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Population Structure and Genetic Diversity of Wheat Landraces from North-western Indian Himalaya

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The present study was aimed at analyzing the genetic diversity of ninety six selected landraces from North-western Himalayan region using 21 microsatellite markers. The PIC value of STMS loci ranged from 0.011 (Xgwm383) to 0.746 (Xgwm369) with mean value of 0.34 for all loci. Total 51 alleles were detected with an average of 2.428 alleles per loci. Clustering of wheat landraces using three different algorithms gave the same kind of results. Lower inbreeding coefficient (F_{ST}) indicated low level of sub-population differentiation. Greater heterozygosity and gene flow among individuals were observed among wheat landraces. The partitioning of total molecular variance also showed similar, maximum variation was contributed by among individuals within populations (83.1%) followed by within individuals (15.12%) and the variation at among populations level was negligible (1.78%). It is concluded that observed number of alleles, effective number of alleles and Shannon's information index were greater in landraces of Kumaon division in comparison to other Himalayan region.

Key Words: Genetic Diversity, Landraces, Microsatellite, North-western Himalaya

Introduction

Triticum aestivum L. em Thell., commonly known as bread wheat is an important cereal globally and its cultivation had evolved with the expansion of major civilizations all over the world. Wheat is being traditionally grown in North-western parts of Indian Himalaya since ancient times. The Himalayan highlands are the reservoir for landraces because of the preponderance of locally developed traditional crop varieties owing to high agro-climatic heterogeneity and local socio-cultural diversity (Partap *et al.*, 2001). Though bread wheat is a globally important crop for human food and nutrition, genome wide molecular studies are very limited majorly because of its large genome size (~16×10^9 bp) and allohexapolyploidy comprising of three closely related genomes (A, B and D) with around 80% repetitive DNA (Gupta *et al.*, 1991; Bennett and Leitch, 1995). These genomic complexities also have been substantial barriers to analyze genetic diversity and population parameters in wheat germplasm. Studies on genetic diversity of economically important traits and population structure are important to conserve and enhance the germplasm use for crop improvement and other plant breeding activities (Uddin *et al.*, 2008). In today's scenario most of the rich plant biodiversity, particularly crop landraces, which sustained agriculture for the last 9000 years is on the verge of extinction due to the introduction of modern high yielding varieties (Brown and Brubaker, 2002). Wheat landraces have played a very important role in the local food security and sustainable development of agriculture as well their significance as genetic resources for wheat improvement. Though wheat breeding programs have made significant progress, use of very few elite germplasm lines as parental stock has led to a decrease in genetic diversity and has narrowed down the wheat genetic base (Hoisington *et al.*, 1999). Improved cultivars have such a great impact that wheat landraces from plains are almost vanished and now landrace diversity can be found only in hilly areas where climatic conditions are least suitable for cultivating high yielding varieties. Therefore, this study aimed at analyzing genetic diversity and population structure of important wheat landraces of Himalayan region of Uttarakhand and Himachal Pradesh. Moreover, there are very limited studies on wheat landraces from North-western Himalayan region. In the previous studies, morphological and agronomic traits as well as physiological indices were widely used for assessing genetic diversity, however these traits are influenced by environment. More recently, molecular markers

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have been increasingly exploited for diversity analysis. Therefore, Simple Sequence Repeats (SSRs), which are among the most versatile molecular markers, abundant, highly polymorphic, genome specific, co-dominant in nature and show a fairly even distribution over the genome (Choudhary *et al.*, 2016), have been used in this study to assess molecular diversity and other population genetics parameters in wheat landraces of Indian Himalayan region.

