RESEARCH

Overexpression of Glutathione Reductase in Cotton Does Not Alter Emergence Rates under Temperature Stress

James R. Mahan,* Dennis C. Gitz, III, Paxton R. Payton, and Randy Allen

ABSTRACT

Cotton (*Gossypium hirsutum* L.) seedlings are considered to be sensitive to temperature stress. The exposure of plants to high and low temperatures can result in the production of reactive oxygen species that contribute to diminished plant performance. Plants have mechanisms that metabolize oxidants into less harmful chemicals, and enhancement of antioxidant metabolism has been shown to be beneficial to plants exposed to acute oxidative stresses. Transgenic overexpression of glutathione reductase (GR) in cotton could result in faster seedling emergence at nonoptimal temperatures. Transgenic GR and 5 nontransgenic cotton lines were planted at four dates in the field in Lubbock, TX, and in constant temperature growth boxes. The temperatures in the field were typical for the cotton planting period in the region, and in growth boxes ranged from 15 to 45°C. Emergence rates were determined for all lines, and the activity of GR and the levels of malondialdehyde in the cotyledons were monitored to verify altered enzyme activity and to detect differences in oxidative damage, respectively. Enhancement of GR activity did not improve emergence or reduce oxidative damage. It is concluded that GR activity did not limit emergence.

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Abbreviations: C312, Coker 312; DOY, day of year; DRR, De Ridder Red Leaf; FM800, Fibermax 800; FM989, Fibermax 989; GR, glutathione reductase; GR+, glutathione reductase overexpressed cotton; GSSG, oxidized glutathione; MDA, malondialdehyde; PM2326, Paymaster 2326; ROS, reactive oxygen species; TBA, thiobarbituric acid.

Early-season low temperatures are thought to limit seedling performance in many crops. This is particularly true for cotton (*Gossypium hirsutum* L.) on the High Plains of Texas. While delaying planting can reduce low temperature stress on the seedlings, a number of factors are responsible for the general interest in planting cotton when low temperature stress is probable. A longer growing season, lower water demand during the early season, and increased opportunity to utilize early rainfall all represent advantages of earlier planting.

Environmental variability experienced by cotton seedlings on the High Plains of Texas is such that early planting dates include temperatures that cover the range from suboptimal to supraoptimal. Juxtaposed to the benefits of early planting, the exposure of the plant to nonlethal lower temperatures generally results in a longer interval between planting and seedling emergence. Delayed emergence can be problematic because increased seedling mortality is positively correlated with longer emergence times (Kerby et

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al., 1996). Thus, the producer must balance the positives and negatives of early planting.

The exposure of plants to high and low temperatures can result in the production of reactive oxygen species (ROS) that contribute to diminished plant performance (Noctor and Foyer, 1998; Grill et al., 2001). Oxidative stress describes the adverse effects of ROS on plants. A variety of enzymatic and nonenzymatic mechanisms exist to metabolize ROS into less harmful chemical species. Recent reviews by Foyer (2001) and Blokhina et al. (2003) summarize much of the current knowledge of antioxidant metabolism in plants. Oxidative damage has been associated with low temperature stress in plants both in the dark (pre-emergence) and in the light (postemergence) (Purvis and Shewfelt, 1993; Anderson et al., 1995; Rikin et al., 1997; Munroa et al., 2004). Diminished performance of cotton seedlings exposed to suboptimal temperatures has been associated with increased oxidative stress (Payton et al., 2001).

The metabolism of oxidative compounds in cotton is accomplished through the coordinated action of a number of enzymes and metabolites as mentioned previously. The system is robust and, in general, quite effective at preventing and alleviating oxidative damage. Antioxidant metabolism in cotton has been the subject of several research studies that have focused on key enzymes involved in antioxidant metabolism: L-ascorbate peroxidase (EC 1.11.1.11), glutathione reductase (EC 1.6.4.2), and superoxide dismutase (EC 1.15.1.1) (Logan et al., 2003; Light et al., 2005). Payton et al. (2001) and others have investigated the possibility that increasing the activity of enzymes involved in antioxidant metabolism could serve to enhance cotton performance in nonoptimal thermal environments. The results of these efforts indicated that under controlled stress conditions, the overexpression of antioxidant enzymes could result in improvement in the performance of the plant. Other research suggests that enhancements might not be as effective under field conditions (Logan et al., 2003).

Malondialdehyde (MDA) is an indicator of oxidative-stress-related peroxidation of membrane lipids. The concentration of MDA has been shown to correlate with peroxides resulting from temperature and water-stress-related membrane damage (Halliwell and Gutteridge, 1986).

