ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCE

PhD DISSERTATION

Ahmad ALSALEH

GENOME WIDE ASSOCIATION MAPPING (GWAS) FOR IMPORTANT AGRONOMIC AND QUALITY TRAITS IN DURUM WHEAT (*Triticum durum* L.)

DEPARTMENT OF BIOTECHNOLOGY

ADANA, 2016

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We certify that the thesis titled above was reviewed and approved for the award of degree of the Doctor of Philosophy (PhD) by the board of jury on 29/06/2016.

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DEDICATED

TO

MY PARENTS

(May their Soul Rest In Eternal Peace)

ABSTRACT

PhD DISSERTATION

GENOME WIDE ASSOCIATION MAPPING (GWAS) FOR IMPORTANT AGRONOMIC AND QUALITY TRAITS IN DURUM WHEAT (*Triticum Durum* L.)

Ahmad ALSALEH

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Agro-morphological, agronomical and quality characteristics are the important traits for durum wheat. Understanding the genetic control of those traits can help breeders to develop varieties with improved characteristics. Fourteen of the above-mentioned characteristics were investigated in 130 durum wheat genotypes, which were divided into four groups, showing a wide range and significant variation. Variance analysis showed significant differences for most of the studied traits. Significant correlations were also observed between those traits. Analysis of molecular variance (AMOVA) revealed that there was variation within and among the groups of the genotypes studied. The weighted Neighbor-joining tree confirmed the groups identified in the PCoA, showing high and wide diversity in the durum genotypes. Structure analysis revealed that the studied genotypes were divided into five groups with respect to the number of tree clusters. The mixed linear model used for accurate marker trait associations revealed that 92 of the 144 MTAs (marker-trait associations) were major MTAs, of which two were significantly associated with plant height (PH) and (vitreous kernel count) VKC. Pleiotropic effects were found in the MTAs. Taken together with the published genetic results, the MTAs determined in this study could be the targets of marker-assisted selection to improve many traits in durum wheat.

Key word: GWAS, agronomy, quality, durum and genetic markers.

ÖZ

DOKTORA TEZİ

MAKARNALIK BUĞDAYDA ÖNEMLI TARIMSAL VE KALITE ÖZELLIKLERI İÇIN GENOM BOYU İLIŞKI HARITALAMASI

Ahmad ALSALEH

ÇUKUROVA ÜNİVERSİTESİ
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: Yrd. Doç. Dr. Faheem SHEHZAD BALOCH

Tarımsal morfolojik, tarımsal ve kalite özellikleri makarnalık buğdayda önemli özelliklerdir. Bu karakterlerin genetik kontrolünün anlaşılması ıslahçılara ıslah edilmiş karakterlere sahip çeşitlerin ıslah edilmesinde yardım edebilir. Dört gruptan oluşan 130 makarnalık buğday genotipi 14 agro-morfolojik, tarımsal ve kalite karakteri açısından incelenmiş ve geniş ve önemli varyasyon saptanmıştır. Varyans analiz sonuçları incelenen özelliklerin çoğunluğu açısından genotipler arasında önemli farklılık olduğunu göstermiştir. İncelenen özellikler arasında önemli ilişkiler olduğu saptanmıştır. Moleküler Varyans analizi sonuçları gruplar arasında ve gruplar içerisinde varyasyon olduğunu göstermiştir. Soyağacı sonuçları temel koordinat analizinde tanımlanan grupları doğrulamıştır. Temel koordinat analizi sonuçları incelenen makarnalık buğday genotiplerinin yüksek ve geniş bir varyasyon gösterdiğini ortaya koymuştur. Strüktür analizi sonuçları incelenen genotiplerin ağaç kümesi sayısı açısından beş gruba ayrıldığını göstermiştir. Doğru markör-özellik ilişkilerinin saptanması için kullanılan karışık doğrusal model 144 markör-özellik ilişkisinin 92'sinin major markör-özellik ilişkisi olduğunu, bunlardan ikisinin bitki boyu ve camsı dane oranı ile çok yakından ilişkili olduğunu göstermiştir. Markör-özellik ilişkilerinde pleiotropik etkiler saptanmıştır. Halen yayınlanmış olan genetik sonuçlarla birlikte değerlendirildiğinde, bu çalışmada saptanan markör-özellik ilişkileri makarnalık buğdayda bir çok özelliğin ıslahında marköre dayalı seleksiyonun hedefleri olabilir.

Anahtar Kelimeler: Genom boyu ilişki haritalaması, tarımsal özellik, kalite, makarnalık buğday, genetik markör

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I forward my thanks to all of thesis committee member for their valuable comments on my thesis. Special thanks go to Prof. Dr. R. HATIPOĞLU and Asst. Prof. F.S. Baloch for their consistent guidance and valuable comments at various occasions. I am grateful to Prof. Dr. Bahattin Tanyolac and Dr. Seda Namli from Ege University for sharing their experience in Tassel software.

Let me stress that this work is not an individual effort, would not have been completed without the joint efforts of a large group who all contributed, in one way or the other, to the completion of this remarkable task. Sincere appreciations extended Dr. Esra Cakir for laboratory activities, Ahmad Can Divitlioglu, Muhsin Avci, Ibrahim Karahan for assistance in phenotypic data evaluation, Burak Ozgoren and Zeyni Dagtekin for sample preparation. I shall be missing something if I do not extend my appreciation to my fellows Dr. Aysun Gedik, Muazzez Derya, Ayse Nuran Koc Cil, Berivan Gungor, Enver E. Endeden, Ali Alpaslan Ezici, Mustafa Topu, Hasan Maral and Rifat Abali for their support, lot of smiles and enjoyable moments.

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Last but not least; I am highly thankful to my wife, sons and my family for their patients to finalize this project where I was busy and away from them for some time.

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LIST OF ABBREVIATONS:

AFLP : Amplified fragment length polymorphism
APS : Ammonium per Sulphate
BARC : Beltsville Agricultural Research Center
BP : Base Pair
CIM : Composite Interval Mapping
CIMMYT : Centro Internacional de Mejoramiento de Maiz y Trigo
cM : Centi Morgan
CSL : Chromosome Substitution Line
CU : University of Cukurova
DArT : Diversity Array Technology
DH : Double haploid
DMF : Di-Metyl-Formamide
Exp : Explanations
GIC : Genotypic Information Coefficient
GWM : Gatersleben Wheat Microsatellites
ICARDA : International Center For Agricultural Research In The Dry Areas
ICC : International Association for Cereal Science and Technology
ISSR : Inter simple sequence repeat
IM : Interval Mapping
ITMI : International Triticeae Mapping Initiative
Kb : Kilo Base Pair
KD : Kernel Dimensions
KL : Kernel Length
KPS : Kernel per Spike
KW : Kernel Width
LOD : Logarithm of the odds ratio
LRT : Likelihood Ratio Test
MAS : Marker Assisted Selection
Mb : Mega Base Pair
ML : Map Length

MD	: Marker Density
MQTL	: Map QTL software
NIL	: Near isogenic lines
NR	: Non Recombinant Haplotypes
PIC	: Polymorphism Information Contents
QTL	: Quantitative Trait Loci
RAPD	: Randomly Amplified Polymorphic DNA
RFLP	: Restriction Fragment Length Polymorphism
RICL	: Recombinant Inbred Chromosome Lines
RILs	: Recombinant Inbred Lines
RSL	: Recombinant Substitution Line
SCRI (SCRs)	: Single-Chromosome Recombinant Lines
SIM	: Simple Interval Mapping
SNP	: Single-Nucleotide Polymorphisms
SSR	: Microsatellites (Simple Sequence Repeat)
SRAP	: Sequence-Related Amplified Polymorphism
STRs	: Simple Tandem Repeats
TBE	:Tris/Borate/EDTA
TCA	:Trichloroacetic acid
TKW	: Thousand kernel weight
TRAP	: Target Region Amplification Polymorphism
USDA	: United States Department of Agriculture
WANA	: West Asia, North Africa.
X^2	:Chi-square test
YrBP	: Years before Present

1. INTRODUCTION

Wheat has evolved through several millennia of cultivation and selection by farming ancestors. Since the time of its domestication in 15,000-10,000 BC, the demand for wheat has continued to rise with the ever increasing global population and it is expected that this demand will have increased by 40% by the year 2030 (Dixon et al., 2009). Wheat is still a traditional staple and major food crop in Mediterranean and many other countries, mainly consumed by the human population as processed products because of the unique functional properties. For one third of the world's population, wheat provides more than half of the required daily calories and nearly half of the protein. Durum wheat (*Triticum turgidum*) or pasta wheat compared with common bread wheat (*Triticum aestivum*) is known for its hardness, protein content, intense yellow color, nutty flavor and excellent cooking quality. The need to fulfil the demand for durum wheat cultivars with superior grain quality and yield is increasingly becoming a priority in all wheat producing areas worldwide.

Durum wheat is one of the most important food crops in Southt West Asia and North Africa (SWANA) region, and it is mainly preferred for the production of pasta or macaroni products. A high grain yield of durum wheat can be obtained by irrigation. Durum wheat generates greater yield than other wheats in areas with low precipitation. The varieties that meet the requirements of high yield and production of high-quality pasta obtain premium prices in the global market. On the other hand, many countries in Europe produce durum wheat commercially in significant quantities (Matz and Samuel 1999). In the Middle East and North Africa, durum wheat is also used in local bread making..

Durum wheat with its production and export potential is an important crop in Turkey, which produces 4. 1 million tons of durum wheat in a growing area of 1.273 million ha (TUIK, 2105); used in the production of bulgur, pasta or for export. In recent years in Turkey, more emphasis has been given to durum wheat research to obtain new cultivars with high yield, good grain quality and biotic-abiotic stress resistantance.

The quality traits of wheat grain are complex and can be determined by various techniques and specialized technological equipment. The majority of grain quality traits are inherited in a polygenic fashion, and the degree of their manifestation depends on environmental conditions; however, phenotypic selection is more time consuming and laborious. Conventional plant breeding methods have made a significant contribution to crop improvement, but have been slow in targeting complex morphological, agronomic and grain quality traits. With the recent development in molecular genetics and genomics, location, separation and exploiting of quantitative trait locus mapping (QTL-mapping) as well as genome-wide association mapping (GWAM) have also been introduced to discover new useful allelic variants through a genome-wide scan. GWAM has become also an important and essential component in crop genetic improvement.

Explaining the phenotypic variation in terms of simple changes in DNA is the goal of many plant breeding programs. Knowing the position of the nucleotide sequence underlying a specific trait offers plant breeders an opportunity to apply marker assisted selection. Most of wheat yield components are controlled by many loci and their molecular characterization and genetic mapping is called quantitative trait loci mapping (QTL-mapping). Family based linkage analysis and Linkage Disequilibrium (LD) based association mapping are two of the most commonly used tools for QTL mapping (Risch and Merikangas, 1996; Mackay, 2001). In linkage analysis, genes are assigned to different chromosomes on the basis of the co-inheritance of markers in a segregating population. The limitations of linkage analysis are the development of bi-parental population, limited allele coverage, low resolution, marker specificity, longer time period and high cost (Flint-Garcia et al., 2003). The relatively new approach of association mapping (AM) overcomes these limitations by using natural germplasm and historical recombination and mutations. The AM has also several advantages over bi-parental mapping such as high-resolution, broader allele coverage, cost effective gene tagging (Flint-Garcia et al., 2003), and reduced research time since it utilizes historic recombination rather than developing new mapping populations, and the ability to detect a greater number of alleles at a particular locus (Yu and Buckler, 2006). Linkage mapping has become a

traditionally employed method to achieve this goal. AM involves searching for genotype-phenotype correlations in unrelated individuals and often it is more rapid and cost-effective than traditional linkage mapping. Myles et al. (2009) emphasized that linkage and the AM are complementary approaches and more similar than is often assumed.

Turkey is part of Fertile Crescent, the primary center of wheat domestication and diversity. Despite the importance of gene pool of this vital area, there is little investigation on gene pool structure of Turkish durum wheat. For this reason, this research was conducted to fill this gap and provide necessary information about the genetic structure and genome distribution of Turkish durum wheat. The objectives of this study are;

- Evaluation of the phenotypic performance of Turkish commercial varieties and landraces of durum wheat for important agro-morphological traits.
- Identification of the genes or the specific nucleotides underpinning trait variation among Turkish durum wheat cultivars and landraces.
- Generation of fundamental knowledge concerning the genetic architecture of extant variation in durum wheat cultivars and landraces using 60,000 DArTseq markers covering the whole genome of durum wheat, and determination of evolutionary phenomena that have led to the existing population structures.
- Association of genotypic data with phenotypic traits in order to identify the QTL by performing marker-trait association in the Turkish durum wheat gene pool and to mine the markers linked to a trait of interest for marker assisted breeding program of durum wheat world wide.

2. REVIEW OF LITERATURE

2.1. Evolutionary history and taxonomy of wheat

Wheat has evolved through several millennia of cultivation and selection by farming ancestors. Since the time of its domestication in 15,000-10,000 BC, the demand for wheat has continued to rise with the ever increasing global population and it is expected that this demand will have increased by 40% by the year 2030 (Dixon et al., 2009). The genetic relationships between einkorn and emmer indicate that the Southeastern part of Turkey and Northern Syria is most likely a key area with regard to plant domestication (Özkan et al., 2002; Dubcovsky and Dvorak, 2007). The genus *Triticum* belongs to the family Poaceae, which consists of approximately 3500 species (Bonnier and Douin, 1990). It is cultivated in a broad range of climates but shows the best adaptation to temperate climate. Cultivated and wild wheat can be classified into three species *T. urartu*, *T. turgidum* and *T. aestivum* on the basis of their genomic constitution AA, AABB, and AABBDD, respectively. Genetic studies have demonstrated that *T. urartu* has donated AA genome to *T. turgidum* (tetraploid wheat, AABB) and the DD genome was donated by *T. tauschii* to form hexaploid wheat (*T. aestivum* L.). Most probably, *Aegilops speltoides* is considered as the donor of BB genome to durum and bread wheat (Huang et al., 2002) (Figure 1). *Triticum urartu*, diploid species, has an AA genome (Harlan and Zohary, 1966) that occurs in central and eastern part of crescent fertile (Zohary and Hopf, 2000). Tetraploid wheat evolved from two closely related diploid species having genomic formula AABB (2n=4x=28, Gill et al., 2004). Then, the domesticated emmer *Triticum dicoccum* hybridized with *Aegilops tauschii* to form the modern hexaploid wheat *Triticum aestivum* (Salamini et al., 2002). Durum wheat (*Triticum turgidum* var *durum* L) is one of important cereal crops cultivated around the globe.

2.2. Economic importance of wheat:

After maize and rice, the most important food crop for human consumption is wheat. Today wheat is grown all over the world, with different varieties sown according to the various climates (Cornell and Hoveling, 1998). The economic importance of wheat triggered intense cytogenetic and genetic studies in past decades resulting in a wealth of information and tools that have been used to develop wheat cultivars with increased yield, improved quality and enhanced biotic and abiotic stress tolerance (Baloch, 2012). Durum wheat (*Triticum turgidum* var *durum* L.), the main tetraploid type, is also important and suitable for pasta, couscous, burghul and other Mediterranean local cereal products. In terms of diploid wheat, there is no economically important cultivation anywhere in the world (Dubcovsky and Dvorak, 2007).

Wheat grain comprises 60-80% carbohydrates, 8-17% protein, 1.5-2% minerals, 1.5-2% fats and 2.2% crude fibers. In addition to all the essential amino acids, it contains vitamins such as B complex and vitamin E (Pena, 2002). Globally, wheat cultivation covers an area greater than any other commercial. In 2013, wheat was grown on approximately 250 million hectares in five continents with total production of 711.1 million tons (Figure 2.1 and 2.2). Asia has had the highest production share by region average for at least the last fifty years from 1960 until 2013 whereas Africa had the lowest level of production. Consequently, wheat prices have also reached the highest level (FAO, 2013).

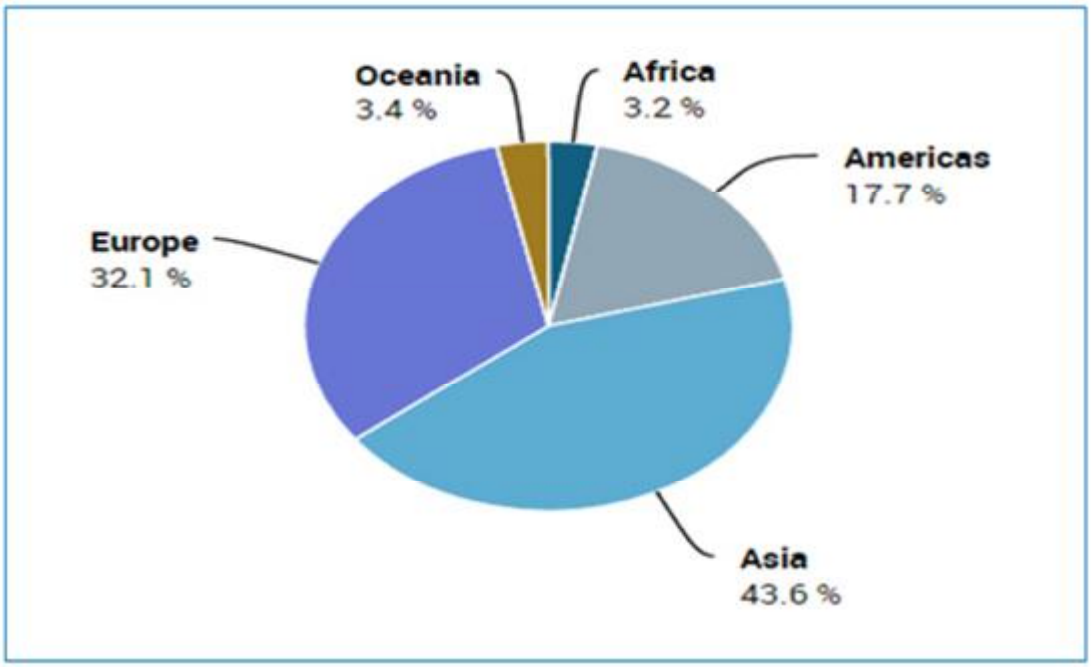

Figure 2.1. Wheat world production share by region from 1961-2013

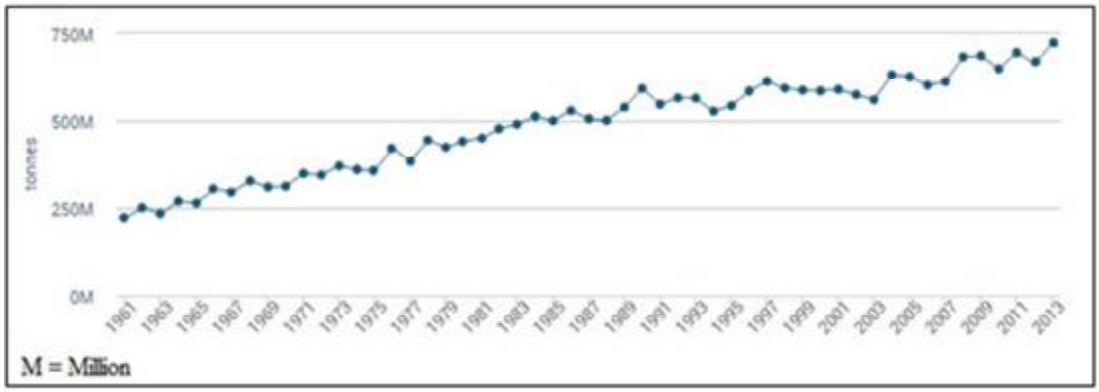

Figure 2.2. World wheat production (1961-2013)

By 2050, the world annual demand for maize, rice and wheat is expected to reach about 3.3 billion metric ton or 800 million metric ton more than the record combined harvest in 2014. Much of the increase in production will need to come from existing farmland; however, one-third of that land is degraded, and farmers' share of water is under growing pressure from other sectors. Furthermore, climate change could have catastrophic effects by reducing wheat and maize yield by 20 % and in Asia, rising sea levels in major river deltas threaten rice production. The potential for increases in cereal production is further constrained by stagnating yields

and diminishing returns from high-input production systems (FAO, http://www.fao.org/3/a-i5318e.pdf). Five countries, Turkey, Iran, Iraq, Syria and Afghanistan, produce approximately 95 % of the wheat in West Asia, with the wheat growing area and production of Turkey and Iran comprising 75 % of the total area and production. Recently in Turkey, the wheat production reached 22 million metric ton Thus, becoming one of the ten top wheat producers in the world (FAO, 2013; Figure 2.3).

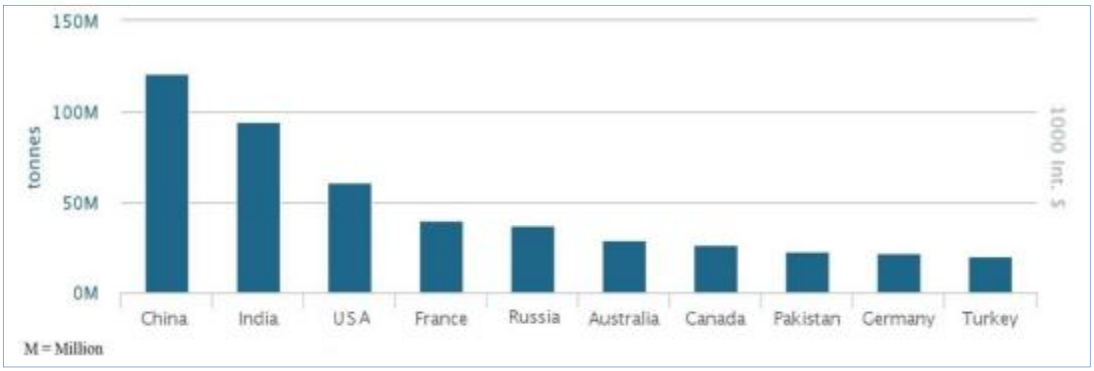

Figure 2.3. Top ten wheat-producing countries in 2013

Since 1961, the importance of wheat for human consumption has resulted in a steady increase in wheat production in Turkey and across the world. As shown in Figure 2.4, wheat production increased from 6.5 metric ton in 1961 to over 20 metric ton in 2013

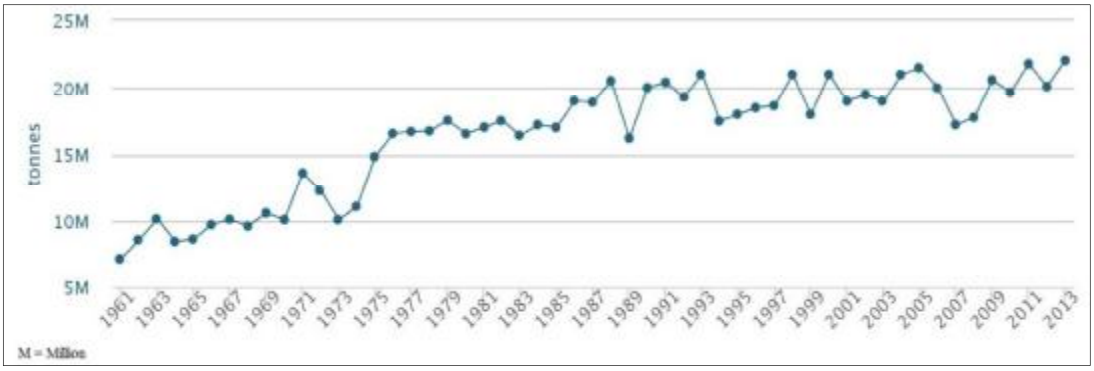

Figure 2.4. Wheat production in Turkey (1961-2013)

The comparison of average production levels of many crops in Turkey for the last five decades, from 1961 until 2013, shows that wheat has the highest production compared with other crops such as sugar beet, barley, forage, silage, alfalfa and tomatoes (FAO, 2013; Figure 2.5). In 2013, wheat production in Turkey reached a significant level taking first place compared with other crops such as sugar beet, barley, grapes and watermelons; 22, 17, 8, 4, 3.2 metric ton, respectively (FAO, 2013).

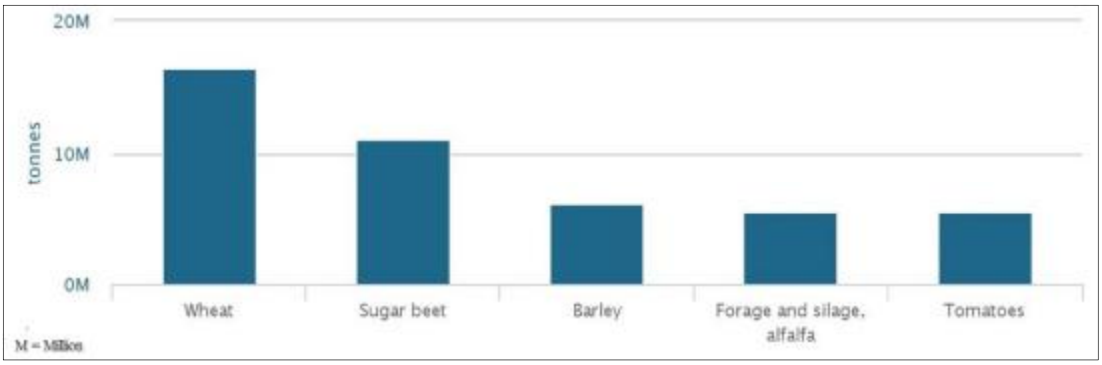

Figure 2.5. Most produced commodities in Turkey 1961-2013

2.3. Durum wheat in Turkey

Durum wheat is grown in more than 50% of the total wheat-growing area in the Mediterranean region (Guzmán et al., 2015). In Turkey, wheat is a traditional crop grown in the high plateau of the Anatolian region. Durum wheat is an important crop and primarily grown where rainfall is more limited (300 to 500 mm). Spring growth habit wheat predominates in the Mediterranean zone and is generally planted in the autumn. Facultative and winter wheats are more often grown in the highland zones. Durum wheat is planted in October and November and harvested in June and July (Zencirci et al., 1995). In recent years, Central Anatolia has become the leading grain-producing region of Turkey. In the 2014/15 season, this region had 240,000 ha of durum wheat under cultivation that yielded an average 3.4 MT/ha. In the same period, the durum wheat yields in Southeast Anatolia from 250,000 ha were 3.8 MT/h. (http://www.agrochart.com/ turkey-wheat-annual-apr-2015). The estimated total of durum wheat output for season 2014/15 was 4.1 MMT

(http://gain.fas.usda.gov). In Turkey, more emphasis has been given to durum wheat research in recent years to better understand the complexities of the relationship between the yield and other traits, and also to obtain a higher yield, maintain the quality and develop biotic-abiotic stress resistant cultivars.

2.4. Wheat Morphological parameters

The morphology parameters of wheat are a great concern of breeding programs. Grain yield is an economically important factor in wheat production. Correlation analysis between grain yield and other yield components in wheat has shown significant correlations of the yield with many plant traits. The advancement in the field of molecular biology using genetic marker technologies and new statistical approaches provide powerful tools for the indirect selection of valuable traits through marker-assisted selection (Landjeva et al., 2007). The detection of specific agronomic traits could be a useful contribution for the selection and generation of new high-yielding wheat varieties over a short period of time. Several of the important traits are explained below.

2.4.1. Days to heading

The heading date (HD) is one of the important components of wheat variety selection, and is governed primarily by temperatures. The variability of the heading date may assist in the adaptation of wheat to local environments Furthermore, the discovery of new heading date determinants is important for cereal improvement. Kiseleva et al. (2016) used common wheat cultivar Chinese Spring (CS) and the substitution line of CS with 5B chromosome from *T. dicoccoides*, which have different heading dates, to detect the determinants of heading date on the 5B chromosome. They reported that the differences in the activity of WRKY, ERF/AP2 and/or the FHY3/FAR1 genes are transcription factors between CS and CS-5Bdic, which can detect a possible reason for the difference in heading dates. Iftikhar et al., (2012) indicated that grain yield had a negative correlation with days to heading.

Gashaw et al., (2007) reported that grain yield had strong negative correlation with the days to heading suggesting the usefulness of selecting early heading durum wheat genotypes with long grain filling period in improving grain yield.

2.4.2. Days to maturity

Plant traits can be used to complement selection based on yield components in wheat. Therefore, understanding the growth stages of wheat is important in making management decisions. The investigation of the heading date and maturation date (MD) has been reported by many of scientists. Tsenov (2009) mentioned that the date to heading could be used as a criterion for distinguishing wheat varieties based on their growing period durability. He also found a high and significant relationship between heading and physiological maturity varied according to temperature. The results reported by Gashaw et al., (2007) indicated a positive correlation between grain yield and days to maturity of durum wheat genotypes.

2.4.3. Plant height

Plant height (PH) is an important trait, affects yield and quality in wheat. Major genes that are discrete and well characterized genetically and phenotypically influence plant height. Although PH is a complex trait, it is far simpler than many other traits (Weiss et al., 2009). In research concerning the genetic control of PH in winter wheat cultivars, Würschum et al., (2015) found that the *Rht-D1* and *Rht-B1* genes had the largest effect on plant height. A genome wide scan for marker trait associations located on chromosomes 6A and 5B showed that the two *Rht-1* semi-dwarfing genes are the major sources of variation in winter wheat cultivars in plant height. Gorjanoviã et al., (2007) studied the gene effects on plant height, spike length and number of spikelets per spike in durum wheat. They showed that non-additive genes play a more important role than additive genes in the inheritance of plant height and number of spikelets per spike. Okuyama et al., (2005) reported that under

non-irrigated conditions, the yield per spike showed a positive direct effect and had a positive correlation with plant height.

2.4.4. Peduncle length

The peduncle length (PL) is considered to be important trait for the grain yield of wheat (Ortiz-Ferrara et al., 1991) and many researchers have investigated the association between grain yield and agronomic traits. The research indicated that peduncle length was the effective component of grain yield. Iftikhar et al., (2012) indicated that grain yield had a positive correlation with peduncle length. Correlation analysis revealed significant positive relationship between grain yield and peduncle length (Zafarnaderi et al 2013).

2.4.5. Peduncle extrusion length

Peduncle extrusion length (PEL) is one of physiological and developmental traits. It is considered to be an adaptive trait and has been studied by many researchers. For instance, Fellahi et al., (2013) and Okuyama et al., (2005) investigated the relationship between peduncle extrusion length and yield per spike in wheat genotypes. All the researchers reported that there are strong positive correlations between peduncle extrusion length and other traits.

2.4.6. Lodging degree

Wheat is susceptible to lodging which is the bending over of the stems near ground level. It is a problem that particularly occurs with high inputs of nitrogen fertilizer and water. Lodging should be prevented since it results in an uneven maturity of the crop, increased moisture content of the grain, decreased grain quality due to grain shriveling as measured by test weight, and increased harvesting costs. Permanent displacement from the vertical can be caused in two distinct ways; root lodging and stem lodging. Root lodging is the displacement of the roots in the soil

and stem lodging is the bending or breaking of the stem base. Plant growth regulators have been used to control lodging in wheat; however, scoring the lodging resistance is difficult under natural field conditions. Lodging is a complex trait comprised of two characters, i.e. stem mechanical elasticity and rigidity; therefore, it is closely associated with stem morphological and anatomical features (Hai et al., 2005). Nine QTLs for lodging resistance were detected by Keller et al., (1999) and a study on rice by Begum et al., (2015) identified 5 QTLs linked to the degree of lodging.

2.5. Spike characteristics

Spike-related traits are significant yield components, which are less environmentally sensitive and exhibit higher heritability than yield (Cuthbert et al., 2008). Analysis has indicated that the number of grains in a spike, thousand-grain weight, number of fertile tiller and peduncle length are the most effective components on grain yield. The analyses of the genetic control of the spike-related characteristics and the individual effects of different genes and quantitative trait loci could provide specific information and be useful for the indirect determination of yield improvement (Ma et al., 2007). One of the first association mapping studies in wheat aimed at identifying significant markers for kernel size and milling quality (Breseghello and Sorrells, 2006). Subsequently, a number of works employed genome-wide association studies (GWAS) to detect marker-trait for a large number of traits, including quality traits in soft wheat (Reif et al., 2011), yield and other agronomic traits in wheat (Liu et al., 2010). In wheat, a number of yield-component QTLs were associated with spike-related and adaptive traits (Neumann et al., 2011). The relationship between plant traits including the length of flag leaf blade, peduncle extrusion, peduncle, spike and sheath, culm diameter and plant height and yield per spike in wheat genotypes were investigated by Okuyama et al., (2005). QTL mapping using various segregating populations was conducted for plant height (PH), spike length (SL), spike number (SN), kernel number per spike (KNS), and thousand kernel weight (TKW) (Börner et al., 2002; Kumar et al., 2007; Cuthbert et al., 2008; Golabadi et al., 2011; Bennett et al., 2012). Yield per spike correlated positively with

spike length and culm diameter. Path coefficient analysis indicated that under irrigated conditions, the spike length and culm diameter had a positive direct effect and a positive correlation with yield per spike and, under non-irrigated conditions, culm diameter, spike length and plant height showed a positive effect and positive correlation with yield per spike. Culm diameter and spike length under irrigated conditions, and plant height under late-season water stress conditions were the plant traits most related to higher grain yield per spike in wheat. Grain yield (GY) is also a product of several contributing morphological factors. GY can be estimated on the basis of the performance of various components such as the flag leaf area, spikelet numbers, seeds per spike, tillers per plant and one thousand grain weight (Bhutta, 2006). The relationships between plant traits and yield per spike in wheat genotypes were also investigated by Okuyama et al., (2005). Their analysis indicated that under irrigated conditions spike length and culm diameter had a positive direct effect and a positive correlation with yield per spike; furthermore, under non-irrigated condition, culm diameter the spike length and plant height showed positive direct effect and positive correlation with yield per spike. Culm diameter and spike length under irrigated condition and plant height under late-season water stress condition were the plant traits most related to higher grain yield per spike in wheat. Iftikhar et al., (2012) reported that grain yield had a positive correlation with spike length and grains per spike whereas there was a negative correlation with days to heading, plant height and tillers per plant. They concluded that traits such as spike length and TKW had a positive correlation and a direct effect on grain yield can be used as suitable selection criteria to develop high yielding genotypes.

2.6. Grain quality

2.6.1. Thousand kernel weight

Wheat grain yield is a complex quantitative trait consisting of various components including thousand-kernel weight (TKW). This is one of the main yield components of wheat having a high and consistent heritability value but is also fairly

strongly affected by growing conditions. TKW is also phenotypically the most stable yield component (Sun et al., 2009), and the effects of most genes affecting the thousand kernel weight are additive. Kernel weight is partially controlled genetically by different loci. In durum wheat, thousand-kernel weight is important trait for measuring end use quality (Patil et al., 2008). . Several QTL mapping studies using various segregating populations have been conducted to assess TKW (Börner et al., 2002; Kumar et al., 2007; Cuthbert et al., 2008; Alsaleh, A. 2011; Golabadi et al., 2011; Bennett et al., 2012). Several QTLs for TKW have been identified in many chromosomes. For instance, Alsaleh (2011) identified ten different QTLs associated with TKW located in chromosomes 2A, 2B, 3A, 3B, 4B, 5A, 7A and 6A. Patil et al., (2013) reported that TKW were influenced by 11 main effect QTLs and 6 digenic epistatic interactions detected on chromosomes 2A, 2B, 4B and 7A. Iftikhar et al., (2012) reported that grain yield had positive correlation with spike length, grains per spike and TKW. Hence, early generation selection for thousand-kernel weight is likely to be most effective (Wang et al., 2012). Ketata et al. (1976) estimated the level heritability of TKW and reported that it could be relatively intermediate to high.

2.6.2. Vitreousness

The percentage of the vitreousness kernels of durum wheat is a major quality attribute not only in durum wheat grading but also traditionally regarded by the wheat industry throughout the world as an important quality factor (Dowell, 2000). The significance of durum wheat has increased worldwide due to shortages of good quality ingredients to be utilized in the food industry and the food shortages occurring in many developing countries (Zencirci and Karagöz, 2005). The best quality durum wheat has high proportion (90-100 %) of vitreous kernels. Understanding the interrelationship among the quality parameters and yield may help breeders create varieties with acceptable quality and high yield . In durum wheat, Elouafi (2001) reported two detected QTLs explaining the vitreous variation on 4BL

and 6BS. The vitreousness trait was influenced by six QTLs, distributed along the following chromosomes 1B, 2A, 2B, 6A, 6B and 7A (Alsaleh,A. 2011).

