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INTER AND INTRA-SPECIFIC VARIATION IN SDS-PAGE OF SEED PROTEINS OF THREE *POA* L. (POACEAE) SPECIES

AHMET TAMKOC[1] AND EMINE ARSLAN[2*]

[1]*Department of Field Crops, Faculty of Agriculture, Selcuk University, 42075 Konya, Turkey.*
[2]*Department of Biology, Faculty of Science, Selcuk University, 42075 Konya, Turkey*
[*]*Corresponding author E-mail: earslan@selcuk.edu.tr*

Abstract

Total seed storage proteins were analyzed in genotypes belonging to three *Poa* species collected from Turkey. In this study, SDS-PAGE method was preferred to inter and intra-specifically determine the genetic relationships of *Poa* species in order to facilitate genotype selection in breeding programs. Individual electrophoregrams of the species obtained with SDS-PAGE can be used as password data for their genetic relationships. Polypeptide patterns ranged from 10 kDa to 128 kDa were calculated according to Nei homology using Bio1D++ computer program. Genetic and morphological differences between the *Poa* species are discussed. The dendrogram obtained with UPGMA clustering method indicated a low intra-specific genetic diversity while *P. pratensis* and *P. trivialis* genotypes stated closer relation as compared to *P. angustifolia* genotypes. It was concluded that seed storage protein profiles could be useful markers in the studies of genetic diversity and genetic relationships of *Poa* species. Additionally, this method can be useful to determine the correct starting material for plant breeding.

Introduction

The *Poaceae* is one of the largest angiosperm families with ca. 10000 species (Doyle *et al*., 1992). It has 142 genera in Turkey. The genus *Poa* is represented by 24 species, of which 3 are endemics in Turkey (Davis *et al*., 1988). Several species of this family are ecologically and economically important. Grasses from these species are natural main feeding resources for wild and domesticated animals. Similarly, cereal crops (wheat, rye, rice, maize, millet) are essential food components for human (Schantz, 1954).

Kentucky bluegrass (*Poa pratensis* L.) is a valuable crop which -under temperate climatic conditions- can be used for many purposes, including livestock feeding (hay harvesting and grazing), lawn planting, and soil improvement (Batygina & Mamet'ev, 1979; Nijs, 1990; Shishkinskaya *et al*., 1994; Baturin *et al*., 1998). Narrow leaved bluegrass (*Poa angustifolia* L.) has been used for the establishment of lawns and soil conservation purposes. Rough bluegrass (*Poa trivialis* L.) is used in lawn and wet areas, and it is preferred due to its capability of growing in cold and lands in shadow (Watson & Dallwitz, 1992).

Since *Poa* species show high level of morphological homogeneity, it is difficult to identify them depending on phenotypical differences. Hence, new procedures are needed to use in genotype identification (Matzk, 1992; Kutlunina, 1992). Genetic markers (exclude biochemical markers) that are not affected by environmental conditions are especially preferred in genetic studies and breeding in *Poa* species. Recently, biochemical methods that include electrophoretic analysis of endosperm proteins are commonly used onto taxa that are phenotypically closely related (Babaoğlu *et al*., 2004). Seed protein electrophoresis has specified as a beneficial method for discrimination of several economically important grass genotypes (Nakamura, 1979; Spoor & Hay, 1979;

Gardiner *et al*., 1986; Gardiner & Forde, 1987; Murphy *et al*., 1990; Van Dreven *et al*., 1990; Freeman & Yoder, 1991; Cooke, 1992). Since these proteins and their complex electrophoretic analysis reveal intra-species polymorphism, certification and identification of genotypes and their offspring as well as making analysis in populations become possible (Konarev, 1983; Sozinov, 1985; Semikhov, 1991; Agafonov & Agafonov, 1992).

Working material that includes *P. angustifolia*, *P. trivialis* and *P. pratensis* species, have been collected from natural pasture for breeding purposes. SDS-PAGE method was used in determination of genetic diversity, at inter- and intra-species level. By applying SDS-PAGE, selection of genotypes which is hard to distinguish from their phenotypes, were carried out in a shorter time by using differences of seed storage protein profiles.

