

BREEDING AND GENETICS

Growth Responses of an Interspecific Cotton Breeding Line and Its Parents to Controlled Drought Using an Automated Irrigation System

Youping Sun, Genhua Niu*, Jinfa Zhang, and Priscilla Del Valle

ABSTRACT

Cotton is an economically important crop with multiple uses as fiber, biofuel, food, and feed, and drought is one of the most limiting factors in cotton production. In this study, *Gossypium hirsutum* ‘Acala 1517-99’, *G. barbadense* ‘PHY 76 Pima’ and their cross-breeding line ‘Q1735-4’ were grown in a greenhouse to characterize their growth and physiological responses to four substrate volumetric water contents (VWC) of 15, 25, 35, and 45%. An automated irrigation system consisting of soil moisture sensors, datalogger, and a relay controller was used. The results from two tests consistently showed that as VWC decreased, leaf area, stem diameter, and total dry weight (DW) decreased linearly for PHY 76 Pima and Q1735-4, but quadratically for Acala 1517-99. However, the reduction in the growth parameters varied among genotypes. As VWC was decreased from 45% to 25%, Acala 1517-99, PHY 76 Pima, and Q1735-4 exhibited reduction in height by 39.2, 32.5, and 23.7%; in leaf area by 70.9, 65.8, and 34.7%; in stem diameter by 33.4, 28.1, and 22.1%; and in total DW by 59.2, 55.6, and 15.1%, respectively. The interspecific cross-breeding line consistently displayed better drought tolerance than its parents. When VWC was further decreased to 15%, the interspecific cross-breeding line still had the lowest reduction in reproductive growth as measured by the total dry fruit weight; but its vegetative growth parameters such as plant height, leaf area, stem diameter, and total DW were similar to Acala 1517-99 and lower than PHY 76 Pima.

Cotton is one of the most economically important crops for the textile and oil industry. Cotton is

grown on more than 11.0 million acres across 17 states from Virginia to California with 16.1 million bales of cotton harvested in 2014 (National Cotton Council of America, 2015). Along with cotton fiber and seed production, cotton wastes (e.g., residues from fields and gins) have been converted into pellets, ethanol, methane, and pyrolytic products for bioenergy usage in recent years (Sharm-Shivappa and Chen, 2008). *Gossypium hirsutum* L. (Upland cotton) and *G. barbadense* L. (Pima cotton) are two extensively cultivated species in the U.S. National Cotton Council of America (2015) reported that Upland cotton planted acres accounted for approximately 98.3% of total cotton planted acres in the U.S. Upland cotton, originating from Mexico, is known for its wide adaptation, fuzzy seed, high lint percentage, and high yield, whereas Pima cotton is known for its superior fiber quality, naked seed, lower lint percentage, and lower yield potential (Zhang and Percy, 2007; Zhang et al., 2014a). *G. barbadense* originated from the coastal region of Peru (Smith and Cothren, 1999) and is bred and grown in arid and semiarid regions including southwestern U.S.

Drought is one of the major environmental stresses affecting cotton production and fiber quality globally. Severe drought slows cotton plant development and causes squares and young small bolls to shed (McWilliams, 2003), and decreases yield (Iqbal et al., 2013). Burke et al. (1985) reported that Upland cotton plants grown under dryland conditions had a reduction of 34 to 83% in plant height, leaf number, leaf size, and total weight per plant. A microscopic examination showed that the reduced leaf area of Upland cultivar Paymaster 266 under water-stress condition resulted primarily from a mitotic sensitivity to water stress (Berlin et al., 1982). Although expansion of palisade cells was not inhibited by water stress, palisade cells from stressed Paymaster 266 had thinner cell walls and larger central vacuoles compared to cells from nonstressed plants. They also observed that the stressed Paymaster 266 had larger mitochondria and smaller, more numerous chloroplasts.

