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Short Communication

Molecular Characterization of Genetic Variants in Bread Wheat through SSR Markers

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Abstract

Gamma Rays, ethyl methane sulfonate and combination of both mutagens were used to develop a diverse population for induction of genetic variability in bread wheat varieties. Thirty stable mutants along with parents were investigated for polymorphism through SSR markers. A total of 269 alleles were amplified, in which 75.46% were polymorphic. Nei's genetic diversity (h) varied from 0.165 to 0.479 with a mean of 0.415. Shanon's index (I) showed a range of 0.23 to 0.672, with an average of 0.598. The proportion of genetic relationship, within populations, was recorded as 16.39% of the whole diversity, and gene flow value was noted as 2.55. The maximum dissimilarity was observed in mutant SE4/12-1 while the minimum was detected in mutant SG1/12-41. Dendrogram based on UPGMA, grouped thirty mutants and three parents into three major and nine sub-clusters "A" to "I". © 2020 Friends Science Publishers

Keywords: Bread wheat; Allo-hexaploid; Mutants; Polymorphism; SSR markers

Introduction

Bread wheat (*Triticum aestivum* L.) is hexaploid (2n=6x=42) comprising of A, B and D genomes which has largest genome of 17 Gb with 80% repeats (Kumar *et al.*, 2016). Nowadays 95% hexaploid wheat is grown in Pakistan which contributes 10% to the value added in agriculture and 2% to GDP, whereas national yield average is 2.5 t/ha (Anonymous 2018). The common yield of wheat is pretty low due to increase in population and also drastic changes in climatic conditions. Though, there is still need to improvement and genetic manipulation is the best tool to increase the production. Therefore, induced new genetic variation is the key factor and mode of inheritance in altered plant traits to initiate constructive wheat breeding programs for sustainable agriculture (Kharestani *et al.* 2016). Hence, induced mutation is applied as a successful tool to increase genetic variability while physical and chemical mutagens induce different mutation spectra and induction of new alleles in crop species.

Molecular characterization of wheat genotypes is also beneficial to assess the loss of genetic polymorphism and detect more variability (Kumar *et al.* 2016). Simple sequence repeat (SSR) markers for genome analysis have many additional properties that evenly disbursed within whole genome, co-dominant and impartial. SSR markers are used effectively to study genetic variation in wheat germplasm (Abbasov *et al.* 2018). In the present study, SSR markers were used to assess the genetic variation among thirty promising wheat mutants, which may possibly help for the development of new variety with wide range of genetic base in wheat breeding.

Materials and Methods

We used 50 g pure basic seed of each variety *i.e.*, Sarsabz, Kiran and TD1 for each treatment/dose for induced mutation by gamma rays (50, 100, 150, 200, 250 and 300 Gy), EMS (0. 4, 0. 8, 1.2, 1.6 and 2.0%) and combined treatment from NIA, Tando Jam and ARI, Tando Jam due to their yield stability and adaptability in different climatic conditions. Control was used as non-mutagenized seeds of each variety and raised the M_1, M_2, M_3, M_4 and M_5 generation. Finally, thirty mutants were selected on the basis of improved agronomical traits, phenotypic diversity and higher yield. Fresh young leaves were collected from field at seedling stage from thirty mutants and DNA was isolated and quantified by using modified CTAB method (Bibi *et al.* 2012).

Forty SSR primers (Table 1) have been used to amplify thirty mutants and three parents. The cocktail was prepared in 10 μL containing 1 μM SSR forward and reverse primer (Gene link),1X Taq buffer, 0.1 u/μL of Taq enzyme, 2.5 m*M* of $MgCl_2$, 0.2 m*M* of dNTPs and 0.8 ng/μL of DNA template for PCR amplification. PCR was programmed for first denaturation for 5 min at 95°C, followed by thirty five repeats for 1 min at 95°C, 1 min at 55°C, 1.30 min at 72°C and one last step of extension at 72°C for 07 min. PCR amplification DNA segment were resolved by 3% agarose gel. Subsequently, gel photograph

Table 1: Simple sequence repeats (SSR) primers for characterization of the wheat mutants

