

**Reciprocal Effect and Inbreeding Depression in Advanced Generations of Cotton
(*Gossypium hirsutum* L.) Bulk Population**

Pamukta İleri Bulk Populasyon Hatlarında Kendileme Depresyonu ve Resiprokal Etki

Şerife BALCI¹, Volkan Mehmet ÇINAR², Aydın ÜNAY^{3*}**Abstract**

Reciprocal differences reflect an unequal contribution of male and female parents in plant breeding. We investigated the reciprocal differences, inbreeding depression, and performance of bulk populations in advanced generations. Seven hybrid combinations and their reciprocals were evaluated in F₄ and F₅ generations in a Randomized Complete Block Design with three replications. The significant reciprocal differences in many combinations for seed cotton yield and fiber length in both generations indicated the importance of selecting parents as male or female at the start of breeding work for these traits. In contrast, fiber fineness, fiber strength, and ginning out-turn were generally traits for which the reciprocal differences of the lines were not significant. The difference in general averages of F₄ and F₅ generations was significant regarding fiber length and fineness. Advanced lines in the F₅ generation were found to have longer and finer fibers. In F₄ generation lines, seed cotton yield per plant ranged between 100.30-160.87 g; ginning out-turn 41.96-47.17%; fiber length 30.04-32.94 mm; fiber fineness 4.52-5.11 mic. and fiber strength 32.50-34.60 g tex⁻¹, while these values were 101.11-137.63 g; 41.45-46.54%; 30.55-33.09 mm; 4.24-4.90 mic. and 32.03-34.30 g tex⁻¹ in F₅ generation, respectively. The higher inbreeding depression estimates for seed cotton yield indicated that non-additive genes were more predominant in the inheritance of this trait compared to ginning out-turn and fiber quality parameters. As a result of the study, it was concluded that it would be beneficial to transfer Carmen × Carisma, Flash × Claudia, and ST-468 × Claudia F₅ advanced lines, which showed superior performance in terms of seed cotton yield and fiber properties, to the F₆ generation.

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Öz

Bitki ıslahında geliştirilen melezlerde ana ve baba olarak kullanılan anaçların eşit olmayan katkıları resiprokal farklılıklar olarak tanımlanmaktadır. Bu çalışmayı ileri pamuk generasyonlarında farklı bulk popülasyonlarının performansını, kendileme depresyonunu ve resiprokal farklılıkları incelemek amacıyla yürüttük. F₄ ve F₅ generasyonlarında yer alan 7 farklı melez kombinasyonunu resiprokal melezleri ile birlikte Tesadüf Blokları Deneme Deseninde üç tekerrürlü olarak değerlendirdik. Kütlü pamuk verimi ve lif uzunluğu için her iki generasyonda yer alan çok sayıda kombinasyonda resiprokal farklılıkların önemli olması, bu özellikler için ıslah çalışmasının başlangıcında anaçların ana veya baba olarak kullanılmasının önemini göstermektedir. Buna karşın lif inceliği, lif dayanıklılığı ve çırcır randımanı yönünden ise hatların çoğunluğunda resiprokal farklılıkların önemli olmadığı tespit edildi. F₄ ve F₅ generasyonlarına ilişkin genel ortalamalarının farkı lif uzunluğu ve lif inceliği yönünden önemli bulundu. F₅ generasyonundaki hatların daha uzun ve daha ince liflere sahip olduğu saptandı. Bunlara ek olarak, F₄ generasyonu hatlarında tek bitki kütlü pamuk verimi 100,30-160,87 g; çırcır randımanı %41,96-47,17; lif uzunluğu 30,04-32,94 mm; lif inceliği 4,52-5,11 mic. ve lif dayanıklılığı 32,50-34,60 g tex⁻¹ arasında değişirken, F₅ generasyonunda tek bitki kütlü verimi 101,11-137,63 g; çırcır randımanı %41,45-46,54; lif uzunluğu 30,55-33,09 mm; lif inceliği 4,24-4,90 mic. ve lif dayanıklılığı ise 32,03-34,30 g tex⁻¹ arasında değişmiştir. Kütlü pamuk verimi için bulunan daha yüksek kendileme depresyonu çırcır randımanı ve lif kalite özelliklerine kıyasla bu özelliğin kontrolünde eklemeli olmayan gen etkilerinin daha baskın olduğunun bir göstergesiydi. Çalışma sonucunda, kütlü pamuk verimi ve lif özellikleri yönünden üstün performans gösteren Carmen × Carisma, Flash × Claudia ve ST-468 × Claudia F₅ ileri hatlarının F₆ generasyonuna aktarılmasının yararlı olacağı kanısına varıldı.

