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ABSTRACT

of the dissertation for the degree of Doctor of Science

GENOTYPING BY SEQUENCING OF WHEAT (*TRITICUM* L.), BARLEY (*HORDEUM* L.) AND THEIR WILD RELATIVES AND SCREENING FOR STRESS RESISTANCE GENES

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INTRODUCTION

Relevance of the topic: Rapid advances in science and technology have led to revolutionary changes in the discovery of genetic potential of living organisms. With the creation of automated sequencing systems, reading of entire human and many other genomes have been made available, and access to large data in billions of nucleotide pairs has been achieved[1]. Thanks to genome data, biology and agrarian sciences have entered a new phase - a period of large-scale data analysis. The development of high-throughput Next Generation Sequencing (NGS) methods has enabled to obtain a reference genome for important plant species in a short time, to reveal all genetic variants, mutations, including common and rare alleles, and in this way, allowed to get a more comprehensive knowledge of the evolution and domestication of plants.

DNA marker technology, which aims to identify polymorphism in the genome, has been widely used for many years for the study of genetic diversity, evolution, the creation and improvement of genetic maps, and molecular selection. Single nucleotide polymorphisms (SNP) are the most widely used molecular markers and are considered the best choice in association analyses, genomic selection, and the study of a population structure. In recent years, new, faster and more efficient methods based on NGS technologies have been developed for the detection of genome-wide SNP markers. NGS methods such as genotyping by sequencing (GBS) and DArTseq based on the reduction of genome complexity using restriction enzymes, Amplicon Sequencing based on PCR amplification of target regions and further sequencing, allow increasing the efficiency of genotyping by facilitating the detection of SNPs.

[1] Mardis, E.R. Next-generation DNA sequencing methods // Annu Rev Genomics Hum Genet., - 2008. №9, - p. 387–402.

Only a few studies have addressed the screening for known genes in local and introduced cereal crop collections conserved in the National Genbank of Azerbaijan while genotyping studies using the Next Generation Sequencers are still at an early stage. Therefore, delivering the most modern genomic approaches - NGS-based genotyping methods to Azerbaijan, its optimization, investigation of the genetic diversity of wheat, barley and egilops collections based on these methods, their passportization, as well as, screening of the collection for genes of agronomic importance are very relevant.

Research Objective: The main objective of the research is genotyping of local and introduced wheat, barley and egilops collections using different NGS methods and molecular markers, assessment of genetic diversity, screening for resistance genes, creation and enrichment of core and trait collections.

The following goals have been set for the purpose of the study:

- Investigation of inter- and intraspecies genetic diversity in wheat and egilops accessions with different ploidy levels, as well as in wild and cultivated barley collections based on NGS technologies;
- Creation of a genome-specific PCR-genotyping panel for wheat based on amplicon sequencing method and its application to the evaluation of genetic diversity in durum and bread wheat collections;
- Genotyping of wild and cultivated barley accessions using amplicon sequencing method, association analysis between SNP markers and traits of agronomic importance;
- Genetic identification of wheat and egilops accessions using microsatellite markers;
- Evaluation of salt resistance of diploid wheat species based on hydroponic system and screening of resistance genes; the creation of trait collection on salt resistance;
- Evaluation of the rust resistance of durum and bread wheat collections in the artificial background, screening of resistance genes and the creation of trait collections;
- Screening of bread wheat collection for genes of agronomic importance using the KASP marker system.

The main provisions to be defended:

- New SNP markers were identified in local and introduced wheat, barley and egilops collection samples using GBS, DArSeq and amplicon sequencing methods and the genetic structure of the collections was determined;
- Core and trait collections for wheat, barley and egilops were created using SNP and gene specific markers;
- Genome-specific panels for quick and easy identification of genetic variants in wheat collections with different ploidy levels were developed;
- Genetic diversity was assessed in the barley collection and SNP markers associated with agronomic traits were identified;
- Genetic polymorphism in wheat and egilops was assessed using microsatellite markers and relevant collection samples were passportizated;
- The salt resistance of diploid wheats of various origin was evaluated, salt resistance genes were screened and their expression was studied;
- In durum and bread wheat collections conserved in the National Genbank, genotypes resistant to stem and leaf rust were found on the basis of artificial background assessment and screening with gene-specific markers.

Scientific novelty: For the first time, genetic diversity in local and introduced wheat, egilops and barley collections were evaluated based on genome-wide single nucleotide polymorphisms using Next Generation Sequencing technologies, intra- and interspecific genetic relationships were determined.

As a result of genotyping by sequencing of durum and bread wheat accessions conserved in the National Genbank 1039 and 411 single nucleotide polymorphisms were revealed, the distribution of SNP markers on the wheat genome (A, B, and D) and chromosomes was determined. This is the first SNP marker data for collections, the vast majority of which were genotypes and varieties of Azerbaijan.

For the first time, in 150 *Aegilops* accessions representing 9 species of Azerbaijan origin, DArTseq technology was applied, and 30433 SNPs and 61574 SilicoDArT markers were identified for the collection. As a result of GBS analysis of *Ae. tauschii* accessions, 384 SNP markers were revealed. Strong genetic differentiation has been found between *Ae. tauschii* genotypes of Azerbaijan and Georgian origin.

For the first time, a PCR-genotyping panel comprising 830 primers covering the A, B and D genomes of wheat has been created and used to evaluate genetic diversity in different wheat collections. The effectiveness, reliability and suitablity of the panel for diversity analysis has been confirmed.

For the first time, a PCR-genotyping panel comprising 365 primers spread across the barley genome was used to genotype 86 *H. spontaneum* and 85 *H. vulgare* accessions collected from 20 different regions of Azerbaijan.

For the first time, association mapping (AM) analyses was performed in the barley collection on bio-morphological traits and disease resistance. Significant marker trait associations were identified for stem rust resistance on chromosome 4H, ~103 cM and for spot blotch on chromosome 7H, ~90 cM.

The screening of diploid wheat species for salt resistance genes revealed the presence of *Nax1* and *Nax2* resistance genes in *T. monococcum* and *T. boeoticum*. Sodium exclusion is controlled by other genes in *T.urartu*.

For the first time, durum and bread wheat varieties and botanical varieties of Azerbaijan were screened for yellow and brown rust resistance genes, as a result, new sources of resistance genes were identified.

For the first time, in the current study, 166 bread wheat accessions conserved in National Genbank were screened for 11 different loci related to productivity, quality and disease resistance using KASP technology and genotypes with favorable alleles on 8 loci were identified.

Scientific and practical significance: In the dissertation work, a large number of new high-quality SNP markers have been identified in cultivated and wild wheat, barley and egilops species, using NGS technology, which also serves an important database and valuable scientific and practical resource for future researches.

The SNP marker set for each species can be used in different collections for population genetics and genome-wide association studies, to identify molecular markers linked with different economic traits, in particular traits of botanical varieties.

Information on the genetic diversity of different plant collections obtained using NGS and molecular marker technology, and genetic profile developed for each sample can be used for the improvement of the conservation strategies of collections, selection of genetically distant forms to enhance heterosis, development of new scientific strategies for wheat and barley breeding by using wild and cultivated genepool.

The first amplicon sequencing panel developed for the wheat plant can be used in any genotyping studies on durum and bread wheat and in future association analyses. SNPs, identified using amplicon sequencing technology and associated with agronomically important traits in barley, will be used as an indispensable source for marker-assisted breeding and genomic selection.

The genotypes with rust resistance genes, especially with rye translocation, as well as genotypes showing resistance to different isolates in the artificial background can be used as a donor for the creation of varieties resistant to stem, leaf and stripe rust.

The results obtained in the study can be used as a source for the development of specialized courses on "Genetics", "Molecular Genetics", "Genomics", "Plant Physiology", "Ecological Genetics", "Breeding and seed production", “Fingerprinting of plant genetic resources using molecular markers”, “Genetic bases of plant resistance to abiotic and biotic stress factors” and other. The results of the dissertation work may serve as a basis for future genomic researches in the country.

Approbation of work: The results of the dissertation work were discussed in The Fifth International Congress on Crop Science (South Korea, 2008), Proceedings of the International Durum Wheat Symposium (Italy, 2008), 8th International Wheat Conference (Russia, 2010), Rust Symposium of Field Plants (USA, 2011), Borlaug Global Rust Initiative, Technical Workshop (China, 2012), ASA, CSSA and SSSA International Annual Meetings (USA, 2012), International Congress on Plant Selection (Turkey, 2013), XI International Scientific and Methodological Conference on Introduction and Conservation of Biological Diversity of Cultured Plants (Russia, 2014), XI International Symposium on New and Non-traditional Plants and Their Utilisation (Russia, 2015), International Conference on Scientific and Practical Investigation of Bioorganic Agriculture (Russia, 2016), International Conference on Plant Genetics, Genomics, Bioinformatics and Biotechnology (Kazakhstan, 2017), Conference dedicated to Academician Belyaev (Russia, 2017), International Vavilov Conference (Russia, 2017), in conferences held inAzerbaijan (2009-2019), in the annual reports of the Biotechnology Department of Genetic Resources Institute of ANAS (2013-2018), in the annual reports of Genetic Resources Institute of ANAS (2016, 2017, 2018), reports of the Department of Biological and Medical Sciences of ANAS (2017, 2018), as weel as in scientific seminars held at the Institute.