Materials and Methods

Plant Material and DNA Extraction

Wheat landraces were collected from fields of different regions of North-western Himalaya where considerable variability are being maintained. These landraces were further grown in field of ICAR-NBPGR Regional Station, Bhowali, Uttarakhand during 2013-14 *rabi* season. The seeds of these landraces were harvested and grown in greenhouse following standard agronomical practices to raise healthy plants during *rabi* season 2014-2015. A set of ninety six landraces were randomly selected for molecular diversity analysis (Table 1). Leaf samples of one month old plant were collected and stored at -80 °C for DNA extraction. Genomic DNA was extracted using the modified cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroof, 1984). The quality and concentration of extracted DNA was estimated by Nano-drop (ND1000, Thermo Scientific, USA). DNA samples concentration was further checked in 0.8% agarose gel electrophoresis using known DNA concentration standards.

Microsatellite Genotyping

Total 21 polymorphic SSR primers were selected from 250 SSRs through polymorphic analysis on eight randomly selected wheat landrace accessions. Primers with multiple bands were also discarded to avoid conflict to decide upon allele whether alternate form is in the same genome or different genome, since wheat genome has multiple copies of similar genomes (A, B, D). These twenty one polymorphic SSR markers (Supplementary Table S1) are randomly distributed across the three genomes. DNA amplification was performed in a 10µl volume containing 20 ng of genomic DNA, 0.2 U DNA polymerase, 1.5 mM Mg, 0.25 mM dNTPs and 0.2 M primer. The PCR cycle consisted of an initial denaturation at 94 °C for 4.5 min, followed by 36 cycles of 94 °C for 55 sec, annealing at 50-60 °C (varied from primer to primer) for 55 sec, 72 °C for 55 sec, and a final extension step of 72 °C for 5 min before cooling at 4 °C. All PCR (polymerase chain reaction) amplifications were carried out in G-Storm thermocycler (Gene Technology Ltd UK). PCR products were electrophoresed in 3.5 % agarose gel.

Statistical Analysis

For estimating different population genetics parameters using PopGene (ver. 1.32 Yeh *et al.*, 2000) wheat landraces were grouped into three sub-populations based on their local niche environment *i.e.* Garhwal and Kumaon divisions of Uttarakhand and Himachal Pradesh. NTSYS-PC (ver. 2.11X; Exeter Software, N.Y., Rohlf, 2000) was used to prepare the dendrogram based on Nei genetic distance obtained from PopGene analysis. NTSYS-PC was also used for hierarchical clustering of wheat landraces based on UPGMA similarity matrix. DARwin version 6.0.10 (Perrier and Jacquemoud-Collet, 2006) was used to create weighted neighbor joining tree based on simple matching (SM) dissimilarity matrix. The Bayesian model-based clustering analysis was used for determining the optimal number of genetic clusters found among wheat landraces using the software STRUCTURE 2.3.4 (Pritchard *et al.,* 2000), which partitions individuals into number of clusters (K) based on the multi-locus genotypic data. The admixture model and correlated allele frequencies were applied for each run with 10,000 iterations and 100,000 Markov Chain Monte Carlo (MCMC) replications. The best K value (genetically distinct clusters) was defined using the Evanno *et al.* (2005) method. The individuals were arranged based on 'Q' (the estimated membership coefficients for each individual, in each cluster). The data set were also subjected to analysis for molecular variation (AMOVA) using Arlequin (ver. 3.5.1.2, Excoffier *et al.*., 2005). Population structure by AMOVA is based on an analysis of variance of gene frequencies, taking into account the number of mutational differences between molecular haplotypes. Fixation indices (Weir and Cockerham, 1984) and population pairwise F_{ST} (pairwise estimates of the correlation of alleles between populations) values were also computed by using the above software. Graphical software R version 3.5.1 (Rcmd function) was embedded with Arlequin version 3.5.1.2 software to generate graphical illustrations of results.

