

GENETIC ANALYSIS OF MORPHOLOGICAL TRAITS THROUGH SIMPLE SEQUENCE REPEATS (SSRS) PRIMERS IN UPLAND COTTON

Javeria Afzal¹*, Waqas Zahid¹, Abdul Rehman¹, Muhammad Iqbal¹, Usman Aziz¹, Annie Irshad²

¹Department of Plant Breeding and Genetics, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur-63100, Pakistan

²Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan

*Corresponding Author's Email: javeria.afzal@outlook.com

ABSTRACT: Cotton is the most important cash crop of Pakistan grown primarily for its fiber. But from last two decades seed cotton yield is stagnant, which might be due to the narrow genetic base of the cultivated cotton varieties. A study was conducted to assess the genetic diversity of 16 cotton genotypes using 50 simple sequence repeats (SSRs) primers. From the genetic diversity analysis, the genotypes, IUB-52, MM-58, IUB-222 and FH-113 were found most diverse which were selected for further analysis. After the assessment of genetic diversity a full diallel analysis was conducted involving four selected genotypes by genetic diversity (IUB- 52, MM-58, IUB-222 and FH-113) to create new variation and to investigate the gene action for different morphological traits i.e. monopodial branches per plant, sympodial branches per plant, number of bolls per plant, boll weight, ginning out turn percentage, fiber length, seed cotton yield per plant and plant height. The parents with their crosses were sown in field with randomized complete block design with four replications. Analysis of variance (ANOVA) showed that significant ($P < 0.5$) differences were present among the genotypes for all the traits. The results of the joint regression analysis proved that data is fit for simple additive-dominance model. The graphical representation of variance (V_r) and covariance (W_r) showed that all the characters were controlled by the additive type of gene action with partial dominance. The genotype IUB-222 contained maximum dominant genes for number of monopodial branches per plant, number of sympodial branches per plant, number of bolls per plant and ginning out turn percentage. Additive type of gene action indicated that selection may be very useful in early segregating generations for the improvements of these traits.

KEY WORDS: *Gossypium hirsutum* L., diallel analysis, gene action, morphological traits, SSRs primers

INTRODUCTION

All around the world cotton is considered an important cash crop (Chary and Leffler, 1984). USA, China, Pakistan, India, Egypt, Australia, Argentina, Greece, and Turkey are considered as major cotton producing countries. Cotton crop has key role in the economy of Pakistan, as annual foreign exchange earnings from cotton is about 62% whereas it accounts approximately 1.4 % to national GDP and about 6.7 % of the value added in agriculture [1].

Cotton is considered as silver fiber in Pakistan. From the independence research has been undertaken to improve the genetics of the cotton, as a result of which a large number of varieties with superior characteristics are being marketed. In Pakistan currently the cotton growing area is 2806 thousand with approximate yield of 773 kg/hectare whereas the total bales production is 12769 [1].

The quality and quantity of the cotton are important factors to meet export as well as domestic requirements. Existing germplasm has been utilized through different breeding techniques to improve the yield and fiber qualities of the cotton [2]. It has reported that currently the average yield potential in cotton varieties is relatively less than the genetic variability existing in the germplasm [3]. The extent of heritable variation passes from generation to generation and a genetic study of these traits is an important tool for plant breeders. Among all the breeding options to attain genetic variability many plant breeders widely used diallel crossing fashion [4,5,6]. Model of genetic variability proposed by [7] is reliable to find out about genetic relationships, i.e. dominance, additive, additive \times additive for most of the quantitative traits in self-pollinated crop plants. For this purpose diallel cross is considered as important tool which can be used to predict genetic variation

among genotypes. Diallel crossing method was used by various researchers [8,9] to find out the inheritance pattern of different agronomic traits i.e. monopodial branches, sympodial branches, no. of bolls and GOT percentage. Their analysis showed significant genotypic differences for all the traits under investigation. The results of dominance gene action with additive effects for lint percentage and boll weight was confirmed [9]. High heritability in broad sense with high genetic variability and genetic advance in plant height, boll retention percentage, nodes per plant and average boll weight were found by [10]. Their study suggested great scope of crop improvement through selection process in segregating generations.

Before the introduction of molecular markers, morphological markers were considered as an important tool to categorize variability in the existing germplasm. In present molecular markers can be used to find out the variability at genetic level without alteration from environment. Among all the options SSR markers (simple sequence repeat) is an important to find out diversity in existing germplasm. It is because they are polymorphic in nature, simple to use and are co-dominant in nature which gives reproducible results. In previous microsatellite markers are the only makers used to find genetic variability in cotton [11].

The purpose of the present study was to investigate genetic diversity of genotypes in cotton (*Gossypium hirsutum*) by using both molecular markers (SSR) and morphological traits. In order to collect significant information to create new recombination that might be used in breeding programs of cotton.

MATERIALS AND METHODS

Evaluation of genetic diversity and selection of parental genotype

In the month of September 2013, in green house sixteen different genotypes were grown (Table 1). Leaves were collected at twenty five days after sowing and used for DNA extraction. CTAB method was used to extract DNA. Fifty SSR markers were used for sorting out the genotypically diverse genotypes. The primers used were NAU 1046, NAU108, NAU 1125, NAU 1189, NAU 1238, NAU 1266, NAU 1356, NAU 2004, NAU 2173, NAU 2508, NAU 2670, NAU 2852, NAU 4926, NAU 5380, NAU 5461, NAU 5244, NAU 3839, NAU 2448, NAU 2121, NAU 1386 and NAU 1200.

Amplification was done in reaction mixture (20 µl) consisting of 2.4 µl of 10 mM dNTPs, 2 µl DNA, 3 µl of 2.5 mM MgCl₂, 2 µl of each reverse and forward primer and 2 µl of 10X PCR buffer. In thermocycler, the amplification was completed,

including the initial step of denaturation containing 5 min at 94°C followed by 35 cycles of 45 s at 94°C for DNA denaturing, 45 s for annealing at 57°C for all the primers and 1 min for DNA expansion at 72°C with a final extension at 72°C for 10 min. After amplification of DNA, gel electrophoresis was used to separate fragments. As staining material 3 µl ethidium bromide was utilized. In the first well of the gel 100 bp DNA ladder with fragment length standard ladder in the amount of 5 µl was used. Ten microliter was loaded in the remaining well from each reaction sample and afterwards photograph was captured under ultraviolet light.

The length of DNA fragments was examined graphically by the comparison with DNA ladder. Data was scored when the snap was taken which was /A for one band A/B for two bands and A/C for three bands and so on. Power Marker V3.25 software was used for sorting out the genetic diversity.

