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THE DETERMINATION OF LEAF ANATOMY, YIELD AND QUALITY CHARACTERISTICS IN F_1 AND F_2 GENERATIONS OF INTERSPECIFIC COTTON HYBRIDS (*Gossypium hirsutum* L. x *Gossypium barbadense* L.)

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ABSTRACT

The breeding between *Gossypium hirsutum* L. and *Gossypium barbadense* L. purpose to increase yield and enhance fiber quality. The cross populations between four hirsutum varieties, Darmi, Helius, LT 4 and LT 64 and barbadense variety, Avesto, were used as material. Interspecific F_1 and F_2 populations were evaluated in terms of heterosis, useful heterosis and F_2 depression for yield, quality parameters and physiological characteristics such as stomatal density and parenchyma thickness in cross section of leaf. All observed characters resulted negative heterotic effects except fiber length and strength. Inbreeding depression for seed cotton yield was highly negative in all hybrid combinations. The differences among genotypes for stomatal density and parenchyma thickness were found to be significant. The significant correlations showed that an increase in stomatal density resulted in an increasing of fiber length and strength, whereas in a decreasing of ginning out turn and fiber fineness. It should be concluded that stomatal density can be used as selection criteria in early generations of interspecific cotton breeding.

Key words: Cotton, F_2 depression, heterosis, parenchyma thickness, stomatal density

INTRODUCTION

The most cultivated species of Gossypium genus are *G. hirsutum* L. (upland cotton) and G. *barbadense* L. (extra-long or long staple cotton) (Chen et al., 2007). Cultivated cotton varieties in Turkey are completely upland cotton, and the least varieties breed by crossing of hirsutum and barbadense are located in very special areas. Turkey is one of the first 5 countries among the world cotton consumption in the 2015/16 season, and the value of its cotton import is above the one million tons, and cotton import of Turkey gradually increased. The cotton varieties are divided into three groups according to fiber length; short (≤29 mm), long (29-32 mm) and extra-long (35- 50 mm).

The spongy parenchyma of *G. barbadense* L. is thicker than the palisade, and it was defined that barbadense varieties have bad performance to moisture deficit (Bhatt and Andal, 1979). *G. hirsutum* L. have high yield, moderately fiber characteristics, more reproductive structures and fewer vegetative, whereas *G. barbadense* L. are considered with high fiber properties and sensitivity to non-suitable conditions. Wise et al. (2000) emphasized that varieties belong to both species have non-identic different characters in terms of anatomic, morphologic and physiologic. The most obvious results of Wise et al. (2000) hirsutum leaves were thicker and smaller than barbadense leaf and thickness was results of palisade layer. In conclusion, they postulated that yield of barbadense varieties were negatively affected by their physiological and anatomical properties.

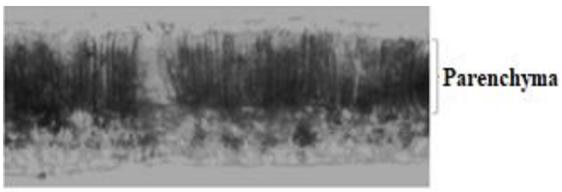
It is necessary to keep in mind that the hirsutum x barbadense hybrids have economic importance in some countries such as China, India and Pakistan (Khan et al., 2017), whereas hirsutum x barbadense crosses were evaluated in pedigree selection of classical breeding. Using seeds of interspecific F_2 hybrids are not considered suitable because inbreeding depression in economical characters such as ginning out-turn, fiber quality and yield is very high and, this creates many problems in filial generations (Natsiou-Vozike et al., 1988). In polyploids crops, inbreeding and abnormal segregation at meiosis because of higher ploidy leads to unfavorable interactions among multiple alleles (Soomro and Kalhoro, 2000). Meredith (1984) revealed that the relationship between lint yield and fiber fineness was high and positive whereas lint yield negatively correlated with fiber length and fiber strength. These paradox limited to interspecific introgression, and infertility, cytological abnormalities and distorted segregation are seen in interspecific crosses (Saha et al., 2004). It was insisted in many studies that traditional breeding methods were unsuccessful in pyramiding target genes for yield and fiber quality in introgression breeding. Interspecific crosses were drawn attention with characteristics such as very late cutout, immature bolls, small boll size, low ginning turn-out and high percentage of mote (Zhang et al., 2014).

The genetic mechanism of yield and quality characteristics in interspecific crosses was well-established. Also, heterotic effects and inbreeding depression of interspecific populations were estimated. There is little information on the heterotic effects of the leaf and stomatal properties of interspecific cross populations in *G. hirsutum* x *G. barbadense*. The objective of the present study was to compare leaf anatomy of hirsutum and barbadense cross populations, to determine whether anatomical differences can be a selection criterion, and to evaluate with yield and quality parameters.

