFORMAN:

Que el trabajo titulado **"Mapa de ligamiento de caracteres relacionados con la calidad de trigo duro (*Triticum turgidum* var. L. *durum*)"**, realizado por D. Ismahane El Ouafi, bajo su dirección, se considera ya finalizado y puede ser presentado para su exposición y defensa como Tesis Doctoral en el Departamento de Genética de la Universidad de Córdoba.

M. M. Nachit　　　　A. Martín Muñoz　　　　L. M. Martín Martín

Córdoba, 29 de Junio de 2001

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A mon oncle Abdou,
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A ceux que j’aime et j’aimerai toujours,

Je dédie ce travail

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Summary

Durum wheat (*Triticum turgidum L.* var *durum*) is mainly produced and consumed in the Mediterranean region; it is used to produce several specific endproducts. Grain quality is important; and simple, rapid, and reliable tests are prerequisite. The aim of this study was to construct a genetic linkage map of durum, to determine QTLs related to grain-quality, and to study their interaction with environments. The population constituted of 114 recombinant inbred lines, derived from the cross: *Omrabi5/ Triticum dicoccoides600545// Omrabi5*. Microsatellites, Amplified Fragment Length Polymorphism (AFLPs), and seed storage proteins (SSP) were used. The traits analyzed over sites x years were protein content (PC), gluten strength (SDS), farinograph parameters, yellow pigment (YP), vitreousness (Vit), test weight (TW), kernel size (TKW), flour yield (FY), and ash content (AC). PC, Vit, and TKW were affected by environments. Transgressive inheritance was shown for SDS, farinograph parameters, TKW, and AC. The traits were grouped in 3 clusters representing protein, gluten strength, and milling. As for the mapping population, a high level of polymorphism was detected (> 60%). The map was constructed with 124 microsatellites, 149 AFLPs, and 6 SSPs; its length was 2288.8cM (8.2cM/ marker). It showed high synteny with previous wheat maps. The microsatellites and AFLPs mapped evenly across the genome, with more markers in B. Thirty-three QTLs showed linkages to different traits, with at least 3 QTLs per trait contributing with more than 30%. The largest contributions were for YP and farinograph stability time (FST); 3 QTLs on 7AL and 7BL explained 62% of YP; and 4 QTLs on 1BL and 2BS 51% of FST. All QTLs showed genetic and QTLxE effects, except Vit where only interaction occurred. Several QTLs also showed pleitropic effect; *Glu-B1* and *Xgwm131b* on 1BL affected SDS and farinograph parameters. Similarly, PC and Vit have 2 QTLs in common (*XMcaaEacg560* and *Xgwm107*), with genetic and interaction effects on PC and only interaction on Vit. Also, *Xgwm518* on 6BS has affected SDS, PC, and TKW. *Omrabi5 Xgwm518*-alleles contributed positively to TKW and negatively to PC and SDS. However, several other TKW-QTLs were not linked to PC and vice versa. Other major genomic regions were linked to quality traits (6B, 3BS, 4BL). Processing traits were on 1AS, 1BL, 2BS, 3AS, 3BS, 4BL, and 6BS. The QTLs on 1AS and 1BL coincided with *Glu-A3* and *Glu-B1*, whereas the other QTLs are new genetic markers. PC-QTLs were distributed over the genome (3BS, 3BL, 4BL, 6AS, 6BS). In contrast, YP-QTLs were confined to 7AL and 7BL telomeres. *Xgwm344*-QTL explained 53% of YP. As for milling characters their QTLs were limited to few regions (3B, 6B, 7A). TKW-QTLs mapped closely to AC, FY, and TW-QTLs. The results show the importance of the introgression of *T. dicoccoides* novel genes to improve durum grain-quality. This improvement was particularly portrayed for PC, SDS, and farinograph parameters. The identified QTLs should be used to enhance selection efficiency for YP, PC, and SDS. The use of QTLs will undoubtedly permit to overcome the difficulties in breaking the negative linkage between TKW and PC. Thus, the outcomes of this study support strongly the use of marker assisted selection in the breeding programs. Furthermore, this population will be used to determine other QTLs of interest, as its parents harbor different genes for diseases and drought tolerance.

Resumen

El trigo duro (*Triticum turgidum L.* var *durum*) se produce y se consume principalmente en la región mediterránea; se utiliza para producir varios productos finales. La calidad del grano es importante; y encontrar métodos simples, rápidos y fiables es un requisito previo. El objetivo de este estudio era construir un mapa genético del trigo duro, determinar QTLs relacionados con la calidad del grano, y estudiar sus interacciones con el ambiente. La población en estudio està constituida por 114 lineas recombinadas derivadas del crecimiento: *Omrabi5/ Triticum dicoccoides600545// Omrabi5* . se han utilizado como marcadores molecualres Microsatellites, AFLPs, y proteínas de reserva de la semilla (SSP). Los caracteres analizados por localidad y ano fueron contenido proteico (PC), fuerza del gluten (SDS), parámetros del farinógrafo, pigmento amarillo (YP), vitrosidad (Vit), peso hectolitrico (TW), tamaño del grano (TKW), rendimiento de harina (FY) y contenido de ceniza (AC). PC, Vit y TKW fueron afectados por el ambiente. La herencia transgresiva fue demostrada para SDS, parámetros del farinógrafo, TKW y AC. Los caracteres fueron agrupados en 3 grupos representando proteínas, fuerza del gluten y molina. En cuanto a la población de mapeo, fue detectado un alto nivel de polimorfismo (>60%). El mapa fue construido con 124 microsatellites, 149 AFLPs, y 6 SSPs; su longitud es de 2288.8cM (8.2cM/ marcador). El mapa demostró gran syntenia con los mapas del trigo. Microsatellites y AFLPs mapean uniformemente en el genoma, con más marcadores en el genoma B. Treinta y tres QTLs tenian asociaciones con diversos caracteres, con por lo menos 3 QTLs por caracter contribuyendo con más del 30%. Las contribuciones más altas fueron para YP y estabilidad del farinógrafo (FST); 3 QTLs en 7AL y 7BL explicaban 62% del YP; y 4 QTLs en 1BL y 2BS 51% del FST. Todos los QTLs demostraron efectos genéticos e interacción, excepto para Vit donde solamente ocurrió la interacción. Varios QTLs demostraron también efectos pleitròpicos; *Glu-B1* y *Xgwm131b* en 1BL han afectado el SDS y los parámetros del farinógrafo. PC y Vit tienen tambien 2 QTLs en común (*XMcaaEacg560* y *Xgwm107*), con efectos genéticos y de interacción sobre PC y solamente de interacción sobre Vit. Tambien, *Xgwm518* en 6BS ha afectado SDS, PC, y TKW. Los allelos *Omrabi5* de *Xgwm518* han contribuido positivamente al TKW y negativamente al PC y SDS. Pero hay varios otros QTLs de TKW que no estan asociados con PC y vice-versa. Otras regiones genòmicas fueron tambien associadas a la calidad (6B, 3BS, 4BL). Caracteres de fabricación estaban en 1AS, 1BL, 2BS, 3AS, 3BS, 4BL, y 6BS. Los QTLs en 1AS y 1BL coincidieron con *Glu-A3* y *Glu-B1*, mientras que los otros QTLs son nuevos marcadores genéticos. Los QTLs del PC estuvieron distribuidos en el genoma (3BS, 3BL, 4BL, 6AS, 6BS). En contraste, los QTLs del YP fueron confinados a los telomeros del 7AL y 7BL. *Xgwm344* explicó 53% del YP. En cuanto a los caracteres de molina, sus QTLs fueron limitados a pocas regiones (3B, 6B, 7A). Los QTLs de TKW fueron mapeados cerca de los QTLs de AC, FY, y TW. Los resultados demuestran la importancia de la introgression de los nuevos genes de *T. dicoccoides* para mejorar la calidad del grano del trigo duro. Esta mejora fue particularmente evidente para PC, SDS, y los parámetros del farinógrafo. Los QTLs identificados deben ser utilizados para aumentar la eficacia de la selección de YP, PC, y SDS. La utilizaciòn de los QTLs permitirà sin duda superar las dificultades en romper el ligamiento negativo entre TKW y PC. Así, los resultados de este estudio apoyan la selección asistida con marcadores en los programas de mejora. Además, esta población seria usada para determinar otros QTLs de interés, porque sus padres poseen diversos genes para la resistencia a enfermedades y a la sequía.

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Acronyms

AC: ash content
AFLPs: amplified fragment length polymorphism
BC-RILs: backcrossed recombinant inbred lines
Br: Breda station
CIMMYT: centro internacional de mejoramiento de maiz y trigo
cM: centimorgam
EP: early planting at Tel Hadya
FAB: farinograph absorbance
FDT: farinograph development time
FMT: farinograph mixing time
FST: farinograph stability time
FY: flour yield
GWM: Gatersleben wheat microsatellites
HMWgs: high molecular weight glutenin subunits
ICARDA: international center for agricultural research in the dry areas
Inc: sowing after legume crop at Tel Hadya
Ir: irrigated at Tel Hadya
LMWgs: low molecular weight glutenin subunits
LOD: logarithm of the odds ratio
LOX: lipoxygenase
LP: late planting at Tel Hadya
MDM: *Omrabi5/ T. dicoccoid es600545// Omrabi5*
MQTL: Multiple Quantitative trait loci
PC: protein content
PPO: polyphenol peroxidase
QTL: Quantitative trait loci
Rf: rainfed at Tel Hadya
RFLPs: restriction fragment length polymorphism
RILs: recombinant inbred lines
sCIM: simplified composite interval mapping
SDS: sedimentation test
SDSni: sedimentation new index
SIM: simple interval mapping
SSP: seed storage proteins
SSR: microsatellites
SUM: summer planting at Tel Hadya
TKW: Thousand-kernel weight
Tr: Terbol station
TW: test weight
Vit: vitreousness
YP: yellow pigment

Introduction

Wheat is one of the most widely grown food grain crop in the world. Understanding its genetics and genome organization using molecular markers is of great scientific value, in order to improve further its performance and quality. Durum and bread wheat (*Triticum durum* and *T. aestivum*) constitute the base for human food, especially in the Mediterranean basin. In West Asia North Africa WANA region, the durum wheat production represents 50% of the world production and the area covered is more than 85% of the total world area in the developing countries (Nachit *et al.* 1995a). The annual world durum wheat production was estimated at 33 million tons (CWB 2001).

Durum wheat (*Triticum turgidum* L. var *durum*) is a tetraploid constituted of A and B genomes (AABB) and is the main source of semolina for the production of pasta, couscous, and grain for burghul. The grain quality is important for processing of these end products, particularly the protein content and the gluten strength. The durum breeding programs are screening annually thousands of lines and populations to identify genetic material with high grain quality. For this reason, it is very important to have simple and rapid tests to screen for desirable traits of grain quality and its end-product. Screening methods based on biochemical analysis have greatly contributed to the improvement of grain quality. During the last two decades, the powerful tool techniques of protein separating in acrylamide gel have allowed to achieve spectacular progress in improving tetra- and hexa-ploid wheats grain quality; and also in studying the phylogenetics, taxonomy, and genetics of several species. The two main poly-acrylamide gel electrophoresis methods used are the A-PAGE (Poly-Acrylamide Gel Electrophoresis at Acidic pH.) for gliadins and, the SDS-PAGE analysis for glutenins. Surely, the electrophoretic procedures have assisted in selection for improvement of some technological properties; however, there is a requirement for more markers to cover other aspects of grain quality.

Because of the need for further rapid methods for exploitation of particular proteins related to quality, the development of fast, reliable, and accurate methods for grain quality determination has become of paramount importance. In this context, molecular markers, in addition to being promising, they seem to be the appropriate choice. Further, because of the need to enhance further the durum grain quality for industrial and nutritional purposes, the durum cultivar *Omrabi5* which has relatively good grain quality pattern, including high gluten strength and adequate milling extraction; was crossed to *T. dicoccoides600545* which possesses high protein content and novel grain quality genes. However, in order to map Quantitative Traits Loci (QTLs) linked to the different parameters of grain quality, a durum genetic linkage map with *T. dicoccoides* introgressions is a prerequisite. The plant materiel used in this study was a population of Recombinant Inbred Lines (RILs) generated from an interspecific cross between the cultivar *Omrabi5* and *Triticum dicoccoides 600545* with a backcross to *Omrabi5*. The population was constituted of 114 RILs developed at CIMMYT/ICARDA durum breeding program for the Mediterranean dryland (Nachit pers. com.). The parents were chosen for their wide polymorphism for several traits

including grain quality, e.g.; *Omrabi5* has an average protein content of 13%, whereas *T. dicoccoides600545* 23%.

The aim of this study was 1) to construct a genetic linkage map of *Omrabi5/ Triticum dicoccoides600545// Omrabi5* population; and 2) to determine Quantitative Traits Loci QTL related to different grain-quality traits, either derived from durum or introgressed from *T. dicoccoides* in the RIL population. To accomplish this study the Polymerase Chain Reaction (PCR) based techniques are mainly used. The markers probed are Microsatellites and Amplified Fragment Length Polymorphism, in addition to seed storage proteins: gliadin and glutenin components. Quality traits such as gluten strength, protein content, yellow pigment, farinograph parameters, kernel size, test weight, and ash content were determined and analyzed over several sites and years in the Mediterranean dryland. This study has also included the analysis and the identification of QTLs for grain quality traits.

Chapter I
Literature Review

I- Wild and Cultivated Wheats

1. Origin of Wheats

The principal center of diversity and origin of the *Triticum* genus is the south-west region of Asia, particularly the mountain areas of the Fertile Crescent between the Mediterranean coast at the west and the plain of Tigris and Euphrates at the east throughout the Syrian desert (Feldman and Sears 1981). In this region, many species of *Triticum*, diploids and polyploids, are widespread. They show a large scale of morphological and ecological variations. In many cases, species grew in polymorphic or mixed populations. This situation generally increases the frequency of spontaneous hybridations and facilitates interspecific mating. These dynamic-connections lead the creation of genetic richness and have made this region a center of active evolution (Feldman and Sears 1981).

Archeological research shows that the center of domestication of actual cultivated wheats coincides with the center of diversity of their wild ancestors. The northeast-east of the Fertile Crescent, more precisely the south-west of Iran, the north-west of Iraq and the south-east of Turkey are the center of diversity of the two wild diploid wheats (*Triticum monococcum* ssp. *boeoticum* Boiss. and *Triticum monococcum* ssp. *urartu* Tumanian ex Gandilyan) and tetraploid wild wheat (*Triticum timopheevii* ssp. *araraticum* Jakubz). This region seems also to be the place of the domestication and evolution of the cultivated wheats *Triticum monococcum* L. ssp. *monococcum* and *Triticum timopheevii* (Zhuk.) Zhuk. ssp. *timopheevii*. The south-west of the Fertile Crescent (north-west of Palestine, south-west of Syria, and the south-east of Lebanon) are believed to be the center of diversity of the tetraploid wild wheat, *Triticum turgidum* ssp. *dicoccoides* Thell., and the place of emergence of the primitive type *Triticum turgidum* ssp. *dicoccum* Schrank ex Schübler. The spontaneous hybridization between *T. dicoccum* "2n=4X" (AABB) and *Triticum tauschii* Coss. "2n=2X" (DD) have resulted in the actual *Triticum aestivum* L. ssp. *aestivum*. This hybridization occurred, probably, in the west of Iran 8000 years ago, when the first cultivated tetraploid wheats (AABB) were introduced in the areas where the diploids wild wheats, holding D genome, were already grown (Feldman and Sears 1981). Wheat was probably domesticated around 10,000-15,000 BC, Epipalaeolithic age (Bozzini 1988), thus determining the evolution of humans from the "shepherd-hunter-gatherer" phase to the "farmer" phase. Genetic and cytogenetic analyses conducted mainly at the hexaploid level have demonstrated that the chromosomes of the three basic genomes in hexaploids (ABD) or two basic genomes in tetraploids (AB) can be grouped into seven basic types. One pair of chromosomes of the genome A is at least partially able to substitute for a specific pair of the genomes B (in tetraploids) or B and D in hexaploids. Very often, similar mutations (or variations) can be found in all of the three types of genomes, in corresponding homoeologous chromosomes. This is easily explained by the fact that the three genomes (A, B, and D) may have had a common ancestor in the past (Bozzini 1988).

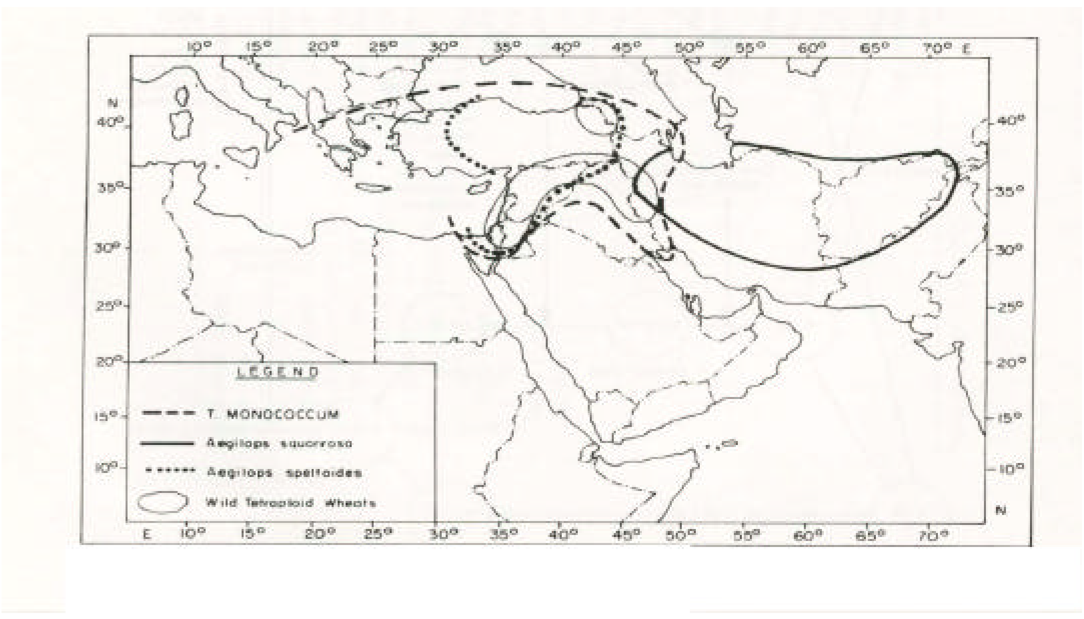


Fig I-1: Distribution of wild wheats, diploid wheat *T. monococcum*, and *Aegilops* species that were genetic donors to the present *Triticum* species.

2. Wild wheats

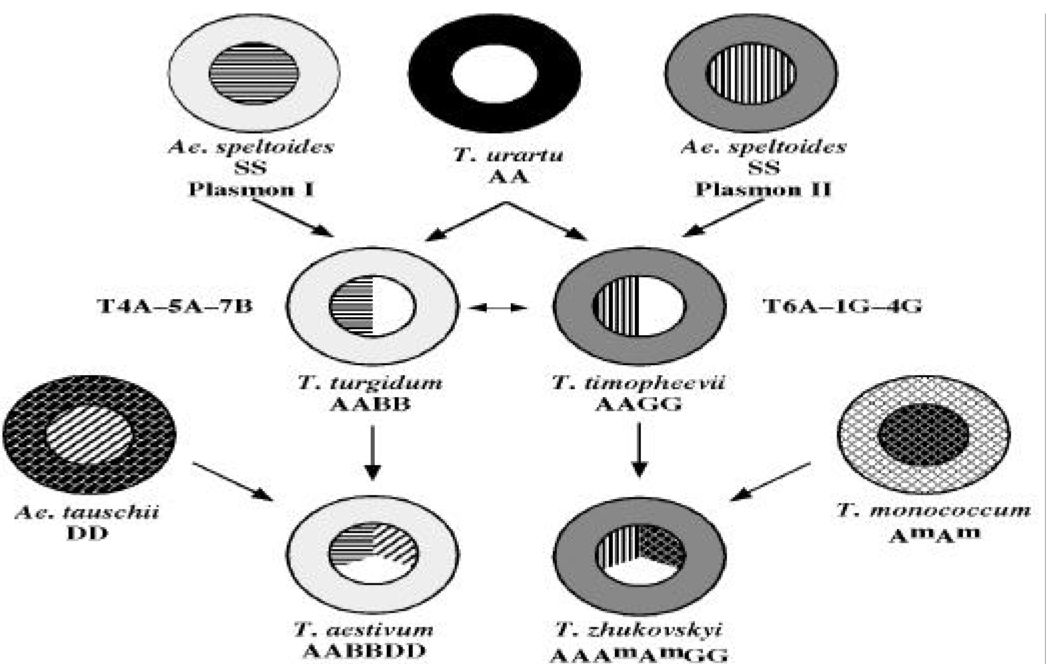
The narrow genetic basic of durum and common wheat is a major constraint for the improvement of these crops (Feldman and Sears 1981). Therefore, it is of great importance to widen the genetic variation of desirable traits, particularly, those affecting yield and quality (Nachit 1998, 2000). Wild relatives of wheat, having a much wider range of genetic variation, could serve as an excellent source for improvement of such desirable traits. In fact, wild relatives hold rich pools of genetic variation and carry many genes of great economic potential (Feldman and Sears 1981). For this reason, many programs are now carrying out hybridization programs, based on interspecific or intraspecific crosses between wild species and cultivated wheats. For instance, the joint ICARDA\CIMMYT durum-breeding project has mainly based its hybridization program on crosses between improved genotypes, Mediterranean landraces and wild relatives to improve and broaden the genetic base for resistance to biotic and abiotic stresses. Thus, landraces and wild relatives from the Middle East have been used to enhance drought tolerance, from Turkey and Algeria to incorporate cold resistance and from Morocco-Iberia region to improve resistance to root rot and Hessian fly (Nachit 1989, Nachit *et al.* 1995b).

Wild species, relatives to actual wheats, belong to *Triticeae* tribe. The genus *Triticum* has three natural groups of species based on chromosome number, namely, diploid, tetraploid, and hexaploid. Wild as well as domesticated species occur in both the diploid and tetraploid groups. Only domesticated species occur in the hexaploid group, although Dekaprelevich (1961) reported hexaploid wheat with fragile rachis growing wild in Georgia, and Sears (1977) synthesized such a hexaploid from wild tetraploid wheat and *T. tauschii* (Sharma and Waines 1980). At the diploid level, basically only one species, *T. monococcum* has been domesticated and possibly has been used by humans for 8,000 to 10,000 years. In general, diploid species are rather

resistant to disease (particularly to rusts) and have profuse tillering with narrow leaves, and good elastic straw and high resistance to lodging. Wild wheats differ from those domesticated mainly in two characteristics: 1) the spikes of the wild wheats disarticulate at maturity while those of domesticated wheats have a tough rachis and remain intact. 2) The kernels in wild wheats are enclosed tightly by the palea, lemma, and glumes, whereas, in the domesticated wheats the kernels are loosely held by the glume and are free-threshing. *Triticum monococcum* L. is not exactly free-threshing, however. These differences between wild and domesticated wheats are apparently caused by gene mutations accompanied by selection under domestication (Sharma and Waines 1980).

The Tribe of *Triticeae* forms an important subdivision of the *Graminea* family. It holds fourteen genus sharing out, according to their morphologic characters, in two sub-classes, the *Hordeinae* and the *Triticinae*. The species such as barley (*Hordeum* ssp.) of the *Hordeinae* sub-class differentiates of the others by the presence of two or three spikelets per rachis. The species as rye (*Secale* ssp.) and wheat (*Triticum* ssp.) of the *Triticinae* under-tribe have usually just one spikelet per rachis (Feldman and Sears 1981). In wheat the inflorescence is a determinate, composite spike. Sessile spikelets are alternate on opposite sides of the rachis of the main axis of the spike, forming a true spike.

Fig I-2: A diagrammatic representation of the current theory of the evolution of wheat (Wheat Genetics Resource Center web site: http://www.ksu.edu/wgrc).



Even if the wild species have, probably, a common ancestor, they differentiate widely from each other, not only by their morphology but also by their geographic and ecological distributions. Cytogenetic analysis has confirmed the taxonomic classification and distinction. Each diploid species has a specific genome that is genetically isolated, in variable degrees, from others species. The polyploid wild species of *Triticum* constitute a classical example of evolution by amphiploidy (Feldman and Sears 1981).

- ***Triticum dicoccoides***

Wild emmer or *T. dicoccoides* (syn. *T. turgidum* ssp. *dicoccoides* Thell.) is the immediate progenitor of all cultivated forms of tetraploid and hexaploid wheats (Feldman and Sears 1981). The wild tetraploid is endemic primarily to the western arc of the Fertile Crescent from the upper Jordan valley to southeastern Anatolia (Kimber and Feldman 1987). It grows on terra rosa or basalt soil in the herbaceous cover of the oak park forest, dwarf shrub formations, pastures, abandoned fields and edges of cultivation.

T. dicoccoides is a tetraploid carrying two genomes A and B. It seems that its cytoplasm is similar to that of *Triticum longissimum* Schweinf. & Muschl. Harlan (1987) reported that *T. dicoccoides* was formed from *T. boeoticum* wild einkorn and the little weedy goat grass *Aegilops speltoides* Tausch. Wild emmer is distributed over the near East Fertile Crescent including parts of Palestine, south Syria, Jordan, southern Iraq and western Iran (Feldman and Sears 1981). Most certainly, wild tetraploid wheats were already largely distributed in the Near East when men started harvesting its grain and using its straw in nature. Their general size and particularly the size of head and the kernels made them much more worthwhile for domestication than diploid wheats. Their large grains attracted pre-agricultural collectors who eventually domesticated it, presumably, in the southern part of the Fertile Crescent (Kimber and Feldman 1987), probably in the Syrian Hauran plain.

Wild relatives of the cultivated wheats hold rich pools of genetic variation and carry many genes of great economic potential. *T. dicoccoides* possesses many useful characters. Among those desirables characters, Pagnotta *et al.* (1995) reported resistance to yellow and leaf rust, photosynthetic yield, tolerance against ecological stresses of drought, salt and herbicides, in addition to high tiller number, earliness, large grains and also high grain protein content. Avivi (1978) found that several collections of *T. dicoccoides* possess an exceptionally high percentage of grain protein (24-29%). Sharma *et al.* (1981) used five accessions of *T. dicoccoides* to compare with *T. monococcum* and *T. araraticum.* They also reported protein contents of up to 33.9 % in *T. dicoccoides*, the highest of the three species. Others also reported that *T. dicoccoides* possesses the higher grain protein content and nutritional value than all cultivated wheats (Nachit *et al.* 1996). An approach to improve pasta and bread making qualities could possibly be the exploitation of the tremendous variation for storage proteins existing in primitive cultivars, landraces and wild relatives of *Triticum* species. A practical attempt to introduce these genes into breeding material has been made. At ICARDA, advanced populations from crosses with *T. dicoccoides* showed high levels of resistance to tan spot (*Pyronophtora tritici repentis*), septoria (*Septoria tritici Blotch*), leaf blotch, yellow rust (*Puccinia striiformis*), heat, and cold (Nachit 1990; Nachit *et al.* 1995b). High protein breeding lines segregating from these crosses, without compromising yield potential of the parental varieties, have been selected. Elouafi *et al.* (1998) reported a positive association between a γ-gliadin subunit belonging to *T. dicoccoides* and the protein content in a RIL population derived from a backcross Korifla/*T. dicoccoides* 600808// Korifla.

The analysis of seed storage protein of *T. dicoccoides* has shown that this species is highly variable for HMW glutenin subunits (gs) encoded by the Glu-A1 and Glu-B1 HMW loci and for the B-group LMW gs (Damania *et al.* 1988; Nachit *et al.* 1995b).

Liu and Shepherd (1996) found 7 different LMW gs patterns in Kushnir's collection and 72 different B subunit patterns in Nevo's collection. These results are in accordance with those reported by Ciaffi *et al.* (1993) on *T. dicoccoides* lines from Jordan and Turkey. They also have observed enormous variation of LMW gs banding patterns beside the usual ω35/γ45/LMW2. The genetic diversity of these *T. dicoccoides* lines appeared geographically structured and partially predictable by ecology and alloenzyme markers (Liu and Shepherd 1996). It should be noted that protein bands with similar molecular weight could be derived from different protein alleles. Nevertheless it appeared that the greater band variation in the old tetraploid wheats than in durum cultivars indicated a greater variation of their protein alleles. The electrophoretic analysis has shown that *T. dicoccoides* alleles by the Glu-A1, Glu-B1, and Glu-B3 loci are uncommon among cultivated durum. Nachit *et al.* (1995b), by evaluating F6 progenies of *T. durum* x *T. dicoccoides*, they found that these progenies have allelic variants at the Glu-A1, Glu-B1, Gli-B1 and Glu-B3 loci, which are not usually present in durum. The presence of a wide polymorphism and the detection of unique subunits in this wild wheat would make worthwhile the assessment of the effect of different gluten components on technological properties (Ciaffi *et al.* 1991).

3. Cultivated wheats

Wheats belong to *Triticum* genus and as mentioned above, the basic chromosome number of this genus is seven (x=7). The cultivated species have different levels of ploidy as follows:

- diploid (2n=2x=14): *T. monococcum*;
- tetraploid (2n=4x=28): *Triticum turgidum* L. ssp. *durum* (Desf.) Husn, ssp. *Turgidum,* ssp. *turanicum* (Jakubz.) A.Löve & D.Löve (Khurasan wheat, Iran), ssp. *carthlicum* (Nevski) Á.Löve & D.Löve (Persian wheat), ssp. *polonicum* L. (Thell.) (Polish wheat), ssp. *dicoccum* and ssp. *T. timopheevii;*
- hexaploid (2n=6x=42): *T. aestivum* L. ssp. *aestivum,* ssp. *compactum* (Host) MacKey (Club wheat), ssp. *spelta* L. Thell. (Feldman and Sears 1981).

Uptoday, *T. monococcum* is still cultivated as feed for poultry and swine in the mountains of some Mediterranean countries (Italy, Spain, Turkey, etc...). Also, until recently *T. dicoccum* was cultivated in Ethiopia, Iran, Turkey, Caucasian region, and in several Mediterranean countries, in India it is called Khapli. Nevertheless, *T. aestivum* is the most widely cultivated form. Most of the actual varieties are suitable for bread making and are adapted to various environmental conditions and to a large scale of latitudes. *T. durum* is also intensively cultivated in dry region (Mediterranean Basin, India, ex-URSS) and regions with low rainfall (wide plains of North America).

- *Triticum durum*

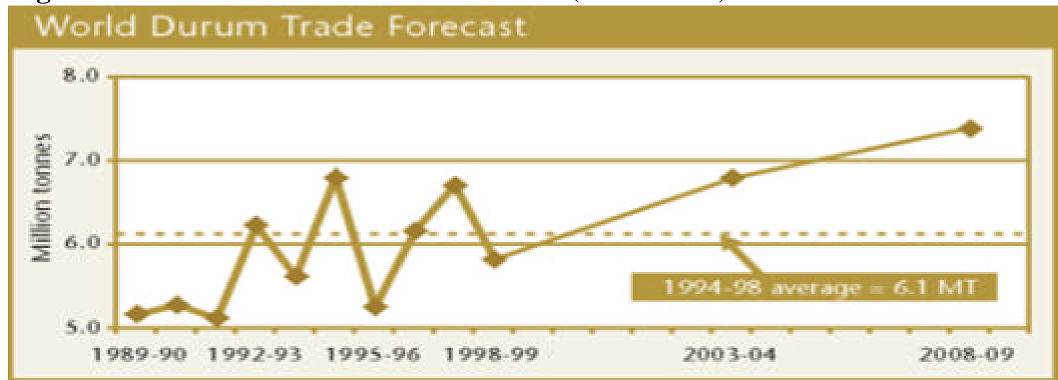
Durum wheat is one of the oldest cultivated plants in the world and is grown mainly in the middle and near East region and North Africa, which are considered the centers of origin and diversification of this crop (Vavilov 1951). Based on archeological evidence it is generally accepted that durum wheat was domesticated at least 2000 years before bread wheat (Morris and Sears 1967) during the late Mesolithic period and the early Neolithic age (Harlan 1986). The adaptation of durum wheat largely overlaps that of bread wheat, but is less widely grown (Autrique *et al.* 1996). On the other hand, durum wheat is better adapted to Mediterranean dryland than bread wheat. This is why

over 80 % of the total world durum wheat area is located in the Mediterranean basin (Porceddu *et al.* 1990) and this is why durum has been concentrated in the driest areas of the West Asia and North Africa (WANA) region. In addition, durum wheat trade expectations for the coming years show an increase of 21% by 2008 (CWB 2001).

Table I-1: Average durum area, yields, and production in selected major durum-producing countries, mean from 1973 to 1996 (Belaid 2000).

NARS	Area (000ha)	Yield (t/ha)	Production (000t)
Algeria	1193	0.71	835
Morocco	1208	1.15	1382
Syria	800	1.59	1252
Tunisia	755	0.97	742
Turkey	2904	1.62	4719
WANA	**6826**	**1.20**	**8917**
USA	1421	2.01	2838
Canada	1672	1.79	3012
Italy	1657	2.20	3646
Spain	238	2.00	459
Greece	394	2.33	947
World	**17800**	**1.48**	**26309**

Fig I-3: World durum wheat trade forecast (CWB 2001).



Durum wheat is not only a food crop, but it also has a broad use in the industrial sector and in animal feeding. However, the most peculiar characteristics of the wheat kernel, elasticity and extensibility of the gluten complex, which confers the viscoelastic properties of the dough, make durum wheat well suited for pasta, as well as bread, couscous, burghul, frike and other local food products. In fact, those end-products have various priorities according to the country (Table I-2). Generally, these end-products require large vitreous kernels with high protein content, good yellow pigment and strong gluten.

Table I-2: Durum use and quality requirements in different part of the world (Nachit *et al.* 1992)

Country/region	Use	Requirement
North Africa, Europe, America, Australia	Pasta	High Vitreousness, medium to strong gluten, high yellow pigments.
North Africa	Couscous, Bread	High Vitreousness, medium to strong gluten, high yellow pigments.
Middle East	Unleavened bread	Medium to strong gluten
Near and Middle East	Burghul	High Vitreousness, medium to strong gluten, yellow pigment.
Andean region	Mote	Hard grain.

Durum wheat kernel is normally hard and similar in protein level to the hexaploid hard wheats. Virtually all varieties have amber, vitreous, and rather large kernels. The protein content is usually about 13%, but may reach 22%. High protein content, however, does not always guarantee optimum cooking quality (Ciaffi *et al.* 1991; Blanco and Giovanni 1996).

Durum wheat is an allotetraploid with an extremely large genome of (16 x 10^9 bp/genome) (Bennett and Smith 1976) with more than 80% repetitive DNA. Understanding its genetic and genome organization using molecular markers is of great interest to geneticists and plant breeders. It will allow more efficiency in selection and in parental choices for crosses in the breeding programs.

III- GENETIC MAPPING

A- WHEAT MAPPING AND MAPS

A primary genetic map, consisting of easily scored polymorphism marker loci spaced through a genome, is an essential prerequisite to detailed genetic studies in any organism. Furthermore saturated linkage maps are essential tools for genetic studies like positional gene cloning, quantitative trait mapping, and marker-assisted selection. Classically, it has been possible to construct such linkage maps only in intensively studied organisms, such as bacteria, yeast or fruit flies, in which many visible mutations were available as genetic markers. Since the beginning of the 80s, this limitation has been overcome because of the development of many molecular marker techniques allowing a visualization of existing polymorphism at DNA level.

Molecular linkage maps have been constructed for many organisms, including human (Schumm *et al.* 1985, White *et al.* 1985; Celera Genomics 2000), maize (Helentjaris *et al.* 1986), the mustard *Arabidopsis thaliana* (http://www.nature.com/genomics/papers/a_thaliana 2001), rice (McCouch *et al.* 1988, Saito *et al.* 1991, Causse *et al.* 1994, Kurata *et al.* 1994, http://www.myriad.com/gdd_rice.html 2001), tomato (Paterson *et al.* 1988, Tanksley *et al*. 1992, de Vicente and Tanksley 1993, Saliba-Colombani *et al.* 2000), barley (Heun *et al*. 1991, Graner *et al.* 1991, 1994; Kleinhofs *et al.* 1993), diploid and hexaploid oat (O'Donoughue *et al.* 1992, 1995), *T. tauschii* (Gill *et al.* 1991, Boyko *et al.* 1999), diploid relatives to wheat (Gill *et al.* 1991, Lagudah *et al.* 1991), hexaploid wheat (Chao *et al.* 1989, Liu and Tsunewaki 1991, Devos *et al.* 1992, Devos and Gale 1993; Xie *et al*. 1993, Nelson *et al.* 1995a,b,c; van Deynze *et al.* 1995a, Jia *et al.* 1996, Röder *et al.* 1998) and more recently durum wheat (Blanco *et al.* 1998, Korzun *et al.* 1999, Nachit *et al*. 2001). The construction of genetic linkage maps relies on the choice of parental lines, segregating population, and markers to reveal polymorphism. Ease in saturating a molecular map is strongly dependent on the polymorphism revealed between the parental lines. The genetic maps can be constructed based on F2, F3 families, backcrosses or Recombined Inbred Lines (RILs). The utilization of RILs have many advantages over the other kind of populations (Saliba-Colombi *et al.* 2000): i) since they can be reproduced easily, RILs favor the genetic analysis of quantitative traits because experiments can be replicated over years and environments; ii) due to their low level of heterozygosity, dominant markers can be used with the same efficiency as the co-dominant ones; and iii) several generation of selfing increases the probability of recombination between genes. Maps constructed with such material could, therefore, facilitate the fine mapping of regions around genes of interest.

Physical maps constructed using C-bands as genetic markers (Curtis and Lukazenwski 1991) and deletion stocks (Werner *et al.* 1992, Kota *et al.* 1993) have given important insights into the relationship between physical and genetic distance in wheat. The deletion stocks are powerful tools for constructing physical maps as they eliminate the requirement for intragenomic polymorphism and can be used to localize agronomically important genes to relatively small chromosomal regions. On the genetic wheat maps, the markers largely clustered around the centromere, indicating that there is more recombination in the distal portions of the chromosomes (Delaney *et al.* 1995). In addition, physical mapping have revealed that markers, which genetically map near

the centromere are physically located at a considerable distance from it (Dvorak and Chen 1984). On the physical maps, in wheat, most markers were tightly clustered in small sized physical segments. These markers were identified to represent expressed genes. Gill *et al.* (1996) compared physical maps with recombination-based maps and found that these gene-rich regions undergo recombination much more frequently than do gene-poor regions. Thurieaux (1977) postulated that recombination is confined to coding regions because different eukaryotic organisms have essentially the same number of genes, and the number of map units per genome is relatively constant even though the physical sizes of the genomes vary. Bread wheat genome is six times larger than that of maize and 35 times larger than the rice genome (Bennett and Smith 1976). Nevertheless, the amount of actively transcribing DNA is probably not much different among the 3 species, implying that <3% of the wheat genome represent genes. Those genes in wheat may be present in uninterrupted clusters, individually interspersed by repetitive DNA blocks, or in a combination of the two arrangements (Gill *et al.* 1996). An individual chromosome contains a single molecule of double stranded DNA. Its length is approximately constant of any given chromosome in a species but varies between chromosomes. Typically, they each consist of something of the order of 10^7 to 10^8 bp. A typical structural gene, coding for polypeptide chain, is between 1 and 2 Kbp long and only 10% of the genome is actually coding, much of the rest being spacer DNA, although the amount of spacer DNA can vary considerably between species. Thus a chromosome probably contains something of the orders of 1000 to 10.000 genes. Therefore Gill and Gill (1994) proposed a mapping strategy to target gene rich regions of wheat. This technique is called a Cytogenetic Ladder Mapping (CLM). The CLM strategy not only efficiently identifies the gene clusters but also preferentially maps them. The gene rich regions in wheat genome may be as amenable to molecular manipulation as are the smaller genomes of plants such as rice (Gill *et al.* 1996, Faris *et al.* 2000).

Dense genetic maps of related crops species provide breeders with multiple choices of markers for tagging desired genes. Moreover, comparison of the chromosomal assignments and orders of marker loci common to several genomic maps may shed light on ancestral chromosomal rearrangements and on evolutionary relationships between different chromosomes. The three species wheat, rice and maize from the grass family *Poaceae* have similar gene composition and colinearity (Ahn and Tanksley 1993, Ahn *et al.* 1993). Therefore, information gained in one crop can be applied directly to the other. The construction of a grass-genome map detailing the gene and DNA sequence similarities between the genomes of the many species of the *Graminea* will enable genetic studies in relatively small genomes, such as rice, to be applied to the much larger wheat genomes, as for instance in identifying and tagging important pest resistance and stress tolerance genes. Comparative maps allow the transfer of information about genetic control of traits from species with small diploid genomes, such as rice (400 millions base pairs/haploid genome), to species with more complex genomic structures, such as durum (tetraploid) and bread (hexaploid) wheat (16.000 Mb/haploid genome) (Sorrells *et al.* 2000). Because of the size and complexity of the genomes, it may not be appropriate to sequence the entire genomes of wheat (*Triticum* ssp.), rye (*Secale cereale* L.), oat (*Avena sativa* L.), or barley (*Hordeum vulgare* L.). However, alternative strategies involving identification of gene-rich regions of the *Triticeae* genome and comparison of the genome structure and genetic colinearity with rice, maize (*Zea mays* L.), sorghum (*Sorghum vulgare* L.), and other species provide *Triticeae* researchers with the knowledge and tools necessary for

genetic parity with simpler genomes. The maps of several members of *Graminea* family were compared and the synteny of these genomes was defined (Moore *et al*. 1995). To date, most comparative mapping among the grasses has relied on RFLP probes (cDNAs or genomic clones) to establish gross gene orders and distance in specific chromosome segments. Only to a limited extent have researchers employed cloned genes, Expressed Sequence Tagged (ESTs), mutant phenotype loci or QTLs in comparative genomics. In wheat, the transfer of information from mapping reference populations to agronomic crosses should take into account homoeology relationships.

Maps comparison have been made in maize (Beavis and Grant 1991; Murigneux *et al*. 1993), barley (Sherman *et al*. 1995), *T. durum* 6A and 6B chromosomes (Chen *et al*. 1994) and wheat (van Deynze *et al*. 1995b) in order to analyze colinearity of markers and to study recombination (Cadalen *et al*. 1997). Comparisons of molecular maps of wheat, barley, *T. tauschii* and *T. monococcum* (Devos *et al*. 1993; Nelson *et al*. 1995ab; van Deynze *et al*. 1995b) indicate that the order of molecular markers on the linkage maps of these species detected with the same probes are largely homosequential. As a result, consensus maps based on species-specific maps from wheat, *T. tauschii*, and barley were developed using wheat as a base for comparison. These consensus maps efficiently combine genetic information accumulated for related grass species (wheat, *T. tauschii* and *Hordeum* species) for comparisons to more distantly related species (rice, maize and oat). They help to circumvent problems with low polymorphism between mapping parents by providing relative marker location information across several maps. For example, 116 markers on the consensus map for group 1 chromosomes provide relative order information for 288 unique markers from the individual linkage maps combined (van Deynze *et al*. 1995b).

Comparative maps can be used for improvement of *Triticeae* species by combining information accumulated in other species. They may be used to saturate species specific maps in a targeted region or simply to develop linkage maps in species such as wheat that may have a low level of polymorphism among parental lines. To construct a new map in durum, for example, both consensus maps and comparative maps can be used to choose the most appropriate probes. Such probes can be chosen to provide genome coverage at the desired resolution and for close linkage to previously mapped agronomic trait loci.

The information gained by identifying orthologous loci for the numerous previously characterized mutants and expressed sequence tags in rice (Kinoshita 1993; Uemda *et al*. 1994; Kurata *et al*. 1994) and wheat (*http://wheat.pw.usda.gov/genome*) may advance genetic research in *Triticeae* species. The relationships between gene products and physiology of plants for particular traits must be known to define orthologous loci between species. Genes affecting what appear to be totally different characters may be the result of orthologous gene loci that differ in expression or interaction with other genes (Darling and Abbott 1992). This technique become even stronger since a major milestone in biology has been achieved with the full genome sequencing of *Arabidopsis thaliana* (http://www.nature.com/genomics/papers/a_thaliana) and the full genome sequencing of rice (http://www.myriad.com/gdd_rice.html).

B- MOLECULAR MARKER TECHNIQUES

During the first half of the last century, most genetic analysis of variation involved looking at fairly gross morphological, anatomical or behavioral differences,

major mutants in other words. Subsequently in the 1950s and 1960s, it was possible to look at more subtle variation in the structure of polypeptides and, more recently, since the 1980s it has become possible to explore variation at the level of DNA itself. Actually, the recent advances in techniques for DNA analysis and subsequent data analysis have greatly increased our ability to understand the genetic relationship among organisms at the molecular level.

The potential usefulness of genetic markers as an instrument for the plant breeder was recognized almost 80 years ago (Sax 1923). However, until the past 20 years its application was largely hindered by the lack of suitable markers. The molecular markers have several advantages over morphological markers (Melchinger *et al.* 1990):

(1). Numerous markers can be identified in breeding materiel.
(2). A relatively large number of alleles can be found.
(3). Most molecular markers show codominant mode of inheritance.
(4). Molecular markers are generally silent in their effect on the phenotype.
(5). Genotypes of most molecular markers can be determined at a very early developmental stage, allowing early screening methods to be applied.

In contrast to hexaploid wheat and to diploid relatives of durum for which several maps have been developed relatively little attention was been given to developing genetic linkage maps for durum wheat (Nachit *et al.* 2001). It was only recently that the first linkage maps of the chromosomes of durum were published. The first map was based on 65 recombinant inbred lines and RFLP markers (Blanco *et al.* 1998); and later microsatellites markers from hexaploid bread wheat were integrated into this genetic linkage map (Korzun *et al.* 1999). The second durum map was developed at our laboratory based on an intraspecific cross Jennah Khetifa x Cham1 with 110 RILS using RFLPs, SSRs, AFLPs, seed storage proteins, and genes (Nachit *et al*. 2001). Furthermore, other *Triticeae* species genetic linkage maps (*T. monococcum, T. tauschii*, rye, barley, etc.) and molecular marker analyses can be of immense help for durum mapping and QTL research using comparative mapping techniques. Comparative genetics research has the general goal of estimating similarity at some level of organization. The discovery of structure or patterns in the relationships among species can lead to new knowledge, hypotheses, and predictions about those species. The evolution of comparative genetics research from the whole plant level to the DNA level will greatly expand our knowledge of genome structure and function because of the diverse approaches scientists take in studying different species.

There are many molecular marker techniques that could be used for genetic linkage mapping. They can easily being divided as based or not on PCR amplification. So I will review the RFLP technique, and the based PCR techniques: Microsatellites and AFLPs.

1- Non- PCR Based Techniques

a. Restriction Fragments Length Polymorphism (RFLP)

One of the techniques derived from the progress in molecular biology research whose application in crop genetics and breeding has already produced very interesting results is the analysis of RFLPs. RFLPs were first proposed by Botstein *et al.* (1980), to

be used as genetic markers. This technique reflects differences in homologous DNA sequences that alter the length of restriction fragments obtained by digestion with a type of restriction enzymes. These differences result from base pair changes or other rearrangements (translocations or inversions) at the recognition site of restriction enzyme or from internal deletion/insertion events. The restriction fragments are separated according to their size by agarose gel electrophoresis .

Furthermore, as genetic markers RFLPs have some convenient properties. They are codominantly expressed; do not have pleiotropic effects on agronomic traits and the number of possible markers provided is virtually infinite (Beckmann and Soller 1986). Another advantage of RFLPs over biochemical markers such as isozymes, which require different staining and electrophoretic techniques for each isozyme, is that the same laboratory method can be used to detect the hybridization patterns of all the available probes. In addition, RFLPs detect much more polymorphism than biochemical markers because many of the probes are non-coding, i.e., less conserved sequences (Vaccino *et al.* 1993).

RFLPs have furnished a very powerful method, which can be used in virtually any plant species, to obtain detailed maps of genetic linkage. In fact, this technique was the most used for genetic mapping. RFLPs have already allowed the construction of high resolution genetic maps for many crops such as barley, maize, tomato, potato, soybean, rice, and lettuce. Such RFLP maps can be used to analyze Quantitative Trait Loci (QTLs) and can increase the selection efficiency for these traits (Heun *et al.* 1991). This technique has also made possible the dissection of quantitative traits into Mendelian factors (Stiles *et al.* 1993).
In addition, RFLPs markers have been used for variety identification in potato, beets, maize, and barley (Vaccino *et al.* 1993). At ICARDA, in collaboration with Cornell University, primers were constructed for many clones, which are available for mapping and marker-assisted selection in the durum breeding project. The obtained clone CDO482, for example, is linked with leaf rust resistance (Nachit *et al.* 1995b).

The main drawbacks of RFLPs assays are that they require expensive laboratory supplies and are rather time consuming. Also, they require manipulation of radioactive isotopes. However, the development of non-radioactive detection methods is reducing the cost and simplifying the procedures (D'ovidio *et al.* 1994). But, still their use remains laborious, and the level of polymorphism can become limiting, especially for crops with a narrow genetic base such as cotton, soybean, wheat, and tomato (Grandillo and Tanksley 1996).

Although the progress in building wheat genetic maps has been steady, the use of RFLP markers in gene mapping has been slow because of the very limited level of polymorphism in wheat (Chao *et al.* 1989; Kam-Morgan *et al.* 1989; Liu *et al.* 1990; Cadalen *et al.* 1997). In cultivated wheats, the polymorphism level of RFLP is low, ranging from 20 to 38% (Liu *et al.* 1990, Chao *et al.* 1989). Because of this limited polymorphism, gene and genome mapping has required the use of populations derived from wide crosses. However, mapping many agronomically important genes or QTL, a major goal in plant breeding, requires informative markers in an intraspecific context. The recently published intraspecific durum map from our laboratory meets this requirement (Nachit *et al.* 2001). This is particularly true for marker-assisted selection. RFLPs detected with single-copy genomic and cDNA clones are extremely powerful for

comparative mapping approaches (Ahn *et al*. 1993; Moore *et al.* 1995; Sherman *et al.* 1995; Yu *et al.* 1996). They are only of limited use for intraspecific molecular analysis of agronomic traits.

2- PCR-Based Techniques

A number of methods for the detection of DNA polymorphism have recently been reported. So far one of the most useful techniques in this respect seems to be PCR (Benito *et al.* 1993). This technique is an effective amplification of target known sequences. PCR has become the standard procedure in plant molecular biology because of its efficiency, ease, and versatility.

In fact, PCR offers a less technically demanding and more rapid methodology. Another particular advantage of this technique is that it does allow for the efficient screening of large populations and in principle, it can be developed for any targeted part of the genome where nucleotide-sequence information is either available or can be readily obtained from RFLP probes. The main advantage of PCR is that primer sequences can be shared and easily synthesized, obviating the need of exchange between labs of biological materials as required by clones for RFLP. The direction of genetic mapping programs is, therefore, tending to be focused on the conversion of an RFLP based to PCR based assay (Koebner 1995). Those PCR primers are called Simple Tagged Sequences (STS). The STS primer is a short, unique sequence that can be amplified by PCR and that identifies a known location on a chromosome. The simple interpretation of the single-locus make STS primers superior to multilocus DNA marker types, especially for map construction. PCR technique is also less expensive because there is no transfer, no hybridization and no radioactive isotopes. The fact that the DNA sequences are amplified with primers of known sequences, make this technique specific, reliable, and repeatable (Lashermes *et al.* 1994).

DNA polymorphism obtained with the PCR were used as genetic markers to tag genes, to fingerprinting viruses, fungi, bacteria, plants, and humans as well as to determine genetic relationships (Yu and Pauls 1993). And more recently, it has been used in genomics to gene identification.

While providing a powerful tool in biological research, PCR has its limitations. For instance, PCR can only efficiently amplify within a certain size range of DNA, and Taq DNA polymerase can introduce errors. The accumulated mutation rate after 20-30 cycles was reported to be as 0,3-0,8% (He *et al.* 1992). Also, a frequent observation has been that results from a particular primer pair may vary between laboratories (Lunz 1990, He *et al.* 1994). A primer pair that produces a product marking a particular chromosome region in one laboratory may not produce the same product when the experiment is repeated in another laboratory. This limits the utility of sharing primer sequences among laboratories. Of course, the relationship of the primer to the target sequence influences reproducibility of PCR. Therefore, a high specificity of the primer to the target sequence decreases mispriming and resultant amplification of extraneous DNA. This is why many studies were conducted on the definition of the ideal STS primer.

In general optimal PCR-primers should have the following criteria:

Specificity:
The primers should be short enough to be specific, preferably between 18 and 22bp. This length will maintain specificity and provide sufficient base pairing for stable duplex formation.

Free of dimers and hairpins:
The primers should be free of dimers and hairpins. Primers should not contain sequences of nucleotides, especially complementarity at the 3' end, that would allow one primer molecule to anneal to itself or to the other primer used in a PCR reaction (primer dimer formation). Once the primer dimer product is formed, it is a competing target for amplification. The primer should not contain complementary (palindromes) within themselves; that is, they should not form hairpins. If this state exists, a primer will fold back and can give rise to stable intrastand structures on itself that limit primer annealing to the template DNA resulting in an unproductive priming event, which decreases the overall signal obtained.

Form stable duplex:
Both primers in a PCR reaction should have similar melting temperature to ensure that they will have the same hybridization kinetics during the template-annealing phase. Primers with an overall G+C of 45-55% are most desirable and a very high GC content result in lower repeatability. The 5' and 3' end stability has to be taken in consideration also, low 3' stability enhances the repeatability.
The latter drawback may be that PCR require information on DNA sequences, which are not always available.

Because of the practicability and the high level of polymorphism found between the parents *Omrabi5* and *T. dicoccoides 600545*, we have opted for the use of microsatellites and AFLPs to construct the map of the population *Omrabi5/ T. dicoccoides // Omrabi5*. The microsatellites markers are used as anchor probes, whereas the AFLPs to saturate the map.

a- Microsatellites (SSR)

Up to 90% of the plant genome consist of repetitive DNA. Tandemly repetitive DNA is classified into three major classes (Tautz 1993):

(i). satellite DNA, which shows repeat units with a length of up to 300 base pairs (bp).

(ii). minisatellite comprised between 9 and 100 bp.

iii). microsatellite or simple sequences that exhibit repeats unit of 1-4 bp in length (Haman *et al.* 1995).

According to Beckmann and Soller (1990), the most informative STS marker appears to be one that amplifies a DNA region containing a microsatellite repeat sequences. Such an STS-based marker has been referred to as a Simple Sequence Length Polymorphism (SSLP) or Sequence Tagged Microsatellite Site (STMS). Microsatellites consist of a small repeat unit, generally less than four nucleotides, that generate repeating regions less than 100 bp (Thomas and Scott 1993). Microsatellites are either dinucleotides as $(GT)_n$; $(CT)_n$; $(GA)_n$, trinucleotides as $(CAC)_n$ or tetranucleotides as $(GACA)_n$ and $(GATA)_n$. It was observed that such microsatellites

show a high frequency of variation in the number of repeats in different individuals or accessions, probably due to slippage during DNA replication (Röder *et al.* 1994). The genomes of all eukaryotes contain microsatellites (Tautz *et al.* 1986). Microsatellites with tandem repeats of a basic motif of <6 bp have emerged as an important source of ubiquitous genetic markers for many eukaryotic genomes (Wang *et al.* 1994). This kind of polymorphism at specific loci is easily detectable using specific primers in the flanking regions of such loci and subsequent amplification via PCR. The high level of polymorphism, combined with a high interspersion rate, makes microsatellites an abundant source of genetic markers. They show, generally, high levels of genetic polymorphism (average of 3,5 alleles per locus) (Bryan *et al.* 1997).

The analysis of microsatellites is based on PCR, which is as mentioned above much easier to perform than RFLP analysis and is highly amenable to automation. In plants, it has been demonstrated that microsatellites are highly informative, locus-specific markers in many species (Condit and Hubbell 1991; Akkaya *et al.* 1992; Lagercrantz *et al.* 1993; Senior and Heun 1993; Wu and Tanksley 1993; Bell and Ecker 1994; Liu *et al.* 1996; Morchen *et al.* 1996; Provan *et al.* 1996; Szewc-Mcfadden *et al.* 1996; Taramino and Tingey 1996; Smulders *et al.* 1997).
So far, extensive genetic maps using microsatellite markers have been constructed on this basis for human and various other mammalian species such as mouse, cattle, and swine (Röder *et al.* 1994). Microsatellites were also used as genetic markers in many other species, such as wheat, rice, barley, maize, *arabidopsis*, grape, and soybean (Xiao *et al.* 1996). Wu and Tanksley (1993) demonstrated that in rice the microsatellites show much more polymorphism than RFLPs markers. This suggests that such markers would be ideally suited to studying genetic diversity in rice (Xiao *et al.* 1996). Microsatellites show also a much higher level of polymorphism and informativness in hexaploid wheat that any other system (Plashke *et al.* 1995, Röder *et al.* 1995, Ma *et al.* 1996, Bryan *et al.* 1997).

Devey *et al.* (1996) used microsatellites markers combined with RFLPs and RAPDs markers to construct a genetic linkage map for *Pinus radiata*. They found that, as in other species, microsatellites are highly polymorphic in pine. For example, in contrast with pine RFLPs, microsatellites only revealed a single locus per primer pair, which makes genetic interpretation much easier (Devey *et al.* 1996). However generally, microsatellites are multiallelic, and therefore have high potential for use in evolutionary studies (Schloetterer *et al.* 1991; Buchanan *et al.* 1994) and genetic relationships. Bread wheat microsatellites were also reported to be successfully used in durum x durum wheat population -Jennah Khetifa x Cham1 (Nachit *et al.* 2001). The majority of microsatellite markers constitute genome-specific markers, with some exception, such as Gwm666 that amplified 5 sites all mapping to the A genome in ITMI (International *Triticeae* Mapping Initiative) population (Röder *et al.* 1998) and amplified 3 sites in *T. durum* x *T. dicoccoides* population mapped to the 3A, 5A and 7A (Korzun *et al.* 1999). But, according to Röder *et al.* (1995) these types of PCR markers may not transfer well to among species as RFLPs do. These findings are confirmed by Bryan *et al.* (1997) who showed a low level of transferability across the 3 wheat genomes and to other cereal genomes.

