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RESEARCH

Identification of Molecular Markers Associated with Genomic Regions Controlling Agronomic Traits in Bread Wheat Genotypes Under Different Moisture Conditions

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Abstract

The study of the association between polymorphism at the DNA level and the diversity of phenotypic traits is an essential tool in breeding programs. To identify informative microsatellite markers related to agronomic traits, this research including 25 bread wheat genotypes was carried out. The experiment was set up in a randomized complete block design with three replications in rainfed and irrigated conditions during two cropping seasons (2018–2020) in the cold Mediterranean climate of Iran. Variance analysis showed significant differences between genotypes for most of the traits. The 16 microsatellite primers out of 20 had considerable polymorphisms, and three markers, namely XCFD168-2D, XGWM350-7D, and XGWM136-1A, were introduced as the most significant markers for subsequent studies. Cluster analysis by the UPGMA method classified 25 wheat genotypes into four groups. Genotypes 1, 3, and 25 have the most significant genetic distance with genotypes 13, 7, and Pishgam. Association analysis by stepwise regression showed that in both years under rainfed conditions, the XGWM350 marker for 1000-grain weight, the XCFD5 marker for spike length, and the XGWM165 and XGWM70 markers for spike dry weight, and under irrigated conditions, the XGWM265 marker for grain yield exhibited significant associations. Also, the XGWM136 and XCFD5 were found to be common markers associated with agronomic traits for all the test environments. In addition, most of the markers were associated with 1000-grain weight, mitt penalty length, and spike grain weight in rainfed conditions and 1000-grain weight in irrigated conditions. After identifying molecular markers related to increased yield and drought tolerance, they can be used as selection criteria to accelerate wheat breeding programs. Also, these marker-trait associations can help wheat improvement programs through marker-assisted selection.

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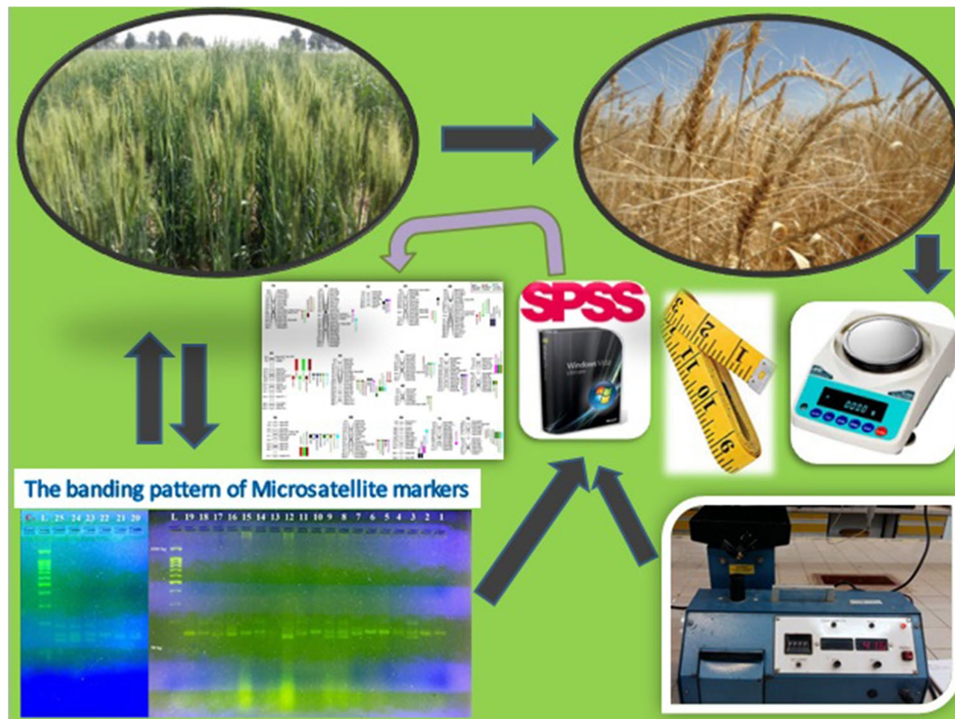
Graphical Abstract



Keywords Drought tolerance · Marker-trait association · Microsatellite marker · Regression · *Triticum aestivum* L

Introduction

Wheat is one of the most important strategic crops in the world, with a cultivated area of 219 million hectares and a production of 808 million tons (FAOSTAT 2022). Providing 25% of the total proteins and calories in the human diet and food supplies worldwide relies on wheat (*Triticum aestivum* L.) (Alipour et al. 2021). The wheat crop is subjected to various biotic and abiotic stresses. Most likely, drought is the most significant abiotic stress affecting wheat yield. Hence, improving drought-tolerant genotypes is a promising strategy for dealing with the world's rapidly diminishing water resources and growing population (Rabieyan et al. 2023). Superior drought-tolerant genotypes could be used as donors in a breeding program (Reddy et al. 2023). The yield and agronomic traits in drought resistance breeding programs are multifaceted characteristics governed by multiple genes (Gupta et al. 2024). Therefore, identifying markers related to these traits can accelerate the development of new wheat cultivars. Morphological markers are valuable indicators of genetic diversity, but they are not the most reliable, as they are highly affected by the environment (Yang et al. 2020). In contrast, DNA markers can pinpoint multiple genes that regulate complex traits without environmental influence (Dagnaw et al. 2023). On the other hand, in drought tolerance, molecular markers can help examine the presence or absence of novel genomic regions associated with drought tolerance and assist in marker-assisted selection (MAS) by allowing focus on the most promising candidates (Ghazy et al. 2021). Simple sequence repeats (SSRs) are high-performance markers primarily used to analyze genetic diversity and population structure and elucidate phylogenetic relationships among plant genetic resources, as such relationships play a crucial role in developing appropriate breeding programs (Belete et al. 2021). SSR markers have many advantages, such as co-dominance, high levels of polymorphism, chromosome specificity, and high reproducibility; they are also excellent for identifying and monitoring target traits within varieties (Alsaleh 2022). Drought tolerance is a multifaceted characteristic governed by multiple genes. Therefore, the breeding challenge for this particular trait is considerable. Identifying of quantitative trait loci (QTLs) associated with drought tolerance marker-assisted is of considerable importance in crop breeding and is a valuable approach to increasing wheat yield and development of drought-tolerant wheat cultivars (Zhao et al. 2023; Gupta et al. 2024). Using novel molecular techniques such as molecular markers and quantitative trait loci (QTL) mapping methods are needed to produce drought tolerance genotypes (Gupta

et al. 2024). One of these procedures in plant breeding is association analysis, which has high resolution and accuracy and is used to identify genomic regions for studying agronomic traits (Mehrabi et al. 2020; Pang et al. 2020; Rabieyan et al. 2023), and drought stress tolerance (Reddy et al. 2023; Zhao et al. 2023) has been used in wheat. A review of quantitative trait loci mapping using regression as a robust, efficient, and effective method concluded that regression has always been an essential tool for quantitative geneticists (Knott 2005). Multiple regression analysis (MRA) based on the relationship between molecular markers (as an independent variable) and morphological traits (as a dependent variable) is a suitable method for identifying trait-dependent markers. This analysis determines the coefficient of explanation (R^2), which shows the degree of relationship between the morphological trait and the molecular marker (Gomez and Gomez 1984). As the value of R^2 increases, the probability of a significant linkage of a trait with the marker also increases. Therefore, it can be hoped that by finding gene loci with high R^2, it will be possible to find agronomic traits related to that marker (Amombo et al. 2018). In a study using association analysis based on multivariate regression, 55 wheat lines, 55 SSR markers, 38 SAMPL markers, and 54 AFLP markers were identified that were associated with at least one of the 14 agronomic traits under study such as biological yield and thousand seed weight. They were introduced as positive markers for marker-assisted selection programs of these traits (Roy et al. 2006). In research, association analysis discussed superior alleles for yield-related traits and their distribution in important wheat cultivars. A significant relationship between the number of desirable alleles and yield indicates that multi-QTL pyramidal production with marker-assisted selection can effectively increase wheat yield. This study is helpful for marker-assisted selection for yield improvement and elucidating the genetic mechanism of important cultivars in wheat (Li et al. 2020). In research, molecular, physiological, and biochemical variability in bread wheat and its wild relative species were investigated under water-deficit stress conditions. The association analysis results showed that 28 and 27 significant marker-trait associations (MTAs) were identified under control and water-deficit stress conditions, respectively. Furthermore, ten MTAs showed sufficiently stable expression across both growth conditions. Of these, five SSR markers were associated with several traits (Khodadadi et al. 2023). The research revealed 44 significant marker-trait associations in durum wheat and 26 of the MTAs were novel. In contrast, the remaining 18 were previously reported and confirmed in this study (Mulugeta et al. 2023). In the study to evaluate the genetic diversity of durum wheat germplasm as revealed by morphological and SSR markers, the results showed significant associations between SSR primers with one or more target phenotypic traits, including spikelets/spike, plant height, spike density, thousand-kernel weight, and grain yield (Dagnaw et al. 2023). Abiotic stresses, especially drought, are associated with changes in various plant characteristics, including agronomic traits, and cause yield loss. Identifying and applying primers related to agronomic characteristics in different moisture conditions can be a useful guide in breeding programs to select superior genotypes. Therefore, this research aims the following: (1) identify genotypes with high yield and tolerance to drought; (2) evaluate the genetic diversity in terms of agronomic traits and microsatellite markers under rainfed and irrigated conditions; (3) investigate the relationship between agronomic traits and SSR molecular markers, and also to identify informative markers related to each trait in rainfed and irrigated conditions.