As fresh water resources become limited, the demand for drought-tolerant plants has become

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increasingly important. Because drought tolerance in cotton is a complex trait and influenced by genotype, environment, and their interaction, the breeding effort for drought-tolerant cotton is highly limited, as compared to breeding for lint yield, quality, and other biotic stress tolerance (Zhang et al., 2014b). Drought tolerance is dependent on plant growth stage. Soil water deficits during critical growth stages, such as reproductive stage, can remarkably affect growth and yield (Kaur and Singh, 1992; Kock et al., 1990). Insufficient irrigation during cotton establishment and prebloom affects total yield (McWilliams, 2003). Cotton water demand increases significantly during the flowering phase (Greigh de Brito et al., 2011), and water deprivation following bloom and into boll development also impacts lint quality (McWilliams, 2003). Insufficient early growth under soil water deficits at pre-flowering reduces the assimilates supply to higher boll demand in high retention cotton (Paytas, 2009). Abiotic stress tolerance including drought tolerance also varies with cotton genotypes (Baloch et al., 2011; Iqbal et al., 2010; Longenberger et al., 2006; Niu et al., 2013). For example, in a greenhouse study where cotton seedlings were subjected to three sequential cycles of drought that consisted of withholding water until an average volumetric water content of 0.07, the Upland variety Deltapine 491 was the most drought tolerant genotype, and none of the converted race stocks (day-sensitive primitive lines, McCarty et al., 1979) were more tolerant than Upland 'Acala 1517-99' (Longenberger et al., 2006). Baloch et al. (2011) reported that Upland cultivars CRIS-477, CRIS-483, and CRIS-486 were highly sensitive to water stress.

There have been few studies on relationships between plant growth and various drought levels. Through interspecific cross breeding between Upland and Pima cotton, Zhang et al. (2014a) have recently developed extremely drought-tolerant interspecific cross-breeding lines including stay-green cotton. Several interspecific cross-breeding lines including 'Q1735-4' showed drought tolerance under reduced irrigation conditions in field trials (Zhang and Hughs, 2012; Zhang et al., 2014a). Some introgressed backcrossed lines also displayed better water osmotic tolerance under polyethylene glycol conditions (Abdelraheem et al., 2014). However, their growth and physiological responses to varying substrate volumetric water contents have not been characterized.

The objective of this study was to determine drought tolerance by characterizing the growth and physiological responses of the breeding line Q1735-4

and its parents (Acala 1517-99 and PHY 76 Pima) to varying substrate volumetric water contents in two tests with different drought periods in a greenhouse. Linear or quadratic relationships between different plant growth characteristics and water moisture level were investigated.

MATERIALS AND METHODS

Plant material and growing conditions. *G. hirsutum* Acala 1517-99 was developed to produce superior fiber quality, high productivity, and strong resistance to bacterial blight and was released by the New Mexico Agricultural Experiment Station in 1999 (Cantrell et al., 2000). *G. barbadense* PHY 76 Pima was released by PhytoGen Seed Company, LLC in 2001 (Bowman et al., 2006). Acala 1517-99 was used as a female in a cross with PHY 76 Pima to improve fiber quality and yield potential. From the subsequent population after many generations of self pollination, a potential drought-tolerant breeding line Q1735-4 of Upland cotton type with high yield and fiber quality was selected by New Mexico State University (Zhang and Hughs, 2012). On 21 August 2013, Acala 1517-99, PHY 76 Pima, and Q1735-4 seeds were sown at a depth of 2.5 cm into 5.8-L black poly-tainer containers (22.5 x 19.5 cm) that were filled with Metro-Mix 360 (Canadian sphagnum peat moss 45-55%, vermiculite, composed bark, dolomite lime; SunGro®, Agawam, MA). Two seeds were sown per container, and thinned to only one seedling after emergence. Seedlings were kept well watered with a water-soluble 15N-2.2P-12.5K fertilizer solution (Peters 15-5-15 Peat-lite special; The Scotts Co., Marysville, OH) at a nitrogen concentration of 105 mg·L⁻¹ using a fertilizer injector (Dosatron International, Inc., Clearwater, FL) to allow seedlings to establish until 15 d after sowing. At each irrigation throughout this study, the same fertilizer solution was applied.

