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BRIEF COMMUNICATION

Physiological response of non-Bt and Bt cotton to short-term drought stress

P. PARIMALA and K. MUTHUCHELIAN+

Department of Bioenergy, School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai, India.

Abstract

Drought stress triggered the accumulation of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) both in non-Bt and Bt cotton with simultaneous production of antioxidant enzymes. And there was no significant difference between non-Bt and Bt cotton under drought stress. In contrast to this, we observed a significant reduction of Bt toxin proteins under 72 h of drought stress in Bt cotton.

Additional key words: antioxidant enzymes; hydrogen peroxide; insecticidal Bt toxin proteins; ion leakage; malondialdehyde.

Environmental stress adversely affects plant performance and results in significant reductions in crop yield and quality worldwide. Drought is considered to be a major environmental factor limiting plant growth and yield (Boyer 1982). Drought can most simply be defined as a period of below normal precipitation that limits plant productivity in a natural or agricultural system (Boyer 1982, Kramer and Boyer 1995).

The exposure of plants to drought stress results in the production of reactive oxygen species (ROS) that leads to diminished plant performance (Noctor and Foyer 1998). ROS are highly toxic and can cause lipid peroxidation and consequently membrane injury and also protein degradation, enzyme inactivation, pigment bleaching, and disruption of DNA strands (Smirnoff 1993). To keep the levels of ROS under control, plants have evolved a series of antioxidative systems (Asada 1999). Compatible solutes are also involved in stabilizing proteins and cell structures, as well as in scavenging ROS (Bartels and Sunkar 2005).

Evaluating the stress responses of mutants and transgenic plants is often the most challenging type of experiment (Verslues *et al.* 2006). With regard to continuing the safe use of Bt crops, the study of critical upper- and lower limits and possible alterations in Bt toxin concentrations from environmental effects should be considered. Interactions of Bt toxin production and abiotic factors such as elevated CO_2, high light, drought, and salt stress could be particularly important to determine whether transgenic crop plants will continue to be effective. The objective of this study was to check whether, under drought stress, there is any significant difference between non-Bt and Bt cotton in the production of antioxidant enzymes. Stable insecticidal Bt toxin protein expression together with endogenous crop fitness is desirable under the future climate conditions. Hence, we tried to find out whether there is any degradation of insecticidal Bt toxin protein in Bt cotton with increasing drought stress.

Bt cotton (RCH2), which contains the toxin gene, *cry*1ac and *cry*2ab from *Bacillus thuringiensis* var. *kursitaki* Berliner, and its parent line, non-Bt cotton (RCH2), were planted in plastic pots with three seeds per pot under controlled laboratory conditions. After 20 days of sowing, drought treatment was started by ceasing watering for 24, 48, and 72 h. Control plants (0 h) were maintained under the same conditions as the drought-stressed plants except that they were well irrigated. Secondary leaves from the control and drought-stressed plants were collected and analyzed respectively. After drought treatment was terminated, some of the plants were rewatered for a further 24 h and all the parameters mentioned above were measured again to determine the recovery of plants from drought stress.

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+Corresponding author; phone: +91 452 2458020, fax: +91 452-2459181, e-mail: drchelian1960@yahoo.co.in
Abbreviations: GR – glutathione reductase; LRWC – leaf relative water content; MDA – malondialdehyde; POD – peroxidase; PPO – polyphenol oxidase; PVP – polyvinyl pyrrolidone; ROS – reactive oxygen species; SOD – superoxide dismutase.
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The leaf relative water content (LRWC) was determined by the method of Slavik (1974) and electrolyte leakage which is used to assess membrane permeability was determined according to Lutts *et al*. (1996). Percentage injury was calculated by using the formula given below:

$$\% \text{ injury} = [(\% L_t - \% L_c)/(100 - \% L_c)] \times 100$$

where % L_t and % L_c are percentage ion leakage data for the treatments and control samples respectively (Arora *et al.* 1992). Lipid peroxidation was measured in terms of MDA content as described by Davenport *et al*. (2003). H_2O_2 content was determined according to Velikova *et al*. (2000).

