

ASSOCIATION MAPPING OF GERMINATION AND SOME EARLY SEEDLING STAGE TRAITS OF A TURKISH ORIGIN OAT COLLECTION

Berk Abdullah KOCAK¹, Fatih Mehmet KILINC¹, Adem BARDAK¹, Huseyin GUNGOR², Tevrican DOKUYUCU³, Aydın AKKAYA⁴, Ziya DURLUPINAR^{1*}

¹Kahramanmaraş Sutcu Imam University, Faculty of Agriculture, Department of Agricultural Biotechnology, Kahramanmaraş, TURKEY

²Düzce University, Faculty of Agriculture, Department of Field Crops, Düzce, TURKEY

³Kahramanmaraş Sutcu Imam University, Faculty of Agriculture, Department of Field Crops, Kahramanmaraş, TURKEY

⁴Mus Alparslan University, Plant Production and Technologies Department, Mus, TURKEY

*Corresponding author: zdurlupinar@ksu.edu.tr

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ABSTRACT

In recent years, oat plant especially for hay yield is on high demand in Turkey. In this study, 167 Turkish oat landraces were evaluated for germination and some early seedling stage traits and genotyped by 6K SNP Chip assay to detect candidate markers using association mapping analysis. The variation in oat genotypes for germination and other investigated traits was significant 5%, except for germination rate (GR). In the research, principal component (PC) 1 and PC2 value was found 41.5% and 21.5%, respectively, explaining the 63% of the total variation. According to the results of the association mapping analysis a total number of 33 candidate markers were observed, eight candidate markers for germination rate, five candidate markers for germination ratio (GP), five markers for radicle length (RL), five markers for coleoptile length (CL), four markers for plumula length (PL), and six markers for seed vigor index (VI). These candidate markers identified in the study for germination and early seedling stage traits could be used in future studies.

Keywords: Association mapping, germination, principal components, oats

INTRODUCTION

Turkey is one of the important gene centers of oats that containing many wild and cultivated varieties (Durlupinar et al., 2011a). Local varieties are important natural genetic resources that offer opportunities and innovations to plant breeders in the development of high yielding and high quality new varieties.

The success of seed germination in a plant and the frequency of normal seedling management are tremendously decisive factors in the production of crop plants, which are of both economic and ecological importance. Germination is considered one of the most critical sages in the life cycle of the plants, since it is under the influence of several biotic and abiotic factors. The process of germination begins with the water uptake of the mature dry seed and ends with the emergence of the radicle from the seed coat (Rajjou et al., 2012). SNP markers identify the locus resulting from single nucleotide differences when two alleles are observed in a population. SNPs represent the most common type of sequence polymorphism in plant and animal genomes. SNP markers have become widely used in many plant species due to

their stability, ease of use, extremely low mutation rate, and high genotyping capacity. The use of SNPs allows for the easy selection of many traits in a large breeding population, turning selection into profit with less risk. Today, many molecular plant-breeding programs are based on SNP markers, and germplasm, gene sources represent the power of genetic interactions essentially to differences in the genome in mapping genes that control certain traits in breeding lines (Morgil et al., 2020).

The complex phenotypic variations or background architecture of many agricultural traits are effected by different environments. Association mapping, also known as linkage disequilibrium (LD), is a powerful and effective tool for revealing the inheritance mechanism of complex traits in identifying candidate genes responsible for the association between phenotypic and genotypic data by revealing historical and ancestral recombination events at the population level. As a new alternative approach to traditional relationship mapping, association mapping has three advantages: 1) increased map density, 2) natural linkage with a wide and unique diversity without the need for the more costly and effort-intensive mapping populations (F₂, BC and RIL) used in traditional linkage

analyses, 3) greater allele diversity (Yu and Buckler, 2006). Based on the size and point of interest of a particular study, association mapping falls into two subcategories. These approaches are candidate gene-based association mapping, which associates causal polymorphisms in candidate genes that are stated to be involved in controlling phenotypic variation for specific traits, and genome-wide association studies known as GWAS, which search for genetic variation in the whole genome to find association peaks and points for various traits.

Association mapping is an application that focuses on LD to examine the relationship between phenotypic variation and genetic polymorphism (Mackay, 2001). Association mapping uses large mixed samples from the natural population sets, which are populations developed from multi-parental hybrids of related species, or collections of breeding material, including cultivars. Samples should be capable of as much genetic variation as the current population collection is useful in practice. In this study, it was aimed i) to reveal germination and early seedling stage traits of Turkish origin oat genotypes obtained from various gene banks, ii) to find out relationships among investigated traits with principal component analysis, iii) to determine candidate markers related with germination traits via association mapping analysis using 6K SNP Chip assay.

MATERIALS AND METHODS

Plant Materials

In the study, 167 local oat genotypes across Turkey obtained from USDA-ARS-National Gene Bank (United States Department of Agriculture- Agricultural Research Service, National Small Grains Collection), and four commercial cultivars, Arslanbey, Kahraman, Kirkklar and Yeniceri were used as plant materials. The pedigree of the landraces were presented in Comertpay et al. (2018).

SNP Genotyping

Single nucleotide polymorphism (SNP) markers developed by Oliver et al. (2011) were used for association mapping analysis in current study. The 6K SNP Chip was applied using Illumina Infinium Technology by General Mills, Inc., Minneapolis, MN, and USDA-ARS. Details about SNP development might be launched in former studies (Oliver et al. 2011; 2013)

Phenotyping

The research was arranged in an augmented experimental design with six replications of commercial cultivars in order to examine germination and some early seedling stage traits under laboratory conditions and to identify candidate markers related to these characteristics in 2020. For this purpose, seeds were surface sterilized with 1% NaOCl (sodium hypochlorite) solution. Twenty-five seeds of each genotype were placed into double-layer sterile filter papers in autoclaved petri dishes and put in the culture room at 25 °C for 8 days for germination.

Seeds were considered germinated when the radicle of the seeds reached at least 2 mm.

Germination rate was measured in the 4th day of the experiment; on the 8th day; germination and some early seedling stage characteristics such as germination ratio, coleoptile length, plumula length, radicle length, seed vigor index were phenotyped and the data were recorded.

Data Analysis

The phenotypic data were subjected to analysis of variance (F test) according to the augmented experiment design. The Duncan test was used to compare mean data. Principal component values were examined using the biplot analysis approach over the average data of the features. All phenotypic data analysis was performed using the JMP 15.1 statistical package program (SAS Institute Inc, 2020). Significance was determined as 5% likelihood level unless else indicated.

