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Population structure and genetic diversity among Indian wheat varieties using microsatellite (SSR) markers

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Abstract

Platform for improvement of crop plants is largely setup by the genetic variability studies. The current work was performed to assess the status of genetic diversity among 319 Indian wheat varieties so that they can be used effectively for future breeding practices. Out of 30 markers applied, 50 polymorphic fragments generated from16 polymorphic primers were selected for diversity studies. Jaccob's similarity model and Bayesian analysis were performed using software package DARwin 5.0 and STRUCTURE 2.3.3 respectively. Grouping obtained from both the analysis were found to be consistent and was helpful in analyzing the distribution of genetic variability across different centres of wheat research in India. The total genetic diversity for all groups (Ht) was 0.38888 and within groups (Hs) was 0.3130 indicating less genetic variability among sub-populations (Gst=0.19). Gene flow (Nm) was also found to be relatively high with a value of 2.0654. Genetic variability parameters computed using POPGENE 1.32 (81%) and AMOVA (78.5%) revealed that the genetic diversity was mainly contained within the populations. This study can complement the breeding programs for identification of parental combinations effectively to maintain maximum genetic variability in progenies.

Keywords: Wheat, genetic diversity, conservation, SSR.
Abbreviations: AMOVA_Analysis of molecular variance; PCR_Polymerase chain reaction; PIC_Polymorphism information content; SSR_Simple sequence repeats; UPGMA_Unweighted pair-group method with arithmatic mean.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple crops which is grown widely across the globe. Continuous breeding practices have led to the reduction in genetic variability (Aremu, 2011). To optimize and conserve the germplasm for plant breeding and other activities, studies on genetic diversity and population sturucture are noteworthy (Uddin et al., 2008). Parental combinations can be recognized effectively for breeding so that segregating population contains maximum genetic divergence (Aremu et al., 2007 ;Barrett et al., 1998; Dje et al., 2000; Liu et al., 2000). Availability of superior and diverse alleles/genes form the starting point of genetic enhancement of crop plants including wheat (Abouzied et al., 2013) which can help in recognition of new cultivars . Different criteria like pedigree analysis, morphological traits or molecular markers can be utilized for the genetic diversity studies (Cox et al., 1985). In contrast to other crop plants like maize, rice or tomato, molecular studies are laborious in wheat due to its hexaploid nature and large genome size (Gupta et al., 1999). In differentiation to cross pollinating species such as tef, azuki, rice, sugarbeet and wild hordeum (Bai et al., 1999; Yee et al., 1999; Maheswaran et al., 1997; Schondelmaier et al., 1996; Pakniyat et al., 1997), genetic variability has also been reportedly found low in wheat. Molecular markers being independent of envioronmental conditions have come up as an effective tool for characterization of genetic material (Cifci andYagdi, 2012; Abouzied et al., 2013; Malik et al., 2013). They provide a new dimension, accuracy and perfection in screening of germplasm (Tar'an et al., 2005). Various molecular assays have evolved in recent years for genetic analysis studies (Chen et al., 1994). Molecular markers, however, based on microsatellite repeats appear most promising as they are accessible in large numbers, detect high level of polymorphism, co-dominant in nature, are highly reproducible and reliable (Li et al., 2009). Across the globe, many researchers have explored the genetic diversity in wheat. Naghavi et al., (2004) conducted comparative analysis of the genetic diversity among bread wheat genotypes based on 17 RAPD and 35 SSR markers. A need to develop wheat varieties with a diverse genetic background and to incorporate new variability into the existing wheat gene pool was revealed by Sud et al. (2005) in their studies among 20 elite wheat genotypes utilizing 25 microsatellite markers and pedigree analysis. Landjeva et al., (2006) by utilizing microsatellite markers noticed that there was no decline in the genetic diversity of 91 old and modern Bulgarian winter wheats. It was suggested by Salem et al., (2008) on their study in seven wheat cultivars that morphological characters and genotypic markers of wheat can be useful for wheat breeders to plan for positive traits. Diverse varieties of wheat that can be utilized in future breeding programs were recognised by Zeb et al., (2009) by utilizing 14 SSR markers on 10 genotypes. Genetic variability analysis on six durum wheat cultivars was carried out in 2010 by Ayed et al. According to them, seed storage protein profiles could be useful markers in the studies of genetic diversity and genotypes classification. Akram et al. (2012) carried out genetic diversity in 40 landrace cultivars of the genus *Triticum* using SSR markers and found greater value of genetic diversity in comparison to other regions obtained from Indian sub-continental germplasm. Level of genetic diversity and population genetic structure of 230

tetraploid wheat accessions was analysed by Laido et al., (2013). Results revealed that genotypes categorised in two groups reflect the evolutionary history of *Triticum turgidum* L. The current work was undertaken with the objective to study the population structure and genetic diversity in wheat cultivars, grown under different regions across India. A large number of genotypes covering different zones of Indian sub-continent were included in the study that can be helpful for breeders in selection of diverse parents for their future breeding programs.