The main findings of the research were published in the following well-known journals: "*Turkish journal of biology*" (2007, 2009), "*Annual Wheat Newsletter*" (2012, 2017), "*Genetic Resources and Crop Evolution*" (2018, 2019, 2020), "*Cereal Communication*" (2018), "*Cereal Chemistry*" (2018), "*Вавиловский журнал генетики и селекции*" (2018), "*Frontiers in Plant Science*" (2019), *Journal of Plant Physioly and Patholohy*" (2021) and others.

Publications: Sixty six scientific works (2 books, 1 monograph, 29 articles, 34 theses) were published on the basis of the dissertation results, 14 of which were in journals with impact factor. Five varieties (durum wheat - Maya, Korifey-88; bread wheat - Start,

Janub; barley - Jamil) have been realized and received a patent and copyright certificate.

Structure and volume of the dissertation: The dissertation consists of an introduction, 9 chapters, conclusion, results, recommendations, references and appendices. 458 references were used in the dissertation, 446 of which are Russian and English. The volume of the dissertation is 332 pages, including 49 tables and 80 figures.

CONTENT OF WORK

Chapter I. Literature review

In the literature review, the results of various studies relevant to the theme of the dissertation have been reported and analyzed, citing sources published in important scientific publications. For the first time, information on Next Generation Sequencing technologies was presented in Azerbaijani, their advantages and disadvantages were analyzed, and the results of genomic and transcriptomic studies on wheat and egilops species using these technologies were discussed. The next sub-headings, summarize the most recent information on the molecular-genetic mechanism of abiotic and biotic stress resistance in wheat and barley, resistance genes and linked markers in various genomes and association mapping based on SNP markers.

Each section of the literature review contains references and comparative analyzes of the researches carried out in Azerbaijan, along with scientific experiments around the world.

Chapter II. Materials and methods of research

The research material consisted of 196 accessions of diploid (*T. monococcum, T. boeoticum, T. urartu*), 350 accessions of durum (*T. durum* Desf.) and bread (*T. aestivum* L.) wheat species, 371 accessions of different *Aegilops* species, and 171 accessions of

barley (*Hordeum* L.) conserved in International (ICARDA, CIMMYT) and National Genbanks.

Seeds of the research material were germinated, DNA was extracted[2], and its quantitative and quality was assessed by Nanodrop (Thermo Scientific, 2000).

Sequencing in GBS and DArTseq analyses was performed using the Illumina HiSeq-2500 platform. The GBS libraries were constructed in 95-plex and genomic DNA was co-digested with the restriction enzymes *Pst*I (CTGCAG) and *Msp*I (CCGG) and barcoded adapters were ligated to individual samples[3]. Samples were pooled by plate into libraries and polymerase chain reaction-amplified. Each 95-plex library was sequenced to 100 bp on a single lane of Illumina HiSeq 2500. Sequence results were analyzed using the UNEAK GBS pipeline, which is part of the TASSEL 3.0 bioinformatics analysis package. Biostatistical analyzes were done using PowerMarker and DARwin 6.0. The genetic structure was investigated using the STRUCTURE 2.3.4 software package and STRUCTURE HARVESTER.

Genotyping by DArTseq was performed by method developed by Sansaloni et al. (2011)[4] and optimized for diversity analysis in wheat. DArT P/L analytical pipeline has generated the allele calls to recognize two kind of polymorphism, SilicoDArT and SNP, a dominant and a co-dominant marker. Then, a set of filtering stringent quality controls parameters, e.g. Call Rate and

[2] Stein, N. A new DNA extraction method for highthroughput marker analysis in a large genome species such as Triticum aestivum / N.Stein, G.Herren, B.Keller // Plant breeding, 2001. №120(4), - p. 354-356.

[3] Poland, J. Genomic selection in wheat breeding using genotyping-by-sequencing / J.Poland, J.Endelman, J.Dawson [et al.] // The Plant Genome, - 2012. №5, - p. 103–113.

[4] Sansaloni, C. Diversity Arrays Technology [DArT] and next-generation sequencing combined: genome-wide, high throughput, highly informative genotyping for molecular breeding of Eucalyptus / C.Sansaloni, D.Jaccoud [et al.] // BMC proceedings, - 2011. №5, - p. 1-2.

reproducibility, was applied to select and provide high quality markers. Cluster and PCoA analyzes based on DART data were performed with the Bio-R software package.

Multiplex amplicon sequencing analysis was performed according to the protocol provided by Schnable Laboratory[5]. A multiplex PCR reaction was performed using SNP specific primers designed for the A, B and D genomes, and all amplicons obtained for each sample were barcoded and pooled.

The clonal amplification of the prepared library was performed using the Ion PGM HI-Q View Template Kit on the PGM OneTouch system. Enriched ISPs were sequenced using the Ion 318 chip on the Ion PGM platform (Thermo Fisher), according to the instruction of the manufacturer. Biostatistical analysis was performed using PowerMarker and DARwin 6.0.

Genotyping of SSR markers, was carried out using fluorescent-dye labeled primers, mapped to A and B genomes followed by separating the dye-labeled fragments on an ABI 3130xl Genetic Analyzer (Applied Biosystems/Thermo Fisher Scientifics) as described previously. Fragment analysis and allele calling were performed using GeneMapper software v.3.7.

Screening of durum wheat accessions with 11 gene specific KASP markers was performed according to the appropriate protocol provided by LGC Genomics[6].

The ability of diploid wheats to exclude Na^+ ions from the leaf was analyzed in a hydroponic system based on a protocol developed

[5] Genotyping by multiplexing amplicon sequencing: [Electronic resource] / Schnable Lab. 2015. URL: http://schnablelab.plantgenomics.iastate.edu/resources/protocols/.

[6] Wheat genotyping library: [Electronic resource] / LGC, Biosearch technologies. – 2016. URL: https://www.researchgate.net/institution/LGC_Biosearch_Technologies2/post/58458fbfdc332d599f0c2991_KASPR_Genotyping_Markers_for_Key_Wheat_Traits

by Mans and James (2003)[7]. Genotypic analysis was performed by screening *Nax1* and *Nax2* resistance genes[8]. In order to assess the resistance of wheat and barley plants to rust, the samples were infected in artificial background with uredinospores of brown and stem rust. Infection types (ITs) were recorded using the Stakman 0 to 4 scale at 12 days (leaf rust) or 14 days (stem rust) post-inoculation.

Chapter III. Study of durum (*T. durum* Desf.) and bread (*T. aestivum* L.) wheat accessions using Next Generation Sequencer technology

3.1. Genotyping by sequensing in durum wheat (*T. durum* Desf.) accessions

Genotyping by sequencing (GBS) is an NGS-based technology and is successfully applied to organisms with large and complex genomes, such as wheat[9]. The advantage of SNP markers detected by GBS is that they cover the whole genome and all chromosomes.

In the current study the genetic diversity of 76 durum wheat (2n = 4x = 28 AABB) accessions was investigated using the GBS method. As a result of GBS analysis, a total of 1039 single nucleotide polymorphisms were obtained for the two genomes (AB). The distribution of SNP markers on durum wheat genome is presented in Figure 3.1.1. Of the 1039 SNP markers obtained, 426 were identified to be located in the A (41%) and 613 in the B (59%)

[7]Munns, R. Screening methods for salinity tolerance: a case study with tetraploid wheat / R.Munns, R.A.James // Plant and soil, - 2003. №253(1), - p. 201-218.

[8] Roder, M.S. A microsatellite map of wheat / M.S.Röder, V.Korzun, K.Wendehake [et al.] // Genetics , - 1998, №149, - p. 2007-2023.

[9] He, J. Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding / J.He, X.Zhao, A.Laroche [et al.] // Frontiers in plant science, - 2014. №5, - p. 1-8.

genome. The number of SNPs per chromosome varied from 49 to 113.

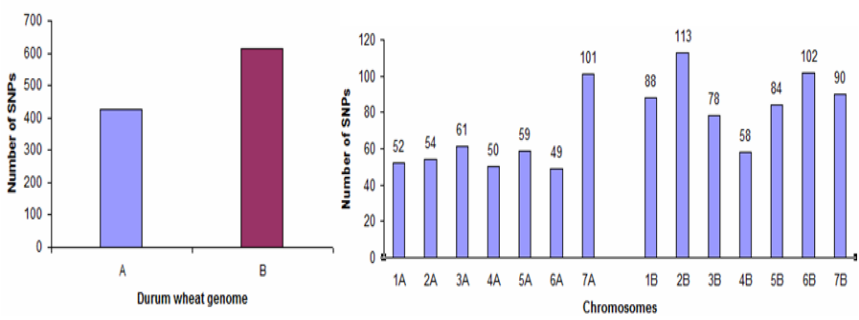

Figure 3.1.1. Distribution of SNP markers across all chromosomes in tetraploid wheat genome (AB)

The number of markers per chromosome within the tetraploid genome was in range 49–101 for genome A and 58–113 for genome B. The maximum number of markers was recorded on chromosome 2B, and the least on the chromosome 6A. Of the single nucleotide mutations detected in the durum wheat collection, 69.2% were transition-type (Ts) and 30.8% were transversion-type (Tv), with a Ts/Tv ratio of 2.25, indicating a high frequency of A↔G and C↔T mutations.