Materials and Methods

Field Experiment

Twenty-five bread wheat genotypes were used, including two cultivars, Pishtaz and Pishgam (check), and 23 accessions of winter bread wheat (Table 1). The genotypes were obtained from Karaj Seedling and Seed Breeding Research Institute. Field experiments in randomized complete block design with three replications were carried out during two consecutive cropping seasons (2018–2019 and 2019–2020) under rainfed and irrigated conditions in the cold Mediterranean climate (34°21′N latitude, 47 9′E Longitude, and 1319 m altitude) in Iran. This climate is classified as semi-arid with mean annual rainfall of 430–460 mm. Each plot consisted of five rows that were 2 m in length. The row distance was 23 cm with a density of 400 seeds per m^2. In rainfed and irrigated conditions, initial irrigation was done for both conditions, due to the lack of rain during cultivation. The first irrigation after planting was considered the planting date (14/11/2018 in the first year and 6/11/2019 in the second year), but no irrigation was done for rainfed conditions during the entire growth period. While for irrigated conditions, in the first year, on May 15th, at the 50% spike stage, the second stage of irrigation was done in late May, after the full spike, and the third stage was done on June 14th, during the milking stage of the seeds; in the second year on May 7th, at the 50% spike stage, the second stage of irrigation was done in late May after the full spike, and the third stage was done on June 6th, during the milking stage of the seeds. Five random samples were selected from each plot, respecting the marginal effect. Harvesting was done for the first year in early July 2018 and the second year in early July 2020. Twenty-four agronomic traits studied are presented as follows: grain yield (GY), the number of spike per m^2 (NSP), the number of grains per spike (NGPS), 1000-grain weight (TGW), biological yield (BY), plant height (PH), spike weight (SW), spike length (SL), peduncle length (PL), xteragen length (XL), mitt penalty length (PML), peduncle length/plant height (PL/PH), hectoliter weight (HW), spike

Table 1 Cultivars and studied genotypes of bread wheat

Genotype no	Genotype name	Origin	Genotype no	Genotype name	Origin	Genotype no	Genotype name	Origin
1	WC-4924	Kalat	10	WC-4987	Unknown	19	Pishtaz	Pishtaz
2	WC-4582	Kermanshah	11	WC-47615	Mexico	20	Pishgam	Pishgam
3	WC-4592	Kermanshah	12	WC-4612	Kordestan Babrar	21	WC-47640	Minnesota
4	WC-47341	Montana	13	WC-5001	Unknown	22	WC-47467	Mexico
5	WC-4965	Kashan	14	WC-4994	Unknown	23	WC-4553	Kerend
6	WC-4840	Sarakhs	15	WC-47638	Peru	24	WC-4583	Kermanshah
7	WC-4958	Badranloo	16	WC-47583	Canada	25	WC-4554	Kerend
8	WC-47399	Bulgaria	17	WC-47522	Mexico			
9	WC-4600	Kermanshah	18	WC-47569	Minnesota			

dry weight (SDW), spike grain weight (SGW), and stem weight (StW), the number of fertile spikelets (NPS), number of infertile spikelets (NNPS), number of spikelets per spike (NSS), harvest index (HI), spike harvest index (SHI), straw yield, other inter nodes length (OIL), and spike density (SD). In spike harvest index (SHI), grain yield is divided by spike yield. Straw Yield (SY) = Biological yield - spike yield. Other Inter nodes Length (OIL) = Plant height - total length of the peduncle, mitt penalty, and spike. Spike Density (SD) was calculated based on five spikes randomly selected for each plot and repetition accession, and calculated by this formula: [10 × (spike length/ number of grains per spike)]. Then, the length of each spike and the number of grains per spike were calculated for each accession in each plot, according to the formula (10×(spike length/number of grains per spike)); the density of each spike was obtained for 10 cm of spike length. The diversity in a population can be evaluated by various methods such as simple measurement of diversity, analysis of variance components, and molecular markers used in this research. To perform statistical analysis, first, the Kolmogorov–Smirnov test was used to test the normality and check the skewness of the data. Variance analysis for traits in rainfed and irrigated conditions from the evaluation of 23 genotypes, and two cultivars to determine the main effects of block and genotype, in such a way that the effect of fixed genotype and random block was considered. Statistical analysis was done using SAS 9,1,3 software. The statistical model of the randomized complete block design is as follows:

$$X_{ij} = \mu + \delta_i + \tau_j + \varepsilon_{ij}$$

The value of each observation (X_{ij}) is obtained from the sum of block effects (δ_i), treatment (τ_j), experimental error (ε_{ij}), and the total population mean (μ).

To estimate the broad-sense heritability ($h^2_{\text{b.s}}$), Kearsey and Pooni's (1996) and SAS software GLM MANOVA analysis were used. The relationships used to estimate the genetic parameters are as follows:

$$\delta_e^2 = MSe \tag{1}$$

$$\delta_p^2 = \delta_g^2 + \frac{\delta_e^2}{r} \tag{2}$$

$$\delta_g^2 = \frac{MSg - MSe}{r} \tag{3}$$

$$h_{b.s}^2 = \frac{\delta_g^2}{\delta_p^2} \tag{4}$$

$$PCV = \frac{\sqrt{Vp}}{\overline{X}}.100 \tag{5}$$

$$GCV = \frac{\sqrt{Vg}}{\overline{X}}.100 \tag{6}$$

$$ECV = \frac{\sqrt{Ve}}{\overline{X}}.100 \tag{7}$$

$$GG = \frac{\left(\left(2.06.\frac{\delta_G^2}{\sqrt{\delta_P^2}}\right).100\right)}{\overline{X}} \tag{8}$$

In the above relationships, V_g and δ_g^2 are the genotypic variance, V_e and δ_e^2 are the environmental variance, V_p and δ_p^2 are the phenotypic variance, MS_e is the mean square of the test error, and MS_g is the mean square of the genotypes. Also, genetic gain (GG), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), and environmental coefficient of variation (ECV) were calculated as the ratio of phenotypic and genotypic standard deviation to the mean of traits (Farshadfar 2010). In this research, genetic parameters were estimated for traits that had significant genetic diversity (according to variance analysis) in the studied genotypes.

Molecular Experiment

The 20 pairs of SSR markers were used for molecular evaluation of the studied genotypes. DNA was extracted from 2 to 3-week-old seedlings by the modified CTAB method (Doyle and Doyle 1987). DNA extraction was done as follows: After transferring 50 mg of the crushed sample with liquid nitrogen to the tube, 800 µl of extraction buffer (4 g CTAB, 16.36 g NaCl, 3.15 g Tris–HCl, 1.48 g EDTA, 400 µl β-mercaptoethanol at pH = 8 to a volume of 100 ml) was added to the tubes and for half an hour. The samples were placed in a water bath at 65 °C. Then, 800 µl of chloroform-isoamyl alcohol solution (24:1) was added to each sample and stirred well for 60 min. After that, the samples were centrifuged for 15 min at 13,000 rpm, and the supernatant phase was transferred to a clean tube. Five hundred microliters of cold isopropanol solution was added to each tube and placed in a freezer at − 20 °C for 2 h. Then, the tubes were centrifuged for 15 min at 13,000 rpm, and after that, the supernatant was slowly emptied, and 500 µl of cold 80% ethanol was added to each tube, and then, a short centrifuge was performed, and the liquid phase was slowly emptied (this step was twice repeated). At the end, the tubes were placed at room temperature to dry, and then, 100 µl of sterile double-distilled water was added to each tube.

To determine the quality and quantity of extracted genomic DNA, electrophoresis on 0.8% agarose gel was used. Five microliters of extracted DNA from each sample was mixed with 2 µl of sampling buffer on the adhesive or clean surface and then loaded. Electrophoresis was performed with a voltage of 90 to 120 V until the blue color reached the end of the gel. Then, after washing the gel for a few seconds with distilled water for 15 to 30 min, the gel was placed inside the ethidium bromide solution for staining. At the end, the gel was put in the gel document device, and the condition of the DNA was examined.

PCR (polymerase chain reaction) is carried out in three steps by temperature. For PCR, DNA samples diluted at a concentration of 10 ng μl^{-1} were used and amplified using 20 pairs of primers (primers were designed from the NCBI site, and the names and sequences of the primers used are given in Table 2). The polymerase chain reaction was performed using a Bio-Rad thermocycler in 20 µl volumes. The volume included reaction buffer 1x, 50 ng DNA, 2 mM $MgCl_2$, 0.05 mM dNTP, 0.2 µM primer (forward and reverse microsatellite primers), and 1 U enzyme of Taq DNA polymerase. The PCR program was 95 °C for 5 min as the primary denaturation, 35 normal PCR cycles, denaturation (95 °C for 30 s), annealing (primer-specific Tm (°C) for 30 s, in this research, ranged from 46 to 60 °C, which was different for each primer), and extension (72 °C for 60 s). The last extension time was 72 °C for 5 min.

After completing the cycles and taking the samples out of the device, they should be kept at 4 °C until electrophoresis. The PCR product was electrophoresed on a 3% agarose gel in 1 × TBE buffer. Ten microliters of safe stain was used for staining. At first, 5 µl of sampling buffer was injected into the amplified DNAs. Then, 15 µl of each sample was loaded into the wells created in 3% agarose gel with a voltage of 90–120 V and run for 2 h. At the end, the gel document device quantum model ST4 was used to highlight the bands. Nevertheless, as mentioned, because all the samples were not placed on the same gel, DNA size marker 100 to 1500 Bb was used in this experiment, which produced 11 bands on 3% agarose gel. After recording the information, the obtained gel was scored for each primer. To perform statistical analysis, the information obtained from the gels and the pattern of band separation during electrophoresis, the resulting data were prepared in a matrix format (zero absence of a band and one presence of a band). For each marker, the number of alleles in the studied genotypes were named as a, b, c, and d.

Statistical Analysis of Genomic Information

The distance between genotypes, cluster analysis by the UPGMA method, and dendrogram drawing was done by NTSYS (ver 2.02). In the UPGMA (Unweighted Paired Group Method using Arithmetic Average) method, the initial cluster is formed based on the similarity between two individuals. Then, the similarity (or distance) of each individual with the other members in a cluster is considered the average similarity of that member among the individuals in the cluster. In this method, two groups are merged when the average distance between them is small enough (Farshadfar 2010).

The molecular indices were calculated as follows:

Polymorphic percentage: To calculate this index, the number of polymorph bands was divided by the total number of bands (Mohammadi and Prasanna 2003)
Polymorphic information content index (PIC): from the relationship of Anderson et al. (1993)
Marker index (MI) and effective multiplex ratio index (EMR): from the relationship of Kumar et al. (2009)
Resolving power (RP): It was obtained from the relationship of Altintus et al. (2008)

Finally, the association between SSR markers and traits measured in field conditions was investigated using stepwise multiple regression by SPSS23 software, so that each

Table 2 SSR primers used to assess the genetic diversity of bread wheat genotypes

