Int. J. Biosci. 2019

INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 1, p. 388-401, 2019

RESEARCH PAPER OPEN ACCESS

Assessing genetic variability index among species of *ACACIA* through morphological and biochemical markers

Sundas Wisal[1], Arshad Khan[1], Karishma Gul[1], Waqar Khan[2], Gul Nigar[4], Murad Ali[3*]

[1] *Department of Botany University of Malakand, KP, Pakistan*

[2] *Nanjing Agriculture University, China*

[3] *Graduate school of Chinese Academy of Agriculture Sciences, China*

[4] *Department of Pharmacy University of Peshawar, KP, Pakistan*

Key words: Fabaceae, Genetic diversity, Morphological, Biochemical characterization.

http://dx.doi.org/10.12692/ijb/14.1.388-401 Article published on January 26, 20199

Abstract

The present research work was conducted to explore and estimate the intra and inter specific genetic variability amongfour species of Family Fabaceae. The method was used morphometric and protein profiling. The four species include *Robina pseudoaccacia*, Accacia modesta, *Vachellia fernesiana* and *Leuceana Leucocephalla* (Genus Leuceana). The twenty genotypes of each four species were collected from different zones of Dir and swat. In *Robina pseudoaccacia* the maximum coefficient of variation (CV %) showed byInternodes length (32.333%) and minimum by pod width (10.181%). *Accacia modesta* maximum coefficient of variation was noted in 100 seed weight (28.957%) and minimum in seed length (14.591%) and in *Accacia Leuceana* the maximum coefficient of variation (CV%) was noted in internodes length (22.959%) and minimum in seed width (7.553%). The comparative variability index of four species shows that, *Robina pseudoaccacia* have highest coefficient of variation (20.157%) fallowed by Accacia modesta (18.467%) and *Vachellia fernesiana* (17.922%) while *Leuceana leucocephalla* had the lowest coefficient of variation (16.07%). Seed storage proteins were detected through SDS-PAGE. In *Accacia modesta* variation (2.3%) was noted, in Loci B8 (0.45 %) followed by B4, B3 and B2 show (0.05%). In *Vachellia fernesiana* high level of genetic variability (6.25%) was noted in Loci B11 (0.85%) followed by B3(0.15%). In *Robina pseudoaccacia* high level of genetic diversity (2.8%) was found in B7 (0.60%) fallowed B3 (0.10%). In *Leuceana leucocephalla* the high level of genetic diversity (1.55%) was found inB4(0.45%) followed by B8 (0.25%).

* **Corresponding Author:** Murad Ali ✉ muradsahil77@gmail.com

Introduction

Family Fabaceae (Leguminosae) includes nearly 697 genera and 3200 species (Polhil l*et al.*, 1981). It is a cosmopolitan family but absent in Antarctica.Many genera are widely distributed in major land biomes,dry to rainy tropical coastal and grassland. It is third biggest family of pheneurogames (flowering plant) and second to the Poaceae in economic importance. Many species in this family are cultured for food, such as peas, beans, peanuts and soyabean (Heywood *et al.*, 2007).

The legumes have strange flower structure,fruit is podded and having nodules with rhizobia (Faria *et al.,* 1989). Fabaceae is an essential economic family having three subfamilies: Mimosoideae, Caesalpinioideae and Faboideae (Takhtajan, 2009). It is generally recognized as pea family, pulse family, bean family and legume family. Sub-family Mimosoideae includes around 79 genera spread throughout warm temperate, tropical and subtropical areas of the world. Mimosoideae is one of the three commonly known subfamilies of Leguminosae (Elias, 1981).The genus Leuceana belongs to family Mimosoideae (sub-family of Fabaceae). It contains around 32 species;that are scattered in most of the tropical areas of the world. (Feria *et al.,* 2011).

It is generally famous as, lead tree, white lead-tree, leuceana, horse tamarind, wildtamarind, white popinac in English and lamtoro,koo babul, subabul,ku-babul in Hindi. Other vernacular names consist of kladingan, ipilipil, palo-maria, kariskis, faux mimosa, lamtoro,tagaraikrathin andto-baoetc (Malik *et al.* 2004). *Vachellia fernesiana,*also called as *Acacia fernesiana*and earliar Mimosa fernesiana,usually famous as sweet acacia,huisache or also needle bush due to various thorns spread beside on its branches.Its origin is Central America and Mexico(Clarke *et al.,* 1989).

The presently known scientific name of the black locust is *Robinapseudoaccacia L.* Extra vernacular names: Locust, False acacia. The genus *Robina* belonging to the family Fabaceae which comprises about 20 deciduous species which live in warm and periodically dry areas of North America and Mexico. The black locust is innate in the inner part of Eastern North America.

The black locust is a tree species which is long living, some representatives can live 200-250 years 1 and the thickness of stem of the old plants approximately 1 meter (Bartha *et al.,*2006).*Acacia modesta* is the member of Mimosaceae which is the sub-family of Fabaceae. Usually it is known as phulai and locally it calledpalosa. It have its distribution in India, Pakistan and Afghanistan. In Pakistan it is present in Khyber Pakhtunkhwa, Punjab and Balochistan(Hook F. 1878 and Ali S.I. 1973).

The *A. modesta*wood is durable and strong and is used to manufacture Persian wheels, cane crushers and agricultural tools and also for the purpose of fuel(Hussain *et al.,*2006). Morphology of seeds of many mimosoid taxa has been treated variously in many studies among them: Manning & Van Staden (1987), Al-Gohary& Mohamed (2007) and Karakish *et al.* (2013). In resolving taxonomic and phylogenetic problems, electrophoresis process is used which become an additional tool applied (George *et al.,* 2001;Burghardt&Espert, 2007).

