Genetic diversity and population structure of wheat in India and Turkey

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Abstract. Genetic diversity among plant species offers prospects for improving the plant characteristics. Its assessment is necessary to help tackle the threats of environmental fluctuations and for the effective exploitation of genetic resources in breeding programmes. Although wheat is one of the most thoroughly studied crops in terms of genetic polymorphism studies, phylogenetic affinities of Indian and Turkish Triticum species have not been assessed to date. In this study, genetic association of 95 tetraploid and hexaploid wheat genotypes originating from India and Turkey was determined for the first time. Combined analysis of random amplified polymorphic DNA and inter-simple sequence repeat markers disclosed 177 polymorphic bands, and both the dendrogram and two-dimensional scatterplot showed similar groupings of the wheat genotypes. Turkish hexaploid varieties were basically divided into two clusters, one group showed its close association with Indian hexaploid varieties and the other with Indian tetraploid varieties. Analysis of molecular variance revealed high (77 %) genetic variation within Indian and Turkish populations. Population structure analysis elucidated distinct clustering of wheat genotypes on the basis of both geographical origin and ploidy. The results revealed in this study will support worldwide wheat breeding programmes and assist in achieving the target of sustainable wheat production.

Keywords: Genetic diversity; molecular markers; ploidy level; population structure; wheat.

Introduction

Genetic variability in natural plant populations holds the potential to deal with multiple biotic and abiotic stresses. The potential to select a superior line increases with genetic diversity, the discovery of which becomes an important tool in plant breeding. On depletion of genetic variability, plants are unable to cope with unfavourable environmental conditions or pathogens and pests. Diversity studies also facilitate the conservation and management aims of a particular plant species. For the effective use of genetic diversity in plant breeding, knowledge of its extent and distribution plays a crucial role. Considering its

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significance, a large number of studies have been performed to estimate genetic diversity employing various methodologies in multiple plant species. Assessment of genetic variability employing molecular markers has proved to be a keystone to understanding the genomic constitution, categorizing the genes responsible for important traits, the classification and conservation of genetic variation in plant germplasm and developing selective proliferation approaches for plant propagation.

Wheat production in developing countries moved from defective to surplus (Pingali 2012) during the Green Revolution (Gollin et al. 2005). Being a good source of carbohydrate, protein, sugar, fat, fibre and minerals, it provides half of the energy requirements of the human population (Simmonds 1989; Shewry 2007; Topping 2007). A constantly rising population demands an increment in wheat production (Ehrlich 1975; Evans 1998; Tilman et al. 2011; Ray et al. 2013). As the crop already covers a wide agricultural area, there is a negligible possibility of area expansion (Young 1999; Bruinsma 2003; Cassman et al. 2003). Wheat faces multiple demands including its growth under warmer conditions (Vermeulen et al. 2012; Wheeler 2012), fighting various diseases (Summers and Brown 2013), reduced energy input for sustainable growth (Ziaei et al. 2015) and high nutritional quality (Shewry 2007, 2009). Considering this situation, Lynch (2007) has suggested a need for a ‘second green revolution’. This second green revolution must place emphasis on the utilization of inherent resources and the thorough understanding of genetic diversity.

