University of Natural Resources
and Applied Life Sciences, Vienna
Department for Agrobiotechnology, IFA-Tulln
Institute of Biotechnology in Plant production
(Head of the institute: Ao.Univ. Prof. Dr. Herman Buerstmayr)

Mapping QTLs for Yield and Yield Components under Drought Stress in Bread Wheat

Dissertation for a
doctorate degree

at the University of Natural Resources and
Applied Life Sciences-BOKU
Vienna

Submitted by:

Alaeddin Kordenaeej

Supervisor:

Ao.Univ. Prof. Dr. **Tamas Lelley**

Co-examiner:
Ao. Univ. Prof. Dr. **Johann Vollmann**

Vienna, March 2008

To

Persia

Acknowledgements

At the end of this work, I would like to thank all the people whose help, attention, and encouragement made this research possible.

The most appreciation and acknowledgment must go to the supervisor of my work Mr. Professor **Tamas Lelley**, a great man that not only his help and guidance are the heart of my achievements but also his personality is always a lesson for me.

I also express my high appreciations to Professor Johann Vollmann, as the second reviewer, and due to his helpful advices.

My next thank goes to Professor Hermann Buerstmayr, the head of the institute, for his help whenever I needed.

My great thanks go to Mrs. Dr. Helga Danhorn for her excellent editorial help.

I would like to give my special appreciation to my dear friends Dr. Aliasghar Nasrollah Nejad, who precisely and friendly conducted the experiment in Iran, and Dr. Abdolali Shojaeiyan who kindly helped me for genotyping the BC2F3 population.

Many appreciations go to Mrs. Getraude Stift and Mr. Martin Pachner for their permanent assistances in the lab from the begging to the end of this work.

I would like to thank Mr. Matthias Fidesser for his help and service in the field and green house, a man who always was ready to help.

I am grateful to my other friends in the Institute of Biotechnology in Plant Production for their aids, supports and encouragements and for providing a friendly atmosphere there during my staying .

Special thanks should also go to our Secretary, Mrs. Gerlinde Kindler, for all of her kind help in administrational affairs.

The big help of Ms. Katharina Schießl for field evaluations is much appreciated.

Dr. László Cseuz and his team in Szeged-Hungary are much appreciated carrying out the field experiments.

The author also wishes to thank Dr. Marion Röder and Dr. Martin Ganal, for their generous supports in the SSR analysis and providing primer sequence information.

I thank my dear friend Dr. Khalil Zaynali Nezhad for his helpful advices.

My great thanks go to Dr. Mohammad Reza Ghanadha from the Tehran University, Mr. Vahab-Zadeh and Mr. Akbari from the Institute of Seed and Plant Improvement in Tehran-Karaj, for providing the parental seeds and also for their recommendations.

Financial support of the Ministry of Science, Research and Technology of Iran and from BOKU-University of Natural Resources and Applied Life Sciences, Vienna, Austria, to the author is greatly appreciated.

Finally, I would like to express my deepest gratitude to my dear wife **Elahe** for her understanding, patience, support, and great help during our staying in Tulln. A lovely little gratitude goes also to my dear son Erfan, who always was a motivation for me.

Abstract

Bread wheat is the major crop, and drought stress is the main factor limiting wheat production in Iran. Identification of quantitative trait loci (QTLs) related to yield and yield components under drought stress is the main objective of this study. Two parental genotypes, contrasting in agronomic and morphological traits of interest, one, ***Tabassi***, a typical Iranian drought tolerant landrace the other, the highly bred and non-drought tolerant European wheat variety ***Taifun***, were crossed to produce a population of 118 F2:7 recombinant inbred lines (RILs). A linkage map, based on 202 polymorphic SSR markers and three morphological traits, i.e. *awnedness*, *spike pubescence* and *flag leaf waxyness* providing 217 loci, was constructed covering 2795 cM of the genome. Phenotypic data for grain yield and number per ten spikes, 1000-kernel weight, and spike length, spikelet per spike, plant height, and ear emergence time were collected under two non-drought, as well as three drought stress conditions in Austria, Iran and Hungary. Out of 146 putative QTLs, 39 were identified as major ones ($R^2 \geq 10\%$), with an average of 5.6 QTLs per trait. Chromosome 4D is strongly suggested as a QTL-rich region for yield, yield components, and other agronomic traits under drought stress. Chromosomes of group 3 are suggested to have major QTLs for grain and spikelet per spike, as well as spike length, under drought. Chromosome 7A was also confirmed to have major QTLs for yield under drought. Application of potassium iodide to simulate a post anthesis drought condition was highly efficient. Tabassi and populations derived from its crossing with Taifun represent an excellent genetic source for any further study on drought, as well as salinity and heat stresses, and for breeding purposes.

Zusammenfassung

Brotweizen ist die wichtigste Getreideart und Trockenheit der größte limitierende Faktor für den Weizenanbau im Iran. Gegenstand der Untersuchungen war die Identifizierung von Genorten für quantitative Eigenschaften, QTLs, des Weizens, die unter Trockenstress Ertrag und ertragrelevante morphologische Merkmale kontrollieren. Zwei Weizengenotypen mit stark divergierenden morphologischen und agronomischen Eigenschaften, ***Tabassi***, eine tockencheitstolerante, iranische Landrasse und ***Taifun***, eine leistungsstarke europäische Zuchtsorte, wurden miteinander gekreuzt. Aus der F2 Generation wurden 118 F2:7 Rekombinante Inzucht-Linien (RILs) entwickelt. Mit 202 polymorphen SSR Markern und den morphologischen Merkmalen Begrannung, Ährenbehaarung und Fahnenblattbereifung wurde eine Kopplungskarte mit 217 Loci und einer Länge von 2795 cM erstellt. Daten wurden unter zwei Nicht-Stress und drei Stress-Umwelten in Österreich, Iran und Ungarn für Samenertrag und Anzahl Körner von 10 repräsentativen Ähren, Tausend Korngewicht, Ährenlänge, Anzahl Ährchen per Ähre, Pflanzenlänge und Ährenschieben erhoben. Von 164 QTLs wurden 39 als Haupt-QTLs ($R^2 \geq 10\%$), Durchschnitts-QTL-Wert von 5,6 per Merkmal, identifiziert. Chromosom 4D erwies sich als besonders reich an QTLs für Ertrag, Ertragskomponenten und andere agronomische Merkmale unter Trockenstress. Chromosomen der homoeologen Gruppe 3 zeigten Haupt-QTLs für Samenertrag, Ährchen pro Ähre und Ährenlänge unter Trockenstress. Chromosom 7A wurde bestätigt als Träger von Haupt-QTLs für Ertrag unter Trockenstress. Die Anwendung von Kaliumjodid war sehr effektiv zur Simulierung von Trockenstress nach der Blüte und um entsprechende QTLs zu finden. Tabassi und seine Kreuzungsnachkommenschaften mit Taifun erwiesen sich als besonders geeignet die Genetik der Trockenheitsresistenz beim Weizen zu erforschen.

Abbreviations

ABA	Abscisic acid
CIM	Composite interval mapping
AFLP	Amplified fragment length polymorphism
cM	CentiMorgan
ANOVA	Analysis of variance
DH	Doubled haploid
ANSHA	ANSHA
Eet	Ear emergence time
EC	Electrical
Gps	Grain number per 10 spikes
GRR	Gene-rich region
H^2	Broad sense heritability
HUN-Ctr	Hungary-Control
HUN-Str	Hungary-Stress
IRN	Iran
KI	Potassium iodide
LOD	Logarithm of odds
LR	Likelihood ratio
LSD	Least significant difference
OA	Osmotic adjustment
Pht	Plant height
QTL	Quantitative trait loci
RCBD	Randomized complete blocks design
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RHKs	Receptor-like histidin kinases
RICL	Recombinant inbred chromosome line
RIL	Recombinant inbred line
IM	Interval mapping
Sln	Spike length
SNP	Single nucleotide polymorphism
Sps	Spikelet per spike
SSD	Single seed descent
SSI	Stress susceptibility index
SSR	Simple sequence repeat
Tab	Tabassi
Tai	Taifun
Tkw	Thousand-kernel weigh
WUE	Water use efficiency
Yld	Grain yield per 10 spikes

Table of contents

	page
Chapter 1: Introduction	
1.1. Wheat, a great fact and a big problem	1
1.2. Objectives of the study	4
1.3. Wheat has a most complex genome	5
1.4. Drought tolerance is an extremely complex trait	6
1.5. Plant responses to drought stress	7
1.5.1. At the level of the whole plant	7
1.5.2. At the cellular level	11
1.6. Traits related to drought stress	13
1.7. Chromosomal regions of the genes controlling traits related to drought	14
1.7.1. Yield and yield components	14
1.7.2. Plant height	16
1.7.3. Ear emergence time	17
1.8. Principles of mapping quantitative trait loci (QTL)	18
1.8.1. Mapping populations	18
1.8.1.1. F2 populations	19
1.8.1.2. Backcross (BC) populations	19
1.8.1.3. Recombinant inbred lines (RILs)	20
1.8.1.4. Doubled haploid (DH) populations	21
1.8.2. Molecular markers and linkage maps	22
1.8.3. Statistical methods for QTL mapping	24
1.9. Evaluation of drought stress	28
Chapter 2: Materials and methods	
2.1. Plant materials	30
2.1.1. Genetic relationship analysis for screening parental genotypes	30
2.1.2. Characteristics of Sardari and Tabassi	30
2.1.3. Characteristics of Taifun and Kärntner Früher (early)	32
2.1.4. Tabassi vs. Taifun	32
2.2. Crossings and construction of mapping populations	35
2.2.1. Recombinant inbred line populations	35
2.2.2. Backcross populations	35
2.2.3. Double haploid production	36
2.3. Field experiments	36
2.3.1. Preliminary field evaluation	36
2.3.2. Final filed evaluation	37
2.3.3. Assessment of post-anthesis drought stress by chemical desiccant	38
2.4. Genotyping	39
2.4.1. DNA isolation	39
2.4.2. PCR reactions	40
2.4.3. Gel electrophoresis and scanning	40
2.4.4. Scoring SSR marker data	40
2.4.5. Linkage map	41
2.5. Field data analysis	43
2.6. QTL analysis	43

Table of contents

	page
Chapter 3: Results	
3.1. Relationship study for screening parental genotypes	44
3.2. Comparison of blocks and check varieties	45
3.3. Comparison of Tabassi and Taifun	46
3.4. Comparison between the experimental environments	48
3.5. Correlation between the traits	50
3.5.1. Correlation between quantitative traits	50
3.5.2. Correlation between quantitative and morphological traits	51
3.6. Phenotypic values of the traits	55
3.7. Characteristics of the linkage map	57
3.8. QTL analysis	59
Chapter 4: Discussion	
4.1. High potential Tabassi	70
4.2. Efficiency of drought stress experiments	
4.3. Linkage map	74
4.4. QTL analysis	79
4.5. QTLs identification and validation	79
4.5.1. QTLs for yield and yield components	79
4.5.1.1. Grain yield per 10 spikes	81
4.5.1.2. Grain number per 10 spikes	82
4.5.1.3. Thousand-kernel weight	82
4.5.1.4. Spike length	83
4.5.1.5. Spikelet per spike	84
4.5.2. Plant height QTLs	85
4.5.3. Ear emergence time QTLs	86
4.6. Chromosome 4D is a QTL-rich region for drought tolerance	87
4.7. Conclusion	88
4.8. Out looks	89
Chapter 5: References	90

* * *

Chapter 1

Introduction

1.1. Wheat, a great fact and a big problem

1.2. Objectives of the study

1.3. Wheat has a most complex genome

1.4. Drought tolerance is an extremely complex trait

1.5. Plant responses to drought stress

- 1.5.1. At the level of the whole plant
- 1.5.2. At the cellular level

1.6. Traits related to drought stress

1.7. Chromosomal regions of the genes controlling traits related to drought

- 1.7.1. Yield and yield components
- 1.7.2. Heading time
- 1.7.3. Plant height

1.8. Principles of mapping quantitative trait loci (QTL)

- 1.8.1. Mapping populations
  - 1.8.1.1. F2 populations
  - 1.8.1.2. Backcross populations (BC)
  - 1.8.1.3. Recombinant inbred lines (RILs)
  - 1.8.1.4. Doubled haploid (DH) population
- 1.8.2. Molecular markers and linkage maps
- 1.8.3. Statistical methods for QTL mapping

1.9. Evaluation of drought stress

1.1. Wheat, a great fact and a big problem

Bread Wheat is the most important staple crop globally. Total cultivation area of wheat in the world is 216.100.018 ha with an average yield of 2.804 kg/ha (FAO, 2006). Drought is accepted as the major abiotic stress reducing yield of wheat and other crops in water-limited areas. The reduction of wheat yield, depending on time and intensity of drought and also additional kinds of biotic and abiotic stresses (Table 1), varies from 10% to 90% of its potential yield under non-stressed conditions (Reynolds et al. 2004). Not only wheat consumers of the world are suffering under this situation, but it also has great economic impact on wheat exporting countries. For example, Australia and the United States, as the world´s two main wheat producers, have lost 70% and 15% of their exports respectively in 2006 due to drought (World bank report, 2006).

Iran, in the Middle East, is part of the geographic area known as the Fertile Crescent, where wheat has been domesticated (Fig. 1). Wheat is providing half of the basic food supply for the Persians.

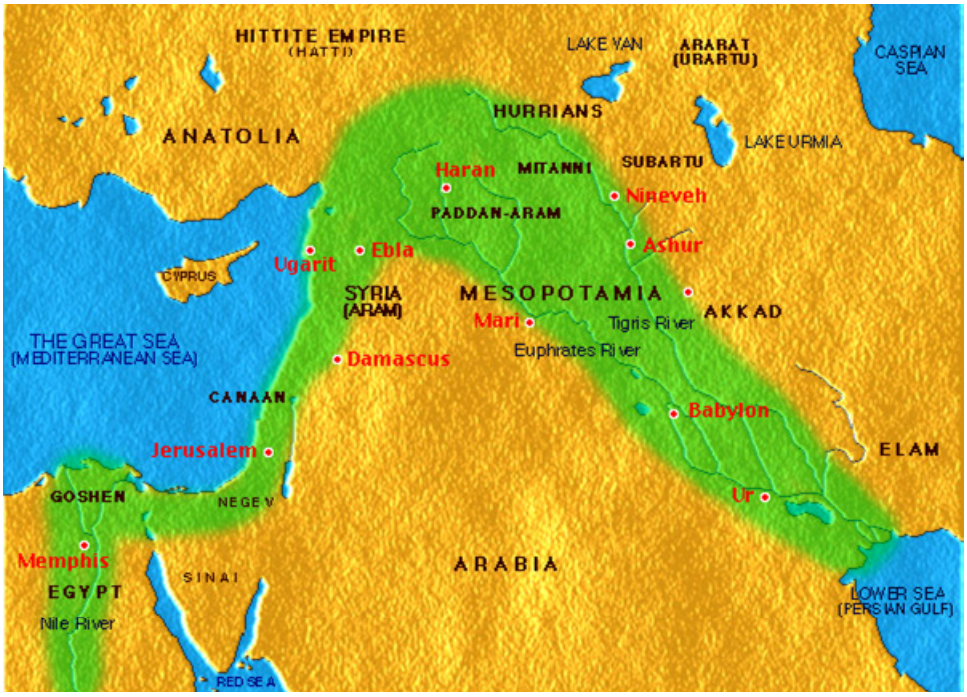


Fig. 1: Approximate geographical location of the Fertile Crescent.

The beginnings of cereal research in Iran date back to the 1930ies. At that time scientists collected seeds of wheat and barley landraces or local varieties, developed and used by farmers over centuries. They molded them by selection and improvement into higher-yielding cultivars. Wheat research was initiated by scientists at the Higher School of Crop Production at Karaj (CIMMYT E-News. November 2007).

Besides highly bred, registered varieties, according to a CIMMYT Report (2002), some 10,000 landraces are still being grown by farmers. The contribution of landraces to the economic and cultural development of Persia ever since cannot be overestimated. The geographic distribution of wheat landraces in Iran, as depicted in Fig. 2, indicates that those landraces have developed their own adaptations to specific environmental conditions. Efforts and research activities to improve wheat production, including field management, disease control, and improvement of irrigation systems made the country independent of wheat import. In September 2007 the first surplus of wheat was exported from Iran.

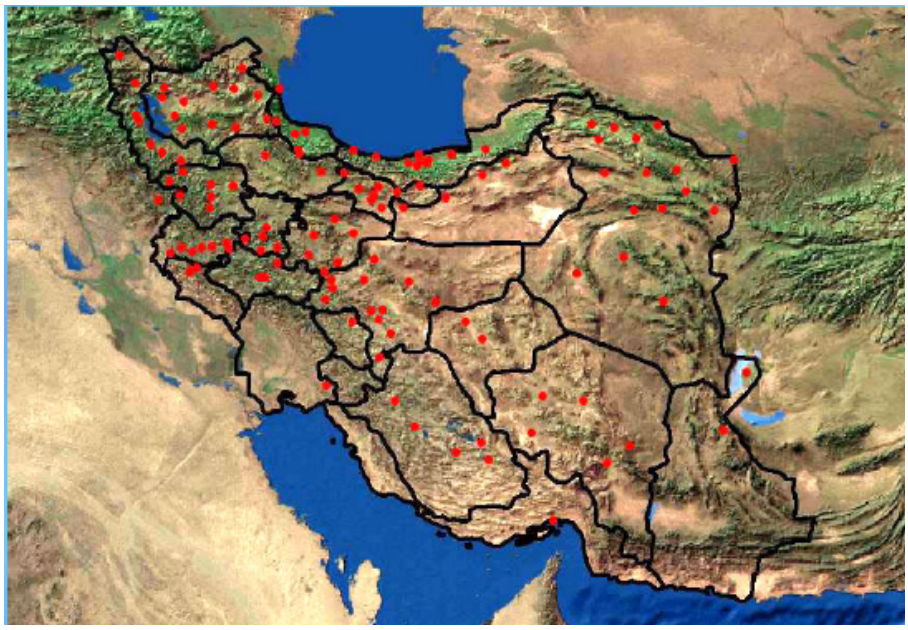


Fig. 2: Geographical distribution of Iranian wheat landraces (CIMMYT Annual Report, 2002).

However, this situation may change year by year. Except for the costal plains by the Caspian Sea and higher valleys in the Elbrus mountain chains (dark blue in Fig. 3), that may receive more than 100 cm of annual precipitation, average annual precipitation in the Western and Northwestern parts of the country is around 25 cm. The situation in most other parts of the country, including Northeastern, East, South, Southeastern, Southwestern and particularly central areas (Fig. 3), is critical, because average annual rainfalls rarely exceed 10 cm.

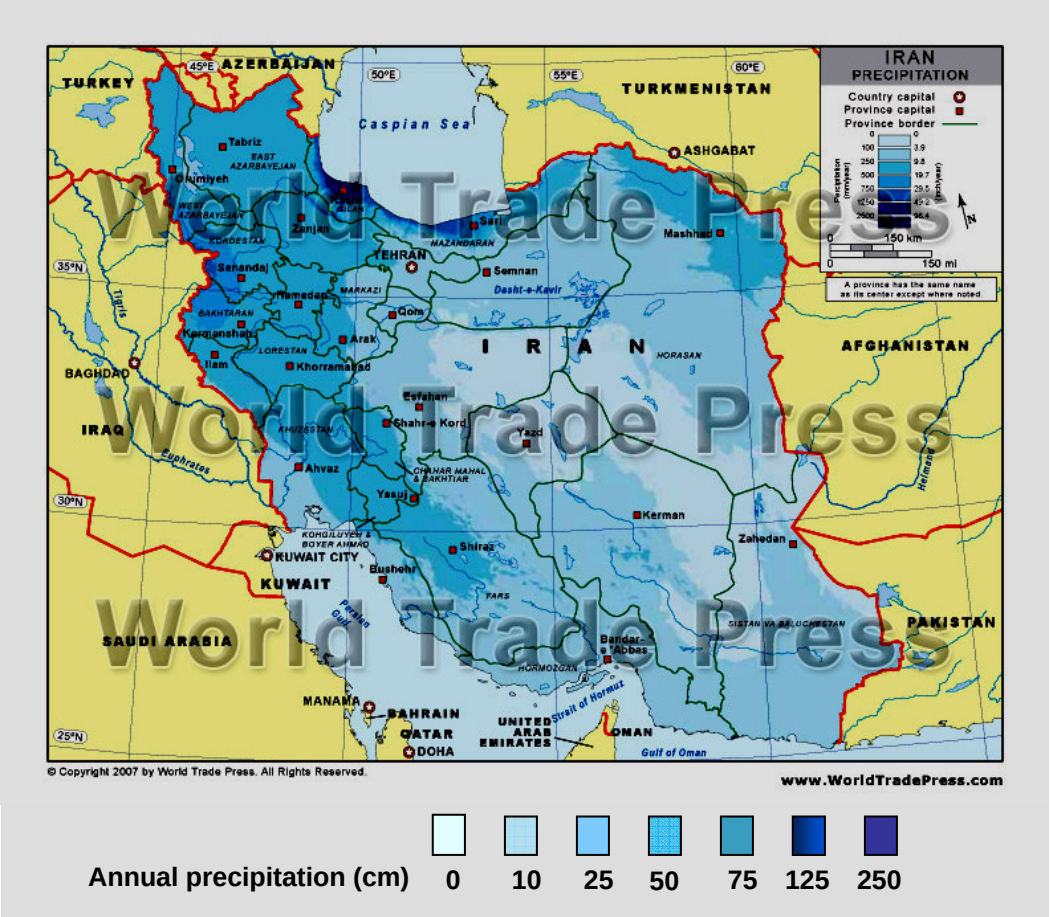


Fig. 3: Precipitation distribution in Iran.

Wheat is cultivated in Iran on 6,000,000 ha with an average yield of 2.417 kg/ha (FAO 2006). Of the production area 60% is located in arid and semi-arid regions. Under the above described conditions, wheat is permanently challenged by drought along with nutrient deficiency of the soil and other kinds of biotic and abiotic stresses. This challenge results, if not always but frequently, in a partial to drastic reduction of yield. For example, a three-year consecutive drought, which occurred between 1999 and 2001, affecting 18 provinces of the country, reduced wheat yields dramatically (Fig. 4), forcing the government to massive wheat imports (Red cross information bulletin,2001).

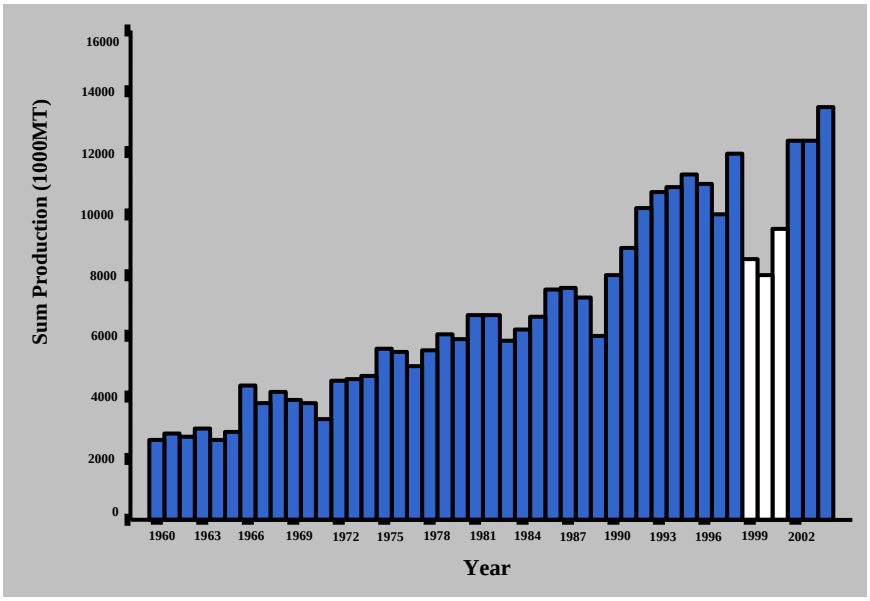


Fig. 4: Total wheat production in Iran between 1960 and 2004. White columns between 1999 and 2001show dramatic yield reduction caused by drought.

1.2. Objectives of the study

Considering these facts about wheat and drought in Iran, the main objective of this project was to study the genetic background of drought tolerance of wheat, and to attempt to identify potential quantitative trait loci (QTLs) related to drought tolerance. To achieve this, the following work had to be done:

- Cross an Iranian wheat genotype selected by nature for drought tolerance and a high yielding European cultivar selected under optimum growing conditions, never exposed to drought.
- Use the F1 to create a mapping population and develop recombinant inbred lines (RILs), using single seed descent (SSD) method (F2:7),
- Back-cross the F1 with the European parent to develop an advanced back-cross population (BC2F4),
- Construct an SSR based linkage map of the F2:7 population,
- Construct an SSR based linkage map of the BC2F4 population,
- Evaluation of the mapping populations under non-drought and drought conditions.

1.3. Wheat has a most complex genome

Bread wheat (*Triticum aestivum*) has one of the largest and most complex genomes among crop plants. It evolved from three closely related diploid species into an allo-hexaploid plant with the genomic formula of AABBDD, having the chromosome number of $2n = 6x = 42$. Extensive genetic and cytogenetic characterization of diploid, tetraploid, and hexaploid wheat contributed greatly to our understanding of the evolution of allopolyploid plants. The total size of the haploid wheat genome contains some 16 Mb, i.e. 16 billion base pairs of DNA which exceeds more than 5 times the human genome (~3 Mb). Due to the close genetic relatedness of the three composing genomes, a large part of the genes are present in triplicate, i.e. present in all three genomes. Such a genomic constitution provides the potential of an enormous genetic variation, allowing wheat world wide adaptation and distribution, to be cultivated from as far north as Finland with almost 200,000 ha to the edge of deserts. This genetic potential of wheat allowed plant breeders to select genotypes for almost all kinds of environmental conditions. On the other hand, the average size of one of the three wheat genomes (~5.3Mb) is almost double of the human genome, containing at least the double amount of DNA with unknown function, e.g. highly repeated sequences, retro elements, etc. Probably this structural difference makes wheat an unrewarding target for molecular genetic or genomic approaches. Both, genes and recombination was found to be highly unevenly distributed on the wheat chromosomes (Dilbirligi et al. 2004). More than 85% of the genes are present in 48 gene-rich regions (GRRs) with varying sizes and densities, encompassing less than 29% of the genome. The remaining 71% is gene-poor, consisting of large blocks of repeated DNA interspersed with very few genes. Recombination was found to occur mainly in GRRs, but various GRRs differ as much as 140-fold in recombination rates. Of the 252 phenotypically characterized useful genes, 241 are physically localized in GRRs, and 21 of them on homoeologous group 3 (Erayman et al. 2004). Wheat has been the last among cereals to be genetically transformed (Khurana 2001).

Successful wheat genotypes, selected over centuries by farmers under drought conditions, prove that genetic variation is available in wheat, making possible to meet this challenge. Now the true challenge facing wheat breeders is to find out how natural selection shaped these plant genotypes to resist drought and other stresses, how a conscious reconstruction of the wheat genome by plant breeders may lead to the production of advanced wheat genotypes, combining drought tolerance with other useful traits.

1.4. Drought tolerance is an extremely complex trait

Of all the abiotic stresses that reduce crop productivity, drought is the most devastating and resistant to breeder's efforts (Tuberosa and Salvi 2006). Its overall complexity originates from the number and complexity of factors, which are involved in plants responses to drought stress. This complexity will be even further increased if other kinds of stresses (Table1) and factors like soil nutrition deficiencies accompany drought.

However, from a conceptual point of view, some believe that drought tolerance may be less complex, considering that most of the crucial traits controlling plant water use and status under stress are basically constitutive. Phenological traits, like flowering time and maturity, are such constitutive traits, usually independent of drought stress (Blum 2000). This may be correct, but when talking about the cellular and molecular aspects of stress responses, much more complexity can be expected.

Terminologically, drought is an extended period when water availability falls below the statistical requirements for a region. Agricultural drought simply occurs when there is not enough soil moisture to meet the needs of a particular crop at a particular time. (Reynolds et al. 2005).

Table1: Biotic and abiotic stresses affecting crop yield in dry environments (Reynolds et al. 2005).

Patterns of Moisture Stress	Temperature Extremes	Nutrient Stress & pH Extremes	Biotic stress	Agronomic Practices
Terminal	Heat Stress Humid	P and N Deficiency/ Efficiency	Root rots	Stubble retention
Pre-Anthesis	Heat Stress Dry	Deficiency (e.g., zinc)	Nematodes	Zero tillage
Residual Moisture	Cold Stress	Toxicity (e.g., boron)	Foliar pathogens	Crop rotations
Reduced Irrigation	Cold Stress – Late Frost	Acid Soils Mineral		Shifting cultivation
General Low Rainfall		Acid Soils Volcanic/Organic		Water harvesting
Shallow, Marginal, Infertile, Eroded Lands		Alkaline Soils		

From a practical point of view, drought tolerance relates to final yield, rather than to the capacity of the plant to survive in water-limited conditions (Tuberosa and Salvi 2006). On the other hand, it can be described as the ability of crop to maintain a constant yield, regardless of any environmental adversity, a concept that is known as yield stability (Cattivelli et al. 2002). To understand these definitions and also the complexities behind stress responses, one needs to know about the mechanisms or strategies by which plants respond to stresses.

1.5. Plant responses to drought stress

Plant responses to drought stress can usually be categorized at the level of the whole plant and at the cellular level (Fig. 5).

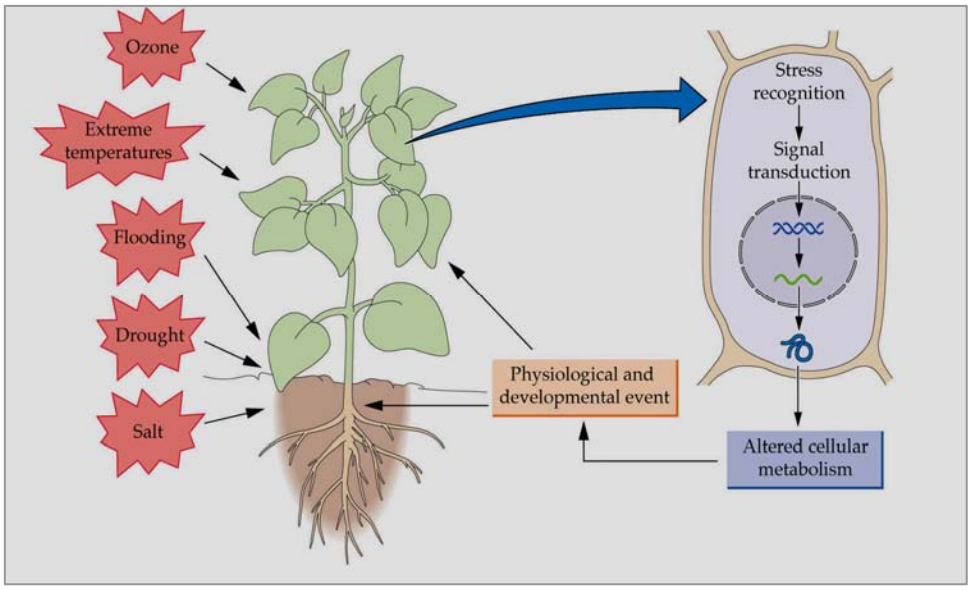


Fig. 5: Plants respond to stresses as whole organism (left) and as individual cells (right) (Chaves et al. 2003).

1.5.1. At the level of the whole plant

Stress responses at the level of the whole plant have classically been divided into escape, avoidance and tolerance mechanisms (Chaves et al. 2003).These mechanisms are not mutually exclusive and, in practice, plants may combine a range of responses

Escape strategy is based on successful completion of the plant life cycle before the onset of severe stress. Plants that escape drought combine short life cycle with a high rate of growth and gas exchange. Maximum use of available water resources and better partitioning of assimilates are two main ways of escape strategy.

Avoidance means to maintain tissue water potential as high as possible, enabling a plant to adjust itself to limited resources of water and minerals under moderate drought stress by minimizing water loss and maximizing water uptake.

Minimizing water loss is achieved by:

- Primary response to water deficit is stomatal closure and reduced cell expansion. Leaf rolling is a consequence of reduced cell expansion (Fig. 6).

- Reduced light absorbance by steeping leaf angle,

- Decreasing canopy leaf area resulting in reduced growth,

- Shedding of older leaves

Stomatal closure plays a very important role in avoidance responses by achieving a balance between water status and carbon uptake in plants (Fig. 7).

Fig. 6: Leaf rolling in wheat (left) and in sorghum (right) under drought (www.plantstress.com).

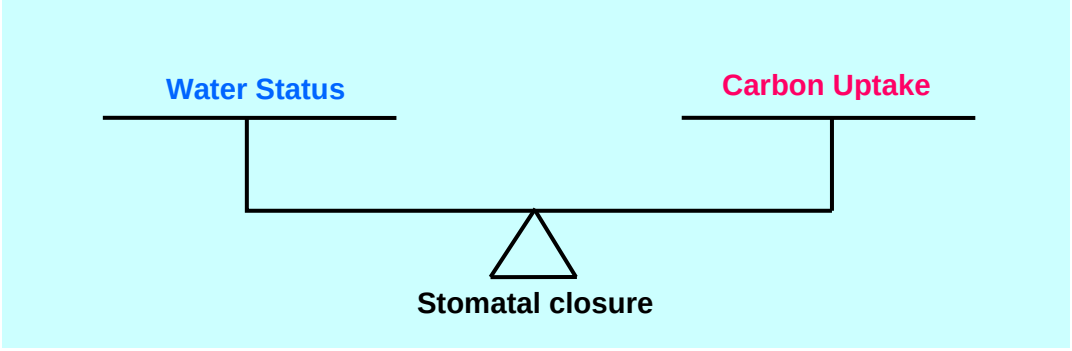


Fig. 7: A trade-off occurs between water status and carbon uptake by stomatal closure.

