



**Dipartimento di Scienze e tecnologie per l'agricoltura, le foreste,
la natura e l'energia
(DAFNE)**

**CORSO DI DOTTORATO DI RICERCA
BIOTECNOLOGIE VEGETALI- XXIV Ciclo**

TUSCIA UNIVERSITY- VITERBO
*DEPARTMENT OF SCIENCE AND TECHNOLOGIES FOR AGRICULTURE, FORESTRY, NATURE AND ENERGY
(DAFNE)*

**PhD Course in
PLANT BIOTECHNOLOGY - XXIV CYCLE
2012**

**Molecular linkage map in an intraspecific recombinant inbred population
of durum wheat
(*Triticum turgidum* L. var. *durum*)**

**Sigla del settore scientifico-disciplinare
AGR/07**

Coordinator

Prof. **Stefania Masci**

Tutors

Prof. **Mario A. Pagnotta**

Prof. **Renato D'Ovidio**

Dottorando:

Iman Yousefi Javan

In the name of God

Acknowledgements

I would be remiss if I did not thank my God and Savior. There were many times that I called on His name for help, comfort, and guidance; He was always right at my side.

I have been truly blessed to work with the best supervisor and scientist: **Prof. M.A. Pagnotta**.

I wish to express my sincerest gratitude to him, for his continuous involvement, supervision, encouragements, patience, devotion, and advices. Not only did he provide professional guidance but extended my horizon to integrate multi-disciplinary approach in research.

I would like to thank him again for his strong support, friendship, and for giving me the opportunity to conduct research and work in his program with scientific freedom; and instill in me the love of Durum. I would like also to express special thanks to my professors/ supervisors Prof. R. D'Ovidio, Pr. M. Nachit, Proffe. S. Masci for their support, kindness, encouragements, constructive criticism, help, and friendship. Their welcoming was always warm, cordial, and friendly. It was a great opportunity to get to know this kind people.

Sincere appreciations and thanks are extended to my colleagues in ENEA and Tuscia University: Dott. L.Mondini, M. Abdolhosseini for their help, friendship, and pleasant way of working. I am thankful to Les Program leader and staff of ICARDA, wheat program.

I gratefully acknowledge ICARDA for their financial support through their Durum Breeding Program in Aleppo/ Syria.

And also so much thanks to my family for they have given everything to me and supported my distance.

LIST OF CONTENTS

pages

Introduction.....	7
Chapter I: Introduction.....	10
1- PLANT OF WHEAT	11
1- 1- The origin of wheat.....	11
1- 1- 1- Natural mutants.....	12
1- 2- Structure and development of Cereal plant	12
1- 3- Wheat plant description	14
1- 3- 1- The grain.....	15
1- 3- 2- Tillers.....	15
1- 3- 3- The roots.....	16
1- 3- 4- The stem	16
1- 3- 5- The ear	16
1- 4- Seed treatment	18
1- 5- Wheat, permitted the rise of Civilization.....	19
1- 5- 1- Emmer	19
1- 5- 2- Einkorn	20
1- 5- 3- Spelt.....	20
1- 5- 4- Bread wheat	20
1- 6- Wild wheat.....	21
1- 6- 1- <i>Triticum dicoccoides</i>	22
1- 7- Cultivated wheats.....	23
1- 7- 1- Diploid wheat	24
1- 7- 1- 1- <i>Triticum monococcum</i>	24
1- 7- 2- Tetraploid Wheat- <i>Triticum durum</i>	25
1- 7- 3- Hexaploid wheat	28
1- 8- ICARDA Center	29
1- 8- 1- Origin of different tetraploid wheat from ICARDA.....	29
2- POPULATION AND MARKER	30
2- 1- The population	30
2- 1- 1- Population size.....	31
2- 1- 2- Segregating populations (F2, F3 and Backcross)	31
2- 1- 3- BIL Population	31
2- 1- 4- DH Population	32
2- 1- 5- RIL Population	33
2- 1- 6- NIL Population	34
2- 2- Developing breeding population.....	34
2- 3- Applications of molecular markers.....	34
2- 4- Molecular marker techniques	36
2- 4- 1- Non- PCR Based Techniques	37
2- 4- 1- 1- Restriction Fragments Length Polymorphism (RFLP)	37
2- 4- 2- PCR-Based Techniques	37
2- 4- 2- 1- Inter simple sequence repeat (ISSR).....	39
2- 5- Microsatellites (SSR).....	39
2- 5- 1- Explanation of the SSR markers.....	39
2- 5- 2- EST- SSR Marker	41

2- 5- 3- SSR markers in the Wheat.....	42
2- 6- Single nucleotide polymorphism (SNP).....	42
2- 6- 1- Explanation of the SNP markers.....	42
2- 6- 2- SNP-EST	44
2- 6- 3- SNP markers in the Wheat.....	44
2- 7- Comparison between SSR and SNP molecular markers	45
2- 8- Target specific genomic regions	46
2- 9- New generation sequencing.....	46
2- 9- 1- Association mapping using natural populations	48
2- 9- 2- Perspectives on genetic mapping and DNA markers.....	49
3- GENETIC MAP.....	51
3- 1- Genetic mapping.....	51
3- 2- Mapping the genome of wheat	52
3- 2- 1- Genetic maps of wheat through molecular markers	55
3- 3- The size of the wheat genome (tetraploid and hexaploid).....	57
3- 4- ITMI map and inter varietal crosses	59
4- SEGREGATION OF MARKERS IN WHEAT CHROMOSOME.....	61
4- 1- Computer software for genetic mapping	61
4- 2- Relationship between DNA marker and cytogenetic maps	62
4- 3- Distorted segregation of molecular markers.....	62
4- 4- Nonrandom segregation of nonhomologous chromosome	63
Chapter II: Material & Methods	66
1- Plant Material.....	67
1- 1- Plant Material.....	67
1- 2- DNA.....	67
1- 2- 1- Station of agricultural species (Tel Hadya Station):.....	69
1- 2- 2- Experimental Design	69
1- 3- SSR Markers.....	69
1- 4- Markers Screening and Amplification.....	72
1- 5- SNPs Identification: Multialignments and primer design	75
1- 5- 1- PCR conditions, sequencing and SNPs characterization	78
1- 6- Data analysis and linkage map construction.....	79
Chapter III: Results & Discussion	81
1- MOLECULAR MARKERS	82
1- 1- Molecular Markers in the population of Jennah Khetifa/ Cham I × Omrabi5/ <i>T. dicoccoides</i> 600545//Omrabi5.....	82
1- 1- 1- Overview of the genetic linkage map	82
1- 2- Microsatellite (SSR) markers analysis.....	83
1- 3- Allele size differences in the SSR markers.....	85
1- 4- The distribution of SSR markers	87
1- 5- Segregation distortion of SSR markers.....	88
1- 6- Clustering of markers (SSR, SNP)	93
1- 7- The RILs distribution between parents for all markers	94
1- 8- Frequency of RILs with parental (non-recombinant) chromosome	96
1- 8- 1- Non-recombinant chromosomes.....	96

1- 9- Characterization of (CT/GA) _n and (GT/CA) _n SSRs in our map	97
1- 10- The distribution of SNP markers	99
2- MAP CONSTRUCTION.....	102
2- 1- Genetic map	102
2- 2- Chromosomes and genomes	103
2- 3- Difference between “A” and “B” genomes	109
2- 4- Changes in location of markers	110
2- 5- Negative crossover interference	111
2- 6- Isogenic line and some trait	112
2- 6- 1- Gene transfer intra specie	113
3- HOMEOLOGOUS GROUP	114
3- 1- Homoeologous group one (1A, 1B).....	114
3- 2- Homoeologous group two (2A, 2B)	115
3- 3- Homoeologous group three (3A, 3B)	116
3- 4- Homoeologous group four (4A, 4B).....	117
3- 5- Homoeologous group five (5A, 5B)	118
3- 6- Homoeologous group six (6A, 6B).....	119
3- 7- Homoeologous group seven (7A, 7B)	120
4- COMPARISON OF PRESENT MAP, WITH THE OTHER MAPS	121
4- 1- Maps comparison.....	121
4- 2- High conserved order of microsatellite loci and structural changes of Chromosomes	124
4- 3- Transferability of the markers	129
4- 4- Parallel mapping in related taxa	130
5- IMPORTANT TRAITS	132
5- 1- The explanation of the traits and characters	132
5- 2- Explanation of the traits associated with markers	132
5- 2- 1- The explanation of Traits - Grain Size and Grain Shape	132
5- 2- 2- The explanation of Trait - PPO (Poly Phenol Oxidases) Pigment Color.....	135
5- 2- 2- 2- Quality of Pasta derivatives from durum wheat	137
5- 2- 3- The explanation of Trait - LOX.....	139
5- 2- 4- The explanation of Traits - GYPC, SC, PC	141
5- 2- 5- The explanation of Trait - GPC	142
5- 2- 6- The explanation of Trait - Rust Disease	144
5- 2- 7- The explanation of Trait - Photoperiod	146
5- 2- 8- The explanation of Trait - Growth.....	148
Chapter IV: General Discussion & Conclusion	152
References.....	156

INDEX OF TABLES

pages

Chapter I

Table 1.1: Shoot structures to identify cereals and selected weeds.	18
Table 1.2: Durum use and quality requirements in different part of the world (Nachit et al. 1992).	27
Table 1.3: The Molecular linkage maps have been constructed in different organisms.	52
Table 1.4: The map of Somers et al. 2004, consensus map.	60

Chapter II

Table 2.1: Origin of Cultivar and wild Species.	69
Table 2.2: The number of SSRs markers used in this work.	70
Table 2.3: The indications for Origins and references of SSR markers.	70
Table 2.4: Probed Polymorphic Microsatellites sequences and their optimal amplification program in Jennah.Ketifa/ChamI × Omrabi5/ <i>T.dicoccoides</i> 600545//Omrabi5.	71
Table 2.5: SNP markers used correctly for the construction of our map came from the two-class.	77

Chapter III

Table 3.1: Number of generated and mapped SSR fragments and their chromosomal assignment in the Omra/dicoccoides//Omrabi5×Jennah.Khetifa/ChamI population in comparison with wheat published maps 85.	85
Table 3.2: Origin of the microsatellites markers used in this study.	87
Table 3.3: Distribution of SSRs markers in each of chromosome for similarity to parents.	90
Table 3.4: Distribution of SSRs markers in the homoeologous group two for similarity to parents, Hetrozigosity and non positive result, in percentage.	91
Table 3.5: Number of durum wheat First Parental (J.K/C1) × durum wheat, Second Parental (O5d/O5) RILs with parental (non-recombinant) chromosomes.	96
Table 3.6: The table indicates the percentage of repeated motifs in the total number of markers, Polymorphic markers and homoeologous groups.	98
Table 3.7: Chromosome assignment, distribution of markers, length of linkage groups, and marker density in genetic map constructed with the J.K/C1 × O5d/O5 recombinant inbred line population.	112
Table 3.8: Islands of Negative Interference along the Chromosomes.	112
Table 3.9: Comparing the SSR markers on the maps of tetraploid and hexaploid wheat, with our markers and shown the similarity between these main maps and our map.	123
Table 3.10: Comparison of allele size differences among mapping parents and microsatellite sources. SE Standard error.	124
Table 3.11: Comparison of map length (cM) of tetraploid wheat genome in various linkage maps generated in different mapping populations, a Mapping populations: (1) durum wheat × wild emmer wheat, (2) durum wheat × durum wheat, (3) bread wheat × bread wheat.	128
Table 3.12: Association of Markers with the characters and Traits. J.K/C: First parental: Jennah Khetifa/ChamI, Od/O: Second parental: Omrabi5//dicoccoides/Omrabi5.	150

INDEX OF FIGURES

pages

Chapter I

Figure 1.1: The distribution of industrial plant in the world (FAO, 2010).....	11
Figure 1.2: Schematic diagram of a mature wheat plant high1	17
Figure 1.3: Schematic diagram of a mature wheat plant high2	17
Figure 1.4: Schematic diagram of a mature wheat plant high3	17
Figure 1.5: Structures of the wheat inflorescence (spike or ear), spikelet and floret of the wheat plant.....	17
Figure 1.6: A diagrammatic representation of the current theory of the evolution of wheat (Wheat Genetics Resource Center web site: http://www.ksu.edu/wgrc).....	22
Figure 1.7: The distribution of the wheat in the world, Top ten wheat producers-2010(million me...)	25
Figure 1.8: World durum wheat trade forecast (CWB, 2008).....	26
Figure 1.9: Center of crop origin, for plant genetic diversity, and ICARDA region contains.....	26
Figure 1.10: Different reaction of different marker on the gel electrophoresis	36
Figure 1.11: Overview of NGS applications in crop genetics and breeding.	48

Chapter II

Figure 2.12: The different type of <i>Triticum turgidum</i> L. subsp. <i>durum</i>	68
Figure 2.13: The figure and the drawing for our cross between the three cultivar varieties of durum wheat and one wild varieties of durum wheat.	68
Figure 2.14: Meteorological data Average of 1999/2009, Min. temp = minimum temperature; Evap. = evaporation; Max. temp = maximum temperature	69
Figure 2.15: Electro program of the GeneScan-500 size standard run under denaturing conditions on the ABI PRISM 310 Genetic Analyzer.....	74
Figure 2.16: Imagines of robot and sequencing.	74
Figure 2.17: The procedure to make the Blast and in the following construction of SNP primers.	75
Figure 2.18: An example of a multi-alignment of sequences used to design primers and different plots of melting curves showing the polymorphism.....	78

Chapter III

Figure 3.1: Identification of SSR markers to be polymorphic and monomorphic between our two parental (J.K/C1) × (O5.d/O5).....	84
Figure 3.2: Graphs on the left came from genemapper V.4.0 evidenced by a peak with a size equal for both parents (Barc263). But the graphs on the right showed two different sizes for screening of relatives (WMS).....	84
Figure 3.3: Analyzed WMS291 Marker on the RIL n.131 and n.113 with FAM florescent trough of Genemapper V.4.0.....	85
Figure 3.4: The differences size between two alleles of homologous chromosomes.	86
Figure 3.5: The average of differences between parents in allele size: (a) different homeologous groups, (b) different SSR markers group, (c) different SSR markers group. Homeologous groups and chromosomes brought together in the same graphical to compare their variability with their average.	86
Figure 3.6: Distribution of the fragments amplified through (a) monomorphic markers, (b) polymorphic markers. For the similarity of parents in the 18 RILs (76-93).....	87
Figure 3.7: Allele frequencies as a function of the genetic linkage map along chromosomes 2A, 2B, 4A and 6A, The x-axis indicates the segregation distortion from the 1:1 ratio observed for each marker, and the y-axis corresponds to the genetic linkage map, Markers marked with crossed square indicate the significance threshold of $P < 0.05$, (a) chromosome 2A that the majority of markers are distributed to the first parental, (b) chromosome 2B that the majority of markers in the short arm are distributed to the first parental, and long arm to the second parental, (c) chromosome 4A that the majority of markers are distributed to the first parental, (d) chromosome 6A that the majority of markers are distributed to the second parental. Segregation distortion between 0.45- 0.55 (45%-55%) frequency of	

alleles,: Frequency of alleles between first and second parental, :Frequency of alleles nearby first parental, Frequency of alleles nearby first parental.	89
Figure 3.8: Distribution of SSRs markers in the homoeologous group two for similarity to parents and Heterozygosity Similar of first parental, Similar of second parental, Heterozygosity.	91
Figure 3.9: Different distribution of SSRs marker in Chromosome, Homoeologous, genome and all the two genomes, // Similar to first parental, // Similar to second parental.	92
Figure 3.10: Distribution of SSR and SNP loci along chromosomes 2A, 2B, and 4B of the of the (J.K/C1) x (O5d/ O5) genetic map.	94
Figure 3.11: The distribution of the 192 RILs the percentage similarity with (a) First Parental J.K/C1, (b) Second Parental O5d/O5,(c) with high similarity to the parental, (4, 5, 27, 62, 147 and 150 RILs number do not exist).	95
Figure 3.12: The dispersion of RILs number in the percentage of similarity (a) to the first Parental (b) to the second Parental. and the equation of the line in this dispersion of 192 RIL.	95
Figure 3.13: The identification numbers of RIL or genotypes for the similarity (a) to the first parental (b) to the second Parental (c) the number of genotypes represented from both parents, These arrows indicated the RIL numbers preferable (superior) in each of the three groups, the similarity of the first parent, the similarity of the second parent.	95
Figure 3.14: Occurrence of parental chromosomes (non-recombinant chromosome) in the mapping data set set as associated with chromosome length (cM).	96
Figure 3.15: The repetition (times) of the SSR motifs (a) in the total and polymorphic markers (b) in differences homoeologous group.	98
Figure 3.16: Distribution of SNPs by the type of nucleotide substitution transitions(C/T; A/G) and transversions (C/A; C/G; T/A; T/G).	100
Figure 3.17: (a) The percentage of polymorphism and monomorphism in the total number of SNP markers, (b) The distribution of SNP markers for the similarity to the two parental and the central part which shows the percentage of heterozygosity.	100
Figure 3.18: Charts to (a) identify Polymorphic SNPs, (b) identify SNPs.	100
Figure 3.19: Indication Genetic map of tetraploid wheat (<i>Triticum durum</i>).	105
Figure 3.20: Location of some markers in our map that changed positions	111
Figure 3.21: (a) The number of total monomorphic and polymorphic SSR markers. (b) The percentage of polymorphic markers (SSR) in different homoeologous group (1-7). (c) The percentage of monomorphic and polymorphic markers (SNP), and the polymorphic markers devising in two class [Group of 4 or other group (1, 2, 3, 5, 6, and 7)]	114
Figure 3.22: Total number of markers used and division between monomorphic and polymorphic in homoeologous group one compared to other homoeologous groups.	115
Figure 3.23: Total number of markers used and those monomorphic or polymorphic in homoeologous group two compared to other homoeologous groups.	116
Figure 3.24: Total number of markers used and those monomorphic or polymorphic in homoeologous group three compared to other homoeologous groups.	117
Figure 3.25: Total number of markers used and those monomorphic or polymorphic in homoeologous group four compared to other homoeologous groups.	118
Figure 3.26: Total number of markers used and those monomorphic or polymorphic in homoeologous group five compared to other homoeologous groups.	119
Figure 3.27: Relationship between the percentage of recombination and distances between markers	120
Figure 3.28: Total number of markers used and those monomorphic or polymorphic in homoeologous group six compared to other homoeologous groups	120
Figure 3.29: Total number of markers used and those monomorphic or polymorphic in homoeologous group seven compared to other homoeologous groups	120
Figure 3.30: (a) The confrontation of our map with the map of Kofa x UC1113 population in chromosome 1A, (b) The confrontation of our map with the map of J.K x Cham II population in chromosome 1A, (c) The confrontation of our map with the map of Messapia x dicoccoides population in chromosome 1A, (d) The confrontation of our map with the map of Omrabi5 x dicoccoides population in chromosome 1A.	125

Figure 3.31: (a) The confrontation of our map with the map of Synthetic × Opata population in chromosome 1A. (b) The confrontation of our map with the map of Synthetic × Opata population in chromosome 2A.....	126
Figure 3.32: (a) The confrontation of our map with the map of Kofa × UC1113 population in chromosome 2A. (b) The confrontation of our map with the map of J.K × Cham II population in chromosome 2A. (c) The confrontation of our map with the map of Messapia × dicoccoides population in chromosome 2A. (d) The confrontation of our map with the map of Omrabi5 × dicoccoides population in chromosome 2A.	126
Figure 3.33: (a) The confrontation of our map with the map of Synthetic × Opata population in chromosome 4A (b) The confrontation of our map with the map of Kofa × UC1113 population in chromosome 4A.....	127
Figure 3.34: The association between A, B, D genome of wheat	130
Figure 3.35: The comparing of our map with the map of Barley, the differences in some inversion in some chromosome.	131
Figure 3.36: The association of some markers with PPO activity trait in several chromosomes (present in our map).....	135
Figure 3.37: The association of some markers with PPO activity trait (present in our map).	137
Figure 3.38: The associations of markers with QTL for gene control.....	137
Figure 3.39: The distribution of Lox gene and LPX locus in the group homoelogenous of 4 and 5	140
Figure 3.40: The association of markers including a short distance with the different traits.	140
Figure 3.41: The association of some markers with GPC trait in several chromosomes(present our map).....	142
Figure 3.42: The association of some markers with GPC trait in several chromosomes(present our map).....	144
Figure 3.43: The association of some markers with temperature-sensitive resistance and FHB traits in several chromosomes (present in our map).	146
Figure 3.44: The association of some markers with APD trait in several chromosomes(present our map).....	148
Figure 3.45: The association of some markers with growth trait in several chromosomes(present our map).....	148

INTRODUCTION

Most projections suggest that wheat, already the most important food source for human-kind, will also be the primary food staple in the developing world in the next 15 years. Demand for wheat in developing countries is expected to grow at around 2.2 percent annually, about the same rate as production growth. By the year 2020, the production-consumption gap that is made up by wheat imports could approach 120 million tons annually, twice today (Rosegrant et al. 1995). It was recently postulated that there would be a need to produce 1 billion tons of wheat by the year 2020 (Braun et al. 1998). These projections assume that increases in global productivity will meet these demands without real price increases, which is highly desirable for consumers. Since increases in the area sown to wheat are likely to be small, productivity increases will have to come almost entirely from the development and application of new technologies. At the same time, greater sustainability and environmental demands on wheat farming systems will have to be met, and yield stability of the world. Genetic improvement of wheat is likely to remain the major source of productivity gains. The crop, yield potential has risen about 25 percent annually over the past 30 years. This trend is expected to continue but may require greater breeding resources, especially as recent gains in efficiency that resulted from computerization and mechanization of breeding begin to dwindle. Innovations in molecular biology appear unlikely to effect an impact on yield progress anytime soon. Despite considerable progress in the last 100 years, huge scope still exists for strengthening and making more durable the resistance of wheat to diseases, viruses and insects. This appears to be the area in which molecular biology will make its first impact on wheat breeding. Molecular biology is also likely to aid conventional breeding in changing the quality of wheat grain by developing it for novel industrial uses and improving its nutritional structure in ways that would clearly benefit consumers (increasing its content of available iron, zinc, vitamin A and certain amino acids).

The last 30 years have witnessed an unprecedented level of international wheat germplasm exchange and the development of a greater degree of genetic relatedness among successful cultivars globally. The concept of broad adaptation has thus been well vindicated. However, this is seen by some as increasing genetic vulnerability to pathogens, although such vulnerability depends more on similarities in resistance genes, which may actually be more diverse now than before. Various new factors, including the growing strength of national breeding programs in the developing world and the advent of breeders' rights, should result in increased diversity among cultivars and perhaps lead to the exploitation of hitherto-overlooked specific adaptation in wheat. This would be especially important if climate change accelerates. Just as increasing nitrogen supply and improving weed

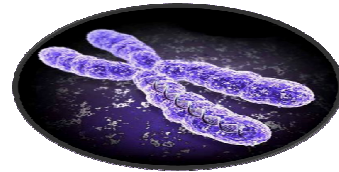
control have been almost universal driving factors of wheat cultivation in the last 50 years, higher atmospheric concentrations of carbon dioxide and global warming with resulting warmer temperatures could significantly influence breeding objectives in the next 50 years.

In the last 10,000 years, the earth's population has doubled ten times, from less than 10m to more than six billion now and ten billion soon. Most of the energy for human nutrition, which made that increase possible, has come from three plants: maize, rice and wheat. Wheat is the oldest and most widespread crop. In the 2007 the wheat world production was 607 million tons, making it the third most-produced cereal after maize (784 million tons) and rice (651 million tons).



CHAPTER I

INTRODUCTION



1- PLANT OF WHEAT

1- 1- The origin of wheat

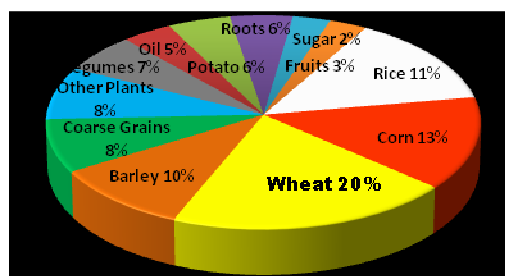
The wheat is derived from three wild ancestral species in two separate inter specific hybridizations. The first took place in the Levant 10,000 years ago, the second near the Caspian Sea 2,000 years later. From the Fertile Crescent extends from Palestine through Syria and southern Turkey into Iraq and western Iran. 7000 BC and then spread quickly as a Neolithic agriculture package to other parts of West Asia, the Nile Valley, and the Balkans. Three millennia later, this wheat-and-barley Neolithic farming system provided food for people living in an extensive area of the Old World from the Atlantic Ocean to the Indian subcontinent and from Scandinavia to the Nile Valley. The Near East crop complex has made a significant contribution to feeding the human population in historical times as well as today (Heun MR et al. 1997).

The hybridization together with domestication resulted in a specie with extra-large seeds incapable of dispersal in the wild, dependent entirely on people to sow them.

The wheat diffusion was parallel with human populations' migrations. The farmers brought not only seeds, but also their habits and agricultural practices: not just sowing, reaping and threshing, but baking, fermenting, owning, hoarding, as well as the wheat use in animal feeding to get meat and milk. The wheat from its origin site in the Middle East spread overall the world, 5,000 years ago reached Ireland, Spain, Ethiopia and India. A millennium later it reached China: paddy rice was still thousands of years in the future (Ozkan et al. 2002).

The wheat plant evolved mainly for three traits to suit its new servants: the seeds grew larger; the “rachis” which binds the seeds together became less brittle so whole ears of grass, rather than individual seeds could be gathered. And the leaf-like glumes that covered each seed loosened, thus making the grains “free-threshing”. In the past twenty years, the different mutations of loci involved in these changes have been located within the wheat plant's genome (Colledge 2007).

Figure: 1.1: The distribution of industrial plant in the world (FAO, 2010).



1- 1- 1- Natural mutants

Eager farmers took it up with astonishing results. By 1974, Indian's wheat production had tripled and India was self-sufficient in food; it has never faced a famine since. In 1970 Norman Borlaug was awarded the Nobel Peace Prize for firing the first shot in what came to be called the “green revolution”. Borlaug had used natural mutants; soon his successors were bringing on mutations artificially.

Scientists used thermal neutrons, X-rays, or ethyl methane sulphonate, a harsh carcinogenic chemical “anything that will damage DNA” to generate mutant cereals. Most of the wheat varieties growing in the field were produced by “mutation breeding”. The irony is that genetic modification (GM) was invented in 1983 as a gentler, safer, more rational and more predictable alternative to mutation breeding-an organic technology. Instead of random mutations, nowadays scientists could add the traits they wanted. In 2004, 200m acres of GM crops were grown worldwide with good effects on yield (up), pesticide use (down), biodiversity (up) and cost (down). Yet, far from being welcomed as a greener green revolution, genetic modification soon ran into fierce opposition from the environmental movement. Around 1998, a century after Crookes and two centuries after Malthus, green pressure groups began picking up public disquiet about GM and rushed the issue to the top of their agendas, where it quickly brought them the attention and funds they crave (Staple food, 2005).

Wheat, because of its unwieldy hexaploid genome, has largely missed out on the GM revolution, as maize and rice accelerate into world leadership. The first GM wheats have only recently been approved for use, their principal advantage to the farmer being so-called “no till” cultivation the planting of seed directly into untilled soil saves fuel and topsoil (Ears of plenty, 2011).

1- 2- Structure and development of Cereal plant

Cereals belonging to the *Graminaceae* family. Worldwide, the most cultivated are wheat, corn, rice, barley, oats and rye. Wheat (*Triticum aestivum*) has been, since prehistoric times, the most important cereal. This feature depends on its adaptability to all types of terrain and different climates. Therefore its cultivation area is between 30°-60° north latitude and 20°-40° south latitude and it is found in culture even at the equator and beyond the Arctic Circle.

Like all of the temperate cereals, wheat undergoes profound changes in structure through its life cycle. The delicate growing point at the shoot apex, at first produces leaves, and then later changes to form the flowering spike or ear. The stem, at first compact and measuring a few millimeters, rapidly expands to a structure that may be a thousand millimeters or longer.

Plant growth and development concerns the length of the plant's life cycle, its subdivision into distinct stages, and the processes of formation of the plant's organs, the leaves, tillers and spikelets. These organs are important because are the basis for the plant adaptation to environments. Cultivars with differing developmental patterns are needed for different sowing dates and regions.

Structural and developmental patterns are also important because many decisions about nutrition and crop management are best made on a developmental rather than a calendar time scale.

The major developmental processes for a cereal are:

- 1-Germination and seedling establishment
- 2-Initiation and growth of leaves
- 3-Tillering
- 4-Growth of the root system
- 5-Ear formation and growth
- 6-Stem extension
- 7-Flowering and grain growth (Lotti 2000).

1- 3- Wheat plant description

**Planting****Soil:** Adequate**Sunlight:** Full**Days to Harvest:** 120 D

The cereals group includes several genera such as wheat (*Triticum*), barley (*Hordeum*), Oats (*Avena*), rye (*Secale*) and the man-made hybrid triticale (*Triticosecale*).

There are about 30 species of wheat, and more than 40,000 cultivars have been produced in the world. Wheat species can be divided into three groups depending on the number of chromosomes present in vegetative wheat plant cells: diploid (14 chromosomes); tetraploid (28 chromosomes); and hexaploid (42 chromosomes). These species can cross breed in nature or by plant breeders. Only three species of wheat are commercially important:

1-*Triticum aestivum*, 2-*Triticum turgidum* cv. *durum* – durum wheats: hard wheats (from Latin, *durum*, meaning ‘hard’), e.g. cv. Yallaroi and Wollaroi. Flour from these wheats holds together well due to high gluten content, cultivars are generally used for pasta and bread products. 3-*Triticum compactum* – club wheats: This species is sometimes considered a subspecies of common wheat. These are usually soft grained wheats often used for cake flour (<http://www.kstate.edu/wgrc/Taxonomy/taxtrit.html>).

Wheat and all other grasses have a common structure which provides the basis for understanding the growth and development of the crop and the reasons for particular management practices.

Wheat is a grass between 50 and 150 cm high. The physical appearance of the grain is familiar to most consumers, with a long stalk that terminates in a tightly formed cluster of plump kernels enclosed by a beard of bristly spikes. Wheat is an annual, which means that at the end of each year, fields must be plowed and prepared again to grow the grass.

Ancestral wheats looked very different, with much smaller kernels. The early domesticators of wheat obviously wanted to select for plants with particularly large kernels, since more nutrition could be eked out from each stalk. Because wheat is generally a self pollinating plant, each plant tends to produce pure lines. When farmers want to hybridize a wheat strain, they must physically pollinate the different plants. Farmers blending wheat for various purposes usually combine different seeds at harvest time and spread them evenly over the field. The wheat grown in general falls into two categories: winter wheat, which is planted in the fall and matures in the summer, and spring wheat, which is planted after the danger of frost is over and also matures in the summer.

Wheat's characteristic golden color at harvest time is well known and often appears in artwork that uses wheat (Dubcovsky and Dvorak 2007).

1- 3- 1- The grain

The grain, in cereals, is the small (3-8 mm long), dry, seed-like "fruit" of a grass, especially a cereal plant. (kernel is an older term for the edible seed of a nut or fruit). Grain is considered as a one-seeded "fruit" (called a caryopsis) rather than a "seed". A seed is a mature ovule which consists of an embryo, endosperm and the seed coat. However, a fruit is a mature ovary which includes the ovule or seed, in addition to the ovary wall that surrounds the seed (pericarp). In wheat, the pericarp is thin and fused with the seed coat (Figure 1.2), and this makes wheat grain a true "fruit". In other plants the pericarp may be fleshy as in berries or hard and dry forming the pod casing of legumes (Setler and Carlton 2000).

Seen in cross-section (Figure 1.4), the main constituents of grain are the bran coat, the embryo or young plant, and the endosperm. In most wheat cultivars, the proportions of grain are: bran 14%, endosperm 83% and embryo 3%. The bran coat, covering the grain, is made up of an outer pericarp derived from the parent plant ovary wall; a testa or seed coat derived from the ovule. And the aleurone layer, important as a source of enzymes and growth factors in germination (Figure 1.2).

The endosperm makes up the bulk of the grain, it provides the energy for the seed germination, and it is the store of starch and protein which is milled for production of white flour.

The whole wheat flour is made of the ground products of the entire grain and therefore naturally contains more vitamins and minerals from the bran and embryo.

The embryos (Figure 1.2) consists of a short axis with a terminal growing point or shoot apex, and a single primary root known as the radical. Around the growing point are the primordial of the first three leaves (Cramb et al. 2000).

1- 3- 2- Tillers

Tillers are basal branches which arise from buds in the axils of the leaves on the main stem. Structurally, they are almost identical to the main stem, and are thus potentially able to produce an ear. Leaves on a tiller are also produced alternately. The tiller is initially enclosed in a modified leaf – the prophyll – which is similar to the coleoptile that encloses the main stem during emergence (Figure 1.3). Reduced tillering in new cereal cultivars is proposed by some scientists to try and increase yield, i.e. by eliminating stems that do not produce ears. However, tillers that have their own roots produce ears. Tillers may also contribute to grain yield as a source or sink for excess sugars and nutrients of the main stem. In the localities where insects, diseases or environmental stresses are common, tillers offer assurance that crop losses are minimal. The wide range of

environments where wheat is grown resulted in a diversity of cereal plant types (Setler and Carlton 2000).

1- 3- 3- The roots

Seminal roots which develop from primordial within the grain. As is the case for leaves and tillers, all the root axes of a plant can be given designations to describe their position, type and time of appearance on the plant. The growing, meristematic tissues of roots are located in the first 2-10 millimeters of the tip of each root. Hence, as roots grow the meristematic tips move further away from the shoot deep into the soil. This contrasts with the structures of blades and sheaths where the meristematic tissue remains close to the stem and pushes older tissues away from the plant (Figure 1.3). Why roots have evolved differently from leaves to have their growing meristematic tissue at the tip is unknown (Cramb et al. 2000). One possibility is that this allows immediate control over root growth in the event of environmental changes such as water or nutrient supply. Hence this enables better control of the direction of root growth in the diverse soil matrix.

1- 3- 4- The stem

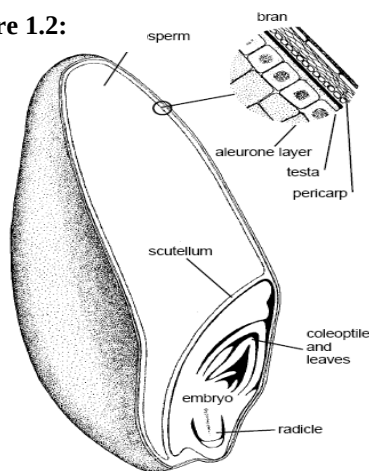
The stem of the wheat plant is made up of successive nodes, or joints, and internodes (Figs 1.2; 1.3). The stem is wrapped in the sheaths of the surrounding leaves. This structure of stem and sheaths gives strength to the shoot, and it is what keeps the cereal shoot erect and reduces lodging. Nodes are the places on stems where other structures such as leaves, roots, tillers and spikelets join the stem. This is also where the vascular channels carrying nutrients into and out of these organs join the vascular connections of the stem. Only when stem elongation begins, do the internodes begin to grow to form the characteristic tall jointed stem of the mature wheat plant (Figure 1.3). As the stem grows, it evolves from a support tissue for leaves, to also become a storage tissue for carbohydrates and nutrients in preparation for subsequent grain filling. At the time of ear emergence, carbohydrates account for 25 to 40% of the dry weight of stems of most wheat. This is an adaptive trait for wheat grown in rainfed environments, since even if severe drought occurs at the end of the season this carbohydrate can be used to fill some grains.

1- 3- 5- The ear

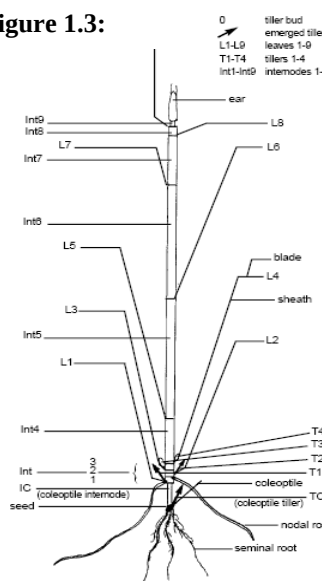
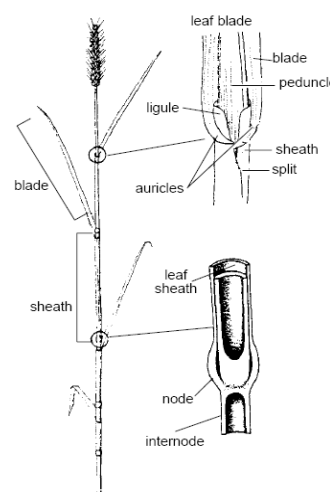
The inflorescence or ear of wheat is a compound spike made up of two rows of spikelets (Figure 1.5) arranged on opposite sides of the central rachis. Like the stem, the rachis consists of nodes separated by short internodes, and a spikelet is attached to the rachis by the rachilla at each node. From the ears structure it is possible to identify the diploid, tetraploid and hexaploid wheat. On each ear there is a single terminal spikelet arranged at right angles to the rest of the spikelets (Figure 1.5). At the base of each spikelet there are two chaffy bracts called sterile or empty glumes

(Fig. 1.5). These enclose up to ten individual flowers called florets, although the upper florets are usually poorly developed. Generally only 2 to 4 florets form grains in every spikelet (Cramb et al. 2000).

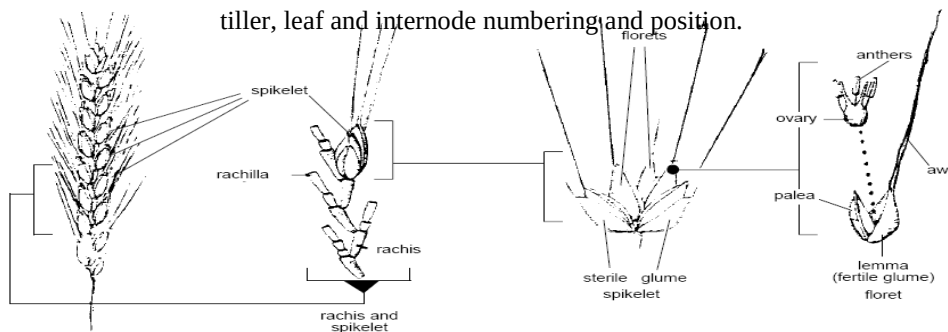
A typical wheat ear will develop 30 to 50 grains. Each flower will produce one grain which grows in the axil of a bract called the lemma, and is enclosed by another bract called the palea. The long awns (sometimes called "beards") found on many modern wheats are extensions of the tip of the lemma (Fig. 1.5), (Kirby and Appleyard 1987).

Figure 1.2:

Structure of the wheat grain.

Figure 1.3:**Figure 1.4:**

Schematic diagram of a mature wheat plant highlighting tiller, leaf and internode numbering and position.

**Figure 1.5:** Structures of the wheat inflorescence (spike or ear), spikelet and floret of the wheat plant**Table 1.1:** Shoot structures to identify cereals and selected weeds.

Grass	Ligule	Auricles	Leaves	Leaf sheath
Wheat	Fringed membrane	Yes-large clasping, with hairs	Usually twist clockwise; no hairs	split
Barley	membrane	Yes-very large, without hairs	Twist clockwise; usually hairless	split
Oats	membrane	none	Twist anticlockwise; no hairs	split
Annual Ryegrass	membrane (<2 mm)	yes – large, clasping	No hairs	Slight split
Barley grass	membrane (<2 mm)	yes – large, pointed	Soft hairs	Slight split
Wild oats	membrane (<2 mm); hairless	none	Twist anti- clockwise; no hairs	split



1- 4- Seed treatment

Seed health is an important attribute of quality, and seed used for planting should be free from pests. Seed infection may lead to low germination, reduced field establishment, severe yield loss or a total crop failure. For example, severely infected wheat grains with Karnal bunt either fail to germinate or produce a greater percentage of abnormal seedlings (Singh 1980; Singh and Krishna 1982). In wheat, fungi (*Fusarium* spp., *Tilletia* spp., *Drechslera* spp., *Septoria* spp. and *Ustilago* spp.), bacteria (*Corynebacterium*, *Pseudomonas* and *Xanthomonas*) and nematodes (*Anguina tritici*) are the most important seed-borne diseases due to their worldwide distribution and losses they incur in crop production (Mamluk and van Leur 1986; Diekmann 1996 a).

Chemical seed treatment is one of the efficient plant protection. But another way more effective than this treatment can be used genetic. These resistance genes protected young seedlings or adult plants against attack from seed-borne, soil-borne or airborne pests. The disease-resistance genes have been transferred from wild grasses into bread wheat using a new pre-breeding technique that allows the genes to be inherited together.

Led by Dr Phil Larkin and Dr Ligia Ayala-Navarrete, the CSIRO team targeted the Lr19, Sr25 and Bdv2 genes that are effective against leaf rust, stripe rust and barley yellow dwarf virus. In tests against all races of the new stem rust pathotype Ug99, the Sr25 gene has provided total resistance while Bdv2 is the first gene to provide true resistance to barley yellow dwarf virus, the most widespread and economically significant cereal virus in Australia. The CSIRO strategy involves two wild relatives of bread wheat: *Thinopyrum ponticum* (the donor of Lr19 and Sr25) and *Th. intermedium* (the donor of Bdv2). For each wild species, the team selected translocations that moved a chunk of wild chromosome (containing the disease-resistance genes) to wheat chromosome 7D. These translocated chromosomes were then crossed so that during sexual reproduction – when chromosomes pair up to exchange DNA – the *Th. intermedium* and *Th. ponticum* fragments were also brought together.

In the hexaploid wheat (*Triticum aestivum* L.) more than 50 leaf rust resistance (Lr) genes against the fungal pathogen *Puccinia triticina* have been identified in the wheat gene pool, and a large number of them have been extensively used in breeding. Of the 50 Lr genes (AWLr10, Lr23), all are known only from their phenotype and/or map position except for Lr21, which was cloned recently (Feuillet et al. 2003).

In wheat, race-specific resistance to the fungal pathogen powdery mildew (*Blumeria graminis* f. sp. *tritici*) is controlled by the Pm genes. There are 10 alleles conferring resistance at the Pm3 locus (Pm3a to Pm3j) localized on chromosome 1AS of hexaploid wheat (*Triticum aestivum* L.).

The diploid *T. monococcum* and the tetraploid *T. turgidum* ssp. *durum*, also carry the gene pm3 (Yahiaoui et al. 2004).

Rong et al. (2000) working in *Triticum turgidum* var. *dicoccoides* have been verified that the powdery mildew resistance gene was designated as Pm26 associated with some markers (WMS149, WMS445).

Seed production in disease-free areas or under effective disease control and field inspection schemes is very important to obtain disease-free seed. So knowing the genes involved in the resistance mechanisms against these diseases will save time in eliminate the damage came from pathogen agents.

1- 5- Wheat, permitted the rise of Civilization

The wheats known today are cereals that evolved in the Middle East through repeated hybridizations of *Triticum* spp. with members of a closely related grass genus, *Aegilops*.

The process which began some ten thousand years ago involved the following major steps. Wild einkorn *T. boeoticum* crossed spontaneously with *Aegilops speltoides* to produce Wild Emmer *T. dicoccoides*; further hybridizations with another *Aegilops*, *Ae. squarrosa*, gave rise to Spelt, Emmer *T. dicoccum* and early forms of Durum Wheat. Bread Wheat finally evolved when cultivated Emmer re-crossed with *Ae. squarrosa* in the southern Caspian plains. This evolution was accelerated by an expanding geographical range of cultivation and by human selection, and had produced Bread Wheats as early as the sixth millennium BC (Gregg 2001).

Modern varieties and Major cultivated species of wheat are selections caused by natural mutation starting with:

1- 5- 1- Emmer

Emmer (*Triticum dicoccum*), a tetraploid species, cultivated in ancient times but no longer in widespread use. A low yielding, tall (2m) awned wheat with small grains and originating from a mutation with no husk. Closely related to the modern durum wheat used for pasta, Emmer dates from approximately 7000 BC. This wheat along with barley has been found on sites, including the Pyramids, all over the near east and Europe from the earliest times. In fact Emmer wheat was the staple cereal of prehistory, the real reason why early agriculture actually worked. Even today it is grown in remote areas of Turkey and Syria (Fleure and Davies 2001; Ozbek et al. 2007).

1- 5- 2- Einkorn

Einkorn (*Triticum monococcum*), a diploid species with wild and cultivated variants. Domesticated at the same time as emmer wheat, but never reached the same importance. It was widely cultivated in Neolithic times and, by the Iron Age, Bread Wheat *T. aestivum* was sustaining populations in much of Europe. A sub species, Club wheat *T. compactum*, was notably grown by Neolithic farmers in Swiss lake side villages. Identification of the types of crops grown in the Iron Age comes from three sources of evidence; carbonized seed, pollen grains and impressions of seed fired into pottery. In proportion related to the climate of the site; Einkorn is more resistant to cold, heat, drought, fungous diseases and bird predation, although its yield is lower than those of emmer, spelt and naked wheat (Chen et al. 2009).

1- 5- 3- Spelt

Spelt (*Triticum spelta*), a hexaploid species cultivated in limited quantities. Similar to Emmer but with a tough husk that cannot be removed. Spelt was probably first sown and harvested in the Bronze Age. Spelt has an appalling yield (by weight, not volume) and even when threshed is mostly husk, consequently it is not surprising that Bronze Age man had very worn teeth. Along with Emmer wheat, Spelt was grown extensively in Britain during the late Iron Age and the Roman period. Its modern use is for specialist bread and breakfast cereals (Fleure and Davies 2001).

1- 5- 4- Bread wheat

Bread wheat (*Triticum aestivum*), a hexaploid species that is the most widely cultivated in the world. Husk free and with (usually) no awns, it is typically short (less than 1m) and stands well in highly fertile situations (Gregg 2001).



1- 6- Wild wheat

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, based on inter specific or intra specific 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. 1995).

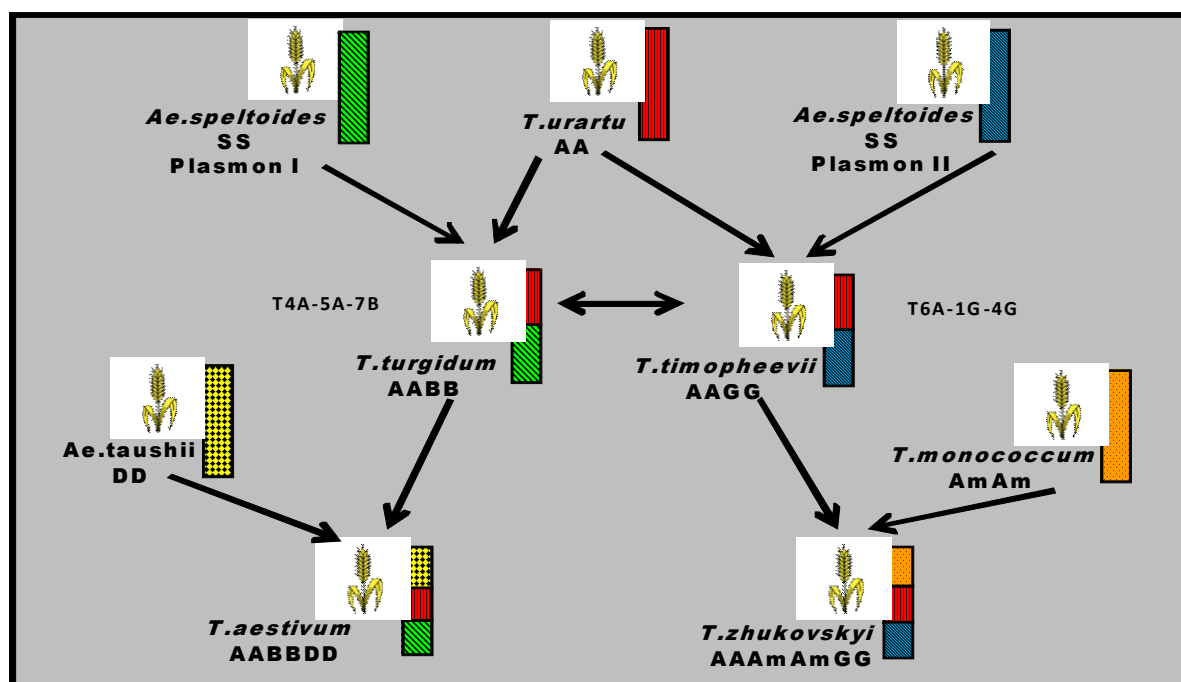
Wild species, relatives to actual wheats, belong to *Triticeae* tribe. The genus has Wild as well as domesticated species occur in both the diploid and tetraploid groups. Only domesticated species occur in the hexaploid group, although Dekaprelevich (1961) reported hexaploid wheat with fragile rachis 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. Nevertheless, *Triticum monococcum* L. is not exactly free-threshing. These differences between wild and domesticated wheats are apparently caused by gene mutations accompanied by selection under domestication (Sharma and Waines 1980).

The *Triticeae* tribe 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* (Fig. 1.6). 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.

Figure 1.6: 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).

1- 6- 1- *Triticum dicoccoides*

Between tetraploid wheat, the first species is *T. turgidum* from var. *dicoccoides* and other wild form of *T. timopheevii*, now these are species, do these have a common origin or separately as there are different. 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 (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. and 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 (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 Haurani plain.

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. 1995). 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 w35/g45/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. (1995 b), by evaluating F6 progenies of *T. durum* × *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).

1- 7- 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:

Genetic and cytogenetic analyses conducted mainly at the hexaploid level have demonstrated that the chromosomes of the three basic genomes in hexaploids (ABD), two in tetraploids (AB) or one in diploid (A^m), 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).

1- 7- 1- Diploid wheat

The A genome of diploid wheat consists of 5 billion bp of DNA organized into seven pairs of chromosomes. Kuspira and his colleagues (Friebe et al. 1990) carried out cytogenetical studies and developed a primary trisomic series that proved to be of limited value due to its high sterility. Friebe et al. (1990) constructed the standard karyotype, and all chromosomes were individually identified. In addition to cytological polymorphism associated with different geographical populations, the diploid wheats contain a translocation involving chromosomes 4A and 5A (Dubcovsky et al. 1996). There has been some differentiation between the A genome of diploid and polyploid wheats as evidenced from the reduced level of pairing, or absence of pairing in the case of chromosome 4A (Gill and Chen 1987), in the amount of C-heterochromatin (Friebe and Gill 1996) and other structural features (Jiang and Gill 1994).

Genetic transfers are feasible from diploid to polyploid wheats by bridging crosses or direct crosses (Cox et al. 1991). However, because of genome differentiation, linkage drag is likely, and methods to enhance recombination may need to be deployed (Dubcovsky et al. 1995). Those who wanted to improve *T. monococcum* by crosses with 4x and 6x wheats were disappointed as such transfers are impossible (Sharma and Waines 1981).

1- 7- 1- 1- *Triticum monococcum*

Is still cultivated as feed for poultry and swine in the mountains of some Mediterranean countries (Italy, Spain, Turkey, Iran, etc.). Diploid cultivated wheat, *Triticum monococcum* L. ssp. *monococcum* L. ($2n = 2x = 14$), is one of the most ancient crops domesticated in the Middle East (Harlan 1980). *T. monococcum* is closely related to *T. urartu* Thum. However, their hybrids are sterile (Johnson and Dhaliwal 1976). *T. monococcum* was assumed to be the ancestor of the A genome of polyploid wheats. Chromosome pairing and recombination between *T. monococcum* chromosomes individually substituted in wheat and the wheat chromosomes of the A genome is low if the wheat Ph1 locus is active (Paull et al. 1994; Dubcovsky et al. 1995a), which indicates that some differentiation has occurred between these genomes. The existence of genome differentiation between *T. monococcum* and to other species is also evident from extensive differences in the restriction profiles of repeated nucleotide sequences and the promoter region of the 18S5.8S-26S rRNA genes, which show very little intraspecific variation in the *Triticum* species (Dvorak et al.

1993). For these reasons, it was proposed to redesignate the genome of *T. monococcum* as (A^m) (Dvo et al. 1993; Dubcovsky et al. 1995 a).

Since the cultivars of *T. aestivum* have low levels of polymorphism, while wild genotypes of *T. monococcum* show high levels of restriction fragment length polymorphism (Castagna et al. 1994; Le corre and Bernardi 1995), *T. monococcum* was used to produce high-density RFLP maps that complemented the genetic maps of *T. aestivum*. A similar rationale was used for the construction of linkage maps of *T. tauschii* (Coss.) Schmalh. (Kam-morgan et al. 1989; Gill et al. 1991; Lacuda et al. 1991).

The fact that linkage groups in this map are longer than the linkage groups in the genetic maps of other species in the tribe *Triticeae* complicates comparisons. A map of chromosome $1A^m$ of *T. monococcum* ssp. *uegilopoides* has been reported and was of a similar genetic length as a map of chromosome 1A of *T. aestivum* and for this reason that A genome is the unnamed (A^m) (Dubcovsky et al. 1995a).

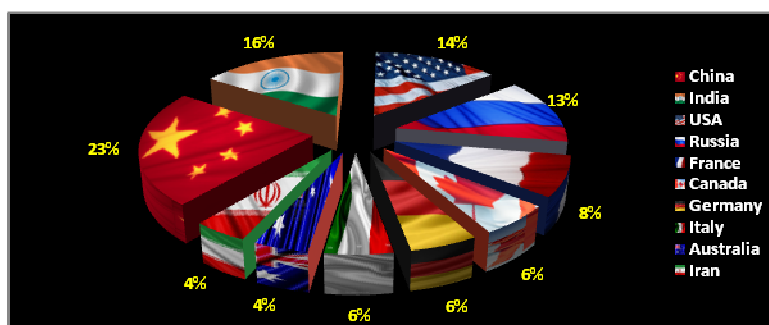
1- 7- 2- Tetraploid Wheat- *Triticum durum*

Durum wheat (*T. turgidum* subsp. *durum*) is a tetraploid species ($2n=28$, genomes AABB), a very important crop for the human diet, particularly in the Mediterranean basin where about 75% of the world's durum grain is produced. With about 21.0 million hectares under cultivation (about 8% of the total wheat cultivated area), durum wheat ranks eighth among all cereals. Except for the small amount used in manufacturing couscous and local bread in some Mediterranean countries, its only significant finished product is represented by alimentary pasta. With wide variation due to drought stress, diseases and pests.

The major exporting countries of the European Union (EU), Canada, and the United States combined, account for 51 percent of total durum production, followed by Syria, Morocco, Russia, Turkey, Mexico.

Figure 1.7:

The distribution of the wheat in the world, Top ten wheat producers-2010 (million metric ton).



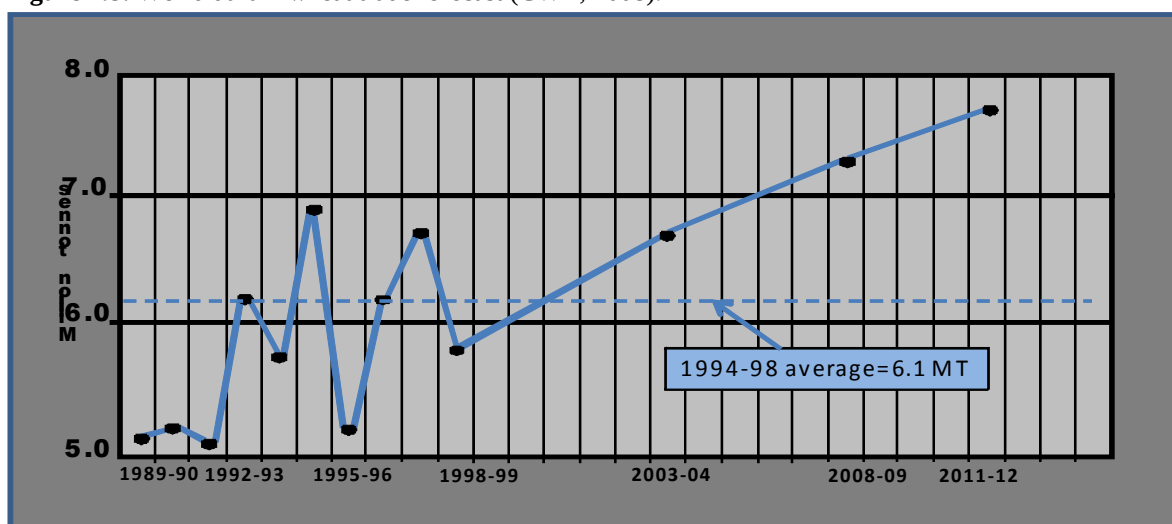
Wheat is the major crop in Syria with annual production of 2.5 million tons. It is planted in different agroclimatic zones, in both rainfed and irrigated areas.

Measurements of genetic diversity in cultivated crops have important implications for plant breeding programs and for the conservation of genetic resources (Tinker et al. 1993).

The EU's 2009/10 durum crop is estimated to be 8.0 million tons. Europe's durum crop is concentrated along the drier Mediterranean Basin area with Italy, France, Spain, and Greece being the largest durum producing countries. Italy is the main market for the European Community durum wheat. With a growth area larger than 1.5 million hectares, and a production of about 4t million tons, Italy is one of the most important durum wheat producing countries in Europe.

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) In addition, durum wheat trade expectations for the coming years show an increase of 21% by 2012 (CWB, 2008).

Figure 1.8: World durum wheat trade forecast (CWB, 2008).



The cause of such differences is mainly due to the different pedology and climatic conditions. In fact, durum wheat is primarily grown under rainfed conditions, and grain yield is strongly limited by the frequent and irregular occurrence of drought combined with heat stress at the late phases of the growth cycle (Araus et al. 2002; Condon et al. 2004). Furthermore, fungal pathogens and other diseases also concur to reduce the field performance with losses reaching 30-50% in grain yield. For these reasons, the improvement of plant capacity to cope with water stress and the accumulation of disease resistance genes into the same genotype are the main objects of the breeding programs for durum wheat in order to reduce the gap existing between potential and actual yield.

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 1.2). Recently, the researchers from some countries (China, Iran, India) to making Nano-Crystalline cellulose, that came from straw of durum wheat. These Nano-Crystalline are cells that contain a natural polymer with microfibrils by highly order, that trough of Plants constantly being produced. Generally, these end-products require large vitreous kernels with high protein content, good yellow pigment and strong gluten.

Table 1.2: 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.

As compared to hexaploid wheat, durum wheat underwent a more limited selection until 1960, when more intense breeding programs based on innovative germplasm introgressions and multi-environment testing for wide adaptation was applied also to durum wheat. Accordingly, the genetic gains obtained after 1970 in grain yield (GY) of durum wheat are comparable to those obtained for hexaploid wheat. These gains have mainly been attributed to a balanced improvement in fertility because of higher allocation of assimilates to the growing tillers and ears concomitant with a general increase in total biomass production, with the harvest index remaining practically unchanged (Slafer and Andrade 1993; Slafer et al. 1996; Pfeiffer et al. 2000; De Vita et al. 2007; Slafer and Araus 2007). As suggested by Pfeiffer et al. (2000), GY components have reached a near-optimal balance in modern elite durum wheat cultivars. While the improvement of GY under optimal growing conditions has prevalingly been attributed to increased spike fertility, under Mediterranean- like conditions the importance of traits at the basis of growth plasticity, such as early vigor and a finely tuned heading date that allows the plant to escape from terminal drought, has been universally recognized (Richards 2000; Spielmeyer et al. 2007).

High-yielding cultivars endowed with drought tolerance and disease resistance, high grain yield, in addition to high commercial and technological value, are therefore highly desirable. Due to the complex genetic basis and the high genotype – environment interaction of quantitative traits as the yield capacity and the tolerance to abiotic and some biotic stresses, the success obtained with traditional breeding approaches have been somehow limited. Therefore, the integration of the traditional breeding methods with modern technologies based on molecular markers is expected to open new opportunities for selection of high yielding durum wheat cultivars in environments characterized by biotic and abiotic constraints. In particular, plant physiology has provided new insights and developed new tools to understand the complex network of drought-related traits, and molecular genetics allows to map disease resistance genes and QTLs affecting the expression of drought tolerance-related traits. This kind of studies makes possible to dissect the genetic mechanisms of analyzed traits and the identification of linked molecular markers that can be used to transfer useful alleles into elite cultivars in order to reduce the gap between yield potential and actual yield (Spielmeyer et al. 2007).

A wide research program is in course at ICARDA. A number of segregating populations, together with the corresponding genetic maps have been developed in the frame of this project, by starting from crosses between durum wheat varieties contrasting for the traits of interest. The work described in this thesis was carried out by using some of these genetic materials, and was focused on studying genetic basis of yield-related traits, and resistance to biotic and abiotic stresses for the improvement of field performance of durum wheat in the Middle East environment.

1- 7- 3- Hexsapioid wheat

Hexsapioid or Bread wheat has a genome size of 16 billion base pairs (bp) of DNA organized into 21 pairs of chromosomes, seven pairs belonging to each of the genomes A, B and D (Sears 1954; Okamoto 1962). Sears (1954) identified individual chromosomes of wheat by monosomy and made some observations on their gross morphology, although most chromosomes were cytologically indistinguishable.

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. It was known as modern wheat, and for this reason that most researchers are to change, with the objectives (1) Production potential: Balanced development between tillering, ear length and density, ear size and number of kernels. (2) Quality: the amount of protein, especially gluten and other nutrients present in grain. (3) Resistance to the following odds: Rusts, Ginning, developing a variety that has very tough husk, Cold, Cultivating a variety that has a delay in the rising, Warm, nurturing species that have early maturing.

1- 8- ICARDA Center

Syria is a producer of durum wheat, this country is a subset of the ICARDA Central. In this project thesis was used species cultivated in Syria and assessment under the ICARDA central. In 1972, the CGIAR commissioned a team of experts to study. The proposed center would focus on sub-tropical (temperate) zones (dry areas). ICARDA center is founded in 1975 in Aleppo (the heart of the Fertile Crescent) and Canada's International Development Research Centre as the executing agency. This region of economic and strategic importance. The ICARDA region contains three of the eight centers of crop origin (see map). Plant genetic diversity in the region is almost unique and this diversity allows scientists to uncover new genes that control vital traits such as drought tolerance, disease resistance or grain quality. The many countries are donors of ICARDA, most important countries are Canada, United States of America, Germany, UK, Italy, Syria, Iran, and European Union).

Figure 1.9: Center of crop Origin, for plant genetic diversity, and ICARDA region contains.



1- 8- 1- Origin of different tetraploid wheat from ICARDA

ICARDA cultivars and advanced materials			ICARDA cultivars and advanced materials	
Anton	1991	Spain	Anton	--
Belik 2	1987	Lebanon	Belik 2	CR/STK
Don Pedro	--	Spain	Don Pedro	CARC/AUK
Heider	1997	Iran	Heider	CAN2 109/2/JO/AA/3/S15/CR
Kabir 1	1993	Algeria	Kabir 1	OVI/CP/2FG
<u>Kofa</u> = Cham 3	1987	Syria	Korifla = Cham 3	DS15/GEIER
<u>Omrabi 3</u> = Cham 5	1993	Syria	<u>Omrabi 3</u> = Cham 5	JO/Haurani
Roqueno	1991	Spain	Roqueno	--
Jabato	1989	Spain	Jabato	--
Waha = <u>Cham 1</u>	1984	Syria	Waha = <u>Cham 1</u>	PLC/RUF/2/GTA/RTTE

2- POPULATION AND MARKER

2- 1- The population

One of the most critical decisions in constructing a linkage map with DNA markers is the mapping population. In making this decision, several factors must be kept in mind, the most important of which is the goal of the mapping project. Is the goal simply to generate a framework map to provide a set of mapped loci for the future, or instead, to identify and orient DNA markers near a target gene for eventual map-based cloning (Young 2000).