GAPIT Version 2 (Tang et al., 2016) software was used to determine the relationship between some germination characteristics in oats and SNP markers in the 6K chipset. In the study, those with a minor allele frequency value of less than 5% (MAF < 0.05) were extracted and high quality robust SNPs were obtained. Heterozygosity frequency was calculated both between individuals and between markers. Afterwards, in the same program, the marker density, which is an important criterion for calculating the LD (linkage disequilibrium) between the markers, was determined, and the comparison between the LD decay at physical distance and the marker density was presented in the plot graphic.

In the LD analysis, the r^2 value of the LD decrease, which indicates the mutual mutation and recombination of the markers with each other, was calculated in the spread graph. In the association study, PCA + K data was used in the Compressed Mixed Linear Model (CMLM) method, which gathers the genotypes into groups and links genetic values of clusters as random effects in the design, thus promotes statistical effectiveness and saves time on large examples, and candidate gene mapping analysis was performed (Zhang et al., 2010). Because of association analysis, the genomic position of the markers on different chromosomes on the x-axis, the logarithmic ($-\log_{10}(p)$) representation of the p-values on the y-axis are presented in the Manhattan plot. In addition, following the method of VanRaden (2008), a heat map showing the genetic and kinship relationship based on the kinship matrix of the genotype individuals and observed and expected and observed a quantile-quantile (Q-Q) plot was created.

RESULTS AND DISCUSSION

Phenotypic Traits

The phenotypic data obtained as a result of the study carried out to determine the performance of local oat genotypes on germination and some early seedling stage traits examined were statistically evaluated and the data for these traits are shown as histogram (Figure 1). The

variation in local oat genotypes was found significant at the 5% significance level, except for germination rate.

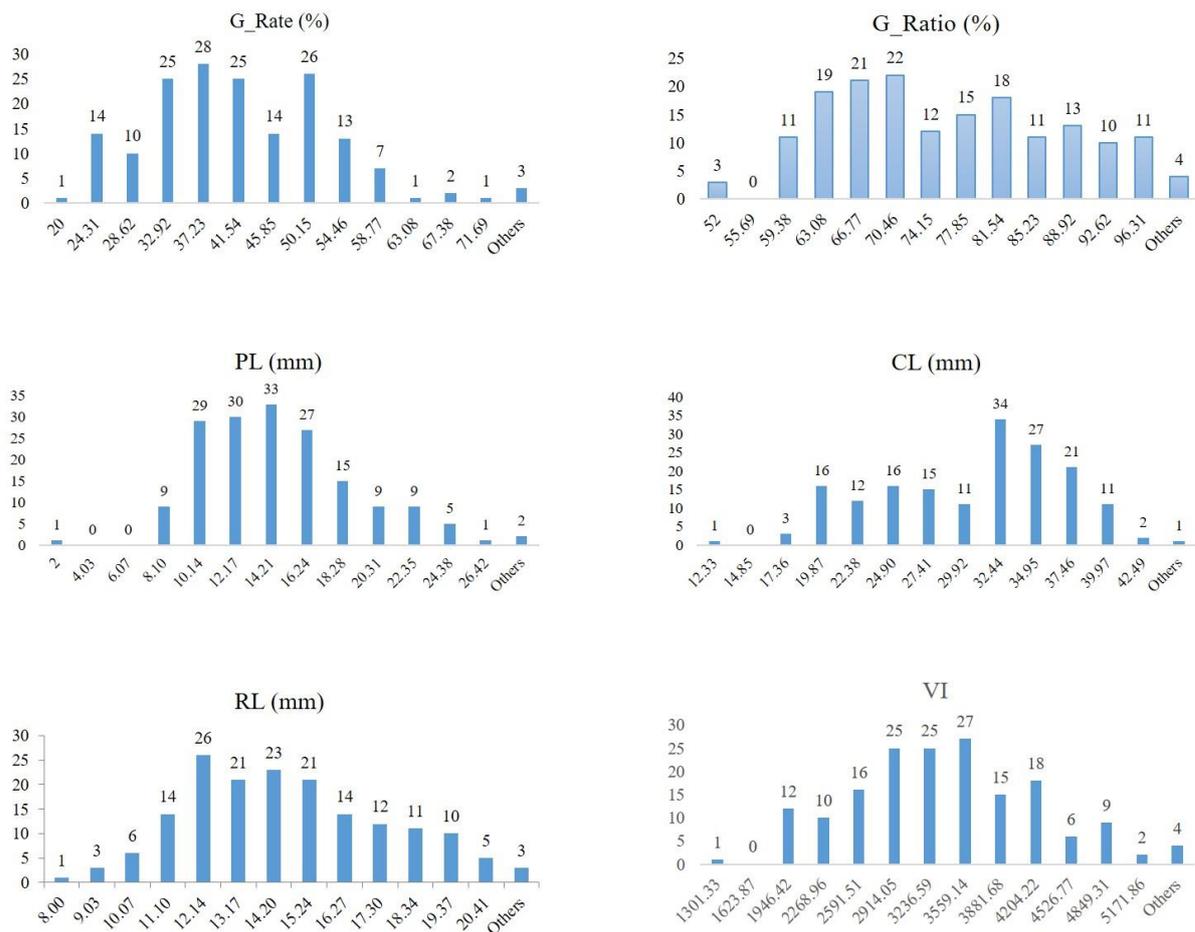


Figure 1. A histogram was created for investigated traits of oat genotypes

Germination rates of local genotypes varied between 20-76%, and between 39-43% among the control varieties (Figure 1). While TL97 reached the highest germination rate value (76%), TL55 got the lowest value (20%). Akyol (2014) reported that there was a significant variation among oat cultivars in terms of germination rate and the germination rate was significantly low, as they require a long period to germinate. Germination ratio among local oat genotypes were found between 52-100% and 80-83% among the control varieties (Figure 1). Genotypes TL10, TL11 and TL149 had the highest germination ratio values (100%), while TL113, TL119 and TL252 had the lowest values (52%). Dumlupinar et al. (2011b) reported significant variations in oat germination ratio and they reported that the hull rates and other seed characteristics of the genotypes played an important role on germination and emergence rates after sowing. Coleoptile length values varied between 12.33-45.00 mm and between 23.65-25.95 mm among the control cultivars (Figure 1). While TL53 genotype reached the highest coleoptile value with 45 mm, TL7 genotype had the lowest value with 12.33 mm. Radicle length among tested local oat genotypes varied between 8.00 and 21.44 mm, and between 12.72 and 13.49 mm in control cultivars.

