



Performances of F₃ and F₄ Bulk Populations in Cotton (*Gossypium hirsutum* L.)

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Abstract: The experiment was laid out in 2020 to compare the performance of thirty-seven F₃ and F₄ multi-parental bulk populations of cotton, including comparative cultivars, and to assess the heritability of traits studied. The differences within generations were significant for seed cotton yield, ginning out-turn, fiber quality, and nep fragments. The mean fiber strength of the F₄ generation showed significant performance compared with F₃. The broad sense heritability was high for ginning out-turn, fiber length, and fiber strength. The normal distribution for F₃ and F₄ generations due to non-significant skewness and kurtosis values indicated that there were no epistatic effects on the heritability of traits studied. Eight F₄ lines were selected for transfer to F₅ generation according to optimization in terms of desired traits.

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1. Introduction

Approximately 95% of the world's cotton production belongs to varieties within the *Gossypium hirsutum* L. (Chen et al., 2007; Fang et al., 2017). Türkiye produces 3.00–3.25% of the world's cotton annually (Anonymous, 2022). Cotton breeders' primary aim is high yield and fiber quality (Abdel-Aty et al., 2023). To improve cotton yield and fiber quality, appropriate breeding strategies are required to produce useful genetic variations and to identify desirable traits (Shahzad et al., 2022). Since high lint yield is the most important goal in cotton breeding programs, the unfavourable association between high yield and fiber quality traits has been ignored (Yang et al., 2023).

The success of the crop-breeding programme is determined by the selection process, and the bulk method is the third preferred method by public and private breeders in cotton (Bowman, 2000). Since the dominant gene variance is higher than the additive gene variance, applying the bulk method as an alternative to the pedigree method in early generations, such as F₂, F₃, and F₄, may be successful for breeding fiber quality traits (May and Green, 1994). It was reported that yield decreased from the F₃ generation to the F₅ generation, and the resulting significant inbreeding depression limited the success of selection in the early generations (Meredith, 1979). Furthermore, it was found that performance in early generations was not a predictor for later generations (Meredith and Bridge, 1973; Galanopoulou-Sendouca and Roupakias, 1999; Basal et al., 2017). In other studies, Khan (2003) and Khan et al. (2009)

determined that the plant families in the F₃, F₄, and F₅ generations gave superior values for fiber quality parameters than the standard varieties due to transgressive expansion and homozygotization.

High values for heritability in a broad sense indicated phenotypic variability due to genetic factors (Elhousary, 2023), whereas the low level of heritability shows the effect of non-additive genes and environment in the inheritance of studied traits. Regarding the broad sense heritability estimated for seed cotton yield, quite different results were found, ranging from low to high. (Balcı et al., 2020; Rehman et al., 2020). Skewness and kurtosis determine the presence of epistatic effects in the inheritance of the trait under study and provide information about the genetic background and structure of the population in the segregating generations (Savitha and Kumari, 2015).

Earlier studies were generally concentrated in early generations with very few hybrid combinations. For this reason, we conducted experiments with 37 multi-parental hybrid combinations in the later generations, such as F₃ and F₄, to estimate heritability and gene action patterns and determine the promising combinations to be transferred to the next generations.

2. Material and Methods

The F₃ and F₄ bulk populations used in the study were the progeny of 37 different cotton multi-parental cross combinations obtained from breeding studies conducted between 2013 and 2019 (Table 1). F₃ and F₄ populations originate from super lines obtained from cycle-1 of recurrent selection introduced by Balcı et al. (2021). We conducted the study at Cotton Research Institute, Nazilli, Aydın/Türkiye, in 2020. F₃ and F₄ generations of these 37 families and comparative cultivars, Gloria and ST-468, were arranged in Randomized Complete Block Design with three replications. Each population was grown in a single row of 12 m in length at 70 × 20 cm spacing. Twenty-five plants were randomly sampled from the middle 10 m section and were recorded seed cotton yield per plant (g) and ginning out-turn (%). Fiber fineness (FF; mic), fiber length (FL; mm), and fiber strength (FS; g tex⁻¹) were determined by HVI analysis. Nep count (number g⁻¹) and seed coat nep count (SCN; number g⁻¹) were measured by USTER® AFIS Pro 2 instrument.

