Full Length Article

Phylogenetic and Taxonomic Relationship of Turkish Wild and Cultivated Emmer (*Triticum turgidum* ssp. *dicoccoides*) Revealed by iPBS-Retrotransposons Markers

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Abstract

Wild emmer (*Triticum turgidum* ssp. *dicoccoides*) is the progenitor of cultivated wheat. Turkey is the main center of wheat and plays a vital role in the spread of various crops among the continents. Karacadağ region is considered as the domestication center of wheat and still, hundreds of landraces are prevalent. A total of 29 wild emmer landraces, 4 cultivated emmer wheat (*T. turgidum* ssp. *dicoccum*) and five durum wheat (*T. turgidum* ssp. *durum*) cultivars were investigated for the genetic diversity and phylogenetic relationship using the iPBS-retrotransposons markers. Mean polymorphism and polymorphic information contents (PIC) were 87.85% and 0.660, respectively. Mean effective numbers of alleles (1.961), Shannon's Information Index (0.682) and gene diversity (0.489) reflected the occurrence of a great level of variations. T17 and Chermik-1 genotypes were found much distinct and breeding valuable genotypes for wheat breeding. The unweighted pair-group method with arithmetic means (UPGMA) divided all genotypes by their genetic makeup and geographical locations. Among 3 species, UPGMA based clustering clearly separated the durum wheat from wild emmer and cultivated emmer wheat. Results are clearly supported by the principal coordinate analysis (PCoA) and model-based structure algorithm. Information provided herein comprehensively reflected the power of iPBS-retrotransposons for the diversity and phylogenetic relationship investigation and reflected that this marker system can be effectively applied to investigate phylogenetic and taxonomic relationship in any crop due to its universal nature. © 2019 Friends Science Publishers

Keywords: Wild emmer; Tetraploid wheat; Genetic diversity; iPBS-retrotransposons

Introduction

Wheat (*Triticum* spp.) is one of the most ancient and an important cereal crop for different countries of the world (Enghiad *et al*., 2017) and taken among the founder crops for the old world agriculture (Weiss and Zohary, 2011). It ranks 1st due to its production and cultivation area among the cereal crops (Reynolds *et al*., 2010). Domestication is a very complex and important process which involves the modification of wild plants into a crop (Gross and Olsen, 2010). Domestication has dramatically promoted human cultural development and considered as an important factor underlying human civilization (Peng *et al*., 2011a, b). Wheat is 1st crop to be domesticated between 12,000 and 10,000 years ago in the Fertile Crescent. 95% of the world wheat

production comes from bread wheat (*T. aestivum* L.) and the rest comes from durum wheat (Peng *et al*., 2012). Mediterranean basin, India, Pakistan, north-central China, southwestern Australia, Argentina, central plains of the US and southern Russia are the main wheat producing regions of the world (Peng *et al*., 2011a). Present day wheat cultivars fall into two groups: (I) tetraploid wheat (*T. turgidum* ssp. *durum*: 2n = 4x = 28, AABB) and (II) hexaploid (*T. aestivum*: 2n = 6x = 42, AABBDD) (Özkan *et al*., 2011). Studies have confirmed three hybridization events in the bread wheat during its evolution (Marcussen *et al*., 2014). Following the divergence of the *Triticum* and *Aegilops* lineages from a common ancestor ~6.5 million ago, these events which were involved in the lineage of A and B genome finally resulted in the formation of diploid D-

genome wheat progenitor *Aegilops tauschii* (2n = 2x = 14, DD genome). The second event occurred back to a few hundred thousand years ago, which led to the development of tetraploid AABB genome of *Triticum turgidum*. This tetraploid genome formed as the result of a hybridization event between *Triticum urartu* (A genome) and *Aegilops speltoides* (B genome) following the whole genome duplication (Özkan *et al.*, 2011). Wheat is considered as a model crop to study adaptation, domestication and evolution in the plants. Domestication of wheat, predominantly due to breeding activities, resulted in the genetic erosion which ultimately increased its susceptibility and vulnerability to biotic and abiotic stresses (Fu and Somers, 2009). High quality food with the higher yield is also a major concern to the world to meet the food requirement for future generations. Therefore, utilization of wild progenitors can serve as a source of variation for the wheat breeding.

Wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) is an annual, predominantly self-pollinated plant which originated as a result of hybridization event between diploid *T. urartu* (genomes AA) with a species closely related to *Aegilops speltoides* (genomes SS, which are closely related to the BB genomes) (Dvorak *et al.*, 1993). Evolutionary studies have confirmed that as a species, wild emmer is 360,000 years old and it originated somewhere in the Fertile Crescent as a result of the hybridization event (Dvorak and Akhunov, 2005). Domestication of wild emmer in western parts of Asia leads in the production of hulled cultivated emmer, however, this hulled emmer was replaced with free-threshing durum wheat during the Greco-Roman times (Arzani and Ashraf, 2017). Currently, wild emmer grows in a discontinuous arc from western Iran to Israel and it is divided into southern (Israel, Lebanon, and southern Syria) and northern (Turkey, Iraq and Iran) populations (Özkan *et al.*, 2002; Luo *et al.*, 2007). In the northern population, *horanum* race is dominant, however, *judaicum* is a robust race which is dominant in southern populations. It is considered that the latter may have originated as a result of hybridization between durum and wild emmer (Blumler, 1998).

Turkey, as an origin center of various crops, represents a great level of biodiversity and played a vital role to connect the continents with each other (Tan, 1998; Baloch *et al.*, 2017). Turkey is considered as the origin center of the wheat and Karacadağ region (southeastern Turkey) is believed where domestication of wild emmer has occurred (Jorgensen *et al.*, 2017). It is believed that cultivation of emmer wheat started in Fertile Crescent about 10,000 – 13,000 years ago where it was the main food source until the beginning of 20th century when its cultivation gradually replaced by durum wheat. Present day, wild emmer is mainly grown on a small area of Kastamonu, Sinop, Karacadağ and Black Sea region in Turkey (Giuliani *et al.*, 2009) and, its diverse wild emmer habitats still survive in cool and humid conditions (Karacadağ Mountains) to hot and dry conditions in Israel (Özkan *et al.*, 2011).

Genetic dissection of any crop remained an utmost desire for the human being to produce higher quantity and quality food and achievement of these goals revolutionized after the advent of molecular markers (Nadeem *et al.*, 2018). Different types of molecular markers have been developed and retrotransposons are one of the most important marker classes (Kalendar *et al.*, 2010). Retrotransposons, which are also known as jumping elements due to their capability to change their locations and copy number, are considered as vital part of the structural evolution of the plant genome (Finnegan, 1989). Retrotransposon content changes individual to individual and normally represents the 50–90% of the plant genome (SanMiguel *et al.*, 1996). Long terminal repeat (LTR) and non-LTR retrotransposons (Non-LTR) are two main groups of retrotransposons. Plant genome reflected the prevalence of LTR retrotransposons as compared to non-LTR. However, various limitations among these retrotransposons markers system demand the development of a new marker system. Inter-primer binding site (iPBS) retrotransposons overcomes all of the limitations present in other retrotransposons markers and emerged as a universal marker system due to easy handling and cost-effective properties (Kalendar *et al.*, 2010).

Investigation of genetic diversity and phylogenetic relationship among the wilds of any crop becomes present day need due to its role as a source of novel genetic variations which can be an asset for the breeders to develop favorable cultivars for farmer and end-user point of interests (Govindaraj *et al.*, 2015; Baloch *et al.*, 2017). In previous studies, many efforts have been put into the description of the genetic diversity of wild emmer using different markers (Peleg *et al.*, 2008; Dong *et al.*, 2009; Kaymaz and İzbırak, 2010). Domb *et al.* (2017) used the Transposons elements (TEs) to evaluate the impact of TEs on the diversity of wild emmer. Tagimanova *et al.* (2015) used the IRAP (Inter-Retrotransposon Amplified Polymorphism) and REMAP (Retrotransposon-Microsatellite Amplified Polymorphism) markers for the investigation of genetic diversity in the Israeli wild emmer germplasm. Very recently Vuorinen *et al.* (2018) aimed to investigate the level of diversity in 14 Israeli wild emmer populations using IRAP and REMAP markers. However, genetic diversity and population structure have not been revealed by the iPBS-retrotransposons markers yet. The present study was aimed to investigate the genetic diversity, population structure and phylogenetic relationship of Turkish tetraploid wheat germplasm using iPBS-retrotransposons markers.