Maximizing water uptake is simply achieved by increased root growth and shedding of older leaves to grow as deep as possible to get access to more water in the soil (Fig. 8).

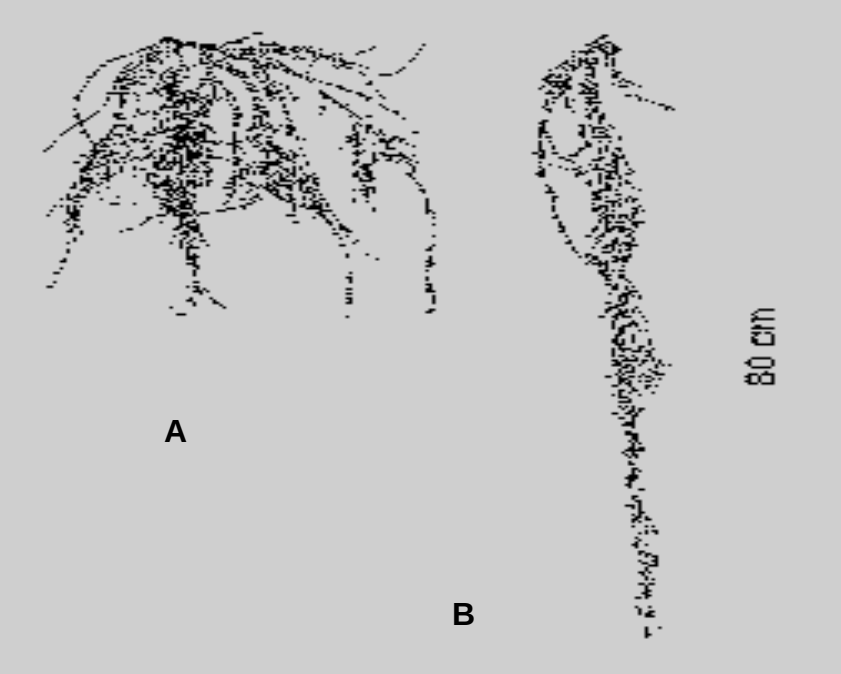


Fig. 8: One month old sorghum roots. A: from a controlled irrigated experiment, B: from dry soil (*www.plantstress.com*).

Tolerance strategy is the ability of the plant to tolerate a long period of low tissue water potential (Sullivan and Aross 1979). It mainly involves osmotic adjustment, which is one of the crucial processes in plant response and adaptation to water deficit, especially in a long period of severe drought. Osmotic adjustment sustains metabolic activities of plant cells to tolerate low water potential and thus enables them to re-grow. During water deficit stomatal conductance will be reduced in order to maintain cell turgor, which is essential for cell expansion (Fig. 9).

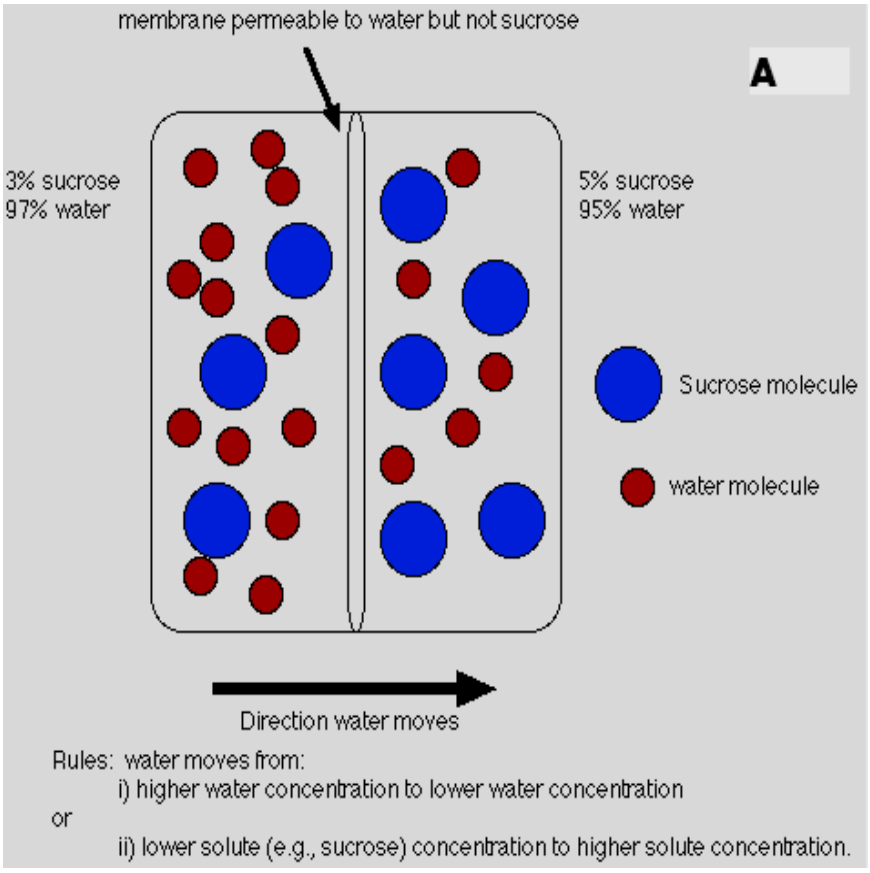


Fig. 9: Osmotic adjustment enables plant to maintain cell turgor.
A: Simple rules of osmotic adjustment, B: Cell turgor (left) and cell Plasmolysis (right) (www.plantphyst.net).

1.5.2. At the cellular level

At the cellular level plants respond to extra-cellular stimuli, i.e. water deficit, by the cell signal transduction pathway (Fig. 5). A signal is any extra- or intracellular factor, chemical or physical, which can be received or sensed by the plant cell and finally initiate a cellular response. A signal is a ligand (in the terminology of cell biologists), meaning a substance that binds specifically (Griffiths et al. 2003). A cell signaling process includes three main steps:

- Recognition
- Transduction
- Response

Recognition, or sensing a signal, means binding the signal to a cell-surface receptor (Fig. 10). This receptor is the extracellular domain of the Receptor-like Histidin Kinases (RHKs), which are the most important receptors in plant cell membrane, mediating recognition of water deficit signals. The receptor-ligand complex acts as a primary message to inform the cell to respond. This message is amplified in the next step which is called transduction.

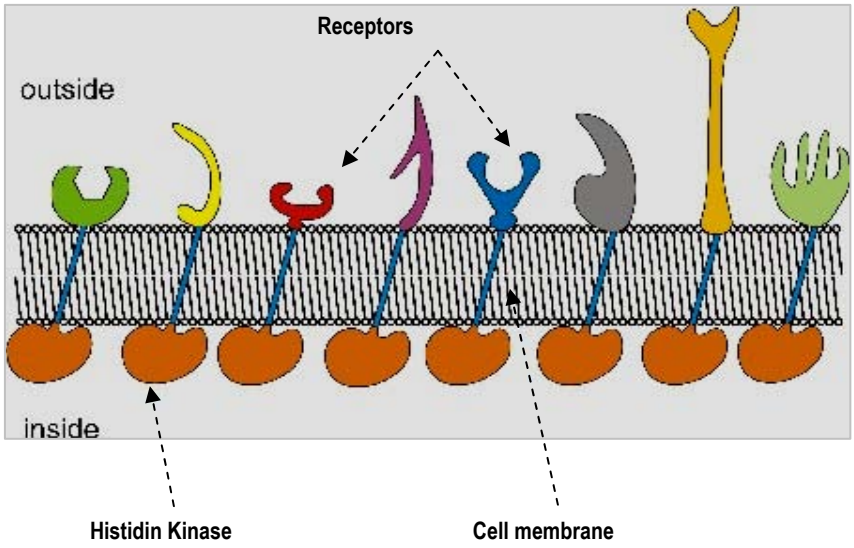


Fig. 10: Plant **receptor-like kinases** display a large variation in their extra cellular domains, which are believed to reflect their affinity for different types of ligands (Griffiths et al. 2003).

Transduction starts after binding the signal ligand to a receptor domain, which will activate internal receptors (RHKs) by phosphorylation. An activated receptor is able to activate molecules, so-called secondary messengers, which can trigger multi step sequential processes, called signal transduction pathways, mediated by calcium ions. The most important secondary messengers in plants are Abscisic acid (ABA), Inositol Phosphates (IPs) and Reactive Oxygen Species (ROS).

Response to the signals, mediated by secondary elements, will result in transcription factors, which are proteins coded elsewhere in the genome. They regulate gene transcription by binding directly, or through an intermediate protein, to the *cis*-regulatory region of that gene (Fig. 11). Target genes are the endpoints of each cascade of events (Gabriela et al. 2002).

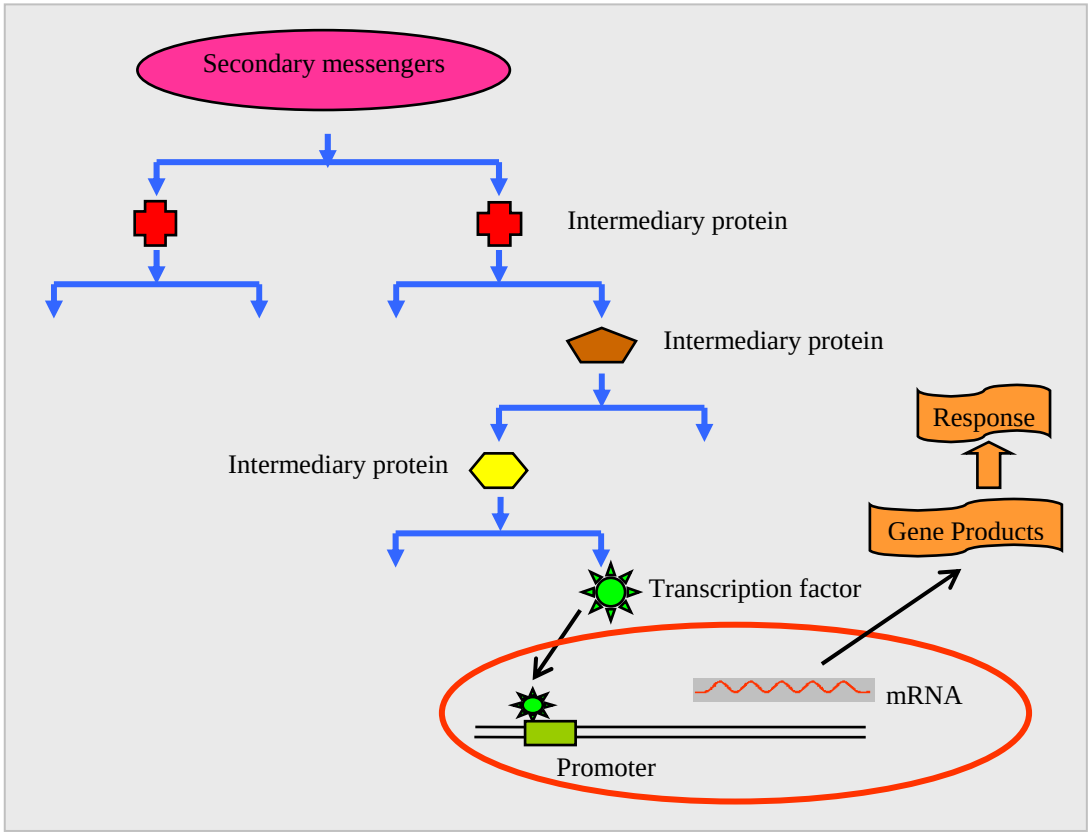


Fig. 11: Schematic diagram of a signal transduction pathway. In each step of the pathway one factor or protein activates the next. Some factors can activate more than one protein in the cell. Final product of each signal transduction pathway is a transcription factor.

1.6. Traits related to drought stress

Grain yield is the most frequently studied and the least understood trait related to agronomic performance of wheat under drought stress. Selection for yield is usually difficult under drought, mostly due to its low heritability and the additional effects of other stresses (Blum 1988). Increasing grain yield can be achieved by increasing either the total biomass produced by the crop, or the proportion of the total biomass that is invested in grains (resulting in increased harvest index). Thus, a gene that increases yield should do so through one of these two fundamental mechanisms.

Grain yield also represents the product of grain number and mean weight per grain. Grain number per se can be broken down into its components: spike number per plant and grain number per spike, the latter being determined by the number of spikelets per spike and grains per spikelet. Therefore, by studying how these yield components vary within a particular genetic background, insight may be gained into the possible function of a gene that influences yield, and how it is likely to exert its effects (Quarrie et al. 2006). Slafer (2003) broke down the determination of yield components into different phases within the plant's life cycle, with some overlapping between phases. Generally speaking, spikelets per spike are determined before grains per spikelet, both overlapping in time with the determination of ears per plant, and with weight per grain being the final component to be determined.

Due to the complexity of drought stress, special attention was given to physiological and molecular aspects of plant response and performance under drought stress. Disregarding constitutive traits, which mostly determine plant adaptation to stress, like growth habit and heading time, traits related to physiological aspects of drought tolerance have mostly been studied, and still are in the center of attention, in plant breeding programs targeting wheat improvement in dry areas (Fig. 12). These traits can be categorized into four groups (Reynolds et al. 2005):

G1. Traits related to pre-anthesis growth
G2. Traits related to access to water
G3. Traits related to water use efficiency
G4. Traits related to photo-protection

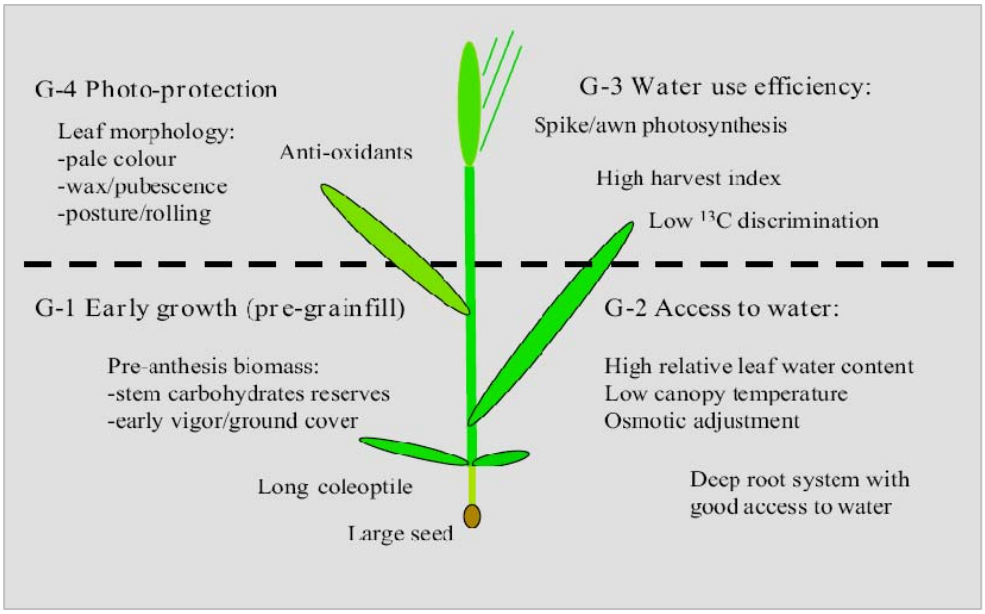


Fig. 12: A conceptual model for drought adaptation. Traits are placed in four groups in a way that the genes and/or physiological effects among groups are likely to be relatively independent. Thus, when parents with contrasting expression in trait-groups are crossed, drought-adaptive genes are more likely to be dissected for a later pyramiding (Reynolds et al. 2005).

1.7. Chromosomal regions of the genes controlling traits related to drought

Understanding the genetic basis of the traits affecting yield under drought condition is crucial for sustainable improvement of wheat in breeding programmes. Enormous advancements in genomic resources and tools in recent years greatly assist mapping and cloning quantitative trait loci (QTLs) and the corresponding genes. (Borevitz et al. 2004). Genome sequences, molecular markers and microarrays are being used for QTL mapping.

1.7.1. Yield and yield components

During the last two decades, several studies in wheat, mostly utilizing intervarietal substitution lines, revealed some of the major chromosomal regions controlling plant response to drought and other abiotic stresses. These fundamental findings could help to better understand the genetic basis of crop performance (yield) under stress. A good review of these works can be found in Cattivelli et al. (2002). Here a brief review:

Osmotic adjustment (OA), as a major cellular stress adaptive response in plants that enhances dehydration avoidance and supports yield under stress (Blum, 1989), was found to be conditioned in wheat by a major locus on the short arm of chromosome 7A (Morgan and Tan

1996). A major QTL, influencing drought induced Abscisic Acid (ABA) accumulation, was localized on the long arm of chromosome 5A (Quarrie et al. 1994).

Water use efficiency (WUE), which simply shows the efficiency of crop to use and manage limited water resource, was another area of interest to be studied. By analyzing D-genome substitution lines (Gorney 1999), it was revealed that chromosome 7D has a positive effect on WUE. Chromosome group 5 of wheat has the highest concentration of QTLs and major loci controlling plant adaptation to the environment, particularly those controlling heading time, frost and salinity tolerance. Moreover, detection of multiple-stress QTLs in wheat and barley indicates the assistance of common mechanisms for coping with different stresses, and consequently common genes controlling different stress tolerance processes (Cattivelli, et al. 2002).

In contrast to those traits, there is general awareness and agreement, that very few studies were carried out targeting QTLs for yield (Quarrie et al. 2005) and particularly yield under drought stress conditions. This, as can be expected, is due to the complexity of this trait and the difficulties of field evaluation and screening under stress conditions.

An initiative and extensive study has been done by Börner et al. (2002), targeting 20 morphological, agronomical and disease-related traits. One hundred and fourteen inbred lines were tested at three non-stressed locations within three seasons. One hundred and twenty one QTLs were found for those traits. This was the highest number of QTLs, which were reported in a QTL analysis. In two consecutive studies 40 and 57 QTLs were identified for yield, yield components, plant height and heading time (Huang et al. 2003 and 2004). Quarrie et al. (2005) evaluated a population of 96 DHs from a cross between Chinese Spring and SQ1 (a high ABA-expressing breeding line) at 24 location-treatment-year combinations, including nutrient, drought and salt stress treatments. Seventeen clusters of QTLs were identified on all seven chromosome groups of wheat. Also Narasimhamoorthy et al. (2006), following an advanced back cross (AB) QTL analysis, found 10 QTLs for yield, its components, plant height and heading time on chromosomes group 2 and 3 of bread wheat. Using a population of 100 RILs and 449 markers (SSR, AFLP, and SAMPL), Kumar et al. (2006) identified 3 clusters of QTLs for 1000-kernel weight on chromosomes 1A, 2B and 7A. McCartney et al. (2005) used a population of 182 DH lines from a cross between two western Canadian marketing wheats. Except for chromosomes 1A, 3A, 5A, 6A, they identified 34 QTLs for yield, test weight, 1000-kernel weight, plant height, time to maturity, and lodging, distributed on all the other chromosome groups of wheat. In a most recent study (Kuchel et al. 2007), a population of 192 DH lines from a cross between two Australian wheat varieties differing in yield was used to dissect QTLs for yield and its components, at 18 site-year combinations. They found 3 clusters of QTLs on chromosomes 1B, 4D, and 7D. This study and the one by Quarrie et al. (2005) can

be considered to be the most valuable QTL studies for yield, regarding the number of location-year combinations, including drought stress conditions.

In some of the studies only one chromosome was targeted for QTL analysis. For example, chromosome 3A was shown to be rich with QTLs controlling agronomic traits. Shah et al. (1999), using a population of 50 recombinant inbred chromosome-3A lines (RICLs-3A) and 13 RFLP markers, found QTLs for plant height, 1000-kernel weight, kernel per spike, number of spike per square meter and plant height under non-stress condition. Campbell et al. (2003), studying the same chromosome using 98 RICLs-3A, found QTLs for yield and its components, as well as plant height. More recently, Dilbirligi et al. (2006) identified 17 QTLs on chromosome 3A related to seven agronomic traits, also under normal conditions. Sishen et al. (2007) dissected 46 putative QTLs for grain yield and its components on 12 chromosomes of wheat. A population of 41 single-break deletion lines and 179 SSR and RFLP markers were used in that study. Spielmeyer et al. (2007) reported a QTL, associated with longer coleoptiles, greater vigour and plant height, on chromosome 6A. Also in a population of 127 RILs from a cross between two bread wheat cultivars ‘Dharwar Dry’ (drought tolerant) and ‘Sitta’ (drought susceptible), utilizing SSR/EST-STS markers, chromosome 4A was shown to have a significant impact on grain yield under drought condition (Kirigwi et al. 2007).

1.7.2. Heading time

Among the three major genetic factors governing heading time in wheat, i.e. vernalization responsive genes, photoperiod responsive genes and narrow-sense earliness or earliness per se (Kato et al. 1998), the first two are environment dependent, while the latter is environment independent. Genes controlling vernalization (Vrn-A1, Vrn-B1 and Vrn-D1) are located on chromosomes 5A, 5B and 5D; those controlling photoperiod sensitivity (Ppd-A1, Ppd-B1 and Ppd-D1) are located on chromosomes 2A, 2B, and 2D.

Narrow–sense earliness is a polygenic trait and several genes are involved in the control of heading time (Shindo et al. 2003). It is interesting to know that in diploid barely, up to now; more than 80 QTLs have been mapped for heading time in different populations (Cattivelli, et al. 2002). In wheat, Islam et al. (1996) found QTLs for late heading located on chromosome group 6. Law et al. (1998) reported QTLs for ear emergence on chromosomes group 1. Sourdlille et al. (2000) detected some QTLs involved in earliness per se on chromosomes 3A, 4A, 4D, 6B, and 7B.

Association between QTLs for heading time and yield and its components was evaluated in some studies, indicating the potential effects of this trait on yield and yield components. For example, Huang et al. (2003) found 8 QTLs for heading time located on 2A, 2D, 3B, 5A, 5B,

6A, and 7B. QTLs on 2A, 2D and 5B showed co-segregation with QTLs for grain yield. In their next study they found five QTLs for heading time located on 2D, 3A, 4A, 7A and 7D (Huang et al. 2004). Quarrie et al. (2006) identified two clusters of QTLs for flowering time, which coincided with QTL clusters for yield on chromosome 7A. Narasimhamoorthy et al. (2006) in a BC2F4 population found 2 QTLs for heading time on chromosome 2D and 3D. The latter coincided with a QTL for kernel per spike.

1.7.3. Plant height

Despite the successes of semi dwarf wheats in dry lands, they have not replaced older, taller wheats in all areas. In Australia for example, the first semi dwarf variety was released in 1968, but less than half of the total area sown with wheat was occupied by semi dwarf varieties (Richard 1992). One reason for the continued success of tall wheats may be their superiority to semi dwarfs in dry areas. As was shown by Butler et al. (2005), taller wheats maintained their higher yield level under post-anthesis drought. The benefits of the dwarfing genes are more pronounced in high yielding winter wheat environments. Under heat and drought stress there may be no benefit of the dwarfing genes for spring wheat (Flintham et al. 1997). Nizam-Uddin and Marschall (1989) concluded that grain yield does not depend on the presence of dwarfing genes per se, but rather on optimum height for given environment. According to the conceptual model for drought tolerance (Reynolds et al. 2002), shorter plants are better adapted to irrigated and high input environments, while taller plants are considered to have better yield stability under adverse conditions. Besides the major dwarfing genes controlling reduced plant height, positioned on chromosomes 2D, 4B, and 4D, there are a number of genetic factors influencing plant height, involving most of the 21 chromosomes of wheat. The first QTLs for plant height were already detected in 1998 by Cadalen et al. on chromosomes 1A, 1B, and 4B. In the other studies a number of QTLs were found for plant height on different chromosomes i.e. 1A, 2D, 4A and 6A (Börner et al. 2002), 1A, 1D, 3A, 3B, 4A, 4B, 5A, 5B, 6A, 6D, 7A and 7D (Huang et al. 2003 and 2004) and 2D, 4B, 4D, 5A, 7A and 7B (McCartney et al. 2005).

Just as in the case of heading time, the association between QTLs for plant height and those related to yield and its components were discussed by Quarrie et al. (2005 and 2006) and Kuchel et al. (2007).

1.8. Principles of mapping quantitative trait loci (QTL)

A quantitative trait locus (QTL) is the location of a gene, or a cluster of genes, affecting a trait that is measured on a quantitative (linear) scale. Examples of quantitative traits are plant height (measured on a ruler) and grain yield (measured on a scale).

The goal of QTL mapping is to establish linkage between a marker allele and an 'allele' of a locus responsible for a significant part of variation of a quantitative trait. A quantitative trait locus may be constituted of several closely linked genes or a group of genes participating in trait expression. Determining the number, location, and interaction of the loci contributing to total phenotypic variation is the penultimate goal of QTL mapping. Final goal could be the identification and isolation of actual genes to determine their functions in trait expression.

1.8.1. Mapping populations

QTL mapping involves just a few basic steps. The primary requirement is a mapping population, which is usually constructed from a cross between two parental genotypes differing in the alleles that effect the variation in the trait of interest. A molecular marker-based linkage map allows each pair of genotypes in the population to be distinguished genetically. The more detailed the linkage map (that is, the larger the number of markers), the better the mapping resolution. The parental alleles are then shuffled in the mapping population, in which the phenotypes of each individual are measured. Finally, by using statistical techniques, QTL mapping results in localization of chromosomal regions that might contain genes contributing to phenotypic variation in a trait of interest (Mauricio 2001).

According to Tanksley (1993), QTLs can be characterized as follows:

- Individually they follow Mendelian segregation,
- Many genes control a given trait,
- Individual gene effects are small,
- The phenotype is subject to considerable environmental variation,
- The genes involved can be dominant or codominant,
- Many different genotypes can produce the same phenotype,
- The genes involved can be subject to epistasis.

Identification of QTLs depends on four main factors:

- How tightly a QTL is linked to the marker,
- The size of the effect of a QTL,
- The size of the mapping population scored,
- The heritability of the involved trait.

Based on the variation and homozygousity expected in a population and the reproductive mode of the plant, most commonly used mapping populations for self-pollinating

crops, like wheat, are: F2, backcross (BC), recombinant inbred lines (RILs), and doubled haploid lines (DHs).

1.8.1.1. F2 populations

The simplest form of a mapping population is a collection of F2 plants. If possible, two parental genotypes should be different in all traits to be studied. The degree of polymorphism can be assessed at the phenotypic level or by molecular markers. Genetically, an F2 population is the product of meiosis of the F1, when the genetic material of the two parents recombines. A big disadvantage of an F2 population is that in the case of dominant markers, like RAPD and AFLP, differentiation between homozygous dominant and heterozygous loci is impossible. Another disadvantage is that they cannot be preserved easily, because F2 plants are further segregating. To produce a genome-wide map, a population of around 100 F2 individuals is recommended as a compromise between resolution of linked loci and costs.

1.8.1.2. Backcross populations (BC)

A backcross population derives from a cross between the F1 hybrid and one of the parental genotypes (recurrent parent). During this process, unlinked fragments of the donor are separated by segregation, and linked donor fragments are reduced in size due to recombination with the recurrent parent. To further reduce the number and size of donor fragments, backcross is repeated. With each round of backcrossing, the proportion of donor genome is reduced by 50% (Fig. 13A).

An F2 population is better than a simple BC population, since QTLs with recessive alleles cannot be detected in a recurrent parent, and when dominance is present, backcross gives biased estimates, because additive and dominant effects are completely confounded (Asins 2002). A common disadvantage for both, F2 and BC populations is their lower level of homozygousity in comparison to RILs and DHs.

A solution for these problems is a method called advanced backcross QTL analysis (AB-QTL), which was suggested by Tanksley and Nelson (1996). The basic idea is simple; it combines backcross and marker analysis in the same population. QTL analysis is delayed until advanced BC generations e.g., BC2, BC3. QTL detection is concentrated on the small fragments of donor parents, which are integrated into the background of recurrent parent genome (Fig. 13B). This technique was established to investigate the potential of less adapted or even wild genotypes, for improving quantitative traits of an elite genotype or variety, representing the recurrent parent. In comparison to RILs and DHs, an advanced backcross population may give us higher resolution of QTL maps, because only small fragments of the donor are integrated into the genome of the recurrent parent. But, regarding the amount of

variation for each trait of interest, it has less resolving power due to the reduced amount of variation from the donor parent. Therefore, to improve QTL detection, an advanced BC population should be larger than RIL or DH populations.

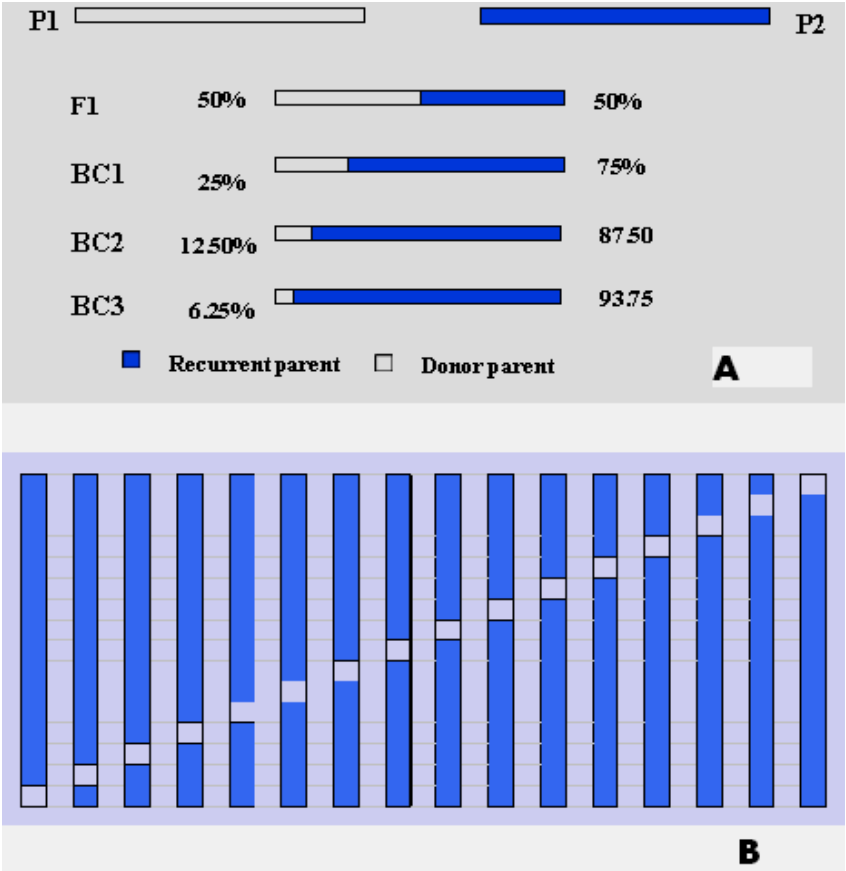


Fig. 13: **A:** Level of heterozygousity-homozygousity in backcross generations, **B:** Theoretical distribution of integrated fragments of donor parent (white sections) into the background of recurrent genome (blue sections) at BC3 generation. It is expected that the 6.25% of the donor genotype are different in each BC3 individual and evenly distributed into the background of the recurrent genome.

1.8.1.3. Recombinant inbred lines (RILs)

Recombinant inbred lines (RILs) are the selfed homozygous progeny of the individuals of an F2 population (Fig. 14). Because, in the selfing process, a single seed of each line is the source of the next generation, RILs are so called single seed descent (SSD) lines. Within six to seven generations theoretically almost complete homozygousity (~1.6% heterozygousity) can be achieved. Because recombination cannot longer change the genetic constitution of RILs, they lack further segregation in the progeny. As a major advantage, this constitution provides a permanent (immortal) genetic resource that can be reproduced indefinitely and shared by many groups of the scientific community. A second advantage of RILs is that, due to several rounds

of meiosis before homozygousity is reached, the degree of recombination is higher, compared to F2 populations. Consequently, RILs yield higher resolution maps than those generated from F2 populations, and the map positions of even tightly linked markers can be identified (Burr and Burr1991).

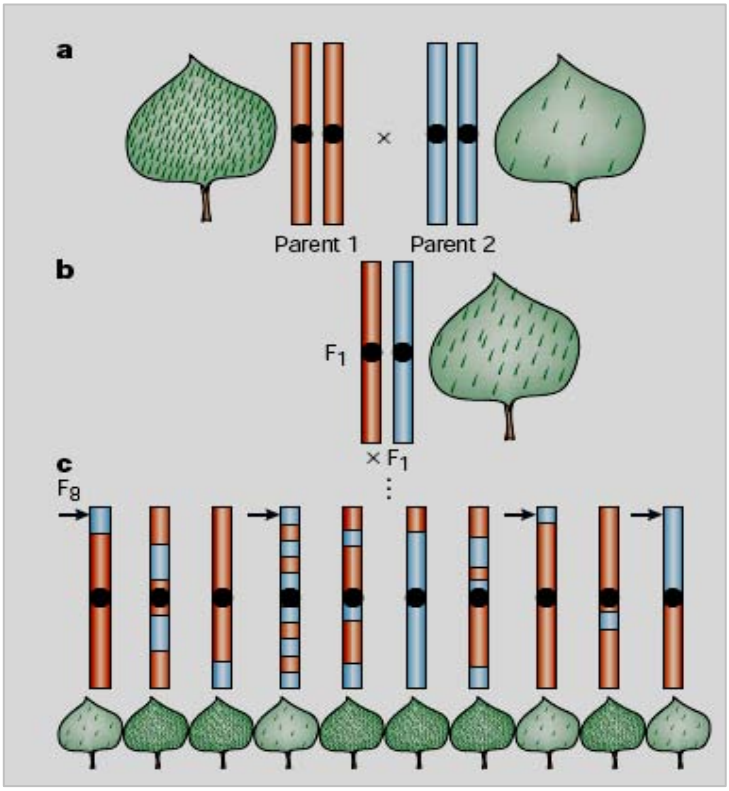


Fig. 14: Schematic diagram showing the procedure to construct a recombinant inbred population using the segregation of trichome density on the leaf surface as a quantitative trait (Mauricio 2001).

