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MOLECULAR BIOLOGY AND PHYSIOLOGY

CPPU (N-(2-chloro-4-pyridinyl)-N'-phenylurea) Enhancement of Cotton Yields

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ABSTRACT

The gradual depletion of the Ogallala Aquifer under the Southern High Plains of Texas during the past fifty years has resulted in reduced well capacities for cotton irrigation. Water table declines have already led some to shift from irrigated to dryland farming, and many of the remaining wells cannot provide enough water for full irrigation. The present study evaluated the effectiveness of the synthetic cytokinin CPPU (N-(2-chloro-4-pyridinyl)-N'-phenylurea) on the stimulation of lateral root production and enhancement of cotton yields under dryland conditions. Seed treatments of 250 µmol/mol or seedling sprays of 5 µmol/mol at the cotyledon stage, increased lateral root production. Cotton yield increases associated with the increased rooting ranged from 8 to 20% in three years of field studies under dryland conditions. The greatest yield increases were associated with dry shallow soils. Evaluation of water-deficit stress levels in untreated and CPPU-treated cotton showed reduced water-deficit stress levels throughout the growing season in the CPPU-treated cotton. This study showed that application of CPPU to cotton, early in development, has the potential to reduce water stress and increase yields.

The Ogallala Aquifer provides one-third of all groundwater used for irrigation in the United States (Dennehy, et al., 2002). A new predevelopment map coupled with a synthesis of annual water levels demonstrates that aquifer storage has declined by approximately 410 km^3 since the 1930s, a 15% larger decline than previous estimates. If current rates of decline continue, much of the Southern High Plains and parts of the Central High Plains will have insufficient water for irrigation within the next 20 to 30 years (Haacker, et al., 2016). Depletion is highly localized with about a third of depletion occurring in 4% of the High Plains land area. Extrapolation of the current depletion rate suggests that 35% of the southern High Plains will be unable to support irrigation within the next 30 years (Scanlon, et al., 2012). Water table declines have already led some to shift from irrigated to dryland farming, as the saturated thickness of the aquifer no longer supports pumping in some areas (Terrell, et al., 2002).

The High Plains region of Texas consists of 27 counties that produce 64% of the state's cotton crop. The loam and sandy soil types make it vital to implement water and soil conservation methods. Currently, less than fifty percent of the cotton is irrigated in this region, and this percentage will decrease with the depletion of the Ogallala Aquifer. The wide year-to-year variation in both total and seasonal rainfall significantly increases the risk of low yields in dryland farming. To mitigate this risk, cotton plants with larger root systems are needed to better extract available soil water. One mechanism to enhance cotton rooting and lessen the drought-induced yield losses was previously reported by Burke (Burke, 2009, Burke, 2011, Burke, 2013). Treatment of cotton seed or seedlings at the cotyledon stage with 6-benzyladenine enhanced root production and yield development under dryland conditions. Unfortunately, the concentrations of commercial 6-benzyladenine required to achieve this response in the field would be cost prohibitive to dryland cotton farmers. The current study investigated a synthetic cytokinin to determine if lower concentrations of active ingredients could induce similar rooting and enhanced yield production similar to that of the 6-benzyladenine. The improved efficacy of the synthetic cytokinin could improve the likelihood of commercialization and make this treatment of cotton a viable method for improving the profitability of dryland cotton production.

The current study evaluated the efficacy of the synthetic cytokinin, CPPU (N-(2-chloro-4-pyridinyl)-N'-phenylurea) (forchlorfenuron) on lateral root production and yield responses in cotton.

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Greenhouse studies were performed to determine optimal treatment concentrations and three years of field trials were performed under a rainfed production system.

MATERIAL AND METHODS

Identification of the Optimal Concentration of CPPU Seed Treatment to Enhance Lateral Root Production in Cotton Seedlings. Commercial cotton seed (FiberMax 9058F) was placed onto screens and submerged in tubs containing either 0, 25, 50, 100, 250, 500, 1000, 4000 or 8000 μmol/mol CPPU (N-(2-chloro-4-pyridinyl)-N'-phenylurea) (forchlorfenuron) [KIM-C1, LLC., Fresno, CA] for 10 seconds. The seeds were placed on paper towels on the lab bench and allowed to air dry. Six days after the seed soak, the seeds were planted in Ray Leach Cone-tainers Single Cell Systems (Stuewe & Sons, Inc., Tangent, Oregon) containing Sunshine Mix #1 soil (Sun Gro Horticulture Distributors Inc., Agawam, MA). Three seeds were planted per cone-tainer and they were placed into racks on benches in a greenhouse set to provide a 31/27°C day/night cycle. Six days after planting, seedlings were thinned to one plant per cone-tainer and grown for an additional five days. High-pressure sodium lights 430 W (P. L. Light Systems, Beamsville. ON Canada) were used to maintain a 16/8 h photoperiod. The soil was removed from the cone-tainer and the roots were rinsed and cleaned by gently dipping the soil into a bucket of water. Roots were photographed and evaluated for lateral root development. Briefly, root lengths were determined from the photographs with a ruler in the frame of the photo serving as a reference.

