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Research Article

Combining ability analysis and breeding for early-maturing cotton (*Gossypium hirsutum L.*) varieties using Line x Tester analysis

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Abstract
The development of early-maturing and high-yielding cotton varieties is a strategic priority in regions with limited growing seasons and increasing climate variability. This study investigates the genetic basis of earliness and evaluates the combining ability of selected cotton lines and cultivars using the Line x Tester hybridisation method. A set of lines (L-158, L-75, L-1703, L-1858, L-257) and testers (L-302, L-597, and the standard cultivar S-6530) were crossed to produce F_1 hybrids and their F_2 and F_3 populations. Field trials were conducted under arid continental conditions in Uzbekistan using standard agronomic practices. The results revealed significant variation in the length of the vegetative period among parental lines and hybrids. General and specific combining ability (*gca* and *sca*) analyses showed that lines L-158 and L-1858 had the highest negative *gca* effects for the vegetative period, indicating their potential for breeding early-maturing genotypes. F_1 hybrids displayed predominance and overdominance effects toward early-maturing original lines, while F_2 and F_3 generations exhibited wider-than-parental variation, leading to the identification of ultra-early recombinant lines. Several new breeding lines, including L-215, L-218, and L-178, demonstrated superior performance in earliness and agronomic traits. These findings provide a valuable framework for optimising selection strategies in cotton breeding programs and highlight the relevance of hybridisation for developing climate-resilient, early-maturing cultivars.

Keywords: Cotton, *gca*, *sca*, genetic variability

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most economically important fibre crops in the world, serving as a key raw material for the textile industry and a livelihood source for millions, especially in agrarian economies. The increasing global demand for natural fibres, coupled with rising concerns over climate change and water scarcity, necessitates the development of early-maturing, high-yielding, and stress-resilient cotton cultivars adaptable to a range of agroecological environments (Abdelraheem *et al.,* 2020; Saleem *et al.,* 2019).

Uzbekistan, situated at the northernmost boundary of the global cotton belt, has long depended on cotton production as a strategic agricultural sector. Historically, the country has devoted more than 1.1 million hectares to cotton cultivation, and significant institutional efforts have led to the zoning of over 40 cultivars since independence. However, many regionally zoned cotton cultivars remain late-maturing, poorly adapted to local microclimatic fluctuations, and susceptible to biotic and abiotic stress factors (Khan *et al.,* 2022). These deficiencies not only limit productivity but also reduce fibre quality, hindering competitiveness in international markets. In response, the Uzbek cotton breeding programs are now prioritising the development of early-maturing cultivars that can reach maturity before October 10, require minimal irrigation, and are compatible with mechanised harvesting techniques (Aishwarya *et al.,* 2025; Iqbal *et al.,* 2021).

Modern cotton breeding approaches increasingly emphasise the strategic selection of parental lines with complementary traits. Among the various breeding tools, Line x Tester mating design has shown promise in assessing the genetic potential of parent lines by evaluating their general combining ability (*gca*) and specific combining ability (*sca*) across diverse genetic backgrounds (Rauf and Naveed, 2018; Sprague and Tatum, 1942; Yadav, 2022). The *gca* reflects additive genetic effects, while *sca* highlights dominance and epistatic interactions. The Line x Tester design offers logistical advantages in breeding programs operating under resource constraints, particularly in short-season regions such as Uzbekistan. This dual analysis aids in selecting optimal parental combinations for breeding programs focused on early maturity and fibre improvement (Jinks, 1956; Zhang *et al.,* 2021; Korabayev *et al.,* 2024). Despite significant advances in marker-assisted selection and genomic breeding in recent years, conventional selection based on phenotypic traits remains crucial for breeding early-maturing cotton, especially in countries where genomic resources are still emerging. The integration of Line x Tester analysis with generation-wise field evaluation offers a viable pathway for capturing both additive and non-additive gene actions that influence earliness, boll number, plant height, and fibre yield.

The current study aims to investigate the inheritance patterns of earliness and to evaluate the *gca* and *sca* of selected cotton lines using Line x Tester hybridisation across F_1, F_2, and F_3 generations. By focusing on the vegetative period as a primary trait of interest, this research seeks to identify genetically stable, early-maturing genotypes suitable for Uzbekistan's continental climate. The findings are expected to inform breeding strategies targeting climate-smart cotton cultivation under resource-constrained conditions.