The determination of genetic diversity among different crop population was done by using different techniques such as SDS_PAGE, DNA marker analysis or morphological characterization (Ghafoor *et al.,* 2008: Nisar *et al.,* 2008).In the few decades researchers estimated genetic assortment in numerous crop species from morphological characters (Nisar *et al.,* 2016). But researchers now believe that evaluation methods through biochemical markers have many benefits over the traditional morphology in genetic diversity (Nidaye *et al.*, 2011). In protein profiling SDS-PAGE are used due to its effortlessness and independence of environmental features it is one of most commonly used biochemical methods to scrutinize the genetic construction of harvest plants (Ghafoor *et al.,* 2005). Many researchers appreciate electrophoresis analysis of seed storage protein revealed SDS-PAGE because it provide evidence for intraspecific and interspecific relationship of variation (Hameed *et al.,* 2009). When the genetic variability is high the chances of improvement in crop will be greater (Daniele *et al.,* 2015). Aims of the study to investigate the genetic divergence among the four species of acacia, and comparison the specie to evaluate the specie with better our environment.

Method and materials

Study duration and area

This study was carried out during November 2017 in Laboratory of Botany University of Malakand, Khyber Pakhtunkhwa, Pakistan.

Morphological characterization

During the present research, for estimation of intraspecific and interspecific diversity among four species of Family Fabaceae are search and assessed on the base of morphometric and SDS PAGE.

The four species in which *Robina pseudoaccacia*(genus Robina) belong to sub-family Faboidecae, and the remaining three species belong to sub- family Mimosoideae, *Accacia modesta* (Genus Accacia), *Vachellia fenesiana* (*Accacia fernesiana*_Genus Vachellia) and *Leuceana Leucocephalla* (Genus Leuceana).

The 20 varieties of each were collected from various parts of district Dir (lower, upper) and swat.The morphometric traits which are noted in the study is divided into 2 categories four(4)(a) Qualitative traits including Leaflet/leaf colour,Leaflet Arrangement, Seed Shape and Seed Colour and b)(9) Quantitative traits.Pod length, Pod widthLeaflet length, Leaflet width, Internode length, Seed per legume, Seed length,Seed width and 100 Seed weight.

Biochemical Analysis/ SDS-PAGE Characterization

Healthy and mature seeds of 20 varieties of each 4 species were used, separately, for the biochemical analysis of protein profiling. For extraction of proteins and SDS-PAGE analysis the following procedure were applied. To find out the level of genetic diversity at Sodium-dodecyl-sulphate poly-acrylamid-gel-electrophoresis (SDS-PAGE) was done. First of all seeds were dried to remove the moisture for better result. Seeds of each species were grinded into fine-powder using conventional method by crushing through pestle and mortar and 0.01 mgof seed flour taken in 1.5 ml Eppendorf tubes. Then added Protein extraction buffer (400 micro litters)(0.2 % SDS, 0.5 ml, Tris-HCL, 5 m Urea, 1 % B-mercaptoethanol, BPB and pH 8.0,), with the help of micropipette to the Eppendrof tube and vortexed for approximately one minute thoroughly to homogenize. After the addition of PEB the samples were centrifuged at 14000rpm for 40min under 40 centigrade then it store in incubator for 3 to 4 hours approximately and after incubation again it were centrifuged at 14000rpm for 20 minute at 40 centigrade. To isolate protein on the base of molecular weight, 12% PAGE separation gel (0.4 % SDS , 3.0 M Tris-HCL, and pH 8.8) was optimized for data recording of protein banding pattern and 4.5% stacking gel (0.4% SDS, 0.493 M Tris-HCl pH 7.0) used for the gel electrophoresis process. Then put 12to 20µl of each sample into each well (left to right). Put the gels in the electrode buffer solution (0.125% SDS, 129 M Glycine, 0.025 M Tris) and Device was attached with continuous electric supply (120 V)until the bromophenol blue (BPB) reached to the bottom of gel plate.

Then the gels were placed in the staining solution (containing 50% distal water,10% glacial acetic acid, 0.2% Commassie Brilliant Blue, 40% methanol with the ratio Of 4:1:5)to stain the protein bands for 30 minutes. After staining process Gels were kept in de-staining solution (5% acetic acid 20% methanol and 75% distal water with ratio of (1:4:15) to de-stained the non-proteinous portion at overnight. Data were evaluated using “0” for absence of protein band and “1” for the presence of protein band. Principal component analysis and cluster analysis was also done for 0, 1data analysis done by using software PCORD. Descriptive statistics and correlation analysis was perform through Microsoft excel 2010.

Results and discussion

Qualitative traits

Frequency distribution for Qualitative agro-morphological traits including Leaflet Arrangement, Leaflet/leaf Colour, Seed Shape and Seed Colour. Among the qualitative traits in the *Robina pseudoaccacia* the green colour of leaf and brown colour of seed was dominant (50%) and seeds shape show no variation. (45%) leaflet is alternately arranged and (55%) were opposite arranged (Table 1).

Table 1. Frequency distribution of 4 qualitative morphological traits of the 20(*Robina pseudo acacia*) studied landraces.

Characters	Categories	Frequency	Percentage
Leaflet Arrangement	Alternate	9	45%
	Opposite	11	55%
Leaf Colour	Green	10	50%
	Light Green	4	20%
	Yellow Green	6	30%
Seed Colour	Brown	10	50%
	Black	7	35%
	Grey	3	15%
Seed Shape	kidney shape	20	100%

Table 2. Frequency distribution of 4 qualitative morphological traits of the 20(*Accacia modesta*) studied landraces.

Characters	Categories	Frequency	Percentage
Leaflet Arrangement	Opposite	20	100
Leaf Colour	Green	12	60
	Light Green	8	40
Seed Colour	Grey		
	Brown	12	60
Seed Shape	Ovule	20	100

In *acacia modesta* the leaf colour green was dominant (60%) and seed colour brown was dominant (60%) and all seeds were of ovule shape (100%) and leaflet show no variation (Table 2). The leaf color of *leuceana leucocephalla* dark green was dominant (65%) and all seed were of dark brown and flat ovule shaped (100%) all leaflet were oppositely arranged (Table3). In *acacia fernesiana* (*Vachellia fernesiana*) green and dark green leaf colour were of equal ratio (50:50) and all seeds were light brown and round ovule shaped (100%) and leaflet were oppositely arranged (100%). The seed color of *Accacia modesta* brown color is (60%) dominant while in *Robina pseudoaccacia* brown color is (50%) dominant, comparing with the seed color of *acacia fernesiana*, the seed color of *fernesiana* specie is (100%) light brown while the *Leuceana leucocephalla* the seed color is (100%) dark brown (Table 1, 2, 3, and 4).

