

Interactive effects of carbon dioxide, low temperature, and ultraviolet-B radiation on cotton seedling root and shoot morphology and growth

David BRAND¹, Chathurika WIJEWARDANA¹, Wei GAO², K. Raja REDDY (✉)¹

¹ Department of Plant and Soil Sciences, Mississippi State University, Starkville, MS 39762, USA

² USDA UVB Monitoring and Research Program, Natural Resource Ecology Laboratory, and Department of Ecosystem Science and Sustainability, Colorado State University, Fort Collins, CO 80523, USA

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Abstract Interactive effects of multiple environmental stresses are predicted to have a negative effect on cotton growth and development and these effects will be exacerbated in the future climate. The objectives of this study were to test the hypothesis that cotton cultivars differ in their responses to multiple environmental factors of (CO₂) [400 and 750 μmol·mol⁻¹ (+CO₂)], temperature [28/20 and 20/12°C (–T)], and UV–B radiation [0 and 10 kJ·m⁻²·d⁻¹ (+UV–B)]. A genetic and molecular standard (TM-1) and three modern cotton cultivars (DP1522B2XF, PHY496W3R, and ST4747GLB2) were grown in eight sunlit, controlled environment chambers with control treatment 400 μmol·mol⁻¹ [CO₂], 28/21°C temperature, and 0 kJ UV–B. The results showed significant differences among the cultivars for most of the shoot and root parameters. Plants grown under low temperature alone or as a combination with +UV–B treatment caused more detrimental effects on root and shoot vigor. Although the elevated CO₂ treatments weakened the damaging effects of higher UV–B levels on cotton growth on all cultivars, increased CO₂ could not mask the negative effects of low temperature. When comparing all cultivars, genetic standard TM-1 produced the smallest values for the majority of traits under CO₂, UV–B, and low temperature either alone or in combination with other treatments. Based on principal component analysis, the four cultivars were classified as tolerant (DP1522B2XF), intermediate (PHY496W3R and ST4747GLB2) and sensitive (TM-1) to multiple environmental stresses. Low temperature was identified as the most damaging treatment to cotton early seedling vigor while elevated CO₂ caused the least. Existing variability of cotton cultivars in response to

multiple environmental stresses could allow for selection of cultivars with the best coping ability and higher lint yield for future climate change environments.

Keywords Cotton, Climate change, winRHIZO, Principal component analysis., Stress tolerance.

Abbreviations: DAP, Days after planting, SPAR- Soil-Plant-Atmosphere-Research, PCA, Principal component analysis, SGR, Seed germination rate, PH, Plant height, NOD, Node number, NAR, Node addition rate, LA, Leaf area, LW, Leaf weight, SW, Shoot weight, RW, Root weight, TD, Total dry weight, RS, Root/shoot ratio, RL, Root length, RSA, Root surface area, RD, Root diameter, RLV, Root length per soil volume, RV, Root volume, RT, Number of tips, RF, Number of forks, RC, Number of crossings, SPAD, Total chlorophyll, PHOL, phenolic content

1 Introduction

Crop physiologists and agronomists all over the world are interested in the depletion of stratospheric ozone layer, increase in atmospheric CO₂ concentration, and temperature fluctuations. Over the past few decades, debate on how projected climatic changes affect crop production and performance has increased tremendously. Current CO₂ level of 400 μmol·mol⁻¹ is projected to further increase by within the next century, thereby affecting the vegetation in both natural and agro-ecosystems. Cotton cultivated in the US Cotton Belt already experiences UV–B radiation of 2 to 11 kJ·m⁻²·day⁻¹ during the summer (Gao et al., 2010). Forecasts indicate that current levels of UV-B radiation will increase by another 2–3 kJ·m⁻²·d⁻¹ due to the emissions of long-lived greenhouse gasses, as well as

human activities (WMO, 2011). According to National Climatic Data Center (NOAA), the average global temperature on earth has increased by about 1.4°F since 1880 (NOAA, 2014). Temperature fluctuations can have both direct and indirect effects on cotton growth and development. Most studies of cotton growth and development have focused on either single or two stress factors, and have given little attention to aspects of cotton root morphology and early seedling vigor. Therefore, studies are needed to understand the interactive effects these environmental factors have on cotton early seedling growth processes thereby improving proper cultivars for future climates.

The rise in atmospheric [CO₂] is considered to have a positive effect on C3 crops, such as cotton, due to increased rate of photosynthesis and vegetative growth (Reddy et al., 1992a, 1995). According to Reddy et al. (2005), elevated [CO₂] weakened apical dominance of the cotton plant resulting in more branches per plant and had little effect on plant height. Moreover, elevated [CO₂] increased total plant leaf area by 27% (Reddy et al., 1998). Previous studies reported that cotton roots grown under elevated [CO₂] conditions increased the volume of tap roots, lateral roots, fine roots, root mass, and lengths (Rogers et al., 1992b; Prior et al., 1994). Supporting this information, in a study of cotton growth under elevated [CO₂]; Reddy et al. (1994) observed an increased length of lateral roots when compared to ambient CO₂ level, but minimal effect on root initiation and development. Previous interactive studies on cotton (Reddy et al., 1995) and other crops (Zhao et al., 2003; Koti et al., 2005) have shown that elevated CO₂ levels do not alleviate the damaging effects of either higher UV–B radiation or high temperature. However, according to recent studies, the harmful effects of drought and higher UV–B levels were hindered under elevated CO₂ treatments in maize hybrids (Wijewardana et al., 2016). Therefore, further research is necessary to clearly understand the interactive effects of elevated CO₂ on cotton seedling growth to determine detrimental or beneficial effects.

Cotton and all other crops cultivated between 40 °N and 40 °S latitudes are already experiencing UV-B doses of 2–10 kJ·m⁻²·d⁻¹ depending on location and season (http://www.toms.gsfc.nasa.gov/ery_uv/ery_uv1.html). The UV-B radiation is projected to further increase in the future. In the US Cotton Belt, low temperatures and UV–B radiation play a significant role in altering the growth and development during early-season cotton growth (Gao et al., 2010; Reddy et al. 1992b, 1996). In cotton, temperature is the key controller of developmental events, plant growth rate, and fruit maturation. Low temperatures can have direct inhibitory effects on growth and yield due to reduction in growth and development processes, chilling injuries, and delayed maturation (Bange and Milroy, 2004a). Cotton seedlings exposed to chilling temperatures early in the season take longer to develop, accumulate biomass at a

slower rate, and may permanently arrest seedling emergence (Christiansen and Thomas, 1969). Late in the season, low temperatures may force bolls to open, thereby affecting lint fiber quality, and severely impeding the effectiveness of chemical harvest aids at defoliation (Bange et al., 2009; Lokhande and Reddy, 2014).

