



## Combining Ability Analysis and Heterotic Effects for Cotton Fiber Quality Traits

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### ABSTRACT

Combining ability and gene action can help breeders to select suitable parents and appropriate breeding strategy. In the present study combining ability analysis and heterotic effects for cotton fiber quality traits were studied in a set of diallel crosses involving six cotton genotypes. The aim of this study was to estimate general combining ability of parents, special combining ability of  $F_1$  diallel crosses, heterotic effects of  $F_1$  diallel crosses in the breeding programme to develop high quality cotton varieties. Randomized complete block design was used to test 15  $F_1$  diallel crosses, and 6 parents. Analysis revealed significant general combining ability effects for all the investigated traits and additive gene effects were important in the inheritance of the traits. Analysis also revealed significant SCA effects for only fiber fineness and spinning consistency index traits and additive and non-additive gene effects were important in the inheritance of the traits. General combining ability was found significant for all investigated traits, revealing the important role of additive gene effects. Specific combining ability was found significant for the Mic and SCI, revealing that non-additive gene effects, as dominant or epistatic are important, but not for Len, Str and SFI.

**Keywords:** cotton, fiber quality, gene action, diallel analysis

### Introduction

Raw cotton and cotton products play an important role in the economy of advanced cotton growing countries. Improvement of fiber quality and cotton fiber quality parameters play a vital role in the cotton price in textile sector. Breeding programs of cotton generally aim to increase fiber quality parameters. The success of cotton breeding programme is based on choice of superior genotypes for hybridization and selection for favorable genes and gene complexes in combination. Since fiber quality traits are quantitatively inherited, a simple genetic model having several genetic parameters needs a lot of work to solve complex relationship of successful breeding (Bolek *et al.*, 2010). Over the years fiber traits were significantly improved by the plant breeders by bringing new allelic recombination and subsequent selection of valuable trans-segregants (Ali *et al.*, 2010).

Combining ability analysis, to compare the performance of  $F_1$  combinations is used in breeding programs (Griffing, 1956) and allow estimation of different genetic parameters (Verhalen and Murray, 1967). Additive-dominance model can direct plant breeder about the validation of data and design as well as usage of data. Heterosis is useful in determining the most appropriate parents for specific traits (Khan *et al.*, 2010).

Cotton fiber quality is expressible by a multitude of measurements (Hake *et al.*, 1996). Fiber length, fiber fineness, fiber strength, short fiber index and the spinning consistency index are the most important fiber quality traits. Because of the high potential for cotton fiber quality *G. barbadence* L. still are used in cotton breeding programs. Thus new cotton varieties with high fiber quality can be obtained. The variation in a fiber trait through plant breeding approaches requires knowledge of the cultivar's genetics

(Aguado *et al.*, 2010). Heterosis is one of the significant techniques in cotton breeding programs (Khan *et al.*, 2010, Bhadate *et al.*, 1980, Basbag *et al.*, 2007). Little work has been reported on the heterosis of fiber quality traits in cotton breeding (Ashokkumar *et al.*, 2013). Estimation of heterotic effects is necessary to identify the new suitable cross combinations.

The aim of this study was to determine the general combining ability of the parents, the specific combining ability of  $F_1$  diallel crosses and the heterotic effects of the hybrids in the breeding programme to develop high quality cotton varieties.

### Materials and Methods

The parent genotypes belonged to *G. hirsutum* L. (Sayar-314, Stoneville-453, Nazilli-84S, and Fantom) and *G. barbadense* L. (Giza-45 and Delcerro) species. During first year of experiment, six cotton genotypes were crossed in a half diallel mating design in the experimental fields of Dicle University of Agriculture Faculty in 2011. In second year, all parents (6) and  $F_1$  crosses (15) were planted in the randomized complete block design with three replicates at the same experimental area in 2012. In all experiments, plot length was 12 m, spacing between and within rows was 70x20 cm. Standard cultural practices were applied as suggested by Diyarbakir ecological region. Fertilizers were 160kg ha<sup>-1</sup> N and 120 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and irrigations were total 9 times in about 8-10 days intervals as furrow irrigation during growing seasons. Data were recorded on fiber obtained from randomly selected boll in each of the three replicates as the fiber length (Len.) (mm) (2.5% Span Length), fiber fineness (Mic.) (micronaire), fiber strength (Str) (gtex<sup>-1</sup>), short fiber index (SFI) and the spinning consistency index (SCI). Investigated fiber quality traits were determined by HVI (High Volume Instruments). The data were analyzed using analysis of variance method by using Dial-98 (Ukai, 2006) and SAS (SAS Institute, Cary, NC). Traits found significant were further analyzed by Griffing's (1956) method-II and model-I. Heterosis (Ht) (%) and heterobeltiosis (Hb) (%) were calculated according to formulas of Hallauer and Miranda (1981).

