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Genetics and Plant Breeding

Identification of Oats (*Avena sativa* L.) Cultivars by Seed and Seedling Protein Electrophoresis

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Abstract

Soluble proteins were extracted from seed and seedlings of eleven oats cultivars to analyze the protein profile through SDS PAGE method. In seed protein electrophoresis totally 14 bands were observed, in these six bands were common to all cultivars. Based on the seed protein banding pattern two of these cultivars were differentiated easily. The unique band at Rf value 0.151 was common for all cultivars, but it was absent in cv. UPO 94. Similarly the band at Rf value 0.662 was present in all cultivars but it was absent in cv. Sabzar. In seedling protein electrophoresis totally nine bands were observed, four bands at Rf value 0.370, 0.524, 0.543 and 0.855 were common for all cultivars. The presence of band at Rf value 0.340 was common in all cultivars, it was absent in UPO 94 and the band at Rf value 0.641 was absent in all cultivars it was present in cv. Sabzar. In both seed and seedling protein cv. Sabzar and UPO 94 expressed the discernible banding pattern when compared to others. The other cultivars are differentiated based on the total numbers of bands, banding pattern, intensity of bands, specific presence or absence of bands.

Highlights

- Easy, quick and reliable method of cultivar identification
- Environmental factors could not affect the results
- Sabzar and UPO 94 were easily differentiated from other cultivars

Keywords: Oats, protein marker, SDS PAGE, cultivar identification

Oats is an important age old cereal cum forage crop, being cultivated from 2000 years ago. It has rich medicinal and nutritional value and being used as food by the diabetic patients, since the grains are filled with cholesterol fighting soluble fibre (Singh *et al.,* 2003). It is native to Asia Minor (Thellung, 1912) and widely distributed in temperate regions of the world. Among the cereals *Avena sativa* ranks fifth position after wheat, rice, maize and barley. At present large number of oats cultivars are available for commercial cultivation. Among the cultivars the identification or discrimination of cultivars is essential for quality seed production through the successful operation of seed certification programme and granting the plant breeders' right under the PVP and FR act. However, with the increase in the number of cultivars of each crop, it is difficult to distinguish the cultivars on the basis of morphological characters alone. This has led to the development of the new stable parameters such as use of their genetic material (nucleic acids and proteins) as a tool for varietal identification. Electrophoresis is a process of separation of different biomolecules under the influence of electric field and has been successfully applied for the identification of cultivars (Vishwanath *et al.,* 2011). Sodium Dodecyl

Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is most economical simple and extensively used biochemical technique for analysis of genetic structure of germplasm (Hameed *et al.,* 2009).

The electrophoresis technique is increasingly utilized as an additional approach for varietal identification and characterization based on storage protein. The uniformity of seed protein profiles is additive in nature and not much influenced by the environmental conditions makes it a unique and powerful tool for genotype identification (Ladizinsky and Hymowitz, 1979). Intrinsic changes in the plant such as chromosomal rearrangements or even doubling of the chromosome numbers leave no or very small effect on the seed proteins profile. They are also independent of cultivar morphology and physiology (Vijayageetha and Balamurugan, 2011), hence proteins can be regarded as markers for the structural genes. The analysis of protein composition can be considered to be an analysis of gene expression and can be used as an ideal means of varietal discrimination (Anitamann *et al.,* 2005). It is a rapid and relatively cheap method and eliminates the plants to grow upto maturity. Keeping in this view the present study was formulated to characterize the oats cultivars through storage seed and seedling protein electrophoresis technique.

Materials and Methods

Seed materials

The genetically pure eleven oats cultivars *viz.*, Sabzar, Kent, HFO 114, OS 6, UPO 212, OS 7, HJ 8, UPO 94, OL 9, JHO 822 and OL 88 obtained from Pant University of Agriculture and Technology, Uttar Pradesh, India were used for characterization based on protein profiles.

Preparation of sample

500 milligram of seed coat removed seed samples were ground using a pestle and mortar. The seed powder of each cultivar was taken in an eppendorf tube and 1 ml of defatting solution (Chloroform, methanol, Acetone - 2:1:1 ratio) was poured in each tube. After thorough shaking, the eppendorf tubes were left for 3 hours. The supernatant was decanted and this procedure was repeated for three times. The samples were then kept overnight at room temperature for drying. Next day, 0.5 ml of extraction buffer (0.1M phosphate buffer) was added and the samples were centrifuged using cooling centrifuge (4^0C) at 12,000 rpm for 20 minutes. In the case of seedling protein 1g of 10 days aged seedlings was taken. The seedlings were ground using a pestle and mortar. 0.5 ml of extraction buffer (0.1M phosphate buffer) was added and the sample was finely ground. It was taken into a clean eppendorf tube and centrifuged in cooling centrifuge (4^0C) at 12,000 rpm for 20 minutes. Both the supernatants were transferred into a fresh eppendorf tubes. To the supernatants, ice cold acetone was added in the ratio of 1: 3 and kept in deep freezer (-20°C) for 3-4 hours. Further, it was centrifuged using cooling centrifuge (4^0C) at 12,000 rpm for 20 minutes and the supernatant was poured off. The precipitated protein was freeze dried and dissolved with required quantity of 0.1M phosphate buffer. The concentrated protein extract was quantified through Bradford's method (1970).