The influence of UV–B radiation on cotton growth and development has been studied extensively. Cotton plants exposure to UV–B radiation reduced the canopy size, internode length, leaf area expansion, and increased the production of wax and phenolic compounds by adopting defensive or repair mechanisms (Kakani et al., 2003; Reddy et al., 2003, 2004). Also, UV–B radiation has been reported to alter leaf anatomy and thickness as well as damage to reproductive processes, resulting in lower yield (Zhao et al., 2003; Kakani et al., 2004). Interactive studies on UV–B and temperature indicated that UV–B damage was higher on plants grown at 30°C than plants grown at 20°C (Nedunchezian and Kulandaivelu, 1996). Moreover, Kakani et al. (2004) reported the early senescence of cotton leaves with exposure to UV–B radiation in an interaction study of UV–B and CO₂. Therefore, studying these interactive effects is critical in developing appropriate management practices for future climate conditions.

The knowledge of root responses to changes in the aerial environment is essential in understanding the cotton responses to predicted changes in climate. Numerous studies have considered the impact of increased CO₂ on cotton root growth and dynamics. Results have suggested that cotton roots become longer, highly branched, and thicker when exposed to high CO₂ as compared to cotton roots grown in ambient air (Singh et al., 2007; Prior et al., 1995). Few studies have reported the interactive effect of elevated CO₂ and temperature (Reddy, 1997), and to date there is no data available on the effects of multiple stresses including [CO₂], low temperature, and UV–B on cotton root growth and architecture during seedling growth and development. In this study, we hypothesized that the genotypic tolerance of cotton to each of the three stressors would be modified by the combined effects of stressors, and root morphology and early seedling vigor could be used to identify tolerance among cotton cultivars during early seedling growth stage. Therefore, the objectives of this study were to understand the interactive effects of CO₂, UV–B radiation, and low temperature on root morphology and early seedling vigor of cotton cultivars and to classify cotton cultivars based on their tolerance to multiple environmental stressors.

2 Materials and methods

2.1 Experimental condition and cotton cultivars

This study was conducted in sunlit plant growth chambers known as Soil-Plant-Atmosphere-Research (SPAR) units

located at the Rodney Foil Plant Science Research facility of Mississippi State University, Mississippi State (33°28' N, 88°47' W), MS, USA. Details of the operation and control algorithms of SPAR chambers have been described by Reddy et al. (1991, 2001, and 2004). The SPAR units are located outdoors and have the capacity to precisely control air temperature, chamber [CO₂], and soil watering by a dedicated computer system. The Plexiglas chambers use natural solar radiation as the light source that allow 97% of the visible solar radiation to pass without spectral variability in absorption while blocking solar UV radiation (100% of UV-B and 88% of UV-A). Variable density shade black cloths placed around the edges of the plant canopy are adjusted frequently to match canopy height, mimicking solar radiation attenuation through the canopy. The conditioned air is passed through the plant canopy with sufficient velocity (4.7 km·h⁻¹) to cause leaf flutter and is returned to the air-handling unit just above the soil level.

Fungicide treated commercial seeds from four cotton cultivars, a genetic standard Texas Marker (TM)-1 (Stelly et al., 2005; Mujahid et al., 2016), and three modern cultivars, (Delta Pine Land (DP)1522 B2XF, PhytoGen (PHY)496W3R, and Stoneville (ST)4747GLB2 were sown in polyvinyl-chloride pots (15.2 cm diameter by 30.5 cm high) filled with a soil medium consisting of a 3:1 of sand and top soil by volume and classified as sandy loam (87% sand, 2% clay, and 11% silt). Each pot contained approximately 500 g of gravel at the bottom and a 6.4-mm hole at the bottom to allow excess water drainage. Twenty four pots (6 pots for each cultivar) were arranged as a completely randomized design in 8 rows with three pots per row in each SPAR chamber. Four cotton cultivars were seeded in alternate rows. Initially, four seeds were sown in each pot and the plants were thinned to one per pot 6 d after emergence. Irrigation was provided for 90 s, three times a day initially, using full-strength Hoagland's nutrient solution (Hewitt, 1952) delivered at 08:00, 12:00, and 17:00 h through an automated and computer-controlled drip system to ensure favorable nutrient and water conditions for plant growth.

2.2 Treatments

Initially, the chambers were maintained at 28/20°C

(day/night), 400 μmol·mol⁻¹ CO₂, and 0 m⁻²·d⁻¹ UV-B until seedling emergence (6 days). Thereafter, each SPAR unit was set to one of the eight treatments until 20 DAS. The treatments included combinations two CO₂ levels of 400 μmol·mol⁻¹ and 750 μmol·mol⁻¹ (+ CO₂), two levels of temperature 28/20°C and 21/12°C (-T) and two daily biologically effective UV-B radiation intensities of 0 and 10 (+ UV-B) kJ·m⁻²·d⁻¹. Control treatment consists of 28/20°C, 400 μmol·mol⁻¹ [CO₂], and 0 kJ UV-B treatments. The treatments conditions are presented in Table 1.

The chamber [CO₂] was maintained either at 400 or 750 μmol·mol⁻¹ by a dedicated infrared model LI-6252 (LI-COR Biosciences, Lincoln, NE, USA) gas analyzer. Pure CO₂ was supplied from a compressed gas cylinder through a system that included a pressure regulator, solenoid and needle valves, and a calibrated flow meter (Reddy et al., 2001). Air temperature in each SPAR chamber was monitored using a type T (copper/constantan) thermocouple connected to an Agilent Technologies 34970A data acquisition unit (Agilent Technologies, Santa Clara, California, USA) and adjusted every 10 seconds throughout the day and maintained within ±0.5°C of the treatment set points. The daytime temperature began at sunrise and returned to the nighttime temperature 1 h after sunset.

The desired UV-B treatment, 10 kJ·m⁻²·d⁻¹, was imposed from 6 DAP to the end of the experiment. The anticipated UV-B radiation dosage was provided by the square-wave UV-B supplementation system under near ambient photosynthetically active radiation (PAR). The UV-B radiation was delivered daily from 08:00 to 16:00 h by eight fluorescent UV-313 lamps (Q-Panel Company, Cleveland, OH, USA) attached horizontally on a metal frame inside each chamber, powered by 40 W dimmable ballasts. Distance from the lamps to the plant canopy was maintained at 0.5 m throughout the experiment. The individual UV lamps were wrapped with solarized 0.07mm cellulose diacetate film (JCS Industries Inc., La Mirada, CA, USA) to filter UV-C (<280 nm) radiation. The UV-B radiation supplied to the top of the plant canopy was checked daily at 08:00 h with a UVX digital radiometer (UVP, Inc., San Gabriel, CA, USA) and calibrated against an Optronic Laboratory (Orlando, FL, USA) Model 754 Spectroradiometer, which was used initially to quantify lamp output. Cellulose diacetate films

Table 1 The treatments, mean day/night temperatures, daily CO₂, and UVB treatments for each chamber.

