Identification of Groundnut (*Arachis Hypogaea* L.) Varieties through Biochemical Markers

BN Motagi, MVC Gowda*, GK Naidu and J Salim

Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, Dharwad-580005, Karnataka

Sixteen groundnut cultivars were assessed for protein (seed and hypocotyl) and glutamate oxaloacetate transaminase (GOT) polymorphism. Seed protein profiles produced six (S1-S6) electrophoretic phenotypes compared to three (G1-G3) of GOT, while, hypocotyl proteins produced twelve (H1-H12) electrophoretic patterns. Individually, the seed and hypocotyl proteins can identify three and nine cultivars, respectively. However, combination of protein (seed and hypocotyl) and GOT isozyme profiles differentiated all the sixteen cultivars.

Key words: Groundnut, Varietal identification, Biochemical markers

Electrophoresis of storage and functional proteins has emerged as an efficient, simple and reliable tool supplementing traditionally used morphological parameters (Picket and Jarman, 1994). ISTA has adopted standard SDS (sodium dodecyl sulphate), PAGE (Poly acryl amide gel electrophoresis) and IEF (Iso Electric Focusing) methods into the international rules (ISTA, 1996) for cultivar identification. Seed proteins and isozymes because of their stability and reproducibility have potential to serve as tools for fingerprinting of genotypes. Therefore, the present study was carried out to determine the protein (seed and hypocotyl) and isozyme (GOT) variation in groundnut cultivars and their potential for finger printing.

Materials and Methods

Sixteen groundnut varieties belonging to Spanish bunch (Spanish Improved, TMV 2, S 206, Dh 3-30, JL 24, KRG 1, K 134, Dh 8, R 8808, Dh 40, TAG 24 and GPBD 4), Virginia runner (S 230 and DSG 1) and Valencia (ICGV 86590 and mutant 28-2) botanical types released for cultivation were assessed for protein (seed and hypocotyl) and isozyme (GOT) polymorphism.

The analysis of proteins and isozyme was performed by polyacrylamide gel electrophoresis (PAGE) technique. Gels were prepared according to the method of Hames and Rickwod (1984). To study seed protein polymorphism, one-dimensional SDS-PAGE (12.5% separating gel and 2.5% stacking gel) was carried out following the procedure given by Laemmli (1970) in a vertical gel system. For this purpose total protein was extracted after suspending seed flour for 1 hour in an extraction buffer (65mM Tris-HCl, pH 6.8) followed by centrifugation at 10,000 rpm at 4 °C for 20 minutes. Then the protein sample was heated for 3 min in boiling water and gradually cooled. 100μg of protein was loaded with micropipette along with 10mL sample buffer containing bromophenol blue in each sample well. The gel was run at 70 Volts for 3 h followed by staining in Coomassie Brilliant Blue R 250 for 6-8 h. Relative mobility (Rm) of the protein band was determined. For hypocotyl protein and GOT, native PAGE was done with 7.5% separating gel. One gm of fresh hypocotyl sample from 6 days old seedling was macerated in phosphate buffer (pH 8.0) at 4 °C. The homogenate was centrifuged at 10,000 rpm for 10 min and the gel was run at 70 Volts for 3 h. Staining was done according to Tankslay and Orton (1983) using Coomassie Brilliant Blue for total protein and Fast Blue BB salt for GOT.

Results and Discussion

Hypocotyl Proteins

A total of 15 bands with Rm values ranging from 0.218 to 0.945 were resolved. Of these, seven bands (0.345; 0.545, 0.600, 0.625, 0.636, 0.691 and 0.945) were polymorphic. Based on presence/absence of these bands, 12 electrophoretic phenotypes (H1-H12) were identified (Plate 1 and Table1). Nine cultivars (K 134, KRG 1, Dh 40, DSG 1, Dh 3-30, TMV 2, ICGV 86590, Mutant and GPBD 4) exhibited unique banding pattern. Two groups had two cultivars each {Spanish Improved and JL 24 (H3), and S 206 and S 230 (H6)} and one group (H12) had three cultivars [Dh 8, R 8808 and TAG 24].

