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***Research Article***

# Genetic Diversity of Emmer Wheats Using IPBS-Retrotranspozon Markers

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**Abstract**

The genetic diversity among totally 21 wheat genotypes (16 *Triticum dicoccum* L. called emmer wheat and 5 *Triticum durum* L. called durum wheat) were investigated using 7 iPBS primers. iPBSs used in the current study generated 134 polymorphic from out of 136 bands for wheat genotypes. Characterization of the iPBS primers were performerd by calculation meanpolymorphism information content (PIC= 0.19), heterozygosity (H= 0.23), polymorphism ratio (P%= 97.92) and number of average polymorphic (19.14). Jaccard's diversity index varied between 0.0222 and 0.7843 with a mean 0.4677 for wheat genotypes. According to the sum of AMOVA, genetic diversity in emmer and durum wheats was significantly high with 77% variation within the population. On the other hand, genetic variation among populations was moderate with a value of 23%. Genetic diversity of populations were calculated by parameters including the mean number of alleles per locus (Na=1.269), effective allele number (Ne=1.31), Shannon information index (I=0.29), Nei's genetic diversity level (h=0.19), and Nei's unbiased genetic diversity level (uh=0.218). Results obtained from this study showed that iPBS molecular markers could be useful in determination of genetic relationships among wheat genotypes.

**Keywords:** Durum wheat, *Triticum dicoccum* L., *Triticum durum* L., AMOVA, Polymorphism, Breeding.

# Bazı Gernik Buğdaylarında IPBS Retrotranspozon Markörleri Kullanılarak Genetik Çeşitlilik Analizi

**Öz**

Toplam 21 buğday genotipi (emmer buğdayı olarak adlandırılan 16 *Triticum dicoccum* L. ve durum buğdayı olarak adlandırılan 5 *Triticum durum* L.) arasındaki genetik çeşitlilik 7 iPBS primeri kullanılarak araştırılmıştır. Mevcut çalışmada kullanılan iPBS'ler, buğday genotipleri için 136 banttan 134'ünü polimorfik olarak üretmiştir. Çalışmada kullanılan 7 iPBS primerinin karakterizasyonu ortalama polimorfizm bilgi içeriği (PIC = 0.19), heterozigotluk (H = 0.23), polimorfizm oranı (P% = 97.92) ve polimorfizm (19.14) değerleri hesaplanarak belirlenmiştir. Jaccard'ın çeşitlilik değeri, buğday genotipleri için ortalama 0.4677 olarak hesaplanmış ve 0.0222 ile 0.7843 arasında değiştiği saptanmıştır. AMOVA sonucuna göre gernik ve makarnalık buğdaylarındaki genetik çeşitlilik, popülasyon içinde %77 varyasyona sahip olduğu tespit edilmiştir. Öte yandan, popülasyonlar arasındaki genetik varyasyon %23 değeriyle orta düzeydeyde olduğu bulunmuştur. Popülasyonların genetik çeşitliliğini belirlemek için lokus başına ortalama alel sayısı (Na = 1.269), etkili alel sayısı (Ne = 1.31), Shannon bilgi indeksi (I = 0.29), Nei'nin genetik çeşitlilik seviyesi (h = 0.19) i parametreleri hesaplanmıştır. Ayrıca, Nei'nin tarafsız genetik çeşitlilik seviyesi (uh) 0.218 olarak saptanmıştır. Bu çalışmadan elde edilen sonuçlara göre iPBS moleküler markörlerinin buğday genotipleri arasındaki genetik ilişkilerin belirlenmesinde faydalı olabileceğini göstermiştir.

**Anahtar Kelimeler:** Ekmeklik buğday, *Triticum dicoccum* L., *Triticum durum* L., AMOVA, Polimorfizm, Islah.

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# 1. Introduction

The cultivated wheat and its close relative species are included in the *Poaceae (Graminea*) family, the genus *Triticum* (Matsuoka, 2011). The genus *Triticum* has species in different polyploidy levels such as diploid, tetraploid and hexaploid species (Provan et al., 2004). While cereal species/types were a single ancestral plant about 13 million years ago, they started to separate from each other as a result of natural mutations and environmental interactions. Diploid wheat species, *Triticum monococcum* and *Triticum urartu*, separated from each other about 0.5-1 million years ago and they existed in the world as two separate species. The results of morphological, cytological and genetic studies have shown that *Triticum turgidum* and *Triticum timopheevii* wheat species having respectively AABB and AAGG genome have emerged after *T. monococcum* and *T.urartu* separation from each other (Matsuoka, 2011; Peng et al., 2011a). After wild wheat (*Triticum urartu*, 2n = 14, $A^uA^u$) and wild grass (*Aegilops speltoides*, 2n = 14, BB) have experienced natural hybridization with each other and chromosome folding approximately 300-500 thousand years ago, wild *Triticum dicoccoides* (2n = 28, $A^uA^uBB$) called as emmer wheat has arisen. Cultured emmer wheat (*Triticum dicoccum*, 2n = 28 chromosomes and $A^uA^uBB$) has been domesticated as a result of natural-artificial selection made from the *Triticum dicoccoides* species (Peng et al., 2011a; Peng et al., 2011b).