Finally, yield and yield-associated traits for wheat cultivars are complex nature and quantitative traits controlled by multiple genes and are highly influenced by environmental conditions. The observation is not easy to conduct, time-consuming and not applicable to large numbers. Therefore, it is useful to use genetic markers to examine morphological traits association to gather specific information about the genetic relationship between related traits crucial for sustained wheat improvement.

2.6.3. Test weight

Test weight (TW) is an important factor in assessing wheat quality and estimation of flour extraction, which is also known to be highly related to semolina yield. Several authors have reported a high and positive correlation between TW and flour yield. TW is affected by the genotype and various environmental factors (Eloaufi,I. 2001). Jia et al., (2013) identified one QTL of TW near the marker wmc167. Alsaleh (2011) reported that test weight was influenced by eight different QTLs; two of which were located on 1A chromosome, with one being found on chromosomes 2A, 2B, 3A, 3B, 4B and 7B. Elouafi (2001) showed significant QTL in two regions, one detected on 7AS next to gwm60, and second in chromosome 6BS flanked by gwm88.

2.7. Molecular Marker Technologies

Understanding the genetic mechanism of agronomic traits is essential for breeding and as well for producing genetic gains through selection in wheat. From the time of Mendel (19^{th} century) until the 1980s, the morphological characters were the major types of markers readily available for genetic mapping. However, during last two decades, scientific progress and recent development in molecular marker technologies has revolutionized the genetic analysis in plants. The availability of

molecular markers can facilitate the selection in the early generations (Ruiz et al., 2005).

Various types of molecular markers have been described in the literature with the first molecular markers being biochemical markers that made a valuable contribution to the development of genetic maps, e.g., in tomatoes (Tanksley and Rick, 1980) and maize (Edwards et al., 1987); however, these kinds of markers have low numbers and limited genome coverage. Later, DNA markers started to be used in various ways such as marker-assisted selection, germplasm characterization, tagging and pyramiding genes, in the study of genetic diversity and the preparation of molecular maps. By using DNA markers, several association maps were initially prepared for rice (Glaszmann, 1986). Subsequently, many maps have been constructed in many different organisms including maize (Helentjaris et al., 1986), barley (Stracke et al., 2003), sorghum (Deu and Glaszmann, 2004), wheat (Elouafi et al., 2001; Maccaferri et al., 2004; Alsaleh et al., 2015 and Baloch et al., 2016) and humans (Schumm et al., 1985). According to Gupta and Vershney (1999, 2000), molecular markers can be divided into three major groups: hybridization based molecular markers, PCR-based molecular markers and sequencing and DNA chip based markers. The most widely used hybridization-based molecular marker is random fragment length polymorphisms (RFLP). The major strength of RFLP markers is high reproducibility, co-dominant inheritance, transferability, not needing sequence information and relatively easy to score. However, there are several limitations including the need for high quantity and quality of DNA and radioactively labeled probes, the dependence on the development of specific probe libraries for the species, no automation ability, and low level of polymorphism. Furthermore, the process is time consuming, laborious, and expensive (Semagn et al., 2006). Therefore, the use of this kind of DNA markers decreased.

2.7.1. PCR-based molecular markers and sequencing

2.7.1.1. Microsatellites

Microsatellites or simple sequence repeats (SSRs) refer to eukaryotes having families of repetitive DNA sequences in their genomes. The strategy for using SSRs as genetic markers is that the repeat region may vary in length between genotypes. Microsatellites in plants were first reported by Condit and Hubbell (1991), after which SSRs markers were intensively used in several research studies. SSR markers appear to be hyper variable in addition to their co-dominance nature, chromosome-specific, repeatability, and reproducibility, which make them ideal for the identification of varieties (Duraa et al., 2013), construction of genetic linkage maps and the QTL analysis of many genome mapping (Plaschke et al., 1995; Ma et al., 1996; Röder et al., 1998; Stephenson et al., 1998; Pestsova et al., 2000; Nachit et al., 2001; Elouafi and Nachit, 2004; Zhang et al., 2008; Maccaferri et al., 2008; Patil et al., 2008; Li et al., 2009; Mason, 2010; Alsaleh, et al., 2015; and Baloch et al., 2016). The use of fluorescent primers in combination with automatic capillary or gel-based DNA sequencers can be found in most advanced laboratories. SSRs are excellent markers for fluorescent techniques, multiplexing and high throughput analysis (Oetting et al., 1995). However, due to the large genome size, the development of microsatellite markers in wheat is extremely time-consuming and expensive; therefore, scientists invented and began to use a new generation of marker technologies.

2.7.1.2. DArT marker and Next Generation Sequencing

Recently, through the use of next-generation sequencing (NGS) technologies, a rapid SNP discovery method known as DArTseq was developed utilizing a DArT marker platform in combination with next-generation sequencing platforms (Sansaloni et al. 2011; Kilian et al. 2012; Cruz et al. 2013; Raman et al. 2014). DArTseq is a novel genotyping-by-next generation sequencing approach,

representing a new implementation of sequencing of complexity-reduced representations (Altshuler et al., 2000) and more recently, this concept has been applied on the next generation sequencing platforms -

DArTseq can be used in genetic diversity assessment, QTL and Genome-Wide Association Studies (GWAS) to decipher the genetic basis of many plant genera. As most of DArTseq methods use methylation sensitive RE, the polymorphism patterns produced by DArTseq include a component of methylation profiling; therefore, they are capable of detecting epigenetic variation (similarly to most of microarray DArT methods). Compared to genome profiling only using SNP assays, the DArTseq method is much more comprehensive in terms of the molecular variation underlying the polymorphism. The technology of DArTseq was optimized for Triticeae and other crops by selecting the most appropriate method to reduce the genome complexity. This method deploys the sequencing of the representations on the Next Generation Sequencing (NGS) platforms. The advantage of DArTseq over the array version of DArT is currently limited to applications requiring very high marker densities (tens of thousands of markers). This technology is therefore positioned in the area of high-resolution mapping and detailed genetic dissection of traits. As modern breeding is rapidly moving in this direction, especially in larger organizations, DArTseq is increasingly used in crop improvement applications but it has disadvantages such as generating dominant markers (Kilian et al., 2012).

2.7.2. Quantitative traits loci

Quantitative traits show the phenotypic variation in a population resulting from the combined allelic effects of many genes and environmental conditions (Falconer and Mackay, 1996). In crop plants, most traits of agricultural and economic significance such as yield, plant maturity, disease and stress tolerance exhibit quantitative inheritance. The genetic loci, which control quantitative traits, are referred to as QTL. The aspect of QTL that is receiving growing attention is the mapping of chromosomal regions affecting qualitative or quantitative traits through molecular mapping and genomic approaches that offer new opportunities and

strategies to dissect major genes and the underlying different interesting traits such as morphology, yield and quality parameters. QTL analysis can be defined as identifying and characterizing genomic segments with a QTL being significantly involved in the expression of a target trait. A QTL comprises two main stems, for example, the construction of a suitable segregation population by crossing two parental lines constructing the target trait(s) and the identification of markers closely linked to the genes(s) of interest by assessing the correlation between the phenotypic value of the different genotypes of the segregating population with the allelic composition at each of the loci used to produce the linkage map (Duraa et al., 2013). The QTL analysis provides information on the number of genes (or QTLs) involved in the expression of the target trait, the additive or dominance effects of the identified regions and their impact on phenotypic expression, and the existence of pleiotropic effects at some genomic regions (Monneveux et al., 2005). Many QTLs recently have been identified in wheat and other crops (Blanco et al., 1998; Lotti et al., 2000; Nachit et al., 2001; Elouafi and Nachit, 2004; Zhang et al., 2008; Maccaferri et al., 2008; Patil et al., 2008; Li et al., 2009; Mason, 2010; Alsaleh, 2011). Once these traits are identified and mapped, marker–assisted selection (MAS) can be used to introduce these traits into a wide variety of populations. Alternatively, a relatively new approach being applied in plants is association mapping, which is based on linkage disequilibrium (LD). In this mapping approach, diverse populations of unrelated material are used to identify associations between allele frequencies and phenotypic variation. While extensive literature is available on identification of QTL from segregating populations, the use of AM in plants remains in a preliminary stage.

2.7.3. Genome wide association studies

Association mapping provides useful insights on the genetic architecture of quantitative traits across a large number of unrelated genotypes (Cane et al., 2014). Association genetics is a multidisciplinary field, involving components of genomics, statistical genetics, molecular biology, and bioinformatics, which together form the basis for selecting, evaluating, and associating genomic regions for correlation with

trait variation (Nnadozie et al., 2006). To detect and identify association of specific genetic functional variants linked to phenotypic differences, association mapping provides a powerful tool that is a complementary strategy to genetic mapping to identify the association between genotype and phenotype, and also complements the existing QTL mapping, and cloning with bi-parental populations, mutational dissection, and transgenic approaches. It has been widely adopted in almost all major crop species for gene identification, QTL validation, and to gain an understanding of the genetic basis of complex traits (Zhu et al., 2008). Association mapping has been widely used in plant research since it was first reported being used in maize. In recent years, association mapping has been applied in *Arabidopsis*, maize, barley, sorghum, sugarcane, sugar beet, soybean, grape, forest tree species and forage grasses as well as durum and spring wheat (Zhang et al., 2014). The basic objective of AM, which is based on linkage disequilibrium (LD), is to detect correlations between genotypes and phenotypes of a sample of individuals. Linkage disequilibrium estimation among a diverse set of accessions within a species typically provides basic information concerning the potential resolution of association mapping and the marker density requirement. One of the major benefits of association mapping is the diversity captured across many different traits. A further attractive feature of AM is that the marker-trait association can be studied using well phenotype germplasm pools and breeding population of locally adapted varieties (Bresegello and Sorrells, 2006). The resulting information is then incorporated into either the candidate-gene or genome-wide association analysis (Tuberosa et al., 2012).

Currently, association mapping is intrinsically more powerful than genetic linkage mapping because it scrutinizes the results of thousands of generations of recombination and selection (Cattivelli et al., 2008); however, future studies of AM in crop plants will further elucidate the structures of plant genomes and also facilitate the use of MAS and map based cloning of genes for difficult traits (Gupta et al., 2005). Nnadozie et al., (2006) compared association genetics and conventional QTL mapping as in Table 2.1.

Table 2.1. A comparison of association genetics and conventional QTL mapping

Attribute	**QTL mapping**	**Association genetics**
Detection goal	Quantitative trait locus, i.e., wide region within specific pedigrees within which a QTL is located	Quantitative trait nucleotide, i.e., Physically as close as possible to causative sequence(s)
Resolution of causative trait polymorphism	Low – moderate density linkage maps only required	High – disequilibrium within small physical regions requiring many markers
Experimental populations for detection	Defined pedigrees, e.g., backcross, F2, RI, three and two generation pedigrees/families, half-sib families	Linkage disequilibrium experiments: unrelated individuals ("unstructured" populations), large numbers of small unrelated families (e.g., transmission disequilibrium tests)
Marker discovery costs	Moderate	Moderate for few traits, high for many traits
Extent of inference	Pedigree specific, except where species has high extant LD	Species or subspecies wide
Number of markers required for genome coverage	102–low 103	105 for small genomes – ~109 for large genomes

In wheat improvement, grain yield is directly determined by yield-component traits, and indirectly affected by other yield-related traits. Therefore, to continue this improvement, it is necessary to combine the measurements of yield-related traits associated with the yield response, and make selections based on the yield components that would result in yield increases. Many successful studies have been undertaken on wheat to dissect the complex quantitative traits including yield and yield components, biotic and abiotic stress tolerance and quality. Some QTLs

observed and identified by Wu et al., (2012) were those for yield per plant (YP) and plant height (PH).

Marker-trait associations were investigated by Bousba, et al., (2013) for certain agronomic traits of wheat genotypes using simple sequence repeat markers. The results showed that landraces were earlier and relatively taller than the improved varieties. Some of the SSR markers showed significant associations with the target agronomic traits in the chromosomes of durum wheat, other markers revealed significant associations with several traits and were associated with the number of grains per main spike, number of spikes per square meter and TKW, as well as with grain yield and its components (Bousba, et al., 2013).

Elite durum wheat accessions were evaluated by Cane` et al. (2014) in order to identify QTLs. The QTLs identified were compared with the QTLs detected for grain yield and its component traits, plant height and peduncle length. Highly significant differences among accessions were detected for all traits, the highest repeatability was observed for seminal root angle, QTLs were also detected for root system architecture.

Recently, Liu et al. (2015) identified marker-trait associations and candidate genes, assessed genetic diversity, and classified the accessions based on phenotypic data and genotypic comparison. The authors conducted a genome-wide association study using single nucleotide polymorphism (SNP) markers across accessions of *Ae. tauschii* that were phenotyped for morphological traits. They identified SNP markers that were significantly associated with morphological traits. In this study, the trait-associated SNPs identified several genes that may be linked to variations in phenotypes.

Hu et al., (2015) studied the associations of single nucleotide polymorphism markers with agronomic traits such as plant height, number of effective spikes, length of main spike, number of spikelets on the main spike, rachis internode length of main spike, number of spikelets per plant, panicle neck length of the main spike, grain number per plant, grain weight per plant and TGW. They reported that significant association pairs were detected and several markers were associated with one or multiple traits.

Tadesse et al., (2015) investigated the genetic basis of yield and grain quality traits in winter wheat genotypes using AM. All the genotypes were genotyped using DArT markers. They identified some molecular markers linked with certain traits and reported that those markers could be used for marker assisted selection.

GWAS studies were reported for cereals (Table 2.2). Ain et al., (2015) used GWAS to identify SNP markers associated with yield and yield related traits in Pakistani historical wheat cultivars evaluated under different environmental conditions. The population was genotyped using high-density SNP assay. Stable marker-trait associations were identified for some yield-related traits. Gene annotation identified that many trait-associated SNPs were linked to genes that had significant importance in plant development. Favorable alleles for days to heading date, plant height, TGW, and grain yield showed minor additive effects.

Major QTLs in wheat have previously been reported (Table 2.2), but there is still a lack of published literature on genome-wide AM studies of important target traits especially in the Turkish pool of wheat genomes.

Table 2.2. Selected reports on association mapping in cereals

Species	Traits identified	References
Rice	Number, length & diameter of culm, tiller number, length and width of flag leaf, stem diameter, plant height and number of tillers, panicle length, grain length, grain thickness, heading date, 1000 grain weight, sheath blight resistance, grain yield, root growth and development	Virk et al., 1996; Zhang et al., 2005; Agrama and Eizenga, 2007; Wen et al., 2009; Yan et al.,2009; Huang et al., 2010; Jia et al., 2012; Clark et al., 2013; Spindel et al., 2015
Barley	Heading and flowering date, plant height, rachil length, resistance to leaf rust and mildew, plant height, yield, yield stability, grain number, thousand kernel weight, starch content, protein content, spot blotch resistance, frost tolerance, kernel and malting quality	Igartua et al., 1999; Ivandic et al., 2003; Kraakman et al., 2004; Kraakman et al., 2006; Roy et al., 2010; Wang et al., 2012; Pasam et al., 2012; Visioni et al., 2013; Matthies et al., 2014

Table 2.2. Contiued

Maize	Plant height, starch production, endosperm color, flowering time, cell wall digestibility, concentration of oleic acid, forage quality, head smut resistance, ear length, ear weight, anthesis silking interval, anthesis date, leaf angle, leaf width, kernel ratio, tocopherol content, leaf blight, and chilling tolerance	Remington et al., 2001; Thornsberry et al., 2001; Guillet- Gluade et al., 2004; Wilson et al., 2004; Anderson et al., 2007; Yang et al., 2010; Strigens et al 2013; Wallace et al., 2014; Ding et al., 2015
Triticale	Aluminum tolerance, and pre-harvest sprouting	Divashuk et al., 2012; Niedziela et al., 2012;
Wheat	Kernel size, HMW glutenin, plant height, milling quality, plant height, protein contents, sedimentation value, weight, insect resistance, disease resistance, starch concentration, leaf rolling, spike length, spikelets/spike stem rust UG99, earliness components, grains per spike, 1000 kernel weight, grain yield, kernels per square mt, test weight, peduncle length, seminal root angle, primary root length, total root length, average root length, total root number, shoot length, carbon isotope discrimination, Psy1-A1 and Psy1-B1, drought and heat stress	Breseghello and Sorrells, 2006; Ravel et al., 2006; Roy et al., 2006, Crossa et al., 2007; Yao, et al., 2009; Adhikari et al., 2012; Reif et al., 2011; Yu et al., 2011; Zhang et al., 2011; Le Gouis et al., 2012; Cane et al., 2014; Hu et al., 2015; Liu et al., 2015; Campos et al., 2016; Mora et al., 2015; Guzman et al., 2016; Mengistu et al., 2016

2.7.4. Population structure

Population structure is the result of selection and admixture in a population and leads to high levels of linkage disequilibrium between unlinked markers (Rostoks et al. 2006). It is used in genetic studies to estimate the relationships among the individuals within a population and between different populations. It also provides an insight into evolutionary relationships of individuals in a population. Several approaches have been suggested for estimating a population structure in AM

studies, including distance-based and model-based methods. Bayesian modeling is the most frequently used model based approach, which uses allele frequencies to estimate the likelihood of an individual belonging to a particular subpopulation. This approach allocates individuals to corresponding populations, which can be incorporated into statistical models to account for the population structure in AM studies (Pritchard et al. 2000). For population structure, the software STRUCTURE has been developed and implemented in AM (Pritchard et al., 2000) in wheat (Breseghello and Sorrells 2006; Chao et al., 2007; Peng et al., 2009; Zhang et al., 2011; Laido et al., 2013; Khan et al., 2015; Liu et al., 2015; Campos et al., 2015; Guzman et al., 2016; Mengistu et al., 2016). Studies on the population structure in wheat among the worldwide accessions have detected a geographic diversity on the basis of molecular marker polymorphism (Balfourier et al. 2007). The impact of plant breeding on wheat diversity among the panels of historical wheat accessions has also been reported (White et al., 2008). Molecular markers are dependable tools for detecting population structure in a collection of wheat genotypes. However, the power to detect the underlying population structure is dependent on the type of molecular markers that are used. SSRs have the best discriminating power and SNP markers are also being extensively used because of the advent of high throughput screening technology and they have a higher frequency in the genome than SSRs. SSRs and SNPs have given similar results in estimating the structure and diversity of a population in maize (Van Inghelandt et al., 2010). The SSR and DArT markers are equally also efficient in revealing the underlying genetic structure (Couviour et al., 2011).

2.7.5. Linkage disequilibrium

LD describes the nonrandom association between alleles at different loci (Flint-Garcia et al., 2003). Genetic, biological, breeding or historical factors may increase or decrease the LD in any crops. These factors affect the population size between genetically distinct individuals as their inter-mating can influence LD (Buckller and Thornsberry, 2002) and rates of recombination and mutation also have

a strong influence on LD (Huttley et al., 1999). Mutation, genetic drift, mating system, population structure, genetic relatedness, admixture, natural or artificial selection and genomic rearrangements will increase LD whereas a high rate of recombination and mutation, recurrent mutation, gene conversion and outcrossing decrease LD (Oraguzie et al., 2007). LD decreases rapidly in outbreeding species compared to inbreeding species (Gupta et al., 2005). If the extent of LD is high, the density of the molecular markers required in a target region will be low while with low LD, the number of markers required will be high but resolution of markers will also be high (Al-Maskri et al., 2012). In self-pollinating plants, higher levels of LD were observed in comparison to outcrossing plant species (Gupta et al., 2005).

A review of previous reports on the extent of LD indicates that it varies with varying wheat populations. Crossa et al. (2007) analyzed LD in a panel of wheat lines derived from CIMMYT elite spring wheat yield trials using DArT, SSRs, AFLPs and RFLs markers. Across all the chromosomes, many LD blocks were observed with an average length of 9.93cM. The LD structure in hexaploid wheat reveals that the LD structure varies with populations, genomic regions and type of marker (Neumann et al., 2011).

Maccaferri et al., (2014) investigated elite durum accessions representing the Mediterranean region with 1200 markers (SSR/STSs and DArT markers. The LD estimates calculated for independent markers showed mean $r^2 = 0.025$ and D' = 0.23. When detectable, LD blocks with sizeable r^2 values (≥ 0.40) were in most cases observed within a 5 cM window; relatively small LD blocks were detectable across the full length of the durum wheat chromosomes, from distal to proximal regions. However, a large number of closely linked adjacent markers showed the LD r^2 values in the range of 0.3 or less.

Laido et al., (2014) used tetraploid wheat lines (*Triticum turgidum*), which included naked and hulled accessions, and analyzed the pattern of LD considering SSRs and DArT as the mostly mapped markers. In addition, to validate the potential for AM in durum wheat, they evaluated the same genotypes for plant height, heading date, protein content, and TKW. Overall, in tetraploid wheat, the pattern of LD is

extremely population dependent and related to the domestication and breeding history of durum wheat.

Spring wheat cultivars and advanced lines were evaluated by Mora et al., (2015) for plant height, kernels per spike, TKW, grain yield and carbon isotope discrimination and tested for genotyping-by sequencing-derived SNP markers across the hexaploid wheat genome. LD among SNPs was calculated for the A, B and D genomes and at the whole-genome level. LD decayed over a longer genetic distance for the D genome than for the A and B genomes. In the A and B genomes, LD declined to 50% of its initial value at about 2 cM. In the D genome, LD was much more extensive, declining to 50% of its initial value only at 22 cM. In the whole genome, LD declined to 50% of its initial value at an average of 4 cM. Important genomic regions associated with complex traits in spring wheat were identified. Selection in these regions may increase the efficiency of the current breeding programs. Although most of the associations were environment specific, some stable associations were detected for carbon isotope discrimination, kernels per spike, plant height and TKW. Environment-specific genomic regions were detected, indicating the presence of QTL-by-environment interaction. Mengistu et al., (2016) tested markers and scored single nucleotide polymorphisms and surveyed the diversity, structure, and genome-specific variation of Ethiopian germplasm using a siding collection of Mediterranean durum wheat accessions. The LD measures between all pairs of SNPs on each chromosome were collapsed in a matrix reporting the inter-marker distances and used to calculate the LD decay in 50 cM with a custom script.

3-MATERIAL

3.1. Plant material and association mapping

The plant material in this study consisted of a panel of 130 durum wheat (*Triticum turgidium* L.) genotypes originating from a wide range of ecological conditions, including 71 cultivars (50 historical cultivars from Turkey and 21 foreign cultivars from different countries) and 59 landraces (44 landraces from the Izmir gene bank and 15 locally adapted landraces collected from different sources). Full details of these genotypes are presented in Appendix 1.

3.2. Field trials:

The field trials were conducted for two consecutive years within a total of five environments. The details of each location/environment and year are given in Table 3.1

Table 3.1. The location/environment details used for growing the genotypes with years and abbreviations.

Year	Environment-Location	Abbreviation
2013/2014	Rainfed; Çukurova Uni station-Adana-Turkey	Ada-14
2014/2015	Rainfed; Low land-Çukurova Uni station-Adana-Turkey	Ada-15-L
	Rainfed; High land-Çukurova Uni station-Adana-Turkey	Ada-15-H
	Rainfed; Field crops vocational school-Kozan-Turkey	Koz-15
	Rainfed;Konya-Turkey	Kon-15

3.2.1. Testing Sites

The experiment was conducted at three locations during the 2013/14 season and two locations during 2014/15. The details for each location are given below.

3.2.1.1. Adana location

This site is located at the research and implementation area of Field Crops Department of Çukurova University, Adana, Turkey (37^0 21'N latitude, 35^0 10'E longitude, 20 m above the sea level). This station has a typical Mediterranean climate with hot and dry summers but with high humidity. However; during winter, this location receives more precipitation than the other locations selected for this study. During the field-experiments in 2013/14 and 2014/15 seasons, the total precipitation amounted to 367.2 and 782 mm, respectively. Nitrogen (180 kg/ha) and phosphorus fertilizers (60 kg/ha) were applied. The experiments were established according to an augmented experimental design (Federer, 1956). During the whole trial, there were a total of 168 entries (130 genotypes plus 5 checks Sham1, Sarıbaşak, Amanos-97 and two times Fuatbay-2000 repeated in each block). The genotypes were divided over 7 blocks, 19 genotypes were included in each block, with five checks using commercial varieties. These checks were replicated and included in every block. Thus, each block consisted of 24 entries (19 genotypes and 5 checks). The genotypes were sown in rows and 30 cm row spacing. The experimental area was kept free from weeds and diseases by spraying with herbicides and fungicides, respectively. Agronomic and plant protection measures were maintained at a normal level during all the e experiments.

3.2.1.2. Kozan/Adana Location

This area is within the research grounds of the vocational school of Cukurova University, Kozan/Adana, Turkey, (37^0 45'N latitude, 35^0 81' E longitude 140 m above sea level). The climate is mild, and generally warm and temperate with the average temperature being 19.2 °C. The rain in Kozan falls mostly in the winter, with relatively little precipitation in the summer. The annually rainfall here averages 795 mm, with the total precipitation of 830 mm during the field-experiments in 2014/15. Nitrogen and phosphorus fertilizers were applied as 180 kg/ha of N and 60 kg/ha of P_2O_5. The experiments were established according to an augmented experimental

design (Federer, 1956). The total number of entries of the whole trial was 168 (130 genotypes plus 5 checks Sham1, Sarbaşak, Amanos-97 and two times Fuatbay-2000 repeated in each block). The genotypes were divided over 7 blocks, each containing 19 genotypes, with five checks. These checks were replicated and included in every block. Thus, each block consisted of 24 entries (19 genotypes and 5 checks). The checks were commercial varieties. Genotypes were sown in rows and 30 cm row spacing. The experiments were kept free from weeds and diseases by spraying with herbicides and fungicides, respectively. Agronomic and plant protection measures were maintained as normal during the entire experimental period.

3.2.1.3. Konya Location

Located at 37° 58'N, 32° 32'E, 1031 m, it has a mid-latitude steppe/ semi-arid cool climate (Köppen-Geiger classification: BSk). According to the Holdridge life zones system of bioclimatic classification, Konya is situated in or near the cool temperate steppe biome. Average monthly temperatures vary by 23.3°C, with total annual precipitation averaging 338.2 mm. During the field experiments in 2014/15 seasons, the total amount of precipitation was 545.5 mm. Nitrogen and phosphorus fertilizers of 180 kg/ha and 60 kg/ha, respectively were applied. The experiments were established according to augmented experimental design (Federer, 1956). The total number of entries of the whole trial was 168 (130 genotypes plus 5 checks Sham1, Sarıbaşak, Amanos-97 and two times Fuatbay-2000 repeated in each block). The genotypes were divided over 7 blocks with 19 genotypes included in each block with five checks. These checks were replicated and included in every block. Thus, each block consisted of 24 entries (19 genotypes and 5 checks) and the checks were commercial varieties. Genotypes were sown in rows and 30 cm row spacing. The experiments were kept free from weeds and diseases by spraying with herbicides and fungicides, respectively. Agronomic and plant protection measures were kept at a normal level during all the experiments.

3.3. Agro-Morphological Traits

Several agro-morphological traits were investigated under different agro-environmental conditions. In each location for some traits, ten plants from each genotype were randomly sampled and all observations were undertaken on these individual specimens. All the plants were harvested by machine at the beginning of June 2015, when grain moisture was about 13. Seed and spikes were kept in a cold and safe place.

Days to heading (day): Number of days from emergence to the day when 75% of main spikes have emerged from the flag leaf.

Days to maturity (day): Number of days from emergence to the day when 75% of peduncle turn to yellow.

Plant height (cm): Length of the plants main culm at maturity from the base of the culm to the tip of the spike; awns were excluded.

Lodging degree: Score stem lodging degree visually estimates the percentage of the lodged area of plot and then estimates the angle of stem lodging. The score is recorded using a scale from (1) no lodging to (9) heavy lodging.

Peduncle length (cm): Length of the top internode of the main culm

Peduncle extrusion length (cm): The extrusion length of the mother shoot was measured at maturity in centimeters from the base of the spike to the end of the flag leaf sheath or the ligule of the flag leaf.

Ten spikes from each plot were collected and visual evaluation of the important spikes traits was scored:

Spike length (cm): Length of main spike measured from the base of the spike to the terminal spikelet.

Spikelet number per spike: Number of spikelets in the main spike

Grain number per spike: Number of grains in the main spike.

Spike weight (g): The weight of spike**.**

Grain weight per spike (g): The weight of number of grains in the main spike.

All grains per spike were kernels were manually counted in five groups and any foreign material or broken kernels were excluded.

Spike Harvest Index: Calculated as ratio of spike yield / spike weight.

3.4. Grain quality traits

Seed samples were cleaned, detritus such as bits of straw and soil were removed and then the following traits were measured:

Hectoliter weight: The weight of one liter of clean seed.

Thousand-kernel weight (g): 200 kernels were manually counted in five groups and any foreign material or broken kernels were excluded.

Vitreousness kernel count (%): The 200 grains counted for kernel weight determination were inspected and manually evaluated for the vitreousness and yellow berry grains. Extra care was taken to avoid confusion between non-vitreous kernels and Suni bug damaged kernels.

3.5. DNA extraction

Total genomic DNA was isolated from young leaves according to CTAB protocol with some modification Özkan et al., (2005). The extracted DNA was evaluated qualitatively in addition to quantity, measured by 0.8 % agarose gel electrophoresis. Before using DNA for molecular analysis, the DNA diluted to a concentration of required concentration, 10 ng/㎖ for SSR applications and 75 ng/㎖ for DArTseq analysis. The DNA of each sample was sent to Triticarte Pty. Ltd, Australia (http://www. triticarte.com.au/) for DArTseq analysis.

3.5.1. Simple sequence repeats (SSRs) or microsatellite analysis

A total of 120 SSR primers covering all the whole wheat genomes were first screened on four wheat genotypes to detect their polymorphism level. Different source of microsatellites were used and their information is briefly described in

Table 3.2. The 87 polymorphic SSR primers were screened for the whole set of genotypes (Appendix 2). The M13-tailed primer method was used for PCR amplification of the SSRs (Schuelke, 2000). PCR was performed using a forward primer with a nucleotide extension at its 5'-end, identical to the sequence of an M13 sequencing primer (5-TGTAAAACGAAGGCCAGT-3), a standard length reverse primer and a fluorescently labeled M13 primer. The SSR fragments were scored and checked twice using the Gene Mapper software v3.7 (Applied Biosystems) as described in the user manual.

Table 3.2. Different sources of microsatellites used in genotyping

Microsatellite developer	**Abbreviation for SSRs**	**SSR used**	**Publishing Reference**
Perry Cregan (USDA)	BARC	7	Song et al., (2005)
Pierre Sourdille (INRA)	CFA	8	http://wheat.pw.usda.gov
Pierre Sourdille (INRA)	CFD	6	http://wheat.pw.usda.gov
Pierre Sourdille (INRA)	CFE	1	http://wheat.pw.usda.gov
Peng, Junhua	CWEM	2	Peng and Lapitan (2005)
Marion Röder (IPK)	GWM	15	Röder et al., (1998)
Peter Isaac	WMC	48	Somer et al., (2004)

PCR amplifications were carried out according to the modified protocol given by Schuelke (2000). M13 tailed-primer PCR amplification of SSRs was performed in a 12µl PCR mix containing 1X buffer, 0.125 mM dNTPs, 0.4 pmol M13 sequence tailed forward primer, 0.3 pmol reverse primer, 3.0 pmol universal M13 primer labeled with one of four (6-FAM, VIC, NED or PET) fluorescent dyes, 0.12U *Taq* DNA polymerase, and approximately 25 ng genomic DNA. PCR amplification was performed with initial denaturation at 94°C for 5 min; 30 cycles of 94 °C for 1 min, 55 to 65 °C (annealing temperature depending on primers) for 1 min, 72 °C for 1

min; followed by 8 cycles of 94°C for 30 s, 53°C for 45 s, and 72°C for 45 s; and final extension at 72°C for 10 min. A set of four PCR products (1 µl each) labeled with a different dye was combined with 0.25µl GeneScan-500 LIZ® size standards (Applied Biosystems) and 9.86µl Hi-Di™ Formamide (Applied Biosystems), denatured at 94°C for 5 min, chilled on ice, and separated on an ABI 3130xl Genetic Analyzer (Applied Biosystems).

3.5.2. Genotyping by sequencing (GBS) analysis.

Genotyping by a sequencing analysis of 130 DNA durum wheat genotypes was performed using a whole genome profiling service. For each genotype, 100 ml of 50-100 ng/ml DNA solution was sent to Diversity Array Technology for SNP and DArTseq analysis (for more information, see www.diversityarrays.com). The protocols of DArTseq have been described by Akbari et al., (2005). DArTseq analysis generated 34,130 SilicoDArT markers and 30,376 SNP markers in 130 durum genotypes.

3.6. Statistical analysis of phenotypic and molecular data

For variance analysis (ANOVA) and molecular variance analysis (AMOVA) the 130 genotypes were categorized into four groups (A, B, C and D) according of the sources of this genotypes. Whereas; group (A) consisted from Turkish released cultivars (Turkish cvs), group (B) is foreign cultivars (foreign cvs) from different countries, group (C) is landraces from Izmir-gene bank (gene bank landraces) and group (D) is landraces locally growing (growing landraces), where the (A, B, C and D) groups have been contained 50, 21, 44 and 15 genotypes, respectively.

The variance analysis for all the investigated morphological traits were undertaken according to Steel et al., (1997) using the JMP 7 software. In addition, the Pearson correlation coefficients (r), the significance of each correlation coefficient and phenotypic frequency distribution were obtained using the same software.

The broad-sense heritability values (h^2) calculated by the Plant Breeding Tool (PBTools) software as ratios of genetic and phenotypic variances (σ^2_g/σ^2_p), where σ^2_p is the phenotypic variance and was estimated as $\sigma^2_g + \sigma^2_e$, with σ^2_g being the genetic variance and σ^2_e is the residual variance (Roff, 1997).

All the images from the DArTseq and SNP platforms were analyzed using DArTsoft v.7.4.7 (DArT P/L, Canberra, Australia). The SSR, DArTseq and SNP markers were scored as binary data (1/0), indicating presence or absence of a marker in genomic representation of each sample as described by Akbari et al., (2005) Scoring the alleles in a binary format simplifies the assessment and statistical analysis for combined analysis using dominant and codominant data (Kaya et al., 2016; Ferrao et al. 2014; Kosman and Leonard 2005). The DArTseq software automatically computes several quality parameters for each marker such as the call rate, PIC value and reproducibility of DArTseq and SNP markers.

The AMOVA is an important statistical procedure that allows the hierarchical partitioning of genetic variation among populations and regions, and the estimation of the widely used *F*-statistics and/or their analogues. In order to compare different ways of partitioning variance, an AMOVA was conducted on the genotypes using the software program GenAlEx 6.1 (Peakall and Smouse, 2001). The program determines the variance components and estimates the total variation within and among the populations. A tree diagram was established using the DARWin 6.0.13 (Perrier, et al., 2003) software according to the Neighbor-Joining method (Saitou and Nei 1987)

Principal coordinate analysis (PCoA) is a platform that provides a spatial illustration of the comparative genetic distances between the genotypes. It also assesses the robustness of the differentiation among the groups classified by a dendrogram (Liu et al. 2013). In this work, the germplasms were divided into four groups; Turkish cultivars, foreign cultivars, Turkish-gene bank landraces, and landraces still commonly grown in local form. PCoA were performed separately on all the groups and as well as the overall population.

The definition of population structure was performed with the STRUCTURE v.2.3.4 software (Pritchard et al., 2000) using a model-based clustering method. The

Structure analysis was carried out with K = 10,5000 burning length and 50,000 reps over 10 iterations, and then the results were run in the Structure Harvester version 0.6.94 software (Earl and Von Holdt, 2012) for an estimated K for the 130 genotypes. Delta K (ΔK) is based on the rate of change in the log probability of data between successive K values. This was used to determine the number of clusters (K) in the population (Evanno et al., 2005).

