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PHYSIOLOGY

Protective Role in Acquired Thermotolerance of Developmentally Regulated Heat Shock Proteins in Cotton Seeds

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INTERPRETIVE SUMMARY

Plants experience high air and soil temperatures during periods of drought and when fields receive limited irrigation. These stresses frequently coincide in areas that have hot arid climates, and where one stress can compound the effects of the others. Just like mature plants, germinating seeds and seedlings also can be subjected to environmental stresses. Even when they imbibe water, seeds may be exposed to elements of a hostile environment, which include extremes of temperature and moisture. Failure to cope with the adversity caused by these extremes results in poor seedling development and, eventually, reduced crop yields. This study evaluated the effect of water-deficit stress on the expression of a high temperature protection system that exists in cotton. The study showed that water-deficit stress reduced overall plant performance, but it also revealed that the level of acquired thermotolerance in stressed plants was proportional to that in well-watered seedlings. The study also showed that heat shock proteins that accumulate in seeds as a part of normal seed development, then are lost from seeds within a few days of germination, remained in the cotyledons of water-stressed cotton seedlings several days longer than in the well-watered seedlings. The presence of these developmentally regulated heat shock proteins failed to enhance inherent or acquired thermotolerance in stressed plants compared with its level in well-watered cotton seedlings. These findings support the hypothesis that the developmentally regulated heat shock proteins may be involved in desiccation tolerance in seeds but are unable to enhance heat tolerance in water-stressed seedlings.

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ABSTRACT

Cotton seeds planted under dryland conditions frequently experience periods of water-deficit stress and elevated temperatures during seedling establishment. The effect of water-deficit stress on the expression of acquired thermotolerance in cotton (*Gossypium hirsutum* L. 'Paymaster HS26')[1] was evaluated. Germinating cotton seeds were water stressed in either polyethylene glycol solutions or vermiculite. Whether exposure to elevated temperatures induced thermotolerance was evaluated with the use of a chlorophyll accumulation assay. The results showed reduced seedling growth under water-deficit stress, yet acquired thermotolerance was not inhibited. Protein analysis at 5 d after planting showed that developmentally regulated heat shock proteins HSP101 and HSP17.6 were present in the cotyledons of water deficit–stressed cotton seedlings and that these proteins were absent in cotyledons from well-watered seedlings. The presence of these heat shock proteins in the water deficit–stressed seedlings failed to enhance their inherent or acquired thermotolerance, compared with that of well-watered seedlings. These results suggest that the developmentally regulated heat shock proteins are not metabolically available to assist in enhancing thermotolerance.

Plants experience high air and soil temperatures during drought and when fields receive limited irrigation. These stresses frequently coincide in areas that have hot arid climates and where one stress can compound the effects of others. This

Abbreviations: EDTA, ethylenediaminetetraacetic acid; HSP, heat shock protein; PEG, polyethylene glycol.

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

problem is exemplified in a study that compared dryland and irrigated cotton, and demonstrated that plants grown under water-stress conditions exhibited up to 85% reduction in leaf area index, plant size, and dry matter accumulation, compared with the same measurements from irrigated controls (Burke et al., 1985). Dryland cotton leaves endured higher daytime canopy temperatures (40°C) than leaves from irrigated plants (30°C). Reduced water availability resulted in stomatal closure, which, in turn, resulted in leaf temperatures rising above that of the surrounding air.

Some or all of the negative impact of damaging high temperatures can be ameliorated by prior exposure to elevated, but nonlethal, temperatures in a process called acquired thermotolerance. Plants, like most organisms, respond to an elevation in temperature by synthesizing heat shock proteins (Miernyk, 1999; Schöffl et al., 1998, 1999; Nover, 1997; Boston et al., 1996; Vierling, 1991). Leaves of dryland cotton have been shown to accumulate significant levels of heat shock proteins in response to heat stress (Burke et al., 1985). During high-temperature stress, low molecular-weight heat shock proteins (15–30 kDa in size) can constitute as much as 1 to 1.5% of total leaf cellular protein content (Hsieh et al., 1992; Mansfield and Key, 1987). Although a correlation between the development of acquired thermotolerance and the appearance of heat shock proteins has been noted, the synthesis of some heat shock proteins is developmentally regulated during seed formation (Wehmeyer and Vierling, 2000; Carranco et al., 1999; Almoguera et al., 1998; Bettey and Finch-Savage, 1998; Bettey et al., 1998; Singla et al., 1998; Sung et al., 1998; Hernandez and Vierling, 1993).

