



COMPARATIVE SEED STORAGE PROFILING OF SELECTED SPECIES OF THE GENUS *VIGNA*

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Abstract: Seed storage profiling of some selected species/varieties of the genus *Vigna* was performed by Native and SDS PAGE. Germplasm of its 10 varieties were biochemically analysed for seed soluble protein content and their electrophoretic characterization. In electrophoretic characterization of seed proteins, Native PAGE and SDS PAGE were performed, respectively. Native PAGE and SDS PAGE were analysed by preparing a zymogram. An UPGMA dendrogram showing the genetic relationships among selected *Vigna* germplasm obtained by using the SDS-PAGE pattern of seed storage proteins was prepared. The germplasm was characterized biochemically by analysing protein polymorphism profile through Native and SDS PAGE. The soluble seed protein content values ranged from 36.23 to 57.53 mg/g. The protein polymorphism revealed a wide variability with respect to the number and mobility of bands. The seed proteins of *Vigna* species studied on Native PAGE exhibited the existence of 42 protein bands located in three zones *namely* A, B and C. All the selected species of the *Vigna* evaluated by SDS-PAGE exhibited a considerable genetic variation and hence the results obtained could be of broader spectrum.

Keywords: Native PAGE, SDS PAGE, Seed storage protein, UPGMA, *Vigna*.

INTRODUCTION

Legumes are valuable sources of proteins, minerals, vitamins and occupy a very important place in human nutrition. The grain legumes occupy a unique position in world agriculture due to their high protein content. These are used as nutritive food, fodder, industrial raw materials and are involved in biological nitrogen fixation. The legumes are important as food plants (beans, gram, peas), as source of edible oil (soybean, ground nut) and also as tanbarks, timber, copal, gums, insecticides, cultivated ornamentals as well as the medicinal plants.

The seeds contain two types of proteins *namely* metabolic and storage proteins. The former is

both enzymatic and structural and concerned with normal cellular activities including the synthesis of storage proteins. The storage proteins, together with reserves of carbohydrates or oils, are synthesized during seed development and provide a source of nitrogen and carbon skeletons for developing seedlings. The storage proteins occur within the cell as discrete protein bodies. The term 'protein body' is used in preference to 'aleurone grain'. Legumes contain relatively low quantity of essential amino acid methionine. A combination of legumes with cereals forms a well-balanced diet for vegetarian section of society. The common examples of such combinations are dal with chawal (pulse-rice) or

dal-roti (pulse-unleavened wheat bread) by the Indians and peanut butter with wheat bread (as sandwiches) in several other cultures, including the Americans. Modern science provides strong support for this practice. The chemical score (*i.e.* biological value) improves greatly when wheat or rice is combined with one of the pulses mainly due to the complementary relationships of their essential amino acids.

The genus *Vigna* contains several species that are of considerable economic importance in many developing countries. Some such species are also valued as forage, cover and green manure crops in many parts of the world. The annual worldwide production of the various *Vigna* species approaches 20 million hectares and virtually all of this production comes from developing countries. Seed storage proteins are the products of gene expression with genetic stability and not being affected by environment. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method is widely used to detect them to study plant taxonomy, affinities and genetic diversity. It is an efficient procedure for differentiating grain legume species.

The analysis of the genetic variation and relationships of legume crops cultivated in China based on seed storage protein, especially in the genus *Vigna* and cultivars of yard long bean have been reported in order to contribute to germplasm collection and breeding purposes (Chen *et al.*, 2006). The electrophoretic banding pattern of polypeptides can be an efficient approach for assessment of cultivar distinctness as part of the legal requirements for the award of plant breeder's rights (Bailey, 1983). The analysis of seed proteins of grain legumes by the SDS-PAGE method has also been useful in identifying and determining the inheritance patterns of insect pest resistance (Fory *et al.*, 1996; Hartweck *et al.*, 1997). Seed storage proteins have been the subject of extensive investigations due to their economic importance as major protein source for humans and their biochemical utility as a model system in study of genetic variability, characterization and expression by molecular techniques. Special emphasis is given to seed proteins because a part of deficiency leading to

malnutrition and under nourishment is related to insufficient protein supply.

As grain legumes provide variety to the diet and contribute proteins, carbohydrates and other valuable proteins, they are regarded as the 'health food' in the developing countries. Proteins are the major seed components in all grain legumes. Legumes occupy the second place after cereals as major source of calories and protein in human diet (Vadivel and Janardhanan, 2005). Many nutritionists have suggested partial replacement of animal food with legumes so as to improve the overall nutritional dietary status (Guillon and Champ, 1996). The amount and composition of seed proteins are widely influenced both by environmental and endogenous factors. The seeds are the storehouse of different bio-molecules, especially the proteins which are of much importance. The high protein content of seeds especially in legumes, has offered scope to the geneticists for undertaking detailed biochemical characterization of that material and understanding the genetic control involved in their synthesis.

Now-a-days the highly versatile polyacrylamide gel electrophoresis technique has become the choice of priority for the biochemical researchers for making its use in characterizing the proteins. Seed storage proteins are the products of gene expression with genetic stability and are not affected by environment.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is most widely used due to its validity and simplicity for describing genetic structure of crop germplasm. SDS PAGE is considered to be a practical reliable method because seed storage proteins are largely independent of environmental fluctuations (Gepts, 1990; Murphy *et al.*, 1990). This technology is widely used to detect the seed polypeptides and to study plant taxonomy, affinities and genetic diversity. Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary problems of crop species (Khan 1992; Rao *et al.*, 1992). Genetic variants showing differences in composition of seed proteins have been reported in many species and genera of

higher plants (Colloda *et al.*, 1991). The seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary problems of several crop plants (Ghafoor *et al.*, 2002). This method can also be used as a promising tool for distinguishing cultivars of particular crop species (Jha and Ohri, 1996).

