The Andhra Agric. J 69 (2): 217-228, 2022

Characterization and Identification of Mungbean *(Vigna radiata L.* Wilczek*)* Varieties using Chemical Tests and Gel Electrophoresis of Soluble Seed Proteins

E Madhavi, P S Rao and K V Radhakrishna

Acharya N.G. Ranga Agricultural University, SRTC, A.P.

ABSTRACT

Variety characterization and identification has become invariably significant for purity maintenance during seed production as well as for the varietal protection under plant variety protection. In the present study, an attempt was made to characterize and identify the sixty-four mung bean genotypes based on reaction of seed to different chemicals (NaOH, KOH) and seedling growth response to GA_3 treatment. Out of which, twenty-five cultivars were taken for electrophoretic identification. Depending on their reaction to KOH, the mung bean genotypes were categorized in to three groups viz., genotype showing orange, orange red and dark orange red reaction. Based on the reaction to NaOH test, the genotypes were categorized into three groups viz., genotype showing orange reaction, genotypes showing orange red and genotype showing dark orange red reaction. Similarly based on the seedling response to GA_3, the genotypes were classified into four groups as very low, low, medium and high response. The protein banding pattern of twenty-five mung bean genotypes obtained through SDS-PAGE were conspicuously genotype specific and also the electrophoregram, dendrogram of seed proteins of twenty-five genotypes revealed their uniqueness in identifying individual cultivars.

Keywords: *Electrophoresis, GA_3 test, Mungbean, Potassium hydroxide test, SDS-PAGE, Sodium hydroxide test and Variety identification.*

Mungbean [*Vigna radiata* (L.) Wilczek) or greengram is the most important legume (Pulse) crop in India after chickpea and pigeon pea. It is a great source of proteins, vitamins, and minerals, particularly in South Asia. India is the primary greengram producer and contributes to about 75 per cent of the world pulses production. It contributes to about 14 per cent of total pulses cultivation area and 7 per cent of total pulses production in India. Maharashtra, Rajasthan, Madhya Pradesh, Bihar, Punjab and Andhra Pradesh are the leading producers of green gram. Being major producer of mung beanm, India has developed a large number of commercial cultivars. These varieties are characterized by high degree of homogenity. The Productivity of mung bean is very low *i.e.* only around 500 kg per ha. The low productivity can be attributed to narrow genetic base and lack of suitable genotypes for different cropping situations (Dikshit *et al*. (2009). Being major producer of mung bean, India has developed a large number of commercial cultivars. These varieties are characterized by high degree of homogeneity. The traditional method of variety characterization is the field sown; grow out test which involves examination of plants from vegetative stage to maturity. This is time consuming since it covers the entire duration of the crop which may extend up to100 days. Moreover, plant morphology characters being polygenic in nature are liable to be influenced by the

environment (Rupinder Kaur *et al*. 2017). Hence there is a need to use alternate descriptors which are rapid, accurate and less affected by environment.

The authorities responsible for this task require stable characters for detecting the performance of registered variations and of new releases. Further characterizations such as laboratory tests like the NaOH or KOH test, response of the variety to the added chemical and protein fingerprinting to characterize the germplasm is considered as a reliable tool of intellectual property protection of crop varieties and germplasm. Genetic markers are used for rapid identification and protection of crop varieties. Electrophoresis of seed proteins in barley and wheat and of isozymes in maize, soybean and sunflower have been used as additional characters for establishing distinctness of varieties (Bhat *et al.* 2001). Among the biochemical techniques, sodium dodycyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is the most widely used technique due to its validity and simplicity for describing genetic structure of plant germplasm. Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary problems of several plants (Pervaiz *et al*., 2011; Emre, 2011). Analysis of seed protein can also provide a better understanding of genetic affinity of the germplasm (Shah *et al*., 2011). This method can also be used as a promising tool for differentiating the cultivars of a particular plant species. SDS-PAGE is also considered to be a practical and reliable method for species identification because seed storage proteins are largely independent of environmental fluctuation. This has led to use the technique of seed protein SDS-PAGE for the characterization of mung bean cultivars in addition to seed and plant morphological characterization.

MATERIAL AND METHODS

The test materials comprising of genetically pure seeds of sixty four mung bean (*Vigna radiata* L.Wilczek) genotypes of different origin (Table 1) which are in demand and in seed production chain was obtained and studied at Seed Research and Technology Centre, A.N.G.R. Agricultural University, Hyderabad, Andhra Pradesh.

Chemical tests

The chemical tests are spot tests and they are useful in identification by representing seed coat colour reaction to chemicals. The differential response of seeds of greengram genotypes to chemical solution tests was used as a tool as per standard procedures given by various workers to identify different genotypes.

Potassium hydroxide (KOH) Bleach test

The test was conducted by soaking the seeds in 1:5 (w/v) solution of Potassium Hydroxide (KOH) and 5% of Naocl at 25°C in test tubes (Agrawal, 1998). After 16 hours the change in the colour of the solution in test tubes was recorded. Based on intensity of colour reaction the green gram genotypes were classified into three groups as Dark Orange Red, Orange Red and Orange (Fig. 1).

