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Estimation of Indian and Turkish Hexaploid Wheat Population Structure Employing Molecular Markers

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Abstract

Bread wheat (*Triticum aestivum*) is the most commonly grown crop due to its adaptation in a wide range of eco-geographical conditions and providing enhanced food assurance to the modern world. A diverse and rich collection is the foundation of each successful wheat improvement program. Therefore, major efforts are in progress worldwide to boost wheat production by broadening genetic diversity. Accepting this issue as a target, present study gives an overview of the major progress in the diversity and population evaluation of Indian and Turkish hexaploid wheat employing ISSR and RAPD primers. Various statistical analyses were employed for determining the hexaploid wheat population structure of India and Turkey. Results of dendrogram, scatterplots, Analysis of Molecular Variance (AMOVA) and population structure analysis were found in accordance with each other. All the experimental genotypes were clustered in two main groups, one group containing Indian varieties and another group containing both Indian and Turkish varieties reflecting the direct or indirect interbreeding among the populations of the two countries. Utilizing the genetic association of Indian and Turkish hexaploid wheat population, based on genetic distance estimated in the study, researchers worldwide may include Indian and Turkish hexaploid varieties in the wheat improvement programs and can evade the likelihood of selected germplasm becoming hereditarily consistent.

Keywords: bread wheat, countries, genetic association, ISSR, RAPD

Introduction

In last 50 years, wheat persisted as one of the most used and stable food grain cereals with 1% yearly gain due to the implementation of advanced agricultural techniques and involvement of productive cultivars. However, to cover up the population needs, it is necessary to raise the annual productivity gains to 2.5% up to 2025 (Curtis, 2002; Dixon *et al*., 2009). Though wheat is already grown in a vast expanse in comparison to any other cereal crop, it cannot be increased further. With no scenario of area expansion under wheat cultivation, the prime prominence would be on escalating the wheat output by adopting the improved cultivation practices. In addition, production in undesirable areas is also putting challenges of biotic and abiotic stresses. Therefore, there is crucial requirement of rigorous research applications and technology modifications to enhance the per unit area wheat production (Alexandratos and Bruinsma, 2012).

From ages of seed selection and modern wheat breeding, bread wheat can be cultivated in moderate climatic conditions across the globe. Among the total area for world wheat production, 90% is allotted to bread wheat. It has been estimated that semi-dwarf varieties are contributing towards 70% of this area, while traditional and tall varieties have occupied 20% and 10% area respectively (Smale *et al*., 1996).

Since 1950's to 2012, wheat in India has recorded an enormous leap in both the production and agricultural land from approximately 6.5 million tones to 90 million tones and

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from nearly 10 million ha to 30 million ha, respectively. With this boost, India has gained second position following China supplying 12% share in global wheat production and is capable to participate in the International Wheat Market by its major export (Nagarajan, 2004; Dronamraju, 2008).

Central Anatolia, in Turkey, has been the centre of wheat for thousands of years, which is the major cereal product and is a component of life in rural vicinity. While Turkey has secured 10th position in the world for the total wheat production, it has become 4th topmost producer of durum wheat contributing 30% of agricultural and industrial output through baking industry (Zencirci and Aktan, 1995; Yildirim *et al*., 2013).

In spite of hefty cultivation of bread wheat after green revolution by developing countries like India and Turkey (Reynolds and Borlaug, 2006), a substantial growth in the production is required. Being actively cultivated, genetic base of bread wheat has been tightened due to the selection towards specific targets during long breeding programs and the cross-made between the common parents leading to the similar qualities in hybrids. In order to evade the chances of genetic uniformity of elite germplasm, thereby threatening the long-term selection gains (Messmer *et al*., 1993), information regarding the association of parents in a breeding program can be used to conserve the genetic resources. Selection of parents for hybridization (Frei *et al*., 1986) and the progeny performance prediction (Hallauer and Miranda, 1988) alleviates on the availability of information about genetic relationships among accessions within the species. Variations in the environmental conditions have a great impact on the accuracy of the data gathered from the parental information, morphological characters, and isozyme studies (Autrique *et al*., 1996; Cox *et al*., 1985; Shamsuddin, 1985). For this problem, a substantial answer has emerged in the form of molecular markers that can be presented as a potential tool for evaluating genetic variability among the intimately linked cultivars of wheat varieties (Davila *et al*., 1998; Kim and Ward, 1997; Plaschke *et al*., 1995). However, the factor determining the scope of their efficacy depends on the type of the marker, the quantity involved, genome coverage and the population under exploration.

