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Research Article

Performance of two cotton interspecific hybrids (*Gossypium hirsutum* L. × *G. barbadense* L.) in Greece: A comparative case study

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Abstract

Cotton (*Gossypium hirsutum* L.) is one of the most prominent and important industrial crops. In Greece, cotton farming occupies a significant share of the agricultural sector. However, the adverse effects of climate change have raised concerns regarding its performance in the near future, thus it is crucial to develop adaptation strategies for the production of cotton in Greece. One such alternative is the adoption of interspecific hybrids (*G. hirsutum* × *G. barbadense*). These hybrids have been suggested to be more resilient and to outperform their parental varieties. In order to evaluate the potential of such hybrids, a field experiment was conducted in two sites in Greece. Two novel cotton hybrids (1432 and 701) and a conventional variety ('Elpida') were farmed in two different plant densities (8 and 13 plants m^2) and their agronomic traits (biomass, LAI), yield and yield components (seed cotton yield, lint yield, seed yield, ration lint/seed cotton, and ratio seed/seed cotton), and quality traits were assessed. The agronomic traits and the yield of the hybrids were equal or superior in comparison to 'Elpida'. Similar findings were also observed in the majority of the quality traits. Based on our results, interspecific cotton hybrids are promising for the European cotton sector.

Keywords: cotton; *G. hirsutum; G. barbadense;* interspecific hybrids

Abbreviation: AA Agioi Anargiroi, AUA Agricultural University of Athens, LAI Leaf Area Index, Mic Micronaire, Mat Maturity, UHML Upper Half Mean Length, UI Uniformity Index, Str Strength, Elg Elongation, CGRD Color Grade, TRcnt Trash count, TrAr Trash Area, TrID TrashGrade, SCI Spinning Consistency Index

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Introduction

Cotton (*Gossypium sp.)* has been cultivated for over five millennia and it is arguably one of the most dominant non-food crops (IISD, 2023). It is mainly cultivated for its fiber (it accounts for nearly a quarter of the global fiber production) (Chen et al., 2023), though it can also be included in animal rations (Kumar *et al.*, 2023), and it is a feedstock for bioethanol production (Nikolić *et al.*, 2017). The widespread distribution of cotton can be partially attributed to its profitability (IISD, 2023) and its adaptability to a wide range of climatic conditions (Tsaliki *et al.*, 2023). Currently, cotton is grown in more than 30 million ha all over the world, with China being the global leader in cotton production (FAOSTAT, 2024).

Another important aspect of its success is the great genetic pool of the Gossypium species (Zhang *et al.*, 2020). The genus *Gossypium* contains 50 different species, with four being cultivated (at least at some point) commercially: *G. hirsutum, G. barbadense, G. arboreum, and G. herbaceum* (Rai *et al.*, 2021). Out of these four species, *G. hirsutum* is the most dominant, contributing to more than 95% of the total cotton production across the world (Nix *et al.*, 2017), followed by *G. barbadense* that is known for its superior fiber quality (Alagarsamy, 2023). Of course, the quality of the fibers is not solely predetermined by the genome of the cotton plants. The literature suggests that agronomic practices affect its quality traits. For instance, plant density affects both the yield, and the quality of the fibers in cotton (Feng *et al.*, 2010; Feng *et al.*, 2011). Low densities decrease fibers' strength and increasing their micronaire (Jalilian *et al.*, 2023), and high densities increase the yield (Khan *et al.*, 2017) but can result to boll shedding, decreased boll size and late maturity (Khan *et al.*, 2017; Jalilian *et al.*, 2023).

Nonetheless, the genetic differences amongst the *Gossypium* species and their effect on their performance are well-known and have been utilized in cotton breeding (Li *et al.*, 2022). Heterosis between different cotton species has been known ever since the late 1890s (Zhang *et al.*, 2023). This phenomenon led to the development of interspecific hybrids that outperform their parental varieties in both quality, and performance (Li *et al.*, 2022). Moreover, these hybrids showcase improved tolerance to biotic and abiotic stress (Zafar *et al.*, 2021), such as heat and drought stress (Baig *et al.*, 2009; Ikram, 2021). This aspect of cotton hybrid breading could constitute an effective adaptation measure that mitigates the adverse effects of climate change on cotton production. Afterall, the available literature dictates that despite the beneficial impact of the elevated atmospheric CO_2 concentration, the climate change-induced rise of temperature and increased drought frequency hinder the development and yields of cotton. The adoption of resilient hybrids in climate-change vulnerable regions could fortify cotton production, whilst simultaneously benefiting the market via the improvement of the produced cotton lint quality.

One such instance, where this hypothesis is applicable, is the European cotton. In the European Union (EU), cotton is mainly grown in Greece and Spain (European Commission, 2024), two mediterranean countries that are susceptible to climate change (Abd-Elmabod *et al.*, 2020; Sebos *et al.*, 2023). In fact, Greece produces more than 80% of the European cotton (Tsaliki *et al.*, 2022). Even though Greek cotton (*G. hirsutum*) holds a small share of the global market compared to the major producing countries (1% of the global cotton production is attributed to the EU), it is an important commodity on a national level. In fact, during the last few years, the total acreage of cotton farms in Greece exceeded 230,000 ha (OPEKEPE, 2023), and its cultivation was the main source of income for approximately 10,000 farmers. The importance of cotton to the Greek and European agricultural sector, as well as the recent predictions that estimate significant cotton losses in southern Europe, call for immediate action in order to safeguard its future.

The aim of the present study is to: i) conduct a preliminary evaluation on the performance of two cotton hybrids in Greece, ii) compare their agronomic traits and yield components with the respective ones of a Greek *G. hirsutum* cultivar, and iii) assess the effect of plant density on the agronomic and quality traits of the two hybrids.