Materials and Methods

Plant Material and DNA Isolation

A total of 29 wild emmer and 4 cultivated emmer wheat landraces were collected from farmers' fields in different provinces of Turkey. Five durum wheats (four of from Syria and one of from Turkey) cultivars were also included as

plant material in this experiment (Table 1). These durum wheat genotypes were used as control group to understand the phylogenetic relationship of wild and cultivated emmer wheat. The seeds were sown into pots under greenhouse conditions to obtain young leave tissues for DNA extraction. The DNA was isolated from bulk leaves of each landrace. Young leaves were taken to the laboratory and frozen at -80°C for later use. Genomic DNA was isolated from young, healthy, and fresh leaves following CTAB protocol (Doyle, 1990) with some modifications of Baloch *et al*. (2016). The DNA concentrations were measured using 0.8% agarose gel and were further confirmed with NanoDrop (DeNovix DS-11 FX, USA) and final concentration was arranged to 5 ng/μL for further use in polymerase chain reaction (PCR). These samples were stored at -25°C until PCR amplification started.

iPBS-retrotransposon Analysis

A total of (70) iPBS-retrotransposons primers were screened on randomly selected (8) wild emmer wheat genotypes for PCR amplifications designed by Kalendar *et al*. (2010). Nine most polymorphic primers were selected in this screening. These 9 primers produced perfect banding profiles which were selected for fingerprinting wheat landraces and cultivars. The name, sequence, and annealing temperature of the primers are described in Table 2. PCR amplifications contained 20 μL reactions with 4 ng/uL template DNA, 2 μL dNTPs (Thermo Scientific), 0.2 μL U Taq DNA polymerase (Thermo Scientific), 4 μL primer, 2 μL 1XPCR buffer (Thermo Scientific), 1,8 μL MgCl$_2$ and 6 μL distilled water PCR conditions were started with denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 15 s, annealing temperature 50–65°C depending on primers used for 1 min; and final extension at 72°C 5 min (Kalendar *et al*., 2010). PCR products were detected by electrophoresis on 1.2% (w/v) agarose gel using 0.5XTBE buffer for 2.5 h; the gel was stained with ethidium bromide before electrophoresis and visualized under UV Imager Gel Doc XR+ system (Bio-Rad, USA) light and it was later photographed. A 100 bp^{+} ladder was used as a molecular weight marker.

Data Analysis

Clear, strong and highly reproductive amplifiable products were undertaken for further analysis. iPBS-retrotransposons is a dominant marker and was scored as binary fashion; 0 or 1 for absence and presence of a band, respectively. Various diversity parameters like a number of effective alleles (Ne), Shannon's Information Index (I), gene diversity (h) and unbiased diversity (uh) were measured using GenAlEx v6.5 (Peakall and Smouse, 2012). The mean polymorphism information contents (PICs) for each primer were calculated by following the criteria of Baloch *et al*. (2015a). Pairwise genetic distance (GDj) was evaluated by applying Jaccard's coefficient (Jaccard, 1908) using R statistical software. To explore the level of diversity more clearly, PopGene ver. 1.32 (Yeh *et al*., 2000) was used to calculate the Nei (1978) genetic distance among the wild and cultivated emmer and durum wheat accessions. To explore the level of genetic diversity in the Turkish wild emmer germplasm, Principle coordinate analysis (PCoA) and the unweighted pair group method with arithmetic mean (UPGMA) was performed. For understanding the phylogenetic relationship among the wild and cultivated emmer and durum wheat accessions, UPGMA based clustering was also achieved through the PopGene ver. 1.32 (Yeh *et al*., 2000). To explore the structure of Turkish wild emmer wheat, Bayesian clustering model was applied in STRUCTURE. Evanno *et al*. (2005) set the criteria for the determination of suitable numbers of the cluster (number of K; the number of subpopulations) by applying the STRUCTURE and we followed this methodology and plotted the number of clusters (K) against logarithm probability relative to the standard deviation (ΔK).

