Vegetable Science (2024) 51(1): 115-119
doi: 10.61180/vegsci.2024.v51.i1.15
ISSN- 0970-6585 (Print), ISSN- 2455-7552 (Online)
www.isvsvegsci.in

RESEARCH ARTICLE

Assessment of genetic purity of F_1 hybrids and their parents through SDS-PASE in brinjal (*Solanum melongena* L.)

Pradeep K. Yadav[1], Rakesh K. Dubey[2*], S. D. Warade[3] and R. C. Shakywar[4]

Abstract

Eight brinjal cultivars/genotypes and their 28 F_1 hybrids were used in the current experiment to determine the genetic purity using SDS-PASE seed protein profiles. When 36 genotypes, including eight parents and 28 F_1 hybrids, were examined using the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), there was a significant variance in the number of protein bands. SDS-PAGE was used to determine which parents (male or female) the F_1s hybrids were more closely linked to as well as the degree of purity between the parents and their hybrids. While Pusa Purple Long, BR-112 and CHFB-7 were weak female parents, Swarna Pratibha, NDB-3, Pant Rituraj and CHFB-6 were powerful female parents. Therefore, it would be much imperative to use parents viz., Swarna Pratibha, NDB-3, Pant Rituraj and CHFB-6 for crossing programmed to transfer desirable traits from parents to offspring through heterosis breeding procedures in brinjal.

Keywords: Brinjal, Heterosis, Hybrids, Genetic Purity, SDS-PAGE.

[1]Defence Institute of Bio-Energy Research (DIBER), DRDO, Nainital, Uttarakhand, India.

[2]ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India.

[3,4]College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh, India.

[*]**Corresponding author;** Email: rksdubey@gmail.com

Citation: Yadav, P.K., Dubey, R.K., Warade, S.D. and Shakywar, R.C. (2024). Assessment of genetic purity of F_1 hybrids and their parents through SDS-PASE in brinjal (*Solanum melongena* L.). Vegetable Science, 51(1), 115-119.

Source of support: Nil

Conflict of interest: None.

Received: 02/01/2024 **Revised:** 14/05/2024 **Accepted:** 27/05/2024

Introduction

Brinjal, Aubergine or Eggplant (*Solanum melongena* L.), a member of the Solanaceae family, is a common vegetable crop grown in tropical and subtropical regions of the world. Since cultivars and hybrids frequently differ in fruit quality and other significant agronomic characteristics that are obscure and challenging to detect, it has become increasingly difficult to identify cultivars and hybrids in brinjal or other crops using this method based on morphological and physiological characteristics. The identification of brinjal cultivars usually relies on morphological, physiological and molecular characteristics. One of these molecular tools is sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which was used to determine the diversity present in total seed proteins of the same and different species and also to differentiate crop germplasm into different varieties (Isemura et al., 2001). SDS-PAGE was used to find out the genetic association between different germplasm. SDS-PAGE procedures were favored because they were less influenced by environmental factors than agro-morphological and phenotypical techniques for examining the genetic diversity of crop germplasm (Tanksley and Jones, 1986). According to the variations in molecular weights discovered, it is mostly used to segregate the total seed proteins (Ullah et al., 2010). Solasodine concentration, total phenol content, anthocyanin content and seed protein