Results

Genetic diversity

A total of 30 SSR markers distributed across different wheat chromosomes were applied. Sixteen markers found polymorphic were selected for further studies that gave fifty reliable polymorphic alleles. Maximum number of alleles per locus were obtained for B genome which counted to be 46 which was followed by A (18) and lastly by D genome with 16 alleles (Table 1). Mean value of allelic polymorphism information content (PIC) was found to be 0.584 with a range of 0.33 to 0.73 for Xgwm265 and Xbarc77 respectively. Percentage of polymorphic bands (PPB) per population was obtained in the range of 96 to 100 with an average value of 98.86%.

Population clusters and sub-structuring

Unrooted neighbor joining tree constructed using DARwin 5.0 broadly divided 319 genotypes into five major groups. Majority of genotypes were found to be clustered in I, III and IV group (Fig.1) while minor groups were formed by genotypes included in II and V. Population structure of 319 accessions was estimated using STRUCTURE ver 2.3.3 software. The number of subpopulations (K) was identified based on maximum likelihood and delta K (ΔK) values. The value of LnP(D) increased from 1 to 15, but showed a knee at a value of seven which implied that there may be seven subpopulations (Fig. 2). Details of seven identified sub-populations (P1-P7) are shown in Table. 2. Maximum percentage of cultivars was found in sub-population P1 with about 20.38% genotypes. It was followed by P6 with 16.30%, P5 (15.67%), P2 (11.28%) and lastly by P3 & P7 (10.65%) with equal number of genotypes in them.

Inter-population genetic diversity

Analysis through Popgene on seven sub-populations depicted an average value of effective number of alleles (Ne) as 1.6753 ± 0.2740. Mean value for observed number of alleles (Na) was 2.0000 ± 0.0000. Assuming Hardy-Weinberg equilibrium, gene diversity (h) varied from 0.0733 to 0.500 with an average value of 0.3854 ± 0.1117 and average value for Shannon's Information Index (I) was obtained as 0.5672 ± 0.1296.

Intra-population genetic diversity

At intra-population level, mean value of effective number of alleles (Ne) and observed number of alleles was 1.5163 and 2.0000 with standard deviation ± 0.0485, 0.2980 respectively. Populations displayed that average value for h and I were 0.3130±0.1387 and 0.4780±0.1731 respectively (Table.3). Among the seven sub-populations, population four exhibited the highest level of variability (h = 0.347, I = 0.522), whereas population one exhibited lowest level of variability (h = 0.260, I = 0.415).

Genetic diversity parameters

Total gene diversity (Ht) and variability within population (Hs) was found to be 0.3888 ± 0.0120 and 0.3130 ± 0.0120 respectively. Intra-variety genetic diversity (Dst=Ht-Hs) determined gave a value of 0.0758. Coefficient of gene differentiation (Gst=0.19) indicated that genetic variation was relatively low among the population with 19% of variability and most of the diversity lies within the populations (81%). Value of Nm depicting gene flow was found to be 2.0654. To assess distinctiveness among and within the sub-populations, an Analysis of Molecular Variance (AMOVA) was carried out. The results were in consent with POPGENE demonstrating that majority of variation prevailed within the populations (78.51%) while among the sub-populations genetic differentiation was relatively less (21.49%) (Table.4).