### Results and Discussion

Mean squares of  $\delta^2$ GCA,  $\delta^2$ SCA,  $\delta^2$ GCA/ $\delta^2$ SCA and GCA for investigated traits in cotton parents are presented in Table 1. Mean squares of general combining ability (GCA) were found significant for all investigated traits, revealing the important role of additive gene effects (Table 1). Specific combining ability (SCA) was found significant for the Mic and SCI,

revealing that non-additive gene effects, as dominant or epistatic are important, but not for the Len, Str and SFI (Table 2).

Variance of GCA effects were higher than variance of SCA effects ( $(\delta^2$ GCA)/ $(\delta^2$ SCA) $>1$ ) for the all investigated traits which indicated that additive gene action is prevailing with non-additive gene actions for the expression of these traits (Table 1). The results are in agreement with earlier reported the findings. (Bolek *et al.*, 2010; Lukonge *et al.*, 2008; Aguiar *et al.*, 2007; Cheatham *et al.*, 2003; Leidi 2003; Tariq *et al.*, 1992; Green and Gulp, 1990; Kanoktip, 1987).

SCA was found highly significant for the Mic. and SCI, revealing an important role of non-additive gene effects (Table 1). (Green and Culp 1990; Bhardwaj and Kapoor; 1998; Cheatham *et al.*, 2003). Additive genetic effects with enough genetic variability were also noticed for the traits permitting for the effective selection (Lukonge *et al.*, 2008). The reason for this difference may be due to different genetic structure of genotypes utilised and the different ecological condition (Bolek *et al.*, 2010).

Positive GCA effects for the Len and SCI were ascertained in Giza-45 and Delcerro, whereas a negative GCA effects were ascertained in Sayar-314, Stoneville-453, Nazilli-84S and Fantom. Positive GCA effects for the Str were obtained from Stoneville-453, Fantom and Delcerro, while on the contrary negative GCA effects were obtained from Sayar-314, Nazilli-84S and Giza-45. Negative GCA effects for the Mic. were detected in Giza-45, Delcerro, and Nazilli-84S, while positive GCA effects were detected in Sayar-314, Stoneville-453, and Fantom. Negative GCA effects for the SFI were ascertained in Giza-45, Delcerro, and Fantom inasmuch as positive GCA effects were ascertained in Sayar-314, Stoneville-453, and Nazilli-84S. In order to do genetic crosses within the parents, Giza-45 and Delcerro were selected for the Len, Mic., SFI, SCI.

These genotypes would be more promising to develop higher fiber quality progenies. The GCA effects of parents indicated that the Giza-45 genotype was greatest general combiner for the Len, Mic., and SFI followed by Delcerro which was the best combiner for the Str and SCI.

Significant GCA effects for all the traits determined suggest at least one parent superior to the others, regarding mean performance in hybrid combinations. Giza-45 and Delcerro had affirmative and significant GCA effects on the Len, Mic., and SFI values, respectively, indicating some dominance genes for the Str, Mic., and SFI in the Giza-45 and Delcerro

genotypes, which are consistent with the results (Aguiar *et al.*, 2007).

Determined SCA effects, Ht (%) and Hb (%) for the investigated traits in cotton  $F_1$  diallel crosses. (Table 2).

The main aim in cotton breeding is obtain a lower value for the Mic. and SFI, while it is referred a high value for the Len, Str and SCI. In this context, higher negative SCA, negative Ht, and negative Hb values are desirable for the Mic. and SFI in cotton breeding studies.