Statistical analysis

Statistical analysis shows significant variation in all calculated characters in *Robina pseudoaccacia* the maximum coefficient of variation (CV%)shown by Internodes length (32.333%) with mean value (2.03) standard deviation (0.658) and sample variance (

0.433) and minimum coefficient showed by pod width (10.181%) mean value (1.310) standard deviation (0.133) and sample variance (0.018) (Table 5).*Accacia modesta* maximum coefficient of variation was noted in 100 seed weight (28.957%) with mean value (6.275),standard deviation (1.817) and sample variance (3.302) and minimum in seed length (14.591%) with mean value (0.82) standard deviation (0.120) and sample variance (0.014) (Table 6).

Table 3. Frequency distribution of 4 qualitative morphological traits of the 20(*Leuceana leucocephala*) studied landraces.

Characters	Categories	Frequency	Percentage
Leaflet Arrangement	Opposite	20	100
Leaf Colour	Green	12	60
	Light Green	8	40
Seed Colour	Grey	8	40
	Brown	12	60
Seed Shape	Ovule	20	100

Table 4. Frequency distribution of 4 qualitative morphological traits of the 20 *Vachellia fernesiana* studied landraces.

Characters	Categories	Frequency	Percentage
Leaflet Arrangement	Opposite	20	100
Leaf Color	Green	7	35
	Dark Green	13	65
Seed Colour	Dark Brown	20	100
Seed Shape	Flate Ovule	20	100

Table 5. Descriptive statistics of 10 quantitative Traits of 20 (*Robina pseudoaccacia)* landraces.

TRAITS	Mean	Standard Error	Standard Deviation	Sample Variance	Range		CV%
					Minimum	Maximum	
PL	0.410	0.024	0.107	0.011	0.200	0.600	26.126
LL	4.635	0.244	1.090	1.188	2.700	6.200	23.512
LW	2.190	0.112	0.503	0.253	1.300	3.300	22.946
IL	2.035	0.147	0.658	0.433	1.000	3.600	32.333
PL	9.285	0.347	1.553	2.412	5.800	11.300	16.726
PW	1.310	0.030	0.133	0.018	1.000	1.500	10.181
S/L	11.000	0.620	2.772	7.684	5.000	15.000	25.200
SL	0.490	0.019	0.085	0.007	0.400	0.800	17.393
SW	0.275	0.010	0.044	0.002	0.200	0.300	16.155
100W	1.253	0.048	0.213	0.045	0.810	1.610	16.966

In *Leuceana leucocephalla* the maximum coefficient of variation (CV%) was noted in internode length (22.959%) with mean value (5) standard deviation (1.148) and sample variance (1.318) and minimum in seed width (7.553%) with mean value (0.48) standard deviation (0.037) and sample variance (0.001) (Table 7). In *Accacia fernesiana, (Vachellia fernesiana)* like maximum coefficient of variation (CV%) shown by 100 seed weight (28.564%) with mean value (7.85) standard deviation (2.243) and sample variance (5.030) and minimum by leaflet width (1.42%)with mean value (0.1)standard deviation (0.044) and sample variance (0.002) (Table 8).

Table 6. Descriptive statistics of 9 quantitative Traits of 20 (*Accacia modesta)* landraces.

Traits	Mean	Standard Error	Standard Deviation	Sample Variance	Minimum	Maximum	CV%
LL	0.64	0.026	0.114	0.013	0.4	1	17.851
LW	0.42	0.019	0.083	0.007	0.2	0.6	19.845
IL	1.845	0.073	0.328	0.108	1.3	2.6	17.801
PL	6.615	0.243	1.086	1.180	4.8	8.4	16.423
PW	1.265	0.052	0.235	0.055	0.5	1.6	18.544
S/LE	5.55	0.185	0.826	0.682	4	7	14.875
SL	0.82	0.027	0.120	0.014	0.6	1.2	14.591
SW	0.66	0.026	0.114	0.013	0.5	1	17.310
100W	6.2753	0.406	1.817	3.302	0.546	8.37	28.957

Table 7. Descriptive statistics of 9 quantitative Traits of 20 20 (*Leuceana leucocephala).*

TRAITS	Mean	Standard Error	Standard Deviation	SampleVariance	Minimum	Maximum	CV%
LL	1.4	0.055	0.245	0.06	1	1.8	17.49636
LW	0.41	0.020	0.091	0.008	0.2	0.5	22.2417
IL	5	0.257	1.148	1.318	2.5	7	22.95992
PL	21.085	0.888	3.973	15.782	7	25.8	18.84139
PW	2.11	0.071	0.319	0.102	1.5	2.5	15.13623
S/L	23.9	0.628	2.808	7.884	19	28	11.74847
SL	0.755	0.018	0.083	0.007	0.6	0.9	10.93481
SW	0.485	0.008	0.037	0.001	0.4	0.5	7.553558
100W	4.86	0.193	0.865	0.748	3.53	6.26	17.79756

Table 8. Descriptive statistics of 9 quantitative Traits of 20 (*Vachellia fernesiana*) landraces.

Traits	Mean	Standard Error	Standard Deviation	Sample Variance	Range		CV%
					Minimum	Maximum	
LL	0.46	0.013	0.060	0.004	0.4	0.6	13.005
LW	0.125	0.010	0.044	0.002	0.1	0.2	35.541
IL	2.525	0.110	0.490	0.240	1.8	3.3	19.397
PL	6.56	0.195	0.874	0.764	5	8.5	13.321
PW	1.065	0.034	0.153	0.023	0.8	1.5	14.378
SLE	17.7	1.054	4.714	22.221	7	25	26.632
SL	0.655	0.037	0.164	0.027	0.5	1.1	25.001
SW	0.51	0.024	0.107	0.011	0.4	0.7	21.003
100W	7.8515	0.501	2.243	5.030	4.07	12.76	28.565

The comparative variability index of Accacia genus shows that , *Robina pseudoaccacia* have highest coefficient of variation (20.157%) with mean value (3.608) fallowed by *Accacia modesta* (18.467%) with mean value (2.676) and *Accacia fernesiana* (17.922%) with mean value (4.15) and *Accacia angustimita* had the lowest coefficient of variation (16.07%) with mean value (6.66) (Table 5, 6, 7 and 8).