Random Amplified Polymorphic DNA (RAPD) molecular marker systems used in this study are significantly quicker and simpler in comparison to few other molecular strategies, making the technique famous for evaluating genetic polymorphism in wheat species (Bibi *et al*., 2009; Cao *et al*., 1999, Cifci and Yagdi, 2012; He *et al*., 1992; Maric *et al*., 2004; Pandey *et al*., 2012). Due to the distinguishing features like abundance, good reproducibility, high polymorphism, vastly informative and quick to use, Inter Simple Sequence Repeats (ISSR) markers can be supportive to RAPD markers (Bornet and Branchard, 2001; Zietkiewicz *et al*., 1994). Similar to RAPD primers, no aforementioned sequence information is necessitated for the genetic studies (Zietkiewicz *et al*., 1994). High annealing temperature contributes significantly towards the elevated reproducibility among ISSR markers as compared to RAPD markers (Bornet and Branchard, 2001; Chowdhury *et al*., 2002). A number of researchers all over the world estimated genetic diversity/genetic similarity among bread wheat varieties using ISSR markers (Carvalho *et al*., 2009; Du *et al*., 2002; Hao *et al*., 2006; Khan *et al*., 2014; Najaphy *et al*., 2011; Rashed *et al*., 2008).

Hence, in present study, the efficacy of RAPD and ISSR markers has been explored in assessing the genetic structure in a collection of Indian and Turkish bread wheat varieties/cultivars and for the identification of genetic relations between the geographical groups. As the relatedness among the wheat varieties from different geographical origin provides an outline regarding variant growth environments and breeding advancement, cultivars can be successfully involved in the wheat development programs.

Materials and methods

A total of 73 wheat genotypes including sixty Indian and thirteen Turkish hexaploid wheat cultivars (*Triticum aestivum* L.) were chosen as the experimental material (Table 1). Varieties involved in the study are good in bread/chapatti making quality (eg. 'C306', 'HD1941', 'HUW213', 'K8434', 'PBW524'), resistant to leaf and stem rust (eg. 'CBW38', 'FLW15', 'HD2733', 'HUW213', 'K7903', 'PBW343'), high yielding ('PBW343', 'Adana', 'Tosunbey', 'Demir2000') and tolerant to several abiotic stresses including salinity, heat and drought ('HD2985', 'HS420', 'K0307', 'K9423', 'K9533', 'KRL210', 'KRL213', 'Karahan99', 'Dağdaş', 'Demir2000'). We tried to include some of the efficient hexaploid wheat varieties from both the countries so that these can be effectively utilized by the breeders for wheat development.

DNA extraction

For the efficient DNA extraction of wheat leaf tissue, as suggested by Burden 2012, tissue lyser has been utilized for grinding. Following this, in the 2 mL microfuge tubes, 0.1 to 0.2 mg leaf samples were lysed in 750 µL of 2% CTAB and 7.5 µL of β-mercaptoethanol for 10 minutes with repeated rotation of lysis blocks after 5 minutes. Frequency of homogenizer was set 25 Hz or 1500 rpm at the time of lysis. After crushing, samples were incubated on a hot block at 65 °C for 30 minutes on addition of 10 µL RNase A for purification of the samples. Further, 25:24:1 phenol:chloroform:isoamyl alcohol followed by chilled isopropanol were added to the sample for additional purification and precipitation. Finally, DNA pellet was washed using 70% ethanol, which on getting desiccated dissolved in 100 µL DNase RNase free water and stored at -20 °C. Wheat genomic DNA was qualified and quantified on 1% agarose gel and spectrophotometer, respectively to prepare homogeneous required concentrations for RAPD and ISSR analysis.

DNA amplification using ISSR primers

The screening of twenty seven ISSR primers (Metabion) against selected wheat genotypes, led to the selection of 10 polymorphic primers (Table 2) for the final analysis. PCR reactions were carried out in 25 µL having 2.5 µL of 10X Taq Buffer containing ammonium sulphate (except ISSRF3), 3 µL of 25 mM $MgCl_2$, 0.4 µL of 25 mM dNTP, 0.5 µL of 10 µM primer, 1.5 units of *Taq* DNA Polymerase (Thermoscientific) and 100 ng of template DNA. The 2-step PCR reactions were performed in Techne-512 Master Cycler by optimizing the number of initial and final step PCR cycles and different reaction conditions for each individual ISSR primer.