The genetic variance was partitioned both within and among the accessions and the three populations suggested by STRUCTURE. Association between markers and traits was tested using a generalized linear model (GLM) and a mixed linear model (MLM) within the program TASSEL version 5.2.22 (http://www.maizegenetics.net) where the marker being tested was considered as a fixed-effects factor and the mapping population was considered as a random-effects factor (Kennedy et al., 1992). The significance of the associations between loci and traits was based on an F-test, at a level αc corresponding to α corrected for multiple testing. Corrected significance levels αc were computed by 1000 permutations within a chromosome. These false associations can be partially corrected with a structured association method that uses a Q-matrix of population membership estimates, and was further reduced by incorporating multiple background QTLs as a random effect in a mixed model based on the premise that the genome of each individual or line is a sample of the gene pool of its population. The average relationship between individuals or lines can be estimated by kinship (K) calculated from a suitable number of random markers across the entire genome. The Q + K method, combining information from both Q and K, was shown to be superior to more conventional linear models in association analyses (Yu et al., 2006). The Q + K method has been implemented in TASSEL as a MLM function.

Significant associations were detected based on a P-value threshold of α=0.01. The use of an adjusted corrective threshold for multiple testing was also investigated. The Bonferroni threshold for multiple testing is defined as α – the probability level - divided by the number of tests; for instance in the current study, a 5% Bonferroni threshold was calculated as 0.05/number of markers tested.

4. RESULTS AND DISCUSSION

4-1- Phenotypic Variations

Wide differences among the study materials were visually observed for many traits during field evaluations. Phenotypic data was collected for a total of 14 traits, but this number varied depending on the year and location. Analysis of variance of augmented design was performed on the 14 traits for the two consecutive years of 2013-2014 and 2014-2015. These genotypes showed significant variation for all of the 14 traits (Table 4.1) as given below:

4.1.1. Agronomic characteristics

4.1.1.1. Days to heading

The average days to heading for all locations varied from 79.7 days to 121.3 days with genotypes mean of 102.9 days. The averaged values of the days to heading for Ada-14, Ada-15-L and Ada-15-H locations were 97 days, 103.1 days, 108.6 days, respectively (Table 4.1). In three locations, the longest HD was found in the genebank landraces, followed by the growing landraces. The average days to heading for genebank landraces was the highest (110.3 days) while foreign cvs was the lowest (94.9 day) (Figure 4.1).

The broad-sense heritability values for HD in Ada-14, Ada-15-L, Ada-15-H and the average of the three locations were 0.98, 0.98, 0.91 and 0.97, respectively (Table 4.1). Higher significant correlation coefficient values were found between HD and MD, number of spikelet per spike, plant height and spike length with *r* values of 0.87**, 0.67**, 0.61** and 0.53**, respectively (Table 4.2). A positive correlation between plant height and days to heading was reported by Ahmad (2013). MacCartney et al., (2005) found also positive significant correlation between days to heading and plant height.

4.1.1.2. Days to maturity

The average days to maturity for all locations varied from 140.5 days to 157.5 days with genotypes mean of 150.0 days. The averaged values of days to maturity for Ada-15-L and Ada-15-H locations were 149.5 days, and 150.6 days, respectively (Table 4.1). The average days to maturity was the highest (153.5 days) for genebank landraces while it was lowest (145.7 day) in the foreign cvs (Figure 4.1).

The broad-sense heritability values for MD in Ada-15-L, Ada-15-H and the averages of the three locations were 0.67, 0.98, and 0.92, respectively (Table 4.1). Days to maturity was significantly correlated with days to heading, spikelets number per spike, plant height and spike length a with r-values of 0.87**, 0.71**, 0.58** and 0.55**, respectively.

4.1.1.3. Plant height

The average plant height for all locations varied from 72.6 cm to 162.0 cm with the genotypes mean being 112.0 cm. The average plant heights for Ada-14, Ada-15-L, Ada-15-H, Koz-15 and Kon-15 locations were 102. 6 cm, 133.8 cm, 115.6 cm, 104.4 cm and 103.1 cm, respectively (Table 4.1). The average plant height for gene bank landraces was the highest (136.0 cm) and lowest for foreign cvs (91.3 cm day) (Figure 4.1).

The broad-sense heritability values for PH in Ada-14, Ada-15-L, Ada-15-H, Koz-15 and Kon-15 locations were 0.98, 0.98, 0.98, 0.98 and 0.97, respectively (Table 4.1). Plant height was significantly positive correlated to peduncle length, peduncle extrusion length, days to heading and days to maturity with *r* values of 0.85**, 0.69**, 0.61** and 0.58**, respectively. Similarly, Baum et al. (2003), reported significant positive correlation between plant height and grain yield at a dry site in Syria.

4.1.1.4. Lodging

The average lodging degree for all locations varied from 0 to 9 cm with a mean value of 1.7 cm. Average lodging degree values for Ada-15-L, and Ada-15-H locations were 0.6 and 2.7, respectively (Table 4.1). The average of the lodging degree for gene bank landraces was the highest (3.5) while for foreign and Turkish cvs, it were the lowest (0.0; 0.4, respectively) (Figure 4.1). The lodging degree score was significantly and positively correlated with plant height, peduncle length, and peduncle extrusion length with *r* values of 0.67**, 0.67** and 0.59**, respectively.

4.1.1.5. Peduncle length

The average peduncle length for all locations varied from 28.2 cm to 56.7 cm with the genotypes mean being 44.5 cm. Average peduncle length values for Ada-15-L, Ada-15-H, Koz-15 and Kon-15 locations were 48.6 cm, 43.8 cm, 44.6 cm and 39.9 cm, respectively (Table 4.1). The average peduncle length for gene bank landraces was the highest (51.7cm) while for foreign cvs it was the lowest (37.8 cm) (Figure 4.1).

The broad-sense heritability values for peduncle length in Ada-14, Ada-15-L, Ada-15-H, Koz-15 and Kon-15 locations were 0.87, 0.95, 0.97 and 0.90, respectively (Table 4.1). Peduncle length was significantly positive correlated to peduncle extrusion length and plant height with *r* values of 0.87**, 0.85**, respectively. The correlation with the test weight was the weakest (-0.06) (Table 4.2). The PL values for genotypes in each environment gave continuous frequency distribution, with the greatest frequency scoring from 37.5 to 40 cm (Figure 4.2).

4.1.1.6. Peduncle extrusion length

The average peduncle extrusion length for all locations varied from 8.4 cm to 37.5 cm with an average of 20.5 cm. The averaged values of peduncle extrusion length for Ada-15-L, Ada-15-H and Koz-15 locations were 23.5 cm, 16.4 cm and

21.5 cm, respectively (Table 4.1). The averaged values of peduncle length for the genebank and growing landraces were higher (24.1 cm and 23.6 cm; respectively) than those for the other groups of genotypes (Figure 4.1).

The broad-sense heritability values for peduncle extrusion length in Ada-15-L, Ada-15-H and Koz-15 locations were 0.93, 0.95 and 0.96, respectively (Table 4.1). In this study, it was observed that genotypes having high peduncle length also had a high peduncle extrusion length. The peduncle extrusion length was significantly positive correlated to peduncle length and plant height with *r* values of 0.87**, and 0.69**, respectively (able 4.2). Similar results were reported by Gupta et al., (2001), Baum et al., (2003), Reynolds et al., (2006, MacCartney et al., (2005), Huang et al., (2006), Yücel et al., (2009), Bogale et al., (2011), Ahmad (2013) and Edae et al., (2013).

Table 4.1. The means, standard deviations, ranges, variance, standard error and broad-sense heritabilities (h^2) of the investigated morphological traits during the 2013/14 and 2014/15 growing seasons together with the different environments and their average.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
HD (day)	Ada-14	Turkish cvs	92.3 ± 11.1	75 -110	122.6	1.6	
	Ada-14	Foreign cvs	94.6 ± 10.9	75 -121	118.8	2.4	
	Ada-14	Gene bank LDs	103.3 ± 11.5	75 -115	132.3	1.7	
	Ada-14	Growing LDs	95.8 ± 12.8	75 -118	164.2	3.3	
	Ada-14	All groups	97.0 ± 12.3	75-121	150.9	1.1	0.98
	Ada-15-L	Turkish cvs	99.6 ± 11.7	81-116	137.8	1.7	
	Ada-15-L	Foreign cvs	92.3 ± 10.1	81 -111	101.3	2.2	
	Ada-15-L	Gene bank LDs	111.4 ± 6.7	86 -120	44.9	1	
	Ada-15-L	Growing LDs	104.7 ± 6.9	93 -115	47.1	1.8	
	Ada-15-L	All groups	103.1 ± 11.6	81-120	135.4	1	0.98
	Ada-15-H	Turkish cvs	106 ± 10.2	91-119	103.4	1.4	
	Ada-15-H	Foreign cvs	97.9 ± 8.6	83-117	73.9	1.9	
	Ada-15-H	Gene bank LDs	116.1 ± 5.4	92-123	29.3	0.8	
	Ada-15-H	Growing LDs	109.3 ± 7.8	92-121	60.4	2	
	Ada-15-H	All groups	108.6 ± 10.4	83-123	107.8	0.9	0.91

Table 4.1. continued.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Average	Turkish cvs	99.3 ± 9.6	82.3-115	92.1	1.4	
	Average	Foreign cvs	94.9 ± 8.5	79.7-116.3	72.1	1.9	
	Average	Gene bank LDs	110.3 ± 5.6	84.3-119.3	31.9	0.9	
	Average	Growing LDs	103.3 ± 5.4	86.7-118	28.7	1.4	
	Average	All groups	102.9 ± 9.6	79.7-121.3	92.5	0.8	0.97
MD (day)	Ada-15-L	Turkish cvs	147.8 ± 5.2	140-156	26.8	0.7	
	Ada-15-L	Foreign cvs	145.7 ± 4.4	141-155	19.1	1	
	Ada-15-L	Gene bank LDs	153.1 ± 1.7	148-156	3	0.3	
	Ada-15-L	Growing LDs	149.5 ± 4.2	143-155	17.7	1.1	
	Ada-15-L	All groups	149.5 ± 4.9	140-156	24	0.4	0.67
	Ada-15-H	Turkish cvs	149.8 ± 5.8	141-159	33.9	0.8	
	Ada-15-H	Foreign cvs	145.8 ± 4.9	141-156	24.3	1.1	
	Ada-15-H	Gene bank LDs	154 ± 2.7	144-158	7.1	0.4	
	Ada-15-H	Growing LDs	150.1 ± 5.2	144-158	26.7	1.3	
	Ada-15-H	All groups	150.6 ± 5.5	141-159	30.1	0.5	0.98
	Average	Turkish cvs	148.9 ± 5.4	140.5-157.5	28.8	0.8	
	Average	Foreign cvs	145.7 ± 4.5	141-155.5	20.2	1	
	Average	Gene bank LDs	153.5 ± 1.9	146-157	3.7	0.3	

Table 4.1. continued

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Average	Growing LDs	150.0 ± 4.4	143.5-155.5	19.1	1.1	
	Average	All groups	150.0 ± 5.0	140.5-157.5	25.3	0.4	0.92
PH (cm)	Ada-14	Turkish cvs	87.7 ± 14.9	50-140	223.3	2.1	
	Ada-14	Foreign cvs	81.8 ± 11.8	65-98	139.7	2.6	
	Ada-14	Gene bank LDs	122.4 ± 17.7	86-160	313.6	2.7	
	Ada-14	Growing LDs	98.2 ± 17.7	70-133	313.4	4.6	
	Ada-14	All groups	102.6 ± 23.2	50-160	536.9	2	0.98
	Ada-15-L	Turkish cvs	114.1 ± 20.4	91-175	414.3	2.9	
	Ada-15-L	Foreign cvs	100.0 ± 8.8	88 -120	77.5	1.9	
	Ada-15-L	Gene bank LDs	168.2 ± 16.5	106-192	270.9	2.5	
	Ada-15-L	Growing LDs	145.9 ± 22.8	12-172	521.4	5.9	
	Ada-15-L	All groups	133.8 ± 32.8	88-192	1072.9	2.9	0.98
	Ada-15-H	Turkish cvs	99.8 ± 16.2	83-144	263.3	2.3	
	Ada-15-H	Foreign cvs	96.3 ± 15.0	80-147	225.3	3.3	
	Ada-15-H	Gene bank LDs	141.1 ± 27.9	84-168	776.7	4.2	
	Ada-15-H	Growing LDs	121.6 ± 15.0	100-141	226.1	3.9	
	Ada-15-H	All groups	115.6 ± 28.4	80-168)	805	2.5	0.98

Table 4.1. continued.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Koz-15	Turkish cvs	92.6 ± 13.5	78-136	181	1.9	
	Koz-15	Foreign cvs	83.2 ± 9.2	74 -111	83.7	2	
	Koz-15	Gene bank LDs	125.9 ± 19.3	76-147	374.1	2.9	
	Koz-15	Growing LDs	108.3 ± 16.8	79-139	281.8	4.3	
	Koz-15	All groups	104.4 ± 22.9	71-147	522.4	2	0.98
	Kon-15	Turkish cvs	94.9 ± 10.8	77-134	115.8	1.5	
	Kon-15	Foreign cvs	86.7 ± 7.9	74-109	63.2	1.7	
	Kon-15	Gene bank LDs	121.5 ± 11.5	93-143	131.6	1.7	
	Kon-15	Growing LDs	105.2 ± 11.7	88-125	136.7	3	
	Kon-15	All groups	103.1 ± 17.1	74-143	292.1	1.5	0.97
	Average	Turkish cvs	97.9 ± 14.4	80.8-143.5	207.9	2	
	Average	Foreign cvs	91.3 ± 8.8	80.8-121.7	78.2	1.9	
	Average	Gene bank LDs	136 ± 11.8	102.5-154.2	139.6	1.8	
	Average	Growing LDs	116.4 ± 13	93-139	168.5	3.4	
	Average	All groups	112 ± 22.4	72.6-162	501.3	2	0.98
Lodging	Ada-15-L	Turkish cvs	0.0 ± 0.0	0 -0	0	0	
	Ada-15-L	Foreign cvs	0.0 ± 0.0	0 -0	0	0	
	Ada-15-L	Gene bank LDs	1.1 ± 2.7	0 -9	7.2	0.4	

Table 4.1. continued.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Ada-15-L	Growing LDs	1.9 ± 3.4	0 -8	11.5	0.9	
	Ada-15-L	All groups	0.6 ± 2.0	0 -9	4.2	0.2	NA
	Ada-15-H	Turkish cvs	0.7 ± 2.3	0 -8	5.2	0.3	
	Ada-15-H	Foreign cvs	0.0 ± 0.0	0 -0	0	0	
	Ada-15-H	Gene bank LDs	5.9 ± 3.7	0 -9	13.5	0.6	
	Ada-15-H	Growing LDs	3.7 ± 4.2	0 -9	17.2	1.1	
	Ada-15-H	All groups	2.7 ± 3.8	0 -9	14.7	0.3	NA
	Average	Turkish cvs	0.4 ± 1.1	0-4	1.3	0.2	
	Average	Foreign cvs	0.0 ± 0.0	0-0	0	0	
	Average	Gene bank LDs	3.5 ± 2.5	0-9	6.5	0.4	
	Average	Growing LDs	2.8 ± 3.3	0-8.5	11.1	0.9	
	Average	All groups	1.7 ± 2.5	0-9	6.3	0.2	NA
PL (cm)	Ada-15-L	Turkish cvs	43.0 ± 5.4	31.0-58.4	28.8	0.8	
	Ada-15-L	Foreign cvs	40.6 ± 4.6	34.6 -53.3	21	1	
	Ada-15-L	Gene bank LDs	57.3 ± 6.6	41.5 -68.0	43.2	1	
	Ada-15-L	Growing LDs	53.2 ± 7.9	40.8 -66.5	62	2	
	Ada-15-L	All groups	48.6 ± 9.3	31.0-68.0	86.1	0.8	0.87
	Ada-15-H	Turkish cvs	38.9 ± 4.9	26.4-49.4	23.5	0.7	

Table 4.1. continued.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Average	All groups	44.5 ± 7.6	28.1-56.7	58.1	0.7	0.99
PEL (cm)	Ada-15-L	Turkish cvs	20.5 ± 4.1	9.0-30.9	16.8	0.6	
	Ada-15-L	Foreign cvs	19.5 ± 4.1	11.4 -26.1	16.5	0.9	
	Ada-15-L	Gene bank LDs	27.7 ± 5.8	15.6 -37.9	34.1	0.9	
	Ada-15-L	Growing LDs	26.8 ± 6.9	13.3 -38.1	47.1	1.8	
	Ada-15-L	All groups	23.5 ± 6.2	9.0-38.1	38.9	0.5	0.93
	Ada-15-H	Turkish cvs	14.1 ± 2.8	8.2-20.2	7.8	0.4	
	Ada-15-H	Foreign cvs	15.2 ± 3.2	8.2 -21.6	10.3	0.7	
	Ada-15-H	Gene bank LDs	18.7 ± 4.7	8.2 -32.0	22.4	0.7	
	Ada-15-H	Growing LDs	18.7 ± 3.9	10.8 -25.3	14.9	1	
	Ada-15-H	All groups	16.4 ± 4.3	8.2-32.0	18.4	0.4	0.95
	Koz-15	Turkish cvs	18.0 ± 3.7	10.8-28.0	13.8	0.5	
	Koz-15	Foreign cvs	18.2 ± 7.1	8.0 -42.5	51.1	1.6	
	Koz-15	Gene bank LDs	26.3 ± 5.5	11.7 -36.0	29.9	0.8	
	Koz-15	Growing LDs	24.5 ± 6.2	13.0 -34.3	38.5	1.6	
	Koz-15	All groups	21.5 ± 6.5	8.0-42.5	42.7	0.6	0.96
	Average	Turkish cvs	17.5 ± 2.9	10.0-24.8	8.6	0.4	
	Average	Foreign cvs	17.7 ± 3.9	10.0 -25.6	15.1	0.8	

Table 4.1. continued.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Average	All groups	44.5 ± 7.6	28.1-56.7	58.1	0.7	0.99
PEL (cm)	Ada-15-L	Turkish cvs	20.5 ± 4.1	9.0-30.9	16.8	0.6	
	Ada-15-L	Foreign cvs	19.5 ± 4.1	11.4 -26.1	16.5	0.9	
	Ada-15-L	Gene bank LDs	27.7 ± 5.8	15.6 -37.9	34.1	0.9	
	Ada-15-L	Growing LDs	26.8 ± 6.9	13.3 -38.1	47.1	1.8	
	Ada-15-L	All groups	23.5 ± 6.2	9.0-38.1	38.9	0.5	0.93
	Ada-15-H	Turkish cvs	14.1 ± 2.8	8.2-20.2	7.8	0.4	
	Ada-15-H	Foreign cvs	15.2 ± 3.2	8.2 -21.6	10.3	0.7	
	Ada-15-H	Gene bank LDs	18.7 ± 4.7	8.2 -32.0	22.4	0.7	
	Ada-15-H	Growing LDs	18.7 ± 3.9	10.8 -25.3	14.9	1	
	Ada-15-H	All groups	16.4 ± 4.3	8.2-32.0	18.4	0.4	0.95
	Koz-15	Turkish cvs	18.0 ± 3.7	10.8-28.0	13.8	0.5	
	Koz-15	Foreign cvs	18.2 ± 7.1	8.0 -42.5	51.1	1.6	
	Koz-15	Gene bank LDs	26.3 ± 5.5	11.7 -36.0	29.9	0.8	
	Koz-15	Growing LDs	24.5 ± 6.2	13.0 -34.3	38.5	1.6	
	Koz-15	All groups	21.5 ± 6.5	8.0-42.5	42.7	0.6	0.96
	Average	Turkish cvs	17.5 ± 2.9	10.0-24.8	8.6	0.4	
	Average	Foreign cvs	17.7 ± 3.9	10.0 -25.6	15.1	0.8	

Table 4.1. continued.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Average	Gene bank LDs	24.1 ± 4.5	13.0 -31.7	20	0.7	
	Average	Growing LDs	23.6 ± 5.0	12.8 -30.3	25.3	1.3	
	Average	All groups	20.5 ± 5.0	8.4-37.5	25.4	0.4	0.98

SD: Standard deviation, SE: Standard Error, HD: Days to heading (day), MD: Days to maturity (day),

PH: Plant Height (cm), Lodging: Lodging degree, PL: Peduncle length (cm), PEL: Peduncle extrusion length (cm).

Table 4.2 Pearson's phenotypic correlation coefficients among agronomic and yield related traits calculated from the mean of the five environments for 130 durum wheat genotypes

	HD	MD	PH	PL	PEL	SL	SW	SY	SHI	SNPS	GNPS	TKW	VCK
HD	1												
MD	0.87**	1											
PH	0.61**	0.58**	1										
PL	0.45**	0.42**	0.85**	1									
PEL	0.26*	0.22	0.69**	0.87**	1								
SL	0.53**	0.55**	0.32**	0.11	-0.05	1							
SW	0.097	0.17*	-0.03	-0.12	-0.16	0.44*	1						
SY	-0.10	-0.01	-0.27**	-0.31**	-0.31**	0.24**	0.79**	1					
SHI	-0.49**	-0.47**	-0.30**	-0.21*	-0.09	-0.30**	0.02	0.14**	1				
SNPS	0.67**	0.71**	0.4**	0.25*	0.08	0.71**	0.40**	0.15*	-0.33**	1			
GNPS	-0.21**	-0.16*	-0.5**	-0.49**	-0.41**	0.27	0.71**	0.66**	0.2*	0.18	1		
TKW	0.17*	0.20**	0.40**	0.34**	0.25**	0.06	0.36**	0.20	0.17	0.08	-0.27**	1	
VCK	0.11**	0.16**	0.25**	0.22**	0.18**	0.04	-0.13	-0.23**	0.01	-0.01	-0.23**	0.11	1
TW	-0.47**	-0.45**	-0.14	-0.06	0.04	-0.40**	-0.06	-0.08	0.38**	-0.40**	0.02	0.08	-0.09

*. Correlation is significant at P≤ 0.05, **. Correlation is significant at P≤0.01

Green and yellow boxes indicate the highest and lowest correlations, respectively.

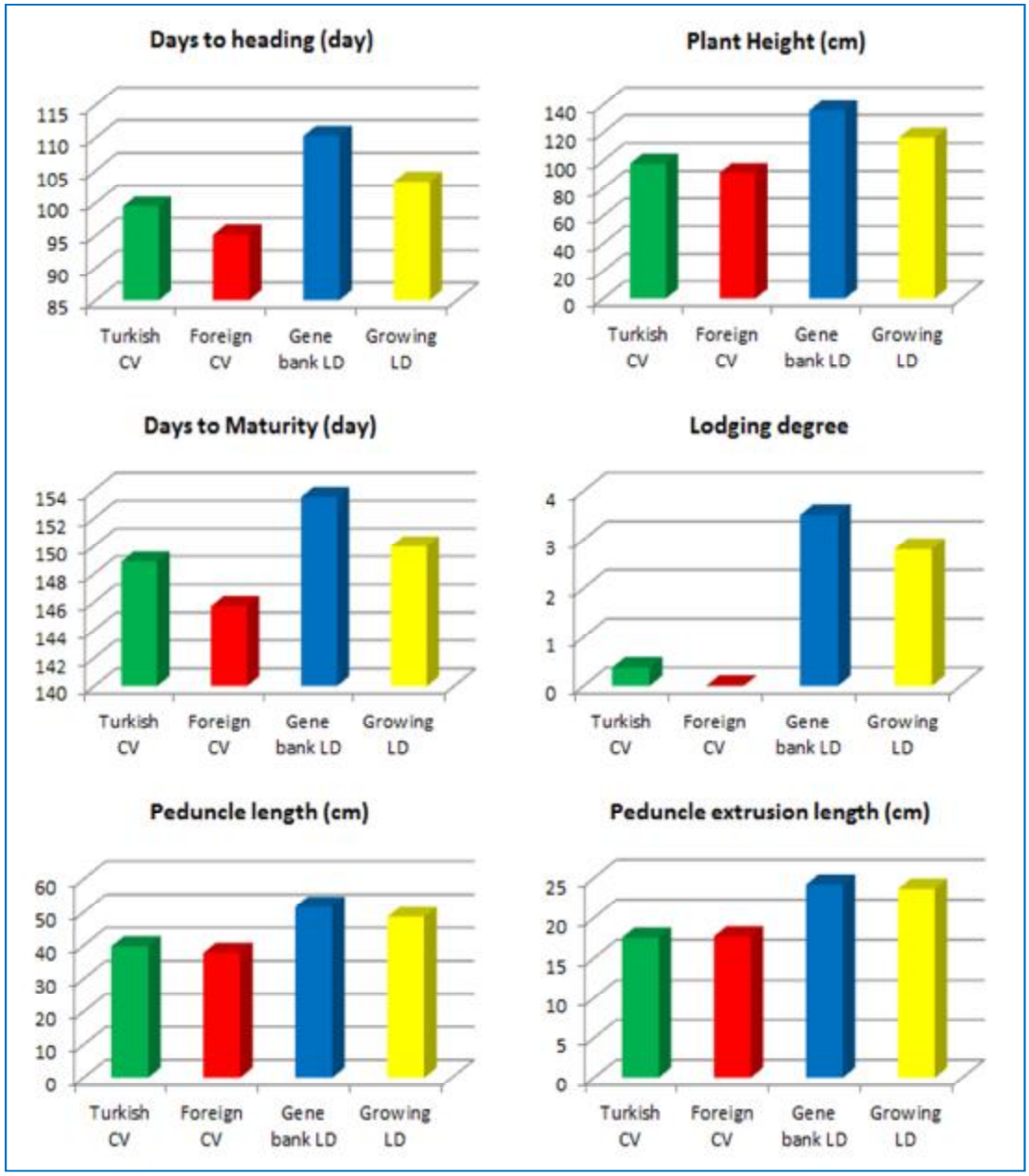

Figure 4.1. Significant variations in the average values of traits found among different groups of the durum wheat diversity panel.

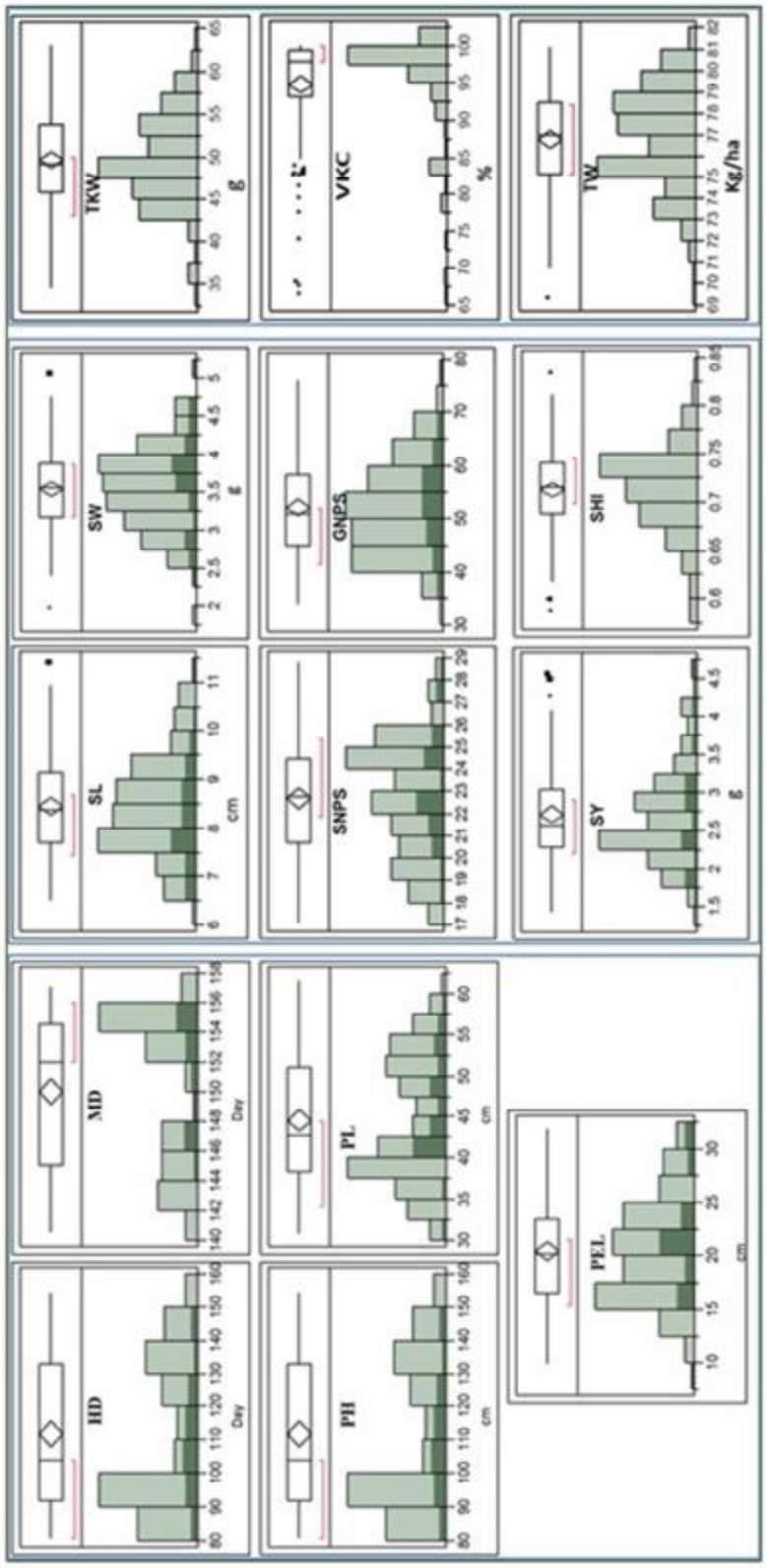

Figure 4.2. Distribution frequency for average of whole studied traits

DH: days to heading (day), DM: days to maturity (day), PH: plant height (cm), PL: peduncle length (cm), PEL: peduncle extrusion length (cm), SL: spike length (cm), SW: spike weight (g), SY: spike yield (g), SHI: spike harvest index, SNPS: spikelet number per spike, GNPS: grains number per spike, TKW: thousand kernel weight (g), VKC: viterousness kernel count (%), and TW: test weight (Kg/ha).

4.1.2. Spike characters

4.1.2.1. Spike length

The average spike length (SL) for all locations varied from 6.2 cm to 11.1 cm with a mean value of 8.5 cm. Average SL values for Ada-15-L, Ada-15-H, and Kon-15 locations were 8.8 cm, 8.8 cm and 7.6 cm, respectively (Table 4.3). The average spike length for the gene bank landraces genotypes was the highest (9.0 cm) while it was the lowest for foreign cvs (8.0 cm) (Figure 4.3).

The broad-sense heritability for spike length in Ada-15-L, Ada-15-H and Kon-15 locations was 0.94, 0.96 and 0.89, respectively (Table 4.3). Spike length was significantly and positively correlated to the number of spikelet per spike, days to maturity and days to heading with r values of 0.71**, 0.55** and 0.53**, respectively. This positive correlation of spike length with other spike traits was also observed by Yücel et al. (2009), and Wang et al. (2011).

4.1.2.2. Spike weight

The average spike weight (SW) for all locations varied from 1.6 g to 5.3 g with an average value of 3.5 g. The averaged SW values for Ada-15-L, Ada-15-H, and Kon-15 locations were 4.2 g, 3.5 g and 2.8 g, respectively (Table 4.3). The average SW of the Turkish cvs was the highest (3.7 g) while it was the lowest for grown landraces (3.2 g) (Figure 4.2).

The broad-sense heritability values for spike weight for Ada-15-L, Ada-15-H, and Kon-15 locations were 0.45, 0.12 and 0.19 in Ada-15-L, Ada-15-H and Kon-15 locations, respectively (Table 4.2). Spike weight was significantly positively correlated to spike yield and grain number per spike with r values of 0.79** and 0.71**, respectively.

4.1.2.3. Spikelet number per spike

The number of spikelets per spike (SNPS) for whole genotypes was evaluated in three locations during the 2014-2015 growing year. The average SNPS for all locations varied from 16.6 to 29.7 with an average of 22.7. The averaged SNPS for Ada-15-L, Ada-15-H, and Kon-15 locations were 24.8, 24.3, and 18.3, respectively (Table 4.3). The average number of SNPS for genebank landraces was the highest (24.1) and lowest for the Turkish cvs and foreign cvs (21.9 and 21.8), respectively (Figure 4.3).

The broad-sense heritability values for spikelet number per spike in Ada-15-L, Ada-15-H and Kon-15 locations were 0.93, 0.68 and 0.56, respectively (Table 4.3). The number of spikelets per spike was significantly positively correlated to the spike length, days to maturity and to heading with *r* values of 0.713**, 0.712**, and 0.67**, respectively.

4.1.2.4. Grain number per spike

The grain number per spike (GNPS) for whole genotypes was evaluated in three locations during the 2014 - 2015 growing year. The average GNPS for all locations varied from 24.7 to 80.7 with a mean value of 52.1. The average GNPS for Ada-15-L, Ada-15-H, and Kon-15 locations were 59.4, 54.2 and 41.3, respectively (Table 4.3). The average of the GNPS for Turkish cvs was the highest (56.5) while it was the lowest for genebank and grown LDs (46.8 and 48.2, respectively) (Figure 4.3).

The broad-sense heritability values for grain number per spike in Ada-15-L, Ada-15-H and Kon-15 locations were 0.55, 0.31 and 0.54, respectively (Table 4.3). Grain number per spike was significantly positively correlated to spike weight and spike yield with r values of 0.71** and 0.66**, respectively, but it was negative correlated to plant height, peduncle length and peduncle extrusion length with *r* values of-0.50,** -0.49** and -0.41**, respectively.

4.1.2.5. Spike yield

Spike yield (SY) or grain weight per spike for whole set of genotypes was evaluated in three location during 2014/15 growing year. The average SY for all locations varied from 1.3 g to 3.8 g with a mean of 2.7 g. The averaged SY values for Ada-15-L, Ada-15-H, and Kon-15 locations were 3.6 g, 2.4 g and 2.0 g, respectively (Table 4.3). The average of SY for Turkish cvs was the highest (2.6 g) while it was the lowest for growing landraces (2.3 g) (Figure 4.3).

The broad-sense heritability values for grain number per spike in Ada-15-L, Ada-15-H and Kon-15 locations were NA, 0.14 and 0.54, respectively (Table 4.3). Spike yield was significantly positively correlated to spike weight and grain number per spike with *r* values of 0.79** and 0.66**, respectively.

4.1.2.6. Spike Harvest Index

Spike harvest index (SHI) for whole genotypes were evaluated in three locations during the 2014 /15 growing year. The average SHI for all locations varied from 0.46 to 0.90 with an average of 0.713. The means of SHI for Ada-15-L, Ada-15-H, and Kon-15 locations were 0.74, 0.70 and 0.70, respectively (Table 4.3). The average of SHI for foreign cvs was the highest (0.739) while it was the lowest for genebank landraces (0.699) (Figure 4.3). Prihar and Stewart (1990) reported that different environmental conditions may result in different harvest index values in the same genotypes.The broad-sense heritability values for spike harvest index in Ada-15-L, Ada-15-H and Kon-15 locations were 0.75, 0.82 and 0.29, respectively (Table 4.3). The Spike harvest index was significantly negatively correlated to HD and MD with *r* values of -0.49** and -0.47**, respectively. The broad-sense heritability estimates for spike characters were lower than agronomy traits, ranging from 97% to 89%, from 48% to 12%, from 93% to 56%, from 66% to 31%, 54% to 14% and from 0.82 to 0.29 for SL, SW, SNPS, GNPS, SY and SHI, respectively (Table 4.3), indicating that less of the phenotypic variance in the genotypes was genetically controlled for spike characters.