Like mature plants, germinating seeds and seedlings can be subjected to environmental stresses. Even when they receive water, seeds may be exposed to elements of a hostile environment, which include extremes of temperature and moisture. Failure to metabolically cope with such adversity results in poor seedling development, poor stand establishment, and reduced crop yields. High soil-surface temperatures reduce seed germination and emergence of pearl millet (*Pennisetum glaucum* [L.] R. Br.; Peacock et al., 1993) and retard the early growth stages of crops such as sorghum (*Sorghum bicolor* [L.] Moench; Ougham et al., 1988), cotton (Ashraf et al., 1994), and potato (*Solanum tuberosum* [L.]; Reynolds and Ewing, 1989). The ability of imbibing seed to respond to heat stress has been linked to seed vigor (Helm et al., 1989). A recent study on lettuce (*Lactuca sativa* L.) seed demonstrated that seed germinated at relatively high temperatures had a much greater rate of germination if matured on the plant at higher temperatures (Sung et al., 1998). To germinate, the seed must be able to survive environmental stresses without aid of a developed root or leaf system to help regulate moisture and temperature levels. Studies have demonstrated that seeds have evolved mechanisms to deal with the potential threat of environmental stresses, including the induction of certain proteins, such as late embryogenic abundant proteins, dehydrins, and heat shock proteins.

In maturing seeds many heat shock proteins are controlled developmentally and expressed in the absence of heat stress (Wehmeyer and Vierling, 2000; Carranco et al., 1999; Almoguera et al., 1998; Bettey and Finch-Savage, 1998; Bettey et al., 1998; Singla et al., 1998; Sung et al., 1998; Wehmeyer et al., 1996; Helm and Abernethy, 1990). Whereas some of these developmentally dependent heat shock proteins are also responsive to elevated temperatures, many appear in the seed during the onset of desiccation and are present several days after seedling emergence (Coca et al., 1994; De Rocher and Vierling, 1995). This response suggests that they possibly have roles in desiccation tolerance or in dormancy during seed development and perhaps during germination. While individual heat shock proteins may perform specific functions, there is growing evidence that some heat shock proteins in plants may be induced by other stresses and, thus, are part of an energy- and resource-saving, cross-tolerance system (Sabehat et al., 1998).

This study examined the impact of water stress on the high temperature protection system of cotton seedlings with the use of a chlorophyll accumulation assay to assess the health of treated cotyledons (Burke, 1998). Under laboratory conditions, the temperature and moisture conditions experienced by cotton seeds germinating at a 5-cm planting depth were simulated. Seedling emergence was significantly retarded under stress conditions, which ultimately can result in delay or failure of the cotyledons to

break the surface and initiate photosynthesis. Although the disappearance of both high and low molecular-weight heat shock proteins was delayed by up to 3 d in water-stressed seedlings, the inherent and acquired thermotolerance in the stressed plants was not enhanced, compared with that of well-watered seedlings.

MATERIALS AND METHODS

Plant Growth Conditions

Seeds of 'Paymaster HS26' were germinated between two layers of water-saturated germination paper surrounded by a layer of wax paper in a glass beaker of water. The were germinated and grown for 24 h in the dark at 28°C in a Model E-30B growth chamber (Percival Scientific, Boone, IA). After 24 h the germinated seeds were transferred to water stress treatments of either germination paper saturated with polyethylene glycol (PEG 4000, 10% w/v), or to vermiculite that contained only enough water to achieve -0.4 MPa (Creelman et al., 1990). Other germinated seeds were kept in the water-moistened paper to serve as controls. Plants were then grown in the dark at 28°C for an additional 2 to 10 d. Effects of the treatments on hypocotyl elongation were determined by evaluating one hundred seedlings for each treatment.

