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VARIABILITY IN EGGPLANT (*Solanum melongena* L.) CULTIVARS AS REVEALED BY SDS-PAGE OF SEED PROTEIN

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ABSTRACT : Fourteen genotypes were distinguished into nine groups on different banding patterns in three zones (A, B and C). PB 64 and PB 66; BARI and Pant Rituraj; KS 331, PB 69 and Punjab Sadabahar; PB 70, SMB 115 and PB 67 fell in four different groups and showed similar banding pattern within the group. PB 64 and Pant Rituraj exhibited similar banding pattern (100%). Similarly, PB 66 and BARI, KS 331 and Punjab Sadabahar, KS 331 and PB 70, Punjab Sadabahar and Pant Samrat, PB 70 and SMB 115 showed 100 percent similarity in seed protein profiles. There were sufficient variability among the PB 69 and PB 67, PB 67 and Pusa Upkar, PB 67 and Pant Samrat. The minimum genetic similarity was observed between PB 69 and PB 67 (44%) followed by Punjab Sadabahar and PB 67 (47%) by PB 67 and Pusa Upkar (47%) and PB 67 and Pant Samrat (47%). The UPGMA analysis showed that PB 60, PB 68 PB 64, Pant Rituraj, PB 66, BARI and PB 69, KS 331, PB 70, SMB 115, Punjab Sadabahar, Pant Samrat, Pusa Upkar formed two different clusters. However, PB 60; PB 68; PB 64 and PB 66; BARI and Pant Rituraj; KS 331, PB 69 and Punjab Sadabahar; PB 70, SMB 115 and PB 70; Pusa Upkar; and Pant Samrat were three different neighbouring groups.

Keywords : *Solanum melongena, variability, SDS-PAGE, seed protein.*

The varieties of *Solanum melongen*a L. display a wide range of fruit shapes and colours, ranging from oval or egg-shaped to long club-shaped; and from white, yellow, green through degrees of purple pigmentation to almost black. In the past, farmers maintained and supplied seeds of eggplant with special type of varieties adapted in the region. Now there are an increasing number of F_1 hybrid varieties bred by private enterprises as well as public enterprises and the seed production of eggplant is shifting from farmers hands to private and public enterprises. Variability refers to the presence of differences among the individuals of plant population. Variability results due to differences either in genetic constitution of the individuals of a population or in the environment in which they are grown. The existence of variability is essential for improvement of quantitative characters *viz.,* higher yield, resistance to biotic and abiotic factors as well as for wide adaptability. Robinson *et. al.* (10) suggested the partition of total variability into genotypic and environmental variance which helps in selection of better genotypes. The present investigation was therefore, undertaken cultivar identification through seed protein profiles of fourteen genotypes using SDS-PAGE.

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MATERIALS AND METHODS

The 14 parental lines were agronomically and morphologically diverse. The genotypes were PB 60, PB 64, PB 66, PB 67, PB 68, PB 69, PB 70, SMB 115, Pant Rituraj (PR), Punjab Sadabahar (Pb. Sad.), KS 331, BARI, Pusa Upkar (PU) and Pant Samrat (PS). SDS PAGE analysis was done a per the standard practices using standard reagents and chemicals. SDS was omitted from any of the reagents in Native-PAGE. The solutions were prepared as follows for SDS PAGE analysis.

Stock Solutions and buffer

All the chemicals used were of Hi Media Laboratories, Mumbai. (4X) Stacking gel buffer having pH-6.8 was prepared by Tris (1.5 g), and Distilled Water (20 ml). The volume was made upto 25 ml with distilled water and filtered through Whatman No. 1 filter paper. (4X) Separating gel buffer having pH 8.8 was made by using 18.15 g Tris and 75 ml Distilled Water. The volume was made upto 100 ml with distilled water, filtered through Whatmann No. 1 and stored at 4°C.

