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PHYSIOLOGICAL, MORPHO-AGRONOMICAL AND MOLECULAR CHARACTERIZATION OF UPLAND COTTON (*GOSSYPIUM HIRSUTUM* L.) GENOTYPES UNDER VARYING WATER SUPPLY

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ABSTRACT
Water deficit limits upland cotton yields across the irrigated semi-arid zone of Central Asia. We grew 13 cotton genotypes under optimal water supply and under simulated drought, in which soil moisture fell to 48–50% of field water-holding capacity during flowering and boll filling. Leaf water content, transpiration rate, photosynthetic pigments, and yield and fiber traits were recorded, and the genotypes were genotyped with 21 simple sequence repeat (SSR) markers. Drought lowered leaf water content by 1.6–8.0%; XiLuZhong-52, T-217, T-4, and T-6 lost the least. Transpiration fell by as much as 59.1% in XiLuZhong-78 but by only 5.3% in T-6. T-2, T-4, and XiLuZhong-78 kept chlorophyll and carotenoid contents close to their well-watered levels. Single-plant yield losses ranged from 14.7% (XiLuZhong-78) to 39.5% (T-17), and XiLuZhong-78, Chuntay-2, and T-6 kept more than 78% of their optimal yield; the standard cultivar Namangan-77 lost 33.2%. Fiber length changed little (0.5–2.4 mm). All SSR loci were polymorphic, with 2–6 alleles per locus and a mean expected heterozygosity of 0.56. In exploratory allele-class comparisons, alleles at BNL3436, NAU3588, JESPR152, and BNL3347 were associated with chlorophyll retention or yield under drought. XiLuZhong-78, Chuntay-2, and T-6 are promising parents for breeding cotton for water-limited conditions, and the four markers deserve testing in larger mapping populations.

KEYWORDS: Drought tolerance; Leaf water content; Photosynthetic pigments; SSR markers; Yield stability; Upland cotton

INTRODUCTION
Upland cotton (*Gossypium hirsutum* L.) supplies about 90% of the world's cotton fiber, and its seed is a source of oil and protein meal (Paterson et al., 2012). In Uzbekistan, cotton occupies roughly 1.2–1.4 million hectares and is still one of the main agricultural exports (Abdullaev and Rakhmatullaev, 2015). Almost all of it is grown under irrigation in the semi-arid Aral Sea basin.

That dependence on irrigation makes the crop vulnerable. Droughts are expected to become more frequent and more severe in many cotton-growing regions, Central Asia among them (Trenberth et al., 2014; Lesk et al., 2016), and the desiccation of the Aral Sea has already reduced the water available to farms in the basin (Micklin, 2007). Breeders therefore need cultivars that hold their yield when irrigation is cut back.

Water deficit lowers leaf turgor, closes stomata, and restricts CO_2 uptake (Chaves et al., 2009). If the stress persists, reactive oxygen species build up, damaging membranes and photosynthetic pigments and hastening leaf senescence (Mittler, 2002; Reddy et al., 2004; Jaleel et al., 2009). In cotton these changes translate into fewer retained bolls, lighter bolls and seeds, and lower lint yield (Pettigrew, 2004; Loka et al., 2011). Tolerant genotypes limit the damage through osmotic adjustment and antioxidant defence (Nayyar and Walia, 2003; Reddy et al., 2004; Farooq et al., 2009).

Some of these responses can be measured quickly in the field. Leaf water content and transpiration rate show how well a plant keeps its leaves hydrated and its stomata open as the soil dries (Barrs and Weatherley, 1962; Turner, 1981). Chlorophyll and carotenoid contents reflect the condition of the photosynthetic apparatus; carotenoids also protect it by dissipating excess light energy (Lichtenthaler, 1987; Demmig-Adams and Adams, 1996; Niyogi, 1999). Expressing each trait as its relative change under stress, as in the indices of Fischer and Maurer (1978), makes genotypes easier to compare.

Simple sequence repeat (SSR) markers are codominant, polymorphic, and reproducible, and they remain a practical tool for describing cotton germplasm (Powell et al., 1996; Temnykh et al., 2001). Many SSR loci have been placed on genetic maps of tetraploid cotton (Lacape et al., 2003; Rong et al., 2004) and used to survey diversity in upland cotton collections (Tyagi et al., 2014). Mapping studies have also tied SSR-tagged regions to cotton performance under water deficit (Saranga et al., 2004; Zheng et al., 2016).

Uzbek breeding lines and cultivars introduced from China are now widely used in local breeding, but they have rarely been compared physiologically, agronomically, and molecularly under the same drought regime. We therefore (1) evaluated 13 genotypes for leaf water status, transpiration, and yield and fiber traits under optimal and simulated drought conditions; (2) measured drought-induced changes in photosynthetic pigments; (3) assessed genetic diversity with 21 SSR markers; and (4) explored whether particular SSR alleles were associated with drought-related traits.

MATERIAL AND METHODS

Experimental site

Field experiments were carried out in 2025 on two experimental fields of the Institute of Genetics and Plant Experimental Biology, Academy of Sciences of the Republic of Uzbekistan, in Zangi-Ota District, Tashkent Region. The fields lie 18–22 km from Tashkent in the upper Chirchik River basin, at 395–400 m above sea level. The climate is sharply continental: summers (June–August) are hot and dry, almost all precipitation falls in autumn, winter, and spring, and cotton has to be irrigated. The frost-free period lasts 200–210 days.

The soil is a typical medium-loam grey-brown soil (sierozem) with relatively low humus content and no signs of salinization, so the stress applied was water deficit alone. Bulk density is 1.32–1.33 g cm^{-3}, field water-holding capacity (FWHC) is about 22%, and the groundwater table lies at 7–9 m. The fields have a gentle slope, which gives even drainage and water distribution during irrigation.