Table 1. Name and origin of 73 Indian and Turkish hexaploid genotypes used in the study (Genotypes 1 to 60 are Indian varieties, while genotypes 61 to 73 are Turkish varieties)

Gel No.	Genotypes	Pedigree
1	'AAI12'	-
2	'AAI16'	-
3	'AAI23'	-
4	'AAI28'	-
5	'AAI347'	-
6	'C306'	RGN / CSK3 // 2*C591 / 3 / C217 / N14 // C281
7	'CBW24'	-
8	'CBW38'	CNDO / R143 // ENTE / Mexi2 / 3 / *Ae. squarrosa* (Taus) / 4 / Weaver / 5 / 2*Pastor
9	'DBW52'	-
10	'DBW77'	-
11	'FLW15'	PBW 343*3 / Tc+Lr32
12	'HD1941'	E 5477 * S64
13	'HD2285'	249 / HD 2150 // HD 2186
14	'HD2687'	CPAN 2009 / HD 2329
15	'HD2733'	Attila / 3 / Tui / Carc // Chen / CHTO / 4 / Attila
16	'HD2781'	BOW / C306 // C591 / HW 2004
17	'HD2888'	C306 / T. sphaeroccum // HW 2004
18	'HD2891'	WL711 // HD 2624
19	'HD2932'	KAUZ / STAR // HD2643
20	'HD2967'	ALD / COC // URES / HD2160M / HD2278
21	'HD2985'	PBW343 / PASTOR
22	'HI1563'	MACS 2496*2 / MC10
23	'HS420'	LAJ3302 // CMH 73A-497 / 3*CNO79
24	'HUW213'	NORTENO / MOTI // HD 2160
25	'HUW234'	HUW12 / SPARROW // HUW12
26	'HUW510'	HD2278 / HUW234 // DL230-16
27	'HW2006'	-
28	'HW2045'	HD 2402*5 / SUNSTAR*6 / C80-1
29	'HW2071'	-
30	'HW4024'	-
31	'HW4060'	-
32	'HW5202'	-
33	'K0307'	K8321 / UP2003
34	'K0402'	HP1731 / UP 2425
35	'K0424'	-
36	'K0607'	-
37	'K0906'	-
38	'K65'	C591 / NP773
39	'K68'	NP773 / K13
40	'K7903'	HD1982 / K816
41	'K8027'	NP875 / 4 / N10B / Y53 // Y50 / 3 / KT54B / 5 / 2*K 852
42	'K816'	CNO // SN64 / KLRE / 3 / 8156
43	'K8434'	HD2160 / K 68
44	'K8962'	K816 SIB / PV18 // HD2160
45	'K9006'	CPAN1687 / HD2204
46	'K9107'	K8101 / K68
47	'K9351'	K72 / K8027 // K72
48	'K9423'	HP1633 / KAL / UP262
49	'K9465'	HD2160 / K68
50	'K9533'	HI1077 / HUW234
51	'CLN5'	-
52	'KRL210'	PBW65 / 2*PASTOR
53	'KRL213'	CNDO / R143 / ENTE / MEXI-1-1 / 3 / *Ae. squarrosa* (TAUS) / 4 / WEAVER / 5 / 28KAUZ
54	'LOK45'	CPAN3066 / KALYANSONA / LOK1 / CIANO79 / CPAN2081 / J24 / SS1063 / CPAN1907 / CC493 // HD2358
55	'MACS6222'	HD2189*2 / MACS2496
56	'NW1014'	CEB148 / KA / 7 / HK / 38MA / 4 / 4777 // REI / Y / 3 / KT / 5 / YR / 6 / TUC
57	'PBW343'	ND / VG9144 // KAL / BB / 3 / YACO / 4 / VEE #5
58	'PBW502'	W-485 / PBW-343 // RAJ-1482
59	'PBW524'	PBW343 / HUW235
60	'WR1451'	-
61	'KARAHAN99'	C126-15 / COFN / 3 / N10B / P14 // P101 / 4 / KRC
62	'EKIZ'	F885 K1.1 / SXL
63	'AT052K2'	-
64	'KAMÇI'	-
65	'SARIBAŞ'	LV-TUR
66	'DAĞDAŞ'	093-44 / AU // SIHHE
67	'ADANA'	PFAU / SERI M 82 // BOBWHITE
68	'TOSUNBEY'	ECVD-12 / KIRAC-66 // (SIB)CROW
69	'MURAT'	KKK / ITD // LOV29
70	'ATILLA12'	MV12 = MIR808 / BEZ1 // BEZ1 / 3 / BEZ1 / PRODUTTORE // BEZ1
71	'AHMETAĞA'	-
72	'DEMIR2000'	21031 / CO-6552142 // MARA / SCOUT / 3 / PAI-YU-PAO
73	'BAĞCI'	HN7 / OROFEN // BEIJING 8 / 3 / SERI M 82 / 4 / 74CB462 / TRAPPER // VONA

Table 2. Characteristics and polymorphism revealed by ISSR primers for 73 Indian and Turkish hexaploid genotypes used in the study