Understanding genetic diversity is essential to improve crop in plant breeding. A popular hypothesis is that a long period of plant breeding and intensive selection further reduces genetic diversity between varieties and narrows the germplasm base available for future breeding advances (Tanksley and McCouch, 1997). Although wheat leads in all cereal grain crops in terms of production, consumption and acreage, its genetic diversity in current varieties is quickly reduced due to the replacement of the landraces having high diversity by pure line varieties (Frankel and Bennett, 1970). This is particularly important problem since narrow genetic variation causes handicaps in breeding to cope with biotic and abiotic stress such as diseases, drought, and climate change (Uddin and Boerner, 2008). Currently, breeders have been mainly focused on increasing genetic diversity of wheat species.

Several molecular marker systems have been used for determine genetic variation in different cereals such as rice, chickpea, lentil, barley, wheat and oat (Tarang et al., 2020; Andeden et al., 2013; Khazaei et al., 2016; Hou et al., 2005; Gurcan et al., 2017; Boczkowska et al., 2014). Recently, iPBS molecular markers developed based on retrotransposons (RTNs) have been used by several researchers for determination of genetic diversity in various plant species (Yıldız et al., 2015; Sipahi and Yumurtacı, 2020; Shirmohammadli et al., 2018; Öztürk et al., 2020; Karık et al., 2019; Hossein-Pour et al., 2019; Borno et al., 2017; Ali et al., 2019). Inter-primer binding site (iPBS) markers based on retrotransposon have advantages compared with other retrotransposon markers because of universal markers depends upon the presence of tRNA as a reverse transcriptase primer binding site, cost-effective and high efficiency (Kalendar et al., 2010; Nadeem et al., 2018).

The aim of the current study was to assess the genetic diversity in totally 21 wheat genotypes including emmer and durum wheat populations using iPBS markers.

# 2. Material and Method

## 2.1. Plant Material

16 emmer wheats (*Triticum dicoccum* L.) and 5 durum wheats (*Triticum durum* L.) which are used as material in the study was provided by Field Crops Department of Agriculture Faculty, Iğdır University.

## 2.2. DNA Isolation and PCR Stages

Total genomic DNA of genotypes was isolated from leaf samples after a modification of the cetyltrimethylammonium bromide (CTAB) extraction protocol (http://primerdigital.com/dna.html). The quality and concentration of genomic DNA was checked spectrophotometrically with Nanodrop (BioSpec-nano Shimadzu Biotech). iPBS analysis was performed using the markers given Table 1 and reported by Kalendar et al. (2010). PCR reactions for iPBS analyzes were performed in 25 µl reactions containing 20 ng genomic DNA, 1x Taq PCR buffer including $MgCl_2$, 1 µM primer, 0.2 µM for each dNTP and 1 U Taq DNA polymerase. PCR reactions were carried out in 0.2 ml PCR tube strips. The amplification profile comprised of an initial denaturation at 94°C for 5 min, followed by 40 cycles at 95°C for 15 s, 45°C to 60°C for 30 s, 72°C for 90 s and a final extension of 5 min at 72°C. After amplification, PCR product were separated by electrophoresis using 2.5% agarose staining with ethidium bromide in 1x TBE buffer with steady voltage of 120 V for 2.5 hour. The pattern of DNA bands was imagined under UV light, photographed with a gel documentation system

## 2.3. Data Analysis

After gel visualized, data matrix was generated by scoring manually the present (1) and absent (0) iPBS bands in individual lines for each primer. The H (gene diversity) and PIC (polymorphism information content) values of iPBS markers used with binary data matrix were calculated using the PowerMarker V3.25 program (Liu and Muse, 2005). The similarity matrix among emmer wheats was calculated with Jaccard's coefficient (Jaccard, 1912). PCA analysis was performed using NTSYS-pc V2.11 program (Rohlf, 2000). Dendrogram to classify genotypes was created using the MEGA program (Kumar et al., 2016). I (Shannon information index), h (diversity) and AMOVA results of the populations were calculated using the GENALEX V6.5 program (Peakall and Smouse, 2006)