Materials and Methods

Plant material: For seed protein profiles, 3 genotyping belonging to *Poa angustifolia* species, 11 genotypes belonging to *P. trivialis* species and 9 genotypes belonging to *P. pratensis* species were examined. Genotypes of *P. pratensis* and *P. trivialis* were collected from an old natural pasture, located at the Kalecik region of the province Ankara and would probably become extinct, genotypes of *P. angustifolia* were collected from Toros Mountains, in 2003-2007.

Protein extraction: Protein extraction was performed according to Saraswati *et al*., (1993). Seeds were ground to fine powder with mortar and pestle. Sample buffer was added to 0.04 g of seed flour as extraction liquid and mixed thoroughly in Eppendorf tube with vortex. The extraction buffer contained the following final concentration: 0.5 M Tris-HCl (pH 6.8), 10% SDS, urea and 5% 2-merkaptoethanol. Before centrifugation at 10.000 g for 5 min (4˚C), the sample buffer was boiled for 5 min.

SDS-PAGE: SDS-PAGE was performed by a standard method on a vertical slab gel. Bromophenol blue was added to the supernatant as tracking dye to watch the movement of protein in the gel. Seed proteins were analyzed using 10% polyacrylamide gel (Laemmli, 1970). After electrophoresis, the protein bands were visualized by staining with Coomassie Brillant Blue G-250. Marker proteins (Fermentas) were used as reference. Molecular weights of the protein bands were estimated by their relative mobilities.

Data analysis: The polymorphic bands were scored visually as present (1) or absent (0). Genetic similarities among genotypes were estimated based on Nei homology using Bio1D++ computer program. Cluster analysis was performed using the UPGMA.

Results and Discussion

Total seed proteins belonging to genotypes of three *Poa* species were analyzed through the slab type SDS-PAGE using 10% polyacrylamide gel. When variations in storage protein banding patterns were investigated, intra-species genetic diversity appeared at a low level. This would be attributed to the fact that *Poa* genotypes worked on, were collected from a narrowly restricted pasture land.

In all, 70 polypeptide bands of different sizes ranging from 10 to 128 kDa were observed in the 23 genotypes that belong to these species (Fig. 1). Cluster analysis of the seed storage proteins of 23 genotypes was performed on the results of SDS-PAGE using unweighted pair-group method using arithmetic averages (UPGMA). The results of cluster analysis are given in the dendrogram (Fig. 2) prepared using Nei homology statistic software.

The dendrogram of total seed protein based on similarity matrix showed that two groups with 28% similarity are formed. First group includes *P. angustifolia* genotypes, while the second group includes *P. trivialis* and *P. pratensis* genotypes with 42% similarity. While in the first group the genotypes assigned as *P. angustifolia* 1 and 2 presented 84% similarity, the genotype *P. angustifolia* 3 was formed with 50% similarity to the genotypes 1 and 2. (Non-common bands of *P. angustifolia* 3 were shown by pale arrow in Fig. 1). This was probably due to natural hybridization between species. In the first clad of the second group, consisted of *P. trivialis* genotypes, similarities ranged from 86% to 100%.

P. trivialis genotypes 1, 2, 3, 9, 10 and 11 were exactly the same. *P. trivialis* 5, 7 and 8 were also exactly same with each other and only 6% genetically different from the remaining ones (someone from common bands of these genotypes are shown by pale arrow in Fig. 1). Genotype 6 shared 93% similarity to these genotypes, while this ratio remained at 87% when the genotype 4 was concerned. Heterogeneity was found to be quite low within *P. trivialis* genotypes. Generally, common band patterns were observed within genotypes with few exceptions. Because these genotypes were grown in a narrow area, intra-spesific natural hybridizations possibly increased their genetic relatedness.

In the second clad of the second group that consisted of *P. pratensis* genotypes, with 84-100% similarity range 1, 3, 5, 9 and 11 numbered *P. pratensis* genotypes formed a subgroup among themselves. The 9 and 11 numbered *P. pratensis* genotypes presented exactly the same protein profiles, while number 1 genotype has also shown close similarity (90% similarity) (Common and non-common bands of 1, 9, 11 genotypes were showed by dark arrow heads, pale arrows and pale arrow heads in Fig. 1). In the same cluster 3 and 5 numbered *P. pratensis* genotypes were 84% similar to these genotypes while they presented 100% similarity among themselves. Another group which has 79% similarity with the above-mentioned group consisted of 2, 4, 6 and 10 numbered *P. pratensis* genotypes with similarity range 86-100%. While genotypes 2, 4 and 6 are exactly similar to each other (Common bands demonstrated with dark arrow in Fig. 1), 10 numbered *P. pratensis* genotype was 14% far away from them.