S. #	Primers	Sequence (5` to 3`)	Temp. (°C)	%GC
1	WMS508	F: GTTATAGTAGCATATAATGGCC	55	36
		R: GTGCTGCCATGATATTT	48	41
2	WMS361	F: GTAACTTGTTGCCAAAGGGG	57	50
		R: ACAAAGTGGCAAAAGGAGACA	56	43
3	WMS193	F: CTTTGTGCACCTCTCTCTCC	59	55
		R: AATTGTGTTGATGATTTGGGG	54	38
4	WMS644	F: GTGGGTCAAGGCCAAGG	58	65
		R: AGGAGTAGCGTGAGGGGC	61	68
5	WMS-71	F: GGCAGAGCAGCGAGACTC	61	67
		R: CAAGTGGAGCATTAGGTACACG	60	50
6	WMS-319	F: GGTTGCTGTACAAGTGTTCACG	60	50
		R: CGGGTGCTGTGTGTAATGAC	59	55
7	WMS-429	F: TTGTACATTAAGTTCCCATTA	50	29
		R: TTTAAGGACCTACATGACAC	53	40
8	Gwm361	GTAACTTGTTGCCAAAGGGG	52	50
		ACAAAGTGGCAAAAGGAGACA	50	43
9	Gwm219	GATGAGCGACACCTAGCCTC	56	60
		GGGGTCCGAGTCCACAAC	55	67
10	Wmc221	ACGATAATGCAGCGGGGAAT	65	50
		GCTGGGATCAAGGGATCAAT	63	50
11	Wmc121	GGCTGTGGTCTCCCGATCATTC	69	59
		ACTGGACTTGAGGAGGCTGGCA	69	59
12	Xcfd68	TTTGCAGCATCACACGTTTT	60	40
		AAAATTGTATCCCCCGTGGT	55	45
13	Gwm325	TTTCTTCTGTCGTTCTCTTCCC	55	45
		TTTTTACGCGTCAACGACG	63	47
14	Gwm179	AAGTTGAGTTGATGCGGGAG	52	50
		CCATGACCAGCATCCACTC	53	58
15	Gwm335	CGTACTCCACTCCACACGG	55	63
		CGGTCCAAGTGCTACCTTTC	54	55
16	Xgwm46	GCA CGT GAA TGG ATT GGA C	51	53
		TGA CCC AAT AGT GGT CA	45	47
17	Xgwm2	CTG CAA GCC TGT GAT CAA CT	52	50
		CAT TCT CAA ATC GAA CA	40	35
18	Xgwm18	TGG CGC CAT GAT TGC ATT ATC ATC TTC	58	44
		GGT TGC TGA AGA ACC TTA TTT AGG	54	42
19	Xgwm33	GGA GTC ACA CTT GTT TGT GCA	52	48
		CAC TGC ACA CCT AAC TAC GTG C	57	55
20	Xgwm5	GCC AGC TAC CTC GAT ACA ACT C	57	55
		AGA AAG GGC CAG GCT AGT AGT	54	52
21	Xgwm44	GTT GAG CTT TTC AGT TCG GC	52	50
		ACT GGC ATC CAC TGA GCT G	53	58
22	Xpsp2999	TCC CGC CAT GAG TCA ATC	50	56
		TTG GGA GAC ACA TTG GCC	50	56
23	Xpsp3000	GCA GAC CTG TGT CAT TGG TC	54	55
		GAT ATA GTG GCA GCA GGA TAC	52	48
24	Xcn15	GGT GAT GAG TGG CAC AGG	53	61
		CCC AAC AGT TGC AGA AAA TTA G	51	41
25	Xcn13	AGA ACA GTC TTC TAG GTT AG	48	40
		CGA GGG ACA GAC GAA TC	49	59
26	DuPw004	GGTCTGGTCGGAGAAGAAGC	56	60
		TGGGAGCGTACGTTGTATCC	54	55
27	DuPw023	ATTAGACACGACCAAACGGG	52	50
		TCAAACAAACAACAGCCAGC	50	45
28	DuPw043	TTTGAACGGAATTTGAGAATTT	46	27
		AGGGTGTGAACATGGAGGAG	54	55
29	DuPw108a	TGAAGAGTGCGATGTGAAGG	52	50
		TGTGACAGAAACTACTAACATTGCG	54	40
30	DuPw108b	TGTTTCTTCCTCGCGTAACC	52	50
		CCTCGAATCTCCCAGTTATCG	54	52
31	DuPw123	CAACGAGAACCAGAAGACCG	54	55
		CCCGTTACACTTGGATGCC	53	58
32	DuPw217	CGAATTACACTTCCTTCTTCCG	53	45
		CGAGCGTGTCTAACAAGTGC	54	55
33	DuPw216	ACAAACCTCTCCCTCTCACG	54	55
		ATGATGATTCAGCGAGTCGG	52	50
34	DuPw210	CGATTTGGATTCTTCCGC	48	50
		AGAGCCTTTGAAGAGCAGGG	54	55
35	DuPw207	GAGAGTATCAATAAAGCTAGATGCCC	56	42
		GCATTTGGAAGGAGATGTGG	52	50
36	DuPw205	ATCCAGATCACACCAAACGG	52	50
		CTTCCGCTTCATCTTCTTGC	52	50
37	DuPw238	TTCATAGACGCAACTAGCCG	52	50
		GACTTTGGTTGTTAAAGGCG	50	45
38	DuPw398	CTGAGCCCTCTTTGCTATGC	54	55
		TCGGTGAGATTGAAAGGTCC	52	50
39	DuPw254	TTAACCATGCAGCAACTTCG	50	45
		GTGTGTACTAACGGCTACGGC	56	57
40	DuPw165	TAGGTCTCGACAACAAGCCG	54	55
		TCACCACTTGGAGGTTACTGC	54	52