Identification of the Optimal CPPU Spray Concentration to Enhance Lateral Root Production of Cotton Seedlings. Commercial cotton seed (FiberMax 9058F) was planted in cone-tainers and grown for six days under greenhouse conditions (described above) prior to spraying with a range of CPPU concentrations. The system used to spray the plants incorporated a fume hood equipped with a mobile spray arm that delivered a spray mist over the seedlings. The nozzle used in this system was identical to those used in field spray applications. Initial concentrations (0, 5, 10, 15, 20, 25, 50, 100, 250, 500, 1000, and 2000 μmol/mol) were evaluated. The plants were grown an additional five days post-spray prior to washing out the roots.

Crop Management: 2012. Eight 15 m rows of FM 9180B2F were planted per replication of a complete block design in a North-South orientation using a John Deere 7300 MaxEmerge 2 VacuMeter Planter. The seeding rate was 10 seeds per meter on 1.02-meter centers. The plots were pre-plant irrigated by furrow irrigation. Following emergence, irrigated plots received 5-mm/day sub-surface drip irrigation and rainfed plots received no further irrigation following the pre-plant irrigation. Three CPPU application options were evaluated: 1) The seedlings in the irrigated and rainfed plots were sprayed with 5 μmol/mol CPPU at the cotyledon stage of growth prior to the complete expansion of the primary leaf; 2) the plants in the irrigated and rainfed plots were sprayed at first square with 5 μmol/mol CPPU; or 3) the seeds planted in the irrigated and rainfed plots were treated with 250 μmol/mol CPPU one week prior to planting. The crop was terminated by spraying the plots with Ginstar (Bayer CropScience, Research Triangle Park, NC) and Prep (Bayer CropScience, Research Triangle Park, NC) according to manufacturer's instructions. The plots were hand harvested approximately four weeks after the termination treatment.

Crop Management: 2013. Eight 25 m rows of FM 9180B2F were planted per replication in an East-West orientation using a John Deere 7300 MaxEmerge 2 VacuMeter Planter. The seeding rate was 10 seeds per meter on 1.02-meter centers. The plots were pre-plant irrigated by furrow irrigation. No subsequent irrigation was applied to any of the plots based upon the 2012 results showing no yield advantage under full-irrigation and published research with 6-benzyladenine not showing yield advantages under full-irrigation. The seedlings were sprayed with 5 μmol/mol CPPU at the cotyledon stage of growth prior to the complete expansion of the primary leaf. The crop was terminated by spraying the plots with Ginstar (Bayer CropScience, Research Triangle Park, NC) and Prep (Bayer CropScience, Research Triangle Park, NC) according to manufacturer's instructions. Four rows per plot with five replications per treatment were hand harvested approximately four weeks after the crop termination.

Crop Management: 2016. Twenty-four 3 m rows of FM 9180B2F were planted per replication of a complete block design in a North-South orientation using a John Deere 7300 MaxEmerge 2 VacuMeter

Planter. The plots were pre-plant irrigated by furrow irrigation. Following emergence, the plots received no further irrigation. The seedlings were sprayed with 5 µmol/mol CPPU at the cotyledon stage of growth prior to the complete expansion of the primary leaf. The crop was terminated by spraying the plots with Ginstar (Bayer CropScience, Research Triangle Park, NC) and Prep (Bayer CropScience, RTP, NC) according to manufacturer's instructions. Fifteen one-meter plots per treatment were hand harvested approximately four weeks after the termination treatment.

Stress Test Bioassay. During the 2016 season, a 1-cm^2 leaf punch was harvested from a source leaf (in cotton this is the fifth main stem leaf from the top) using a leaf tissue punch. This was repeated on five separate plants of the untreated and CPPU-treated cotton every three to four days throughout the growing season. The punches were transferred to a well in a Costar® 3524 24-well cell culture cluster (Corning Inc., Corning, NY) that had been half-filled with water. The lid was returned to the cell culture plate immediately following addition of the leaf punches. This process was repeated until samples from all treatments had been harvested. Upon returning to the lab, the punches were placed on moistened Model 583 Gel Dryer Filter Paper (Bio-Rad Laboratories, Hercules, CA) in a Pyrex baking dish. The leaf punches and filter paper were covered with Glad® ClingWrap (CO_2 permeable) (The Glad Products Company, Oakland, CA) and pressed flat with a speedball roller for Microseal film (MJ Research, Inc., Waltham, MA) to remove air bubbles and ensure good contact between the tissue and filter paper. Initial chlorophyll fluorescence yield of quantum efficiency (Fv/Fm') levels were determined using an Opti-Science OS1-FL Modulated Fluorometer and then samples were placed in the dark in a VWR Model 2005 incubator (Sheldon Manufacturing, Inc., Cornelius, OR) set to 40°C. The samples were heat treated for 30 min at 40°C, and then removed from the incubator and placed on the bench top at 25°C for 30-min. Chlorophyll fluorescence yield of quantum efficiency (Fv/Fm') levels were again determined to evaluate the sensitivity of the leaf punches to the heat treatment. The decline in fluorescence yield (Fv/Fm') was used as a relative measure of the stress level of the plant (a slow decline occurring in leaf tissue from stressed plants, and a more rapid decline occurring in leaf tissue from less stressed plants).

