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# Characterization of Advanced Breeding Lines and Assessment of Genetic Diversity in Bottle Gourd (*Lagenaria siceraria* (Mol.) Standl.) through SDS–PAGE

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## ABSTRACT

Seed proteins of eight advanced breeding lines and two $F_1$ hybrids of bottle gourd were resolved by SDS-PAGE. An electrophorogram for each line was scored and Jaccard's similarity coefficient was calculated. The round-fruited cultivar, PBOG 13 had a distinguishable protein profile. All the eight advanced breeding lines and two $F_1$ hybrids were found to be grouped into four major groups. Leaf morphological variation (normal leaf vs. segmented leaf) was not the major component of overall diversity. We conclude that even though seed protein profiles are potentially useful markers in the study of genetic diversity of bottle gourd, the accuracy with which the information is linked to leaf morphology is low.

**Keywords:** cluster analysis, genetic diversity

## INTRODUCTION

Reliable identification of genotypes using classical methods based on morphological and physiological characters has become increasingly difficult. Time and resource requirements of field tests and their dependence on normal environmental conditions make such procedures impractical for routine screening (Weeden 1984). Electrophoresis of seed proteins is based on the concept that each cultivar/genotype is distinct and relatively homogenous at the genetic level. Seed protein electrophoresis was frequently utilized 20-30 years ago as an additional approach for species identification and as a useful tool for back-tracking the evolution of various groups of plants (Ladizinsky and Hymowitz 1979). Seed protein and isozyme variants have been extensively used as molecular genetic markers for characterization of plant species and cultivars (Breetting and Widrelechner 1995; Sharma and Ram 2003). Seed proteins have the advantage of being scorable from unviable organs or tissues and the electrophoretic protocol for bulk protein assay is generally simpler than that for isozymes (Cooke 1984). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) provides the best resolution in the identification of germplasm/cultivars by protein patterns (Khan *et al*. 2002).

The present study is an attempt to characterize eight advanced breeding lines and two $F_1$ hybrids in bottle gourd and to estimate genetic diversity through SDS-PAGE.

## MATERIALS AND METHODS

The chemicals used in protein electrophoresis were purchased from Sisco Research Laboratory Pvt. Ltd, Mumbai and Banglore Genei, Banglore, India.

### Plant samples

The experimental materials for the present investigation consists of eight advanced breeding lines viz., PBOG13, PBOG 22, PBOG 54, PBOG 61, PBOG 76, PBOG 117, PBOG 119 and Pusa Naveen were collected from different parts of Uttar Pradesh, Uttarakhand and Delhi (India) and two $F_1$ hybrids i.e. PBOG 13× PBOG 61 and PBOG 13×PBOG 76, which were derived from parental lines PBOG 13, PBOG 61 and PBOG 76. Out of these, PBOG 13 is round-fruited. The remainder had long fruit. PBOG 54 has segmented leaves in contrast to normal leaves in other types.

### Protein extraction and electrophoresis

The variability of seed storage proteins was analyzed by using SDS-PAGE (Laemmli 1970). Seeds of eight advanced breeding lines and two $F_1$ hybrids evaluated in the field were collected and 0.1 g seed was taken in pestle and mortar and adding 1000 µl extraction buffer (1 M Tris-HCL-pH 8.0, 2% SDS, 10% glycerol, 1 mM PMSF-phenyl methyl sulphonyl fluoride and 2% β-mercaptoethanol). The sample was homogenized and heated in a boiling water bath for 5 min at 100°C. The contents were centrifuged for 30 min at 10,000 rpm in a refrigerated centrifuge at 4°C and the supernatant (protein fraction) was stored at -20°C. Sample buffer (Tris-pH 7.4, 2% SDS, 2% mercaptoethanol and bromophenol blue) heated in boiling water bath for 5 min at 65°C just before loading in the gel. A standard SDS gel was made. Protein samples (7 µl/well) were loaded and run at a constant 100 V with electrode buffer (Tris-glycine and SDS, pH 8.6) using a protein molecular weight marker.

### Staining and distaining of the gel

The gel was stained overnight in staining solution (0.25 g Coomassie brilliant blue R-250, 60 g TCA, 180 ml methanol, 60 ml glacial acetic acid), then replaced with distaining solution (3% NaCl) and lightly shaken to remove the blue background. The gel was visualized on a Syngene Documentation System.