Seed Proteins

SDS-PAGE of seed proteins showed differences in number and intensity of bands for different groundnut cultivars. A total of 17 bands appeared with Rm values

*Corresponding Author: E-mail: mvcgowda@satyam.net.in

Table 1. Pattern of Polymorphic bands for proteins (hypocotyls and seed) and Glutamate Oxaloacetate Transaminase (GOT) isozyme in groundnut cultivars

Variety	Hypocotyl protein							Seed protein				GOT	
	0.345	0.545	0.600	0.625	0.636	0.691	0.945	0.529	0.567	0.739	0.962	0.338	0.385
Spanish Improved	+	+	+	+	+	+	–	–	+	–	–	–	+
TMV 2	+	+	–	+	–	+	–	–	+	–	–	–	–
S 206	+	+	+	–	+	+	–	–	+	–	–	–	+
Dh 3-30	+	+	–	–	+	+	–	–	+	–	–	–	+
JL 24	+	+	+	+	+	+	–	–	+	–	–	–	–
KRG 1	+	+	–	+	+	+	+	–	+	–	+	–	+
K 134	+	–	+	+	+	+	+	–	+	–	–	–	+
Dh 8	+	+	–	–	–	–	–	–	+	–	–	–	–
S 230	+	+	+	–	+	+	–	–	+	+	+	+	+
R 8808	+	+	–	–	–	–	–	+	–	–	+	+	+
Dh 40	+	–	–	+	+	+	+	–	+	–	–	–	+
DSG 1	+	–	+	–	+	+	+	–	+	+	+	+	+
TAG 24	+	+	–	–	–	–	–	–	+	+	–	+	+
ICGV 86590	–	+	–	–	+	–	+	–	+	+	+	–	+
Mutant (28–2)	–	+	–	–	–	–	+	+	+	+	+	+	+
GPBD 4	–	+	–	+	–	–	–	–	+	–	+	–	+

ranging from 0.300 to 0.970. Of these, only four bands with Rm values 0.529, 0.567, and 0.962 were polymorphic forming six electrophoretic phenotype patterns (S1-S6; Plate 1 and Table 1). Among the 16 cultivars, three (Mutant, R.8808 and TAG 24) exhibited unique banding patterns and thus could be identified solely by the cultivar specific electrophoretogram. Pattern I (S1) had Valencia Mutant; Pattern II (S2) had Virginia runner (S 230 and DSG 1) and Valencia (ICGV 86590) cultivars. While, other electrophoretic patterns (S3-S6) had Spanish bunch cultivars. GPBD 4 and its female parent KRG1 showed similar banding pattern and formed a separate group (S5). Hartzook *et al.* (1969) also observed differences in seed protein content among three botanical types and concluded that PAGE of seed proteins could be used as tool in establishing cultivar identification.

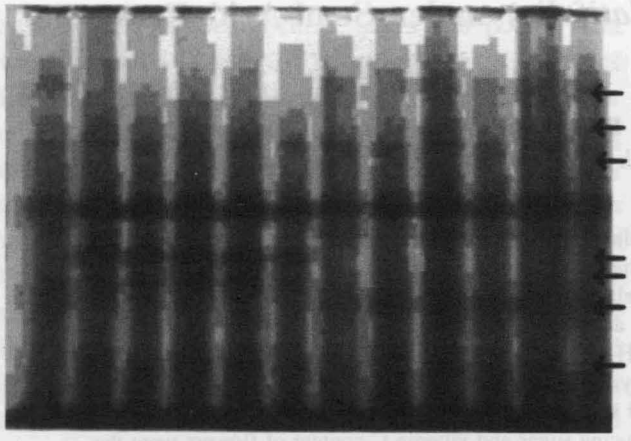
GOT Isozyme

GOT revealed four bands (Rm values 0.338, 0.385, 0.431 and 0.492). Presence and/or absence of first two bands resulted in three banding patterns (G1-G3; Plate 1 and Table1). The pattern I (G1) comprised five cultivars having both the polymorphic bands (0.338 and 0.385). Grieshammer and Wynne (1990) and Lacks and Stalker (1993) reported that, these two polymorphic bands were present only in Virginia types. Pattern II (G2) with eight genotypes had only one polymorphic band (0.385); while, pattern III (G3) comprised three genotypes lacked both the polymorphic bands.

