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Research Article

Analysis of Genetic Diversity in Wheat (*Triticum Aestivum* L.) Using Simple Sequence Repeats Marker

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Abstract

Today, the ecological cycle is changing day by day due to some environmental changes, therefore efforts should be made to screen or develop improved crops which can give better yield with quality. All the major cereal crops like wheat, rice etc. need to develop a better improved varieties by developing vital traits. The present investigation was carried out at experimental field station of the Department of Agricultural Biotechnology, SVPUA&T, Meerut (U.P.). For this study the genomic DNA of 60 wheat varieties were taken to analyze the genetic diversity by using 50 SSR wheat specific primers. Results from 50 SSR primers had generate total of 66 reproducible and clearly scorable bands across the 60 wheat varieties with an average of 1.78 bands per primer. Out of fifty SSR primers, 37 SSR primers had shown polymorphism. The average number of polymorphic bands was 1.56 per primer. The PIC value of 37 SSR markers out of fifty is varies from primer 0.08 to 0.99. The obtained SSR profile was utilized for estimating pair wise genetic similarity matrix and further used for dendrogram construction and cultivator differentiation using UPGMA clustering algorithm program. Similarity value for all the 60 varieties ranged from 0.72 to 0.98. The minimum similarity exhibited by variety WH711 and SL1. Whereas the maximum similarity was shown by variety KRL19 and W13. The varieties bearing the desired values from different clusters can be exploited in future breeding programme for the improving the wheat varieties for yield and physiological traits.

Keywords: Genetic Diversity, Algorithm, Similarity matrix, Physiological traits, SSR, Dendogram

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Introduction

Among the various major cereal crops wheat (*Triticum aestivum* L.) is one of the most important cereal crop worldwide and grown under both irrigated and rain fed conditions. It is the staple food for 40% of the world's population [1, 2]. Currently it is growing as most important cereal crop on a large scale [3], because of its significance as cash crop and major role in supplying the dietary requirements of the society. It provides 21% of the total food calories and 20% of the protein for more than 4.5 billion people in 94 developing countries [4, 5]. Global wheat grain production must increase 2% annually to meet the requirement of consistently increasing world population (around 9 billion) till 2050 [6-8]. India is second largest producer of wheat in the world. The area, production, and productivity of wheat in India in 2017-18 was 29.58 million ha, 99.7 million ton and 33.71 qtls/ha, respectively [9]. It is grown in all the regions of the country and the states, namely, Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan, Bihar, Maharashtra, Gujarat, West Bengal, Uttarakhand and Himachal Pradesh together contribute about 98% to the total wheat production of the country.

Quantitative and qualitative characters of grain are the principal characters of a cereal crop [10, 11]. The quantitative characters generally are affected by a number of yield contributing characters. Information regarding mutual association between yield and yield components is necessary for effective utilization of the genetic stock for crop improvement. The presence of genetic diversity and genetic relationships among genotypes is a prerequisite and paramount important for successful wheat breeding programme. The success of a breeding program depends largely upon the amount of genetic variability present in the population and the extent to which the desired traits are heritable [12]. Genetic divergence refers to the genetic distance between species or between populations within a species. Genetic distance can be used to compare the genetic dissimilarity between different species. Developing hybrid wheat varieties with desirable traits require a thorough knowledge about the existing genetic variability [13]. The statistical analysis revealed that the diverse clusters. Out of those, closely internal cluster distances, may generate a wide range of transgressive segregants for development of high yielding wheat varieties [14]. Several genetic variability studies

have been conducted on different crop species based on quantitative and qualitative traits in order to select genetically distant parents for hybridization [15].