Plant material

Thirteen genotypes were studied: eight Uzbek breeding lines, three cultivars introduced from China, the local standard cultivar Namangan-77, and the long-staple cultivar Marvarid (*Gossypium barbadense* L.), which was included as a reference (Table 1). Seed came from the genebank of the Uzbek Cotton Research Institute (UzPITI) and from the seed collection of the Institute of Genetics and Plant Experimental Biology.

Table 1. Cotton genotypes evaluated in this study.

Genotype	Species	Origin	Type
T-2	*G. hirsutum* L.	Uzbekistan	Breeding line
T-4	*G. hirsutum* L.	Uzbekistan	Breeding line
T-6	*G. hirsutum* L.	Uzbekistan	Breeding line
T-7	*G. hirsutum* L.	Uzbekistan	Breeding line
T-17	*G. hirsutum* L.	Uzbekistan	Breeding line
T-217	*G. hirsutum* L.	Uzbekistan	Breeding line
T-218	*G. hirsutum* L.	Uzbekistan	Breeding line
T-1003	*G. hirsutum* L.	Uzbekistan	Breeding line
Namangan-77	*G. hirsutum* L.	Uzbekistan	Standard cultivar
Chuntay-2	*G. hirsutum* L.	China	Introduced cultivar
XiLuZhong-52	*G. hirsutum* L.	China	Introduced cultivar
XiLuZhong-78	*G. hirsutum* L.	China	Introduced cultivar
Marvarid	*G. barbadense* L.	Uzbekistan	Long-staple reference

Experimental design and water supply treatments

Field trials and agronomic assessments followed the methodological guidelines of UzPITI (2014). The field was divided into two water supply backgrounds. Within each, genotypes were arranged in randomized blocks with three replications; each plot was a single row of 15 hills. Seeds were sown by hand at 4–5 cm depth in a 90 × 20 × 1 cm scheme (row spacing × plant spacing × seeds per hill).

Under optimal water supply (OF), the crop received four irrigations in a 1:2:1 scheme across the vegetative, flowering–boll formation, and maturation phases, a total of 4800–5000 m^3 ha^{-1}. Under simulated drought (SD), it received two irrigations in a 1:1:0 scheme (2800–3000 m^3 ha^{-1}): the number of irrigations during flowering and boll filling was reduced, and no water was given during maturation. In June–July, when the physiological measurements were made, soil moisture was 70–72% FWHC in OF and 48–50% FWHC in SD. Mineral fertilizer was applied at the standard rates for the region (N 250, P_2O_5 180, and K_2O 115 kg ha^{-1} as pure nutrients), partly before autumn ploughing and partly as three top-dressings at the start of budding, at mass budding, and at flowering. Inter-row cultivation and weeding were timed to the irrigations, and Bi-58 was applied against aphids at the seedling stage. All practices other than irrigation were the same in both treatments.

Physiological measurements

Physiological traits were measured in both treatments on the same days during flowering and boll formation, on the fully expanded third leaf from the apex of three representative plants per plot. Leaf water content (LWC, %)

was calculated from the fresh, dry, and fully turgid leaf masses following Tretyakov (1990). Transpiration rate (TR, mmol H_2O m^{-2} s^{-1}) was measured with a TPQK-1000 leaf porometer between 09:00 and 11:00 under natural light.

Photosynthetic pigments were extracted from 0.1 g of fresh leaf tissue in 96% ethanol. After centrifugation at 3000 *g* for 10 min, absorbance was read at 665 and 649 nm for chlorophylls and at 470 nm for carotenoids on a Cary 60 UV-Vis spectrophotometer (Agilent, USA). Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents were calculated with the equations of Lichtenthaler and Wellburn (1983) and expressed per gram of fresh weight (mg g^{-1} FW).

For each trait, the drought response was expressed as an adaptability coefficient, Cad (%) = [(SD − OF)/OF] × 100, where OF and SD are the trait means under optimal water supply and simulated drought (adapted from Fischer and Maurer, 1978). Negative values denote a reduction under drought; values close to zero denote stability.

Morpho-agronomical traits

At maturity, boll weight (g), single-plant seed cotton yield (g), fiber output (lint percentage, %), fiber length (mm), and 1000-seed weight (g) were recorded for each genotype in both treatments (UzPITI, 2014). Fiber length was measured with a High Volume Instrument at the Republican "Sifat" Centre (Tashkent) in accordance with standard UzDSt 604-2001.

DNA extraction

Genomic DNA was isolated from 50–100 mg of young leaf tissue ground in liquid nitrogen, using the PureLink™ Genomic Plant DNA Purification Kit (Thermo Fisher Scientific, USA). Because cotton leaves are rich in phenolics, 1% polyvinylpyrrolidone and 1–2% β-mercaptoethanol were added to the lysis buffer when needed. Lysis with proteinase K was carried out at 55°C for 10–30 min, RNA was removed with RNase A (10 mg mL^{-1}, 37°C, 10 min), and the lysate was cleared at 12,000 *g* for 5 min. DNA was bound to the spin column, washed with Wash Buffers 1 and 2, dried by a further 2-min spin, and eluted in 50–100 µL of elution buffer pre-warmed to 65°C. DNA concentration and purity were checked on a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific); A260/A280 ratios were about 1.8 and A260/A230 ratios exceeded 2.0.

SSR genotyping

A panel of 21 SSR markers from the BNL, NAU, JESPR, and TMB series was assembled from published molecular-genetic studies of fiber quality, drought tolerance, and salt tolerance in cotton, taking map positions into account (Rong et al., 2004; Tyagi et al., 2014).