Treatments	Mean Temperature/°C	[CO ₂]/(μmol·mol ⁻¹)	UV-B/(kJ·m ⁻² ·d ⁻¹)
Control	24.41±0.15	458.35±2.08	0
-T	17.50±0.17	434.24±2.70	0
+ UV-B	24.28±0.18	417.23±2.29	10
+ CO ₂	24.37±0.16	737.64±1.11	0
+ CO ₂ + UV-B	24.48±0.18	730.98±1.32	10
+ CO ₂ -T	17.59±0.17	729.42±1.23	0
-T + UV-B	17.55±0.17	457.32±2.77	10
+ CO ₂ -T + UV-B	17.35±0.17	740.16±1.22	10

were replaced and lamp output was adjusted as needed in order to maintain the respective UV–B radiation levels. In the control units, unilluminated lamps and frame were used to simulate equivalent shading. The actual UV–B radiation was measured at three different locations, 50-cm apart in each SPAR chamber to make certain plants received the exact UV–B dosage. The average daily biologically affective UV–B radiation was $10 \pm 0.15 \text{ kJ} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ during the experimental period.

3 Measurements

3.1 Seedling growth

The seedling emergence was recorded as the number of days from sowing to 50% emergence in each pot. Plant heights were measured and nodes were counted on all plants at final harvest, 20 DAP. Leaf area was measured using the LI-3100 leaf area meter (LI-COR, Biosciences). Plant total dry weights (TD) including leaves, stems, and roots were recorded after oven drying for 5 days at 75°C.

3.2 Root morphology

After separating the stem from individual root systems of each plant, roots were carefully washed by placing the pot on sieves and gently spraying with water. The cleaned individual root systems were floated in approximately 5 mm of water in a 0.3- by 0.2-m Plexiglas tray and gray-scale root images were acquired according to the procedure described by Wijewardana et al. (2015). Roots were untangled and separated with a plastic paintbrush to minimize root overlap. The tray was placed on top of a specialized dual-scan optical scanner (Regent Instruments, Inc.), linked to a computer. Gray-scale root images were acquired by setting the parameters to high accuracy (resolution 800 by 800 dpi). Acquired images were analyzed for root length, root surface area, average root diameter, root volume, and number of tips, forks, and crossings using WinRHIZO Pro software (Regent Instruments, Inc.).

3.3 Phenolics and chlorophyll content using SPAD meter

The UV–B absorbing compounds (Phenolics) were extracted from five 0.38 cm² leaf discs placed in a vial with 10 mL of phenol reagent (79:20:1 V/V of methanol, distilled water, and HCl) at 18 DAP. The vials were incubated at room temperature for 24 h in dark to allow for complete extraction of UV–B absorbing compounds. The absorbance of these extracts from different treatments was measured using a Bio-Rad ultraviolet/VIS spectrophotometer (Bio-Rad Laboratories) at 320 nm using phenol

reagent as the check. The content of UV–B absorbing compounds was calculated using the equation: $y = 16.05 \times A$, where y is concentration of UV–B absorbing compounds ($\mu\text{g} \cdot \text{mL}^{-1}$ of extract) expressed as equivalents of p-coumaric acid and A is absorbance at 320 nm (Koti et al., 2007). The relative leaf chlorophyll content was assessed with a hand-held chlorophyll meter, SPAD-502 (Minolta Canada Inc., Ontario, Canada). Five SPAD readings were taken from two fully expanded leaves per plant, between 10:00 and 12:00 h.

3.4 Statistical analyses

Data analysis for all measured and calculated variables were conducted using ANOVA procedures in SAS (SAS Institute). The single stress factors, combinations of two factors, and the combination of all three factors were regarded as treatments. The data was analyzed as a complete randomized design. Fisher protected least significant difference tests at

$P = 0.05$ was used to test the differences among treatments for measured parameters, and the standard errors of the mean were calculated and presented in the figures as error bars.

Principal component analysis (PCA) was used to separate 4 cotton cultivars into multiple stress tolerant groups. The objective of the PCA is to identify principal variables, or factors that explain the pattern of correlations within a set of observed variables or traits. The PCA tends to accomplish this by developing a new set of uncorrelated variables called principal components (PC scores), thereby reducing the number of variables. The concept of PCA and the implementation of this function to separate cultivars into tolerant groups have been described previously for other crops (Singh et al., 2008; Wijewardana et al., 2015, 2016b).

In summary, index values for each of the seven different treatments were initially calculated by assessing the response of each shoot, root, and physiological trait compared to its control value. Afterwards, the summation of responses of all traits which developed under each treatment was used as the index values for PCA analysis. The analysis was performed using the PRINCOMP procedure in SAS. The PCA allows seven different index values, also known as eigenvectors and for cultivars termed as eigenvalues (PC scores). These index values were used to identify the correlation of response variable vectors and cultivars across the ordination space. The results were summarized in biplots using SigmaPlot 13 (Systat Software, Inc.), which are the plots of mean principal component scores for the first two principal components. Compared to other analytical tools, the scores plot from PCA in the data table produced a clearer grouping of the cultivars by creating a new set of uncorrelated variables.

4 Results

4.1 Above-ground traits

Elevated CO₂, low temperature, and UV-B radiation as well as interaction between CO₂ and UV-B showed no significant effects on plant height. Only the interactions of UV-B × -T ($P < 0.001$), and CO₂ × T × UV-B ($P < 0.001$) interactions were significant for plant height (Table 2). Taller plants were observed when plants were grown under +CO₂ conditions (Fig. 1a). Plants grown under -T condition were the shortest among the other treatments, either alone, or in combination with +UV-B. The greatest reduction in plant height (56%) was observed in TM-1 while the reduction was only 5% in DP1522B2XF compared to the plants grown in control conditions.

A significant reduction of leaf area was observed under -T condition (Fig. 1b). Plants grown under elevated CO₂ had significantly more leaf area than control plants. Although increased CO₂ produced more leaf area either alone or together with UV-B, it had minimal positive effect on low temperature stressed plants. Over all the genotypes -T alone or together with UV-B had the most negative effect on leaf area as compared to the plants grown under the control conditions. When the plants were

subjected to all the stressors (+CO₂-T+UV-B), the reduction of leaf areas were 78% (DP1522B2XF) to 85% (TM-1) less than the plants grown under control conditions.

There were no significant effects of the interactions of +CO₂ × -T, +UV-B × -T, and +CO₂ × +UV-B on total plant dry matter content tested. However, effects of individual stresses on total dry matter were significant. Elevated CO₂ increased total plant dry weight by 33%, while low temperature and +UV-B decreased weight by 72% and 6% accordingly (Fig. 1c). Even though [CO₂] did not alleviate the negative effect of low temperature for total dry matter in the -T treatment, elevated [CO₂] treatment in combination with UV-B treatment caused 6% increase in total dry matter with respect to the control (Fig. 1c). The highest reduction of total dry matter was observed under -T + UV-B treatment, 75% compared to the control. The two cotton cultivars, TM-1 and ST4747GLB2 had the least dry matter content compared to the other two cultivars under +CO₂ -T + UV-B treatment.

4.2 Below-ground traits

Statistically significant reduction of root length due to low temperature was observed among all the cotton cultivars

Table 2 The analysis of variance across the treatments of carbon dioxide concentration ([CO₂]), temperature (T), UV-B radiation (UV-B), and Hybrid (Hy), and their interactions on corn vegetative, physiological, and photosynthetic traits; seed germination rate (SGR), plant height (PH), node number (NOD), node addition rate (NAR), leaf area (LA), leaf weight (LW), shoot weight (SW), root weight (RW), total dry weight (TD), root/shoot ratio (RS), root length (RL), root surface area (RSA), root diameter (RD), root length per soil volume (RLV), root volume (RV), number of tips (RT), number of forks (RF), number of crossings (RC), total chlorophyll (SPAD), phenolic content (PHOL).

