Research Article **AJBB (2021) 4:9**

American Journal of Biotechnology and Bioinformatics
(ISSN:2575-999X)

The intravarietal relationship among different accessions of *Dolichos biflorus* was checked based on protein profiling using SDS-PAGE.

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ABSTRACT

In this study, the phylogenetic relationship within the selected Eleven Indian (*Dolichos biflorus* (horse gram) varieties was analyzed for total soluble seed protein. Twenty-five bands were documented through SDS PAGE based on 100 seed weight of each variety and were studied for genetic diversity. Jaccard's similarity matrix was acquired and used in UPGMA cluster analysis based on the polymorphism generated by the presence (1) or absence (0) of protein bands. Thus, the dendrogram showed four major groups that corre-spond to an earlier study on polymorphisms of 11 accessions of Indian Dolichos. Signifi-cant correspondence between the clustering pattern and the pedigree was observed; thus, a high genetic diversity could be kept within the Dolichos varieties. A similarity matrix among the targeted genotypes and phylogenetic analysis is considered a unique feature in the present work. Therefore, the current investigation was carried out to analyse Protein diversity of unexplored Dolichos genotypes at the molecular level, construct a dendro-gram based on similarity band matrix and generate efficiency in genetic divergence analysis among Dolichos. This study underlines the importance of using genetic diversity based in the Dolichos breeding program.

Keywords: Cluster analysis;*Dolichos biflorus*; Dendrogram; Seed proteins; SDS-PAGE;

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How to cite this article:
Smita Srivastava and Prem Kumar Dantu. The intravarietal relationship among different accessions of *Dolichos biflorus* was checked based on protein profiling using SDS-PAGE. American Journal of Biotechnology and Bioinformatics, 2021; 4:9.

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Introduction

Legumes have been a source of protein, starch oil, mineral, vitamins, and health-protecting compounds from the beginning of human history (Duranti *et al.,* 2008). Dolichos bean or Hyacinth bean, or Indian bean (Lablab purpureus L.) Sweet is a multi-utility and multi-beneficial leguminous crop. It is grown for vegetables, pulse, fodder, green manure, cover crop, medicine, and ornamental purpose (Ayyangar and Nambiar, 1935). It is native to Southeast Asia and tropical Africa, but the origin of cultivated species is considered southern India (Vavilov, 1951; Zohary, 1970). India is the only country developing Dolichos on a large acreage, used as human food. However, Dolichos is a versatile crop and can be grown from near sea level to 1800 m, and it is a drought-tolerant plant. It can be grown with rainfall as low as 380 mm, being a leguminous crop valued nitrogen to the soils where it increases and improves soil fertility. The cultivated horse gram's protein content is 16.9–30.4% (Chahota *et al.,* 2013). It also has high lysine content, an essential amino acid (Gopalan, Ramashastri, & Balasubramanyan, 1989). Dolichos is also rich in phosphorus, iron, and vitamins such as carotene, thiamine, riboflavin, niacin, and vitamin C (Sodani, Paliwal, & Jain, 2004). It contains many medicinal and therapeutic benefits, although many of them are yet to be proven scientifically. It can be an ayurvedic medicine used to treat enema, piles, renal stones, etc. It has polyphenols with high antioxidant properties, molybdenum that regulates calcium intake, and iron that helps transport oxygen to cells and forms part of hemoglobin in the blood (Mohar *et al.,* 2013). Dolichos is a rich source of Haemagglutinin, an agent or substance responsible for red blood cells and agglutinate. Besides, it contains anti-nutrients, i.e., as a protease inhibitor (Kuhar *et al.,* 2013), and it is an excellent example of such a legume.

Seed proteins can be broadly classified into viz, storage, structural, and biologically active proteins. (Mandal & Mandal, 2000). Seed storage and protein electrophoresis as dominant tools in solving the taxonomic problem and explaining the source and evolution of several cultivated plants (Kmael *et al.,* 2009; Haider *et al.,* 2010; Karihaloo *et al.,* 2002). It is used to study a single charged species' properties and a separation technique of polypeptides of different molecular weights. Simultaneously, SDS-PAGE became a powerful technique for resolving a mixture of insoluble proteins, especially membrane protein, solubilized by SDS. Any changes in the composition of seed storage protein directly reproduce numerous genetic variations (Masood *et al.,* 2004; Yu-Xia *et al.,* 2008;s Sharma *et al.,* 2015).