PCR was run in 15 µL reactions containing 7.5 µL Phusion™ Plus PCR Master Mix (2×; Thermo Fisher Scientific), 0.3 µL of each primer (10 µM stock; 0.2 µM final), 0.5 µL template DNA (10–100 ng), and 6.4 µL nuclease-free water. The thermal profile was 98°C for 30 s; 30 cycles of 98°C for 10 s, 55–65°C for 15–30 s (annealing temperature set for each primer pair according to its T_m), and 72°C for 15–30 s per kb; and a final extension at 72°C for 5 min. Three to five microlitres of each product were separated on 1.5–2.0% agarose gels in 1× TBE at 90–110 V for 45–55 min, stained with ethidium bromide, and sized against a 100-bp ladder (Thermo Fisher Scientific). For selected markers, fragment sizes were determined on an ABI 3500 Genetic Analyzer (Applied Biosystems, USA) with FAM- or HEX-labelled forward primers and Peak Scanner 2.0 software.

Data analysis

Means and standard errors were calculated for each genotype and treatment, and the least significant difference at $P = 0.05$ ($LSD_{0.05}$) was used to compare genotypes for 1000-seed weight. For each SSR locus, the observed (Na) and effective (Ne) numbers of alleles and the expected heterozygosity (H_e) were calculated with POPGENE 1.32 (Yeh et al., 1999) and GenAlEx 6.5 (Peakall and Smouse, 2012). To explore marker-trait associations, genotypes were split into carriers and non-carriers of each allele, and trait means under SD were compared by one-way ANOVA. No correction for multiple testing was applied, so these comparisons are exploratory.

RESULTS

Leaf water content

With full irrigation, LWC ranged from 71.1% in T-217 to 76.9% in Namangan-77. Drought lowered LWC in every genotype, but the size of the drop varied about fivefold, from 1.6% in XiLuZhong-52 to 8.0% in T-1003 (Figure 1).

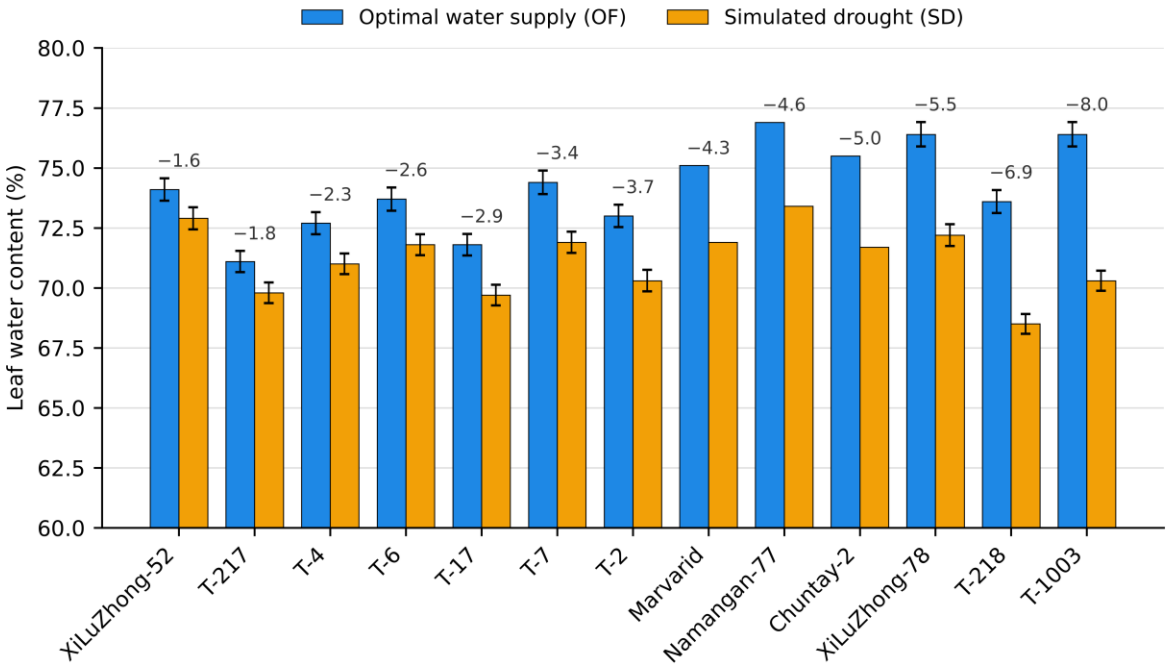

Figure 1. Leaf water content (LWC, %) of 13 cotton genotypes under optimal water supply (OF) and simulated drought (SD). Values are means ± SE (N = 3). The number above each pair of bars is the adaptability coefficient (Cad, %); genotypes are ordered from the least to the most affected.

Four genotypes stood out for keeping their leaves hydrated: XiLuZhong-52 (Cad = −1.6%), T-217 (−1.8%), T-4 (−2.3%), and T-6 (−2.6%), followed closely by T-17 (−2.9%). At the other end, T-1003 (−8.0%), T-218 (−6.9%), and XiLuZhong-78 (−5.5%) lost the most leaf water.

Transpiration rate

Transpiration responded more strongly to drought than any other physiological trait. Under OF, TR ranged from 257.97 ± 5.4 mmol H_2O m^{-2} s^{-1} in XiLuZhong-52 to 445.01 ± 7.9 mmol H_2O m^{-2} s^{-1} in XiLuZhong-78 (Figure 2).

