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RESEARCH ARTICLE

INTRA-SPECIES PROTEIN VARIATION STUDIES ON *DOLICHOS LABLAB* LINN.

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ABSTRACT

Legumes are a source of wholesome protein, alimentary fiber, and bioactive substances displaying antioxidant activity together with anti-inflammatory and antineoplastic properties. Cereals and Pulses form an integral part of the human diet.Eight accessions of *Dolichos lablab* Linn. were procured from Pulses and Castor Research station, Navsari. Total proteins were extracted in chilled 100 mM Tris-HCl buffer (pH 8) containing 1% SDS and 0.01% β- mercaptoethanol. Albumins and Globulins were extracted based on differential solubility in water and dilute salt solution respectively. The genotype of an organism expresses itself through the phenotype - through proteins. So, protein profiling of the plant is essential to reveal the role of proteins. Electrophoresis serves as an imperative tool for studying the protein profiling of the plants. Therefore, Extracted samples were subjected to electrophoretic separation by SDS-PAGE. Proteins were stained using Coomassie blue and silver staining technique. The binary data was subjected to Statistical analysis using MVSP software. The genetic similarity matrix was calculated using Jaccard's co efficient and the dendrogram was based on Jaccard's distance matrix is obtained using UPGMA. Thus three different Phylogeny trees for 8 accessions of *D. lablab* L. were made based on protein profile of Total Proteins, Albumins, and Globulins. Present work provides the protein variation and phylogenetic relationships between eight accessions of *Dolichos lablab* L.

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INTRODUCTION

Cereals and Pulses form an integral part of the human diet. Pulses are important food crops that can play a major role in addressing future global food security and environmental challenges, as well as contributing to healthy diets. (Calles, 2016) Pulses have sustained during human evolution for thousands of years as an indispensable portion of the nutrition. Due to their imperative role in diet and nutrition, the 68th UN General Assembly declared 2016 the International Year of Pulses (IYP). *Dolichos lablab* Linn. also known as *Lablab purpureus* is a species of bean in the family Fabaceae. The plant is used as anti-inflammatory, aphrodisiac, antispasmodic, antidiabetic, febrifuge and for flatulent, bilious, stomachic and phlegmatic. (Gętek *et al.,* 2014) The planet displays enormous diversity in the flora and fauna. The distinctions of characters originate in same species of plants (wild and cultivated) can be cited as intra-specific variations. However these variations need not be correlated with subspecies and varieties of classical taxonomy. (Acquaah, 1992) Methodically it can be quoted as the difference in various qualitative and quantitative characters showed by the diverse individuals of same species. Against an increasingly complex and interesting array of variations in plants, these variations are congregated into two simple categories, Genetic and Epigenetic.Genetic variations, are firmly heritable i.e., truthfully passed from one generation to another and do not alter under cultivated conditions. Genetic variants are ascertained by isozyme techniques and Random Amplified polymorphic DNA assays.Epigenetic variations, on the other hand, are mostly induced by the environment in which plants are growing and are also affected by the developmental efforts. Epigenetic variations in plants usually comprise morphological, chemical as well as physiological variations. Therefore a great deal of information on the morphological, biochemical, physiological, and genetic variation is necessary before an observed pattern of variation is interpreted. It is also true that underneath these intra-specific variations, there exists a fixed spectrum of unchangeable characteristics their by making these species unique (Chen, 2007). The genotype of an organism expresses itself through the phenotype - through proteins. Protein molecular markers provide indirect information about plant genome structure. Plant proteomics is the rapidly progressing field in the current

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era of functional genomics. The proteins encoded in a plant cell play significant role in survival and adaptation of the plant to external stresses. There are enormous proteins in each organelle of the plant cell where they exhibit substantial and diverse functions. The manifestation of certain proteins is observed in specific developmental phase of life cycle of the plant. Study of plant proteome is more multifaceted due to further metabolic cellular or organ based compartmentalization in metabolism. So, protein profiling of the plant is essential to reveal the role of proteins. Electrophoresis serves as an imperative tool for studying the protein profiling of the plants. (Iqbal et al., 2005)

MATERIALS AND METHODS

Total eight authentic accessions namely Gujrat Waal-2, Gujrat Wal-1, Gujrat waal-125-36, GNIB-21, Katargam, Gujarat papdi, Manchi Waal, Kapasi, obtained from Agricultural Research Station are the collected varieties native to the various villages of Gujarat. Intraspecific variation in Phenotype profile observed in the accessions of *D. lablab* L. is provided in Table 1. Chemicals and reagents for SDS-PAGE were procured from sigma Aldrich and the protein marker was purchased from In-vitrogen. Intra-species Genotype variation was studied using the SDS PAGE (Laemmli,1970) Protein profiling for accessions of *D. lablab* L. Total proteins, Albumins and globulins were extracted from eight accessions were subjected to electrophoresis analysis to obtain protein profiles.