1.8.1.4. Doubled haploid (DH) population

Doubled haploid populations contain absolutely true-breeding lines, which are homozygous in every single locus of each gene. In bread wheat, two major routes of DH production have been developed: microspore culture (Zheng, et al. 2001) and wheat-maize cross system (Suenaga 1994). Although wheat and maize belong to separate sub-families of the Gramineae, the fertilization of the wheat egg-cell with pollen of maize is successful. After naturally elimination of the maize chromosomes, following fertilization of the wheat egg cells result in haploid embryos. To achieve diploidy, haploid seedlings have to be treated with colchicine. Fig.15 illustrates this procedure. Besides absolute homozygousity, which can be

utilized in DH populations for a high resolution of QTL mapping, they also provide a permanent genetic constitution.

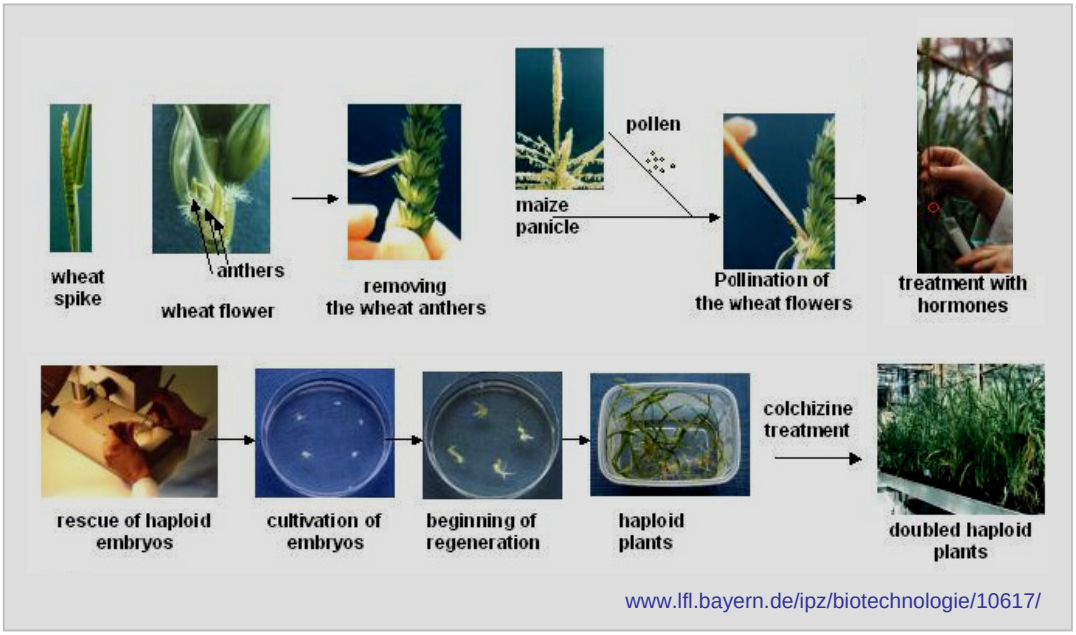


Fig. 15: Wheat-maize cross system for doubled haploid (DH) production.

Incompatibility of some wheat genotypes to wheat-maize cross system, low rate of embryo regeneration and, particularly, viability of haploid plants are limiting factors to utilize DHs in comparison to RILs.

1.8.2. Molecular markers and linkage maps

Once a mapping population is developed, it can be genotyped to construct a linkage map. Genotyping means evaluation of the individuals in a segregating population using molecular markers. The data can be used to identify the relative orders and distances between the markers in linkage groups. Regardless of the technical aspects, accuracy and efficiency of genotyping is highly dependent on the type of markers and their ability in differentiating individuals. During the past two decades a large number of different DNA markers have been developed. The most commonly used markers today are Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and Simple Sequence Repeats (SSR). Table 2 summarizes their major differences and characteristics.

Table 2: Comparison of different DNA-based markers.

Features	RFLP	RAPD	AFLP	SSR
DNA amount required (µg)	10	0.02	0.5-1.0	0.05
Required DNA quality	high	high	moderate	moderate
PCR-based	no	yes	yes	yes
polymorphic loci/marker	1-3	several	several	1-3
Ease of use	not easy	easy	easy	easy
Amenable to automation	low	moderate	moderate	high
Reproducibility	high	unreliable	high	high
Development cost	low	low	moderate	high
Cost per analysis	high	low	moderate	low
Alleles per locus	few	two	two	several
Type of segregation	Co-dominant	Dominant	Dominant	Co-dominant

Among these markers, SSRs are known as anchored markers, meaning that they have sequence-specific physical locations. Generally they are co-dominant. Therefore it is possible to distinguish heterozygous individuals or loci in mapping populations. In contrast, AFLPs, just like RAPDs, are anonymous markers. Due to their dominant segregation pattern they are not able to differentiate heterozygous individuals. But, while SSRs are usually single locus markers, AFLPs are multi-locus markers, therefore more efficient than SSRs. They may produce several polymorphic loci per PCR reaction (Table 2). In comparison to SSRs and AFLPs, the other two markers, RAPDs and RFLPs, are less popular. RFLPs are laborious to be used, and RAPDs are considered not to be robust enough.

Once the marker data have been evaluated for all individuals of the mapping population, a linkage map can be constructed. A linkage map is indeed a linear order of linked markers with their relative genetic distances. There are two important points to be considered. The first one is to test the segregation of a marker according to an expected Mendelian ratio. This will be done by a chi-square test. Table 3 shows expected segregation ratios in different segregating populations. The second step is a linkage analysis determining the strength of linkage between two markers. This is usually done using odds ratios (i.e. the ratio of linkage versus no linkage). This ratio is more conveniently expressed as the logarithm of the ratio, and is called a logarithm of odds (LOD) value or LOD score (Risch 1992). Typically LOD values of > 3 are used. A LOD value of 3 between two markers indicates that linkage is 1000 times more likely (i.e. 1000:1) than no linkage (Null hypothesis). LOD values may be lowered in order to

detect more linkages, even if they are less likely, or to place additional markers on the map already constructed (Collard et al. 2005).

Table 3: Expected segregation ratios in different mapping populations.

Population type	Codominant markers	Dominant markers
F2	1:2:1(AA:Aa:aa)	3:1 (B-:bb)
BC	1:1(Cc:cc)	1:1 (Dd:dd)
RIL & DH	1:1 (EE:ee)	1:1 (FF:ff)

Relative distances between the markers located on the same chromosome depend on the recombination frequencies by which they segregate (Paterson 1996a). Because recombination frequency and the frequency of crossing-over are not linearly related, mapping functions are required to convert recombination fractions into centiMorgan (cM). Two commonly used mapping functions are the Haldane and Kosambi mapping functions. Haldane's mapping function assumes that there is no interference, which would increase or decrease the proportion of double crossovers. Kosambi's mapping function is based on empirical data, taking into consideration the proportion of double crossovers as the physical distance varies. Kosambi's function adjusts the map distance based on interference, which changes the proportion of double crossovers (Hartl and Jones 2001).

All these processes, including linkage analysis and genetic distance calculation, can easily be done by mapping softwares. Most commonly used programs are Mapmaker/EXP (Lander et al. 1987), MapManager QTX (Manly et al. 2001) and JoinMap (Stam 1993).

1.8.3. Statistical methods for QTL mapping

The final step of QTL mapping is, after constructing a linkage map based on the segregation of marker alleles, to utilize this map for finding association between segregation of markers and segregation of traits. Segregation data of traits are collected on the same population in field experiments. This evaluation is basically a statistical procedure. In the simplest way, an ANOVA can be used to see whether there is a significant association between a marker locus and a trait, but this does not identify the exact location of a QTL. We have to identify an interval, which is flanked by the markers, where, with the highest probability, a QTL related to a trait of interest can be found. Based on this concept, known as confidence interval, several techniques were developed for QTL mapping. Fig. 16 illustrates the most commonly used methods (Mauricio 2001).

A- In the regression technique or single marker analysis method, the phenotype is correlated with each marker genotype (Haley and Knott 1992). The middle part in Fig. 16 shows the differential migration of a DNA fragment on a gel. In this case, a single marker 'A' is scored. Individuals that are homozygous for the A allele have high trichome density, individuals that are homozygous for the 'a' allele have low trichome density, and heterozygotes have intermediate trichome density. A linear regression of trichome density on the number of A alleles shows a significant relationship between the marker and the phenotype, which indicates that a QTL for trichome density is probably linked to that marker. The simple regression method described is of limited use in localizing the chromosomal segment that contains a QTL. The method underestimates the effect of the QTL. The further the QTL is from the marker, the weaker the effect.

B- In the interval mapping (IM) method, one pair or two pairs of flanking markers at a time are used simultaneously (Lander and Botstein 1989), and a QTL is located within a chromosomal interval, defined by the flanking markers. The technique involves scoring a large number of markers, as illustrated on the top of Fig. 16, and then assessing the probability that an interval between two markers is associated with a QTL affecting the trait of interest. The results of the analysis are plotted as a 'Likelihood-ratio(LR)-test-statistic' against the chromosomal map position, measured in centiMorgan (cM). The LR can be defined as:

$$LR = 2Ln\left[\frac{\textit{Maximum likelihood of the presence of a QTL (H1)}}{\textit{Maximum likelihood of the absence of a QTL (H0)}}\right]$$

The dotted line in Fig. 16B represents a significance threshold above which a likelihood-ratio test provides a statistically significant fit to a model of the data. The best estimate of the location of the QTL is given by the chromosomal location that corresponds to the highest significant likelihood ratio. Although the interval mapping method was an important improvement, statistically it is too biased. In particular, a QTL outside the interval under consideration can affect the ability to find a QTL within the interval. In addition, false identification of a QTL can arise, if other QTLs are linked to the interval of interest (the false 'ghost peak' on the right of Fig. 16).

C- Composite interval mapping (CIM) combines interval mapping technique with multiple regression analysis (Zeng 1993, 1994 and Jansen 1993). The major problem with standard interval mapping is that linked and unlinked QTLs affect the result of the analysis. In the best case this modifies the outcome, and in a worst case 'ghost QTLs' will be detected. Composite interval mapping assesses the probability that an interval between two markers is associated with a QTL affecting the trait of interest, as well as controlling the effects of other

QTLs on the trait, providing more accurate results. This method is a combination of regression analysis and interval mapping. Additional markers are incorporated as cofactors in the regression. By selecting and including other markers in the analysis, the efficiency of QTL mapping can be improved. Methodologically, the first step is to divide the population according to the marker with the best marker-trait association. Permutation tests are then performed on these subdivided groups to test for the next best association and so on (Jansen 1993).

This method was extended by Zeng (1994) to a multiple interval mapping (MIM) technique. Multiple interval mapping combines multiple QTL mapping analysis with the analysis of genetic architecture by using an algorithm to search for number, positions, effects and interactions of significant QTLs (Kao et al. 1999 and Zeng et al. 1999).

Several computer programs, such as Mapmaker (Paterson et al. 1988), QTLMapManager (Manly et al. 2001), Windows QTL Cartographer (Wang et al. 2007), QGene (Nelson 1997) etc., are available for QTL mapping.

Dissected QTLs usually are evaluated according to their contribution to the phenotypic variation of the trait of interest. This contribution is calculated based on R^2 value, which is a fraction of 1 or 100%. By this way, an individual QTL can be described as '*major*' or '*minor*'. A major QTL will account for a relatively large amount (e.g. > 10%) and a minor QTL will usually account for < 10% of phenotypic variation of the trait observed in a mapping population. Sometimes, the term 'major QTL' refers to one that is stable across different experimental locations, whereas a minor QTL may be environmentally specific. In more formal terms, QTLs may be categorized as suggestive, significant and highly significant (Lander and Kruglyak 1995).

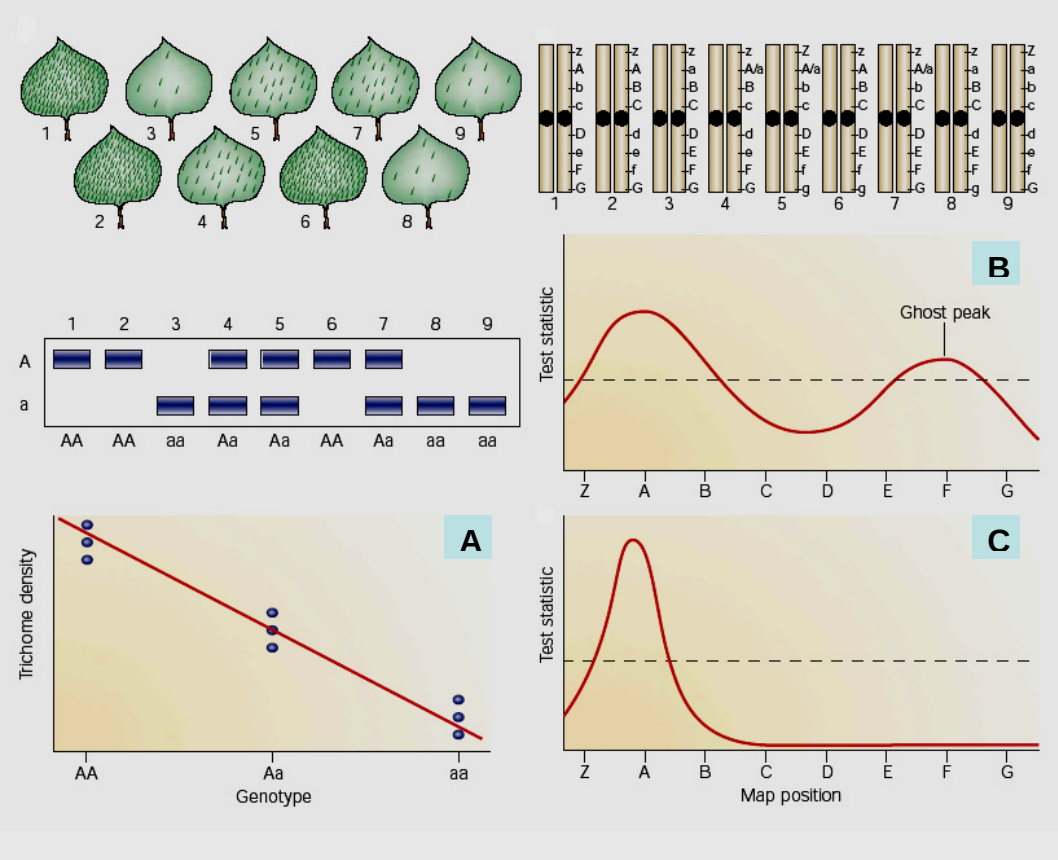


Fig. 16: A schematic diagram of the statistical method for QTL mapping. Trichome density of the leaf is considered here as a quantitative trait (Mauricio 2001).

Finally, the accuracy of any mapping procedure does not depend only on the ability of the statistical method to determine the location and to estimate the genetic effect of the QTL. Other factors also have influence on this accuracy: the type and size of the segregating population, the heritability of the trait, the number and contribution of each quantitative trait locus to the total genotypic variance, their interactions, their distribution over the genome, the number and distance between consecutive markers, the reliability of the order of markers in the linkage map, the evaluation of the trait, etc (Asins 2002).

1.9. Evaluation drought stress

An accurate phenotyping of a mapping population in the field for a trait as complex as drought tolerance is certainly a very important and limiting factor in detecting a QTL. For this reason, one needs to know about the pattern of drought in terms of time, duration, and severity. CIMMYT defines the three most distinct drought stress patterns as (A) pre-flowering stress, (B) grain-filling stress, and (C) continuous (seasonal) stress (Reynolds et al. 2005). Pre-flowering stress has a negative impact on spikelet number and kernel per spikes (Schpiler and Blum 1991). Grain filling stress, or so-called terminal (end-season or post-anthesis) drought, is the most common drought stress in many cultivation areas of wheat, including Iran (Golabadi et al. 2006).

Development of grain in wheat depends on three sources:

- Carbohydrate produced after anthesis and translocated directly to the grains,
- Carbohydrate produced after anthesis, but stored temporarily in the stem before being re-mobilized to the grains
- Carbohydrate produced before anthesis, stored mainly in the stem, and remobilized to grains during grain filling (Ehdaie et al. 2006).

Under terminal drought, there is a rapid decline of photosynthesis after anthesis, limiting the contribution of current assimilates to the grain (Johnson et al. 1981). Flag leaf photosynthesis alone cannot support both respiration and grain growth under terminal stress (Rawson et al. 1983). Therefore, a substantial amount of the carbohydrates used during grain filling, must come from reserves assimilated before anthesis (Gent 1994). While root storage is important in some legumes and other species, there is no evidence that roots or leaves are as important as stems for reserve storage in small grain species (Blum 1998). Therefore, in dry areas wheat yield may highly depend on stem reserves used for grain filling, which do not play a significant role under well-watered conditions.

To what extend stored reserves in the stem may contribute to grain yield in wheat depends on the plant's ability to store assimilates in the stem, and on the efficiency with which the stored reserves are *mobilized* and translocated to the grain. The latter is indeed a function of the genotype's sink strength, which depends on the number of *grains per spike* and *grain weight* (Ehdaie and Waines 1996).

Stem length is an important character affecting stem reserve storage. The *Rh1* and *Rh2* dwarfing genes of wheat were found to reduce stem length by 21%, as a consequence reserve storage decreased by 35% and 39% respectively (Borrell et al. 1993).

The capacity of stem reserve utilization for grain filling, when the photosynthetic source is completely inhibited by stress, can be assessed by destroying the photosynthetic source at the onset of grain filling. Grain weight, with no current photosynthesis, is then measured in

comparison with normal plants (Blum 1998). Spraying the plants with an oxidizing chemical, such as magnesium chlorate or potassium iodide (KI), destroys the photosynthetic sources. The chemical is applied by spraying the whole plant, or just the leaf canopy, about two weeks after anthesis. Work is therefore scheduled according to the different dates of anthesis of different genotypes. Non-treated control plants are required. Capacity for stem reserve support of grain filling is measured by the difference in final kernel weight between treated and non treated plants of any given genotype. An important thing to be considered is that the chemical desiccant does not simulate drought stress, but only the effect of stress is simulated by inhibiting current assimilation. The correlation between the rate of reduction in grain weight by chemical desiccation and the rate of reduction by actual drought stress was found to be significant and reasonably high (Blum 1993b, Nicolas and Turner 1993).

* * *

Chapter 2

Materials & methods

2.1. Plant materials
- 2.1.1. Genetic relationship analysis for screening parental genotypes
- 2.1.2. Characteristics of Sardari and Tabassi
- 2.1.3. Characteristics of Taifun and Kärntner Früher (early)
- 2.1.4. Tabassi vs. Taifun

2.2. Crossings and construction of mapping populations
- 2.2.1. Recombinant inbred line populations
- 2.2.2. Backcross populations
- 2.2.3. Double haploid production

2.3. Field experiments
- 2.3.1. Preliminary field evaluation
- 2.3.2. Final filed evaluation
- 2.3.3. Assessment of post anthesis drought stress by chemical desiccant

2.4. Genotyping
- 2.4.1. DNA isolation
- 2.4.2. PCR reactions
- 2.4.3. Gel electrophoresis and scanning
- 2.4.4. Scoring SSR marker data
- 2.4.5. Linkage map

2.5. Field data analysis

2.6. QTL analysis

2.1. Plant materials

This project was started by selecting five Iranian and two European wheat genotypes as potential parents to establish mapping populations. The Iranian wheat genotypes are three landraces, Sardari (Sar.), Sorkh-Tokhm (S.T.), and Tabassi (Tab.), and two registered varieties Hyrmand (H.), and Roshan (R.). Today, all of them are cultivated across the country. The seeds of the Iranian wheats were provided by the Institute of Seed and Plant Improvement in Tehran-Karaj and were strongly recommended for the purpose of the present study. The two European wheat varieties were Taifun (Tai.), a high yielding, healthy spring type wheat, registered in Germany and Austria, and Kärntner Früher (K.F.), an old Austrian variety registered since 1959.

2.1.1. Genetic relationship analysis for screening parental genotypes

In order to find the best cross combinations of contrasting parents, a genetic relationship study was done in Dr. Marion Röder's lab at IPK-Gatersleben, Germany, in February-March 2004.

Four hundred SSR markers were used to evaluate genetic distance (Nei and Li, 1979). and polymorphism between the five Iranian and the two European parental genotypes. Based on the results of this study and information about Sardari and Tabassi (section 2.1.2), the crossing program was focused mainly on the combinations Sardari x Taifun and Tabassi x Taifun. However, the other cross combinations, also considered important, were used too, obtaining the maximum genetic potential of this plant material for future breeding programs in Iran, e.g. for bread making quality or disease resistance.

2.1.2. Characteristics of Sardari and Tabassi

Sardari is basically a winter type land race originating from the Northwest of Iran (Fig. 17), which is a semi-arid to arid region with hard winter. In this region, early spring frost and drought stress, particularly terminal drought during grain filling, are the main problems for wheat cultivation. Due to the tolerance of Sardari to frost and drought, it is at present the best choice available to Iranian farmers living in this area. As a good genetic source, Sardari has frequently entered plant breeding programs. For example, cultivar 'Azar 2', a variety now widely grown in Iran, was developed in 1999 from a cross involving Sardari. Azar2 has the excellent drought tolerance of 'Sardari', together with other advantageous agronomical traits like resistance to lodging and to yellow rust, to which Sardari is sensitive (ICARDA, Annual Report 2000). Most recently, Pirseyedi et al. (2006) evaluated 35 morphotypes of Sardari for morphological and protein characteristics under dry condition.

Tabassi is an old local variety, which had been selected from a collection of wheat in the Northeast of Iran (Khoda- Bandeh 1992). It entered yield trials in 1955 and has been tested at locations such as Karaj, Bidestan, Ghazwin, Varamin, Isfahan, Shiraz, Ahvaz, Mashhad, Gorgan, Tabriz, Mian-do-Ab, Moghan, and Kermanshah (Fig. 18). These locations are commonly semi-arid or arid, differing in annual temperatures and precipitations. Passing those yield trials successfully indicates the high genetic potential of Tabassi for adaptation to different stresses like drought, heat, and salinity. Indeed, the name Tabassi comes from the city Tabass, which is located at Kavir-e-Namak (desert of salt) in the Northeast of Iran. This area has less than 50 mm rainfall and high temperatures during the growing season. Salinity is also a main stress there (Fig. 18). Like Sardari, Tabassi and its mutants are good genetic sources for plant breeding and research activities, targeting drought, heat, and salinity stress in Iran (Poustini and Aboutalebian 2001, Fazel-Najafabadi et al. 2004). In a most recent study Naserian et al. (2007) evaluated Tabassi and its irradiation-induced seventeen mutants for yield and yield components under irrigated and rain fed conditions. While Sardari can be a good genetic source for cold and drought tolerance, Tabassi has good genetic potential for drought, heat, and salinity tolerance.

Agronomically, Tabassi is early maturing spring type wheat with a tall and strong stem. It has awned spikes and oval pubescent glumes, turning brownish at the time of maturity. The grains are long and yellow (Fig. 19).

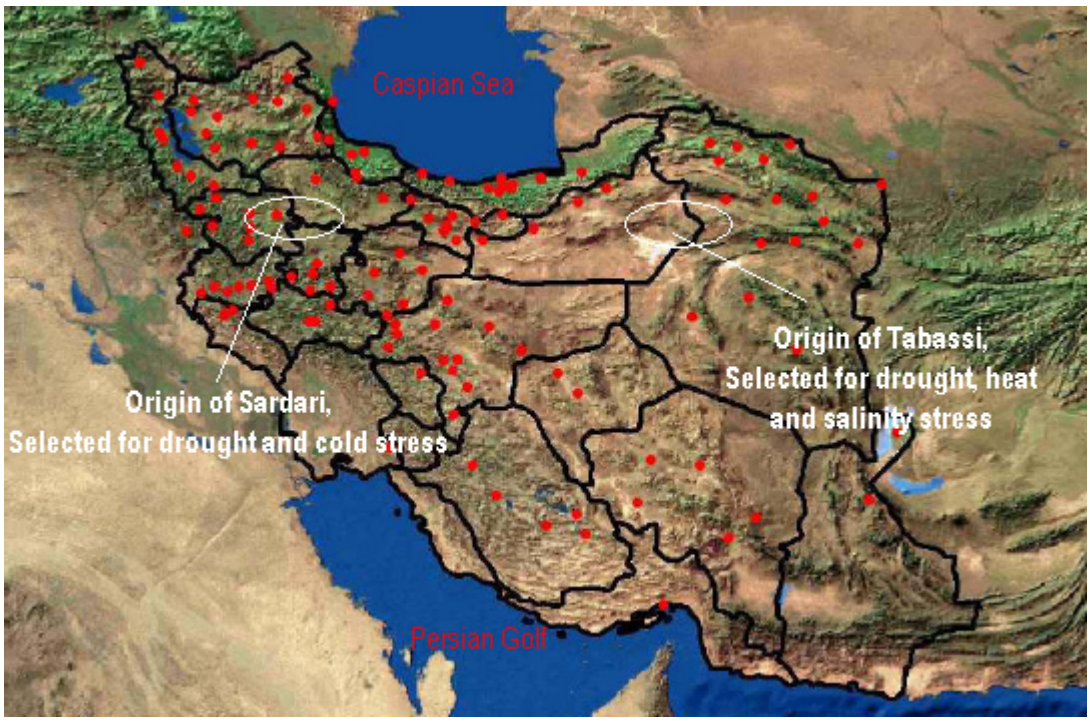


Fig. 17: Origins of Iranian landraces, with special indication of the regions where Sardari and Tabassi originated.

Fig. 18: Locations of field trials in Iran (encircled), where Tabassi has been tested since 1955.

2.1.3. Characteristics of Taifun and Kärntner Früher (early)

Taifun is a high yielding, German spring wheat variety, with short straw. It matures one week to 10 days later than Tabassi. In contrast to Tabassi it shows good resistance to powdery mildew, rust, and septoria, having moderate tolerance to fusarium head blight. It has high bread making quality (http://www.lochow-petkus.de).

Kärntner Früher is, as suggested by its name, an early maturing, old Austrian wheat variety, cultivated since 1959. It matures a few days earlier than Tabassi. It is tall, with good bread making quality, but highly susceptible against all kinds of foliar diseases, except fusarium head blight (http:// www.saatbau.at).

2.1.4. Tabassi vs. Taifun

In nearly all aspects, including potential tolerance to drought stress, which is the main reason for this research, in plant height, heading (flowering) time, awnedness, waxyness of the flag leaf, spike pubescence, spike length, average seed number per spike, 1000-kernel weight and grain size and colour, these two parental wheats are clearly differing from each other (Fig. 20). These phenotypic differences between the two parental genotypes produced amazing variation between the lines in the F2:7 population.

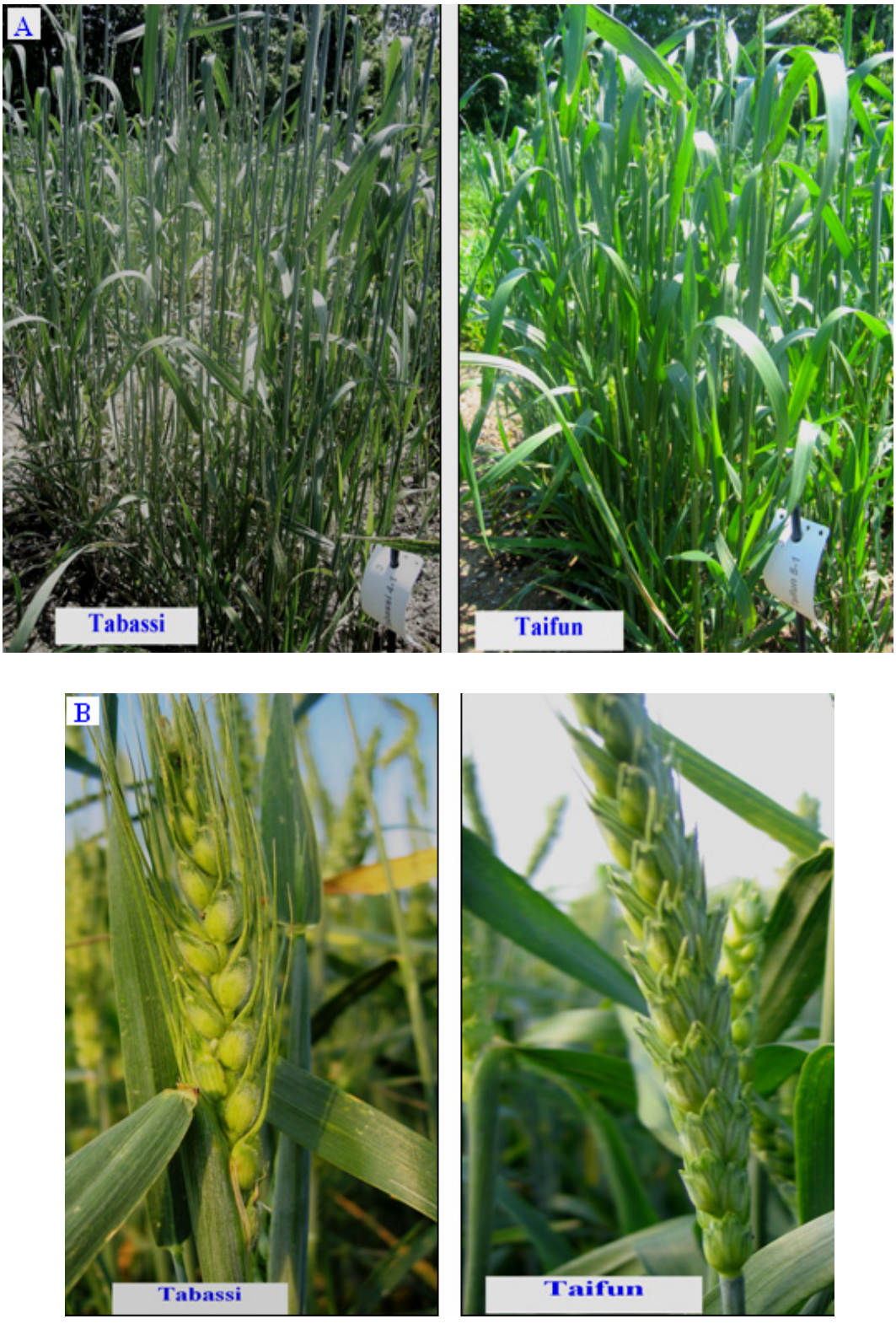
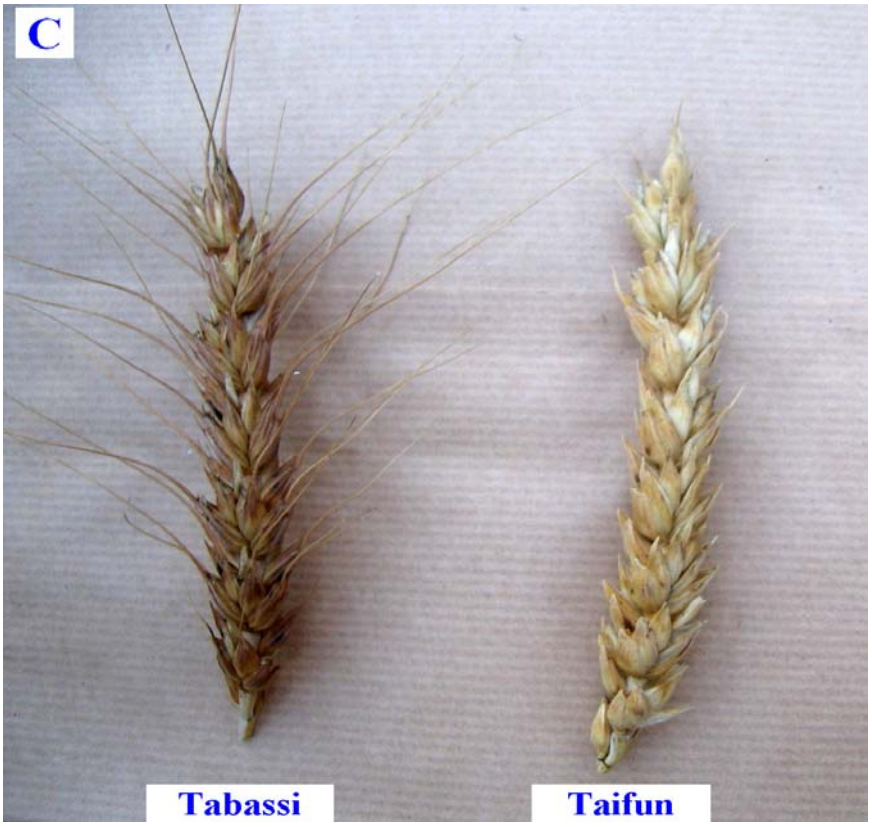


Fig. 19: Major morphological and agronomic differences between Tabassi and Taifun
A: Waxyness of flag leaf **B**: Spike pubescence **C**: Awnedness **D**: Grain size and color



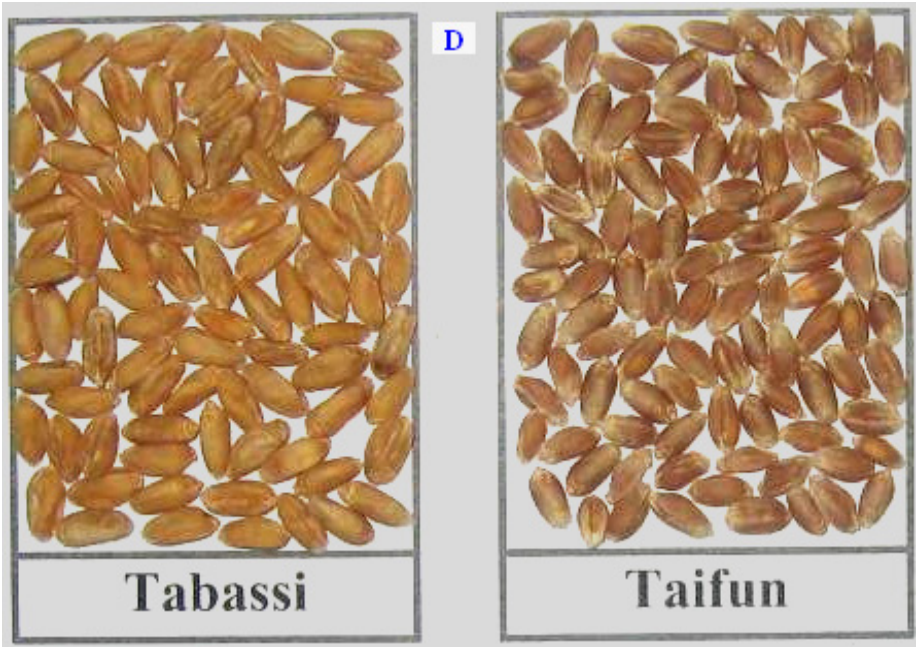


Fig. 19 (continued)

2.2. Crossings and construction of mapping populations

First, crosses between all five Iranian genotypes and the two European varieties were done in winter season 2003/2004, in the green house. All 10 different F1 progenies were sown the next spring, for selfing and back-crossing in the field. Crossing the Iranian genotype Roshan with Taifun yielded only very few F1 seeds. Because of this and the relatively close genetic relatedness of this genotype to Tabassi (see later in the Results 3.1), Roshan was excluded from the program. The genotype Hyrmand showed close relationship to Kärntner Früher (see later). Therefore this genotype, after selfing and back crossing the F1, was set aside too, like Roshan. For both genotypes F1, F2, and reciprocal BC1F1 seeds are available for later considerations (Table 5). The genotype Sorkh-Tokhm turned out to be the least adapted to the environmental conditions in Tulln, showing less vigour compared to the others. It was the earliest genotype, even earlier than Kärntner Früher. Moreover, plants were highly susceptible to all kinds of diseases, and therefore eventually work with this genotype was also terminated. Material available for later considerations of this genotype is shown in Table 5.