As already mentioned, these regions are highly interspersed throughout eukaryotic genomes. Some of them have a microsatellite sequence once every 10kb (Thomas and Scott 1993). In plants, the frequency and number of several microsatellite

types have been estimated for many plant species. The results from these studies indicate that $(AT)_n$ is far more abundant in plants than in mammalian species, where the dinucleotide repeat $(AC)_n$ is the most abundant microsatellite type followed by (AG)n (Ma *et al.* 1991). In wheat, the total number of $(GA)_n$ blocks was estimated to be $3{,}6 \times 10^4$ and the number of $(GT)_n$ blocks to be $2{,}3 \times 10^4$ per haploid wheat genome. It seems that wheat microsatellites are relatively long containing up to 40 dinucleotide repeats (Röder *et al.* 1994). The smaller insert libraries tended to give higher estimates of the abundance of $(AC)_n$ and $(AG)_n$ sites than libraries with larger inserts (Condit and Hubbell 1991, Röder and Sorrells 1996). In addition, the wheat genome is also rich in trinucleotide repeats. For $(TCT)_n$ and $(TTG)_n$ together 2.3×10^4 sites were estimated while the tetranucleotide repeats appear to be rare (Röder and Sorrells 1996). The abundance of trinucleotide repeats should be given special attention, because it has been demonstrated that trinucleotide microsatellites are highly polymorphic and are stably inherited in human genome (Hearne *et al.* 1992). Nevertheless, most developed microsatellites so far are based on dinucleotide repeats.

The majority of microsatellites are inherited in a codominant manner and, in most cases, they are chromosome-specific. This mode of inheritance permits an easy transfer of markers between genetic maps of different crosses. In contrast to the dominant PCR marker type based on arbitrary primers, which requires the generation of a new map for each cross (Thomas and Scott 1993). Microsatellites besides being abundant, highly polymorphic are evenly distributed over the genome and require only small amounts of genomic DNA for analysis. Therefore they are highly suitable as genetic markers in wheat for mapping agronomically important genes. Furthermore, the analysis of microsatellites can easily be automated and applied to large plant numbers, as has been shown for microsatellites analysis in the human genome (Mannfield *et al.* 1994).
It seems that microsatellites in plants can be up to tenfold more variable than other marker system such as RFLPs. Microsatellites, therefore, may represent a very useful genetic marker system for the genetic mapping of species with little intraspecific polymorphism, as it is the case in most of the breeding crops. As mentioned before, in *T. aestivum*, microsatellites show a much higher level of polymorphism and informativness than any other marker system. However, due to the large genome size, the development of microsatellite markers in wheat is extremely time-consuming and expensive. Only 30% of all primer pairs developed from microsatellite sequences are functional and suitable for genetic analysis (Röder *et al.* 1995; Bryan *et al.* 1997). This low percentage is currently the major limitation on large-scale development of microsatellite markers for wheat. These problems may be related to the complex genome of wheat, which contains a large fraction of repetitive DNA. Obviously, with the high levels of repetitive sequences present in the wheat genome, it is likely that a relative large number of microsatellites will be present in flanking DNA sequences that are themselves repetitive sequences (Bryan *et al.* 1997). Isolation of microsatellites from libraries, which are enriched in single copy and low copy number sequences, may improve the success rate (Röder *et al.* 1994). The use of methylation sensitive restriction digests for the initial library construction may reduce the proportion of non-useful amplification products (Bryan *et al.* 1997). Röder *et al.* (1998) confirmed that an effective way to increase the efficiency of functional primer pairs is to use the under-methylated fraction of the wheat genome as a source for microsatellite isolation. She reported an increase of the success rate of functional primers from 31 to 68% by using a predigestion with PstI and subsequent isolation of the fragments in the size range of 2-5

Kb before digestion with a 4bp restriction enzyme (MboI or Sau3A) and cloning. Thus, as has been shown for the isolation of single-copy RFLP clones from plants with large genomes, predigestion with the CNG methylation-sensitive restriction enzyme *Pst*I creates a fraction that is highly enriched for low- and single-copy DNA.

The amplification of microsatellite markers has been also limited by the high costs during marker development, mainly due to excessive sequencing and primer analysis (Schondelmaier *et al.* 1996). So far microsatellites were developed for some crops such as: Olive (Rallo *et al.* 2000), barley (Liu *et al.* 1996) Soybean (Akkaya *et al.* 1992) and others.

b- Amplified Fragment Length Polymorphism (AFLP)

A more recently developed method, which is equally applicable to all species and is highly reproducible (Vos *et al.* 1995), is termed Amplified Fragment Length Polymorphism (AFLP). AFLP technique has become a synonym for a new powerful marker technology. This technique is based on the selective amplification of a limited number of DNA restriction fragments cut out of complex plant genomic DNA by restriction enzymes. AFLP analysis involves three steps:

- restriction of the DNA and ligation of oligonucleotide adapters,

- selective amplification of sets of restriction fragments, and

- gel analysis of the amplified fragments.

Then the first step involves restriction digestion of the genomic DNA with two specific enzymes, one a rare cutter and the other a frequent cutter. Adaptors are then added to the ends of the fragments to provide known sequences for PCR amplification. These adaptors are necessary, because the restriction site sequence at the end of the fragments is insufficient for primer design. Short stretches of known sequence are added to the fragment ends through the use of a ligase (binding) enzyme. If the PCR amplification of the restricted fragments was then carried out, all the fragments would not be resolvable on a single gel. Primers are thus designed so that they incorporate the known adaptor sequence plus 1, 2 or 3 additional base pairs, (any one out of the four possible: A, G, C or T) depending on the genome size (Table I-3). PCR amplification will only occur where the primers are able to anneal to fragments, which have the adaptor sequence plus the complementary base pairs to the additional nucleotides. The additional base pairs are thus referred to as selective nucleotides. If one selective nucleotide is used, more fragments will be amplified than if two are used, and even fewer fragments will be amplified with three selective nucleotides. For some technological reasons, addition of more than three selective nucleotides result in some non-specific PCR amplification. Normally two separate selective rounds of PCR are carried out. In the first round only one selective nucleotide is used, whereas in the second round the same selective nucleotide plus one or two additional ones are used. In practice this results in 50-100 fragments being amplified, which can be separated on a polyacrylamide gel by electrophoresis.

Table I-3: Selective nucleotide selection

Type	Genome Size (bp X 10^6)	No. of EcoRI/MseI Fragments	Total n. of selective nucleotides	Primer pairs
Bacteria	5	10^2 - 10^4	2-3	+1/+1
Fungi	15	3 x 10^2 - 3 x 10^4	3-4	+1/+2 or +2/+2
Arabidopsis	100	5 x 10^4	5-6	+2/+3
Crop plants	400-6000	2 x 10^5	6	+3/+3
Large genome plants	15000-50000	2 x 10^5	6-7	+3/+3

The amplified products are either visualized after exposure to X-ray film, or by silver staining procedures. AFLPs provides an effective means of detecting several polymorphisms in a single assay. All the evidence so far indicates that they are as reproducible as RFLPs. AFLPs technique require more DNA (0.3-1.0 µg per reaction) and are more technically demanding than RAPDs, but their automatisation and the recent availability of kits means that geneticists and breeders can more extensively use this technology. A great advantage of AFLP technique is that it permits simultaneous identification of polymorphism at a large number of loci. Most AFLP fragments correspond to unique positions on the genomes, and hence can be exploited as landmarks in genetic and physical maps. AFLP technique can, then, bridge the gap between genetic and physical maps (Vos *et al.* 1995). But it is less efficient than RFLP for synteny studies (Tanksley *et al.* 1988). Rouppe *et al.* (1997) reported that AFLPs markers are locus specific but only at species level. Since the technique provides simultaneous coverage of many loci in a single assay and can be used to generate DNA fingerprints of the complexity required by altering the number of selective bases employed, it is proving to be an invaluable tool for studies of diversity, particularly in species where other generations markers, such as microsatellites, are not yet available (Donini *et al.* 1997). The AFLP markers can be used to detect corresponding genomic clones, e.g. Yeast Artificial Chromosomes (YACs), an AFLP marker will detect a single corresponding YAC clone in pools of as much as 100 YAC clones. Also, this technique may be used for fingerprinting of cloned DNA segments like cosmids, P1 clones, Bacterial Artificial Chromosomes (BACs) or YACs (Vos *et al.* 1995).

Using gel scanners heterozygotes can be identified. Otherwise AFLPs are dominant markers. AFLP technique is robust and reliable because stringent reaction conditions are used for primer annealing: the reliability of the RFLP technique is combined with the power of the PCR technique.

AFLPs have proven to be very important in the analysis of crop species and genetic diversity studies (Winfield *et al.* 1998; Paran *et al.* 1998). Donini *et al.* (1997) suggest the utilization of AFLP technique for the studies of temporal and spatial variation in DNA methylation as they show AFLP distinct organ amplification. They suggest that theses different AFLPs pattern obtained from different organs of the same plant is likely due to differences in DNA methylation between organs. So far, AFLP

technique contributed to the construction of many plant genome maps like melon (Wang *et al.* 1997), potato (Rouppe van der Voort *et al.* 1997) barley (Becker *et al.* 1995) eucalyptus (Marques *et al.* 1998), and durum wheat (Nachit *et al.* 2001).

III- Quality QTLs Determination

Phenotypes of the majority of traits in nature and agriculture are continuous variables. This continuous distribution has been attributed to the collective action of many genes -termed quantitative trait loci (QTLs) (Geldermann 1975) interacting with environment (Johanssen 1909). Therefore quantitative traits are those controlled by naturally occurring allelic variation at several genes, which are influenced variably by environmental conditions. The wheat grain quality traits are considered to be inherited as quantitative traits as it is known to be controlled by a group of genes and being very affected by environmental variations (Kuspira and Unran 1957, Diehl *et al.* 1978, Nachit *et al.* 1995a, Porceddu *et al.* 1990). The quality cannot be expressed in terms of a single property, but depend on several milling, chemical, baking, processing, and physical dough characteristics; each one of them is important in the production of each end-product. Positioning QTLs on genetic maps is a powerful technique to portion quantitative traits on Mendelian genes, especially that more genetic maps are available now (Paterson *et al.* 1991). Several QTLs associated with important traits have been identified in many crops including QTLs related to yield, Heading date, pollen sterility, and root morphology in rice (Ray *et al.* 1996; Doi *et al.* 1998; Lin *et al.* 1996; Moncada *et al.* 2001); QTLs associated with agronomic performance, grain and malt quality, and disease characters in barley (Backes *et al.* 1995; Tinker *et al.* 1996; Mather *et al.* 1997; Zhu *et al.* 1999; Igartua *et al.* 2000); QTls for amylose content, disease resistance, and grain-yield in wheat (Araki *et al.* 1999; Waldron *et al.* 1999; Kato *et al.* 2000).

Durum wheat is mainly grown in the Mediterranean region for the production of pasta, couscous, burghul, frike, kichk, and bread (Nachit 1989). Each end-product requires its own special quality parameters. Although the processing of those end-products has been practiced for thousands of years, there is currently an increased need to understand the relationship between protein composition and grain flour functionality, to define the most critical criteria and their effect on the end-product quality. Durum wheat normally has an amber vitreous kernel that produces a yellow milling product (semolina). In contrast to bread wheat, the durum is used mainly for its semolina production, which is the desired main end-product in durum, not flour. The major milling fractions are bran, embryo, semolina, and flour. Semolina is defined as the purified middling of durum wheat that will pass through a N°20 US sieve, of which not more than 3% will pass through a N°100 US sieve. This fraction is used to produce pasta, couscous, and other local end-products. Whereas, durum flour is the purified endosperm of durum wheat that is ground fines enough to pass through a N°100 US sieve (Heyare *et al.* 1987). The flour is used mainly for noodles and durum-bread. Color is of prime importance in semolina, since the consumer generally expects yellow pasta products. The source of color in durum semolina is the xanthophylls, especially lutein (Nachit *et al.* 1995a). As for burghul, whole grain is used after debraning and cracking.

Therefore, the quality of end product is related to the quality of the durum grain, which, in turn, is mainly determined by the genotype, but also by the environment (weather and nutrition) and crop management. For pasta, couscous, and burghul, the commercial value of the end-product depends on the total amount of storage proteins and their amino acid composition. Seed storage protein content has an important effect on these endproducts making technology characteristics. Starch, vitamins and mineral substances, although of importance, are less critical since they are generally also found in other human foods. Neverthe less, the amylose/amylopectin ratio of starch is extremely important because it affects the quality of some bread wheat end-uses, e.g.; noodles (Araki *et al.* 1999). Indeed, the starch is the major component of the wheat grain, making up 65-70% of the dry weight of mature grain and its potential use is still subject to debate (Rahman *et al.* 1995; Araki *et al.* 1999).

Table I-4: Wheat Proteins

Groups	Solubility	Structure	Properties	Content (%)	Origin
Albumins	Water		Enzymic	15–20	Cytoplasmic proteins
Globulins	Neutral salt		Foaming, emulsifying		
Gliadins	70% ethanol	Low molecular weight	Extensibility	40	Storage proteins
Glutenins	Acids, bases, hydrogen and hydrophobic disrupting agents	High molecular weight (>100,000)	Elasticity, tenacity	40	

A- Seed Storage Proteins

Functional properties of durum flour products are mainly determined by their composition of seed storage proteins. Seed storage proteins are accumulated in the endosperm of the developing grain through compartmentalization within subcellular organelles known as protein bodies (Pernollet 1978). These endosperm proteins constitute more than 80% of the total proteins and consist, predominantly, of two classes termed gliadins and glutenins, so classified on the basis of their solubility in different solvents and their amino-acid composition. The gliadins are alcoho-solubles and do not form intermolecular disulphide bonds (Wall 1979), whereas glutenins are alcoho-insolubles and are very aggregated. During the processing these proteins are converted to gluten.

The two protein groups have very different biophysical properties: the glutenins are largely responsible for gluten viscoelasticity whereas the gliadins act to plasticise the gluten mass (Shewry *et al.* 1999). The viscoelasticity being the property of a substance that enables it to change its length, volume, or shape in direct response to a force effecting such a change and to recover its original form upon the removal of the force, and being plastic is the capability of being modelled. Liu and Shepherd (1996)

reported that the rate of proteins is the major contributor to durum dough strength. In fact, the wheat gluten consists of over 50 individual proteins (Sabelli and Shewry 1991).

Other than the utilization of the solubility criteria to classify wheat proteins, Shewry *et al.* (1986) proposed to classify them according to their chemical and therefore genetic similarities. So the wheat proteins are divided into two main classes, generally referred as monomeric and polymeric proteins, depending on whether they are consisted of single or multiple-chain polypeptides (MacRitchie and Lafiandra 1997).

A-1- Monomeric Proteins

The monomeric (or single) chain protein comprises 2 main groups: the gliadins and the albumins/globulins. The gliadins are structural proteins, whereas albumins and globulins are metabolic and include various enzymes.

A-1-1 Gliadins

The gliadin group has received a great deal of attention from scientists in the past. This was because of their technological importance and the ease of the screening technique. Gliadins are readily soluble in alcohol/water mixtures. They are composed of monomeric polypeptides (Table I-4) stabilized by intrachain disulphide bridges, except in ***w***-gliadins, which have no cysteine in their primary structure (Kasarda 1989). Gliadins are characterized by a high content of glutamine (30-55% of all amino-acid residue), proline (15-30%), and a small amount of lysine (Kasarda *et al.* 1976) (Table I-5).

Electrophoresis in acidic buffers separates wheat gliadins into four fractions: alpha (α), beta (β), gamma (γ), and omega (ω) gliadins, in order of decreasing mobility (Woychik *et al.* 1961). Molecular weight of gliadins range from 30 to 80 Kilodalton (Kd), the ω gliadins are clearly separated from other wheat polypeptides because their molecular weight (70 – 80 Kd) does not overlap with others. The ω gliadins are deficient in sulfur, but the other gliadins normally have an even number of cysteine residues, which form intramolecular disulphide bonds. The α and β gliadins are grouped in the same class designated as alpha-type because of their structural similarities in their N-terminal sequences (Kasarda *et al.* 1987). Synthesis of gliadin polypeptides shows both temporal and tissue specific regulation. Their synthesis begins in the endosperm 10-15 days after anthesis and continues until grain maturity (Reeves and Okita 1987).

In durum wheat most attention has been focused on the gliadin proteins. This follows the discovery by Damidaux *et al.* (1978) of a relationship between certain gliadins (γ–42 and γ–45) and gluten strength. Gamma 42 is associated with poor elastic recovery and γ–45 with high elastic recovery. Usually, the γ-42 component is associated with ω components 33, 35, 38 whereas γ-45 is linked to the ω-35. By molecular tools, the amplification of γ-gliadin showed the presence of 5 major bands, ranging between 750 and 1000 base pairs (bp). Genotypes with 900 bp correspond to γ42-gliadin genotype (e.g. Cham1) and with 950 bp to γ45 genotypes (*Omrabi5*) (Nachit *et al.* 1995a). Further work had shown that this very strong relationship is independent of the gliadin gene pool (Ducros 1982). In fact, there is no functional correlation between gliadin alleles and grain quality. They could only be considered as biochemical markers, while the glutenins play the major role for pasta quality (Damidaux *et al.* 1978, Payne *et al.* 1984). This idea was emphasize by the new combination ω 33-35-38/γ45/LMW1

found in Australian poulard landrace (ICDW12060) which has a poor grain quality even if it carry a γ45 (Table I-6) (Impiglia *et al.* 1996). Nevertheless, α–gliadins were also found to have different effects on gluten firmness (Pogna *et al.* 1990).

A number of cDNA or genomic clones coding for α-type gliadins from *T. aestivum* and in one case from *T. urartu* have been cloned and sequenced (Okita *et al.* 1985; Reeves and Okita 1987; Anderson 1991; Anderson *et al.* 1997). D'Ovidio *et al.* (1992), based on PCR amplification, reported a very high level of structural differentiation of the genes coding for α-type gliadins. Although a high level of conservation of the coding regions of the α-type gliadins in *T. durum*, *T. aestivum* and *T. urartu* was found. Similar results have already been obtained for ω and γ-gliadins sequences (D'Ovidio *et al.* 1990). In all genotypes containing the A genome, a high level of sequence conservation has been shown in the 5' and 3' flanking regions of a α-type gliadin gene located on chromosome 6A. Using NcoI digestion the same conservation in the 5' and 3' region was shown in genotypes containing D (*T. tauschii*) and S (*T. speltoides*) genomes (D'Ovidio *et al.* 1992).

Although the genetic control of seed storage proteins involves limited number of loci, which consequently cover only a small part of genome, a comparative analysis of glutenins and gliadins could provide additional information about the phylogenetic relationship among the three-*Triticum* diploid species and between them and the polyploid wheats. Ciaffi *et al.* (1997) reported that the gliadin composition of *T. urartu* resembled more that of A genome of polyploid wheats more than did *T. boeoticum* or *T. monococcum*, supporting the hypothesis that *T. urartu*, rather that *T. boeoticum*, is the donor of the A genome in cultivated wheats.

The first studies on the chromosomal location of genes coding for gliadin components in wheat were conducted mainly in hexaploid wheat because the tetraploid have only two pairs of homoeologous chromosomes, and it was, therefore, more difficult to produce aneuploids from them. Gliadin proteins are controlled by complex gene families located on the short arms of chromosomes of the homoeologous group 1 and 6 of A and B genomes (Payne *et al.* 1984). The genes coding for α and β gliadins are named Gli-A2 and Gli-B2 on chromosomes group 6 and most of the genes coding for γ and ω gliadins (named Gli-A1 and Gli-B1) on chromosomes 1A and 1B (Lafiandra *et al.* 1989). Gli-A1 and Gli-B1 loci are closely linked (about 2 cMs) to genes for glume hairiness (Hg1) and glume color (Rg1), respectively, permitting selection for high gluten strength, which is related to LMW-GS, whose coding loci are genetically linked to Gli-1, on the basis of head phenotype in segregating populations (Leisle *et al.* 1981).

Table I-5: Comparison of amino-acid analyses for glutenin, gliadins, and monomeric albumins (MacRitchie and Lafiandra 1997).

Amino-acid	Glutenin (mol%)	Gliadin (mol%)	Albumin, monomeric (mol%)
Cys(half)	2.6	3.3	8.1
Met	1.4	1.2	2.6
Asp	3.7	2.8	7.6
Thr	3.4	2.4	2.4
Ser	6.9	6.1	6.4
Glu	18.9	34.6	10.8
Pro	11.9	16.2	7.5
Gly	7.5	3.1	8.3
Ala	4.4	3.3	8.4
Val	4.8	4.8	11.3
Ile	3.7	4.3	1.7
Leu	6.5	6.9	7.6
Tyr	2.5	1.8	3.4
Phe	3.6	4.3	0.1
Lys	2.0	0.6	5.0
His	1.9	1.9	0.02
Arg	3.0	2.0	5.7
Trp	1.3	0.4	3.0

A-1-2- Monomeric Albumins/Globulins:

Albumins (soluble in water) and globulins (soluble in dilute salt solution) are a mixture of low molecular weight compounds. Albumins and globulins are cytoplasmic proteins with enzymatic activities with foaming and emulsifying properties (Table I-4). They are mainly located in the germ and aleurone layers and amount to 20% of the total proteins in the caryopsis, but the higher the protein content; the lower is the percentage (Feillet 1976). They are mostly of lower molecular weight than the gliadins (20000-30000 daltons). Their amino-acid composition is distinctly different from gluten proteins (gliadins and glutenins) (Table I-5).

Gluten proteins are characterized by unusually high contents of glutamic acid and proline, whereas the albumins and globulins have much lower glutamic acid content but a higher content in the essential amino acid lysine.

A-2- Polymeric proteins

Three main groups of protein constitute the multi-chain or polymeric proteins: glutenins, high molecular weight albumins and triticins.

A-2-1- Glutenins

Glutenins form the major portions (80%) of the polymeric proteins. Glutenins are similar to gliadins in their chemical composition (Table I-5), indicating similarities in genetic ancestry (MacRitchie and Lafiandra 1997). They represent about 30-40% of total flour proteins and are considered the most important contributors to the

viscoelastic properties of dough. The glutenins contain different polypeptides connected by intermolecular disulphide bonds. The polypeptides are called subunits and are subdivided into low molecular weight LMW (42-51 Kd) and high molecular weight HMW (95-136 Kd) according to their molecular weight when separated on sodium dodecyl sulphate polyacrylamide gel (SDS- PAGE) (Payne 1987). Glutenin is consisted of approximately 20% HMW glutenin subunits (gs) and 80% LMW gs.

a- High Molecular Weight Glutenins (HMW)

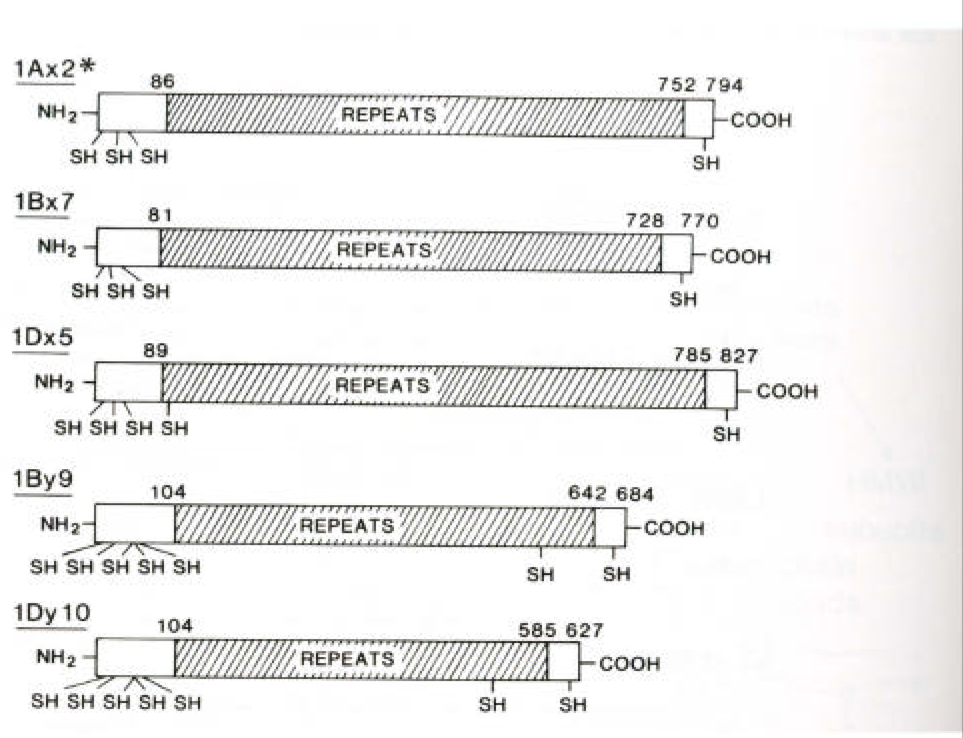
The HMW subunits of glutenin are one of the most widely studied groups of wheat prolamins, mainly because of their role on determining the breadmaking quality of bread wheat (Payne *et al.* 1984). The HMW gs can easily be distinguished from other storage proteins by their low mobility in sodium dodecyl sulphate polyacrylamide gel electrophoresis, SDS-PAGE, (Miflin *et al.* 1983).

The HMW gs are coded by the complex Glu-1 loci present on the long arm of the group 1 homoeologous chromosomes (Bietz *et al.* 1975, Lawrence and Shepherd 1981). Molecular analyses have indicated that each locus comprises two tightly linked genes encoding a lower y-type subunit and a higher x-type subunit (Harberd *et al.* 1986). Therefore bread wheat could in theory contain six different subunits. In fact only 3, 4, or 5 subunits are present in bread wheat cultivars. There are 2 coded by GluD1, either 1 or 2 by GluB1 and either 1 or none coded by GluA1 (Payne *et al.* 1981). In durum wheat, each genotype can carry from one to three HMW gs, only. There are several structural changes, such as point mutations, deletions, and duplications in HMW (Reddy and Appels 1993). Reddy and Appels (1993) also have reported that 1Ay silent gene exhibited maximum change in its amino-acid composition than other y genes. Mutations were localized mainly in the central repetitive region. Branlard *et al.* (1989) studied 502 varieties of durum wheat from 23 countries and found that most of them do not have HMW gs coded by locus Glu-A1, and that more than 83% of them carry the null allele (Glu-A1a). Vallega and Waines (1987) reported many *dicoccum* accessions carrying both null A and null B alleles, which indicates that the HMW gs, or at least those detected by SDS-PAGE, are not critical for the survival of cultivated tetraploid wheats. They reported also that *dicoccums* were found to be markedly different from both common and durum wheats in the frequency of each Glu-1 allele (Vallega and Waines 1987). In durum the variation at the Glu-B1 locus was found to be more extensive than that observed at Glu-A1 (13 Glu-B1 and 6 Glu-A1 alleles), and the same held true for *T. dicoccum* (14 Glu-B1 and 6 Glu-A1 alleles) (Vallega 1988). And also for durum wheat landraces, five allelic variants at the *Glu-A1* locus and 9 at the *Glu-B1* locus, with 19 possible *Glu-A1*/*Glu-B1* combinations were reported in a durum wheat world collection composed by 81 landraces (Impiglia *et al.* 1996). This phenomenon, which was also noticed amongst hexaploid cultivars, has been interpreted as supportive evidence to the hypothesis of a polyphyletic origin of the B genome of polyploid wheats (Vallega 1988). For a long time durum wheat has been regarded as unsuitable for bread making largely due to its poor gluten strength, attributed either to the absence of the D-genome chromosomes (Morris *et al.* 1966) or to the almost complete absence of *Glu-A1* alleles (Ducros 1987, Boggini and Pogna 1989).

Amino acid sequences deduced from genomic clones for subunits Ax2*, Bx7, Dx5, Dy10, Dx2, and Dy12 have shown that the typical x-type and y-type are made up of a large central domain, composed of repeated amino-acid sequences, which is flanked by

unique N- and C-terminal regions. A central repeated domain adopts a β-spiral structure (Fig I-4) (Reddy and Appels 1993).

Fig I-4: Schematic sequences of the five HMW subunits present in the good quality bread wheat cv. Cheyenne, deduced from the nucleotide sequences of cloned genes. Cysteine residues are indicated by SH (Shewry *et al.* 1995).



The differences in HMW subunit size result mainly from variation in the repeat structure, and in particular from differences in the number of hexapeptide and tripeptide motifs (Shewry *et al.* 1992). At GluB1, two alleles (7 and 20) were reported to possess only the x-type subunit, but the reversed phase high performance liquid chromatography technique (RP-HPLC) revealed that the subunit 20 is actually composed by two fragments: 20x and 20y (Margiotta *et al.* 1993).

The distribution of cysteine residues is of particular interest in relation to potential covalent cross-linking gluten. All subunits have a single cysteine in their C-terminal domain, while the x-type and the y-type subunits have 3 and 5 cysteines,

respectively in their N-terminal domains. This is due to a deletion of two adjacent cysteine residues in the x-type subunits. Furthermore, several subunits have an additional cysteine residue in their repetitive domain (Shewry *et al.* 1992). Kasarda (1989) reported that for elongation of glutenin polymers the presence of at least 2 cysteine residues available is necessary to form intermolecular disulphide bonds. A comparative study between subunit 20y and subunit 17 showed a lack of 2 cysteine residues, the second and third cysteine in the N-terminal region, in 20y (Buonocore *et al*. 1996). These results are confirming Kohler *et al.* (1993) findings related to the fact that the first and second cysteine in the x-type subunits are involved in an intra-molecular disulphide bond, meaning no effect on glutenin polymer formation, while the third and the fourth cysteines, present in the N and C-terminal regions, respectively, are available to form intermolecular disulphide linkages.

The most common HMW gs in durum wheat are coded by Glu-B1, they are 7+8, 20, 6+8. Autran and Feillet (1987) found that the HMWgs at the GluB1 locus are correlated with dough strength in the order 6+8 > 20 > 13+16. Boggini and Pogna (1989) reported another order (7+8 >> 20 > 6+8). In contrast, Liu and Shepherd (1996), reported that the correlation with dough strength fellows rather an order similar to that of bread wheat's order: 13+16 > 7+8 > 6+8 > 20. In fact, several recent studies have shown that band 20 is associated with the lowest strength; this finding was confirmed by our analysis on the durum cultivar "Korifla" (data not published). Pogna *et al.* (1990) found that genotypes containing LMW-2 and HMW gs 7+8 had the best gluten properties. These finding are in accordance with those of Payne (1987) who showed the additive effects of allelic variation at the Glu-A1 and Glu-A3 loci on dough quality, and with those of Liu and Shepherd (1996) who emphasize this results. "The protein genes at Glu1 and Glu3 loci influence quality characteristics predominantly in linear, cumulative fashion".

An increase in the number of HMW gs in durum wheat might produce an increase in gluten strength (Ciaffi *et al.* 1991, Nachit pers. com.). It has been reported that the introduction, in hexaploid wheats, of a Glu-A1 locus encoding two subunits from the A genome diploid relative, *Triticum thaoudar*, increases gluten strength (Rogers *et al.* 1989 by Ciaffi *et al.* 1991). Ciaffi *et al.* (1995) reported a 4% increase in the glutenin due to an introgression of a Glu-A1 y-type from *T. dicoccoides*. Thus, the increase in gluten strength and bread making properties associated with the presence of both 1Ax and 1Ay subunits may results from an increase of polymeric proteins due to the greater amount of the HMW subunits. But in fact, the association between HMW gs and quality characteristics of durum wheat has not been shown to be as close as that in the bread wheat, although certain HMW alleles can influence the viscoelastic properties of durum wheat dough (Autran and Feillet 1987). One approach to developing new cultivars of durum wheat with improved pasta-making quality would be to introduce one of the GluA1 alleles which codes for a HMW gs and to transfer from bread wheat one of the Glu-B1 alleles shown to promote gluten strength, e.g. GluB1i or GluB1c (Ng *et al.* 1989).

b- Low Molecular Weight Glutenins (LMW)

The LMW glutenins differ from HMW in molecular weight and chromosomal location of genes. The LMW gs are subdivided into B, C, and D subunits based on their SDS page mobility (C being faster, B intermediate, and D slower). The LMW gs are more difficult to detect because of their similarity in molecular weight to some gliadins,

albumin, and globulins. Some LMWgs, especially C and D-type, are similar to gliadins in their biochemical characteristics and even their amino-acid sequences. The B and C subunits are encoded mainly at the Glu-3 loci on the short arm of the group 1 chromosomes (Payne 1987), but some are encoded by the Gli-1 loci (Lafiandra *et al.* 1984), both loci being located on the short arm (SA) of the group 1 chromosomes. The D subunits are encoded by genes on the SA of chromosomes 1B (Lafiandra *et al.* 1984) and 1D (Jackson *et al.* 1983). Metakovsky *et al.* (1997) suggested that only B-type LMWgs are encoded by Glu3 loci while other glutenins are in fact, former gliadins and are controlled by Gli-1 and Gli-2 complex loci. The D subunits appear to be derived from ω-gliadins since they have similar electrophoretic properties and have ω-gliadins type N-terminal amino acid sequences but with an additional cysteine residue, acquired presumably by mutation (Lafiandra *et al.* 1984). Those gliadin-type glutenin subunits have an odd number of cysteines residues and furthermore, have one cysteine residue that is likely to be involved in intermolecular disulphide banding. They may act as chain terminator to prevent elongation of developing glutenin polymers. This would result in a decrease in the average molecular weight of the gluten fraction and negative effects on dough strength and on viscoelastic properties (Masci *et al.* 1999). Consequently, the D subunits although they may only be present in small amounts compared with other gluten subunits, they may contribute negatively to gluten strength. Similarly, some C subunits show amino-acid sequence homology with α and γ gliadin and could derive from spontaneous mutations, which affect the numbers and position of cysteine residues (Lafiandra *et al.* 1984, Shewry *et al.* 1994).

In durum wheat, DNA sequence analysis of PCR fragments corresponding to LMW showed that the coding regions are uninterrupted by introns and possess a proline- and glutamine rich domain. Those two last domains are encoded by a tandem array of irregular repeats followed by a unique sequence (C domain) often interspersed with several stretches of glutamine codons (van Campenhout *et al.* 1995). The 3' terminal position ought to be unique among the known *Glu-3* loci Isolation, characterization, and sequencing of some LMWgs genes indicate that the repetitive domain is the most variable region and that the repeat motif is the major unit leading to variation in LMWgs genes (D'Ovidio *et al.* 1997, 1999). The basic motif was suggested to be 18-27 bp, which went through several mutational events such as deletion, duplications, and substitutions (D'Ovidio *et al.* 1999).

In durum, two major types of LMW glutenin patterns known as LMW-1 and LMW-2 were first reported by Payne *et al.* (1984) with some new patterns observed by Margiotta *et al.* (1987). Using a simple one-dimensional two-step SDS page procedure developed by Singh and Shepherd (1988), Carrillo *et al.* (1990) described 3 other types of LMW glutenin patterns along with the two major patterns reported previously among 139 durum landraces of Spain and other 38 durum cultivars. Gupta and Shepherd (1988) also observed two possible variants coded by Glu-A3 and three variants coded by Glu-B3 in a survey of only 11 durum wheat cultivars. Generally the new genotypes occurred more frequently in the North Africa and Mediterranean region. However, because of the known difficulties of resolving these patterns, there has been no systematic study on the extent of genetic variation of LMWgs in durum wheat. Recently, a total of 27 different LMW gs banding patterns were reported in a durum wheat world collection composed by 81 landraces (Impiglia *et al.* 1996) showing different association with gluten strength. Each banding pattern appear to hold three to six subunits showing differences in the relative mobility of the bands and in the staining intensity of the subunits. If

considering the old classification, the authors defined having 23.27% LMW1 and 76.73% LMW2. Liu and Shepherd (1996) found that the Pattern LMW IIt was associated with the higher specific SDS.

The Glu-A3 and Glu-B3 loci are tightly linked with the Gli-1 loci of ω and γ gliadins, especially, the Glu-B3 locus that is tightly linked to Gli-B1. The map distance is estimated about 2 cM (Pogna *et al.* 1990). More simply, γ45 are usually associated with a subunit termed LMW2 and they confer a good quality whereas γ42 are usually linked to LMW1 and confer a bad quality. The amplification of LMW glutenin sequences showed the presence of 3 bands, ranging between 900 and 1200 bp. The two smaller fragments were present in all genotypes. The size of the long fragment differed between genotypes possessing LMW1 or LMW2 gs. The amplification product of LMW2 genotypes (*Omrabi5*) was about 50 bp longer than that obtained in LMW1 genotypes (*Cham1*) (Nachit *et al.* 1995a).

Table I-6: Different recombination of associations between Glu-B3 and Gli-B1 loci in different varieties (Nachit *et al.* 1992).

Glu-B3		**Gli-B1**	
LMW	**Quality**	**w-Gli**	**g-Gli**
1	bad (Waha)	33-35-38	42
2	good (Korifla)	35	45
2	good (Berillo)	35	42
2	good (ICD12060)	33-35-38	45

Ruiz and Carrillo (1993) reported a new gene coding for a LMW gs called LMW9 (later on redesignated as Glu-B2), this gene was found to be tightly linked to two ω-gliadin genes situated at the Gli-B3 locus, showing that Gli-B3 is a complex locus, as is Gli-1/Glu-3 with tightly linked genes coding for gliadins and LMW glutenins. A further study of the effect of the Glu-B2 on durum wheat quality showed that the Gli-B3/Glu-B2 alleles had no effects on the grain quality (Ruiz and Carrillo 1996).

Recently, the inadequacy of the association between glutenin quality and LMW patterns has been shown largely, because durum wheat quality seems to depend on specific LMW gs encoded at the Glu-A3; Glu-B3 and Glu-B2 loci. So, what was considered as LMW-models are actually a mixture of subunits controlled by different alleles at Glu-A3; Glu-B3 and Glu-B2 loci (Table I-7).

Table I-7: Equivalence between LMW models and their allelc composition (b) and diagram showing the *Glu-A3*-, *Glu-B3*- and *Glu-B2*-encoded B-LMW gs (a) (Nieto-Taladriz *et al.* 1997)

Mex Lgn a b c d e f g h a b c d e f g h i a b

References *Glu-A3* *Glu-B3* *Glu-B2*

Mex = mexicali, Lgn = langdom

(a)

Model	Glu-A3	Glu-B3	Glu-B2
LMW-1	B	b	a
	b	b	b
	b	i	b
LMW-1 $^{-}$	e	i	b
	h	b	b
LMW-2	a	a	a
	c	a	a
	d	a	b
	c	c	b
	f	a	b
	c	f	a
	g	g	a
LMW-2 $^{-}$	h	a	a
	h	c	b
	e	d	a
	e	e	a
	e	f	a
LMW-2*	d	h	b

(b)

As it was demonstrate that the glutenin subunits encoded at the Glu-B3 were responsible for the differences in quality, Nieto-Taladriz *et al.* (1997) established a rank of alleles based on their effect on gluten strength: Glu-B3a = Glu-B3f > Glu-B3g > Glu-B3b = Glu-B3i. So the wheats carrying γ45 can exhibit six different alleles at the Glu-B3 locus (a, c, d, e, f, g) and this variability can explain why cultivars with γ45 have pasta quality ranging from medium to high depending on the allele present at the Glu-B3 locus.

A-2-2- High Molecular Weight (HMW) Albumins

The polymeric proteins next in abundance are the HMW albumins. These are mainly β–amylase and do not occur in the protein bodies. It seems probable, therefore, that their subunits form polymers with themselves and not with the glutenins.

A-2-3- Triticins

The third group of polymeric proteins is the triticins, which are globulin-type proteins. They also appear to consist of polymers among their own subunits (Singh and Shepherd 1983).

B- Protein content (PC)

Durum wheat is generally used to produce pasta products that include various shapes and sizes of macaroni, spaghetti, flat or corrugated sheets used in lasagna and noodles, and other pasta shapes developed from extrusion of dough through a die. Other products produced from durum wheat include leavened and unleavened bread, couscous, burghul and frike. These diverse products require different quality characteristics, however, all require minimum level of protein, high milling yield, yellow flour color and moderately strong gluten. Durum technological properties are determined on the basis of cooking characteristics of the pasta it produces, where cooking quality is the capacity of the cooked product to maintain good texture and not become mush or a sticky mass, and to maintain a correct firmness after some overcooking. So, in general good cooking quality is related to a high level of protein and gluten content or an intermediate content of protein but high gluten quality (D'Egidio *et al.* 1990). Wheat protein content higher than 13% was reported to yield a satisfactory final product, whereas protein content lower than 11% gave a very poor product. Considering the different pasta drying systems applied, D'Egidio *et al.* (1994) indicate that at a low drying temperature, protein content and gluten quality were both of importance in determining pasta-cooking quality whereas at high temperature only protein content was essential. The total protein content of durum wheat may be as high as 22% (dry weight basis) or more, but the average level is around 12-14%. From analysis by electron microscopy, Banasik *et al.* (1976) concluded that most of the protein in durum wheats could be found within the cell structure, surrounding the starch granules as enveloping proteins, with less amounts found as fibril proteins or deposited as protein mass, proteins are deposited in the matrix differently in different varieties.

Findings on the nature of genetic control are rather controversial, although all scientists agree that this is undoubtedly a complex subject and one that is difficult to study, due to the strong influence of the environment upon its expression. Protein content is a typical quantitative trait controlled by a complex genetic system and highly influenced by environmental factors. Nachit *et al.* (1995a) showed that environmental effects override the effects of genotype for protein content and that GxE interaction appears negligible. Those finding are in agreement with Mariani *et al.* (1995). Therefore, the major influences on grain crude protein concentration are environmental, particularly climate and nitrogen (Nachit *et al.* 1995a, Trocoli *et al.* 2000). Grain yield is also affected by nitrogen fertilization (Langer and Liew 1973). However a negative correlation has been found between yield and grain protein concentration in wheat (Stenram *et al.* 1990) and in *T. dicoccoides* derived crosses (Maali 1991). This may be

because more energy is required to produce protein than carbohydrate (Penning de vries *et al.* 1994). Thus in the selection of higher yielding genotypes, it is easier to increase carbohydrates than proteins. However, the seed protein percentage may be increased by genetic manipulation without concurrent reduction in productivity (Day *et al.* 1985, Nachit *et al.* 1995b). Most information concerning grain nitrogen relations in cereals are obtained from field, where the significance is imprecise because of variations in environmental conditions, including nitrogen availability. A reliable comparison of the protein concentration and composition in developing wheat grains during different nitrogen regimes accordingly requires a precise control of the nutrient input during vegetative and generative growth (Johanson and Lundborg 1994).

Under severe drought conditions the grain tends to be shriveled with low starch accumulation and therefore a relatively high content of bran and nitrogen are obtained. Using 171 Mediterranean landraces, Nachit *et al.* (1995a) reported a protein content mean of 10.9 under irrigated conditions and 12.4 under dryland conditions. An increase in grain nitrogen concentration would normally be expected to result in increases in the proportion of vitreous grains, sedimentation volumes, gluten concentration and dough strength (Blumenthal *et al.* 1993). In addition, several studies reported that excessively high temperatures and low relative humudities during grain filling resulted in increased grain nitrogen concentration, but in reduced dough strength and gluten quality (Borghi *et al.* 1995, Blumenthal *et al.* 1993). Johansson and Lundborg (1994) showed that nitrogen addition over time decided the final protein concentration in the grain, and that this final concentration was established rather early during grain development.

Semolina protein content generally is about 1% less than the whole-wheat protein (Nachit *et al.* 1995a). Therefore, they suggest that the actual grain protein concentration is the result of two parameters. First, grain growth, which mainly is controlled by the genotype, and secondly, nitrogen transport and accumulation, which is mainly controlled by the applied nitrogen regime. Protein content was completely independent of LMW gs (Impiglia *et al.* 1996). This finding was expected, as factors other than storage proteins are the cause of high protein content.

Therefore, kernel protein is controlled by a complex genetic system and is strongly influenced by several environmental factors. Some results (Kuspira and Unran 1957, Diehl *et al.* 1978) indicate that protein content is a quantitative trait governed by several genes distributed throughout the genome. Halloran (1975) and Konzak (1977), on the other hand, claimed that the character is governed by a few major genes, without however excluding the action of many other genes of minor effect. These different results are also reflected in heritability estimation, which was reported to be ranging from 0.15-0.26 (Sunderman *et al.* 1965) to 0.49-0.72 (Nachit *et al.* 1995a) to 0.90 (Kaul and Susulki 1965). In general, genes for low grain protein content show weak dominance over those for high protein content (Halloran 1981), though in some cases the direction is the opposite (Cowley and Wells 1980). Millet and Pinthus (1980) found that the genes for low protein of the cultivated parents showed a weak dominance over the high protein of the wild parents, and that the protein content in wheat is mainly determined by the mother plant. It seems there is no cytoplasmic effect on the grain protein content (Millet and Pinthus 1980; Levy and Feldman 1989). Other findings reported a transgressive segregation for PC, indicating the presence of different genes controlling this complex trait (Levy and Feldman 1989, Maali 1991). More recent

studies reported a control of two partially dominant genes with additive effect (Dhaliwal *et al.* 1994) whereas other studies suggested 4 major genes (Levy *et al.* 1988).

In bread wheat, genes for protein content have been located on chromosomes 3D, 4D, 5A, 5B, and 7B by Kuspira and Unran (1957) and on chromosome 5A and 5D by Morris *et al.* (1973); and on 5D by Law *et al.* (1978). A gene was also identified by Stein *et al.* (1992) on 5B on the variety "Wichita" and on 1B, 1A, and 7A on "Plainsman". Levy and Feldman (1989) suggested that chromosomes 1AS, 1BS, 5A, 5B, 7A, and 7B of *T. dicoccoides* carry at least 4 genes associated with grain protein content linked to 27 morphological and biochemical markers. The gene on 1AS was indirectly confirmed by Elouafi *et al.* (1998) who reported a *dicoccoides* omega gliadin subunit (Rm = 44) highly associated with protein content. Joppa and Cantrell (1990) using *T. durum* cv. *Langdon-T. dicoccoides* chromosome substitution lines, reported that chromosomes 2A, 3A, 6A, 3B, 4B, 6B, and 7B all affect protein content. Steiger *et al.* (1996) crossed these substitution lines with a durum variety and reported that lines with chromosome 5B and 6B explained over 70% of the total genotypic variation of grain protein content. In durum wheat, Blanco *et al.* (1996) reported that 20 significant marker loci were detected, which identified at least 6 QTLs for protein content on the chromosome arms 4AS, 5AL, 6BS, 7BS, and 6AS. Joppa *et al.* (1997) identified a major QTL accounting for 66% variation in grain protein content on chromosome 6B. This locus is located on the short arm of chromosome 6B near the centromere, flanked by Xmwg79 and Xabg387. Chee *et al.* (1998), reported a grain protein content QTL on the 6BS, and suggested it to be the same as the one reported by Joppa *et al.* (1997). They also advanced that the protein content trait is controlled by a single genetic factor. More recently, a microsatellite was reported to be related to grain protein content. Prasad *et al.* (1999) used a bulk segregant analysis on a population presenting high variation on protein content and identified WMC41 on the 2DL as being linked to grain protein content and explaining 18.73% of the total variation between the two parents. Therefore, it is obvious that several QTLs control grain protein content in wheat and that is controlled by fertilizer, environment, and genotype.

Several works suggested the utilization of *T. dicoccoides* as source for high protein content genes (Feldman and Sears 1981, Nachit *et al.* 1990). In fact, high protein content in *dicoccoides* derived crosses in comparison with cultivated wheat derived crosses was reported by many studies (Feldman and Sears 1981, Nachit *et al.* 1990, Maali 1991; Nachit and Maali 1997).

C- Kernel Quality Characteristics

Yellow Pigment (YP)

Durum wheat normally has an amber vitreous kernel that produces a yellow milling product. Color is of prime importance in semolina. Therefore many studies were conducted to define their biochemical pathways and genetic control. This color is the result of the natural carotenoid pigments present in the seed and of their residual contents after the storage of the grain or semolina and after milling. The oxidative effect of the lipoxygenase enzymes during pasta processing (McDonald 1979) and the conditions of the processing itself affect also the durum endproduct color. The carotenoid pigments are classified into carotenes, unsaturated hydrocarbons, and

xanthophylls. Xanthophylls being the most abundant, which possess one or more oxygen-bearing functional groups. Lepage and Sims (1968) using chromatography methods reported that the pigments in durum variety were composed of 84.8% free lutein (a xanthophylls), 9.8% lutein monoester, and 5.3% lutein diester. Other xanthophylls have been reported such as triticoxanthin, taraxanthin, flavoxanthin, and canthaxanthin (Laignelet 1983). Carotenoids are antioxidant compounds that reduce the oxidative damage to biological membranes by scavenging peroxyradicals, such as those involved in certain human disease and in the aging process and in the degradation of food quality. These criteria contribute to an increase in the nutritional value of durum products. The carotenoids are mainly located in the outer layers of the kernel, with the embryo, bran, and endosperm containing smaller amounts (Trocoli *et al.* 2000). The carotene content trait is mainly affected by genotypic effect (heritability ranging from 0.90-0.97) and therefore easy to select for (Nachit *et al.* 1995a). In contrast, in bread wheat cultivars the flour color was reported to be difficult to manipulate as it is expressed as a quantitative character (Moss 1967). In tritordeums, the high carotene content is under the control of the *Hordeum chilense* genome (chromosome $7H^{ch}$) and is not dependent on tritordeum yield or grains size (Alvarez *et al.* 1998). While in durum wheat, the color is highly heritable and is controlled by additive gene effects. Some studies suggest that the major genes are probably on chromosomes 2A and 2B (Joppa and Williams 1988). Others reported the BCD1 on chromosome 6D and BCD828 on chromosome 3B as being linked to bread wheat flour color (Parker *et al.* 1996), whereas, more recently Parker *et al.* (1998) reported a major locus on 7A in bread wheat explaining 60% of the genetic variation.

Nevertheless, a high level of carotenoid pigments in semolina does not guarantee a high color pasta as the color is also affected by the level of lipoxygenase (LOX) activity (McDonald 1979) and polyphenol oxidase activity (Dexter *et al.* 1984).

Lipoxygenase (LOX)

Lipoxygenase enzymes are a family of enzymes that catalyze the breakdown of lipids, polyunsaturated fatty acids containing a cis-cis-1,4- pentadiene system, producing firstly free radicals and subsequently, after oxygenation, conjugate cis, trans-diene hydroperoxides. Radical forms produced during the intermediate steps of poly-unsaturated fatty acid peroxidation by LOX are responsible for the oxidative degradation of pigments, such as β-carotene, xanthophylls, and chlorophylls (Trocoli *et al.* 2000). The formed hydroperoxides are cleaved by the hydroperoxidase lyase to form aldehydes, causes of the off-flavor.

Lipoxygenase enzymes are found in plants, animals, and microorganisms. In plant, they are found in seed, seedling and leaves (Prigge *et al.* 1996). In higher plants, LOX react with the substrate linoleic acid or linolenic acid, and form hydroperoxides. The product is though to be involved in plant defense, wound response, senescence and development (Shibata 1996). Nevertheless, they are responsible for yellow color destruction of pasta by oxidation. Indeed, some of their volatile compounds, such as n-hexanal, are implicated in the production of aroma or undesirable flavors and odors. The reaction can be inhibited by processing in a vacuum or by adding oxidation inhibitors such as L-ascorbic acid. Barone *et al.* (1999) reported a monomeric structure of wheat LOX (95000 ± 5000). They also confirmed its affinity with linoleic acid as substrate and with optimal pH values. Therefore, β-carotene bleaching occurred only in presence of

linoleic acid, confirming the co-oxidative nature of enzymatic pigment degradation. β-carotene also acts as an inhibitor of LOX activity preventing semolina bleaching and improving pasta quality (Trono *et al.* 1999). The combined mechanism of oxidation favorizes a loss of semolina sulphydryl groups (Tsen and Hlynka 1963). This is the cause of an increase in the amount of free lipids in dough and an increase in the mixing tolerance and relaxation times of dough itself (Hosoney *et al.* 1980). Furthermore, lipoxygenase activity is directly affected by storage conditions (Kankovirta *et al.* 1998). Borrelli *et al.* (1999) showed a genotypic and environmental control of the LOX levels in durum wheat. Many genes were isolated, and some molecular analysis showed that the genotypic variation is due to different transcriptional levels of the relative genes (Manna *et al.* 1998). The isoenzyme LOX1 in barley for example is exclusively responsible for the lipoxygenase activity in seeds (Hotlman *et al.* 1996). Some LOX-free mutations have been developed in soybean and could be of great help to promote soybean human consumption.

Polyphenol oxidase (PPO) and peroxidase

Undesirable dough and product discoloration that may be caused by enzymatic browning was reported (Dexter *et al.* 1984). Hatcher and Kruger (1993) showed that polyphenol oxidase (PPO) distribution in millstreams closely parallels the efficiency of the milling process. Milling to elevated extraction yield (>70%) causes the enzyme levels to rise dramatically. Phenolic acids, potential substrates of this enzyme, are endogenous to the wheat plant (Bose 1972) and flour (Sosulski 1982). PPO is believed to be involved in the oxidation of such endogenous wheat phenolics, resulting in the production of labile quinones. The quinones produced can react with a number of compounds, amines and thiols or undergo self-polymerization to produce highly colored products (Pierpoint 1969). The close relationship between the enzyme activity and its potential substrates suggests a role for such simple phenolic acids in the complex process of enzymatic darkening in end-products (Hatcher *et al.* 1997). Durum wheats have lower polyphenoloxidase activity than other classes of wheat. Further, the peroxidase activity is much higher in low-grade flour than in purified semolina (Feillet 1988). Some studies showed that peroxidase activity is affected by the drying conditions of pasta (Kobrehel and Abecassis 1985).

D- Milling Quality Characteristics

Test Weight (TW)

Another aspect of milling that is considered as an important quality characteristic is test weight, which is known to be highly related to semolina yield. Test weight is the weight of grain, which fills a specified volume under standard packing conditions. Traditionally, low-test weight (TW) in wheat has been associated with low flour yield. Several works have reported a high and positive correlation between high TW and high flour yield (Marshal *et al.* 1986; Barmore and Bequette 1965). A high TW is commonly associated with sound plump kernels. TW is affected by the genotype (seed shape and size) and various environmental factors such as disease and lodging, which can cause shriveling of grain (Roth *et al.* 1984; Blum *et al.* 1991; Saadalla *et al.* 1990).

Test weight is highly heritable. Ghaderi and Everson (1971) estimated its broad-sense heritability in winter wheat to be 0.48, while Teich (1984) estimated it to be 0.98 ± 0.08 and Jalaludin and Harrison (1989) estimated to 0.78. In durum wheat, Nachit *et al*. (1995a) reported a heritability of be 0.94-0.97 showing a very high genotypic control (explaining up to 80% of the total variation). In contrast, Schuler *et al.* (1994) reported a major contribution of environmental conditions to TW variation (≈70%) and a moderate GxE interaction (≈20%). Because, cultivar differences in TW are likely due to the effect of many characters of the kernel, several studies have been conducted, mainly on soft bread wheat, to find simple factors that correlated with TW and can be used in the early generation of breeding programs.

Yamazaki and Briggle (1969) described the components of TW as kernel volume (size and shape) and kernel weight. Kernel volume affects the packing efficiency or the percent volume of a given container that is occupied by grain. Kernel weight affects the total weight of grain within that volume. Packing efficiency is considered to be a cultivar characteristic while kernel density, which influences kernel weight, is considered to be more environmentally influenced. Ghaderi and Everson (1971) concluded that differences in TW among cultivars were related to cultivar differences in packing efficiency and influenced very little by density, variation in density being due, at least in part, to airspace within the kernel (Yamazaki and Briggle 1969). Ghaderi and Everson (1971) determined also that kernel width and thickness were related to TW. They also found that width and length were correlated to kernel volume but that width was more highly correlated. Therefore, the spike characteristics of a wheat cultivar may be important to TW due to possible effects on kernel shape. Ghaderi and Everson (1971) noted that shriveling reduced TW by introducing plane and concave surfaces to the otherwise normal contours of the grain and decreasing packing efficiency. It is also likely that environment is capable of altering wheat kernels in ways not easily detected by visual inspection (Schuler *et al.* 1994). On the other hand, Yamazaki and Briggle (1969) did not find any relationship between grain dimensions, size, or size distribution and TW. More recently, Trocoli and DiFonzo (1999) showed that TW is related more to kernel shape than to kernel size.
Flour protein was reported to be significantly correlated to TW but not to TKW and kernel density (Schuler *et al.* 1994). As flour protein increased, TW tended to increase. Yamazaki and Briggle (1969) hypothesized that as protein content increased more protein would be available to fill voids among the large starch particles of the endosperm, increasing kernel weight by increasing density and ultimately increasing TW. While a significant negative correlation between protein content and TW was reported by Matsuo and Dexter (1980). Concerning grain yield, other studies suggested that TW and grain yield are not genetically correlated (Jalaludin and Harrison 1989). In practice, the milling industry prefers large kernels because of the strong relationship with milling yield.