profiling using SDS-PAGE were among the biochemical analyses that are commonly employed to explore the genetic diversity and evolutionary relationships among brinjal. By using SDS-PAGE of the soluble seed proteins, the genotype of eggplant, its hybrids, and nearby species are to be identified. The study included 54 accessions of both cultivated and wild eggplant species. The findings of electrophoresis banding patterns revealed that there was a lot of variation in the group of eggplant in terms of the quantity, shape, location, staining intensities and presence or absence of protein bands in the profile, which could be used to characterize and identify different cultivars of eggplant. The cultivars of elongated fruit eggplant had nine characteristic group-specific bands that set them apart from the other groups. The round fruit type, primitive cultivars, weedy and wild groups of eggplant, including the really wild *Solanum incanum*, were also found to have very variable seed protein profiles, indicating that these groups consist of many genotypes (Hasan and Isai, 1998). Using SDS-PAGE of seed proteins extracted in Tris-HCl buffer, several kinds of vegetables (7 in brinjal, 6 in French bean, and 9 in pea) were characterized. Numerous bands ranging from 7 (French bean) to 18 (pea) were found in the protein profiles of various vegetable crops. The protein composition of brinjal revealed eight bands. In Brinjal, Pant Rituraj, Pant Brinjal 4 and Pant Brinjal Hybrid 1 may be distinguished from Pant Samrat, T-3, DBL 21, and Punjab Sadabahar. Five species of the Solanaceae family (*Solanum melongena*, *Solanum xanthocarpum*, *Datura alba*, *Lycopersicon esculentum*, and *Capsicum annuum*) were examined for their cytological characteristics and protein profiles using polyacrylamide gel electrophoresis and cytological indicators including mitotic index and pollen viability. SDS-PAGE was used to separate the seed proteins of two F_1 bottle gourd hybrids and eight advanced breeding lines. To identify Jaccard's similarity coefficient, an electrophorogram for each line was evaluated. The cultivar of PBOG 13 having spherical fruits, had a unique protein profile. There were discovered to be four main groupings comprised of all eight advanced breeding lines and two F_1 hybrids. There was no difference between segmented leaves and regular leaves in the primary factor while determining overall diversity and authors concluded that, despite the potential utility of seed protein profiles as genetic diversity markers for bottle gourds, the information's accuracy in relation to leaf shape was limited (Dubey and Ram, 2008).The genetic diversity in 7 genotypes of *Brassica napus* was examined using SDS-PAGE to measure total seed protein content. To quantify the genetic diversity and perform a cluster analysis, ten repeatable bands were used. Five of these bands were found to be polymorphic. The genotypes were sorted into two major groups, which included clusters, and a dendrogram was created. These clusters' findings revealed genetic diversity in these accessions at the SDS-PAGE level. The findings indicated that it was possible to discriminate between the several genotypes of *Brassica napus* species using the SDS-PAGE technique. Two F_1 interspecific hybrids (H_1 and H_2) of the chili pepper (Capsicum) were analyzed by Kumar and Tata (2015). They were produced by crossing *C. annum* var. X-235 and *C. frutescens* L. reciprocally, and they were examined for identification and genetic purity using cytomorphological and SDS-PAGE seed protein profiles. The parental genomes of F_1 hybrids revealed by cytogenetic analysis differ from one another by one inversion, two or more translocations, and a few small structural changes. Compared to H_2, H_1 had higher levels of meiotic abnormalities, pollen, and seed sterilities. F_1 hybrids were shown to have lower seed protein profiles, according to Choudhary et al., (2015). Therefore, the present study was carried out to clarify the identification and genetic purity of eight brinjal cultivars, including one wild (*Solanum gilo*) and their 28 F_1 hybrids, on the basis of banding pattern of seed protein profiles.

Materials and Methods

The chemicals used in seed protein electrophoresis were purchased from Sigma Aldrich Chemical Private Limited, Bommasandra, industrial area, Bangalore, Karnataka (India).

Plant Samples

The experimental materials for the present investigation was consist of eight homozygous pure lines viz., Swarna Pratibha, NDB-3, Pant Rituraj, Pusa Purple Longe, BR-112, CHFB-6, CHFB-7 and *S. gilo* and were collected from different parts of Uttar Pradesh, Uttarakhand, Jharkhand, New Delhi, Arunachal Pradesh, Haryana, and 28 F_1 hybrids i.e., Swarna Pratibha x NDB-3, Swarna Pratibha x NDB-3, Swarna Pratibha x NDB-3, Swarna Pratibha x Pant Rituraj, Swarna Pratibha x Pusa Purple Long, Swarna Pratibha x BR-112, Swarna Pratibha x CHFB-6, Swarna Pratibha x CHFB-7, Swarna Pratibha x *S. gilo*, NDB-3 x Pant Rituraj, NDB-3 x Pusa Purple Long, NDB-3 x BR-112, NDB-3 x CHFB-6, NDB-3 x CHFB-7, NDB-3 x *S. gilo*, Pant Rituraj x Pusa Purple Long, Pant Rituraj x BR-112, Pant Rituraj x CHFB-6, Pant Rituraj x CFHB-7, Pant Rituraj x *S. gilo*, Pusa Purple Longe x BR-112, Pusa Purple Longe x CHFB-6, Pusa Purple Longe x CHFB-7, Pusa Purple Longe x *S. gilo*, BR-112 x CHFB-6, BR-112 x CHFB-7, BR-112 x *S. gilo*, CHFB-6 x CHFB-7, CHFB-6 x *S. gilo* and CHFB-7 x *S. gilo* were derived from the eight pure homozygous lines, details about all the breeding lines are given in table number 1.