ISSR Primer	Sequence	Melting Temperature (Tm)	Total Number of Bands	Polymorphic Bands	Percent Polymorphism Detected
ISSRF3	5'- $(AG)_8$ CG - 3'	56.0	11	7	63.6
ISSRF4	5'- $(AG)_8$ TG - 3'	53.7	8	7	87.5
ISSRF9	5'- $(GAA)_5$ - 3'	39.6	7	3	42.8
ISSRM1	5'- $(AGC)_6$ G - 3'	63.1	6	5	83.3
ISSRM2	5'- $(ACC)_6$ G - 3'	63.1	10	8	80.0
ISSRM3	5'- $(AGC)_6$ C - 3'	63.1	23	22	95.6
ISSRM8	5'- $(AC)_9$ G - 3'	56.7	12	9	75.0
ISSRM9	5'- $(AC)_8$ CG - 3'	56.0	10	8	80.0
ISSRM12	5'- $(GACAC)_4$ - 3'	61.4	5	4	80.0
ISSRM17	5'- CAG $(CA)_8$ -3'	56.7	4	1	25.0
Total			96	74	77.0

Table 3. Characteristics and polymorphism revealed by RAPD primers for 73 Indian and Turkish hexaploid genotypes used in the study

RAPD Primer	Sequence	Melting Temperature (Tm)	Total Number of Bands	Polymorphic Bands	Percent Polymorphism Detected
cRAPD1	5' – GAAACGGGTG - 3'	32	6	4	66.6
cRAPD2	5' - GTG ACGTAGG - 3'	32	10	5	50.0
RAPDB3	5' - GTG ACGTAGG - 3'	34	6	6	100.0
RAPDB4	5' - CTCACCGTCC - 3'	34	12	8	66.6
RAPDB5	5' – GACGGATCAG - 3'	32	9	6	66.6
RAPDB10	5' – CTACTGCGCT - 3'	32	7	4	57.1
RAPDB13	5' – TTCAGGGTGG - 3'	32	4	2	50.0
RAPDL2	5' – GTTTCGCTCC - 3'	32	6	3	50.0
RAPDL4	5' – AAGAGCCCGT - 3'	32	8	3	37.5
RAPDL6	5'- CCCGTCAGCA - 3'	34	7	4	57.1
Total			75	45	60.0

DNA amplification using RAPD primers

In a preliminary screening of 43 primers (MWG Biotech-AC), based on the polymorphism pattern, 10 primers were selected. RAPD reactions, were carried out in Techne-512 Master Cycler with a 15 µL amplification reaction volume comprising 1.5 µL of 10X Taq buffer with ammonium sulphate, 2.5 µL of 25 mM $MgCl_2$, 3 µL of 1 mM dNTP, 1.5 µL of 5 µM RAPD primer, 3 units of *Taq* DNA polymerase (Thermoscientific) and 50 ng of template DNA (Table 3). The PCR conditions were optimized for each RAPD primer according to their annealing temperature. Both RAPD and ISSR based amplified products were separated on 1.5% agarose gel (containing 10 μgml^{-1} ethidium bromide) in 1 X TBE buffer at 80 V for 5-6 hours in Thermoscientific Gel Electrophoresis System. The image of DNA fragments in the gel was captured with Vilber Lourmat Documentation system. Thermo Scientific Generuler 100 bp Plus DNA ladder was used as molecular size marker for both marker systems.

Statistical analysis

The data analysis of gel has been done by preparing the binary data matrix with 0 and 1 characters for the absence and presence of bands, respectively. Reproducible amplified bands have been identified on three times repetition of PCR reactions. Relative polymorphism information content of two primers was compared on the basis of total number of bands and polymorphic bands. Simqual analysis of NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System) version 2.02e software (Rohlf, 1992) was used to obtain the simple matching coefficients and preparing the similarity matrix. A combined RAPD and ISSR dendrogram has been constructed based on this similarity matrix using the R software.

Authentication of dendrograms and verification of grouping of cultivars on the basis of originating countries have been made by constructing Scatterplots. Two dimensional scatterplots were drawn via R software by double centering and performing the Eigen analysis with similarity matrix based on Simple Matching coefficients.

Indian and Turkish hexaploid wheat population segregation was estimated utilizing AMOVA executed in GenAlEx 6.5 software (Peakall and Smouse, 2012; Peakall and Smouse, 2006). One thousand permutations have been made to establish the significance of variance components among the populations.

Bayesian model-based clustering algorithm employed in STRUCTURE version 2.3.4 (Falush *et al*., 2007; Falush *et al*., 2003; Hubisz *et al*., 2009; Pritchard *et al*., 2000) has been exploited for the determination of most selective genetic clusters among Indian and Turkish hexaploid wheat population. Admixture model and correlated allele frequencies were used for assumed number of populations (K) ranging from 1 to 4. For each assumed population, 10 independent runs were implemented with burn-in period of 50000 and Markov Chain Monte Carlo (MCMC) replications, 100000. Utilizing the parameters depicted by Evanno *et al*. (2005), Structure Harvester v6.0 (Earl and vonHoldt, 2011) software program have been used for validating the optimal K value that signify the distinguishing groups.