Materials and Methods

Experimental design

In 2023, two cotton hybrids (*Gossypium hirsutum* × *G. harbadense*) and a *G. hirsutum* L. cultivar were grown in two different experimental sites in Greece. The first site is located in the experimental field of the Laboratory of Agronomy, in the Agricultural University of Athens (37°59′ N and 23°42′ E), and the second in Thessaly, Agioi Anargiroi (39°29' N and 22°20' E). The experiment in both sites was set up as a split-plot design with 4 replicates (per site), with the location being the main plot and the cotton varieties and the plant density being the sub-plots (Figure 1). Two hybrids (1432 and 701) and a cultivar ('Elpida') that is mainly cultivated in Greece (ICAC, 2023) were assessed in this study. The different plant densities included D1 (8 plants per m^2) and D2 (13 plants per m^2) (Vardoulis *et al.*, 2006).

Site description and crop establishment

The soil properties in both sites are presented in the table below (Table 1).

Table 1. Soil properties of both experimental sites[1]

Site	Property	Value	Site	Property	Value
AUA	Soil	Clay Loam	**AA**	Soil type	Clay
	Clay (%)	29.4		Clay (%)	53
	Silt (%)	35.1		Silt (%)	24
	Sand (%)	35.5		Sand (%)	23
	pH (1:1 H_2O)	7.39		pH (1:1 H_2O)	7.4
	Nitrogen content (%)	0.143		Nitrogen content (%)	0.11
	Phosphorus (P_{Olsen}) content (mg kg^{-1} soil)	13.6		Phosphorus (P_{Olsen}) content (mg kg^{-1} soil)	15
	Potassium content (mg kg^{-1} soil)	233		Potassium content (mg kg^{-1} soil)	390
	Calcium carbonate (%)	15.34		Calcium carbonate (%)	1.0
	Soil organic matter (%)	1.67		Soil organic matter (%)	1.4

[1] AUA – Agricultural University of Athens (37°59′ N and 23°42′ E); AA – Agioi Anargiroi, Thessaly (39°29' N and 22°20' E)

The acreage of the experimental site in the AUA was 960 m^2. Before sowing, 160 kg ha^{-1} of nitrogen (N) fertilization [350 kg ha^{-1} of ENTEC 46 (46-0-0), EUROCHEM AGRO HELLAS S.A., 249 Mesogeion Street 15451, Neo Psychiko, Athens] were broadcasted and incorporated in the soil through ploughing. Cotton seeds were sown in 8th of May. Seeds were sowed by hand, in two different densities (D1 & D2). The distance from row to row was 95 cm. The emergence started 8 Days After Sowing (DAS) and completed in 12 DAS. Six irrigations were performed, with a dropping system that was installed in the field, right after sowing. The total amount of water was 600 mm. Weeds were managed weekly, by hand and hoeing. Harvest took place in 3rd of October, when the boll moisture was 9.5%. The duration of the experiment was 148 days.

In AA the experimental area was 1,296 m^2. Overall, the site establishment followed the same protocol as the respective one in the AUA (fertilization, irrigation, dates, row spacing etc.). The only difference amongst the sites were the sowing and harvesting time. In contrast to the AUA site, in AA the seeds were sown in 2nd of May, whilst the harvest was performed in 26th of September. Cotton crop was harvested when boll's moisture was 9.5%. Experiment's duration was 147 DAS.

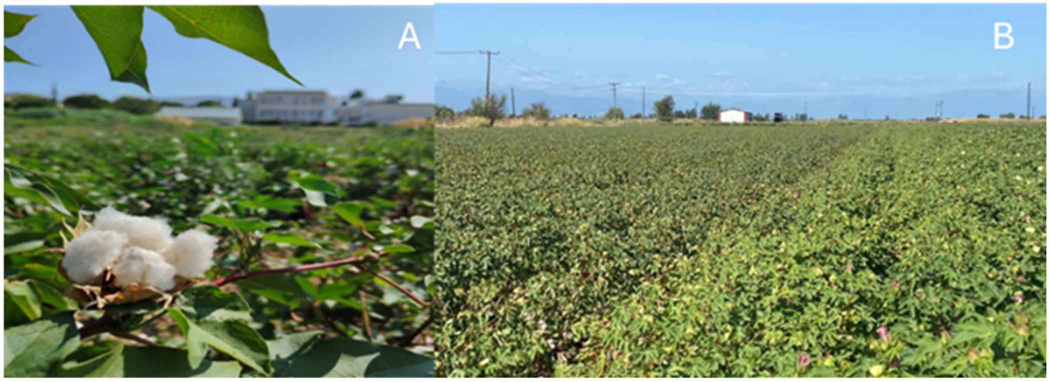


Figure 1. Cotton experiments in AUA (A) and AA (B)

Meteorological data

Mean Temperature (°C) and precipitation (mm) were measured throughout the duration of the study, in both sites. For the AUA, data meteorological data were collected from the meteorological station of Institute for Environmental Research, National Observatory of Athens (IERSD/NOA). For Agioi Anargiroi, the meteorological data were collected from a meteorological station, that is located close to the field. All meteorological data are presented in the figure below (Figure 2).

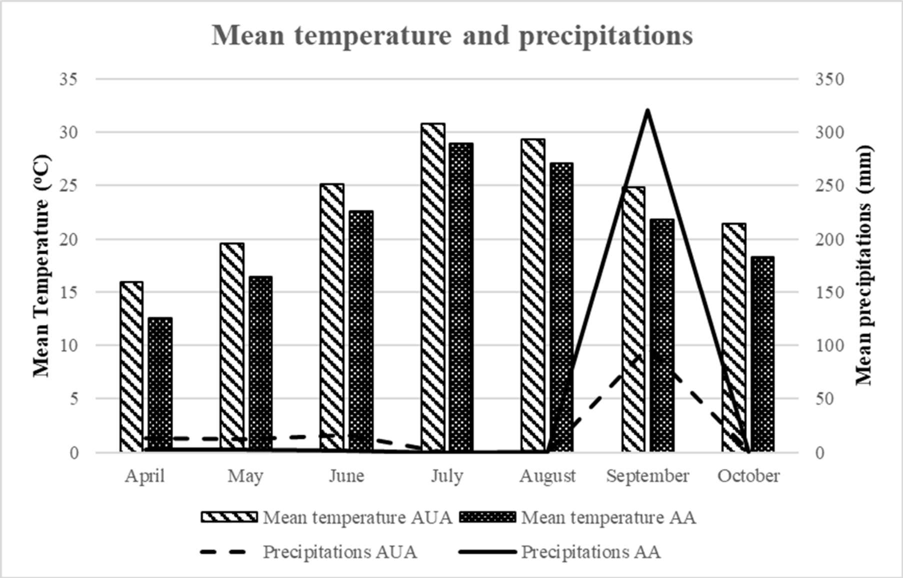


Figure 2. Mean temperatures and precipitations in both experimental sites throughout the duration of the study