The conventional breeding methods are not highly reliable as they are affected by environmental conditions [16]. However, molecular markers methods are more stable and they can provide detailed characterization of genetic resources. The main applications of molecular markers can be classified into two categories: (a) assessment of genetic diversity and (b) identification and characterization of genomic regions controlling the trait of interest [17-20]. Microsatellite markers are short repeating nucleotide DNA sequences [21]. They have high reproducibility, are multi-allelic, specific to genes, co-dominant and highly polymorphic [22]. SSR markers have been used to characterize genetic diversity in wild relatives [23]. The SSR marker platform, which can be developed using genomes (SSRs) or transcriptomes (EST-SSRs), is a powerful tool for examining population genetic diversity [18]. Generally, SSR markers are suitable for parental identification, pedigree analysis, and development of newly improved varieties as they are independent of environmental conditions and plant development stage. Thus, SSR markers are a useful tool to select a desirable alleles or traits. A tight linkage relationship may be established between the SSR marker and the gene controlling the trait for better understanding [24]. Microsatellites (simple sequence repeat [SSR]) were used in the this study DNA based molecular markers are the direct source to measure the genetic diversity.

Material and Methods

Plant Material

This investigation was carried out at the experimental field station of the Department of Biotechnology, SVPUA&T, Meerut. To analyse the genetic diversity of Indian wheat cultivars a total of sixty varieties were taken from Department of genetics and plant breeding, SVPUA &T, Meerut and NBPGR, New Delhi. All varieties were grown and maintained in the field under normal condition.

DNA isolation

Genomic DNA was isolated from fresh leaves of each 60 varieties viz. K-9423(UNNATHALNA),AAI-12,SL-1, LN-26P,LN-15B,UP-2425, LN-15C, K-9644, K-9162, W-7,HUW-533,SL-2,UP-2565,DBW-835,WCW-953,K-710,K-9397,K-616,NW-1076, SL-7, W-3,K-617,LN-16B,SL-4,W-4,K-424, WCW-984, AAI-2, HUW-516,SL-5,K-8962,HUW-846,HUW-825,K-7903(HALNA),NW-2036,HUW-637, K-9533(NAINA), NW-1014, HUW-638,HUW-234,HUW-213, AAI-336,SL-15,K-712, DBW-17, DBW-16, KRL-213, RAJ-3765,KHARCHIA-65, KRL1-4, KRL-19, HD-2009,WH-1021, PBW-226, PBW-343, PBW 373,PBW-502,PBW-550,WH-711, KRL-210 and preserved under -80 deep fridge. The genomic DNA from the leaves were isolated using the standard protocol [25], with slight modification. In this method Cetyl Trimethyl Ammonium Bromide (CTAB) was used as a detergent to lyse the wall of cells for release of DNA.

Polymerase Chain Reaction

For SSR analysis a total of 50 wheat specific primers were used (**Table 1**). DNA amplification reaction for SSR primers were carried out according to standard procedure in 20µl. The SSR-PCR, the reaction profile consisted of an initial denaturation step of 4 min at 95ºC, followed by a 1 min denaturation step at 95ºC, annealing for 1 min 40 sec. at 40ºC and extension for 2 min at 72ºC. A total of 35 cycles were performed followed by 10 min extra extension step after the last cycle. Amplified PCR products were separated on 1.5 % (w/v) agarose gel in standard 1 X TBE (pH 8).

SSR data analysis

The ability of primers to resolve the different varieties, the resolving power (Rp) for each primer was calculated following [26] method as Rp = Ib (band information). Resolving Power is calculated as 1-[2 x (0.5-p)], p being the proportion of the 60 varieties containing the bands and Gene Diversity is calculated as $1-\sum pi^2$ [27]. The bands were scored as present (1) or absent (0) for each DNA sample with the all 50 SSR for wheat specific primers. Amplification was performed twice and only reproducible amplifications products were included in the data analysis. Similarity matrix using the similarity coefficient of [28] was constructed from the whole data. Pair wise distances between DNA accessions were calculated and analysed using the Unweighted Pair Group Method Arithmetic average (UPGMA) [29]. Clusters were analysed using the computer program NTSYS-PC, version 2.11s [30]. In some cases no band were observed, possibly due to insufficient homology between the primer and DNA template. There is also the possibility that this situation might have occurred by failure of the PCR caused by some other region as well.