Perhaps the goal is mapping quantitative trait loci (QTL), or the monitoring of several disease resistance loci in the process of pyramiding them into a single background.

Whichever goal is the motivating factor behind mapping, it will have a critical influence on which parents are chosen for crossing, the size of the population, how the cross is advanced, and which generations are used for DNA and phenotypic analysis (Young 2000).

To obtain a linkage map it is required a segregating population obtained by parents sufficiently genetically diversified, in order to have a great amount of polymorphism between them. The high genetic polymorphism allows to evaluate the linkage between the different markers and determine their disposition along the chromosome and to measure the recombination distances between them. The markers could be of different typologies (RFLP, AFLP, ISSR, SSR, S-Sap, SNP, etc.), moreover could be sequence of express gene, but more commonly the markers is linked to a target gene. As the area in which it was identified the presence of the target gene is limited to a small portion of the genome, it is appropriate to restrict the analysis to markers located in that region. In case there are sufficiently detailed linkage map, it's possible locate the available gene of interest. However, if the maps are not available to locate the gene of interest, or to increase the degree of resolution of the map with other markers, it will be necessary to find new markers located near the gene.

The near isogenic lines (near isogenic Lines, NILs), differing only in the region around the gene. If the isogenic lines aren't available, it is possible to adopt the strategy proposed by Michelmore et al. (1991), which includes the performance analysis on DNA pools from different individuals of a segregating population, distinguished according to phenotype resistant or susceptible to the character of interest (Bulk segregant Analysis, BSA). Finally, it is possible to use resistant materials derived from interspecific crosses, to introduce into the genome portions of the genome of a resistant genotype.

The genetic maps can be obtained using different types of segregating populations: F₂, F₃, BC, RILs, DHLs (Burr et al. 1988; Burr and Burr 1991; Liu et al. 2005; Naghi et al. 2007).

2- 1- 1- Population size

Once an appropriate mapping population has been chosen, the appropriate population size must be determined. Since the resolution of a map and the ability to determine marker order is largely dependent on population size, this is a critical decision. Clearly, population size may be technically limited by how many seeds are available or by the number of DNA samples that can reasonably be prepared. One population will be more numerous and large size will be more useful.

For example, Messeguer et al. (1991) examined over 1000 F₂ plants to construct a high resolution map around the Mi gene of tomato, Stuber et al. (1987) analyzed over 1800 maize F₂s to find QTLs controlling as little as 1% of the variation in yield components, and Alpert and Tanksley in 1996, analyzed more than 3,400 individuals to obtain a detailed map around a fruit weight locus (Alpert and Tanksley 1996).

2- 1- 2- Segregating populations (F₂, F₃ and Backcross)

The most basic and traditional system to obtain a segregating population is the intersection between two inbred lines showing between them a sufficient degree of polymorphism. F₁ progeny will be genetically homogeneous and heterozygote and in meiotic the recombination events occur, which will be detected by self-fertilization in the subsequent generation (F₂) or in backcross (BC).

In this last case, the recombination occurred in individual gametes can be shown directly. In the F₂ populations, however, since the recombination may involve both gametes that give rise to the zygote, consideration must be given for each marker, three genotype categories: homozygous similar to a parent, like the other parent and heterozygote. Where the characters in question are the dominant type, as in much of the resistance genes, the heterozygote can't be distinctive from the dominant homozygote. The F₂ and BC populations have the great disadvantage of not being able to be preserved over time and each sample to be analyzed is represented from a single plant, then it should repeat the intersection to get new segregating progeny in each experiment.

Analysis of the F₃, obtained by selfing of each F₂ individual, it is possible to determine the genotype (homozygous / heterozygous) of F₂, dominant. The homozygous genotypes, in fact, produce only individuals with the dominant phenotype, while heterozygous individuals will give a segregation ratio of 3 dominant: 1 recessive.

2- 1- 3- BIL Population

Recently, some breeding programs have adopted advanced backcross inbred line (BIL) populations as a method for the identification and the introgression of useful genes from wild relatives or unadapted germplasm with the potential to improve the agronomic performance of elite cultivated lines (Fulton et al. 1997; Bernacchi et al. 1998; Talamè et al. 2004). The potential benefits of these

lines versus more balanced populations (for example: F2, F3, BC1, etc.) depends on the possibility of reducing the introgressed chromosomal regions and fine mapping loci linked with quantitative traits (QTLs). Epistatic QTLs or QTLs with gene actions ranging from recessive to additive can be detected in BILs and each QTL is treated as a single Mendelian factor (Fulton et al. 1997). The application of the backcrossing method to the improvement of quantitative traits has been limited mainly because of the low heritability of these characters and the difficulty of simultaneously transferring a relatively large number of genes.

In contrast to more traditional procedures of wheat breeding, the backcross inbred line method, first described by Wehrhahn and Allard, (1965), produces BILs that can be tested in replicated trials over environments prior to selection. BILs are characterized by the low proportion of the donor parent in each of the population members and therefore are ideally suited for mapping inter specific variation (Eshed and Zamir 1995).

The wild tetraploid wheat *Triticum turgidum* var. *dicoccoides* shows particular promise as a donor of useful genetic variation for several traits, including disease resistance, drought tolerance, grain protein quantity and quality. Despite this, little is known at the genetic level about the introgression of DNA from wild relatives into cultivated wheat.

2- 1- 4- DH Population

In some plant species have developed systems for the production of double haploid plants through regeneration from haploid tissue (another culture or ovaries) or by inter specific crosses and subsequent selective chromosome elimination. These systems were resulted efficient in some grass, for example rice as a bear, here in breeding programs are now used correctly, in wheat, however, the possibility of producing haploid is less efficient and limited to certain genotypes.

These systems were more efficient in some grass, for example rice and barley here in breeding programs are now used correctly, in wheat, however, the possibility of producing haploid is less efficient and limited to certain genotypes.

Following the doubling of the chromosomal inducible with colchicine, an alkaloid that inhibits the formation of the spindle, it's possible quickly obtain homozygous at all locus. The advantages of this strategy is the ability to immediately highlight the genotypes derived from the F1 gametes segregating in virtually limitless multiplication of the material, recombinant lines genetically homogeneous, with analysis replicated, allowing a more accurate assessment of the characters more influence by environment, such as the dissection of quantitative traits into Mendelian components or QTL (Quantitative Trait Loci). Another advantage is the ability to distribute dihaploids populations in different research groups, allowing to work independently on the same segregating material. A disadvantage of this technique is the frequent deviation from the normal ratio of 1:1

segregation, caused by gamete selection (Graner et al. 1991; Kintzios et al. 1994; El kharbotly et al. 1996).

2- 1- 5- RIL Population

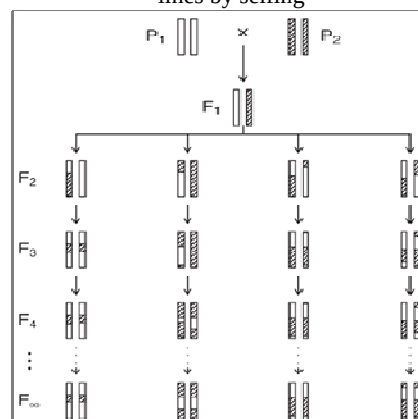
The RIL require considerable time and a major financial commitment, but once obtained are stable and can be used indefinitely, when they have an unlimited number of recombinant plants for each configuration. Recombinant Inbred Lines (RILs) can serve as powerful tools for genetic mapping. An RIL is formed by crossing two inbred parents followed by repeated selfing or sibling mating to create a new Inbred Line (Broman, 2005).

In species in which it was not possible to develop techniques to obtaining diaploidi, it is still possible to obtain segregating populations stabilized through several generations of selfing from F₂ individual seeds, a technique called SSD (Single Seed Descent). From the F₆, when only 3% of the characters will still be in the heterozygous state, the population may be considered genetically fixed and used for genetic analysis. In this case, the analysis of the frequency of recombination will be considered including those that occur in subsequent generations to F₁. Considering recombinant inbred population as genetically determined and fixed, the frequency of recombination is: $r = (\text{Recv.}) / 2x (\text{not rec.})$ (Haldane and Waddington 1931; Burr and Burr 1988). The main disadvantage compared to diaploid lines, derived from the time needed for the many generations of selfing to the achievement of homozygosity.

The time required to build up the genetic material can be reduced, however, raising plants in a controlled environment, so could be run multiple generations in a year. As each RIL is an inbred strain, and so can be propagated eternally, a panel of RILs has a number of advantages for genetic mapping: one need genotype each strain only once. One can phenotype multiple individuals from each strain to reduce individual, environmental, and measurement variability. Multiple invasive phenotypes can be obtained on the same set of genomes. And, as the breakpoints in RILs are more dense than those that occur in any one meiosis, greater mapping resolution can be achieved. (Threadgill et al. 2002; Churchill et al. 2004).

Such a panel would serve as a valuable community resource for mapping the loci that contribute to complex phenotypes in the wheat. The use of such a panel requires a detailed understanding of the relationship between alleles at linked loci on a Recombinant Inbred (RI) chromosome, particularly for the reconstruction of the parental origin of DNA (the haplotypes) on the basis of less-than-fully informative genetic markers [such as single-nucleotide

Panel: The production of recombinant inbred lines by selfing



polymorphisms (SNPs)]. The haplotype reconstruction will likely make use of the standard hidden Markov model (HMM) technology, and a primary ingredient to the HMM will be the two-point haplotype probabilities on an RIL chromoby some, such as the probability that the RIL is fixed at allele A at one locus and allele E at a second locus, as a function of the recombination fraction (per meiosis) between the two loci.

Methodologies for the identification and mapping of genes underlying quantitative traits in plants have advanced considerably in recent years since the advent of marker-saturated linkage maps (Tanksley 1993). The developments in marker technology were not accompanied by corresponding progress in RIL population structures. Are suitable for DNA-based mapping, but recombinant inbred (Burr and Burr 1991) provide permanent mapping resources. These types of populations are also better suited for analysis of quantitative traits.

2- 1- 6- NIL Population

Near Isogenic Lines are obtained with a program of backcrosses and selection for resistance, so that after a sufficient number of generations, you will get a line with genotype substantially equal to the improved varieties, but with the resistance gene. Because of the "linkage drag" on the sides of the transferred gene will still be a portion of the donor genotype. Each polymorphism analysis revealed differential markers between the susceptible varieties and near isogenic lines will be attributed, with a high degree of probability, the region surrounding the gene (Michelmore et al. 1991).

2- 2- Developing breeding population

A number of authors have noted the difficulty of adequately evaluating hybrid performance when seed quantities are small. When only limited numbers of crosses can be evaluated, it is difficult to sample a large number of parents and select those that combine well and have the most heterosis. Wilson (1984) pointed out that developments of diverse populations that combine well are desired for the initiation of a hybrid wheat breeding program. He mentioned as examples the Reid and Lancaster maize populations and the Kafir and Milo sorghums.

2- 3- Applications of molecular markers

The development of molecular techniques for genetic analysis has led to a great increase in our knowledge of cereal genetics and our understanding of the structure and behavior of cereal genomes. 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 material.
- (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 has been given to developing genetic linkage maps for durum wheat (Nachit et al. 2001; Peleg et al. 2008; Zhang et al. 2008).

Scientists are constructing genetic linkage maps composed of DNA markers for a wide range of plant species (O'Brien 1993). Several types of DNA markers have been widely used (Fig. 1.10).

All types of DNA markers detect sequence polymorphisms and monitor the segregation of a DNA sequence among progeny of a genetic cross in order to construct a linkage map. While the theory of linkage mapping is the same for DNA markers as in classical genetic mapping, special considerations must be kept in mind. This is primarily a result of the fact that potentially unlimited numbers of DNA markers can be analyzed in a single mapping population (Young 2000).

The analysis of DNA sequence variation is of major importance in genetic studies. In this context, molecular markers are a useful tool for assaying genetic variation, and have greatly enhanced the genetic analysis of crop plants. A variety of molecular markers, including restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites or simple sequence repeats (SSRs), have been developed in different crop plants (Philips et al. 2001; Varshney et al. 2004).

These molecular techniques, in particular the use of molecular markers, have been used to monitor DNA sequence variation in and among the species and create new sources of genetic variation by introducing new and favorable traits from landraces and related grass species.

It gives a theoretical measure of genetic diversity relatedness among cultivars based on the assumption of equal parent contributions. Genetic diversity in wheat has been estimated using markers such as isozyme (Asins and Carbonell 1989), seed storage proteins (Branlard et al. 1989; Metakovsky et al. 1989). However DNA markers have the advantage of directly detecting sequence variation among cultivars. DNA based markers such as Restriction Fragment Length

Polymorphisms (RFLPs) (Autrique et al. 1996; Paull et al. 1998), Random Amplified Polymorphic DNAs (RAPDs) (Fahima et al. 1999), were used to study genetic diversity in wheat. Both isozyme and RFLPs show low level of polymorphism due to a high amount of repetitive DNA in wheat (Flavell and Smith 1976; Ranjekar et al. 1976). Data analysis and linkage map construction.

Improvements in marker detection systems and in the techniques used to identify markers have been the basis for most work in crop plants, valuable markers have been generated from RAPDs and AFLPs. Simple sequence repeats (SSR) or microsatellite markers have been developed more recently for major crop plants and this marker system is predicted to lead to even more rapid advances in both markers linked to useful traits has been based on complete linkage maps and bulked segregant analysis. However, alternative methods, such as the construction of partial maps and combination of pedigree and marker information have also proved useful in identifying marker/trait associations.

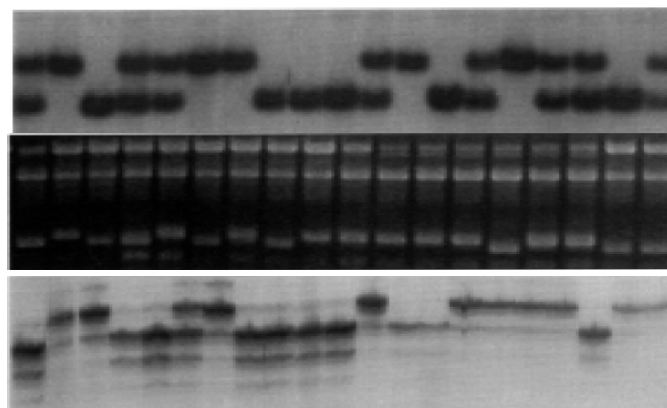
A revision of current breeding methods by utilizing molecular markers in breeding programs is, therefore, crucial in this phase. This review provides an overview of current stage of these developments, examines some of special issue that have used in applying molecular techniques to molecular breeding of cereals species (Korzun 2003).

Figure 1.10: Different reaction of different marker on the gel electrophoresis

RFLP
Restriction Fragment
Length Polymorphism

RAPD
Random Amplified
Polymorphic DNA

SSR
Simple Sequence Repeats or
“Microsatellites”



2- 4- Molecular marker techniques

Genetic linkage maps are a fundamental tool for several purposes, such as evolutionary genomics, understanding the biological basis of complex traits, dissection of genetic determinants underlying the expression of agronomically important traits. The construction of a genetic map requires a segregating plant population and molecular markers. Genetic linkage maps indicate the position and relative genetic distances between markers along chromosomes. Genes or markers that are closing together or tightly-linked will be transmitted together from parent to progeny more frequently than genes or markers that are located further apart (Korzun 2003).

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 be divided as based or not on PCR amplification. So I will review the based PCR techniques: Microsatellites and SNPs marker.

2- 4- 1- Non- PCR Based Techniques

2- 4- 1- 1- 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.

Although it is well recognized that RFLPs are suitable to estimate phylogenetic relationships among related taxa, the detection of RFLPs is both laborious and tedious, especially in plants with a great genome size such as wheat, and more than 40 RFLP markers are required to estimate accurate genetic relationships (Liu et al. 1990).

2- 4- 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 (Koeber 1995 a, b). 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 a, b).

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 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.

The microsatellites markers are used as anchor probes, whereas the AFLPs to saturate the map.

On the other hand, RAPD markers are more easily handled, and thus are becoming more desirable to estimate genetic relationships among related taxa. Analysis of allelic variations in RAPD markers. But RAPD markers belong to another group of techniques markers, means PCR based techniques (Devos and Gale 1992).

2- 4- 2- 1- Inter simple sequence repeat (ISSR)

Taking these situations into account (knowing the disadvantages of the markers in the first group), the variability of ISSR markers in wheat species has been investigated, and has solved some problems on the part of other markers. The ISSR markers are also useful for detecting genetic polymorphisms (Zietkiewicz et al. 1994). Thus, ISSR DNA has been proposed as a new source of genetic marker that overcomes many of the technical limitations of RFLP (Rafalski et al. 1991) and RAPD (Devos and Gale 1992) analyses, even in plants (Tsumura et al. 1996). to the SSR-PCR method that amplifies with primers located on the flanking single-copy DNA, microsatellite anchored primers that anneal to an SSR region can amplify regions between adjacent SSRs (intersimple sequence repeats; ISSR). Reasonably is used only one primer.

Molecular marker technologies, however, are currently undergoing a transition from largely serial technologies based on separating DNA fragments according to their size (e.g. SSR, AFLP) to highly parallel, hybridization-based technologies that can simultaneously assay hundreds to tens of thousands of markers.

2- 5- Microsatellites (SSR)

2- 5- 1- Explanation of the SSR markers

Recently, all these molecular markers have fulfilled a vital role in modern breeding programs and their application has become a standard practice for the selection of several alleles of interest. Among the different classes of molecular markers available, microsatellites (SSR, Simple Sequence Repeats) include an important role. and we will be able to give a great help in the genetic programs. Up to 90% of the plant genome consists 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.

SSRs are tandemly arranged arrays of short repeats comprising a few nucleotides (Hamada et al. 1982; Tautz 1989; Weber and May 1989), and there is estimated to be a total of 500 to 30,000 microsatellites per plant genome (Condit and Hubbell 1991). SSRs are known to be hyper variable and distributed throughout the genome even in plants (Lagercrantz et al. 1993; Morgante and Olivieri 1993; Senior and Heun 1993; Wu and Tanksley 1993; Bell and Ecker 1994).

The SSR are DNA sequences consisting of 2-6 bases repeated in a straight line (tandem) for a variable length from 20 to 150 base pairs. These repeated sequences are distributed randomly and abundantly in the genome of eukaryotes (Hearne et al. 1992; Wu et al. 1993). Their length has a high degree of polymorphism (Levinson and Gutman 1987). Morgante and Olivieri (1993) and Wang et al. (1994) have identified in (AT)_n and (ATA)_n repeat sequences, respectively, dinucleotide and trinucleotide more abundant in plants.

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 (Bryan et al. 1997).

Lagercrantz et al. (1993) have identified the presence of mainly (GT)_n (CT)_n in *Brassica napus*, while Ma et al. (1996) found that in wheat the frequency of (AC)_n (AG)_n is greater than in rice and in *Arabidopsis*, and is less than in corn. The flanking regions of microsatellites that are generally unique, so it's possible can clone the SSR flanking sequences determine the specific locus and

synthesize primers that allow to analyze the polymorphism through PCR amplification (Karp et al. 1997).

The benefits of SSR are: i) have a high length polymorphism, and therefore are very informative (Morgante and Olivieri 1993), ii) high reproducibility determined by the use of locus specific primers, which facilitates the comparison of results obtained in different laboratories, iii) are inherited in a codominant, thus allowing to distinguish homozygous individuals from that heterozygotes.

SSR markers are useful for a variety of applications in plant genetics and breeding because of their reproducibility, multiallelic nature, codominant inheritance, relative abundance and good genome coverage (Powell et al. 1996). SSR markers have been useful for integrating the genetic, physical and sequence-based physical maps in plant species, and simultaneously have provided breeders and geneticists with an efficient tool to link phenotypic and genotypic variation (Gupta and Varshney 2000).

Furthermore, the microsatellite because of their hyper variability and ease of handling in comparison to restriction fragment length polymorphisms (RFLPs), SSRs can provide a powerful tool to construct linkage maps (Senior and Heun 1993; Wu and Tanksley 1993; Bell and Ecker 1994).

2- 5- 2- EST- SSR Marker

Expressed sequence tag (EST) projects have generated a vast amount of publicly available sequence data from plant species. These data can be mined for simple sequence repeats (SSRs). These SSRs are useful as molecular markers because their development is inexpensive, they represent transcribed genes and a putative function can often be deduced by a homology search. Because they are derived from transcripts, they are useful for assaying the functional diversity in natural populations or germplasm collections. These markers are valuable because of their higher level of transferability to related species, and they can often be used as anchor markers for comparative mapping and evolutionary studies. They have been developed and mapped in several crop species and could prove useful for marker-assisted selection, especially when the markers reside in the genes responsible for a phenotypic trait. Applications and potential uses of EST-SSRs in plant genetics and breeding are discussed.

2- 5- 3- SSR markers in the Wheat

Recently, simple sequence repeats (SSRs, also known as microsatellites) have become the markers of choice for cereal genetic analysis and mapping (Varshney et al. 2007 a, b). The development of informative microsatellite markers in wheat is difficult and time-consuming due to its large genome size, polyploidy, and the high level of repetitive sequences in its genome. Many SSR markers publicly available wheat microsatellite primer sequences have been reported (Devos et al. 1995; Korzun et al. 1997; Röder et al. 1998 a, b; Pestsova 2000 a, b, c; Salina et al. 2000; Song et al. 2002; Gao et al. 2004; Nicot et al. 2004), which is a small number relative to the genome size of wheat. Until now have been published more than 2,100 SSR markers in 21 chromosomes of hexaploid wheat (Röder et al. 2007). Most of these SSR are also common in the 14 chromosomes of tetraploid wheat. The construction of a genetic map with molecular markers is a key step in the analysis of agronomically important traits, or biologically. The genetic linkage maps are essential tools for different purposes, such as evolutionary genomics, understanding the biological basis of complex traits, the dissection of genetic determinants underlying the expression of agronomically important characters and to facilitate assisted selection with markers (MAS) (Fahima et al. 2008). These maps are allowing the dissection of complex traits in the individual components and to assign to each locus, the effect on the expression of character can be assessed (Dilbirligi et al. 2006; Song et al. 2007).

2- 6- Single nucleotide polymorphism (SNP)

2- 6- 1- Explanation of the SNP markers

The development of high-throughput methods for the detection of single nucleotide polymorphisms (SNPs) and small indels (insertion/deletions) has led to a revolution in their use as molecular markers. SNPs are increasingly becoming the marker of choice in genetic analysis and are used routinely as markers in agricultural breeding programs (Gupta et al. 2001). They also have many uses in human genetics, such as for the detection of alleles associated with genetic diseases and the identification of individuals (Nikiforov et al. 1994). SNPs are invaluable as a tool for genome mapping, offering the potential for generating very high-density genetic maps, which can be used to develop haplotyping systems for genes or regions of interest (Rafalski 2002). The low mutation rate of SNPs also makes them excellent markers for studying complex genetic traits and as a tool for the understanding of genome evolution (Syvanen 2001).

The SNPs are single base pair substitutions that occur within and outside genes, and they are the most common form of sequence variation (Ching et al. 2002). a substantial private and public effort has been undertaken to characterize the SNPs responsible for genetic diversity. The SNPs are identified in expressed sequence tag (EST) sequences, thus the polymorphisms can be directly used

to map functional and expressed genes, rather than DNA sequences derived from conventional RAPD and AFLP techniques, which are typically not genes (Zhu et al. 2003; Huang et al. 2002). SNPs are found in both the coding and non-coding regions of the genome, and randomly distributed markers as well as markers clustered in genes can be discovered (Dvorak et al. 2005).

Unlike random amplified polymorphic DNAs and RFLPs, SNPs are direct markers because sequence information provides the exact nature of the allelic variants. They are far more prevalent than microsatellites and, therefore, may provide a high density of markers near a locus of interest.

Limited work has been carried out to examine the occurrence of SNPs in plants, although these preliminary studies have indicated that SNPs appear to be even more abundant in plant systems than in the human genome.

SNPs occur normally throughout a plant's DNA. They occur once in every 300 nucleotides on average, which means there are roughly more than 10 million SNPs in the plant genome. Most commonly, these variations are found in the DNA between genes. Most of the variability of SNP markers contain two alleles, and are appointed Biallelic loci. For this group of marker is using Haplotype Instead of alleles.

Germano and Klein (1999) identified five SNPs in 1 kb of nDNA of *Picea rubens* and *Picea mariana*, and also discovered SNPs in the chloroplasts of these species. Coryell et al. (1999) identified two SNPs in approximately 400 bp of sequence in soybean (*Glycine max*). In maize (*Zea mays*), they have been found even more frequently, with one SNP approximately every 48 bp and every 130 bp in 3' untranslated regions and coding regions, respectively (Tenaillon et al. 2001; Rafalski 2002). Mogg et al. (2002) amplified and sequenced the flanking region of 97 previously characterized microsatellite primer sets in 11 maize inbred lines. The sequencing results indicated that the flanking regions of maize microsatellites show increased levels of polymorphism when compared with other regions of the genome, with SNPs in these regions found on average every 40 bp.

While nucleotide diversity studies and the development and deployment of single nucleotide polymorphism (SNP) markers are straightforward in diploid and polyploid species, such as maize or soybean (Ching et al. 2002; Wright et al. 2005), they are complicated in recently evolved polyploid species by high levels of orthologous gene similarity. Sequence similarity makes sequencing of single genes and allocation of sequences into respective genomes difficult. Special strategies are therefore required for nucleotide diversity studies and the development of SNP markers for young polyploid species, which include wheat and other economically important plants.

Single-nucleotide polymorphisms may fall within coding sequences of genes, non-coding regions of genes, or in the intergenic regions (regions between genes). SNPs within a coding sequence do not

necessarily change the amino acid sequence of the protein that is produced, due to degeneracy of the genetic code.

Until recently, an allele was defined as a single gene having a specific sequence, while a haplotype is defined as a collection of alleles which, due to their proximity, are usually inherited together. With SNPs it is possible for several alleles to share some SNPs but not others. In this case the current consensus is that each defined sequence type is a single haplotype and not a single allele.

Over half of all known plant disease mutations come from replacement polymorphisms (Ching et al. 2002).

greatest importance of SNPs markers in research biotechnology is for comparing regions of the genome between cohorts (such as with matched cohorts with and without a disease) in genome-wide association studies. They are usually biallelic and thus easily assayed (Wright et al. 2005). SNPs do not usually function individually, rather, they work in coordination with other SNPs to manifest the traits.

2- 6- 2- SNP-EST

With the development of high-throughput sequencing technology, large amounts of data have been submitted to the various DNA databases that may be suitable for data mining and SNP discovery. In particular, expressed sequence tag (EST) sequencing programs have provided a wealth of information, identifying novel genes from a broad range of organisms and providing an indication of gene expression level in particular tissues (Adams et al. 1995). EST sequence data may provide the richest source of biologically useful SNPs due to the relatively high redundancy of gene sequence, the diversity of genotypes represented within databases, and the fact that each SNP would be associated with an expressed gene (Picoult-Newberg et al. 1999).

2- 6- 3- SNP markers in the Wheat

A possible strategy for nucleotide diversity studies and SNP discovery in young polyploid species, such as wheat, is to find diverged regions in orthologous genes and use them for the design of polymerase chain reaction (PCR) primers that anneal to only a single DNA target. These genome-specific primers (GSPs) amplify DNA from only a single genome and facilitate gene sequencing and SNP discovery (Ching et al. 2002). An alternative strategy is to shotgun-sequence cDNAs and then allocates each sequence to a genome. Both approaches have been used in polyploid wheat (Blake et al. 2004; Ravel et al. 2006) although those studies were of limited scope and genome coverage (Somers et al. 2003; Rustgi et al. 2009) and none mapped the markers. A domestication bottleneck at the tetraploid level and a polyploidy bottleneck during the transition from the

tetraploid to hexaploid level are expected to have reduced the diversity of polyploid wheat compared to wild emmer.

Nucleotide diversity $\theta\pi$ (Tajima et al. 1983) was reported to be 2.7×10^{-3} in 24 A- and B-genome wild emmer genes (Haudry et al. 2007). For comparison, $\theta\pi$ was estimated to be 9.7×10^{-3} in teosinte genes (*Zea mays* ssp. *parviglumis*) (Wright et al. 2005) and 7.7 to 8.1×10^{-3} in wild barley genes (*Hordeum vulgare* ssp. *spontaneum*) (Morrell et al. 2005, 2006).

The diversity of emmer was reduced by the domestication bottleneck but, curiously, no further diversity loss took place in the A and B genomes during the polyploidy bottleneck accompanying the evolution of *T. aestivum* from domesticated tetraploid wheat (Haudry et al. 2007). Levels of diversity in the *T. aestivum* D genome are unknown.

2- 7- Comparison between SSR and SNP molecular markers

As the SNP markers include only two alleles at a locus, comparing with SSR markers that including more alleles in the one locus, There will have less information, with respect to other markers. A number of reviews have highlighted the difficulties of using a biallelic SNP-based marker system in place of multi-allelic systems such as RFLPs and microsatellites. Xiong and Jin (1999) demonstrated that, whereas the biallelic nature of SNPs makes them less informative per locus examined than multiallelic microsatellites, this limitation is easily overcome by using more loci. For instance, Kruglyak (1997) determined that a 4 cM map of 750 SNP-based markers was equivalent in the information content to a 10 cM map of 300 microsatellite markers. Given the greater abundance of SNPs, such a requirement should not prove excessive. The relatively high frequency of SNPs in the genome brings about the possibility of cloning genes via linkage disequilibrium (Brookes 1999). In essence linkage disequilibrium is the non-random association of alleles at different loci. Logically this will occur when two loci occur close together on the same chromosome. The dense genetic maps (up to one marker per 1 kb) possible with SNPs allow genome scans for linkage disequilibrium (of SNPs) to be studied in association with complex phenotypes (Xiong and Jin 1999). In the future it may be possible to use SNPs in combination with linkage disequilibrium to screen directly the coding polymorphisms of genes derived from complex populations to identify directly candidates for the agronomic trait under investigation (Brookes 1999).

As with the majority of molecular markers, one of the limitations of SNPs is the initial cost associated with their development. A variety of approaches have been adopted for the discovery of novel SNP markers. The conversion of microsatellite markers, identifying the relatively abundant nucleotide polymorphisms surrounding simple sequence repeats has the advantage that many of these markers have already been characterized (Mogg et al. 2002).

2- 8- Target specific genomic regions

More general strategy makes it possible to target specific genomic regions without the need for developing specialized genotypes, generally known as bulked segregant analysis (Michelmore et al. 1991; Giovanonni et al. 1995). The strategy is to select individuals from a segregating population that are homozygous for a trait of interest and pool their DNA. In the pooled DNA sample, the only genomic region that will be homozygous will be the region encompassing the genomic region of interest, which can then be used as a target for screening DNA markers rapidly.

This means that any trait that can be scored in an F₂, backcross, or RI population can now be rapidly targeted with DNA markers (Zhang et al. 1994). Used in conjunction with AFLP markers, it is possible to identify large numbers of DNA markers in a region of interest in a short time.

Moreover, pooled DNA samples can also be generated based on homozygosity for a DNA marker (as opposed to a phenotypic trait). In this way, any genomic region of interest that has been previously mapped in terms of DNA markers can be rapidly targeted with new markers. This may be especially useful in trying to fill in gaps on a genetic map. All that is required is a pooled DNA sample selected on the basis of DNA markers flanking the genomic region of interest (Giovanonni et al. 1995).

2- 9- New generation sequencing

Next generation genomic sequencing technologies have made it possible to directly map mutations responsible for phenotypes of interest via direct sequencing. However, most mapping strategies proposed to date require some prior genetic analysis, which can be very time-consuming even in genetically tractable organisms (Austin et al. 2011). The detection and exploitation of genetic variation have always been an integral part of plant breeding. DNA-based molecular markers are useful for detecting the genetic variation available in germplasm collections and/or breeding lines. During the past two decades, many different molecular markers have been developed for most major crop species. These markers have been used extensively for the development of saturated molecular genetic and physical maps and for the identification of genes or quantitative trait loci controlling traits of economic importance for marker-assisted selection (MAS) (Varshney et al. 2005, 2006). In addition to traditional trait or QTL mapping using biparental populations, new approaches such as association mapping (Ersoz et al. 2007), advanced back-cross QTL analysis (Tanksley and Nelson 1996), functional genomics (Schena 1998), genetical genomics (Jansen and Nap 2001), allele mining (Varshney et al. 2005), tilling and Ecotilling (Till et al. 2007) have become available in recent years. Genomics-assisted breeding is a holistic approach using different genomic strategies and tools (Varshney et al. 2005).

The prediction of phenotype from genotype using different genomic tools and strategies is the basis of genomics assisted breeding (Varshney et al. 2006). By improving the precision and efficiency of predicting phenotypes from genotypes, the development of improved cultivars with enhanced resistance or tolerance to biotic and/or abiotic stresses and higher agronomic performance can be greatly accelerated.

Indeed, successful examples of genomics-assisted breeding have been demonstrated for several cereals (Varshney et al. 2006, 2007). Genomics-assisted breeding approaches have greatly advanced with the increasing availability of genome and transcriptome sequence data for several model plant and crop species. Complete and/or draft genome sequences have become available for several plant species such as wheat (<http://www.wheatgenome.org>) and barley (http://www.public.iastate.edu/_imagefpc/IBSC%20Webpage/IBSC%20Template-home.html).

Complementary to genome sequencing is the widespread application of transcriptome sampling strategies, which has resulted in large collections of expressed sequence tags (ESTs) for nearly all economically important plant species (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). Previously, most genome and transcriptome sequencing projects used Sanger sequencing methodology (Sanger et al. 1977).

However, owing to growing interest in human genome resequencing, a new generation of sequencing technologies has emerged. These next-generation sequencing (NGS) technologies are able to generate DNA sequence data inexpensively and at a rate that is several orders of magnitude faster than that of traditional technologies.

Advances in sequencing technologies are driving down sequencing costs and increasing sequence capacity at an unprecedented rate, making whole-genome resequencing by individual laboratories possible (Hudson 2008; Gupta 2008). As a result, genomics-assisted breeding should gain momentum, with the potential for significant improvements in the precision and efficiency for predicting phenotypes from genotypes.

This review article discusses the concept and potential implications of NGS technologies for crop genetics and breeding. Sanger dideoxy sequencing (Sanger et al. 1977) and its modifications (Prober et al. 1987; Madabhushi 1998) dominated the DNA sequencing field for nearly 30 years and in the past 10 years the length of Sanger sequence reads has increased from 450 bases to more than 1 kb. The limitations of Sanger sequencing are: (i) the necessity to separate elongation products by size before scanning, requiring one capillary or gel lane per sample, and (ii) the need to produce clonal populations of DNA using *Escherichia coli*, which is labor, robotics and space intensive for large-scale operations.

Another method is Next Generation Mapping (NGM), that method for rapidly and robustly mapping the physical location of EMS mutations by sequencing a small pooled F2 population. This method, called uses a chastity statistic to quantify the relative contribution of the parental mutant and mapping lines to each SNP in the pooled F2 (Austin et al. 2011).

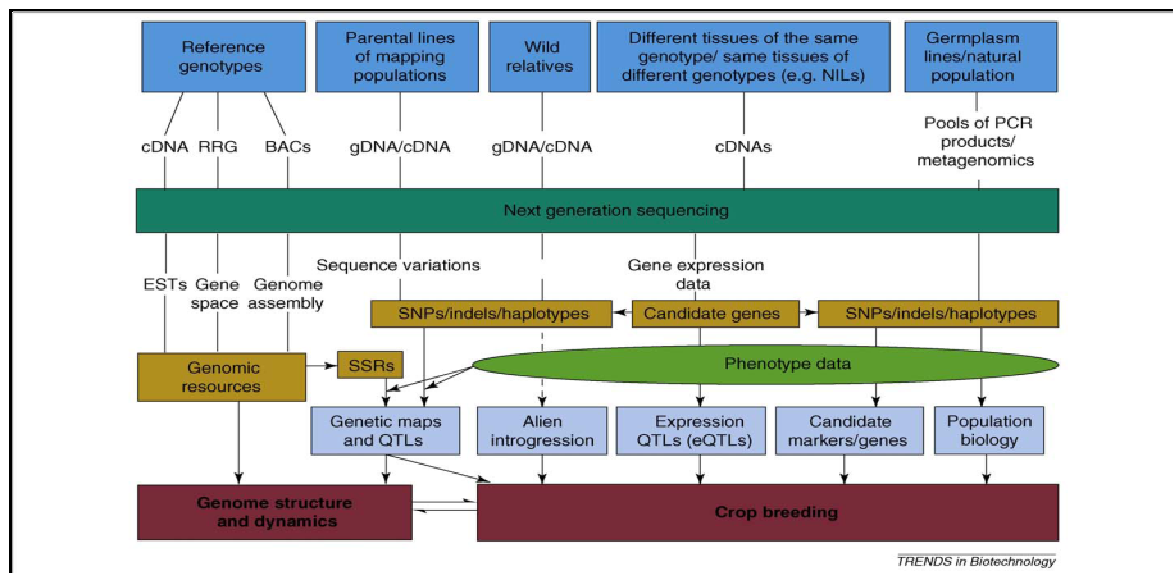


Figure 1.11: Overview of NGS applications in crop genetics and breeding.

In the figure 1.11, the NGS technologies have several potential applications in crop genetics and breeding, including the generation of genomic resources, marker development and QTL mapping, wide crosses and alien gene introgression, expression analysis, association genetics and population biology, as shown here. For instance, sequencing of genomic DNA including bacterial artificial chromosomes (BACs), reduced representation of genome (RRG) or cDNA from the reference genotypes using NGS technologies can provide genomic resources such as ESTs, gene space and genome assembly. These resources have a direct impact on understanding the genome architecture for crop genetics. Another application of NGS is in parental genotyping of mapping populations or of wild relatives, which can accelerate the development of molecular markers, e.g. SSR and SNP markers. These markers can be used to construct genetic maps, to identify QTLs and to monitor alien genome introgression in the case of wide crosses. These QTL-associated markers for a trait of interest can then be used in selecting progenies carrying favorable alleles via marker-assisted selection (MAS).

2- 9- 1- Association mapping using natural populations

Association mapping uses one of two approaches, candidate gene sequencing (CGS) or whole genome scanning (WGS) of natural populations (Rafalski 2002). Population surveys for haplotypes identified based on either CGS or WGS can take advantage of past recombination events to identify trait, marker relationships on the basis of linkage disequilibrium (LD). NGS technology has the

potential to accelerate both CGS- and WGS-based association mapping approaches (Nordborg and Weigel 2008). In general, CGS-based approaches involve Sanger sequencing of PCR amplicons for selected candidate genes across hundreds of genotypes of the natural population, which is time-intensive and expensive. NGS approaches, in particular Solexa approach, offer the possibility to sequence pools of PCR amplicons for a larger number of candidate genes generated for several hundred genotypes of the natural population with the help of barcodes. Thus, in a single Solexa run, sequence data (SNPs and haplotypes) will be available for a larger number of candidate genes in the natural population within a short time and at considerably lower cost compared to Sanger sequencing. By contrast, WGS approaches require the screening of natural populations with a large set of genome-wide markers, which is not possible in many crop species.

However, as mentioned above, NGS technologies can facilitate the rapid development of genome-wide markers (Barbazuk et al. 2007; Ossowski et al. 2008) that could be subsequently used for WGS approaches to association mapping (Nordborg and Weigel 2008).

2- 9- 2- Perspectives on genetic mapping and DNA markers

Finally, DNA marker technology is still so technically complex that it is practically impossible for it to be applied where it is needed most in less developed countries.

Simple sequence repeat markers have played a critical role in merging disparate linkage maps (Akkaya et al. 1995; Bell and Ecker 1994). Because they are nearly always single locus markers, even in complex genomes like the grasses and soybean, SSRs define specific locations in a genome unambiguously. This makes them suitable to tie multiple maps together. Moreover, being PCR-based, the information necessary to map SSR loci can be shared among labs simply by sharing primer sequence data.

However, better types of DNA genetic markers are on the horizon. The most important are SNPs that can be assayed through DNA chip technology. Already, researchers working with the human genome have constructed a map of more than 2000 SNP markers, many assayed by chip technology. It may also be possible to create the equivalent of “radiation-hybrids” for plants. This is an extremely powerful resource that has routinely been used in animal genome mapping for decades. Research have recently shown that it is possible to create stable lines of oats that contain a small segment of maize genome (Ananiev et al. 1997). If enough oat lines carrying overlapping maize segments can be generated, extremely fast and efficient high resolution mapping may be achievable.

Even as DNA marker technology advances, parallel achievements are essential in complementary technologies. As the number of markers, genetic resolution, and amount of mapping information grows, so does the need for better computer algorithms and databases. Finally, genetic linkage

maps, even those based on DNA markers, are still limited by the range of sexual crosses that can be made. To make the most of genes uncovered through genetic mapping, improvements in making wide crosses, somatic hybrids, and plant transformation will be essential. In the future, better DNA markers, along with advances in these complementary technologies, will enable linkage mapping, one of the oldest genetic techniques, to become one of the most powerful.

3- GENETIC MAP

3- 1- Genetic mapping

The mapping of the wheat genome was really important in speeding up the breeders work in improving this important food resource, and in understanding the transmissions mechanism of genetic information. The new dawn of agriculture and the revolution in agriculture should start from the practice of mapping and deciphering the genome of the wheat plant. This important discovery in the agricultural sector, will surely bring the news relevant. The genome mapping of wheat, will allow the creation of plants with greater resistance in a way that can be grown and developed even in the toughest conditions on the planet, especially in areas with a greater "risk of hunger".

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.

A.H. Sturtevant (American geneticist) constructed the first genetic map of a chromosome in 1913. Throughout his career he worked on the *Drosophila melanogaster* with T.H. Morgan. By watching the development of flies in which the earliest cell division produced two different genomes, he measured the embryonic distance between organs in a unit which is called the sturt in his honor. 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 (Varshney et al. 2007 a, b), (Table 1.3).

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. Comparative mapping in plants has provided evidence for conservation of markers and gene order (colinearity) between related genomes. Rice is a particularly valuable reference for the grass family because most of its small diploid genome has been sequenced.

British researchers (Steinberg et al. 2010) have been able to decode the genome of wheat, one of the most difficult to decipher, five times the size of the human genome, contains 17 billion letters with a very complex internal structure. Bread wheat genome is six times larger than that of maize and 35 times larger than the rice genome (Bennett and Smith 1976).

Table 1.3: The Molecular linkage maps have been constructed in different organisms.

Plant	Year	References
Diploid wheat	1991	Gill et al.
	1991	Lagudah et al.
	1996	Dubcovsky J et al.
	2007	Singh K et al.
Tetraploid wheat	1998	Blanco et al.
	1999	Korzun et al.
	1999	Korzun V et al.
	2001	Nachit et al.
	2008	Zhang W et al.
	2004	Elouafi I & Nachit
Hexaploid wheat	2008	Peleg et al.
	1989	Chao et al.
	1991	Liu and Tsunewaki.
	1992	Devos et al.
	1993	Devos and Gale.
	1995	Nelson et al.
	1995	Van Deynze et al.(a)
	1996	Jia et al.
	1998	Röder et al.
	2004	Sourdille P et al.
	2008	Xue S et al.
	2008	Paux E et al.

Plant	Year	References
Barley	1991	Heun et al.
	1991	Graner et al.
	1993	Kleinhofs et al.
	1994	Graner et al.
Rice	1988	McCouch et al.
	1991	Saito et al.
	1994	Causse et al.
	1994	Kurata et al.
maize	1986	Helentjaris et al.
Oat	1992	O'Donoughue et al.
	1995	O'Donoughue et al.
	2005	Portyanko et al.
Tomato	1988	Paterson et al.
	1992	Tanksley et al.
	1993	De Vicente & Tanksley
	2000	Saliba-Colombani et al.

3- 2- Mapping the genome of wheat

The initial checks on the offspring, more certain and reliable, they begin to spread throughout Europe in order to develop genetic tests by which we can identify individuals likely to develop any hereditary disease primary, yet it is manifested clinically. Although this work is a complex and very expensive in terms of money and time, some international programs (ITMI, CIP) are developing these methods. In a cell of a higher organism, such as durum wheat, the number of genes is very high, and studies that bring to the identification and location on the DNA chain is very complex. Many research centers in Europe and the U.S. are making this effort, although it must be admitted that the genetic mapping of wheat proceeds much more slowly than that of other cereals (i.e. rice). Because from a genetic point of view they are studied more thoroughly for obvious economic reasons. Despite this, more genetic map will be complete in the future, more applications will result in practical matters.

With those elaborate techniques (Introduction, paragraph 2- 9-) of bioengineering, will be possible to know the sequences of chromosomes within the DNA in different polyploids wheat and then interpret them and can then take action where we have genetic abnormalities. This should help to stabilize and then reduce the prices of wheat, which have surged recently due to allay fears about the impact of climate change and water scarcity. Then develop new types of wheat with high productivity will be key to achieving that goal goal (Hall and Hall 2011).

Despite this "It took fifteen years to map the human genome sequence and only one year to the genome of wheat, thanks to huge advances in DNA technology". Now sequence analysis are to detect genetic variation among different types of natural grain which will serve to speed up breeding programs and to address the problem of shortage of food globally (Hall and Hall 2011).

Decoding of wheat and most important state in the decoding of the human genome that was worked in 2000, the rice genome was decoded in 2005, maize in 2009 and that of soybean in 2010.

The mapping of these three plants are very simpler than that of wheat, which has a complex structure due to its origins, knowledge of the genome, that is, the genetic material carried by the chromosomes that pass through a mapping, that is, a precise localization of DNA sequences that comprise it. 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, each consists of about 107 to 108 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 1,000 to 10,000 genes. Therefore Gill and Gill (1994) proposed a mapping strategy to target genes in the 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. Through this mapping will be possible to get to know, the modern methods of bioengineering, all the genes and their position. 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 of these crops 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 *Graminaceae* 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 *Graminaceae* 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. 1995 b) 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. 1995 a, b; Van Deynze et al. 1995 b) 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.

Size and complexity, Up to now it has been proven a huge challenge for scientists. The mapping almost complete - 95% of the genes - will now allow creating new types of plants resistant to pests and diseases that can grow in wide weather and climate conditions and produce higher yields.

Tetraploid wheat are available in the detailed genetic maps (*Triticum taushii*, 2n "4x" 28) which include about 2100 loci mapped either directly or inferred from genome locations aligned with related *Triticum* species, and this mapping will demand is possible only and only using the knowledge had come back from the genetic maps of various molecular markers.

Details of the loci mapped in wheat can be found in the gene catalog (Macintosh et al. 1995) and further updates published in the annual report of wheat newsletter or in the database Grain Genes. Reporting the distances between markers on a genetic map of wheat, it has been possible to identify the association between the various genes with markers, the positions of genes and markers.

On the genetic wheat maps, the markers largely clustered around the telomeric, indicating that there is not more recombination in the distal portions of the chromosomes (Delaney et al. 1995). And on the contrary, the markers close clustered around the centromere, there is more recombination.

3- 2- 1- Genetic maps of wheat through molecular markers

The allocation of RFLP and SSR markers to specific regions of wheat chromosomes enables alignment of genetic and cytogenetic maps, thus providing a comprehensive assessment on the extent of genetic map coverage across the cytogenetic map of wheat. Moreover, integration of deletion and genetic mapping facilitates the analysis of recombination in defined regions of wheat chromosomes (Gill et al. 1996 a, b).

Although RFLP, AFLP and SSR markers have made substantial contributions to the wheat genome mapping effort, novel DNA-based markers are allowing further resolution of wheat genetic maps. Wheat genetic maps were first comprised of restriction fragment length polymorphisms (RFLPs) and later on PCR-based markers were adopted, including random amplified polymorphic DNA and amplified fragment length polymorphism (AFLP) (Gale et al. 1995; Blanco et al. 1998; Messmer et al. 1999; Peng et al. 2000; Paillard et al. 2003). In contrast to hexaploid wheat (AABBDD), for which several linkage maps have been developed (Table 1.3), relatively little attention has been given to developing genetic linkage maps for durum wheat. The first full genetic linkage map for this species, based on RFLP markers, was presented by Blanco et al. (1998), and subsequently integrated with SSRs from hexaploid wheat (Table 1.3). Later on, other inter-specific linkage maps based on RFLPs, SSRs and AFLPs were developed (Peng et al. 2000; Maccaferri et al. 2008), and the first intra-specific linkage map (Nachit et al. 2001).

A number of studies have assigned RFLP and SSR markers to wheat chromosomes by nullisomic-tetrasomic (NT) and deletion lines (Kota et al. 1993; Delaney et al. 1995 a, b; Hohmann et al. 1995 a, b; Mickelson-Young et al. 1995; Gill et al. 1996 a, b; Weng et al. 2000; Sourdille et al. 2004).

SSRs, also known as microsatellites, have become the markers of choice for cereal genetic analysis and mapping (Röder et al. 1998 b; Song et al. 2005; Gupta et al. 2002). To date, over 2,000 SSR markers on 21 hexaploid wheat chromosomes have been published (Ganal and Röder 2007). Microsatellite markers generally exhibit a higher level of polymorphism that is critical for detecting differences among related crop cultivars, and they can even discriminate among closely related wheat breeding lines (Plaschke et al. 1995).

It seems that microsatellites in plants can be up to tenfold more variable than other marker system such as RFLPs. 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 is functional and suitable for genetic analysis (Röder et al. 1995; Bryan et al. 1997).

This high percentage is no longer the major limitation on a large scale the development of microsatellite markers for wheat, however, increasing this percentage will bring up a map with high precision. 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).

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 *Pst*I and subsequent isolation of the fragments in the size range of 2-5 Kb before digestion with a 4bp restriction enzyme (*Mbo*I or *Sau*3A) 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.

Nevertheless, more recently new molecular markers types have been developed in order to satisfy the need of high-throughput assays, able to analysis a great number of markers and genotypes with a reduced cost particularly in the genome of wheat that contains a large number of genes, on the genome compared to other plants.

Diversity Array Technology (DArT) is an array-based platform for high-throughput analysis of DNA polymorphism and molecular markers for genetic mapping in the wheat (Jaccoud et al. 2001).

The genotyping technology involves the use of methylation sensitive restriction enzymes to digest genomic DNA, thereby reducing genome complexity and enriching for low copy sequences for marker development. DNA samples enriched for low copy DNA sequences from parents and individuals of mapping populations are hybridized to a microarray panel of clones representing low copy sequences from the same plant species. Restriction site polymorphisms between individuals from mapping populations are detected through differences in hybridization signal, with clones on the microarray panel scored as dominant markers and providing for allele attribution to one or the other parental genotype. DArT and other DNA markers have been used to produce genetic maps for a range of crop species including rice by (Jaccoud et al. 2001), and barley with (Wenzl et al. 2004).

3- 3- The size of the wheat genome (tetraploid and hexaploid)

We concentrated on both, tetraploid and hexaploid wheat, because they have a use in Commercial, so genetic changes are based on these two types. For knowledge of a genetic map it is need to identify the distances between the markers and the length of the various chromosomes and the entire genome.

The exact genetic size of the hexaploid wheat genome is still unknown, but data from several published maps suggest it is greater than 3,500 cM. The size of the map constructed in the International *Triticeae* Mapping Initiative (ITMI) population is about 3,700 cM (reviewed by Gupta et al. 1999), and the map generated in the intervarietal cross between Courtot and CS is 3,685 cM in length (Sourdille et al. 2003).

Recently, Quarrie et al. (2005) used a doubled haploid population of CS × SQ1 and 567 markers to construct a wheat genetic map covering all 21 chromosomes, with a total length of 3,522 cM. In the study of Liu et al. in 2005 the genetic map spans 3,045.8 cM of genetic distance and is lacking information for chromosome 3D, which accounts for roughly 170 cM on the ITMI map. Although the map of (Liu et al. 2005) contains over 600 markers, some regions with inadequate coverage and large gaps still exist. The short arms of chromosomes 1A, 2A, 4A, and 5D, and the long arms of 4D, 6D, and 7A contain few markers, or no markers at all. In addition, gaps of significant size exist on 1D, 2B, 2D, 3A, 4A, 5A, 6A, and 7D. The addition of more markers, particularly for A- and D-genome chromosomes will provide more complete genome coverage. However, previously published consensus genetic (Somers et al. 2004) maps allowed us to draw some comparisons with SSR loci on the map of (Liu et al. 2005). Of the SSRs that they (Somers) used for mapping, 71 were in common with those on the maps presented by (Somers et al. 2004). Seven of these (Xbarc24, Xbarc90, Xbarc91, Xbarc95, Xbarc101, Xbarc140, and Xbarc143) detected loci on chromosomes different from those reported by (Somers et al. 2004).

For example Xbarc95-2D was assigned to chromosome 2D by nullisomic-tetrasomic (NT) analysis, and the remaining six markers were each closely linked to other markers that were assigned to chromosomes also by nulli-tetrasomic analysis. The order of common markers along (Liu et al., 2005). Maps agreed well with those presented by Somers et al. (2004) and Sourdille et al. (2004) with only a few notable exceptions.

Instead on the map (Peng et al., 2011) The total map length exceeded 3000 cM and possibly covered the entire tetraploid genome (AABB). clusters of molecular markers were observed on most of the 14 chromosomes. In the genetic map of (Peng et al. 2011). Peng et al. 2011 the using 203 microsatellite primer pairs, than 187 (91%) generated various levels of polymorphism between the parental lines of the mapping population. In the map of Peng et al. 2011 The dominant markers of the H and L maps (the parental genotype derived from the Hermon population [H], and *T. durum* Langdon [L]), were based on PCR bands amplified from genomic segments of *T. dicoccoides* correspondingly. The (H) and (L) maps span a distance of 3169 cM and 3180 cM, respectively. The mean interval lengths of H and L maps were 9.9 cM and 10.7 cM, respectively.

It is estimated that the genome sizes of tetraploid wheat (*T. dicoccoides* and *T. turgidum durum* cultivar, Langdon, Ldn) and hexaploid wheat (*T. aestivum*) are $\sim 12 \times 10^9$ and 17×10^9 bp, respectively (Bennett et al. 1998). If the average chromosome length is assumed to be 200 cM (Messmer et al. 1999), the total map size of hexaploid wheat is estimated as 4200 cM. The sizes of published maps for the populations Chinese Spring $\times$ *T. spelta* (Liu and Tsunawaki 1991), Chinese Spring $\times$ Synthetic (Gale et al. 1995), Forno $\times$ Oberkulmer (Messmer et al. 1999) and W7984 $\times$ Opata 85 (Faris et al. 2000) in hexaploid wheat were 1801, 2575, 2469, and 3700 cM, respectively. These maps cover 43%, 61%, 59%, and 88% of the entire genome of hexaploid wheat, respectively. The total map size of tetraploid wheat (*T. dicoccoides* and *T. turgidum durum* cultivar, Langdon, Ldn) could be estimated as 2800 cM if the size of hexaploid wheat is 4200 cM (Messmer et al. 1999). In tetraploid durum wheat, the total length of published RFLP-based genetic map is 1352 cM (Blanco et al. 1998), and has been extended to 2035 cM after integrating 79 microsatellite markers (Korzun et al. 1999). These two maps cover 48% and 73% of the entire genome of tetraploid wheat, respectively.

In the work of Akhunov et al. (2010) after the process of GSP (Genetic Screening Processor) and SNP. A total of 6,045 wheat ESTs was downloaded from the wEST database into the pipeline and CPs anchored in exons and flanking one or two introns were developed. The Southern hybridization profiles of the ESTs were examined in the wEST database and CPs for those that showed a complex profile was eliminated. Speltoides genes, and 1,574 *Ae. tauschii* genes and were sequenced.

A total of 11,764 GSPs was designed and tested for genome specificity by PCR amplification of *T. aestivum* nullisomic-tetrasomic (NT) lines. GSPs derived from 1,102 EST unigenes (705 in the A genome, 703 in the B genome, and 706 in the D genome) were validated by PCR with NT lines.

Target DNA was PCR amplified in 32 wheat lines using GSPs. A total of 41,065,555 bp of the amplicons was sequenced (14,734,124 bp in the A genome, 14,554,737 bp in the B genome, and 11,776,694 bp in the D genome) using GSP pairs as sequencing primers, and 5,471 SNPs at 1,791 loci were discovered.

3- 4- ITMI map and inter varietal crosses

A large proportion of genetic mapping studies in wheat (*Triticum aestivum*) involved inbred populations derived from genetically diverse parents. These include the International Triticeae Mapping Initiative (ITMI) population derived from cultivated (Opata-85) and synthetic (W- 7984) accessions (Van Deynze et al. 1995 a) and populations derived from interspecific crosses involving winter and spelt wheats (*T. aestivum* subsp. *spelta*) (Messmer et al. 1999).

The ITMI population is of particular importance as it is a publicly available resource, providing the research community with the capability to determine the chromosomal location of DNA-based markers with respect to existing wheat molecular markers on a single genetic map (Song et al. 2005).

The effective application of the ITMI population and its genetic map in breeding studies however, is limited by the lack of trait variation of commercial relevance (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi).

ITMI was founded in 1989 by the core-group with the specific goal to develop genetic maps of wheat and its relatives. Founding of this international organization, which has been managed from the University of California since its conception, was a response to the realization that the development of detailed comparative genetic maps for wheat and its relatives required close coordination, collaboration, and sharing of resources and results. The success of ITMI is evident from the fact that after eight years of ITMI activity, wheat and related species are becoming among the best-mapped plant species. The steady progress made by ITMI is clearly evident from the voluminous progress reports prepared annually by the coordinators of individual homoeologous groups in the tribe (Mc Guire and Qualset 1997).

The existence of this formal, well established collaborative organization will can possible rapid dissemination of the results of this project in the ITMI workshops that are held annually in different countries.

The wheat EST has one of the principal projects complementary in other countries participating in ITMI as well as preliminary efforts in the USA. In the summer of 1998, ITMI fostered foundation

of the International *Triticeae* EST Cooperative (ITEC) to initiate collaborative studies on gene function in wheat and the other members of the tribe *Triticeae* (see Wheat EST sub-section in the section below). The ITEC effort illustrates the role ITMI plays in fostering development of international links.

Been identified and calculated distances only between markers microsatellites on chromosomes in the first map of the wheat that was valid reference in 2004 by Somers et al., the corresponding with (table 1.4).

Table 1.4: The map of Somers et al. 2004, consensus map.

Chromosome	No. of markers	Genetic length	Marker density	Chromosome	No. of markers	Genetic length	Marker density	Chromosome	No. of markers	Genetic length	Marker density
1A	44	126	2.9	4D	34	91	2.6	Group 1	170	354	2.0
1B	75	111	1.5	5A	62	184	3.0	Group 2	198	373	1.9
1D	51	117	2.3	5B	77	173	2.2	Group 3	194	343	1.6
2A	62	143	2.3	5D	76	120	1.5	Group 4	136	238	2.3
2B	76	123	1.6	6A	38	156	4.1	Group 5	215	477	2.6
2D	61	107	1.8	6B	50	82	1.6	Group 6	123	348	2.3
3A	49	116	2.4	6D	35	110	3.1	Group 7	199	436	2.2
3B	88	148	1.7	7A	62	131	2.1	A genome	369	944	2.6
3D	57	79	1.4	7B	68	151	2.2	B genome	483	847	1.7
4A	53	88	1.7	7D	69	154	2.2	D genome	383	778	2.1
4B	49	59	1.2					Total.geno	1235	2569	2.0

Genetic maps developed from populations derived from inter varietal crosses are therefore being developed for genetic analysis and marker-trait associations relevant to breeding programs, despite low level of DNA polymorphism in cultivated wheat accessions (Chao et al. 1989; Bryan et al. 1997; Varshney et al. 1998; Liu et al. 2005; Somers et al. 2004). Examples include inter varietal genetic maps developed from populations involving crosses within spring wheat Australian cultivars (Chalmers et al. 2001), spring wheat varieties from North and South America (Liu et al. 2005) and European winter wheat varieties (Paillard et al. 2003). Furthermore, comparison of genetic maps between studies have identified ambiguities, and combining single population genetic maps into a consensus map can resolve disagreements in marker order. This approach was taken by Somers et al. (2004) who joined four genetic maps developed from populations derived from three inter varietal crosses involving Canadian wheat varieties and the ITMI population, into a single consensus map. Similarly, other consensus maps have been developed using other inter varietal populations (<http://www.shigen.nig.ac.jp/wheat/komugi/maps/markerMap.jsp>). The consensus maps are particularly useful references for targeting additional markers in specific chromosomal regions for fine mapping of quantitative trait loci (QTL) without the need to cross-reference independent maps.

4- SEGREGATION OF MARKERS IN WHEAT CHROMOSOME

4- 1- Computer software for genetic mapping

The concepts and theories previously developed for classical genetic mapping can still be applied to mapping with DNA markers. The difference between classical and DNA-based mapping lies in the number of markers that are mapped in a single population. With DNA-based genetic maps, this number can easily reach into the thousands, and so there is a close connection between progress in DNA markers and advances in computer technology.

In the simplest situations, all that is required to construct a linkage map from DNA marker data are statistics software packages capable of running Chi-squared contingency table analysis. This statistical test determines two-point linkage between markers, which can then form a basis for constructing linkage groups. Unfortunately, as the number of markers begins to grow, this approach becomes increasingly unsuited for comparing possible orders and choosing the best. Still, in research situations where computer power is limiting and where linkage analysis is based on relatively few markers, this strategy is perfectly suitable.

For most mapping projects the most widely-used genetic mapping software is Mapmaker (Lander et al. 1987). Mapmaker is based on the concept of the LOD score, the “log of the odds-ratio” (Morton 1955). A LOD score indicates the log (10) of the ratio between the odds of one hypothesis (for example, linkage between two loci) versus an alternative hypothesis (no linkage in this example). Through the use of the LOD score, data from different populations can be pooled, one reason that the program has gained so much popularity among human and animal geneticists where population sizes can be limiting. Yet even in plants, Mapmaker has become a virtual standard for constructing genetic linkage maps, as indicated by its widespread use.

Mapmaker’s popularity for genetic analysis is based on the ease with which it performs multipoint analysis of many linked loci. Most plant genetic linkage maps have at least one hundred markers, and sometimes one thousand markers or more.

Therefore, fast and simple multipoint analysis is absolutely essential to sort out the many different possible marker orders. Mapmaker has several routines that simplify multipoint analysis, including an algorithm that quickly gathers markers into likely linkage groups and another for guessing the best possible order. Once a plausible order has been established, another algorithm compares the strength of evidence for that order compared to possible alternatives in a routine called “ripple”. The power of this routine is that it enables the user to confirm the best order in a way that increases only arithmetically with increasing number of loci (as opposed to factorial, if all possible orders must be compared).

40 N.D. Young Just building a linkage map of DNA markers is generally just a first step in genetic marker analysis. As noted earlier, it is often essential to relate one's map to those derived from other mapping populations. The computer program JoinMap is specifically suited for this application (Stam 1993). Often one wishes to apply information about a linkage map to QTL analysis. Indeed, Mapmaker has even been modified to carry out quantitative trait locus (QTL) analysis using mathematical models and an interface very much like the original program (Lander and Botstein 1989).

