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GENETIC VARIATIONS OF *ROBINIA PSEUDOACACIA* PLANT USING SDS-PAGE

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Abstract

The biochemical analysis using SDS-PAGE has great contribution for the estimation of genetic diversity. We estimated the genetic diversity of *R. pseudoacacia* germ plasm protein. A total of 19 varieties were collected from different areas of Dir lower were investigated for the level of genetic divergence and genetic linkages. The total germ plasm grouped were separated at 20% distance into two linkages based on Euclidean distances the 19 cultivars were further divide at 45% distance into three clusters, cluster I, cluster 2 and cluster 3. Cluster 1 was comprised of Munda 3, Munda 4, Talash 2 and UOM 1. Cluster 2 was comprised of Maidan 1 and Gulabad 1. Cluster 3 was comprised Maidan 2, UOM 3, Talash 1, Maidan 4, Maidan 3, Gulabad 2, Gulabad 3 and Gulabad 4. A total of range 00% to 88% variation recoded among 19 varieties. . The result obtained after SDS-PAGE were computed for the construction of phylogenetic diversity, geographic relationship, Euclidian distance, genetic distance and linkage distance .This plant show a lot of variation in germ plasmic level. It is concluded that it is possible to improve and produce new varieties of this plant.

Key words: *Robinia pseudoaacacia*, SDS-PAGE, Polygenetic diversity.

Introduction

A *Robinia pseudoacacia* locally known as kekar is perennial plant. It is a single trunked with several erect twigs, lightly brown. The shoot olive-brown changing to brown, properly slender, twist and turn, borne with single or double prickles at some nodes. Leaves are dark blue-green, deciduous, alternate, pinnately, composite, leaf 1-2' long, leaflets may be irregular at apex, autumn color yellowish green to green. Flowers are white overhanging inflorescences that are showy white and aromatic but last only a couple of weeks. Fruit shapes are peapod, fruit length are 3 to 6 inches, dry or hard, fruit color are black or red (Edward *et al*., 1994).

There is a significant connection between historic cultural centers and centers of biodiversity (Shinwari, 2010). Some Factors endorsing high biodiversity, such as constant water availability, environmental heterogeneity and productive soils have also preferred human settlement (Balmford *et al*., 2001) To estimate the genetic diversity and relationship of germplasmic collections, identification of genetic of diversity in desirable traits so many different methodologies are now approved in germplasm evaluation that may include morphological characterization, biochemical markers evaluation at protein level (SDS-PAG) (Nisar *et al*., 2009; Shah *et al*., 2011; Akbar *et al*., 2012). The importance of electrophoretic evidence in plant systematics has been discussed in detail by many workers (Kamel, 2005; Zada *et al*., 2013; Khan *et al*., 2013). In Leguminosae many studies have been carried out based on the electrophoresis of seed proteins (Hussein & George, 2002; Hussein *et al*., 2005). Electrophoretic patterns of total seed proteins as revealed by polyacrylamide gel electrophoresis (PAGE) with sodium dodecyl sulphate (SDS) have been successfully used to resolve the taxonomic and evolutionary problems of some plant species (Ladizinsky & Hymowitz, 1979; Potokina *et al*., 2000; Ghafoor & Arshad, 2008; Ayten *et al*., 2009). Plant breeding, the induced evolution change the phyto history in the recent past and the improvement in plants are mainly based on the presence of genetic variation either natural or induced through gene recombinant, mutation etc. cereals are more researched as compared to legumes, the scope of plant genetic improvement through the manipulation of available genetic variability is still equally believed by all the plant scientists. Sound breeding program in any field crop depends mainly upon the availability of genetic variability either existing mutation, gene recombination etc. (Ghafoor, 1999). Among biochemical techniques, SDS-PAGE is the most widely used due to its validity and simplicity for determination genetic structure of crop germ plasm (Ghafoor, 1999). SDS-PAGE protein analysis has been used widely in study of several plant species as identification of seed protein by electrophoresis has indicated that seed protein profile is highly stable and species specific. Moreover, seed protein profile is hardly affected by experimental conditions. Because of relative ease, fast and cheaper cost per assay. SDS-PAGE has been used by various workers for estimation of genetic diversity (Ferreira *et al*., 2000; Valizadeh, 2001). These studies on seed storage protein, not only helped in the identification and characterization of diversity in the varieties, cultivars and their wild relatives but also in determining the out crossing rate and phylogenetic relationships(Asghar *et al*., 2003).