Table. 1: Name and origin of cotton genotypes used in Genetic Diversity Study

Sr. No.	Genotype	Origin
1	IUB-222	Department of Plant Breeding & Genetics UCA&ES IUB BWP
2	IUB-13	Department of Plant Breeding & Genetics UCA&ES IUB BWP
3	IUB-63	Department of Plant Breeding & Genetics UCA&ES IUB BWP
4	QMIUB-65	Department of Plant Breeding & Genetics UCA&ES IUB BWP
5	IUB-52	Department of Plant Breeding & Genetics UCA&ES IUB BWP
6	IUB-09	Department of Plant Breeding & Genetics UCA&ES IUB BWP
7	IR-3701	NIBGE Faisalabad
8	IR-1524	NIBGE Faisalabad
9	FH-113	AARI Faisalabad
10	FH-142	AARI Faisalabad
11	CIM-598	CCRI Multan
12	CIM-599	CCRI Multan
13	CEMB-22	CEMB Lahore
14	MNH-886	CRS Multan
15	MNH-786	CRS Multan
16	MM-58	Department of Plant Breeding & Genetics UCA&ES IUB BWP

Design of field experiment

In full diallel fashion, the selected genotypes were crossed during the month of February 2014. Plant material from different sources was used for this study (Table 1). The parents used were IUB-52, MM-58, IUB-222 and FH-113 and crosses were IUB-222 × IUB-52, IUB-222 × MM-58, IUB-222 × FH-113, IUB- 52 × IUB-222, IUB-52 × MM-58, IUB-52 × FH-113, MM-58 × IUB-222, MM-58 × IUB-52, MM-58 × FH-113, FH-113 × IUB-222, FH-113 × IUB-52 and FH-113 × MM-58.

At maturity, seeds of hybrids and parents were collected for further sowing. Parents, direct crosses and reciprocals were sown in the trial area of Department of Plant Breeding and

Genetics University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur during the crop season of 2014. After land preparation sowing was done on bed by dibbling method a triplicated randomized complete block design (RCBD). Distance was maintained between plant to plant (30 cm) and Row to row (75 cm). For all genotypes, identical management and cultural practices were applied throughout the growing season. Ten plants were selected randomly to collect the data for traits mentioned below.

Measures of morphological traits

Data was recorded for ten plants and then average was calculated.

Monopodial branches per plant

Branches arise from the base of the main stem and resembles like main stem were counted.

Sympodial branches per plant

These are the direct fruit bearing branches. At maturity the sympodial branches were counted.

Bolls per plant

Number of bolls was calculated and then average was calculated.

Boll weight

Boll weight was obtained by dividing seed cotton yield per plant on total number of bolls per plant.

Ginning out turn percentage

Ginning out turn is the ratio of lint to seed cotton expressed as a percentage and calculated by the following formula:

Ginning out – turn (%)

$$= \frac{\text{Weight of lint (g)}}{\text{Weight of Seed Cotton (g)}} \times 100$$

Fiber length

Fiber length was measured in millimeters by tuft method.

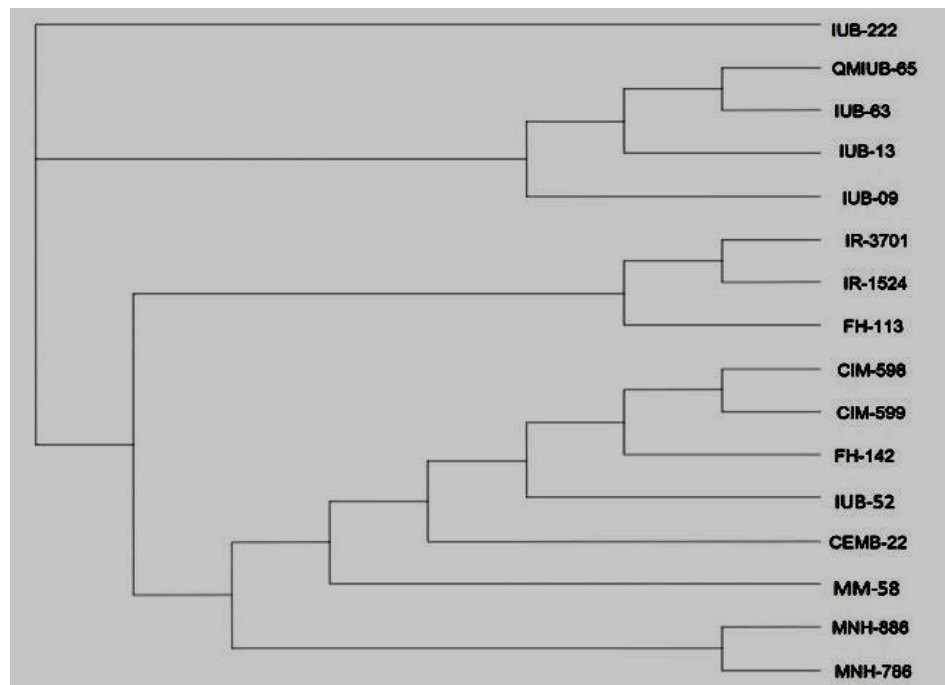


Fig. 1 Dendrogram constructed for 16 genotypes based on genetic distance by using SSR primers

Seed cotton yield per plant

Seed cotton of the plants was picked at maturity and weighed through electric balance in grams.

Plant height

The final height was calculated with measuring rod from first cotyledon node of the plant to apical bud, when growth ceased.

Statistical Analysis

The significant differences between the mean values of the parents and F1 were estimated by applying the analysis of variance [12]. Only that parameters were further analyzed showing the significant differences for genotypes. Genetic study was carried out by subjecting the data to technique of diallel analysis as devised by [13] and [14].

Graphical analysis

The parabola limits which help to draw parabola were found by the following formula.

$$W_{ri} = (V_{ri} \times V_{oL_o})^{1/2}$$

Where V_{ri} = expected V_r values and V_{oL_o} = variance of parents

For drawing regression line, expected W_{rei} values were calculated as below. $W_{rei} = (W_r - b)(V_r + b)V_{ri}$

The point of interception of the regression line with W_r axis is obtained by following formula.

$$a = (W_r - b) V_r$$

Where W_r = mean of covariances and V_r = mean of variances

RESULTS & DISCUSSION**Genetic Diversity Study**

The current study was planned to investigate genetic diversity by the use of SSR markers between the cotton genotypes. Among 16 cotton genotypes, 50 primers were used to test out polymorphism in the present study. By the use of UPGMA method, dendrogram was created for 16 cotton varieties by data generated of 50 SSR markers. Dendrogram divided the genotypes into four clusters (Fig 1).