# 3. Results and Discussion

iPBS markers have been used for revealing genetic diversity in different plants, such as wheat (Ghonaim et al., 2020), safflower (Ali et al., 2019), guava (Mehmood et al., 2016), patato (Demirel et al., 2018), chickpea (Andeden et al., 2013), pea (Baloch et al., 2015) and rice (Shirmohammadli at el., 2018). Here we have used iPBS markers for determination of genetic variation

among 21 wheat genotypes. Totally 7 iPBS primers were used and they generated robust and reproducible band patterns. The primers produced 136 bands and 134 of them were polymorphic. The number of polymorphic amplification products per iPBS primer varied from 9 for iPBS-2375 to 29 for iPBS-2219 with an average 19.14. The average polymorphic alleles number per locus is quite higher than demonstrated in wild emmer wheats by Fahima et al. (1998). Average of polymorphism ratio (P%) of iPBS primers was 97.92. Average of gene diversity (H) and polymorphism information content (PIC) belonging to markers were 0.23 and 0.19, respectively. The iPBS-2377 and iPBS-2271 have the highest (0.31) and lowest (0.14) gene diversity (H), respectively (Table 1). Khaled et al. (2015) reported that 46 of 117 bands of ISSR markers and 50 of 95 bands RAPD markers were polymorphic in bread wheat lines. Moreover, they demonstrated that average PIC values of ISSR and RAPD markers were 0.10 and 0.15, respectively. In another study of ISSR markers in bread wheat lines in Iranian, mean of poliymorphic loci, P% value and H value were 9.7, 83% and 0.36 (Dashchi et al., 2012). In the present study, the total number of bands (136) was higher than reported by Andeden et al. (2013), but was lower than shown by Arystanbekkyzy et al. (2019). The efficiency of polymorphic loci to search genetic diversity with markers is evaluated by the PIC value (Mir et al., 2012). The average PIC value determined by using 7 iPBS marker in 16 emmer and 5 durum wheats was lower than previous studies conducted using different marker systems Salem et al. (2008), Najaphy et al. (2011), Yagdi (2012) and Moragues et al. (2007). However, average PIC value in the present study was higher than reported by Khaled et al. (2015). The variation in PIC values may be due to the use of different marker systems or the different number of genotypes and markers.

*Table 1. Seven iPBS markers used to determine diversity among 21 wheat genotypes*

| | | | Number of Bands | | Diversity Parameters | | |
|---|---|---|---|---|---|---|---|
| **Marker Name** | **Sequence** | **Temperature** | **TB** | **PB** | **P%** | **H** | **PIC** |
| **2378** | GGTCCTCATCCA | 45 | 20 | 20 | 100 | 0.27 | 0.22 |
| **2377** | ACGAAGGGACCA | 45 | 19 | 19 | 100 | 0.31 | 0.25 |
| **2375** | TCGCATCAACCA | 45 | 10 | 9 | 90 | 0.20 | 0.17 |
| **2278** | GCTCATGATACCA | 45 | 18 | 18 | 100 | 0.24 | 0.20 |
| **2271** | GGCTCGGATGCCA | 55 | 22 | 21 | 95.45 | 0.14 | 0.12 |
| **2270** | ACCTGGCGTGCCA | 60 | 18 | 18 | 100 | 0.20 | 0.16 |
| **2219** | GAACTTATGCCGATACCA | 57 | 29 | 29 | 100 | 0.24 | 0.20 |
| **Total** | | | 136 | 134 | | | |
| **Average** | | | 19.42 | 19.14 | 97.92 | 0.23 | 0.19 |

TB: Total band number, PB: Polymorphic band number, P%: Polymorphism ratio, H: Gene diversity, PIC: Polymorphism information content