Table 1. Designation of F₃ and F₄ bulk population

Code	Pedigree	Code	Pedigree
1	(Julia × ST-468) × (Gloria × Carisma) - I	44	(Carmen × ST-468) × (Gloria × Flash) - II
10	(Julia × ST-468) × (Gloria × Carisma) - II	3	(Carisma × Carmen) × (Gloria × Flash) - I
6	(Julia × ST-468) × (Gloria × Carisma) - III	18	(Carisma × Carmen) × (Gloria × Flash) - II
5	(Gloria × Flash) × (Gloria × Carisma) - I	20	(Carmen × ST-468) × (Gloria × Carisma) - I
13	(Gloria × Flash) × (Gloria × Carisma) - II	22	(Carmen × ST-468) × (Gloria × Carisma) - II
34	(Gloria × Flash) × (Gloria × Carisma) - III	8	(ST-468 × Claudia) × (Gloria × Flash) - I
15	(Julia × ST-468) × (Gloria × Flash) - I	38	(ST-468 × Claudia) × (Gloria × Flash) - II
24	(Julia × ST-468) × (Gloria × Flash) - II	4	(Carmen × ST-468) × (ST-468 × Claudia)
11	(Julia × ST-468) × (Gloria × Flash) - III	7	(Gloria × Flash) × (Gloria × Carisma)
16	(Julia × ST-468) × (Gloria × Flash) - IV	9	(Julia × ST-468) × (Carmen × Carisma)
31	(ST 468 × Claudia) × (Gloria × Carisma) - I	12	(Gloria × Flash) × (Gloria × Flash)
32	(ST 468 × Claudia) × (Gloria × Carisma) - II	14	(Julia × ST-468) × (ST-468 × Claudia)
42	(Carmen × ST-468) × (Gloria × Flash) - I	17	(Carmen × ST-468) × (Carmen × Carisma)
19	(Carmen × ST-468) × (Carisma × Carmen)	36	(ST-468 × Claudia) × (Julia × ST-468)
21	(Julia × ST-468) × (Carisma × Carmen)	37	(Carmen × Carisma) × (Gloria × Carisma)
26	(Carmen × Carisma) × (ST-468 × Claudia)	39	(Carmen × ST-468) × (Julia × ST-468)
29	(Julia × ST-468) × (Julia × ST-468)	40	(Gloria × Carisma) × (Gloria × Carisma)
30	(Carisma × Carmen) × (Gloria × Carisma)	41	(Carmen × Carisma) × (Gloria × Flash)
35	(Julia × ST-468) × (Carmen × ST-468)		

ANOVA was performed to compare the means of observed traits between and within generations. Distribution curves were formed for all observed traits, and skewness and kurtosis were estimated using the frequency distribution (Kapur, 1981). According to Singh and Chaudhary (1985), descriptive statistics were performed using the JMP® 14 statistical program (JMP, 2018). We calculated broad-sense heritability (h²bs) using variance component analysis as follows;

Environmental variance (σ^2_e) = Mean square of error
 Genotypic variance (σ^2_g) = (mean square of genotype - mean square of error) / replication
 Phenotypic variance (σ^2_p) = $\sigma^2_g + \sigma^2_e$

$$h^2_{bs} = \frac{\sigma^2_g}{\sigma^2_p} \times 100 \text{ (Allard, 1960).} \quad (1)$$

Heritability was considered low ($\leq 30\%$), moderate (30-60%), and high ($60\% \leq$) (Srinivas et al., 2014).

3. Results and Discussion

Significant differences for all traits except seed coat neps within F₃ and F₄ indicated the opportunity for efficient selection among populations (Table 2). The effectiveness of breeding programs depends on the performance and variability among segregating populations (El-Shazly et al., 2023). Similar variability emphasized by El-Mansy (2005) and El-Shazly (2013) in later generations of cotton. The mean differences between F₃ and F₄ generations were significant for ginning out-turn, fiber fineness, fiber length, fiber strength, and nep fragments. Fiber strength increased in F₄ generations compared with F₃, whereas ginning out-turn and fiber length reduced, and fibers coarsed and nep fragments unfavourable increased in F₄ generations. In terms of the mentioned traits, it can be said that inbreeding depression is clearly manifested in the F₄ generation (Meredith, 1979; Wu et al., 2010; Basal et al., 2017).

