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Short Communication

Evaluation of root traits and water use efficiency of different cotton genotypes in the presence or absence of a soil-hardpan

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ABSTRACT

Cotton (*Gossypium* spp.) is an important fiber and oil crop grown worldwide. Water and nutrient stresses are major issues affecting cotton production globally. Root traits are critical in improving water and nutrient uptake and maintaining plant productivity under optimal as well as drought conditions. However, root traits have rarely been utilized in cotton breeding programs, a major reason being the lack of information regarding genetic variability for root traits. The objective of this research was to evaluate ten selected cotton genotypes for root traits and water use efficiency. The tested genotypes included germplasm lines (PD 1 and PD 695) and cultivars that are currently grown in the southeastern USA (PHY 499WRF, PHY 444WRF, PHY 430W3FE, DP 1646B2XF, DP 1538B2XF, DP 1851B3XF, NG 5007B2XF, and ST 5020GLT). Experiments were conducted under controlled environmental conditions in 2018 and 2019. A hardpan treatment was included in the second year to evaluate the effect of a soil hardpan on root traits and water use efficiency. Genotype PHY 499WRF ranked at the top and NG 5007B2XF ranked at the bottom for root morphological traits (total and fine root length, surface area, and volume) and root weight. PHY 499WRF was also one of the best biomass producers and had high water use efficiency. PHY 444WRF, PHY 430W3FE, and PD-1 were the other best genotypes in terms of root traits and water use efficiency. All genotypes had higher values for root traits and water use efficiency under hardpan conditions. This trend indicates a horizontal proliferation of root systems when they incur a stress imposed by a hardpan. The genotypic differences identified in this research for root traits and water use efficiency would be valuable for selecting genotypes for cotton breeding programs.

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1. Introduction

Cotton (*Gossypium* spp.) is the most important fiber crop in the world, accounting for about 25% of total world fiber use [1]. Furthermore, it is one among the five most important oil crops grown worldwide [2]. India, USA, China, Brazil, and Pakistan are the top five cotton producers in the world [3]. Most cotton growing regions are located in the arid and semi-arid regions worldwide [4]. Increasingly variable rainfall patterns, scarcity of water resources [5,6], and low nutrient availability [7,8] are major issues affecting cotton production worldwide.

Root system architecture and morphology are critical in improving resource (water and nutrients) capture and maintaining

plant productivity under drought conditions [5,9,10]. Cotton root system consists of a tap root, branch roots, and root hairs [11]. Chen et al. [10] reported that root morphology, particularly, surface fine root length and middle root length, strongly influences phosphorus uptake of cotton under phosphorus-deficient conditions. Water stress has been found to increase the ratio of root dry weight to shoot dry weight in cotton as roots elongate or proliferate horizontally to seek additional water within the soil profile [12–15]. Ball et al. [12] found that root elongation was less sensitive to drought stress than leaf expansion, probably relating to their relative importance in maintaining productivity under drought conditions [16].

Developing a germplasm pool with sufficient genetic variability in root traits is essential for cotton breeders for developing cultivars with root systems that improve resource capture under optimal and stress conditions [5]. However, genetic variability in root

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traits under optimal as well as stress conditions is not well-studied in cotton. As a result, little progress has been made in improving root system architecture and morphology of this crop that will increase resource acquisition. A major reason for why roots remain under-exploited plant organs is the challenging nature of root systems as they have high phenotypic plasticity in response to physical, chemical, and biological factors in the soil [17]. Expensive, time-consuming, and labor-intensive traditional methods for root harvest and measurements pose additional challenges to root studies [17–19]. Evaluating cotton genotypes, including germplasm lines and adapted varieties, for root traits will identify contrasting genotypes that can be included in crop improvement programs and help develop varieties with drought tolerance and/or improved resource capture.

Depletion of water resources has increased the importance of improving agronomic water use efficiency (the amount of biomass produced per unit water used) of cotton through cultivar selection and management practices [20]. Water use efficiency results in greater yield per unit rainfall and is often associated with crop drought tolerance [21]. It is an important parameter in determining crop performance in many production systems. Limited research has been conducted to document genetic variability for water use efficiency in cotton. DeTar [22] tested two cultivars for water use efficiency in California, USA, and found that the cultivars did not differ for that trait. In contrast, Snowden et al. [23] did find differences in water use efficiency among seven cotton cultivars that were tested in Texas, USA. Similarly, Saranga et al. [24], reported variation in water use efficiency among six cotton cultivars tested in the western Negev in Israel. Further research is required to better explore genetic variability in cotton for water use efficiency.

Several cotton growing areas in the world have soils with a compacted zone or hardpan. For example, the majority of the southeastern cotton growing regions in the USA, which occupy the major portion of its 'cotton belt', contain soils that have an inherent hardpan. Plant roots often fail to penetrate these compacted soil layers. Thus, the hardpan restricts root exploration and reduces water- and nutrient-uptake, and consequently, crop yields [25–27]. Furthermore, soil hardpans worsen the impact of drought on plants as they reduce the extent to which plant roots can exploit stored soil water in deep horizons [28]. Genotypic differences exist in cotton for root penetration of soil hardpan [29]. Thus, a viable approach to manage compacted soil would be the use of cultivars with root systems that penetrate the hardpan to extract resources from deeper soil layers or proliferate horizontally when they sense the hardpan in order to better explore more horizontal areas.

The objective of this research was to evaluate ten selected cotton genotypes (germplasm lines and cultivars) for root traits and water use efficiency. Experiments were conducted under controlled environmental conditions in two years. A hardpan treatment was included in the second year to evaluate the root traits and water use efficiency of cotton genotypes with and without the presence of a hardpan.