First cluster contain five genotypes and divided into three sub-clusters include one genotype IUB-222 that is diverse from other genotypes of second and third sub-clusters. Second sub-cluster having two genotypes QM IUB-63 and IUB-63 are more

close to each other. Third sub-cluster contains two genotypes namely IUB-13 and IUB-09 that are similar with each other, but different from the genotypes of sub clusters one and two. Cluster two include of three genotypes in which IR-3701 and IR-1524 are genetically close to each other but diverse from FH-113.

Cluster three is the biggest cluster in dendrogram divided into two sub-clusters containing six genotypes, former sub-cluster includes three genotypes in which CIM-598 and CIM-599 are genetically more close to each other but different from the genotype of same sub-cluster that name is FH-142. Second sub-cluster consist of three genotypes namely IUB-52, CEMB-22 and MM-58, these genotypes are similar with each other but different from the genotypes of first sub-cluster.

Cluster four of the dendrogram has two varieties, MNH-886

and MNH-786. These varieties are more close to each other but diverse from the other varieties of dendrogram. After the dendrogram construction we find out four diverse genotypes i.e.IUB-222, IUB-52, MM-58 and FH-113. The amount of genetic variation was studied by the use of DNA markers which produce varying amounts of data in different crops and mainly differ in their principles. The present study was designed to explore genetic diversity among the cotton genotypes using SSR markers as shown in Fig 2 to Fig 7.

[15] reported that in genotypes of cotton, the genetic diversity through SSRs is higher. The present results of study are according to the results of [16]. Similarly, narrow genetic diversity in the cultivated upland cotton was reported by [17].

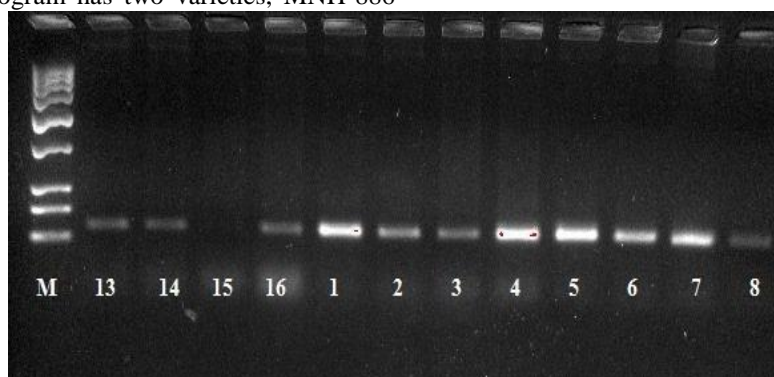


Fig. 2 Banding pattern of 12 cotton varieties obtained by using NAU1266

Where; M stand for Marker, 1 for IUB-222, 2 for IUB-13,3 for IUB-63, 4 for QM-IUB-65, 5 for IUB- 52, 6 for IUB-09,7 for IR-3701, 8 for IR-1524, 9 for FH-113, 10 for FH-142, 11 for CIM-598 and 12 for CIM-599.

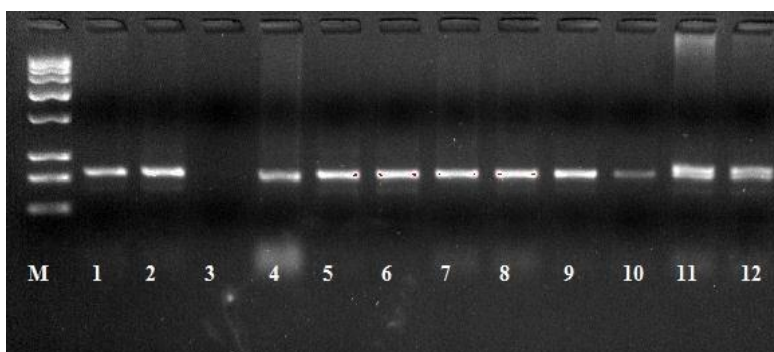


Fig. 3 Banding pattern of 12 cotton varieties obtained by using NAU1356.

Where; M stand for Marker, 13 for CEMB-22, 14 for MNH-886, 15 for MNH-786, 16 for MM-58, 1 for IUB-222, 2 for IUB-13, 3 for IUB-63, 4 for QM-IUB-65, 5 for IUB-52, 6 for IUB-09, 7 for IR-3701 and 8 for IR-1524

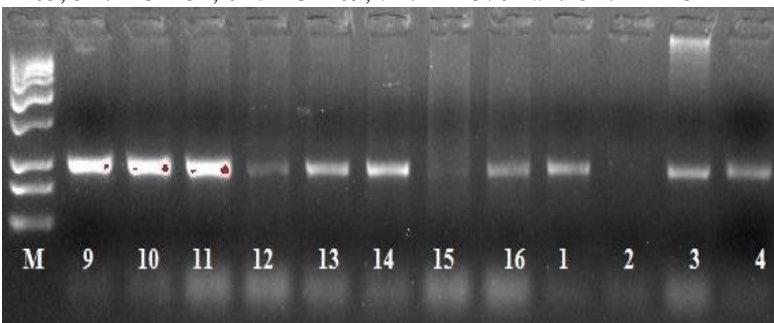


Fig. 4 Banding pattern of 12 cotton varieties obtained by using NAU2173

Where; M stand for Marker, , 9 for FH-113, 10 for FH-142, 11 for CIM-598, 12 for CIM-599, 13 for CEMB-22, 14 for MNH-886, 15 for MNH-786, 16 for MM-58, 1 for IUB-222, 2 for IUB-13, 3 for IUB-63 and 4 for QM-IUB-6

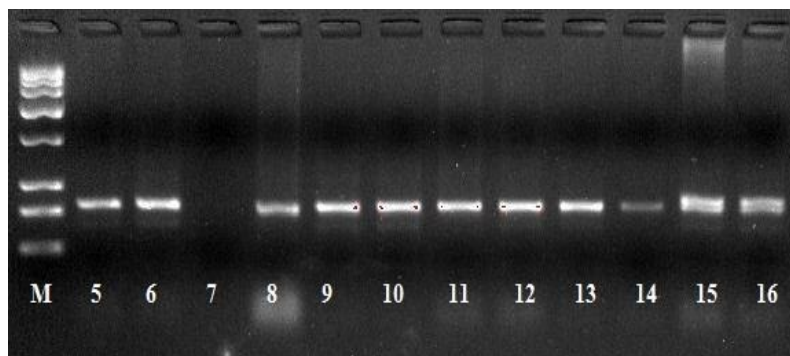


Fig. 5 Banding pattern of 12 cotton varieties obtained by using NAU2508

Where; M stand for Marker, 5 for IUB-52, 6 for IUB-09, 7 for IR-3701, 8 for IR-1524, 9 for FH-113, 10 for FH-142, 11 for CIM-598, 12 for CIM-599, 13 for CEMB-22, 14 for MNH-886, 15 for MNH-786 and 16 for MM-58.

