

Dongxiao LI, Cundong LI, Hongchun SUN, Wenxin WANG, Liantao LIU, Yongjiang ZHANG

Effects of drought on soluble protein content and protective enzyme system in cotton leaves

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Abstract The effects of soil drought on soluble protein content and protective enzyme system of cotton leaves at different positions were studied in the transgenic cotton cultivar, Lumianyan28. The results indicated that the soluble protein content in main stem leaves and in middle-fruit branch leaves under drought treatment were higher than that of CK, the normal soil water management treatment. Lower fruit branch leaves under drought treatment had higher protein and enzyme levels than CK treatments. From June 25 to July 22, the activity of superoxide dismutase (SOD) in main stem leaves under drought treatment was lower than that of CK. Other time periods had varying results. The peroxidase (POD) activity in main stem leaves and in fruit branch leaves was lower at early stage and then higher at late stage, showing a trend of descending first, and then ascending obviously under the drought condition. The catalase (CAT) activity showed an increase-decrease trend, higher in main stem leaves under drought treatment than that of CK. However, it was opposite in the lower fruit branch leaves, and there were no significant differences between the two CAT treatments in the middle branch leaves. It is suggested that the soluble protein and cellular protection enzymes, such as superoxide dismutase, peroxidase activity, and catalase in main stem leaves and fruit branching leaves play important physiological functions in the early growth stage under drought stress.

Keywords drought, cotton, soluble protein, protective enzyme system

1 Introduction

Agricultural production is frequently influenced by drought, which is becoming more persistent in China since 1990. Cotton, one of the most important economic crops in the North China Plain, has been up to a 30% yield reduction from the drought stress every year (Liu, 2000). Previous research has shown various physiological and biochemical changes in cotton plants when drought stressed, such as the regulation of stomatal aperture, osmotic pressure of cells, and protein synthesis (Dasgupta and Beweley, 1984; Marie Pierre et al., 1992; Bray, 1993; Lu et al., 1994; Cheng, 1995).

Under abiotic stresses, (such as salt, osmotic stress, low and high temperatures) reactive oxygen species (ROS) are generated rapidly in cells, resulting in the peroxidation of cellular membranes and finally in the disruption of plant growth and development. The plants, however, have initiated a distinct protection response to the abiotic stresses. The chief components in the cellular protection system includes the enzyme activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT); all of which have been widely investigated in rice, wheat, and maize (Wang et al., 2002; Wei et al., 2003; Wang et al., 2007). The listed research suggests that the above enzymes play important roles in cellular protection when plants are exposed to stresses by scavenging the excess ROS. In cotton, several studies have been conducted to elucidate the relationship between the cellular protection enzymes and the certain abiotic stresses, especially salt, or biotic stresses, such as pathogenic infection (Liang et al., 2008; Ma et al., 2008). However, to date, few studies have been done to explore the cellular protection enzyme characterizations when plants are exposed to drought stress.

In the past several years, the effects of drought on fiber yield, plant growth and development, and physiological parameters, such as chlorophyll content and photosynthetic rate in cotton have been studied (Liu et al., 2007; Cai et al., 2008; Liu et al., 2008). Also, there have been researches about the variety laws of soluble leaf protein under water

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Dongxiao LI, Cundong LI (✉), Hongchun SUN, Wenxin WANG, Liantao LIU, Yongjiang ZHANG
Department of Crop Cultivation and Farming System, Key Laboratory of Crop Growth Regulation of Hebei Province, Agricultural University of Hebei, Baoding 071001, China
E-mail: nxylcd@hebau.edu.cn

stress (Zhang, 1998; Shi et al., 2007). In our study, the regulation effects of drought on soluble protein content and protective enzyme systems in cotton were elucidated, aiming to provide a theoretical foundation for drought-stressed cotton cultivation and drought-resistant cultivar breeding.

2 Materials and methods

2.1 Experimental design

The experiment was carried out in the waterproof installations (arc-shape steel prop covered with plastic film) located at the teaching experiment base at the Agricultural University of Hebei. The soil type was loamy. Lumianyan28, a transgenic cotton cultivar was used as experimental material. Two different water treatments including drought and control (CK) were set up. In drought treatment, the plants were grown in waterproof installations, and the relative water content (RWC) in 0–80 cm soil layer was kept at less than 60% of optimum over the entire growth season. This condition was realized by avoidance of the natural rainfall and regular low level irrigation based on the soil moisture monitoring. The plants treated as CK were grown in a near-by field. In the CK plants, the RWC in 0–80 cm soil layer was sustained at 60–80% during the experiment by natural rainfall and regular irrigation. The drought treatment and CK had three replicates each, with each plot area of 73.5 m², a density of 45000 plants per ha and a paired row space of 80 cm in wide row and 70 cm in narrow row. Except for the water treatment, all other soil and plant management conditions during the experiment were the same between the treatments, which mimicked conventional cotton fields with high yield.