Cluster analysis

The genetic similarity matrix and phylogram tree were constructed with a Tree View Software to estimate the phylogenetic relation between the seven sub-populations. The genetic distance and genetic identity was calculated using Nei's unbiased measure (Nei, 1978). The matrix constructed revealed that least genetic distance (0.084) occured between sub- pop2 & pop6 and the maximum distance (0.213) was found to be in sub-pop2 and sub-pop4 (Table.5). Dendrogram (Fig. 3) distributed the populations into three sub-groups. Sub-pop4 as depicted from the data shows maximum genetic diversity and forms a distinct group. On the basis of genetic identity results, Sub-pop 4 and 6 were found to have least genetic identity of 0.822. Sub-pop 2 and 5 were however, found to have maximum genetic identity of 0.908. Different level of variability was obtained for research centres distributed across India. Relatively higher level of genetic diversity was revealed by genetic markers in the cultivars belonging to research station: JNKVV Powarkheda, IARI RRS Shimla, DWR RRS Shimla, DWR Karnal, CCSHAU Hisar, IARI New Delhi and PAU Ludhiana. Next was the category consisting of cultivars from centres such as UAS Dharwad, SKRAU Durgapur, MPKV Niphad, IARI RRS Wellington and BHU Varanasi which were distributed predominately across two groups implying that genetic background was relatively less. Four local landraces, included in the present study, got evenly distributed to 2, 3, 4 and 5th group. Since, number of genotypes put to study from the centres, AAU RRS ARNEJ, IARI PUSA Bihar, CSKHPKV Malan, ARS Niphad, SKUAST Jammu and VPKAS Almora were few, it was difficult to conclude the panoramas (Fig. 4). Principal component analysis was performed in order to determine the relationships among wheat genotypes in terms of their position relative to two coordinate axes. The plot of the first and second component accounted for 32.22 and 14.55% of the variation, giving a cumulative variation of 46.77%. High degree of overlapping was observed in the plot that grouped accessions together without distinguishing them (Fig. 5).

Discussion

Genetic diversity in wheat has been increasingly narrowed down, due to modern breeding practices (Sear, 1981). For effective conservation and improvement of existing germplasm,

Table 1. Microsatellite markers, chromosomal location, sequence and results generated in wheat genotypes.

Sr.No.	Primer Designation	Chromosomal Location	No. of alleles	Primer Sequence (5'-3')	Allele Size Range (bp)	Gene Diversity
1	Xgwm312	2A	2	5' ATCGCATGATGCACGTAGAG 3' 5' ACATGCATGCCTACCTAATGG3'	200-220	0.462
2	Xgwm46	7B	3	5' GCACGTGAATGGATTGGAC 3' 5' TGACCCAATAGTGGTGGTCA 3'	160-200	0.651
3	Xgwm273	1B, 6B	2	5' ATTGGACGGACAGATGCTTT 3' 5' AGCAGTGAGGAAGGGGATC 3'	170-200	0.500
4	Xgwm265	2A, 4A, 4D	2	5' TGTTGCGGATGGTCACTATT 3' 5' GAGTACACATTTGGCCTCTGC3'	160-210	0.331
5	Xgwm44	4A, 7D	3	5' GTTGAGCTTTTCAGTTCGGC 3' 5' ACTGGCATCCACTGAGCTG 3'	120-200	0.580
6	Xgwm493	3B	3	5' TTCCCATAACTAAAACCGCG 3' 5' GGAACATCATTTCTGGACTTTG3'	150-200	0.630
7	Xgwm111	4A, 6D, 7B	5	5' TCTGTAGGCTCTCTCCGACTG 3' 5' ACCTGATCAGATCCCACTCG 3'	150-300	0.696
8	Xgwm382	2A	4	5' GTCAGATAACGCCGTCCAAT 3' 5' CTACGTGCACCACCATTTTG 3'	100-120	0.545
9	Xgwm371	5B	3	5' GACCAAGATATTCAAACTGGCC 3' 5' AGCTCAGCTTGCTTGGTACC 3'	120-200	0.634
10	Xgwm374	1B	3	5' ATAGTGTGTTGCATGCTGTGTG 3' 5' TCTAATTAGCGTTGGCTGCC 3'	190-210	0.575
11	Xgwm219	6B	3	5' GATGAGCGACACCTAGCCTC 3' 5' GGGGTCCGAGTCCACAAC 3'	150-200	0.610
12	Xgwm191	1D, 2B, 3D, 5B, 6B	3	5' AGACTGTTGTTTGCGGGC 3' 5' TAGCACGACAGTTGTATGCATG 3'	110-160	0.449
13	Xgwm153	1B	3	5' GATCTCGTCACCCGGAATTC 3' 5' TGGTAGAGAAGGACGGAGAG 3'	170-220	0.652
14	Xbarc267	7B	3	5' GCGTGCTTTTTATTTTTGTGGACATCTT 3' 5' GCGAATAATTGGTGGGTGAAACA 3'	140-180	0.591
15	Xbarc77	3B	4	5' GCGTATTCTCCCTCGTTTCCAAGTCTG 3' 5' GTGGGAATTTCTTGGGAGTCTGTA 3'	150-250	0.735
16	Xbarc4	5B	3	5' GCGTGTTTGTGTCTGCGTTCTA 3' 5' CACCACACATGCCACCTTCTTT 3'	150-190	0.705
Total			50			9.347
Mean			3.1			0.584