Table 2. Mean values, heritability and comparisons of F₃ and F₄ populations

Gen.	SCY		GOT		FL		FF		FS		SCN		Neps	
	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4
1	93.0	109.0	39.8	42.0	32.2	30.4	4.3	4.7	32.1	32.7	13.0	16.3	49.1	53.8
10	95.9	111.0	46.8	41.4	31.2	30.8	4.8	4.7	34.0	35.4	14.7	9.0	36.5	35.1
6	91.7	102.9	43.7	44.6	31.2	30.9	4.5	5.0	33.7	34.3	10.0	8.3	27.8	54.5
5	95.5	107.7	41.9	40.9	30.7	32.5	4.5	4.3	32.8	34.1	6.0	12.3	34.5	67.1
13	108.6	107.5	43.9	42.9	30.4	31.1	4.5	4.5	31.9	35.2	9.7	7.0	37.1	45.8
34	69.1	90.4	43.7	42.7	31.0	30.5	4.3	4.7	31.2	33.8	24.3	6.0	98.5	37.1
15	98.1	95.2	44.5	41.4	31.1	32.2	4.6	4.5	33.2	34.6	19.0	15.0	42.5	51.8
24	100.1	103.0	43.4	42.4	31.1	30.2	4.8	4.6	34.1	32.0	10.0	16.0	24.5	46.5
11	94.0	118.6	41.3	41.7	32.3	31.3	4.5	4.6	33.9	32.5	15.7	11.0	47.1	45.8
16	108.2	112.7	42.4	40.3	30.7	32.0	4.7	4.7	32.0	34.4	14.3	12.7	41.8	49.1
31	92.0	103.1	40.5	41.7	31.0	31.0	4.3	4.5	32.5	33.0	5.0	13.0	33.1	59.8
32	83.3	107.9	45.7	42.2	30.5	30.8	4.7	4.7	32.4	34.9	5.0	3.0	29.8	14.8
42	85.7	96.5	42.3	43.6	31.9	30.6	4.5	4.8	33.8	34.3	9.5	15.3	41.1	42.5
44	100.6	108.0	44.5	41.3	30.7	31.1	4.6	4.6	32.1	33.5	7.7	30.8	38.5	58.5
3	107.9	87.8	46.9	42.5	31.0	30.7	4.8	4.6	32.4	34.0	8.3	17.0	34.5	26.5
18	100.7	110.7	45.5	40.4	31.7	31.5	4.5	4.5	33.8	34.4	17.0	25.3	54.5	72.5
20	100.0	99.8	43.8	42.8	30.7	30.3	4.7	4.7	32.4	32.0	4.0	36.0	46.5	99.1
22	130.9	80.8	42.2	41.1	31.4	30.8	4.6	4.6	33.0	32.2	15.7	19.0	69.8	103.1
8	100.1	102.5	42.9	41.2	31.4	31.1	4.5	4.6	33.7	33.6	7.0	7.0	29.1	85.8
38	84.4	90.2	43.8	45.4	30.4	31.0	4.7	4.7	32.2	33.9	5.7	16.7	32.5	39.1
4	102.6	97.0	42.8	40.8	30.9	32.3	4.6	4.5	33.0	33.9	11.0	19.1	31.8	69.8
7	90.7	100.7	43.6	42.3	30.8	30.8	4.8	4.7	31.2	33.9	13.0	19.7	71.1	37.1
9	106.9	84.2	41.9	43.6	31.8	31.3	4.4	4.7	35.2	33.7	14.7	5.0	41.1	30.5
12	101.4	105.3	43.8	43.1	30.7	30.9	4.7	4.6	31.7	34.3	9.0	3.0	45.8	36.5
14	114.0	115.8	43.8	41.4	31.1	30.5	4.5	4.6	33.7	32.7	10.0	18.3	25.1	27.1
17	102.4	111.0	42.8	41.9	30.5	30.5	4.6	4.7	31.6	33.8	11.3	15.0	74.5	48.5
19	81.1	113.5	44.6	40.8	31.4	30.6	4.5	4.8	33.0	32.4	19.0	19.0	103.8	58.5
21	98.1	99.3	42.5	43.1	32.0	30.9	4.4	4.7	33.3	32.4	7.0	14.0	29.8	65.8
26	96.3	94.9	42.5	43.5	30.5	30.9	4.5	4.9	33.5	33.0	13.0	13.0	37.8	35.1
29	97.5	123.6	42.7	43.2	31.4	30.7	4.5	4.7	33.8	33.7	5.7	21.0	28.5	37.1
30	92.5	101.8	44.4	42.1	31.9	30.6	4.7	4.8	32.5	33.3	3.0	7.7	57.8	32.5
35	115.6	112.6	44.8	43.0	31.1	30.9	4.6	4.8	33.1	33.9	8.3	6.3	19.8	25.8
36	120.1	91.0	42.5	43.5	31.6	30.3	4.4	4.6	33.0	31.6	22.0	7.2	76.8	51.1
37	130.0	95.9	43.6	41.6	31.2	30.9	4.3	4.5	34.5	31.8	17.0	12.3	56.5	52.5
39	81.6	100.4	42.6	42.3	31.2	31.2	4.5	4.6	32.1	35.0	3.7	14.3	26.8	64.5
40	95.5	84.8	43.0	43.5	30.7	30.6	4.5	4.7	32.0	33.8	3.7	11.5	21.8	27.1