Anahtar Kelimeler: Atasal kalıtım, F₄ ve F₅, Lif kalitesi, Kütlü pamuk verimi, Seleksiyon

1. Introduction

Türkiye is a leading cotton producer (Ağazade, 2021). The most important fiber crop, cotton (*Gossypium hirsutum* L.), is cultivated in a total area of 500 thousand ha in the Southeastern Anatolia, Aegean, and Mediterranean regions of Türkiye. Cotton is extensively used in the textile industry and oil sector, and its cottonseed meal is also utilized in animal nutrition. Environmental factors, crop management, and genetic factors affected the seed cotton yield and quality parameters (Xiao and Tao, 2014; Rizzo et al., 2022). Plant breeders constantly try to develop new cotton varieties with higher yields and quality (Bilgin and Korkut, 2005). Choosing a suitable parent in the cotton breeding program is one of the most important processes (Lukonge et al., 2008). In other words, many crosses are required to determine suitable parents based on progeny performance in cotton, as in all cultivated plants. The mean performance is the primary criterion for choosing better hybrids because it exposes their real value.

The reciprocal effect, cytoplasmic effects, and parent-of-origin effect are epigenetic phenomena, and they are defined as the difference between the hybrid performance obtained when classical hybrids and parents are replaced. Thus, the choice of which parent will be used as male or female in breeding studies is determined. Numerous genetic factors influence reciprocal differences, perhaps the most important of which is that two sets of chromosomes of the endosperm, which contains three sets of chromosomes, come from the maternal parent (Gonzalo et al., 2007). Moreover, non-allelic reciprocal effects were reported to lead to invalidation of the additive-dominant model (Abedi et al., 2015).

In studies on cotton, the results of the full diallel analysis indicated different reciprocal effects in terms of significance. Shaukat et al. (2013) emphasized significant specific combining ability effects and reciprocal effects for fiber fineness, length, and strength, whereas non-significant reciprocal effects for the said traits were recorded by Hussain et al. (2010). Non-significant differences between reciprocal crosses in the F₁ and F₂ generations were recorded for yield, yield components, and fiber quality parameters, whereas significant reciprocal differences were detected in the F₃ generation by Zhang et al. (2016). Similarly, it was stated that the effects of combining ability for boll number per sympodial branch and plant, boll weight, ginning out-turn, and seed cotton yield differ in reciprocal crosses (Khan et al., 2015). It was emphasized that using elite cultivars as female parents had higher performance than their reciprocals because direct cross hybrids outperformed their respective reciprocal crosses for most of the traits evaluated (AnandaLekshmi et al., 2023).

Studies on the reciprocal effect have concentrated on early generations. However, more accurate predictions can be achieved in later generations when homozygosity and homogeneity increase. In addition, in cases where reciprocal lines are used, their performance in later generations is unknown. Therefore, we tested the hypothesis that the reciprocal effect decreases in advanced generations to determine whether the reciprocal effect is important not only in hybrid cultivar breeding and pedigree and bulk breeding processes. For this purpose, we evaluated seven advanced lines and their reciprocals in F₄ and F₅ bulk population generations regarding fiber properties and yield.