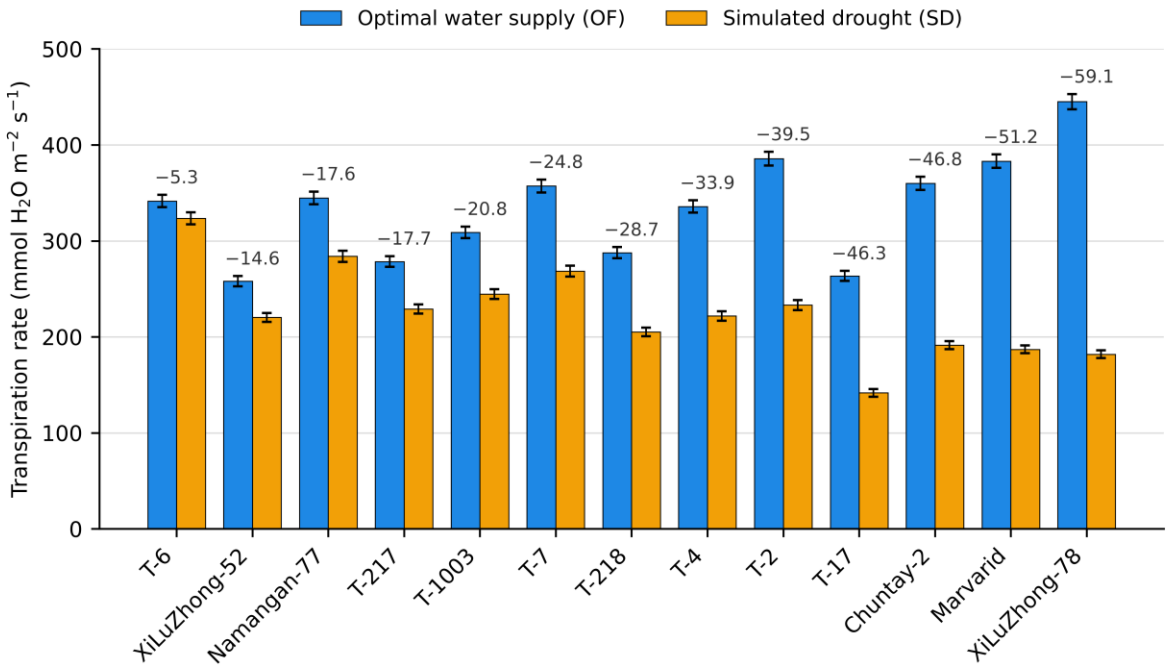

Figure 2. Transpiration rate (TR, mmol H_2O m^{-2} s^{-1}) of 13 cotton genotypes under optimal water supply (OF) and simulated drought (SD). Values are means ± SE (N = 3). The number above each pair of bars is the adaptability coefficient (Cad, %); genotypes are ordered from the least to the most affected.

Under SD, XiLuZhong-78 cut its transpiration by 59.1%, Marvarid by 51.2%, Chuntay-2 by 46.8%, and T-17 by 46.3%. T-6 (−5.3%), XiLuZhong-52 (−14.6%), Namangan-77 (−17.6%), and T-217 (−17.7%) changed much less.

Photosynthetic pigments

Chlorophyll *a* under OF ranged from 0.75 ± 0.03 mg g^{-1} FW in T-1003 to 1.85 ± 0.06 mg g^{-1} FW in XiLuZhong-52. Drought affected the genotypes very differently (Figure 3A). T-2, T-4, and XiLuZhong-78 kept chlorophyll *a* at, or slightly above, their OF levels, whereas T-6, T-7, T-217, T-218, Namangan-77, and Chuntay-2 lost a good deal of it. The largest losses were in T-6 (0.65 mg g^{-1} FW) and T-218 (0.55 mg g^{-1} FW).

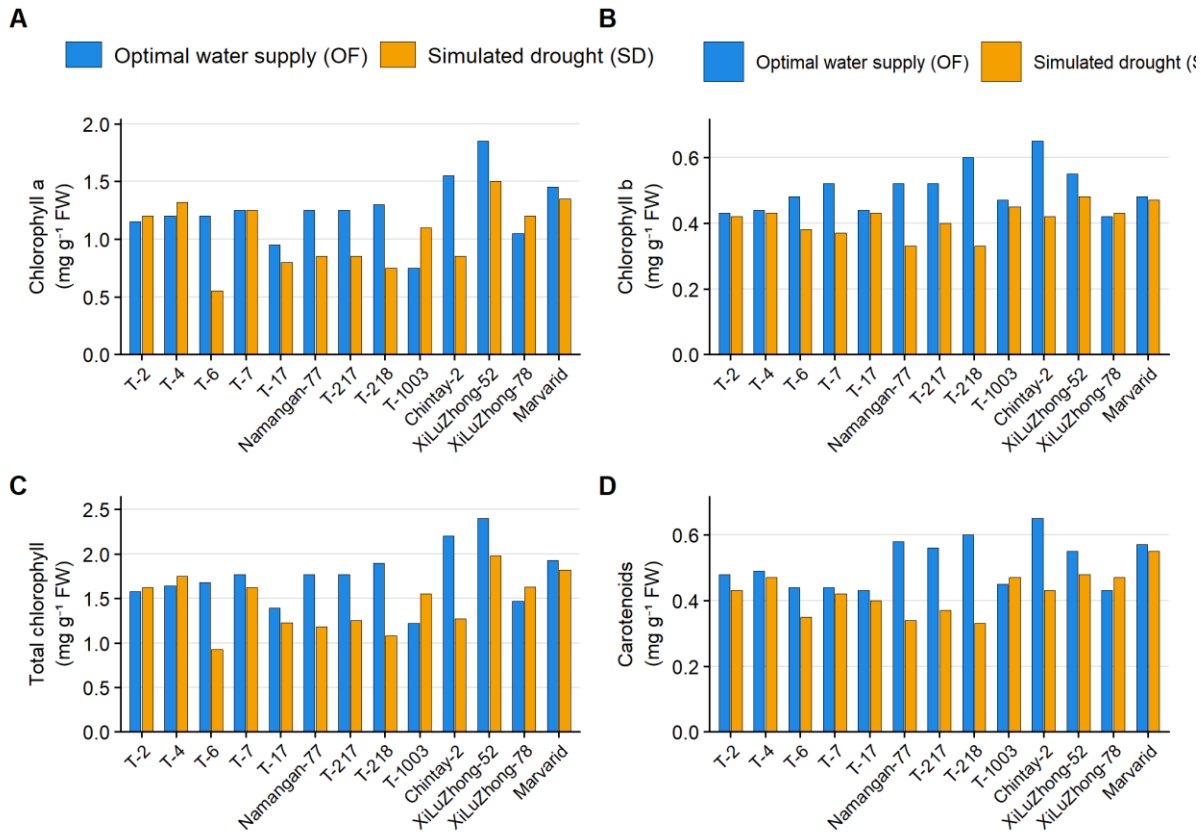

Figure 3. Contents of chlorophyll *a* (A), chlorophyll *b* (B), total chlorophyll (C), and carotenoids (D) (mg g^{-1} fresh weight) in 13 cotton genotypes under optimal water supply (OF) and simulated drought (SD).

