# Variability in Seed Protein of Two Cultivated Species of Barnyard Millet Through SDS-PAGE

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Seed protein variability of four barnyard millets belonging to two cultivated species viz., *Echinochloa frumentacea* and *E. crusgalli* ssp. *utilis* were examined through SDS-PAGE. Morphological traits and potentiality of high seed setting at different environments (March, April, May and June sowing) indicated that the latter showed resistance/ tolerance to cold and smut disease of grain at high altitudes (2100 m and above) of Himalayas. Differences in banding patterns and intensity in colour of bands revealed that characterization and identification of genotypes belonging to these two cultivated species is possible through analysis of protein subunits.

**Key Words: Barnyard Millet, Cold Tolerance, Seed Protein, SDS-PAGE**

Among different minor crops, barnyard millet (*Echinochloa* species) possesses distinct position for its cultivation and consumption in India, particularly in hills of Uttaranchal. Very little attention has, however, been paid for the genetic improvement of this crop towards augmenting its yield potential. Heterogeneity of environment and exposure to low temperature at higher elevation cause economic loss in both grain and fodder yield of barnyard millet cultivars. Moreover, in absence of wide genetic diversity among local cultivars and released varieties, the selection for adaptation to cold temperature is discouraging (Gullord *et al.*, 1975). In order to explore superior genetic potential for both grain and fodder yield, SDS-PAGE analysis of total seed protein was carried out among four genetically diverse cultivars of barnyard millet for identification, characterization (Dontsova and Pausheva, 1979; Dolinsek, 1980; Shevchuk, 1985; Hussain *et al.*, 1989; Nishiyama *et al.*, 1991; Rogl and Javornik, 1996) and tolerance to biotic and biotic stresses (Padmavathy *et al.*, 1999; Pattnaik and Kole, 2002).

## Materials and Methods

Four barnyard millet genotypes belonging to two different cultivated species viz., *Echinochloa frumentacea* (Local-1) and *E. crusgalli* ssp. *utilis* (PRB 9402, PRB 9403 and PRB 9404) of Asiatic origin (Halaswamy *et al.*, 2001) were grown in March, April, May and June in two consecutive years, 1997 and 1998 in randomized block design with four replications at Hill Campus (21000 m and above), G.B Pant University of Agriculture and Technology, Ranichauri (30° 15' N latitude and 7°02' E longitude). Twenty-five morphological and yield characters were recorded for respective sowings. Mean values of two years' field observations were subjected to statistical analysis.

About 200 seeds of each genotype were milled. Total seed protein was extracted at room temperature using 0.14 M Tris-HCI sample buffer (pH 6.8), 4% SDS, 3% 2-mercaptoethanol, 20% glycerol, 0.02% bromophenol blue (Rogl and Javornik, 1996). Prior to centrifugation at 10,000 rpm for 15 minutes, 0.5 g flour with 500 µl extraction buffer was incubated in boiling water for 2-3 minutes. About 25 µl of extract containing protein concentration of 0.050, 0.054, 0.057 and 0.060 in Local-1, PRB 9403, PRB 9402 and PRB 9404, respectively were loaded in individual well for one run.

SDS-PAGE was conducted following the procedure of Laemmli (1970) with a few modifications. The final concentration of separating gel was 15% acrylamide, 0.375 M Tris-HCI (pH-8.8), 0.1% SDS and 0.8 bisacrylamide. The stacking gel contained 3% acrylamide, 0.125 M Tris-HCI (pH 6.0), 0.1% SDS and 0.8% bisacrylamide. The electrode buffer contained 0.025 M Tris-HCI, 0.192 M glycline and 0.1% SDS. Electrophoresis was carried out for 5 hours at constant current of 20 mA per gel. The gels (1.0 mm thick) were stained in 0.1% coomassie brilliant blue R staining solution with 45% methanol and 0.2% acetic acid. The intensity of bands was measured in terms of optical density (O.D.) at 660 nm with spectrophotometer. Intensity of bands was classified as dark, medium dark and light with optical density range of 1.5 to 1.8, 1.0 to 1.4 and < 1.0, respectively. Five molecular weight markers viz., phosphorylase B (97.4 kD), bovine serum allbumin (67.0 kD), egg albumin (45.0 kD), carbonic

**Table I. Morphological characters and yield performance of barnyard millet genotypes**

| Species | Domesticated | Chromosome no. (2n) | Genotype | Special features |
|---|---|---|---|---|
| *Echinochloa* | Asia/China | 36 | Local-1 | Plant height about 150 cm, susceptible to cold and smut disease, seed sets only in March sown, no seed setting in June sown, Yield ranges from 6 to 12 q/ha. |
| *Echinochloa crusgali* ssp. *utilis* | Asia/Japan | 54 | PRB-9402 | Plant height about 80 cm. Medium maturing ear head small, compact and awnless, cold tolerant, seeds sets in March to June sowing, yield 12-16 q/ha. Resistant to smut disease. |
| | | | PRB-9403 | Plant height about 190 cm, Medium maturing, Inflorescence large awned type club shaped, pendent to erect, cold tolerant, seed sets in March to June sown crop, resistant to smut disease, yield 28-36 q/ha. |
| | | | PRB-9404 | Plant height about 190 cm, early maturing, ear head large, erect, awnless and lax type, cold tolerant, resistant to smut disease, seed sets in March to June sowing, grain yield 28-36 q/ha. |

