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Full Length Research Paper

Genetic diversity of Ethiopian emmer wheat *Triticum dicoccum* Schrank landraces using seed storage proteins markers

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Emmer wheat [*Triticum dicoccum* (Schrank)] cultivation in Ethiopia has been reducing and the genetic diversity maintained in the germplasm collection needs to be determined. The objective of this study was to assess the level of genetic diversity among populations of Ethiopian emmer wheat using seed storage protein analysis. Seeds of 85 accessions representing seven administrative regions of Ethiopia were obtained from the Ethiopian Biodiversity Institute and grown at Sinana Agricultural Research Center. Seed storage proteins were extracted from a single seed and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Total genetic diversity, intra-population genetic diversity and inter-population genetic diversity were 0.33, 0.2903 and 0.0391, respectively. Low genetic differentiation (G_{ST} = 0.119) and high gene flow (N_m = 3.697) were observed among populations. The genetic distance (D) between populations ranged from 0.0424 to 0.1128. Cluster analysis revealed two main clusters and one outlier. The results provide important baseline for future germplasm conservation and improvement programs.

Key words: Cluster analysis, emmer wheat, genetic distance, genetic diversity, seed storage protein.

INTRODUCTION

Emmer wheat [*Triticum dicoccum* (Schubler)] was first domesticated in the Near East (Charmet, 2011), which was followed by subsequent hybridization and introgression from wild emmer wheat (*Triticum diccocoides*) in southern Levant (Lebanon, Syria and Israel) (Özbek et al., 2011; Cіváň et al., 2013; Fuller et al., 2011; Riehl et al., 2013). It is speculated that early immigrants of Hamites brought emmer wheat to Ethiopia, some 5,000 years ago and was introduced into the Ethiopian Highlands from Egypt along the Nile River

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(Fuller et al., 2011 and Oliveira et al., 2012). Emmer wheat comprises about 7% of Ethiopia's entire wheat production (BOSTID, 1996). The major production areas in Ethiopia include Bale, Arsi, Shewa, Harerge, Wollo, Gojam and Gondar. It is known by different vernacular names such as "Aja" (in Amharic), "Hayssa" or "Matajebo" (in Afaan Oromo) and "Arras" (in Tigrigna).

Emmer wheat landraces are locally adapted to diverse ecological zones as a result of natural selection and farmers' cultivation methods. They are an important genetic resource for breeding novel genetic diversity into bread wheat and hence their genetic analysis is of importance (Oliveira et al., 2014). The diversity of emmer wheat is seriously threatened by genetic erosion due to the increase in bread wheat production. The urgent need to preserve and utilize landrace genetic resources as a safeguard against an unpredictable future is evident (Jaradat, 2011; Haile, 2012).

Genetic diversity can be estimated by different methods such as morphological traits, end-use quality traits, and molecular markers (Hailu, 2011; Oliveira et al., 2012). Seed storage proteins are suitable for diversity studies, cultivar identification and as excellent markers of the gluten content wheat (Özbek et al., 2011). According to solubility properties, seed storage proteins are classified into four classes: albumins, globulins, gliadins and glutelins. Gluten, comprising 78 to 85% of total wheat endosperm protein, is a very large complex composed mainly of polymeric and monomeric proteins known as glutenins and gliadins, respectively (Hu et al., 2012 and Konvalina et al., 2011). Glutenins confer elasticity to dough, whereas gliadins are viscous and give extensibility to dough (Riefolo et al., 2011). Due to extensive polymorphism, these proteins have been widely used for cultivar identification in hexaploid and tetraploid-wheats (Geleta and Grausgruber, 2013; Konvalina et al., 2013). Allelic variants differ in the number, mobility, and intensity of their components and can be characterized through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Colomba and Gregorini, 2011). The present study was undertaken to evaluate the genetic diversity of Ethiopian emmer wheat using total seed storage proteins markers.