The average PIC for the collection based on GBS data was 0.329, which is a high value for biallelic SNP markers with a maximum PIC value of 0.5.

The principal component (PCA) and STRUCTURE analyzes identified 3 subpopulations in the collection (Figure 3.1.2). The first subpopulation had the highest, and the third subpopulation had the least admixture. All varieties that have common parent are included into the same group. Six clusters were identified in the dendrogram created based on SNP data. The grouping pattern observed in the cluster analysis was in agreement with the PCA and STRUCTURE analyses. Some relationship was noted between grouping of genotypes and their classification into botanical varieties.

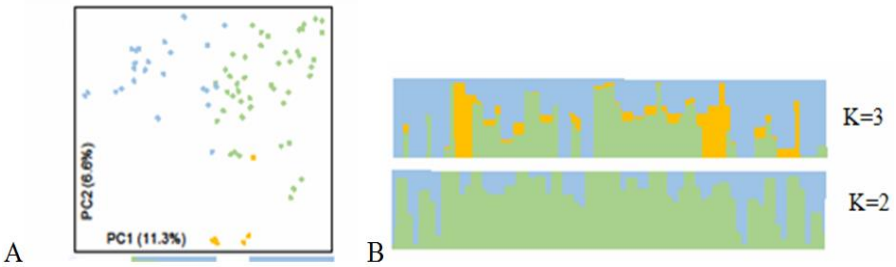

Figure 3.1.2. A) PCA analysis for 76 *T. durum* genotypes. The colors correspond to the colors in the population structure analysis. B) STRUCTURE analysis in *T. durum* collection

The results show that GBS technology is a powerful tool for creating new markers in plants with polyploid genomes such as wheat. These results will open the door for future association mapping studies of many traits, including traits of botanical varieties.

3.2. Genotyping by sequencing in bread wheat (*T. aestivum* L.) accesions

As a result of GBS analysis of 87 local and introduced wheat varieties and accessions a total of 411 single nucleotide polymorphisms (SNPs) were found for the hexaploid genome (AABBDD). The highest number of markers was recorded in genome B (48.8%) and the lowest in genome D (14%). Uneven distribution of markers has been found in both the hexaploid genome and different homeological groups. The number of SNPs on the chromosomes varied widely (3-36), the maximum number was recorded on 5B and minimum on 5D chromosomes.

It was found that 70.2% of TNPs were transition-type, and 29.8% were transversion-type, whereas the Ts/Tv ratio for the three genomes of hexaploid wheat was 2.36.

The average genetic diversity index and the polymorphism information content for the 87 accessions were 0.422 and 0.331, respectively. The vast majority of SNPs were characterized by high

values of GDI, and 42% of SNPs had maximum value (0.5), indicating the rich genetic diversity of the bread wheat collection which mainly (78.2%) consisted of Azerbaijani varieties.

The dendrogram created by cluster analysis was consistent with STRUCTURE and PCoA analyses. Several regularities were noted in the grouping nature of accessions: a) differentiation of local and introduced accessions (Figure 3.2.1); b) joint grouping of genotypes of the same botanical varieties; c) joint grouping of varieties; and d) the link between the grouping of varieties and genealogy.

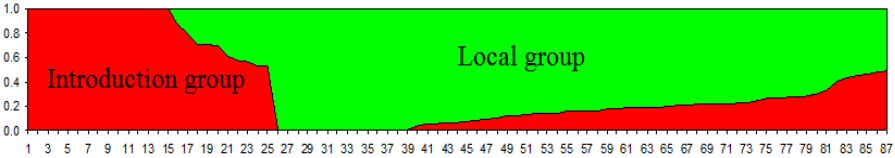

Figure 3.2.1. STRUCTURE analysis in bread wheat collection

Summarizing the results of the analyzes, it can be concluded that the use of same seed material from the same sources in various eco-geographical regions across the country for many years has led to their joint grouping with similar genetic backgrounds and, on the other hand, to the differentiation of local germplasm from other introduced materials. The presence of similar phenotypic features among the botanical varieties grouped together based on the SNP data confirms that the traits of botanical varieties are important and greatly affect the overall grouping of bread wheat genotypes and the genetic structure of the local collection.

Chapter IV. Single nucleotide polymorphism analysis of aegilops (*Aegilops* l.) collection

4.1. Assessment of genetic diversity of *Aegilops* species of Azerbaijan origin based on DArTseq and SNP markers

Diversity Arrays Technology (DArT) is a high-throughput and low-cost genotyping method for genome-wide polymorphism

determination[10]. For the first time in the dissertation, DArTseq technology was applied to *Aegilops* species of Azerbaijan origin, and the genetic diversity of 150 accessions belonged to 9 species was investigated using two types of marker systems - SNP and SilicoDArTseq. In total, 30433 SNPs and 61574 SilicoDArTseq markers were obtained for *Aegilops* samples using DArTseq technology. The number of effective alleles (Ne), the expected heterozygosity and the observed heterozygosity for the studied collection were 1.334, 0.222, and 0.029, respectively. The average Shannon Genetic Diversity Index for the 150 Aegilops accessions was 0.809.

4.2. Determination of the genetic relationship among *Aegilops* accessions

Two dendrograms were prepared and compared on the basis of the SNP and DArTseq markers to determine the genetic relationship between and within *Aegilops* species.

The clustering pattern in dendrograms coincided with each other and with the taxonomy of the genus. Both SNP and DArTseq markers were able to identify *Aegilops* species and grouped them into separate clusters and groups (Figure 4.2.1).

[10] Jaccoud, D. Diversity arrays: a solid state technology for sequence information independent genotyping / D. Jaccoud, K.Peng, D.Feinstein [et al.] // Nucleic Acids Res., - 2001. №29(4), - p. 1-7.

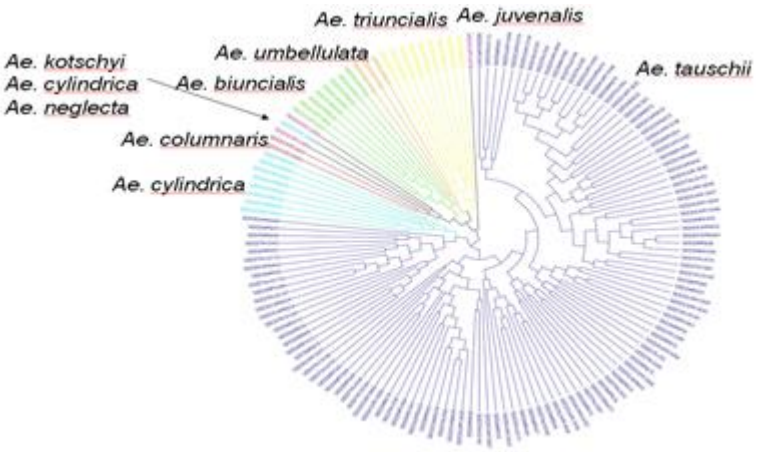

Figure 4.2.1. Dendrogram of 150 *Aegilops* accessions based on SNP data using Rocers genetic distance index

Unlike the SNP dendrogram, DArTseq markers were more effective in the identification of *Ae. tauschii*. In dendrograms the closest specie to *Ae. tauschii* (D) was *Ae. cylindrica* (DC) - the other source of the D genome. This similarity between species is expected as *Ae. tauschii* is thought to be a donor of the D genome of *Ae. cylindrica*. The results of our research revealed that *Ae. juvenalis* is more similar to its father parent *Ae. umbellulata*, rather than to *Ae. tauschii*. In general, DArTseq technology was able to distinguish the *Aegilops* species with the U genome from D genome species with high accuracy.

The PCoA analysis confirmed the subgrouping obtained by cluster analysis; the first two principal coordinates explained a very large portion of the total variation - 82.34% (Figure 4.2.2). STRUCTURE analysis identified two distinct groups within the *Ae. tauschii*.

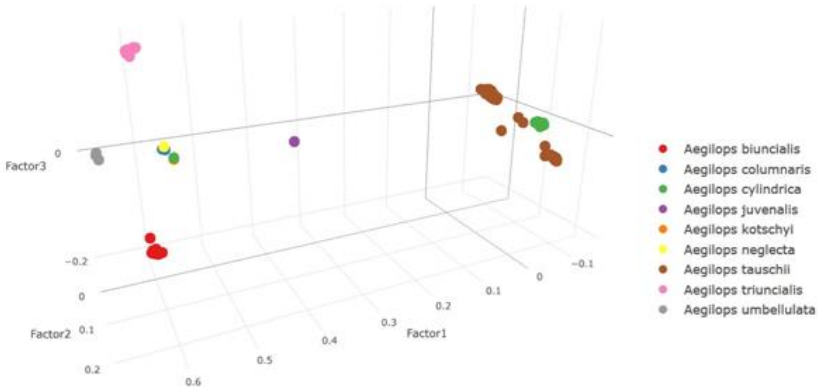

Figure 4.2.2. PCoA analysis for 150 *Aegilops* accessions based on SNP markers obtained by DArTseq technology

The high diversity found in *Aegilops* species of Azerbaijan can be very useful for profitable diversifying the gene pool of hexaploid wheat.