Determination of Optimum Temperature for Chlorophyll Accumulation

Cotyledons were excised from dark-grown seedlings and exposed to selected heat and light treatments. Chlorophyll accumulation was evaluated in cotyledons from seedlings grown 3, 5, 7, 9, or 11 d at a range of temperatures from 10 to 45°C. Cotyledons were placed on moist filter paper on temperature blocks of an electronically controlled eight-position thermal plate system, the CELTEC (Burke and Mahan, 1993), set to the desired temperatures. The cotyledons were covered with Glad cling wrap (First Brands Corp., Danbury, CT) to prevent drying while still allowing gas exchange. Chlorophyll accumulation was determined (Arnon, 1949) after 20 h under continuous light at 115 mmol m^{-2} s^{-1} (two F40/AGRO-LITE fluorescent bulbs and two 75-W incandescent bulbs; Philips Analytical, Chandler, AZ). Five replications were analyzed for each day after planting.

Determination of the Challenge Temperature That Results in Maximum Inhibition of Chlorophyll Accumulation

Cotyledons from dark-grown seedlings were placed on the CELTEC at temperatures from 30 to 54°C at 3, 5, and 7 d after planting. The cotyledons were challenged for 30 min at a treatment temperature. They were placed in the light on the moist filter paper at 28°C for 20 h before determination of their chlorophyll content. The 30-min challenge temperature that prevented subsequent chlorophyll accumulation at the optimum temperature was determined and used in subsequent experiments. Five replications were analyzed for each d after planting.

Developmental Change in Chlorophyll Accumulation

Cotyledons from both control and water-stressed dark-grown seedlings were excised 3, 5, 7, 9, and 11 d after planting. Excised cotyledons on moist filter paper were placed on the temperature blocks of the CELTEC and covered with Glad cling wrap. They were exposed to continuous light for 20 h. Chlorophyll content was determined after the 20 h light treatment at 10, 15, 20, 25, 30, 35, 40, or 45°C. Five replications were analyzed for each day after planting.

Protein Isolation and Western Blot Analysis

Protein was isolated from an equal number of cotyledons by pulverizing the tissue in extraction buffer (0.5 M Tris-Cl, pH 8.65; 50 m*M* EDTA; 0.1 M KCl and 2% β-mercaptoethanol). The pulverized tissue was centrifuged in 1.5-mL microcentrifuge tubes in a benchtop microcentrifuge at 10,000 g for 5 min at 4°C to remove cell debris. The clear supernatant was removed to fresh microcentrifuge tubes. Protein concentrations were estimated by the BioRad protein assay (BioRad, Hercules, CA), using bovine serum albumin as a standard. Proteins were fractionated by gel electrophoresis (sodium dodecylsulfate–polyacrylamide, 12% w/v) on the BioRad minigel system. Fractions were

transferred in buffer (5.8 g L^{-1} Tris base, 2.9 g L^{-1} glycine, and 200 mL L^{-1} 100% methanol) to polyvinylidene difluoride membranes (Pierce, Rockford, IL) for 1 h at 0.2 A on the BioRad TransBlot.

Membranes were blocked for 1 h in Tris-buffered saline that contained 0.1% (v/v) Tween 20 and 5% (w/v) non-fat dried milk. Primary antibody at the desired dilution was incubated with the membrane in Tween–Tris-buffered saline for 1 h at room temperature. Following three 5-min washes in Tween–Tris-buffered saline, membranes were diluted 1:10,000 in Tween-Tris buffered saline and incubated 1 h with goat-anti-rabbit horseradish peroxidase–conjugated secondary antibody (Pierce, Rockford, IL). After three more 5-min washes in Tween–Tris-buffered saline, signals were detected with the SuperSignal Substrate system (Pierce).

Determination of Inherent and Acquired Thermotolerance

To determine the level of acquired thermotolerance (inducible protection of subsequent chlorophyll synthesis after a 50°C, 30-min challenge), cotyledons from etiolated cotton seedlings were pre-incubated for 4 h at 40°C and then exposed to 50°C for 30 min. The seedlings were placed at 30°C and chlorophyll was allowed to accumulate for 16 to 20 h under continuous light. Inherent thermotolerance was determined as above in cotyledons that had not received the pre-incubation at 40°C.