Materials and Methods

Nineteen seeds samples of *R. pseudoacacia* were collected from district Dir lower. Matured seeds were collected, dried and used to extract total seed storage protein.

For Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis, the seeds were placed in oven over night at 37^{o}C to remove the water content of the seed. The dry seed of each genotype were grounded in Eppendorf tube with large needle. Then 400ml of the protein extraction buffer (PEB) was added to 0.01g of seed flour and vortexes (using Gyro mixer vortex) thoroughly to homogenize. The proteins were

extracted at room temperature for 20 minutes. In order to purify, the homogenate samples were centrifuged at 12,000rpm for 10 minutes at room temperature. The extracted crude proteins were recovered as clear supernatant and were transferred to a new 1.5ml Eppendorf tube and stored at 4^{o}C until they were run on the polyacrylamide gel.

To optimize the Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) protocol suitable for *R. pseudoacacia*, different protocols were tested. For example the protocols used previously by Laemmli (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage, Payne (1987) for protein analysis of wheat and Lioi *et al*. (1999), for protein analysis of *R. pseudoacacia* using modifications.

Results and Discussion

A total of 19 genotypes of *R. psedouacacia* were collected from different areas of Dir lower for the investigation of genetic differences intra-genetically. The genetic diversity was based upon on the proteomics assay.

In various combination the SDS-PAGE was carried out and was found that 15% acryl amide gel concentration give best resolution. For checking the reproducibility, all the 19 genotypes were run in two replicates. It was found that 8 bands were produce (Figs. 1 and 2).

Dendogram was constructed for proteomic of *R. pseudoacacia*, by using software STATISTICA 6.0 in Windows XP2005 (www.statsoft.com). Dendogram (Fig. 3) divided 19 combinations into tow linkage at 20% linkage distance, linkage 1 and linkage 2. At 45% distance linkage 1 and Linkage 2 are divide into three cluster, cluster 2 and cluster 3. Cluster 1 further divide into two sub cluster, 1st cluster consist of Talash 2, UOM 1 and Munda 3, 2nd sub cluster consist of Munda 4. Cluster 2 was consist of Gulabad 1 and Maidan 1. Cluster 3 were further divide into two sub cluster,1st sub cluster consist of Maidan 2, UOM 3 and Talash 1 and 2nd sub cluster consist of Maidan 4, Maidan 3, Gulabad 2, Gulabad 3, Gulabad 4, Talash 4, UOM 4, Talash 3, UOM 2 and Munda 1.

The band by SDS- PAGE among the 19 lines of *R. pseudoacacia* were varied among 1st band present in Gulabad 1 and maidan 1, 2nd band also present in Gulabad 1 and maidan 1, 3rd band present in Munda 1, Talash 2, UOM 1, Maidan 1, Gulabad 1, Maidan 2, UOM 3, Talash1, Maidan 4, Maidan 3, Gulabad 4, Gulabad 2, Talash 4, UOM 4, Talash 3, UOM 2 and Munda 1,band 4th absent only in Munda 4,band 5th absent in Munda 4 ,Maidan 2,UOM 3 and Talash1,band 6th absent in Maidan 3 and Maidan 4,band 7th absent in Munda 3 Talash 2 UOM1 and band 8th absent in Munda 3 ,Talash 2 and Munda 4 (Table 1).