2.2.1. Recombinant inbred line populations

From the selfing of the F1 generation of cross combinations between Iranian and European parental genotypes 160 to 220 F2 progenies were grown. Single spikes per progeny were used to produce F3, F4, F5, F6, and F7 generations of recombinant inbred lines, so called RILs. At present, F2:6 populations of the combinations Sardari-Taifun and Sardari Kärntner Früher and F2:8 population of Tabassi-Taifun are available (Table 5).

Table 5: Currently available generations of different cross combinations between five Iranian and two European parental wheats

	Tabassi		Sardari		Sorkh-Tokhm		Hyrmand		Roshan	
Taifun	F2:8	BC3F3	F2:6	BC3F2	F2:4	BC3F1	F1:2	BC1F1	F1:2	BC1F1
	-	BC2F4	-	BC2F2	-	-	-	-	-	-
Kärntner Früher	F2:6	BC1F1	F2:6	BC3F1	F1	BC1F1	F1:2	BC1F1	F1:2	BC1F1

2.2.2. Backcross populations

The F1 plants of each different cross combination were back crossed on the field in summer 2004 with both Iranian and European parental genotypes to create first generations of back crosses. Back crossing continued during 2005 and 2006 to obtain BC2F1 and BC3F1 generations of the combinations Tabassi-Taifun and Sardari-Taifun in both directions. Parallel, selfing was done to produce BC1F2, BC2F2, BC2F3, BC3F2, and BC3F3 generations to achieve higher homozygousity in advanced back cross generations (Table 5).

2.2.3. Double haploid production

An attempt was made to obtain doubled haploid lines from BC2F1 and BC3F1 families of the cross combination Sardari-Taifun during the years 2005 and 2006, using wheat-maize crosses (Suenaga, 1994). A large number of embryos were obtained, but only a few plants could be regenerated. This program had to be terminated because of the high time-requirement and low yield of plantlets.

2.3. Field experiments

2.3.1. Preliminary field evaluation

In summer 2006 a preliminary experiment was carried out to study the following four populations: F2:5 of Sardari -Taifun, Tabassi -Taifun, and Sardari - Kärntner Früher, as well as the BC3F1 population of Tabassi –Taifun. The aim of this experiment was to find the best cross combinations of RILs and BC populations for final field evaluation. A total of 711 lines were phenotypically studied for different morphological and agronomical traits, mostly related to yield, in the research field of IFA–Tulln, Austria. Based on this experiment, the cross combination Sardari x Taifun was also excluded from further evaluations, even though originally Sardari was the main candidate for development of the mapping population. As mentioned before, Sardari is internationally known as a highly drought tolerant wheat genotype, and is intensively used for developing new drought tolerant wheat varieties. The major reason for this decision was that the F2:5 lines of the cross combination Sardari -Taifun still showed considerable variation, especially with respect to growth habit ranging from winter to spring type (Fig. 20). This heterogeneity also was clearly visible, when studying the Gliadin fraction of storage proteins electrophoretically (Fig. 21). This material, however, is of great value and will be evaluated later under Iranian conditions. From now on, work was focused on the combination Taifun x Tabassi, developing F2:7 and BC2F3 generations for a final field evaluation.

Fig. 20: Single spike progenies of the cross combination Sardari x Taifun segregating for growth type in F2:5.

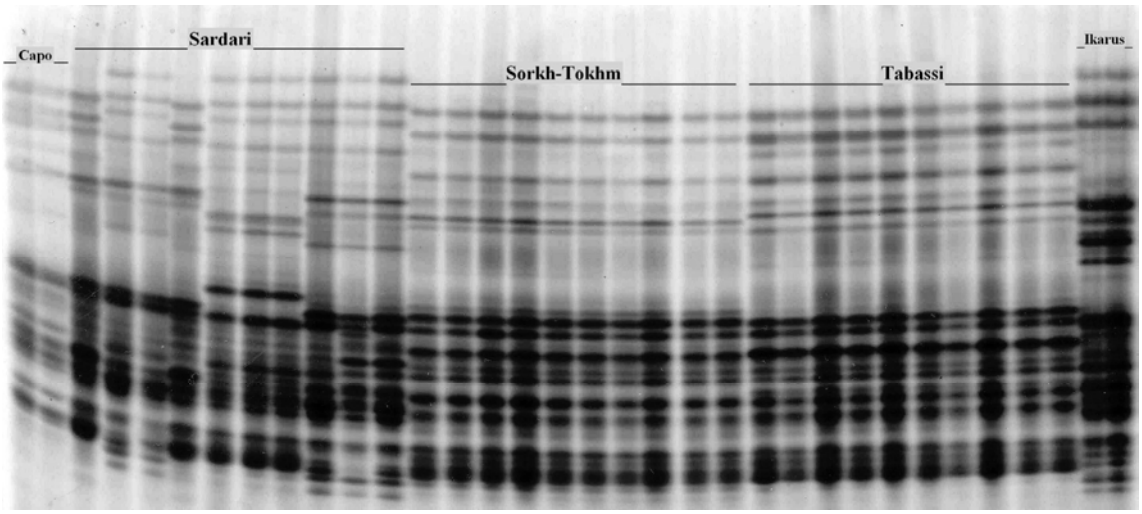


Fig. 21: Gliadin banding patterns of ten seeds of each of the three landraces: Sardari, Sorkh-Tokhm and Tabassi, revealing in an A-PAGE considerable heterogeneity in Sardari.

2.4.2. Final field evaluation

Final field experiments were conducted in three locations: Tulln-Austria, Ilam-Iran, and Szeged-Hungary. Fifty seeds of each F2:7 and BC2F3 line were planted 2007 at the recommended time for spring wheats in each location, in double rows with 17 cm between the rows and 30 cm between the double rows, with a plant distance of 5 cm within the rows. To determine the experimental error, five check varieties: the two parents of the populations Tabassi and Taifun, the varieties Kärntner Früher, the German spring wheat variety Trappe, and one variety according to the location of the experiment were included in four replications, randomized, representing an augmented experiment, based on a randomized complete block design with four blocks.

The experiments in Hungary were conducted under two environmental conditions. One was grown under rain fed conditions, the other under an electroautomated shelter preventing plants to receive any rain, providing a controlled drought stress condition starting after tillering.

At all locations, the experiments were kept weed- and disease-free by the use of herbicides and fungicides, as required. Fertilization was applied as customary in the site. In Iran the experiments were irrigated until anthesis has started. The idea was to provide the condition of terminal (post anthesis) drought stress, which is the dominant pattern of drought stress in most of the wheat cultivation areas in Iran.

Table 6: Agronomic and morphologic traits of the F2:7 population derived from the cross Tabassi x Taifun, evaluated in field experiments.

Trait	Abbreviation	Time and way of scoring	Zadok's code
Total grain yield of 10 spikes (g)	Yld	After harvest Ten representative spikes were measured.	92
Number of grains of 10 spikes	Gps	After harvest Ten representative spikes were counted.	92
Thousand kernel weight (g)	Tkw	After harvest Calculated from total grain weight and number of seeds.	92
Spike length (cm)	Sln	After harvest Ten representative spikes were measured.	92
Number of spikelet / spike	Sps	After harvest Ten representative spikes were used.	92
Plant height (cm)	Pht	At grain filling Five main stems per double row were measured from ground to the tip of the spikes, without awns.	85-89
Ear emergence (heading) time (days after sowing)	Eet	Recorded, when 50% of the spikes were completely out of the flag leaf sheath, i.e. last spikelet visible,	55
Awnedness (Yes/No)	Awn	At flowering	65-69
Spike pubescence (Yes/No)	Pub	At flowering	65-69
Waxyness of flag leaf (Yes/No)	Wax	At flowering	65-69

Seven agronomic, as well as three morphologic traits were evaluated in all locations. Table 6 shows these traits and the time of scoring, according to Zadok's wheat growth stages (Zadok et al. 1974).

2.5.3. Assessment of post anthesis drought stress by chemical desiccant

Potassium Iodide (KI) was applied to the F2:7 and BC2F3 populations of the Tabassi-Taifun cross combination to destroy photosynthetic sources in leaves during grain filling. Plants were labelled at flowering (anthesis) time. Two weeks later, whole plants were sprayed by 0.5 %. KI (Fig. 21 and 22). Grain yield, grain number per spike, as well as 1000-kernel weight was measured. The experimental design used was the same as for all other locations.

Fig. 21: Spraying plants with Potassium Iodide (KI), two weeks after flowering (anthesis)

Fig. 22: The experimental field after spraying with Potassium Iodide. Plants were labelled at their flowering time.

2.4. Genotyping

2.4.1. DNA isolation

Two to three fresh, 4-5 cm long, young leaves of each F2:7, as well as each BC2F3 line were dried by a lyophylizer and then ground with an electric mortar. Promega Wizard

Genomic DNA Purification Kit (Promega Crop) was used to isolate genomic DNA. Concentrations of samples were determined by the GenQuant RNA/DNA Calculator (Amersham Bioscience) and finally adjusted to 100 ng/µl for stock solution. The quality of DNA samples was tested in 1.5 % agaros gel. Isolation of samples with low amount of, or unclean DNA was repeated.

2.4.2. PCR reactions

PCR components and reaction conditions are shown in Table 7:

Table 7: PCR components and reaction conditions for SSR primers

PCR Component	Volume(1X-10µl)	Temperature (°C)	Time	Cycle
ddH_2O	4.02	95	2′	1
10X PCR buffer (+$MgCl_2$)	1.0	95	45″	7
dNTPs (2mM)	1.0	68	45″	
Forward primer (10µM)	0.025	72	1′	
Fluorescent label (10µM)	0.225	95	45″	30
Reverse primer (10µM)	0.250	50, 55, 60	45″	
Taq DNA polymerase (8U/µl)	0.08	72	1′	
Template DNA (10ng/µl)	3.4	72	5 ′	1
Total	**10.0**			

Primer sequence information was kindly provided by Dr. Marion Röder and Dr. Martin Ganal, IPK Gatersleben, Germany. Forward primers were extended by the M13 Table “GCCCAGTCACGACGTT” at the 5’ end. Before PCR, fluorescent dye oligos, either FAM or HEX with the excitation values of 488 and 532 respectively, were added to the PCR mix to label the PCR products (Amersham Biosciences, 2004).

2.4.3. Gel electrophoresis and scanning

PCR products were loaded on 12% polyacrylamide gel, using 1XTBE buffer in a C.B.S chamber (C.B.S. Scientific Inc). Electrophoresis conditions were set at a constant current of 400V and 10°C for 2 hours. Using the two fluorescent-labeled oligos, FAM and HEX, it was possible to load two different PCR products on the same gel. After electrophoresis, the glass plates containing the gels were placed on the laser scanner Typhoon (Typhoon Trio., Variable Mode Imager, Amersham Biosciences, 2004), and were scanned with different emission filters for FAM and HEX with emission values of 520 BP 40 and 555 BP 20 respectively.

2.4.4. Scoring SSR marker data

Images of gels were transferred to an excel sheet and scored, based on the position of the parental fragments, the size of which were known. Tabassi-type fragments were scored as 1, Taifun-type fragments as 2. Heterozygous, unknown and missing individuals were scored as 3, 5, and 0 respectively. A DNA sample of Chinese Spring was used as standard for fragment analysis, because its fragment sizes were known for each marker. Score 4 was used for the respective Chinese spring fragment (Fig. 23).

2.4.5. Linkage map

A total of 204 wheat SSRs together with 3 morphological markers, awnedness, waxyness and pubescence, resulting in 217 polymorphic loci, were used to construct a linkage map with 118 F2:7 lines of the Tabassi-Taifun cross combination, applying JionMap version 3.0 (Stam 1993). Marker data on the BC2F3 population were collected with the same markers used for the F2:7 lines. Unfortunately, because of time limitation, their linkage analysis and QTL mapping has not been carried out yet. In the F2:7 population, locus genotype frequencies were set according to co-dominant and dominant segregation patterns. The Chi-square test at significance level 0.01 was applied to test the deviations of segregation ratios from the expectation. Similar loci or individuals were excluded before grouping. Recombination fractions were converted to centiMorgan with Kosambi's mapping function (Kosambi, 1944). Minimum LOD threshold of 3.0 was chosen to group marker loci. Mapping was first done for each chromosome by itself to find fixed linkage groups, and later with all markers to finalize the map.

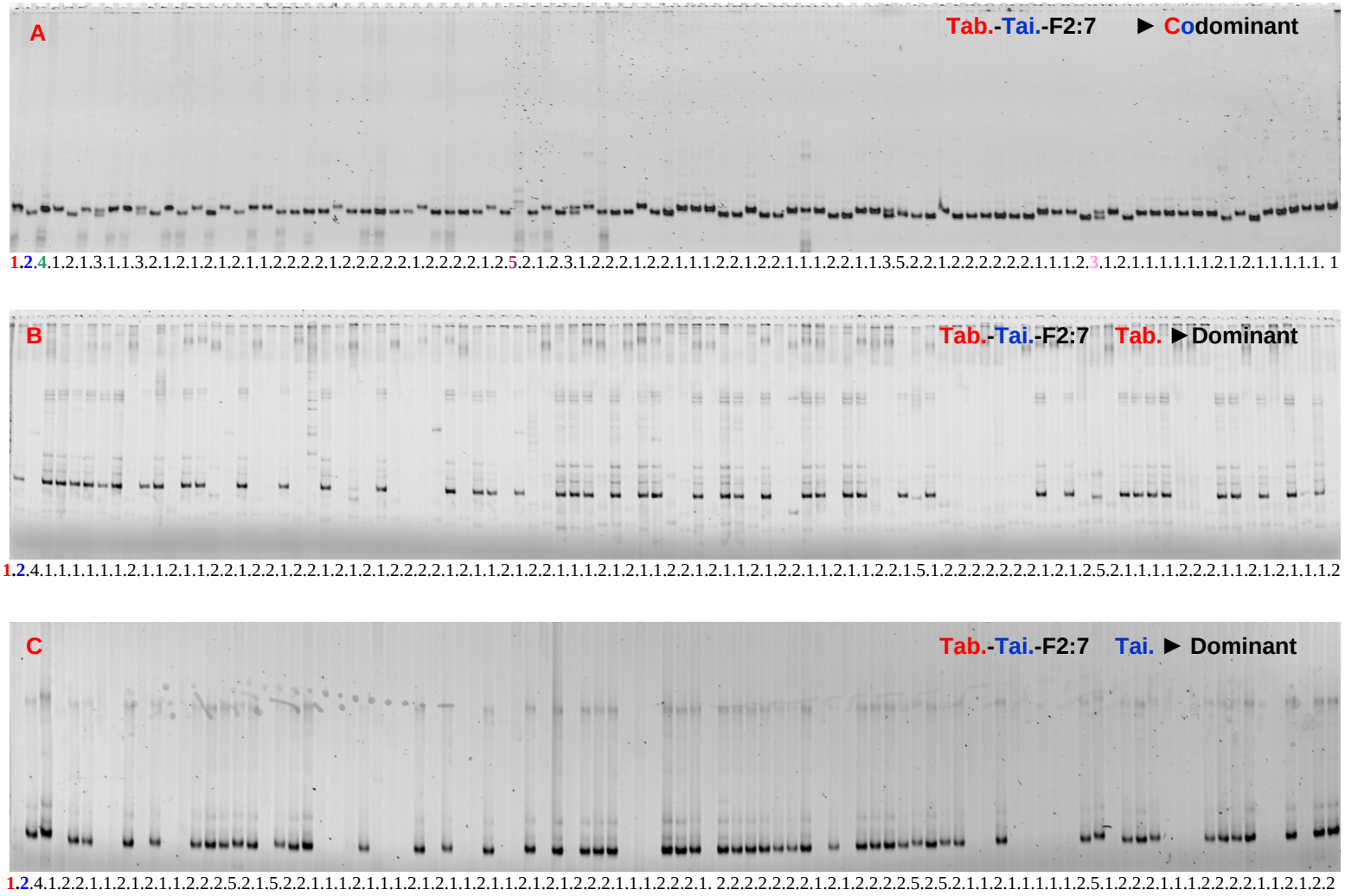


Fig. 23: SSR fragments in the Tabassi x Taifun F2:7 population on a 12% polyacrylamide gel. A: Codominant alleles, B and C: dominant alleles of Tabassi and Taifun, respectively. Numeric score 1 corresponds to Tabassi-type, 2 to Taifun-type, 3 to heterozygous, 4 to Chinese Spring-type and 5 to unknown alleles.

2.5. Field data analysis

Field data were analysed using SPSS version 12.0 (SPSS Inc, 2003). Analysis of variance (ANOVA) was done to estimate the effects of blocks. Augmented experiment based on a randomized complete block design for check varieties. Pearson's correlation coefficient was used to calculate the correlation between seven quantitative traits of the F2:7 lines. To calculate correlations between quantitative and categorical (morphological) traits, Spearman's nonparametric coefficient was applied. Broad sense heritability (H^2) was calculated by regression of the F2:7 means on mid-parental values for all agronomic traits, according to Falconer and Mackay (1996).

2.6. QTL analysis

QTL analysis was done applying composite interval mapping (CIM) (Zeng 1993, 1994). Windows QTL Cartographer version 2.5 software (Wang et al. 2007) was used for the analysis. Permutation test (Doerge and Churchill, 1996) for the traits was performed at significance level ($P<0.05$), resulting in an average value of LR=14 to reach a minimum LOD value of 3. Forward and backward regression was used to select cofactors for CIM. The p (F_{in}) and p (F_{out}) thresholds were 0.05 and the maximum number of 20 cofactors was used according to the formula $2(\sqrt{n}) - 2$, where n is the population size (Piepho,). CIM model 6 was used with a 10-cM window size and 1-cM walk speed. A QTL was declared when the LOD score was greater than 3. Graphical maps of chromosomes were generated using MapChart version 2.1 software (Voorrips, 2002).

* * *

Chapter 3

Results

3.1. Relationship study for screening parental genotypes
3.2. Comparison of blocks and check varieties
3.3. Comparison of Tabassi and Taifun
3.4. Comparison between the experimental environments
3.5. Correlation between the traits
  3.5.1. Correlation between quantitative traits
  3.5.2. Correlation between quantitative and morphological traits
3.6. Phenotypic values of the traits
3.7. Characteristics of the linkage map
3.8. QTL analysis

3.1. Relationship study for screening parental genotypes

Out of 400 SSR markers tested, 206 were polymorphic and used for a genetic relationship study between the five Iranian and two European genotypes. Cluster analysis using UPGMA (Fig. 25) revealed the greatest genetic distance between the two closely related Iranian genotypes Tabassi and Roshan and the two European genotypes Taifun and Kärntner Früher (Table 7). The variety Hyrmand showed close genetic relationship to the European varieties.

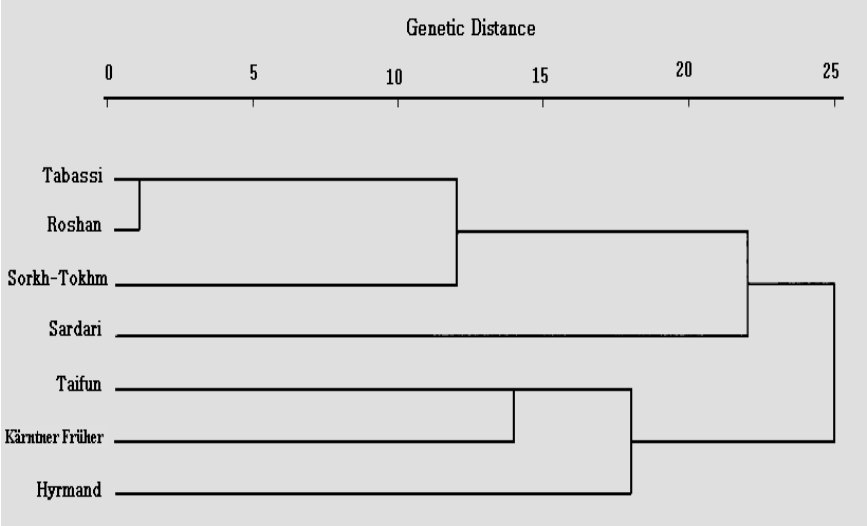


Fig. 25: Dendrogram of genetic distance resulting from a cluster analysis of the selected seven parental genotypes. Data obtained with 206 polymorphic SSR markers.

Table 7: Genetic distance matrix of the parental genotypes Tabassi and Taifun, showing the greatest distance.

			Iranian			European	
	Hyrmand	**Roshan**	**Sardari**	**Sorkh-Tokhm**	**Tabassi**	**Kärntner Früher**	**Taifun**
Hyrmand							
Roshan	0.849						
Sardari	0.728	0.779					
Sorkh-Tokhm	0.810	0.771	0.791				
Tabassi	0.793	0.482	0.811	0.708			
Kärntner Früher	0.772	0.843	0.810	0.825	0.846		
Taifun	0.740	0.838	0.794	0.804	0.863	0.661	

3.2. Comparison of blocks and check varieties

The effects of blocks were not significant at any of the experimental locations. For this reason there was no need to adjust the data of the F2:7 lines. Thus, mean values of the traits were used for QTL analysis. The results of ANOVA are summarized in Table 8.

Table 8: A summary of ANOVA, showing the effects of blocks and check varieties, as the sources of variation over different locations. AUT: Austria, IRN: Iran, KI: KI-induced drought stress, HUN-Ctr: Hungary - control experiment, HUN-Str: Hungary- stress experiment;

Source of variation	AUT	IRN	KI	HUN- Ctr	HUN- Str
Grain yield per 10 spikes					
Block	ns	ns	ns	ns	ns
Check	*	*	**	*	ns
Grain number per 10 spikes					
Block	ns	ns	ns	ns	ns
Check	*	*	**	*	**
1000- kernel weight					
Block	ns	ns	ns	ns	ns
Check	*	**	**	**	**
Spike length					
Block	ns	ns	–	ns	ns
Check	**	*	–	*	*
Spikelet per spike					
Block	ns	ns	–	ns	ns
Check	**	**	–	*	*
Plant height					
Block	ns	ns	–	ns	ns
Check	**	**	–	**	**
Ear emergence time					
Block	ns	ns	–	ns	ns
Check	**	**	–	**	**

ns: non-significant, *: significant at α=0.05, and **: significant at the α=0.01 level.

As Table 8 shows, except for grain yield in HUN-Str, there were significant differences between check varieties for all traits at the different locations. In the KI-experiment only three traits were measured.

3.3. Comparison of Tabassi and Taifun

Table 9 and Fig. 26 show the differences between Tabassi and Taifun as the parental genotypes of the mapping population. There were no significant differences between Tabassi and Taifun for grain yield in Austria and both experiments in Hungary. But under two stress experiments in Iran and KI-induced stress, Tabassi yielded significantly higher than Taifun.

There was no significant difference between Tabassi and Taifun for grain number per spike under rain fed conditions in Austria and Hungary, but Taifun showed a significantly higher number of grains in all stress conditions.

Thousand-kernel weight was significantly different at all locations of the experiment, Tabassi showing higher 1000-kernel weight than Taifun, both under rain fed and stress conditions.

Tabassi and Taifun did not differ significantly in spike length under stress condition in Iran, but the differences were significant at the other locations. For the other traits, including spikelet per spike, plant height and ear emergence time, the difference was significant at all locations.

Table 9 and Fig. 26: Tabulated mean values with significance levels (left) and graphic representations (right) of the traits of Tabassi (Tab.) and Taifun (Tai.) of all locations of the experiment.

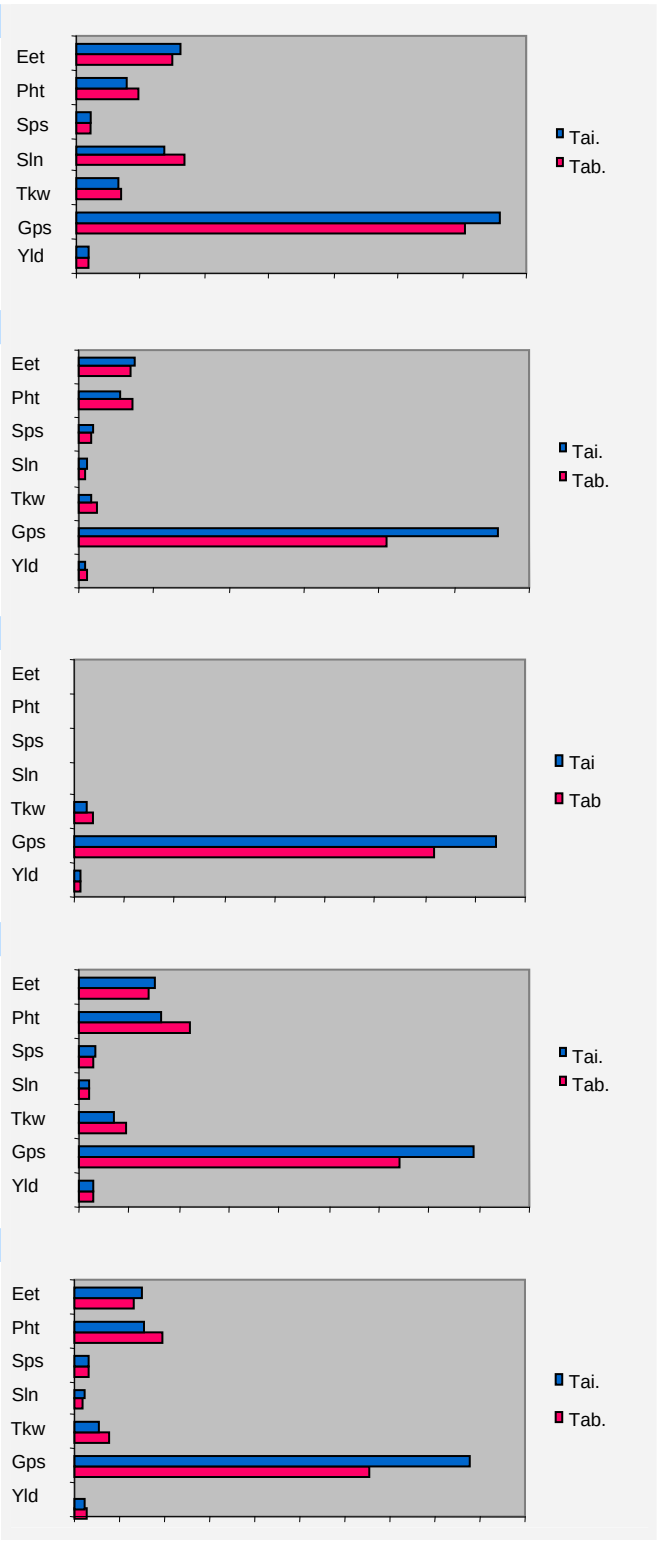
	AUT		
Traits	Tab.	Tai.	Dif.
Yld	11,1	10,2	ns
Gps	302,0	349,0	ns
TKW	36,8	29,2	**
Sln	10,5	11,9	**
Sps	17,1	19,4	**
Pht	83,8	68,0	**
Eet	74,1	81,3	**

	IRN		
	Tab.	Tai.	Dif.
Yld	10,4	8,5	*
Gps	409,8	559,0	**
TKW	25,4	15,2	**
Sln	9,5	9,7	ns
Sps	17,7	19,1	**
Pht	72,5	55,0	**
Eet	67,3	74,5	**

	KI- experiment		
	Tab.	Tai.	Dif.
Yld	6,3	5,4	*
Gps	358,5	421,0	*
TKW	17,7	12,7	**
Sln	—	—	—
Sps	—	—	—
Pht	—	—	—
Eet	—	—	—

	HUN-Ctr.		
	Tab.	Tai.	Dif.
Yld	13,3	13,5	ns
Gps	319,8	394,3	ns
TKW	46,8	34,3	**
Sln	9,8	10,8	*
Sps	14,9	16,4	*
Pht	103,3	82,5	**
Eet	70,0	77,0	**

	HUN-Str.		
	Tab.	Tai.	Dif.
Yld	12,5	11,4	ns
Gps	327,0	438,3	*
TKW	38,3	26,7	**
Sln	8,8	10,8	*
Sps	15,7	16,3	*
Pht	97,0	76,3	**
Eet	67,0	75,0	**



3.4. Comparison between the experimental environments

Principle components analysis (PCA) defined three factors, explaining 82.7 % of variation within the F2:7 population for all agronomic traits at each location. Factors are plotted on a three-dimensional graph (Fig. 27).