MATERIALS AND METHODS

Plant sample

Eighty-five emmer wheat accessions were obtained from the Ethiopian Biodiversity Institute, Addis Ababa (Table 1). The accessions were selected evenly from the collections obtained from the main emmer wheat growing regions based on their abundances. Accessions were planted under rain fed condition in July to December of 2013 at the Sinana Agricultural Research Center (2400 m.a.s.l). Sinana Agricultural Research Center is located 463 km southeast of Addis Ababa (capital of Ethiopia). This site was selected because of its agro-ecology and its national recognition as the major emmer wheat producing regions of Ethiopia. Twenty seeds from each accession were planted in two rows with 10 cm spacing between plants and 20 cm spacing between rows. Seeds were bulked from the rows.

Table 1. Emmer wheat accessions representing seven geographical regions of Ethiopia.

Region	State/region	Number of accessions
Arsi	Oromia	15
Bale	Oromia	16
Gondar	Amhara	12
Hararghe	Oromia	11
Shewa	Oromia	11
Tigray	Tigray	9
Wollo	Amhara	9

SDS-PAGE electrophoresis

The variability of seed storage-proteins was analyzed using SDS-PAGE (Damania et al., 1983). Six seeds from each accession were randomly selected**.** Each seed was ground to a fine powder using a mortar and pestle. The grounded seed sample was transferred to 1.5 mL microcentrifuge tube and 400 µL protein extraction buffer [1.0M Tris-HCl (pH=6.8), 2.5% (w/v) SDS, 10% (v/v) glycerol, 0.02% (w/v) bromophenol blue and 5% (v/v) β-mercaptoethanol] was added. After brief vortex, the extracts were heated at 90°C for 5 min and centrifuge for 5 min at 12,000 g. The supernatant containing the dissolved seed storage proteins was transferred to a new 1.5 microcentrifuge tube and stored at 4°C. Resolving gel (10%) and stacking gel (4%) were prepared based on Laemmli protocol. The electrophoretic trays were assembled and filled with the running buffer (1.44% Glycine, 0.3% Tris-base and 0.1% SDS), the comb was removed, the wells were cleaned with running buffer and the sample (20 µl) and standard wheat (Alcatal) sample (25 µl) were loaded at the bottom of each well using micropipette. The power supply was connected at 100 V at room temperature for the time required for the tracking marker dye in extraction buffer to migrate off the gel. After electrophoresis, the gel was transferred to tray containing staining solution (125 ml distilled water, 25 ml acetic acid, 100 ml ethanol (absolute) and 0.25g coomassie blue R-250) and kept on a shaker for overnight, followed by distaining with distilled water or distaining solution (125 ml distilled water, 25 ml acetic acid, 100 ml ethanol (absolute) without commasie-blue) until the background of the gel became clear. Picture was taken by digital Panasonic photo camera (Lumix, model No. DMC-S3) (Laemmli, 1970).

Data analysis

Electrophoregrams for the accessions were scored for the presence (1) or absence (0) of each band noted. Data were entered in a binary data matrix. POPGENE version1.32 (Yeteh et al., 1999) was used to calculate genetic diversity for each population as percentage of polymorphism, gene diversity and Shannon-Weaver diversity index (H). NTSYS-pc version 2.02 (Rohlf, 2000) and Free Tree 0.9.1.50 (Pavlicek et al., 1999) were used to calculate Jaccard's similarity coefficient. The Unweighted Pair Group Method with Arithmetic mean (UPGMA) (Sneath and Sokal, 1973) was used to analyze and compare individual samples and regional populations, and generate dendrograms. The neighbor joining (NJ) method (Studier and Keppler, 1988) was used to compare individual genotypes and evaluate patterns of genotype clustering using Free Tree 0.9.1.50 (Payne et al., 2001).