Measurements

Agronomic traits

The agronomic traits that were studied during the experiment, were the dry weight, the Leaf Area Index (LAI), the seed cotton yield, the seed yield, and the lint yield. For the dry weight measurements, five random plants were selected from each plot and cut at ground level. The collected plant-samples were then put in an oven for 48 hours (at 65 °C) and their biomass was measured. LAI was measured with an automatic leaf area meter (Delta-T Devices Ltd) following the equation (1):

(1) LAI = leaf area (m^2)/ ground area (m^2).

After harvesting, bolls were weighted, in order to estimate crop's yield. Bolls from the whole plot were collected. Plot size was 40 and 54 m^2 for AUA and AA, respectively. The yield was estimated (kg ha^{-1}) according to the following equation (2):

(2) Yield = plot's bolls weight * 10.000 / plot's size

After ginning, seed and lint yield were estimated, by weighting seeds and lint. Seed/weight and lint/weight ratios (%), were also calculated.

Quality traits

After harvest, 500 g unginned cotton per plot were used in order to evaluate the quality of the fibers. The samples were measured at the National Centre for Quality Control, Classification and Standardization of Cotton (1st km Karditsa-Mitropolis, 43100 Karditsa, Greece) which is the division of the Institute of Industrial and Forage Crops of the Hellenic Agricultural Organization "DEMETER" (Theofrastou 1, 41335 Larissa, Greece). Quality measurements were carried out via a High-Volume Instrument (HVI) (HVI SW Version 3.2.4.21) (Table 2).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the SPSS software (SPSS Inc. version 22.0, IBM Corp, Armonk, NY, USA). Significant differences between means were determined by Tukey's Honestly Significant Difference test (HSD) at the 5%, 1% and 0.1% levels of significance. Results regarding the environmental effects are graphically depicted via PCA biplot analysis. Graphs were exported using GGEBiplot software (GGEBiplot version 4.1, Weikai Yan).

Table 2. The cotton quality traits that were assessed in the present study, a short description of each trait, the methodology that was used in each assessment, and the corresponding reference of each methodology

Trait	Description	Method	Reference
Micronaire (Mic)	An indicative measurement of the air permeability of compressed cotton fibers	Via evaluating the resistance of airflow in a specific amount of cotton fibers	Pei *et al.* (2021)
Maturity (Mat)	The probability of breakage and entanglement	Measure of secondary walls' thickness	Liu *et al.* (2023)
Upper Half Mean Length (UHML)	Fiber's length	The mean length of fiber's longer half	Darawsheh *et al.* (2022)
Uniformity Index (UI)	The proportion of the Upper-Half Mean Length to the mean length of the fibers	Uniformity Index = (Mean Length)/(Upper Half Mean Length)	Günaydin *et al.* (2018)
Short fiber index	The amount of fibers < 12.7 mm	HVI	Sahar *et al.* (2021)
Strength (Str)	The needed strength for fiber's breakage	Exert force until the breakage of a bundle of fibers	Mathangadeera *et al.* (2020)
Elongation (Elg)	The percentage of fiber's extension before the breakage	Pull fiber's and measure the length right before the breakage	Gundim *et al.* (2020)
Color Grade (CGRD)	The value of reflectance (Rd) and yellowness (+b)	Measure the reflectance and yellowness of the fiber, using the Nickerson-Hunter color chart	Çopur *et al.* (2018)
Trash count (TRcnt)	Trash that exceeds the grayness threshold set in the camera	Counting an amount of trash if it exceeds the grayness threshold set in the camera	Darawsheh *et al.* (2022)
Trash Area (TrAr)	The area covered with trash	Counting the pieces of trash that are exposed to the glass window	
TrashGrade (TrID)	The trash grade	Determined by calibrating HVI with known samples	
Spinning Consistency Index (SCI)	Indicator that classifies fibers, according to their quality and spinnability	$SCI = -414.67 + 2.9 \times Str - 9.32 \times Mic + 49.17 \times UHML + 4.74 \times UI + 0.65 \times Rd + 0.36 \times (+b)$	Yusupaliyeva & Yuldashev (2022)

Results

Crop performance and yield components

Both the performance, and the yield of the cotton were mainly affected by the genotype. In both sites, the two hybrids (1432 and 701) reported statistically significantly greater above-ground biomass, compared to the respective one of 'Elpida'. The differences between the biomass of 1432 and 701 plants were insignificant, yet their dry matter was 13-20% higher compared to the one of 'Elpida's plants (Table 3). Similarly, 'Elpida's' yield was significantly lower in both sites, and the yield amongst the two hybrids reported insignificant differences.