Tank buffer with pH 8.3 was prepared using Tris (3.28 g), Glycine (14.41 g) and 10 % SDS (10 ml). The final volume was made upto 1 l and stored at room temperature. Ammonium per sulphate (APS) (10% w/v) was prepared by mixing 0.1 g APS, & 1 ml Distilled Water. This solution was freshly prepared. Sodiumdodecyl sulphate (SDS) solution was prepared by using 1.0 g SDS in 10 ml Distilled Water. Acrylamide-bis-acrylamide (30% w/v) was made by 30 g Acrylamide and 0.8 g Bis-acrylamide. The final volume was made upto 100 ml and stored at 4 0C in a dark bottle. Sample buffer, with pH6.8 (100 ml) was prepared taking 25 ml Stacking gel buffer, 2 ml of 20 % Glycerol, 4 ml of 10 % SDS, 20 mg Bromophenol blue & 0,312 g of 0.2 mM DTT. The final volume was made upto 100 ml. Stacking gel mixture was prepared by mixing 1.33 ml Acrylamide stock solution, 2.5 ml of (4X) Separating gel buffer, 0.1 ml of 10% SDS solution, 6.0 ml of Distilled water, 50 µl of 10% APS and 5.0 µl of TEMED.

Separating gel mixture was prepared as follows :

Components	Components of resolving gel mixture (ml)		
	10 %	12.5 %	15 %
Water	12.1	9.8	6.8
Acrylamide stock (30 %)	10	12.5	15
Separating gel buffer (1.5 M, pH 8.8)	4.6	7.5	7.6
SDS (10 %)	0.3	0.64	0.64
APS (10%)	0.1	0.1	0.1
N,N,N`,N-Tetramethylethylenediamine (TEMED)	0.02	0.02	0.02

Silver Staining Solutions was made using 40 % Methanol and 10% Acetic acid. The final volume was made upto 100 ml with distilled water. 30 per cent Ethanol was used as Washing Solution. Sensitizer was prepared by 0.02 % Sodiumthiosulfate and Sodium thiosulfate (20mg). Final volume was made upto 100 ml with distilled water. Staining solution was prepared by mixing 200 g of Silver nitrate and 20 µl Formaldehyde solution. Final volume was made upto 100 ml with distilled water. Developer was made by using 6.0 g Sodium bicarbonate, 50 µl of 0.05 % Formaldehyde and 2 ml Sodium thiosulfate. The final volume is made upto 100 ml with distilled water. Stop Solution comprised of 6 ml of 6 % Acetic acid. The final volume was made upto 100 ml with distilled water.

Procedure

The glass plates were cleaned using detergent and water and then wiped with ethanol. Gel casting tray was set. The upper plate was fitted and then the gasket was fixed and the other plate was kept over it and fixed. Water was poured between the glass plates to check leakage. Separating gel was poured in the space between the plates leaving about 2.5 cm space from the top. One ml water was carefully layered over the separating gel to seal off the gel from air, which inhibited gel polymerization. The gel could set in 30 minutes. The gel polymerization was detected in the volume left in the beaker. During this gel setting period, electrode buffer (1X) was prepared. Once the gel was fully set, a clear interface was visible between the top of the gel and the water layered earlier on the gel. The excess water was removed from the top of the gel by using a 5 ml syringe followed by immediate rinsing of the syringe. Stacking gel was poured over the separating gel. The comb was inserted in place leaving a 10 mm gap between the comb and the separating gel.

The stacking gel could set in about 15 min. The gel was left for one hour for complete polymerization. Now the sample was ready for loading. The comb was removed once the gel had set. The wells were cleaned with a syringe. The plates were removed from the casting set. The plates were placed on the assembly Air bubbles, if any, were removed carefully by agitating the buffer with glass rod. Electrode buffer was filled to the upper tank and the samples were loaded with a micro-syringe. 70 µl of prepared samples and 15 µl of molecular marker were mixed with 30 µl and 85 µl sample buffer, respectively, in eppendorf tubes. These tubes were than placed in floating tray and the tray was floated in boiling water for 3 minutes. This step was omitted in case of Native-PAGE. The mixture was than loaded in individual wells. The run was performed at 80 V. Native-PAGE was performed at 40 C inside a refrigerator. After completion of the run the gel sliver staining was performed.

