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Aastha
Department of Agriculture Biotechnology, College of Agriculture, SVPUA&T, Meerut, (U.P.) India

Vaishali
Department of Agriculture Biotechnology, College of Agriculture, SVPUA&T, Meerut, (U.P.) India

Khyati Lehari
Department of Agriculture Biotechnology, College of Agriculture, SVPUA&T, Meerut, (U.P.) India

Vishakha Burman
Department of Agriculture Biotechnology, College of Agriculture, SVPUA&T, Meerut, (U.P.) India

Chetan Chauhan
Department of Horticulture, College of Agriculture, SVPUA&T, Meerut, (U.P.) India

Rajendra Singh
Department of Entomology, College of Agriculture, SVPUA&T, Meerut, (U.P.) India

Akash Singh
Department of Genetics and Plant Breeding, College of Agriculture, SVPUA&T, Meerut, (U.P.) India

Correspondence
Aastha
Department of Agriculture Biotechnology, College of Agriculture, SVPUA&T, Meerut, (U.P.) India

Molecular characterization of wheat genotypes using eSSR Markers

Aastha, Vaishali, Khyati Lehari, Vishakha Burman, Chetan Chauhan, Rajendra Singh and Akash Singh

Abstract
Wheat is an important Rabi Crop, which is grown between October and December and harvested between April and May. Globally, wheat is the leading source of protein than maize or rice. A study was undertaken to examine the genetic diversity of 20 wheat (*Triticum aestivum* L.) using designed EST-SSR markers. The similarity coefficient value for all the 20 genotypes ranged from 0.23 to 0.63. The minimum similarity exhibited by genotype PBW550 and DBW17. Whereas, the maximum similarity was shown by genotype DBW16 and RAJ4246. The pair wise genetic similarities among all pairs of samples were estimated and subjected to cluster analysis. The UPGMA based clusters shows that all the genotypes are interlinked with each other and exhibited high genetic similarity. The UPGMA based clustering of 20 wheat genotypes grouped the wheat genotypes into three distinct clusters at 70% similarity coefficient.

Keywords: *Triticum aestivum* L., simple sequence repeats, genetic diversity, cluster analysis.

Introduction
Common wheat (Triticum aestivum) (2n = 6x = 42) belongs to family Poaceae, the most diverse and important family of the plant kingdom. It produces large edible grains and provides about one-half of humans' food calories and a large part of their nutrient requirements. The majority of the cultivated wheat varieties belong to four main species of the genus *Triticum*. These are the *T. aestivum* (hexaploid), *T. durum (tetraploid)*, *T. dicoccum (diploid)* and *T. monococcum*. *Aestivum* wheat, popularly known as bread wheat, is the most important species which covers ninety per cent of the area. Second popular wheat being durum wheat which covers about 9 percent of the total area while *T. diccoum* wheat and *T. monococcum* wheat cover less than the one percent of the total area. The world acreage under wheat crop is 215.26 million ha. World wheat production was 711 million tonnes in 2013-14, 730 million tonnes in 2014-15, 733 million tones in 2015-16 and 760 million tones in 2016-17 (Food and Agriculture Organization of the United state, 2017) [3]. Wheat has good nutrition profile with 12.1 percent protein, 1.8 percent lipids, 1.8 percent ash, 2.0 percent reducing sugars, 6.7 percent pentosans, 59.2 percent starch, 70 percent carbohydrates and provides 314K cal/100g of food (Lafiandra *et al*., 2014) [6]. It is also a good source of minerals and vitamins *viz*., calcium (37mg/100g), iron (4.1mg/100g), thiamine (0.45mg/100g), riboflavin (0.13mg/100g) and nicotinic acid (5.4mg/100mg) (Lorenz and Kulp, 1991) [7, 8]. Unlike other cereals, wheat contains a high amount of gluten, the protein that provides elasticity that is necessary for excellent bread making (Breiman and Graur, 1995).
Assessment of genetic diversity at the molecular level is more meaningful than at the phenotypic level as the later involves data on morphological traits which are environmental dependent. Though, they significantly contribute towards phenotypic variation but cannot give accurate phenotype. So the study of polymorphism is best done at the level of arrangement of nucleotide bases in DNA, the primary source of all biological information. SSRs are the primary assay for detecting molecular polymorphism and well-developed SSR linkage maps are available for a number of species (Sverdlov *et al*., 1998) [12]. The genomic SSRs have neither genic function nor close linkage to transcriptional regions, while EST-SSRs are potentially tightly linked with functional genes that perhaps control certain important genetic characters (Zeng *et al.*, 2010) [13]. In addition, EST-SSR markers contain high transferability because EST-SSRs are derived from expressed sequences that are more conserved than the non-genic sequences and easily found in other relative species (Scott *et al.*, 2000) [11].

