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Analysis Of Population Structure and Genetic Diversity and Relationships in Saudi Arabia and Exotic Genotypes of Bread Wheat (*Triticum Aestivum* L.) Using Genomic Microsatellites Markers

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Abstract

To increase the economic value of crops, understanding population structure and genetic diversity is a very important objective. Loss of genetic diversity has been noted in wheat over the last few decades. The collection of thirteen genotypes from three separate regions, Saudi Arabia, Egypt and the USA were used to determine genetic diversity and population structure characteristics to answer this challenge. Using 121 SSR alleles that showed strong polymorphism among genotypes, these genotypes were analyzed. Of the 17 SSR loci, a total of 121 SSR alleles were identified and used to analyze population structure and perform principal coordinate analysis (PCoA). Large range of number of different alleles extended from 2 alleles for marker *Xgwm631* to 11 alleles for marker *Xtaglgap, Xgwm458*, *Xgwm190*, *Xgwm46* with an average of 7.12 for all 17 SSR loci. With an average of 0.317, 0.401 and 0.315, respectively, polymorphism information quality (PIC), gene diversity (GD) and minor allele frequency (MAF) ranged from 0.230 to 0.375, 0.260 to 0.500 and 0.150 to 0.460. Results of principal coordinate analysis (PCoA) agreed with structural analysis. Structure grouped thirteen wheat genotypes into three subpopulations with substantial subpopulation divergence (P > 0.001). Index of fixation (Fst), a population substructure indicator, was 0.283, 0.658 and 0.291 for G1, G2 and G3, respectively. Also, 34% variation among and 66% within populations was recognized by AMOVA. Current investigation has shown high GD among genotypes that can be used in Saudi Arabia to produce superior wheat cultivars with significant agronomic characteristics.

Keywords: Bread wheat, Genetic diversity, Genotypes, SSRs, Population structure

Introduction

Bread wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is a major crop with two distinct varieties; wild emmer wheat (*T. dicoccoides*, 2n = 4x = 28, AABB) and tausch's goatgrass (*Aegilops tauschii*, 2n = 2x = 14, DD) (Kihara 1944, Zohary 1970, Feldman et al. 1995). It was one of the first domesticated food crops and it was the staple food of major civilizations in West Asia, North Africa and Europe for 8,000 years (Van deWouw et al. 2010). Its genome has been limited since domestication. In many crop species, including wheat, domestication has resulted in the reduction of genetic variability (Reeves et al. 1997). The small genetic base is a major concern affecting the success of crop genetic enhancement (Hoisington et al. 1999). There has also been a substantial decrease in the genetic diversity of the various crop cultivars recorded in the last century (Raman et al. 2010 and Alsharari and Okasha 2021).

Decreased genetic diversity can be increased by the introduction of new alleles from different plant genetic resources. Consequently, the characterization and utilization of germplasm maintained in plant genetic resource centers are necessary to increase crop yield and resistance to pathogens in order

to meet the needs of the increasing human population (Reeves et al. 1997). In the case of imbalances, modern breeding practices and genetic drift have also led to the creation of large genomic masses in the wheat genome. This can be due to the targeted choice of preferred alleles in agronomic interest loci that control characteristics. As a result of the need to propagate unique characteristics of target environments, genetic diversity within breeding programs has been reduced. The exploitation of untapped alleles from genetically diverse germplasm gathered in genetic resource centers is critical for coping with an increasing population (Reeves et al. 1997, Hoisington et al. 1999, Morgante and Salamini 2003, Raman et al. 2010, Van deWouw et al. 2010, Novoselovic et al. 2016).

Genetic diversity and phylogenetic relationship characterization offer useful knowledge required to extend the narrow genetic base as well as strengthen crop breeding and conservation strategies (Salem and Sallam 2016). Population structure research also promotes a deeper understanding of germplasm diversity and studies on association mapping (Novoselovic et al. 2016, Salem and Sallam 2016). Therefore, different methods have been used to determine the extent and structure of genetic variation in many crops, including wheat, rice, and barley (Varshney et al. 2008, El Rabey et al. 2013). Environmental factors are influenced by the study of genetic diversity based on these phenological, morphological and biochemical characteristics (Caballero et al. 2004). Therefore, in genetic diversity research, molecular DNA markers were developed and proved effective (Röder et al. 2002, Eltaher et al. 2018).