Chlorophyll *b* behaved in much the same way (Figure 3B). It ranged from 0.42 ± 0.02 mg g^{-1} FW in T-2 to 0.65 ± 0.03 mg g^{-1} FW in Chuntay-2 under OF; under SD it stayed stable in T-2, T-4, T-17, XiLuZhong-78, and Marvarid and fell most in T-218, Namangan-77, and Chuntay-2. Total chlorophyll ranged from 1.22 ± 0.04 mg g^{-1} FW (T-1003) to 2.40 ± 0.08 mg g^{-1} FW (XiLuZhong-52) under OF and declined in all genotypes under SD (Figure 3C). The same five genotypes (T-2, T-4, T-17, XiLuZhong-78, and Marvarid) stayed close to their OF values, while T-218 and Namangan-77 showed the steepest declines.

Carotenoids under OF ranged from 0.43 ± 0.02 mg g^{-1} FW in T-17 to 0.65 ± 0.03 mg g^{-1} FW in Chuntay-2 (Figure 3D). Again, T-2, T-4, T-17, XiLuZhong-78, and Marvarid held their carotenoid levels or raised them slightly under SD, whereas T-6, T-7, Namangan-77, T-217, T-218, and Chuntay-2 lost carotenoids.

Yield components and yield

Boll weight was the most stable yield component. Its Cad ranged from −1.3% in Chuntay-2 to −18.0% in T-217 (Table 2), and Chuntay-2, T-4 (−3.3%), T-1003 (−4.3%), and T-17 (−6.4%) were affected least.

Table 2. Boll weight (g) and adaptability coefficient (Cad, %) of 13 cotton genotypes under optimal water supply (OF) and simulated drought (SD).

Genotype	OF ± SE (g)	SD ± SE (g)	Cad (%)
T-2	6.03 ± 0.17	5.12 ± 0.44	−15.1
T-4	5.82 ± 0.17	5.63 ± 0.49	−3.3
T-6	6.60 ± 0.19	6.07 ± 0.53	−8.0
T-7	5.54 ± 0.16	5.01 ± 0.43	−9.6
T-17	5.64 ± 0.16	5.28 ± 0.46	−6.4

Genotype	OF ± SE (g)	SD ± SE (g)	Cad (%)
Namangan-77	5.50 ± 0.16	5.00 ± 0.43	−9.1
T-217	5.90 ± 0.17	4.84 ± 0.42	−18.0
T-218	5.79 ± 0.17	5.18 ± 0.45	−10.5
T-1003	5.62 ± 0.16	5.38 ± 0.47	−4.3
Chuntay-2	5.38 ± 0.16	5.31 ± 0.46	−1.3
XiLuZhong-52	5.61 ± 0.16	4.76 ± 0.41	−15.2
XiLuZhong-78	5.53 ± 0.16	4.85 ± 0.42	−12.3
Marvarid	3.40 ± 0.10	3.17 ± 0.27	−6.8

SE = standard error (N = 3). Cad (%) = [(SD − OF)/OF] × 100.

Single-plant seed cotton yield differed most among genotypes in its response to drought (Table 3). With full irrigation, Chuntay-2 (90.33 ± 2.61 g), XiLuZhong-78 (86.25 ± 2.49 g), T-6 (84.62 ± 2.44 g), and T-17 (83.00 ± 2.40 g) yielded most. Under SD, yield losses ranged from 14.7% in XiLuZhong-78 to 39.5% in T-17, a 2.7-fold difference. XiLuZhong-78, Chuntay-2 (−17.0%), T-6 (−17.3%), and T-218 (−21.9%) kept more than 78% of their OF yield. T-17, T-2 (−35.5%), Namangan-77 (−33.2%), and T-217 (−32.9%) lost a third or more. In T-17 the large yield loss came with fairly stable boll weight (−6.4%), which suggests that the plants retained fewer bolls rather than producing lighter ones.

Table 3. Single-plant seed cotton yield (g) and adaptability coefficient (Cad, %) under optimal water supply (OF) and simulated drought (SD).

Genotype	OF ± SE (g)	SD ± SE (g)	Cad (%)
T-2	82.50 ± 2.38	53.18 ± 4.60	−35.5
T-4	75.44 ± 2.18	50.67 ± 4.39	−32.8
T-6	84.62 ± 2.44	70.00 ± 6.06	−17.3
T-7	78.09 ± 2.25	57.50 ± 4.98	−26.4
T-17	83.00 ± 2.40	50.23 ± 4.35	−39.5
Namangan-77	78.64 ± 2.27	52.52 ± 4.54	−33.2
T-217	81.36 ± 2.35	54.58 ± 4.72	−32.9
T-218	74.17 ± 2.14	57.89 ± 5.00	−21.9
T-1003	71.25 ± 2.06	51.67 ± 4.48	−27.5
Chuntay-2	90.33 ± 2.61	75.00 ± 6.49	−17.0
XiLuZhong-52	81.46 ± 2.35	62.22 ± 5.39	−23.6
XiLuZhong-78	86.25 ± 2.49	73.57 ± 6.37	−14.7
Marvarid	50.53 ± 1.46	38.01 ± 3.29	−24.8

SE = standard error (N = 3). Cad (%) = [(SD − OF)/OF] × 100.