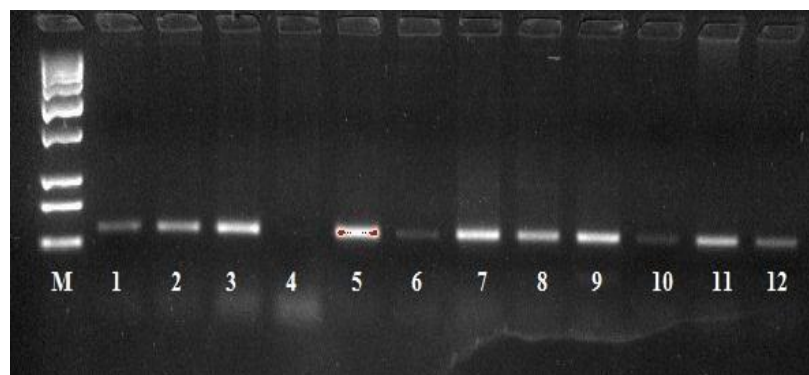


Fig. 6 Banding pattern of 12 cotton varieties obtained by using NAU2852

Where; M stand for Marker, 1 for IUB-222, 2 for IUB-13, 3 for IUB-63, 4 for QM-IUB-65, 5 for IUB-52, 6 for IUB-09, 7 for IR-3701, 8 for IR-1524, 9 for FH-113, 10 for FH-142, 11 for CIM-598 and 12 for CIM-599

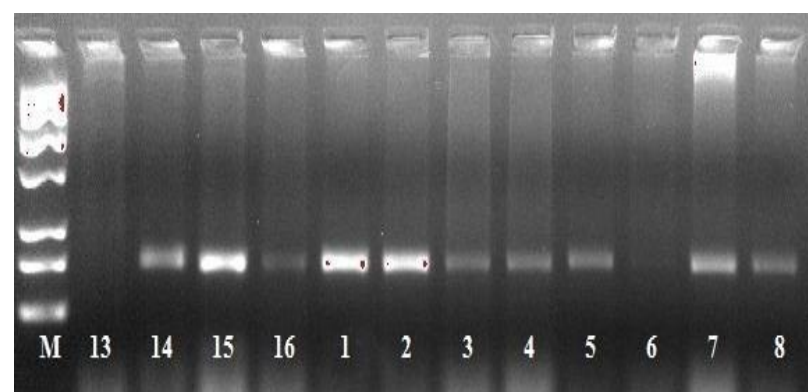


Fig. 7 Banding pattern of 12 cotton varieties obtained by using NAU4926.

Where; M stand for Marker, 13 for CEMB-22, 14 for MNH-886, 15 for MNH-786, 16 for MM-58, 1 for IUB-222, 2 for IUB-13, 3 for IUB-63, 4 for QM-IUB-65, 5 for IUB-52, 6 for IUB-09, 7 for IR-3701 and 8 for IR-1524.

Biometrical Study

The obtained data for all traits (i.e. plant height, monopodial branches/plant, number of bolls per plant, sympodial branches/plant, seed cotton yield per plant, G.O.T %, fiber length, and boll weight) were processed to obtain analysis of variance using [12]. The ANOVA proved significant differences between genotypes for the traits under observation

excluding boll weight. To evaluate gene action of the collected data, diallel analysis was carried out by help of joint regression analysis following additive-dominance model to confirm the data capability for analysis. Following approach proposed by [13,14], for simple additive-dominance model showed that data will be applicable in joint regression analysis in which the value of b must deviate from zero in significant manner but not from the unity. It is possible when statements underlying model

of simple additive-dominance are satisfied. Joint regression analysis results explained (Table 2) that the b value for monopodial branches /plant, number of bolls per plant, seed cotton yield per plant, sympodial branches/plant, boll weight, plant height

G.O.T % and fiber length considerably deviated from zero but not from unity. Vr/Wr graphs for traits were assembled by variance (Vr) and co-variance (Wr) values. The gene action mode was discovered by Vr/Wr graph with the division for dominant and recessive genes.

The outcomes from analysis of variance and join regression analysis proved that among the genotypes significant differences are present for all the traits and data is able to progress for additive-dominance model. Vr/Wr graph showed that an additive type of gene action with partial dominance was present for all the traits as regression line cut wr-axis above origin. As the regression line did not deviate from the unit slop therefore the non-allelic interactions were not present and through selection these traits can be improved. The additive type of gene action with partial dominance for these traits were also confirmed by the findings of different scientists which support our results [18,19,20].

Number of monopodial branches per plant

On regression line, the array point's position indicated that genotype IUB-222 possessed more dominant genes for the characters because it occupies nearest position from the origin, while the genotype FH-113 possessed lowest number of dominant genes because it gained farthest position from origin (Fig.8). The genotype IUB-222 showed the highest value of array mean (1.61) followed by FH-113 (1.57) and the genotypes MM-58 and IUB-52 had lowest array mean

values (1.34) and (1.05) respectively. While within array, the cross IUB-222 × FH-113 (1.77) scored highest value for monopodial branches per plant (Table 3).

Sympodial branches per plant

On regression line the position of array points indicated that genotype IUB-222 possessed more dominant genes for the characters because it occupies nearest position from the origin while the genotype FH-113 possessed lowest number of dominant genes because it gained farthest position from origin (Fig.8). The genotype IUB-222 showed the highest value of array mean (38.69) followed by MM-58 (34.44) and the genotypes IUB-52 and FH-113 had lowest array mean values (32.23) and (30.46) respectively. While within array, the cross FH-113 × IUB-222 (37.33) scored highest value for monopodial branches per plant (Table 4).

Number of bolls per plant

On regression line, array points location for this trait discovered that genotype IUB-222 have the dominant genes in maximum amount because it occupies nearby position of the origin. Next to it is MM-58 and FH- 113 respectively, and lastly IUB-52 occupied the farthest position on regression line which showed that it possessed maximum number of recessive genes (Fig.9). The genotype IUB-222 showed the highest value of array mean value (114.42) for anumber of bolls per plant followed by IUB-52 (109.25) and the genotypes FH-113 and MM-58 had lowest array mean values (106.96) and (105.04) respectively. While within the array the cross IUB-222 × FH-113 (113.00) scored highest value for no. of bolls per plant (Table 5).