Among the crosses, the determined SCA effects varied from -0.65 to 0.72 for the Len, -0.10 to 0.03 for the Mic., -1.65 to 0.85 for the Str, -0.44 to 0.78 for the SFI, -1.30 to 2.34 for the SCI. Stoneville-453xGiza-45, Sayar-314xDelcerro, Sayar-314xGiza-45, Stoneville-453xFantom, Nazilli-84SxDelcerro and FantomxDelcerro for the Len were determined to be greater specific combinations. Stoneville-453xDelcerro, Sayar-314xFantom, Sayar-314xDelcerro, Stoneville-453xFantom, Nazilli-84SxDelcerro for Str were found to be the best specific combinations. Significant and positive SCA effects for the SCI were observed in the three of the fifteen cross combinations; Sayar-314xDelcerro, DelcerroxGiza-45, Nazilli-84SxGiza-45. On other hand, Sayar-314xGiza-45, Nazilli-84SxFantom, Stoneville-453xGiza-45, Nazilli-84SxDelcerro for the SFI were found to be the greater specific combinations. Negative and significant SCA effects were determined for FantomxGiza-45, Sayar-314xGiza-45, and Stoneville-453xGiza-45 diallel crosses for the Mic.

Among the crosses the determinate Ht values varied from -1.77% to 3.81% for the Len, -3.43% to 10.58% for the Mic., -1.33% to 7.85% for the Str, -10.48% to 24.86% for the SFI, -12.82% to 7.06% for the SCI. In addition among the crosses the estimated Hb values varied from -7.87% to -0.03% for the Len, -17.98% to 5.19% for the Mic., -5.06% to 3.80% for the Str, -26.51% to 19.24% for the SFI, -18.22% to 4.63% for the SCI. Similar results were reported by Rauf *et al.*, (2005). High and positive Hb (%) values were determined for the crosses Sayar-314xGiza-45,

Sayar-314xDelcerro, Stoneville-453xGiza-45 and Nazilli-84SxGiza-45 for the Len, Sayar-314xDelcerro, Stoneville-453xFantom, Stoneville-453xDelcerro, Nazilli-84SxDelcerro for the Str, FantomxGiza-45, Stoneville-453xGiza-45, Sayar-314xGiza-45 for the SCI, while high and negative Ht values were determined for the crosses DelcerroxGiza-45, FantomxGiza-45, Nazilli-84SxGiza-45, Stoneville-453xGiza-45, Sayar-314xGiza-45 and Stoneville-453xDelcerro for the Mic., Sayar-314xGiza-45 Stoneville-453xGiza-45 and Nazilli-84SxGiza-45 for the SFI. The lower Hb values were determined for the crosses Sayar-314xGiza-45 (-17.98%) for the Mic, and Sayar-314xGiza-45 for the SFI (-26.51%) (Table. 2). Performance of parents and crosses could vary widely with genetic background and growing conditions (Ashokkumar *et al.*, 2013; Bolek *et al.*, 2010; Ehsan *et al.*, 2008; Copur, 2006).

### Conclusion

As a result of this study it may be concluded that additive genetic effects were noticed for the Len, Str, SFI. On the other hand, both additive and non-additive genetic effects were defined for the Mic. and SCI. Hence, selection in early generations may be more preferred for the Len, Str, SFI, while selection in the late generations may be more preferable for the Mic. and SCI. Suitable parents were determined to improve fiber quality traits of cotton. Namely Giza-45 for the Len, Mic., and SFI; Delcerro for the Str and SCI were selected as the thriving parents. These results implied that Stoneville-453xGiza-45, Sayar-314xDelcerro, Sayar-314xGiza-45, Stoneville-453xFantom, Nazilli-84SxDelcerro and FantomxDelcerro for the Len; FantomxGiza-45, Sayar-314xGiza-45, Stoneville-453xGiza-45 for the Mic, Stoneville-453xDelcerro, Sayar-314xFantom, Sayar-314xDelcerro, Stoneville-453xFantom, Nazilli-84SxDelcerro for the Str; Sayar-314xDelcerro, DelcerroxGiza-45, Nazilli-84SxGiza-45 for the SCI may be proposed as the utmost promising cross combinations.

