

Analysis of gliadin patterns and diversity in *Triticum polonicum* L. accessions from Ethiopia

Eleni SHIFERAW^{1,2}

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Abstract: Gliadins from 25 accessions represented by 350 individual seed samples were analysed by acid-polyacrylamide gel electrophoresis (A-PAGE) with the objective of identifying gliadin band patterns and examine the extent of diversity in *Triticum polonicum* L. collections from Ethiopia. Seventy polymorphic bands and 68 different patterns were identified. Eighteen different mobility bands and 16 patterns were identified in ω -gliadin region, 22 bands and 20 patterns in γ -gliadin region, 12 bands and 22 patterns in β -gliadin region and 18 bands and 10 patterns in α -gliadin region. The average genetic diversity calculated from the data of the four gliadin zones of the analysed samples was 0.15. The γ region have the highest diversity ($H = 0.193$), followed by ω regions ($H = 0.177$) and β region ($H = 0.168$) and the lowest diversity was observed in α region ($H = 0.127$). Cluster analysis based on genetic distances resulted in grouping of the analysed accessions in to seven main groups. Though the level of diversity was relatively lower than other tetraploid wheat species from Ethiopia, the findings are indicative of the existence of variation in the collections which can be exploited for wheat improvement.

Key words: A-PAGE; genetic diversity; gliadin; *Triticum polonicum*

Analiza vzorcev gliadinov in raznolikosti akcesij poljske pšenice (*Triticum polonicum* L.) v Etiopiji

Izvleček: V raziskavi so bili s kisljo poliakrilamidno gelsko elektroforezo (A-PAGE) analizirani gliadini 25 akcesij poljske pšenice v 350 vzorcih semen z namenom določiti vzorce gliadinskih prog in ugotoviti obseg raznolikosti te pšenice v Etiopiji. Določeno je bilo 70 polimorfni prog in 68 različnih vzorcev. Osemnajst različnih mobilnih prog in 16 vzorcev je bilo določenih v območju ω -gliadinov, 22 prog in 20 vzorcev v območju γ -gliadinov, 12 prog in 22 vzorcev v območju β -gliadinov in 18 prog in 10 vzorcev v območju α -gliadinov. Poprečna genetska raznolikost, izračunana na osnovi podatkov analiziranih vzorcev za ta štiri območja gliadinov je bila 0,15. Območje γ je imelo največjo raznolikost ($H = 0,193$), sledili sta mu območji ω ($H = 0,177$) in β ($H = 0,168$), najmanjša raznolikost je bila ugotovljena v območju α ($H = 0,127$). Analiza grozdov na osnovi genetske oddaljenosti je združila analizirane akcesije v sedem glavnih skupin. Čeprav je raven raznolikosti nekoliko manjša kot pri drugih tetraploidnih vrstah pšenice v Etiopiji, so odkrita pokazala obstoj spremenljivosti v zbirkah, ki bi jih lahko uporabili pri izboljšanju pšenice.

Ključne besede: A-PAGE; genetska raznolikost; gliadin; *Triticum polonicum*

¹ Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia

² Corresponding author, e-mail: teleni08@gmail.com

1 INTRODUCTION

Focus given mainly to increasing grain yield and crop productivity has resulted in an increase in micronutrient deficiency in food grains (Garg et al., 2018). Agronomic and genetic biofortification are being used as a means to improve the deficiency of micronutrients in modern varieties. Exploitation of underutilized species is one option to meet the demand for enhanced nutrition requirements. Evaluation of minor wheat species may provide breeders with important sources of genes suitable for biotic and abiotic stress tolerance and nutrition improvement. Among the many underutilized crops *Triticum polonicum* L., commonly known as Polish wheat, offers valuable agronomical and nutritional benefits that can be incorporated in to wheat varieties currently in use. *Triticum polonicum* L., $2n = 28$, AABB), is a species which is grown sporadically in Africa (Ethiopia and Algeria), Europe (Spain, Italy, Ukraine and Russia) and some countries in the Asia temperate (USDA, 2020). Lately, *T. polonicum* is gaining attention due to its desirable traits. In comparison with durum and bread wheat, it has abundant protein and ash content and low fat content (Bieńkowska et al., 2019a) and it has good content of iron phosphorus, zinc, sulphur, magnesium, and boron, in addition to a lower content of aluminum (Bieńkowska et al., 2019b). Starch with up to 43.2 % amylase content was also reported in the grain of *T. polonicum* (Rodríguez-Quijano et al., 2003). Foods with high amylase content take longer to digest hence to improve diet, cereals with high amylase content are needed (Vaziri et al., 2014). It is also shown to have potential and useful attributes in durum wheat breeding for drought tolerance (Hakimi et al., 1998).