The gel was transferred to 100 ml fixer and left for 60 - 90 minutes on gentle shaker. The fixing solution was removed and the gel was washed with 30 % ethanol for 20 minutes. Sensitization was performed with sodium thiosulfate reagent for 1 minute. Rinsed the gel with millipore distilled water for 20 seconds. Staining solution was added and kept in shaker for 20 minutes. The gel was then washed with millipore distilled water for 20 sec. Developer was added for 2- 3 minutes and then developments of band were observed. After the development of bands the stop

solution was added to stop the reaction. The electrophoregrams were photographed in a gel documentation system.

RESULTS AND DISCUSSION

The advantages of using electrophoresis as a tool in taxonomical and breeding work have been discussed by Allard and Kehler (1) and Ladizinski and Hymowitz (4). The methods which are usually used for identifying different cultivars of crop plant are mainly based on the phenotypic expressions of different plant parts. The technique is based on the concept that each cultivar is distinct and relatively homogenous at the genetic level. Thus, by screening enough loci, one should be able to uniquely define each cultivar (Weeden 13). This technique was therefore, employed in the present investigation to identify fourteen genotypes (parents) of Eggplant, some of which otherwise look similar on the basis of growth habit, flower colour, leaf colour, number of flowers per inflorescence, color, shape and size of fruits and were indistinguishable on the basis of overall phenotypic expression. The seed proteins of fourteen Eggplant lines were subjected to sodium dodecyl sulphate polyacrylamide disc gel electrophoresis. The results obtained in the present study (Fig. 1) demonstrated the existence of sixteen protein bands located in three zones (A, B and C).

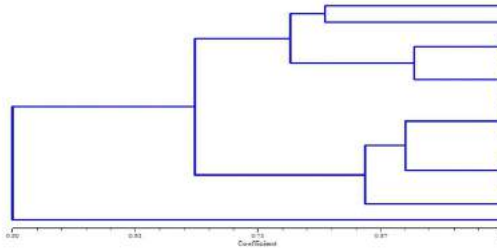

Fig. 1 : Dendogram showing different cluster groups of eggplant genotypes on the basis of protein profiles.

The cultivars which were indistinguishable on the basis of simple identification through morphological traits like growth habit, flower colour etc could be distinguished through electrophoretic patterns. For example, the fourteen genotypes were distinguished into nine groups on different banding patterns in three zones (A, B and C). PB 64 and PB 66; BARI and Pant Rituraj; KS 331, PB 69 and Punjab Sadabahar; PB 70, SMB 115 and PB 67 fell in four different groups and showed similar banding pattern within the group. The degree of darkness and thickness of various bands in different cultivars are the most commonly reported types of variation, suggesting that formulation of many of the bands in the seed protein profile are under the control of quantitative gene systems. This kind of variation may be due to the lack of separation on the gels of several proteins having similar migration rates. In any case, no attempt has been made to estimate the number of genes causing quantitative variation in seed protein bands (Ladizinski and Hymowitz, 3 & 4).

The index of similarity is the second way of expressing variation in the banding patterns between two gels. Using this index, the similarity in the banding patterns of fourteen Eggplant genotypes was analyzed in this investigation (Table 1). A number of genotypes pairs have SI values 100 per cent indicating very close relationship between them. Low value of similarity index was shown by PB 69 and PB 67 (44%) followed by Punjab Sadabahar and PB 67 (47%) by PB 67 and Pusa Upkar (47%) and PB 67 and Pant Samrat (47%) depicting that this was the most diverse groups in evolutionary study.

