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Biochemical characterization of parental lines and F_1 hybrids in smooth gourd (*Luffa cylindrica* Roem.)

VBS Chauhan, DK Singh and Harshwardhan Choudhury

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Smooth gourd (*Luffa cylindrica* Roem.) a member of the family Cucurbitaceae is commonly grown for its tender fruits as well as for sponge, which is used for scrubbing purposes. For an appropriate breeding strategy carried out for the exploitation of existing genetic variability, it is equally important to characterize the genetic polymorphism at the molecular level This will not only help in hybrid development but also for registration as per legislative requirements, as morphological differences only cannot be interpreted to provide accurate estimates of genetic differences. Besides, it is also useful in testing purity of hybrid seed lot. Thus, by screening enough loci one should be able to uniquely differential each cultivar. Biochemical methods using storage proteins, isozymes have been widely used in such studies (Smith and Smith, 1992) and these methods continue in routine testing of parentage monitoring of genetic purity and as additional descriptors in DUS (distinct, uniformity and stability) testing. Identification of cultivars using protein profiles is more suitable as this is expression of genetic makeup of plants and protein profiles are also species specific. Seed proteins have the advantage of being scorable and the electrophoretic protocol for bulk protein assay is also simpler than that of isozymes. Keeping the above considerations in view, this experiment was carried out to characterize the six al lines PSG-93, PSG-82, PSG-07-04, PSG-115, PSG-199 and PSG-161 parent and hybrids of smooth gourd developed following a half diallel mating system, through seed protein electrophoresis.

The 15 F_1s plants along with their six parents were evaluated during summer season 2008 at the Vegetable Research Centre of composite seeds were collected from each genotype for Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The electrophoretic details were broadly as described by Laemmli (1970) with slight modification. Single seed was ground in 1ml extraction buffer (0.0625 N Tris-HCL + 2% SDS + 10% Glycerol + 1 mM Phenylmethane sulphonyl fluoride + 2% Mercaptoethanol). The mixture was heated at 100^{0}C for 5 minutes. It was centrifuged at 10,000 rpm for 15 minutes. Supernatant was stored at 4^{0}C for further use. Equal volume (25ul) of protein sample (supernatant) and sample buffer (1.5g Tris + 20ml Glycerol + 2g SDS + 2ml Mercaptoethanol + 2% Bromophenol blue) in final volume of 100 ml with distilled water) were mixed and heated at 64^{0}C for 5 minutes. 5µl of prepared samples were loaded in different wells of the gel (15% consistency). A standard medium range protein molecular weight marker of known molecular weight (14.3 KD to 97.4 KD) was used along with samples. Gel was stained in staining solution (0.25g Coomassie Brilliant blue R + 6g Trichloro acetic acid + 180ml Methanol + 60g Glacial acetic acid and final volume made up to 1 litre), was destained in 3% NaCl solution and the electrophoregrams of the seed protein profiles were prepared.

The protein profile is depicted in Fig.1, which comprised of seventeen protein bands, distributed into three major zones A (29 to 97.4 KD), B (20.1 to 29 KD) and C (14.3 to 20.1 KD) and each zone consisted of a number of bands or subzones. For genotype discrimination, the presence and absence of protein bands was the criteria for characterization of diallel progeny. Zone A comprised of six sharp protein bands of high molecular weight and were designated as A1, A2, A3, A4, A5 and A6. The protein band A1was present in all parents and F_1 except two parents PSG-93 and PSG-82 and only one F_1 PSG-93 x PSG-07-04. The subzone A2 was present in three parents PSG-115, PSG-199 and PSG-161 and six F_1 s plants PSG-93 x PSG-82, PSG-93 x PSG-115, PSG-93 x PSG-199, PSG-82 x PSG-161, PSG-115 x PSG-199 and PSG-199 x PSG-161. The A3 protein band was