Sodium hydroxide (NaOH) test

The test was conducted by soaking the seeds in 5% NaOH solution at 25°C. After four hours, the change in the colour of solution and seed coat was recorded. Based on change in colour of the solution the genotypes were categorized into three groups as Orange, Orange red and Dark orange red (Fig. 2).

Seedling growth response to Gibberellic acid (GA_3)

Four hundred randomly selected seeds (100 seeds in each in four replications) were placed with hillum side down on the two layers of moistened germination towels with GA_3 15 ppm solution prepared in distilled water. These rolled towels were placed in vertical position in seed germinator at 25^0C for 7 days. Hypocotyl length was measured in centimeters. The response of GA_3 on seedling growth was measured on the basis of per cent increase over control and the genotypes were classified into four groups as Very low increase, Low increase, Medium increase and High increase.

Electrophoretic technique of total soluble seed proteins

SDS-PAGE of total soluble seed proteins was carried out by using 12 per cent gels according to the methods prescribed by Laemmli (1970) with slight modifications.

Protein was extracted from single seed after defatting (Chloroform, methanol and acetone in 2:1:1 ratio), by adding 0.2 ml Tris glysine extraction buffer (25 mM, pH 8.5). The suspension was centrifuged at 10000 rpm for 15 minutes. The extract was dissolved in equal amount of working buffer (Tris-HCl 0.0625 M, pH 6.8, 2% SDS, 5% 2- mercaptoethanol, 15% glycerol and 0.001% bromophenol blue) and kept in boiling water for 2 minutes, again centrifuged and the supernatant was used for loading on to the gels. A current of 1.5 mA per well with a voltage of 80V was applied until the tracking dye crossed the stacking gel. Later the current was increased to 2 mA per well and voltage up to 120 V. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel. Then the gel was stained using coomaasie brilliant blue solution overnight and destained using a mixture of 227 ml of methanol, 46 ml of acetic acid and 227 ml of distilled water until the bands were clearly visible. Gels were scored for presence and absence of bands for each variety for comparison.

Gel Analysis of Protein

The protein profiles were recorded by placing gel on a transilluminator and the migration values for each of the bands were measured from the point of loading. Relative mobility (Rm) of each band was calculated as:

$$Rm = \frac{\text{Distance traveled by protein sample (cm)}}{\text{Distance traveled by tracking dye (cm)}}$$

Scoring of bands

The protein bands scored visually and traced onto the graph. The 'Rm` value and the banding patterns were drawn. The banding patterns thus obtained were used to detect the differences among the genotypes. These differences were assessed as qualitative and quantitative variations among genotypes (Wouters and Booy 2000).

Qualitative variation

When a particular protein band (as designated by its 'Rm` values) was present in the electrophorgram of one cultivar but absent in that of another, the variation was referred as qualitative variation.

Quantitative variation

When a particular band was observed in the electrophoregram of two or more different cultivars, but differs in band size or staining intensity, the variation was referred as quantitative variation. The band intensity was assessed visually and categorized as faint, light, medium and dense bands and represented by electroporegram (Cherry *et al*. 1970).

RESULTS AND DISCUSSION

With release of large number of crop varieties it is becoming increasingly difficult to distinguish genotypes on the basis of morphological features alone. Hence, a number of chemical tests have been developed for varietal identification. These tests are very quick, easy and reproducible (Agrawal and Sharma, 1989, Aswani kumar *et al.* 1995).

KOH Bleaching Test

Based on the KOH test, genotypes could be grouped into three categories: Orange (19 genotypes), Orange red (28 genotypes) and Dark Orange red (17 genotypes).

NaOH Test

On the basis of colour reaction with Sodium hydroxide solution, the mung bean genotypes could be grouped into three categories: Orange (32 genotypes), Orange Red (PANTM-5) and Dark Orange Red (31 genotypes).

Gibberellic acid (GA_3) Test

The present study, GA_3 (15 ppm) was used and its effect on increase in hypocotyls length over control was studied (Bansal *et al.* 1992). The per cent increase in hypocotyle length over control was ranged from 0.32 (HUM-1) to 55.46 (COGG-912). Based on the response to GA_3, it was possible to categorize the genotypes into Very Low (< 10%), Low (10% to 30%), medium (30% to 50%) and high (> 50 %) increase over control. Among 64 genotypes, 25 genotypes showed very low increase, 27 genotypes showed low increase, 11 genotypes showed medium increase and only COGG-912 showed high increase over control (Fig. 2). The difference between varieties should be based on gene difference which can be measured by comparing the product of gene activity i.e. by using protein as genetic marker.