4- 2- Relationship between DNA marker and cytogenetic maps

The most common method to relate DNA marker maps to specific chromosomes is the use of aneuploids, such as monosomics (Helentjaris et al. 1986; Rooney et al. 1994), trisomics (Young et al. 1987), and substitution lines (Sharp et al. 1989). In species where aneuploid lines for each chromosome are available, nucleic acid hybridization with a mapped DNA clone indicates its chromosome location by observing the loss of a band (in the case of nulli-somics) or a change in the relative signal on an autoradiogram (Mc Couch et al. 1988). This type of analysis may require "withinlane" standards (such as a second DNA clone of previously determined chromosome location), so that subtle changes in the relative intensity of a band can be compared between lanes. Using substitution lines to associate mapped DNA markers to specific chromosomes is similar in concept to aneuploid mapping. In cereal species where this approach is most common, lines with known chromosomes or chromosome arms substituted with homoeologous segments from alien species have been developed. Probing a DNA clone onto a blot containing restriction digested DNA from a complete set of substitution lines easily identifies the chromosome location of that clone (Sharp et al. 1989). This is because the substitution line corresponding to the location of a clone shows a different restriction fragment pattern compared to the other substitution lines.

4- 3- Distorted segregation of molecular markers

Segregation distortion is defined as a deviation of observed genetic ratios from the expected Mendelian ratios in a given phenotypic or genotypic class within a segregating population.

Distorted segregation may be caused by competition between gametes for preferential fertilization or from abortion of gamete or zygote (Lyttle 1991). Meiotic drive is another phenomenon affecting segregation ratios through a variety of molecular and cytogenetic mechanisms resulting in non-equal representation of homologous alleles (or chromosomal segments) among the functional gametes (Lyttle 1991).

It is possible to discern whether the cause of distorted segregation is gametic competition or zygotic selection by estimating the frequencies of two alleles for a codominant locus.

In general, normal Mendelian segregation can be viewed as a product of evolutionary co-adaptation, and of adjustment of genomic components within a species rather than an automatic outcome of the eukaryote meiotic mechanics (Korol et al. 1994). Indirect evidence for this is provided by the fact that segregation distortions frequently occur in the progeny of interspecific hybrids and are similar in manifestation to meiotic drive systems.

Distorted segregation of molecular markers has been observed in mapping populations derived from intra and interspecific hybrids in many plants and many crop species, including potato (Gebhardt et al. 1989), corn and maize (*Zea mays*; Gardiner et al. 1993; Wendel et al. 1987; Lu et al. 2002), rice (*Oryza sativa*; Causse et al. 1994; Xu et al. 1997), common bean (Vallejos et al. 1992), and barley (*H. vulgare*; Heun et al. 1991; Graner et al. 1991; Devaux et al. 1995).

In wheat, this phenomenon has also been reported repeatedly (Liu and Tsunewaki 1991; Devos et al. 1993; Nelson et al. 1995 a, b; Blanco et al. 1998; Messmer et al. 1999; Blanco et al. 2004; Peng et al. 2000; Quarrie et al. 2005).

In the project of (Peng et al. 2011), the portion of segregation-distorted marker loci (5.9%) but was relatively low compared with the previous studies in wheat (Liu and Tsunewaki. 1991; Blanco et al. 1998; Messmer et al. 1999). The possible causes for segregation deviation of molecular markers are chromosomal rearrangement (Tanksley 1984) and gametic or zygotic selection (Nakagahra 1986). Further indications of causes of deviation such as presence of lethal, meiotic drive, and chromosomal rearrangements could be obtained from the analysis of additional populations with segregation distortion (Blanco et al. 1998).

Backing again on the work of (Peng et al. 2011) the obtained data indicate that the gametes carrying *T. durum* alleles have stronger vigor and higher competition ability than those with *T. dicoccoides* alleles.

It is noteworthy that *T. dicoccoides* was a pollen-parent of the F1 hybrid, hence the cytoplasm was provided by *T. durum*. We could speculate that some regions with presumably gametic selection carry loci involved in nuclearcytoplasmic interaction. While segregation distortion is a common phenomenon in different types of mapping populations, such as F2, RILs or double haploid (DH), RIL populations have the highest probability for distortions due to repeated 5–6 generations of selection forces (Korol et al. 1994; Singh et al. 2007).

4- 4- Nonrandom segregation of nonhomologous chromosome

Fifty years ago, a departure from random segregation of markers on nonhomologous chromosomes observed in crosses between different strains of house mouse, was explained by a mutual attraction of the segregating chromosomes of the same origin to migrate to the same pole during meiosis (the “affinity” hypothesis) (Michie 1953; Wallace 1953). Consequently, gametes with parental

combinations of alleles at loci of nonhomologous chromosomes should appear with a higher frequency than the recombinant gametes. As the different QTLs, such as various markers WMC349, WMS149, WMS251 situated on chromosomes not homologous have effects on Lox gene.

The departure from independent segregation of unlinked genes was termed quasi-linkage. Reviewing the data in the literature, Wallace (1960 a) postulated possible chromosome affinity in cotton. Even earlier, Malinowsky (1927) explained, based on the chromosome affinity hypothesis, a number of cases of abnormal segregation in hybrids of lettuce, peas, tobacco, beans, and wheat. He was probably the first to introduce the term “affinity”. A pronounced effect of quasi-linkage was reported in an interspecific hybrid of *Coix* (Sapre and Deshpande 1987). There is evidence for nonrandom segregation of nonhomologs during the first meiotic division in wheat (Driscoll et al. 1979). Quasi-linkage has also been established in tomato (Wallace 1960 b; Zhuchenko et al. 1977; Korol et al. 1989, 1994).

Besides nonrandom assortment (or affinity), quasi-linkage can also be explained by recourse to mechanisms of differential viability. Let us consider the MiMj/mimj heterozygote resulting from a cross between MiMj/MiMj and mimj/mimj. Suppose that parental combinations of whole chromosomes (MiMj and mimj) and recombinant combinations (Mimj and miMj) have differential selective values. Experimental evidence of this type has been obtained in *Drosophila* (Dobzhansky et al. 1965). Selective differences between parental and recombinant combinations may be reflected in the differential viability of zygotes, embryos, and adult individuals. The effect can also be expressed at the gamete stage in the form of differential fertilizing capacity of spermatozoa, differential rates of pollen tube growth, etc. (Korol et al. 1989, 1994).

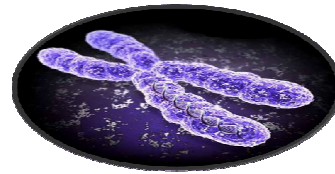
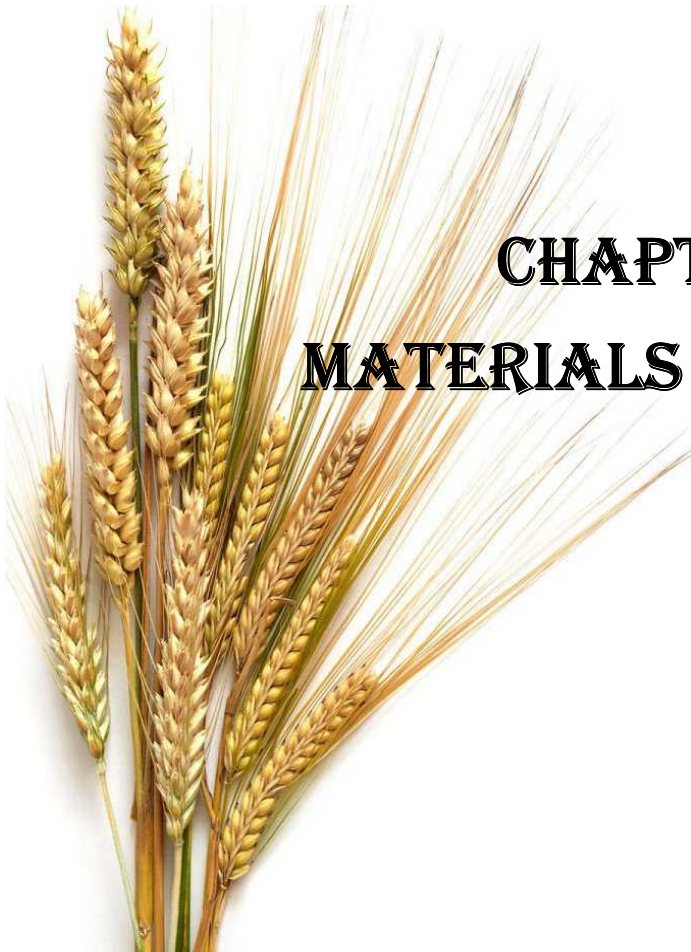
The major problem with the old data on quasi-linkage was poor genome coverage of the morphological markers that could be followed up in one cross. This strongly limited the possibilities of discriminating among different explanatory hypotheses. Molecular markers solve this problem, but at the price of another one: The size of the available segregating populations is usually very small, restricting the detection power of the tests, so that only big deviations could be declared significant. In the data of (Peng et al. 2011), together with the few examples on molecular marker segregation in tetraploid wheat, hexaploid wheat, rice, maize, and *Arabidopsis* indicate that quasi-linkage may be a much more common phenomenon in plants (especially, cereals) than ever thought before. One practical aspect is related to a possible effect of quasi-linkage on the rate of false positive detection in QTL mapping. The widespread approach of multi locus (composite) interval mapping (Zeng 1994; Jansen and Stam 1994) may be helpful in such cases.

With current procedures, the number of plant samples and DNA markers that can reasonably be processed limits the widespread application of mapping technology.

Even the most efficient DNA extraction techniques handle only a few hundred samples each day, and once samples have been isolated, significant investments in time and effort are still required to obtain genotypic information. Given the fact that a typical breeding project might include several thousand, or even tens of thousands of individuals, and since information is needed as quickly as possible to make breeding decisions, the current technical limitations are significant. These limitations also constrain the application of DNA marker technology in QTL mapping to genetic factors with relatively major effects.

CHAPTER II

MATERIALS & METHODS



1- PLANT MATERIAL

1- 1- Plant Material

In this study 192 individuals of *Triticum turgidum* subsp. *durum* belonging to the recombinant inbred lines (RILs) utilized to generate a genetic linkage map. The RIL population was developed and cultivated at ICARDA (International Center for Agricultural Research in the Dry Areas), Aleppo, Syria.

The population was in F8-F9 (seven generations of selfing) by single-seed decent (SSD) after a cross between 2 durum wheat (*Triticum turgidum* ssp. *durum*) cultivars.

First parental was the genotype 112 of the RIL from a previous cross between two durum wheat lines Jennah Khetifa and ChamI. It was chosen the RIL number 112, for its traits: (i) high quality in the production of pasta, couscous, and burghul; and (ii) resistance against the abiotic stresses, such as drought, cold and heat. The ChamI and Jennah Ketifa genotypes were drought-resistant, and had a good tillering.

The second parental was genotype 101 of the RIL from Backcross between two durum wheat lines (Omrabi5 × *T.dicoccoides*) × Omrabi5. This Recombinant Inbred Line (BC-RIL) developed at CIMMYT/ICARDA durum breeding program for the Mediterranean dryland from a cross between the durum cultivar Omrabi5 and the accession number 600545 of *T. dicoccoides*. The F1-cross was backcrossed to the maternal parent Omrabi5. The Omrabi5 cultivar is a cross between Haurani × Jori-C, bred for Mediterranean dry land conditions by CIMMYT/ICARDA durum program (Nachit pers. com.). It is released in Turkey, Algeria, Iran, and Iraq for commercial production in dry areas. It combines drought tolerance with yield and yield stability as a high weight for the thousands of seeds, and a high content of proteins. As for *Triticum dicoccoides* 600545, it was collected from Jordan at 25 km west of Amman on the Amman-Dead Sea highway. It shows resistance to yellow rust and tolerance to drought. Parents' pictures and cross schemes are reported in Figure (2.13).

1- 2- DNA

No matter what type of population or DNA marker one plans to use, DNA must first be isolated from the plants in the mapping population. Such as morphological or disease resistance traits, whose expression tends to be highly dependent upon growth conditions.

After having crossed these two relatives, and after the last selfing (the seven generation), every line was bulk-harvested to provide seed for field experiments and DNA extraction. DNA was extracted from fresh leaf tissue. High-quality genomic DNA from parents and RILs was extracted from young leaves using CTAB method (Hoisington et al. 1994; Nachit et al. 2001). The DNA extracted from different individuals, they had different concentration, hence was adjusted by adding water to arrive

at a constant concentration of 100 ng/ul for the all individuals. The DNA concentration was also adjusted to 7 ng/ul and DNA samples were stored at 4°C to be used for the analysis of microsatellites, and SNP markers (Tai and Tanksley 1990).

Figure 2.12: The different type of *Triticum turgidum* L. subsp. *durum*.



Figure 2.13 : The figure and the drawing for our cross between the three cultivar varieties of durum wheat and one wild varieties of durum wheat.

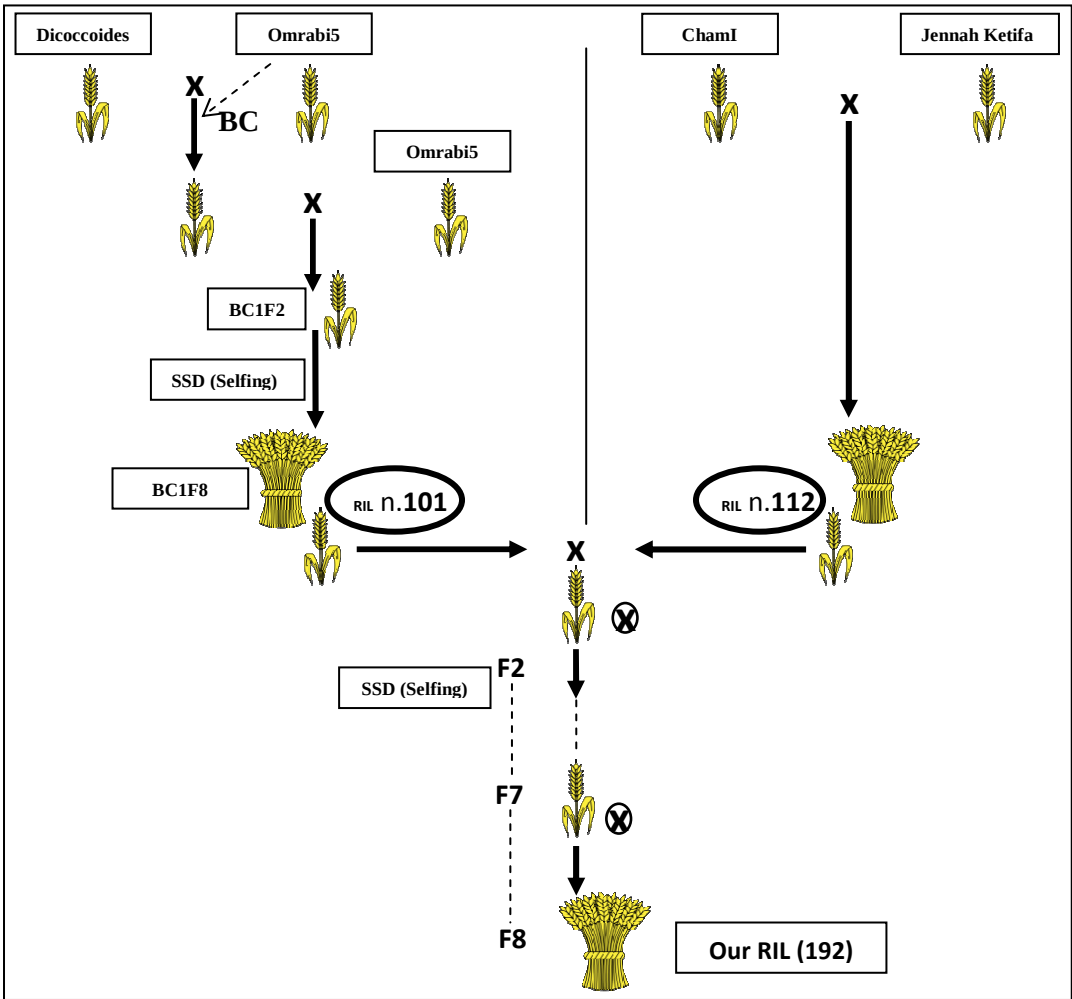


Table 2.1 : Origin of Cultivar and wild Species.

Species	Name of cultivar	Cultivar's code	Region
<i>T. turgidum ssp. durum</i> (Desf.) Husn. (cultivars)	<i>Cham1</i>	Ch1	Syria (Developed by SGCASR)
<i>T. turgidum ssp. durum</i> (Desf.) Husn. (cultivars)	<i>Jennah Ketifa</i>	J.K	Syria (Developed by SGCASR)
<i>T. turgidum ssp. durum</i> (Desf.) Husn. (cultivars)	<i>Omrabi</i>	Om	Syria/Alraka, Syria (Developed by SGCASR)
Emmer and Durum Wheats	<i>T.Dicoccoides</i>	Dc	Turkey, Iran

The Syrian Commission of the Agricultural Scientific Research (SGCASR)

1- 2- 1- Station of agricultural species (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. 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 50 mm (Fig. 2.14).

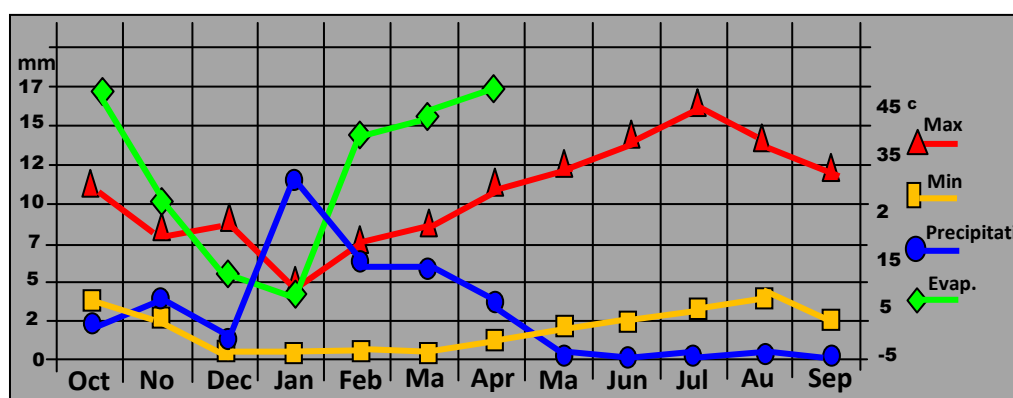


Figure 2.14 : Meteorological data Average of 1999/ 2009, Min. temp = minimum temperature; Evap. = evaporation; Max. temp = maximum temperature.

1- 2- 2- Experimental Design

For collection of phenotypic and genotypic data for grain quality traits, the 192 RILs were divided over 6 blocks where 32-test RILs were included in each block. The field design used was the augmented design (Federer 1956; Peterson 1985). The trial was sown in 8 rows of 2,5 m long spaced by 30cm.

1- 3- SSR Markers

A total of 216 genomic SSR primer pairs were screened using the two parental lines. Markers were prevalently chosen within the public SSRs (<http://wheat.pw.usda.gov>) (Table 2.4 presents the list of the screened SSR markers).

These markers consisted primarily of Gatersleben Wheat Microsatellites (GWM; Ganal and Röder, 2007) and a few additional markers from Wheat Microsatellite Consortium (WMC; Gupta et al. 2002), INRA Clermont-Ferrand (CFA and CFD; Sourdille et al. 2004; Guyomarc'h et al. 2002), and Beltsville Agriculture Research Center (BARC; Song et al. 2005) this 216 markers showing codominant alternative alleles between the two parental lines and covering all 14 chromosomes of tetraploid wheat were used to genotype the 192 RILs (Table 2.2).

To have a complete understanding of the origins of these markers refer to Table 2.3, for example Barc markers were been built from wheat and barley scab initiative to map and characterize genes for fusarium resistance.

Some of the SSRs used in this study were mapped in previous published maps, i.e. a durum wheat mapping population (249 RILs from the cross 'Kofa × Svevo'; Jurman et al. unpublished data), herein indicated as 'K × S', as well as on the bread wheat Ta-SSR-2004 consensus SSR map (Somers et al. 2004) and on the Ta-Synthetic/Opata- BARC map (Song et al. 2005), hereafter referred to as ITMI map. SSR primer sequences of BARC, CFA, CFD, GWM, GPW and WMC primer's sets are publicly available on the GrainGenes Triticeae database (<http://wheat.pw.usda.gov/>). Most of these primers generated SSR loci that were not previously mapped either in the Ta-Synthetic/Opata-SSR or in the Ta-SSR-2004 (Tables 2.2 and 2.4).

Table 2.2: The number of SSRs markers used in this work.

SSR	Class Number	Reference
Barc (Xbarc)	39	Song et al. (2002, 2005)
Cfa (Xcfa)	13	Sourdille et al. (2003); Guyomarc'h et al. (2002)
Cfd (Xcfd)	10	Sourdille et al. (2003); Guyomarc'h et al. (2002)
GPW (Xgpw)	2	Sourdille et al. (2001)
Wmc (Xwmc)	46	Gupta et al. (2002); http://wheat.pw.usda.gov/ggpages/SSR/WMC
Wms (Xwms)(gwm)	100	Pagnotta et al. (2006); Röder et al. (1998); Martin Ganal, IPK, Gatersleben, Germany
Total	210	

Table 2.3: The indications for Origins and references of SSR markers.

Name of Markers	Origin	Microsatellite code	Polyploid wheat	Microsatellite developer
WMS	Gatersleben wheat microsatellites	GWM	Tetraploide, Hexaploide wheat	Marion Röder (IPK)
WMC	Gatersleben wheat microsatellites	XWMC	Tetraploide wheat	Wheat Microsatellite Consortium
BARC	Wheat and Barley Scab Initiative to map and characterize genes for fusarium resistance	XBARC	Tetraploide, Hexaploide wheat	Perry Cregan (USDA)
CFA	A and B genome diploid donors	XCFA	Diploide wheat	Pierre Sourdille (INRA)
CFD	D genome diploid donors	XCFD	Diploide wheat	Pierre Sourdille (INRA)
MST	A, B and D genome diploid donors	XMST	Diploide wheat	---
GPW	A, B and D genome diploid donors	XGW	Diploide wheat	Marion Röder (IPK)

Table 2.4: Probed Polymorphic Microsatellites sequences and their optimal amplification program in *Jennah.Ketifa/ChamI* × *Omrabi5/T.dicoccoides600545//Omrabi5*.

SSRs	Forward (Left) →	Sequence (5'-3')	← Revers (Right)	PCR- Program
Barc 52	GCGCCATCCATCAACCGTCATCGTCATA	GCGAGGAAGGCGGCCACCAGAATGA	65°-36c	
Barc 78	CTCCCCGGTCAAGTTTAATCTCT	GCGACATGGGAATTTCAGAAGTGCCTA	65°-36c	
Barc 79	GCGTTGGAAAGGAGGTAATGTTAGATA	TCGTGGGTTACAAGTTTGGGAGGTCA	57°-36c	
Barc 101	GCTCCTCTCACGATCACGCAAAG	GCGAGTCGATCACACTATGAGCCAATG	55°-36c	
Barc 104	GCGCTTCCAAGGCTTAGAGGCT	GCGCTTGGAAACGCTAAGAGCGG	55°-36c	
Barc 107	CGAACTGAACTGAAAACACAGACAT	AGGGAGAGTGGACGTCATAATTTGTG	60°-36c	
Barc 119	CACCCGATGATGAAAAT	GATGGCACAAGAAATGAT	55°-36c	
Barc 167	AAAGGCCCATCAACATGCAAGTACC	CGCAGTATTCTTAGTCCCTCAT	55°-36c	
Barc 170	CGTTGACTTTGAATGGCTGAACA	CGCCACTTTTTACCTAATCCTTTTGAA	59°-36c	
Barc 171	GCGGGGTCATCTTAGTAACTCAAATA	ACTGTCAACGTTGGTTCACATTCA	56°-36c	
Barc 198	CGTGAAAAGAAGTGCCGCATTATGA	CGCTGCCTTTTCTGGATTGCTTGTC	55°-36c	
Barc 213	GCGTAGATTCTCGGTTTGTTGGCTTGC	CCGTCCCTCCTTCTGGTCT	59°-36c	
Barc 309	AAGCCGAGAGAAGGTCAGC	GCGAAAGCCCTAAAGTTACAA	60°-36c	
Barc 318	CGACTAACAATTTTTCATTT	TGATTTGCTAACAAGGAG	55°-36c	
Barc 343	GGCCTAATTACAAGTCCAAAA	GCTCAAAGTAAAGTTCACGAATAT	52°-36c	
Barc 349	CGAATAGCCGCTGCACAAG	TATGCATGCCTTCTTTACAAT	60°-36c	
Barc 354	CGTTGTTTGCCTAGAAGGAGGTT	GCGAATGCGGGCGATAAAGTGG	55°-36c	
Barc 361	TGCAGGCTAGATTGGGCGACG	GTGGGGCCTAGGCAGTGGACG	60°-36c	
Cfa 2043	CAGCCGAAGAAGGATTCTG	GAGGCAGGAACCTAGGGGAG	60°-36c	
Cfa 2086	TCTACTTTCAGGGCACCTCG	TCTCTCAAACCTCCCTGTAA	60°-36c	
Cfa 2153	TTGTGCATGATGGCTTCAAT	CCAATCCTAATGATCCGCTG	60°-36c	
Cfa 2201	CAAACCAACCTCATTGACCC	CCACCAGAACTTCAACCTGG	60°-36c	
Cfa 2114	ATTGGAAGGCCACGATACAC	CCCGTCGGGTTTTATCTAGC	60°-36c	
Cfa 2121	TAAATGGCCATCAAGCAATG	GCTTGTGAACTAATGCCTCCC	60°-36c	
Cfa 2263	GGCCATGTAATTAAGGCACA	CTCCAGGAGTACAGAAGAGGA	60°-36c	
Cfa 2278	GCCTCTGCAAGTCTTTACCG	AAGTCGGCCATCTTCTTCT	60°-36c	
Cfd 70	GTCGGCATAGTCGCACATAC	ACTATGCCAAGGGGAGTGTG	60°-36c	
Cfd 73	GATAGATCAATGTGGGCCGT	AACTGTTCTGCCATCTGAGC	60°-36c	
Cfd 88	TAGGCATAGTTTTGGGCCTG	GGTAGAAGGAAGCTTCGGGA	60°-36c	
Cfd 267	GTGCGTCGTGTAGCAGCTC	CTCTCTGTCTCCAGGTCGT	60°-36c	
Mst 101	CCACCATGAAGACCTTCCTC	ACCTTGCATGGGTTTAGCTG	60°-36c	
Gpw 95010	CCATTCCGCAATGATGATA	GTGTCTTCACCAACTTCACACTCCAGG	60°-36c	
Wmc 24	GTGAGCAATTTTGATTATACTG	TACCCTGATGCTGTAATATGTG	59°-36c	
Wmc 49	CTCATGAGTATATACCGCACA	GACGCGAAACGAATATTCAAGT	51°-36c	
Wmc 63	GTGCTCTGGAAACCTTCTACGA	CAGTAGTTTAGCCTTGGTGTGA	60°-36c	
Wmc 104	TCTCCCTCATTAGAGAGTTGTCCA	ATGCAAGTTTAGAGCAACACCA	61°-36c	
Wmc 125	ATACCACCATGCATGTGGAAGT	ACCGCTTGTCAATTCCTTCTGT	61°-36c	
Wmc 163	TTACACCCATCAGGGTGGTCTT	GTCCGTCTATCCATACGACAAA	60°-36c	
Wmc 175	GCTCAgTCAAACCGTACTTCT	CACTACTCCAATCTATCGCCGT	61°-36c	
Wmc 177	GGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA	55°-36c	
Wmc 201	CATGCTCTTCACTTGGGTTCTG	GCGCTTGCAGGAATTCAACACT	61°-36c	
Wmc 219	TGCTAGTTTGTATCCGGGCGA	CAATCCCGTTCTACAAGTTCCA	60°-36c	
Wmc 262	GCTTTAACAAAGATCCAAGTGGCAT	GTAACATCCAAACAAAGTCGAACG	61°-36c	
Wmc 272	TCAGGCCATGTATTATGCAGTA	ACGACCAGGATAGCCAATTCAA	60°-36c	
Wmc 278	AAACGATAGTAAAATTACCTCGGAT	TCAAAAAATAGCAACTTGAAGACAT	53°-36c	
Wmc 310	TGTGAGGCTGGGAGGAAAAGAG	GCTAGGTTGTGCCACAATGC	61°-36c	
Wmc 317	TGCTAGCAATGCTCCGGGTAAC	TCACGAAACCTTTTCTCTCTCC	59°-36c	
Wmc 332	CATTTACAAAGCGCATGAAGCC	GAAAACCTTGGGAACAAGAGCA	61°-36c	
Wmc 349	ACACACACTCGATCGCAC	GCAGTTGATCATCAAAACACA	57°-36c	
Wmc 360	TATGTGTGTGAAAAATGG	AATTTCTGTAGAACCGA	50°-36c	
wmc 361	AATGAAGATGCAAAATCGACGGC	ATTCTCGCACTGAAAACAGGGG	61°-36c	
Wmc 397	AGTCGTGCACCTCCATTTTG	CATTGGACATCGGAGACCTG	61°-36c	
Wmc 407	GGTAATTCTAGGCTGACATATGCTC	CATATTTCCAAATCCCCAACTC	60°-36c	
Wmc 441	TCCAGTAGAGCACCTTTCATT	ATCACGAAGATAAACAAACGG	56°-36c	
Wmc 453	CTTGTGTCCATAACCGACCTT	ATCTTTTGAGGTTACAACCCGA	65°-36c	
Wmc 477	CGTCGAAAACGTACACTCTCC	GCGAAACAGAATAGCCCTGATG	65°-36c	
Wmc 522	AAAAATCTCACGAGTCGGGC	CCCGAGCAGGAGCTACAAAT	65°-36c	

SSRs	Forward (Left) 	Sequence (5'-3') 	Revers (Right)	PCR- Program
Wms 16	GCTTGGACTAGCTAGAGTATCATAC	CAATCTTCAATTCTGTGCGACGG		50°-36c
Wms 18	TGGCGCCATGATTGCATTATCTTC	GGTTGCTGAAGAACCTTATTTAGG		50°-36c
Wms 24	FCACACAAGGCACCATTCG	CAATGGACATAGTTGTGTGCG		60°-36c
Wms 46	GCACGTGAATGGATTGGAC	TGACCCAATAGTGGTGGTCA		60°-36c
Wms 47	TTGCTACCATGCATGACCAT	TTCACCTCGATTGAGGTCTT		60°-36c
Wms 67	ACCACACAAACAAGGTAAGCG	CAACCCTCTTAATTTTGTGGG		60°-36c
Wms 92	CCGGCGACAACTATAGCTC	TTCTGGTGATTCAAACCTGAACC		59°-36c
Wms 95	GATCAAAACACACACCCCTCC	AATGCAAAAGTGAAAAACCCG		55°-36c
Wms 113	TTCGAGGTTAGGAGGAAGAGG	GAGGGTCGGCCTATAAGACC		55°-36c
Wms 120	GATCCACCTTCTCTCTCTC	GATTATACTGGTGCCGAAAC		60°-36c
Wms 136	GACAGCACCTTGCCCTTG	CATCGGCAACATGCTCATC		60°-36c
Wms 169	ACCACTGCAGAGAACACATACG	GTGCTCTGCTCTAAGTGTGGG		60°-36c
Wms 191	AGACTGTTGTTTGCGGGC	TAGCACGACAGTTGTATGCATG		60°-36c
Wms 198	CTTTGTGCACCTCTCTCTCC	AATTGTGTTGATGATTGGGG		60°-36c
Wms 200	TCAACGGAACAGATGAGCG	GACCTGATGAGAGCAAGCAC		60°-36c
Wms 218	CGGCAAACGGATATCGAC	AACAGTAACTCTGCCATAGCC		60°-36c
Wms 234	FGAGTCCTGATGTGAAGCTGTTG	CTCATTGGGGTGTGTACGTG		55°-36c
Wms 249	CAAAATGGATCGAGAAAGGGA	CTGCCATTTTCTGGATCTACC		58°-36c
Wms 269	TGCATATAAACAGTCACACACCC	TTTGAGCTCCAAAGTGAGTTAGC		60°-36c
Wms 291	CATCCCTACGCCACTCTGC	AATGGTATCTATTCCGACCCG		60°-36c
Wms 304	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG		55°-36c
Wms 311	FTCACGTGGAAGACGCTCC	CTACGTGCACCACCATTTTG		60°-36c
Wms 312	ATCGCATGATGCACGTAGAG	ACATGCATGCCTACCTAATGG		60°-36c
Wms 319	GGTTGCTGTACAAGTGTTACG	CGGGTGCTGTGTGTAATGAC		65°-36c
Wms 328	CGGGTGCTGTGTGTAATGAC	CACAACTCTTGACATGTGCG		55°-36c
Wms 339	ATTTTCTTCTCACTTATT	AAACGAACAACCACTCAAT...		55°-36c
Wms 371	GACCAAGATATTCAAACTGGCC	AGCTCAGCTTGCTTGGTACC		60°-36c
Wms 372	AATAGAGCCCTGGGACTGGG	GAAGGACGACATTCCACCTG		60°-36c
Wms 425	GACGAGCCCAAGCTGGCA	TCGTTCTCCCAAGGCTTG		60°-36c
Wms 427	AAACTAGAACTGTAATTCAGA	AGTGTGTTCAATTTGACAGTT		50°-36c
Wms 459	ATGGAGTGGTCACACTTTGAA	AGCTTCTCTGACCAACTTCTCG		55°-36c
Wms 495	GAGAGCCTCGCGAAATATAGG	TGCTTCTGGTGTTCTTCG		60°-36c
Wms 499	ACTTGTATGCTCCATTGATTGG	GGGGAGTGGAACTGCATAA		60°-36c
Wms 501	FGGCTATCTCTGGCGCTAAAA	TCCACAAACAAGTAGCGCC		55°-36c
Wms 512	AGCCACCATCAGCAAAATT	GAACATGAGCAGTTTGGCAC		60°-36c
Wms 537	ACATAATGCTTCTGTGCACC	GCCACTTTTGTGTCGTTCT		60°-36c
Wms 558	GGGATTGCATATGAGACAACG	TGCCATGGTTGTAGTAGCCA		55°-36c
Wms 570	TCGCCTTTTACAGTCGGC	ATGGGTAGCTGAGAGCCAAA		60°-36c

1- 4- Markers Screening and Amplification

polymerase chain reaction (PCR)-based markers, which all require smaller amounts of starting material and simpler extraction technologies (Lange et al. 1998). With these methods, the goals are simplicity, speed, and a small amount of starting material. Simplicity and speed are absolutely essential for processing large numbers of individuals an obvious necessity when large populations of several hundred, or even thousands, of individuals need to be examined. Small amounts of starting material are advantageous if larger quantities are hard to obtain, such as seeds. DNA used for this genetic mapping to be highly purified. As long as an extraction provides DNA in sufficient

quantity and quality for restriction enzyme digestion for any samples, through template for PCR. SSR markers were screened to test their polymorphisms between the parents.

The SSRs markers were amplified by PCR in the plates of 96, in 10 µl of reaction containing:

3 µl genomic DNA with a concentration of 5 ng /µl, 5 µl of H₂O Mol Bio grade autoclaved, 1X PCR Buffer Promega (100 mM Tris-HCL – pH 8.3, 500 mM KCL, 1.5 mM MgCl₂), 0.002 µl Forward primer (→) labeled with 0.02 µl colored fluorescent dyes or M13 tail (FAM, VIC or Hex, NED and PET) with a size respectively 494, 538, 535, 546 and nm (absorbance maximum), (M13 appointed a DNA fragment with the sequence complement to a tail added into 5' of the Forward primer to give the relevance the gene or interest fragment with the two primers), 0.02 µl unlabeled Reverse primer (←), 0.6 µl each dNTP 100 mM(25µmol), e 0.05 µl di AmpliTaq-DNA Polymerase (Applied Biosystems, Foster City, USA).

PCR amplifications were performed in a thermocycler Eppendorf (Master cycler) and Biometra using the following conditions according to Röder et al. (1998), 2 min at 94°C, followed by 35 cycles of 30 sec 94°C, 30 sec annealing (between 50 and 65°C, depending on the optimal annealing temperature of the primers), 1 min 72°C, and a final extension of 10 min at 72°C, and finally the samples were kept at 8°C.

In general, the reactions of SSR markers for the parental screening were grouped on the basis of the annealing temperature. This was verified for each marker through the formula:

$69.3 + [0.41 \times (\% \text{ GC}) - 650 / L]$, and the site of GrainGenes. After amplification the samples have been loaded into a sequence for amplification reading. Up to 4 amplification, labeled with different dyes were bulk together in a total 16 µl of a liquid comprising: 2.5 µl of amplification, 0.5 µl of LIZ (GeneScan™_500LIZ size standard) and 13µl of Hi-Di™ Formamide (Grenetic Analysis Grade).

This Liz developed for the Applied Biosystems fluorescence-based DNA electrophoresis systems. The use of an internal lane size standard during electrophoresis enables automated data analysis and precise DNA fragment size comparisons between electrophoresis runs. LIZ is designed for sizing DNA fragments in the 35-500 bp range and provides 16 single-stranded labeled fragments of 35 of 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490 and 500 bases. Each of the DNA fragments is labeled with the LIZ fluorophore, which results in a single peak when run under denaturing or native conditions (Fig. 2.15).

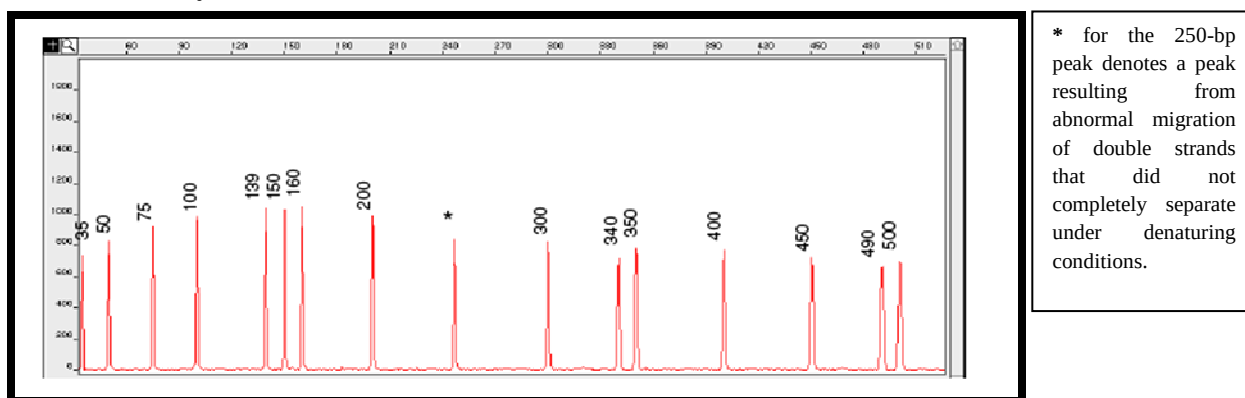
The amplification products were analyzed by means of capillary electrophoresis (ABI-3130, Fig 2.16), multiplexing different fluorescent dyes. Then later we observed the peaks specificity, shown by the program Genemapper version 4.0. Indeed, Electropherograms were analyzed with GeneMapper V.4. The program Genemapper was used to identify the size of the fragments amplified with the primers used (Singer and Burke 2003; Takuya et al. 2002).

It is critical to the sizing method employed by the GeneMapper software that there are at least two size standard fragments larger than amplified largest unknown fragment.

The size standards are loaded in the same capillary as the experimental samples. The sample and size standard fragments undergo the same electrophoretic forces, therefore the relative electrophoretic mobility of any sample fragment is a good indicator of its molecular weight, as there is no capillary-to-capillary variation.

The SSR of the 2 parental lines (Parental Screening) were evaluated in both high-resolution Agarose gel, which electrophoresis capillary sequencer ABI3130.

Figure 2.15: Electro program of the GeneScan-500 size standard run under denaturing conditions on the ABI PRISM 310 Genetic Analyzer.



The 16ul of the above mentioned mix were load into the wells of the plate 384 or 96 and sent for Sequencing (ABI3130).

For simplification and for the spread of DNA in 96-well or 384-well plates an automatic machine, Robot (Beckman Coulter TM) was used (Fig. 2.16), it has several pipettes and 16 places for the plates.

Figure 2.16: Imagines of robot and sequencing.



1- 5- SNPs Identification: Multialignments and primer design

Two different typologies of SNP mutations have been analyzed: (i) unspecific SNPs on genes with different functions localized on chromosomes 2 and 4 and (ii) SNPs specific for genes involved in drought and salinity stresses tolerance (Table 2.5).

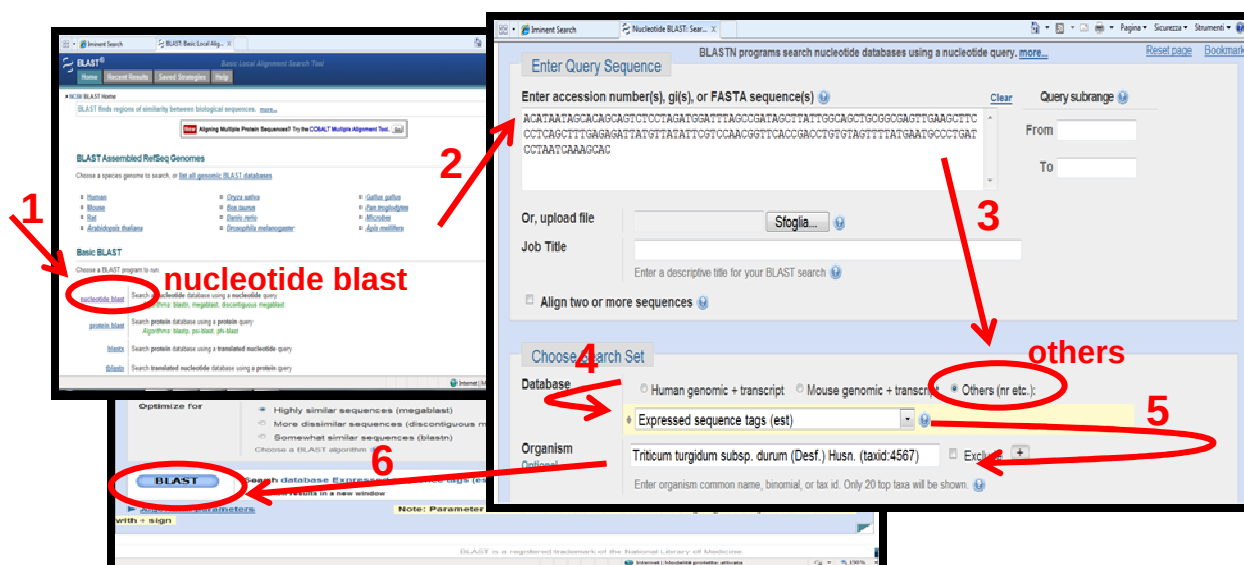
The first class of SNPs has been developed employing the SNP mutations available on database “snpdb/ Haplotype Polymorphism in Polyploid Wheats and their Diploid Ancestors”.

An online SNP database (<http://probes.pw.usda.gov:8080/snpworld/Search>) was constructed. It contains sequences of GSPs for the amplification and sequencing of 2114 loci and other relevant information about the ESTs and SNPs (such as deletion-bin mapping of each EST), top 38 blast hits of each EST, alignments of nucleotide sequences generated with primers derived from each EST, a reference sequence for a locus and its source, and graphical and numerical displays of each SNP. Reference sequences were used to specify the positions of SNPs.

For the majority of the loci, the CV *Triticum aestivum*, or from China or from Iran sequence was used as a reference sequence.

The sequences of putative genes have been analyzed and SNP mutation were identified that will be repeated the same SNP (similarity SNP) in Durum wheat *Triticum dicoccoides* and must had less than 1000 nucleotides (bases). SNPs sequence were studied to know if the SNP belonged to the Exon or Intron. The SNPs that were present in Exon Ranges were chosen, to be sure to have the SNPs in the c-DNA segment, after amplification. The Exon Ranges is chosen and copied and brought to the website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). With nucleotide blast option the sequence was compared to other in the database. Automatically the blast option opens a page with a blank picture above, for paste of our sequence. It was chosen others for the database option, under this section is written Expressed sequence tags(est) in the gap, for Organism option, was chosen the specie: *Triticum turgidum* subsp. durum (Desf.) Husn. (taxid:4567).

Figure 2.17 : The procedure to make the Blast and in the following construction of SNP primers.



We choose BLAST option to get the percentage of identity between the fragments which include SNPs with the original fragments of our species studied.

Utilizing in particular the mutations localized on exon sequences on chromosomes 2 and 4. These SNPs have been analyzed using HRM (High Resolution Melting) technology and since this technique requires the employment of amplification fragment no longer than 100 bp, new primer pairs for all the SNPs considered have been designed in order to obtain fragments with the right length. For the development of the second class of SNPs, several sequences deriving from UniGene (gene-oriented clusters of transcript sequences) and “mRNA complete cds” from wheat, *Arabidopsis*, barley and rice species were multi-aligned using ClustalW2 (Multiple Sequence Alignment, EMBL-EBI, website: <http://www.ebi.ac.uk/Tools/msa/clustalw2>) software and conserved portions (overlapping the substrate binding sites of proteins) were selected and loaded on Primer3 software (website: http://biotools.umassmed.edu/bioapps/primer3_www.cgi) for primer design. Also in this case, primers were chosen in order to obtain amplification fragments no longer than 100 bp as requested in HRM procedure (Fig 2.17; Table 2.5).

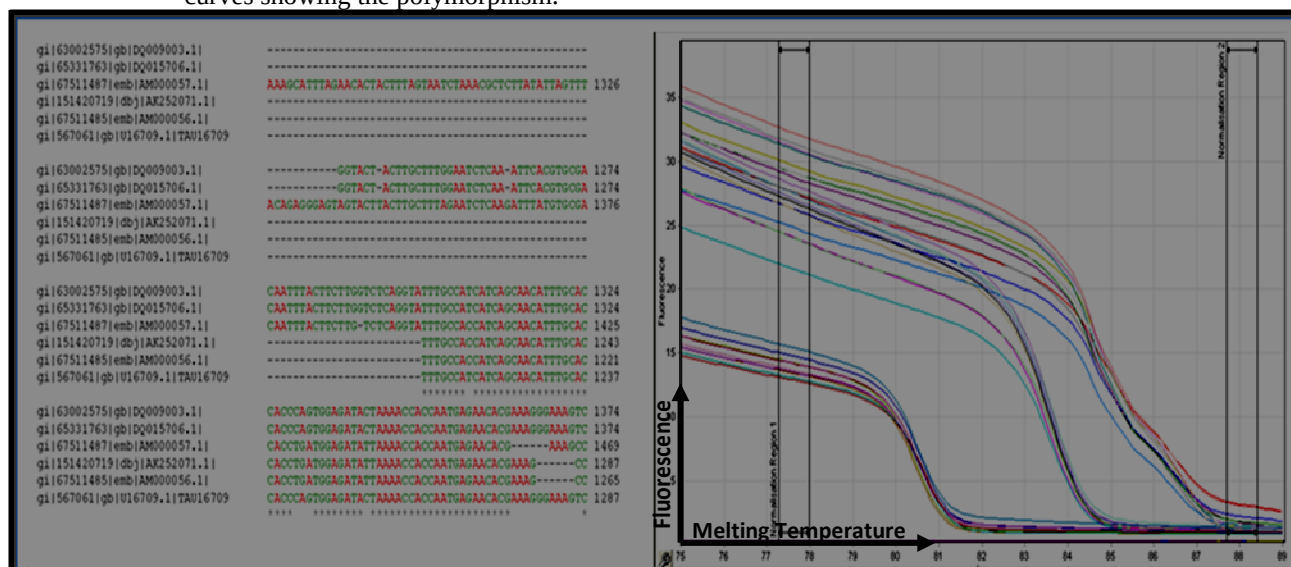
Table 2.5: SNP markers used correctly for the construction of our map came from the two-class.

Group 1: Markers non-specific gene								
N.	Gene	Cod of Sequence	BLAST with <i>T. durum</i>	SNPs	Locus	Fragment lenght	Tm	Mono/Poli
1	-	BE403352	no	2+ins of A, T/C-T/G	2A	141 bp	60°C	Mono
2	-	BE403516	yes-ok	2, C/T-A/G	2A	155 bp	60°C	Mono
3	-	BE405045	no	C/T	4B	180 bp	60°C	Poli
4	-	BE442788-a	yes-100%	A/C	4B	134 bp	60°C	Poli
5	-	BE442788-b	yes-100%	C/T	4B	123 bp	60°C	Poli
6	-	BE443094-a	no	C/A-A/T	2A	150 bp	60°C	Mono
7	-	BE443094-b	no	T/C	2A	150 bp	60°C	Mono
8	-	BE444541-a	yes-100%	G/T-T/G-A/T	2A	107 bp	60°C	Mono
9	-	BE444541-b	yes-100%	C/T-C/T-C/T	2A	134 bp	60°C	Mono
10	-	BE445278	no	G/A	2B	122 bp	60°C	Mono
11	-	BE471201	yes-98%	C/T-T/A-G/A	2A	155 bp	60°C	Mono
12	-	BE490267-a	no	C/T	2A	164 bp	60°C	Mono
13	-	BE490267-b	no	G/A	2A	169 bp	60°C	Mono
14	-	BE490763	no	T/C	2A	151 bp	60°C	Mono
15	-	BE500206	no	G/A-T/G	4B	149 bp	60°C	Mono
16	-	BE585760-a	no	T/C-G/A	2A	150 bp	60°C	Mono
17	-	BE585760-b	yes	G/A	2A	150 bp	60°C	Poli
18	-	BE403637-a	yes 100%	C/T-T/A-G/T-A/G	4A	144 bp	60°C	Mono
19	-	BE403637-b	yes 100%	A/T-ATG/GGC-C/T	4A	150 bp	60°C	Mono
20	-	BE404553	yes 96%	A/C	4A	140 bp	59°C	Poli
21	-	BE404717	yes	T/C-T/C	4A	102 bp	60°C	Mono
Group 2: Markers specific for Drought tolerance								
		Cod. UniSTS	Description					
22	OST1A	UniSTS:273614	MYB transcription factor	not sequenced	PMC92356P1	134 bp	60°C	Mono
23	OST1B	UniSTS:18383	zinc finger (C3HC4-type RING finger) family protein	not sequenced	D11S2591	113 bp	59°C	Mono
24	OST1C	UniSTS:239878	LTP2 (LIPID TRANSFER PROTEIN 2); lipid binding	not sequenced	DXMit3	86 bp	60°C	Mono
25	OST1D	UniSTS:152073	Zinc finger (C3HC4-type RING finger) family protein	not sequenced	D11S2920	120 bp	60°C	Poli
26	RD26	UniSTS:498527	Beta glucanasi HALF-1	not sequenced	Ndufb11	200-300 bp	59°C	Mono
27	SDIR1	UniSTS:258524	SDIR1 (SALT- AND DROUGHT-INDUCED RING FINGER1)	not sequenced	MASC_STS12221	336 bp	61°C	Mono
28	DREB1A	UniSTS 1	Dehydration responsive elements-1	not sequenced	no	100 bp	60°C	Mono
29	DREB1B	UniSTS 2	Dehydration responsive elements-1	not sequenced	no	100 bp	61°C	Mono
30	DREB2A	UniSTS 3	Dehydration responsive elements-2	not sequenced	no	100 bp	59°C	Poli
31	DREB2B	UniSTS 4	Dehydration responsive elements-2	not sequenced	no	100 bp	59°C	Mono
32	DREB3B	UniSTS 5	Dehydration responsive elements-3	not sequenced	no	100 bp	60°C	Poli
33	DREB4B	UniSTS 6	Dehydration responsive elements-4	not sequenced	no	100 bp	60°C	Poli
34	DREB5A	UniSTS 7	Dehydration responsive elements-5	not sequenced	no	100 bp	60°C	Mono
35	DREB5B	UniSTS 8	Dehydration responsive elements-5	not sequenced	no	100 bp	60°C	Mono
36	ZIP3	UniSTS 9	Zinc finger family protein	not sequenced	no	100 bp	59°C	Poli
37	HKT1	UniSTS 10	High Affinity Potassium uptake TF-1	not sequenced	no	100 bp	61°C	Mono
38	WRKY1	UniSTS 11	WRKY Transcription Factor-1	not sequenced	no	100 bp	60°C	Mono

1- 5- 1- PCR conditions, sequencing and SNPs characterization

PCR amplifications were performed on a Rotor-Gene 6000 real-time PCR Thermocycler (Corbett Research, Australia) using 1.25 units of TaKaRa Taq DNA polymerase (TaKaRa Ex Taq R-PCR Custom) in a total volume of 25 µl containing 10 ng of cDNA, 1X RT PCR buffer, 100 µM of each dNTP 5 pmoles of each PCR primer, Mg^{2+} 1.5 mM and 1.25 µl of EvaGreen dye 20X. PCR reaction was performed with a cycling of 45 cycles at 95°C for 5 seconds (denaturation) and 60°C (annealing) for 10 seconds, followed by a pre-melting hold of 72°C for 2 minutes and 50°C for 30 seconds, and a melting step with a ramp temperature of 75-95°C. For data quality control, PCR amplifications were analysed through the assessment of the C_t (*Threshold cycle*) value, end point fluorescence level, and the amplification efficiency. The melting data were normalized by adjusting the beginning and end fluorescence signals to the same level. High resolution melting curve analysis was performed using the HRM analysis module. Different plots of the melting data were visualized by selecting a genotype for comparison and negative first-derivative melting curves were produced from the fluorescence versus temperature plots (Fig. 2.18). An initial screening were performed probing all the primers designed on the two parentals J.K/CI and O5/O5 in order to identify polymorphic primers. Replicates of the amplification fragments resulted polymorphic, have been sequenced in order to identify the typology of SNP mutation occurring, using an ABI 3130xl sequencing platform. Sequences obtained were analysed using DNAMAN (www.Lynnon.com) software, validated employing RealSNP (Sequenom, Inc.) software, confirmed blasting them with the corresponding sequences present in genes databases through BLAST (Basic Local Alignment Search Tool; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) program, and multi-aligned to identify SNPs mutations. For each couple of peaks resulting polymorphic, respective bins have been assigned in order to assess the polymorphism on the progeny.

Figure 2.18 : An example of a multi-alignment of sequences used to design primers and different plots of melting curves showing the polymorphism.



1- 6- Data analysis and linkage map construction

Advances in computer technology have been essential for progress in DNA marker maps. While the theory behind linkage mapping with DNA markers is identical to mapping with classical genetic markers, the complexity of the problem has increased dramatically (Young 2000).

For each segregating marker, a Chi-square (X^2) analysis was performed to test for deviation from the 1:1 expected segregation ratio. Linkage maps of SSR, SNP were constructed using MapMaker1 described in Mester et al. (2003 a, b, 2004) and Ronin et al. (2008).

With a linkage criterion of $P = 0.001$, the Kosambi function was used to calculate genetic distances from recombination fractions and the best order of markers within linkage groups was determined using the Ripple command. First, the pairwise recombination fractions (rf) were calculated for all pairs of markers using maximum likelihood estimation procedure.

The Kosambi function was used to calculate genetic distances in CentiMorgan units from recombinant fraction (Kosambi 1944). Linkage groups were assigned to the chromosomes by comparison with the other published durum genetic maps (Korzun et al. 1999; Elouafi et al. 2001; Nachit et al. 2001; Elouafi and Nachit 2004; Blanco et al. 1998, 2004) and the bread wheat SSR consensus map developed by (Somers et al. 2004).

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.

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 Jennah Ketifa/ ChamI × Omrabi5/ *T. dicoccoides*600545// Omrabi5 map to the previously published wheat maps, especially to Peleg et al. (2008) and Zhang et al. (2008) wheat microsatellites map.

Then, the number of clusters (linkage groups, LG) was evaluated as a function of the threshold (maximal) value rf 0, allowing for preliminary assignment of a marker to a certain LG.

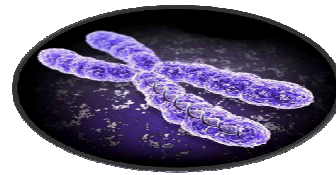
For example, marker BARC309 may be assigned to an (LGj) if recombination between BARC309 and at least one marker from (LGj) is lower than the threshold rf_0 and is the lowest compared to its distances to any other LG (Peleg et al. 2008).

The number of scored markers may considerably exceed the number of practically resolvable markers by recombination for the given population size. Thus, only a small portion of markers (here referred to as delegate markers) can be included in the skeleton map, with the remainder of markers being attached to the delegates. Besides non-resolvable linkage caused by small sample size, the necessity for selection of representative markers for the skeleton map derives from non-random (clustered) recombination distribution in the genome (Korol et al. 1994), varying information content of markers (missing data, distorted segregation, and scoring errors), and negative interference (Peng et al. 2000; Esch and Weber 2002). The reliability of the obtained multilocus map order was tested using the jackknife resampling procedure (Mester et al. 2003 b).



CHAPTER III

RESULTS & DISCUSSION



1-MOLECULAR MARKERS

1- 1- Molecular Markers in the population of Jennah Khetifa/ Cham I × Omrabi5/ *T. dicoccoides*600545//Omrabi5

The construction of genetic linkage maps relies mainly on the choice of parental lines for the segregating population and markers to reveal polymorphism (Saliba-Colombi et al. 2000). But also the choice of the crossing system and segregation analysis has practical importance, nowadays the most used segregant populations are Recombinant Inbred Lines obtained by single seeds method. 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. The cross of present research was developed with the aim of studying a number of morpho-phenological and agronomic traits for which the two parents were contrasting. Maps constructed with such populations could afterward facilitate the fine mapping of regions around genes of interest. The two parents Jennah.Khetifa/ChamI and Omrabi5/*T. dicoccoides*//Omrabi5 are very distant genetically (Pool) and the segregant population was developed using SSD (Single Seed Descent) method. The genetic map was constructed utilizing microsatellites and Single Nucleotide Polymorphism (SNP). Since most of the microsatellite markers used here, were already used and mapped in previous works they were also used as milestones or anchor primers for chromosomal arm and centromer assignments.

1- 1- 1- Overview of the genetic linkage map

The first parental (J.K/C1) is a modern cultivar (released in 2000) and the second parental (O5d/O5) is a new (year of release 2001) high-yielding durum wheat variety selected by Dr. Miloudi Nachit (ICARDA) from a late-maturing pure line (Recombinant inbred lines N.101).

Whenever possible, the larger is the mapping population much precise are the results. As been revealed that populations with less than 50 individuals generally provide too little mapping resolution to be useful. In our case the population size is more than doubled the minimum level. The relatively population size used for construction of the genetic map presented in this work (192 RILs) is higher as compared with other studies (62-160 by Blanco et al. 1998, 2004; Nachit et al. 2001; Elouafi and Nachit 2004; Akbari et al. 2006; Panio et al. 2011). This high number of RILs can be give markers distances more accurate and it is highly advantageous to improve the resolution of QTL mapping for the agronomic traits taken into consideration.

1- 2- Microsatellite (SSR) markers analysis

Sufficient DNA polymorphisms between parents must be present. This cannot be overemphasized in the absence of DNA polymorphism segregation analysis and linkage mapping are impossible.

The high level of polymorphism in the microsatellite markers, combined with their high interspersed rate, makes the SSR suitable markers for genetic mapping (Wu and Tanksley 1993; Plashke et al. 1995; Röder et al. 1995; Ma et al. 1996; Bryan et al. 1997).

We analyzed 254 molecular markers to have a total of 115 polymorphic loci which were used to assemble the genetic linkage map including 105 SSR and 10 SNP markers. The identification of polymorphism and monomorphism for the used markers was process by screening all the markers on our two parents. We read and analyzed the peaks came from Genemapper software. The monomorphic markers shown a single band with a size equal in both parents. Maybe the height of peaks could be different (y-axis), but the size of the amplified fragments was the same (x-axis). In contrast to the polymorphic markers carrying peaks with the same shape but with different sizes.

The level of monomorphism in the chosen microsatellite markers were 51.4%. As expected, microsatellite markers were more polymorphic (48.6%) than SNP markers (26.3%). This percentage show polymorphism between first and second parental (Fig. 3.1).

In the (Fig. 3.2) is reported an example showing the marker Barc263 which amplified a fragment with same size in the two Parents and marker WMS120 which, instead, amplified two different fragments in each parental.

Looking to the SSR markers origin come out that polymorphism was more present in WMS (41%) and WMC (25%) SSR. Other SSR groups: BARC, CFA, CFD, and MST/GPW contained 19%, 8%, 5%, and 2% respectively. Looking at the polymorphism distribution among the 7 homeologous groups, group 2 has the majority of polymorphism by 45%.

Figure 3.1: Identification of SSR markers to be polymorphic and monomorphic between our two parental (J.K/C1) × (O5.d/O5).

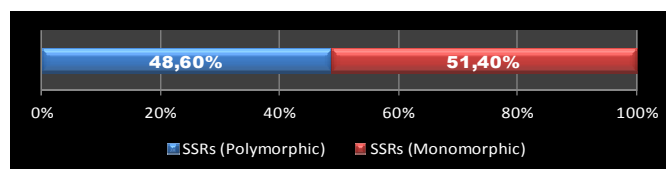
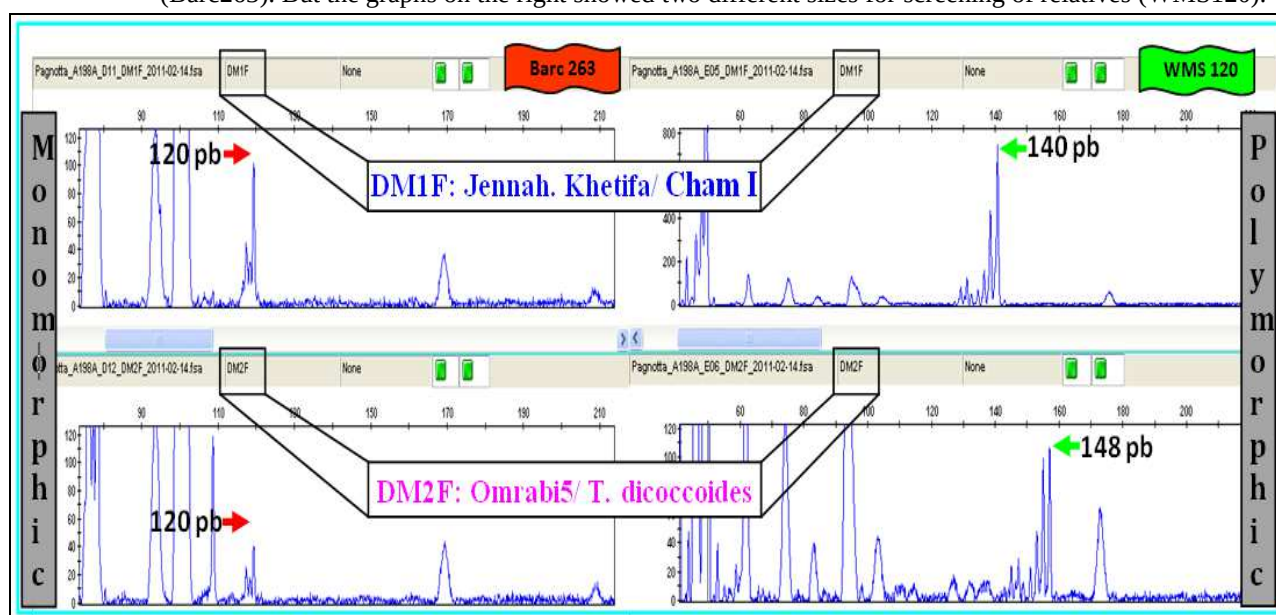


Figure 3.2 : Graphs on the left came from genemapper V.4.0 evidenced by a peak with a size equal for both parents (Barc263). But the graphs on the right showed two different sizes for screening of relatives (WMS120).