was documented *via* gel documentation system of Vilber Lourmat, France.

Data were recorded as presence of allele and absence of allele through UVi Band Map software. The genetic attributes were created by software of population genetic structure named "POPGENE" (Yeh *et al.* 1997). Genetic kinship among the populations was calculated by the Nei's formula and also used to find phylogenetic relationship through un-weight pair group method with the arithmetic averages (UPGMA) (Nei and Li 1979).

Results

Estimation of genetic variability among promising mutants

Out of 40 primers, fourteen alleles produced polymorphic amplification from the genomic DNA of wheat mutants with parents. The total number of the amplified alleles was 269 across the set of 33 mutants with parent. The share of the polymorphic alleles with a mean was 75.46% (Table 2). The individual genotype of 33 mutants and parents created polymorphism and among these few monomorphic alleles were also ascertained (Fig. 1). Primer WMS-644 amplified six DNA fragments, in which five were polymorphic and varied from 200 bp to 1.25 kb.

Genetic variation within population

Genetic variation between the mutants and parents is given in Table 1. In individual mutants along with parent, the percentage of P allele per population varied from 66.7–87%, with a mean of 78.96%. Number of alleles (Na) ranged from 1.3 to 2.0, while number of effective alleles (Ne) ranged from 1.325 to 1.925. Heterozygosity (H) varied from 0.165 to 0.479 to with a mean of 0.415. Shanon Index (I) showed a range of 0.23 to 0.672, with an average of 0.598. In 30 mutants and three parents of bread wheat, various levels of genetic dissimilarity were observed. The maximum dissimilarity was observed in mutant SE4/12-1, while the minimum was detected in mutant SG1/12-41 (Table 3). Dendrogram based on UPGMA (Fig. 2), the varieties were classified into three groups and nine clusters A to I.

Population genetic structure and differentiation

Wheat mutants and their parent exhibited different levels of genetic variation among the populations in Table 2. The total genetic diversity (H_T) and observed genetic diversity (Hs) within the populations were estimated about 0.50 and 0.42, respectively. The genetic diversity within populations (Ds) was recorded as 16.39% of the whole diversity which showed that high genetic diversity was observed among the populations. The Nm (gene flow) value was 2.55 showing that number of genes migrating between the populations was maximum (Table 4).

Table 2: Genetic variation statistics for all alleles of mutants and their parents