The hypothesis of this study was that transgenic overexpression of glutathione reductase in cotton will result in faster seedling emergence at nonoptimal temperatures in the field. Transgenic cotton overexpressing the antioxidant enzyme glutathione reductase (GR) was planted at four dates in the field in Lubbock, TX, to assess the effect of the enhanced antioxidant activity on seedling emergence under variable temperatures. The wild type of the transgenic variety and four nontransgenic varieties were also included in the study for comparative purposes. Seedling emergence was also monitored under controlled temperature conditions in the laboratory. The activity of GR and the levels of MDA in the cotyledons were monitored to verify altered enzyme activity and to detect differences in oxidative damage, respectively.

MATERIALS AND METHODS

Plant Material

Transgenic cotton plants (*Gossypium hirsutum* L. cv. Coker 312) that expressed GR from *Arabidopsis thaliana* (L.) Heynh. were used in this study. Wild-type Coker 312 (C312) served as the control for comparisons. A mutated GR cDNA was amplified from *Arabidopsis* leaf mRNA using primers designed from a cDNA described by Kubo et al. (1993). The predicted polypeptide contains a 74-amino-acid N-terminal leader sequence that has features of chloroplast-targeting peptides. The mutated cDNA was developed with an *Nco*I site immediately preceding the transcription start site (AGATGG to CCATGG) to allow for ligation into the tobacco etch virus leader sequence start codon in the pRTL2 expression vector. The coding sequence was under the control of a cauliflower mosaic virus 35S promoter. Transgenic cotton plants were produced by hypocotyl inoculation with *Agrobacterium tumefaciens* and regeneration via somatic embryogenesis. Kanamycin-resistant plants were regenerated essentially as described by Horsch et al. (1985). To determine whether significant differences existed among the independently transformed lines, a Kruskal–Wallace nonparametric ANOVA was performed with respect to total leaf GR activity and photosynthetic rate (Sokal and Rohlf, 1995; Payton et al., 2001). No significant differences were seen ($\alpha = 0.05$) among six independently transformed lines ($n \geq 5$ plants line^{-1}). Seed used for this study represents bulked lot from all independent lines.

For comparison, the following three commercial varieties common for the region were planted: Fibermax 989 (FM989), Fibermax 800 (FM800), and Paymaster 2326 (PM2326). Additionally, De Ridder Red Leaf (DRR), a circa-1950s obsolete cultivar (Rhyne, 1954) exhibiting enhanced foliar anthocyanin content, was also evaluated based on the observation that anthocyanin expression is often correlated with low temperature tolerance (Thomashow, 1999) and to take advantage of the little remaining potential genotypic diversity contributed by earlier developed lines (Iqbal et al., 2001). All seeds were treated with fungicide (Captan; Arysta LifeScience, Cary, NC) before planting. Seeds of all six lines were analyzed in field plantings where temperature was variable and in a constant-temperature system in the laboratory.

Field Study Planting Dates

Four planting dates were used in this study: 18 April (day of year [DOY] 108), 27 April (DOY 117), 10 May (DOY 130), and 23 May (DOY 143). The dates were chosen to provide for the occurrence of less than optimal temperatures and higher than optimal temperatures during the emergence period. The first and second plantings represent earlier than normal planting dates, while the third and fourth plantings are representative of typical planting dates for the region.

Seedling Emergence in the Field

Emergence was defined as the protrusion of the cotyledon above the soil surface. Cotton was planted in seedbeds on a 1-m row spacing in Lubbock, TX, at latitude 33°35.6′ N, longitude 101°54.1′ W. The soil series for the area is an Amarillo sandy loam

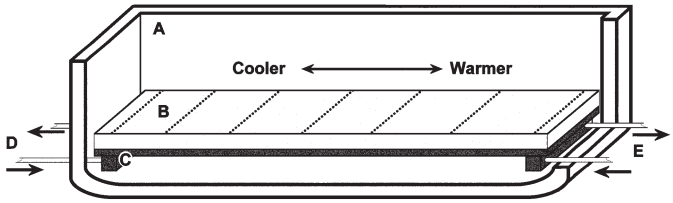

Figure 1. Cutaway schematic of temperature gradient apparatus used in cotton seedling emergence experiments. (A) Commercially available thermoplastic urethane foam core insulated chest-type cooler. For clarity, the lid is not shown. (B) Water-saturated peat mix. Dotted lines represent isotherms that develop along the length of the plate into which seeds were planted. (C) Aluminum plate with hollow headers into which constant-temperature water was introduced. (D) Flow from constant-temperature cold-water source through header at one end of gradient plate. (E) As for D but from warm-water source. Note the direction of temperature gradient developed across the plate is shown on the back wall of the insulated chest.

(fine-loamy, mixed, superactive, thermic Aridic Paleustalfs). Each cotton line was planted in randomized block with four replicates. Each replicate consisted of a row of 1-m length for each line. Seeds were planted by hand at a depth of 5 cm and spaced 10 cm apart. Approximately 25 mm of water was applied by surface drip irrigation (sufficient to wet the upper 10 cm of soil) on the day of planting provided moisture for seedling establishment. Plots were irrigated weekly following planting with 25 mm of water (no rain fell during the experimental interval). Plots were cultivated by hand following irrigations to prevent crusting of the surface. The number of emerged seedlings was determined daily for 15 d.