Seed Protein Extraction and SDS-PASE

For the assessment of genetic purity, the storage seed proteins were analyzed by using SDS-PASE (Laemmli, 1970). Eight advanced breeding lines of seeds were collected, along with 28 F_1 hybrids that were examined in the field. 0.1 g of the obtained seeds were then put in a pestle and mortar with 1000 µL of extraction buffer (1 M Tris-HCL-pH 8.0, 2%

Table 1: Source and morphological features of eight pure homozygous lines of brinjal collected from different sources

S. No.	*Parent*	*Source of collection*	*Morphological features*
1	Swarna Pratibha	Central Horticulture Experimental Station, Ranchi, Jharkhand	Semi-erect plant, solitary fruit bearing habit and long fruit with purple colour.
2	NDB-3	Narendra Deva University of Agricultureand Technology Kumarganj Faizabad, U.P.	Erect plant, solitary fruit bearing habit, long fruit with purple colour.
3	Pant Rituraj	Govind Ballabh Pant University of Agri. & Tech., Pantnagar, Uttarakhand.	Determinate plant, solitary fruit bearing habit, round fruit with purple colour.
4	Pusa Purple Long	Indian Agriculture Research Institute, Pusa, New Delhi.	Erect plant, cluster fruit bearing habit, long fruit with dark purple colour.
5	BR-112	Department of Vegetable Science, HAU, Hisar, Haryana.	Determinate plant, solitary fruit bearing habit, round fruit with purple colour.
6	CHFB-6	Department of Vegetable Science, CFH, CAU, Pasighat, Arunachal Pradesh	Erect plant, solitary fruit bearing habit, oblong fruit with dark purple colour.
7	CHFB-7	Department of Vegetable Science, CHF, CAU, Pasighat, Arunachal Pradesh	Determinate plant, solitary fruit bearing habit, round fruit with purple colour.
8	S. gilo	Department of Vegetable Science, CHF, CAU, Pasighat, Arunachal Pradesh	Erect plant, cluster fruit bearing habit, round fruit with green colour.

SDS, 10% glycerol, 1 mM PMSF-phenyl methyl sulphonyl fluoride, and 2% -mercaptoethanol). The material was homogenized and heated for 5 minutes at 100°C in a water bath. The mixture was spun in a refrigerated centrifuge at 10,000 rpm for 30 minutes at 4°C, and the supernatant (protein fraction) was kept at -20°C. Just before loading the sample buffer into the gel, it was heated in a boiling water bath for five minutes at 65°C using Tris-pH 7.4, 2% SDS, 2% mercapto ethanol, and bromophenol blue. A standard SDS gel was made. Using a protein molecular weight marker, protein sample (7 µL/well) was loaded and ran at a constant 100 V with electrode buffer (Tris-glycine and SDS, pH 8.6).

Staining and Distaining of the Gel

Staining and distaining of the gel were done as per the standard protocol developed by Mortz et al., (2001) for silver staining. The gel was initially placed in a tray with a fixing solution and left there for 30 minutes before being washed for 1 minute with twice-distilled water. The gel was then exposed to a 0.02% sodium thiosulphate solution for 5 minutes. The gel was then rinsed with double distilled water for one minute. After that, it was put in a staining solution and left on a gel rocker in the dark for 20 minutes. The gel was thereafter rinsed twice with distilled water for 45 seconds each time. The gel was then moved to a developing solution, and the reaction was finally stopped using 12% glacial acetic acid. Before envisioning the dark brown band, the gel was cleaned with twice-distilled water. The gel was visualized on a Syngene Documentation System.

Results and Discussion

The SDS-PASE (Fig. 1) of storage seed protein was performed to assess the genetic purity among the pure homozygous breeding lines (eight breeding lines/varieties and 28 F_1 hybrids)with differences in morphological features (Table 1) of brinjal seeds. The cluster analysis (Fig. 2 and Table 2) separated the eight homozygous pure and 28 F_1 hybrids into two major groups (IA & IB) and four subgroups (IA1, IA2, IB1 and IB2).