Thousand-Kernel Weight (TKW)

Durum wheats vary greatly in TKW from 20 to over 60 grams. High 1000-kernel weight is desirable for easy processing, milling, and semolina traits. Kernel weight is affected by the environment during grain filling and by the number of heads and number of fertile florets per spike. The genetic of kernel weight is unknown, but

estimates of gene number vary from 1 to 4 or more genes in different crosses (Joppa and Williams 1988). Sun *et al.* (1972) and Ketata *et al.* (1976) estimates of TKW heritability in the broad sense were relatively intermediate to high. This indicates the involvement of high genetic effect and suggested that selection will be valuable in early generations. Theses finding were confirmed by (Maali 1991). They reported a high heritability coupled with high genetic advance in crosses with *T. dicoccoides*, confirming a high additive effect. Using durum varieties crosses, they also suggested a cytoplasmic effect on TKW.

In *T. dicoccoides*, a significant positive correlation between seed weight and protein content was reported (Maali 1991). Significant correlations between semolina yield and TW and semolina yield and kernel size were reported (Ghaderi and Everson 1971, Matsuo and Dexter 1980). They reported a highly significant overall correlation (r=0.75) between TW and TKW, suggesting that the 2 traits were affected by environment factors in a similar manner. However several other researchers failed to find any correlation between TW and TKW (Schuler *et al.* 1994; Trocoli and Di Fonzo 1999; Yamazaki and Briggle 1969). Consequently, although these 2 parameters affect the same character they seem to behave differently with respect to milling yield. Actually, TW and TKW measure different features of the grain. While the former can be a reflection of the soundness of grain, and therefore high TW may be expected when the grain is undamaged by unfavorable weather conditions, kernel weight will be a measure of average kernel size and thus larger kernels, resulting from unrestricted grain ripening are expected to have a greater endosperm to bran ratio (Trocoli *et al.* 2000). TKW was strongly correlated with kernel length, width and volume (Schuler *et al.* 1994). And as the TW, the TKW is highly heritable (0.94-0.97) and therefore, is more influenced by genotypes than by environments (Nachit *et al.* 1995a).

<u>Vitreousness (Vit)</u>

The vitreousness measurement is used only for durum wheat. The vitreousness of the kernel is often considered to be important in milling. Actually, a high percentage of vitreous kernels is required for all products (Williams *et al.* 1984). A vitreous grain implies that the endosperm has a glassy translucent appearance rather than a mealy or starchy nature. Nonvitreous kernel is also designed as starchy kernel.

Vitreousness gives distinct physical properties to the whole kernels and the best quality durum wheat has high proportions (90-100%) of vitreousness. The starchy or non-vitreous kernel has a negative effect on semolina yield. As the proportion of starchy grains increases, the proportion of semolina decreases, the proportion of flour increases, and the protein content decreases (Matsuo and Dexter 1980). In fact, vitreous areas of the endosperm are known to be higher in protein than mealy areas. When milled, nonvitreous kernels produce total milling yield similar to that of vitreous kernels, but less semolina and more flour is produced. Vitreousness was significantly associated with protein content of grain particularly under zero-nitrogen application (Nachit and Asbati 1987). They reported that zero-nitrogen environment was the best environment for high selection efficiency for vitreousness. The incidence of starchy grain in durum wheat can increase when normally vitreous grain varieties are grown in low protein environments (Trocoli *et al.* 2000). Vitreousness does not affect the milling yield, but rather the semolina yield. Some heritability studies suggest that selection for

vitreousness in early segregating populations is possible and the most successful crosses are made when the female parent has highly vitreous kernels (Nachit and Asbati 1987). The broad sense heritability of vitreousness was estimated to 0.59 under irrigated conditions and 0.79 under dry conditions (Nachit *et al.* 1995a). The environmental and GE interaction effects were respectively of 58.1% and 23% under irrigated conditions and 70.0% and 16.9% under dry conditions (Nachit *et al.* 1995a).

Ash Content (AC)

The quality for milling is mainly represented by high extraction rate. Many studies are conducted on ash content in order to maximize the semolina yield and to minimize the flour yield, because semolina has a higher price.

The ash content is considered as the most important aspect of durum wheat quality for semolina milling (Trocoli *et al.* 2000). Several reports have already pointed out the environmental effects (Cubadda *et al.* 1969), while Peterson *et al.* (1986) noted a high genotypic influence. Other studies proved that there is a strong genotype-by-environment interaction on the ash content (Fares *et al.* 1996). Therefore, favorable growing conditions result in higher ash content in whole grain due to increased uptake of minerals from the soil. A lower semolina yield can therefore be expected, because the ash content of semolina is correlated with both ash content of the whole kernel and the extraction rate (Dexter and Matsuo 1978). High semolina ash content is normally associated with a longer extraction time, which can produce a duller semolina color because of the presence of high ash outer endosperm particles giving a brown hue to pasta products (Kobrehel *et al.* 1974), Borrelli *et al.* 1999). Borrelli *et al.* (1999) showed also an indirect effect of semolina ash content on pigment degradation during pasta processing. Furthermore, this parameter is especially important in several European countries where the ash content of durum wheat for human consumption is regulated by law.

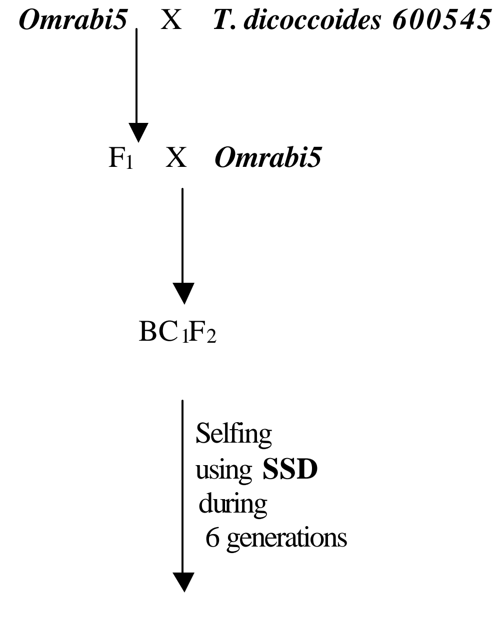
Morris *et al.* (1945) using a micro dissection technique on wheat kernels, obtaining 4 endosperm and 2 bran fractions reported an ash gradient in the kernel. Using other techniques, theses finding were confirmed by Matsuo and Dexter (1980), Dexter *et al.* (1994) and lately by Fares *et al.* (1996) using a successive debraning levels. Fares *et al.* (1996) reported that a high value of removed ash level (RAL), starting from level 1 could prove the presence of lower ash content in the inner layers of endosperm. Therefore, with the same ash content, an outer ash concentration is more desirable and allows a lower ash content in semolina.

Chapter II
Material & Methods

Plant material and experimental design and sites

1- Plant Material

The plant materiel used is the population stemming from the cross between *"Omrabi5/ T. dicoccoides600545// Omrabi5"* with the pedigree ICDMN91XMP (Nachit pers. com). The population is constituted of 114 backcrossed Recombinant Inbred Lines (bc-RIL) developed at CIMMYT/ICARDA durum breeding program for the Mediterranean dryland from a cross between the durum cultivar *Omrabi5* and the *T. dicoccoides* accession number: 600545. The F1-cross was backcrossed to the maternal parent *Omrabi5*. The *Omrabi5* cultivar is a cross between *Haurani* x *Jori-C*, bred for Mediterranean dryland conditions by CIMMYT/ICARDA durum program (Nachit pers. com.). It is released in Turkey, Algeria, Iran, and Irak for commercial production in dry areas. It combines drought tolerance with yield and yield stability. As for *Triticum dicoccoides600545*, it was collected from Jordan at 25 km west of Amman on the Amman-Dead See highway; it shows resistance to yellow rust and tolerance to drought. Our population was advanced up to F8 generation. This population was developed by using single seed descent method (SSD) up-to F6 generation. Afterward, the seeds of the BC-RILs were bulked in order to produce F7-seed and the same to have the actual population, F8 BC-RILs constituted of 114 lines. These recombinant backcrossed inbred lines (bc-RILs) present a valuable and significant polymorphism for most of the quality traits: protein content, carotene (yellow pigment) content, vitreousness, ash content, kernel weight, hectoliter weight, sedimentation test, and farinograph parameters. The *T. dicoccoides* appears to be a potential donor for quality genes since it holds a valuable polymorphism for gliadins and glutenin subunits and it has a very high protein content.



Pedigree of *Omrabi5/ T. dicoccoides600545// Omrabi5* mapping population.

2- Sites description

The bc-RILs trial was grown in three locations for the 4 consecutive seasons: 1996/97; 1997/98; 1998/99, and 1999/2000. The main locations are Tel Hadya, Breda, and Terbol.

2-1- Tel Hadya station:

Tel Hadya is the main research station at the head quarters of the International Center of Agricultural research in the Dray Areas (ICARDA). It is at 35 Km south west of Aleppo city/Syria and located at 36°01' N latitude; 36°56' E longitude, and at 284 m above the sea level. The soil at Tel Hadya is fine to very fine clay, montmorillonitic (the dominant clay mineral), thermic (mean annual temperature at 5cm of 15 to 22°C), Calcixerollic Xerochrept or Chromic Calcixerert. In older soil science classification, the soil was classified as Terra Rosa (USA) or Red Mediterranean soils (France). This station is characterized by the following climatic conditions: wet and cold in winter and warm and dry summer, a typical Mediterranean climate. The average annual precipitation is 335 mm. During the field-testing seasons of *Omrabi5/ T. dicoccoides600545// Omrabi5* population the precipitation amounts were as follows: 427mm in 1996/97; 411mm in 1997/98, 309mm in 1998/99, and 261mm 1999/2000 (Fig II-1). More details on precipitation, evapotranspiration, and average, minimum, and maximum temperatures are reported in Fig II-1.

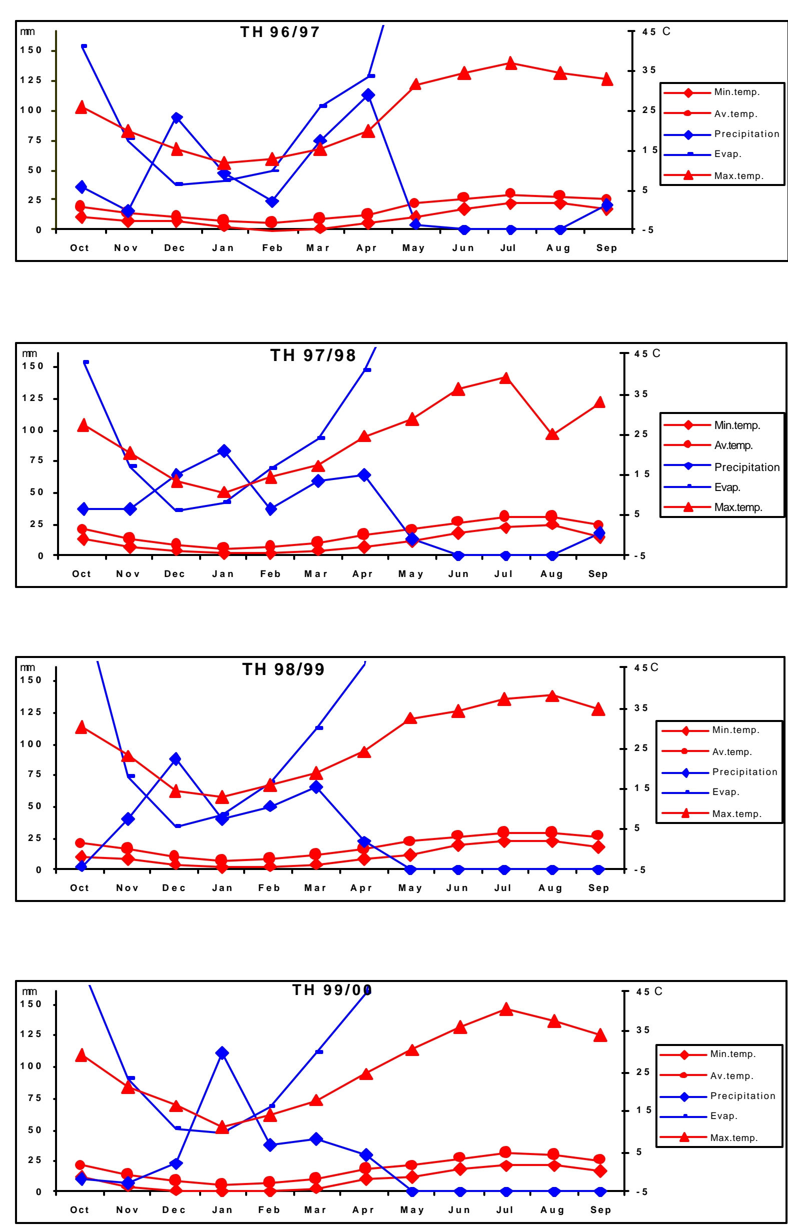


Fig II-1: Meteorological data of Tel Hadya station for: 1996/97; 1997/98; 1998/99 and 1999/2000. *Min. temp = minimum temperature; Av. temp. = Average temperature; Evap. = evaporation; Max. temp = maximum temperature.*

To create different environmental conditions, the double gradient screening technique (Nachit *et al.* 1995b) was used for all testing seasons. These environments are described below:

Early Planting (EP):

This environment is characterized by very early planting time (mid October). This environment is used mainly for resistance screening to cold and yellow rust. The amounts of fertilizers are added at sowing date as follows: 60Kg/ha nitrogen unit (NH_4NO_3) and 40 Kg/ha of P_2O_5. Irrigation (40mm) is applied after sowing to allow germination.

RainFed (Rf):

The rain-fed environment is used as test for the Mediterranean continental dryland. In addition to screening for adaptation, screening is also made for drought and the other associated biotic and abiotic stresses prevalent in this environment. The date of sowing is usually mid-November and of harvesting is around mid-June. As in the case of Early Planting at Tel Hadya, similar amounts of nitrogen and phosphorus fertilization are applied.

Irrigated (Ir):

The sowing date is also conducted mid-November and the harvest mid-June. In this environment, additional 30 Kg of N-fertilization are supplemented, and also supplementary irrigation 30-40 mm is applied. This environment is also used for yield potential testing.

Late Planting (LP):

The sowing date is beginning of April while the date of harvest around mid-June. This environment is useful for screening to terminal stress (heat and drought) tolerance.

Summer planting (Sum):

The summer planting is characterized by sowing in mid-July and harvesting in mid-October. This special environment is used for heat tolerance screening during the vegetative stage and for identifying population carrying vernal genes, in addition, it is used to speed the breeding cycle.

Sowing after legume crop (Inc):

This environment is used to grow the durum crop after a legume crop and to study the effect of slow release of nitrogen on grain quality. The dates of sowing and harvesting are similar to those of the normal planting at Tel Hadya station.

2-2- Breda station:

Breda station is located 30 Km east of Tel Hadya, 85 Km south east of Aleppo city. It is at 35°56' N latitude, 37°10' E longitude, and 300 m above the sea level. The soil of Breda is classified as a clayey (high clay content), montmorillonitic, thermic, Calcixerollic Xerochrept. In the older classification system it was classified as reddish brown soils (USA) or a Brown Steppe Soils or Sierozems (France). Breda has an average annual precipitation of 260mm, the precipitation was230mm in 1996/97, 229mm in 1997/98, 198mm in 1998/99, and 229 mm in 1999/2000 (Fig II-2). Nitrogen and phosphorous were added at sowing date (mid of November) as follows: 50Kg/ha

nitrogen unit (NH_4NO_3) and 40 Kg/ha of P_2O_5. This station is also characterized by harsh continental climatic conditions. It is used for screening to drought, cold, and terminal heat resistance.

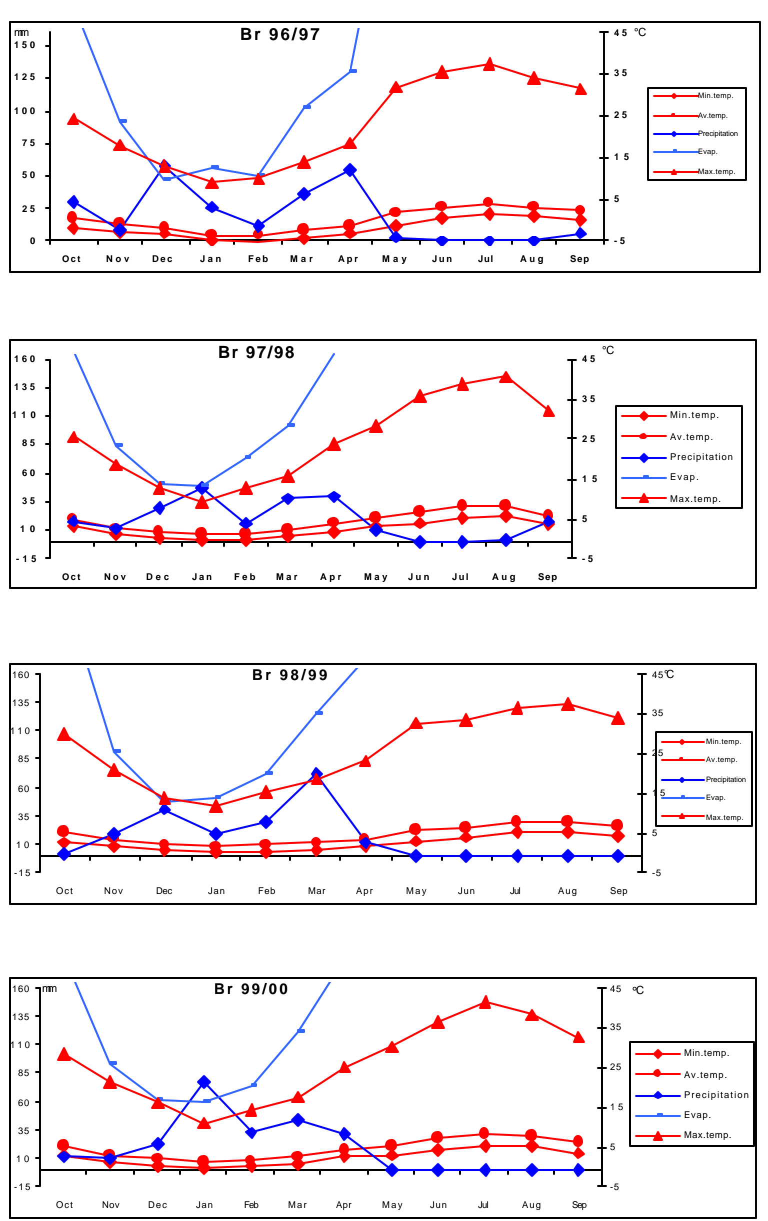


Fig II-2: Meteorological data of Breda station in: 1996/97; 1997/98; 1998/99, and 1999/2000. *Min. temp = minimum temperature; Av. temp. = Average temperature; Evap. = evaporation;*

Max. temp = maximum temperature.

2-3- Terbol station:

Terbol station is located in Lebanon in the Beka'a valley. It is at 10Km south east of Riyak, 33°33' N latitude, 35°59' E longitude, and 890 m above the sea level. This station is characterized with cold winters, favorable seasons, and high soil fertility. The soil is a fine clay, montmorillonitic, thermic, Chromic Haploxerert. The average annual precipitation is around 524mm and was 501mm in 1996-97, 526mm in 1997-98, 292mm in 1998-99, and 339mm in 1999-2000 (Fig II-3).

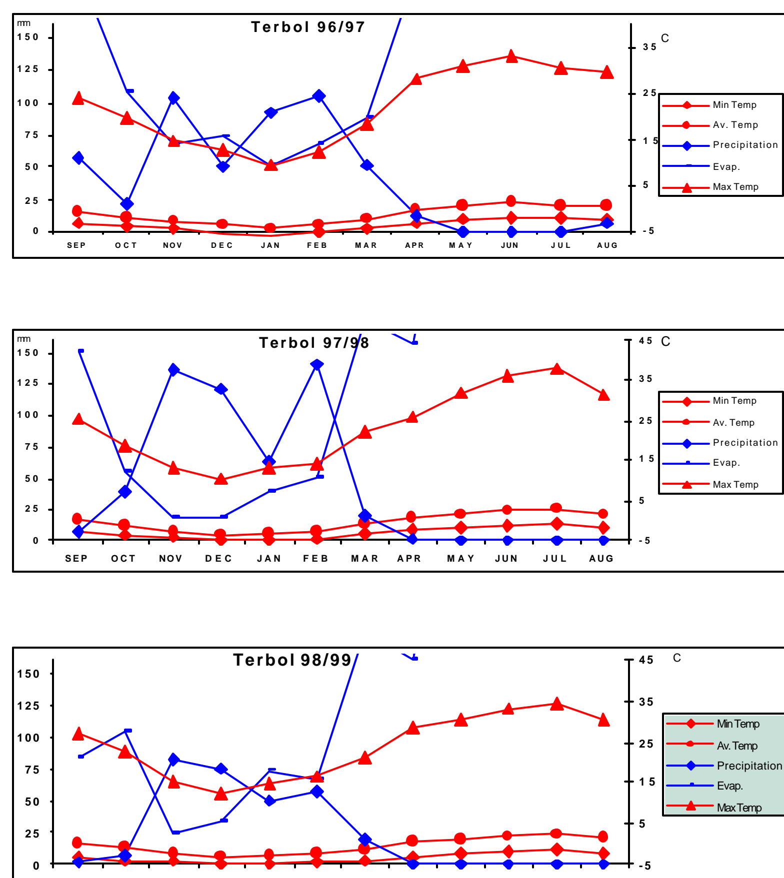


Fig II-3: Meteorological data of Terbol station in: 1996/97; 1997/98; 1998/99, and 1999/2000. *Min. temp = minimum temperature; Av. temp. = Average temperature; Evap. = evaporation; Max. temp = maximum temperature.*

3- Experimental Design

For collection of phenotypic data for grain quality traits, the 114 BC-RILs were divided over 6 blocks where 19-test BC-RILs were included in each block, with 5

commercial and well-known durum genotypes as checks (*Omrabi5, Haurani, Korifla, Cham1,* and *Gidara2*). Thus each block was constituted of 24 entries (19 test BC-RILs and 5 Checks). The field design used was the augmented design (Federer 1956; Peterson 1985). The total number of entries of the whole trial was 144 (114 BC-RILs + 5 checks repeated in each block). The 114 BC-RILs were tested over several sites (Tel Hadya, Breda, Terbol) and seasons (1996/97, 1997/98, 1998/99, 1999/2000).

The trial was sown in 2 rows of 2,5m long spaced by 30cm at Terbol station (Tr) in 1996/97 and 1998/99; in 8 rows of 2.5 m long spaced by 22.5cm at rain-fed (Rf) 1998/99; late planting (LP) 1997/98 and 1998/99; early planting (EP) 1997/98 and 1998/99; Breda station (Br) 1997/98 and 1998/99; Tel Hadya after Vetch rotation (Inc) 1997/98 and 1998/99; and Terbol 1997/98. The sowing in Tel Hadya-late planting, after Vetch rotation, and Breda 1996/97 was in 6 rows of 2.5m long spaced by 30cm and in 1row in irrigated (Ir) 1998/99 and Tel Hadya Summer Planting (Sum) 1998/99. In early planting 1996/97, the sowing was in 2 rows of 2.5m long spaced by 30cm.

B- Biochemical analysis

B-1- Quality traits assessment

The conducted grain quality analyses are related to technological end-products milling, processing, and nutritional qualities. The quality traits probed on the 114 inbred lines of *Omrabi5/ T. dicoccoides600545// Omrabi5* are as follows:

- ***Protein content (PC):***

  Generally, high protein content is associated with good pasta, burghul, and couscous making values. The protein content was conveniently determined in all cereals by Near-Infra-Red (NIR) of the reflectance spectrometry, due to its rapidness and accurateness. Further, it was monitored by the standard Kjeldahl test (AACC 1976) to ensure continued accuracy. Therefore, we adjust our data by testing randomly 20% of our population using Kjeldahl method.

- ***Sodium-Dodecyl-Sulfate (SDS) or Sedimentation Test:***

  Sedimentation test is a method to estimate the strength of wheat gluten, it is based on the hydratation capacity of a flour in a low acidity media. Grains from each line are tempered at 16% moisture and milled using Udy Cyclone mill equipped with a 100-mesh sieve. Three grams of the wheat meal sample are then used for SDS-test (Zeleny 1947). The durum flour is suspended with bromophenol blue solution (1%). The protein hydration is facilitated by the addition of sodium dodecyl sulfate, which is a mild detergent, and lactic acid with a determined shaking and inversions. Results are expressed in milliliters of the interface line between solid (ground sample) and liquid (solution) into a measuring cylinder. The gluten strength is measured for all environments and years.

  The SDS measurements of the season 1996/97 (irrigated and rainfed) were performed using the NIRs procedure, whereas for remaining environments/sites, during 1997/98, 1998/99, and 1999/2000, the measurements was performed using the above described chemical procedure.

- ***Firmness (SDSni):***

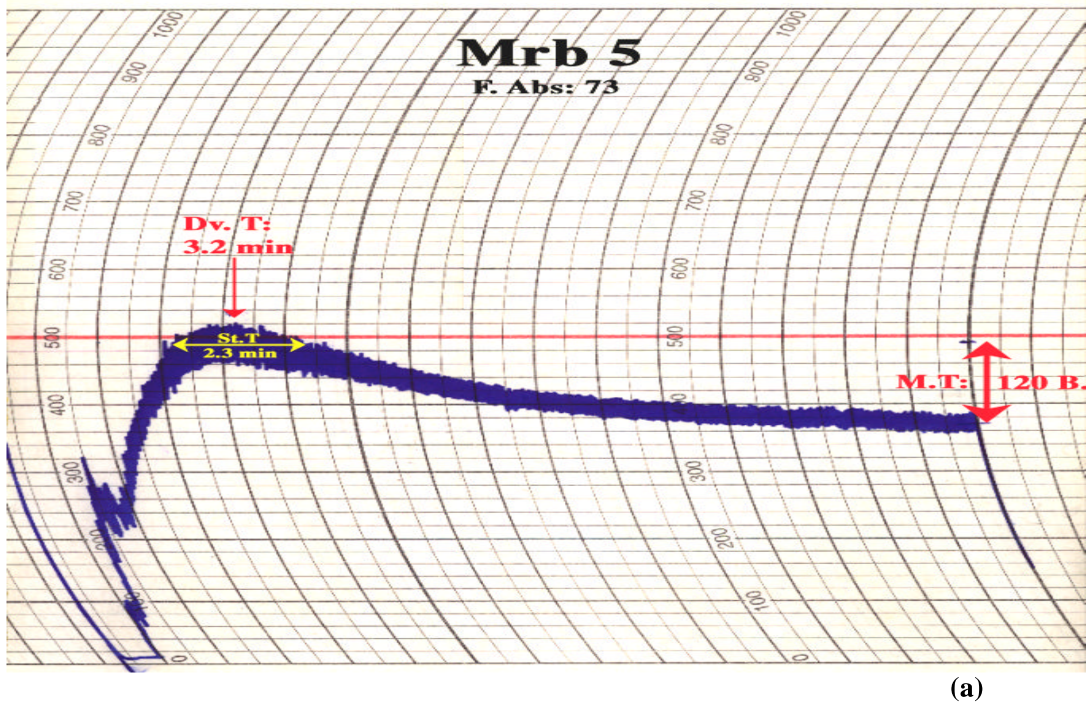
Firmness is the force required to cut cooked pasta. Good quality pasta and couscous should have the correct firmness or chewiness after cooking or steaming, respectively. The SDS index is used as surrogate for firmness test. The SDS index is measured as follows (Nachit *et al.* 1992):

SDSni = (SDS x Protein content) / 100

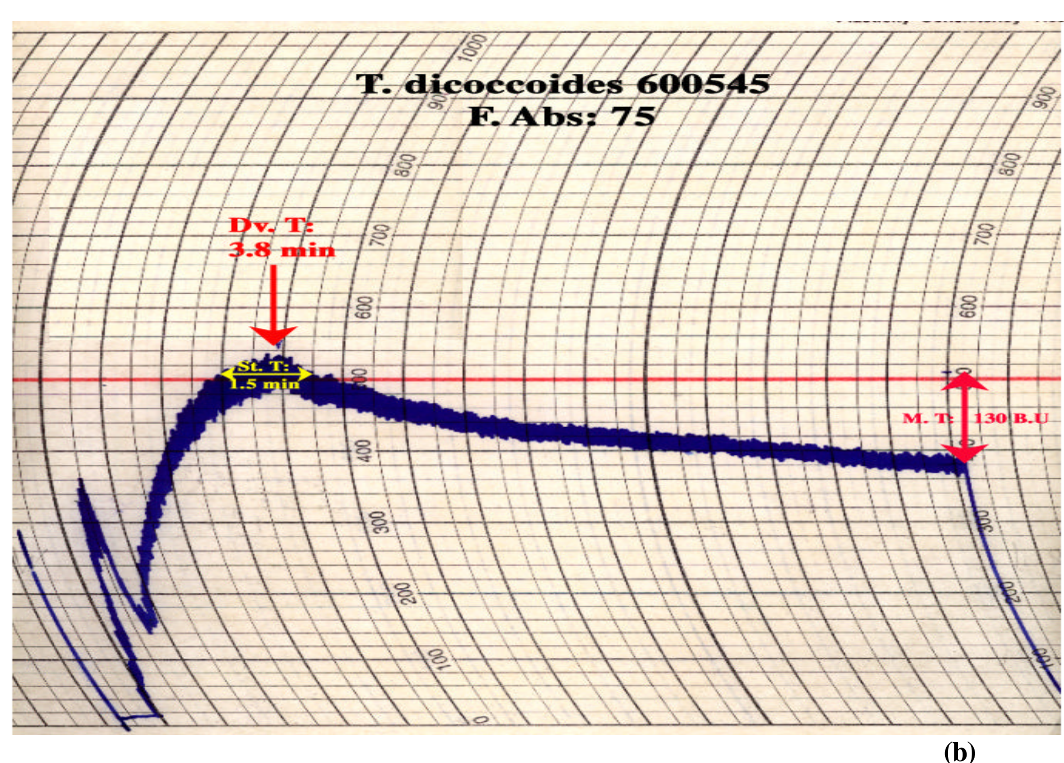
Before, the used SDS index was as follow: *SDSi = (SDS / Protein content) * 100*
But, in that way the SDS index value is affected by protein content and protein quality, and sometimes weak wheat with high protein content can give a fairly high sedimentation index value just because of its high protein content. This can be misleading, and can be overcome by SDSni.

- ***Farinograph:***

Farinograph is one of the most widely used physical dough testing instruments in the world. It measures plasticity and mobility of dough that is subjected to prolonged relatively gentle mixing at constant temperature. Resistance offered by the dough to mixing blades is transmitted trough a dynamometer to a pen that traces a curve on a kymograph chart (Brabender 1965). The resistance to mixing could also be measured with mixograph or resistogram. The farinograph used in this study is a Brabender OHG Duisburg/Germany with two mixing arms (Brabender 1965). Grains from each line were tempered at 17% moisture and milled using Udy Cyclone mill equipped with a 100-mesh sieve. The flour humidity is determined by weight before and after incubation for one hour at 130°C. Depending on the flour humidity, forty five to fifty grams of flour is used for farinograph test. The farinograph test indicates basically two important physical dough characteristics: 1) the absorption or amount of water (ml) required for a dough to have definite consistency (FAB); and 2) the behavior of the dough during mixing in graphical form, showing Farinograph Stability Time (FST), Farinograph Development Time (FDT), and Farinograph Mixing Time (FMT) (Fig II-4). For FMT, it measured using Brabender Units (BU), the highest BU values are related with weak dough strength, whereas small values are associated with strong dough. For correlation, clustering, and QTL analysis, the obtained data from Brabender graph were inversed by multiplying by "–1", as the small values are the desirable ones.



(a)



(b)

Fig II-4: Farinograph scan of the two parents: *Omrabi5* (a) and *T. dicoccoides600545* (b).

- ***Yellow Pigment (YP):***

The color of durum wheat is more or less yellow or amber; and is caused by the presence of carotenoid pigments, mainly xanthophylls and lutein. Color can be

estimated visually in the semolina, or instrumentally by reflectance spectroscopy (NIR). However, under our conditions, we find out that the more accurate measure remain the pigment chemical extraction. Yellow pigment contents were determined on 8 gram of semolina extracted overnight with 40 ml of water-saturated n-butyl alcohol. After filtering the extract through a whatman No.1, light transmission was determined in a spectrophotometer at a wavelength of 440 nanometers (nm). The determined values were calculated according to the concentration scale based on β-carotene (AACC 1976).
The yellow pigment measurements done for the season 1996-97 (irrigated and rainfed) were performed using the NIRs procedure.

- ***Vitreousness (Vit):***

A high value for vitreousness is related to high semolina extraction. The vitreousness is expressed as percentage of vitreousness and it is determined visually. The vitreous kernel has to be 100% free of yellow berry sections.

- ***Test Weight (TW):***

The test weight or the weight per hectoliter reflects the density and the volume occupied by the grains (packing efficiency), it was determined with a Schopper Chondrometer, using a 500-mL container. The grain weight of the 500-mL container was multiplied by 2, in order to have the weight of 1 liter.

- ***Thousand-Kernel Weight (TKW):***

The high weight of 1000-kernel and test weight are considered to be related to semolina yield, while the small kernels are linked to higher bran percentage, i.e. low endosperm: bran ratio. The weight per thousands was determined with an electronic seed counter (Numigral Chopin S.A.) using 200 kernels; broken kernels and foreign material had been removed previously by handpicking. The weight of 200 kernels was multiplied by 5, in order to have the 1000 kernels weight.

- ***Milling extraction:***

The durum grain milling output is suitable for semolina, pasta, and couscous processing. Because durum grains have greater hardness, its endosperm is less friable and produces a higher yield of coarse ground stock (semolina). From the durum grain about 75% of the kernel becomes milled product and the remaining 25% is considered feed. Of the milled product, 80 to 85% is semolina. The milling process is a series of physical manipulations involving essentially, cleaning, grinding grain, separating the bran and germ from the endosperm, and reducing the maximum amount of endosperm to flour fineness. One kilogram of each progeny was milled in a Buhler mill machine. This automatic mill grinds small quantities of wheat and generates flour test samples very similar to the ones used in industry. The Buhler mill has three break and three reduction passages that give a total of six passages of flour samples. The six obtained flours are mixed and weighted.

- ***Ash content (AC):***

Ash content is important in the assessment of semolina quality. It is chemically determined using the ignition method where 4-g sample are overnight incinerated at 585°C in a silica dish. After cooling, the dish and ash are weighed and the ash is brushed out, the dish reweighed, and the weight of the ash determined by difference.

In this study the ash content was measured using the Near Infra-Red (NIRs) procedure.

B-2- Protein electrophoretic analysis

The method of separating seed storage protein in acrylamide gel proved to be a powerful tool for seed storage protein characterization. This biochemical analysis technique is based on relative mobility of the protein fractions (bands), through an electrical field. This relative mobility depends on several factors including the strength of the field, the net charge, the size of the molecules, the ionic strength, the viscosity and the temperature of the medium in which the molecules are moving. Electrophoresis is a simple, rapid and sensitive analytical tool.

Up to day the most common analytical procedures for seed storage proteins are A-PAGE (Acid Poly-Acrylamide Gel Electrophoresis) for gliadins and, the SDS-PAGE analysis for glutenins.

a- Gliadin assay

Single seeds were crushed in mortar. Gliadin proteins were extracted from single seeds with 1,5 M dimethylformamide (100 µl per 20 mg of seed), and the mix was shaken by vortex. After an incubation for one hour at room temperature, and centrifugation for 15 min at 8000 tr/min, the supernatant (5µl) were fractionated on polyacrylamide-gel electrophoresis at 8.5% (C = 2.7) in aluminum lactate buffer at pH 3.1 (A-PAGE), a modified procedure of Tkachuk and Metlish (1980) was used.

b- Glutenin assay

To obtain good separation of Low Molecular Weight glutenin subunits (LMW) in a background free from gliadins, albumins, and globulins, an extraction with alkylation and a simple one step one-dimensional (1-D SDS-PAGE) procedure were adopted (Alvarez *et al.* 1999a). The residue from the samples used for gliadin extraction was cleaned 3 times with 50% propanol-1 at 60°C to remove monomeric proteins overlapping in SDS-PAGE with LMW glutenins subunits. Then the residue was reduced in 50% propanol-1, 80mM Tris-HCl pH: 8.5, 2% DL-dithiothreitol (DTT); incubated for 30min at 60°C, and centrifuged for 10min at maximum speed. 2.8µl of 4-vinylpiridine was added to the supernatant and incubated for 30min at 60°C. Finally the glutenins were precipitated with 1 ml of cold acetone (-20°C), dried under a fume hood and finally stored at –20°C till use.

In contrast with the gliadins A-PAGE, the SDS-PAGE procedure uses a discontinuous buffer system. In this system two different gels are used, a non-restrictive large pore gel (10%), called stacking gel, which is layered on top of the separating or main gel. While in continuous system, we used just a single separating gel and the same buffer in the chambers and in the gel.

The extracted glutenins were separated in a 8-14% gradient gel at C=1.28% in Tris-Glycine buffer (Alvarez *et al.* 1999b), and stained with a solution of Coomasie Brilliant Blue R250 in 1% ethanol, 12% trichloroacetic acid (TCA).

C- Molecular Analysis

C-1- DNA extraction

The method used is a SDS (Sodium-Dodecyl-Sulfate) procedure published in International Triticeae Mapping Initiative (ITMI) Wheat mapping workshop (1994). Young leaves were collected from young plants (20 to 30 days old), quick-frozen in liquid nitrogen and ground. The powdered leaves were transferred to tubes and 20-25 ml of heated extraction buffer (65°C) (500mM NaCl; 100mM Tris-HCI pH 8.0; 50mM EDTA; 0:84 SDS; sodium bisulfite) at pH 8.0 was added to each tube. This mix was incubated 30 min at 65°C, with a shacking after each 5 min. Twenty ml of chloroform-isoamyl alcohol (24:1) was added and the whole content was shaken vigorously to produce an emulsion. After the centrifugation for 15 min at 2,800 rpm, the supernatant was recuperated, and 2 volumes of cold 95% EtOH (-20°C) was added. The DNA was precipitated and kept at -20°C for 30-60 min or for overnight. The precipitated DNA was afterward recuperated, and washed twice with cold 70% ethanol and dissolved in 500µl of TE (10mM Tris pH.8; 1mM EDTA pH.8). Afterward, the DNA was treated by 2µg/ml stock solution of RNAse for 30min at 37°C and stored at -20°C until used. The concentration of the extract DNA was determined by spectrophotometer.

C-2- Microsatellites (SSR)

The Gatersleben wheat microsatellites (*gwm*) were used (Röder *et al.* 1998). The Gwm-PCR amplifications were performed in 10µl 10mM Tris-HCl (pH 9.0), 50mM KCl, 2.5 mM $MgCl_2$, 0.2mM dNTPs, 5% glycerol, 0.25µM primer, 0.5 unit of *Taq* DNA polymerase (Boehringer, Inc.) and 25ng of durum genomic-DNA. Taq DNA polymerase is a thermostable polymerase enzyme isolated from the thermophilic eubacterium Thermus aquaticus BM. It has a stable activity under high temperature (95°C) and can therefore be used for DNA-fragment amplification by PCR. For most of the primers, the PCR cycling program used was a touchdown from 63°C to 56°C (-1°C/cycle) or from 64°C to 57°C (-1°C/cycle), except for some microsatellites that required amplification under specific fixed temperatures (Table II-1).

The population parents were screened using 195 gwm microsatellites. Sixty six percent of the screened microsatellites were polymorphic. Therefore, the 129 polymorphic microsatellites were probed on the whole 114 recombined inbred lines and 170 polymorphic scored bands were generated.

Table II-1: Probed Microsatellites sequences and their optimal amplification program in *"Omrabi5/ T. dicoccoides600545//Omrabi5"*.

SSR	Sequence (5'-3')		PCR- Program
	Left	Right	
Gwm 2	CTGCAAGCCTGTGATCAACT	CATTCTCAAATGATCGAACA	TD:64-56
Gwm 5	GCCAGCTACCTCGATACAACTC	AGAAAGGGCCAGGCTAGTAGT	fix55
Gwm 11	GGATAGTCAGACAATTCTTGTG	GTGAATTGTGTCTTGTATGCTTCC	TD:63-56
Gwm 18	TGGCGCCATGATTGCATTATCTTC	GGTTGCTGAAGAACCTTATTTAGG	fix50
Gwm 30	ATCTTAGCATAGAAGGGAGTGGG	TTCTGCACCCTGGGTGAT	fix55
Gwm 33	GGAGTCACACTTGTTTGTGCA	CACTGCACACCTAACTACCTGC	fix55
Gwm 43	CACCGACGGTTTCCCTAGAGT	GGTGAGTGCAAATGTCATGTG	TD:63-56
Gwm 46	GCACGTGAATGGATTGGAC	TGACCCAATAGTGGTGGTCA	TD:64-56

Gwm	47	TTGCTACCATGCATGACCAT	TTCACCTCGATTGAGGTCCT	TD:64-56
Gwm	58	TCTGATCCCGTGAGTGTAAC A	GAAAAAAATTGCATATGAGCCC	TD:63-56
Gwm	60	TGTCCTACACGGACCACGT	GCATTGACAGATGCACACG	TD:64-56
Gwm	63	TCGACCTGATCGCCCCTA	CGC CCTGGGTGATGAATAGT	TD:65-56
Gwm	66	CCAAAGACTGCCATCTTTCA	CATGACTAGCTAGGGTGTGACA	TD:63-56
Gwm	68	AGGCCAGAATCTGGGAATG	CTCCCTAGATGGGAGAAGGG	TD:63-56
Gwm	71	GGCAGAGCAGCGAGACTC	CAAGTGGAGCATTAGGTACACG	TD:63-56
Gwm	88	TCCATTGGCTTCTCTCTCAA	CACTACAACTATGCGCTCGC	TD:65-56
Gwm	95	GATCAAACACACACCCCTCC	AATGCAAAGTGAAAAACCCG	Fix60
Gwm	99	GCCATATTTGATGACGCATA	AAGATGGACGTATGCATCACA	TD:63-56
Gwm	107	GGTCTCAGGAGCAAGAACAC	ATTAATACCTGAGGGAGGTGC	TD:64-56
Gwm	108	CGACAATGGGGTCTTAGCAT	TGCACACTTAAATTACATCCGC	TD:63-56
Gwm	111	TCTGTAGGCTCTCTCCGACT G	ACCTGATCAGATCCCACTCG	TD:63-56
Gwm	113	GAGGGTCGGCCTATAAGACC	ATTCGAGGTTAGGAGGAAGAGG	TD:63-56
Gwm	114	ATCCATCGCCATTGGAGTG	ACAAACAGAAAATCAAAACCCG	TD:64-56
Gwm	118	GATGTTGCCACTTGAGCATG	GATTAGTCAAATGGAACACCCC	fix55
Gwm	120	GATCCACCTTCCT CTCTCTC	GATTATACTGGTGCCGAAAC	TD:63-56
Gwm	122	GGGTGGGAGAAAGGAGATG	AAACCATCCTCCATCCTGG	TD:63-56
Gwm	124	GCCATGGCTATCACCCAG	ACTGTTCGGTGCAATTTGAG	TD:63-56
Gwm	129	TCAGTGGGCAAGCTACACAG	AAAACTTAGTAGCCGCGT	FIX:55
Gwm	131	AATCCCCACCGATTCTTCTC	AGTTCGTGGGTCTCTGATGG	TD:63-56
Gwm	136	GACAGCACCTTGCCCTTTG	CATCGGCAACATGCTCATC	TD:64-56
Gwm	140	ATGGAGATATTTGGCCTACAAC	CTTGACTTCAAGGCGTGACA	TD:63-56
Gwm	144	TTTGCTGTGGTACGAAACAT AC	ACTCACAAATGTCTAATAAAAC	TD:63-56
Gwm	148	GTGAGGCAGCAAGAGAGAAA	CAAAGCTTGACTCAGACCAAA	TD:64-56
Gwm	149	CATTGTTTTCTGCCTCTAGC C	CTAGCATCGAACCTGACCAAG	TD:64-56
Gwm	153	TGGTAGAGAAGGACGGAGAG	GATCTCGTCACCCGGAATTC	TD:63-56
Gwm	154	TCACAGAGAGAGAGGGAGGG	ATGTGTACATGTTGCCTGCA	Fix44
Gwm	155	CAATCATTTCCCCCTCCC	AATCATTGGAAATCCATATGCC	fix55
Gwm	156	CCAACCGTGCTATTAGTCATTC	CAATGCAGGCCCTCCTAAC	TD:64-56
Gwm	164	ACATTTCTCCCCCATCGTC	TTGTAAACAAATCGCATGCG	TD:63-56
Gwm	165	TGCAGTGGTCAGATGTTTCC	CTTTTCTTTCAGATTGCGCC	TD:64-56
Gwm	169	ACCACTGCAGAGAACACATACG	GTGCTCTGCTCTAAGTGTGGG	TD:63-56
Gwm	181	TCATTGGTAATGAGGAGAGA	GAACCATTCATGTGCATGTC	fix50
Gwm	186	GCAGAGCCTGGTTCAAAAAG	CGCCTCTAGCGAGAGCTATG	TD:64-56
Gwm	191	TAGCACGACAGTTGTATGCATG	AGACTGTTGTTTGCGGGC	TD:63-56
Gwm	193	CTTTGTGCACCTCTCTCTCC	AATTGTGTTGATGATTTGGGG	TD:63-56
Gwm	205	CGACCCGGTTCACTTCAG	AGTCGCCGTTGTATAGTGCC	TD:64-56
Gwm	213	TGCCTGGCTCGTTCTATCTC	CTAGCTTAGCACTGTCGCCC	TD:63-56
Gwm	218	CGGCAAACGGATATCGAC	AACAGTAACTCTCGCCATAGCC	Fix:60
Gwm	219	GGGGTCCGAGTCCACAAC	GATGAGCGACACCTAGCCTC	TD:63-56
Gwm	234	GAGTCCTGATGTGAAGCTGT TG	CTCATTGGGGTGTGTACGTG	TD:64-56
Gwm	247	GCAATCTTTTTTCTGACCAC G	ATGTGCATGTCGGACGC	TD:63-56
Gwm	249	CTGCCATTTTTCTGGATCTACC	CAAATGGATCGAGAAAGGGA	TD:64-56
Gwm	251	GGGATGTCTGTTCCATCTTAG	CAACTGGTTGCTACACAAGCA	TD:63-56
Gwm	259	CGACCGACTTCGGGTTC	AGGGAAAAGACATCTTTTTTTTC	TD:63-56
Gwm	260	GCCCCCTTGCACAAATC	CGCAGCTACAGGAGGCC	TD:64-56
Gwm	264	GCATGCATGAGAATAGGAAC TG	GAGAAACATGCCGAACAACA	TD:64-56
Gwm	265	TGTTGCGGATGGTCACTATT	GAGTACACATTTGGCCTCTGC	TD:63-56
Gwm	268	AGGGGATATGTTGTCACTCCA	TTATGTGATTGCGTACGTACCC	TD:64-56
Gwm	269	TTTGAGCTCCAAAGTGAGTT AGC	TGCAT ATAAACAGTCACACACCC	TD:64-56
Gwm	273	ATTGGACGGACAGATGCTTT	AGCAGTGAGGAAGGGGATC	TD:64-56
Gwm	274	TATTTGAAGCGGTTTGATTT	AACTTGCAAAACTGTTCTGA	TD:63-56
Gwm	275	AATTTTCTTCCTCACTTATT CT	AACAAAAAATTAGGGCC	fix46
Gwm	276	ATTTGCCTGAAGAAAATATT	AATTTCACTGCATACACAAG	TD:63-56
Gwm	282	TTGGCCGTGTAAGGCAG	TCTCATTCACACACAACACTAGC	TD:63-56
Gwm	297	ATCGTCACGTATTTTGCAAT G	TGCGTAAGTCTAGCATTTTCT	TD:63-56
Gwm	299	ACTACTTAGGCCTCCCGCC	TGACCCACTTGCAATTCATC	TD:63-56
Gwm	302	GCAAGAAGCAACAGCAGTAAC	CAGATGCTCTTCTCTGCTGG	TD:63-56
Gwm	311	TCACGTGGAAGACGCTCC	CTACGTGCACCACCATTTTG	TD:63-56
Gwm	319	GGTTGCTGTACAAGTGTTCACG	CGGGTGCTGTGTGTAATGAC	TD:63-56
Gwm	332	AGCCAGCAAGTCACCAAAAC	AGTGCTGGAAAGAGTAGTGAAGC	TD:63-56
Gwm	334	AATTTCAAAAAGGAGAGAGA	AACATGTGTTTTTAGCTATC	Fix50
Gwm	335	CGTACTCCACTCC ACACGG	CGGTCCAAGTGCTACCTTTC	TD:64-56
Gwm	339	AATTTTCTTCCTCACTTATT	AAACGAACAACCACTCAATC	Fix50
Gwm	340	GCAATCTTTTTTCTGACCAC G	ACGAGGCAAGAACACACATG	TD:63-56
Gwm	344	CAAGGAAATAGGCGGTAACT	ATTTGAGTCTGAAGTTTGCA	TD:63-56
Gwm	356	AGCGTTCTTGGGAATTAGAGA	CCAATCAGCCTGCAACAAC	TD:63-56
Gwm	357	TATGGTCAAAGTTGGACCTCG	AGGCTGCAGCTCTTCTTCAG	TD:64-56
Gwm	368	CCATTTCACCTAATGCCTGC	AATAAAACCATGAGCTCACTTGC	Fix50

Gwm 369	CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC	TD:64-56
Gwm 371	GACCAAGATATTCAAACTGGCC	AGCTCAGCTTGCTTGGTACC	TD:65-56
Gwm 372	AATAGAGCCCTGGGACTGGG	GAAGGACGACATTCCACCTG	TD:63-56
Gwm 374	ATAGTGTGTTGCATGCTGTGTG	TCTAATTAGCGTTGGCTGCC	TD:63-56
Gwm 375	ATTGGCGACTCTAGCATATACG	GGGATGTCTGTTCCATCTTAGC	TD:63-56
Gwm 376	GGGCTAGAAAACAGGAAGGC	TCTCCCGGAGGGTAGGAG	TD:63-56
Gwm 382	GTCAGATAACGCCGTCCAAT	CTACGTGCACCACCATTTTG	TD:63-56
Gwm 391	ATGTGCATGTCGGACGC	ATAGCGAAGTCTCCCTACTCCA	TD:65-57
Gwm 400	TGTAGGCACTGCTTGGGAG	GTGCTGCCACCACTTGC	TD:64-56
Gwm 403	CGACATTGGCTTCGGTG	ATAAAACAGTGCGGTCCAGG	Fix50
Gwm 408	TCGATTTATTTGGGCCACTG	GTATAATTCGTTCACAGCACGC	TD:63-56
Gwm 410	GCTTGAGACCGGCACAGT	CGAGACCTTGAGGGTCTAGA	Fix:55
Gwm 413	TGCTTGTCTAGATTGCTTGGG	GATCGTCTCGTCCTTGGCA	TD:63-56
Gwm 415	GATCTCCCATGTCCGCC	CGACAGTCGTCACTTGCCTA	TD:64-56
Gwm 427	AGTGTGTTCATTTGACAGTT	AAACTTAGAACTGTAATTTCAGA	TD:63-56
Gwm 443	GGGTCTTCATCCGGAACTCT	CCATGATTTATAAATTCCACC	Fix55
Gwm 448	CACATGGCATCACATTTGTG	AAACCATATTGGGAGGAAAGG	TD:65-57
Gwm 480	TGCTGCTACTTGTACAGAGGAC	CCGAATTGTCCGCCATAG	TD:63-56
Gwm 493	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	TD:63-56
Gwm 494	ATTGAACAGGAAGACATCAGGG	TTCCTGGAGCTGTCTGGC	TD:64-56
Gwm 497	GTAGTGAAGACAAGGGCATT	CCGAAAGTTGGGTGATATAC	TD:63-56
Gwm 498	TTTGCATGGAGGCACATACT	GGTGGTATGGACTATGGACACT	Fix55
Gwm 499	ACTTGTATGCTCCATTGATTGG	GGGGAGTGGAAACTGCATAA	TD:63-56
Gwm 508	GTTATAGTAGCATATAATGGCC	GTGCTGCCATGATATTT	TD:64-56
Gwm 512	AGCCACCATCAGCAAAAATT	GAACATGAGCAGTTTGGCAC	Fix60
Gwm 513	GGTCTGTTCATGCCACATTG	ATCCGTAGCACCTACTGGTCA	TD:64-56
Gwm 518	CAGGGTGGTGCATGCAT	AATCACAACAAGGCGTGACA	TD:64-56
Gwm 526	CAATAGTTCTGTGAGAGCTGCG	CCAACCCAAATACACATTCTCA	Fix50
Gwm 537	GCCACTTTTGTGTCGTTCCT	ACATAATGCTTCCTGTGCACC	TD:64-56
Gwm 538	GTTGCATGTATACGTTAAGCGG	GCATTTCGGGTGAACCC	TD:64-56
Gwm 540	TCTCGCTGTGAAATCCTATTTC	AGGCATGGATAGAGGGGC	TD:63-56
Gwm 550	CCCACAAGAACCTTTGAAGA	CATTGTGTGTGCAAGGCAC	TD:63-56
Gwm 554	TGCCCACAACGGAACTTG	GCAACCACCAAGCACAAAGT	TD:64-56
Gwm 558	GGGATTGCATATGAGACAACG	TGCCATGGTTGTAGTAGCCA	TD:63-56
Gwm 570	TCGCCTTTTACAGTCGGC	ATGGGTAGCTGAGAGCCAAA	TD:63-56
Gwm 573	TTCAAATATGTGGGAACTAC	AAGAGATAACATGCAAGAAA	TD:63-56
Gwm 582	TCTTAAGGGGTGTTATCATA	AAGCACTACGAAAATATGAC	Fix48
Gwm 601	TTAAGTTGCTGCCAATGTTCC	ATCGAGGACGACATGAAGGT	TD:63-56
Gwm 604	TATATAGTTCAATATGACCCG	ATCTTTTGAACCAAATGTG	Fix50
Gwm 610	CTGCCTTCTCCATGGTTTGT	AATGGCCAAAGGTTATGAAGG	TD:63-56
Gwm 611	CGTGCAAATCATGTGGTAGG	CATGGAAACACCTACCGAAA	TD:63-56
Gwm 613	CCGACCCGACCTACTTCTCT	TTGCCGTCGTAGACTGG	TD:63-56
Gwm 614	TTTTACCGTTCCGGCCTT	GATCACATGCATGCGTCATG	TD:64-56
Gwm 617	GATCTTGGCGCTGAGAGAGA	CTCCGATGGATTACTCGCAC	TD:65-57
Gwm 626	GATCTAAAATGTTATTTTCTCTC	TGACTATCAGCTAAACGTGT	TD:63-56
Gwm 630	GTGCCTGTGCCATCGTC	CGAAAGTAACAGCGCAGTGA	TD:63-57
Gwm 635	TTCCTCACTGTAAGGGCGTT	CAGCCTTAGCCTTGGCG	TD:63-56
Gwm 636	CGGTAGTTTTTAGCAAAGAG	CCTTACAGTTCTTGGCAGAA	Fix48
Gwm 639	CTCTCTCCATTCGGTTTTCC	CATGCCCCCCTTTTCTG	TD:64-56
Gwm 644	GTGGGTCAAGGCCAAGG	AGGAGTAGCGTGAGGGGC	TD:63-56
Gwm 674	TGACCGAGTTGACCAAAACA	TCGAGCGATTTTTCCTGC	TD:63-56

The PCR was carried out in Perkin-Elmer 9700 Thermal Cycle. The PCR amplified fragments were either separated in 12% acrylamide gel (39:1) or a 6% denaturing-acrylamide gel (19:1) depending on the number of base pairs differentiating the two parents *Omrabi5* and *T. dicoccoides600545*. The fragments were visualized by silver staining.

C-3- Amplified Fragment Length Polymorphism Analysis (AFLPs)

The AFLP method used was as described by Vos et *al.* (1995). The Life Technology Kit with EcoRI as rare cutter and MseI as frequent cutter was used. The DNA (80ng) was first digested in 2 units of MseI and 2 units of EcoRI in 50mM NaCl

at 37°C for 2 hr. The enzyme was after on inactivated by incubation at 70°C for 15 min and cooled down in ice. The digestion step was checked in 1% agarose gel.

The Digested DNA was ligated to the EcoRI adapter:
5'-CTCGTAGACTGCGTACC
CATCTGACGCATGGTTAA-5'
And the MseI adapter:
5'-GACGATGAGTCCTGAG
TACTCAGGACTCAT-5'
at 20°C for 2 hr. The resulting ligated product was therefore diluted 5 times with TE.

The ligated DNA was preamplified in 20µl 10mM Tris-HCl (pH 9.0), 50mM KCl, 1.5 mM $MgCl_2$, 0.8mM dNTPs, 0.56µM pre-amp primer MseI, 0.56µM pre-amp primer EcoRI, 1 unit of *Taq* DNA polymerase (Boehringer, Inc.), and 2µl of the diluted ligated DNA. The PCR cycling program used was 20 cycles: 95°C for 30 seconds, 56°C for 1 minute, and 72°C for 1min. The preamplified product was also diluted 5 times and checked in 1% agarose gel.

A final selective amplification was carried out in 10µl of 10mM Tris-HCl (pH 9.0), 50mM KCl, 1.5 mM $MgCl_2$, 0.8mM dNTPs, 0.25µM selective primer MseI, 0.25µM selective primer EcoRI, 0.75 unit of *Taq* DNA polymerase (Boehringer, Inc.), and 2.5µl of the preamplified DNA. The PCR cycling program used was a touchdown from 65°C to 56°C (-0.7°C each cycle) and it was carried out in Perkin-Elmer 9700 Thermal Cycle. The PCR amplified fragments were separated in 6% denaturing-acrylamide gel (19:1) and the fragments were visualized by silver staining. The gels were dried and scanned.