Since most of the new varieties of wheat are notably poorer in mineral content breeders have specified *T. polonicum* as a genetic resource with good potential to enhance wheat's nutrition, specifically for genetic biofortification of durum and bread wheat (Bieńkowska et al., 2019b). The species is also reported to show satisfactory resistance to *Fusarium* head blight (Wiwart, 2013), and could be a potential source of semi-dwarfing genes for the development of durum wheat cultivars (Watanabe, 2004). *T. polonicum* can be used in wheat breeding programs since it can be crossed and produce fertile and genetically stable hybrids with *T. aestivum* L. and *T. durum* Desf. (Akond et al., 2006; Hakimi et al., 1997).

Gliadins are mainly monomeric proteins that are not affected by environment (Lookhart & Finney, 1984) and coast effective hence they make good tool for identifying genetic differences and determine quality (Bushuk & Zillman, 1978; Payne, 1987). In prior studies high and low molecular weight glutenin subunits composition of

Ethiopian tetraploid wheat (Hailu et al., 2006) and allelic variation of gliadin coding loci of improved durum wheat varieties from Ethiopia (Hailegiorgis et al., 2017) have been reported. *T. polonicum* is one of the minor tetraploid wheat species cultivated in Ethiopia with limited importance, usually grown mixed with *T. durum* and *T. aestivum* (Demissie & Habtemariam, 1991) with earlier reports of cultivation in pure stands as well (Engels & Hawkes, 1991). It is a neglected crop in terms of conservation, research and utilization. The diversity of gliadin patterns in Ethiopian tetraploid wheat has not been reported so far. Genetic diversity study in conserved genetic resources of the species is important to better utilize and manage the resource. This study examined gliadin pattern and diversity of some *T. polonicum* collections from Ethiopia.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL AND GLIADIN EXTRACTION

Twenty five *T. polonicum* accessions from Ethiopia obtained from the Ethiopian Biodiversity Institute were used for this study. Fourteen seeds from each accession (a total of 350 individual seed samples) were used for gliadin profiling and diversity assessment. Each wheat grain was crushed using mortar and pestle and gliadin was extracted with 70 % (v/v) ethanol allowing the mixture to stand at 40 °C for 45 minutes and at +4 °C until electrophoresis. Aliquot of the supernatant was mixed with sample buffer (18 % sucrose containing methyl green tracking dye) before loading 25 µl of the extract on the gel.

2.2 A-PAGE

Glidins were fractionated using acid -polyacrylamide gel electrophoresis (A-PAGE), containing acrylamide (7.5 %) and bis-acrylamide (0.375 %) with ferrous sulfate (0.009 %) and ascorbic acid (0.00018 %) polymerized by the addition of hydrogen peroxide (15 %). Electrophoresis was performed at 50 V, 150 V and 250 V for 10 min each, at 350 V for 60 min and at 550 V for 80 minutes in 0.05 M aluminum lactate (pH 3.1) buffer. The cultivar *Icaro* was used as a standard in each gel run to compare electrophoretic bands and patterns. At the end of the run, gels were fixed using 12 % trichloroacetic acid for 15 minutes and stained overnight in staining solution containing 0.04 % (w/v) Coomassie brilliant blue R-250 in 100 ml ethanol and 80 ml 12 % trichloroacetic acid.

After destaining using tap water, gels were photographed on a white illumination source for documentation. Each sample was run twice to confirm the gliadin profile.

2.3 DATA ANALYSIS

Banding pattern of each individual seed was compared within and among accessions and the standard cultivar to distinguish the different gliadin bands and patterns. Each different band of individual seeds displayed by gliadin loci was scored for its presence (1) or absence (0). The data obtained were entered as a binary data matrix and used to perform genetic diversity measures using GenALEX 6.5 (Peakall & Smouse, 2012). Gene diversity was computed as the expected heterozygosity (H) based on band frequencies of gliadins. Genetic distance matrix was used to perform cluster analysis using the unweighted pair-group method for the arithmetic average (UPGMA) (Sneath & Soakal, 1973) clustering method by TFPGA version 1.3 (Miller, 1997).

3 RESULTS AND DISCUSSION

3.1 GLIADIN ELECTROPHORETIC PATTERNS

Twenty five different accessions of *T. polonicum* represented by 350 samples were analyzed by A-PAGE. Electrophoretic profile for representative accessions is shown in Fig.1. Assuming that the bands with the same relative mobility represent the same subunit, a total of 70 different bands and 68 gliadin patterns were detected. The average number of gliadin bands per accession was 24 and it ranged from 14 to 37. These bands were grouped into

patterns at each of the four gliadin zones (α , β , γ and ω gliadins). The patterns within each gliadin group of α , β , γ and ω were identified by comparing banding patterns of each sample with each other and the standard cultivar.