Table 1 List of SSR primers

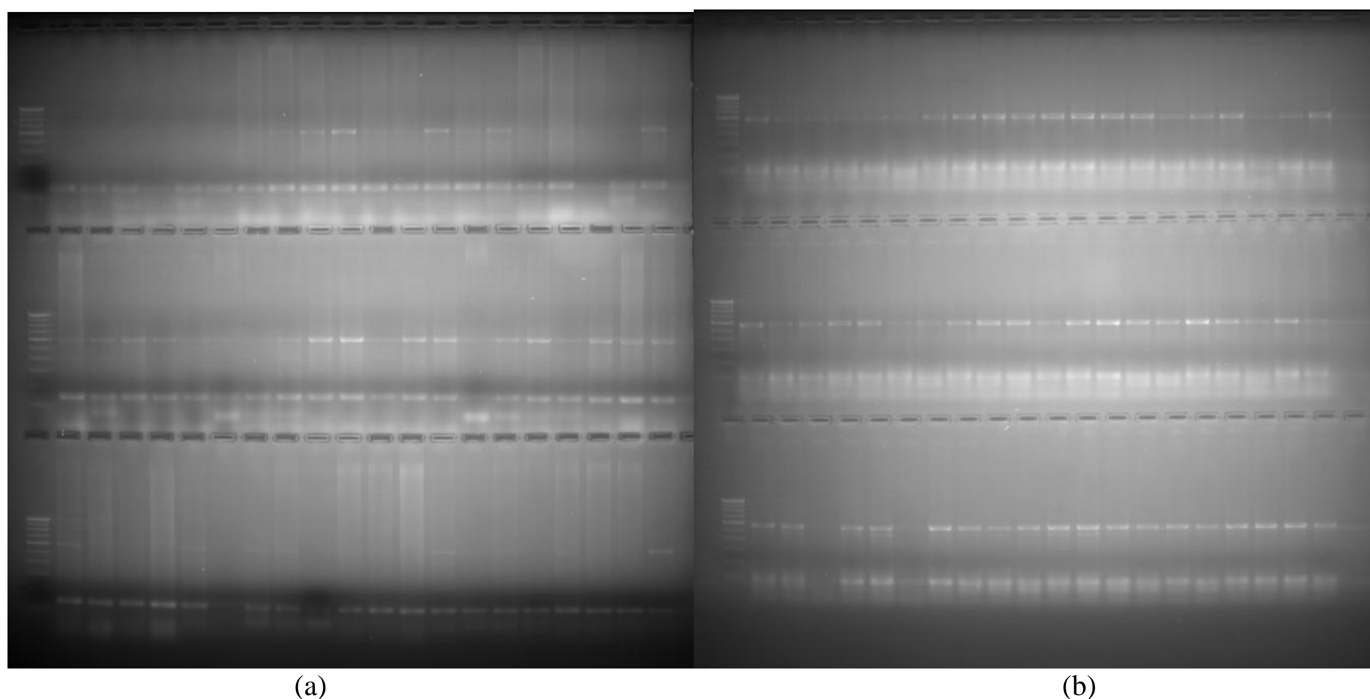
S. N.	Locus	Forward Primer	Reverse Primer
1	Xgwm425	GAG CCC ACA AGC TGG CA	TCG TTC TCC CAA GGC TTG
2	Xgwm445	TTT GTT GGG GGT TAG GAT TAG	CCT TAA CAC TTG CTG GTA TGG A
3	Xgwm601	ATC GAG GAC GAC ATG AAG GT	TTA AGT TGC TGC CAA TGT TCC
4	Xgwm609	GCG ACA TGA CCA TTT TGT TG	GAT ATT AAA TCT CTC TAT GTG TC
5	Xgwm674	TCG AGC GAT TTT TCC TGC	TGA CCG AGT TGA CCA AAA CA
6	Xgwm630	GTG CCT GTG CCA TCG TC	CGA AAG TAA CAG CGC AGT GA
7	Xgwm569	GGA AAC TTA TTG ATT GAA AT	TCA ATT TTG ACA GAA GAA TT
8	Xgwm582	AAG CAC TAC GAA AAT ATG AC	TCT TAA GGG GTG TTA TCA TA
9	Xgwm565	GCG TCA GAT ATG CCT ACC TAG G	AGT GAG TTA GCC CTG AGC CA
10	Xgwm624	TTG ATA TTA AAT CTC TCT ATG TG	AAT TTT ATT TGA GCT ATG CG
11	Xgwm495	GAG AGC CTC GCG AAA TAT AGG	TGC TTC TGG TGT TCC TTC G
12	Xgwm415	GAT CTC CCA TGT CCG CC	CGA CAG TCG TCA CTT GCC TA
13	Xgwm547	GTT GTC CCT ATG AGA AGG AAC G	TTC TGC TGC TGT TTT CAT TTA C
14	Xgwm513	ATC CGT AGC ACC TAC TGG TCA	GGT CTG TTC ATG CCA CAT TG
15	Xgwm456	TCT GAA CAT TAC ACA ACC CTG A	TGC TCT CTC TGA ACC TGA AGC
16	Xgwm341	TTC AGT GGT AGC GGT CGA G	CCG ACA TCT CAT GGA TCC AC
17	Xgwm312	ATC GCA TGA TGC ACG TAG AG	ACA TGC ATG CCT ACC TAA TGG
18	Xgwm349	GGC TTC CAG AAA ACA ACA GG	ATC GGT GCG TAC CAT CCT AC
19	Xgwm473	TCA TAC GGG TAT GGT TGG AC	CAC CCC CTT GTT GGT CAC
20	Xgwm358	AAA CAG CGG ATT TCA TCG AG	TCC GCT GTT GTT CTG ATC CT
21	Xgwm339	AAT TTT CTT CCT CAC TTA TT	AAA CGA ACA ACC ACT CAA TC
22	Xgwm376	GGG CTA GAA AAC AGG AAG GC	TCT CCC GGA GGG TAG GAG
23	Xgwm319	GGT TGC TGT ACA AGT GTT CAC G	CGG GTC CTG TGT GTA ATG AC
24	Xgwm320	CGA GAT ACT ATG GAA GGT GAG G	ATC TTT GCA AGG ATT GCC C