MATERIALS AND METHODS

Four cotton varieties (*Gossypium hirsutum* L.), Darmi, Helius, LT 4 and LT 64 known as early maturity and Avesto (*Gossypium barbadense* L.) with best fiber characteristics were crossed to develop interspecific hybrid at Nazilli Cotton Research Institute. Four hirsutum x barbadense interspecific hybrids were selfed in 2012. Totally 14 genotypes; 5 parents, 4 F_1, 4 F_2 and Flash used as check variety, were planted in University of Adnan Menderes, Agricultural Faculty's experimental area according to the Randomized Complete Block Design with three replicates. The plot size was two rows and 5 m long. After thinning, the distance between rows and plant to plant was 0.70 x 0.20 m. Sowing date was on 8 May 2013. A basal fertilizer recommended dose of 60-60-60 NPK kg ha-1 was applied in the form of 15.15.15 compose fertilizer. The remaining nitrogen (116 kg ha-1) was applied as top dressed at the before first irrigation in the form of ammonium nitrate (% 33 N). The required cultural practices such as irrigation, weeding and plant protection were applied according to cotton growing practices of Aegean Region. The experimental area was furrow irrigated 4 timely during growing season.

Data were recording for ginning out turn (%), fiber fineness (mic.), fiber length (mm), fiber strength (g tex^{-1}) and plant yield (kg $plant^{-1}$). Seed cotton yield per plant was measured as average of randomly selected 10 plants after first and second picking. All the fiber characteristics were analyzed via High Volume Instrument (HVI). From each parcel, 10 plants were randomly selected and 5 youngest fully developed leaves were sampled at stage of boll maturity in the morning, and transported to the lab in plastic bags over ice. The central section of each leaf was evaluated (Wise et al., 2000). Leaf palisade thickness (PL) was measured from Scanning Electron Microscopy's (SEM) of leaf cross-sections. Stomatal length (SL) and width (SW) were measured from abaxial surface in ten stomata by screening of SEM (Figure 1). The number of stomata (SD) was counted as per µm2 in leaf surface area. Similarly, chlorophyll content (CC) was measured by chlorophyll content meter (Opti-sciences CCM 200, USA) as chlorophyll content index (CCI) at noon (Shakeel et al., 2017).



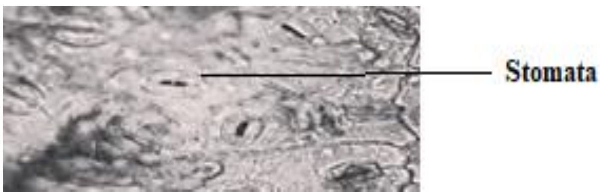


Figure 1. Scanning electron micrographs of parenchyma (left) and stomata (right)

The data of all measured characteristics were analyzed by JMP (5.0.1) statistical program. The genotypic means were compared using LSD test at 0.05 probability level. Heterosis, useful heterosis and inbreeding depression were calculated with following formulas;

Heterosis (%) = [F_1 – Mean of Parent /Mean of Parent] x 100

Useful Heterosis (%) = [F_1 – Check Variety/Check Variety] x 100

Inbreeding Depression (%) = [F_2 – F_1 / F_1] x 100

RESULTS AND DISCUSSION

The mean and statistical comparisons of studied characteristics were estimated to evaluate the performance of F_1 and F_2 generations, and all characteristics showed significantly broad range of variation except SW, SL and CC (Table 1).

The highest ginning out-turns (GOT), seed cotton yield and coarsest fibers was obtained in check variety Flash and this variety followed by mean of parents. For these three characteristics, F_1 and F_2 populations were between parents and check variety. Especially, seed cotton yield of

F_2 populations were the lowest level. In segregated populations, F_1 and F_2, genotypes exhibited lower GOT, micronaire, moderate fiber length and strength. Interspecific intogression breeding in cotton is limited success in pyramiding desirable genes between hirsutum and barbadense genotypes (Zang et al., 2014; Coban and Unay, 2017).

The barbadense parent, Avesto, had superior fiber length and strength while fiber characteristics of early matured parents (*G. hirsutum* L.) and check variety were lower than other genotypes. Fiber length values of F_1 and F_2 populations were between Avesto and hirsutum parents, whereas fiber strength of some F_1 and F_2 populations were superior to Avesto (Table 1).