Table. 2 Joint regression analysis of different traits of upland cotton plant (*Gossypium hirsutum* L.)

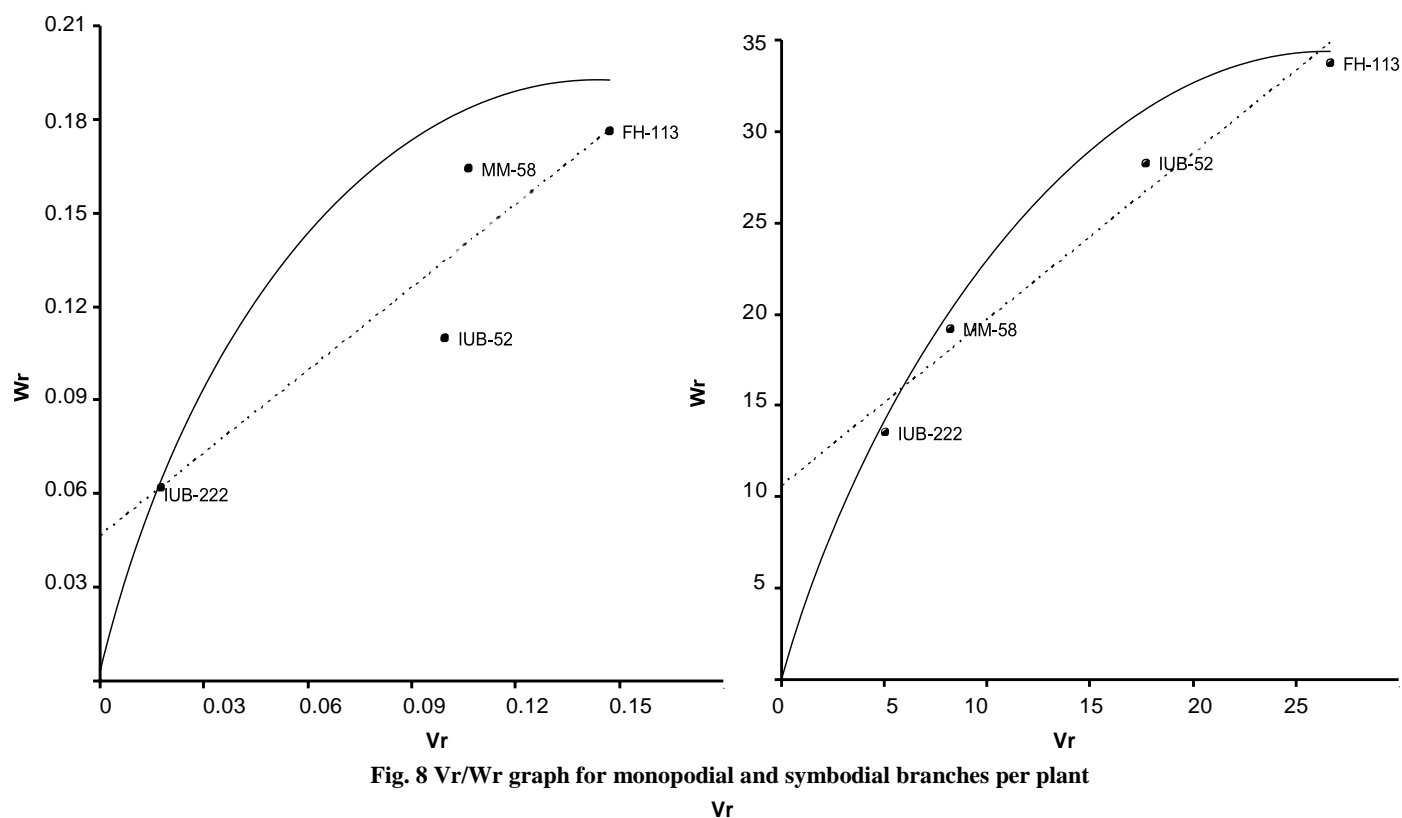
Sr. No.	Characters	Regression coefficient		Conclusions
		b = 1	b = 0	
1	No of monopodial branches/plat	0.5 N.S	3.501**	Data was fit for diallel analysis
2	No of sympodial branches / plant	0.83N.S	7.96**	Data was fit for diallel analysis
3	No of bolls per plant	0.26N.S	6.923**	Data was fit for diallel analysis
4	Boll wight	0.79N.S	5.72**	Data was fit for diallel analysis
5	G.O.T %	-.47N.S	5.81**	Data was fit for diallel analysis
6	Fiber length	7.3N.S	7.04**	Data was fit for diallel analysis
7	Seed cotton yield	0.37N.S	6.60**	Data was fit for diallel analysis
8	Plant height	0.59N.S	16.09**	Data was fit for diallel analysis

Table. 3 Array mean table for monopodial branches per plant in 4×4 diallel cross of cotton

Genotypes	IUB-222	IUB-52	MM-58	FH-113
IUB-222	1.63	1.47	1.57	1.77
IUB-52	1.47	0.70	1.02	1.00
MM-58	1.57	1.02	1.10	1.67
FH-113	1.77	1.00	1.67	1.83
Array mean	1.61	1.05	1.34	1.57

Table. 4 Array mean table for sympodial branches per plant in 4×4 diallel cross of cotton

Genotypes	IUB-222	IUB-52	MM-58	FH-113
IUB-222	42.00	37.25	38.18	37.33
IUB-52	37.25	31.40	33.17	27.10
MM-58	38.18	33.17	34.93	31.48
FH-113	37.33	27.10	31.48	25.93
Array mean	38.69	32.23	34.44	30.46



Boll weight

The array point location on regression line visibly showed that the genotype IUB-52 possessed the maximum quantity of dominant genes and it occupied the nearest position near the origin. The genotype IUB-222 occupied the farthest position from origin as it possessed a maximum quantity of recessive genes. Genotypes MM-58 and FH-113 have the frequency of both dominant and recessive genes because these two genotypes occupied central position on regression line (Fig.9). The results from array mean table for the trait boll weight revealed that genotype IUB-52 showed the highest value of array mean value (2.87) for boll weight and genotype IUB-222 possessed the lowest array mean value (2.67). While within array the, cross IUB-52× IUB-52 (2.90) scored the highest value for boll weight (Table 6).