4.3. Assessment of genetic diversity in *Aegilops tauschii* collection using GBS-based SNP markers

Of the single nucleotide polymorphism markers obtained for the *Aegilops tauschii* collection of Azerbaijani and Georgian origin using GBS technology, 348 high quality SNP markers with a minor allele frequency of higher than 10% and a heterozygosity of less than 5% were selected and used for analysis. All types of single nucleotide substitutions (4 transitions and 8 transversions) were found in 106 *Aegilops* genotypes, which indicates that the variability of the *Ae. tauschii* genome is higher than that of the wheat genome. Out of 348 markers 19 were unique and found in one or two *Ae. tauschii* genotypes, the number of unique alleles per genotype varied from 1 to 12.

The average GDI and PIC values in the *Ae. tauschii* collection were 0.386 and 0.303, respectively. Nei genetic distance index varied between 0-1 and averaged 0.64.

The cluster, PCoA, and STRUCTURE analyzes revealed a sharp genetic differentiation between the *Ae. tauschii* collections of Azerbaijan and Georgia. In addition, within each country subpopulations that differed significantly were identified (Figure 4.3.1). The lack of gene flow among *Ae. tauschii* populations located in different countries, geographical regions and coordinates has led to their isolation from other populations and, ultimately, to such differentiation.

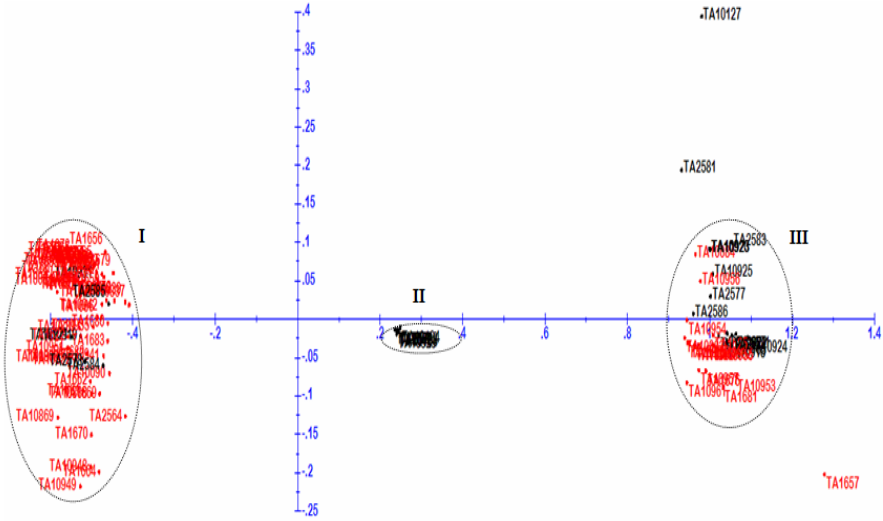

Figure 4.3.1. Distribution pattern of 106 *Ae. tauschii* genotypes of Azerbaijan (red) and Georgia (black) origin based on GBS-derived SNP markers

The genetic variation, as well as geneotypes with unique alleles identified by GBS analysis in the *Ae. tauschii* collection can be effectively used in order to create new, higher quality wheat varieties.

Chapter V. Genotyping of wheat and barley accessions based on amplicon sequencing

5.1. Development of amplicon sequencing panel on wheat genome

PCR-GBS or amplicon sequencing is an alternative method of reducing genome complexity using selective primers and is an invaluable tool for plant breeding and genome studies.

For the first time, an amplicon sequencing panel comprising 830 primers spread across the wheat genome (A, B and D) was developed for more efficient identification of genetic variants in wheat. Sequencing of 11 libraries was performed on the Ion PGM platform using the Ion 318TM v2 chip, the number of reads per circle varied between 3.8-4.5x10^5 (Figure 5.1.1).

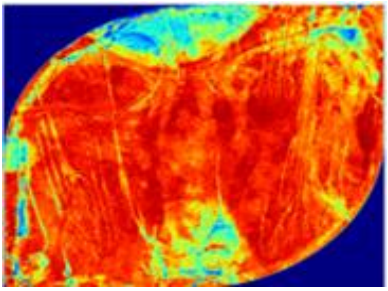

Figure 5.1.1. Pseudo-color image of Ion 318TM v2 chip. The red color indicates 100% loading, and the blue color indicates that the wells in that area are empty. Each 318 chip contains 11 million wells.

Based on the bioinformatic analysis, 401 high-quality markers were selected and the panel was examined in different bread wheat (*T. aestivum*) and durum wheat (*T. durum*) collections. The distribution of markers across the genome is consistent with GBS analysis, which proves the effectiveness of the panel created for the wheat genome. Among the three wheat genomes, the B genome had the highest (151 SNP) and the D genome (88 SNP) had the lowest marker density. The number of SNPs mapped in the A and B genomes was 1.6 and 1.7 times higher than in the D genome. Of the 21 chromosomes, 1B and 3B chromosomes were characterized with the highest number of SNPs, while chromosome 3D and the smallest wheat chromosome 4D had the least number of SNPs. The most commonly encountered transition-type and transversion-type substitutions were C/T (177) and G/T (31), respectively.

5.2. Study of genetic diversity in durum wheat collection based on amplicon sequencing

The created amplicon sequencing panel was first examined in two durum wheat (*T. durum* Desf.) collections. A number of statistical parameters were measured, and genetic relationship was assessed.

The first collection of durum wheat consisted of 69, and the second of 96 accessions. The sequencing results of amplicons obtained with SNP specific primers were compared with GBS analysis. The coefficients of genetic diversity in both collections had average values and amounted to 0.276 and 0.226, respectively. Sixteen of the SNP markers were unique, and 12 were rare alleles; the number of unique alleles per genotype varied from 1 to 4. Four unique alleles (54728 (T), 20572 (G), 27819 (G) and 66294 (G)) were obtained for the Maya variety which certificated and realized in 2019.

Cluster analysis based on the amplicon sequencing results was able to identify the vast majority of accessions in the first collection and all accessions in the second collection. Comparison of the grouping patterns in the clusters based on the amplicon sequencing and the GBS data revealed the presence of very large homologies. Thus, although the botanical varieties were to some extent distributed throughout the dendrogram, most samples that were genetically close to each other, belonged to the same botanical varieties as in the GBS analysis (v. *leucurum*: 6093, 6098 and 6086; v. *lecomelan*: 6138 and 6139). In addition, a joint grouping of varieties with common parents (for example, Shiraslan 23 and Mirbashir 50) was observed in the dendrogram. This, on the one hand, confirms the validity of the results obtained from different sequencing methods, and on the other hand, provides the basis for studies to reveal the association between the newly established SNP markers and traits of botanical varieties in durum wheat.

5.3. Study of genetic diversity in bread wheat collection based on amplicon sequencing

Verification of the amplicon sequencing panel on bread wheat was carried out in collections comprising 69 and 88 *T. aestivum* varieties and accessions. As with the GBS data, within each genome the chromosomes of 4th homoelogical group (4A, 4B, and 4D) were characterized by the least number of markers. Most of the single nucleotide substitutions were C/T and G/A transitions. The GDI and PIC parameters in the first collection, averaged 0.252 and 0.205, while in the other collection were 0.305 and 0.246, respectively. The highest polymorphism among bread wheat botanical varieties was recorded for var. *ferrugineum*, var. *milturum* and var. *erythrospermum*.

Genotypes with unique alleles were found in both collections. A total of 11 unique and 38 rare alleles were obtained. Two unique alleles were recorded in accession number 22 (v. *lutescens*) and the variety Nurlu 99. Cluster analysis allowed to differentiate and identify all samples. The new varieties Leyla and Start were genetically close to Parzivan 2 and Aran, respectively.

The new genotyping panel allowed to identify genotypes with unique profiles, as well as to generate a general picture of genetic diversity in the whole collection. The compliance of the results with the GBS analysis confirms the reliability of panel. The created panel can be used with confidence in future genotyping and association analyzes on durum and bread wheats.

5.4. Genotyping of barley collection based on amplicon sequencing

For the first time, genotyping of 86 wild (*H. spontaneum*) and 85 cultivated barley (*H. vulgare*) samples from 20 different regions of Azerbaijan was performed using a panel consisting of 365 SNP specific primers distributed throughout the barley genome. As a result of the sequencing $4.72x10^4$ reads were obtained, with a median length of 117 bp.

No significant variation in the number of SNP markers was observed along the H chromosomes, with the highest SNP number on 6H (45) and the least marker number on the 7H (29) chromosome. The SNP markers found in the barley genome included all types of substitutions, except A/T, transitions accounted for 66% and transversions for 34% of the total variants. A high genetic diversity on SNP markers was found in the studied barley collection (GDI=0.347; PIC=0.280). Cultivated barley accessions (GDI=0.343; PIC=0.272) has been shown to be more genetically rich than wild barleys (GDI=0.247; PIC=0.200).

The Nei genetic distance index among barley accessions varied between 0-0.76 and averaged 0.42. Accessions were combined into 3 major groups in the dendrogram. Cluster analysis allowed to completely differentiate wild and cultural barley genotypes. Further diversification of the dendrogram showed the existence of two genetically distinct groups within both species. STRUCTURE and PCoA analyzes confirmed the obtained results from the cluster analysis (Figure 5.4.1).