Protein profiling through SDS PAGE

The extracted seed and seedling proteins were separated using vertical PAGE in a discontinuous buffer system at a monomer concentration of 30 per cent stock gel solution using 10 per cent separating gel containing 1.5 M Tris HCl (pH 8.8) and 4 per cent stacking gel containing 0.5 M Tris HCl (pH 6.8) method prescribed by Laemeli (1970) with slight modifications. Concentration of protein sample was adjusted to 150µg in a volume of 50µl, by mixing with sample loading buffer, boiling was done for 2-3 minutes to ensure complete interaction between proteins and SDS. The sample was cooled and carefully injected in each well with a micro syringe. Then electrophoresis was done under room temperature at 100V for 5 hours in an electrode buffer containing 0.5 M Tris and 0.192 M Glycine.

Fixing and staining

At the end of the electrophoresis the gels were removed and immersed in staining solution (0.1g Comassie Brilliant Blue R 250, 40% methanol, 10% glacial acetic acid, 50% distilled water) for overnight. After proper staining the gel was transferred to destainer (40% methanol, 10% glacial acetic acid, 50% distilled water) with gentle shaking. The destainer was changed frequently till appropriate visibility of the bands on gel and it was photographed.

Evaluation and documentation

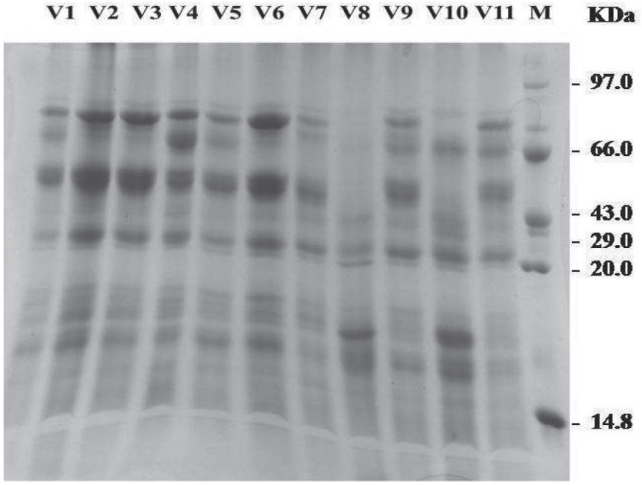
The distance moved by the tracking dye from the point of loading was measured on the gel. Then the distance traveled by each band was also measured. The complete gel imprints were made on the transparency sheets to determine their intensity. Based on the relative front value and intensity of band, electrophoregram were prepared for each gel. Relative front (Rf) of each band was calculated as follows

$$Rf = \frac{\text{Distance travavelled by the band}}{\text{Distance travelled by the tracking dye}}$$

Bands were numbered in the order of increasing Rf values. Apart from these, recording presence or absence of a band and the intensity of the band in each cultivar was critically observed for discriminating the cultivars.

Results and Discussion

Seed protein patterns can be used as a promising tool for distinguishing cultivars of particular crop species (Seferoglua *et al.*, 2006). The present study revealed that in seed protein electrophoresis the wider variation was observed in banding pattern Figure 1. The cultivars were differs among in number of bands, relative front value and intensity of bands, totally 14 low, medium, high and very high intensity bands were appeared. The detailed electrophoregram of total soluble seed protein has been presented in Figure 2. The entire protein profile was divided into five regions starting from A to E (97 KDa to 14.8 KDa). This was in the order of increasing Rf values and decreasing molecular weight of proteins. Among the five regions A (Rf value 0.151 to 0.173), C (Rf value 0.314 to 0.337) and E (Rf value 0.454 to 0.762) were most useful to differentiate the cultivars due to clear banding appearance also very high intensity bands were noticed in Rf value 0.173, 0.314, 0.382 and 0.613. The Rf value for all the bands found in the entire profile have been presented in Table 1. Maximum number of 14 bands was observed in



V1- Sabzar; V2- Kent; V3- HFO 114; V4- OS 6; V5- UPO 212; V6- OS 7; V7- HJ 8; V8- UPO 94; V9- OL 9; V10- JHO 822; V11- OL 88