Seedling Emergence at Constant Temperature

Emergence was defined as the protrusion of the cotyledon above the soil surface. Defined constant temperatures were generated using a temperature gradient plate technique (Fig. 1) essentially described by Walter Heydecker (Garcia-Huidobro et al., 1982). Briefly, the temperature gradient plate consists of an aluminum plate (1 m by 0.5 m), which is heated at one end and cooled at the other by pumping constant-temperature water through manifolds at either end of the plate. The underside of the plate is insulated with closed-cell insulation and the apparatus is placed in a closed, plastic, foam-filled chest-type cooler. To initiate an experiment, the plate was covered with a commercial potting soil (Sunshine #1; Sun Gro Horticulture Canada Ltd., Vancouver, BC) mix to a depth of 5 cm, the peat mix brought to field capacity with several irrigations. The soil temperature was measured with a thermocouple along the length of the plate at a depth of 2 cm over a few days until a stable linear temperature gradient (~12 to ~50°C) formed along the length of the plate. Zones of constant temperature from 15 to 45°C (±1°C) were defined in each box perpendicular to thermal gradient, and seeds were planted at 2-cm depth along these constant temperature zones. Thirty seeds each of two cotton lines were planted at each temperature on three dates in a minimum of two different temperature boxes. Boxes were closed at planting to provide darkness and were opened daily, under low-light conditions, for <2 min to count emerged seedlings. Emergence was recorded daily for up to 15 d, with *no emergence* denoted as >15 d. In this experiment, each gradient plate represents a block of discrete temperature treatments (plots), replicated either with multiple gradient plates or in separate runs (temporally). The four boxes used in the study were located in a controlled temperature room (22 ± 2°C).

Percent Germination

To investigate whether differences in germination percentage could mask results seen in emergence and to ensure that seedling vigor was not a factor related to the percent germination of the seed lots used in this study, we examined germination in controlled environment chambers (model I-30NL; Percival Scientific, Perry, IA) by the method described by Williams et al. (2000). Briefly, six replications of 10 seeds each were imbibed and germinated in 3-mm-filter paper towels, rolled, and incubated at a cycling temperature of 20°C for 16 h and 30°C for 8 h. Germination counts were taken 1 wk after imbibition. Germination data were analyzed by a one-way ANOVA to determine whether significant differences existed between any of the lines.

Temperature Monitoring

Soil temperature in the field was monitored at 5 cm below the soil surface with a thermistor temperature probe (TMCx-HD Onset Computer, Pocasset, MA). The temperature was measured every 5 min over the experimental interval. Under constant temperature conditions in growth boxes, the temperature at 2-cm seed depth was monitored with a thermocouple at each measurement.

Statistical Analysis of Emergence

All data were analyzed by selected routines within the general linear model set (proc GLM;) in SAS version 9.1 (SAS Institute, Cary, NC. Cotton cultivar effects on days to 50% emergence were evaluated by Dunnet's multiple range test with Bonferroni correction assuming statistical significance at the 0.05 level (means cultivar/bon;). Effect of GR overexpression was additionally evaluated by multiple range test using C312 as a control, with Bonferroni correction to control experiment-wide error (means line/deponly DUNNETT ('C312') alpha = .05;). To fully assess differences between cultivars at each planting date, means were obtained with LSMEANS with separation by Tukey's Honestly Significant Difference range procedure (lsmeans line/adjust = tukey pdiff;). Cultivar effects on seed box emergence were similarly evaluated at each temperature.

Plant Sampling

Seedling tissues for MDA determinations were sampled 2 d after emergence. Cotyledons of emerged seedlings (three plants from each of four plots) were collected in the field and immediately frozen in liquid nitrogen and stored at −80°C before extraction and assay. Glutathione reductase activity was determined in cotyledons of seedlings 2 d after emergence following planting in the greenhouse in a commercial potting soil (Sunshine #1; Sun Gro Horticulture Canada Ltd.).

Glutathione Reductase Extraction

Frozen tissue (0.1 g) was placed in a 2-mL flat-bottom microcentrifuge tube with four tungsten steel balls (three of 3-mm diam., one of 5-mm diam.). Tubes were placed in a liquid nitrogen bath

and repeatedly agitated on a vortex mixer until the plant material was ground to a fine powder. The powder was homogenized with 20% (w/w) polyvinylpolypyrrolidone and 1 mL of the extraction buffer containing 0.1 mM ethylenediaminetetraacetic acid, 0.2% (v/v) Triton X, and 2% (w/v) polyvinylpyrrolidone in 0.1 M Tris-HCl (pH 7.0) until the solution was thawed. The extract was decanted and centrifuged at 15,000 × *g* for 2 min in a microcentrifuge. The supernatant was placed on ice and used immediately for enzyme assays. The small size of the extract made desalting the extract impractical.