Results

Amplification products of the 73 hexaploid wheat cultivars with 10 ISSR and 10 RAPD primers yielded a total of 96 and 75 scorable bands, out of which 74 and 45 bands were polymorphic respectively (Tables 2 and 3). The size of the amplification products ranged from 200 bp to 2000 bp (Fig. 1 and 2). Average

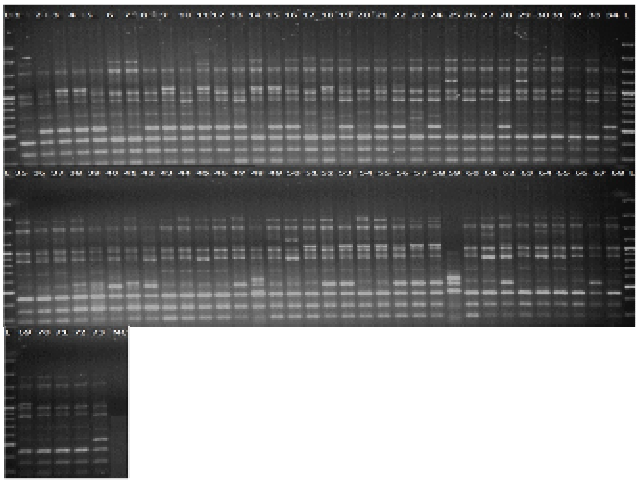

Fig. 1. ISSRM3 profile for 73 Indian and Turkish hexaploid wheat varieties. L = molecular size markers; Generuler 100 bp plus DNA Ladder. Lane numbers correspond to serial numbers in Table 1

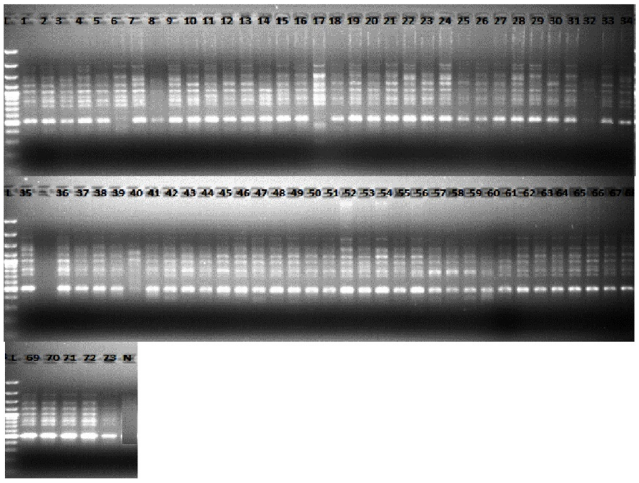

Fig. 2. RAPDB5 profile for 73 Indian and Turkish hexaploid wheat varieties. L = molecular size markers: Generuler 100 bp plus DNA Ladder. Lane numbers correspond to serial numbers in Table 1

Table 4. Analysis of Molecular Variance (AMOVA) in different Indian and Turkish hexaploid wheat populations

Source of Variation	Degree of Freedom	Square Sum	Variance Component	Percentage	Probability
Geographic Origin					
Among Pops	1	59.820	2.186	14%	P < 0.001
Within Pops	71	929.865	13.097	86%	

number of polymorphic bands per primer was found, 7.4 and 4.5 while percentage polymorphism was 77 and 60% in case of ISSR and RAPD bands, respectively (Tables 2 and 3).

Determining the genetic association among Indian and Turkish wheat population

Indian and Turkish wheat varieties have shown differentiated clustering in the combined ISSR and RAPD dendrogram on the basis of origin. As ISSR primers cover up the region among two microsatellites and RAPD is known to amplify entire genome, employing combined ISSR and RAPD polymorphic bands data will augment the legitimacy of both the relatedness among the genotypes and dendrogram. As per the origin countries, wheat varieties were basically divided into two clusters, cluster 1 was containing all the Indian hexaploid varieties and further, Cluster 2 possessing all the Turkish Hexaploid varieties (Fig. 3). Four Indian hexaploid wheat varieties 'HW2071', 'HW4024', 'HUW213' and 'PBW524' were separated as outgroups from the other hexaploid varieties. Mutual ISSR and RAPD data was examined on the basis of Simple Matching similarity coefficients that were obtained in the series of 0.39 to 0.99 and 0.72 to 0.96 among Indian and Turkish wheat varieties, respectively.