The temperature in the greenhouse was measured using T thermocouples (Omega Engineering, Inc., Stamford, CT) connected to a 21X datalogger (Campbell Scientific Inc., Logan, UT). Day and night temperature was 29.0 ± 4.5°C (mean ± standard deviation) and 23.6 ± 2.3°C, respectively. Photosynthetic photon flux (PPF) was measured with a quantum sensor (Apogee Instruments, Logan, UT). Daily light integral inside the greenhouse was 14.1 ± 3.6 mol·m⁻²·d⁻¹. During the experiment, 1.32 mL·L⁻¹ AVID® 25% EC (2% Abamectin, Syngenta Crop Protection Inc., Greensboro, NC) was sprayed three times to control thrips and/or aphids.

Treatments. When cotton seedlings had two true leaves on 3 September, plants were divided into two tests (see Data collection, below). Containers were irrigated using an automated irrigation system similar to that described by Nemali and van Iersel (2006). One 10HS capacitance sensor (Decagon, Pullman, WA) was inserted perpendicularly into the substrate in a randomly selected container in each treatment. The sensors were connected to a CR10 datalogger (Campbell Scientific, Logan, UT) through a AM416 multiplexer (Campbell Scientific), and the substrate moisture was measured every 5 min. A substrate-specific calibration equation converted voltage readings from the soil moisture sensors to substrate volumetric water content (VWC, $L \cdot L^{-1}$) ($VWC = -20.99 + 25.349 \times \text{voltage} + 46.55 \times \text{voltage}^2$, $R^2 = 0.984^{***}$). The datalogger compared the VWC in each treatment with the VWC set point for that particular treatment. As soon as the VWC in a container dropped below the set point for irrigation, the datalogger sent a signal to the 16-channel SDM-CD16AC relay controller (Campbell Scientific), which opened the solenoid valve (X-13551-72; Dayton Electric Co., Niles, IL) corresponding to that treatment for 40 sec. Each container was watered with one dribble ring (Dramm, Manitowoc, WI) at a diameter of 15 cm with five emitter holes per ring. The dribble ring was connected to a pressure-compensated drip emitter (8 LPH; Netafim USA, Fresno, CA) with an average flow rate of $133.3 \text{ mL} \cdot \text{min}^{-1}$. Irrigation thresholds were VWC values of $0.15 \text{ L} \cdot \text{L}^{-1}$ (15%), $0.25 \text{ L} \cdot \text{L}^{-1}$ (25%), $0.35 \text{ L} \cdot \text{L}^{-1}$ (35%), and $0.45 \text{ L} \cdot \text{L}^{-1}$ (45%).

Data collection. Cotton is a relatively long growth cycle crop, and drought tolerance could be screened up to 50 d from sowing (Greigh de Brito et al., 2011). In Test 1, 37 d after treatment (DAT) (10 October 2013), cotton plants began to flower and the plants were harvested due to space constraints (~6 plants per square meter). Plant height (cm), from the pot rim to the top of shoot, was recorded at the initiation of treatments on 3 September and again at harvest on 10 October. Stem diameter (mm) was measured at the first node of each plant. The number of nodes and squares were counted. Leaf area (cm^2) was determined using a LI-3100C area meter (LI-COR® Biosciences, Lincoln, NE). Shoots were cut off at the substrate surface, and roots were removed by carefully breaking apart the soil and cleaned with running tap water. The shoots and roots were oven dried for 10 d at 65°C , after which dry weight (DW, g) was determined. In Test 2, 68 DAT (8 November), the plants were harvested. Data were collected on

the same parameters as described above. In addition, fruit dry weight was also collected.

Experimental design and data analysis. The experimental design was a randomized complete block design with four treatments (VWC thresholds), two blocks, and four replications in each block. As stated earlier, the greenhouse study was composed of two tests under the same treatment conditions in that plants were destructively harvested and measured at 37 and 68 DAT, respectively. All data were analyzed by a two-way analysis of variance (ANOVA). When the effects of volumetric water content were significant, linear and quadratic regression analysis was performed. All statistical analyses were performed using SAS software (Version 9.1.3, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

The daily average substrate VWC was above the set point over the course of the experiment. Clear differences in the average VWC among the four treatments were maintained throughout the experiment, although daily fluctuations in VWC occurred, especially at three lower set points (Fig. 1). That VWC fluctuated more at lower set points was observed previously (Burnett and van Iersel, 2008; Garland et al., 2012; Nemali and van Iersel, 2006). This could be the result of lower substrate hydraulic conductivity when substrates have a lower VWC (Naasz et al., 2005). The averaged VWC, representing measurements from 11 to 56 DAT, was 17.6, 27.9, 39.5, and 45.7% in treatments with irrigation set points of 15, 25, 35, and 45%, respectively.