Table 1. Mean performance of parental genotypes, F_1 and F_2 populations for evaluated characteristics

	Genotypes	GOT (%)		FF (mic.)		FL (mm)		FS (g tex^{-1})		SCY (g)	
Parents	Avesto(1)	36.44	b	3.50	bcd	34.88	a	39.27	ab	20.75	efg
	Darmi (2)	35.59	bcd	4.10	ab	26.52	fg	34.10	cd	37.73	abcd
	Helius (3)	35.90	bc	4.50	a	26.68	efg	35.36	bcd	50.40	a
	Lt 4 (4)	33.66	cde	3.69	bcd	30.11	cde	37.63	abc	47.42	ab
	Lt 64 (5)	37.56	b	4.64	a	26.12	g	30.86	d	38.88	abc
F_1	1 x 2	33.26	de	3.55	bcd	34.02	ab	40.56	a	34.96	bcd
	1 x 3	33.54	cde	3.32	d	33.53	abc	40.50	a	25.43	def
	1 x 4	33.48	cde	3.64	bcd	30.48	cd	39.03	abc	35.12	bcd
	1 x 5	33.35	de	3.22	d	34.38	a	39.23	ab	33.48	cde
F_2	1 x 2	31.41	e	3.41	cd	30.63	bcd	37.43	abc	17.61	fg
	1 x 3	33.50	cde	4.03	abc	30.71	bcd	38.96	abc	12.04	g
	1 x 4	32.10	e	3.33	d	32.21	abcd	40.53	a	14.84	fg
	1 x 5	33.51	cde	3.75	bcd	29.93	def	36.63	abc	18.11	fg
Check	Flash	42.15	a	4.55	a	29.93	def	36.86	abc	37.60	abcd
LSD $_{(0.05)}$		2.54		0.66		3.44		4.98		13.36	

Table 1. Continued

	Genotypes	SW (µm)	SL (µm)	SD		PL (µm)		CC (CCI)
Parents	Avesto	13.92	26.46	276.13	bcd	256.51	bcde	29.48
	Darmi	13.58	24.54	258.00	bcde	265.65	bcde	23.82
	Helius	14.92	25.29	216.33	f	281.46	abcd	22.67
	Lt 4	14.69	25.87	253.33	bcdef	305.16	ab	23.61
	Lt 64	13.87	25.58	233.46	ef	220.76	e	31.69
F_1	1 x 2	12.39	21.79	377.33	a	303.65	abc	21.16
	1 x 3	13.00	24.22	277.33	bcd	295.03	abcd	21.25
	1 x 4	14.01	25.85	280.00	bc	246.05	de	25.99
	1 x 5	13.28	25.13	246.00	cdef	286.79	abcd	20.29
F_2	1 x 2	14.13	25.93	236.66	def	245.92	de	23.24
	1 x 3	15.24	25.64	288.33	b	307.28	ab	21.49
	1 x 4	12.93	24.33	283.66	bc	248.36	cde	24.14
	1 x 5	14.23	24.93	252.00	bcdef	244.41	de	23.62
Check	Flash	14.92	25.41	221.73	ef	321.39	a	25.35
LSD $_{(0.05)}$				41.31		55.37		

In terms of leaf characters, the differences among genotypes for SD and PL were found to be significant. Stomatal density was found between 221.73 and 377.33 per mm^2 of leaf. The SD values of Avesto were the highest in the all parents and check variety (Table 1). It was seen that all the F_1's formed more stomatal densities and some F_2 populations (1x3; 1x4) had higher values than the Avesto. Parenchyma length was the highest in Check variety, Flash. The some F_1 and F_2 populations had lower PL values than their parents. The ranges of abaxial stomatal density (per mm^2) and palisade layer thickness (µm) were parallel to Wise et al. (2000) in terms of hirsutum and barbadense values. In the characteristics that were insignificant differences between genotypes, stomatal width, stomatal length, stomatal density and chlorophyll content changed between 12.39-14.92 µm, 21.79-26.46 µm and 20.29-28.48 CCI, respectively.

The heterosis and useful heterosis data for ginning out-turn indicated that all interspecific F_1 hybrids gave lower values below mid-parent and check variety, Flash (Table 2). Similarly, all F_2 hybrids, except Avesto x LT 64, inbreeding depression for ginning out-turn were negative direction. The results of ginning out-turn showed that the genetic divergence among parents was limited and this character was affected by several factors such as linkage, epistasis etc. (Baloch et al., 1993; Munir et al., 2016).

Fiber fineness, fiber length and fiber strength are important quality characters in cotton. In case of FF, heterosis were generally negative direction and between -21.07-1.24 % indicating reduction in fiber fineness on crossing between hirsutum and barbadense. Similarly, it was seen that fineness mean values of F_1 and F_2 populations were thinner than commercial variety, Flash (Table 2). Munir et al. (2016) stated that heterosis for fineness should be negative direction in interspecific hybrids. The positive and highest inbreeding depression were recorded in 1x3 and 1x5 F_1 populations. When the homozygosity increases and dominant allelic factors cause the high heterosis value, inbreeding depression is high level (Komal et al., 2014). It's not possible to say the same things for fiber length because of high heterosis and middle-low inbreeding depression. It is speculated that the desired genes for fiber length are assembled in this populations. Heterosis and useful heterosis values for fiber strength were found positively and middle-low level whereas inbreeding depression were negative and low level.