Table 3. Combined analysis of variance (F values) for the agronomic traits and yield components of three cotton genotypes (1432, 701, and 'Elpida') cultivated in two different sites (AUA and AA), and in two different sowing densities (D1 and D2)

Genotype	**DM (kg ha^{-1})**		**Yield (kg ha^{-1})**		**Lint yield (kg ha^{-1})**		**Ratio seed/ total weight %**	
	D1	**D2**	**D1**	**D2**	**D1**	**D2**	**D1**	**D2**
AUA								
1432	6207.00^{b}	6226.50^{b}	5083.28^{b}	4893.53^{b}	1806.80^{ns}	1629.78^{ns}	63.83^{ab}	65.45^{b}
701	6144.50^{b}	6440.00^{b}	4890.33^{b}	5084.18^{b}	1695.35^{ns}	1721.60^{ns}	64.67^{b}	65.15^{b}
'Elpida'	5420.75^{a}	5369.00^{a}	4391.64^{a}	4261.85^{a}	1666.33^{ns}	1672.89^{ns}	61.00^{a}	59.77^{a}
AA								
1432	6308.3^{b}	6383.58^{b}	5133.86^{b}	5061.21^{b}	1826.06^{ns}	1754.55^{ns}	63.85^{b}	64.52^{b}
701	6301.5^{b}	6534.50^{b}	4969.78^{b}	4977.30^{b}	1704.49^{ns}	1707.04^{ns}	65.16^{b}	65.05^{b}
'Elpida'	5589.5^{a}	5529.50^{a}	4501.98^{a}	4432.45^{a}	1729.19^{ns}	1775.86^{ns}	60.70^{a}	59.05^{a}
Site (F_s)	6.43^{**}		*ns*		*ns*		*ns*	
Genotype (F_v)	103.93^{***}		73.26^{***}		*ns*		46.25^{***}	
Density (F_d)	*ns*		*ns*		*ns*		*ns*	
$F_s \times F_v$	*ns*		*ns*		*ns*		*ns*	
$F_s \times F_d$	*ns*		*ns*		*ns*		*ns*	
$F_v \times F_d$	*ns*		*ns*		*ns*		*ns*	
$F_s \times F_v \times F_d$	*ns*		*ns*		*ns*		*ns*	

F-test ratios originated from ANOVA. ns: non-significant; *, ** and ***: Significant at the 5%, 1% and 0.1% levels, respectively

The difference in the yield between the hybrids and 'Elpida' ranged from 11% to 19%, and the highest yield was observed in 1432 (D1 in the AA). The ratio of the harvested seed weight/seed cotton weight was notably lower in 'Elpida', and the differences between the two hybrids were once again insignificant (Table 3). In the AUA, this ratio was 2.8-3.7% and 5.4-5.7% higher in the hybrids in the D1 and D2 (respectively), and in the AA it was lower in 'Elpida' (compared to 1432 and 701) by 3.2-4.5% and 5.4-6% in D1 and D2, respectively. The lint yield did not report any significant differences amongst the different cotton genotypes, sites, or plant densities.

Even though the location and the plant density did not affect the results in most aspects of the performance and the yield components of the crop, the interaction site x genotype was significant in the LAI. In particular, in D2 the differences amongst the LAI values reported in both sites and regardless of the genotype, were insignificant. On the contrary, in D1 the lowest LAI values were observed in 'Elpida' in both sites (AUA and AA) (Figure 3). Similarly, in both sites the differences in the LAI values of the two hybrids were insignificant. However, both 1432 and 701 reported significantly higher LAI values in AA, compared to the respective ones recorded in the AUA.

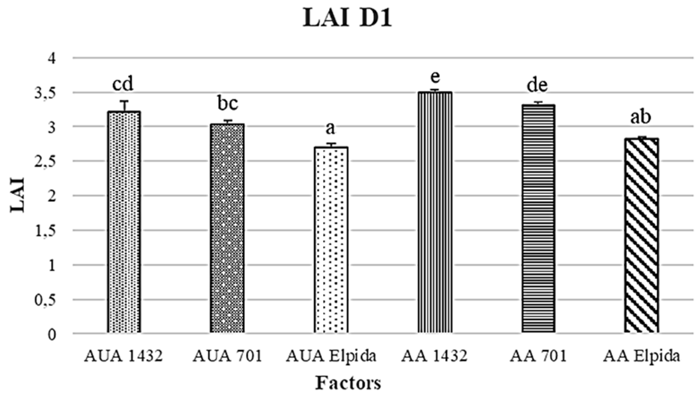


Figure 3. LAI values as affected by the combination of genotype x site

Sites: AUA and AA; Genotypes: 1432, 701, and 'Elpida'. Statistically significant differences are depicted by the different letters (a, b, c, d, and e)

The interaction genotype x density affected only the ratio of the lint weight/seed cotton weight and the seed yield. Regarding the ratio, in both sites "Elpida" reported significantly higher results in both densities (38-40%). This ratio in 1432 and 701, in both sites and in both D1 and D2, noted insignificant differences and ranged between 33-35% (Figure 4). The seed yield of the hybrids was significantly higher than the respective one of 'Elpida' and exceeded 3 tons per ha, regardless of the site and the density (Figure 4). No statistically significant differences were observed between the seed yields of 1432 and 701, nor between D1 and D2 in 'Elpida'.

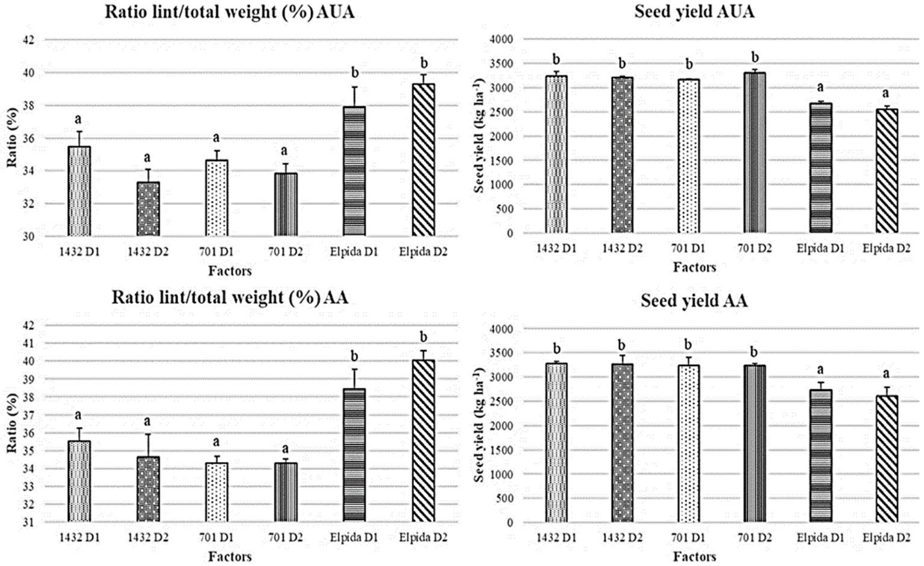


Figure 4. Lint yield to seed cotton yield ratio and seed yield as affected by the combination of genotype x density

Genotypes: 1432, 701, and 'Elpida'; Densities: D1 and D2. Statistically significant differences are depicted by the different letters (a and b).