Dolichos is still an underutilized and unexplored crop in the area under cultivation and efforts towards its genetic improvement. Adaptation and mitigation strategies against climate change-induced threats to global food security, biodiversity, and sustainable development require climate-resilient crops like Dolichos. It is a potential crop for sustainable agriculture in dryland ecosystems assuring food and income security to small and marginal farmers. Thus, attention should be given to comprehensive genetic and protein improvement and conservation of Dolichos' plant genetic resources. However, the present research aims to divide total seed protein using electrophoresis through SDS-PAGE in eleven cultivars of Dolichos, optional for growing in different Indian regions. A dendrogram was developed based on the protein band using SDS-PAGE. However, the genetic information available for much-researched Dolichos species could be useful in linkage map construction and tagging and valuable mapping genes.

Materials and Methods:

Plans Materials

Seeds of different accession of Dolichos representing various geographic areas of India. The seeds were kindly supplied by the NBPGR New Delhi (Table I).

Table I: In present study were used different accession of dolichos, their Genotypes and area of adaptation.

S. No.	Accession no.	Genotypes	Area of adaptation	Species
1.	IC-426949	MR-04-08	Tamil nadu	*Labla purpureus*
2	IC-426957	MR-04-09	Tamil nadu	*Labla purpureus*
3.	IC-426962	MR-04-35A	Tamil nadu	*Labla purpureus*
4.	IC426985	MR-04-57	Tamil nadu	*Labla purpureus*
5.	IC-427425	MR-04-20A	Tamil nadu	*Labla purpureus*
6.	IC-426975	MR-04-45A	Tamil nadu	*Labla purpureus*
7.	IC-426988	MR-04-62	Tamil nadu	*Labla purpureus*
8.	IC-426991	MR-04-64	Tamil nadu	*Labla purpureus*
9.	IC-426994	MR-04-69	Tamil Nadu	*Labla purpureus*
10.	IC-446560	PSR-11475	Telangana	*Labla purpureus*
11.	IC-393739	ARKA-VIJAY	Other	*Labla purpureus*

Seed valuation

Eleven accessions of Dolichos seeds were studied for the difference in their size and weight. The final comparison with total soluble protein based on the hundred seed weight of eleven accessions is given below (Table II).

Extraction of Proteins

Protein extractions were carried out by 0.2gm defatted powder in 2ml of 0.1M Tris-Cl (pH-8.0) and kept overnight at room temperature to extract the proteins. Incubated samples were centrifuged at 12000rpm at room temperature; the supernatant was collected. Lowry's methods used the collected supernatant for protein quantification (Lowry *et al.,* 1951).

SDS-Polyacrylamide gel electrophoresis:

Analysis of proteins was carried out by one dimensional discontinuous SDS-PAGE through 15% polyacrylamide gel, using Laemmli (1970) formulation. Before loading, the samples were boiled for 5min and 2% SDS, 1%Dithiothreitol, 105 glycerol, and 0.1% Bromophenol Blue. The gel's staining was done in 0.1% Coomassie Brilliant Blue (CBB) containing 45%, methanol: 10%, acetic acid: 45%, distilled water; while distaining was in the same solution but without the dye.

Statistical Analysis

Phylogenetic relationships of eleven accessions of Dolichos were analyzed based on their total seed protein profile on SDS- gel under reducing conditions. The dendrograms were scored as binary numbers, presence (1) and absence (0). A dendrogram based on the genetic distance matrix was constructed by the unweighted pair groups' method with arithmetic mean (UPGMA). For Jaccord's coefficient and dendrogram construction, the NTSYS- pc version 2.02 was used (Exeter Software, New York, USA).

Results

As shown in (Table. II), the seed weight and total protein content in all the accession of Dolichos were relatively high and found to vary between heaviest MR-04-69 (447g/100 seeds) and weighed the least MR-04-62 (294g/ 100 seeds). Protein content in the eleven cultivars tested diverse from as low as 5µmol/µl in MR-04-35 to as high as 37µg/µl in MR-04-20A (Fig. 1).