They generated 115 polymorphic fragments, i.e. 1.13 fragment per each microsatellite, the vast majority of the microsatellite primer pairs (92%), amplified only one polymorphic locus (polymorphic fragment) in agreement with earlier results. Which have shown that the majority of microsatellite markers are genome-specific and usually amplify only a single locus (Roder et al. 1998; Korzun et al. 1999). Nevertheless, other SSRs generated 2 or 3 fragments, some amplifying orthologous loci (Table 3.1). For example, WMS291 and WMC201 amplified two fragments, mapped to the two homoeologous sites on the A and B genome (Fig. 3.3). This confirms earlier findings for WMS291, while for WMC201 amplified only one fragment placed on 6A (Röder et al. 1998 b). All markers used in present research that had two fragments belonged to this group (homoeologous sites). But in other cases SSRs could amplified in non-homoeologous regions such as gwm554, gwm264, gwm131 and gwm537 (Elouafi and Nachit 2004) or gwm154 produced four polymorphic fragments two mapped in the O5d/O5 population, one on 3BL and one on 7AS (Röder et al. 1998 b).

Figure 3.3 : Analyzed WMS291 Marker on the RIL n.131 and n.113 with FAM florescent trough of Genemapper V.4.0.

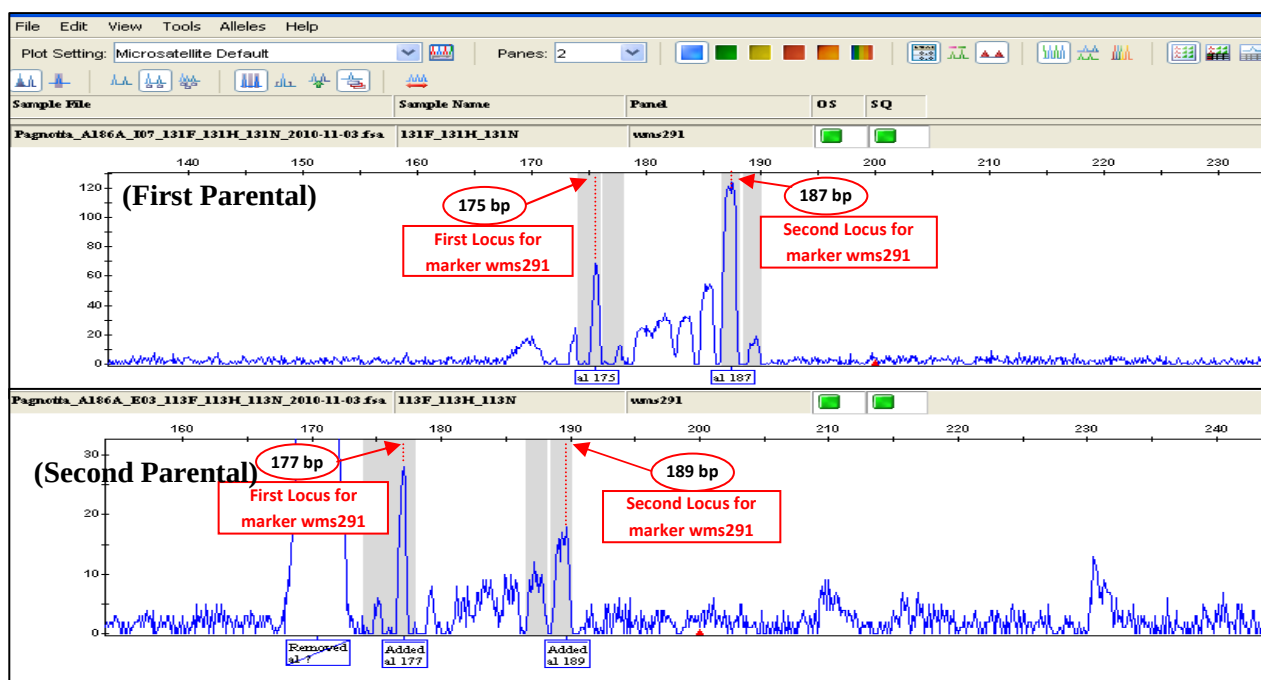


Table 3.1: Number of generated and mapped SSR fragments and their chromosomal assignment in the Omrabi5/*dicoccoides*//Omrabi5 × Jennah.Khetifa/ChamI population in comparison with wheat published maps.

SSRs	Am.Frag	Mapped	Loc.Popu	Kno.Loc
Wms 136	2	1	1AS	1A
Wms 311	3	1	7AL	7A
Wms 95	2	1	2AS	2AS
Wms 249	2	1	2AS	2AS
Wms 113	2	1	4BS	4B
Barc 309	2	2	2AS	2AL
WMS 291	2	1	5AL	5A
Wmc 262	2	2	4AL	4A
WMC 201	2	2	6AS	6A
CFD 88	2	2	4A	4B

Am.Frag: amplified fragments
Loc.Popu: localization in J.K/C × Od/O population
Kno.Loc: known localization

1- 3- Allele size differences in the SSR markers

The allele size differences between parents have been computed, excluding the M13 tail. With the aim to identify difference in allele, which means the increase of SSR motifs in one allele compared to another. A comparison was made among the individual RILs with respect to the differences in parent allele size across all the mapped loci.

CFD markers had the greatest parent allele size differences (28.2 bp), compared to WMC markers with the lowest parent allele-size difference (9.3 bp). The average parent allele size difference using SSRs markers was (12.4 bp). Allele pairs differing by (9 bp) or less between mapping parents occurred on average at 25% of the mapped loci with a tendency of genetically narrow crosses (O5d/O5 × J.k/C1) to have a larger fraction of parent allele pairs at (9 bp) or less. The MST and

CFD markers had the largest fraction of markers with parent allele pairs of (25 bp and 28 bp). An allele pair of (10 bp) or less between parents from different crosses was observed for 69 (66%) of the 102 microsatellite loci.

The difference size of fragments identified by SSR markers, between the two parents, were longer in chromosome two and shorter in chromosome five compared to other. This low difference size between the two alleles in chromosome five indicates that alleles are similar (Figs 3.4; 3.5). Difference in size between two alleles amplified through the markers of the WMC group is on average 9 nucleotides (Table 3.2).

Studies for the differences allele size, also brings an understanding on the origin of the mapped markers in our map. Explains that difference size between two alleles amplified through the markers of the WMC group, is an average of 9 nucleotides, in the regions or genes that contain this fragments and alleles, show a little differences (Table 3.2).

Sometimes mapping of inbreeding species requires that parents be as distantly related as possible, which can often be inferred from geographical, morphological, or isozyme diversity. In some cases, suitable wide crosses may already be available because a frequent goal in plant breeding in the past has been the introduction of desirable characters from wild relatives into cultivars.

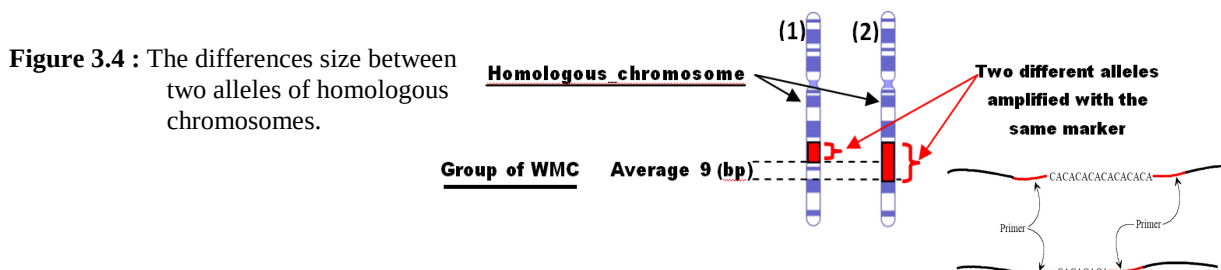
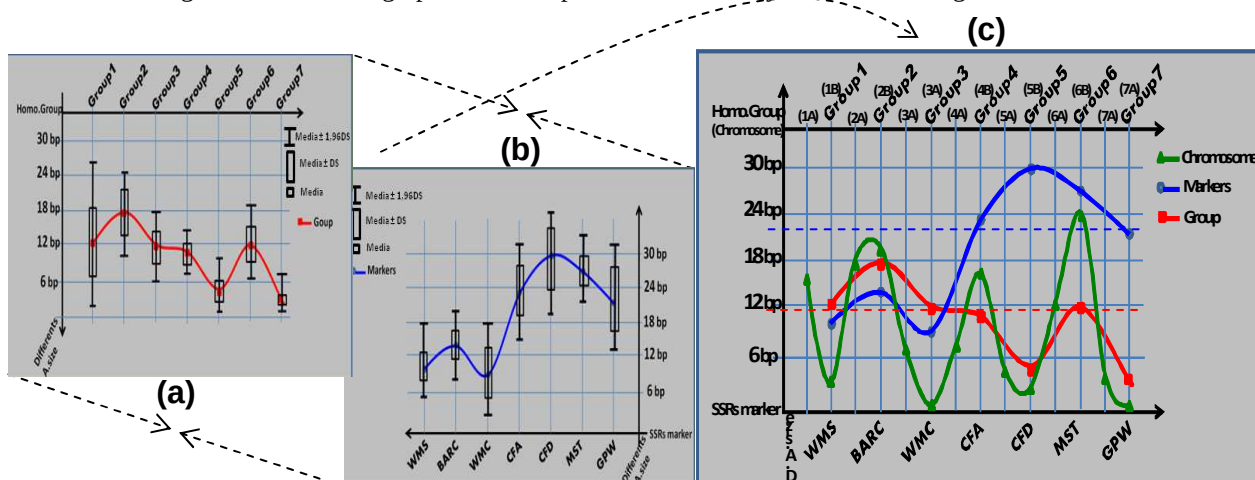


Figure 3.5 : The average of differences between parents in allele size: (a) different homeologous groups, (b) different SSR markers group, (c) different SSR markers group. Homeologous groups and chromosomes brought together in the same graphical to compare their variability with their average.



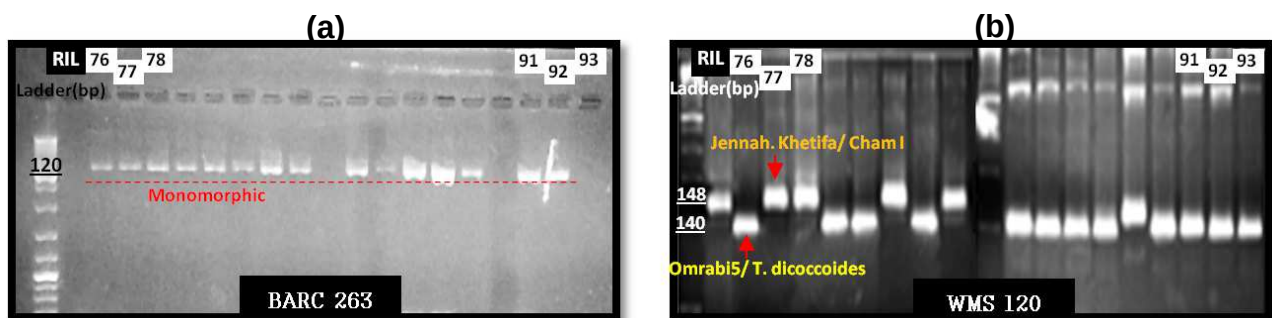
Comparing the markers and homology groups was observed that the great variability was between marker groups while the homology groups are similar. Then there was a similarity nucleotide numbers of the differences size in the homology groups compared to markers group.

Table 3.2: Origin of the microsatellites markers used in this study.

Name of Markers	Origin	Microsatellite code	Polyploid wheat	dimensional differences	Microsatellite developer
WMS	Gatersleben wheat microsatellites	GWM	Tetraploide, Hexaploide wheat	10 bp	Marion Röder (IPK)
WMC	Gatersleben wheat microsatellites	XWMC	Tetraploide wheat	9 bp	Wheat Microsatellite Consortium
BARC	Wheat and Barley Scab Initiative to map and characterize genes for fusarium resi stance	XBARC	Tetraploide, Hexaploide wheat	14 bp	Perry Cregan (USDA)
CFA	A and B genome diploid donors	XCFA	Diploide wheat	18 bp	Pierre Sourdille (INRA)
CFD	D genome diploid donors	XCFD	Diploide wheat	28 bp	Pierre Sourdille (INRA)
MST	A, B and D genome diploid donors	XMST	Diploide wheat	25 bp	
GPW	A, B and D genome diploid donors	XGW	Diploide wheat	20 bp	Marion Röder (IPK)

1- 4- The distribution of SSR markers

Figure 3.6 : Distribution of the fragments amplified through (a) monomorphic markers, (b) polymorphic markers. For the similarity of parents in the 18 RILs (76-93).



The proportion of BARC, WMC, WMS, CFA and CFD markers amplifying paralogous loci ranged from 13% to 41%. The proportion of GPW/MST markers amplifying paralogous loci was 2% (Figs 3.8(2); 3.8). In the analyzed RIL the SSR markers BARC, WMC, WMS, CFA, CFD and GPW/MST markers contain 49%, 52%, 56%, 53%, 61% and 62% respectively of alleles as the first parental (J.K/C1).

The BARC markers, compared to other SSR markers, were almost unique marker to be shared with same percentage between the two parents. Therefore these markers contain a maximum diversity and difference, between the first and second parental.

A significant segregation distortion was found in 105 (48%) out of 216 markers analyzed on 192 RILs. Fifty four markers (52.6%) showed distortion in favor of the first parental (J.K/C1) allele whereas 42 (40.5%) showed distortion in favor of the second parental (O5d/O5) allele (at $P \leq 0.05$). The rest of the markers (6) had a rate almost equal between the two Parental.

1- 5- Segregation distortion of SSR markers

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.

Most of the molecular markers, SSR and SNP (57.9%), showed Mendelian segregation in the 192 RILs. χ^2 test was used to check whether the marker segregation in F2 fitted the Mendelian model (1:2:1 for codominant and 3:1 for dominant markers) in F7-8 fitted the Mendelian model (1:1 ratio; $\alpha = 0.01$). However in total (42.1%) showed significant ($P < 0.05$) deviation from the expected ratio which is close to the number one would expect to get by chance in a test including many loci even if no real distortion for all (Figs 3.8(2); 3.8).

These markers with skewed segregation occur in all chromosomes except in 3B, 7A and 7B. The chromosomes with the most mapped skewed markers are 1A, 2A, 2B, 4A, 6A and 6B. Distorted markers favoring the first parental were found on 1A, 1B, 2A, 2B, 4B and 6B. Those favoring the second parental only on 6A (Figs 3.8(2); 3.8). Additionally, distortions favoring both first or second parental in the same chromosome were also found and therefore couldn't be segregation to one of the two parents (Figs 3.8(2); 3.8). The 54 markers that showed distortion in favor of the first parental (J.K/C1) allele were distributed among seven chromosomes as follows: 1B (4), 2B (21), 3A (3), 4A (11), 5A (3), 5B (3) and 7B (1). The markers that showed distortion in favor of the second parental (O5d/O5) allele were distributed as follows: 6A (12) and 6B (4). Chromosome 1A showed segregation distortion in favor of the second parental allele in the long arm and in favor of the first parental allele in the short arm. Also chromosome 2B showed opposite patterns in the two chromosome arms: markers on 2AS were in favor of the second parental alleles while markers on 2AL were in favor of the second parental alleles (Fig. 3.7).

Notably, all markers mapped on homeologous groups of 2 (chromosomes 2A, 2B), showed skewed segregation in favor of the first allele parental, but the distorted segregation in chromosome 2A a frequency nearby the first Parental, compared distorted segregation showed in chromosome 2B. Out of the 14 chromosomes of genome A and B of tetraploid wheat, only three chromosomes (3B, 4B, and 7B) showed no segregation distortion (Fig. 3.7). Most of our RILs had the similarity with the first parental (J.K/C1). The reason for this majority, could be containing the alleles or fragments, that belonged to the first parental.

Figure 3.7: Allele frequencies as a function of the genetic linkage map along chromosomes 2A, 2B, 4A and 6A. The x-axis indicates the segregation distortion from the 1:1 ratio observed for each marker, and the y-axis corresponds to the genetic linkage map. Markers marked with crossed square indicate the significance threshold of $P < 0.05$. (a) chromosome 2A that the majority of markers are distributed to the first parental, (b) chromosome 2B that the majority of markers in the short arm are distributed to the first parental, and long arm to the second parental, (c) chromosome 4A that the majority of markers are distributed to the first parental, (d) chromosome 6A that the majority of markers are distributed to the second parental.

▣ Segregation distortion between 0.45- 0.55 (45%-55%) frequency of alleles, ◆: Frequency of alleles between first and second parental, □: Frequency of alleles nearby first parental, ⊗ Frequency of alleles nearby first parental.

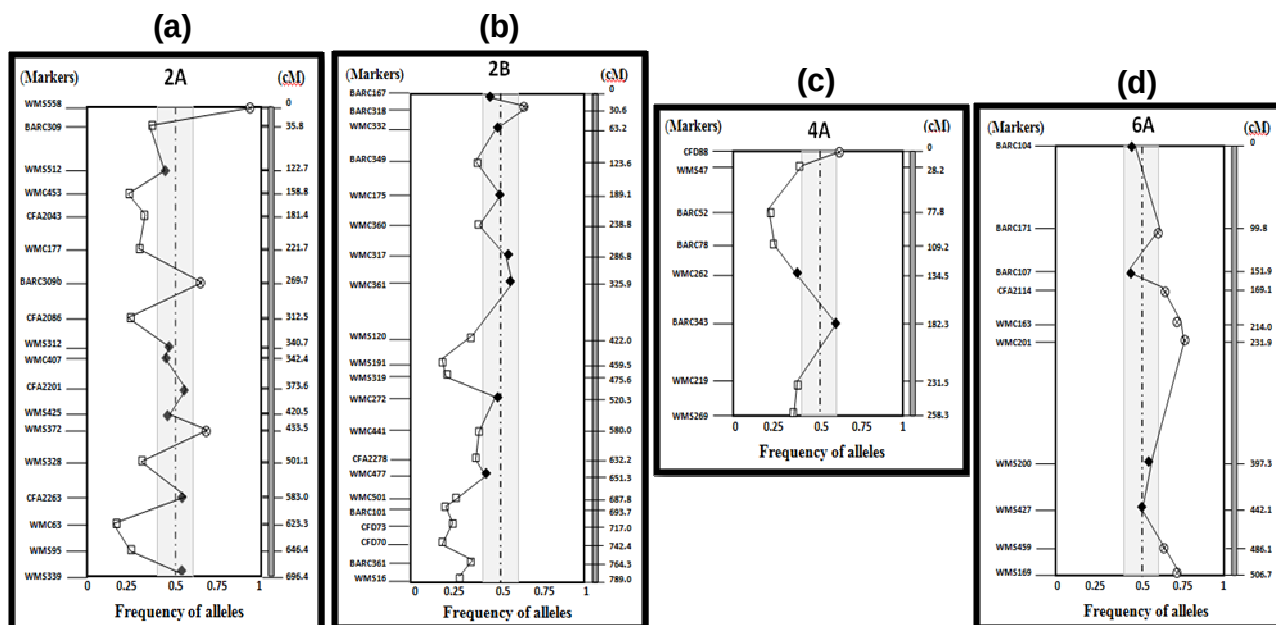


Table 3.3; Figure 3.8 (2) : Distribution of SSRs markers in each of chromosome for similarity to parents.

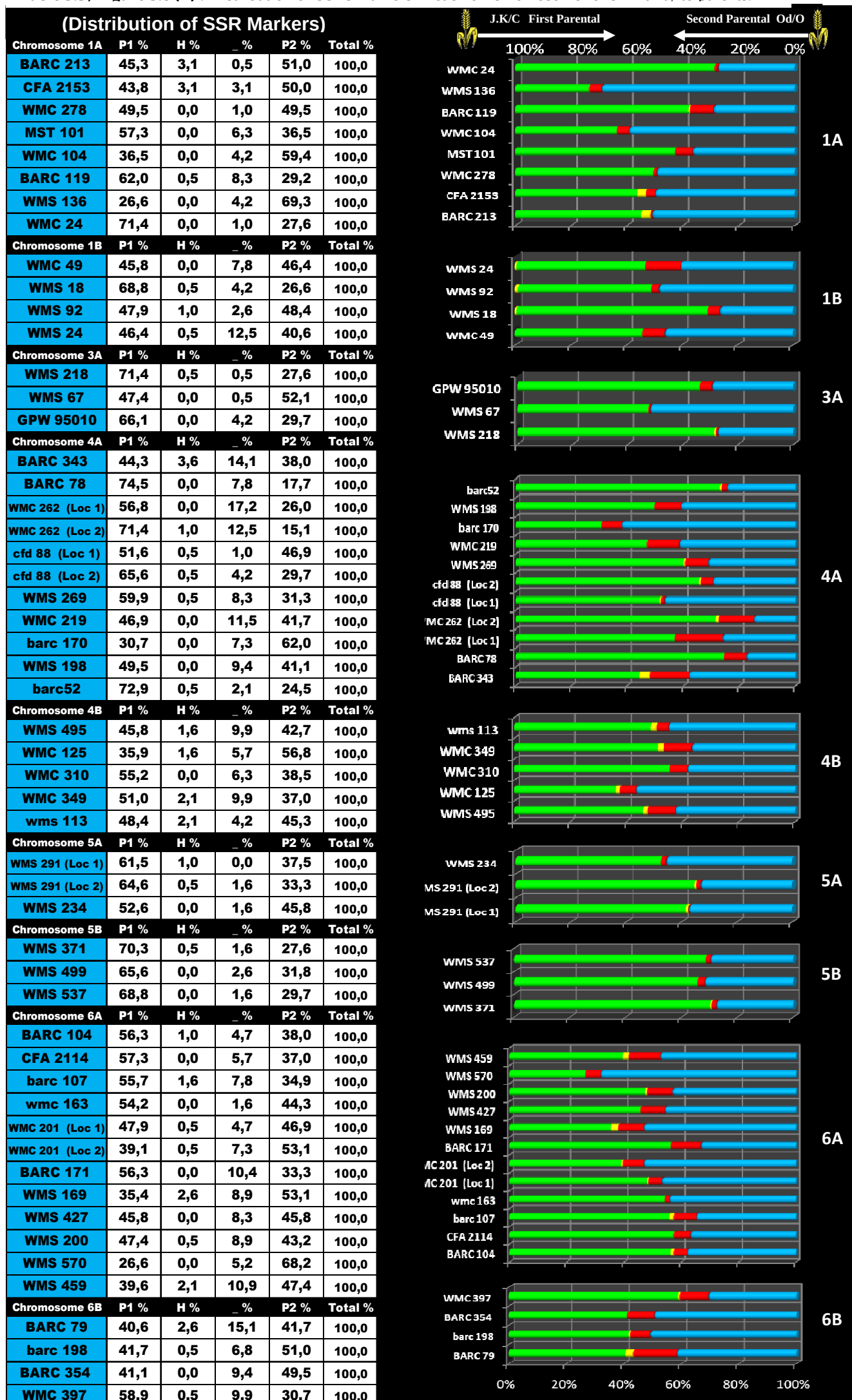
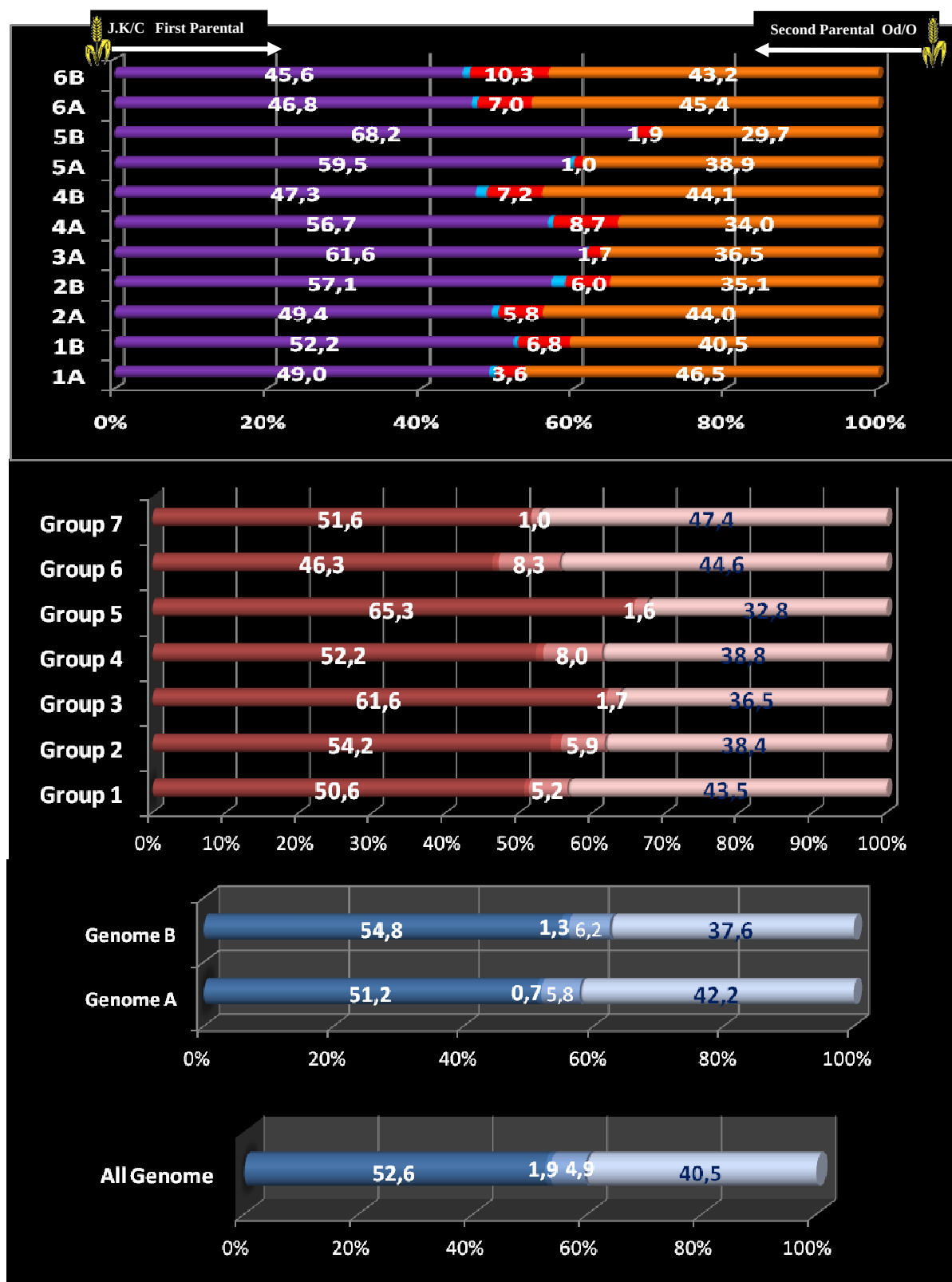


Table 3.4: Distribution of SSRs markers in the homoeologous group two for similarity to parents, Heterozygosity and non positive result, in percentage.

2 A					2 B				
P1 %	P1 %	H %	_%	P2 %	P1 %	P1 %	H %	_%	P2 %
BARC 309	56.3	0.0	7.8	35.9	WMS 501	67.2	3.1	4.7	25.0
WMC 522	46.9	1.6	17.2	34.4	barc 318	45.8	0.0	5.2	49.0
WMC 63	70.8	0.0	2.1	27.1	WMC 332	50.5	9.9	9.9	29.7
CFA 2086	67.7	1.6	3.1	27.6	WMC 441	51.0	3.6	13.0	32.3
CFA 2201	46.4	0.0	6.8	46.9	WMC 477	52.6	1.6	6.3	39.6
WMC 407	49.5	3.6	9.4	37.5	BARC 349	53.6	1.6	5.2	39.6
WMS 425	51.6	1.6	4.2	42.7	BARC 167	47.9	2.6	3.1	46.4
WMS 339	50.5	0.0	1.0	48.4	WMC 317	50.5	1.6	9.4	38.5
BARC 309	37.5	0.0	7.3	55.2	wmc 360	52.1	0.5	3.6	43.8
CFA 2043	55.7	0.5	1.0	42.7	WMC 361	47.9	0.5	1.6	50.0
WMC 453	68.2	1.6	1.0	29.2	cfcd 73	69.3	1.0	9.4	20.3
WMS 304	5.2	0.0	2.6	92.2	cfcd 70	76.6	2.1	0.0	21.4
CFD 267	46.4	0.0	2.1	51.6	BARC 361	57.8	0.0	15.6	26.6
CFA 2121	43.2	1.0	2.1	53.6	wms 16	63.5	0.0	1.6	34.9
WMS 328	59.9	0.5	8.3	31.3	BARC 101	71.4	3.1	3.6	21.9
WMS 312	48.4	3.6	15.1	32.8	wms 120	56.3	0.5	6.3	37.0
CFA 2263	51.6	1.0	3.6	43.8	WMC 175	44.3	2.1	9.4	44.3
WMS 47	55.7	0.0	2.1	42.2	WMS 191	75.0	1.6	5.2	18.2
WMS 311	53.6	1.0	11.5	33.9	wms319	62.0	1.6	4.7	31.8
WMS 372	46.9	1.0	6.8	45.3	cfa 2278	56.3	0.0	4.2	39.6
WMC 177	58.3	0.0	7.3	34.4	wmc 272	48.4	0.0	4.2	47.4
WMS 512	53.1	1.6	14.6	30.7	cfa 2278	56.3	0.0	4.2	39.6
WMS 95	63.0	0.5	2.6	33.9	wmc 272	48.4	0.0	4.2	47.4
WMS 558	6.8	0.0	0.0	93.2					
WMS 249	40.6	0.5	5.2	53.6					

Figure 3.8: Distribution of SSRs markers in the homoeologous group two for similarity to parents and Heterozygosity
■ Similar of first parental, ■ Similar of second parental, ■ Heterozygosity.

Figure 3.9 : Different distribution of SSRs marker in Chromosome, Homoeologous, genome and all the two genomes,
 ■/■/■ Similar to first parental, ■/■/■ Similar to second parental.



1- 6- Clustering of markers (SSR, SNP)

The genetic markers were distributed non-randomly (Px^2 (df 114) ≤ 0.0001) along the chromosomes. Clusters of markers were observed on most of the chromosomes of the A and B genomes. As said before, the X^2 test was used to check whether the marker segregation in F2 fitted the Mendelian model (1:1 for dominant and codominant markers). The SSR markers used in the current study were selected, according to previously published maps, to cover all 14 chromosomes, whereas SNP markers scored in the mapping population were not targeted to specific genomic regions. Therefore, clustering of markers was tested for the two types of markers separately, revealing significant clusters of SNP markers on most chromosomes.

In our map most of markers are located on the centromeric regions, while few markers far apart each other, were located in the telomeric regions. The cluster of markers in the 11 chromosome from these 14 chromosomes (1A, 1B, 2B, 3A, 4A, 4B, 5A, 5B, 6A, 6B and 7A) was increased the number of markers with the length of the chromosomes up to centromeric regions and then in some chromosomes begins to increase and in some starts to decrease (Fig 3.10).

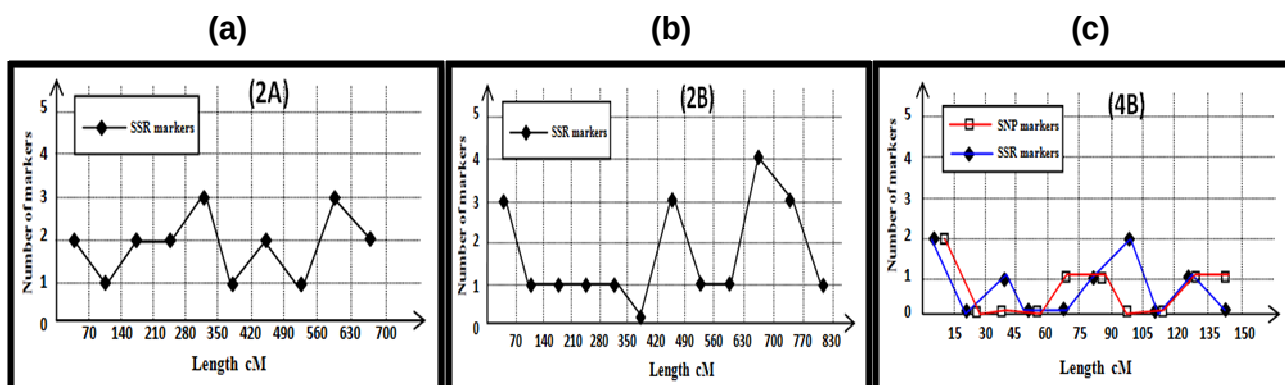
In wheat and in many other organisms, recombination is unevenly distributed with “hot-spots” and “cold-spots” across chromosomes (Dvorák and Chen 1984; Gill et al. 1996 a, b; Faris et al. 2000). Clustering around centromeres is a well known phenomenon with all types of markers, resulting from centromeric suppression of recombination (Tanksley et al. 1992; Korol et al. 1994). Contrary to other wheat mapping populations (Röder et al. 1998 b; Peng et al. 2000).

In the current study, SSRs showed only a moderate tendency to cluster around centromeres, presumably due to the selection of markers based on their known location. However, a remarkable clustering of SNP markers was found in telomeric regions (Fig. 3.10). Zhang et al. (2006) reported for durum wheat that SNP markers showed a stronger tendency than SSR markers in particular to map to gene-rich telomeric regions.

High-density physical maps in wheat revealed that more than 85% of wheat genes are present in gene-rich regions, physically spanning only 5-10% of the genome (Gill et al. 1996 a, b; Faris et al. 2000). These regions are strongly associated with recombination rate in wheat (Gill et al. 1996 a, b; Weng and Lazar 2002) and are predominantly located in telomeres (Qi et al. 2004).

For example, the clusters of SNP markers in homoeologous group 4 (Figs 3.10) are associated with a reported gene-rich region near chromosome telomeric ends (Gill et al. 1996a). The high proportion of clustering SNP markers may, therefore, be indicative of gene-rich regions. If this is indeed the case, SNP markers may be unique for fine mapping of genes/ QTLs residing in gene-rich regions, thereby facilitating positional cloning.

Figure 3.10: Distribution of SSR and SNP loci along chromosomes 2A, 2B, and 4B of the (J.K/C1) x (O5d/ O5) genetic map.



1- 7- The RILs distribution between parents for all markers

The RIL were been divided in the three groups looking the similitude with the parents:

- 1- 71 RILs (35.9%) include almost half of the characters from our first parent, and almost other half of the traits come from our second parental, therefore included a range of similarity between 47% to 53% with the first or second parental, were the most contrasting RILs: 16.40 and 103 (Fig. 3.13, b).
- 2- Corresponds the first parent, means these RILs included a majority of the characters or loci with the similarity of the first parental, were the most contrasting RILs: 45 and 125 (Fig. 3.13, a).
- 3- Corresponds the second parental, were the most contrasting RILs: 132 and 134 (Fig. 3.13, c).

The distribution of the 192 RILs analysis of the segregation data has shown that throughout the length of the genome, 192 genotypes (53.6%) were similar to the first parental (J.K/C) and 41.8% percent to the second parental (O5d/O5). The rest, belongs to heterozygote (1.4%), or non-results, having a light peak (3.2%) (Fig. 3.11 a and b).

After 7 generations of selfing, in F8, two genes will have 98.45% of homozygosity and 1.55% of heterozygosity trough of the equation $[(1-1/2)^7]^2$ (Wricke 1986). The calculated heterozygosity and the heterozygosity detected in present experiment are very similar (1.4 vs 1.55), hence the RIL population utilized is very stable with an high number of homozygous genotypes.

Figure 3.11: The distribution of the 192 RILs the percentage similarity with (a) First Parental J.K/C1, (b) Second Parental O5d/O5,(↓) with high similarity to the parental, (4, 5, 27, 62, 147 and 150 RILs number do not exist).

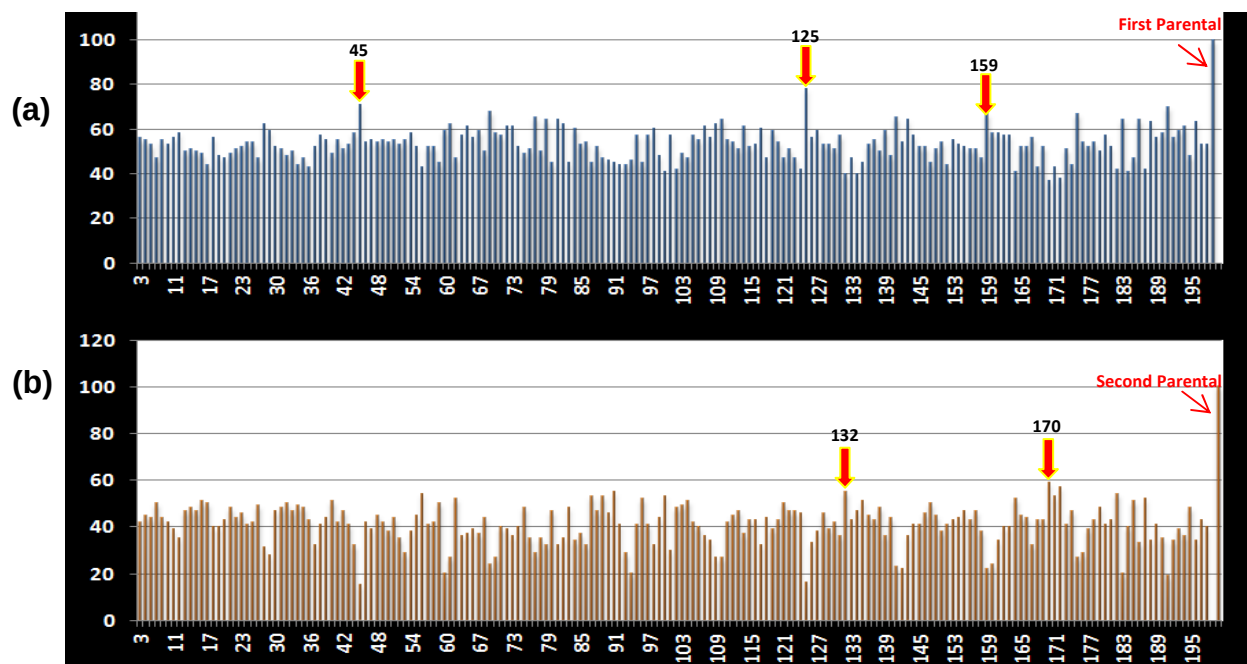


Figure 3.12: The dispersion of RILs number in the percentage of similarity (a) to the first Parental, (b) to the second Parental. And the equation of the line in this dispersion of 192 RIL.

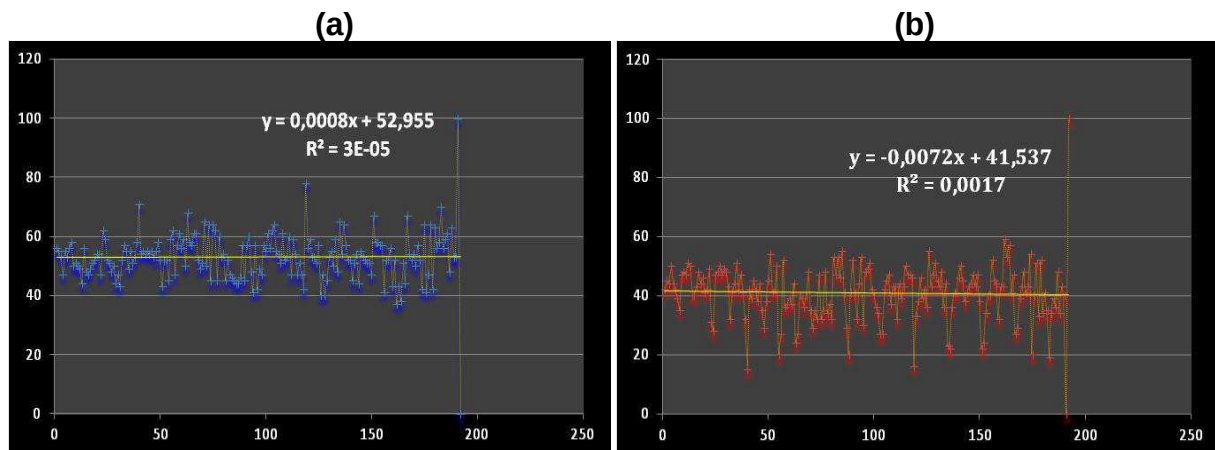
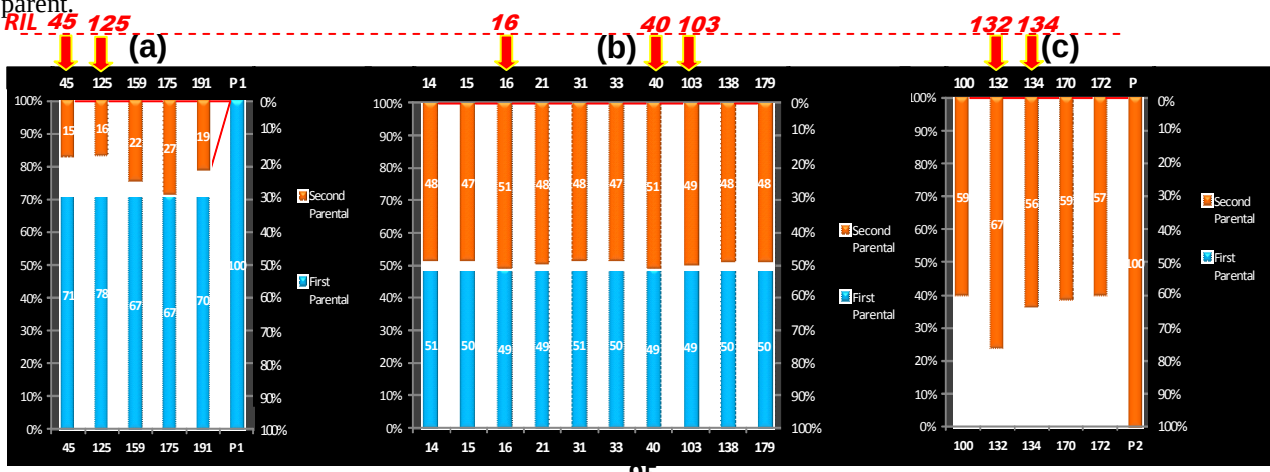


Figure 3.13: The identification numbers of RIL or genotypes for the similarity (a) to the first parental (b) to the second Parental (c) the number of genotypes represented from both parents,(↓)These arrows indicated the RIL numbers preferable (superior) in each of the three groups, (■) the similarity of the first parent, (■) the similarity of the second parent.



1- 8- Frequency of RILs with parental (non-recombinant) chromosome

Out of the 2,688, RIL × chromosome combinations, 429 RIL × chromosomes (15.9%) remained non-recombinant (parental) and the number remained (2,259 RIL × chromosome) belonged to the recombined chromosomes even after seven cycles of recombination (Table 3.5). The lowest frequency of parental chromosomes was observed in group 2 chromosomes (2 and 3 for 2A and 2B, respectively), while the highest frequency of parental chromosomes was observed in group 5 chromosomes (62 and 117 for 5A and 5B, respectively). The recovery rate of non-recombinant chromosomes correlated ($R^2 = 0.815$, $P = 0.001$; Fig. 3.14) with chromosome length (cM) but was unrelated ($R^2 = 0.43$, $P = 0.31$) to the number of markers per chromosome. The probability of recovering non-recombinant chromosomes was similar for both parental lines J.K/C1 and O5d/O5.

1- 8- 1- Non-recombinant chromosomes

A relatively higher frequency (15.9%) of non-recombinant chromosomes was observed in this RIL. The highest frequency of non-recombinant chromosomes was found in group 5 chromosomes (41.1%), which is close to the theoretical proportion expected for the estimated length of chromosome 5 (~ 40 cM). While, Singh et al. (2007) reported the highest frequency of RILs with non-recombinant chromosome in *T. boeoticum* × *T. monococcum* RIL population, was in chromosome 4A. Chromosome 4A is known to be involved in cyclical interchange with chromosome 5A (Devos et al. 1995). Also in the study of Pleng et al. (2008), the highest frequency of non-recombinant chromosomes was found in group 4 chromosomes (34%), but in our case, only 14.3% of the non-recombinant chromosomes were found in group 4 chromosomes (Table 3.5).

Table 3.5: Number of durum wheat First Parental (J.K/C1) × durum wheat, Second Parental (O5d/O5) RILs with parental (non-recombinant) chromosomes.

Chromosome	Number of RILs with non-recombinant chromosomes		Total	total percentage
	P1 (J.K/C1)	P2 (O5d/O5)		
1A	4	4	8	1.88
1B	11	21	32	7.52
2A	1	1	2	0.47
2B	1	2	3	0.70
3A	16	51	67	15.76
3B	-	-	-	0
4A	1	5	6	1.41
4B	27	28	55	12.94
5A	14	48	62	14.58
5B	28	85	113	26.58
6A	1	2	3	0.70
6B	16	20	36	8.47
7A	2	36	38	8.94
7B	-	-	-	0
Total	120	271	433	100%

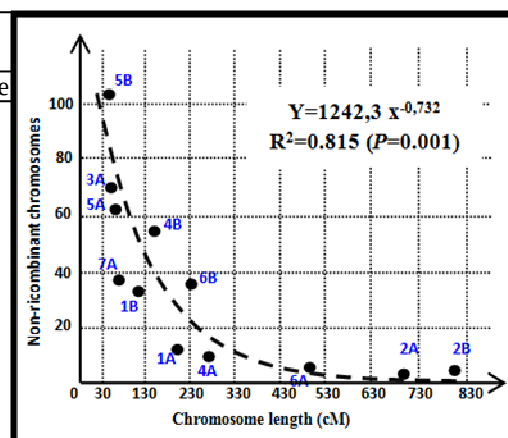


Figure 3.14: Occurrence of parental chromosomes (non-recombinant chromosome) in the mapping data set as associated with chromosome length (cM).

The parental genotypes of our cross cannot be considered as highly divergent in evolutionary terms. The slightly reduced proportion observed for recombinant chromosomes compared to the theoretical expectation could explain as a result of the known general trend of decrease in recombination in hybrids (Fig. 3.14).

1- 9- Characterization of (CT/GA)_n and (GT/CA)_n SSRs in our map

The most common classes of SSR motifs are. mono-, di-, and tri-nucleotide repeats. The relative frequencies of 14 microsatellite motifs in the tetraploid wheat (*T. durum*) genome was estimated as a basis for efficient development of a microsatellite map. Nine di-nucleotide, four tri-nucleotide, and two tetra-nucleotide repeat motifs were end labeled. The sequences of SSR markers was computed to identify the amount, and also to identify the majority of repeats sequences.

The analyzed 216 SSRs marker, used to screen Parent, contained di-nucleotide repeats by 29.4% for (CA)_n, 23.4% for (GA)_n, 15.8% for (CT)_n and 12.1% (GT)_n, and tri-nucleotide repeats by 6.8% for (ATT/TAA)_n (Table 3.6). After the screening procedure was performed, the 102 polymorphic markers contained 87.5% of the di-nucleotide (the most (CT)_n), 9.5% of the tri-nucleotide and 3.0% of the tetra-nucleotide. The SSR markers mapped have the highest repeats sequence of di-nucleotide (CA/GT)_n by 30.4% (Fig. 3.15, a).

This information could be useful in subsequent studies in mapping to select the markers that contain some SSR motifs. For example, the distribution of this motifs have indicated that in group one comprised the majority repeating of (CT) motifs, in group two comprised the majority repeating of (CA) motifs. In group four comprised the majority repeating of (TATC) motifs. In group five comprised the majority repeating of (GA) motifs. In group six comprised the majority repeating of (ATT) motifs (Fig. 3.15, b).

Therefore, an estimate for the frequency of (CT/GA)_n and (GT/CA)_n SSRs in durum wheat was assessed by the screening of genomic libraries.

The frequency of this SSR motives, (CT) and (GT), in the study of Saal and Wricke, (1999) was in contrast to our results, because their obtained the frequency of this two sequence 0.15% and 23% respectively. The loss of about half of the positive clones may have been due to unspecific hybridization or wrong selection. A similar drop in the yield of positive clones was reported by Bryan et al. (1997), a (CT/GA)_n or a (GT/CA)_n SSR theoretically occurs every 268 kbp at most, Bennett and Smith (1976) have determined the length, of haploid cereal genome size (Rye), that corresponded to 9120 Mbp. Consequently, between 17,500 and 34,000 (CT/GA)_n and (GT/CA)_n SSRs would exist in the rye genome. Comparing these results with other cereal species 23,000 SSR for (CT/GA)_n and (GT/CA)_n would exist in barley (Liu et al. 1996), whereas in durum wheat more than 60,000 would be commuted, and about 130,000 of these motifs have been estimated (Röder et

al. 1995; Ma et al. 1996). Surprisingly, the predominance of the (CT/GA)_n motif reported for most plant species was not confirmed in Saal and Wricke (1999).

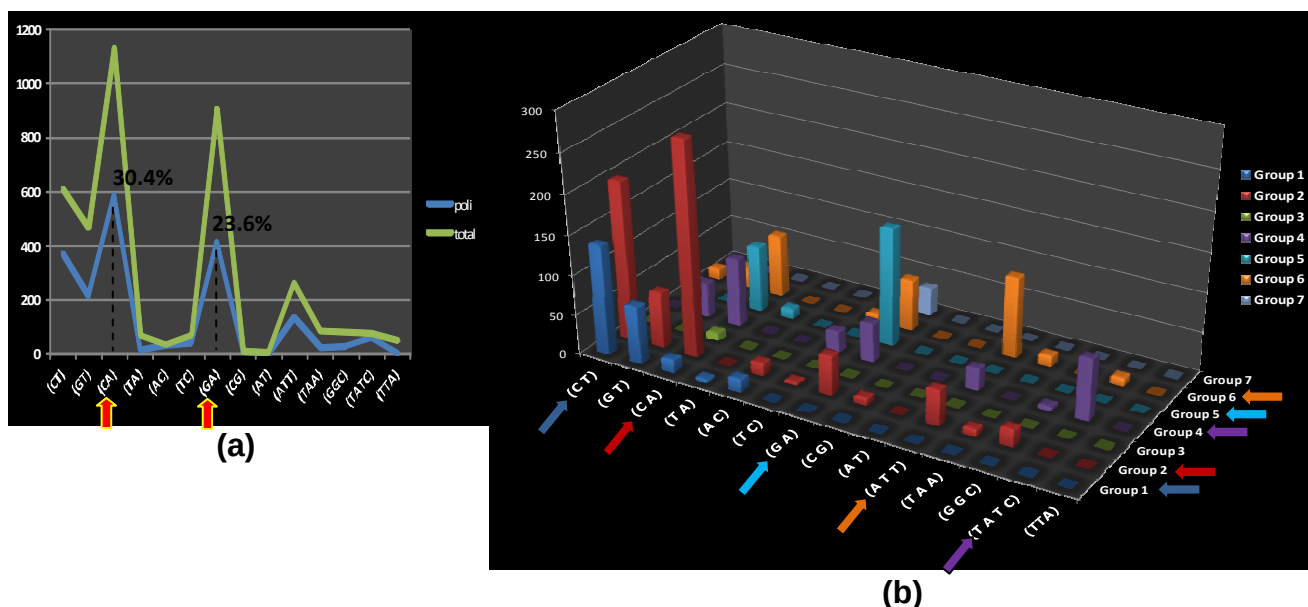
In the work of Röder et al. (1998) microsatellite-containing clones were purified from various genomic phage λ libraries containing small inserts primer pairs could be designed for 54% of the sequenced clones containing (GA)_n or (GT)_n microsatellites based on hybridization of the plasmid clones.

The sequences of microsatellite is non-stable and reducing or increased repeat units is cause of great changes in length, Litt and Luty (1989) and Weber (1990) have done much research on the sequence of (CA)_n. Akkaya et al. (1992) on the sequence of (AT)_n, (ATT)_n. These groups have verified the results that there is a positive correlation between the repeated sequences and the number of alleles.

Table 3.6: The table indicates the percentage of repeated motifs in the total number of markers, Polymorphic markers and homoeologous groups.

SSRs motifs	(CT) _n	(GT) _n	(CA) _n	(TA) _n	(AC) _n	(TC) _n	(GA) _n	(CG) _n	(AT) _n	(ATT) _n	(TAA) _n	(GGC) _n	(TTA) _n	(TATC) _n
Polimorphich	17.2	11.1	30.4	0.9	1.7	2.1	23.6	0.5	0.0	7.0	1.2	1.4	0.0	3.0
Monomorphich	12.5	13.1	28.4	2.6	0.0	1.5	25.3	0.0	0.3	6.6	3.3	2.8	2.5	1.0
Total Markers	15.8	12.1	29.4	1.8	0.9	1.8	23.4	0.2	0.1	6.8	2.3	2.1	1.3	2.0
Group 1	55.6	29.2	6.8	1.6	6.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Group 2	23.6	8.4	31.8	0.0	1.9	0.5	23.8	1.1	0.0	5.3	1.1	2.6	0.0	0.0
Group 3	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Group 4	0.0	15.3	30.0	0.0	0.0	9.4	17.1	0.0	0.0	9.8	0.0	1.7	0.0	16.7
Group 5	9.1	0.0	47.2	7.4	0.0	0.0	36.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Group 6	4.4	8.2	37.3	0.0	0.0	2.8	20.4	0.0	0.0	19.4	100	0.0	0.0	3.1
Group 7	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Figure 3.15: The repetition (times) of the SSR motifs (a) in the total and polymorphic markers (b) in differences homoeologous group.



1- 10- The distribution of SNP markers

In wheat genome, SNPs were classified into transitions (C/T and A/G) and transversions (A/C, A/T, C/G and G/T) according to their substitution types, and the transitions were more common than the transversions among the sequence changes (Wang et al. 2005). Since, 41% of the substitutions were found to occur at a CpG site, a dinucleotide known for its high mutability (Wang et al. 2005 b).

It was verified in the first class (Refer to materials and methods 1-5-) of our SNP, the SNPs transitions were higher than SNPs transversions, being in agreement with study of (Wang et al. 2005; Batley et al. 2003). The high frequency of transitions detected has been observed in previous SNP discovery programs (Garg et al. 1999; Picoult-Newberg et al. 1999; Deutsch et al. 2001) and reflects the high frequency of the C to T mutation after methylation (Coulondre et al. 1978), The relative abundance of the C/A and its reverse complement, T/G transversions compared with C/G and A/T transversions, was unexpected and remains to be explained (Fig. 3.16).

In the online SNP database (<http://probes.pw.usda.gov:8080/snpworld/Search>), 2114 putative SNPs had been found in 274 EST sequences.

A total of 38 candidate SNPs from 32 loci were validated using direct sequencing of PCR products, The SNPs were chosen based on a range of redundancy and cosegregation scores and predicted expression of multiple genes (Fig. 3.17, a). To identify the polymorphism of SNP markers we used High Resolution Melting (HRM) that is a novel, homogeneous, to analyze genetic variations (SNPs, mutations, methylations) in PCR amplicons. It goes beyond the power of classical melting curve analysis by allowing to study the thermal denaturation of a double-stranded DNA. Identification of polymorphism is verified through the movement of a peaks in the graphics that determined the melting temperature of SNP markers.

Our samples (38 markers) are discriminated on the basis of GC content and, according to their sequence, length or strand complementarily. Even single base changes such as SNPs can be readily identified. Mutations in PCR products are detectable by High Resolution Melting because they change the shape of DNA melting curves. A combination of new-generation DNA dyes, high-end instrumentation and sophisticated analysis software allows to detect these changes and to derive information about the underlying sequence constellation.

Out of the 38 candidate SNPs, after sequencing them 10 (26%) were shown to be true polymorphic (Table 2.5), and 28 were shown to be false.

There were also some fragments that contained 2, 3 or 4 SNPs in the same sequence.

The distribution of the 10 SNP markers similarity between parents in all 192 genotypes, have shown that 44.27% were similar to the first parental, and 40.52% to the second, while the remaining 15.22% were heterozigote. From these 10 SNP markers only 6 markers (2 markers from first class

and 4 markers from second class) are mapped in chromosome 4B, and the remaining 4 markers, have not been mapped. Chromosome 4B has shown a high variability between the two parents of our population through the verification of SNP markers (Fig. 3.17).

Figure 3.16 : Distribution of SNPs by the type of nucleotide substitution transitions (C/T; A/G) and transversions (C/A; C/G; T/A; T/G).

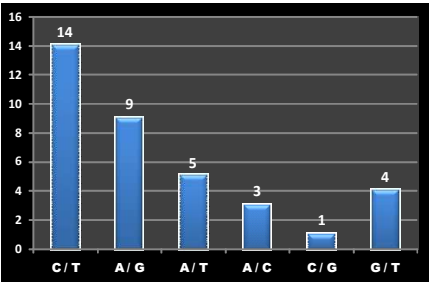
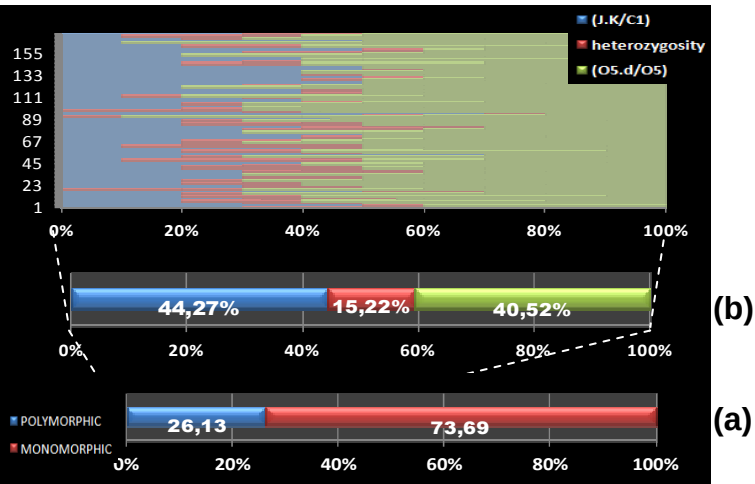
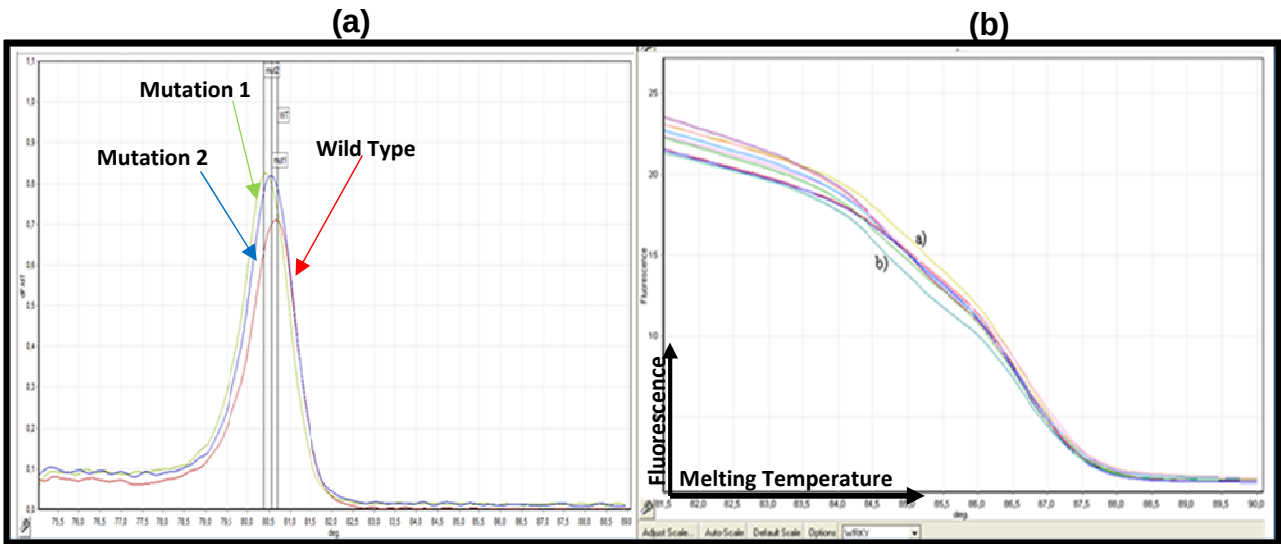


Figure 3.17 : (a) The percentage of polymorphism and monomorphism in the total number of SNP markers, (b) The distribution of SNP markers for the similarity to the two parental and the central part which shows the percentage of heterozygosity.



In the second class of our SNPs the identification of multiple cosegregating SNPs within an alignment of EST sequences allows the accurate prediction of sequence haplotypes. Comparison of predicted haplotypes with known wheat lines from which the sequences were derived allows the identification of predicted orthologous genes.

Figure 3.18 : Charts to (a) Identify Polymorphic SNPs, (b) Identify SNPs.



SNPs are becoming the marker to be chosen for molecular genetic analysis, even if with other molecular markers, their discovery and characterization is both expensive and laborious. The methods of the mining sequence data sets applied to discovery of SNPs, should provide the cheapest source of abundant SNPs (Gu et al. 1998; Taillon-Miller et al. 1998; Buetow et al. 1999; Picoult-Newberg et al. 1999). Although every effort is made to produce and submit sequence of only the highest quality, the high-throughput nature of the sequencing programs inevitably leads to the submission of inaccuracies. The electronic filtering of these data to identify potentially biologically relevant polymorphisms is thereby hampered by the false calling of these bases. Previous methods used to identify SNPs in aligned sequence data has relied on the comparison of sequence trace files to filter out polymorphisms, where the base calling within one or more of the traces is of dubious quality and, therefore, likely to be due to sequence error rather than representative of a true polymorphism (Kwok et al. 1994; Garg et al. 1999; Marth et al. 1999). This method, although suitable for comparing genomic sequence, is limited by the requirement of sequence trace file data and does not distinguish errors incorporated during the reverse transcription of mRNA. For highly redundant data sets compiled from a variety of sources with a limited availability of sequence trace files, this means of filtering sequence errors from true polymorphisms is not feasible. However, the redundant nature of these EST data sets does permit the selection of polymorphisms that occur multiple times within a set of aligned sequences.

The frequency of occurrence of a polymorphism at a particular locus provides a measure of confidence in the SNP representing a true polymorphism and is referred to as the SNP redundancy score. By examining SNPs that have a redundancy score of two or greater, i.e. two or more of the aligned sequences represent the polymorphism, the vast majority of sequencing errors are removed. Although some true genetic variation is also ignored due to its presence only once within an alignment, the high degree of redundancy within the data permits the rapid identification of large numbers of SNPs from data collated from a variety of sources.

2- MAP CONSTRUCTION

2- 1- Genetic map

Genetic map is an essential prerequisite to detailed genetic studies in any organism. Furthermore dense and saturated genetic maps provide scientists with essential tool for genetic studies like positional gene cloning, quantitative trait mapping, and marker-assisted selection. Genetic map with complete coverage of the genome and the confidence to locus is required for detection, mapping, and estimation of gene effects on phenotypic traits. The present map, based on a cross between two durum wheat, probably covers most of the genome of tetraploid wheat. In addition, this map is probably one of map based on a wider population (192 RILs).