RESULTS

Growth Rates of Well-Watered and Water-Stressed Seedlings

Growth rates for control seedlings (grown in water), as estimated by hypocotyl length over time, exceeded those for water-stressed seedlings (Fig. 1), which shows that growth in water containing polyethylene glycol did create a stress for the emerging seedlings. One hundred seedlings were evaluated for each treatment and day after planting. Differences in hypocotyl length were observed as early as 3 d after planting, and by 11 d after planting, the polythylene glycol-treated seedlings had hypocotyl lengths equivalent to those of the well-watered seedlings at 5 d after planting. The horizontal line in Fig. 1 represents the 5-cm distance that seedlings in the field would grow through to reach the soil surface.

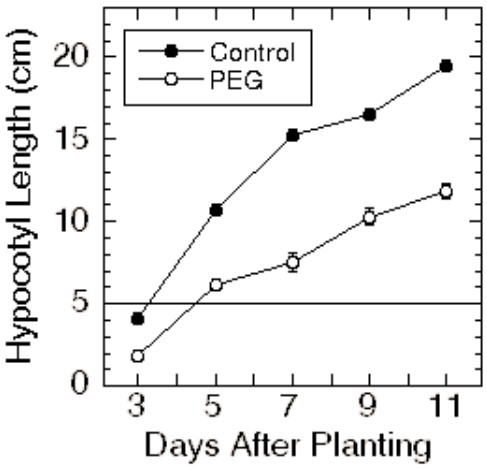

Fig. 1. Hypocotyl elongation in well-watered (control) vs. water deficit–stressed (PEG, polyethylene glycol) cotton 'Paymaster HS26.' Significant reduction in hypocotyl elongation is apparent in water deficit–stressed seedlings. The horizontal line represents the soil surface at a 5-cm distance from each planted seed. Error bars give standard errors.

Optimum Temperatures for Chlorophyll Accumulation

Cotton seedling emergence usually occurs 3 to 5 d after planting in the field. The objectives were to determine both the optimum temperature for chlorophyll accumulation during seedling emergence, and whether there were developmental constraints on the optimum temperature. Also, the effect of water stress on the optimum temperature for chlorophyll accumulation as the seedling emerged was studied. Cotyledons were selected at 3, 5, 7, 9, and 11 d after planting, and the ability to accumulate chlorophyll was determined over temperatures ranging from 10 to 45°C (Fig. 2). Cotyledons growing in water accumulated maximum chlorophyll levels at 30°C for all but the 11-d-old plants, which accumulated very low levels of chlorophyll at any temperature. In these plants the absolute levels of chlorophyll accumulation progressively decreased, such that at 11 d after planting chlorophyll accumulation was barely detectable.

Younger seedlings accumulated most of the chlorophyll from 3 (Fig. 2A) to 5 d (Fig. 2B) after planting, which coincides with the period in the