Fig. 1: Total seed protein profile of oats cultivars through SDS PAGE

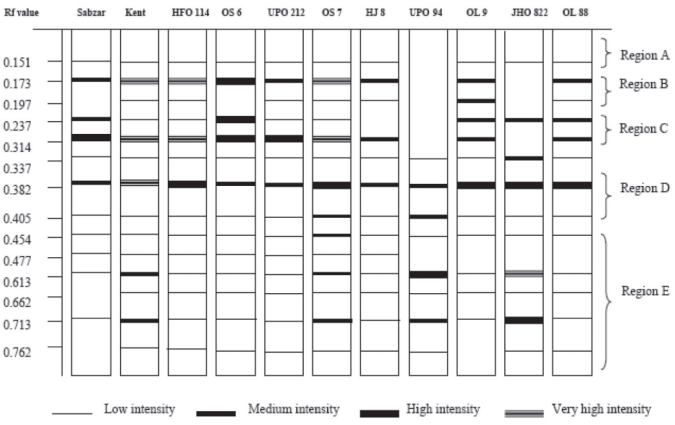
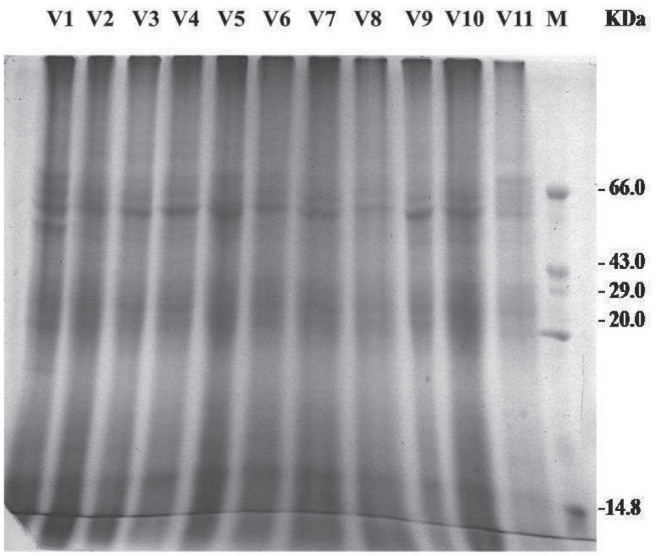


Fig. 2: Electrophoregram of total seed protein profile



V1- Sabzar; V2- Kent; V3- HFO 114; V4- OS 6; V5- UPO 212; V6- OS 7; V7- HJ 8; V8- UPO 94; V9- OL 9; V10- JHO 822; V11- OL 88

Fig. 3: Total seedling protein profile of oats cultivars through SDS PAGE

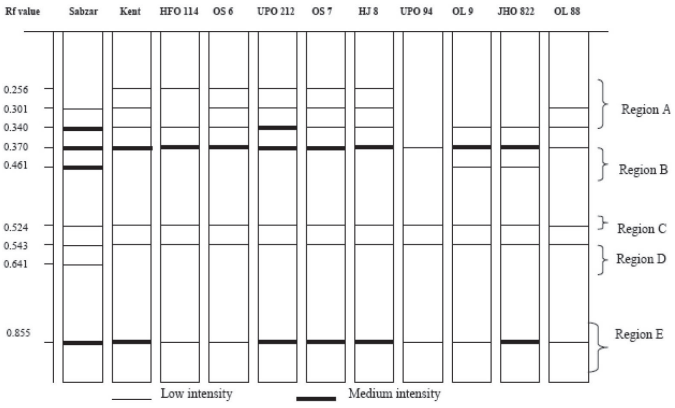


Fig. 4: Electrophoregram of total seedling protein profile

Kent, HFO 114, UPO 212 and OS 7. UPO 94 expressed minimum number of eight bands, where as in OS 6, HJ8 and OL 88 expressed 13 bands. OL 9 expressed 12 bands, Sabzar expressed 11 bands and JHO 822 expressed 10 bands. The six common bands (Rf value 0.337, 0.382, 0.405, 0.454, 0.613 and 0.713) for seed proteins were detected for all the eleven cultivars. In some cultivars the number of bands remained same, hence the presence or absence of bands were used for varietal characterization. The band at Rf value 0.151 was common for all cultivars, but it was absent in cv. UPO 94. Similarly the band at Rf value 0.662 was absent in cv. Sabzar.