2.2 Sampling and measurement

2.2.1 Sampling

The representative leaves of main stems and fruit branches were sampled for analysis. The leaves of main stems at different growth stages were picked (seven times), packed in the ice box at 7:00–8:00 in the morning, quickly frozen in liquid nitrogen, and stored at –70°C for further analysis. The fruit branch leaves were sampled by labeling the boll on the first fruit node of lower and middle fruit branches with small cards on the first flowering day and then sampling the same fruit branch leaves at 10-day intervals. The leaves of first or the second branch were referred to the lower position (sampling three times), whereas the leaves of sixth or the seventh branch were referred to the middle position (sampling four times).

2.2.2 Measurement of soluble protein content

The soluble protein content of leaves was measured

according to coomassie brilliant blue G250 method described by Read, etc. (Read and Northcote, 1981). The leaves about 0.5–1.0 g were ground into homogenate in the mortar with quartz sand, then transferred into a volumetric flask, and then dissolved in 100 mL distilled water. The solution was centrifuged for 5 mins at 3000 r·min⁻¹ by low-speed table centrifuge (TD5A-WS). The supernatant was used for assay of soluble protein content using Colorimetric Determination with U-2001 spectrophotometer (HITACHI) at 595 nm colorimetric wavelength.

2.2.3 Measurement of SOD activity

The superoxide dismutase (SOD) activity was measured based on the nitroblue tetrazolium (NBT) reduction method provided by Sunque (Zou, 2000b). About 0.5 g leaves were grounded into powder in the precooled mortar, followed by 1 mL phosphate buffer (0.1 mmol·L⁻¹, at pH 7.8). When the sample was sufficiently homogenized, 4 mL phosphate buffer was added to wash the mortar, and thereafter, all solution was transferred into a plastic centrifuge tube, centrifuged at 10000 r·min⁻¹ for 20 min by refrigerated centrifuge (SIGMA). The supernatant after centrifugation was used for SOD activity assay by using Colorimetric Determination with U-2001 spectrophotometer (HITACHI) at 560 nm colorimetric wavelength. The activity unit of SOD is given as U·g⁻¹·min⁻¹. This same supernatant was also used in separate tests for the measurement of POD and CAT activity.

2.2.4 Measurement of POD activity

The peroxidase (POD) activity was determined according to the description of East China Normal University (East China Normal University, 1980). The POD reaction system consisted of the following components: 2.91 mL phosphate buffer (10 mmol·L⁻¹, pH 7), 0.05 mL guaiacol (20 mmol·L⁻¹), 40 mmol·L⁻¹ H₂O₂, and 0.02 mL enzyme solution. The POD activity was determined by U-2001 spectrophotometer (HITACHI) at 470 nm colorimetric wavelength, and the units represented are U·g⁻¹·min⁻¹.

2.2.5 Measurement of CAT activity

The catalase (CAT) activity was determined based on the description of Zou (2000b). The reaction system for CAT included the following components: 0.2 mL reserved supernatant, the same as that for SOD activity assay, 1.5 mL phosphate buffer (pH 7.8, 0.2 mol·L⁻¹), and 1 mL distilled water. The colorimetric determination of CAT was conducted by U-2001 spectrophotometer (HITACHI) at 240 nm colorimetric wavelength. The biochemical reaction was started by adding 0.3 mL 0.1 mol·L⁻¹ H₂O₂ in the reaction system. The activity of CAT was calculated by the alteration of the OD₂₄₀ values at 2 min intervals after the

initial biochemical reaction, and the units shown are $U \cdot g^{-1} \cdot min^{-1}$.

2.3 Data analysis

The statistical analysis of all data was performed using Excel 2003, DPS v2.00, and SPSS17.0 for Windows system. If a particular F test was significant, the differences of the averages which derived from three replicates in the figures were labeled based on the logarithm spectral ratio (LSR) method analysis.