VBS Chauhan, DK Singh and Harshwardhan Choudhury
Department of Vegetable Science, G. B. Pant University of Agriculture and Technology, Pantnagar-263145

present in all genotypes except four F_1 PSG-93 x PSG-07-04, PSG-82 x PSG-199, PSG-115 x PSG-161 and PSG-199 x PSG-161. The subzone A4 was common in all parents and F_1s plants. The subzone A5 was found in three parents PSG-82, PSG-115 and PSG-199 and six F_1 PSG-07-04 x PSG-115, PSG-07-04 x PSG-199, PSG-07-04 x PSG-161, PSG-115 x PSG-199, PSG-115 x PSG-161 and PSG-199 x PSG-161. The protein band A6 was specific to two parents PSG-115 and PSG-199 which exhibited similarity in fruit flesh thickness, fruit diameter and number of fruit per plant and two F_1s plants involving them as one of parents like PSG-115 x PSG-161 and PSG-199 x PSG-161. On the basis of morphological characters it was found that among all parents PSG-115 had club shape fruit with dark green colour and PSG-161 is only parent which had light green fruit colour with cylindrical shape. F_1 of cross PSG-115 x PSG-161 showed cylindrical shape fruit with dark green colour. The subzone B1 was present in only one parent PSG-82 and one F_1 PSG-115 x PSG-161, on the basis of observation on quantitative traits, it was found that parent PSG-82 was the lowest yielder among all genotype and appearance of first male and female flower were at higher nodes. The subzone B2 was common in all parents and F_1s plants. The subzone B3 was common in all parents and F_1 except two parents PSG-07-04 and PSG-115 and in only one F_1 PSG-93 x PSG-161. The subzone B4 was present in all parents and F_1 except only one parent PSG-93 and three F_1 PSG-93 x PSG-161, PSG-82 x PSG-07-04 and PSG-199 x PSG-161. The subzone B5 was found in all parents and F_1 except two parents PSG-93 and PSG-82 and three F_1 PSG-93 x PSG-161, PSG-115 x PSG-161 and PSG-199 x PSG-161. The protein band B6 was present in all genotypes except only one F_1 PSG-93 x PSG-161. The subzone B7 was present in only two F_1, PSG-93 x PSG-115(less fruit thickness) and PSG-93 x PSG-161 (low fruit weight, less flesh thickness, less fruit diameter and low yield). This hybrid PSG-93 x PSG-161 having all traits at lower extreme was uniquely grouped in a separate cluster IV of dendrogram. The C zone comprised of four bands C1, C2, C3 and C4. The subzone C1 was common in all parents and F_1 except two parents PSG-93 and PSG-82 and two F_1s PSG-93 x PSG-115 and PSG-93 x PSG-161. The protein band C2 was found in all parents and F_1 except two parents PSG-93 and PSG-82 and four F_1 PSG-93 x PSG-115, PSG-93 x PSG-161, PSG-82 x PSG-07-04 and PSG-82 x PSG-115. The subzone C3 was present in all parents and F_1 except three F_1 PSG-93 x PSG-161, PSG-115 x PSG-161 and PSG-199 x PSG-161. The subzone C4 was common in all parents and F_1s plants. These protein bands were distinctly categorized in three different zones

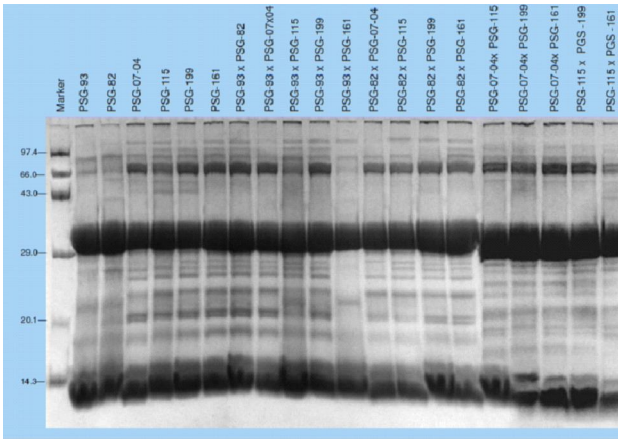

Fig. 1: Seed Protein profiles of smooth gourd parents and their corresponding hybrids