25	Xgwm153	GAT TCT GTC ACC CGG AAT TC	TGG TAG AGA AGG ACG GAG AG
26	Xgwm154	TCA CAG AGA GAG AGG GAG GG	ATG TGT ACA TGT TGC CTG CA
27	Xgwm113	ATT CGA GGT TAG GAG GAA GAG G	GAG GGT CGG CCT ATA AGA CC
28	Xgwm159	GGG CCA ACA CTG GAA CAC	GCA GAA GCT TGT TGG TAG GC
29	Xgwm148	GTG AGG CAG CAA GAG AGA AA	CAA AGC TTG ACT CAG ACC AAA
30	Xgwm135	TGT CAA CAT CGT TTT GAA AAG G	ACA CTG TCA ACC TGG CAA TG
31	Xgwm10	CGC ACC ATC TGT ATC ATT CTG	TGG TCG TAC CAA AGT ATA CGG
32	Xgwm5	GCC AGC TAC CTC GAT ACA ACT C	AGA AAG GGC CAG GCT AGT AGT
33	Xgwm120	GAT CCA CCT TCC TCT CTC TC	GAT TAT ACT GGT GCC GAA AC
34	Xgwm107	ATT AAT ACC TGA GGG AGG TGC	GGT CTC AGG AGC AAG AAC AC
35	Xgwm122	GGG TGG GAG AAA GGA GAT G	AAA CCA TCC TCC ATC CTG G
36	CWM-112	TGC AGC CAC AAA ATC CAT C	TGC TGC AAT ACA ACA TCC AT
37	CWM-114	GGG CCC ATT GGA GAA CCT	GCA GTG AGC GCC CGT AAT A
38	CWM-118	TTT CGC AGC CGC AAC TAC C	TGA TCT TCC ACG CCG CTA TG
39	CWM-101	GCC TTC GCC ACC AAC TTC	GGC GCG TAA ATC CCC TCT C
40	CWM-107	GCC GGC TCG CCA TGT TCT CCA	CTC ATC ATC TCG ACT CGC CCT
41	CWM-119	GTC AAC AAC AAC GCC TGG	TAA GCG GAA GAA AGA TG
42	CWM-115	CCT TTC TCA TCC TTG CCA TCC	GTT GTT GTG GAA ATG GTT
43	XGWM276-7A	ATT TGC CTG AAG AAA ATA TT	AAT TTC ACT GCA TAC ACA AG
44	XGWM-260-7A	GCC CCC TTG CAC AAT C	CGC AGC TAC AGG AGG CC
45	XGWM-635-7D	TTC CTC ACT GTA AGG G	CAG CCT TAG CCT TGG CG
46	CWM-103	ATG CAG CAA TCC CCT CCC	CCA GTC CCG AGC TTG TAA AA
47	XGWM-293-7A	TAC TGG TTC ACA TTG GTG CG	TCG CCA TCA CTC GTT CAA G
48	CWM-105	GCT GAA GCC ATG CAT AAT AGT	CCA GGG GTT TTC CAT CTC C
49	XGWM-332-7A	AGC CAG CAA GTC ACC AAA AC	AGT GCT GGA AAG AGT AGT GAA
50	CWM-110	TCA GGG AAG CAG CGT GTA GAG	CGG CCA GTC AGC GCG GGT AAT