Electrophoresis of total soluble seed protein through SDS PAGE was used in conjunction with chemical tests in order to characterize the varieties as banding pattern for each genotype will be unique and specific. The banding pattern represented by electrophoregram is presented in Fig. 3. In the present study, 25 genotypes having wide variability for various morphological traits, exhibited a total 25 protein bands. None of the varieties showed all the 25 bands. The total number of bands present in these 25 genotypes ranged from 10 to 25. The genotypes PANTM-5 and TAP-7 produced same number of bands (10) but varied in Rm values and their intensities which indicated the distinct relationship between them. These two genotypes could also be distinguished based on specific dark band (Rm 0.22) in TAP-7 whereas it was absent in PANTM-5. The PANTM-5 had five dark bands (Rm 0.27, 0.32, 0.75, 0.82 and 0.85) which were absolutely absent in TAP-7. The genotypes RMG-429 and Pratap had same band numbers (13) but certain differences were observed in Rm values and intensity of bands which were aided in identifying them individually. Out of 13 bands, 9 bands were with common Rm values (0.15, 0.20, 0.32, 0.47, 0.52, 077, 0.82, 0.85 and 0.95) but differ with intensity except one band with Rm value 0.32 showed dark stains in both genotypes. These two varieties could also be distinguished from one another by qualitative differences in four band Nos. 1, 2, 5, 9 (Rm – 0.02, 0.07, 0.27 and 0.70) which were absent in Pratap. Band No. 5 (Rm-0.27) was specific to RMG-429 and bands at Rm values 0.04, 0.10, 0.50, 0.67 were absent in RMG-429. Electrophoresis of two genotypes viz., PDM-139 and RMG-268 revealed fourteen bands. From the present study, it is evident that there existed both qualitative and

quantitative differences in the protein banding pattern among the two genotypes. The genotype PDM-139 can be distinguished from RMG-268 by presence of band Nos. 1, 3, 4, 5 and 13 (RM – 0.04, 0.10, 0.15, 0.27 and 0.85) which were absent in RMG-268. While one band Nos. 2, 3, 5, 8 and 13 (RM – 0.17, 0.20, 0.35, 0.60 and 0.89) were present in RMG-268 which were absent in PDM-139. Nine bands were Rm values 0.07, 0.32, 0.44, 0.54, 0.67, 0.70, 0.77, 0.82 and 0.95 were present in both genotypes but with different intensities. PKVM-8802, Shalimarmung and MH-96-1 each had 15 bands. One dark band with Rm value 0.32 was common to all the three genotypes and remaining 14 bands differed in banding pattern (Rm values) and as well as intensity. All the three genotypes have no bands with Rm values 0.30, 0.35, 0.57, 0.62, 0.87, 0.92 and 1.00. The genotype MH-961 deferred from other genotypes by the presence of 7 specific band numbers 1,2,4,6,7,10,15 at Rm values 0.02, 0.05, 0.17, 0.22, 0.25, 0.60, 0.89 respectively which were absent in remaining genotypes in this group. The genotype PKVM 8802 can be distinguished from other two genotypes in this group by the absence of 3 bands at Rm values 0.07, 0.44, 0.75 and the presence of 3 specific band Nos.7, 9 and 11 with Rm value 0.47, 0.52 and 0.72 which were absent in remaining genotypes of this group. Shalimarmung is differed from MH-961‘ and PKVM 8802 by the presence of band No.9 (Rm.0.54) and 11 (Rm.0.70) which were absent in other two genotypes. The band was absent at Rm value 0.82 while present in remaining genotypes in this group. Seventeen bands were observed in three genotypes viz., Pusa Ratan, RMG-344 and ML-818. Out of 17 bands only one faint band (Rm value 0.72) was common in all the three genotypes. Remaining 16 bands differed in banding pattern and as well as intensity. Pusaratan is distinguished from RMG -344 and ML-818 by the presence of specific band Nos. 1(Rm 0.04), 9 (0.54) and 10 (Rm 0.62) which were absent in remaining two genotypes. It was also differed by the absence of band at Rm Value 0.35 whereas this band was present in other two genotypes. RMG-344 differed from ML-818 and Pusa Ratan by the presence of band Nos.1 (Rm 0.02), 9 (RM 0.50), 10 (Rm 0.52) and 11 (Rm 0.60) and absence of two bands at Rm 0.44 and 0.89.ML-818 can be identified from other two genotypes of this group by presence of seven specific band Nos.3, 4, 5, 8, 11, 15 and 17 (Rm 0.22, 0.25, 0.30, 0.57, 0.75, 0.87 and 1.00) and absence of bands at Rm values 0.07, 0.15, 0.27, 0.32 and 0.95. Twenty bands were observed in two genotypes viz., Dholi and Hum-12. Out of 20 bands, six bands were common in both genotypes with Rm values 0.10, 0.17, 0.22, 0.47, 0.70 and 0.85. Dholi could be distinguished from HUM-12 by the presence of Dark band Nos.6, 7, 12, 13, 15 and 18 (Rm 0.27, 0.32, 0.60, 0.65, 0.72 and 0.82) and absence of bands at Rm values 0.04, 0.25, 0.30, 0.89, 0.92 and 1.00 which were present in HUM-12. HUM-12 can be identified by the presence of band Nos. 1, 7, 8, 18, 19, 20 (Rm 0.04, 0.25, 0.30, 0.89, 0.92 and 1.00) and absence of bands at Rm values 0.27, 0.32, 0.60, 0.65 and 0.72. Twenty-one bands were observed in two genotypes viz., COGG-912 and Kopergoan. These genotypes had five common bands at Rm values 0.10, 0.22, 0.35, 0.60 and 0.89. COGG-912 could be distinguished by the presence of specific band Nos. 3,6,7,9,10,14 and 18 (Rm 0.15, 0.25, 0.32, 0.44, 0.54, 0.67 and 0.82) and by absence of band at Rm values 0.27, 0.47, 0.62, 0.70, 0.92, 0.95 and 1.00). Twenty-three bands were recognized in Ganga-8 and IPM-99125. Out of 23 bands, eleven bands were found common with Rm values 0.22, 0.25, 0.27, 0.30, 0.35, 0.57, 0.65, 0.77, 0.87, 0.92 and 1.00 and much variation was observed in banding pattern of remaining 12 bands among the two genotypes. Ganga-8 can be distinguished by the

