Indian J. Plant Physiol., Vol. 8, No. 4, (N.S.) pp. 402-404 (Oct.-Dec., 2003)

SHORT COMMUNICATION

BIOCHEMICAL APPROACH FOR IDENTIFICATION OF CLUSTERBEAN VARIETIES

K.C. GOYAL* AND S.N. SHARMA

Biochemistry and Plant Physiology Laboratory, STR, Rajasthan Agricultural University, Agricultural Research Station, Durgapura, Jaipur - 302 018

Received on 7 June, 2003

Clusterbean varieties, viz. RGC 936, RGC 986, RGC 1003, RGC 1033, RGC 1055, RGC 1059, were distinguished by SDS-PAGE profiles of seed proteins. The band profile for one variety was clearly distinguishable from another. Such a biochemical approach offers a useful and rapidly performed adjunct to the more traditional methods of varietal identification in clusterbean.

***Key words*: Clusterbean, electrophoresis, varietal identification**

Clusterbean (guar) is an important cash crop in India. The seeds are rich in protein and gum contents. Traditionally, the identification of clusterbean varieties is carried out on the basis of morphological descriptors (grow out test) and/or with the help of certain additional physiological traits. Identification, only on the basis of morphological characters sometimes becomes quite difficult, because same variety can reveal striking morphological differences under variable environmental and agronomical conditions. Proteins being the direct gene products, reflect the genomic composition of varieties accurately and therefore, are ideal for genotypic distinctness. Biochemical approach like gel electrophoresis of proteins and isozymes is a powerful tool to distinguish among varieties in crops (Bushuk and Zillman 1978, Goyal, *et al.* 1986, Cooke 1984, 1992, 1995, Dadlani *et al.* 1994, Goyal and Sharma 1998, Rao *et al.* 2001). In the present investigation, an attempt has been made to identify clusterbean varieties on the basis of electrophoregrams of seed proteins using SDS-PAGE (Sodium dodecylsulphate polyacrylamide gel electrophoresis) method.

Seeds of six varieties of clusterbean, namely RGC 936, RGC 986, RGC 1003, RGC 1033, RGC 1055, RGC 1059, were procured from clusterbean breeder, Agricultural Research Station, Durgapura, Jaipur. Clusterbean seeds were crushed to powder using pestle and mortar. The powdered seed (0.5g) was vigorously mixed in ethyl acetate for 20 min with the simultaneous addition of sodium sulphite (1 mg) and sodium metabisulphite (1 mg) and centrifuged at 5000 rpm for 10 min in a refrigerated centrifuge. The residue was treated with CMA solution (Chloroform: Methanol: Acetone - 2: 1:1, respectively) for 20 min with vortexing and then solution was decanted off. The treatment was repeated one more time as above and centrifuged at 5000 rpm for 10 min. The final residue was kept in suitable volume of extraction buffer (0.1 M Tris-HCl buffer pH 7.5) for 16 to 18 hrs in cold. The contents were centrifuged at 10,000 rpm for 15 min. The clear supernatant was used as protein sample (soln. I). During protein extraction, treatments with ethyl acetate and CMA solution removed most of the lipids, as presence of lipids interferes during isolation of proteins (Stegemann and Pietsch 1983). To prevent auto-oxidation of phenols, sodium sulphite and sodium metabisulphite were added. The loss of proteins by above treatments was negligible.

SDS - Polyacrylamide slab gel electrophoresis was carried out according to Laemmli (1970) using 12% acrylamide separating gel (1.5 M Tris-HCl buffer pH 8.8) with top layer of 6% acrylamide stacking gel (0.5 M Tris-

* Corresponding author.

HCl buffer pH 6.8). Aliquot (100 µl) of protein sample (soln. I) was treated with equal volume of sample buffer (containing 0.5 M Tris-HCl buffer pH 7, 9.6 g urea, 1 g SDS, 4 ml glycerol, 1 ml bromophenol blue tracking dye) and kept at 90°C water bath for 10 min (soln. II). The standardized volume of soln. II was loaded on slab gel well. An electric current of 36 mA was passed for 45 min and then raised to 58 mA until the tracking dye migrated to the anode end of the slab gel. The samples were applied in duplicate using two slab gel plates at a time in an electrophoresis assembly (Make: ATTO Corpn. Japan). All the operations were carried out in cold environment. Staining was carried out in Coomassie brilliant blue R (CBBR) solution (0.5 g CBBR dissolved in 250 ml methanol, 10 ml acetic acid and 240 ml water). The gels were destained by repeated washings with methanol: acetic acid: water (50: 70: 880 v/v/v respectively) until the bands became prominent and resolutive. The destained gels were image scanned densitometrically using densitometer (Bio-Rad make, USA, Model: GS 700) and protein electrophoregrams were analysed for presence and/or absence of protein bands. The protein band profile for each variety was obtained.

Table 1. Protein banding pattern of clusterbean varieties.