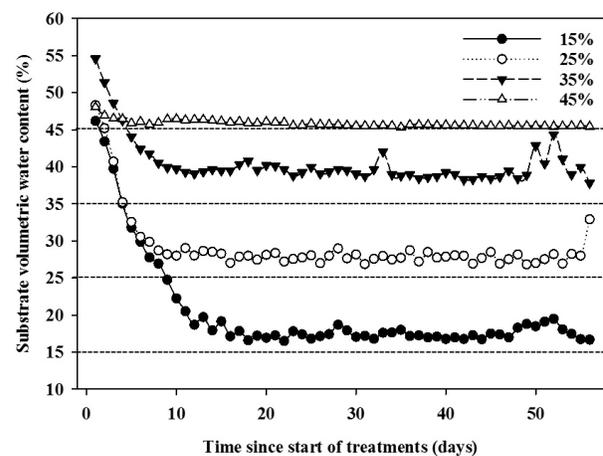


Figure 1. Average substrate volumetric water content (VWC) as maintained by a soil moisture sensor-controlled automatic irrigation system. Dashed lines indicate the VWC threshold at which the containers in the various treatments were irrigated.

Plant height, stem diameter, and number of nodes of the three cotton genotypes increased linearly and/or quadratically with increasing VWC set points in both Test 1 (37 DAT) and Test 2 (68 DAT) (Table 1, Fig. 2A-C, Fig. 3A-C). In Test 1, when VWC set points decreased from 45 to 25%, the slope of the regression curve of Q1735-4 was less steep than that of Acala 1517-99 and PHY 76 Pima for plant height, stem diameter, and number of nodes. As VWC decreased from 45 to 25%, Acala 1517-99, PHY 76 Pima, and Q1735-4 plants showed reductions in plant height of 21.5, 19.3, and 18.3%, respectively; reductions in stem diameter of 10.7, 17.5, and 12.2%, respectively; and a decrease in the number of nodes of 9.5, 10.6, and 10.6, respectively (Fig. 2A-C). In Test 2 (68 DAT), similar trends were observed. As VWC decreased from 45 to 25%, Acala 1517-99, PHY 76 Pima, and Q1735-4 plants had reduction in height by 39.2, 32.5, and 23.7%, respectively; stem diameter decreased by 33.4, 28.1, and 22.1%, respectively; and number of nodes decreased by 26.3, 23.5, and 10.4%, respectively (Fig. 3A-C). The interspecific cross-breeding line consistently performed better than both its parents. Surprisingly, under severe water stress when the VWC set point was further decreased to 15%, Q1735-4 had similar height, stem diameter, and number of nodes to its Upland parent Acala 1517-99, but lower than its Pima parent PHY 76 Pima, indicating the Pima cotton became more drought tolerant than Upland cotton and Q1735-4 at the lowest VWC (15%).