Among the oat genotypes, TL343 had the longest radicle length with 21.44 mm, while TL7 genotype had the shortest radicle length with 8.00 mm. Oner et al. (2018) reported that radicle length showed a significant change depending on to the severity of stress conditions. Plumula length values were observed between 2.00-28.45 mm, and between 14.79-17.13 mm in control cultivars. While TL326 genotype had the highest plumula length with 28.45 mm, TL128 genotype had the lowest value with 2 mm. Oner et al. (2018) found significant variations in oat genotypes in terms of plumula length, in agreement with our results. Seed vigor index ranged from 1301.33-5360.00 and 2986.26-3171.88 among the control varieties (Figure 1). In terms of seed vigor indices, TL149 genotype had the highest indices with 5360, while TL 7 genotype had the lowest with 1301.33. Gungor et al. (2017) stated that oat genotypes showed significant differences in terms of seed vigor index, and they reported that the cultivar Yeniceri had the highest seed vigor index.

Principal Component Analysis

Principal component (PC) biplot analysis plays an important role in selecting genotypes with superior

adaptability in plant breeding programs, but they also actively benefit not only in the adequate evaluation of genotypes, but also in determining the positive or negative relationships of traits with each other. (Yan and Tinker, 2006). In our study, the PC1 value was found 41.5% and the PC2 value was 21.5%, explaining 63% of the total variation. It was determined that there were strong positive relationships between germination rate and germination ratio, radicle length and coleoptile length,

seed vigor index and coleoptile length, and between seed vigor index and radicle length in the local oat genotypes investigated in the study. In addition, significant and negative correlations were determined for plumula and coleoptile lengths, and plumula and radicle lengths (Figure 2). Gungor et al. (2021) stated a 50.3% of the total variation, with 33.9% PC1 and 16.4% PC2 values, which is consistent with our findings.

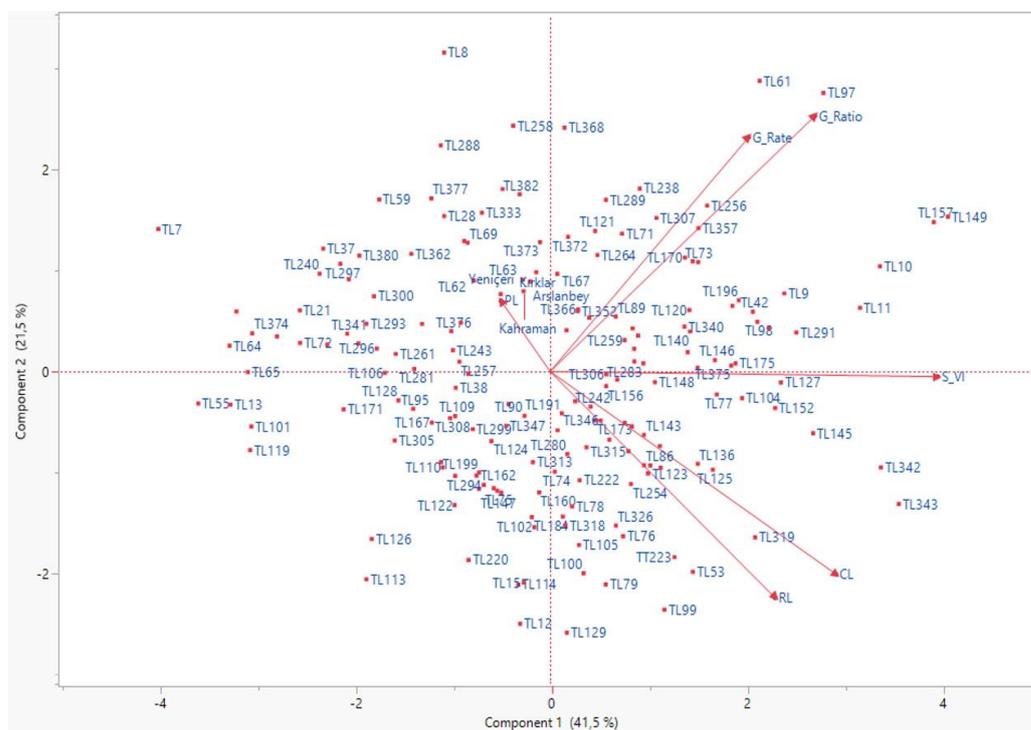


Figure 2. Principal component biplot analysis of oat genotypes and investigated traits

Association Mapping Analysis

In the study, the 4599 SNP markers obtained from 6K SNP assay were filtered down to 2306 SNP markers and the heterozygosity frequencies of both were determined. (Figure 3a). Next, the marker density, which is an important criterion for calculating the LD between marker pairs, was tested and the histogram plot showed whether the markers were adequately suited to a good LD density by comparison between the marker density and LD degradation (Figure 3b). When the distribution states of the SNPs in the spread graph are examined, their frequencies continue in an increasing trend after 1000 and in 2000; the accumulation frequencies were determined to start from 0.6 and end at 1.0. In order to determine the population structure, the eigenvalue, where the PC value is determined as optimal K, by entering the R scripts with the current parameter settings in GAPIT, the heat map plot based on the kinship matrix was created with the EMMA algorithm based on the model of 3D PC and Van Raden (2008). While there was a significant change in the break point of the 4th PC from the variance components, this

situation showed that the first 4 main components reflected the accumulation frequency well and explained 50% of the variance components (Figure 3c). In the 3D PC pilot, it was determined that a significant part of the genotypes were tightly clustered close to PC2, while the other parts were located on the left side close to PC3 and diverged from each other (Figure 3d). Considering the mating status of genotypes in the bidirectional kinship matrix; It was determined that the population structure was divided into 4 different groups considering their ancestral origins, and a similar result was obtained from the heat map of the kinship matrix analysis (Figure 4). Wang et al. (2017) observed a significant change in the first 8 PC values of the variance components in the population structure and kinship analyzes performed as a result of genotyping with 90K Illumina SNP markers. It was determined that their findings were different from ours by revealing that their wheat genotypes were separated into different clusters in the three-dimensional plot and that the panel was classified according to three groups in the dendrogram of the heat map of the kinship matrix.