Table 1. List of mungbean genotypes used in the present study

S.No.	Name of the genotype	S.No.	Name of the genotype
1	AKM-8803	33	PANTM-4
2	AKM-9910	34	PANTM-5
3	ASHA	35	PDM-11
4	BM-4	36	PDM-139
5	CO-1	37	PDM-54
6	CO-6	38	PKVM-8802
7	COGG-912	39	PRATAP
8	DHOLI	40	PS-10
9	GANGA-1	41	PS-16
10	GANGA-8	42	PUSA BAISAKHI
11	GM-3	43	PUSA RATAN
12	GM-4	44	PUSA VISHAL
13	HUM-1	45	PUSA-105
14	HUM-12	46	PUSA-9072
15	HUM-2	47	PUSA-9531
16	HUM-6	48	RMG-268
17	IPM-99-125	49	RMG-344
18	K-851	50	RMG-429
19	KOPERGOAN	51	RMG-62
20	MGG-295	52	SHALMAR MUNG
21	MH-96-1	53	SML-134
22	ML-267	54	SML-32
23	ML-5	55	SML-668
24	ML-613	56	SONA
25	ML-818	57	SUJATA
26	MUM-2	58	T-44
27	NDM-1	59	TAP-7
28	OBGG-52	60	TARM-1
29	OUM-11-5	61	TARM-18
30	PANTM-1	62	TARM-2
31	PANTM-2	63	VAMBAN-1
32	PANTM-3	64	WGG-37