Results

Within the wild and cultivated emmer and durum wheat accessions; nine most polymorphic iPBS-retrotransposons primers were yielded a total of 171 scoreable bands and 19 was the average number of bands per primer. Among the 171 bands, 86.54% (148) were found to be polymorphic with an average of 16.44 fragments per primer (Table 3). iPBS2238 resulted in maximum (25) number and iPBS2375 in minimum (14) number of total bands. iPBS2238 was found much informative by resulting maximum (23) of polymorphic bands and iPBS2375 and iPBS2399 resulted in a minimum of (12) polymorphic bands. These nine iPBS-retrotransposons primers reflected a higher polymorphism with an average of 87.85%. Average PIC value was 0.66 ranging 0.913 to 0.306 in iPBS2378 and iPBS2399 primers respectively. Maximum band frequency/locus was 0.514 with iPBS2241 and 0.471 was the minimum band frequency/locus resulted by iPBS2230 and 0.489 was the average band frequency/locus. 1.982 and 1.942 were the maximum and minimum effective numbers of alleles resulted with iPBS2295 and iPBS2241 respectively and 1.961 were the average effective numbers of alleles. 0.682 was the average, 0.688 and 0.679 were the minimum Shannon's Information Index resulted with iPBS2295 and iPBS2399 respectively. Maximum (0.495) and minimum (0.486) gene diversity were observed with iPBS2295 and iPBS2399 primers respectively, while 0.489 was the average gene diversity. Average unbiased diversity was 0.502 and 0.509 and 0.499 were the maximum and minimum values of unbiased diversity resulted with iPBS2295 and iPBS2399 primers respectively.

The pairwise genetic distance among the wild and cultivated emmer and durum wheat accessions was calculated by applying Jaccord coefficient using R statistical software and 0.398 was the average genetic distance.

Table 1: Passport data of wild, cultivated emmer and durum wheat accessions

No	Genotype names	Scientific names	Collected sites	Geographical coordinates	Altitude (m)
1	Kaynak	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N: 37.44.040; E: 39.38.160	1200
2	Çermik1	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.57.104; E: 39.42.991	906
3	Karacadağ1	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.44.510; E: 39.39.887	1110
4	Karacadağ2	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.46.250; E: 39.43.904	1202
5	Karacadağ3	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.46.250; E: 39.43.904	1202
6	Karacadağ4	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.46.250; E: 39.43.904	1202
7	Karacadağ5	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.46.584; E: 39.44.565	1228
8	Karacadağ6	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.50.699; E: 39.48.008	1252
9	Çermik2	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.57.104; E: 39.42.991	906
10	Karacadağ7	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.44.510; E: 39.39.887	1110
11	Karacadağ8	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.44.510; E: 39.39.887	1110
12	Karacadağ9	*T. turgidum* ssp. *dicoccoides*	Diyarbakır		
13	Karacadağ10	*T. turgidum* ssp. *dicoccoides*	Diyarbakır		
14	Karacadağ11	*T. turgidum* ssp. *dicoccoides*	Diyarbakır		
15	Karacadağ12	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.49.819; E: 39.46.590	1251
16	Karacadağ13	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.49.819; E: 39.46.590	1251
17	Karacadağ14	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.50.400; E:39.47.671	1251
18	Karacadağ15	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.50.400; E:39.47.671	
19	Karacadağ16	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.45.970; E: 39.43.350	1285
20	Karacadağ17	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.45.970; E: 39.43.350	1285
21	Karacadağ18	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.45.970; E: 39.43.350	1285
22	Karacadağ19	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.45.970; E: 39.43.350	1285
23	Karacadağ20	*T. turgidum* ssp. *dicoccoides*	Diyarbakır	N:37.45.970; E: 39.43.350	1285
24	Eruh1	*T. turgidum* ssp. *dicoccoides*	Siirt	N:37.44.217; E: 42.13.359	985
25	Eruh2	*T. turgidum* ssp. *dicoccoides*	Siirt	N:37.44.217; E: 42.13.359	985
26	Eruh3	*T. turgidum* ssp. *dicoccoides*	Siirt	N:37.44.217; E: 42.13.359	985
27	Eruh4	*T. turgidum* ssp. *dicoccoides*	Siirt	N:37.41.429; E: 42.17.965	
28	Tunceli1	*T. turgidum* ssp. *dicoccoides*	Tunceli		1300
29	Tunceli2	*T. turgidum* ssp. *dicoccoides*	Tunceli		1200
30	25G	*T. turgidum* spp. *dicoccum*	Sinop	N:41.27'15; E:35.16'57	1247
31	32G	*T. turgidum* spp. *dicoccum*	Sinop	N:41.26'15; E:35.22'54	1111
32	33G	*T. turgidum* spp. *dicoccum*	Sinop	N:41.25'58; E:35.22'48	1155
33	S48	*T. turgidum* ssp. *durum*	Dayr Azzaur (Syria)		
34	34G	*T. turgidum* spp. *dicoccum*	Sinop	N:41.27'55; E:35.16'50	1250
35	T17	*T. turgidum* ssp. *durum*	Maraş		
36	S41	*T. turgidum* ssp. *durum*	Dara (Syria)		
37	S43	*T. turgidum* ssp. *durum*	Dara (Syria)		
38	S45	*T. turgidum* ssp. *durum*	Damascus (Syria)		