S. #	Mutants	No of alleles	P	% of alleles	P	Na	Ne	H	I
1	SE4/12-1-1	9		77.8		2.0000	1.9252	0.4794	0.6722
2	SE4/12-1-2	4		66.7		2.0000	1.7333	0.4213	0.6118
3	SE4/12-3	7		77.8		2.0000	1.8667	0.4630	0.6554
4	SE4/12-4	10		83.3		2.0000	1.8394	0.4529	0.6445
5	SE4/12-5	6		75		2.0000	1.8218	0.4488	0.6406
6	SE4/12-6	8		80		2.0000	1.5509	0.3450	0.5254
7	SE5/12-7	11		85		2.0000	1.6687	0.3773	0.5561
8	SE5/12-8	9		82		2.0000	1.8218	0.4488	0.6406
9	SE5/12-9	9		82		2.0000	1.8218	0.4488	0.6406
10	SE5/12-10	9		82		2.0000	1.7000	0.3944	0.5779
11	TCT4/12-1	10		83		1.6667	1.5551	0.3007	0.4284
12	TCT4/12-2	10		83		2.0000	1.8218	0.4488	0.6406
13	SE5/12-12	10		83		2.0000	1.7628	0.4266	0.6164
14	SE5/12-13	11		85		2.0000	1.7632	0.4324	0.6238
15	SE5/12-15	8		80		2.0000	1.8533	0.4596	0.6520
16	SE5/12-17	5		71		2.0000	1.8533	0.4596	0.6520
17	SE5/12-19	4		66.7		2.0000	1.6727	0.3994	0.5882
18	SG3/12-20	8		80		2.0000	1.9119	0.4760	0.6688
19	SG3/12-21	10		83.3		2.0000	1.8218	0.4448	0.6406
20	SG3/12-23	7		77.8		2.0000	1.9119	0.4760	0.6688
21	SG3/12-25	6		75		2.0000	1.8218	0.4488	0.6406
22	SG2/12-26	9		77.8		2.0000	1.7632	0.4324	0.6238
23	SG2/12-27	6		75		2.0000	1.8533	0.4596	0.6520
24	SE2/12-29	4		67		2.0000	1.7632	0.4324	0.6238
25	SG4/12-35	8		80		2.0000	1.7632	0.4324	0.6238
26	SG1/12-38	12		86		2.0000	1.5509	0.3450	0.5254
27	SG1/12-41	6		75		1.3333	1.3252	0.1646	0.2290
28	SG1/12-43	13		87		1.6667	1.4060	0.2513	0.3760
29	KCT7/12-44	8		80		2.0000	1.7632	0.4324	0.6238
30	SCT6/9-	10		83		2.0000	1.8533	0.4596	0.6520
31	Sarsabz	7		77.8		2.0000	1.5509	0.3450	0.5254
32	Kiran-95	7		77.8		2.0000	1.8533	0.4596	0.6520
33	TD-1	8		80		2.0000	1.7632	0.4324	0.6238

Abbreviations: P: Polymorphic allele; Na: Observed number of alleles; Ne: Effective number of alleles; h: Nei's gene diversity; I: Shannon's index

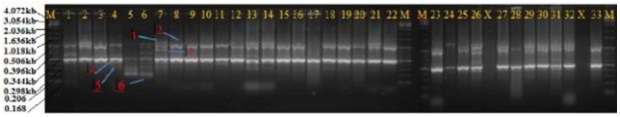

Fig. 1: Amplification profile of 33 wheat genotypes with primer WMS-644 by SSR makers (Number are correspondent to names of the genotypes presented in Table 1).

Discussion

In Pakistan, wheat genotypes such as Sarsabz, kiran-91 and TD1 are high yielding popular varieties but due to climate change these varieties are susceptible to biotic and abiotic stress. To address this issue, we developed mutants to create new genetic variation for the improvement of these varieties. This genotypic variation is useful for the parental selection, breeder rights, and varietal development (Abbasov *et al* 2018). Our results revealed that the genetic variability appeared in all the mutants/parents which produced 75.46% polymorphic fragments. Our promising mutants exhibited the genetic polymorphism through their banding pattern. SSR markers confirmed that the polymorphism might be a result of variations in nucleotides because of addition or deletion between two priming positions (Kumar *et al* 2016).