Materials and Method
The experiment was conducted in Random Block Design (RBD) with three replications in two

crop season of year 2014-15 and 2015-16. For this purpose a total of 20 Wheat genotype viz K1205, PBW435, PBW533, DBW16, PBW550, DBW17, UP2425, K9351, HD3076, K9465, PBW502, HD2735, HUW234, HD2864, PBW590, RAJ4246, HD2987, PBW373, WH1021, PBW226 were collected from Chiraudi Farm, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. Seeds of all 20 genotypes of wheat were germinated in petri dishes under three different concentrations (5%, 10% and 15%) of Polyethylene Glycol (PEG) along with control (no PEG only distilled water) for imposing drought stress condition. After germination, the 15 days old seedlings were transferred to the field.

Extraction of DNA and SSR analysis

For genomic DNA isolation, CTAB method was used (Doyle and Doyle, 1987) [1]. CTAB (Cetyl Trimethyl Ammonium Bromide) is a cationic detergent, which solubilizes cell membranes and forms a complex with DNA. PCR technique was performed in 25 µl volume containing: master mix beads, 10 µl buffer (10 X), 1 µl primer (100 pmol), 1 µl DNA template (50 ng) and 13 µlH O sterile. The amplification was carried out in a thermocycler programmed as follows: 1 cycle of 94°C/2 min, 35 cycles of (94°C/1min, 48°C/2 min and 72°C/2 min), 1 cycles of 72°C/7 min. The primer annealing degrees were varied according to the melting point of each primer. Agarose gel was used for separating the PCR products of amplified DNA fragments by electrophoresis. Agarose gel electrophoresis of the isolated genomic DNA was performed to carry out quantitative as well as qualitative analysis of DNA.

PCR amplification of genomic DNA using eSSR primers

A set of 29 eSSR primers, custom synthesized by Banglore Genei Pvt. Ltd. were used in the present study. EST mining and EST-SSR primer designing: EST sequences for designing the EST SSR primers were searched from the sequences available in public domain at (http://www.ncbi.nlm.nih.gov). All wheat ESTs were downloaded from NCBI in FASTA format. Downloaded EST sequences were assembled in the form of contigs using an online available EG assembler program (http://www.genome.jp/tools/egassembler). Primers were designed for the identified eSSR sequences using online available software PRIMER 3 (http://www.frodo.wi.mit.edu).

Result and Discussion

In the present study, we attempted to characterize 20 wheat genotypes at molecular level, using 29 EST- simple sequence repeat (eSSR) markers. A total of 29 eSSR primers resulted in scorable and reproducible result hence they were considered for the analysis of genetic diversity of wheat genotypes (Figure 1).

29 primers generated a total of 193 bands of which 166 were polymorphic and 27 bands were monomorphic. The expected gene diversity was calculated for all the polymorphic bands and was found to be varing from 0.07 of primer 7 to 0.96 value of primer 1 with a mean diversity of 0.61 (Table 1). The higher mean PIC value indicated the informative ness of the primers pairs in detecting genetic diversity. Hence, the primer2, primer11, primer13, primer21 and primer22 seem to be more informative as they showed the expected gene diversity value higher than 0.80 similar result was shown by (Röder *et al*., 1995), Eujayl *et al.*, (2002) [2] and Nicot *et al*., (2004) [9] and can be used in future studies in the field of taxonomical and genetic resource management.

Resolving power of the 29 EST-SSR primers ranged from 0.34 to 1.92 with an average of 1.0 resolving power for all polymorphic primers (Table 1). The highest resolving power 1.92 was recorded for the primer 7. On the other hand the lowest resolving power 0.34 was recorded with the primer1. Based on resolving power and ability of primers to differentiate all accessions, the primer pair, the primer4, primer 18, primer 20 and primer29 seems to be more informative as they show the resolving power value higher than 1.50. Thus the significant value of resolving power indicated the ability of primers to resolve the different closely related genotypes of wheat.

Table 1: Expected gene diversity and resolving power of eSSR primer used to amplify 20 wheat genotype.