Glutathione Reductase Assay

The assay for GR was modified from Gossett et al. (1994). A 1.0-mL assay contained 0.5 mM of oxidized glutathione (GSSG) and 0.15 mM of reduced nicotinamide adenine dinucleotide phosphate (NADPH) in 0.05 M Tris-HCl (pH 7.5). The assay was initiated with the addition of 0.05 mL of leaf extract. If the activity in the extract was >0.03 units, the extract was diluted with buffer (0.05 M Tris-HCl [pH 7.5]) and assayed again. Enzyme activity was monitored by the decrease in absorbance at 340 nm at 25°C for 30 s and the initial rate determined. To ensure the oxidation of the NADPH was due to GR, we used a control assay omitting the GSSG. A unit of activity is the amount of enzyme that will catalyze the reduction of 1.0 μmol of GSSG min^{-1} at 25°C.

Malondialdehyde Extraction

Frozen tissue (0.1 g) was homogenized as described above for glutathione reductase extraction. The powder was homogenized with 1.5 mL of 85% ethanol until the solution was thawed. The extract was decanted and centrifuged at 15,000 × *g* for 2 min in a microcentrifuge. The supernatant was used immediately in the MDA assays.

Malondialdehyde Assay

The lipid peroxidation assays were performed according to a modification of the method of Hodges et al. (1999), which corrects for the presence of other compounds that absorb at 532 nm. The extract (0.5 mL) was reacted with 20% (w/v) trichloroacetic acid, 0.01% (w/v) butylated hydroxytoluene, and 0.65% (w/v) thiobarbituric acid (TBA) at 95°C for 25 min in a total volume of 1.5 mL. A control reaction consisted of the reaction mixture with water substituting for the TBA. The assay mixture was cooled to room temperature and centrifuged (15,000 × *g*) for 10 min. The absorbance of each sample was determined at 440 nm, 532 nm, and 600 nm. The concentration of MDA was calculated using the following equations as developed by Hodges et al. (1999):

$$A = [(\text{Abs } 532_{+TBA}) - (\text{Abs } 600_{+TBA}) - (\text{Abs } 532_{-TBA} - \text{Abs } 600_{-TBA})]$$

$$B = [(\text{Abs } 440_{+TBA} - \text{Abs } 600_{+TBA}) \times 0.0571]$$

$$\text{MDA equivalents (nmol/mL)} = (A - B/157{,}000) \times 10^6$$

RESULTS

Thermal Environments

The field planting dates covered an interval from earlier than normal to normal for the region. It is important to note that the focus of this study was on the effects of nonlethal temperatures on the seedlings in the field. Constant temperature emergence in the study was measured across the range of temperatures representative of those experienced by seedlings in the field and at the upper and lower extremes included temperatures that were lethal for the seedlings.

Soil temperature during the study period is shown in Fig. 2. For comparative purposes among plantings, soil temperatures were categorized as suboptimal (<26°C), optimal (26–36°C), and supraoptimal (>36°C) as previously proposed on the basis of optimal function of a cotton enzyme by Mahan and Gitz (2007). The shaded section of the Fig. 2 indicates the range of optimal temperatures.

The observation interval was divided into four planting periods for analysis. Each period began on the planting date and included a subsequent 15-d interval. In general, the thermal environment became progressively warmer across the four planting dates with respect to the fraction of optimal temperatures and mean temperature. Table 1 indicates the distribution of temperatures over all of the planting periods. The temperature variation observed over the experimental interval was typical for the cotton planting period in the region, with a mixture of suboptimal, optimal, and supraoptimal temperatures.

Transgenic Enhancement of Glutathione Reductase Activity

The GR activity was increased approximately 25-fold in the glutathione reductase overexpressed cotton (GR+) line compared with the nontransgenic lines (Fig. 3). Glutathione reductase activity in nontransgenic lines was in the range previously reported (Mahan and Mauget, 2005; Mahan and Wanjura, 2005). While altered antioxidant metabolism can diminish the effects of acute oxidative challenges under controlled stress conditions (Foyer et al., 1995; Allen et al., 1997; Roxas et al., 2000; Payton et al., 2001), their ability to improve performance in the field is less well established (Logan et al., 2003). Mahan and others (Mahan and Mauget, 2005; Mahan and Wanjura, 2005) investigated antioxidant metabolism in cotton in the field in response to chronic water deficits. Seedling antioxidant metabolism was found to be variable over time, though the variation was not clearly associated with low or high temperature and indicated a sufficiency of antioxidant metabolism.