Table 3: Nei's Original Measures of Genetic Identity and Genetic distance

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1	****	0.9559	0.9223	0.9425	0.9027	0.8069	0.8203	0.9027	0.9027	0.9271	0.7400	0.8950	0.8625	0.8744	0.9659	0.9892	0.8795	0.9180	0.8950	0.9967	0.9027	0.9652	0.9892	0.9652	0.8971	0.8704	0.6771	0.6954	0.8971	0.8961	0.8704	0.9194	0.8744
2	0.045	****	0.8969	0.8101	0.9267	0.8286	0.7499	0.8002	0.8002	0.7858	0.6522	0.7783	0.8030	0.7554	0.9154	0.9816	0.9296	0.8848	0.7783	0.9312	0.9267	0.8747	0.9390	0.9993	0.8101	0.6954	0.7679	0.5741	0.8101	0.8066	0.8888	0.8728	0.8701
3	0.081	0.1088	****	0.8385	0.9644	0.8222	0.8219	0.9644	0.9644	0.8551	0.9166	0.9417	0.8788	0.9056	0.8594	0.9281	0.9674	0.9658	0.9417	0.9193	0.9644	0.9056	0.9281	0.9056	0.9727	0.7598	0.8386	0.8275	0.9727	0.9281	0.7598	0.9968	0.9056
4	0.059	0.2106	0.1761	****	0.7950	0.7566	0.8653	0.9045	0.9045	0.9608	0.7442	0.9265	0.8802	0.9348	0.9512	0.8843	0.7193	0.8755	0.9265	0.9581	0.7950	0.9348	0.9212	0.8269	0.8635	0.9847	0.5327	0.7760	0.8695	0.9212	0.8173	0.8543	0.8269
5	0.102	0.0761	0.0363	0.2294	****	0.9308	0.8779	0.8889	0.8889	0.7486	0.8488	0.8815	0.9222	0.8612	0.8896	0.9122	0.9723	0.9800	0.8815	0.8808	1.0000	0.8171	0.8748	0.9165	0.8833	0.6993	0.9270	0.8011	0.8833	0.9200	0.8691	0.9427	0.9707
6	0.215	0.1880	0.1957	0.2790	0.0718	****	0.9513	0.7609	0.7609	0.6131	0.7303	0.7952	0.9578	0.8173	0.8897	0.7860	0.8261	0.9317	0.7952	0.7753	0.9308	0.6553	0.7288	0.8227	0.7161	0.6838	0.8981	0.7939	0.7161	0.8948	0.9435	0.7911	0.9847
7	0.198	0.2878	0.1961	0.1446	0.1303	0.0500	****	0.8430	0.8430	0.7329	0.8046	0.8922	0.9940	0.9276	0.9103	0.7613	0.7468	0.9389	0.8922	0.8117	0.8779	0.7200	0.7496	0.7543	0.7823	0.8400	0.7833	0.9041	0.7823	0.9624	0.8933	0.8134	0.9616
8	0.102	0.2229	0.0363	0.1004	0.1177	0.2732	0.1707	****	1.0000	0.9252	0.9474	0.9925	0.8859	0.9707	0.8522	0.8748	0.8659	0.9420	0.9925	0.9187	0.8889	0.9265	0.9122	0.8171	0.9928	0.8621	0.7165	0.8964	0.9928	0.9574	0.6993	0.9800	0.8612
9	0.102	0.2229	0.0363	0.1004	0.1177	0.2732	0.1707	0.0000	****	0.9252	0.9474	0.9925	0.8859	0.9707	0.8522	0.8748	0.8659	0.9420	0.9925	0.9187	0.8889	0.9265	0.9122	0.8171	0.9928	0.8621	0.7165	0.8964	0.9928	0.9574	0.6993	0.9800	0.8612
10	0.076	0.2411	0.1565	0.0399	0.2895	0.4892	0.3108	0.0777	0.0777	****	0.7645	0.9181	0.7708	0.8977	0.8658	0.8874	0.7308	0.8209	0.9181	0.9543	0.7486	0.9819	0.9469	0.8079	0.9187	0.9420	0.4604	0.7205	0.9187	0.8605	0.6719	0.8821	0.7236
11	0.301	0.4274	0.0871	0.2955	0.1639	0.3143	0.2175	0.0540	0.0540	0.2685	****	0.9408	0.8450	0.9205	0.6963	0.7164	0.8259	0.8975	0.9408	0.7545	0.8488	0.7638	0.7496	0.6666	0.9401	0.7169	0.7815	0.9494	0.9401	0.9102	0.5661	0.9303	0.8234
