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Multi-Season Evaluation of ELS *Bt* Cotton Hybrids for Yield, Fibre Quality, Biotic Stresses and Genotype × Environment Interaction

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Abstract

The major objective of extra-long staple (ELS) cotton breeding is development of high yielding H×B (*G. hirsutum* × *G. barbadense*) hybrids with stable performance in different environments. A study was conducted where seven H×B ELS cotton hybrids were evaluated with a commercial check for three consecutive years (2023-24, 2024-25 and 2025-26) to assess their stability in yield and quality traits. Various biometric and quality observations were recorded such as number of sympodia (NS), number of bolls (NB), single boll weight (SBW), seed cotton yield (SCY), ginning outturn (GOT), fibre length (FL), fibre strength (FS) and micronaire (Mic) and a significant variability was noted among the hybrids for all the traits. Among the evaluated hybrids, CH25 × CBL5 recorded the highest seed cotton yield (5,459.22 kg ha^{-1}), followed by CH4 × CBL6 (4,758.84 kg ha^{-1}) and MS4A × CBL4 (4,559.76 kg ha^{-1}). CH6 × CBL7 exhibited superior fibre quality with maximum fibre length (38.6 mm) and fibre strength (42.9 g tex^{-1}). PCA analysis revealed that PC$_1$ contributed 89.88% of the total variation. AMMI analysis indicated significant genotype × environment interaction effects and identified CH6 × CBL7 as a stable genotype across environments. Biotic stress screening revealed moderate resistance in most hybrids against sucking pests, whereas MS4A × CBL3 was susceptible. Disease screening identified CH25 × CBL5, CH4 × CBL6 and MS4A × CBL4 as tolerant entries against major diseases. Overall, CH25 × CBL5 and CH4 × CBL6 emerged as promising hybrids combining high seed cotton yield, desirable fibre quality and tolerance to major biotic stresses.

Keywords: AMMI analysis, *Bt* hybrids, ELS cotton, PCA biplot, Stability analysis

Introduction

Cotton (*Gossypium* spp.) is considered as one of the important commercial crops worldwide and contributes significantly to the agricultural and textile economy. Extra-long staple (ELS) cotton is highly valued for its superior fibre length, strength and spinning quality required for premium textile production. Cotton fibers measuring an upper half mean length (UHML) of more than 32.5 mm come under the category Extra-Long Staple (ELS) (Baghyalakshmi *et al.*, 2024a). The development of quality extra-long fibre with high-yielding hybrids is increasing as the industrial demand for such cotton is continuously increasing. The productivity and fibre quality of cotton are strongly influenced by genotype, environment and genotype × environment (G×E) interaction. The hybrids stability and adaptability varies with the changing environment and hence the selection of genotypes gets complicated (Kang, 1997, Campbell *et al.*, 2008). Therefore, testing hybrids across multiple cropping seasons becomes essential to identify superior genotypes combining yield, fibre quality and stability (Purchase *et al.*, 2000). Advanced statistical tools such as Principal Component Analysis (PCA) and Additive Main effects and Multiplicative Interaction (AMMI) analysis are widely used for studying G×E interaction and stability in crop plants (Gauch Jr., 1992; Yan and Kang, 2002). PCA facilitates understanding of trait contribution towards total variation, while AMMI analysis effectively explains genotype and environment interactions.

Article History

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Along with the yield and fibre quality, for sustainable cotton production, resistance against sucking pests and major diseases also plays a major role. Sucking pests, such as jassids, thrips, aphids and whiteflies significantly reduce cotton productivity, while diseases like *Alternaria* leaf spot, Tobacco Streak Virus (TSV), boll rot and rust adversely affect crop performance (Patil *et al.*, 2018). Hence, the present study was carried out to evaluate seven ELS H×B *Bt* cotton hybrids over three years with different cropping seasons for yield, fibre quality, stability and biotic stress tolerance using PCA and AMMI approaches.

The objectives of this study were to: (i) evaluate the performance of ELS *Bt* cotton hybrids for yield and fibre quality, (ii) assess genotype × environment interaction and stability using PCA and AMMI analyses, and (iii) identify promising hybrids with stable performance and tolerance to major pests and diseases.

