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Short Communication

Identification of putative hybrids between pigeon pea (*Cajanus cajan*) and *C. cajanifolius* using seed protein markers

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Pigeon pea [*C. cajan* (L.) Millsp.] is an important grain legume crop in the semi-arid tropics. Breeding tools employed for genetic improvement in this crop are limited to selection and intervarietal hybridization. Productivity in pigeon pea is constrained mainly by biotic and abiotic stresses. *C. cajanifolius* (Haines) Van der Maesen, the putative progenitor of pigeon pea, can be used as a potential donor for genes conferring resistance to bruchid, pod borer and tolerance to drought besides hardiness, high protein content with minimum protease inhibitors and high methionine content [see [1], for review]. These genes could be effectively introgressed into pigeon pea genotypes by backcross breeding using *C. cajanifolius* as the male parent, as crosses in reciprocal combination were not successful [2]. Use of molecular markers unique to *C. cajanifolius* could be useful for verification of hybridity in such breeding programmes.

Seed protein markers have been effectively employed for varietal differentiation in several crop plants [3]. However, these have been used for verification of hybridity only in a few crops [4-6]. Variation in electrophoretic banding patterns of seed proteins have also been effectively used in pigeon pea for generating seed protein profiles [7] and elucidating phylogenetic relationship [8, 9]. Detection of seed protein markers could be done by a relatively simple and low-cost technique and can be practiced even in small laboratories with reasonable accuracy.

Available literature, however, reveals no attempts on electrophoretic analysis of seed protein fractions and its utilization in this genus for verification of hybridity in wide crosses. We report here on electrophoretic analysis of seed glubulins for detection of suitable markers for use in verification of hybridity in two separate inter-specific crosses in *Cajanus* involving two pigeon pea varieties as female parents and *C. cajanifolius* as the male.

The study included two pigeon pea cultivars, AKPH 1156 and AKT 9013, *C. cajanifolius* and the putative F_1 hybrids derived from the crosses, AKPH 1156 × *C. cajanifolius* and AKT 9013 × *C. cajanifolius*.

Analysis of seed globulins was done by Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Extraction of the globulin fraction from the seed flour and its denaturation were done following the procedures as described by Mohanty *et al.*, [3].

Protein samples (approximately 500 µg) were electrophoresed in a discontinuous SDS-PAGE following Laemmli [10] using a 12% resolving gel (0.375 Tris. HCl, pH 8.8) and 4% stacking gel (0.125 M Tris. HCl, pH 6.8) in Tris-Glycine buffer (0.025 M Tris, pH 8.3; 0.192 M Glycine; 0.1% SDS) for 16h, constantly at 20 mA.

Seed protein expression is usually controlled by homologous multigene families exhibiting monogenic segregation with codominance for molecular weight variants and presence of polypeptide bands being completely dominant over absence [11]. Deletion or mutation of structural genes coding for the polypeptides or their regulatory loci results in inhibition of transcription or translation of polypeptides [12] leading to the lack of expression of the concerned polypeptides. This kind of variation, revealed in electrophoretic banding patterns, could lead to the detection of genotype or species-specific bands. Therefore, the polypeptides varying for

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their presence could be used as reliable molecular markers for verification of hybridity of inter-varietal [4, 5] and inter-specific [6] crosses.

SDS-PAGE of seed globulins of five genotypes including two pigeon pea cultivars, *C. cajanifolius* and their two putative F_1 hybrids led to the detection of 14 polypeptide bands with R_m values ranging from 0.248 to 0.634 (Fig. 1). Out of these, four polypeptides