Molecular marker-based genome analysis is a more rigorous method for assessing species diversity than casual morphological or physiological-level analysis [9]. For the measurement of genetic variation, molecular markers such as randomly RAPD, RFLP, AFLP, SSRs, EST-SSRs were used to estimate the proportion of similar or different alleles in landraces and cultivated wheat genotypes (Paull et al. 1998, Soleimani et al. 1998, Eujayl et al. 2001). The most commonly applied approaches in the study of plant genetic diversity were microsatellites among these markers, as they are abundant, codominant, highly polymorphic and widely distributed along chromosomes (Röder et al. 1998). SSRs have been used in many crops, including wheat, for genome profiling, association mapping, fingerprinting and genetic diversity and structure assessment (Salem 2015, Salem and Mattar 2014). For further wheat genetic diversity studies, SSR markers were further used and proved effective (Salem et al. 2015).

In this study, genome-level molecular diversity was estimated in thirteen landraces using eighteen SSRs markers and collected comparative diversity indicators and trends from various geographic regions of Saudi Arabia in order to i) identify specific sources of alleles for wheat improvement programs, ii) characterize the allelic, genetic diversity and population structure of Saudi Arabi and exotic bread wheat genotypes using SSRs markers, iii) compare the genetic properties among wheat genotypes subpopulation and iii) evaluate the potential application of current results for future studies on the evaluation and establish a wheat genetic resource conservation strategy. In order to determine the genetic diversity of traits of agricultural interest, more research may be performed on different genetically determined germplasm and used to study the origin and transmission of specific alleles.

Material and Methods

Plant material

In total, thirteen distinct genotypes of landrace and exotic bread wheat (*Triticum aestivum* L.) were used in this analysis (**Table 1**). All genotypes were extracted from the germplasm collection of the

National GenBank, Agricultural Research Center (ARC), Minister of Environment, Agriculture and Water, Riyad, Saudi Arabi and Agricultural Research Center (ARC), Egypt.

Table 1. List of thirteen bread wheat landraces used in this study.

Serial	Accession ID number	Species	Pedigree	Source of seeds	Local Name	Collected location status
1	110	*T. aestivum*	Landrace	National GenBank	Al Ahsa Burr	Al Ahsa (Al Ahsa Province)
2	129	*T. aestivum*	Landrace	National GenBank	Al Ahsa Burr, Salt Tolerant	Al Ahsa (Al Ahsa Province)
3	302	*T. aestivum*	Landrace	National GenBank	Baldy Maya Burr	Al-Namas (ِِAsir Province)
4	552	*T. aestivum*	Landrace	National GenBank	Baldy BurrYamani	Tabuk (Tabuk Province)
5	18	*T. aestivum*	Landrace	National GenBank	Alssamaa Burr	Almajmah (Riyadh Province)
6	296	*T. aestivum*	Landrace	National GenBank	Maya Balady Burr	Asir (ِِAsir Province)
7	20	*T. aestivum*	Landrace	National GenBank	Hanta Asmer	Tamir, Almajmah (Riyadh Province)
8	6	*T. aestivum*	Landrace	National GenBank	Halibah Burr	Buraydah (Al-Qassim Province)
9	--	*T. aestivum*	Landrace	National GenBank	Alssamaa Burr	---
10	--	*T. aestivum*	Cultivar	National GenBank	Classic Burr	---
11	--	*T. aestivum*	Cultivar	National GenBank	Yecora Rojo	USA wheat
12	--	*T. aestivum*	Cultivar	ARC	Gemmiza 11	Egyptian wheat
13	--	*T. aestivum*	Cultivar	ARC	Gemmiza 10	Egyptian wheat

Genomic DNA extraction

DNA was isolated and purified from the wheat leaves Per each genotype. Young leaves from the thirteen genotypes of 2-3 leaf seedlings (two-week-old seedlings) as defined by (Salem 2004).