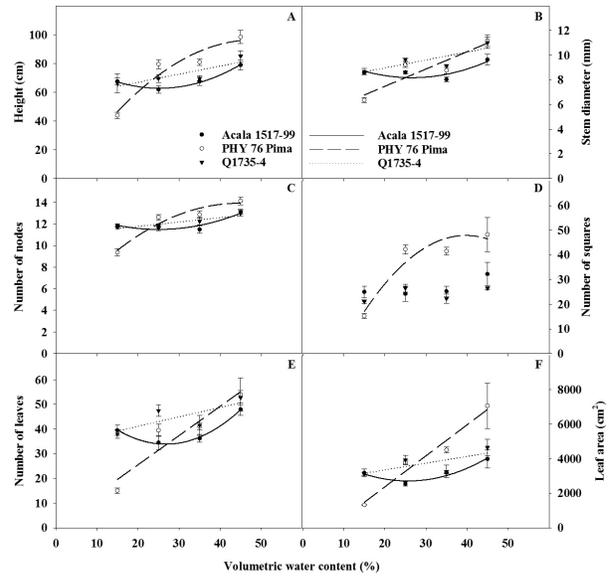


Figure 2. Plant height, stem diameter, number of nodes, number of squares, number of leaves, and leaf area of three cotton genotypes (Acala 1517-99, PHY 76 Pima, and Q1735-4) irrigated with a soil moisture sensor-controlled automatic irrigation system as a function of the substrate volumetric water content threshold from the start of the irrigation treatments to 37 d after treatment.

Boll number per plant and seed cotton yield have been recommended as the selection criteria in breeding programs for drought tolerance (Yagmur et al., 2014). In the present study, cotton plants were harvested before bolls were developed due to the nature of the greenhouse conditions. The number of squares, the first stage in cotton boll formation, was counted and

Table 1. A summary of analysis of variance for effects of substrate volumetric water content (VWC), genotype (Gt), and their interactions on height, stem diameter, number (No.) of nodes, squares, and leaves, leaf area, shoot DW, root DW, fruit DW, and total dry weight (DW) at the harvest of cotton genotypes (Acala 1517-99, PHY 76 Pima, and Q1735-4).

Source	Height	Stem diameter	No. of nodes	No. of squares	No. of leaves	Leaf area	Shoot DW	Root DW	Fruit DW	Total DW
Test 1, 37 d after treatment										
Gt	ns	***	ns	***	**	*	ns	**	-	ns
VWC	***	***	***	***	***	***	***	*	-	***
Gt * VWC	***	***	***	***	**	***	**	ns	-	**
Acala 1517-99	L ^{**} , Q ^{**}	L ^{ns} , Q ^{**}	L ^{**} , Q ^{**}	L ^{ns} , Q ^{ns}	L ^{ns} , Q ^{**}	L ^{ns} , Q [*]	L ^{ns} , Q ^{ns}	L ^{ns} , Q ^{ns}	-	L ^{ns} , Q ^{ns}
PHY 76 Pima	L ^{***} , Q [*]	L ^{***} , Q ^{ns}	L ^{***} , Q ^{**}	L ^{***} , Q [*]	L ^{***} , Q ^{ns}	L ^{***} , Q ^{ns}	L ^{***} , Q ^{**}	L ^{**} , Q ^{**}	-	L ^{***} , Q ^{***}
Q1735-4	L ^{**} , Q ^{ns}	L ^{***} , Q ^{ns}	L ^{**} , Q ^{ns}	L ^{ns} , Q ^{ns}	L [*] , Q ^{ns}	L [*] , Q ^{ns}	L ^{ns} , Q ^{ns}	L ^{ns} , Q ^{ns}	-	L ^{ns} , Q ^{ns}
Test 2, 68 d after treatment										
Gt	ns	*	*	ns	**	ns	**	***	**	**
VWC	***	***	***	***	***	***	***	***	***	***
Gt * VWC	***	**	***	*	ns	***	***	***	**	***
Acala 1517-99	L ^{**} , Q ^{***}	L ^{**} , Q ^{**}	L ^{***} , Q ^{***}	L ^{**} , Q ^{***}	L [*] , Q ^{**}	L ^{***} , Q ^{***}	L ^{**} , Q ^{***}	L ^{**} , Q ^{***}	L ^{ns} , Q ^{**}	L ^{**} , Q ^{***}
PHY 76 Pima	L ^{***} , Q ^{ns}	L ^{***} , Q ^{ns}	L ^{***} , Q [*]	L ^{***} , Q ^{ns}	L ^{***} , Q ^{ns}	L ^{***} , Q ^{ns}	L ^{***} , Q [*]	L ^{***} , Q ^{ns}	L ^{**} , Q ^{ns}	L ^{***} , Q ^{ns}
Q1735-4	L [*] , Q [*]	L ^{**} , Q ^{ns}	L [*] , Q ^{ns}	L ^{ns} , Q ^{ns}	L ^{**} , Q ^{ns}	L ^{**} , Q ^{ns}	L ^{**} , Q ^{ns}	L ^{ns} , Q ^{ns}	L ^{ns} , Q ^{ns}	L [*] , Q ^{ns}

ns, *, **, ***: nonsignificant or significant at $p < 0.05$, 0.01, or 0.001, respectively.