Extraction of Total Proteins

0.1 g of seed powder of each accession of *D. lablab* L. was macerated 5 mL of chilled 100 mM Tris-HCl buffer (pH 8) containing 1% SDS and 0.01% β- mercaptoethanol using precooled mortar and pestle. This step was performed for complete solubilization of proteins. The extract was transferred in to clean dry falcon tube (capacity 15 mL) and centrifuged at 8000 rpm for 10 minutes at 4^0C to obtain clear supernatant containing solubilized proteins. Proteins were precipitated using 2 folds of ice cold acetone. The tubes were kept undisturbed at -20^0C for 12-18 hours for complete precipitation of proteins. Further, the precipitate was collected by centrifugation.The precipitate was then air dried for 20 minutes to ensure the elimination of traces of Acetone from precipitate and reconstituted in 0.1 mL of 0.125 M Tris buffer (pH 8). Reconstituted total proteins were used for electrophoretic analysis.

Fractionation ofAlbumins and Globulins

Albumins and globulin extraction was carried out on the basis of their differential solubility in water and dilute salt solutions respectively. To enable complete extraction of albumins and globulins the precipitate of total proteins was reconstituted in 5 folds of water followed by 2 folds of 0.5 N sodium chloride solution. The washings were given and supernatants were collected separately by centrifugation. These clear supernatant solutions of albumins and globulins were used for Electrophoretic analysis of Seed proteins of *D. lablab* L.

Separation of Proteins

Completely solubilized Total proteins, Albumins and globulins were subjected to SDS-PAGE. 15 □L of sample was boiled for 3 minutes with same volume of gel loading buffer (Laemmli, 1970). 20 µL of the samplewas loaded on 12 % Gel to obtain better resolution and separation. Resolved proteins were stained Coomassie blue as well as silver staining (Chevallet et al., 2006). Comparison of Protein profiles for Total Proteins, Albumins, Globulins obtained by SDS PAGE analysis of 8 accessions of *D. lablab* L. was carried out on the basis of bands relative mobility. The presence of bands was recorded in binary data matrix taking 1 as the presence of the band on the gel and 0 as the absence of the band. The binary data was subjected to Statistical analysis using MVSP software. The genetic similarity matrix was calculated using Jaccard's co efficient and the dendrogram was based on Jaccard's distance matrix is obtained using UPGMA. Thus three different Phylogeny trees for 8 accessions of *D. lablab* L. were made based on protein profile of Total Proteins, Albumins, and Globulins.

RESULTS AND DISCUSSION

The scoring for SDS PAGE analysis of Total Protein, Albumin and Globulin was done for the presence or absence of protein bands, which are identical by their respective relative mobility.

