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RESEARCH ARTICLE

ASSESSMENT OF THE GENETIC DIVERGENCE AND POPULATION STRUCTURE WITH SSR MARKERS IN ADVANCED LINES OF BREAD WHEAT (TRITICUM AESTIVUM L.).

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ABSTRACT

Evaluating genetic divergence and population structure across bread wheat cultivars are crucial in breeding initiatives, and SSR markers possess some characteristics that render them appropriate for those objectives. Sixty-five wheat cultivars by employing 17 SSR markers that amplified a total of 68 alleles and there was a range of 2 to 7 alleles per locus, with an average of 4 alleles each locus. The polymorphism information content (PIC) varied between 0.21 and 0.84, with a mean value of 0.66. The PIC values of the markers exhibited a range of 0.66 to 0.84, showing a significant degree of diversity among the wheat genotypes. The genotyping data were used for population structure analysis which grouped into three (K=3) main populations in which population I, II, III contained 26, 20 and 19 genotypes respectively. The UPGMA dendrogram showed higher genetic variation in the wheat genotypes. The genotypes were categorized into three clusters and six sub-clusters using genetic distance-based clustering, which relied on SSR markers. The PCA graphical representations indicated that the P3-2-2-P9 and P3-18-10-P15 lines were positioned significantly distant from the centroid, suggesting a higher level of genetic diversity and potential utility for future breeding initiatives. The findings of the study indicate that the SSR markers examined in this research exhibit a satisfactory level of polymorphism and reproducibility in their fingerprinting patterns, making them suitable for assessing the genetic diversity of wheat genotypes which could potentially contribute to future selection and breeding efforts.

KEYWORDS

Genetic diversity, Population structure, SSR markers, Advanced lines (wheat)

1. INTRODUCTION

Wheat (*Triticum aestivum L.*) holds the third most significant cereal crop on a global scale, and belongs to the genus *Triticum* and the family "*Gramineae (Poaceae)*"(Shewry, 2009). Cultivated wheat (2n = 6x = 42, AABBDD) is an allohexaploid, composed of three distinct genomes viz., A, B and D. Total worldwide wheat production was 778.2 million tonnes in 2021-22 and it was forecast that world wheat production in 2022-23 at 781.1 million tonnes, up 2.91million tonnes from previous years (FAO, 2022). In 2022, an increase of 1.26 million tonnes or 0.16% in wheat production around the globe (USDA 2022).

Genetic diversity is an important factor in any successful wheat breeding program. The presence of genetic variation within plant species presents opportunities for enhancing the characteristics of plants. They involve establishing a core collection through removing insignificant accessions and discovering lines that could be beneficial for future breeding programmes (Govindraj et al., 2010). According to a study, the process of wheat breeding through hybridization necessitates the careful selection of a wide range of genotypes, regardless of whether the resulting product is a pure line or a hybrid variety (Zeb et al., 2009). The requirement and primary relevance for a successful wheat breeding program lies in the presence of genetic diversity and genetic interactions among genotypes. To create hybrid wheat varieties with desirable characteristics, it is necessary to have a comprehensive understanding of the current genetic diversity (Kahrizi et al., 2010).

The fundamental prerequisite for molecular wheat breeding involves the selection of a diverse range of genotypes (Raj et al., 2017). The utilization of molecular markers for the evaluation of genetic variability has been demonstrated to be a fundamental aspect in comprehending the genomic composition, classifying the genes accountable for significant characteristics, and categorizing and preserving genetic diversity in plant germplasm (Khan et al., 2015). Single-sequence repeats (SSRs) are a valuable tool in diversification research for accurately determining the level of genetic similarity. Microsatellite markers are well-suited for detecting allele frequency within a population and assessing population structure due to their high rate of polymorphism, high Polymorphic Information Content (PIC), co-dominant character, selective neutrality, distribution across the genome, and cost and labor efficiency (Khaled et al., 2015). This approach is suitable for determining the frequency of alleles in the population and assessing the structure of the population (Kumar et al., 2016). SSR are widely distributed throughout eukaryotic genomes and can be utilized as versatile and multi-allelic genetic markers by polymerase chain reaction (PCR). It has proven to be highly valuable for various crop improvement applications due to their significant polymorphism and ease of handling (Gupta et al., 2009).

Across the globe, many researchers have explored the genetic diversity in wheat. Very few studies have investigated genetic diversity and molecular characterization in wheat genotypes in Bangladesh. However, SSR markers are the best choice because of having some advantages over other markers to check the genetic divergence. Considering these facts, the

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research hypothesis might be characterizing the wheat cultivars and advanced lines using SSR markers. This will produce suitable genes and genotypes together with diverse parents for future breeding.

Therefore, the present study should be conducted under the following specific objectives.

I. To identify genetic divergence in advanced lines with SSR markers.

II. To study population structure in advanced lines of wheat using SSR markers.

2. Materials and Methods

2.1 Site of Experiment and Collection of Plant Materials

The experiment was executed at the experimental field of the Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur (HSTU), during the rabi season. The molecular marker work was carried out in the laboratory of genetics and plant breeding at HSTU and Bangladesh Wheat and Maize Research Institute (BWMRI). In this research study, sixty-five wheat genotypes were utilized as plant materials. Three lines were designated as parents, two were classified as check varieties, while the remaining sixty lines were advanced line (Table 1). The seed materials of these genotypes were procured from BWMRI.

Table 1: List of 65 wheat genotypes used in this study

Sl No	Line Name	Sl No	Line Name	Sl No	Line Name
1	P3-2-2-P3	23	P3-18-10-P15	45	P3-29-4-P8
2	P3-2-2-P6	24	P3-21-10-P9	46	P3-29-4-P9
3	P3-2-2-P9	25	P3-21-10-P14	47	P3-29-4-P10
4	P3-2-2-P12	26	P3-28-3-P2	48	P3-29-4-P15
5	P3-2-2-P13	27	P3-28-3-P3	49	P3-29-10-P1
6	P3-2-6-P1	28	P3-28-3-P5	50	P3-29-10-P2
7	P3-2-6-P5	29	P3-28-3-P9	51	P3-29-10-P3
8	P3-2-6-P14	30	P3-28-3-P10	52	P3-29-10-P4
9	P3-3-3-P8	31	P3-28-3-P15	53	P3-29-10-P5
10	P3-3-3-P14	32	P3-29-2-P1	54	P3-29-10-P6
11	P3-4-6-P11	33	P3-29-2-P2	55	P3-29-10-P8
12	P3-4-6-P14	34	P3-29-2-P5	56	P3-29-10-P9
13	P3-5-2-P4	35	P3-29-2-P11	57	P3-29-10-P10
14	P3-5-2-P6	36	P3-29-2-P12	58	P3-29-10-P11
15	P3-5-2-P12	37	P3-29-2-P13	59	P3-29-10-P14
16	P3-5-2-P13	38	P3-29-4-P1	60	P3-29-10-P15
17	P3-11-3-P5	39	P3-29-4-P2	61	BARI Gom - 30 (check)
18	P3-11-3-P12	40	P3-29-4-P3	62	BARI Gom - 33 (check)
19	P3-11-3-P15	41	P3-29-4-P4	63	AGHRANI (parent)
20	P3-18-3-P7	42	P3-29-4-P5	64	SHATABDI (parent)
21	P3-18-3-P12	43	P3-29-4-P6	65	BARI Gom - 25 (parent)
22	P3-18-10-P5	44	P3-29-4-P7		

2.2 Genomic DNA isolation, purification and Quantification

The genomic DNA was extracted from a small quantity of fresh leaf tissue (5.0 g) from each variety using the CTAB procedure, as described by (Saghai-Maroof et al., 1984; Xu et al., 1994). According to a study, the concentration and amount of DNA were assessed using a UV spectrophotometer (Jiang, 2013).

2.3 PCR Amplification and Electrophoresis

Polymerase chain reaction (PCR) amplifications were conducted in a 10 µL tube utilizing a Veriti™ 96-Well Fast Thermal Cycler manufactured by Applied Biosystems, USA. Each tube was supplemented with 2 µL of template DNA and 8 µL of the reaction mixture, consisting of 0.5 µL of Forward primer, 0.5 µL of Reverse primer, 2 µL of nuclease-free water, and 5 µL of G2 Green Master Mix. The optimization of the thermocycling program involved an initial denaturation step at a temperature of 95°C for a duration of 4 minutes. This was followed by 40 cycles of denaturation at 95°C for 1 minute, followed by annealing at temperatures ranging from 52 to 63°C. Expansion was achieved by annealing at 72°C for 1 minute, and a final cycle of 72°C for 10 minutes, followed by a hold at 4°C (Kumar et al., 2016). On 0.8% agarose gel electrophoresis, the amplified product was resolved. The gels were subjected to a voltage of 100V for a duration of 45 minutes. After electrophoresis was completed, DNA bands were observed using a UV trans-illuminator and gel dock (Shuaib et al., 2010). A selection of 17 primer pairs for microsatellite (SSR) markers, encompassing 17 chromosomes, was made for the purpose of conducting diversity analysis on a set of 65 wheat genotypes.

2.4 Data analysis

The mean PIC was computed for each SSR based on the formula provided by some researcher, PIC = 1- $P(Pi)^2$, where Pi is the fraction of the population bearing the i^{th} allele, measured for each SSR locus (Powell et al., 1996; Botstein et al., 1980). The presence (1) or absence (0) of SSR bands was assigned to each genotype, and the resulting binary data matrix was analyzed using the software STRUCTURE V2.3.4 to determine genetic relationships among the genotypes (Pritchard et al., 2000). The researchers computed simple matching similarity coefficients for all pairwise comparisons between the genotypes. The researchers included all the loci that could be scored in order to create a bivariate 1-0 data matrix. To estimate genetic diversity, the analysis was conducted using the MetaboAnalyst program (Online Version) developed (Chong and Xia, 2018).