Band size	GC%	T_M	(5′-3′) Sequence	Name	No
150 bp	47.6	57	5′ ACCTCATCCACATGTTCTACG 3′	XGWM350-7D-F	1
	64.7		5′ GCATGGATAGGACGCCC 3′	XGWM350-7D-R	
100 bp	30	50	5′ AATTTCAAAAAGGAGAGAGA 3′	XGWM334-6A-F	2
	30		5′ AACATGTGTTTTTAGCTATC 3′	XGWM334-6A-R	
100 bp	55.6	58	5′ CAATCATTTCCCCCTCCC 3′	XGWM155-3A-F	3
	36.4		5′ AATCATTGGAAATCCATATGCC 3′	XGWM155-3A-R	
150 bp	31.8	56	5′ ATGGCATAATTTGGTGAAATTG 3′	XGWM577-7B-F	4
	36.4		5′ TGTTTCAAGCCCAACTTCTATT 3′	XGWM577-7B-R	
200 bp	55	52.5	5′ AGTGGCTGGGAGAGTGTCAT 3′	XGWM70-6B-F	5
	61.6		5′ GCCCATTACCGAGGACAC 3′	XGWM70-6B-R	
180–200 bp	45	58	5′ ACGGCGAGAAGGTGCTC 3′	XGWM642-1D-F	6
	64.7		5′ CATGAAAGGCAAGTTCGTCA 3′	XGWM642-1D-R	
250 bp	57.9	52	5′ GACAGCACCTTGCCCTTTG 3′	XGWM136-1A-F	7
	52.6		5′ CATCGGCAACATGCTCAT 3′	XGWM136-1A-R	
200 bp	61.1	57.5	5′ GCCATGGCTATCACCCAG 3′	XGWM124-1B-F	8
	45		5′ ACTGTTCGGTGCAATTTGAG 3′	XGWM124-1B-R	
150 bp	45	58.5	5′ TGTTGCGGATGGTCACTATT 3′	XGWM265-2A-F	9
	52.4		5′ GAGTACACATTTGGCCTCTGC 3′	XGWM265-2A-R	
250 bp	61.6	51	5′ GCTTGAGACCGGCACAGT 3′	XGWM410-2B-F	10
	55		5′ CGAGACCTTGAGGGTCTAGA 3′	XGWM410-2B-R	
200 bp	50	50.6	5′ TGCAGTGGTCAGATGTTTCC 3′	XGWM165-4B-F	11
	45		5′ CTTTTCTTTCAGATTGCGCC 3′	XGWM165-4B-R	
250 bp	40	52.5	5′ GCTGATGCATATAATGCTGT 3′	XGWM4-4A-F	12
	47.6		5′ CACTGTCTGTATCACTCTGCT 3′	XGWM4-4A-R	
100 bp	45	50.7	5′ GGTTTTCTTTCAGATTGCGC 3′	XGWM192-5D-F	13
	47.6		5′ CGTTGTCTAATCTTGCCTTGC 3′	XGWM192-5D-R	
100 bp	26.1	46.7	5′ TCAAAACATAAATGTTCATTGGA 3′	XGWM233-7A-F	14
	40.9		5′ TCAACCGTGTGTAATTTTGTCC 3′	XGWM233-7A-R	
250 bp	50	49.4	5′ CTGCAAGCCTGTGATCAACT 3′	XGWM2-3D-F	15
	35		5′ CATTCTCAAATGATCGAACA 3′	XGWM2-3D-R	
200 bp	57.9	59.5	5′ TGCCCTGTCCACAGTGAAG 3′	XCFD5-5B-F	16
	45		5′ TTGCCAGTTCCAAGGAGAAT 3′	XCFD5-5B-R	
250 bp	55	50.6	5′ TCAGTGGGCAAGCTACACAG 3′	XGWM129-5A-F	17
	44.4		5′ AAAACTTAGTAGCCGCGT 3′	XGWM129-5A-R	
250 bp	45	56	5′ CTTCGCAAATCGAGGATGAT 3′	XCFD168-2D-F	18
	50		5′ TTCACGCCCAGTATTAAGGC 3′	XCFD168-2D-R	
220–230 bp	50	54	5′ GAGTCCTGATGTGAAGCTGTTG 3′	XGWM234-5B-F	19
	55		5′ CTCATTGGGGTGTGTACGTG 3′	XGWM234-5B-R	
100 bp	47.6	59	5′ GGAGTCACACTTGTTTGTGCA 3′	XGWM33-1A-F	20
	45.5		5′ CACTGCACACCTAACTACCTGC 3′	XGWM33-1A-R	

quantitative trait was considered a dependent variable and SSR markers as independent variables.

Results

The results of the analysis of variance (Table 3) showed significant differences between genotypes for most of the traits in both irrigated and rainfed conditions.

Estimation of Genetic Parameters of Studied Traits in 25 Bread Wheat Genotypes Under Rainfed and Irrigated Conditions

The estimation of genetic parameters for agronomic traits in rainfed conditions (Table 4) showed that the highest broad-sense heritability in the first year was respectively related to the TGW (0.766) with low PCV (12.29), PH (0.586), PML (0.575), and PL (0.571) with medium to low

PCV and (13.64), (15.66), and (16.5), respectively, NNPS (0.567) with high PCV (38.98) and SL (0.559) with low PCV (12.84). In addition, the contribution of GCV in these traits was higher than that of ECV. In the second year, the estimation of genetic parameters was related to HW (0.765) with high PCV (30.87), SHI (0.765), OIL (0.657), and SGW (0.545) with average PCV and (21.82), (20.51), and (18.05), respectively. In addition, the contribution of GCV in these traits was higher than that of ECV. The TGW (0.392) with low PCV (11.28) and the contribution of ECV in this trait were more than GCV.

Based on the more significant genetic gain (Table 4) in the first year, respectively, NNPS (45.54), XL (42.66) with high PCV (39.78), SD (22.39) with average PCV (19.58). In addition, the contribution of GCV in these traits was more than that of ECV. The StW (20.31) and OIL (19.96) have medium to high PCV (23.01) and (26.96), respectively. In addition, the contribution of ECV in these traits was higher than that of GCV. The PL was (19.42), and TGW and PML were 19.39 and 18.56, respectively. In the second year; parameters including HW (48.64), SHI (34.4), OIL (27.76), and SGW (20.27). The StW (18.54) has a medium to high PCV (23.54). The contribution of the ECV in the StW trait was higher than that of GCV. Also, in the first year, for the traits of PH and SD, and in the second year, for the traits of HW and SHI, the contribution of V_g was high. Therefore; these traits are likely to respond to the selection.

The estimation of genetic parameters for agronomic traits in irrigated conditions (Table 5) showed that the highest broad-sense heritability in the first year was respectively related to the TGW (0.872) with low PCV (14.15), OIL (0.638) with medium to high PCV (23.37), NNPS (0.638) with high PCV (41.05), SL (0.623) with low PCV (13.60), GY (0.604) with medium to high PCV (26.68), PML (0.542) with low PCV (13.62), and SD (0.514) with medium PCV (18.64). In addition, the contribution of GCV in these traits was more than that of ECV. The HI (0.5) and SGW (0.49) have medium to high PCV (21.63) and (23.4), respectively. In these traits; the contribution of GCV and ECV were almost equal. In the second year; parameters including OIL (h =0.866) with high PCV (27.03), SHI

Table 3 Mean squares (MS) and coefficient of variation (CV) derived from analysis of variance of different traits in bread wheat genotypes under rainfed and irrigated conditions

Second year				First year				Traits
Irrigation		Rainfed		Irrigation		Rainfed		
C.V%	MS	C.V%	MS	C.V%	MS	C.V%	MS	
16.65	29,295.60**	14.63	8919.70**	16.78	24,612.73**	23.85	9874.99**	Grain yield
12.39	23,563.89**	14.97	15,069.44**	11.32	8926.39 ^{ns}	7.23	11,756.71**	Hectoliter weight
8.30	49.14**	8.80	34.30**	5.07	87.36**	5.94	31.44**	1000-grain weight
15.84	64.47 ^{ns}	13.79	49.31 ^{ns}	14.32	54.06*	13.61	63.27**	Number of grains per spike
20.95	21,916.94*	25.22	11,378.52 ^{ns}	26.93	16,968.14 ^{ns}	18.14	6419.54 ^{ns}	Number of spike per m^2
18.87	641,492.05**	22.85	134,829.28 ^{ns}	18.60	56,599.15 ^{ns}	21.27	64,537.71 ^{ns}	Biological yield
19.95	59,593.70 ^{ns}	23.42	36,024.24 ^{ns}	18.77	34,846.11**	21.32	19,866.34**	Spike weight
24.28	309,389.17**	29.27	72,698.98 ^{ns}	22.42	21,734.83 ^{ns}	19.95	16,258.84 ^{ns}	Straw yield
9.52	286.98**	10.58	208.68**	6.35	42.71**	6.61	63.92**	Spike harvest index
10.95	194.50 ^{ns}	13.32	431.51*	8.33	247.94**	8.78	326.30**	Plant height
22.65	59.55 ^{ns}	18.37	45.93*	22.97	34.40**	27.53	46.75**	Xteragen length
14.15	60.56 ^{ns}	13.93	46.35 ^{ns}	10.35	42.93**	10.80	61.50**	Peduncle length
12.45	26.23 ^{ns}	12.45	26.38*	9.22	22.35**	10.20	26.34**	Mitt penalty length
16.01	3.37 ^{ns}	12.19	3.48**	8.35	4.41**	8.52	3.41**	Spike length
9.88	108.29**	12.02	84.95**	14.06	67.55**	21.58	93.39**	Other inter nodes length
15.42	37.03 ^{ns}	15.31	32.94 ^{ns}	12.99	102.11**	13.06	101.07**	Spike density
5.86	1.55 ^{ns}	5.23	1.21 ^{ns}	7.54	5.37**	7.16	5.86**	Number of fertile spikelets
13.37	0.05 ^{ns}	11.34	0.05 ^{ns}	24.79	1.07**	25.45	1.43**	Number of infertile spikelets
5.03	1.34 ^{ns}	5.47	1.10 ^{ns}	6.43	3.08*	6.03	2.26*	Number of spikelets per spike
21.45	0.32 ^{ns}	20.12	0.28*	17.21	0.44**	17.27	0.17**	Spike dry weight
25.13	0.32 ^{ns}	18.58	0.22**	15.08	0.12**	17.90	0.13**	Stem weight
8.10	0.19**	12.20	0.17**	16.21	0.27**	18.01	0.08**	Spike grain weight
12.14	0.004 ^{ns}	11.69	0.003 ^{ns}	13.46	0.002 ^{ns}	11.05	0.002 ^{ns}	Peduncle length/plant height
13.81	60.30**	20.90	24.85*	15.35	85.12**	13.76	34.28**	Harvest index

^{ns}, *, and **; not significant and significant at 5% and 1% probability levels, respectively

Table 4 Estimation of genetic parameters of agronomic traits in 25 bread wheat genotypes under rainfed conditions in two cropping seasons