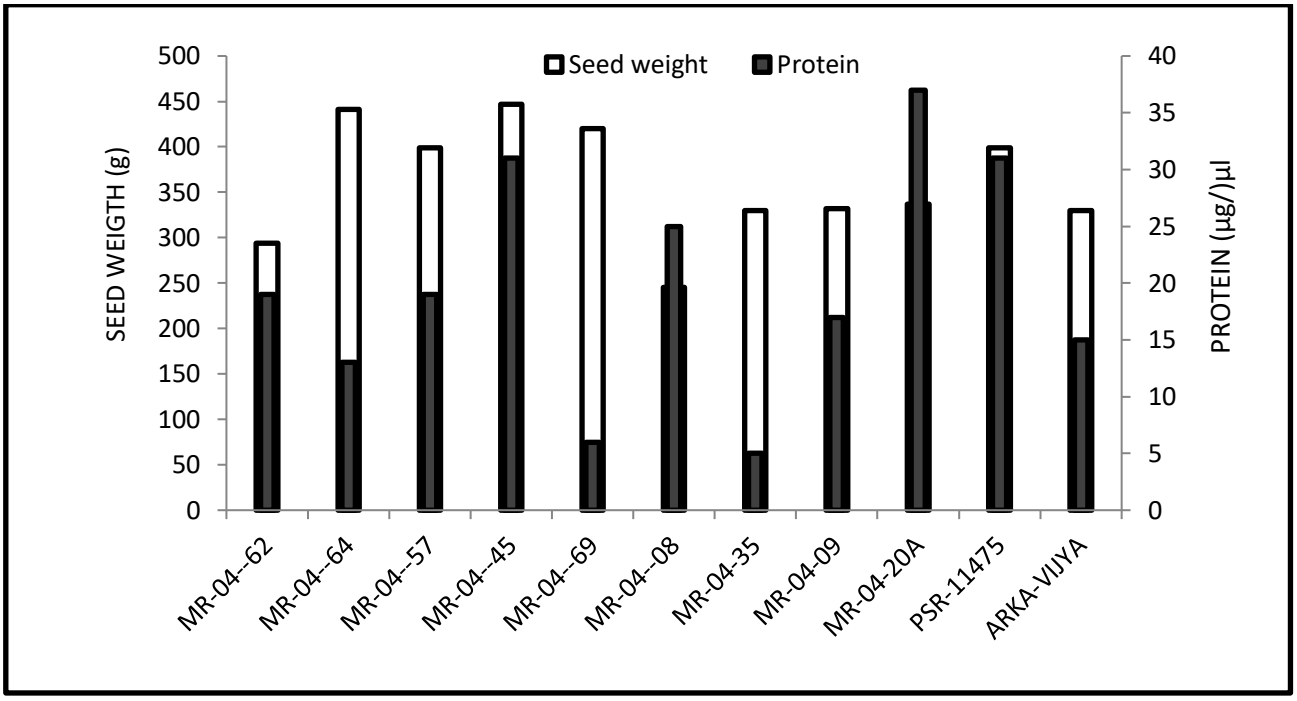

Fig. 1: Seed weight opposed to protein in the eleven accessions of Dolichos used in this study.