3. Results

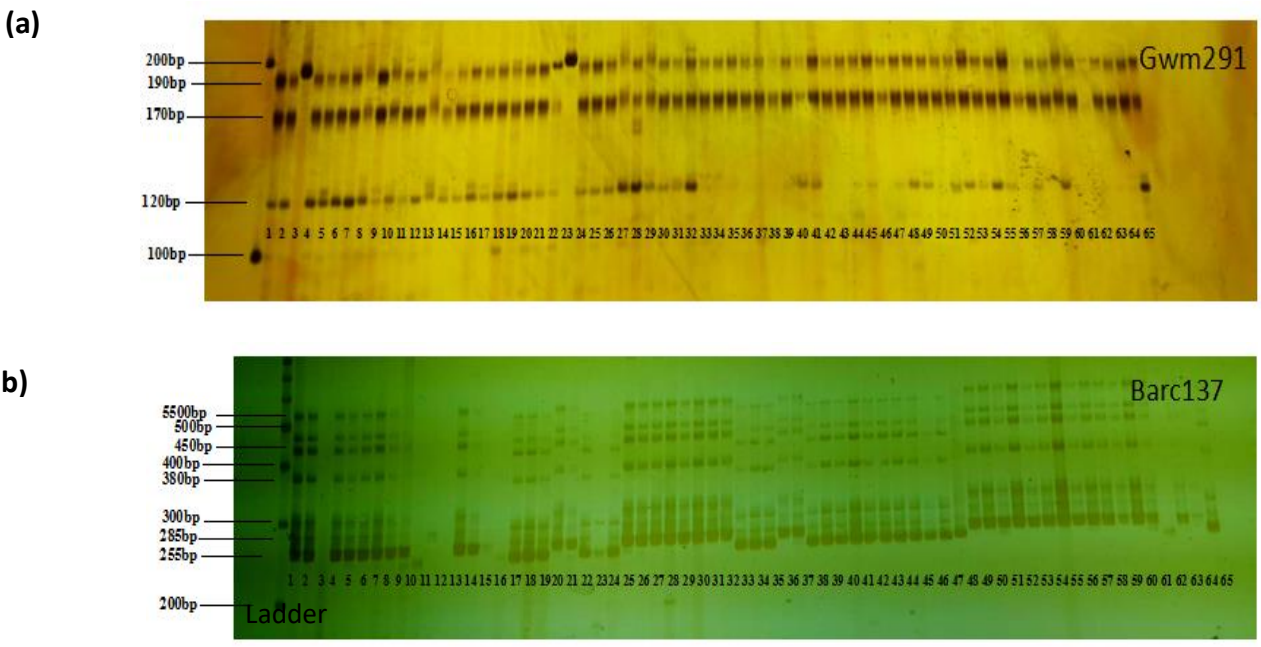
3.1 Assessment of polymorphism from SSR Profiles

After PCR and PAGE analysis, a total of 68 alleles were identified at 17 SSR markers over 65 wheat genotypes (Figure 1). In the case of TaXbarc137, a maximum range of band (255-550bp) was found and which was followed by Xwmc112 (200-400bp) and Gwm495 (160-300bp) respectively (Table 2). The minimum range of bands was found in TaBarc271 (100-110). There was a range of 2 to 7 alleles per locus, with an average of 4 alleles observed across the 68 loci. The marker TaXbarc137 produced the highest number of polymorphic alleles of seven (7) followed by TaXcfd43, TaXgwm294, Xgwm296 and TaXcfa2129 with six (6) each, respectively, while TaBarc271, Barc20 and TaGwm293 markers produced the least number of polymorphic alleles per locus of two (2). The estimation of polymorphic information content (PIC) was used to measure the genetic diversity. The Polymorphic Information Content (PIC) values of Simple

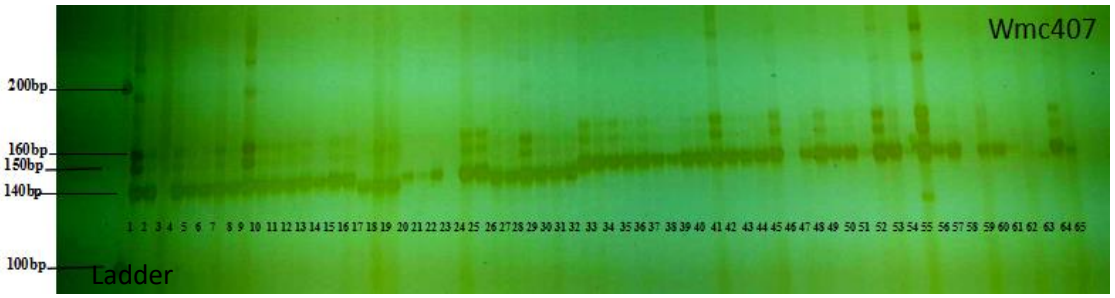
Sequence Repeats (SSRs) varied between 0.21 and 0.84, with an average of 0.66. The TaXbarc137 had the highest PIC value of 0.84, followed by TaXcfd43 (0.83), TaXgwm294 (0.82), Xgwm296 (0.81), TaXcfa2129 (0.81), Gwm513 (0.74), Tagwm1037 (0.74), Xwmc112 (0.73), Gwm495 (0.72), TaGwm291 (0.66), WMS0691 (0.66), TaBarc101 (0.63), TaBarc68 (0.59), TaXwmc407 (0.54), TaGwm293 (0.44), Barc20 (0.38) and the lowest TaBarc271 (0.21) respectively. Eleven (11) out of seventeen (17) primers showed PIC values between 0.66 and 0.84.

Table 2: List of 17 SSR markers used for diversity analysis of wheat genotypes

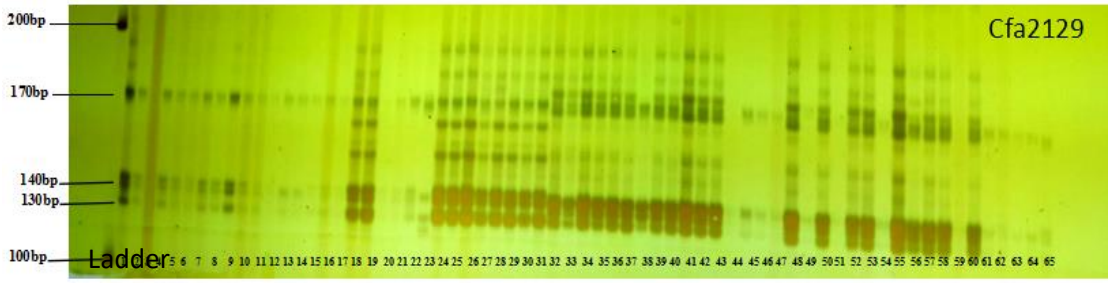
Primer name	Forward Sequence	Reverse Sequence	Annealing Temp.(C)	Number of alleles	Range of allele size (bp)	PIC
TaGwm291	AATGGTATCTATTCCGACCCG	CATCCCTAGGCCACTCTGC	57.5	3	120-200	0.66
TaXbarc137	CCAGCCCCTCTACACATTTT	GGCCCATTTCCCACTTTCCA	55	7	255-550	0.84
TaXwmc407	CATATTTCCAAATCCCCAACTC	GGTAATTCTAGGCTGACATATGCTC	56	3	140-160	0.54
TaXcfa2129	ATCGCTCACTCACTATCGGG	GTTGCACGACCTACAAAGCA	56	6	130-170	0.81
TaXcfd43	CCAAAAACATGGTTAAAGGGG	AACAAAAGTCGGTGCAGTCC	54.5	6	145-300	0.83
TaXgwm294	GCAGAGTGATCAATGCCAGA	GGATTGGAGTTAAGAGAGAACCG	56.5	6	100-175	0.82
TaGwm293	TCGCCATCACTCGTTCAAG	TACTGGTTCACATTGGTGCG	55	2	145-210	0.44
TaBarc 271	CGCACCTAATATCGTAAAACAATGTA	CGCTTTCCCAGAATATTATTTGTATTGT	55	2	100-110	0.21
TaBarc68	CGATGCCAACACACTGAGGT	GCCGCATGAAGAGATAGGTAGAGAT	58	3	100-150	0.59
TaBarc101	GCTCCTCTCACGATCACGCAAA	GCGAGTCGATCACACTATGAGCCAATG	62	3	120-170	0.63
Xgwm296	AATTCAACCTACCAATCTCTG	GCCTAATAAACTGAAAACGAG	52	6	140-240	0.81
Xwmc112	TGAGTTGTGGGGTCTTGTTTGG	TGAAGGAGGGCACATATCGTG	58	4	200-400	0.73
WMS0691	GGGAGGATATGAGGGCTCAC	GCACGTGATTGGTGAAAATG	56	3	140-165	0.66
Tagwm1037	CTTCATCTGCGACCTTCCAT	CTTTATTCCTGGTTATTGCC	53	4	90-160	0.74
Barc20	GCGATCCACACTTTGCCTCTTTTACA	GCGATGTCGGTTTTCAGCCTTTT	59	2	200-280	0.38
Gwm513	ATCCGTAGCACCTACTGGTCA	GGTCTGTTCATGCCACATTG	56	4	130-210	0.74
Gwm495	GAGAGCCTCGCGAAATATAGG	TGCTTCTGGTGTTCCTTCG	56	5	160-300	0.72
Average				4		0.66