The seed storage proteins are used as genetic markers to: (1) analyse genetic diversity within and between accession, (2) domesticate plant in relation to genetic resources conservation and breeding, (3) establish the genome relationship and (4) improve the crop (Ghafoor *et al.*, 2001). Ghafoor and Ahmad (2005) reported the variation in storage protein banding pattern by SDS-PAGE, found in selected black gram species, but the magnitude was low. They reported only 46 exhibited variations out of 105 genotypes tested for SDS-PAGE, which was about 44 % of the total genotypes under investigation.

The SDS-PAGE polypeptide profiles have been studied in different legumes like *Vigna* and its cultivars grasses, forage legumes, soybean and different other grain legumes (Valizadeh, 2001; Chanyou *et al.*, 2006). Seed protein patterns

obtained by electrophoresis have been successfully used as a promising tool for distinguishing cultivars of particular crop species (Jha and Ohri, 1996). Biochemical analyses including seed protein profiling provide consistent results as they are less likely to be altered by the environment (Roy *et al.*, 2001). SDS PAGE and RAPD profiles have been successfully used for analysis of diversity in *Vigna unguiculata* (L.) Walp (Mignouna *et al.*, 1998). In the present investigation, protein profile and polypeptide profile of selected *Vigna* species were analysed on NATIVE and SDS-PAGE to understand genetic variability.

MATERIALS AND METHODS

Seed material

The seed materials used in the present exploration were obtained from Department of Pulses, Dr. Punjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.), India and Agricultural Research Station, Badnapur (M.S.), India. The seed material comprised five varieties of *Vigna radiata*, two varieties of *Vigna mungo* and one variety each from *Vigna umbellata*, *Vigna aconitifolia* and *Vigna unguiculata*, denoted by specific code names which are given in table 1.

Table 1: The list of selected species / varieties of the genus *Vigna*.

| S. No. | Selected species/ varieties of <i>Vigna</i> | Code |
|--------|---|--------|
| 1. | <i>Vigna mungo</i> (L.) Hepper - Tau 1 | Sam 1 |
| 2. | <i>Vigna umbellata</i> (Thunb.) Ohwi and Ohashi | Sam 2 |
| 3. | <i>Vigna radiata</i> (L.) Wilczek AKM 8802 | Sam 3 |
| 4. | <i>Vigna radiata</i> (L.) Wilczek - BPMR 145 | Sam 4 |
| 5. | <i>Vigna mungo</i> (L.) Hepper - BDU 1 | Sam 5 |
| 6. | <i>Vigna unguiculata</i> (L.) Walp | Sam 6 |
| 7. | <i>Vigna radiata</i> (L.) Wilczek - NVL 1 | Sam 7 |
| 8. | <i>Vigna radiata</i> (L.) Wilczek - BM 4 | Sam 8 |
| 9. | <i>Vigna radiata</i> (L.) Wilczek - BM 2002-01 | Sam 9 |
| 10. | <i>Vigna aconitifolia</i> (Jacq.) Marechal | Sam 10 |

Native PAGE protein molecular weight marker

Native PAGE protein molecular weight marker is a set of five proteins with molecular weight ranging from 240 kD to 18.4 kD, to characterize the proteins separated in polyacrylamide gels in their

native state, manufactured by Genei, Bangalore, India was used in present research work.

Extraction of seed proteins

Mature seeds were washed with water, dried and

ground to make fine powder. The mature seed powder was defatted with hexane, air dried and stored at 4°C. Seed powder was kept for extraction in 1:6 proportions with 1% PVP (polyvinyl polypyrrolidone). The suspension was centrifuged at 12000 rpm at 4°C for 20 minutes to remove the particulate matter and clear supernatant was used for protein estimation, analysis of the trypsin inhibitor and the native PAGE. Similarly, the protein extracted in 10 mM Tris-HCl buffer, pH 8.0 containing 1% SDS and 25% glycerol was used for SDS-PAGE.

Protein estimation

The protein estimation was carried out by the Lowry's method (Lowry *et al.*, 1951).

Standardization of Lowry's assay by using BSA

The stock solution of BSA was prepared in distilled water. The protein was estimated by measuring the absorbance at 660 nm. The stock solution was diluted to prepare 1mg/ml working solution of BSA in distilled water. Reagent A was prepared by dissolving 2% sodium carbonate in 0.1 N sodium hydroxide. Reagent B was prepared by dissolving 0.5% copper sulphate in 1% potassium sodium tartarate. The alkaline copper solution was prepared by mixing 50 ml of reagent A and 1ml of reagent B prior to use. The commercially available Folin- Ciocalteu reagent was diluted with equal volume of water prior to use. BSA (100 µg – 1000 µg) was taken and the volume was made up to 1000 µl with distilled water. Then 5 ml of alkaline copper solution was added to each tube, mixed thoroughly and allowed to stand at room temperature for 10 minutes. Later on, 0.5 ml of Folin's reagent was added to it, mixed well and incubated at room temperature in the dark for 30 minutes. The absorbance was read at 660 nm. A graph of absorbance versus concentration of BSA was plotted and this graph was used for estimating the protein concentration in sample. Suitable quantity of extract was assayed by Folin Lowry's method and respective protein value was expressed in mg/g.

Electrophoresis

The water-soluble proteins were analysed by using vertical slab polyacrylamide gel electrophoresis (PAGE) apparatus. Electrophoresis was performed

at 100 V for half an hour and then at 150 V until the bromophenol blue marker reached the bottom of the gel approximately for 4 hours. After electrophoresis, the gels were fixed in 10% fixing solution for 30 minutes. Then gels were stained with staining solution (ix) and destained in destained solution (X).