Source of variation	SGR	PH	NOD	NAR	LA	LW	SW	RW	TD	RS	RL	RSA	RD	RLV	RV	RT	RF	RC	SPAD	PHOL
CO ₂	NS	NS	NS	NS	NS	**	NS	***	***	NS	NS	*	**	NS	***	NS	NS	NS	NS	***
T	NS	NS	***	NS	***	***	***	***	***	**	***	***	*	***	***	***	***	***	***	NS
T*CO ₂	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
UV-B	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	***
UV-B*CO ₂	NS	NS	NS	NS	**	**	NS	NS	**	NS	**	NS	***	NS	NS	NS	NS	NS	NS	NS
T*UV-B	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	***
T*UV-B*CO ₂	*	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**
Cultivar (Cul)	***	***	NS	***	***	***	NS	NS	**	NS	***	***	***	***	***	***	***	***	NS	NS
Cul*CO ₂	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**
Cul*T	NS	NS	NS	**	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	*	NS	NS
Cul*T*CO ₂	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cul*UV-B	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cul*UV-B*CO ₂	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	***	NS	NS	NS	NS	NS	NS	NS
Cul*T*UV-B	*	**	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	*	NS	**	NS	**	NS	NS
Cul*																				
T*UV-B*CO ₂	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

†Significance levels are indicated by ***, **, * and NS representing $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P > 0.05$, respectively.

The observations of plant height, node No., leaf area, and total dry matter were recorded at 38 DAP, physiological measurements at 33 DAP, and all other photosynthesis measurements at 30 DAP.

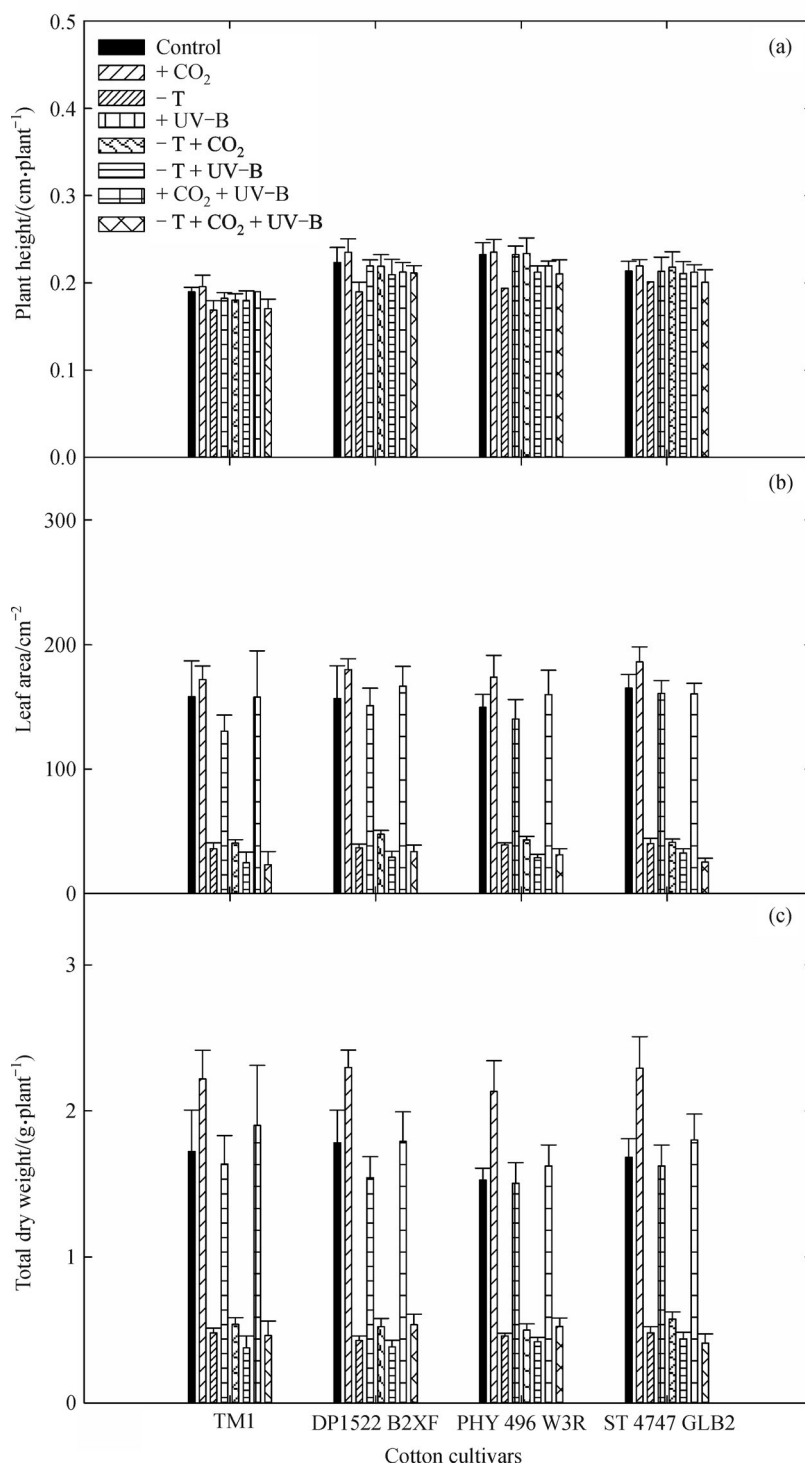


Fig. 1 Impact of CO₂ concentration (control, 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ and + CO₂, 750 $\mu\text{mol}\cdot\text{mol}^{-1}$), low temperature day/night (control, 28/20°C and -T, 20/12°C), and UV-B radiation (control, 0 and + UV-B, 10 $\text{kJ}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$), and their interactions on (a) plant height, (b) leaf area, and (c) total dry weight for four cotton cultivars (TM-1, DP1522 B2XF, PHY496W3R, and ST4747GLB2). Bars indicate standard errors of the mean ± 6 replications.