(c)


Wmc407
200bp
160bp
150bp
140bp
100bp
Ladder

(d)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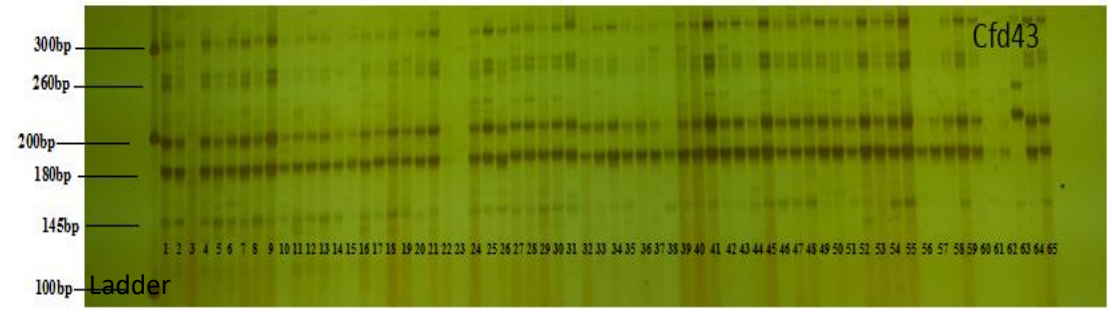
140bp
130bp
100bp
Ladder

(e)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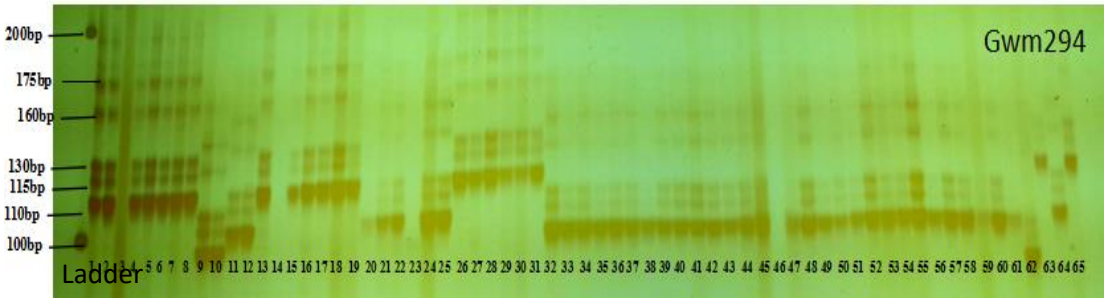
145bp
100bp
Ladder

(f)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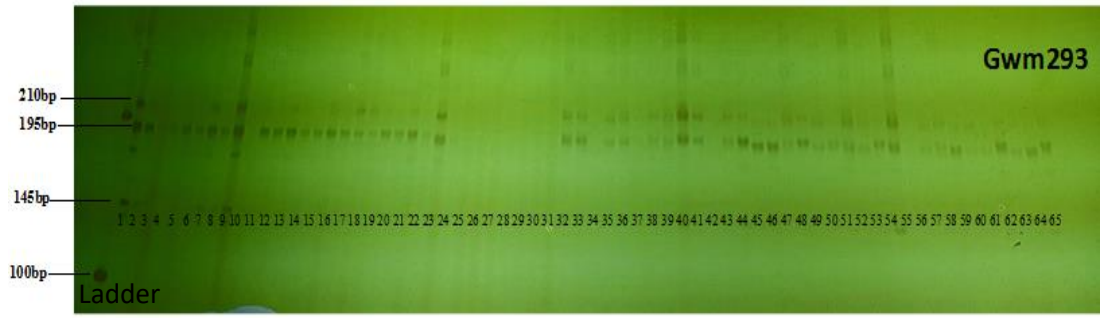
Ladder

(g)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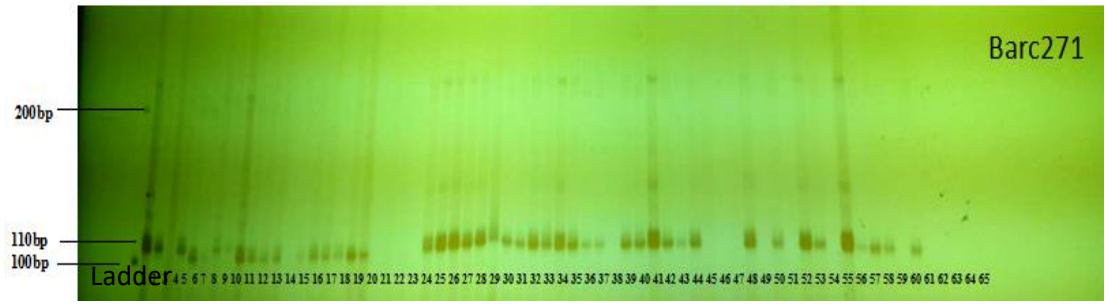
100bp
Ladder

(h)


Barc271
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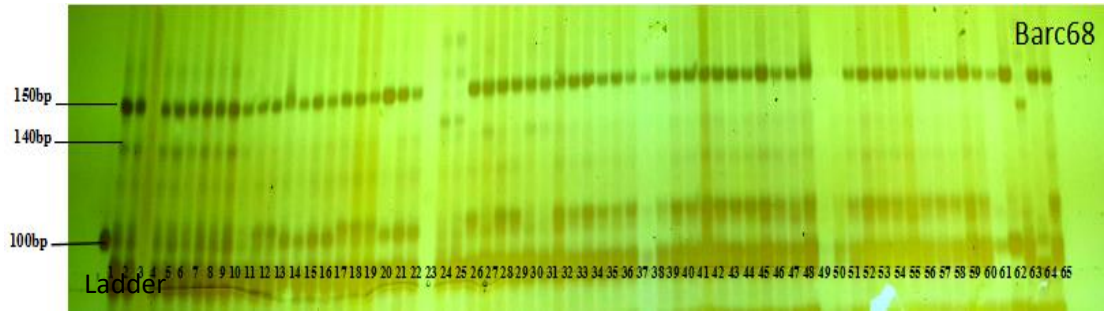
110bp
100bp
Ladder

(i)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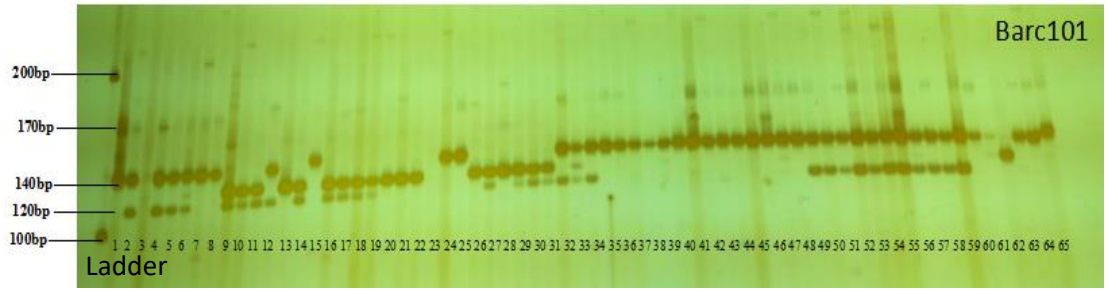
100bp
Ladder

(j)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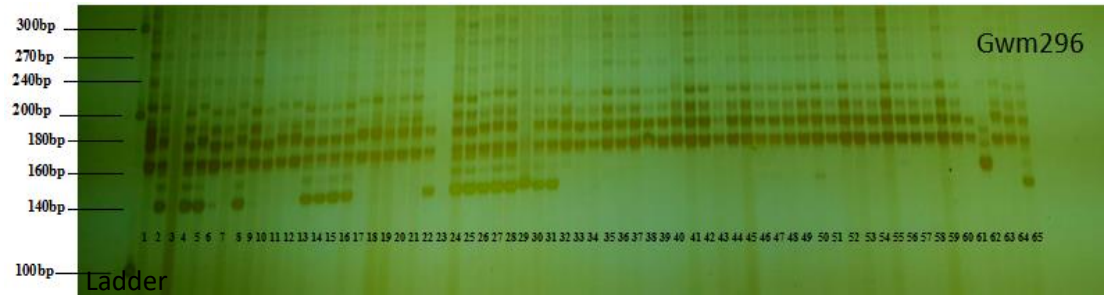
140bp
120bp
100bp
Ladder

(k)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200bp
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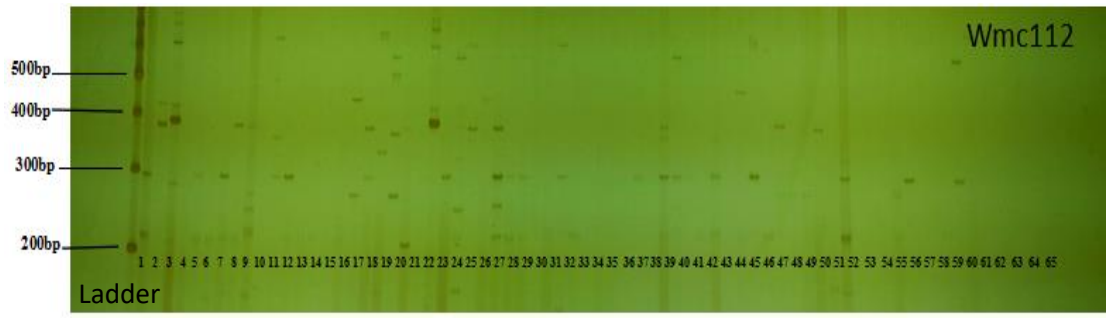
160bp
140bp
100bp
Ladder