During the course of evolution, wheat gained sufficient genetic diversity along the road from einkorn to bread wheat. Today, however, its diversity is weakening due to repeated cultivation of landraces for specific characters, narrow adaptation, farmers’ varietal selection and the requirement of uniform varieties in industrial seed grain processing (Bellon 1996; Smale 1997; Heal et al. 2010). Implementation of high-yielding commercial varieties is responsible for maintaining its genetic diversity. In a review, Wendel (2000) shed light on several aspects of the genome duplication and divergence leading to the development of evolutionary genetic diversity. Polyploidy resulting from hybridization leads to gene duplication across the entire genome and thus underlies the emergence of genetic variation. The agriculturally important phenomenon of hybrid vigour in polyploids is a consequence of genetic variability. As wheat is a polyploid species, it is beneficial to include tetraploid and hexaploid varieties in genetic variability assessment programmes. Such assessment programmes are imperative for managing populations by identifying the breeding genotypes. However, due to low reproducibility of the RAPD system, molecular marker techniques vary from each other in data generation efficiency and the genome area covered in the study. Selection of the type of marker tool for a study depends on the target crop and the issue. For example, random amplified polymorphic DNA (RAPD) markers are known for their simplicity, cost efficiency, fast polymorphism assessment, no prior information of DNA sequences being required and extensive coverage of the intact genome being possible. However, due to low reproducibility of the RAPD system, expense of the amplified fragment length polymorphism (AFLP) marker system and requirement of prior information about DNA sequences in SSR analysis, another dominant marker system, inter-simple sequence repeats (ISSRs), was included in the study. Due to high annealing temperature and extended sequence in comparison to RAPD markers, ISSR primers can produce more reproducible and reliable band patterns. Inter-simple sequence repeat markers are employed for distinguishing DNA on the basis of single base variation or insertions and deletions, and are equivalent to the SSR system in reproducibility. These markers are widely implemented for DNA fingerprinting, identification of species association, genetic variability studies and for recognizing the geographic origin of different plant species along with their ploidy.
| Sl. no. | Name of genotype | Genotype number | Ploidy | Origin |
|--------|------------------|----------------|--------|--------|
| 30     | KR-8             | G1             | 6X     | India  |
| 2      | AAI_2            | G2             | 6X     | India  |
| 3      | AKAW_4006        | G3             | 6X     | India  |
| 4      | AKDW_2997        | G4             | 4X     | India  |
| 5      | DBW_14           | G5             | 6X     | India  |
| 6      | DBW_39           | G6             | 6X     | India  |
| 7      | DDK_1025         | G7             | 6X     | India  |
| 8      | DT_132           | G8             | 4X     | India  |
| 9      | GW_03-12         | G9             | 6X     | India  |
| 10     | GW_03-2          | G10            | 6X     | India  |
| 11     | GW_03-3          | G11            | 6X     | India  |
| 12     | GW_03-4          | G12            | 6X     | India  |
| 13     | GW_03-9          | G13            | 6X     | India  |
| 14     | HD_2177          | G14            | 6X     | India  |
| 15     | HD_2236          | G15            | 6X     | India  |
| 16     | HD_2270          | G16            | 6X     | India  |
| 17     | HD_2307          | G17            | 6X     | India  |
| 18     | HD_2329          | G18            | 6X     | India  |
| 19     | HD_2380          | G19            | 6X     | India  |
| 20     | HD_2402          | G20            | 6X     | India  |
| 21     | HD_2501          | G21            | 6X     | India  |
| 22     | HD_2643          | G22            | 6X     | India  |
| 23     | HD_2881          | G23            | 6X     | India  |
| 24     | HUW_12           | G24            | 6X     | India  |
| 25     | HUW_251          | G25            | 6X     | India  |
| 26     | HUW_37           | G26            | 6X     | India  |
| 27     | HUW_468          | G27            | 6X     | India  |
| 28     | HUW_533          | G28            | 6X     | India  |
| 29     | HUW_55           | G29            | 6X     | India  |
| 30     | K_01006          | G30            | 6X     | India  |
| 31     | K_0204           | G31            | 6X     | India  |
| 32     | K_616            | G32            | 6X     | India  |
| 33     | K_8020           | G33            | 6X     | India  |
| 34     | K_86             | G34            | 6X     | India  |
| 35     | K_88             | G35            | 6X     | India  |
| 36     | K_911            | G36            | 6X     | India  |
| 37     | KALYANSONA       | G37            | 6X     | India  |
| 38     | KBD_65           | G38            | 4X     | India  |