Population size has a great effect on the estimation of QTL number and genetic effects (Sourdille et al. 1996; Beavis 1998; Mackay 2001; Schon et al. 2004; Vales et al. 2005; Zou et al. 2005; Buckler et al. 2009).

This is the first genetic map of a cross involving two different previous RIL came from durum wheat, therefore, could serve as a new source of identifying new markers (clones) that have not been mapped previously. This map provides a valuable resource for wheat genetic research and genetic resource of which we have additional SNP markers with chromosomal location, which will increase the pool of markers available for wheat research.

The skeleton map accounted for a total length of 3170.3 cM, with an average density of one marker per 31.7 cM (Table 3.7). The length of individual chromosomes ranged between 789 cM for chromosome 2B and 32 cM for chromosome 3A (Table 3.7).

Few chromosome arms 2AL, 2BL, 4AS and 6BL were partly covered and two arm 3AL and 5BS was not covered (Table 3.7). Lack of complete genome coverage of homoeologous group 7 was observed in a few other wheat mapping populations (Röder et al. 1998 b; Paillard et al. 2003; Elouafi and Nachit 2004).

A total of 216 markers were tested for genome specificity by PCR amplification of *T. turgidum* Nullisomic-Tetrasomic (N-T) lines. The results derived from these markers showed that 124 loci were mapped in the A genome, and 92 in the B genome.

This map could be used for further QTL detection (for example: drought response, grain minerals concentration, resistance to diseases, increase tolerance, increase wheat yield and powdery mildew resistance) and as a tool for marker-assisted selection and map-based breeding for resistance to biotic and abiotic stresses.

After elimination of fifteen unlinked SSRs loci, the remaining 100 SSR and SNP loci resulted in 14 linkage groups ranging from 2 loci on chromosome 3A to 25 loci on chromosome 2A (Table 3.7).

Table 3.7: Chromosome assignment, distribution of markers, length of linkage groups, and marker density in genetic map constructed with the J.K/C1 × O5d/O5 recombinant inbred line population.

Linkage group	SSR (Monomorphic)	SSR (Polymorphic)	SNP (Polymorphic)	Total Polymorphic Markers (SSRs + SNPs)	Skeleton markers	Length (cM)	cM/marker	CV %
1A	3	8	---	8	7	214.969	30.70	69.22
1B	4	4	---	4	4	123.075	30.76	47.22
2A	21	25	---	25	19	696.251	36.65	58.75
2B	15	21	2	23	21	788.877	37.57	53.02
3A	6	2	---	2	2	32.130	16.07	---
3B	3	1	---	1	---	---	---	---
4A	18	11	---	11	10	258.258	25.82	30.78
4B	8	5	8	13	13	140.395	10.79	106.35
5A	2	3	---	3	2	46.326	23.17	---
5B	2	3	---	3	3	37.250	12.41	25.09
6A	7	12	---	12	10	506.821	50.68	85.42
6B	16	5	---	5	4	233.440	58.36	32.00
7A	3	3	---	3	3	45.201	15.06	33.16
7B	3	2	---	2	2	47.300	23.65	---
Group 1	7	12	---	12	11	338.044	30.73	62.70
Group 2	36	46	2	46	40	1485.125	37.13	55.00
Group 3	8	3	---	3	2	32.130	16.07	---
Group 4	26	16	8	26	23	398.653	17.33	81.65
Group 5	4	6	---	6	5	83.576	16.72	58.67
Group 6	23	17	---	17	14	740.261	52.88	70.48
Group 7	6	2	---	5	5	92.501	18.50	49.34
A genome	60	64	---	62	53	1799.956	33.96	69.20
B genome	51	41	10	50	47	1370.337	29.15	77.75
Total	111	105	10	115	100	3170.293	31.70	72.90

2- 2- Chromosomes and genomes

The map covered 3170.3 cM, and contains linkage gaps. There were several large linkage distances, notably on the B genome (Fig. 3.19). Included on the map are several ESTs mapped via a PCR that interrogates a single nucleotide polymorphism (SNP) between two mapping parents. The ESTs are indicated by Genebank accession number-nucleotide position of the SNP (Somers et al. 2003 b). Genes for disease resistance, storage proteins, and plant height are indicated since these loci segregated as Mendelian factors.

There are characteristic clusters of microsatellites near the centromere of each chromosome. A shaded zone on each chromosome indicates the approximate position of the centromere based on comparative mapping to both physical and genetic maps of microsatellite markers available at GrainGenes (<http://wheat.pw.usda.gov>).

In a separate study, approximately 250 WMC and GWM microsatellites from the consensus map were physically mapped into chromosome bins, using the disomic deletion stocks of Chinese Spring

CS (Endo and Gill 1996). This facilitated an improved ordering of markers around the centromere and helped with correct positioning of the centromere (Fig. 3.19).

The density of markers on the map ranged from the little homoeologous group 3, up to much of the homoeologous group 2. The seven homologous groups of the tetraploid wheat genome varied in the number of markers, map length, and marker density. There were 12, 46, 3, 16, 6, 17, and 2 markers mapping in the group 1 through 7 chromosomes, respectively. There were some markers mapping to at least two non-homoeologous (paralogous) positions in the genome.

Total marker number and density was highest in homoeologous group 2 (total 46 loci, 40 skeleton loci with 37.13 cM per marker), whereas total map length was the highest (1485.1 cM) in group 2. Homoeologous group 7 had the lowest marker number and density (total 10 loci, 9 loci were monomorphic and 1 loci was polymorphic for our two Parental). Group of 6 after group of 2 had the longest map length (740.3 cM).

The average distance between marker pairs is 31.7 cM, ranging from 10.8 cM for chromosome 4B to 58.4 cM for 6B (Fig. 3.19), with some gaps wider than 93 cM and 99 cM on all linkage groups, except for 6B and 6A (Fig. 3.19).

Differences were also found between the two sub-genomes, with 45 (52.6 %) markers mapped to the A genome (average 8 markers per chromosome) and 50 (47.4 %) to the B genome (average of 7 markers per chromosome). The A-genome skeleton map was denser, with 53 markers that accounted for 1800.0 cM of genetic distance (34.0 cM per marker). The B genome skeleton map spanned 1370.3 cM with 47 markers (29.2 cM per marker).

The number of polymorphic markers was greater than the number of loci on our skeleton map. After analyzing all polymorphic markers with the Mapmaker program, some markers were not present in our map, the reason to be absent was that included a long distance from other markers. Eleven polymorphic markers (1, 6, 1 and 3 in group of 1, 2, 5 and 6 respectively) were not associated with the other markers, therefore not taken on our map. Eleven microsatellites showed a significant skewness from the expected heritability ratio (or distribution) and therefore were eliminated from the map.

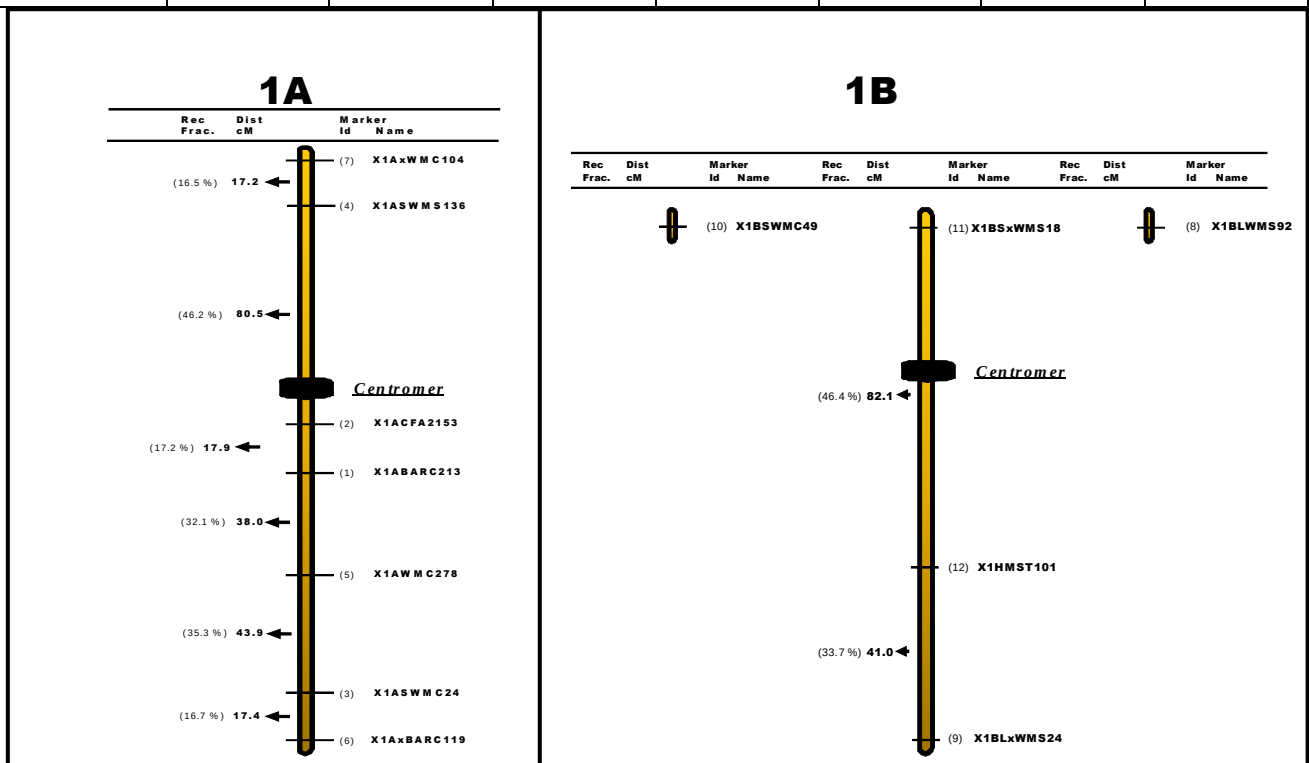
Chromosomes 1A, 2A, 2B and 6A showed marker clustering around the centromeric region. Marker clusters are usually associated with reduced recombination in the proximal region of chromosome arms. However, in this mapping population the marker distribution was relatively adequate and few clusters of tightly linked loci were revealed, as most of the SSRs and SNPs marker locations in our map were known and the markers were selected to avoid closely linked multiple loci.

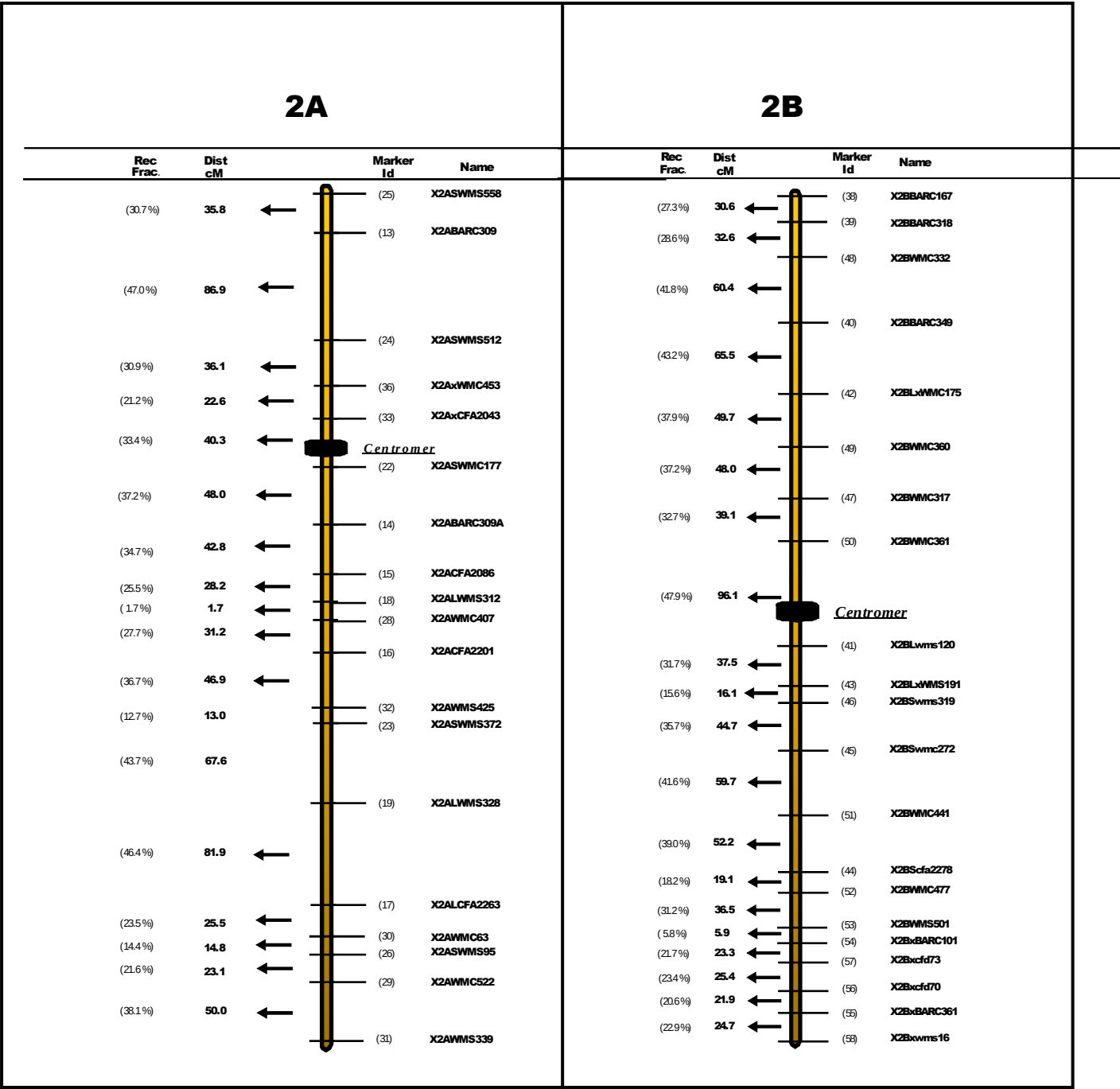
Figure 3.19: Indication Genetic map of tetraploid wheat (*Triticum durum*).**Genome A**

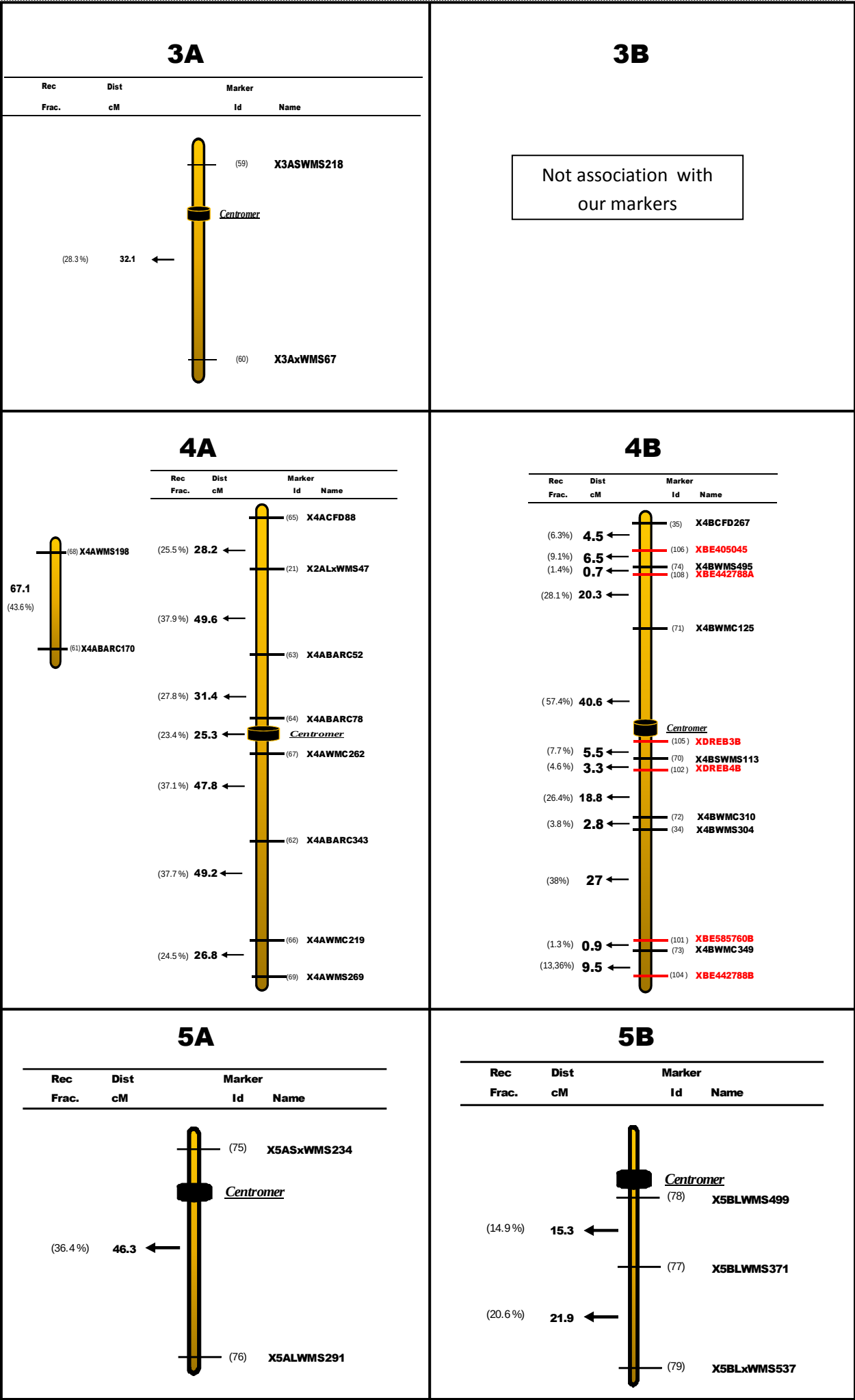
Chromosome	1A	2A	3A	4A	5A	6A	7A
Data File	J.Kcmdmitar	J.Kcmdmitar	J.Kcmdmitar	J.Kcmdmitar	J.Kcmdmitar	J.Kcmdmitar	J.Kcmdmitar
Map Scale	10.0 cM per cm	10.0 cM per cm	10.0 cM per cm	10.0 cM per cm	10.0 cM per cm	10.0 cM per cm	10.0 cM per cm
Kosambi Mapping Function	Kosambi Mapping Function	Kosambi Mapping Function	Kosambi Mapping Function	Kosambi Mapping Function	Kosambi Mapping Function	Kosambi Mapping Function	Kosambi Mapping Function
Segment Break Dist	> = 999.9 cM	> = 999.9 cM	> = 999.9 cM	> = 999.9 cM	> = 999.9 cM	> = 999.9 cM	> = 999.9 cM
Segment Break Frac	> = 50.0%	> = 50.0%	> = 50.0%	> = 50.0%	> = 50.0%	> = 50.0%	> = 50.0%
Log-Likelihood	-293.71	-855.24	-53.43	- 446.86	- 54.70	- 438.42	- 87.54
Longest Seg cM	214.969	696.251	32.130	258.258	46.326	506.821	45.201
Loop Tolerance	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Inner Tolerance	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Iterations	3.00	3.00	3.00	3.00	3.00	3.00	3.00

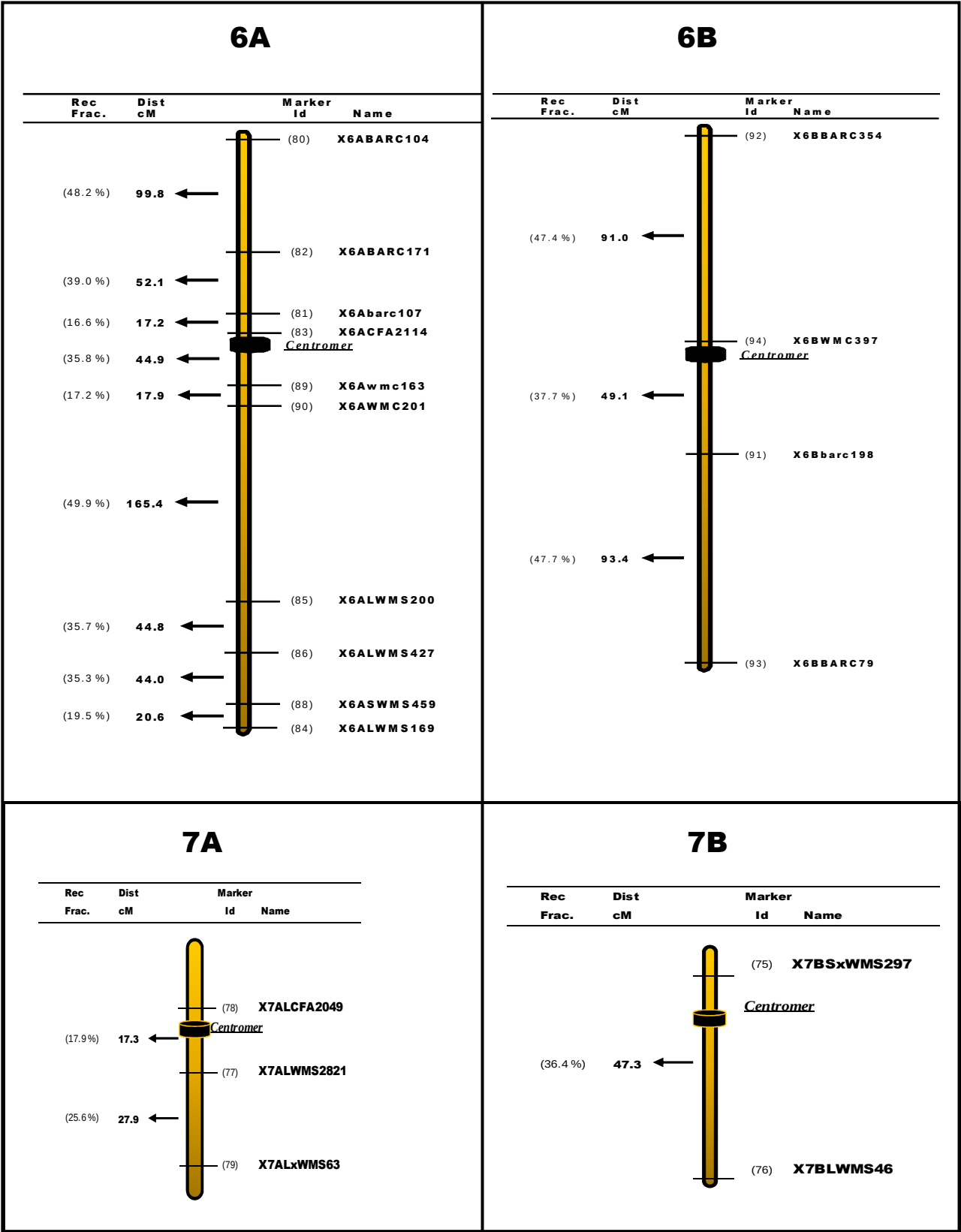
Genome B

Chromosome	1B	2B	3B	4B	5B	6B	7B
Data File	J.Kcmdmitar	J.Kcmdmitar	J.Kcmdmitar	J.Kcmdmitar	J.Kcmdmitar	J.Kcmdmitar	J.Kcmdmitar
Map Scale	10.0 cM per cm	10.0 cM per cm	10.0 cM per cm	10.0 cM per cm	10.0 cM per cm	10.0 cM per cm	10.0 cM per cm
Kosambi Mapping Function	Kosambi Mapping Function	Kosambi Mapping Function	Kosambi Mapping Function	Kosambi Mapping Function	Kosambi Mapping Function	Kosambi Mapping Function	Kosambi Mapping Function
Segment Break Dist	> = 999.9 cM	> = 999.9 cM	> = 999.9 cM	> = 999.9 cM	> = 999.9 cM	> = 999.9 cM	> = 999.9 cM
Segment Break Frac	> = 50.0%	> = 50.0%	> = 50.0%	> = 50.0%	> = 50.0%	> = 50.0%	> = 50.0%
Log-Likelihood	-206.98	-966.84	—	- 186.94	- 91.98	- 144.98	- 109.7
Longest Seg cM	123.075	788.877	—	140.395	37.250	233.440	47.300
Loop Tolerance	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Inner Tolerance	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Iterations	3.00	3.00	3.00	3.00	3.00	3.00	3.00









Interval length obviously varied within and among chromosomes with a coefficient of variation (CV) ranging from 25% to 106% in the entire genome. The weighted mean CV of B genome was larger than that of A genome (Table 3.7).

The coefficient of variation, in present work showed that the markers are located more precise in the chromosomes 4A by CV: 30.78 and 6B by CV: 32.00. The coefficient of variation was also calculated between the two genomes, which has resulted in the A genome by CV: 68.63 was localized more correctly than B genome by CV: 77.75. This accuracy of A genome may reflect, to some extent, marker clustering observed on most of the chromosomes for A and B genomes (Fig. 3.19). The significance of clustering (on the levels of A, B, and the entire genome for our maps) was tested by comparison of the observed distribution of intervals with different marker numbers with Poisson distribution (Korol et al. 1994). The clustering phenomenon for the B genome and the entire genome proved to be highly significant, but not for A genome even though marker clusters were observed on a few B chromosomes (Table 3.7). As an example, the distribution pattern of various marker intervals on A, B, and the entire genome for our map is shown in Table 3.7. The observed distribution on A genome basically fits the expected Poisson model.

On B and the entire genome, the observed distribution pattern greatly deviated from the Poisson model due to a significant excess of highly populated and/or empty or sparsely populated intervals and a deficit of moderately populated intervals.

2- 3- Difference between “A” and “B” genomes

In present project there are 57 SSR polymorphic loci located on A genome (57%), and 37 SSR+ 6 SNP polymorphic loci on the B genome (43%). Length of the A genome and B genome has been calculated 1799.956 and 1370.337, respectively. The density of markers on the A genome was 33.96 cM/marker that ranged from 16.07 cM/marker on 3A to 50.60 cM/marker on 6A. The lengths of our first parental (C1/J.K) and second parental (O5d/O5) for A genome were 895.9 cM and 1615 cM respectively, but those for B genome were slightly longer, 1341.5 cM and 1982.8 cM respectively.

In the mapping work of first parental (Jennah Ketifa/ Cham1) was used 140 molecular marker (46%) for A genome and 166 marker (54%) for B genome from a total 306 molecular marker that included 138 RFLPs, 26 SSRs, 134 AFLPs, 3 Genes, 5 SSPs. (Nachit et al. 2001; Elouafi and Nachit 2004).

In the studying of Elouafi and Nachit (2004) mapped our second parental, i.e. Omrabi 5/*Triticum dicoccoides* 600545// Omrabi 5. In this genetic mapping was used 273 markers (124 SSRs, 149

AFLPs, 6 Genes), in these markers 102 (37%) belonged to A genome, and 171 (63%) to B genome. The common thing in this work with ours, was that a majority of markers mapped were SSRs. In the study of Peng et al. (2011) among the 549 loci, 231 (42%) were located on A genome and 318 (57.8%) on B genome. The total lengths of *T. dicoccoides* domesticated descendant (H) and wild emmer domesticated descendant (L) maps for A genome were 1589 cM and 1607 cM respectively, and those for B genome were slightly shorter, 1580 cM (H) and 1573 cM (L), respectively. The difference of map length between A and B genome was not significant.

2- 4- Changes in location of markers

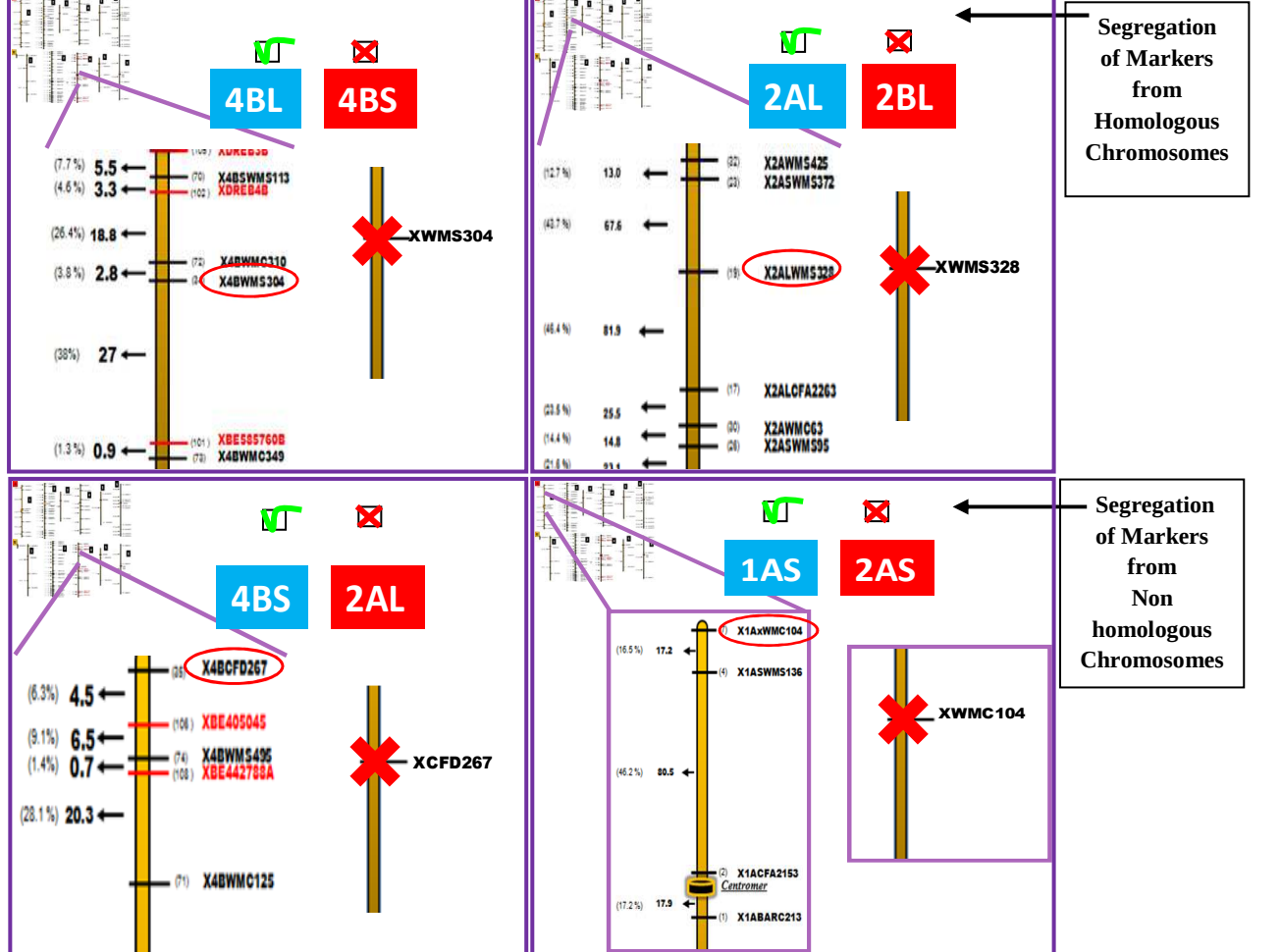
As mentioned above there were some markers with more loci, in this case there were also some markers that, in the analysis of Nulli-Tetrasomic lines have demonstrated a position different from brought on mapping that been calculated with mapmaker. The reason for this difference in position among different maps could be explained by the fact that some markers have two or more loci, but sometimes one locus remains monomorphic, and the other locus polymorphic differently in the different RILs. For example Wmc310 marker in the Nulli-Tetrasomic lines showed that been localized on the chromosome 4A, but in present map it is localized on the 4B.

The changes of chromosomal location are divided in two groups: 1- segregation of markers from homologous chromosomes, or 2- segregation of markers from nonhomologous chromosomes.

In the first class the changes of the map location of Xgwm304, mapped in the long arms of chromosome 4B, instead of be located in the short arms of chromosome 4B; or Xgwm328 that located in the long arm of chromosome 2A instead of be located in the long arm of chromosome 2B. Also in the second class: the markers wmc104 and cfd267 were located on the chromosomes 1AS and 4BS respectively instead of 2AS and 2AL (Fig. 3.20).

Moreover, a chromosome segment inversion could be also possible, but for this we should assume that the inversion is present in both *T. dicoccoides* and *T. durum*. Joppa et al. (1995) found that a high proportion (70%) of *T. dicoccoides* genotypes had translocations.

Nonhomoeologous translocations have been repeatedly reported in hexaploid wheat (Liu et al. 1992; Devos et al. 1993) and tetraploid wheat (Blanco et al. 1998). Translocation may be one of the forces maintaining the high genetic diversity of durum wheat under the diverse natural environments (Joppa et al. 1995; Kawahara and Nevo 1996). Translocation frequencies (TF) of various populations were correlated with environmental variables, primarily with water availability and humidity, and possibly also with soil type. In general, TF was higher in peripheral populations in ecologically heterogeneous frontiers of species distribution than in the central populations located in the catchment area of the upper Jordan valley (Joppa et al. 1995).



The maximum likelihood (ML) estimates the coefficient of coincidence (c) indicating that negative

The maximum likelihood (ML) estimates the coefficient of coincidence (c) indicating that negative crossover interference (i.e., $c > 1$) is characteristic of our *T. durum* $\times$ *T. durum* hybrid. In fact, it is manifested in some regions of all chromosomes in both genomes A and B (Table 3.8). Deviations from the ‘no interference’ Haldane recombination scheme were highly significant. The maximum (per chromosome) values of X^2 (df = 1) ranged, correspondingly, from 55.79 (with $c = 4.48$) for one of the islands of chromosome 2B. Clearly, the deviations from Kosambi interference in such cases should be even more significant. Each chromosome manifested a few islands of negative interference, from 1 to 2,3. As a rule, these islands were located in proximal regions, either spanning the centromere (chromosomes 1A, 3B, 4A, 6B, 7A, 7B), or excluding it. In the latter cases, the location of the islands could be to one (chromosomes 1B, 2A, 3A, 4B) or both (5A, 5B) sides of the centromere. When two or more islands were found per chromosomal arm, the location of extra islands was either median (1B) or terminal/ subterminal (5A, 5B).

both sides of the central point of any considered interval pair and by variation of the position of the

central point. Correspondence between the results of this Map, was employed as an additional important test for existence of a tract of negative interference (Table 3.8). As in our previous results with chromosome 1B, a tendency for a higher level of negative crossover interference was found in regions proximal to or spanning the centromere. Likewise, alternation of negative interference by segments with strong positive interference found earlier in chromosome 1B (Peng et al. 1999 a) appears to be a general phenomenon. In particular, for many such regions with positive interference, the estimated value of C (Coefficient of coincidence) was significantly smaller than predicted by Kosambi mapping function (Peng et al. 2011) (Table 3.8).

Table 3.8: Islands of Negative Interference along the Chromosomes

Island 1		
Chromosome	c^2	$x^{2,3}$
1A	WMC278	
	7.74	13.82****
1B	MST101	
	>20	23.65****
2A	WMC177	
	6.24	11.21****
2B	GWM120	
	4.48	55.79****
3A	GWM218	
	2.44	35.31****
3B	---	---
4A	BARC78	
	4.63	22.34****
4B	WMC125	
	4.53	18.53 ****
5A	GWM234	
	2.86	15.23****
5B	GWM499	
	4.21	32.02****
6A	CFA2114	
	3.68	14.32****
6B	WMC397	
	5.93	12.43****
7A	GWM2821	
	3.24	14.37****
7B	---	---

1. The central marker for two adjacent intervals with maximum x^2 is presented for each island of negative interference.
2. (c) Coefficient of coincidence.
3. (****) Significance at the level of 0.001, respectively.

2- 6- Isogenic line and some trait

It is usually difficult to precisely determine the effects of specific genes on the plant performance, because these effects are usually limited and often influenced by the environment in which the plants are grown. However, these effects can be determined accurately using isogenic lines, which are not usually available in tetraploid wheat and it takes time to develop them. It is necessary to create the primary genetic collection and to study inheritance of individual traits for development of near-isogenic lines. In comparison with hexaploid wheat species, aneuploid analysis was not applied in tetraploid wheat until Joppa. Williams (1988) who made Langdon durum substitution lines. The establishment of a genetic collection in tetraploid wheat was restricted because the

knowledge of inheritance of specific characters was scarce. Hence, the utilization of genetic collections of hexaploid wheat may be beneficial. To develop near-isogenic lines in tetraploid wheat, the genes to be introduced were located on a specific chromosome using aneuploid stocks of LD222 (Nishikawa, unpublished) and Langdon (Joppa 1993), Landgdon D-genome chromosome substitution lines (Joppa and Williams 1988). The linkage maps of these genes were developed using microsatellite markers (Röder et al. 1998 b; Song et al. 2005; Torada et al. 2006).

2- 6- 1- Gene transfer intra specie

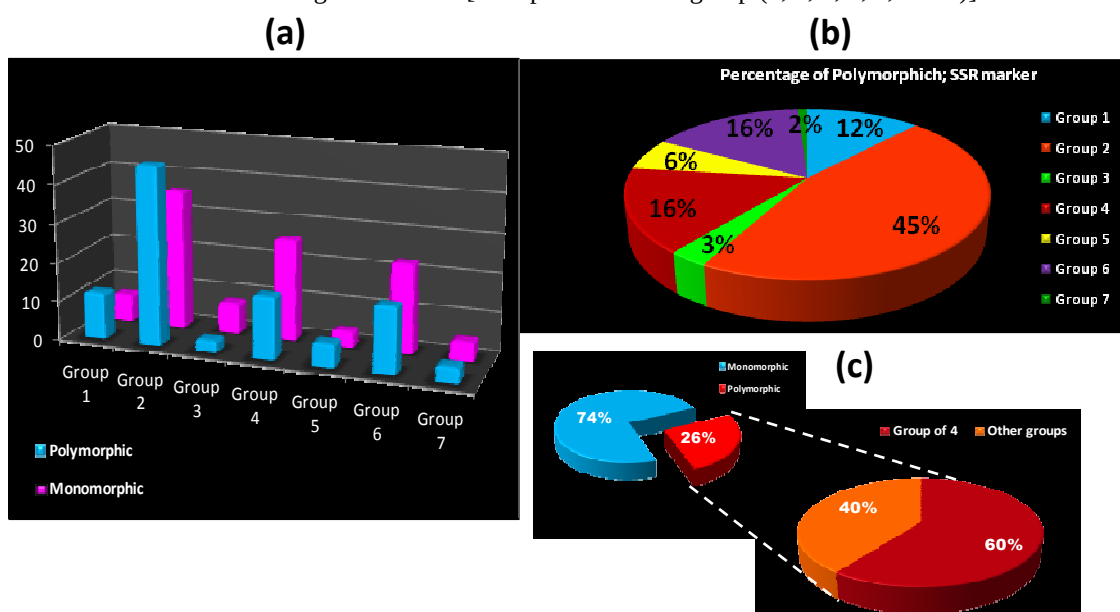
Normally *Thinopyrum intermedium* and *Th. ponticum* do not exchange DNA because bread wheat contains a gene (called Ph1, located on group of 7) that prevents DNA from different sources from pairing and exchanging DNA.

For example the scientists allowed *Th. intermedium* and *Th. ponticum* DNA to recombine and then screened for new arrangements that bring together the desirable resistance genes while losing a deleterious gene that causes an extreme yellow color in flour. The upshot now is that we have beautiful new combinations of these valuable disease-resistance genes (Larkin 1960). This recombination between two species also the exist in Parents with species of other work (Nachit et al. 2001; Peleg et al. 2011).

3- HOMEOLOGOUS GROUP

The 14 chromosomes are covered by 105 SSRs and 10 SNPs markers, shared with various degrees in different groups. Some groups have higher polymorphic, while other are mainly monomorphic. That depended on the similarity between the parental. A range of 1 to 46 microsatellites per chromosome were located on genetic maps (Fig. 3.21).

Figure 3.21 : (a) The number of total monomorphic and polymorphic SSR markers.
(b) The percentage of polymorphic markers (SSR) in different homoeologus group (1-7).
(c) The percentage of monomorphic and polymorphic markers (SNP), and the polymorphic markers devising in two class [Group of 4 or other group (1, 2, 3, 5, 6, and 7)].



3- 1- Homoeologous group one (1A, 1B)

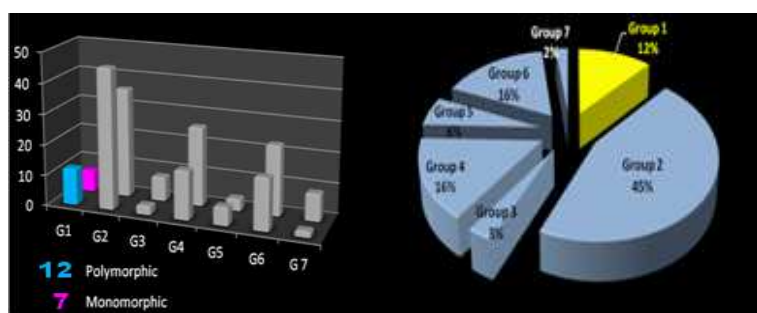
In group of homoeologous 1, were polymorphic the 66% and 34% markers for chromosome 1A and 1B respectively. SNP markers were absent on this group as well as for homoeolog groups 3, 5 and 7. The centromere is located between the markers Xcfa2153 (in short arm) and Xbarc213 (in long arm) of chromosome 1A. This chromosome has the highest level of polymorphism, which allowed locating, 12% of all mapped markers, this is in agreement with previously published data (Van Deynze et al. 1995 a, b).

It was observed a uniform distribution of markers on chromosome 1A, while in the chromosome 1B was detected clustering of markers near the centromere and around the Gli1-Glu3 loci, encoded for storage proteins, in agreement with the data of the firsts molecular studies on wheat (Snape et al. 1985; Dvorak and Appels 1986; Curtis and Lukaszewski 1991; Devos et al. 1992; Werner et al.

1992). The saturation of the homoeologous group 1 is particular important for the presence of genes coding for storage proteins, for the transfer of technological and nutritional quality, as well as of several genes for resistance to biotic stress (diseases cryptogams as rust and powdery mildew and some insects).

The Cham 1 and Jennah Khetifa relatives of our first parental, were different for the strength of the gluten and resistance to rust and powdery mildew, therefore they are presented on genetic analysis for these characters. Markers on these chromosomes may be useful for assisted selection in breeding programs of several important traits.

Figure 3.22: Total number of markers used and division between monomorphic and polymorphic in homoeologous group one compared to other homoeologous groups.



3- 2- Homoeologous group two (2A, 2B)

The highest density of markers is represented on homoeologous group 2. where 45% of the total SSR and SNP markers are distributed on the map.

The majority of SSR and SNP markers for chromosomes 2A and 2B showed significant ($P < 0.05$) segregation distortion. Nevertheless, chromosome 2A showed highly diversity marker order between the two parents (54% polymorphic). The present map was adjusted comparing this chromosome with to other work (Francki et al. 2009) in the study of Francki et al. (2009) chromosome 2A showed highly similar marker in tree genetic maps derived from tree different cross (P92201D5-2-2/P91193D1-10, Ajana/WAWHT2074, Blanco/Millewa). Markers order was also well conserved across the genetic map of chromosome 2B with 21 markers. A large number of markers are concentrated in the centromeric region, which probably represents an extreme case of the suppression of recombination in the vicinity of the centromere that is apparent from the clustering of markers in the centromeric regions of all *T. durum* linkage maps (Dubcovsky et al. 1996).

Also the long arm (2AL) and the markers relative position was in agreement with previously published work (Nelson et al. 1995 a; Blanco et al. 1998; Roder et al. 1998; Korzun et al. 1999). The centromere has been located between WMC177 markers (short arm) and BARC309 (long arm).

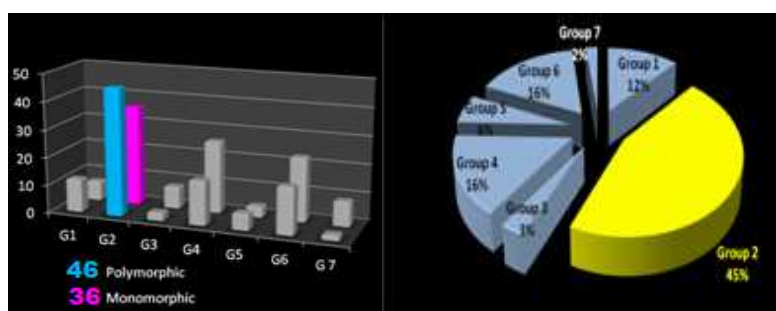
The centromere in chromosome 2B has been located between WMS191 markers (long arm) and WMS319 (short arm).

The map of chromosome 2A and 2B is the longest, 696.3 cM and 788.9 cM (Figure 3.19), among the other 14 chromosome. The difference of our study with the other was the high number of polymorphic markers on chromosome 2A of our map, compared to the work of Francki et al. (2009), Peleg et al. (2008). Five markers were on the short arm and seven on the long arm of chromosome 2A.

Most linkage groups were aligned on the long arms, indicating a good coverage across the wheat cytogenetic map. However, the order of some markers on linkage groups for chromosome 2B did not coincide with the order determined by other study mapping. The chromosomes 2 are determinants for wheat adaptation, containing loci that control important physiological characteristics such as: responses to photoperiod, vernalization, and the size of the plant (Nelson et al. 1995 a) and times of flowering (Scarath and Law 1984). This group is also important for resistance to certain diseases in fact, several genes for rust, powdery mildew, and *Stagonospora nodorum*, *Septoria nodorum* blotch, and bunt resistance, are located on chromosome 2A and 2B (Anderson et al. 1993). De Felice (2002) have verified that the parents of first parental Jennah Khetifa and Cham1 show different needs for photoperiod, different height, earliness, and resistance to different diseases.

A saturated map of these chromosomes, together with phenotypic data of major complex traits, could be used to determine the positions of loci for the control quantitative characters (Quantitative Trait Loci, QTL) in this group of chromosomes.

Figure 3.23 : Total number of markers used and those monomorphic or polymorphic in homoeologous group two compared to other homoeologous groups.



3- 3- Homoeologous group three (3A, 3B)

The map of chromosome 3A is the shorter, 32.130 cM (Figure 3.19), among the other 14 chromosome. Most of the markers located on chromosome 3B were monomorphic, then the map of chromosome 3B was absent. Although 8 SSR markers were bin mapped to chromosome 3A. 35% of these 8 markers were polymorphic, but the density of this chromosome was low. Between the

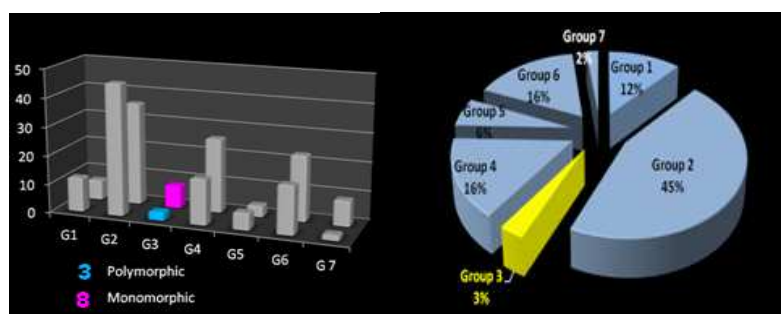
various works Francki et al. (2009) showed a high density of markers on chromosome 3A. Francki et al. (2009) showed interesting features across the genetic maps, with 75 SSR and DArTs ordered in the Ajana/WAWHT2074 map of which 34 were redundant, the most of any chromosome group across the four maps. In some map in particularly the order of markers gwm493, gwm389 and gwm533 was different (Peleg et al. 2008) (Fig 3.24).

A study by Adhikari et al. (2004) identified inconsistencies in order of SSRs gwm389, gwm533 and gwm493 on chromosome 3BS comparing four genetic maps, similar observations of the same markers for chromosome 3B (see Francki et al. 2009).

The microsatellites have an association with trait locus of *Fusarium* head blight (FHB) resistance (Matus-Cádiz et al. 2006), that were polymorphic for other study and unfortunately monomorphic in the screening of our parentals. Francki et al. (2009) showing significant ($P < 0.05$) segregation distortion for chromosome 3A in the P92201D5-2-2/P91193D1-10 genetic map as in this study, that have significant ($P < 0.05$) segregation distortion.

The group 3 chromosome contains genes for resistance to rusts, *Septoria nodorum* blotch, decay, mildew and nematodes, and genes for the red color of the kernels are located on the short arms of this group (RA1 and RB1). The parental genotypes of the RIL were used for the contrasting color of the kernels: Jennah Khetifa kernels have red, and Cham 1 white.

Figure 3.24 : Total number of markers used and those monomorphic or polymorphic in homoeologous group three compared to other homoeologous groups.



3- 4- Homoeologous group four (4A, 4B)

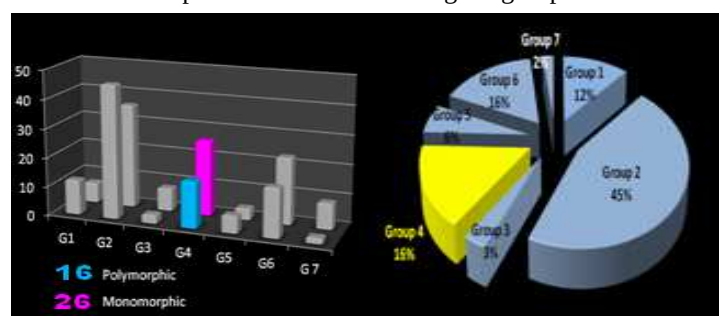
Homoeologous group 4 was one of the groups that we have focused, even if did not include a high number of markers. This group contained markers associated with some important traits (Drought tolerance). Chromosome 4A and 4B was covered by ten and seven SSR markers, also zero and six SNP markers, respectively.

Interestingly, in the work of Francki et al. (2009) the linkage groups 4A and 4B were aligned to this region for all three maps (Blanco/Millewa, P92201D5-2/P91193D1-10, and Cadou/Reeves), and few markers were identified on the this two chromosome (4A, 4B) for either the genetic maps. Comparing the same SSRs markers of our map in this group, with these three maps.

The order of markers on chromosome 4A is different. In some instances, SSR markers did not align with the expected regions of the cytogenetic map, and duplicated marker loci or segregation distortion may have contributed to inaccurate or ambiguous ordering of markers in the genetic map. Chromosomes reside had a two genes for dwarfism and had a chromosomal region associated with the resistance of the kernel to remain on the ear (Anderson et al. 1993). Dwarfism in tetraploid wheat is associated effects on yield. The genetic system consists of two loci, Gai/Rht1 on chromosome 4A (Gale and Marshall 1981).

The centromere of chromosome 4A was placed between BARC78 (short arm) and WMC262 (long arm). Chromosome group 4 carries several genes on the short arms for resistance to leaf and stem rust and for reduced plant height. Chromosome 4D, collinear with 4B, also carries resistance to several abiotic stresses. On the chromosomes of group 4 are present several genes for resistance to leaf rust and stem, and to reduce the size of the plant. Some of the markers on the 4A or 4B were previously mapped to 4D in the synthetic population M6 × Opata (Nelson et al. 1995 a; Roder et al. 1998).

Figure 3.25 : Total number of markers used and those monomorphic or polymorphic in homoeologous group four compared to other homoeologous groups.



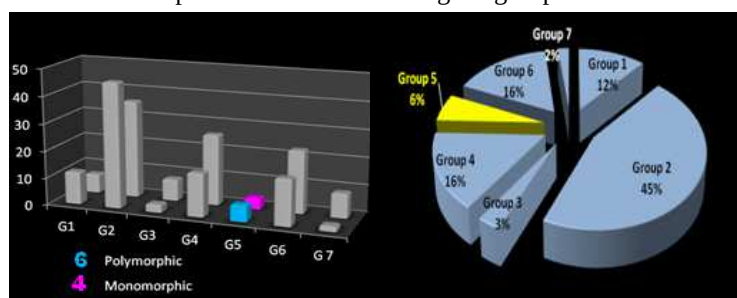
3- 5- Homoeologous group five (5A, 5B)

The homoeologous group 5 had the lowest representation of SSR markers. Even if it was reported (Sourdille et al. 2004) that groups 2 and 5 has best covered (126 and 125 microsatellites, respectively). In our case chromosomes 5A and 5B had included a total of 6 markers.

For chromosome 5B, only 1 marker, Xgwm234, was located on the short arm with LOD 3, whereas one other marker is on the long arm (Nachit et al. 2001). The gene Lox11-1 for α -lipoxygenase mapped in the same position as Lpx-B2.

Many useful genes present in group 5, were associated with molecular markers: resistance to copper, the absence of hair on the kernels, the presence of lipoxygenase, abscisic acid accumulation, reduced size, response to vernalization, Cochliobolis resistance, leaf rust and to yellow rust.

Figure 3.26 : Total number of markers used and those monomorphic or polymorphic in homoeologous group five compared to other homoeologous groups.



3- 6- Homoeologous group six (6A, 6B)

The homoeologous group 6 was the other one that had the high representation of SSR markers. The 16% of all polymorphic SSR markers mapped in the wheat genome were assigned to this chromosome group. Similar to homoeologous group 4, the majority of SSRs markers were mapped to 6A the markers density was highest on the long arm of the 6A (Fig. 3.27).

This group, particularly on chromosome 6A, showed a greater number of markers grouped in the centromeric region. About this group chromosomal intervals included a greater distance than other groups of homology. Linkage order is still based on maximum likelihood, in other words, the order of markers that yields the shortest distance and requires the fewest multiple crossovers between adjacent markers. Likewise, genetic distance between markers is measured in centimorgans, which is based on the frequency of genetic crossing-over (Young 2000).

With increasing intervals between markers, it also increases the percentage of recombination, with values up to 20 cM approximate linearly, but the percentage recombination after 20 cM is to underestimate (Fig. 3.27). Unlike the work of Francki et al. (2009) the majority of SSRs markers were mapped on 6B with linkage groups from P92201D5-2-2/P91193D1-10 and Ajana/WAWHT2074 maps having the highest density of markers. On group of 6 are located a few genes for resistance to biotic stresses such as leaf rust, stem rust, yellow rust. Homoeologous group 6 also contains important genes, such as those for enzymes α -amylase and for α -/ β -gliadins. Gliadin is a glycoprotein present in wheat and several other cereals within the grass genus *Triticum*. Gliadins are prolamins and are separated on the basis of electrophoretic mobility and isoelectric focusing (types: α -, β -, γ -, ω - gliadins).

Also in the study of (Castro et al. 2008) in the cross (*Triticum dicoccoides* $\times$ *Aegilops tauschii*) was discovered genes for resistance to parasites (Greenbug and Russian wheat aphid - RWA), associated with markers located on the long arm of chromosome 6A (Xwms1009, Xwms1185, Xwms1291).

Two of these four markers were the same used in our map and located on chromosome 6A, with the similarity of 66% with the first parental.

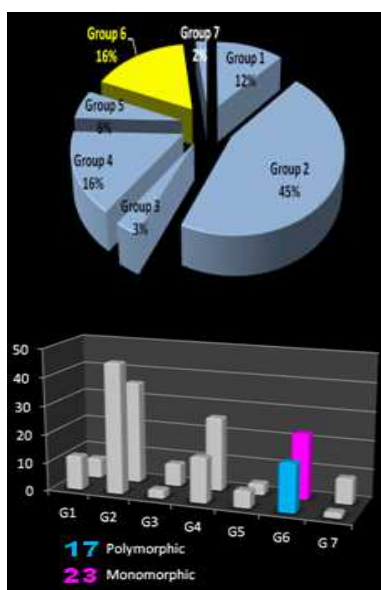


Figure 3.28 : Total number of markers used and those monomorphic or polymorphic in homoeologous group six compared to other homoeologous groups .

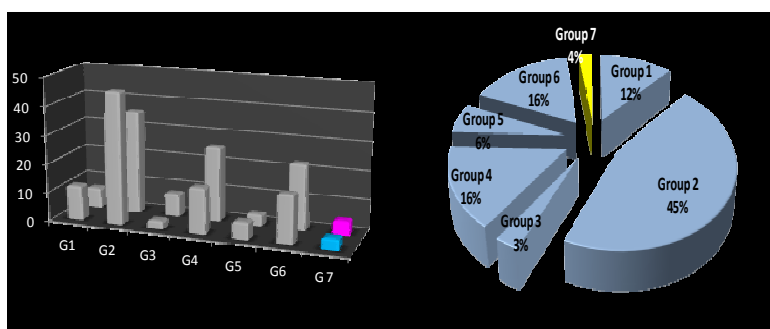
3- 7- Homoeologous group seven (7A, 7B)

The homoeologous group 7 had the lowest representation of SSR markers. Out of 8 SSR markers present in this group only five was polymorphic. Probably the traits that transport from chromosomes 7A and 7B, will not have a big difference in our RIL.

A marker was located on the short arm and 2 on the long arm. The centromere was positioned between Xcfa2049 and Xgwm2821 (Wms2821).

Chromosomes 7A and 7B carry loci that control the reduction of the size, the response to vernalization, in addition to genes for resistance to rust, powdery mildew, virus BYDV (Barley Yellow Dwarf Virus), aphids red wheat. The parental genotypes of RIL showed a contrasting level of resistance to aphids.

Figure 3.29 : Total number of markers used and those monomorphic or polymorphic in homoeologous group seven compared to other homoeologous groups.



4- COMPARISON OF PRESENT MAP, WITH THE OTHER MAPS

4- 1- Maps comparison

In the most important plant species there are often multiple efforts to construct DNA based genome maps. This has led to have several maps for the same species with little or no information about the relationships among maps (Young 2000). Of course this makes difficult to relate the reported location of a gene on one map to its location on another map. It also means that the maps are less saturated, and therefore less powerful, than they could be. Even where there is no proprietary barrier to relating maps each another, there are often practical and theoretical problems, but also some advantage. The most obvious is that markers polymorphic in one mapping population may not show variation in a second population, so the utilization of several mapping populations enlarges the markers which could be mapped. The firsts genetic maps were based on mapping populations optimized for DNA polymorphisms, often including parents from distinct, but cross-compatible species. As researchers move to more narrow crosses, previously excellent genetic markers will be useless for lack of polymorphism (Young 2000).

Present map, constructed with SSRs and SNPs markers, is compared with other maps: 1) maps of the relatives of our RIL; 2) main maps of ITMI built by SSR markers (Consensus map); 3) main maps already published on the website grain gene (<http://wheat.pw.usda.gov/GG2/index.shtml>); and 4) other maps published in the last 7 years.

Comparing of these genetic maps with present genetic map, we observed that in the screening of microsatellite markers, the percentage of polymorphism varies from 21% to 84%. This percentage indicates the degree of difference and diversity between their parental. Hexaploid bread wheat shows relatively low levels of polymorphism between the two Parental for SSRs loci, most likely as a result of its narrow genetic base (Chao et al. 1989). Usually <10% of all RFLP loci are polymorphic in an intraspecific cross, but ≤ 98 SSRs loci are polymorphic (Röder et al. 1998 b). However, in the genetically distant cross of hexaploid bread wheat, Opata 85 $\times$ W7984, 72% of the RFLP probes (Mingeot and Jacquemin 1999) and 80% of the microsatellite primers (Röder et al. 1998 a, b) exhibited polymorphism between the parents. The high percentage of polymorphism (80%) on the map of Somers et al. (2004) shows that between the two parental populations exist e genetic distance (Table 3.9).

In a distant cross of tetraploid wheat, Messapia $\times$ MG4343, which is similar to our mapping population, 70.1% RFLP probes (Blanco et al. 1998) and 84.4% microsatellites (Korzun et al. 1999) detected polymorphism between the parents. Therefore, for distant crosses of both hexaploid

tetraploid wheats, about 70% RFLP probes and 80% microsatellite primer sets can detect polymorphism.

The level of polymorphism of microsatellite primer sets in the present mapping population was even (>45%). Furthermore, and 26% SNPs primer combinations screened detected polymorphism between the parents. This clearly shows that is significant in normal level genetic differentiations occurred between *T. durum* (J.K/C1) and another *T. durum* (O5.d/O5) during their evolutionary divergence caused by domestication, even though they share the same A and B genomes and display no genetic or reproductive obstacles. Remembering again that this difference depends on the choice of markers. Therefore, present map is in accord with most published maps.

The percentage of polymorphism between parents is 48.6% in present map that showed the high diversity between parents through the markers used for this work. The two parents were also highly polymorphic in morphological and agronomic traits, i.e. plant height, growth duration, leaf shape, grain characteristic, spike fragility, stripe-rust resistance, and so on (Peng et al. 2011).

These results confirm the transferability of microsatellites between two durum wheat. The same result was reported in durum $\times$ durum wheat cross (Nachit et al. 2001), bread $\times$ durum wheat (Elouafi et al. 2004) and in *T. durum* $\times$ *T. dicoccoides* cross (Korzun et al. 1999). 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).

Unfortunately on the map of Nachit et al. (2001) and Elouafi et al. (2004), that were the parents of our RILs, were only few SSR markers identical with our. However, these few markers were compared to verify the differences and similarities of their position in the maps. The mapping of these microsatellites was almost in agreement with previous publications. Therefore, 90 percent of mapped microsatellites mapped as expected in the consensus map (ITMI map), this result was verified by Somers et al. (2004) results. Whereas, on the comparison between these two maps, 65% of SSR markers were identical, that only some (about 20%) microsatellites were found to map in other localization (Table 3.9). Most of these microsatellites have been mapped on the same chromosome, but different homologous as Gwm122 (2B instead of 2A), Gwm156 (5A instead of 5B), Gwm415 (5A instead of 5B). This could be explained by the presence of additional loci in the wheat genome.

Table 3.9 : Comparing the SSR markers on the maps of tetraploid and hexaploid wheat, with our markers and shown the similarity between these main maps and our map.

Map of Species	N. of SSRs marker (Total)	N. of SSRs marker Polymorphic (%)	N. of other markers (Type)	N. of SSR markers identical	Identity between our map	Length of Map cM	Majority of identity (Chromosome)	Refrence	Relations hip with our map
<i>Triticum turgidum</i> (Jennah.Khetifa × ChamI)	45	26 (57.8 %)	280 AFLP, RFLP	3	80 %	3598	1A, 6A	Nachit et al. (2001)	First Parental
<i>Triticum turgidum</i> (Omrabi5 × <i>dicoccoides</i>)	195	124 (63.6 %)	155 QTL, AFLP	14	72 %	2289	2A, 5B	Elouafi et al. (2004)	Second Parental
<i>Triticum aestivum</i> (Synthetic × Opata)	1543	1235 (80 %)	0	47	65 %	2569	2A, 2B	Somers et al. (2004)	Consensus map
<i>Triticum turgidum</i> (Kofa × UC1113)	1235	230 (18.6 %)	39 QTL, SNP	20	85 %	2140	6A, 6B	Zhang et al. (2008)	The Graingene
<i>Triticum turgidum</i> (Messapia × <i>dicoccoides</i>)	96	81 (84.4 %)	215 RFLP	7	88 %	---	2A	Korzun et al. (1999)	The Graingene
<i>Triticum turgidum</i> (Jennah.Khetifa × ChamII)	87	31 (35.6 %)	433 AFLP, RFLP	2	94 %	4673	1A	Diab (2001)	The Graingene
<i>Triticum turgidum</i> <i>Durum</i> (Desf.) × <i>dicoccoides</i>	914	196 (21.4 %)	478 Dart	10	73 %	2317	5B, 2B	Peleg et al. (2008)	The publication

Several genetic maps were constructed for tetraploid wheat, either for *T. durum* × *T. dicoccoides* (Peng et al. 2000; Blanco et al. 1998, 2004; Elouafi and Nachit 2004) or *T. durum* × *T. durum* (Nachit et al. 2001), revealing map sizes of 2,237 to 3,598 cM. Other maps constructed for hexaploid wheat populations reported (for genomes A and B) map sizes of 1,791.0 to 2,356 cM (Röder et al. 1998 a, b; Quarrie et al. 2005; Akbari et al. 2006).