Fig 1. Response of mung bean genotypes to NaOH and KOH tests

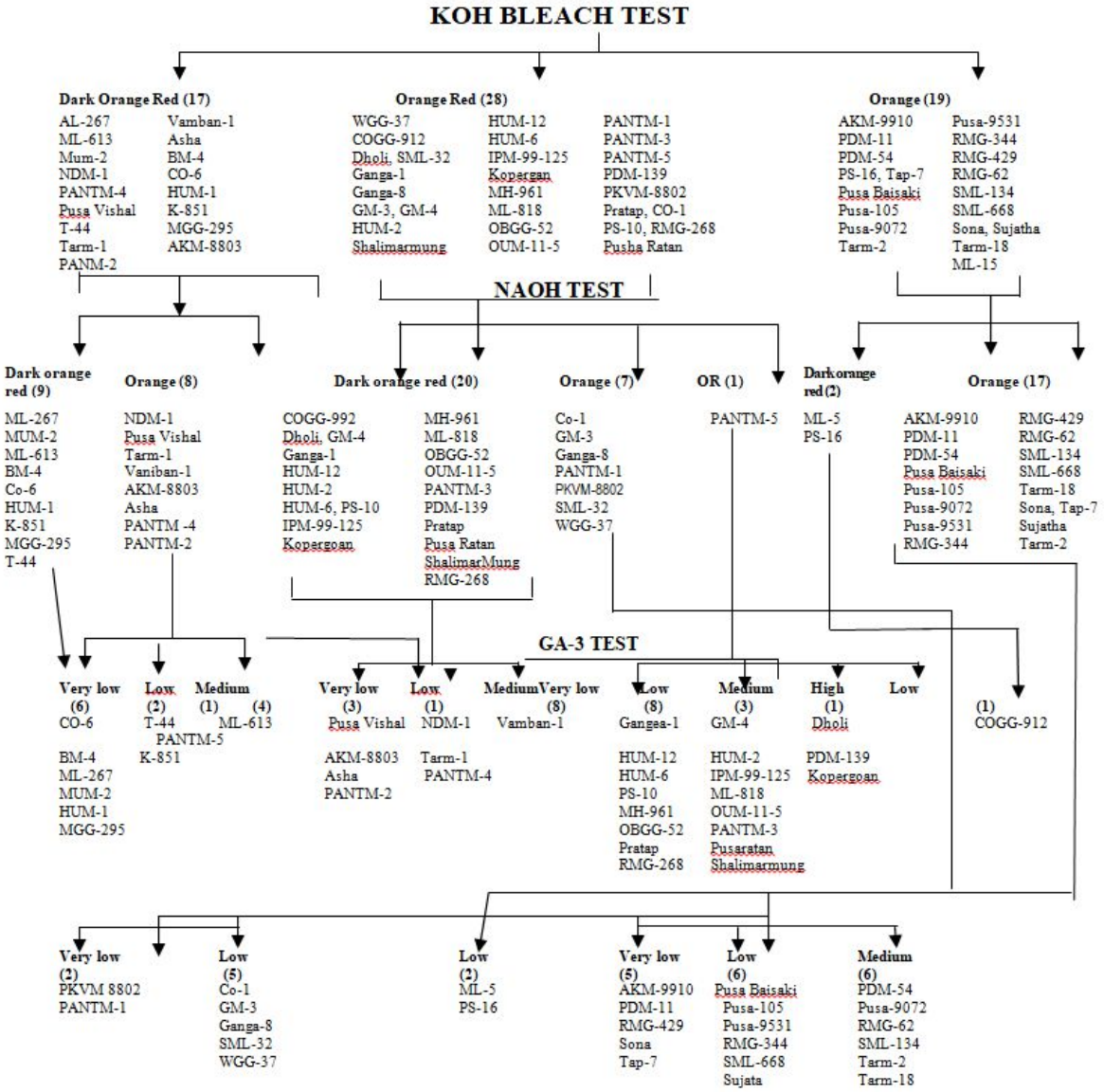

Fig 2. Mung bean genotypes identification based on seed and seedling response to chemical tests

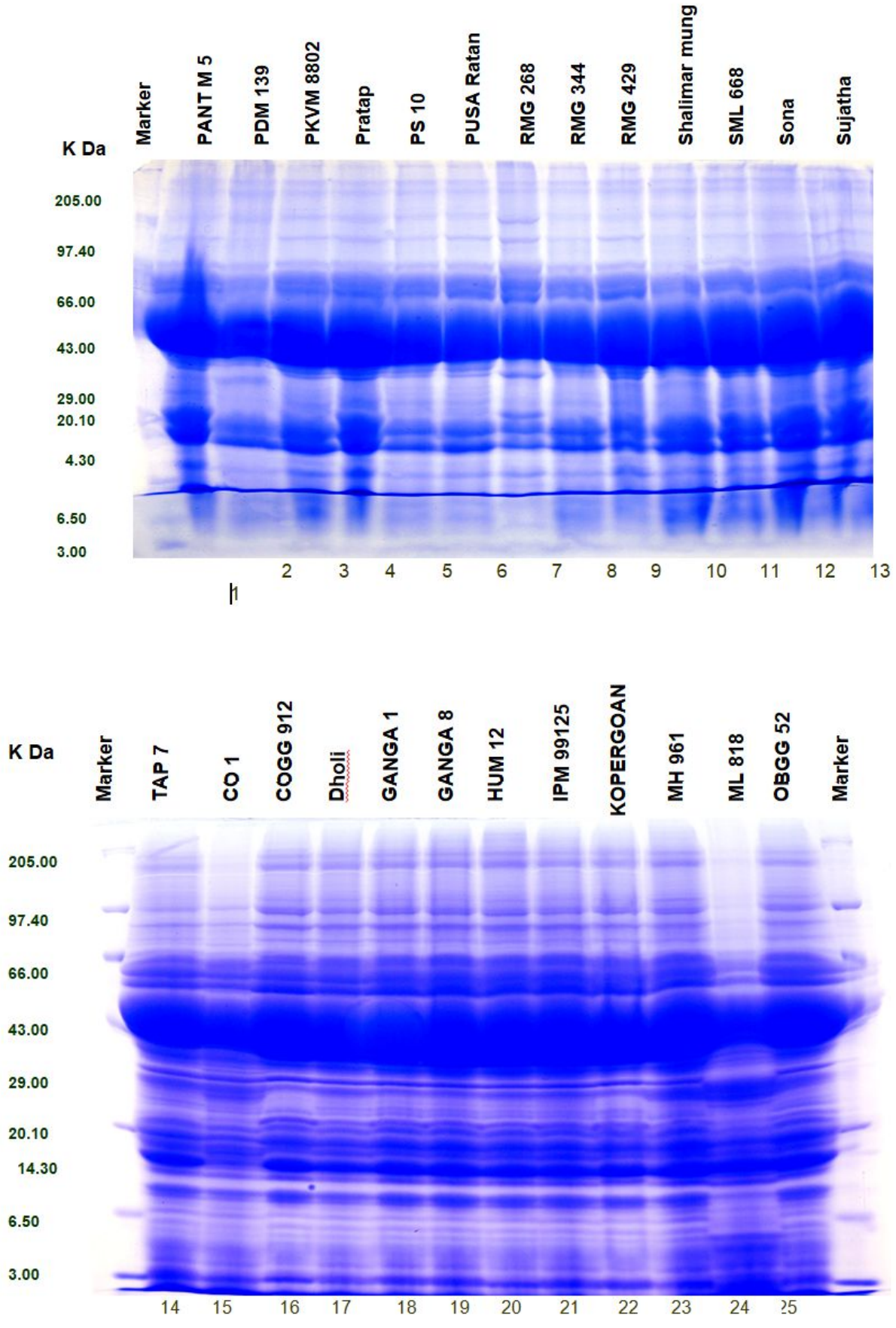

Fig 3. SDS-PAGE profile (banding patterns) of soluble seed proteins in mung bean varieties