(l)


Wmc112
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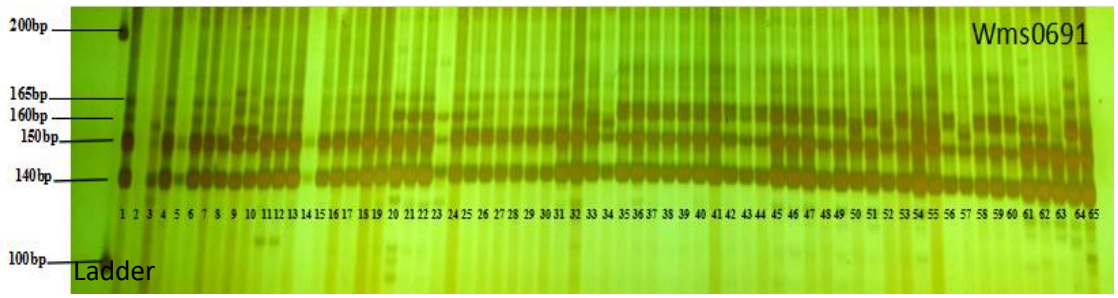
400bp
300bp
200bp
Ladder

(m)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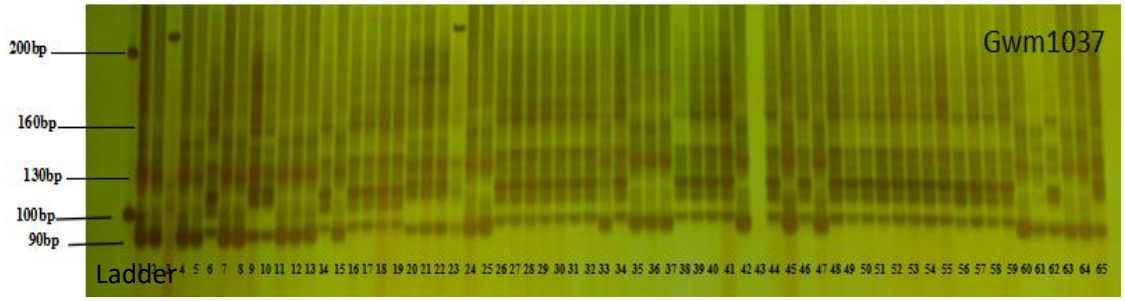
100bp
Ladder

(n)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Ladder

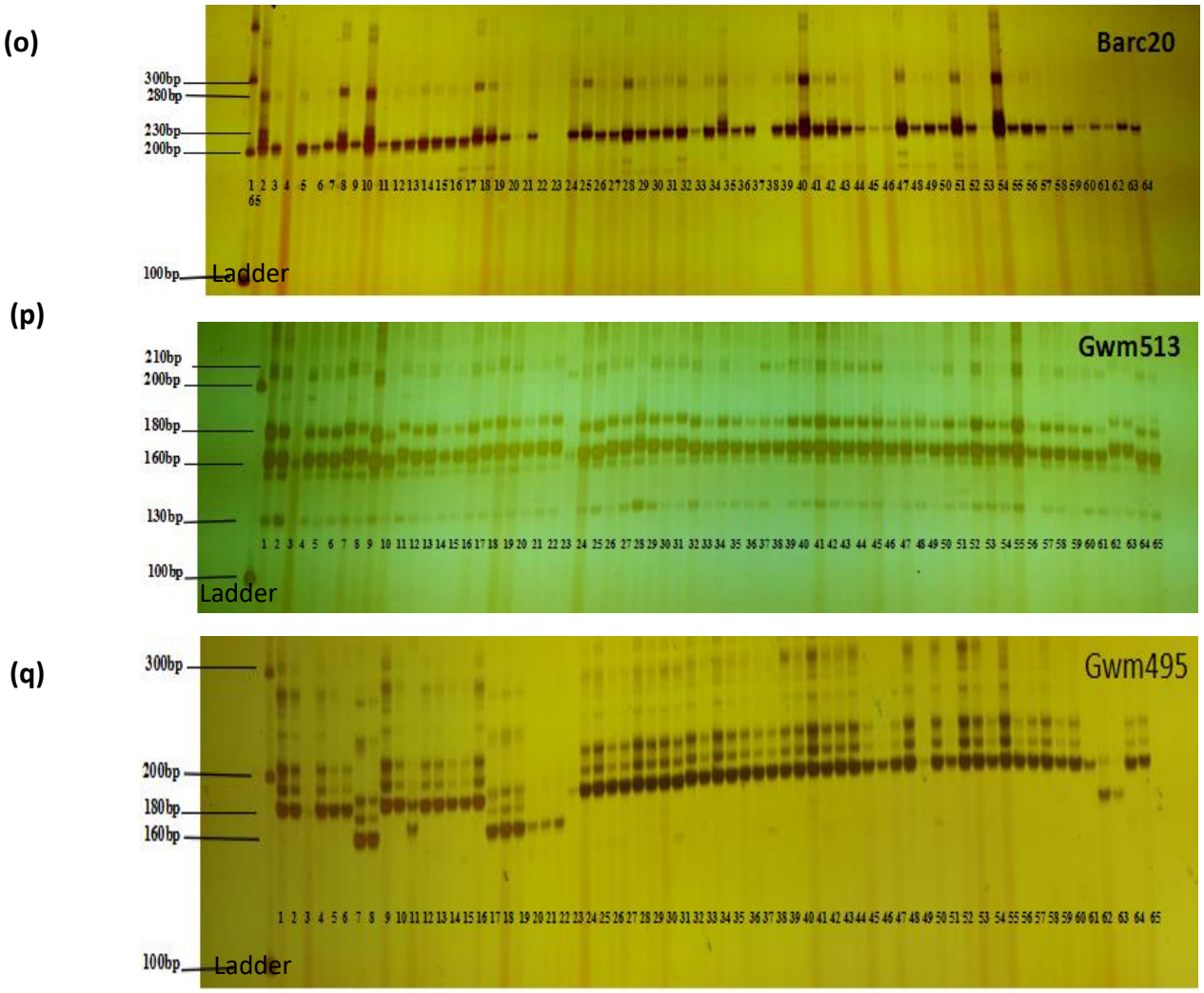

Figure 1: DNA band profile of 65 wheat genotypes using 17 polymorphic SSR markers (a) to (q) showing different polymorphism patterns within wheat genotypes.

3.2 Analysis of population structure

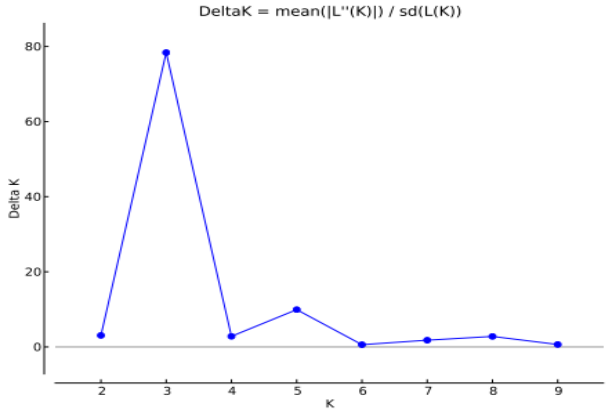

Figure 2: Representation of population structure dividing the wheat genotypes into three subgroups based on K value

Evaluating population structure is an important initial step in association analysis. Sixty-five advance lines of wheat genotypes was assessed using STRUCTURE V2.3.4 software. The analysis of population structure revealed that the log-likelihood value (ΔK) reached its maximum value at K=3 (Figure 2). This indicates a distinct peak that represents the classification of genotypes into three distinct sub-groups, referred to as Population I, Population II, and Population III (Figure 3). These sub-groups accounted for 40.8%, 29.8%, and 29.3% of the total genotypes, respectively. Population I (77.8%), Population II (57.9%), and Population III (68.4%) all had a substantial percentage of pure population. In each sub-population, the remaining sections were combined. The optimal value for population genetic structure analysis, denoted as "K = 3," was within the range of 1 to 10. The FST population values were 0.51 for Population I, 0.19 for Population II and 0.49 for Population III (Table 3) with an average of alpha 0.13. Average distances (expected heterozygosity) between individuals in the same populations (Table 3). The average intra-population distances in the same cluster were 0.1982 for Population I, 0.3628 for Population II and 0.2172 for Population III. Net nucleotide distance between Population I and Population II, Population I and Population III, Population II and Population III was found 0.1229, 0.1302 and 0.1555, respectively (Table 4).