Total Protein Analysis

SDS-PAGE profile of Total Proteins was studied in 8 accessions of *D. lablab* L. (Figure 1) and migration velocities of the same were calculated.Substantial variation was observed in protein profiles of accessions of *D. lablab* L. studied. There was a monomorphic band having relative migration (RM) 0.18. On comparison, it was found that several proteins were differentially expressed with respect to protein profile of each accession. Protein band with RM 0.2 was found to be restricted to Gujarat Waal-1, Gujarat waal-2, Gujarat papdi, Gujrat waal-125-36 whereas protein band RM 0.22 was observed only in Gujarat Waal-1, Gujarat papdi, GN1B-21, NPS-1 and Gujrat waal-125-36. A unique band with RM 0.24 was observed in Katargam, 0.42 and 0.86 in Gujarat Waal -2 and GN1B-21, NPS-1 showed two unique bands with RM 0.92 and 0.96. Band with RM 0.62 was present in all the accessions studied except for Kapasi, similarly band with RM 0.76 was present in all and was missing in Manchi Waal. Expression of some specific protein bands were observed in few accessions like Gujarat waal-2 having protein band RM 0.42, 0.86, whereas accession GN1B-21, NPS-1 showed protein band RM 0.52 and Gujarat waal-1, Manchi waal both the accessions showed protein band RM 0.58. On the basis of similarity matrix calculated using UPGMA Jaccard's Coefficient, maximum similarity of 50 % was observed between Gujarat Waal-1 and Gujarat Papdi, similarly Gujrat waal-125-36 showed 50% similarity with Gujarat Papdi, Katargam and also with Kapasi. Manchi Waal and Kapasi showed least similarity of 22.7%. Based on UPGMA Jaccard's Coefficient of similarity (Jaccard, 1901) for Total Protein analysis Dendrogram was made to study the phylogenetic relationship of eight accessions of *D. lablab* L. The dendrogram showed three major clusters connected to each other at different nodes with variation in similarity index, as shown in Fig I. The first cluster consists of Gujarat Waal-1 and Gujarat Papdi at node-1 with 50% similarity. Second cluster has three plants, Katargam and Gujarat Waal-125-36 at node -2 with 50% similarity, to this minor cluster Kapasi is attached at node-3 with 47.9 % similarity.

Table 1. Phenotypic characters of Accessions of *D. lablab* L

Sr. No.	Character	G. Wal-2	G. Wal-1	GW125-36	GNIB-21	Katargam	Gujarat papdi	Manchi Waal	Kapasi
1.	Days to 50 % Flowering	62	64	64	40 - 45	69 – 73	65-72	68 – 72	69 – 74
2.	Days to 80 % maturity	120	135	145	90	>145	>145	>145	95
3.	Plant height (cm)	69	74	72	39.7	65.1	65	74	72
4.	Avge. no. of branches/plant	3	3	2.5	4.2	6.0	4.3	3.5	4
5.	Avge. no. of. pods/plant	41	37	33	31.7	47	42	44	35
6.	Pod length (cm)	4.5	5	4.5	3.3	3.5	4.5	3.3	3.5
7.	No. of. seeds/pod	4	4-5	4	3.4	4-5	4	5	4
8.	100-grain wt (g.)	20	22	20	22	20	20	18	17.5
9.	Seed color	Chocolaty White	White	White	White	White	White	White	Greenish brown
10.	Plant type	Erect	Spreading	Spreading	Erect	Spreading	Spreading	Erect	Spreading
11.	Foliage	Dark green	Light green	Light green	Dark green	Green	Light green	Dark green	Green
12.	Flower colour	White	Pink	White	White	White	Pink	White	White
13.	Stem colour	Green	Purple	Green	Green	Green	Green	Green	Green
14.	Bearing habit	DT	NDT	NDT	DT	NDT	NDT	DT	NDT
15.	Pod type	Green pods	Green pods	Green pods	Curvy pods	Curvy pods	Green pods	Curvy pods	Curvy pods
14.	Grain yield (kg/ha)	1348	1157	822	3145	2857	3645	2887	3574

