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SSR-based molecular profiling reveals genetic diversity and interspecific admixture between *Triticum aestivum* L. and *Triticum durum* Desf

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ABSTRACT

Wheat (*Triticum* spp.) is a global staple, with hexaploid bread wheat (*Triticum aestivum* L., $2n=6x=42$) and tetraploid durum wheat (*Triticum durum* Desf., $2n=4x=28$) accounting for the vast majority of global production. Characterizing genetic diversity and population structure across these species is essential for germplasm conservation and parental selection in breeding programs. In this study, 25 wheat genotypes (13 from *T. aestivum* and 12 from *T. durum*) were evaluated using 28 simple sequence repeat (SSR) markers to assess genetic variation and interspecific population admixture. Twelve polymorphic SSR loci yielded 33 total alleles, ranging from 2 to 4 alleles per locus (mean = 2.75). The mean polymorphic information content (PIC) and observed heterozygosity were 0.41 and 0.32, respectively, indicating moderate genetic diversity. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis based on Nei's genetic distance effectively stratified the germplasm into two major species-specific clusters corresponding to hexaploid bread wheat and tetraploid durum wheat. Notably, two genotypes-GDW-1255 and DBPY-08-22-positioned between the primary clusters, revealing significant interspecific admixture. Furthermore, highly divergent entries such as GW-2013-503 and 18th-SBWOM-HT-85 were identified as valuable candidates for hybrid development. These findings demonstrate the utility of microsatellite markers in resolving ploidy-level population architecture, identifying admixed lines to control for structure in genome-wide association studies (GWAS), and selecting diverse parental combinations to maximize heterosis.

Keywords: Molecular diversity; Population structure; *Durum wheat* Bread wheat microsatellites; interspecific admixture

INTRODUCTION

The hexaploid wheat is staple food in major parts of the world, feeding more than one-third of the globe (Bhatta *et al.*, 2018). Wheat is the world's most widely cultivated staple food crop that possesses second important position in India. The cultivation of wheat in India was initiated more than 5,000 years back during the Indus valley civilization era, where the *Triticum sphaerococcum* found. However, nowadays *Triticum sphaerococcum* has disappeared and replaced by present species viz., *Triticum aestivum* L. (Bread wheat) (Hexaploid, $2n=6x=42$, AABBDD), *Triticum durum* Desf. (Macaroni wheat) (Tetraploid,

$2n=4x=28$, AABB), and *Triticum dicoccum* Schub L. (Emmer wheat) (Tetraploid, $2n=4x=28$, AABB) covering 87%, 12%, and 1% of the total wheat-growing area, respectively (Kulshreshtha, 1985). The bread wheat is cultivated in all the wheat-growing areas of the country, the macaroni or *durum* wheat is primarily grown in the Northern (Punjab) and Southern states. In contrast, the emmer wheat is confined to the Southern states (mainly Karnataka) and some parts of Gujarat (Das, 2008).

At the time of independence (1947), India could not produce sufficient food for the fulfillment of population demand. Now, India produces surplus production and export wheat in the global market. The production level of wheat in India

had a quantum jump from 6.46 million tons with 663 kg/ha productivity from an area of 9.75 million ha in 1950-51 to more than 103.6 million tons with 3507 kg/ha productivity from an area of about 29.14 million hectares during 2018-19 (Anonymous, 2020). The seed-storage proteins of wheat represent an essential source of food and energy, is also involved in determining bread-making quality (Cooke and Law, 1998). Wheat varieties are qualified into different classes, which exhibit various applications and differ in quantity and quality of proteins (*i.e.*, gluten). The gluten, comprising roughly 78 to 85% of total wheat endosperm protein, is a vast complex composed mainly of polymeric (multiple polypeptide chains) and monomeric (single chain polypeptides) proteins known as glutenins and gliadins, respectively (MacRitchie, 1994). Glutenins confer elasticity to dough, whereas gliadins are viscous and give extensibility to a dough (Payne *et al.*, 1984).