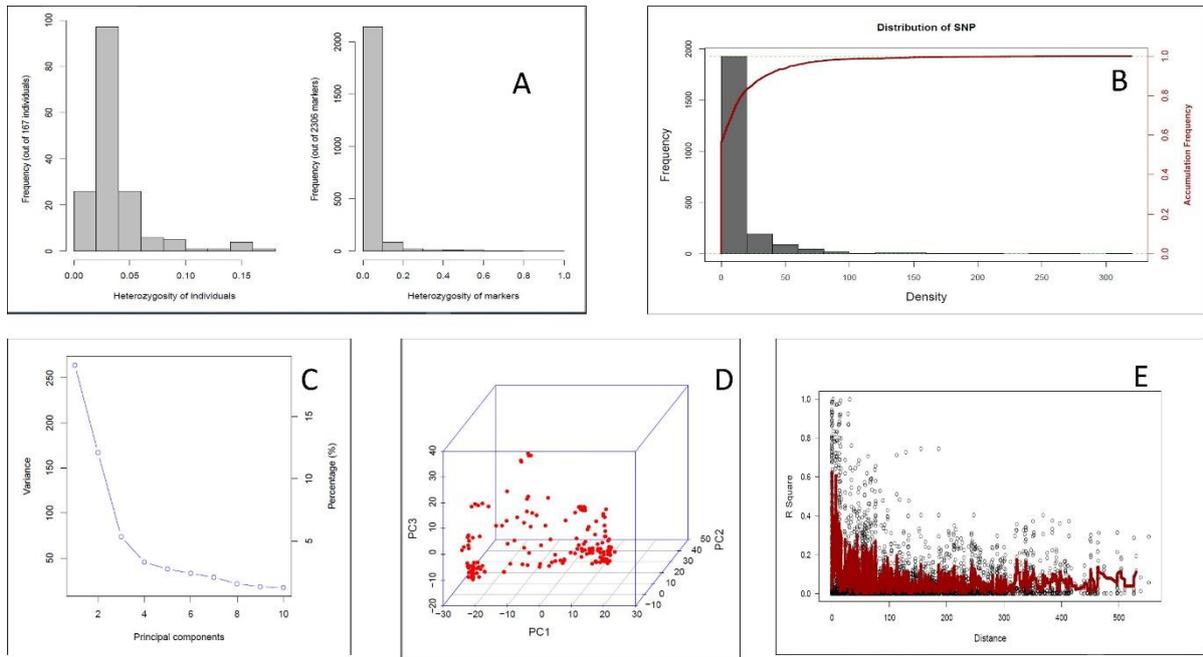


Figure 3. Marker properties of SNP markers (a) Heterozygosity frequencies of 6K SNP chip markers and 167oat individuals, (b) Histogram of marker densities and degradations, (c) principal components of the marker variables, (d) 3D principal components of the SNP markers, (e) graphic of the LD degradations

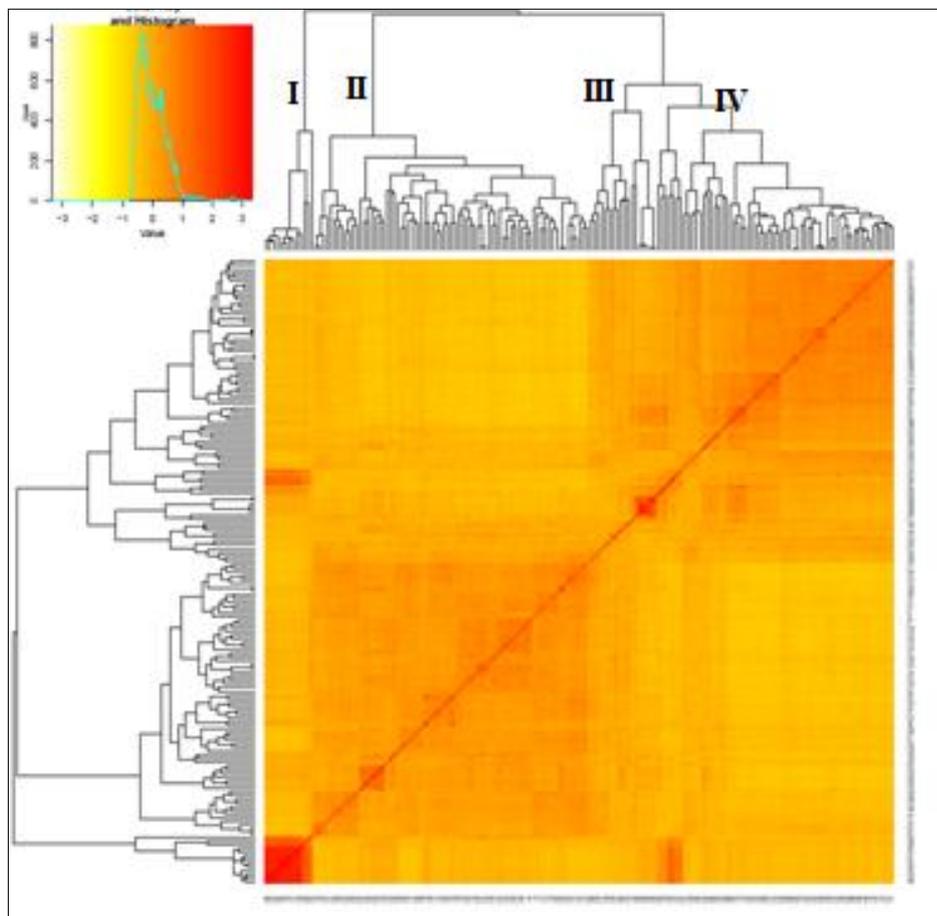


Figure 4. Heat map of genetic and kinship relationship based on the kinship matrix

The concept of LD, first described by Jennings in 1917 and first expressed numerically by Lewtonin in 1964, is a phenomenon that describes the non-random relationship of alleles at different loci in individuals in a population (Abdurakhmonov and Abdukarimov, 2008). Genetic drift, selection, mutation rate, autogamy, epistatic interaction, genetic isolation, population size, genomic rearrangements in chromosomes are important factors responsible for LD increase, as well as being followed in an attitude that varies from population to population, from individual to individual, from species to species. It has different traces as a shareholder in the success of association mapping (Flint-Garcia et al., 2003; Nadeem et al., 2018).

In the spread graph of LD degradation shown here, the coverage of the SNP markers in the area covered and their relationship with each other might be seen. The LD decrease was found to average when it fell to half of the maximum r^2 value. In the research findings, the LD decreased from 0.6 to 0.3 at a short distance (Figure 3e).

While establishing the relationship between phenotype and genotype in candidate gene association mapping analysis, the single locus-based compressed mixed linear model statistical method, which is one of the most popular methods reduces the type-I error (false positives) caused by kinship and population structure. As it is a common problem, and ensures the reliability of the test with the inevitability of a large amount of computational power, it has opened the door to the search for different algorithms (Sakiroglu, 2020). By taking the average of the data of the six traits examined in the study, the correlation analysis was carried out in the CMLM model. The candidate markers associated with each trait, the locations of the QTLs in the chromosomal regions were observed in the Manhattan plot graphs. The observed and expected P-values were presented in Q-Q plot graph (Figure 5). The significance threshold range of each of these markers was determined on the basis of $-\log_{10}(p) \geq 2.50$, and a false discovery rate (FDR) described by Benjamini and Hochberg (1995) was used to determine statistical significance threshold ($\alpha=0.05$) using SAS Multtest procedure (SAS Institute Inc, 2020).