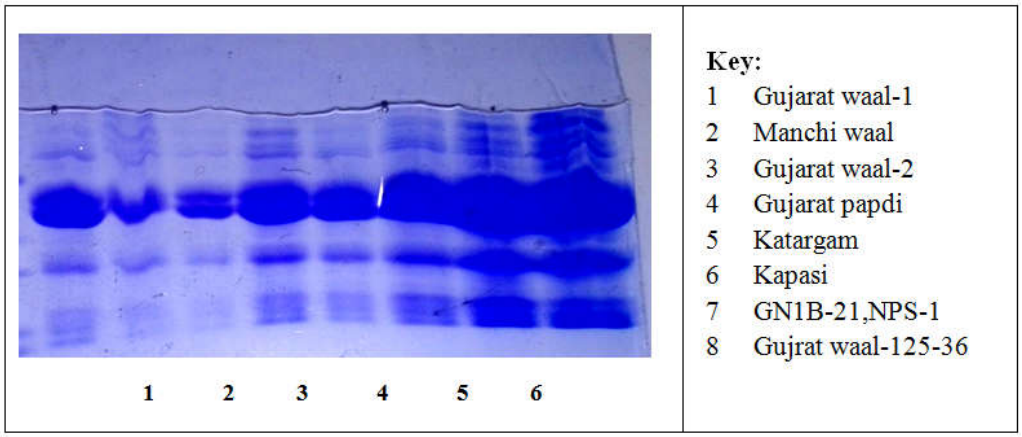


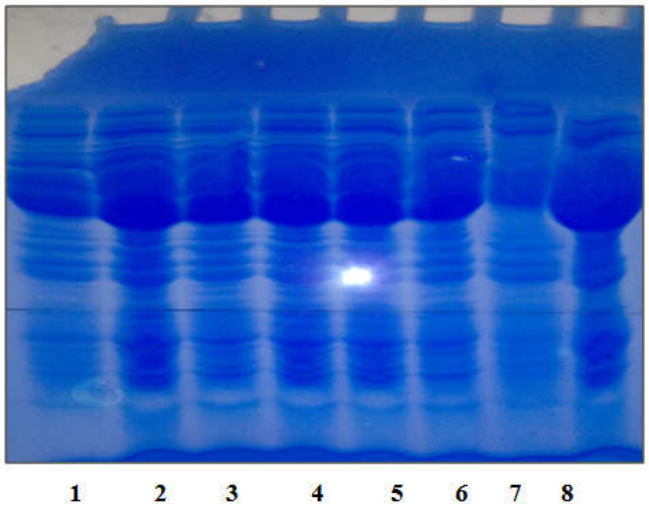
Fig. 1. SDS-PAGE profile of Total Proteins from D. lablab L.

Table 2. Jaccard's Coefficient Similarity matrix based on SDS PAGE Profiles of Total Proteins from Accessions of *D. lablab L* obtained by SDS-PAGE

	Gujarat Waal-1	Manchi Waal	Gujarat Waal -2	Gujarat Papdi	Katargam	Kapasi	GN1B-21NPS-1	Gujarat Waal-125-36
Gujarat Waal-1	1							
Manchi Waal	0.4	1						
Gujarat Waal -2	0.44	0.304	1					
Gujarat Papdi	0.5	0.263	0.391	1				
Katargam	0.385	0.429	0.407	0.333	1			
Kapasi	0.375	0.227	0.25	0.45	0.458	1		
GN1B-21NPS-1	0.346	0.318	0.423	0.409	0.37	0.36	1	
Gujarat Waal-125-36	0.478	0.4	0.385	0.5	0.5	0.5	0.4	1

Table 3. Grouping of Accessions of *D. lablab L* into clusters based on SDS PAGE ofTotal proteins

Node	Group 1	Group 2	Similarity	Objects in group
1	Gujarat Waal-1	Gujarat Papdi	0.5	2
2	Katargam	125-36	0.5	2
3	Node 2	Kapasi	0.479	3
4	Gujarat Waal -2	GN1B-21NPS-1	0.423	2
5	Node 1	Node 3	0.42	5
6	Node 5	Node 4	0.376	7
7	Node 6	Manchi Waal	0.335	8



Key
1 Gujarat waal-1
2 Manchi waal
3 Gujarat waal-2
4 Gujarat papdi
5 Katargam
6 Kapasi
7 GN1B-21,NPS-1
8 Gujrat waal-125-36

Fig. 2. SDS-PAGE profile of Albumins from *D. lablab* L.

Table 4. Jaccard's Coefficient Similarity Matrix based on SDS PAGE Profiles of Albumins from Accessions of *D. lablab* L. obtained by SDS-PAGE

	Gujarat Waal-1	Manchi Waal	Gujarat Waal -2	Gujarat Papdi	Katargam	Kapasi	GN1B-21NPS-1	GW-125-36
Gujarat Waal-1	1							
Manchi Waal	0.364	1						
Gujarat Waal -2	0.458	0.476	1					
Gujarat Papdi	0.478	0.5	0.944	1				
Katargam	0.4	0.409	0.44	0.4	1			
Kapasi	0.391	0.12	0.269	0.231	0.435	1		
GN1B-21NPS-1	0.36	0.304	0.4	0.36	0.346	0.333	1	
Gujratwaal-125-36	0.5	0.381	0.417	0.375	0.478	0.476	0.435	1

Table 5. Grouping of Accessions of *D. lablab L* into clusters based on SDS PAGE of Albumins