2. Materials and methods

2.1. Plant material

The cotton genotypes evaluated in this study were PD 1, PD 695, PHY 499WRF, PHY 444WRF, PHY 430W3FE, DP 1646B2XF, DP 1538B2XF, DP 1851B3XF, NG 5007B2XF, and ST 5020GLT. PD 1 and PD 695 are germplasm lines developed by the USDA-ARS Pee Dee Cotton Germplasm Enhancement Program at the Clemson University Pee Dee Research and Education Center (REC), Florence,

SC. PD 1 (PI 606805; pedigree: PD 4381/PD 8623) was released in 1985 [30]. It produces a deep root system, which possesses the ability to penetrate compacted soil layers [29]. PD 695 (PI 529615; pedigree: LA Frego 2/2 × PD 8562) was released in 1976 [31]. It produces a shallow root system and often fails under compacted soils [29]. The Pee Dee cotton germplasm program is one of the most significant, long-term public cotton germplasm enhancement programs and has been recognized for developing new cotton germplasm adapted to the southeastern USA [32]. Thus, PD 1 and PD 695 were included in this study as reference genotypes and to test their root morphological traits and water use efficiency under the presence and absence of a hardpan. The PhytoGen (Dow AgroSciences) varieties: PHY 499WRF, PHY 444WRF, and PHY 430W3FE, the Deltapine (Bayer CropScience) varieties: DP 1646B2XF, DP 1538B2XF, and DP 1851B3XF, the Stoneville (Bayer CropScience) variety, ST 5020GLT, and the Americot Seed Company's NexGen (NG) 5007B2XF are current popular high yielding varieties in the southeastern USA [33]. Furthermore, PHY 499WRF has excellent yield stability across many environments and appears to maintain yields under drought conditions (personal observation, M.A. Jones). Whether this yield increase was due to any mechanisms related to its root system was unclear.

2.2. Experimental details

This research was conducted under controlled environmental conditions in a greenhouse at the Department of Plant and Environmental Sciences, Clemson University, Clemson, SC, USA. Independent experiments were conducted in 2018 and 2019. Root traits were measured in both years, whereas, plant water use and water use efficiency were measured only in 2019.

The practices used to grow and maintain plants followed the methods given by Fried et al. [34,35]. The methods are briefed below with any modifications described in detail. The cotton plants were grown in mesocosms made up of polyvinyl chloride (PVC) with an inside diameter of 15 cm (Fig. S1). Each mesocosm was sealed at the bottom with a plastic cap, which had a central hole of 0.5 cm diameter for drainage. The mesocosms used in 2018 were of 71 cm height. In 2019, the mesocosms were constructed of two stacked PVC columns; the heights of the bottom and top columns were 74 and 25 cm, respectively. In both years, the mesocosms were filled with saturated Turface MVP (Burnett Athletics, Campobello, SC, USA). Turface is calcined, non-swelling illite and silica clay, and it allows for easy separation of roots [36,37]. In 2019, we wanted to examine the root morphology and water use efficiency of cotton genotypes when their root systems incur the stress resulting from a hardpan and in the absence of that. Therefore, in 2019, a synthetic hardpan was placed on top of the bottom column in half of the mesocosms. The synthetic hardpan was made up of 85% paraffin wax (Royal Oak Enterprises LLC, Roswell, GA, USA) and 15% petroleum jelly (Vaseline; Unilever, Englewood Cliffs, NJ, USA) by weight, and had a diameter of 16.5 cm, thickness of 1 cm, and strength (penetration resistance) of 1.5 MPa at 30 °C (see Fig. S1 of Fried et al. [34] for more information). The wax-petroleum jelly system is an efficient approach to measure the penetrability of roots as described by previous researchers in several field crops [27,34,35,38–43]. The top column was placed on top of the wax-petroleum jelly synthetic hardpan (in half of the mesocosms that contained a synthetic hardpan) or directly on top of the bottom column (in the other half of the mesocosms that did not contain a synthetic hardpan) in 2019. In this way, the synthetic hardpan was imposed at 25 cm depth in half of the mesocosms. The top and bottom columns along with the synthetic hardpan in between (if the mesocosm contained one) were tightly sealed together with a duct tape. In both years, the turface in all mesocosms was fertilized with a controlled-release fertilizer,

Osmocote with 18:6:12, N:P₂O₅:K₂O (Scotts, Marysville, OH, USA) at a rate of 20 g per column before planting. A systemic insecticide Marathon (a.i.: Imidacloprid:1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine; OHP, Inc., Mainland, PA, USA) was applied at 1.7 g per column before sowing to control sucking insect pests in both years. Three seeds of a single genotype were sown at 2.5 cm depth in each column on September 12, 2018 (year-1) and July 16, 2019 (year-2). In both years, thinning was performed at 14 days after planting (DAP) by retaining only the healthiest plant out of the three in each column and removing the other two. In 2019, after thinning, the top of each mesocosm was covered with aluminum foil to prevent evaporation [44,45]. A small slit was made in the aluminum foil to allow the cotton plant to grow through. Immediately after covering the top with aluminum foil, each mesocosm was weighed to record their initial weight, which was later used for the estimation of plant water use. Each mesocosm was watered at 51 days after covering (DAC) it with aluminum foil (10 mL), 53 DAC (20 mL), 76 DAC (20 mL), 88 DAC (20 mL), 94 DAC (40 mL), 101 DAC (60 mL), 116 DAC (60 mL), and 126 DAC (60 mL) in 2019 for preventing plants from wilting. All mesocosms were weighed before and after each watering in order to estimate plant water use at multiple time points during the season. In 2018, all mesocosms were watered at 28 DAP with 10 mL and at 44, 63, 69, 78, 84, and 91 DAP with 20 mL in order to prevent plants from wilting. Mesocosms were not weighed in 2018.