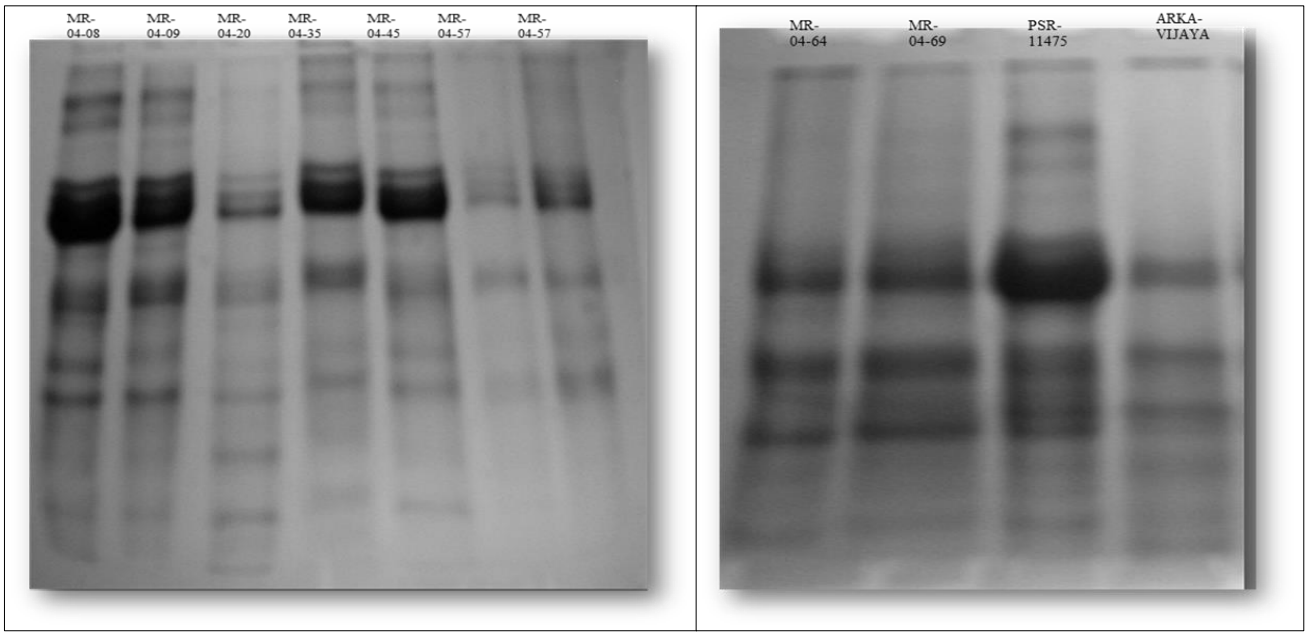

Fig. 2: Electrophoregrams showing seed storage proteins band of eleven dolichos varieties (MR-04-08, MR-04-09, MR-04-20, MR-04-35,MR-04-45, MR-04-57,MR-04-62 MR-04-64 MR-04-69, PSR 11475 and Arka –vijaya) used in present study.

The intragenic relationship between the 11 Indian Dolichos varieties was analyzed for total soluble seed proteins using SDS-PAGE, and the protein bands thus generated were utilized to study genetic diversity. The similarity coefficients were used for UPGMA cluster analysis and to build up the dendrogram (Fig. 3). A total of 25 distinct protein bands could be identified in the SDS-PAGE gel (Fig. 2). PSR-11475 and MR-04-08 were characterized by two different bands of the relatively heavy molecular band. It shows more diversity based on the protein profile. Four distinct clusters, Group I, II, III, and IV, could be identified. Cluster I is made up of MR-04-45A. However, Cluster II is made up of three sub-clusters made up of six

accessions, viz.MR-04-08, MR-04-09, MR-04-35A, MR-0420A, and PSR-11475. The first sub-cluster consists of two accessions: MR-04-08 and MR-04-09 do not show any variation in protein profile; these two accessions are separated from the third accession MR-04-35A, by the shortest distance. The second sub-cluster is made up of single accession PSR-11475, and the third is made up of two accession MR—04-20A and MR-04-35. Cluster III comprises three accessions, MR-04-64, MR-04-69, and MR-04-62, of which MR-04-64 and MR-04-69 are related while MR-04-62 is quite distinct. Cluster IV is made up of two accessions Arka-Vijay and MR-04-57. Accession MR-04-45A and PSR-1145 are very similar concerning their protein profiling and differ from all other accessions. Incidentally, PSR-1145 and MR-04-45A have the 31µg/ µl protein in both varieties. They are most similar; this indicates that accession can be used as a parent for hybridization with either of the accessions belongs to group IV and I.

Discussion

Statistical distance represents the extant of protein diversity among clusters. Cluster II displayed minimum intra cluster, while the maximum intracluster distance was recorded in cluster IV, which Maximum intracluster distance was observed cluster I and cluster IV and fallowed by Cluster II and Cluster IV may be due to selection practice among the genotype for diverse seed character which is indicating wider genetic diversity among the genotype included in these groups. Similar results in Dolichos beans have been reported by (Upadhyay *et al.,* 2008) based on the morphological characters. Simultaneously, Sammour *et al.* (2007) observed that SDS-PAGE could disclose relationships between eight Lathyrus species collected from different geographical areas. Other seed storage protein functions as a positive tool for exclusive cultivars of particular crop science (Jha and ohri, 1996). According to Ghafoor e*t al.* (2002), the SDS-PAGE-based cluster analysis is a dominant tool for differentiating *Vigna radiata and Vigna mungo.* Genetic diversity within the 60 Ghanian Cowpea (vigna unguiculate L.) germplasm is based on seed storage.

Table II. In present study were used different genotypes of Dolichos, Seed morphology and weight of seeds.

S.No.	Genotypes	100 grain Wt (gm)	Size	Seed color
1.	MR-04-08	245	Small	Light yellow
2.	MR-04-62	294	Small	Light yellow
3.	MR-04-35A	330	Small	Light yellow
4.	ARKA-VIJAYA	330	Small	Light yellow
5.	MR-04-09	332	Large	Light yellow
6.	MR-04-20A	337	Large	Light yellow
7.	MR-04-57	399	Large	Light yellow
8.	PSR-11475	399	Large	Light yellow
9.	MR-04-64	414	Small	Light yellow
10.	MR-04-69	420	Small	Light yellow
11.	MR-04-45A	447	Large	Light yellow