The 115 markers cover a total of 3170.3 cM, almost like the linkage map reported by Nachit et al. 2001 for the cross Jennah Khetifa × Cham 1, that had calculated 3597.8 cM. This is due mainly to the intra specific cross durum × durum and to the greater number of mapped markers.

Some microsatellite markers (WMS) used have known chromosomal assignments and were previously mapped in several mapping populations, including the bread wheat population ITMI (Röder et al. 1995, 1998) and durum populations, Messapia × *T. dicoccoides* MG4343 (Korzun et al. 1999), Jennah Khetifa × Cham1 (Nachit et al. 2001), Omrabi 5/*T.dicoccoides* × Omrabi5 (Elouafi et al. 2004). Therefore, it was decided to use these codominant and very informative markers as a main framework for our genetic map. The majority of our SSR markers were not identical to the SSRs in the other maps, these markers were positive results, because have been localized on chromosomes, that aren't located until now in durum wheat. The SSR markers named CFD (which are D genome specific markers) and provided an improved marker density on the D genome in the maps of Röder et al. (1998) and Pestsova et al. (2000 c) (Table 3.10).

In the work of Samers et al. (2004) the comparison of eight mapping parents are included in a database that records the allele size, excluding the M13 tail, for each parent for each mapped marker.

A comparison was made among the four populations with respect to the differences in parent allele size across all the mapped loci. Genetically, wide crosses such as SO ('Synthetic'/'Opata', 68 RILs) and RD ('RL4452'/'AC Domain', 91 DH lines) with >40% polymorphism had greater differences in parent allele size compared to genetically narrow crosses such as SB ('Superb'/'BW278', 186 DH lines) and WM ('Wuhan'/'Maringa', 93 DH lines) with <40% polymorphism. Over all populations, SSR named WMC had the greatest parent allele size differences (12.2 bp) compared to BARC ones with the lowest parent allele-size difference (4.0 bp) (Table 3.10). Also in our map, most of the WMC markers includes a level of polymorphism above 40%, then this population brings a similarity with the populations of SO, RD, (>45% polymorphism) (Samers et al. 2004). Allele size differences between the SSR markers used in present map with the map of Samers et al. (2004), were similar between the parents of present population with the Synthetic or Opata, even if they are hexaploid wheat.

Table 3.10: Comparison of allele size differences among mapping parents and microsatellite sources. SE Standard error.

Marker source	Population ^a	Allele pair difference(bp)				Marker source (average±SD)	Markers ≤ 4bp (%)
	SO	RD	SB	WM			
WMC	13.9	16.1	10.1	8.7	12.2±3.4		36
BARC	11.8	6.3	8.8	ND ^b	9.0±2.8		34
CFA/CFD	14.2	ND	7.7	ND ^b	11.5±3.8		40
GDM	11.2	10.6	11.8	10.6	10.0±1.6		27
GWM	14.3	5.8	11.8	11.8	10.9±3.6		41
Population (average±SD)	±13.9	±27.1	±11.2	±10.9	11.2±2.2		38
Markers≤4bp(%)	31	42	46	44	38		

4- 2- High conserved order of microsatellite loci and structural changes of Chromosomes

Most of the markers position was in agreement with the one published in other seven studies. In these comparisons, some chromosomes present the inversions between the order of the markers, or between short and long arms. Some markers were new and never previously mapped in *T. durum* and then those regions of the chromosomes carry a low identity with maps of other species studied (Table 3.9; Fig. 3.30, 3.31). In particularly the chromosomal locations and map orders for most of the microsatellite loci in present molecular map of *T. durum* (Table 3.9; Fig. 3.19) are the same as those in *T. aestivum* (Röder et al. 1998 a, b; Somers et al. 2004) and in *T. durum* (Korzun et al. 1999). This result indicates that the macrochromosomal organization is highly conserved among various species in the genus *Triticum*. It also further corroborates that wheat microsatellite markers are mainly genome-specific, and the microsatellite markers developed in hexaploid wheat are easily transferred to other species in the genus (Röder et al. 1998 a, b; Peng et al. 2011).

However, some changes of chromosomal location and map order were also observed for a few microsatellite markers in the present map in comparison with the maps of (Röder et al. 1998 a, b; Somers et al. 2004; Peng et al. 2011). The inconsistency of map order may be explained by the presence of additional loci and structural changes of chromosomes in the wheat genome (Peng et al. 2011), and limitations caused by the population size [70 and 150 recombinant inbred lines in Röder et al. (1998) and Peng et al. (1999 a), respectively, as against 192 F8 individuals in the present study] (Table 3.11). For example, the microsatellite locus Xwmc104 was mapped to 1BL by (Korzun et al. 1999). In contrast, Xwmc104 was mapped to 1BS by Röder et al. (1998) and in the present study mapped to 1AS. Thus, the Xwmc104 locus in Korzun et al. (1999) is different from that in Röder et al. (1998) and in the present study (Fig. 3.30, c).

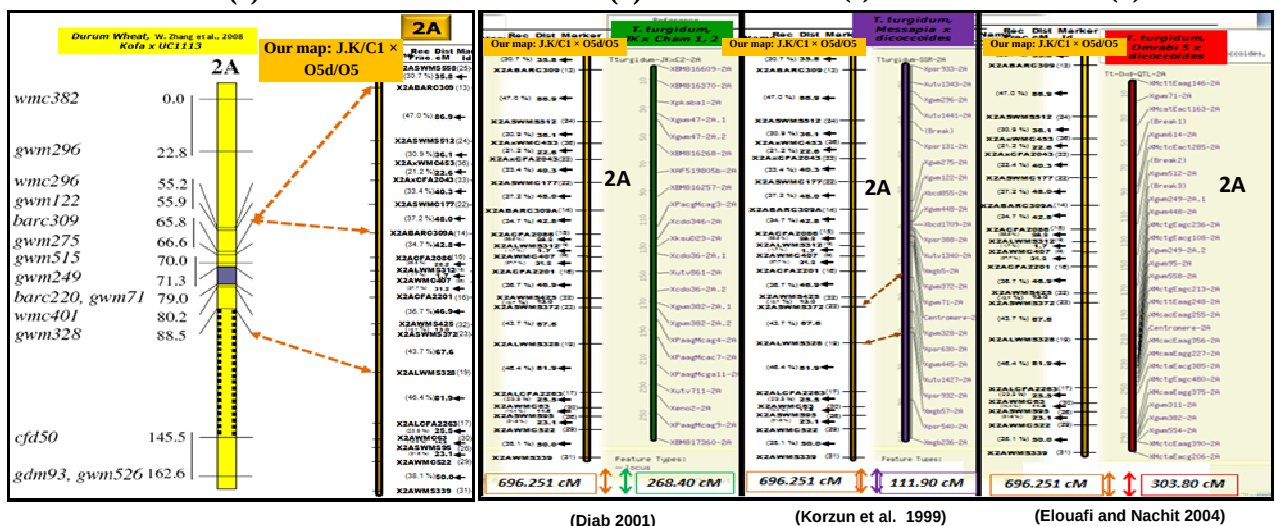
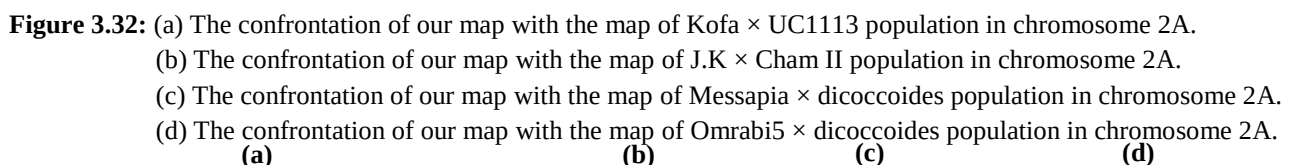
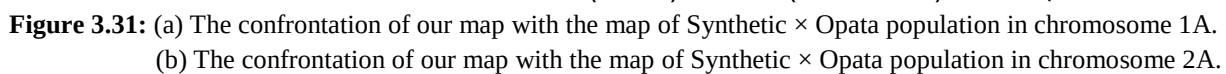
The Xgwm498 was mapped to 1A and 1B by Korzun et al. (1999) and Röder et al. (1998), respectively (Fig. 3.30). So there should be two loci for this microsatellite and these two loci were mapped to chromosome 1A and 1B, respectively. In present study the localization of this marker is in agreement with the work of Röder et al. (1998).

In wheat, paracentric and pericentric inversions have been repeatedly observed on chromosome 4A (Liu et al. 1992; Devos et al. 1995). Peng et al. (2011) observed an inversion within a small segment at the proximal region of *T. dicoccoides* 5B map involving several closely linked markers. Thus, the changes of the map order of Xgwm278, Xcfa2153, Xbarc213, and Xwmc278, genetically near the centromere of chromosome 1A, are possibly due to the inversion of this chromosome segment, but for this we should assume that the inversion is present in both *T. durum*, first parental and *T. durum* second parental.

Joppa et al. (1995) found that a high proportion (70%) of *T. dicoccoides* genotypes had translocations, as was evident in the study of Kawahara and Nevo (1996). Compared with the map of Somers et al. (2004), the changes of chromosomal location of Xgwm304, Xgwm328, Xcfd267, and Xwmc104 may be explained by the translocations of chromosomal segments between 4BS versus 4BL, 2BL versus 2AL, 2AL versus 4BS, and 2AS versus 1AS, respectively.

Non-homoeologous translocations have been repeatedly reported in hexaploid wheat (Liu et al. 1992; Devos et al. 1993) and tetraploid wheat (Blanco et al. 1998). As before said, this translocation may be depends of different reasons (Results, paragraph 2- 4-).

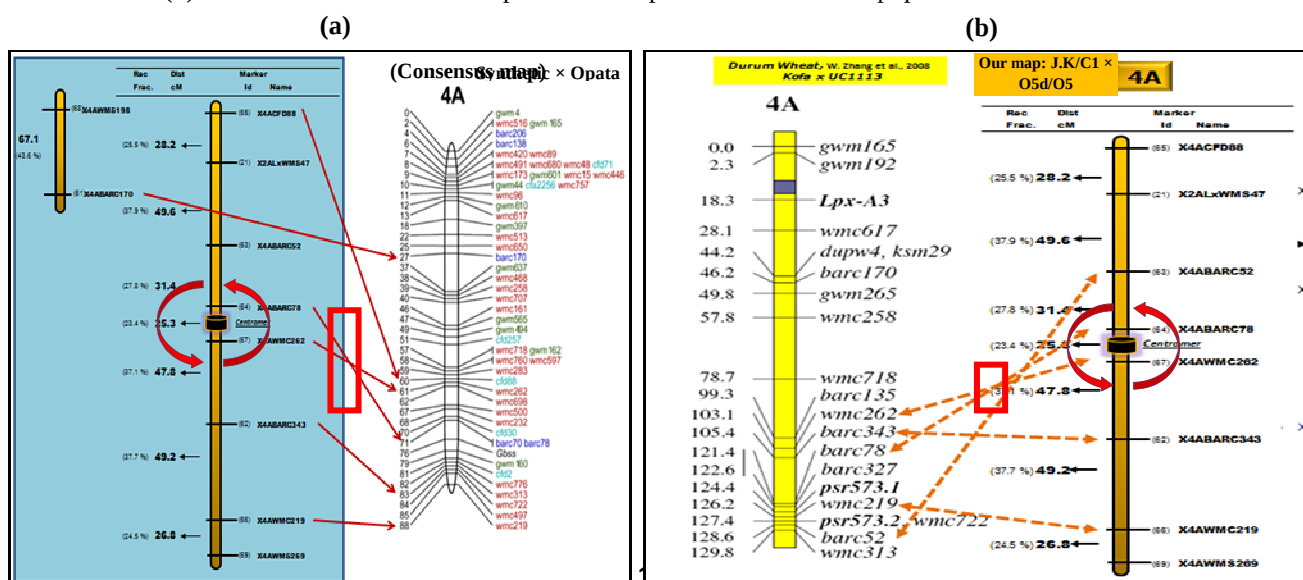
Figure 3.30: (a) The confrontation of our map with the map of Kofa × UC1113 population in chromosome 1A.
 (b) The confrontation of our map with the map of J.K × Cham II population in chromosome 1A.
 (c) The confrontation of our map with the map of Messapia × dicoccoides population in chromosome 1A.
 (d) The confrontation of our map with the map of Omrabi5 × dicoccoides population in chromosome 1A.



Comparing each of the 14 chromosomes with other maps gave us the knowledge to know the correct and precisely positions of the markers on chromosomes. This comparison in most of the cases indicates the inversion of chromosome arms and also the position or region of centromere in different chromosome. This comparing can give much information on the differences in the positions of the markers between the different species of wheat (Van Deynze et al. 1995 a; Peng et al. 2011). For example, there is an inversion from the long arm and short arm in 1A, and so the range of this distance will likely be an artifact that generated from the elaboration of the data with the Map maker program. Finally, could be theoretical problems in relating linkage data order from one map to another, since each map is based on a different set of segregating individuals. However, the use of appropriate computer algorithms can potentially overcome this problem (Qui et al. 1996; Stam 1993).

For Chromosome 2A there is high identity between the markers mapped on our map and those markers, mapped on the reference maps particularly consensus map (85%) (Fig. 3.31, a and b). In chromosome 4A there is a change between the markers, and then an inversion position between microsatellite markers (barc78-wmc262) on the centromeric regions. The distance between these markers probably represents an extreme case of the suppression of recombination in the vicinity of the centromere. An alternative explanation of the absence of recombination in the centromeric region of chromosome 4A is that barc78 in the short arm and wmc262 in the long arm differ by a pericentric inversion (Table 3.11; Figure 3.33, a and b). This alternative explanation seems, however, unlikely because similar genetic distances among markers in the vicinity of the centromere are observed on a map of chromosome 4A in the mapping population Synthetic × Oyata (distance between of barc78-wmc262 was 19.6cM), (Somers et al. 2004) and Kofa × UC1113 (distance between of barc78-wmc262 was 18.3cM), (Zhang et al. 2008).

Figure 3.33: (a) The confrontation of our map with the map of Synthetic × Oyata population in chromosome 4A.
(b) The confrontation of our map with the map of Kofa × UC1113 population in chromosome 4A.



The total length of the maps showed a number almost equal in crosses between the same species. The length of present map is almost identical to the map of Nachit et al. published in 2001, because the both of the map were crossed between *T. durum* × *T. durum*, (3,170.293cM ≈ 3,597.8 cM). This similarity, also exists in the length of the chromosomes and genomes between the these two maps.

Table 3.11: Comparison of map length (cM) of tetraploid wheat genome in various linkage maps generated in different mapping populations, a Mapping populations: (1) durum wheat × wild emmer wheat, (2) durum wheat × durum wheat, (3) bread wheat × bread wheat.

Chromosome	Röder et al.(1998)	Peng et al.(2000)	Nachit et al. (2001)	Paillard et al. (2003)	Blanco et al. (2004)	Elouafi and Nachit (2004)	Quarrie et al.(2005)	Liu et al.(2005)	Akbari et al.(2006)	Peleg et al. (2008)	Iman et al.(2011) Current map
Cross ^a	3	1	2	3	1	1	3	3	3	1	2
Type	RIL	F2	RIL	RIL	RIL	BC1F8	DH	RIL	DH	RIL	RIL
Number	70	150	110	240	65	114	95	118	62	152	192
1A	155.8	152.0/138.6	201.2	65	180.9	147.8	131	166.9	149.3	183.8	214.9
1B	151.8	200.4/200.2	459.4	180.0	162	208.6	167	124.1	119.4	181.5	123
2A	138.3	207.8/250.3	138.8	204.0	318	153.9	170.2	123	73.9	188.8	696.2
2B	180	256.2/257.9	320.8	178.0	260.7	167.3	184	197.6	143.4	182.9	788.8
3A	160.3	267.4/261.7	300.2	103.0	170.2	137.4	158.3	217.3	111.4	118.8	32.1
3B	265.5	250.5/266.1	345.7	182.0	181	239.4	190.1	272.7	199.8	217.2	-
4A	101.6	193.3/192.5	302.6	105.0	236.6	118.6	179.2	167.2	170.6	121.5	258.2
4B	66.0	82.9/104.6	133.9	80.0	200.8	180.1	145.2	83.1	91.6	112.0	140.395
5A	175.1	271.6/274.9	172.3	198.0	266.4	90.0	190.7	138.3	190.7	144.0	46.3
5B	165.6	310.0/276.7	239.5	45.0	253.4	209.4	222.2	213	147.7	209.8	37.2
6A	134.1	180.2/179.4	325.3	192.0	178.6	111.7	151.7	196.2	148.5	161.2	506.8
6B	98.7	237.1/239.2	251.3	50.0	148.8	180.2	125.1	141.9	57.8	175.3	233.4
7A	178.2	317.0/309.8	174.6	242.0	281.3	136.5	167.1	131.9	156.4	138.0	45.201
7B	191.4	234.0/227.8	232	141.0	199.7	156.5	173.8	117.3	179.4	183	47.300
A genome	1,043.4	1,589.3/ 1,607.2	1,615	1,109.0	1,632	895.9	1,148.2	1,140.8	1,000.8	1,056.1	1799.956
B genome	1,119	1,580.1/ 1,572.5	1,982.8	856.0	1,406.4	1,341.5	1,207.4	1,149.7	939.1	1,261	1370.337
Total	2,164	3,169.4/ 3,179.7	3,597.8	1,965.0	3,038.4	2,237.4	2,355.6	2,290.5	1,939.9	2,317.1	3170.293

The present study adds to the repertoire of molecular markers, so far used for the construction of molecular maps in durum wheat. These maps were extensively used for comparative genomics, although all classes of mapped markers were not found to be equally useful for this purpose (Devos and Gale 1997). It is also known that the resolution available in the existing molecular genetic maps in durum wheat is not satisfactory either for map-based cloning or for gene tagging that is required for marker-aided selection. Therefore, there is a need to add more molecular marker loci to the available maps. The present study is an effort in this direction. The results of genetic mapping in the present study were largely in agreement with those of chromosome assignment done using nullisomic-tetrasomic and ditelocentric lines, although this exercise of chromosome assignment

could not be undertaken for all the microsatellite primer pairs used in the present study (only 15 primer pairs could be used for chromosome assignment). For some of the microsatellite markers, the mapping results of the present study involving the ITMI pop were also confirmed either by using another mapping population (at CIMMYT by Khairallah, 2008) or in another independent study using ITMI pop (at Agriculture Canada, in Winnipeg by D. Somers, 2008).

Respect of part (4- 2-), In this case, the microsatellite markers maybe prove very useful for comparative genomics to resolve the conservation of colinearity.

4- 3- Transferability of the markers

The several SSR markers used in the present study were developed from hexaploid wheat (WMS and SWM), A genome of *T. urartu* (CFA), and the D genome of *Ae. Tauschii* (CFD, GDM). These markers helps us to know about our population the percentage of genomes transfer. *T. urartu* genome, that contributed A genome of hexaploid wheat (Dvorak et al. 1988), shows considerable differences from the A genome of *T. monococcum* or *T. boeoticum* (Johnson and Dhaliwal, 1976). About 65–70% of SSRs markers developed from hexaploid wheat and diploid *Ae. Tauschi*, showed amplification in *T. durum* from Jennah Ketifa/ChamI and *T. durum* Omrabi5/ *dicoccoides*.

A high level of conservation exists between A and B genomes of tetraploid wheat. As many as 46.3% of the D genome, 32% of the B genome specific, 8% of the A genome specific SSR markers showed amplification in our population. In agreement with the work of Bai et al. (2004) the SSR markers isolated from hexaploid wheat are transferable for about 67%, in *T. monococcum*.

Only about 50% of markers is transferable from the A genome of hexaploid wheat to tetraploid, A and B genome. The majority of the markers that did not show transferability in the study of Bai et al. (2004) did not show transferability in the present study as well, with few exceptions like GWM136, GWM614 and GWM154 that did show amplification either in *T. monococcum*. On the other hand, Sourdille et al. (2001) reported transferability of 93% of the SSR markers derived from hexaploid wheat on the corresponding ancestral A genome of diploid species. However, they used only 12 A genome specific primers, which is much less a number than used in the present study. Guyomarc'h et al. (2002 b) reported about 50% the transferability of *Ae. tauschii* derived SSR markers in the A genome of diploid species. Genomic relationship among A, B and D genomes of hexaploid wheat has been established by studying chromosome pairing in hexaploids having the recessive mutant allele ph1b and nullihaploids that lack chromosome 5B (Jauhar et al. 1991).

They found (Jauhar et al. 1991) that A and D genomes showed 80% association against only 11.5% between A and B and 7% between B and D. Thus, A and D genomes are much more closely related to each other than either one of the two is to the B genome. Evolutionary relationships between A,

B and D genomes of wheat and its progenitor species have been studied by gene comparisons as well (Huang et al. 2002; Petersen et al. 2006) (Fig. 3.34).

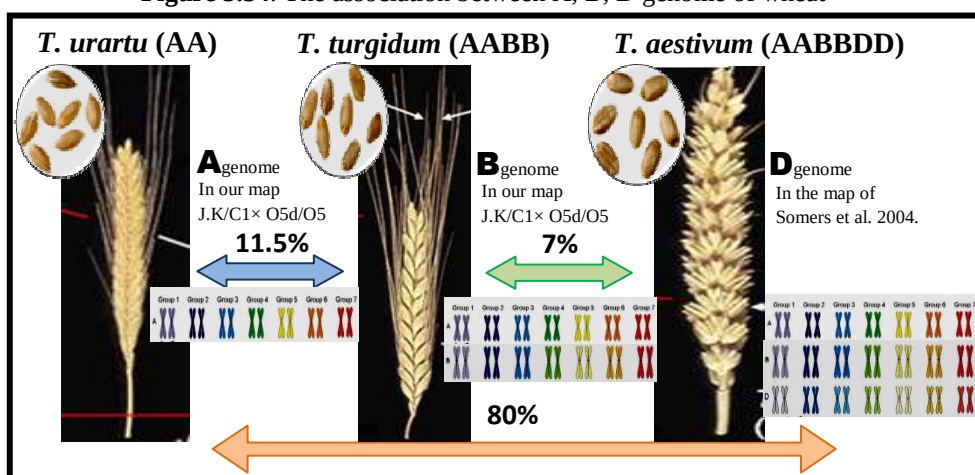
Based on genes sequence homology, it was concluded that the A and D genomes are more closely related than is the S genome of *Sitopsis* section or the B genome of tetraploid and hexaploid cultivated and wild wheats. Thus, SSR markers derived from *Aegilops* species of *Sitopsis* section (excluding *Ae. speltoides*) should also show high transferability to A genome diploid wheat (Petersen et al. 2006).

Triticum durum and *T. monococcum* harbour desirable variability, which may meet some of the future requirements for wheat improvement.

Although relatively high-density linkage maps are now available in hexaploid wheat (Sourdille et al. 2003; Somers et al. 2004; Quarrie et al. 2005; Torada et al. 2006), gene cloning in tetraploid wheat is still complex due to the polyploid genome, large genome size and limited polymorphism. A and D genome of diploid species could be used for cloning orthologous genes from tetraploid and hexaploid wheat.

T. monococcum is easily cultivated in the field, also *T. durum* compared to *Ae. tauschii*, which is extremely difficult to handle in the field due to shattering and hard threshing. So those species of diploid and tetraploid wheat have a potential for becoming a model *Triticeae* species for understanding wheat genomics and cloning of genes because of its relatively small genome size in *T. monococcum* (Lijavetzky et al. 1999).

Figure 3.34: The association between A, B, D genome of wheat



4- 4- Parallel mapping in related taxa

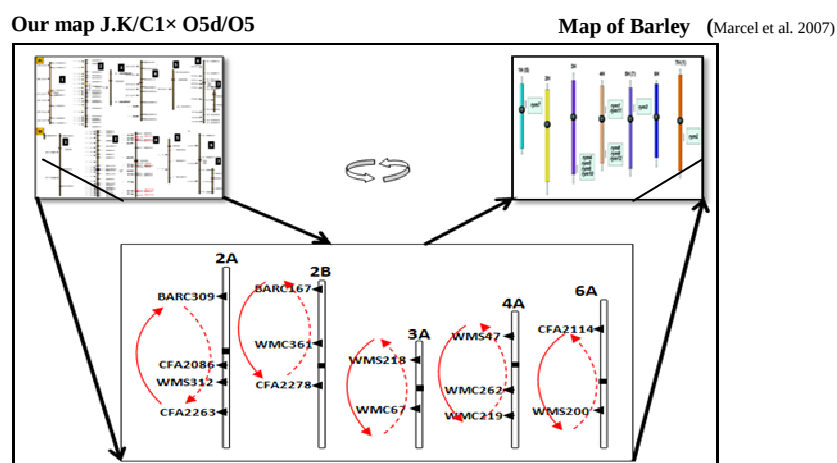
One of the most powerful aspects of genetic mapping with DNA markers is the fact that markers mapped in one genus or species can often be used to construct parallel maps in related, but genetically incompatible, taxa. For this reason, a new mapping project can often build on previous mapping work in related organisms. Examples include a barley map constructed with wheat

markers (Roder et al. 1998; Somers et al. 2004; Rostoks et al. 2005), a potato map constructed with tomato markers (Bonierbale et al. 1988; Gebhardt et al. 1991; Tanksley et al. 1993), sorghum maps constructed with maize markers (Hulbert et al. 1990; Pereira et al. 1994), a turnip map constructed with markers from cabbage (McGrath and Quiros 1991), and a mungbean map constructed with markers from both soybean and common bean (Menancio-Hautea et al. 1993).

Not only does a pre-existing map provide a set of previously tested DNA markers, it also gives an indication of linkage groups and marker order. Comparing A genome of our map with the 7 chromosomes of barley map (Rostoks et al. 2005), only five paracentric of inversions involving complete chromosome arms was detected (Stein et al. 2007; Marcel TC et al. 2007) (Fig. 3.35). But changed also the distances between them, as also found among most of the grasses (Bennetzen and Freeling 1993) and legumes (Boutin et al. 1995).

In cases like the present markers can be added to a new map in an optimum manner, either by focusing on markers evenly distributed throughout the genome, or by targeting specific regions of interest (Concibido et al. 1996). Even though the wheat and barley maps are nearly homosequential (synthetic) in marker order, both differ significantly from the linkage map of maize, despite the fact that some were constructed with the same SSR markers (Prince et al. 1993).

Figure 3.35: The comparing of our map with the map of Barley, the differences in some inversion in some chromosome.



5- IMPORTANT TRAITS

5- 1- The explanation of the traits and characters

In most cases, genome mapping is directed toward a comprehensive genetic map covering all chromosomes evenly. This is essential for effective marker-assisted breeding, QTL mapping, and chromosome characterization. However, there are special situations in which specific regions of the genome hold special interest. One example is the primary goal of a research project based on cloning specific gene. In this case, markers that are very close to a target gene and suitable as starting points for chromosome walking are needed, so the goal is to generate a high density linkage map around that gene as quickly as possible (Young 2000).

This introgressed region, often highly polymorphic at the DNA sequence level, provides a target for rapidly identifying clones located near the gene of interest (Muehlbauer et al. 1991).

The rapid advance in molecular marker and linkage mapping technologies exponentially increases the number of marker loci assigned to genetic maps. Dense genetic maps are a very useful tool in the identification of molecular markers closely linked to genes or QTLs of interest, isolation of genes via map based cloning, comparative mapping, and genome organization studies (Varshney et al. 2007 a, b).

The genetic linkage map presented in the current study is the first published map between two different RIL from durum wheat population. This genetic map spans over 3170.3 cM, with each chromosome represented by one linkage group. The two parental lines were found polymorphic in various morphophysiological traits, such as Length, Germplasm color, Grain protein content (Landi 2004).

Breeding new varieties of wheat with improved end uses quality is a key aim of wheat breeding programs. The end-use quality of wheat is dependent on a large complex of genes that are greatly influenced by environment conditions. Grain protein content (GPC), wet gluten content (WGC), kernel hardness (KH), water absorption (Abs) and dough stability time (DST) are all important grain quality traits in durum wheat.

5- 2- Explanation of the traits associated with markers

5- 2- 1- The explanation of Traits - Grain Size and Grain Shape

TGW is highly positively correlated with grain area, width, and FFD in all populations and years ($r \geq 0.75$, $P < 0.001$) and moderately correlated with grain length ($r \geq 0.23$, $P < 0.001$). Interestingly, the L/W ratio shows no significant or a very weak correlation, with either of the two main grain size variables (TGW and grain area), suggesting that the relative proportions of the main

growth axes of the grain, which largely describe grain shape, is independent of grain size (Gegas et al. 2010).

In the work of Gegas et al. (2010) a principal component analysis (PCA) was performed to identify the major sources of variation in the morphometric data sets of the length traits in DH population. PCA does that by identifying orthogonal directions, namely principal components (PCs), along which the trait variance is maximal (Jolliffe 2002). The substantive importance of a given variable for a given factor can be gauged by the relative weight of the component loadings (Field 2005). In study of (Gegas et al. 2010), only variables with loading values of > 0.4 were considered important and therefore used for interpretation following the criteria proposed by Stevens (2009) that take into account both the sample size and the percentage of shared variance between the variable and the component (Stevens 2009). In the work of Stevens et al. (2009) two significant PCs, PC1 and PC2, were extracted for each DH population that explain 90.9% of the variation apparent in these populations. Both PCs showed analogous organization in all populations used in their work (six), with PC1 (55.6 to 67.1%) and PC2 (23.8 to 30.1%) explaining primarily variation in grain size and grain shape, respectively. Furthermore, PCA on a population-identified two PCs, comparable to the ones identified for the individual DH and RIL populations, each of which explained 68.7 and 23.3% of the variation, respectively.

Therefore, PC1 describes grain size differences, where a proportional increase along both the longitudinal (length) and proximodistal (width) axes positively associates with an increase in grain area and subsequently grain weight. On the other hand, PC2 captures primarily grain shape differences with L/W ratio and grain length being the main explanatory factors. The phenotypic model for the grain size and shape parameters suggests that these two traits are probably under the control of distinct genetic components.

Quantitative Trait Loci (QTL) analysis was performed on the DH population (Gegas et al. 2010). The strong positive correlations between the grain size variables (i.e., TGW, area, width, and FFD) and between the grain shape variables (i.e., L/W and length) can be attributed to cosegregating QTL with the same allelic effect. Indeed, in the study of (Gegas et al. 2010) QTL for the grain size variables cosegregated consistently in all populations and years. The same holds true for the QTL for grain length and L/W.

Gegas et al. (2010) used high-throughput morphometric analysis to quantify grain size and shape variation and determine its underlying genetic basis in an extensive collection that included DH mapping populations, ancestral wheat species, and commercial varieties.

Quantitative analyses of the morphometric data revealed that grain size and shape are largely independent traits. This is unlikely to be the result of artificial selection during breeding since size

and shape are also independent variables in primitive wheat. At the developmental level, this phenomenon may reflect differential modulation in growth (or growth arrest) along the main axes of the grain at different developmental stages. In tomato (*Solanum lycopersicum*), for example, loci that affect fruit shape have been identified, but not for the fruit size and vice versa (Frary et al. 2000; Tanksley 2004). Specifically, the *fw2* gene, that probability will present in our map, for associated with some markers, was shown to be a major determinant for fruit size but not shape variation (Frary et al. 2000), whereas the *ovate* (Liu et al. 2002).

The notion that certain developmental constraints during fruit or grain growth could lead to morphological changes is further corroborated by recent studies on grain size/shape genes in wheat (Fan et al. 2006; Song et al. 2007; Shomura et al. 2008; Takano-Kai et al. 2009).

The *GS3* locus was found to have major effects on grain length and weight and smaller effects on grain width (Fan et al. 2006), and the longer grains can be attributed to relaxed constraints during grain elongation (Takano-Kai et al. 2009).

The *GW2* gene was shown to alter grain width and weight and to lesser extend grain length owing to changes in the width of the spikelet hull (Song et al. 2007).

Several QTL for grain weight and length (Thomson et al. 2003; Li et al. 2004) were reported on durum wheat chromosome 3 and correspond to QTL and markers for related traits identified in this study. Specifically, wheat QTL for grain weight correspond to *TGW* and grain width QTL on the synthetic regions of wheat chromosomes 5A and 6A. The *GS3* genes effect on grain size is attributed primarily to alterations in grain length and less so in width (Fan et al. 2006). Is been verified with one QTL located on the chromosome 4B. So the chromosome 4B is chromosome that is referring to this character.

A total of 25 QTL for PC1 and PC2 were identified in the mapping populations (Gegas et al. 2010) with LOD scores ranging between 2.9 and 10.6. The majority of the QTL identified are located on five chromosomes, 1A, 3A, 4B, 5A, and 6A.

The three QTLs for PC2 were detected on chromosome 1A, each of which explained 29.2, 11.3, and 18.5% of the variation in grain shape.

Meta-analysis identified two QTLs at close proximity to each other, MQTL1 between markers *psp3027* and *wPt5374* and MQTL2 between *Wmc24* and *Wmc275* on the consensus map for 1A.

In present map, were used some markers in common that carry effects on QTL1 and QTL2 for PC2 located in same chromosome, as *psp3027*, *Wmc275* and *Wmc24*. Unfortunately the *psp3027* marker in our two relatives was monomorphic, but the other two polymorphic.

Other QTLs for PC2 were detected on chromosome 3A populations around markers *barc19* and *wmc264*. Always being in common with the markers used on the same chromosome in present map (Fig. 3.36).

While the QTLs for PC1 was identified around of marker *gwm2*, and revealed one other meta-QTL that spanned the interval *s635ACAG*-*barc19*.

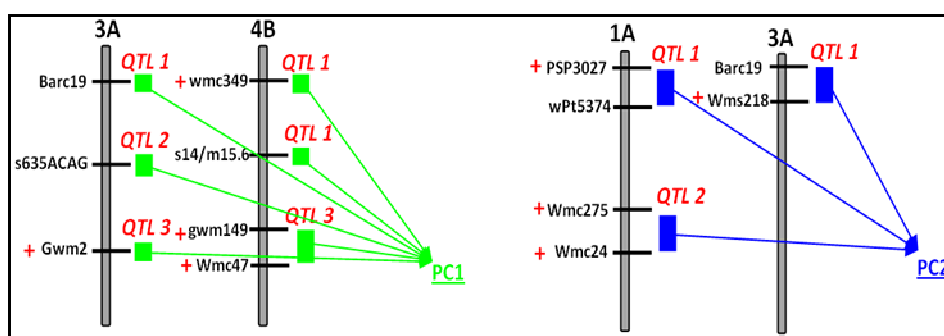
Two QTLs for PC1 were detected in DH populations on chromosome 4B (Gegas et al. 2010), around markers *s14/m15.6* and *wmc349*. Another meta-QTL was identified between the markers *Gwm149* and *Wmc47* on the wheat map of Gegas et al. (2010). And also the markers *Wmc47*, and *Gwm149* *Gwm2* are present in our map (Fig. 3.36).

The QTL for grain size and shape on chromosome 3A identified in this analysis are of particular interest since their mapping intervals coincide with the approximate position of the *sphaerococcum* locus (Salina et al. 2000). The *sphaerococcoid* mutation reduces grain length, thus significantly altering grain shape and size. The three genes S1, S2, and S3 of the *sphaerococcoid* mutation were mapped on chromosomes 3D, 3B, and 3A, respectively (Salina et al. 2000). The S3 locus was shown to be located between the centromeric markers *gwm2* and *gwm720* on 3A (Salina et al. 2000). This position corresponds to the mapping interval (highest LOD scores) of QTL for grain length and L/W and grain width in the population the work of (Gegas et al. 2010).

The distribution of these markers has shown a majority of percentage (56%-62%) to the first parental the only exception of the marker *Wms218* that with 48% was to the second parental. These dates bring the results that in present study the first parental contained the traits of grain size and grain shape, most probably the first parental could be useful in plant breeding for these both characters (Figs. 3.8, 3.10).

Figure 3.36:

The association of some markers with PPO activity trait in several chromosomes (+ present in our map).



5- 2- 2- The explanation of Trait - PPO (Poly Phenol Oxidases) Pigment Color

Poly phenol oxidases catalyze the oxidation of endogenous phenolic acids, and produce short-chain polymers that decrease the whiteness of noodles made from flours of many bread wheat and durum wheat varieties. This darkening process in noodles is associated with PPO activity and its

corresponding substrates (Miskelly 1984; Kruger et al. 1992; Baik et al. 1995). A major genetic effect on PPO activity has been reported to be located on chromosomes 2A and 2D in hexaploid wheat (Zeven 1972; Wrigley and McIntosh 1975; Souza et al. 1998; Jimenez and Dubcovsky 1999; Raman et al. 2005; Sun et al. 2005).

A major locus responsible for high PPO activity was mapped on the long arm of chromosome 2A, by using a set of RI lines derived from a cross between the cultivars Jennah Khetifa and Cham 1. Wrigley and McIntosh (1975) found that the gene influencing the phenol color reaction of grain was located on the long arm of chromosome 2A. In tetraploid wheat, Jimenez and Dubcovsky (1999) also found that the gene affecting PPO activity was located on the long arm of chromosome 2A. Nair and Tomar (2001) found that the genes conferring the phenol color reaction in grain and in glumes were not linked, and at least 2 genes determined the phenol color reaction of grains in tetraploid wheat.

Watanabe et al. (2004) mapped the genes (Tc1 and Tc2) responsible for the high phenol color reaction of kernels on the long arms of chromosomes 2A and 2B. The map distances were estimated to be 46.8 cM for Tc1 and 40.7 cM for Tc2 from the centromere in durum wheat.

In present work we have identified the markers which could facilitate selection of genotypes with lower PPO activity in durum wheat. Reducing PPO activity in varieties having other high-quality attributes is highly desirable to maintain fresh quality.

PPO activity is measured by spectrophotometric absorbance at 405 nm; it was 0.185 ± 0.01 for Cham 1 and 0.302 ± 0.02 for Jennah Khetifa. PPO activity of lines in the RI population ranged from 0.15 to 0.4. The mean value of the population was 0.239 ± 0.005 .

In the work of Watanabe et al. (2006) the chromosome segment exceeded LOD = 3 for chromosome 2A. The stepwise multiple regression analysis revealed that these loci were responsible for 49.1% of the variation in PPO activity among RI lines. The loci for PPO activity were significantly associated with the SSR marker Xgwm312 (wms312), which has been mapped on the long arm of chromosome 2A (Nachit et al. 2001). It indicated that the loci for PPO activity linked with Xgwm312 can be treated as a major gene. Many researchers have found that genes for PPO activity were located in homeologous group 2 play a major role in PPO activity in wheat.

Sun et al. (2005) developed co-dominant STS markers for PPO activity associated with SSR markers, Xgwm312 (wms312), Xgwm294 (wms294) and Xcfa2086 (cfa2086) in durum and bread wheat. These molecular markers could be used for a faster and easier selection of plants during breeding for low PPO activity.

Substitution lines of chromosome 2A of the hexaploid varieties Cheyenne, Thatcher and Timstein in Chinese Spring showed significantly higher PPO activity than all other substitution lines. Moreover,

substitution lines of chromosome 2A of *Triticum turgidum* var. *dicoccoides* and of chromosome 2D of Chinese Spring in the durum wheat variety Langdon showed a significant increase in PPO activity (Jimenez and Dubcovsky 1999; Watanabe et al. 2004). Jimenez and Dubcovsky (1999) found that the gene(s) responsible for high PPO activity in chromosome 2D from Chinese Spring on the long arm was within a deletion that represents 24% of the distal part of the arm.

However, also Li et al. (1999) found loci for PPO activity on chromosomes 5B and 7D and on chromosomes of homeologous group 6 by using nullisomic-tetrasomic lines of *T. durum*, Chinese Spring and a PPO maize probe.

The identification of a molecular marker (Xgwm312) linked to high PPO activity has the potential to accelerate selection for reduced pasta darkening by carrying out negative selection for this trait in durum wheat breeding for the WANA region in Syria (Simeone et al. 2002; Sun et al. 2005).

In present work wms312 marker is located on chromosome 2A, the major locus responsible for high PPO activity was mapped on the long arm of chromosome 2A. Also the one other marker that included the PPO activity, Xcfa2086 that was located with a distance of 28.2 cM to wms312 (Fig. 3.37; Table 3.12).

The distribution of Wms312 and Cfa2086 markers (present in our map for this character) has shown a majority of percentage (49% and 68%) to the first parental (J.K×CI/ O5d×O5), that indicates the first parental contained the traits of grain size and grain shape, most probably will be useful the first parental in plant breeding for these both character (Figs. 3.8, 3.10).

Figure 3.37: The association of some markers with PPO activity trait (+ present in our map).

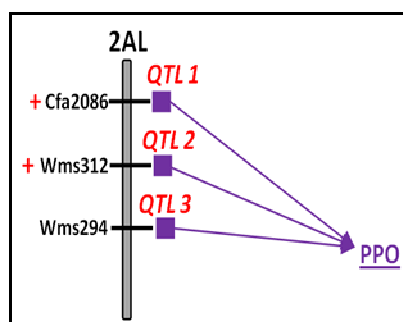
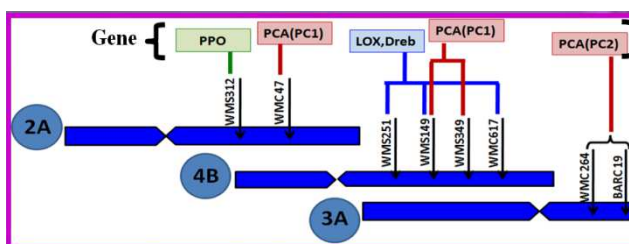


Figure 3.38: The associations of markers with QTL for gene control.



5- 2- 2- 2- Quality of Pasta derivatives from durum wheat

The qualitative factors of Pasta can be briefly summarized in 5 points: (1) the type of place of origin of the durum wheat from which the flour is produced; (2) the characteristics of the flour; (3) the manufacturing processes of kneading, drawing and drying, and (4) possible added ingredients.

Countries of pasta makers, as Italy, are very strict about its production procedure and follows stringent methods. The law of 1967 clearly states that and use of common wheat flour in place of

durum wheat flour in pasta is a fraudulent act. So, manufacturing of pasta, especially dry pasta, with common wheat is considered to be against law. The standard measure for pasta mixture is flour with 30% water. To maintain the protein structure and tightness of the dough, the flour must contain particles of uniform dimensions.

When raw, good quality dry pasta must have the following characteristics:

- 1- It must have a uniformly smooth appearance and texture.
- 2- No spots or dark shades must be visible when light shines through it.
- 3- It must have a clear and unmistakable amber yellow color.
- 4- It must be odorless.
- 5- it must taste slightly sweet.
- 6- when broken it must make a dry sound and the fracture must appear smooth and glassy with no air bubbles.

After the balanced proportion of water and flour, then the two major components of pasta, starch and gluten comes. They play a big role in maintaining the proportion and consistency of the mixture.

Starch and gluten comes to play during cooking, as the pasta takes a particular shape and texture depending on them. Starch defines the component of carbohydrate found at 60-70% in the wheat grain. In raw pasta, starch is found in the granules. On the other hand, the gluten is a viscous substance resembling the Latin gluten = glue. These are not really wheat-based components but it is formed through the interaction of two proteins, gliadin and glutelin when these are hydrated.

These appear in dough of pasta when water is added to the flour and kneaded thoroughly. This gluten blends with granules of starch and form a consistent and regular arrangement. While cooking, these two ingredients play completely different roles. The starch granule absorbs water and rapidly swells up until it breaks and frees its content in the water. The two gluten proteins on the other hand coagulate forming a very compact lattice those envelopes the starch granules and try to hold them as much as it can.

In poorly prepared pasta, the starch out balances the protein element and the pasta turn out to be a rigid piece of wheat with whitish water. Pasta that is prepared with well balance of the elements is a soft and spongy one as the gluten has managed to stop the starch from absorbing water. Thus the internal balance, nutritional value and taste of pasta are preserved.

Since durum wheat is mainly used for pasta, the varieties that meet the requirements of high-quality pasta products receive premium prices in the global market. These requirements include bright yellow color, high protein content and pasta firmness, and small cooking loss (for a review see Troccoli et al. 2000). Pasta color is determined by grain carotenoid content and carotenoid

degradation by lipoxygenases during pasta processing (Troccoli et al. 2000). The main carotenoid pigment in the durum grain is lutein, which contributes to both pasta quality and nutritional value (Hentschel et al. 2002).

Yellow pigment is mainly controlled by additive gene effects and has high heritability (Clarke et al. 2006; Clarke et al. 2000; Elouafi et al. 2001). Equally important parameters for pasta quality are pasta firmness and cooking loss, which are associated with grain protein content (GPC) and gluten strength (Sissons et al. 2005).

5- 2- 3- The explanation of Trait - LOX

The bright yellow color is an important parameter involved in the definition of pasta quality and is, therefore, a targeted for durum wheat breeding programs. This character depends on i) carotenoid accumulation in the endosperm, due to carotenoid biosynthesis during caryopsis development, mainly regulated by the rate-limiting Phytoene synthase (PSY) reaction, ii) carotenoid loss during pasta processing, due to pigment oxidation catalysed by *Lipoxygenase* (LOX). genetic variability of LOX activity and Lpx genes. As far as carotenoid accumulation in the endosperm, modern cultivars showed significantly higher values of YPC compared to old cultivars and wild ssp. *dicoccum* and ssp. *dicoccoides* accessions (Trono et al. 2010). Total carotenoid concentration varied between 1.18 and 4.42 mg/g with an average of 2.46 mg/g. Lutein was the main component of carotenoids, followed by zeaxanthin and beta-carotene, whereas alfa-carotene and beta-cryptoxanthin were minor components.

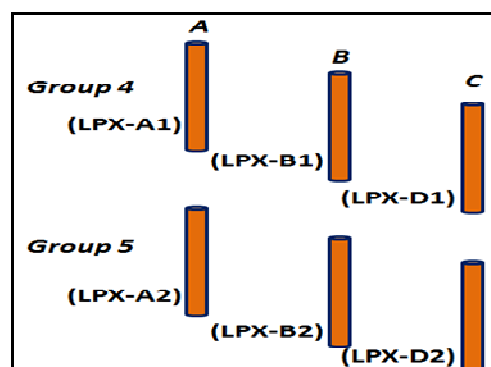
In the study of Trono et al. (2010) the construct linkage map and used 150 SSR markers for subsequent QTL analysis. Five QTLs were identified on chromosomes 2A, 3B, 5A and 7A in the Latino × Primadur population, accounting for a large proportion of the phenotypic variation for YPC and Yellow Index (YI). The locus encoding Psy1 was co-segregating with the QTL on 7AL, thus confirming that allelic differences at Psy1 are associated with differences in YPC.

As far as carotenoid degradation during pasta processing, LOX activity showed a large variability in our collection (from 0.02 and to 7.91 EU/g dw) in their work (Trono et al. 2010) with old cultivars showing significantly higher activity values compared to modern ones. The expression analysis carried out on four genotypes contrasting for LOX activity showed that the Lpx genes, namely Lpx-1, Lpx-2 and Lpx-3, were differently expressed during seed maturation with the Lpx-1 transcripts being the most abundant in mature grains (Yoneyama et al. 2003).

LOX is regulated by three genes, Lox A, Lox B, Lox C. The wheat LOX isoanzymes were assigned to chromosomes 4A (LPX-A1), 4B (LPX-B1), 4D (LPX-D1), 5A (LPX-A2), 5B (LPX-B2), and 5D (LPX-D2) (Fig. 3.39).

For these markers located in the group of five, the distortion should have been in favour of *T. durum*, Jennah Ketifa/ Cham1 alleles, because in the field of *T. durum* (first parental), The bright yellow color in the final product (Pasta) is higher compared to the *T. durum*, Omrabi5/ *T. dicoccoides*600545// Omrabi5 (Second parental).

Figure 3.39: The distribution of Lox gene and LPX locus in the group homoelogous of 4 and 5.

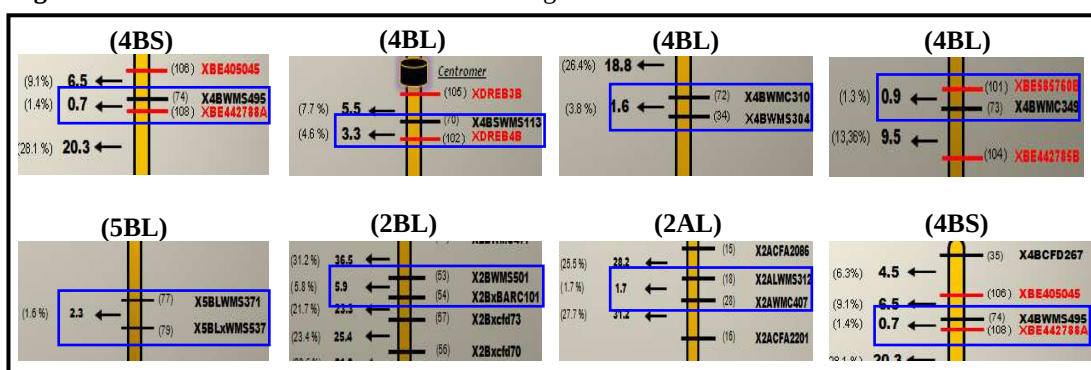


In our study there were associations between Wmc617, Wms251, Wms149 and Wmc349 markers (SSR markers) and the Lpx-B1 locus, located on the short and long arm of chromosome 4B. Between these four microsatellite markers also existed a proximity of the marker Wms349 with the BE585760B (SNP marker), this short distance between these two markers (0.9 cM) verifies that the BE585760B marker, will be associated with the LOX trait (Yoneyama et al. 2003).

This closeness between the two markers was also present in other chromosomes for example: there is a short distance between Wms312 and Wmc407 (1.7 cM) on the long arm of chromosome 2A, Wms312 includes an association with the trait of grain color (PPO) and most probably Wmc407 will be associated with the traits of grain color (PPO). We identified this relationship between different markers with short distance and the association of them with the traits, in our map.

As between the Wms304 and wmc310 markers (1.6 cM) on the chromosome 4B for trait of longitudinal (length). Between the Wms371 and Wms537 (2.3 cM) on the chromosome 5BL for trait of Stripe rust resistance (Feng 2010). Between the Wms495 and BE442788A (0.7 cM) on the chromosome 4BS for trait of Pasta color (Lox). Between the Wms113 and DREB4B (3.3 cM) on the chromosome 4BL for trait of Pasta color (Lox). Between the Wms501 and Barc101 (5.9 cM) on the chromosome 2BL for trait of grain color (Nachit et al. 2001).

Figure 3.40: The association of markers including a short distance with the different traits.



5- 2- 4- The explanation of Traits - GYPC, SC, PC

Yellow pigment (GYPC), Semolina Color (SC), and Pasta Color (PC) are the most important factors determining the end-use quality of wheat for pasta-making (Jun Li et al. 2011). In the work of Zhang et al. (2008) Yellow pigment (GYPC), semolina color (SC) and pasta color (PC) traits were highly correlated. All three color parameters showed a significant ($P < 0.001$) negative correlation with TKW (GYPC $R = -0.28$, SC $R = -0.22$, PC $R = -0.17$) and TWT (GYPC $R = -0.24$, SC $R = -0.28$, PC $R = -0.25$) suggesting that differences in grain size and shape might have affected grain pigment concentration in this segregating population.

They identified four major overlapping QTL for GYPC, SC, and PC on chromosomes 1B, 6AL, 7AL and 7BL and a smaller one with a larger effect on pasta color on chromosomes 4B. The QTL peaks for GYPC, SC and PC on chromosome 6A were all mapped between BARC113 and GWM570. This QTL showed LOD scores above the 2.5 threshold. These two markers are located on chromosome 6A also on present map, with this difference that in the parental screening, BARC113 was monomorphic and GWM570 was polymorphic and associated to these three traits.

The overlapping QTL for GYPC, SC and PC on chromosomes 7A and 7B were both located on the telomeric region of the long arm. In chromosome 7B for GYPC between WMC311 and CFA2257, for SC between WMC276 and GWM146, for PC between BARC1073 and GWM146. The marker WMC311 in present map, is polymorphic and localized on the long arm of chromosome 2A. This difference in position, to be located in another chromosome, indicates that the PC trait in our map will be able to another QTL configuration on the long arm of chromosome 2A.

A more variable QTL was detected for GYPC and SC on chromosome 1B, between markers WMC626 and BARC302.

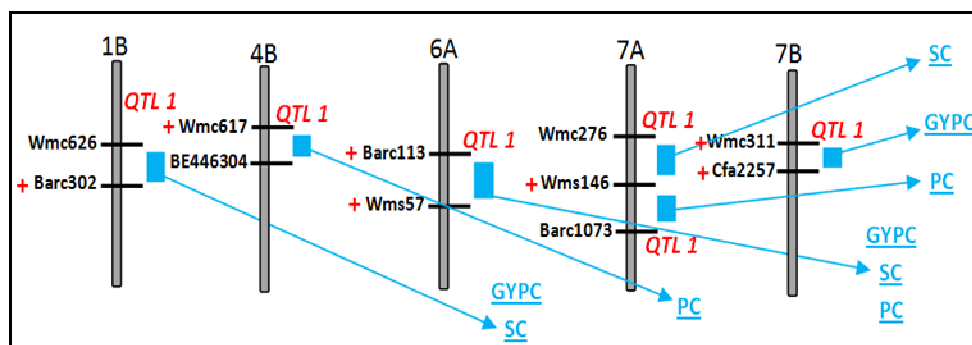
Unfortunately both of these markers are monomorphic in present parental. The QTL on chromosome arm 4BS showed a significant peak at the *Lpx-B1* locus for PC but not for YP or SC. This peak was positioned between WMC125 and BE446304. That in our map, WMC125 was located in on the same chromosome (4BS), also was polymorphic between parental.

The second Parental showed higher GYPC, SC and PC values than first Parental ($P < 0.01$), in the study of Zhang et al. (2008) contributed for improved GYPC, SC and PC and identify the QTL located on chromosomes 1B, 6A, and 7B. In present results the same markers were associated with these characters and localized in same chromosome 1B, 6A, but instead of 7B there was 4A. These characteristics of quality pasta for GYPC, SC and PC, had association with WMS570, WMC276 and WMC617, located on chromosome 6AL, 7AL and 4BS, respectively.

Granule-bound starch synthase, as the waxy protein, catalyses the synthesis of amylose in wheat endosperm starch. In durum wheats, the genes encoding GBSS are present at the two fix loci on chromosome 7A and 4A.

Figure 3.41:

The association of some markers with GPC trait in several chromosomes (+ present in our map).



5- 2- 5- The explanation of Trait - GPC

Grain protein content (GPC) is an important factor determining the end-use quality of wheat for pasta-making (Li et al. 2011). It is well known that WGC, Abs, DDT and DST are also important quality traits strongly correlated with GPC (Matsuo et al. 1982; Autran et al. 1996). Moreover, it determines nutritional value and quality. The concentration and the quality of grain protein are the two major characteristics determining the quality of pasta, the major end-product made from durum wheat. Several studies have demonstrated a strong relationship between GPC and pasta cooking quality, better cooking firmness and tolerance to overcooking (Matsuo et al. 1972, 1982; Wasik 1975; Autran et al. 1996; D'Egidio et al. 1990; Feillet and Dexter 1996).

The amount of wheat protein (GPC) can be divided into four groups, Hard 1 (minimum protein 13.0%), Hard 2 (minimum protein 11.5%), Premium White (minimum protein 10.5%) and Standard White (No minimum protein).

Many recent reports (Joppa and Cantrell 1990; Sourdille et al. 1999; Zanetti et al. 1999; Perretant et al. 2000; Blanco et al. 1996, 2002, 2006) concluded that genetic factors impacting GPC in both cultivars and wild wheat were distributed over all 14 wheat chromosomes.

Chee et al. (2001) detected a high grain protein QTL, QGpc.ndsu.6Bb, from *Triticum turgidum* L. var. *dicoccoides*, and deduced that the high GPC locus was insensitive to environmental conditions. The hardness (Ha) locus on chromosome 5D is the main determinant of grain texture in hexaploid wheat, and this locus also controlled the production of puroindoline proteins (Morris 2002).

Recently, Chantret et al. (2004, 2005) sequenced the Ha locus. The recent emphasis on end-use quality has increased the economic value of these traits (Dohlman and Hoffman 2000).

One approach to increase the GPC is to utilize the high grain protein alleles from wild relatives of wheat. Wild emmer (*Triticum turgidum* ssp. *dicoccoides*) is a potential source of high GPC alleles (Avivi 1978). This wild emmer was also, one of our first parents’.

Recombinant substitution lines of *T. dicoccoides* FA15-3 chromosome 6B in the cultivar ‘Langdon’ (*Triticum turgidum* var *durum*, LDN hereafter) showed that a QTL for high GPC was present between SSR marker Xbarc354 and RFLP marker Xpsr113 on chromosome arm 6BS (Joppa et al. 1997). These markers were mapped also on rice chromosome 2 and therefore define a 30 cM collinear region between the two species (Fig. 3.42).

Distelfeld et al. in (2004) used information from the rice genomic sequence to saturate with markers the wheat chromosome 6B region of the QTL for high grain protein content.

In their work approximately 40 pairs of EST specific primers were designed, from 20 PCR products and used as probes, only eleven of these probes showed polymorphism between the parental lines and were further used for mapping. The conservation of gene order (micro-collinearity) between wheat and rice in the GPC region was well established.

The GPC gene was mapped into a 0.9-cM interval defined by flanking loci Xucw70 and Xucw73. The wheat region between Xucw70 and Xucw73 corresponds to a 100-Kb segment in rice located, in this identify region, and on the 6B chromosome of wheat also were presented SSR markers Xgwm219 (WMS219) and Xwmc397 (WMC397) associated with GPC locus (Fig. 3.42).

Also Joppa et al. (1997) on ‘Langdon’ durum wheat line with a pair of 6B chromosomes from an accession of *Triticum turgidum* L. var. *dicoccoides* previously shown to have a gene (s) for high grain protein content (GPC). They identified closely linked markers for use in marker-assisted breeding for map the gene(s) for high GPC.

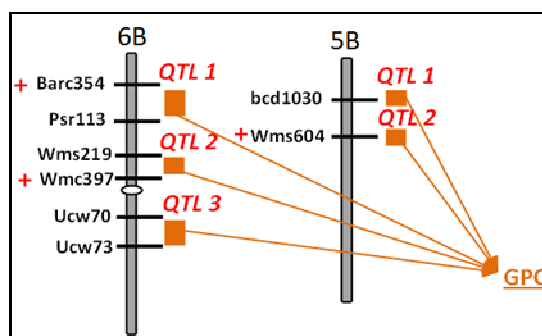
Provided strong evidence that a gene(s) for high GPC is located near the centromere of 6B. The most likely location for the gene(s) is in the short arm between Xabg387-6B and Xwmc397-6B. The LOD score for this interval is 18.9. Segregation in this segment accounted for 66% of the variation in GPC. Eleven additional markers have been mapped within seven cM of the midpoint of Xabg387-6B and Xwmc397-6B. One or more of these markers should be useful in marker-assisted breeding for high GPC in durum wheat.

Due to the strong environmental influence on GPC, molecular markers linked to quantitative trait loci (QTL) affecting GPC have the potential to be valuable in wheat breeding programs.

In the work of Gonzalez-Hernandez (2004), studying a population of 133 recombinant inbred lines in replicated trials at three locations in North Dakota, have verified in *Triticum turgidum* (L.) var. *dicoccoides* that there is an interval of QTL for GPC trait on the 5BL chromosome. Their analysis, performed with MQTL, indicates the presence significant peak affecting GPC (threshold value for main effect = 16.4). This peak was in the interval Xbcd1030-Xgwm604 (wms604). The closest locus was Xgwm604 (wms604) (TS = 155.7). This peak explained 32% of the total phenotypic variance (variance QTL main effect/phenotypic variance = 32%). the marker wms604 was present in my map therefore also our RILs they had association with this character; the similarity of this marker has been distributed to the second parental.

This shows the value of chromosome 5B from *Triticum turgidum* (L.) var. *dicoccoides*, alone or in combination with other genes mapped in *Triticum turgidum* (L.) var. *dicoccoides* or other sources, to increase GPC in breeding lines.

Figure 3.42: The association of some markers with GPC trait in several chromosomes (+ present in our map).



5- 2- 6- The explanation of Trait - Rust Disease

Stripe (yellow) rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. (*Pst*), is an important disease of wheat (*Triticum aestivum* L.) globally. Use of host resistance is an important strategy to manage the disease. The cultivar Flinor has temperature-sensitive resistance to stripe rust. To map quantitative trait loci (QTLs) for these temperature-sensitive resistances of Feng, in 2011, confirmed crossed between Flinor and susceptible cultivar Ming Xian 169. The seedlings of the parents, and F1, F3 progeny were screened against Chinese yellow rust race CYR32 in controlled temperature growth chambers under different temperature regimes. Genetic analysis confirmed two genes for temperature-sensitive stripe rust resistance.

A linkage map of SSR markers constructed through (Feng 2011), that using 130 F3 families derived from the cross. Two temperature-sensitive resistance QTLs were detected on chromosome 5B, designated QYr-tem-5B.1 and QYr-tem-5B.2, respectively, and are separated by a genetic distance of over 50 cM. In the work of Feng 2011, the loci contributed 33.12% and 37.33% of the total phenotypic variation for infection type, respectively, and up to 70.45% collectively.

Favorable alleles of these two QTLs came from Flinor. Of the 978 SSR primer pairs used to screen for polymorphism between the parents, 282 were polymorphic. A molecular marker linkage map was constructed using 229 marker loci exhibiting non-distorted segregation. That in this group of polymorphic markers, there are so many of our markers in common with the work of their (Feng 2011). QYr-tem-5B.1, QYr-tem-5B.2 They were both located on chromosome 5B.

QYr-tem-5B.1 was flanked by SSR markers Xwms67 and XBarc89, and QYr-tem-5B.2 was flanked by SSR markers Xwmc235 and Xwms604. QYr-tem-5B.1 and QYr-tem-5B.2 explained up to 33.12% and 37.33% of the phenotypic variation of infection type, respectively (Fig. 3.43).

The Xwms67 was present on our map with the difference, to be located on chromosome 3A for association of this character with this marker in our linkage map, there's probably another QTL on chromosome 3A. Instead Wms235 the marker was present on our map on chromosome 5B, with the similarity of 52% from the first parental (J.K/CI). The association of this character with this marker shows that this trait comes from our first parental.

Fusarium head blight (FHB) is one of the most important fungal wheat diseases worldwide. Understanding the genetics of FHB resistance is key to facilitate the introgression of different FHB resistance genes into adapted wheat.