The genetic diversity of crops determines their potential for breeding efficiency and hence their use for the development of new varieties, which eventually may result in enhanced food production. Genetic diversity in the plant could result from geographical impact through evolution. Hence, traits could be considered as a function of variety. Estimation of genetic distance is one of the appropriate tools for parental selection of genotypes in breeding programs. Proper selection of the parents is essential to be used in crossing nurseries to enhance the genetic recombination for potential yield increase (Islam *et al.*, 2004). The traditional method for estimation of genetic diversity and relationships among groups of plants by using morphological characters, agronomic information and biochemical variations may influence by the environment and represent only visible diversity. Besides, the collection of such data is laborious. Hence, molecular markers are an attractive alternative (Storfer, 1996) widely for the evaluation of genetic diversity. Molecular markers are a relatively new breeding technology and are widely used by breeders to select variations in genotypes. Molecular markers are also helpful

to confirm gene flow, admixture present in cultivated lines received via human mediated breeding either natural or due to transformation from wild relatives. Out of various molecular markers, Simple sequence repeats (SSRs) (Tautz and Renz, 1984) has been widely exploited in wheat due to its high level of polymorphisms, co-dominant inheritance and equal distribution across the plant genome (Parker *et al.*, 2002, Röder *et al.*, 1995). SSR is more abundant, ubiquitous in presence, hypervariable in nature and has high polymorphic information content (Gupta *et al.*, 1996). SSRs markers may link with functional genes and such kind of markers may helpful for characterization of genotypes in respect to character specific diversity. The marker-based approach can determine the smallest possible allelic variation in coding regions.

With the above facts, objectives of the present investigation were i) to investigate and compare genetic diversity among 25 genotypes using 28 SSR markers, ii) to decipher the presence of population structure and cluster among population and iii) estimate the admixture proportion of individuals from the population to facilitate the hybrid breeding program

MATERIAL AND METHOD

SSR Genotyping and Cluster analysis

A set of 25 genotypes comprising of thirteen **bread wheat** (*T. aestivum* L.) and twelve **durum wheat** (*T. durum* Desf.) **genotypes** (Table. 1) was sown **under normal conditions** in a Randomized Block Design (RBD) with three replications. For genomic DNA extraction, the seedlings were collected at 15 days old and stored at -80 °C till use. Genomic DNA was extracted from the collected leaves using liquid nitrogen and the CTAB procedure with minor modifications (Doyle and Doyle, 1987). Based on quantification data from the spectrophotometer, a portion of DNA samples were diluted with an appropriate quantity of TE buffer to yield a working concentration of 50 ng/μl and stored at -20° C for further molecular analysis.

Table 1 : List of wheat genotypes used for the study

Sr. No.	Genotypes	Sr. No.	Genotypes
1	GW-451	14	GDW-1255
2	GW-496	15	DBPY-08-22
3	GW-366	16	GW-09-246
4	GW-11	17	GW-1287
5	GW-173	18	KLM-1005
6	GW-322	19	NIDW-295
7	15 th -HTWYT-9	20	VD-2013-02
8	15 th -HTWYT-12	21	GW-2010-286
9	15 th -HTWYT-21	22	QLD-23
10	15 th -HTWYT-28	23	GW-2015-691
11	15 th -SBWOM-60	24	GW-2013-503
12	18 th -SBWOM-HT-85	25	GW-1286
13	18 th -SBWOM-HT-86		

Based on the published literature (Ganal & Röder, 2007, Gupta *et al.*, 2002, Sourdille *et al.*, 2004, Guyomarc'h *et al.*, 2002, Song *et al.*, 2005) 28 SSR markers (Table 2.) were used for diversity analysis. PCR reaction was performed constituting of 15µl volume; 1.5µl of PCR buffer (10X), 200µM of dNTPs, 0.3 Unit *Taq* DNA polymerase, 5.0 pmoles/primer and 1.0 µl of template DNA (50ng). The PCR condition was: 5 min at 94°C; 35 cycles of 60s denaturation at 94°C, 60s annealing at 54°C to 58°C (depending on the melting temperature (T_m) of respective primer) and 2 min extension at 72°C in each cycle followed by 10 min at 72°C for final product extension and hold at 4°C. All the PCR amplified products were resolved on 3 per cent agarose gel.

Total number of alleles, Polymorphism Information Content (PIC) and heterozygosity (H) were analyzed by PowerMarker software version 3.25 (Liu and Muse, 2005). Genetic similarity was calculated by using Nei's similarity coefficient, and cluster analysis was performed using the UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) method. Relationships between the wheat genotypes were graphically represented in a dendrogram using the PowerMarker software.