Preparation of Zymogram

Zymogram is the diagrammatic representation of the enzyme or protein band location in the strip of a gel. It is extrapolated on the basis of relative mobility of each in the gel. The relative mobility (REM) of each band was calculated according to the following formula:

$$\text{REM} = \frac{\text{Distance between the origin and band}}{\text{Distance between the origin \& tracking dye}}$$

SDS PAGE

In SDS PAGE, polypeptides are separated according to their molecular weight, not by intrinsic electrical charge. Sodium dodecyl sulphate (SDS) is an anionic detergent that denatures proteins by wrapping around the polypeptide backbone. In doing so, the SDS confers a negative charge to the polypeptide in proportion to its length. When proteins are treated with SDS and reducing agent 2 ME (2-Mercaptoethanol), the polypeptides become rods of negative charges with equal charge unit per length. Here, denaturing discontinuous PAGE system was used as described by Laemmli (1970). This system is almost similar to the native PAGE (Davis, 1964) except for the presence of SDS.

Preparation of Dendrogram

The genetic relationships among the 10 *Vigna* genotypes were viewed in two dendrograms *viz.*, rooted and unrooted respectively, using Neighbor-Joining and Unweighted pair group method of arithmetic means -UPGMA (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

The selected germplasm of different varieties of *Vigna* was characterized biochemically by analysing protein polymorphism profile through NATIVE and SDS PAGE along with other most

important biochemical aspects. The results obtained are as per the following:

1. Estimation of extractable/soluble seed protein content

A good amount of variability in the soluble seed protein content could be evidently noticed in the

selected germplasm of the genus *Vigna*. The soluble seed protein content values ranged from 36.23 to 57.53 mg/g. The highest amount of soluble seed protein content (57.53 mg/g) was noticed in *Vigna unguiculata* (L.) Walp. The lowest soluble seed protein content (36 mg/g) was shown by

Table 2: Seed protein content (soluble) in selected species/varieties of the genus *Vigna*.

| S. No. | Name of <i>Vigna</i> varieties studied | Extractable protein content (mg/g)(x) | S.D. | ± S.E. |
|--------|---|---------------------------------------|------|--------|
| 1. | <i>Vigna mungo</i> (L.) Hepper - Tau 1 | 38.20 | 0.82 | 0.47 |
| 2. | <i>Vigna umbellata</i> (Thunb.) Ohwi and Ohashi | 49.23 | 0.45 | 0.26 |
| 3. | <i>Vigna radiata</i> (L.) Wilczek AKM 8802 | 45.73 | 0.25 | 0.14 |
| 4. | <i>Vigna radiata</i> (L.) Wilczek - BPMR 145 | 42.63 | 0.40 | 0.23 |
| 5. | <i>Vigna mungo</i> (L.) Hepper - BDU 1 | 36.23 | 0.49 | 0.28 |
| 6. | <i>Vigna unguiculata</i> (L.) Walp | 57.53 | 0.50 | 0.29 |
| 7. | <i>Vigna radiata</i> (L.) Wilczek - NVL 1 | 49.93 | 0.80 | 0.46 |
| 8. | <i>Vigna radiata</i> (L.) Wilczek - BM 4 | 55.27 | 0.57 | 0.33 |
| 9. | <i>Vigna radiata</i> (L.) Wilczek - BM 2002-01 | 51.20 | 0.62 | 0.36 |
| 10. | <i>Vigna aconitifolia</i> (Jacq.) Marechal | 53.77 | 0.32 | 0.18 |

variety *Vigna mungo* (L.) Hepper-BDU 1. Summary of the different values are given in table 2.

2. Seed protein characterization by Native PAGE

The protein polymorphism of ten varieties of *Vigna* revealed a wide variability with respect to the number and mobility of bands (fig.1). A total of 130 bands were resolved on the native gel, which were categorized into 42 polypeptide bands. The relative mobility (REM) values of these 42 bands ranged from 0.04 to 0.69. The selected germplasm varied in the presence of various protein bands. Maximum number of bands *i.e.*, 16 bands were found in *Vigna radiata* (L.) Wilczek- NVL 1 and *Vigna radiata* (L.) Wilczek- BM 2002-01 whereas minimum number of bands *i.e.*, 8 bands in *Vigna mungo* (L.) Hepper - Tau 1. The least number of bands were present in lane 1, while nine bands could be seen in lane 2, twelve bands in lane 3, thirteen bands in lane 4 and 6, fifteen bands in lane 5, 8 and 10 and maximum number of bands could be recorded in lane 7 and 9.

3. Zymogram analysis of seed protein separated by Native PAGE

The seed proteins of *Vigna* species studied on Native PAGE exhibited the existence of 42 protein bands located in three zones A, B and C (fig. 2). Zone A representing the heaviest molecular weight protein was sub divided into 18 sharp and distinct bands *i.e.*, A₁, A₂, A₃, A₄, A₅, A₆, A₇, A₈, A₉, A₁₀, A₁₁, A₁₂, A₁₃, A₁₄, A₁₅, A₁₆, A₁₇ and A₁₈. Similarly, zone B representing mostly medium dark sharp bands, was sub divided into 12 bands *i.e.*, B₁, B₂, B₃, B₄, B₅, B₆, B₇, B₈, B₉, B₁₀, B₁₁ and B₁₂. The next zone C representing lighter bands with few faint bands was subdivided into 12 bands *viz.*, C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, and C₁₂. The two bands *i.e.* A₁ and B₆ were common in nine and eight genotypes respectively. These bands can serve as a source of reference for inter-gel or inter-laboratory comparison. Thicker band A₁ was absent in *Vigna mungo* (L.) Hepper -Tau 1. The sharp dark band was absent in the lines of *Vigna mungo* (L.) Hepper -Tau 1, *Vigna umbellata* (Thunb.) Ohwi and Ohashi and *Vigna unguiculata* (L.) Walp. A single distinguishing

band was present in lines of *Vigna radiata* (L.) Wilczek- BM 2002-01, *Vigna umbellata* (Thunb.) Ohwi and Ohashi, *Vigna unguiculata* (L.) Walp.,