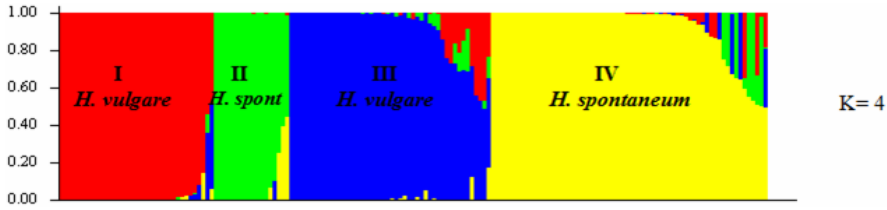

Figure 5.4.1. STRUCTURE analysis based on 255 SNP markers in 169 barley genotypes

For the first time in the study, an association analysis of SNP markers with a number of morphobiological traits and disease resistance was performed in the cultivated barley collection. Association mapping (AM) analyses using principal components was performed on 14 bio-morphological traits and the best performing analysis was for spike length. In addition, significant marker trait

association was identified for stem rust (*P. graminis* f. sp. *tritici*, race QCCJ) resistance on chromosome 4H (~103 cM) and for spot blotch (*C. sativus*; isolate ND85F) on chromosome 7H (~90 cM).

Chapter VI. Fingerprinting of wheat and *Aegilops* accessions using molecular markers

6.1. SSR analysis of diploid wheats

6.1.1. Assessment of genetic diversity of *T. urartu* accessions using SSR markers

The genetic diversity of *T. urartu* accessions of various origin was evaluated using SSR marker technology. A total of 83 alleles were synthesized with 11 SSR primers for the 74 *T. urartu* genotype representing 8 countries. The highest allele number was recorded with barc-213 (15), while the lowest allele number was found for barc-200 and barc-209 primers (4). The expected heterozygosity (H_E) for the collection was 0.56, and the polymorphism information content was 0.52. The genotypes were grouped into 3 clusters based on cluster analysis; no linkage was observed between countries and grouping patterns. Molecular variance analysis (AMOVA) showed that most of the genetic diversity (90%) was explained by country variation and a small part (10%) by inter-country variation. Low F-Statistic (Fst) values calculated across geographical regions confirmed this fact.

6.1.2. Assessment of genetic diversity of *T. boeoticum* accessions using SSR markers

As a result of the SSR marker analysis of the 63 wild *T. boeoticum* accessions, 83 alleles were amplified using 11 primers, with an average of 7.5 alleles per locus. Unique alleles were recorded for all loci. He and PIC for the collection were 0.52 and 0.49, respectively. The highest diversity among the countries, included into the study, was observed for Syria (PIC=0.49; 6 genotypes), while the lowest (PIC=0.26) was found for 4 samples from Iraq.

Samples were clustered in 5 groups by cluster and PCoA analysis, with a low genetic differentiation among the samples. The link between genetic distance and geographic region was recorded only for Iranian genotypes.

6.1.3. Comparative SSR analysis of diploid wheat

A total of 139 accessions (14 *T. monococcum*, 71 *T. urartu*, and 54 *T. boeoticum*) were investigated with SSR markers in order to establish inter-specific relationship in diploid wheats. Of the 111 alleles amplified for the collection, 26 were specific for *T. urartu*, 20 for *T. boeoticum*, and 4 for *T. monococcum*. Among the three species, *T. urartu* is characterized by the highest and *T. monococcum* by the lowest total allele number and genetic diversity index (Table 6.1.1).

Table 6.1.1

Statistical parameters obtained with 11 SSR markers for diploid wheat species

Species	Number of accessions	Number of total alleles	Npa	Ho	He	PIC
T. urartu	71	81	15	0.28	0.58	0.54
T. boeoticum	54	78	6	0.13	0.52	0.50
T. monococcum	14	35	4	0.13	0.40	0.36
Total	139		25			

The genetic distance index between samples varied from 0-1, with an average of 0.64. The average genetic distance for *T. boeoticum* was 0.54, for *T. urartu* 0.53, and 0.40 for *T. monococcum*. The NJ dendrogram identified 13 clusters with a value of 1000 bootstrap. Cluster analysis was able to distinguish diploid wheat accessions at a species level, and genotypes were grouped according to taxonomic classification, with certain exceptions (Figure 6.1.1).

Thus, clusters I and III were represented mainly by *T. urartu*, clusters VII and X with *T. boeoticum*, whereas *T. monococcum* samples formed independent subclusters in clusters VI and IX.

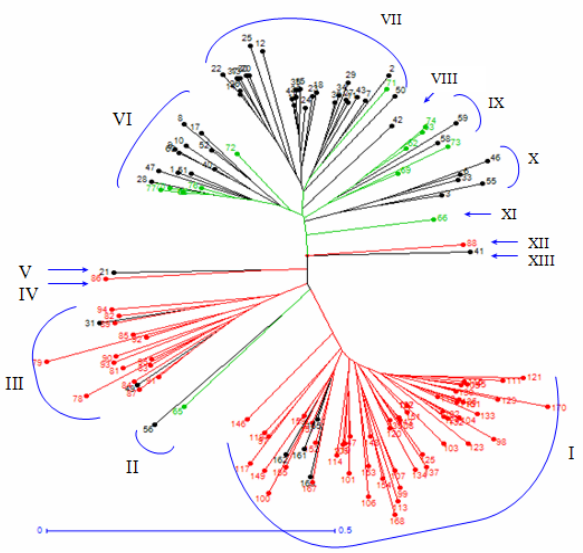

Figure 6.1.1. Nei's genetic distance based dendrogram generated using SSR data that show relationships among 139 diploid wheat accessions. Genotypes indicated with red color are *T. urartu*, with black are *T. boeoticum* and with green color are *T. monococcum*. Roman numerals indicate the cluster numbers.

As it is seen in the dendrogram *T. monococcum* accessions were placed close to *T. boeoticum* genotypes, indicating that these species share alleles. Moreover, among three species the least genetic distance was also noted between *T. boeoticum* and *T. monococcum* (GD=0.16), while *T. urartu* and *T. monoccocum* were revealed to be genetically distant (GD=0.54). Our findings are in good agreement with previous works on the origin of wheat species, and support the idea of domestication of *T. monococcum* from *T. boeoticum*.

6.2. Assessment of genetic diversity in durum wheat (*T. durum* Desf.) collection using SSR markers

Genetic diversity of 145 durum wheat accessions representing 29 botanical varieties was studied using SSR marker system. A total of 104 amplicons were amplified for 145 accesions using 13 SSR primer pairs, the number of alleles per primer was 8. Mean values for H_E and PIC in the collection were 0.62 and 0.58, respectively, which indicates high genetic diversity of durum wheat acessions of Azerbaijan. The genetic distance values between accessions ranged from 0 to 1, and averaged 0.60. The dendrogram did not reveal a

clear grouping pattern on botanical varieties, the highest similarity was noted between v. *hordeiforme* and v. *melanopus.* The general topology of the SSR dendrogram, especially the grouping pattern of the species, coincided with the results of GBS and amplicon sequencing. The rich genetic diversity found on the durum wheat botanical varieties suggests that the collection could be used as a donor of new alleles to further expand the genetic base of *T. durum* in future breeding studies.

6.3. Estimation of genetic diversity of bread wheat (*T. aestivum* L.) collection using SSR markers

Genetic variation of 158 bread wheat accessions, including local varieties and accessions of Azerbaijan was investigated using SSR markers. In total, 35 alleles were synthesized for the collection and the number of alleles per primer was 8.8. The study revealed a rich genetic diversity (GDI = 0.654) in the collection. Genotypes were grouped into 3 groups based on cluster analysis; in each cluster genetically distant and close accessions were identified. Of all the botanical varieties, var. *ferrugineum* (0.66) was characterized by the highest and var. *albidum* (0.22) by the lowest genetic diversity index. Thus, the results obtained can be used for the certification of bread wheat accessions and for the selection of parent combinations to be crossed out in breeding programs.

6.4. Study of genetic polymorphism of *Aegilops* species using SSR markers

6.4.1. Estimation of genetic variation of *Aegilops* accessions based on microsatellite loci

In the study the genetic diversity of 88 accessions representing 8 *Aegilops* species of different origin was investigated using SSR marker technology. The study is the first SSR evaluation study on *Aegilops* species of Azerbaijan and Georgia origin.

A total of 58 alleles were synthesized by seven SSR primer pairs and the average number of amplicons per primer was 8. All primers demonstrated 100% transferability among the 8 species,

except gwm210. In total, 33% of the synthesized alleles were specie-specific and 21% were accession-specific. Two separate alleles were sufficient to identify all accessions of *Ae. cylindrica*, whereas a combination of two alleles could distinguish *Ae. triuncialis* accessions analyzed. The highest genetic diversity among species was recorded for *Ae. speltoides* (PIC = 0.591) and the lowest for *Ae. cylindrica* (PIC = 0.044). The results of the study show that along with diploid *Ae. tauschii*, accessions of the tetraploids *Ae. biuncialis* and *Ae. triuncilais* from Azerbaijan were also highly diverse. The results provide a comprehensive information on molecular-genetic diversity of *Aegilops* species, that can be used for their effective conservation, management and use in wheat improvement programs as a source of adaptive alleles.