12	0.111	0.2506	0.0601	0.0763	0.1262	0.2292	0.1141	0.0075	0.0075	0.0854	0.0610	****	0.9225	0.9928	0.8748	0.8522	0.8301	0.9497	1.0000	0.9111	0.8815	0.9045	0.8896	0.7950	0.9707	0.9034	0.7104	0.9285	0.9707	0.9800	0.7335	0.9574	0.8833
13	0.148	0.2195	0.1292	0.1276	0.0810	0.0431	0.0061	0.1211	0.1211	0.2604	0.1684	0.0807	****	0.9451	0.9265	0.8156	0.8134	0.9710	0.9225	0.8535	0.9222	0.7720	0.8034	0.8078	0.8369	0.8419	0.8223	0.9109	0.8369	0.9808	0.8974	0.8699	0.9809
14	0.134	0.2938	0.0992	0.0675	0.1494	0.2017	0.0751	0.0298	0.0298	0.1079	0.0828	0.0073	0.0565	****	0.8843	0.8174	0.7828	0.9434	0.9928	0.8902	0.8612	0.8695	0.8543	0.7617	0.9348	0.9239	0.6941	0.9465	0.9348	0.9880	0.7566	0.9212	0.8921
15	0.035	0.0884	0.1516	0.0501	0.1170	0.1168	0.0940	0.1600	0.1600	0.1441	0.3620	0.1338	0.0763	0.1230	****	0.9315	0.8089	0.9228	0.8748	0.9564	0.8896	0.8843	0.9189	0.9212	0.8174	0.8948	0.6877	0.7312	0.8174	0.9189	0.9520	0.8503	0.9212
16	0.011	0.0186	0.0746	0.1230	0.0919	0.2408	0.2727	0.1338	0.1338	0.1194	0.3335	0.1600	0.2038	0.2016	0.0710	****	0.9172	0.8996	0.8522	0.9796	0.9122	0.9512	0.9874	0.9880	0.8843	0.7911	0.7061	0.6341	0.8843	0.8503	0.9482	0.9189	0.9543
17	0.128	0.0730	0.0332	0.3294	0.0281	0.1911	0.2920	0.1440	0.1440	0.3136	0.1913	0.1862	0.2066	0.2449	0.2121	0.0864	****	0.9242	0.8301	0.8585	0.9723	0.8251	0.8814	0.9299	0.8885	0.6043	0.8997	0.7061	0.8885	0.8381	0.7670	0.9464	0.8876
18	0.086	0.1224	0.0384	0.1330	0.0202	0.0708	0.0631	0.0597	0.0597	0.1974	0.1081	0.0516	0.0294	0.0583	0.0804	0.1058	0.0789	****	0.9497	0.9085	0.9800	0.8528	0.8868	0.8902	0.9207	0.8104	0.8726	0.8871	0.9207	0.9796	0.8685	0.9564	0.9808
19	0.111	0.2506	0.0601	0.0763	0.1262	0.2292	0.1141	0.0075	0.0075	0.0854	0.0610	0.0000	0.0807	0.0073	0.1338	0.1600	0.1862	0.0516	****	0.9111	0.8815	0.9045	0.8896	0.7950	0.9707	0.9034	0.7104	0.9285	0.9707	0.9800	0.7335	0.9574	0.8833
20	0.003	0.0713	0.0842	0.0428	0.1270	0.2545	0.2086	0.0847	0.0847	0.0468	0.2818	0.0931	0.1585	0.1163	0.0446	0.0206	0.1526	0.0960	0.0931	****	0.8808	0.9808	0.9924	0.9434	0.9129	0.8966	0.6389	0.7094	0.9129	0.8996	0.8385	0.9228	0.8528
21	0.102	0.0761	0.0363	0.2294	0.0000	0.0718	0.1303	0.1177	0.1177	0.2895	0.1639	0.1262	0.0810	0.1494	0.1170	0.0919	0.0281	0.0202	0.1262	0.1270	****	0.8171	0.8748	0.9165	0.8833	0.6993	0.9270	0.8011	0.8833	0.9200	0.8691	0.9427	0.9707
22	0.035	0.1339	0.0992	0.0675	0.2020	0.4226	0.3285	0.0763	0.0763	0.0183	0.2695	0.1004	0.2588	0.1398	0.1230	0.0501	0.1923	0.1592	0.1004	0.0194	0.2020	****	0.9880	0.8921	0.9348	0.8834	0.5506	0.6814	0.9348	0.8543	0.7161	0.9212	0.7617