Result and Discussion

Molecular characterization using SSR markers

The characterization of wheat varieties using microsatellite molecular markers systems is important to find out genetically diverse wheat varieties useful for breeding programmes for crop improvement. [18, 19, 31]. Fifty SSR primers, were used to amplify the genomic DNA of 60 wheat varieties to analyze the genetic diversity (**Figure 1 a,b**). A total of fifty SSR primers generate 66 reproducible and clearly scorable bands across the sixty wheat genotype with an average of 1.78 bands per primer. Out of fifty SSR primers, 37 SSR primers were showed polymorphism. The average number of polymorphic bands was 1.56 per primer (**Table 2**). Among the tested SSR primers, thirty seven amplified polymorphic SSR loci, polymorphism range from 33.33% to 100 %. Various studies have used SSR markers to investigate genetic diversity in cultivated hexaploid wheat varieties of *T. aestivum* L. [32-34]. The PIC values derived from allelic diversity. In this study the PIC value of 37 SSR markers out of fifty is varies from primer 0.08 to 0.99 with a mean PIC value for SSR primers was 0.619 (Table 2, **Figure 2A**). The higher PIC value depicted the diverse varieties and the lower one depicted closely related genotype. Our results are in accordance with [35] who studied the genetic diversity in wheat genotypes using SSR markers. [36] reported comparatively lower range of PIC value from 0.27 to 0.54 with an average of 0.38 for 12 wheat genotypes. Resolving power of 37 polymorphic SSR primers varies between 0.22 to 5.76 with an average value of 3.13 (Table 2, Figure 2B). The primer Xgwm276-7A showed the lowest resolving power, while Xgwm319 showed maximum resolving power. the significant value of resolving power indicated the ability of primers to resolve the different closely related varieties of wheat.

[37] reported 02 to 10 alleles per locus in 122 durum wheat genotypes, with 19 SSR markers. [38] reported an average of 4.8 alleles per primers in 10 wheat genotypes, using 12 SSR markers that supports the present study results. [39] reported a mean of 2.31 amplified bands (alleles) per marker among 17 coffee genotypes with 16 SSR markers and a mean PIC value of 0.43 [40]. [41] found a good correlation between genetic diversity and the morphological variability of *Moringa* genotypes. [42] reported resolving power of the 16 SSR primers ranged from 1.0 to 5.2 with an average 2.46 on 20 wheat genotypes also.



(a) (b)

Figure 1(a&b) SSR profiling pattern of 60 wheat varieties wit primer Xgwm319 and CWM-103.The samples were loaded in the sequence: 1. K-9423, 2. AAI-12, 3.SL-1, 4. LN-26P, 5. LN-15B, 6. UP-2425, 7. LN-15C, 8.K-9162, 9.K-9644, 10.W-7, 11.HUW-533, 12.SL-2, 13.UP-2565, 14.DBW-835, 15.WCW-953, 16.WCW-984, 17.K-710, 18.K-9397, 19.K-616, 20.NW-1076, 21.SL-7, 22.W-3, 23.K-617, 24.LN-16B, 25.SL-4, 26.W-4, 27.K-424, 28.AAI-2, 29.HUW-516, 30.SL-5, 31.K-8962, 32.HUW-846, 33.HUW-825, 34.K-7903, 35.NW-2036, 36.HUW-637, 37.K-712, 38.K-9533, 39.NW-1014, 40.HUW-638, 41.HUW-234, 42.UW-213, 43.AAI-336, 44.SL-15, 45.HD-2967, 46.DBW-17, 47.DBW-16, 48.KRL-213, 49.RAJ-3765, 50.KHARCHIA-65, 51.W-13, 52.KRL-19, 53.KRL-210, 54. WH-1021, 55.PBW-226, 56. PBW-343, 57.PBW-373, 58.PBW-502, 59. PBW-550, 60. WH-711