Node	Group 1	Group 2	Similarity	Objects in group
1	Gujarat Waal -2	Gujarat Papdi	0.944	2
2	Gujarat Waal-1	Gujrat waal-125-36	0.5	2
3	Manchi Waal	Node 1	0.488	3
4	Node 2	Katargam	0.439	3
5	Node 4	Kapasi	0.434	4
6	Node 5	GN1B-21NPS-1	0.369	5
7	Node 6	Node 3	0.36	8

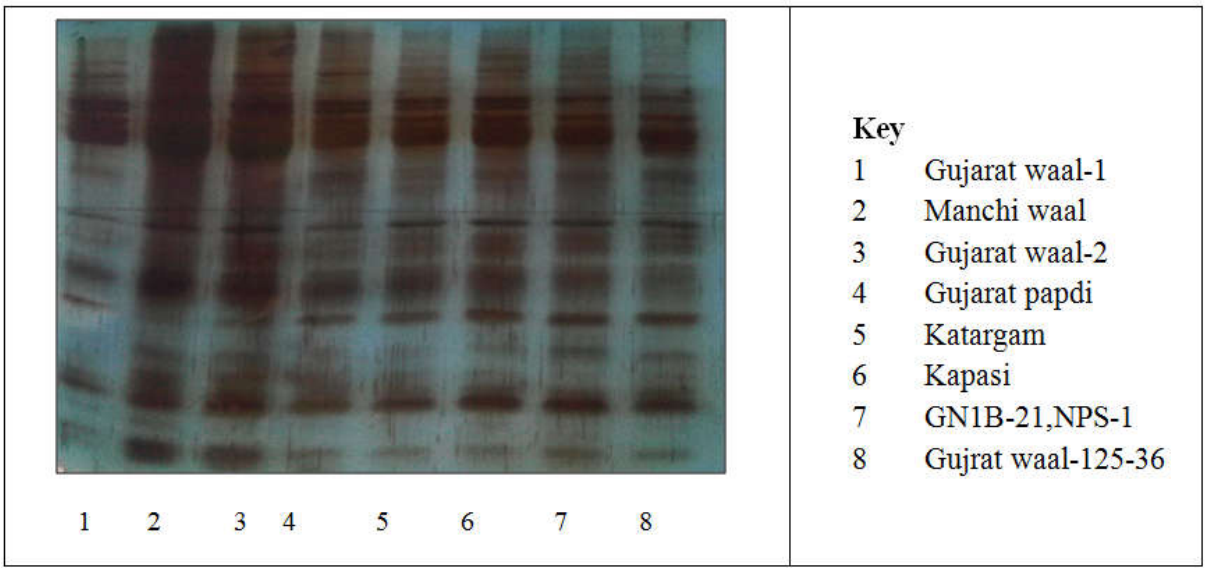


Fig.3. SDS-PAGE profile of Globulins from *D. lablab* L.

Third cluster consists of Gujarat Waal -2 and GN1B-21NPS-1 at node-4 with 42.3% similarity. First cluster is connected to Kapasi of cluster 2 at node-5 with 42% similarity. Third cluster is connected to earlier two clusters at node-6 with 37.6% similarity. Lastly, at node-7 Manchi Waal is added to the dendrogram with least similarity of 33.5%.

Albumin Analysis

SDS-PAGE profile of Albumins was studied in 8 accessions of *D. lablab* L. and migration velocities of the same were calculated. Variation of expression of albumins was clearly seen in protein profiles of different accessions studied.

Table 6. Jaccard's Coefficient Similarity Matrix based on SDS PAGE Profiles of Globulins from Accessions of *D. lablab* L. obtained by SDS-PAGE

	Gujarat Waal-1	Manchi Waal	Gujarat Waal -2	Gujarat Papdi	Katargam	Kapasi	GN1B-21NPS-1	Gujarat Waal-125-36
Gujarat Waal-1	1							
Manchi Waal	0.391	1						
Gujarat Waal -2	0.458	0.571	1					
Gujarat Papdi	0.478	0.6	0.944	1				
Katargam	0.4	0.375	0.44	0.4	1			
Kapasi	0.4	0.375	0.44	0.4	1	1		
GN1B-21NPS-1	0.36	0.333	0.4	0.36	0.346	0.346	1	
GW-125-36	0.4	0.375	0.44	0.4	1	1	0.346	1