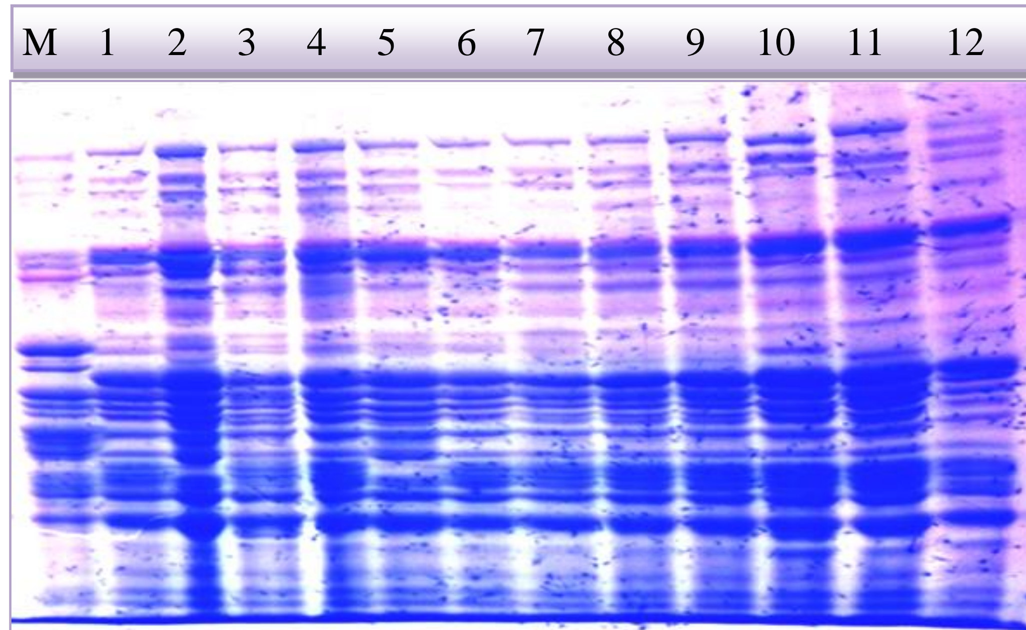

Figure 1. SDS-PAGE protein banding patterns of two *T. dicoccum* accessions. Lane M: bread wheat variety, alcatal used as a control, Lanes 1-6: Accession 238855 and Lanes 7-12: Accession 238856. Six seeds were individually analyzed for each accession.

Table 2. Percentage of polymorphic loci (PPL), Nei's mean gene diversity (H), intra-population genetic diversity (Hs), inter-population diversity (D_{ST}), gene differentiation (G_{ST}) and gene flow (N_m) for seven Ethiopian emmer wheat populations.

Population	PPL	H ± SD	Hs	D_{ST}	G_{ST}	N_m
Bale	78.79	0.301 ± 0.193				
Arsi	81.82	0.288± 0.176				
Shewa	84.85	0.318 ± 0.179				
Hararge	72.73	0.281± 0.199				
Wollo	72.73	0.308 ± 0.205				
Gondor	72.73	0.274 ± 0.192				
Tigray	69.70	0.261 ± 0.210				
Average	76.19	0.290 ± 0.240	0.290±0.240	0.039		
Total Population	90.91	0.329 ± 0.172			0.119	3.696

RESULTS

Genetic diversity analysis

In this study, SDS-PAGE of grain storage proteins was performed in order to investigate genetic diversity among different Ethiopian Emmer wheat landraces. An example of an electrophoregram showing protein banding pattern for two accessions is presented in Figure 1. A total of 33 seed storage proteins were resolved by SDS-PAGE with protein bands numbered 1, 10 and 32 in common for the accessions. However, the remaining bands showed good pattern of variation to study the diversity of this crop. Bread wheat variety Alcatal was used as standard for comparison.

The highest percentage of polymorphic loci was observed for the population from Shewa (PPL= 84.85%), while the Tigray population showed the lowest percentage of polymorphic loci (PPL= 69.70%) (Table 2). The Shewa population was also the most diverse (H =0.318).