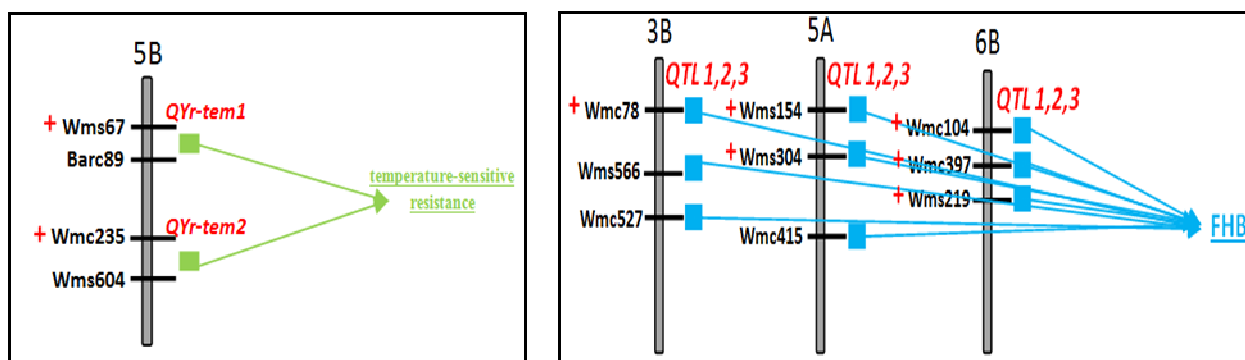
In the project of Cuthbert et al. (2007) was studied the FHB resistance QTL on chromosome 6B, quantify the phenotypic variation, and qualitatively map the resistance gene has a Mendelian factor. The FHB resistant parent BW278 was used as the source of the resistance allele. A large recombinant inbred line (RIL) mapping population was developed from the cross BW278/AC. The population segregated for three known FHB resistance QTL located on chromosomes 3BS, 5A, and 6B. Molecular markers on chromosome 6B (WMC104, WMC397, GWM219), 5A (GWM154, GWM304, WMC415), and 3BS (WMC78, GWM566, WMC527) were amplified on approximately 1,440 F2:7 RILs (Fig. 3.43).

Disease response was evaluated on 89 RILs and parental checks in the greenhouse and field nurseries. Dual floret injection (DFI) was used in greenhouse trials to evaluate disease severity (DS). Macroconidial spray inoculations were used in field nurseries conducted at two locations in southern Manitoba, to evaluate disease incidence, disease severity, visual rating index, and *Fusarium-damaged* kernels (Cuthbert et al. 2007). The phenotypic distribution for all five-disease infection measurements was bimodal, with lines resembling either the resistant or susceptible checks or parents. All of the four field traits for FHB resistance mapped qualitatively to a coincident position on chromosome 6BS, flanked by GWM133 and GWM644, and is named Fhb2. Qualitative mapping of Fhb2 in wheat provides tightly linked markers that can reduce linkage drag associated

with marker assisted selection of Fhb2 and aid the pyramiding of different resistance loci for wheat improvement.

Even on our RILs, WMC104, WMC397, GWM219 markers on chromosome 6A, GWM154, WMC415 markers on chromosome 5A, and WMC78, GWM566, WMC527 markers on chromosome 3B, contain the association with *Fusarium head blight tract* (FHB), but with some differences. In our map WMC104 marker is located on chromosome 1A, instead of 6A. WMC219 and WMS154 markers were monomorphic in the screening of our parental. The two markers (GWM566, WMC527) taken on the chromosome 3B in the map of Cuthbert et al. (2007) were absent in our map.

Figure 3.43: The association of SOME markers with temperature-sensitive resistance and FHB traits in several chromosomes (+ present in our map).



5- 2- 7- The explanation of Trait - Photoperiod

Variation in photoperiod response plays an important role in adapting crops to agricultural environments. The timing of flowering is an important component of adaptation that affects cereal yield and grain quality. In hexaploid wheat, mutations conferring photoperiod insensitivity (flowering after a similar time in short or long days) have been mapped on the 2B (*Ppd-B1*) and 2D (*Ppd-D1*) chromosomes. In collinear positions to the 2H *Ppd-H1* gene of barley. No A genome mutation is known. On the D genome, photoperiod insensitivity is likely to be caused by deletion of a regulatory region that causes miss expression of a member of the pseudo-response regulator (PRR) gene family and activation of the photoperiod pathway irrespective of day length. Photoperiod insensitivity in tetraploid (durum) wheat is less characterized.

The *Ppd-B1* locus is well defined genetically in hexaploid wheat, also was verified on 2B chromosome (Worland and Snape 2001). But for chromosome 2A the situation is less clear (Law et al. 1978; Scarth and Law 1984). A PRR gene homologous to *Ppd-H1* is present on 2A but sequencing revealed no mutation likely to cause photoperiod insensitivity (Beales et al. 2007).

Furthermore, analysis of a 'Mercia' single chromosome substitution line thought to carry a photoperiod insensitive allele on chromosome 2A from 'C591' in fact carried a *Ppd-B1a* allele (Mohler et al. 2004). Photoperiod insensitivity exists in tetraploid (AB genome) wheat (Motzo and Giunta 2007) but its genetic basis is poorly understood compared to the hexaploid.

However, a QTL that is a candidate for a *Ppd* locus has recently been mapped on chromosome 2A of tetraploid wheat by Maccaferri et al. (2008).

For verify if, the *Ppd-A1* locus will be mapped in wheat, and to determine if, it could be located in the near-isogenic Lines. Wilhelm (2008) screened the parents of the F2 populations with 24 SSRs markers for the short arm of chromosome 2A. In their study (Maccaferri et al. 2008) one marker (*wmc177*) was polymorphic and could be scored in both crosses. Comparison with the phenotype scores and genotype scores from the deletion assays placed WMC177, 2 and 2.8 cM from "*Ppd-A1*" in the GS-100 × GS-101 and GS-105 × GS-104 crosses, respectively.

This places "*Ppd-A1*" in the central region of the 2A short arm (Somers et al. 2004)(Fig. 3.44).

As in the preliminary test of the parental lines, GS-105 plants flowered about five days later than GS-100 plants in the second experiment. This could be due to a difference between the "*Ppd-A1*" alleles or to additional background genetic variation.

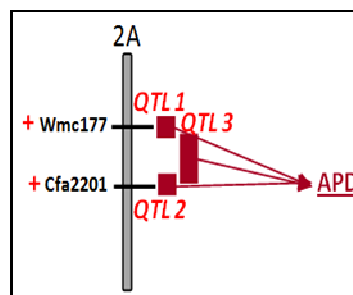
In Analysis of Kofa and Svevo, the parents of the population described by Maccaferri et al. (2008) showed that Kofa carries the GS-100 type allele and Svevo carries the GS-105 type allele. No major flowering time gene segregates in the Kofa × Svevo cross but a QTL was found with a peak in the 8.6 cM XWMC177-XCFA2201 interval with 'Svevo' providing the later flowering allele (Maccaferri et al. 2008) (Fig. 3.44).

As they said Photoperiod insensitivity exists in tetraploid (AB genome) wheat (Motzo and Giunta 2007) but its genetic basis is poorly understood compared to the hexaploid. However, a QTL that is a candidate for a *Ppd* locus has recently been mapped on chromosome 2A of tetraploid wheat by (Maccaferri et al. 2008). Clearly any photoperiod insensitivity in durum wheat must result from a mutation different to that on 2D (*Ppd-D1a*) Wilhelm (2008) explored PRR gene variation in five pairs of near-isogenic tetraploid wheat lines developed by Clarke et al. (1998) that differ in photoperiod sensitivity. They show that photoperiod insensitivity is associated with mutation of the PRR gene on chromosome 2A, and localized with short distance from the WMS425 marker.

Previously described Photoperiod loci in barley (*Ppd-H1*) and wheat (*Ppd-B1* and *Ppd-D1*) have been mapped to collinear positions on the respective group 2 chromosomes (Laurie 1997; Börner et al. 1998). *Ppd-H1* was identified as a member of the pseudo-response regulator (PRR) gene family by (Turner et al. 2005). Beales et al. (2007) described the cloning and sequencing of orthologous PRR genes from the A, B and D genomes of hexaploid wheat. The nearby this gene with the

WMS425 marker, will be able to identify the presence of this character on the groups of two in our map. This marker has been polymorphic between our two parental, and in our RIL had the similarity of 62% with the first Parental (J.K/C1), so this character is recessive in the first parental (J.K/C1).

Figure 3.44: The association of some markers with APD trait in several chromosomes (+ present in our map).



5- 2- 8- The explanation of Trait - Growth

Watanabe (2008) presented that the assessments of characteristics near-isogenic lines are being progressed. The Rht-B1b allele reduced plant height and caused deep seed dormancy most severely, whereas Rht-B1c allele resulted weak, less number of tillers at the early stage of growing. The effects of other alleles were similar to that of Rht-B1b.

It should be noted that the gibberellic acid sensitively reduced height genes, Rht14, Rht16 and Rht18, were induced independently. These genes were allelic to each other, and linked with XBARC3 on chromosome 6AS. Microsatellite mapping indicated that they were located at the same locus on the short arm of chromosome 6A (Fig. 3.43).

The Rht-B1b allele encodes a mutant form of the protein, a gibberellic acid (GA) signaling repressor (Peng et al. 1999 b). Rht-B1b allele is associated with a single base-pair change leading to a TAG stop codon. Variation among multiple alleles at Rht-B1b locus may suggest that there is nucleotide sequence polymorphism in the semi-dwarfing genes.

The BARC3 marker also was present among our markers, in our map this marker with the near distance by other two markers, which until now not identified for this character. For this short distance, the two markers BARC3 and CFA2114 will carry the same genes, from parents.

These two Markers on our RIL had the similarity of 56% and 58% with the first Parental (J.K/C).

Figure 3.45: The association of some markers with growth trait in several chromosomes (+ present in our map).

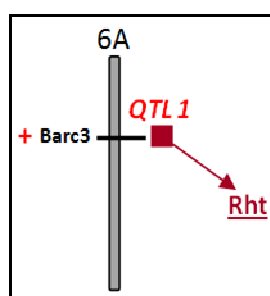
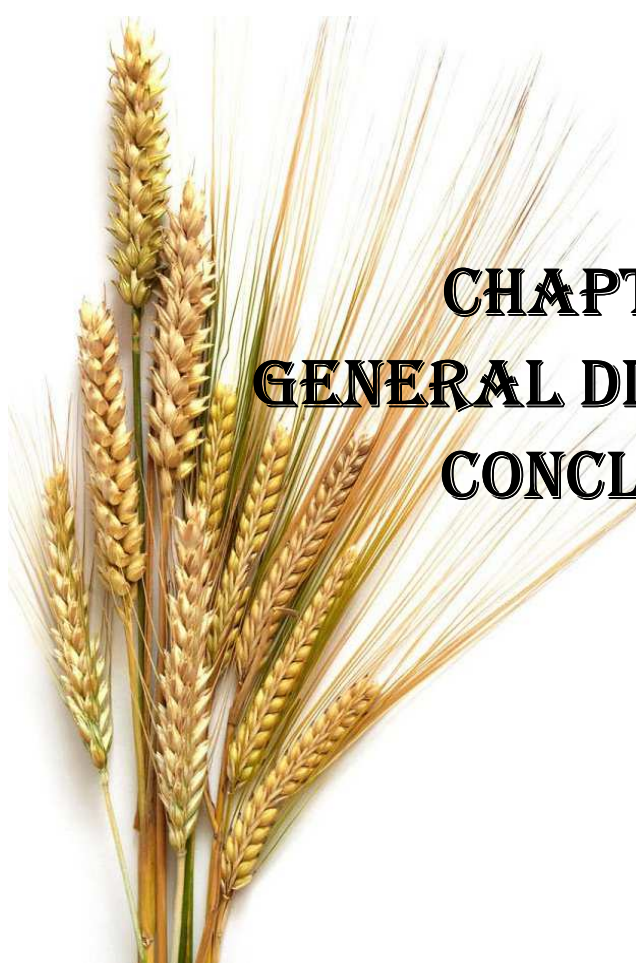


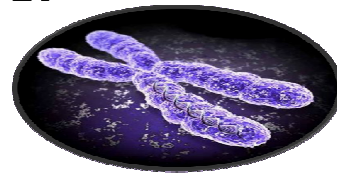
Table 3.12: Association of Markers with the characters and Traits.

J.K/C: First parental: Jennah Khetifa/ChamI, Od/O: Second parental: Omrabi5//dicoccoides/Omrabi5.

Marker	Symbol of Traits	Location	Poli/Mono	Traits (Characters)	Gene	Allele high	References
WMS570	GYPC	6AL	poli	Yellow pigment		Od/O	(Zhang et al. 2008)
WMC276	SC	7AL	mono	semolina color,		---	(Zhang et al. 2008)
WMC617	PC	4BS	mono	pasta color	Lpx-B1	---	(Zhang et al. 2008)
BARC113	GYPC, SC and PC	6A	mono	Yellow pigment , semolina color, pasta color	Lpx-B1	---	(Zhang et al. 2008)
WMS219	GPC	6BL	mono	Grain protein content		---	(Zhang et al. 2008)
WMC311	GYPC	2A	poli	Yellow Pigment		J.K/C	
WMC177	PHT	2A	poli	Photoperiod insensitivity	Ppd-A1	J.K/C	(Somers et al. 2004)
CFA2201		2A	poli	Later Flowering		Od/O	(Maccaferri et al. 2008)
WMS312	PPO (Polyphenol oxidases)	2A	poli	Pigment Color		J.K/C	(Nachit et al. 2001)
WMS249	PPO (Polyphenol oxidases)	2A	poli	Pigment Color		Od/O	(Sun et al. 2005)
WMC349	PC1	4B	poli	longitudinal (length) and proximodistal (width)	GS3	J.K/C	(Gegas et al. 2010)
WMS149	PC1	4B	mono	longitudinal (length) and proximodistal (width)		---	(Gegas et al. 2010)
WMC264	PC2	3A	poli	grain shape	GW2	Od/O	(Salina et al. 2000)
BARC19	PC2	3A	mono	grain shape		---	(Gegas et al. 2010)
MWG79	GPC	6B	mono	Grain protein content	GPC locus	---	(Distelfeld et al. 2004)
WMC104	FHB	1A/6A	poli	FHB resistance (Fusarium head blight)		Od/O	(Cuthbert et al. 2007)
WMS67		3A/5B	poli	Stripe rust (Diseases of Rust)	QYr-tem-5B.1	Od/O	(Feng 2011)
Barc89		5B	mono	Stripe rust (Diseases of Rust)	QYr-tem-5B.1	---	(Feng 2011)
WMC235		5B	mono	Stripe rust (Diseases of Rust)	QYr-tem-5B.2	---	(Feng 2011)
WMS604		5B	mono	Stripe rust (Diseases of Rust)	QYr-tem-5B.2	---	(Feng 2011)
WMC397	FHB	6B	poli	<i>Fusarium head blight</i>		J.K/C	(Cuthbert et al. 2007)
GWM219	FHB	6A	mono	<i>Fusarium head blight</i>		---	(Cuthbert et al. 2007)
GWM154	FHB	5A	mono	<i>Fusarium head blight</i>		---	(Cuthbert et al. 2007)
WMC415	FHB	5A	poli	<i>Fusarium head blight</i>		J.K/C	(Cuthbert et al. 2007)
WMC468	GI	4A	mono	germination index (GI), (Grain Dormancy)		---	(Mares et al. 2004)
WMC262	GI	4A	poli	germination index (GI), (Grain Dormancy)		loc1,2 J.K/C J.K/C	(Mares et al. 2004)
WMC161	GI	4A	mono	germination index (GI), (Grain Dormancy)		---	(Mares et al. 2004)
WMC256	Ash	6A	mono				



CHAPTER IV GENERAL DISCUSSION & CONCLUSION



CONCLUSION

The current consensus map shows the position of microsatellites at a density that makes the map useful in plant breeding. In smaller populations, the accurately position markers is lower than in larger populations. The disagreements in marker order of closely linked markers between genetic maps and derivation of the most correct marker order can be facilitated by construction of the consensus map.

The exact fine marker order may differ slightly in other populations, and users should be prepared to establish the order for closely linked markers in their mapping and breeding populations. This population is derived from a cross between genetically diverse parents and is therefore ideal for identifying loci controlling both qualitative and quantitative traits.

The traits associated with high priority for quantitative trait loci (QTL) analysis, like the grain quality traits, linked to the end products (pasta, couscous, and burghul), such some disease are extremely useful in plant breeding activities. The genetic linkage map presented in the current study is the first published map utilizing a RIL population derived by durum wheat Parents belonging to two RIL populations and using of SSRs and SNPs markers. The two parental lines were found polymorphic in various morphophysiological traits, such as productivity, heading date, plant height, drought resistance and others.

However, a few chromosome arms (2AS, 4AS, 6AS and 6BS) were partly covered and one arm (4BS) was not covered. Lack of complete genome coverage of homoeologous group 4 was observed in a few other wheat mapping populations (Röder et al. 1998 a, b; Paillard et al. 2003; Elouafi and Nachit 2004).

The primary use of our map is in molecular mapping of traits and plant breeding. The precise marker order over short chromosome intervals (<90 cM) may not be that important to make plant selections. But our map is approached at this distance, and drawing up for chromosome intervals with 90 cM. The marker order between 90 cM bins along the chromosome is more important in order to detect recombination events in gametes and make proper plant selections.

This map provided a normal number of markers along the length of the chromosome that can be used to genotype individuals for detecting recombinants, fixing loci, restoring a recurrent genetic background, or assembling complex genotypes in complex crosses (Gupta et al. 1999; Huang et al. 2003).

Grain quality traits were found to be affected by environmental fluctuations, this is particularly true for protein content, and 1000-kernel weight. Further, the mapping population Jennah Ketifa/ ChamI × Omrabi5/ *T. dicoccoides*600545// Omrabi5 showed significant transgressive inheritance for several

traits (e.g.: Length of the wheat, PPO activity, Yellow pigment) for had associations of markers with genes that control these traits.

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 this population (First (J.K/CI) and second (O5d/O5) Parental), a high level of polymorphism was detected (> 45%), although the mapped population is a RIL. The (J.K/CI) × (O5d/O5) map was constructed with 100 microsatellites and 10 SNPs as framework markers. The length of the map was 3170.293 cM, with an average of marker per 31.70cM. The map showed a high synteny with former published durum and bread wheat maps.

The SSR markers were evenly mapped across the whole genome. The microsatellites proved to be good as anchor probes and were very useful in arms and chromosomes identification. SNPs proved also to be good markers for map saturation in one of chromosome 4B. Most of the combinations were mapped in different genomic regions, which amplified numerous fragments that were mapped in 13 different linkage groups. In agreement with earlier published data (Elouafi, 2004).

More genetic markers were mapped in the A genome comparatively to the B genome. In conclusion, Wms and Wmc from Bread and durum wheat microsatellites amplified and mapped perfectly in durum/ durum genetic background.

Indeed, the mapping population showed a fixed genetic structure, as it was developed using single seed descent (SSD) method. This makes Jennah Ketifa/ChamI × Omrabi5/ *T. dicoccoides*600545//Omrabi5 population ideal for assessing the environmental impact on trait expression. In the future this population will be 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. It is important the introgression of these identified desirable genes from *Triticum dicoccoides* to improve durum genetic material. This positive contribution was noticed for protein content and gluten strength. The durum cultivar Omrabi5, showed a positive and significant positive effect on test weight, thousand-kernel weight, flour yield, and yellow pigment. The durum cultivar Jennah.Ketifa/Cham I, on the other hand, showed a positive effect for quality of Pasta, had a high PPO (Poly phenol oxidases) activity.

In this study, it was also demonstrated the importance of mapping and association of some markers with some traits, that are of economic and nutritional interest. The genetic 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, final product-quality, biotic stress resistance, etc. The results also suggest strongly the use of identified markers 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.

The marker density in our map can provide a better choice of markers for specific breeding populations to ensure adequate polymorphic marker coverage in regions of interest. Further, the marker density in this map is likely sufficient to perform association mapping and possibly linkage disequilibrium (LD) studies on germplasm collections. There were a sufficient number of markers distributed over the genome, which amplified single loci only or low complexity profiles that are well suited for association mapping and LD studies.

These types of markers would avoid the confusion of scoring alleles amplified from paralogous or homoeologous loci, which is a concern in a polyploidy species such as wheat.

A database of allele sizes is provided at (<http://wheat.pw.usda.gov>) so users can consider the approximate size of DNA fragments in their breeding parents and populations and how best to use the map to develop molecular breeding strategies.

In summary, the microsatellite consensus map brings together large collections of publicly available microsatellites onto a single genetic map derived by joining this genetic maps. There are still hundreds of microsatellite markers in the public domain that could be added to the present consensus map. Future prospects include adding more microsatellite and SNP-based markers to the consensus map, a thorough alignment to the physical map of wheat, as well as implementation of the map in haplotype diversity studies of durum wheat.

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. The construction of a molecular map using a population of RILs derived from intraspecific cross of durum wheat.
2. High level of polymorphism between the two parents was detected. The genetic linkage map of Jennah Ketifa/ ChamI × Omrabi5/ *T. dicoccoides*600545// Omrabi5 population was constructed.
3. Identification of loci associated to genomic traits implicated in productivity for MAS utilization.
4. The identification of the percentage of recombination between different markers in the RIL lines derived by crossing tetraploid wheat.
5. Study and analyze the similarity and the differences between the RIL in various loci, our genotypes showed a similarity with the high percentage to the first parent.
6. Quantitative and transgressive inheritances were revealed for most grain quality traits.
7. SSRs used as anchor probes to identify chromosomal arms and SNPs as markers to saturate the map of chromosom 4B.
8. More genetic markers mapped to A than to B genome.
9. This present study provided insight into the variation in allele size between mapping parents, which may be extended to other parents and breeding populations. as the CFA markers 28.2 bp have shown the maximum differences dimensional and WMC markers 9.3 bp minimum differences in our population.
10. The Jennah Ketifa/ ChamI × Omrabi5/ *T. dicoccoides*600545// Omrabi5 mapping population is a potential source for grain quality improvement and for studying QTLs use in durum breeding.
11. Resulted in a comprehensive analysis of marker order and distance of DNA markers for use in molecular breeding.

REFERENCES

- Adams M.D.**, Kerlavage A.R, Fleischmann R.D, Fuldner R.A, Bult C.J, Lee N.H, Kirkness E.F, Weinstock K.G, Gocayne J.D, White O. (1995). Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence. (The Genome Directory, Suppl.) *Nature*, 377: 3-174.
- Adhikari T.B.**, Wallwork H, Goodwin S.B. (2004). Microsatellite Markers Linked to the Stb2 and Stb3 Genes for Resistance to *Septoria tritici* Blotch in Wheat. *Crop Sci*, 44: 1403-1411.
- Ahn A.**, Anderson J.A, Sorrells M.E, Tanksley S.D. (1993). Homoeologous relationships of rice, wheat and maize chromosomes. *Mol. Gen. Genet*, 241: 483-490.
- Ahn S.N.**, Tanksley S.D. (1993). Comparative linkage maps of rice and maize genomes. *Proc Natl. Acad. Sci. USA*, 90: 7980-7984.
- Akbari M.**, Wenzl P, Caig V, Carlig J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden M.J, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A. (2006). Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. *Theoretical and Applied Genetics*, 113: 1409-1420.
- Akhunov D.E.**, Akhunova A.R, Anderson O.D, Anderson J.A, Blake N, Clegg M.T, Coleman-Derr D, Conley E.J, Crossman C.C, Deal K.R, Dubcovsky J, Gill B.S, Gu Y.Q, Hadam J, Heo H, Huo N, Lazo G.R, Luo M.C, Ma Y.Q, Matthews D.E, McGuire P.E, Morrell P.L, Qualset C.O, Renfro J, Tabanao D, Talbert L.E, Tian C, Toleno D.M, Warburton M.L, You F.M, Zhang W, Jan Dvorak J. (2010). Nucleotide diversity maps reveal variation in diversity among wheat genomes and chromosomes, *BMC Genomics* 2010, 11: 702. DOI:10.1186/1471-2164-11-702.
- Akkaya M.S.** (1995). Integration of simple sequence repeat DNA markers into a soybean linkage map. *Crop Science* 35: 1439-1445.
- Alpert K.B.**, Tanksley S.D. (1996). High resolution mapping and isolation of a yeast artificial chromosome contig containing fw2.2: A major fruit weight quantitative trait locus in tomato. *Proc Natl Acad Sci*, 93: 15503-15507.
- Ananiev E.V.**, Riera-Lizarazu O, Rines H.W, Phillips R.L. (1997). Oat-maize chromosome addition lines: a new system for mapping the maize genome. *Proc. Natl. Acad. Sci. USA*, 99: 3524-3529.
- Anderson J.A.**, Churchill G.A, Autrique J.E, Tanksley S.D, Sorrells M.E. (1993). Optimizing parental selection for genetic linkage maps. *Genome*, 36: 181-186.
- Araus J.L.**, Slafer G.A, Reynolds M.P, Royo C. (2002). Plant breeding and drought in C-3 cereals: What should we breed. *Annals of Botany*, 89: 925-940.
- Asins M.J.**, Carbonell E.A. (1989). Distribution of genetic variability in a durum wheat world collection. *Theoretical and Applied Genetics*, 77: 287-294.
- Austin R.S.**, Vidaurre D, Stamatiou G, Breit R, Provart N, Bonetta D, Zhang J, Fung P, Gong Y, Wang P.W, Court M.P, Guttman D.S. (2012). Next-generation mapping of Arabidopsis genes, *The Plant Journal*, 67: 715-725.
- Autran J.C.**, Abecassis J, Feillet P. (1996). Statistical evaluation of different technological and biochemical tests for quality assessment in durum wheats. *Cereal Chem*, 63: 390-394.
- Autrique E.**, Nachit M.M, Monneveux P, Tanksley S.D, Sorrells M.E. (1996). Genetic diversity in durum wheat based on RFLPs, morphological traits, and coefficient of parentage. *Crop Sci*, 36: 735-742.
- Avivi L.** 1978. High protein content in wild tetraploid *Triticum dicoccoides* Korn. In: S. Ramanujam (ed) 5th Int. Wheat Genet. Symp. New Delhi, India. pp. 372-380.
- Bai J.**, Liu K, Jia X, Wang D. (2004). An analysis of homoeologous microsatellites from *Triticum urartu* and *Triticum monococcum*. *Plant Sci*, 166: 341-347.
- Baik B.K.**, Czuchajowska Z, Pomeranz Y. (1995). Discoloration of dough for oriental noodles. *Cereal Chem* 72: 198-205.
- Barbazuk W.B.** (2007). SNP discovery via 454 transcriptome sequencing. *Plant J*, 51: 910-918.

- Batley J**, Mogg R, Edwards D, O'Sullivan H, Edwards K.J. (2003). A high-throughput SNUPE assay for genotyping SNPs in the flanking regions of *Zea mays* sequence tagged simple sequence repeats. *Mol Breed*, 11: 111-120.
- Beavis W.B.** (1998). QTL analyses: power, precision, and accuracy. In: Patterson AH (ed) *Molecular dissection of complex traits*. CRC Press. Boca Raton.
- Beavis W.D**, Grant D. (1991). A linkage map based on information from four F₂ populations of maize (*Zea mays* L.). *TAG*, 82: 636-644.
- Beckman J.S**, Soller M. (1990). Toward a unified approach to genetic mapping of eukaryotes based sequence tagged microsatellites. *Biotechnology*, 8: 930-932.
- Bell C.J**, Ecker J.R. (1994). Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. *Genomics*, 19 : 137-144.
- Bennett M.D**, Cox A.V, Leitch I.J. (1998). Angiosperm DNA C-values database. <http://www.rbgekew.org.uk/cval/database1.html>.
- Bennett M.D**, Smith J.B. (1976). Nuclear DNA amounts in angiosperms. *Philosophical Transactions of the Royal Society of London*, B 274: 227-274.
- Bennetzen J.L**, Freeling M. (1993). Grasses as a single genetic system: genome composition, collinearity and compatibility. *Trends Genet*, 9: 259-261.
- Bernacchi D**, Beck-Bunn T, Emmatty D, Eshed Y, Inai S, Lopez J, Petiard V, Sayama H, Uhlig J, Zamir D, Tanksley S. (1998). Advanced backcross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single donor introgression for desirable wild QTL-alleles derived from *Lycopersicon hirsutum* and *L. pimpinellifolium*. *Theor Appl Genet*, 97: 170-180.
- Blake N.K**, Sherman J.D, Dvorak J, Talbert L.E. (2004). Genome-specific primer sets for starch biosynthesis genes in wheat. *Theoretical and Applied Genetics*, 109: 1295-1302. DOI: 10.1007/s00122-004-1743-4.
- Blanco A**, Bellomo M.P, Cenci A, De Giovanni C, D'Ovidio R, Iacono E, Laddomada B, Pagnotta M.A, Porceddu E, Sciancalepore A, Simeone R, Tanzarella O.A. (1998). A genetic linkage map of durum wheat. *TAG*, 97: 721-728.
- Blanco A**, De Giovanni C, Laddomada B, Sciancalepore A, Simeone R, Devos K.M, Gale M.D. (1996). Quantitative trait loci influencing grain protein content in tetraploid wheats. *Plant Breed*, 115: 10-316.
- Blanco A**, Pasqualone A, Troccoli A, Di Fonzo N, Simeone R. (2002). Detection of grain protein content QTL across environments in tetraploid wheats. *Plant Mol Biol*. 48: 615-623.
- Blanco A**, Simeone R, Cenci A, Gadaleta A, Tanzarella O.A, Porceddu E, Salvi S, Tuberosa R, Figliuolo G, Spagnoletti P, Roder M.S, Korzun V. (2004). Extension of the -Messapia x dicoccoides linkage map of *Triticum turgidum* (L.). *Cellular and Molecular Biology Letters* 9: 529-541.
- Blanco A**, Simeone R, Gadaleta A. (2006). Detection of QTL for grain protein content in durum wheat. *Theor Appl Genet* 112: 1195-1204.
- Bonierbale M.W**, Plaisted R.L, Tanksley S.D. (1988). RFLP maps based on common sets of clones reveal modes of chromosome evolution in potato and tomato. *Genetics*, 120: 1095-1103.
- Botstein D**, White R.L, Skolnick M, Davis R. (1980). Construction of a genetic linkage map in man using restriction fragment polymorphisms. *Am. J. Hum. Genet*, 32: 314-331.
- Boutin S**, Young N.D, Olson T, Yu Z.H, Shoemaker R.C, Vallejos C.E. (1995). Genome conservation among three legume genera detected with DNA markers. *Genome*, 38: 928-937.
- Bozzini A.** (1988). Origin, distribution and production of durum wheat in the world. In: *Durum wheat: Chemistry and Technology*, G. Fabriani and C. Lintas (ed.) AACC. St Paul, MN: 1-16.
- Branlard G**, Autran J.C, Monneveux P. (1989). High molecular weight glutenin subunits in durum wheat (*T. durum*). *Theoretical and Applied Genetics*, 78: 353-358.
- Brookes A.J.** (1999). The Essence of SNPs. *Gene*, 234: 177-186.

- Bryan G.J**, Collins A.J, Stephenson P, Orry A, Smith J.B, Gale M.D. (1997). Isolation and characterisation of microsatellites from hexaploid bread wheat. *Theoretical and Applied Genetics*, 94: 557-563.
- Buckler E.S**, Holland J.B, Acharya C.B, Brown P.J, Browne C, Ersoz E, Flint-Garcia S, Garcia A, Glaubitz J.C, Goodman M.M, Harjes C, Guill K, Kroom D.E, Larsson S, Lepak N.K, Li H.H, Mitchell S.E, Pressoin G, Peiffer J.A, Rosas M.O, Rocheford T.R, Romay M.C, Romero S, Salvo S, Villeda H.S, Sliva H.S.D, Sun Q, Tian F, Upadaya N, Ware D, Yates H, Yu J, Zhang Z, Kresovich S, McMullen M.D. (2009). The genetic architecture of maize flowering time. *Science*, 325: 714-718.
- Buetow K.H**, Edmonson M.N, Cassidy A.B. (1999). Reliable identification of large numbers of candidate SNPs from public EST data. *Nat Genet*, 21: 323-325.
- Burr B**, Burr F. (1988). I polimorfismi dei frammenti generate da enzimi di restrizione e il loro impiego nel miglioramento genetic. *Agricoltura e ricerca, Speciale Genetica agraria, Atti XXXII Convegno Societa Italiana Genetica Agraria*, pp: 61-68.
- Burr B**, Burr F.A, Thompson K.H, Albertson M.C, Stuber C.W. (1988). Gene mapping with recombinant inbreds in maize. *Genetics* 118: 519-526.
- Burr B**, Burr F.A. (1991). Recombinant inbreds for molecular mapping in maize. *Trends Genet*, 7: 55-60.
- Cadalen T**, Boeuf C, Bernard S, Bernard M. (1997). An interval molecular marker map in *Triticum aestivum* L. Em. Thell and comparison with a map from a wide cross. *TAG*, 94: 367-377.
- Castagnar G**, Maga M, Parenzini M, Heun F, Salahini F. (1994). RFLP-based genetic relationships of einkorn wheats. *Theor Appl Genet*, 88: 818-823.
- Catherine F**, Travella S, Stein N, Albar L, Nublat A.L, Keller B. (2003). Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome, *PNAS journal*, Edited by Robert Haselkorn, University of Chicago, Chicago, IL, received for review August 4, 2003.
- Causse M**, Fulton T.M, Cho Y.G, Ahn S.N, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald P.C, Harrington S.B. (1994). Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics*, 138: 1251-1274.
- Causse M.A**, Fulton T.M, Cho Y.G, Ahn S.N, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald P.C, Harrington S.E, Second G, McCouch S.R, Tanksley S.D. (1994). Saturated molecular map of rice genome based on an interspecific backcross population. *Genetics*, 138: 1251-1274.
- Chalmers K.J**, Campbell A.W, Kretschmer J, Karakousis A, Henschke P.H, Pierens S, Harker N, Pallotta M, Cornish G.B, ShariXou M.R, Rampling L.R, McLauchlan A, Daggard G, Sharp P.J, Holton T.A, Sutherland M.W, Appels R, Langridge P. (2001). Construction of three linkage maps in bread wheat, *Triticum aestivum*. *Aust J Agric Res*, 52: 1089-1119.
- Chantret N**, Cenci A, Sabot F, Anderson O. (2004). Sequencing of the *Triticum monococcum* hardness locus reveals good microcolinearity with rice. *Mol Genet Genomics*, 271: 377-386.
- Chantret N**, Salse J, Sabot F, Rahman S, Bellec A, Laubin B, Dubois I, Dossat C, Sourdille P, Joudrier P, Gautier M.F, Cattolico L, Bechert M, Aubourg S, Weissenbach J, Caboche M, Bernard M, Leroy P, Chalhou B. (2005). Molecular basis of evolutionary events that shaped the hardness locus in diploid and polyploidy wheat species (*Triticum* and *Aegilops*). *Plant Cell*, 17: 1033-1045.
- Chao S**, Sharp P.J, Worland A.J, Warham E.J, Koebner R.M.D, Gale M.D. (1989). RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. *Theoretical and Applied Genetics*, 78: 495-504.
- Chee P.W**, Elias E.M, Anderson J.A, Kianian S.F. (2001). Evaluation of a high protein QTL from *Triticum turgidum* L.v ar. *dicoccoides* in an adapted durum wheat background. *Crop Sci*, 41: 295-301.
- Chen Q.P.F.Q**, Ming Wei Y, Wang J.R, Zheng Y. (2009). Molecular Characterization of the *pina* Gene in Einkorn Wheat. *Biochemical Genetics*, Volume 47, Numbers 5-6: 384-396. DOI: 10.1007/s10528-009 9239-1.
- Chen Z.J**, Devey M.E, Tuleen N.A, Hart G.E. (1994). Use of recombinant substitution lines in the construction of RFLP-based genetic maps of chromosomes 6A and 6B of tetraploid wheat (*Triticum turgidum*). *TAG*, 89: 703-712.

- Ching A**, KS C, Jung M, Dolan M, Smith O.S, Tingey S, Morgante M, Rafalski A.J. (2001). SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. *BMC Genetics*, 3: 19. DOI: 10.1186/1471-2156-3-19.
- Churchill G.A**, Airey D.C, Allayee H. (2004). Complex Trait Consortium. The Collaborative Cross, a community resource for the genetic analysis of complex traits *Nat. Genet*, 36: 1133-1137.
- Ciaffi M**, Benedettelli S, Giorgi B, Porceddu E, Lafiandra D. (1991). Seed storage proteins of *Triticum turgidum* ssp. *dicoccoides* and their effect on the technological quality in durum wheat. *Plant Breeding*, 107: 309-319.
- Ciaffi M**, Lafiandra D, Porceddu E, Benedettelli S. (1993). Storage protein variation in wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) from Jordan and Turkey. I. Electrophoretic characterization of genotypes. *TAG*, 86: 474-480.
- Clarke F.R**, Clarke J.M, Mc Caig T.N, Knox R.E, De Pauw R.M. (2006). Inheritance of yellow pigment concentration in seven durum wheat crosses. *Can J Plant Sci*, 86: 133-141.
- Clarke J.M**, Clarke F.R, Ames N.P, Mc Caig T.N, Knox R.E. (2000). Evaluation of predictors of quality for use in early generation selection. In: Royo C, Nachit M.M, DiFonzo N, Arauz J.L. (eds) *Durum wheat improvement in the Mediterranean region: new challenges*. International Centre for Advanced Mediterranean Agronomic. Studies (CIHEAM), Zaragoza, pp 439-446.
- Colledge S**. (2007). University College, London. Institute of Archaeology. The origins and spread of domestic plants in southwest Asia and Europe. Left Coast Press, Pp: 40. ISBN 9781598749885. (http://books.google.com/books?id=D2nym35k_EcC&pg=PA40).
- Concibido V.C**, Young N.D, Lange D.A, Denny R.L, Danesh D, Orf J.H. (1996). Targeted comparative genome analysis and qualitative mapping of a major partial-resistance gene to the soybean cyst nematode. *Theor Appl Genet*, 93: 234-241.
- Condit R**, Hubbell S.P. (1991). Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. *Genome*, 34 : 66-71.
- Condon A.G**, Richards R.A, Rebetzke G.J, Farquhar G.D. (2004). Breeding for high water-use efficiency. *Journal of Experimental Botany*, 55: 2447-2460.
- Coryell V.H**, Jessen H, Schupp J.M, Webb D, Keim P. (1999). Allele-specific hybridisation markers for soybean. *Theoretical and Applied Genetics*, 101: 1291-1298.
- Coulondre C**, Miller J.H, Farabaugh P.J, Gilbert W. (1978). Molecular basis of base substitution hot spots in *Escherichia coli*. *Nature*, 274: 775-780
- Cox T.S**, Harrell L.G, Chen P, Gill B.S. (1991). Reproductive behavior of hexaploid/diploid wheat hybrids. *Plant Breed*, 107: 105-118.
- Cramb J**, Courtney J, Tille P. (2000). Chapter Coordinator: Ian Foster, *Principles and Practice*, Compiled by W.K. Anderson and J.R. Garlinge, Agriculture Western Australia, Funded by the Grains Research and Development Corporation, Bulletin 4443, Replaces Bulletin 4196, ISSN 1326-415X, Agdex 112/01, Chief Executive Officer, Department of Agriculture, Western Australia, *The Wheat Book*
- Curtis C.A**, Lukaszewski A.J. (1991). Genetic linkage between Cbands and storage protein genes in chromosome 1B of tetraploid wheat. *Theor Appl Genet*, 81: 245-252.
- Cuthbert P.A**, Somers D.J, Brulé-Babel A. (2007). Mapping of Fhb2 on chromosome 6BS: a gene controlling Fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). *Theor Appl Genet*, 114: 429-437. DOI 10.1007/s00122-006-0439-3.
- D'Egidio M.G**, Mariani B.M, Nardi S. (1990). Chemical and technological variables and their relationships: A predictive equation for pasta cooking quality. *Cereal Chem*, 67: 275-281.
- Damania A.B**, Tahir M, Somaroo B.H. (1988). Improvement of *Triticum durum* proteins utilizing wild gene resources of *T. dicoccoides* Koern. ICARDA.

- De felice S.** (1999). Analisi Genetica di marcatori RFLP e ISSR-PCR e loro inserimento in una mappa cromosomica di frumento duro, Teses of laurea (Bh.c) in the Plant Genetic, University of Tuscia (Universita della Tuscia), Anno accademico 1998-1999, Matr: A 3016.
- De Vicente M.C,** Tanksley S.D. (1993). QTL analysis of transgressive segregation in an interspecific tomato cross. *Genetics*, 134: 585-596.
- De Vita P,** Li Destri Nicosia O, Nigro F, Platani C, Riefolo C. (2007). Breeding progress in morpho-physiological, agronomical and qualitative traits of durum wheat cultivars released in Italy during the 20th century. *European Journal of Agronomy*, 26: 39-53.
- Delaney D.E,** Nasuda S, Endo T.R, Gill B.S, Hulbert S.H. (1995 a). Cytologically based physical maps of the group 2 chromosomes of wheat. *Theoretical and Applied Genetics*, 91: 568-573.
- Delaney D.E,** Nasuda S, Endo T.R, Gill B.S, Hulbert S.H. (1995 b). Cytologically based physical maps of the group 3 chromosomes of wheat. *Theoretical and Applied Genetics*, 91: 780-782.
- Deutsch S,** Iseli C, Bucher P, Antonarakis S.E, Scott H.S. (2001). A cSNP map and database for human chromosome 21. *Genome Research*, 11: 300-307
- Devaux P,** Kilian A, Kleinhofs A. (1995). Comparative mapping of the barley genome with male and female recombination-derived, doubled haploid populations. *Mol Gen Genet*, 249: 600-608.
- Devos K.M,** Atkinson M.D, Chinoy C.N, Liu C.J, Gale M.D. (1992). RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. *Theor Appl Genet*, 83: 931-939.
- Devos K.M,** Atkinson M.D, Chinoy C.N, Liu C.J, Gale M.D. (1993). RELP-based genetic map of the homoeologous group-2 chromosomes of wheat, rye and barley. *Theoretical and Applied Genetics*. 85: 784-792.
- Devos K.M,** Bryan G.J, Stephenson P, Gale M.D. (1995). Application of two microsatellite sequences in wheat storage proteins as molecular markers. *Theoretical and Applied Genetics*, 90: 247-252.
- Devos K.M,** Dubcovsky J, Dvůrák J, Chinoy C.N, Gale M.D. (1995). Structural evolution of wheat chromosomes 4A, 5A, and 7B and its impact on recombination. *Theor. Appl. Genet*, 91:282-288.
- Devos K.M,** Gale M.D. (1992). The use of random amplified polymorphic DNA markers in wheat. *Theoretical and Applied Genetics*, 84: 567-572.
- Devos K.M,** Gale M.D. (1993). Extended genetic maps of the homoeologous group 3 chromosomes of wheat, rye and barley. *TAG*, 85: 649-652.
- Devos K.M,** Gale M.D. (1997). Comparative genetics in the grasses. *Plant Mol Biol*, 35: 3-15.
- Devos K.M,** Millan T, Gale M.D. (1993). Comparative RFLP maps of homoeologous group 2 chromosomes of wheat, rye and barley. *TAG*, 85: 784-792.
- Diab A.** (2001). 110 F9 recombinant inbred lines (RILs) derived by single-seed descent from the cross ICD-MN91-0012 between Jennah Khetifa (Tamgurt) and Cham1 were used. GrainGenes Map Data Report, <http://wheat.pw.usda.gov/cgibin/graingenes/report.cgi?class=mapdata&name=T.+turgidum,+J.K+x+Cham1+2>.
- Diekmann M.** (1996 a). Diseases in seed production. In A.J.G. Van Gastel, M.A. Pagnotta & E. Porceddu, eds. *Seed Science and Technology. Proc. Train-the- Trainers Workshop Sponsored by Med-campus Programme (EEC)*, 24 Apr.-9 May 1993, Amman. Aleppo, Syria, ICARDA.
- Dilbirligi M,** Erayman M, Campbell B, Randhawa H, Baenziger P, Dweikat I, Gill K. (2006). High-density mapping and comparative analysis of agronomically important traits on wheat chromosome 3A. *Genomics*, 88: 74-87.
- Distelfeld A,** Olmos S, Uauy C, Schlatter A.R, Dubcovsky J, Fahima T. (2004). Microcolinearity between the grain protein content QTL region in wheat chromosome arm 6BS and rice chromosome 2. project was supported by USDA-BARD grant US-3224-01 and USDA-IFAFS, 2001-04462.
- Dobzhansky T.H,** Spassky B, Anderson W. (1965). Bichromosomal synthetic semilethals in *Drosophila pseudoobscura*. *PNAS*, 53: 345-348.

- Dohlman E**, Hoffman L. (2000). The new agricultural trade negotiations: background and issues for the U.S. wheat sector. Wheat yearbook. Economic Research Service, USDA.
- Driscoll D.J**, Palmer C.G, Melman A. (1979). Nonhomologous associations of C-heterochromatin at human male meiotic prophase. Cyt. Cell Genet, 23: 23-32.
- Dubcovsky J**, Dvorak J. (2007). Genome plasticity: a key factor in the success of polyploid wheat under domestication. Science, 316: 1862-1866.
- Dubcovsky J**, Luo M.C, Dvorak J. (1995 a). Differentiation between homoeologous chromosomes 1A of wheat and 1Am of *Triticum monococcum* and its recognition by the wheat Phl locus. Proc Natl Acad Sci USA, 92: 6645-6649.
- Dubcovsky J**, Luo M.C, Zhong G.Y, Bransteitter R, Desai A, Kilian A, Kleinhofs A, Dvorak J. (1996). Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. Genetics, 143: 983-999.
- Dvolák J**, Chen K-C. (1984). Distribution of nonstructural variation between wheat cultivars along chromosome arm 6B: evidence from the linkage map and physical map of the arm. Genetics 106: 325- 333.
- Dvorak J**, Appels R. (1986). Investigation of homologous crossing over and sister chromatid exchange in the wheat Nor-B2 locus coding for rRNA and Gliadins. Genetics, 113: 1037-1056.
- Dvorak J**, di Terlizzi P, Zhang H.B, Resta P. (1993). The evolution of polyploid wheats: identification of the A genome donor species. Genome, 36: 21-31.
- Dvorak J**, Mc Guire P.E, Cassidy B. (1988). Apparent sources of the A genome of wheats inferred from polymorphism in abundance, and restriction fragment length of repeated nucleotide sequences. Genome, 30: 680-689.
- Dvorak Z**, Ulrichova J, Modriansky M. (2005 a). Role of microtubules network in CYP genes expression. Curr Drug Metab, 6: 545-552.
- Ears of plenty**, 2011 (<http://www.heirloomorganics.com/guide/va/guidetogrowingwheat.html>) (How to Grow Wheat, Guide to Growing Wheat) and <http://www.economist.com/node/5323362> The Economist The story of wheat, Ears of plenty, The story of man's staple food.
- El Ouafi I**. (2001). Quantitative Trait Loci (QTL) determination of grain quality traits in durum wheat (*Triticum turgidum* L. var. durum), CORDOBA UNIVERSITY. ETSIAM, GENETIC DEPARTMENT CIMMYT/ICARDA DURUM JOINT PROGRAM. High Technical School of Agronomy and Forestall Engineering, Genetic Department.
- El-Kharbotly A**, Palomino-Sanchez C, Salamini F, Jacobsen E, Gebhardt C. (1996). R6 and R7 alleles of potato conferring race-specific resistance to *Phytophthora infestans* (Mont.) de Bary identified genetic loci clustering with the R3 locus on chromosome XI. Theoretical and Applied Genetics, 92: 880-884.
- Elouafi I**, Nachit M.M, Martin L.M. (2001). Identification of a microsatellite on chromosome 7B showing a strong linkage with yellow pigment in durum wheat (*Triticum turgidum* L. var. durum). Hereditas, 135: 255–261.
- Elouafi I**, Nachit M.M. (2004). A genetic linkage map of the Durum x *Triticum dicoccoides* backcross population based on SSRs and AFLP markers, and QTL analysis for milling traits. Theor Appl Genet, 108: 401-413.
- Endo T.R**, Gill B.S. (1996). The deletion stocks of common wheat. J Hered, 87: 295-307.
- Ersoz E.S**, Yu J.J, Buckler E.S. (2007). Applications of linkage disequilibrium and association mapping in crop plants. In Genomics-assisted Crop Improvement, in press. Eds. Varshney R, Tuberosa R. Springer.
- Esch E**, Weber W.E. (2002). Investigation of crossover interference in barley (*Hordeum vulgare* L.) using the coefficient of coincidence. Theor Appl Genet, 104:786-796.
- Eshed Y**, Zamir D. (1995). An introgression line population of *Lycopersicon pennelli* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics, 141: 1147-1162.
- Fahima T**, Peleg Z, Saranga Y, Suprunova T, Ronin Y, Marion S, Röder Andrzej K, Abraham B, Fahima T. (2008). High-density genetic map of durum wheat x wild emmer wheat based on SSR and DArT markers, Theoretical and Applied Genetics, 117: 103-115. DOI 10.1007/s00122-008-0756-9.

- Fahima T**, Sun G.L, Beharav A, Krugman T, Beiles A, Nevo E. (1999). RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel. Theoretical and Applied Genetics, 98: 434-447.
- Fan C**, Xing Y, Mao H, Lu T, Han B, Xu C, Li X, Zhang Q. (2006). GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112: 1164-1171.
- FAO**. (2010). Major Food And Agricultural Commodities And Producers - Countries By Commodity". Fao.org. <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567-ancor>. Retrieved 2010-05-21.
- Faris J.D**, Haen K.M, Gill B.S. (2000). Saturation mapping of a gene-rich recombination hot spot region in wheat. Genetics, 154:823-835.
- Federer W.T**. (1956). Augmented designs. In: Hawaiian planters, Record, 55: 191-208.
- Feillet P**, Dexter J.E. (1996). Quality requirements of durum wheat for semolina milling and pasta production. In: J.E. Kruger, R.B. Matsuo & J.W. Dick (Ed.). AACC, St. Paul, MN, USA. Pasta and Noddle Technology, pp. 95-131.
- Feldman M**, Sears E. (1981). The wild genes ressources of wheat. Sci Annu, 244: 98-109.
- Feng J**, Zuo L.L, Zhang Z.Y, Lin R.M, Cao Y.Y, Xu S.C. (2011). Quantitative trait loci for temperature-sensitive resistance to *Puccinia striiformis* f. sp. *tritici* in wheat cultivar Flinor. Euphytica, 178: 321-329. DOI 10.1007/s10681-010-0291-z.
- Field A**. (2005). Exploratory factors analysis. Discovering Statistics Using SPSS, Wright D.B, editor London: SAGE Publications, pp. 619-680.
- Flavell R.B**, Smith D.B. (1976). Heredity, 37: 231.
- Fleure F**, Davies T. (2001). story of Man in Britain. <http://www.farm-direct.co.uk/farming/stockcrop/wheat/wheatist.html>.
- Foolad M.R**, Arulsekhar S, Becerra V, Bliss F.A. (1995). A genetic map of *Prunus* based on an interspecific cross between peach and almond. TAG, 91: 262-269.
- Francki M.G**, Walker E, Crawford A.C, Broughton S, Ohm H.W, Barclay I, Wilson R.E, Mc Lean R. (2009). Comparison of genetic and cytogenetic maps of hexaploid wheat (*Triticum aestivum* L.) using SSR and DArT markers. Mol Genet Genomics, 281: 181-191. DOI 10.1007/s00438-008-0403-9.
- Frary A**, Nesbitt T.C, Grandillo S, Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert K.B, Tanksley S.D. (2000). fw2.2: A quantitative trait locus key to the evolution of tomato fruit size. Science, 289: 85-88.
- Friebe B**, Gill B.S. (1996). Chromosome banding and genome analysis in diploid and cultivated polyploid wheats. In P.P. Jauhar, ed. Methods of genome analysis in plants, Boca Raton FL USA, CRC Press: 39-60
- Friebe B**, Kim N.S, Kuspura J, Gill B.S. (1990). Genetic and cytogenetic analyses of the A genome of *Triticum monococcum*. VI. Production and identification of primary trisomics using the C-banding technique. Genome, 33: 542-555.
- Fulton T.M**, Beck-Bunn T, Emmatty D, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley S.D. (1997). QTL analysis in an advanced backcross of *Lycopersicon peruvianum* to the cultivated tomato and comparisons with a QTL found in other wild species. Theor Appl Genet, 95: 881-894.
- Gale M.D**, Atkinson M.D, Chinoy C.N, Harcourt R.L, Jia J, Li Q.Y, Devos K.M. (1995). Genetic maps of hexaploid wheat. In: Li Z.S, Xin Z.Y. (eds) Proceedings of 8th international wheat genetics symposium, China Agricultural Sciencetech Press, Beijing, 29-40.
- Gale M.D**, Marshall G.A, Gregory R.S, Quick J.S. (1981). Norin 10 Semi-Dwarfism in Tetraploid wheat and associated effects on Yield, Plant Breeding Institute, Maris Lane, Trumpington. Cambridge. CB2 2LQ, England.
- Ganal M.W**, Röder M.S. (2007). Microsatellite and SNP markers in wheat breeding. In: Varshney RK, Tuberosa R (eds) Genomics assisted crop improvement, volume 2. Genomics applications in crops. Springer, Berlin.

- Gao L.F**, Jing R.L, Huo N.X, Li Y, Li X.P, Zhou H.R, Chang X.P, Tang J.F, Ma Z.Y, Jia J.Z. (2004). One hundred and one new microsatellite loci derived from ESTs (EST-SSRs) in bread wheat. *Theoretical and Applied Genetics*, 108: 1392-1400.
- Gardiner J.M**, Coe E.H, Melia-Hancock S, Hoisington D.A, Chao S. (1993). Deveopment of a core RFLP map in maize using an immortalized F2 population. *Genetics*, 134: 917-930.
- Garg K**, Green P, Nickerson D.A. (1999). Identification of candidate coding region single nucleotide polymorphisms in 165 human genes using assembled expressed sequence tags, *Genome Research*, 9: 1087- 1092.
- Gebhardt C**, Ritter E, Barone T, Debener T, Walkemeier B, Schachtschabel U, Kaufman H, Thompson R.D, Bonierbale M.W, Ganal M.W, Tanksley S.D, Salamini F. (1991). RFLP maps of potato and their alignment with the homoeologous tomato genome. *Theor Appl Genet*, 83: 49-57.
- Gebhardt C**, Ritter E, Debener T, Schachtschabel U, Walkemeir B, Uhrig H, Salamini F. (1989). RFLP analysis and linkage mapping in *Solanum tuberosum*. *Theoretical and Applied Genetics*. 78: 65-75.
- Gegas V.C**, Nazari A, Griffiths S, Simmonds J, Fish L, Orford S, Sayers L, Doonan J.H, Snape J.W. (2010). A Genetic Framework for Grain Size and Shape Variation in Wheat. *Plant Cell*, 22(4): 1046-1056. DOI: 10.1105/tpc.110.074153.
- Germano J**, Klein A.S, (1999). Species specific nuclear and chloroplast single nucleotide polymorphisms to distinguish *Picea glauca*, *P. mariana* and *P. rubens*. *Theoretical and Applied Genetics*, 99: 37-49.
- Gill B.S**, Chen P.D. (1987). Role of cytoplasm-specific introgression in the evolution of the polyploid wheats. *Proc. Natl Acad Sci*, 84: 6800-6804.
- Gill I**, Lubber E.L, Gill B.S, Raupp W.J, Cox T.S. (1991). A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat. *Genome*, 34: 362-374.
- Gill K.S**, Gill B.S, Endo T.R, Boyko E.V. (1996 a). Identification of highdensity mapping of gene-rich regions in chromosome group 5 of wheat. *Genetics*, 143:1001-1012
- Gill K.S**, Gill B.S, Endo T.R, Taylor T. (1996 b). Identification and highdensity mapping of gene-rich regions in chromosome group 1 of wheat. *Genetics*, 144:1883-1891.
- Gill K.S**, Gill B.S. (1994). Mapping in the realm of polyploidy: the wheat model. *Bio Essays*, 16: 841-846.
- Gill K.S**, Lubbers E.L, Gill B.S, Raupp W.J, Cox T.S. (1991). A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). *Genome*, 34: 362-374.
- Giovanonni S. J**, Mullins T.D, Field K.G. (1995). Microbial diversity in oceanic systems: rRNA approaches to the study of unculturable microbes, p. 217-48. Zn I. Joint [ed.], *Molecular ecology of aquatic microbes*. Springer-Verlag.
- Giura A**, Saulescu N.N. (1996). Chromosomal location of genes controlling grain size in a large grained selection of wheat (*Triticum aestivum* L.). *Euphytica*, 89: 77-80.
- Gonzalez-Hernandez J.L**, Elias E.M, Kianian S.F. (2004). Mapping genes for grain protein concentration and grain yield on chromosome 5B of *Triticum turgidum* (L.) var. *dicoccoides*. *Euphytica*, 139: 217-225.
- Graner A**, Bauer E, Kellerman A, Kirchner S, Muraya J.K, Jahoor A, Wenzel G, (1994). Progress of RFLP map construction in winter barley. *Barley Genet Newslet*, 23: 53-61.
- Graner A**, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann R.G. (1991). Construction of an RFLP map of barley. *Theoretical and Applied Genetics*, 83: 250-256.
- Gregg T**. (2001). Crops of Britain. A Field Guide to the Crops of Britain and Europe - G M de Rougemont Foragers and Farmers . <http://www.farm-direct.co.uk/farming/stockcrop/wheat/wheathist.html>.
- Gu Z**, Hillier L, Kwok P.Y. (1998). Single nucleotide polymorphism hunting in cyberspace. *Hum Mutat*, 12: 221-225.
- Gupta P.K**, Balyan H.S, Edwards K.J, Isaac P, Korzun V, Röder M, Gautier M.F, Joudrier P, Schlatter A.R, Dubcovsky J, De la Pena R.C, Khairallah M, Penner G, Hayden M.J, Sharp P, Keller B, Wang R.C.C, Hardouin J.P,

- Jack P, Leroy P. (2002). Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. *Theoretical and Applied Genetics*, 105: 413-422
- Gupta P.K**, Roy J.K, Prasad M. (2001). Single nucleotide polymorphisms: a new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Curr Sci*, 80: 524-535.
- Gupta P.K**, Varshney R.K, Sharma P.C, Pamesh B. (1999). Molecular markers and their applications in wheat breeding. *Plant Breed*, 118: 369-390.
- Gupta P.K**. (2008). Ultrafast and low-cost DNA sequencing methods for applied genomics research. *Proc. Natl. Acad. Sci. India*, 78: 91-102.
- Gupta P.K**. Varshney R.K. (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113: 163-185.
- Guyomarc'h H**, Sourdille P, Edwards K.J, Bernard M. (2002). Studies of the transferability of microsatellites derived from *Triticum tauschii* to hexaploid wheat and to diploid related species using amplification, hybridization and sequence comparisons. *Theor Appl Genet*, 105: 736-744.
- Haldane J.B.S**, Waddington C.H. (1931). Inbreeding and linkage. *Genetics*, 16: 357-374.
- Hall N**, Hall A. (2011). Wheat's Genetic Code Cracked: Draft Sequence Coverage of Genome to Aid Global Food Shortage. *Biotechnology and Biological Sciences Research Council* (2010, August 27). Wheat's genetic code cracked: Draft sequence coverage of genome to aid global food shortage.
- Hamada H**, Petrino M.G, Kakunaga T. (1982). A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. *Proc Natl. Acad. Sci. USA*, 79: 6465-6469.
- Haman A**, Zink D, Nagl W. (1995). Microsatellite fingerprinting in the genus *Phaseolus*. *Genome*, 38: 507-515.
- Harlan J.C**. (1986). Plant domestication: Diffuse origin and diffusion. In: *The origin and domestication of cultivated plants*. Barigozzi C ed Elsevier, Amsterdam: 22-34.
- Harlan J.R**. (1987). *The living fields*. Cambridge university, press.
- Haudry A**, Cenci A, Ravel C, Bataillon T, Brunel D, Poncet C, Hochu I, Poirier S, Santoni S, Glemin S, David J. (2007). Grinding up wheat: a massive loss of nucleotide diversity since domestication. *Mol Biol Evol*, 24: 1506-1517. DOI: 10.1093/molbev/msm077.
- He Q**, Marjamaki M, Soini H, Mersola J, Viljanen M.K. (1994). Primers are decisive for sensitivity of PCR. *Bio. Techniques*, 17: 82-87.
- Hearne C.M**, Ghosh S, Todd J.A. (1992). Microsatellites for linkage analysis of genetic traits. *Trends Genet*, 8: 288-294.
- Helentjaris T**, King G, Wright S, Schaeffer A, Nienhuis J. (1986). Construction of linkage maps in maize and tomato based on isozymes and random cDNA sequences. *TAG*, 72: 761-769.
- Helentjaris T**, Weber D.F, Wright S. (1986). Use of monosomics to map cloned DNA fragments in maize. *Proc. Natl. Acad. Sci. USA*. 83: 6035-6039.
- Helentjaris T**. (1987). A genetic linkage map for maize based on RFLPs. *Trends Genet*, 3: 217-221.
- Hentschel V**, Kranl K, Hollmann J, Lindhauer M.G, Bohm V, Bitsch R. (2002). Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. *Agric Food Chem*. 50: 6663-6668.
- Heun M**, Kennedy A.E, Anderson J.A, Lapitan N.L.V, Sorrells M.E, Tanksley S.D. (1991). Construction of a restriction fragment length polymorphism map for barley (*Hordeum Vulgare*). *Genome*, 34: 437-447.
- Heun M.R**. (1997). Site of Einkorn Wheat Domestication Identified by DNA Fingerprinting. *Science*, 278: 1312- 1314. DOI: 10.1126/science.278.5341.1312.
- Hohmann U**, Graner A, Endo T.R, Gill B.S, Herrmann R.G. (1995). Comparison of wheat physical maps with barley linkage maps for group 7 chromosomes. *Theoretical and Applied Genetics*, 91: 618-626.