Ginning out turn percentage

The array point location on regression line indicated that the genotype IUB-222 possessed the maximum amount of dominant genes and it occupied the neighboring position near the origin and the genotype IUB-52 occupied the outermost position from origin as it possessed maximum quantity of recessive genes. Genotypes FH-113 and MM-58 had the equal frequency of dominant and recessive genes because these two genotypes occupied central position on regression line (Fig.10). The results from array mean table for

G.O.T percentage revealed that genotype IUB-52 showed the highest value of array mean (40.51) followed by FH-113 (40.24) and the genotypes MM-58 (40.02) and IUB-222 (39.02) had mean values respectively. While within array the cross FH-113 X IUB-52 (40.73) scored the highest value for G.O.T percentage (Table.7).

Fiber length

On regression line, place of the array points showed that the genotype FH-113 possessed the maximum amount of dominant genes and it occupied the neighboring position near the origin and the genotype IUB-222 occupied outermost position from origin as it possessed maximum quantity of recessive genes. Genotypes IUB-52 and MM-58 had the

equal frequency of dominant and recessive genes because these two genotypes occupied central position on regression line (Fig.10). The results from array mean table for the trait fiber length revealed that genotype IUB-222 showed the highest value of array mean (27.84) followed by IUB-52 (27.75) and the genotypes MM-58 (27.50) and FH-113 (26.99) had mean values respectively. While within array the cross FH-113× IUB-52 (27.32) and FH-113× IUB-222 (27.10) scored the highest value fiber length (Table.8).

Seed cotton yield per plant

On regression line, Array point's position explained that the genotype FH-113 possessed the maximum amount of dominant genes and it occupied the nearby position near the origin and the genotype IUB-222 occupied outermost position from origin as it possessed maximum quantity of recessive genes. Genotypes MM-58 and IUB-52 had the frequency of dominant and recessive genes because these two genotypes occupied central position on regression line (Fig.11). The results from array mean table for the trait seed cotton yield per plant revealed that genotype IUB-222 showed the highest value of array mean (308.69) next to it was MM-58 (307.27), IUB-52 (302.88) and FH-113 (292.61) respectively while within array the cross MM-58 × FH-113(299.51) scored the highest array value for seed cotton yield per plant (Table.9).

Plant Height

Array point's position on regression line showed that the genotype IUB-52 possessed the maximum amount of dominant genes as it occupied the closest position near the origin and the genotype MM-58 occupied outermost position from origin as it possessed maximum quantity of recessive genes. Genotypes IUB-222 and FH-113 had the frequency of dominant and recessive genes because these two genotypes occupied central position on regression line (Fig.11).The results from array mean table for the trait seed cotton yield per plant revealed that genotype IUB-222 showed the highest value of array mean (158.07) next to it was MM-58(147.82), IUB-52 (141.99) and FH-113 (138.21) respectively while within array the cross FH-113 × IUB-222(152.42) scored the highest array value for seed cotton yield per plant (Table 10).

Table. 5 Array mean table for No. of bolls per plant in 4×4 diallel cross of cotton

Genotypes	IUB-222	IUB-52	MM-58	FH-113
IUB-222	118.67	115.83	110.17	113.00
IUB-52	115.83	108.33	106.33	106.50
MM-58	110.17	106.33	100.33	103.33
FH-113	113.00	106.50	103.33	105.00
Array mean	114.42	109.25	105.04	106.96

Table.6 Array mean table for boll weight in 4×4 diallel cross of upland cotton

Genotypes	IUB-222	IUB-52	MM-58	FH-113
IUB-222	2.52	2.85	2.73	2.60
IUB-52	2.85	2.90	2.87	2.87
MM-58	2.73	2.87	2.79	2.76
FH-113	2.60	2.87	2.76	2.75

Table. 7 Array mean table for ginning out turn percentage in 4×4 diallel cross of upland cotton

Genotypes	IUB-222	IUB-52	MM-58	FH-113
IUB-222	38.20	39.08	39.45	39.35
IUB-52	39.08	41.67	40.57	40.73
MM-58	39.45	40.57	39.50	40.55
FH-113	39.35	40.73	40.55	40.33
Array mean	39.02	40.51	40.02	40.24

Table. 8 Array mean table for fiber length in 4×4 diallel cross of upland cotton

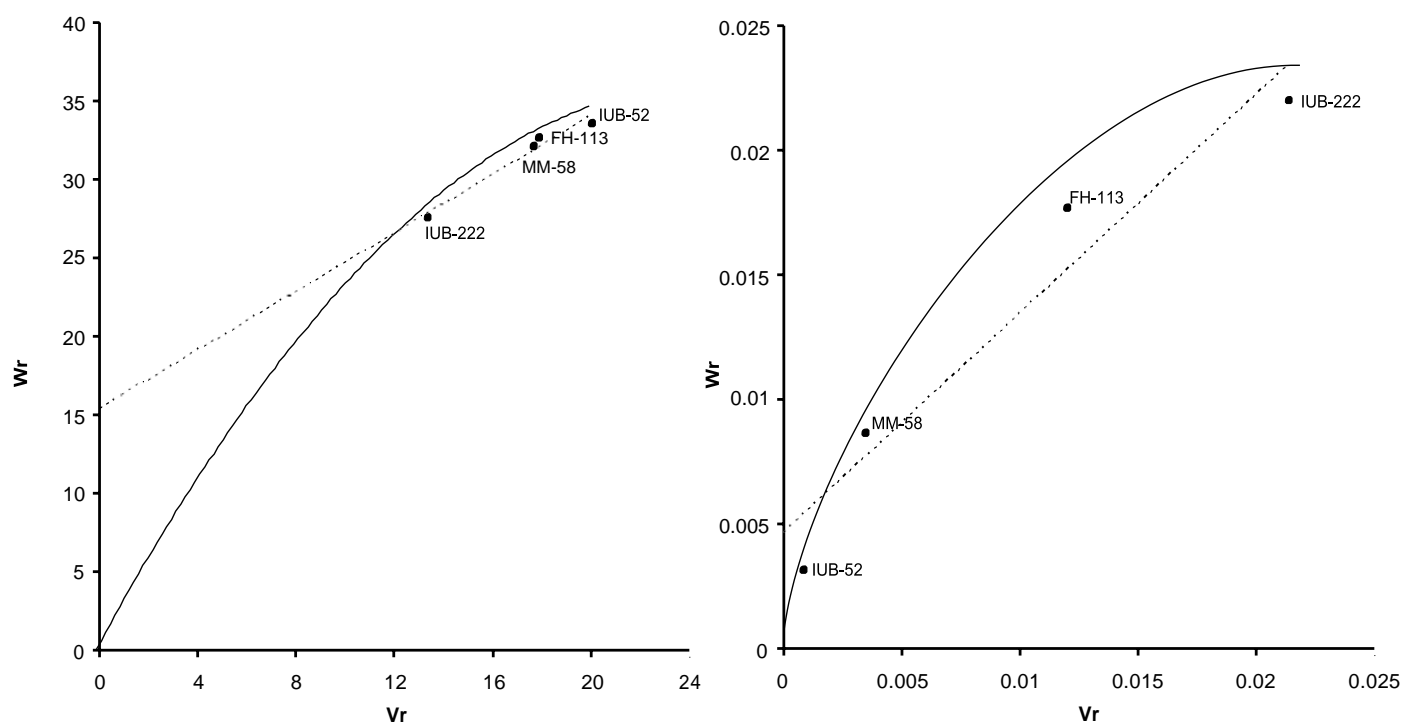
Genotypes	IUB-222	IUB-52	MM-58	FH-113
IUB-222	28.37	28.07	27.82	27.10
IUB-52	28.07	27.83	27.77	27.32
MM-58	27.82	27.77	27.37	27.03
FH-113	27.10	27.32	27.03	26.50
Array mean	27.84	27.75	27.50	26.99