Vigna radiata (L.) Wilczek- NVL 1, *Vigna mungo* (L.) Hepper -Tau 1, *Vigna mungo* (L.) Hepper - BDU 1, *Vigna conitifolia* (Jacq.) Marechal, *Vigna*

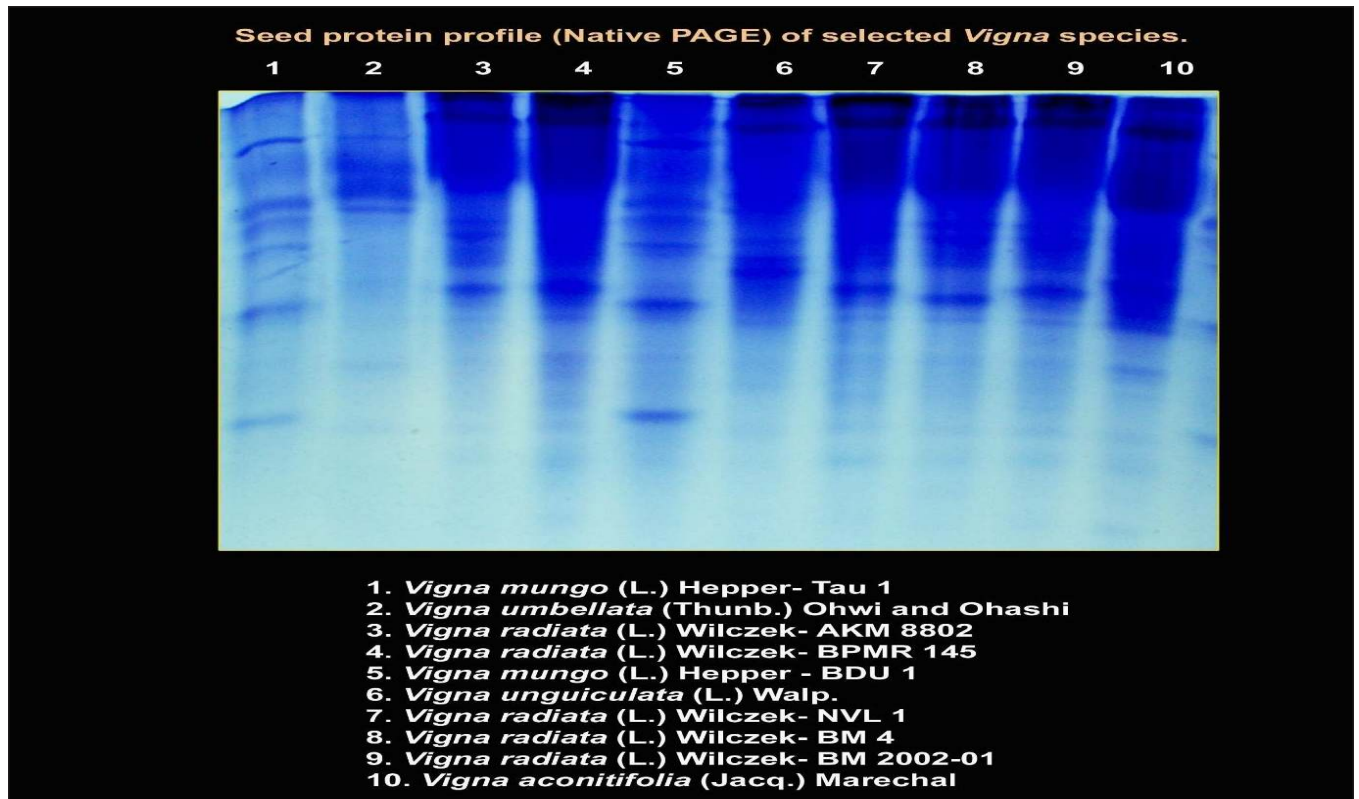


Fig.1: Seed protein profile by Native PAGE of selected *Vigna* species/varieties.