Materials and Methods

Seven ELS H×B *Bt* cotton hybrids and one commercial check were evaluated during 2023-24, 2024-25 and 2025-26 under open field conditions. The experimental material consisted CH4 × CBL6, CH5 × CBL2, CH6 × CBL7, CH25 × CBL5, MS1A × MCBL1, MS4A × CBL3, MS4A × CBL4 and a commercial check. Geographically, the experimental location is situated at ICAR-CICR, Regional Station Coimbatore, at an elevation of 427 meters above mean sea level at a latitude of 11.0122° N and a longitude of 76.9354° E and consisted of red loam soil. The weather parameters prevailing during the three seasons were given in the table 1. The crop was raised under well-irrigated conditions. The experiment was conducted in a randomized block design with three replications following standard agronomic practices. Various observations were recorded for number of sympodia, number of bolls, single boll weight, seed cotton yield, ginning outturn, fibre length, fibre strength

Table 1: Weather parameters for the three cropping seasons

Year & Month	T_{max} (°C)	T_{min} (°C)	Relative Humidity (%)		Rainfall (mm)
			7.22	14.22	
2024-24					
June	33.8	24.0	83.4	50.9	0.5
July	31.2	23.4	83.4	57.2	1.5
August	33.5	23.5	83.0	48.3	0.0
September	32.5	23.8	83.2	55.0	0.4
October	32.9	23.6	84.8	46.7	1.1
November	30.0	22.9	91.0	61.1	13.1
December	28.9	22.2	90.3	62.5	2.0
January	29.2	21.5	87.0	55.1	1.1
February	32.9	22.0	83.1	41.3	0.0
2024-25					
June	32.1	24.3	82.0	60.0	27.0
July	30.6	23.6	84.0	63.0	89.3
August	31.8	23.9	87.0	57.0	31.8
September	32.9	23.7	84.0	55.0	11.2
October	30.8	22.7	92.0	68.0	369.4
November	29.8	22.0	91.0	60.0	64.2
December	29.6	21.0	92.0	60.0	39.1
January	30.1	22.6	85.0	52.0	0.0
February	31.6	21.0	82.0	43.0	0.0
2025-26					
June	29.4	21.2	80.0	62.0	13.2
July	29.9	22.1	81.0	65.0	65.2
August	31.9	22.9	90.0	52.0	4.3
September	32.5	22.5	86.0	56.0	56.6
October	31.9	22.2	91.0	58.0	126.2
November	30.1	21.7	88.0	68.0	32.6
December	29.9	18.6	83.0	41.0	22.0
January	32.6	21.6	84.0	49.1	0.0

and micronaire. Fibre quality parameters were analyzed using standard High Volume Instrument (HVI) techniques following standard fibre testing procedures. PCA and AMMI analyses were used to study genotype × environment interaction and stability. The recommended fertilizer dose and plant protection measures were followed throughout the crop growth period to maintain the plant health. To evaluate differences between genotypes, environments (years) and genotype × environment interactions, yield and fibre quality were subjected to ANOVA.

Screening for Sucking Pest Incidence and Disease Incidence

The hybrids were screened under field conditions for various sucking pests including jassids (*Amrasca biguttula biguttula*), thrips (*Thrips tabaci*), aphids (*Aphis gossypii*) and whiteflies (*Bemisia tabaci*) following standard screening procedures. The hybrids were evaluated for Tobacco Streak Virus (TSV), Alternaria Leaf Spot (ALS), boll rot and rust under natural field conditions. Five plants were randomly selected from each replication and tagged for pest observation. Pest population and disease scoring was recorded during three intervals (vegetative stage, flowering stage and boll formation stage) from three fully expanded leaves representing top, middle and bottom canopy portions. Mean pest population per plant was calculated. Jassid injury was assessed using a 1-4 injury grading scale as suggested by Patil *et al.* (2018). Disease severity was assessed using Percent Disease Index (PDI) following standard disease scoring methods (Mohan *et al.*, 2014).

Statistical Analysis

The mean data across years were subjected to analysis of variance. PCA biplot analysis and AMMI analysis were performed to assess genotype × environment interaction and stability following procedures described by Gauch Jr. (1992) and Yan and Kang (2002). Statistical analyses were performed using R software. PCA and AMMI analyses were conducted using the packages “agricolae” (De Mendiburu, 2015) and “ammistability.”