The polymorphism information content (PIC) was estimated as per the given formula (please provide reference)

$$\widehat{PIC}_1 = 1 - \sum_{u=1}^k \tilde{p}_{lu}^2 - \sum_{u=1}^{k-1} \sum_{v=u+1}^k \tilde{p}_{lu}^2 \tilde{p}_{lv}^2$$

Where,

P_{lu} = An allele A_u having population frequency for l th locus

P_{lv} = An allele A_v having population frequency for l th locus

At a single locus, heterozygosity (H) is estimated as

$$\hat{H}_l = 1 - \sum_{u=1}^k \tilde{p}_{lu}^2$$

Population Structure Analysis

The population structure of 25 genotypes was analysed using a model based on a clustering algorithm using the software package STRUCTURE Version 2.1 (<http://pritch.bsd.uchicago.edu/software.html>) (Pritchard and Wen, 2004). The optimum number of K was selected after five independent runs with burn-in of 50,000 iterations, followed by 50,000 iterations for each value of K (tested from $K=2$ to $K=4$). Finally, output of analysis was retrieved using the STRUCTURE harvester (version?) (Earl & VonHoldt, 2012) and the optimum number of $K=2$ was determined by calculating Evanno's (ΔK) (Evanno *et al.*, 2005). For each subpopulation identified, the mean population differentiation (F_{ST}) and H_e (Nei, 1978) were determined based upon optimal K value. The F_{ST} value (Nei, 1977) helps in providing estimating the degree of fixation of alleles within the population while, H_e (Nei, 1978) depicts the average distance between individuals within the same population. Principal coordinate Analysis (PCoA) was performed based on genetic distances among the genotypes computed with DARwin 6.0.14 software (Dissimilarity Analysis and Representation for Windows) (Perrier & Jacquemoud, 2006).

Table 2 : List of SSR primers found polymorphic in present study

Sr. no.	Primer name		Sequence (5'→3')	T_m value (°C)	%GC
1	CFA 2114	F	ATTGGAAGGCCACGATACAC	57.30	50.00
		R	CCCGTCGGGTTTATCTAGC	59.35	55.00
2	CFD 65	F	AGACGATGAGAAGGAAGCCA	57.30	50.00
		R	CCTCCCTTGTTTTGGGATT	55.25	45.00
3	CFD 71	F	CAATAAGTAGGCCGGGACAA	57.30	50.00
		R	TGTGCCAGTTGAGTTTGCTC	57.30	50.00
4	GWM 160	F	TTCAATTCACTCTTGGCTTGG	55.92	42.86
		R	CTGCAGGAAAAAAGTACACCC	58.39	45.45
5	GWM 148	F	GTGAGGCAGCAAGAGAGAAA	57.30	50.00
		R	CAAAGCTTGACTCAGACCAAA	55.92	42.86
6	GWM 181	F	TCATTGGTAATGAGGAGAGA	53.20	40.00
		R	GAACCATTTCATGTGCATGTC	55.25	45.00
7	GWM 397	F	TGTCATGGATTATTGGTCGG	55.92	42.86
		R	CTGCACTCTCGGTATACCAGC	61.78	57.14

Sr. no.	Primer name		Sequence (5'→3')	Tm value (°C)	%GC
8	GWM 407	F	CATATTTCCAAATCCCCAACTC	56.53	40.91
		R	GGTAATTCTAGGCTGACATATGCTC	61.34	44.00
9	WMC 154	F	ATGCTCGTCAGTGTCATGTTTG	58.39	45.45
		R	AAACGGAACCTACCTCACTCTT	58.39	45.45
10	WMC 170	F	ACATCCACGTTTATGTTGTTGC	56.53	40.91
		R	TTGGTTGCTCAACGTTTACTTC	56.53	40.91
11	WMC 28	F	ATCACGCATGTCTGCTATGTAT	56.53	40.91
		R	ATTAGACCATGAAGACGTGTAT	54.66	36.36
12	WMC 44	F	GGTCTTCTGGGCTTTGATCCTG	62.12	54.55
		R	TGTTGCTAGGGACCCGTAGTGG	63.98	59.09

RESULTS AND DISCUSSION

The wheat research activities in Gujarat were initiated with the establishment of Wheat Research Station at Vijapur in 1962. Multi-disciplinary and multi-locational activities of varietal improvement, resources management, crop protection and quality improvement has been done by this research centre. Along with this, the centre has prominent share in wheat improvement in Central and Peninsular India too. To assess the molecular diversity present in this centre, 25 varieties/breeding lines were collected and molecular characterization of these lines were conducted which could provide a clear view to breeders for further crop improvement.