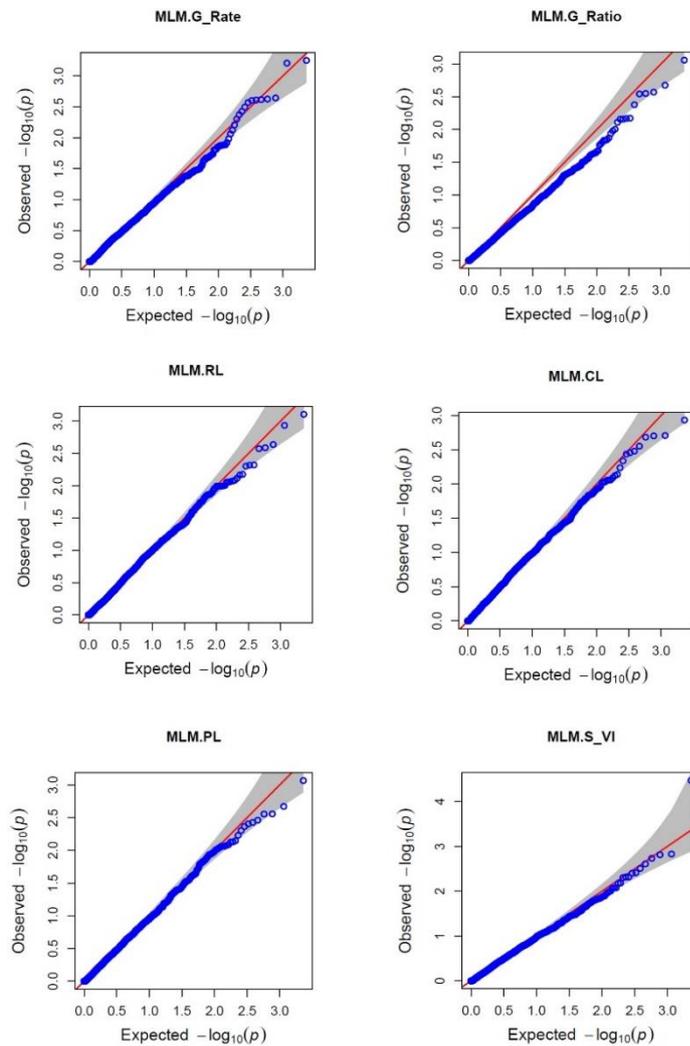


Figure 5. Q-Q Plot of the observed and expected $-\log_{10}(p)$ values

As a result of the candidate gene association analysis conducted out with the CMLM model using the mean data for the germination rate of the germination traits, eight candidate markers (values $-\log_{10} \geq 2.50$ and $p < 0.01$) were determined (Table 1 and Figure 6a). The phenotypic variance (r^2) of the germination rate, which explains the percentage of the marker, varied between 0.10 and 0.12, and the minor allele frequency (MAF) value between 0.01 and 0.40 for all markers (Table 1). The candidate markers were located on chromosomes 19, 15, 6, 11, 6, 8, 11 and 6 located in positional distances of 221, 164, 1023, 88, 1023, 1628, 37 and 1002 cM longs, respectively.

GMI_DS_LB_1076 marker was taken place in Mrg19 linkage group which is consisted with Chaffin et al. (2016). The GMI_GBS_50325 marker was localized in the Mrg15 linkage group, which was positioned in the Mrg15 linkage group in framework consensus map and in the Mrg11 linkage group in oat reference consensus map (Bekele et al., 2018). The locations of candidate markers previously reported that associated with germination rate in Mrg6, Mrg8, Mrg11, Mrg15 and Mrg19 linkage groups were also in harmony with our findings (Lin et al., 2014; Chaffin et al., 2016; Brooke, 2017; Bekele et al., 2018).

Table 1. Associated loci with germination and root traits identified in 167 local oat genotypes

	SNP	Chromosome	Position	P Value	r^2	MAF	FDR
Germination Rate	GMI_DS_LB_1076	19	221	0.00562	0.12	0.03	0.0031
	GMI_GBS_50325	15	164	0.00622	0.11	0.24	0.0031
	GMI_ES14_c2965_193	6	1023	0.002275	0.10	0.06	0.0033
	GMI_ES02_c24507_561	11	88	0.002364	0.10	0.22	0.0033
	GMI_ES_LB_9817	6	1023	0.002415	0.10	0.05	0.0033
	GMI_GBS_13916	8	1628	0.002454	0.10	0.12	0.0033
	GMI_ES14_c5428_351	11	37	0.002503	0.10	0.40	0.0033
	GMI_GBS_95238	6	1002	0.002694	0.10	0.01	0.0033
Germination Ratio	GMI_ESI_c12749_234	21	1720	0.000875	0.07	0.43	0.0028
	GMI_ES05_c1006_442	23	232	0.00212	0.06	0.17	0.0028
	GMI_ES05_c2760_657	1	866	0.002698	0.06	0.41	0.0028
	GMI_GBS_95238	6	1002	0.00283	0.06	0.01	0.0028
	GMI_ES14_lrc18344_662	20	2491	0.002873	0.06	0.46	0.0028
Radicle Length	GMI_ES_LB_11026	20	2035	0.000782	0.11	0.46	0.0026
	GMI_ES_LB_11028	20	2035	0.001162	0.11	0.45	0.0026
	GMI_ES01_c10216_255	13	714	0.002302	0.10	0.46	0.0026
	GMI_ES05_c2715_265	20	2051	0.002573	0.10	0.48	0.0026
	GMI_GBS_50940	9	983	0.002665	0.10	0.41	0.0026
Coleoptile Length	GMI_ES15_c5315_156	6	1045	0.001165	0.08	0.14	0.0036
	GMI_ES_15_lrc19156_98	18	231	0.001961	0.07	0.41	0.0036
	GMI_ES22_c9827_183	33	380	0.001986	0.07	0.26	0.0036
	GMI_DS_LB_9600	23	1106	0.002074	0.07	0.07	0.0036
	GMI_GBS_78545	8	814	0.002805	0.07	0.13	0.0036
Plumula Length	GMI_DS_LB_4204	21	777	0.000858	0.11	0.06	0.0039
	GMI_ES01_c3435_183	5	1251	0.002128	0.10	0.26	0.0039
	GMI_ES15_c1855_452	8	1296	0.002768	0.10	0.18	0.0039
	GMI_DS_LB_10835	21	777	0.002786	0.10	0.11	0.0039
Seed Vigor Index	GMI_ESI_c12749_234	21	1720	3.39E-05	0.11	0.43	0.00027
	GMI_ES22_c7747_621	21	1747	0.001483	0.06	0.39	0.0037
	GMI_ES14_lrc18344_662	20	2491	0.001536	0.06	0.46	0.0037
	GMI_ES22_c9230_196	33	820	0.00185	0.06	0.21	0.0037
	GMI_GBS_95238	6	1002	0.002459	0.05	0.01	0.0039
	GMI_ES_CC10682_318	21	1783	0.003098	0.05	0.23	0.0039