Table 7. Grouping of Accessions of *D. lablab L* into clusters based on SDS PAGE of Globulins

Node	Group 1	Group 2	Similarity	Objects in group
1	Katargam	Kapasi	1	2
2	Node 1	Gujarat Waal-125-36	1	3
3	Gujarat Waal -2	Gujarat Papdi	0.944	2
4	Manchi Waal	Node 3	0.586	3
5	Gujarat Waal-1	Node 4	0.443	4
6	Node 5	Node 2	0.404	7
7	Node 6	GN1B-21NPS-1	0.356	8

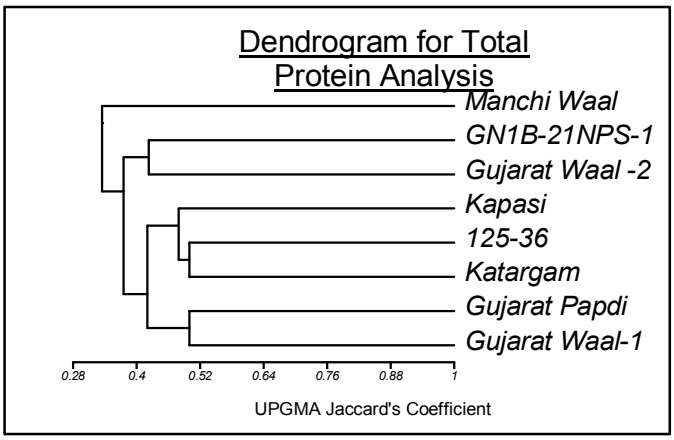


Fig. 4 Dendrogram showing phylogenetic relationship of the eight accessions of *D. lablab* L. based on SDS PAGE Data of Total Proteins

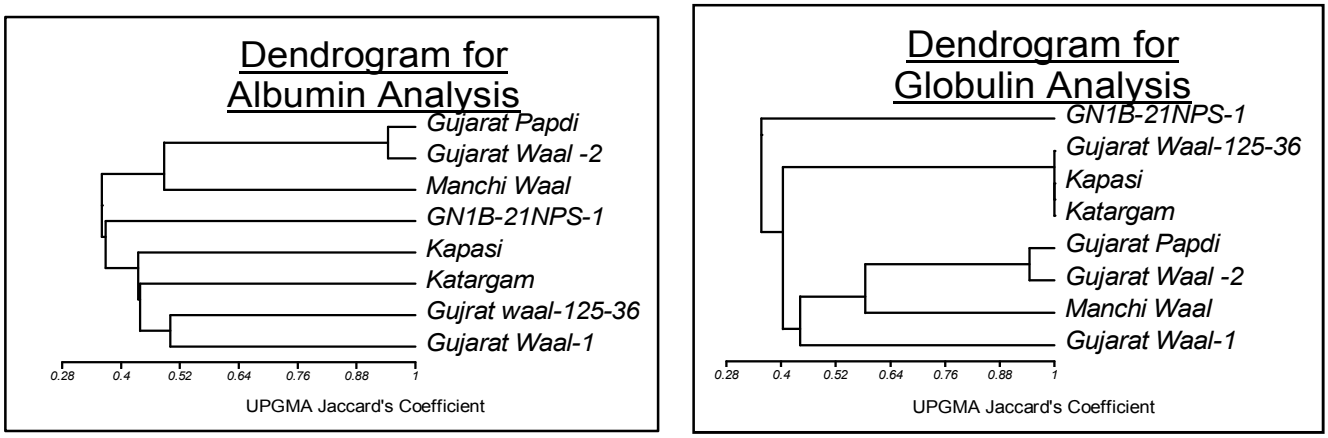


Fig. 5 Dendrogram showing phylogenetic relationship of the eight accessions of *D. lablab* L. based on SDS PAGE Data of Albumins& Globulin