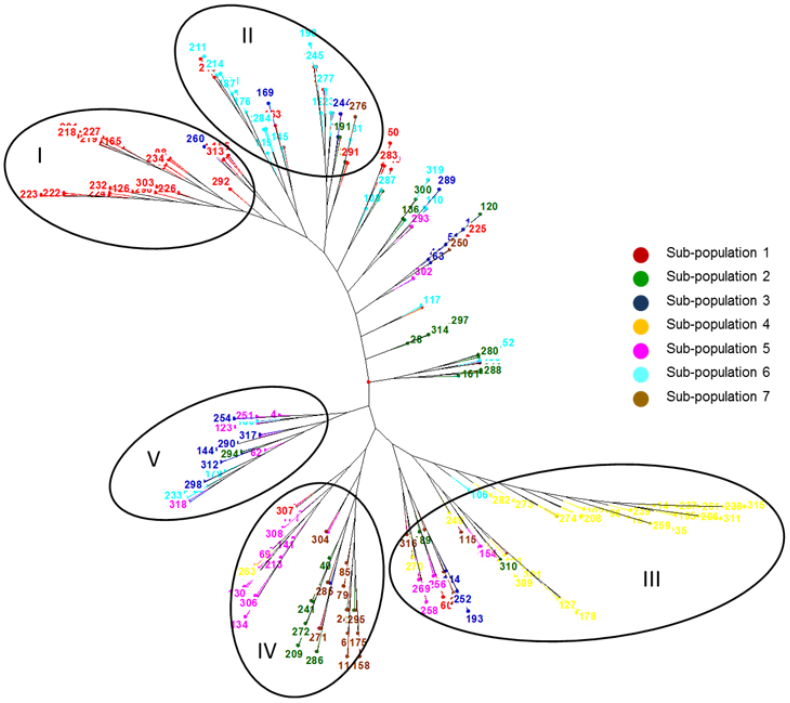

Fig 1. Unrooted neighbour joining based on SSR markers (Coloured according to the grouping obtained through STRUCTURE analysis): I. Sub-population P1; II. Sub-population P6; III. Sub-population P4; IV. Sub-population P2, P5, P7; V. Sub-population P3.

Table 2. Details of sub-populations identified by STRUCTURE.

Population ID	No. of genotypes	All zones	Central Zone	North Western Plain Zone	North Eastern Plain Zone	Peninsular Zone	Southern Hill Zone	Northern Hill Zone	Northern Zone
1	65	1	4	33	11	5	0	11	0
2	36	1	1	18	1	3	0	11	0
3	34	0	4	15	2	1	2	10	0
4	48	0	10	24	0	9	1	2	2
5	50	1	5	23	6	4	7	4	0
6	52	0	3	21	5	10	2	10	1
7	34	0	5	15	3	3	3	5	0

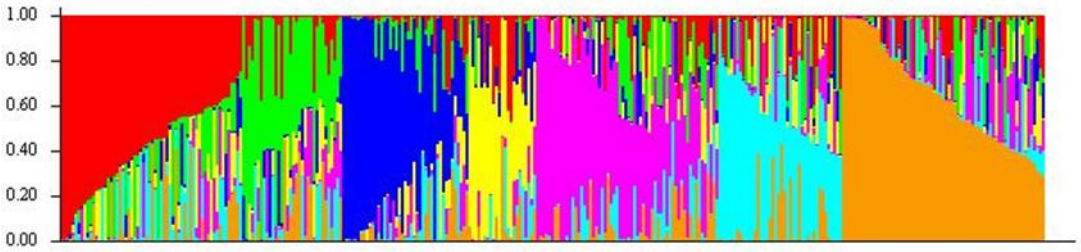

Fig 2. Population structure of 319 wheat genotyopes for k=7 estimated using STRUCTURE. Colour represent seven different sub-populations identified with each bar representing the estimated membership of single genotype in each of the seven clusters.