SSRs genotyping

Selected seventeen wheat microsatellite (SSRs) markers for sixteen loci representing chromosomes 1A, 1B, 1D, 2A, 2D, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5B, 5D, 7A and 7B for genotyping (Table 2). Devos et al. (1995) defined the primer sequence of *Xtaglut* and *Xtaglgap*. Following Röder et al. (1998) and Devos et al. (1995), microsatellite amplifications were performed (**Table 2).**

Table 2. Description of SSR markers, chromosomal location, motif, distance from the centromere, expected fragment size (bp) and annealing temperature (ºC)

Locus	Chromosomal location	Motif	Distance % from Centromere	Expected fragment size (bp)	Annealing Tm (ºC)
Xtaglut	1A	(CAG)5(CAA) 8	-	100	50
Xgwm018	1BS	(CA)17GA(TA)4	5.2	183	55
Xtaglgap	1BS	(CAA)15	76.92	282	60
Xgwm458	1D(c)	(CA)13	0	112	60
Xgwm095	2AS	(AC)16	2.09	122	60
Xgwm261	2DS	(CT)21	51.39	189	55
Xgwm155	3AL	(CT)19	57.31	144	60
Xgwm389	3BS	(CT)14 (GT)16	98.91	129	60
Xgwm003	3DL	(CA)18	42.89	79	55
Xgwm160	4AL	(GA)21	100	182	60
Xgwm513	4BL	(CA)12	12.26	140	60
Xgwm165	4DL	(GA)20	-	187	60
Xgwm186	5AL	(GA)26	22.66	135	60
Xgwm408	5BL	(CA)>22(TA)(CA)7(TA)9	63.13	173	55
Xgwm190	5DS	(CT)22	100	209	60
Xgwm631	7AS	(GT)23	4.02	196	60
Xgwm046	7B(c)	(GA)3GC(GA)33	0	179	60

Microsatellite markers analysis

Genetic properties of markers

PowerMarker software V 3.25 was used to measure the statistical description of all eighteen SSRs markers, such as gene diversity, polymorphism information material (PIC) and minor allele frequency (MAF) (Liu and Muse 2005). The informativeness of the PIC microsatellite markers was measured for each marker according to Nei (1973). Gene diversity (GD), the probability that two randomly selected population alleles are different, was calculated by DeGiorgio and Rosenberg (2008).

Population structure

To determine the number of subpopulations among all genotypes with the STRUCTURE 3.4.0 program, a model-based (Bayesian) approach was used (Pritchard et al. 2000). Using k-values (assumed fixed number of subpopulations), the structure was evaluated from 1 to 10 in the whole population. For each k-value, three independent tests were carried out and the software was set at 100,000 as a burn-in iteration, followed by 100,000 replications of the Markov chain Monte Carlo (MCMC) after burn-in. The principal co-ordinates analysis (PCoA) was performed based on the genetic distance between the genotypes using NTSYS-pc version 2.1 software (Rohlf 2000) to determine the relationship between the genotypes under investigation.

Analysis of molecular variance (AMOVA)

A molecular variance (AMOVA) analysis was conducted using GeneAlEx 6.411 with 1000 permutations and the number of different alleles (Peakall and Smouse 2006).

Results

Genetic Diversity, PIC and MAF

With an average of 0.317, 0.401 and 0.315, respectively, polymorphism information quality (PIC), gene diversity (GD) and minor allele frequency (MAF) ranged from 0.230 to 0.375, 0.260 to 0.500 and 0.150 to 0.460 (**Fig. 1a**). A total of 121 alleles were detected from a set of 17 SSR loci on a panel of thirteen wheat genotypes. The number of alleles ranged from 2 alleles for marker *Xgwm631* to 11 alleles for marker *Xtaglgap, Xgwm458, Xgwm190, Xgwm46*, with an average of 7.12 for all 17 SSR loci **(Fig. 1b).**