Heterosis value for seed cotton yield per plant was between -28.51%-19.56% (Table 2). The all populations were under check variety, Flash and useful heterosis varied from -32.37% to -6.61%. The highest inbreeding depression among observed characters was estimated for SCY and SCY values of populations were lower than the lowest yielded, Avesto. It should be concluded that mentioned populations were lack the promising segregation. It should be concluded that high inbreeding depression was results of linkage disequilibrium, epistasis and ploidy level (Baloch et al., 1993).

Table 2. Heterotic effects and inbreeding depression for evaluated characteristics.

	GOT	FF	FL	FS	SCY	SW	SL	SD	PL	CC
					Heterosis (%)					
1 x 2	-4.56	-6.70	10.81	10.59	19.56	-9.89	-14.54	41.28	16.30	-20.60
1 x 3	-7.27	-17.20	30.03	8.52	-28.51	-9.84	-6.39	12.63	13.87	-18.50
1 x 4	-4.50	1.24	-6.17	1.50	3.03	-2.06	-1.20	5.76	-9.10	-2.09
1 x 5	-10.97	-21.07	12.70	11.86	12.29	-4.42	-3.45	-3.45	12.63	-33.66
					Useful Heterosis (%)					
1 x 2	-21.09	-21.97	13.62	10.03	-7.02	-16.95	-14.24	70.17	2.49	-16.52
1 x 3	-20.42	-27.03	11.99	9.84	-32.37	-12.86	-4.68	25.07	-0.41	-16.17
1 x 4	-20.59	-19.78	1.83	5.85	-6.61	-6.09	1.73	26.27	-16.95	2.52
1 x 5	-20.90	-29.23	14.82	6.40	-10.95	-10.99	-1.10	10.94	-3.19	-19.96
					Inbreeding Depression (%)					
1 x 2	-5.59	-3.94	-9.96	-7.73	-49.62	14.04	18.99	-37.27	-19.01	9.82
1 x 3	-0.11	21.38	-8.41	-3.77	-52.65	17.23	5.86	3.96	4.15	1.12
1 x 4	-4.03	-8.76	5.64	3.84	-57.74	-7.70	-5.88	1.31	0.94	-7.11
1 x 5	0.47	16.45	-12.94	-6.62	-45.90	7.15	-0.79	2.43	-14,77	16.41

Stomatal width and stomatal length gave similar results for all three studied heterotic parameters. The main differences in leaf characteristics were found for stomatal density. Heterosis and useful heterosis were positive and high levels, and inbreeding depressions were very low except 1x2 population. When the mean values of populations evaluated together, it should be said that leaf characteristics were moving in the direction of Avesto (*Gossypium barbadense* L.).

Palisade thickness (PL) of Avesto was the lowest among the other parents and check variety. Heterosis values were mostly positive whereas useful heterosis was in negative direction. The inbreeding depressions of especially two F_2 populations (1x2 and 1x5) were high and negative (Table 2). Genotypic differences for stomatal density and parenchyma length were significant and, these parameters were the most important criteria of this study because these leaf characteristics could be selection criteria in hirsutum x barbadense breeding. The correlation coefficients between stomatal density and GOT, FF, FL, FS and SCY were estimated as -0.41**, -0.45**, 0.55**, 0.60** and -0.26 whereas correlations with PL were found non-significant (non-tabulated data). The relationships showed that an increase in stomatal density resulted in increases in the values of

FL and FS, whereas GOT and FF reduced. The stomatal density of *G. barbadense* L. varieties and interspecific hybrid populations with *G. barbadense* L. is more remarkable.

CONCLUSIONS

Avesto (*G. barbadense* L.) x Darmi, Helius, Lt4 and Lt64 (*G. hirsutum* L.) hybrid populations exhibited that genotypes having lower fineness and higher fiber length and strength can be breed by classical breeding method, but lower ginning out-turn and yield are major problems for interspecific hybrid populations. The remarkable heterotic performance for mid parent and useful heterosis were higher and negative directions for ginning out-turn and yield. The negative heterotic effects for fiber fineness but positive direction heterosis mid parent and useful heterosis for fiber length and strength showed that fiber characteristics move in the direction of barbadense. It could be suggested that recurrent selection can be successful at desirable combination through the breakdown of undesirable linkage groups. The significant correlations between stomatal density and GOT, yield and quality parameters showed that stomatal density should be used as selection criteria in early generations of classical cotton breeding.

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