diversity studies are very important (Rao and Hodgkin, 2002; Zeb et al., 2009). Present work was thus, carried out to study the genetic diversity and population structure among 319 Indian wheat varieties, so that they can be utilized effectively by breeders in selection of diverse parents for future crossing programmes. SSR markers were utilized in the study considering their high polymorphism, specificity (Pestova et al., 2000), reproducibility (Stachel et al., 2000) and high variability (Brown et al., 1996). Considering their high level of polymorphism, the choosen primers were very informative. Analysis of molecular data obtained was carried out using Jaccob's similarity model and Bayesian statistics. The grouping obtained through UPGMA unrooted neighbour joining tree was found to be comparable to the Bayesian clusters obtained through STRUCTURE analysis. Since, the genotypes belong only to Indian sub-continent, clear distinction cannot be made about the distribution of genotypes in different groups on the basis of their region. North-Western Plain zone and Northern hill zone contributed maximum number of genotypes in the present work leading to their high number in each group. In groups one, two and three, genotypes belonging to these regions were found predominately. However, in group four, genotypes from central zone and peninsular zone were contributing more as compared to NWPZ. Almost equal number of genotypes was obtained from all zones in sub-population five and seven. PZ and NHZ found better representation in group six. Results obtained, however from intrapopulation genetic diversity analysis revealed that the majority of genotypes included in sub-pop1 were hexaploid in nature which might have led to its lower variability. Higher variation in sub-pop 4 may be due to the presence of different type of wheat varieties falling under categories of aestivum, durum and triticale. Nei's genetic diversity (h) is considered as an important parameter for molecular analysis of the 'genetic distance' between sub-populations and is considered as equivalent to PIC, Nei's (1973) genetic diversity (h) or expected heterozygocity is considered as one of the common indicator in population genetics. Presence of considerable level of genetic differentiation in populations is depicted by the value of gene diversity (h=0.3130) and Shannon's information index (I=0.4780). Inter-population genetic variability obtained in our study was in accordance with the results of Mahjoub et al (2012), but, it was higher than that observed by Abouized et al. (2013) in *Triticum*. Culley et al. (2002) explained that coefficient of gene differentiation (Gst), is an important indicator of proportion of variation among populations and is directly proportional to the amount of variation among them. Intra-variety genetic diversity (Dst=0.0758) obtained in the present study was relatively lower, consequently the coefficient of genetic differentiation was also low (Gst=0.19) depicting that only 19% of the variability was present among the populations and most of the variation (81%) lies within the population. Similar level of variability was obtained by Abouzied et al. (2013) in their studies. However, the values were relatively higher than observed by Carvalho et al. (2009) in which they interpretated the genetic diversity of 48 wheat cultivars based on their ITS-rDNA variation. Comparing the results with Carmona et al (2010) on their genetic variability studies on khorasan and rivet wheat, the coefficient of genetic differentiation reported by them was relatively more. For comparison of the group of populations and to assess their distinctiveness, AMOVA was utilized. The results of AMOVA were also found comparable to Gst, depicting that the variability was prominently conserved within the sub-populations (78.51%) in comparison to among the sub-populations (21.49%). These results were in accordance with Mahjoub et al. (2012) and Ehtemam et al. (2009) in wheat. Low level of variability among population can be explained by the high value of gene flow obtained. As a general indicator of the magnitude of genetic exchange (Abouzied et al., 2013), level of differentiation among population is inversely proportional to the value of gene flow (Wang et al., 2012; Matallana et al., 2009). According to Wright (1965), Nm value less than one indicates restricted gene flow among populations which leads to low variablity among groups. Since the Nm obtained (2.0654) was high, genetic exchange among the populations might have led to low genetic differentiation among them. To understand the

Table 3. Summary of genetic variation statistics within populations.