used as an indicator of cotton yield potential. Drought stress reduced the number of squares of PHY 76 Pima quadratically at Test 1 (37 DAT) (Table 1, Fig. 2D), but there were no significant linear or quadratic trends for Q1735-4 and Acala 1517-99. A reduction of 22.5, 68.4, and 20.8% in number of squares was recorded for Acala 1517-99, PHY 76 Pima, and Q1735-4, respectively, as VWC decreased from 45 to 15%, indicating better reproductive growth in the interspecific cross-breeding line and its Upland parent under the drought conditions. In Test 2 under longer drought conditions (68 DAT), a greater reduction in reproductive growth was detected in the three genotypes, as expected. The number of squares of Acala 1517-99 and PHY 76 Pima decreased quadratically and/or linearly, respectively, with decreasing VWC (Table 1, Fig. 3D). The number of squares of Acala 1517-99 and PHY 76 Pima was reduced by 72.1 and 86.3%, respectively, when VWC set points decreased from 45 to 15%. Although no significant linear or quadratic trend occurred, the actual number of squares in Q1735-4 decreased by 62.1% when VWC decreased from 45 to 15%. Once again, the interspecific cross-breeding line showed less reduction than its parents in reproductive growth, indicating better drought tolerance than both its parents. The yield of these three cotton genotypes would be expected to decrease because water stress reduced yield via decreasing boll number (Alishah and Ahmadikah, 2009).

Photosynthetic surface area commonly is decreased when plants are under drought conditions (Burnett and van Iersel, 2008). Number of leaves and leaf area of the three cotton genotypes were reduced as VWC decreased (Fig. 2E-F, Fig. 3E-F). In both tests, linear regression relations of the number of leaves and leaf area with VWC were observed for both PHY 76 Pima and Q1735-4, whereas quadratic regression relations were observed for Acala 1517-99 (Table 1, Fig. 2E-F, Fig. 3E-F). The number of leaves and the leaf area of Q1735-4 decreased more slowly than that of both its parents, Acala 1517-99 and PHY 76 Pima, as the VWC set point decreased from 45 to 25%. In Test 1 (37 DAT), as VWC decreased from 45 to 25%, the number of leaves of Acala 1517-99, Pima Phy 76, and Q1735-4 was reduced by 28.1, 26.9, and 10.0%, respectively; and leaf area decreased by 36.3, 46.5, and 15.3%, respectively. In Test 2 (68 DAT), a similar trend was noted, although reduction in growth was more profound. As VWC decreased from 45 to 25%, the number of leaves of Acala 1517-99, PHY 76 Pima, and Q1735-4 was reduced by 63.0, 57.6, and 27.7%, respectively; whereas leaf area was reduced by 70.9, 65.8 and 34.7%, respectively. As VWC further decreased to 15%, Q1735-4 had a similar number of leaves and leaf area to its Upland parent Acala 1517-99, but lower than the Pima parent PHY 76 Pima. This result is similar to those for plant height, stem diameter, and number of nodes.

As photosynthetic surface area is reduced, plants are expected to accumulate less biomass. Shoot, root, and total dry weight of PHY 76 Pima decreased quadratically with decreasing VWC set points in Test 1 (37 DAT) (Table 1, Fig. 4A-C). The actual shoot, root, and total DW of Acala 1517-99 and Q1735-4 tended to decrease, but no significant linear or quadratic trend was detected. In Test 1 (37 DAT), as VWC decreased from 45 to 25%, Acala 1517-99, PHY 76 Pima, and Q1735-4 decreased their shoot DW by 20.8, 39.9, and 3.0%, respectively; root DW by 1.9, 15.8, and 0%, respectively; and total DW by 19.7, 38.4, and 2.3%, respectively. When VWC set points reached 15%, Q1735-4 had similar shoot, root, and total dry weights to its Upland parent Acala 1517-99, but much lower than its Pima parent PHY 76 Pima. Once again, the result is similar to these for leaf number, leaf area, plant height, stem diameter, and number of nodes.