The polymorphism information content (PIC) value described by Botstein *et al.* (1980) and modified by

Table 1. Prominent wheat landraces used in the diversity analysis

S.No.	District	IC Number	Landrace name	S.No.	District	IC Number	Landrace name
Himachal Pradesh				Kumaon, Uttarakhand			
1	Chamba	IC381111	Sherwan	48	Almora	IC260848	Mishrygahun
2		IC381124	Gandham	49		IC260854	Mishrygahun
3		IC381190	Amreek Gandham	50		IC595395	Thanga gehun
4	Hamirpur	IC208899	Unknown	51	Bageshwar	IC260857	Dhudgahun
Garhwal, Uttarakhand				52		IC260858	Dhudia
5	Chamoli	IC260865	Gahun	53		IC266976	Thangi
6		IC260866	Gahun	54		IC266977	Bhati
7		IC260868	Gahun	55		IC266978	Rati
8		IC260869	Gahun	56		IC393109	Gehun
9		IC260871	Gahun	57		IC393110	Gehun
10		IC260877	Gahun	58		IC393116	Gehun
11		IC260890	Chudi Gahun	59		IC393117	Gehun
12		IC383581	Lakha Gainhu	60		IC393118	Gehun
13		IC383592	Wheat	61		IC398292	Lal Gehun
14		IC383593	Lakha Gainhu	62		IC398294	Juinsi Ninsa
15	Dehradun	IC345589	Ghaon	63		IC398296	Setta
16		IC345598	Ghawn	64		IC398297	Tank
17		IC345604	Ghaon	65		IC398298	Sathi
18		IC345620	Ghaun	66		IC398302	Thaenkna
19		IC345671	Gahoun	67		IC398303	Chini
20		IC345673	Gahoun	68		IC398305	Munnar
21		IC345687	Gahoun	69		IC398307	Thull
22		IC345688	Gahoun	70		IC398309	Safed Jhusial
23		IC345690	Gahoun	71	Champawat	IC266764	Gehun
24	Pauri	IC430330	Gahun	72		IC266789	Jhausa
25		IC430369	Donia	73		IC266791	Gerua
26		IC430373	Chudiya	74		IC392578	Guhu
27		IC564090	Mundari	75		IC406688	Mishri Gehun
28		IC564096	Bareek lal	76		IC406690	Jusia Gehun
29		IC564113	Lal mundiya	77		IC406715	Dolat kani
30		IC564114	Safed mundiya	78		IC406724	Safed Gehun
31		IC564159	Safed mundari	79		IC595382	Ratuva gehun
32	Rudraprayag	IC260880	Gahun	80	Nainital	IC260845	Jhusia
33		IC260887	Mundarigaun	81		IC573137	Ryat gaun
34		IC260888	Gahun	82		IC573138	Syat gyan
35		IC260894	Lal Gahun	83		IC573140	Chnosi
36		IC260895	Lal Gahun	84		IC573157	Munda
37		IC260901	Gahun	85	Pithoragarh	IC266831	Munara
38		IC260902	Gahun	86		IC266847	Gerua
39		IC382649	Cheuri	87		IC266852	Dudh Gehun
40		IC382653	Muneri	88		IC266854	Unknown
41		IC382658	Muneri	89		IC266872	Dapati Gehun
42		IC382664	Deshigenhun	90		IC266884	Mota gehun
43		IC393131	Gehun	91		IC266921	Mota Gehun
44	Tehri	IC589303	Baniya gehun	92		IC406697	Mundia
45	Uttarakashi	IC589276	Hasia Gehun	93		IC444217	Daapti Gehun
46		IC589278	Lal mishri	94		IC444226	Raje Gehun
47		IC589300	Lal Mishri Gehun	95		IC444229	Bhotta Gehun
				96		IC444232	Bhotia Gehun

Anderson *et al.* (1993) for self-pollinated species was calculated as follows:

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2 PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

where, P*ij* is the frequency of the *j*th allele for the *i*th marker, and summed over n alleles. PIC is also an estimate of the discriminatory power of a SSR marker locus.

Results and Discussion

Landraces are naturally a rich genetic resource evolved along with continuous selection against various adverse biotic and abiotic stresses, and yield parameters over long period of time. These are the major source of trait specific genes for crop improvement and broadening the wheat genetic base. The present study was concentrated on finding out the genetic diversity, allelic richness, allelic abundance and many other important population parameters in randomly selected wheat landraces from Himalayan region. Our findings revealed that significant genetic diversity exists in wheat landraces of the region.