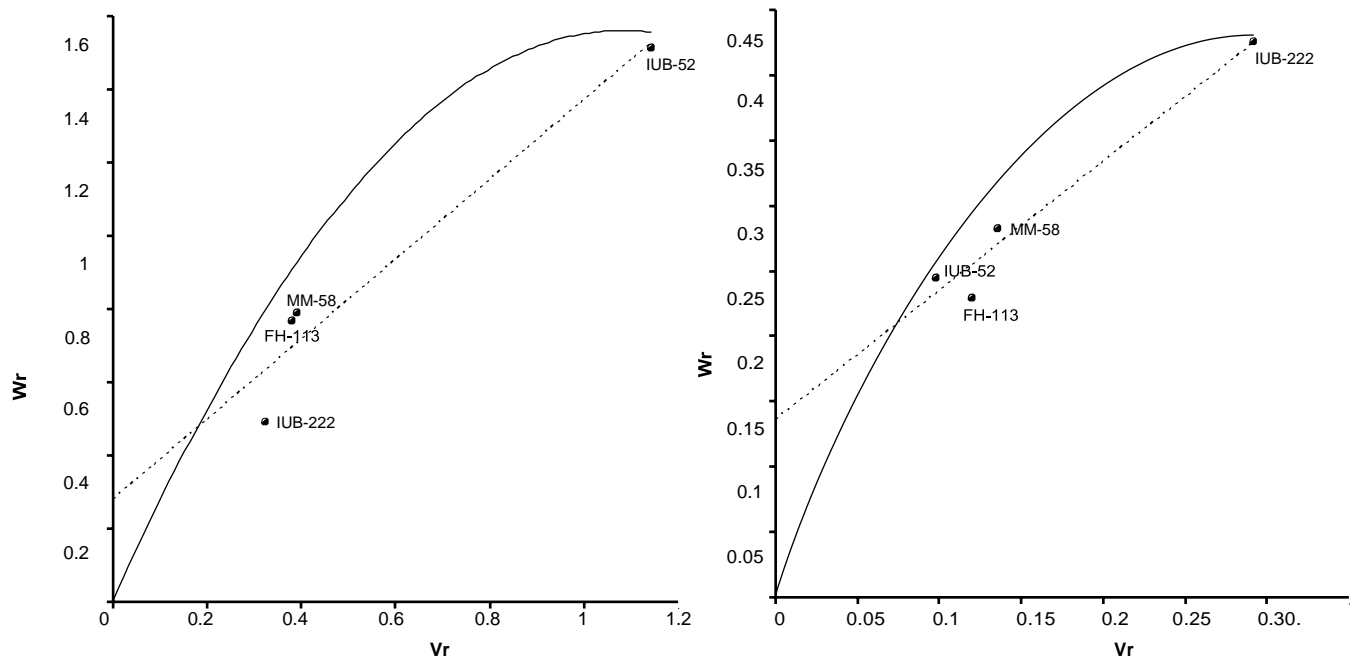
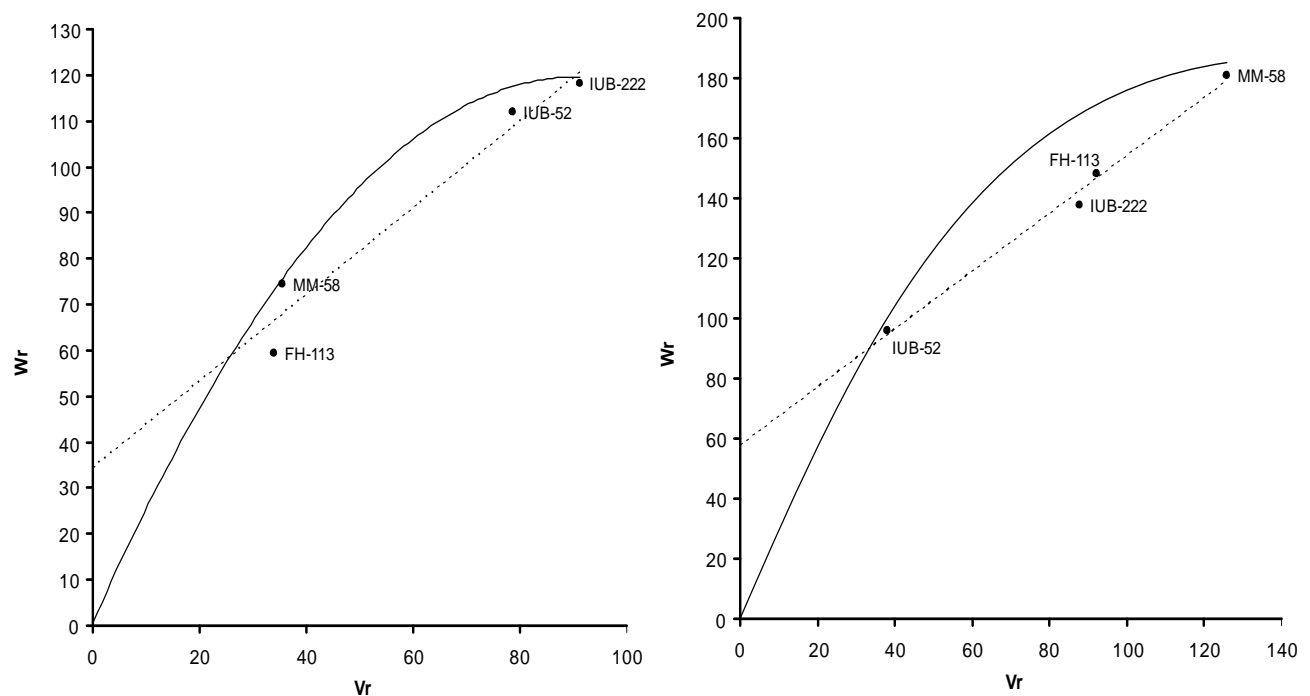
Table. 9 Array mean table for seed cotton yield per plant in 4×4 diallel cross of upland cotton