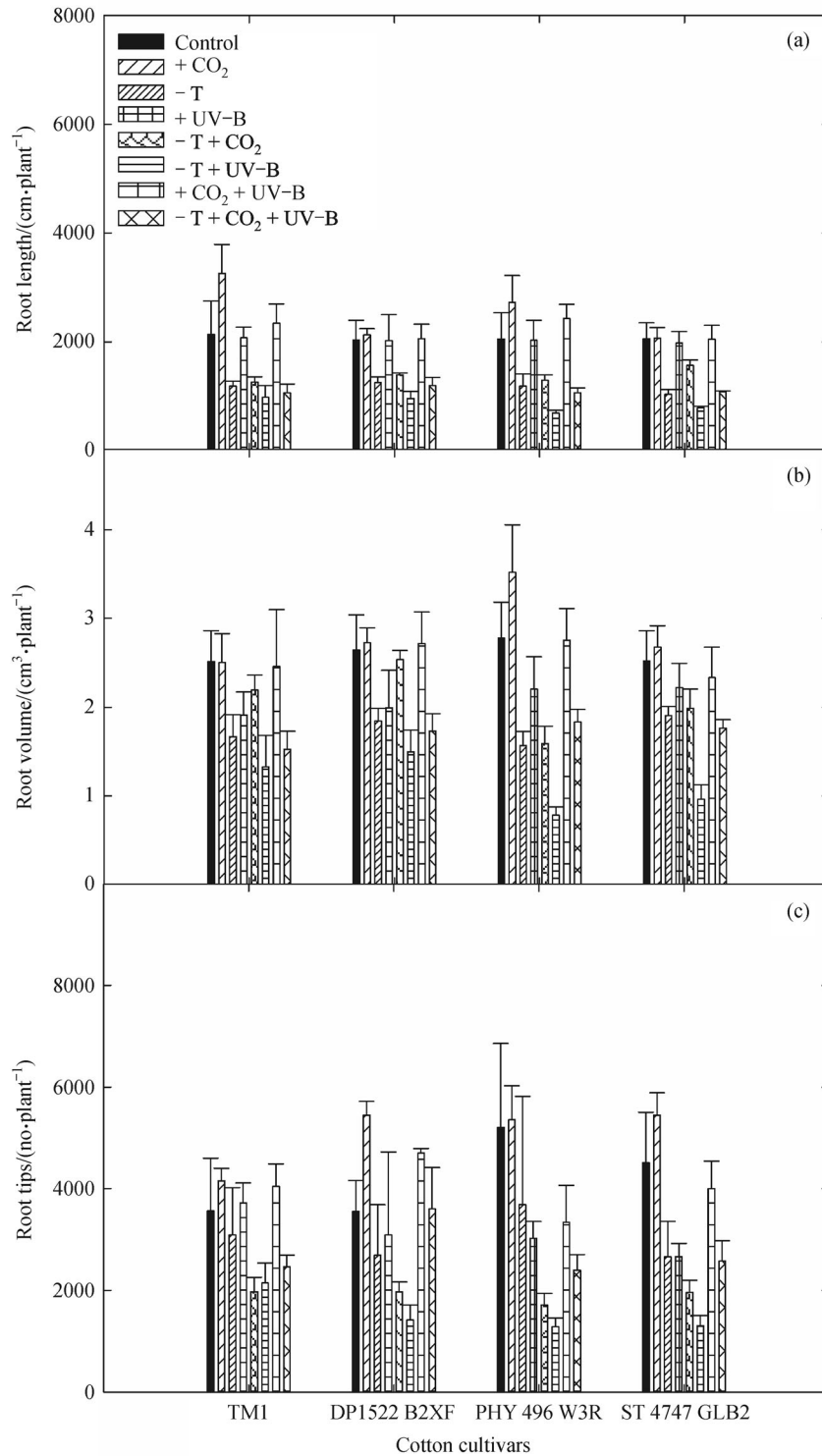


Fig. 2 Impact of CO₂ concentration (control, 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ and + CO₂, 750 $\mu\text{mol}\cdot\text{mol}^{-1}$), low temperature day/night (control, 28/20°C and -T, 20/12°C), and UV-B radiation (control, 0 and + UV-B, 10 $\text{kJ}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$), and their interactions on (a) root length, (b) root volume, and (c) number of tips for four cotton cultivars (TM-1, DP1522 B2XF, PHY496W3R, and ST4747GLB2). Bars indicate standard errors of the mean ± 6 replications.

under $-T + UV-B$ and $-T$ treatments. Elevated CO_2 increased total root length by 23% and 8% either alone or together with $+CO_2 + UV-B$ treatments (Fig. 2a). Even though the combined effect of $+CO_2 -T + UV-B$ reduced the root length by 46% compared to the control, the greatest reduction was observed under $-T + UV-B$, which was about 59%. The average reduction of root length was 44% and 2% under $-T$ or $+UV-B$ treatments, whereas cultivar ST4747GLB2 had the shortest root length under these treatments.

Growth at elevated CO_2 alone significantly increased subsequent root volume from 2% to 9% when averaged across $+CO_2$ treatment (Fig. 2b). Cultivar PHY496W3R showed the greatest increase in root volume, followed by ST4747GLB2, under increased CO_2 treatment. Low temperature significantly affected root volume decreasing it by 40% in ST4747GLB2 and 13% in TM-1 as compared to their control treatments. Treatments with the combination of $-T$ and $+UV-B$ produced a 60% reduction of root volume when compared to the control.

Effects of low temperature on cotton cultivars varied significantly with the number of root tips. A decrease in temperature either alone or combined with $+UV-B$ treatment caused a reduction in root tips (Fig. 2c). Under $-T$ treatment, the reduction of tips varied from 13% (TM-1) to 41% (ST4747GLB2), whereas $-T + UV-B$ treatment caused 75% reduction in PHY496W3R and 40% in TM-1 cultivars. Except for DP1522B2XF cultivar, the interaction of all three stresses, $+CO_2 -T + UV-B$, resulted in a 31% (TM-1), 54% (PHY496W3R), and a 43% (ST4747GLB2) decrease in tips compared to their respective control treatments. The decrease in root tips due to increased $UV-B$ radiation was offset by increased CO_2 for all cultivars. The cultivar DP1522B2XF had the greatest increase for root tips (53%).

4.3 Physiological traits

No significant interactions of $+CO_2 \times -T$ and $+UV-B \times -T$ were observed for the total chlorophyll at 20 DAP (Fig. 3a). However, either $-T$ or $+UV-B$ alone produced significant ($P < 0.001$) reductions in total chlorophyll for any of the cultivars. Compared with plants grown under $-T$, plants grown at $-T + UV-B$ had 3% less total chlorophyll concentration. Elevated CO_2 alleviated the negative effect of $+UV-B$ treatment and it increased chlorophyll by 3% (DP1522B2XF) to 9% (PHY496W3R) with respect to their control treatments. Compared to the control condition, $+CO_2$ alone produced a 10% increase in average total chlorophyll in the three commercial cultivars as well as the genetic standard cultivar tested. The negative effect of low temperature did not negate the increased CO_2 treatment.

A significant difference was observed for $UV-B$ -absorbing compounds (phenolic) under $-T + UV-B + CO_2$ treatment. The increase in phenolic compounds with

elevated $UV-B$ levels was offset by increased CO_2 conditions (Fig. 3b). Except for DP1522B2XF, CO_2 individually or together with $UV-B$ decreased the production of phenolic compared to their respective control treatments. The $-T + UV-B$ treatment created the greatest production of phenolic compounds compared to all other treatments with an increase of phenolic compounds ranging from 19% in DP1522B2XF to 55% in PHY496W3R. The $UV-B$ treatment alone produced an average 28% increase of phenolic content in the leaves, with the greatest increase observed in ST4747GLB2. Among the treatments, the highest increase of phenolic compounds content in cotton leaves was observed in TM-1.