Cotton quality traits

Mat was the only fiber quality trait that was completely unaffected and did not report any significant differences amongst the different genotypes, sites, and densities. On the contrary, Mic, UHML, and SF were significantly affected only by the cotton genotype (Table 4). Mic values were higher in 'Elpida' (by 20-25%

compared to the hybrids' Mic values), but only in D2 (no significant differences were reported amongst 'Elpida' and the hybrids in D1). Similarly, SF was notably higher in 'Elpida'. In particular, in the AUA the SF values in 'Elpida' were 2.5-2.8% higher compared to the ones in 701, and 1.3-2.6% higher compared to the ones of 1432. In the AA the respective differences ranged between 2-2.6% and 1.5-2.4%. The UHML values were notably higher in the cotton hybrids in both sites, and particularly in the 701 that reported the best UHML results (ranging between 32.64-32.87 mm).

Table 4. Combined analysis of variance (F values) for the measured quality traits Mic, Mat, UHML, and SF of three cotton genotypes (1432, 701, and 'Elpida') cultivated in two different sites (AUA and AA), and in two different sowing densities (D1 and D2)

Variety/hybrid	**Mic**		**Mat**		**UHML (mm)**		**SF (%)**	
	D1	**D2**	**D1**	**D2**	**D1**	**D2**	**D1**	**D2**
AUA								
1432	3.48^{ns}	3.31^{a}	0.82^{ns}	0.82^{ns}	30.58^{a}	31.78^{b}	7.84^{ab}	7.30^{a}
701	3.43^{ns}	3.26^{a}	0.82^{ns}	0.82^{ns}	32.83^{b}	32.66^{b}	6.66^{a}	7.09^{a}
'Elpida'	3.71^{ns}	4.06^{b}	0.82^{ns}	0.83^{ns}	28.89^{a}	27.44^{a}	9.15^{b}	9.90^{b}
AA								
1432	3.44^{ns}	3.30^{a}	0.82^{ns}	0.83^{ns}	31.46^{b}	32.17^{b}	7.75^{a}	7.52^{a}
701	3.45^{ns}	3.32^{a}	0.84^{ns}	0.82^{ns}	32.87^{b}	32.64^{b}	7.20^{a}	7.31^{a}
'Elpida'	3.80^{ns}	3.93^{b}	0.83^{ns}	0.83^{ns}	28.44^{a}	28.21^{a}	9.24^{b}	9.91^{b}
Site (F_s)	*ns*		*ns*		*ns*		*ns*	
Genotype (F_v)	11.35^{***}		*ns*		68.07^{***}		69.95^{***}	
Density (F_d)	*ns*		*ns*		*ns*		*ns*	
F_s x F_v	*ns*		*ns*		*ns*		*ns*	
F_s x F_d	*ns*		*ns*		*ns*		*ns*	
F_v x F_d	*ns*		*ns*		*ns*		*ns*	
F_s x F_v x F_d	*ns*		*ns*		*ns*		*ns*	

F-test ratios originated from ANOVA. ns: non-significant; *, ** and ***: Significant at the 5%, 1% and 0.1% levels, respectively.

Both SCI, and UI, were affected by the interaction of genotype x density. In both sites and in both D1 and D2, the SCI was higher in 701 and 4123. The differences amongst these hybrids though were insignificant (Figure 5). The highest SCI mean value was observed in AUA, in 1432 D2 (187.94) and the lowest in AA, in 'Elpida' D2 (132.14). The same subplots (1432 D2 and 'Elpida' D2) also reported the highest and lowest UI values (respectively). In general, the best UI results were noted in the two hybrids (Figure 5).

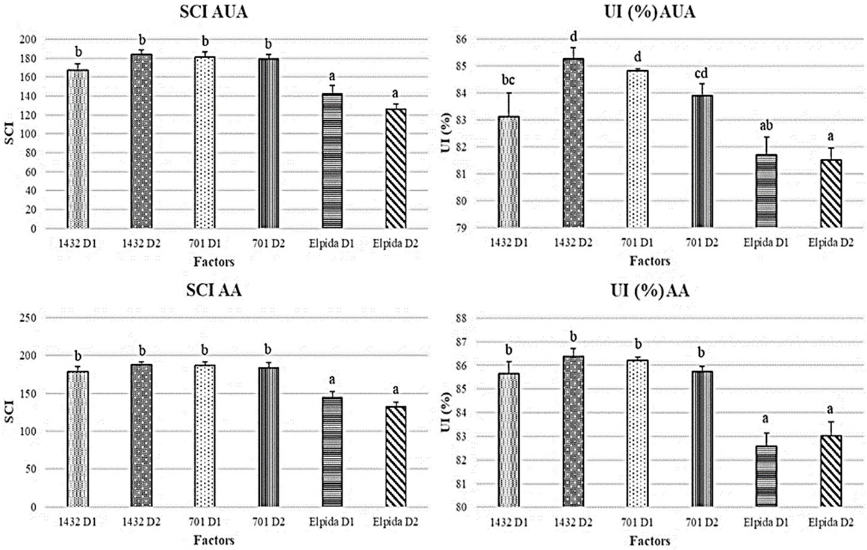


Figure 5. SCI and UI as affected by the combination of genotype x density
Genotypes: 1432, 701, and 'Elpida'; Densities: D1 and D2. Statistically significant differences are depicted by the different letters (a, b, c, and d).