2. Materials and Methods

The initial material of the study was developed by crossing seven cotton (*Gossypium hirsutum* L.) varieties (Gloria, Claudia, Carmen, Julia, ST-468, Carisma, and Flash) according to the reciprocal line × tester method (4 × 3 and versus) at Cotton Research Institute, Nazilli-Aydın. F₄ bulk populations were developed from selected seven cross combinations and their reciprocals. Half of the seeds of the F₄ populations were saved for the following year. In 2020, F₄ and F₅ bulk populations were grown in a Randomized Complete Block Design with three replications.

The soil structure of the trial field is slightly alkaline and non-saline, with very high lime content, high nitrogen content, medium phosphorus levels, and low potassium levels. According to climatic data in 2020, the highest maximum daily temperatures were recorded in July (39.7 °C) and August (39.1 °C). May and October had the lowest minimum daily temperatures (14.7 and 14.3 °C, respectively). The average daily temperatures varied from 20.6 °C (October) to 28.9 °C (July), and the average monthly rainfall was below 1.0 mm.

Plots comprised six 12-m-long rows with a 70 × 20 cm sowing norm. Agricultural practices such as fertilization, weed control, irrigation, and insect control were standard practices for the Aegean Region, Türkiye. We observed 20 competitive plants to detect biometrical traits, seed cotton yield per plant (g), ginning out-turn (%), and fiber quality parameters for each genotype at each plot. Fiber fineness (mic.), fiber length (mm), and fiber strength (g tex⁻¹) were also determined using Uster® HVI-1000 at the fiber quality laboratory at Nazilli Cotton Research Institute.

The observed data were subjected to analysis of variance (Steel et al., 1997) using the ‘agricolae’ package (Mendiburu and Mendiburu 2019) in R software. The least significant difference (LSD) test and orthogonal contrast technique were used to separate genotype and generation means, respectively. Reciprocal cross advantages (R) were calculated according to the formula proposed by Bulant et al. (2000);

$$R (\%) = \frac{\text{Normal cross mean} - \text{Reciprocal cross mean}}{\text{Normal cross mean}} \times 100 \quad (\text{Eq.1}).$$

Inbreeding depression and critical value for significance were calculated according to the following method (Singh, 2003);

$$\text{Inbreeding depression} (\%) = \frac{F_4 - F_5}{F_4} \times 100 \quad (\text{Eq.2}).$$

$$\text{Critical Value} = \frac{\sqrt{2MSE}}{r} \times 5\% \text{ and } 1\% \text{ t table value.} \quad (\text{Eq.3}).$$

where; MSE is the mean square for error and r is the number of replications.

3. Results

The significant reciprocal effects for seed cotton yield were recorded in Carmen × Carisma, Carmen × Flash, Flash × Gloria, and Carisma × Julia in both generations (*Table 1*). The difference between the grand means of the F₄ and F₅ populations was non-significant. Carmen × Carisma in F₄; Carmen × Carisma and ST-468 × Claudia in F₅ produced the highest seed cotton yield. The negative and significant inbreeding depression was observed in Carmen × Carisma and its reciprocal and Gloria × Flash, whereas F₅ lines of Flash × Carmen, Flash × Claudia and its reciprocal, Julia × ST-468 and ST-468 × Claudia had higher performance than that of F₄ lines (*Table 1*).

Table 1. Reciprocal differences, mean value, and inbreeding depression for seed cotton yield.

Genotype	Reciprocal	Reciprocal Advantage (%) F ₄	Reciprocal Advantage (%) F ₅	Seed Cotton Yield (g)		Inbreeding Depression
				F ₄	F ₅	
Carmen × Carisma	A × B	-20.60**	-20.43**	160.87	137.63	14.44**
Carisma × Carmen	B × A			127.73	109.52	14.26*
Carmen × Flash	A × B	-19.65**	-17.66*	124.83	122.79	1.63
Flash × Carmen	B × A			100.30	101.11	-0.81
Flash × Gloria	A × B	26.70**	18.97*	109.87	103.75	5.57
Gloria × Flash	B × A			139.20	123.44	11.32*
Flash × Claudia	A × B	-4.14	-2.98	112.03	130.14	-16.16*
Claudia × Flash	B × A			107.40	126.27	-17.57*
Carisma × Julia	A × B	16.80*	24.18**	106.77	103.55	3.01
Julia × Carisma	B × A			124.70	128.59	-3.12
ST-468 × Julia	A × B	6.93	1.93	111.13	104.57	5.90
Julia × ST-468	B × A			118.83	106.59	-10.30*
ST-468 × Claudia	A × B	2.85	-3.73	118.23	136.40	-15.36*
Claudia × ST-468	B × A			121.60	131.31	-7.99
Mean				120.25	125.41	-5.33
LSD (0.05)				16.70	13.89	
F ₄ vs F ₅				ns		