Clustering of Wheat Landraces

Cluster analysis based on different algorithms (Figures 2 & 3) revealed that genetic differentiation in these wheat landraces was not the effect of geographical distance. This may probably be because of isolation by distance is not enough to hinder gene flow among landraces of different regions. This result reveals that sampling distance for wheat landraces can be further increased and tested to distinguish among wheat landraces based on their sampling distance. This information also indicated that by increasing both the number of samples collected from a single sampling site and distance between sampling sites can maximize efficiency of sampling with maximum collected diversity.Wheat landraces were also clustered based on the Bayesian model-based clustering analysis using the software STRUCTURE 2.3.4 (Pritchard *et al.,* 2000). The programme partitions individuals into number of clusters (K) based on the multi-locus genotypic data. Individuals in the sample are probabilistically assigned to populations, or jointly to two or more populations if their genotypes indicate that they are admixed (Figures 3, 4 and 5). All ninety six landraces were grouped into four major clusters (K = 4). Substantial amount of admixture among the wheat landraces at genetic level was observed, which was in accordance with the results obtained by the above clustering methods. Overall, if we look at all the three methods of clustering with different algorithms

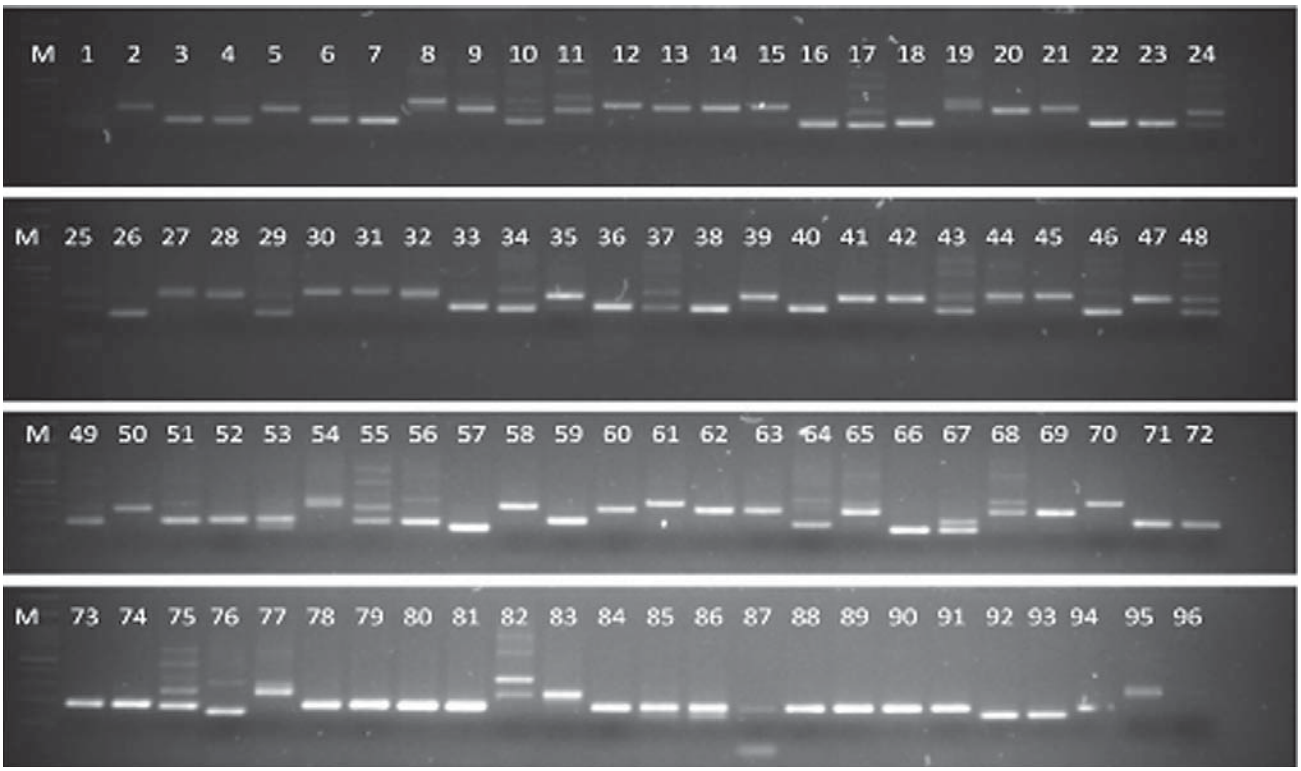

Fig. 1. SSR primer (Xgwm-369) profile of wheat landraces obtained using agarose gel electrophoresis. (M= 50bp marker; wheat landraces are labelled from 1 to 96 as given in the Table No. 1)

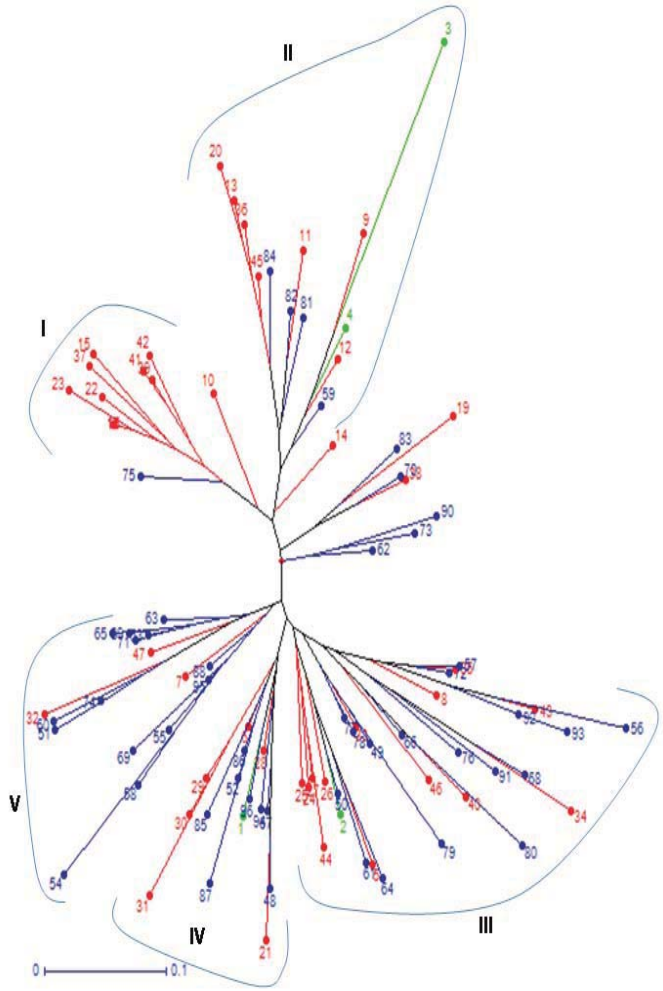

Fig 2. Unrooted neighbour joining tree generated by DARwin v. 6.0.10. Colour indicates geographical location [green: H.P. (1-4); red: Garhwal division (5-47); blue: Kumaon division (48-96)]. The numerical values representing the different landraces are the serial number of the Table 1.

and population genetic parameters, similar pattern was revealed, which strengthen the results. Though different clustering methods from software like STRCUTURE, NTSYSpc and DARwin have grouped the landraces in distinct clusters, but the individual populations were distributed randomly irrespective of their geographical locations. After partitioning the variation in different categories, the AMOVA results also indicated that major source of variation is individual genotypes not populations defined based on their geographical locations (Table 4). The pairwise F_{ST} estimates also suggest the same i.e. divergence at population level is negligible (Table 3).