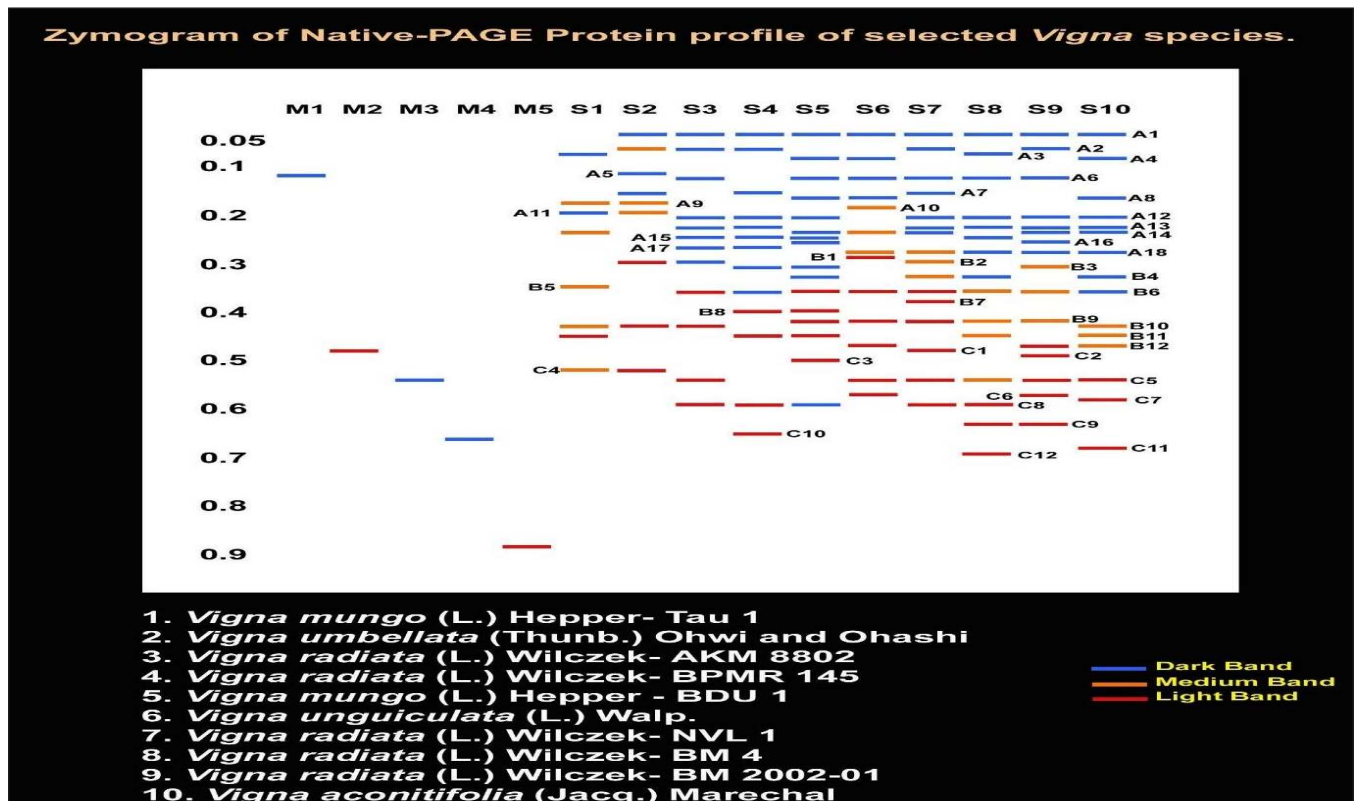


Fig. 2: Zymogram of Native PAGE protein profile of selected *Vigna* species/varieties.

radiata (L.) Wilczek- BPMR 145 and *Vigna radiata* (L.) Wilczek- BM 4. Therefore, the dissimilar groups were mainly due to the absence or presence of bands.

4. Seed protein characterization by SDS-PAGE

All the selected species studied for the analysis of SDS-PAGE exhibited a considerable genetic variance in the analyses of total germplasm and hence the results obtained by this study could be of broader spectrum. SDS denatured protein gels could resolve a total of 62 bands, which were grouped as 33 distinct SDS protein bands (fig. 3). These SDS protein bands belonged to different molecular weight ranging from 17 KDa to 97 KDa. The relative mobility of these bands varied from

0.06 to 0.80. Low, medium and high mobility bands were observed in all the cases. *Vigna mungo* (L.) Hepper -Tau 1 exhibited maximum number of bands *i.e.* all the 9 bands were visible, followed by *Vigna radiata* (L.) Wilczek-BPMR 145 with 8 bands. A high molecular weight polypeptide band of medium to high intensity with REM 0.09 (MW 91.2 KDa) was unique to six germplasm *viz.*, *Vigna radiata* (L.) Wilczek- BPMR 145, *Vigna mungo* (L.) Hepper - BDU 1, *Vigna unguiculata* (L.) Walp, *Vigna radiata* (L.) Wilczek- NVL 1, *Vigna radiata* (L.) Wilczek- BM 4 and *Vigna radiata* (L.) Wilczek- BM 2002-01. *Vigna aconitifolia* (Jacq.) Marechal could be further differentiated from other germplasm by the presence of a strong protein band of highest molecular weight *i.e.*, 97.7 KDa.