SSR genotyping and genetic divergence analysis

Total of twenty-eight microsatellite primers were used for molecular diversity analysis of twenty-five genotypes of wheat, out of which twelve primers were found to be polymorphic. The result obtained using twelve polymorphic primers have been presented in Table 3. SSR marker analysis of all twelve primers generated total number of 33 alleles with an average of 2.75 alleles. A total number of alleles varied from 2 (GWM 181) to 4 (WMC 170). The molecular size of bands/alleles was observed from 100 bp (GWM 181) to 296 bp (WMC 44). The PIC values in present study ranged from 0.30 (primer GWM 407) - 0.64 (primer CFD 65) with an average PIC value of

0.41. The co-dominant nature of SSR markers facilitates the analysis of the genotypic heterozygosity. The heterozygosity in the present study ranged from 0.00 (GWM 397) – 1.00 (CFD 65) with an moderate heterozygosity of 0.32. Higher heterozygosity depicted presence of higher ploidy level in genotypes. The average Major Allele Frequency was found to be 0.63 in present study.

Total number of alleles from present study were 2 to 4 with average of 2.75 which found to be in accordance with data recorded by Sharma *et al.*, 2021 as total number of alleles 2 to 6 and average of 2.58 whereas, higher total number of alleles (4-40) compared with present study has been reported previously by Roussel *et al.*, 2005 which indicated that it could be due to the separation of PCR products using a fragment analyzer. The average PIC value obtained in present study (0.41) has been in accordance with average PIC value obtained (0.35) by Sharma *et al.*, 2021. Primer pairs GWM 181, WMC 170 and CFD 65 found to be the most informative primers based upon PIC values greater than 0.5 among which WMC 170 has been previously reported to be associated with salinity tolerance in bread wheat (Gupta *et al.*, 1996) which makes it a marker of choice for trait specific studies as well as diversity analysis. Nei's genetic distances were used to generate the cluster analysis from which the highest genetic distance was noted between genotypes GW-2013-503 AND 18th-SBWOM-HT-85 supporting the fact that this both genotypes belong to different species of wheat.

Table 3 : Results of SSR analysis in twenty-five genotypes of wheat

Sr. No.	Primer	Molecular band/allele size (bp) (Approx.)	No. of polymorphic alleles	PIC	Heterozygosity	Major Allele Frequency
1	CFA 2114	209 – 229	2	0.37	0.05	0.52
2	CFD 65	155 – 216	3	0.55	1.00	0.50
3	CFD 71	146 – 209	3	0.46	0.56	0.64
4	GWM 148	145 – 169	2	0.35	0.04	0.66
5	GWM 160	181 – 204	2	0.33	0.20	0.70
6	GWM 181	100 – 162	4	0.64	0.88	0.46
7	GWM 397	188 – 232	2	0.37	0.00	0.52
8	GWM 407	131 – 174	2	0.30	0.10	0.76
9	WMC 28	158 – 198	4	0.37	0.48	0.76
10	WMC 44	207 – 296	2	0.30	0.08	0.75
11	WMC 154	114 – 162	3	0.29	0.13	0.81
12	WMC 170	198 – 230	4	0.59	0.28	0.50
Total			33	-	-	
Average			2.75	0.41	0.32	0.63

Clustering pattern obtained from SSR data analysis

Based on the SSR data, cluster analysis was performed using Nei's genetic distances, and a dendrogram was generated, showing genetic relationships among twenty-five genotypes. Nei's genetic distances for twelve primers ranged from 0.07 to 0.78. The highest genetic distance (0.78) was observed between genotypes GW-2013-503 AND 18th-SBWOM-HT-85 indicating higher genetic diversity between the genotypes on allelic level. The minimum similarity value (0.07) was observed between genotypes 15th-HTWYT-9 and GW-366 indicating the relatedness between the genotypes.

The clustering pattern of the dendrogram (Fig.1) generated using the pooled molecular data of twelve SSR loci indicated two clusters, namely 1 and 2 (Fig.2). Cluster 1 (Red circle) included all the durum wheat (*T. durum*) genotypes, while cluster 2 (Green circle) included all the bread wheat (*T. aestivum*) genotypes. The dendrogram clearly shows genotypes 14 (GDW-1255) and 15 (DBPY-08-22) admixtures from the clusters while DBPY-08-22 genotype more aligning towards cluster 1 consisting of bread wheat.

Population structure and PCoA

Population structure (Fig.2) divided 25 genotypes into two main populations. Population 1 (Pop₁-Red) and population 2 (Pop₂-Green) contain 11 and 14 genotypes, respectively. Pop₁ consisted of durum wheat genotypes while Pop₂ shows presence of bread wheat genotypes. Further, based on membership fractions, the genotypes under different

populations were categorized as pure or admixture. The genotypes with a probability of more than ≥ 0.80 were considered as pure (88%) and less than 0.80 as an admixture (12%). Pop₁ showed 10 pure (90 %) and 1 admixed (10 %) individual while, Pop₂ showed 12 pure (86 %) and 2 admixed (14 %) individuals. Genotypes GDW-1255 and DBPY-08-22 has shown higher percent of admixture among all the genotypes. Both the genotypes have been reported to be from durum wheat background but showing per cent admixtures from bread wheat.