Measurement of Relative Root Length. Seedling Root Measurements: The seedling photographs were opened in Photoshop and a new layer was added to trace the lateral roots. A second layer was added to trace the taproot, and a third layer was added to trace a 1-centimeter line using the ruler in the photo. The histogram tool was utilized to determine the number of pixels drawn in each layer. Lengths were calculated by dividing the total number of pixels in the taproot and lateral root layers, respectively, by the number of pixels in the 1-cm line layer. This method allowed for the accurate determination of lateral and taproot lengths.

Mature Plant Root Measurements: In an effort to evaluate relative rooting patterns in the upper 0.5-meter of soil, the root systems of twenty field-grown plants per treatment were harvested using "The Uprooter" (Grants Pass, OR) at harvest during the 2013 study. Twenty plants from control (untreated) and CPPU-treated blocks from Plot 1 were evaluated because this plot exhibited the highest yield increase of the five replicate plots due to shallow soil on the west end of the field. The root systems were photographed on a 2-inch by 2-inch grid and root lengths determined by tracing the roots in Photoshop and comparing the total pixel number to the number of pixels in a 2-inch line as described above.

Meteorological Measurements. The United States Department of Agriculture-Plant Stress and Water Conservation (USDA – PSWC)- Meteorological Tower is located immediately adjacent to the experimental plots. Five-minute measurements of rainfall (mm), and temperature (˚C) were collected and hourly averages calculated.

Experimental Design and Statistical Analysis. The field experiment in 2012 was a combination of a split plot (based on water levels) with the randomized block design as indicated (to randomize the CPPU levels).. The field experiment in 2013 used a uniform block design with five replications. The 2016 experiment used a complete block design with fifteen replications per treatment. Statistical significance among treatments was analyzed with a student's *t* test through the statistical applications of Excel software and the T-test calculator for two independent means located at http://www.socscistatistics.com/tests/studentttest/Default2.aspx. Graphs were created using KaleidaGraph Version 4.1.3 (Synergy Software, Reading, PA, http://www.synergy.com/wordpress_650164087/kaleidagraph/).

RESULTS

Initial seed treatments with CPPU concentrations of 0, 5, 10, 15, 20, 25, 50, 100, 250, 500, 1000, and 2000 µmol/mol were evaluated for enhancement of cotton seedling lateral root development. Tests revealed enhanced root production from 25 µmol/mol through 250 µmol/mol , with optimal lateral root production at 250 µmol/mol (Figure 1). Seed treatments above 250 µmol/mol exhibited reductions in lateral root production. The test was repeated with a second set of ten plants per treatment comparing only the 0 and 250 µmol/mol CPPU treatments. Representative photographs of control (left) and CPPU-treated (right) root systems are shown in the upper right side of the figure. A significant increase in seedling lateral root development was observed in subsequent studies of the seedlings from CPPU-treated seeds (bar graph, *p*-value = 0.047668. The results are significant at $p < .05$).

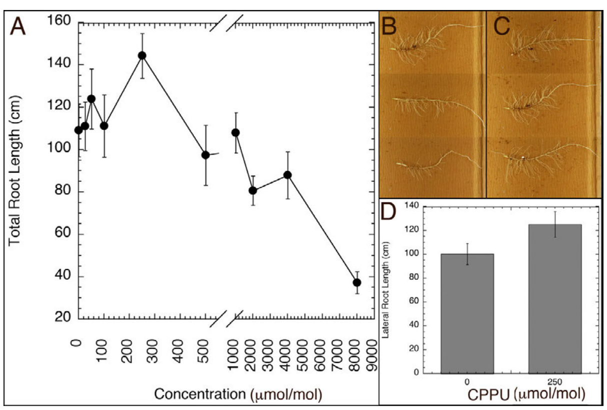

Figure 1. A) Graph showing the affect of the concentration of CPPU cotton seed treatment on subsequent seedling lateral root development. B) Representative photographs of root systems from three seedlings of control (left) and CPPU-treated (right) seeds. C) Bar graph comparing lateral root development in seedlings from control and 250 µmol/mol CPPU-treated seeds.