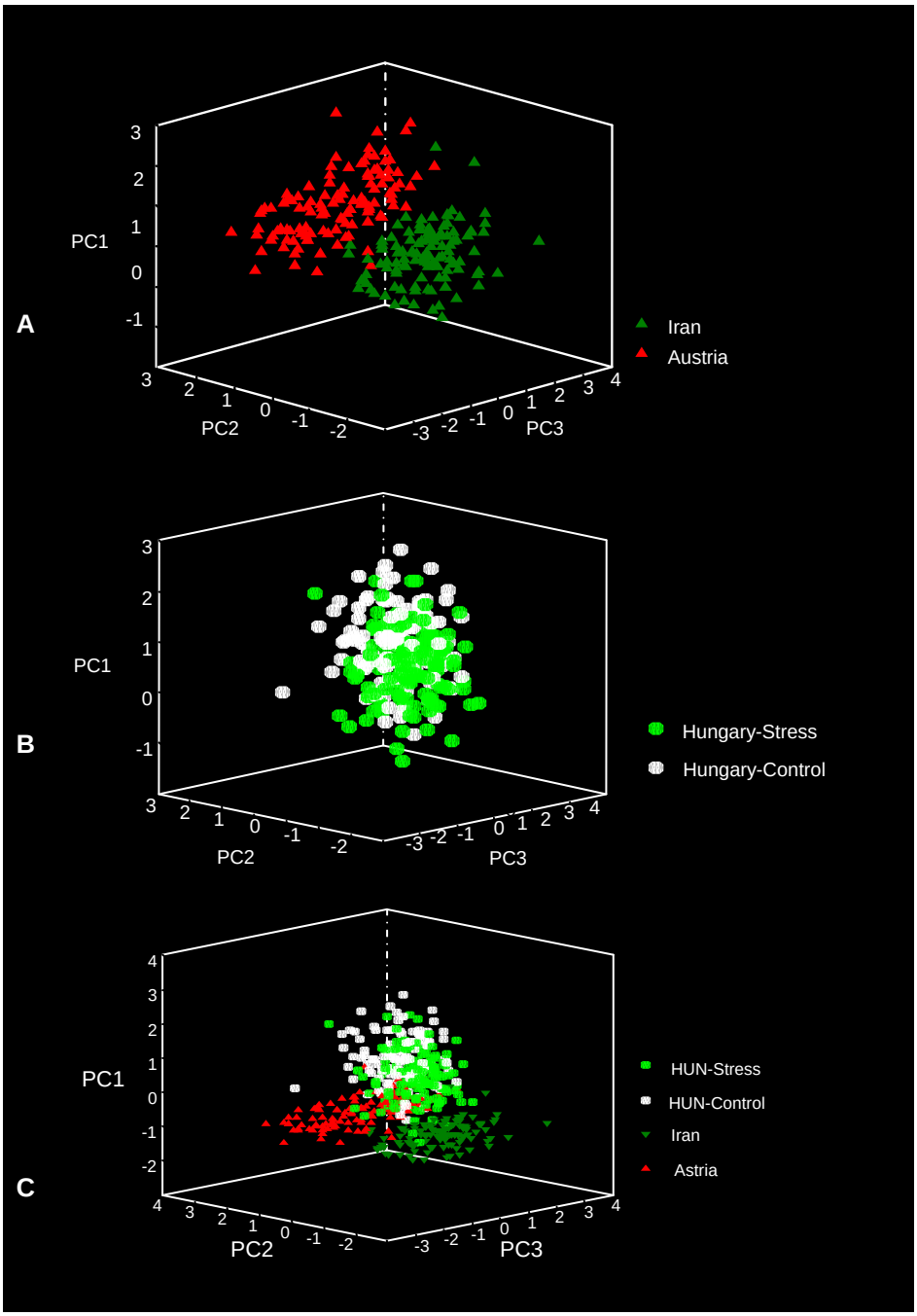


Fig. 27: Scatter plots of principle components explaining major variations of agronomic traits in F2:7 lines in the four environments:
A: Non- stress, Austria vs. Stress, Iran
B: Non- stressed and stressed conditions, Hungary
C: All environments

Distribution of data shows that F2:7 lines under stress in Iran are clearly separated from those in Austria, indicating that drought stress resulted in an obvious reduction in all traits. But, data from the two experiments in Hungary showed overlapping, indicating that drought under rain shelter was not severe enough to induce significant phenotypic differences. For this reason, the data of this experiment were only used to confirm the presence of QTLs found in other experiments.

As shown in the three dimensional scatter plots of Yld, Gps and Tkw (Fig. 28), drought stress, induced by potassium iodide (KI), resulted in visible differentiation of this F2:7 population from the one under non-stress condition in Austria. Similar effect resulted from natural stress condition in Iran. Yield reduction under natural stress in Iran and stress induced by KI showed a highly significant correlation (r = 0.690).

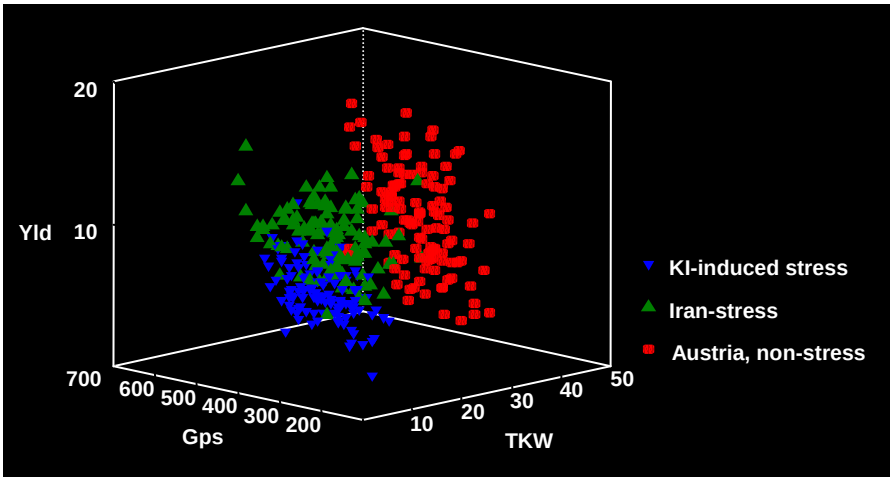


Fig. 28: Three dimensional scatter plot of Yld, Gps and Tkw of the F2:7 population under KI-induced stress condition in comparison to the non-stress and stress conditions In Austria and Iran.

3.5. Correlations between the traits

3.5.1. Correlations between quantitative traits

Correlations between the traits were calculated for each environment independently (Table 10).

Table 10: Correlation coefficients between agronomic traits in different experimental locations;

Trait	Locations	Yld	Gps	Tkw	Sln	Sps	Pht
Gps	AUT	0.896 **					
	IRN	0.640 **					
	KI	0.716 **					
	HUN-Ctr	0.054					
	HUN-Str	– 0.132					
Tkw	AUT	0.670 **	0.279				
	IRN	0.593 **	– 0.218 *				
	KI	0.652 **	0.118				
	HUN-Ctr	0.654 **	– 0.688 **				
	HUN-Str	0.835 **	– 0.610 **				
Sln	AUT	0.111	0.142	0.025			
	IRN	0.254 **	0.301 **	0.019			
	HUN-Ctr	0.137	0.379 **	– 0.164			
	HUN-Str	0.025	0.164	– 0.087			
Sps	AUT	0.084	0.175	– 0.113	0.519 **		
	IRN	0.296 **	0.491 **	– 0.110	0.355 **		
	HUN-Ctr	– 0.050	0.532 **	– 0.395 **	0.522 **		
	HUN-Str	0.004	0.424 **	– 0.204 *	0.586 **		
Pht	AUT	0.277 **	0.114	0.406 **	0.139	– 0.098	
	IRN	0.460 **	0.210 *	0.376 **	0.315 **	0.156	
	HUN-Ctr	0.208 *	– 0.014	0.124	– 0.043	0.007	
	HUN-Str	– 0.040	0.227 *	– 0.147	0.157	0.253 **	
Eet	AUT	– 0.377 **	– 0.426	– 0.106	0.226 *	0.378 **	– 0.196 *
	IRN	– 0.367 **	0.258 **	– 0.188 **	– 0.139	0.076	– 0.554 **
	HUN-Ctr	– 0.139	0.287 **	– 0.300 **	0.454 **	0.692 **	– 0.067
	HUN-Str	0.012	0.067	– 0.026	0.247 **	0.335 **	– 0.020

*: Correlation is significant at the 0.05 level; **: Correlation is significant at the 0.01 level.

Grain yield per ten spikes (Yld) was significantly correlated to grain number per ten spikes (Gps) under non-stress conditions in Austria, and both stress conditions, in Iran and in the KI experiment. Correlation under non-stress condition was stronger. In contrast, there were no significant correlations between these traits at both experiments in Hungary. Thousand-kernel weight (Tkw) showed significant positive and high correlation with Yld in all experiments, the strongest correlation was observed under stress condition in Hungary (r = 0.835).

Except under stress environment in Iran, where the correlation between Yld and spike length (Sln) showed weak significance (r = 0.254), there were no significant correlations

between these two traits at other locations. The same was found for spikelet per spike (Sps), which only in Iran showed positive significant correlation with Yld (r = 0.296).

Taller plants yielded significantly more than shorter plants, both under stress in Iran and non-stress conditions in Austria and Hungary, although the correlations were not strong. Plants which emerged earlier also showed higher yield than those which headed later. This was observed in Austria and Iran, where there were significant negative correlations between Yld and ear emergence time (Eet). No significant correlations were observed in Hungary.

While Gps and Tkw showed positive significant correlations under non-stress conditions in Austria and Hungary, this correlation was negatively significant under stress.

Correlations between Gps and Sln were significant in Iran and under non-stress condition in Hungary, but the influence of Sln on Gps seems to be weak.

Except in Austria, Gps was significantly correlated to Sps in all other locations. However, correlations were again not very strong. While there were no significant correlations between plant height (Pht) and Gps under non stress conditions, taller plants produced slightly more grains under drought stress conditions. Gps was slightly decreased in plants which emerged earlier.

At none of the locations Sln and Sps showed a significant correlation with Tkw.

Tkw was significantly higher in taller plants under stress condition in Iran, while there were no significant differences between plants with different heights in other experiments.

Correlations between Sln and Sps were significant in all experiments.

While taller plants produced longer spikes under stress in Iran, there was no significant difference in Sln between tall and short plants at all other locations. Plants which emerged later produced significantly longer spikes under non-stress conditions, both in Austria and Hungary.

Negative significant correlation between Pht and Eet was observed in Austria and Iran.

3.5.2. Correlations between quantitative and morphological traits

Non-parametric correlation coefficient of Spearman (Table 11) was calculated between quantitative traits, i.e. yield, and yield components, plant height and ear emergence time on the one hand and the morphological traits: awnedness (Awn), spike pubescence (Pub), and waxyness of flag leaf (Wax).

Table 11: Correlation coefficients between agronomic and morphological traits in different experimental locations.

	Location	Yld	Gps	Tkw	Sln	Sps	Pht	Eet	Awn	Pub
Awn	AUT	0.187 *	0.033	.0324 **	0.017	0.025	0.069	0.049		
	IRN	– 0.084	– 0.227 *	0.104	– 0.088	– 0.299 **	– 0.083	– 0.082		
	KI	0.068	– 0.079	0.128						
	HUN-Ctr	0.019	0.105	– 0.064	– 0.070	0.020	– 0.032	0.083		
	HUN-Str	0.079	0.046	0.047	– 0.065	– 0.087	0.038	– 0.021		
Pub	AUT	– 0.144	– 0.251 **	0.078	– 0.310 **	– 0.333 **	– 0.036	– 0.031	– 0.002	
	IRN	0.066	– 0.045	0.121	– 0.066	– 0.050	0.036	0.067	– 0.002	
	KI	– 0.118	– 0.208 *	0.045						
	HUN-Ctr	– 0.076	– 0.118	0.059	– 0.203 *	– 0.173	– 0.183	– 0.089	– 0.002	
	HUN-Str	0.025	– 0.109	0.048	– 0.250	– 0.181	– 0.106	– 0.004	– 0.002	
Wax	AUT	0.239 **	0.122	0.224 *	0.068	0.048	– 0.016	– 0.057	0.302 **	– 0.036
	IRN	0.119	– 0.054	0.184 *	0.063	– 0.157	0.173	– 0.196 *	0.302 **	– 0.036
	KI	0.054	0.080	– 0.007						
	HUN-Ctr	0.021	– 0.003	0.007	0.013	0.012	– 0.043	– 0.042	0.302 **	– 0.036
	HUN-Str	0.022	– 0.163	0.084	– 0.026	– 0.075	– 0.030	– 0.202 *	0.302 **	– 0.036

*: Correlation is significant at the 0.05 level, **: Correlation is significant at the 0.01 level.

Awnedness of plants showed significant positive correlation with Yld and Tkw under non-stress condition in Austria. There was a significant negative correlation between the presence of awns and both Gps and Sps under stress condition in Iran.

Spike pubescence (Pub) correlated negatively with Gps, Sln and Sps in Austria, with Gps under KI-induced stress in Austria and with Sln under non-stress condition in Hungary.

Similar to the correlation with awnedness, plants with waxy flag leaves yielded more under non stress condition in Austria. They also had higher Tkw, both in Austria and Iran. Plants with waxy flag leaves emerged earlier than those with non-waxy leaves under stress in Iran and in Hungary. They yielded more than plants without waxy flag leaves (Table 12 and Fig. 29).

Mean values of the traits correlating significantly with morphological traits are shown in Table 12 and Fig. 29.

Table 12: Statistics of agronomic traits correlated with morphological traits over experimental locations.

		Nr.	Mean	S.D.	Min	Max	Nr.	Mean	S.D.	Min	Max
Trait	**location**			**Awn. +**					**Awn. –**		
Yld	AUT	62	10,9	2,7	4,5	17,1	56	9,7	2,9	4,8	16,6
Gps	IRN	62	413,9	62,9	295	559	56	452,3	68,3	312	646
Tkw	AUT	62	31,9	3,5	23,6	39,9	56	29,4	4,1	21,1	40,5
Sps	IRN	62	17,3	1,2	14,8	20,9	56	17,9	0,9	15,7	19,7
				Pub. +					**Pub. –**		
Gps	AUT	42	308,6	308,6	77,4	157	76	348,6	67,3	170	520
	KI	42	363,7	67,9	247	499	76	394,1	62	190	522
Sln	AUT	42	10,6	1,1	8,7	13	76	11,3	1.0	9,2	13,4
	HUN-Ctr	42	10	1,2	8,3	13,1	76	10,4	1.0	8,5	13,2
Sps	AUT	42	17,6	1,3	14,2	20,9	76	18,5	1,2	15,7	21
				Wax. +					**Wax. –**		
Yld	AUT	73	10,9	2,7	4,5	17,1	45	9,5	3,0	4,8	16,6
Tkw	AUT	73	31,5	3,7	24,2	40,5	45	29,3	4,2	21,1	37,5
	IRN	73	21,6	3,4	14	33,8	45	20,4	3,1	14,4	29
Eet	IRN	73	70,6	3,8	64	83	45	71,8	3,1	65	79
	HUN-Str	73	71,3	2,9	65	77	45	72,5	2,9	67	79

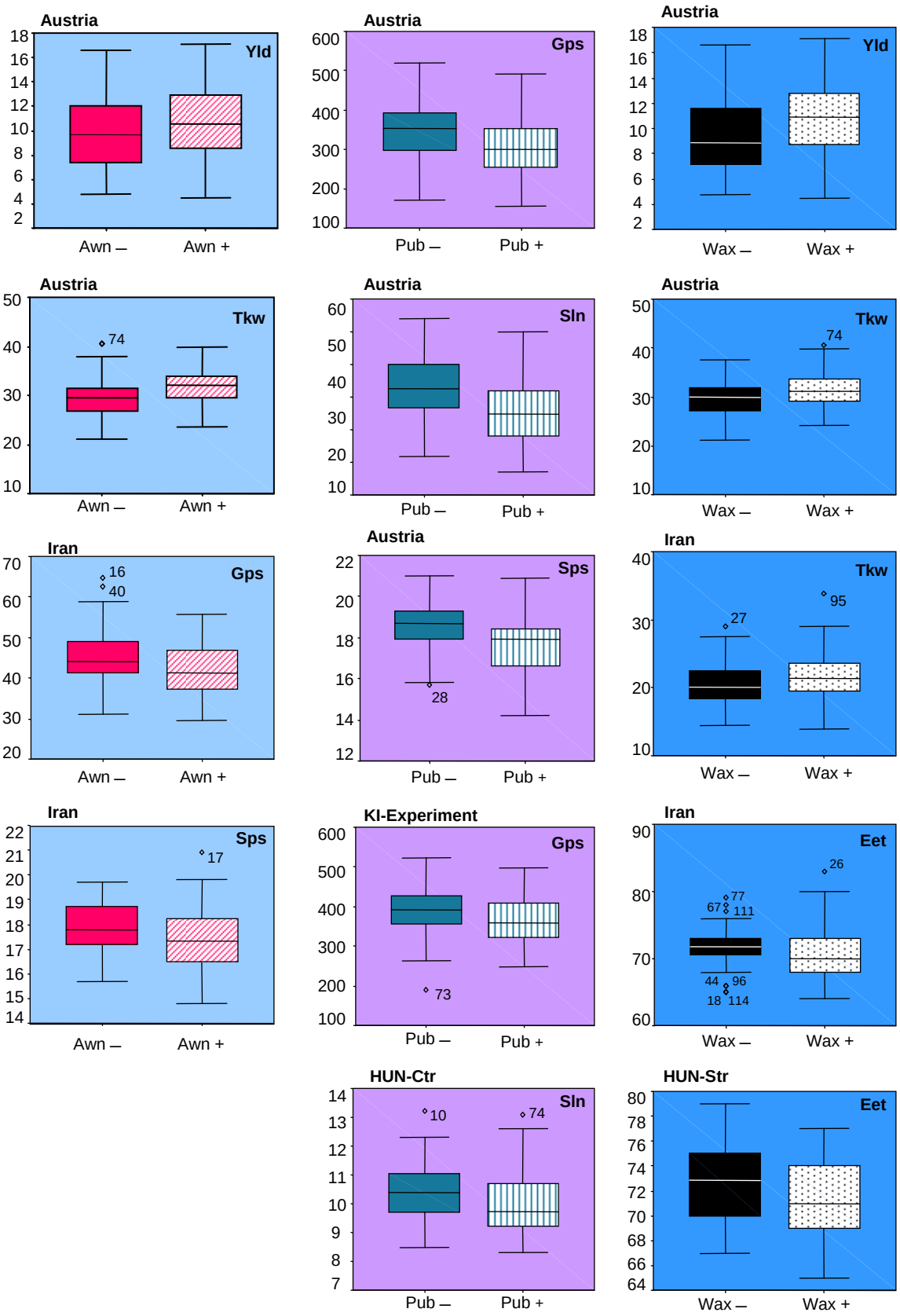


Fig. 29: Box plots of trait means. Only those, which showed significant correlations with morphological traits under stress and non- stress conditions, are presented.

3.6. Phenotypic values of the traits

Mean values, maximum, minimum, and standard deviations of the traits are shown in Table 13 and Fig. 31. Among all traits, Eet showed the lowest and Yld the highest variation. Drought generally caused decrease in Yld, Tkw, Sln, Pht, and Eet. Under drought stress conditions Sps was reduced, while Gps was increased, resulting in an increased ratio of Gps to Sps (Table 14).

Broad sense heritability (H^2) for all agronomic traits was calculated by regression of the F2:7 means on the mid-parent values (Falconer and Mackay 1996). All heritability estimates were high, ranging from 0.73 for Sps to 0.98 for Pht. Traits with means of the F2:7 lines lower than the mid-parent value (Gps-F7 and Sps-F7 in Fig. 30) had lower heritabilities.

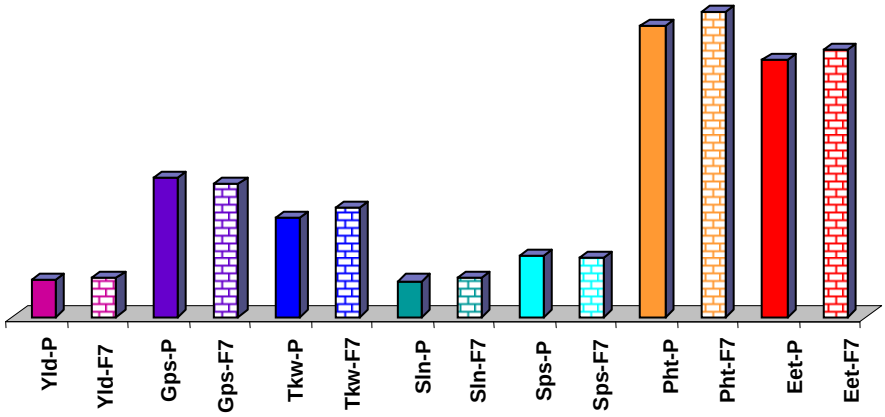


Fig. 30: Mean values of F2:7 lines vs. mid-parent values of the traits from pooled data of different locations.

Table 13 and Fig. 31: Mean values, standard deviations and heritability estimates of the traits in the Tab. x Tai. F2:7 population in the different experiments

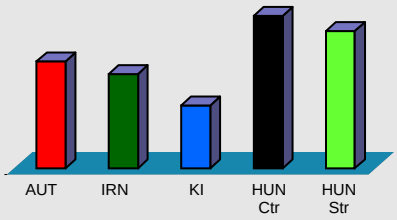
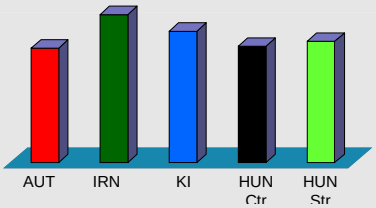
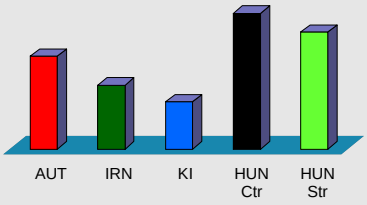
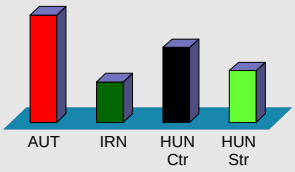
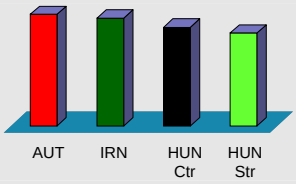
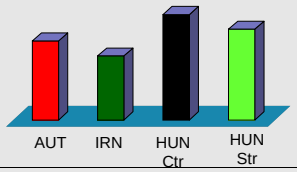
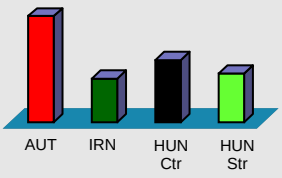
Trait	Location	Mean	S.D.	Heritability	S.E.	
Yld	AUT	10,4	2,9	0.92	± 0.62	
	IRN	9,1	1,7			
	KI	6,1	1,5			
	HUN-Ctr	14,8	2,7			
	HUN-Str	13,3	3,5			
Gps	AUT	334,4	73,3	0.84	± 13.02	
	IRN	432,2	68,3			
	KI	383,3	65,5			
	HUN-Ctr	340,8	66,8			
	HUN-Str	354,4	57,4			
Tkw	AUT	30,7	4,0	0.90	± 2.26	
	IRN	21,2	3,4			
	KI	15,7	2,5			
	HUN-Ctr	45,0	5,9			
	HUN-Str	38,8	7,3			
Sln	AUT	11,0	1,1	0.95	± 0.98	
	IRN	9,5	1,1			
	HUN-Ctr	10,3	1,1			
	HUN-Str	9,7	1,1			
Sps	AUT	18,2	1,3	0.73	± 0.71	
	IRN	17,6	1,1			
	HUN-Ctr	16,1	1,7			
	HUN-Str	15,2	1,4			
Pht	AUT	78,7	7,7	0.98	± 1.24	
	IRN	63,8	9,1			
	HUN-Ctr	105,0	13,3			
	HUN-Str	90,4	12,8			
Eet	AUT	78,5	3,7	0.96	± 0.43	
	IRN	71,2	3,6			
	HUN-Ctr	73,4	3,5			
	HUN-Str	71,8	3,0			

Table 14: Increase in Gps and decrease in Sps under drought stress.

	Grain/spike	Spikelet/spike	Gps : Sps
AUT	33,4	18,2	**1,8**
IRN	43,2	17,6	**2,5**
HUN-Ctr	34,1	16,1	**2,1**
HUN-Str	35,4	15,2	**2,3**

3.7. Linkage map

The genetic linkage map (Fig. 33) consists of 217 loci on 25 linkage groups (Table 15), including 214 loci from 204 SSR markers, as well as 3 loci for morphological traits, i.e. spike pubescence (Pub), waxyness of flag leaf (Wax), and awnedness (Awn). These loci were mapped on chromosomes 1A, 2A, and 5A, respectively. Total map coverage, resulting from 202 linked loci, is 2795 cM, excluding chromosome 7D, which contained only 4 unlinked markers. This map provides an average distance of 14.3 cM between loci. The map length, divided among the three genomes, is 937 cM, 949 cM and 909 cM for the A-, B-, and D-genome, respectively. The average distance for the loci is 14.5 cM in the A-, 13.2 cM in the B-, and 16.0 cM in the D-genome. The distribution of markers on chromosomes is not uniform, with gaps of more than 50 cM on chromosomes 1D, 2B, 4A, 4D, 5A, 6A, 6B, and 7B (Fig. 32).

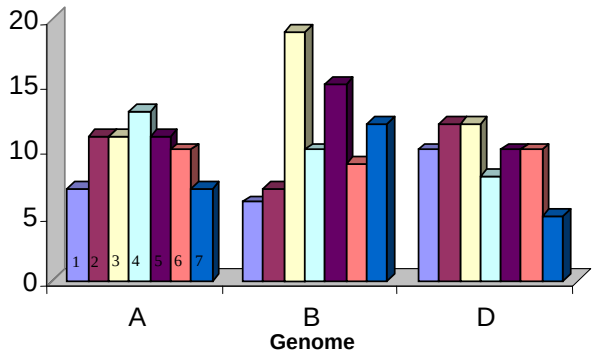


Fig. 32: SSR marker loci distribution in the Tab. x Tai. F2:7 population

Eight markers are present in duplicate and 5 markers in triplicate loci (loci with alphabetic letters at the end of their names in Fig. 33). Thirteen markers having duplicate or triplicate loci in the reference map of Röder et al. (1998) show only one locus in the present map. They are named based on their names in the reference map. Out of 17 SSR marker loci, which show dominant segregation patterns, 9 originated from Tabassi and 8 from Taifun. The

three loci responsible for the morphological traits were considered as dominant alleles from Tabassi, because they are absent in Taifun.

Chromosomes 2A, 3B, 5A, 5D, and 6D are divided into two sub-linkage groups. Out of 214 SSR marker loci, 15 showed segregation distortion and were not linked to the expected linkage groups. Of the 18 new loci (underlined in Fig. 33), which were localized on this map, 11 were not reported even in the latest reference map, and therefore they can be introduced as new loci. The remaining 7 loci occupy different locations on the present map in comparison to the reference map.

Table 15: SSR marker loci information on a linkage map of the Tab. x Tai. F2:7 population

Chr.	Linked loci	Unlinked(distorted) loci	Sub groups	New loci	Loci on reference map	Allele loci
1A	6 + pub.	Xgwm750				
1B	6			Xgwm1171a	5A	1171b-7A
1D	9			Xgwm4122	2B	
2A	12 + wax.		2	Xgwm630	2B	
				Xgwm957b	not reported	957a- 1D
2B	6	Xgwm1027				
2D	12			Xgwm294b	not reported	294- 2A
				Xgwm834	7A	
3A	11					
3B	18	Xgwm533b	2	Xgwm181	not reported	
				Xgwm340	not reported	
				Xgwm1005	not reported	
3D	10	Xgwm4056, Xbarc135				
4A	13			Xgwm4450b	not reported	4450a- 4B
4B	10					
4D	8					
5A	8 + awn.	Xgwm186, 4916	2			
5B	14	Xgwm408				
5D	8	Xgwm902	2			
6A	10			Xgwm169b	not reported	169a- 6A
				Xgwm1103b	not reported	1103a- 6D
6B	9			Xgwm1016b	not reported	1016a- 5B
6D	11	Xgwm1630	2	xgwm539	2D	
				xgwm719b	not reported	719a- 7A
				xgwm815b	not reported	815a- 2D
7A	6	Xgwm746a				
7B	12			Xgwm790b	6B	
				Xgwm3036	1D	
7D		Xgwm746b, 3102				
		Xbarc172, 352				
Total	202	15				

3.8. QTL analysis

Fig. 33 shows the ultimate locations of the major and minor QTLs and the markers associated with them, identified under stress and non-stress conditions.

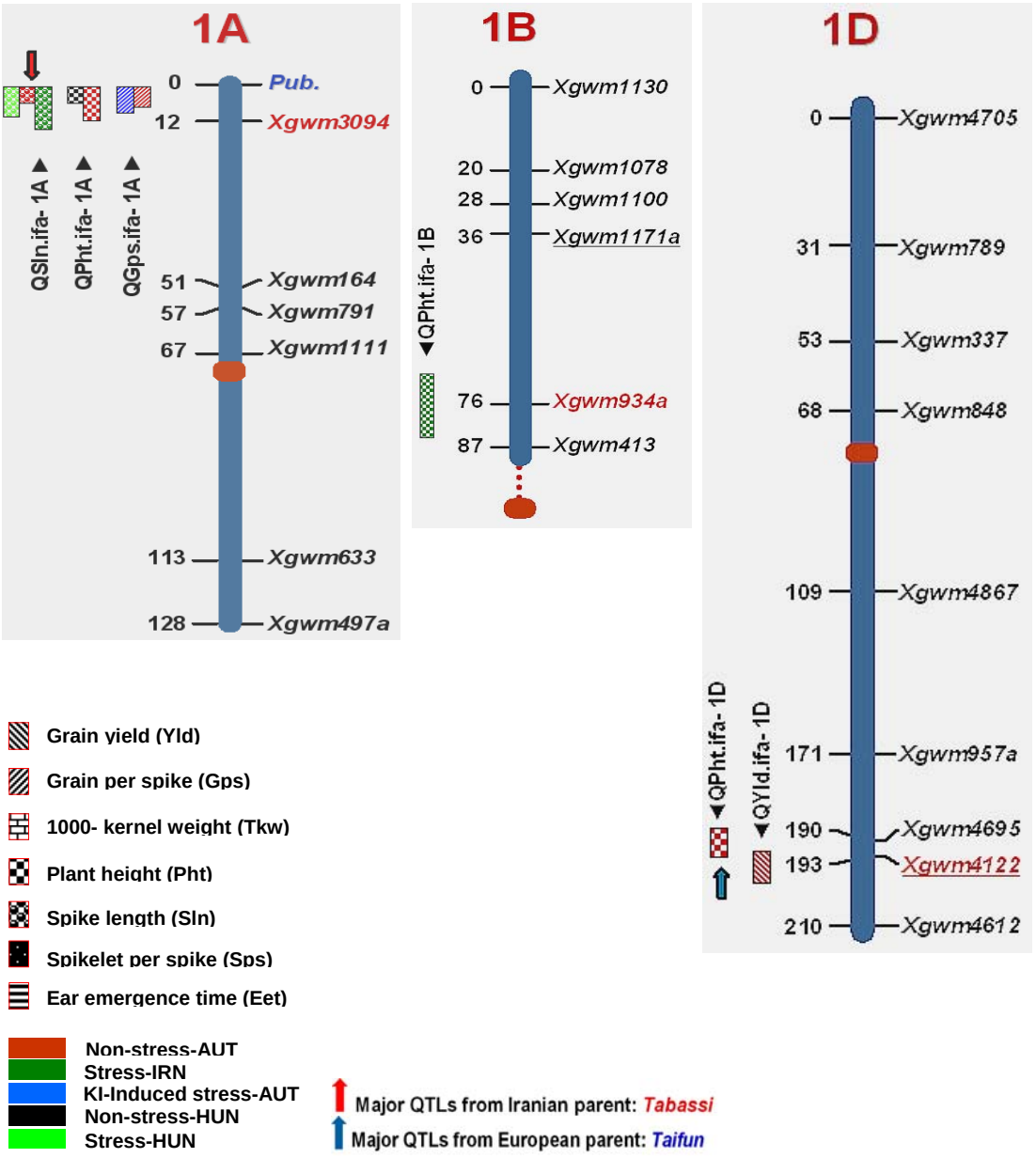


Fig. 33: Major and minor QTLs corresponding to yield, yield components, plant height and ear emergence time in an SSR-based linkage map of Tabassi x Taifun F2:7 RIL population, identified under drought and non-drought conditions. The distances between the loci are in centiMorgan. Red fonts indicate markers most closely associated to the identified QTLs. Blue fonts show morphological marker loci. *Underlined* marker loci represent new ones, detected in the present study.