6.4.2. Assessment of genetic inter- and intraspecific relationship in *Aegilops* collection

Genetic distance index in *Aegilops* collection based on SSR markers ranged from 0 to 1 and averaged 0.51. Cluster and PCoA analyzes based on SSR data revealed a marked genetic differentiation and strong genetic discontinuities among the *Aegilops* species. The dendrogram obtained from seven SSR markers allowed distinguishing all *Aegilops* species that belonged to different sections and had different genomic structure at the cluster or subcluster levels.

The UNJ clustering algorithm based on SSR data grouped *Aegilops* accessions into nine clusters, clustering based on genetic distance was in agreement with taxonomy (Figure 6.4.1).

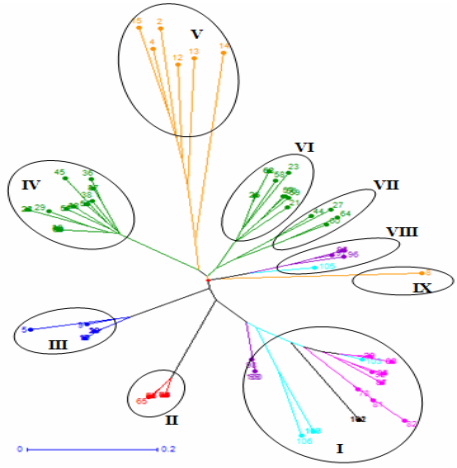

Figure 6.4.1. Dendrogram showing the genetic relationship among 88 *Aegilops* accessions. Different colors indicate different species (*Ae. umbellulata* (black), *Ae. tauschii* (green), *Ae. cylindrica* (red), *Ae. biuncialis* (violet), *Ae. geniculata* (blue), *Ae. speltoides* (yellow), *Ae. neglecta* (light blue), and *Ae. triuncialis* (pink).

All *Ae. cylindrica* accessions were in cluster II and all *Ae. geniculata* were in cluster III. *Ae. tauschii* genotypes were shared among homogeneous clusters IV, VI and VII and despite the large number did not interfere with other species. This on one hand indicated a high diversity within the species and, on the other hand, the uniqueness of the D genome.

Chapter VII. Estimation of salt resistance of diploid wheat accessions of diverse origin

7.1. Assessment of salt resistance of dipolid wheats in hidroponic systems

Salt resistance of 196 diploid wheat accessions (31 - *T. monococcum*, 87 - *T. urartu*, 78 - *T. boeoticum*) of different origin from ICARDA genbank was evaluated. For this purpose, first of all the ability of the accessions to exclude sodium from roots was tested using a hydroponic system based on the method developed by Muns and James. Resistant varieties - *T. monococcum*, CV 68-101, Line 149 and sensitive variety - Tamoroi were used as a control.

7.1.1. Detection of salt resistance of *T. monococcum* genotypes

Leaf sodium in studied *T. monococcum* accessions ranged from 11.5 to 175.6 mM. The accessions were divided into 3 groups based on sodium exclusion ability. The first group consisted of 12 *T. monococcum* accessions of diverse origin. Sodium exclusion ability of the genotypes fell into group 1 was higher than that of Line 149, and the amount of leaf Na^+ in these accessions was below 40 mM. The selected accessions can be used successfully in breeding for salt resistance. The amount of Na^+ ions accumulated in the leaves of the accessions from the second group varied between 43.3 and 64.5 mM, and the group was estimated as moderately resistant. Seven samples of the third group were considered to be more sensitive with high leaf sodium ranging from 75.4 to 175.6 mM.

7.1.2. Assessment of salt resistance of *T. boeoticum* genotypes

In the study, the salt stress resistance of 77 *T. boeticum* wheat accessions collected from different countries at different times was investigated. A significant differentiation of genotypes for salt resistance has been identified. Of the 77 genotypes, 47 were able to exclude a large amount of Na^+ ions, and the leaf sodium in these accessions varied in the range of 7.69 - 40.9 mM. The above-mentioned samples were selected for salt resistance selection and included in the collection. These accessions were selected for breeding on salt resistance and included in the trait collection.

7.1.3. Determination of salt resistance of *T. urartu* accessions

The salt resistance of 87 *T. urartu* accessions was also studied, of which 20 genotypes were highly and 44 moderately resistant. Twenty-three accessions were considered as sensitive. The amount of Na^+ ions accumulated in the leaves of highly resistant accessions ranged from 15.9 mM to 39.9 mM. Selected genotypes can be used in both breeding programs on salt resistance and as a useful trait collection for identification of new genes.

7.2. Screening of *Nax* genes in diploid wheat genotypes

At the next stage of the study, the diploid wheat samples were screened with the gwm312 SSR marker linked with the *Nax1* gene (Figure 7.2.1). Donors of *Nax* genes - *T. monococcum* CV 68-101 and Line149 were taken as positive and susceptible Tamaroi variety was taken as a negative control.

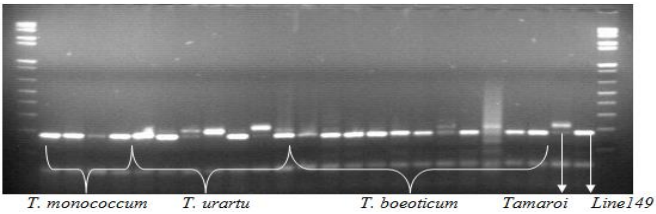

Figure 7.2.1. Screening of diploid wheats for *Nax1* gene with gwm 312

As a result of the screening for *Nax1* gene, all *T. monococcum* accessions except for one genotype were found to have resistance genes, whereas polymorphism was observed in *T. boeoticum* and *T. urartu* genotypes. As a result of fragment analysis 199 bp fragment was found in 25 *T. monococcum* samples, as in control accessions, suggesting that the above mentioned samples had a *Nax1* gene. In 5 *T. monococcum* accessions the length of amplicons differed. Wide polymorphism for *Nax1* gene was observed in *T. boeoticum*, whereas in *T. urartu* accessions the *Nax1* gene was not detected.

During the screening of diploid wheats for the *Nax2* gene, the gene was only detected in *T. monococcum* and *T. boeoticum* species. In *T. urartu* genotypes *Nax2* was absent (Figure 7.2.2).

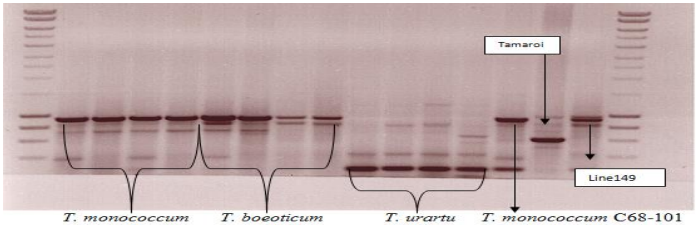

Figure 7.2.2. Screening of *Nax2* gene in diploid wheats

The results from the molecular analysis and hydroponic system were compared and systematized. In 25 *T. monococcum* accessions, where the presence of *Nax1* and *Nax2* genes was confirmed, the amount of Na^+ and K^+ ions was close to that of the resistant Line 149 variety. Thus, the average Na^+ and K^+ ions in these samples was 55 mM and 213 mM, respectively. In one *T. monococcum* accession collected from Montenegro, with lack of the *Nax* genes, the amount of Na^+ ions was 175 mM and K^+ ions 93 mM.

In 18 *T. boeoticum* accessions with both salt resistance genes (*Nax1, Nax2*), the mean for Na^+ and K^+ ions was 34 mM and 235 mM, respectively. A similar result was also observed in 47 accessions with only *Nax2* gene. In two accessions with *Nax1* gene, the amount of leaf sodium was 127 mM. Although none of the *Nax* genes were detected in 7 *T. boeoticum* accessions, the amount of Na^+ ions was low - 53 mM. This result provided the basis for future studies by showing that sodium exclusion can be controlled by other resistance genes in these *T. boeoticum* accessions.

Interesting results were also obtained for the studied *T. urartu* accessions. No *Nax* genes were detected in 43 accessions, distinguished by high resistance, and the amount of Na^+ ions accumulated in them varied in the range of 15–59 mM. In 37 genotypes this value was between 61 and 250 mM. Selected resistant accessions can be used in the identification of new resistance genes as well as in the breeding for salt resistance.

7.3. Determination of expression of salt resistance genes

In the study, 3 resistant and 3 sensitive genotypes of each species, including control accessions, were planted in small hydroponic systems and RNA was extracted from root tissue. Subsequently, cDNAs were synthesized on RNA and expression of *Nax* genes was investigated.

Studies have shown that in *T. monococcum* genotypes with low Na^+ accumulation, the expression of *Nax1* gene was intense, whereas

genotypes with high leaf sodium were characterized by low expression. The same results were also reported for the *Nax2* gene.

As there were no *Nax2* gene in *T. urartu* accessions, no expression was observed in *T. urartu* accessions for this gene. In wild diploid species - *T. boeoticum* and *T. urartu* genotypes, the expression of *Nax1* genes was not related to the accumulation of Na^+ ions (Figure 7.3.1).