Cotton seedlings were sprayed with CPPU concentrations of 0, 5, 10, 15, 20, 25, 50, 100, 250, 500, 1000, and 2000 µmol/mol, and lateral root development was evaluated. Phytotoxicity was observed on the cotyledons sprayed with CPPU applications above 25 µmol/mol (Figure 2). Analysis of lateral root development revealed that the 5-µmol/mol concentration exhibited the highest lateral root development of the seedling spray treatments. The CPPU enhancement of lateral root development was also observed in FM 9180B2F, a line previously used in the characterization of 6-benzyladenine enhancement of cotton root development.

Figure 2. Photographs for cotton seedlings five days after being sprayed with a 0, 25, 50, 100, 250, 500, 1000, or 2000 µmol/mol of CPPU. Downward view (top), side view (middle) and close up of seedlings sprayed with 50 µmol/mol CPPU (bottom). The white arrows highlight regions exhibiting spray damage.

Field studies of the response of cotton to CPPU-treatment were performed in 2012, 2013, and 2016. Rainfall patterns for July, August, and September (the peak flowering and boll filling period) are shown in Figure 3. The least amount of rainfall occurred during the 2012 study with a 29.6 mm rain event occurring on DOY 256. The 2013 study received rain events of 41.8 and 35.5 mm on DOY 200 and DOY 227, with no further significant rain events for the remainder of the season (Figure 3). Finally, the 2016 study was dry during July and August, followed by rain events of 34.5 mm, 54.6 mm and 34.4 mm on DOY 243, 244, and 246. An additional rain event of 18.3 mm occurred on DOY 259 (Figure 3).

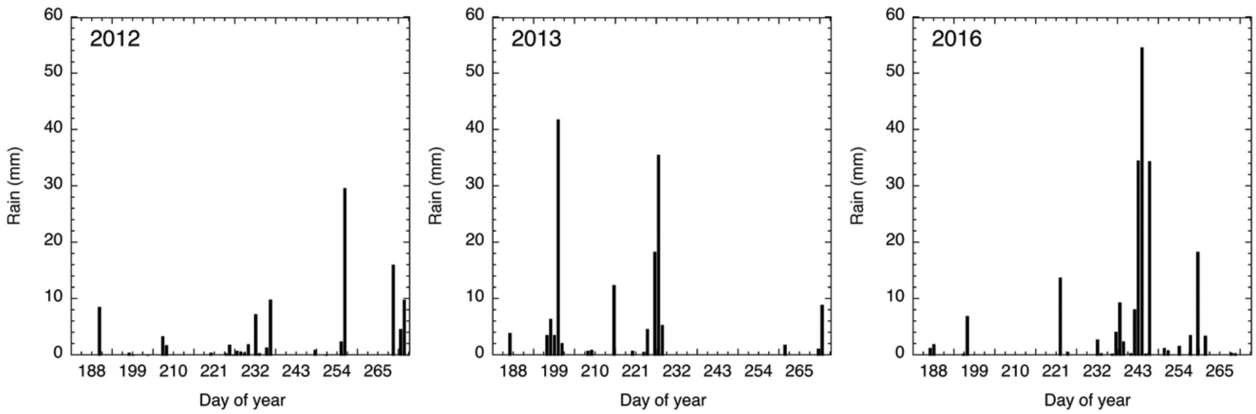

Figure 3. Graphs showing the rainfall patterns during July, August and September for the 2012, 2013 and 2016 field studies.

The low rainfall experienced during the 2012 growing season provided an excellent environment for evaluating water-deficit stress responses in the presence or absence of a CPPU treatment. Figure 4 shows a photograph of the FM 9180B2F plants grown under a pre-plant irrigated + 5 mm/day irrigation (left) or pre-plant irrigated only (right). The severe water-deficit stress resulted in short plants that yielded approximately one-fourth that of the irrigated plots. No significant differences in seed cotton yields were observed among the control and CPPU treatments under full irrigation (Figure 4). The seed cotton yields of the rainfed plots did show significant differences in yield among the treatments. The plants sprayed with 5 µmol/mol CPPU at first square showed yields equal to the untreated control. The plots with the seed treated with 250 µmol/mol CPPU one week prior to planting exhibited a 14.4% yield increase, and the plots sprayed at the cotyledonary stage exhibited 13.3% yield increase in seed cotton yields compared with the untreated control.

The 2013 growing season had timely rain events on DOY 200 and DOY 227 that resulted in higher yields compared with the 2012 study. Figure 5 shows a photograph of the rainfed plots following harvest of the four-row plots. The increased size of the plants compared to the 2012 study is apparent from the photograph. Additionally, the rainfed cotton yields were approximately three times higher than those of 2012. The graph in Figure 5 shows seed cotton yields for untreated controls (solid circles), and seedlings sprayed at the cotyledon stage (open circles). The bar graph inset shows average yield of the plots of the five untreated and five CPPU treated plots. Each data point is an average of the yields of four rows harvested per replication.