Table 1. Mean values, heritability and comparisons of F₃ and F₄ populations (continued)

Gen.	SCY		GOT		FL		FF		FS		SCN		Neps	
	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4	F3	F4
41	94.7	105.8	42.6	41.8	31.2	30.2	4.5	4.6	33.5	31.1	5.7	17.0	62.1	28.5
ST-468	72.7		44.6		27.4		5.4		30.2		14.8		51.5	
Gloria	102.2		42.5		28.9		5.3		32.8		10.5		41.8	
F3 Mean	98.9		43.4		31.2		4.6		32.9		10.8		44.9	
F4 Mean	102.2		42.3		30.9		4.7		33.5		14.1		49.1	
LSD_(0.05)	25.9	23.6	1.8	1.9	0.7	0.8	0.3	0.3	1.6	1.6	ns	ns	40.8	36.4
F₃ vs F₄	ns		**		*		**		**		*		ns	
h²bs (%)	36.76		76.88		76.40		52.38		71.34		19.32		53.41	

*; P < 0.05; **; P < 0.01; ns: non-significant, SCY: Seed cotton yield (g plant⁻¹); GOT: Ginning Out-Turn (%); FL: Fiber length (mm); FF: Fiber fineness (mic.); FS: Fiber Strength (g tex⁻¹).

F₃ and F₄ means showed that seed cotton yield per plant of both generations was similar to Gloria, whereas F₃ and F₄ generations produced significantly higher seed cotton yield than ST-468 (Table 1). Although the mean ginning out-turn of segregating populations in both generations was significantly lower than ST-468 cultivar, both generations had significantly stronger fibers than ST-468. Similarly, Azam et al. (2013), Kumar et al. (2014), and Fawad et al. (2022) compared F₃ populations with their parents and reported significant differences. In addition, both generations had significantly longer and finer fibers compared to the standard cultivars. The differences between populations and standard cultivars were non-significant for SCN and neps.

Heritability was estimated as low for seed cotton yield and seed coat neps; medium for fiber fineness and neps; and high for ginning out-turn, fiber length, and fiber strength (Table 1). It was concluded that environmental effects were higher than genetic factors in the inheritance of traits with low heritability. The high variance values observed in both generations for seed cotton yield and nep fragments confirmed the low and medium heritability levels. However, genetic variation in a broad sense of heritability can be due to additive as well as dominant and epistatic variation (Falconer and Mackay, 1996). Similar to the results of our study, non-additive gene effects were found to be higher for seed cotton yield (Lingaswamy et al., 2013; Usharani et al., 2016; Monicashree et al., 2017; Prakash et al., 2018). Zeng and Meredith (2010) found a moderately broad sense heritability for seed coat neps and reported that more than 50% of the variation was due to environment with genotype-environment interaction. In previous studies, broad sense heritability of 70% and above was found for fiber length (Fang et al., 2014; Gore et al., 2015; Huang et al., 2017; Ma et al., 2018) and ginning out-turn (Rehman et al., 2020; Ishaq et al., 2021). These results are in agreement with our study findings. Heritability ranging from high to low for different traits showed that bulk or modified bulk method should be applied for combination breeding. Evaluating breeding methods and selection criteria for improving fiber quality, May and Green (1994) concluded that the selection of F₂ bulk populations for fiber traits is more beneficial than selecting individual F₂ plants when initiating a line breeding program for quality improvement.