Fiber traits and seed weight

Fiber traits changed only moderately (Table 4). Fiber output rose under SD in several genotypes, most in T-6 (+2.3 percentage points) and T-4 (+1.8), and fell most in T-218 (−3.0) and T-17 (−1.5). Fiber length decreased in every genotype, by 0.5 mm in T-1003 up to 2.4 mm in Namangan-77, that is, by 1.6–7.2%.

Table 4. Fiber output (FO, %) and fiber length (FL, mm) under optimal water supply (OF) and simulated drought (SD).

Genotype	FO OF (%)	FO SD (%)	ΔFO (pp)	FL OF (mm)	FL SD (mm)	ΔFL (mm)
T-2	43.5	43.2	−0.3	32.3	31.3	−1.0
T-4	42.3	44.1	+1.8	32.5	31.5	−1.0
T-6	43.5	45.8	+2.3	32.4	30.7	−1.7
T-7	43.1	44.0	+0.9	32.5	31.0	−1.5

Genotype	FO OF (%)	FO SD (%)	ΔFO (pp)	FL OF (mm)	FL SD (mm)	ΔFL (mm)
T-17	40.1	38.6	−1.5	33.0	31.7	−1.3
Namangan-77	41.2	42.1	+0.9	33.3	30.9	−2.4
T-217	42.2	41.0	−1.2	32.3	30.8	−1.5
T-218	41.9	38.9	−3.0	33.0	32.1	−0.9
T-1003	38.6	38.8	+0.2	32.2	31.7	−0.5
Chuntay-2	45.5	45.8	+0.3	32.7	31.5	−1.2
XiLuZhong-52	43.6	42.4	−1.2	32.2	30.3	−1.9
XiLuZhong-78	42.0	43.4	+1.4	33.4	31.4	−2.0
Marvarid	35.7	35.9	+0.2	38.2	37.5	−0.7

ΔFO = change in fiber output (percentage points); ΔFL = change in fiber length (mm); both calculated as SD − OF.

1000-seed weight fell in all genotypes under SD (Table 5). Losses were smallest in T-4 (−7.2%), T-6 (−7.7%), T-218 (−8.3%), T-2 (−8.5%), and Marvarid (−8.7%), and largest in T-1003 (−17.4%), T-7 (−15.9%), and Namangan-77 (−15.7%).

Table 5. 1000-seed weight (g) and adaptability coefficient (Cad, %) under optimal water supply (OF) and simulated drought (SD).

Genotype	OF (g)	SD (g)	Cad (%)
T-2	99.9	91.4	−8.5
T-4	110.1	102.2	−7.2
T-6	104.5	96.5	−7.7
T-7	104.1	87.6	−15.9
T-17	100.0	87.4	−12.6
Namangan-77	95.9	80.8	−15.7
T-217	101.8	87.8	−13.8
T-218	92.7	85.0	−8.3
T-1003	123.2	101.8	−17.4
Chuntay-2	113.3	102.2	−9.8
XiLuZhong-52	93.2	84.0	−9.9
XiLuZhong-78	95.4	86.7	−9.1
Marvarid	116.6	106.4	−8.7

Cad (%) = [(SD − OF)/OF] × 100. $LSD_{0.05}$ = 3.1 (OF) and 3.7 (SD).

Across all agronomic traits, Chuntay-2, XiLuZhong-78, and T-6 combined the highest yields under full irrigation with the smallest losses under drought. The standard cultivar Namangan-77, by contrast, lost a third of its yield.

SSR diversity

All 21 SSR loci were polymorphic in the panel (Table 6). The number of alleles per locus ranged from 2 to 6 (mean 3.05), and the effective number of alleles from 1.25 at NAU2679 to 5.72 at BNL3436 (mean 2.58). Mean expected heterozygosity was 0.56. Ten loci had H_e above 0.50, and four (BNL3436, 0.825; NAU3588, 0.781; BNL4028, 0.750; NAU5434, 0.712) exceeded 0.70. NAU2679 was the least informative locus (H_e = 0.198) because one allele dominated.

Table 6. Allelic diversity of 21 SSR loci in 13 cotton genotypes.

Locus	Na	Ne	H_e
BNL1151	2	2.00	0.500
BNL1421	3	1.75	0.430
BNL1604	3	2.41	0.586
BNL1672	2	1.99	0.498
BNL3347	3	2.47	0.595

Locus	Na	Ne	H_e
BNL3436	6	5.72	0.825
BNL4028	4	4.00	0.750
JESPR152	4	3.18	0.685
JESPR157	2	1.99	0.498
JESPR204	3	2.24	0.554
NAU2277	3	2.28	0.562
NAU2679	2	1.25	0.198
NAU3084	2	1.92	0.480
NAU3204	3	3.00	0.667
NAU3292	2	1.99	0.498
NAU3588	6	4.56	0.781
NAU3826	2	2.00	0.500
NAU3917	2	1.99	0.498
NAU5433	2	1.97	0.492
NAU5434	5	3.47	0.712
TMB1963	3	1.98	0.494
Mean	3.05	2.58	0.560

Na = observed number of alleles; Ne = effective number of alleles; H_e = expected heterozygosity.

Exploratory marker-trait associations

Allele classes at four markers differed in trait means under SD (Table 7). Genotypes without the 135-bp allele of BNL3436 had 35.5% more total chlorophyll than carriers (1.64 vs 1.21 mg g^{-1} FW). Carriers of the 240/260-bp allele of NAU3588 yielded 21.2% more than non-carriers (63 vs 52 g). The 400-bp allele of JESPR152 was linked to both higher total chlorophyll (1.70 vs 1.42 mg g^{-1} FW) and higher yield (63 vs 51 g), and carriers of the 150-bp allele of BNL3347 yielded 22% more than non-carriers (61 vs 50 g). These comparisons were made in only 13 genotypes and were not corrected for multiple testing, so we regard them as leads to be checked rather than as established associations.