3 Results

3.1 The effect of drought on main stem leaves

3.1.1 Soluble protein content

With growth progression, the soluble protein contents of stem leaves showed a decreasing trend in both drought treatments and CK (Fig. 1). In the control (CK) leaves, the soluble protein content slowly declined and remained at a low level until the final leaf harvest (Sept. 5). However, in the drought treatment leaves, the soluble protein content also declined over time but then significantly increased above the CK leaf content at the final harvest, September 5 ($P < 0.05$). Additionally, on all previous sampling days except during August, the soluble protein contents were also significantly ($P < 0.05$) higher than those of CK. These results demonstrate that drought stress may lead to soluble leaf protein content increase. This may result from the decrease of water content in plant tissues, as well as the increase in osmotic substances when cotton plants suffered from drought stress.

3.1.2 SOD activity

Figure 2 shows that the activity of SOD in stem leaves under drought conditions had a decreasing-and-then-

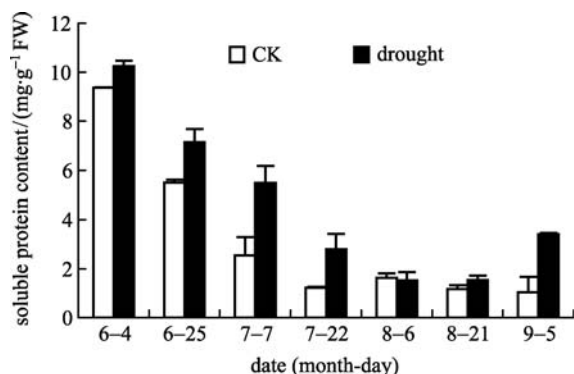


Fig. 1 The content of soluble protein in stem leaves at different stages

increasing trend. From June 25 to July 22, the SOD activity in drought treatment was lower than that of CK, and there was a very significant difference between the two treatments on July 7 ($P < 0.05$), which illustrates that the SOD activity of stem leaves in drought treatment was not higher than that of CK in the early and middle leaf growth stages but in the late stage after Aug. 6, the treatments varied significantly. This may indicate that SOD played an important role in the regulation of O_2^- scavenging when plants suffered from drought stress.

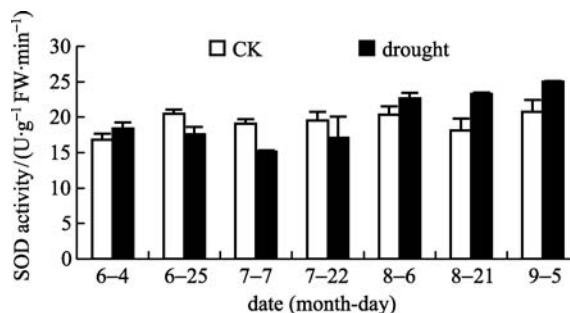


Fig. 2 The activities of SOD in stem leaves at different stages

3.1.3 POD activity

Figure 3 shows that the POD activities in both CK and drought treatments gradually increased with the growth progression but then rapidly increased from July 7 to July 22, with a significant difference between the two treatments. This was followed by relatively little changes on Aug. 6 or a little decrease in the POD activity of the drought treatment on Aug. 21 until it reached a peak value. The POD activities of stem leaves in drought were significantly ($P < 0.05$) lower than those of CK on June 4 and August 21 but significantly ($P < 0.05$) higher than those of CK on the other days, and both showed very significant differences on July 22 ($P < 0.01$).

3.1.4 CAT activity

The CAT activity of stem leaves increased before June 25

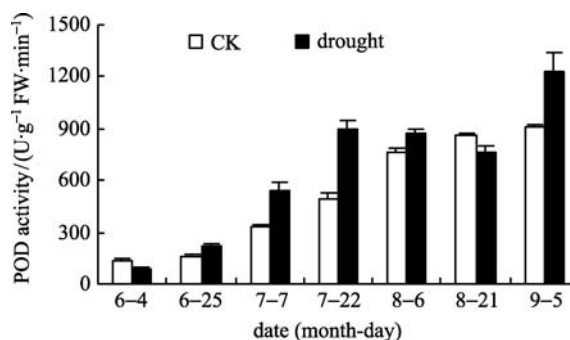


Fig. 3 Changes of activities of POD in stem leaves at different stages

and then descended quickly (Fig. 4). The CAT activities in both CK and drought treatment showed a similar pattern. After July 22, the CAT activity was hardly detected in the leaves. On June 4 and June 25, the CAT activity of stem leaves in drought was significantly higher than that of CK ($P < 0.05$), respectively, and after June 25, there was no significant difference, showing that CAT mainly took effect at early growth stages.