MATERIALS AND METHODS

The primary objective of this study was to investigate the inheritance of earliness traits and identify valuable donors, as well as to optimise selection methods for the development of early-maturing cotton varieties. The research employed a Line x Tester system to assess the general and specific combining abilities (*gca* and *sca*) of the initial and newly developed lines and hybrids of American cotton. The class boundaries for frequency distribution of vegetative period were determined based on the observed range of data across all populations to ensure consistent and comparable intervals. The F_3 generation was evaluated as individual plant progenies. Each number in **Table 3** represents the number of progenies falling within a specific class boundary. A total of 300 plants were screened per F_3 progeny.

Experimental design and location: Field experiments were conducted at the Uzbek Scientific Research Institute of Cotton Breeding, Seed Production, and Agrotechnology, located northeast of Tashkent (41°22′N, 60°54′E) at an altitude of 584 m. The soil was classified as typical sierozem on loess-like loams, non-saline, with deep groundwater (below 7–8 meters). The region features a sharply continental climate, with sunny days and an annual precipitation of approximately 360 mm, primarily in autumn, winter, and spring.

Plant materials and hybridisation: The initial genetic material included self-pollinated lines of *G. hirsutum* L. and the zoned cultivar C-6530. The maternal lines used were L-158, L-75, L-1703, L-1858, and L-257. The testers included L-597, L-302, and cultivar C-6530. Hybridisation followed standard breeding protocols with pre-flowering bud emasculation, isolation, and artificial pollination of 80–100 flowers per cross.

The F_1 hybrid nursery was established from F0 seeds with three replicates using 10-hole row plots. Phenological data collection during the growing season included dates of emergence, flowering, and boll opening. At harvest, raw cotton yield was measured, and boll size, lint percentage, and fibre length were determined in laboratory conditions. General combining ability and specific combining ability were estimated using the fixed-effects linear model proposed by Sprague and Tatum (1942), suitable for Line x Tester analysis. The analysis of variance (ANOVA) was performed to partition the total variability into components attributable to *gca* and *sca*. The significance of *gca* and *sca* effects was tested using Fisher's F-test. Variance components (GCA, SCA) were calculated to assess the relative importance of additive and non-additive genetic effects. Broad-sense heritability (H^2) for vegetative period length was estimated as:

$$H^2 = \sigma^2G / (\sigma^2G + \sigma^2E)$$

where,

σ^2G = genetic variance (GCA + SCA)
σ^2E = environmental variance

Software and tools: Statistical analyses were conducted using Microsoft Excel for descriptive statistics and GenStat v12.1 for ANOVA and combining ability estimation. Graphs and visualisations were created in R (version 4.2.2) using the ggplot2 package.

Trait evaluation: The trait of vegetation period length was particularly emphasised, characterised from sowing to boll opening. The vegetative period length (physiological maturity) was defined as the number of days from sowing to the opening of the first boll on the plant. This standard metric for assessing earliness in cotton breeding has been consistently employed in both regional and international research, including studies conducted under Egyptian conditions (Kandil and El-Hashash, 2019), line × tester analyses in Egyptian cotton (Abdel-Moneam and Sultan, 2020), and evaluations of cotton genotypes in Uzbekistan (Khodjaev *et al.,* 2022). The selected parental lines differed in this trait by 2–10 days, with L-158, L-75, and C-6530 being the earliest (127.7–132.6 days), while L-302 was the latest (137.5 days). Although the absolute difference in maturity among parental lines was modest (2-10 days), this variation was sufficient to generate significant transgressive segregation in the hybrid populations, highlighting the potential for genetic recombination.

RESULTS AND DISCUSSION

Combining ability and inheritance of vegetative period length: Uzbekistan represents the northernmost cotton-growing region globally, where early maturity is critical to achieving high-quality pre-frost cotton yields. Accelerated boll opening by 3–5 days can increase early harvest yield by 30–50%. Since earliness is a complex polygenic trait, parental lines with contrasting vegetative period lengths were selected for hybridisation. Parental lines differed by 2–10 days, with L-158, C-6530, and L-75 being the earliest (127.7–132.6 days), while L-302 was the latest (137.5 days).