The combinations used were as follow:

- MseI+cag and EcoRI+agc (*McagEagc*);
- MseI+cag and EcoRI+agg (*McagEagg*);
- MseI+cta and EcoRI+acg (*MctaEacg*);
- MseI+ctc and EcoRI+agg (*MctcEagg*);
- MseI+ctg and EcoRI+aca (*MctgEaca*);
- MseI+ctg and EcoRI+acc (*MctgEacc*);
- MseI+ctg and EcoRI+acg (*MctgEacg*);
- MseI+ctg and EcoRI+agg (*MctgEagg*);
- MseI+ctt and EcoRI+agg (*McttEagg*);
- MseI+caa and EcoRI+aca (*McaaEaca*);
- MseI+caa and EcoRI+acc (*McaaEacc*);
- MseI+caa and EcoRI+acg (*McaaEacg*);
- MseI+caa and EcoRI+agg (*McaaEagg*);
- MseI+cac and EcoRI+aag (*McacEaag*);
- MseI+cat and EcoRI+act (*McatEact*);
- MseI+ctc and EcoRI+aag (*MctcEaag*);
- MseI+ctg and EcoRI+aag (*MctgEaag*);
- MseI+ctg and EcoRI+agc (*MctgEagc*);
- MseI+ctt and EcoRI+aag (*McttEaag*); and
- MseI+ctc and EcoRI+act (*MctcEact*).

The 20 AFLPs combinations used generated 279 polymorphic bands. These polymorphic bands were scored as present / absent (1/0) using the Crosscheker program (Buntjer, Wageningen University, 1999).

D- Statistical Analysis

The field design used was an augmented design (Federer 1956). The design divides the experimental area into a number of blocks of test plots, and three or more check varieties are chosen to be used as checks inside each block. The checks were replicated but not the experimental test lines. Measurements of the experimental lines were adjusted for block differences, which were measured by the check varieties, which were included in every block.

For the mapping population, the 114 BC-RILs were divided over 6 blocks where 19 BC-RILs with 5 commercial checks were included in each block. Thus each block was constituted of 24 entries (19 test BC-RILs + 5 Checks). The total number of entries of the whole trial was 144 (114 + 5 checks repeated in each block). To adjust data and to perform analysis of variance (ANOVA) a software developed at our program was used (Nachit, unpublished).

The heritability at broad sense for the BC-RIL population was calculated as follows:

$h^2 = [(VRIL - \sqrt{(VP1 \times VP2)}) / VRIL] \times 100$

VRIL = variance of the RIL population
VP1 = variance of the first parent
VP2 = variance of the second parent

The heritability estimation was performed only for the environments where the checks were included in planting and grain quality assessment. Therefore, the environments 97Ir and 00Ir were excluded from heritability analysis, as the grain quality data of the checks were not available. When the parents showed low environmental interaction, the mean of the variances of the 5 checks used in the RIL trials (*Omrabi5, Haurani, Korifla, Cham1,* and *Gidara2*) was used to estimate the environmental variance.

The SYSTAT program version 7.0 was used to perform general statistics parameters including: mean, standard error, range, and coefficient of variability for the population *Omrabi5/ T. dicoccoides600545// Omrabi5*. SYSTAT was also used to determine distribution, Pearson correlation matrix, and clustering between traits over years and over sites.

E- Map Construction

The map was constructed using MAPMAKER version 2.0 (Lander *et al.* 1987) based on the method of maximum likelihood and the Lander-Green algorithm to calculate the best map of any given order of loci. Likelihood of the map is the probability that the constructed map (consisting of an order for the loci and recombination fractions between them) would give rise to the observed data. This method is widely favored because it can be applied even if the modes of inheritance and amounts of data vary among loci. Kosambi function

(Kosambi, 1944) was used to convert the recombination frequency to genetic distances in centimorgams (cM). The map was constructed at LOD of 3.0 (Logarithm of the odds ratio), except for some segment fragments that were joined at LOD 2.5. Comparison was made to earlier published Triticeae maps.

Two-point analysis was first applied to get groups of related markers at maximum LOD score of at least 3.0 and the minimum recombination ratio at more 40%. Those formed groups were afterward ordered using "First Order" command whenever it was possible (usually the "first order" command was found to cause the computer to crush, especially when it is used to analyze large number of markers). Usually, the first order was aided with LOD table correlations between markers to figure out the most linked markers. The obtained order is then fine-tuned using a Three-Point linkage analysis "Ripple" command. Other markers are added using "Place" command and fine-tuned using again the "Ripple" command.

Both chromosome assignment and centromere localization were determined by comparing the *Omrabi5/ T. dicoccoides600545// Omrabi5* map to the previously published wheat maps, especially to Röder *et al.* (1998) wheat microsatellites map.

F- Multiple Quantitative Trait Loci Analysis (MQTL)

The QTLs analysis was performed with the software package Multiple Quantitative Trait Loci (MQTL) (Tinker and Mather 1995). MQTL program consist of 4 steps:

(i) Performing interval mapping to find evidence of QTL,
(ii) Estimating thresholds for inferring QTL presence,
(iii) Inferring the presence of QTL and estimating their positions, and
(iv) Estimating the additive allelic effects at putative QTL.

(i) Interval Mapping:

Genome-wide QTL searches were performed by Simple Interval Mapping (SIM) and Simplified Composite Interval Mapping (sCIM) (Tinker and Mather 1995), each with a test for QTL main effects and a test for QTL x Environment interaction. Therefore, for each trait we got four scans (plots of the test statistic against map position). Environments were assumed to have fixed effects. The difference between the two interval mapping methods is that SIM uses genotype information only for markers in the region being tested, whereas sCIM also includes genotype information for background markers elsewhere in the genome. Therefore, sCIM analysis adjusts for the possible effects of QTL elsewhere in the genome and then improves the QTL precision (Mather *et al.* 1997). However, if we are dealing with more than one environment, there is no reliable threshold that can be used for sCIM (Tinker and Mather 1995).

(ii) Estimating Thresholds:

All four scans were based on a test statistic for linear models described by Haley and knott (1992). Although this test statistic is an approximation of the likelihood ratio, no assumptions were made about its distribution. Instead, significance thresholds were estimated separately for each trait by permutation (Churchill and Doerge 1994) as follows. Phenotypic values were assigned randomly to bc-Recombinant Imbred lines, and then SIM was applied to the permuted data to determine the maximum values of the test statistics for QTL main effect and

QTLxE interaction. This procedure was repeated 5000 times to approximate the distributions of the maximum test statistics under the null hypothesis (no QTL). These distributions were then used to estimate thresholds to maintain the genome-wise type-error rate below 5%. In simulation studies, Tinker and Mather (1995) found this procedure provided good control of type-I error rate for SIM, even when environments had different amounts of residual variance. Thresholds for sCIM could not be estimated because it is not clear how to control the Type I error rate when sCIM is applied to multiple environments data (Tinker and Mather 1995).

(iii) Making Inferences and Estimating QTL positions:

Two levels of QTL inference were made. Primary QTL were declared at positions where SIM peaks were significant for either QTL main effects or QTLxE interaction and where sCIM peaks were also strong. Secondary QTL were declared where either SIM or sCIM, but not both, gave evidence for a QTL. Estimates of the positions of primary and secondary QTL corresponded to the peaks of the sCIM scans. When evidence for a QTL main effect and a QTLxE interaction were found near the same position, a single QTL was inferred based on the effect that seemed strongest.

(iv) Estimating Allelic Effects:

Main effects and QTLxE interactions were estimated in multi-locus linear models. Each estimated main effect corresponded to the average difference between homozygous classes for a given QTL. Estimates were made for a model that included only primary QTL, as well as for a model that included both primary and secondary QTL. Estimates were also made for each elected QTL to determine his effect on the trait.

Reduction in variance (R^2), relative to a model that included only the environmental main effects, was estimated for models with four levels of complexity: model 1, primary QTL main effects, model 2, primary QTL main effects and primary QTLxE interactions, Model 3, main effects and QTLxE interactions for both primary and secondary QTL, and Model 4 effects for all background markers estimated separately by environment. The model with all background markers did not contain terms for specific QTL. For *Omrabi5/ T. dicoccoides600545// Omrabi5* map the forth model is excluded as the background markers did not cover the whole genome and therefore did not represent the whole genetic variance.

In the MQTL manual, the background markers should be selected in a way to cover the whole genome with two conditions, the total background marker number should be lower than the population size and the distance between each two-background marker should be around 25 cM. But in the present study, the sCIM analysis run based on such background markers have mostly resulted in an interminable and uninterpretable scan. Therefore, the CIM program approach (Yiang pers.com.) using few background markers corresponding to the significant SIM peaks was adopted.

The population was grown in several locations and years; and the quality traits were determined for all those environments.

Chapter III
Results & Discussion

Molecular Markers & Mapping
of
Omrabi5/ T. dicoccoides600545// Omrabi5

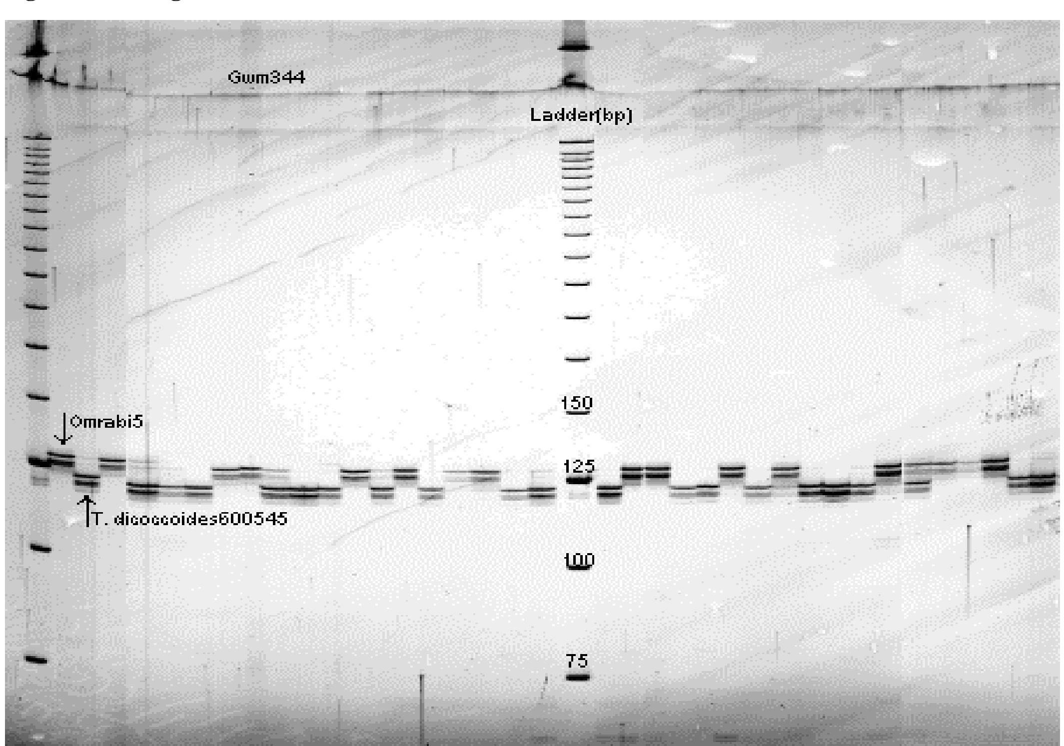
Omrabi5/ T. dicoccoides600545// Omrabi5 population consisted of 114 BC-RILs at F8. The construction of genetic linkage maps relies mainly on the choice of parental lines, segregating population, and markers to reveal polymorphism populations (Saliba-Colombi *et al.* 2000). Moreover, the utilization of RILs is known to be more suitable for genetic analysis of quantitative traits. In addition, single seed descent populations gave a predominantly fixed genetic structure, which make the population valuable for assessing the environmental impact on trait expression. Maps constructed with such populations could, afterward, facilitate the fine mapping of regions around genes of interest. The two parents *Omrabi5* and *T. dicoccoides600545* are very distant genetically (Pool II) and the population was developed using SSD method. The genetic map of *Omrabi5/ T. dicoccoides600545// Omrabi5* population was constructed based on microsatellites (SSR), amplified fragment length polymorphism markers (AFLP), and seed storage proteins. The microsatellite markers were used as milestones or anchor primers for chromosomal, arm, and centromere assignments. They allow the construction of a high-confidence framework map. Whereas, AFLPs were used for map saturation as they are supposed to amplify fragment all over the wheat genomes. The seed storage proteins are thigthly related to wheat grain quality and therefore are of great importance in the present study.

A- <u>Microsatellites</u>

It is widely accepted that microsatellites are one of the most informative markers (Tautz *et al.* 1986; Beckmann and Soller 1990; Condit and Hubbell 1991; Akkaya *et al.* 1992; Lagercrantz *et al.* 1993; Senior and Heun 1993; Thomas and Scott, 1993, Röder *et al.* 1994, Wang *et al.* 1994, Bell and Ecker 1994; Liu *et al.* 1996; Morchen *et al.* 1996; Provan *et al.* 1996; Szewc-McFadden *et al.* 1996; Taramino and Tingey 1996; Smulders *et al.* 1997). Their high level of polymorphism, combined with their high interspersion rate, makes them an abundant source of genetic markers (Wu and Tanksley 1993; Plashke *et al.* 1995; Röder *et al.* 1995; Ma *et al.* 1996, Bryan *et al.* 1997). They are highly suitable as genetic markers in wheat for mapping agronomic important genes. The microsatellites used (Gwm = Gatersleben wheat microsatellites) have known chromosomal assignments and were previously mapped in many mapping populations including the International Triticeae Mapping Initiative population (ITMI) (Röder *et al.* 1995; Röder *et al.* 1998); and the durum populations: *Messapia* x *T. dicoccoides*

MG4343 population (Korzun *et al.* 1999) and Jennah Khetifa x Cham1 population (Nachit *et al.* 2001). Therefore, it was decided to use these codominant and very informative markers as a main framework for our genetic map (see resolution in Fig. III-1).

Fig. III-1: Probing of *Omrabi5/ T. dicoccoides600545// Omrabi5* with Gwm344



In this study, the parents *Omrabi5* and *T. dicoccoides600545* were first screened using 192 gwm microsatellites, out of which 122 were polymorphic and were applied to the whole mapping population (114 RILs). Thus, 63.5% of the microsatellites used show polymorphism between *Omrabi5* and *T. dicoccoides600545*. These results confirm the transferability of microsatellites between bread and durum wheat. The same result was reported in a *T. durum* x *T. dicoccoides* cross (Korzun *et al.* 1999) and in durum x durum wheat cross (Nachit *et al.* 2001). Whereas, other findings suggested that microsatellites may not transfer well among species and showed a low level of transportability across the 3 wheat genomes and to other cereal genomes (Röder *et al.* 1995, Bryan *et al.* 1997). Twenty-one microsatellites showed a significant skewness from the expected heritability ratio (or distribution) and therefore were eliminated from the mapping analysis. Molecular markers representing skewed segregation have already

been reported in other *Triticeae* species (Heun *et al.* 1991; Liu and Tsunewaki 1991; Blanco *et al.* 1998; Nachit *et al.* 2001). Chromosomal rearrangements (Tanksley 1984), alleles inducing gametic or zygotic selection (Nakagarha 1986), parental reproductive differences (Foolad *et al.* 1995), presence of lethal genes (Blanco *et al.* 1998), wide genetic background of the parents and the single seed descend method (Nachit *et al.* 2001) have been suggested as potential causes of distortion. The mapping analysis was made based on the non-skewed 101 polymorphic microsatellites. Seventy eight percent of the scored microsatellite fragments were mapped (Table III-3 & Fig. III-5): 45 fragments on the A genome and 79 on the B genome (Table III-4). They generated 161 polymorphic fragments, i.e. 1.59 fragment per each microsatellite.

Most microsatellites generated one polymorphic fragment (Table III-1). These findings confirm earlier results showing that the majority of microsatellite markers are genome-specific and usually amplify only a single locus (Röder *et al.* 1998, Korzun *et al.* 1999). Nevertheless, some of them did generate 2 to 3 fragments. Some microsatellites amplified orthologous loci (Fig. III-5), for instance, Gwm165 and Gwm234 amplified two fragments where each mapped to the two homoeologous sites on A and B genome. The result for Gwm165 fragments confirmed earlier findings (Röder *et al.* 1998); while Gwm234 was reported to amplify only one fragment and to be mapped on chromosome 5B (Röder *et al.* 1998). In other cases, microsatellites amplified non-homoeologous regions (Table III-1); such as Gwm554, Gwm264, Gwm131, and Gwm537 (Table III-1, Fig. III-5). Gwm154 produces the highest number of polymorphic fragments (4). Two out of these fragments were mapped in the *Omrabi5/ T. dicoccoides600545// Omrabi5* map, one on 3BL and one on 7AS, whereas the two other fragments did not fit in any linkage group. This chromosomal localization is in disagreement with ITMI map where Gwm154 amplified just one fragment mapped to the short arm of 5A (Röder *et al.* 1998).

Most microsatellites mapping was in agreement with earlier publications. Therefore, ninety percent of mapped microsatellites did map as expected, whereas, only twelve microsatellites were found to map in other localization (Table III-1). Some of these microsatellites were mapped on the homoeologous chromosome such as Gwm122 (2B instead of 2A), Gwm156 (5B instead of 5A), Gwm415 (5B instead of 5A), Gwm443 (5A instead of 5B), and Gwm635 (7B instead of 7A), while Gwm265 was

mapped in 4A instead of 2A; Gwm299 in 2B instead of 3B; Gwm513 in 7B instead of 4B, and Gwm582 in 6B instead of 1B. This could be explained by the presence of additional loci in the wheat genome. In addition, certain microsatellites generate fragments that were mapped in different chromosomes. For instance, Gwm554a, b, and c fragments were mapped in 2A, 1B, and 5B, respectively. Gwm264 generated 3 fragments that mapped to 1A, 1B, and 7B. The same was also observed for Gwm131, Gwm537, and Gwm644 (Table III-1). The same behavior was reported for Gwm666 that amplified 5 sites all mapping to the A genome in ITMI population (Röder *et al.* 1998) and 3 sites in *Messapia* x *MG4343* population mapped to the 3A, 5A and 7A (Korzun *et al.* 1999). As mentioned in the material and methods most of the linkage groups were formed at a maximum LOD score of at least 3.0 and the minimum recombination ratio at more 40%. Therefore, the localization of these microsatellites is quite reliable.

Table III-1: Number of generated fragments per polymorph microsatellite and their chromosomal assignment in *Omrabi5/ T. dicoccoides600545// Omrabi5* population in comparison with wheat published maps.

Microsatellites	Nr. of fragments	Nr of map. fragments	Ch. assign in MDM	known Chr. Assign
Gwm 154	**4**	**2**	**3BL, 7AS**	**5AS**
Gwm 311	3	1	2AL	2A
Gwm 63	3	1	7AL	7A
Gwm 88	3	2	6BS	6BL
Gwm 276	3	2	7AL	7A
Gwm 554	**3**	**3**	**1BL, 2AL, 5BL**	**5B**
Gwm 630	3	2	2BS	2B
Gwm 335	3	1	5B	5B
Gwm 124	3	2	1B L	1B
Gwm 149	3	2	4BL	4B
Gwm 264	**3**	**3**	**1AL, 1BS, 7BS**	**1B, 3B**
Gwm 448	3	1	2AS	2A
Gwm 493	3	2	3BS	3B
Gwm 408	2	2	5BL	5B
Gwm 639	2	1	5BL	5A,5B
Gwm 131	**2**	**2**	**1BL, 7BL**	**7BL, 1B, 3B**
Gwm 249	2	2	2AS	2A
Gwm 282	2	2	7AL	7A
Gwm 371	2	2	5BL	5B
Gwm 570	2	1	6AL	6A
Gwm 219	2	1	6BL	6B
Gwm 2	2	1	3AS	3AS (2AS)
Gwm 60	2	2	7AS	7AS
Gwm 205	2	2	5AS	5A
Gwm 33	2	2	1AS	1BL, 1AS
Gwm 43	**2**	**1**	**4AL**	**7BL**
Gwm 95	2	1	2AS	2AS
Gwm 113	2	1	4BS	4B
Gwm 114	2	2	3BL	3B
Gwm 136	2	1	1AS	1A

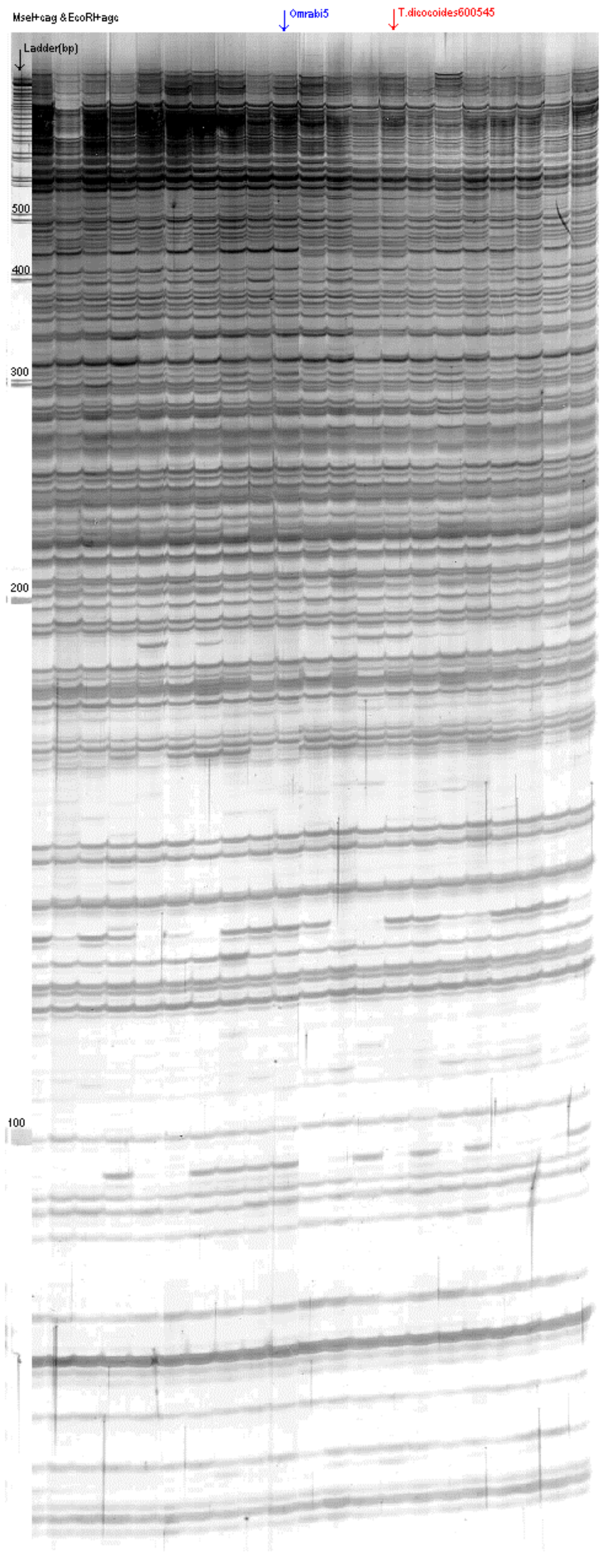
Gwm 148	2	1	2BS	2BS
Gwm 155	2	1	3AL	3AL
Gwm 165	**2**	**2**	**4AS, 4BL**	**4BS, 4A**
Gwm 213	2	2	5BS	5B
Gwm 234	**2**	**2**	**5AS, 5BS**	**5BS**
Gwm 251	2	1	4BL	4B
Gwm 259	2	2	1BL	1B
Gwm 268	2	2	1BL	1B
Gwm 319	2	1	2BS	2B
Gwm 340	2	1	3BL	3BL
Gwm 368	2	1	4BS	4B
Gwm 376	2	2	3BS	3B
Gwm 403	2	1	1BL	1B
Gwm 494	2	2	6A	6A
Gwm 537	**2**	**2**	**5BL, 7BS**	**7B**
Gwm 644	**2**	**2**	**1BL, 3BS**	**6B, 7B**
Gwm 558	1	1	2AL	2A
Gwm 118	1	1	5BS	5BL
Gwm 260	1	1	7AS	7AS
Gwm 626	1	1	6BL	6B
Gwm 674	1	1	3AL	3A
Gwm 11	1	1	1B	1BL
Gwm 30	1	1	3AL	1AS, 3A
Gwm 46	1	0	-	7BS
Gwm 58	1	1	6BS	6BL
Gwm 66	1	0	-	4B, 5B
Gwm 71	1	1	2AS	2A
Gwm 99	1	1	1AL	1A
Gwm 107	1	1	4BL	4B
Gwm 129	1	1	2BS	2B, 5AS
Gwm 122	**1**	**1**	**2BS**	**2A**
Gwm 140	1	1	1BL	1B
Gwm 144	1	1	3BS	3B
Gwm 156	**1**	**1**	**5BS**	**5A**
Gwm 181	1	1	3BL	3B
Gwm 186	1	1	5AL	5AL
Gwm 191	1	1	2BL	2B,5B,6B
Gwm 193	1	1	6BS	6BS
Gwm 265	**1**	**1**	**4AL**	**2A**
Gwm 269	1	0	-	5D
Gwm 273	1	0	-	1B
Gwm 274	1	1	1BL	1B, 7B
Gwm 299	**1**	**1**	**2BS**	**3B**
Gwm 302	1	0	-	7B
Gwm 332	1	1	7AL	7A
Gwm 344	1	1	7BL	7B
Gwm 369	1	1	3AS	3A
Gwm 372	1	0	-	2A
Gwm 374	1	1	2BS	2B
Gwm 375	1	1	4BL	4BL
Gwm 382	1	1	2AL	2A,2B
Gwm 400	1	1	7BS	7B
Gwm 413	1	1	1B	1B
Gwm 415	**1**	**1**	**5BS**	**5A**
Gwm 443	**1**	**1**	**5AS**	**5B**
Gwm 497	1	1	1AL	1A, 2A
Gwm 498	1	1	1BL	1B
Gwm 499	1	1	5BL	5B
Gwm 508	1	1	6BS	6B
Gwm 512	1	1	2AS	2A
Gwm 513	**1**	**1**	**7BS**	**4B**

Gwm 518	1	1	6BS	6B
Gwm 538	1	1	4BL	4B
Gwm 573	1	1	7BS	7A,7B
Gwm 582	**1**	**1**	**6BL**	**1B**
Gwm 601	1	1	4AS	4A
Gwm 604	1	1	5BL	5B
Gwm 610	1	1	4AS	4A
Gwm 613	1	1	6BS	6B
Gwm 614	1	1	2AS	2A
Gwm 635	**1**	**1**	**7BS**	**7A**
Total	**161**	**124**		

B- AFLPs

AFLP technique is a new powerful marker technology. It is highly reproducible and can be applicable to all species (Vos *et al.* 1995). It is a robust and reliable technique, since stringent reaction conditions are used for primer annealing. In this study, AFLP markers were used for map saturation. Twenty AFLP combinations were used with EcoRI as the rare cutter and MseI as the frequent cutter (MseI+cxx and EcoRI+axx with "x" being one of the four possible nucleotides: A, C, G, or T). The 20 used AFLP combinations generated 279 polymorphic fragments (see resolution in Fig. III-2). These results confirm that AFLPs provides an effective means of detecting simultaneous polymorphism at a large number of loci in a single assay (Vos *et al.* 1995). Fifty four percent of these polymorphic fragments were mapped in *Omrabi5/ T. dicoccoides600545// Omrabi5* population, of which 34.7% were mapped in the A genome and 61% in the B genome (Table III-4). Therefore, as for the microsatellites, more AFLP markers were mapped in the B genome than in the A genome. More markers mapped to the B genome in comparison to the other wheat genomes have been observed in *T. durum* and *T. aestivum* (Chao *et al.* 1989, Xie *et al.* 1993, Nelson *et al.* 1995b,c; van Deynze *et al.* 1995a, Röder *et al.* 1998, Nachit *et al.* 2001). Chao *et al.* (1989) suggested that the reasons are either the high mutation rate in the B genome or the high genetic variability of the B genome progenitor species. The first hypothesis is supported by the fact that B genome chromosomes contain more heterochromatin and repeated sequences than the A and D genomes, whereas the second one is supported by the fact that the B genome donor was an outbreeding species. In addition, another potential cause of polymorphism level differences between wheat genomes could be that the A genome donor was domesticated very early whereas the B donor was never domesticated as separate species (Nachit pers.com.).

Fig. III-2: Probing of *Omrabi5/ T. dicoccoides600545// Omrabi5* with the combination MseI+cag & EcoRI+agc.



The AFLP combinations used showed different behavior in both number of generated polymorphic fragments and percentage of mapped fragments (Table III-2). *MctgEagc* and *MctcEact* generated the highest number of polymorphic fragments, 31 and 25 respectively, followed by *MctgEacc* with 20 polymorphic fragments, whereas, *MctgEagg* generated only 5 polymorphic fragments. Nevertheless, only 3 fragments were mapped from the 20 generated ones by the primer *MctgEacc* combination (13.6%), whereas 2 fragments were also mapped from the 5 generated by *MctgEagg* combination (40%).

Table III-2 shows the chromosomal localization of different AFLP fragments derived either from the same combination or from different combinations. The majority of AFLP fragments were mapped in several chromosomes. These mapping findings consolidated earlier suggestions for using AFLP technique as DNA fingerprints or for studies of diversity since this technique provides simultaneous coverage of many loci in a single assay (Donini *et al.* 1997; Winfield *et al.* 1998; Paran *et al.* 1998). Sixteen combinations out of the 20 used were mapped in both A and B genomes. Only 3 combinations were showed to be specific to the B genome (Table III-2). It was reported that AFLPs markers are locus specific at the species level (Rouppe *et al.* 1997) and that they correspond to unique positions on the genomes (Vos *et al.* 1995). For instance, *MctgEagc* combination generated 31 polymorphic fragments. Nineteen were mapped in different chromosomes: 4 in chromosome 1A, one in 1B, three in 2A, two in 2B, one in 3A, three in 3B, one in 4A, one in 5A, one in 5B, one in 6B, and one in group15. Therefore, the AFLP technique proved to be a very good technique for a random amplification across the whole genome and also for map saturation (Becker *et al.* 1995; Rouppe van der Voort *et al.* 1997; Wang *et al.* 1997; Marques *et al.* 1998; Nachit *et al.* 2001).

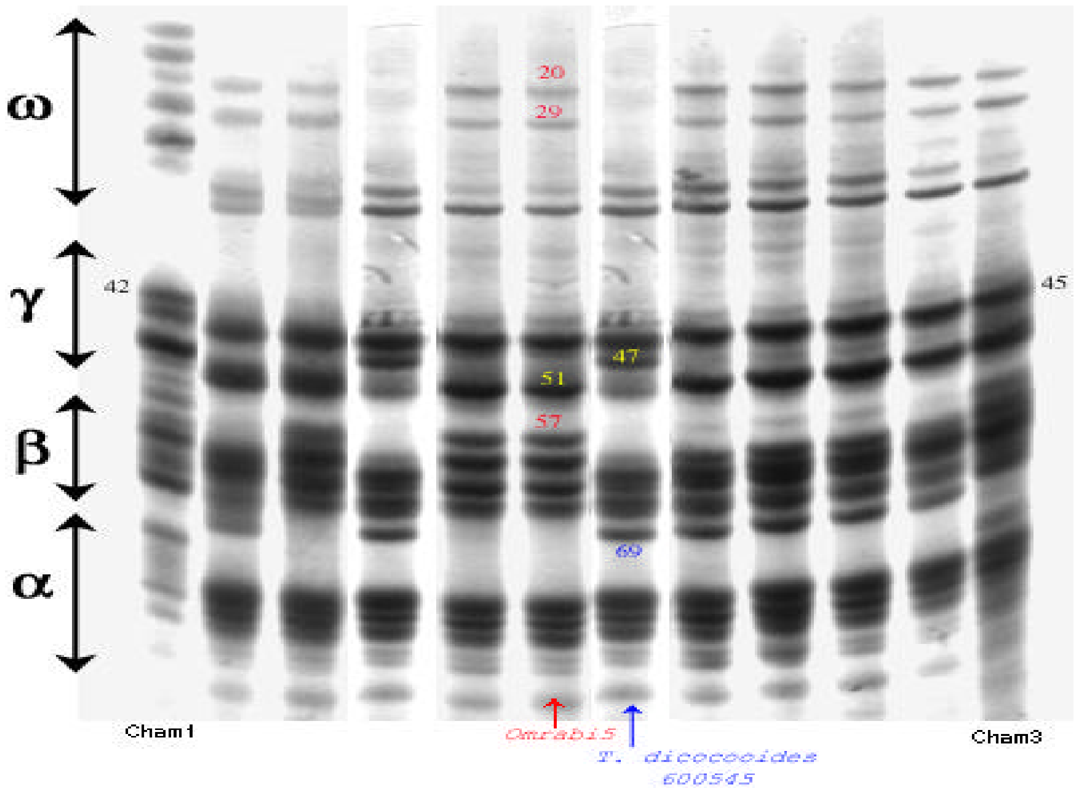
Table III-2: Scored polymorphic fragments generated by the used AFLP combinations and their mapping in *Omrabi5/ T. dicoccoides600545// Omrabi5* map.

Combination	Poly. fragments	Map. fragments	Chromosomal location	% Mapped
MctgEagc	**31**	**19**	1A (4), 1B, 2A (3), 2B (2), 3A, 3B (3), 4A, 5A, 5B, 6B, g15	61.3
MctcEact	**25**	**18**	1A (2), 2A, 2B, 3B (4), 4A (2), 5B, 6B (4), 7A (2), 7B	72
McaaEacg	15	12	1B (2), 3A, 3B (2), 5B (3), 6B (2), 7A (2)	80
MctaEacg	19	11	1A, 1B (2), 2A (2), 4A, 4B (2), 6A, 6B, g15	57.9
MctcEagg	13	10	1B (2), 2B, 3B, 4B, 5A, 5B (3), 6B	76.9
McttEagg	16	9	1B, 2B, 3B, 4B, 5B, 6B (2), 7B (2)	56.3
McaaEagg	15	9	1B, 2A (2), 2B (2), 3B (2), 6A, 7B	60
McaaEaca	16	8	1A (2), 3B (3), 7A, g15 (2)	50
MctcEaag	17	8	2A, 3B (3), 6A, 6B, 7A, g15	47.1
McagEagg	18	6	3A, 5A, 5B, 6A, 6B, 7B	33.3
McaaEacc	7	6	1B, 2B, 3B, 5B, 6B (2)	85.7
McagEagc	9	5	1B, 3A, 6A, 7A, g15	55.6
McacEaag	7	5	2A (2), 5B (2), 6B	71.4
MctgEaag	9	5	1A, 1B, 2B, 4B (2),	55.6
McttEaag	13	5	2A, 3B (3), 7B	46.2
MctgEacg	**5**	4	1B, 2A, 3B, 5A	80
MctgEaca	9	**3**	1B, 6A, 7B	33.3
MctgEacc	20	**3**	3A (2), 5B	15
MctgEagg	**5**	**2**	6B, 7B	60
McatEact	10	1	2A	10
Total	**279**	**149**		**54.1**

M = MseI and E = EcoRI

C- Seed Storage Proteins

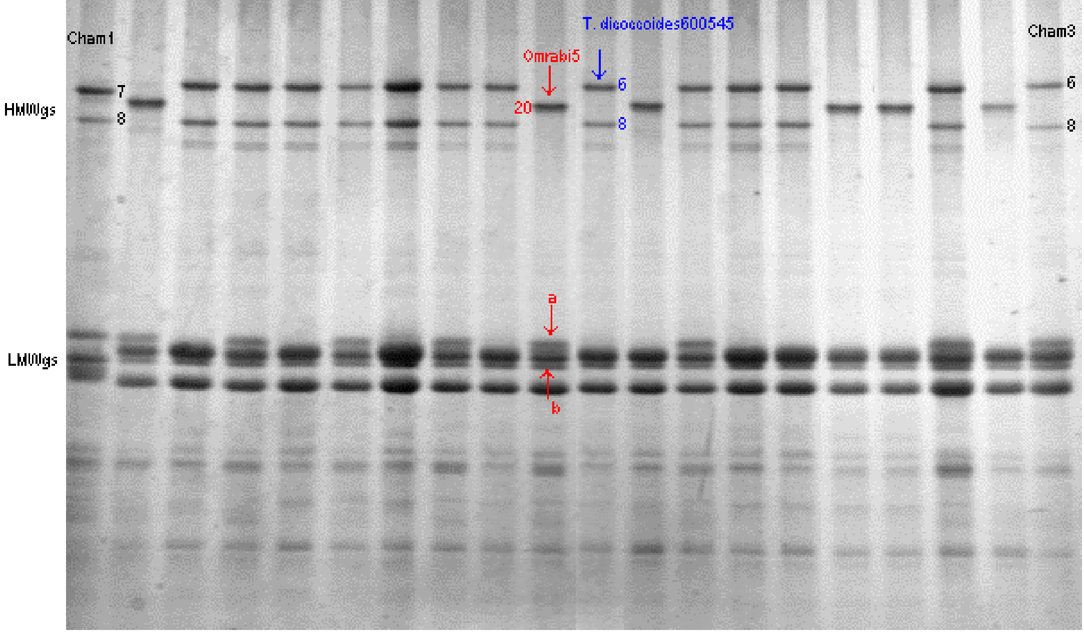
Fig. III-3: A-PAGE gel of *Omrabi5/ T. dicoccoides600545// Omrabi5* gliadin subunits separated on 10% acrylamide



The two parents of the RIL population have different patterns for the four fractions of gliadin proteins: α, β, γ, and ω subunits (Fig. III-3). For the ω-gliadins, three polymorphic fragments were scored in the mapping population: a null form with a relative mobility of 20; a medium size ω-gliadin with a relative mobility of 29, and a small size ω-gliadin with a relative mobility of 38. The second and third scored ω-gliadin were skewed, and therefore were not considered in our mapping analysis, whereas the first null form was mapped on the short arm of chromosome 1A (*Gli-A1*), concording with previous mapping of *Gli-A1* loci (Payne *et al.* 1984, Lafiandra *et al.* 1989). For γ–gliadins, however *Omrabi5* and *T. dicoccoides600545* showed different patterns, they were monomorphic for the γ-45 reported to be tightly and positively linked to gluten strength (Damidaux *et al.* 1978; Ducros 1982; Pogna *et al.* 1990). They showed two polymorphic γ–gliadin subunits with relative mobility of 47 and 51. γ-47 and γ-51 were co-segregating and mapped at the same location as ω-20 on 1AS (*Gli-*

A1). For β–subunits, two polymorphic fragments were scored, the first with a relative mobility of 57 and the second at 69. Both were skewed but did map very well on the short arm of chromosome 6A (*Gli-A2*) at 3.9 cM from each other. For α–gliadin subunits, out of the seven revealed subunits, only one subunit (73) was polymorphic. It was reported that there is high level of conservation of the coding regions of the α–type gliadin gene located on chromosome 6A in *T. durum*, *T. aestivum* and *T. urartu* (D'Ovidio *et al.* 1992). This α–gliadin subunit was fairly distributed in the mapping population but did not map. The α–gliadins were reported to have different effects on gluten firmness (Pogna *et al.* 1990; Elouafi *et al.* 1998).

Fig. III-4: SDS-PAGE gel of *Omrabi5/ T. dicoccoides600545// Omrabi5* glutenin subunits separated on gradient acrylamide (14 to 8%).



The two parents Omrabi5 and T. dicoccoides600545 presented different patterns for both LMW and HMW glutenin subunits (Fig. III-4). For HMW subunits, both parents showed null allele at Glu-A1x and Glu-A1y loci, whereas for Glu-B1x and Glu-B1y loci they were polymorphic. Earlier studies showed a high frequency of the null allele at Glu-A1 loci, especially the Glu-A1x locus, in both durum varieties and dicoccum accessions (Ducros 1987, Boggini and Pogna, 1989, Branlard et al. 1989, Vallega and Waines 1987). In contrast, T. dicoccoides has displayed high variability for HMW glutenin subunits encoded by the Glu-A1 (Damania et al. 1988; Nachit et al. 1995b).

Omrabi5 carries HMW-20 while T. dicoccoides600545 HMW-6+8. In general, HMW subunits were extensively studied because of their correlation with breadmaking quality of bread wheat (Payne et al. 1984). Nevertheless, in durum wheat this correlation is less strong although certain HMW alleles affect the viscoelastic properties of durum wheat dough (Autran and Feillet, 1987, Rogers et al. 1989, Ciaffi et al. 1991, Ciaffi et al. 1995).

For LMW, the known LMW-models are lately recognized to be a mixture of subunits controlled by different alleles (Payne *et al.* 1984, Margiotta *et al.* 1987, Gupta and Shepherd 1988, Carrillo *et al.* 1990, Ruiz and Carrillo 1993, Impglia *et al.* 1996, Ruiz and Carrillo 1996, Nieto-Taladriz 1997). Therefore, instead of scoring the B-LMW as a pattern, it was preferred to score them as separate fragments. Two polymorphic fragments called a and b were scored (Fig. III-4). The distribution of the LMWa (*Glu-A3*) subunit in the RILs showed an expected Mendelian behavior and mapped to the short arm of chromosome 1A at 0.9 cM from the *Gli-A1* locus (ω-20 and γ-47/51). *Glu-3* loci coding for B-LMW subunits are also known to be localized on the short arm of the group 1 chromosomes (Payne 1987, Lafiandra *et al.* 1984, Jackson *et al.* 1983, Metakovsky *et al.* 1997, Nachit *et al.* 2001). In the mapping population, the genetic distance (0.9 cM) between *Glu-A3* and *Gli-A1* was closer than the estimated distance between *Glu-B3* and *Gli-B1* (2 cM) reported by Pogna *et al.* (1990). The second scored subunit, LMWb was skewed and it has not been assigned to any linkage group.

D- Genetic Map Construction

A primary genetic map is an essential prerequisite to detailed genetic studies in any organism. Furthermore dense and saturated genetic maps provide scientists with essential tools for genetic studies like positional gene cloning, quantitative trait mapping, and marker-assisted selection. *Omrabi5* and *T. dicoccoides600545* showed a high level of polymorphism (63.5% of microsatellites). This can be explained by the genetic difference between *T. durum* and *T. dicoccoides* that has been used for mapping. The *Omrabi5/ T. dicoccoides600545// Omrabi5* map was constructed using 478 markers (192 microsatellites, 279 AFLPs, and 7 SSP). Two hundred seventy nine markers (124 microsatellites, 149 AFLPs, and 6 SSP) were mapped and formed 15 linkage groups, 14

durum chromosomes plus one unknown group called g15 that is not yet assigned to any chromosome (Fig. III-5). This group 15 is composed by only 6 AFLPs fragments. In the *Omrabi5/ T. dicoccoides600545// Omrabi5* map, the mapped markers were evenly distributed with no significant clustering except in the centromeric regions of some chromosomes, such as 1B, 2A, 3B, 5B and 6B (Fig. III-5). The length of the map was 2288.8cM, i.e.; an average of a marker per each 8.2cM. The longest chromosomes were 3B, 5B, 1B, and 6B with 239.4, 209.4, 208.6, and 180.2cM, respectively, whereas the smallest chromosome was 5A with 90cM (Table III-3). The centromeres were positioned at the midpoint between the most proximal microsatellites on the short and long arm according to previously published maps: ITMI population microsatellite map (Röder *et al.* 1998), *Messapia* x *MG4343* map (Korzun *et al.* 1999), and Jennah Khetifa x Cham1 map (Nachit *et al.* 2001).

Table III-3: Distribution of molecular markers, assignment, and cM coverage across the 14-durum chromosomes in the A and B genomes

Chromosome	SSR	AFLPs	Genes	Markers		Size (cM)	cM/Marker
				Nbre	%		
1A	6	10	3	19	6.8	147.8	7.8
1B	**16**	14	1	31	11.1	208.6	6.7
2A	11	15	0	26	9.3	153.9	**5.9**
2B	9	8	0	17	6.1	167.3	9.8
3A	5	6	0	11	3.9	137.4	12.5
3B	11	**24**	0	**35**	12.5	**239.4**	6.8
4A	5	**4**	0	**9**	3.2	118.6	**13.2**
4B	9	6	0	15	5.4	180.1	12.0
5A	5	**4**	0	**9**	3.2	**90.0**	10.0
5B	**16**	14	0	30	10.8	209.4	7.0
6A	**3**	6	2	11	3.9	111.7	10.2
6B	10	17	0	27	9.7	180.2	6.7
7A	10	7	0	17	6.1	136.5	8.0
7B	8	8	0	16	5.7	156.5	9.8
g15	0	6	0	6	2.2	51.4	8.6
Total	**124**	**149**	**6**	**279**	**100**	**2288.8**	**8.2**

Table III-3 shows the distribution of mapped molecular markers, their assignment, and their cM coverage across the 15 formed linkage groups. Chromosome 3B, 1B, and 5B showed the highest number of mapped markers: 35, 31, and 30 markers

respectively. Indeed, chromosome 5B and 1B showed the highest number of mapped microsatellites, 16 Gwm each. For chromosome 3B, it showed the highest number of mapped AFLPs (Table III-3). The lowest number of mapped markers was noted in chromosomes 4A, 5A, 6A, and 3A. Chromosomes 6A, 4A, and 5A showed the lowest mapped microsatellites, only 3, 5, and 5 Gwm fragments, respectively. In addition, chromosomes 4A and 5A showed the lowest mapped AFLPs (Table III-3).

Furthermore, chromosome 2A showed the highest level of saturation. It did have one marker for each 5.9cM. Nevertheless, this mean coverage is biased as chromosome 2A showed 3 gaps that need to be saturated with other markers. The centromere and long arm regions of chromosome 2A showed a good agreement with ITMI microsatellite map, whereas for the short arm many rearrangements were noticed (Röder *et al.* 1998). Gwm249 was reported to map to the two homoeologous sites on 2A and 2D (Röder *et al.* 1998), while in MDM mapping population the two amplified Gwm249 fragments were both mapped on 2A at 35.5cM distance from each other. The same behavior was noticed for Gwm630 on the short arm of 2B. In JK x Cham1, the microsatellite Gwm382 amplified two fragments mapped on the telomeric region of 2AL (Nachit *et al.* 2001). In ITMI population, 3 fragments were reported mapping to the three homoeologous long arm telomeres (Röder *et al.* 1998), whereas, in MDM population only one fragment was amplified and mapped to the telomere region of 2AL. The homoeologous chromosome 2B was constructed with 17 markers (9 SSRs and 8 AFLPs) (Table III-3). In comparison with ITMI map, chromosome 2B showed few rearrangements involving Gwm129, 630, 148, 319, and 374. It appears like a double inversion involving *Xgwm374-319* on one hand and *Xgwm148-630* on the other hand. However, in ITMI population these microsatellites were only assigned to the most likely interval on the RFLP framework map (Röder *et al.* 1998).

The longest chromosomes 3B, 5B, 1B, and 6B showed very good coverage with 6.8, 7.0, 6.7, and 6.7 cM/marker, respectively (Table III-3). Chromosome 1B is well formed and showed a full order-agreement with ITMI microsatellite map (Röder *et al.* 1998), *Messapia* x *MG4343* map (Korzun *et al.* 1999), and JKxCham1 map (Nachit *et al.* 2001) (Fig. III-5). Gwm268 in the long arm of 1B amplified two fragments mapped at 15.4cM from each other, flanking *Xgwm274a*. In addition, Gwm124 and Gwm259 did also amplify two fragments that mapped at 3.5 and 9.6cM, respectively, whereas,

both earlier published maps reported only one fragment (Röder *et al.* 1998; Korzun *et al.* 1999). *Glu-B1* gene was mapped on 1BL at 45.5cM from the centromere, as expected. Korzun *et al.* (1999) reported a distance of 62cM between *Glu-B1* and the centromere. The homoeologous chromosome 1A was constructed by 6 microsatellites, 10 AFLPs, and 3 seed storage proteins (*Gli-A1*: γ-47 + ω-20 and *Glu-A3*). The two-gliadin subunits co-segregated and mapped at 0.9cM from *Glu-A3* locus. In general, chromosome 1A shows good agreement with earlier reported markers order (Röder *et al.* 1998; Korzun *et al.* 1999). Nevertheless, an inversion was revealed involving Gwm33 and Gwm136. Röder *et al.* (1998) reported that Gwm33 amplified 3 fragments mapped to the three homoeologous sites of bread wheat, whereas in the *Omrabi5/ T. dicoccoides600545// Omrabi5* map, Gwm33 amplified 2 fragments mapped both to 1AS at 0.5cM from each other. *Xgwm33a* and *Xgwm33b* cosegregate except for only one recombined inbred line (number 84).

Chromosome 3B is also well saturated and showed a full order-agreement with ITMI microsatellite map except for the telomere region of the long arm where an inversion including Gwm340, Gwm181, and Gwm114 was revealed (Röder *et al.* 1998). Gwm376 amplified two fragments spaced by 4 other markers covering 9.4cM. This is in agreement with the *Messapia* x *MG4343* map where two Gwm376 fragments were reported to map at 1.9cM from each other (Korzun *et al.* 1999). Gwm493 and Gwm114 amplified two fragments, which mapped at 9.9 and 2cM from each other, respectively. In ITMI population, Röder *et al.* (1998) reported that Gwm114 amplified two fragments, which mapped to 3BL and 3DS. The homoeologous chromosome 3A showed a relatively loose construction, it did present 1 marker each 12.5cM (Fig. III-5). The long arm showed an inversion involving *Xgwm155* and *Xgwm674*, as Gwm674 was reported to be localized close by the centromere and Gwm155 on the long arm (Röder *et al.* 1998; Korzun *et al.* 1999; Nachit *et al.* 2001).

The short arm of chromosome 5B showed a very interesting translocation-inversion rearrangement with chromosome 5A (Fig. III-5). Therefore, Gwm156 and Gwm415 were mapped on 5BS instead of 5AS whereas Gwm443 was mapped on 5AS instead of 5BS. The microsatellite Gwm234 amplified two fragments a and b, one mapped to 5AS and one to 5BS. Otherwise, the remaining parts of chromosome 5B (centromeric and long arm regions) showed a perfect agreement with ITMI map (Röder

et al. 1998) and *Messapia* x *MG4343* map (Korzun *et al.* 1999). Gwm639 amplified two fragments, only one fragment was mapped to 5BL. Whereas Röder *et al.* (1998) and Korzun *et al.* (1999) reported 3 and 2 fragments, respectively, mapped on the homoeologous sites. This could be explained by the short size of the 5A chromosome in MDM map in comparison with other wheat maps (Röder *et al.* 1998; Korzun *et al.* 1999; Nachit *et al.* 2001). In the centromeric region of chromosome 5B, *Xgwm213a* and *Xgwm335a* mapped on the same point at 0 cM from each other. Röder *et al.* (1998) has reported that these two microsatellites cosegregate and consist of identical or almost identical sequences. On the long arm, *Xgwm554c* was mapped as expected, flanked by *Xgwm639c* and *Xgwm408a*. This microsatellite inspite of its behavior in ITMI and *Messapia* x *MG4343* maps has amplified in *Omrabi5/ T. dicoccoides600545// Omrabi5* map 3 fragments mapped to 3 different chromosomes: 1B, 2A and 5B (Fig. III-5). In the short arm of chromosome 5A, two fragment of Gwm205 were mapped at 2.1cM from each other. Röder *et al.* (1998) reported two fragments mapped on 5AS and 5DS. The centromere of chromosome 5A was positioned at the midpoint between *Xgwm205d* and *Xgwm186*, according to ITMI map (Röder *et al.* 1998). However, there is only 0.37 recombination fraction between these two microsatellites.

Other cosegregating microsatellites were revealed in the centromeric region of chromosome 6B between *Xgwm193*, *Xgwm58*, and *Xgwm88b*. Gwm193 and Gwm88 were assigned to the most likely interval on the RFLP framework map of ITMI population, where no cosegregation was reported (Röder *et al.* 1998). Chromosome 6B is well saturated and showed a full order-agreement with ITMI microsatellite map, except for a small inversion on the segment involving *Xgwm508* and *Xgwm518* (Röder *et al.* 1998) (Fig. III-5), whereas, chromosome 6A did not show any rearrangements but it presented only 4 microsatellites, 6 AFLPs, and 2 β-gliadins (*Gli-A2*). Several colinearity analysis and recombination studies were conducted between *T. durum* 6A and 6B chromosomes and *T. aestivum* chromosomal group 6 (Chen *et al.* 1994, Cadalen *et al.* 1997). Gwm494 amplified 2 fragments that were mapped close by each other. The centromere was positioned on Gwm494 as reported by Röder *et al.* (1998). Gwm582 was reported to map to 1BS (Röder *et al.* 1998), nevertheless in MDM mapping population it did map to 6BL. These new fragments, which mapped to other chromosomal locations, could be explained by the presence of additional loci in the wheat genome.

Another microsatellites co-segregation was noticed on the long arm of chromosome 7A between *Xgwm282a* and *Xgwm332* (Fig. III-5). This cosegregation was not reported earlier. In fact, Gwm282 amplified two fragments: a and b. They flanked *Xgwm63e* on the long arm of 7A at 9.4cM from each other. In general chromosome 7A showed a good agreement to previous published maps (Röder *et al.* 1998; Korzun *et al.* 1999; Nachit *et al.* 2001). A new microsatellite (*Xgwm154e*) was incorporate in 7AS at 4.6cM from *Xgwm60b*. This microsatellite was reported to map on 5AS (Röder *et al.* 1998), whereas in MDM map the two amplified fragments mapped to 3BL and 7AS. In chromosome 7B new localizations were found for microsatellite-fragments *Xgwm264a* and *Xgwm537a*. Gwm264 was reported to map to 1BS and 3BS, while in MDM map it mapped to 1AL, 1BS, and 7BS. Indeed, Gwm537 was reported to map to 7BS, whereas in *Omrabi5/ T. dicoccoides600545// Omrabi5* map two fragments were mapped: one to 7BS and one to 5BL (Röder *et al.* 1998). As for Gwm635, it was assigned to the short arm of chromosome 7B, while in ITMI population the two amplified fragments were localized on 7AS and 7DS (Röder *et al.* 1998).

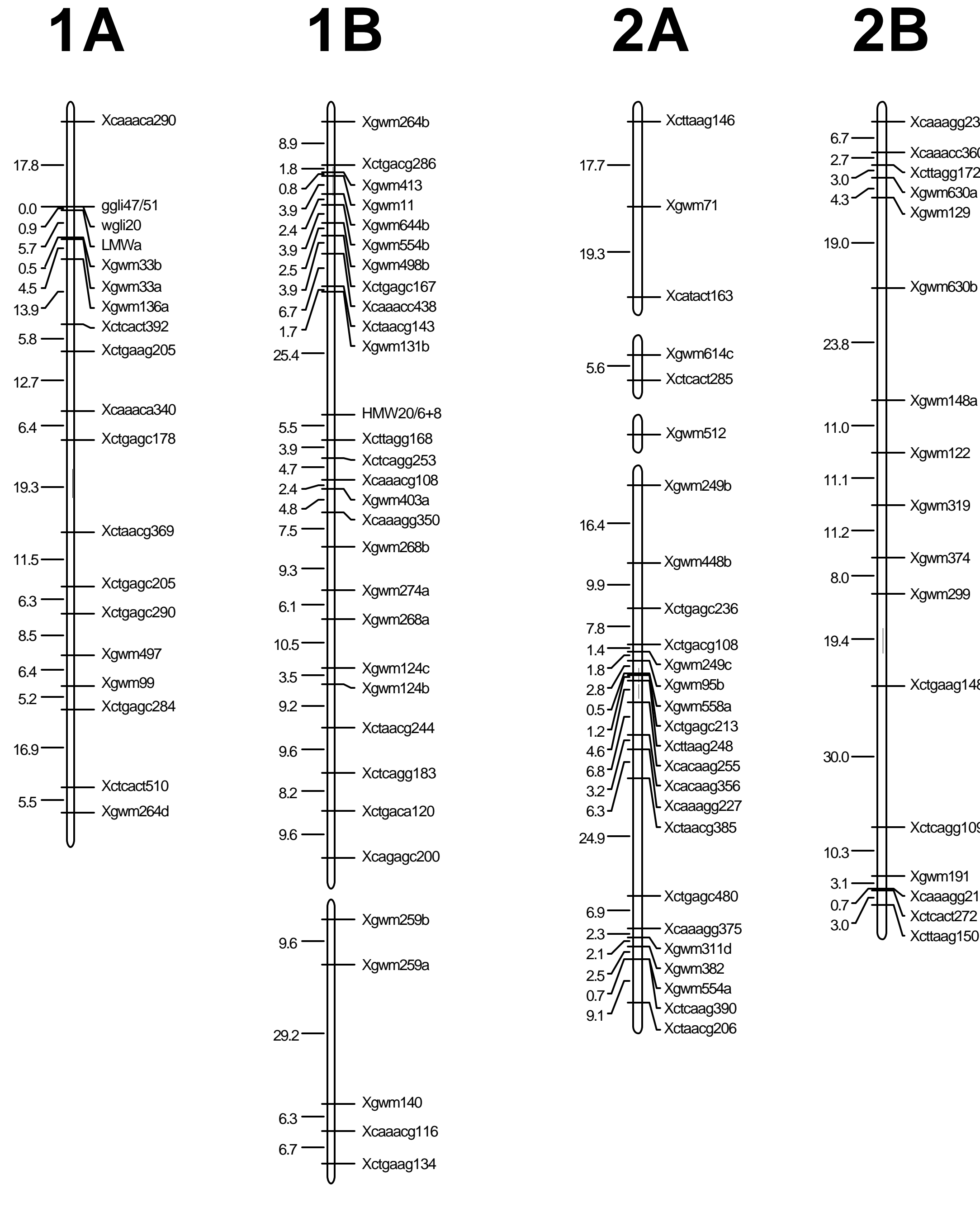
Chromosome 4A was the linkage group with the least markers coverage. It consisted of 1 marker each 13.2cM. This chromosome was constituted of 5 SSRs and 4 AFLPs. Four microsatellites followed the expected order (Gwm165, 601, 610, and 265), whereas Gwm43 was mapped in 4A instead of 7B. In MDM mapping population, Gwm265 was mapped on the long arm of chromosome 4A. This is in agreement with *Messapia* x *MG4343* map (Korzun *et al.* 1999) whereas is in disagreement with ITMI microsatellite map where Gwm265 was reported to map to 2AL (Röder *et al.* 1998). Analysis of chromosome locations using nullisomic-tetrasomic lines of wheat has shown that this microsatellite has loci on chromosomes 2A, 4A, and 4D of bread wheat (Korzun *et al.* 1999). The chromosome 4A was constructed at LOD 3.5 except for *Xgwm165b* on the short arm that was joined at LOD 1.8. In MDM mapping population Gwm165 amplified two fragments mapping to 4AS and 4BL. This is in agreement with bread wheat ITMI population map (Röder *et al.* 1998). Chromosome 4B displays some gaps and more markers need to be added. The long arm of this linkage group was constructed at a high LOD (>3) whereas the telomere of the long and short arms were linked at low LOD value (<1.5).