4.4 Classification of cotton cultivars

Results of the principal component analysis of treatment profiles are summarized in Table 3, with the seven different treatments listed in descending order of least harmful to most harmful effects. The PC1 was dominated by $-T$ and $-T + UV-B$ explaining 72% of the variability, followed by PC2, $+CO_2$ and $+CO_2 + UV-B$ treatment for tested cotton cultivars.

Principal component analysis separated the four cotton cultivars associated with the shoot, root, and physiological trait responses into a tolerant, intermediate, and sensitive group (Fig. 4). Over 90% of the variability could be explained using the first two principal components as shown in Fig. 4. The cultivar with the largest loadings on the X and Y axes was DP1522B2XF (loading factors; PC1 and PC2, 0.2178 and 1.6606) in the tolerant quadrant of the PCA plot. The two cultivars, PHY496W3R and ST4747GLB2, classified under the intermediate category, represented higher positive loadings for PC1 (X axis) and lesser loadings towards Y axis (PC2). The TM-1 cultivar separated out into multiple stress sensitive category bearing the smallest loadings for both PC1 and PC2. Despite the tolerant and sensitive cultivars showing a large variation, little difference was noticed in the PHY496W3R and ST4747GLB2 cultivars as determined by PCA.

Fig. 5 shows the distribution of the parameters only under the multiple stress treatment ($+CO_2 -T + UV-B$). Based on all 19 traits measured, the first two PCs are effective in capturing the combined variance of all the traits, thereby removing the effects of scale. The PCA plot was developed in such a way that the score of each trait is plotted reflecting the maximum amount of variance on the first and second principal components (PC1 and PC2). The right side of the plot along the X axis of PC1 there are higher coefficients for LA (2.57), RL (2.56), TD (2.56), RF (2.55), and RC (2.55). Therefore, the higher the PC1 score for a cultivar, the higher the values would be for these traits. Along the Y axis, NOD (4.68), SGR (4.08), PH (4.08), PHOL (3.05), and RT (3.09) show higher coefficients for PC2; therefore PCA technique allowed to

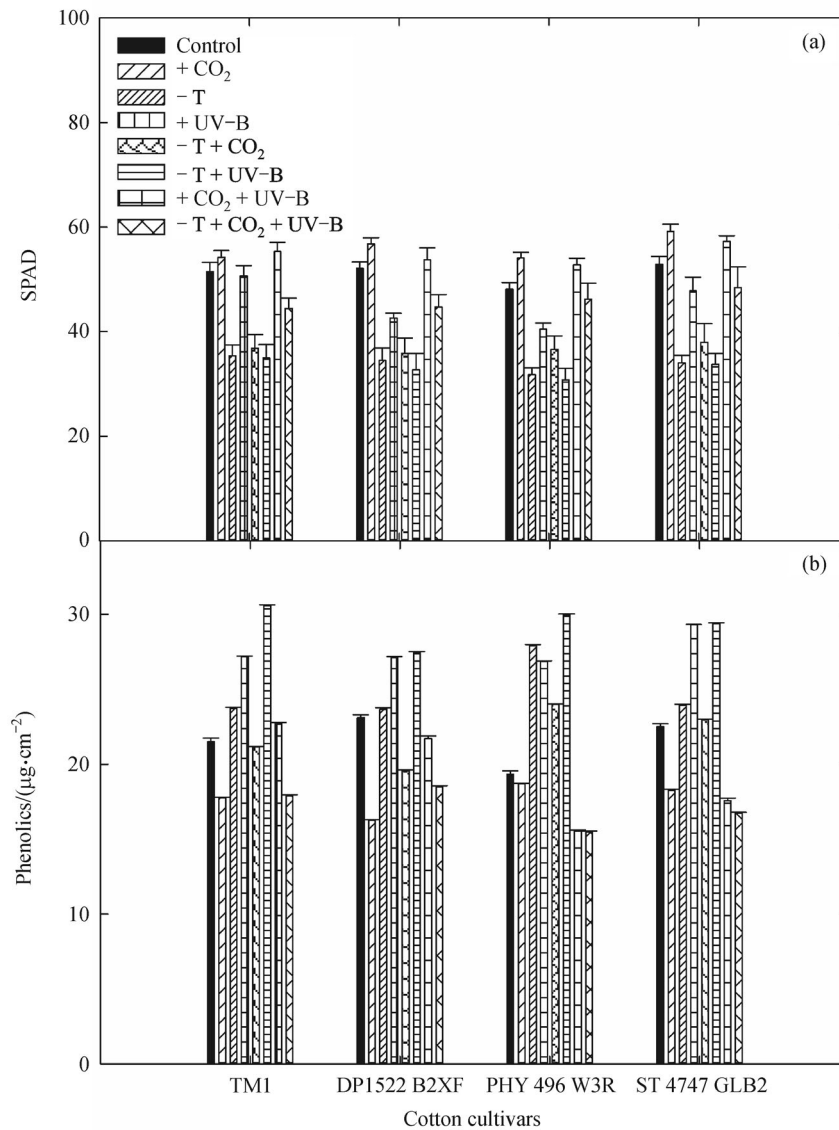


Fig. 3 Impact of CO_2 concentration (control, $400 \mu\text{mol}\cdot\text{mol}^{-1}$ and + CO_2 , $750 \mu\text{mol}\cdot\text{mol}^{-1}$), low temperature day/night (control, $28/20^\circ\text{C}$ and $-T$, $20/12^\circ\text{C}$), and UV-B radiation (control, 0 and + UV-B, $10 \text{ kJ}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$), and their interactions on (a) SPAD, and (b) phenolics, for four cotton cultivars (TM-1, DP1522 B2XF, PHY496W3R, and ST4747GLB2). Bars indicate standard errors of the mean ± 6 replications.

identify the parameters that best describe the multiple stress tolerance based on the relative magnitude of the coefficient of each trait relating it to the first and second principal components. Hence, by analyzing the results in the present study, one can obtain a visual, nonnumeric grasp of the amount of genetic variability that exist across the four cotton cultivars studied.

5 Discussion

The main objective of this study is to identify changes in root morphology and early shoot growth of cotton plants

and the interactive effects of expected future climate changes in low temperature, increased UV-B radiation, and elevated CO_2 environments. Though many studies have reported two factor interactions on cotton vegetative and reproductive growth (Reddy et al., 1995, 1998, 2004; Zhao et al., 2003), no studies to date have reported three factor interactions. Therefore, this study is very instrumental in identifying existing genetic variation that may provide clues for developing new cultivars, which would perform in current and projected future variable climatic conditions. Moreover, this is the first study to investigate multiple environmental stressors on several root and shoot morphological traits during seedling growth stages.

Table 3 Principal component analysis eigenvectors of PC1, PC2, PC3, and PC4 of 4 cotton cultivars for seven different treatments and the variation accounted by each eigenvector.

Treatments	Eigenvectors			
	PC1	PC2	PC3	PC4
+ CO ₂	0.073	0.866	0.121	0.090
+ UV-B + CO ₂	0.360	0.227	-0.590	0.303
+ UV-B	0.394	0.033	0.532	0.114
-T + UV-B	0.445	0.104	0.179	0.205
-T + CO ₂	0.393	-0.287	-0.412	0.246
-T + UV + CO ₂	0.396	-0.314	0.362	0.114
-T	0.448	0.074	-0.149	-0.878

†Treatments are listed in order of top is the best for causing less harmful effects as determined by the first principal component for the root growth and cotton seedling vigor.