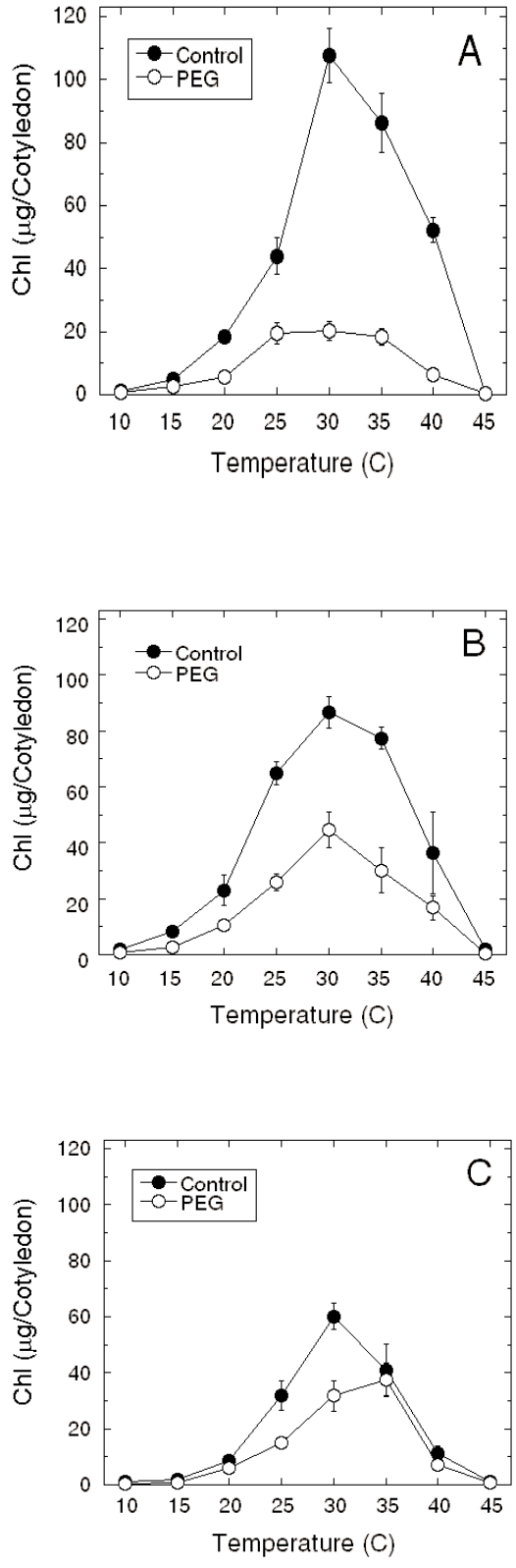

Fig. 2. Developmental changes in the temperature sensitivity of chlorophyll (Chl) accumulation in well-watered and water deficit–stressed cotton seedlings: A, 3 d after planting; B, 5 d after planting; C, 7 d after planting; D, 9 d after planting; E, 11 d after planting. Error bars give standard errors

field in which cotton would be emerging and initiating photosynthesis. In water-stressed plants chlorophyll accumulation was consistently lower than in irrigated plants. Optimum temperature for chlorophyll accumulation ranged from 25 to 35°C, and chlorophyll accumulation was still evident in cotyledons from 11-d-old plants (Fig. 2). We also found, however, that at temperatures <25°C and >30°C chlorophyll accumulation in both irrigated and water-stressed plants was significantly diminished; therefore, subsequent experiments were carried out at the optimum 30°C.

Optimum Temperatures for Inhibition of Chlorophyll Accumulation

To determine the effects of elevated temperature stress on subsequent chlorophyll accumulation at the optimal temperature,

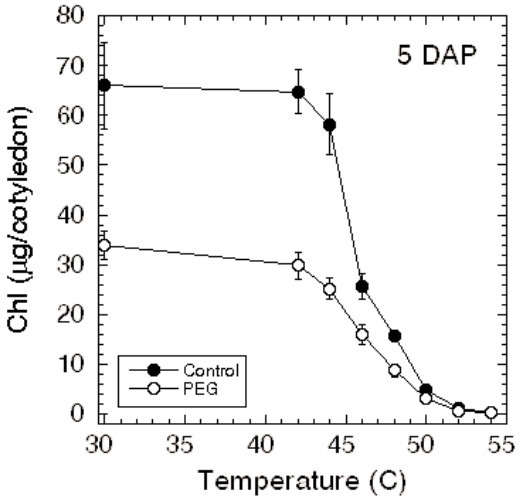

Figure 3. Temperature sensitivity of subsequent chlorophyll (Chl) accumulation 5 d after planting (DAP) at 30°C following a 30-min challenge at the indicated temperatures. Similar temperature inhibition curves are apparent in the well-watered (control) and water deficit–stressed (PEG, polyethylene glycol) cotton. Error bars give standard errors.

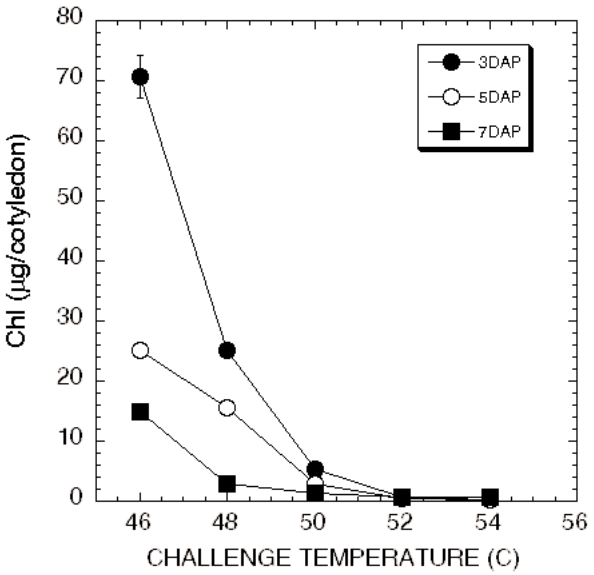

Figure 4. Chlorophyll (Chl) accumulation in cotyledons of well-watered cotton seedlings at 3, 5, and 7 d after planting (DAP) when challenged for 30 min at 46, 48, 50, 52, or 54°C prior to exposure to continuous light at 30°C. Error bars give standard errors.