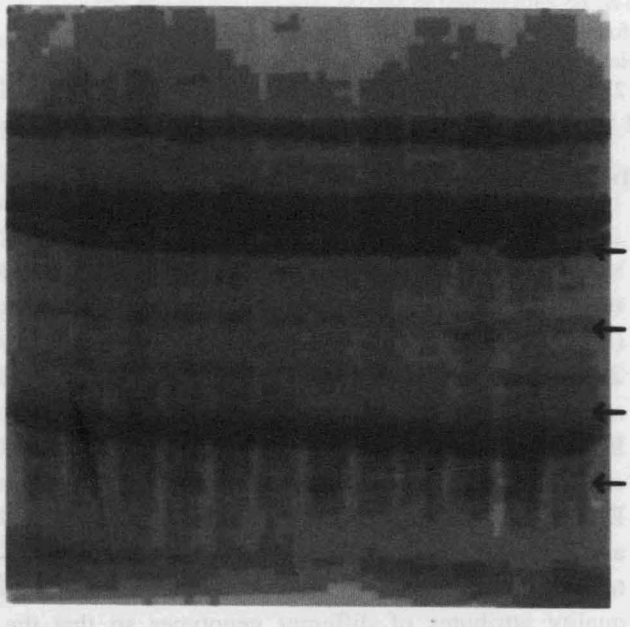
Pattern I that is specific to Virginia botanical type had cultivars belonging to Virginia (S 230 and DSG 1) with some Spanish cultivars (R 8808, TAG 24 and Mutant 28-2). TAG 24 is a cross derivative from two mutants TGE 1 and TGS 2, which in turn derived from Virginia cultivars *viz.*, TG 1 and M 13, respectively. R 8808 had ICGS 11 in its pedigree, which is a selection from Robut 33-1, a Virginia bunch cultivar. 28-2 is a secondary mutant of Dharwad Early Runner (DER). While pattern II and III had Spanish bunch cultivars.

Cultivar Fingerprinting

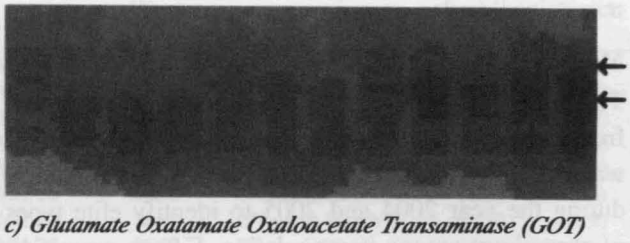
Hypocotyl protein profile has an advantage of twelve (H1-H12) electrophoretic phenotypes compared to six (S1-S6) of seed protein and three (G1-G3) of GOT, which can be used to identify the groundnut cultivars. Hypocotyl and seed protein together identified 14 cultivars. While, Spanish Improved could not be differentiated from JL 24 as they formed a separate group (H3S6). Combination of hypocotyl protein and GOT profiles formed fifteen groups and identified 14 cultivars. Cultivars R 8808 and TAG 24 formed a single group (H12G1). Seed protein and GOT profiles together resulted in eight groups identifying four cultivars. Two groups (S2G1 and S5G2) had two cultivars each, one



a) Hypocotyl proteins



b) Seed proteins



c) Glutamate Oxatamate Oxaloacetate Transaminase (GOT)

Fig. 1: Banding patterns with polymorphism for protein (hypocotyl and seed) and GOT Isozyme

Table 2. Protein fingerprint of groundnut cultivars

Protein fingerprint	Cultivar
H1 S6 G2	K 134
H2 S5 G1	KRG 1
H3 S6 G2	Spanish Improved
H3 S6 G3	JL 24
H4 S6 G2	Dh 40
H5 S2 G1	DSG 1
H6 S2 G1	S 230
H6 S6 G2	S 206
H7 S6 G2	Dh 3-30
H8 S6 G3	TMV 2
H9 S2 G2	ICGV 86590
H10 S1 G1	Mutant (28-2)
H11 S5 G2	GPBD 4
H12 S3 G1	R 8808
H12 S4 G1	TAG 24
H12 S6 G3	Dh 8

group each with three (S6G3) and five (S6G2) cultivars. However, proteins (seed and hypocotyl) and GOT profiles together identified all the sixteen groundnut varieties (Table 2). The multiple enzyme system was used for complete cultivar fingerprinting in soybean. Thus, electrophoresis can be used successfully as a tool for varietal identification in groundnut.

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