Allelic Diversity

The microsatellite molecular markers were selected for genotyping of landraces in this study considering their high polymorphism, specificity (Pestova *et al.*, 2000), reproducibility (Stachel *et al.*, 2000) and high variability (Brown *et al.*, 1996). Genotyping and analysis of wheat landraces differentiated the intra and inter-population diversity and confirmed the robustness of microsatellite loci for genetic diversity studies of wheat landraces (Figure 1) reported in earlier studies (Plaschke *et al*., 1995; Eujayl *et al*., 2002; Maccaferri *et al*., 2005; Arora et al 2014). Number of private alleles specific to particular populations was very low (Supplementary Table S2), which indicates that the evolutionary period for population divergence after separation from recent common ancestor of the wheat landraces is very short or genetic divergence in bread wheat might be slow. Rare alleles (frequency < 0.05%) are those alleles which got introduced in to the population in very recent past

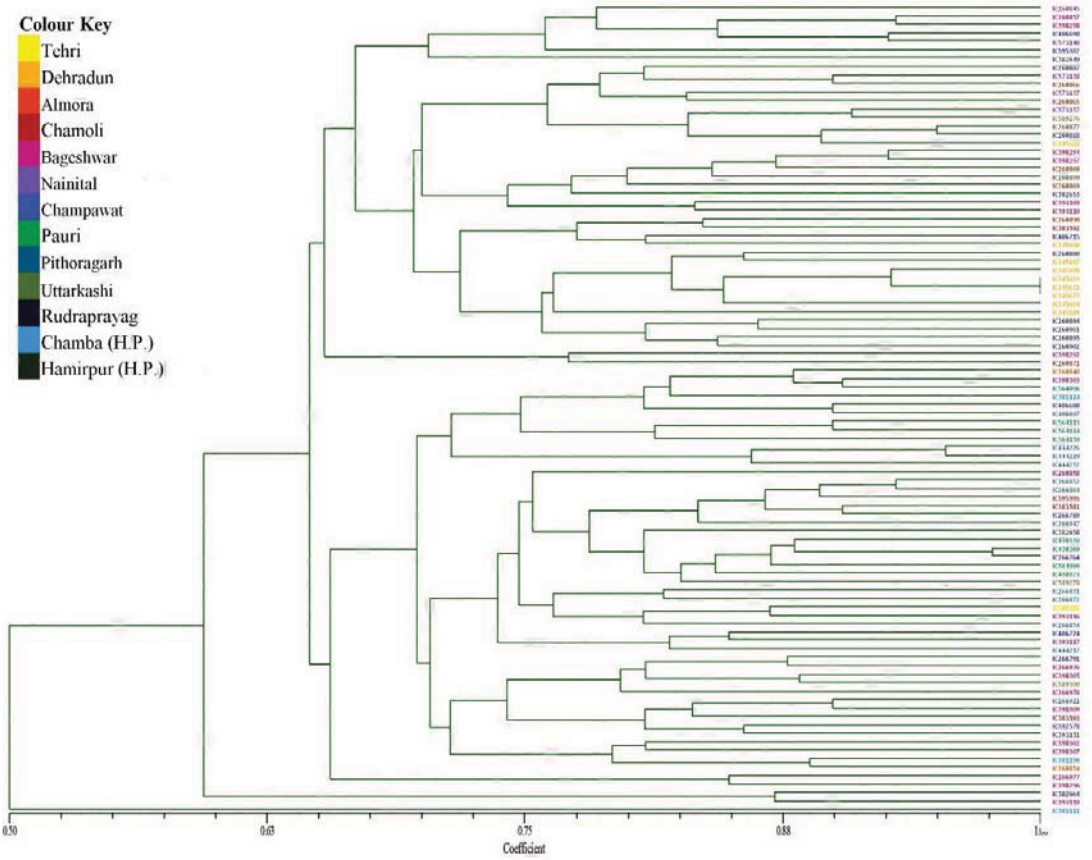

Fig. 3. SAHN dendrogram based on UPGMA clustering method from similarity matrix of DICE coefficient generated by NTSYSpc v. 2.11X