Population Structure and relationships

In order to research the population structure and relationships between certain genotypes, structure analysis software was used. By plotting the number k against the estimated likelihood value [lnP(D)] derived from STRUCTURE runs, the best number of subpopulations was determined. Obviously, for all the values observed, lnP(D) proved to be an increasing function of k (**Fig. 2a and Fig. 2b**). The rate of change of the likelihood distribution is shown in Fig. 2a and the absolute values of the 2nd order rate of change of the likelihood distribution are shown in Fig. 2b using STRUCTURE analysis software. Structure simulation showed that the best k was the estimated average of lnP(D) against k= 3, suggesting that three subpopulations could comprise all the thirteen most probable wheat genotypes. This can also validate by plotting the number k against the number delta k. For k = 3, a sharp peak was found for **(Fig. 2c**). To show the genetic structure of the thirteen wheat genotypes, a k value of two was therefore chosen **(Fig. 3)**. The projected population structure indicated that partial member-ship genotypes displayed distinctive personalities. The findings of PCoA agreed with structure studies by grouping the genotypes of thirteen wheat into three distinct groups **(Fig.4).** The structure and principal coordinate analysis showed that thirteen genotypes of wheat were split into three subpopulations (G1, G2 and G3). Subpopulation G1 contained five genotypes (Al-Ahsa Burr, Maya Balady Burr, Alssamaa Burr, Classic Burr and Yecora Rojo). Although two genotypes were in subpopulation G2 (Al-Ahsa Burr salt-tolerant and Gemmiza 10) and six genotypes (Baldy Maya Burr, Baldy, Alssamaa Burr, Hanta Asmer, Halibah Burr and Gemmiza 11) were in subpopulation G3. There was a major divergence between subpopulations and the mean distance between genotypes in the same subpopulations **(Table 3**). The mean distance between G1 and G3

individuals was found to be similar to each other, 0.308 and 0.307, respectively. The mean distance between individuals in G1 was found to be 0.168. The index of fixation (Fst) is a population substructure indicator and is most useful for analyzing the overall genetic divergence between subpopulations. Thus, for G1, G2 and G3, the Fst value was 0.283, 0.658 and 0.291, respectively. In G3 six genotypes, the maximum number of samples was found, followed by G1 five genotypes and then G2 two genotypes.

The AMOVA, Fst and Nm or a haploid number of migrants showed that genotypes within subgroups are strongly genetically differentiated between groups in comparison to genotypes, indicating a higher percentage of variation within groups than between groups (Table 4). Sixty-six percent of the genetic variation existed within subgroups of wheat genotypes, while 34% of the genetic variation was among subgroups. The haploid (Nm) was 0.49, suggesting low gene flow (exchange) between the subgroups and high differentiation within subgroups.

Table 3. STRUCTURE analysis of thirteen genotypes showing fixation index (Fst), predicted heterozygosity and number of genotypes in each subpopulation

Subpopulation groups	Fst	Expected heterozygosity	Number of landraces
G1	0.283	0.308	5
G2	0.658	0.168	2
G3	0.291	0.307	6

Table 4. Molecular variance analysis using 121 SSRs of the genetic differentiation among and within three subpopulations of thirteen Saudi Arabia and exotic wheat genotypes

Source of variation	df	SS	MS	Est. Var.	%	*P-value*
Among subgroups	2	16.887	8.444	1.429	34%	0.001
Within subgroups	10	27.267	2.727	2.727	66%	0.001
Total	12	44.154		4.156	100%	0.001
Fixation Index	0.34					
Nm (Haploid)	0.49					

AMOVA: analysis of molecular variance; df: degrees of freedom; SS: sum of squares deviation; MS: mean squared deviation; Est. Var.: estimates of variance components; %: percentage of total variance contributed by each component.