Results and Discussion

One of India’s most significant cash crops and the world’s most significant crop for natural textile fibre is cotton. *Gossypium hirsutum* and *Gossypium barbadense*, two allotetraploid species that have developed separately, are responsible for more than 90% of the annual production of commercial fiber (Baghyalakshmi *et al.*, 2024b). The commercial exploitation of heterosis between *G. hirsutum* × *G. barbadense* is the main strategy for increasing ELS cotton yield (Kannan and Saravanan, 2016; Baghyalakshmi *et al.*, 2024a). The identified hybrids were tested over three seasons/years to study its stability in terms of yield and quality. The weather parameters showed that there was fluctuation of temperature and rainfall over the years. Variations in rainfall pattern and quantity among years created contrasting crop-growing conditions. As the H×B hybrids are confined to the southern zone especially in parts of Tamil Nadu and Karnataka, the crop was evaluated over years to study the stability to represent the varying climatic conditions across the environments.

Mean Performance of Hybrids

The mean performance of ELS *Bt* cotton hybrids over three years for fibre yield and quality traits is given in table 2. For all evaluated traits significant differences among hybrids were observed, indicating the presence of substantial variability. Seed cotton yield together with fiber quality is the primary economic trait in cotton improvement programmes. Among the evaluated hybrids, CH25 × CBL5 recorded the highest seed cotton yield (5,459.22 kg ha^{-1}), followed by CH4 × CBL6 (4,758.84 kg ha^{-1}) and MS4A × CBL4 (4,559.76 kg ha^{-1}) (Table 2). These hybrids significantly outperformed the commercial check (3,140.62 kg ha^{-1}). Similar variability for yield traits among *Bt* cotton hybrids was reported by Wagh *et al.* (2024). The superior performance of CH25 × CBL5 may be attributed to higher boll number and improved plant productivity. CH4 × CBL6 combined high yield with superior fibre quality traits, making it suitable for commercial cultivation. ELS

Table 2: Mean performance of hybrids over three years for yield and fiber traits

Genotype	NS	NB	SBW (g)	SCY (kg ha^{-1})	GOT (%)	FL (mm)	FS (g tex^{-1})	Mic (µ)
CH4 × CBL6	25	56.5*	5.03	4,758.84*	35.98	37.1*	40.2*	3.1
CH5 × CBL2	20	20.5	6.36*	1,986.84	37.02*	32.2	36.5	3.7
CH6 × CBL7	25.5	39	5.47	3,482.28	37.61*	38.6*	42.9*	3.0
CH25 × CBL5	24.5	61.5*	5.26	5,459.22*	31.53	36.5	40.4*	5.0
MS1A × MCBL1	25	46.5	5.43	4,182.12*	36.82	35.0	34.9	3.8
MS4A × CBL3	23.5	27	5.41	2,270.88	40.08*	34.0	32.5	4.5
MS4A × CBL4	27	57.5*	4.75	4,559.76*	32.04	32.8	33.9	4.5
Check	22	21	5.02	3,140.62	33.24	35.3	35.7	3.2
Mean	24.36	44.07	5.39	3,814.28	34.87	35.17	37.33	3.94
SE (±)	1.21	2.86	0.19	185.42	1.02	0.84	1.35	0.28
CD (P=0.05)	2.64	6.23	0.41	404.06	2.22	1.83	2.94	0.61
CV (%)	8.92	9.18	6.71	10.82	5.14	4.86	6.39	9.56

Values are means of three cropping seasons. *indicates significant superiority over the commercial check at $p \leq 0.05$

cotton breeding primarily focuses on fibre quality traits. CH6 × CBL7 exhibited the highest fibre length (38.6 mm) and fibre strength (42.9 g tex^{-1}), indicating excellent spinning quality. Similar findings regarding fibre quality variability in cotton were reported by Bradow and Davidonis (2000). The hybrid CH4 × CBL6 recorded desirable fibre quality with fibre length of 37.1 mm and fibre strength of 40.2 g tex^{-1}. Micronaire values ranged from 3.0 to 5.0, which fall within the acceptable range for ELS cotton. Lower micronaire values in CH4 × CBL6 and CH6 × CBL7 indicated the finest fibres appropriate for premium textile industries. Similarly higher GOT contributes to increased lint productivity and economic returns. The highest GOT was observed in MS4A × CBL3 (40.08%), followed by CH6 × CBL7 (37.61%) and CH5 × CBL2 (37.02%). The hybrid MS4A × CBL3 had fewer monopodial branches and lesser plant height which may have contributed for such high GOT.