r^2 : Variation explained by the marker, P Value: Significant threshold of the markers, MAF: Minor Allele Frequency, FDR: False Discovery Rate

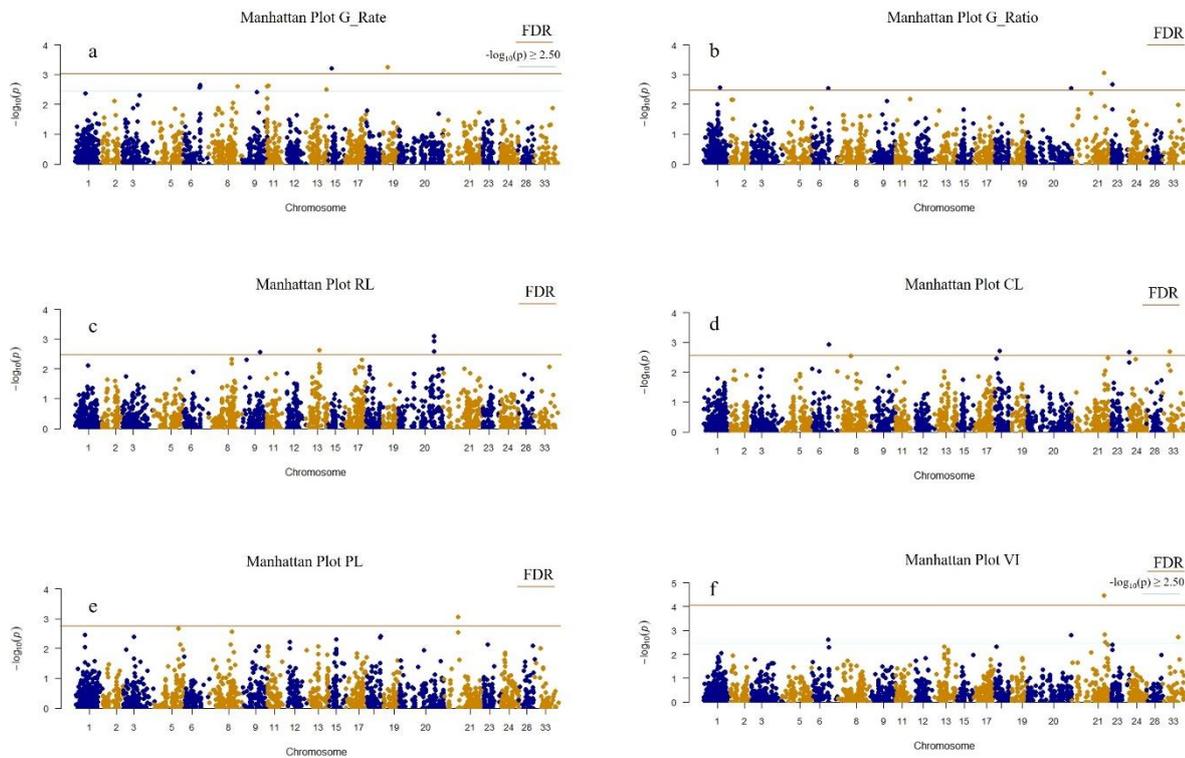


Figure 6. Manhattan plots for germination and root traits using the compressed mixed linear model (CMLM). The orange horizontal lines represent the false discovery rate (FDR) of 5%. (a) Germination rate (FDR=0.0031), (b) germination ratio (FDR=0.0028), (c) radicle length (FDR=0.0026), (d) coleoptile length (FDR=0.0036), (e) plumula length (FDR=0.0039), (f) seed vigor index (FDR=0.0027).

Five candidate markers at $-\log_{10} \geq 2.50$ and $p < 0.01-0.001$ were determined for germination ratio (Table 1 and Figure 6b). The phenotypic variance (r^2) of the germination ratio, changed between 0.06 and 0.07, and the MAF value was between 0.01 and 0.46 for all markers. The candidate markers were located on chromosomes 21, 23, 1, 6 and 20 fell into positional distances of 1720, 232, 866, 1002 and 2491 cM longs, respectively (Table 1). Of the candidate markers, GMI_ES01_c12749_234 was located in the Mrg21, GMI_ES05_c2760_657 was located in Mrg01, and GMI_GBS_95238 was located in Mrg06 linkage group. Those findings are in consistent with the previous works, while GMI_ES05_c1006_442 took place in the Mrg23 in our study in contrast to previously reported Mrg05 linkage group maps (Lin et al., 2014; Chaffin et al., 2016; Bekele et al., 2018). On the other hand, there was no report for the GMI_ES14_lrc18344_662 candidate marker.

In terms of radicle length, five candidate markers at the significance of $-\log_{10} \geq 2.50$ and $p < 0.01-0.001$ were determined via association analysis (Table 1 and Figure 6c). The phenotypic variance (r^2) ranked between 0.10 and 0.11, and the MAF value ranged from 0.41 and 0.48 for all markers (Table 1). These candidate markers were located on chromosomes 9, 13 and 20 and with the positional distances of 2035, 2035, 714, 2051 and 983 cM longs. GMI_ES_LB_11026, GMI_ES_LB_11028 and GMI_ES05_c2715_265 markers were located in Mrg20 linkage group in consistent with Oat Consensus map 2018 (Bekele et al., 2018). GMI_ES01_c10216_255 marker is

located in Mrg13 as reported by Chaffin et al. (2016). GMI_GBS_50940 marker was also reported in Oat Consensus maps 2016 and 2018 located in Mrg9 linkage group, which is similar with our results (Chaffin et al., 2016; Bekele et al., 2018).