Table 1. Values of  $\delta^2$ GCA,  $\delta^2$ SCA,  $\delta^2$ GCA/ $\delta^2$ SCA and GCA for investigated traits in cotton parents (Griffing, 1956).

Parameters	Genotypes	Len	Mic.	Str	SFI	SCI
GCA	1 Sayar-314	-1,28**	0,62**	-0,281	0,58**	-8,39**
	2 Stoneville-453	-0,82**	0,0004	0,054	0,23*	-0,03
	3 Nazilli-84S	-0,81**	-0,15**	-1,302**	0,4**	-5,54**
	4 Fantom	-0,75**	0,26**	1,171**	-0,03	-2,66**
	5 Delcerro	1,20**	-0,30**	<b>1,841**</b>	-0,35**	<b>10,00**</b>
	6 Giza-45	<b>2,44**</b>	<b>-0,43**</b>	-1,484**	<b>-0,85**</b>	6,62**
	$\delta^2$ GCA	52,42**	3,65**	41,97**	6,68**	1210,13**
	$\delta^2$ SCA	0,64	0,07**	1,8	0,54	112,96**
	$(\delta^2$ GCA)/ $(\delta^2$ SCA)	81,16	51,51	23,32	12,18	10,71

$\sigma^2$ GCA: variance of general combiningability,  $\sigma^2$ SCA: variance of specific combiningability \* and \*\*: significant at  $P \leq 0.05$  and  $P \leq 0.01$  respectively; Len: Fiber Length (mm), Mic: Fiber Fineness (micronaire), Str: Fiber Strength (g/tex), SFI: Short Fiber Index, SCI: Spinning Consistency Index

Table 2. Determinated SCA effects, Ht (%) and Hb (%) for the investigated traits in cotton F<sub>1</sub> diallel crosses.