*, **, significant at 5% and 1% probability level, respectively. ns: non-significant.

Ginning out-turn values of ST-468 × Claudia, Flash × Claudia, and their reciprocals significantly differed in both generations (Table 2). The grand mean of the F₄ generation was significantly superior to F₅. Flash × Claudia in F₄ and ST-468 × Claudia lines in both generations had the higher ginning out-turn. Gloria × Flash, Flash × Claudia, Carisma × Julia, and Claudia × ST-468 exhibited negative and significant inbreeding depression.

Table 2. Reciprocal differences, mean value, and inbreeding depression for ginning out-turn.

Genotype	Reciprocal	Reciprocal Advantage (%) F ₄	Reciprocal Advantage (%) F ₅	Ginning out-turn (%)		Inbreeding Depression
				F ₄	F ₅	
Carmen × Carisma	A × B	1.48	-0.71	43.95	44.18	-0.51
Carisma × Carmen	B × A			43.43	43.86	-0.99
Carmen × Flash	A × B	1.45	2.39	41.96	42.18	-0.52
Flash × Carmen	B × A			42.57	43.19	-1.46
Flash × Gloria	A × B	2.63	-0.45	42.12	41.98	0.32
Gloria × Flash	B × A			43.23	41.79	3.32*
Flash × Claudia	A × B	-7.11**	-5.25**	47.17	45.12	4.34**
Claudia × Flash	B × A			43.81	42.75	2.43
Carisma × Julia	A × B	-3.43	-3.75	44.72	43.06	3.71*
Julia × Carisma	B × A			43.19	41.45	4.03*
ST-468 × Julia	A × B	1.11	0.54	43.84	44.24	-0.90
Julia × ST-468	B × A			44.33	44.48	-0.34
ST-468 × Claudia	A × B	-6.18**	-9.28**	46.97	46.54	0.92
Claudia × ST-468	B × A			44.07	42.22	4.19**
Mean				44.17	43.36	2.27
LSD _(0.05)				1.97	1.72	
F ₄ vs F ₅				ns		

*, **, significant at 5% and 1% probability level, respectively. ns: non-significant.

Table 3. Reciprocal differences, mean value, and inbreeding depression for fiber length

Genotype	Reciprocal	Reciprocal Advantage (%) F ₄	Reciprocal Advantage (%) F ₅	Fiber Length (mm)		Inbreeding Depression
				F ₄	F ₅	
Carmen × Carisma	A × B	2.12 ^{ns}	1.60 ^{ns}	30.59	31.18	-1.92
Carisma × Carmen	B × A			31.24	31.68	-1.40
Carmen × Flash	A × B	-0.64 ^{ns}	1.69 ^{ns}	30.96	31.23	-0.88
Flash × Carmen	B × A			30.76	31.76	-3.24
Flash × Gloria	A × B	-5.60**	-4.76**	31.83	32.13	-0.96
Gloria × Flash	B × A			30.04	30.60	-5.19**
Flash × Claudia	A × B	4.87**	4.31*	30.61	31.28	-2.18
Claudia × Flash	B × A			32.10	32.62	-1.63
Carisma × Julia	A × B	-3.70*	-6.57**	32.38	33.09	-2.19
Julia × Carisma	B × A			30.22	30.91	-2.29
ST-468 × Julia	A × B	4.54**	4.42*	29.87	30.55	-2.29
Julia × ST-468	B × A			31.22	31.90	-2.17
ST-468 × Claudia	A × B	4.79**	3.29*	31.43	31.12	1.00
Claudia × ST-468	B × A			32.94	32.14	2.42
Mean				31.09	31.66	-1.87
LSD _(0.05)				0.99	1.01	
F ₄ vs F ₅				**		

*, **, significant at 5% and 1% probability level, respectively. ns: non-significant.