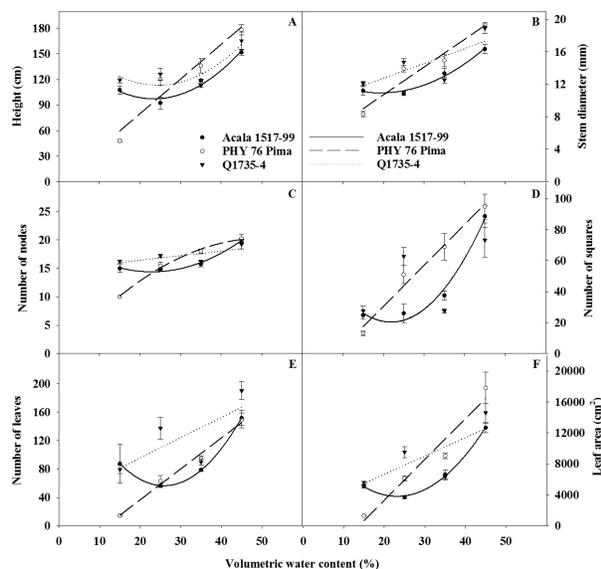


Figure 3. Plant height, stem diameter, number of nodes, number of squares, number of leaves, and leaf area of three cotton genotypes (Acala 1517-99, PHY 76 Pima, and Q1735-4) irrigated with a soil moisture sensor-controlled automatic irrigation system as a function of the substrate volumetric water content threshold from the start of the irrigation treatments to 68 d after treatment.

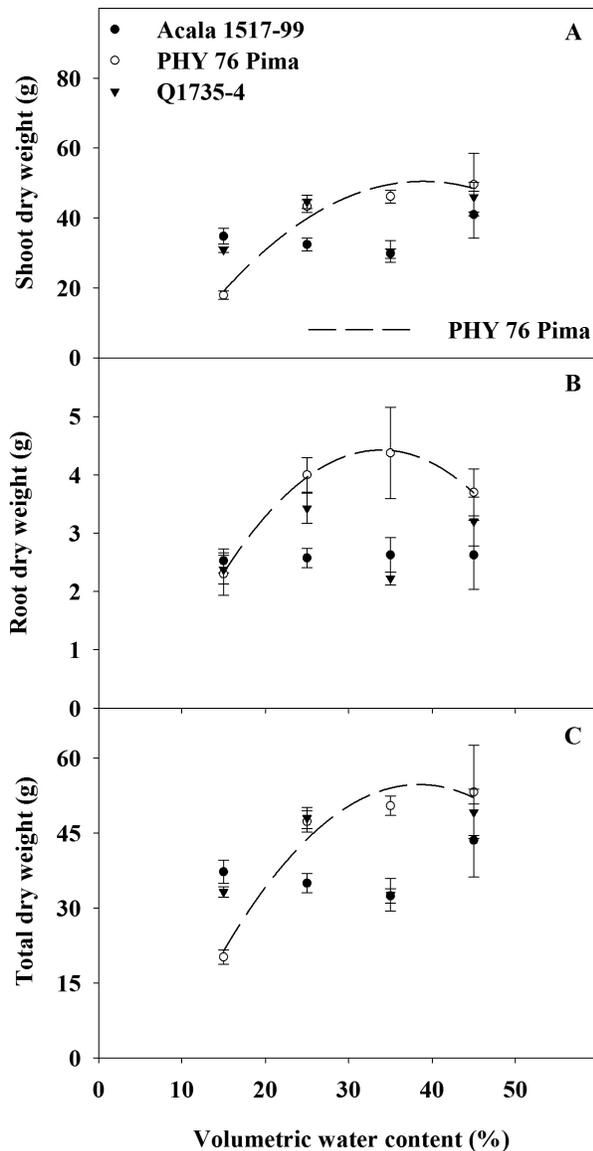


Figure 4. Plant shoot, root, and total dry weight of three cotton genotypes (Acala 1517-99, PHY 76 Pima, and Q1735-4) irrigated with a soil moisture sensor-controlled automatic irrigation system as a function of the substrate volumetric water content threshold from the start of the irrigation treatments to 37 d after treatment.