Seedling Emergence in the Field under Variable Temperatures

While the pattern of temperatures (Fig. 2) indicates the potential for both high and low temperature stress on metabolic and structural levels, none were lethal to emerged seedlings. Lethal effects aside, the question remains as to the extent to which nonoptimal temperatures result in oxidative damage. Enhanced antioxidant activity could be predicted to provide protection against both low and high temperature oxidative damage. Two possible indicators of

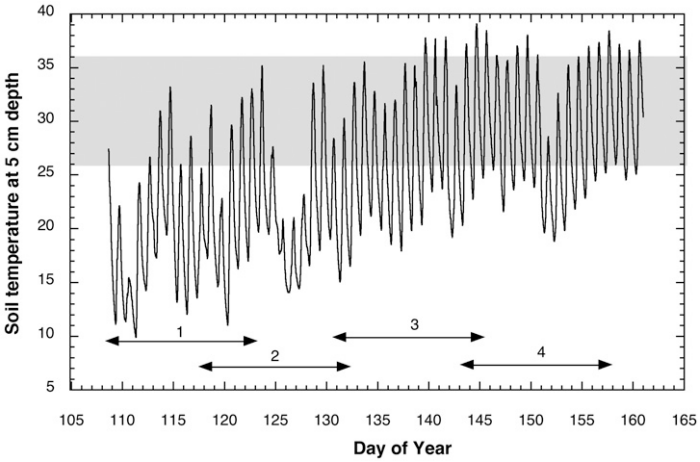

Figure 2. Soil temperatures (°C) at 5-cm depths during the experimental interval. Arrows indicate cotton planting dates and observation periods. Shaded portion indicates optimal temperature range (26–36°C).

Table 1. Soil temperatures at 5-cm depth during cotton planting periods 1 through 4 over the experimental interval (number in parentheses is planting date). Categories of suboptimal (subopt), <26°C; optimal (opt), 26 to 36°C; and supraoptimal (supraopt), >36°C indicate fraction of 15-d period within each temperature (temp.) range.

Planting period†	Subopt	Opt	Supraopt	Mean temp.	Min. temp.	Max. temp.
	% of time			°C		
1 (DOY 108)	80	19	1	20	10	34
2 (DOY 117)	75	23	2	22	10	35
3 (DOY 130)	49	41	10	27	14	39
4 (DOY 143)	33	52	15	29	19	37

†DOY, day of year.

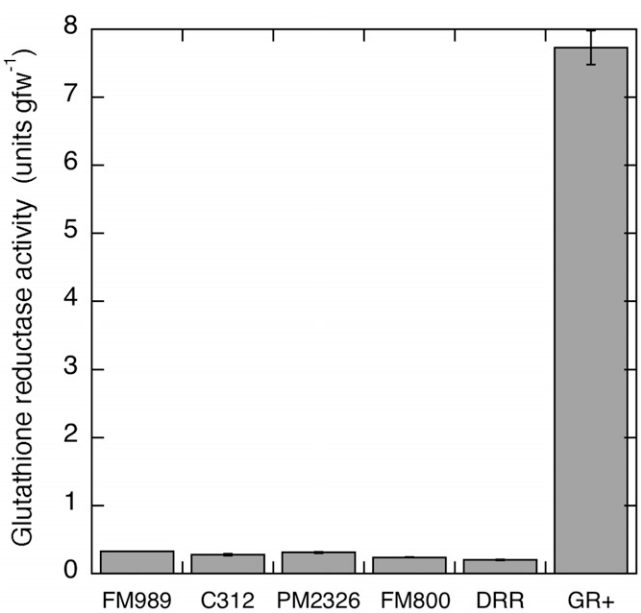

Figure 3. Glutathione reductase activity in cotyledons collected from greenhouse-grown cotton plants 2 d after emergence. Error bars indicate the standard error of the mean. C312, Coker 312; DRR, De Ridder Red Leaf; FM800, Fibermax 800; FM989, Fibermax 989; GR+, glutathione reductase overexpressed cotton; PM2326, Paymaster 2326.

enhanced oxidative protection are faster emergence and reduced production of MDA, a commonly used indicator of oxidative damage.

The time required for emergence in the field varied with planting date in all the cotton lines of the study (Fig. 4). Emergence within a line was similar for all cotton lines, with the variation within treatments relatively small. Emergence times among the lines appear similar with the slowest for all lines associated with Planting Date 1 (earliest planting) and the fastest with Planting Date 4 (latest planting). Emergence times varied significantly with planting date for all cultivars (ANOVA, $P < 0.001$). The fastest emergence time was associated with the DRR line for Planting Dates 1, 2, and 4 while shortest emergence time was spread among three lines: C312 (Planting Date 1), FM989 (Planting Dates 2 and 3), and GR+ (Planting Date 4). Within each planting date, the range among lines from shortest to longest emergence time was approximately 2 d. The patterns of field emergence indicate that the enhancement of GR activity in the transgenic line (GR+) did not result in a functionally significant reduction in emergence time under field conditions.