Genotypes	IUB-222	IUB-52	MM-58	FH-113
IUB-222	315.40	310.79	313.92	294.65
IUB-52	310.79	303.53	306.85	290.33
MM-58	313.92	306.85	308.78	299.51
FH-113	294.56	290.33	299.51	285.93
Array mean	308.69	302.88	307.27	292.61

Table.10. Array mean table for plant height in 4×4 diallel cross of upland cotton

Genotypes	IUB-222	IUB-52	MM-58	FH-113
IUB-222	169.33	148.62	161.90	152.42
IUB-52	148.62	140.40	144.70	134.23
MM-58	161.90	144.70	149.73	134.95
FH-113	152.42	134.23	134.95	131.23
Array mean	158.07	141.99	147.82	138.21

**Fig. 9 Vr/Wr graph for number of bolls and boll weight per plant**

Fig.10 V_r / W_r graph for ginning out turn percentage and fiber lengthFig.11 V_r / W_r graph for seed cotton yield per plant and plant height

CONCLUSION

After calculations for genetic diversity, among sixteen genotypes only four genetically diverse cultivars were selected for genotypic studies. These four genotypes were IUB-222, IUB-52, MM-58 and FH-113. The Genotype

IUB-222 contained maximum dominant genes for no. of monopodial branches per plant, no. of sympodial branches per plant, no. of bolls per plant and ginning out turn percentage while it contained maximum recessive genes for boll weight, fiber length and seed cotton yield per

plant. The genotype IUB- 52 possessed maximum dominant genes for boll weight, fiber length and plant height while it possessed maximum recessive genes for bolls per plant and GOT%. The genotype FH-113 possessed maximum dominant genes for seed cotton yield and maximum recessive gene for monopodial and sympodial branches per plant. The genotype MM-58 carried the maximum recessive gene for plant height. From the above mentioned findings, it was suggested that all plant trait were showing additive type of gene action with partial dominance, so they could be improved by simple selection procedures during the early segregation generations.

Competing Interest

“The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.”

REFERENCES:

1. Anonymous. Economic Survey of Pakistan. (2013-14). Finance Division, Economic Advisory Wings Islamabad, Pakistan, 2013.
2. Nadeem K, Azhar F.M. Genetic analysis of seed cotton yield and its components *G.hirsutum* L. Int. J. Agri. Bio. 6:865-868, 2004.
3. Ali M.A, Khan I.A. Assessment of genetic variation and inheritance mode in some metric traits of cotton (*Gossypium hirsutum* L.). J. Agri. Social Sci. 3: 112-116, 2007.
11. Zhang Y, Lin Z, Xia Q, Zhang M, Zhang X. Characteristics and analysis of simple sequence repeats in the cotton genome based on linkage map constructed from a BC. Population between *Gossypium hirsutum* and *barbadense* Genome. Theore. Appl. Genet. 51:534-54, 2008.
12. Steel R.G.D, Torrie J.H, Dickey D. Principles and Procedure of Statistics. A Biometrical Approach 3rd Ed. McGraw Hill Book Co. Inc., New York. pp. 352-358, 1997.
13. Hayman I. The analysis of variance of diallel tables. – Biometrics **10**: 235-244, 1954a.
14. Jinks J.L. The analysis! of continuous variation in a diallel cross of *Nicotian rustica* varieties. - Ibid. 39: 767-788, 1954
15. Li Z.X, Wang Y, Zhang G, Zhang L, Wu J.C, Ma Z. Assessment of genetic diversity in glandless cotton germplasm resources by using agronomic traits and molecular markers. Frontiers of Agriculture in China 2:245-252, 2008.
4. Iqbal M, Chang M.A, Iqbal M.Z. Breeding behavior effects for yield, its component and fiber quality in intra-specific crosses of cotton (*G.hirsutum* L.). J. Bio. Sci. 4:451-459, 2003.
5. Basal, H, Turgut I. Genetic analysis of yield components and fiber strength in upland cotton (*Gossypium hirsutum* L.). Asian J. Plant Sci. 4:293-298, 2005.
6. Ali M.A, Awan S.I. Inheritance pattern of seed and lint traits in cotton (*Gossypium hirsutum*). Int. J. Agri. Bio. 11:44-48, 2009.
7. Stuber C.W. Estimation of genetic variances using inbred relatives. Crop Sciences. 10,129-135, 1970.
8. Dhivya R, Amalabalu P, Pushpa R, Kavithamani D. Variability, heritability and genetic advance in upland cotton (*Gossypium hirsutum* L.). African J. Plant Sci. 8(5): 1-5, 2014.
9. Siraj A.C, Abdul W.B, Baloch J.B, Imran A, Humaira W. Genetic analysis of fiber characters of upland cotton (*Gossypium hirsutum* L.). Global Journal of Biodiversity Science and Management. 3:264-268, 2013.
10. Aziz U, Afzal J, Iqbal M, Naeem M, Khan M.A, Nazeer W, Aslam M.T, Zahid W. Selection Response, Heritability and Genetic Variability Studies in Upland Cotton.J. Appl. Environ. Biol. Sci., 4(8S) 400-412, 2014.
16. Tatineni V, Cantrell R.G, Davis D.D Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPDs. Crop Sci. 36:186-192, 1996
17. Iqbal M.J, Aziz N, Saeed N.A, Zafar Y. Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. Theore. Appl. Genet. 94:139-144, 1997.
18. Paxasia M.J, Shukla P.J, Poshiya V.K. Combining ability for vegetative characters over environments in upland cotton (*G. hirsutum* L.). Gujarat Agri. Uni. Res. J. Ind. 23: 28-32, 1998.
19. Iqbal M. Inheritance and combining ability studies for earliness, yield and yield components in 6 x 6 intra-specific hybrids of *Gossypium hirsutum* L. Ph.D. Thesis submitted through the department of Plant Breeding and Genetics to the Sindh Agri. Univ. Tando Jam, 2002.
20. Khan I.A, Ali A, Zubair M, Khan I.A. Genetic analysis of morphological traits in upland cotton. The Pak. cotton. 44:25-26, 2003.