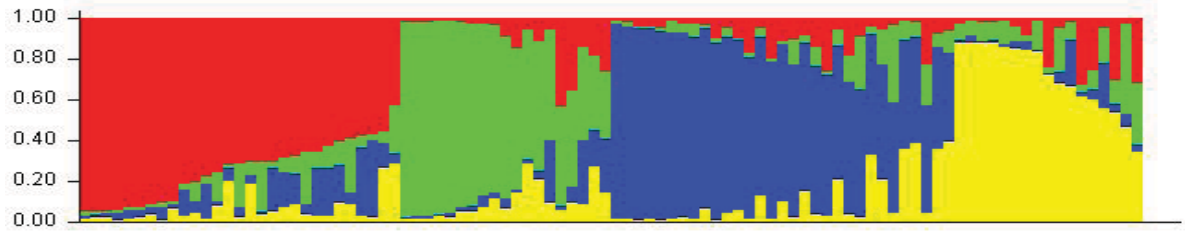

Fig. 4. Population structure of 96 wheat landraces for k=4 in a single line plot using STRUCTURE ver. 2.3.4 software. Four different colours represent four sub-populations of wheat identified with each bar representing the estimated membership (0 - 1) of single genotype in each of the four clusters

(Supplementary Table S2). Rare alleles may get chance to established or lost based on its functionality and role in fitness of the individual. Moderate number of rare alleles (18) has been observed, indicating that the genotypes are dynamically evolving in their local niches with moderate level of selection forces (Supplementary Table S2).

Effective number of alleles (Ne) which measures the number of equally frequent alleles that would take to achieve a given level of gene diversity (expected heterozygosity), allows us to compare populations where the number and distributions of alleles differ drastically, because it is measured locus by locus rather than by mean gene diversity. In simple term, when the heterozygosity is high, effective number of alleles is also high. Therefore, it measures the distribution of allele frequencies among alleles of the same locus, equal distribution leads to highest Ne. From the analysis, lower level of Ne has been observed for wheat landraces from all the regions (Table 2). Shannon's information

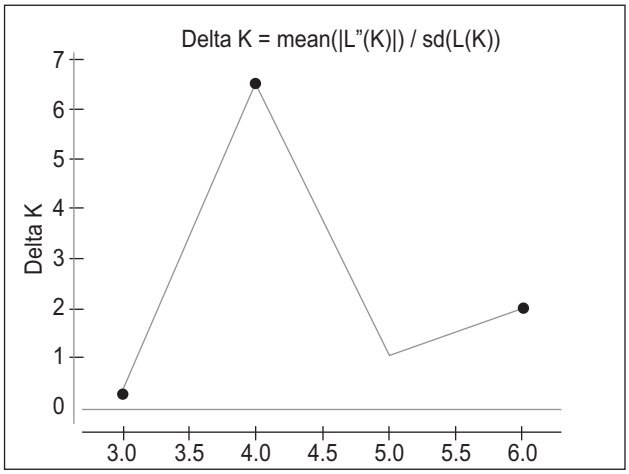

Fig. 5. Best K was determined by Evanno *et al.*, 2005 method. K value is plotted against delta K. K value at highest delta K is selected as best K which is 4.

index (I) is an important measure of diversity similar to Ne. The highest value for 'I' is obtained when each allele is present only once in the entire sample being measured; it penalizes redundancy at the allelic level, with respect to the entire sample. Therefore, this index is important when rare alleles are abundant and are of much importance for selecting a genotype especially in developing core or mini-core. Shannon information index also estimated lower for these landraces (Table 2). Level of Levene's (1949) expected heterozygosity, Nei's (1973) expected heterozygosity and average expected heterozygosity was observed similar for all landraces irrespective of their geographic location which further fit with the above results (Table 2). Comparatively observed number of alleles, effective number of alleles, and Shannon's information index were greater in landraces of Kumaon division in comparison to landraces from H. P. and Garhwal Division, even though Kumaon and Garhwal division populations grouped together and H. P. population grouped separately probably because of heterozygosity level within populations across loci.