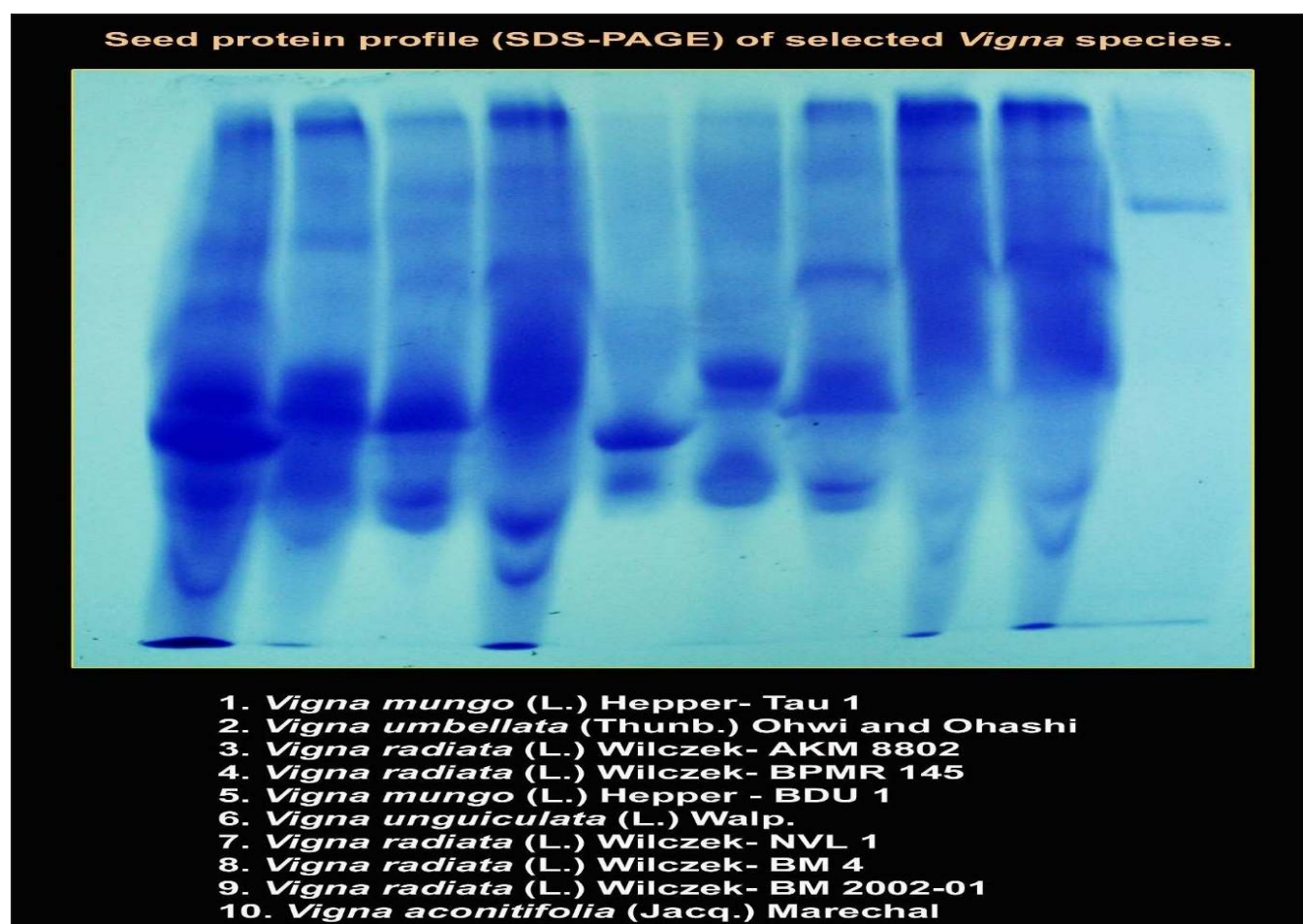


Fig. 3: Seed protein profile by SDS-PAGE of selected *Vigna* species/varieties.

Variability of protein bands was well expressed in the entire gel. The presence or absence type of polymorphism of SDS proteins was revealed and the different germplasm showed both homology and diversity in their banding pattern.

5. Zymogram analysis of seed protein separated by SDS-PAGE

Lot of variability was observed in polypeptide seed protein profiles of different species studied on SDS PAGE (fig. 4). The seed proteins on SDS

gels exhibited the existence of 33 protein bands located in four zones namely A, B, C and D. Zone A representing the heaviest molecular weight protein was sub divided into 6 sharp and distinct bands *i.e.* A₁, A₂, A₃, A₄, A₅ and A₆. Among these A₅ and A₆ were comparatively darker bands. Similarly, Zone B representing mostly sharp and dark bands was sub divided into 11 bands *i.e.* B₁, B₂, B₃, B₄, B₅, B₆, B₇, B₈, B₉, B₁₀ and B₁₁. The zone B had thicker and darker bands. The next zone C representing dark to lighter bands with few faint bands was sub divided into 8 bands *viz.*, C₁, C₂, C₃, C₄, C₅, C₆, C₇ and C₈. The next zone D characterized by lighter to faint bands was sub divided into 8

bands *i.e.* D₁, D₂, D₃, D₄, D₅, D₆, D₇ and D₈. The band A₂ was common in 6 genotypes while A₆ and D₆ were common in 5 genotypes each. These bands can serve as a source of reference for inter-gel or inter-laboratory comparison. A single distinguishing band was present in lines of *Vigna aconitifolia* (Jacq.) Marechal, *Vigna mungo* (L.) Hepper -Tau 1, *Vigna radiata* (L.) Wilczek- BM 4, *Vigna umbellata* (Thunb.) Ohwi and Ohashi, *Vigna mungo* (L.) Hepper - BDU 1, *Vigna radiata* (L.) Wilczek- BM 2002-01, *Vigna radiata* (L.) Wilczek- BPMR 145, *Vigna unguiculata* (L.) Walp., *Vigna radiata* (L.) Wilczek AKM 8802 and *Vigna radiata* (L.) Wilczek- NVL 1. Thus, an UPGMA