Present research work was undertaken for morphological, biochemical and phylogenetic characterization of 19 samples of *R. pseudoacacia* collected from Dir lower. Results presented in the present study is the first documented attempt for estimation of genetic diversity present in *R.pseudoacacia* found in various locations of Dir lower Pakistan. Sodium Dodycyle Sulphate Polyacrylamid Gel Electrophoresis (SDS-PAGE) was used to separate various alleles of seed storage proteins extracted from *R. pseudoacacia* seeds. The technique of SDS-PAGE was selected because it is easy, reliable and cheap procedure and has been widely used in characterization of various plant species in the past (Bretting & Widrlechner, 1995; Nisar *et al*., 2009b). Morphological characterization of the *R.pseudoacacia* germ plasm collected during present study was carried out and key for identification of species was developed. The technique of SDS-PAGE was optimized which was suitable for seeds of *R. pseudoacacia*. Profiling of the germplasm using SDS-PAGE was done and genetic differences among the germ plasm were estimated. Scoring of alleles (protein bands) was carried out using unweighted consideration of alleles principle. Genetic distance (GD) estimates showed (Table 2) high values among the germ plasm agreements (GD ranging from 0-100%). Phylogenetic relationship among the R.pseudoacacia accessions was studied using cluster analysis. It is suggested that more research work should be conducted for better understanding of the genome structure of *R. pseudoacacia*.

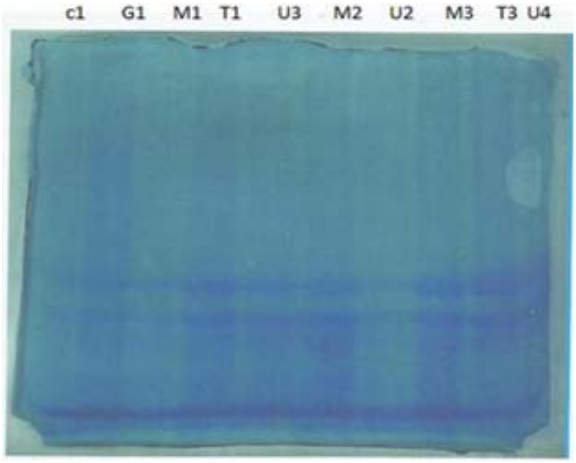


Fig. 1. Proteomic assay of 10 genotype of *R. pseduoacacia* germ plasm based on SDS-PAGE.