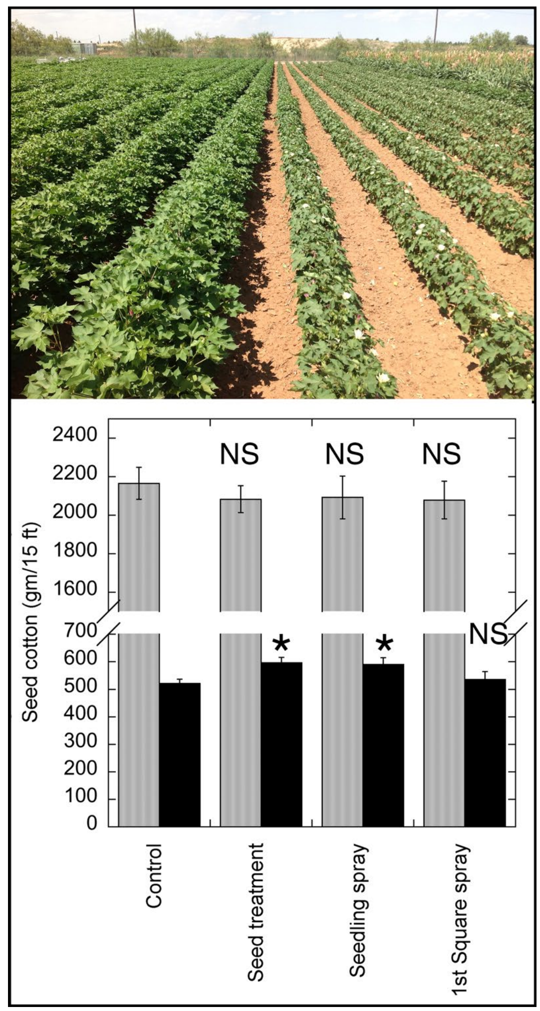

Figure 4. Photograph of the pre-plant irrigated + 5 mm/day irrigation (left) or pre-plant irrigated only (right) cotton during the 2012 field study. The graph shows seed cotton yields for untreated controls, seed treated with 250 µmol/mol CPPU, seedlings sprayed at the cotyledon stage with 5 µmol/mol CPPU, and seedlings sprayed at the first square stage with 5 µmol/mol CPPU. NS= not significant, *= significant at the <0.05 level. Irrigated plots have gray bars and rainfed plots have black bars.

The observed increase in plot yields from plot 1 to plot 5 reflects differences in soil depths. In 1999, this field was laser planed to level the field. This treatment resulted in the movement of topsoil from the west end of the field (Plot 1) to the east end of the field (Plot 5). This resulted in Plot 1 having shallow soils and plot 5 having the deepest soils in this field. The average cotton seed yield of the five plots per treatment is shown in the inset in Figure 5. The increase in yield for the entire field was 8%, and the increase in yield in the shallow soils of plot 1 was 20%.

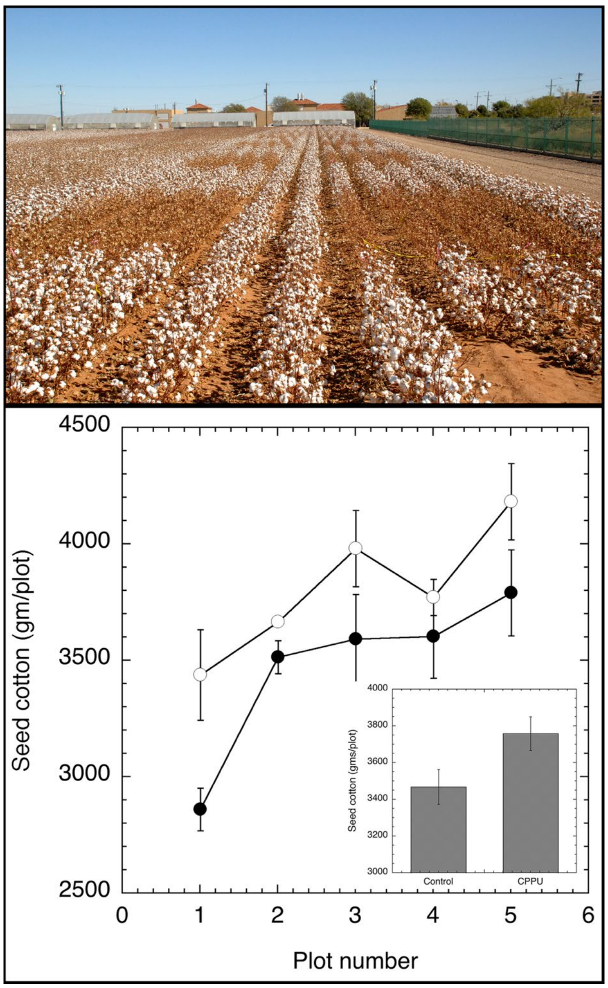

Figure 5. Photograph of the with 5 µmol/mol CPPU-treated cotton sprayed at the cotyledon stage (left) and untreated control (right) cotton under rainfed conditions during the 2013 field study. The graph shows seed cotton yields for untreated controls (solid circles), and seedlings sprayed at the cotyledon stage (open circles). The bar graph inset shows average yield of the five untreated and five CPPU treated plots.. NS= not significant, *= significant at the <0.05 level.