Five candidate markers ($-\log_{10} \geq 2.50$ and $p < 0.01$) were determined for coleoptile length (Table 1 and Figure 6d). The phenotypic variance (r^2) for the coleoptile length was determined between 0.06 and 0.08, and the MAF value between 0.03 and 0.41 for all markers. Those candidate markers were located on chromosomes 6, 18, 33, 23 and 8 positional distances were 1045, 231, 380, 1106 and 814 cM longs, respectively.

The GMI_ES15_c5315_156 candidate marker is located in Mrg06, GMI_ES22_c9827_183 in Mrg18, GMI_DS_LB_9600 in Mrg23 and, GMI_GBS_78545 in Mrg8 linkage groups, which were also previously reported (Oat-2014-CrownRust, Oat-2016-AxM, Oat-2016-Consensus, Oat-2016-PxB, Oat-2018-Consensus, 1045 cM long) (Lin et al., 2014; Chaffin et al., 2016; Bekele et al., 2018). On the other hand, GMI_ES_15_lrc19156_98 marker located in Mrg18 was not reported previously in the literature.

Four candidate markers ($-\log_{10} \geq 2.50$ $p < 0.01-0.001$) were determined as a result of the candidate gene association mapping analysis for plumula length (Table 1 and Figure 6e). The phenotypic variance (r^2) varied between 0.10 and 0.11, and the MAF value between 0.06 and 0.26 for all markers (Table 1). The candidate markers

are located on chromosomes 21, 5, 8, and 21 and fell into positional distances of 777, 1251, 1296 and 777 cM longs respectively. GMI_DS_LB_4204 candidate marker was located in the Mrg21 linkage group (Lin et al., 2014; Chaffin et al., 2016; Bekele et al., 2018). Kilinc (2020) reported a pleiotropic effect for GMI_DS_LB_4204 on vegetative period and days to maturity in oat. GMI_ES01_c3435_183 marker was located in Mrg05 linkage group, which is indicated by Chaffin et al. (2016) related with *P_{C71}* gene. Mrg05, Mrg08 and Mrg21 linkage groups related to plumula length were detected in our study, which is also reported by Mohler, (2021). GMI_ES15_c1855_452 (Mrg8) and GMI_DS_LB_10835 (Mrg21) markers were also indicated in Oat Consensus Map 2018 (Bekele et al., 2018).

Six candidate markers ($-\log_{10} \geq 2.50$ and $p < 0.01-0.0001$) were determined for the seed vigor index (Table 1 and Figure 6e). The phenotypic variance (r^2), varied between 0.05 and 0.11, and the MAF value between 0.01 and 0.46 for all markers (Table 1). The candidate markers are located on chromosomes 21, 21, 20, 33, 6 and 21 with the positional distances of 1720, 1747, 2491, 820, 1002 and 1783 cM longs, respectively. The candidate marker GMI_ES01_c12749_234, GMI_ES22_c7747_621 and GMI_ES14_lrc18344_662 were located in the Mrg21, Mrg21 and Mrg20 linkage groups, respectively and, they were presented in previously published oat reference consensus maps, which is in agreement with our results (Oat-2014-CrownRust, Oat-2016-AxM, Oat-2016-Consensus, Oat-2018-Consensus, 1720 cM long) (Lin et al., 2014; Bekele et al., 2018). GMI_ES22_c9230_196 and GMI_GBS_95238 markers were located in the same linkage groups (Mrg33 and Mrg6, respectively) which was reported by Chaffin et al. (2016), while GMI_ES_CC10682_318 marker was located in Mrg21 linkage group, which was also reported by Kilinc (2020).

In addition, Huang et al. (2020) reported robust approach associated (FarmCPU) with seed vigor traits in two different locations by establishing a new phenotyping pipeline in 650 elite oat breeding lines from CORE (Collaborative Oat Research Enterprise) material. They determined the candidate marker and QTL for the first time by GWAS mapping based on strong statistical method, 2 of 41 SNP genomic regions determined for root traits affecting seed vigor were not mapped, other markers were distributed in 16 linkage groups, 16 associated with stem traits, which are other seed vigor factor, were not mapped. They stated that although one of the SNPs could not be mapped, the other markers fell into 10 linkage groups.

CONCLUSION

As a result of the principal component and biplot analysis based on the average data of the examined traits and genotypes, the PC1 component value was determined as 41.5% and the PC2 component value was determined as 21.5%, and it was determined that it explained 63% of the total variation, reflecting a value above the average. In terms of germination parameters examined in oat

genotypes, strong positive relationships were determined between germination rate and germination ratio, radicle length and coleoptile length, seed vigor index and coleoptile length and radicle length.

According to candidate gene association mapping analysis performed with the CMLM model, 33 genomic regions were identified consisted of eight QTLs associated with germination rate, five QTLs associated with germination ratio, five QTLs associated with radicle length, five QTLs associated with coleoptile length, four QTLs associated with plumula length, and six for seed vigor index. Some of these QTLs are similar to those in the studies in the literature, and newly identified genomic regions have also been found for the first time. As a result, the candidate markers of these QTLs determined in the study were validated in future studies both in multi-field trials and with the currently developed multi-locus model algorithms and integrated into approaches such as haplotype analysis and genomic selection by determining whether they are true QTLs.

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Conflict of Interest

The authors declare no conflicts of interest.

LITERATURE CITED

- Abdurakhmonov, I., A. Abdukarimov. 2008. Application of association mapping to understanding the genetic diversity of plant germplasm resources. *International Journal of Plant Genomics* 2008:1-18.
- Akyol, Z. 2014. The effect of different pH level and soaking time on germination of oats types. MSc Thesis. Kahramanmaraş. pp. 50.
- Bekele, W.A., C.P. Wight, S. Chao, C.J. Howarth, N.A. Tinker. 2018. Haplotype-based genotyping-by-sequencing in oat genome research. *Plant Biotechnology Journal* 16: 1452-1463.
- Benjamini, Y., Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal statistical society: series B, Methodological* 57(1): 289-300.
- Brooke, J. 2017. Genetic analysis of loose smut (*Ustilago avenae* Pers. (Rostr.)) resistance in oat. Master Thesis. University of Saskatchewan Plant Science. Saskatchewan. pp 123.
- Chaffin, A.S., Y.F. Huang, S. Smith, W.A. Bekele, E. Babiker, B.N. Gnanesh, B.J. Foresman. 2016. A consensus map in cultivated hexaploid oat reveals conserved grass synteny with substantial sub-genome rearrangement. *Plant Genome* 9(2):1-21. doi: 10.3835/plantgenome2015.10.0102.
- Comertpay, G., E. Habyarimana, F.S. Baloch, H. Gungor, T. Dokuyucu, A. Akkaya, Z. Dumlupinar. 2018. Geographical description and molecular characterization of genetic structure and diversity using a 6K SNP array in Turkish oat germplasm. *Canadian Journal of Plant Science* 99(1): 12-21.