In general, microsatellites were more mapped than AFLP markers (77% versus 53.4%). Indeed, more markers were mapped in the B genome comparatively to the A genome (Table III-4). This statement was true for microsatellites and AFLPs, both markers showed preference mapping to the B genome. Thus, there is one marker for each 8.4cM in the A genome while one per 7.8cM in the B genome (Table III-4). Furthermore, 63.7% of total mapped microsattelites and 61.1% of total mapped AFLPs were localized in the B genome while only 39.2 and 34.7% were mapped in the A genome. This is in agreement with earlier published maps (Röder *et al.* 1998; Nachit *et al.* 2001). Probably, it reflects the amount of revealed polymorphism within the A and B genomes of *Omrabi5/ T. dicoccoides600545// Omrabi5* population. In addition, the Gwm microsatellites used were isolated from Chineese Spring genome and not from a wild relative, as it was proposed by Röder *et al.* (1998) to increase the number of microsatellites in A and D genome. They reported that using the diploid ancestors as a source of microsatellite isolation enriched the microsatellites from the D genome.

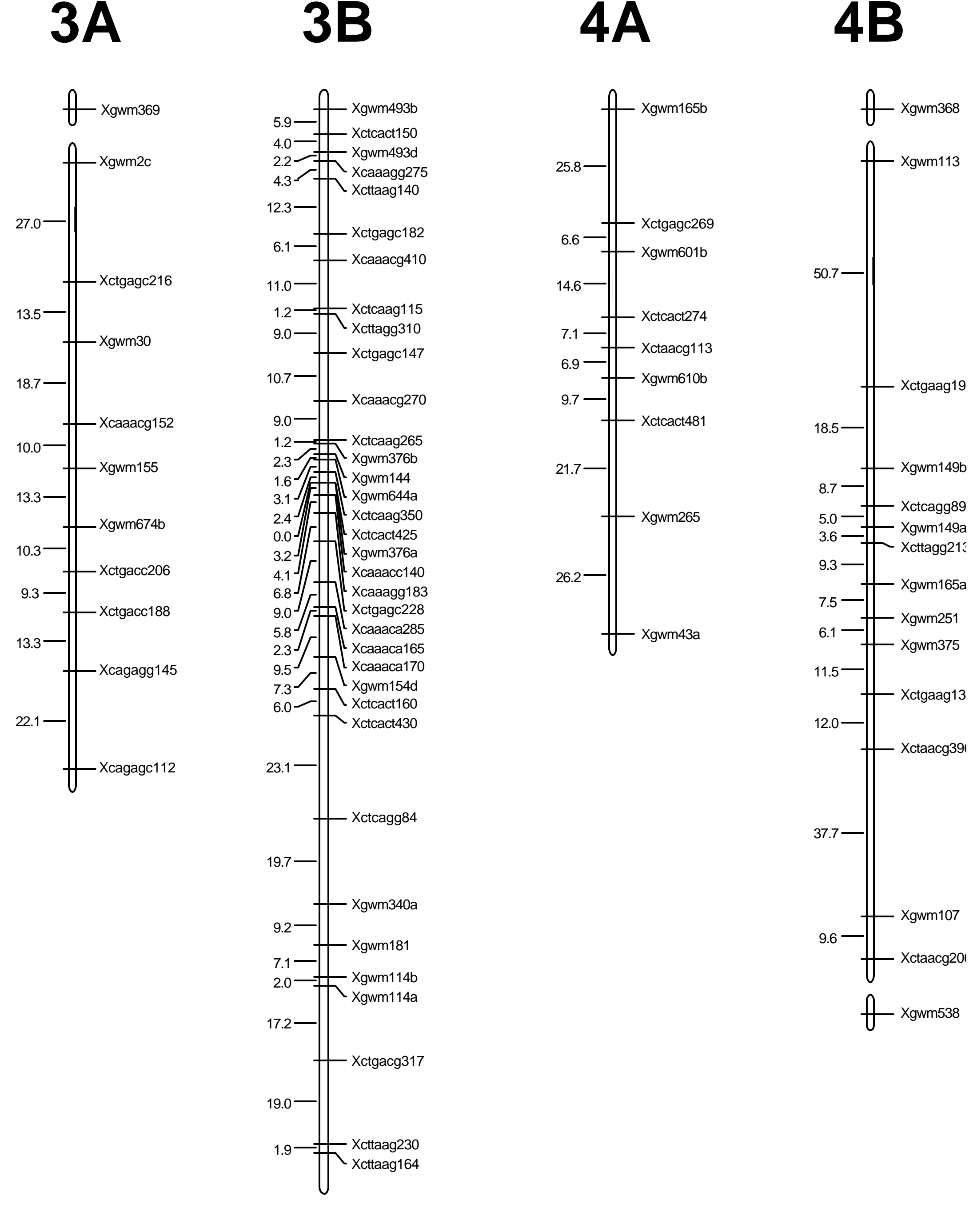
Table III-4: Assigned markers on the A and the B genome

Genome	SSR	Aflps	Genes	Markers		Size (cM)	cM/Marker
				Nbre	%		
A	45	52	5	**102**	36.6	859.9	**8.4**
B	79	91	1	**171**	61.3	1341.5	**7.8**

Fig. III-5: Molecular linkage map of *Omrabi5/ T. dicoccoides600545// Omrabi5* population. Short arms of chromosomes are at the top. The approximate locations of centromeres are indicated *in black*. On *the left* are map distances in centiMograms (cM) calculated by Kosambi function, and on *the right* are DNA-markers. Markers preceded *by X* are DNA markers of unknown function, whereas markers *without X* are genes controlling specific traits. *AFLP* markers are designated by 6 nucleotides (the first 3 are *MseI+3*; and the other 3 are *EcoRI+3*) followed by basepair size of the scored fragment.



1A
Xcaaaca290
17.8
0.0
ggli47/51
0.9
wgli20
5.7
LMWa
0.5
Xgwm33b
4.5
Xgwm33a
13.9
Xgwm136a
Xctcact392
5.8
Xctgaag205
12.7
Xcaaaca340
6.4
Xctgagc178
19.3
Xctaacg369
11.5
Xctgagc205
6.3
Xctgagc290
8.5
Xgwm497
6.4
Xgwm99
5.2
Xctgagc284
16.9
Xctcact510
5.5
Xgwm264d
1B
Xgwm264b
8.9
1.8
Xctgacg286
0.8
Xgwm413
3.9
Xgwm11
2.4
Xgwm644b
3.9
Xgwm554b
2.5
Xgwm498b
3.9
Xctgagc167
6.7
Xcaaacc438
1.7
Xctaacg143
Xgwm131b
25.4
HMW20/6+8
5.5
Xcttagg168
3.9
Xctcagg253
4.7
Xcaaacg108
2.4
Xgwm403a
4.8
Xcaaagg350
7.5
Xgwm268b
9.3
Xgwm274a
6.1
Xgwm268a
10.5
Xgwm124c
3.5
Xgwm124b
9.2
Xctaacg244
9.6
Xctcagg183
8.2
Xctgaca120
9.6
Xcagagc200
Xgwm259b
9.6
Xgwm259a
29.2
Xgwm140
6.3
Xcaaacg116
6.7
Xctgaag134
2A
Xcttaag146
17.7
Xgwm71
19.3
Xcatact163
Xgwm614c
5.6
Xctcact285
Xgwm512
Xgwm249b
16.4
Xgwm448b
9.9
Xctgagc236
7.8
1.4
Xctgacg108
1.8
Xgwm249c
2.8
Xgwm95b
0.5
Xgwm558a
1.2
Xctgagc213
4.6
Xcttaag248
6.8
Xcacaag255
3.2
Xcacaag356
6.3
Xcaaagg227
Xctaacg385
24.9
Xctgagc480
6.9
2.3
Xcaaagg375
2.1
Xgwm311d
2.5
Xgwm382
0.7
Xgwm554a
9.1
Xctcaag390
Xctaacg206
2B
Xcaaagg23
6.7
Xcaaacc36
2.7
Xcttagg172
3.0
Xgwm630a
4.3
Xgwm129
19.0
Xgwm630b
23.8
Xgwm148a
11.0
Xgwm122
11.1
Xgwm319
11.2
Xgwm374
8.0
Xgwm299
19.4
Xctgaag14
30.0
Xctcagg10
10.3
Xgwm191
3.1
Xcaaagg21
0.7
Xctcact272
3.0
Xcttaag150



3A
Xgwm369
Xgwm2c
27.0
Xctgagc216
13.5
Xgwm30
18.7
Xcaaacg152
10.0
Xgwm155
13.3
Xgwm674b
10.3
Xctgacc206
9.3
Xctgacc188
13.3
Xcagagg145
22.1
Xcagagc112
3B
Xgwm493b
5.9
Xctcact150
4.0
Xgwm493d
2.2
Xcaaagg275
4.3
Xcttaag140
12.3
Xctgagc182
6.1
Xcaaacg410
11.0
Xctcaag115
1.2
Xcttagg310
9.0
Xctgagc147
10.7
Xcaaacg270
9.0
Xctcaag265
1.2
Xgwm376b
2.3
Xgwm144
1.6
Xgwm644a
3.1
Xctcaag350
2.4
Xctcact425
0.0
Xgwm376a
3.2
Xcaaacc140
4.1
Xcaaagg183
6.8
Xctgagc228
9.0
Xcaaaca285
5.8
Xcaaaca165
2.3
Xcaaaca170
9.5
Xgwm154d
7.3
Xctcact160
6.0
Xctcact430
23.1
Xctcagg84
19.7
Xgwm340a
9.2
Xgwm181
7.1
Xgwm114b
2.0
Xgwm114a
17.2
Xctgacg317
19.0
Xcttaag230
1.9
Xcttaag164
4A
Xgwm165b
25.8
Xctgagc269
6.6
Xgwm601b
14.6
Xctcact274
7.1
Xctaacg113
6.9
Xgwm610b
9.7
Xctcact481
21.7
Xgwm265
26.2
Xgwm43a
4B
Xgwm368
Xgwm113
50.7
Xctgaag19
18.5
Xgwm149b
8.7
Xctcagg89
5.0
Xgwm149a
3.6
Xcttagg21
9.3
Xgwm165a
7.5
Xgwm251
6.1
Xgwm375
11.5
Xctgaag13
12.0
Xctaacg39
37.7
Xgwm107
9.6
Xctaacg20
Xgwm538

5A

Xcagagg315
11.4
Xctgacg298
9.4
Xgwm234a
5.9
Xgwm443
3.5
Xgwm205c
2.1
Xgwm205d
31.6
Xctgagc156
13.1
Xgwm186
12.9
Xctcagg366

5B

Xctgacc358
13.6
Xgwm156
11.0
Xctcagg236
10.5
Xctcact122
12.4
Xcagagg132
9.8
Xgwm234b
5.7
Xgwm118
2.5
Xgwm415
19.7
Xgwm213b
5.4
Xgwm213a
0.0
Xgwm335a
2.7
Xctgagc196
5.6
Xgwm371b
4.2
Xgwm371a
6.5
Xgwm499
4.9
Xctcagg222
3.8
Xctcagg224
5.5
Xcttagg254
7.9
Xcaaacg297
4.2
Xgwm639a
7.1
Xgwm554c
19.9
Xgwm408a
2.5
Xgwm408b
3.8
Xcacaag202
5.1
Xcaaacc446
1.8
Xcacaag418
3.1
Xgwm604
1.1
Xcaaacg385
3.5
Xcaaacg340
25.7
Xgwm537b

6A

Xcagagg107
8.3
Xcagagc450
3.7
Xctaacg234
11.8
Xctgaca134
20.8
bgli57
3.9
bgli69
29.2
Xgwm494b
4.7
Xgwm494a
16.1
Xcaaagg330
10.2
Xgwm570b
2.9
Xctcaag285

6B

Xcagagg63
14.4
Xgwm613
29.8
Xctaacg24
9.0
Xcttagg40
8.7
Xgwm508a
8.8
Xcaaacg39
8.2
Xgwm518
3.9
Xctcact19
5.2
Xctcact11
4.8
Xcacaag2
6.2
Xctcaag17
5.3
Xctgagg17
4.4
Xcaaacg5
6.6
Xctcact47
4.7
Xctcagg38
2.8
Xcaaacc3
0.5
Xgwm193
0.0
Xgwm58
0.0
Xgwm88a
2.8
Xgwm88d
3.9
Xcaaacc4
7.7
Xcttagg40
4.8
Xctgagc41
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Xgwm582a
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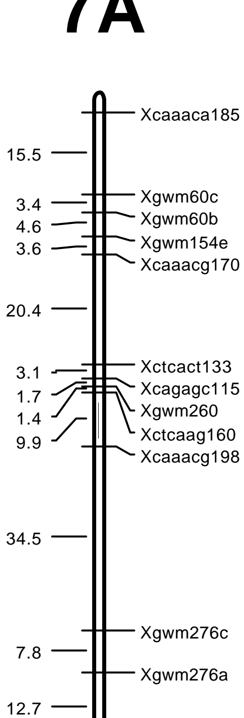
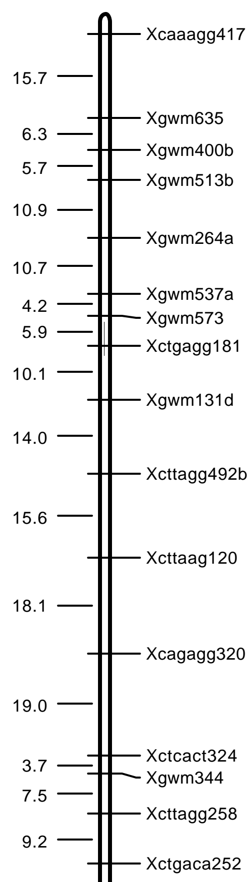
11.6
Xctcact75

7A





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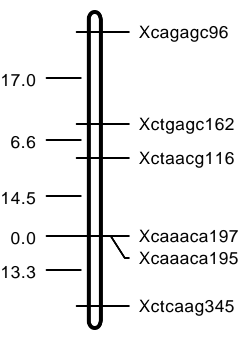


Chapter IV
Results & Discussion

Grain Quality Characteristics

I- Grain Quality Characteristics

1- Protein Content (PC)

The protein content mean, minimum, maximum, heritability, variance, and coefficient of variability for the recombined inbred lines and their parents are shown in table IV-1. The protein content mean of the RILs over the 18 studied environments was 17.1%, ranging from 10.6 to 23.5%, whereas the mean of the two parents: *Omrabi5* and *T. dicoccoides* was 16.1 and 18.5, respectively. These results show a moderate to high transgressive inheritance in both negative and positive senses as the RILs data display lower and higher protein content than the parents (Fig IV-2). The frequency distributions for protein content in the 18 studied environments have shown a good fitting to normal distribution, confirming that the protein content has quantitative inheritance (Fig IV-2). Several researchers agree that protein content is a typical quantitative trait controlled by a complex genetic system and highly influenced by environmental factors (Nachit *et al.* 1995a, Mariani *et al.* 1995, Trocoli *et al.* 2000). The average frequency distribution for the RILs and parents illustrated in figure IV-1, show clearly this transgressive inheritance. This is also in accordance with earlier reported studies on *T. dicoccoides* derived crosses where a significant transgressive inheritance was highlighted (Maali 1991). A significantly high protein content in *dicoccoides* derived crosses in comparison with cultivated wheat derived crosses was also reported by Feldman and Sears (1981) and Nachit *et al.* (1990).

The protein content means found in MDM mapping population are higher than the usual durum crosses. The total protein content of durum wheat is usually around 12-14%. However, the durum parent *Omrabi5* gave a higher value 16.1%. These results show the progress made to increase durum grain protein content by using *T. dicoccoides* in the breeding; and also the suitability of the Mediterranean continental dryland for producing high grain quality of durum (Nachit pers.com.). In general good cooking quality is related to a high level of protein and gluten content (D'Egidio *et al.* 1990, D'Egidio *et al.* 1994, Ciaffi *et al.* 1991; Blanco and Giovanni, 1996). Wheat protein content higher than 13% is considered as satisfactory for the final product, whereas protein content lower than 11% gives a poor end-product. Additionally, the various

durum wheat end-products require different quality characteristics; however, all require a minimum level of protein (13%), high milling yield, yellow flour color and moderately strong gluten.

In most environments, the effect of transgressive inheritance was detected (mean $\pm$ 2σ). This transgressive inheritance was particularly significant in the environments: Tel-Hadya EP00, Sum00, and Ir00 (Fig IV-2). These results corroborate the utilization of *T. dicoccoides* as source for high protein content genes (Feldman and Sears 1981, Nachit *et al.* 1990).

The broad sense heritability estimation for protein content was performed for 16 environments; the mean heritability was of 0.64 ranging from 0.05 to 0.96. The 97Ir environment was excluded from heritability analysis because no checks were included. In general, a high variation was noticed across environments. For instance, the highest heritability values were recorded in the stressed environments (99Br, 99Inc, 00Br, etc.), except for 99Kf (a site with favorable conditions during the vegetative stage but with extreme hot and dry conditions during the grain filling stage). Further, theses environments have also shown the highest values for protein content, e.g.; the minimum protein content of RILs in the dry-cold environment Breda were of 17; 15.1; and 19.8% in the seasons 1997/98, 98/99, and 99/00, respectively. While the lowest heritability values were obtained in irrigated/favorable sites such as: 00Sum and 00EP (Table IV-1). This is in agreement with earlier findings showing that under severe drought conditions a relatively higher content of protein is obtained (Johansson and Lundborg 1994, Nachit *et al.* 1995a). The average genetic coefficient of variability for the RILs was of 5.8%, varying from 2.9% (PC00Br) to 8.9 (PC98Rf).

Table IV-1: Protein content mean, minimum, maximum, heritability, variance, and coefficient of variability in *Omrabi5/ T. dicoccoides600545// Omrabi5*.

Trait-year-site	RILs Mean	RILs Min	RILs Max	P1	P2	Heritability	Variance (s^2)	Genetic C.V. (100)
PC97Ir	13.0	10.6	15.4	14.1	16.6	-	1.1	8.1
PC97Rf	15.8	13.0	17.8	15.0	19.0	0.29	1.0	6.3
PC98Br	19.3	17.0	21.4	18.5	21.4	0.93	0.8	4.6
PC98EP	15.4	12.0	18.8	14.3	17.7	0.48	1.7	8.5
PC98LP	18.5	16.7	20.9	16.7	18.6	0.84	0.8	4.8
PC98Rf	15.5	13.0	18.5	15.0	19.3	0.32	1.9	8.9
PC99Br	18.4	15.1	20.5	16.5	19.9	0.96	0.7	4.5
PC99Inc	17.4	14.9	19.7	16.4	20.2	0.94	1.1	6.0
PC99Kf	21.7	19.9	23.5	21.4	22.3	0.05	0.6	3.6
PC99LP	17.9	15.3	20.8	16.1	19.1	0.92	1.5	6.8
PC99Rf	14.2	11.6	17.6	14.2	15.6	0.47	1.1	7.3
PC99Tr	18.8	16.8	20.7	18.9	20.3	0.92	0.8	4.7
PC00Tr	15.5	14.0	17.8	14.4	17.4	0.67	0.6	5.0
PC00Rf	17.2	15.4	20.3	14.8	18.3	0.74	0.8	5.2
PC00Br	21.6	19.8	23.4	20.4	21.4	0.93	0.4	2.9
PC00EP	14.9	12.3	18.2	14.0	14.3	0.42	1.2	7.3
PC00Sum	17.5	14.7	20.0	16.0	16.6	0.24	1.4	6.8
PC00Ir	15.0	12.9	17.7	13.4	14.4	-	0.9	6.3
Mean	**17.1**	**14.7**	**19.6**	**16.1**	**18.5**	**0.64**	**1.0**	**5.8**

P1 = *Omrabi5*; P2 = *T. dicoccoides600545*

Fig IV-1: Distribution frequency for average protein content in *Omrabi5/ T. dicoccoides600545// Omrabi5* (18 environments).

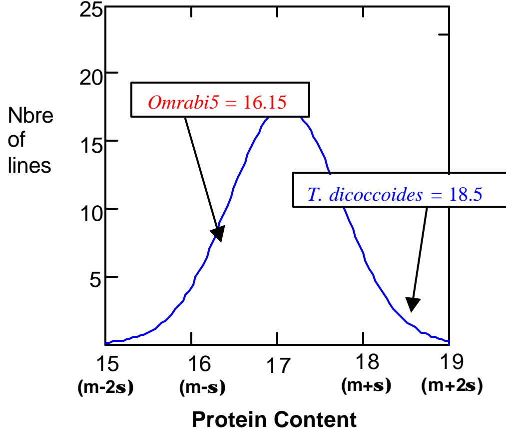
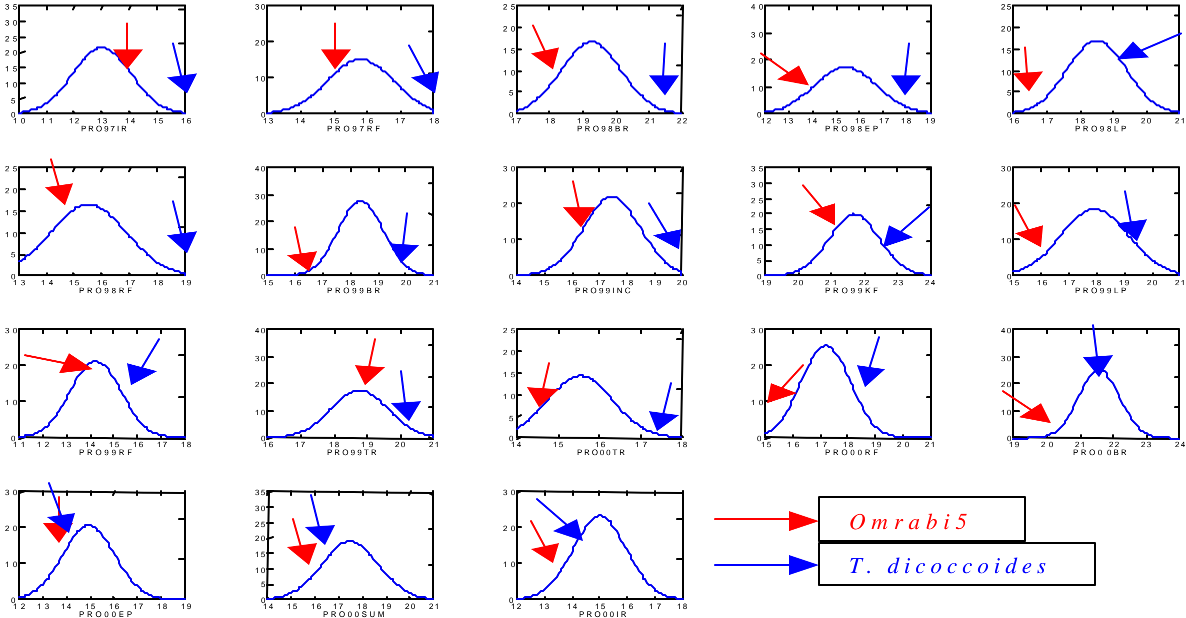


Fig IV-2: Distribution of protein content in the 18 studied environments in *Omrabi5/ T. dicoccoides600545// Omrabi5*



2- Sedimentation test (SDS)

Table IV-2 shows sedimentation test values for the mean, minimum and maximum, heritability, variance, and coefficient of variability for the recombined inbred lines and their parents. The sedimentation test mean for the RILs was 40.2 ml; this value represents the average of 18 environments (sites x years), whereas the means for P1 *Omrabi5* and P2 *T. dicoccoides* were 34.9 and 35.4 ml, respectively. This result also demonstrates the effect of the transgressive inheritance; the RILs were significantly higher than the two parents. Figure IV-3 shows the frequency distribution for the RILs and parents with also the clear effect of transgressive inheritance. When taking into consideration only the mean values, it was found that the positive transgressive inheritance was highly significant. Also when 3σ was computed, still transgressive inheritance could be detected (Fig IV-3). Earlier studies on sedimentation volume inheritance with the durum crosses Haurani x *T. dicoccoides* and Stork x *T. dicoccoides* have shown similar results (Maali 1991).

Furthermore, when the results are examined separately for each environment, the transgressive inheritance showed larger values; e.g.; SDS in the environment SDS99Inc (Table IV-2 and Fig IV-4). However, transgressive inheritance was also revealed for sedimentation test low value, e.g.; SDS99Inc, SDS99Rf, SDS99Tr, and SDS00Tr.

In Figure IV-4 the frequency distributions for sedimentation test in 18 environments are shown. All environments have shown a good fit to the normal distribution, suggesting the involvement of polygenes for this trait. Considering different environments, it was detected that transgressive inheritance occurred in 16 out of 18 environments used for sedimentation test. These results are of importance to broaden and introgress new genes to improve further the durum gluten strength. As the usefulness of the sedimentation test in breeding has been confirmed by other authors (Nachit 1992, Nachit *et al.* 1995). Several durum-breeding programs rely on the sedimentation test to assess gluten strength. These results are corroborating earlier results for improving durum quality processing by using the *dicoccoides* in crosses (Nachit and Maali 1997). However, in our earlier studies with the population Korifla/ *T. dicoccoides600808,* the seed storage protein subunits α–a, γ-a, ω-a, and LMWdc have shown no significant effects on sedimentation volume (Elouafi *et al.* 1998). This indicates that different *T. dicoccoides* accessions can contribute differently to gluten strength.

Furthermore, heritability values for sedimentation test were estimated for 16 environments. The mean heritability was 0.70 with a range from 0.17 to 0.97. In the environments where the checks were not included, the heritability analysis was excluded. Earlier studies made on sedimentation test have also revealed similar values for broad sense heritability, 0.94 in dryland and 0.93 in irrigated conditions (Nachit *et al*. 1995a). Similar results were also reported in bread wheat by Kaul and Sosulski (1964) where heritability was found to be between 0.92 and 0.98 and the sedimentation trait was controlled by two partially dominant genes.

In addition, the average genetic coefficient of variability of sedimentation test for the RILs was 16.6%, varying from 9.8% (SDS97Rf, SDS99LP, SDS00Br) to 26% (SDS98LP). In table IV-2, environments with moisture and heat stress have shown the highest values for SDS-sedimentation. Therefore, abiotic stressed environments of late planting (LP) and summer planting (Sum) induced a positive effect on gluten strength (Table IV-2). In contrast, the rain-fed environment (98Rf) which was relatively wet in 1998 season during the grain filling period and combined with low nitrogen fertiliser (60 Kg/ha) application had negative effect on gluten strength (Table IV-2). Similar

effects were also revealed in the early planting environment of 1997/98 where in addition to high precipitation; supplementary irrigation was applied (Table IV-2). These results clearly demonstrate the importance of the effect of environmental conditions (precipitation, nitrogen fertilisation, and drought) on gluten strength. These findings are fully in agreement with the sedimentation study in JK x Cham1 (Elouafi *et al.* 2000). Similar findings were also reported by Nachit *et al.* (1995a), where it was reported that the SDS-sedimentation volume is affected by environmental conditions; 27% in the Mediterranean dryland and 27.5% in the irrigated conditions.

Table IV-2: Sedimentation test mean, minimum, maximum, heritability, variance, and coefficient of variability in *Omrabi5/ T. dicoccoides600545// Omrabi5*.

Trait-year-site	**RILs Mean**	**RILs Min**	**RILs Max**	**P1**	**P2**	**Heritability**	**Variance (s^2)**	**Genetic C.V.** (100)
SDS97Ir	34.7	24	45	27	46	-	28.5	15.4
SDS97Rf	41.1	33	52	36	46	0.64	25.3	12.2
SDS98Br	48	27	64	38	42	0.90	60.2	16.2
SDS98EP	14.9	**9**	25	8	12	0.17	13.7	24.8
SDS98LP	48.9	28	**65**	39	44	0.96	40.9	13.1
SDS98Rf	27.6	**13**	47	20	28	0.60	51.5	26.0
SDS99Br	44.2	33	53	44	38	0.29	18.9	9.8
SDS99Inc	51.5	31	**70**	46	38	0.85	58.9	14.9
SDS99Kf	38.5	17	59	33	33	0.45	60.4	20.2
SDS99LP	55.9	34	**68**	51	46	0.93	42.5	11.7
SDS99Rf	41.8	21	63	39	30	0.77	53.9	17.6
SDS99Tr	44.6	24	61	41	33	0.76	56.6	16.9
SDS00Tr	36.1	18	49	31	29	0.91	46.7	18.9
SDS00Rf	40.6	23	60	33	37	0.97	40.6	15.7
SDS00Br	35.2	24	45	32	34	0.81	16.9	11.7
SDS00EP	30.6	**15**	40	28	26	0.30	23.9	15.9
SDS00Sum	51.8	23	**73**	48	44	0.86	119.3	21.1
SDS00Ir	38	17	55	33	29	-	41.7	16.9
Mean	**40.2**	**23.0**	**55.2**	**34.9**	**35.4**	**0.70**	**44.5**	**16.6**

P1 = *Omrabi5*; P2 = *T. dicoccoides600545*

Fig IV-3: Distribution frequency for average sedimentation test in *Omrabi5/ T. dicoccoides600545// Omrabi5* (18 environments).

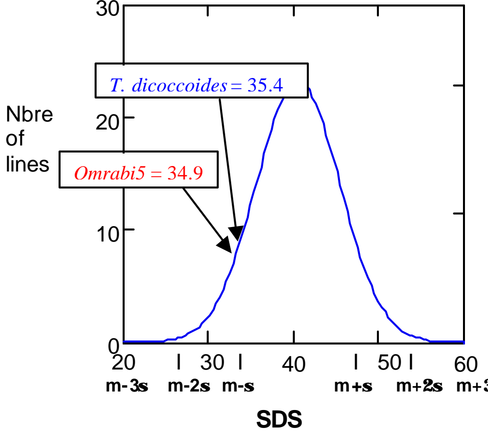
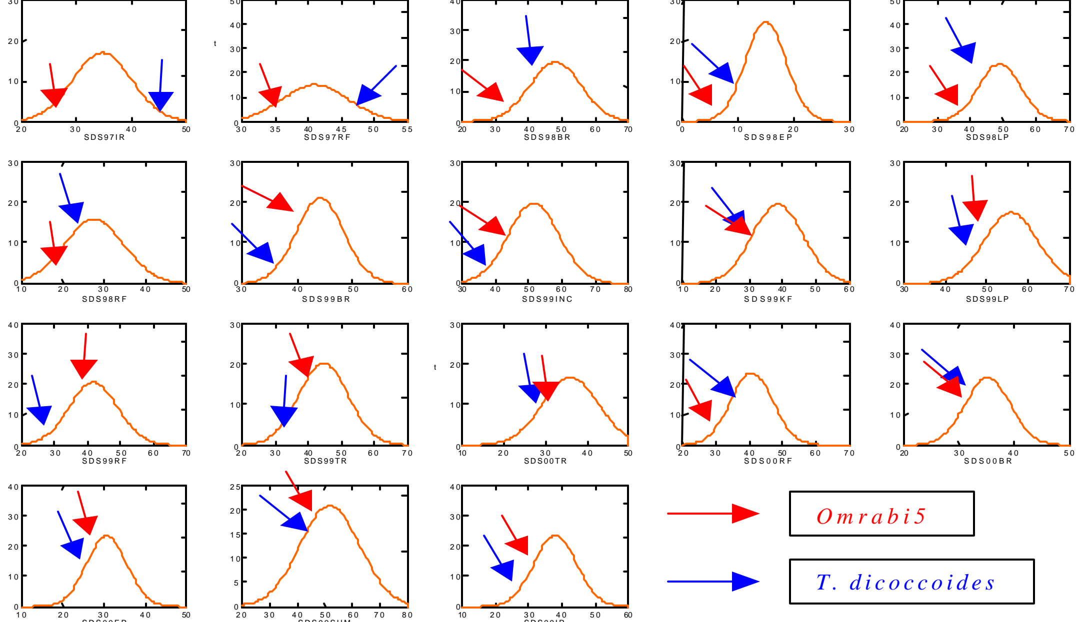


Fig IV-4: Distribution of sedimentation test (SDS) in the 18 studied environments in *Omrabi5/ T. dicoccoides600545// Omrabi5*.



3- Firmness (SDSni)

Durum technological properties are determined on the basis of cooking characteristics of the pasta it produces, where cooking quality is the capacity of the cooked product to maintain good texture (al diente) and an adequate firmness after cooking. The sedimentation index SDSni is used as surrogate to test for firmness of durum endproducts; pasta, couscous, and burghul (Nachit, pers. com.). The table IV-3 shows the statistical and genetic parameters for SDSni. As this index is a combination

of protein content and sedimentation test, its shows the same high transgressive inheritance and strong heritability as gluten strength and protein content traits (Table IV-3). Concerning heritabilities (Table IV-3), the highest values for SDSni heritability were achieved in several environments (98Br, 99Inc, 99LP, 00Tr, 00Rf, and 00Sum), whereas the medium values were in 00EP and 99Br. Also the highest genetic coefficients of variability were found in the stressed environments.

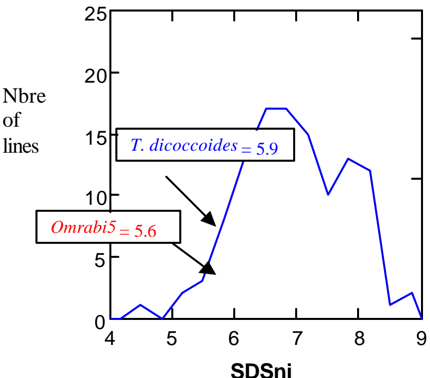
Figure IV-5 shows the distribution frequency for average SDSni. These results indicate that *T. dicoccoides600545* shows better values for SDSni than *Omrabi5*. Indeed, the majority of the RILs showed improvement in SDSni.

Table IV-3: Sedimentation test index (SDSni) mean, minimum, maximum, heritability, variance, and coefficient of variability in *Omrabi5/T. dicoccoides600545//Omrabi5*.

Trait-year-site	**RILs Mean**	**RILs Min**	**RILs Max**	**P1**	**P2**	**Heritability**	**Variance (s^2)**	**Genetic C.V. (100)**
SDSni97Ir	4.5	2.9	6.8	0.1	0.1	-	0.8	19.9
SDSni97Rf	6.5	4.5	8.8	5.4	8.8	0.55	1.1	16.1
SDSni98Br	9.2	5.3	12.7	8.1	9.4	0.93	2.4	16.8
SDSni98EP	2.3	1.2	4.2	1.1	2.1	0.57	0.5	30.7
SDSni98LP	9.1	4.7	12.7	6.5	8.2	0.96	2.1	15.9
SDSni98Rf	4.3	1.9	8.4	3.0	5.4	0.69	1.8	31.2
SDSni99Br	8.1	6.2	10.3	7.2	7.5	0.53	0.8	11.0
SDSni99Inc	9.0	5.6	13.3	7.6	7.7	0.86	2.4	17.2
SDSni99Kf	8.4	4.0	13.3	7.1	7.4	0.03	2.9	20.27
SDSni99LP	10.0	6.0	13.1	8.2	8.8	0.94	2.1	14.5
SDSni99Rf	6.0	2.7	9.5	5.6	4.7	0.64	1.7	21.7
SDSni99Tr	8.4	4.1	11.6	7.7	6.7	0.79	2.2	17.6
SDSni00Tr	5.6	2.9	8.8	4.5	5.1	0.89	1.4	21.1
SDSni00Rf	7.0	4.3	10.2	4.9	6.8	0.96	1.4	16.9
SDSni00Br	7.6	5.5	10.1	6.5	7.3	0.81	0.8	11.8
SDSni00EP	4.6	2.2	6.9	3.9	3.7	0.45	0.9	20.6
SDSni00Sum	9.1	4.6	14.1	7.7	0.5	0.89	4.3	22.8
SDSni00Ir	5.7	2.3	8.9	4.4	4.2	-	1.4	20.7
Mean	**7.0**	**4.0**	**10.3**	**5.6**	**5.9**	**0.75**	**1.7**	**19.3**

P1 = *Omrabi5*; P2 = *T. dicoccoides600545*

Fig IV-5: Distribution frequency for average SDSni index in *Omrabi5/ T. dicoccoides600545// Omrabi5* (18 environments).



4- Farinograph

The farinograph parameters: FAB, FDT, FST, FMT are used as tests to study the gluten strength quality. The mean, minimum and maximum, heritability, variance, and coefficient of variability of the farinograph parameters for the recombined inbred lines and their parents are shown in table IV-4. The farinograph parameters means of the RILs over the 4 test environments were as follows: FAB (mean: 74.3; range: 69.3-78.2), FDT (mean: 3.5; range: 2.3-5.2), FST (mean: 2.3; range: 0.9-4.6), FMT (mean: 121.1; range: 60.3-221.6), whereas the means of the two parents: *Omrabi5* and *T. dicoccoides600545* were 73.5 and 74.5 for FAB, 3.03 and 3.37 for FDT, 2.18 and 1.30 for FST, 106.25 and 166.67 for FMT, respectively. Transgressive inheritance was revealed for all parameters (Fig. IV-6), and the distributions for all environments showed normal patterns.

The broad sense heritability estimated showed very low value for FAB (0.08) and medium values for FDT (0.47), FST (0.66), and FMT (0.46). These results are in agreement with the values presented earlier for the sedimentation test. Further, the average genetic coefficient of variability of farinograph parameters for the RILs was of 1.94% (FAB); 15.8% (FDT); 42.1% (FST), and 27.6% (FMT). Besides, high values (>3 min) for FDT and FST and low values (less than 120 BU) for FMT are desirable for farinograph parameters. The results are of interest for identifying RIL with desirable combination to be used in the hybridization program on the durum-breeding program.

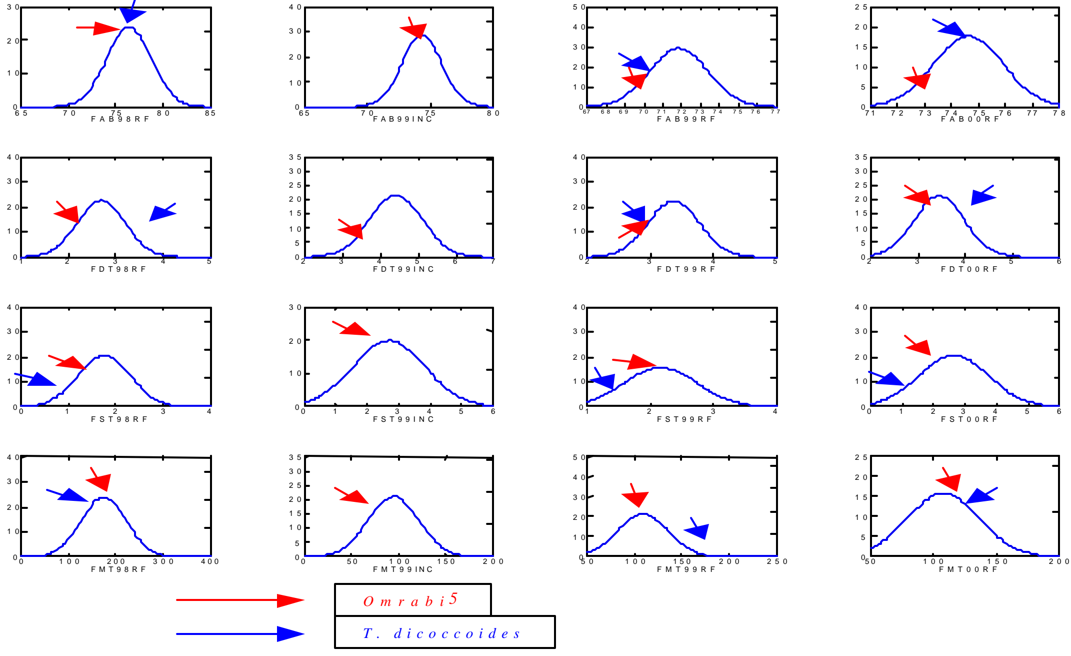
Table IV-4: Farinograph parameters mean, minimum, maximum, heritability, variance, and coefficient of variability in *Omrabi5/ T. dicoccoides600545// Omrabi5.*

Trait-year-site	RILs Mean	RILs Min	RILs Max	P1	P2	Heritability	Variance (s^2)	Genetic C.V. (100)
FAB98Rf	76.4	68.5	80.5	76	77.5	-	-	-
FAB99Inc	74.3	69.5	78.5	75	-	-	2.5	2.13
FAB99Rf	71.8	67.5	76.0	70	71	-	-	-
FAB00Rf	74.6	71.6	77.8	73	75	0.08	1.7	1.75
Mean	**74.3**	**69.3**	**78.2**	**73.5**	**74.5**	**0.08**	**2.1**	**1.94**
FDT98Rf	2.7	1.7	4.7	2.3	3.3	-	-	-
FDT99Inc	4.4	2.9	6.5	3.6	-	-	0.5	16.1
FDT99Rf	3.4	2.5	4.3	3	3	-	-	-
FDT00Rf	3.5	2.1	5.1	3.2	3.8	0.47	0.3	15.6
Mean	**3.5**	**2.3**	**5.2**	**3.03**	**3.37**	**0.47**	**0.4**	**15.8**
FST98Rf	1.7	0.7	3.3	1.7	1.1	-	-	-
FST99Inc	2.7	0.9	5.9	2.5		-	1.3	42.2
FST99Rf	2.2	1.2	3.9	2.2	1.3	-	-	-
FST00Rf	2.6	0.8	5.4	2.3	1.5	0.66	1.2	42.1
Mean	**2.3**	**0.9**	**4.6**	**2.18**	**1.30**	**0.66**	**0.8**	**42.1**
FMT98Rf	174.0	80.0	335.0	100	185	-	-	-
FMT99Inc	94.3	45.0	160.0	85		-	726.5	28.6
FMT99Rf	108.5	65.0	210.0	120	185	-	-	-
FMT00Rf	107.7	51.3	181.3	120	130	0.46	820.7	26.6
Mean	**121.1**	**60.3**	**221.6**	**106.25**	**166.67**	**0.46**	**515.7**	**27.6**

P1 = *Omrabi5*; P2 = *T. dicoccoides600545.*

FAB: are in ml. FDT, FST values are scored as follows: 1 = weak; 2 = weak-medium; 3 = medium; 4 = medium-strong; 5 = strong. FMT: 200-250 very weak; 150-199 weak; 100-149 medium strong; 50-99 well balanced-strong; <50 very strong-over stable.

Fig IV-6: Distribution of farinograph absorbance, development, stability, and mixing time in the 4 studied environments in *Omrabi5/ T. dicoccoides600545// Omrabi5*.



5- Yellow Pigment (YP)

Carotene content is a trait of great commercial importance in durum wheat end-products. It has also nutritional value. Durum wheat grain is normally amber and vitreous, and its milling produces yellow semolina. The mean, minimum and maximum, heritability, variance, and coefficient of variability of yellow pigment content for the recombined inbred lines and their parents are shown in table IV-5. The yellow pigment mean of the RIL over the 18 studied environments was 5.5ppm, ranging from 2.1 to 9.3ppm, whereas the mean of the two parents: *Omrabi5* and *T. dicoccoides600545* were 6.6 and 5.3ppm, respectively. This indicates some dominant inheritance by the *T. dicoccoides* (or of low YP values). Negative transgressive inheritance was detected in some stressed environments such as Breda 97/98 and 99/00, Early Planting 97/98, and Rainfed 97/98 (Fig IV-8). The average frequency distribution for the RILs and parents illustrates the transgressive inheritance for yellow pigment (Fig IV-7).

The broad sense heritability estimated in 16 environments, showed values varying from 0.48 to 0.99 with a mean of 0.87. These heritabilities values indicate the strong genotypic effect on yellow pigment content. This confirms earlier published

studies on carotenoid content heritability in durum wheat (Nachit *et al.* 1995a) and in *Tritordeums* (Alvarez *et al.* 1998). In durum, the carotenoid content was reported to be highly heritable and controlled by additive gene effects (Joppa and Williams 1988, Parker *et al.* 1998, Borreli *et al.* 1999). In tritordeums, the carotene content was reported to be independent of grain yield and grain size (Alvarez *et al.* 1998). Further, the average genetic coefficient of variability for the RILs was high (24.2%), varying from 18.3% (00Br) to 31.0% (00EP, 99LP).

Table IV-5: Yellow pigment mean, minimum, maximum, heritability, variance, and coefficient of variability in *Omrabi5/ T. dicoccoides600545// Omrabi5*.

Trait-year-site	RILs Mean	RILs Min	RILs Max	P1	P2	Heritability	Variance (s^2)	Genetic C.V. (100)
YP97Ir	4.5	2.6	7.3	6.1	4.5	-	1.5	27.2
YP97Rf	5.4	2.7	7.4	6.5	5.6	0.77	1.3	21.1
YP98Br	4.9	3.1	7.7	5.7	5.2	0.58	1.2	22.4
YP98EP	5.5	3.3	8.4	6.3	5.8	0.48	1.4	21.5
YP98LP	5.9	3.2	9.1	7.0	5.7	0.98	2.1	24.5
YP98Rf	5.3	2.9	8.4	6.0	5.5	0.97	1.4	22.3
YP99Br	5.9	3.6	8.7	6.6	5.5	0.87	1.7	22.8
YP99Inc	5.9	3.2	9.2	7.3	5.9	0.98	2.4	26.2
YP99Kf	5.6	3.1	8.9	7.2	5.8	0.76	1.8	23.9
YP99LP	5.3	2.8	8.0	6.9	5.5	0.99	1.5	23.1
YP99Rf	5.6	3.1	8.8	6.7	4.9	0.96	2.3	27.0
YP99Tr	5.2	2.9	8.7	6.9	4.8	0.95	2.1	27.8
YP00Tr	5.5	2.9	9.3	6.6	5.0	0.97	2.2	26.9
YP00Rf	5.4	3.2	8.0	6.4	5.6	0.96	1.7	24.1
YP00Br	6.2	4.1	8.8	6.9	6.1	0.91	1.3	18.3
YP00EP	5.3	3.1	7.9	7.1	5.5	0.99	1.8	25.3
YP00Sum	6.2	2.1	8.8	6.4	4.2	0.85	1.7	21.0
YP00Ir	5.1	2.8	8.9	5.9	3.6	-	2.5	31.0
Mean	**5.5**	**3.0**	**8.5**	**6.6**	**5.3**	**0.87**	**1.8**	**24.2**

P1 = *Omrabi5*; P2 = *T. dicoccoides600545*

Fig IV-7: Distribution frequency for average yellow pigment content in *Omrabi5/ T. dicoccoides600545// Omrabi5* (18 environments).

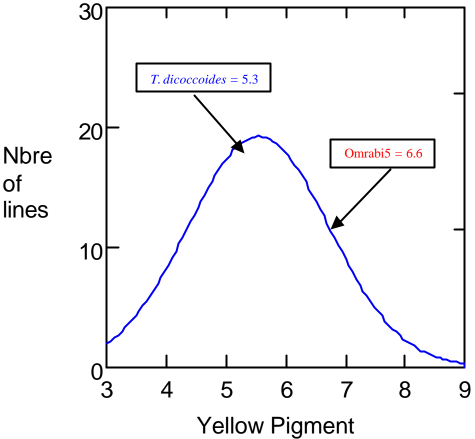
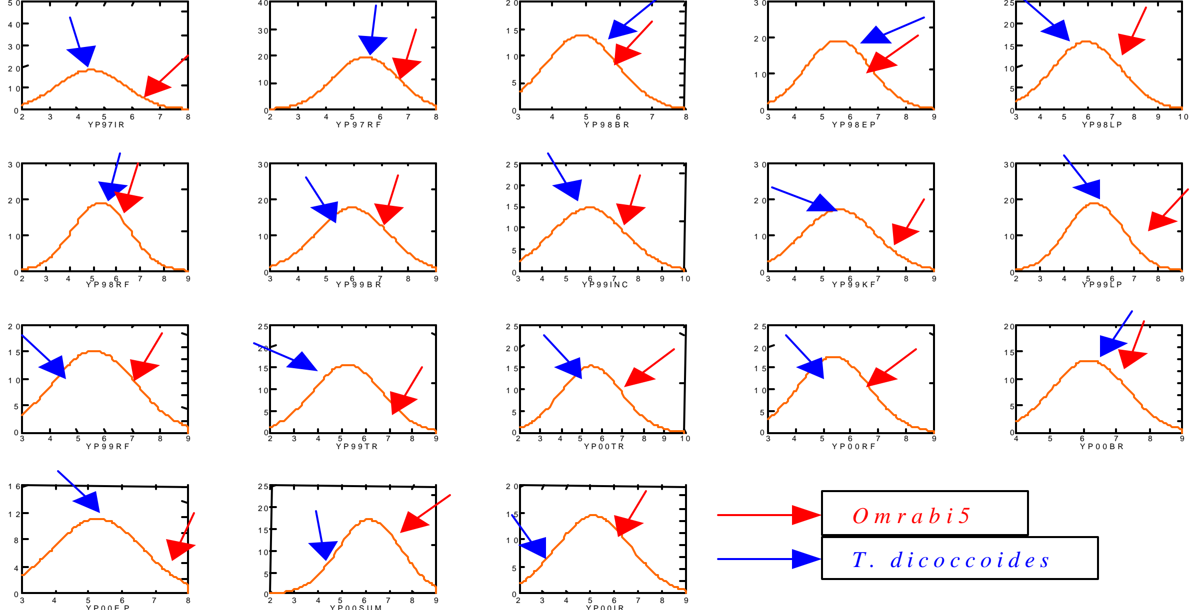


Fig IV-8: Distribution of yellow pigment in the 18 studied environments in *Omrabi5/ T. dicoccoides600545// Omrabi5* .



6- Vitreousness (Vit)

The mean, minimum, maximum, heritability, variance, and coefficient of variability for the recombined inbred lines and their parents for vitreousness is shown in Table IV-6. The vitreousness mean for the RILs was 96.6%, this mean represent the average of 18 environments (sites x years). Most of the RILs had high values for vitreousness except for 98EP where the mean was of 77.1, ranging from 29.0 to 90%. These very low vitreousness values could be explained by the high precipitation received during this season 1997/98 plus the applied supplementary irrigation. The

means for *Omrabi5* and *T. dicoccoides600545* were 95.8% (68-100) and 97.8% (80-100), respectively. The lowest values for both parents, 68 and 80% respectively, were also found in the Tel-Hadya 98EP.

The figure IV-9 shows the distribution frequency of the average of vitreousness in the 18 tested environments. This graph shows a moderate transgressive inheritance. However, when the analysis were done for each environment separately, as shown in Figure IV-10, a strong negative transgressive inheritance was detected, e.g.; 97Rf; 98Br; 98LP; 99Br; 99Inc; 00Sum, and 00Ir. In spite of this, positive transgressive inheritance was also revealed in few environments, e.g.; 99LP and 00EP. All environments have shown a good fit to the normal distribution (Fig IV-10).

Broad sense heritability estimation in 16 environments ranged from 0.00 to 0.92 with a mean of 0.42. These heritability values suggest the strong environmental effect on vitreousness trait. In comparison with earlier studies in similar environments, the broad sense heritability of vitreousness was estimated at 0.59 for irrigated conditions and 0.79 for dry dryland (Nachit *et al.* 1995a).

Furthermore, the estimations of genetic coefficient of variability for the RILs had a mean of 2.7%, varying from 0.4% to 11.9%. The highest vitreousness values were observed in stressed environments. Therefore, the minimum vitreousness value for RIL population in 98Br, 99Inc, and 00Br was 98%, whereas the lowest vitreousness value was observed in 98EP. The results also show that the screening for vitreousness require to be conducted under high rainfall conditions with low nitrogen fertilization (Nachit and Asbati 1987), as it was shown for 98EP environment. Moreover, vitreousness is a desirable character in durum wheat processing; high percentage (90-100%) of vitreous kernel is a major requirement for all durum products (Matsuo and Dexter 1980, Williams *et al.* 1984; Nachit and Asbati 1987). The present results confirm the importance of *T. dicoccoides* for the improvement of vitreousness in durum wheat.

Table IV-6: Vitreousness mean, minimum, maximum, heritability, variance, and coefficient of variability in *Omrabi5/ T. dicoccoides600545// Omrabi5*.

Trait-year-site	RILs Mean	RILs Min	RILs Max	P1	P2	Heritability	Variance (s^2)	Genetic C.V. (100)
Vit97Ir	98.1	82.0	100.0	96.0	100.0	-	10.9	3.4
Vit97Rf	96.3	88.0	100.0	98.0	99.0	0.00	6.0	2.5
Vit98Br	99.6	98.0	100.0	100.0	100.0	0.00	0.3	0.5
Vit98EP	76.1	29.0	90.0	68.0	80.0	0.84	82.1	11.9
Vit98LP	98.6	95.0	100.0	99.0	99.0	0.55	1.2	1.1
Vit98Rf	96.7	86.0	100.0	95.0	99.0	0.01	6.0	2.5
Vit99Br	99.6	97.0	100.0	100.0	100.0	0.13	0.4	0.6
Vit99Inc	99.7	98.3	100.0	100.0	100.0	0.75	0.2	0.4
Vit99Kf	94.0	77.1	100.0	93.0	97.0	0.51	17.0	4.4
Vit99LP	99.2	91.7	100.0	96.0	99.0	0.51	1.6	1.3
Vit99Rf	98.2	88.2	100.0	99.0	99.0	0.92	5.5	2.4
Vit99Tr	96.8	89.0	100.0	97.0	97.5	0.49	12.8	3.7
Vit00Tr	98.9	95.5	100.0	97.0	100.0	0.73	1.2	1.1
Vit00Rf	97.7	89.5	100.0	97.0	99.0	0.00	4.3	2.1
Vit00Br	99.8	98.1	100.0	99.0	100.0	0.50	0.2	0.4
Vit00EP	98.0	90.3	100.0	92.0	96.0	0.00	2.9	1.7
Vit00Sum	92.0	68.1	100.0	98.0	96.0	0.86	41.0	6.9
Vit00Ir	99.5	96.0	100.0	100.0	100.0	-	0.7	0.8
Mean	**96.6**	**86.5**	**99.4**	**95.8**	**97.8**	**0.42**	**10.8**	**2.7**

P1 = *Omrabi5*; P2 = *T. dicoccoides600545*

Fig IV-9: Distribution frequency for average of vitreousness in *Omrabi5/ T. dicoccoides600545// Omrabi5* (18 environments).

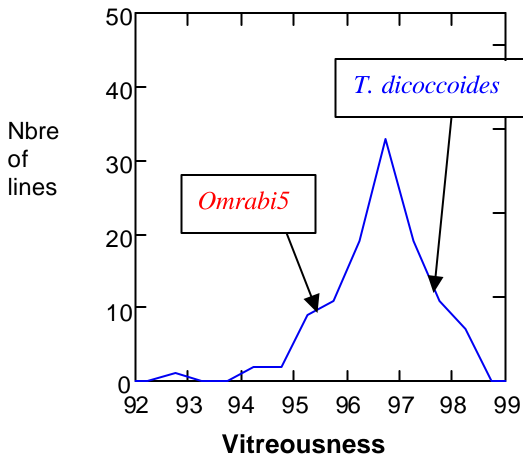
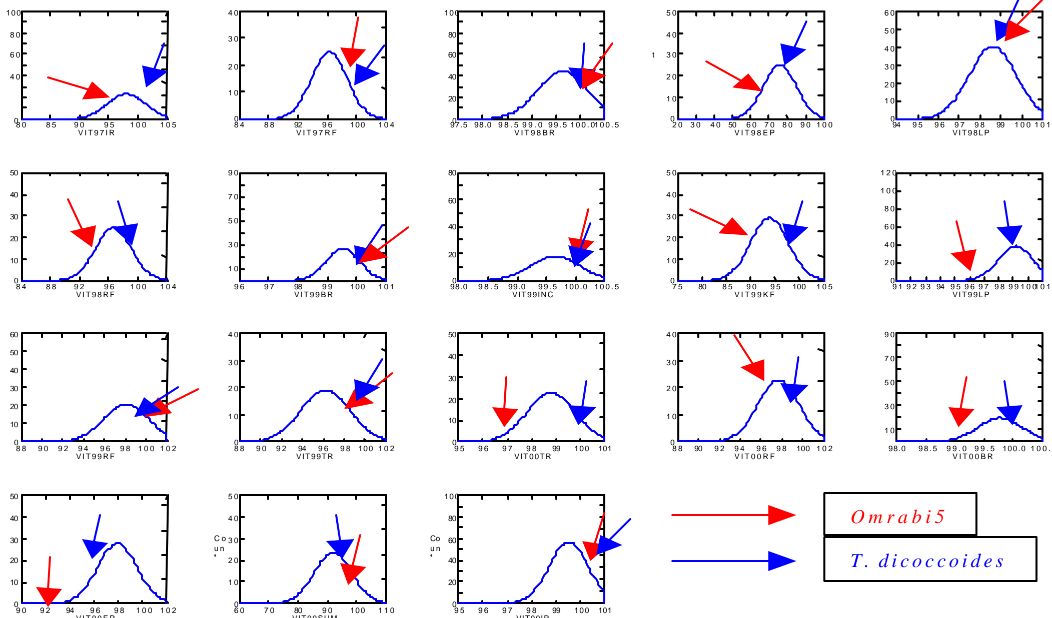


Fig IV-10: Distribution of vitreousness in the 18 studied environments in *Omrabi5/ T. dicoccoides600545// Omrabi5*.