**Table 2. Mean performance of different quantitative characters in four barnyard millet genotypes over four dates of sowing in two years.**

| Characters | Local-1 | PRB 9402 | PRB 9403 | PRB 9404 | C.D. 0.05 |
|---|---|---|---|---|---|
| Plant height (cm) | 98.08 | 80.00 | 118.29 | 104.75 | 15.50 |
| Days to 50% flowering | 116.00 | 10.3.6 | 105.45 | 93.26 | 9.53 |
| Grain filling (days) | 48.62 | 45.17 | 43.94 | 3.94 | 1.23 |
| Grain yield (q/ha) | 4.50 | 8.00 | 22.20 | 20.63 | 3.71 |
| Straw yield (t/ha) | 3.50 | 1.20 | 8.85 | 8.20 | 1.58 |
| Harvest | 0.11 | 0.40 | 0.19 | 0.20 | 0.26 |
| Diseases and pests | Grain smut disease appeared | Nil | Nil | | |

anhydrase (29.9 kD) and ribonuclease A (14.0 kD) supplied by E-Merk (Nidna) Ltd. were used as standards for determining the molecular weight of seed protein submits.

## Results and Discussion

Seed protein profiles of four barnyard millet genotypes viz., Local 1 (*Echinochloa frumentacea)* and PRB 9402, PRB-9403 and PRB0\9404 (*E. crusgalli* ssp. *utilis*) differed considerably for presence and absence of various protein subunits and intensity of colour of bands (Fig. 1). The gels could be divided into three zones viz., A, B and C. Zone A showed five protein bands ($A_1$, $A_2$, $A_3$, $A_4$ and $A_5$), in a range of 62.00 kD to 105.68 kD molecular weight. Zone B consisted of only one band with a molecular weight of 43.00 kD whereas, zone C had maximum 6 bands i.e., $C_1$, $C_2$, $C_3$, $C_4$, $C_5$ and $C_6$ falling in a range of molecular weight of 10.43 kD to 29.00 kD. The genotypes of two species did not show many differences in banding pattern of protein subunits at zone C except intensity of bands. This indicated that both the species possessed common protein subunits of low molecular weight while the differences existed only in high and low concentration of protein bands $A_3$, $A_4$ and $A_5$. A major variable protein subunit was observed in zone B. Variation in the band may be helpful in distiction between two species of barnyard millets. Some of the differences in morpho-physiological characters (Tables 1, 2) such as acclimation to cold, resistance to grain smut disease (Bandyopadhyay, 1998) and cross incompatibility (de Wet *et al.,* 1983) between two species may be governed by this protein subunit.

PRB-9402, PRB-9403 and PRB-9404 exhibited similar pattern of bands at zone A with minor differences. Dwarf genotypes PRB 9402 possessed high expression of all the subunits of seed protein depicted in profile. B and $A_1$ was absent in PRB 9403 while in PRB 9404 both $A_1$ and $A_2$ subunits were found missing. This leads to suggest that differences in banding pattern at zone A might be associated with identification and characterization of cultivars belonged to species *E. crusgalli* ssp. *utilis.*

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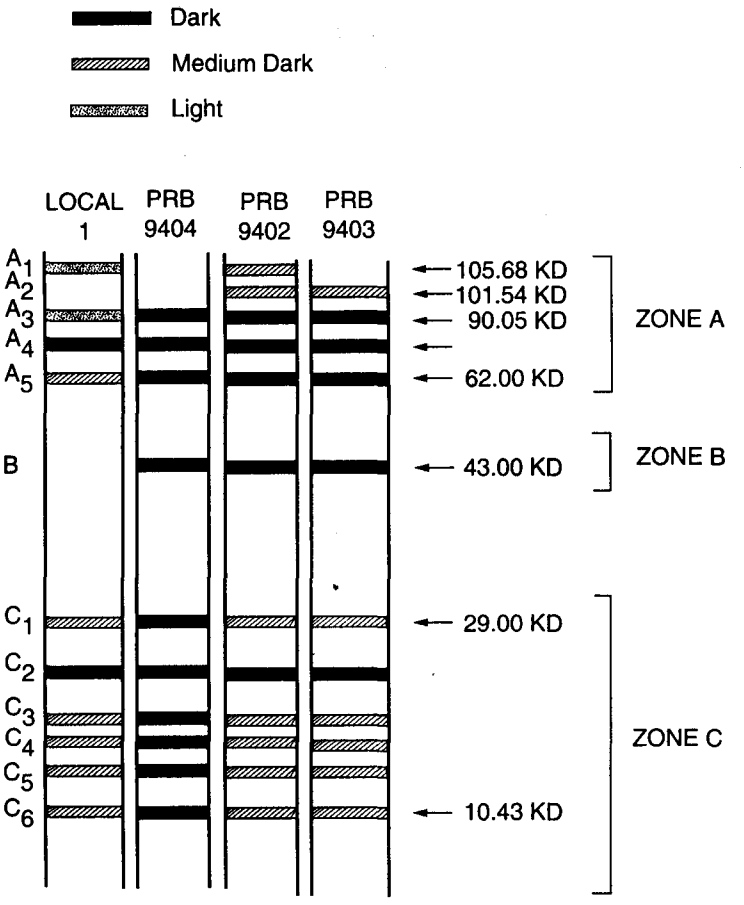


*Fig. 1. Variability in seed protein subunits in barnyard millet*

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