Table 7. Exploratory associations between SSR alleles and drought-related traits under simulated drought (SD) in 13 cotton genotypes.

Marker	Allele	Trait under SD	Carriers	Non-carriers	Difference	Sig.
BNL3436	135 bp	Total chlorophyll (mg g^{-1} FW)	1.21	1.64	+35.5% in non-carriers	**
NAU3588	240/260 bp	Single-plant yield (g)	63	52	+21.2%	**
JESPR152	400 bp	Total chlorophyll (mg g^{-1} FW)	1.70	1.42	+19.7%	*
JESPR152	400 bp	Single-plant yield (g)	63	51	+23.5%	**
BNL3347	150 bp	Single-plant yield (g)	61	50	+22.0%	**

Carriers = genotypes with the indicated allele; non-carriers = genotypes without it. ** $P < 0.01$; * $P < 0.05$ (one-way ANOVA, no correction for multiple testing).

DISCUSSION

No single trait ranked the 13 genotypes the way yield did. Genotypes that kept their leaves hydrated were not always the ones that kept their pigments, and the genotype with the smallest yield loss cut its transpiration the most. This is what one would expect if drought tolerance is built from several partly independent components (Saranga et al., 2004; Blum, 2011; Ashraf and Harris, 2013), and it argues for screening on more than one trait.

Leaf water status and transpiration

XiLuZhong-52, T-217, T-4, and T-6 lost only 1.6–2.6% of their leaf water under drought. Osmotic adjustment or tighter control of water loss would explain this (Nayyar and Walia, 2003; Farooq et al., 2009); we did not measure osmolytes, so the mechanism remains to be confirmed. Comparable variation in leaf water status has been used to screen upland cotton for drought tolerance (Pettigrew, 2004; Basal et al., 2005).

The transpiration data point to two strategies. XiLuZhong-78, Marvarid, Chuntay-2, and T-17 roughly halved their transpiration, saving soil water at the expense of gas exchange (Chaves et al., 2009). T-6, XiLuZhong-52, T-217, and Namangan-77 kept transpiring at close to their OF rates. Neither strategy guaranteed yield: XiLuZhong-78 closed its stomata hardest and lost the least yield, whereas T-17 did the same and lost the most. Water saving, it seems, pays off only in combination with other traits.

Photosynthetic pigments

T-2, T-4, and XiLuZhong-78 kept their chlorophyll and carotenoid contents under drought, which suggests that their photosynthetic apparatus was well protected, probably by antioxidant defence (Reddy et al., 2004; Jaleel et al., 2009). In T-218 and Namangan-77, the sharp fall in chlorophyll *b* fits the known tendency of light-harvesting complexes to be dismantled first under stress (Lichtenthaler, 1987). Carotenoid retention in the tolerant genotypes is consistent with the role of these pigments in dissipating excess light energy (Demmig-Adams and Adams, 1996; Niyogi, 1999). Pigment loss and uneven carotenoid responses are not specific to drought: in soybean leaves infected with *Fusarium solani*, chlorophyll *a* and *b* declined at budding and flowering, and carotenoids rose in some cultivars and fell in others (Yuldashov et al., 2023). This points to genotype-specific pigment behaviour under both biotic and abiotic stress.

Chuntay-2 does not fit this pattern. It lost chlorophyll under drought, yet its boll weight hardly changed and it kept 83% of its yield. Its tolerance must rest on something other than pigment stability, perhaps osmotic adjustment or remobilization of stored assimilates to the bolls. A screen based on pigments alone would have discarded one of the best genotypes in the trial.

Yield and fiber traits

Yield losses varied 2.7-fold. XiLuZhong-78, Chuntay-2, and T-6 combined high yield under full irrigation with losses below 18% under drought, the combination breeders look for in water-limited environments (Saranga et al., 2004; Blum, 2011). T-17 lost yield mainly through fewer bolls, consistent with the high sensitivity of boll retention to water deficit during flowering (Loka et al., 2011). For genotypes of this type, selection for boll retention rather than boll weight would be more useful.

Namangan-77, the local standard, lost a third of its yield. If irrigation supplies in Uzbekistan continue to shrink, this cultivar is likely to perform poorly, and the more tolerant genotypes identified here are worth testing as replacements or as parents.

Fiber length fell by no more than 2.4 mm, so moderate drought did little harm to this quality trait. The small gains in fiber output in some genotypes more likely reflect lighter seeds than extra lint, given that seed weight declined in every genotype (Pettigrew, 2004).

SSR diversity and candidate markers

With all loci polymorphic and a mean H_e of 0.56, the panel holds enough variation for breeding use, similar to that found in SSR surveys of other upland cotton collections (Tyagi et al., 2014). The physiological contrasts among genotypes also suggest useful crosses, for instance combining the leaf water retention of XiLuZhong-52 with the yield stability of XiLuZhong-78 or Chuntay-2.

The four candidate markers relate to chlorophyll retention (BNL3436, JESPR152) and to yield under drought (NAU3588, JESPR152, BNL3347). That JESPR152 was associated with both traits makes biological sense, since pigment retention sustains the assimilate supply to developing bolls (Chaves et al., 2009). Still, with 13 genotypes and no multiple-testing correction, false positives are likely. These markers should be tested in larger biparental or association mapping populations before they are used in selection (Saranga et al., 2004; Zheng et al., 2016).