Fresh leaves (0.3 g) were homogenized with 3 ml of the ice-cold extracting buffer in an ice bath. The homogenized slurry was centrifuged at 10,000 × *g* for 15 min at 4°C and the supernatant was collected. 0.05 M potassium phosphate buffer (pH 7.0) containing 1% (w/v) polyvinyl pyrrolidone (PVP) was used as extraction buffer. Protein concentration in the homogenate was determined according to Lowry *et al*. (1951) using bovine serum albumin as standard. The superoxide dismutase (SOD) activity was assayed by monitoring the inhibition

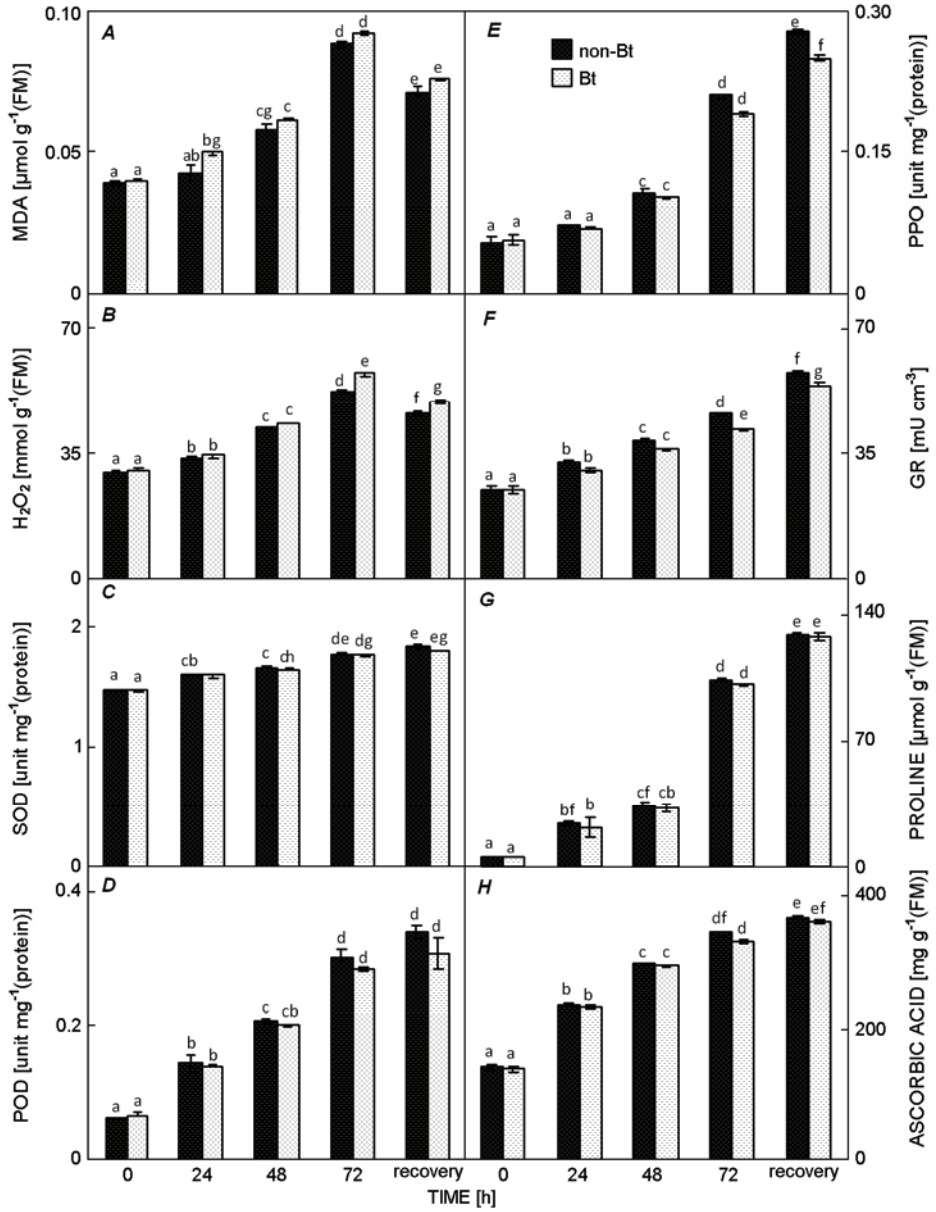

Fig. 1. Changes in malondialdehyde (MDA) (*A*), H_2O_2 (*B*), superoxide dismutase (SOD) (*C*), peroxidase (POD) (*D*), polyphenol oxidase (PPO) (*E*), glutathione reductase (GR) (*F*), proline (*G*) and ascorbic acid (*H*) in the leaves of non-Bt and Bt cotton under drought stress (0, 24, 48, and 72 h) and recovery. Mean ± SE (n = 5). The different letters indicate significant differences at P<0.01 as determined by *Tukey* test. FM – fresh mass.