Table 2: iPBS-retrotransposons primer names, sequence and annealing temperature used in this study

Primer name	Sequence	Annealing temp (ºC)
iPBS2075	CTCATGATGCCA	50
iPBS2375	TCGCATCAACCA	52
iPBS2399	AAACTGGCAACGGCGCCA	52
iPBS2378	GGTCCTCATCCA	53
iPBS2230	TCTAGGCGTCTGATACCA	53
iPBS2383	GCATGGCCTCCA	53
iPBS2238	ACCTAGCTCATGATGCCA	55
iPBS2241	ACCTAGCTCATCATGCCA	55
iPBS2295	AGAACGGCTCTGATACCA	60

The maximum genetic distance was 0.6639 present between T17 and Chermik-1 genotypes, followed by T17 and Karacadağ-1 genotypes having 0.641 genetic distance. The minimum genetic distance was 0.115 present between Karacadağ-17 and Karacadağ-18, followed by Chermik-1 and Kaynak genotypes having 0.1481 genetic distance. To explore the level of diversity more clearly, genetic distance among the wild, cultivated emmer and durum wheat accessions was also calculated (Table 4). Wild emmer wheat reflected maximum genetic distance of 0.121 with the durum wheat, cultivated emmer wheat reflected maximum

genetic distance 0.1338 with the durum wheat. The minimum genetic distance of 0.119 was present between wild emmer wheat and cultivated emmer wheat.

The UPGMA based clustering divided (38) wheat genotypes into two main groups A and B (Fig. 1). Group A was smaller than group B and it clustered only (5) genotypes belonging to durum wheat. Group B was the larger and diverse group and clustered a total of 33 wheat genotypes. Origin center wise and geographical provinces played effective role in the clustering.

To understand the phylogenetic relationship among

Table 3: Various diversity parameters evaluated in this study for wild and cultivated emmer and durum wheat accessions using iPBS-retrotransposons

Primer	Total	Polymorphic bands	Monomorphic bands	Polymorphism (%)	PIC	Band frequency/ locus	Ne	I	h	uh
2075	19	19	0	100	0.786	0.483	1.952	0.680	0.487	0.500
2230	20	19	1	95	0.690	0.471	1.968	0.685	0.492	0.505
2238	25	23	2	92	0.667	0.480	1.961	0.683	0.490	0.503
2241	15	13	2	86.66	0.662	0.514	1.949	0.680	0.487	0.500
2295	17	17	0	100	0.680	0.497	1.982	0.688	0.495	0.509
2375	14	12	2	85.71	0.628	0.511	1.954	0.681	0.488	0.501
2378	18	17	1	94.44	0.913	0.474	1.962	0.683	0.490	0.503
2383	18	16	2	88.88	0.611	0.499	1.972	0.686	0.493	0.506
2399	25	12	13	48	0.306	0.48	1.950	0.679	0.486	0.499
Total	171	148	23							
Average	19	16.44	2.55	87.85%	0.660	0.489	1.961	0.682	0.489	0.502

PIC=Polymorphic information contents; Ne; No. of effective alleles, I = Shannon's Information Index, h = Diversity, uh = Unbiased Diversity

Table 4: Genetic distance among wild and cultivated emmer and durum wheat accessions using iPBS-retrotransposons

Species	*T. turgidum* ssp. *dicoccoides*	*T. turgidum* spp. *dicoccum*	*T. turgidum* ssp. *durum*
T. turgidum ssp. *dicoccoides*	****	0.8876	0.8855
T. turgidum spp. *dicoccum*	0.1192	****	0.8748
T. turgidum ssp. *durum*	0.1216	0.1338	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

three different species of wheat, UPGMA based clustering was performed which clearly discriminated durum wheat species in the same group of wild emmer and cultivated emmer wheat (Fig. 2).