Each genotype was grown in four mesocosms in 2018 and in four mesocosms containing the hardpan and four other mesocosms containing no hardpan in 2019. Plants were maintained under optimum temperature conditions (30/22 °C, daytime maximum/night-time minimum; [46]) until harvest. A systemic insecticide Safari (Dinotefuran, [N-methyl-N'-nitro-N''-(tetrahydro-3-furanyl)methyl]guanidine); Valent USA Corporation (Sumitomo Chemical), Walnut Creek, CA, USA) was applied at 0.63 g L⁻¹ before sowing to control sucking insect pests at 84 DAP in 2018 and 79 DAP in 2019. Plants were harvested at 93 DAP in 2018 and 153 DAP in 2019. During harvest in both years, plants were cut at the base to separate shoots from the roots. Shoots were packed in paper bags and dried to constant weight at 70 °C for determining dry weight.

2.3. Data collection

Root harvest, processing, and further analysis followed the protocol given by Fried et al. [34,35], which is briefed below. Since no root systems penetrated the hardpan, only the top columns contained roots in 2019 under hardpan condition. In both years, roots were separated from the surface carefully to eliminate root loss and breakage. After harvest, root system of each plant was washed, placed between wet paper towels, sealed in Ziploc bags (S.C. Johnson & Sons, Inc. Racine, WI, USA), and stored at 4 °C. Afterward, each root system was scanned separately using an Epson Perfection V600 scanner (6400 dpi resolution) (Epson, Long Beach, CA, USA). The scanned images of roots were analyzed using WinRHIZO Pro image analysis system (Regent Instruments, Inc., Quebec City, QC, USA) to estimate total root length (sum of the lengths of all roots in the root system), total root surface area, total root volume, fine root (diameter < 0.25 mm) length, and fine root surface area. After scanning, each root system was packed in a paper bag and dried to constant weight at 70 °C for determining dry weight.

In 2019, water used by the cotton plants during the growth season was estimated in order to determine their water use efficiency. For this purpose, each mesocosm was weighed when its top was covered with aluminum foil (0 DAC, which was 14 DAP), at 51, 53, 76, 88, 94, 101, 116, and 126 DAC, and at final harvest (139 DAC, which was 153 DAP). Plant water use in each mesocosm

between two successive weighing dates was estimated as the difference in weights of the mesocosm on those dates plus weight of supplemental water added. Cumulative plant water use was calculated for each mesocosm by adding individual plant water use values. Plant water use efficiency was estimated as the ratio of total biomass (sum of shoot and root dry weights) to the total water used in the season [44,45].

2.4. Experimental design and data analysis

The experiments were conducted using a randomized complete block design in 2018 and a split plot design in 2019. Hardpan was the whole plot factor [two levels- presence and absence of a hardpan in the plant growth columns (mesocosms)] and genotype as the sub plot factor (ten levels, ten different genotypes) in 2019. The hardpan and genotype combinations were arranged in a 2 × 10 factorial treatment design. All treatments had four replications. Analysis of variance was performed using the GLIMMIX procedure in SAS (Version 9.4, SAS Institute, Cary, NC, USA) in both years. Separation of least squares means was done using the LSD test. The probability threshold level (α) for statistical significance was set at 0.05. The CORR procedures in SAS was used to find the relationships of water use efficiency with water use, biomass, shoot weight, and root traits. The JMP software (a statistical analysis software from SAS institute) was used to carry out a principal component analysis on root traits of all genotypes and to generate a biplot.

3. Results

When plants were harvested at 153 DAP in 2019, genotypic differences were significant for all root traits (Table S1). In 2019, even though a wax hardpan was introduced at 25 cm depth in the mesocosms, none of the genotypes penetrated the hardpan. However, the effect of hardpan was significant on all traits except cumulative water use (Table S1). The effect of hardpan-by-genotype interaction was not significant on any traits (Table S1) indicating that the presence of a hardpan did not much alter the relative performance of genotypes.

3.1. Root and shoot traits

Hardpan treatment significantly affected all root traits [morphological traits (Fig. 1) and weight (Fig. 2)]. All genotypes had higher values for all root traits under hardpan conditions (Figs. 1, 2). This trend indicates a significant proliferation or horizontal growth of root systems when they incur a stress imposed by a hardpan.

PHY 499WRF was one of the genotypes with greatest length, surface area, and volume of total roots and fine roots (roots with a diameter < 0.25 mm) in 2018 and under both hardpan-treatments (presence or absence of a hardpan) in 2019 (Fig. 1). This genotype, in fact, had the largest numerical values for most root morphological traits in 2018 and under both hardpan-treatments in 2019 (Fig. 1). Other PhytoGen varieties (PHY 444WRF and PHY 430W3FE) and PD-1 were also ranked among the top genotypes for total and fine root length, surface area, and volume in 2018 and under both hardpan-treatments in 2019 (Fig. 1). Genotype NG 5007B2XF had the lowest values for most root morphological traits in 2018 and under both hardpan-treatments in 2019 (Fig. 1). Genotype PD 695 had the lowest values for all root morphological traits in 2018, whereas it had high (under hardpan conditions) or intermediate (under no-hardpan conditions) values for those traits in 2019 (Fig. 1).