Variability among the species

The variation among the four specie of family Acacia on the basis of morphological traits are found among the species of Acacia in case of leaflet length, leaf width, pod length,seed width pod width, seed per legume, seed length, 100 seed weightand internodes length.

Table 9. Correlation among the 9 quantitative traits of 20 landraces of (*Robina pseudoaccaci)a*.

Traits	PL	LL	LW	IN	PL	PW	S/L	SL	SW	100W
PL	1									
LL	.466*	1								
LW	.520*	.777**	1							
IL	0.137	0.014	0.388	1						
PL	0.387	0.044	-0.179	-0.171	1					
PW	0.066	0.251	0.002	-.568**	0.227	1				
S/L	0.443	0.174	0.068	0.101	.661**	-0.085	1			
SL	0.012	0.1	0.071	-0.069	0.277	-0.222	0.245	1		
SW	0.277	0.084	0.035	0.266	0.177	-0.311	0.299	.487*	1	
100 W	.625**	0.387	0.262	0.061	0.167	0.105	0.024	0.054	0.382	1

Table 10. Correlation among the 9 quantitative traits of 20 landraces of (*Accacia modesta*).

Traits	LL	LW	IL	PL	PW	S/L	SL	SW	100W
LL	1								
LW	.851**	1							
IL	-0.191	-0.035	1						
PL	-0.031	0.124	-0.07	1					
PW	0.114	0.226	0.056	0.341	1				
S/L	-0.301	-0.015	0.234	.788**	0.349	1			
SL	0.092	-0.201	-0.158	0.087	0.251	0.043	1		
SW	0.129	-0.133	-0.02	0.052	-0.212	-0.033	.755**	1	
100W	0.11	-0.133	-0.024	0.229	-0.041	0.064	0.273	0.349	1

In *Robina pseudoaccacia* the quantitative traits are categories through average mean value of traits which are; the petiole length mean value is 0.48, leaf length 4.635, leaf width 2.19, internodes length 2.035,pod length 9.285, pod width 1.31, seed/legume 11, seed length 0.49, seed width 0.275 and 100 seed weight mean value is 1.253.In *Acacia modesta* the quantitative traits are categorized through average mean value of traits which are; petiole length mean value is 0.2; leaf length 0.64, leaf width 0.42, internodes length1.845, pod length 6.615, pod width 1.265, seed/legume 5.55, seed length 0.82, seed width 0.66, and 100 seeds mean value is 6.2753. In *Leuceana leucocephalla* the quantitative traits are categorized through average mean value of traits which are; petiole length mean value is 0.655, leaf length 1.4, leaf width 0.41,internode length 5, pod length 21.085, pod width 2.11, seed /legume 23.9, seed length 0.755, seed width 0.485, and 100 seeds weight mean value is 4.86.

In *Vachellia fernesiana* the quantitative traits are categories through average values of the traits which is; the petioles mean value are 0.1, leaf length 0.46, leaf width 0.1, internodes length 2.525, pod length 6.56, pod width 1.065, seed/legume 17.7,seed length 0.655,seed width 0.51, and 100 seeds weight mean value are 7.8515. (Table 5, 6, 7 and 8).

Correlation analysis

Correlation analysis of 20 landraces of *Robina pseudoaccacia* was done for 10 quantitative traits by

SPSS 16.0. Total observed correlation coefficient values were 55, in which 48 were positive and 7 were negative. The correlation of leaflet length was significant positive correlated to petiole length (0.466) while strongly positive correlated with leaflet width (0.777**).Leaf width was significant positive correlated with petiole length (0.520*) while negative correlated to pod length (-0.179).

Table 11. Correlation among the 9 quantitative traits of 20 landraces of (*Leuceana leucocephalla)*.

Traits	LL	LW	IL	PL	PW	S/L	SL	SW	100W
LL	1								
LW	.730**	1							
IL	0.208	0.015	1						
PL	-0.081	-0.072	-0.114	1					
PW	-0.087	-0.058	-0.323	.729**	1				
S/L	0.138	0.004	-0.183	0.341	0.318	1			
SL	-0.052	0.133	-0.4	0.126	0.178	0.366	1		
SW	-0.411	-0.425	-0.15	.678**	.508*	0.292	.461*	1	
100W	-0.191	0.135	-0.343	0.279	.535*	0.01	0.38	0.218	1

Table 12. Correlation analysis of 20 landraces of (*Vachellia fernesiana)*.

Parameter	LL	LW	IL	PL	PW	SLE	SL	SW	100W
LW	0.396	1							
IL	-.503*	-0.369	1						
PL	-0.213	-0.203	0.236	1					
PW	0.414	-0.097	-0.121	-0.054	1				
SLE	-0.101	-0.088	-0.031	0.415	0.123	1			
SL	.505*	0.307	-0.116	-0.16	.627**	-0.012	1		
SW	0.394	-0.055	0.035	-0.018	0.44	-0.379	.447*	1	
100W	0.096	-0.118	-0.327	-0.026	-0.055	-0.151	-0.142	-0.116	1

NOTE: LL =Leaflet length, LW=Leaflet width, IL=Internodes Length, PL=Pod length, PW=Pod width, S/L=Seed per legume, SL=Seed length, SW=Seed width, 100 W=100 Seeds Weight.

Table 13. Total Genetic diversity present in seven (7) bands of 20 genotypes of (*Robina pseudoaccacia)*.