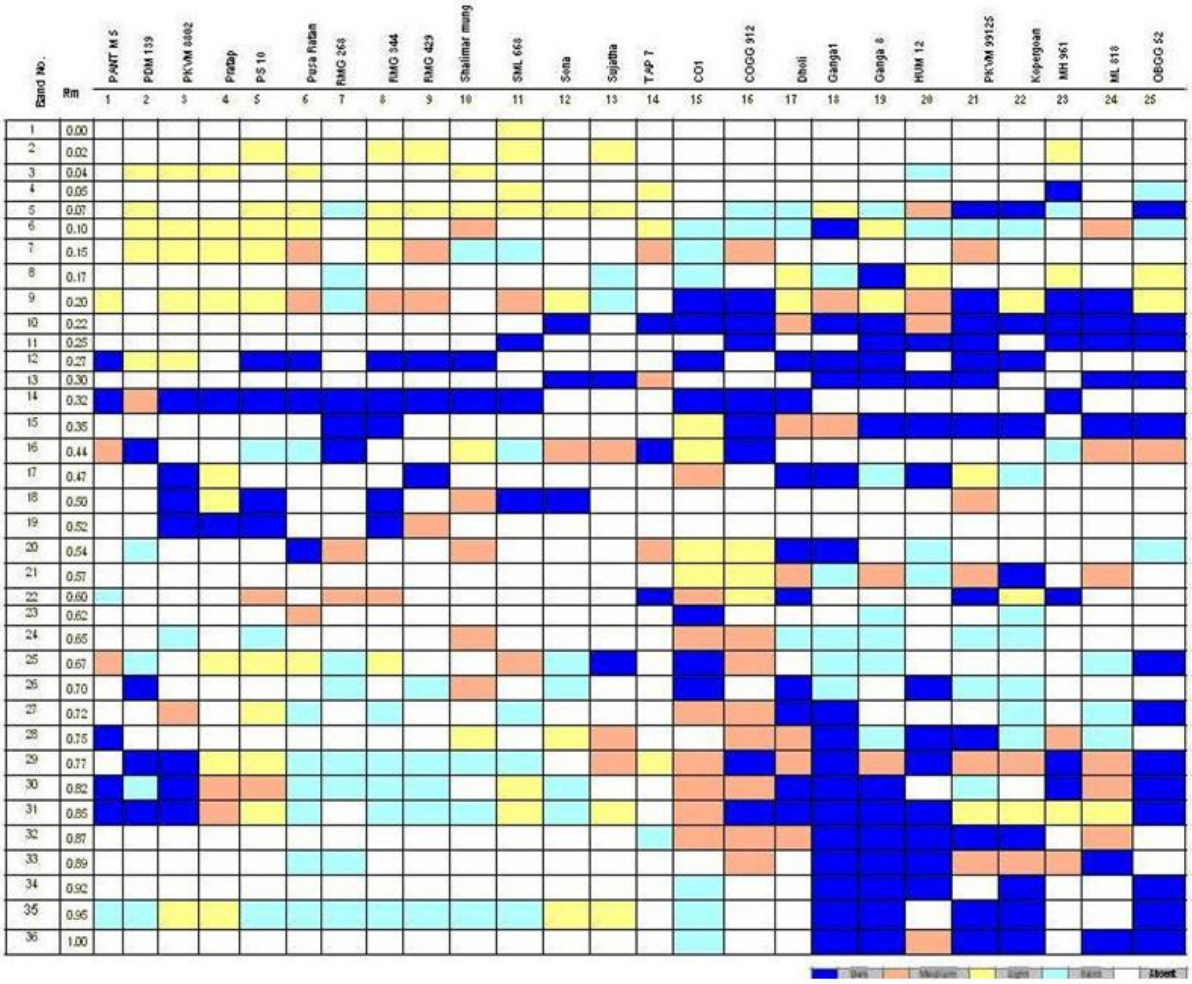

Fig 4. Electrophoregram of soluble seed protein profile in mung bean varieties

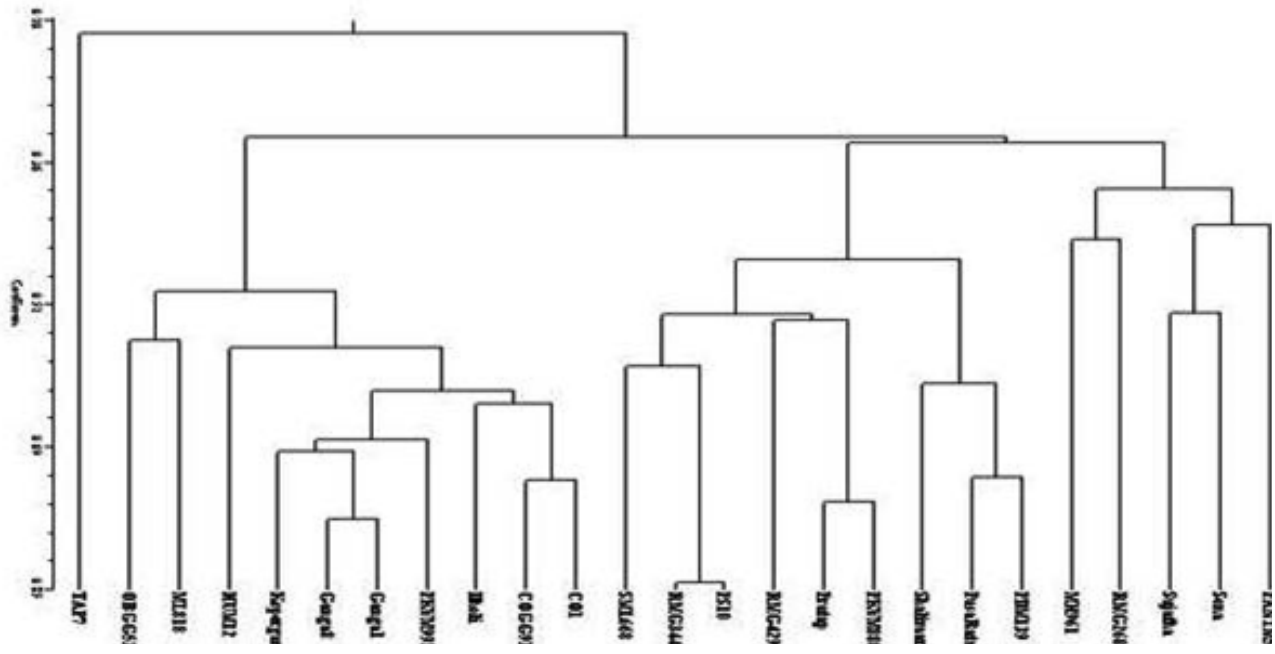

Fig 5. Dendrogram of mung bean varieties generated using SDS-PAGE of soluble seed proteins

presence of specific band Nos.3, 12, 14, 17, and 21 (Rm 0.17, 0.62, 0.67, 0.82 and 0.92) and absence of bands at Rm values 0.15, 0.50, 0.60 and 0.70) which were present in IPM- 99-125 and vice versa. Cluster analysis was performed by means of the unweighted pair group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973).The coefficient value (0.20 to 0.89) derived from the soluble protein pattern indicated the wide diversity of the greengram varieties. The dendogram revealed that 25 genotypes were clustered into 4 groups and no significant association was found among them (Fig. 4 and Fig.5).

Several scientists reported the similar results in differentiating the genotypes by protein banding pattern in Rice (Iwasaki *et al*. 1982, Savich and Peruski, 1986, Malik 1988 and Rao PS *et al. 2012*) and Groundnut (Rao PS *et al*. 2013) Sunflower (Anuradha *et al.* 1992), Cotton (Nerker and Rao, 1993), Maize (Wang chun *et al*. 1994) and Soybean (Orf *et al*. 