Combining ability analysis revealed that lines L-1858 and L-158 had the most favourable general combining ability (GCA) effects, indicated by negative gi values. In the context of breeding for earliness, negative GCA values are desirable, reflecting reduced vegetative period. These findings are consistent with those of Zhang *et al.* (2021), who observed high negative GCA effects for vegetative traits in Chinese upland cotton lines under continental climates. In contrast, the highest specific combining ability (SCA) effects for earliness were observed in the cross L-158 × L-302, indicating a strong non-additive genetic interaction between these two parents. This aligns with the findings of Rauf *et al.* (2017), who emphasised the relevance of non-additive effects in early maturity under drought-prone conditions.

Table 1 presents GCA and SCA estimates for F_1 hybrids. Out of the 15 combinations, 12 showed dominance or overdominance in favour of early-maturing original lines, especially L-158 × L-302 and L-158 × C-6530 hybrids, which exhibited the shortest vegetative periods. These patterns align with findings from Singh and Sharma (2020), where topcross hybrids consistently expressed shorter vegetative durations than mid-parent values.

Variability and extended expression beyond parent values in F_2 generation: The F_2 segregants derived from late-maturing tester L-302 and early-maturing line L-158 displayed intermediate maturity but leaned toward the later parent. Crosses involving L-1858 and L-257 were closer to the early-maturing maternal lines. Interestingly, the L-302 × L-1703 hybrid maintained parity with both original lines in maturity duration. A reverse trend was noted in L-302 × L-75 hybrids, where the later-maturing trait dominated.

The mean vegetative period in F_2 hybrid populations ranged from 130.2 to 133.4 days. Variation patterns depended on the genotypes of both lines and testers. The F_2 hybrids involving dwarf lines and testers such as C-6530 and L-597 were 2–3 days later than their original lines, showing intermediate inheritance or slight regression. Such variability is in agreement with the observations by Ahmad *et al.* (2020), who noted broader-than-parental distribution in segregating populations of cotton crosses with diverse maturity genes.

Table 2 shows variability measures in F_2 hybrids. The variance ranged from 5.1 to 6.8 days (3.8%–5.2%), indicating moderate genetic divergence among parental forms. Most F_2 hybrids were distributed in the 126–137 day range, consistent with parental norms. However, wider-than-parental variation was observed in all combinations. Transgressive segregants were identified using a threshold of +1 and +2 standard deviations (SD) from the population mean for earliness. This led to the identification of recombinants that were significantly earlier than the earliest parent. Similar transgressive segregants were identified by Ali *et al.* (2022), highlighting their potential for creating novel early-maturing phenotypes.

Table 1. Combining ability of parental lines and F_1 hybrids for vegetative period length (sowing to maturity)

S.No.	Lines	C-6530	L-302	L-597	*gca* effect (gi)	*sca* effect (Sij)			SCA variance	GCA variance
						1	2	3		
1	L-158	127.77	125.60	129.80	-2.5844	0.4177	1.8556	1.6244	1.1006	16.5815
2	L-75	130.70	132.60	129.50	0.6267	0.1399	1.9333	1.8867	1.3657	0.8661
3	L-1703	132.50	130.90	133.37	1.9490	0.6177	1.0890	0.6577	0.0365	9.3802
4	L-1858	128.80	130.10	130.93	-0.3621	0.7712	0.4221	0.5355	-0.1986	0.212
5	L-257	129.90	131.00	130.20	0.0601	0.0934	0.8999	0.6201	-0.1627	-0.1068
6	*gca* effect (gi)	0.3733	0.2666	0.4534						
7	*SCA* variance (σ^2si)	0.0208	0.1251	0.0766	gi – gj = 0.7616,gi – gj = 0.6808,gi – gj = 0.5896 Sij – Sg = 1.1289					
8	*GCA* variance (σ^2gi)	0.1260	4.2139	3.1876						

Table 2.Variability for vegetative period length (sowing to first boll opening) in plants of F_2 populations and parents