Gliadin patterns in the four gliadin regions observed in the analysed samples is depicted in Fig. 2. Based on the observed gliadin profiles, 10 different gliadin patterns and 18 different mobility bands were identified in the α -gliadin region which showed limited variation compared to the other zones. Each α -gliadin pattern showed two to five bands. The most frequent pattern was pattern 1, observed in 84 % of the analysed accessions followed by pattern 2, 3 and 5 each observed in 16 % of the accessions. Twenty two different gliadin patterns were identified in the β -gliadin zone each pattern showing three to six bands. The most frequent pattern was pattern 1 detected in 30 % of the accessions followed by pattern 20 observed in 20 % of the accessions. In γ -gliadin zone, 20 different patterns having one to five bands in each pattern were detected. Pattern 1 was the most frequent one appearing in 68 % of the accessions. In ω -gliadin zone, 16 different patterns having three to seven bands were observed. Pattern 1 was the most frequent pattern encountered in 44 % of the accessions followed by pattern 11 appearing in 20 % of the accessions. The number of gliadin bands and patterns, 70 and 68 respectively, detected in the present study is higher than that of Pan et al. (2007) who reported 48 gliadin bands and 65 patterns in *T. polonicum* accessions from different countries. In other studies, Zaefizadeh et al. (2010) reported 66 polymorphic bands and 81 patterns in durum wheat landraces and Ojaghi & Akhundova, (2010) reported 48 bands and 47 patterns in double haploid *T. aestivum*. When comparing the different gliadin regions, α -gliadin region showed the lowest number of band patterns which is similar to find-

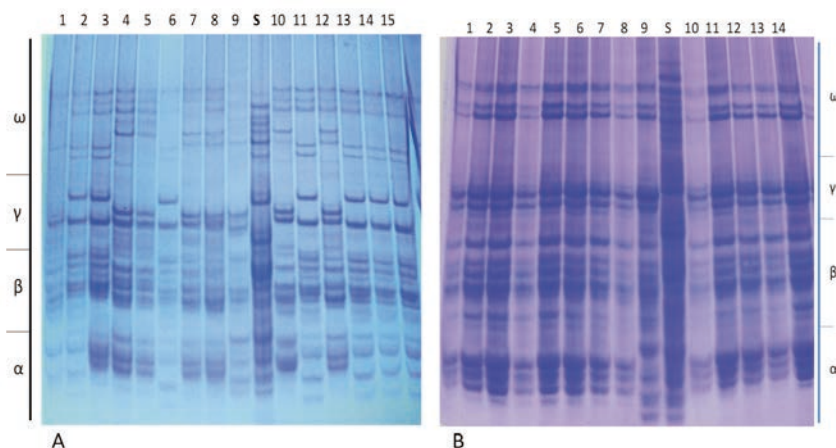


Figure 1: Gliadin electrophoretic patterns on A-PAGE for representative *T. polonicum* accessions showing within accession variation (A) and no variation within accession (B).

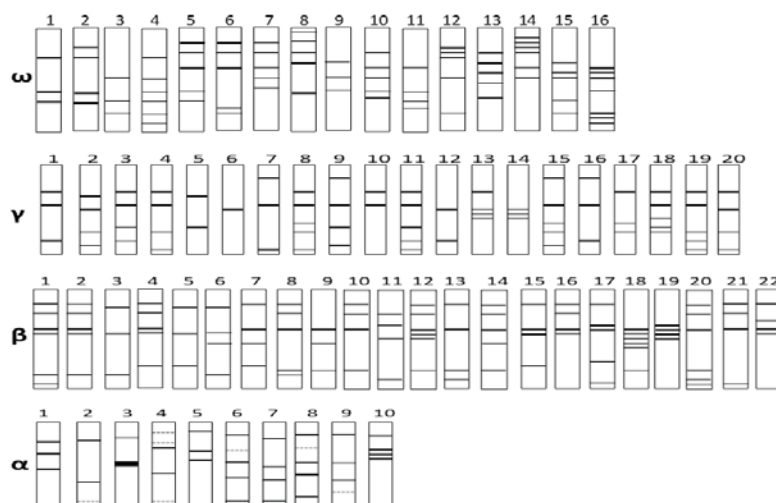


Figure 2: Electrophoregram of different gliadin patterns in α , β , γ , and ω gliadin regions observed in the analysed samples.

ings in studies on other wheat species (Ram et al., 2005; Aliyeva et al., 2012).