Non-significant skewness and kurtosis indicated that all traits had a normal distribution. Tabachnick and Fidell (2013) defined kurtosis and skewness values between -1.5 and +1.5 as normal distribution (Figures 1 and 2). Positive and negative skewness are indicators of complementary gene and duplicate (additive × additive) gene interactions, respectively. Negative or near-zero kurtosis showed the absence of gene interaction, while positive values are a signal of gene interaction (Choo and Reinbergs, 1982; Savitha and Kumari, 2015). In another study, Percy et al. (2006) found positive and high kurtosis values for seed cotton yield and fiber length but insignificant kurtosis values for fiber fineness. Our findings clearly showed that epistatic effects are not important in controlling all traits. Verhalen et al. (1971), Khan et al. (2003), and Khan et al. (2009) reported that epistatic gene effects were not significant in the genetic control of fiber quality traits. Although the longer right tail for seed cotton yield indicated that the expansion in the direction of high seed cotton yields continues, this aspect of the distribution is problematic in terms of nep fragments (Figure 1). These results show that simultaneous improvement between yield and neps is not possible, contrary to what was stated by Zeng and Meredith (2010). Therefore, seed cotton yield should be limited to a certain level.

In the F₄ generation, among 1174 plants, there were 247 superior plants with yield values 3 standard deviations above the population mean for seed cotton yield. This finding indicated that

approximately 21.04% of the population was suitable for high-yielding selection. The percentages of superior plants were 8.52% for ginning out-turn, 11.93% for fiber length, and 9.97% for fiber strength. 20.10% of the population was 3 standard deviations below the mean fiber fineness. Transgressive segregation could be said to cause this performance (Khan et al., 2009). Since the variance and, therefore, the standard deviation was high, this evaluation could not be made in terms of nep fragments. When optimization is desired in terms of all traits, 8 F₄ lines were selected for transfer to F₅ generation according to seed cotton yield above 90 g; ginning out-turn 42.2%; fiber length 30.2 mm; fiber strength 32.0 g tex⁻¹; fiber fineness between 4.5-4.7 mic.; seed coat neps below 21.0 and neps 64.5. Similar thresholds were proposed in the decision tree model developed by Çakmak et al. (2023). These lines were (Gloria × Flash) × (Gloria × Carisma); (Gloria × Flash) × (Gloria × Flash); (Gloria × Flash) × (Gloria × Carisma) – II; (Julia × ST-468) × (Gloria × Flash) – II; (Julia × ST-468) × (Julia × ST-468); (ST-468 × Claudia) × (Gloria × Carisma) – II; (Carmen × ST-468) × (Julia × ST-468) and (ST-468 × Claudia) × (Gloria × Flash) – II. These advanced lines were also the genotypes in which the negative association between yield and fiber quality traits was broken (Clement et al., 2015; Mızrak et al., 2020).