Principal coordinate analysis (PCoA) also divided the genotypes according to their phylogenetic group and their collection points (Fig. 3). Wild emmer genotypes in Karacadağ mostly clustered together and the same pattern was observed for genotypes from Tunceli and Eruh. Durum genotypes from Turkey and Syria clustered separately but were present very close to each other. Cultivated emmer wheat made its separate group than wild emmer thus confirming its uniqueness from wild emmer wheat. Bayesian based clustering algorithm divided the genotypes into two population; A and B (Fig. 4). The maximum observed (ΔK) value was (2) and most of the genotypes in population A belong to durum and cultivated emmer wheat and population B clustered genotypes from the wild emmer.

Discussion

The iPBS-retrotranposons becomes a marker of choice for the scientific community due to its low cost and higher efficiency (Nadeem *et al*., 2018). This marker system overcomes the limitations of other retrotransposons based markers and has been successfully applied for the investigation of genetic diversity in various crops like pea (*Pisum sativum* L.) (Baloch *et al*., 2015a), lentil (*Lens culinaris* L.) (Baloch *et al*., 2015b), common bean (*Phaseolus vulgaris* L.) (Nemli *et al*., 2015), okra (*Hibiscus esculentus* L.) (Yıldız *et al*., 2015), and recently in tobacco (*Nicotiana tabacum* L.) (Yaldiz *et al*., 2018). In wheat, different retrotransposons based markers (Domb *et al*., 2017; Vuorinen *et al*., 2018) have been applied to evaluate
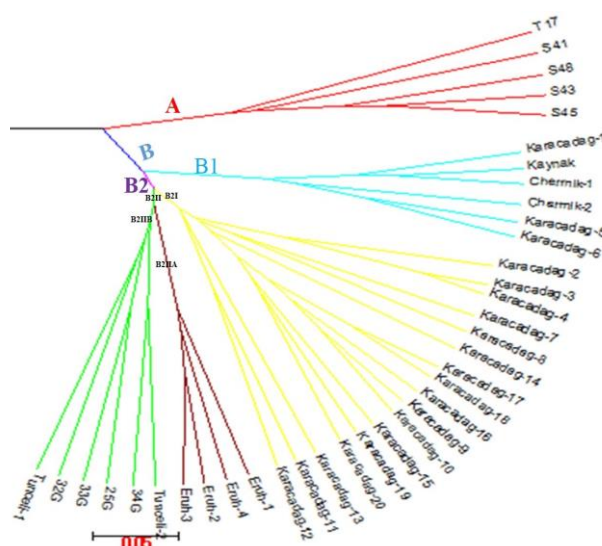

Fig. 1: UPGMA based clustering of 38 wheat genotypes using iPBS-retrotransposons markers

Fig. 2: UPGMA based clustering of 3 wheat species using iPBS-retrotransposons markers

the level of diversity and to our best knowledge, no study has yet been conducted in wild emmer wheat to evaluate the level of diversity using iPBS-retrotransposons markers. Mostly, iPBS-retrotransposons have been utilized for the identification of genetic diversity in various studies for
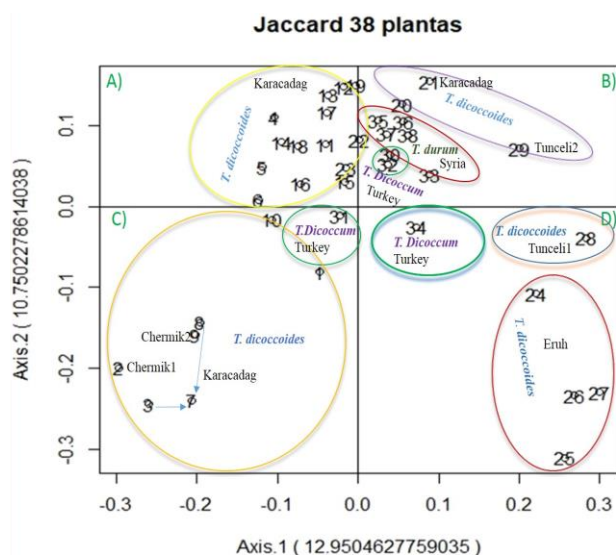
Fig. 3: Principal coordinate analysis (PCoA) for wild and cultivated emmer and durum wheat accessions using iPBS-retrotransposons markers
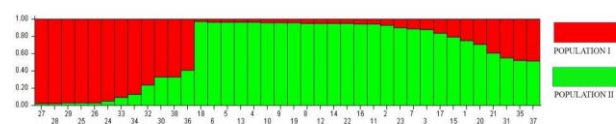