of photochemical reduction of nitroblue tetrazolium (Beauchamp and Fridovich 1971). Peroxidase (POD) and polyphenol oxidase (PPO) activities were assayed by the method of Kumar and Khan (1982). Glutathione reductase (GR) activity was measured according to Carlberg and Mannervik (1985) by following the oxidation of NADPH spectrophotometrically at 340 nm.

Proline and ascorbic acid contents were measured according to the method of Bates *et al.* (1973) and Omaye *et al.* (1979), respectively. The levels of *Cry*1Ac and *Cry*2Ab were determined in freeze-dried leaf material by *ELISA*, using a kit from *Desigen Diagnostics* (*Mahyco Research Centre*, Jalna, India). All data were subjected to *ANOVA* test and means were compared by the *Tukey* test using *SigmaPlot 11*. Comparisons with P values < 0.01 were considered significantly different.

Drought stress caused significant decline in LRWC under drought stress in both non-Bt and Bt cotton. LRWC decreased with increasing drought stress with no significant difference between the non-Bt and Bt cotton. Relative to controls the drought-stressed leaves (24, 48, and 72 h) exhibited reduced LRWC by 17, 37, and 64% in non-Bt cotton and 17, 42, and 70% in Bt cotton, respectively. The recovery was about 68% in non-Bt and 66% in Bt cotton with respect to their controls. It could be interpreted as a mechanism that concentrates solutes in the cell sap, thereby lowering the osmotic potential and contributing to osmotic adjustment (Lissner 1999).

Under environmental stresses plant membranes are subjected to changes often associated with increased permeability and reduced integrity (Blokhina *et al.* 2003). In the present study, the electrolytic leakage and percentage injury increased with increasing drought stress both in non-Bt and Bt cotton and there was no significant difference between the two ones. The percentage injury was higher in Bt cotton (18, 69, and 96%) than in non-Bt cotton (9, 62, and 87%) under 24, 48, and 72 h of the drought stress. On exposure to 24-h recovery, the percentage injury observed in Bt and non-Bt cotton were 63 and 57%, respectively, based on their controls. Both non-Bt (46%) and Bt cotton (43%) recovered from drought stress and there was no significant difference between non-Bt and Bt cotton at P<0.01.

MDA and H_2O_2 contents significantly increased in both non-Bt and Bt cotton under drought stress (Fig. 1*A*,*B*). There was no significant difference between the non-Bt and Bt cotton in MDA content under drought stress and recovery. But in H_2O_2 content there was a small but significant difference between the two at 72 h of drought stress and after 24 h of recovery, with the Bt cotton having slightly higher levels than the non-Bt cotton. Similar results have also been detected in olive trees (Sofo *et al*. 2004). H_2O_2 as a ROS can damage the membrane lipids, proteins and DNA (Bowler *et al.* 1992). Therefore, it is important for plant cells to keep the levels of H_2O_2 low or to scavenge it efficiently (Loggini *et al.* 1999).

In order to cope with continuous ROS production, plants have a battery of enzymatic and nonenzymatic antioxidants, which function as an extremely efficient cooperative system (Noctor and Foyer 1998, Loggini *et al*. 1999, Willekens *et al*. 1997). Accordingly, our results show that the antioxidant enzymes such as SOD, PPO, POD, and GR significantly increased with increasing drought stress both in non-Bt and Bt cotton (Fig. 1*C*,*D*,*E*,*F*). Our results are in a good agreement with those of Khan *et al.* (2009) who observed similar results in mustard under salt stress. The role of antioxidant enzymes under stressful conditions has already been reported by many earlier studies (Duan *et al*. 2005, Yin *et al.* 2005). The observed increase in SOD activity could increase the ability of plants to scavenge ROS. Bray *et al*. (2000) and Sheen and Calvert (1969) also specified that superoxide dismutase is a major scavenger of superoxide and its enzymatic action results in the formation of H_2O_2 and O_2. POD and PPO decomposes H_2O_2 by oxidation of cosubstrates such as phenolic compounds and/or antioxidants. The increased POD and PPO both in non-Bt and Bt cotton might reduce the phenol accumulation in plants under drought stress. Glutathione reductase is involved in scavenging the products of oxidative stress such as H_2O_2 (Gamble and Burke 1984, Bowler *et al.* 1992) and thus helps in ameliorating the adverse effects of oxidative injury. Glutathione also takes part in the removal of