Genotypes	LEN			MIC			STR			SFI			SCI		
	SCA	Ht (%)	Hb (%)	SCA	Ht (%)	Hb (%)	SCA	Ht (%)	Hb (%)	SCA	Ht (%)	Hb (%)	SCA	Ht (%)	Hb (%)
1 x 2	-0.12	0.17	-2.25	0.01	2.84	-8.43	-0.028	0.89	-0.16	0.78	<b>24.86</b>	13.25	-7.07**	-12.82	<b>-18,22</b>
1 x 3	-0.18	0.45	-1.56	0.16**	5.84	-8.43	-0.304	-1.29	-1.68	-0.05	2.07	1.72	-9.31**	-12,72	-15,76
1 x 4	-0.11	0.29	-2.28	0.25**	8.43	1.12	0.388	-0.84	-4.79	0.16	9.30	-1.89	-0.73	-5,65	-7,27
1 x 5	0.60	3.11	-4.78	-0.01	0.77	-14.42	0.684	4.16	-0.76	0.19	9.31	-7.06	<b>5.29*</b>	1,45	-5,84
1 x 6	0.63	<b>3.81</b>	-6.60	-0.07**	-1.57	<b>-17.98</b>	0.044	-0.62	-2.15	<b>-0.44</b>	<b>-10.48</b>	<b>-26.51</b>	-4.73**	5,47	1,36
2 x 3	-0.27	-0.78	-1.21	-0.04	0.62	-2.64	-0.607	<b>-1.33</b>	-2.74	0.57	17.90	7.28	-4.77*	<b>-12,48</b>	-15,03
2 x 4	0.10	0.12	<b>-0.03</b>	0.34**	<b>10.58</b>	<b>5.19</b>	0.419	5.32	2.16	0.45	20.64	19.24	-5.43**	-5,67	-10,04
2 x 5	-0.65	<b>-1.77</b>	-7.17	-0.07	-1.77	-6.95	<b>0.848</b>	<b>7.85</b>	<b>3.80</b>	0.03	11.14	3.38	-5.64**	-10,88	-11,90
2 x 6	<b>0.72</b>	3.20	-5.05	-0.06*	-2.46	-9.59	-0.025	1.04	-1.53	-0.43	-7.09	-16.91	-4.31**	3,19	0,63
3 x 4	0.008	0.25	-0.33	-0.003	2.58	-5.41	-0.557	0.34	-4.01	-0.29	-2.41	-12.13	-1.30	-11,11	-12,74
3 x 5	0.30	1.68	-4.30	0.03	0.39	-1.79	0.372	3.93	-1.34	-0.14	-0.61	-15.25	0.47	-6,11	-9,85
3 x 6	0.12	2.94	-5.67	-0.06	-2.95	-7.18	-1.642	0.33	-0.83	0.09	-6.53	-23.05	4.97*	2,39	1,93
4 x 5	0.27	1.25	-4.18	-0.10	-1.32	-10.82	0.132	1.76	0.94	0.26	11.85	5.19	2.34	-6,34	-11,65
4 x 6	-0.36	0.00	<b>-7.87</b>	<b>-0.15**</b>	-3.42	-14.50	0.025	0.91	-4.53	0.01	-1.80	-11.26	-5.06*	<b>7,06</b>	<b>4,63</b>
5 x 6	0.01	1.49	-1.35	-0.01	<b>-3.43</b>	-5.63	-0.229	1.12	<b>-5.06</b>	0.06	-1.54	-5.65	0.96*	2,50	-1,16
S. Error	<b>0.37</b>	<b>1.55</b>	<b>2.46</b>	<b>0.13</b>	<b>4.19</b>	<b>5.90</b>	<b>0.59</b>	<b>2.55</b>	<b>2.47</b>	<b>0.33</b>	<b>10.42</b>	<b>12.72</b>	<sup>4.24</sup>	<b>6.91</b>	<b>7.02</b>
Means	<b>0.07</b>	<b>1.08</b>	<b>-3.64</b>	<b>0.01</b>	<b>1.01</b>	<b>-7.16</b>	<b>-0.03</b>	<b>1.57</b>	<b>-1.51</b>	<b>0.08</b>	<b>5.11</b>	<b>-4.64</b>	<sup>-2.29</sup>	<b>-4.11</b>	<b>-7.39</b>
Max.	<b>0.72</b>	<b>3.81</b>	<b>-0.03</b>	<b>0.34</b>	<b>10.58</b>	<b>5.19</b>	<b>0.85</b>	<b>7.85</b>	<b>3.80</b>	<b>0.78</b>	<b>24.86</b>	<b>19.24</b>	<sup>5.29</sup>	<b>7.06</b>	<b>4.63</b>
Min.	<b>-0.65</b>	<b>-1.77</b>	<b>-7.87</b>	<b>-0.15</b>	<b>-3.43</b>	<b>-17.98</b>	<b>-1.64</b>	<b>-1.33</b>	<b>-5.06</b>	<b>-0.44</b>	<b>-10.48</b>	<b>-26.51</b>	<sup>-9.31</sup>	<b>-12.82</b>	<b>-18.22</b>

Where: SCA: variance of specific combining ability, Ht: Heterosis (%), Hb: Heterobeltiosis (%), \* and \*\*: significant at P ≤ 0.05 and P ≤ 0.01 respectively; LEN: Fiber Length (mm), MIC: Fiber Fineness (micronaire), STR: Fiber Strength (g/tex), SFI: Short Fiber Index, SCI: Spinning Consistency Index 1: Sayar-314, 2: Stoneville-453, 3: Nazilli-84S, 4: Fantom, 5: Delcerro, 6: Giza-45