Oxidative Damage (MDA Levels) in the Field

The amount of MDA in cotyledon was sampled at seedling emergence (Fig. 5). The amount of MDA varied among the sampling dates, with the highest values associated with the earliest (and coolest) sampling and the lowest at the final (and warmest) planting date. The range of MDA content (between 20 and 60 nmol g^{-1} fresh wt.) and pattern of variation are comparable with those previously reported by Mahan and Mauget (2005). The variation in MDA in the GR+ line is not fundamentally different from that in the other lines. The increase in GR activity by expression of a transgene in the GR+ line apparently did not reduce oxidative damage (measured as MDA content).

Seedling Emergence under Constant Temperatures

Continuously variable temperatures in the field might mitigate the effects of temperature-related oxidative stress because stressful temperatures were invariably followed by an interval of optimal temperatures. Transient exposure to stress temperatures followed by nonstress temperatures may prevent detectable oxidative damage. In an effort to address this possibility, the emergence of the lines was assessed over a range of constant temperatures in a controlled temperature environment in which there was no respite from temperature-related damage.

Emergence rate varied temperature for all the lines (Fig. 6) over the gradient of 15 to 45°C. The percent emergence at 30°C for the six lines was determined to be C312, 77%; FM800, 84%; FM989, 98%; PM2326, 97%; GR+, 77%; and DRR, 70%. The time interval for

50% emergence and percent emergence are not correlated ($r = 0.07$), indicating that differences in emergence time were not an artifact of reduced emergence percentage among the cotton lines. All lines have identifiable optimal and nonoptimal temperatures with respect to emergence that can be assessed in terms of the following parameters: the minimum days required for 50% emergence; the temperature associated with the minimum emergence time (optimal temperature); the range of optimal emergence temperatures; and, finally, the optimal temperature range for emergence (2× increase in time compared with minimum). Table 2 summarizes the thermal dependence results. The thermal dependencies among the lines are similar at less than optimal temperatures. Four lines, FM800, DRR, C312, and GR+, had an optimal range with a width ≥15°C and that extended to a maximum of 40°C, while the PM2326 and FM989 showed a narrower range optimal temperatures (≤12°C) that did not extend above 35°C. While the differences among the lines are statistically significant at constant temperatures 35°C and above ($P < 0.0001$), the GR+ and C312 were remarkably similar. In this study there was no functionally significant reduction in emergence time under constant temperature regimes that could be associated with the enhancement of GR activity in the transgenic line.

Comparison of Field and Constant Temperature Emergence Results

Among all the lines the minimum emergence times were similar under constant temperature and field conditions, with emergence times varying by 1.2 d (from 1.8 to 3 d) under the constant temperature and by 1.8 d (from 3 to 4.8 d) under field conditions. The maximum emergence times were 4 d (from 9 to 13 d) under constant temperature conditions and 1.75 d (from 8.75 to 10.5 d) under field conditions.

The lines PM2326 and FM989 showed contrasting responses between the field and constant-temperature portions of the study. Under field conditions the PM2326 emerged quickly in all four plantings, while the FM989 emerged slowly at all four planting dates. This is in contrast to the constant-temperature emergence results in which the PM2326 and FM989 were similar in terms of a narrower optimal range for emergence. To ensure that differences in the thermal dependence of germination among the lines (GR+ and C312 in particular) did not mask differences in emergence, the germination of all lines was determined (Table 2). Percent germination, as measured according to Williams et al. (2000), exhibited no significant difference between the lines (ANOVA, $P = 0.61$), indicating that germination did not mask differences in emergence. Thus, while differences were evident in response of the lines within and between constant temperature and field

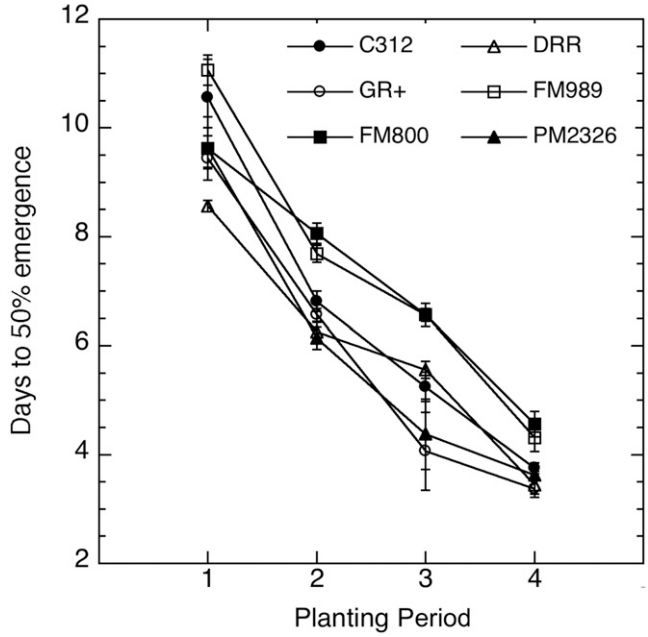

Figure 4. The time required for 50% emergence for each of four cotton planting dates in the field. Each value represents the mean of 4 replications of each planting. Error bars indicate the standard error of the mean. C312, Coker 312; DRR, De Ridder Red Leaf; FM800, Fibermax 800; FM989, Fibermax 989; GR+, glutathione reductase overexpressed cotton; PM2326, Paymaster 2326.