In Test 2 (68 DAT), as the VWC decreased, shoot, root, and total dry weight of Acala 1517-99 and PHY 76 Pima decreased linearly and/or quadratically (Table 1, Fig. 5A-B, D). Shoot and total dry weight of Q1735-4 decreased linearly, but no significant regression relation of root dry weight was detected for this genotype. When VWC set points decreased from 45 to 25%, Acala 1517-99, PHY 76 Pima, and Q1735-4 decreased their shoot DW by 66.6, 64.9, and 24.3%, respectively; root DW by 46.6, 54.1, and 0%, respectively; and total DW by 59.2, 55.6, and 15.1%, respectively. As VWC reached 15%, Q1735-4

had similar shoot, root, and total dry weights to its Upland parent Acala 1517-99, but lower than the Pima parent PHY 76 Pima. The result is similar to these measurements on leaf number, leaf area, plant height, stem diameter, and number of nodes. Additionally, in Test 2, fruit dry weight of Acala 1517-99 and PHY 76 Pima decreased quadratically and linearly, respectively, with decreasing VWC (Table 1, Fig. 5C), but no significant trend occurred for Q1735-4. A reduction in fruit dry weight of Acala 1517-99, PHY 76 Pima, and Q1735-4 was 27.1, 80.0, 10.9%, respectively, when VWC set points decreased from 45 to 15%. Therefore, the interspecific cross-breeding line showed better drought tolerance in reproductive growth.

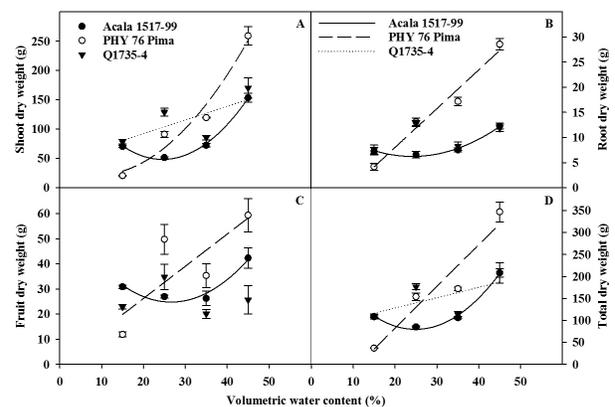


Figure 5. Plant shoot, root, fruit, and total dry weight of three cotton genotypes (Acala 1517-99, PHY 76 Pima, and Q1735-4) irrigated with a soil moisture sensor-controlled automatic irrigation system as a function of the substrate volumetric water content threshold from the start of the irrigation treatments to 68 d after treatment.

In conclusion, the controlled drought provided by the automated irrigation system negatively impacted the growth and development of the three cotton genotypes used in the present study. Reductions in all the growth parameters measured in this study varied with genotypes, and a linear or quadratic relationship between a growth parameter and soil water content might exist depending on genotype, trait, and growth stage. The interspecific cross-breeding line Q1735-4 was consistently more tolerant to drought than its parents Acala 1517-99 and PHY 76 Pima based on smaller reductions in growth parameters when substrate moisture content decreased from 45 to 25% in both tests (i.e., 37 and 68 DAT). However, when the soil water content was further reduced to 15%, the interspecific cross-breeding line still had the lowest reduction in reproductive growth as measured by fruit weight, but the Pima cotton par-

ent was the most drought tolerant when vegetative growth parameters in leaves, roots, and shoots was measured. The physiological and structural changes and the genetic and molecular basis of these different genotypic responses to varying drought regimes are currently unknown. Although gene expression in different genotypes might be different under different drought conditions (Rodriguez-Urbe et al., 2014), the present study provides useful information and a practical technique for further studies of drought tolerance in cotton.

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