**Table 1. Continued**

| Sl. no. | Name of genotype | Genotype number | Ploidy | Origin |
|---------|------------------|----------------|--------|--------|
| 39      | KBD_821          | G39            | 4X     | India  |
| 40      | KBD_921          | G40            | 4X     | India  |
| 41      | KBD_922          | G41            | 4X     | India  |
| 42      | KBD_925          | G42            | 4X     | India  |
| 43      | KBD_9452         | G43            | 4X     | India  |
| 44      | KBD_9915         | G44            | 4X     | India  |
| 45      | KD_9851          | G45            | 4X     | India  |
| 46      | KLP_306          | G46            | 6X     | India  |
| 47      | KLP_307          | G47            | 6X     | India  |
| 48      | KLPD_1106        | G48            | 4X     | India  |
| 49      | NAW_1448         | G49            | 6X     | India  |
| 50      | NIDW_295         | G50            | 4X     | India  |
| 51      | NW_1076          | G51            | 6X     | India  |
| 52      | NW_2036          | G52            | 6X     | India  |
| 53      | PBW_550          | G53            | 6X     | India  |
| 54      | RAJ_1482         | G54            | 6X     | India  |
| 55      | RAJ_1555         | G55            | 4X     | India  |
| 56      | RAJ_3072         | G56            | 6X     | India  |
| 57      | RAJ_3077         | G57            | 6X     | India  |
| 58      | RAJ_3777         | G58            | 6X     | India  |
| 59      | RAJ_4027         | G59            | 6X     | India  |
| 60      | RAJ_4037         | G60            | 6X     | India  |
| 61      | RAJ_4120         | G61            | 6X     | India  |
| 62      | RAJ_6560         | G62            | 4X     | India  |
| 63      | RD_1008          | G63            | 4X     | India  |
| 64      | RD_1063          | G64            | 4X     | India  |
| 65      | RD_1093          | G65            | 4X     | India  |
| 66      | RD_1097          | G66            | 4X     | India  |
| 67      | SAW_327          | G67            | 6X     | India  |
| 68      | SAW_337          | G68            | 6X     | India  |
| 69      | SAW_94           | G69            | 6X     | India  |
| 70      | SONALIKA         | G70            | 6X     | India  |
| 71      | UP_2338          | G71            | 6X     | India  |
| 72      | UP_2511          | G72            | 6X     | India  |
| 73      | UP_2525          | G73            | 6X     | India  |
| 74      | UP_2696          | G74            | 6X     | India  |
| 75      | VEERI            | G75            | 6X     | India  |
| 76      | VL_832           | G76            | 6X     | India  |
two developing countries would be of immense benefit to wheat improvement programmes.

Association and contrast among the wheat cultivars from different countries can provide a useful overview on the evolutionary record of the genotypes and, hence, can facilitate the reach of breeding improvement. Although a number of genetic similarity studies were conducted on diverse wheat germplasm using ISSR and RAPD marker systems, phylogenetic association of Indian and Turkish Triticum species has not been documented to date. The present study represents the first effort in this direction, its objectives being to gain a better understanding of the genetic association and population structure of Indian and Turkish wheat on the basis of both geographical origin and ploidy. The share of the genetic variations within and among populations was also revealed so that the information provided can be effectively used by scientists for the development of genetically diverse, promising and healthier wheat varieties.

Methods

Study materials

The object of the present diversity study was a collection of 95 Indian and Turkish wheat genotypes including tetraploid (Triticum turgidum ssp. durum) and hexaploid (Triticum aestivum L.) wheat cultivars (Table 1). Well-known varieties were chosen for the experiment to facilitate the use of results in future breeding programmes.

Plant genomic DNA extraction

Two to three weeks grown seedlings were utilized for total wheat DNA extraction following the cetyltrimethylammonium bromide (CTAB) method (Doyle 1990) with some modifications. Initially, cells were disrupted and purified with 2 % CTAB buffer and 10 μL RNase A, respectively, followed by incubation at 65 °C. This was followed by protein extraction employing phenol : chloroform : isoamyl alcohol and finally the CTAB–DNA complex was precipitated with isopropanol. The DNA pellet was twice washed with 70 % ethanol, dried and ultimately, dissolved in 1 % agarose gel electrophoresis, respectively. The DNA quality were verified using spectrophotometry and a concentration of 50 ng μL−1 as templates for polymerase chain reactions (PCRs).