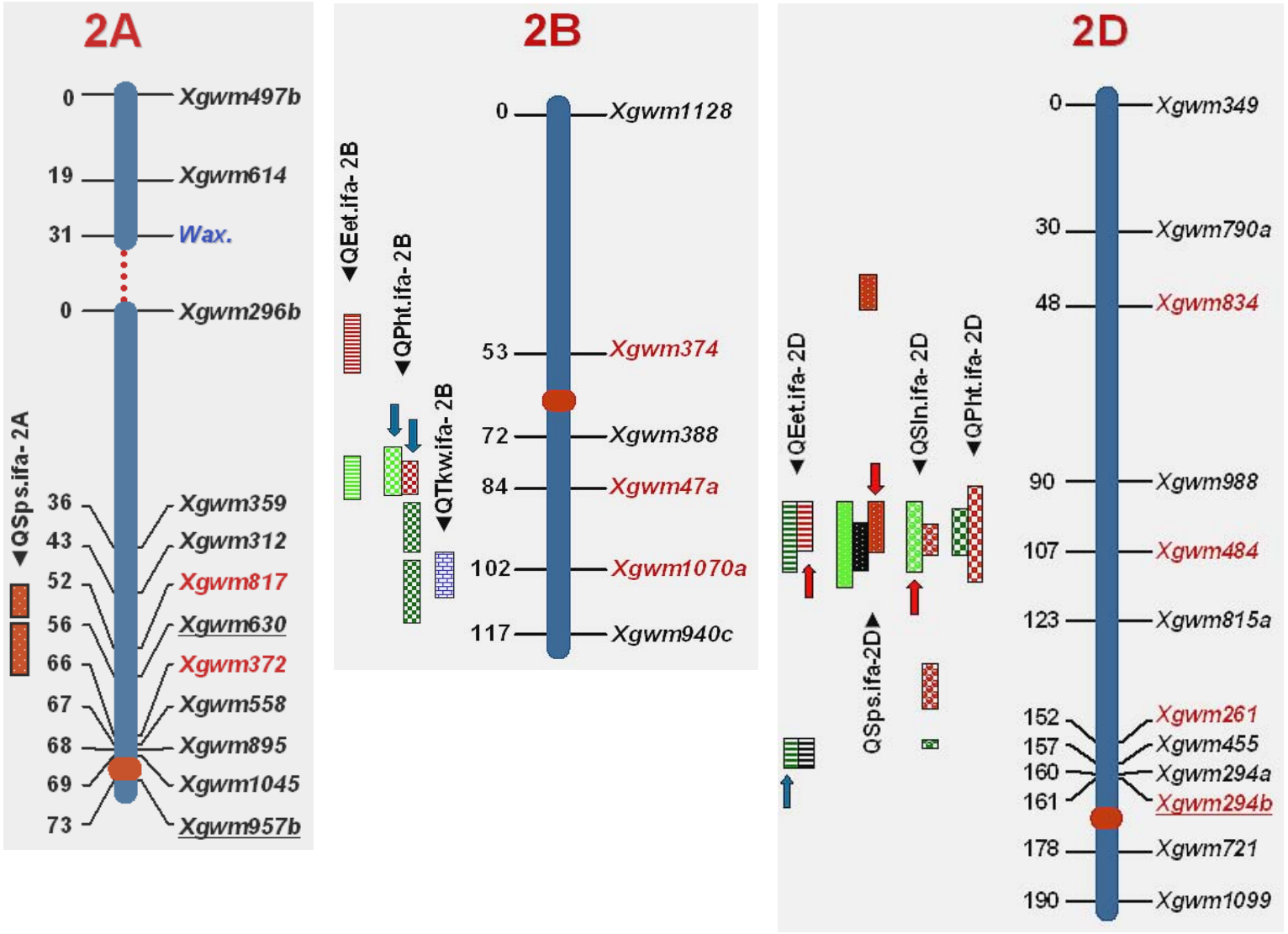


Fig. 33(Continued)

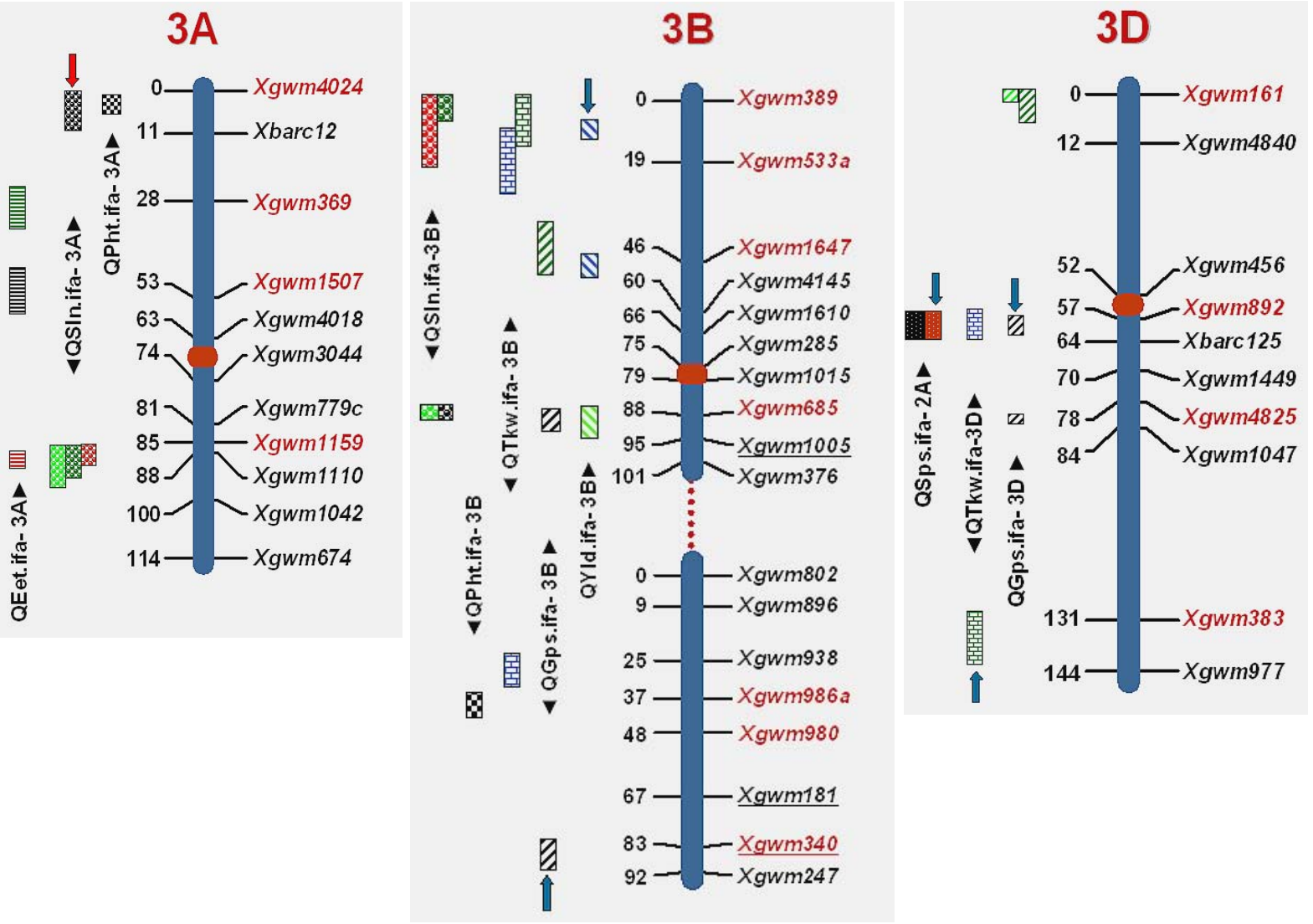


Fig. 33(Continued)

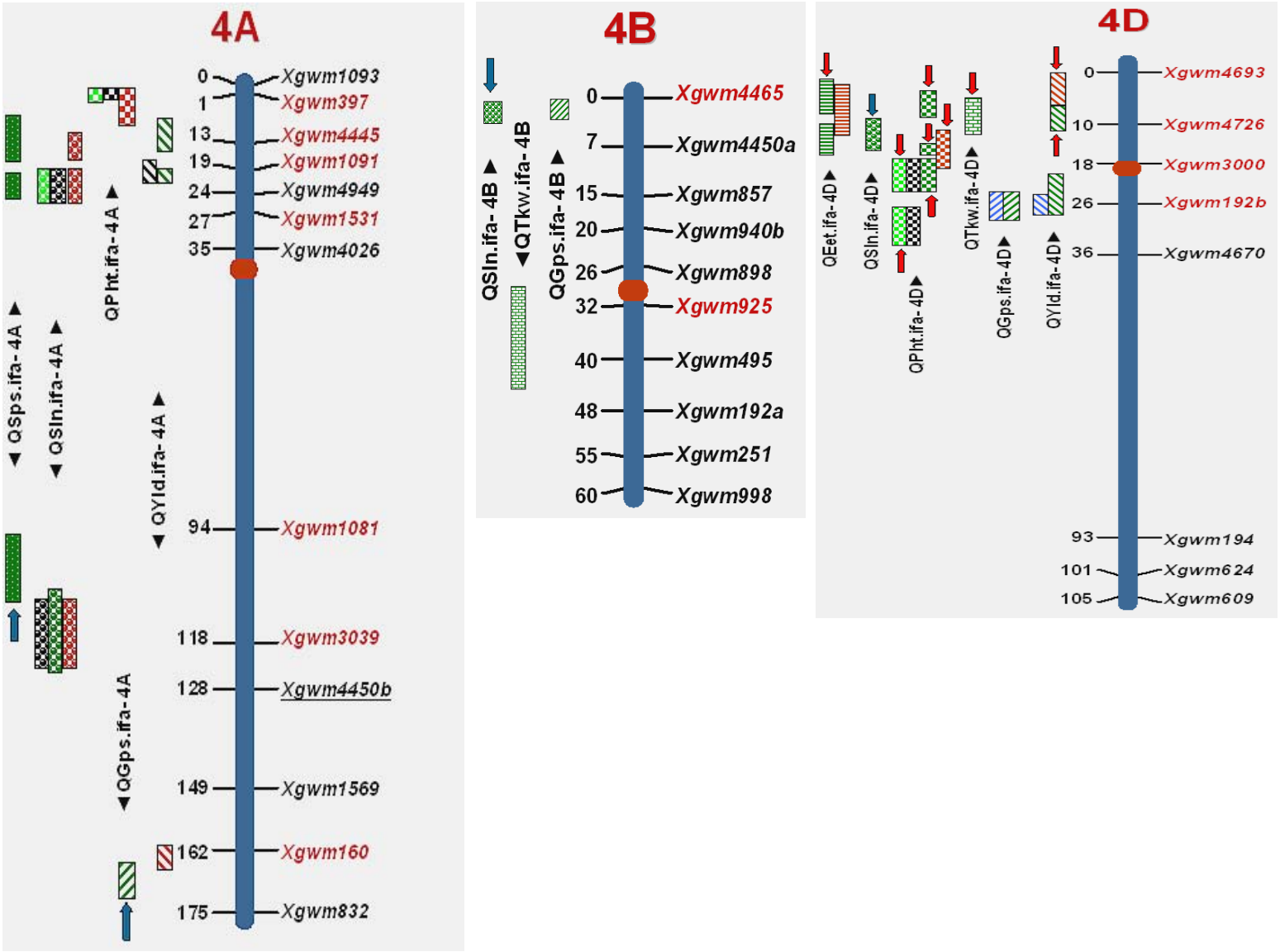


Fig. 33(Continued)

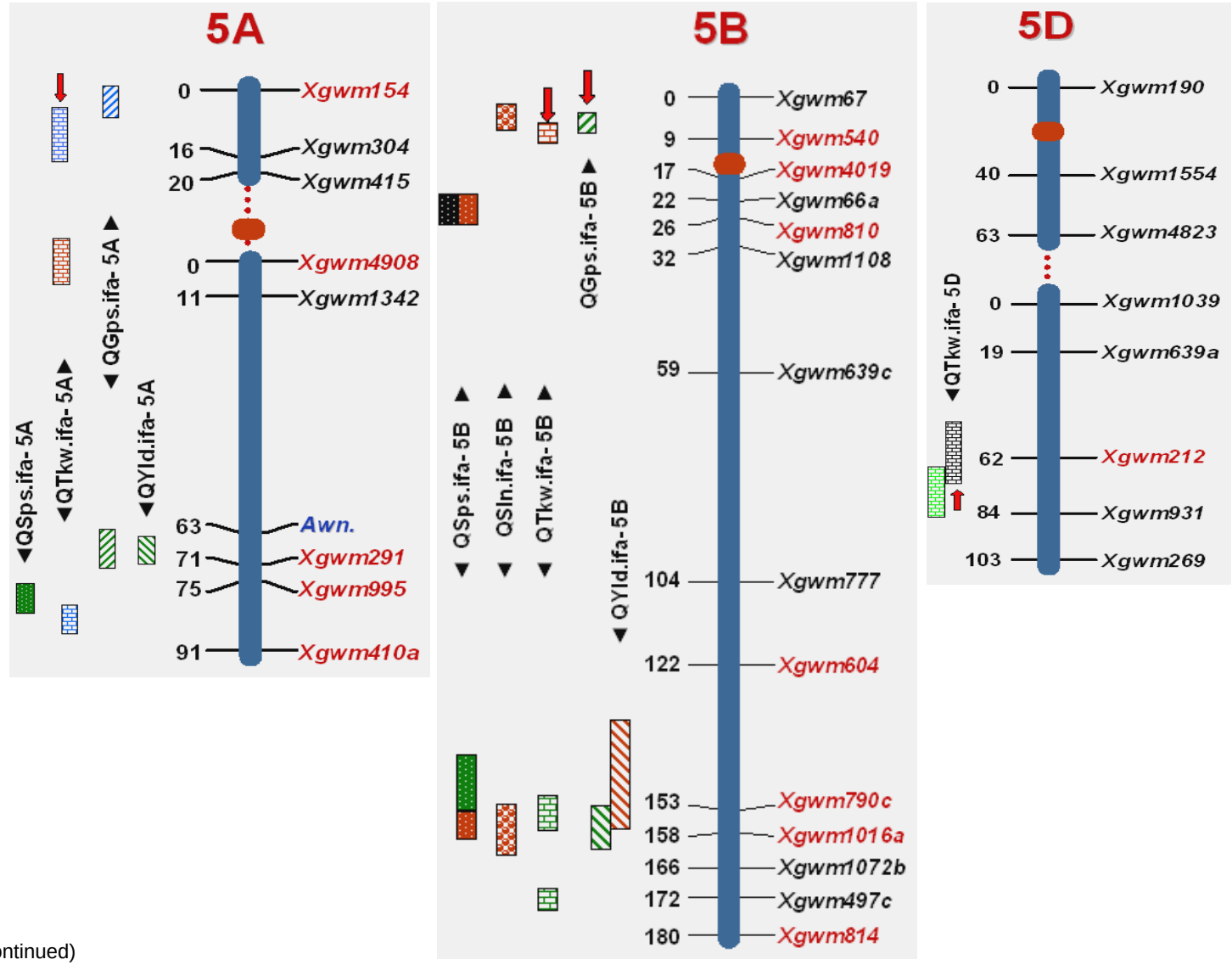


Fig. 33(Continued)

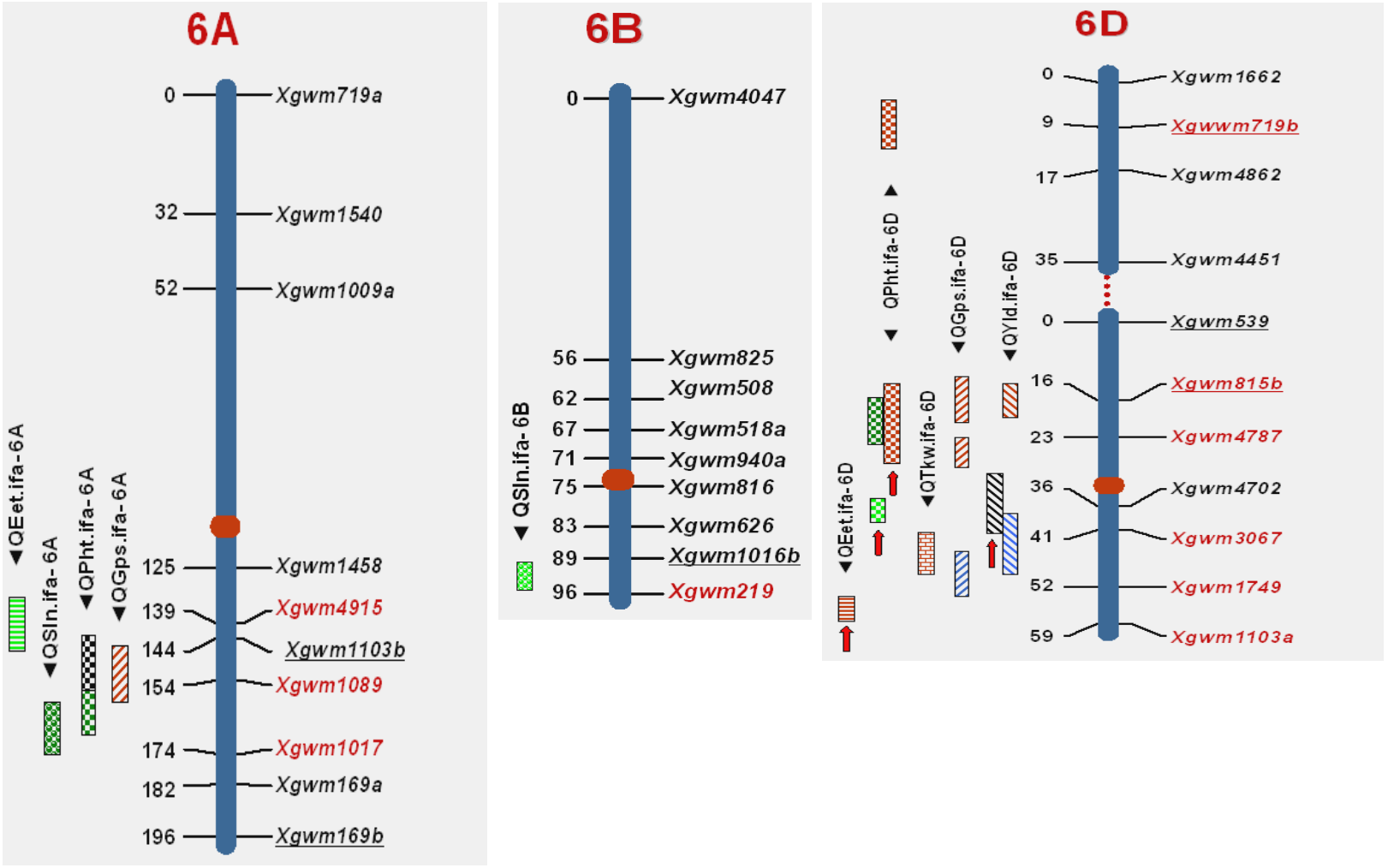


Fig. 33(Continued)

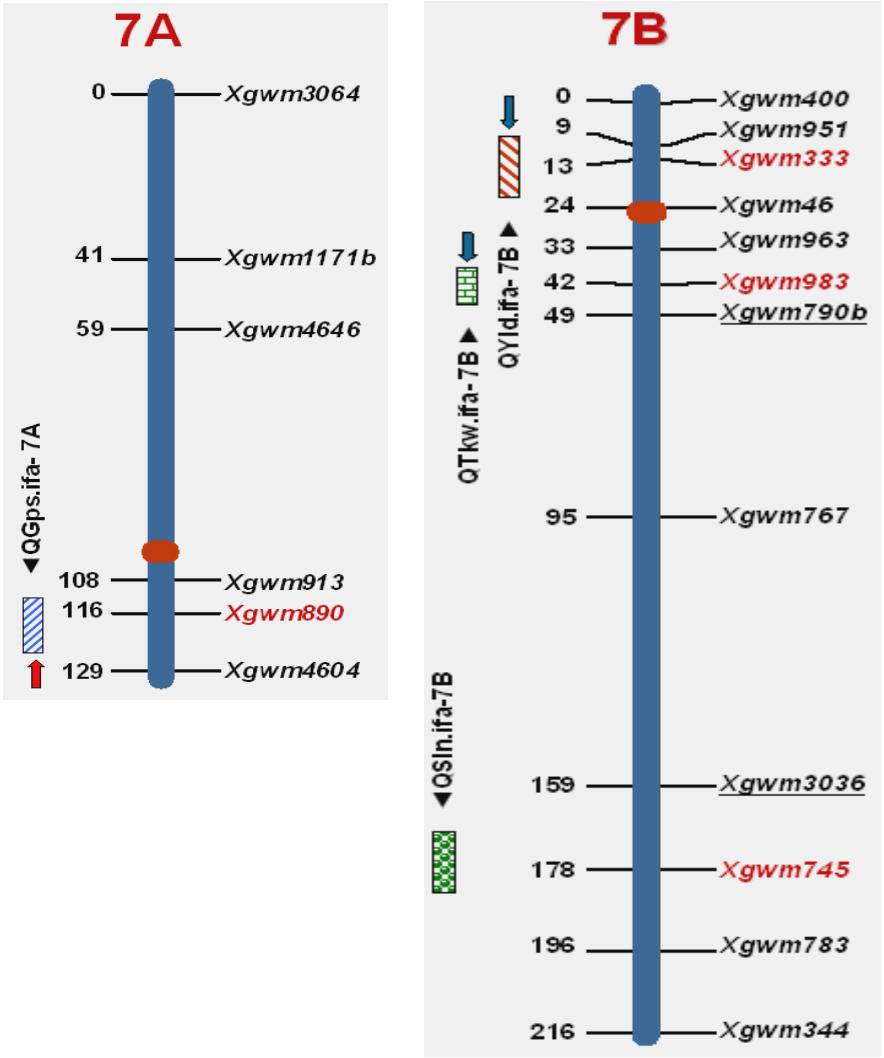


Fig. 33(Continued)

Table 16: Distribution of QTLs among the 20 chromosomes in the F2:7 lines. Alleles contributed by Tabassi are indicated by **Red** fonts, those from Taifun by **blue** fonts. **Highlighted** fonts show major QTLs.

	1A	1B	1D	2A	2B	2D	3A	3B	3D	4A	4B	4D	5A	5B	5D	6A	6B	6D	7A	7B
AUT	Gps		Yld	Sps	Pht	Sln	Sln	Sln	Sps	Yld		Yld	Tkw	Yld		Gps		Yld		Yld
	Sln		Pht	Sps	Pht	Sln	Eet			Sln		Pht		Tkw				Gps		
	Pht					Sps				Sln		Eet		Sln				Gps		
						Sps				Sln				Sln				Tkw		
						Pht				Pht				Sps				Pht		
						Eet								Sps				Pht		
																		Eet		
IRN	Sln	Pht			Pht	Sln	Sln	Gps	Gps	Yld	Gps	Yld	Yld	Yld		Sln				Tkw
					Pht	Pht	Eet	TKW	Tkw	Yld	Tkw	Gps	Gps	Gps		Pht		Pht		Sln
						Eet		Sln		Gps	Sln	Tkw	Sps	Tkw						
						Eet				Sln		Sln		Tkw						
										Sps		Pht		Sps						
										Sps		Pht								
										Sps		Pht								
												Eet								
												Eet								
KI	Gps				Tkw			Yld	Tkw			Yld	Tkw					Yld		
								Yld				Gps	Tkw					Gps	Gps	
								Tkw					Gps							
								Tkw												
HUN-Ctr	Pht					Sps	Sln	Gps	Gps	Yld		Yld		Sps	Tkw	Pht		Yld		
						Eet	Pht	Gps	Gps	Sln		Pht								
							Eet	Sln	Sps	Sln		Pht								
								Pht		Pht										
HUN-Str	Sln				Pht	Sln	Sln	Yld	Gps	Sln		Pht			Tkw	Eet	Sln	Pht		
					Pht	Sps		Sln		Pht		Pht								

General distribution of QTLs on chromosomes is shown in Table 16. Out of 146 putative QTLs, identified for 7 agronomic traits in five experiments, 54.1% were found under stress and 45.9% under non-stress conditions (Fig. 34). Parental QTLs were not contributed to the F2:7 lines equal shares in the different experiments, among the traits and genomes. In total, 56.8% of QTLs were Tabassi-type and 43.2% Taifun-type.

While the two parents contributed almost equally under drought stress conditions, 50.6% of Tabassi vs. 49.4% of Taifun, Tabassi contributed significantly more QTLs than Taifun under non-stress conditions, 60.2% from Tabassi vs. 38.8 from Taifun (Fig. 34).

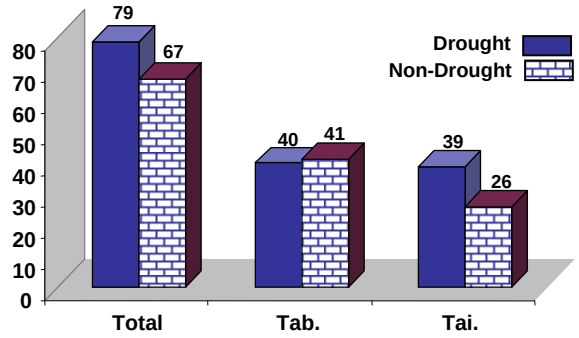


Fig. 34: Parental QTL contributions under drought and non-drought stress conditions in absolute numbers.

Among the experiments, these contributions were not uniform for the two parents (Fig. 35). While in Austria Tabassi contributed twice as many QTLs as Taifun, their contributions were the same in Iran and Hungary-non stress.

Taifun presented more alleles in KI-experiment, but Tabassi presented more alleles under stress condition in Hungary. It is important to mention that all the 15 QTLs found in the KI-experiment were identified only for three traits i.e. Yld, Gps and Tkw. The other traits could not be measured in this experiment.

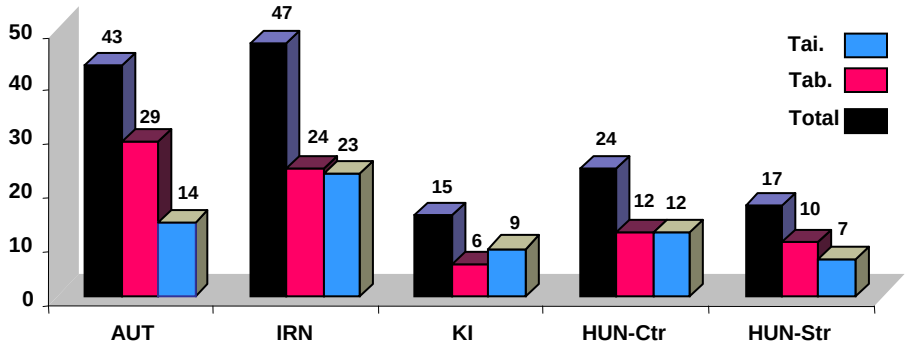


Fig. 35: Distribution of parental QTLs over experimental locations.

Within the genomes, QTLs were unevenly distributed (Fig. 36 and Fig. 37), i.e. 32.9%, 28.1%, and 39.0% for genome A, B, and D, respectively. The highest number of Taifun-type

QTLs were present in genome B and lowest in genome D (56.1% vs. 26.3%). For Tabassi-type QTLs, genome D carried the highest (73.7%) and genome B the lowest (43.9%) number. Contributions of the two parents in genome A were similar (52.1% for Taifun and 47.9% for Tabassi). The highest percentage of QTLs identified under drought stress was on genome B (63.4 %). Genome D contributed a similar number of QTLs under stress and under non-stress conditions (49.1% vs. 50.9%). In genome A, 52.1% of QTLs were found under drought stress and 47.9% under non-drought stress conditions.

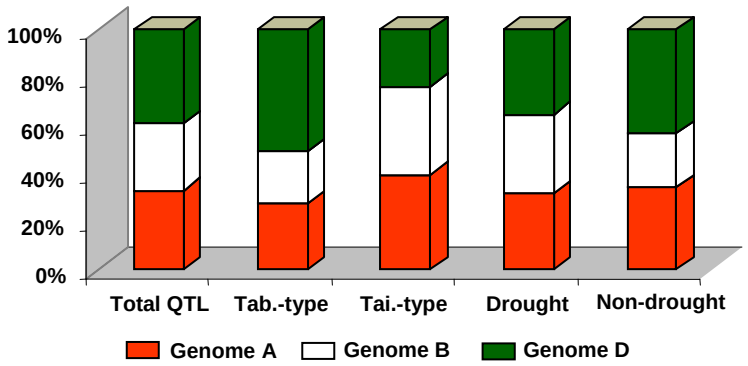


Fig. 36: Distribution of all QTLs within the genomes.

The chromosomes contributing the highest number of QTLs were 4D with 19 and 4A with 18 QTLs (Fig. 37).These two chromosomes also contain the highest number of QTLs from Tabassi (16 on 4D) and Taifun (11 on 4A). In contrast, chromosomes 1B and 7A contain only 1 QTL from Tabassi, and chromosome 6B one QTL from Taifun.

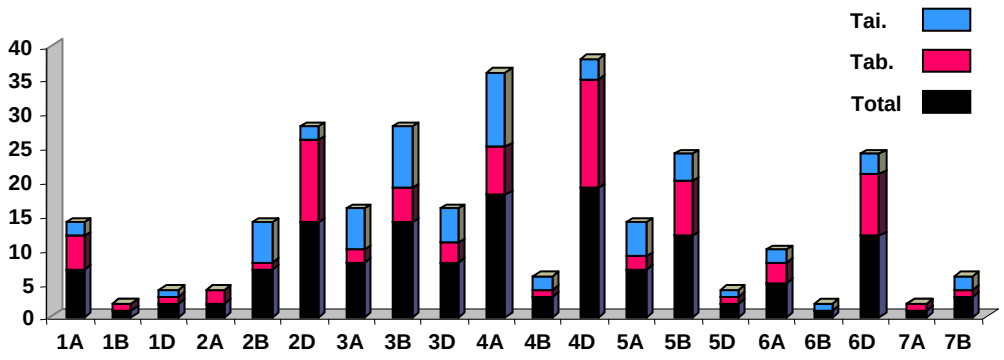


Fig. 37: Fractions of parental QTLs among the different chromosomes.

Among the traits, the highest number of QTLs (31 and 29) was identified for Pht and Sln (Fig. 38). For both of these traits, Tabassi contributed more QTLs than Taifun. The lowest number of QTLs (11) was found for Eet. QTLs identified for Pht showed the highest phenotypic variation (R^2 = 10.4%) and those for Sln, the lowest (R^2 = 6.8%).

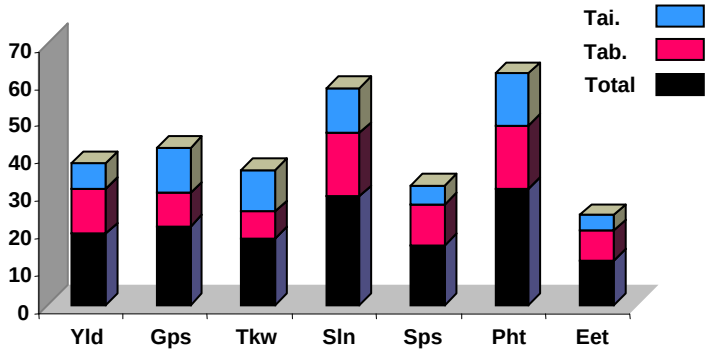


Fig. 38: Contribution of parental QTLs to different traits.

* * *

Chapter 4

Discussion

4.1. High potential Tabassi
4.2. Efficiency of drought stress experiments
4.3. Linkage map
4.4. QTL analysis
4.5. QTL identification and validation
4.5.1. QTLs for Yield and yield components
4.5.1.1. Grain yield per 10 spikes (Yld)
4.5.1.2. Grain per 10 spikes (Gps)
4.5.1.3. 1000-kernel weight (Tkw)
4.5.1.4. Spike length (Sln)
4.5.1.5. Spikelet per spike (Sps)
4.5.2. QTLs for plant height and ear emergence time
4.6. Chromosome 4D is a QTL-rich region for drought tolerance
4.7. Conclusion
4.8. Out looks

4.1. High potential Tabassi

There is general agreement that, under drought stress conditions, old cultivars and landraces yield higher and have more stability in comparison to improved varieties (Ceccarelli et al. 1991 and Blum et al. 1996). This idea is supported by the results of the present study, which show that, while under non-drought conditions there was no significant difference in average grain yield between the Iranian landrace Tabassi and the western European cultivar Taifun, under drought conditions Tabassi significantly out-yielded Taifun (Table 9 and Fig. 26). As can be seen in Table 7 and Fig. 25, among the five Iranian landraces and varieties Tabassi showed the largest genetic distance to Taifun. This was visible in a morphological comparison of the two plants (Fig. 19), and resulted in remarkable phenotypic variation of the F2:7 RIL-population. We expect this genetic difference also to provide a large number of allelic combinations of agronomic traits. As a consequence, the mean values of the F2:7 lines were generally higher than the mid-parent values for Yld, Tkw, Sln, Pht and Eet (Fig. 30). This genetic difference also explains the high heritability estimates, the presence and validation of QTLs in this study.

Phenotypic characteristics of Tabassi are clearly differing from those of Taifun, as shown in section 2.1.4, Fig. 20. They support the idea that Tabassi has a high potential for drought tolerance. For example, Tabassi was 15.0 cm taller than Taifun under non-stress condition in Austria and 17 cm under stress condition in Iran. The latter difference resulted from a lesser reduction of plant height of Tabassi (11 cm) than of Taifun (13.0 cm) under stress condition (Table 29). As a consequence Tabassi can be expected to have more stem reserves, supporting grain development during a post anthesis drought stress. Basically, grain development in wheat depends on three sources:

- Carbohydrate produced after anthesis and translocated directly to the grain,
- Carbohydrate produced after anthesis, but stored temporarily in the stem, before being re-mobilized to the grain,
- Carbohydrate produced before anthesis, stored mainly in the stem, and remobilized to grain during grain filling (Ehdaie et al. 2006).

Therefore, a substantial amount of the carbohydrates, translocated to the seeds during grain filling, must come from reserves assimilated before anthesis (Gent 1994). Stem length is an important character affecting stem reserve storage (Borrell et al. 1993). Significant positive correlations of Pht with Yld and Tkw under drought and non-drought stress (Table 10) support this idea.

Tabassi also has much higher Tkw compared to Taifun (more than 30% under both non-drought and drought conditions). This is mainly due to its larger seed size (Fig. 19-D). In return, Tabassi produces less Sps and Gps under both stress and non-stress conditions than Taifun. The similar yield capacity under non-stress conditions indicates genetic differences of

the yield components (more or larger seeds). Under stress, the larger seeds (sink) of Tabassi may excert a stronger absorbing power to remobilize stem reserves.

Besides these phenotypic differences, Tabassi and Taifun are different in terms of plant phenology and constitutive characteristics. Plant phenology is timing the developmental stages of plants, i.e. the time of heading, flowering and ripening. Early heading or flowering is a trait enabling the plant to complete its life cycle in general, or developmental stages in particular, during stress.

On average, Tabassi was 7 days earlier for ear emergence than Taifun, both in stress and non-stress environments (Table 9). This earliness showed a significant negative correlation with Yld and Tkw both under stress and non-stress conditions, implying that plants, which emerged earlier, yielded more.

Root structure, as a constitutive trait, can play an important role in plant response to drought stress. In cereals, dry topsoil inhibits the formation and establishment of new roots. Under these conditions, assimilates preserved in the stem (stem reserves) will be transferred to the roots, for further growth deeper into the soil.

In association with this project, in a pilot experiment, the development of shoots and roots of Tabassi and Taifun was studied under two water regimes, i.e. 80% and 40% of field capacity, in the green house of the Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary (data by courtesy of Dr. **János Györgyei**).