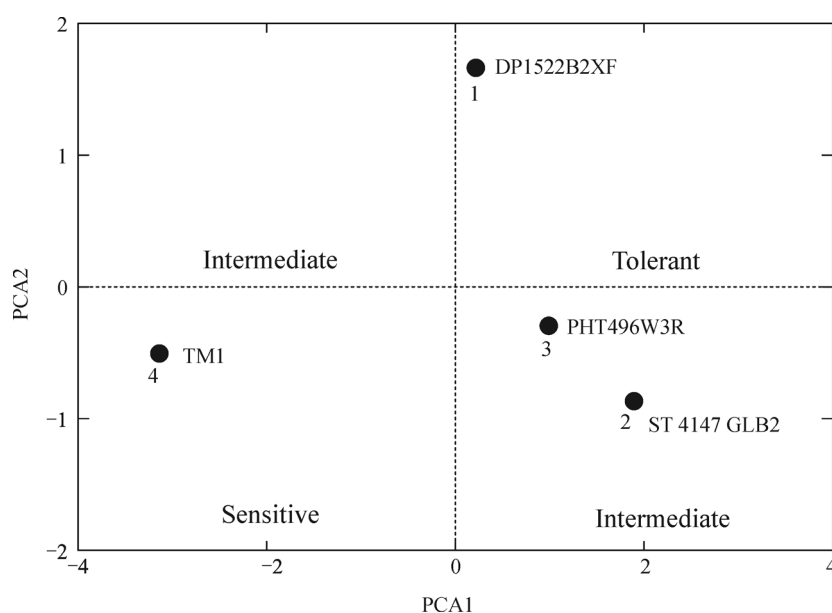


Fig. 4 A plot showing principal component analysis (PCA) for the first two principal component (PC) scores, PCA1 vs. PCA2 related to the classification of 4 cotton cultivars (solid symbols) for multiple stress tolerance. Cotton cultivars were classified mainly into 3 groups as tolerant, intermediate, and sensitive depending on the contribution of all the parameters tested under each of the treatment. One to four numbers given under each of solid circle represent the relative ranking of each hybrid for multiple stress tolerance.

Vegetative growth of all cotton cultivars including root morphology traits was negatively influenced by low temperature and increased UV-B treatment, but it was positively influenced by elevated CO₂. The [CO₂] enrichment increased the total biomass production significantly in all four cotton cultivars tested. Supportive to these findings, Kimball and Mauney (1993) reported a 65% increase in above-ground biomass in cotton on average, by CO₂ enrichment to 650 μmol·mol⁻¹ under open-top experiments. Similar increases in dry matter production and growth under elevated [CO₂] have been observed in soybeans (Koti et al., 2005, 2007), corn (Wijewardana et al., 2016a), and cotton (Reddy et al., 1995, 1998, 2004) in sunlit plant growth chamber conditions. The increase in biomass may be due to

modifications in phenotype, increase in leaf and stem growth (Reddy et al., 1998), and increase in photosynthetic rates (Reddy et al., 1995, 2004). It has been reported in the literature that through a wide range of temperatures favorable to cotton growth, increased [CO₂], encouraged a higher production of carbohydrate than in ambient [CO₂] due to the reduction in photorespiration and faster carboxylation (Reddy et al., 2005). According to Bowes (1996), the present ratio of [CO₂] and [O₂] causes about 35% decrease in photosynthesis of C₃ plants at 25°C through O₂ inhibition and photorespiration. Additionally, he reported that [CO₂] enrichment can reduce photorespiration by 50%, which could lead to greater photosynthetic rates in C₃ plants.

In the present study, enrichment of CO₂ stimulated root

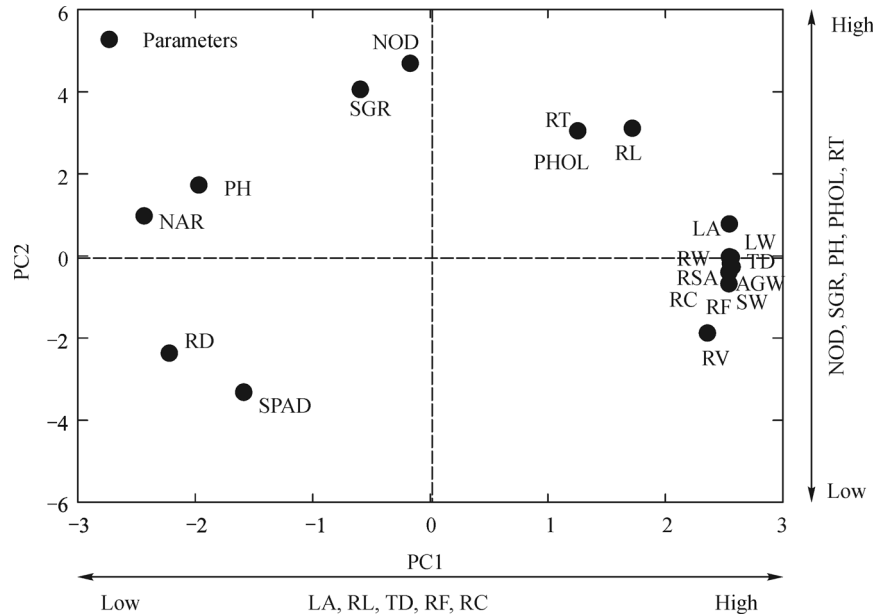


Fig. 5 A plot showing principal component analysis (PCA) for the first two principal component (PC) scores, PC1 vs. PC2 related to the distribution of 19 parameters (solid circles) for multiple stress tolerance. The coefficients (PC1 and PC2) for the parameters are distributed across the ordination space that reflects the contribution of the parameters in determination of multiple stress tolerance. The coefficients used in the analysis were seed germination rate (SGR), plant height (PH), node number (NOD), node addition rate (NAR), leaf area (LA), leaf weight (LW), shoot weight (SW), root weight (RW), total dry weight (TD), root/shoot ratio (RS), root length (RL), root surface area (RSA), root diameter (RD), root volume (RV), number of root tips (RT), number of root forks (RF), number of root crossings (RC), total chlorophyll (SPAD), and phenolic content (PHOL). The coefficient values for parameters were multiplied by ten to obtain a clear and superimposed figure.