Among the three studied material, while the most genetic diversity has occurred in *P. angustifola* genotypes, the least genetic diversity revealed in *P. trivialis* genotypes. Genetic relationship was lower among the species according to seed storage protein. Although it is declared by some researchers (Funk *et al*., 2003) that *P. angustifolia* is similar to *P. pratensis* as morphology, in this research it was observed that *P. pratensis* is closer to *P. trivialis*. Though these *Poa* genotypes were similar in morphology, the obvious differences between these genotypes include the presence of stolon in *P. trivialis,* rhizome in *P. pratensis* and the longer length of rhizome in *P. angustifolia* as compared with *P. pratensis*. However, according to protein profiles while it is expected that *P. pratensis* and *P. angustifolia* genotypes should be more familiar by having rhizome, *P. pratensis* and *P. trivialis* stated closer than *P. angustifolia*. This situation would be attributed to the fact that *P. pratensis* and *P. trivialis* genotypes are grown in the same location which allows them to achieve gene-flow while *P. angustifolia* grows in location, 350 km away, hence no gene-flow.

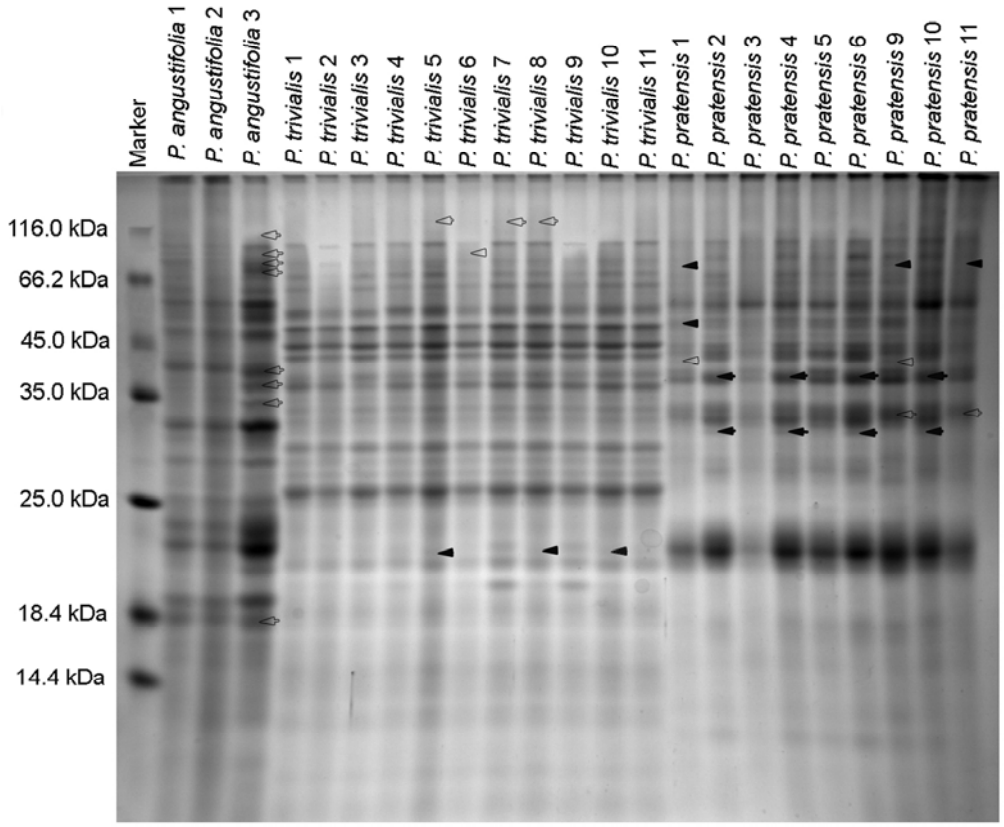

Fig. 1. SDS-PAGE protein profiles of genotypes belong to three *Poa* species.
Pale arrows showing non-common bands of *P. angustifolia* 3 genotype, pale arrows and dark arrow heads showing common bands of some genotypes of *P. trivialis*, and dark arrow showing common bands of 2, 4, 6, 10 genotypes of *P. pratensis*, pale arrows heads and dark arrow heads showing common and non-common bands of 1, 9, 11 genotypes of *P. pratensis.*

