Short communication

Diversity of blackgram (*Vigna mungo* L. Hepper) genotypes assessed through morphological and biochemical approaches

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Received 2 June 2009; received in revised form 3 July 2009; accepted 6 July 2009.

Abstract

Morphological and seed protein variations in 12 blackgram genotypes from the eastern, northern, and southern parts of India were assessed. The morphological response of the genotypes over two years (2006 and 2007) in four consecutive environments (*rabi* and pre-*kharif*) revealed three clusters with overlapping genotypes. Five clusters obtained through SDS-PAGE seed protein analysis expressed better grouping of genotypes. The clustering pattern, however, did not reflect the geographical distribution of genotypes.

Keywords: Euclidean distance matrix, Geographic distribution, Seed proteins.

Blackgram (*Vigna mungo* L. Hepper), an important protein–rich (21.8–26.3%) grain legume, has limited diversity in the Indian subcontinent, which impedes its yield and protein content improvement programmes (Roy, 1995). Moreover, only few studies have assessed genetic variations of blackgram in India. Diversity is generally assessed based on morphological characters using multivariate analysis (Gantait and Das, 2009). The biometric characters, however, are known to be greatly influenced by the environment that makes such studies unreliable (Dasgupta and Das, 1984). Long-term variability studies, therefore, may be necessary to overcome such problems. Biochemical analyses including seed protein profiling provide consistent results as they are less likely to be altered by the environment (Roy et al., 2001). The present study aims to profile the variations in 12 blackgram genotypes from the northern, southern, and eastern states of India based on long term morphological assessments and seed protein analysis using SDS-PAGE.

Based on yield performance and local popularity, 12 blackgram genotypes (seven from Andhra Pradesh in southern India: LBG 708, 709, 719, 731, 738, 611, and 697; four from West Bengal in eastern India: B 76, WBG 26, WBU 705, and WBU 108; and one from Uttar Pradesh in northern India: T 9) were selected and grown in a randomized block design experiment with three replications. A plant to plant distance of 15 cm and a row to row spacing of 30 cm were maintained in 3 × 1 m plots. The experiment was conducted during *rabi* (first week of November) and pre-*kharif* (first week of April) seasons of 2006 and 2007 (four consecutive seasons). Normal intercultural operations were followed. Fertilizers such as single super phosphate and muriate of potash were applied @ 20 kg·ha^{-1} during land preparation. However, no nitrogen fertilizers were used. From each plot, 10 plants without any infection were taken randomly. Observations were recorded on the number of pods/plant, number of seeds/pod, 100 seed weight and seed yield/plant. Data were pooled

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and subjected to Euclidean distance matrix analysis (NTSyspc software package). For each genotype, bulked seeds of 5 g were crushed using mortar and pestle. Protein extraction was done with 100 mg seed powder and a solution containing 25 ml 0.5 M tris-HCl (pH 6.8), 20 ml glycerol, 10 ml 10% SDS, 1 ml 2-mercaptoethanol, and 44 ml double distilled H_2O. The extracts were centrifuged at 10000 rpm for 15 min. and the supernatants were heated at 95°C for 5 min. in a water-bath before loading on the gel. Electrophoresis was performed twice each year following the method of Laemmli (1970) and the gels were kept in the staining solution with 0.2% Coomassie Brilliant Blue G-250 in 45:45:10 % methanol: water: acetic acid overnight. De-staining was performed in a solution of 25: 65: 10% methanol: water: acetic acid with continuous gentle shaking at 67°C for 3–4 h (Emre, 2009). Protein bands were scored for their presence as *1* and absence as *0* from the de-stained gels. Relative mobility (Rm) values for each band was calculated using the distance travelled by individual protein/total distance run by the solvent front (Kole et al., 2006). A distance matrix was established from the banding patterns of 12 genotypes (Yuzbasioglu et al., 2008).

Dendrogram constructed from the Euclidean distance matrix comparing morphological responses (Fig. 1) revealed three distinct groups with B 76 and T 9 in cluster *1*; LBG 697, WBG 26, WBU 105, and WBU 108 in cluster *2*, and LBG 611, LBG 708, LBG 709, LBG 719, LBG 731 and LBG 738 in cluster *3*. Although no overlapping was observed between the first and second clusters, geographic distribution of the genotypes was not a major determinant of the clustering pattern, which is consistent with the observations of Dasgupta and Das (1984) in wheat.

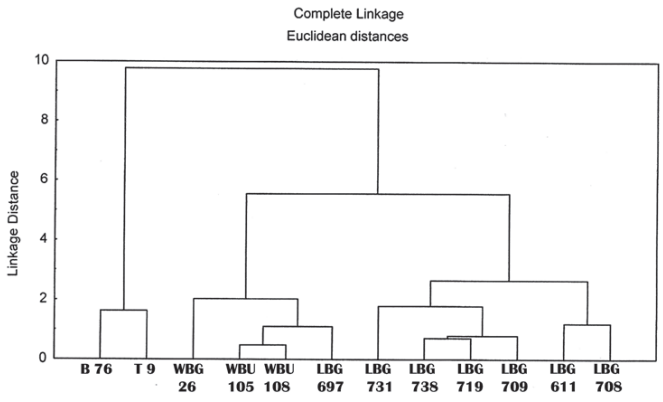

Figure 1. Dendrogram of 12 blackgram genotypes of northern, eastern and southern India based on Euclidean distance matrix of morphological responses.