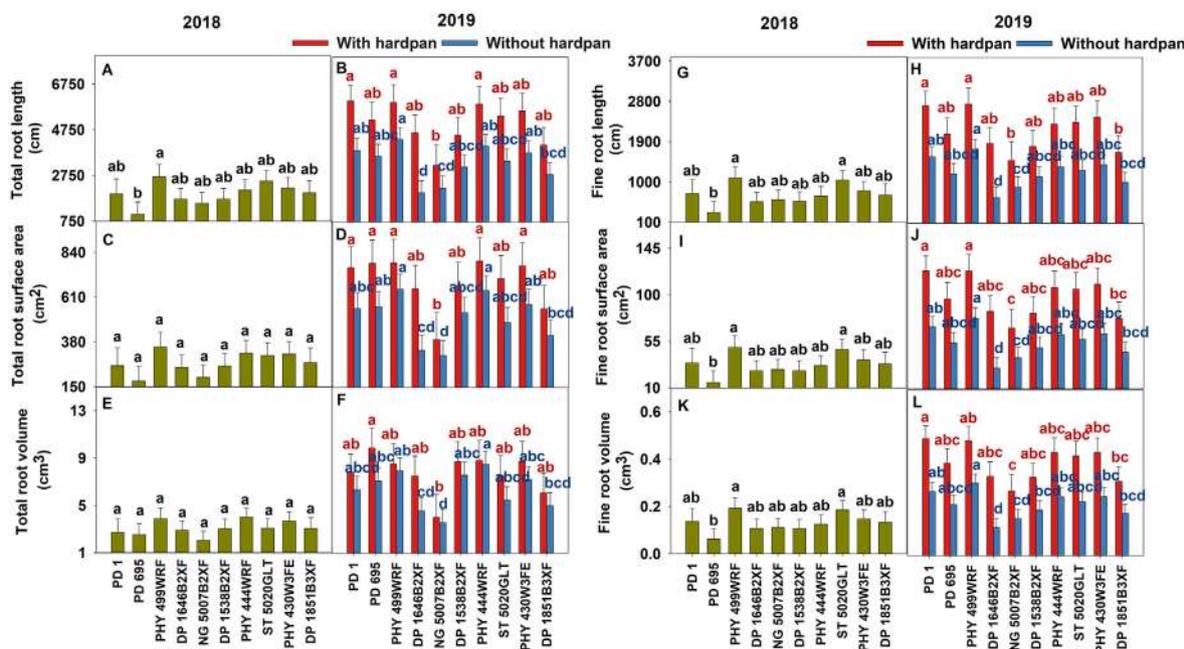


Fig. 1. Morphological traits of total root system (A–F) and fine roots (roots with a diameter < 0.25 mm) (G–L) of cotton genotypes in 2018 and 2019. A hardpan treatment was included when experiment was conducted in 2019. For this purpose, a synthetic hardpan was placed at 25 cm depth in half of the mesocosms used to grow cotton plants. Bars (least squares means) with different letters are significantly different according to the LSD test at $\alpha = 0.05$. In figure B, D, F, H, J, and L, the red letters compare genotypes under hardpan condition and the blue letters compare genotypes under no-hardpan condition.

PHY 444WRF and PHY 430W3FE were also ranked at the top for root dry weight in 2018 and under both hardpan-treatments in 2019 (Fig. 2A, B). PHY 499WRF was one among the top genotypes for root weight in 2018, but it had only intermediate values for root weight under both hardpan-treatments in 2019. NG 5007B2XF ranked low for root weight under both hardpan-treatments in 2019, whereas, PD 695 ranked low for root weight in 2018. Genotype PHY 499WRF ranked at the top for shoot weight in both years (Fig. 2C, D). Similar to the case of root weight, NG 5007B2XF also ranked low for shoot weight under both hardpan-treatments in 2019, whereas, PD 695 ranked low for shoot weight in 2018. In case of total biomass (root + shoot weight), genotypes PHY 499WRF, ST 5020GLT, PD-1, NG 5007B2XF, DP 1538B2XF, PHY 444WRF, and PHY 430W3FE were ranked as high, DP 1646B2XF and DP 1851B3XF as intermediate, and PD 695 as low in 2018 (Fig. 2E, F). In 2019, genotypes PHY 444WRF, PHY 430W3FE, PHY 499WRF, ST 5020GLT, PD-1, PD 695, and DP 1851B3XF were ranked as high, DP 1538B2XF as intermediate, and DP 1646B2XF and NG 5007B2XF as low for total biomass under hardpan conditions. Under no-hardpan conditions in 2019, the ranking remained more or less similar for total biomass, except that DP 1646B2XF was ranked as intermediate.

We conducted a principal component analysis (PCA) based on all root traits evaluated in this study to group the genotypes based on root traits. The PCA separated the genotypes into clusters (Fig. 3). In 2018, PHY 499WRF, which had higher values for root traits were separated from other genotypes into cluster-1. Similarly, NG 5007B2XF and PD 695, which had lower values for root traits were separated from other genotypes into cluster-3 and 4, respectively. All other genotypes were included in cluster-2. In 2019, PHY 499WRF and PD 1, which had higher values for root traits were separated from other genotypes into cluster-1 under both hardpan-treatments (Fig. 3B, C). As in 2018, NG 5007B2XF, which had lower values for root traits was separated from other genotypes into cluster-3 under both hardpan-treatments. All other genotypes were included in cluster-2 under both hardpan-treatments except that DP 1646B2XF, which had lower values for root traits was grouped into cluster-4 under no-hardpan condition.