The three components *i.e.*, that environments, genotypes and genotype × environment (G×E) interactions were found to be highly significant ($p<0.01$) through combined ANOVA analysis representing the presence of variability among the genotypes and the environments tested (Table 3). Further the significant G×E interactions for all the tested traits indicate the necessity for stability analysis as their performance vary according to the seasons.

PCA Biplot Analysis

Principal Component Analysis showed that PC1 accounted for 89.88% of the total variation, indicating that the first principal component explained most (89.88%) of the observed variation and similar results have already been reported (Meera *et al.*, 2025). The two hybrids CH25 × CBL5 and MS4A × CBL4 were located on the positive side of PC1 indicating strong association with higher yield and favourable trait combinations (Figure 1). The hybrid CH4 × CBL6 was distinctly located, reflecting superiority for fibre quality traits and GOT. Substantial environmental influence on genotype performance was demonstrated by the PCA results which emphasized the importance of testing genotypes across multiple cropping seasons. A genotype and environment have a minor interaction influence when their PCA1 score is close to zero. Our results were comparable to those of Elizabeth (2018) and Khan *et al.* (2021).

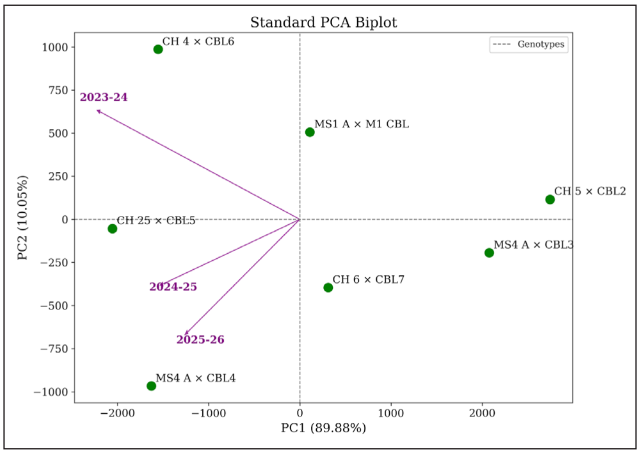

Figure 1: PCA Biplot Yield vs. Environment

AMMI1 Biplot

The AMMI model's strength depends on its ability to divide the GEI into principal components, thereby providing insights into the interaction patterns of genotypes with varying environments. In this study, the AMMI analysis revealed significant variation among the genotypes, environments and their interactions, with the first two principal components PC1 and PC2 accounting for the majority of the GEI variance. The AMMI1 biplot exhibited mean yield on X-axis and Y-axis indicated interaction principal component axis (IPCA1) scores. Genotypes which were more stable across environments were located near the horizontal axis. CH25 × CBL5, positioned on the right side of the biplot and close to the horizontal axis, combined high productivity with moderate stability (Figure 2). Due to its significant contribution to the GEI sum of squares and its ability to distinguish between main and interaction effects, the AMMI model is successful (Shahriari *et al.*, 2018). MS1A × MCBL1, located close to the zero interaction line, exhibited greater stability and are in line with the findings of Zeng and Meredith Jr. (2009).

AMMI2 Biplot

Further genotype × environment interaction and stability patterns were explained by the AMMI2 biplot. Genotypes were considered to be stable and having minimal interaction

Table 3: ANOVA for G×E interaction

Source of Variation	df	PH	NM	NS	NB	SBW	SCY	GOT	FL	FS	Mic
Environments	2	215.46**	0.82*	18.71**	145.62**	0.52**	1456820.5**	22.54**	8.72**	32.41**	0.41**
Replications within Envt.	6	12.35	0.15	1.62	9.88	0.08	34562.2	0.98	0.92	1.85	0.06
Genotypes	6	185.71**	2.11**	11.28**	210.54**	0.74**	1689205.4**	38.62**	12.44**	46.92**	0.82**
Genotype × Environment	12	52.63**	0.64**	4.85**	71.92**	0.19**	412865.7**	8.74**	3.96**	14.28**	0.21**
Error	36	18.42	0.18	1.94	16.71	0.07	68255.6	1.64	1.02	3.25	0.08
Total	62	-	-	-	-	-	-	-	-	-	-