Fig. 4: Structure based clustering of wild and cultivated emmer and durum wheat accessions using iPBS-retrotransposons markers

different crops. Therefore, it was also important to check the effectiveness and versatility of this marker system for the investigation of phylogenetic and taxonomic studies and there is no more crop better than the wheat to study and understand the phylogenetic relationship among the tetraploid wheat.

A good level of diversity was obtained using iPBS-retrotransposons and 87.85% was the mean polymorphism achieved in this study. Mean polymorphism obtained in this study was higher than the obtained by the Domb *et al.* (2017) using IRAP and REMAP markers. Our results reflected the higher efficiency of iPBS-retrotransposons markers which could be used to investigate the novel variations. By comparing this marker system with others for the wild emmer wheat, mean polymorphism obtained in this study was found higher than the obtained by Dong *et al.* (2009) using EST-SSR markers, Fahima *et al.* (1999) using RAPD markers, Khan *et al.* (2015) using RAPD markers in wheat. The mean PIC value, an indicator of genetic diversity was found higher than the achieved by Dong *et al.* 2009; Ren *et al.* (2013) and much closer with the Peleg *et al.* (2008) using EST-derived SSR markers. Average number and range of effective numbers of alleles were found higher than obtained by Arora *et al.* (2014) using SSR markers in Indian wheats. Very recently Eltaher *et al.* (2018) used GBS identified SNPs in Nebraska winter wheat to investigate the

genetic diversity and found a lesser number of an effective number of alleles than those of this study. This reflects the existence of higher variations in Turkish wild and cultivated wheat germplasm and possible efficient usage of them for wheat breeding. Shannon information index (I), commonly used to characterize the level of diversity in a population by accounting both the abundance and evenness of specie present was found higher in this study as compared to obtained by Syouf *et al.* (2006), Dong *et al.* (2009) for the wild emmer wheat. Average genetic diversity was in line with Fahima *et al.* (2002) and found higher than that of Fahima *et al.* (1999), Dong *et al.* (2009), Ren *et al.* (2013) in wild emmer wheat using different types of markers. Mean unbiased diversity resulted by Eltaher *et al.* (2018) for wheat was found much lower as compared to the one obtained in this study, however was found lower as compared to obtained by Laidò *et al.*, 2013 using SSR and DArT markers to evaluate the genetic diversity and population structure of tetraploid wheats. Our results confirmed the efficiency, uniqueness of iPBS-retrotransposons markers, and confirm it as a much powerful marker system which can be utilized for the evaluation of diversity in any plant species due to its universal nature.

To explore the diversity level, pairwise genetic distances (GDj) among the wild, cultivated emmer and durum wheat accessions were also calculated. The maximum genetic distance was present between T17 and Chermik-1 genotypes. T17 belongs to durum wheat from Maraş (Turkey) province and Chermik-1 is a wild emmer landrace from Diyarbakır (Turkey). Karacadağ-17 and Karacadağ-18 were found genetically much similar by reflecting minimum (0.115) genetic distance and belongs to same province (Diyarbakır) of Turkey and much closer collection point. To obtain plants with favorable traits remained the main focus of breeder and genetically distinct genotypes always act as a source of breeding material. T17 and Chermik-1 genotypes are genetically distinct genotypes which can be used as candidate parents for possible breeding activities in Turkey. To explore the level of diversity among the emmer and durum wheat accessions, the genetic distance was also investigated and found to be 0.1216 between wild emmer wheat and durum wheat species. Wild emmer and cultivated emmer wheat species reflected minimum genetic distance. This reflect the close relationship of both wheat species and Peng *et al.* (2011a, b) stated that wild emmer is the progenitor of cultivated wheats and they suggested a hypothesis that hunter-gatherers started to cultivate wild emmer wheat about 10,000 BP ago and subconscious plant selection by the peoples resulted in the development of cultivated emmer wheat.