Table 3: FST population values and average distances (expected heterozygosity) between individuals in same cluster of 65 wheat genotypes obtained from population structure analysis

Mean value of FST			Average distances (expected heterozygosity) between individuals in same cluster		
1	2	3	1	2	3
0.5105	0.1855	0.4967	0.1982	0.3628	0.2172

Table 4: Allele-frequency divergence among populations (Net nucleotide distance) of 65 wheat genotypes obtained from population structure

	Population I	Population II	Population III
Population I	-	0.1229	0.1302
Population II	0.1229	-	0.1555
Population III	0.1302	0.1555	-

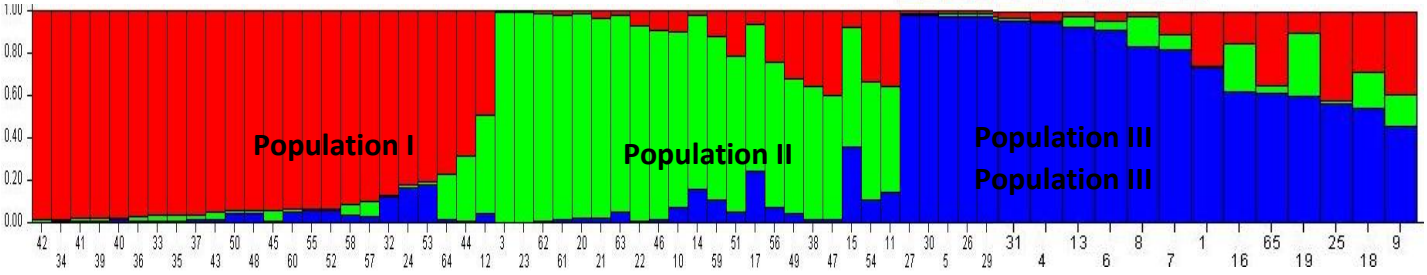

Figure 3: Population structure at K = 3 of 65 wheat genotypes based on genotypic data using 17 (seventeen) microsatellite markers. Color codes are- Population I = Red, Population II = Greenand Population III=Blue.

Legend: 1.P3-2-2-P3, 2.P3-2-2-P6, 3.P3-2-2-P9, 4.P3-2-2-P12, 5.P3-2-2-P13, 6.P3-2-6-P1, 7.P3-2-6-P5, 8.P3-2-6-P14, 9.P3-3-3-P8, 10.P3-3-3-P14, 11.P3-4-6-P11, 12.P3-4-6-P14, 13.P3-5-2-P4, 14.P3-5-2-P6, 15.P3-5-2-P12, 16.P3-5-2-P13 17.P3-11-3-P5, 18.P3-11-3-P12, 19.P3-11-3-P15, 20.P3-18-3-P7, 21.P3-18-3-P12, 22.P3-18-10-P5, 23.P3-18-10-P15, 24.P3-21-10-P9, 25.P3-21-10-P14, 26.P3-28-3-P2, 27.P3-28-3-P3, 28.P3-28-3-P5, 29.P3-28-3-P9, 30.P3-28-3-P10, 31.P3-28-3-P15, 32.P3-29-2-P1, 33.P3-29-2-P2, 34.P3-29-2-P5, 35.P3-29-2-P11, 36.P3-29-2-P12, 37.P3-29-2-P13, 38.P3-29-4-P1, 39.P3-29-4-P2, 40.P3-29-4-P3, 41.P3-29-4-P4, 42.P3-29-4-P5, 43.P3-29-4-P6, 44.P3-29-4-P7, 45.P3-29-4-P8, 46.P3-29-4-P9, 47.P3-29-4-P10, 48.P3-29-4-P15, 49.P3-29-10-P1, 50.P3-29-10-P2, 51.P3-29-10-P3, 52.P3-29-10-P4, 53.P3-29-10-P5, 54.P3-29-10-P6, 55.P3-29-10-P8, 56.P3-29-10-P9, 57.P3-29-10-P10, 58.P3-29-10-P11, 59.P3-29-10-P14, 60.P3-29-10-P15, 61.BARI Gom - 30 (check), 62.BARI Gom - 33 (check), 63.AGHRANI (parent), 64. SHATABDI (parent), 65.BARI Gom - 25 (parent).

3.3 Genetic distance-based analysis

The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis showed genetic distance-based results that identified two primary groups among the 65 wheat genotypes. These groups exhibited similarity coefficients ranging from 0.00 to 2.5 (Figure 4). The two groups were further subdivided into three clusters: cluster I (consisting of 47 genotypes), cluster II (consisting of 15 genotypes), and cluster III (consisting of 3 genotypes) (Table 5). Cluster I consists of three sub-clusters, with Sub-cluster A including nine genotypes that were further classified into two distinct classes. In the first class, the highest similarity was seen between G18 (P3-11-3-P12)-G19 (P3-11-3-P15), while in the second class, the highest similarity was found between G20 (P3-18-3-P7)-G21 (P3-18-3-P12) and the lowest similarity was found between G22 (P3-18-10-P5)-G63 (AGHRANI). The sub-cluster B consisted of a total of 24 genotypes, which were subsequently categorized into three distinct classes. Among them highest and lowest similarity found between G15 (P3-5-2-P12)-G16 (P3-5-2-P13) and G11 (P3-4-6-P1)-G56 (P3-29-10-P9) in 1st class; G51(P3-29-10-P3)-G54(P3-29-10-P6) and G51(P3-29-10-P3)-G65 (BARI Gom-25) in 2nd class and G55(P3-29-10-P8)-G52(P3-29-10-P4) and G32(P3-29-2-P1)-G37(P3-29-2-P13) in 3rd class respectively. Within Sub-cluster C, a total of 14 genotypes were identified. Among these genotypes, the G39 (P3-29-4-P2)-G40 (P3-29-4-P3) group exhibited the most resemblance, while the G24 (P3-21-10-P9)-G44 (P3-29-4-P7) group shown the lowest similarity. Cluster II consisted of 4 genotypes, with Sub-cluster A containing 9 genotypes and Sub-cluster B including 6 genotypes. In Sub-cluster A, the maximum similarity was seen between G2 (P3-2-2-P6)-G4 (P3-2-2-P12) and G8 (P3-2-6-P14)-G25 (P3-21-10-P14). In Sub-cluster B, the highest similarity was identified between G30 (P3-28-3-P10)-G31 (P3-28-3-P15) and G28 (P3-28-3-P5)-G31 (P3-28-3-P15). Cluster III comprised a collection of three distinct genotypes, namely G3 (P3-2-2-P9), G23 (P3-18-10-P15), and G61 (BARI Gom-30).

Table 5: Cluster groups and their containing 65 wheat genotypes name based on UPGMA dendrogram

Clusters	Number of genotypes	Genotypes
I	47	G9(P3-3-3-P8), G10(P3-3-3-P14), G11(P3-4-6-P1), G12(P3-4-6-P14), G14(P3-5-2-P6), G15(P3-5-2-P12), G16(P3-5-2-P13), G17(P3-11-3-P5), G18(P3-11-3-P12), G19(P3-11-3-P15), G20(P3-18-3-P7), G21(P3-18-3-P12), G22(P3-18-10-P5), G24(P3-21-10-P9), G32(P3-29-2-P1), G33(P3-29-2-P2), G34(P3-29-2-P5), G35(P3-29-2-P11), G36(P3-29-2-P12), G37(P3-29-2-P13), G38(P3-29-4-P1), G39(P3-29-4-P2), G40(P3-29-4-P3), G41(P3-29-4-P4), G42(P3-29-4-P5), G43(P3-29-4-P6), G44(P3-29-4-P7), G45(P3-29-4-P8), G46(P3-29-4-P9), G47(P3-29-4-P10), G48(P3-29-4-P15), G49(P3-29-10-P1), G50(P3-29-10-P2), G51(P3-29-10-P3), G52(P3-29-10-P4), G53(P3-29-10-P5), G54(P3-29-10-P6), G55(P3-29-10-P8), G56(P3-29-10-P9), G57(P3-29-10-P10), G58(P3-29-10-P11), G59(P3-29-10-P14), G60(P3-29-10-P15), G62(BARI Gom - 33), G63(AGHRANI), G64(SHATABDI), G65(BARI Gom - 25)
II	15	G1(P3-2-2-P3), G2(P3-2-2-P6), G4(P3-2-2-P12), G5(P3-2-2-P13), G6(P3-2-6-P1), G7(P3-2-6-P5), G8(P3-2-6-P14), G13(P3-5-2-P4), G25(P3-21-10-P14), G26(P3-28-3-P2), G27(P3-28-3-P3), G28(P3-28-3-P5), G29(P3-28-3-P9), G30(P3-28-3-P10), G31(P3-28-3-P15)
III	3	G3(P3-2-2-P9), G23(P3-18-10-P15), G61(BARI Gom - 30)