Results of SDS-PAGE analysis of total seed proteins are shown in Fig. 2. A total of 16 distinctly scoreable polypeptide bands were detected with Rm values ranging from 0.09 to 9 (Fig. 3). Nine polypeptide bands were polymorphic and hence used to discriminate the genotypes. Between WBU 105 and WBU 108 there were two polymorphic bands with different Rm values (Table 1). Six polymorphic bands were scored between LBG 708 and LBG 709 as well as LBG 731 and LBG 738 (Fig. 3). Rm values indicated differences in proteins of higher molecular weight for majority of the

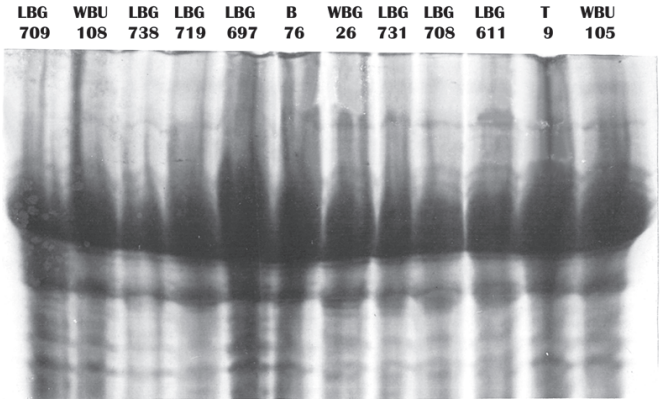

Figure 2. Electropherogram showing seed protein banding pattern in 12 blackgram genotypes form northern, eastern and southern India.

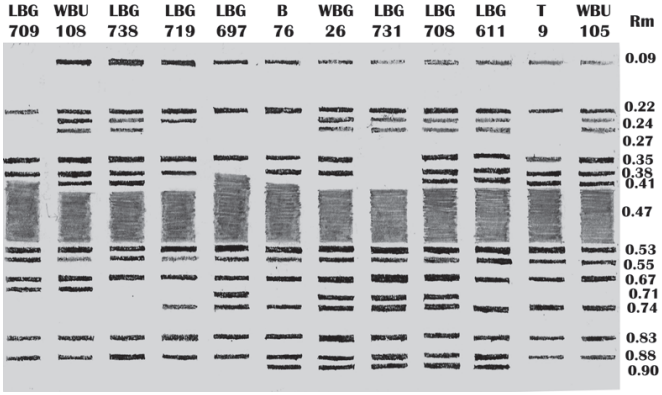

Figure 3. Zymogram showing seed protein profile of 12 blackgram genotypes from SDS-PAGE.

Table.1. Electrophoresis banding pattern of 12 genotypes from northern, eastern, and southern India based on SDS-PAGE where presence of band is denoted by "+" and absence is by "–".

Band No.	Rm Value	LBG 709	WBU 108	LBG 738	LBG 719	LBG 697	B 76	WBG 26	LBG 731	LBG 708	LBG 611	T 9	WBU 105
1	0.09	–	+	+	+	+	+	+	+	+	+	+	+
2	0.22	+	+	+	+	+	+	+	+	+	+	+	+
3	0.24	–	+	+	+	–	–	+	+	+	+	–	+
4	0.27	–	+	+	–	–	–	+	+	+	+	–	+
5	0.35	+	+	+	+	+	+	+	–	+	+	+	+
6	0.38	+	+	+	+	–	+	+	–	+	+	+	+
7	0.41	–	+	+	–	–	–	–	–	+	+	+	+
8	0.47	+	+	+	+	+	+	+	+	+	+	+	+
9	0.53	+	+	+	+	+	+	+	+	+	+	+	+
10	0.55	+	+	+	+	+	+	+	+	+	+	+	+
11	0.67	+	+	+	+	+	+	+	+	+	+	+	+
12	0.71	+	+	–	–	+	–	+	+	+	–	–	–
13	0.74	–	–	–	+	+	+	+	+	+	+	+	+
14	0.83	+	+	+	+	+	+	+	+	+	+	+	+
15	0.88	+	+	+	+	+	+	+	+	+	+	+	+
16	0.90	–	–	–	–	–	+	+	+	+	+	–	–

genotypes. The dendrogram of SDS-PAGE distance matrix recorded five distinct groups (Fig. 4). Seed protein analysis distinguished the genotypic groupings more clearly than morphological characters, although it did not reflect the geographical distribution pattern. Pariya et al. (1997) also noted lack of positive association between geographic distribution and genetic diversity in this crop.

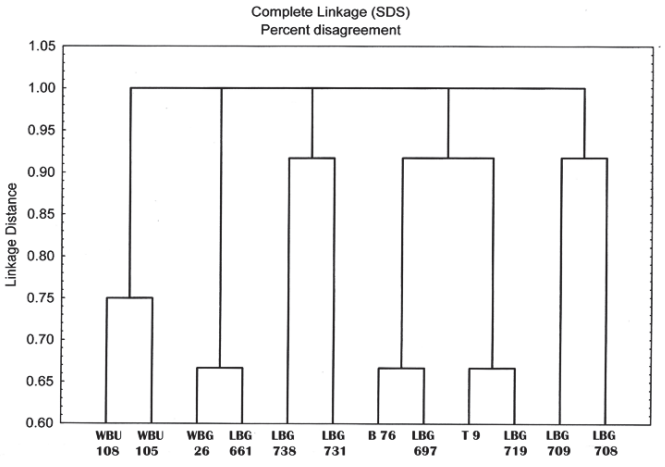

Figure 4. Dendrogram of 12 blackgram genotypes of northern, eastern and southern India based on dissimilarity index of seed protein.

Overall, diversity analysis based on biochemical approaches is a better discriminator than morphological attributes. However, genotypes showing close affinity for morphological characters within a certain geographical location stood distinctly apart in seed protein grouping meaning that clustering pattern based on morphological character and seed proteins may not result in identical results.

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