To understand the level of diversity and phylogenetic relationship, UPGMA based clustering was achieved which divided all genotypes of wheat into (2) main groups A and B (Fig. 1). A was the small but genetically pure group by clustering (5) genotypes belonging to durum wheat. B was

the large and diverse group by clustering (33) genotypes. Main group B was further divided into B1 and B2. Only (6) genotypes belonging to wild emmer wheat were clustered into B1 subgroup. B2 was further subdivided into B2I and B2II. B2I was found the largest subgroup of the study by clustering a total of (17) wild emmer genotypes. B2II was further subdivided into two groups B2IIA and B2IIB. B2IIA contained wild emmer genotypes from Eruh province of Turkey and B2IIB was found to be the most diverse subgroup by clustering different genotypes from different provinces and species. All genotypes of durum clustered in this subgroup and two genotypes from Tunceli province also clustered in this subgroup. We observed a clear grouping of genotypes into their respective groups on the basis of their genetic makeup, as all three species of wheat made their own separate groups. Similarly, geographical provinces also played their role effectively as genotypes from Karacadağ and Eruh made their own groups. Clustering on the basis of geographical regions in wheat has been proven in earlier studies (Khan *et al*., 2015; Baloch *et al*., 2017) and our results were found to be in line with these studies. To understand the phylogenetic and taxonomic relationship among the wild, cultivated emmer and durum wheat accessions, the UPGMA based clustering was achieved which divided them into two groups (Fig. 2). The wild emmer wheat and cultivated emmer wheat were found to prevail under the same group and durum wheat clearly made divergence from both species and confirmed the hypothesis that wild emmer was the progenitor of cultivated emmer wheat (Shizuka *et al*., 2015) and confirmed that durum wheat originated from the domesticated emmer wheat (Arzani and Ashraf, 2017). To confirm the clustering evaluated by UPGMA, PCoA was performed which confirmed the results of UPGMA based clustering (Fig. 3). Genotypes from Karacadağ region and belonging to wild emmer, mostly clustered together. All the genotypes from durum made their separate group as present in the UPGMA based clustering. However, genotypes belonging to cultivated emmer wheat made two groups on the basis of their geographic and origin, but both of these groups were found to be much closer to each other by confirming similarity in their genetic makeup. To evaluate the population structure of 38 wheat genotypes, structure algorithm was performed which divided genotypes into two populations (Fig. 4) on the basis of membership coefficient equal or more than 0.5 as suggested by Habyarimana (2016). Population A (red) was found much diverse by clustering genotypes from the durum wheat and cultivated emmer wheat. Population B (green) was found the largest population and it clustered wild emmer genotypes. Structure algorithm strongly supported the results of UPGMA and PCoA by clearly discriminating wild emmer wheat from the durum wheat and cultivated emmer wheat. In structure clustering, genotypes from cultivated emmer wheat were found much closer with the wild emmer wheat and confirmed the close relationship of wild emmer with

cultivated emmer wheat. Results of this study showed a good potential of variations in the wild emmer wheat using the iPBS-retrotransposons which can be used for the wheat breeding in near future. There is need to collect a good numbers of einkorn, wild and cultivated emmer and durum wheat genotypes from their center of origin and domestication and their characterization with iPBS-retrotransposons markers will be a very effective tool to understand the evolutionary processes of the wheat crop.

Conclusion

This study comprehensively explained the level of diversity and their phylogenetic relationships in Turkish wild and cultivated emmer wheat germplasm. About 88% polymorphism was present which reflected the occurrence of good level of variations. T17 and Chermik-1 were found to be two distinct genotypes belonging to durum wheat and wild emmer wheat, respectively and these genotypes can be used as candidate parents for the development of new and improved wheat cultivars. The iPBS-retrotransposons clearly discriminated durum wheat from the cultivated wheat and wild emmer wheat and confirms the hypothesis that wild emmer is the progenitor of cultivated emmer wheat and durum wheat originated from cultivated emmer wheat.

Acknowledgments

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