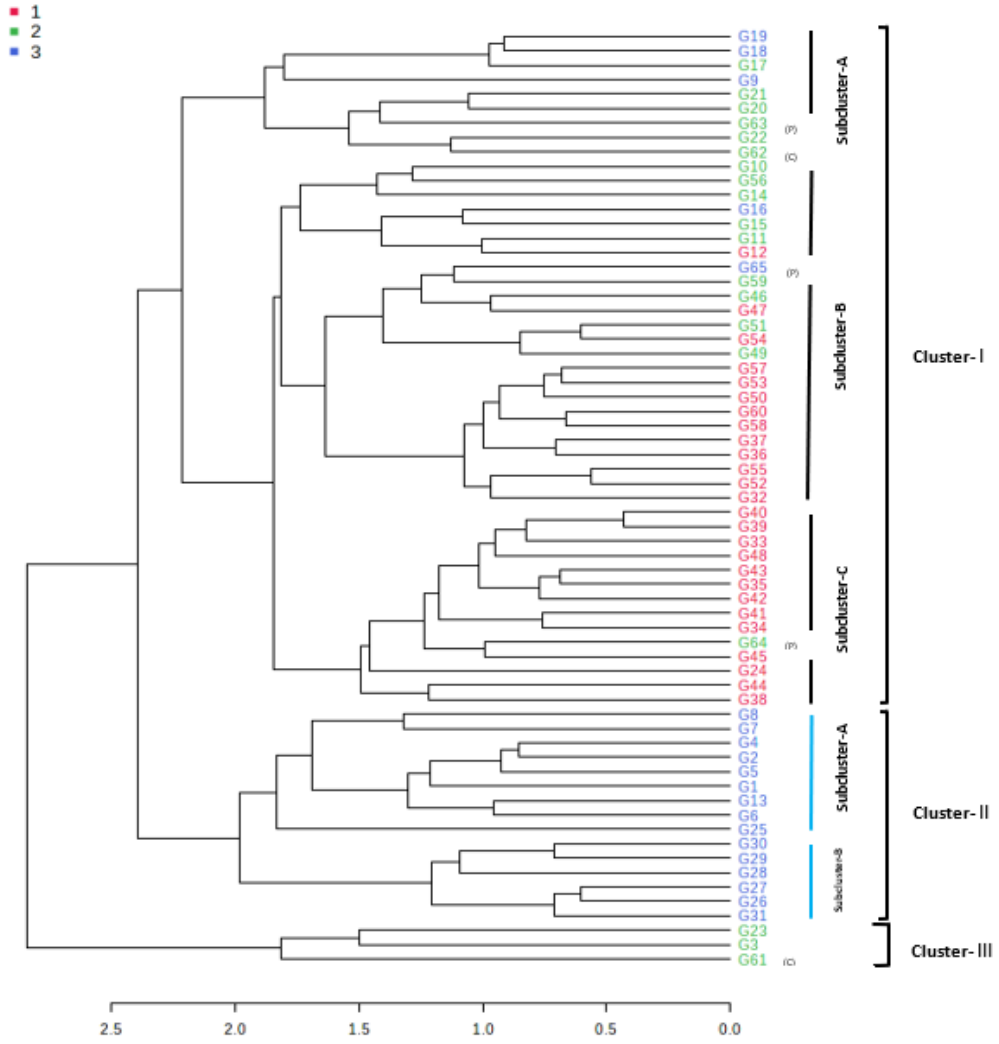

Figure 4: UPGMA tree displaying the distribution of the 65 wheat genotypes in four groups, and presenting the genetic similarities and dissimilarities within and between the clusters. Names of the genotypes are given on the terminal of branches. (Here, P= Parents, C= Check varities)

3.4 Principal component analysis

The utilization of Principal Component Analysis Associations (PCA) aids in the identification of key variables that significantly influence the phenotype of various wheat landraces. The PCA scree plot displayed the cumulative variance explained by the green line at the top, while the blue line underneath represented the variance explained by each individual principal component (Figure 5). PCA scatter plots were used to analyse the diversity of genotypes. The first three eigenvalues, which accounted for 37.4% of the cumulative variation, were shown for this purpose. The first three principal components exhibited eigenvalues of 16.1%, 13.1%, and 8.2%, resulting in a total cumulative variation of 37.4% when considering these three components in principal coordinates.

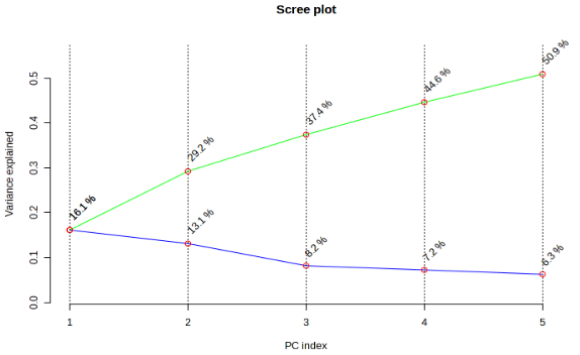

Figure 5: The scree plot displays the Principal coordinate analysis of the top 5 PCs for 65 wheat genotypes based on SSR marker data.

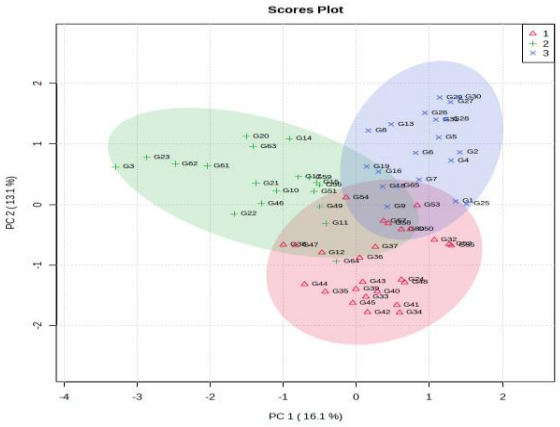

Figure 6: Two-dimensional principal component analysis (PCA) based on SSR polymorphism in the 65 wheat genotypes.

The PCA demonstrates that the first and second principal components (PC1) and (PC2) accounted for 16.1% and 13.1% of the total variation, respectively (Figure 6). The localization of genotypes in the 2D PCA plot provides insights into the genetic distances among the wheat genotypes. Through the utilization of visualization techniques and color coding, it was noted that the three groups derived from the structure and cluster analysis were also evident in the principal component analysis (PCA). The genotypes G11 (P3-4-6-P11), G12 (P3-4-6-P14), G37 (P3-29-2-P13), G3 (P3-29-4-P1), G4 (P3-29-4-P10), G49 (P3-29-10-P1), G50 (P3-29-10-P2), G54 (P3-29-10-P6), G57 (P3-29-10-P10), G58 (P3-29-10-P11), and G60 (P3-29-10-P15) were found to be mixed with both population I and population II. The genotypes G9 (P3-3-3-P8), G16 (P3-5-2-P13), G18 (P3-11-3-P12), G19 (P3-11-3-P15), and G65 (BARI Gom - 25) were shown to

be present in both population II and population III. The following genotypes, namely G1 (P3-2-2-P3), G9 (P3-3-3-P8), G18 (P3-11-3-P12), G50 (P3-29-10-P2), G53 (P3-29-10-P5), G54 (P3-29-10-P6), G57 (P3-29-10-P10), G58 (P3-29-10-P11), G60 (P3-29-10-P15), and G65 (BARI Gom - 25), were found to be mixed with both population I and population III. The genotypes G9 (P3-3-3-P8), G18 (P3-11-3-P12), G54 (P3-29-10-P6), G57 (P3-29-10-P10), G58 (P3-29-10-P1)1, G60 (P3-29-10-P15), and G65 (BARI Gom - 25) were combined with their respective populations, namely Population I, Population II, and Population III. The genotypes G3(P3-2-2-P9), G10(P3-3-3-P14), G13(P3-5-2-P4), G14(P3-5-2-P6), G20(P3-18-3-P7), G21(P3-18-3-P12), G22(P3-18-10-P5), G23(P3-18-10-P15), G26(P3-28-3-P2), G27(P3-28-3-P3), G28(P3-28-3-P5), G29(P3-28-3-P9), G30(P3-28-3-P10), G34(P3-29-2-P5), G35(P3-29-2-P11), G38(P3-29-4-P1), G41(P3-29-4-P4), G44(P3-29-4-P7), G46(P3-29-4-P9), G47(P3-29-4-P10), G61(BARI Gom - 30), G62(BARI Gom - 33) and G63(AGHRANI) found far away from the centroid of the cluster and the rest of the genotypes were placed more or less around the centroid (Figure 7).

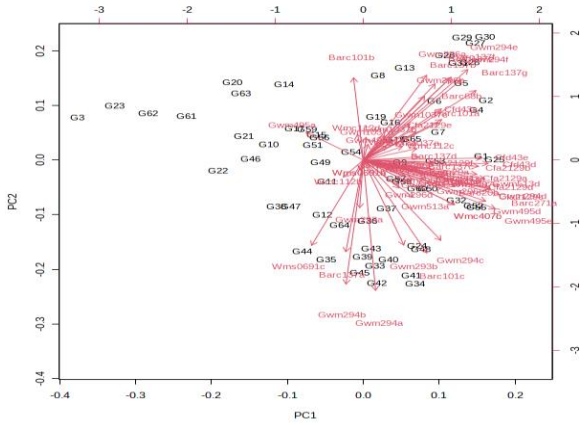

Figure 7: Two dimensional view of Principal Coordinate Analysis of SSR markers over 65 wheat genotypes.

4. Discussion

SSR markers have been widely applied for the purpose of assessing their genetic diversity in wheat genotypes. In this study, an average of 4 alleles observed across the 68 loci ranged from 2 to 7. The number of alleles ranged from 2 to 8, with an average of 3.8 alleles per locus and identified total 166 alleles by analyzing 26 markers (Shafi et al., 2021). Other researchers have reported averages of 2.68, 6.88 and 7.6 alleles per locus in various wheat collections (Sharma et al., 2021; Christov et al., 2022; Ahmed et al., 2020). PIC value is divided into 3 classes: PIC>0.5 = highly informative; 0.25> PIC> 0.5 = moderately informative; and PIC <0.25 = slightly informative (Sagwal et al., 2022; Rohmawati et al., 2021). All markers showed highly informative PIC values with the average of 0.84 reported by (Rohmawati et al., 2021). The highest and lowest PIC values 0.84 and 0.21 were estimated for the markers TaXbarc137 and TaBarc271 respectively. The average PIC value is 0.66 and the 14 Primers viz. TaXbarc137, TaXcfd43, TaXgwm294, Xgwm296, TaXcfa2129, Gwm513, Tagwm1037, Xwmc112, TaGwm291, WMS0691, Tabarc101, TaBarc68 and TaXwmc407 with PIC values respectively showed highly informative. The marker Tagwm293, and Barc20 obtained PIC values of 0.44 and 0.38 showed moderately informative. The TaBarc271 marker showed slightly informative with a PIC value of 0.21. The PIC value is a good measure of a marker's usefulness for linkage analysis and detected a marker's probability of the marker being in the progeny.