Table 1: Similarity Index (SI %) for seed protein profiles in different genotypes of Eggplant

Genotypes	PB 60	PB 68	PB 64	PB 66	BARI	PR	KS 331	PB 69	Pb. Sad.	PB 70	SMB 115	PB 67	PU	PS
PB 60	100													
PB 68	82	100												
PB 64	83	82	100											
PB 66	75	73	91	100										
BARI	75	73	91	100	100									
PR	83	82	100	91	91	100								
KS 331	62	73	75	67	67	75	100							
PB 69	62	58	75	82	82	75	82	100						
Pb. Sad.	54	64	67	73	73	67	90	90	100					
PB 70	62	73	75	67	67	75	100	82	90	100				
SMB 115	62	73	75	67	67	75	100	82	90	100	100			
PB 67	60	57	50	44	44	50	53	44	47	53	53	100		
PU	54	64	67	73	73	67	90	90	100	90	90	47	100	
PS	54	64	67	73	73	67	90	90	100	90	90	47	100	100

The UPGMA analysis showed that there are two major cluster I and II at the demarcation of 50% similarity. The major cluster I could be separated into two subclusters IA and IB at the demarcation of nearly 70% similarity. The subcluster IA could further be divided at the demarcation of nearly 78% similarity. The subgroup IA_1 comprised of genotype PB 60 and PB 68 while sub group IA_2 comprised of PB 64 and Pant Rituraj and PB 66 and BARI these genotypes showed maximum similarity between and within the subcluster.

Similarity subgroup IB could further be divided into two subclusters IB_1 and IB_2 at the demarcation of nearly 75% similarity. The subcluster IB_2 comprise of only one genotype PB 69. The subcluster IB_1 could further divided into two minor groups IB_1 A and IB_1 B at demarcation of nearly 90% similarity.The minor group IB_1 A comprised of genotypes KS 331, PB 70 and SMB 115 showing maximum genetic similarity within the minor group. Likewise minor group IB_1 B comprised of four genotypes. Punjab Sadabahar, Pant Samrat and Pusa Upkar which also showed maximum genetic similarity within the minor group.

The importance of this experiment for the characterization of germplasm lines could be realized from the fact that for some genotypes the cultivars which were dissimilar based on morphological features could be easily distinguished through electrophorosis of proteins/ isoenzymes. Similar findings have also been reported in bottle gourd (Upadhyay *et al.,* 12) ; muskmelon (Yadav *et al.,* 15), and in capsicum (PeddaKasim *et al.,* 8). Similar results in indigenous germplasm lines of Eggplant have also been reported by many investigators (Karihaloo and Gotlieb 2; Mennella *et al.,* 5; Noli *et al.,* 6; Patel *et al.,* 7; Ram *et al.,* 9). It can therefore, be concluded that the electrophoretic resolution of seed protein in Eggplant was successful in germplasm identification in most of the cases. Sometimes, the protein profile failed to differentiate between the genotypes which were morphologically dissimilar. The most typical example was the presence of green fruited cultivars PB 67 and PB 69 in different mega groups along with other purple types. These two groups contain different cultivars which were distinct from each other with respect to fruit shape and other morphological traits. Similar findings, where the protein profile showed similar banding pattern between morphologically dissimilar genotypes has earlier been supported in Eggplant (Karihaloo and Gottlieb, 2; Kumar and Tata, 3; Sammour *et al.,* 11) and in different cucurbitaceous crops (Upadhyay *et al.,* 12; Yadav *et al.,* 15).

CONCLUSION

It can therefore, be concluded that the electrophoretic resolution of seed protein in Eggplant was successful in germplasm identification in most of the cases. Sometimes, the protein profile failed to differentiate between the genotypes which were morphologically dissimilar. The most typical example was the presence of green fruited cultivars PB 67 and PB 69 in different mega groups along with other purple types. These two groups contain different cultivars which were distinct from each other with respect to fruit shape and other morphological traits.

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