Bands	F	p%	A%	TGD%
B1	10	50	50	0.50
B2	13	65	35	0.35
B3	18	90	10	0.10
B4	10	50	50	0.50
B5	10	50	50	0.50
B6	15	75	25	0.25
B7	8	40	60	0.60

Internodes length was strongly negative correlated to pod width (-0.568**) while seed per legume was strongly positive correlated to pod length (0.661**).seed length is positive correlated to seed width (0.487*) and 100 seed weight was strongly positive correlated to petiole length(0.625**) (Table 9)Correlation analysis of 20 landraces of *Accacia Modesta* was done for 9 quantitative traits by SPSS 16.0. Total observed correlation coefficient values were 45, in which30 were positive and 15 were negative. Leaf length was strongly positive correlated with leaf width (0.851**) while negative correlated with internodes length (-0.191), pod length (-0.031) and seed per legume (-0.301) and positive correlated with pod width (0.114), seed length (0.092),seed width (0.129) and 100 seed weight (0.11).

Pod length was strongly positive correlated with seed per legume (0.788**) and Seed length was also strongly positive correlated with seed width (0.755**) (Table 10).

Table 14. Total Genetic diversity present in eight (8) bands of 20 genotypes of Acacia (*modesta*).

Bands	F	P%	A%	GD%
B-1	20	100	0	0
B-2	19	95	5	0.05
B-3	19	95	5	0.05
B-4	10	50	50	0.5
B-5	17	85	15	0.15
B-6	20	100	0	0
B-7	13	65	35	0.35
B-8	11	55	45	0.45

Table 15. Total Genetic diversity present in eight (8) bands of 20 genotypes of (*Leuceana leucocephala*).

Bands	f	P%	A%	TGD%
B1	15	75	25	0.25
B2	13	65	35	0.35
B3	14	70	30	0.3
B4	11	55	45	0.45
B5	13	65	35	0.35
B6	15	75	25	0.25
B7	18	90	10	0.1
B8	15	75	25	0.25

Table 16. Total Genetic diversity present in eleven (11) bandsof 20 genotypes of (*Vachelia fernesiana*).

Bands	F	P%	A%	GD%
B-1	10	50	50	0.5
B-2	5	25	75	0.75
B-3	17	85	15	0.15
B-4	13	65	35	0.35
B-5	11	55	45	0.45
B-6	10	50	50	0.5
B-7	7	35	65	0.65
B-8	8	40	60	0.6
B-9	6	30	70	0.7
B-10	5	25	75	0.75

Correlation analysis of 20 landraces of *Leuceana leucocephalla* was done for 9 quantitative traits by SPSS 16.0. Total observed correlation coefficient values were 45, in which 31were positive and 14 were negative.Leaf length was strongly positive correlated with leaf width(.851**) while negative correlated with pod length (-0.081), pod width (-0.087), seed length (-0.052), seed width (-0.411) and 100 seed weight(-0.191) and positive correlated with internodes length (0.208) and seed per legume (0.138).Pod length was significant positive correlated with pod width (.729**) and seed width (678**) and positive correlated with seed per legume (0.341),seed length (0.126) and 100 seed weight (0.279).Pod width was significant positive correlated with seed width (.508*) and 100 seed weight (.535*) .Seed length was significant positive correlated with seed width (.461*) and positive correlated with100seed weight(0.38) (Table 11).

Correlation analysis of 20 landraces of *Vachellia fernesiana* was done for 9 quantitative traits by SPSS 16.0. Total observed correlation coefficient values were 45, in which 22 were positive and 23 were negative. Leaflet length was significant negative correlated with internodes length (-.503*) while significant positive correlated with seed length (.505*).Seed length highly positive correlated with pod width (.627*) and seed length was significant positive correlated with seed width (.447*) (Table 12).

The present panel was closely related to, Legumes, broadly defined by their unusual flower structure, podded fruit, and the ability of 88% of the species examined to date to form nodules with rhizobia (Faria *et al.,* 1989), are second only to the Graminiae in their importance to humans. Economically Fabaceae (or Leguminosae) is an important family having three subfamilies: Mimosoideae (actinomorphic flower with valvate arrangement of petals), Caesalpinioideae (zygomorphic flower with imbricate arrangement of petals) and Faboideaeor Papilionoideae, zygomorphic flower with papilionaceous corolla) (Takhtajan, 2009).).

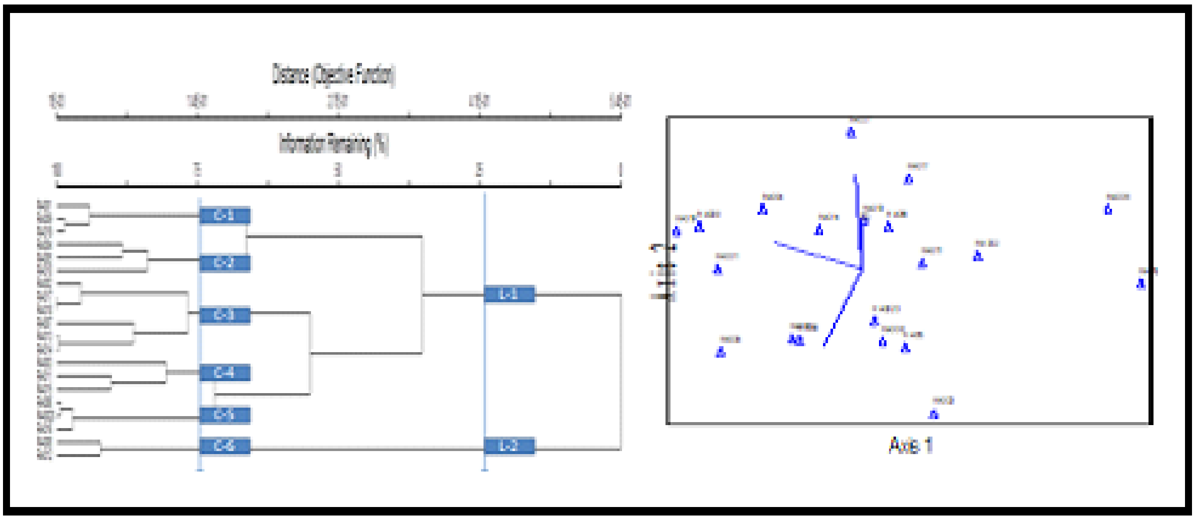

Fig. 1. Dendogram and PCA of 40 genotype Robina pseudoaccacia used PCORD.