Analysis of Molecular Variance (AMOVA) is one of the most extensively employed configurations for the assessment of whole distribution of variation among and within populations. Less (14%) but greatly momentous ($P < 0.001$) genetic variations were obtained among Indian and Turkish hexaploid wheat populations featured to countries of origin. However, noteworthy variability (86%) was obtained within Indian and Turkish populations ($P < 0.001$) (Table 4).

Clustering of populations in to subgroups as per the geographical origin

Principal Coordinate Analysis (PCoA) represented two dimensional scaling of genotypes on the basis of phylogenetic similarity matrices and visualized the dispersion of genotypes on the basis of origin and justified the outliers obtained in dendrograms (Fig. 4). Two-dimensional plots showed that the first and second principal coordinate accounts for 9% and 8% of total variation, respectively. Indian and Turkish hexaploid cultivars clustered into separate groups justifying the grouping obtained from the dendrogram. The outgrouped accessions ('PBW524', 'HUW213', 'HW2071', 'HW4024') in the dendrogram were splitted in principal coordinate graph also. Turkish hexaploid wheat varieties, 'Karahan 99' and 'Ekiz' were clustered in close association with Indian hexaploid wheat varieties. As Indian wheat varieties, 'K7903'; 'K65'; 'K68' and 'K8027' shared a closer branch with Turkish wheat genotypes in the dendrogram, similarly, in PCoA analysis, these varieties were grouped with the Turkish wheat cluster. As varieties, 'PBW343' and 'PBW502' were present in the same branch of dendrogram, likewise they overlapped in coordinate graph.

STRUCTURE 2.3.4 software based on Bayesian clustering has been utilized to scrutinize genetic structure and relatedness of Indian and Turkish hexaploid wheat populations. Binary data producing 119 polymorphic ISSR and RAPD bands contributed towards the formation of distinct subpopulation clusters according to the origin countries. Probable number of clusters during analysis was set from 1 to 4, while ΔK value proposed by Evanno test justified $K = 2$ representing maximum log likelihood. Thus, presence of two clusters was noticed in hexaploid wheat population of the two geographical origins (Figs. 5 a,b,c).

The two genetically variant groups identified by STRUCTURE analysis were found in concurrence with the clusters distinguished in dendrogram and PCoA analysis. First group in red coloured partitions covered a major part of Indian population, whereas second group in green coloured partitions resemble most of the Turkish population and a considerable share of Indian population. However, a number of Indian and Turkish genotypes have revealed admixture grouping. Individuals within first and second clusters were anticipated as 24% and 25% heterozygous, respectively. Sixty Indian accessions showed 59% and 41% membership in first and second cluster respectively, whilst thirteen Turkish accessions have scored 10% and 90% participation in first and second cluster (Table 5).

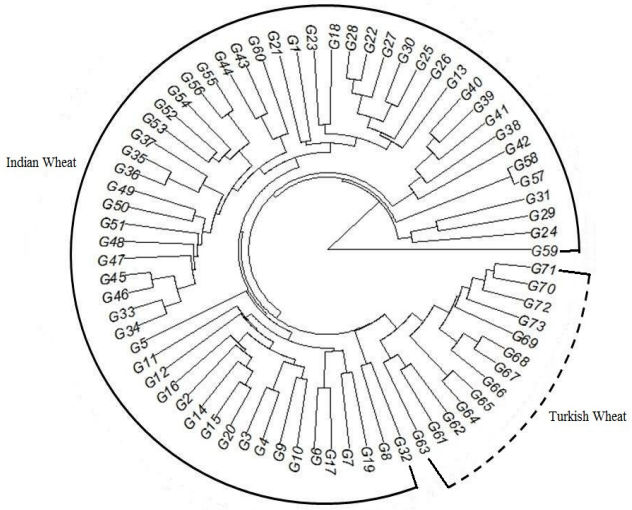

Fig. 3. Simple Matching Coefficient based dendrogram of 73 Indian and Turkish hexaploid wheat genotypes using NTSYS - PC and R software package

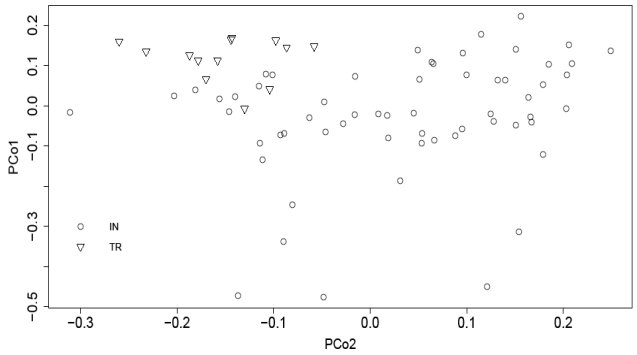

Fig. 4. Principal Coordinate Analysis of 73 Indian and Turkish wheat genotypes based on geographic origin of the genotypes

Table 5. Proportion of membership of each pre-defined population in each of the 2 clusters obtained from STRUCTURE analysis

Given Pop	Inferred Clusters		Number of Individuals
	1	2	
1	0.592	0.408	60
2	0.096	0.904	13

Discussion

In the 20th century, wheat research has been progressed to its peak for revealing its origin, variability, genetics, physiology, biotic and abiotic stresses and the strategies for its production augmentation through a number of crop improvement programs. Extent of genetic diversity is basically held responsible for the successful crop improvement strategy.