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Research Note

**Table 1** Source and morphological features of the eight advanced breeding lines of bottle gourd collected in India.

| Name of the parents | Place of collection | Morphological features |
|---|---|---|
| PBOG 13 | Uttarakhand | Flat round, moderate dense pubescence and whitish green fruit |
| PBOG 22 | Uttar Pradesh | Long, smooth, pale green with very low dense pubescence on fruit |
| PBOG 54 | Uttar Pradesh | Long, bottle shaped, moderate dense pubescence and dark green fruit with segmented leaf |
| PBOG 61 | Uttarakhand | Long, smooth, green with high dense pubescence on fruit surface |
| PBOG 76 | Uttar Pradesh | Long, smooth and white green fruit high dense pubescence on fruit surface |
| PBOG 117 | Uttar Pradesh | Long, smooth, pale green fruit with moderate dense pubescence on fruit surface |
| PBOG 119 | Uttar Pradesh | Long, smooth, pale green fruit with high dense pubescence on fruit surface |
| Pusa Naveen | New Delhi | Cylindrical and straight light green with high dense pubescence on fruit surface |

## Cluster analysis

Cluster analysis and the generation of a hierarchical dendrogram was achieved through UPGMA (Sokal and Michener 1958) using the software package NTSYSpc (Rohlf 2000).

## RESULTS AND DISCUSSION

SDS-PAGE (**Fig. 1**) of seed storage protein was performed to investigate the genetic diversity among different advanced breeding lines (eight breeding lines/cultivars and two $F_1$ hybrids) with morphological differences (**Table 1**) of bottle gourd seed. The cluster analysis (**Fig. 2**) separated the eight advance breeding lines and two $F_1$ hybrids into four major groups. Despite the fact that PBOG 54 has a distinct leaf trait i.e. a segmented leaf (novel genotype) cluster analysis did not separate it into a separate group, showing the weakness of SDS-PAGE in correlating protein profile data with morphological traits. This result also demonstrates that an obviously distinct type of breeding lines based on a particular morphological variation, for example leaf shape conditioned by qualitative inheritance may not necessarily show wide diversity based on SDS-PAGE. The weakness of the correlation of the method to SDS-PAGE banding patterns is further demonstrated by the fact that long fruit types, e.g. PBOG 117, PBOG 22, PBOG 54, PBOG 61, PBOG 76, PBOG 119 and Pusa Naveen were clustered into different groups and had different banding patterns. Yadav *et al*. (1998) and Choudhary and Ram (2000) also used SDS-PAGE to distinguish muskmelon genotypes while Sharma and Ram (2003) could distinguish bottle gourd by using seed storage protein. Seed storage protein profiles are not reliable markers which makes them inappropriate for cultivar identification, registration of new varieties, pedigree analysis, and in the studies of genetic diversity, classification and improving the efficiency of bottle gourd breeding programme in cultivar/hybrids development. Alternative, more reliable molecular markers need to be sought.

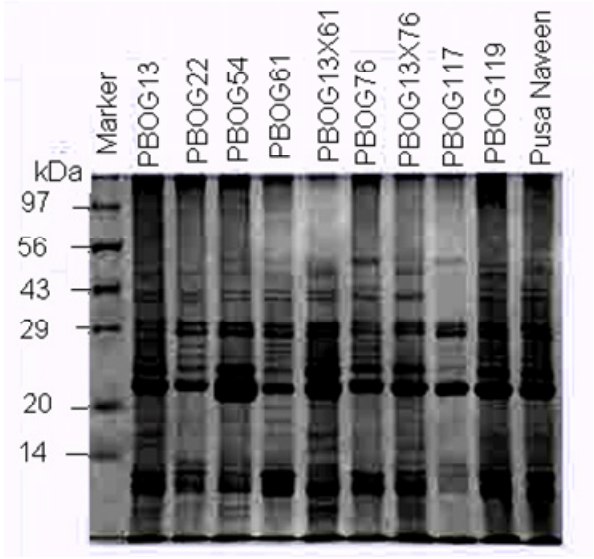


**Fig. 1** SDS-PAGE of bottle gourd seed storage proteins.

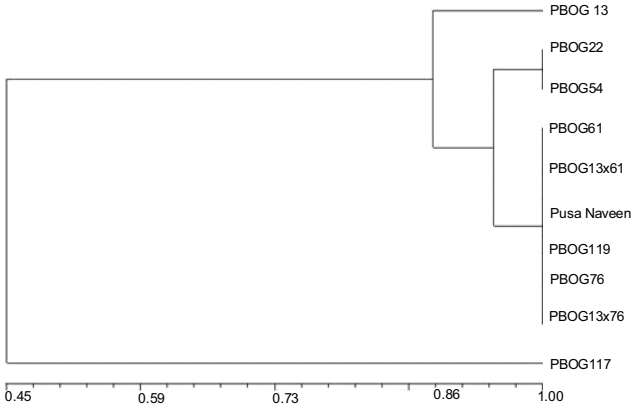


**Fig. 2** UPGMA Jaccard's cofficient and dendrogram of eight advanced breeding lines and two $F_1$ hybrids of bottlegourd.

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