Genetic differentiation and gene flow

The average gene diversity among populations was lower

Table 3. Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Pop	Bale	Arsi	Shewa	Hararge	Wollo	Gondar	Tigray
Bale	*****	0.9549	0.9441	0.9526	0.9220	0.9415	0.9363
Arsi	0.0461	*****	0.9040	0.9208	0.8933	0.9585	0.9278
Shewa	0.0575	0.1010	*****	0.9281	0.9343	0.9199	0.9515
Hararge	0.0486	0.0825	0.0747	*****	0.9374	0.9573	0.9512
Wollo	0.0812	0.1128	0.0679	0.0646	*****	0.9253	0.9314
Gondar	0.0603	0.0424	0.0834	0.0437	0.0777	*****	0.9539
Tigray	0.0658	0.0749	0.0498	0.0500	0.0710	0.0472	*****

Table 4. Similarity matrix for the seven Ethiopian emmer wheat populations based on seed storage protein markers.

	Bale	Arsi	Shewa	Hararge	Wollo	Gondar	Tigray
Bale	…….						
Arsi	0.676	…….					
Shewa	0.699	0.643	…….				
Hararge	0.694	0.663	0.644	…….			
Wollo	0.691	0.640	0.642	0.688	…….		
Gondar	0.668	0.681	0.672	0.690	0.677	…….	
Tigray	0.679	0.644	0.678	0.701	0.683	0.705	…….

than the average gene diversity within populations. The extent of gene differentiation relative to the total population (G_{ST}) was about 0.1191. The extent of gene flow (N_m) among populations of Ethiopian emmer wheat landraces was high (Table 2).

Inter-population genetic distance and cluster analysis

Inter-population genetic distance (D) showed that the Arsi populations were the most distantly related. Genetic distance between the other pairwise combinations of populations was very low with the least genetic distance between populations from Tigray and Gondor (Table 3).

From Jaccard genetic similarity of emmer wheat landraces, comparatively the highest similarity was observed between Tigray and Gondor than other landraces combination. The lowest similarity was found in Wollo versus Arsi landraces pair. All landraces showed an average of 0.674 genetic similarities, which could mean that the landraces share an average of 67.4% of their protein band, fragments (Table 4).

UPGMA analysis of Ethiopia emmer wheat populations revealed two major clusters and one outlier (Arsi). Tigray and Gondar, which are highly, related populations (Figure 2).

DISCUSSION

Knowledge of genetic diversity within and among populations from different geographic areas is expected to have a significant impact on the conservation and utilization programs of emmer wheat germplasm. So, understanding the level and structure of the genetic diversity of a crop is a prerequisite for the conservation and efficient use of the available germplasm for plant breeding (Laido` et al., 2013).

The genetic diversity parameters: percent of polymorphic loci, gene diversity (GD), and Shannon's diversity index (I) indicated that the genetic diversity in the emmer wheats populations is indeed high. Shannon diversity index result showed that populations of emmer wheat from Shewa were more diverse. This result is also in agree with the findings of Laido` et al. (2013) in their study on 18 species of *Dicoccum* analyzed by using biochemical markers (H=0.45) and Özkan et al. (2011) on emmer wheat using HMW-gs (H=0.31). In this study, an average value of gene diversity (H=0.33) was obtained. Laido` et al. (2013) obtained exactly the same with mean genetic diversity result of the subspecies *Dicoccoides* accessions (H=0.33) collected from different parts of the world by using molecular marker and higher than that of Oliveira et al. (2014) using SNP marker (H=0.27). However, this level of gene diversity is still considered substantial for a dominantly self-pollinating and rarely out-crossed plant species such as wheat. The genetic diversity in the Ethiopian emmer landraces is most likely the result of its long cultivation history and different climatic and topographic factors which enhanced the effects of natural and artificial selection on germplasm