Population	No.of polymorphic loci	PPB	Na	Ne	h	I
1	50	100	2.00±0.00	1.40±0.29	0.26±0.14	0.42±0.17
2	49	98	1.98±0.14	1.58±0.30	0.34±0.13	0.51±0.16
3	50	100	2.00±0.00	1.53±0.30	0.32±0.14	0.49±0.17
4	50	100	2.00±0.00	1.58±0.28	0.35±0.12	0.52±0.15
5	49	98	1.98±0.14	1.45±0.29	0.30±0.14	0.45±0.17
6	50	100	2.00±0.00	1.53±0.32	0.31±0.16	0.48±0.20
7	48	96	1.96±0.20	1.54±0.31	0.32±0.15	0.49±0.19
Mean		98.86		1.52±0.30	0.31±0.14	0.48±0.17

where; PPB: Percentage of polymorphic loci; Na: Observed number of alleles; Ne: Effective number of alleles; h: Nei's (1973) genetic diversity; I: Shannon's information index.

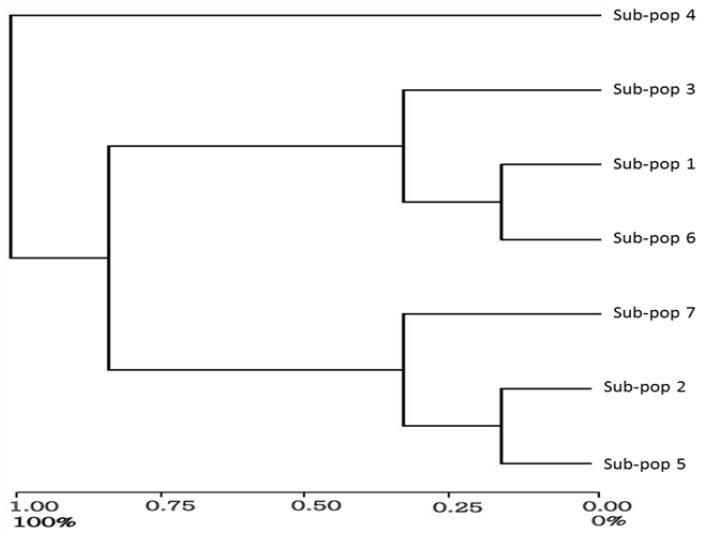

Fig 3.UPGMA dendrogram based on Nei's genetic distance. Branches represent the sub-populations as obtained through STRUCTURE analysis,

extent and distribution of genetic diversity, genotypes were categorised on the basis of their origin by utilizing the clustering obtained (Fig.4). The range of distribution obtained can help the breeding programs in selection of parents with good genetic diversity as background. Diverse parental genotypes can be selected for favourable combinations with the aim to broaden the genetic base and progeny performance for complex traits such as yield and partial disease resistance (Bohn et al., 1999). Present study on genetic diversity will thus be useful for planning future studies on wheat genetic resources.

Material and Methods

Plant materials

The present study was carried out at Directorate of Wheat Research, Karnal and included 319 wheat genotypes (Supplementary Table 1) representing released varieties, elite wheat genotypes and genetic stocks from the Indian Wheat Program. Seeds were procured from Germplasm Resource Unit, Directorate of Wheat Research, Karnal.

Genomic DNA extraction and Molecular marker assay

DNA was extracted from the leaves of all the accessions grown in the field using the modified CTAB method (Saghai-Maroof et al., 1984). DNA samples were analyzed both quantitatively and qualitatively using 0.8% agarose gel electrophoresis. A total of 30 SSR markers (Roder et al., 1998) distributed on different wheat chromosomes were evaluated (Annexure-2). Out of 30 markers, 16 primers found to be polymorphic were selected for diversity studies (Table.1). PCR reaction was conducted in a 15µl reaction containing 1X PCR buffer, 200mM dNTPs, 0.25 µl of primer, 2mM $MgCl_2$, 1µl Taq polymerase and 50ng template DNA. PCR amplification was performed using BIORAD S 1000 thermocycler following the PCR protocol: Initial denaturation at 94^0C for 4min, 35 cycles at 94^0C for 1min, X^0C (X^0C depending upon the primer pair) for 1min, 72^0C for 1min and final extension step at 72^0C for 6min. Amplified products were resolved on 3% agarose gels (HiMedia) at 4v/cm in 1X TAE buffer and fragment sizes were calculated by interpolation from the migration distance of marker fragments of 100-bpDNA ladder (Invitrogen, USA).