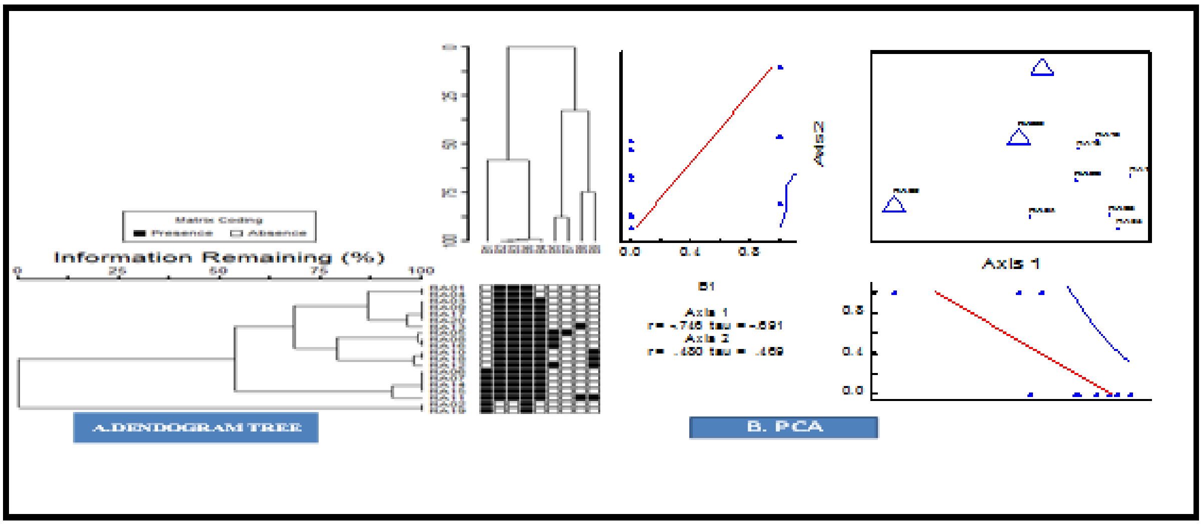

Fig. 2. A. SDS PAGE dendogram tree and B. PCA (PCORD) for bands of 20 landraces of *Robina pseudoaccacia*.

Another member of family Fabaceae is L-leucocephalla belong to the Sub-family Minosoideae.

It has a non-protein, amino acid, named mimosine in their leaves and foliage, usually toxic to other trees as they inhibits their growth, but not its own seedlings (Ferguson and Rathinasabapathi, 2009).

Comparison on the base of SDS-PAGE

To report questions about the biology of Acacia, such as identification and relationships among Acacia species, botanists have revealed interest in using molecular techniques from the last few years (Harrier *et al.,* 1996; Byrne *et al.,* 2001), and also in conservation and population genetic structure

(*Shrestha et al., 2002*). To find out the relationship among germplasm and its genetic diversity or identification of desirable characters of the germplasm, different methods are used such as morphological characterization and biochemical markers at protein level (Nisar *et al.,* 2009;Shah *et al.,* 2011; Akbar *et al.,* 2012). The determination of genetic diversity among different crop population was done by using different techniques such as SDS-PAGE analysis, DNA marker analysis and morphological characterization (Nisar *et al.,* 2008; Ghafoor*et al.,* 2008; murad*et al.,* 2017).

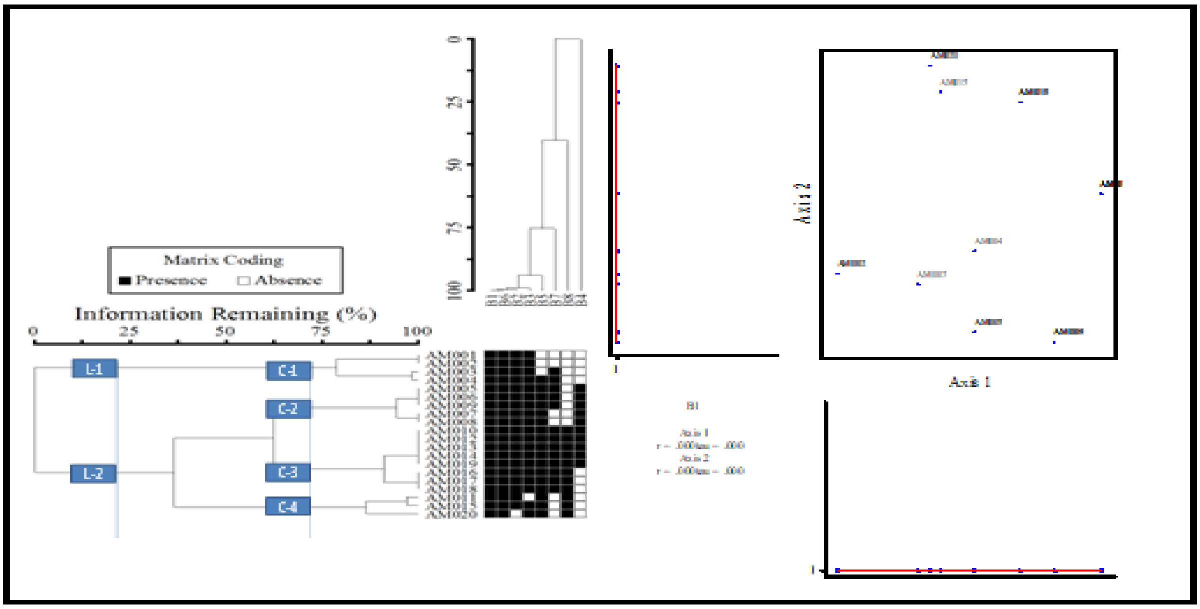

Fig. 3. SDS PAGE dendogram tree for bands of 20 landraces of *Accacia modesta.*

On the basis of variation present in their protein profiling, the 20 landraces of *Robina pseudoaccacia* divided into multiple clusters (Fig. 2). The dendogram tree delineated landraces into two lineages i-e L-I and L-II and 4 clusters. The lineage 1 consists of 3 clustres and lineage 2 consists of 1 cluster.