Table 4.3. The means, standard deviations, ranges, variance, standard error and broad-sense heritabilities (h^2) of Spike traits during the 2014/15 growing seasons for the different environments/locations

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
SL (cm)	Ada-15-L	Turkish cvs	8.7 ± 1.1	6.5-11.5	1.3	0.2	
	Ada-15-L	Foreign cvs	8.6 ± 1.1	7.0 -11.2	1.1	0.2	
	Ada-15-L	Gene bank LDs	9.2 ± 0.9	7.2 -11.6	0.8	0.1	
	Ada-15-L	Growing LDs	8.5 ± 1.3	6.5 -11.4	1.7	0.3	
	Ada-15-L	All groups	8.8 ± 1.1	6.5-11.6	1.2	0.1	0.94
	Ada-15-H	Turkish cvs	8.5 ± 1.3	6.6 -11.7	1.6	0.2	
	Ada-15-H	Foreign cvs	8.1 ± 1.0	6.5 -10.3	0.9	0.2	
	Ada-15-H	Gene bank LDs	9.6 ± 0.9	7.2 -11.3	0.8	0.1	
	Ada-15-H	Growing LDs	8.3 ± 1.5	6.2 -11.4	2.3	0.4	
	Ada-15-H	All groups	8.8 ± 1.3	6.1 -11.7	1.6	0.1	0.96
	Kon-15	Turkish cvs	7.6 ± 0.9	6.0 -9.9	0.8	0.1	
	Kon-15	Foreign cvs	7.1 ± 0.6	6.3 -7.8	0.3	0.1	
	Kon-15	Gene bank LDs	7.9 ± 0.6	6.8 -9.7	0.4	0.1	
	Kon-15	Growing LDs	7.5 ± 1.3	5.9 -9.9	1.7	0.3	
	Kon-15	All groups	7.6 ± 0.9	5.9 -9.9	0.8	0.1	0.89
	Average	Turkish cvs	8.3 ± 1.1	6.6 -11.4	1.2	0.2	

Table 4.3. Continued.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Average	Foreign cvs	8.0 ± 0.9	6.7 -10.8	0.9	0.2	
	Average	Gene bank LDs	9.0 ± 0.7	7.7 -10.6	0.5	0.1	
	Average	Growing LDs	8.1 ± 1.2	6.5 -10.9	1.5	0.3	
	Average	All groups	8.5 ± 1.0	6.2 -11.1	1.1	0.1	0.97
SW (g)	Ada-15-L	Turkish cvs	4.3 ± 0.8	2.6 -6.1	0.6	0.1	
	Ada-15-L	Foreign cvs	4.1 ± 0.8	2.2 -5.5	0.6	0.2	
	Ada-15-L	Gene bank LDs	4.2 ± 0.8	2.9 -6.1	0.6	0.1	
	Ada-15-L	Growing LDs	3.9 ± 0.7	1.7 -4.8	0.5	0.2	
	Ada-15-L	All groups	4.2 ± 0.8	1.9 -6.1	0.6	0.1	0.45
	Ada-15-H	Turkish cvs	3.6 ± 0.7	1.6 -5.4	0.5	0.1	
	Ada-15-H	Foreign cvs	3.3 ± 0.6	2.1 -4.3	0.4	0.1	
	Ada-15-H	Gene bank LDs	3.6 ± 0.6	2.4 -5.0	0.4	0.1	
	Ada-15-H	Growing LDs	2.8 ± 0.7	2.0 -3.8	0.5	0.2	
	Ada-15-H	All groups	3.5 ± 0.7	1.6 -5.4	0.5	0.1	0.12
	Kon-15	Turkish cvs	3.0 ± 0.5	2.1 -4.1	0.2	0.1	
	Kon-15	Foreign cvs	2.9 ± 0.5	2.3 -4.5	0.3	0.1	
	Kon-15	Gene bank LDs	2.5 ± 0.6	1.4 -3.8	0.4	0.1	

Table 4.3. Continued.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Kon-15	Growing LDs	2.7 ± 0.6	1.8 -4.1	0.3	0.2	
	Kon-15	All groups	2.8 ± 0.6	1.4 -4.5	0.3	0.1	0.19
	Average	Turkish cvs	3.7 ± 0.5	2.5 -5.1	0.3	0.1	
	Average	Foreign cvs	3.5 ± 0.5	2.8 -4.6	0.3	0.1	
	Average	Gene bank LDs	3.5 ± 0.5	2.4 -4.3	0.2	0.1	
	Average	Growing LDs	3.2 ± 0.7	2.0 -4.4	0.4	0.2	
	Average	All groups	3.5 ± 0.5	1.6 -5.3	0.3	0	0.48
SNPS	Ada-15-L	Turkish cvs	24.2 ± 3.2	18.1-29.6	10.5	0.5	
	Ada-15-L	Foreign cvs	22.6 ± 2.4	19.0 -27.9	5.9	0.5	
	Ada-15-L	Gene bank LDs	26.4 ± 1.8	21.2 -28.9	3.3	0.3	
	Ada-15-L	Growing LDs	25.4 ± 2.4	21.4 -30.8	5.9	0.6	
	Ada-15-L	All groups	24.8 ± 2.9	18.1-30.8	8.5	0.3	0.93
	Ada-15-H	Turkish cvs	22.9 ± 4.4	16.7-29.5	19.1	0.6	
	Ada-15-H	Foreign cvs	23.7 ± 4.5	17.0 -33.4	20.6	1	
	Ada-15-H	Gene bank LDs	26.1 ± 2.2	21.7 -30.9	4.8	0.3	
	Ada-15-H	Growing LDs	23.7 ± 2.9	17.1 -28.9	8.2	0.7	
	Ada-15-H	All groups	24.3 ± 3.8	16.7-33.4	14.7	0.3	0.68

Table 4.3. Continued.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Kon-15	Turkish cvs	18.1 ± 1.8	15.1-23.5	3.1	0.2	
	Kon-15	Foreign cvs	18.6 ± 1.7	15.6 -22.0	3	0.4	
	Kon-15	Gene bank LDs	18.8 ± 1.9	15.7 -25.0	3.8	0.3	
	Kon-15	Growing LDs	17.6 ± 1.9	15.0 -21.9	3.7	0.5	
	Kon-15	All groups	18.3 ± 1.8	15.0-25.0	3.4	0.2	0.56
	Average	Turkish cvs	21.9 ± 2.8	17.0-27.5	8.1	0.4	
	Average	Foreign cvs	21.8 ± 2.2	17.8 -26.0	5	0.5	
	Average	Gene bank LDs	24.1 ± 1.8	20.6 -28.8	3.4	0.3	
	Average	Growing LDs	22.7 ± 2.4	19.9 -27.3	5.7	0.6	
	Average	All groups	22.7 ± 2.6	16.6-29.7	6.7	0.2	0.9
GNPS	Ada-15-L	Turkish cvs	63.3 ± 11.0	29.4-83.8	121.4	1.6	
	Ada-15-L	Foreign cvs	61.1 ± 15.6	19.0 -88.6	242.5	3.4	
	Ada-15-L	Gene bank LDs	54.8 ± 7.7	40.2 -77.6	59.3	1.2	
	Ada-15-L	Growing LDs	56.6 ± 11.4	29.1 -76.1	130.5	2.9	
	Ada-15-L	All groups	59.4 ± 11.6	19.0-88.6	133.6	1	0.55
	Ada-15-H	Turkish cvs	59.4 ± 10.0	38.9-85.6	100.7	1.4	
	Ada-15-H	Foreign cvs	54.7 ± 8.7	38.6 -70.3	75.9	1.9	

Table 4.3. Continued.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Ada-15-H	Gene bank LDs	49.7 ± 5.4	37.3 -64.0	29.1	0.8	
	Ada-15-H	Growing LDs	48.4 ± 12.1	32.3 -73.2	146.8	3.1	
	Ada-15-H	All groups	54.2 ± 9.8	32.3-85.6	96.7	0.9	0.31
	Kon-15	Turkish cvs	45.8 ± 8.6	23.9-62.0	73.5	1.2	
	Kon-15	Foreign cvs	48.5 ± 8.1	36.5 -67.9	66.2	1.8	
	Kon-15	Gene bank LDs	33.3 ± 6.5	23.1 -53.9	42.6	1	
	Kon-15	Growing LDs	36.8 ± 8.9	22.8 -58	79.6	2.3	
	Kon-15	All groups	41.3 ± 10.1	22.8-67.9	101.2	0.9	0.54
	Average	Turkish cvs	56.5 ± 7.8	35.8-76.0	61.5	1.1	
	Average	Foreign cvs	55.2 ± 8.8	40.7 -70.7	77.8	1.9	
	Average	Gene bank LDs	46.8 ± 5.9	38.1 -63.8	35.2	0.9	
	Average	Growing LDs	48.2 ± 9.4	33.8 -69.1	88	2.4	
	Average	All groups	52.1 ± 8.8	24.7-80.7	77.9	0.8	0.66
SY (g)	Ada-15-L	Turkish cvs	3.2 ± 0.6	1.9 -4.5	0.3	0.1	
	Ada-15-L	Foreign cvs	3.1 ± 0.6	1.4 -4.3	0.4	0.1	
	Ada-15-L	Gene bank LDs	3.0 ± 0.6	2.0 -4.3	0.4	0.1	
	Ada-15-L	Growing LDs	2.9 ± 0.5	1.5 -3.6	0.3	0.1	

Table 4.3. Continued

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Ada-15-L	All groups	3.6 ± 1.5	1.4-4.5	2.3	0.1	NA
	Ada-15-H	Turkish cvs	2.6 ± 0.6	1.1-4.0	0.3	0.1	
	Ada-15-H	Foreign cvs	2.4 ± 0.5	1.3 -3.2	0.2	0.1	
	Ada-15-H	Gene bank LDs	2.4 ± 0.5	1.6 -3.4	0.2	0.1	
	Ada-15-H	Growing LDs	2.0 ± 0.6	1.3 -2.8	0.3	0.1	
	Ada-15-H	All groups	2.4 ± 0.5	1.1-4.0	0.3	0	0.14
	Kon-15	Turkish cvs	2.1 ± 0.4	0.9-2.8	0.2	0.1	
	Kon-15	Foreign cvs	2.1 ± 0.4	1.5 -3.2	0.2	0.1	
	Kon-15	Gene bank LDs	1.8 ± 0.5	1.1 -2.9	0.2	0.1	
	Kon-15	Growing LDs	1.8 ± 0.5	1.0 -2.9	0.3	0.1	
	Kon-15	All groups	2.0 ± 0.5	0.9-3.2	0.2	0	0.54
	Average	Turkish cvs	2.6 ± 0.5	1.3-3.8	0.3	0.1	
	Average	Foreign cvs	2.5 ± 0.5	1.4 -3.6	0.3	0.1	
	Average	Gene bank LDs	2.4 ± 0.4	1.6 -3.5	0.1	0.1	
	Average	Growing LDs	2.3 ± 0.5	1.3 -3.1	0.3	0.1	
	Average	All groups	2.7 ± 0.6	1.3-3.8	0.4	0.1	0.47
SHI	Ada-15-L	Turkish cvs	0.750 ± 0.04	0.7 -0.8	0	0.01	

Table 4.3. Continued.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Ada-15-L	Foreign cvs	0.759 ± 0.04	0.6 -0.8	0	0.01	
	Ada-15-L	Gene bank LDs	0.709 ±0.05	0.6 -0.9	0	0.01	
	Ada-15-L	Growing LDs	0.712 ± 0.04	0.7 -0.8	0.04	0.01	
	Ada-15-L	All groups	0.74 ± 0.04	0.6 -0.8	0.002	0.004	0.75
	Ada-15-H	Turkish cvs	0.706 ± 0.09	0.4 -1.0	0.01	0.01	
	Ada-15-H	Foreign cvs	0.726 ± 0.04	0.6 -0.8	0	0.01	
	Ada-15-H	Gene bank LDs	0.672 ± 0.05	0.6 -0.8	0	0.01	
	Ada-15-H	Growing LDs	0.698 ± 0.07	0.6 -0.8	0.04	0.02	
	Ada-15-H	All groups	0.70 ± 0.06	0.4 -1.0	0.005	0.006	0.82
	Kon-15	Turkish cvs	0.692 ± 0.09	0.3-0.9	0.01	0.01	
	Kon-15	Foreign cvs	0.733 ± 0.09	0.5 -0.9	0.01	0.02	
	Kon-15	Gene bank LDs	0.719 ± 0.1	0.5 -0.9	0.01	0.01	
	Kon-15	Growing LDs	0.657 ± 0.1	0.5 -0.8	0.04	0.02	
	Kon-15	All groups	0.70 ± 0.1	0.3 -0.9	0.009	0.008	0.29
	Average	Turkish cvs	0.716 ± 0.07	0.46 -0.90	0.01	0.01	
	Average	Foreign cvs	0.739 ± 0.07	0.56 -0.80	0	0.01	
	Average	Gene bank LDs	0.699 ± 0.07	0.56 -0.87	0	0.01	

Table 4.3. Continued.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Average	Growing LDs	0.700 ± 0.07	0.6 -0.8	0.04	0.02	
	Average	All groups	0.713 ± 0.05	0.46 -0.9	0.005	0.006	0.52

SD: Standard deviation SE: Standard Error , SL: Spike length (cm), SW: Spike weight (g), SNPS: Spikelet number per spike, GNPS: Grain number per spike, GWPS: Spike Yield (g), SHI: Spike Harvest Index.

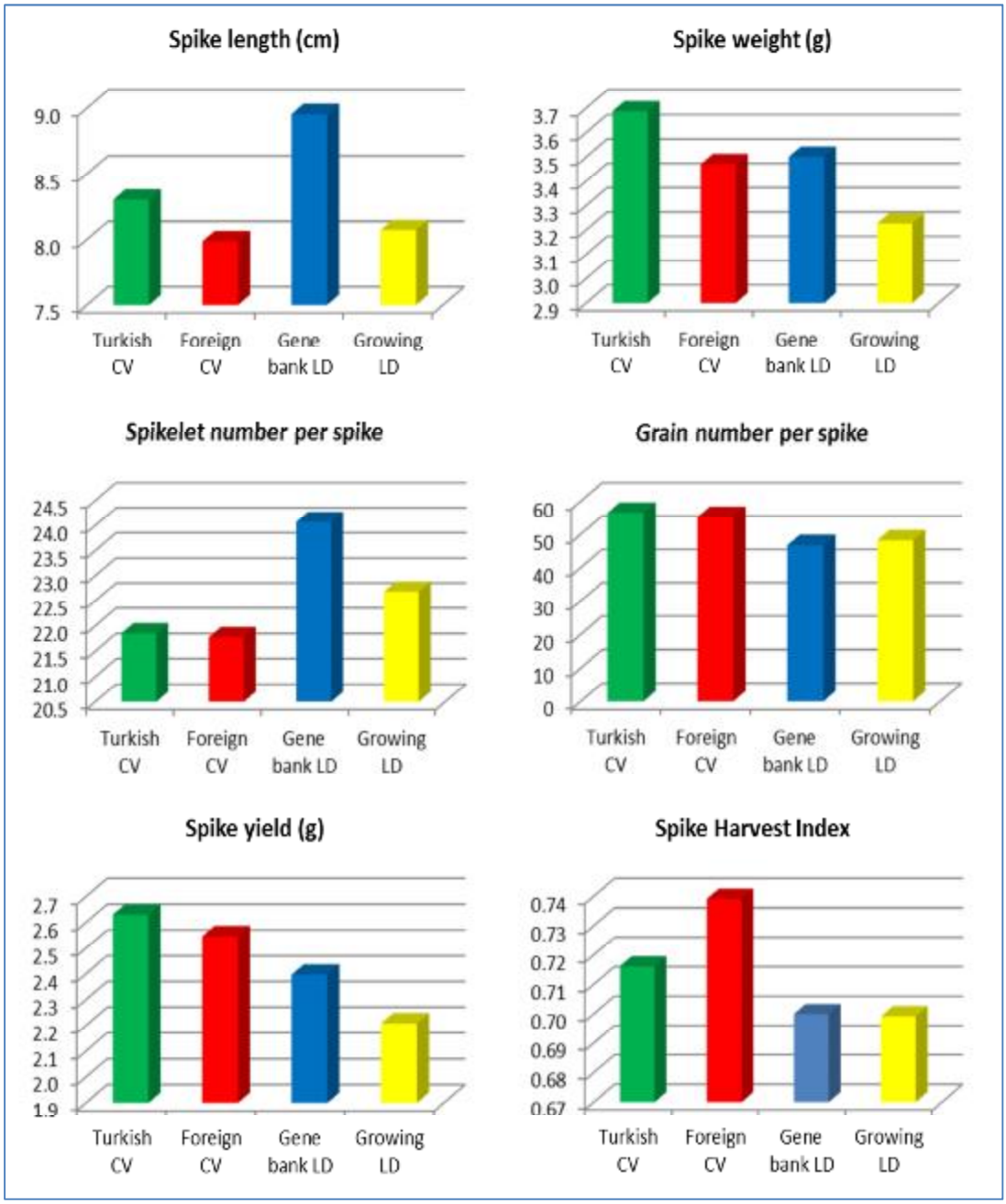

Figure 4.3. Significant variations in average values of spikes characteristics found among the different groups of the durum wheat diversity panel

4.1.3. Quality characters

4.1.3.1. Thousand kernel weight

The average thousand kernel weight (TKW) for all locations varied from 31.4 g to 70.4 g with a mean value of 49.5 g. The averaged values of TKW for Ada-15-L, Ada-15-H, and Kon-15 locations were 53.1 g, 46.3 g cm and 48.6 g, respectively (Table 4.4). The average of the TKW for genebank LDs genotypes was the highest (52.2 g) while for foreign cvs it was the lowest (47.7 g) (Figure 4.4). The broad-sense heritability values for spike length in Ada-15-L, Ada-15-H and Kon-15 locations were 0.78, 0.74 and 0.49, respectively (Table 4.4). TKW was positively correlated to plant height and spike length with *r* values of 0.40** and 0.36** , respectively.

4.1.3.2. Vitreousness kernel count

The average vitreousness kernel count (VKC) for all locations varied from 54% to 100% with a mean value of 94.8. The averaged values of VKC for Ada-15-L, Ada-15-H, and Kon-15 locations were 86.8%, 99.1% and 98.7%, respectively (Table 4.4). The average of VKC for the genebank LDs genotypes was the highest (97.2%) and lowest for growing LDs (91.8 %) (Figure 4.4). The broad-sense heritability for VKC was 0.84 in Ada-15-H location (Table 4.4). Although the VKC was correlated to plant height, it was not significant.

4.1.3.3. Test weight

The average test weight (TW) for all locations varied from 68.8 kg/ha to 81.9 kg/ha with a mean value of 76.7 kg/ha. The averaged values of the test weight for Ada-15-L, and Ada-15-H locations were 78.1 kg/ha and 75.3 kg/ha, respectively (Table 4.4). The average of the test weight was the highest for foreign cvs (78.1 kg/ha) while it was the lowest for genebank LDs (75.8 kg/ha) (Figure 4.4). The broad-sense heritability values for test weight for Ada-15-L, and Ada-15-H locations

were 0.92 and 0.88, respectively (Table 4.4). The TW was correlated to plant height, but not significantly. The test weight was negatively correlated to days to heading, days to maturity, spike length and spikelet number per spike with *r* values of -0.47**, -0.45**, -0.40**, and -0.40**, respectively.

Finally, although the prime purpose of this study was to identify marker-trait associations for some of important agronomy, spike characters and some quality traits, the opportunity was also taken to evaluate the phenotypic traits of durum wheat genotypes. Selection of the appropriate characteristics will ensure the improvement of more than one characteristic simultaneously due to the correlation among different traits. All studied characteristics for both seasons showed continuous distributions suggesting that these traits were complex in nature and quantitatively inherited.

Table 4.4. The means, standard deviations, ranges, variance, standard error and broad-sense heritabilities (h^2) of quality traits during the 2014/2015 growing season in different environments.

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
TKW (g)	Ada-15-L	Turkish cvs	53.0 ± 7.1	34.4-69.0	50.1	1	
	Ada-15-L	Foreign cvs	52.5 ± 5.7	41.6 -67.0	32.6	1.2	
	Ada-15-L	Gene bank LDs	54.7 ± 9.0	37.7 -75.2	80.7	1.4	
	Ada-15-L	Growing LDs	51.0 ± 6.4	33.7 -60.1	40.7	1.6	
	Ada-15-L	**All groups**	**53.1 ± 7.4**	33.7-75.2	**55.1**	**0.7**	**0.78**
	Ada-15-H	Turkish cvs	45.0 ± 6.5	25.5-55.7	42.2	0.9	
	Ada-15-H	Foreign cvs	44.5 ± 5.1	33.2 -52.8	25.6	1.1	
	Ada-15-H	Gene bank LDs	49.8 ± 9.4	25.6 -73.2	87.5	1.4	
	Ada-15-H	Growing LDs	43.0 ± 6.5	34.1 -55.7	41.7	1.7	
	Ada-15-H	**All groups**	**46.3 ± 7.8**	25.5-73.2	**60.6**	**0.7**	**0.74**
	Kon-15	Turkish cvs	46.5 ± 5.4	35.1-57.0	28.9	0.8	
	Kon-15	Foreign cvs	46.0 ± 3.8	37.4 -52.2	14.8	0.8	
	Kon-15	Gene bank LDs	52.3 ± 6.2	35.7 -62.7	38.2	0.9	
	Kon-15	Growing LDs	49.6 ± 3.9	41.8 -53.8	14.9	1	
	Kon-15	**All groups**	**48.6 ± 5.9**	35.1-62.7	**34.7**	**0.5**	**0.49**

Table 4.4 Continued

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Average	Turkish cvs	48.4 ± 4.9	36.5-59.8	24.1	0.7	
	Average	Foreign cvs	47.7 ± 3.8	39.6 -55.9	14.6	0.8	
	Average	Gene bank LDs	52.2 ± 6.0	34.6 -62.9	36.2	0.9	
	Average	Growing LDs	48.4 ± 4.7	37.3 -57.3	22.4	1.2	
	Average	**All groups**	**49.5 ± 5.4**	31.4-70.4	**29**	**0.5**	**0.83**
VKC (%)	**Ada-15-L**	**Turkish cvs**	**83.9 ± 21.7**	**3.0-100.0**	**469.9**	**3.1**	
	Ada-15-L	Foreign cvs	85.2 ± 18.8	41.0 -100.0	353.4	4.1	
	Ada-15-L	Gene bank LDs	93.5 ± 17.9	0.0 -100.0	320.6	2.7	
	Ada-15-L	Growing LDs	79.4 ± 24.1	26.0 -100.0	581.5	6.2	
	Ada-15-L	**All groups**	**86.8 ± 20.8**	0.0-100.0	**430.9**	**1.8**	**NA**
	Ada-15-H	Turkish cvs	99.4 ± 1.0	96.0-100.0	1.1	0.1	
	Ada-15-H	Foreign cvs	98.3 ± 3.0	86.0 -100.0	9.1	0.7	
	Ada-15-H	Gene bank LDs	99.2 ± 1.2	95.0 -100.0	1.4	0.2	
	Ada-15-H	Growing LDs	99.3 ± 1.1	96.0 -100.0	1.3	0.3	
	Ada-15-H	**All groups**	**99.1 ± 1.6**	86.0-100.0	**2.6**	**0.1**	**0.84**
	Kon-15	Turkish cvs	98.4 ± 4.0	76.0-100.0	15.9	0.6	
	Kon-15	Foreign cvs	99.0 ± 1.6	94.0 -100.0	2.7	0.4	

Table 4.4 Continued

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Kon-15	Gene bank LDs	98.9 ± 1.5	94.0 -100.0	2.3	0.2	
	Kon-15	Growing LDs	98.9 ± 1.7	95.0 -100.0	2.7	0.4	
	Kon-15	**All groups**	**98.7 ± 2.8**	76.0-100.0	**7.9**	**0.2**	**NA**
	Average	Turkish cvs	93.9 ± 7.5	68.0-100.0	56.6	1.1	
	Average	Foreign cvs	94.1 ± 6.5	79.7 -100.0	41.6	1.4	
	Average	Gene bank LDs	97.2 ± 5.9	67.0 -100.0	35.3	0.9	
	Average	Growing LDs	91.8 ± 8.6	74.0 -100.0	73.1	2.2	
	Average	**All groups**	**94.8 ± 7.2**	**54-100.0**	**51.3**	**0.6**	**NA**
TW (Kg/ha)	**Ada-15-L**	**Turkish cvs**	**78.4 ± 2.4**	**72.8-82.0**	**5.6**	**0.3**	
	Ada-15-L	Foreign cvs	79.3 ± 1.9	75.9 -82.5	3.5	0.4	
	Ada-15-L	Gene bank LDs	77.2 ± 2.5	71.57 -82.9	6.1	0.4	
	Ada-15-L	Growing LDs	78.5 ± 1.4	76.4 -82.2	2	0.4	
	Ada-15-L	**All groups**	**78.1 ± 2.3**	71.5-82.9	**5.5**	**0.2**	**0.92**
	Ada-15-H	Turkish cvs	75.3 ± 3.6	66.0-80.9	13.3	0.5	
	Ada-15-H	Foreign cvs	76.9 ± 2.1	73.2-79.6	4.4	0.5	
	Ada-15-H	Gene bank LDs	74.6 ± 2.7	69.2 -79.2	7.2	0.4	
	Ada-15-H	Growing LDs	75.4 ± 1.8	72.3 -78.3	3.3	0.5	

Table 4.4 Continued

Trait	Environment	Group	Mean ± SD	Range	Variance	SE	h^2
	Ada-15-H	**All groups**	**75.3 ± 3.0**	66.1-80.9	**9.2**	**0.3**	**0.88**
	Average	Turkish cvs	76.9 ± 2.8	69.4-81.1	8.1	0.4	
	Average	Foreign cvs	78.1 ± 1.8	74.6 -81.0	3.4	0.4	
	Average	Gene bank LDs	75.8 ± 2.0	71.6 -80.1	4.2	0.3	
	Average	Growing LDs	77.1 ± 1.3	75.0 -79.5	1.6	0.3	
	Average	**All groups**	**76.7 ± 2.4**	**68.8-81.9**	**5.8**	**0.2**	**0.95**

SD: Standard deviation, SE: Standard Error, TKW: Thousand kernel weight (g), VKC: Viterousness kernals count (%), TW: Test weight (Kg/ha)

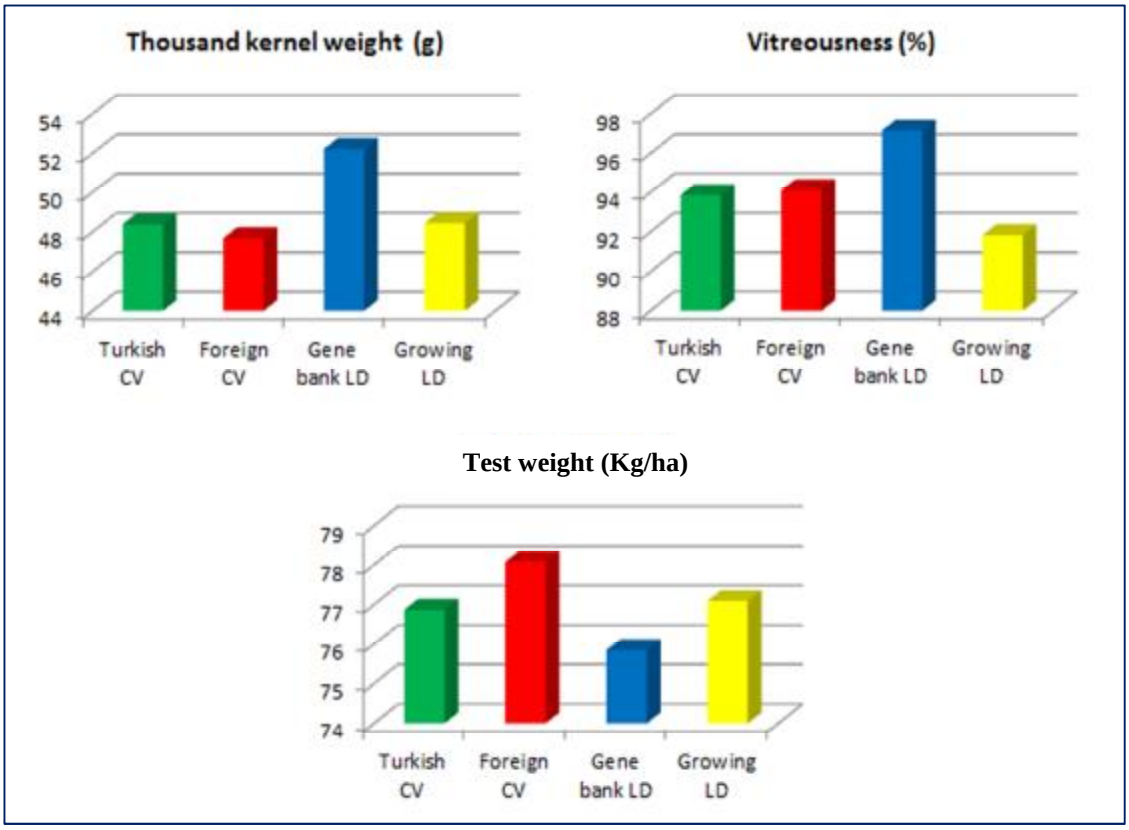

Figure 4.4. Significant variations in average values of quality traits found among the different subpopulations of the durum wheat diversity panel for thousand kernel weight (TKW), vitreousness (VKC) and test weight (TW).

4.2. Genetic variation

The 130 durum wheat genotypes were genotyped with 87 SSR primers and amplified 801 alleles. Then, 129 of these genotypes were genotyped using SNPs and DArTseq; however, one genotype, cv Güney Yıldızı, was not genotyped by SNPs and DArTseq because of poor DNA quality. All genotypes were allocated to one of four groups; Group A containing 49 Turkish cultivars, 21 foreign cultivars in Group B, 44 genebank landraces in C group and 15 growing landraces in Group D. After carrying out DArTseq and SNP analysis, 34130 SilicoDArT and 30376 SNP markers were obtained from 129 genotypes.

In the SSR, DArTseq and SNP analyses, each band was considered to represent a locus and was scored as binary data, with the presence of bands denoted as ‘1’ and their absence as ‘0’. Scoring the alleles in a binary format simplifies the

assessment and statistical analysis for combined analysis using dominant DArT and codominant SSR & SNP data (Kaya et al., 2016; Ferrao et al., 2014; Kosman and Leonard 2005).

The analysis of our data consisted of at least three steps for per-marker quality control of GWAS: 1) identification of markers with zero and missing score, 2) identification of markers demonstrating a significant deviation from Hardy-Weinberg equilibrium and 3) the removal of all makers with a very low minor allele frequency.

A subset of 1274 SNP markers were distributed through the A and B wheat genome chosen from a total of 30376 SNP markers based on a high proportion (>0.98) of technical replicate assay pairs, for which the marker score was consistent (RepAvg), and high of Call Rate values (>0.98); the proportion of samples for which the genotype call was either "1" or "0", rather than "-"; in addition, there was no missing score. In addition to the SNP markers, 4282 SilicoDArT markers were selected based on the high value of “CallRate” and reproducibility, which is the proportion of technical replicate assay pairs for which the marker score is consistent (>0.98), and there were no missing scores. Finally, further processing eliminating genetic markers with a minor allele frequency (MAF) < 0.05% generated 6357 markers for this study.

4.2.1. Analysis of molecular variance

To detect population differentiation utilizing molecular markers, the analysis of molecular variance (AMOVA) was undertaken with 6357 markers including 801 SSR, 4282 silicoDArTs and 1274 SNP markers using GenAlex 6.5. (Excoffier et al., 1992). As mentioned above, the germplasms were divided into the four groups of Turkish cultivars, foreign cultivars, Turkish-gene bank landraces, and landraces still commonly grown in local farmers’ fields. All data was analyzed for allele frequency and its overall distribution among and within suggested groups. Although the difference among groups was significant ($P \leq 0.001$), revealing 86% and 14% of variation presented within and among groups, respectively, the results showed a large

and significant variation within groups. Our results largely matched those of Maccaferri et al. (2005; 2014), and Khan et al. (2015). This indicates that there was a significant pattern of diversity in the studied durum wheat collection. The results of AMOVA also showed that all variance components were significant (P <0.001) at any level of group comparison (Table 4.5), (Figure 4.5).

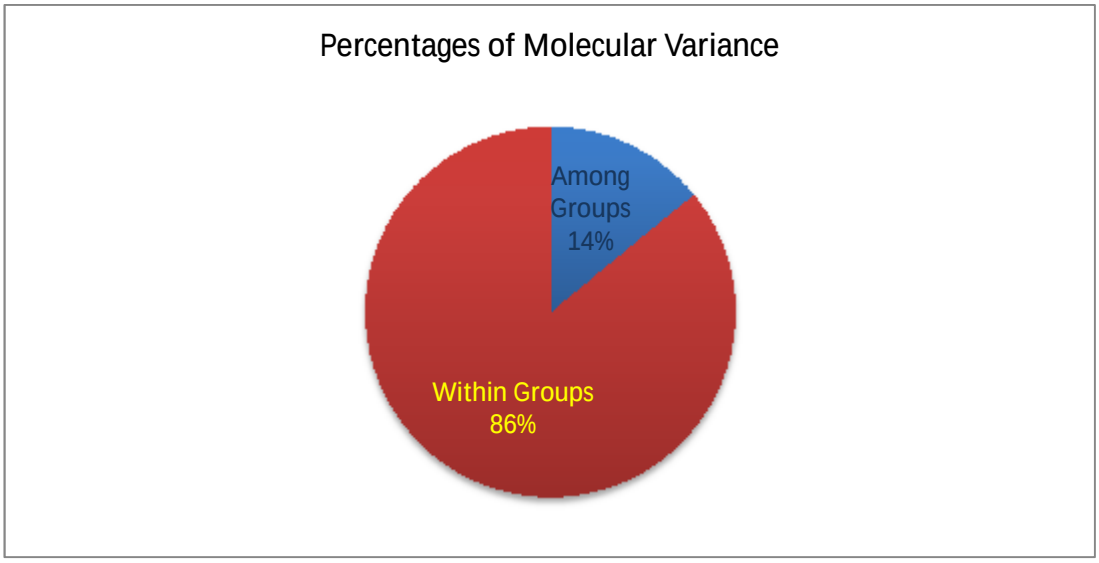

Figure 4.5. The results of AMOVA analysis revealing variation within and among groups.

Table 4.5. Analysis of molecular variance for Turkish cvs, foreign cvs, Genebank LDs and growing LDs groups

Source	df	SS	MS	Est. Var.	%
Among Groups	3	9526.41	3175.47	87.888	14%
Within Groups	125	66559.25	532.474	532.474	86%
Total	128	76085.66		620.362	100%

df: degree of freedom, SS: square sum, MS: mean square, Est. Var.: Estimated Variance

The effective number of alleles, which is the number of alleles with equal frequencies considered the same value of expected heterozygosity in populations, was studied. Alleles with low frequencies contribute very little to the effective number of alleles. The growing LDs showed the highest effective number of the alleles “genetic variability” (Ne= 1.208) according to the Shannon Information Index (I= 0.227), which indicates high diversity and high uniformity. In foreign cvs,

the values of the number of alleles (Na), effective number of alleles (Ne), Shannon Information Index (I), heterozygosity He) and unbiased expected heterozygosity (uHe) were 1.012, 1.189, 0.184, 0.116 and 0.119, respectively. The highest number of alleles (Na) was found at genebank LDs (1.510). Unbiased expected heterozygosity devised an estimator of gene diversity showed that the growing LDs had the highest value for uHe = 0.143, in addition of heterozygosity He = 0.138 (Table 4.6) (Figure 4.6).