Inter-simple sequence repeats analysis

Twenty-seven ISSR primers (Metabion) were examined as templates for polymerase chain reactions (PCRs). Those 10 primers showed positive outcomes (Table 2) against chosen wheat varieties. Every PCR mixture of 25 μL contained 2.5 μL of 10× Taq buffer containing ammonium

| Sl. no. | Name of genotype | Genotype number | Ploidy | Origin |
|--------|-----------------|----------------|--------|--------|
| 77     | WR_1381         | G77            | 6X     | India  |
| 78     | WR_1408         | G78            | 6X     | India  |
| 79     | WR_1421         | G79            | 6X     | Turkey |
| 80     | BAYRAKTAR 2000  | G80            | 6X     | Turkey |
| 81     | SEVAL           | G81            | 6X     | Turkey |
| 82     | KENANBEY        | G82            | 6X     | Turkey |
| 83     | BEZOSTAJA 1     | G83            | 6X     | Turkey |
| 84     | GÜN_91          | G84            | 6X     | Turkey |
| 85     | KONYA_2002      | G85            | 6X     | Turkey |
| 86     | AKBUĞDAY        | G86            | 6X     | Turkey |
| 87     | GEREK_79        | G87            | 6X     | Turkey |
| 88     | KIRAC_66        | G88            | 6X     | Turkey |
| 89     | ESER            | G89            | 6X     | Turkey |
| 90     | SÖNMEZ 2001     | G90            | 6X     | Turkey |
| 91     | HARMANKAYA 99   | G91            | 6X     | Turkey |
| 92     | KINACI 97       | G92            | 6X     | Turkey |
| 93     | YUREGİR 89      | G93            | 6X     | Turkey |
| 94     | ALTAY 2000      | G94            | 6X     | Turkey |
| 95     | LÜTFIBEY        | G95            | 6X     | Turkey |
sulfate (except ISSR F3 where KCl was used), 3 μL of 25 mM MgCl₂, 0.4 μL of 25 mM dNTP, 0.5 μL of 10 μM primer, 1.5 units of Taq DNA Polymerase and 100 ng of template DNA. The two-step ISSR–PCR reactions were performed in an Eppendorf Master Cycler. The physical reaction conditions and the number of initial and final PCR cycles were optimized for each individual ISSR primer.

**Random amplified polymorphic DNA analysis**

For RAPD reactions, a total of 43 primers (MWG Biotech-AC) were screened for polymorphism using selected genotypes, and 10 primers were selected for the final reactions (Table 3). The PCR mixture contained 1.5 μL of 10× Taq buffer with ammonium sulfate, 2.5 μL of 1 mM dNTP, 3 μL of 1 mM dNTP, 3 units Taq DNA polymerase (Thermo-scientific), 1.5 μL of 5 μM RAPD primer and 50 ng of template DNA in a total volume of 15 μL. Polymerase chain reaction amplifications were carried out utilizing an Eppendorf Master Cycler with initial denaturation at 94 °C for 3 min, followed by repeated cycles of denaturation at 94 °C for 45 s, annealing as per the primer’s melting temperature for 1 min and primer extension at 72 °C for 1 min. On completion of the repeated number of cycles, final extension was performed at 72 °C for 10 min.

**Data analysis**

All the ISSR- and RAPD-based PCRs were repeated three times for the identification of reproducible amplified

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**Table 2.** Characteristics and polymorphism revealed by ISSR primers for 95 wheat genotypes used in the study.

| ISSR primer | Sequence      | Melting temperature (Tₘ) | Total number of bands | Polymorphic bands | Per cent polymorphism detected |
|-------------|---------------|--------------------------|-----------------------|-------------------|--------------------------------|
| ISSR F3     | 5′-(AG)₆ CG-3′ | 56.0                     | 8                     | 7                 | 87.5                           |
| ISSR F4     | 5′-(AG)₆ TG-3′ | 53.7                     | 12                    | 12                | 100                            |
| ISSR F9     | 5′-(GAA)₄ G-3′ | 39.6                     | 13                    | 12                | 92.3                           |
| ISSR M1     | 5′-(AGC)₆ G-3′ | 63.1                     | 12                    | 11                | 91.6                           |
| ISSR M2     | 5′-(ACC)₆ G-3′ | 63.1                     | 14                    | 14                | 100                            |
| ISSR M3     | 5′-(AGC)₆ C-3′ | 63.1                     | 17                    | 16                | 94.1                           |
| ISSR M8     | 5′-(AC)₆ G-3′  | 56.7                     | 13                    | 12                | 92.3                           |
| ISSR M9     | 5′-(AC)₆ CG-3′ | 56.0                     | 13                    | 13                | 100                            |
| ISSR M12    | 5′-(GACAC)₄ C-3′ | 61.4                     | 6                     | 6                 | 100                            |
| ISSR M17    | 5′-CAG (CA)₈ C-3′ | 56.7                     | 8                     | 7                 | 87.5                           |
| **Total**   |                |                          | **116**               | **110**           | **94.8**                       |