Table 2 Polymorphic SSR primers with their Polymorphic Information Content and Resolving Power

S.N	Primer Code	Amplified	Polymorphic bands	Monomorphic band	Polymorphism %	PIC Value	Resolving power
1	Xgwm425	2	2	0	100	0.62	3.76
2	Xgwm601	2	2	0	100	0.56	1.12
3	Xgwm609	1	1	0	100	0.35	1.80
4	Xgwm674	3	3	0	100	0.92	3.54
5	Xgwm630	2	2	0	100	0.60	3.80
6	Xgwm569	1	1	0	100	0.27	1.86
7	Xgwm582	2	2	0	100	0.52	3.96
8	Xgwm495	1	1	0	100	033	1.82
9	Xgwm415	2	2	0	100	0.88	2.62
10	Xgwm513	1	1	0	100	0.72	1.46
11	Xgwm456	2	2	0	100	0.91	2.12
12	Xgwm341	2	2	0	100	0.58	3.84
13	Xgwm312	2	1	1	50	0.93	3.76
14	Xgwm349	2	1	1	50	0.81	3.02
15	Xgwm473	3	3	0	100	0.62	5.64
16	Xgwm339	5	5	0	100	0.94	5.18
17	Xgwm376	2	2	0	100	0.85	2.76
18	Xgwm319	3	2	1	66.66	0.72	5.76
19	Xgwm320	1	1	0	100	0.08	1.96
20	Xgwm153	3	1	2	33.33	0.72	5.02
21	Xgwm113	2	1	1	50	0.62	3.72
22	Xgwm159	1	1	0	100	0.19	1.9
23	Xgwm135	2	1	1	50	0.52	3.96
24	Xgwm10	1	1	0	100	0.08	1.96
25	Xgwm5	2	2	0	100	0.87	3.84
26	Xgwm120	2	2	0	100	0.85	3.78
27	Xgwm107	1	1	0	100	0.16	1.92
28	Xgwm122	1	1	0	100	0.08	1.96
29	CWM-112	1	1	0	100	0.67	1.82
30	CWM-114	1	1	0	100	0.6	1.06
31	CWM-118	1	1	0	100	0.99	1.97
32	XGWM276-7A	2	2	0	100	0.99	0.22
33	CWM-103	1	1	0	100	0.98	0.80
34	XGWM-293-7A	2	2	0	100	0.58	3.84
35	CWM-105	2	2	0	100	0.58	3.84
36	XGWM-332-7A	1	1	0	100	0.26	1.86
37	CWM-110	1	1	0	100	0.99	0.76
	Total	66	58	07			
	Average	1.78	1.56	0.18	91.89	0.619	3.127

Genetic similarity matrix and cluster analysis

The SSR profile was utilized for estimating pair wise genetic similarity matrix. which was further analyzed using UPGMA clustering algorithm program by software programme NTSYS-PC for dendrogram construction and cultivator differentiation. Similarity value for all the 60 varieties ranged from 0.72 to 0.98. The minimum similarity exhibited by genotype WH711 and SL1. Whereas the maximum similarity was shown by genotype KRL19 and W13. The UPGMA based clustering grouped 60 wheat varieties in to four major groups i.e. A, B, C and D groups (**Figure 3**). The group A includes 8 varieties namely UP2565, SL2, W7, K9644, HUW533, K9162, HUW638 and K9423. The group B subdivided into four sub groups. The sub group B1 includes 15 varieties namely K616, HUW825, HUW637, LN16B, K617, WCW953, K712, NW1076, K710, DBW16, HD2009, Kharchia65, K9397, WCW984 and DBW835. The sub group B1 further divided into small clusters. The sub cluster B2 includes 9 varieties namely HUW516, K424, HUW213, HUW234, W4, SL4, HUW846, K8962 and W3 which are further grouped into small clusters. The sub cluster B3 includes only 4 varieties namely AAI-2, NW1014, NW2036 and SL5. The sub cluster B4 includes 6 varieties namely RAJ3765, KRL19, W13, KRL213, DBW17 and SL5. The three varieties also