7- Test Weight (TW)

Table IV-7 shows the mean, minimum, maximum, heritability, variance, and coefficient of variability of the recombined inbred lines and their parents for test weight. The test weight was measured for only three environments, two during the season 1998/99 (Inc and Rf) and one during the season 1999/00. The test weight mean for the RILs was 72.4g ranged from 66.6 to 77.6g, whereas the means for P1 *Omrabi5* and P2 *T. dicoccoides600545* were 79.2 and 70.6, respectively. There is a clear dominance of the low values test weight of the *dicoccoides* parent. Fig IV-11 shows the average frequency distribution for the RILs and parents. The results are explicit and demonstrate that the RILs test weights are either equal or lower than the two parents test weights. In the three studied environments, the maximum test weight of the RILs was less that *Omrabi5* test weight. However, few lines were not significantly different from *Omrabi5* (Table IV-7).

In Figure IV-13, the frequency distributions for test weight are shown. All environments have shown a good fitting to normal distribution. In our trials, the test weight did not show a large variation across environments. However, it was pointed out

that test weight can be affected by various environmental factors such as disease and lodging (Roth *et al.* 1984; Blum *et al.* 1991; Saadalla *et al.* 1990).

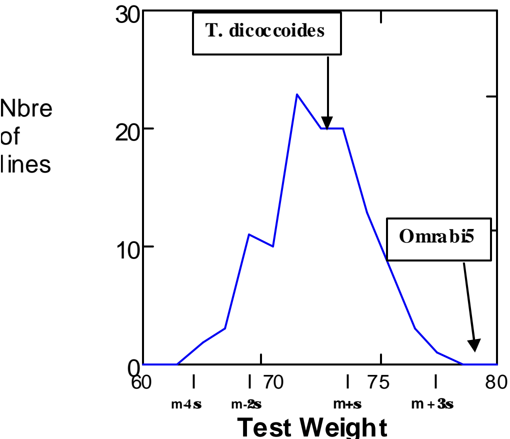
Furthermore, heritability value was estimated only for one environment, and it was 0.91 in 00Rf; and the GCV was 3.0%. This high value confirms the importance of the genotypic control on test weight trait. This is in agreement with earlier published broad sense heritability values (Teich 1984; Roth *et al.* 1984; Blum *et al.* 1991; Jallaludin *et al.* 1989; Saadalla *et al.* 1990; Nachit *et al.* 1995a). However, it is in disagreement with other studies showing a low heritability values suggesting a major contribution of environmental conditions to test weight variation (Ghaderi and Everson 1971, Schuler *et al.* 1994). From a breeding point of view, it is very important to find simple factors that correlated with test weight and can be used in the early generation of breeding programs. Because, it is a critical trait for yield components. For this, some research divides the components of test weight to the size and shape (width and thickness) of the grain and the weight of this grain (Yamazaki and Briggle 1969, Ghaderi and Everson 1971, Schuler *et al.* 1994). They suggest the spike characteristics as an indirect selection for test weight, specially the kernel shape (Trocoli and DiFonzo, 1999). In other breeding program it is considered in combination with vitreousness as a good test for high semolina extraction (Nachit pers. com.). In fact, it was reported that test weight is highly related to semolina yield and flour yield (Marshal *et al.* 1986), although earlier works did not find this association (Barmore and Bequette 1965).

Table IV-7: Test weight mean, minimum, maximum, heritability, variance, and coefficient of variability in *Omrabi5/T. dicoccoides600545//Omrabi5*.

Trait-year-site	**RILs Mean**	**RILs Min**	**RILs Max**	**P1**	**P2**	**Heritability**	**Variance (s^2)**	**Genetic C.V.** (100)
TW99Inc	71.9	67.1	77.9	78.3	73.0		5.4	3.2
TW99Rf	72.0	66.6	76.4	78.6	66.8		4.5	2.9
TW00Rf	73.5	67.9	78.5	80.6	72.1	0.91	4.3	2.8
Mean	**72.4**	**67.2**	**77.6**	**79.17**	**70.63**	**0.91**	**4.7**	**3.0**

P1 = *Omrabi5*; P2 = *T. dicoccoides600545*

Fig IV-11: Distribution frequency for average of test weight in *Omrabi5/ T. dicoccoides600545// Omrabi5* (3 environments).



8- Thousand Kernel Weight (TKW)

In this study the mean, minimum, maximum, heritability, variance, and coefficient of variability for the recombined inbred lines and their parents is shown in table IV-8. The RILs mean over the 18 studied environments (sites x years) was 29.9g, while the means for the parents, *Omrabi5* and *T. dicoccoides600545* were 32.1 and 28.6, respectively. The average minimum of the RILs over all sites x years was 22.5g and the maximum was 38.4g. However, the environment 00Ir generated maximum value of 47.4g, and 10 environments out of 18 produced maximum values higher than 40g. This result shows clearly the effect of transgressive inheritance. The figure IV-12 showing the average frequency distribution for the RILs and their parents, illustrate very well this transgressive inheritance. RILs exhibit either higher or lower TKW values than both parents. The RILs values showed a wide range, varying from 15.1g (98EP) to 47.4g (00Ir).

Furthermore, if the results were examined considering each environment the variation was very significant across environments (Fig IV-13). All environments have shown a good fitting to normal distribution, suggesting quantitative trait inheritance. In some environments such as: 97Ir; 97Rf; 99Lp, and 99Tr a significant positive transgressive inheritance was clearly noticed, whereas in other environments such as: 99Br; 00Tr; 00Br, and 00Ir a significant negative transgressive inheritance was revealed, especially in 00Ir. This suggests an environmental effect on this trait. It is well known that kernel weight is affected by the environment during grain filling and by the number of heads and number of fertile florets per spike.

In addition, broad sense heritability estimation in 16 environments shows a mean of 0.60 varying from 0.00 to 0.96. Most of the environments shows heritabilities higher than 0.5 except for the extreme stressed environments 99Kf and 98EP. This intermediate to high heritability values suggests a high genetic effect on TKW. This is in agreement with earlier studies (Sun, 1972; Ketata *et al.* 1976; Maali 1991). Higher heritability values (0.94-0.97) were also reported (Nachit *et al.* 1995a).

Furthermore, the average genetic coefficient variability for the RILs was 10.9%. Across the studied environments, the genetic C.V. was stable and was around 10%. The lowest was found in 98Br (8%), and the highest during the same year 98 in Early planting (18.4%). Similar studies using durum cultivars x *T. dicoccoides* population reported high heritability coupled with high genetic advance, confirming a high additive effect (Nachit and Maali 1997).

Table IV-8: Thousand-kernel weight mean, minimum, maximum, heritability, variance, and coefficient of variability in *Omrabi5/ T. dicoccoides600545// Omrabi5*.

Trait-year-site	RILs Mean	RILs Min	RILs Max	P1	P2	Heritability	Variance (s^2)	Genetic C.V. (100)
TKW97Ir	33.6	24.8	40.7	31.6	27.6	-	11.2	9.9
TKW97Rf	28.9	21.7	35.9	28.6	26.0	0.54	8.8	10.3
TKW98Br	33.4	26.5	41.9	34.7	30.4	0.96	7.2	8.0
TKW98EP	24.1	15.1	37.0	26.0	19.4	0.17	19.7	18.4
TKW98LP	26.2	19.8	32.4	28.2	26.4	0.81	7.5	10.4
TKW98Rf	28.7	21.0	40.2	36.7	20.9	0.93	15.9	13.9
TKW99Br	20.7	16.1	28.7	25.1	22.5	0.47	5.9	11.7
TKW99Inc	31.0	23.9	40.8	36.2	29.4	0.93	12.6	11.4
TKW99Kf	25.3	20.9	31.8	23.2	26.0	0.00	5.3	9.1
TKW99LP	28.8	19.9	40.2	29.0	26.2	0.28	15.3	13.6
TKW99Rf	34.3	25.9	42.4	37.1	31.8	0.80	12.1	10.1
TKW99Tr	30.6	23.9	38.8	29.8	29.4	0.62	8.1	9.3
TKW00Tr	35.5	27.8	42.2	38.1	35.1	0.65	10.1	8.9
TKW00Rf	29.4	21.4	38.4	33.0	26.0	0.42	8.6	9.9
TKW00Br	21.8	16.1	27.9	23.5	24.0	0.80	5.1	10.4
TKW00EP	32.8	25.7	40.9	32.0	36.0	0.92	11.0	10.1
TKW00Sum	34.8	27.9	43.0	39.2	32.9	0.40	10.4	9.3
TKW00Ir	38.0	27.2	47.4	45.4	44.2	-	15.8	10.5
Mean	**29.9**	**22.5**	**38.4**	**32.1**	**28.6**	**0.60**	**10.6**	**10.8**

P1 = *Omrabi5*; P2 = *T. dicoccoides600545*

Fig IV-12: Distribution frequency for average of 1000-kernel weight in *Omrabi5/ T. dicoccoides600545// Omrabi5* .

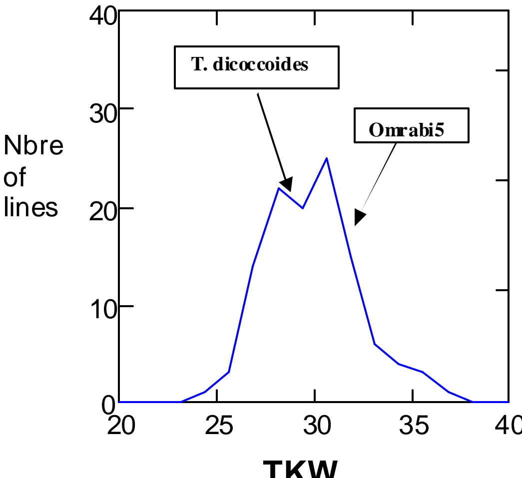
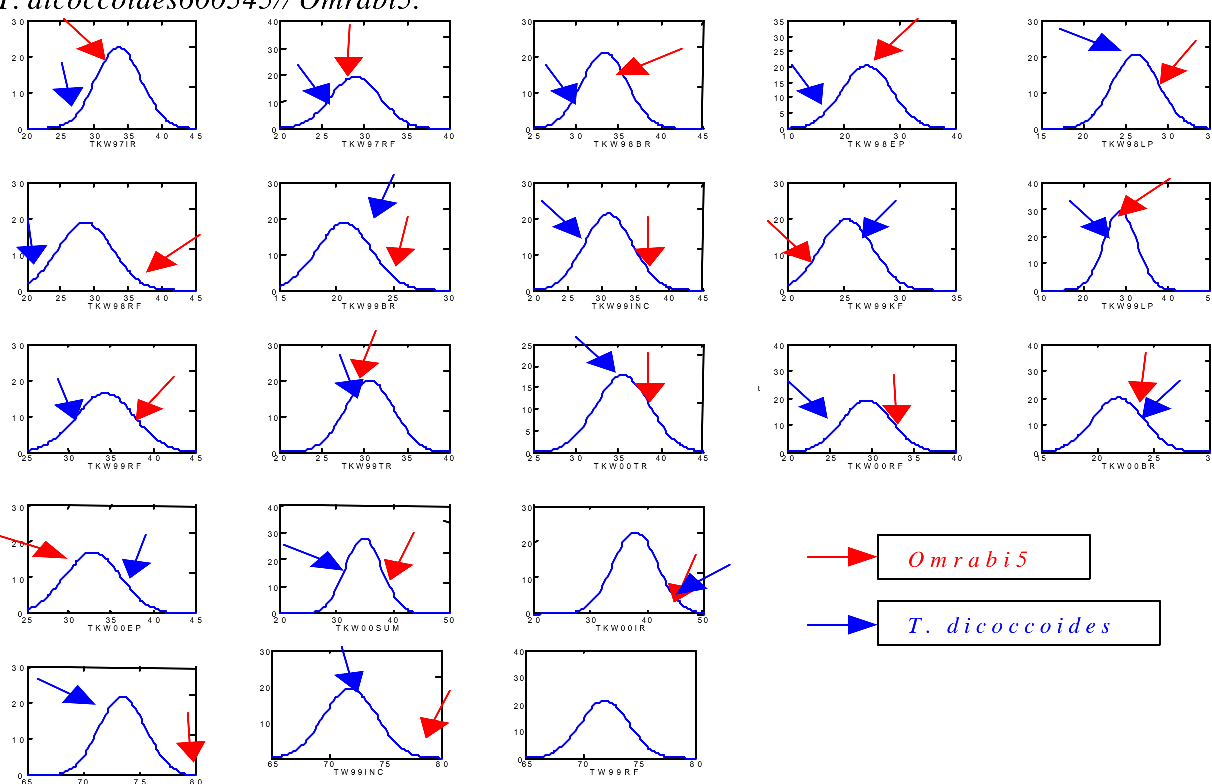


Fig IV-13: Distribution of 1000-kernel weight and test weight in *Omrabi5/ T. dicoccoides600545// Omrabi5.*



9- Milling Extraction

The flour yield (FY%) test is used to assess to milling extraction. The mean, minimum and maximum, heritability, variance, and coefficient of variability of the flour yield for the recombined inbred lines and their parents are shown in table IV-9. The flour yield mean of the RILs over the 3 test environments was 69 with a range from 62.3 to 73.4, whereas the means of the two parents: *Omrabi5* and *T. dicoccoides600545* were 71.0 and 63.1, respectively. Transgressive segregants were revealed in all 3 environments (Fig. IV-14). The distributions for all environments showed normal patterns.

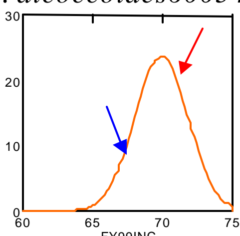
The broad sense heritability estimated for flour yield was 0.43. The calculated average genetic coefficient of variability showed low value (2.9%).

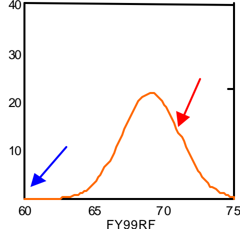
Table IV-9: Flour yield mean, minimum, maximum, heritability, variance, and coefficient of variability in *Omrabi5/ T. dicoccoides600545// Omrabi5*.

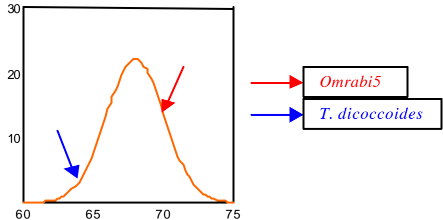
Trait-year-site	RILs Mean	RILs Min	RILs Max	P1	P2	Heritability	Variance (s^2)	Genetic C.V. (100)
FY99Inc	70.0	64.6	74.7	71.5	67.4		3.7	2.7
FY99Rf	69.0	60.2	73.5	70.4	58.7	-	4.4	3.0
FY00Rf	68.0	62.2	71.9	70.2	64.6	0.43	4.2	3.0
Mean	**69.0**	**62.3**	**73.4**	**71.0**	**63.1**	**0.43**	**4.1**	**2.9**

P1 = *Omrabi5*; P2 = *T. dicoccoides600545*

Fig IV-14: Distribution of flour yield in the 3 studied environments in *Omrabi5/ T. dicoccoides600545// Omrabi5*.







10- Ash Content (AC)

Semolina is actually the targeted product in durum wheat. A high extraction rate, and therefore low ash content, is of extreme importance in durum wheat production (Kobrehel *et al.* 1974, Dexter and Matsuo 1978, Borrelli *et al.* 1999, Trocoli *et al.*

2000). Therefore, measurement techniques to be applied in early generation selection were extensively studied (Morris *et al.* 1945, Matsuo and Dexter 1980, Dexter *et al.* 1994, Fares *et al.* 1996). However, pasta with low ash content is not nutritionally appropriate. Table IV-10 shows ash content mean, range, heritability, variance, and coefficient of variability for the recombined inbred lines and their parents. The ash content mean for the RILs over the 18 studied environments was 2%, the RILs minimum mean being 1.8% and the RILs maximum mean 2.1%. The lowest value was found in 97Ir (1.4%) and the highest in 99Kf, 99Lp and 99Tr, (2.4%). The highest values were therefore obtained in favorable growing conditions. Dexter and Matsuo (1978) reported a higher ash content in whole grain due to increased uptake of minerals from the soil in favorable growing conditions. The means for both parents: *Omrabi5* and *T. dicoccoides600545* were the same (2%). This result shows a transgressive inheritance as the RILs was higher than the two parents. This transgressive inheritance is clearly noticed in the average frequency distribution figure (Fig IV-15). Both parents show similar values while the RILs exhibit strong positive or negative transgressive inheritance values. When the 18 studied environments were examined one by one (Fig IV-16), the transgressive inheritance was found to be larger; e.g.; 98LP, 99Kf, 00Br, and 00Sum. However, transgressive inheritance was also revealed for low value also; e.g.; 99Tr and 00EP. Figure IV-16 shows also the good fitting to normal distribution for all studied environments.

The estimated heritability values were, in general, very low. Five out of 16 environments showed heritabilities with nil (0.00) values (Table IV-10). This suggests a high environmental effect on ash content trait. This is in agreement with Cubadda *et al.* (1969) work, but it disagrees with Peterson *et al.* (1986) findings. However, in our study other environments show high heritability values, e.g. 99Inc (0.81) and 00Sum (0.7). Those high values suggest a high genetic effect, and are in accordance with Peterson *et al.* (1986) findings. More recent studies suggest a strong genotype-by-environment interaction on the ash content (Fares *et al.* 1996). The heritability mean over the 16 environments, used in the heritability estimation, was 0.39%. This high variability in the heritability values means that some environments were conducive for ash content differences of RILs whereas others were not.

On the contrary of heritability estimations, the average genetic coefficient variability for the RILs was more or less stable over environments. The mean was 33.9%, varying from 26.1% (00Br) to 48.4% (97Ir).

Table IV-10: Ash content mean, minimum, maximum, heritability, variance, and coefficient of variability in *Omrabi5/ T. dicoccoides600545// Omrabi5*.

Trait-year-site	RILs Mean	RILs Min	RILs Max	P1	P2	Heritability	Variance (s^2)	Genetic C.V. (100)
AC97Ir	1.6	1.4	1.8	1.7	1.8	-	0.6	48.4
AC98Br	1.6	1.5	1.8	1.6	1.7	0.21	0.2	27.9
AC98EP	1.8	1.6	2.0	1.8	2.0	0.39	0.6	43.0
AC98LP	1.9	1.7	2.0	1.8	1.8	0.32	0.4	33.3
AC98Rf	1.7	1.5	1.9	1.7	1.8	0.77	0.5	41.6
AC99Br	2.1	1.9	2.3	2.1	2.2	0.00	0.3	26.1
AC99Inc	2.0	1.8	2.1	2.0	2.1	0.81	0.4	31.6
AC99Kf	2.2	2.0	2.4	2.2	2.2	0.00	0.5	32.1
AC99LP	2.2	2.0	2.4	2.1	2.3	0.00	0.5	32.1
AC99Rf	2.0	1.8	2.2	2.0	2.1	0.00	0.4	31.6
AC99Tr	2.2	2.0	2.4	2.3	2.3	0.37	0.4	28.7
AC00Tr	2.0	1.9	2.2	2.0	2.1	0.58	0.4	31.6
AC00Rf	2.0	1.9	2.2	2.0	2.2	0.14	0.5	35.4
AC00Br	2.2	2.0	2.3	2.2	2.2	0.00	0.3	24.9
AC00EP	2.0	1.7	2.1	2.0	2.0	0.41	0.5	35.4
AC00Sum	2.1	1.9	2.3	2.1	2.1	0.70	0.6	36.9
AC00Ir	1.9	1.7	2.1	2.0	1.9	-	0.5	37.2
Mean	**2.0**	**1.8**	**2.1**	**2.0**	**2.0**	**0.31**	**0.4**	**33.9**

P1 = *Omrabi5*; P2 = *T. dicoccoides600545*

Fig IV-15: Distribution frequency for average ash content in *Omrabi5/ T. dicoccoides600545// Omrabi5* (17 environments).

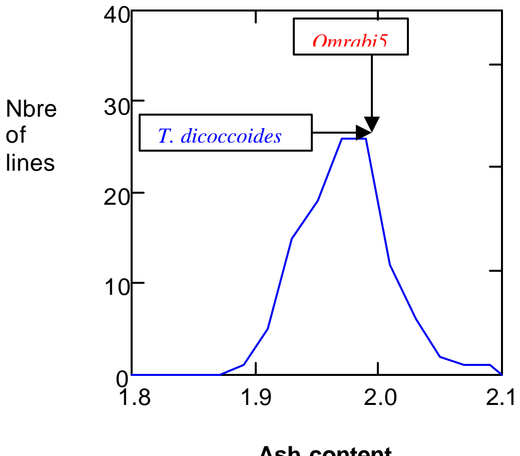
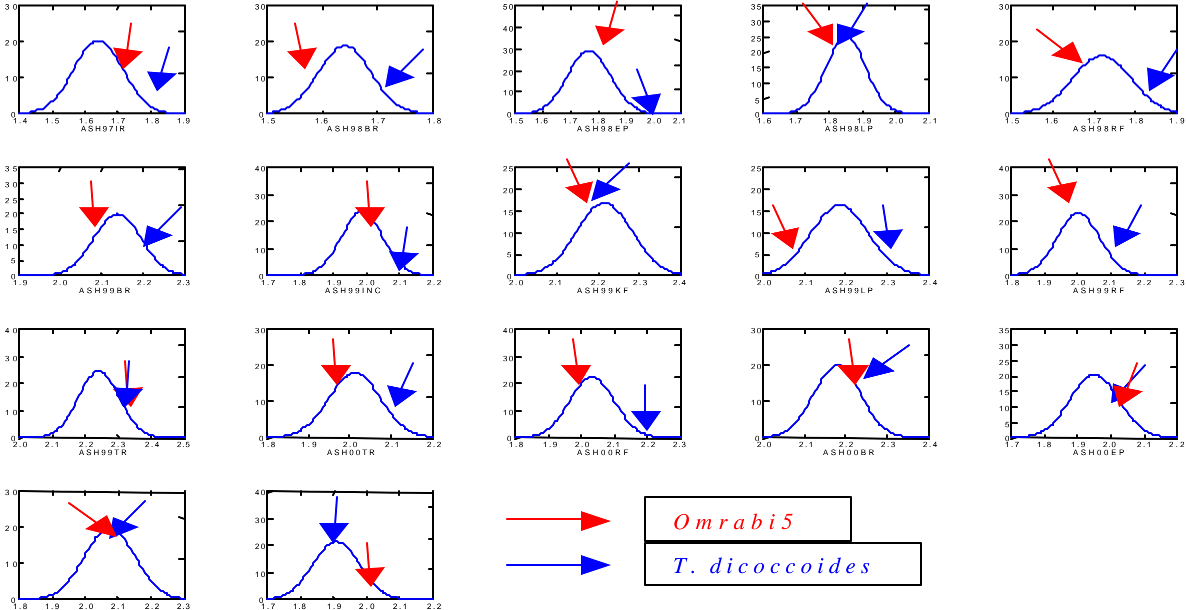


Fig IV-16: Distribution of ash content in the 17 studied environments in *Omrabi5/ T. dicoccoides600545// Omrabi5*.



II- Correlation and Clustering of grain-quality characteristics

Mean data for the grain quality traits were analyzed to study the relationship among the traits studied. Two analyses were conducted: correlation and clustering. Pearson correlation matrix and the cluster show the correlation between the averages of different studied grain-quality traits in Table IV-11 and Fig IV-17. Very strong correlation was noticed between SDS and SDSni (0.96) and a significantly positive correlation between protein content and SDSni (0.44). These are logical associations since the SDS index calculation is based on SDS and protein content (SDSni = (PC x SDS)/100). The three correlated parameters showed a negative correlation to TKW (Table IV-11).

A strong correlation was found between sedimentation test and its index with farinograph parameters. The large values for SDS and SDSni were positively and highly correlated with the larger time (min) required for farinograph development time (FDT), and stability time (FST); and with the lower Brabender units (strong dough) for the mixing time (FMT).

Protein content shows a positive correlation with vitreousness (0.42). This is in accordance with earlier published association between both traits (Matsuo and Dexter 1980, Nachit and Asbati 1987). They reported that when the proportion of vitreous grains decreases, the proportions of semolina and protein content decrease, while that of flour increases. In addition, protein content shows a positive non-linear correlation with FAB (0.43). To less extend, FAB was also found to be associated with SDS. Moreover, for the FAB prediction and calibration for farinograph test, protein content measurements are used. In addition, it is known that durum flour water absorption is higher than that of hard bread wheat. Further, durum flour has a very slow absorption; but a high water binding capacity which is linked to longer shelf life. On the other hand, protein content shows a negative correlation with test weight and TKW. The correlation values were similar for both TW and TKW (-0.56). The correlation between protein content and TW was reported to be negative by Matsuo and Dexter (1980) and significantly positive by Yamazaki and Briggle (1969) and Schuler *et al.* (1994). A lack of association between protein content and TKW in durum wheat was reported (Schuler *et al.* 1994).

However, the correlation between TKW and TW was not very high, it was of 0.38. These findings are in agreement with Ghaderi and Everson (1971) and Matsuo and Dexter (1980). Actually, they found a higher overall correlation (r = 0.75) between TW and TKW, suggesting that the 2 traits were affected by environment factors in a similar manner. However our findings disagree with other researchers that failed to find any correlation between TW and TKW (Yamazaki and Briggle 1969; Schuler *et al.* 1994; Trocoli and Di Fonzo 1999). A significant association (at 5%) was revealed between TW and flour yield. This confirms Barmore and Bequette (1965) findings. Even though other findings pointed out a strong correlation between TW and flour yield (Marshal *et al.* 1986). In this study the cluster analysis (Fig IV-17) grouped closely together TW, TKW, and flour yield with yellow pigment. In the same trend as protein content, vitreousness shows a negative moderate correlation to TKW, whereas FAB shows a negative correlation to flour yield.

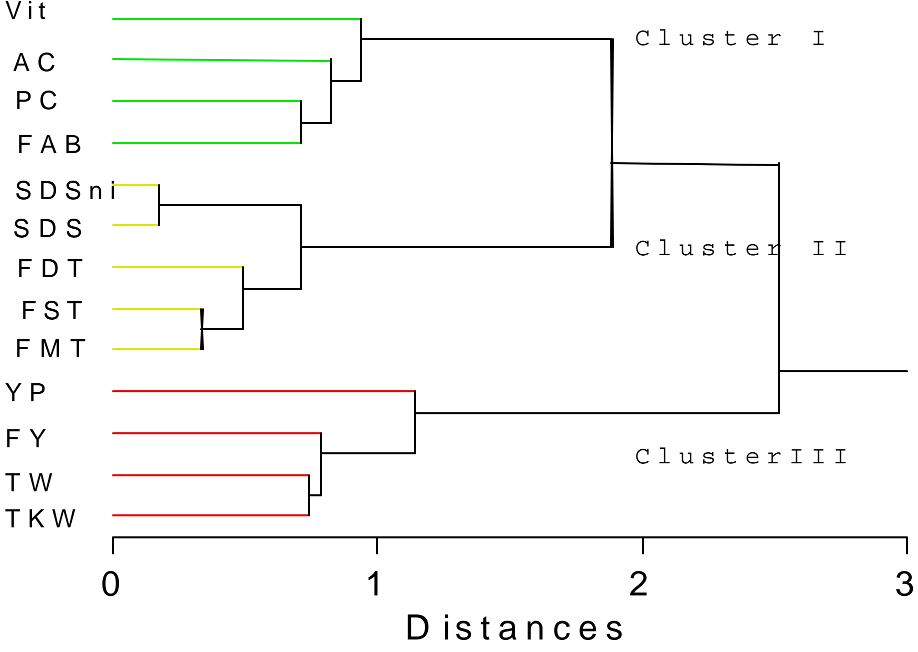
Table IV-11: Pearson correlation matrix between grain-quality traits in *Omrabi5/ T. dicoccoides600545// Omrabi5*, using the average data of 1996/97, 97/98, 98/99 and 99/2000. (*P<0.05 and **P<0.001).

Trait	PC	SDS	SDSni	FAB	FMT	FDT	FST	YP	Vit	TW	TKW	FY	AC
PC	1												
SDS	0.18	1											
SDSni	**0.44****	**0.96****	1										
FAB	**0.43****	0.21	0.32*	1									
FMT	-0.06	**0.72****	**0.63****	-0.07	1								
FDT	0.14	**0.75****	**0.72****	0.24	**0.68****	1							
FST	-0.05	**0.76****	**0.67****	0	**0.82****	**0.77****	1						
YP	-0.19	-0.1	-0.14	0.03	0.12	-0.08	-0.03	1					
Vit	**0.42****	0.23	**0.33****	0.03	0.08	0.06	0.12	-0.12	1				
TW	**-0.56****	-0.11	-0.25	-0.28*	-0.01	-0.28*	-0.11	0.01	-0.22	1			
TKW	**-0.56****	**-0.38****	**-0.50****	-0.19	-0.18	-0.24	-0.22	-0.04	**-0.41****	**0.38****	1		
FY	-0.14	-0.09	-0.13	**-0.43****	0.1	-0.13	-0.09	0.04	0.03	0.28*	0.3*	1	
AC	**0.34****	0.14	0.23	0.30*	0.11	0.15	0.17	0.19	0.13	-0.22	-0.3*	-0.2	1

The clustering analysis shows a strong differing 3 major sub-clusters (Fig IV-17). Sub-cluster I constituted by FAB, protein content, ash content and vitreousness. The second sub-cluster could be called gluten strength sub-cluster. In this sub-group, farinograph parameters (FDT, FMT and FST) and sedimentation test parameters (SDS and SDSni) are clustering together. As for the sub-cluster III, the flour yield, test weight and 1000-kernel weight were grouped with the yellow pigment. These results are fully in agreement with earlier works, and show clearly the relationship between the different

traits for the different qualities. Several traits are used as surrogate for parameters that are difficult to test. For instance, test weight and 1000-kernel weight for flour yield.

Fig IV-17: Clustering of durum grain-quality traits in *Omrabi5/ T. dicoccoides600545// Omrabi5* population



Chapter V
Results & Discussion

Multiple Quantitative Trait Loci Analysis (MQTL)

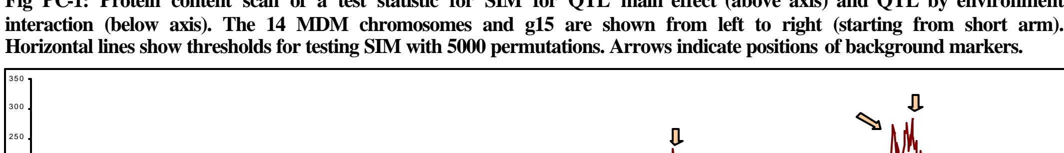
I- Protein Content (PC)

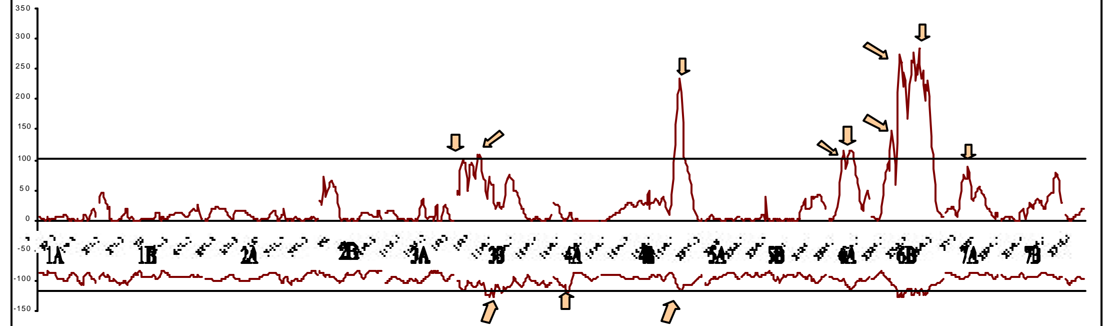
I-a- Estimation of QTL positions by simple interval mapping for PC

The test statistic of SIM analysis for protein content trait in *Omrabi5/ T. dicoccoides600545// Omrabi5* population showed many peaks across the genome, especially on the chromosomes: 3B, 4B, 6A, and 6B. The highest and widest peak was observed on chromosome 6B, it covers almost 100cM including the centromeric region and part of the short arm of 6B. Most of the detected peaks were showing both main and interaction effects (Fig. PC-1).

These significant peaks across several chromosomes showing all main and GxE effects suggest that the protein content is a quantitative trait with a complex genetic control and a high environmental influence. These results are in agreement with several studies on protein content and confirm that protein content is a quantitative trait governed by several genes distributed throughout the genome (Kuspira and Unran 1957, Diehl *et al.* 1978; Levy and Feldman 1989, Maali 1991). Other findings reported that this character is governed by a few major genes, but with the action of many other minor genes (Halloran 1975; Konzak 1977; Dhaliwal 1994; Levy *et al.* 1988). Furthermore, our findings are also in agreement with other research studies reporting that the environmental influences on grain protein content are more important than the genetic ones (Nachit *et al.* 1995a, Mariani *et al.* 1995; Trocoli *et al.* 2000). It seems that durum wheat grain under severe drought conditions, especially under excessively high temperatures and low relative humidity during grain filling, tends to be shriveled with low starch accumulation and therefore with a relatively high content of bran and nitrogen (Borghi *et al.* 1995, Blumenthal *et al.* 1993).

Fig PC-1: Protein content scan of a test statistic for SIM for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting from short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows indicate positions of background markers.





I-b- Estimation of QTL positions by simplified composite interval mapping for PC

For the simplified composite interval mapping analysis of protein content in our mapping population, 93 markers covering the whole genome were used as background markers. They were chosen as recommended by Tinker and Mather (1995), i.e.; the total background marker number to be lower than the population size (114 RILs in our case) and the distance between each two-background marker around 25 cM. Unfortunately, the resulting output spectrum was very inconsistent (Fig PC-2); it revealed too many peaks all over the 14 linkage-chromosomes. This inconsistency could be explained by the fact that in sCIM mapping, the adjustment for other possible QTL could create noise, generating spurious peaks, and raising significance thresholds (Tinker and Mather, 1995).

Therefore, the more evident peaks of test statistic for SIM were chosen as background markers (see arrows in Fig PC-1), the resulted scan for the sCIM gave smaller peaks for the main effect, whereas it gave much higher peaks for GxE interaction (Fig PC-3). sCIM analysis, in contrast to SIM analysis, adjusts the tested QTL considering the genotype information and possible effects of QTL in the genome. As a result, it has an ability to improve QTL precision and to detect QTL with smaller effects (Mather *et al.*, 1997). As mentioned before, chromosome 6B presented a large and wide peak. In order to assess the contribution of this wide peak, regression models including one, two or more markers were applied. Two markers were chosen: *Xgwm518* on the short arm of 6B and *XMcaaEacg560* close by the centromere region. The sCIM analysis was useful in determining the precise location of these QTLs. Furthermore, by performing QTL analyses across environments; we should have gained precision of estimation of the QTL position. The QTLs on 6B identified in this study are in agreement with earlier works on protein content-QTLs. Joppa and Cantrell (1990) using Langdom durum-*dicoccoides* chromosome substitution lines, reported a QTL on chromosome 6B. Later, Steiger *et al.* (1996) had confirmed the 6B-QTL by crossing these substitutions lines with a durum variety. Moreover, a QTL on 6BS was also reported in durum wheat (Blanco *et al.* 1996). Indeed, a major QTL explaining 66% of total protein content variation was reported on 6BS near the centromere, flanked by Xmwg79 and Xabg387

(Joppa *et al.* 1997). This QTL was confirmed by Chee *et al.* (1998), who also suggested that the protein content trait is controlled by a single genetic factor. In *Omrabi5/ T. dicoccoides600545// Omrabi5* population mapping, RFLP probes were not used as genetic markers, hence it is difficult to compare the QTL close by the centromere of 6BS to previous reported QTLs on the same genomic region. Yet, more research should be conducted in this important chromosomal region for protein content.

Protein content was also found to be linked to chromosome 3B. On 3BS, the SIM analysis detected a main effect on *XMcttEaag140* and *Xgwm154d*, while by sCIM scan, the same markers showed an interaction effect instead of a main effect. Similar differences in the QTLs-effect detected by SIM and sCIM were reported by Mather *et al.* (1997) in barley. These QTLs on 3BS are in agreement with the QTL reported on 3B in Langdom durum-*dicoccoides* chromosome substitution lines (Joppa and Cantrell 1990), whereas in bread wheat, a protein content QTL was reported on the homoeologous chromosome 3D (Kuspira and Unran 1957).
Further, on the long arm of chromosome 4B, another QTL (*Xgwm107*) was detected. As for the QTLs on 3B, the QTL on 4BL showed a very significant main effect with SIM analysis, whereas with sCIM it did show only an interaction effect. Similarly, a QTL on 4B was reported in Langdom durum-*dicoccoides* chromosome substitution lines (Joppa and Cantrell 1990); and on 4D in bread wheat (Kuspira and Unran 1957).

As for chromosome 6A, β-gli69 (*Gli-A2*) showed a main and QTLxE effect on protein content. This result is also in agreement with previously reported QTL on 6A (Joppa and Cantrell 1990; Blanco *et al.* 1996). Actually, this β-gliadin is the only seed storage protein showing relationship with protein content. It is well known that other factors than seed storage proteins are the cause of high protein content. Impglia *et al.* (1996) reported that protein content was independent of LMW glutenin subunits in a durum core collection, whereas, Elouafi *et al.* (1998) reported a positive association between a γ-gliadin subunit belonging to *T. dicoccoides* and the protein content in a RIL population, developed from a backcross between a *Korifla/ T. dicoccoides600808// Korifla*.

In addition to the genomic regions found associated with protein content in this studying, several other genomic localizations were reported in the literature, including

chromosomes 1A and 1B (Levy and Feldman 1989; Stein *et al.* 1992); chromosomes 2A and 3A (Joppa and Cantrell 1990); short arm of chromosome 4A (Blanco *et al.* 1996); chromosome 5A (Kuspira and Unran 1957; Morris *et al.* 1973; Levy and Feldman 1989; Blanco *et al.* 1996); chromosome 5B (Kuspira and Unran 1957; Levy and Feldman 1989; Stein *et al.* 1992; Steiger *et al.* 1996); chromosome 5D (Morris *et al*. 1973; Law *et al.* 1978); and chromosomes 7A and 7B (Kuspira and Unran 1957; Levy and Feldman 1989; Joppa and Cantrell 1990; Stein *et al.* 1992; Blanco *et al.* 1996). Recently, a new chromosomal region was added to this long list. Prasad *et al.* (1999), using bulk segregant analysis, reported that the microsatellite WMC41 on the 2DL was linked to protein content.

Fig PC-2: Protein content scan of a test statistic for SIM (solid red lines) and sCIM using 93 background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations.

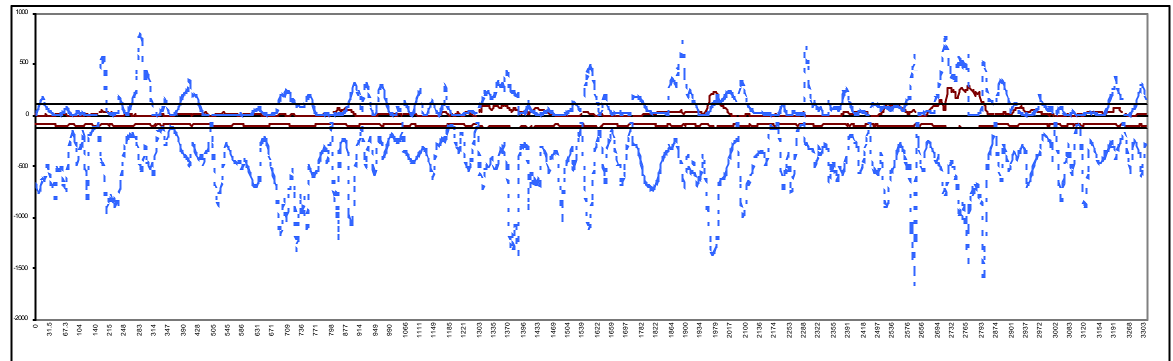
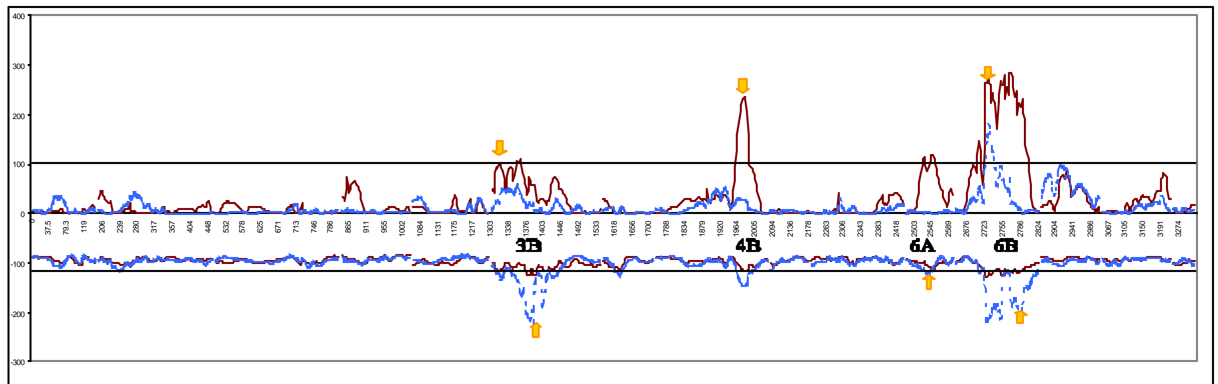


Fig PC-3: Protein content scan of a test statistic for SIM (solid red lines) and sCIM using few background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs.



I-c- Estimation of the additive effects of the putative QTLs for PC

The six detected QTLs explained 27% of the total protein content variation. As it was expected, all the QTLs showed that *T. dicoccoides600545* alleles had a significant positive effect on protein content (Fig PC-4). In this figure the strong effect of the *T. dicoccoides600545* alleles and of the environments are shown to be of large magnitude. This positive effect did reach +2.2% in early planting in 1998. Theses results corroborate earlier suggestion of utilization of *T. dicoccoides* as source for high protein content genes (Damania *et al.* 1988, Feldman and Sears 1981, Nachit *et al.* 1990; Maali 1991; Nachit and Maali 1997). *Xgwm518* and *XMcaaEacg560* on 6BS explained 14% each, while *Xgwm107* on 4BL 12%; and the two other markers *XMcttEaag140* and *Xgwm154d* on 3BS explained 6 and 5%, respectively (Table PC-1). As for the *Gli-A2* locus on 6AS, it has a low explanation rate (5%). All the detected QTLs showed QTLxE interaction effects. These QTLxE interactions on 3B, 4BL and 6BS were clearly due to changes in magnitude across environments. On the other hand, the QTLxE interaction effect of *Gli-A2* gene on 6AS was a crossover interaction; it showed that *Omrabi5* allele has a positive effect on RIL-protein content in some environments (specially in 99LP), whereas in other environments the *T. dicoccoides600545* allele was more favourable, e.g.; 00Rf and 00Ir. Protein content is an important trait; it holds also a nutritional aspect, which is very important, particularly in the areas where the consumption of wheat products is high, as it is the case of the southern and eastern Mediterranean countries. Therefore, understanding these fluctuations would help in improving protein content in durum, in order to improve the nutrition in the areas where high amount of durum grain is consumed.

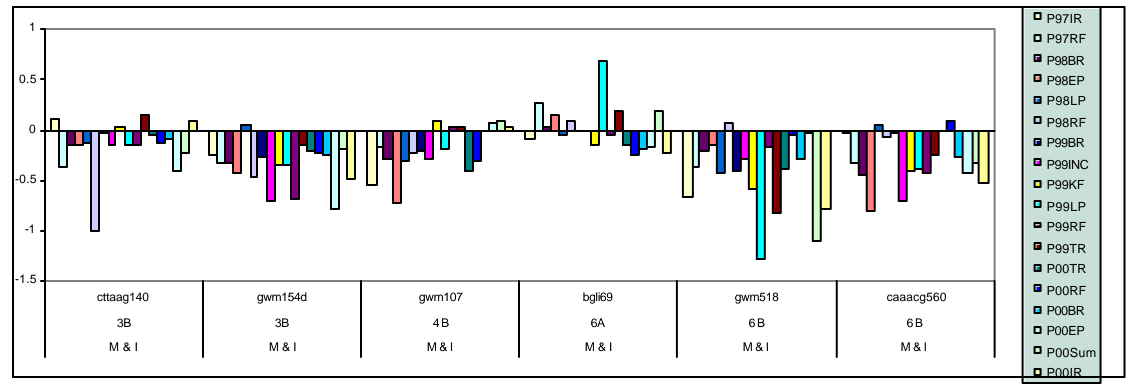
The QTLs effect was especially more exhibited in stressed environments than in non-stressed ones. This has been evident with *XMcttEaag140* who in 98Rf; with *Xgwm154d* in 00EP; with *Gli-A2* in 99LP; with *Xgwm518* in 99LP; and with *XMcaaEacg560* in 98EP. These environments (Tel-Hadya rainfed, early planting, and late planting) are subjected usually to drought, cold, and terminal stresses (drought and heat), respectively. This is confirming that under severe drought conditions a relatively high content of protein is obtained (Johansson and Lundborg 1994; Nachit *et al.* 1995a).

Table PC-1: Protein Content QTLs and their contributions

Chromosomal localization	QTL marker	cM	V_g/V_{ph}	$V_g + V_{QTLxE} / V_{ph}$
3BS	*XMcttEaag140*	0	5	6
3BL	*Xgwm154d*	5	4	5
4BL	*Xgwm107*	0	11	12
6AS	*Gli-A2*	0	4	5
6BS	*Xgwm518*	0	12	14
6BS	*XMcaaEacg560*	0	12	14
Total explanation (R^2)			**20%**	**27%**

V_g = genetic variance; V_{QTLxE} = QTLxE variance; V_{ph} = phenotypic variance.

Fig PC-4: Relative magnitude, chromosomal location and effect-nature of estimated protein content QTLs in each environment.

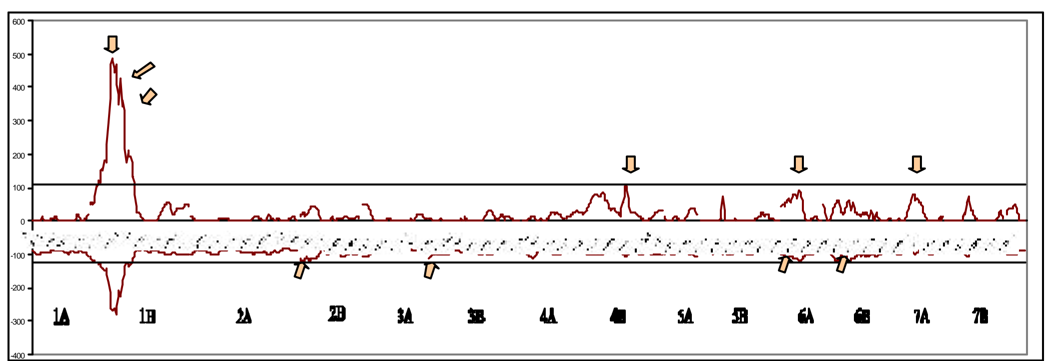


E- Sedimentation test

II-a- Estimation of QTL positions by simple interval mapping for SDS

In the simple interval mapping analysis for gluten strength, the test statistic showed a large and wide peak in the chromosome 1B. This peak corresponded to both main and interaction effects. From the scan alone, it is not clear whether there was one, two or more QTL affecting the sedimentation test around this region. The highest peak is obvious and it is 20cM apart from *Xgwm131b*, but it is not clear if adding more QTLs corresponding to the other peaks around *Xgwm131b* will improve further the total explanation (Fig SDS-1). On chromosome 2B, the AFLP marker *XMcttEagg172* showed a low significance interaction effect.

Fig SDS-1: SDS scan of a test statistic for SIM for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting from short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows indicate positions of background markers.



II-b- Estimation of QTL positions by simplified composite interval mapping for SDS

Similarly to protein content, the use of the 93 background markers covering the whole genome resulted in a noisy scan with numerous peaks (Fig SDS-2). It did show too many peaks all over the genome, sometimes even higher than the large and wide peak on chromosome 1B observed in SIM scan. While the more evident peaks of test statistic for SIM were chosen as background markers (arrows in Fig SDS-1), the resulted scan for the simplified composite interval mapping confirmed the wide and large peak in the chromosome 1B with both main and interaction effects (Fig. SDS-3).

As mentioned in SIM analysis, it was not sure that adding more QTLs corresponding to the other peaks around *Xgwm131b* would explain further additional variation. Therefore, regression models including one, two or three of the highest peaks were applied and consequently, the *Glu-B1* gene was added. In this way, the QTL is surrounded by both chosen QTLs: *Xgwm131b* and *Glu-B1*. These two QTLs on the long arm of chromosome 1B corroborate earlier suggestions of HMW glutenin effect on gluten strength (Autran and Feillet, 1987, Rogers *et al.* 1989, Ciaffi *et al.* 1991, Ciaffi *et al.* 1995). Even though, most of these studies noticed that in durum wheat the association between HMW-gs and gluten strength is not as strong as the correlation observed for breadmaking quality in bread wheat (Payne *et al.* 1984). Moreover, more studies showed rather LMW-gs being more related to gluten strength than HMW-gs (Damidaux *et al.*, 1978; Payne *et al.* 1984; Pogna *et al.* 1990; Nachit *et al.* 1995b; Liu and Shepherd 1996; Impiglia *et al.* 1996; Elouafi *et al.* 1998). One of the two scored

LMW-gs in *Omrabi5/ T. dicoccoides600545// Omrabi5* population was mapped on 1AS but did not give any evidence of effect on gluten strength. In addition, no QTL affecting SDS was detected on the genomic region of *Glu-B3* (1BS), though the 1B short arm needs further saturation. Unfortunately all the developed microsatellites on this arm, except Gwm33, were not polymorphic in the present mapping population. In JKxCham1 population a major QTL was detected at 0 cM of *Glu-B3* contributing up to 77% of the total variability found, i.e.; it explained 27% of the total SDS variability (Elouafi *et al.* 2000). In contrast to *Omrabi5/ T. dicoccoides600545// Omrabi5* population, the *Glu-B1* gene in JKxCham1 population did not show any effect on the gluten strength. This lack of effect has been explained by the fact that HMW7+8 and HMW20 (for Jennah Khetifa and Cham1, respectively) have similar effect on gluten strength (Elouafi *et al.* 2000).

The sCIM analysis did also confirm the interaction peak on *XMcttEagg172* on 2BS and revealed a new QTL on 4BL at 5cM of *Xgwm375* (Fig SDS-3). *Xgwm375*-QTL showed a main effect, whereas, in JKxCham1 population a QTLxE interaction-QTL have been identified on 4BS. This former QTL have shown a positive interaction with high nitrogen fertilization and highland conditions (Elouafi *et al.*, 2000). On the basis of the sCIM scan, another new interaction effect QTL was detected on 6BS (Fig SDS-3). It is localized at 0 cM of *Xgwm518*, the microsatellite *Xgwm518* was mapped at 12.5cM of *Gli-B2* in *Messapia* x *MG4343* population (Korzun *et al.*, 1999). Accordingly the QTL identified in *Omrabi5/ T. dicoccoides600545// Omrabi5* population may be the same as the QTL reported by Elouafi *et al.* (2000) in JKxCham1 population next to *XPstIaag&MseIcga9* marker (at 8.4cM from *Gli-B2*).

Fig SDS-2: SDS scan of a test statistic for SIM (solid red lines) and sCIM using 93 background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations.

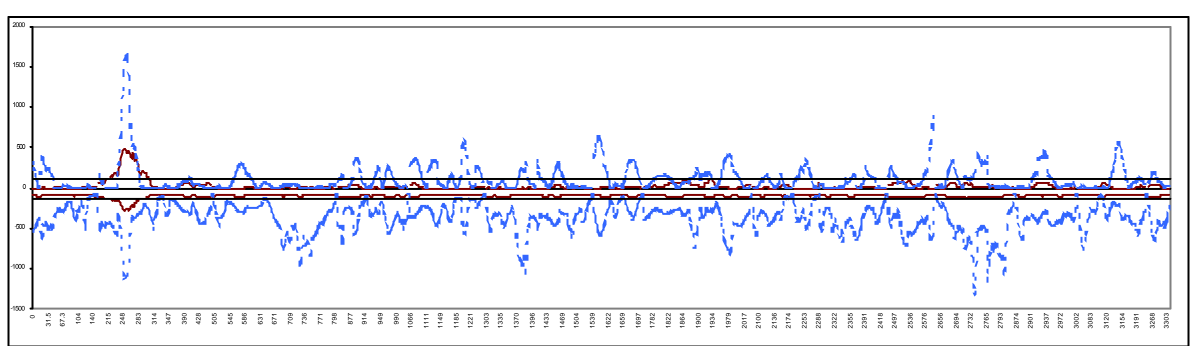
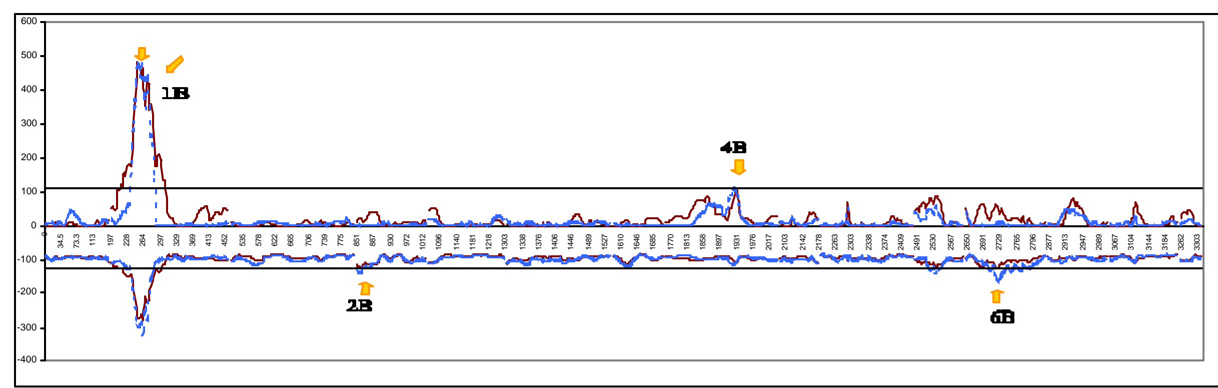


Fig SDS-3: SDS scan of a test statistic for SIM (solid red lines) and sCIM using few background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs.



II-c- Estimation of the additive effects of the putative QTLs for SDS

In *Omrabi5/ T. dicoccoides600545// Omrabi5* population, the five chosen QTLs explained 41% of the total gluten strength variation. The major peak QTLs *Xgwm131b* and *Glu-B1* explained 30 and 28%, respectively (Table SDS-1), while together the total explanation is not significantly increased (32%). These two major QTLs on 1BL showed a clear positive effect of *T. dicoccoides600545* alleles on gluten strength (Fig SDS-4). This effect was continuously positive across environments but with varying magnitudes. The highest *T. dicoccoides600545* alleles positive effect (+9.74ml) was particularly noticed in Tel-Hadya Summer Planting 99/00 (Fig SDS-4).

Omrabi5 at *Glu-B1* locus hold the HMW-20 whereas *T. dicoccoides600545* hold the HMW-6+8. These findings show that the HMW-6+8 gave higher gluten strength than HMW-20. Our findings are in agreement with earlier reported *Glu-B1* alleles order in durum wheat (Autran and Feillet 1987; Liu and Shepherd 1996), but in disagreement with Boggini and Pogna (1989) who reported that the *Glu-B1* is correlated with dough strength in the following order: 7+8 >> 20 > 6+8. Although, in MDM mapping population the HMW-20 did show a positive effect on sedimentation test in two environments: Tel-Hadya 98EP and 00Ir. The interpretation of the positive expression of HMW-20 under these special environments is beyond this study.

Therefore, other that improving durum wheat gluten strength by the introgression of new *Glu-A1* alleles, from wild relatives, or by the increase of the number of *Glu-A1* HMW glutenin subunits as suggested by several research (Rogers *et*

al. 1989; Ciaffi *et al.* 1991; Ciaffi *et al.* 1995), the gluten strength improvement could be achieved also by the introgression of some *Glu-B1* alleles.

The QTL on the short arm of 2B explained just 3% of the total gluten strength variation. This QTL showed a clear crossover interaction effect. Its effect was positive in some environments and negative in others, nevertheless its general trend gave the impression of a positive *Omrabi5* allele effect on sedimentation test especially in high input environment such as: 99Kf and 99Tr.

The QTL *Xgwm375* on 4BL chosen for its main effect explained 6%, whereas the 5th chosen QTL (*Xgwm518*) for its GxE interaction on 6BS explained 5% of the total variation. These determined QTLs on 4BL and 6BS showed also that *T. dicoccoides600545* alleles had significant positive effect (Fig SDS-4), suggesting that emmer wheat alleles enhance the gluten strength through other QTL/genes than seed storage protein genes. The interaction-QTL *Xgwm518* showed rather a magnitude changes across environments ranging from +5.5ml in 00Ir to +0.3ml in 98Br.

In general, most of the selected QTLs present an obvious interaction effect, suggesting that the gluten strength criterion is widely affected by environmental conditions. They showed also that most of the positive effect on gluten strength is due to *T. dicoccoides600545* alleles. However, a high to moderate trangressive inheritance was noticed in the progeny across the 18 environments, suggesting rather a new alleles-combination.