[Note: PH = Plant height; NM = Number of monopodia; NS = Number of sympodia; NB = Number of bolls; SBW = Single boll weight; SCY = Seed cotton yield; GOT = Ginning outturn; FL = Fibre length; FS = Fibre strength; Mic = Micronaire]

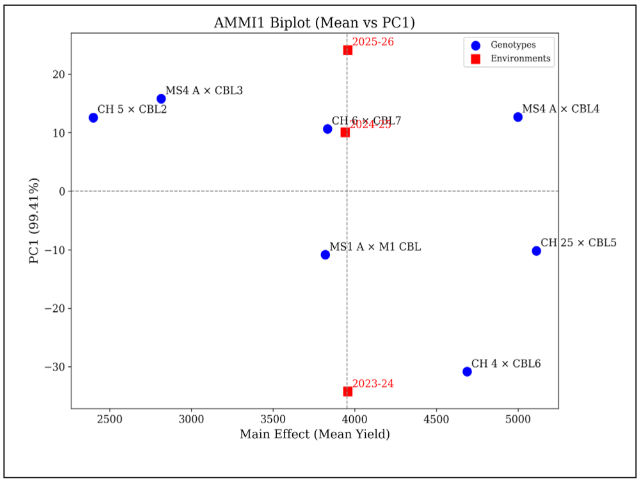

Figure 2: AMMI2 Biplot (Stability)

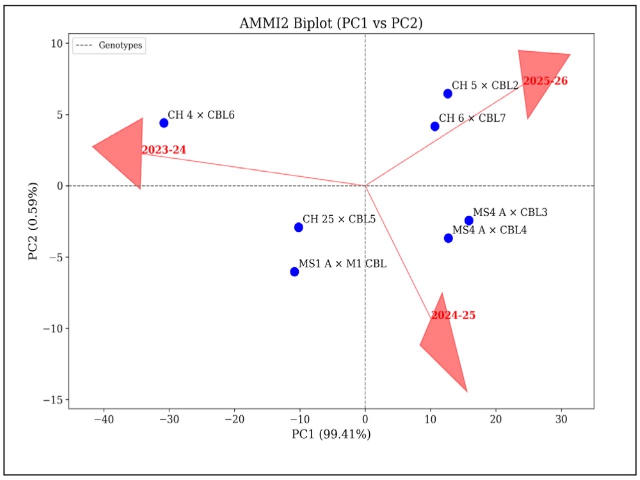

Figure 3: AMMI2 Biplot for stability

with environments if they are located close to the origin. CH6 × CBL7 was among the most stable hybrids, exhibiting relatively consistent performance across the three years under different cropping seasons. This method has been employed for selection of hybrids with stable performance by Yaşar (2023). On the other hand, CH5 × CBL2 exhibited higher interaction effects, indicating specific adaptation rather than broad adaptability to particular environmental conditions. The environmental vectors representing 2023-24, 2024-25 and 2025-26 indicated substantial environmental variation influencing genotype performance (Figure 3). These findings are in agreement with reports of Maleia *et al.* (2017) and Satish and Reddy (2018), which highlighted the effectiveness of AMMI analysis in identifying stable cotton genotypes.

Biotic Stress Screening

Screening for Sucking Pest Resistance

The seven ELS H×B *Bt* cotton hybrids along with the commercial check were screened under field conditions for their reaction against major sucking pests including aphids, jassids, thrips and whiteflies. Considerable variation was observed among the hybrids for pest incidence and resistance reaction, indicating differential levels of tolerance among the genotypes. The aphid population ranged from 1.33 to 2.78 insects plant^{-1}. The lowest aphid incidence was recorded in CH5 × CBL2 (1.33 plant^{-1}), followed by CH4 × CBL6 and MS1A × MCBL1 (1.67 plant^{-1} each). The highest aphid population was observed in CH6 × CBL7 (2.78 plant^{-1}). Jassid population ranged from 1.00 to 1.67 insects plant^{-1}. CH25 × CBL5 and the commercial check recorded the lowest jassid population. Most hybrids recorded a jassid injury grade of 2.00 and were categorized as moderately resistant (MR) (Amutha, 2022). However, MS4A × CBL3 recorded a grade of 3.00 and was categorized as susceptible. Thrips population ranged from 0.33 to 1.33 insects plant^{-1}. CH25 × CBL5 recorded the lowest thrips incidence (0.33 plant^{-1}). Whitefly incidence remained very low across hybrids. The moderate resistance observed in most hybrids corroborates earlier findings reported by Patil *et al.* (2018).