S.No.	Parents and F_2 populations	Class boundaries (days)										X±Sx	S
		117-119	120-122	123-125	126-128	129-131	132-134	135-137	138-140	141-143	144-146		
1	C-6530	-	-	-	15	26	17	2	-	-	-	130.3±0.3	2.4
2	L-302	-	-	-	2	18	24	16	-	-	-	132.7±0.3	2.5
3	L-597	-	-	-	19	26	15	-	-	-	-	129.8±0.3	2.3
4	L-158	-	2	19	23	16	-	-	-	-	-	126.6±0.3	2.6
5	L-75	-	-	3	15	28	14	-	-	-	-	129.6±0.3	2.5
6	L-1703	-	-	-	-	17	23	18	2	-	-	133.2±0.3	2.5
7	L-1858	-	-	-	15	18	24	3	-	-	-	130.8±0.3	2.7
8	L-257	-	-	3	16	22	19	-	-	-	-	129.9±0.3	2.7
9	L-158 x C-6530	8	10	18	45	81	61	33	20	15	9	131.5±0.3	5.7
10	L-158 x L-302	-	14	27	32	67	58	50	29	14	9	132.2±0.3	5.7
11	L-158 x L-597	10	23	31	43	65	44	40	24	15	5	130.7±0.4	6.3
12	L-75 x C-6530	16	35	34	40	33	52	51	19	17	3	130.2±0.4	6.8
13	L-75 x L-302	5	20	29	30	31	55	46	40	31	13	132.9±0.4	6.8
14	L-75 x L-597	2	18	18	36	46	67	45	30	24	14	133.3±0.4	6.6
15	L-1703 x C-6530	-	16	35	28	42	56	50	43	22	8	132.7±0.4	6.2
16	L-1703 x L-302	-	4	13	34	64	79	45	33	18	10	133.2±0.3	5.1
17	L-1703 x L-597	-	14	30	38	40	77	70	29	22	10	132.7±0.3	5.8
18	L-1858 x C-6530	3	20	26	42	55	50	39	33	23	9	132.0±0.4	6.3
19	L-1858 x L-302	8	17	28	49	64	51	36	23	17	7	131.1±0.4	6.1
20	L-1858 x L-597	2	18	24	43	39	72	37	31	28	6	132.3±0.4	6.1
21	L-257 x C-6530	4	18	22	41	49	57	57	21	21	10	132.2±0.4	6.2
22	L-257 x L-302	4	21	40	46	53	62	39	18	11	6	130.6±0.3	5.9
23	L-257 x L-597	7	18	36	47	51	48	41	26	22	4	131.2±0.4	6.3

Stabilisation and trait diversity in F_3 generation: F_3 families displayed variability amplitudes similar to F_2 populations, but their distribution curves approached normality, whereas F_2 distributions were flatter. To quantitatively assess the reduction in genetic variation and the extent of transgressive segregation, we calculated the proportion of progenies falling beyond ±1SD and ±2SD from the population mean. The frequency of transgressive segregants for earliness (defined as progenies with values < mean - 1SD and < mean - 2SD) was lower in the F_3 generation compared to the F_2, confirming the stabilisation of segregating traits. Most F_3 progenies fell into the 123–134 day maturity class, further indicating a convergence towards the mean. The observed reduction in both the range and the standard deviation of the phenotypic variation strongly suggests the stabilisation of segregating traits. This trend corroborates earlier work by Simongulyan (1971; 1977), who reported that stabilisation of maturity traits begins from F3 and strengthens in subsequent generations.

Table 3 illustrates that 11 out of 15 combinations exhibited slight overdominance for earliness (1–2 days earlier than original lines), while two showed standard dominance, and one displayed late-maturity dominance. The increased frequency of exceptional recombinant lines in early-maturing families (117–122 days) compared to late-maturing segregants indicates the potential for selecting ultra-early recombinant lines in F_3. Comparable results were obtained by Kaya and Basbag (2021) in Mediterranean environments, where F_3 lines retained early maturity traits under fluctuating temperature regimes. Overall, the combined GCA/SCA analysis and generation-wise trait distribution patterns highlight the role of both additive and non-additive gene actions in determining the vegetative period. These findings provide compelling evidence that Line x Tester, when properly implemented and statistically monitored, offers a robust strategy for improving earliness and stability in upland cotton breeding programs.