The TrID and Elg, much alike the Mic, were only affected by the genotype and reported significant differences only in D2. 'Elpida' fibers had the higher Elg% by approximately 0.8-1.3% (Table 5). TrID on the contrary was higher in 1432, and particularly in D2 (of both sites) where it was significantly increased (by two times fold in AUA and by 60% in AA) compared to the lowest values reported in 'Elpida'. Rd measurements indicated improved reflectance in 'Elpida', and particularly in D2 (Table 5). The increment of Rd values compared to the respective ones of the hybrids ranged between 5-10%. Contrasting the Rd measurements, +b values were significantly higher in 1432, followed by 701. The most notable differences in +b was observed in AA and particularly between 'Elpida' and 1432 (by 9% and 10% in D1 and D2, respectively).

Str did not differ significantly between 1432 and 701. However, it was significantly higher in the hybrids when compared to 'Elpida' (Figure 6). Interestingly, this trait reported significant differences also between D1 and D2 in 'Elpida' (D1 was improved by 2.5-3% in both sites). The best Str results were noted in 1432, and the worst in 'Elpida'.

Table 5. Combined analysis of variance (F values) for the measured quality traits Elg, Rd, +b, and TrID of three cotton genotypes (1432, 701, and 'Elpida') cultivated in two different sites (AUA and AA), and in two different sowing densities (D1 and D2)

Variety/hybrid	**Elg (%)**		**Rd**		**+b**		**TrID**	
	D1	**D2**	**D1**	**D2**	**D1**	**D2**	**D1**	**D2**
AUA								
1432	9.46^{ns}	8.91^{a}	70.60^{ns}	68.99^{a}	9.75^{b}	9.37^{ns}	6.25^{ns}	7.25^{b}
701	9.53^{ns}	9.32^{a}	72.63^{ns}	72.76^{b}	9.28^{ab}	9.17^{ns}	5.50^{ns}	5.50^{ab}
'Elpida'	9.86^{ns}	10.19^{b}	74.58^{ns}	76.23^{c}	8.85^{a}	8.50^{ns}	5.00^{ns}	3.50^{a}
AA								
1432	9.46^{ns}	9.17^{a}	71.10^{a}	71.62^{a}	9.51^{b}	9.53^{b}	6.50^{ns}	6.75^{b}
701	9.57^{ns}	9.61^{a}	72.28^{a}	73.30^{a}	9.49^{b}	9.39^{b}	6.25^{ns}	5.50^{ab}
'Elpida'	9.84^{ns}	10.44^{b}	76.87^{b}	77.93^{b}	8.71^{a}	8.66^{a}	5.25^{ns}	4.25^{a}
Site (F_s)	*ns*		*ns*		*ns*		*ns*	
Genotype (F_v)	*15.44****		*31.92****		*14.69****		*10.88****	
Density (F_d)	*ns*		*ns*		*ns*		*ns*	

$F_s \times F_v$	*ns*	*ns*	*ns*	*ns*
$F_s \times F_d$	*ns*	*ns*	*ns*	*ns*
$F_v \times F_d$	*ns*	*ns*	*ns*	*ns*
$F_s \times F_v \times F_d$	*ns*	*ns*	*ns*	*ns*

F-test ratios originated from ANOVA. ns: non-significant; *, ** and ***: Significant at the 5%, 1% and 0.1% levels, respectively

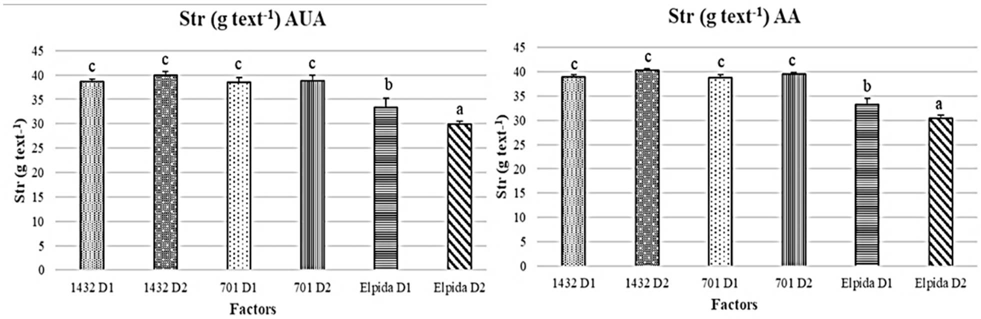


Figure 6. Str as affected by the combination of genotype x density

Genotypes: 1432, 701, and 'Elpida'; Densities: D1 and D2. Statistically significant differences are depicted by the different letters (a, b, and c).

Lastly, TrAr and TrCnt were notably higher in the hybrids, and particularly in 1432 D2 (Figure 7). These traits were significantly affected by genotype x density, with 1432 showcasing the highest values in both traits, and 'Elpida' the lowest. &01 and 'Elpida' did not report significant differences in TrAr and TrCnt in D1.