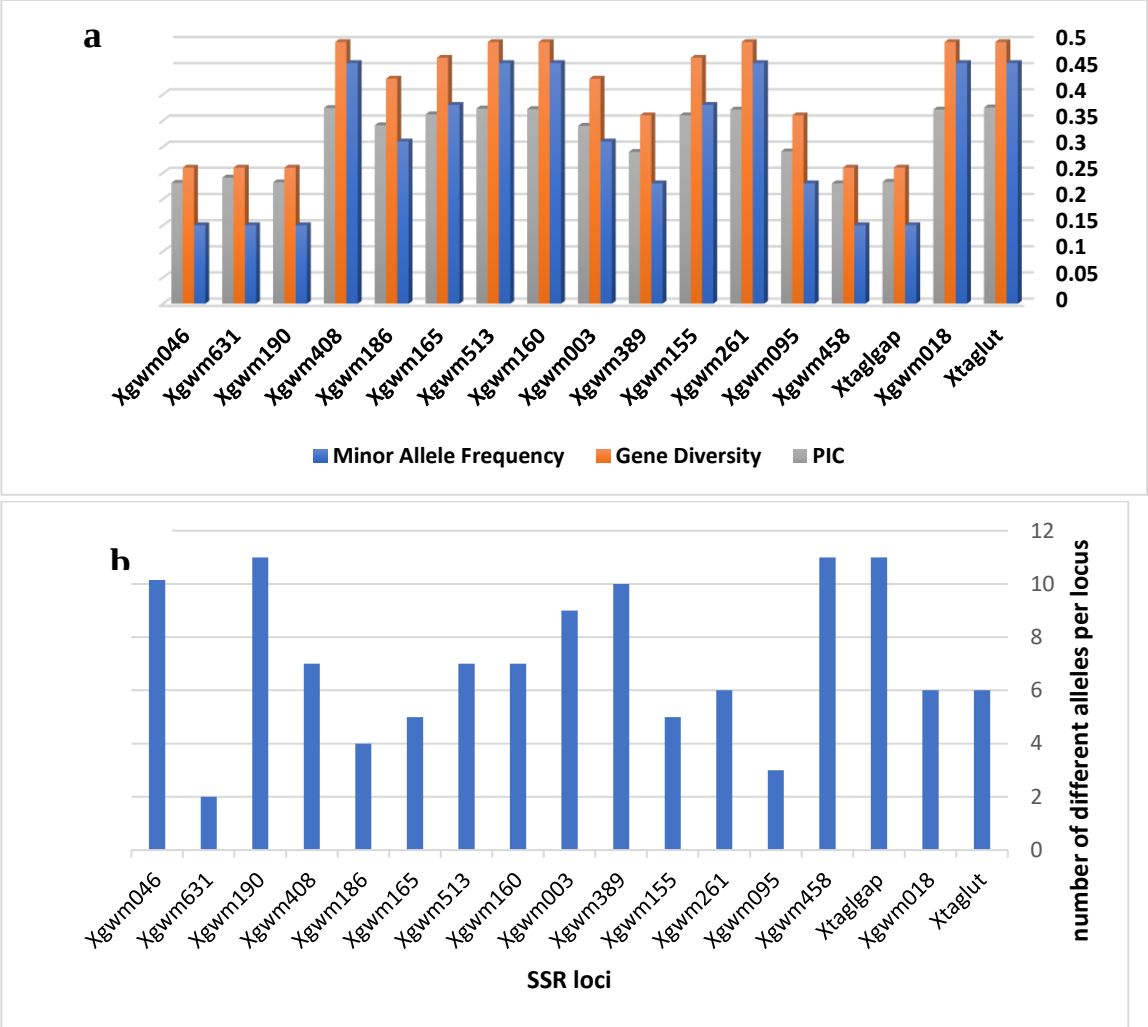
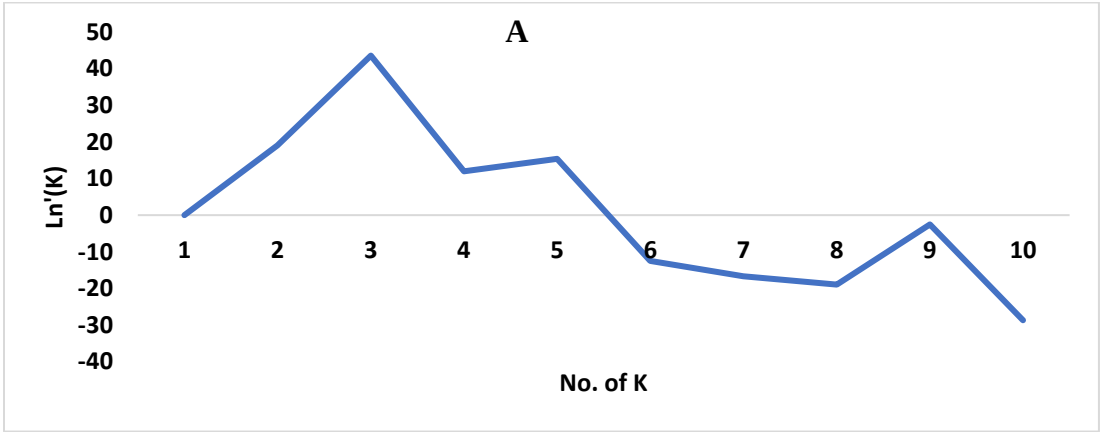
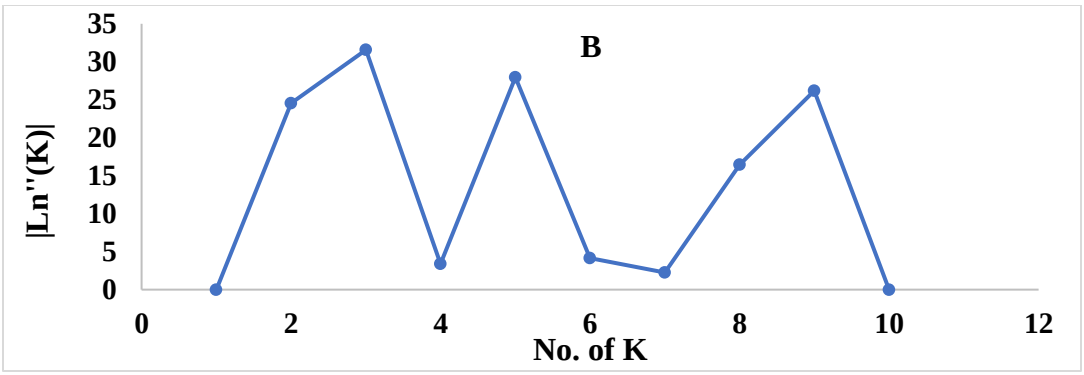