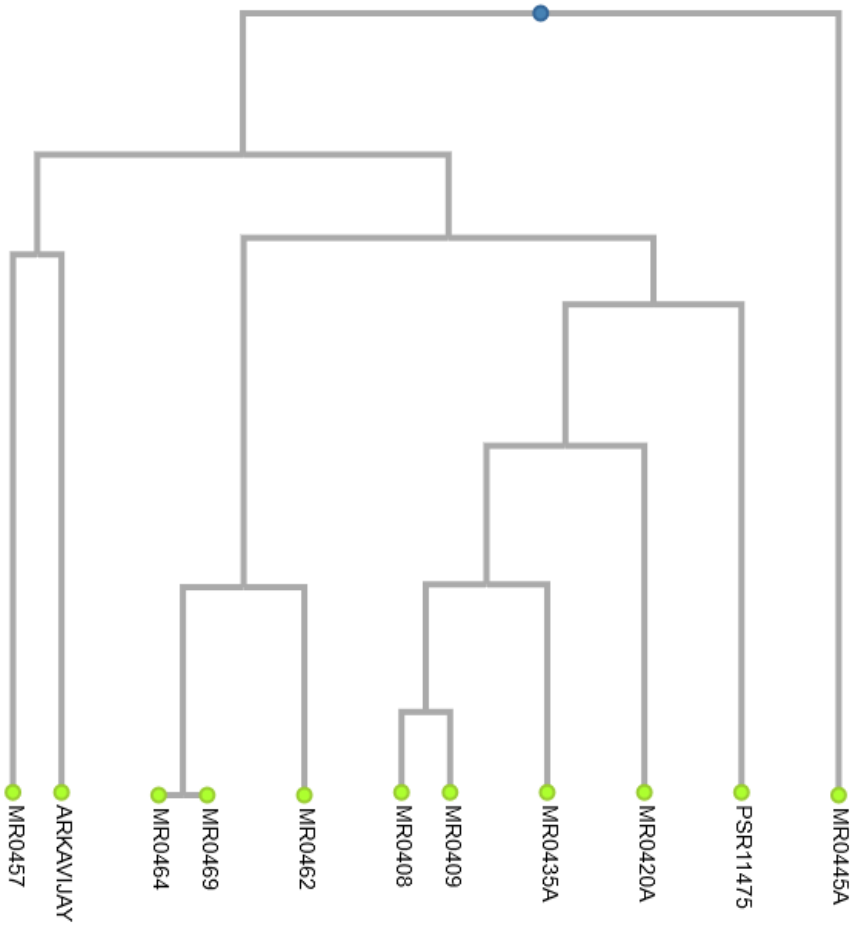

Fig. 3: Dendrogram showing genetic distance among studies to Eleven accessions of Dolichos varieties.

The present study of the intragenic relationship between the 11 Indian Dolichos varieties was analyzed for total soluble seed proteins using SDS-PAGE. The protein bands, thus generated, were utilized to study genetic diversity. This study only indicates that genetic similarity or dissimilarity goes much deeper than one generation of parental lineages in the present study and the previous reports (Kleim *et al.*, 1992; Satyavathi *et al.*, 2006; Dantu *et al.;* 2011). Dolichos occupies a unique position for vegetable purposes among the legumes vegetables (Biju *et al.,* 2001; Rai *et al*., 2009). It is evident from the limited area of cultivation under this crop and its genetic enhancement (Vaijayanthi *et al.,* 2018). There is appreciable variation between the accessions; hence there is ample scope for genetic improvement through breeding. In conclusion, electrophoresis (SDS-PAGE) of seed storage proteins can be economically used to assess genetic variation and relation in germplasm and differentiate mutants from their parent genotypes.

Conclusion

Dolichos considered an underutilized and unexplored crop in terms of cultivated area and efforts to genetic improvement. However, it has a vast potential to play a crucial role in sustainable agriculture, nutritional and income security of small and marginal farmers in dry and semi-arid regions of tropic and subtropics shortly. There are considerable variations between the present study's accessions; therefore, sampling scope for genetic improvement through the breeding. In the finale, SDS-PAGE of seed storage proteins can be cheaply used to assess genetic variation and relation in germplasm and differentiate mutants from their parent genotypes. The difference in

the existing gene pool could be of enormous value to breeders for developing new cultivars. As well, PSR-1145 and MR-04-45A helps the breeders to design their hybridization program with a greater probability of success. Furthermore, seed storage protein-based markers can be used for the identification of genotypes in the future. It is suggested that genotypes with similar banding patterns should be further characterized by Swath analysis.

Acknowledgments

The authors wish to acknowledge the Director of the Institute for the facilities provided to carry out the research work. We accepts the help of NBPGR, Delhi, for providing seeds for the study; Dr. Juhi Chaudhary, Plant Biotechnology Laboratory, Department of Botany, Dayalbagh Educational Institute (Deemed University) Dayalbagh, Agra, India, for analyzing a part of the data.

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