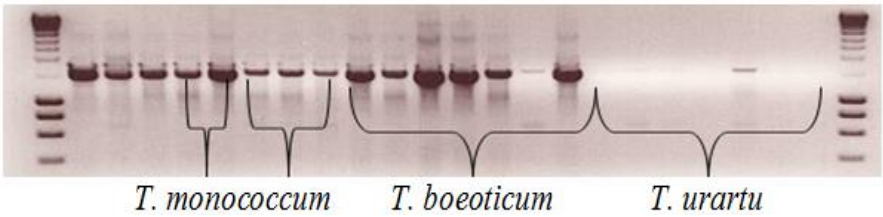

Figure 7.3.1. Results of RT-PCR for *Nax2* gene

Thus, the results of the reverse transcriptase PCR (RT-PCR) method confirmed that *Nax* genes were more specific to *T. monococcum*, and that the expression rate of genes was directly related to the resistance level of the samples. No significant difference in expression was observed in *T. boeoticum* accessions, whereas the absence of *Nax* genes, especially *Nax2* genes, in *T. urartu* genotypes was confirmed.

Chapter VIII. Estimation of rust resistance of durum and bread wheat accessions

8.1. Assessment of resistance of durum wheat collection to leaf and stem rust

Rust resistance of durum and bread wheat accessions conserved in the National Genebank was assessed in artificial background and for resistance genes.

The studied collection consisted of 18 varieties and 64 accessions belonged to 15 botanical varieties. Plants were inoculated

with 4 leaf rust (BBBDB, MFBJG, TTRSD and MRDSD) and 3 stem rust (MCCFC, TPMKC and RKQQC) races. Susceptible Morocco variety was taken as a control. According to the results of phytopathological studies in the durum wheat collection, 18 of the 82 *T. durum* accessions were estimated as high-resistant, 23 moderate resistant, and 41 sensitive.

Fourteen resistant and 12 moderate resistant accessions were identified in the studied collection. As a result of molecular screening, the Lr19 gene was detected in 2 accessions (6129 - *T. durum* var. *melanopus* and Jafari), and both samples showed high resistance to 4 races of the rust. In wheat accessions of Azerbaijan origin assessed in the artificial background a direct correlation between leaf rust resistance and the spike color was detected. Thus, 83.3% of the resistant and medium-resistant accessions had white spike, whereas 70.7% of the sensitive genotypes were characterized by red spike. Spike color related to leaf rust resistance at P<0.05* level (Figure 8.1.1).

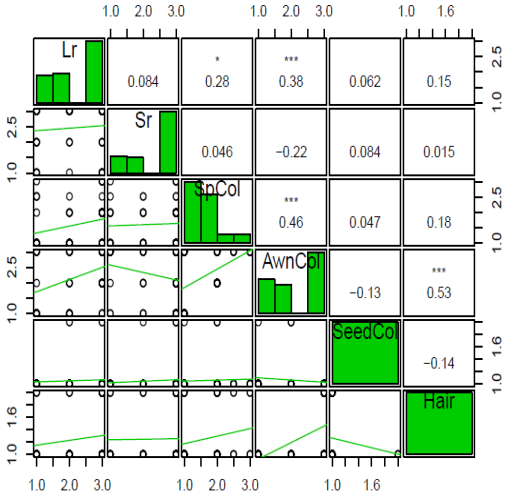

Figure 8.1.1. The correlation matrix between studied phenotypic traits. The upper triangle of the matrix shows Pearson's correlation coefficients, and the below-triangle displays scatter plots. Lr – leaf rust resistance; Sr – stem rust resistance; SpCol – spike color; AwnCol – awn color; SeedCol – seed color; Hair – hairness; *p < 0.05; *** p < 0.001.

8.2. Assessment of leaf and stem rust resistance in bread wheat collection

As a result of assessment of stem and leaf rust resistance of bread wheat collection in the artificial background 29 accessions were found to be resistant to stem rust and only 3 genotypes to leaf rust. For the first time, *Lr34* gene located on 7D chromosome was detected in 12 bread wheat accessions (6959, 6960, 6961, 7010, Grekum 75/50, Arzu, Zardabi, Gurgane 1, Mirbashir 128, Akinchi 84, Guneshli and Yegane), and T1RS·1BL rye translocation in 9 bread wheat (6945, 6983, 6984, 6987, 7014, 7033, Akinchi 84, Guneshli, Mirbashir 128) genotypes (Figure 8.2.1).

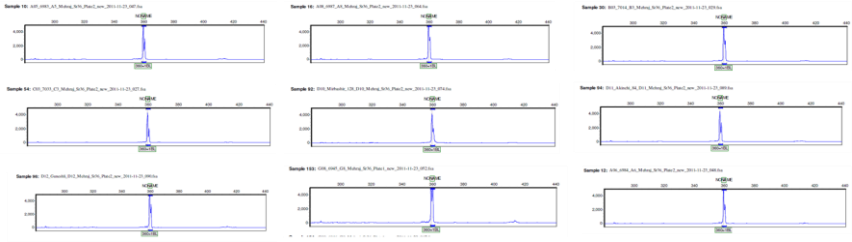

Figure 8.2.1. Fragment analysis of accessions with rye translocation

As a result of the study, a trait collection of 25 bread wheat accessions with a high resistance to leaf rust was created.

Chapter IX. Genotyping of bread wheat (*T. aestivum* L.) varieties and accessions of Azerbaijan using KASP technology

9.1. Screening for genes associated wity productivity

For the first time, in 166 bread wheat accessions stored in the National Genbank screening of 11 different loci related to productivity, quality, and disease resistance was carried out using KASP technology.

Plant height and photoperiod-insensitivity are considered one of the most important phenotypic features associated with productivity. The *Rht* genes associated with dwarfing and high-yield phenotype were discovered in 1935 and have been widely used in wheat breeding following the Green Revolution. Of the 166 bread

wheat genotypes studied, *Rht-B1b* allele of the *Rht-B1* gene was detected in 43 accessions, and the *Rht-D1b* allele of the *Rht-D1* gene in 5 accessions. Six samples were found to be heterozygous for *Rht-B1* and 4 for the *Rht-D1* gene (Figure 9.1.1). Among the accessions with the *Rht-B1b* allele, along with the botanical varieties collected from different regions of Azerbaijan, varieties such as Arzu, Karabakh, Zardabi, Akinchi 84, Ruzi 84, Azamatli 95, Gobustan, Yegane were also found. Analysis revealed that 53% of bread wheat samples were photoperiod-insensitive and 41.5% were photoperiod-sensitive.

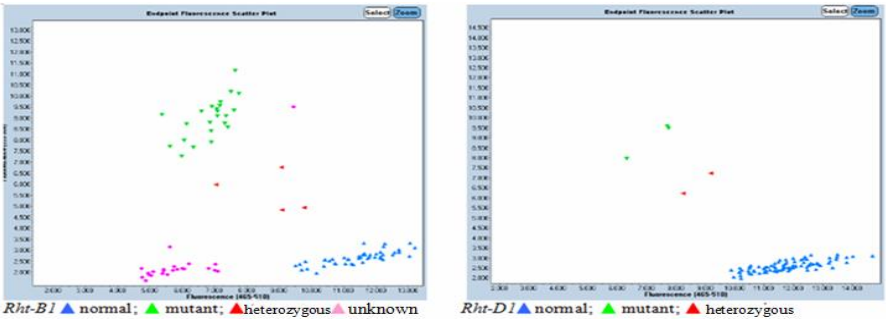

Figure 9.1.1. Screening of bread wheat accessions for *Rht-B1* gene. Results of one plate (96 accessions)

9.2. Screening for genes associated with grain quality

The *Glu-D1a* allele of the *Glu-D1* gene, encoding the glutenin subunits and located on I homeological chromosome, is widely distributed in bread wheat and has a positive effect on baking quality. As a result of the study of bread wheat varieties and accessions, a total of 34 genotypes were identified with the *Glu-D1a* allele, 31 accessions including 7 varieties were homozygous and 3 were heterozygous. In one accession collected from Agdam (var. *milturum*), an allele associated with high protein content was found. This genotype can be used as a donor in high protein content breeding programs.

9.3. Screening for disease resistance genes

As a result of the screening of accessions for stem (*Sr*) and leaf rust (*Lr*) resistance genes, 17 genotypes were found to be resistant to leaf rust (*Lr34*), while 33 genotypes were heterozygous for *Lr34* gene. Of the varieties, *Lr34* was only recorded in Saba and Guneshli. Ten out of 17 accessions belonged to var. *alborubrum*. The mentioned genotypes can be used as valuable genetic resources to increase leaf rust resistance in breeding.

The results of the screening for FHB 5A locus associated with resistance to fusarium showed that 47 genotypes were resistant to the disease, and the rest were susceptible. Heterozygosity was observed in 4 genotypes. Along with old varieties Bol bughda, Yerli, Birlik, Gurgane 1, Gizil bughda, the newly created Start and Leila varieties were also resistant. During the screening of the *Fhb1* gene that provides type II resistance to fusarium a positive (resistance) allele was identified in one var. *barbarossa* accession and one other genotype (var. *alborubrum* - Aghsu) was heterozygous.

SUMMARY

For the first time in Azerbaijan, a large number of wheat, barley and *Aegilops* collections were genotyped using the most up-to-date methods of genomics, biotechnology and molecular genetics, the inter- and intraspecific genetic polymorphism was identified using molecular marker technology, and the collection was screened for genes of salt and rust resistance. The obtained results were analyzed using the latest bioinformatic and biostatistic software packages. Summary of the results of all the analyzes confirmed that Next Generation Sequencing technologies are indispensable for the study of the genetic diversity of plant genetic resources. Genotyping by sequencing (GBS), amplicon sequencing and molecular markers are complementary techniques for the detection of SNPs and other variations in the genome, and for evaluation of genetic diversity. By using these technologies, it is possible to identify new genes and significantly improve the quality and speed of breeding. The results of the dissertation are of great scientific and practical importance.