S.No	Primer name	Sequence	Total no. of bands	No. of polymorphic bands	Expected gene diversity/PIC	Resolving power	Product size
1	PWeSSR1A F	AGATCTAGAGCAGAGGACGAG	8	8	0.96	0.34	176
	PWeSSR1A R	CTGCATCAACCTTTCCAC					
2	PWeSSR2A F	GATCCTCTACTCCCCGTCT	11	11	0.81	0.62	134
	PWeSSR2A R	ACTCCACACCCTTGATAGTCT					
3	PWeSSR3A F	GAGGAAGGAGAAGGAGGAG	10	8	0.61	1.06	135
	PWeSSR3A R	GGTCTCCACGATGTCCAC					
4	PWeSSR4A F	TCCTAAATCCTGAGGAGAGAC	2	2	0.40	1.54	178
	PWeSSR4A R	CCTCCTACATCATTGCTCTTA					
5	PWeSSR5A F	TTGATGCTCTGTAAAACCAAT	4	4	0.75	0.70	142
	PWeSSR5A R	TGCATTTTAGTCCCTTATTTCT					
6	PWeSSR6A F	TAGGTGCGCAGTACTAGATGT	4	2	0.43	1.30	177
	PWeSSR6A R	GAAAGCGTATACTCGATTGAA					
7	PWeSSR7A F	CATGAAACTGCATTTTAGTCC	3	1	0.07	1.92	138
	PWeSSR7A R	ACCAATCTTGATGGTTGTTTA					
8	PWeSSR8A F	ACTACCTCCCCTTCATGGT	7	7	0.62	1.00	124
	PWeSSR8A R	TCCACTCCTCCAGCATCT					

9	PWeSSR9A F	CTTCGACAACCACCTCAG	10	10	0.74	0.80	175
	PWeSSR9A R	CATCTCTTAGCATGCAACATC					
10	PWeSSR10A F	TGCGCAGTACTAGGTGTTTAT	3	1	0.34	1.36	150
	PWeSSR10A R	CTGAAAGCGTATACTACTGGAA					
11	PWeSSR11A F	GAACGAGAGGAAGAGGAAAG	8	8	0.87	0.50	170
	PWeSSR11A R	CTGGTTCTCGACCTCACC					
12	PWeSSR13A F	GACCAAGCACCAACAGTG	8	7	0.73	0.80	124
	PWeSSR13A R	TTGCCTAGCAAGACATATACC					
13	PWeSSR14A F	ACCATGATGACCATAGCATT	10	10	0.91	0.38	135
	PWeSSR14A R	CTACCTAGCTAGCCTGATCCT					
14	PWeSSR15A F	GTGCGTGACAGAGAGATGA	8	8	0.65	0.68	165
	PWeSSR15A R	GGGTACTTGAACTCGATGAC\					
15	PWeSSR16A F	CTAGGGTTTCGTCCAATTC	8	6	0.70	0.76	141
	PWeSSR16A R	GTCGAACCCCATGTACAGAT					
16	PWeSSR17A F	CCTGATCCTACCCATATATCA	3	3	0.51	1.36	168
	PWeSSR17A R	CCTAATTTAACCACAGGGAAC					
17	PWeSSR18A F	CAACAAGAGCAGGAGCAG	8	8	0.75	0.82	162
	PWeSSR18A R	GAGTAGTCGGATCTGGAGGT					
18	PWeSSR19A F	CATCATGGTGGTTTTTACAAT	2	1	0.29	1.64	135
	PWeSSR19A R	ATTAGCAGCTGCAACTTAGTG					
19	PWeSSR20A F	GCAAGCAACTAATGGAGTTTA	2	1	0.49	1.14	158
	PWeSSR20A R	TTCAGAGAACCAATACAAAACA					
20	PWeSSR21A F	CCGTCGTCAGTTCAAATGG	3	3	0.40	1.52	124
	PWeSSR21A R	TCCAGGAATGGGTTTACTGC					
21	PWeSSR22A F	GGAAGAACAAGGGCAATGG	9	9	0.95	0.38	172
	PWeSSR22A R	CGGCACCCTGATGTCCTC					
22	PWeSSR23A F	GATCTGTGACCGAGGCAGA	10	10	0.87	0.54	168
	PWeSSR23A R	GCTGTGGAGGTCCAAAATGT					
23	PWeSSR24A F	CTACCCGCCGCAGCTCTAC	8	7	0.49	1.20	151
	PWeSSR24A R	GGTTCTTGAAGTCGGTGGTG					
24	PWeSSR25A F	CTCCCTCTGCCCCTCTTG	12	11	0.69	0.80	162
	PWeSSR25A R	CAGCTCGCCTGTATCCATCT					
25	PWeSSR26A F	CCTGCTCTGCCATTACTTGG	5	4	0.66	0.94	168
	PWeSSR26A R	TGCACCTCCATCTCCTTCTT\					
26	PWeSSR27A F	ACGGCGTGTTGAGTTTTTCT	8	7	0.70	1.00	142
	PWeSSR27A R	CAACTGCAACAACAAAACAGT					
27	PWeSSR28A F	TGTGCTTCAAGCCTCAAGTG	7	5	0.37	1.36	152
	PWeSSR28A R	GCTCGCACTCGAGTACACTG					
28	PWeSSR29A F	CACCATCACCGAGATCCAA	9	8	0.63	0.94	164
	PWeSSR29A R	GGAGCTCCTCCACCTTGTC					
29	PWeSSR30A F	GAACATTTTTGCGTCCTGTG	3	3	0.35	1.60	134
	PWeSSR30A R	TGGTGATCCAGAAGCCATTT					