Furthermore, it serves as an indication of the allelic variety present among various varieties (Meti et al., 2013). In addition, the average PIC values observed in this study were mostly similar to 0.64 and 0.67 reported by using 22 SSR markers, respectively (Tsonev et al., 2021). The present study reported a lower mean PIC value compared to a 0.79 reported by using 25 SSR markers (Jlassi et al., 2021). On the contrary, a higher mean PIC value in this study was found compared to 0.48 and 0.32 reported by most of researcher respectively (Belete et al., 2021; Pour-Aboughadareh et al., 2022). So, SSR markers with high PIC value imply that were highly informative for future breeding programs.

The genetic population of the complete sample assembly was stratified based on all marker systems, revealing the presence of a unique structure. The analysis for K = 3 was provided, as its populations demonstrated a substantially greater degree of co-linearity (80%) with neighbor-joining clusters. Using K = 3, the population structures study categorized the 65 wheat genotypes into three subpopulations (Mohi-Ud-Din et al., 2022). A group researcher also observed a comparable range that Populations I, II, and III were comprised of 26, 20, and 19 genotypes, respectively (Christov et al., 2022). Out of the total, 21 genotypes were pure in Population I, 11 genotypes were pure in Population II, and 13 genotypes were pure in Population III. The remaining 20 genotypes were mixed among these three groups.

A group researcher demonstrated that out of the 20 wheat genotypes examined, only 4 exhibited admixtures (Sihag et al., 2021). A group researcher identified a total of 53 bread wheat genotypes among these genotypes, population I comprised 20 genotypes, population II consisted of 14 genotypes, and population III comprised 17 genotypes (Mohi-Ud-Din et al., 2022). FST quantifies the extent to which population structure may account for genetic variation, as determined by Wright's F-statistics (Wright, 1965). The population in this study was determined using pairwise FST values, which varied from 0.1855 to 0.5105. The pairwise FST analysis indicated that these three populations were considerably distinct from each other. The fixation index, a measure of population substructure, ranged from 0.283 to 0.658 as detected by (Alsharari, 2021).

The cluster analysis was conducted using the UPGMA method to determine the genetic diversity among 65 wheat genotypes. The genotypes were classified into 3 groups such as cluster I, II, an III, which was consistent with the findings (Wang et al., 2017). Many scientists have implemented these strategies in wheat breeding schemes and have achieved informative results (Tsonev et al., 2021; Zatybekov et al., 2021; Ahmed et al., 2017; Salehi et al., 2018). In this study, the observed cluster III with genotypes G3 (P3-2-2-P9), G23 (P3-18-10-P15) and G61 (BARI Gom-30) exhibited a greater genetic divergence in comparison to the remaining two groups. The individuals within this cluster had a higher degree of dissimilarity compared to the other two cluster members.

The genotypes within this cluster exhibited greater genetic diversity compared to the genotypes within other clusters. A group researcher classified 20 Egyptian wheat landraces and two cultivars based on 10 SSR markers into four clusters based on the UPGMA method for genetic diversity analysis and clearly revealed the significant molecular diversity of the Egyptian wheat landraces and cultivars (Al-Naggar et al., 2020). Based on the degree of diversity, the UPGMA analysis can result in the formation of two clusters or four clusters (El-Bakatoushi, 2019; Haque et al., 2021). Furthermore, genetic diversity studies have indicated as many as 9 clusters and 13 clusters (Naceur et al., 2012; Schuster et al., 2009).

The PCA has considerable importance in the selection phase of molecular breeding projects. PC1 and PC2 had a greater degree of variation, exceeding 25%. Utilising the higher variation (>25%) in conjunction with cluster analysis can be employed to identify appropriate and correlated genotypes. A group researcher found that the first three principal components (PC1-PC3) had eigen-values greater than 1 (Ali et al., 2019). These components accounted for individual variance values of 30.93%, 18.44%, and 17.84%, respectively. Together, they explained 67.21% of the cumulative variation in grain yield. The initial two principal components (PCs) were graphed on PC axis 1 and 2, revealing significant diversity among the current wheat lines and control samples. Some researcher utilised a Pearson-based PC1 and PC2 biplot to project genotypes in three subpopulations of spring wheat (Sajjad et al., 2018). The structure analysis revealed three distinct sub-populations, which were also differentiated based on the first two principal components. This finding is consistent with the results reported (Tascioglu et al., 2016).

The genotypes positioned at a greater distance from the centroid exhibited greater genetic diversity, whereas the genotypes positioned close to the centroid displayed very similar genetic origins. G3 (P3-2-2-P9) and G23 (P3-18-10-P15) were positioned much further from the centroid compared to the others, resulting in the observation of the most diverse genotypes. The two principal components exhibited comparable major grouping patterns to those examined by UPGMA clustering, with consistently clustered 65 wheat genotypes. This aligns with the findings of who reported three principal components that displayed similar major grouping patterns to UPGMA clustering (Pathaichindachote et al., 2019). These genotypes exhibit no genetic similarity among themselves or among the complete set of sixty-five genotypes examined. Consequently, they possess the potential to serve as parental individuals in future breeding initiatives.

5. Conclusion

The purpose of this study was to evaluate the genetic diversity and population structure of 65 wheat lines through the utilisation of 17 simple sequence repeat (SSR) markers. The SSRs marker has exhibited notable

patterns characterised by significant variability, hence enabling the differentiation of all cultivars. The study identified a total of 68 alleles. Each marker locus included a range of alleles, varying from two to seven, with an average of four. The average value for genetic diversity and polymorphism information content was 0.21 and 0.84, respectively. The examination of the structure indicated the existence of three distinct subpopulations, which align with the clustering pattern based on genetic distance. The cluster analysis categorised the data into three primary genetic groups, regardless of the origin of the data collection. Cluster III, consisting of P3-2-2-P9 and P3-18-10-P15, exhibited distinct genetic patterns and relationships, indicating the possibility of divergent genetic compositions among them. Novel genes can be derived from these sources and utilised in wheat breeding programmes.

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Conflict of Interest

The authors have declared that no competing interests exist.

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Author Contributions

Conceptualization, M.H. and M.A.H..; Data collection and analysis, A.S., and M.M.H.P.; Original draft, A.S.; Review and editing, M.H., A.S., M.M.H.P., and M.A.H.; Funding acquisition, A.S. All authors provided intellectual inputs, read the manuscript, approved for submission and agreed to the published version of the manuscript.

Ethics Approval

There is no ethical approval required in this study.

Availability of Data and Materials

The availability of data and materials in this research is subject to the policy set by the corresponding author upon reasonable request.

References

Ahmed, H.G.M.D., Kashif, M., Rashid, M.A.R., Sajjad, M., and Zeng, Y., 2020. Genome wide diversity in bread wheat evaluated by SSR markers. Int. J. Agric. Biol., 24, Pp. 263-272.

Ahmed, H.G.M.D., Khan, A.S., Khan, S.H., and Kashif, M., 2017. Genome wide allelic pattern and genetic diversity of spring wheat genotypes through SSR markers. Int. J. Agric. Biol., 19, Pp. 1559-1565.

Ali, Y., Khan, M.A., Hussain, M., Atiq, M., and Ahmad, J.N., 2019. An assessment of the genetic diversity in selected wheat lines using molecular markers and PCA based cluster analysis. Appl. Ecol. Environ. Res., 17 (1), Pp. 931-950.

Al-Naggar, A.M.M., El-Shafi, M.A.E.M.A., El-Shal, M.H., and Anany, A.H., 2020. Molecular assessment of genetic diversity among Egyptian landraces of Wheat (Triticum aestivum L.) Using Microsatellite Markers. Asian J. Biochem. Genet. Mol. Biol., 3 (4), Pp. 46-58.

Alsharari, S.F., 2021. 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. Nveo- Nat. Vol. Essent. Oil, 9960-9973.

Batool, N., Ilyas, N., Shahzad, A., Hauser, B.A., and Arshad, M., 2018. Quantitative trait loci (QTLs) mapping for salt stress tolerance in wheat at germination stage. Pak. J. Agric. Sci., 55 (1)

Belete, Y., Shimelis, H., Laing, M., and Mathew, I., 2021. Genetic diversity and population structure of bread wheat genotypes determined via phenotypic and SSR marker analyses under drought-stress conditions. J. Crop Improv., 35 (3), Pp. 303-325.

Chong, J., and Xia, J., 2018. MetaboAnalystR: an R package for flexible and reproducible analysis of metabolomics data. Bioinformatics, 34 (24), Pp. 4313-4314.

Christov, N.K., Tsonev, S., Dragov, R., Taneva, K., Bozhanova, V., and Todorovska, E.G., 2022. Genetic diversity and population structure of modern Bulgarian and foreign durum wheat based on microsatellite and agronomic data. Biotechnol. Biotechnol. Equip.", 36 (1), Pp. 637-652.

Drikvand, R.M., Najafian, G., and Salahvarzi, A., 2015. Investigation of genetic diversity of some durum and bread wheat genotypes using SSR markers. J. Bio. Env. Sci., 6 (3), Pp. 24-32.

El-Bakatoushi, R. 2010. Genetic diversity of winter wheat (Triticum aestivum L.) growing near a high voltage transmission line. Rom. J. Biol. - Plant Biol., 55 (2), Pp. 71-87.