Year	Trait	Mean	σ^2_G	σ^2_p	σ^2_e	h^2_{bs}	PCV	GCV	ECV	GG
First	Grain yield	283.75	1764.5	6346.03	4581.6	0.278	28.08	14.8	23.86	16.08
	Hectoliter weight	760.15	2912.9	5930.98	3018.1	0.491	10.13	7.10	7.23	10.25
	1000-grain weight	28.68	9.51	12.41	2.9	0.766	12.29	10.75	5.94	19.39
	Number of grains per spike	34.8	13.62	36.04	22.42	0.378	17.25	10.6	13.61	13.43
	Spike weight	459.31	3425.03	13,016.3	9591.26	0.263	24.84	12.74	21.32	13.46
	Spike harvest index	60.4	15.99	31.93	15.93	0.501	9.36	6.62	6.61	9.66
	Plant height	89.88	88.02	150.25	62.23	0.586	13.64	10.44	8.78	16.46
	Xteragen length	12.03	11.92	22.9	10.98	0.521	39.78	28.7	27.55	42.66
	Peduncle length	32.47	16.4	28.7	12.3	0.571	16.5	12.47	10.8	19.42
	Mitt penalty length	22.35	7.05	12.25	5.2	0.575	15.66	11.88	10.2	18.56
	Spike length	9.88	0.9	1.61	0.71	0.559	12.84	9.6	8.53	14.79
	Other inter nodes length	27.34	19.53	54.34	34.81	0.359	26.96	16.16	21.58	19.96
	Spike density	35.35	26.59	47.9	21.31	0.555	19.58	14.59	13.06	22.39
	Number of fertile spikelets	16.96	1.46	2.93	1.47	0.499	10.1	7.13	7.15	10.38
	Number of infertile spikelets	2.1	0.38	0.67	0.29	0.567	38.98	29.35	25.64	45.54
	Number of spikelets per spike	18.92	0.32	1.62	1.3	0.198	6.73	2.99	6.03	2.74
	Spike dry weight	1.61	0.03	0.11	0.08	0.273	20.57	10.74	17.54	11.55
	Stem weight	1.15	0.03	0.07	0.04	0.429	23.01	15.06	17.39	20.31
	Spike grain weight	1.08	0.01	0.053	0.04	0.25	21.4	10.7	18.53	11.02
	Harvest index	25.61	7.28	19.71	12.43	0.369	17.34	10.54	13.77	13.2
Second	Grain yield	385.2	1915.02	5089.7	3174.6	0.376	18.52	11.36	14.63	14.36
	Hectoliter weight	250	4556.3	5956.9	1400.7	0.765	30.87	27	14.97	48.64
	1000-grain weight	38.86	7.54	19.23	11.69	0.392	11.28	7.06	8.8	9.11
	Spike harvest index	41.61	63.11	82.47	19.36	0.765	21.82	19.09	10.57	34.4
	Plant height	112.7	68.78	293.9	225.2	0.234	15.22	7.36	13.32	7.33
	Xteragen length	27.44	6.84	32.25	25.41	0.212	20.7	9.53	18.37	9.04
	Mitt penalty length	28.49	4.6	17.18	12.58	0.268	14.55	7.53	12.45	8.02
	Spike length	9.48	0.71	2.05	1.34	0.347	15.12	8.91	12.21	10.82
	Other inter nodes length	29.54	24.12	36.72	12.6	0.657	20.51	16.62	12.02	27.76
	Spike dry weight	1.99	0.04	0.2	0.16	0.2	22.47	10.05	20.1	9.26
	Stem weight	1.5	0.05	0.12	0.08	0.382	23.54	14.56	18.5	18.54
	Spike grain weight	1.58	0.04	0.08	0.04	0.545	18.05	13.33	12.17	20.27
	Harvest index	17.56	3.8	17.26	13.46	0.22	23.66	11.1	20.89	10.72

(0.797) with medium PCV (21.54), HW (0.796) with high PCV (27.4), SGW (0.708) with low PCV (15.08), and HI (0.65) with medium to high PCV (23.33). In addition, the contribution of GCV in these traits was more than that of ECV.

Genetic gain (Table 5) for the following traits in the first year consisted of NNPS (53.98), GY (33.2), OIL (30.73), TGW (25.4), SGW (23.51), and HI (22.11). The XL (21.14) has a high PCV (28.66). In addition, the contribution of the ECV in XL trait was more than that of GCV. In the second year, genetic gain for the following traits consisted of OIL (48.23), HW (44.91), SHI (35.35), HI (31.22), GY (22.99), and SGW (21.98). So, in the first year, the V_g for the traits of GY, TGW, and SD, and in the second year for the traits of HW and SHI, was also high. Therefore, it is possible to choose the optimal breeding method for these traits.

Evaluation of the Genetic Diversity of the Wheat Genotypes Using SSR Markers

In this research, 16 out of the 20 primers showed suitable polymorphism and were selected to estimate genetic diversity. Table 6 shows the results of the primer indices. The total number of bands was 35 bands, of which 33 bands were polymorphic, and the percentage of total polymorphism was estimated at 93.75. Most primers had 100% polymorphism, and the lowest polymorphism (50%) belonged to primers XGWM334 and XGWM642. Therefore, all the primers of this research were found to be useful for genetic diversity study in wheat. The average number of bands and average polymorphism were 2.188 and 2.063, respectively. The highest number of alleles was related to primer XGWM136 with

Table 5 Estimation of genetic parameters of agronomic traits in 25 bread wheat genotypes under irrigated conditions in two cropping seasons

Year	Trait	Mean	σ^2_G	σ^2_p	σ^2_e	h^2_{bs}	PCV	GCV	ECV	GG
First	Grain yield	395.7	6733.9	11,145	4411.2	0.604	26.68	20.74	16.78	33.2
	1000-grain weight	39.9	27.8	31.9	4.1	0.872	14.15	13.21	5.07	25.4
	Number of grains per spike	38.2	8.03	38	29.97	0.211	16.13	7.41	14.32	7.02
	Spike weight	583.4	2617.7	19,610.6	11,992.9	0.388	24	14.96	18.77	19.21
	Spike harvest index	68.48	7.94	26.84	18.9	0.296	7.57	4.11	6.35	4.61
	Plant height	96.04	61.3	125.3	63.96	0.489	11.66	8.15	8.33	11.75
	Xteragen length	15.62	7.18	20.05	12.87	0.358	28.66	17.15	22.97	21.14
	Peduncle length	36.21	9.63	23.68	14.05	0.407	13.44	8.57	10.35	11.26
	Mitt penalty length	24.04	5.81	10.72	4.91	0.542	13.62	10.03	9.22	15.21
	Spike length	10.3	1.22	1.96	0.74	0.623	13.60	10.74	8.35	17.46
	Other inter nodes length	23.31	18.84	29.67	10.73	0.638	23.37	18.67	14.05	30.73
	Spike density	38.08	25.88	50.36	24.48	0.514	18.64	13.36	12.99	19.73
	Number of fertile spikelets	17.61	1.2	2.96	1.76	0.406	9.78	6.23	7.53	8.18
	Number of infertile spikelets	1.67	0.3	0.47	0.17	0.638	41.05	32.8	24.69	53.98
	Number of spikelets per spike	19.36	0.51	2.06	1.55	0.248	7.41	3.69	6.43	3.78
	Spike dry weight	2.21	0.1	0.24	0.14	0.42	22.22	14.34	16.97	19.07
	Stem weight	1.29	0.027	0.067	0.04	0.4	20.02	12.66	15.5	16.49
	Spike grain weight	1.58	0.067	0.14	0.07	0.49	23.4	16.34	16.75	23.51
	Harvest index	30.22	21.2	42.72	21.52	0.5	21.63	15.24	15.35	22.11
Second	Grain yield	527.8	7191.6	14,912.5	7720.9	0.482	23.14	16.07	16.65	22.99
	Hectoliter weight	348	7234.7	9094.4	1859.7	0.796	27.4	24.44	12.39	44.91
	1000-grain weight	43.15	12.1	24.93	12.83	0.485	11.57	8.06	8.3	11.57
	Number of spike per m^2	515.1	3426.3	15,064.3	11,637.9	0.227	23.83	11.36	20.95	11.17
	Biological yield	2581.9	134,724.3	372,043.4	237,319	0.362	23.62	14.22	18.87	17.62
	Straw yield	1520.8	57,681.2	194,026.9	136,345.7	0.297	28.96	15.79	24.28	17.74
	Spike harvest index	48.84	88.16	110.66	22.5	0.797	21.54	19.22	9.71	35.35
	Other inter nodes length	23.29	34.33	39.63	5.3	0.866	27.03	25.16	9.88	48.23
	Spike grain weight	1.86	0.06	0.08	0.02	0.708	15.08	12.68	8.15	21.98
	Harvest index	21.95	17.04	26.23	9.19	0.65	23.33	18.8	13.81	31.22

five, and the lowest number of alleles was related to primers XGWM155, XGWM410, and XGWM234 with two alleles. The highest index of polymorphism information content was related to primers XGWM155, XGWM234, XCFD168, XGWM577, XGWM642, and XCFD5, so these primers have more power in creating polymorphism among the investigated genotypes, and according to this issue, these primers can be introduced in the program of genetic diversity among bread wheat genotypes. Among the investigated molecular indices, the highest marker index belonged to primers XGWM136, XCFD168, and XGWM350, and the lowest belonged to primers XGWM334 and XGWM642. The highest index of effective multiplex ratio (EMR) was related to primers XGWM136, XGWM350, XCFD168, and XGWM165, and the lowest belonged to primers XGWM334 and XGWM642. The highest resolving power index (RP) was related to primers XGWM4, XCFD168, and XGWM350, and the lowest belonged to XGWM334. In total, XCFD168, XGWM350, and XGWM136 markers had 100% polymorphism. The banding pattern of SSR markers using the XGWM350 primer in bread wheat genotypes is shown in in Fig. 1.

Classification of the Bread Wheat Genotypes Based on SSR Markers

Cluster analysis using the UPGMA method based on the Jaccard similarity coefficient and high cophenetic correlation (74%) classified wheat genotypes in four different groups (Fig. 2). The first group includes genotypes number 1, 3, 25, 11, 17, 16, 22, 4, 12, 10, 15, 8, 9, 23, 24, 5, 6, and Pishtaz cultivar. The second group includes genotypes number 2 and 21; the third group consists of genotypes number 7, 13, and Pishgam cultivar, and the fourth group includes genotypes number 14 and 18. The results of the principal coordinates analysis based on the SSR marker also showed a similar pattern with the cluster analysis and the two-dimensional distribution diagram. The genotypes were placed in four groups. Finally, genotypes 1, 3, and 25 have the most significant

Table 6 Diversity indicators of microsatellite (SSR) markers in the studied wheat genotypes in this experiment

Marker	Chromosome	Number of reproduced fragments	Number of polymorphic bands	Polymorphic percentage	Marker index	Effective multiplex ratio	Resolving power	Polymorphic information content
XGWM350	7D	3	3	100	1.011	3	3.12	0.337
XGWM334	6A	2	1	50	0.074	0.5	0.16	0.147
XGWM155	3A	1	1	100	0.499	1	0.96	0.499
XGWM577	7B	2	2	100	0.934	2	1.60	0.467
XGWM70	6B	2	2	100	0.557	2	1.28	0.278
XGWM642	1D	2	1	50	0.23	0.5	0.72	0.461
XGWM136	1A	5	5	100	1.76	5	2.32	0.352
XGWM124	1B	2	2	100	0.768	2	1.92	0.384
XGWM265	2A	2	2	100	0.614	2	1.68	0.307
XGWM410	2B	1	1	100	0.365	1	0.48	0.365
XGWM165	4B	3	3	100	0.973	3	2.96	0.324
XGWM4	4A	2	2	100	0.422	2	3.52	0.211
XGWM2	3D	2	2	100	0.755	2	2.24	0.378
XCFD5	5B	2	2	100	0.883	2	1.76	0.442
XCFD168	2D	3	3	100	1.466	3	3.20	0.489
XGWM234	5B	1	1	100	0.493	1	0.88	0.493
Total	-	35	33	-	-	-	-	-
Mean	-	2.188	2.063	93.75	0.738	2	1.8	0.371

genetic distance with genotypes 13, 7, and the Pishgam cultivar, and these two groups can be introduced for breeding programs to obtain heterosis achievements.