Table 4. Reciprocal differences, mean value and inbreeding depression for fiber fineness.

Genotype	Reciprocal	Reciprocal Advantage (%) F ₄	Reciprocal Advantage (%) F ₅	Fiber Fineness (mic.)		Inbreeding Depression
				F ₄	F ₅	
Carmen × Carisma	A × B	6.73*	3.46 ^{ns}	4.70	4.43	5.74*
Carisma × Carmen	B × A			5.02	4.59	8.63**
Carmen × Flash	A × B	-1.37 ^{ns}	-5.82*	5.11	4.76	6.98**
Flash × Carmen	B × A			5.04	4.48	11.17**
Flash × Gloria	A × B	0.35 ^{ns}	1.11 ^{ns}	4.74	4.49	5.14*
Gloria × Flash	B × A			4.75	4.54	4.42
Flash × Claudia	A × B	-6.06*	-8.51**	4.95	4.90	1.14
Claudia × Flash	B × A			4.65	4.48	3.72
Carisma × Julia	A × B	-4.84 ^{ns}	4.71 ^{ns}	4.75	4.46	6.04*
Julia × Carisma	B × A			4.52	4.67	-3.39
ST-468 × Julia	A × B	2.41 ^{ns}	-1.55 ^{ns}	4.57	4.31	5.69*
Julia × ST-468	B × A			4.68	4.24	9.33**
ST-468 × Claudia	A × B	-0.89 ^{ns}	2.84 ^{ns}	4.87	4.46	8.29**
Claudia × ST-468	B × A			4.82	4.59	4.84
Mean				4.80	4.53	5.55
LSD (0.05)				0.27	0.21	
F ₄ vs F ₅					**	

*, **, significant at 5% and 1% probability level, respectively. ns: non-significant.

Table 5. Reciprocal differences, mean value, and inbreeding depression for fiber strength.

Genotype	Reciprocal	Reciprocal Advantage (%) F ₄	Reciprocal Advantage (%) F ₅	Fiber Strength (g tex ⁻¹)		Inbreeding Depression
				F ₄	F ₅	
Carmen × Carisma	A × B	-2.70 ^{ns}	4.58 ^{ns}	33.65	32.03	4.79
Carisma × Carmen	B × A			32.74	33.50	-2.33
Carmen × Flash	A × B	1.25 ^{ns}	3.63 ^{ns}	34.17	33.10	3.13
Flash × Carmen	B × A			34.60	34.30	0.86
Flash × Gloria	A × B	-1.12	-3.72	33.10	34.07	-2.93
Gloria × Flash	B × A			32.73	32.80	-3.59
Flash × Claudia	A × B	2.64 ^{ns}	3.83 ^{ns}	32.67	32.20	1.45
Claudia × Flash	B × A			33.54	33.43	0.31
Carisma × Julia	A × B	2.72 ^{ns}	-3.05 ^{ns}	33.64	33.53	0.32
Julia × Carisma	B × A			32.50	32.51	-0.02
ST-468 × Julia	A × B	0.20 ^{ns}	1.30 ^{ns}	33.29	33.23	0.17
Julia × ST-468	B × A			33.36	33.67	-0.93
ST-468 × Claudia	A × B	1.78 ^{ns}	4.08 ^{ns}	33.83	32.67	3.44
Claudia × ST-468	B × A			34.43	34.00	1.26
Mean				33.44	33.20	0.59
LSD (0.05)				1.70	1.71	
F ₄ vs F ₅					ns	

*, **, significant at 5% and 1% probability level, respectively. ns: non-significant.