Monomorphic protein band of relative migration 0.18 was observed. A unique band was observed in Katargam with RM 0.24, in GN1B-21NPS-1 with RM 0.92, 0.96. Accessions Katargam and Kapasi showed three unique bands with RM 0.66, 0.68 and 0.84. Protein band of RM 0.3 was observed in all the accessions except in GN1B-21NPS-1, similarly band with RM 0.6 was observed in all but was absent in Manchi Waal. Also band with RM 0.62 was observed in all but was missing in Kapasi. Variation of expression of albumins was clearly seen in protein profiles of accessions Gujarat waal-1(RM 0.2, 0.22, 0.58) Manchi waal (RM 0.42,0.48) Gujarat waal-2(RM 0.2, 0.24, 0.34) Gujarat papdi(RM 0.20, 0.42) Katargam (RM 0.34,0.84) Kapasi (RM 0.34,0.84) GN1B-21,NPS-1 (RM 0.28, 0.92, 0.96) Gujrat waal,125-36(RM 0.22). On the basis of similarity matrix calculated for SDS PAGE profile of Albumins using UPGMA Jaccard's Coefficient, maximum similarity of 94 % was observed between Gujarat Waal-2 and Gujarat Papdi. Whereas Manchi Waal and Kapasi showed least similarity of 12%. Based on UPGMA Jaccard's Coefficient of similarity (Jaccard, 1901) for Albumin analysis Dendrogram was made to study the phylogenetic relationship of eight accessions of *D. lablab* L. The dendrogram revealed two major clusters connected to each other at different nodes with variation in similarity index, as shown in Fig.5. The first cluster consists of Gujarat Waal-2 and Gujarat Papdi at node-1 with 94.4% similarity. To this cluster Manchi Waal is added at node-3 with 48.8% similarity. Second cluster has five plants, initially Gujarat Waal-1 and Gujrat waal-125-36 are connected at node-2 with 50% similarity, at node-4 Katargam is added with 43.9% smilarity and Kapasi is added to this cluster at node -5 with 43.4% similarity, finally to this minor cluster GN1B-21NPS-1is attached at node-6 with 36.9 % similarity. Lastly, both the clusters are connected at node-7 with least similarity of 36%.

Globulins Analysis

SDS-PAGE profile of Globulin proteins was studied in 8 accessions of *D. lablab* L. and migration velocities of the same were calculated. Differential expression of Globulins was evident in different accessions studied. (Fig.5) Monomorphic protein bands of relative migration 0.18 and 0.62 were observed. A unique band was observed in Gujarat Waal -1 with RM 0.22, 0.74 in GN1B-21NPS-1 with RM 0.52, 0.92, 0.96. Accessions Manchi Waal, Gujarat Waal -2 and Gujarat Papdi showed four unique bands with RM 0.34, 0.36, 0.4 and 0.42. Protein band of RM 0.3 was observed in all the accessions except in GN1B-21NPS-1, similarly band with RM 0.6,0.76 was observed in all but wasn't there in Manchi Waal, so also band with RM 0.64 was observed in all but was missing in Gujarat Waal-1 and GN1B-21NPS-1, band 0.72, 0.8 was also present in all except in GN1B-21NPS-1. On the basis of similarity matrix calculated for SDS PAGE profile of Globulin proteins using UPGMA Jaccard's Coefficient. 100% similarity was observed between Katargam, Kapasiand Gujarat Waal--125-36. Whereas Manchi Waal andGN1B-21NPS-1showed least similarity of 33.3%. Based on UPGMA Jaccard's Coefficient of similarity (Jaccard, 1901) for Globulin proteins analysis Dendrogram was made to study the phylogenetic relationship of eight accessions of *D. lablab* L. The dendrogram revealed two major clusters connected to each other at different nodes with variation in similarity index, as shown in Fig.5. The first cluster consists of three plants together with 100 % similarity viz. Katargam, Kapasiand GW-125-36. Second cluster has four plants, initially Gujarat Waal-2 and Gujrat papdi are connected at node-3 with 94.4% similarity, at node-4 Manchi Waal is added to the cluster with 58.6% smilarity and Gujarat Waal-1 is added to this cluster at node -5 with 44.3% similarity. Cluster 1 and Cluster 2 are connected at node -6 with 40.4% similarity. Finally GN1B-21NPS-1 is added to the dendrogram externally at node-7 with 35.6% similarity indicating its distant relatedness.

Conclusion

The current work reveals the protein profile of the leguminous plant *D. lablab* L.which can further be extended for the analysis of Specific proteins. Present research work also demonstrates Phylogenetic relationshipsbetween eight accessions as well as differential expression of proteins in the accessions of *Dolichos lablab* L.

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