To better understand why the cotton seed yield of the CPPU-treated cotton was 20% higher than the untreated control we pulled 20 plants from the untreated control and CPPU treated cotton in Plot 1. Figure 6 is a graph of cotton root lengths of twenty plants randomly harvested from Plot 1 of the 2013 field study. Plant root lengths have been sorted from the least to the greatest for comparison purposes. On average, greater lateral root production was observed in the CPPU-treated cotton. These results are consistent with the increased lateral root production in the greenhouse studies.

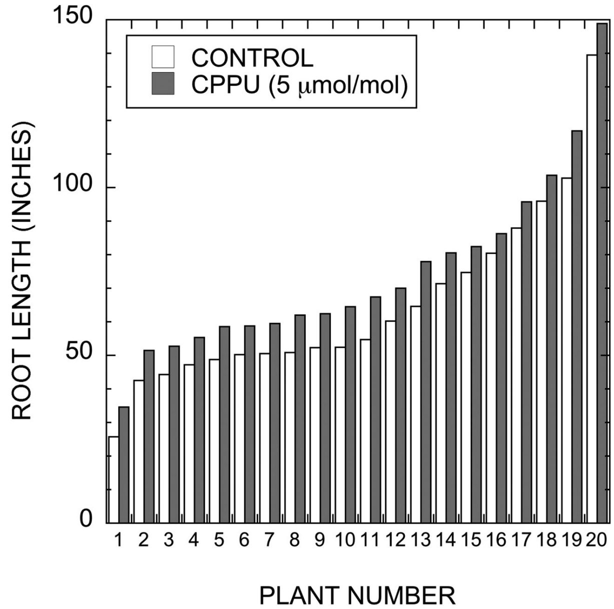

Figure 6. Graph of cotton root lengths of twenty plants randomly harvested from Plot 1 of the 2013 field study (See Figure 5). Plant root lengths have been sorted from the least to the greatest. Untreated plants = open bars; CPPU-treated seedlings = solid bars.

The increased yield and increased rooting of the CPPU-treated cotton in 2013 suggested that the increased rooting may have lessened the deleterious effects of water-deficits on growth and yield development. To test this, we utilized a water-deficit stress bioassay developed by Burke (Burke, 2007, Burke, et al., 2010) to determine stress levels throughout the 2016 growing season. Figure 7 is a photograph of FM 9180B2F cotton roots harvested on DOY 214 in the 2016 study from rainfed plots of control (untreated) and CPPU-treated seedlings. The graph shows the efficiency of quantum yield (Fv/Fm) of control (solid circles) and CPPU-treated (open circles) FM 9180B2F cotton throughout the 2016 growing season. In this bioassay, the higher the efficiency of quantum yield the greater the water-deficit stress level of the plant. Areas highlighted in green show times when the CPPU-treated cotton was less stressed than the untreated controls, and red areas represent times when the CPPU-treated plants were more stressed than the untreated controls. Clearly the increased lateral root production provides greater access to available soil water and reduces the deleterious effects of water-deficit stress. The improved seedling growth of the CPPU-treated FM 9180B2F is apparent in the photograph taken on DOY 182 in the rainfed plots of the 2016 study (Figure 8). The graph shows higher cotton yields in the CPPU-treated plants. Although yield differences were obtained across the field because of the soil depth, the CPPU-treated cotton consistently yielded more cotton than the untreated control.