Table 4.6. Mean and SE over Loci for each group, Grand Mean and SE over Loci and Groups

Population		N	Na	Ne	I	He	uHe
Turkish cvs	**Mean**	49	1.263	1.196	0.204	0.126	0.128
	SE		0.012	0.004	0.003	0.002	0.002
Foreign cvs	**Mean**	21	1.012	1.189	0.184	0.116	0.119
	SE		0.012	0.004	0.003	0.002	0.002
Gene bank LDs	**Mean**	44	1.510	1.190	0.203	0.122	0.124
	SE		0.011	0.004	0.003	0.002	0.002
Growing LDs	**Mean**	15	1.333	1.208	0.227	0.138	0.143
	SE		0.012	0.003	0.003	0.002	0.002
Grand Mean and SE over Loci and Populations:							
Total	**Mean**	32	1.279	1.196	0.205	0.126	0.128
	SE		0.006	0.002	0.001	0.001	0.001

N: Samples number, Na:No. of Alleles, Ne: No. of Effective Alleles, I: Shannon Information Index , He: Expected Heterozygosity and uHe: Unbiased Expected Heterozygosity.

The banding pattern graph for allelic data depicted that among all groups, number of bands and its frequency was equal for foreign cvs and growing LDs, while gene bank LDs and Turkish cvs, the number of bands was greater. Gene bank LDs group showed the highest unique allele loci to single population followed by growing LDs than Turkish cvs with foreign cvs having the lowest level (Figure 4.6)

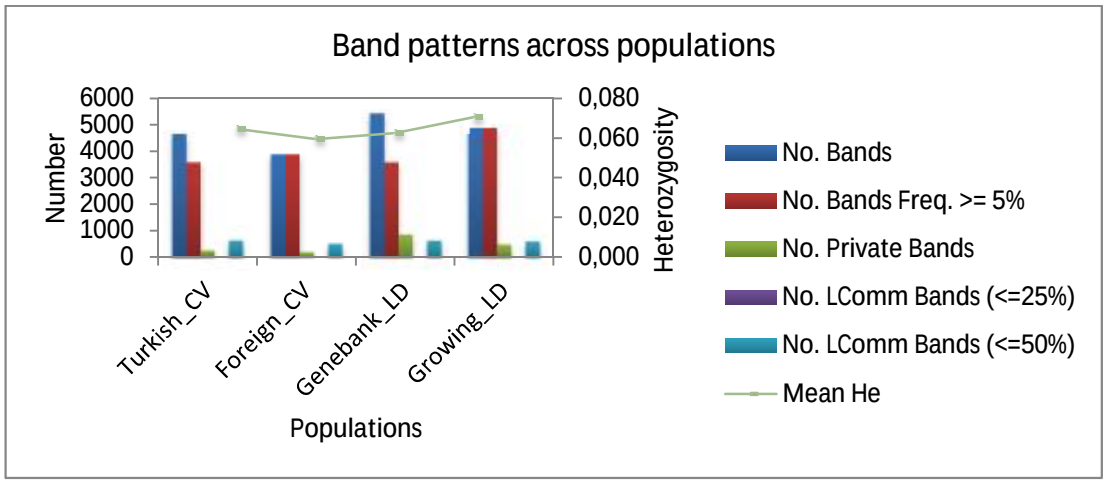

Figure 4.6. Band patterns for binary data of the studied four populations

The mean percentage of polymorphic loci was 62.49. The percentage for the polymorphic loci for genebank LDs, growing LDs, Turkish cvs and foreign cvs were: 74.37%, 65.13%, 61.87% and 48.58%, respectively.

The genetic divergence between groups or within a group were measured as genetic distance (Table 4.7), which can also be expressed in terms of the arithmetic mean of gene identity. The pairwise population matrix of the Nei genetic identity is given in Table 4.7 showing that the Turkish cvs and foreign cvs were very close to each other (0.990) while the foreign cvs and genebank LDs were distant from each other (0.957) (Table 4.8).

Table 4.7. Pairwise Group Matrix of the Nei Genetic Distance

	No	Turkish cvs	Foreign cvs	Genebank LDs	Growing LDs
Turkish cvs	49	0.000			
Foreign cvs	21	0.010	0.000		
Gene bank LDs	44	0.020	0.044	0.000	
Growing LDs	15	0.013	0.026	0.018	0.000

Table 4.8 Pairwise Group Matrix of the Nei Genetic Identity

	No	Turkish cvs	Foreign cvs	Gene bank LDs	Growing LDs
Turkish cvs	49	1.000			
Foreign cvs	21	0.990	1.000		
Gene bank LDs	44	0.980	0.957	1.000	
Growing LDs	15	0.988	0.974	0.983	1.000

The highest value of the pairwise group matrix of Nei unbiased genetic identity was calculated between all groups, and it was found that Turkish cvs and foreign cvs were very close to each other (0.992) while foreign cvs and gene bank LDs were very distant to each other (0.960) (Table 4.10).

Table 4.9. Analysis of Pairwise Group Matrix of the Nei Unbiased Genetic Distance.

	No	Turkish cvs	Foreign cvs	Gene bank LDs	Growing LDs
Turkish cvs	49	0.000			
Foreign cvs	21	0.008	0.000		
Gene bank LDs	44	0.018	0.041	0.000	
Growing LDs	15	0.009	0.021	0.014	0.000

Table 4.10. Analysis of Pairwise Group Matrix of the Nei Unbiased Genetic Identity.

	No	Turkish cvs	Foreign cvs	Gene bank LDs	Growing LDs
Turkish cvs	49	1.000			
Foreign cvs	21	0.992	1.000		
Gene bank LDs	44	0.982	0.960	1.000	
Growing LDs	15	0.991	0.979	0.986	1.000

For all population comparisons of AMOVA, it was evident that groups within a given comparison were more genetically differentiated than when contrasted among groups. This is reflected by the higher proportion of total variance for the ‘within’ analyses. According to the pairwise population matrix of the Nei genetic distance given in Table 4.7, Turkish cvs and Foreign cvs groups are closely related (0.008) and it is almost certain or there is high probability that they have a

recent common ancestor. The second pairwise groups closest to each other are Turkish cvs and growing LDs (0.009), followed by the genebank LD and grown LD groups (0.014); however, the foreign cvs and gene bank LDs showed farther Nei genetic distance (0.041). Figure 4.7 reveals that Turkish CVs was located almost at the middle of groups.

4.2.2. Principal coordinate analysis

It is critically important to assess the accuracy of the imputed molecular markers (SSR, SNP and SilicoDArT) before using them for further analysis. Therefore, genetic relationships among the durum panel genotypes were also investigated graphically via principal coordinate analysis (PCoA). Initially, the analysis was performed separately for each marker types (SSR, SNP and Silico DArT) and then the analysis was also carried out using combined marker data (Figure 4.8).

PCoA was performed with the GenALEX V6.5 software using the co-dominant genotypic distance and the covariance-standardized methodology to elucidate the patterns of the population structure. In particular, it maximizes the linear correlation between the distances in the distance matrix, and the distances in the space of low dimension. The first two principal coordinates were drawn in a graph in two-dimensional space to show the clustering of different genotypes

PCoA was carried out first on the 801 SSR markers in order to identify relationships within and among the groups. The first three coordinates explained 10.24%, 5.32% and 4.52% of the variance, respectively with a cumulative total of 20.07 %. Using PCoA, the genotypes can be divided into four groups. Foreign cultivars showed close association with each other, the gene bank LDs also showed a close association with each other except for four landraces admixture with Turkish cvs, in same way an admixture was observed between Turkish and foreign cultivars confirming their relatedness within the diverse gene pool. The growing LDs were distributed between Turkish and foreign cultivars.

PCoA was carried out on 1274 SNP, 4282 SilicoDArT and 6357 combined all markers, in order to identify relationships within and among groups. Table 4.11 demonstrated the percentage of variation explained by the first 3 axes. PCoA plots were illustrated using the first two coordinates using data from 129 tetraploid wheat genotypes (Figures 4.7 and 4.8).

Table 4.11. Percentage of variation explained by the first 3 axes

Markers	**Axis**	**1**	**2**	**3**
SSR	%	10.24	5.32	4.52
	Cum %	10.24	15.56	20.07
SNP	%	18.22	6.69	4.25
	Cum %	18.22	24.91	29.16
SilicoDArT	%	20.90	5.44	3.73
	Cum %	20.90	26.34	30.06
Whole markers	%	18.29	4.99	3.47
(SSR, SNP& DArT)	Cum %	18.29	23.28	26.75

The eigenvalue-one criterion was used to retain the principal components that contributed considerable variability. The eigenvalues were calculated for the first 3 principal coordinate for all populations, and are given in Table 4.12.

Table 4.12. Eigenvalues by axis and sample eigen vectors explained by the first 3 principal components of the studied four groups

Axis No.	**No**	**1**	**2**	**3**
Eigenvalue		**0.013**	**0.004**	**0.001**
Turkish cvs	49	-0.020	-0.012	0.030
Foreign cvs	21	-0.080	-0.011	-0.018
Gene bank LDs	44	0.077	-0.030	-0.010
Growing LDs	14	0.022	0.054	-0.002

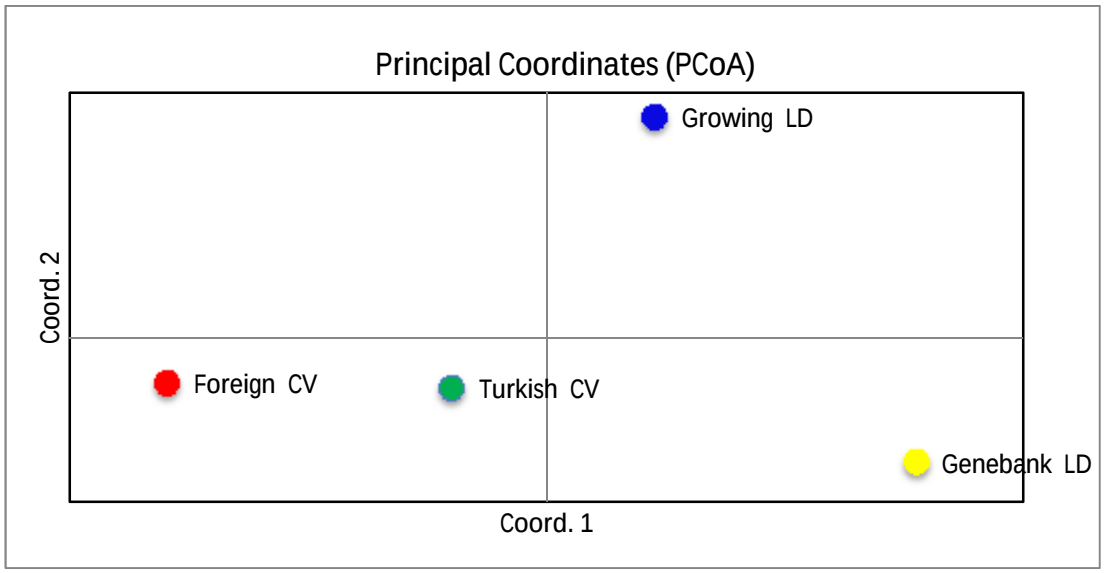

Figure 4.7. Principal Coordinates Analysis of the four studied groups.

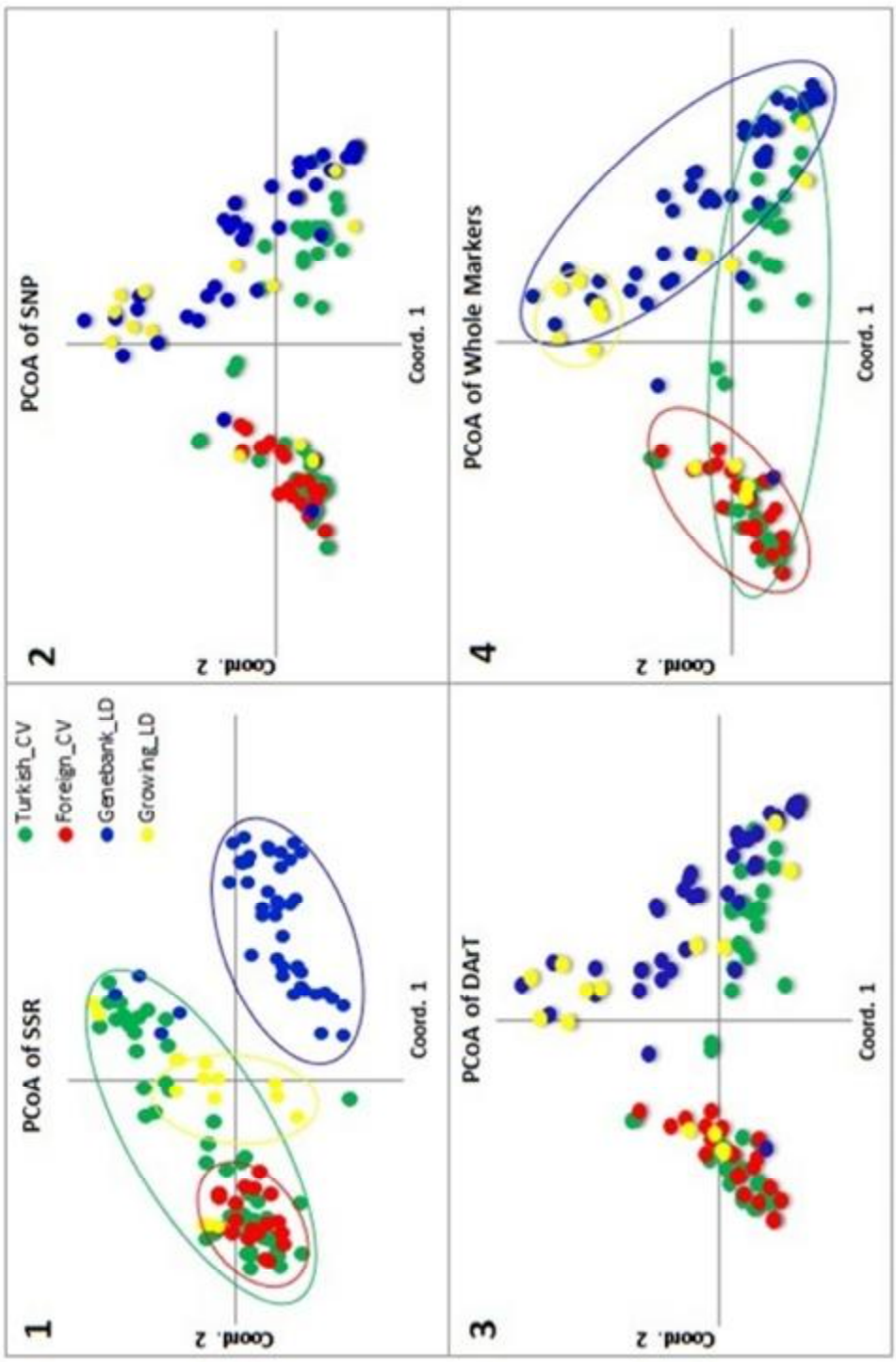

Figure 4.8. Relationships between individuals visualized by PCoA using: (1) SSR data, (2) SNP data, (3) DArT data and (4) Combined data

PCoA was carried out with the 129 genotypes using 1274 SNP, 4282 silicoDArTs and 6357 SNP in order to identify relationships within and among the groups. The results showed a clear match between the results of SNPs, DArT and the combined markers; therefore, for the combined markers where the results are compatible with the results of each of the genetic markers SNP and DArT, will be discussed only.

The first three coordinates of combined markers explained 18.29.%, 4.99% and 3.47% of the variation, respectively. PCoA analysis divided genotypes into two main groups: all foreign cvs were centered together and were included in first group; the second group contained the majority of the gene bank LDs, and the Turkish cvs and growing LDs were distributed across the two main groups of the foreign cvs" and "gene bank LDs". Foreign cvs also showed a close association with each other. In addition, genebank LDs revealed an association with each other; however, this was relatively less close except for one landrace (TR81304-Malatya) which had an admixture with the foreign cvs group. Thus, admixture was observed between Turkish cvs and growing LDs within the main groups. Some genotypes from the Turkish cvs were mixed with the first group and distributed relatively closer to the foreign cvs; however, most of the growing LDs were distributed on a larger scale within the second group. Except five landraces from enebank LDs group (Karadere, Şırnak Alkaya, Kurtulan, Havrani, and similar to Levante) existed together with the first group.

The PCoA of SNP, SilicoDArT and combined markers (SSR, SNP and SilicoDArT) showed similar results. High diversity was noticed between the genotypes belonging to Turkish cvs or the genotypes from the gene bank landraces or even the genotypes from growing LDs group. This is considered to be due to durum wheat originating in the Fertile Crescent and thought to be the primary center of wheat domestication and diversity. Wheat has been cultivated in this region for the last 12,000 years, contributing these genotypes, the ancestors of which can be traced back to high diversity of the Fertile Crescent gene pool.

To develop new varieties with improved phenotypes, foreign varieties from other countries were used in Turkish wheat breeding programs. Genotypes from

Asia, Europe and America were introduced into the Turkish wheat breeding programs and based on PCoA, the result indicated that there is high contribution from foreign genotypes to the Turkish breeding process. Although the sources of the foreign cvs are geographically distant from Asia, Europe and America, there was a clear convergence. The reason for this is probably the small number of foreign cvs used (21 only).

The results also confirm the relatedness between the foreign cvs within the diverse of Turkish cvs gene pool. The results also indicates the kinship between growing LDs with gene bank LDs. PCoA also revealed that these individuals were relatively separated for the spring and winter type varieties (Figure 4.8).

4.4.3 Genetic distance and population diversity analysis

To check and confirm genetic variations for each group, the weighted Neighbor-joining dendrogram based on the Dice dissimilarity index tree was constructed with 6357 loci separately for each group, and the results showed clear diversity among genotypes from each group (Figure 4.9).

Relationships between genotypes were detected. Dice dissimilarity index values were calculated among the genotypes for each DNA marker type, and the weighted Neighbor joining tree was constructed four times, first using SSRs (801 markers), second using SNPs (1274 markers), third using 4282 markers of DArTs and last using overall 6357 loci (Figure 4.10).

Comparing the results from each type of marker with those obtained from a set of whole markers, a high match and very rare discrepancies in the clustering of genotypes were observed. However, it should be considered that the nature of each kind of marker in addition to the total numbers used for each type in this analysis might have led to a slightly different genetic structure. Therefore, it was necessary to undertake a deeper detailed analysis using the weighted Neighbor joining tree of the whole markers combined.

Using all the DNA markers in analyses, the population of 129 genotypes were splatted into the three main clusters: A, B and C containing 43, 80 and 6 genotypes,

respectively (Figure. 4.10). Cluster A consisted of 43 genotypes included 27 from genebank LDs, 4 from growing LDs and 12 from Turkish cvs; Cluster B was composed of 80 genotypes divided into two sub-clusters as B-1 and B-2, with 67 and 13 genotypes, respectively; C-luster B-1 contained two subclusters B-1-1 and B-1-2 with 58 and 9 genotypes, respectively. While 88% of the subcluster B-1-1 were from cultivars with all the whole foreign cultivars (21) being included, 30 genotypes were from Turkish cvs, two from the gene bank LD and five from the growing LD. Cluster B-1-2 was composed mainly of 7 Turkish cvs and two gene bank LDs. Clusters B-2 and C consisted mainly of LDs, 8 from gene bank LDs and 5 from growing LDs belonging to B-2, and for cluster C, there were 5 genotypes from the gene bank and one from growing LDs.

It is noted from the tree that Clusters A, B-1-2, B-2 and C did not contain any genotypes from foreign cvs; these cvs were mainly located in the B-1-1 cluster which showed the highest admixture cluster. The B-1-1 cluster included genotypes from the 4 main groups.

The overall genetic marker data was used to produce the Dice genetic distance among 129 genotypes. The result revealed that the Dice average genetic distance among all genotypes was 0.4441, and the lowest genetic distance was 0.015 between genotypes 8 and 30 (Şölen with Tuten-2002) from the Turkish cvs. The second lowest genetic distance was found between genotypes 96 and 101 (0.016) (TR32167-Yozgat with TR35148-Yozgat) from the genebank LD group while the highest distance (0.621) was recorded between genotypes 51 or 48, and 82 and 127 (Zenit, Tunca-79, TR81259-Malatya and Karakilcik) (Table 4.13).

The weighted Neighbor joining tree confirmed the groups identified in PCoA, showing a high and wide diversity of the durum genotypes whether from the genebank or growing LDs or even from Turkish cvs while foreign cvs were clustered together and mostly intertwined with the Turkish cvs (Figure 4.10).

Table 4.13. Lowest and highest Dice genetic distance scored between 129 genotypes over all the genetic markers

Genotype			Genotype			Genetic
No	Genotype name	Group	No	Genotype name	Group	distance
Lowest Dice genetic distance scored between 129 genotypes over all the genetic markers :						
8	Solen-2002	Turkish CV	30	Tuten-2002	Turkish CV	0.015
96	TR32167-Yozgat	Gene bank LD	101	TR35148-Yozgat	Gene bank LD	0.016
43	Ceylan-95	Turkish CV	120	Kurtulan	Growing LD	0.022
19	Akbasak-073-44	Turkish CV	116	Menceki	Growing LD	0.024
76	TR81371-Nigde	Gene bank LD	100	TR31893-Malatya	Gene bank LD	0.024
23	Diyarbakir-81	Turkish CV	43	Ceylan-95	Turkish CV	0.025
23	Diyarbakir-81	Turkish CV	120	Kurtulan	Growing LD	0.026
80	TR45305-Yozgat	Gene bank LD	101	TR35148-Yozgat	Gene bank LD	0.026
80	TR45305-Yozgat	Gene bank LD	96	TR32167-Yozgat	Gene bank LD	0.027
22	Aydin-93	Turkish CV	47	Alibaba	Turkish CV	0.031
66	Inide	Foreign CV	67	Dylan	Foreign CV	0.033
43	Ceylan-95	Turkish CV	121	Karadere	Growing LD	0.036
120	Kurtulan	Growing LD	121	Karadere	Growing LD	0.037
100	TR31893-Malatya	Gene bank LD	107	TR81258-Malatya	Gene bank LD	0.039

Table 4.13. Continued.

Genotype No	Genotype name	Group	Genotype No	Genotype name	Group	Genetic distance
23	Diyarbakir-81	Turkish CV	121	Karadere	Growing LD	0.039
76	TR81371-Nigde	Gene bank LD	107	TR81258-Malatya	Gene bank LD	0.042
75	TR81249-Elazig	Gene bank LD	100	TR31893-Malatya	Gene bank LD	0.043
91	TR54973-Yozgat	Gene bank LD	101	TR35148-Yozgat	Gene bank LD	0.045
2	Cesit-1252	Turkish CV	74	TR72025-Konya	Gene bank LD	0.045
75	TR81249-Elazig	Gene bank LD	76	TR81371-Nigde	Gene bank LD	0.046
75	TR81249-Elazig	Gene bank LD	107	TR81258-Malatya	Gene bank LD	0.046
91	TR54973-Yozgat	Gene bank LD	96	TR32167-Yozgat	Gene bank LD	0.046
80	TR45305-Yozgat	Gene bank LD	91	TR54973-Yozgat	Gene bank LD	0.048
102	TR81277-Ankara	Gene bank LD	114	TR81338-Ankara	Gene bank LD	0.048
3	Yilmaz-98	Turkish CV	77	TR71914-Konya	Gene bank LD	0.049
15	Sham-1	Turkish CV	69	Cham-1	Foreign CV	0.049
Highest Dice genetic distance scored between 129 genotypes overall the genetic markers:						
54	Clavdio	Foreign CV	82	TR81259-Malatya	Gene bank LD	0.618
38	Gokgol-79	Turkish CV	127	Karakilcik	Growing LD	0.618

Table 4.13. Continued.

Genotype No	Genotype name	Group	Genotype No	Genotype name	Group	Genetic distance
79	TR81381-Sivas	Gene bank LD	82	TR81259-Malatya	Gene bank LD	0.618
54	Clavdio	Foreign CV	127	Karakilcik	Growing LD	0.618
79	TR81381-Sivas	Gene bank LD	127	Karakilcik	Growing LD	0.619
82	TR81259-Malatya	Gene bank LD	127	Karakilcik	Growing LD	0.619
51	Zenit	Foreign CV	82	TR81259-Malatya	Gene bank LD	0.621
48	Tunca-79	Turkish CV	127	Karakilcik	Growing LD	0.621
51	Zenit	Foreign CV	127	Karakilcik	Growing LD	0.621

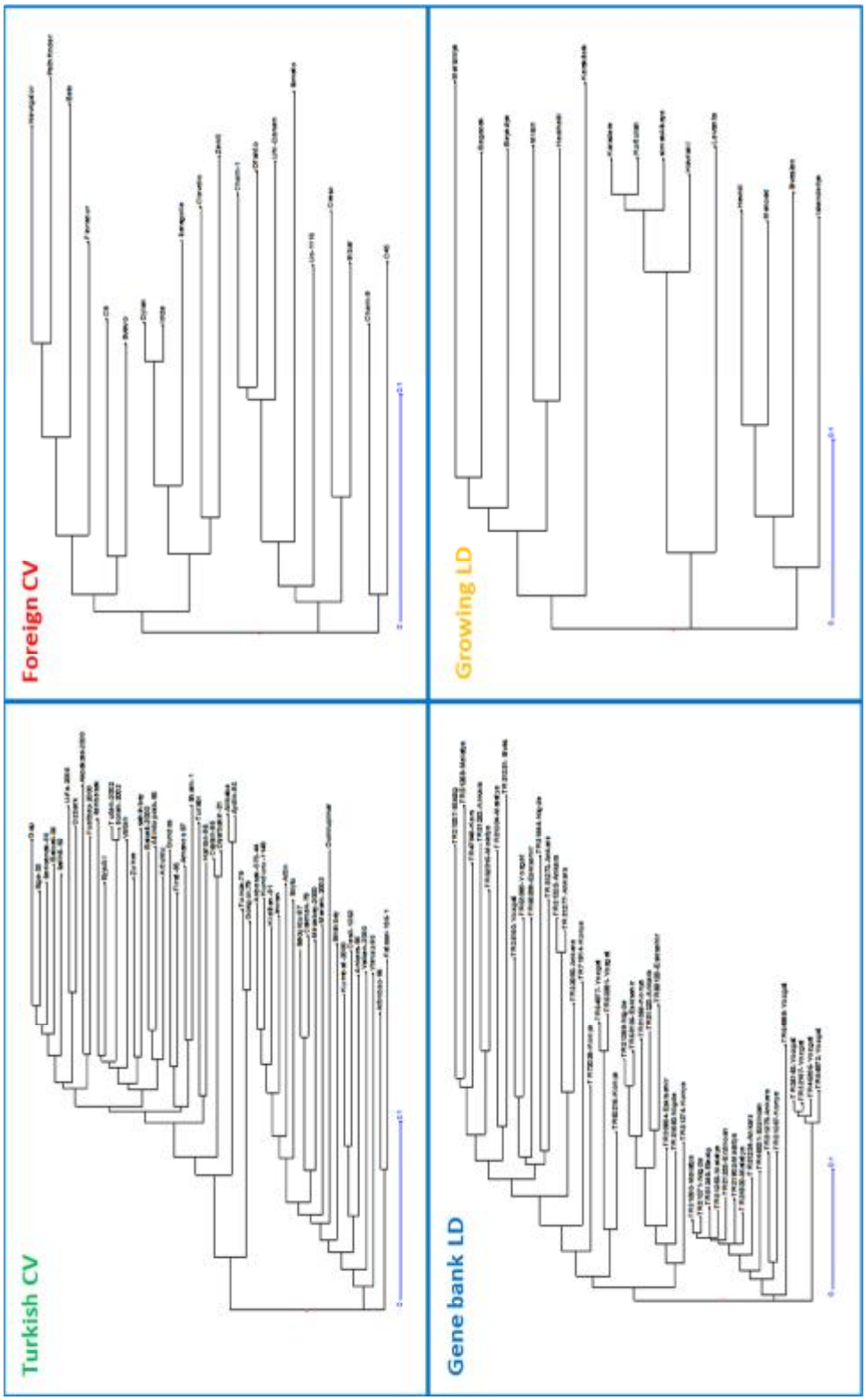

Figure 4.9. Neighbor joining dendrogram based on the Dice dissimilarity index showing the relationships between genotypes within each group separately, with 6357 loci for each.

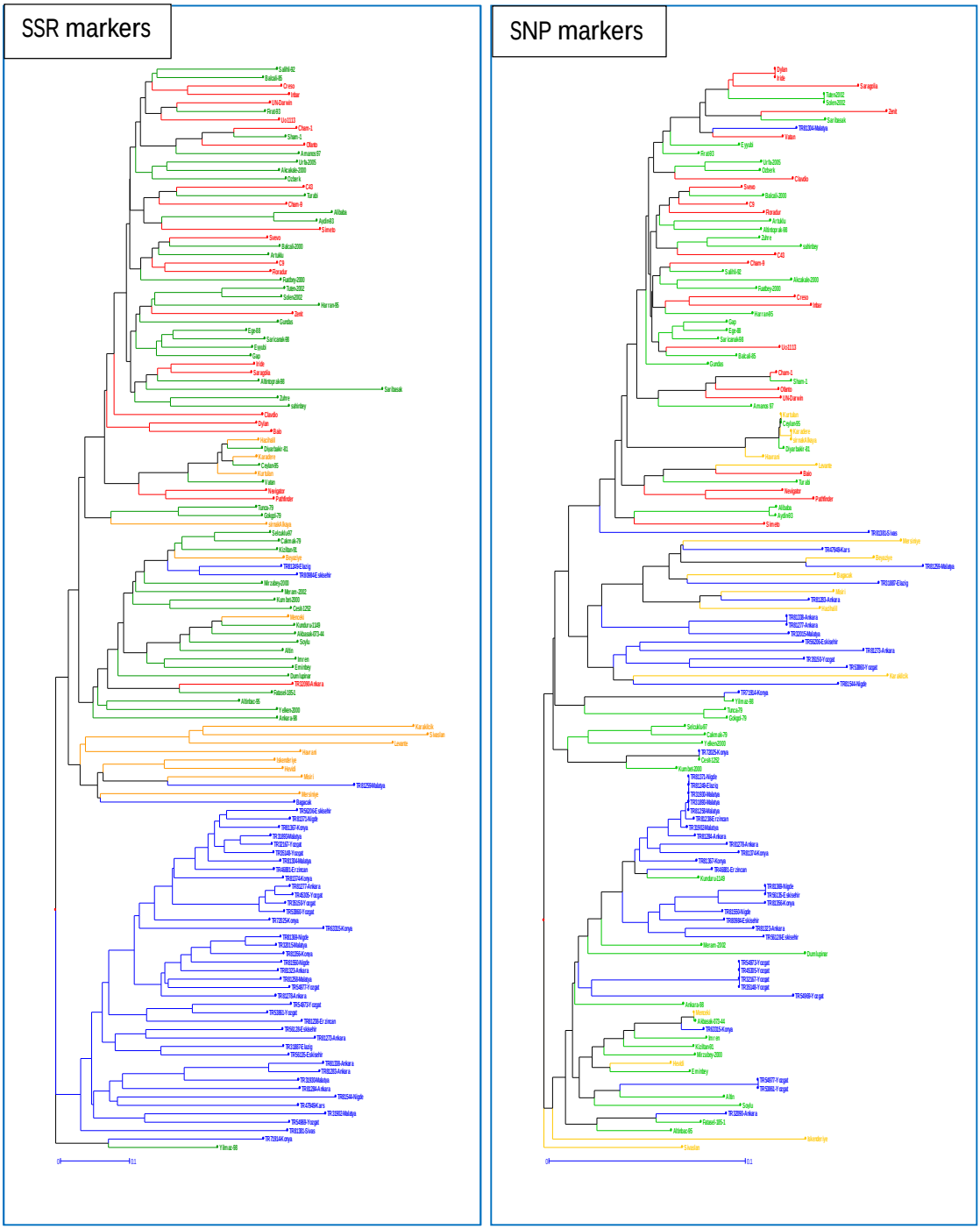

Figure 4.10 Neighbor-Joining tree showing relationships between 129 wheat genotypes revealed by overall genetic markers. The colors correspond to the groups; Green branches and names for Turkish cvs, red for foreign cvs, blue for gene bank LDs and yellow for growing LDs.

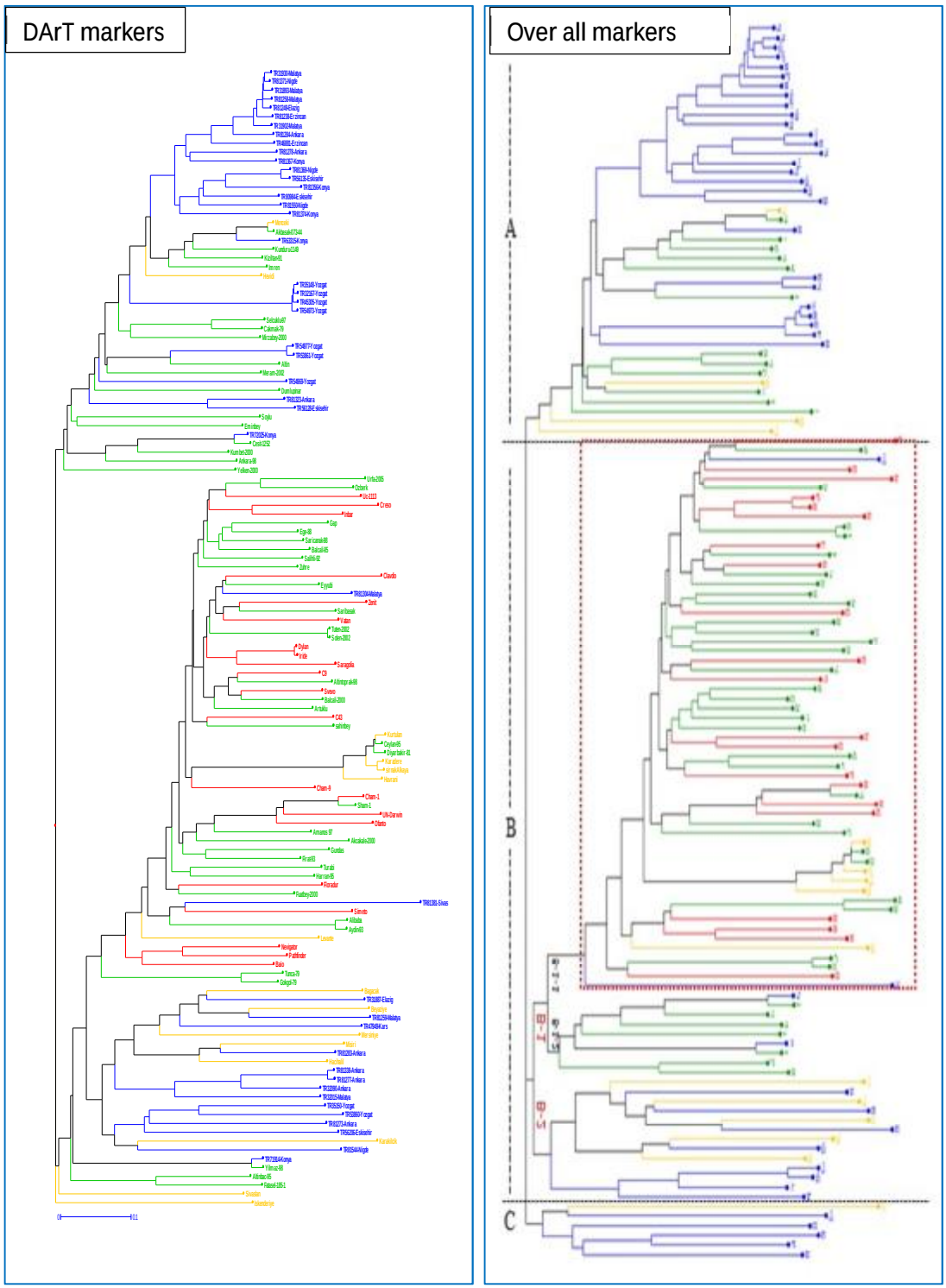

Figure 4.10. Continued.

4.3. Defining population structure

A potential problem in association studies is that population identification may lead to the discovery of many false positives (Zhao et al., 2007). For this reason, several biometrical models have been developed for population stratification (Zhu et al., 2008). A popular method to detect population structure is proposed by Pritchard et al. (2000), in which, molecular marker information is used to assign group membership probabilities to the genotypes utilizing a Bayesian framework.