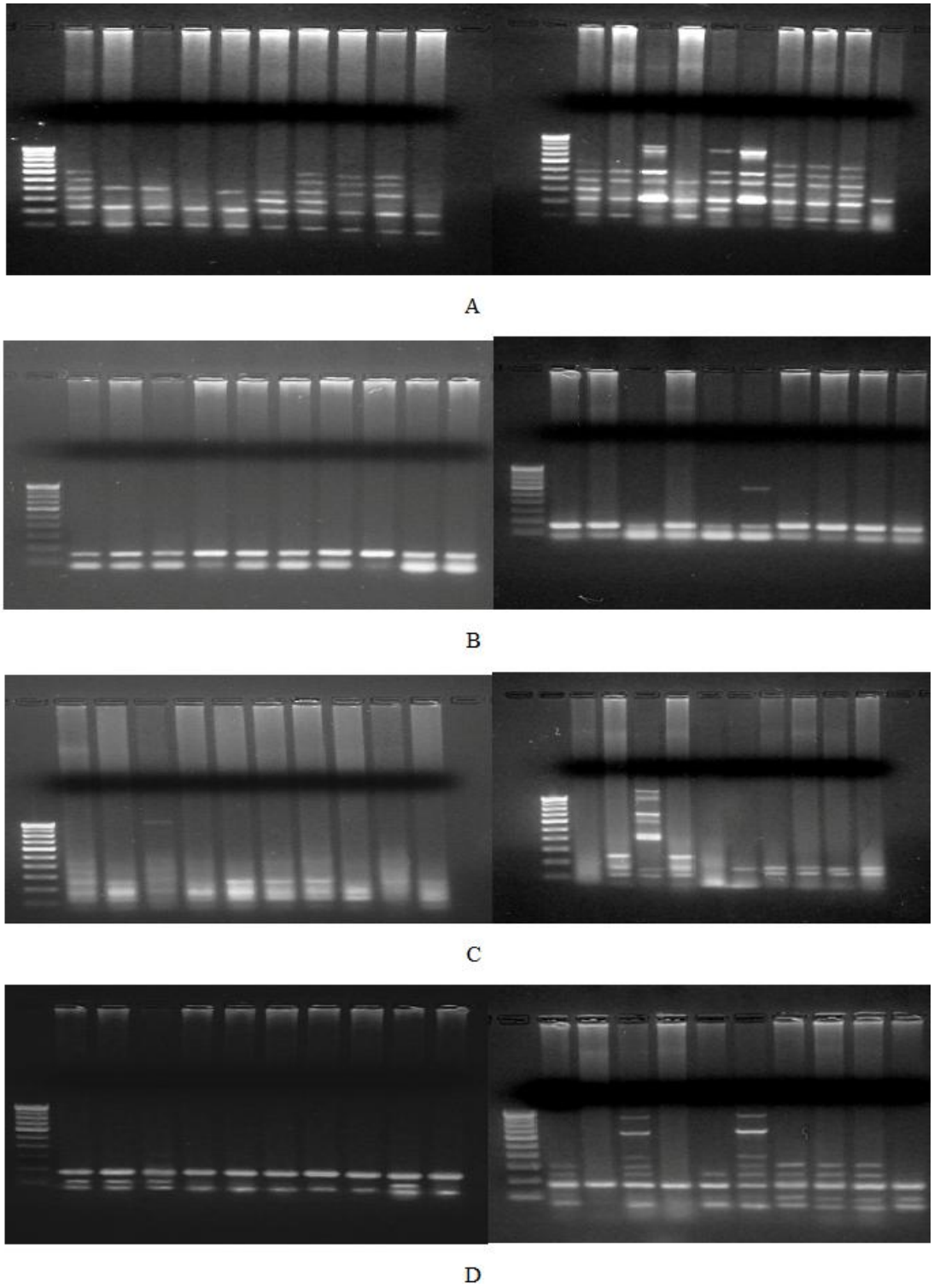

Fig 1: (A) Amplification of 20 wheat genotype using eSSR primer9 **(B)** Amplification of 20 wheat genotype using eSSR primer10 **(C)** Amplification of 20 wheat genotype using eSSR primer11, **(D)** Amplification of 20 wheat genotype using eSSR primer12

Genetic similarity matrix and cluster analysis

The SSR profile was utilized for estimating pair wise genetic similarities among various entries using Jaccard's coefficient (1908) [5] method. All the polymorphic bands were scored as 0-1 and the genetic similarity matrix was generated using UPGMA clustering algorithm program by software programme NTSYS-PC version 2.02e (Table 2). A dendogram was also constructed for grouping of closely related genotypes of wheat.

Based on the distance matrix expressed as similarity coefficient a dendrogram was generated by the UPGMA method. Similarity value for all the 20 genotypes ranged from 0.23 to 0.63 (Figure 2). The minimum similarity exhibited by genotype DBW17 and UP2425. Whereas, the maximum similarity was shown by genotype RAJ4246 and HUW234 similar result shown by (Goswami and Ranade, 1999) [4].

	K-1205	PBW435	PBW533	DBW16	PBW550	DBW17	UP-2425	K-9351	HD3076	K-9465	PBW502	HD2735	HUW234	HD2864	PBW590	RAJ4246	HD2987	PBW373	WH1021	PBW226	Average D^2
K-1205																					0.45