cotyledons were examined 5 d after planting from seedlings grown under control and water-stressed (polyethylene glycol) conditions (Fig. 3). Etiolated cotyledons were exposed to temperatures ranging from 30 to 54°C for 30 min and subsequently allowed to accumulate chlorophyll for 20 h in the light. Chlorophyll levels for water-stressed cotyledons were consistently lower than for those of the control. At challenge temperatures of 44°C and greater, cotyledons from both irrigated and water-stressed seedlings began to exhibit a decline in ability to accumulate chlorophyll. Whereas absolute chlorophyll accumulation was reduced more in water-stressed cotyledons, the sensitivity to extreme temperature of both types of plants was similar. In addition, although chlorophyll accumulation was inhibited by temperatures of 44°C and greater, the older the tissue, the greater the rate of inhibition as temperatures exceeded 44°C (Fig. 4).

Effects of Water Stress on Acquired Thermotolerance and Levels of Individual Heat Shock Proteins in Emerging Seedlings

Heat shock proteins accumulate at various stages during seed development and maturation; some are also heat shock–inducible (Wehmeyer and Vierling, 2000; Carranco et al., 1999; Almoguera et al., 1998; Bettey and Finch-Savage, 1998; Bettey et al., 1998; Singla et al., 1998; Sung et al., 1998; Hernandez and Vierling, 1993). To explore the effect of water stress on seed content of heat shock proteins and the possibility that they provide some resistance to elevated temperatures, the levels of HSP101 and HSP17.6 were studied by western blot analysis (antisera provided by Dr. Elizabeth Vierling, Dep. of Biochemistry, Univ. of Arizona, Tucson). The data in Fig. 5 demonstrate that cotyledons from well-watered seedlings retain

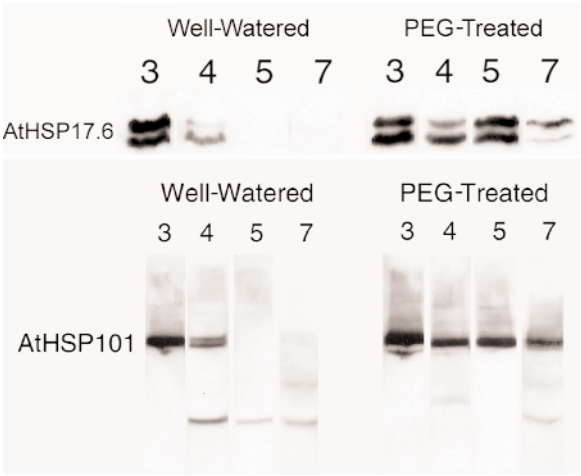

Figure 5. Western blot analysis of HSP17.6 and HSP101 levels in well-watered (control) and water deficit– stressed (PEG, polyethylene glycol) cotton 3,4, 5, and 7 d after planting using antibodies raised against Arabidopsis thaliana (L.) Heynh. HSP 17.6 and HSP 101. Note the loss of HSP 17.6 and HSP 101 in well-watered seedlings but not in waterdeficit–stressed seedlings.

HSP101 and HSP17.6 up to 4 d after planting; whereas, water-stressed cotyledons retain these heat shock proteins for as long as 7 d after planting. To ensure that the heat shock proteins were developmental and not present as a result of metal contaminants in the PEG solution, cotyledons were subjected to similar water-stress conditions from two sources — polyethylene glycol or vermiculite moistened to -0.4 MPa. After 5 d of growth, cotyledons were analyzed for presence of HSP101 and HSP17.6, which revealed little difference between the treatments, thereby suggesting that the heat shock protein turnover was related to the polyethylene glycol-induced water stress.