The cluster 1 in lineage 1 composed of 7 landraces such as (RA001, RA004, RA003 and AM009 RA0017, RA0020 and RA 0013). And cluster 2 of lineage 1 consist of 6 landraces (RA005, RA008, RA0016, RA0010 and RA0018 and RA 0012), cluster 3 had 5 landraces (RA006, RA007, RA014, RA015 and RA011) and cluster 4 had 2 landraces (RA002 and RA019). The high level of genetic diversity was found in B7 (0.60%) fallowed by B1,B4 and B5 (0.50%) B2(0.35%) B6 (0.25%) And B3 (0.10%) (Table13).

In the present research work, for SDS-PAGE analysis all the 20 landraces of *Accacia modesta* were selected. The information collected about seed storage protein from the protein bands in gels, shows in clustersusing PCORD. In *Accacia modesta* the high level of variation was noted in B4 (0.5 %) followed by, B8 (0.45 %), B7 (0.35 %), B5 (0.15 %), B2 and B3(0.05%) and B1 and B6 have no variation (0%).

On the basis of variation present in their protein profiling the 20 landraces of *Accacia modest*a divided into multiple clusters (Fig. 3). The dendogram tree delineated landraces into two lineages i-e L-I and L-II and 4 clusters. The lineage 1 consists of 1 cluster and lineage 2 consists of 3 clusters. The cluster 1 in lineage 1 composed of 4 landraces such as (AM001, AM002, AM003 and AM004).And cluster 2 of lineage 2 consist of 5 landraces (AM005,AM006,AM009, AM007 and AM008), cluster 3 had 8 landraces (AM008, AM010, AM012, AM013, AM014, AM019, AM016, AM017 and AM018) and cluster 4 had 3 landraces (AM011,AM015 and AM020) (Table 14).

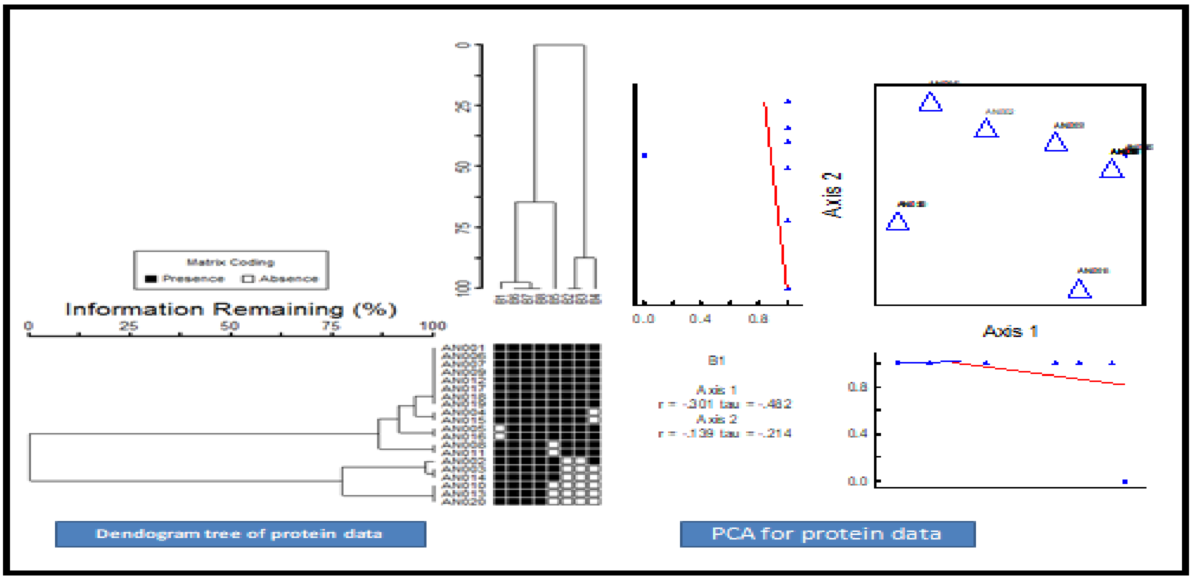

Fig. 4. SDS PAGE dendogram tree and PCA for bands of 20 landraces of *Leuceana leucocephala*.

In the present research work, for SDS-PAGE analysis, all the 20 landraces of (*Leuceana leucocephalla*) were selected. The information collected about seed storage from the protein bands in gels was shows to cluster analysis on the basis of presence and absence of seed proteins using PCORD. On the basis of variation present in seed storage protein the 20 landraces of *leuceana leucocephalla* divided into multiple clusters (Fig. 4).

It was detected that dendogram delineated landraces into two linkagesi-e L-I and L-II and 2 clusters. The lineage 1 consists of 1clustre and lineage 2 also consists of 1 cluster.

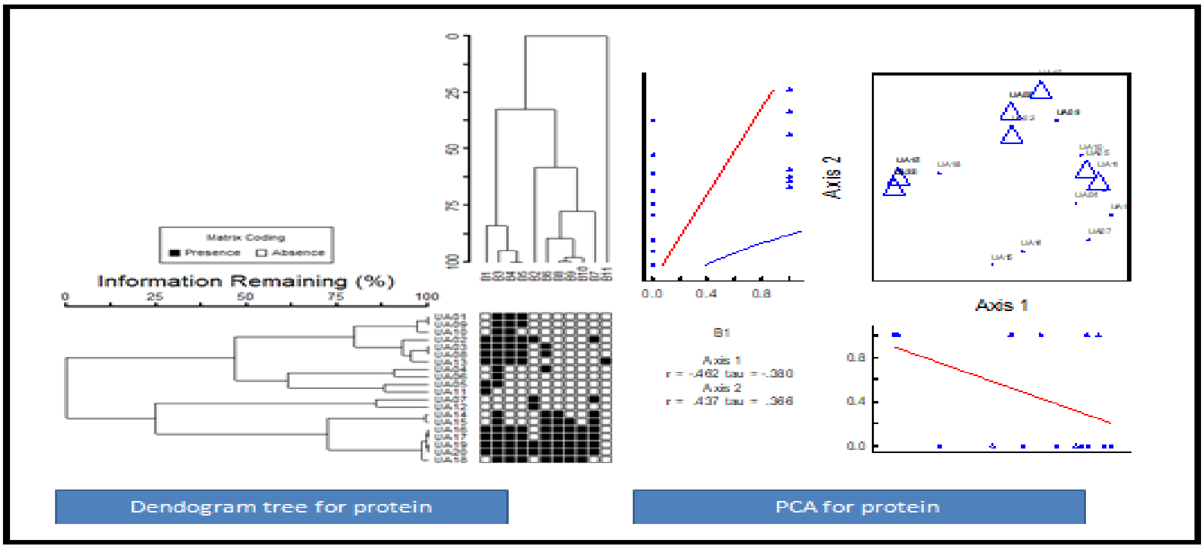

Fig. 5. SDS PAGE dendogram tree PCA for protein bands data of *Vachellia fernesian*.