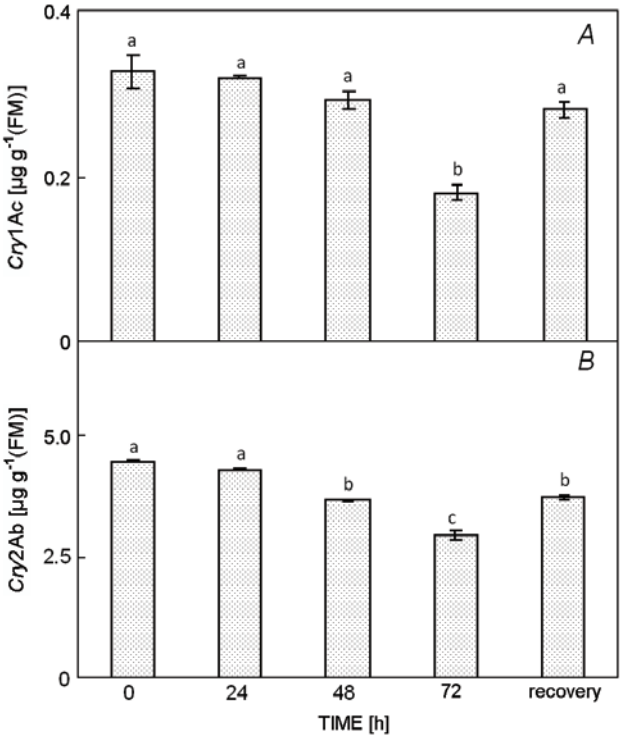

Fig. 2. Production of insecticidal Bt toxin proteins *Cry*1Ac (*A*) and *Cry*2Ab (*B*) in Bt cotton under drought stress (0, 24, 48, and 72 h) and recovery. Mean ± SE (n = 5). The *different letters* indicate significant differences at P<0.01 as determined by *Tukey* test. FM – fresh mass.

excess H_2O_2 (Noctor and Foyer 1998) and lipid peroxides, keeping ROS under control (Rausch *et al.* 2007).

Proline and ascorbic acid accumulated in both non-Bt and Bt cotton under drought stress and recovery (Fig. 1*G*,*H*). There was no significant difference between the non-Bt and Bt cotton. One of the potentially important mechanisms of drought tolerance is osmotic adjustment which can be achieved from the accumulation of compatible solutes in protoplasm (Bartels and Sunkar 2005). And ascorbic acid can directly scavenge ROS, thus providing membrane protection (Thomas *et al*. 1992).

In the present study, the contents of *Cry*1Ac and *Cry*2Ab concentrations in Bt cotton plants declined with the degree of drought stress (Fig. 2). But then under recovery conditions, we found a pronounced increment of Bt toxin proteins. A similar reduction in toxin concentration with elevated CO_2 was found in a Bt transgenic cotton cultivar CK-12 (Coviella *et al.* 2002). However, decline in endotoxin proteins in cotton tissues could also result from degradation of the proteins or remobilization (translocation) of total N for further growth and development (Chen *et al.* 2005).

Taken together, the data obtained from this study indicated that, even though, there was not a great difference in antioxidant enzyme activities between non-Bt and Bt cotton under drought stress and the insecticidal Bt protein content which is necessary for destroying boll worms was degraded markedly in Bt cotton. Bt toxin protein has the prime importance in Bt cotton production and therefore introduction of unstable Bt toxin protein will be ineffective. From our results, we suggest that the biotechnologists should focus on the stability of insecticidal Bt toxin protein to abiotic stresses.

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