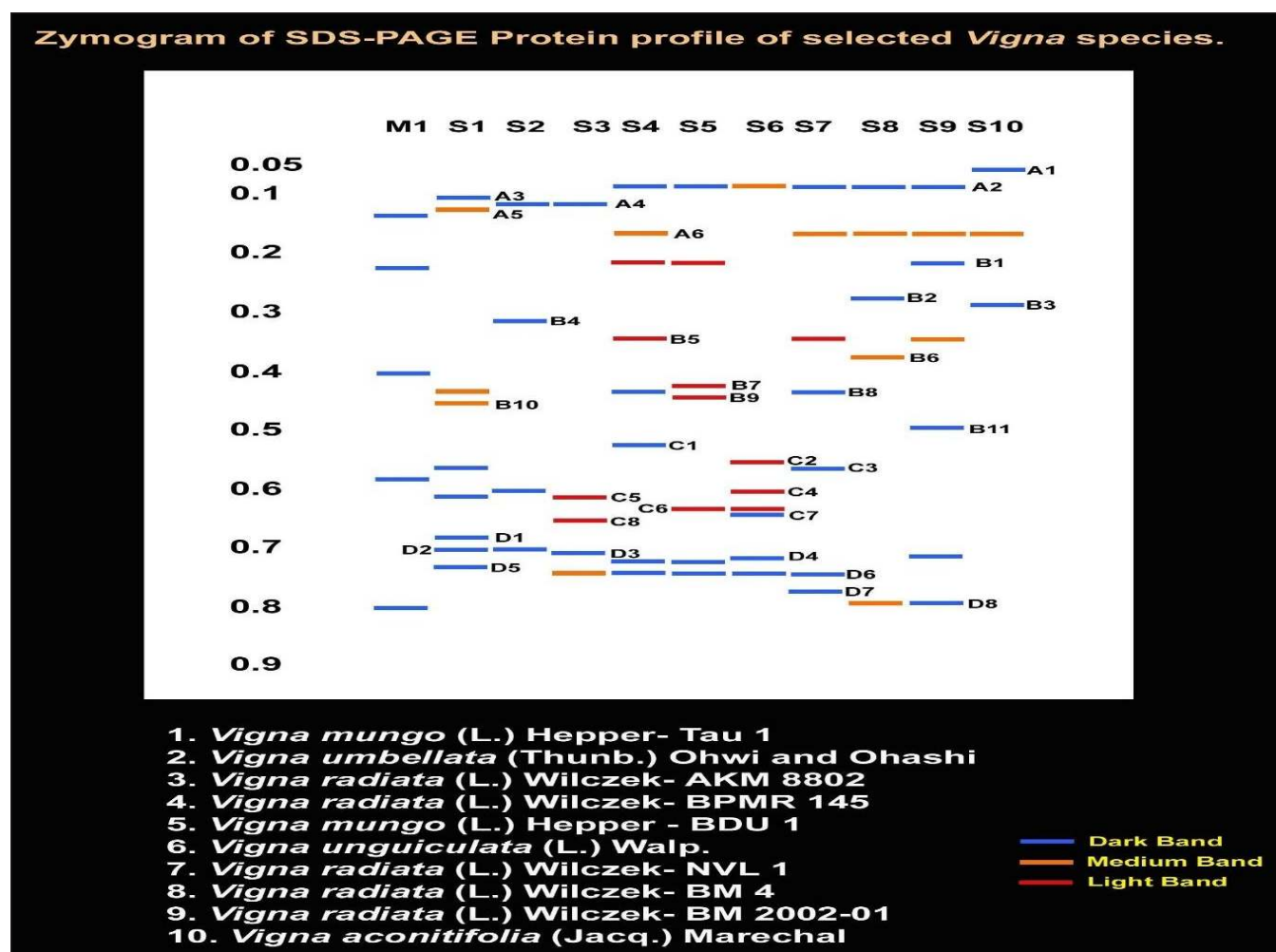


Fig. 4: Zymogram of SDS-PAGE protein profile of selected *Vigna* species/varieties.

dendrogram based on ten *Vigna* germplasm data was established.

6. Cluster analysis of dendrogram based on SDS-PAGE (Rooted)

According to the statistical analysis data of the presence and absence of each band, the Jaccard's

similarity index between each germplasm accession was evaluated (table 3). An UPGMA dendrogram was established (fig. 5). Thus, the dendrogram of total seed proteins based on distance coefficient using UPGMA revealed three distinct clusters: A, B and C. The cluster A included 6 germplasm, cluster B included 4 and

cluster C included 1 germplasm as the most divergent genotype. The cluster A included four *Vigna radiata* varieties along with *Vigna mungo* (L.) Hepper-BDU 1 and *Vigna aconitifolia* (Jacq.) Marechal whereas other three species of *Vigna* were included in cluster B. The cluster A was divided into 2 sub-clusters AI and AII. In the first sub-cluster (AI), *Vigna radiata* (L.) Wilczek- BM 2002-01 and *Vigna radiata* (L.) Wilczek- BM 4, have shown more closeness with *Vigna aconitifolia* (Jacq.) Marechal. In the second sub-cluster (A II), *Vigna radiata* (L.) Wilczek- NVL 1

and *Vigna radiata* (L.) Wilczek- BPMR 145 was close to *Vigna mungo* (L.) Hepper - BDU 1. It may be mentioned that the four varieties of *Vigna radiata* have shown more closeness with *Vigna mungo* (L.) Hepper - BDU 1 and *Vigna aconitifolia* (Jacq.) Marechal in the same cluster A. In cluster B, *Vigna unguiculata* (L.) Walp. and *Vigna umbellata* (Thunb.) Ohwi and Ohashi were close to *Vigna radiata* (L.) Wilczek AKM 8802. Of the different *Vigna* species, *Vigna mungo* (L.) Hepper - Tau 1 was observed as a distinct node and joined with rest of the 9 germplasm in the cluster C.