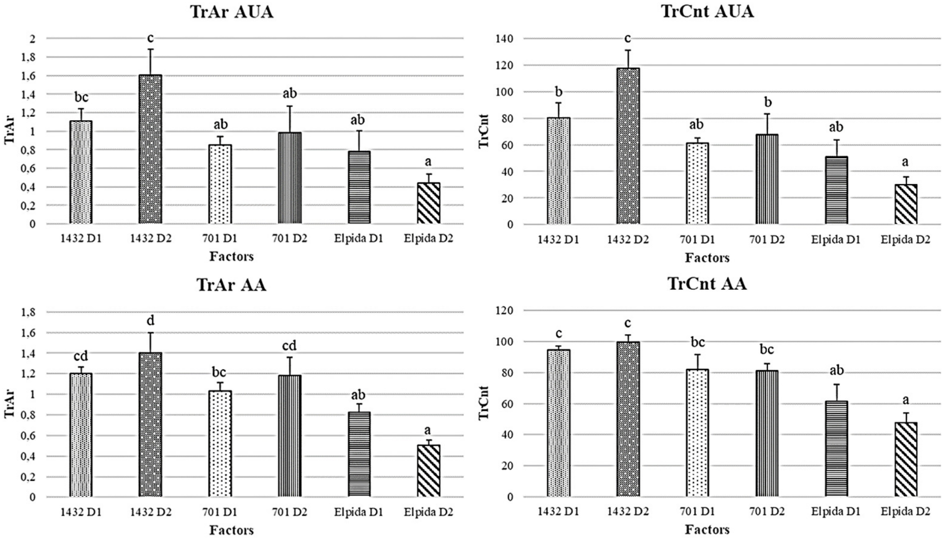


Figure 7. TrAr and TrCnt as affected by the combination of genotype x density

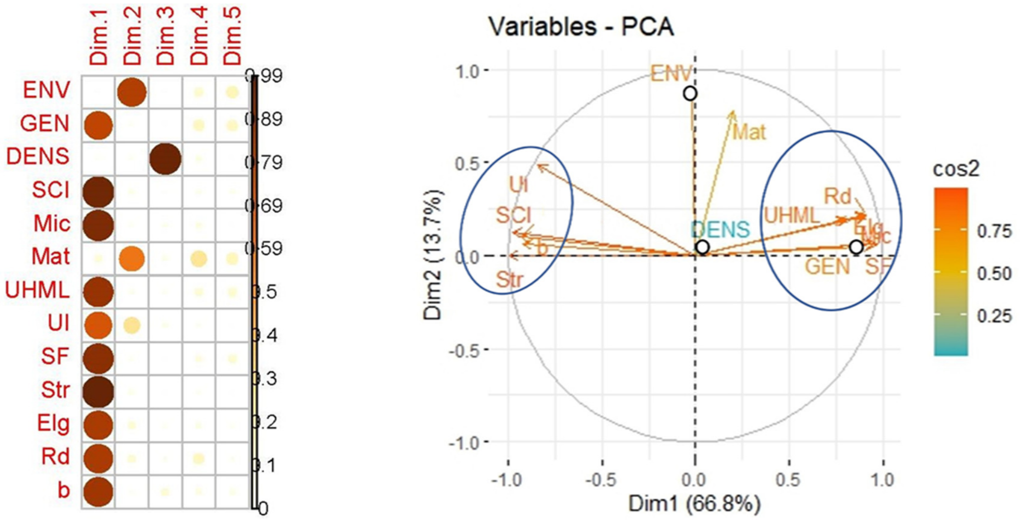
Genotypes: 1432, 701, and 'Elpida'; Densities: D1 and D2. Statistically significant differences are depicted by the different letters (a, b, c, and d).

PCA analysis

Figure 8 showcases plots depicting the contribution of ten fiber quality attributes viz. Spinning consistency index (SCI), Micronaire (Mic), Maturity index (MAT), Upper half mean length (UHML), Uniformity index (UI), Short fiber (SF), Fiber strength (STR), Elongation (%) (ELG), Reflectance (RD) and

Yellowness (b) across two environments for three cotton genotypes. As illustrated in Figure 8a, apart from the Density, all other descriptors made nearly equal contributions to the first dimension, while Environment and Mat to the second dimension. Density is a significant contribution in the third dimension. The goodness-of-fit of the first two dimensions of the analysis explained a significant 80.5% of the overall variation, as presented in Figure 8b. Regarding the Fiber quality attributes there are two distinct clusters. The first cluster (right) is formed by UHML, Rd, Elg, SF, Mic which have a positive correlation with the Genotype since their vectors form acute angles with the Genotype vector. The second cluster presented in Figure 8b is formed by four fiber quality measurements, i.e., UI, SCI, Str and b with their biplot axes pointing in similar directions and a positive correlation among them.

Further, it is notable that the Environment has the same contribution in nearly all of the fiber quality measurements except from Mat and UI that seem to have a stronger correlation. In addition, the Density had a minor but positive correlation to all fiber quality traits dew to the minor nature of its projection on the horizontal axes.



(a) (b)

Figure 8. PCA results visualized graphically

(a) A correlation plot that highlights the most contributing variables for each dimension (b) A plot with the percentage of the variation explained by the first two principal components (80.5%) and the contribution of the measurements to each dimension. Dots are factors and arrow are Fiber quality attributes.

Discussion

In their majority, the results regarding the performance of cotton plants did not differ significantly in the two sites. The most significant differences were noted in the biomass measurements and the LAI. In a study conducted by Karydogianni *et al.* (2020), the authors assessed the effect of fertilization in cotton, in two different sites located close to respective ones of the present study. In their work, Karydogianni *et al.* (2020) did not observe any significant differences in the dry matter and the LAI values of cotton, though in their research they used another cotton variety (ST 402), and the fertilizers and fertilization rates were also different. In our study, notable differences in the dry matter (only in 701) and the LAI values amongst the AUA and the AA plants were only reported in the D1. This observation could be attributed to the combination of the different soil properties in the two sites (Table 1) and the improved solar radiation transmission in the lower densities (Yang *et al.*, 2014), that improved the vigorousness of the hybrids. This probably resulted to a slightly better canopy structure, that could enhance the photosynthetic activity and increase the biomass of the plants (Mutsaers, 1982). This hypothesis coincides with the specific characteristics of 701 phenotype (CPVO, 2023).

Similarly, the different densities had little to none effect on the cotton's agronomic characteristics, and the yield. This was unexpected as the literature suggests that the density of cotton plants is closely correlated to their performance (Zhi *et al*., 2016). However, it should be noted that some agronomic traits of cotton (e.g. LAI) have been proposed to be unaffected by the sowing density Chapepa *et al.* (2020). In Greece, optimal sowing density for cotton usually ranges from 10-20 plants m^2, depending on the variety, the soil characteristics, and other environmental factors (Kosmidou and Rousopoulos, 1986), though is some instances higher sowing rates were also possible (Bartzialis *et al*., 1998). Our results suggest that as the yields of 701 and 1432 did not differ significantly in D1 and D2, lower densities could be preferable as they reduce the production cost (Khan *et al*., 2017). In their study, Altundag and Karademir (2021) have also concluded that plant density does not nessecerily affect the yield components of cotton.