3.2 GENETIC DIVERSITY

A total of 70 bands were scored among the studied samples. The overall frequencies of these bands ranged from 0.007 to 0.871 among all the populations. The band with the highest frequency was detected in γ region while bands with the lowest frequency were detected in γ and α gliadin regions. The majority of the bands (74.28 %) showed frequency of < 25 %. The number of bands within each accession ranged from 14 to 37. Within accessions, the percentage of polymorphic bands ranged from 0.00 to 47.14 and the gene diversity (H) ranged from 0.00 to 0.153. The highest value was recorded in accession 23 (accession no. 226725) while five accessions (209774, 222726, 222632, 222242 and 226441) showed no within accession variation.

The overall gene diversity in the samples based on the patterns observed for each of the four gliadin regions showed that the γ region has the highest diversity ($H = 0.193$), followed by ω regions ($H = 0.177$) and β region ($H = 0.168$) and the lowest diversity was observed in α region ($H = 0.14$). In all the *T. polonicum* samples (350 entries) all bands were polymorphic and the mean gene diversity estimate was 0.167. The genetic diversity is relatively lower than other tetraploid wheat from Ethiopia examined using seed storage protein (Hailu et al., 2006) and microsatellite markers (Teklu et al., 2006; Yifru et al., 2006). Assessment of agromorphological characteristics of *T. polonicum* from Ethiopia had also indicated that phenotypic variation is relatively small except for the

apical tooth and awn length (Demissie & Habtemariam, 1991). Limited cultivation of the species might have caused genetic erosion resulting in low genetic variation. Hailu et al (2006) reported 100 % genetic erosion of *T. polonicum* in Tigray and Gojam regions of Ethiopia and 84.4 % genetic erosion throughout the country. Yifru et al. (2006) also reported a much localized use of *T. polonicum* which might result in its loss. Tetraploid wheat used to occupy 60 % of the total wheat area (Tessema and Belay 1991). However, because of broader adaptability and yield advantage, bread wheat has been expanding in area of production substituting traditional durum and emmer wheat (Tsegaye & Berg, 2007). Recent reports estimate that tetraploid wheat accounts for around 40 % of the total wheat production (Brascesco et al., 2019), however, detail data on production of the different tetraploid wheat species is lacking.

3.3 ANALYSIS OF RELATIONSHIP

Nei's genetic distance (1972) among the accessions ranged from 0.00 to 0.538. The lowest genetic distance was observed among accessions 18, 19 and 21 (0.00) followed by accession 2 and 3 (0.0002). The highest distance was observed between accessions 22 and 24 (0.538) and accession 22 and 15 (0.529). The UPGMA clustering, based on Nei's genetic distance, for the 25 accessions revealed seven major groups (Fig. 3) containing one to 10 accessions per group. Group A comprises of accessions that showed no within accession diversity while group C contains accessions with the relatively high values of gene diversity, percentage of polymorphic bands and number of bands per accession.

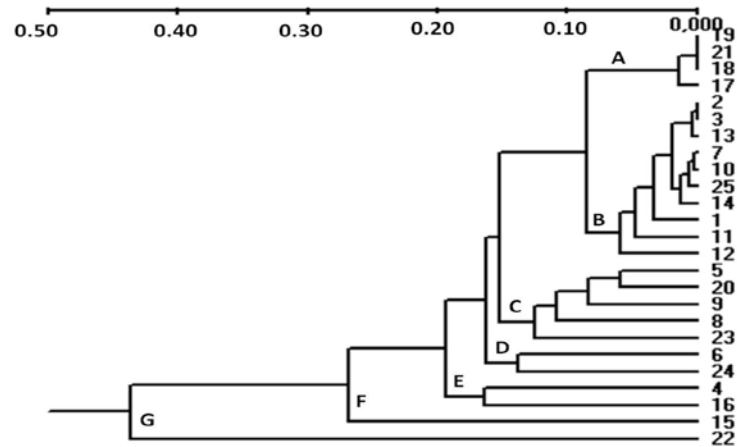


Figure 3: Dendrogram showing relationship among 25 *T. polonicum* accessions based on Nei's genetic distance.

4 CONCLUSION

Despite lower diversity revealed in the analysed accessions, the findings reported here have important implications for *T. polonicum* conservation and utilization in the country. In the Ethiopian genebank there are very few *T. polonicum* accessions. However farmers cultivate *T. polonicum* mixed with other wheat species hence wheat accessions conserved as *Triticum* spp also include *T. polonicum* as shown by activities that splits these mixed accessions in to their respective species. Splitting mixed species and additional collection of the species from potential growing areas need to be conducted so that the species can be used in improving wheat varieties in nutrient composition and disease resistance. Analysis of nutrient content and diversity analysis using molecular markers need also be carried out in order to reveal further the potential value of the species.

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