PBW435	0.44																				0.40
PBW533	0.36	0.37																			0.43
DBW16	0.38	0.36	0.36																		0.40
PBW550	0.44	0.34	0.44	0.29																	0.41
DBW17	0.38	0.35	0.33	0.30	0.23																0.39
UP-2425	0.37	0.34	0.31	0.28	0.37	0.24															0.38
K-9351	0.46	0.31	0.42	0.33	0.30	0.29	0.29														0.39
HD3076	0.33	0.35	0.35	0.29	0.37	0.35	0.26	0.33													0.39
K-9465	0.52	0.31	0.41	0.31	0.35	0.39	0.37	0.30	0.31												0.42
PBW502	0.41	0.41	0.46	0.48	0.46	0.38	0.40	0.43	0.40	0.49											0.45
HD2735	0.46	0.33	0.42	0.37	0.39	0.36	0.35	0.34	0.42	0.41	0.37										0.41
HUW234	0.59	0.59	0.56	0.60	0.60	0.55	0.56	0.57	0.55	0.60	0.59	0.58									0.56
HD2864	0.54	0.37	0.53	0.43	0.39	0.44	0.44	0.41	0.46	0.40	0.43	0.34	0.60								0.45
PBW590	0.55	0.49	0.52	0.50	0.40	0.50	0.50	0.44	0.46	0.44	0.51	0.50	0.53	0.48							0.49
RAJ4246	0.57	0.62	0.60	0.63	0.61	0.61	0.61	0.61	0.58	0.61	0.58	0.60	0.40	0.61	0.52						0.58
HD2987	0.40	0.43	0.42	0.47	0.49	0.46	0.40	0.44	0.35	0.46	0.36	0.43	0.55	0.45	0.54	0.54					0.44
PBW373	0.46	0.38	0.45	0.39	0.37	0.42	0.39	0.38	0.41	0.41	0.44	0.29	0.54	0.42	0.42	0.58	0.40				0.41
WH1021	0.42	0.41	0.44	0.36	0.43	0.35	0.33	0.37	0.37	0.44	0.38	0.33	0.54	0.41	0.50	0.59	0.31	0.27			0.40
PBW226	0.51	0.39	0.49	0.40	0.43	0.44	0.46	0.40	0.45	0.42	0.52	0.40	0.62	0.39	0.52	0.61	0.47	0.37	0.34		0.45