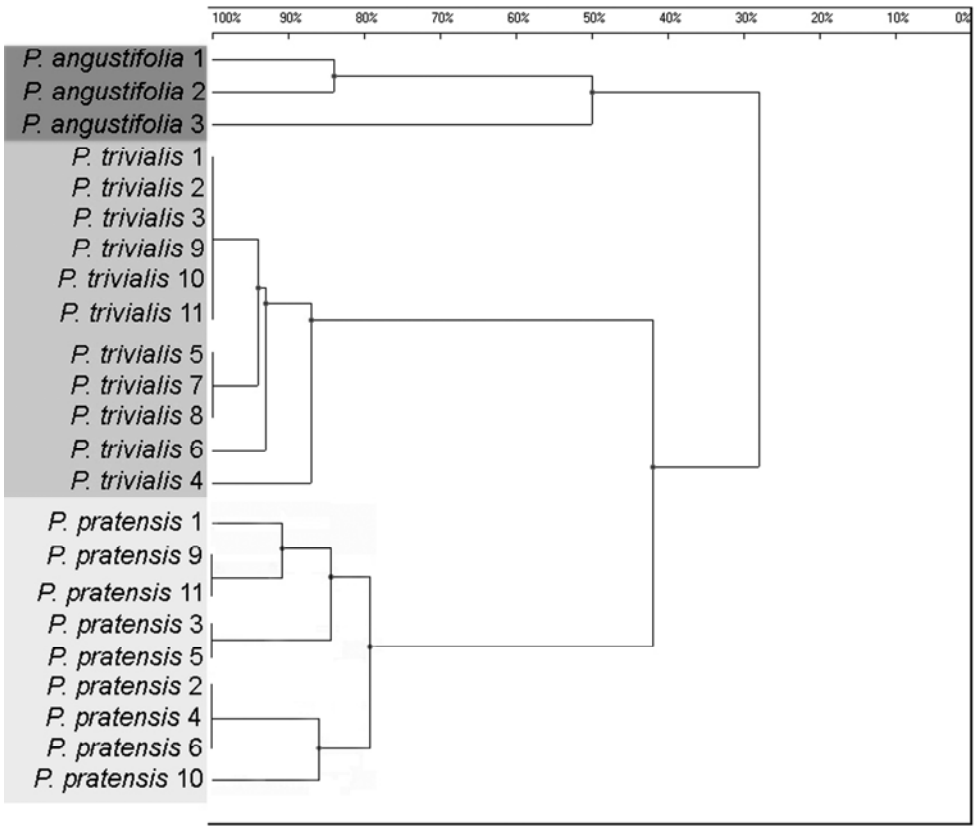

Fig. 2. UPGMA dendrogram showing genetic homology among genotypes belong to three *Poa* species.

Seed storage proteins are good markers for assessing taxonomic and phylogenetic relationships at various levels from species to subfamilies. Hence, seed proteins are easy to use, widely applicable markers for various systematic problems (Chen *et al*., 1997). Also seed protein characters were successfully used in the systematic and phylogeny of the grasses (Shotwell & Larkins, 1989). According to Agafonov *et al*., (2001), the data obtained from SDS-PAGE analysis of eight standard samples of *P. pratensis* cultivars showed that this method was adequate to determine and certificate the maternal genotype by analyzing the progeny phenotypes.

Our results have demonstrated that selection of distinct genotypes within a species increases the chance of a successful breeding program. At the same time by selecting dissimilar genotypes, no time, effort and money are wasted. It is concluded that, seed storage protein electrophoresis is a practical and successful method in rapidly selecting suitable genotypes to be used in breeding programs.

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