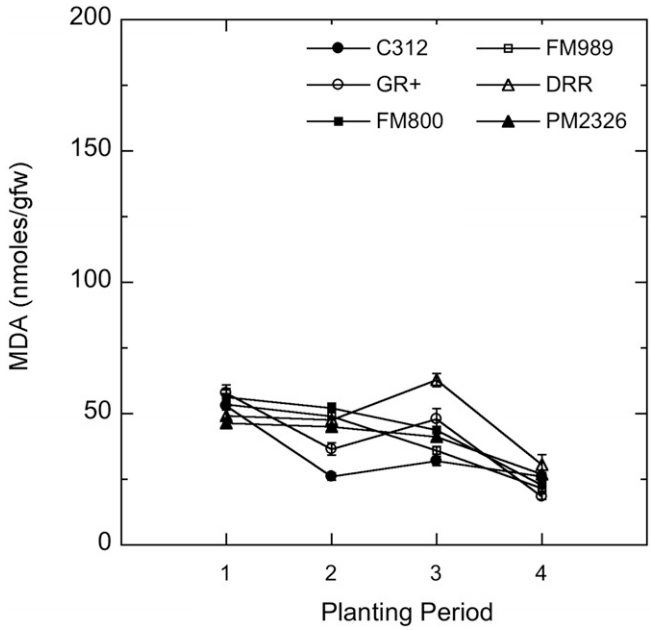

Figure 5. The amount of malondialdehyde (MDA) in cotyledons at the time of emergence for each of four cotton plating dates. Each value represents the mean of a minimum of three assays of samples from four replications for each planting. Error bars indicate the standard error of the mean. C312, Coker 312; DRR, De Ridder Red Leaf; FM800, Fibermax 800; FM989, Fibermax 989; GR+, glutathione reductase overexpressed cotton; PM2326, Paymaster 2326.