In order to elevate the understanding of wheat domestication and breeding for the efficient development of modern cultivars and to cope with the climate change and sustainable agriculture conditions, exploring the genetic diversity of hexaploid wheat cultivars is an important requisite. This study portrays genetic variation of hexaploid wheat genotypes from both the countries, India and Turkey, which are contributing significantly in fulfilling the wheat requirement of the world being 2nd and 10th producer, respectively employing ISSR and RAPD markers.

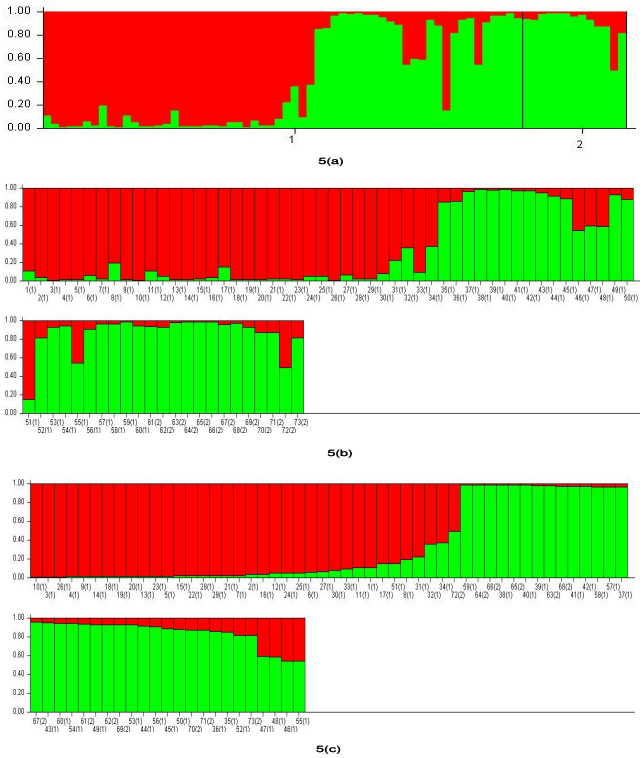

Fig. 5. (a) Two clusters obtained from population STRUCTURE analysis of 73 Indian and Turkish hexaploid wheat genotypes based on geographical origin; Red Zone consists of basically Indian varieties, Green Zone include both Indian and Turkish Varieties. (b) For the distinguishing clusters, vertical coordinates symbolize membership coefficients and every vertical line all along with the horizontal coordinate denotes individual genotypes. Numbers in the bracket indicate their major population group, India and Turkey (c) This outline signifies the compilation of genotypes on the basis of Q that elucidate the share of each individual genome that fit in two distinct clusters

Although a number of advanced marker systems, both dominant and codominant as well as high throughput marker systems have developed, ISSR and RAPD have still maintained its credibility in wheat diversity studies due to some specific features like reliability and reproducibility of ISSR and cost-effectiveness of RAPD. Several workers have advocated RAPD and ISSR markers to estimate the genetic divergence in hexaploid wheat from different regions of the world (Bhutta *et al*., 2006; Cao *et al*., 1998; Du *et al*., 2002; Freitas *et al*., 2000; Hao *et al*., 2006, Khan *et al*., 2010; Khavarinejad and Karimov, 2012; Malik *et al*., 2008; Maric *et al*., 2004; Mukhtar *et al*., 2002; Najaphy *et al*., 2011; Rehman *et al*., 2013).

As earlier mentioned, in this study, 60 and 77% average polymorphism was obtained using RAPD and ISSR primers, respectively across 73 hexaploid wheat cultivars which was in accordance with a number of earlier studies performed on hexaploid genotypes. We have obtained 4.5 polymorphic bands per primer in case of RAPD primers, while Joshi and Nguyen (1993) have obtained only 1.8 per primer with 65% polymorphism among 15 bread wheat varieties. Similarly, Mukhtar *et al*. (2002) observed 64.38% polymorphism among the selected 20 genotypes with 50 random primers, which is close to the presented experiment values whereas

Ahmed *et al*. (2010) assessed 32 advanced wheat breeding lines and 61.4% polymorphism was generated by 15 random decamer primers. In 2013, Fadoul *et al*., assessed the genetic diversity among locally cultivated Sudan's hexaploid wheat cultivars using 21 arbitrary 10-mer and found 65.5% polymorphism. In 2012, in a study performed by Cifci and Yagdi, on 16 Turkish bread wheat varieties using RAPD primers, genetic similarity ranged from 0.316 to 0.860, while in our study, varieties were found more similar with similarity coefficients in the series of 0.72-0.96.