1980). All these results suggest that the electrophoretic differences in protein banding pattern of different genotypes enable us to identify a particular genotype with the presence or absence of specific position of band and also the intensity of the band which could be used as a powerful technique for ascertaining genetic homology at the molecular level.

CONCLUSION

Characterizations such as laboratory tests like the NaOH or KOH test, response of the variety to the added chemical and protein fingerprinting to characterize the germplasm is considered as a reliable tool of intellectual property protection of crop varieties and germplasm. Electrophoretic differences in protein banding pattern of different genotypes enable us to identify a particular genotype which could be used as a powerful technique for ascertaining genetic homology at the molecular level.

LITERATURE CITED

Agrawal R L and Sharma B L 1989. Identification of mungbean varieties on the basis of seedling growth response to GA_3 and DDT treatment. *Seed Research* **17**(1): 84-87.

Anamika Nath S R, Maloo K K, Barman B L, Meenal A , Gangarani Devi1 G S, Yadav and Sheetal Tak 2017 Molecular Characterization of Green Gram [*Vigna radiata* L.) Wilczek] for Future Breeding Programmes. *Int.J.Curr.Microbiol.App.Sci* 6 (6): 1385-1398

Anitalakshmi V R Gowda, C S Sathisha and S Rajendra Prasad 2014 Varietal response to various chemical tests for their characterization in rice (*Oryza sativa* L.). *Indian J. of Plant Sci*., 3 (2): 177-179.