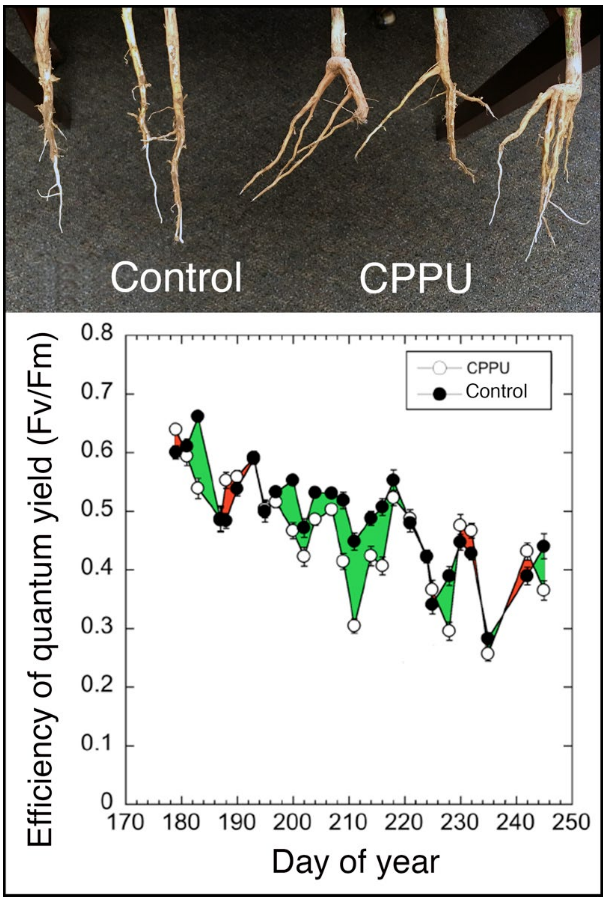

Figure 7. Photograph of FM9180 cotton roots harvested on DOY 214 in the 2016 study from rainfed plots of control (untreated) and 5 µmol/mol CPPU sprayed at the cotyledon stage. Graph of the efficiency of quantum yield of control (solid circles) and CPPU-treated (open circles) FM9180 cotton throughout the 2016 growing season. In this bioassay, the higher the efficiency of quantum yield the greater the water stress level of the plant. Areas highlighted in green show times when the CPPU-treated cotton was less stressed than the untreated controls, and red areas represent times when the CPPU-treated plants were more stressed that the untreated controls.

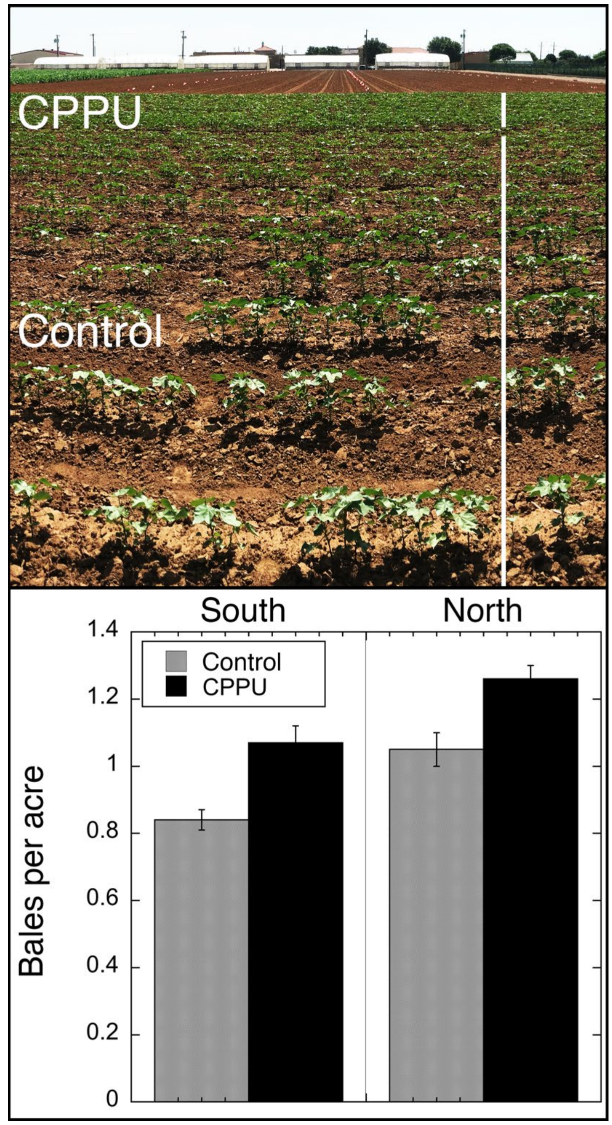

Figure 8. Photograph of FM9180 cotton on DOY 182 in the rainfed plots of the 2016 study. Control (untreated) cotton is shown in the lower portion of the photograph (see the lower white line on the right), and the 5 µmol/mol CPPU sprayed at the cotyledon stage seedlings are shown in the upper section of the photograph (see the upper white line on the right). The graphs shows cotton yields from the south (shallow soils) and north (deeper soils) regions of the field. Control untreated FM9180 a represented by the gray bars and the CPPU-Treated FM9180 are represented by the black bars.

DISCUSSION

The current study evaluated the efficacy of the synthetic cytokinin, CPPU (N-(2-chloro-4-pyridinyl)-N'-phenylurea) (forchlorfenuron) on lateral root production and yield responses in cotton. The determination of optimal CPPU concentrations for treating cotton seedlings at the cotyledon stage in the field showed that concentrations of 5 µmol/mol CPPU provided enhanced root development, thickening of the hypocotyl, and improved seedling development. Previous studies of 6-benzyladenine treatment of field-grown cotton reported that concentrations from 100-300 µmol/mol 6-benzyladenine were required to maximize root development (Burke, 2011, Burke, 2013).