The genotype of the cotton was the most influential factor in the present study. The DM, LAI, and the yield of the two hybrids were significantly higher than the respective ones of 'Elpida'. This was partially anticipated, as the literature suggests that the agronomic traits and the yield of interspecific hybrids is often superior to the respective ones of *G. hirsutum* (Li *et al*., 2022). Interestingly, the differences in the lint yield of 701, 1432, and 'Elpida' were insignificant, and the yields of the hybrids were higher due to their increased seed yields. This finding is on par with the literature (Sharma, 1979; Shahzad *et al*., 2020) and it implies that these hybrids could have an improved nitrogen uptake efficiency (NUE), as NUE has been suggested to regulate seed yield in cotton (Gospodinova and Panayotova, 2019). This is also supported by the observed improved agronomic traits of 701 and 1432 in both sides. Overall, the yields of 'Elpida', 701, and 1432 were similar to the yields reported by other studies conducted in Greece, with similar fertilization and irrigation regimes, and sowing densities (Avgoulas *et al*., 2005; Patsiali *et al*., 2014; Karydogianni *et al*., 2020).

Most of the quality traits assessed in the present study were not affected by the environment and the sowing density. In particular, Mst, Mic, Mat, UHML, SF, Elg, Rd, +b, and TrID were solely affected by the genotype. This result is in accordance with some studies suggesting that plant densities do not affect cotton fiber's quality traits (Arunvenkatesh and Rajendran, 2013), yet it contradicts the significant impact of spatial variability on them (He *et al*., 2021; Darawsheh *et al*., 2022), as proposed by the literature. Possibly, the similar edaphoclimatic conditions in the two sites reduced the effect of environmental factors on the performance of the cotton. This has also been observed in a study by Karydogianni *et al.* (2020).

Higher Mic values (within a range) characterize the fibers as more mature and thicker, facilitating spinning process. According to Pei *et al.* (2021), Mic values 3.7 - 4.2 correspond to premium fibers, whilst values exceeding 5.0 cause a decrease in cotton's quality and price. In the present study, 'Elpida's Mic was within the optimal range (3.9 and 3.7 for AUA and AA, respectively), and the hybrids were significantly inferior. This seems to agree with other studies, where hybrid's Mic was lower than the respective of conventional cultivars (Zhi *et al*., 2016). On the contrary, our results regarding the Mat contradict the literature. Mat did not report any significant differences amongst the different sites, genotypes, and densities, in contrast to the findings of Long *et al.* (2021), though it is worth mentioning that according to Feng *et al.* (2014) plant density does not affect this trait. Interestingly, Mat values in all three studied genotypes ('Elpida', 701, and 1432) ranged 0.82-0.84, constituting their fibers immature (Darawsheh *et al*., 2022).

Fibers' length differs between different cotton genotypes. This trait is also affected by abiotic and biotic factors (Delhom *et al*., 2020). In the present study, UHML was affected only by the genotype. Moreover, SF was statistically significantly lower in the hybrids. A higher content of short fibers can cause a decrease in UI (Günaydin *et al*., 2018). Here, UI was lower in 'Elpida' in both sites (81.6 and 82.81 for AUA and AA, respectively) compared to the hybrids (84.28 and 85.99 for AUA and AA, respectively). Based on our results, 'Elpida's SF rate is characterized as average and the respective ones of 701 and 1432 as high – very high. Plant density once again did not affect this trait (UI). This seems to agree with the study of Khan et al. (2019), where the UI of cotton fibers did not differ in three different densities. Overall, 701 and 1432 reported longer fibers than ‘Elpida’. The results from the present study seems to agree with other studies (Bednarz *et al*., 2006; Arunvenkatesh *et al*., 2013). Elg on the contrary was lower in the hybrids compared to ‘Elpida’. Nonetheless, Elg was exceptional in all three genotypes (Darawsheh *et al*., 2022).

Similarly, all three genotypes showcased high Str values. In the present study, Str was affected by the combination of genotype x density. Notably, increasing plant densities caused a decrease in fiber’s Str in both sites. The Str results seem to agree with the ones presented by Khan *et al*. (2019), where it was affected by plant density, and by Fang *et al*. (2021), where the authors concluded that it partially depends on the genome of cotton. The hybrids also recorded higher TrID, TrAr and TrCnt than ‘Elpida’, indicating higher amount of trash. According to Darawsheh *et al*. (2022) the amount of trash is highly affected by the environment, however in the present study, there were insignificant differences between the two sites. Identically to the other quality traits, trash amount was affected only by the plant’s genotype.

Rd and +b are used in order to estimate cotton’s lint color. Rd measures the brightness of the sample, and +b refers the yellowness (Matusiak *et al*., 2010; Çopur et al. 2018). In the present study, both Rd and +b were affected only by plant’s genotype. For Rd, higher values were recorded on ‘Elpida’. For +b, higher values were noted on hybrids. On the contrary, Çopur *et al*. (2018) found that these traits are affected by the environment. Lastly, the SCI was unaffected by any factor other than the cotton’s genotype. Hybrids’ SCI was statistically significantly higher than the respective one of ‘Elpida’. Their fibers are characterized as A++ because of the high value of SCI (higher than 150) and ‘Elpida’’s are characterized as A+ (Yusupaliyeva and Yuldashev, 2022).

Conclusions

In their majority, the quality traits of the 701 and 1432 were notably improved in comparison to ‘Elpida’s. The differences amongst the traits of the two hybrids’ fibers in both sites and densities were insignificant and inconsistent, therefore we failed to distinguish one of these two as superior regarding its quality. The overall performance of the hybrids was promising as their agronomic characteristics, yield components, and fiber quality were on average equal or greater than the respective ones of ‘Elpida’s. The findings of the present study suggest that they could constitute a dynamic alternative for the EU cotton sector. However, much is needed in order to optimize their performance. D1 seems suitable for their cultivation, yet the optimal fertilization rates have not been identified. Further research is also required in order to assess their adaptability to margin and low-fertility soils, and to heat and drought stress.

Authors’ Contributions

IK think up the idea; DB and ET designed the experiments; The validation was performed by AT, ATs and IR; The formal analysis was performed by AM, ET, and DV; PS conducted the investigation; DB, ET and DV curated the data; The original draft was prepared by AM and PS; Writing—review and editing was performed by I.K., AM and PS; IK visualized, supervised and administrated the project. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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