Anuradha Varier, Malavika Dadlani and Robbert J Cooke 1992 Identification of sunflower (*Helianthus annmus*. L) Hybrids and inbreds by SDS- PAGE of seed proteins. *Seed Research* 20 (2) 138-141

Ashwanikumar K, Chowdary R K , Kapoor R L and Dahiya O S 1995 Identification of pearlmillet hybrids and their parental lines using seeds and seedling characters, chemical ests and gel electrophoresis. *Seed Science and Technology*, 23: 21-32

Bansal R, Malik C P and Thind S K 1992 Effect of GA_3 in some early cultivars at early seedling stage. *Oryza* 29: 51-55

Bhat K V 2001 DNA fingerprinting and cultivar identification. *National research centre on DNA fingerprinting.N.B.P.G.R*, New Dehli-110 012

Chandu Singh, Jeevan Kumar S P, Sripathy KV, Somasundaram G, Udaya Bhaskar K, Ramesh KV, Madan Kumar and Rajendra Prasad S 2017 Characterization and Identification of Rice Germplasm Accessions Using Chemical Tests. *Seed Research* 45 (1): 75-83

Cherry J P, Katterman F R H and Endrizzi J E 1970 Seed esterases, leucine aminopeptidases and catalases of species of the genus Gossypium. *Theoretical and Applied Genetics* 42: 218-226

Dikshit H K, Sharma T R, Singh B B and Kumari J 2009 Molecular and morphological characterization of fixed lines from diverse cross in Munbean (*Vigna radiata* (L.) Wikzek). *Genetics*, 88: 3

Emre I 2011 Determination of genetic diversity in the *Vicia L.* (section vicia) by using SDS-PAGE. *Pak. J. Bot*., 43 (3):1429-1432

Iwasaki T N, Shibuya T, Suzuki and Chikubu S 1982 Gel filtration and electrophoresis and soluble rice protein extracted from long, medium and short grain varieties. *Cereal Chem*. 59(3): 192-195.

Laemmali U K 1970 Sodium dodecyl sulphate poly acrylomide gel electrophoresis. *Nature* (London) 227:680

Kumar S P, S Rajendra Prasad, R Banerjee and C Thammineni 2015 Seed birth to death: dual functions of reactive oxygen species in seed physiology. *Ann. Bot.,* 116 (4): 663-668

Malik S S 1988 Identification of rice varieties by electrophoretic analysis of proteins. *Seed Science and Technology*, 83-86

Masuthi D, Vyakarnahal B S S , Deshpande VK, Jagadeesha R C, Mukesh L, Chavan and K Rubina 2015 Characterization of traditional aromatic rice cultivars by chemical markers. *I. J. Ag. Res.* 3 (5): 103- 107

Nerkar Y S and Rao T N 1993 Use of seed protein and enzyme polymorphism in the identification of cultivars of cotton. *Seed Research* 375-392

Orf J H, Kaizuma N and Hymowitz T 1980 Six soyabean seed protein electrophoretic variants. *Seed Science and Technology* 8: 404-406

Pervaiz Z H, Tehrim S, Rabbani M A, Masood M S and Malik S A 2011 Diversity in major seed storage proteins of rice landraces of Pakistan. *Pak. J. Bot*., 43 (3): 1607-1612.

Rao P S, Bharathi M , Bayyapu K, Keshavulu K, Subba Rao L V and Neeraja C N 2012 Varietal identification in rice (*Oryza sativa* L.) through chemical tests and gel electrophoresis of soluble seed proteins. *I. J. Ag.Sci.,* 82 (4): 304-11.

Rao P S, Bharathi M and Bayyapu Reddy K 2013 Identification of peanut (*Aarachis hypogaea* L.) varieties through chemical tests and electrophoresis of soluble seed proteins; *Legume Res.,* 36 (6): 475 – 483.

Rupinder Kaur A K Toor L, Geeta Bassi and T S Bains 2017 Characterization of Mungbean (*Vigna radiata* L. Wilczek) Varieties using Morphological and Molecular Descriptors *Int .J. Curr. Microbiol. App. Sci* 6 (6): 1609-1618

Savich I M and Peruanskii Yu-V 1986 Pattern of cereal storage proteins and its use in identifying varieties. *Fiziologiya-i- Biokhemiya-kul-turnykh-Rastenii*-lriz- 131-138

Shah S M A, Hidayat-ur-Rahman, F M Abbasi, M A Rabbani, I A Khan, Z K Shinwari and Z Shah (2011). Interspecific variation of total seed protein in wild rice germplasm

using SDS-PAGE. *Pak. J. Bot.,* 43(4): 2147-2152

Sneath P M A and Sokal R R 1973 *Numerical taxonomy, the principles and practice of numerical classification* San Francisco. W N Freeman, pp.776.

Wang Chun, Bian K E, Zhang, Huaxiao, Zhouzhanming and Wang Jian 1994 Polyacrylamide gel electrophoresis of salt soluble proteins for maize variety identification and genetic purity assessment. *Seed Science and Technology* 22: 51-57

Wouters T C A and Booy G 2000 Stability of esterases and total proteins during seed storage of perennial ryegrass (*Lolium perenne* L.) and their use for cultivar discrimination. *Euphytica* 111:161-8.