included in the main group B but not are the part of any sub group. The present as an individual member as stay at one end of the sub cluster. The Main cluster C includes 9 varieties namely AAI336, PBW343, WH711, PBW550, PBW502, PBW373, PBW226, WH1021, KRL210 which are further arranged in small clusters. The main cluster D grouped 6 varieties namely LN15C, LN15B, UP2425, SL1, LN26 and AAI12.

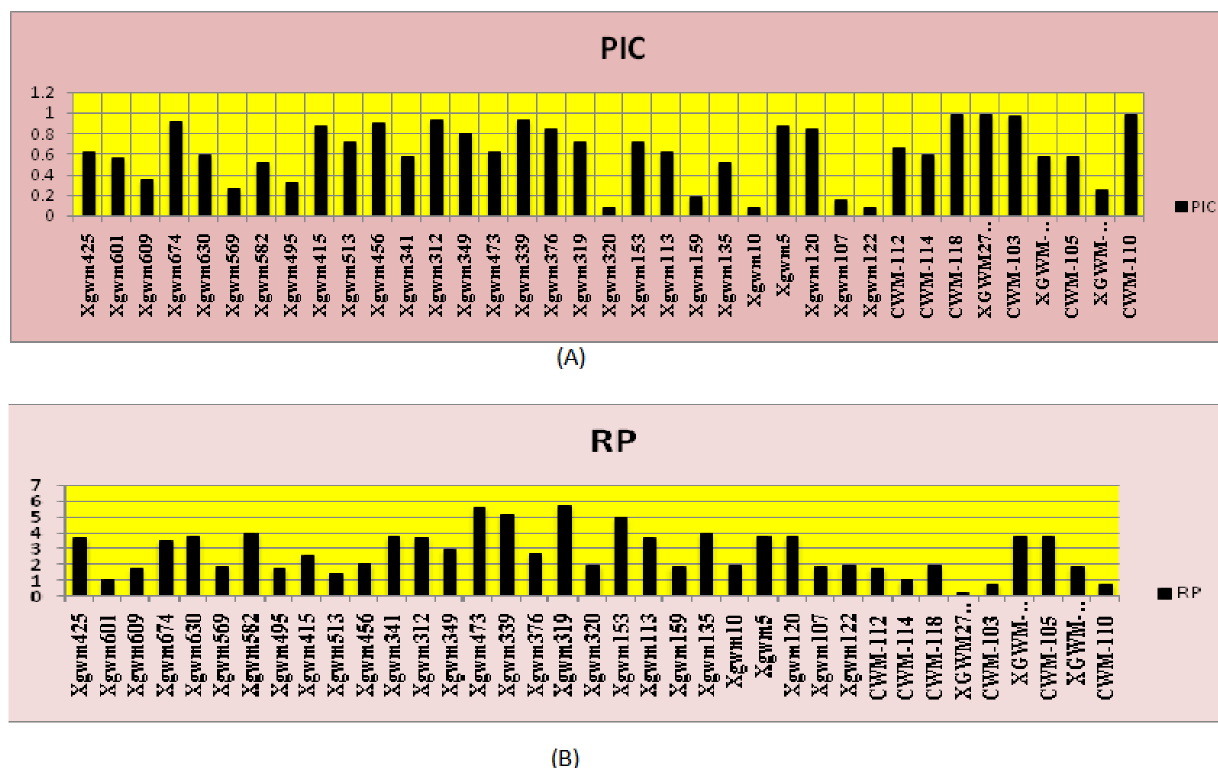


Figure 2 Graphical representation of (A) Polymorphic Information Content of SSR primers used in wheat genotypes (B) Resolving Power of SSR primers used in wheat genotypes