All the genotypes are divided into two main clusters based on the dendrogram. Table 2 and Fig. 2 show the distribution of the different genotypes among the dendrogram's different groups. With the possible exception of the cross between Swarna Pratibha x BR-112, which was originally found in cluster 2, the first cluster contained almost all the genotypes. When Swarna Pratibha is crossed with NDB-3, Pant Rituraj, BR-112, CHFB-7, and *S. gilo*, all of the hybrids that result are closely related to Swarna Pratibha, the female parent. However, when Swarna Pratibha is crossed with Pusa Purple Long and CHFB-6, the resulting hybrids are closely related to their respective male parents rather than Swarna Pratibha. When NDB-3 was crossed with Pusa Purple Long, BR-112, CHFB-7 and *S. gilo*, the hybrids were more closely related to their male parents (Pant Rituraj and CHFB-6) than to their female parent (NDB-3), whereas when NDB-3 was crossed with Pant Rituraj and CHFB-6, the hybrids are more closely related to their female parent (NDB-3).

An affordable, straightforward, and widely used biochemical technique for characterizing the seed protein diversity of crop germplasm is SDS-PAGE (Cook, 1995; Das and Mukherjee, 1995; Fufa et al., 2005; Iqbal et al., 2005). Over the course of the past 25 years, it has been observed that variations in seed proteins have been the most frequently utilized biochemical genetic markers. The fact that seed and seedling proteins are polymorphic, primary gene products and generally unaffected by environmental interactions

Table 2: Major cluster produced by SDS-PAGE analysis in 36 genotypes and 28 hybrids of brinjal

I	I A	I A 1	Pusa Purple Long, BR-112, CHFB-7, *S. gilo*, NDB-3, Pant Rituraj, Swarna Pratibha x Pusa Purple Long and Pant Rituraj x BR-112
		I A 2	Swarna Pratibha x CHFB-6, NDB-3 x Pant Rituraj, Pusa Purple Long x CHFB-7, CHFB-6 x CHFB-7, BR-112 x *S. gilo*, CHFB-6 x *S. gilo*, BR-112 x CHFB-7, CHFB-6, CHFB-7 x *S. gilo* and Pusa Purple Long x *S. gilo*
	I B	I B 1	Swarna Pratibha x *S. gilo* and NDB-3 x Pusa Purple Long
		I B 2	Swarna Pratibha, Swarna Pratibha x NDB-3, Pant Rituraj x CHFB-7, Pusa Purple Long x BR-112, Swarna Pratibha x Pant Rituraj, Pant Rituraj x CHFB-6, NDB-3 x CHFB-7, Swarna Pratibha x CHFB-7, NDB-3 x *S. gilo*, Pant Rituraj x Pusa Purple Long, BR-112 x CHFB-6, Pant Rituraj x *S. gilo*, Pusa Purple Long x CHFB-6, NDB-3 x BR-112 and NDB-3 x CHFB-6
II			Swarna Pratibha x BR-112

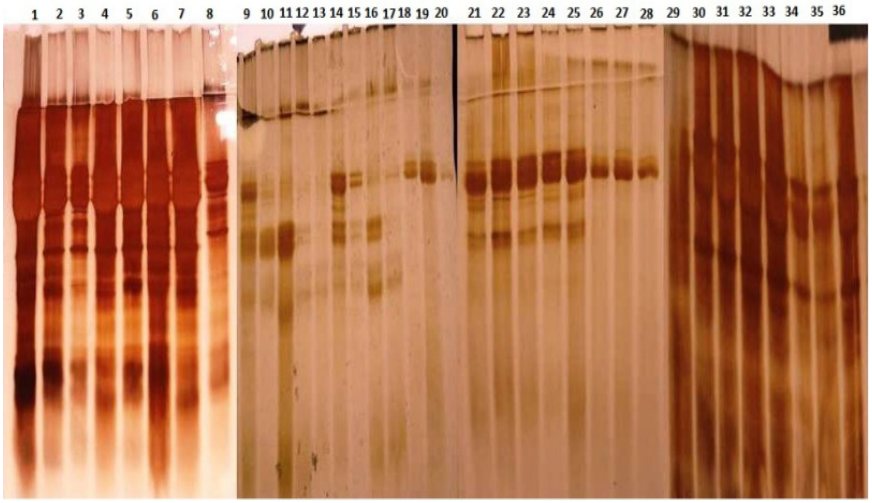

Figure 1: The sodium dodiayl sulphate polyacrylamide gel electrophoresis (SDS-PASE) of storage seed protein