The cluster 1 in lineage 1 composed of 14 landraces such as (AN001, AN006, AN007 and AN009 AN0012, AN0017 AN18, AN0019AN004,AN0015,AN005, AN0016, AN0018 and AN0011).And cluster 2 of lineage 1 consist of 6 landraces (AN002,AN003,AN0014, AN0010 and AN0013 and AN0020).In *Leuceana leucocephalla* the high level of genetic diversity was found in B4(0.45%) followed by B2 and B5 (0.35%),B1,B6 and B8 (0.25%),B3 (0.3%) and B7 (0.1%) (Table 15 and Fig.4).SDS PAGE was used to find out of the genetic divergence existed in the landraces**.** It was found that genotypes shown significant level of genetic divergence on the basis of seed storage protein.In the present research work, for SDS-PAGE analysis for seed storage proteins profile all the 20 landraces of *Vachellia fernesiana* were selected. Information collected about seed storage protein from the protein bands in gels using PCORDwas shows to cluster analysis.In *Vachellia fernesiana* the high level of genetic diversity was noted in B11 (0.85%) followed B2 and B10 (0.75%) respectively and followed B9 (0.7%), B7 (0.65%), B8 (0.6%), B1 and B6 (0.5%),B5(0.45%),B4(0.35%) and B3(0.15%).

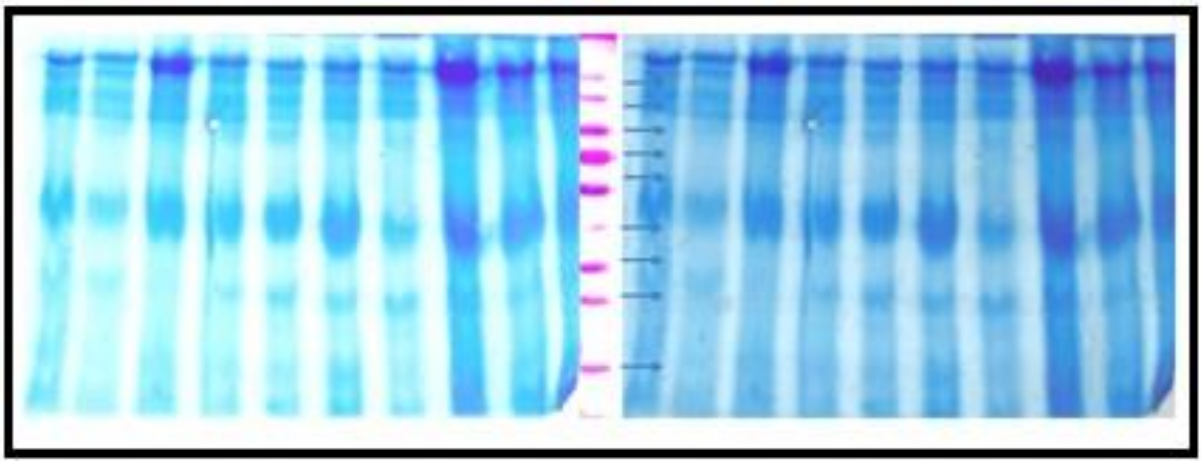

Fig. 6. The gel show the protein bands pattern and their existing, of landraces of Acaciaspecies.

On the basis of variation present in their protein profile the 20 landraces of *Vachellia fernesiana* delineated into multiple clusters (Fig. 5). It was noted that dendogram divided landraces into two linkages i-e L-I and L-II and 6 clusters. The lineage 1 consists of 3 clusters and lineage 2 also consists of 3 clusters. The cluster 1 in lineage 1 composed of 7 landraces such as (AF001, AF009, AF0010 and AF002, AF003, AF008 and AF0013) and cluster 2 in lineage 1 consist of 2 landraces (AF004 and AF006)and cluster 3 also had 2 landraces(AF005 and AF0011).While cluster 4 of lineage 2 had also 2 landraces (AF007 and AF0012).Cluster 5 had 2 landraces (AF0014 and AF0015) and cluster 6 had 5 landraces (AF0016, AF0017, AF0019, AF0020 and AF0018) (Fig. 5 and Table 16).

The results of the present study are closely related with that of (Zahoor*et al.,* 2015) who estimated the genetic diversity in 19 varieties of *Robina pseudoaccacia* through SDS-PAGE were collected from different areas of Dir lower were investigated for the level of genetic divergence and genetic linkages. The total germplasm 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, swat1, and Swat3. Cluster 2 was comprised of Maidan 1 and Gulabad 1. Cluster 3 was comprised Maidan 2, Swat 2, Talash 1, gumbir, Maidan 3, Gulabad 2, shagokas 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, Euclidian distance, genetic distance, linkage distance andgeographic relationship.This plant show a lot of variation in germplasm level, our findings are totally deviated from the findings of (Zahoor*et al.,* 2015) in which the detected variation only on the base of protein proofing pattern while in our present investigation we detected allelic base variation in seed color of *Robina pseudoaccacia*, the shape of seed is kidney shape, leaf color is light green and we also detected 7 loci bands in *Robina pseudoaccacia* through polyacrylamide gel electrophoresis similarly with the findings of (Zahoor*et al.,* 2015). While in our panel we only determine the genetic diversity in four different species of acacia through their morphometric and biochemical markers which asses the variation and conformation of these two reliable techniques for the assortment of genetic diversity is mostly conform through SDS-page.

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