- Hoisington D**, Khairallah M, Gonzalez-De-Leon D. (1994). Laboratory protocols: CIMMYT Applied Molecular Genetics Laboratory. CIMMYT, Mexico.
- Huang F.C**, Klaus S.M.J, Herz S, Zuo Z, Koop H.U, Golds T.J. (2002). Efficient plastid transformation in tobacco using the *aphA-6* gene and kanamycin selection. *Mol Genet Genomics*, 268: 19-27.
- Huang S**, Sirikhachornkit A, Su XJ, Faris J, Gill B, Haselkorn R, Gornicki P. (2002). Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the *Triticum/Aegilops* complex and the evolutionary history of polyploid wheat. *Proc Natl Acad Sci USA*, 99: 8133-8138.
- Hudson M**. (2008). Sequencing breakthroughs for genomic ecology and evolutionary biology. *Mol. Ecol. Resour.* 8: 3-17.
- Hulbert S.H**, Richter T.E, Axtell J.D, Bennetzen J.L. (1990). Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. *Proc Natl Acad Sci USA*, 87: 4251-4255.
- Jaccoud D**, Peng K, Feinstein D, Kilian A. (2001). Diversity arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research* Feb 15, 29 (4): E25.
- Jansen R.C**, and Nap J.P. (2001). Genetical genomics: the added value from segregation. *Trends Genet.* 17: 388-391.
- Jansen R.C**, Stam P. (1994). High resolution of quantitative traits into multiple loci via interval mapping. *Genetics*, 136: 1447-1455.
- Jauhar P.P**, Riera-Lizarazu O, Dewey W.G, Gill B.S, Crane C.E, Bennett J.H. (1991). Chromosome pairing relationships among the A, B, and D genomes of bread wheat. *Theor Appl Genet*, 82: 441-449.
- Jia J**, Devos K.M, Chao S, Miller T.E, Reader S.M, Gale M.D. (1996). RFLP -based maps of the homoeologous group 6 chromosomes of wheat and their application in the tagging of *pm12*, a powdery mildew resistance gene transferred from *Aegilops speltoides* to wheat. *TAG*, 91: 559-565.
- Jiang J**, Gill B.S. (1994). New 18S-26S ribosomal RNA gene loci: chromosomal landmarks for the evolution of polyploid wheats. *Chromosoma*, 103: 179-185.
- Jimenez M**, Dubcovsky J. (1999). Chromosome location of genes affecting polyphenol oxidase activity in common and durum wheat seeds. *Plant Breeding*, 118: 395-398.
- Johnson B.L**, Dhaliwal H.S. (1976). Reproductive isolation of *Triticum boeoticum* and *Triticum urartu* and the origin of the tetraploid wheat. *Am J Bot*, 63: 1088-1094.
- Jolliffe I.T**. (2002). Principal Component Analysis. New York Springer.
- Joppa L.R**, Cantrell R.G. (1990). Chromosomal location of genes for grain protein content of wild tetraploid wheat. *Crop Sci*, 30: 1059-1064.
- Joppa L.R**, Du C, Hart G.E, Hareland G.A. (1997). Mapping a QTL for grain protein in tetraploid wheat (*Triticum turgidum* L.) using a population of recombinant inbred chromosome lines. *Crop Sci*, 37: 1586-1589.
- Joppa L.R**, Nevo E, Beiles A. (1995). Chromosome translocations in wild populations of tetraploid emmer wheat in Turkey. *Theor Appl Genet*, 91: 713-719.
- Joppa L.R**, Williams N.D. (1998). Langdon durum substitution lines and aneuploid analysis in tetraploid wheat. *Genome*, Volume 30: 222-228.
- Joppa L.R**. (1993). Chromosome engineering in tetraploid wheat. *Crop Sci*, Volume 33: 908-913.
- Joppa L.R.**, Nevo E, Beiles A. (1995). Chromosome translocations in wild populations of tetraploid emmer wheat in Turkey. *Theor Appl Genet*, 91: 713-719.
- Kam-morgan N.W**, Gill B.S, Muthukrishnain S. (1989). DNA restriction fragment length polymorphisms: a strategy for genetic mapping of D genome of wheat. *Genome*, 32: 724-732.
- Karl W**, Broman T. (2005). The Genomes of Recombinant Inbred Lines, Department of Biostatistics, Johns Hopkins University, Baltimore, Maryland 21205, the Genetics Society of America. DOI: 10.1534/genetics.104.035212.

- Karp A**, Kresovich S, Bhat K.V, Ayad W.G, Hodgkin T. (1997). Molecular tools in plant genetic resources conservation: A guide to the technologies; In: IPGRI Technical Bulletin No. 2. International Plant genetic Resources Institute, Rome, Italy.
- Kawahara T**, Nevo E. (1996). Screening of spontaneous major translocations in Israeli populations of *Triticum dicoccoides* Koern. Wheat Inf Serv, 83: 28-30.
- Khairallah M.M**, Ortiz-Monasterio I, Skovmand B, Reynolds M.P, Smale M, Warburton M, Singh R.P, Crossa J, Almanza Pinzon M.I, Trethowan R.M. (2008). Dimensions of diversity in CIMMYT bread wheat, CIMMYT, International Maize and Wheat Improvement Center.
- Kimber G**, Feldman M. (1987). Wild wheat. An introduction. Special Report 353 College of Agriculture. University of Missouri, Columbia, USA.
- Kintzios S**. (1994). Distorted segregation for mildew resistance in double haploid lines of spring barley Plant Breeding 112: 248-251.
- Kirby E.J.M**, Appleyard M. (1987). Cereal development guide. Stoneleigh, Kenilworth, UK, NAC Cereal Unit. 85pp.
- Kleinhofs A.A**, Kilian A, Saghai-Maroo M.A, Biyashev R.M, Hayes P, Chen F.Q, Lapitan N, Fenwick A, Balke T.K, Kanazin V, Ananiev E, Dahlleen L, Kudrna D, Bollinger J, Knapp S.J, Liu B, Sorrells M, Heun M, Franckowiak J.D, Hoffman D, Skadsen R, Steffenson B.J. (1993). A molecular, isozyme and morphological map of the barley (*Hordeum Vulgare*) genome. TAG, 86: 705-712.
- Kobner R.M.D**. (1995 a). Generation of PCR-based markers for the detection of rye chromatin in a wheat background. Theoretical and Applied Genetics, 90: 740-745.
- Kobner R.M.D**. (1995 b) Predigestion of DNA template improves the level of polymorphism of RAPDs in wheat. Genetic Analysis-Biomolecular Engineering, 12, 63-67.
- Korol A.B**, Preygel I.A, Nyutin Y.I. (1989). Quasi-linkage in tomato: Formal analysis. Genetika 25: 2225-2233.
- Korol A.B**, Preygel I.A, Preygel S.I. (1994). Recombination variability and evolution - algorithms of estimation and population genetics models. Chapman and Hall, London, UK.
- Korzun V**, Boerner A, Worland A.J, Law C.N, Roder M.S. (1997). Application of microsatellite markers to distinguish inter-varietal chromosome substitution lines of wheat L. *Triticum aestivum*. Euphytica, 95: 149-155.
- Korzun V**, Röder M.S, Wendekake K, Pasqualone A, Lotti C, Ganai M.W, Blanco A. (1999). Integration of dinucleotide microsatellites from hexaploid bread wheat into a genetic linkage map of durum wheat. TAG, 98: 1202-1207.
- Korzun V**. (2003). Molecular Markers and their applications in cereals breeding, Marker assisted selection: a fast track to increase genetic gain in plant and animal breeding, Lochow-Petkus GmbH, Grimsehlstr. 24, 37574Einbeck, Germany.
- Kosambi D.D**. (1944). The estimation of map distances from recombination values. Annu Eugen, 12: 172-175.
- Kota R.S**, Gill K.S, Gill B.S, Endo T.R. (1993). A cytogenetically based physical map of chromosome 1B in common wheat. Genome, 36: 548-554.
- Kruger J.E**, Morgan B, Matsuo R.R, Preston K.R, 1992. A comparison of methods for the prediction of Cantonese noodle color. Can J Plant Sci, 72: 1021-1029.
- Kruglyak L**. (1997). The use of a genetic map of biallelic markers in linkage studies. Nat Genet, 17: 21-24. DOI: 10.1038/ng0997-21.
- Kurata N**, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio B.A, Shomura A, Shimizu T, Lin S.Y, Inoue T, Fukuda A, Shimano T, Yuboki Y, Tomaya T, Miyamoto Y, Kiriara T, Hayasaka K, Miyao A, Monna L, Zhong H.S, Tamura Y, Wang Z.X, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y. (1994). A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nat genet, 8: 365-372.
- Kwok P.Y**, Carlson C, Yager T.D, Ankener W, Nickerson D.A. (1994). Comparative analysis of human DNA variations by fluorescence-based sequencing of PCR products. Genomics, 23: 138-144.

- Lagercrantz U**, Ellegren H, Andersson L. (1993). The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. *Nucleic Acids Res*, 21: 1111-1115.
- Lagudah E.S**, Appels R, Brown A.H.D, Mc Neil D. (1991). The molecular-genetic analysis of *Triticum tauschii*, the D genome donor to hexaploid wheat. *Genome*, 34: 375-386.
- Lander E**, Botstein D. (1989). Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, 121: 185-199.
- Lander E.S**, Green P, Abrahanson J, Barlow A, Daly M.J, Lincon S.E. (1987). MAPMAKER: an interactive computing package for constructing primary genetic linkages of experimental and natural populations. *Genomics*, 1: 174-181.
- Lange D.A**, Peuela S, Denny R.L, Mudge J, Orf J.H, Young N.D. (1998). A plant DNA isolation protocol suitable for polymerase chain reaction marker-assisted selection. *Crop Science*, 38: 217-220.
- Lashermes P**, Couturon E, Charrier A. (1994 a). Combining ability of doubled haploids in *Coffea canephora* P. *Plant Breed*, 112: 330-337.
- Lashermes P**, Couturon E, Charrier A. (1994 b). Doubled haploids of *Coffea canephora*: development, fertility and agronomic characteristics. *Euphytica* 74:149-157.
- Le Corre E**, Bernardi M. (1995). Assessment of the type and degree of restriction fragment length polymorphism (RFLP) in diploid species of the genus *Triticum*. *Theor Appl Genet*, 90: 311-325, 273-284, 1063-1067.
- Li J**, Cui F, Ding A, Zhao C, Wang X, Wang L, Bao Y, Qi X, Li X, Gao J, Feng D, Wang H. (2011). QTL detection of seven quality traits in wheat using two related recombinant inbred line populations. *Euphytica*, Springer Science+Business Media B.V. 2011. DOI 10.1007/s10681-011-0448-4.
- Li J**, Thomson M, McCouch S.R. (2004). Fine mapping of a grain-weight quantitative trait locus in the pericentromeric region of rice chromosome 3. *Genetics*, 168: 2187-2195.
- Li W.L**, Faris J.D, Chittoor J.M, Leach J.E, Hulbert S.H, Liu D.J, Chen P.D, Gill B.S. (1999). Genomic mapping of defense response genes in wheat. *Theor Appl Genet*, 98: 226-233.
- Lijavetzky D**, Muzzi G, Wicker T, Keller B, Wing R, Dubcovsky J. (1999). Construction and characterization of a bacterial artificial chromosome (BAC) library for the A genome of wheat. *Genome*, 42: 1176-1182.
- Litt M**, Luty J.A. (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet*, 44:397-401.
- Liu C.J**, Devos K.M, Chinoy C.N, Atkinson M.D, Gale M.D. (1992). Non-homoeologous translocations between group-4, 5 and 7 chromosomes in wheat and rye. *Theor Appl Genet* 83: 305-312.
- Liu C.Y**, Shepherd K.W. (1996). Variation of B subunits of glutenin in durum, wild and less-widely cultivated tetraploid wheats. *Plant breeding*, 115: 172-178.
- Liu J**, Van Eck J, Cong B, Tanksley S.D. (2002). A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. *Proc Natl Acad Sci USA*, 99: 13302-13306.
- Liu S.C**, Kowalski S.P, Lan T.H, Feldmann K.A, Paterson A.H. (2005). Genome-wide high-resolution mapping by recurrent intermating using *Arabidopsis thaliana* as a model. *Genetics*, 142: 247-258.
- Liu Y.G**, Mori N, Tsunewaki K. (1990). Restriction fragment length polymorphism (RFLP) analysis in wheat. I. genomic DNA library construction and RFLP analysis in common wheat. *Jpn J Genet*, 65: 367-380.
- Liu Y.G**, Tsunewaki K. (1991). Restriction fragment length polymorphism analysis of wheat. II. Linkage maps of the RFLP sites in common wheat. *Jap J Genet*, 66: 617-633.
- Liu Z.H**, Anderson A.J, Hu J, Friesen T.L, Rasmussen J.B, Faris J.D. (2005). A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. *Theoretical and Applied Genetics*, 111: 782-794.
- Liu Z.W**, Biyashev R.M, Saghai Maroof M.A. (1996). Development of simple sequence repeat DNA markers and their integration into a barley linkage map. *Theor. Appl. Genet*, 93: 869-876.

- Lotti C.** (2000). Integrazione di marcatori AFLP e SSR in una mappa di frumento duro e mappatura di QTL per il contenuto proteico delle Cariossidi. PhD Teses, university of Bari, Dipartimento di Biologia e Chimica Agroforestale ed Ambientale. Triennio accademico 1998-2000.
- Lu H,** Romero-Severson J, Bernardo R. (2002). Chromosomal regions associated with segregation distortion in maize. *Theoretical and Applied Genetics*, 105: 622-628.
- Lunz U.** (1990). Thermocycler temperature variation invalidates PCR results. *Bio. Techniques*, 9: 286-291.
- Lyttle T.W.** (1991). Segregation distorters. *Annu Rev Genet*, 25: 511-557.
- Ma Z.Q,** Röder M, Sorrells M.E. (1996). Frequencies and sequence characteristics of di-, tri-, and tetra-nucleotide microsatellites in wheat. *Genome*, 39: 123-130.
- Maccaferri M,** Sanguineti M.C, Corneti S, Araus Ortega J.L, Ben Salern M, Bort J, De Ambrogio E, del Moral L.G, Demontis A, El-Ahmed A, Maalouf F, Machlab H, Martos V, Moragues M, Motawaj J, Nachit M.M, Nserallah N, Ouabbou H, Royo C, Slama A, Tuberosa R. (2008). Quantitative trait loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability. *Genetics*, 178: 489-511.
- Mackay T.F.C.** (2001). The genetic architecture of quantitative traits. *Annu Rev Genet*, 35: 303-339.
- Madabhushi R.S.** (1998). Separation of 4-color DNA sequencing extension products in noncovalently coated capillaries using low viscosity polymer solutions. *Electrophoresis*, 19: 224-230.
- Malinowsky E.** (1927). The hypothesis of chromosome affinity and the phenomenon of suppression of characters on crossing. *J. Genet*, 18: 223-231.
- Mamluk O.F,** Van Leur J. (1986). Transmission of seed-borne diseases of wheat and barley. In J.P. Srivastava and T.L. Simarski, eds. *Seed production technology*. Aleppo, Syria, ICARDA.
- Marcel T.C,** Varshney R.K, Barbieri M, Jafary H, De Kock M.J.D, Graner A, Niks R.E. (2007). A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Puccinia hordei* and of defence gene homologues. *Theor Appl Genet*, 114: 487-500.
- Markers,** *Theor Appl Genet* 107: 1002-1112.
- Marth G.T,** Korf I, Yandell M.D, Yeh R.T, Gu Z.J, Zakeri H, Stitzel N.O, Hillier L, Kwok P.Y, Gish W.R. (1999). A general approach to singlenucleotide polymorphism discovery. *Nat Genet*, 23: 452-456.
- Matsuo R.R,** Bradley J.W, Irvine G.N. (1972). Effect of protein content on the cooking quality of spaghetti. *Cereal Chem*, 49: 707-711.
- Matsuo R.R,** Dexter J.E, Kosmolak F.G, Leslie D. (1982). Statistical evaluation of tests for assessing spaghetti-making quality of durum wheat. *Cereal Chem*, 59: 222-228.
- Matus-Cádiz M.A,** Pozniak C.J, Hughes G.R, Hucl P. (2006). Multiplexed PCR to screen for a major QTL carrying fusarium head blight resistance in Sumai-3 wheat, *Canadian Journal of Plant Science*, pubs.aic.ca by 77.95.137.67 on 08/25/11.
- Mc Couch S.R,** Kochert G, Yu Z.H, Wang Z.Y, Khush G.S, Coffman W.R, Tanksley S.D. (1988). Molecular mapping of rice chromosomes. *Theoretical and Applied Genetics*, 76: 815-829.
- Mc Grath J.M,** Quiros C.F. (1991). Inheritance of isozyme and RFLP markers in *Brassica campestris* and comparison with *B. oleracea*. *Theor Appl Genet*, 82: 668-673.
- Mc Guire P.E,** Qualset C.O. (1997). Progress in genomemapping of wheat and related species. Joint Proceedings of the 5th and 6th Public Workshops of the International Triticeae Mapping Initiative, Genetic Resources Conservation Program Report No. 18, Univ. California, Div. Agric. Natural Resources.
- McIntosh R.A,** Hart G.E, Gale M.D. (1995). Catalogue of gene symbols for wheat. In: Li ZS, Xin ZY (eds) *Proc 8th Int Wheat Genet Symp*. China Agricultural Sciencetech Press, Beijing, pp: 29-40.

- Melchinger A.E**, Lee M, Lamkey K.R, Hellauer R, Woodman W.L. (1990). Genetic diversity for restriction fragment length polymorphisms and heterosis for two diallel sets of maize inbreds. *Theoretical and Applied Genetics*, 80: 488-496.
- Menancio-Hautea D**, Kumar L, Danesh D, Young N.D. (1993). RFLP linkage map for mungbean (*Vigna radiata* (L.) Wilczek) in: *Genetic Maps, 1992*. S. J. O'Brien, ed. Cold Spring Harbor Press, Cold Spring Harbor, NY, pp. 6.259-6.260.
- Messeguer R**, Ganai M, de Vicente M.C, Young M.D, Bolkan H, Tanksley S.D. (1991). High resolution RFLP map around the root knot nematode resistance gene (Mi) in tomato. *Theor Appl Genet*, 82: 529-536
- Messmer M.M**, Keller M, Zanetti S, Keller B. (1999). Genetic linkage map of wheat x spelt cross. *Theoretical and Applied Genetics*, 98: 1163-1170.
- Mester D.I**, Ronin Y.I, Hu Y, Peng J, Nevo E, Korol A.B. (2003a). Eycient multipoint mapping: making use of dominant repulsionphase markers. *Theor Appl Genet*, 107: 1002-1112.
- Mester D.I**, Ronin Y.I, Minkov D, Nevo E, Korol A.B. (2003 b). Constructing large-scale genetic maps using an evolutionary strategy algorithm. *Genetics*, 165: 2269-2282.
- Mester DI**, Ronin YI, Nevo E, Korol A.B. (2004) Fast and high precision algorithms for optimization in large scale genomic problems. *Comput Biol Chem*, 28: 281-290.
- Michelmore R.W**, Paran I, Kesseli R.V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc Natl Acad Sci USA*, 88: 9828-9832.
- Michie D**. (1953). Affinity: A new genetic phenomenon in the house mouse. *Nature*, 171: 26-27.
- Mickelson-Young L**, Endo T.R, Gill B.S. (1995). A cytogenetic ladder-map of the wheat homeologous group 4 chromosomes. *Theoretical and Applied Genetics*, 90: 1007-1011.
- Mingeot D**, Jacquemin J.M. (1999). Mapping of RFLP probes characterized for their polymorphism on wheat. *Theor Appl Genet*, 98: 1132-1137.
- Miskelly D.M**. (1984). Flour components affecting paste and noodle colour. *J Sci Food Agric*, 35: 463-471.
- Mogg R**, Batley J, Hanley S, Edwards D, O'Sullivan H, Edwards K.J. (2002). Characterising the flanking regions of Zea mays microsatellites reveals a large number of useful sequence polymorphisms. *Theor Appl Genet*, 105: 532-543.
- Moore G**, Devos K.M, Wang Z, Gale M.D. (1995). Grasses, line up and form a circle. *Curr Biol*, 5: 737-739.
- Morgante M**, Olivieri A.M. (1993). PCR-amplified microsatellites as markers in plant genetics. *Plant J*, 3: 175-182.
- Morrell P.L**, Roleno D.M, Lundy K.E, Clegg M.T. (2005). Low levels of linkage disequilibrium in wild barley (*Hordeum vulgare* ssp. *spontaneum*) despite high rates of self-fertilization. *Proc Natl. Acad. Sci. USA*, 102: 2442-2447. DOI: 10.1073/pnas.0409804102.
- Morrell P.L**, Toleno D.M, Lundy K.E, Clegg M.T. (2006). Estimating the contribution of mutation, recombination and gene conversion in the generation of haplotypic diversity. *Genetics*, 173: 1705-1723. DOI: 10.1534/genetics.105.054502.
- Morris C.F**. (2002). Puroindolines: the molecular genetic basis of wheat grain hardness. *Plant Mol Biol*, 48: 633-647.
- Morris R**, Sears E.R. (1967). The cytogenetics of wheat and its relatives. In: Quinsberry and Reitz (eds). Madison, 19-87.
- Morton N.E**. (1955). Sequential tests for the detection of linkage. *American Journal of Human Genetics*, 7: 277-318.
- Muehlbauer G.J**, Staswick P.E, Specht J.E, Graef G.L, Shoemaker R.C, Keim P. (1991). RFLP mapping using near-isogenic lines in the soybean [*Glycine max* (L.) Merr.]. *Theor Appl Genet*, 81: 189-198.
- Murigneux A**, Baud S, Becket M. (1993). Molecular and morphological evaluation of double-haploid lines in maize. 2. Comparison with single-seed-descent lines. *TAG*, 87: 278-287.

- Nachit M.M.**, Asbati A, Azrak M, Rbeiz N, El Saleh A. (1995). Durum breeding research to improve dryland productivity in the Mediterranean region. Cereal Improvement Program Ann Report, 1995: 95-96.
- Nachit M.M.**, El ouafi I, Pagnotta M.A, El saleh A, lacono E, Labhilili M, Asbati A.P, Azrak M, Hazzam H, Benscher D, Khairallah M, Ribaut J.M , Tanzarella O.A, Porceddu E, Sorrells M.E. (2001). Molecular linkage map for an intraspecific recombinant inbred population of durum wheat (*Triticum turgidum* L. var. durum). TAG, 102: 177-186.
- Nachit M.M.**, Monneveux P, Araus J.L, Sorrells M.E. (2000). Relationship of dryland productivity and drought tolerance with some molecular markers for possible MAS in durum (*Triticum turgidum* L. var. durum). Seminaires Mediterraneens CIHEAM/ Udl-IRTA/ CIMMYT/ ICARDA, Serie A, 40: 191-201.
- Nachit M.M.** (1989). Moisture stress tolerance and yield stability in durum wheat (*Triticum turgidum* L. var. durum) under Mediterranean conditions, XII EUCARPIA Congress, Feb. 27- March 4, Goettingen, Germany.
- Nachit M.M.** (1992). Durum wheat breeding for Mediterranean dryland of North Africa and West Asia. Paper presented at Durum Wheat Workshop "Discussion on Durum Wheat: Challenges and Opportunity". CIMMYT, Ciudad Obregon, Mexico. March 23 – 25: 14-27.
- Nachit M.M.** (1998). Durum dryland breeding for productivity improvement in the Mediterranean region. In: VI Giornate Internazionali sul grano Duro, Apr 30 –May 2, Foggia, Troccoli, De Vita and Di Fonzo (eds): 179-192.
- Naghi T.M.**, Kiani K, Mazlouman S.J, Taheri A, Kamrani R.S, Panjavi B, Harandi B.A. (2008). Musculoskeletal injuries associated with earthquake. A report of injuries of Iranian December 26, 2007 Bam earthquake casualties managed in tertiary referral centres. Injury, 36 (1): 27-32.
- Nair S.K.**, Tomar S.M.S. (2001). Genetics of phenol colour reaction of grains and glumes in tetraploid and hexaploid wheats. J Genet Breed, 55: 369-373.
- Nakagahra M.** (1986). Geographic distribution of gametophyte genes in wide crosses of rice cultivars. In Rice genetics-Proceedings of the international rice genetics symposium (ed. G.S. Khush). IRRI, Manila, pp: 73-82.
- Nelson J.C.**, Allen E, Van Deynze K, Autrique E, Sorrells E, Hall Lu Y, Merlino M, Atkinson M, Leroy P. (1995 a). Molecular mapping of wheat, homoeologous group two. TAG, 38: 516-524.
- Nelson J.C.**, Van Deynze A, Autrique E, Sorrells M, Hai Lu Y, Negre S, Bernard M, Leroy P. (1995b). Molecular mapping of wheat, homoeologous group 3. Genome, 38: 525-533.
- Nicot N.**, Chiquet V, Gandon B, Amilhat L, Legeai F, Leroy P, Bernard M, Sourdille P. (2004). Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags (ESTs). Theoretical and Applied Genetics 109: 800-805.
- Nikiforov T.T.**, Rendle R.B, Goelat P, Rogers Y-H, Kotewicz M.L, Anderson S, Trainor G.L, Knapp M.R. (1994). Genetic bit analysis: a solid phase method for typing single nucleotide polymorphisms. Nucleic Acids Res, 22: 4167-4175.
- Nordborg M.**, Weigel D. (2008). Next-generation genetics in plants. Nature, 456: 720-723.
- O'Donoghue L.S.**, Kianian S.F, Rayapati P.J, Penner G.A, Sorrells M.E, Tanksley S.D, Phillips R.L, Rines H.W, Lee M, Fedak G, Molnar S.J, Hoffman D, Salas C.A, Wu B, Autrique E, Van Deynze A. (1995). A molecular map of cultivated oat. Genome, 38: 368-380.
- O'Donoghue L.S.**, Wang Z, Röder M, Kneen B, Leggett M, Sorrells M.E, Tanksley S.D. (1992). An RFLP –based map of oat on a cross between two diploid taxa (*Avena atlantica* x *A. hirtula*). Genome, 35: 765-771.
- Okamoto S.**, Okamoto U. (1962). Keio journal of Medicine, pp: 11-105.
- Ossowski S.**, Richard S.K, Clark M, Christa L, Warthmann N, Weigel D. (2008). Sequencing of natural strains of *Arabidopsis thaliana* with short reads. Genome Res, 12: 2024-2033.
- Ozbek O.**, Millet E, Anikster Y, Arslan O, Feldman M. (2007). Spatio-temporal genetic variation in populations of wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides* , as revealed by AFLP analysis. TAG Theoretical and Applied Genetics, Volume 115, Number 1: 19-26. DOI: 10.1007/s00122-007-0536-y.

- Ozkan H**, Brandolini A, Schäfer-Pregl R, Salamini F. (2002). AFLP analysis of a collection of tetraploid wheats indicates the origin of emmer and hard wheat domestication in southeast Turkey. *Molecular Biology and Evolution*, 19 (10): 1797-801. PMID 12270906.
- Pagnotta M.A**, Farina A., Santangelo F., Porceddu E. (2006), Analisi del linkage disequilibrium e della biodiversità per tolleranza a stress idrici in frumento duro, Frumisis, U.O. Viterbo, Università degli studi della Tuscia.
- Paillard S**, Schnurbusch T, Winzeler M, Messmer M, Sourdille P, Abderhalden O, Keller B, Schachermayr G. (2003). An integrative genetic linkage map of winter wheat (*Triticum aestivum* L.). *Theor Appl Genet*, 107: 1235-1242.
- Panio G**. (2011). Study of genetic bases of traits involved in resistance to biotic and abiotic stresses in durum wheat (*T. turgidum* L. subsp. *durum* (Desf.) Husn.), PhD Tesis in Plant Biotechnology XXIII Cycle, Agrobiology and agrochemistry department.
- Paterson A.H**, Lander E.S, Hewitt J.D, Peterson S, Lincon S.E, Tanksley S.D. (1988). Resolution of quantitative traits into Mendelian factors, using a complete linkage map of restriction fragment length polymorphism. *Nature*, 335: 721-726.
- Paull J.G**, Chalmers K.J, Karakousis A, Kretschmer J.M, Manning S, Langridge P. (1998). Genetic diversity in Australian wheat varieties and breeding material based on RFLP data. *Theoretical and Applied Genetics*, 96: 435-446.
- Paull J.G**, Pallotap M.A, Langridge T, THE T.T. (1994). RFLP markers associated with Sr22 and recombination between chromosome 7A of bread wheat and the diploid species *Triticum boeoticum*. *Theor Appl Genet*, 89: 1039-1045.
- Paux E**, Sourdille P, Salse J, Saintenac C, Choulet F, Leroy P, Korol A, Michalak M, Kianian S, Spielmeier W, Lagudah E, Somers D, Kilian A, Alaux M, Vautrin S, Berges H, Eversole K, Appels R, Safar J, Simkova H, Dolezel J, Bernard M, Feuillet C. (2008). A physical map of the 1–gigabase bread wheat chromosome 3B. *Science* 322: 101-104.
- Peleg Z**, Saranga Y, Suprunova T, Ronin Y.W, Marion T, Röder M.S, Kilian A, Abraham B.K, Fahima T. (2008). High-density genetic map of durum wheat x wild emmer wheat based on SSR and DArT markers, *Theoretical and Applied Genetics*, 117: 103-115. DOI 10.1007/s00122-008-0756-9.
- Peng J.H**, Korol A.B, Fahima T. (2011). Molecular Genetic Maps in Wild Emmer Wheat, *Triticum dicoccoides*: Genome-Wide Coverage, Massive Negative Interference and Putative Quasi-Linkage.
- Peng J**, Richards D.E, Hartley N.M. (1999 b). ‘Green Revolution’ genes encode mutant gibberellin response modulators. *Nature*, Volume: 400: 256-261.
- Peng J.H**, Fahima T, Roder M.S, Li Y.C, Dahan A, Grama A, Ronin Y.I, Korol A.B, Nevo E. (1999 a). Microsatellite tagging of stripe-rust resistance gene YrH52 derived from wild emmer wheat, *Triticum dicoccoides*, and suggestive negative crossover interference on chromosome 1B. *Theoretical and Applied Genetics* 98: 862-872.
- Peng J.H**, Fahima T, Röder M.S, Li Y.C, Grama A, Ronin Y.I, Korol A.B, Nevo E. (2000). Molecular genetic maps in wild emmer wheat, *Triticum dicoccoides*: genome-wide coverage, massive negative interference, and putative quasi-linkage. *Genome Research*, 10:1059-1031.
- Pereira M.G**, Lee M, Bramel-Cox P, Woodman W, Doebley J, Whitkus R. (1994). Construction of an RFLP map in sorghum and comparative mapping in maize. *Genome*, 37: 236-243.
- Perretant M.R**, Cadalen T, Charmet G, Sourdille P, Nicolas P, Boeuf C, Tixier M.H, Branlard G, Bernard S, Bernard M. (2000). QTL analysis of bread-making quality in wheat using a doubled haploid population. *Theor Appl Genet* 100: 1167-1175.
- Pestsova E**, Ganai M.W, Röder M.S. (2000 c) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. *Genome* 43: 689-697.
- Pestsova E**, Korzun V, Goncharov N.P, Hammer K, Ganai M.W, Röder M.S. (2000 b) Microsatellite analysis of *Aegilops tauschii* germplasm. *Theoretical and Applied Genetics*, 101: 100-106.

- Pestsova E**, Salina E, Borner A, Korzun V, Maystrenko O.I, Röder M.S. (2000 a). Microsatellites confirm the authenticity of intervarietal chromosome substitution lines of wheat (*Triticum aestivum* L). *Theoretical and Applied Genetics*, 101: 95-99.
- Petersen G**, Seberg O, Yde M, Berthelsen K. (2006). Phylogenetic relationships of *Triticum* and *Aegilops* and evidence for the origin of the A, B, and D genomes of common wheat (*Triticum aestivum*). *Mol Phylogenet Evol*, 39: 70-82.
- Pfeiffer W.H**, Sayre K.D, Reynolds M.P. (2000). Enhancing genetic grain yield potential and yield stability in durum wheat in Options Mediterraneennes, Serie A, Seminaires Mediterraneennes, edited by C. Royo C, Nachit M.M, Di Fonzo N, Araus J, Zaragoza I.L, Spain. Volume. 40: 83-93.
- Phillips R.L**, Vasil I.K. (2001). *DNA-Based Markers in Plants*, 2nd Edition, Kluwer Academic Publishers. The Netherlands.
- Picoult-Newberg L**, Ideker T.E, Pohl M.G, Taylor S.L, Donaldson M.A, Nickerson D.A, M, Boyce-Jacino M. (1999). Mining SNPs from EST databases, *Genome Research*, 9: 167- 174.
- Plaschke J**, Ganai M.W, Röder M.S. (1995). Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theoretical and Applied Genetics*, 92: 1078–1084.
- Portyanko V.A**, Chen G, Rines H.W, Philips R.L, Leonard K.J, Ochocki G.E, Stuthman D.D. (2005). Quantitative trait loci for partial resistance to crown rust, *Puccinia coronata*, in cultivated oat, *L. Avena sativa*. *Theoretical and Applied Genetics*, 111: 313-324.
- Powell W**. (1996). Polymorphism revealed by simple sequence repeats. *Trends Plant Sci*, 1: 215-222.
- Prince J.P**, Pochard E, Tanksley S.D. (1993). Construction of a molecular linkage map of pepper and a comparison of synteny with tomato. *Genome*, 36: 404-417.
- Prober J.M**, Prober J.M, Trainor G.L, Dam R.J, Hobbs F.W, Robertson C.W, Zagursky R.J, Cocuzza A.J, Jensen M.A, Baumeister K. (1987). A system for rapid DNA sequencing with fluorescent chain terminating dideoxynucleotides. *Science*, 238: 336-341.
- Qi L.L**, Echalié B, Chao S, Lazo G.R, Butler G.E, Anderson O.D, Akhunov E.D, Dvolák J, Linkiewicz A.M, Ratnasiri A, Dubcovsky J, Bermudez-Kandianis C.E, Greene R.A, Kantety R, La Rota M, Munkvold J.D, Sorrells S.F, Sorrells M.E, Dilbirligi M, Sidhu D, Erayman M, Randhawa H.S, Sandhu D, Bondareva S, Gill K.S, Mahmoud A.A, Ma X.F, Miftahudin, Gustafson J.P, Conley E.J, Nduati V, Gonzalez-Hernandez J.L, Anderson J.A, Peng J.H, Lapitan N.L.V, Hossain K.G, Kalavacharla V, Kianian S.F, Pathan M.S, Zhang D, Nguyen H.T, Choi D.W, Close T.J, McGuire P.E, Qualset C.O, Gill B.S. (2004). A chromosome bin map of 16,000 EST loci and distribution of genes among the three genomes of polyploidy wheat. *Genetics*, 168:701-712.
- Quarrie S.A**, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusi T.D, Waterman E, Weyen E.J, Schondelmaier J, Habash D.Z, Farmer P, Saker L, Clarkson D.T, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti M-C, Hollington P.A, Aragués R, Royo A, Dodig D. (2005). A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring x SQ1 and its use to compare QTLs for grain yield across a range of environments. *Theor Appl Genet*, 110:865-880.
- Qui X**, Stam P, Lindhout P. (1996). Comparison and integration of four barley genetic maps. *Genome*, 39: 379-394.
- Rafalski A**. (2002 a). Applications of single nucleotide polymorphisms in crop genetics. *Curr. Opin. Plant. Biol.* 5: 94-100.
- Rafalski A**. (2002 b). Novel genetic mapping tools in plants: SNPs and LD-based approaches. *Plant Sci*, 162: 329-333.
- Rafalski J.A**, Tingey S.V, Williams J.G.K. (1991). RAPD markers a new technology for genetic mapping and plant breeding. *Ag. Biotech. News. Info*, 3: 645-648.
- Raman R**, Raman H, Johnstone K, Lisle C, Smith A, Martin P, Allen H. (2005). Genetic and silico comparative mapping of the polyphenol oxidase gene in bread wheat (*Triticum aestivum* L.). *Funct Integ Genom*, 6: 185-200.
- Ranjekar P.K**, Pallotta and Lafontaine J.G. (1976). *Biochim. Biophys. Acta*, 425: 30.

- Ravel C**, Praud S, Murigneux A, Canaguier A, Sapet F, Samson D, Balfourier F, Dufour P, Chalhoub B, Brunel D, Beckert M, Charmet G. (2006). Single-nucleotide polymorphism frequency in a set of selected lines of bread wheat (*Triticum aestivum* L.) Genome, 49: 1131-1139. DOI: 10.1139/G06-067.
- Richards R.A.** (2000). Selectable traits to increase crop photosynthesis and yield of grain crops. Journal of Experimental Botany, 51: 447-458.
- Röder M.S**, Korzun V, Wendehake K, Gill B.S, Ganal M.W. (1998 a). The physical mapping of microsatellite markers in wheat. Genome, 41: 278-283.
- Röder M.S**, Korzun V, Wendehake K, Plaschke J, Tixier M.H, Leroy P, Ganal M.W. (1998 b). A microsatellite map of wheat. Genetics, 149: 2007-2023.
- Röder M.S**, Plaschke J, König S.U, Börner A, Sorrells M.E. (1995). Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Gen. Genet, 246: 327-333.
- Röder M.S**, Plaschke J, König S.U, Börner A, Sorrells M.E, Tanksley S.D, Ganal M.W. (1994). Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Gen. Genet. 225: 445-454.
- Röder S**, Korzun V, Wendehake K, Plaschke J, Tixier M.H, Leroy P, Ganal M. (2007). A Microsatellite Map of Wheat. Genetics, Volume 149: 2007-2023.
- Rong W**, Burnstock G, Spyer K.M. (2000). P2X purinoceptor-mediated excitation of trigeminal lingual nerve terminals in an in vitro intra-arterially perfused rat tongue preparation. Journal of Physiology, 524: 891-902.
- Ronin Y**, Mester D, Minkov D, Korol A.B. (2008). A new strategy for building reliable dense genetic maps. Genetics, In press.
- Rooney E.K**, Gross J.D, Satre M. (1994). Characterization of an intracellular Ca²⁺ pump in Dictyostelium. Cell Calcium, 16: 509-522.
- Rostoks N**, Mudie S, Cardle L, Russell J, Ramsay L, Booth A, Svensson J.T, Wanamaker S.I, Walia H, Rodriguez E.M, Hedley P.E, Liu H, Morris J, Close T.J, Marshall D.F, Waugh R. (2005). Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. Molecular Genetics and Genomics, 274: 515-527.
- Rustgi S**, Bandopadhyay R, Balyan H.S, Gupta P.K. (2009). EST-SNPs in bread wheat: discovery, validation, genotyping and haplotype structure. Czech J Genet Plant Breed, 45: 106-116.
- Saal B**, Wricke G. (1999). Development of simple sequence repeat markers in rye (*Secale cereale* L.), 1999 NRC Canada Genome, 42: 964-972.
- Saito A**, Yano M, Kishimoto N, Nakagahra M, Yoshimura A, Saito K, Kuhara S, Ukal Y, Kawase M, Nagamine T, Yoshimura S, Ideta O, Ohsawa R, Hayano Y, Iwara N, Sugiura M. (1991). Linkage map of restriction fragment length polymorphism loci in rice. Japan J Breed, 41: 665-670.
- Saliba-Colombi V**, Causse M, Gervais L, Philouze J. (2000). Efficiency of RFLP, RAPD, and AFLP markers for the construction of an intraspecific map of the tomato genome. Genome, 43: 29-40.
- Salina E**, Börner A, Leonova I, Korzun V, Laikova L, Maystrenko O, Röder M.S. (2000). Microsatellite mapping of the induced sphaerococcoid mutation genes in *Triticum aestivum*. Theoretical and Applied Genetics, 100: 686-689.
- Sanger F**, Coulson A.R, Friedmann T, Air G.M. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA, 74: 5463-5467.
- Sapre A.B.** and Deshpande D.S. (1987). Spontaneous emergence of parents from the F1 interspecific hybrids of Coix L. J. Hered, 78: 357-360.
- Sax K.** (1922). Sterility in wheat hybrids. II. Chromosome behavior in partially sterile hybrids. Genetics, 7: 513-550.
- Scarth R**, Law C.N. (1984). The control of the daylength response in wheat by the group 2 chromosomes. J Plant Breed 92: 140-150.
- Schena M.** (1998). Microarrays: biotechnology's discovery platform for functional genomics. Trends Biotechnol, 16: 301-306.

- Schon C.C.**, Utz H.F, Groh S, Truberg B, Openshaw S, Melchinger A.E. (2004). Quantitative trait locus mapping based on resampling in a vast maize testcross experiment and its relevance to quantitative genetics for complex traits. *Genetics*, 167: 485-498.
- Sears E. R.** (1954). II Aneuploids of bread wheat, The Aneuploids del grano tenero. Bull. 572, University of Missouri Agricultural Experiment Station, Columbia, MO.
- Senior M.L.**, Heun M. (1993). Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a ct primer. *Genome*, 36: 884-889.
- Setler T.L.**, Carlton G. (2000). Chapter Coordinator: T.L. Setter, Principles and Practice, Compiled by W.K. Anderson and J.R. Garlinge. Agriculture Western Australia, Funded by the Grains Research and Development Corporation, Bulletin 4443, Replaces Bulletin 4196, ISSN 1326-415X, Agdex 112/01, Chief Executive Officer, Department of Agriculture, Western Australia, The Wheat Book.
- Sharma H.C.**, Wainies J.G. (1980). Inheritance of though rachis in crosses of *Triticum monococcum* L and *Triticum boeoticum* (wheat). *Heredity*, 71 (3): 214-216.
- Sharma H.C.**, Wainies J.G. (1981). Attempted gene transfer from tetraploids to diploids in *Triticum*. *Can. J. Genet. Cytol*, 23: 639-645.
- Sharp P.J.**, Chao S, Desai S, Gale M.D. (1989). The isolation, characterization and application in the Triticeae of a set of wheat RFLP probes identifying each homoeologous chromosome arm. *Theoretical and Applied Genetics*, 78: 342-348.
- Sherman J.D.**, Fenwick A.L, Namuth D.M, Lapitan N.L.V. (1995). A barley RFLP map: alignment of the three barley maps and comparisons to gramineae species. *TAG*, 91: 681-690.
- Shomura A.**, Izawa T, Ebana K, Ebitani T, Kanegae H, Konishi S, Yano M. (2008). Deletion in a gene associated with grain size increased yields during rice domestication. *Nat Genet*, 40: 1023-1028.
- Simeone R.**, Pasqualone A, Clodoveo M.L, Blanco A. (2002). Genetic mapping of polyphenol oxidase in tetraploid wheat. *Cell Mol Biol Lett*, 7: 763-769.
- Singh A.**, Hamme J.D, Ward O.P. (2007). Surfactants in microbiology and biotechnology. Part 2. Application aspects. *Biotechnol Adv*, 25: 99-121.
- Singh D.V.** (1980). A note on the effect of Karnal bunt infection on the vigour of wheat seed. *Seed Res*, 8: 81-82.
- Singh K.**, Ghai M, Garg M, Chhuneja P, Kaur P, Schnurbusch T, Keller B, Dhaliwal H.S. (2007). An integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum* x *T. monococcum* RIL population. *Theoretical and Applied Genetics*, 115: 301-312.
- Singh R.A.**, Krishna A. (1982). Susceptible stage of inoculation of and effect of Karnal bunt on viability of wheat seed. *Ind. Phytopathol*, 35: 54-56.
- Sissons M.J.**, Egan N.E, Gianibelli M.C. (2005). New insights into the role of gluten on durum pasta quality using reconstitution method. *Cereal Chem*, 82: 601-608.
- Slafer G.A.**, Andrade F.H. (1993). Physiological attributes to the generation of grain yield in bread wheat cultivars released at different eras. *Field Crops Research*, 31: 351-367.
- Slafer G.A.**, Araus J.L. (2007). Physiological traits for improving wheat yield under a wide range of environments. In *Scale and Complexity in Plant Systems Research (Wageningen UR Frontis Series)*, edited by J. H. J. Spiertz, P. C. Struik and H. H. van Laar. Springer Dordrecht, Netherlands, Volume 21: 147-156.
- Slafer G.A.**, Calderini D.F, Miralles D.J. (1996). Yield components and compensation in wheat: opportunities for further increasing yield potential. In *Increasing Yield Potential in Wheat: Breaking the Barriers*, edited by Reynolds M.P, Rajaram S, Mc Nab A. CIMMYT El-Batan Mexico: 101-134.
- Snape J.W.**, Flavell R.B, O'Dell M, Hughes W.G, Payne P.I. (1985). Intrachromosomal mapping of the nuclear organiser region relative to three marker loci on chromosome 1B of wheat (*Triticum aestivum*). *Theor Appl Genet*, 69: 263-270.

- Somers D.J**, Isaac P, Edwards K. (2004). A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.), Theoretical and Applied Genetics, 109: 1105-1114.
- Somers D.J**, Kirkpatrick R, Moniwa M, Walsh A. (2003). Mining single-nucleotide polymorphisms from hexaploid wheat ESTs. Genome, 46: 431-437. DOI: 10.1139/g03-027.
- Song Q.J**, Fickus E.W, Cregan P.B. (2002). Characteristics of trinucleotide markers in wheat. Theoretical and Applied Genetics, 104: 286-293.
- Song Q.J**, Shi J.R, Singh S, Fickus E.W, Costa J.M, Lewis J, Gill B.S, Ward R, Cregan P.B. (2005). Development and mapping of microsatellite (SSR) markers in wheat. Theoretical and Applied Genetics, 110: 550-560.
- Song QJ**, Fickus EW, Cregan PB (2002) Characterization of trinucleotide SSR motifs in wheat. Theor Appl Genet 104:286–293
- Song X.J**, Huang W, Shi M, Zhu M.Z, Lin H.X. (2007). A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet, 39: 623-630.
- Song Y.C**, Kawas E, Good B.M, Wilkinson M.D, Tebbutt S.J. (2007). DataBiNS: a BioMoby-based data-mining workflow for biological pathways and non-synonymous SNPs, Bioinformatics. University of British Columbia, Vancouver, Canada, PubMed, 15; 23 (6): 780-2.
- Sorrells M.E**, Diab A, Nachit M.M. (2000). Comparative genetics of drought tolerance. Seminaires Mediterraneens Ciheam/Udl-IRTA/ CIMMYT/ ICARDA, Serie A, N.40: 191-202.
- Sourdille P**, Cadalen T, Guyomarc'h H, Snape J.W, Perretant M.R, Charmet G, Boeuf C, Bernard S, Bernard M. (2003). An update of the courtot x Chinese spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor Appl Genet, 106: 530-538.
- Sourdille P**, Perretant M.R, Charmet G, Cadalen T, Tixier M.H, Joudrier P, Gautier M.F, Branlard G, Bernard S, Boeuf C, Bernard M. (1999). Detection of QTL for bread making quality in wheat using molecular markers. Kluwer dordrecht, pp 361-366.
- Sourdille P**, Perretant M.R, Charmet G, Leroy P, Gautier M.F, Joudrier P, Nelson J.C, Sorrells M.E, Bernard M. (1996). Linkage between RFLP markers and gene affecting kernel hardness in wheat. Theor Appl Genet, 93: 580-586.
- Sourdille P**, Singh S, Cadalen T, Brown-Guedira G, Gay G, Qi L, Gill B, Dufour P, Murigneux A, Bernard M (2004) Microsatellitebased deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genom, 4: 12-25. DOI:10.1007/s10142-004-0106-1.
- Sourdille P**, Tavaud M, Charmet G, Bernard M. (2001). Transferability of wheat microsatellites to diploid triticeae species carrying the A, B and D genomes. Theor Appl Genet, 103: 346-352.
- Souza E**, Windes J, Udall J. (1998). Inheritance of polyphenol oxidase reaction in three recombinant inbred populations of wheat. In: Agronomy Abstracts. Amer Soc Agron Madison, WI: 67.
- Spielmeyer W**, Hyles J, Joaquim P, Azanza F, Bonnet D. (2007). A QTL on chromosome 6A in bread wheat (*Triticum aestivum*) is associated with longer coleoptiles, greater seedling vigour and final plant height. Theoretical and Applied Genetics, 115: 59-66.
- Stam P**. (1993). Construction of integrated genetic linkage maps by means of anew computer package, JoinMap. Plant Journ, 3: 739-744.
- Staple food**, The story of man's staple food Dec 20th 2005 | from the print edition Related topics Consumer non-cyclicals Thomas Malthus United States Wheat farming Grain and oilseed farming.
- Stein N**, Prasad M, Scholz U, Thiel T, Zhang H, Wolf M, Kota R, Varshney R.K, Perovic D, Grosse I, Graner A. (2007). A 1000-loci transcript map of the barley genome: New anchoring points for integrative grass genomics. Theor Appl Genet, 114: 823-829.
- Steinberg B.E**, Huynh K.K, Brodovitch A, Jabs S, Stauber T, Jentsch T.J, Grinstein S. (2010). A catio counterflux supports lysosomal acidification, JCB volume 189 no. 7: 1171-1186.

- Stevens J.P.** (2009). Exploratory and confirmatory factor analysis. *Applied Multivariate Statistics for the Social Sciences*. New York: Routledge, Taylor & Francis Group, pp. 325-381.
- Stuber C.W**, Edwards M.D, Wendel J.F. (1987). Molecular markers facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its component traits. *Crop Sci*, 27: 639-648.
- Sun D.J**, He Z.H, Xia X.C, Zhang L.P, Morris C.F, Appels R. (2005). A novel STS marker for polyphenol oxidase activity in bread wheat. *Mol Breed*, 16: 209-218.
- Syvanen A.C.** (2001). Genotyping single nucleotide polymorphisms. *Nat Rev Genet*, 2: 930-942.
- Tai Q**, Tanksley C. (1990). A rapid and inexpensive method for isolation of total DNA from dehydrated plant. *Plant Molecular Biology Reporter*, 8: 297-303.
- Taillon-Miller P**, Gu Z.J, Li Q, Hillier L, Kwok P.Y. (1998). Overlapping genomic sequences: a treasure trove of single-nucleotide polymorphisms. *Genome Research*, 8: 748-754.
- Tajima F.** (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics*, 105: 437-460.
- Takano-Kai N**, Jiang H, Kubo T, Sweeney M, Matsumoto T, Kanamori H, Padhukasahasram B, Bustamante C, Yoshimura A, Doi K, McCouch S. (2009). Evolutionary history of GS3, a gene conferring grain length in rice. *Genetics*, 182: 1323-1334.
- Talamè V**, Sanguineti M.C, Chiapparino E, Bahri H, Ben Salem M, Forster B.P, Ellis R.P, Rhouma S, Zoumarou W, Waugh R, Tuberosa R. (2004). Identification of *Hordeum spontaneum* QTL alleles improving field performance of barley grown under rainfed conditions. *Ann Appl Biol*, 144: 309-319.
- Tanksley S.D**, Ganai M.W, Prince J.P, De Vicente M.C, Bonierbale M.W, Broun P, Fulton T.M, Giovannoni J.J, Grandillo S, Martin G.B, Messeguer R, Miller J.C, Miller L, Paterson A.H, Pineda O, Röder M.S, Wing R.A, Wu W, Young N.D. (1992). High density molecular linkage maps of tomato and potato genomes. *Genetics Society of America*. 132:1141-1160.
- Tanksley S.D**, Nelson J.C. (1996). Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theoretical and Applied Genetics*, 92: 191-203.
- Tanksley S.D.** (1984). Linkage relationships and chromosomal locations of enzyme-coding genes in pepper, *Capsicum annum*. *Chromosoma*, 89:352-360.
- Tanksley S.D.** (1993). Genetic and physical mapping of barley telomeres. *Mol Gen Genet*, 238: 294-303.
- Tanksley S.D.** (2004). The genetic, developmental, and molecular bases of fruit size and shape variation in tomato. *Plant Cell*, 16: S181-S189.
- Tautz D**, Trick M, Dover G.A. (1986). Cryptic simplicity in DNA is a major source of genetic variation. *Nature*, 322: 652-656.
- Tautz D.** (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res*, 12: 6463-6471.
- Tautz D.** (1993). Notes on the definition and nomenclature of tandemly repetitive DNA sequences. In: Pena Chakraborty S.D.J, Epplen R, Jeffreys A.J (eds) *DNA fingerprinting: state of the science*. Birkhäuser, Basel, pp: 21-28.
- Tenaillon M.I**, Sawkins M.C, Long A.D, Gaut R.L, Doebley J.F, Gaut B.S. (2001). Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp *mays* L.). *Proc Natl. Acad. Sci. USA*, 98: 9161-9166.
- Thomas M.R**, Scott N.S. (1993). Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). *Theoretical and Applied Genetics*, 86: 985-990.
- Thomson M.J**, Tai T.H, McClung A.M, Lai X.H, Hinga M.E, Lobos K.B, Xu Y, Martinez C.P, McCouch S.R. (2003). Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. *Theor Appl Genet* 107: 479-493.

- Threadgill D.W.**, Hunter K.W, Williams R.W. (2002). Genetic dissection of complex and quantitative traits: from fantasy to reality via a community effort. *Mamm. Genome*, 13: 175-178.
- Till B.J.** (2007). Tilling and eco tilling for crop improvement. In *Genomic Assisted Crop Improvement: Genomics Approaches and Platforms* (Varshney R.K, Tuberosa R. eds). Springer, pp: 333-349.
- Tinker A.**, Marjorie A, Koblinsky A, Daly P, Rooney C, Leighton C, Griffiths M, Zahidul Huque A.A, Kwast B. (1993). "Making Motherhood Safe". World Bank Discussion Paper No. 202. Washington, DC: The World Bank, 14-15.
- Torada A.**, Koike M, Mochida K, Ogihara Y. (2006). SSR-based linkage map with new markers using an intraspecific population of common wheat. *Theor Appl Genet*, Volume 112: 1042-1051.
- Troccoli A.**, Borrelli G.M, De Vita P, Fares C, Di Fonzo N. (2000). Durum wheat quality: a multidisciplinary concept. *J Cereal Sci*, 32: 99-113.
- Trono D.**, De Vita V, Mastrangelo A.M, Verlotta A, De Simone V, Di Gesu A.M, De Santis G, Cattivelli L, Blanco A. (2010). Genetic Variability of carotenoid content and lipoxygenase among durum wheat genotypes. *Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress* Matera. Italy 27/30 September, 2010. ISBN: 978-88-904570-0-5.
- Tsumura Y.**, Ohba K, Strauss S.H. (1996). Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglasfir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). *Theoretical and Applied Genetics*, 92: 40-45.
- Vales M.I.**, Schon C.C, Capettini F, Chen X.M, Corey A.E, Mather D.E, Mundt C.C, Richardson K.L, Sandoval-Islas J.S, Utz H.F, Hayes P.M. (2005). Effect of population size on the estimation of QTL: a test using resistance to barley stripe rust. *Theor Appl Genet*, 111: 1260-1270.
- Vallejos C.E.**, Sakiyama N.S, Chase C.D. (1992). A molecular marker-based linkage map of *L. Phaseolus vulgaris*. *Genetics*, 131: 733-740.
- Van Deynze A.E.**, Dubcovsky J, Gill K.S, Nelson J.C, Sorrells M.E, Dvorak J, Gill B.S, Lagudah E.S, McCouch S.R, Appels R. (1995 a). Molecular-genetic maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. *Genome*, 38: 45-59.
- Van Deynze A.E.**, Nelson J.C, Harrington S.E, Yglesias E.S, Braga D, McCouch S.R, Sorrells M.E. (1995 b). Comparative mapping in grasses: wheat relationships. *Mol Gen Genet*, 248: 744-754.
- Varshney R.K.**, Beier U, Khlestkina E, Kota R, Korzun V, Röder M, Graner A, Börner A. (2007 a). Single nucleotide polymorphisms in rye: discovery, frequency and applications for genome mapping and diversity studies. *Theoretical and Applied Genetics*, 114: 1105-1116.
- Varshney R.K.**, Langridge P, Graner A. (2007 b). Application of genomics to molecular breeding of wheat and barley. *Adv Genet*, 58: 121-155.
- Varshney R.K.**, Prasad M, Graner A. Molecular marker maps of barley: a resource for intra and interspecific genomics a In: G. Wenzel, L. Horst (eds.), *Molecular markers in Improvement of Agriculture and Forestry*, Springer Verlag, Germany.
- Varshney R.K.**, Sharma P.C, Gupta P.K, Balyan H.S, Ramesh B, Roy J.K, Kumar A, Sen A. (1998). Low level of polymorphism detected by SSR probes in bread wheat. *Plant Breed*, 117: 182-184.
- Varshney R.K.** (2005). Genomics-assisted breeding for crop improvement. *Trends Plant Sci*, 10: 621-630.
- Varshney R.V.**, Hoisington D.A, Tyagi A.K. (2006). Advances in cereal genomics and applications in crop breeding. *Trends Biotechnol*, 24: 490-499.
- Vavilov N.I.** (1951). The origin, variation, immunity and breeding of cultivated plants. The Ronald Press Co, New York.
- Wallace M.E.** (1953). Affinity: A new genetic phenomenon in the house mouse. Evidence from within laboratory stocks. *Nature*, 171: 27-28.
- Wallace M.E.** (1960 a). Possible cases of affinity in cotton. *Heredity* 14: 263-274.

- Wallace M.E.** (1960 b). Possible cases of affinity in tomatoes. *Heredity*, 14: 275-283.
- Wang J.R.**, Wei Y.M, Yan Z.H, Zheng Y.L. (2005). Detection of single nucleotide polymorphisms in 24 kDa dimeric α -amylase inhibitors from cultivated wheat and its diploid putative progenitors, *Biochimica et Biophysica Acta*, 1723 : 309- 320.
- Wang R.**, Guegler K, LaBrie S.T, Crawford N.M. (2000). Genomic analysis of a nutrient response in *Arabidopsis* reveals diverse expression patterns and novel metabolic and potential regulatory genes that are induced by nitrate. *Plant Cell*, 12: 1491-1510.
- Wang Z.**, Weber J.L, Zhong G, Tanksley D.S. (1994). Survey of plant short tandem DNA repeats. *Theoretical and Applied Genetics*, 88: 1-6.
- Wasik R.J.A.W.B.** (1975). Relation between molecular-weight distribution of endosperm proteins and spaghetti-making quality of wheats. *Cereal Chem* 52: 322-328.
- Watanabe N.**, Akond A.S.M.G.A, Nachit M.M. (2006). Genetic mapping of the gene affecting polyphenol oxidase activity in tetraploid durum wheat. *J Appl Genet*, 47(3): 201-205.
- Watanabe N.**, Takeuchi A, Nakayama A. (2004). The inheritance and chromosomal location of the genes for phenol colour reaction of grain in durum wheat. *Euphytica*, 139: 87-93.
- Watanabe N.** (2008). Genetic collection and development of near-isogenic Lines in durum wheat. *Becmhuk BOTuC*, 2008, Tom 12. Number 4.
- Weber J.L.**, May P.E. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am J Hum Genet*, 44: 388-396.
- Weber J.L.** (1990) Informativeness of human (dC-dA)~.(dG-dT),, polymorphisms. *Genomics*, 7 : 524-530
- Wehrhahn C.**, Allard R.W. (1965). The detection and measurement of the effects of individual genes involved in the inheritance of a quantitative character in wheat. *Genetics*, 51: 109-119.
- Wendel J.F.**, Edwards M.D, Stuber C.W. (1987). Evidence for multilocus genetic control of preferential fertilization in maize. *Heredity*, 58: 297-302.
- Weng Y.**, Lazar M.D. (2002). Comparison of homoeologous group-6 short arm physical maps of wheat and barley reveals a similar distribution of recombinogenic and gene-rich regions. *Theor Appl Genet*, 104:1078-1085
- Wenzl P.**, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A. (2004). Diversity arrays technology (DArT) for whole-genome profiling of barley. *Proceedings of the National Academy of Sciences of the United States of America*, 101: 9915-9920.
- Werner J.E.**, Endo T.R, Gill B.S. (1992). Toward a cytogenetically based physical map of the wheat genome. *Proc Natl Acad Sci USA*, 89: 11307-11311.
- Wricke G.**, Weber W.E. (1986). *Quantitative Genetics and Selection in Plant Breeding*. Walter de Gruyter. Berlin. New York.
- Wright S.I.**, Bi I.V, Schroeder S.G, Yamasaki M, Doebley J.F, Mc Mullen M.D, Gaut B.S. (2005).The effects of artificial selection on the maize genome. *Science*, 308: 1310–1314. DOI: 10.1126/science.1107891.
- Wrigley C.W.**, McIntosh R.A. (1975). Genetic control of factors regulating the phenol reaction of wheat and
- Wu K.S.**, and S.D. Tanksley S.D. (1993). Abundance, polymorphism and genetic mapping of microsatellites in rice. *Mol. Gen. Genet*, 241: 225-235.
- Xiong M.**, Jin L. (1999). Comparison of the power and accuracy of biallelic and microsatellite markers in population-based gene-mapping methods. *Am. J. Hum. Genet*, 64: 629-640.
- Xu Y.**, Zhu L, Xiao J, Huang N, Mc Couch S.R. (1997). Chromosomal regions associated with segregation distortion of molecular markers in F2, backcross, doubled haploid, and recombinant inbred populations of rice (*Oryza sativa* L.). *Mol Gen Genet*, 253: 535-545.

- Xue S**, Avvakumov G.V, Walker J.R. (2008). A high-density intervarietal map of the wheat genome enriched with markers derived from expressed sequence tags. *Theoretical and Applied Genetics*, 117: 181-189.
- Yahiaoui N**, Srichumpa P, Dudler R, Keller B. (2004). Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene Pm3b from hexaploid wheat, *The Plant Journal*, Volume 37, Issue 4, pages: 528-538.
- Yoneyama T**, Kasuya H, Onda H, Akagawa H, Jinnai N, Nakajima T, Hori T, Inoue I. (2003). Association of positional and functional candidate genes FGF1, FBN2, and LOX on 5q31 with intracranial aneurysm. *J Hum Genet*, 48: 309-314. DOI 10.1007/s10038-003-0030-6.
- Young N.D**, Miller J.C, Tanksley S.D. (1987). Rapid chromosomal assignment of multiple genomic clones in tomato using primary trisomics. *Nucleic Acids Research*, 15: 9339-9348.
- Young N.D.** (2000). Constructing a plant genetic linkage map with DNA markers, Kluwer Academic Publishers. Printed in the Netherlands, Phillips R.L, Vasil J.K. (eds.), volume:32 3445-3457.
- Yousefi Javan I**, Mondini L, Nachit M.M, Pagnotta M.A. (2011). Intra-specific genetic structure of durum wheat based on SSR and SNP markers. *Minerva Biotechnologica*, 23 (2 Suppl 1): 1-3.
- Yu K.F**, Pauls K.P. (1993). Segregation of random amplified polymorphic DNA markers and strategies for molecular mapping in tetraploid alfalfa. *Genome*, 36: 844-851.
- Zanetti S**, Keller M, Winzeler M, Saurer W, Keller B, Messmer M. (1999). QTL for quality parameters for bread-making quality in a segregating wheat by spelt population. In: Scarascia Mugnozza G.T, Porceddu E, Pagnotta M.A (eds) *Genetics and breeding for crop quality and resistance*. Kluwer Dordrecht, pp 357-360.
- Zeng Z.B.** (1994). Precise mapping of quantitative trait loci. *Genetics*, 136: 1457-1468.
- Zeven A.C.** (1972). Identification of chromosomes carrying a locus for a gene conditioning the production of tyrosinase in wheat grains. *Wheat Inf Serv*, 35: 3-8.
- Zhang Q.S**, Manche L, Xu R.M, Krainer A.R. (2006). hnRNP A1 associates with telomere ends and stimulates telomerase activity. *RNA*, 12: 1116-1128.
- Zhang W**, Chao S, Manthey F, Chicaiza O, Brevis C, Echenique V, Dubcovsky J. (2008). QTL analysis of pasta quality using a composite microsatellite and SNP map of durum wheat. *Theoretical and Applied Genetics*, 117: 1361-1377. DOI 10.1007/s00122-008-0869-1.
- Zhang W.** (1994). Positional Cloning of the mouse obese gene and its human homologue. This author first identified leptin. *Nature*, 372: 425-432.
- Zhu W**, Schlueter S.D, Brendel V. (2003). Refined annotation of the *Arabidopsis thaliana* genome by complete EST mapping. *Plant Physiology*, 132: 469-484.
- Zhuchenko A.A**, Andryuschenko V.K, Nyutin Y.I, Korol A.B, Vyrodov D.A. (1977). Genetic effects of mutagenic treatment of tomato hybrids. III. Induced changes in recombination frequency between unlinked markers. *Genetika*, 13: 1922-1932.
- Zietkiewicz E**, Rafalski A, Labuda D. (1994). Genomic fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, 20: 176-183.
- Zou F**, Gelfond J.A.L, Airey D.C, Lu L, Manly K.F, Williams R.W, hreadgill D.W. (2005). Quantitative trait locus analysis using recombinant inbred intercross (RIX): theoretical and empirical considerations. *Genetics*, 170: 1299-1311.
- Zvi Peleg Z**, Saranga Y, Suprunova T, Ronin Y.W, Röder M.S, Kilian A, Korol A.B, Fahima T. (2008). High-density genetic map of durum wheat x wild emmer wheat based on SSR and DArT markers, *Theor Appl Genet*, 117:103–115, DOI 10.1007/s00122-008-0756-9.