Table 4. Analysis of molecular variance (AMOVA) for the clusters of wheat accessions detected by STRUCTURE analysis

Sources of variation	df	Sum of squares	Variance components	Percentage of variation
Among populations	6	567.642	1.93674***	21.29
Within populations	312	2234.029	7.16035***	78.71
Total	318	2801.671	9.09709	

*** Significant at $p < 0.001$

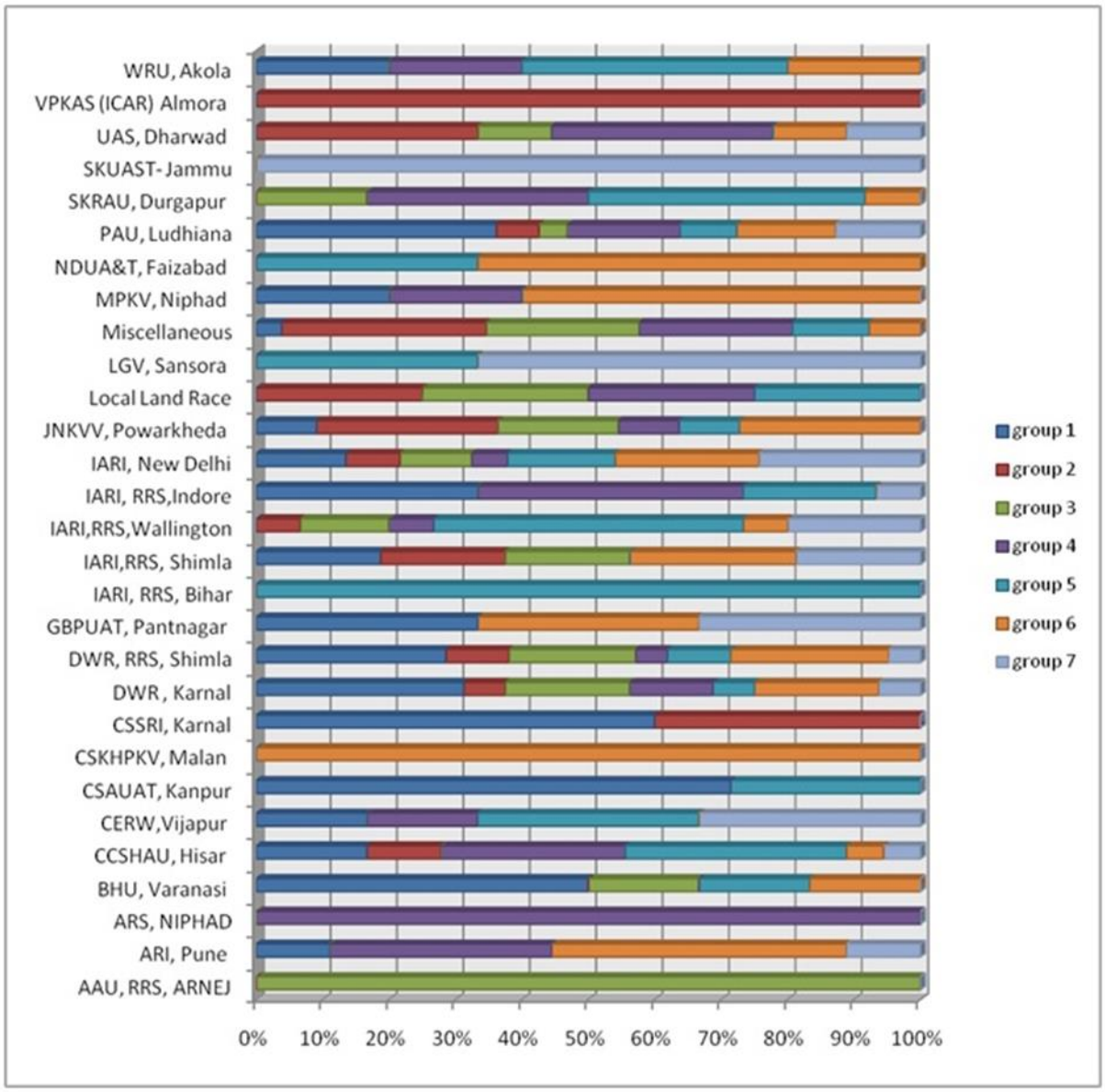

Fig 4. Distribution of genotypes across different sub-groups identified by STRUCTURE analysis**.**