The intraspecific differentiation and population structure detection within and among groups of the durum wheat collection were performed with the STRUCTURE v.2.3.4 software (Pritchard et al., 2000), which uses model-based clustering. To estimate the mean likelihood for a number of populations (*K*) the admixture model was used to correlate the allele frequencies. This structure analysis was performed for at least 10 runs, 20,000 burn in length and 50,000 repetitions. The results were run in the Structure Harvester program (Earl and von Holdt, 2012) in order to estimate suitable K values for the 129 genotypes. Delta K (ΔK), based on the rate of change in the log probability of data between successive K values was used to determine the number of clusters (K) in the population. The optimal number of populations (*K*) was inferred by Δ*K* values (Evanno et al., 2005), and the calculation of Δ*K* based on the STRUCTURE output indicated an optimal *K* value of 2 (Table 4.14) (Figure 4.11).

Table 4.14 The STRUCTURE output for estimated K value for the 129 genotypes

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-235182.84	7.874318	—	—	—
2	10	-197134.49	26.525227	38048.35	26184.61	987.15875
3	10	-185270.75	71.112044	11863.74	7338.91	103.20207
4	10	-180745.92	281.90669	4524.83	59645.95	211.58047
5	10	-235867.04	189389.8806	-55121.12	714924.11	3.77488
6	10	-1005912.27	638315.069	-770045.23	249534.6	0.390927
7	10	-1526422.9	3467466.227	-520510.63	1206989.2	0.34809
8	10	-839944.33	1243072.55	686478.57	846236.77	0.680762
9	10	-999702.53	757780.1511	-159758.2	5852567.3	7.723305
10	10	-7012028.05	5007400.37	-6012325.52	—	—

K = number of populations assumed; Reps = number of times the simulation was run for a given K; mean LnP(K) = mean log likelihood of K over all reps for that K; Stdev LnP (K) = SD for LnP(K) over all reps for that K; Lni(K) = first-order rate of change of mean LnP (K), defined as LnP (K) – LnP (K−1); ¦Ln"(K)¦ = second-order rate of change, defined as Lni(K + 1) − Ln'(K); ΔK = ¦Ln"(K)¦ divided by Stdev LnP (K). The highest Δ*K* is highlighted in yellow (*K* = 2).

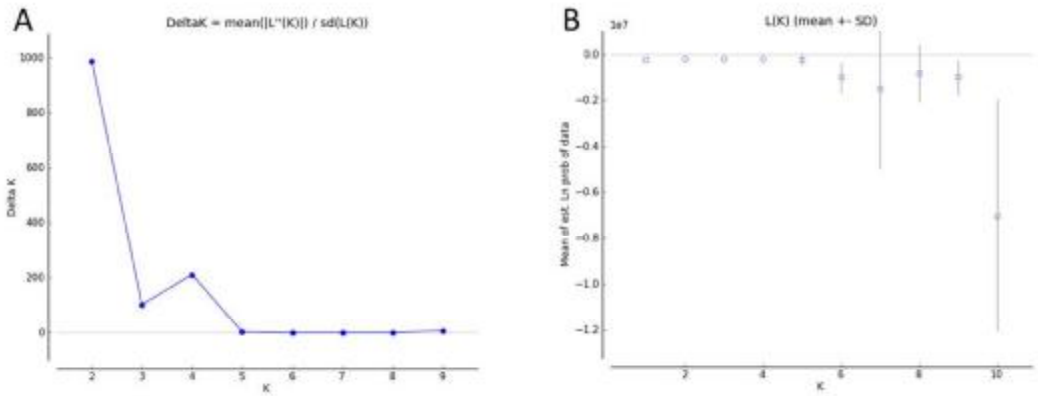

Figure 4.11. Inference of the optimal K value. Plots of ΔK (A) and mean likelihood (B) at each K obtained from the STRUCTURE analysis of a dataset containing 129 durum genotypes

At K = 2, - the genotypes were classified into four groups (I, II, III and IV). Groups I and III had a simple genetic structure while Groups II and IV were admixtures (Figure 4.12).

Groups I, II, III and IV contained 34, 24, 24 and 47 genotypes, respectively. Group I was mainly composed of 22 genotypes of Turkish cvs; in addition, there were 10 genotypes from foreign cvs and two from growing LDs. Group II was complex and highly diverse with 11 genotypes from foreign cvs and 8 from Turkish cvs, three from growing LDs and two from genebank LDs. Group III mainly

consisted of 21 genotypes of genebank LDs and two from Turkish cvs and one from growing LDs. The last group mainly comprised 21, 17 and 9 genotypes from genebank LDs, Turkish cvs, and growing LDs, respectively. These results indicated that Turkish cvs contributed to all groups, no contribution by foreign cvs was noticed in groups III and-IV . And for gene bank LDs group no contribution noticed aslo at group I.Groups I and II showed an exact correspondence to Cluster B-1-1 of the tree analysis results, and Groups III and IV matched Clusters A, B-1-2, B-2 and C.

For the K value, the Q-matrix (population membership estimates) was extracted from the STRUCTURE runs. This matrix provides the estimated membership coefficients for each genotype in each of the subgroups. The delta (K) criterion suggested by Evanno et al., (2005) gave the highest value, this method is known to give rise to the first structure level (Lia et al., 2009), which appears to principally discriminate the genotypes . In the present study panel, we confirm the opinion that using only one covariate in the association model would not fully regulate the genotypes stratification (Mezmouk et al., 2011). And (Evanno et al., 2005) reported partial sampling of genotypes would lead to a lower ΔK at the true K. In addition, in 2011, Kalinowski published a paper showing that unbalanced sample sizes may lead to further errors.

Evanno et al., (2005) showed that the real number of groups is best detected by the modal value of ΔK, a quantity based on the second order rate of change with respect to K of the likelihood function. However, we emphasize that while ΔK helps in identifying the correct number of clusters in most situations, it should not be used exclusively. We agree with the suggestion of Evanno that this criterion is another ***ad hoc*** criterion, and that it should be used together with the battery of other information provided by the structure L(K) itself, the value of alpha and individual assignment patterns. Taken together the results of the neighbor joining tree with the bar plot with K = 5, which was inferred as the optimal K value based on the highest of log-likelihood value (Figure 4.12), this shows the population structure of durum core collection in more detail, revealing that this collection can simply be divided into five groups. We find that ΔK and ***ad hoc*** quantity related to the correct order rate of change of the log probability of data with respect to the number of clusters were a

good predictor of the real number of clusters. Structure analysis identifies groups of genotypes corresponding to the uppermost hierarchical level, and performs well on both the dominant and codominant markers. This was also confirmed by Evanno et al., (2005).

The overall proportion of membership of the sample in each of the 5 inferred clusters were 0.281, 0.102, 0.438, 0.093 and 0.087, respectively. Table 4.15 shows allele-frequency divergence among pops (Net nucleotide distance), computed using point estimates of P.

Table 4.15. Allele-frequency divergence among groups (Net nucleotide distance), computed using point estimates of P.

Group	**1**	**2**	**3**	**4**
2	0.0895			
3	0.1234	0.0695		
4	0.0947	0.0717	0.1071	
5	0.0864	0.0405	0.0679	0.0607

Group I comprised a simple genetic structure and was composed of six genotypes (75, 76, 100, 19, 116 and 95) mainly from the genebank LDs except the two genotypes, 19 and 116, from Turkish cvs and growing LDs, respectively.

Group II was complex and diverse, and contained 29 genotypes; 17 from genebank LDs, 11 from Turkish cvs and one from growing LDs. Groups I and II were mainly parallel with Cluster A inferred from the Neighbor Joining analysis. It was also noted that there was a membership coefficient represented by a red segment in genebank LD.

Group III consisted of 12 genotypes mainly composed of 6, 4, 2 genotypes from Turkish cvs, genebank LDs and growing LDs, respectively. This group was similar to B1-2 of the neighbor joining analysis.

Group IV was complex and composed of 12 evenly distributed genotypes from genebank and growing LDs. Group IV was in agreement with Cluster B-2 of the neighbor joining analysis.

Group V was simple in genetic structure and composed of 23 genotypes 15 from Turkish cvs, 6 from foreign cvs and two from growing LD.

Group VI was complex and highly diverse, consisting of 32 genotypes; 15 from foreign cvs, 13 from Turkish cvs, 3 from growing LDs and one from genebank LDs.

Groups V and VI were in good agreement in terms of the result of the neighbor joining analysis and correspond to the sub cluster B-1-1.

Group VII was complex and diverse, containing 6 genotypes 5 of which were from genebank LDs and one from Turkish cvs except for genotype number 12 located in B-1-2 the genotypes corresponded to sub-cluster A in the tree.

Finally, Group VIII was complex and highly diverse, consisting of 9 genotypes; 7 from genebank LDs and two from Turkish cvs, and mainly corresponded to the sub-cluster C in partnership with B-2 and B-1-1.

The five genotypes from genebank LD (75, 76, 95, 100) and two genotypes, one from Turkish group (no: 19) and one from growing LD (no: 116), showed a perfect match to the genetic structure carrying the same red area for membership coefficient, and formed a small cluster in the neighbor joining analysis (C). Similar case was also noted for the following genotypes; 8, 20, 24, 30, 32, 61, 66, 67, 9, 17, 23, 35, 43, 44, 50, 120, 121, 14, 29 and 39. A complete match with the genetic structure was found in 14 genotypes from Turkish cvs, three from Foreign cvs, and two from growing LDs. This is shown in the blue area for membership coefficient in Figure 4.12, but distributed to the branches of B-1-1. In the same context, two genotypes (82, 125) showed a similar genetic structure as denoted in the green area for membership coefficient. Genotypes 101 and 96, for 15 and 69 and for 102 and 114 showed a similar genetic structure revealed in the colored segments for membership coefficient, and separately formed small clusters in the tree. (Figure 4.12)

Generally, neighbor joining cluster analyses and PCoA are used as complementary approaches to confirm the results obtained using STRUCTURE. In PCoA, the genotypes showed high diversity especially those in the landrace groups. The Turkish and foreign varieties also showed close association with each other, and

similar varieties demonstrated admixture in the structure analysis confirming their relatedness within the diverse gene pool. The four groups showed similar grouping to the neighbor joining tree clustering. The substructure in the durum wheat collection using different methodologies was compared and the final K value using STRUCTURE was ascertained. The tree clustering basically divided groups into similar clusters to those produced by the structure bar plot at K2, and the results indicated a high correspondence. Population structure analyses indicated that durum wheat genotypes can also be efficiently categorized on the basis of both geographical origin. However, it is necessary to keep in mind that structure and all other Bayesian methods are model-based, with strong priors and hypotheses and it also important to consider all the limitations and restriction of these approaches in terms of ensuring a correct analysis of the results. Thus, it may be interesting to crosscheck the outputs from such analysis with distance-based (usually factorial analysis) methods that make no assumptions on the data. When constructing the model and choosing the parameters, many factors should be taken into consideration; for example, 1) likelihood plot of models, 2) stability of grouping patterns across 10 runs, 3) germplasm information or “breeder’s knowledge,” 4) Cluster analysis (NJ tree), and 5) Principal component analysis (PCA) or Principal coordinate analysis (PCoA). On the basis of this information, the selection of optimal structure grouping will be facilitated, while we found that the real difficulty lies for K estimating, which generally works well in datasets with a small number of discrete populations.

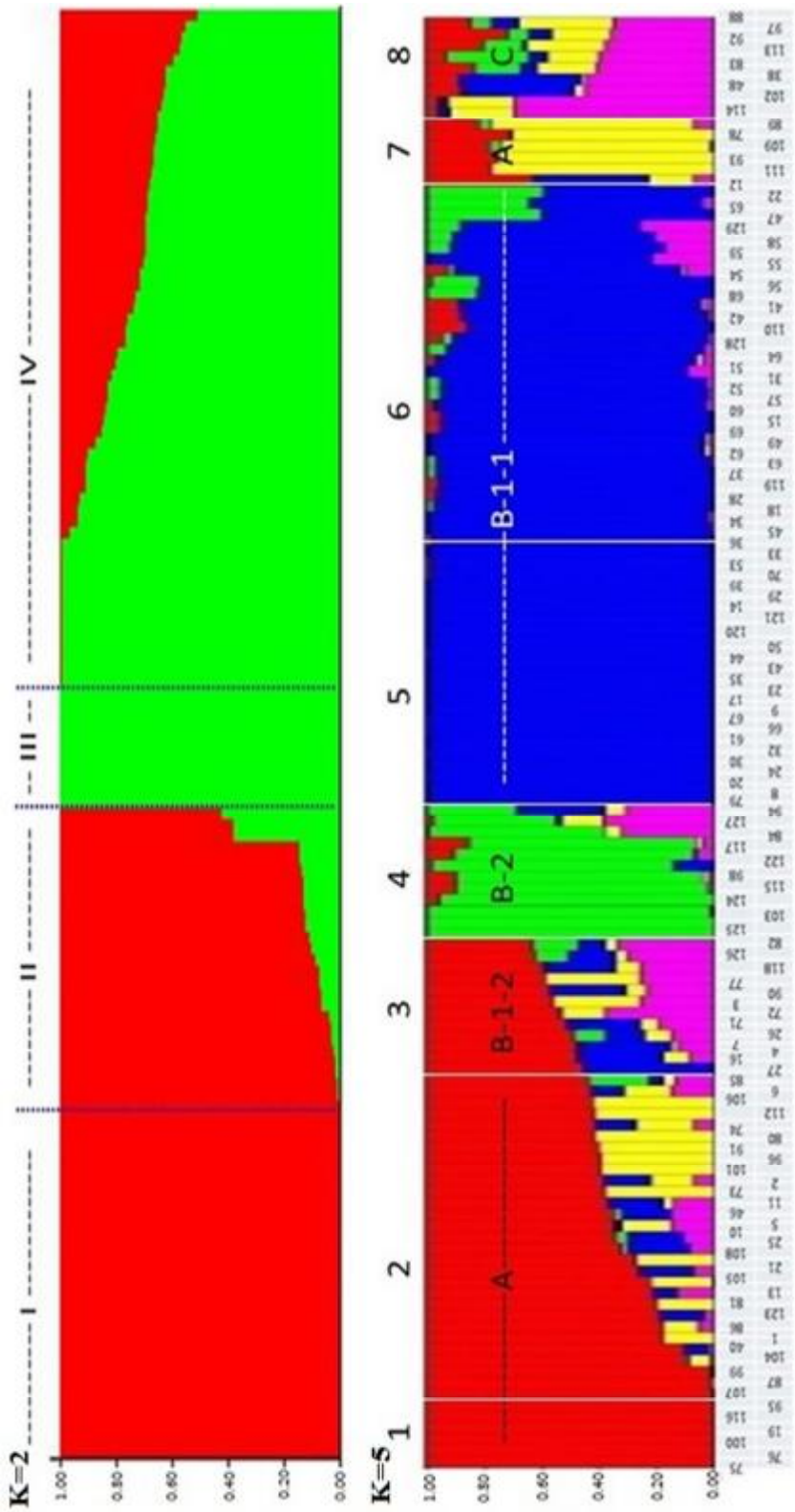

Figure 4.12. The survey of the genetic relationship among 129 durum wheat genotypes using STRUCTURE. Models with $K = 2$ and $K = 5$ showed the ΔK value and the highest log-likelihood value, respectively. A vertical axis indicates a membership coefficient in each optimum number of K. For the individual bar plots, the corresponding genotypes number, as classified by the neighbor joining tree, are indicated at the bottom of the plot.

4.4. Linkage disequilibrium

From 6357 markers, only 4853 were chosen to estimate the linkage disequilibrium (LD) in durum wheat. To calculate pair-wise r2 values for markers within each linkage group and the visualization of plotted LD decay, Tassel 3 software was used. LD was measured from the combined results of all the markers as well as LD was measured also with 410 SSRs, 3751 DArTs and 692 SNPs separated, due to size limitations, the TASSEL output (LD) files could not be included in the supplement but they are available upon request.

Generally, LD can be measured using R^2, which summarizes both the recombination and mutation history (Flint-Garcia et al., 2005). The LD in the entire durum collection can be calculated separately for locus pairs according to each of the marker types (SSR, DArT, SNP) and the combined markers. For the SSR markers, there were 1504 (7.82%) of the 19225 marker pairs of loci that showed a significant level of LD ($p < 0.01$), and 219 (3.89%) of the 5624 marker pairs of loci showing a significant level of LD which had $R2 > 0.2$. For the DArT markers, of 186276 marker pairs of loci, 4330 (2.32%) showed a significant level of LD ($p < 0.01$), and 3987 (43.1%) of 9260 marker pairs of loci had $R2 > 0.2$. For the SNP markers, of 33326 marker pairs of loci, 2822 (8.47%) showed significant LD ($p < 0.01$), and 1195 (74.3%) of 1609 marker pairs of loci had $R2 > 0.2$. For the combined set of markers, of 241376 marker pairs of loci, 7954 (3.30%) showed significant LD ($p < 0.01$), and 7954 (33.3%) of 23864 marker pairs had $R^2 > 0.2$. The results showed that the highest LD was observed with SNPs markers (8.47%), while the DArTseq markers were the lowest (2.32%). Figure 4.13 shows the distribution of the r2 values of the markers for the 129 durum genotypes. Approximately, 95.1% of the r2 values were below 0.2, and 6.52% of markers have an r^2 value ranging from 0.05 to 0.10. The patterns of LD were also visualized across the genome from the diagonal of the heat maps (Table 4.16) (Figure 4.14).

Table 4.16. Significant level of LD, and percentage of marker pairs of loci

		Total of chromosome pairs of loci	pDisq (p<0.01)	%
SSR		19225	1504	7.82
	R2 >0.2	5624	219	3.89
DArT		186276	4330	2.32
	R2 >0.2	9260	3987	43.1
SNP		33326	2822	8.47
	R2 >0.2	1609	1195	74.3
Combined		241376	7954	3.30
	R2 >0.2	23864	7954	33.3

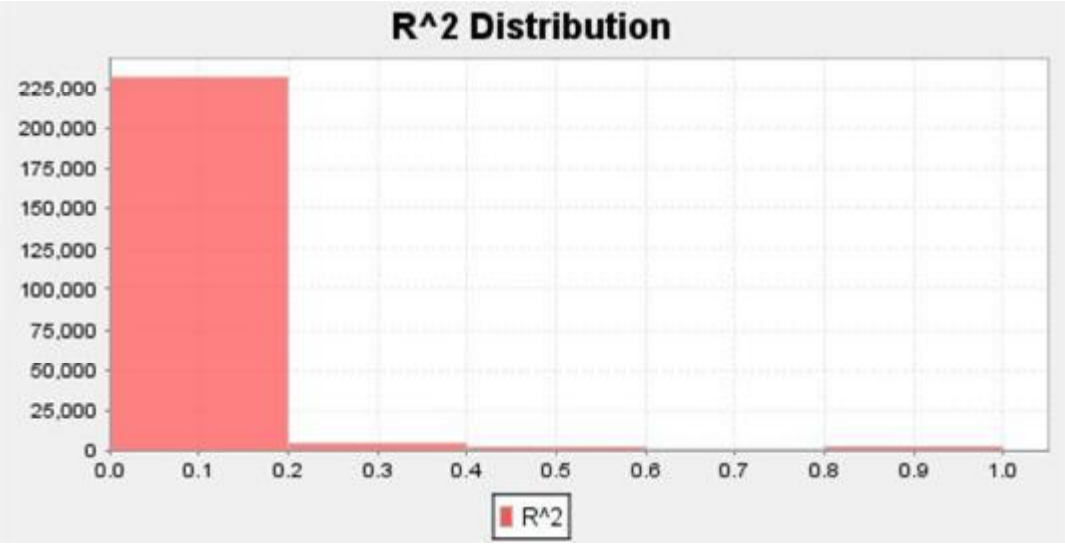

Figure 4.13. Distribution of the r2 values of the markers for the 129 durum genotypes.

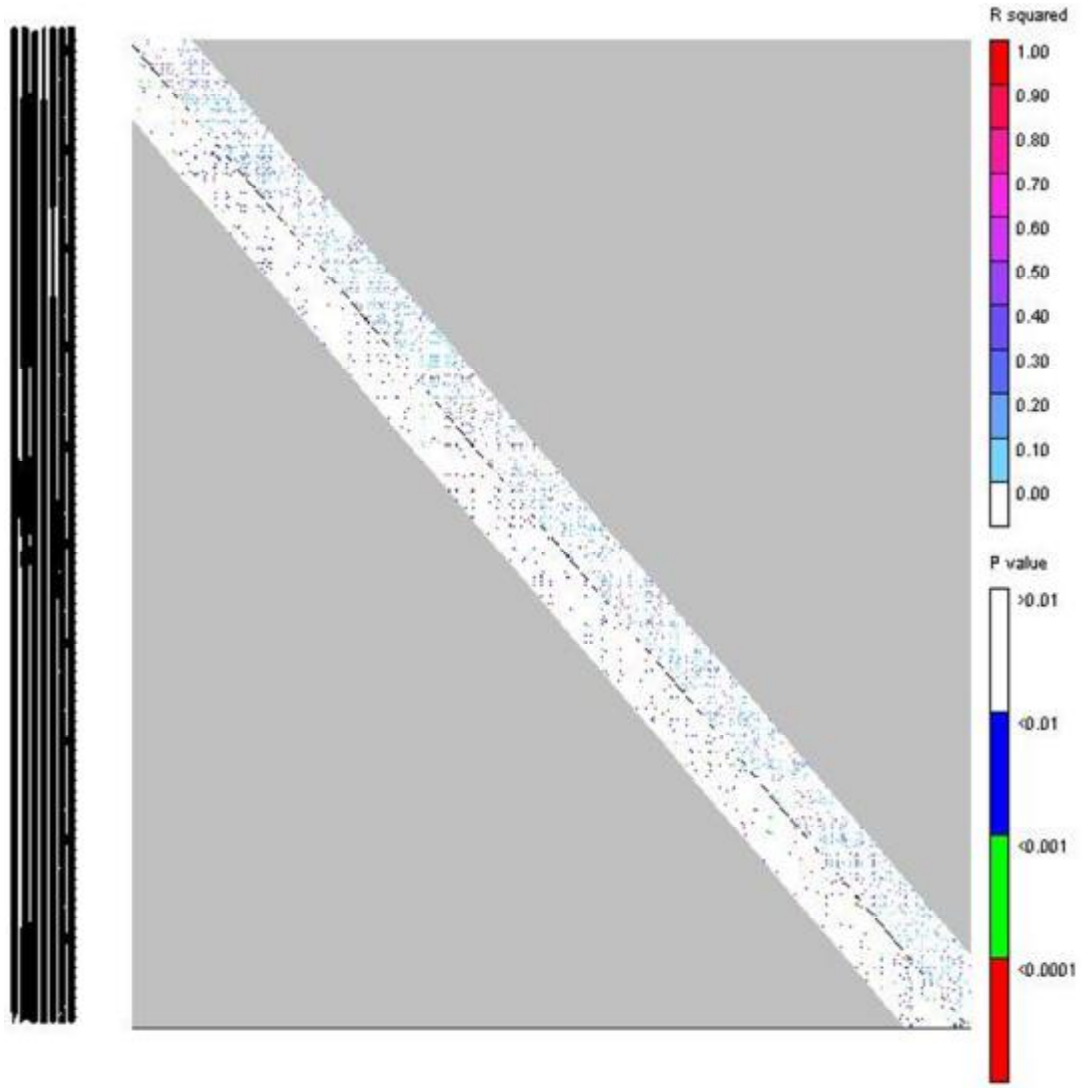

Figure 4.14. LD measurements (r2, above the diagonal line) and probability values (P, below the diagonal line) for the 3073 markers located in the 129 durum genotypes. The markers were ordered on the x and y axes based on their genomic location, with each cell of the heat maps representing a single marker pair.

The levels of LD varies in different populations or species due to many genetic and breeding factors such as genetic drift, recombination and the mating system, mutation, population structure, amount of inbreeding, migration and selection (Kaya et al., 2016; Al-Maskri et al., 2012; Yao et al., 2009). Since wheat is autogamous, its LD extent is greater by three orders than the LD extent in maize, which is an outcrossing species (Breseghello and Sorrels, 2006). A higher level of LD in the centromere region may be due to a low frequency of recombination around

centromere (Jones et al., 2002) and loss of variability during domestication. Peng et al. (2003) revealed that domestication-related QTLs for plant height, yield per plant and days were detected in the centromere region of chromosome 2A. These levels observed in wheat were higher than the LD level observed in maize that decayed at about 1kb distance (Remington et al., 2001; Palaisa et al., 2003). Accurate estimates of LD are very important for marker trait association and the criterion that establishes the critical values to test the significance of syntenic LD as a statistical factor may also affect the analysis.

The extent of LD differs in various wheat populations and these patterns of the LD extent depict the selection pressure on respective genomic regions (Sajjad et al., 2013). Liu et al. (2010) observed a very low level of LD (3cM) in a set of 103 Chinese wheat accessions assayed with 31 SSR markers. In a survey of 96 diverse wheat accessions assayed with 874 DArTs, highly variable results were observed with Chromosome 7A showing very high values of LD of about 45cM while few very closely linked markers showed no significant LD (Ahmad et al., 2014). It is obvious that different populations of wheat selected from diverse germplasm exhibit different levels of resolution for association mapping studies. Therefore, LD patterns in wheat may vary with genomic regions, marker types and populations.

Unfortunately, in the current study, appropriate chromosome location and position of the makers were not found to allow the exact LD extent by cM to be determined in the 129 durum wheat genotypes.

4.5. Allele frequencies and significant markers

For the 6357 markers scored across the 129 genotypes, the frequency for allele “1” ranged from 0.008 to 0.992 and the frequency for allele “0”, many of markers had a minimum allele frequency inferior to 0.05 and were not used; therefore, only 3073 markers remained for the further association analysis. The software program TASSEL (Bradbury et al. 2007) was used for the association analysis. To reduce false or spurious associations, population structure (Q) and kinship (K) were calculated first. They were used as covariates in a mixed linear

model (MLM) for the associations. A generalized linear model (GLM) was also used, in which only the Q was used as a covariate. The significance levels were modified using the Bonferroni correction, with each significance value being divided by 3073 (the number of markers used). Any result below the corrected <0.05 p-value was considered significant. Based on the corrective model, the significant marker-trait associations that were detected varied from 4 to 144 at a value threshold of α= 0.01 (Table 4.17). Using the highly conservative 5% Bonferroni correction threshold (0.05/3073) for multiple comparisons, only 2 markers were significantly associated.

Table 4.17. Number of markers with significant marker traits associations

Model	α =0.05	α =0.01	Bonferroni 5%
GLM	26358	1399	650
MLM+K	8229	181	2
MLM+K+Q	842	144	2

□: the threshold probability level, GLM: generalized linear model, MLM: mixed linear model, K: Kinship, Q: refers to the structure matrix from STRUCTURE

4.6. Marker-trait associations

A total of 6357 high quality markers (SNPs and DArTs) with no missing data and SSR markers with missing data (<5%) were used in this AM study, in order to identify markers associated with agro-morphological traits including spike traits and also quality traits in a diverse panel of durum wheat cultivars and landraces.

The software program TASSEL (Bradbury et al., 2007) was used for the association analysis. A generalized linear model (GLM) was first used where only the Q was used as a covariate (Appendix 3). However, the mixed linear model (MLM) is more powerful compared to the general linear model (GLM) or any other model developed so far. Theoretically, kinship creates LD between genetically linked loci but it can also create LD between genetically unlinked loci. To reduce false or spurious associations, population structure (Q) and kinship (K) were calculated, and used as covariates in a mixed linear model approach (MLM). The kinship matrix (K) and probability of membership of each line (Q) in the model for each trait were

calculated (Yu et al., 2006). The kinship matrix was calculated as implemented in the TASSEL software and calculated as the proportion of alleles shared between each pair of lines.

The significance levels were modified using the Bonferroni correction, in which each significance value was divided by 3073 (the number of markers used). Any result the corrected <0.05 p-value was considered significant. The GLM generated more significant associations. The addition of the Q and K matrix resulted in a reduction in the number of marker-trait associations in both MLM (K) and MLM (Q+K) demonstrating that covariates were necessary to decrease and eleminate false positive associations.

SSRs, DArTs and SNPs markers were significantly associated with investigated traits at different rates. The output data was filtered on p-value, whereas p-value $\leq 1e^{-3}$ and loci with $R^2 \geq 0.1$ was taken into consideration, except for some segment fragments that joined at $R^2 \geq 0.09$. The MTAs were additionally scored based on the corrective Bonferroni threshold of 5% to identify the highly significant markers. Among the 144 MTAs detected in this study, 88 were major MTAs and two only were significantly associated.

4.6.1. Marker-Agronomy trait associations

In our investigation for marker traits association (MTA) based on the MLM (Q+K) model, of 144 associations detected, 77, 54, 13 were associated with agronomy, spike character and quality traits, respectively. Days to heading scored in two environments during 2015 was found to be associated with nine loci in different chromosomes. Four (*wPt* - 1258749, *wPt* - 1698914, *wPt* - 1769376 and *wPt* - 7353451, 3A) were associated and considered as major MTAs with an explanation range of R^2 value from 10% to 15% of the phenotypic variation. While the other 5 minor MTAs were observed in 3A, 5A, 5B and 7A, in addition to the associated markers, there were unknown chromosome loci. The highest R^2 value (0.15) was detected in the Ada-15-L environment with the marker *wPt* - 1258749 (Appendix 4).

Many of the previous studies published the associated marker traits or identified location of QTLs for the HD trait. Kiseleva et al., (2016) found seventy-eight markers for heading date in the pericentromeric region of the 5B chromosome and they were significantly associated with heading date variation. Kobayashi et al., (2016) reported eleven significant SNP markers associated with "days to heading in autumn sowing". These were grouped into 6 QTLs located in 2A, 2B, 2D, 3B, 5A, 6D, and 7D. Ain et al., (2015) also reported five MTAs, which were detected for HD on 1B and 2B. Lopes et al., (2015) reported three SNPs located in chromosome 5A associated with the HD trait. Laido et al., (2014) identified 43 markers being specific for the HD trait. In the association analysis conducted by Neumann et al., (2011) marker-trait associations shared between heading date and flowering time were located in chromosomes 1B, 5D, 6A and 7A. Dodig et al., (2012) reported two MTAs with the HD trait located in 2BS and 6DS. Others markers located in 3B were reported by Dura et al., (2013). Bousba et al., (2013) reported 3 MTAs with the HD trait. Many researchers also found numerous QTLs for HD (Würschum et al., 2015; Zhang et al., 2009; Wang et al., 2009; Maccaferri et al., 2008; Marza et al., 2006).

Days to maturity was scored in two environments during 2015 and was found to be associated with five loci in different chromosomes. Three were major MTAs observed in chromosomes 2A, 3A & 5B by SNP-982956, ***wPt*** -7353451 and ***wPt*** -7353451 with a R^2 value ranging from 10% to 12% of the phenotypic variation. The remainder of the MTAs had a minor effect. The highest R2 value (0.12) was detected at mean value environment by the SNP-982956 marker (Appendix 4). Ain et al., (2015) reported three MTAs being detected in the 1B and 2B for the MD trait. Study on the grain size of wheat undertaken by Su et al., (2011) demonstrated the association of a haplotype of earlier heading date and maturity in hexaploid wheat. Many other authors reported and published information about QTLs (Hanocq et al., 2007; Marza et al., 2006).

The highest number of marker trait associations was shown in plant height. This trait was scored in five environments, 35 MTAs were detected in chromosomes 1A, 1B, 2B, 3A, 4A, 4B, 5A, 5B, 6B and 7A in addition to 16 associated markers in unknown chromosomes location. Of the 35 MTAs, 32 were considered to be major,

one, *wPt* – 3950570, had a significant score based on the corrective Bonferroni threshold of 5%, confirmed in four environments in addition to the mean of environment values with the explanation range of R^2 from 10% to 17% of the phenotypic variation. The second marker, *wPt* – 4004275, was detected in Ada-15-H, Koz-15 and the mean value with R^2 values of 0.11, 0.11 and 0.10, respectively. In addition, to the five different markers detected in Ada-14-L and Koz-15 the third flanked marker was SNP-1229434 detected in Ada-14-L, Koz-15 R^2 values 0.14, and 0.12 respectively. Furthermore, the *wPt-* 3944345 was detected in Ada-15-L and the mean value explained the R^2 value as 13% and 11% of the phenotypic variation respectively (Appendix 4).

Mengistu et al., (2016) reported 8 MTAs in chromosmes 1B, 2A, 3B, 4A, 4B and 6B, and suggested a complex genetic control for PH. Ain et al., (2015) reported ten MTAs on 2B for the plant height (PH) trait. Lopes et al., (2015) detected major effects for PH associated with *Rht-B1* and *Rht-D1* and several SNP markers in chromosomes 4B and 6A. Ain et al., (2015) reported 10 MTAs for the PH trait. Laido et al., (2014) identified 26 markers as being specific for the PH trait. Yu et al., (2014) identified seven MTAs located in 3B, 5B and 7B for the PH trait. Hu et al., (2014) detected six significantly associated SNPs for PH in chromosomes 1A, 2A, 4B, 6A and 6B. Maccaferri et al., (2014) reported 50 chromosome regions containing evidence of association with the PH trait. Zanke et al., (2014) reported a total of 153 significant marker-trait associations for plant height in chromosomes 1A, 2A, 3A, 3B, 5B, 5D and 7B. Dura et al., (2013) detected two significantly associated markers Xwmc177 and Xwmc24 located in chromosomes 2A and 1A, respectively. Bousba et al., (2013) reported one MTA with the PH trait. Neumann et al. (2011) investigated marker-trait associations common to plant height and peduncle length, and detected the MTAs in chromosomes 1A, 2B, 4A and 7B for peduncle length and in chromosomes 1B, 4A, 6B and 7A for plant height. A significant association of the DArT marker wpt730772 with plant height in elite soft winter lines was detected in chromosome 6AS by Kulwal et al. (2012). Furthermore, many additional QTLs were identified by various authors (Zhang et al., 2011; Yao et al., 2009; Rebetzke et al., 2008; Marza et al., 2006; Quarrie et al., 2005).

The peduncle length was measured in four environments during 2015. Twelve MTAs were detected with peduncle length in chromosomes 1B and 4B. In addition of the detected twelve MTAs, there were MTAs that were in unknown chromosome loci, explaining 9% to 13 % of the phenotypic variation. The MTA SNP1229434 detected in Ada-15-L, Koz-15 gave a mean of 12–13% for the phenotypic variation in these study areas. Of the 12 MTAs, five were major MTAs explaining 10% to 13% of the phenotypic variation (See Appendix 4). Yu et al., (2014) identified 7 MTAs in chromosomes 1B, 2B and 5B for the peduncle length trait. Hu et al., (2014) also reported several markers being significantly associated with peduncle length PL trait in chromosomes 1B, 5A, 6A and 6B. Neuman et al., (2011) found six common QTLs for PL. Dura et al., (2013) reported five putative markers being significantly associated with PL. These markers were detected in chromosomes 1B, 2B, 3A, 3B, 4A, 5A, 6A, 6B, and 7A. In addition, Borner et al., (2002) identified QTLs for peduncle length in chromosome 6A. Marza et al., (2006) found 10 yield QTLs in chromosomes 1AL, 1B, 2BL, 4AL, 4B, 5A, 5B, 6B, 7A and 7D. Rao et al. (2007) identified QTLs for peduncle length in chromosome 1H.

Peduncle extrusion length measured during 2015 in three environments. Sixteen MTAs were detected with peduncle extrusion length in many chromosomes (1B, 2A, 2B, 3B, 4A, 4B, 6A and 7A). In addition of the sixteen MTAs detected markers were associated with the loci of unknown chromosomes, which explained 9% to 15 % of the phenotypic variation. Two markers, ***wPt*** -7903270 and SNP1229434, detected at Ada-15-L, Koz-15 respectively explained the phenotypic variation at the highest level at 11–12 % and 12–15 %, respectively. Of the16 MTAs, seven were considered to be major (Appendix 4).