Tabassi and Taifun showed clear differences in shoot and root development (Fig. 39), and also significant differences in shoot and root dry matter (Fig. 40). Under 80% of field capacity, considered as optimum water level, Tabassi produced 20% and 40% more dry matter of shoot and root, respectively, compared to Taifun. Under 40% of field capacity, considered as water deficit level, Tabassi produced 40% and 30% more dry matter of shoot and root than Taifun. The ratio of root to shoot dry weight increases, as water stress develops (Blum, www.plantstress.com, last update 2005). Taifun behaves accordingly in this experiment. In the case of Tabassi there is no significant difference of this ratio between the two water regimes (Fig. 40). This can be taken as an obvious sign for a constitutional difference between a non-drought tolerant and a drought tolerant genotype. We may speculate that, while Taifun invested a large fraction of its assimilates into root development resulting in a weak shoot development, Tabassi, as a genotype selected under drought conditions, developed a strong root system constitutionally, without loosing assimilates for shoot development. This preliminary result strongly suggests that a constitutional difference in plants' root development may play a major role in drought tolerance.

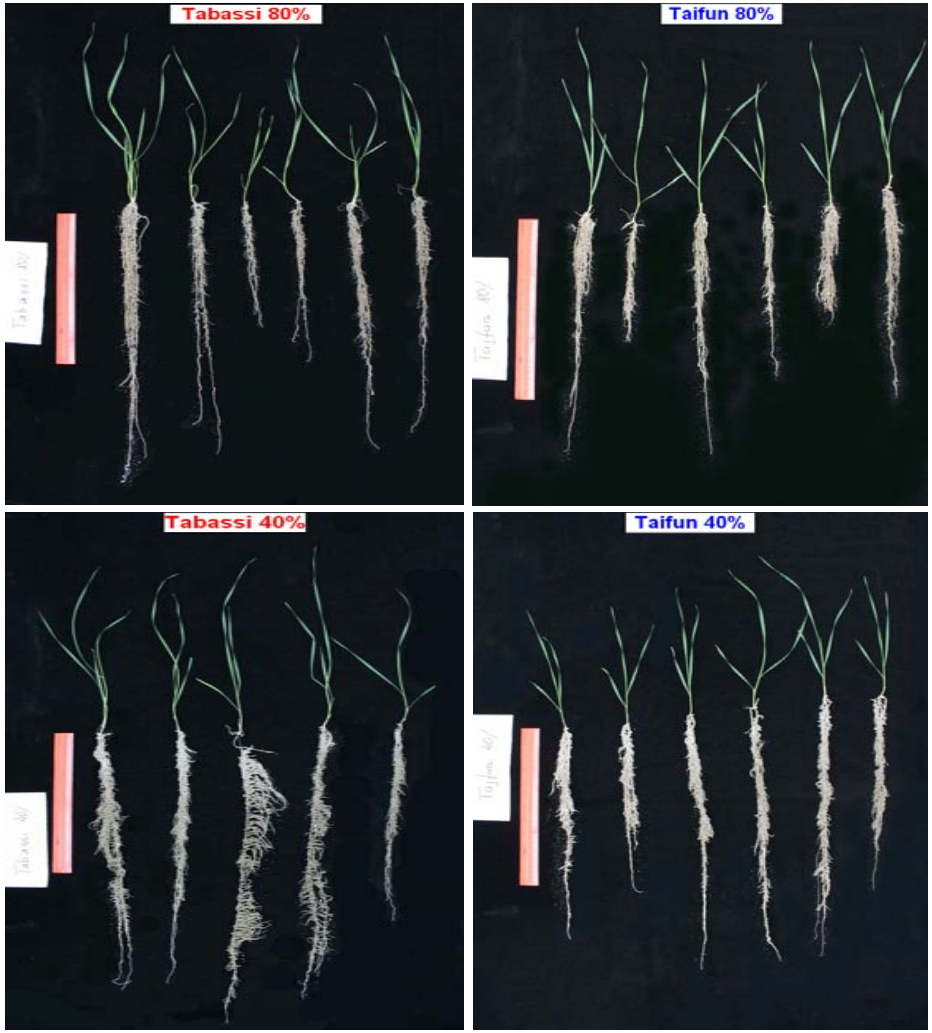


Fig. 39: Root and shoot mass and development in Tabassi (left) and Taifun (right) under two water regimes: 80% of field capacity (above) and 40% of field capacity (below) in a green house experiment.

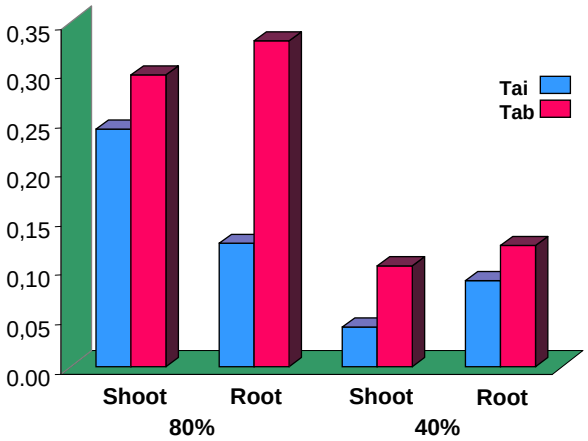


Fig. 40: Difference of shoot and root dry weight between Tabassi and Taifun at two water regimes, i.e. 80 and 40% of field capacity.

Another evidence for the superiority of Tabassi to Taifun in tolerating drought stress is the stress susceptibility index (SSI), calculated according to Fischer and Maurer (1978). For all traits, except for Sps, Tabassi showed less susceptibility to drought stress (Table 17). Interestingly, Sps had the lowest heritability (H^2=0.73) among the agronomic traits (Table 13). This is in agreement with the fact that traits with low heritability are more influenced by the environment. The trait of Taifun, most sensitive to drought, was Gps in the KI-experiment. Gps showed the second lowest heritability (H^2=0.84).

Table 17: Drought stress susceptibility index of Tabassi and Taifun calculated for agronomic traits. The lowest and highest SSIs for Tabassi and Taifun are highlighted by yellow and blue colors, respectively.

Trait	Parent	AUT vs. IRN	AUT vs. KI
Yld	Tabassi	0.56	0.95
	Taifun	1.50	1.11
Gps	Tabassi	0.73	1.10
	Taifun	1.24	1.64
Tkw	Tabassi	0.80	0.95
	Taifun	1.25	1.10
Sln	Tabassi	0,67	
	Taifun	1.30	
Sps	Tabassi	4,27	
	Taifun	-1.88	
Pht	Tabassi	0.84	
	Taifun	1.20	
Eet	Tabassi	0.84	
	Taifun	1.04	

Although grain yield of Tabassi was the least susceptible trait compared to Taifun, this result has to be treated with some care, because grain yield was measured only on 10 representative spikes. For this reason, 1000-kernel weight is a more reliable yield component to judge the differences between the parents and the lines in the experiments. As mentioned before, Tabassi showed significantly higher Tkw (almost 30%) under both drought and non-drought conditions.

Decrease in yield and 1000-kernel weight under stress seems to be the result of decrease in grain weight in all stress environments. It was, however, accompanied with a parallel increase in Gps. Since Sps did not change under drought conditions, the higher seed number under stress is only possible through a higher fertility of tertiary florets. Since this was true for both genotypes, it can be interpreted as an adaptation of the species to survive via more seeds under stress conditions. The higher yield loss of Taifun under stress indicates that, for a non tolerant plant, increasing seed number under stress is probably not enough for survival.

Morphological traits i.e. awnedness, waxyness, and pubescence which are typically present in Tabassi and absent in Taifun, are supposed to have positive influence on yield under stress. But the results of the present study do not support this. While the effect of awns on yield was weakly significant under non-stress conditions in Austria, but not in Hungary, there was no positive correlation between awnedness and yield under stress conditions. It seems that awns, as a photosynthetic source, would partially support yield under non-stress condition, but they did not play a role in the time of grain filling, when photosynthesis was inhibited and plants could only resort to assimilates, stored as reserves in the stem. This may explain the absence of significant correlation between Awn and Yld under drought stress.

Pubescence and waxyness basically decrease light-radiation induced evapo-transpiration from the leaf surface. But on the other hand, they may also reduce radiation use efficiency, which can reduce yield (Richard 1996). The negative correlation between Pub and Yld indicates this negative influence of pubescence on yield. Waxyness showed a positive correlation with Yld and Tkw under stress and non-stress conditions, but it was not strong enough to conclude a large effect of this trait on yield. The same can be said about the relationship between Awn and Yld (Table 11).

4.2. Efficiency of drought stress experiments

Drought tolerance can only be evaluated, if drought stress causes significant yield reduction (Blum 1993). Distribution of data, resulting from principle components analysis of 7 agronomic traits, evaluated in different experiments in Austria, Iran and Hungary as shown in Fig. 27, and also distribution of data for Yld, Gps and Tkw in the KI experiment (Fig. 28), proove the efficiency of both the drought experiments in Iran and the KI-experiment.

Concerning the KI experiment, it is important to mention that a chemical desiccant, which has been applied two weeks after anthesis, only simulates the effect of drought in a final stage of seed filing. The highly significant correlation (r = 0.690) of yield reduction under natural stress in Iran and stress induced by KI in Tulln, Austria, is in full agreement with data reported by Blum (1993b) and Nicolas and Turner (1993), who found high correlation between the rate of reduction in grain weight by chemical desiccation and the rate of reduction by actual drought stress. The KI experiment was intended to verify the capability of plants to remobilize reserves stored in the stem, when photosynthesis is inhibited during the grain filling period by drought. The correlation found by Blum (1993b) and Nicolas and Turner (1993), as well as in the present experiment, strongly suggests that plants' capability of stem reserve remobilization plays a very important role in terminal drought.

Table 18: Comparison of correlation coefficients of yield reductions caused by chemical desiccation and natural drought stress.

	Chemical desiccant experiment		
	Blum 1993	Nicolas and Turner 1993	present study
Natural drought stress	0.81**	0.79**	0.69**

Moreover, the KI-experiment showed high efficiency in QTL identification. The number of QTLs, which were identified under drought stress in Iran and in the KI-experiment, is shown in Fig. 41. For the three traits, i.e., Yld, Gps, and Tkw, which were evaluated in both experiments, 19 QTLs were found in Iran and 15 in the KI-experiment, indicating the high efficiency of the latter to provide a condition similar to post anthesis drought, as occuring naturally in Iran.

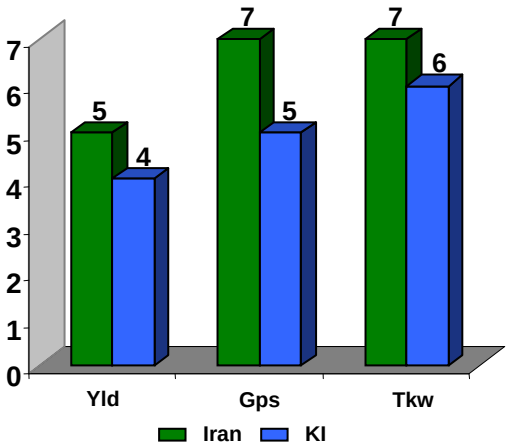


Fig. 41: Comparison of the number of QTLs under drought stress in Iran and the KI-experiment.

The two experiments in Hungary, one in non-drought condition and the other under a rain shelter providing drought stress (Fig. 31 of section 3.6, and Fig. 41), showed significant differences for yield and yield components. According to expectation, there was a clear reduction of Yld and Tkw. The use of the water shelter created the expected stress conditions with respect to the water supply for the plants during plant growth (Table 19). However, the mean values of Yld, Tkw, and Pht under stress in Hungary were higher than under non-stress condition in Austria (Table 13, Fig. 31). According to field observations in Hungary (Fig. 43), plants were more vigorous even under the rain shelter than the plants in Austria. The difference can probably be explained by the difference in the yield potential of the experimental fields. In Tulln, where the depth of the topsoil is only about 60 cm, on a deep layer of gravel deposited

by the Danube, and with an average yield potential of 6 t/ha, the nursery in Szeged possess a rich deep chernozem with a yield potential of 10 t/ha. For this reason data of the two experiments in Hungary were used with care for QTL analysis.

As mentioned in section 1.9, providing a proper field condition for drought stress is difficult, due to the complexity of the traits and environmental effects. In this study even such a sophisticated equipment to control rain fall did not provide the expected environment.

Fig. 42: The author expresses his high appreciations for Dr. László Cseuz and his team for carrying out the experiments in Szeged-Hungary.

Table 19: Total precipitation and water potential (pressure) in the stress and non-stress experiments in Hungary from 10.03.07 to 05,07,07

	HUN-Ctr	HUN-Str
Total precipitation (mm)	249.60	5.90
Water potential (Pa*)	-51,52	-77,62

*Pa= Pascal

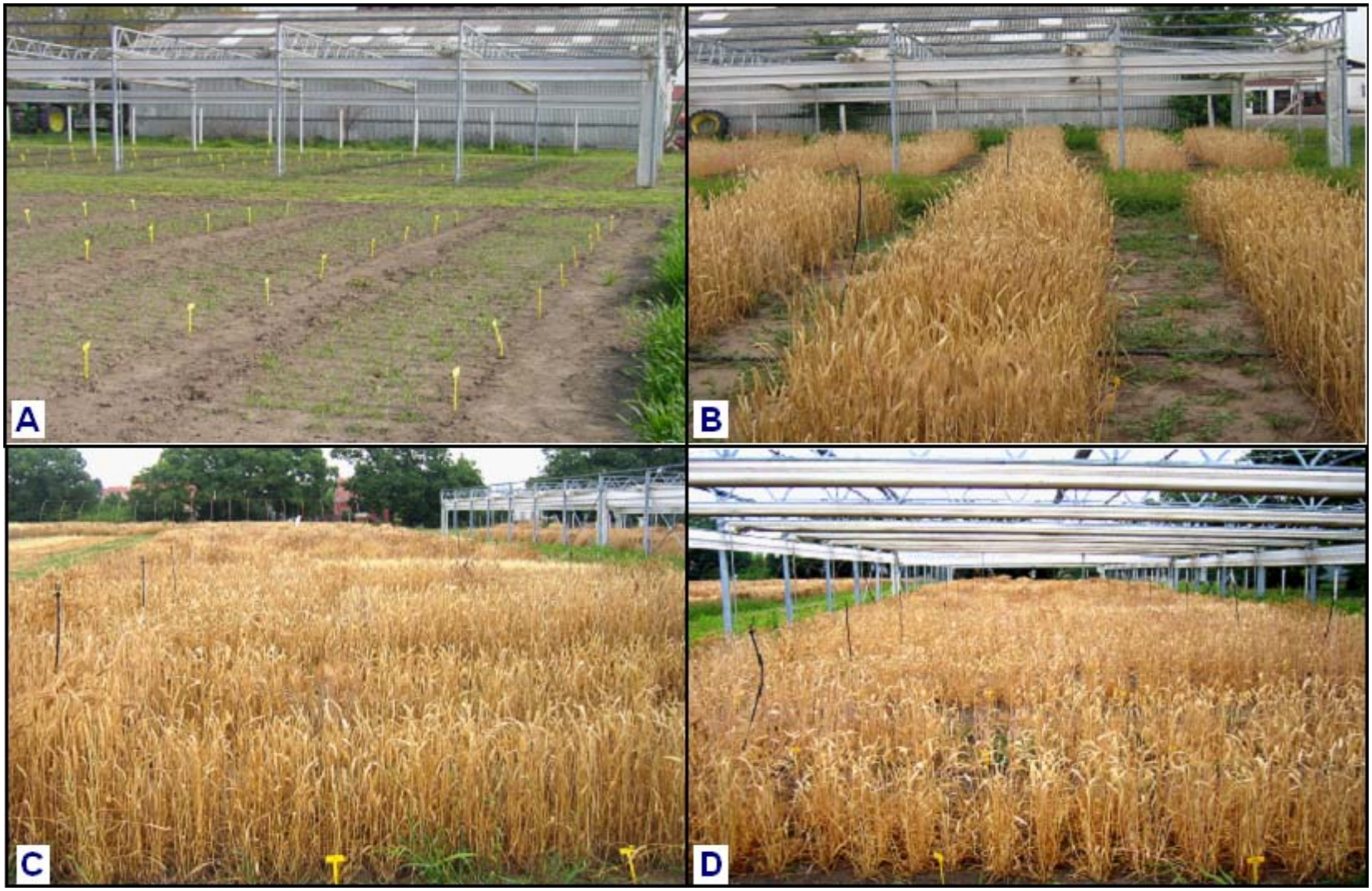


Fig. 43: The experimental fields in Hungary; A: Control plots (non-stress) in the front and plots under the rain shelter (stress) in the back, at the time of tillering. B: The experiments at the time of harvesting. C: The control (non-stress) experiment, D: Rain sheltered experiment, showing lesser plant density and shorter plants.

4.3. Linkage map

There is no absolute value for the number of DNA markers required for a genetic linkage map, since the number of markers varies with the number and length of chromosomes in the organism (Collard et al. 2005). For QTL detection a relatively sparse map, consisting of evenly spaced markers, is adequate. However, this depends on the size of the genome of the species. Our present study supports this idea. For example, as shown in Table 20, chromosome 3B with the highest number of markers (18) carried 0.8 QTL per marker. Chromosome 4B, with 10 markers and the smallest average genetic distance between its marker loci (6 cM), had only 0.3 QTL per marker. In contrast chromosome 4D with 8 markers and an average map distance of 13.1 cM showed the highest number of QTLs per marker (2.4).

Table 20: Marker density and number of QTLs identified on different linkage groups in the Tab. x Tai. F2:7 population.

Chr.	Nr. of linked loci	Map length	Average distance	Nr. of associated markers	Total QTLs	QTL per 10cM	QTL per Marker	Average QTL intervals	Average LOD	Average R^2 %
1A	7	128	18,3	1	7	0,5	1,0	6,3	4,5	6,5
2A	13	104	8,0	2	2	0,1	0,2	6,5	3,4	5,9
3A	11	114	10,4	4	8	0,7	0,7	7,6	4,3	6,3
4A	13	175	13,5	7	18	1,0	1,4	8,3	4,2	7,2
5A	9	91	10,1	5	7	0,8	0,8	11,0	4,4	7,7
6A	10	196	19,6	3	5	0,3	0,5	10,0	4,7	7,3
7A	6	129	21,5	1	1	0,1	0,2	10,0	5,1	10,8
Genome A	**69,0**	**937,0**	**14,5**	**23**	**48,0**	**0,5**	**0,7**	**8,5**	**4,4**	**7,4**
1B	6	87	14,5	1	1	0,1	0,2	14,0	4,9	7,3
2B	6	117	19,5	3	7	0,6	1,2	11,1	4,7	7,8
3B	18	193	10,7	7	14	0,5	0,8	9,9	4,3	7,4
4B	10	60	6,0	2	3	0,5	0,3	7,0	6,3	9,2
5B	14	180	12,9	7	12	0,7	0,9	8,4	4,0	6,7
6B	9	96	10,7	1	1	0,1	0,1	6,0	3,1	4,7
7B	12	216	18,0	3	3	0,1	0,3	9,0	2,1	9,1
Genome B	**75,0**	**949,0**	**13,2**	**24**	**41,0**	**0,4**	**0,5**	**9,3**	**4,2**	**7,4**
1D	9	210	23,3	1	2	0,1	0,2	6,5	6,8	10,2
2D	12	190	15,8	4	14	0,7	1,2	12,4	5,3	8,5
3D	10	144	14,4	4	8	0,6	0,8	6,6	5,3	9,2
4D	8	105	13,1	4	19	1,8	2,4	6,2	6,7	13,1
5D	8	166	20,8	1	2	0,1	0,3	17,5	3,3	10,2
6D	11	94	8,5	6	12	1,3	1,1	8,3	5,2	9,7
Genome D	**58,0**	**909,0**	**16,0**	**20**	**57,0**	**0,6**	**1.0**	**9,6**	**5,5**	**10,1**

Out of the 205 linked SSR-loci on the map, 32.7% showed significant association with the QTLs (loci with red fonts in Fig. 33). This observation also indicates that genes are not evenly distributed in the wheat genome. Uneven distribution was observed for parental QTLs within and between the genomes, as described in section 3.7. Unfortunately, in the present study only four polymorphic markers were found on chromosome 7D, which is supposed to have important QTLs for agronomic traits. However, this map can be further improved by using more SSR and/or AFLP markers.

4.4. QTL analysis

Table 21 summarizes the locations of major QTLs ($R^2 \geq 10.0$) on the map, with their relative interval lengths, the closest associated marker loci, as well as additive effects and phenotypic variations (R^2), which can be explained by these QTLs.

4.5. QTL identification and validation

QTL analysis was done using composite interval mapping (CIM). QTLs with LOD values less than 3.0 were not considered. A permutation test at a significance level of 0.01 (Doerge and Churchill 1996) was applied for all traits to achieve a minimum LOD=3. QTLs were categorized, based on R^2 values, as major QTLs with a minimum R^2=10, and minor QTLs with R^2 values of less than 10. A QTL was valid, when it was identified at least in two locations and confirmed by other studies. As exceptions we also considered those few QTLs, which were identified in only one experiment, but met all statistical requirements, i.e. high R^2 and LOD values, to be valid. QTLs, which were found in the two experiments in Hungary, were only considered, if they occurred in any other experiment of the present study, i.e. either in Austria, Iran, or in the KI experiment. Most of the QTLs found confirm QTLs identified in previous investigations, as described in the next sections. Some of the QTLs, however, can be introduced as new.

4.5.1. Yield and yield components QTLs

Grain yield and its components are quantitative in their effects and were subjected to QTL analysis. **T**here is general agreement that very few studies were carried out targeting QTLs for yield (Quarrie et al. 2005), and particularly yield under drought. Normal distribution of yield and yield components among RILs in each of the five experiments, and significant RILs x environment interaction, confirmed the polygenic inheritance of yield and its components, involving several QTLs as stated by Campbell et al. (2003) and Huang et al. (2003). Nevertheless, the high estimates of heritability (Table 13) give support to the validity of the QTLs. This issue was discussed earlier by Rasyad and Van Stanfold (1992).

Table 21: Summary of major QTLs with associated marker loci, interval length, LOD, additive effects and phenotypic variation values in the Tabassi x Taifun F2:7 population. All 146 QTLs are presented in Fig. 33.

Trait	Location	Chr.	Closest Marker	Interval length(cM)	LOD	Add. eff.	R^2 %	Parental allele
Yld	KI	3B	Xgwm533a	6	7,4	-0,7	18,2	Tai.
	AUT	4D	Xgwm4693	6	6,8	1,2	13,4	Tab.
	IRN	4D	Xgwm4693	5	8,7	1,3	20	Tab.
	HUN-Ctr	6D	Xgwm3067	7	6,5	1,5	18,1	Tab.
	AUT	7B	Xgwm333	10	6,2	-1,1	11,3	Tai.
Gps	HUN-Ctr	3B	Xgwm340	10	5,2	23,5	10	Tai.
	HUN-Ctr	3D	Xgwm892	4	7,2	33,1	13,7	Tai.
	IRN	4A	Xgwm832	8	3,6	32,8	10	Tai.
	IRN	5B	Xgwm640	6	3,1	-36,9	12,7	Tab.
	KI	7A	Xgwm890	10	5,1	22,2	10,8	Tab.
Tkw	IRN	3D	Xgwm383	13	6,7	-1,7	10,1	Tai.
	IRN	4D	Xgwm4726	7	6,7	1,8	12,6	Tab.
	KI	5A	Xgwm154	11	5,2	-0,9	11,5	Tai.
	AUT	5B	Xgwm540	5	5,8	-1,4	10,7	Tai.
	HUN-Ctr	5D	Xgwm212	25	3,4	4,5	12,3	Tab.
	IRN	7B	Xgwm983	6	6,1	-1,8	11,8	Tai.
Sln	AUT	1A	Xgwm3094	4	8,9	-0,5	13,2	Tab.
	HUN-Str	2D	Xgwm484	18	7,6	-0,4	10,9	Tab.
	HUN-Ctr	3A	Xgwm4024	9	4,4	-0,4	10,5	Tab.
	IRN	4B	Xgwm4465	3	10,2	0,9	14,9	Tai.
	IRN	4D	Xgwm4726	6	6,3	0,7	10,7	Tai.
Sps	AUT	2D	Xgwm484	13	7,5	-0,5	11,1	Tab.
	AUT	3D	Xgwm892	7	7,4	0,5	11,5	Tai.
	IRN	4A	Xgwm1081	14	5	1,2	13	Tai.
Pht	AUT	1D	Xgwm4695	6	8,1	-3,1	12,4	Tai.
	HUN-Str	2B	Xgwm47a	11	8,4	-5	13,4	Tai.
	AUT	2B	Xgwm47a	7	6,7	-3,4	13,1	Tai.
	HUN-Str	4D	Xgwm3000	6	11,1	6,3	21,8	Tab.
	AUT	4D	Xgwm3000	8	5,7	2,6	10,6	Tab.
	IRN	4D	Xgwm4726	2	13,8	5,4	27,8	Tab.
	IRN	4D	Xgwm4693	5	10,5	5,1	24,1	Tab.
	HUN-Str	4D	Xgwm192b	8	8	5,7	17,8	Tab.
	IRN	4D	Xgwm3000	6	10,3	4,9	21,3	Tab.
	HUN-Str	6D	Xgwm3067	4	7,9	5,3	14,5	Tab.
	AUT	6D	Xgwm3067	15	7,8	3,3	15,3	Tab.
Eet	AUT	2D	Xgwm484	12	9,1	-1,8	18,7	Tab.
	IRN	2D	Xgwm294b	7	5,7	1,3	10	Tai.
	IRN	4D	Xgwm4693	7	4,5	-1,5	11,4	Tab.
	AUT	6D	Xgwm1103a	4	5,7	-1,5	11,3	Tab.

4.5.1.1. Grain yield per 10 spikes (Yld)

Five major and 16 minor QTLs were identified for Yld (Fig. 33 in Results 3.8, Table 21), explaining from 4.3% to 18.2% of the phenotypic variation with a LOD of 3 to 7.4 and an average additive effect of 1.3.

The first major QTL is QYld.ifa-3B with an R^2=18.2% and an allele from Taifun. It is associated with Xgwm533a and was identified in the KI-experiment. Along with this QTL, two minor QYld.ifa-3B were found in the KI- and the Hungary-stress experiments. The three together explain 33.9% of variation for Yld on chromosome 3B. The presence of these QTLs on 3B is in agreement with QTLs for grain weight per spike, which were found in previous studies by Huang et al. (2003, 2004) and Sishen et al. (2007). QYld.ifa-3B in the KI-experiment co-segregated with two minor QTLs for Tkw, identified in KI and Iran, as well as two minor QTLs for Sln found in Austria and Iran. This region seems to be gene-rich, controlling Yld, Tkw, and Sln.

Two major QTLs for Yld were found on chromosome 4D in Iran (R^2=20%) and Austria (R^2=13.4%). QYld.ifa-4D found in Iran is located in close proximity to QYld.ifa-4D found in Austria, both associated with the same marker, Xgwm4693. A minor QYld with exactly the same interval was found in the non-stress experiment in Hungary. QYld.ifa-4D found in Iran has co-segregated with the major QTLs QTkw.ifa-4D, QPht.ifa-4D, and QSln.ifa-4D. This region showed the highest number of major QTLs under drought stress conditions. The presence of QYld.ifa-4D is confirmed by 3 minor QYld.ifa-4A on chromosome 4A, being the homoeologous region. In the study of McCartney et al. (2005) and Kuchel et al. (2007) Yld - QTLs were detected on chromosome 4A in a similar position as in the present study. In comparison to QYld.ifa.4D, co-segregating with Xgwm4693, Kuchel et al. (2007) found a major QTL for Yld on 4D (*QGyld.agt-4D*), which was associated with Xgwm194 and located on the opposite arm of this chromosome. Homoeologous group 4 seems to harbour a number of QTLs affecting yield.

A fourth major QTL for Yld on chromosome 6D, QYld.ifa-6D, was identified in HU-ctr with R^2=18.1% and an allele from Tabassi. The presence of this QTL is confirmed by two minor QTLs found in KI and AUT. Huang et al. (2004), McCartney et al. (2005), and Kuchel et al. (2007) reported QTLs for grain yield on chromosome 6D.

A fifth major QTL for Yld is located on chromosome 7B with R^2=11.3% and an allele from Taifun, identified in AUT. Chromosome 7B was shown by Quarrie et al. (2005 and 2006) to be a main region for yield QTLs under non-drought condition. All QTLs found for Yld in the present study are confirmed by the results of Quarrie et al. (2005), who identified QTLs for yield almost on every chromosome of wheat under drought and non-drought stress conditions.

4.5.1.2. Grain per 10 spikes (Gps)

Similar to Yld, 5 major and 16 minor QTLs were found for Gps, with R^2 values ranging from 4.6% to 12.7%, with a LOD score from 3.1 to 7.2, and with an average additive effect of 6. Major QGps.ifa-3B, associated with Xgwm340, was found in HUN-Ctr and carries an allele from Taifun. The presence of this QTL, of two minor QTLs for Gps, three minor QTLs for Tkw and 4 minor QTLs for Sln indicate that chromosome 3B is an important region for yield and its components. In HUN-Ctr a second major QTL for Gps was found on 3D. This QTL, QGps.ifa-3D, which carries a Taifun-type allele, is associated with Xgwm892, and can be considered as a homoeo-allele of QGps.ifa-3B. The existence of QGps.ifa-3D is confirmed by 3 minor QTLs for Gps, identified in IRN, HUN-Ctr, and HUN-Str. Interestingly, Huang et al. (2004) found a QTL for grain number per spike (*QGne.ipk-3D*) exactly in the same region where two minor QGps.ifa-3Ds were found in IRN and HUN-Str, fairly close to Xgwm161.

The three remaining major QTLs for Gps were found under stress conditions. QGps.ifa-4A, explaining 10% of phenotypic variation, co-segregated with Xgwm160. The presence of this QTL is confirmed by a minor QTL for Gps found in AUT, and by those two found on 4D under stress conditions in IRN and KI experiment. All these QTLs carry Taifun alleles and increase the number of grain per spike. A fourth major QTL for Gps, QGps.ifa-5B, found in IRN, carries a Tabassi allele and explains 12.7% of the phenotypic variation. Two minor QTLs for Gps, identified on 5A, under stress condition in IRN and KI, one from Tabassi and one from Taifun, can be considered as the homoeologous alleles of the major QTL on 5B. Kuchel et al. (2007) also reported a major QTL for grain number on chromosome 5B (*QGno.agt-5B*) under dry condition in Australia. QGps.ifa-7A, the fifth major Gps QTL, was found in KI-experiment, explaining 10.8% of phenotypic variation and carrying an allele from Tabassi. This QTL is the only one identified on chromosome 7A. Quarrie et al. (2006) mentioned that 7A can be considered an important chromosome for yield and yield component QTLs under drought stress.

4.5.1.3. 1000-kernel weight (Tkw)

Out of 18 QTLs for Tkw, with R^2 values ranging from 5.1% to 12.6% and with a LOD from 3.1 to 6.7, six, on chromosomes 3D, 4D, 5A, 5B, 5D and 7B, are considered major ones. The average additive effect of these major QTLs is 2.3. The first major QTL for Tkw, QTkw.ifa-3D, which is co-segregating with Xgwm383, was found in IRN, and carries the allele from Taifun. This and three minor QTLs, which were identified on chromosome 3B under stress conditions, prove the importance of chromosomes 3D and 3B for QTLs associated with yield components, e.g. Tkw. Except for the minor QTkw.ifa-3D, detected in KI experiment, carrying a Tabassi allele, all other Tkw-QTLs on chromosomes 3D and 3B are from Taifun. The location of the major QTkw.ifa-3D, associated with Xgwm383, shows high synteny with the location of the Tkw QTL, *QGwt.crc.3D,* reported by McCartney et al. (2005). Huang et al. (2004) also

found a QTL for Tkw on 3D (*QTgw.ipk-3D*), but associated with Xgwm161.

A second major QTL for Tkw, QTkw.ifa-4D, located on chromosome 4D and carrying an allele from Tabassi, was found in IRN. This QTL showed co-segregation with major QTLs for Yld, Pht, and Eet in IRN. QTkw.ifa-4D explains the highest phenotypic variation for Tkw (R^2=12.6%) with an additive effect of 1.8. The location of this QTL is also in high synteny with *QGwt.crc-4D,* identified by McCartney et al. (2005).

A third major QTL for Tkw is located on chromosome 5A, QTkw.ifa-5A, and was found in KI experiment, carrying an allele from Taifun. Interestingly, the highest number of QTLs for Tkw, 8 out of 18, was found on chromosomes of group 5. Three of these 8 QTLs are major ones, one on each of the group 5 chromosomes: QTkw.ifa-5A, QTkw.ifa-5B and QTkw.ifa-5D. They were identified in KI, AUT and HUN-Ctr, respectively. QTkw.ifa-5D, carrying a Tabassi allele, showed the highest additive effect for Tkw, 4.5. It seems that group 5 chromosomes are of major importance with respect to Tkw, both under drought stress and non-drought conditions. Tkw QTLs found on group 5 chromosomes are in agreement with the results of Börner et al. (2002), Huang et al. (2003), Jun-Ying Su et al. (2006), and Sishen Li et al. (2007).

The last major QTL for Tkw, QTkw.ifa.7B, is co-segregating with Xgwm983 and was found in IRN. It is adjacent to a major QYld.ifa-7B. The same major Tkw QTL on chromosome 7B (*QTgw.ipk-7B.2*), associated with the same marker, *Xgwm983*, was reported by Huang et al. (2003).