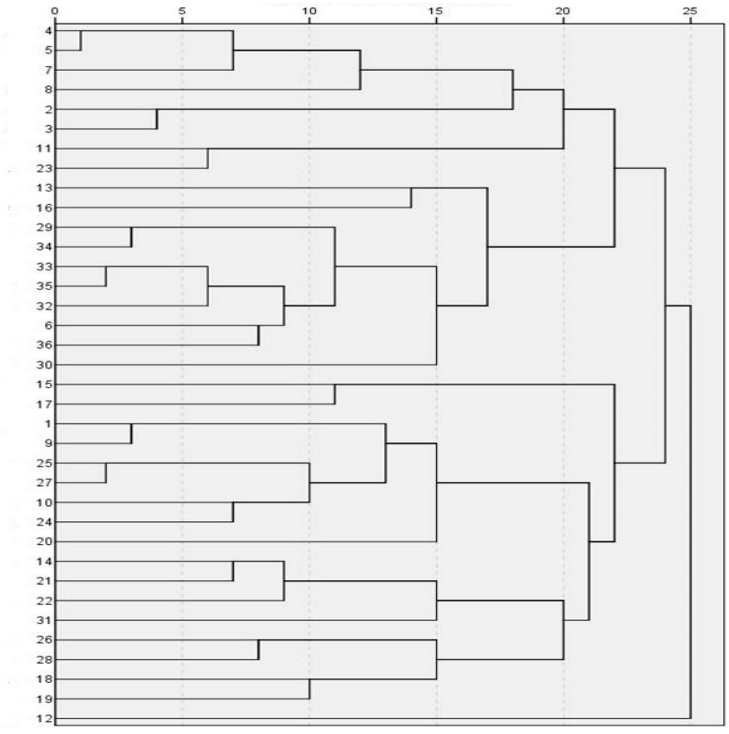

Figure 2: Dendrogram showing cluster groups of brinjal genotypes based on protein profiles through SDS-PAGE

is crucial to the system's success (Smith and Smith, 1992). When interpreting darkness and thickness, care should be taken because the lack of separation on the gels of several proteins with similar migration rates may cause this kind of variation. Studies are needed to determine how many genes are responsible for the quantitative variation in seed protein bands. To identify the protein banding patterns of 36 brinjal genotypes (28 F_1 hybrids and eight parents), SDS-PAGE was used. In the current study, the genotypes varied significantly in the protein band number, which ranged from 8 to 33. According to Kumar and Tata (2015), it was shown that F_1s hybrids had lower seed protein profiles. SDS-PAGE was also utilized by Yadav et al. (1998) and, Choudhary and Ram (2000) and Dubey and Ram (2008) to differentiate among the bottle gourd genotypes and hybrids.

Conclusion

About 36 genotypes (8 parents and 28 F_1 hybrids) with the greatest variance in protein band counts were identified using SDS-PAGE. In order to determine which parents (male or female) the F_1 hybrids were most closely linked, SDS-PAGE was employed to examine the genetic purity and genetic variability among the parents and their hybrids. More closely hybrids to their female parents were Swarna Pratibha, NDB-3, Pant Rituraj and CHFB-6 and less closely hybrids to their parents were Pusa Purple Long, BR-112, and CHFB-7. Therefore, it was recommended that parents Swarna Pratibha, NDB-3, Pant Rituraj and CHFB-6 be used for a crossing program to transfer the desired traits to offspring through heterosis breeding in brinjal.

References

Bhat, T. M. and Kudesia, R. (2011). Evaluation of genetic diversity in five different species of family Solanaceae using cytological characters and protein profiling. Gen Eng Biotech J, 20, 1-8.

Chaudhary, H. and Ram, H. H. (2000). Characterization of indigenous muskmelon germplasm lines based on SDS-PAGE of seed proteins. Veg Sci, 27, 35-38.

Choudhary, R., Rai, G. K., Parveen, A., Rai, P. K. and Malhotra, R. K. (2015). Genetic diversity of *Brassica napus* using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). SABRAO Journal of Breeding and Genetics, 47(1), 14-20.

Cook, R. J. (1984). The characterization and identification of crop cultivars by electrophoresis. Electrophoresis, 5, 59-72.

Das, S. and Mukarjee, K. K. (1995). Comparative study on seed proteins of *Ipomoea*. Seed Sci Tech, 23, 501-509.

Dubey, R. K. and Ram, H. H. (2008). Characterization of advanced breeding lines and assessment of genetic diversity in Bottle Gourd (*Lagenaria siceraria* (Mol.) Standl.) through SDS-PAGE. International Journal of Plant Breeding, 2(2), 85-86.

Fufa, H., Baenziger, P. S., Beecher, B. S., Dweikat, I., Graybosch, R. A. and Eskridge, K. M. (2005). Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. Euphytica, 145, 133-146.