In seedling protein electrophoresis, totally nine low and medium intensity bands were observed (Figure 3). The electrophoregram of all cultivars has been presented in Figure 4. The entire protein profile was divided into five regions starting from A to E, high intensity bands were observed in regions A (Rf value 0.370 to 0.461) and E (Rf value 0.855). The Rf value for all the bands found in the entire profile have been presented in Table 2. Maximum numbers of eight bands were observed in cv. Sabzar and minimum numbers of four bands were noticed in UPO 94, whereas cv. Kent, OS 6, UPO 212, OS 7 and HJ 8 expressed seven numbers of bands and cv. HFO 114, OL 9, JHO 822 and OL 88 expressed six numbers of bands. The four bands (Rf value 0.370, 0.524, 0.543 and 0.855) were common for all cultivars. The band at Rf value 0.340 was common for all cultivars, whereas, it was absent in UPO 94 and the band at Rf value 0.641 was absent in all cultivars it was present in cv. Sabzar were effectively used for discriminating these cultivars.

Table 1: Intensity and relative mobility of seed proteins of oats cultivars through SDS PAGE

Band No.	Rf value	Cultivars										
		Sabzar	Kent	HFO 114	OS 6	UPO 212	OS 7	HJ 8	UPO 94	OL 9	JHO 822	OL 88
1.	0.151	+	+	+	+	+	+	+	-	+	+	+
2.	0.173	++	++++	++++	+++	++	++++	++	-	++	-	++
3.	0.197	-	+	+	-	+	+	++	-	++	-	+
4.	0.237	++	+	+	+++	++	+	-	-	++	++	++
5.	0.314	+++	++++	++++	+++	+++	++++	++	-	++	-	++
6.	0.337	+	+	+	+	+	+	+	+	+	++	+
7.	0.382	++	++++	+++	++	++	+++	++	++	+++	+++	++
8.	0.405	+	+	+	+	+	++	+	++	+	+	+
9.	0.454	+	+	+	+	+	++	+	+	+	+	+
10.	0.477	+	+	+	+	+	+	+	-	-	-	-
11.	0.613	+	++	+	+	+	++	+	+++	+	++++	+
12.	0.662	-	+	+	+	+	+	+	+	+	+	+
13.	0.713	+	++	+	+	+	++	+	++	+	+++	+
14.	0.762	-	+	+	+	+	+	+	+	-	+	+

+ = Low intensity ++ = Medium intensity +++ = High intensity ++++ = Very high intensity

Table 2: Intensity and relative mobility of seedling proteins of oats cultivars through SDS PAGE

Band No.	Rf value	Cultivars										
		Sabzar	Kent	HFO 114	OS 6	UPO 212	OS 7	HJ 8	UPO 94	OL 9	JHO 822	OL 88
1.	0.256	-	+	+	+	+	+	+	-	-	-	-
2.	0.301	+	+	-	+	+	+	+	-	-	-	+
3.	0.340	++	+	+	+	++	+	+	-	+	+	+
4.	0.370	++	++	++	++	++	++	++	+	++	++	+
5.	0.461	++	-	-	-	-	-	-	-	+	+	-
6.	0.524	+	+	+	+	+	+	+	+	+	+	+
7.	0.543	+	+	+	+	+	+	+	+	+	+	+
8.	0.641	+	-	-	-	-	-	-	-	-	-	-
9.	0.855	++	++	+	+	++	++	++	+	+	++	+

+ = Low intensity ++ = Medium intensity

Electrophoresis of proteins is a powerful tool for identification of genetic diversity and the SDS PAGE is particularly considered as a reliable technology because seed storage proteins are highly independent of environmental fluctuations (Javid *et al.,* 2004). In mature seeds type and amount of proteins are more constant than other plant tissues (Magni *et al.,* 2007) therefore, the SDS PAGE pattern of seed storage proteins showed polymorphism on the basis of difference in protein intensity among cultivars (Sadia *et al.,* 2009). Numerous studies have already been conducted for examining the protein banding pattern in important crops for varietal identification (Dadlani and Varier, 1993). The protein banding patterns are specific to each cultivar. When compared to seed protein profiles, seedling protein profiles lacked clarity and sharpness. This might probably due to the interference of fat with the proteins or due to the diffusion of proteins (Sandhya, 2000). Similarly seed protein electrophoresis was successfully employed to discriminate the cultivars was reported by Varma *et al.*, (2005) in maize, Ehsanpour *et al,*. (2010) in Iranian Pistachios, Vijayageetha and Balamurugan (2011) in mustard and Singh *et al,*. (2013) in Coriander. The present investigation revealed electrophoresis (SDS PAGE) of seed and seedling protein can be economically used to assess the genetic purity of the cultivars.

Conclusion

Based on the results of this study, it can be concluded that Sabzar and UPO 94 cultivars were easily identified based on specific banding pattern in both seed and seedling protein electrophoresis and the reaming cultivars are discriminated from other cultivars based on individual banding patterns.

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