## References

- Aguado, A, De Los Santos B, Gamane, D, Garcí'a del Moral, LF, and Romero F, (2010). Gene effects for cotton-fiber traits in cotton plant (*Gossypium hirsutum* L.) under *Verticillium* conditions. *Field Crops Research*. 116 (2010) 209-217.
- Aguiar PAD, Penna JCV, Freire EC, and Melo LC, (2007). Diallel analysis of upland cotton cultivars. *Crop Breeding and Applied Biotechnology* 7: 353-359.
- Ali Z, Khan TM, and Noorka IR, (2010). Diallel Analysis to Determine Gene Action for Lint Percentage And Fibre Traits in Upland Cotton. *Int. J. Agric. Appl. Sci.* Vol. 2, No.1.
- Ashokkumar K, Senthil Kumar K, and Ravikesavan R, (2013). Heterosis Studies for Fibre Quality of Upland Cotton in Linextester Design. *African Journal of Agricultural Research*. Vol. 8(48), pp. 6359-6365.
- Basbag S, Ekinçi R and Gencer O, (2007). Combining ability and heterosis for earliness characters in line  $\times$  tester population of *Gossypium hirsutum* L. *Hereditas* 144:185-190.
- Bhardwaj R P, and Kapoor CJ, (1998). Genetics of Yield and its Contributing Traits in Upland Cotton (*Gossypium hirsutum* L). *Proceedings of the World Cotton Research Conference-2*, Athens, Greece, pp. 214-216.
- Bhatade SS, Shobhane MR, and Unchegaonkar PK, (1980). Heterosis and Combining Ability in *G. arboreum* L. *Indian J. Agri. Sci.*, 50:310-16.
- Bolek Y, Cokkizgin H, Bardak A, (2010). Combining Ability and Heterosis for Fiber Quality Traits in Cotton. *Plant Breeding and Seed Science*. Volume 62, Issue , Pages 3-16, ISSN (Online) 2083-599X, ISSN (Print) 1429-3862, DOI: 10.2478/v10129-011-0001-6, March 2011.
- Cheatham CL, Jenkins JN, Mc Carty JC, Watson C, and Wu J, (2003). Genetic Variances and Combining Ability of Crosses of American Cultivars, Australian Cultivars and Wild Cottons. *J. Cotton Sci.*, 7: 16-22.
- Copur O, (2006). Determination of Yield and Yield Components of Some Cotton Cultivars in Semi-arid Conditions. *Pak. J Biol. Sci.* 9(14):2572-2578.
- Ehsan F, Nadeem A, Tahir MA, Majeed A, (2008). Comparative Yield Performance of New Cultivars of Cotton (*Gossypium hirsutum* L.). *Pak. J. Life Soc. Sci.* 6(1):1-3.
- Green CC, and Culp TW, (1990). Simultaneous Improvement of Yield, Fiber Quality and Yarn Strength in Upland Cotton. *Crop Sci.*, 30: 66-69.
- Griffing B, (1956). Concept of General and Specific Combining Ability in Relation to Diallel Crossing Systems. *Aust. J. Biol. Sci.* 9:463-493.
- Hake KD, Bassett DM, Kerby TA, Mayfield WD, (1996). Producing Quality Cotton. In: Hake, S.J., Kerby, T.A., Hake, K.D. (Eds.), *Cotton Production Manual*. University of California Publication 3352, pp. 134-149.
- Hallauer AR, Miranda JB, (1981). *Quantitative Genetics in Maize Breeding*. Iowa State Uni. Press Ames. USA.
- Kanoktip K, (1987). Study on the Inheritance of Certain Agronomic Characteristics in Cotton. *Field Crops Abs.* No: 92-073564.
- Khan N, Basal H, Hassan G, (2010). Cotton Seed Oil and Yield Assessment via Economic Heterosis and Heritability in Intraspecific Cotton Populations. *Afr. J. Biotechnol.* 9(44):7418-7428.
- Leidi EO, (2003). Combining Ability of Yield and Yield Components in Upland Cotton (*Gossypium hirsutum* L.) Under Drought Stress Conditions. *Word Cotton Research Conference 3, Abstracts of Paper and Poster Presentations*. s.337. Cape Town. South Africa.
- Lukonge EP, Labuschagne MT, and Herselman L, (2008). Combining Ability for Yield and Fibre Characteristics in Tanzanian Cotton Germplasm. *Euphytica*, 161 :3 83-3 89.
- Rauf S, Khan TM, and Nazır S, (2005). Combining Ability and Heterosis in *Gossypium hirsutum* L. *International Journal of Agriculture and Biology*. 7(1)109-113.
- Tanq M, Khan MA, Sadaqat HA, and Jamil T, (1992). Genetic Component Analysis. In *Upland Cotton*. *J. Agric. Res.*, 30: 439-445.
- Ukai Y, (2006). DIAL98. User's Guide. Ver. 6. DIAL98. Japan.
- Verhalen LM, Murray JC, (1967). A Diallel Analysis of Several Fiber Properties Traits in Upland Cotton (*G. hirsutum* L.). *Crop. Sci.* 7:501-505.