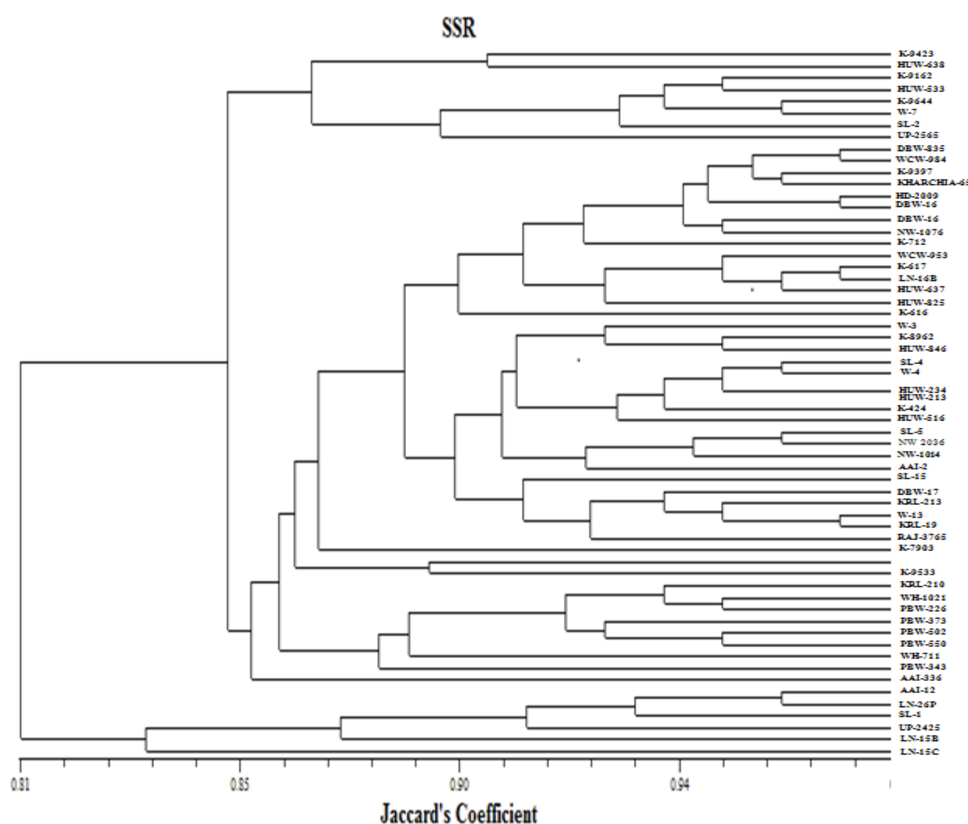


Figure 3 Cluster based grouping of 60 wheat varieties on the basis of SSR primers

The findings of [43] supported that the cluster obtained based on the similarity matrix, using the UPGMA algorithm, in 55 wheat varieties with 20 SSR primers. [44] reported that the UPGMA dendrogram separated the six durum wheat varieties into three clusters with 19 sequence-related amplified polymorphic (SRAP) primers. [38] reported Similarity value for all the 10 accessions ranged from 0.1 to 0.66 using 12 SSR markers data that supports the present study results. [45] found that the cluster analysis broadly grouped 54 genotypes into four clusters. [42] reported the similarity value for the 20 varieties taken ranged from 0.35 to 0.90. These all results showed that climatic conditions may affect the plant genome which are inherited through genome generation to generation [46, 47].

Conclusion

The present study suggests that microsatellite markers are appropriate to study of genetic diversity among the varieties and also able to amplify the different loci of all the 60 wheat varieties. The primer Xgwm276-7A showed the lowest resolving power, while Xgwm319 showed maximum resolving power which indicated the ability of primers to differentiate the different closely related varieties of wheat. The minimum similarity exhibited between genotype WH711 and SL1, whereas the maximum similarity indicated in genotype KRL19 and W13. This will help to identify the diverse genotypes and can be helpful for breeding to get good genotypes.

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