Table 2: Similarty matrix based Jaccard Coefficient of similarty obtained from eSSR primers in wheat genotypes

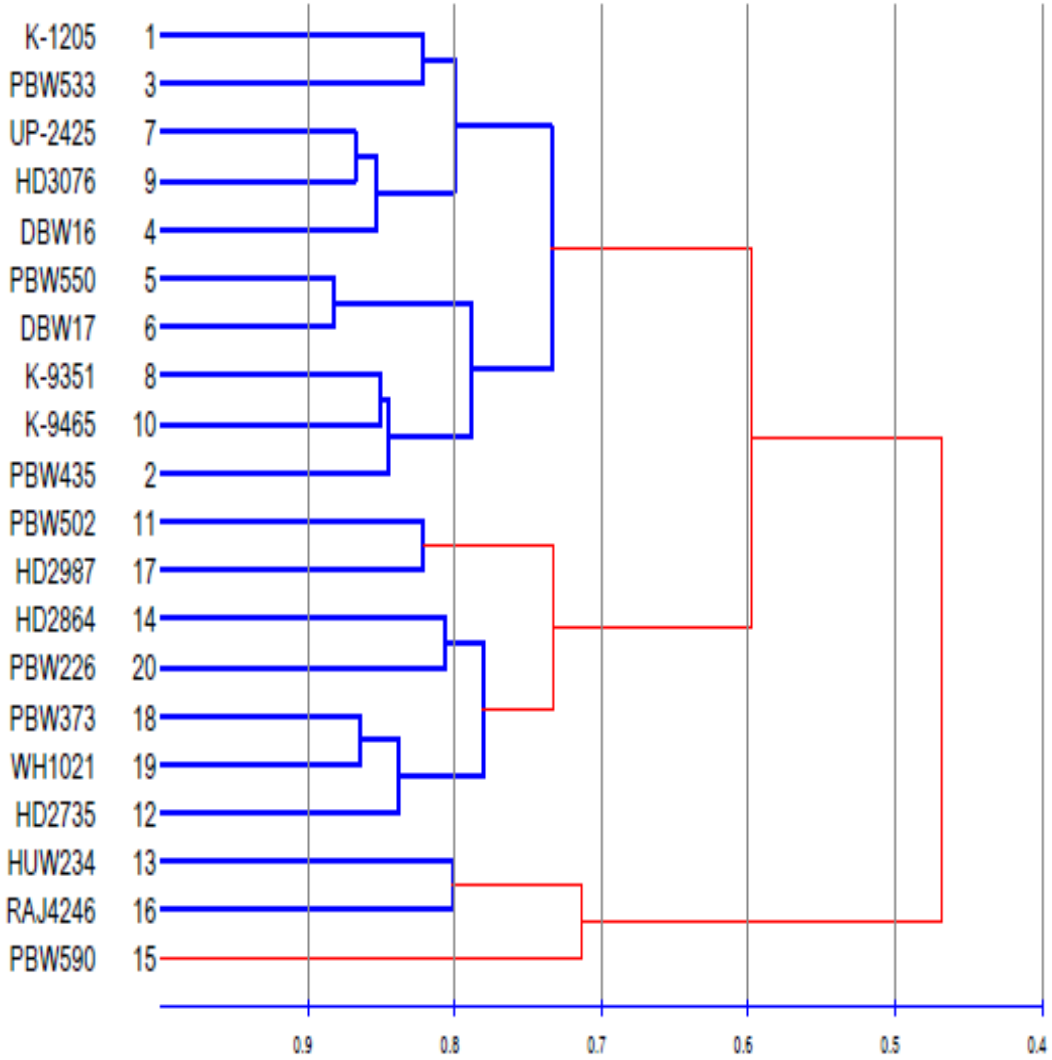

Fig 2: Dendrogram of twenty wheat genotypes.

Conclusion

On analyzing the molecular data it was observed that similarity coefficient value for all the 20 genotypes ranged from 0.23 to 0.63. The minimum similarity exhibited by genotype PBW550 and DBW17. Whereas, the maximum similarity was shown by genotype DBW16 and RAJ4246.The pair wise genetic similarities among all pairs of samples were estimated and subjected to cluster analysis. The UPGMA based clusters shows that all the genotypes are interlinked with each other and exhibited high genetic similarity. The UPGMA based clustering of 20 wheat genotypes grouped the wheat genotypes into three distinct clusters at 70% similarity coefficient.

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