(A, B, and C) and three protein bands A4, B2 and C4 were unambiguously resolved in all genotypes. However, few bands were specific to certain genotypes such as A6 specific to PSG-115, PSG-199, PSG-115 x PSG-161 and PSG-199 x PSG-161 and B7 specific to PSG-93 x PSG-115 and PSG-93 x PSG-161. Ladizinsky and Hymowitz (1979) reported such variation as the commonly reported ones, suggesting that the formation of many of the bands in the seed protein profile are under control of quantitative gene system and such variation may be due to lack of separation of several proteins having similar migration rates on the gels. In any case, no attempt has been made to estimate the number of genes causing quantitative variation in seed protein bands. PSG-199 and PSG-161 produced morphologically similar fruits but differed in protein banding pattern on the basis of band A5 and A6, which were present only in PSG-161and showed the utility of this method to differentiate morphologically similar genotypes on the basis of their protein profile. The morphologically indistinguishable genotypes were identified on the basis of their seed protein profiles by Choudhary and Ram, (2000) in muskmelon and Singh and Ram, (2001); Singh *et al* (2010) in cucumber. Tolentino *et al*. (1997) studied the taxonomic relationship and genetic diversity between *Luffa cylindrica* and *L. acutangula* based on total seed protein using SDS-PAGE.

Similarity index was calculated to study the genetic relationship among the parents, hybrids of smooth gourd and lines found to range from 23% to 100% among the genotypes. Among the parental lines, PSG-93 showed minimum similarity (47%) with PSG-115 and their cross showed 43.32% mid parent heterosis for yield which gave 107.8 q./ ha. fruit yield. Among all crosses, PSG-82 x PSG-07-04 recorded maximum yield of 152.76q./ ha. and exhibited 106.31% mid parent heterosis for yield. The parental lines involved in PSG-82 x PSG-07-04

cross showed 50% similarity to each other. The parental lines PSG-82 and PSG-199 showed 56% similarity and their cross PSG-82 x PSG-199 exhibited maximum mid parent heterosis (125.74%) for fruit yield and able to produce 125.11q/ha. fruit. The lines PSG-115 and PSG-199 showed maximum similarity among all parental lines (93%) and their cross PSG-115 x PSG-199 exhibited very low mid parent heterosis (42.69%) and gave 101.85q/ha fruit yield. Thus, it was evident that parental lines with more dissimilarity produced better heterotic combination for different traits and vice versa. The minimum similarity index 23% was recorded for cross PSG-93 x PSG-07-04 with PSG-93 x PSG-161 and the first cross exhibited lower node number of first male flower, first female flower anthesis and higher yield while other cross PSG-93 x PSG-161showed low fruit weight, less flesh thickness, less fruit diameter and low yield among hybrids. On the basis of protein profile of twenty one smooth gourd genotypes, the dendrogram generated through unweighted pair group method using arithmetic average (UPGMA) analysis is presented in Fig. 2, which shows four major clusters. First cluster had three genotypes comprising of two parents PSG-93, PSG-82 and one cross PSG-82 x PSG-07-04 the second cluster consisted of fifteen genotypes while the third cluster had two crosses PSG-115 x PSG-161 and PSG-199 x PSG-161. Only one cross PSG-93 x PSG-161 included in fourth cluster which was most diverse from other genotypes and it exhibited low fruit weight, less flesh thickness, less fruit diameter among all genotype and low yield among F_1 hybrids. Depending on protein pattern banding pattern, different genotype of ash gourd were distinguished in different group through electrophoresis by Zaporteza *et al*. (1999) and in sponge gourd by Pandey *et al*. (2007) and in cucumber by Singh *et al* (2010).

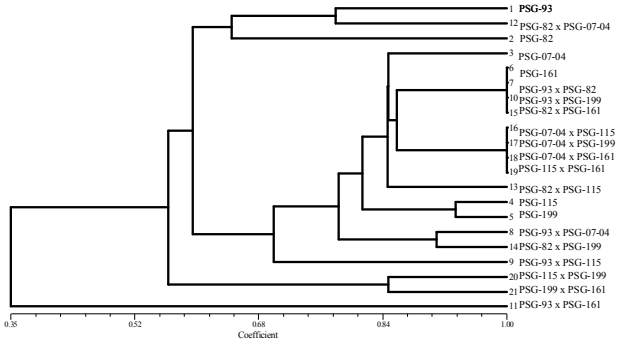

Fig 2: Dendrogram for smooth gourd parents and their corresponding hybrids

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