Evaluation of genetic diversity is one of the critical steps in breeding studies to be carried out to improve different crop characteristics such as quality and yield. To determine genetic relationships among emmer genotypes, Jaccard similarity index was calculated (Table 2). The dendrogram and PCA analysis were also performed as based on this similarity coefficient. According to the Jaccard similarity index, G5 and G8 genotypes were found to have the highest degree of relationship with 0.7843. G1 and G16 genotypes are the most distant genotypes with a Jaccard similarity index of 0.0222. The average of Jaccard similarity index was 0.4677. Given Jaccard coefficient of all genotypes revealed significantly variation between 16 emmer and 5 durum genotypes. Carvalho et al. (2009), reported that Jaccard similarity values ranged from 0.32 to 0.85 in their study to reveal genetic diversity using ISSR markers in Portuguese bread and durum wheats. Burkhamer et al. (1998) reported that similarity values between wheat accessions varied between 0.34 and 0.81. Hazen et al. (2002) showed that the mean Jaccard coefficient based on genetic distance matrix was 0.507 among all 46 wheat accessions. Results of similarity analysis (Jaccard's coefficient) showed that there is considerably genetic variation among all genotypes used in present study.

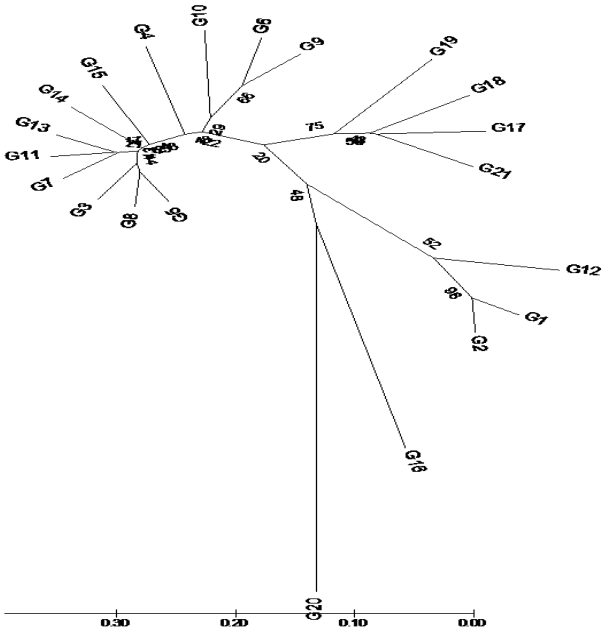


***Figure 1.*** *Dendrogram of cluster analysis for all wheat genotypes with bootstrap*

A dendrogram based on Jaccard similarity index was generated with Bootstrap analysis by using MEGA7 version to visualize relationships among all wheat populations (Figure 1). The dendrogram classified into six groups all genotypes, the first group consist of twelve genotype (G5, G8, G3, G7, G11, G13,

G14, G15, G4, G10, G6 and G9). The second group includes three emmer wheat genotype; G1, G2, and G12. While G17 (Aydın93), G18 (Şahinbey), G19 (Diyarbakır81) and G21 (Fırat93) of durum wheat genotypes formed clusters among themselves (fourth group), G20 (Sarıçanak) created a separate cluster by separating from all other genotypes (fifth group). Finally, the sixth group involve one emmer wheat called as G16.

*Table 2. Jaccard similarity coefficients based on the similarity matrix of 21 wheat genotype used*

| | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 | G13 | G14 | G15 | G16 | G17 | G18 | G19 | G20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **G2** | 0.4762 | 1 | | | | | | | | | | | | | | | | | | |
| **G3** | 0.2308 | 0.2619 | 1 | | | | | | | | | | | | | | | | | |
| **G4** | 0.3056 | 0.3000 | 0.5536 | 1 | | | | | | | | | | | | | | | | |
| **G5** | 0.2368 | 0.3000 | 0.7400 | 0.5088 | 1 | | | | | | | | | | | | | | | |
| **G6** | 0.2778 | 0.2439 | 0.6585 | 0.4255 | 0.7632 | 1 | | | | | | | | | | | | | | |
| **G7** | 0.1905 | 0.1957 | 0.6667 | 0.6481 | 0.6481 | 0.5909 | 1 | | | | | | | | | | | | | |
| **G8** | 0.2093 | 0.2667 | 0.7358 | 0.5424 | 0.7843 | 0.6364 | 0.6786 | 1 | | | | | | | | | | | | |
| **G9** | 0.1944 | 0.2632 | 0.6250 | 0.4545 | 0.6842 | 0.6154 | 0.5952 | 0.5682 | 1 | | | | | | | | | | | |
| **G10** | 0.2000 | 0.2045 | 0.5370 | 0.4643 | 0.4643 | 0.4783 | 0.5455 | 0.5000 | 0.6250 | 1 | | | | | | | | | | |
| **G11** | 0.2308 | 0.2326 | 0.6604 | 0.5818 | 0.7400 | 0.7436 | 0.7308 | 0.7358 | 0.7105 | 0.5370 | 1 | | | | | | | | | |
| **G12** | 0.2692 | 0.2667 | 0.2941 | 0.4444 | 0.2745 | 0.3947 | 0.3333 | 0.2727 | 0.3889 | 0.3864 | 0.3469 | 1 | | | | | | | | |
| **G13** | 0.2500 | 0.2791 | 0.6852 | 0.6071 | 0.6981 | 0.5909 | 0.7547 | 0.6964 | 0.6341 | 0.5926 | 0.7500 | 0.3019 | 1 | | | | | | | |
| **G14** | 0.2174 | 0.1961 | 0.6842 | 0.5833 | 0.6379 | 0.5833 | 0.7500 | 0.7241 | 0.4898 | 0.5424 | 0.6552 | 0.2542 | 0.7069 | 1 | | | | | | |
| **G15** | 0.2041 | 0.2308 | 0.6500 | 0.5806 | 0.6610 | 0.6122 | 0.6557 | 0.6885 | 0.6522 | 0.5161 | 0.7368 | 0.2833 | 0.7288 | 0.6719 | 1 | | | | | |
| **G16** | 0.0222 | 0.0408 | 0.3387 | 0.2615 | 0.3226 | 0.2692 | 0.3710 | 0.3385 | 0.3696 | 0.3220 | 0.3175 | 0.1731 | 0.3438 | 0.3382 | 0.3239 | 1 | | | | |
| **G17** | 0.1667 | 0.2000 | 0.5172 | 0.4032 | 0.4746 | 0.5000 | 0.5517 | 0.4839 | 0.5714 | 0.4821 | 0.4667 | 0.2941 | 0.4918 | 0.5000 | 0.5000 | 0.3387 | 1 | | | |
| **G18** | 0.1364 | 0.1957 | 0.4590 | 0.3333 | 0.4194 | 0.4583 | 0.4677 | 0.4308 | 0.5952 | 0.4483 | 0.4590 | 0.2642 | 0.4839 | 0.4265 | 0.4706 | 0.3770 | 0.6182 | 1 | | |
| **G19** | 0.1591 | 0.2444 | 0.5254 | 0.3692 | 0.5085 | 0.4490 | 0.4603 | 0.4688 | 0.6190 | 0.4167 | 0.5000 | 0.2364 | 0.5246 | 0.4412 | 0.5303 | 0.3710 | 0.5517 | 0.5965 | 1 | |
| **G20** | 0.1061 | 0.2000 | 0.4805 | 0.3951 | 0.4487 | 0.4091 | 0.5263 | 0.4750 | 0.4063 | 0.4342 | 0.4074 | 0.2267 | 0.4810 | 0.4699 | 0.4881 | 0.2529 | 0.5405 | 0.4935 | 0.4146 | 1 |
| **G21** | 0.1707 | 0.2619 | 0.4333 | 0.3281 | 0.4407 | 0.4783 | 0.4426 | 0.4516 | 0.5116 | 0.4464 | 0.4333 | 0.2800 | 0.4833 | 0.3824 | 0.4058 | 0.3279 | 0.6226 | 0.6111 | 0.5172 | 0.5342 |

The principal component analysis (PCA) of 21 wheat genotypes was performed based on Jaccard similarity matrix to establish relationships among genotypes. Two-dimensional and three-dimensional graphs generated by PCA showed result of according with dendrogram to a large extent (Figure 2).