Population Differentiation

Lower F_{ST} indicated the very low level of sub-population differentiation and existence of substantial level of heterozygosity reflects the gene flow among individuals might be through inbreeding or admixture among populations. Gene flow (Nm) value lesser than one indicates restricted gene flow among populations as per Wright (1965). Higher level of gene flow (2.36) among populations explains existence of higher variability with low level of sub-population differentiation (Table 2). The same also has been established from previous studies that level of population differentiation is inversely proportional to the magnitude of gene flow among sub-populations (Díaz-Matallana *et al.*, 2009; Wang *et al.*, 2012; Abouzied *et al.*, 2013).The pairwise F_{ST}

Table 2. Overall summary of genetic variation and heterozygosity statistics across the loci

	Allelic variation across the loci			Heterozygosity across the loci		
	Parameters	Mean	St. Dev	Parameters	Mean	St. Dev
Landraces from Himachal Pradesh	na	1.76	0.77	Obs Het	0.02	0.08
	ne	1.56	0.56	Exp_Het	0.32	0.27
	I	0.43	0.38	Nei	0.28	0.24
Landraces from Garhwal Division	na	1.76	0.77	Obs Het	0.06	0.08
	ne	1.56	0.56	Exp_Het	0.34	0.25
	I	0.43	0.38	Nei	0.33	0.24
landraces from Kumaon Division	na	2.38	0.74	Obs Het	0.04	0.06
	ne	1.72	0.78	Exp_Het	0.32	0.26
	I	0.52	0.41	Nei	0.32	0.25
Over all	na	2.43	0.68	Obs Het	0.05	0.06
	ne	1.77	0.78	Exp_Het	0.34	0.25
	I	0.55	0.4	Nei	0.34	0.25
	FST	0.1				
	Nm*	2.36				

na = Observed number of alleles; ne = Effective number of alleles [Kimura and Crow (1964)]; I = Shannon's Information index [Lewontin (1972)], Observed heterozygosity; Expected heterozygosity was computed using Levene (1949); Nei's (1973) expected heterozygosity, $F_{ST=}$ inbreeding coefficient (population structure at subpopulation to total population), Nm = Gene flow estimated from $F_{ST} = 0.25(1 - F_{ST})/F_{ST}$.

Table 3. Population pair wise F_{STs}

	Himachal Pradesh	Garhwal Division	Kumaon Division
Himachal Pradesh	0.0000		
Garhwal Division	0.04955	0.0000	
Kumaon Division	0.05190	0.03113	0.0000

(inbreeding coefficient total to sub-grouped individuals) comparison among populations was done to measure population's divergence. F_{ST} was recorded very low to negligible indicating the no or very less divergence among populations grouped based on the geographical origin of landraces (Table 3).

Analysis of Molecular Variance (AMOVA)

For assessment of source of variation among landraces total molecular variance was partitioned in categories *viz*. among populations, among individuals within populations and within individuals. The results revealed maximum variation was contributed by among individuals within populations (83.1%) followed by within individuals (15.12%). The variation level at among populations was negligible (1.78%) (Table 4). Management of landrace diversity by the farmers has been conferring vitality and viability to traditional agricultural systems. The dominance of only a few wheat varieties in production system poses a major threat to the genetic diversity of the crop. As Himalayan highlands are the reservoir for wheat landraces due to preponderance of locally developed traditional crop varieties owing to high agro-climatic heterogeneity, this study was taken up as a model in Uttarakhand and its adjoining parts of HP. The landraces in the region are being managed by subsistence growers that possess lower risk of failure under marginal production environment and are also key to food and nutritional security of isolated communities. Large extent of variation and low level of differentiation was observed among the landraces studied. These landraces can be a potential resource for trait specific crop improvement and as well in restoring lost wheat genetic base by germplasm enhancement activities.

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Table 4. Analysis of Molecular Variance (AMOVA)

Source of Variation	d.f.	Sum of squares	variance components	Percent of Variation
Among Populations	2	17.686	0.05761 Va	1.78
Among individuals within populations	93	546.132	2.69140 Vb	83.1
Within individuals	96	47	0.48958 Vc	15.12
Total	191	610.818	3.23859	
Fixation Indices	FIS : 0.84609 FST : 0.01779 FIT : 0.84883			

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