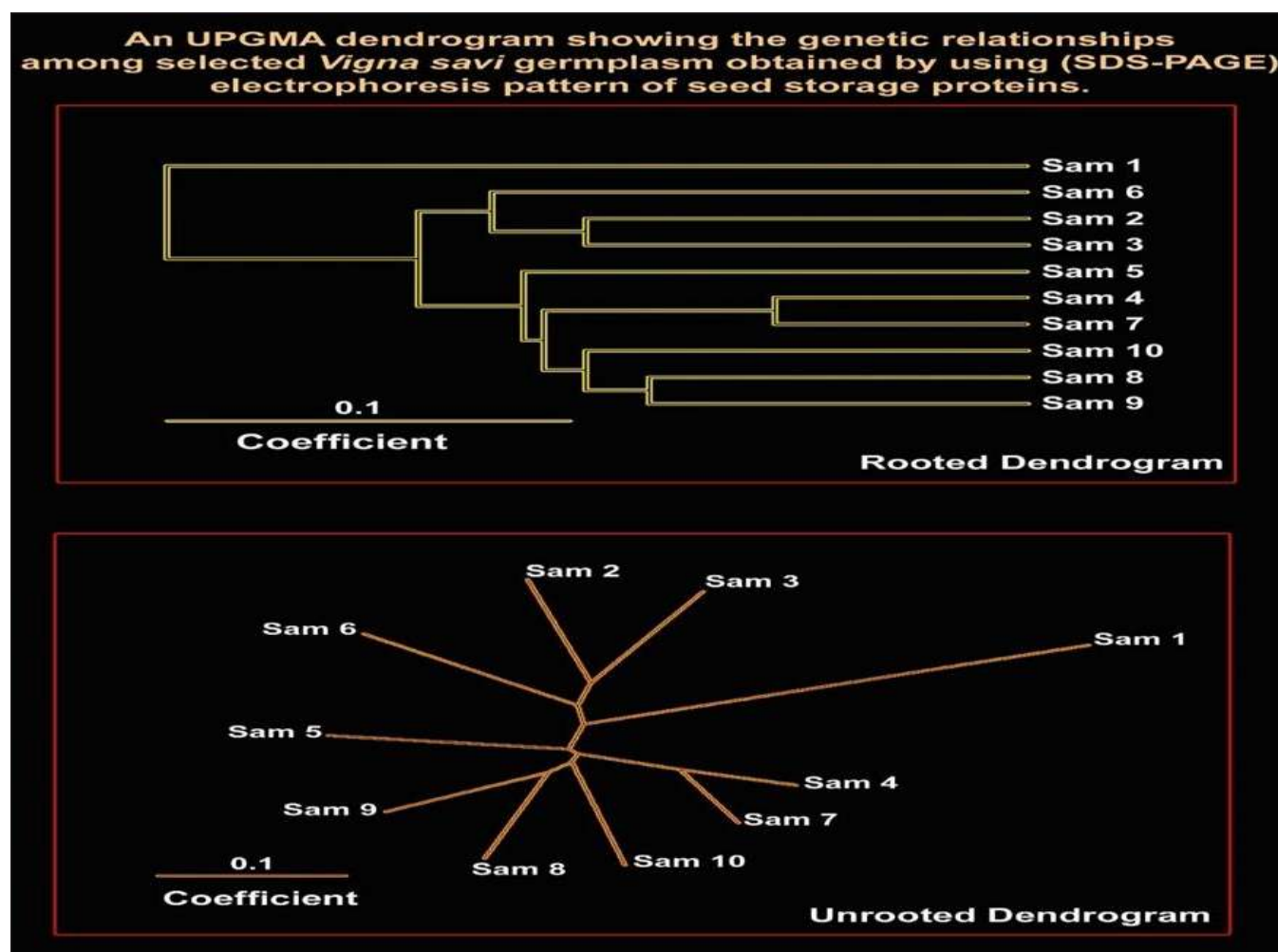


Fig.5: An UPGMA dendrogram obtained by SDS-PAGE of selected *Vigna* species/varieties.

Vigna mungo (L.) Hepper-Tau 1 was the most genetically divergent as compared to the rest of the *Vigna* germplasm.

7. Cluster analysis of dendrogram based on SDS-PAGE (Unrooted)

An UPGMA dendrogram (unrooted) based on seed protein analyses data was constructed (fig. 5). Although each species contained different

number of germplasm, it was convenient to do so through adjusting the program. Results showed that six species/varieties of *Vigna* had close affinities to form one cluster firstly then they cluster with other *Vigna* germplasm. *Vigna radiata* (L.) Wilczek-BPMR 145 was relatively close to *Vigna radiata* (L.) Wilczek- NVL 1, so was *Vigna radiata* (L.) Wilczek- BM 4 to *Vigna radiata* (L.) Wilczek-BM 2002-01 and *Vigna umbellata*

(Thunb.) Ohwi and Ohashi to *Vigna radiata* (L.) Wilczek AKM 8802. Similarly, like rooted dendrogram, *Vigna mungo* (L.) Hepper -Tau 1 was observed as a most distinct accession than those of others. The results obtained after Native and SDS-PAGE electrophoresis showed that the method provided a powerful tool for reliable

germplasm discrimination based on genetic differences in seed storage protein compared to selected germplasm of *Vigna*. Thus, the present study explores the existing polymorphism of total proteins through SDS and Native PAGE to facilitate characterization of selected germplasm of *Vigna*.

Table 3: Cluster based on SDS PAGE protein peptides in selected *Vigna* species/varieties.

| Cluster | Sub-cluster | Frequency | Name of selected <i>Vigna</i> species |
|---------|-------------|-----------|--|
| A | A I | 03 | <i>Vigna radiata</i> (L.) Wilczek - BM 2002-01 <i>Vigna radiata</i> (L.) Wilczek - BM 4 <i>Vigna aconitifolia</i> (Jacq.) Marechal |
| A | A II | 03 | <i>Vigna radiata</i> (L.) Wilczek - NVL 1 <i>Vigna radiata</i> (L.) Wilczek - BPMR 145 <i>Vigna mungo</i> (L.) Hepper - BDU 1 |
| B | -- | 03 | <i>Vigna radiata</i> (L.) Wilczek AKM 8802 <i>Vigna umbellata</i> (Thunb.) Ohwi and Ohashi <i>Vigna unguiculata</i> (L.) Walp |
| C | -- | 01 | <i>Vigna mungo</i> (L.) Hepper - Tau 1 |

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