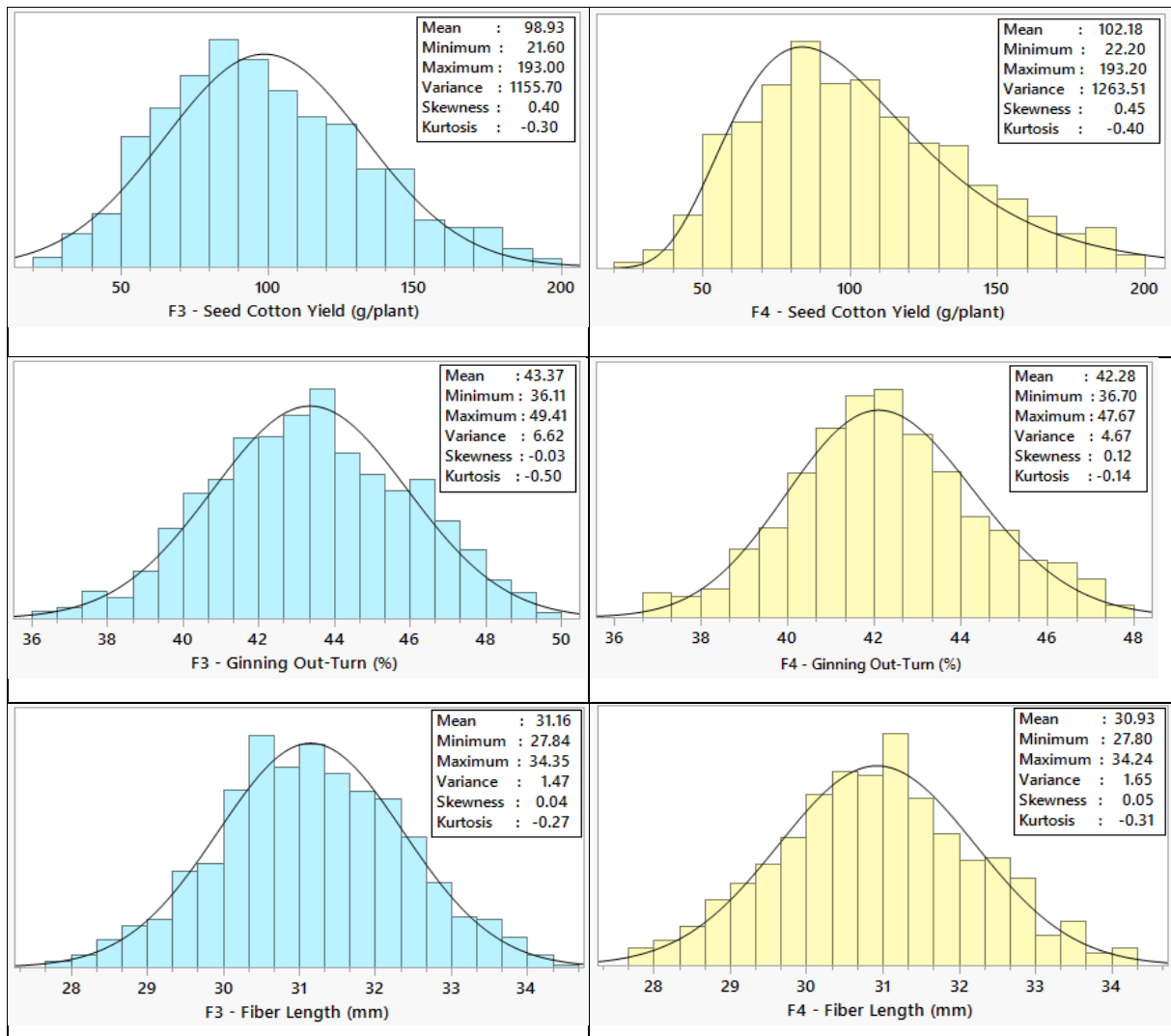


Figure 1. Normality distribution of yield and fiber quality in F₃ and F₄ generations.