The increased yield and increased rooting of the CPPU-treated cotton in the 2012 and 2013 seasons suggested that the increased rooting lessened the deleterious effects of water-deficits on growth and yield development. To test this, we utilized a water-deficit stress bioassay developed by Burke (Burke, 2007, Burke, et al., 2010) to determine stress levels throughout the 2016 growing season. Figure 7 shows the efficiency of quantum yield (Fv/Fm) of control (solid circles) and CPPU-treated (open circles) FM 9180B2F cotton throughout the 2016 growing season. In this bioassay, the higher the efficiency of quantum yield the greater the water-deficit stress level of the plant. Areas highlighted in green show times when the CPPU-treated cotton was less stressed than the untreated controls, and red areas represent times when the CPPU-treated plants were more stressed that the untreated controls. Clearly the increased lateral root production provided greater access to available soil water and reduced the deleterious effects of water-deficit stress.

The benefits of water stress avoidance on cotton growth have been reported previously (Burke, et al., 1985). The effects of the soil water deficit were analyzed by monitoring changes in growth parameters of photoperiodic cotton strains T185 and T25 at 106 DAP. When comparing growth under irrigated and dryland conditions, cotton strain T185 had a greater reduction in plant height, leaf area index, plant dry weight, and leaf number as compared to T25. T25 was identified as a drought resistant cotton line exhibiting enhanced stress avoidance because of elevated lateral root production (Quisenberry, et al., 1981). Significant correlations between root characteristics and dry-matter yield under dryland conditions suggested that overall root vigor allowed T25 to be a better competitor for limited soil water. They concluded that root morphology and root growth potentials appear to be important for the adaptation of cotton to conditions where limited soil-water availability is a major growth constraint. In the present study, CPPU resulted in an enhancement of lateral root development, and the treated plants exhibited reduced water-deficit stress levels based upon the chlorophyll fluorescence bioassay.

Because of the continued pumping of the Ogallala, much of the Southern High Plains and parts of the Central High Plains will have insufficient water for irrigation within the next 20 to 30 years (Haacker, et al., 2016). Water table declines have already led some to shift from irrigated to dryland farming, as the saturated thickness of the aquifer no longer supports pumping in some areas (Terrell, et al., 2002). With the reduced availability of irrigation, it is essential that producers maximize soil water capture by the plant. This study has demonstrated that treatment of modern cotton cultivars with CPPU enhances early season root development that results in a greater exploration of the soil volume. The exploration of a greater soil volume helps the plant delay the onset of water-deficit stress and allows the plant to develop higher yields than untreated controls. This yield enhancement technology provides producers transitioning from irrigated to rainfed production systems a tool to maximize cotton production in water-limited environments.

DISCLAIMER

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REFERENCES

Burke, J.J. 2007. Evaluation of source leaf responses to water-deficit stresses in cotton using a novel stress bioassay. Plant Physiol. 143(1): 108-121. doi:10.1104/pp.106.087783.

Burke, J.J. 2009. Cytokinin enhancement of cotton. United States Patent and Trademark Office US 7,634,870 B1.

Burke, J.J. 2011. 6-Benzyladenine enhancement of cotton. J. Cotton Sci. 15: 206-214.

Burke, J.J. 2013. 6-Benzyladenine enhancement of cotton yields. J. Cotton Sci. 17: 245-252.

Burke, J.J., C.D. Franks, G. Burow, and Z. Xin. 2010. Selection system for the stay-green drought tolerance trait in sorghum germplasm. Agron. J. 102(4): 1118-1122.

Burke, J.J., P.E. Gamble, J.L. Hatfield, and J.E. Quisenberry. 1985. Plant morphological and biochemical responses to field water deficits. I. Responses of glutathione reductase activity and paraquat sensitivity. Plant Physiol. 79: 415-419.

Dennehy, K.F., D.W. Litke, and P.B. McMahon. 2002. The High Plains Aquifer, USA: Groundwater development and sustainability. Geological Society, London, Special Publications 193: 99-119.

Haacker, E.M., A.D. Kendall, and D.W. Hyndman. 2016. Water level declines in the High Plains Aquifer: Predevelopment to resource senescence. Ground Water 54: 231-242.

Quisenberry, J.E., W.R. Jordan, B.A. Roark, and D.W. Fryrear. 1981. Exotic cottons as genetic sources of drought resistance. Crop Sci. 21: 889-895.

Scanlon, B.R., C.C. Faunt, L. Longuevergne, R.C. Reedy, W.M. Alley, V.L. McGuire, and McMahon, P.B. 2012. Groundwater depletion and sustainability of irrigation in the US High Plains and Central Valley. Proc. National Acad. Sci. 109: 9320-9325.

Terrell, B.L., P.N. Johnson, and E. Segarra. 2002. Ogallala Aquifer depletion: Economic impact on the Texas High Plains. Water Policy 4: 33-46.