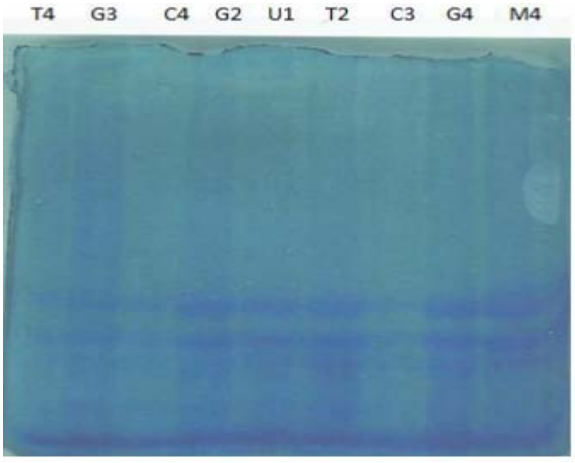
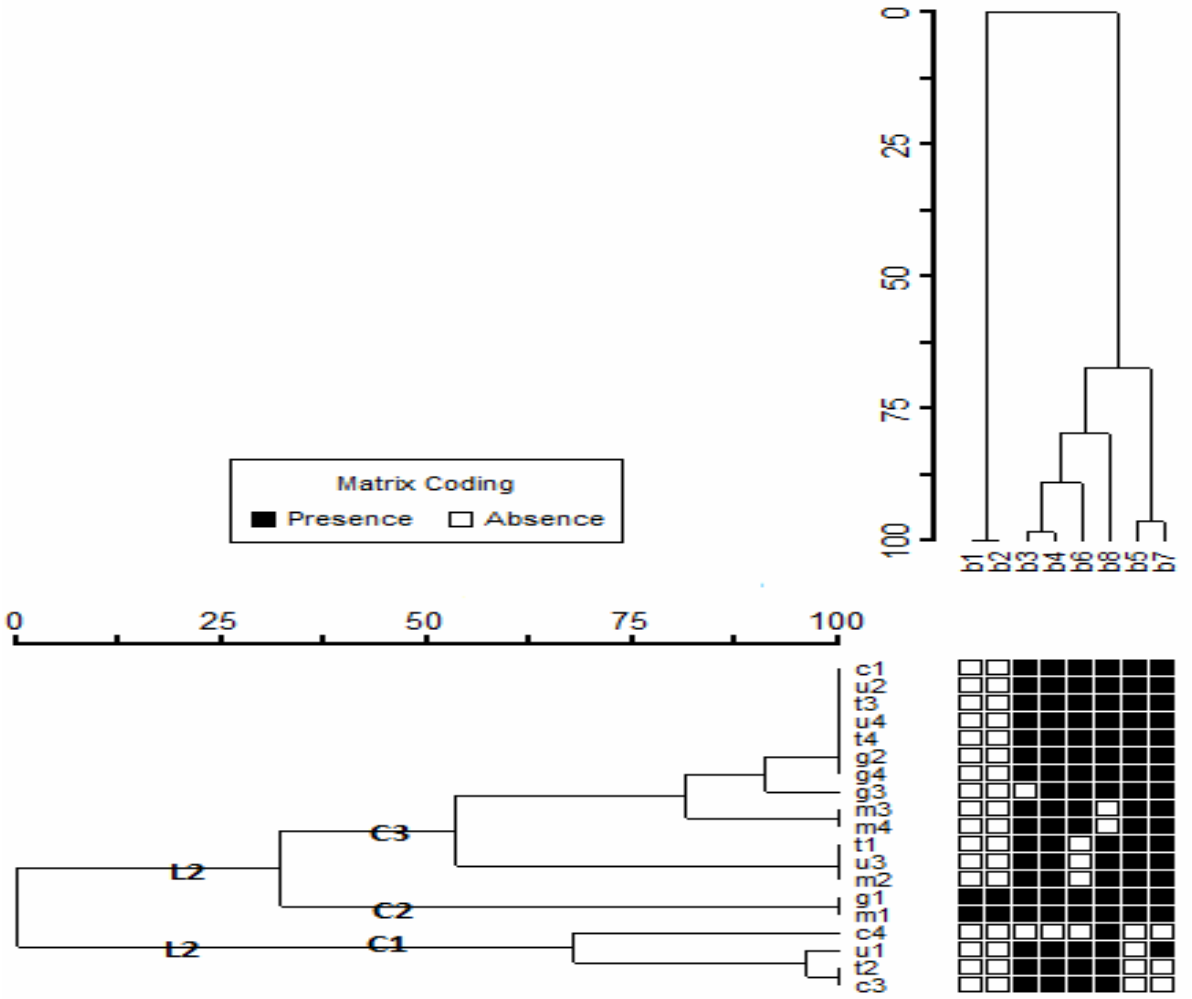


Fig. 2. Proteomic assay of 9 genotype of *R. pseduoacacia* germ plasm based on SDS-PAGE.



A: cluster analysis of 19 among 19 cultivars of *robinia pseudoacacia*. **B**: genetic polymorphism based on protein polypeptide distributed in 19 cultvars of *robinia pseudoacacia*. **C**: zygomorph of 8 bands repoted in *robinia pseudoacacia* seed.

Fig. 3. Two way cluster analysis of molecular traits matrix coding indication the presence and absence of protein band using PCA

Table 1. Binary matrix data profile.