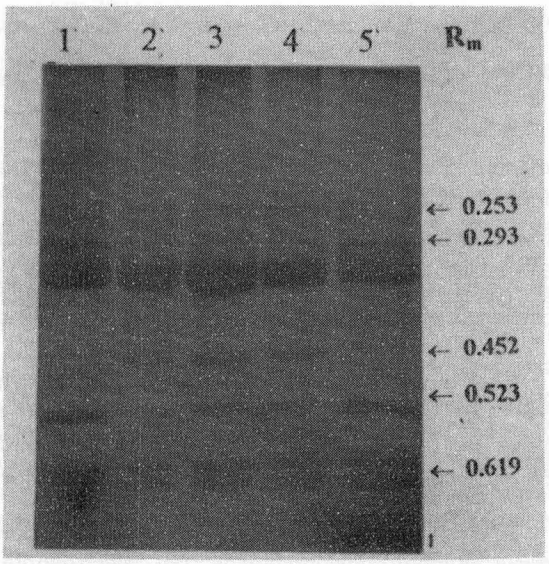

Fig. 1. Electrophoretic banding patterns of two pigeon pea (*Cajanus cajan*) cultivars, AKPH 1156 (lane 1), AKT 9013 (lane 5), *C. cajanifolius* (lane 3) and their two interspecific hybrids AKPH 1156 × *C. cajanifolius* (lane 2) and AKT 9013 × *C. cajanifolius* (lane 4) derived from SDS-PAGE of seed globulins; Male parent-specific polypeptide markers are indicated on the right with their R_m values

were universally present in all the five genotypes. Four polypeptide bands (R_m 0.248, 0.269, 0.309 and 0.530) were expressed only in the two pigeon pea varieties and lacked expression in *C. cajanifolius* and the two putative hybrids. No conclusion, however, could be made from the present study on the genetic reasons for the failure of expression of these four polypeptides in the hybrids.

Six polypeptides of R_m values 0.253, 0.293, 0.373, 0.452, 0.523 and 0.619 were expressed in *C. cajanifolius* but failed to express in the two pigeon pea cultivars. Out of these, five polypeptides (R_m 0.253, 0.293, 0.452, 0.523 and 0.619) were inherited by both the putative F_1 hybrids. These five polypeptides specific to *C. cajanifolius* can be potentially used as markers for identification of hybrids in wide hybridization programmes involving *C. cajanifolius* as the male donor parent.

References

1. **Panigrahi J.** 1999. Genetic Improvement of Pigeon pea. Unpubl. M. Phil. Thesis, Berhampur University, Berhampur.
2. **Mohanty M and Patnaik S. N.** 1989. Cytomorphological analysis of F_1 hybrids between *Cajanus cajan* (L.) Millsp. and *Atylosia cajanifolia* (Haines). Cytologia, **54**: 121-128.
3. **Mohanty J. B., Naik B. S., Panigrahi J and Kole C.** 2001. Identification of mungbean cultivars based on electrophoretic banding patterns of seed proteins. Crop Res., **21**: 134-138.
4. **Smith S. C. and Wych R. D.** 1986. The identification of female selfs in hybrid maize. Seed Sci. Technol., **14**: 1-8.
5. **Bennet M., Sajid G. M., Chattern N. J. and Asaif K. H.** 1991. Electrophoretic characterization of quack grass and bluebunch wheat grass hybrid seeds. Seed Sci. Tech., **19**: 355-362.
6. **Parani M., Singh K. N., Rangasamy S. and Ramalingam R. S.** 1997. Identification of *Sesamum alatum* × *Sesamum indicum* hybrid using protein, isozyme and RAPD markers. Indian J. Genet., **57**: 381-388.
7. **Ladizinsky G. and Hamel A.** 1980. Seed protein profiles of pigeon pea (*Cajanus cajan*) and some *Atylosia* species. Euphytica, **29**: 313-317.
8. **Pundir R. P. S. and Singh R. B.** 1985. Cytogenetics of F_1 hybrids between *Cajanus* and *Atylosia* species and its phylogenetic implications. Theor. Appl. Genet., **71**: 216-220.
9. **Jha S. S. and Ohri D.** 1996. Phylogenetic relationships of *Cajanus cajan* (L.) Millsp. (pigeon pea) and its wild relatives based on seed protein profiles. Genet. Res. Crop Evol., **43**: 275-281.
10. **Laemmli U. K.** 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophages T4. Nature, **227**: 680-685.
11. **Osborn T. C.** 1988. Genetic control of bean seed protein. CRC Critical Rev. Plant Sci., **7**: 93-116.
12. **Brown J. W. S., Bliss F. A. and Hall T. C.** 1981. Linkage relationships between genes controlling seed proteins in French bean. Theor. Appl. Genet., **60**: 251-259.