The delay in removal of some heat shock proteins from water-stressed cotyledons led to questions about whether water-stressed cotton seedlings might exhibit enhanced thermotolerance. To test this possibility, cotyledons from water-stressed and nonstressed seedlings were treated 5 d after planting at 40°C for 4 h and subsequently subjected to a 50°C challenge for 30 min, which was followed by a 20-h light treatment for chlorophyll accumulation (Fig. 6). Cotyledons from control well-watered seedlings accumulated 64 ug chlorophyll/ cotyledon and cotyledons from well-watered seedlings that had been preincubated at 40°C and challenged at 50°C exhibited chlorophyll accumulation levels ≈58% that of the nontreated control. Cotyledons from water-stressed seedlings accumulated 50 ug chlorophyll/cotyledon, while cotyledons from water-stressed seedlings that had been preincubated at 40°C and challenged at 50°C exhibited ≈50% chlorophyll accumulation levels of the unchallenged control (well-watered). No chlorophyll accumulation was observed in cotyledons from well-watered or water-stressed seedlings challenged directly at 50°C for 30 min. This finding suggests that the presence of heat shock proteins in the cotyledons of water-stressed seedlings failed to provide heat tolerance beyond the level found in well-watered samples. The thermotolerance data showed proportional levels of acquired thermotolerance, as measured by chlorophyll accumulation, under well-watered and water-stressed conditions, despite the presence of elevated levels of HSP101 and HSP17.6 in the water-stressed cotyledons.

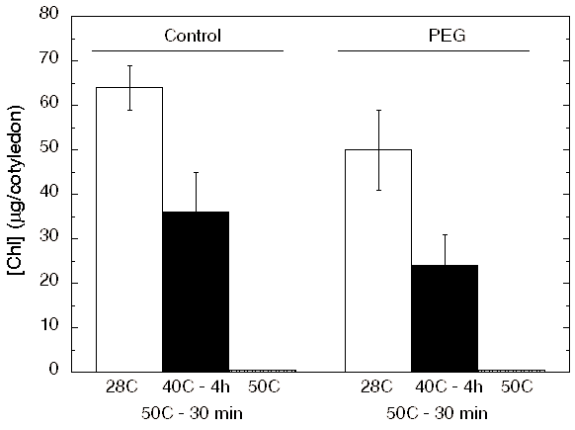

Figure 6. Chlorophyll (Chl) accumulation in cotyledons of well-watered (control) and water-stressed (PEG, polyethylene glycol) cotton seedlings, as well as in well-watered (heat shock) and water-stressed (PEG + heat shock) cotton seedlings that had been preincubated at 40°C for 4 h and challenged at 50°C for 30 min before exposure to continuous light at 30°C. Error bars give standard errors.

DISCUSSION

This study simulated water-stress conditions and temperature profiles that cotton in both dryland and irrigated farming systems frequently experiences. The objective was to assess the subsequent health and heat shock protein patterns of stressed plants with the chlorophyll accumulation assay and monospecific antibodies. Water stress visibly retarded growth and development of cotton seedlings, compared with those grown under well-watered conditions, and resulted in reduced cotyledon surface area and reduced hypocotyl length. This effect was observed even when different types of polyethylene glycol and vermiculite were used to simulate water-stress conditions. This outcome demonstrated that the seedling response to stress was independent of the method employed to simulate the stress. Chlorophyll accumulation in cotyledons from well-watered seedlings was controlled developmentally, with maximum levels observed between 3 and 5 d after planting. These circumstances correspond closely to observations in the field under irrigated conditions in which cotton seedlings emerge from the soil 3 to 5 d after planting. This comparison suggests that there are sufficient seed reserves to sustain the plant for at least 5 and even up to 9 d after planting to allow the plant to reach the surface and initiate photosynthesis. The ability to accumulate chlorophyll in cotyledons from water-stressed plants was reduced, compared with nonstressed

plants; the maximum accumulation occurred between 5 and 7 d after planting. Much as the growth and development of water-stressed seedlings was slowed, the rate of mobilization and utilization of stored nutrients may also have been retarded, because cotyledons from these plants still accumulated some chlorophyll even up to 11 d after planting, whereas controls did not. Thus, it appears that although irrigated cotton seedlings may break the soil surface and initiate photosynthesis more quickly than dryland seedlings, the latter may possess enough reserves to achieve the same goal, albeit at a slower rate.