**Table 3.** Characteristics and polymorphism revealed by RAPD primers for 95 wheat genotypes used in the study.

| RAPD primer | Sequence      | Melting temperature (Tₘ) | Total number of bands | Polymorphic bands | Per cent polymorphism detected |
|-------------|---------------|--------------------------|-----------------------|-------------------|--------------------------------|
| cRAPD1      | 5′-GAA ACG GGT G-3′ | 32                      | 6                     | 4                 | 66.6                           |
| cRAPD2      | 5′-GTG ACG TAG G-3′ | 32                      | 12                    | 11                | 91.6                           |
| RAPD B3     | 5′-GTG ACG TAG G-3′ | 34                      | 9                     | 7                 | 77.7                           |
| RAPD B4     | 5′-CTC ACC GTC C-3′ | 34                      | 6                     | 5                 | 83.3                           |
| RAPD B5     | 5′-GAC GGA TCA G-3′ | 32                      | 11                    | 10                | 90.9                           |
| RAPD B10    | 5′-CTA CTG CGC T-3′ | 32                      | 7                     | 6                 | 85.7                           |
| RAPD B13    | 5′-TTC AGG GTG G-3′ | 32                      | 5                     | 3                 | 60.0                           |
| RAPD L2     | 5′-GTT TCG CTC C-3′ | 32                      | 8                     | 7                 | 87.5                           |
| RAPD L4     | 5′-AAG AGC CCG T-3′ | 32                      | 11                    | 9                 | 81.8                           |
| RAPD L6     | 5′-CCC GTC AGC A-3′ | 34                      | 7                     | 5                 | 71.4                           |
| **Total**   |                |                          | **82**                | **67**            | **81.7**                       |
bands. Amplified fragments were counted from smaller to larger size. A binary data matrix was obtained by scoring the gel as 1 and 0 to show the presence and absence of bands, respectively. Information capacity of the primers and polymorphism content of the genotypes were estimated by calculating the total number of bands and of polymorphic bands. The binary data matrix was used to obtain the similarity matrix depending on simple matching (SM) coefficient by Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC) version 2.02e software (Rohlf 1997). This similarity matrix was utilized in R software for constructing a combined dendrogram of RAPD and ISSR.

On the basis of SM coefficients, the similarity matrix was double centred using the DCENTER module of NTSYS-PC. Then eigen analyses were performed using the EIGEN module of NTSYS-PC to construct two-dimensional scatterplots by the R package. Scatterplots were drawn for the substantiation of the dendrograms and verification of genotypes clustering according to both ploidy and geographical origin.

To explain the population structure of Indian and Turkish wheat genotypes, analysis of molecular variance (AMOVA) was performed using GenAlEx 6.5 software (Peakall and Smouse 2006, 2012) with 1000 permutations. The programme was used for the determination of variance components and estimating the total variation within and among the populations.

Bayesian model-based clustering with assumed K populations was employed for genetically homogenous group estimation in Indian and Turkish wheat germplasm. A parameter of 50 000 burn-in period and 100 000 Markov Chain Monte Carlo replication, along with the admixture model and correlated allele frequencies, was used in STRUCTURE, version 2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009). A total of 10 independent runs were performed for each value of K (from 1 to 4 assumed) (Evanno et al. 2005). For the determination of the best possible K value elucidating the genetically distinctive clusters in the data, the Structure Harvester v6.0 (Earl and vonHoldt 2012) programme was used implementing parameters described by Evanno et al. (2005).