Fig. 1. Genetic diversity distribution of the 121 SSR markers used in the genetic diversity study for thirteen genotypes of wheat.(a) diversity of genes and the polymorphic information content (PIC) for each marker and (b) the number of different alleles per loci.





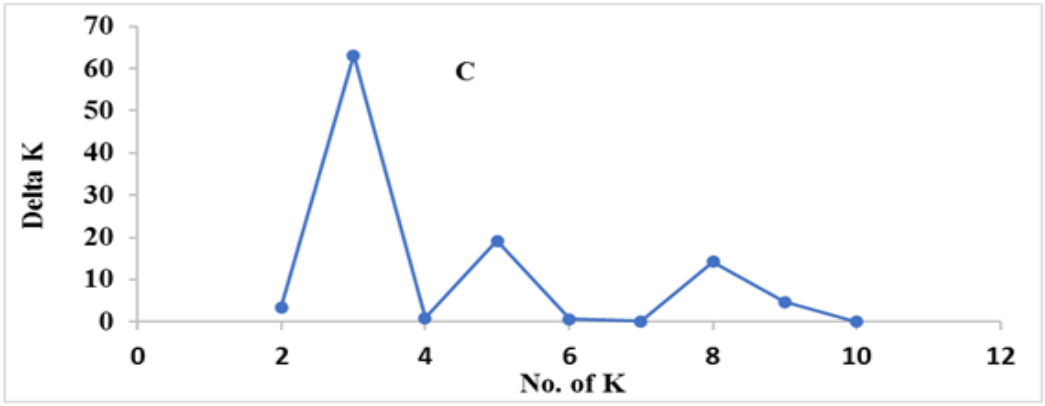


Fig. 2: Population structure analysis of wheat genotypes using 121 SSRs; (a) shows Rate of change of the Likelihood distribution (using STRUCTURE), (b) shows the absolute values of the 2nd order rate of change of the Likelihood distribution (c) DK for differing numbers of subpopulations (k). Within the population (using STRUCTURE). Unfilled square point refers to the best k = 3