Similar level of polymorphisms among wheat genotypes was also reported for ISSR based PCR marker analyses. Du *et al*. (2002) observed 87% polymorphism employing 11 ISSR primers for diversity assessment of 47 hybrid wheat. Malik *et al*. (2008) studied the genetic diversity in bread wheat (*Triticum aestivum* L.) varieties released for high yield, quality and abiotic stress in India and found 68.42% polymorphism using 20 UBC series ISSR markers. Najaphy *et al*. (2011) utilized 10 ISSR primers that generated 80.2% polymorphism among 30 wheat accessions. Genetic similarity data reported in this study confirmed the results from preceding researchers and can be further correlated with the agronomic and physiological responses of these varieties that may be supportive in the oblique assortment of efficient bread wheat genotypes (Liu *et al*., 2012). Although, single usage of RAPD marker system is questionable due to its sensitivity, its alliance with ISSR primers can produce more reliable outcomes and thus the close association among the Indian and Turkish wheat genotypes determined in the experiment can lead to secure genetic upgrading by developing distinct genotypes (Zarkti *et al*., 2010).

The cluster analysis done by combining RAPD and ISSR markers has revealed some interesting results as a number of varieties from same geographical origin were grouped together.

The information obtained from the dendrogram also exposed the parental information for a number of varieties and similarity percentage was also in accordance with the clustering data (Fig. 3). Hexaploid wheat variety 'PBW343' is parent for both, 'PBW502' and 'HD2985' possessing 99% and 74% similarity and so grouped with the two offsprings in same and separate branch, respectively. While varieties 'HD2781' and 'HD2888', shared the two common parents 'C306' and 'HW2004' with 81% and 87% similarity with the first one but these were grouped separately. Similarly, the hexaploid wheat varieties 'HUW510' and 'K9533' were clustered near to each other as these belong to one common parent 'HUW234' and both of these cultivars possess 88% similarity with it. Some of the varieties like 'K8434', 'K9107' and 'K9465' were clustered together in the similar branch as these belong to same parent, 'K68'. It was observed that these offspring cultivars were clustered together but these were far from their parent in UPGMA clustering. The genotypes like 'K7903' has parent 'K816' which was also included in the analysis and exhibited 82% similarity, but did not show the common clustering. Varieties like 'K8434' were clustered together with 'K8962' and possessed 89% similarity with one of the identical parent, 'HD2160'. As the Indian hexaploid varieties were split into two separate clusters, most of the parent varieties were present in one of the clusters from which both the Turkish varieties and rest of the Indian varieties group was emerged.

Cluster analysis and AMOVA results in the experiment were in accordance with each other revealing high genetic variation within Indian and Turkish hexaploid wheat samples in comparison to less but significant diversity between the two countries samples. It can be interpreted from the results that high within countries variations influenced the genetic association among countries that may be developed due to variant agricultural conditions and selective breeding.

The results of the two methods, cluster analysis and principle coordinate analysis were comparable. Both of them classified the 73 wheat genotypes in mainly 2 groups and presented similar grouping of the genotypes with some minor disagreements. Both of them clustered Indian varieties and all the Turkish varieties in separate groups. Some of the Indian varieties separated as outliers in Dendrogram were also far from all the genotypes in Principal Coordinate graph.

Bayesian clustering presented admixture between Indian and Turkish hexaploid wheat genotypes indicating either migration or interbreeding between the cultivars. Membership probability of Indian and Turkish populations obtained through STRUCTURE was found in accordance with UPGMA and PCoA clustering. When clustered according to the countries, significant genetic variation was apparent among the groups obtained. Elevated intensity of genetic diversity has been obtained in Indian cultivars may be because of their involvement in the breeding strategies throughout the world.

It has been well familiar that bread wheat is continuously losing its genetic diversity mainly due to the selection and repeated production of improved high yielding varieties by farmers. Present study providing an outline of the genetic diversity and population structure of Indian and Turkish hexaploid wheat will be helpful for the inclusion of these varieties in different breeding programs around the world for attaining utmost diversity. Utilizing this data, accessions with advantageous characteristics and genetically distant from each other can be selected for selective and diverse breeding.

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