FAO, 2022. Online sources FAO Cereal Supply and Demand Brief, World Food Situation, Global cereal production and trade Forecast to Fall to three-year lows, release date: 02/12/2022 https://www.fao.org/worldfoodsituation/csdb/en/

Govindaraj, M., Shanmugasundaram, P., Sumathi, P., and Muthiah, A.R., 2010. Simple, rapid and cost effective screening method for drought resistant breeding in pearl millet. Electron. J. Plant Breed. 1. Pp. 590-599.

Gupta, P.K., Kumar, J., Mir, R.R., and Kumar, A., 2010. Marker-assisted selection as a component of conventional plant breeding. Plant Breed. Rev., 33, Pp. 145.

Jiang, G.L., 2013. Molecular Markers and Marker-Assisted Breeding in Plants. In Plant Breeding from Laboratories to Fields, edited by Sven Bode Andersen. InTech. https://doi.org/10.5772/52583.

Jlassi, I., Bnejdi, F., Saadoun, M., Hajji, A., Mansouri, D., Ben-Attia, M., and El-Bok, S., 2021. SSR markers and seed quality traits revealed genetic diversity in durum wheat (Triticum durum Desf.) Mol. Biol. Rep., 48 (4), Pp. 3185-3193.

Haque, M.S., Saha, N.R., Islam, M.T., Islam, M.M., Kwon, S.J., Roy, S.K., and Woo, S.H., 2021. Screening for drought tolerance in wheat genotypes by morphological and SSR markers. J. Crop Sci. Biotechnol., 24, Pp. 27-39.

Kahrizi, D., Maniee, M., Mohammadi, R., and Cheghamirza, K., 2010. Estimation of genetic parameters related to morpho-agronomic traits of Durum Wheat (Triticum turgidum var. durum). Biharean Biologist, 4 (2), Pp. 93-97.

Khaled, F.M.S., Marion, S.R., and Andreas, B., 2015. Assessing genetic diversity of Egyptian hexaploid wheat (Triticum aestivum L.) using microsatellite markers. Genet. Resour. Crop Evol. 62, Pp. 377-385.

Khan, M.K., Pandey, A., Thomas, G., Akkaya, M.S., Kayis, S.A., Ozsensoy, Y., and Hakki, E.E., 2015. Genetic diversity and population structure of wheat in India and Turkey. AoB Plants, 7.

Kumar, S., Kumar, V., Kumari, P., Kirti, Singh, A.K., and Singh, R., 2016. DNA fingerprinting and genetic diversity studies in wheat genotypes using SSR markers. J. Environ. Biol., Pp. 319-326.

Kumar, P., Ramesh, K.Y., Sandeep, K., and Pritam, K., 2016. Molecular Diversity Analysis in Wheat Genotypes Using SSR Markers. Electron. J. Plant Breed., 7 (2), Pp. 464. https://doi.org/10.5958/0975 928X.2016.00060.0.

Meti, N., Samal, K.C., Bastia, D.N., and Rout, G.R., 2013. Genetic diversity analysis in aromatic rice genotypes using microsatellite based simple sequence repeats (SSR) marker. Afr. J. Biotechnol., 12 (27), Pp. 4238.

Mohi-Ud-Din, M., Hossain, M.A., Rohman, M.M., Uddin, M.N., Haque, M.S., Dessoky, E.S., and Aloufi, S., 2022. Assessment of genetic diversity of bread wheat genotypes for drought tolerance using canopy reflectance-based phenotyping and SSR marker-based genotyping. Sustainability, 14 (16), Pp. 9818.

Naceur, A.B., Chaabane, R., El-Faleh, M., Abdelly, C., Ramla, D., Nada, A., and Sakr, M., 2012. Genetic diversity analysis of North Africa's barley using SSR markers. J. Genet. Eng. and Biotechnol., 10 (1), Pp. 13-21.

Pathaichindachote, W., Panyawut, N., Sikaewtung, K., Patarapuwadol, S., and Muangprom, A., 2019. Genetic diversity and allelic frequency of selected Thai and exotic rice germplasm using SSR markers. Rice Science, 26 (6), Pp. 393-403.

Pour-Aboughadareh, A., Poczai, P., Etminan, A., Jadidi, O., Kianersi, F., and Shooshtari, L., 2022. An Analysis of Genetic Variability and Population Structure in Wheat Germplasm Using Microsatellite and Gene-Based Markers. Plants, 11 (9), Pp. 1205.

Pritchard, J.K., Stephens, M., and Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics, 155 (2), Pp. 945-959.

Raj, R.S., Yama, S.V., Viral, K.M., Baranda, M.N.J., ShradhaN, Tyagi, and Snehal, B.B., 2017. Ascertaining Narrow Genetic Base in Commercial Accessions of Wheat Commonly Grown in Gujarat via Molecular Markers." Electron. J. Plant Breed., 8 (2), Pp. 558. https://doi.org/10.5958/0975- 928X.2017.00084.9.

Rohmawati, I., Nursusilawati, P., and Abdullah, S., 2021. Genetic diversity of Some Indonesian Local Rice Varieties based on Simple Sequence Repeat (SSR) marker related to Aromatic Genes. In IOP Conference Series: Earth and Environmental Science (Vol. 715, No. 1, p. 012046) IOP Publishing.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., and Allard, R.W., 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proceedings of National of Academy Science (USA) 81, Pp. 8 014–8.

Sagwal, V., Sihag, P., Singh, Y., Mehla, S., Kapoor, P., Balyan, P., and Kumar, U., 2022. Development and characterization of nitrogen and phosphorus use efficiency responsive genic and miRNA derived SSR markers in wheat. Heredity, Pp. 1-11.

Salehi, M., Arzani, A., Talebi, M., and Rokhzadi, A., 2018. Genetic diversity of wheat wild relatives using SSR markers. Genetika, 50 (1), Pp. 131-141.

Schuster, I., Vieira, E.S.N., Silva, G.J.D., Franco, F.D.A., and Marchioro, V.S., 2009. Genetic variability in Brazilian wheat cultivars assessed by microsatellite markers. Int. J. Genet. Mol. Biol., 32, Pp. 557-563.

Sajjad, M., Khan, S.H., and Shahzad, M., 2018. Patterns of allelic diversity in spring wheat populations by SSR-markers. Cytology and Genetics, 52 (2), Pp. 155-160.

Shafi, S., Tahir, M., Khan, M.A., Bhat, M.A., Kumar, U., Kumar, S., and Mir, R.R., 2021. Trait phenotyping and SSR markers characterization of wheat (Triticum aestivum L.) germplasm for breeding early maturing wheat's for Western-Himalayas. Genet. Resour. Crop. Evol., Pp. 1-16.

Sharma, P., Mehta, G., Muthusamy, S.K., Singh, S.K., and Singh, G.P., 2021. Development and validation of heat-responsive candidate gene and miRNA gene based SSR markers to analysis genetic diversity in wheat for heat tolerance breeding. Mol. Biol. Rep., 48 (1), Pp. 381-393.

Shewry, P.R. 2009. Wheat. Journal of Experimental Botany, 60 (6), Pp. 1537-1553.

Shuaib, M., Jamal, M., Akbar, H., Khan, I., Khalid, R., 2010. Evaluation of Wheat by Poly-Acrylamide Gel Electrophoresis. African Journal of Biotechnology, 9 (2) Pp. 243-247.

Sihag, P., Sagwal, V., Kumar, A., Balyan, P., Mir, R.R., Dhankher, O.P., and Kumar, U., 2021. Discovery of miRNAs and development of heat-responsive miRNA-SSR markers for characterization of wheat germplasm for terminal heat tolerance breeding. Front. genet., 12, Pp. 699420.

Tomar, R.S.S., Tiwari, S., Naik, B.K., Chand, S., Deshmukh, R., Mallick, N., 2016. Molecular and morpho-agronomical characterization of root architecture at seedling and reproductive stages for drought tolerance in wheat. PloS one, 11 (6), Pp. e0156528.

Tascioglu, T., Metin, O.K., Aydin, Y., Sakiroglu, M., Akan, K., and Uncuoglu, A.A., 2016. Genetic diversity, population structure, and linkage disequilibrium in bread wheat (Triticum aestivum L.). Biochem. Genet., 54, Pp. 421-437.

Tsonev, S., Christov, N.K., Mihova, G., Dimitrova, A., and Todorovska, E.G., 2021. Genetic diversity and population structure of bread wheat varieties grown in Bulgaria based on microsatellite and phenotypic analyses. Biotechnol. Biotechnol. Equip., 35 (1), Pp. 1520-1533.

United States Department of Agriculture (USDA), World Wheat Production 2022/2023. December 2022, online source: http://www.worldagriculturalproduction.com/crops/ wheat.aspx

Xu, Y., Shimoro, X., Hofstra, H., 1994. Plant DNA isolation protocol. Nucleic Acid Res. 22, Pp. 2399-2403.

Zatybekov, A., Anuarbek, S., Abugalieva, S., and Turuspekov, Y., 2020. Phenotypic and genetic variability of a tetraploid wheat collection grown in Kazakhstan. Vavilov Journal of Genetics and Breeding, 24 (6), Pp. 605.

Zeb, B., Khan, I.A., Ali, S., Bacha, S., Mumtaz, S., and Swati, Z.A., 2009. Study on genetic diversity in Pakistani wheat varieties using simple sequence repeat (SSR) markers. Afr. J. Biotechnol., 8 (17), Pp. 4016-4019.