23	0.011	0.0629	0.0746	0.0821	0.1338	0.3164	0.2882	0.0919	0.0919	0.0546	0.2882	0.1170	0.2189	0.1575	0.0846	0.0127	0.1262	0.1201	0.1170	0.0076	0.1338	0.0121	****	0.9512	0.9212	0.8482	0.6352	0.6662	0.9212	0.8629	0.7911	0.9315	0.8174
24	0.035	0.0007	0.0992	0.1901	0.0763	0.1952	0.2820	0.2020	0.2020	0.2134	0.4056	0.2294	0.2135	0.2723	0.0821	0.0121	0.0727	0.1163	0.2294	0.0583	0.0763	0.1141	0.0501	****	0.8269	0.7161	0.7581	0.5874	0.8269	0.8174	0.8834	0.8843	0.8695
25	0.109	0.2106	0.0277	0.1398	0.1241	0.3340	0.2456	0.0073	0.0073	0.0848	0.0617	0.0298	0.1780	0.0675	0.2016	0.1230	0.1182	0.0826	0.0298	0.0912	0.1241	0.0675	0.0821	0.1901	****	0.8227	0.7120	0.8518	1.0000	0.9212	0.6553	0.9880	0.8269
26	0.139	0.3633	0.2747	0.0155	0.3577	0.3801	0.1743	0.1403	0.1403	0.0598	0.3229	0.1016	0.1721	0.0791	0.1112	0.2344	0.5037	0.2102	0.1016	0.1092	0.3577	0.1240	0.1646	0.3340	0.1952	****	0.4260	0.7810	0.8227	0.8897	0.7404	0.7860	0.7566
27	0.390	0.2640	0.1760	0.6298	0.0758	0.1075	0.2443	0.3334	0.3334	0.7757	0.2466	0.3419	0.1957	0.3652	0.3744	0.3480	0.1057	0.1363	0.3419	0.4480	0.0758	0.5967	0.4538	0.2769	0.3397	0.8533	****	0.7396	0.7120	0.7822	0.7479	0.8006	0.9015
28	0.363	0.5550	0.1894	0.2536	0.2217	0.2308	0.1008	0.1093	0.1093	0.3278	0.0519	0.0742	0.0934	0.0550	0.3131	0.4555	0.3480	0.1198	0.0742	0.3433	0.2217	0.3837	0.4061	0.5320	0.1604	0.2472	0.3016	****	0.8518	0.9379	0.6353	0.8409	0.8526
29	0.109	0.2106	0.0277	0.1398	0.1241	0.3340	0.2456	0.0073	0.0073	0.0848	0.0617	0.0298	0.1780	0.0675	0.2016	0.1230	0.1182	0.0826	0.0298	0.0912	0.1241	0.0675	0.0821	0.1901	0.0000	0.1952	0.3397	0.1604	****	0.9212	0.6553	0.9880	0.8269
30	0.110	0.2150	0.0746	0.0821	0.0833	0.1112	0.0383	0.0435	0.0435	0.1502	0.0941	0.0202	0.0194	0.0121	0.0846	0.1621	0.1767	0.0206	0.0202	0.1058	0.0833	0.1575	0.1474	0.2016	0.0821	0.1168	0.2457	0.0641	0.0821	****	0.8326	0.9315	0.9512
31	0.139	0.1179	0.2747	0.2017	0.1403	0.0582	0.1129	0.3577	0.3577	0.3976	0.5690	0.3099	0.1083	0.2790	0.0492	0.1646	0.2653	0.1410	0.3099	0.1761	0.1403	0.334	0.2344	0.1240	0.4226	0.3005	0.2905	0.4537	0.4226	0.1833	****	0.7288	0.9239
32	0.084	0.1361	0.0032	0.1575	0.0591	0.2344	0.2065	0.0202	0.0202	0.1254	0.0722	0.0435	0.1393	0.0821	0.1621	0.0846	0.0551	0.0446	0.0435	0.0804	0.0591	0.0821	0.0710	0.1230	0.0121	0.2408	0.2224	0.1733	0.0121	0.0710	0.3164	****	0.8843
33	0.134	0.1392	0.0992	0.1901	0.0298	0.0155	0.0388	0.1494	0.1494	0.3235	0.1944	0.1241	0.0193	0.1141	0.0821	0.1575	0.1192	0.0194	0.1241	0.1592	0.0298	0.2723	0.2016	0.1398	0.1901	0.2790	0.1037	0.1595	0.1901	0.0501	0.0791	0.1230	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Table 4: Nei's Analysis of Gene Diversity in Subdivided Populations