Evaluating the Association of Studied Traits with Molecular Markers

In this research, the association of studied traits in 25 bread wheat genotypes with SSR markers using stepwise multiple regression analysis to identify genomic regions involved in yield control and drought tolerance in bread wheat by considering marker locations as independent variables and agronomic traits as dependent variables was investigated using SPSS software in Tables 7 and 8. It should be noted that the association with SSR markers was estimated only for significant traits in the analysis of the variance.

Agronomic Traits Under Rainfed Conditions

The results of stepwise regression analysis to identify informative markers related to agronomic traits in rainfed conditions are presented in Table 7. It was found that the XGWM124$_{(a_2)}$ and XGWM410$_{(a_1)}$ markers in the first year and XGWM577$_{(a_2)}$ marker in the second year have a significant association with

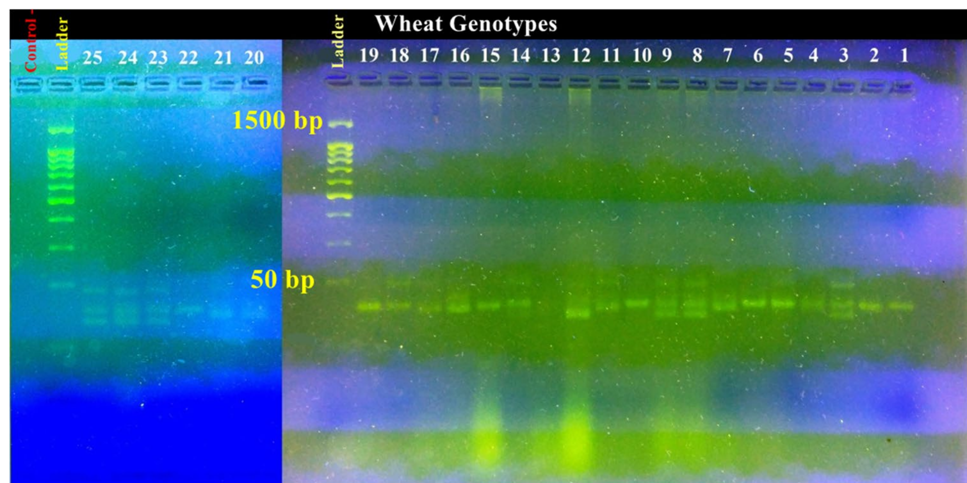


Fig. 1 The banding pattern of SSR markers using XGWM350 primer in bread wheat genotypes

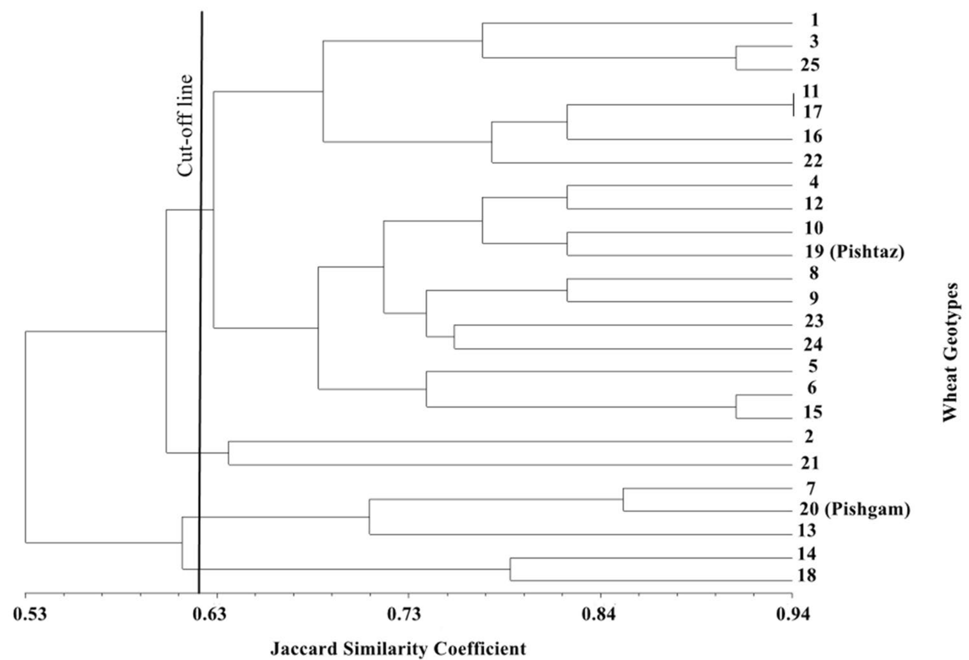


Fig. 2 Grouping of bread wheat genotypes based on Jaccard similarity coefficient and UPGMA method

the grain yield trait in rainfed conditions, which explained 34% of the total variations in the first year and 17% in the second year. Traits associated with yield, i.e., 1000-grain weight in the first year, have a significant association with XGWM577$_{(}$a$_{2)}$, XGWM350$_{(}$a$_{3)}$, and XGWM165$_{(}$a$_{3)}$ markers, and in the second year with XGWM350$_{(}$a$_{2)}$, XGWM2$_{(}$a$_{2)}$, and XGWM642$_{(}$a$_{1)}$ markers, which could justify 64 and 57% of the total variations, respectively. The number of grains per spike has a significant association with XGWM350$_{(}$a$_{3)}$, XGWM165$_{(}$a$_{2)}$, and XGWM136$_{(}$a$_{1)}$ markers in the first year, which could justify 51% of the total variation. The most significant and positive effect was associated with the locus of XGWM136$_{(}$a$_{1)}$, the number of grains per spike, and relatively the 1000-grain weight. The results of the rest traits are observed in Table 7. Eventually, it can be expressed that 64 gene loci for agronomic traits in rainfed conditions were identified. It can be concluded that 26 gene loci covered all measured traits. In the first year, the markers XGWM577$_{(}$a$_{2}$,a$_{1)}$, XGWM124$_{(}$a$_{2)}$, XGWM165$_{(}$a$_{1)}$, XGWM334$_{(}$a$_{1)}$, XGWM136$_{(}$a$_{3)}$, and XGWM155$_{(}$a$_{1)}$ were the most repeated, and in the second year, the markers XGWM577$_{(}$a$_{2}$,a$_{1)}$, XGWM70$_{(}$a$_{2)}$, XGWM136$_{(}$a$_{3)}$, XGWM334$_{(}$a$_{1)}$, XGWM642$_{(}$a$_{1)}$, and XCFD5$_{(}$a$_{2)}$ were the most repeated, and jointly, in both years, XGWM577$_{(}$a$_{2}$,a$_{1)}$, XGWM334$_{(}$a$_{1)}$, and XGWM136$_{(}$a$_{3)}$ markers were the most repeated. So, these markers can be used more in breeding programs.

Agronomic Traits Under Irrigated Conditions

The results of stepwise regression analysis to identify informative markers related to agronomic traits in irrigated conditions are presented in Table 8. It was concluded that the grain yield in the first year has a significant correlation with seven amplified loci, which include XGWM577$_{(}$a$_{2}$,a$_{1)}$, XGWM136$_{(}$a$_{3}$,a$_{4)}$, XGWM265$_{(}$a$_{1)}$, XGWM410$_{(}$a$_{1)}$, and XGWM2$_{(}$a$_{2)}$ markers and in the second year with three amplified loci, which include XGWM124$_{(}$a$_{1}$,a$_{2)}$ and XGWM265$_{(}$a$_{1)}$ markers. They justified 84 and 52% of the total variations, respectively. The most significant and positive effects in the first year were associated with the three loci XGWM136$_{(}$a$_{3)}$, XGWM577$_{(}$a$_{2)}$, and XGWM410$_{(}$a$_{1)}$, and in the second year, they were associated with the locus XGWM265$_{(}$a$_{1)}$. For associated traits with yield, i.e., 1000-grain weight in the first year, five markers, including XCFD5$_{(}$a$_{2)}$, XGWM577$_{(}$a$_{2)}$, XGWM155$_{(}$a$_{1)}$, XGWM350$_{(}$a$_{3)}$, and XGWM165$_{(}$a$_{2)}$ markers, and, in the second year, the XGWM2$_{(}$a$_{2)}$ marker are informative and explained 82% and 24% of the total variability, respectively. In the case of this trait, all the mentioned markers in the first and second years had a significant and positive effect. The number of grains per spike trait in the first year was significantly associated with two markers XGWM350$_{(}$a$_{3)}$ and XCFD5$_{(}$a$_{1)}$ and could justify 44% of the total variation. The number of spikes per m^2 in the second year includes markers XGWM234$_{(}$a$_{1)}$ and XGWM136$_{(}$a$_{4)}$, which justified 38% of the total variation. The results of the rest traits are observed in Table 8. Eventually, it can be expressed that 51 gene loci for agronomic traits in irrigated conditions were identified. It can be noted that 23 gene loci covered all measured traits. The markers XGWM124$_{(}$a$_{2)}$, XGWM410$_{(}$a$_{1)}$, XGWM350$_{(}$a$_{3)}$, XGWM577$_{(}$a$_{2)}$, and XCFD5$_{(}$a$_{2)}$ in the first year, the markers XGWM136$_{(}$a$_{2)}$, XGWM70$_{(}$a$_{2)}$, and XGWM124$_{(}$a$_{2)}$ in the

Table 7 Markers with significant association with agronomic traits of bread wheat genotypes under rainfed conditions