The data show that even at optimum temperatures, cotyledons from water-stressed seedlings had a reduced ability to accumulate chlorophyll, compared with cotyledons from well-watered seedlings. On the other hand, cotyledons from both well-watered and water-stressed seedlings displayed similar patterns of sensitivity to the 30-min high temperature challenge. Both lost much of their ability to accumulate chlorophyll between 45 and 55°C. Although chlorophyll accumulation generally was inhibited at 45°C, the older the tissue, the greater the rate of inhibition as temperatures exceeded 44°C (Fig. 5). This result suggests that cotyledons become more sensitive to elevated temperatures as development proceeds. A similar phenomenon has been observed in imbibing wheat seed (Abernethy et al., 1989), found to be substantially more tolerant to high temperature early in imbibition as opposed to later in the process. This finding indicates some form of protection was lost with age or that recovery from thermal damage possibly was hampered by the depletion of nutrients in older cotyledons.

The developmental regulation of the heat shock response has received increased scrutiny in recent years (Wehmeyer and Vierling, 2000; Carranco et al., 1999; Almoguera et al., 1998; Bettey and Finch-Savage, 1998; Bettey et al., 1998; Singla et al., 1998; Sung et al., 1998; Hernandez and Vierling, 1993). A review by Schöffl et al. (1998) concluded that the expression of subsets of heat shock genes during gametogenesis and embryogenesis suggests that the developmentally expressed heat shock proteins serve certain functions that may differ from those required for coping with environmentally stressed vegetative tissue. Expression of HSP17.4 during seed maturation parallels the acquisition of dormancy and desiccation tolerance, and it has been hypothesized that it might be important for one of these processes. Wehmeyer et al. (1996) used seed dormancy mutants and showed that HSP17.4 was not sufficient for dormancy; however, they could not rule out its possible involvement in this process. Wehmeyer and Vierling (2000) evaluated desiccation-intolerant mutants and showed that they all had greatly reduced or undetectable HSP17.4 levels. These results support the possible involvement of HSP17.4 in the ability of seed to survive desiccation. The small, class I, heat shock proteins from plants have molecular chaperone activity *in vitro* (Lee et al., 1997). Wehmeyer and Vierling (2000) suggest *in vivo* HSP17.4 may have a similar role in preventing irreversible aggregation of other proteins during desiccation or may assist in refolding of denatured proteins during imbibition. HSP101 also has been shown to play a crucial role in thermotolerance in *Arabidopsis thaliana* (L.) Heynh. (Queitsch et al., 2000) and to be developmentally regulated during seed development (Singla et al., 1998).

CONCLUSIONS

In this study, water-stressed cotyledons were shown to have acquired thermotolerance levels 5 d after planting that were similar to those of the well-watered cotyledons of the same age. These patterns of thermotolerance did not match the profiles of HSP101 and HSP17.6 in the cotyledons. Characterization of HSP101 and HSP17.6 protein patterns showed significant levels of protein in cotyledons from water-stressed cotton seedlings, yet no detectable levels of HSP101 or HSP17.6 were detected in cotyledons from well-watered seedlings 5 d after planting. Evaluation with the chlorophyll accumulation assay of thermotolerance levels in these tissues failed to show any differences in acquired thermotolerance levels despite the elevated levels of HSP101 and HSP17.6 in the cotyledons of the water-stressed seedlings. This result indicates that, although HSP101 and HSP17.6 are known to aid in acquisition of thermotolerance, their increased presence in water-stressed cotyledons did not enhance acquired thermotolerance. It is unclear whether the longer retention of these proteins in water-stressed cotyledons is caused by a response to water stress or by a slower mobilization of

stored reserves in the cotyledons. The data suggest that water stress does not induce cross-tolerance that would cooperate in protecting the seedling from damage caused by elevated temperature. Such cross-tolerance has been observed in plants (Sabehat et al, 1998), whereby a response to one stress also helps protect the plant from another coincident or subsequent environmental stress. Our findings, however, are in agreement with a recent report that in the pupa of the flesh fly *Sarcophaga crassipalpis* (Tammariello et al., 1999), two heat shock protein transcripts (HSP23 and HSP70) accumulated in response to desiccation; a corresponding enhancement of tolerance to high or low temperatures was not observed.

Our results suggest that water-deficit stress during the early growth of dryland cotton adds yet another impediment to the successful emergence of cotton, and the presence of developmentally regulated heat shock proteins does not boost either inherent or acquired thermotolerance.

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