Resistance Categorization

Based on sucking pest incidence and jassid injury grading, most hybrids were categorized as moderately resistant (MR). Among the evaluated entries, CH25 × CBL5 showed comparatively lower infestation of jassids, thrips and whiteflies, indicating better tolerance against sucking pests. In contrast, MS4A × CBL3 showed higher jassid infestation and was categorized as susceptible.

Disease Screening

The *Bt* hybrids were screened for Alternaria Leaf Spot (ALS), Tobacco Streak Virus (TSV), boll rot and rust under natural field conditions. Disease incidence was given as Percent Disease Index (PDI). Among the hybrids the Alternaria Leaf Spot (ALS) incidence ranged from PDI of 3.00 to 6.00 which indicated mild to moderate infection across all entries. The hybrids CH25 × CBL5 and CH4 × CBL6 showed relatively lower disease incidence, indicated better tolerance to Alternaria leaf spot. The evaluated entries showed 0.75 to 3.70 PDI for Tobacco Streak Virus (TSV) incidence. The hybrids, namely MS4A × CBL4, CH25 × CBL5 and CH4 × CBL6, showed no visible TSV symptoms under field conditions. The remaining hybrids exhibited varying levels of susceptibility to TSV, which may adversely affect productivity and quality of the fibre. Boll Rot incidence PDI was ranging from 0.00 to 2.50 PDI which indicated the low disease incidence among the tested entries. Some hybrids were not affected by boll rot during the cropping period, indicating favorable boll health and better field resistance. Rust Incidence ranged from 4.00 to 10.00 PDI among the hybrids. Although rust symptoms were observed in all entries, the severity remained within manageable limits. Lower rust incidence was recorded in CH25 × CBL5 and CH4 × CBL6 compared to susceptible entries. Similar variability for disease resistance in *Bt* cotton hybrids was reported by Patil *et al.* (2018).

Disease-Tolerant Hybrids

Among the tested hybrids, MS4A × CBL4, CH25 × CBL5 and CH4 × CBL6 were identified as superior entries due to their tolerance against major diseases, particularly TSV and boll rot. These hybrids remained free from all diseases except mild incidence of ALS and rust. The combined expression of higher yield, superior fibre quality and tolerance to major biotic stresses makes CH25 × CBL5 and CH4 × CBL6 promising candidates for commercial cultivation and future cotton breeding programmes aimed at developing stress-tolerant ELS *Bt* cotton hybrids.

Ethical Statement

The authors declare that no generative artificial intelligence tools were used in drafting or preparing this manuscript. The manuscript was written, interpreted and revised solely by the authors, who take full responsibility for its content.

Conclusion

The present investigation demonstrated significant variability among the evaluated ELS H×B *Bt* hybrids for seed cotton yield, fibre quality, stability and biotic stress tolerance. Among the tested hybrids, CH25 × CBL5 emerged as the best stable genotype with the highest seed cotton yield and superior tolerance to major sucking pests and diseases. CH4 × CBL6 also exhibited desirable combination of high fibre yield with superior quality traits such as fibre length and fibre strength. The hybrid CH6 × CBL7 exhibited greater stability across environments and had the best fibre quality parameters based on AMMI analysis. The combined stability analysis through PCA and AMMI effectively explained genotype × environment interactions and aided in identifying stable and adaptable hybrids suitable across multiple cropping seasons. Most of the hybrids exhibited moderate resistance against sucking pests, while MS4A × CBL3 was found susceptible due to higher jassid injury. Disease screening identified MS4A × CBL4, CH25 × CBL5 and CH4 × CBL6 as tolerant entries against most of the diseases, particularly TSV and boll rot. Overall, the two hybrids, CH25 × CBL5 and CH4 × CBL6 were identified as promising hybrids for commercial cultivation owing to their combined superiority in yield, fibre quality and biotic stress tolerance, whereas CH6 × CBL7 can serve as a valuable genotype for stability and fibre quality improvement programmes.

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