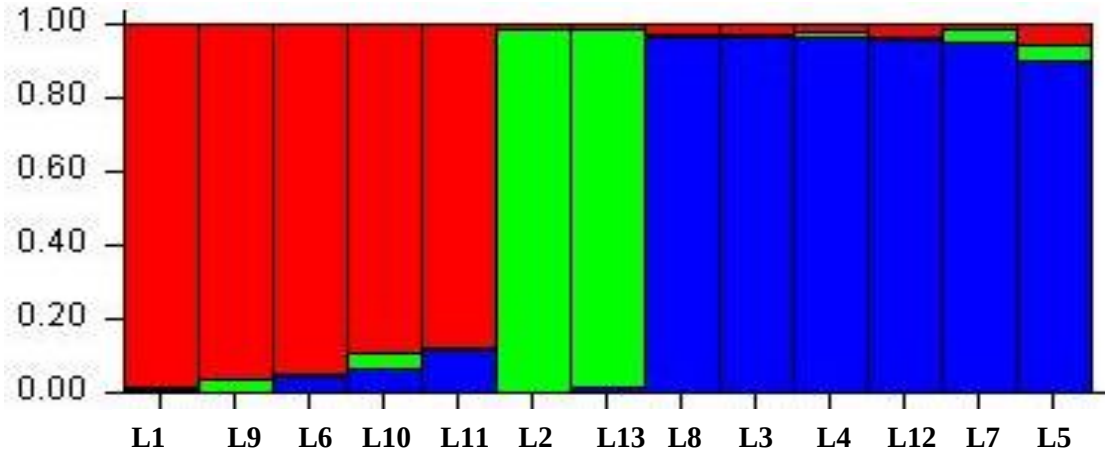


Fig. 3. The approximate population structure of wheat landraces as shown by 17 SSR markers for (k = 3), red indicates group 1, green indicates group 2 and blue indicates group 3.

Discussion

It is a prerequisite for the conservation and productive use of usable germplasm for plant breeding to consider the extent and structure of genetic diversity of a crop (Laidò et al. 2013). In addition, its monitoring will help us select parents with preferred alleles and determine changes in allelic frequencies (Christiansen et al. 2002). Eighteen microsatellite markers revealing 121 alleles from the thirteen local and exotic wheat genotypes were enough in the current study to discriminate against wheat genotypes in Saudi Arabia. With an average of 7.12, the number of alleles per marker ranged from 2 to 11. Using microsatellite markers, several average allele numbers have been found in wheat. In 55 genotypes of wheat, Prasad et al. (2000) found an average of 7.4 allele numbers. Out of nineteen wheat microsatellites of 502 European varieties, Röder et al. (2002) found an average allele number of 10.5 alleles per marker. In comparison, Huang et al. (2002) analyzed 998 accessions from 68 countries with 26 microsatellites and thus found an average of 18.1. In 54 varieties, Khlestkina et al. (2004) found an average of 6.6. An average allele number of 3.9 in seven Turkish wheat genotypes was identified by Akfirat and Uncuoglu (2013). In 33 Egyptian wheat genotypes, Salem et al. (2015) identified 95 alleles with an average of 5.59 alleles per locus. In 55 landrace populations, Kyratzis et al. (2019), 224 alleles were identified with an average of 11.79 alleles per locus. A comparison of the results found in the current study with those previously published shows that the average number of alleles per locus recorded during the current study was lower than some previous studies, but comparable to the results of Salem et al. 2015, Prasad et al. 2000, Khlestkina et al. 2004, which found 5.59, 7.4.6 and 6.6 alleles per locus in wheat genotypes, respectively.