3.2. Water use and water use efficiency

In 2019, cotton genotypes were evaluated for water use and water use efficiency under hardpan- and no-hardpan- conditions. Under hardpan condition, PHY 430W3FE, DP 1538B2XF, DP 1851B3XF, and NG 5007B2XF used the least amount of water, PD 1, PHY 499WRF, DP 1646B2XF, and ST 5020GLT used an intermediate amount of water, and PHY 444WRF and PD 695 used the maximum amount of water (Fig. 4A). Under no-hardpan condition, DP 1538B2XF, NG 5007B2XF, DP 1646B2XF, PHY 499WRF, and ST 5020GLT used the least amount of water, PD 695, PHY 444WRF, and DP 1851B3XF used an intermediate amount of water, and PD 1 and PHY 430W3FE used the maximum amount of water (Fig. 4A). The above trends in water use were consistent throughout the season under both hardpan-treatments (Fig. 4C, D).

For all genotypes, water use efficiency was higher under hardpan condition than under no-hardpan condition (Fig. 4B). Genotypes PHY 430W3FE, PHY 499WRF, PHY 444WRF, ST 5020GLT, DP 1851B3XF, PD 1, PD 695, and DP 1538B2XF were ranked high and DP 1646B2XF and NG 5007B2XF were ranked low for water use efficiency under hardpan condition (Fig. 4B). Under no-hardpan condition, PHY 499WRF, PHY 444WRF, ST 5020GLT, and PD 1 were ranked high and NG 5007B2XF was ranked low for water use efficiency. Genotypes PHY 430W3FE, DP 1851B3XF, DP 1538B2XF, DP 1646B2XF, and PD 695 had intermediate value for water use efficiency. Under no-hardpan condition, water use efficiency, which is the ratio of biomass to water use, was not correlated with water use, but with total biomass, root weight, total root length, total root surface area, fine root length, fine root surface area, and fine root volume (Table 2). Under hardpan condition, water use efficiency was not correlated with any traits ($P > 0.05$).

4. Discussion

The genotypic differences in root traits were not apparent in 2018 as they were in 2019. This is likely due to the time of root

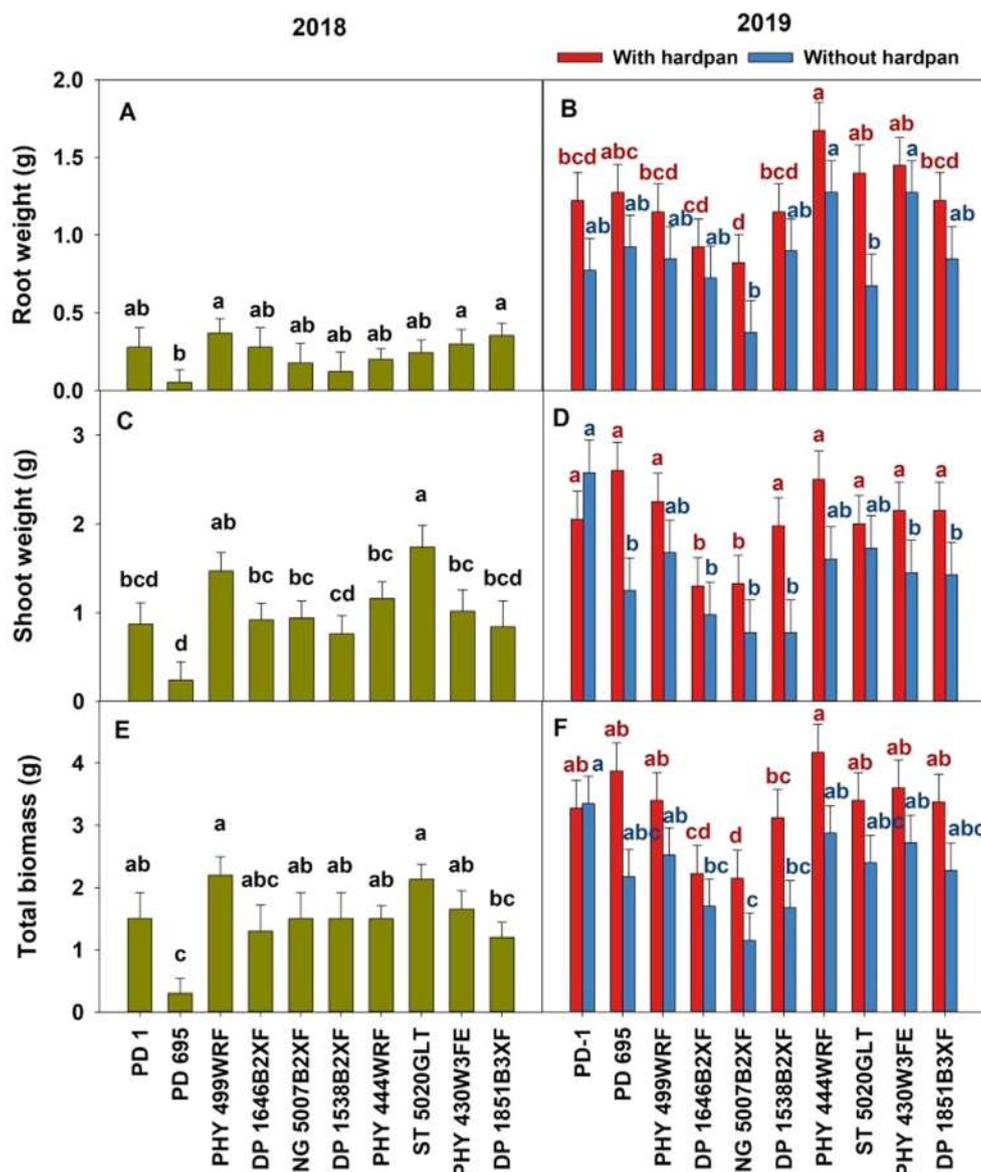


Fig. 2. Root weight, shoot weight, and total biomass (root + shoot weight) of cotton genotypes in 2018 and 2019. A hardpan treatment was included when experiment was conducted in 2019. For this purpose, a synthetic hardpan was placed at 25 cm depth in half of the mesocosms used to grow cotton plants. Bars (least squares means) with different letters are significantly different according to the LSD test at $\alpha = 0.05$. In figure B, D, and F, the red letters compare genotypes under hardpan condition and the blue letters compare genotypes under no-hardpan condition.