Genetic diversity and Population structure analysis

Gene diversity for markers was measured by calculating polymorphism information content (PIC) according to following formulae:

$$PIC=1-\sum_{i=1}^{k} Pi^2$$

where k is the total number of alleles detected for a locus of a marker and Pi the frequency of the ith allele. To infer the population structure, model-based (Admixture) cluster software STRUCTURE 2.3.3 was chosen. To infer the number (K) of the subpopulations, software was run using a burn-in of 50,000, run length of 100,000 and a model allowing for admixture and correlated allele frequencies was used. The number K was set from 1 to 15 and ten independent STRUCTURE runs were made for each K and an average likelihood value across ten runs was calculated. Sub-groups were established according to the criterion proposed by Pritchard et al. (2000). Cluster analysis and Neighbour-joining tree was constructed using software package DARwin 5.0 (Perrier and Jacquemoud-Collet 2006). Genetic dissimilarity matrix was constructed according to Langarica et al. (2011).

Population genetic analysis

POPGENE 1.32 program was used to calculate various genetic parameters including the percentage of polymorphic

Table 5. Nei's unbiased genetic identity (above diagonal) and genetic distance (below diagonal).

pop ID	1	2	3	4	5	6	7
1	****	0.8797	0.9054	0.8083	0.8502	0.9194	0.8492
2	0.1281	****	0.9006	0.8550	0.9075	0.9052	0.9029
3	0.0994	0.1047	****	0.8311	0.8885	0.8952	0.8896
4	0.2128	0.1566	0.1850	****	0.8695	0.8226	0.8917
5	0.1623	0.0971	0.1182	0.1398	****	0.8612	0.9049
6	0.0840	0.0996	0.1107	0.1953	0.1494	****	0.8715
7	0.1634	0.1022	0.1169	0.1147	0.0999	0.1375	****

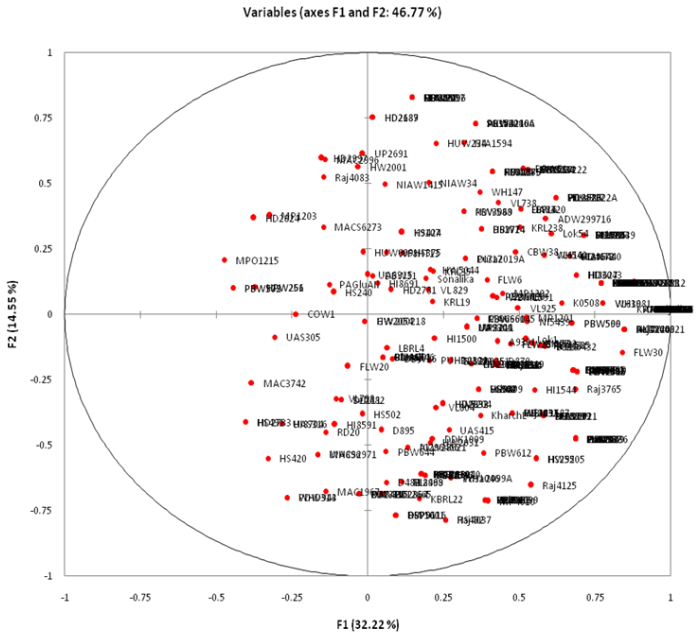

Fig 5. Principal component analysis of SSR data showing the genetic relationships among 319 wheat accessions.

loci (PPB), the effective number of alleles (ne), observed number of alleles per locus (na), Nei's (1973) gene diversity (h) and Shannon's information measure(I), (Lewontin, 1972). At the species level, total heterozygosity (Ht), heterozygosity within population (Hs), coefficient of gene differentiation (Gst), inter-variety genetic diversity (Dst) and gene flow (Nm) were calculated using Nei's (1973) gene diversity statistics. To examine the genetic relationship, a dendrogram was constructed using UPGMA of POPGENE software (version 1.32; Yeh et al. 1997). To evaluate the differentiation among populations and within populations, an analysis of molecular variance (AMOVA) was carried out using Arlequin 3.11 software (Excoffier et al., 2006). XLSTAT was used to perform Principal component analysis (PCA).

Conclusion

In order to make the best utilizaton of genetic potential of genotypes for improvement of traits and for adaptation to various stress conditions, genetic study is very crucial (Salem et al., 2008). In this study, we identified the level of diversity present in the genotypes distributed across different agro-climatic regions of India. Continuous genetic diversity assessment will help to maintain the diverse species for conservation and crop improvement. The results can also help the breeders so that they can effectively select the parents leading to progenies with high differentiation among them.

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