4.6.2. Marker-Spike trait associations:

The most conservative model (MLM + K + Q) was also used to search for marker trait associations in the six spike traits that were evaluated. After multiple comparison adjustments, 54 MTAs for spike traits were found, explaining 9–15 % of the phenotypic variation. Of these MTAs, 35 were considered to be major in terms

of the spike length trait in chromosomes 2A, 3A, 4B and 5BL explaining 9% to 13% of the phenotypic variation. In addition, there were markers associated with unknown chromosome locations (Appendix 4). Of the 54 MTAs, eight were detected as having a spike length trait explaining 9% to 13% of the phenotypic variation. Four markers (*wPt* - 1699756, SNP-1070815, SNP-2252454 and SNP-999325) were confirmed in three environments. Multi-trait marker-trait associations were identified for spike length by many researchers, and our results conform to those of several published studies on chromosome location.

Mengistu et al., (2016) reported relevant MTAs in chromosome 2B for spike length. Yu et al., (2014) published several MTAs in 1B, 3B and 5B where SL was involved. Dura et al., (2013) reported that five markers associated with SL were located in chromosomes 1B, 4B and 7B. Dodig et al., (2012) reported two MTAs with SL located in 2DS and 6DS. Cui et al., (2013) identified four major QTLs and 20 minor additive QTLs in two RIL populations affecting spike length. Neumann et al., (2011) reported many MTAs on chromosomes 2B, 2D, 3A, 3B, 5B, 6B and 7A, but spike length-specific MTAs were also located in chromosomes 3A, 4A, 5B and 7B. One of the MTAs in chromosome 7B was significantly associated with spike length. Liu et al., (2010) detected marker-trait associations for spikelet number and spike length in chromosome 4AL by conducting an association analysis with 116 SSR markers mapped in chromosome 4A for 103 Chinese spring wheat. In addition, many QTLs were reported; Marza et al., (2006) identified 10 QTLs for spike length on 1AL, 1B, 1AS, 2BL, 2BS, 3B, 4B, 5B, 7A and 7B. Ma et al., (2007) mapped one QTL for spike length on chromosome 5A. Kumar et al., (2007) identified one QTL in chromosome 2B and 2DS. Yao et al., (2009) found another locus in chromosome 2A. Yao et al., (2009) found marker-trait associations for spike length both on the short and long arms of chromosome 2A. Ten MTAs were found for spike weight explaining 9% to 13% of the phenotypic variation at chromosomes 3A & 5BL in addition to markers associated with the loci of unknown chromosomes. Among eight MTAs, seven were considered to be major(Appendix 4).

Four markers with significant association for spike weight located in chromosomes 1A, 1BL, 1BS, 2A, 2B, 4B, 6B and 7B were reported by Dura et al.,

(2013). Marza et al., (2006) detected five QTLs for mean spike weight in chromosomes 1B, 2B, 3B, 5A and 6B.

For spike yield, only four MTAs were found explaining 9–12% of the phenotypic variation, of which one was major (Appendix 4). Campbell et al., (1999) detected QTLs for grain weight on chromosomes 1A, 2A and 2B. Quarrie et al., (2006) found another locus on chromosome 7A. Kumar et al., (2007) found a QTL for grain yield trait. Pushpendra et al., (2007) identified three QTLs on chromosomes 1A, 2B and 7A. Sun et al., (2009) detected QTLs on chromosomes 2A and 6A. Yao et al., (2009) found marker-trait associations using SSR markers on both arms of chromosome 2A for grain per spike. Another study on the grain size of wheat also demonstrated the association of haplotype of a grain size gene with larger grain size in hexaploid wheat (Su et al., 2011). Marone et al., (2012) reported that among the environment-specific unmapped markers, wpt0866 was associated with kernel number. Bousba et al., (2013) reported 6 MTAs with grain per spike traits.

Six MTAs were found, of which four were major for spike harvest index explaining 9% to 11% of the phenotypic variation at chromosomes 2A & 4B. In addition, many markers were associated with unknown chromosome loci. (Appendix 4). Marza et al., (2006) found 10 yield QTLs on chromosomes 1AL, 1B, 2BL, 4AL, 4B, 5A, 5B, 6B, 7A and 7D. Kumar et al., (2007) reported a QTL for harvest index on chromosome 4AL. For GWPS, Yu et al., (2014) found one MTA on chromosome 2B.

For spikelet number per spike (SNPS), the lowest MTAs were detected in spike traits, with only two MTAs, one of which was major, explaining a total of 9% to 15 % of the phenotypic variation at 5BL. In addition, there were markers associated with unknown chromosome loci (Appendix 4). Hu et al., (2014) found a total of 22 SNPS markers having significant associations on 2A, 2B, 3A, 5A, 7A and 7B. On chromosomes 1B, 2B, 5B and 7B Yu et al., (2014) found thirteen markers significantly associated with spikelet number per spike. A very strong MTA was obtained for spikelet number per spike on chromosome 2BS (wpt8492), and two unmapped markers wpt666595 and wpt667101 for spike number per m2 were reported by Edae et al., (2014). Cui et al., (2012) reported up to 25 putative additive

QTLs for the number of spikelets per spike, and they were covered all the 21 wheat chromosomes except 1B, 3D and 6B. Kumar et al., (2007) identified three QTLs on chromosomes 2B, 4A and 6A. Yao et al., (2009) mapped one QTL on chromosome 2A. Kumar et al., (2007) found a QTL for grain yield and harvest index traits on chromosome 2DS, and another multi-trait QTL for biological yield, harvest index and spikelet per spike on chromosome 4AL.

For grain number per spike (GNPS), the highest number of MTAs (twenty six) that explained 9% to 15 % of the phenotypic variation, and among 26 MTAs, 16 were considered to be major (Appendix 4). Mengistu et al., (2016) reported an MTA on chromosome 2B for seeds per spike. Yu et al., (2014) also found five associated markers for GNPS on chromosomes 3B, 4B, 6B and 7B. Similarly, Hu et al., (2014) reported several markers significantly associated with the GNPS trait located on chromosomes 1B, 5A, 6A and 6B. Neumann et al., (2011) identified trait-specific marker-trait association on chromosome 5B for spikelet number. Yao et al., (2009) detected four different QTLs on chromosome 4A for spikelet number per spike using SSR markers. Liu et al., (2010) found six marker-trait associations for kernel number per spike on chromosome 4A. McIntyre et al., (2010) detected three putative QTLs which explained 5-8% of the variation on chromosomes 1D, 4D and 6B for high kernel number per spike. All three QTLs were co-located with the QTL for high harvest index, and two were also co-located with QTL for high kernel weight. The spike number is strongly related with the kernel number per unit area, the main yield component of wheat. Quarrie et al., (2005) mapped three QTLs for grain number per spike on chromosomes 5A, 7A and 7B. Marza et al., (2006) detected seven QTLs on chromosomes 1A, 1B, 2B, 3B, 4B, 6A and 7B. Quarrie et al., (2006) found another locus on chromosome 7A. Kumar et al., (2007) reported and identified one genomic region on each of chromosome 1A and chromosome 1D controlling grain number per spike in wheat.

4.6.3. Marker-quality trait associations:

Quality traits were scored in three environments. The implementation of the MLM using the Q+K model showed that of the 3073 markers, only 13 showed significant associations (p-value $\leq 1e^{-3}$) with the studied quality traits. These markers were located on the 1A, 2A, and 7B chromosomes. In addition, there were associated markers that did not have known positions.

Thousand kernel weight trait was found to be associated with 5 markers explaining 9% to 16 % of the phenotypic variation, of which three were major MTAs (Appendix 4). Mengistu et al., (2016) reported one relevant MTA on a chromosome for TKW. Ain et al., (2015) detected ten MTAs for the TKW trait on several chromosomes. Hu et al., (2014) reported that seven SNPs had significant associations with TKW located in chromosomes 1B, 2A, 4A, 5B, 6A, 6B and 7B. Yu et al., (2014) reported that TKW was significantly associated with 13 markers spread over chromosomes 1B, 2B, 3B, 4B, 5B, 6B and 7B. Laido et al., (2014) identified 45 markers being specific for the TKW trait. Dura et al., (2013) reported three significantly associated markers , namely Xwmc322 on 3A and 3B, Xwmc475, on 5A and 7B and Xbarc100 on 2A, 2B and 5AL. Bousba et al., (2013) reported 8 MTAs with the TKW trait. Huang and Brule-Babel (2012) reported that a few SNPs and haplotypes were associated with thousand kernel weight. In addition, several QTLs were reported by Jochen et al., (2011); Alsaleh (2011); Sun et al., (2009); Maccaferri et al., (2008); Quarrie et al., (2006) and Quarrie et al., (2005) .

Vitreousness kernel count showed the highest number of MTAs with the R^2 value (0.23) detected by wmc469-bp158 on 1A at Kon-15 environment and based on the Bonferroni correction, this association was significant. Another major association detected by wmc407-bp190 was in 2A in the Koz-15 environment explaining 11% of the phenotypic variation (Appendix 4). There were seven QTLs, distributed along chromosomes 1B, 2A, 2B, 3A, 6A, 6B and 7A, with 7A having the largest effect according to Alsaleh, (2011). Also Elouafi (2001) reported two QTLs on 4BL and 6BS for the vitreousness kernel count trait.

Test weight was only associated with the two markers, *wPt* -1386615 and *wPt* -991875, in the Ada-15-H environment explaining 9% of the phenotypic variation (Appendix 4). Twelve MTAs for the TW located chromosomes 1A, 1D, 1B, 3A, 3B, 3D, 5A, 6B, 7A and 7D were reported by Jochen et al., (2012). In winter wheat under Colorado environments, test weight QTLs were detected on chromosomes 1B, 6B, 7A and 7D by El-Feki (2010). Seven QTLs located on chromosomes 1A, 2A, 2B, 3A, 3B, 4B and 7B were reported by Alsaleh, (2011). Elouafi, (2001) reported two QTLs for TW on chromosomes 6BS and 7AS.

This study confirmed the association of many traits with phenotypic variation in the investigated traits. Taken together with the published genetic results, these MTAs could be the target of marker-assisted selection to elevate many traits in durum wheat. However, due to the high number of markers with an unknown chromosome location found in this study, it was not possible to determine whether the MTAs are located in QTL regions.

4.7. Pleiotropic effect

The pleiotropic effect or closely linked traits allowed the unravelling of the origin of genetic correlations among the morphological traits. The significant association of the same genetic marker with multiple traits may be the result of the pleiotropic effect. Thirteen MTAs affecting many traits were detected overlapping with other traits; thus, confirming that pleiotropic effects can be found in MTAs. We observed that three markers were significantly associated with three different traits; SNP-1229434 and the DArT marker *wPt*-1258425 with PH, PL and PEL r = 0.85**, 0.69**, and 0.87**, respectively And SNP-4404598 with the PH, PL and GNPS traits with r = 0.85**, 0.69**, and -0.49**, respectively (Table 4.18). These associations were also supported by Pearson's correlation analysis (Table 4.2).

The highest significant associations detected with four MTAs were found between PL and PEL with r = 0.87**. High significant associations were also detected with 4 MTAs found between PH and PL with r = 0.85**. While three MTAs were detected to have significant associations between PH and PEL with r =

0.69**. In addition, three MTAs were found to have significant associations with GNPS and SY with r = 0.66**. Furthermore, several different genetic markers were significantly associated with the same trait (Table 4.18).

Many researchers have reported on pleiotropic effects, in which many MTAs are in the same position within a region in numerous wheat traits (Würschum et al., 2015; Lopes et al., 2015; Liu et al., 2015; Ain et al., 2015).

This study confirms that the detection of multi-trait chromosome regions, some of them were major MTAs for many traits and should be further proper validation in association with the traits investigated in the current study may be useful in MAS.

In the present study, significant markers were identified using GLM or MLM. Many MTAs were related to the selected traits that were investigated. The genetic linkages map is yet to be finished, and therefore little information on the chromosome locations can be provided. Hence, the loci given here as being associated with morphological traits cannot be directly compared with the QTL reported by other researchers.

This study has confirmed the association of many traits with phenotypic variation in the investigated traits. Taken together with the published genetic results, these MTAs could be the target of marker-assisted selection to improve many traits in durum wheat.

Table 4.18. Pleiotropic effect regions in the investigated durum collection

Marker	**Chr.**	**Trait-1**	**Trait-2**	**Trait-3**	**Pearson's correlation**		
SNP-1229434		PH	PL	PEL	0.85**	0.69**	0.87**
wPt-1258425		PH	PL	PEL	0.85**	0.69**	0.87**
SNP-4404598		PH	PL	GNPS	0.85**	0.69**	-0.49**
wPt-7353451	3A	HD	MD		0.87**		
wPt-3944345		PH	PEL		0.69**		
wPt-3950570		PH	PL		0.85**		
Table 4.18.Continued.							
SNP-998647	4B	PL	PEL		0.87**		
wPt-7903270		PL	PEL		0.87**		
gwm369-bp255	3A	SW	SY		0.79**		
wPt-1721268		GNPS	SY		0.66**		
wPt-4409344		GNPS	SY		0.66**		
wPt-5567724		GNPS	SY		0.66**		
wPt-1122735		GNPS	VKC		-0.23**		

5. CONCLUSION

This study has provided unprecedented insights through the large-scale examination of the genetic and phenotypic diversity of a large collection of Turkish commercial cultivars and landraces of durum wheat. To date, there is almost no investigation that has been conducted in this detail to quantify the variation within these genotypes.

This phenotypic investigation showed that there is a wide range and significant variation among the genotypes and across the years for all the traits. All characteristics that were studied for both years showed continuous distributions suggesting that these traits are complex in nature and quantitatively inherited.

A variance analysis of augmented design was performed on the fourteen agronomic and quality traits evaluated for two consecutive years in five different environments. The results showed that the broad-sense heritability estimates for quality traits were less than those for agronomy traits, but higher than the spike characters. Significant correlations were also observed between days to heading (HD) and days to maturity (MD), and between the peduncle length and peduncle extrusion length.

The genetic variation and structure of Turkish durum collection and the germplasm were genotyped using high-density genetic markers; SSR, DArT and SNPs. These genetic markers separated the genotypes into four different groups. For all groups, the comparisons of molecular variance (AMOVA) proved that there was high intra-diversity among groups when compared across the groups. This is reflected by the higher proportion of total variance for the ‘within’ groups analyses.

The positioning of Turkish cvs close to foreign cvs and a little close to the center of the PCoA diagram indicated high contribution by foreign genotypes to the Turkish breeding process. Foreign varieties were introduced into the Turkish breeding programs to develop new varieties with improved phenotypes. Many foreign cultivars were used as parents in the Turkish durum wheat-breeding program reflecting the association between foreign cultivars and Turkish cultivars. Although

the foreign cultivars were from geographically distant sources in Asia, Europe and America, there was a clear convergence.

The results showed that the Turkish landraces gene pool has greater richness in allelic diversity than other groups, especially the foreign cultivars. PCoA also indicated high diversity of Turkish cvs and landraces. According to pairwise population matrix of Nei genetic distance, Turkish cvs and foreign cvs groups were closely related. Taken together the results of the Neighbor-Joining tree with the bar plot with K = 5, which was inferred as the optimal K value based on the highest of log-likelihood value, the genotypes were classified into eight groups (1, 3 and 5) that had simple genetic structure, and groups 2, 4, 6, 7 & 8 that were an admixture.

First, a generalized linear model (GLM) was used to identify markers associated with fourteen traits. In addition, to reduce false associations, population structure (Q) and kinship (K) were used as the covariates in a mixed linear model approach mixed linear model. The significance levels were modified using the Bonferroni correction generated among 144 MTAs, 92 were major markers trait associated and two only were significant MTAs with plant height and Vitreous Kernels count traits. The association mapping analysis confirmed that investigated traits in durum wheat are controlled by a number of gene loci. These traits are important in the understanding of the genetic basis of durum wheat and will serve to promote and enhance breeders' knowledge to select germplasms for future breeding programs. Thus, a breeding strategy based on marker-assisted selection would need to incorporate those markers that were found to be significantly linked to the traits that can be used efficiently for the improvement of these traits through marker-assisted selection.

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CURRICULUM VITAE

I was born on 21 March 1963 in Aleppo, Syria. I received my primary and secondary school education in Aleppo. I got my BSc degree from the Department of Applied Chemistry, Faculty of Science, Aleppo University in 1989. I obtained my degree of M.Sc. from biotechnology department, Cukurova University, in 2011.

I worked with the International Center of Agricultural Research in the Dry Areas (ICARDA) for 21 years (1992-2013) as a Research Assistant. During this period, I joined Cornell University as a visiting fellow to work for durum wheat. I received also short training on DArT technique in durum wheat in 2005, when I was as a visiting fellow at Diversity Arrays Technology Pty Limited center at Canberra, Australia. In 2006, I received the Generation Challenge Program Award. I worked on Diversity Analysis of wheat accessions in CIMMYT, Mexico. I co-authored of a number of scientific papers. Recently, I was admitted to PhD in Department of Biotechnology at the University of Cukurova, Adana, Turkey in 2012, and now I hope to see the peace is prevailing everywhere especially at my lovely country to go back and start build it.

APPENDIX

Appendix 1. The list of cultivars and landraces selected for association mapping, country, release year, pedigree and group name

No	Name	Country	Release year	Pedigree	Group
1	Kunduru-1149	Turkey	1967	(S)LV-TUR	Turkish CV
2	Çeşit-1252	Turkey	1999	61-130/KUNDURU-414-44//377-2[1987]	Turkish CV
3	Yılmaz-98	Turkey	1998	DF-9-71/3/V-2466//ND-61-130/414-44/4/ERGENE	Turkish CV
4	Yelken-2000	Turkey	2000	ZF/LEEDS//FORAT/3/ND-61-130/LEEDS/4/(TR.SE)AU-107/5/GERARDO	Turkish CV
5	Altın (ALTIN-40-98)	Turkey	1998	BARRIGON-YAQUI-ENANO/2*TEHUACAN-60//2B//LONGSHANKS/3/BERKMEN-469	Turkish CV
6	Meram-2002	Turkey	2002		Turkish CV
7	Dumlupınar	Turkey	2006	BERKMEN/G75T181	Turkish CV
8	Şölen-2002	Turkey	2002	STERNA,MEX/ALTAR/3/GANSO//CANDO	Turkish CV
9	Altıntoprak-98	Turkey	1998	ALTAR-84/ARAOS	Turkish CV
10	Çakmak-79	Turkey	1979	UVEYIK-162/ND-61-130	Turkish CV
11	Eminbey	Turkey	2007	ÇMK79"S"//414-44/OVİ/3/BERK/OVİ/4/KND1149/5/LDS//DWARFMUTANT/SARIBAŞAK	Turkish CV
12	Kümbet-2000	Turkey	2000	61-130//414-44/377-2/3/DF15-72	Turkish CV
13	İmren	Turkey	2009		Turkish CV
14	Balcalı-2000	Turkey	2000	MAGHREBI-72/(SIB)FLAMINGO,MEX//CRANE(SIB)/ND-USA-2299/3/(SIB)YAVAROS-79/4/DACKIYE/(SIB)RABICORNO//(SIB)WINGET; (SIB)STERNA,MEX	Turkish CV
15	Sham-1	Turkey	1984	PELICANO/RUFF//GAVIOTA/ROLETTE; PELICANO(SIB)/(SIB)RUFF//GAVIOTA(SIB)/(SIB)ROLETTE	Turkish CV
16	Ankara-98	Turkey	1998	KOBAK-2916/LEEDS//6783/3/BERKMEN-469/7/CRANE/GANSO//APULICUM/3/DF-17-72/4/DI-165137/GEDIZ-75/5/ANHINGA/6/CASTELPORZIANO/G2//2*TEHUACAN-60/TEHUACAN-60	Turkish CV
17	Balcalı-85	Turkey	1985	JORI-69(SIB)/(SIB)ANHINGA//(SIB)FLAMINGO,MEX	Turkish CV
18	Fuatbey-2000	Turkey	2000		Turkish CV
19	Akbaşak-073144	Turkey	1970	(S)LV-TUR	Turkish CV
20	Artuklu	Turkey	2008	Topdy-4 CD84785-3B-030 YRL-040OPAP-1Y-OPAP	Turkish CV
21	Mirzabey-2000	Turkey	2000	GD-2/D-1184528	Turkish CV
22	Aydın-93	Turkey	1993	JORI-69/HAURANI	Turkish CV
23	Diyarbakır-81	Turkey	1981	LD-393//BELADI-116-E/2*TEHUACAN-60/3/COCORIT-71	Turkish CV
24	Eyyubi	Turkey	2008	Podiceps-11-cd86608-7M-030YRC-040PAP-4Y-1PAP-0Y	Turkish CV
25	Selçuklu-97	Turkey	1997	073-44*2/OVI/3/DF-21-72//ND-61-130/UVEYIK-162	Turkish CV
26	Fatasel-185/1	Turkey	1964	Selected from FATA bring from Burdur in 1952	Turkish CV
27	Altınbaç-95	Turkey	1995	KUNDURU//D-68111/WARD	Turkish CV
28	Harran-95	Turkey	1995	KORIFLA//DS-15/GEIGER	Turkish CV
29	Sarıçanak-98	Turkey	1998	DACKIYE/GEDIZ-75//USDA-575	Turkish CV
30	Tüten-2002	Turkey	2002	ALTAR/AVETORO/3/GANSO/FLAMINGO,MEX//CANDO	Turkish CV
31	Turabi	Turkey	2004	CRESO/CRANE	Turkish CV
32	Ege-88	Turkey	1988	JORI-C-69/ANHINGA//FLAMINGO,MEX	Turkish CV
33	Güney yıldızı	Turkey	2010	RASCON-39/TILD-1	Turkish CV
34	Fırat-93	Turkey	2002	SNIPE/3/JORI-C-69/CRANE/GANSO/ANHINGA; ANHINGA(SIB)/(SIB)VOL//(SIB)FLAMINGO,MEX/3/	Turkish CV

				SHAW	
35	Şahinbey	Turkey	2008	Lagost-2 ICD.86-0471-ABL-OTR-8AP-0TR-20AP-OTR	Turkish CV
36	Zühre	Turkey	2011	SNTURK-M-183-84-375/(SIB)NIGRIS//TANTLO-1	Turkish CV
37	Gündaş	Turkey	2012	LGT3/BICRECHAM-1	Turkish CV
38	Akçakale-2000	Turkey	2002	SCHELLENTE//CORMORANT/RUFFOUS/3/AJAIA	Turkish CV
39	Gökgöl-79	Turkey	1979	BUCK-BALCARCE//BARRIGON-YAQUI-ENANO*2/TEHUACAN-60	Turkish CV
40	Amanos 97	Turkey	1997	OSTRERO//CELTA/YAVAROS,AUS	Turkish CV
41	Kızıltan-91	Turkey	1991	UVEYIK-162/61-130//BARRIGON-YAQUI-ENANO*2/TE	Turkish CV
42	Özberk	Turkey	2005	FLAMINGO,MEX/GARZA//CANDEAL-1/GREBE/3/CENTRIFEN/FLAMINGO,MEX/PETREL/5/AKBASAK-073-44/YERLI/6/CAR	Turkish CV
43	Urfa-2005	Turkey	2005	Fg'S'/Gr'S'//CandeaI I/4/Grebe 'S'/3/Ctfn/Fg'S'//Ptl 'S'/5/Akb.073.44/ye rli/6/Carc'S	Turkish CV
44	Ceylan-95	Turkey	1995	STORK(SIB)/(SIB)RABICORNO	Turkish CV
45	Salihli-92	Turkey	1992	SHWA//21563/ANHINGA/3/EGE-88; B.BAL//BARRIGON-YAQUI-ENANO*2/TEHUACAN-60	Turkish CV
46	Gap	Turkey	2004	GEDIZ-75(SIB)/(SIB)FLAMINGO,MEX//(SIB)TEAL,MEX	Turkish CV
47	Soylu	Turkey	2012		Turkish CV
48	Ali baba	Turkey	2010	AWALI-2/BITTERN	Turkish CV
49	Tunca-79	Turkey	1979	FATA(SEL.181-1)/ND-61-130//LEEDS	Turkish CV
50	Saribasak	Turkey	1970	LV-TUR	Turkish CV
51	Vatan	Turkey	1978	TADZHIKSKAYA-CHERNOKOLOSAYA/KHORANKA-46	Foreign CV
52	Zenit	Italy	1992	VALRICCARDO/VIC	Foreign CV
53	Saragolıa	Italy	2004	IRIDE/LINEA-PSB-0114	Foreign CV
54	Svevo	Italy	1996	CIMMYT-SELECTION/ZENIT	Foreign CV
55	Clavdio	Italy	2011	Sel.CIMMT35/Durango/IS 1938/Grazia	Foreign CV
56	Baio	Italy	1998	DUILLO/F-21//G-76	Foreign CV
57	UN Darwin	USA	2006	IDO-445/MANNING	Foreign CV
58	Uc.1113	USA	2005	KIFS//RSS/BD-1419/3/MEXIS-CP/4/WAHAS/5/YAVAROS-79	Foreign CV
59	Pathfinder	Canada	1999	WESTBRED-881/DT-367; DT-367/WESTBRED-881	Foreign CV
60	Nevigator	Canada	1999	KYLE/WESTBRED-881	Foreign CV
61	Floradur	Austria	2003	HELIDUR/CIMMYT-4833	Foreign CV
62	C9	West bank			Foreign CV
63	C43	West bank			Foreign CV
64	Inbar	West bank	1978	D-27534/3/JORI(SIB)//LD-357-E/2*TEHUACAN-60; LD-357-E/2*TEHUACAN-60//JORI-69; D-27534-13-M-4-Y-1-M/3/JORI(SIB)//LD-357-E/2*TEHUACAN-60	Foreign CV
65	Creso	Italy	1974	YAKTANA-54/N10B//2*CAPELLI-63/3/3*TEHUACAN-60/4/CPB-144; CAPELLI-B-144/5/YAKTANA-54//(SELECTION-14)NORIN-10/BREVOR/3/CAPELLI-63/4/3*TEHUACAN-60; MARINGA/ZENATI/CPB-144	Foreign CV
66	Simeto	Italy	1988	CAPEITI-8/VALNOVA	Foreign CV
67	Irıde	Italy	1996	ALTAR-84/IONIO; ALTAR-84/(SIB)ARES	Foreign CV
68	Dylan	Italy	2002	NEUDUR/ULISSE	Foreign CV
69	Ofanto	Italy	1990	ADAMELLO/APPULO	Foreign CV
70	Cham-1	Syria	1984	PELICANO/RUFF//GAVIOTA/ROLETTE; PELICANO(SIB)/(SIB)RUFF//GAVIOTA(SIB)/(SIB)ROLETTE	Foreign CV

71	Cham-9	Syria	2010	Foreign CV
72	TR 32090 - Ankara			Gene bank LD
73	TR 53861 - Yozgat			Gene bank LD
74	TR 80984 - Eskişehir			Gene bank LD
75	TR 72025 - Konya			Gene bank LD
76	TR 81249 - Elaziğ			Gene bank LD
77	TR 81371 -Niğde			Gene bank LD
78	TR 71914 -Konya			Gene bank LD
79	TR 81356 -Konya			Gene bank LD
80	TR 81381 -Sivas			Gene bank LD
81	TR 45305 -Yozgat			Gene bank LD
82	TR 46881 - Erzincan			Gene bank LD
83	TR 81259 -Malatya			Gene bank LD
84	TR 81273 -Ankara			Gene bank LD
85	TR 47949 -Kars			Gene bank LD
86	TR 54969 -Yozgat			Gene bank LD
87	TR 63315 -Konya			Gene bank LD
88	TR 81238 - Erzincan			Gene bank LD
89	TR 56206 - Eskişehir			Gene bank LD
90	TR 56128 - Eskişehir			Gene bank LD
91	TR 54977 -Yozgat			Gene bank LD
92	TR 54973 -Yozgat			Gene bank LD
93	TR 53860 -Yozgat			Gene bank LD
94	TR 56135 - Eskişehir			Gene bank LD
95	TR 32015 -Malatya			Gene bank LD
96	TR 31930 -Malatya			Gene bank LD
97	TR 32167 -Yozgat			Gene bank LD
98	TR 35150 -Yozgat			Gene bank LD
99	TR 31887 -Elaziğ			Gene bank LD
100	TR 31902 -Malatya			Gene bank LD
101	TR 31893 -Malatya			Gene bank LD
102	TR 35148 -Yozgat			Gene bank LD
103	TR 81277 -Ankara			Gene bank LD
104	TR 81283 -Ankara			Gene bank LD
105	TR 81284 -Ankara			Gene bank

			LD
106	TR 81367 -Konya		Gene bank LD
107	TR 81374 -Konya		Gene bank LD
108	TR 81258 -Malatya		Gene bank LD
109	TR 81278 -Ankara		Gene bank LD
110	TR 81323 -Ankara		Gene bank LD
111	TR 81304 -Malatya		Gene bank LD
112	TR 81369 -Niğde		Gene bank LD
113	TR 81550 -Niğde		Gene bank LD
114	TR 81544 -Niğde		Gene bank LD
115	TR 81338 -Ankara		Gene bank LD
116	Bağacak	Turkey	Growing LD
117	Menceki	Turkey	Growing LD
118	Mersiniye	Turkey	Growing LD
119	Sivaslan	Turkey	Growing LD
120	Şırnak Alkaya	Turkey	Growing LD
121	Kurtulan	Turkey	Growing LD
122	Karadere	Turkey	Growing LD
123	Hacıhalil	Turkey	Growing LD
124	Hevidi	Turkey	Growing LD
125	Beyaziye	Turkey	Growing LD
126	Mısrı	Turkey	Growing LD
127	İskenderiye	Turkey	Growing LD
128	Karakılçık	Turkey	Growing LD
129	Havrani	Turkey	Growing LD
130	Similar of Levante	Turkey	Growing LD

Appendix 2. SSR primers used for screening of polymorphic.

	Primer Name	5'……3'	Chromosomal Location	Repeat Motif
1	WMC120F	GGAGATGAGAAGGGGGTCAGGA	1A	(CA), (GA), (GT)
	WMC120R	CCAGGAGACCAGGTTGCAGAAG		
2	WMC231F	CATGGCGAGGAGCTCGGTGGTC	3B	GA)10 , (GT)8
	WMC231R	GTGGAGCACAGGCGGAGCAAGG		
3	WMC406F	TATGAGGGTCGGATCAATACAA	1B	(CA)16
	WMC406R	CGAGTTTACTGCAAACAAATGG		
4	WMC477F	CGTCGAAAACCGTACACTCTCC	2B	(GT)16
	WMC477R	GCGAAACAGAATAGCCCTGATG		
5	WMC1F	ACTGGGTGTTTGCTCGTTGA	3B/6A	(CT)(CA)
	WMC1R	CAATGCTTAAGCGCTCTGTG		
6	WMC361F	AATGAAGATGCAAATCGACGGC	2B	(CA)10
	WMC361R	ATTCTCGCACTGAAAACAGGGG		
7	WMC107F	GAATTCAGGCCCTTCTCGGA	7A	(GT)15
	WMC107R	CATTGAACCTCGCATAACGG		
8	CFA2147F	TCATCCCCTACATAACCCGA	1B/1D	(CATC)4
	CFA2147R	ATCGTGCACCAAGCAATACA		
9	GWM156F	CCAACCGTGCTATTAGTCATTC	3B/5AL/5BS	(GT)14
	GWM156R	CAATGCAGGCCCTCCTAAC		
10	WMC296F	GAATCTCATCTTCCCTTGCCAC	2A	(GA)11 & , (GT)28
	WMC296R	ATGGAGGGGTATAAAGACAGCG		
11	WMC145F	GGCGGTGGGTTCAAGTCGTCTG	6A	GTGGCCG (CGG)3TGG(CGG)2C
	WMC145R	GGACGAGTCGCTGTCCTCCTGG		
12	GWM304F	AGGAAACAGAAATATCGCGG	2A/5A	(CT)22
	GWM304R	AGGACTGTGGGGAATGAATG		
13	WMC218F	TCTCCTGTCGGCTGAAAGTGTT	7B	(TG)7CGTGC(GT)7
	WMC218R	CCATGGAGGTTCACCTAGCAAA		
14	WMC219F	TGCTAGTTTGTCATCCGGGCGA	4AL	(CA)57
	WMC219R	CAATCCCGTTCTACAAGTTCCA		
15	WMC47F	GAAACAGGGTTAACCATGCCAA	4BL/5A/5B	(CA)8
	WMC47R	ATGGTGCTGCCAACAACATACA		
16	WMC238F	TCTTCCTGCTTACCCAAACACA	4BS	(CA)22
	WMC238R	TACTGGGGGATCGTGGATGACA		
17	WMC128F	CGGACAGCTACTGCTCTCCTTA	1B	(GA)10 &, (GT)16
	WMC128R	CTGTTGCTTGCTCTGCACCCTT		
18	WMC262F	GCTTTAACAAAGATCCAAGTGGCAT	4AL	GA)29
	WMC262R	GTAAACATCCAAACAAAGTCGAACG		
19	WMC469F	AGGTGGCTGCCAACG	1A/6D	(CT)
	WMC469R	CAATTTTATCAGATGCCCGA		
20	WMC291F	TACCACGGGAAAGGAAACATCT	3BL	(GT)26
	WMC291R	CACGTTGAAACACGGTGACTAT		

21	WMC307F	GTTTGAAGACCAAGCTCCTCCT	3B	GT)8 (GA)13
	WMC307R	ACCATAACCTCTCAAGAACCCA		
22	WMC312F	TGTGCCCGCTGGTGCGAAG	1A	(GA)14
	WMC312R	CCGACGCAGGTGAGCGAAG		
23	WMC317F	TGCTAGCAATGCTCCGGGTAAC	2BL	(GT)23
	WMC317R	TCACGAAACCTTTTCCTCCTCC		
24	WMC31F	GTTCACACGGTGATGACTCCCA	1B	(GA)11, (GT)19
	WMC31R	CTGTTGCTTGCTCTGCACCCTT		
25	WMC323F	ACATGATTGTGGAGGATGAGGG	7B	(CA)11, (CA)11, (CT)11
	WMC323R	TCAAGAGGCAGACATGTGTTCG		
26	WMC327F	TGCGGTACAGGCAAGGCT	5AL	(GT)25
	WMC327R	TAGAACGCCCTCGTCGGA		
27	WMC332F	CATTTACAAAGCGCATGAAGCC	2B	(CT)12
	WMC332R	GAAAACTTTGGGAACAAGAGCA		
28	GWM369F	CTGCAGGCCATGATGATG	3A/4B/7B	(CT)11(T)2(CT)21
	GWM369R	ACCGTGGGTGTTGTGAGC		
29	WMC476F	TACCAACCACACCTGCGAGT	7B	(GT)7 118, (GT)25
	WMC476R	CTAGATGAACCTTCGTGCGG		
30	WMC511F	CGCACTCGCATGATTTTCCT	4BS	(GT)7, CGTG
	WMC511R	ATGCCCGGAAACGAGACTGT		
31	WMC612F	GAGGTCAGTACCCGGAGA	3B	
	WMC612R	CCACCCCAATTCAAAAAG		
32	WMC626F	AGCCCATAAACATCCAACACGG	1B	
	WMC626R	AGGTGGGCTTGGTTACGCTCTC		
33	WMC657F	CGGGCTGCGGGGGTAT	4B	
	WMC657R	CGGTTGGGTCATTTGTCTCA		
34	WMC662F	AGTGGAGCCATGGTACTGATTT	7B	
	WMC662R	TGTGTACTATTCCCGTCGGTCT		
35	WMC727F	CATAATCAGGACAGCCGCAC	5AL	
	WMC727R	TAGTGGCCTGATGTATCTAGTTGG		
36	WMC75F	GTCCGCCGCACACATCTTACTA	5B	(GT)13
	WMC75R	GTTTGATCCTGCGACTCCCTTG		
37	BARC354F	CGTTGTTTGCGTAGAAGGAGGTT	6B	
	BARC354R	GCGAATGCGGGCGATAAAGTGG		
38	CFE143F	CGACTAACGACCAAAGCACA		(CAGG)4
	CFE143R	CATCCACACCCACAAGGAG		
39	CFA2191F	AGAGCAGGAGGTTGGGTTCT	3B	(TCCC)4
	CFA2191R	CCGGAATTTCACTACCAGGA		
40	BARC85F	GCGAACGCTGCCCGGAGGAATCA	7B	(CAT)8
	BARC85R	GCGTCGCAGATGAGATGGTGGAGCAAT		
41	CFA2114F	ATTGGAAGGCCACGATACAC	6A	(CA)32
	CFA2114R	CCCGTCGGGTTTTATCTAGC		