growth while low temperature and +UV-B either individually or in combination, suppressed most root traits. Previous root studies on soybeans (Rogers et al., 1992b), cotton (Prior et al., 1994; Reddy, 1997), and sorghum (Pritchard et al., 2006) have reported significant increases in root diameter, root length, and dry weight densities due to CO₂ enrichment. Along with scanned root images of control treatments, there is a clear evidence that the variability exists between the tolerant DP1522B2XF and sensitive TM-1 cotton cultivars to either single, two, and three factor interactions of +UV-B, +CO₂, and -T on root growth (Fig. 6). Under elevated atmospheric [CO₂]-enriched treatments, roots were longer, thicker and highly branched compared to the root systems in the respective control treatments. With respect to TM-1 (multiple stress sensitive) root system, DP1522B2XF (multiple stress tolerant) cotton cultivar had a prolific and more abundant root structure (Fig. 6) under all the treatments. This implies that compared to TM-1 cultivar, DP1522B2XF may ensure a greater absorption of accessible nutrients and water during plant establishment. Low temperature, combined with increased UV-B radiation, produced shorter root length and poorly branched root systems in all cultivars (Fig. 6). Earlier studies reported 35–36°C (Arndt, 1945; McMichael and Burke, 1994) as the optimum temperature for the growth of cotton roots during the early seedling development. Low temperature can affect growth in a number of root

components such as reduced root elongation rate, lateral root development, root branching, and root dry matter accumulation (Kasper and Bland, 1992). Reduced import of photosynthetic compounds from the shoots may restrict growth and development of roots under low temperature. Sanders and Markhart (2001) reported that under that low temperatures reductions in water and nutrient uptake in root systems, probably due to lower activities of enzymes associated with changes in the structure of membrane lipids in cotton roots. This will create major implications for crop performance because of the reduction in water movement through the roots which, in turn, causes decline in photosynthesis and thus affecting plant component dry matter accumulations.

This study confirmed previous reports (Zhao et al., 2003; Nedunchezian and Kulandaivelu, 1996; Mark and Tevini, 1996) in that UV-B radiation reduced the canopy size by decreasing leaf area, stem extension, and branch length. While higher UV-B radiation increased phenolic accumulation, total biomass production and chlorophyll synthesis were also affected. Differences in accumulation of phenolic are dependent on both UV-B irradiance levels and genotype (Kakani et al., 2004). The highest amount of phenolic concentration was recorded in TM-1 leaves exposed to +UV-B-T treatment and lowest was for PHY496W3R under +UV-B radiation alone. Even though these cultivars were intermediate and sensitive with respect to multiple stresses, they may be tolerant to

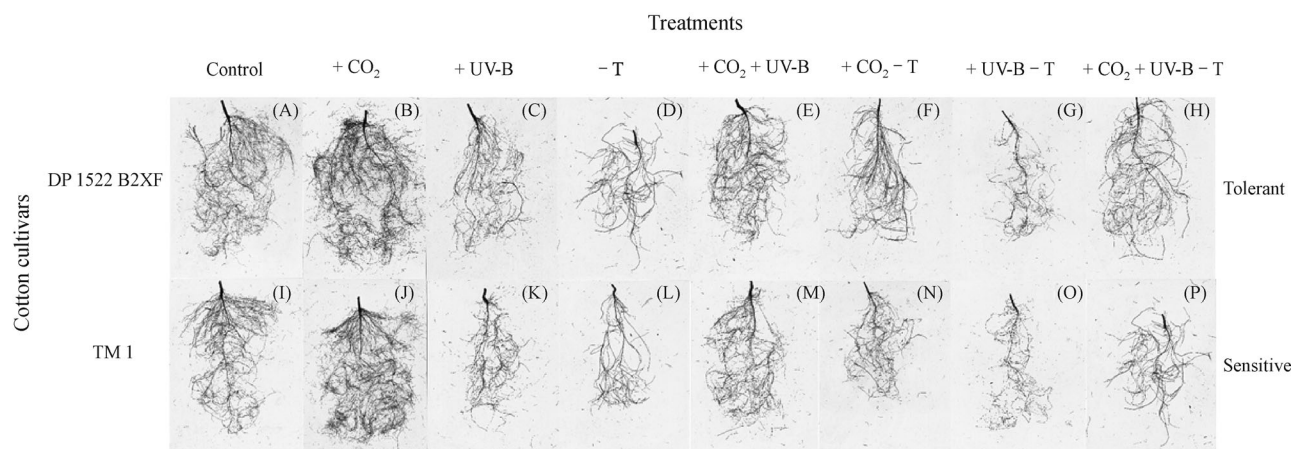


Fig. 6 Representative scanned root images from single and combined stress treatments for multiple stress tolerant DP1522B2XF and sensitive TM-1 cotton cultivars harvested at 20 d after planting. Images represent (A and I) control condition (CO_2 –400 $\mu\text{mol}\cdot\text{mol}^{-1}$, T –28/20°C, and UV–B, 0 $\text{kJ}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$), (B and J) + CO_2 (750 $\mu\text{mol}\cdot\text{mol}^{-1}$), (C and K) + UV–B (10 $\text{kJ}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$), (D and L) –T (20/12°C), (E and M) + CO_2 + UV–B, (F and N) + CO_2 – T, (G and O) + UV–B – T, and (H and P) + CO_2 + UV–B – T.

increased UV–B doses. A decreased total dry weight was observed under increased UV–B radiation. This lack of total dry matter may be attributed to reduction in leaf area expansion, lower photosynthesis and diverting photoassimilates to UV–B screening compounds under increased UV–B conditions. Exposure to UV–B radiation resulted in a loss of chlorophyll similar to that of plants exposed to low temperature. This can be attributed to the breakdown of structural integrity of chloroplasts (Tevini et al., 1991). Compared to other treatments, leaf chlorophyll showed a greater reduction after exposure to elevated UV–B radiation and –T treatments. This suggests that cotton leaves are more sensitive to + UV–B–T than treatments with increased CO_2 levels.

The CO_2 enrichment increased plant height, leaf area, total dry weight, total root length, volume, and dry weight respectively, either alone or combined with UV–B stress. This suggests that CO_2 enrichment could reduce adverse effects of increased UV–B radiation in cotton during seedling development and growth stages. However, increased CO_2 could not offset the negative effects of low temperature. This implies that most cotton growth and developmental events are highly sensitive to low temperature stress, as compared to increased UV–B and CO_2 . Further research is needed to confirm this in additional cotton cultivars and other crop species.

6 Conclusions

Low temperature was the major influencing factor on root morphology and shoot growth of cotton cultivars. Elevated UV–B exposure and low temperature caused significant reduction in most shoot, root and physiological parameters. This indicates that low temperature and + UV–B could be

major environmental stressors that impact cotton growth and development during the early-season in the US Cotton Belt. The interaction between low temperature and UV–B radiation increased the accumulation of phenolics, the UV–B screening compounds in the leaves, diverting the photoassimilates away from growth. The adverse effects of + UV–B irradiance on cotton vegetative traits may be diminished by rising atmospheric [CO_2] in the predicted changing climate. Based on the principal component analysis, leaf area, root length, total plant dry weight, and numbers of root forks and crossings best described the variability among the 19 traits measured under multiple stress conditions. Moreover, based on PCA analysis, cultivar DP1522B2XF was identified as tolerant, PHY496W3R, and ST4747GLB2 as intermediately tolerant, and TM-1 as sensitive to multiple environmental stresses. Given that genotypic differences were observed among the cotton cultivars examined suggests that existing genetic variation may provide opportunity for improvements in early seedling growth traits. Improved cultivars able to withstand multiple stress environments would produce a more vigorous canopy and a better root system which could lead to greater yield stabilization.

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