4.5.1.4. Spike length (Sln)

Few QTLs were reported for spike length. Börner et al. (2002) found QTLs on 1B, 4A, and 5A, Mohammadi et al. (2005) reported a major QTL for Sln on chromosome 2D. In this study we found, only under stress in Iran, a significant correlation between Sln and Yld, although it was rather low. Maybe the lack of correlation between Yld and Sln is the reason, why this trait was not studied more intensively compared to other yield components. However, in the present work the number of QTLs for Sln was the second highest after Pht. Out of 29 QTLs identified for Sln, with R^2 values ranging from 3.5% to 14.9% and LOD values between 3.0 and 10.2, five QTLs were considered to be major ones, located on chromosomes 1A, 2D, 3A, 4B, and 4D. QSln.ifa-1A, identified in AUT and linked to Xgwm3094, carries an allele from Tabassi with R^2=13.2. In the same interval, 2 minor QTLs were found on 1A under stress condition in IRN and HUN, associated with the same marker, Xgwm3094, and fairly close to the locus of spike pubescence. These QTLs can be introduced as new ones for Sln on 1A. The three QTLs confirm each other, and the one reported by Börner et al. (2002) on 1B.

Chromosome 2D also presented a major QTL for Sln, QSln.ifa-2D, under stress in HUN, carrying a Tabassi allele, and co-segregating with Xgwm484. QSln.ifa-2D coincides with two major QTLs, one for Sps and another for Eet, which were found in AUT. The presence of QSln.ifa-2D is confirmed by the occurrence of a minor QTL for Sln under non-stress condition

in AUT co-segregating with the same marker, Xgwm484. In addition two minor Sln QTLs were found in AUT and IRN on the same chromosome arm, but more proximal to the centromere.

A third major QTL for Sln is located on 3A, QSln.ifa-3A, and was found in HUN-Ctr, carrying a Tabassi allele and co-segregating with Xgwm4024. This QTL is also co-segregating with a minor QTL for Pht, also found in HUN-Ctr. Chromosome 3D did not contain any QTL for Sln. But the presence of the major QSln.ifa-3A and further 3 minor QTLs for Sln on 3A, as well as 4 minor QTLs on chromosome 3B, indicate the importance of this homoeologous group for spike length, both under drought and non-drought conditions. This can also be true for homoeologous group 4, which contributed 9 QTLs for Sln including two major ones: QSln.ifa-4B and QSln.ifa-4D, both identified under drought in IRN, co-segregating with Xgwm4465 and Xgwm4726, respectively, as well as 7 minor QTLs with an average R^2=6.0, both in drought and in non-drought environments. Four of these minor QTLs are located nearly at the same interval to, and co-segregated with, the same marker, Xgwm4949. Three QTLs also were located in the same interval to and co-segregating with Xgwm3039. The other minor QTLs are localized on 5B, 6A, 6B and 7B.

4.5.1.5. Spikelet per spike (Sps)

Three major and 12 minor QTLs were identified for Sps with R^2 values ranging from 3.3% to 13.0% and LOD score between 3.0 and 7.5. For this trait also only few QTLs have been reported, more attention was paid to Gps than to Sps. Similarly to Sishen et al. (2007), who reported a major QTL for Sps on 2A, we localized two minor QTLs on 2A.

The first major QTL for Sps, QSps.ifa-2D, was identified on chromosome 2D in AUT, carrying an allele from Tabassi. It coincides with two minor QSps.ifa-2Ds at the same locus, found in HUN-Ctr and HUN-Str, and co-segregating with the same marker, Xgwm484. Moreover, it coincides with two major QSln.ifa-2Ds and QEet.ifa-2D found in HUN-Str and AUT, respectively.

A second major QTL for Sps was found on chromosome 3D, QSps.ifa-3D, which is co-segregating with Xgwm892 and was found in AUT with a Tabassi allele. Interestingly, a minor QTL for Sps was found in HUN-Ctr at the same locus and interval. Coincidence of the major QSps.ifa-3D, the major QGps.ifa-3D and the minor QTkw.ifa-3D indicate the importance of this region for QTLs influencing yield components.

A third major QSps.ifa-4A, associated with Xgwm1081 and explaining 13.0 % of phenotypic variation, was found in IRN. It carries an allele of Taifun. In the same linkage group, in two different loci, two minor QTLs for Sps were found on 4A. All three QSps on chromosome 4A occurred under drought stress in IRN. Locating major QSps.ifa-4A adjacent to the three minor QTLs for Sln found in AUT, IRN, and HUN-Ctr indicates the importance of this region for Sln and Sps QTLs. These findings are in agreement with Börner et al. (2002), who reported 9 QTLs for grain number per spike, grain weight, and spike length on chromosome 4A, and

Sishen et al. (2007), who found a QTL for Sps on 4A.

4.5.2. Plant height (Pht) QTLs

Major genes, controlling reduced plant height, are positioned on chromosomes 2D, 4B, and 4D. Alleles located on 4B are known as *Rht-B1a*, *Rht-B1b* and those located on 4D, as *Rht-D1*a and *Rht-D1b*. Alleles *Rht-B1b* and *Rht-D1b* reduce sensitivity to gibberellic acid (GA), a substance necessary for stem elongation (Butler et al. 2005). The consequence of this reduced sensitivity will be the reduction of stem elongation and finally plant height. These alleles usually occur in dwarf or semi-dwarf wheats. In contrast, the presence of alleles *Rht-B1a* and *Rht-D1a* in tall wheats do not result in reduced height. Besides these major genes, a number of QTLs influencing plant height, involving most of the 21 chromosomes of wheat, was identified (Snape et al. 1977), indicating the quantitative nature of this trait. This idea is supported by the present study, where the highest number of QTLs (31) was found for this trait. Moreover, the highest LOD value (13,8), R^2=27.8%, and heritability estimate (98.0%) were calculated for Pht. Out of 11 major QTLs, carrying 8 alleles from Tabassi and 3 from Taifun, 7 QTLs were identified under stress and 4 under non-stress conditions.

The first major QTL for plant height found in AUT, QPht.ifa-1D, co-segregating with Xgwm4122 and with a minor QTL for Yld. Three minor QTLs, which were identified for Pht on homoeologous chromosomes 1A and 1B, confirm the presence of QPht.ifa-1D. McCartney et al. (2005) reported a major QTL for test weight on 1D, which coincides with QPh.ifa-1D and a minor QYld.ifa-1D found in this study. Also by Huang et al. (2004) a QTL for Pht was found on 1D.

The next two major QTLs for Pht were localized on chromosome 2B, QPht.ifa-2B, under HUN-Str, and non-stress conditions in AUT. They were located nearly in the same intervals close to Xgwm47a. Both of these QTLs carry alleles from Taifun reducing plant height by 3.4 to 5 cm in AUT and HUN-Str, respectively. Adjacent to these QTLs towards the telomere, two Pht QTLs were found in IRN. All these findings indicate that this region on 2B harbors QTLs important for Pht. Moreover, two minor QTLs for Pht on 2D, which were found in AUT and IRN, seem to be homoeologous alleles to those on 2B.

Out of the 6 major QTLs on 4D, Qpht.ifa-4D, five were found in IRN and HUN-Str carrying Tabassi alleles. The positive effects of these QTLs on plant height, in average 5.5 cm under drought stress, suggest that these QTLs are positioned in the location of *Rht-D1a*. Börner et al. (2002), Huang et al. (2003), McCartney et al. (2005) and Mohammadi et al. (2005) reported QTLs for Pht on chromosome 4D. Chromosome 4B, although expected (Butler et al. 2005), did not contain any QTLs for Pht. However, 3 minor QTLs were identified on chromosome 4A, which carried Taifun alleles, in contrast to QPht.ifa-4D carrying alleles from Tabassi. So far chromosome 4A has not been reported to contain Pht genes. The three QTLs reported here, as well as those described by Börner et al. (2002) and Huang et al. (2004),

makes, however, the existence of such a gene on this chromosome possible.

Chromosome 6D also contributed two major QTLs for plant height, QPht.ifa-6D, one localized in AUT and one in HUN-Str. Two minor QTLs, on 6D and on 6A each, confirm the presence of major QPht.ifa-6D. Huang et al. (2004) found a QTL for Pht on 6D nearly at the same position as the major QPht.ifa-6D found in AUT. Also Spielmeyer et al. (2007) reported a QTL for Pht on 6A as was found in this study, QPht.ifa-6A, under stress and non-stress conditions.

4.5.3. Ear emergence time (Eet) QTLs

Three major genetic factors are governing heading time in wheat, i.e. vernalization responsive genes, photoperiod responsive genes and narrow-sense earliness or earliness per se (Kato et al. 1998). The first two are environment dependent, while the latter is environment independent. Genes controlling vernalization (*Vrn-A1, Vrn-B1* and *Vrn-D1*) are located on chromosomes 5A, 5B and 5D, those controlling photoperiod sensitivity (*Ppd-A1, Ppd-B1* and *Ppd-D1*) on chromosomes 2A, 2B, and 2D.

Earliness per se is a polygenic trait, and several genes are involved in the control of heading time (Shindo et al. 2003). In the present study, out of 12 QTLs identified for Eet, with R^2 values ranging from 4.0% to 9.1% and LOD scores between 3.0 and 9.1, four QTLs were considered as major ones, localized on chromosomes 2D, 4D and 6D.

The first two major QTLs, QEet.ifa-2D, identified in AUT and IRN, were localized in different intervals. Major QEet.ifa-2D, carrying Tabassi allele, found in AUT, seems to be positioned in the expected location of *Ppd-D1*. It coincides with a minor QTL for Eet found in IRN and co-segregating with Xgwm484. The location of this QTL is in complete agreement with the location of *QEet.ipk-2D* reported by Huang et al. (2003) in association with Xgwm484. The presence of this major QTL coincides with seven major and minor QTLs corresponding to Pht, Sln, Sps in this area and indicates a major region of QTLs for a number of different traits.

A major QEet.ifa-2D, found in IRN, carrying an allele from Taifun, is in complete concurrence with a minor QTL for Eet, which was identified in HUN-Ctr. Both co-segregate with Xgwm294b and partially coincide with a minor QTL for Pht found in IRN. Narasimhamoorthy et al. (2006) found a major QTL for Eet (*QHd.ksu-2D*) associated with Xgwm261, in an interval very close to QEet.ifa-2D.

A third major QTL for Eet, QEet.ifa-4D, was positioned on chromosome 4D, found in IRN. It is co-segregating with Xgwm4963 and carries an allele from Tabassi. QEet.ifa-4D occurs together with a minor QEet.ifa-4D in AUT and adjacent to another minor QTL in IRN. The coincidence of these QTLs for Eet with those related to major and minor QTLs for Sln, Pht, Tkw, Gps and Yld indicate a gene-rich region. QEet.ifa-4D coincides with *QMat.crc-4D* found by McCartney et al. (2005).

Chromosome 6D also contributed a major QTL for Eet, QEet.ifa-6D, which was identified in AUT. A minor QTL on 6A, found in HUN-Str, must be a homoeologous allele of the major QEet.ifa-6D, considering that both of these QTLs carry alleles from Tabassi. Huang et al. (2003) reported a QTL for Eet on 6A (*QEet.ipk-6A*), in the same chromosomal position near the centromere.

In agreement with Huang et al. (2004), chromosome 3A contributed in the present study 3 minor QTLs for Eet, in the expected location of genes affecting earliness per se (Miura and Worland 1994) under both stress and non-stress conditions.

4.6. Chromosome 4D is a QTL-rich region for drought tolerance

Chromosome 4D can be introduced as a very gene-rich region, containing QTLs detected mainly under drought stress. It contributes the highest number of QTLs (19) within the genome, with an average of 1.8 QTL per 10cM. Indeed, out of 19 QTLs, 14 were identified under drought stress i.e. 3 QTLs for Yld and 2 for Gps in IRN and KI, one for Tkw and Sln each, both in IRN, 5 QTLs for Pht in IRN and HUN-Str, and 2 QTLs for Eet in IRN. As shown in Fig. 44, the positions of these QTLs found in the present study are in complete synteny with the same QTLs identified by McCartney et al. 2005. The distance between the common marker, *Xgwm194*, and the common QTLs for Yld, Tkw, Pht and Eet is nearly the same in the two maps.

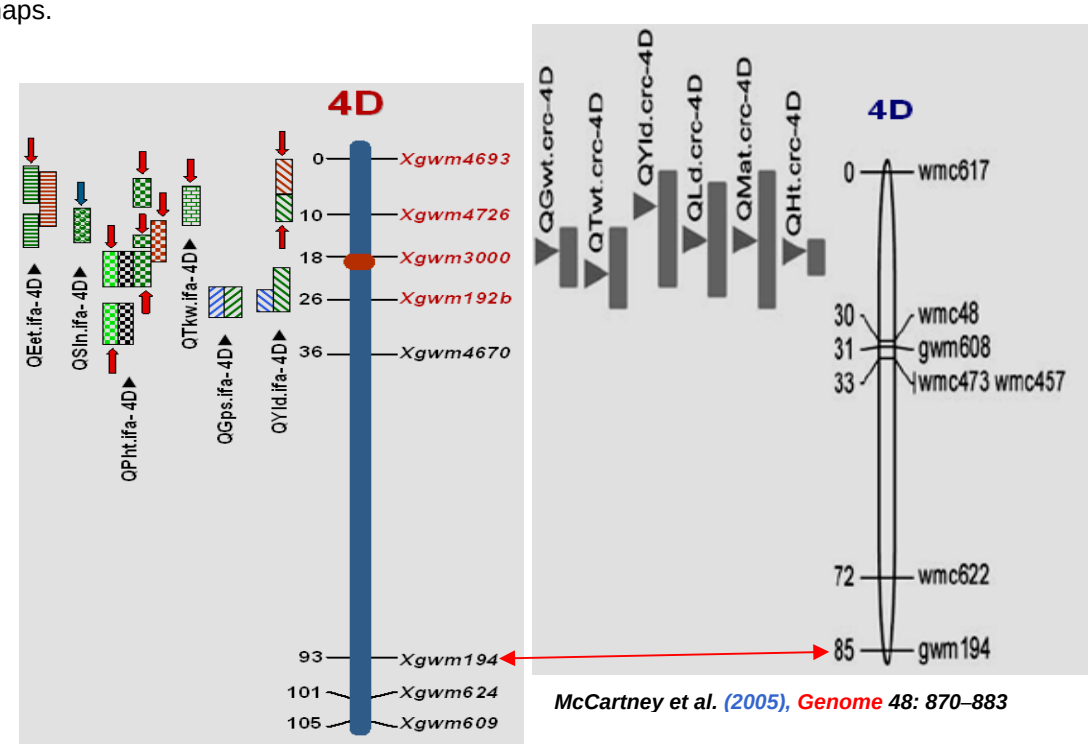


Fig. 44: Synteny of the locations of QTLs for Yld, Tkw, Pht and Eet on chromosome 4D in the present study (left) and the study of McCartney et al. 2005 (right). Marker *Xgwm194* is indicated by arrow as a benchmark between the two maps.

4.7 Conclusions

Tabassi, as drought tolerant landrace, appears superior to Taifun, a non-drought tolerant cultivar, under drought stress in total yield and 1000-kernel weight. These advantages can mainly be due to the clear differences between the two genotypes in (1) root mass and development, as a major constitutive trait, (2) early emergence, as a phenological event, and (3) the difference in plant height. All these characteristics allow Tabassi to have a significant higher and more stable yield under drought stress compared to Taifun. From this point of view, Tabassi and the RIL- and back-cross populations developed from the cross with Taifun are valuable genetic sources for further studies on drought stress tolerance. Moreover, as mentioned in section 2.1.2. regarding the origin of Tabassi and its utilization in salinity and heat tolerance studies, this genotype can also be of great value to investigate the genetics of these traits. Finally, the material may enter practical breeding programs targeting tolerance of drought, salinity and heat stresses.

The KI-experiment has proven very successful to provide conditions for studying the effect of post-anthesis drought, simulating closely to the natural condition of drought in Iran. Moreover, it was very efficient in QTL detection with respect to number and their synteny to those found under natural stress condition in Iran.

Chromosome 4D, containing the highest QTL number per cM unit (1,8 per 10cM), appears to be a QTL-rich region for yield, yield components, and traits related to yield, i.e. plant height and ear emergence time, under drought stress. A synteny of QTLs found on 4D in the present study and those identified by McCartney et al (2005), as well as the confirmation of each single QTL on 4D by other studies, proves this claim.

In addition to the importance of chromosome 3A for QTLs for agronomic traits, mentioned in section 1.7.1, we emphasize the importance of 3B, along with 3A, as the main region for spike length, and together with 3D, as the main region for grain per spike.

The importance of 7A showing QTLs for yield under stress and 7B under non-stress condition is strongly emphasized, and in complete agreement with results reported by Quarrie et al. (2006).

4.8. Outlooks

- Enhancing the F2:7 SSR linkage map, using AFLP markers,
- Construction of a linkage map of the BC2F3 population derived from the same parents Tabassi and Taifun,
- Construction of a joint map of the F2:7 and BC2F3,
- Construction a BC2F3 QTL map,
- Evaluation of the F2:8 and BC2F4 populations for agronomic traits for validation of QTLs,
- QTL analysis for salinity and heat tolerance utilizing the same populations.

* * *

Chapter 5

References

References

- Amersham Biosciences (2004) Handbook, Fluorescence Imaging, Principles and methods.

- Asins, M.J. (2002) Present and future of quantitative trait locus analysis in plant breeding, Plant Breeding 121: 281-291

- Blum, A. (1988) plant breeding for stress environments. CRC press, Boca Raton, 38-78.

- Blum, A. (1989) Osmotic adjustment and growth in barely genotypes under drought stress. Crop Sci. 29:230-233.

- Blum, A. (1993) Selection for sustained production in water deficit environments. Inter. Crop Sci. Soc. of America, Chapter 44, pp.343-347.

- Blum, A., Mayer J., and G., Golan (1993b) Chemical desiccation of wheat plants as a simulator of post-anthesis stress. Field Crops Res. 6:149-155.

- Blum, A. (1996) Yield potential and drought resistance: are they mutually exclusive? Breaking the barriers, pp. 99-10.

- Blum A. (1998) Improving wheat grain filling under stress by stem reserve mobilization. Euphytica 100:77-83.

- Blum, A. (2000) Is drought a complex trait? *Proceedings of an International Workshop:* Field Screening for Drought Tolerance, ICRISAT, Patancheru, India.

- Borevitz, J.O. and J. Chory (2004) Genomic tools for QTL analysis and gene discovery. Current Opinion in Plant Biology 7:132-136.

- Borrell, A.K., Lincoln, L.D., and M.G., Dalling (1993) The influence of the *Rh1* and *Rh2* alleles on the deposition and use of stem reserve in wheat. Ann Bot 71:317-326.

- Börner A., Schumann E., Furste A. et al. (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum L.*). Theor Appl Genet 105:921-936.

- Burr, B., and F.A. Burr (1991) Recombinant inbreds for molecular mapping in maize: theoretical and practical considerations. Trends in Genetics 7:55-60

- Butler, J.D., Byrne, P.F., Mohammadi, V., Chapman, P.L., and S.D. Haley (2005) Agronomic performance of Rht alleles in a spring wheat population across a range of moisture levels. Crop Science 45:939-947.

- Cadalen, T., Sourdlille, P., Charmet, G., et al. (1998) Molecular markers linked to genes affecting plant height in wheat using a doubled-haploid population. Theor Appl Genet 96:933-940.

- Campbell, B.T., Baenziger, P.S., Gill, K.S., et al. (2003) Identification of QTLs and environmental interactions associated with agronomic traits on chromosome 3A of wheat. Crop Sci. 43:1493-1505.

- Cattivelli, et al. (2002) Chromosome regions and stress related sequences involved in resistance to abiotic stress in *Triticeae*, Plant Mol Boil 48:649-665.

- Ceccarelli, S., Acevedo, E., and S., Grando (1991) Breeding for yield stability in unpredictable environments-single traits, interaction between traits and architecture of genotypes. Euphytica 56:169-185.

- Chaves, M.M., Maroco, J.P., and J.S. Pereira (2003) Understanding plant responses to drought: from genes to the whole plant. Functional Plant Biology 30:239-264.

- Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B. and F.C.K. Pang (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142:169-96.

- Dilbirligi, M. et al. (2004) Identification of wheat chromosomal regions containing expressed resistance genes. Genetics 166:461-481.

- Dilbirligi, M., Erayman, M., Campbell, B.T., et al. (2006) High density mapping and comparative analysis of agronomically important traits on wheat chromosome 3A. Genomics 88:47-87.

- Doerge, R.W., and G.A., Churchill (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285-294.
- Ehdaie, B., and J.G., Waines (1996) Genetic variation for contribution of pre-anthesis assimilates to grain yield in spring wheat. Jour Genet Breed 50:47-56.
- Ehdaie, A., Alloush, B.G., Madore, M.A., and J.G., Waines (2006) Genotypic variation for stem reserves and mobilization in wheat: I. Pos-tanthesis changes in internode dry matter. Crop Sci. 46:735-746.
- Erayman, M., Sandhu, D., Sidhu, D., et al. (2004) Demarcating the gene-rich regions of the wheat genome, Nucleic Acids Res. 32:3546-3565.
- Falconer, D.S., and T.F.C. Mackay (1996) Introduction to quantitative genetics, 4th Edd. Addison-Wesley Longman LTD, London.
- Fazel-Najafabadi, M., Ghanadha, M.R., Zali, A.A. and B. Yazdi-Samadi (2000) Genetic analysis of seedling characters in bread wheat. 14th International crop science congress, Australia.
- Fischer, R.A., and R. Maurer (1978) Drought resistance in spring wheat cultivars: I. Grain yield responses. Aus Jour Agric Res 29: 897-912.
- Flintham, J.E., Borner, A., Worland, A.J., and M.D. Gale (1997) Optimizing wheat grain yield, : Effects of Rht gibberellin-insensetive dwarfing genes. Jour Agric Sci 128:11-25
- Gabriela, M.P., and C.H., Foyer (2002) Common components, networks and pathways of cross-tolerance to stress. The central role of “Redox” and Abscisic Acid-mediated controls. Plant physiology, 122:460-468.
- Gent, M.P.N. (1994) Photosynthate reserves during grain filling in winter wheat. Agron. J. 86:159-167.
- Golabadi, M., Arzani, A., and S.A.M. Mirmohammadi- Maibodi (2006) Assessment of drought tolerance in segregating population in durum wheat. African Journal of Agricultural Research 55:162-171.
- Griffiths A., Gelbart, W.M., Miller J.H., and R.C., Lewontin (2003) Modern genetic analysis. W.H. Freeman and Company.

- Haley, C. S. and S. A. Knott 1992 A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315-324.

- Hartl, D., and E., Jones (2001) Genetic analysis of genes and genomes. Jones and Bartlett publisher.

- Huang, X.Q., Göster, H., Ganal, M.W. and M.S. Röder (2003) Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat(*Triticum aestivum L*.) Theor Appl Genet 106:1379-1389.

- Huang, X.Q., Kempf, H., Ganal, M.W. and M.S. Röder (2004) Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum L*.), Theor Appl Genet 109:933-943.

- Islam-Faridi, M.N., Worlanf, A.J. and C.N., Law, (1996) Inhibition of ear emergence time and sensitivity to day length determined by group 6 chromosome of wheat, Heredity 77:572-580.

- Jansen, R.C. (1993) Interval mapping of multiple quantitative trait loci. Genetics 135:205-211

- Johnson, R.C., Witters, R.E., and A.J., Ciha (1981) Daily patterns of apparent photosynthesis and evapotranspiration in a developing winter wheat crop. Agron. J., 73:414-418.

- Jun-Ying Su, Yi-Ping Tong, Quan-You Liu, et al. (2006) Mapping quantitative trait loci for post-anthesis dry matter accumulation in wheat. Journal of Integrative plant Biology, 48:938-944.

- Kao, C.H., Zeng, Z.B., and R.D. Teasdale (1999) Multiple interval mapping for quantitative trait loci. Genetics 152:1203-1216.

- Kato, K., Miura H., Akyama, M. et al. (1998) RFLP mapping of three major genes, Vrn1, Q and B1, on the long arm of chromosome 5A of wheat. Euphytica 101:91-95.

- Kirigwi, F.M., Van Ginkel, M., Brown-Guedira G., et al. (2007) Markers associated with a QTL for grain yield in wheat under drought. Mol Breeding 20:401-413

- Khoda-Bandeh, N. (1992) Cereals, Tehran university publications, pp: 506.

- Khurana, P. (2001) Wheat biotechnology. Electronic Journal of Biotechnology, 2:74-102

- Kosambi, D.D. (1944) The estimation of map distances from recombination values. Ann. Eugen. 12:172-175.

- Kuchel, H., Williams, J.K., Langridge, P., Eagles, H.A. and S.P. Jefferies (2007) Genetic dissection of grain yield in bread wheat. I. QTL analysis, Theor Appl Genet 115:1029-1041.

- Kumar, N., Pawan, L., Gupta, K., et al. (2006) QTL analysis for grain weight in common wheat. Euphytica 151:135-144.

- Lander, E.S., Green, P., Abrahamson, J. et al. (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

- Lander, E. S. and D. Botstein (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

- Lander, E.S. and D. Kruglyak (1995) Genetic dissection of complex traits; Guidelines for interpreting and reporting linkage results. Nat Genet 11:241-247.

- Law, C.N., Suarez, E., Miller, E., and A.J. Worland (1998) The influence of the group 1 chromosome of wheat on ear emergence times and their involvement with Vernalization and day length. Heredity 80:83-91.

- Liu ZH, Anderson J.A., Friesen T.L. et al. (2005) A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. Theor Appl Genet 111:782-794

- Manly, K.F., Cudmore R.J., and J.M. Meer (2001) MapManager QTX, cross-platform software for genetic mapping. Mammalian Genome 12:930-932.

- Mauricio, R. (2001) Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology, Nature Genetics 2:370-381.

- McCartney C.A., Somers D.J., Humphreys, D.G. et al. (2005) Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross RL4452 X 'AC Domain. Genome 48:870-883.

- Miura, H., and A.J., Worland (1994) Genetic control of vernalization, day-length response, and earliness per se by homoeologous group 3 chromosomes in wheat. Plant Breeding 113:160-169.

- Mohammadi, V., Ghanadha, M.R., Zali, A.A., Yazdi-Samadi, B., and P. Byrne (2005) Mapping QTLs for morphological traits in wheat. Iranian journal of agricultural sciences 36:145-157.
- Morgan, J.M., and M.K. Tan (1996) Chromosomal location of a wheat osmoregulation gene using RFLP analysis. Aust. Jour. Plant Physiol. 23:803-806.
- Narasimhamoorthy, B., Gill, B.S., Fritz, A.K., et al. (2006) Advanced backcross QTL analysis of a hard winter wheat x synthetic wheat population. Theor Appl Genet. 112:787-796.
- Naserian, B., Asadi, A., Rahimi, M., and R., Ardakani (2007) Evaluation of wheat cultivars and mutants for morphological and yield traits and comparing of yield components under irrigated and rain fed conditions. Asian journal of Plant Sciences 6: 214-224.
- Nei, M., and W.H., Li, (1979) Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc Natl Acad Sci 76:5269-5273.
- Nelson J.C. (1997) QGENE: software for marker-based genomic analysis and breeding. Molecular Breeding 3:239-245.
- Nicolas, M.E., and N.C. Turner (1993) Use of chemical desiccants and senescing agents to select wheat lines maintaining stable grain size during post anthesis drought. File Crops res. 31:155-171.
- Nizam Uddin, M., and D.R., Marschall (1989) Effects of dwarfing genes on yield and yield components under irrigated and rainfed conditions in wheat. Euphytica 42:127-134.
- Paterson A.H., Lander, E., Hewitt, J.D., et al. (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:7231-726.
- Paterson, A.H. (1996a) Making genetic maps, In: A.H. Paterson (Ed.), Genome mapping in plants. R.G. Landes Company, pp. 23-39.
- Piepho, H.P. (2001) A quick method for computing approximate thresholds for quantitative trait loci detection. Genetics 157**:**425-43.
- Pirseyedi S.A., Mardi, M., Nagavi, M.R. et al. (2006) Evaluation of genetic diversity and identification of informative markers of morphological characters in Sardari derivative wheat lines. Pakistani Journal of Biological Sciences, 13:2411-2418.

- Poustini, K., and M.A., Aboutalebian (2001) Phosphorus uptake and distribution responses of two wheat cultivars to salinity stress. Iranian Journal of Agricultural Sciences 3:599-606.

- Quarrie, S.A., Guilli, M., Calestani, C., Steed, A., and N., Marmiroli (1994) Location of a gene regulation drought-induced abscisic acid production on the long arm of chromosome 5A of wheat. Theor Appl Genet. 89:794-800.

- Quarrie S.A, Steed A., Calestani C. et al. (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.

- Quarrie, S.A., Quarrie, S.P., Radosevic, R., et al. (2006). Dissecting a wheat QTL for yield present in a range of environments: From the QTL to candidate genes. Journal of Experimental Botany 11:2627-2637.

- Rasyad, A., and D.A., Van Stanfold (1992) Genetic and maternal variances and covariances of kernel growth traits in winter wheat. Crop Sci. 32:1139-1143.

- Rawson, H.M., Hindmarsh, J.H., Fischer, R.A., and Y.M., Stockman (1983) Changes in leaf photosynthesis with plant ontogeny and relationships with yield per ear in wheat cultivars and 120 progeny. Aust Jour Plant Physiol. 10:503-514.

- Red Cross information bulletin (2001) Iran drought.

- Reynolds, M.P., Acevedo, E., Sayre, K.D., and R.A. Fischer (1994) Yield potential in modern wheat varieties: Its association with a less competitive ideotype. Filed Crop Res 37:149-160.

- Reynolds M.P. Mujeeb-Kazi A. and M. Sawkins (2005) Prospects of utilizing plant adaptive mechanisms to improve wheat and other crops in drought and salinity prone environments, Annal. of Appl. Biol. 146:239-259.

- Richard, R.A. (1992) The effect of dwarfing genes in spring wheat in dry environment. I. agronomic characteristics. Aust Jour Agric Res 43:517-527.

- Richards, R.A. (1996) Defining selection criteria to improve yield under drought. Plant Growth Regulation 20:157-166

- Risch, N. (1992) Genetic linkage: Interpreting LOD scores. Science 255:803-804

- Röder, M.S., Korzun, V., Wendehake, K., et al. (1998) A microsatellite map of wheat. Genetics 149:2007-2023.
- Schpiler, L., and A., Blum (1991) Differential relation of wheat cultivar to hot environments. Euphytica 35:483-492.
- Shah, M.M., Gill, K.S., Baenziger, P.S. et al. (1999) Molecular mapping of loci for agronomic traits on chromosome 3A of bread wheat. Crop Sci. 39:1728-1732
- Shindo, S., Tsujimoto, H. and T., Sasakuma (2003) Segregating analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines. Heredity 90:56-63
- Sishen, Li., Jizeng, Jia, Xyanyun, Wei, L. et al. (2007) An interval genetic map and QTL analysis for height in wheat using a doubled-haploid population. Theor Appl Genet 96:933-940.
- Slafer, G.A. (2003) Genetic basis of yield as viewed from a crop physiologist's perspective. Annals of Applied Biology 142:117-128.
- Sourdlille, P., Snape, J.W., Cadalen, T., et al. (2000) Detection of QTLs for heading time and photoperiod response in wheat using doubled haploid population, Genome 43:487-494.
- Spielmeyer, W., Hyles, J., Joaquim, P., et al. (2007) A QTL on chromosome 6A in bread wheat is associated with longer coleoptiles, greater vigour and final plant height. Theor Appl Genet 115:59-66
- Stam, P. (1993) Construction of integrated genetic linkage maps by means of a computer package: JOINMAP. Plant J. 5:739-744.
- Suenaga, K. (1994) Doubled haploid system using the intergeneric crosses between wheat and maize. Bull. Natl. Inst. Agrobiol. Res. 9:83-139.
- Sullivan, C.Y., and W.M., Aross (1979) Stress physiology in crop plants, Wiley, New York, 263-281
- Tanksley, S.D. (1993) Mapping poly genes, Annual Review of genetics 27:205-233.
- Tanksely, S.D., and J.C. Nelson (1996) Advanced backcross QTL analysis: a method of simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191-203.

- Tuberosa, R. and S. Salvi (2006) Genomics-based approaches to improve drought tolerance of crops, Trends in Plant Science 8:405-412.
- Voorrips, R.E. (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. Heredity 93:77-78.
- Wang, S., Basten, C.J., and Z.B., Zeng (2007) Windows QTL Carthographer, new version, Statistical Genetics, North Carolina State University.
- Zadoks, J.C., Chang, T.T. and C.F.; Konzak (1974) A Decimal code for the growth stages of cereals. Weed Research 14:415-421.
- Zeng Z.B. (1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc. Nat. Acad. Sci. US, 90:10972-10976.
- Zeng, Z.B. (1994) Precision mapping of quantitative trait loci. Genetics 136:1457-1468.
- Zeng, Z.B., Kao, C.H., and C.J., Basten (1999) Estimating the genetic architecture of quantitative traits. Genet Res 74:279-289.
- Zheng, Y.W., Weng, L.Y., Polle, E., and C. F., Konzak (2001) Culture of freshly isolated wheat (Triticum aestivum L.) microspores treated with inducer chemicals. Plant Cell Report 20:685-690.

* * *