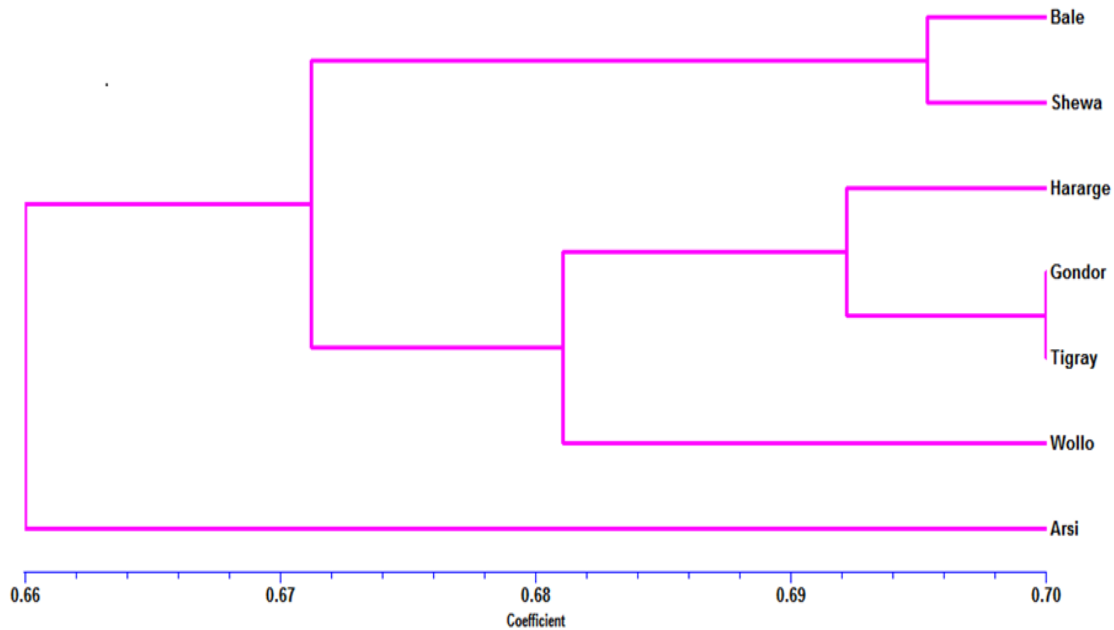

Figure 2. A dendrogram of 7 Ethiopian Emmer wheat populations derived by UPGMA from Nei's (1972) standard genetic distance based on data generated using storage protein.

diversity.

The coefficient of gene differentiation showed that the genetic variation within and among the 7 geographical regions was 88 and 12%, respectively. This result is to some extent in agreement with 73 emmer wheat accessions from Ethiopia highlands that have been recently studied with SSR markers (D=0.27) (Teklu et al., 2007). Therefore, low genetic differentiation between populations was observed due to migration or selection. The values obtained from gene flow (N_m) show the approximate number of individuals migrating from one population to the other. The highest gene flow (N_m=3.6) observed in this study may suggest that the seed exchanging system might be high among the farmers, resulting in low genetic differentiation observed among the populations (D=0.12). Considering the genetic similarity values, the results indicate that the landraces were slightly genetically different from each other. These results are comparable to mean genetic similarity coefficients reported such as 0.65 by Salunkhe et al. (2013) for emmer wheat accessions of India using SSR marker.

In general, the cluster analysis showed a considerable variation among the populations. The formation of solitary cluster might be due to intensive natural or human selection for diverse adaptive complexes.

Therefore, it is concluded that seed storage protein profiles could be useful markers in cultivar identification, registration of new varieties, pedigree analysis, and in the studies of genetic diversity currently conserved in the germplasm of landraces both in *in situ* and *ex situ* collections, thereby improving the efficiency of wheat breeding programs in cultivar development. Finally, seed storage protein analysis permits grouping of peptides patterns and of group based patterns, and is a simple, repeatable and economic procedure.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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