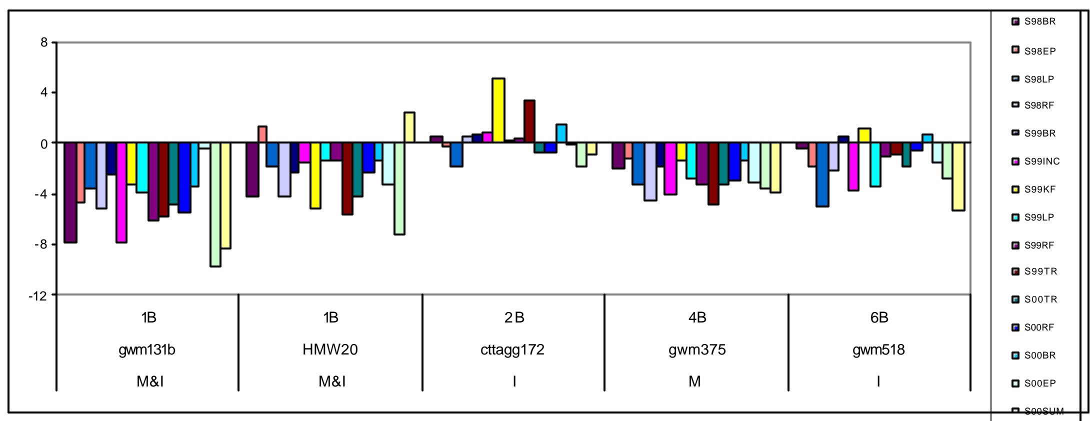
As the sedimentation test assessment during the 1997 trail was conducted using NIRS while during 98, 99 and 2000 using chemical test, the environments: 97Ir and 97Rf have been dropped. The elimination of these two environments has improved the total explanation from 35% to 41% .

Table SDS -1: Sedimentation Test QTLs and their contributions

Chromosomal localization	QTL marker	cM	V_g/V_{ph}	$V_g + V_{QTLxE} / V_{ph}$
1BL	*Xgwm131b*	20	25	30
1BL	*Glu-B1*	5	23	28
2BS	*XMcttEagg172*	0	1	3
4BL	*Xgwm375*	5	5	6
6BS	*Xgwm518*	0	3	5
Total explication			**31%**	**41%**

V_g = genetic variance; V_{QTLxE} = QTLxE variance; V_{ph} = phenotypic variance.

Fig SDS-4: Relative magnitude, chromosomal location and effect-nature of estimated SDS QTLs in each environment.



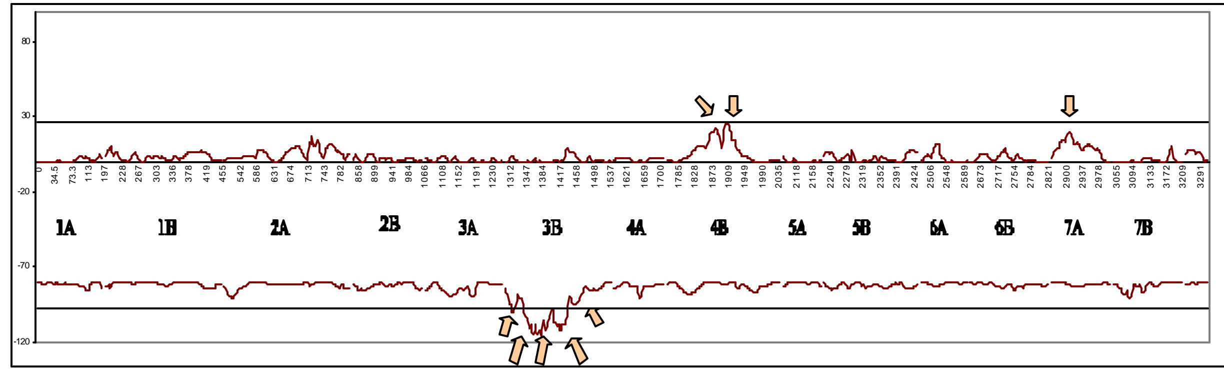
F- Farinograph

III-1- Farinograph Absorbance (FAB)

1-a- Estimation of QTL positions by simple interval mapping for FAB

For farinograph absorbance, the test statistic scan for SIM analysis exceeded slightly the significance threshold of the main effect only in one region in chromosome 4B, and it exceeded 3 times the QTLxE threshold across the chromosome 3B. The detected peak on 3B covered most of the chromosome (Fig FAB-1).

Fig FAB-1: FAB scans of a test statistic for SIM for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting from short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows indicate positions of background markers.



1-b- Estimation of QTL positions by simplified composite interval mapping for FAB

Similarly to sedimentation test and protein content, the farinograph absorbance sCIM using 93 background markers covering the whole genome has resulted in large

peaks (Fig. FAB-2). This scan is confirming that, at least for our mapping population, the background markers should be more defined and localized rather than spread all over the genome. Therefore, it has been decided that the background markers should be chosen according to SIM results as it was suggested in CIM program (Yiang pers.com.) and confirmed by Elouafi *et al.* (2000) on JKxCham1 population.

Thus, few background markers corresponding to the few peaks observed in SIM scan were chosen (arrows in Fig FAB-1). In general and contrary to protein content, the simplified composite interval mapping analysis for FAB gave a higher main effect scan and relatively lower interaction scan (Fig FAB-2). Furthermore, it confirmed the main effect peak on the long arm of chromosome 4B (*Xgwm165a*). For the multiple peaks showed by SIM on chromosome 3B, the sCIM analysis determined the peak more accurately. For more accurate estimation of the QTL position, a regression models were used combining the different possible markers detected either by SIM or/and sCIM. Consequently, *Xgwm144* marker on the short arm of 3B emerged as the best indicator, and the other markers did not show any further explanation. As the sCIM analysis did not show any new QTL for this trait, then the SIM might have been sufficient. However, sCIM was useful in improving the accuracy of estimation of QTL position on 3BS.

Fig FAB-2: FAB scans of a test statistic for SIM (solid red lines) and sCIM using 93 background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations.

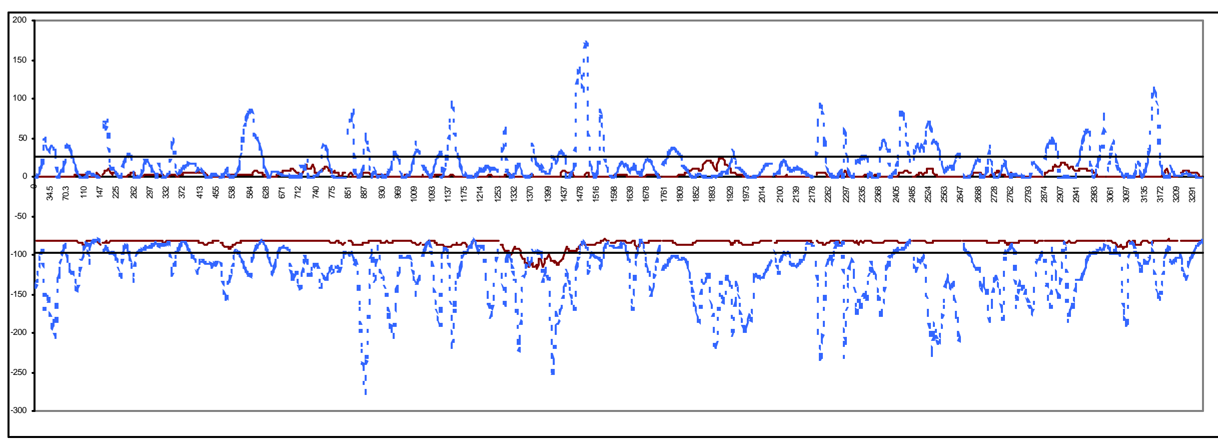
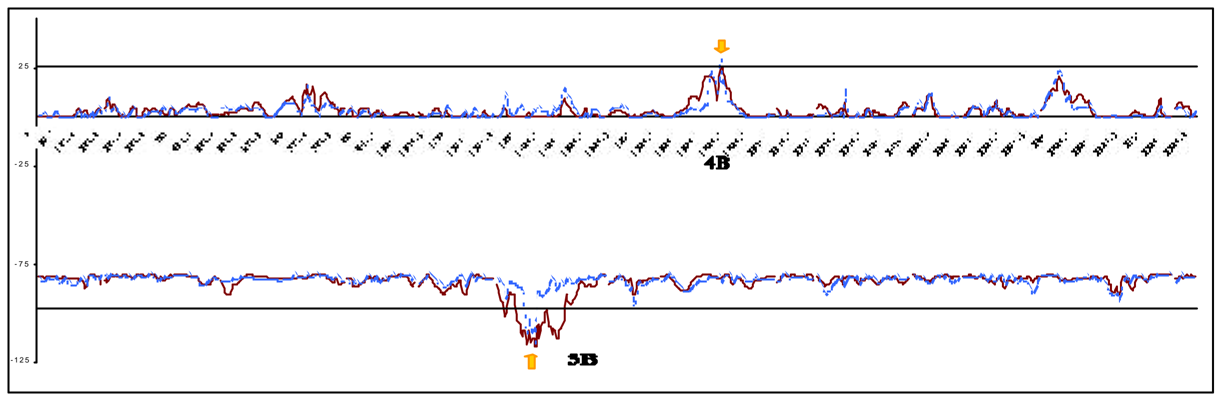


Fig FAB-3: FAB scans of a test statistic for SIM (solid red lines) and sCIM using few background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs



1-c- Estimation of the additive effects of the putative QTLs for FAB

The two detected QTLs, *Xgwm144* and *Xgwm165a*, explained 14% of the total absorbance variation, each one of them contributing with a small effect (Table FAB-1). They explained 8 and 6%, respectively. The QTL on 3BS showed a full interaction variation (8%) while it did not show any genetic variation (0%). Negative effect on FAB was shown in Tel-Hadya Rainfed environment (98Rf) by *T. dicoccoides600545 Xgwm144*-allele; but in 99Inc, 99Rf, and 00RF *Omrabi5 Xgwm144*-allele affected negatively the FAB. The environment 98Rf was relatively wet during the grain-filling period compared with 99Inc, 99Rf, and 00Rf. The environmental conditions of 98Rf were relatively wet during the grain-filling period, whereas those of 99Inc, 99Rf, and 00Rf were relatively moisture stressed during the same developmental stage (grain filling). Consequently, it appears that the environmental conditions during the grain-filling period, which affect grain protein quality and quantity and other components, have also influence on flour water absorption. However, the physiological and chemical mechanisms are unknown and their study goes beyond the aim of this subject.

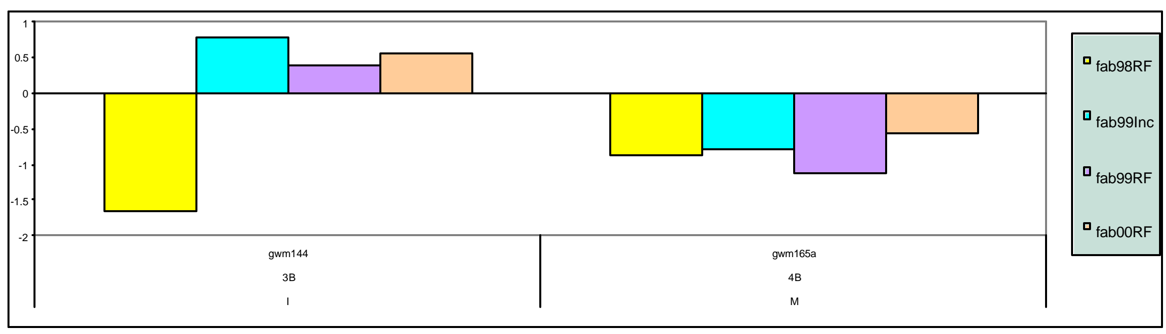
The *Xgwm165a*-QTL on 4BL presents only genetic variation. This main effect showed that the wild parent *T. dicoccoides600545* alleles have an obvious negative effect on farinograph absorbance trait. The general effect of both QTLs is a mixture of main and interaction effects (Fig FAB-4).

Table FAB -1: Farinograph absorbance QTLs and their contributions

Chromosomal localization	QTL marker	cM	V_g/V_{ph}	$V_g + V_{QTLxE} / V_{ph}$
3BS	*Xgwm144*	0	0	8
4BL	*Xgwm165a*	0	5	6
Total explication			**5%**	**14%**

V_g = genetic variance; V_{QTLxE} = QTLxE variance; V_{ph} = phenotypic variance.

Fig FAB-4: Relative magnitude, chromosomal location and effect-nature of estimated FAB QTLs in each environment.

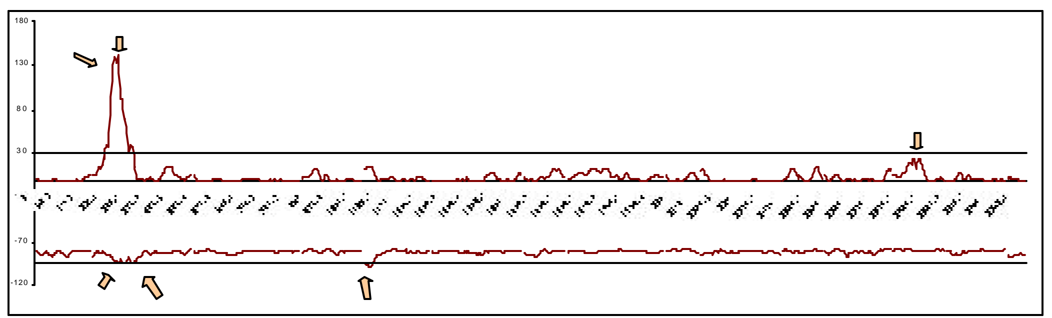


III-2- Farinograph Development Time (FDT)

2-a- Estimation of QTL positions by simple interval mapping for FDT

In the simple interval mapping analysis of farinograph development time, the test statistic showed a large and wide peak in the chromosome 1B at the same position as in sedimentation test (Fig FDT-1). This peak presents mainly main effect with a very low interaction effect. From the scan analysis alone, it is not clear whether there was one or more QTL in this genomic region. So, few peaks were selected as background markers for the sCIM analysis. In addition, the SIM scan analysis showed also a small interaction effect peak on the telomere of 3AS.

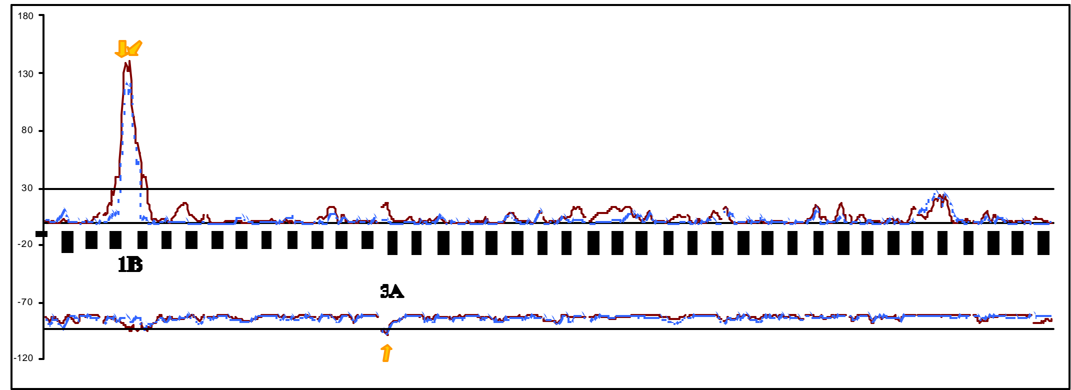
Fig FDT-1: FDT scan of a test statistic for SIM for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting from short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows i ndicate positions of background markers.



2-b- Estimation of QTL positions by simplified composite interval mapping for FDT

Following the same strategy developed for protein content, sedimentation test, and farinograph absobance the significant peaks of test statistic for SIM were chosen as background markers (see arrows in Fig FDT-1). In general, the simplified composite interval mapping was not different from the SIM scan. It confirmed the main effect of the wide peak in the chromosome 1B (Fig FDT-2). Using regression models, instead of having just one marker far from the detected QTL, two markers: *Xgwm131b* and the gene *Glu-B1* were chosen to flank this QTL of interest. sCIM scan confirmed also the interaction effect of *Xgwm369* on the telomeric region of 3AS. As for farinograph absorbance, the SIM for FDT might have been sufficient, since sCIM did neither detect new QTL nor improve QTL-SIM positions. This may be due to the chosen background number, which could be insufficient for new-QTLs detection.

Fig FDT-2: FDT scan of a test statistic for SIM (solid red lines) and sCIM using few background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs.



2-c- Estimation of the additive effects of the putative QTLs for FDT

The three determined QTLs explained 33% of the total development time variation. The major QTLs were *Xgwm131b* and *Glu-B1*, which accounted for 28 and 27% of the total variability; as for the other interaction QTL (*Xgwm369*), it explained 7% (Table FDT-1).

The two main effect QTLs, *Xgwm131b* and *Glu-B1*, showed that the *T. dicoccoides600545* alleles had a positive effect on development time (Fig FDT-3),

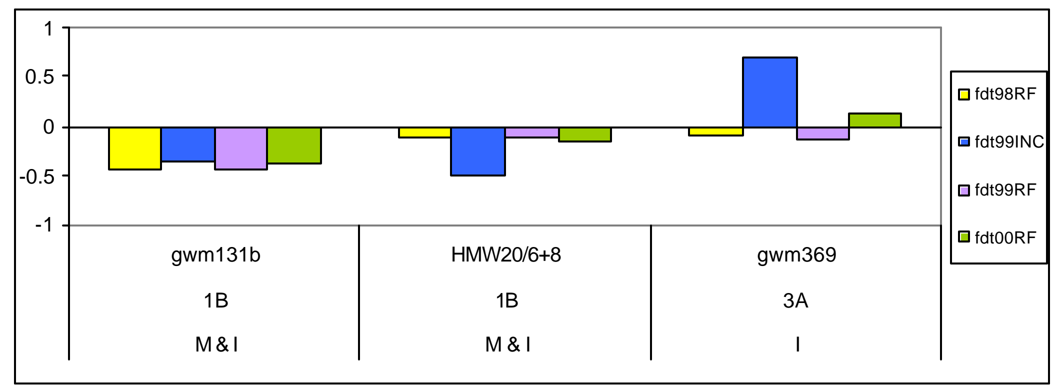
indicating that *T. dicoccoides600545* alleles do not only enhance the gluten strength, but also the development time trait. The QTL *Xgwm369* was positive in some environments and negative in others.

Table FDT-1: Farinograph development time QTLs and their contributions

Chromosomal localization	QTL marker	cM	V_g/V_{ph}	$V_g + V_{QTLxE} / V_{ph}$
1BL	***Xgwm131b***	20	26	28
1BL	***Glu-B1***	5	24	27
3AS	***Xgwm369***	20	3	7
Total explication			**28%**	**33%**

V_g = genetic variance; V_{QTLxE} = QTLxE variance; V_{ph} = phenotypic variance.

Fig FDT-3: Relative magnitude, chromosomal location and effect-nature of estimated FDT QTLs in each environment.

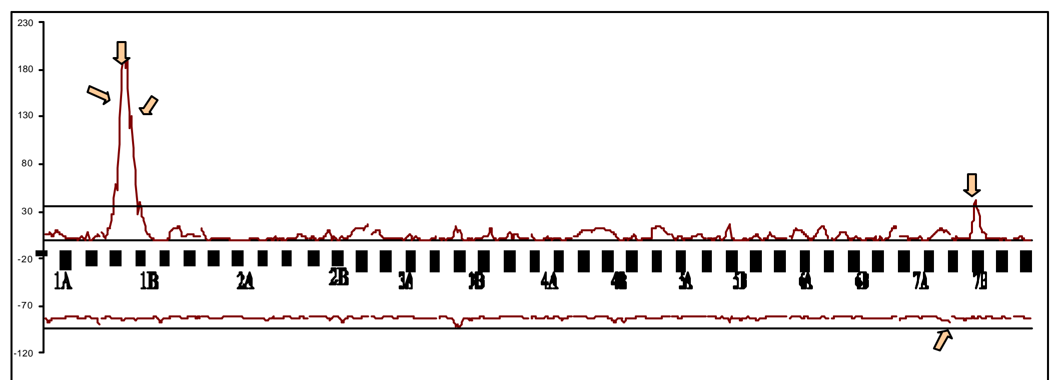


III-3- Farinograph Mixing Time (FMT)

3-a- Estimation of QTL positions by simple interval mapping for FMT

The scan of the simple interval mapping analysis of mixing time is very similar to the scan of development time on chromosomal group 1, showing a large and wide peak around the *Glu-B1* gene (Fig FMT-1). Nevertheless, for development time, the SIM analysis showed also a small GxE interaction peak on the short arm of 3B and a small main effect peak on chromosome 7B.

Fig FMT-1: FMT scan of a test statistic for SIM for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting from short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows indicate positions of background markers.

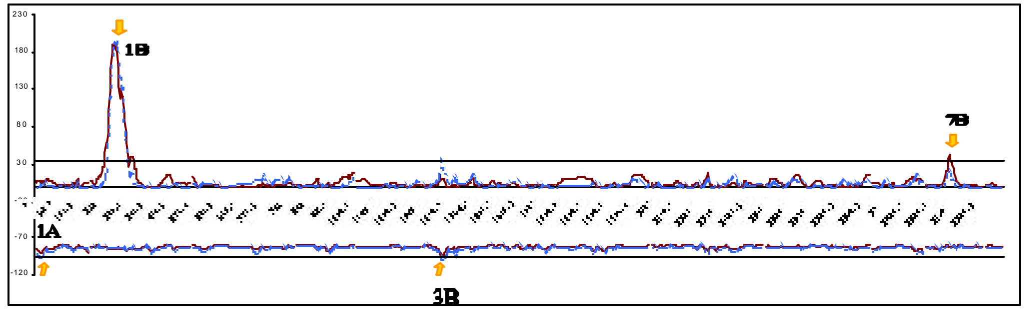


3-b- Estimation of QTL positions by simplified composite interval mapping for FMT

The simplified composite interval mapping analysis for mixing time, based on few background markers, shows the same large peak on chromosome 1B around *Glu-B1* (Fig FMT-2). *Xgwm131b* was chosen as it shows the highest explanation. sCIM did also confirm the suggested QTL on 3BS (*XMctcEaag115*); it showed also that this QTL has a small main effect in addition to its interaction effect. On chromosome 7B, the main effect QTL (*XMctgEagg181*) suggested by SIM was dismissed by sCIM analysis. Such kind of behavior was explained by the fact that sCIM has eliminated sampling variance that contributed to type-I error for SIM. This dismissing could also indicate that the SIM scan was influenced by 2 separate QTLs that were not strong enough to manifest themselves as 2 separate peaks in the sCIM scan (Tinker *et al.*, 1996).

New interaction-QTL was suggested by the sCIM on chromosome 1AS corresponding to gliadin/ LMW genes (*Gli-A1*/ *Glu-A3*). Tinker *et al.* (1996) advanced that sCIM show new QTLs because it has the ability to detect QTL with small effects as it accounts for the genetic component of the background variance. Indeed, sCIM could show regions where there is evidence for multiple linked QTLs.

Fig FMT-2: FMT scan of a test statistic for SIM (solid red lines) and sCIM using few background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs



3-c- Estimation of the additive effects of the putative QTLs for FMT

The major detected QTL is *Xgwm131b*; this microsatellite explained 35% out of the total mixing time variation (Table FMT-1). The other detected QTLs have minor contributions such as: 3% for *Gli-A1/Glu-A3* genes; 6% for *XMctcEaag115*, and 9% for *XMctgEagg181*.

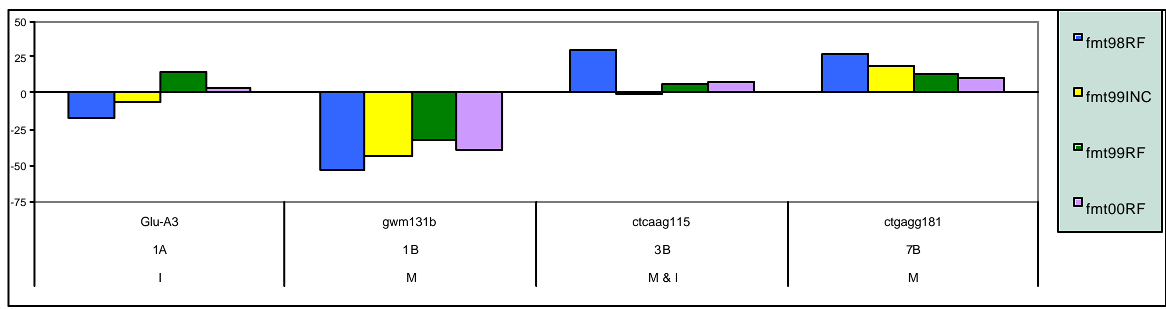
T. dicoccoides600545 alleles had a significantly positive effect on mixing time, which is related to strong dough strength, in the RILs especially through *Xgwm131b* (Fig FMT-3). This effect is consistent across the 4 studied environments whereas, other *Omrabi5* alleles (*XMctcEaag115* and *XMctgEagg181*) affected also positively the mixing time, but their effect remain smaler as *XMctgEagg181* explained only 9% of the variation, whereas *Xgwm131b* explained 35%. The 4th identified QTL showed rather environmental variation through *Gli-A1/Glu-A3* genes on 1AS. Therefore, both parents contribute alleles with favourable effects, resulting in larger amounts of transgressive inheritance.

Table FMT-1: Farinograph Mixing Time QTLs and their contributions

Chromosomal localization	QTL marker	cM	V_g/V_{ph}	$V_g + V_{QTLxE} / V_{ph}$
1AS	*Glu-A3*	0	1	3
1BL	*Xgwm131b*	0	35	35
3BS	*XMctcEaag115*	0	3	6
7B	*XMctgEagg181*	5	9	9
Total explanation			**39%**	**45%**

V_g = genetic variance; V_{QTLxE} = QTLxE variance; V_{ph} = phenotypic variance.

Fig FMT-3: Relative magnitude, chromosomal location and effect-nature of estimated FMT QTLs in each environment.

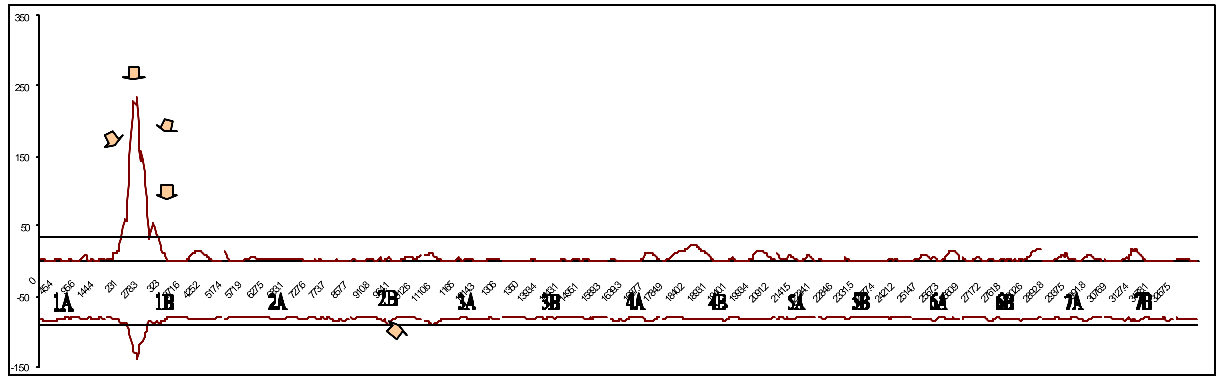


III- 4- Farinograph Stability Time (FST)

4-a- Estimation of QTL positions by simple interval mapping for FST

The SIM scan for stability time is very explicit and straightforward. Its peaks did exceed the threshold in two chromosomal regions (Fig FST-1). The first one is a large peak, similar to the ones of mixing time and development time on chromosome 1B around the *Glu-B1* gene; and the second is a small peak on chromosome 2B showing a QTLxE interaction effect.

Fig FST-1: FST scan of a test statistic for SIM for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting from short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows indicate positions of background markers.

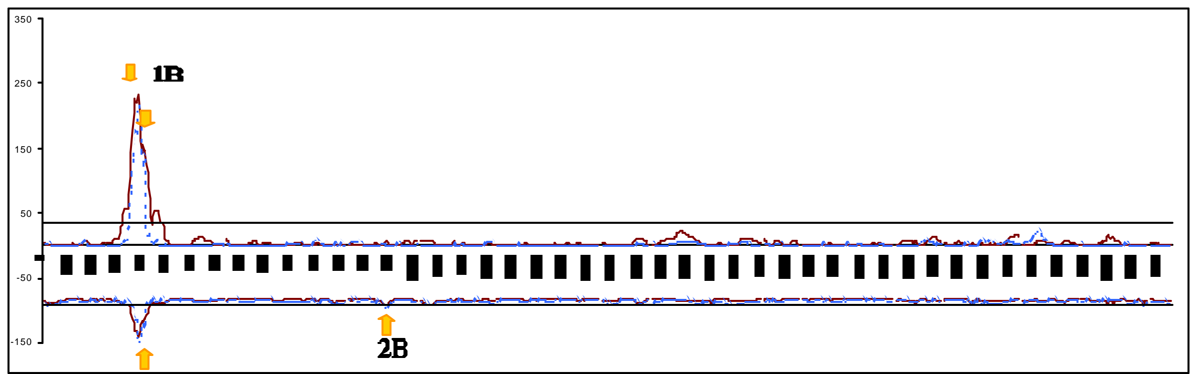


4-b- Estimation of QTL positions by simplified composite interval mapping for FST

Using the more evident peaks of test statistic for SIM as background markers (see arrows in Fig FST-1), the resulted scan analysis for the simplified composite interval mapping gave almost the same scan as the SIM (Fig FST-2). Therefore, it confirmed the

two observed peaks. For chromosome 1B, regression models were used and *Xgwm131b*, *Glu-B1* gene, and *XMcaaEacg108* were chosen as QTLs expressing both main and interaction effects. *Xgwm374a* was the closest marker to the GxE QTL identified on chromosome 2BS.

Fig FST-2: FST scan of a test statistic for SIM (solid red lines) and sCIM using few background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs



4-c- Estimation of the additive effects of the putative QTLs for FST

The three QTLs on 1B, *Xgwm131b*, *Glu-B1*, and *XMcaaEacg108*, are expressing main and interaction effects. The *Xgwm131b* microsatellite explained 45% of the total variation, 39% being genetic and 6% QTLxE (Table FST-1). The two other markers explained 44 and 32%, respectively. The 3rd minor QTL on the centromeric region of 2BS, *Xgwm374*, showed only an interaction effect and explained 4% of the total stability time variation.

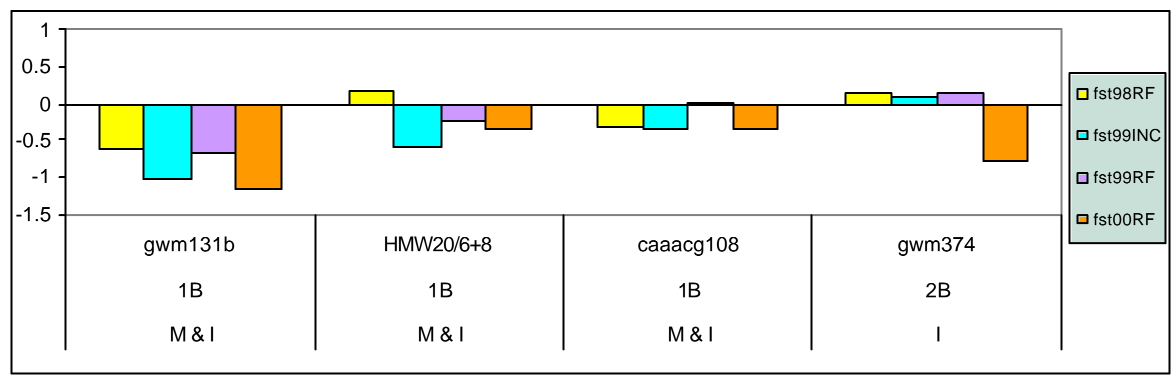
As for development and mixing time, the *T. dicoccoides600545* alleles had a positive effect on stability time (Fig FST-3), especially the QTLs identified on 1B. This positive effect of *dicoccoides* alleles reached up to +1.2min in 00RF environment.

Table FST-1: Farinograph stability time QTLs and their contributions

Chromosomal localization	QTL marker	cM	V_g/V_{ph}	$V_g + V_{QTLxE} / V_{ph}$
1BL	*Xgwm131b*	20	39	45
1BL	*Glu-B1*	5	37	44
1BL	*XMcaaEacg108*	0	27	32
2BS	*Xgwm374*	0	1	4
Total explanation			**42%**	**51%**

V_g = genetic variance; V_{QTLxE} = QTLxE variance; V_{ph} = phenotypic variance.

Fig FST-3: Relative magnitude, chromosomal location and effect-nature of estimated FST QTLs in each environment.

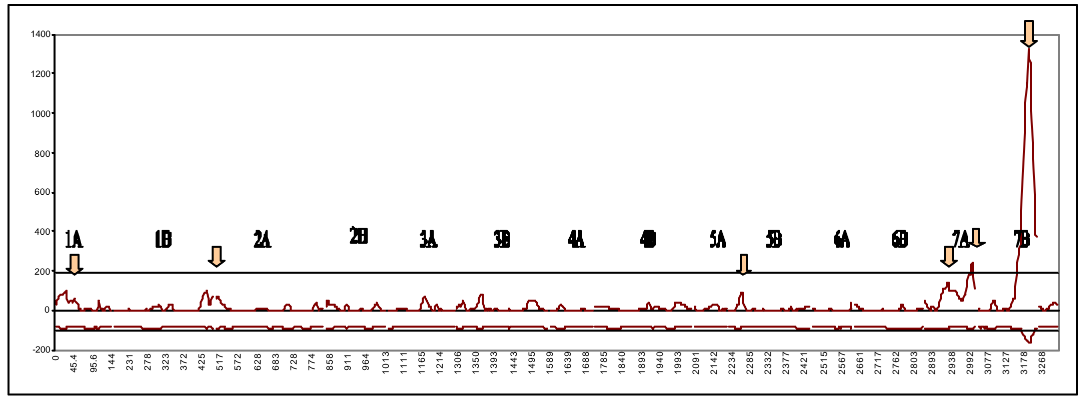


IV- Yellow Pigment

IV-a- Estimation of QTL positions by simple interval mapping for YP

In the simple interval mapping analysis of yellow pigment content, the test statistic exceeded the significance threshold in 2 regions, first on chromosome 7A, and secondly on chromosome 7B. In general, the scan showed that there is strong genotypic effect on this trait. Therefore, the small peak on 7A presented only a main effect and the very high peak on chromosome 7B presented a strong main effect and weak QTLxE interaction effect. These results corroborate earlier studies on durum wheat carotene content (Nachit *et al.* 1995a), where it was reported that yellow pigment trait is mainly affected by genotypic effect in an additive manner. Furthermore, The peak on 7B was very high suggesting a major QTL on this region (Fig YP-1).

Fig YP-1: Yellow pigment scans of a test statistic for SIM for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting from short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows indicate positions of background markers.



IV-b- Estimation of QTL positions by simplified composite interval mapping for YP

Following the same strategy developed for protein content, sedimentation test, and farinograph absorbance the significant peaks for the test statistic for SIM were chosen as background markers (see arrows in Fig YP-1). The simplified composite interval mapping scan confirmed the peak on *Xgwm344* on the telomeric region of 7BL with both main and interaction effects.

The peak detected by SIM, which was close to *Xgwm63e* on the telomeric region of 7AL, has completely vanished by sCIM analysis. Instead of this locus, a new small QTL peak close to the centromeric region emerged by sCIM (Fig YP-2). This new peak

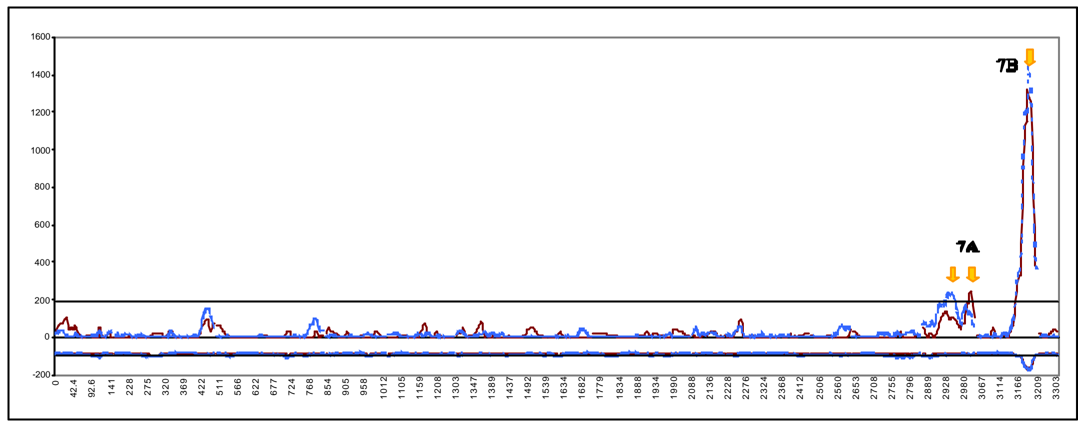
appeared in an area where there had been a small non-significant peak by SIM scan. It corresponded to the AFLP marker *XMcaaEacg198*.

Therefore, in *Omrabi5/ T. dicoccoides600545// Omrabi5* population all yellow pigment QTLs were detected on the homoeologous regions of chromosomal group 7. A major QTL on 7BL and 2 minor QTLs on 7AL were identified. This is in agreement with Parker *et al.* (1998) work on bread wheat. They reported a major locus on 7A explaining 60% of the genetic variation. The chromosomal-group localization is also in agreement with the findings in tritordeums (Alvarez *et al.* 1998), where it was reported that carotene content is controlled by the chromosome $7H^{ch}$. Nevertheless, earlier studies reported rather QTLs on chromosomes 2A and 2B in durum wheat (Joppa and Williams 1988) and on chromosomes 6D and 3B in bread wheat (Parker *et al.* 1996). In addition, in *Jennah Khetifa* x *Cham1* durum mapping population, YP-QTLs were mapped to the chromosome 4B (Nachit, pers. com.).

Durum wheat color is known to be affected by the level of lipoxygenase (LOX) activity (McDonald 1979). The LOX is responsible for oxidative degradation of yellow color of durum endproducts and production of undesirable odors (Tsen and Hlynka 1963; Hosoney *et al.* 1980; Borreli *et al.* 1999; Trocoli *et al.* 2000). Nevertheless, in *Omrabi5/ T. dicoccoides600545// Omrabi5* population no QTL was detected on chromosomal group 4 and 5, known to hold LOX genes. Actually, LOX genes were reported to map in bread wheat on 4AL (*Lpx-A1*); 4BS (*Lpx-B1*); 5AL (*Lpx-A2*); 5BL (*Lpx-B2*); 4DS (*Lpx-D1*); and 5DL (*Lpx-D2*) (Hart and Langstone 1977; Nachit *et al.* 2001).

As well as lipoxygenase, polyphenol oxidase activity did also affect durum wheat color (Dexter *et al.* 1984). The peroxidase isozymes are high tissues specificity. They were localized on many chromosomes: 1BS, 1DS, 2AS, 2BS, 2DS, 3AL, 3BL, 3DL, 4AL, 7AS, 7DS (McIntosh *et al.*, 1998 Catalogue of gene symbols, 9IWGS). In MDM mapping-population, no QTL was found to affect yellow pigment near any of those mentioned above genomic regions.

xFig YP-2: Yellow pigment scans of a test statistic for SIM (solid red lines) and sCIM using few background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs.



IV-c- Estimation of the additive effects of the putative QTLs for YP

The three detected QTLs explained 62% of the total yellow pigment variation (Table YP-1). A major QTL was detected at 0 cM of *Xgwm344* on the long arm of chromosome 7B by both SIM and sCIM analysis. This microsatellite by itself explained 53% of the total variation, out of which 52% is genetic variation and only 1% is QTLxE variation. The detected QTL look like the homoeologous locus of the QTL detected in bread wheat by Parker *et al.* (1998) on 7AL. This former QTL was reported to explain 60% of the total genetic variation in bread wheat.

The other two QTLs on the long arm of chromosome 7A: *XMcaaEacg198* and *Xgwm63e*, were showing only main effect, and have explained 6 and 13% of the total variation, respectively. This is a good example of the complementarity of SIM and sCIM analysis, as the QTL on *Xgwm63e* was detected only by SIM, whereas *XMcaaEacg198* was rather detected by sCIM analysis.

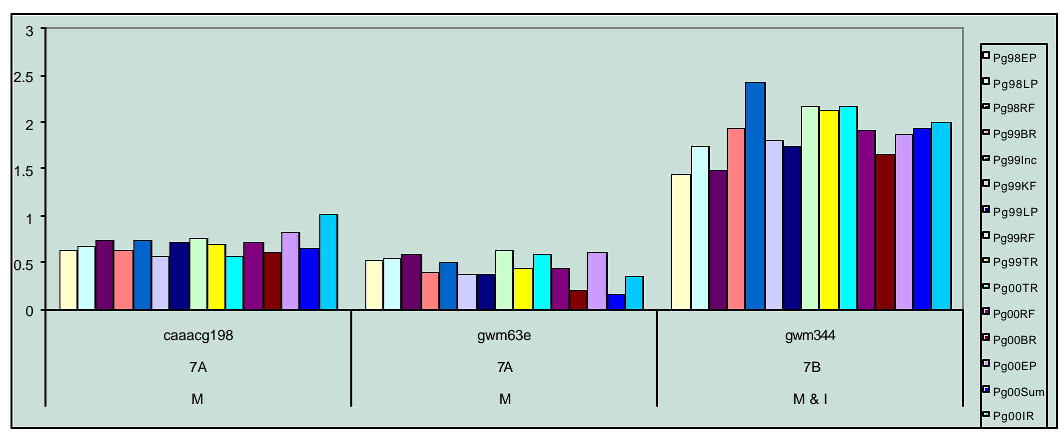
All yellow pigment QTLs showed that the *Omrabi5* alleles had a significant positive effect (Fig YP-3), indicating that the durum variety *Omrabi5* alleles increased the yellow pigment content. This positive effect was consistent with minor magnitude changes across sites and years; this consistency was evident as shown in the Fig YP-3. This positive effect can go up to +2.5ppm in Tel-Hadya 98/99 (Inc 99).

Table YP-1: Yellow pigment QTLs and their contributions

Chromosomal localisation	QTL marker	cM	V_g/V_{ph}	$V_g + V_{QTLxE} / V_{ph}$
7BL	***Xgwm344***	0	52	**53**
7AL	*Xgwm63e*	0	13	13
7A	*XMcaaEacg198*	5	6	6
Total explanation			**60%**	**62%**

V_g = genetic variance; V_{QTLxE} = QTLxE variance; V_{ph} = phenotypic variance.

Fig YP-3: Relative magnitude, chromosomal location and effect-nature of estimated YP-QTLs in each environment.

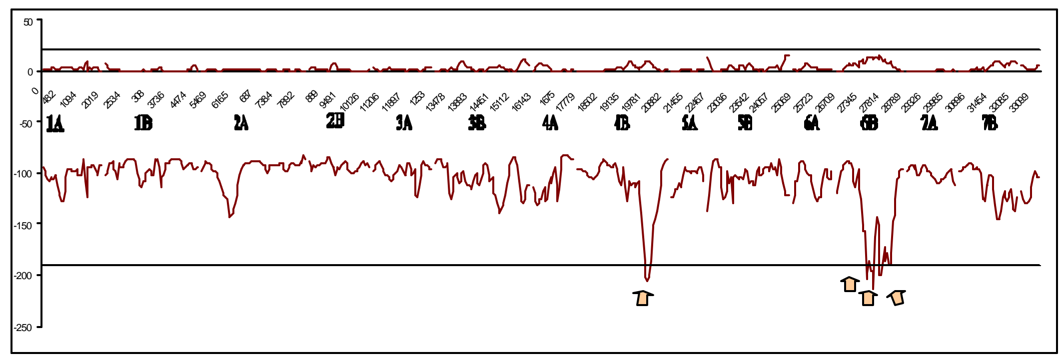


V- *Vitreousness*

V-a- Estimation of QTL positions by simple interval mapping for Vitreousness

For vitreousness, the test statistic scan for SIM analysis was very specific. In contrast to the other studied traits, the vitreousness interaction peaks were much higher than the main effect peaks. This indicates that this trait is highly affected by environmental conditions. Therefore, the SIM did not present any peak above the main effect threshold, whereas it showed few peaks with interaction effects on chromosomes 4B and 6B (Fig Vit-1).

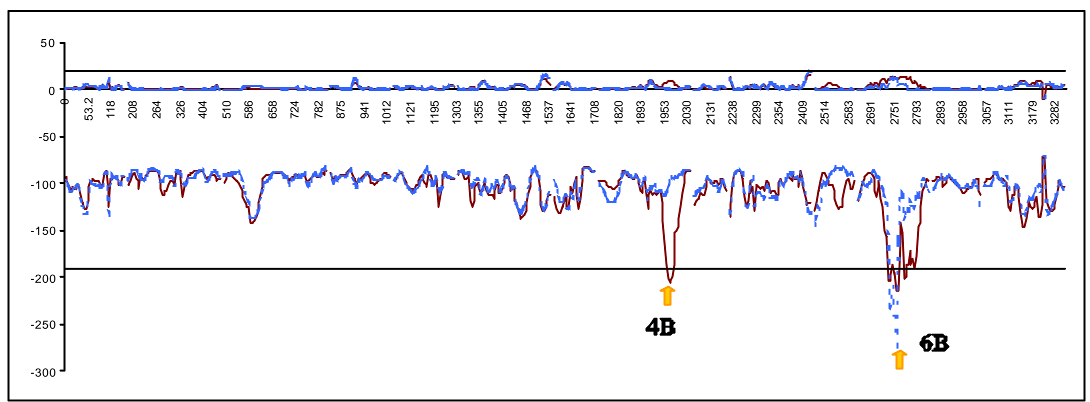
Fig Vit-1: Vitreousness scan of a test statistic for SIM for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting from short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows indicate positions of background markers.



V-b- Estimation of QTL positions by sCIM mapping for Vitreousness

The simplified composite interval mapping scan of vitreousness, using few background markers, was very similar to the SIM scan (Fig Vit-2). There was just one difference between the two analyses concerning the peak on 4B, SIM showed a weak significance peak on 4B, whereas sCIM dismissed it completely. Otherwise, The peak on 6B was narrowed by sCIM analysis and becomes more defined to *XMcaaEacg560* on 6BS. Both peaks showed only an interaction effect. This is in agreement with earlier findings highlighting strong environmental effect on vitreousness (Nachit and Asbati, 1987; Nachit *et al.* 1995a; Trocoli *et al.* 2000).

Fig Vit-2: Vitreousness scan of a test statistic for SIM (solid red lines) and sCIM using few background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs.



V-c- Estimation of the additive effects of the putative QTLs for Vitreousness

The two detected QTLs, *Xgwm107* on 4BL and *XMcaaEacg560* on 6BS, explained only 9% of the total vitreousness variation, each one of them contributing with a small effect (Table Vit-1). Both QTLs, as suggested by the scans, show full interaction variation (8%) with minimal genetic variation (1%). These two QTLs showed clear crossover interaction with positive effect in some environments and negative in others (Fig Vit-3). Nachit *et al.* (1995a) reported environmental and GxE interaction effects on vitreousness of 70.0% and 16.9%, respectively, under dry conditions; this result also suggests low genetic effect on vitreousness trait. These two QTLs were also found to affect protein content. As protein content and vitreousness are linked in their biochemical pathways, we can confidently suggest that these two QTLs are single locus with a pleitropic effect. These findings were expected as in the mapping-population data, Pearson correlation between protein content and vitreousness was found to be positive (0.43); similar association was reported by Matsuo and Dexter (1980) and Nachit and Asbati (1987). In fact, vitreous areas of the endosperm are known to be higher in protein than mealy-whitish areas (Trocoli *et al.* 2000).

The present experiment did not allow the detection of vitreousness-QTL. This could be due to the nitrogen application in the trials, as the zero-nitrogen environment was reported to be the best environment for an efficient selection for vitreousness (Nachit and Asbati, 1987).

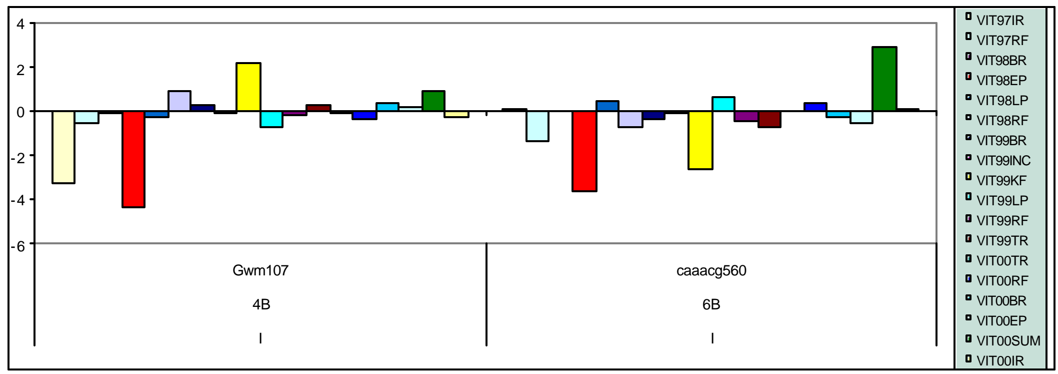
As no genetic variation was detected in MDM mapping population, figure Vit-3 did not determine the alleles, either *Omrabi5* or *T. dicoccoides600545,* that have the positive effect on vitreousness. The environment Tel-Hadya early planting 97/98 showed a significant positive effect of *T. dicoccoides600545* alleles on vitreousness in the RILs (Fig Vit-3). As mentioned before, this environment had experienced a high precipitation, in addition to supplementary irrigation; therefore the RILs vitreousness values obtained in 98EP were particularly low (down to 29%), whereas in the other 18 environments the values were all above 85%.

Table Vit-1: Vitreousness QTLs and their contributions

Chromosomal localisation	QTL marker	cM	V_g/V_{ph}	$V_g + V_{QTLxE} / V_{ph}$
4BL	*Xgwm107*	0	0	6
6BS	*XMcaaEacg560*	0	1	7
Total explanation			**1%**	**9%**

V_g = genetic variance; V_{QTLxE} = QTLxE variance; V_{ph} = phenotypic variance.

Fig Vit-3: Relative magnitude, chromosomal location and effect-nature of estimated Vit-QTLs in each environment.

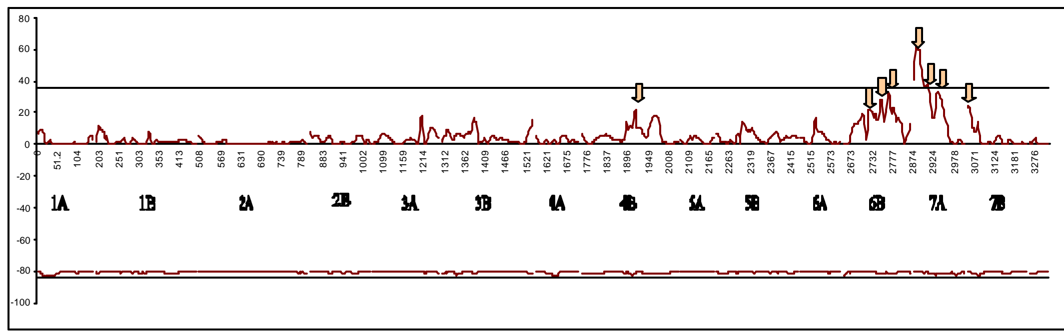


VI- Test Weight (TW)

VI-a- Estimation of QTL positions by simple interval mapping for TW

For test weight, the test statistic scan for SIM analysis was simple and showed only few peaks on the main effect. No interaction effect was shown across the whole genome, suggesting a high genotypic effect on this milling related trait. This is in agreement with earlier findings (Ghaderi and Everson 1971; Teich 1984; Jallaludin *et al*. 1989; Nachit *et al*. 1995a). The SIM scan exceeded the significance threshold on the main effect only in 2 regions both localized on chromosome 7A (Fig TW-1).

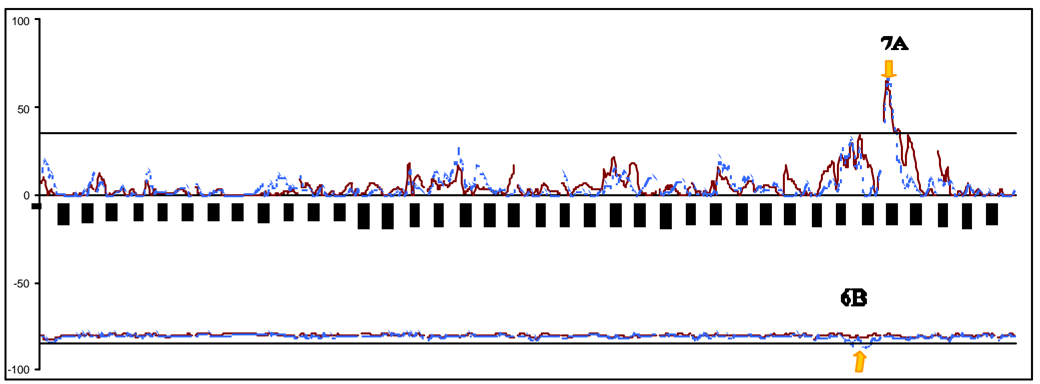
Fig TW-1: TW scan of a test statistic for SIM for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting from short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows indicate positions of background markers.



VI-b- Estimation of QTL positions by sCIM mapping for TW

The simplified composite interval mapping analysis confirmed the first high peak on chromosome 7A and deleted the second low peak on the same chromosome (Fig TW-2). Therefore, on 7AS a QTL was detected next to *Xgwm60c*. The sCIM scan identified also two new peaks on chromosome 6B both presenting only a QTLxE effect, in an area where SIM scan showed non-significant small main effect peaks. Across this genomic region, a regression model was applied using either one of the two detected peaks or both of them. Only *Xgwm88a* on the centromeric region of 6BS was selected as QTL, as adding the second QTL did not contribute further in explaining the total variation.

Fig TW-2: TW scan of a test statistic for SIM (solid red lines) and sCIM using few background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs.



VI-c- Estimation of the additive effects of the putative QTLs for TW

The QTL detected on 7AS explained by itself 17% of the total test weight variation, whereas, *Xgwm88a* around the centromeric region of 6BS explained 9% (Table TW-1). The total explanation was of genetic nature, no interaction effect was revealed. Even the 9% explanation of the QTL on 6BS was fully found to be due to genetic effect, however sCIM scan identified it as being of QTLxE interaction effect. These findings are in agreement with earlier studies showing a high genotypic effect on test weight, explaining up to 80% of the total variation (Ghaderi and Everson 1971; Teich 1984; Jallaludin *et al.* 1989; Nachit *et al.* 1995a). However, other conducted research showed a medium to high environmental effect on test weight (Roth *et al.* 1984; Blum *et al.* 1991; Saadalla *et al.* 1990; Schuler *et al.* 1994). It was stipulated it that test weight is correlated to kernel size, shape, and weight (Yamazaki and Briggle 1969; Schuler *et al.* 1994; Trocoli and DiFonzo 1999).

The two detected test weight QTLs: *Xgwm88a* and *Xgwm60c* showed an epistasic effect (Fig TW-3). As their individual effect explained just 9 and 17%, respectively, while together the explanation rate was found to be higher (30%). The difference between the lines comprising AAAA (p1-p1) and AABB (p1-p2) comparatively with the difference between BBAA (p2-p1) and BBBB (p2-p2) was obviously large (Fig TW-3). Therefore, the shown improvement is not only due to the additional effect of *Xgwm88a* and of *Xgwm60c* (*Omrabi5* alleles), but rather to their additive and multiplicative effects (epistasis).

Omrabi5 alleles had a significant positive and consistent effect on the TW trait across all environments (Fig TW-4). The magnitude changes between different environments were very small (Fig TW-4). This positive effect on TW of *Omrabi5* alleles was expected, as the wild relatives are known to hold smaller and shrivelled grain in comparison with cultivated wheat. Even-though, *T. dicoccoides* grain among the other wild relatives is one of the largest after *T. polonicum* (Nachit, pers. com.). In fact, this was one of the major reasons for its early adoption in the southern part of the Fertile Crescent (Kimber and Feldman, 1987).

Table TW-1: Test weight QTLs and their contributions

Chromosomal localisation	QTL marker	cM	V_g/V_{ph}	$V_g + V_{Q\ TLxE} / V_{ph}$
6BS	*Xgwm88a*	0	9	9
7AS	*Xgwm60c*	0	17	17
Total explanation			**29%**	**30%**

V_g = genetic variance; V_{QTLxE} = QTLxE variance; V_{ph} = phenotypic variance.

Fig TW-3: Evidence of epistasic effect between test weight QTLs.