Traits	Year	Marker†	Regression coefficient (*B*)	Standard error (SE)	*t*-value	Significance level	R^2	Adjusted R^2
Grain yield	First	Constant	320.44	17.871	17.931	**	0.397	0.342
		$XGWM124_{(a_2)}$	−68.482	20.944	−3.27	**		
		$XGWM410_{(a_1)}$	52.57	22.02	2.39	*		
	Second	Constant	368.704	12.072	30.541	**	0.201	0.167
		$XGWM577_{(a_2)}$	51.40	21.341	2.41	*		
1000-grain weight	First	Constant	22.844	1.31	17.46	**	0.686	0.641
		$XGWM577_{(a_2)}$	4.624	0.856	5.40	**		
		$XGWM350_{(a_3)}$	5.89	1.212	4.46	**		
		$XGWM165_{(a_3)}$	3.563	1.26	2.83	**		
	Second	Constant	31.97	1.571	20.35	**	0.622	0.568
		$XGWM350_{(a_2)}$	5.62	1.72	3.28	**		
		$XGWM2_{(a_2)}$	2.64	0.973	2.712	*		
		$XGWM642_{(a_1)}$	2.45	0.949	2.58	*		
Hectoliter weight	First	Constant	781.49	11.55	67.673	**	0.719	0.662
		$XGWM165_{(a_1)}$	63.99	29.994	2.133	*		
		$XGWM124_{(a_1)}$	−65.10	17.75	−3.67	**		
		$XGWM265_{(a_1)}$	64.34	24.14	2.67	*		
		$XGWM155_{(a_1)}$	−38.65	15.43	−2.51	*		
	Second	Constant	293.53	15.59	18.83	**	0.461	0.412
		$XGWM136_{(a_3)}$	−82.941	26.02	−3.19	**		
		$XGWM577_{(a_1)}$	−49.22	22.243	−2.213	*		
Harvest index	First	Constant	25.36	0.658	38.54	**	0.479	0.432
		$XGWM165_{(a_1)}$	−6.56	1.92	−3.42	**		
		$XGWM577_{(a_2)}$	2.422	1.12	2.171	*		
	Second	Constant	22.26	1.31	17.06	**	0.509	0.439
		$XGWM124_{(a_2)}$	−6.514	1.46	−4.47	**		
		$XCFD5_{(a_1)}$	2.96	1.062	2.79	*		
		$XGWM124_{(a_1)}$	−3.48	1.43	−2.44	*		
Xteragen length	First	Constant	13.76	0.73	18.84	**	0.757	0.708
		$XGWM577_{(a_2)}$	7.12	1.07	6.683	**		
		$XGWM577_{(a_1)}$	−5.621	1.021	−5.51	**		
		$XGWM155_{(a_1)}$	−3.80	0.91	−4.184	**		
		$XGWM334_{(a_1)}$	6.484	1.88	3.45	**		
	Second	Constant	28.32	0.838	33.791	**	0.165	0.129
		$XGWM136_{(a_3)}$	−3.65	1.711	−2.131	*		
Mitt penalty length	First	Constant	20.93	0.665	31.46	**	0.636	0.583
		$XGWM577_{(a_2)}$	4.34	0.906	4.79	**		
		$XGWM577_{(a_1)}$	−2.85	0.864	−3.30	**		
		$XGWM165_{(a_2)}$	2.19	0.887	2.47	*		
	Second	Constant	27.081	0.708	38.235	**	0.639	0.566
		$XGWM70_{(a_1)}$	3.78	0.838	4.51	**		
		$XGWM334_{(a_1)}$	−4.69	1.48	−3.173	**		
		$XGWM70_{(a_2)}$	6.742	2.164	3.12	**		
		$XGWM642_{(a_1)}$	−2.09	0.878	−2.38	*		
Spike length	First	Constant	10.064	0.17	59.064	**	0.643	0.592
		$XCFD5_{(a_1)}$	−1.16	0.32	−3.611	**		
		$XGWM136_{(a_2)}$	1.564	0.377	4.15	**		
		$XGWM265_{(a_1)}$	−1.47	0.485	−3.023	**		
	Second	Constant	8.89	0.31	28.702	**	0.208	0.173
		$XCFD5_{(a_2)}$	0.981	0.40	2.454	*		

Table 7 (continued)

Traits	Year	Marker†	Regression coefficient (*B*)	Standard error (SE)	*t*-value	Significance level	R^2	Adjusted R^2
Spike dry weight	First	Constant	1.70	0.055	30.821	**	0.671	0.605
		XGWM136$_{(a_3)}$	0.268	0.073	3.66	**		
		XGWM334$_{(a_1)}$	− 0.383	0.113	− 3.38	**		
		XGWM165$_{(a_1)}$	− 0.372	0.113	− 3.281	**		
		XGWM70$_{(a_1)}$	− 0.148	0.062	− 2.37	*		
	Second	Constant	2.063	0.08	25.644	**	0.739	0.670
		XGWM70$_{(a_2)}$	0.743	0.19	3.904	**		
		XGWM577$_{(a_2)}$	0.346	0.091	3.83	**		
		XGWM165$_{(a_2)}$	− 0.275	0.081	− 3.38	**		
		XCFD168$_{(a_3)}$	− 0.283	0.083	− 3.43	**		
		XCFD5$_{(a_2)}$	0.206	0.084	2.46	*		
Stem weight	First	Constant	1.09	0.042	26.044	**	0.245	0.213
		XGWM124$_{(a_1)}$	0.234	0.085	2.734	*		
	Second	Constant	1.502	0.044	33.971	**	0.469	0.421
		XGWM70$_{(a_2)}$	0.698	0.212	3.30	**		
		XGWM334$_{(a_1)}$	− 0.418	0.153	− 2.73	*		
Spike grain weight	First	Constant	1.04	0.034	30.233	**	0.205	0.171
		XGWM136$_{(a_3)}$	0.171	0.07	2.44	*		
	Second	Constant	1.54	0.063	24.26	**	0.638	0.587
		XGWM577$_{(a_2)}$	0.29	0.066	4.412	**		
		XGWM350$_{(a_1)}$	0.224	0.064	3.50	**		
		XGWM265$_{(a_2)}$	− 0.187	0.069	− 2.73	*		
Number of grains per spike	First	Constant	39.502	1.41	28.10	**	0.571	0.510
		XGWM350$_{(a_3)}$	− 6.624	1.64	− 4.05	**		
		XGWM165$_{(a_2)}$	− 5.81	1.57	− 3.70	**		
		XGWM136$_{(a_1)}$	4.32	1.76	2.453	*		
Spike weight	First	Constant	520.622	24.08	21.623	**	0.438	0.387
		XGWM165$_{(a_1)}$	− 136.463	47.78	− 2.86	**		
		XGWM124$_{(a_2)}$	− 69.992	28.87	− 2.43	*		
Plant height	First	Constant	88.932	2.38	37.38	**	0.418	0.365
		XGWM577$_{(a_2)}$	14.904	3.84	3.89	**		
		XGWM577$_{(a_1)}$	− 7.953	3.582	− 2.22	*		
Peduncle length	First	Constant	32.561	0.798	40.82	**	0.653	0.621
		XGWM577$_{(a_2)}$	7.682	1.29	5.972	**		
		XGWM577$_{(a_1)}$	− 5.322	1.201	− 4.431	**		
Number of fertile spikelets	First	Constant	18.98	0.518	36.612	**	0.468	0.419
		XGWM124$_{(a_2)}$	− 2.19	0.505	− 4.331	**		
		XCFD168$_{(a_1)}$	− 1.01	0.457	− 2.202	*		
Number of infertile spikelets	First	Constant	1.88	0.14	13.41	**	0.288	0.257
		XGWM350$_{(a_3)}$	0.81	0.265	3.052	**		

*, **significant at 5% and 1% probability levels, respectively

†a_1, a_2, a_3, a_4, and a_5 are the average alleles 1, 2, 3, 4, and 5, respectively

Table 8 Markers with significant association with agronomic traits of bread wheat genotypes under irrigated conditions

Traits	Year	Marker†	Regression coefficient (*B*)	Standard error (SE)	*t*-value	Significance level	R^2	Adjusted R^2
Grain yield	First	Constant	374.72	12.76	29.37	**	0.885	0.838
		XGWM577$_{(a_2)}$	145.70	19.04	7.653	**		
		XGWM136$_{(a_3)}$	171.744	33.40	5.143	**		
		XGWM265$_{(a_1)}$	− 122.541	26.084	− 4.7	**		
		XGWM410$_{(a_1)}$	89.64	18.29	4.902	**		
		XGWM2$_{(a_2)}$	− 62.80	18.43	− 3.41	**		
		XGWM577$_{(a_1)}$	− 58.85	16.294	− 3.612	**		
		XGWM136$_{(a_4)}$	− 79.04	29.13	− 2.713	*		
	Second	Constant	678.42	40.99	16.552	**	0.583	0.523
		XGWM124$_{(a_2)}$	− 193.603	42.131	− 4.60	**		
		XGWM265$_{(a_1)}$	106.924	42.131	2.54	*		
		XGWM124$_{(a_1)}$	− 100.35	44.40	− 2.26	*		
1000-grain weight	First	Constant	31.193	1.082	28.83	**	0.858	0.821
		XCFD5$_{(a_2)}$	3.68	1.19	3.1	**		
		XGWM577$_{(a_2)}$	5.532	1.171	4.73	**		
		XGWM155$_{(a_1)}$	2.622	1.051	2.494	*		
		XGWM350$_{(a_3)}$	4.562	1.193	3.823	**		
		XGWM165$_{(a_2)}$	3.41	1.12	3.05	**		
	Second	Constant	41.75	0.858	48.641	**	0.267	0.236
		XGWM2$_{(a_2)}$	4.40	1.52	2.90	**		
Harvest index	First	Constant	35.19	1.21	29.19	**	0.696	0.653
		XGWM124$_{(a_2)}$	− 7.15	1.45	− 4.95	**		
		XGWM165$_{(a_1)}$	− 7.79	2.40	− 3.244	**		
		XGWM410$_{(a_1)}$	3.322	1.514	2.194	*		
	Second	Constant	20.591	0.806	25.56	**	0.381	0.354
		XGWM136$_{(a_2)}$	6.78	1.801	3.763	**		
Spike grain weight	First	Constant	1.62	0.11	15.09	**	0.581	0.543
		XCFD5$_{(a_2)}$	0.29	0.09	3.30	**		
		XGWM124$_{(a_2)}$	− 0.30	0.1	− 3.101	**		
	Second	Constant	1.84	0.048	38.50	**	0.160	0.123
		XGWM70$_{(a_2)}$	0.498	0.238	2.09	*		
Number of grains per spike	First	Constant	41.38	0.96	43.262	**	0.488	0.442
		XGWM350$_{(a_3)}$	− 6.69	1.534	− 4.362	**		
		XCFD5$_{(a_1)}$	− 4.58	1.534	− 2.984	**		
Spike weight	First	Constant	655.914	30.423	21.56	**	0.505	0.459
		XGWM124$_{(a_2)}$	− 140.21	35.66	− 3.932	**		
		XGWM410$_{(a_1)}$	118.65	37.49	3.17	**		
Plant height	First	Constant	92.87	1.93	48.214	**	0.269	0.237
		XGWM577$_{(a_2)}$	9.90	3.41	2.91	**		
Xteragen length	First	Constant	16.80	0.671	25.05	**	0.324	0.294
		XGWM350$_{(a_3)}$	− 4.204	1.27	− 3.32	**		
Peduncle length	First	Constant	37.40	0.781	47.884	**	0.265	0.233
		XGWM350$_{(a_3)}$	− 4.25	1.48	− 2.88	**		
Mitt penalty length	First	Constant	26.164	0.94	27.923	**	0.741	0.704
		XGWM577$_{(a_2)}$	2.93	0.75	3.901	**		
		XGWM124$_{(a_2)}$	− 3.124	0.81	− 3.874	**		
		XCFD168$_{(a_2)}$	− 1.36	0.642	− 2.11	*		