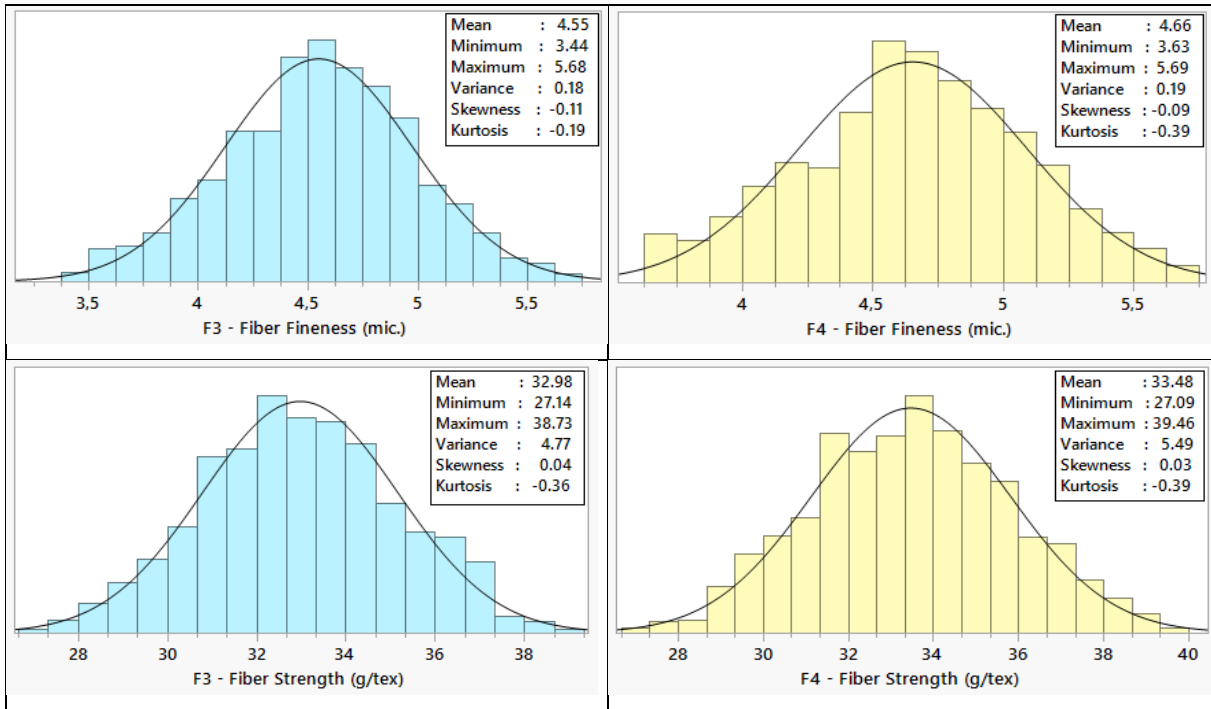


Figure 1. Normality distribution of yield and fiber quality in F₃ and F₄ generations (continued).

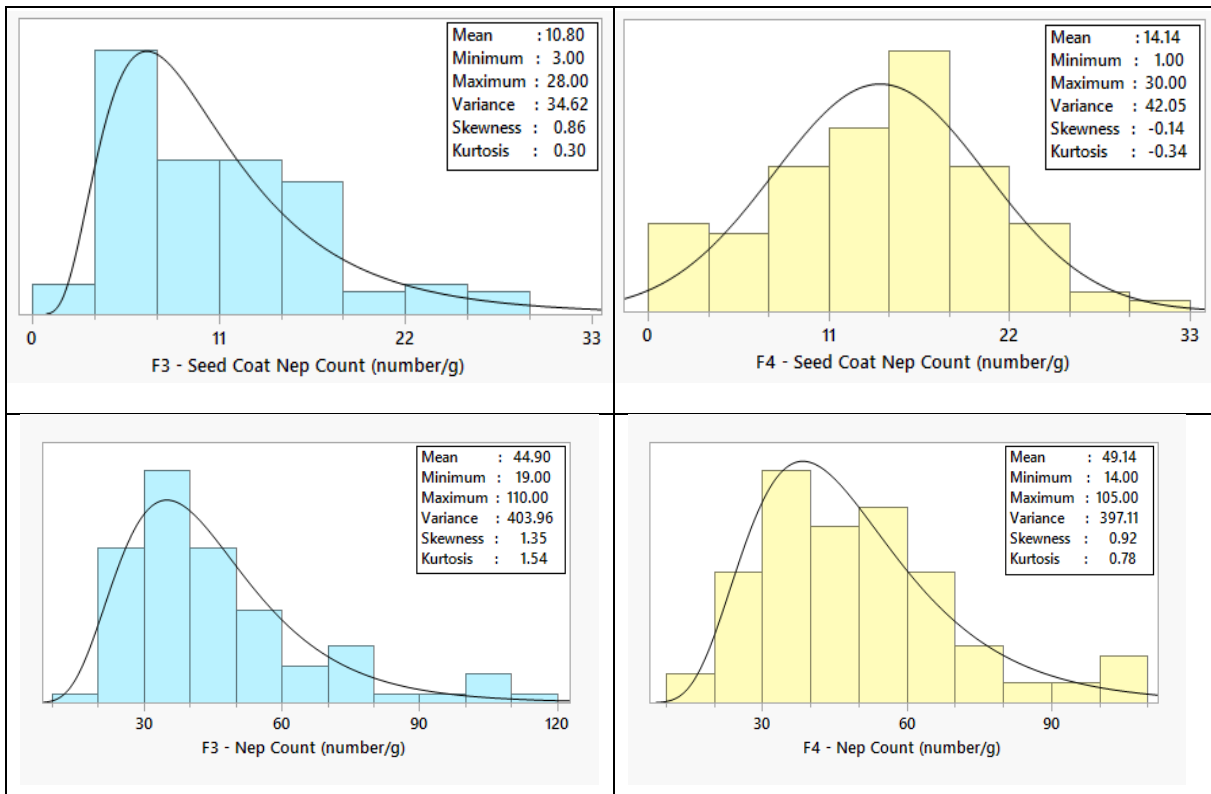


Figure 2. Normality distribution of nep fragments in F₃ and F₄ generations.

4. Conclusion

The results from the present study indicated the existence of sufficient variation for selection in multi-parental populations. Due to the continued high variation in seed cotton yield and nep fragments, single plant selection could be delayed until the F₆ generation. The most important goal for cotton breeders should be to combine high yields with improved fiber quality.

Contribution of the Authors

The authors declare that the contribution of the authors is equal.

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