The reciprocal differences of ST-468 × Claudia, ST-468 × Julia, Carisma × Julia, and Flash × Claudia in both generations and Flash × Gloria in F₄ were significant for fiber length (Table 3). It was shown that the F₅ bulk populations produced higher fiber lengths than those of the F₄. This was generally reflected positively in inbreeding depression, except for ST-468 × Claudia and its reciprocal.

Flash × Claudia in both generations, Carmen × Carisma in F₄, and Carmen × Flash in F₅ differed from the reciprocals for fiber fineness (Table 4). The difference between the grand mean of the generations was significant.

Julia $\times$ Carisma and ST-468 $\times$ Julia in F₄, ST-468 $\times$ Julia and its reciprocal in F₅ had positive performance. The generally positive and significant inbreeding depression indicated that F₅ bulk populations exhibited finer fibers.

The reciprocal differences of all bulk populations in both generations were non-significant for fiber strength (Table 5). The grand mean of F₄ and F₅ lines were similar, whereas Julia $\times$ Carisma, Flash $\times$ Claudia, Gloria $\times$ Flash, and Carisma $\times$ Carmen in F₄; Carmen $\times$ Carisma and Flash $\times$ Claudia in F₅ had significantly lower fiber strength than that of other lines. Non-significant inbreeding depression values indicated that the fiber strength of F₅ lines was unchanged.

4. Discussion

Most hybrid combinations showing reciprocal significance were recorded in fiber length and seed cotton yield, whereas fiber strength was the character for which there was no reciprocal difference. The fact that the reciprocal differences for seed cotton yield and fiber length in cross combinations were significant in both generations gives the impression that the reciprocal differences will not change from generation to generation. In parallel with our study results, Khan et al. (2009) and Zangi et al. (2010) pronounced the reciprocal differences for yield and yield components, whereas significant reciprocal effects for days to flowering, seeds/locule, and lint percentage (Khan et al., 2011) and fiber fineness (Wells and Meredith, 1984) were estimated in an earlier generation.

The grand mean of F₄ and F₅ for observed characters indicated that seed cotton yield, ginning out-turn, and fiber strength remained stable, while the F₅ bulk population had significantly finer and longer fiber. This showed that the breeding program carried out was generally successful. According to Uster cotton classification, fibers between 4.0 and 4.9 mic.; 29.5 and 32.5 mm; and above 30.0 g tex⁻¹ are defined as average, long, and strong, respectively (USTER, 2024). The fiber properties of all F₅ bulk populations in our study meet these standards. Carmen $\times$ Carisma, Flash $\times$ Claudia, and ST-468 $\times$ Claudia combinations were also performed for seed cotton yield and ginning out-turn.

Ginning out-turn and fiber quality characteristics compared to yield presented lower inbreeding depression, corroborating the results reported by Khan et al. (2007), Khan et al. (2009), and Tigga et al. (2017). The high inbreeding depression for seed cotton yield indicated that dominance persists even in advanced generations such as F₅ (Carvalho et al., 2018). The combinations such as Carmen $\times$ Carisma, Flash $\times$ Claudia, and ST-468 $\times$ Claudia with high performance in terms of yield showed high and significant inbreeding depression. It was concluded that continuing the bulk selection method would be useful, especially in these combinations.

5. Conclusions

It is concluded that reciprocal differences persist in advanced generations. For this reason, at the beginning of the breeding program, selecting suitable parents and determining whether they will be used as males or females would be useful. The higher inbreeding depression for seed cotton yield indicates continued dominance compared with other traits. It was concluded that Carmen $\times$ Carisma, Flash $\times$ Claudia, and ST-468 $\times$ Claudia F₅ lines should be transferred to the F₆ generation. The fact that the negative associations between yield and ginning out-turn and fiber quality characteristics found in other combinations were broken in these three combinations shows the success of suitable parent selection.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Ünay, A.; Design: Balcı, Ş., Ünay, A.; Data Collection or Processing: Balcı, Ş.; Statistical Analyses: Çınar, V.M.; Literature Search: Çınar, V.M., Ünay, A.; Writing, Review and Editing: Balcı, Ş., Çınar, V.M., Ünay, A.

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