Results

Genetic diversity

Ninety-five Indian and Turkish wheat varieties were amplified using 43 and 27 ISSR and RAPD markers, respectively. The 10 most polymorphic ISSR and RAPD primers generated 116 and 82 genetic loci, respectively, with a total of 198 loci. Among ISSR primers, ISSR M3 generated the maximum number of polymorphic fragments (16) and cRAPD2 was the most prolific RAPD primer (11). In total, 94.8 and 81.7 % bands were found to be polymorphic among ISSR and RAPD markers. The average number of polymorphic bands per primer was 11.0 and 6.7 for ISSR and RAPD primers, respectively (Tables 2 and 3). For both primer types, the main amplified region was in the range of 300–2000 bp (Figs 1 and 2).

Genetic relationships/association

A Fan dendrogram of the combined RAPD and ISSR data showed clear groupings of genotypes on the basis of both ploidy and origin (Fig. 3). On combining both RAPD and ISSR data, individual errors of either marker system are reduced and combined the dendrogram provided a more robust overview of the relatedness of Indian and Turkish populations.

On the basis of ploidy, wheat varieties were divided into three clusters, containing 18 tetraploid and 77 hexaploid varieties. Among hexaploid varieties, two Indian genotypes, NW 2036 and RAJ 4027, were separated as outliers from the rest. However, all the hexaploid genotypes were basically divided into two groups, and six Turkish genotypes were clustered with the Indian Hexaploid group. Similarity coefficients among Indian hexaploid varieties ranged from 0.71 to 0.98 while among Turkish hexaploid varieties ranged from 0.42 to 0.95.

Furthermore, the molecular variance factor in both Indian and Turkish populations was compared as a further measure of genetic diversity. Results from AMOVA for geographical origin indicated 77 % genetic variation within populations, while the variation between the populations was 23 % (PhiPT = 0.232; P = 0.010). On the basis of ploidy, AMOVA detected higher genetic variation within tetraploid and hexaploid populations (92 %); however, the genetic variation between ploidies was only 8 % (PhiPT = 0.078; P = 0.010) (Table 4).

Population structure

Principal coordinate analysis (PCoA) serves as a platform to provide a spatial illustration of the comparative genetic distances between the individuals. It also assesses the robustness of the differentiation among the groups classified by the dendrogram (Liu et al. 2013). In our scatterplots, the first two principal components explained 17.6 and 10.7 % of the total variation, respectively. In accordance with the dendrogram, hexaploid individuals were clearly separated from tetraploid varieties by the first principal coordinate (Fig. 4A). Similarly, the second principal coordinate (10.71 % of total variation) divided the Turkish populations from the Indian ones (Fig. 4B).

For population genetic structure analysis, Bayesian clustering modelling was executed in the STRUCTURE software using genotyping data generated by 177 RAPD
and ISSR loci. As the clustering model presumes the underlying existence of $K$ clusters, an Evano test was performed and yielded $K = 3$ as the highest log-likelihood. This means that 3 was the optimum number of subpopulations, indicating that the two major population groups actually represent three distinct clusters.

The analysis of structure according to the geographical origin was performed by setting the range of possible number of subpopulations ($K$) from 1 to 4. Indian and Turkish populations involved in this procedure showed separation from each other in accordance with clusters obtained in PCoA. At $K = 3$, wheat genotypes were divided into three clusters with two main populations, 1 and 2 (Fig. 5A) representing Indian and Turkish wheat gene pool, respectively. Red colour bars represent individuals belonging to the Indian wheat gene pool while those in green belong to the Turkish gene pool. The Indian wheat gene pool was again distributed into subclusters with blue representing the tetraploid wheat population (Fig. 5B). The Indian population consisted of 79 accessions, of which 72 % belonged to the first cluster, 4 % to the second cluster and 24 % to the third; whereas the Turkish group consisted of 16 accessions with 13, 86 and 1 % belonging to the first, second and third cluster, respectively (Table 5). Some of the Indian and Turkish hexaploid genotypes, including NW_2036, RAJ_4027, Bayraktar_2000, Seval, Gün_91, Konya_2002, showed admixture clustering (Fig. 5C). Within the first, second and third clusters, expected heterozygosity within individuals was found to be 0.18, 0.15 and 0.16, respectively.