Locus	Sample Size	Ht	Hs	Gst	Nm*
Mean	210	0.4959	0.4146	0.1639	2.5506
St. Dev		0.0000	0.0004		

* Nm = estimate of gene flow from Gst or Gcs. E.g., Nm = 0.5(1 - Gst)/Gst
See McDermott and McDonald, Ann. Rev. Phytopathol. 31:353-373 (1993)

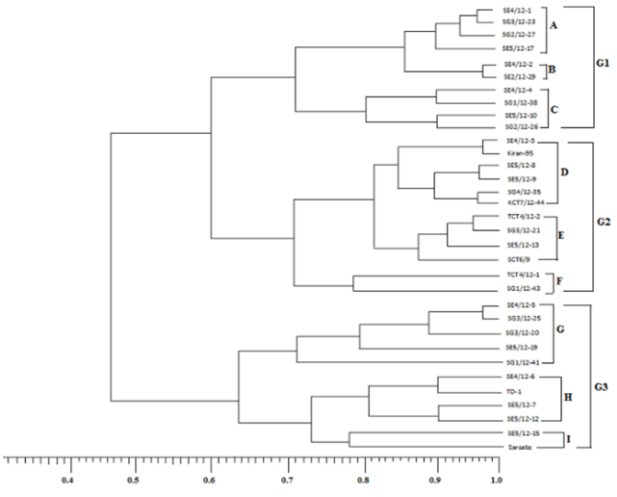

Fig 2: Dendrogram showing Nei's genetic distance by UPGMA method

The present, results showed large differentiation, based on the Nei's analysis of gene diversity and a significant degree of genetic differences was exhibited among all the wheat genotypes. It is the correlation of gametes in subpopulations relative to gametes moved at indiscriminately from the complete population and studies the overall genetic divergence among subpopulations (Aboughadareh *et al*. 2018). It describes expected degree of heterozygosity within a population. Results showed that the gene flow among the mutants was high enough. The migration of genes in distinct populations is high in comparison to those two populations which have the same or less genetic diversity. The population divergence may be explained in terms of genetic drift when one migrant per generation is received (Aboughadareh *et al*. 2018). It could be one of the reasons that gene flow constraints phylogeny by combining the gene pools of the populations and accordingly prevents the event of differences in genetic diversity. Moreover, high genotypic variations are recognized to control gene flow.

Results showed genetic relationship among the promising mutants with their parents and proved that mutation is valuable technique to create the new alleles in bread wheat. Previously, Bibi *et al.* (2012) recorded that crop plant improvement depends on the data about the genetic kinships among plants within or between crop species. The information regarding the genetic similarity is useful to prevent any possible risk of elite genotypes developing genetically uniform. It was also reported that breeders usually use the exotic material from ICARDA/CIMMYT crossed with indigenous cultivars to develop the variety which may cause the narrow genetic stock for wheat (Sundeep *et al*. 2016). Thus, conscious struggles have to be generated to expand the parental genetic makeup to create assured high genetic variability among the genotypes of the crop plants. In the present study, among 30 mutants, ten mutants were grouped together in one group (71%). Though, eleven mutants and a single parent Kiran-95 in group two was observed the most distinguishable one and these eleven mutants in the same group showed the sharing of the same blood among the mutants (70%). However, nine mutants and two parents Sarsabz and TD1 formed another distinguished group which exhibited the 37% distinctness among the mutants. Phylogenetic relationship not only gives the information regarding genetic similarity but also provides a chance to

find new and helpful genes (Sajjad *et al.* 2018). Thus, conscious struggles have to be generated to expand the parental genetic makeup to create assured high genetic variability among the genotypes of the crop plants.

Conclusion

Our mutants manifested significant degree of genetic differences among the genotypes with 16.4% of the total variation among the mutants whereas heterozygosity Hs and Ht was recorded 0.4146 and 0.4959, respectively while gene flow among the mutants was high enough (2.55). It also provides a better gene flow of wheat mutants and a source of variation for the selection of the parents to speed up the breeding program.

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Author Contributions

Sajida bibi as a first author contribution is 70% and second author rubina has 30% contribution in this research paper. I tried to write in a correction grid but I could not write on it.

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