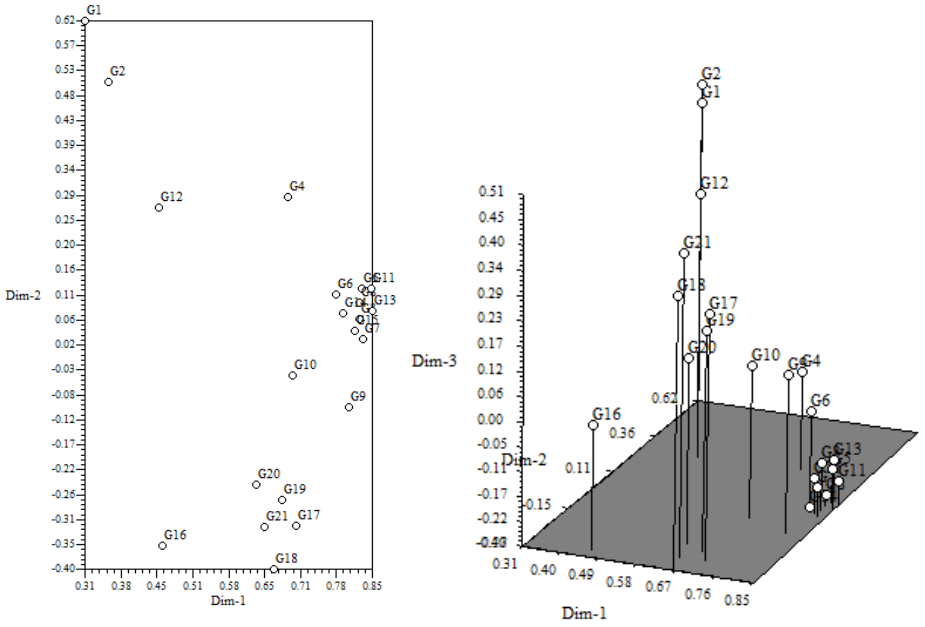


***Figure 2.*** *Two-dimensional and three-dimensional graphs generated by PCA analysis*

In the study, emmer and durum wheats were examined as two separate populations. The genetic similarity value between the emmer and durum populations was determined as 0.886 in our study according to the method reported by Nei (1987). According to the results of molecular variance analysis (AMOVA), while variation within the population was 77%, the variation between populations was 23% (Table 3). Etminan et al. (2016) reported that the result of AMOVA analysis using ISSR primers in durum wheat are that the variation among populations is low (10%) and the variation within population is high (90%). The estimated variation value among population and within population was 4.269 and 14.588, respectively. In present study, genetic variation within populations was significantly high.

*Table 3. Analysis of molecular variance (AMOVA) based on 134 iPBS polymorphic loci*

| Source | df | SS | MS | EV | Value (%) |
|---|---|---|---|---|---|
| **Among population** | 1 | 47.111 | 47.111 | 4.269 | 23 |
| **Within population** | 19 | 277.175 | 14.588 | 14.588 | 77 |
| **Total** | 20 | 324.286 | | 18.857 | 100 |

df: Degrees of freedom, SS: Sum of square, MS: Mean squared deviations, EV: Estimated variation

Sum of genetic diversity parameters in two different populations consisting of emmer and durum wheats is given in Table 4. Observed alleles number (Na) changed from 1.127 with 0.078 standard deviation for durum population to 1.410 with 0.079 for emmer population. The highest effective alleles number (Ne) belonged to the emmer population with 1.331. The highest and the lowest Shannon's information index (I) for emmer and durum populations was 0.317 and 0.024 respectively. The maximum Nei's genetic diversity (h) detected for the emmer population was 0.204. Nei's unbiased measure of genetic diversity (uh) was slightly different for two populations. This genetic diversity parameters of populations were moderately lower than defined by Etminan et al., (2016) in landraces and breeding lines of durum wheat. A study examining the variation between *Triticum turgidum* and *Triticum durum* species showed that h and I values was 0.1739 and 0.2552 in *T. turgidum* populations, respectively, was 0.3030 and 0.4539 in *T. durum* populations (Carvalho et al., 2009).

*Table 4. Genetic diversity values for populations*

| | | N | Na | Ne | I | h | uh |
|---|---|---|---|---|---|---|---|
| **Emmer population** | **Average** | 16 | 1.410 | 1.331 | 0.317 | 0.204 | 0.217 |
| | **SE** | | 0.079 | 0.030 | 0.022 | 0.016 | 0.017 |
| **Durum population** | **Average** | 5 | 1.127 | 1.289 | 0.263 | 0.176 | 0.219 |
| | **SE** | | 0.078 | 0.029 | 0.024 | 0.017 | 0.021 |
| **Total** | **Average** | 10.50 | 1.269 | 1.310 | 0.290 | 0.190 | 0.218 |
| | **SE** | 0.337 | 0.056 | 0.021 | 0.016 | 0.011 | 0.013 |

N: Sample number, Na: Number of observed alleles, Ne: Number of effective alleles, I: Shannon's index, h: Genetic diversity, uh: Unbiased genetic diversity, SE: Standard error

# 4. Conclusions and Recommendations

Knowledge related to the level of genetic variation and relationships in crops is prerequisite in breeding programs to improve them. The iPBS marker system generated adequate polymorphism and reproducible band profiles to assess genetic diversity in wheat genotypes. The current study illustrated that iPBS markers are a powerful tool to detect the genetic variation in emmer wheats. The genetic diversity data obtained from present study can be used by breeders to develop new varieties and in selection of parents for crossing in breeding programs.

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