The dissertation is the first research activity where the 3 most modern methods of genomics and genetics based on NGS technologies have been used and the results were consisted with each other. Through scientific manuscripts published in prestigious international journals, the fact that Azerbaijan has rich genetic resources of wheat, barley and their wild relatives and is one of the origin centers for these crops has been confirmed and reported to the world scientific community. The dissertation can play an important scientific and practical base for future researches on genomics and genetics. The results obtained in the dissertation will enable the biodiversity research and breeding in Azerbaijan to move to a new quality level.

RESULTS

1. Genotyping by sequencing of 76 durum (*T. durum* Desf.) and 87 bread wheat (*T. aestivum* L.) accessions, revealed 1039 and 411 single nucleotide polymorphisms (SNP), respectively. 69.2% of SNP markers in durum wheat collection was transition-type and 30.8% transversion-type, whereas 70.2% of SNPs in bread wheat was transition-type and 29.8% transversion-type. The distribution of SNPs in the tetraploid and hexaploid wheat genomes has shown that B genome has higher marker density.
2. The average PIC and genetic diversity index for the durum and bread wheat collections based on SNP markers were 0.33 and 0.42, respectively. An association was revealed between the grouping of both species and their genealogy. It has been established that the D genome of the bread wheat varieties and accessions of Azerbaijan is genetically more polymorphic compared to gene pools of some other countries.
3. The first DArTseq analysis of *Aegilops* species produced 30433 SNPs and 61574 SilicoDArT markers for 150 *Aegilops* acccessions of Azerbaijan origin. The Shannon Genetic Diversity Index in the collection by DArTseq technology was 0.809. The topology of the dendrogram was consistent with the taxonomy of the genus, and both markers were able to distinguish the *Aegilops*

species with the U genome from D genome species and to identify each specie within the genus. Two genetically distinct groups were found within the *Ae. tauschii* of Azerbaijan. Compared to SNP markers, the SilicoDArT markers were found to be more effective in identification of *Ae. tauschii*.

4. For the first time, using the Next Generation Sequencer (NGS) technology the genetic diversity of *Ae. tauschii* collection of Azerbaijan and Georgian origin was evaluated and 348 SNP markers were identified. The averages for GDI and PIC in the collection were 0.386 and 0.303, respectively. Unique SNP markers have been identified for certain *Ae. tauschii* accessions. Significant genetic differentiation on single nucleotide polymorphism has been identified between Azerbaijani and Georgian *Ae. tauschii* collections.
5. For the first time, based on NGS technology an amplicon sequencing panel consisting of 401 primers spread across the entire wheat genome was created. As a result of the application of a genotyping panel in four different wheat collections new single nucleotide polymorphism markers were found and all wheat accessions and varieties were genetically identified. Comparison of amplicon sequencing and GBS methods showed the effectiveness of the panel for rapid and reliable determination of genetic variations in wheat.
6. For the first time in Azerbaijan, the genetic diversity of 85 *H. vulgare* and 84 *H. spontaneum* accessions collected from 20 different regions of the republic were evaluated using amplicon sequencing panel comprising 255 primers. GDI and PIC value for the barley collection were 0.347 and 0.280, respectively. The highest number of SNP markers were recorded in 6H and the least number in 7H chromosomes. The existence of two genetically distinct groups was found within each species. The cultivated barley accessions were genetically more diverse than wild barleys.
7. For the first time in the cultivated barley collection, association mapping analyses were performed between 14 morphobiological

traits, diseases resistance and SNP markers. Significant marker-trait associations were identified for stem rust resistance on chromosome 4H at ~103 cM and for spot blotch on chromosome 7H at ~90 cM.

8. A total of 111 alleles were amplified for 139 diploid wheat accessions, using 11 polymorphic SSR markers. 45% of alleles were species-specific. High transferability of the SSR markers across wheat species has been found, indicating the conservation of the studied SSR regions on the diploid species. High genetic diversity was identified (GRI = 0.65) for the studied collection. Among the diploid wheat species, *T. urartu* was characterized by the highest genetic variation (PIC = 0.54) and the number of unique alleles. *T. boeoticum* and *T. monoccocum* were genetically close, whereas *T. urartu* and *T. monoccocum* were the most genetically distant.
9. A total of 145 durum and 158 bread wheat accessions were fingerprinted, using microsatellite markers. The polymorphism information content for the *T. durum* and *T. aestivum* collections was 0.58 and 0.62, respectively. The general topology of the SSR dendrogram was consistent with the results of GBS and amplicon sequencing. Var. *leucurum* of durum and var. *ferrugineum* of bread wheat were characterized by higher genetic diversity. The study confirmed that the SSR primers used in the study were effective for the study of genetic polymorphism and genetic relationships in tetraploid and hexaploid wheats.
10. A total of 58 alleles were obtained as a result of the SSR evaluation of *Aegilops* species, using 7 microsatellite markers. The transferability of SSR markers across eight species was 100%, with exception of gwm210. It was established that 33% of the bands were species-specific, while 21% were accession-specific. A high level of polymorphism was recorded for the collection (PIC = 0.540). Along with the diploid *Ae. tauschii*, accessions of the tetraploid species *Ae. biuncialis* and *Ae. triuncilais* of Azerbaijan origin were also highly diverse. The highest genetic similarity was noted between *Ae. neglecta* and

Ae. biuncialis, and the lowest between *Ae. speltoides and Ae. umbellulata*.

11. The study of salt tolerance of 196 diploid wheat accessions of diverse origin in hydroponic system showed that 12 *T. monococcum*, 47 *T. boeoticum* and 20 *T. urartu* genotypes have a high ability of Na^+ exclusion from the roots and genotypes were estimated as a salt resistant. The salt tolerance genes *Nax1* and *Nax2* were only found in *T. monococcum* and *T. boeoticum*.
12. The results of the reverse transcriptase PCR (RT-PCR) confirmed that *Nax* genes are more specific to *T. monococcum*, and the gene expression was directly related to the salt tolerance of accessions. No significant difference was observed in expression of *Nax* genes in *T. boeoticum*, while the absent of *Nax* genes, especially *Nax2* gene in *T. urartu* genotypes was confirmed.
13. The results of phytopathological studies showed that, of the 82 durum wheat genotypes, 18 were highly resistant, 23 moderately resistant, and 41 sensitive to leaf rust. 14 highly and 12 moderately resistant accessions to stem rust were identified in the studied collection. During the molecular screening, the *Lr19* gene was detected in 2 accessions (6129 - *T. durum* var. *melanopus* and Jafari), and both genotypes showed high resistance to 4 isoforms of the rust. A direct relationship between resistance to leaf rust and spike color was found in durum wheat genotypes of Azerbaijan origin in the artificial background. In 83.3% of high- and medium-resistant genotypes, the spike color was white, and 70.4% of susceptible genotypes had red spikes.
14. As a result of the evaluation of leaf and stem rust resistance in bread wheat collection in artificial background 29 accessions were resistant to stem rust and only 3 to leaf rust. For the first time 12 bread wheat genotypes (6959, 6960, 6961, 7010, Grekum 75/50, Arzu, Zardabi, Gurgene 1, Mirbashir 128, Akinchi 84, Guneshli və Yegane) were found to have *Lr34* resistance gene located on 7D chromosome, and 9 bread wheat accessions (6945, 6983, 6984, 6987, 7014, 7033, Akinchi 84, Guneshli, Mirbashir 128) had T1RS·1BL rye translocation.

15. For the first time, as a result of the screening of 166 bread wheat genotypes for 11 loci using KASP genotyping technology new, valuable gene sources have been identified in the collection. The mutant (dwarf) alleles of the *Rht-B1* and *Rht-D1* genes were found in 43 and 5 bread wheat genotypes, respectively. Analysis revealed that 53% of bread wheat samples were photoperiod-insensitive and 41.5% were photoperiod-sensitive. A total of 31 genotypes had *Glu-D1a* allele, which has a positive effect on baking quality. As a result of the screening of bread wheat genotypes for biotic stress resistance genes, 17 genotypes were found to be resistant to leaf rust based on *Lr 34* gene, and 48 genotypes to fusarium based on *Fhb5* and *Fhb1*.

RECOMMENDATIONS

1. The amplicon sequencing panel comprised of 401 primers created within the dissertation study can be used for rapid and accurate determination of genetic variants, for genotype-phenotype association analyzes in durum and bread wheat collections, as well as in modern scientific breeding programs.
2. The use of SNP markers associated with stem rust and spot blotch resistance in marker-assisted selection and genome selection studies of barley is recommended.
3. The salt-resistant diploid wheat varieties and accessions selected as a result of the screening of *Nax* genes and hydroponic system can be used to create new salt-resistant varieties and to identify new resistance genes.
4. It is recommended to use genotypes with rust resistant genes and T1RS·1BL rye translocation as the primary breeding material and as a genetic source to create resistant varieties to brown, yellow and stem rusts.
5. The bread wheat accessions with favorable alleles of *Rht-B, Rht-D, Glu-D1, Lr 34, Fhb5* and *Fhb1* genes can be used as a donor in breeding for resistance, productivity and grain quality.

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