Hasan, S. M. Z. and Isai, M. L. (1998). Variability in eggplant *(Solanum melongena* L.*)* and its nearest wild species as revealed by polyacrylamide gel electrophoresis of seed protein. Pertanica J Tropical Agri Sci, 21(2), 113-122.

Iqbal, S. H., Ghafoor, A. and Ayub, N. (2005). Relationship between SDS-PAGE markers and *Ascochyta* blight in chickpea. Pakistan Journal of Botany, 37, 87-96.

Isemura, T., Shiyo, N., Shigeyuki, M., Michihiro, Y., Hiroo, N., Masayoshi, I. and Osamu, K. (2001). Genetic variation and geographical distribution of Azuki bean *(Vigna angularis)* landraces based on the electropherogram of seed storage proteins. Breeding Sciences, 51, 225-230.

Kumar, O. A. and Tata, S. S. (2015). Assessment of genetic purity of F_1 interspecific hybrids of Chili pepper. Asian Journal of Plant Sciences Research, 5(5), 47-51.

Ladizinski, G. and Hymowitz, T. (1979). Seed protein electrophoresis in taxonomic and evolutionary studies. Theoretical and Applied Genetics, 54, 145-151.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-685.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the folin phenol reagent. J Bio Chem, 193, 265-275.

Mortz, E., Krogh, T. N., Worm, H. and Gorg, A. (2001). Improved silver staining protocols for high sensitivity protein identification using matrix-assisted laser distortion/ionization time flight analysis. Proteomics, 1(11), 1349-1363.

Ram, H. H., Sharma, K. and Jaiswal, H. R. (2005).Distinguishing certain released varieties of brinjal, French bean and pea by SDS-PAGE. Vegetable Science, 32(1), 15-18.

Tanksley, S. D. and Jones, R. A. (1986). Application of alcohol dehydrogenase allozymes in testing the genetic purity of F_1 hybrids of tomato. Hort Sci, 16, 179-181.

Ullah, I., Khan, I. A., Ahmad, H., Gafoor, S. U., Gul, S., Muhammad, I. and Ilyas, M. (2010). Seed storage protein profile of rice varieties commonly grown in Pakistan. Asian Journal of Agricultural Science, 2, 120-123.

Yadav, R. K., Ram, H. H. and Kumar, A. (1998). Electrophoretic characterization of indigenous germplasm lines of muskmelon. Vegetable Science, 25, 8-10.

सारांश

वर्तमान प्रयोग में आठ बैंगन प्रजातियों/ जनन्द्र्यों और उनके 28 एफ1 हाइब्रिड्स का उपयोग जीन सुशुद्धता की जाँच के लिए किया गया था, जिसमें सोडियम डोडेसिल सल्फेट पॉलीएक्रिलेमाइड जेल इलेक्ट्रोफोरेसिस बीज प्रोटीन प्रोफ़ाइल का उपयोग किया गया। जिसमें 36 जेनोटाइप्स (आठ पेरेंट्स और 28 एफ1 हाइब्रिड्स)-सोडियम डोडेसिल सल्फेट पॉलीएक्रिलेमाइड जेल इलेक्ट्रोफोरेसिस (एसडीएस-पीएजी) का उपयोग करके जाँचे गए, तो प्रोटीन बैंड्स की संख्या में महत्वपूर्ण भेद था । एसडीएस-पीएजी का उपयोग किया गया था ताकि यह निर्धारित किया जा सके कि एफ1 हाइब्रिड्स माता-पिता (पुरुष या मादा) से कितना मजबूत रूप से जुड़े हुए हैं और माता-पिता और उनके हाइब्रिड्स के बीच शुद्धता का स्तर का लेवल क्या है । पूसा पर्पल लॉन्ग, बीआर-112 और सीएचएफबी-7 कमजोर मादा पैरेंट थे, जबकि स्वर्ण प्रतिभा, एनडीबी-3, पैंट ऋतुराज और सीएचएफबी-6 मजबूत मादा पैरेंट थे। इसलिए, बैंगन में हेटेरोसिस ब्रीडिंग प्रक्रिया के माध्यम से पेरेंट्स से अवंतन करने के लिए स्वर्ण प्रतिभा, एनडीबी-3, पन्त ऋतुराज और सीएचएफबी-6 जैसे पेरेंट्स का उपयोग करना अत्यंत लाभकारी होगा।