Localities	Band-1	Band-2	Band-3	Band-4	Band-5	Band-6	Band-7	Band-8
Munda1	0	0	1	1	1	1	1	1
Gulabad1	1	1	1	1	1	1	1	1
Maidan1	1	1	1	1	1	1	1	1
Talash1	0	0	1	1	1	0	1	1
Uom3	0	0	1	1	1	0	1	1
Maidan2	0	0	1	1	1	0	1	1
Uom2	0	0	1	1	1	1	1	1
Maidan3	0	0	1	1	1	1	1	0
Talash3	0	0	1	1	1	1	1	1
Uom4	0	0	1	1	1	1	1	1
Talash4	0	0	1	1	1	1	1	1
Gulabad3	0	0	0	1	1	1	1	1
Munda4	0	0	0	0	0	0	0	1
Gulabad2	0	0	1	1	1	1	1	1
Uom1	0	0	1	1	0	1	1	1
Talash2	0	0	1	1	0	1	0	1
Munda3	0	0	1	1	0	1	0	1
Gulabad4	0	0	1	1	1	1	1	1
Maidan4	0	0	1	1	1	1	1	0

Table 2. Genetic disagreement of *R. pseudoacacia.*

Localities	Munda1	Gulabad1	Maidan1	Talash1	UOM3	Maidan2	UOM2	Maidan3	Talash3	UOM4	Talash4	Gulabad3	Munda4	Gulabad2	UOM1	Talash2	Munda3	Gulabad4	Maidan4
Munda1	0.00																		
Gulabad1	0.25	0.00																	
Maidan1	0.25	0.00	0.00																
Talash1	0.13	0.38	0.38	0.00															
UOM3	0.13	0.38	0.38	0.00	0.00														
Maidan2	0.13	0.38	0.38	0.00	0.00	0.00													
UOM2	0.00	0.25	0.25	0.13	0.13	0.13	0.00												
Maidan3	0.13	0.38	0.38	0.25	0.25	0.25	0.13	0.00											
Talash3	0.00	0.25	0.25	0.13	0.13	0.13	0.00	0.13	0.00										
UOM4	0.00	0.25	0.25	0.13	0.13	0.13	0.00	0.13	0.00	0.00									
Talash4	0.00	0.25	0.25	0.13	0.13	0.13	0.00	0.13	0.00	0.00	0.00								
Gulabad3	0.13	0.38	0.38	0.25	0.25	0.25	0.13	0.25	0.13	0.13	0.13	0.00							
Munda4	0.63	0.88	0.88	0.50	0.50	0.50	0.63	0.75	0.63	0.63	0.63	0.50	0.00						
Gulabad2	0.00	0.25	0.25	0.13	0.13	0.13	0.00	0.13	0.00	0.00	0.00	0.13	0.63	0.00					
UOM1	0.13	0.38	0.38	0.25	0.25	0.25	0.13	0.25	0.13	0.13	0.13	0.25	0.50	0.13	0.00				
Talash2	0.25	0.50	0.50	0.38	0.38	0.38	0.25	0.38	0.25	0.25	0.25	0.38	0.38	0.25	0.13	0.00			
Munda3	0.25	0.50	0.50	0.38	0.38	0.38	0.25	0.38	0.25	0.25	0.25	0.38	0.38	0.25	0.13	0.00	0.00		
Gulabad4	0.00	0.25	0.25	0.13	0.13	0.13	0.00	0.13	0.00	0.00	0.00	0.13	0.63	0.00	0.13	0.25	0.25	0.00	
Maidan4	0.13	0.38	0.38	0.25	0.25	0.25	0.13	0.00	0.13	0.13	0.13	0.25	0.75	0.13	0.25	0.38	0.38	0.13	0.00

R. pseduoacacia germ plasm shows from 00% to 88% genetic disagreement

Conclusion

In the present study high degree of gerem plasm protein variation recoded from range 0% to 88%. Thus it is possible to improve the plant and develop more new varieties of *R. pseudoacacia*. It is believed that more diverse plant can survive in environment more easily, because diverse plant adopted in environment. Due to high degree of diversity it is suggested that *R.pseudoacacia* plant can be produce in new varieties.

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