**Discussion**

The complex nature and huge size of the wheat genome pose serious challenges towards genetic means of increasing its production. Hence, furthering our understanding of
the wheat genome utilizing a variety of analyses has assisted efforts towards the genetic improvement of modern cultivars. Our examination of the literature to date found no prior genotypic characterization of the Indian and Turkish wheat varieties, simultaneously using RAPD and ISSR markers (Khan et al. 2014). The present study constitutes the first attempt to better understand jointly the origin, evolution and molecular diversity of Indian and Turkish wheat varieties at different ploidy levels.

Evaluating genetic diversity in Indian and Turkish wheat
Since their introduction, ISSR and RAPD markers have been broadly utilized for variability estimation of wheat genotypes. Several RAPD- and ISSR-based diversity studies including diploid, tetraploid and hexaploid wheat have been published (Castagna et al. 1997; Nagaoka and Ogihara 1997; Pujar et al. 1999, 2002; Barcaccia et al. 2002; Teshale et al. 2003; Mantzavinou et al. 2005; Thomas et al. 2006; Aliyev et al. 2007; Grewal et al. 2007; Anand et al. 2008; Cenkci et al. 2008; Sawalha et al. 2008; Tahir 2008; Carvalho et al. 2009; Pandey et al. 2012). Due to the possession of diverse (A, B, D) genomes of wheat, tetraploid and hexaploid varieties were involved in the study.

Although Indian and Turkish wheat germplasms were not simultaneously used earlier, the average RAPD- and ISSR primer-based polymorphism, 81.7 and 94.8 %, respectively, revealed in this study, were comparable with several prior diversity studies. The very first attempt made by other researchers among Indian tetraploid wheat varieties revealed high genetic variability in durum released cultivars (50.6 %) in comparison to landraces (44.8 %) (Pujar et al. 1999). Teshale et al. (2003) found 79.6 % polymorphism among 27 tetra- and hexaploid Indian genotypes using RAPD markers. A detailed study on 96 commercial Indian wheat genotypes, including tetraploids, triticale and hexaploids, indicating 78.8 % polymorphism, revealed a narrow genetic base of tetraploid cultivars in comparison to hexaploids (Thomas et al. 2006).

The similarity coefficient values range among Indian hexaploid varieties observed in our study (0.71–0.98)
was found to be higher than that in a previous study by Grewal et al. (2007) (0.52–0.82) using RAPD markers. In the present work, the average count of polymorphic bands per primer was higher in the case of ISSR (11) compared with that in RAPD (6.7). These results were consistent with a previous study by Pujar et al. (2002) on Indian tetraploid wheat varieties. Although limited studies have been performed on diversity assessment of Turkish wheat, Akar and Ozgen (2007) assessed the genetic variability of 100 durum wheat varieties using RAPD markers and observed higher genetic diversity in landraces than in cultivars. Cifci and Yagdi (2012) distinguished 16 Turkish bread wheat varieties using RAPD markers with product size in the range of 300–2800 bp, which was similar to our results.

### Analysis of genetic relationships among wheat genotypes

The dendrogram obtained in this study clearly clustered the genotypes according to their ploidy level, consistently with the evolution of wheat (Alamerew et al. 2004). Furthermore, Indian and Turkish varieties were grouped separately. The information revealed by the dendrogram highlighted the parentage association of the varieties. Varieties HD_2177 and HD_2329 grouped together in the dendrogram with 95 % similarity share three common parents, HD_1962, E_4870, K_65. HD_2402 also grouped with its parent variety HD_2236 and showed 96 % similarity. Varieties Raj_1482, Raj_3072 and Raj_3077 were clustered together. Within this cluster, Raj_1482 is the parent