harvest for both years, as the roots were harvested ~ 2 months earlier in 2018 than in 2019. At the time of root harvest, most plants were at the vegetative stage and the remaining few were at the flowering stage in 2018. On the other hand, most plants were at the flowering stage and a few were at the boll development stage at the time of root harvest in 2019. Cotton actively develops its root system during the vegetative stages and attains the peak root mass during the flowering stage [47,48]. Root mass starts to decline once boll filling begins as the cotton plant redirects assimilates from root to developing bolls [47,48]. Thus, the genotypic differences in root traits become apparent at the time plants reach their maximum root development as in 2019. The relative performance of genotypes with respect to root traits were similar in most cases in both years. Overall, PHY 499WRF ranked at the top and NG 5007B2XF ranked at the bottom for root morphological traits and root weight in 2018 and under both hardpan-treatments in 2019.

Genotype PD 695 had the lowest values for all root morphological traits in 2018, whereas it had high (under hardpan conditions)

or intermediate (under no-hardpan conditions) values for those traits in 2019 (Fig. 1). Similarly, for root weight, this genotype had the lowest value in 2018 and high values under both hardpan-treatments in 2019 (Fig. 2). These results suggest that PD 695 does not produce a large root system during earlier part of life cycle but can increase root production during the later growth stages.

It was interesting to note that all genotypes increased total and fine root length, surface area, and volume and root weight when they were grown under hardpan conditions (Figs. 1, 2). This indicates that all genotypes increased root growth in a horizontal plane above the hardpan when their root systems could not go deeper after penetrating the hardpan. This may be for better exploration of water and nutrients from upper soil surface, i.e., above the hardpan (0–25 cm depth), when the vertical root growth was prohibited. It appears that when cotton plants are grown in compacted or dense soil, roots become more branched in the upper part of the soil [11]. Furthermore, a ‘topsoil foraging’ ideotype (i.e. high