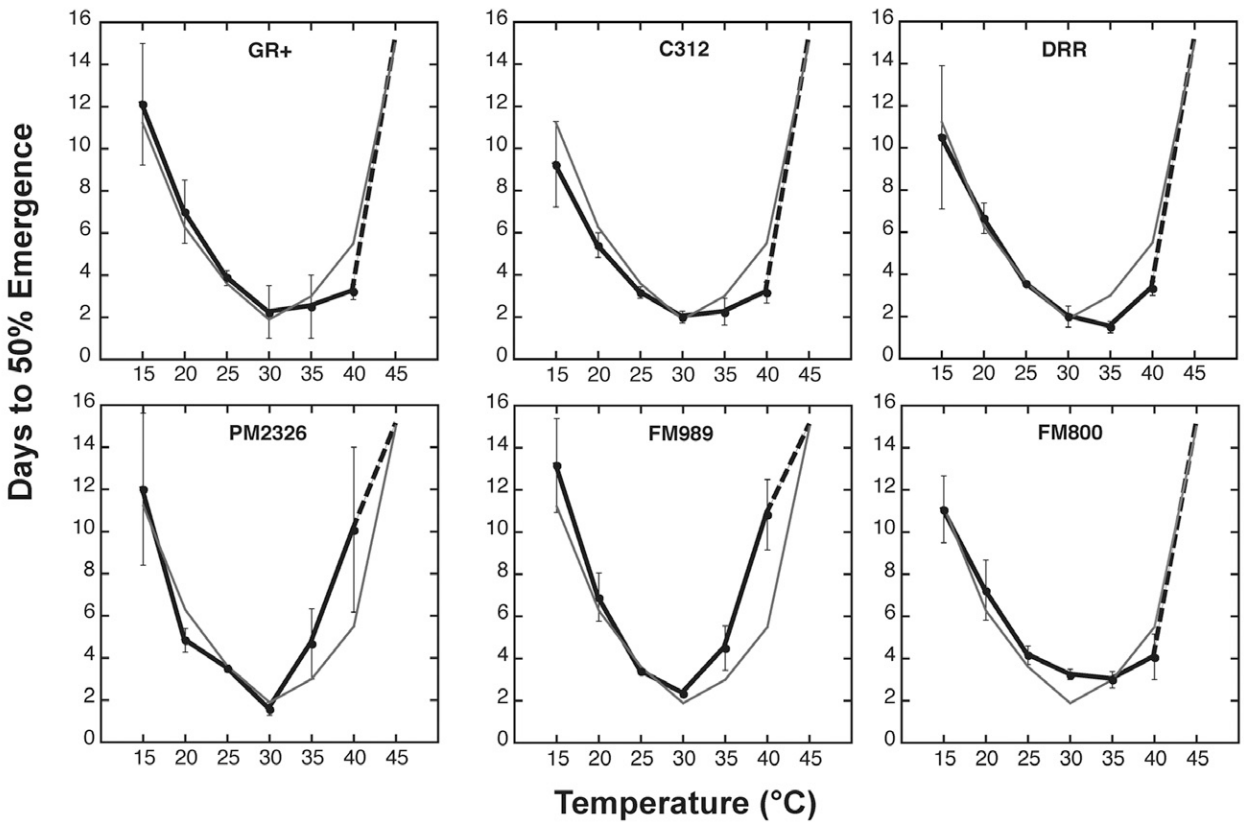

Figure 6. The time required for 50% emergence for cotton at constant temperatures. Seeds were planted in thermal gradient boxes (15–45°C). Three replications of 30 seeds at each temperature were planted in two thermal gradient boxes. Emergence was determined daily. Each value is the mean of three replicates. Dashed black line indicates no emergence occurred above 40°C within the 15-d observation period. The error bars indicate the standard error of the mean. Gray line without data points is the mean of the six cotton lines in the study. C312, Coker 312; DRR, De Ridder Red Leaf; FM800, Fibermax 800; FM989, Fibermax 989; GR+, glutathione reductase overexpressed cotton; PM2326, Paymaster 2326.

environments, the lack of a functional advantage in emergence or plant performance in the GR+ plants is apparent.

DISCUSSION

It was hypothesized that enhancement of GR activity in cotton through the overexpression of a transgene for GR would result in a decrease in oxidative damage in the germinating/emerging seedling that would be evidenced by faster emergence and decreased levels of the oxidative stress indicator MDA.

On the basis of field studies of cotton under environmental stresses, Mahan and others (Mahan and Mauget, 2005; Mahan and Wanjura, 2005) suggested that antioxidant metabolism in cotton seedlings was only modestly responsive to water deficit and thermal stresses. In their analysis they did not rule out the possibility that an inability to increase antioxidant metabolism, even at stressful temperatures, might contribute to diminished seedling performance. Of the enzymes involved in antioxidant metabolism, GR has been postulated to play a unique role that suggests that limitations in its function might be particularly harmful (Tausz et al., 2004). In this study the effect of transgenic overexpression of GR on the thermal dependence of seedling performance was investigated under field and controlled-temperature conditions.

Table 2. Emergence and percent germination of six cotton lines under constant temperatures. Seeds were planted in thermal gradient boxes (15–45°C). Standard error of mean is in parentheses. 2× range indicates temperature range over which emergence time ≤ 2× minimum value.

Line†	Days to 50% emergence	Fastest emergence	2× range	2× range width	Germination
	d	°C			%
PM2326	2 (0.30)	30	22–34	12	80 (12.6)
FM800	3 (0.25)	30	25–40	15	83 (6.1)
FM989	2.5 (0.18)	30	24–35	11	83 (6.1)
DRR	1.8 (0.50)	30	25–40	15	93 (4.2)
C312	2 (0.29)	30	22–40	18	87 (4.2)
GR+	3 (1.25)	30	24–41	17	93 (4.2)

†C312, Coker 312; DRR, De Ridder Red Leaf; FM800, Fibermax 800; FM989, Fibermax 989; GR+, glutathione reductase overexpressed cotton; PM2326, Paymaster 2326.

Glutathione reductase activity was found to be increased approximately 25-fold in the GR+ transgenic seedlings as compared with both the C312 wild-type and the commercial lines. The combination of variable-temperature field and constant-temperature conditions analyzed in this study allowed an assessment of emergence rates of several cotton lines over a range of temperatures that were representative of those typical for cotton seedlings in the region. A range of constant-temperature plantings provided emergence data over a thermal range of 15 to 45°C. Four planting periods produced thermal environments in the field with a range of temperatures that subjected the seedlings to suboptimal, optimal, and supraoptimal temperatures from a metabolic point of view. Differences among the lines in emergence times under both constant temperature and field emergence were statistically significant, though the functional relevance of the differences was not readily apparent.

The results of this study indicate that enhanced GR activity did not appreciably improve seedling performance under low suboptimal or supraoptimal temperatures, and it is therefore postulated that antioxidant metabolism in cotton seedlings might be generally considered to be sufficient. The modification of antioxidant metabolism as a means of improving cotton performance under adverse temperatures typical for the plant is probably not a fruitful exercise. The results of this study suggest that, under typical field conditions, rate-related limitations on metabolism may be of greater importance than those related to metabolic damage. Improvement of the thermal dependence of metabolic rates may be a fruitful avenue of research.

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