Table 8 (continued)

Traits	Year	Marker†	Regression coefficient (*B*)	Standard error (SE)	*t*-value	Significance level	R^2	Adjusted R^2
Spike length	First	Constant	10.14	0.251	40.411	**	0.680	0.616
		XCFD5$_{(a_1)}$	– 1.06	0.38	– 2.80	*		
		XGWM136$_{(a_2)}$	1.88	0.431	4.351	**		
		XGWM265$_{(a_1)}$	– 1.441	0.54	– 2.69	*		
		XGWM350$_{(a_3)}$	0.90	0.38	2.37	*		
Number of fertile spikelets	First	Constant	18.761	0.37	50.881	**	0.528	0.485
		XGWM124$_{(a_2)}$	– 1.99	0.432	– 4.593	**		
		XGWM410$_{(a_1)}$	1.14	0.454	2.503	*		
Number of infertile spikelets	First	Constant	0.721	0.21	3.48	**	0.535	0.493
		XGWM124$_{(a_2)}$	0.964	0.202	4.77	**		
		XCFD168$_{(a_1)}$	0.58	0.183	3.154	**		
Spike dry weight	First	Constant	2.45	0.191	12.834	**	0.579	0.519
		XCFD5$_{(a_2)}$	0.34	0.12	2.84	**		
		XGWM124$_{(a_2)}$	– 0.36	0.13	– 2.762	*		
		XGWM265$_{(a_2)}$	– 0.26	0.124	– 2.082	*		
Stem weight	First	Constant	1.494	0.061	24.53	**	0.397	0.370
		XGWM124$_{(a_2)}$	– 0.28	0.072	– 3.89	**		
Biological yield	Second	Constant	2700.34	90.057	29.99	**	0.273	0.241
		XGWM136$_{(a_2)}$	– 591.86	201.37	– 2.94	**		
Hectoliter weight	Second	Constant	388.084	15.864	24.464	**	0.554	0.490
		XGWM136$_{(a_5)}$	– 100.724	32.332	– 3.12	**		
		XCFD5$_{(a_1)}$	– 93.40	30.323	– 3.08	**		
		XGWM70$_{(a_2)}$	155.314	69.22	2.244	*		
Number of spike per m^2	Second	Constant	574.27	19.764	29.06	**	0.434	0.382
		XGWM234$_{(a_1)}$	– 78.89	27.163	– 2.904	**		
		XGWM136$_{(a_4)}$	– 76.571	28.91	– 2.65	*		

*, **significant at 5% and 1% probability levels, respectively

†a_1, a_2, a_3, a_4, and a_5 are the average alleles 1, 2, 3, 4, and 5, respectively

second year, and jointly, in both years, the XGWM124$_{(a_2)}$ marker were the most repeated. So, these markers can be used more in breeding programs.

Discussion

High significant differences for most of the traits indicated the genetic diversity among genotypes, and it is possible to select superior genotypes based on agronomic traits in rainfed and irrigated conditions. High significant variation ($p < 0.01$) among the spring wheat for all morphological traits measured under drought stress was reported (Ahmed et al. 2022). In the research on genome-wide association mapping of genomic regions associated with drought stress tolerance at seedling and reproductive stages in bread wheat, analysis of variance (ANOVA) indicated highly significant differences among the wheat genotypes for all the studied seedling traits, including shoot weight, plant height, thousand-kernel weight, and grain yield under drought stress (Reddy et al. 2023), which was consistent with the results of this research.

Plant breeding programs mainly focus on genetic diversity, inheritance, conservation, and evolution (Peterson et al. 2014). The diversity in a population can be evaluated by various methods such as simple measurement of diversity, analysis of variance components, and molecular markers applied in this research. In this study, the estimation of genetic parameters showed that the phenotypic variation coefficient values were more significant than the genotypic variation coefficient in both conditions, which shows the existence of environmental effect on the expression of traits, according to researchers like Kumar et al. (2023b) and Amare (2023). Also, it is likely evidence of genotype-environment interactions (Kaur et al. 2023). Therefore, selecting these genotypes based on phenotype may help increase grain yield (Kobir

et al. 2023). Also, in the case of most studied traits, the genotypic variation was more than the environmental variation. Therefore, if a significant part of the phenotypic diversity is genetic, it indicates that the effect of the environment on the emergence of this trait is small; if the contribution of the genetic variation coefficient is small, it means that the resulting diversity is not only due to genotypes but also due to the effects of the environment. A low broad-sense heritability value indicates more phenotypic variation due to the effect of the environment on the traits, and breeding through selection is problematic because it covers genotypic effects. A high amount of genetic advance or genetic gain indicates that the trait is controlled by additive genes, and selection helps improve such a trait. The low genetic advance or genetic gain indicates that the trait is controlled by non-additive genes and heterosis breeding will be helpful (Farshadfar 2010). In this research, concerning the issue of high heritability along with high genetic gain in traits indicating the additive effects of genes and the selection of early generations may be more favorable for these traits, with the opinions of researchers such as Kumar et al. (2023a) and Kumar et al. (2023b). Therefore, heritability and genetic gain are essential parameters for the selection of breeding methods. In the present research, by comparing the results obtained in each environmental condition, it was observed that stress has led to an increase in heritability and advanced or genetic gain in some traits investigated in this research. Traits with h^2_b ($>$ 60.0%) and GG ($>$ 20.0%) together indicate that the variation is mainly due to genetic factors, making them reliable candidates for a selection process (Sallam et al. 2024; Kobir et al. 2023), which is similar to the results of this study.

Heritability is a powerful indicator that explains the transfer of traits from parents to their progeny. Heritability estimation helps the plant breeder to select elite genotypes from a genetically diverse and segregating population (Farshadfar 2010). The genetic advance is the mean genotypic value over the parental population, a measure of genetic gain under selection (Kumar et al. 2023b). Therefore, in this research, the estimation of the broad-sense heritability of traits showed that in both years, in rainfed conditions, the TGW trait; in irrigated conditions, the GY, TGW, OIL, SGW, and HI traits; and so on. Common in both environmental conditions, the TGW trait (according to the opinions of Mulugeta et al. (2023) in durum wheat and Krishnappa et al. (2023) in bread wheat) had high broad-sense heritability. Also, based on the genetic gain in both years, in rainfed conditions, OIL and StW traits, in irrigated conditions, in addition to GY, traits of OIL, SGW, and HI, and jointly in both environmental conditions, the OIL trait had the highest genetic gain, which was more suitable for the selection of traits. The results of genetic parameters, in terms of GY, TGW, and HI traits, were consistent with the opinion of Choudhry and Khaliq (1992) concerning bread wheat. In a study, evaluating the genetic diversity of Ethiopian durum wheat landraces under water-stressed and nonstressed conditions, intermediate to high estimates of the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in a broad sense (h^2_b), and genetic advance in percent of the mean (GAPM) were observed for all the studied traits except for 1000 seed weight at stress. The estimation of variability parameters showed that genotypic variation was higher than environmental variation for most traits. Also, the intermediate to high estimate of h^2_b and relatively high estimate of GAPM were observed in spike length and grain yield at normal conditions and in spike length at stressed conditions (Dukamo et al. 2023), which was consistent with the results of this study. Due to the high heritability and genetic gain of the measured traits, high genetic efficiency will be expected. Selection based on these characteristics leads to the release of new cultivars.

In evaluating the genetic diversity of bread wheat genotypes studied using SSR markers (Table 4), it can be concluded that XCFD168, XGWM350, and XGWM136 markers with 100% polymorphism, the highest number of alleles, high amount of polymorphism information content indices, marker index, effective polymorphism ratio index and resolving power index, and considering the high reproduction of bands and the production of high polymorphism bands to the most appropriate primers for wheat were introduced in subsequent studies. They have the necessary efficiency to investigate the relationships between genotypes and genetic structures. The XCFD168 marker was introduced as the superior marker in the study of Ramya et al. (2010), which was also consistent with the results of this study. Also, the large number of amplified sites in this experiment shows that a small number of SSR primers with high polymorphism information can distinguish many samples and different sample populations. Already, various researchers have reported the high efficiency of SSR markers in studies related to determining genetic diversity and evolutionary relationships in different plant species (Dagnaw et al. 2023; Jabari et al. 2023; Khodadadi et al. 2023). Markers with high polymorphic information content values can reveal variation between alleles and effectively be used for molecular mapping and analysis of genetic variation in populations (Kalivas et al. 2011). In research on morphological characteristics using 12 spike-related traits and analysis of genetic diversity of durum wheat (*Triticum turgidum* var. durum), high genetic diversity using 10 SSR markers, with polymorphic information content (PIC), was obtained as 0.69 (Ouaja et al. 2020). An evaluation of the genetic diversity of 17 bread wheat hexaploid genotypes using 16 microsatellite markers (SSR) concluded that only 11 markers had the most polymorphism and reproducibility (Kara et al. 2020). In a study to investigate the genetic diversity of Iranian wild relatives of bread wheat using ISSR and

SSR markers, the polymorphism information content (PIC), marker index (MI), and resolving power (Rp) in SSR marker were 0.830 to 0.919, 1.326 to 3.167, and 3.169 to 5.692, respectively (Jabari et al. 2023). Therefore, based on the results of molecular markers in this research and the results of other researchers, the application of molecular markers, including SSR as indirect selection criteria, plant genetic improvement, and cultivar release operations, is performed faster and more accurately in breeding programs.

In this research, the high value of the cophenetic correlation coefficient showed that the cluster analysis using Jaccard's similarity coefficient based on the data obtained from the SSR marker was very suitable in the grouping of the studied wheat genotypes (Fig. 2). The results of the principal coordinate analysis based on the SSR marker also showed a high agreement with the results of the cluster analysis using the UPGMA method based on the Jaccard similarity coefficient. Finally, genotypes 1, 3, and 25 have the most significant genetic distance with genotypes 13, 7, and the Pishgam cultivar. Therefore, it is recommended to use superior genotypes in breeding programs and genotypes with high genetic distance based on molecular studies to use heterosis in genetic diversity programs.