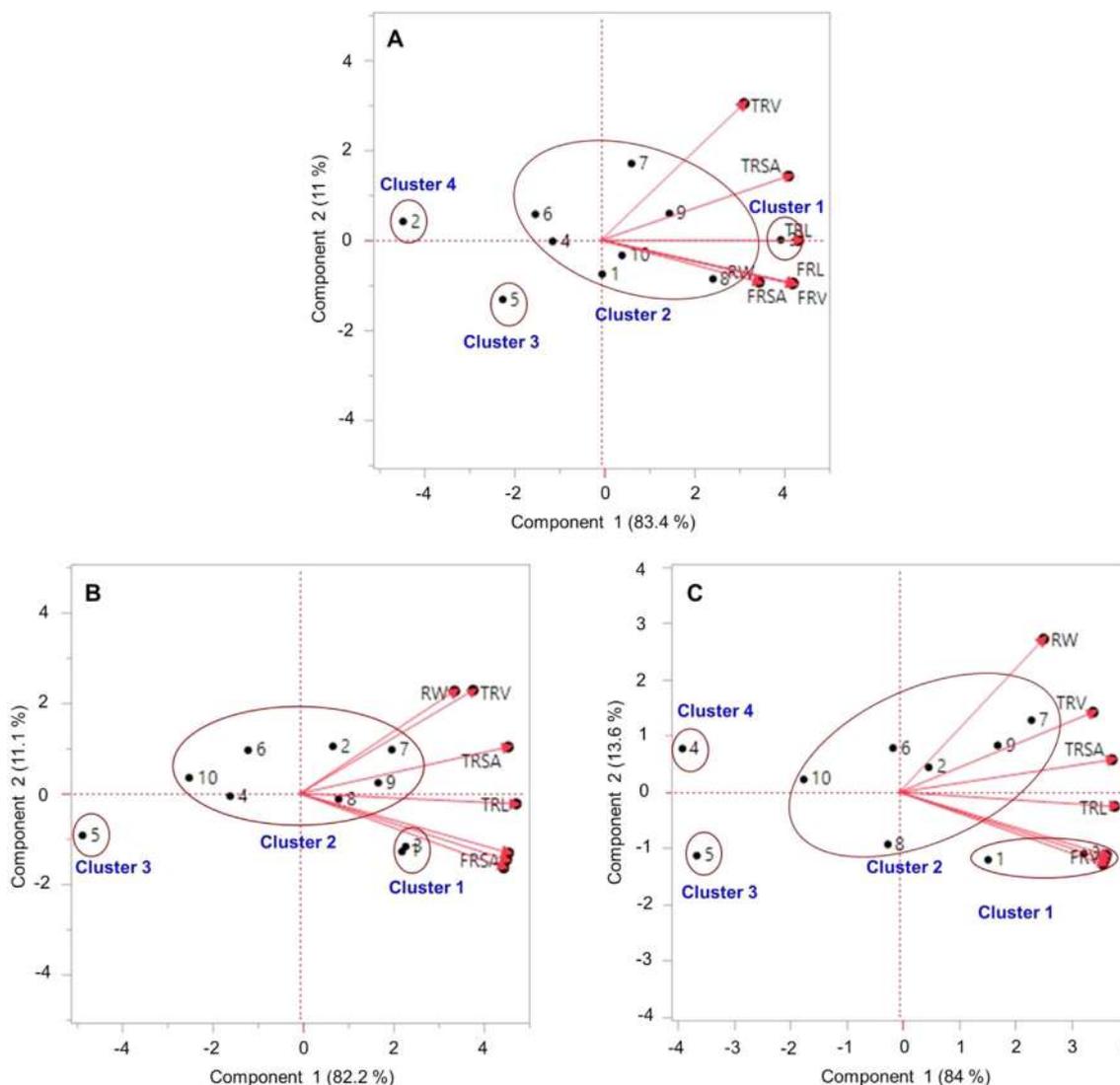


Fig. 3. Principal component analysis biplot that separated the cotton genotypes into clusters based on root traits in 2018 (A), under hardpan condition in 2019 (B), and under no-hardpan condition in 2019 (C). TRL, total root length; TRSA, total root surface area; TRV, total root volume; FRL, fine root (diameter < 0.25 mm) length; FRSA, fine root surface area; FRV, fine root volume; RW, root weight. Genotypes 1–10 are marked on the biplots; 1, PD 1; 2, PD 695; 3, PHY 499WRF; 4, DP 1646B2XF; 5, NG 5007B2XF; 6, DP 1538B2XF; 7, PHY 444WRF; 8, ST 5020GLT; 9, PHY 430W3FE; 10, DP 1851B3XF.

root growth, particularly surface area, in the topsoil) has been proposed to improve the acquisition of immobile nutrients such as phosphorus, manganese, and copper [49,50]. Consequently, breeding for this root ideotype has been proposed to increase phosphorus-uptake efficiency, which has been shown to promote the growth of crops in low-phosphorus soils [51–53]. Thus, PD 1, PHY 499WRF, PHY 444WRF, PHY 430W3FE, and ST 5020GLT, which had high total and fine root length and surface area in the upper soil (0–25 cm depth) under hardpan condition offer useful genetic backgrounds for breeding for improved nutrient foraging from upper soil layers.

Water use efficiency values of all genotypes were higher under hardpan conditions than under no-hardpan conditions (Fig. 4B). When this trend is assessed along with increased values of root length, surface area, volume, and weight under hardpan condition of all genotypes, it appears that some root-related mechanisms resulted in better biomass production per unit amount of water used by these genotypes. Further studies are warranted to elucidate these mechanisms. Notably, DP 1646B2XF had the lowest water use efficiency value among all genotypes under hardpan

condition, while it was one among the top genotypes for water use efficiency under no-hardpan condition. This shows that this genotype was more affected than other genotypes by a hardpan stress in terms of water use efficiency.

Cotton genotypes differed for water use and water use efficiency. Water use efficiency can be increased with decreased water use, increased biomass production, or a combination of both [54]. Water use efficiency associated with reduced water use often results in lower yield [54]. Increased water use efficiency could be a useful selection criterion in crop breeding programs only if it is associated with high biomass and/or yield [55,56]. Water use efficiency of cotton genotypes tested in this study was not correlated with water use, but with biomass, shoot weight, and root traits (root weight, total root length, total root surface area, fine root length, fine root surface area, and fine root volume). This increases the usefulness of the high water use efficient genotypic backgrounds for cotton breeding programs.

The reduced water use of NG 5007B2XF (Fig. 4A) did not result in increased water use efficiency under both hardpan-treatments (Fig. 4B); the reason was its decreased biomass production