CONCLUSION

The 13 genotypes responded to simulated drought in quite different ways. XiLuZhong-52, T-217, T-4, and T-6 best maintained leaf water content; T-2, T-4, and XiLuZhong-78 best retained photosynthetic pigments; and XiLuZhong-78, Chuntay-2, and T-6 combined high yield potential with the smallest yield losses, which makes them the most promising materials for breeding under limited water. The standard cultivar Namangan-77 proved comparatively sensitive. The SSR panel revealed ample diversity, and alleles at BNL3436, NAU3588, JESPR152, and BNL3347 are candidates for marker-assisted selection once they are validated. Next steps are to test these associations in segregating populations and to evaluate the best genotypes over several seasons and locations in Uzbekistan's cotton-growing regions.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Abdullaev I and Rakhmatullaev S (2015). Transformation of water management in Central Asia: from State-centric, hydraulic mission to socio-political control. *Environ. Earth Sci.* 73: 849-861.
2. Ashraf M and Harris PJC (2013). Photosynthesis under stressful environments: an overview. *Photosynthetica* 51: 163-190.
3. Barrs HD and Weatherley PE (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci.* 15: 413-428.
4. Basal H, Smith CW, Thaxton PS and Hemphill JK (2005). Seedling drought tolerance in upland cotton. *Crop Sci.* 45: 766-771.
5. Blum A (2011). Plant breeding for water-limited environments. Springer, New York.
6. Chaves MM, Flexas J and Pinheiro C (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.* 103: 551-560.
7. Demmig-Adams B and Adams WW (1996). The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends Plant Sci.* 1: 21-26.
8. Farooq M, Wahid A, Kobayashi N, Fujita D, et al. (2009). Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev.* 29: 185-212.
9. Fischer RA and Maurer R (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Aust. J. Agric. Res.* 29: 897-912.
10. Jaleel CA, Manivannan P, Wahid A, Farooq M, et al. (2009). Drought stress in plants: a review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.* 11: 100-105.
11. Lacape JM, Nguyen TB, Thibivilliers S, Bojinov B, et al. (2003). A combined RFLP-SSR-AFLP map of tetraploid cotton based on a *Gossypium hirsutum* × *Gossypium barbadense* backcross population. *Genome* 46: 612-626.
12. Lesk C, Rowhani P and Ramankutty N (2016). Influence of extreme weather disasters on global crop production. *Nature* 529: 84-87.
13. Lichtenthaler HK (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol.* 148: 350-382.
14. Lichtenthaler HK and Wellburn AR (1983). Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochem. Soc. Trans.* 11: 591-592.
15. Loka DA, Oosterhuis DM and Ritchie GL (2011). Water-deficit stress in cotton. In: Stress physiology in cotton (Oosterhuis DM, ed.). The Cotton Foundation, Cordova, 37-72.
16. Micklin P (2007). The Aral Sea disaster. *Annu. Rev. Earth Planet. Sci.* 35: 47-72.
17. Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 7: 405-410.
18. Nayyar H and Walia DP (2003). Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and abscisic acid. *Biol. Plant.* 46: 275-279.
19. Niyogi KK (1999). Photoprotection revisited: genetic and molecular approaches. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50: 333-359.
20. Paterson AH, Wendel JF, Gundlach H, Guo H, et al. (2012). Repeated polyploidization of *Gossypium* genomes and the evolution of spinnable cotton fibres. *Nature* 492: 423-427.
21. Peakall R and Smouse PE (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics* 28: 2537-2539.
22. Pettigrew WT (2004). Physiological consequences of moisture deficit stress in cotton. *Crop Sci.* 44: 1265-1272.
23. Powell W, Morgante M, Andre C, Hanafey M, et al. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed.* 2: 225-238.
24. Reddy AR, Chaitanya KV and Vivekanandan M (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.* 161: 1189-1202.
25. Rong J, Abbey C, Bowers JE, Brubaker CL, et al. (2004). A 3347-locus genetic recombination map of sequence-tagged sites reveals features of genome organization, transmission and evolution of cotton (*Gossypium*). *Genetics* 166: 389-417.
26. Saranga Y, Jiang CX, Wright RJ, Yakir D, et al. (2004). Genetic dissection of cotton physiological responses to arid conditions and their inter-relationships with productivity. *Plant Cell Environ.* 27: 263-277.
27. Temnykh S, DeClerck G, Lukashova A, Lipovich L, et al. (2001). Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. *Genome Res.* 11: 1441-1452.
28. Trenberth KE, Dai A, van der Schrier G, Jones PD, et al. (2014). Global warming and changes in drought. *Nat. Clim. Change* 4: 17-22.
29. Tretyakov NN (1990). Practicum on plant physiology. Agropromizdat, Moscow (in Russian).
30. Turner NC (1981). Techniques and experimental approaches for the measurement of plant water status. *Plant Soil* 58: 339-366.
31. Tyagi P, Gore MA, Bowman DT, Campbell BT, et al. (2014). Genetic diversity and population structure in the US Upland cotton (*Gossypium hirsutum* L.). *Theor. Appl. Genet.* 127: 283-295.

32. UzPITI (2014). Methodological guidelines for field experiments with cotton. Uzbek Cotton Research Institute, Tashkent (in Uzbek).
33. Yeh FC, Yang RC and Boyle T (1999). POPGENE version 1.32: Microsoft Windows-based freeware for population genetic analysis. University of Alberta, Edmonton.
34. Yuldashov U, Matniyazova H, Tillaboyeva D, Nabiyev S, et al. (2023). Physiological and biochemical properties of soybean cultivars infected with phytopathogenic fungi. *SABRAO J. Breed. Genet.* 55: 2025-2036.
35. Zheng JY, Oluoch G, Riaz Khan MK, Wang XX, et al. (2016). Mapping QTLs for drought tolerance in an F2:3 population from an inter-specific cross between *Gossypium tomentosum* and *Gossypium hirsutum*. *Genet. Mol. Res.* 15: gmr.15038477.