Genotype DBPY-08-22 was received as a durum wheat, however in STRUCTURE analysis this genotype appeared in *T. aestivum* population group with higher level of admixtures of durum (40%) while genotype GDW-1255 received as a durum wheat showed admixture from aestivum group (20%). The molecular study is giving actual genotypic representation of particular genotype. Admixture study is indicating that due to human mediated breeding bread and durum type of admixtures gets appeared which may give opportunity for combining superior character from bread and durum for developing superior varieties. By using this admixture varieties further improvement is possible and they may work as bridging varieties for crossing between aestivum and durum. Similar studies on the admixture genotypes from the wheat genotypes has been reported by Khan *et al.*, 2015 using ISSR and RAPD markers to gain a better understanding of the genetic association and population structure of Indian and Turkish wheat on the basis of both geographical origin and ploidy.

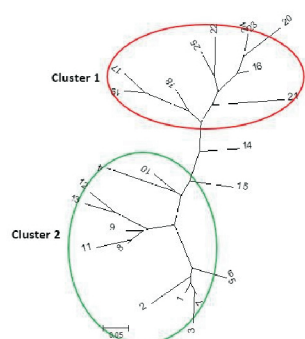
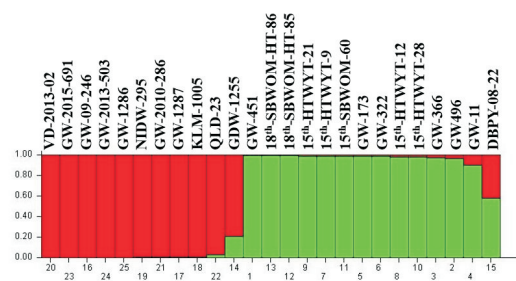
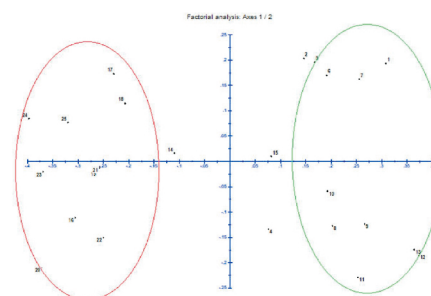
Table 4 : Results of STRUCTURE analysis of for the fixation index (F_{st} ; indicating significant divergence) and average distances (expected heterozygosity)

Population	F_{st}	Exp. Heterozygosity (H_e)	No. of genotypes
Pop ₁	0.36	0.35	11
Pop ₂	0.29	0.39	14

The degree of genetic differentiation and average distance (H_e) in each subpopulation suggested that highest degree of genetic differentiation was detected in population 1 ($F_{st} = 0.36$), whereas lowest value was observed in population 2 ($F_{st} = 0.29$) (Table.4). Along with this, the lowest expected heterozygosity (H_e) was observed in population 1 ($H_e = 0.35$) and the highest H_e was observed in population 2 ($H_e = 0.39$). The results of sub-populations generated by STRUCTURE analysis based on $K=2$ value (Fig.1), UPGMA cluster analysis (Fig.2) and PCoA (Fig.3) showed a high degree of similarity with genotypes *GDW-1255* and

DBPY-08-22 showing no presence in any of the cluster specifically and thus appreciable admixtures with the bread wheat genetic constitution.

Superior F_{st} values denotes the population is fixed with the alternate alleles and this has been supported by the previous studies by Robbana *et al.*, 2019 while studying durum wheat landraces. Distinct clustering pattern in *T. durum* and *T. aestivum* genotypes of Gujarat based upon PCoA analysis has also been reported by Zala *et al.*, (2014) by employing RAPD and SSR markers showing importance of such studies in selection of genotypes for further crop improvement programme.

**Fig.1 Dendrogram showing clustering of twenty-five wheat genotypes based on microsatellite markers****Fig.2 Assignment of Wheat germplasms into two populations based on population structure analysis****Fig.3 Principal coordinate analysis (PCoA) based on genetic distance among the bread and durum wheat genotypes based on microsatellite markers data**

Conflict of interest: There is no conflict of interest

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