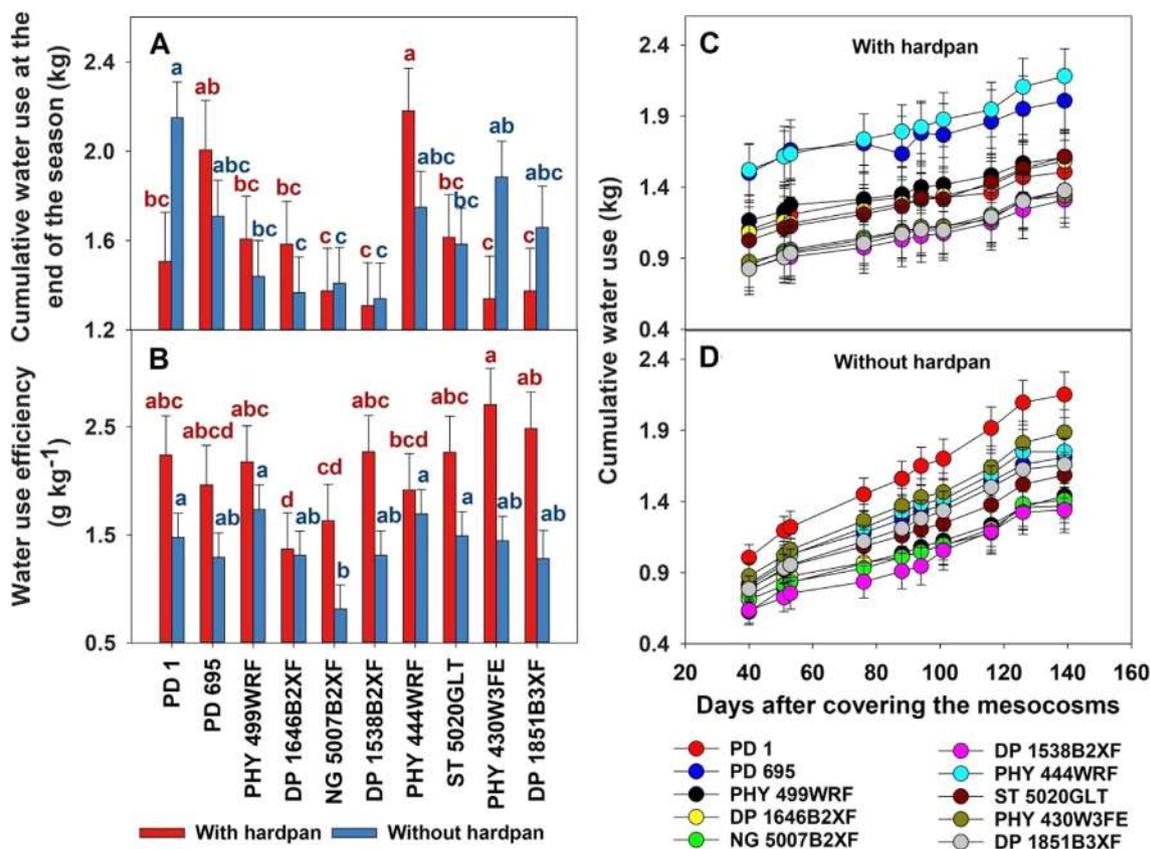


Fig. 4. Cumulative water use at the end of the season (for a period of 0–139 days after covering the mesocosms) (A), water use efficiency (ratio between total biomass and water use) (B) and season-long water use (C) of cotton genotypes in 2019. A hardpan treatment was included when experiment was conducted in 2019. For this purpose, a synthetic hardpan was placed at 25 cm depth in half of the mesocosms used to grow cotton plants. All mesocosms were covered by aluminum foil at 14 days after planting to prevent evaporative loss of water. In figure A and B, bars (least squares means) with different letters are significantly different according to the LSD test at $\alpha = 0.05$. In figure A and B, the red letters compare genotypes under hardpan condition and the blue letters compare genotypes under no-hardpan condition. Plant water use was not measured in 2018.

(Fig. 2F), which even offset the effect of decreased water use on water use efficiency. The same genotype also showed poor performance in terms of root morphological traits (Fig. 1). Thus, the reduced biomass production of this genotype might be a result of decreased resource (water and nutrients) capture through a poor root system, which ultimately resulted in decreased water use efficiency as well.

Even though, genotype PHY 444WRF used the maximum (under hardpan condition) or intermediate (under no-hardpan condition) amounts of water (Fig. 4A), it could also utilize that for increasing biomass production (Fig. 2F). This genotype had the numerically greatest biomass production under hardpan condition in 2019. The increased biomass production enabled this genotype to possess high water use efficiency as well (Fig. 4B). The same genotype was ranked at the top for root morphological traits and root weight (Figs. 1, 2). Thus, in contrast to NG 5007B2XF, the increased biomass production of PHY 444WRF, which might be a result of increased resource capture through an efficient root system, might have resulted in its increased water use efficiency. These results demonstrate that PHY 444WRF would produce high biomass under optimal water conditions. Whether the increased water use of PHY 444WRF will be a disadvantage for this genotype under water stress conditions needs to be tested in future studies.

Genotype PHY 499WRF exhibited many beneficial traits. It utilized only intermediate (under hardpan condition) or least (under no-hardpan condition) amounts of water (Fig. 4A) but was ranked among the top biomass producers (Fig. 2F). Increased biomass production without much increase in water use ranked this genotype

at the top for water use efficiency (Fig. 4B). This genotype was also ranked at the top for root morphological traits and root weight (Figs. 1, 2). These results indicate that an efficient root system and a root-related mechanism of PHY 499WRF might have helped this genotype to increase nutrient absorption without much increase in water use, which might also have resulted in its increased biomass production and water use efficiency. The high biomass production and water use efficiency of PHY 499WRF without increased water use suggest that this genotype would perform well under water limited conditions. This result also supports the yield maintenance of PHY 499WRF during drought years in the southeastern USA.

Although root traits are critical in improving yield and water use efficiency of crop plants, root traits are difficult to be used in breeding programs as their current estimation methods are not high throughput. Identification of molecular markers associated with root traits can help minimize expensive or laborious phenotyping methods. Recent literature suggests that marker assisted selection based on QTL mapping and GWAS or genomic selection will help breed crop varieties with useful root traits [57].

5. Conclusions

The present research evaluated ten selected cotton genotypes for root traits and water use efficiency. Overall, PHY 499WRF ranked at the top and NG 5007B2XF ranked at the bottom for root morphological traits and root weight. PHY 499WRF also produced

increased amount of biomass and had high water use efficiency. The genotypic differences identified for root and shoot traits and water use efficiency would be valuable for selecting genotypes for cotton breeding programs. Since all genotypes tested in this study, except PD 1 and PD 695, are patented commercial varieties, public breeders cannot use them in their breeding programs. However, their genetic background (parental lines) could be used instead. Current results were obtained when the cotton genotypes were grown under controlled environmental conditions. Future studies should verify the results under field conditions. Furthermore, future studies should evaluate lint yield in relation to root traits and estimate water use efficiency based on lint yield.

CRedit authorship contribution statement

Ricardo St Aime: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Grace Rhodes:** Investigation. **Michael Jones:** Conceptualization, Resources, Writing - review & editing. **B. Todd Campbell:** Conceptualization, Resources, Writing - review & editing. **Sruthi Narayanan:** Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing - original draft, Visualization, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data for this article can be found online at <https://doi.org/10.1016/j.cj.2020.12.001>.

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