Protein band No.	Rm value	Varieties (presence/absence of protein band and intensity)					
		RGC 936 (16)	RGC 986 (19)	RGC 1003 (18)	RGC 1033 (18)	RGC 1055 (17)	RGC 1059 (20)
1	0.054	-	-	-	-	-	++
2	0.081	-	-	-	-	-	++
3	0.140	+++	+++	+++	+++	++	++
4	0.163	-	-	-	-	+	+
5	0.195	+++	+++	+++	+++	++	++
6	0.213	+	+	+	+	+	-
7	0.250	++	++	++	+	++	+++
8	0.272	-	+	+	+	-	-
9	0.300	-	-	-	-	-	++
10	0.327	++	++	++	++	++	+++
11	0.354	-	-	-	+	+	-
12	0.409	+++	+++	+++	+++	++	+++
13	0.477	+	+	+	+	-	+
14	0.495	+	+	+	+	+	+
15	0.545	+++	+++	+++	+++	++	+++
16	0.572	+++	+++	+++	+++	+++	+++
17	0.595	+	+	+	+	+	+
18	0.636	+	+	+	+	+	+
19	0.654	+	+	+	+	+	+
20	0.695	++	++	++	++	++	+++
21	0.727	++	++	++	+	++	+++
22	0.754	-	+	-	-	-	-
23	0.863	+++	+++	+++	+++	++	++
24	0.954	-	++	++	-	-	+++

(+++: High, ++; Medium, +: Low intensity. The values in parenthesis indicate the total number of protein bands)

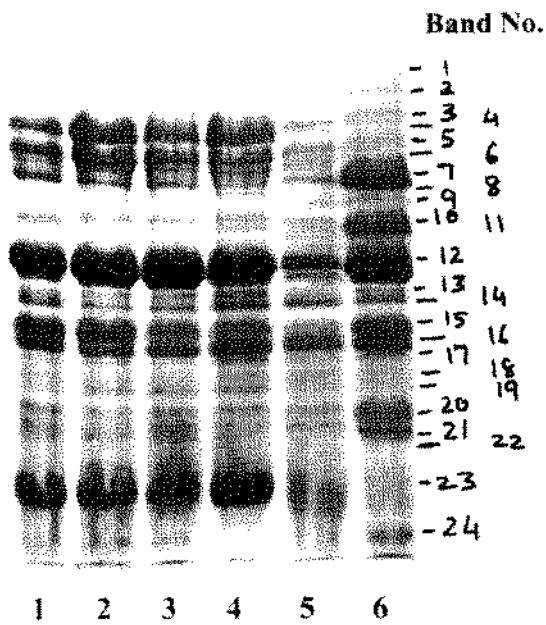

Fig. 1. Protein banding patterns of clusterbean varieties (From left to right: 1. RGC 936, 2. RGC 986, 3. RGC 1003, 4. RGC 1055, 5. RGC 1059, 6. RGC 1033.)

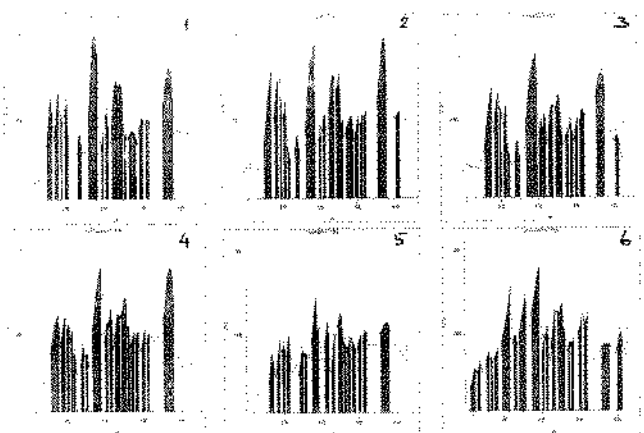

Fig. 2. Protein band profiles of clusterbean varieties after densitometric image scanning (1. RGC 936, 2. RGC 986, 3. RGC 1003, 4. RGC 1055, 5. RGC 1059, 6. RGC 1033.)

The protein electrophoregrams of all six clusterbean varieties are shown in Fig. 1. The varieties were distinguishable from each other by determining the presence and/or absence of specific protein band/s in their electrophoregrams and the results are depicted in Table 1. The protein band profiles of all six varieties are depicted in Fig. 2 which clearly showed a distinguishable pattern for each variety. In all 108 protein bands were observed among six varieties, being maximum (20) in RGC 1033 and minimum (16) in RGC 936. The similarities of certain protein bands among varieties obviously are due to the genetic relationships among them.

This biochemical approach for varietal identification in clusterbean is quick and reliable as compared to normally carried out and time-consuming grow out test.

REFERENCES

Bushuk, W. and Zillman, R.R. (1978). Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature. *Can. J. Plant Sci.* **58**: 505-515.

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

Cooke, R.J. (1992). Handbook of Variety Testing: Electrophoresis Handbook: Variety Identification. The International Seed Testing Association, Switzerland.

Cooke, R.J. (1995). Gel electrophoresis for the identification of plant varieties. *J. Chromatography.* **698**: 281-289.

Dadlani, M., Vashisht, V., Singh, D.P. and Varier, A. (1994). A comparison of field grow out and electrophoresis methods for testing genetic purity of cotton hybrid seed. *Seed. Res.* **22**: 160-162.

Goyal, K.C., Singh, G. and Chaturvedi, S.N. (1986). Protein electrophoregram: An identification tool for cotton seed varieties. *Agril. Biol. Res.* **2**: 17-20.

Goyal, K.C. and Sharma, S.N. (1998). Biochemical tool (Discontinuous PAGE) for varietal identification in soybean (*Glycine max* L.) *Seed Res.* **26**: 204-206.

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

Rao Prasada, K.S.S.V., Varier, A., Mahapatra, T., Kumari, K.A. and Sharma, S.P. (2001). Electrophoresis of seed esterases and RAPD analysis for identification of hybrids and parental lines of pearl millet. *Plant Varieties and Seeds* **14**: 41-52.

Stegemann, H. and Pietsch, G. (1983). Methods for characterization of seed proteins in cereals and legumes. In: W. Gottschack and H.P. Mueller (eds.), Seed Proteins: Biochemistry, Genetics, Nutritive Value, pp. 45-75. Martinus Nijhoff, The Netherlands.