- Dumlupinar, Z., H. Maral, R. Kara, T. Dokuyucu, A. Akkaya. 2011a. Evaluation of Turkish oat landraces based on grain yield, yield components and some quality traits. *Turkish Journal of Field Crops* 16: 190-196.
- Dumlupinar, Z., T. Dokuyucu, A. Akkaya. 2011b. Determination cold tolerance of Turkish and USA origin oat genotypes under Kahramanmaraş-Afsin condition. *KSU J. Nat. Sci.* 14(2): 35-42.
- Flint-Garcia, S.A., J.M. Thornsberry, E. Buckler. 2003. Structure of linkage disequilibrium in plants. *Annual Review of Plant Biology* 54(1): 357-374.
- Gungor, H., Y. Cikili, Z. Dumlupinar. 2017. The effects of salt stress on germination and seedling development of some commercial and landraces of oat genotypes. *KSU J. Nat. Sci.* 20 (Special Issue): 263-267.
- Gungor, H., Y. Cikili, Z. Dumlupinar. 2021. Screening of oat varieties and landraces at early vegetative stage under salt stress conditions: Morpho-physiological and PCA biplot analysis. *Cereal Research Communications* 49(4): 587-597.
- Huang, C.T., K.E. Klos, Y.F. Huang. 2020. Genome-wide association study reveals the genetic architecture of seed vigor in oats. *G3 (Bethesda)* 10(12): 4489-4503.
- JMP. 2020. Version 15.1. SAS Institute Inc., Cary, NC, 1989–2020.
- Kilinc, M.F. 2020. Association mapping analysis and determination of some agronomical traits for Turkey origin oat genotypes. PhD Thesis Kahramanmaraş. pp. 225.
- Lin, Y., B.N. Gnanesh, J. Chong. 2014. A major quantitative trait locus conferring adult plant partial resistance to crown rust in oat. *BMC Plant Biol.* 14: 250.
- Mackay, T. 2001. The genetic architecture of quantitative traits. *Annu. Rev. Genet.* 35: 303-339.
- Mohler, V. 2021. Allocation of the oat powdery mildew resistance gene Pm3 to oat chromosome 1A. *Cereal Research Communications*. 1-8.
- Morgil, H., Y.C. Gercek, I. Tulum. 2020. Single nucleotide polymorphisms (SNPs) in plant genetics and breeding. In *The Recent Topics in Genetic Polymorphisms*. IntechOpen. pp. 137-145.
- Nadeem, M.A., M.A. Nawaz, M.Q. Shadid, Y. Dogan, G. Comertpay, G. Chung, F.S. Baloch. 2018. DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment* 32(2): 261-285.
- Oliver R.E., G.R. Lazo J.D. Lutz, M.J. Rubenfield, N.A. Tinker J.M. Anderson, N.H. Wisniewski Morehead, D. Adhikary, E.N. Jellen, P.J. Maughan, G.L. Brown Guedira, S. Chao, A.D. Beattie, M.L. Carson, H.W. Rines, D.E. Obert, J.M. Bonman, E.W. Jackson. 2011. Model SNP development for complex genomes based on hexaploid oat using high-throughput 454 sequencing technology. *BMC Genomics* 12: 77.
- Oliver R.E., N.A. Tinker, G.R. Lazo, S. Chao, E.N. Jellen, M.L. Carson, H.W. Rines, D.E. Obert, J.D. Lutz, I. Shackelford, A.B. Korol, C.P. Wight, K.M. Gardner, J. Hattori, A.D. Beattie, A. Bjørnstad, J.M. Bonman, J.L. Jannink, M.E. Sorrells, G.L. Brown-Guedira, J.M. Fetch, S.A. Harrison, C.J. Howarth, A. Ibrahim, F.L. Kolb, M.S. McMullen, J.P. Murphy, H.W. Ohm, B.G. Rossnagel, W. Yan, K.J. Miclaus, J. Hiller, P.J. Maughan, R.R. Redman Hulse, J.M. Anderson, E. Islamovic, E.W. Jackson. 2013. SNP discovery and chromosome anchoring provide the first physically-anchored hexaploid oat map and reveal synteny with model species. *PLoS ONE* 8(3): e58068.
- Oner, F., F. Ozkorkmaz, N. Yilmaz. 2018. The effect of gibberellic acid applications on some germination parameters in oats under salt stress. *International Journal of Agricultural and Natural Sciences* 1(1): 33-35.
- Rajjou, L., M. Duval, K. Gallardo, J. Catusse, J. Bally, C. Job, D. Job. 2012. Seed germination and vigor. *Annu. Rev. Plant Biol.* 63: 507-533.
- Sakiroglu, M. 2020. Bioinformatic methods for Genomic analysis. ISBN: 978-605-282-541-9, Turkey, pp 245.
- SAS. 2020. Version 9.3. SAS Institute Inc., Cary, NC, 1989–2020.
- Tang, Y., X. Liu, J. Wang, M. Li, Q. Wang, F. Tian. 2016. GAPIT Version 2: an enhanced integrated tool for genomic association and prediction. *Plant Genome* 9:0120. <http://dx.doi.org/10.3835/plantgenome2015.11.0120>.
- VanRaden, M.P. 2008. Efficient methods to compute genomic predictions. *J. Dairy. Sci.* 4414-4423. <http://dx.doi.org/10.368/jds.2007-0980>.
- Wang, S., X., Zhu, Y.L. Zhang, D.X. Shao, H. Liu, P. Hu. 2017. Genome-wide association study for grain yield and related traits in elite wheat varieties and advanced lines using SNP markers. *PLoS One*, 12(11): e0188662. <https://doi.org/10.1371/journal.pone.0188662>.
- Yan, W., N.A. Tinker. 2006. Biplot analysis of multi-environment trial data: Principles and applications. *Can. J. Plant. Sci.* 86: 623-645. <http://dx.doi.org/10.4141/P05-169>.
- Yu, J., E.S. Buckler. 2006. Genetic association mapping and genome organization of maize. *Curr. Opin. Biotechnol* 17: 155-160.
- Zhang, Z., E. Ersoz, C.Q. Lai, R.J. Todhunter, H.K. Tiwari, M.A. Gore, 2010. Mixed linear model approach adapted for genome-wide association studies. *Nat. Genet.* 42: 355–360. doi: 10.1038/ng.546