42	CFD238F	GTTGAGGAGGACAAAGAGGC	2B	(GGGA)3
	CFD238R	GATACGAGCGAGCCCATAAA		
43	CFD242F	CCAGTTTGCAGCAGTCACAT	7A	(GTT)15(AGC)5
	CFD242R	CAGACCTTAACGGGGTTGAA		
44	GWM456F	TCTGAACATTACACAACCCTGA	1B/3D	(GA)21
	GWM456R	TGCTCTCTCTGAACCTGAAGC		
45	GWM375F	ATTGGCGACTCTAGCATATACG	4B	
	GWM375R	GGGATGTCTGTTCCATCTTAGC		
46	GWM513F	ATCCGTAGCACCTACTGGTCA	4BL/5B/7BS	(CA)12
	GWM513R	GGTCTGTTCATGCCACATTG		
47	GWM547F	GTTGTCCCTATGAGAAGGAACG	2BS/3B	(CA)12
	GWM547R	TTCTGCTGCTGTTTTCATTTAC		
48	GWM70F	AGTGGCTGGGAGAGTGTCAT	6B	(GT)7GC(GT)11
	GWM70R	GCCCATTACCGAGGACAC		
49	GWM77F	ACCCTCTTGCCCGTGTTG	3BS	(CA)10 (GA)40
	GWM77R	ACAAAGGTAAGCAGCACCTG		
50	WMC553F	CGGAGCATGCAGCTAGTAA	6A	(CA)24
	WMC553R	CGCCTGCAGAATTCAACAC		
51	BARC77F	GCGTATTCTCCCTCGTTTCCAAGTCTG	3B	(ATCT)6
	BARC77R	GTGGGAATTTCTTGGGAGTCTGTA		
52	BARC78F	CTCCCCGGTCAAGTTTAATCTCT	4A	(TC)27(TATC)43
	BARC78R	GCGACATGGGAATTTCAGAAGTGCCTAA		
53	CWEM48F	TCTGTTGTCGGCATTTCAGT	6DL	(TGT)5
	CWEM48R	TGGCGTTACATTCATTTGGA		
54	CWEM51F	CGACAAGAACAAAGCCTGAG		(CCAT)6
	CWEM51R	CCTCTATCGCGCTGTTGATT		
55	CWEM52F	CCTACCTACGACGCAAGTCC	7BL	(GCAAAC)5
	CWEM52R	AGCGAGCAGAAAGCATCAAG		
56	CFA2141F	GAATGGAAGGCGGACATAGA	5A/5D	(GA)18
	CFA2141R	GCCTCCACAACAGCCATAAT		
57	CFD7F	AGCTACCAGCCTAGCAGCAG	5B/5DL	(TC)27
	CFD7R	TCAGACACGTCTCCTGACAAA		
58	CFD168F	CTTCGCAAATCGAGGATGAT	2A/2D	(CTG)20
	CFD168R	TTCACGCCCAGTATTAAGGC		
59	CFD2F	GGTTGCAGTTTCCACCTTGT	2A/2D/3A/3D/4A/5B/5D	(CA)21
	CFD2R	CATCTATTGCCAAAATCGCA		
60	CFD6F	ACTCTCCCCCTCGTTGCTAT	2A/3B/7A	(GA)6(GCTA)4
	CFD6R	ATTTAAGGGAGACATCGGGC		
61	CFD71F	CAATAAGTAGGCCGGGACAA	4A/4D	(CA)10(GA)30
	CFD71R	TGTGCCAGTTGAGTTTGCTC		
62	GWM293F	TACTGGTTCACATTGGTGCG	5AL/5B/5D/7B	(CA)24
	GWM293R	TCGCCATCACTCGTTCAAG		

63	WMC407F	GGTAATTCTAGGCTGACATATGCTC	2A	(GA)16
	WMC407R	CATATTTCCAAATCCCCAACTC		
64	WMC486F	CCGGTAGTGGGATGCATTTT	6B	(GT)28
	WMC486R	ATGCATGCTGAATCCGGTAA		
65	WMC50F	CTGCCGTCAGGCCAGGCTCACA	3A	(GT)10
	WMC50R	CAACCAGCTAGCTGCCGCCGAA		
66	WMC517F	ATCCTGACGTTACACGCACC	7B	(CA)
	WMC517R	ACCTGGAACACCACGACAAA		
67	WMC522F	AAAAATCTCACGAGTCGGGC	2A	(CT)
	WMC522R	CCCGAGCAGGAGCTACAAAT		
68	WMC524F	TAGTCCACCGGACGGAAAGTAT	5A	(GT)
	WMC524R	GTACCACCGATTGATGCTTGAG		
69	WMC532F	GATACATCAAGATCGTGCCAAA	3A	(GA)
	WMC532R	GGGAGAAATCATTAACGAAGGG		
70	WMC592F	GGTGGCATGAACTTTCACCTGT	2B	
	WMC592R	TGTGTGGTGCCCATTAGGTAGA		
71	WMC596F	TCAGCAACAAACATGCTCGG	7A	
	WMC596R	CCCGTGTAGGCGGTAGCTCTT		
72	WMC598F	TCGAGGAGTCAACATGGGCTG	2A	(XXXX)
	WMC598R	ACGGTCGCTAGGGAGGGGAG		
73	WMC607F	ATATATGCCCATGAAGCTCAAG	7A	(XX)
	WMC607R	GATCGAGCTAAAGCTGATACCA		
74	WMC616F	TAAAGCTAGGAGATCAGAGGCG	5B	(XX)
	WMC616R	TAATCCCATCTTGAGAAGCGTC		
75	WMC619F	TTCCCTTTCCCCTCTTTCCG	1B	(XX)
	WMC619R	TACAATCGCCACGAGCACCT		
76	WMC633F	ACACCAGCGGGGATATTTGTTAC	7A	(XX)
	WMC633R	GTGCACAAGACATGAGGTGGATT		
77	WMC664F	GGGCCAACAAATCCAAT	3A	(XX)
	WMC664R	TCTACTTCCTTCATCCACTCC		
78	WMC163F	TTACACCCATCAGGGTGGTCTT	6AL	(GT)8
	WMC163R	GTCCGTCTATCCATACGACAAA		
79	WMC274F	AAGCAAGCAGCAAAACTATCAA	3B	(GAAAA)9
	WMC274R	GAATGAATGAATGAATCGAGGC		
80	GWM124F	GCCATGGCTATCACCCAG	1B	(CT)27(GT)18
	GWM124R	ACTGTTCGGTGCAATTTGAG		
81	WMC335F	TGCGGAGTAGTTCTTCCCCC	7B	(CA)5G(CA)12
	WMC335R	ACATCTTGGTGAGATGCCCT		
82	WMC339F	CCGCTCGCCTTCTTCCAG	1D	(GA)8
	WMC339R	TCCGGAACATGCCGATAC		
83	WMC364F	ATCACAATGCTGGCCCTAAAAC	7B	(CA)18
	WMC364R	CAGTGCCAAAATGTCGAAAGTC		

84	WMC658F	CTCATCGTCCTCCTCCACTTTG	2A	(XX)
	WMC658R	GCCATCCGTTGACTTGAGGTTA		
85	GWM635F	TTCCTCACTGTAAGGGCGTT	7A/7B/7D	(CA)10(GA)14
	GWM635R	CAGCCTTAGCCTTGGCG		
86	WMC73F	TTGTGCACCGCACTTACGTCTC	5B	(CA)9
	WMC73R	ACACCCGGTCTCCGATCCTTAG		
87	WMC764F	CCTCGAACCTGAAGCTCTGA	2B	(XX)
	WMC764R	TTCGCAAGGACTCCGTAACA		
88	WMC766F	AGATGGAGGGGATATGTTGTCAC	1B	(XXXX)
	WMC766R	TCGTCCCTGCTCATGCTG		
89	WMC770F	TGTCAGACTTCCTTTGATCCCC	2B	(XX)
	WMC770R	AAGACCATGTGACGTCCAGC		
90	WMC776F	CCATGACGTGACAACGCAG	4A	
	WMC776R	ATTGCAGGCGCGTTGGTA		
91	WMC83F	TGGAGGAAACACAATGGATGCC	7A	(GT)28
	WMC83R	GAGTATCGCCGACGAAAGGGAA		
92	BARC89F	GGGCGCGGCACCAGCACTACC	5B	(TCA)11
	BARC89R	CTCCGAGGCCACCGAAGACAAGATG		
93	BARC74F	GCGCTTGCCCCTTCAGGCGAG	5B	(GA)13(GATA)7(GA)9
	BARC74R	CGCGGGAGAACCACCAGTGACAGAGC		
94	CFA2028F	TGGGTATGAAAGGCTGAAGG	7A	(CA)21
	CFA2028R	ATCGCGACTATTCAACGCTT		
95	GWM130F	AGCTCTGCTTCACGAGGAAG	2B/7A/7D	(GT)22
	GWM130R	CTCCTCTTTATATCGCGTCCC		
96	CFA2058F	CCCATTGCCATCTCAGTCTT	2A	(TC)28
	CFA2058R	ATAGTAGGCCCAAAGCGATG		
97	CFA2183F	TCTTGGATGGATTTGTGAGC	3A	(CA)26
	CFA2183R	TTCCTTCTCCTTCATTAGCTGC		
98	CFA2234F	AATCTGACCGAACAAAATCACA	3A	(CA)17
	CFA2234R	TCGGAGAGTATTAGAACAGTGCC		
99	CFA2241F	TTGGCCATCAGGCTCTAGTT	1B	(CA)21
	CFA2241R	GTGATGCTGTTCTCAAGCCA		
100	CFA2263F	GGCCATGTAATTAAGGCACA	2AL	(CA)24
	CFA2263R	CTCCCAGGAGTACAGAAGAGGA		
101	WMC397F	AGTCGTGCACCTCCATTTTG	6B	(CA)
	WMC397R	CATTGGACATCGGAGACCTG		
102	BARC181F	CGCTGGAGGGGGTAAGTCATCAC	1B	(CT)17
	BARC181R	CGCAAATCAAGAACACGGGAGAAAGAA		
103	CFD61F	ATTCAAATGCAACGCAAACA	1D	(CACAA)3(AC)22
	CFD61R	GTTAGCCAAGGACCCCTTTC		
104	WMC311F	GGGCCTGCATTTCTCCTTTCTT	7B	(GT)12
	WMC311R	CTGAACTTGCTAGACGTTCCGA		

105	WMC41F	TCCCTCTTCCAAGCGCGGATAG	2D	(GA)25 & (GCC)7
	WMC41R	GGAGGAAGATCTCCCGGAGCAG		
106	WMC181F	TCCTTGACCCCTTGCACTAACT	2A	(GT)19, (GT)10
	WMC181R	ATGGTTGGGAGCACTAGCTTGG		
107	WMC11F	TTGTGATCCTGGTTGTGTTGTGA	3A/3D	(CT)
	WMC11R	CACCCAGCCGTTATATATGTTGA		
108	WMC128F	CGGACAGCTACTGCTCTCCTTA	1B	(GA)10, (GT)16
	WMC128R	CTGTTGCTTGCTCTGCACCCTT		
109	WMC110F	GCAGATGAGTTGAGTTGGATTG	5A	(GT)
	WMC110R	GTACTTGGAAACTGTGTTTGGG		
110	CFD20F	TGATGGGAAGGTAATGGGAG	1B/5B/7A	(GGAA)3(CTAC)3
	CFD20R	ATCCAGTTCTCGTCCAAAGC		
111	GWM388F	CTACAATTCGAAGGAGAGGGG	2B	(CT)4(CA)11(CA)12
	GWM388R	CACCGCGTCAACTACTTAAGC		
112	WMC76F	CTTCAGAGCCTCTTTCTCTACA	7B	(GT)
	WMC76R	CTGCTTCACTTGCTGATCTTTG		
113	GWM333F	GCCCGGTCATGTAAAACG	7B	(GA)19
	GWM333R	TTTCAGTTTGCGTTAAGCTTTG		
114	GWM335F	CGTACTCCACTCCACACGG	5B	(GA)14(GCGT)3
	GWM335R	CGGTCCAAGTGCTACCTTTC		
115	WMC313F	GCAGTCTAATTATCTGCTGGCG	3A	(CA)18
	WMC313R	GGGTCCTTGTCTACTCATGTCT		
116	GWM294F	GGATTGGAGTTAAGAGAGAACCG	2AL	(GA)9TA(GA)15
	GWM294R	GCAGAGTGATCAATGCCAGA		
117	BARC232F	CGCATCCAACCATCCCCACCCAACA	5A/5B/5D	(CT)18
	BARC232R	CGCAGTAGATCCACCACCCCGCCAGA		
118	GWM630F	GTGCCTGTGCCATCGTC	2A/2B	(GT)16
	GWM630R	CGAAAGTAACAGCGCAGTGA		
119	CFD60F	TGACCGGCATTCAGTATCAA	5B/6D	(CA)25
	CFD60R	TGGTCACTTTGATGAGCAGG		
120	CFD73F	GATAGATCAATGTGGGCCGT	2B/2D	(CT)19
	CFD73R	AACTGTTCTGCCATCTGAGC		

Appendix 3. Environment, Marker name, P and R2 values significantly associated markers with studied traits under different conditions in 2014 and 2015 using (MLM +K) models.

Trait	Environment	Marker name	p	markerR2
HD	Ada-15-L	wPt-1258749	2.24E-05	0.15
	Mean	wPt-1258749	6.07E-04	0.10
	Mean	wPt-1382625	9.99E-04	0.09
	Mean	wPt-1698914	3.11E-04	0.11
	Ada-15-L	wPt-1769376	3.96E-04	0.10
	Ada-15-H	wPt-4404438	6.37E-04	0.10
	Ada-14-L	wPt-4409679	6.97E-04	0.10
	Ada-15-H	wPt-4992555	5.05E-04	0.10
	Ada-15-L	wPt-4992555	8.14E-04	0.09
	Mean	wPt-4992555	5.75E-04	0.10
	Ada-15-L	wPt-7353451	2.81E-04	0.11
	Ada-15-H	gwm335-bp252	9.98E-04	0.09
MD	Ada-15-H	wPt-1216651	4.44E-04	0.10
	Mean	wPt-1699005	8.01E-04	0.09
	Ada-15-H	wPt-7353451	5.43E-04	0.10
	Mean	SNP-4008660	9.88E-04	0.09
	Ada-15-L	SNP-982956	1.58E-04	0.12
	Mean	SNP-982956	1.16E-04	0.12
PH	Kon-15	wPt-1030541	7.60E-04	0.10
	Mean	wPt-1258425	3.58E-04	0.11
	Mean	wPt-1392491	6.57E-04	0.10
	Koz-15	wPt-1699984	9.90E-04	0.09
	Ada-14-L	wPt-3936470	6.84E-04	0.11
	Ada-15-L	wPt-3944345	7.54E-05	0.13
	Mean	wPt-3944345	3.50E-04	0.11
	Ada-14-L	wPt-3950570	1.79E-04	0.14
	Ada-15-L	wPt-3950570	4.76E-04	0.10
	Kon-15	wPt-3950570	3.32E-04	0.12
	Koz-15	wPt-3950570	8.65E-06	0.17
	Mean	wPt-3950570	1.63E-05	0.16
	Ada-15-H	wPt-4004275	2.78E-04	0.11
	Koz-15	wPt-4004275	2.73E-04	0.11
	Mean	wPt-4004275	5.08E-04	0.10
	Ada-15-L	wPt-4005091	3.98E-04	0.10
	Ada-15-L	wPt-4404359	1.86E-04	0.12
	Kon-15	wPt-7352465	5.55E-04	0.11
	Ada-15-H	cfa2147-bp306	5.44E-04	0.10
	Ada-15-H	cfa2147-bp310	4.09E-04	0.11

	Ada-15-H	cfa2263-bp146	3.89E-05	0.15
	Ada-15-H	cfd60-bp154	2.94E-05	0.16
	Ada-15-H	cfd60-bp156	4.06E-05	0.15
	Ada-15-H	cfd71-bp166	4.30E-05	0.15
	Ada-15-H	cfd71-bp168	2.19E-04	0.12
	Kon-15	cfd73-bp296	7.17E-04	0.10
	Ada-15-H	gwm130-bp120	4.72E-05	0.17
	Ada-15-H	gwm304-bp212	3.10E-04	0.11
	Ada-15-H	gwm456-bp122	3.46E-05	0.16
	Ada-15-H	gwm456-bp124	8.79E-05	0.14
	Ada-15-L	gwm77-bp166	8.11E-04	0.09
	Koz-15	SNP-1007226	3.82E-04	0.10
	Ada-14-L	SNP-1007226	7.86E-04	0.11
	Kon-15	SNP-1009535	5.73E-04	0.11
	Ada-14-L	SNP-1075915	6.84E-04	0.11
	Ada-14-L	SNP-1125660	6.84E-04	0.11
	Koz-15	SNP-1229434	2.05E-04	0.11
	Ada-14-L	SNP-1229434	2.07E-04	0.14
	Koz-15	SNP-4404598	2.33E-04	0.11
	Mean	SNP-4404598	6.10E-04	0.10
	Koz-15	SNP-4409281	3.82E-04	0.10
	Ada-14-L	SNP-4409281	7.86E-04	0.11
	Ada-14-L	SNP-5580485	3.67E-04	0.12
	Koz-15	SNP-985312	3.82E-04	0.10
	Ada-14-L	SNP-985312	7.86E-04	0.11
	Kon-15	wmc312-bp242	4.62E-04	0.12
	Ada-15-H	wmc83-bp114	3.33E-04	0.11
PL	Mean	wPt-1120369	6.03E-04	0.10
	Ada-15-H	wPt-1258425	9.30E-04	0.09
	Mean	wPt-1399704	9.02E-04	0.09
	Ada-15-H	wPt-1720274	9.64E-04	0.09
	Ada-15-H	wPt-2291099	4.44E-04	0.10
	Mean	wPt-2291099	2.60E-04	0.11
	Mean	wPt-3534135	6.03E-04	0.10
	Koz-15	wPt-3950570	8.21E-04	0.10
	Kon-15	wPt-4005446	2.70E-04	0.12
	Ada-15-L	wPt-7903270	3.50E-04	0.11
	Koz-15	SNP-1229434	1.19E-04	0.13
	Ada-15-L	SNP-1229434	1.71E-04	0.12
	Ada-15-L	SNP-4404598	9.48E-04	0.09
	Ada-15-L	SNP-998647	1.70E-04	0.12
PEL	Mean	wPt-984082	6.74E-04	0.10

	Mean	wPt-1008985	6.74E-04	0.10
	Koz-15	wPt-1053199	6.65E-04	0.10
	Ada-15-H	wPt-1258425	8.57E-04	0.09
	Koz-15	wPt-1270682	8.42E-04	0.10
	Ada-15-L	wPt-1721368	8.86E-04	0.09
	Mean	wPt-1721368	3.71E-04	0.11
	Ada-15-H	wPt-3533440	1.68E-04	0.12
	Mean	wPt-3944345	9.26E-04	0.09
	Ada-15-H	wPt-4995434	5.31E-04	0.10
	Ada-15-H	wPt-5004781	5.89E-04	0.10
	Ada-15-L	wPt-7903270	1.61E-04	0.12
	Mean	wPt-7903270	2.45E-04	0.11
	Ada-15-L	SNP-1229434	6.99E-04	0.10
	Mean	SNP-1229434	1.02E-04	0.13
	Ada-15-L	SNP-3026467	8.32E-04	0.09
	Koz-15	SNP-4410750	9.29E-04	0.10
	Mean	SNP-4989020	6.74E-04	0.10
	Ada-15-L	SNP-998647	3.27E-05	0.15
	Mean	SNP-998647	1.87E-04	0.12
SL	Ada-15-L	wPt-1267592	7.49E-04	0.09
	Ada-15-L	wPt-1699756	3.99E-04	0.10
	Kon-15	wPt-1699756	3.72E-04	0.12
	Mean	wPt-1699756	1.83E-04	0.12
	Kon-15	wPt-4008204	4.90E-04	0.11
	Mean	wPt-4008204	4.79E-04	0.10
	Ada-15-L	SNP-1070815	3.99E-04	0.10
	Kon-15	SNP-1070815	3.72E-04	0.12
	Mean	SNP-1070815	1.83E-04	0.12
	Ada-15-L	SNP-1210075	5.93E-05	0.14
	Kon-15	SNP-12772312	4.90E-04	0.11
	Mean	SNP-12772312	4.79E-04	0.10
	Ada-15-L	SNP-2252454	3.99E-04	0.10
	Kon-15	SNP-2252454	3.72E-04	0.12
	Mean	SNP-2252454	1.83E-04	0.12
	Ada-15-L	SNP-999325	3.99E-04	0.10
	Kon-15	SNP-999325	3.72E-04	0.12
	Mean	SNP-999325	1.83E-04	0.12
SW	Ada-15-H	wPt-1084815	8.57E-05	0.13
	Ada-15-H	wPt-1150954	8.57E-05	0.13
	Mean	wPt-1234753	8.54E-04	0.10
	Ada-15-H	wPt-1671034	8.57E-05	0.13
	Ada-15-H	wPt-3952156	8.57E-05	0.13

	Mean	wPt-4017906	7.18E-04	0.10
	Mean	gwm369-bp255	2.53E-04	0.13
	Ada-15-L	SNP-1110572	2.14E-04	0.11
	Mean	SNP-1110572	9.40E-04	0.10
SNPS	Ada-15-H	wPt-1273237	2.16E-04	0.12
	Ada-15-H	wPt-1745356	9.77E-04	0.09
	Mean	wPt-1745356	6.19E-04	0.10
GNPS	Ada-15-L	wPt-1082459	3.38E-04	0.11
	Kon-15	wPt-1122735	8.39E-04	0.10
	Ada-15-L	wPt-1164339	3.38E-04	0.11
	Ada-15-H	wPt-1260443	7.72E-04	0.10
	Mean	wPt-1260443	5.17E-04	0.10
	Ada-15-L	wPt-1261579	4.31E-04	0.10
	Ada-15-L	wPt-1721268	6.24E-04	0.10
	Ada-15-H	wPt-1861064	7.72E-04	0.10
	Mean	wPt-1861064	5.17E-04	0.10
	Ada-15-L	wPt-2279057	3.41E-04	0.11
	Kon-15	wPt-2279162	9.43E-04	0.10
	Ada-15-H	wPt-2335181	7.72E-04	0.10
	Mean	wPt-2335181	5.17E-04	0.10
	Ada-15-L	wPt-3384895	6.48E-04	0.10
	Ada-15-L	wPt-3944344	3.38E-04	0.11
	Ada-15-L	wPt-4394232	3.38E-04	0.11
	Ada-15-L	wPt-4404283	6.38E-04	0.10
	Ada-15-L	wPt-4409344	6.24E-04	0.10
	Ada-15-L	wPt-4537344	2.46E-04	0.11
	Ada-15-L	wPt-5567724	6.24E-04	0.10
	Ada-15-L	wPt-12465585	3.38E-04	0.11
	Ada-15-L	SNP-1053788	3.38E-04	0.11
	Ada-15-L	SNP-1083267	3.38E-04	0.11
	Ada-15-L	SNP-1215845	3.38E-04	0.11
	Ada-15-L	SNP-1264542	3.38E-04	0.11
	Ada-15-L	SNP-2276567	3.38E-04	0.11
	Ada-15-L	SNP-4006184	3.38E-04	0.11
	Ada-15-L	SNP-4404598	9.76E-04	0.09
	Ada-15-L	SNP-990178	3.38E-04	0.11
SY	Ada-15-L	wPt-5567724	7.24E-04	0.09
	Ada-15-L	wPt-4409344	7.24E-04	0.09
	Ada-15-L	wPt-1721268	7.24E-04	0.09
	Mean	gwm369-bp255	2.84E-04	0.12
SHI	Ada-15-L	wPt-1126150	7.19E-04	0.09
	Ada-15-L	wPt-1764585	3.05E-04	0.11

	Mean	wPt-4004214	8.18E-04	0.09
	Ada-15-L	SNP-2277865	3.34E-04	0.11
	Mean	SNP-986179	4.16E-04	0.10
	Mean	wmc522-bp236	7.35E-04	0.11
TKW	Kon-15	wPt-1235015	3.23E-05	0.16
	Ada-15-H	wPt-4407952	6.05E-04	0.10
	Ada-15-H	wPt-4535983	8.89E-04	0.09
	Kon-15	SNP-1090158	4.54E-04	0.11
	Kon-15	wmc339-bp226	3.98E-04	0.12
VKC	Ada-15-L	wPt-1122735	9.44E-04	0.09
	Ada-15-L	wPt-2301430	7.99E-04	0.09
	Kon-15	wPt-3574929	9.23E-04	0.10
	Kon-15	SNP-4992399	9.23E-04	0.10
	Kon-15	wmc407-bp190	8.54E-04	0.11
	Kon-15	wmc469-bp158	5.62E-06	0.23
TW	Ada-15-H	wPt-1386615	9.68E-04	0.09
	Ada-15-H	wPt-991875	9.66E-04	0.09

Appendix 4. Marker name, chromosome location, MAF, P and R2 values significantly associated markers with studied traits under different conditions in 2014 and 2015 using MLM (Q+K) models.

					MarkerR2					
Trait	**Marker**	**Locus**	**MAF**	**p**	**Ada-14-L**	**Ada-15-L**	**Ada-15-H**	**Koz-15**	**Kon-15**	**Mean**
HD	*wPt-1258749*		0.49	2.24E-05 , 6.07E-04		0.15				0.10
	wPt-1382625	7A	0.25	9.99E-04						0.09
	wPt-1698914		0.28	3.11E-04						0.11
	wPt-1769376		0.08	3.96E-04		0.10				
	wPt-4404438		0.33	6.37E-04			0.10			
	wPt-4409679		0.36	6.97E-04	0.10					
	wPt-4992555	5A	0.09	8.14E-04 , 5.05E-04 , 5.75E-04		0.09	0.10			0.10
	wPt-7353451	3A	0.34	2.81E-04		0.11				
	gwm335-bp252	5B	0.18	9.98E-04			0.09			
MD	*SNP-4008660*		0.47	9.88E-04						0.09
	SNP-982956	2A	0.26	1.58E-04 , 1.16E-04		0.12				0.12
	wPt-1216651	5B	0.11	4.44E-04			0.10			
	wPt-1699005		0.22	8.01E-04						0.09
	wPt-7353451	3A	0.34	5.43E-04			0.10			
PH	*wPt-1030541*	6B	0.34	7.60E-04					0.10	
	wPt-1258425		0.50	3.58E-04						0.11
	wPt-1392491		0.50	6.57E-04						0.10
	wPt-1699984		0.43	9.90E-04				0.09		
	wPt-3936470		0.18	6.84E-04	0.11					
	wPt-3944345		0.16	3.50E-04 , 7.54E-05		0.13				0.11
	wPt-3950570		0.21	1.79E-04 , 4.76E-04 , 8.65E-06, 3.32E-04 , 1.63E-05	0.14	0.10		0.17	0.12	0.16
	wPt-4004275		0.38	5.08E-4 , 2.73-04 , 2.78E-04			0.11	0.11		0.10
	wPt-4005091		0.37	3.98E-04		0.10				
	wPt-4404359		0.36	1.86E-04		0.12				
	wPt-7352465		0.32	5.55E-04					0.11	
	cfa2147-bp306	1B	0.28	5.44E-04			0.10			
	cfa2147-bp310	1B	0.45	4.09E-04			0.11			
	cfa2263-bp146	1B	0.19	3.89E-05			0.15			
	cfd60-bp154	5B	0.30	2.94E-05			0.16			
	cfd60-bp156	5B	0.33	4.06E-05			0.15			
	cfd71-bp166	4A	0.25	4.30E-05			0.15			
	cfd71-bp168	4A	0.38	2.19E-04			0.12			
	cfd73-bp296	2B	0.16	7.17E-04					0.10	

	gwm130-bp120	2B	0.11	4.72E-05			0.17			
	gwm304-bp212	5A	0.26	3.10E-04			0.11			
	gwm456-bp122	1B	0.31	3.46E-05			0.16			
	gwm456-bp124	1B	0.32	8.79E-05			0.14			
	gwm77-bp166	3B	0.07	8.11E-04		0.09				
	SNP-1007226		0.40	7.86E-04 , 3.82E-04	0.11			0.10		
	SNP-1009535		0.05	5.73E-04					0.11	
	SNP-1075915	6B	0.18	6.84E-04	0.11					
	SNP-1125660	6B	0.18	6.84E-04	0.11					
	SNP-1229434		0.50	2.07E-04 , 2.05E-04	0.14			0.11		
	SNP-4404598		0.39	6.10E-04 , 2.33E-04				0.11		0.10
	SNP-4409281		0.40	7.86E-04 , 3.82E-04	0.11			0.10		
	SNP-5580485		0.10	3.67E-04	0.12					
	SNP-985312	4B	0.40	7.86E04 , 3.82E04	0.11			0.10		
	wmc312-bp242	1A	0.40	4.62E-04					0.12	
	wmc83-bp114	7A	0.29	3.33E-04			0.11			
PL	*wPt-1120369*	1B	0.29	6.03E-04						0.10
	wPt-1258425		0.50	9.30E-04			0.09			
	wPt-1399704	1B	0.10	9.02E-04						0.09
	wPt-1720274		0.49	9.64E-04			0.09			
	wPt-2291099	1B	0.28	4.44E-04 , 2.60E-04			0.10			0.11
	wPt-3534135		0.29	6.03E-04						0.10
	wPt-3950570		0.21	8.21E-04				0.10		
	wPt-4005446		0.34	2.70E-04					0.12	
	wPt-7903270		0.19	3.50E-04		0.11				
	SNP-1229434		0.50	1.71E-04 , 1.19E-04		0.12		0.13		
	SNP-4404598		0.39	9.48E-04		0.09				
	SNP-998647	4B	0.21	1.70E-04		0.12				
PEL	*wPt-984082*	7A	0.09	6.74E-04						0.10
	wPt-1008985	7A	0.09	6.74E-04						0.10
	wPt-1053199	4A	0.10	6.65E-04					0.10	
	wPt-1258425		0.50	8.57E-04			0.09			
	wPt-1270682	1B	0.22	8.42E-04				0.10		
	wPt-1721368		0.20	3.71E-04 , 8.86E-04		0.09				0.11
	wPt-3533440	2A	0.08	1.68E-04			0.12			
	wPt-3944345		0.16	9.26E-04						0.09
	wPt-4995434	6A	0.23	5.31E-04			0.10			
	wPt-5004781		0.22	5.89E-04			0.10			

	wPt-7903270		0.19	1.61E-04 , 2.45E-04	0.12			0.11
	SNP-1229434		0.50	6.99E-04 , 1.02E04	0.10			0.13
	SNP-3026467	3B	0.25	8.32E-04	0.09			
	SNP-4410750		0.05	9.29E-04		0.10		
	SNP-4989020		0.09	6.74E-04				0.10
	SNP-998647	4B	0.21	3.27E-05 , 1.87E-04	0.15			0.12
SL	*wPt-1267592*		0.42	7.49E-04	0.09			
	wPt-1699756		0.05	3.99E-04 , 3.72E-04, 1.83E-04	0.10		0.12	0.12
	wPt-4008204		0.06	4.79E-04 , 4.90E-04			0.11	0.10
	SNP-1070815		0.05	3.99E-04 , 3.72E04 , 1.83E-04	0.10		0.12	0.12
	SNP-1210075		0.32	5.93E-05	0.14			
	SNP-12772312		0.06	4.79E-04 , 4.90E-04			0.11	0.10
	SNP-2252454	2A	0.05	3.99E-04 , 3.72E-04, 1.83E-04	0.10		0.12	0.12
	SNP-999325		0.05	3.99E-04 , 3.72E-04, 1.83E-04	0.10		0.12	0.12
SW	*gwm369-bp255*	3A	0.05	2.53E-04				0.13
	SNP-1110572		0.09	2.14E-04 , 9.10E04	0.11			0.10
	wPt-1084815		0.16	8.57E-05		0.13		
	wPt-1150954		0.16	8.57E-05		0.13		
	wPt-1234753		0.29	8.54E-04				0.10
	wPt-1671034	5B	0.16	8.57E-05		0.13		
	wPt-3952156	5BL	0.16	8.57E-05		0.13		
	wPt-4017906		0.16	7.18E-04				0.10
SNPS	*wPt-1273237*		0.09	2.16E-04		0.12		
	wPt-1745356	5BL	0.15	9.77E-04 , 6.19E04		0.09		0.10
GNPS	*wPt-1082459*		0.06	3.38E-04	0.11			
	wPt-1122735		0.50	8.39E-04			0.10	
	wPt-1164339		0.06	3.38E-04	0.11			
	wPt-1260443		0.08	7.72E-04, 5.17E-04		0.10		0.10
	wPt-1261579		0.16	4.31E-04	0.10			
	wPt-1721268		0.32	6.24E-04	0.10			
	wPt-1861064		0.08	7.72E04 , 5.17E-04		0.10		0.10
	wPt-2279057		0.31	3.41E-04	0.11			
	wPt-2279162		0.23	9.43E-04			0.10	
	wPt-2335181		0.08	7.72E-04 , 5.17E-04		0.10		0.10
	wPt-3384895		0.21	6.48E-04	0.10			
	wPt-3944344		0.06	3.38E-04	0.11			
	wPt-4394232		0.06	3.38E-04	0.11			
	wPt-4404283		0.31	6.38E-04	0.10			

	wPt-4409344		0.32	6.24E-04	0.10			
	wPt-4537344		0.13	2.46E-04	0.11			
	wPt-5567724		0.32	6.24E-04	0.10			
	wPt-12465585		0.06	3.38E-04	0.11			
	SNP-1053788		0.06	3.38E-04	0.11			
	SNP-1083267	2A	0.06	3.38E-04	0.11			
	SNP-1215845	2A	0.06	3.38E-04	0.11			
	SNP-1264542		0.06	3.38E-04	0.11			
	SNP-2276567		0.06	3.38E-04	0.11			
	SNP-4006184		0.06	3.38E-04	0.11			
	SNP-4404598		0.39	9.76E-04	0.09			
	SNP-990178	2A	0.06	3.38E-04	0.11			
SY	*wPt-1721268*		0.32	7.24E-04	0.09			
	wPt-4409344		0.32	7.24E-04	0.09			
	wPt-5567724		0.32	7.24E-04	0.09			
	GWM369-bp255	3A	0.05	2.84E-04				0.12
SHI	*wPt-4004214*		0.17	8.18E-04				0.09
	wPt-1126150		0.10	7.19E-04	0.09			
	SNP-986179	4B	0.11	4.16E-04				0.10
	wmc522-bp236	2A	0.09	7.35E-04				0.11
	SNP-2277865		0.12	3.34E-04	0.11			
	wPt-1764585		0.12	3.05E-04	0.11			
TKW	*wPt-4535983*		0.07	8.89E-04		0.09		
	wPt-4407952		0.08	6.05E-04		0.10		
	SNP-1090158	7B	0.12	4.54E-04			0.11	
	wmc339-bp226		0.11	3.98E-04			0.12	
	wPt-1235015		0.35	3.23E-05			0.16	
VKC	*wPt-1122735*		0.50	9.44E-04	0.09			
	wPt-2301430		0.36	7.99E-04	0.09			
	wPt-3574929		0.08	9.23E-04			0.10	
	SNP-4992399		0.08	9.23E-04			0.10	
	wmc407-bp190	2A	0.06	8.54E-04			0.11	
	wmc469-bp158	1A	0.06	5.62E-06			0.23	
TW	*wPt-1386615*		0.25	9.68E-04		0.09		
	wPt-991875		0.15	9.66E-04		0.09		

MAF: minor allele frequency, p: The values of the association effect and significance. R^2 : phenotypic variance imparted by each marker