A dendrogram was made based on the similarity matrix to evaluate bread wheat cultivars using SSR markers. The cultivars were divided into three main groups, which showed the similarity between cultivars based on morphological characteristics of a limited range. In contrast, the similarity between cultivars based on the SSR range was comprehensive. Therefore, SSR markers were reported to be more accurate than morphological traits (Feltaous 2019). In a study using SSR markers and structure analysis, 105 bread wheat genotypes were classified into four clusters. This study showed the reliability of SSR markers, which can be helpful for future genetic studies, such as genetic mapping, comparative genomic analysis, genetic variation, and molecular marker-assisted breeding in wheat to select promising genotypes (Ahmed et al. 2020). In another study to assess genetic diversity and population structure based on SSR markers, 63 genotypes were classified into three clusters (Türkoglu et al. 2023).

Identifying QTLs controlling traits compatible with drought tolerance in breeding drought tolerance is necessary to understand how these traits are controlled and expressed (Sallam et al. 2019). In evaluating the association between studied traits in bread wheat genotypes with SSR markers in this research, the results showed a relationship between studied traits and primers. Although mapping based on quantitative trait loci (QTL) is suitable for tracking traits-related genes, this process is time-consuming and laborious (Rakshit et al. 2010). To overcome these limitations, it seems appropriate to identify markers related to traits through regression. Multiple regression analysis determines the explanation coefficient R^2, which indicates the degree of relationship between the trait and the molecular marker (Gomez and Gomez 1984).

Therefore, according to the results of this research (Tables 7 and 8), in both environmental conditions, in the first year, the markers XGWM410$_{(a_1)}$, XGWM577$_{(a_2)}$, XGWM350$_{(a_3)}$, XGWM165, XCFD5$_{(a_1)}$, XGWM136$_{(a_2)}$, XGWM265$_{(a_1)}$, and XGWM124 were common, and in the second year, the markers XGWM2$_{(a_2)}$, XGWM136 and XCFD5 about agronomic traits were common. Therefore, the presence of a significant association between several markers with one trait shows the existence of a quantitative and multigenic nature. The identification of common markers is essential in plant breeding because they enable the simultaneous selection of several traits (Tuberosa et al. 2002). Also, due to the high association of these markers, scanning in their adjacent chromosomal regions can effectively identify the genes controlling agronomic traits in wheat genotypes. In addition, the most positive markers in the first year, in rainfed conditions, respectively, are related to the traits of thousand grain weight, mitt penalty length, hectoliter weight, xteragen length, and spike grain weight, and in irrigated conditions, respectively, related to the traits of thousand grain weight, grain yield, spike length, and number of infertile spikelets, and in the second year, in rainfed conditions, related to thousand grain weight, spike dry weight, spike grain weight, and mitt penalty length, and in irrigated conditions, related to thousand grain weight trait. In this study, some markers related to traits were different in irrigated and rainfed conditions, indicating environmental conditions' influence on these traits. For this reason, different QTLs were identified in this experiment. In a study regarding stress tolerance using SSR markers, the XGWM577 marker was introduced as the superior marker (Younis et al. 2020). According to the results obtained from the researchers, there was a significant association between the grain yield trait and XGWM124 marker (Jlassi et al. 2021), between grain yield with the XGWM410 marker and 1000-grain weight and peduncle length with XGWM577 marker (Maccaferri et al. 2011), and between the grain yield with XGWM577 marker (Barakat et al. 2011; Rahmati et al. 2018) that was consistent with the results of this research. According to the opinion of Ali et al. (2011), the marker XGWM155 was related to the agronomic traits, and according to the results of this research, this marker was associated with the agronomic traits of 1000-grain weight, hectoliter weight, and xteragen length and had a significant association. In this research, the significant association between SSR markers and 14 agronomic traits on bread wheat was investigated using stepwise multiple regression analysis. The results showed that, for example, one marker with the trait of plant height, two markers with the trait of peduncle length, four markers with the trait of the number of spikelets per spike, two markers with the trait of the number of spikes, two markers with the trait of the number of seed per

spike, one marker with the trait biological yield, three markers with grain yield trait, and four markers with harvest index trait had significant association (Roy et al. 2006), which was consistent with the results of this research in terms of traits used with SSR markers. Sequencing markers produced by primers with high R^2 and comparing them with the sequences in the selection programs with the marker-assisted selection and gene matching based on the map increases their ability. Some markers were associated with more than one trait, which can be caused by pleiotropic effects or the continuity of related QTLs in different traits. Multivariate regression analysis identifying the quantitative trait loci (QTL) requires less time and cost (Ruan et al. 2009) and does not require the formation of a population for mapping. In an evaluation, 34 continuous SSR markers were identified with plant height trait, grain yield, and fodder yield in millet through association analysis (Kannan et al. 2014). Stepwise regression analysis was used to evaluate bread wheat genotypes in salt-tolerant conditions (Al-Ashkar et al. 2020). Population structure and analysis of the association between morphological traits in some landraces of Egyptian bread wheat were investigated using SSR, ISSR, and AFLP markers under heat stress. In total, 57 and 60 significant MTAs were obtained at the 1% probability level for ten traits under heat stress. Spike length (SL) had the highest (35) significant MTAs distributed across 23 loci, followed by 14 MTAs for the number of seeds per spike (NKSp) at both loci. SSR locus (Xgwm369) on chromosome 3A is pleiotropic with grain weight per spike (GWP), spike length (SL), and plant height (PH), and common to both loci, which were potentially important targets for selection. The results showed that the identified MTAs were related to the genes controlling important heat stress tolerant traits (Muhammad et al. 2020). In the study related to association analysis for agronomic traits in wheat under terminal heat stress, highly significant MTAs under heat stress conditions were identified for spikelet per spike (xwmc553), grains per spike (cxfa2147, xwmc418 and xwmc121), biomass (xbarc7), and grain yield (xcfa2147 and xwmc671). The identified markers in this study could facilitate MAS and gene pyramiding against heat stress in wheat (Khan et al. 2021). Genetic diversity and phylogenetic relationships of wheat (*T. aestivum* L.) genotypes using phenological, molecular, and DNA barcoding markers, the 16 wheat genotypes showed significant genetic variation using the markers assayed. Desirable phenological parameters with molecular markers were convenient for evaluating Egyptian wheat genotypes (El-Esawi et al. 2023). Based on the results of this research and other researchers, wheat, drought-tolerant superior genotypes with high grain yield, and yield components are desirable as donors parent in the breeding program. Identifying molecular markers related to agronomic traits can accelerate the screening of drought-tolerant genetic materials in breeding programs. The presence of molecular markers with a high explanatory coefficient for the examined traits allows breeders to select QTLs related to drought tolerance, regardless of the effect of environmental factors. We reached this achievement that marker-trait associations (MTAs) are key elements for identifying genomic regions associated with agronomic traits in wheat under drought stress. The identified MTAs could be applied in marker-assisted breeding, fine mapping, and cloning of the underlying genomic regions in wheat germplasm.

Conclusion

Using molecular markers along with regression analysis is a valuable tool in applying the genetic diversity of crops. It will make it possible to predict the state of plant growth by combining different methods before conducting the field experiment. In addition, some germplasms of a plant species are evaluated in terms of quantitatively important traits to drought tolerance stress using molecular markers. Also, the relationship of these markers with different traits is investigated; this will be a markable contribution to the breeding programs. In this research, the agronomic traits of wheat seedlings in two environments (irrigated and rainfed) were investigated during two consecutive cropping seasons. Significant differences between the genotypes were revealed for most traits, indicating the genetic diversity between the genotypes. The estimation of genetic parameters showed that the phenotypic variation coefficient was greater than the genotypic variation coefficient in both conditions. In the case of most studied traits, the genotypic variation was more than the environmental variation. Besides, almost high broad-sense heritability ($h^2_{b.s}$) and genetic gain were observed for many traits in this study, indicating their potential in wheat breeding. Therefore, due to the high genetic diversity, heritability, and genetic gain, it is possible to produce desirable cultivars through selection and hybridization. The XCFD168-2D, XGWM350-7D, and XGWM136-1A primers were identified as the most suitable primers for wheat in subsequent studies. These microsatellite primers used in this study can provide a high level of polymorphism and separate groups from each other—also, the XCFD168-2D marker in the first year with mitt penalty length and the number of infertile spikelets traits in irrigated conditions, the number of fertile spikelets in rainfed conditions and in the second year with spike dry weight trait in rainfed conditions; the XGWM350-7D marker in the first year with traits of thousand grain weight and number of grains per spike in both environmental conditions, number of infertile spikelets in rainfed conditions, xteragen length, peduncle length, and spike length in irrigated conditions and in the second year with the traits of thousand grain weight and spike grain weight in rainfed conditions; and the XGWM136-1A marker in the first year with spike length in both environmental conditions, grain yield in irrigated conditions, spike dry weight, spike grain weight, and number of

grains per spike traits in rainfed conditions and in the second year with hectoliter weight in both environmental conditions, xteragen length in rainfed conditions and harvest index, biological yield, and number of spike per m^2 traits in irrigated conditions had a high significant association. Cluster analysis classified 25 bread wheat genotypes into four different groups. Finally, genotypes 1, 3, and 25 have the most significant genetic distance with genotypes 13, 7, and Pishgam, and these two groups can be introduced for breeding programs to release superior cultivars. In general, the results of association analysis by stepwise regression showed that in both years under rainfed conditions, the XGWM350 marker about 1000-grain weight, the XCFD5 marker about spike length trait, and the XGWM165 and XGWM70 markers about spike dry weight trait, and under irrigated conditions, the XGWM265 marker about grain yield trait jointly had a significant association. In addition, the XGWM136 and XCFD5 markers were common in agronomic traits in all environments. Meanwhile, the most common positive markers in both years are related to 1000-grain weight in both conditions, mitt penalty length, and spike grain weight in rainfed conditions. The association of these markers with the traits showed that their location inside the genome is probably the regions adjacent to the genes controlling the desired traits. Drought stress reduces genetic reserves, increases genetic erosion, and finally causes a decrease in yield production and a negative effect on human livelihood. Consequently, there is an urgent need to develop drought-tolerant and high-yielding genotypes to ensure sustainable production and global food security. Therefore, selection based on molecular markers, including SSR, and investigating their relationship with agronomic traits can be precious for accelerating classical breeding programs to select superior genotypes in different moisture conditions, produce high-yielding and drought-tolerant cultivars, and help the global production of wheat and create human food security.

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Data Availability I attached the original data in the excel form as suplementary file.

Declarations

Competing Interests The authors declare no competing interests.

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