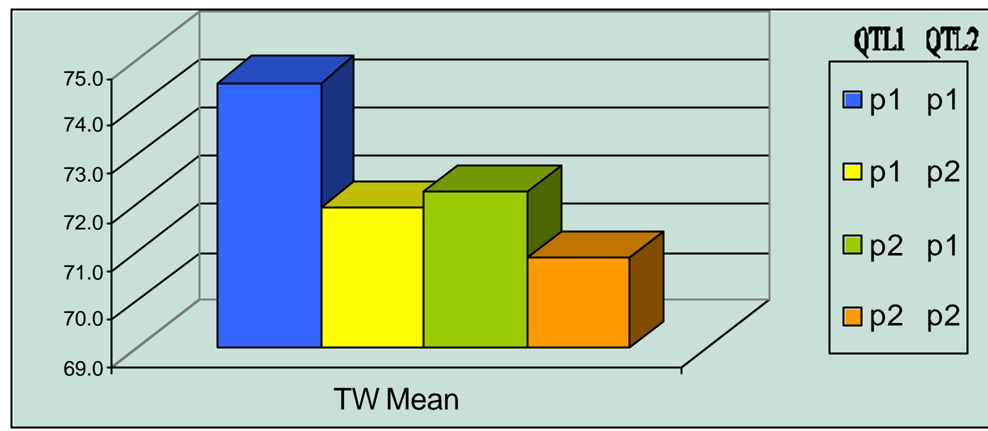
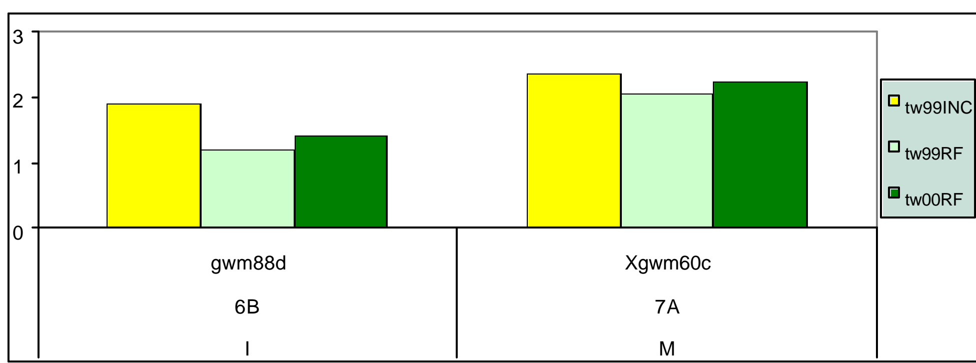


Fig TW-4: Relative magnitude, chromosomal location and effect-nature of estimated TW-QTLs in each environment.



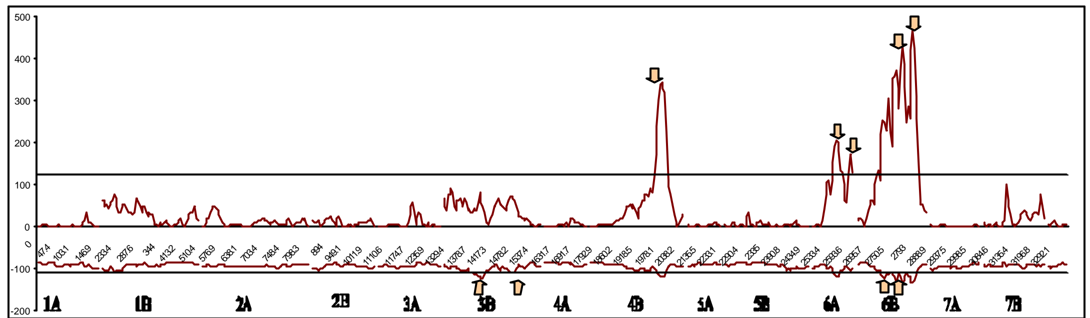
VII- Thousand-Kernel Weight (TKW)

VII-a- Estimation of QTL positions by simple interval mapping for TKW

The test statistic of the SIM analysis showed interesting peaks in chromosomes 4B, 6A, and 6B with main and QTLxE interaction effects (Fig TKW-1). Another low QTLxE interaction peaks were also observed in 3B. The major peak on chromosome 6B

was wide covering around 50cM with both effects, it did present 5 prominent peaks, four out of them were taken as background markers for elaboration of sCIM analysis.

Fig TKW-1: TKW scan of a test statistic for SIM for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting from short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows indicate positions of background markers.



VII-b- Estimation of QTL positions by sCIM mapping for TKW

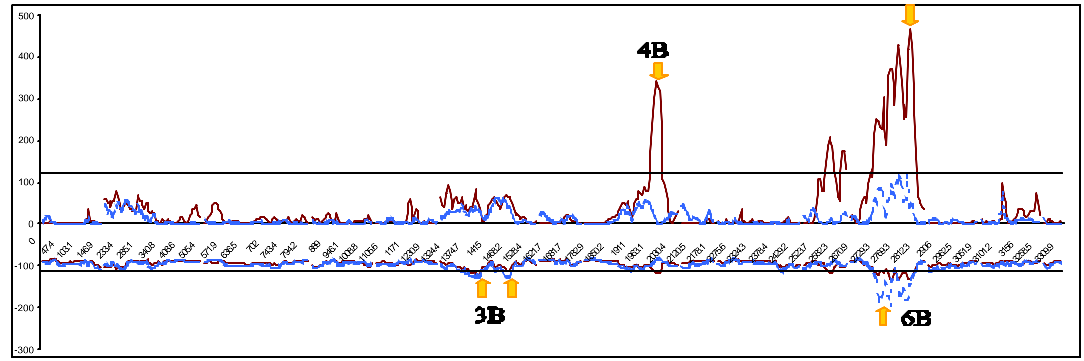
As for protein content, the simplified composite interval mapping analysis for TKW gave smaller peaks for the main effect, whereas it gives higher peaks for QTLxE interaction (Fig TKW-2). Therefore, all main effect peaks detected on 4B, 6A, and 6B by SIM analysis were totally eliminated by the sCIM analysis, even though they all presented a valuable peak on SIM analysis scan. The fact that sCIM analysis discard these QTLs suggest them as potential ghost QTLs. Nonetheless, they all kept their QTLxE interaction effects, and therefore should be considered as real QTLs.

Therefore, on chromosome 6B, *Xgwm518* on the short arm and *Xgwm582a* on the long arm were selected for their main effect (detected by SIM) and their interaction effects (as suggested by sCIM). Actually, their explanation was mainly genetic (Table TKW-1). The microsatellite *Xgwm518* was also showed to affect protein content and gluten strength. Our study could not determine whether this QTL is a single pleitropic locus or rather 2 or more tightly linked loci. Moreover, the identified SDS-, protein content-, and TKW-QTL were localized at 0cM of *Xgwm518*. However, this localization did not assure that they are at the same point physically, as the physical map could be very different than the genetic

map (Thurieaux 1977; Dvorak and Chen 1984; Werner *et al.* 1992; Kota *et al.* 1993; Delaney *et al.* 1995; Gill *et al.* 1996).

As for chromosome 4B, *XMcttEagg213* was selected for the same reasons (SIM-main effect and sCIM-interaction effect). While, the peaks detected on 6A by SIM were discarded because they did not enhance the total variation explanation. Using regression models on the interaction peaks on 3B, two markers *XMctcEaag350* on the short arm and *XMctcEagg84* on the long arm were selected, being the best contributors.

Fig TKW-2: TKW scan of a test statistic for SIM (solid red lines) and sCIM using few background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs.



VII-c- Estimation of the additive effects of the putative QTLs for TKW

The five detected QTLs affecting TKW explained 32% of the total variation, 25% out of it was representing the genetic variation. The QTLs detected around the centromeric region of chromosome 6B explained the majority of thousand-kernel weight total explanation (28%), whereas, the other QTLs on 3BS, 3BL, and 4BL showed additive minor effects (Table TKW-1). Their respective contributions were of 5, 5, and 3%. These findings are in agreement with earlier studies reporting an intermediate to high heritability rates indicating the involvement of high additive genetic effect (Sun 1972; Ketata *et al.* 1976; Maali 1991; Nachit *et al.* 1995a). Indeed, TKW genes number was estimated to range from 1 to 4 or more genes in different crosses (Joppa and Williams 1988). It was also reported that kernel weight is affected by the environment during grain filling and by the number of heads and number of

fertile florets per spike as well as kernel length, width and volume (Ghaderi and Everson 1971; Matsuo and Dexter 1980; Schuler *et al.* 1994).

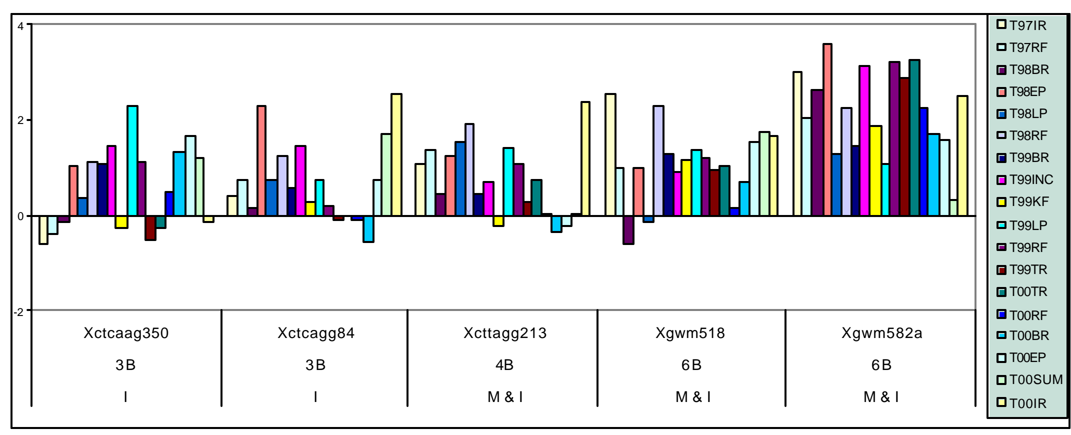
As for the test weight, *Omrabi5* alleles had a significant positive effect on the TKW trait (Fig TKW-3). In general, this positive effect is quite consistent across environments, especially the QTLs detected around 6B-centromere. Therefore, QTLxE interactions of *Xgwm582a* and *Xgwm518* are mainly due to changes in magnitude, while QTLxE interactions of *XMctcEaag350*, *XMctcEagg84*, and *XMcttEagg213* to crossover interactions.

Table TKW-1: Thousand kernel weight QTLs and their contributions

Chromosomal localisation	QTL marker	cM	V_g/V_{ph}	$V_g + V_{QTLxE} / V_{ph}$
3BS	*XMctcEaag350*	0	3	5
3BL	*XMctcEagg84*	0	3	5
4BL	*XMcttEagg213*	5	2	3
6BS	*Xgwm518*	0	12	13
6BL	*Xgwm582a*	5	20	22
Total explanation			**25%**	**32%**

V_g = genetic variance; V_{QTLxE} = QTLxE variance; V_{ph} = phenotypic variance.

Fig TKW-3: Relative magnitude, chromosomal location and effect-nature of estimated TKW -Q TLs in each environment.



VIII- Milling extraction

VIII-a- Estimation of QTL positions by simple interval mapping for FY

The flour yield is the only criteria where no peaks were observed. The test statistic scan of the simple interval mapping analysis showed small and non-significant peaks (Fig FY-1). Therefore, it was difficult to rely on the developed-strategy with few peaks as background markers for the elaboration of the sCIM analysis. The resulted scan with few ones was very similar to the SIM one, showing no-significant peaks (Fig FY-2). Therefore, more background markers were chosen in the genome; precisely 47 background markers were chosen with an average distance of 40cM between each two BM.

Fig FY-1: Flour yield scan of a test statistic for SIM for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting from short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows indicate positions of background markers.

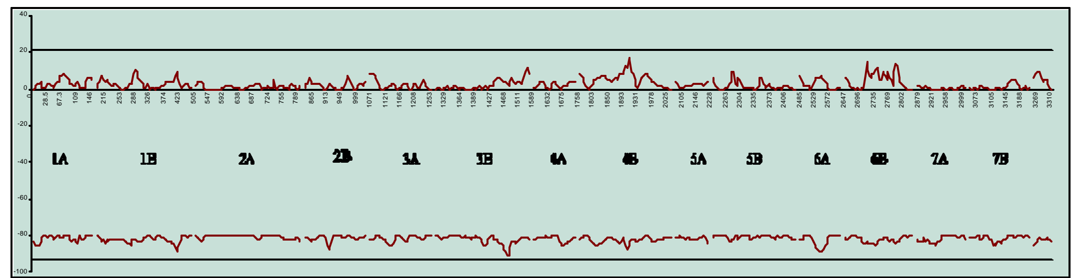
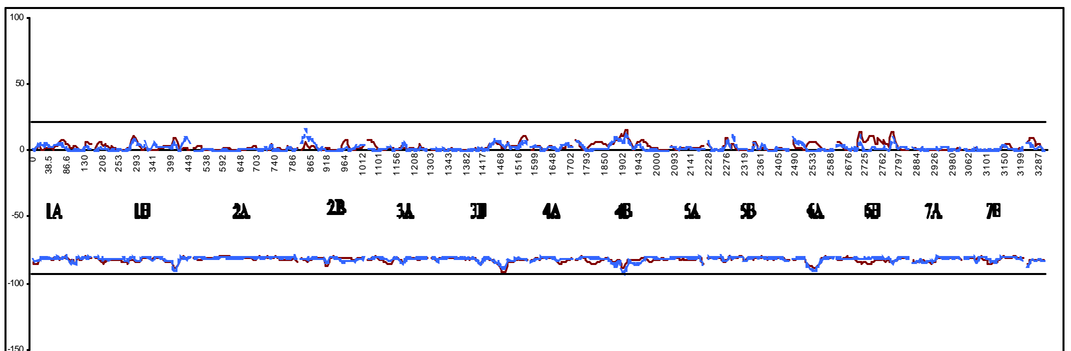


Fig FY-2: Flour yield scan of a test statistic for SIM (solid red lines) and sCIM using few background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs.



VIII-b- Estimation of FY-QTL positions by sCIM and their additive effects

The resulted scan from the simplified composite interval mapping analysis with the 47 background markers was more informative than SIM and sCIM-few BM scans (Fig FY-3). The test statistic showed few peaks with low-significance for both main and interaction effects. Thus, some main-effect QTLs were identified on 5BS and 6BS, whereas other interaction-effect QTLs were identified on 3A and 7AS.

On 5BS, *Xgwm415b*-QTL was selected for its Main effect. Its explanation was very low (2%). On 6BS, *XMcaaEacg390* was also chosen for its presumably main effect and its effect was also low (5%). The contribution of the third GE-QTL *Xgwm154e* was even lower (1%). All the selected QTLs have explained only 10% of the total flour yield variation (Table FY-1). This suggests that *Omrabi5* alleles have a slightly positive effect on flour yield trait (Fig FY-4).

Fig FY-3: Flour yield scan of a test statistic for SIM (solid red lines) and sCIM using 47 background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs.

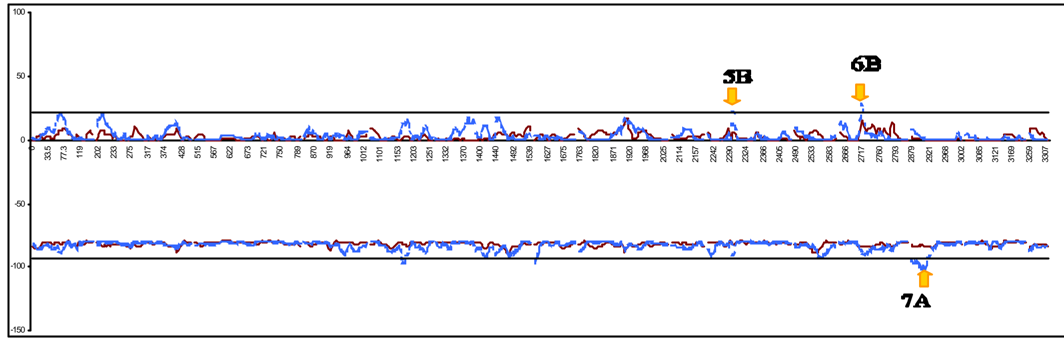
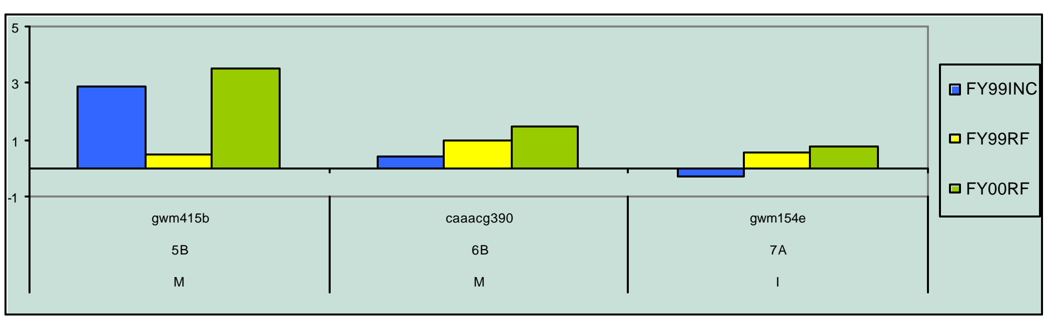


Table FY-1: Flour yield QTLs and their contributions

Chromosomal localisation	QTL marker	cM	V_g/V_{ph}	$V_g + V_{QTLxE} / V_{ph}$
5BS	*Xgwm415b*	0	2	2
6BS	*XMcaaEacg390*	0	4	5
7AS	*Xgwm154e*	0	0	1
Total explanation			**7%**	**10%**

V_g = genetic variance; V_{QTLxE} = QTLxE variance; V_{ph} = phenotypic variance.

Fig FY-4: Relative magnitude, chromosomal location and effect-nature of estimated FY-QTLs in each environment.

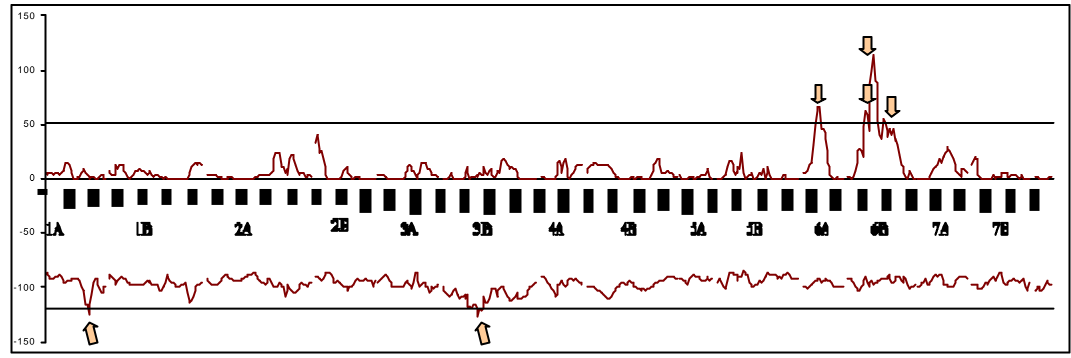


IX- Ash content

IX-a- Estimation of QTL positions by simple interval mapping for AC

For ash content, the test statistic scan for SIM analysis has exceeded several times the threshold for main effect on the homoeologous group 6 (Fig AC-1). On 6A, it shows one small and defined peak, whereas on 6B, the peak was wider with many gradient heads. For the QTLxE interaction effect, the scan exceeded the threshold once on chromosome 1A and twice on chromosome 3B .

Fig AC-1: Ash content scan of a test statistic for SIM for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting from short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows indicate positions of background markers.



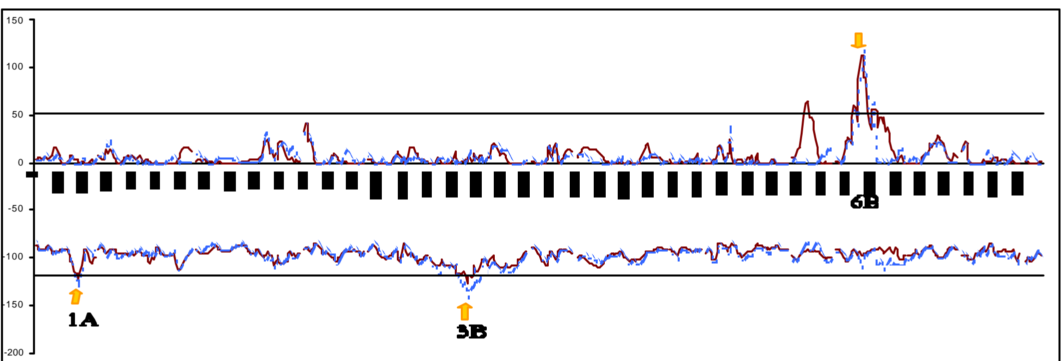
IX-b- Estimation of QTL positions by sCIM for AC

The simplified composite interval mapping analysis resulted in almost the same main-effect scan (except for chromosome 6A) but with higher interaction peaks (Fig AC-2). The peak on chromosome 6A was completely eliminated by sCIM analysis, and

is likely a ghost QTL, whereas on chromosome 6B, the gradient heads shown on SIM scan became more defined and corresponded to the marker *XMctcEact198* on the short arm.

The interaction peaks on 1A (*XMctgEagc290*) and 3BS (*Xgwm644a*) were confirmed with sCIM analysis. These findings suggest that ash content trait is under the control of both genetic and environment effects. Peterson *et al.* (1986) reported a high genotypic influence, whereas, others reported rather an environmental effect (Cubadda *et al.* 1969) or genotype-by-environment interaction (Fares *et al.* 1996). They showed that favorable growing conditions result in higher ash content in whole grain, due to increased uptake of minerals from the soil.

Fig AC-2: Ash content scan of a test statistic for SIM (solid red lines) and sCIM using few background markers (broken blue lines) for QTL main effect (above axis) and QTL by environment interaction (below axis). The 14 MDM chromosomes and g15 are shown from left to right (starting with short arm). Horizontal lines show thresholds for testing SIM with 5000 permutations. Arrows show positions of selected QTLs.



IX-c- Estimation of the additive effects of the putative QTLs for AC

The total explanation determined for the ash content variation was very low. The 3 determined QTLs explained only 11% of the total variation (Table AC-1). They showed minor additive effects, *XMctcEact198* on 6BS showed the highest genetic explanation rate (6%), while *XMctgEagc290* on 1A and *Xgwm644a* on 3BS showed trivial interaction effects (2% each).

Generally, the total found effects on ash content were small. The QTL on 1AS and 3BS are expressing crossover interaction effects, and therefore showed high

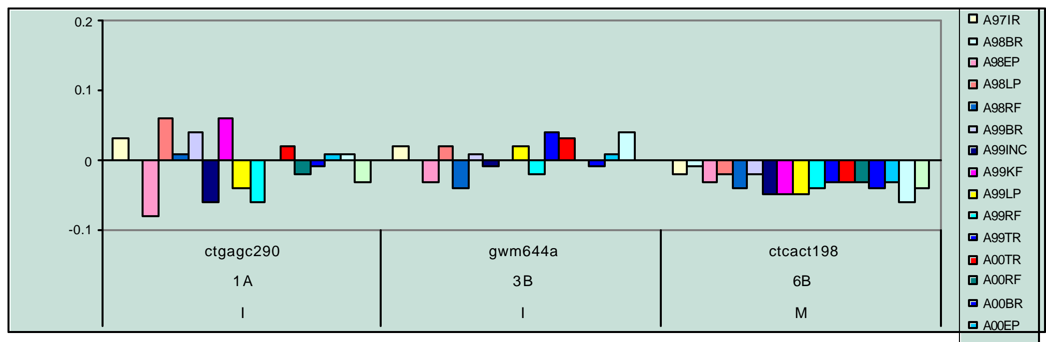
fluctuation across environments (Fig AC-3). On the other hand, the main effect *XMctcEact198*-QTL on 6BS showed a positive effect of *T. dicoccoides600545* alleles.

Table AC-1: Ash content QTLs and their contributions

Chromosomal localization	QTL marker	cM	V_g/V_{ph}	$V_g + V_{QTLxE} / V_{ph}$
1A	***XMctgEagc290***	5	0	2
3BS	***Xgwm644a***	0	0	2
6BS	***XMctcEact198***	0	6	6
Total explanation			**6%**	**11%**

V_g = genetic variance; V_{QTLxE} = QTLxE variance; V_{ph} = phenotypic variance.

Fig AC-3: Relative magnitude, chromosomal location and effect-nature of estimated AC-QTLs in each environment.



X- Genetic variance explained by different QTL-models

Comparison between primary and secondary QTLs was conducted to assess their contributions and identify the most suitable model for QTL analysis. Figure VI-1 shows the estimation allelic effects of the detected QTLs for each grain-quality trait. The model 1 represents the estimation of main effect of primary QTLs, the model 2 the estimation of main and QTLxE interaction effects of primary QTLs, and the model 3 the estimation of main and QTLxE interactions effect for both primary and secondary QTLs. Primary QTLs are those detected by both SIM and sCIM analysis, whereas secondary QTLs are those detected by only one of them.

The model I was significantly informative for YP, FDT, FST, FMT and SDS; meaning that the majority of the identified QTLs for these traits are of genetic effect and

were detected by both QTL-analyses. In contrast, the model I was very weak for flour yield, vitreousness, FAB, and ash content. The explanation rate ranged from 0 to 6%. For model 2 (addition of GxE primary QTLs effect), all traits showed increase values for genetic variance except FMT, TW, and FY. As for the full QTLs model (model 3), the secondary QTLs have improved moderately the estimation effects (Table V-1). Nevertheless, in some cases the obtained improvement was extremely large. This was the case of the secondary-QTLs detected for the milling characters: TW, TKW, and FY, whereas in other traits (i.e.: PC, FAB, FDT) no secondary QTLs were included due to their low contributions.

Table V-1: Allelic estimation of detected grain-quality QTLs considering different statistical models (1, 2, and 3).

	Model		
Trait	**1**	**2**	**3**
Protein content	20	27	27
SDS	26	32	41
FAB	5	14	14
FDT	28	33	33
FMT	35	35	45
FST	42	49	51
Yellow pigment	52	53	62
Vitreousness	1	7	9
Test Weight	17	17	30
1000-Kernel Weight	14	18	32
Flour Yield	0	0	10
Ash Content	6	11	11

Chapter VI
General Discussion & Conclusion

- *General discussion*

Thirty-three QTLs were detected in *Omrabi5/ T. dicoccoides600545// Omrabi5* population exhibiting linkage with different grain-quality traits (Table VI-1). Most of the traits showed more than 3 QTLs explaining more than 30% of the total variation. The highest total contributions were found for yellow pigment and FST traits. Three QTLs were determined on the group 7 explaining 62% of the total YP variation; and 4 QTLs on 1BL and 2BS explaining 51% of the total FST variation. The large amount of YP variation explained by Xgwm344 would suggest that this QTL or this genomic region predominantly controls YP inheritance. The lowest explanation values were shown for vitreousness, flour yield, and ash content. All the grain-quality QTLs showed both genetic and QTLxE effects, except, for the vitreousness trait where only interaction effect was revealed (Table VI-1).

Table VI-1: All grain-quality determined QTLs in *Omrabi5/ T. dicoccoides600545// Omrabi5*.

Trait	N. QTLs	Total contribution	Involved chromosomes	Effect
PC	6	27%	3BS, 3BL, 4BL, 6AS, 6BS	G + QTLxE
SDS	5	41%	1BL, 2BS, 4BL, 6BS	G + QTLxE
FAB	2	14%	3BS, 4BL	G + QTLxE
FDT	3	33%	1BL, 3AS	G + QTLxE
FMT	4	45%	1AS, 1BL, 3BS, 7B	G + QTLxE
FST	4	51%	1BL, 2BS	G + QTLxE
YP	3	62%	7AL, 7BL	G + QTLxE
Vit	2	9%	4BL, 6BS	QTLxE
TW	2	30%	6BS, 7AS	G + QTLxE
TKW	5	32%	3BS, 3BL, 4BL, 6BS, 6BL	G + QTLxE
FY	3	10%	5BS, 6BS, 7AS	G + QTLxE
AC	3	11%	1AS, 3BS, 6BS	G + QTLxE

N. QTLs = number of detected QTLs, G = Genetic, QTLxE = QTL x Environment Interaction.

Several QTLs have shown to affect two or more traits (Table VI-2). This was evident in the case of the *Glu-B1* gene region on 1BL, where the two identified QTLs *Glu-B1* and *Xgwm131b* were found to affect gluten strength, FDT, FST, and FMT. Further, protein content and vitreousness showed also two QTLs in common, *XMcaaEacg560* on 6BS and *Xgwm107* on 4BL. However, these two QTLs were not

affecting PC and vitreousness in the same way. Both of them showed only QTLxE effect on vitreousness whereas both showed genetic and QTLxE effects on PC.

A QTL on 6BS (*Xgwm518*) was found to affect gluten strength, PC, and TKW. Actually, this QTL showed that *Omrabi5 Xgwm518*-alleles contributed positively to TKW in the RILs, whereas *T. dicoccoides600545 Xgwm518*-alleles to PC and SDS. The present study could not determine whether this QTL is a single pleitropic locus or rather a complex of 2 or more tightly linked loci; as a genetic positioning at 0 cM does not secure a positioning at the same physical-point. Anyway, either the detected QTL is one single-locus or many tightly linked loci; it is evident that breaking such linkage at this genetic position is difficult if not impossible. However, this QTL explains partially why it is difficult from the breeding point of view to combine high protein content with high TKW. In contrast to *Xgwm518*-QTL, there are several other QTLs linked to TKW (3B, 4BL, and 6BL) and independent from PC. As well as other QTLs linked to PC (3B, 4BL, 6AS, and 6BS) and independent from TKW. These TKW and PC independent chromosomal controlling regions are suggested as candidate QTLs to be combined in the same genetic background. As they are genetically independent, their introgression in the same genotype should be easy to accomplish.

Furthermore, flanking *Xgwm518*-QTL, which is linked to PC, SDS, and TKW, two other QTLs were found to be linked with FY and AC (Fig V-1). These results reveal with strong evidence that several important candidate genes for grain quality are localized in this chromosomal-genomic region. Other major genomic regions linked to grain quality traits were also detected in 3BS and 4BL. On the short arm of 3B, close by the centromeric region, 3 QTLs were linked to FAB, AC, and TKW. All of them showed only an interaction effect on these grain-quality traits and were mapped at 1.6 and 3.1 cM from each other. Similarly, on the long arm of 4B, three other QTLs linked to TKW, FAB, and SDS were mapped close to each other. These 3 QTLs showed a genetic effect on TKW, FAB, and SDS, *Omrabi5 XMcttEagg213*-alleles contributed positively to TKW, whereas *T. dicoccoides600545 Xgwm165a* and *Xgwm375*-alleles to FAB and SDS, respectively.

Table VI-2: QTLs in common between grain-quality traits in *Omrabi5/ T. dicoccoides600545// Omrabi5*.

Marker	Trait	Effect
Glu-B1 (1BL)	SDS	G + QTLxE
	FDT	G + QTLxE
	FST	G + QTLxE
XMcaaEacg560 (6BS)	PC	G + QTLxE
	Vit	QTLxE
Xgwm107 (4BL)	PC	G + QTLxE
	Vit	QTLxE
Xgwm131b (1BL)	SDS	G + QTLxE
	FDT	G + QTLxE
	FMT	G
	FST	G + QTLxE
Xgwm518 (6BS)	SDS	QTLxE
	PC	G + QTLxE
	TKW	G + QTLxE

When studying the distribution of the grain quality traits QTLs in *Omrabi5/ T. dicoccoides600545// Omrabi5* genetic linkage map, several traits have clustered together specifically in some chromosomal segments (Fig VI-1). Further, it has been revealed that the traits that are important for processing were found to be localized on the chromosome-arms: 1AS, 1BL, 2BS, 3AS, 3BS, 4BL, and 6BS. Consequently, these chromosome-arms are harboring genes of interest for gluten strength (SDS, FDT, FMT, FST). Moreover, on 1AS and 1BL, the processing-QTLs concurred with the seed storage protein *Glu-A3* and *Glu-B1* genes, whereas the other QTLs on the remaining chromosomal locations are new genetic markers.

The QTLs linked to protein content were distributed across the whole genome. Several chromosome-arms, including 3BS, 3BL, 4BL, 6AS, and 6BS were found to control this highly important nutritional trait. In contrast, yellow pigment QTLs were found to be more restricted in one chromosomal group; they were all mapped to the group 7; precisely on the telomere-regions of 7AL and 7BL. For yellow pigment, a major QTL was detected at 0cM of *Xgwm344* on 7BL. This SSR marker has explained a large proportion of the total variation (53%); which most of it was due to genetic effect. In addition to *QTL-Xgwm344* high contribution, it agrees with previous findings in bread wheat and tritordeums. These results are of great importance of adoption in marker assisted breeding. In addition, as the YP-QTLs in Jennah Khetifa x Cham1 population were mapped in different chromosomes, this will have undoubtedly positive

effect on pyramiding YP genes in durum through the use of targeted transgressive inheritance breeding.

As for milling characters, the detected QTLs were confined to few genetic regions. Thousand-kernel weight QTLs were mapped closely to ash content, flour yield, and test weight QTLs. The TKW-QTL detected on 3BS was mapped at 3.1 cM from *QAC-3B*; similarly the TKW-QTL detected on 6BS was mapped at 8 and 4cM from *QFY-6B* and *QAC-6B*, respectively. Another two genomic regions controlling milling characters were determined around 6B centromere and the short arm of 7A. Two QTLs linked to TW and TKW were flanking the 6B centromere (*QTW-6BS* and *QTKW-6BL*); whereas the two other QTLs linked to TW and FY were detected on 7AS.

Fig VI-1: Estimated locations of significant grain-quality QTLs detected in *Omrabi5/ T. dicoccoides600545// Omrabi5* population. *PR* designates protein content; *SDS*: sedimentation test; farinograph parameters (*FAB:* absorbance; *FDT:* development time; *FMT:* mixing time; *FST:* stability time); *YP:* yellow pigment; *Vit:* vitreousness; *TW:* test weight; *TKW:* 1000-kernel weight; *FY:* flour yield; and *AC:* ash content. QTL codes are followed by first the *chromosome-symbol* and secondly by the *number of the QTL on the chromosome*. *In parenthesis (m or d)* indicates which parental-alleles are the favorable ones for the trait.

1A

Xcaaaca290
17.6
QAC-1A
0.0 ggli47/5?
0.9 wgli2?
QFMT-1A
5.7 LMWa
0.5 Xgwm33b
4.5 Xgwm33a
13.9 Xgwm136a
Xctcact392
5.8
Xctgaag205
12.7
Xcaaaca340
6.4
Xctgagc178
19.?
Xctaacg369
11.5
Xctgagc205
6.3
Xctgagc290
8.5
Xgwm497
6.4
Xgwm99
5.2
Xctgagc284
16.9
Xctcact510
5.5
Xgwm264d

1B

Xgwm264b
8.9
1.8 Xctgacg286
0.8 Xgwm413
3.9 Xgwm11
2.4 Xgwm644b
3.9 Xgwm554b
2.5 Xgwm498b
3.9 Xctgagc167
6.7 Xcaaacc438
1.7 Xctaacg143
Xgwm131b
25.4

QSDS-1B.1 (d)
QFMT-1B (m)
QFDT-1B.1 (d)
QFST-1B.1 (d)

HMW20/6+8
5.5
3.9 Xcttagg168
4.7 Xctcagg253
2.4 Xcaaacg108
4.8 Xgwm403a
7.5 Xcaaagg350

QSDS-1B.2 (d)
QFDT-1B.2 (d)
QFST-1B.2 (d)

QFST-1B.3 (d)

Xgwm268b
9.3
Xgwm274a
6.1
Xgwm268a
10.5
Xgwm124c
3.5
Xgwm124b
9.2
Xctaacg244
9.6
Xctcagg183
8.2
Xctgaca120
9.6
Xcagagc200

Xgwm259b
9.6
Xgwm259a
29.2
Xgwm140
6.3
Xcaaacg116
6.7
Xctgaag134

2A

Xcttaag146
17.5
Xgwm71
19.?
Xcatact163
Xgwm614c
?.6
Xctcact285
Xgwm512
Xgwm249b
16.4
Xgwm448b
9.9
Xctgagc236
7.8
1.4 Xctgacg108
1.8 Xgwm249c
2.8 Xgwm95b
0.5 Xgwm558a
1.2 Xctgagc213
4.6 Xcttaag248
6.8 Xcacaag255
3.2 Xcacaag356
6.3 Xcaaagg227
Xctaacg385
24.9
Xctgagc480
6.9
2.3 Xcaaagg375
2.1 Xgwm311d
2.5 Xgwm382
0.7 Xgwm554a
9.1 Xctcaag390
Xctaacg206

2B

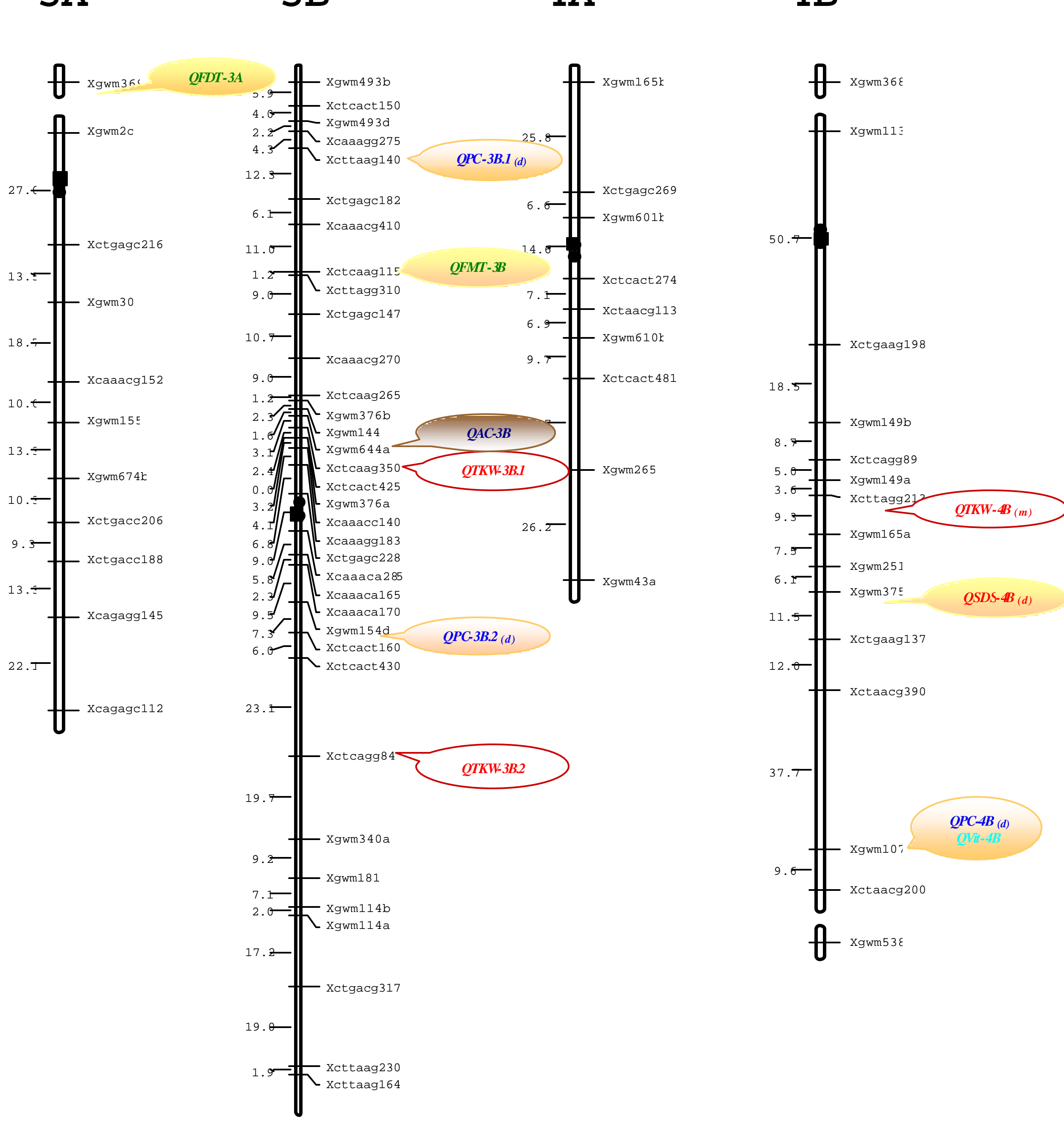
Xcaaagg237
6.7
2.7 Xcaaacc360
3.6 Xcttagg172
4.3 Xgwm630a
Xgwm129

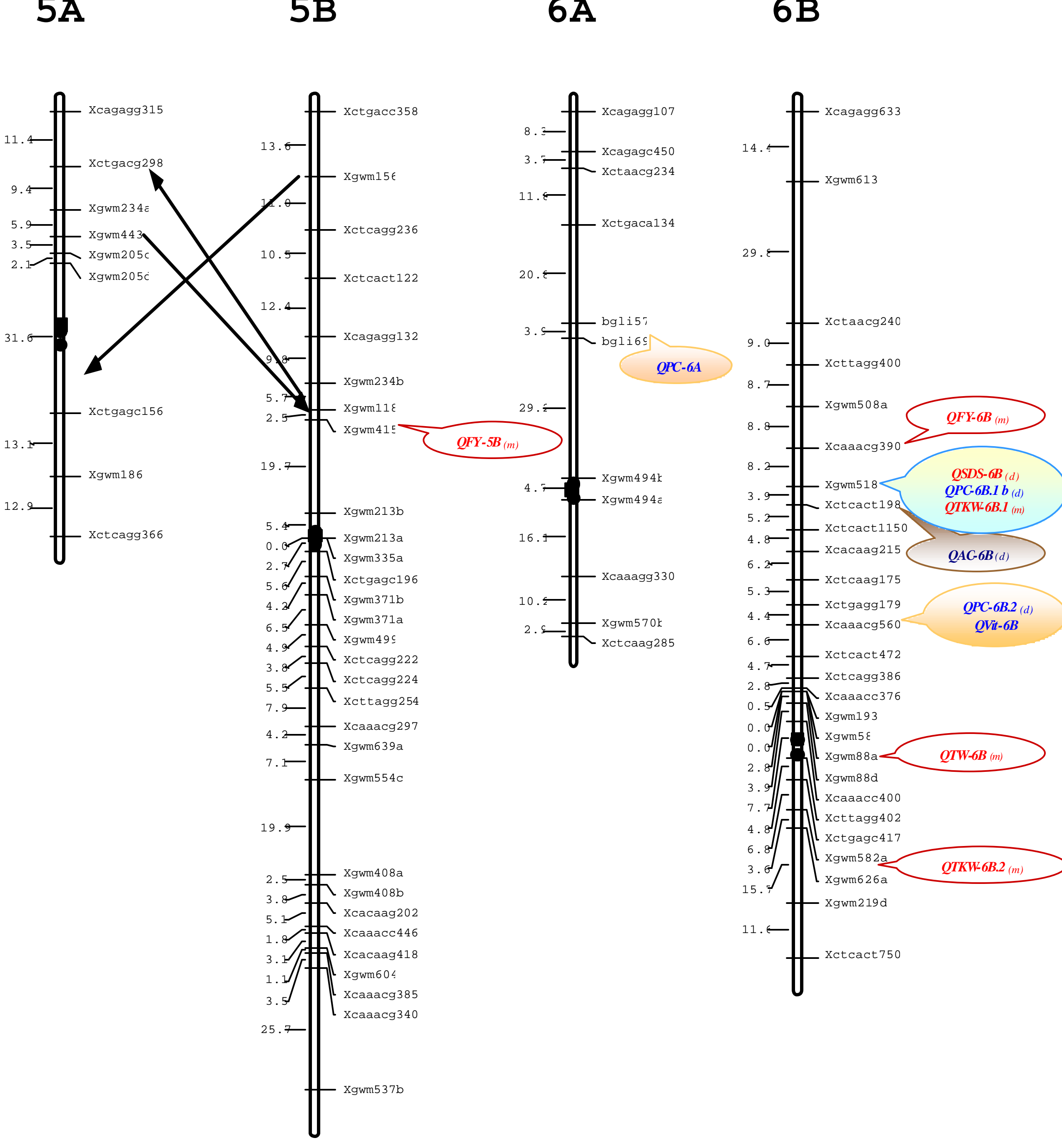
QSDS-2B

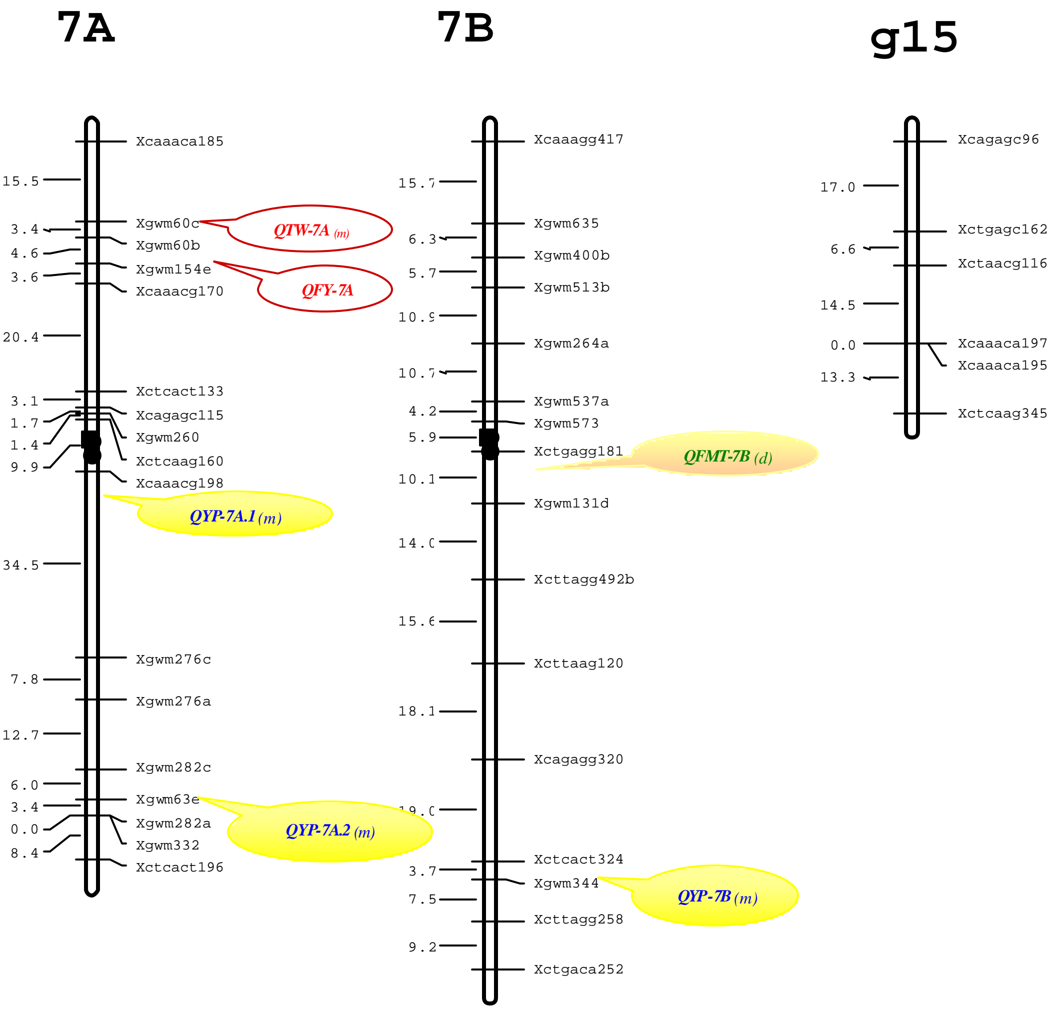
19.0
Xgwm630b
23.8
Xgwm148a
11.0
Xgwm122
11.1
Xgwm319
11.2
Xgwm374
8.6
Xgwm299

QFST-2B

19.4
Xctgaag148
30.0
Xctcagg109
10.3
Xgwm191
3.1
0.7 Xcaaagg217
3.6 Xctcact272
Xcttaag150


3A
3B
4A
4B
QFDT-3A
Xgwm2c
Xctgagc216
Xgwm30
Xcaaacg152
Xctgacc206
Xctgacc188
Xcagagg145
Xcagagc112
Xgwm493b
Xctcact150
Xgwm493d
Xcaaagg275
Xcttaag140
QPC-3B.1 (d)
Xctgagc182
Xcaaacg410
QFMT-3B
Xcttagg310
Xctgagc147
Xcaaacg270
Xctcaag265
Xgwm376b
Xgwm144
Xgwm644a
QAC-3B
Xctcaag350
QTKW-3B.1
Xctcact425
Xgwm376a
Xcaaacc140
Xcaaagg183
Xctgagc228
Xcaaaca165
Xcaaaca170
Xgwm154d
QPC-3B.2 (d)
Xctcact160
Xctcact430
Xctcagg84
QTKW-3B2
Xgwm340a
Xgwm181
Xgwm114b
Xgwm114a
Xctgacg317
Xcttaag230
Xcttaag164
Xctgagc269
Xctcact274
Xctaacg113
Xctcact481
Xgwm265
Xgwm43a
Xctgaag198
Xgwm149b
Xctcagg89
Xgwm149a
QTKW-4B (m)
Xgwm165a
Xgwm251
QSDS-4B (d)
Xctgaag137
Xctaacg390
QPC-4B (d)
QVit-4B
Xctaacg200


5A
Xcagagg315
11.4
Xctgacg298
9.4
Xgwm234a
5.9
Xgwm443
3.5
Xgwm205c
2.1
Xgwm205d
31.6
Xctgagc156
13.1
Xgwm186
12.9
Xctcagg366
5B
Xctgacc358
13.6
Xgwm156
11.0
Xctcagg236
10.5
Xctcact122
12.4
Xcagagg132
9.8
Xgwm234b
5.7
Xgwm118
2.5
Xgwm415
QFY-5B (m)
19.7
Xgwm213b
5.4
Xgwm213a
0.0
Xgwm335a
2.7
Xctgagc196
5.6
Xgwm371b
4.2
Xgwm371a
6.5
Xgwm499
4.9
Xctcagg222
3.8
Xctcagg224
5.5
Xcttagg254
7.9
Xcaaacg297
4.2
Xgwm639a
7.1
Xgwm554c
19.9
Xgwm408a
2.5
Xgwm408b
3.8
Xcacaag202
5.1
Xcaaacc446
1.8
Xcacaag418
3.1
Xgwm604
1.1
Xcaaacg385
3.5
Xcaaacg340
25.7
Xgwm537b
6A
Xcagagg107
8.3
Xcagagc450
3.7
Xctaacg234
11.8
Xctgaca134
20.6
bgli57
3.9
bgli69
QPC-6A
29.2
Xgwm494b
4.7
Xgwm494a
16.1
Xcaaagg330
10.2
Xgwm570b
2.9
Xctcaag285
6B
Xcagagg633
14.4
Xgwm613
29.8
Xctaacg240
9.0
Xcttagg400
8.7
Xgwm508a
QFY-6B (m)
8.8
Xcaaacg390
8.2
Xgwm518
QSDS-6B (d)
QPC-6B.1 b (d)
QTKW-6B.1 (m)
3.9
Xctcact198
5.2
Xctcact1150
4.8
Xcacaag215
QAC-6B (d)
6.2
Xctcaag175
5.3
Xctgagg179
4.4
Xcaaacg560
QPC-6B.2 (d)
QVit-6B
6.6
Xctcact472
4.7
Xctcagg386
2.8
Xcaaacc376
0.5
Xgwm193
0.0
Xgwm58
0.0
Xgwm88a
QTW-6B (m)
2.8
Xgwm88d
3.9
Xcaaacc400
7.7
Xcttagg402
4.8
Xctgagc417
6.8
Xgwm582a
3.6
Xgwm626a
QTKW-6B.2 (m)
15.7
Xgwm219d
11.6
Xctcact750



7A
7B
g15
Xcaaaca185
15.5
Xgwm60c
QTW-7A (m)
3.4
Xgwm60b
4.6
Xgwm154e
QFY-7A
3.6
Xcaaacg170
20.4
Xctcact133
3.1
Xcagagc115
1.7
Xgwm260
1.4
Xctcaag160
9.9
Xcaaacg198
QYP-7A.1 (m)
34.5
Xgwm276c
7.8
Xgwm276a
12.7
Xgwm282c
6.0
Xgwm63e
QYP-7A.2 (m)
3.4
Xgwm282a
0.0
Xgwm332
8.4
Xctcact196
Xcaaagg417
15.7
Xgwm635
6.3
Xgwm400b
5.7
Xgwm513b
10.9
Xgwm264a
10.7
Xgwm537a
4.2
Xgwm573
5.9
Xctgagg181
QFMT-7B (d)
10.1
Xgwm131d
14.0
Xcttagg492b
15.6
Xcttaag120
18.1
Xcagagg320
Xctcact324
3.7
Xgwm344
QYP-7B (m)
7.5
Xcttagg258
9.2
Xctgaca252
Xcagagc96
17.0
Xctgagc162
6.6
Xctaacg116
14.5
Xcaaaca197
0.0
Xcaaaca195
13.3
Xctcaag345

Conclusion

Grain quality traits were found to be affected by environmental fluctuations, this is particularly true for protein content, vitreousness, and 1000-kernel weight. Further, the mapping population *Omrabi5/ T. dicoccoides600545// Omrabi5* showed significant transgressive inheritance for several traits, e.g.; SDS, farinograph parameters, TKW, and ash content. Using correlation and clustering analysis, the grain quality traits have been grouped in 3 distinct clusters representing protein quantity (PC, Vit, AC), quality (SDS, farinograph parameters), and milling (FY, TKW, TW). Yellow pigment has been clustered with milling characteristics.

For basic genetic studies, development of primary genetic linkage maps is of paramount importance. Furthermore, saturated genetic maps provide geneticists and breeders with powerful tools for quantitative trait mapping, positional gene cloning, and marker-assisted selection. It is of interest to notice, that due to the large genetic distance between the parents of *Omrabi5/ T. dicoccoides600545// Omrabi5* population, a high level of polymorphism was detected (> 60%), although the mapped population is a backcross RIL. The *Omrabi5/ T. dicoccoides600545// Omrabi5* map was constructed with 124 microsatellites as framework markers, 149 AFLPs as saturating markers, and 6 SSPs. The length of the map was 2288.8cM, with an average of marker per 8.2cM. The map showed a high synteny with former published durum and bread wheat maps. The SSR and AFLP markers were evenly mapped across the whole genome. Further microsatellites proved to be good as anchor probes and were very useful in arms and chromosomes identification. AFLP proved also to be good markers for map saturation; most of the combinations were mapped in different genomic regions, particularly the combination *MctgEagc,* which amplified numerous fragments that were mapped in 11 different linkage groups. In agreement with earlier published data, more genetic markers were mapped in the B genome comparatively to the A genome. In conclusion, Gwm-Bread wheat microsatellites amplified and mapped perfectly in durum/ *dicoccoides* genetic background. Generally, they tend to map more than AFLPs.

Indeed, the mapping population showed a fixed genetic structure, as it was developed using single seed descent (SSD) method. This makes *Omrabi5/*

T. dicoccoides600545// Omrabi5 population ideal for assessing the environmental impact on trait expression. In the present study, it was used for determination of QTLs associated with grain-quality traits, but in the future this population will be also used for identification of QTLs linked to agronomic, physiologic, and biotic traits.

For several grain-quality traits, *Triticum dicoccoides* was found to hold novel genes that could be used in durum improvement. These results show to great extend how important is the introgression of these identified desirable genes from *Triticum dicoccoides* to improve durum genetic material. This positive contribution was noticed for protein content; gluten strength; farinograph development, mixing, and stability time; and weakly for ash content. The durum cultivar *Omrabi5*, on the other hand, showed a positive and significant positive effect on test weight, thousand-kernel weight, flour yield, and yellow pigment. The results of this study have concurred perfectly with the theoretical and scientific background for grain quality in the durum breeding programs and with the results generated through conventional methods. These results have also clarified the linkage among traits and identified avenues to break the negative linkages such as of protein content and kernel weight. This will have also an agronomic implication on improving protein content and grain yield.

In this study, it was also demonstrated the importance of mapping and assessing the QTLs of grain quality traits, that are of economic and nutritional interest. The genetic and QTL mapping also have strong implication to comprehend the underlying basic mechanisms of different research disciplines. They can be used to investigate the interaction between different research fields; e.g.; grain-quality, stress-physiology, biotic stress resistance, etc… The results also suggest strongly the use of identified QTLs to enhance the efficiency in plant breeding, especially those showing high explanation rates. In our case the determined QTLs will be subject to immediate validation on diverse genetic backgrounds and use in marker assisted breeding. They could be used as indirect selection criteria for quantitative traits, as breeders will be able to select at the genetic level rather than relying on phenotypic expression. The advantage of working at DNA level is that the molecular marker tests are not affected by environmental fluctuations; and as consequence the use of QTLs as surrogate for the phenotypic expression of the trait is more reliable than the conventional trait testing. As

the molecular marker techniques require small amounts of test material, they could also be applied at very early stage and to large number of populations/genotypes.

Further, molecular biology techniques are less time-consuming and more precise than conventional techniques. Also, their research outputs are highly repeatable, reliable, and less subject to mistakes. Moreover, the attractiveness of these powerful and useful tools is the use of the same protocols, equipments, and reagents for any given trait. Therefore, similar approaches could be used for screening for different characters. This uniformity of working will have definitely positive consequences on research efficiency and speediness.

The concluding remarks could be summarized as follows:

1. Quantitative and transgressive inheritances were revealed for most grain quality traits.
2. Cluster analysis detected 3 major groupings:1) protein content and vitreousness, 2) processing quality, and 3) milling quality.
3. High level of polymorphism between the two parents was detected. The genetic linkage map of *Omrabi5/ T. dicoccoides600545// Omrabi5* population was constructed.
4. SSRs used as anchor probes to identify chromosomal arms and AFLPs as markers to saturate the map.
5. More genetic markers mapped to B than to A genome.
6. *T. dicoccoides600545* alleles contributed positively to protein related traits (PC, SDS, FDT, FST); whereas those of *Omrabi5* to milling traits (TW, TKW, FY) and YP.
7. Thirty-three QTLs found to control grain quality traits. A major gene for yellow pigment was detected on 7BL.
8. Promising QTLs to improve simultaneously TKW and PC in durum are identified.
9. The usefulness of SIM and sCIM analyses to study QTLs genetic and interaction effects has been shown.
10. The *Omrabi5/ T. dicoccoides600545// Omrabi5* mapping population is a potential source for grain quality improvement and for studying QTLs use in durum breeding.

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