Polymorphism information quality, gene diversity and minor allele frequency

The values of GD, PIC and MAF are extremely useful for studying the degree of polymorphisms between genotypes and are particularly useful in wheat breeding programs (Eltaher et al. 2018, Huang et al. 2002). In this analysis, the average of (PIC), (GD) and (MAF), 0.317, 0.401 and 0.315, respectively, was lower than that reported in SSR marker-based studies (Salem et al. 2015, Huang et al. 2002) and moderate according to Würschum et al. (2013). PIC value > 0.5 is considered to be an extremely informative marker, while 0.5 > PIC > 0.25 is a fairly informative marker, and PIC value < 0.25 is a marginally informative marker as reported by Botstein et al. (1980). The main reason for this type of SSR marker is descriptive markers and moderate PIC values due to the number of alleles per marker than other SSR markers (SSR motif). An inverse relationship exists between the number of alleles and the SSR marker is informativeness. In comparison, as the number of alleles increase, the PIC values increased (Salem et al. 2015, Huang et al. 2002).

Population Structure and relationships

In this analysis, 121 SSR alleles were used to estimate the population structure of thirteen wheat genotypes from three regions: Saudi Arabia, Egypt and the USA, obtained from the 17 SSR loci. In previous studies, SSRs have been identified as DNA markers that show a high level of polymorphism in plants Powell et al. (1996). The 13 genotypes of wheat were divided into three subpopulations with substantial subpopulation divergence (*P > 0.001*). Similar results have been published by Salem et al. 2015 and Huang et al. (2002). The AMOVA study showed a high degree of genetic diversity within populations (66%). At 34%, the genetic diversity among subpopulations was low. This low genetic differentiation between genotypes may be attributable to gene flow resulting from seed movement Dhanapal et al. (2014). To

increase the diversity of local germplasms, farmers prefer to exchange seeds. This leads to an increase in the distribution of alleles, irrespective of their geographical distance, among different populations Louette et al. (1997).

The results of the PCoA analysis were in agreement with the population structure. All the thirteen genotypes were clustered into three groups with the same genotypes as revealed by STRUCTURE. The G3 that represented population 3 showed higher genetic diversity than G2 and G1, respectively. This is not surprising, since G3 included 6 genotypes covering two different regions (Saudi Arabia and Egypt), while G2 showed low genetic diversity because it contained two genotypes from one region (Saudi Arabia and Egypt). Previous studies have also found various subpopulations according to collection districts, such as durum wheat landraces, to constitute nine subpopulations (Ruiz et al. 2012); six subpopulations had a durum wheat elite collection (Maccaferri et al. 2005). The G3 offers a valuable source of wheat genetic diversity since genotypes from two different sections were included in it (Saudi Arabia and Egypt). In potential breeding programs, these genotypes will be used to increase genetic diversity in wheat. In the marker-assisted selection and genome-wide association studies, such diversity could be very helpful by creating a multi-parent advanced generation inter-cross (MAGIC).

Within subgroups, the AMOVA showed a moderate degree of diversity. Although the variation between populations was lower compared to the variation within the population, it was important. Selection for morphological traits can be due to this mild variance within classes. The low value of gene flow (0.49) or exchange between lines within subpopulations can explain the 34% degree of heterogeneity among the population. The degree of differentiation among the population is inversely proportional to the value of gene flow, as a general indicator of the magnitude of genetic exchange (Arora et al. 2014). The value of Nm (Haploid) less than 1 indicates restricted gene exchange between populations, leading to low variability between groups (Eltaher et al. 2018). The gene flow is called low gene flow in the present research, which led us to moderate genetic differentiation between populations (Wang et al. 2012). In subpopulations 1, 2 and 3, hybridization between genotypes may cause the required variation to boost genetic benefit through active selection.

Conclusion

Polymorphism was explained by wheat microsatellite markers and considered in local and exotic bread wheat to be adequately informative in the present investigations. In order to enhance the wheat breeding program, the genetic diversity levels found in in local and exotic bread wheat genotypes may be useful for the wheat breeder. The estimated parameters of genetic diversity indicate that these genotypes may be a potentially useful source for the selection of diverse parents in wheat breeding programs for heterotic combinations.

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Compliance with ethical standards

Conflict of interest

The author declares no conflict of interests.

Ethics approval and consent to participate

Not applicable

Availability of data and materials

All data generated or analyzed during this study already exist in this published article.

Authors' contributions

SFA contributed to the design, performance of the lab experiment, data analysis, writing of the manuscript and following up the publication with the journal (correspondence).

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