Table 3. Variability in vegetative period length in F_3 progenies of hybrids and parents

S.No.	Parents and F3 families	Class Boundaries (days)										X±Sx	S
		117-119	120-122	123-125	126-128	129-131	132-134	135-137	138-140	141-143	141-143		
1	C-6530	-	-	10	18	16	16	-	-	-	-	128.9±0.4	3.2
2	L-302	-	-	-	-	4	17	11	8	-	-	135.2±0.3	2.4
3	L-597	-	-	-	14	25	15	6	-	-	-	133.7±0.4	2.8
4	L-158	-	-	10	30	16	4	-	-	-	-	127.7±0.3	2.4
5	L-75	-	-	6	14	23	17	-	-	-	-	129.6±0.4	2.8
6	L-1703	-	-	4	12	19	25	-	-	-	-	130.3±0.4	2.9
7	L-1858	-	-	5	18	21	16	-	-	-	-	129.4±0.4	2.8
8	L-257	-	-	-	8	20	24	8	-	-	-	131.6±0.3	2.7
9	L-158 x C-6530	4	13	40	101	116	14	8	4	-	-	128.1±0.2	3.4
10	L-158 x L-302	6	17	18	35	56	145	76	14	6	4	131.9±0.3	4.8
11	L-158 x L-597	9	28	43	96	68	32	15	6	3	-	127.9±0.3	4.6
12	L-75 x C-6530	4	9	12	37	108	66	52	12	-	-	131.1±0.3	4.2
13	L-75 x L-302	6	27	31	43	107	52	22	7	-	-	129.05±0.3	4.8
14	L-75 x L-597	4	23	41	56	93	65	12	6	-	-	128.8±0.3	4.3
15	L-1703 x C-6530	7	24	32	79	108	37	9	4	-	-	128.2±0.2	4.1
16	L-1703 x L-302	11	25	32	73	96	46	17	3	-	-	127.9±0.2	3.8
17	L-1703 x L-597	9	22	31	59	103	30	12	5	-	-	128.3±0.3	4.3
18	L-1858 x C-6530	7	17	44	68	92	54	14	-	-	-	128.4±0.2	4.1
19	L-1858 x L-302	4	11	35	72	85	73	12	8	-	-	129.3±0.2	4.1
20	L-1858 x L-597	6	36	47	71	93	36	9	2	-	-	127.6±0.2	4.2
21	L-257 x C-6530	9	23	41	78	103	21	15	10	-	-	128.2±0.3	4.4
22	L-257 x L-302	3	24	41	62	86	61	15	8	-	-	128.8±0.3	4.4
23	L-257 x L-597	7	16	41	54	93	60	24	5	-	-	129.1±0.3	4.4

The results of this study provide compelling evidence for the effectiveness of Line x Tester hybridisation in identifying and selecting early-maturing cotton genotypes. Through the analysis of general and specific combining abilities (*gca* and *sca*), it was established that certain parental lines—particularly L-158 and L-1858—demonstrated high breeding potential for reducing the vegetative period. Their negative *gca* values, in the context of maturity traits, are indicative of additive gene effects that contribute to the development of early-maturing cultivars. Among the F_1 hybrids, dominance and overdominance toward earliness were observed in 80% of combinations, with crosses such as L-158 × L-302 and L-158 × C-6530 standing out. In the F_2 generation, most hybrids exhibited intermediate inheritance with a tendency toward the earlier parent, while moderate phenotypic variability suggested relative genotypic proximity between parental lines. Significantly, the F_3 generation demonstrated a narrowing of variability and a normal distribution curve, indicating the stabilisation of early maturity traits. The appearance of outlier progenies in both F_2 and F_3 populations—some exceeding the earliness of both original lines—confirms the potential of recombination for producing ultra-early lines with new genetic configurations. These findings emphasise the value of combining ability analysis in optimising parental selection and predicting hybrid performance for earliness. The identification of early-maturing, genetically stable recombinants lays a strong foundation for the development of climate-resilient cotton varieties with reduced input requirements and enhanced economic efficiency. The strategic use of genotypes with strong *gca* and *sca* effects can greatly accelerate breeding programs targeting high-yield, early-maturing cotton cultivars suitable for mechanised and energy-efficient agriculture in Uzbekistan and similar agroclimatic regions.

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