### Table 4. Analysis of molecular variance in Indian and Turkish wheat populations.

| Source of variation | df | Square sum | Variance component | Percentage | Probability |
|---------------------|----|------------|--------------------|------------|-------------|
| Geographic origin   |    |            |                    |            |             |
| Among populations   | 1  | 133.52     | 4.46               | 23         | <0.001      |
| Within populations  | 93 | 1370.776   | 14.74              | 77         |             |
| Ploidy              |    |            |                    |            |             |
| Among populations   | 1  | 54.21      | 1.32               | 8          | <0.001      |
| Within populations  | 93 | 1450.09    | 15.59              | 92         |             |

Figure 3. Simple matching coefficient-based Fan dendrogram using NTSYS-PC and R software package of 95 Indian and Turkish wheat genotypes.

Figure 3.
of Raj_3077, with 93 % similarity. HD_2307 and HD_2501, which were grouped separately from other ‘HD’ varieties, share as a common parent HD_2160 and consistently exhibited 93 % similarity. Not only hexaploids, but also some of the tetraploid varieties like AKDW_2997 were also allocated in the same subcluster with its parent Raj_1555 and showed 88 % similarity (Fig. 3).

Analysis of molecular variance results disclosed in the study were in agreement with the UPGMA clustering and supported a high level of diversity within-country samples. Although the variation between Indian and Turkish populations was lower in comparison to within-population variation, it was significant according to the partitioning value ($P = 0.010$) (Table 4). The results suggest that similarity association between the countries was affected by within-country inconsistencies of the varieties. This high variation within groups can be attributed to selective adaptation towards the growth conditions at the time of breeding.

**Investigating the Indian and Turkish wheat population structure**

Similar separation of Indian and Turkish wheat varieties was observed by PCoA on the basis of ploidy and geographical region. The outcomes of the two methods (cluster analysis and PCoA) were comparable. Both of them classified 95 wheat genotypes mainly into three clusters and offered similar alignment of the genotypes with a few negligible discrepancies. The groups attained were in agreement with the recognized geographical origin as well.

Population structure analyses indicated that wheat accessions can be efficiently categorized on the basis of both geographical origin and ploidy. Using the maximum membership probability in STRUCTURE, Indian and Turkish populations showed similar grouping to the UPGMA and PCoA clustering. The PCoA clustering divided Indian and Turkish populations basically into similar clusters to those produced by the Structure bar plot at $K = 3$. In PCoA, some of the Indian and Turkish varieties showed close association with each other, and similar varieties demonstrated admixture in Structure analysis confirming their relatedness within the diverse gene pool. In dendrogram also, these varieties showed distinct clustering with the main population groups. Closeness of some of the Turkish hexaploid genotypes with Indian hexaploid genotypes in PCoA was also supported by the population structure as well as the dendrogram. Similar and mutually supportive results from all the statistical analyses demonstrated the capability of RAPD and ISSR markers to distinguish Indian and Turkish wheat varieties efficiently.

**Conclusions**

Genetic diversity evaluation serves as a crucial platform in plant improvement. The present study provides a detailed understanding of the genetic association of Indian and Turkish hexaploid and tetraploid wheat. The Turkish hexaploid populations showed their closeness to Indian genotypes, confirming their alliance within the diverse gene pool. The present genetic diversity study of wheat
material obtained from diverse regions will support breeders in expanding the genetic variation of breeding accessions and utilizing the studied wheat resources more effectively.

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Contributions by the Authors
M.K.K. initiated and obtained the funding for the research work. M.K.K. and A.P. contributed in performing the research work and preparation of the manuscript. M.K.K.,

| Given population | Inferred clusters | Number of individuals |
|------------------|------------------|----------------------|
|                  | 1                | 2                    | 3                    |
| 1                | 0.720            | 0.039                | 0.241                |
| 2                | 0.133            | 0.857                | 0.009                |

Table 5. Proportion of membership of each pre-defined population in each of the three clusters obtained from STRUCTURE analysis.
A.P., S.A.K. and Y.O. carried out all statistical analyses. All other authors have provided suggestions and guidance for the successful completion of the work. All authors read and approved the final manuscript.

Conflicts of Interest Statement
None declared.

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