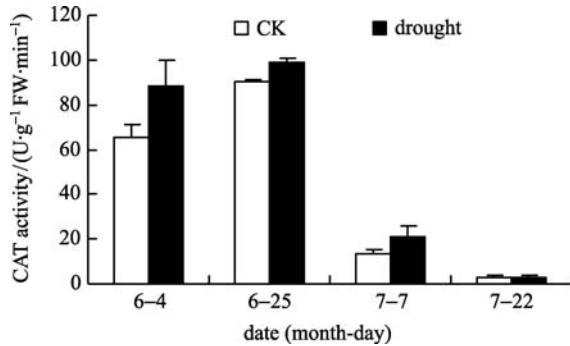


Fig. 4 Changes of activities of CAT in stem leaves at different stages

3.2 Effect of drought on fruit branch leaves

3.2.1 Soluble protein content

Figure 5 shows that in the lower fruit branch leaves of the drought treatment, the soluble protein content was unchanged within the 30-day boll age. However, in the CK treatment leaves, the protein level was initially significantly higher but dropped rapidly over 20 days. At the final date, boll age 30 days, both treatments showed the same level of soluble leaf protein.

For the middle fruit branch leaves under the conditions of drought and CK, the soluble protein contents both showed the trend of first fall and then rise within 40-day boll age, which was similar to that of stem leaves. At every sampling date, the value in drought was higher than that of control, and at the 40-day boll age, the drought treatment was significantly greater ($P < 0.05$).

3.2.2 SOD activity

The SOD activities in the lower and middle fruit branch leaves in both drought treatment and CK showed rising trends along with the development process (Fig. 6). For lower fruit branch leaves, there were significant differences between two treatments at boll age 20 days and 30 days ($P < 0.05$). However, for middle fruit branch leaves, no significant differences existed in two treatments but in boll age 10 days ($P < 0.05$), illustrating SOD resisting drought stress in a certain extent and taking effects for the time-space characteristics.

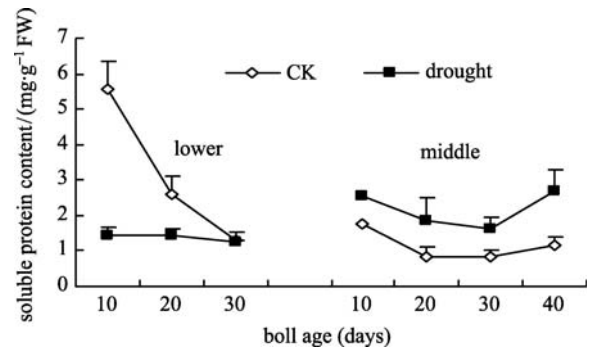


Fig. 5 Effect of water stress on soluble protein in the bottom and middle interior branch leaves

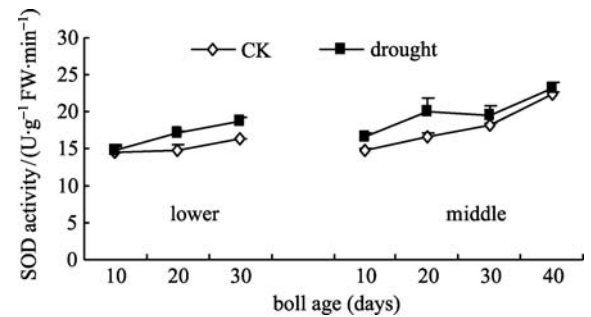


Fig. 6 Changes of activities of SOD in the bottom and middle interior branch leaves

3.2.3 POD activity

Figure 7 shows that under drought treatment, POD activities in both the lower and middle fruit branch leaves first fell and then rose, which was similar to that of stem leaves after July 22. It was possibly related to the action characteristics of POD under drought stress. In contrast, in the CK treatment, the values in the lower and middle fruit branch leaves both showed a regular ascending trend, which may be for the lignifications of cotton plant with growth progress.

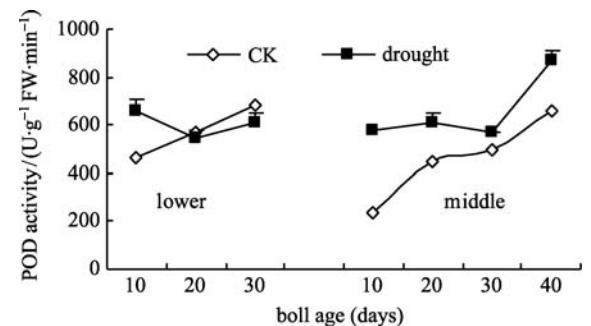


Fig. 7 Changes of activities of POD in the bottom and middle interior branch leaves

3.2.4 CAT activity

The CAT activity in the CK treatment of the lower fruit branch leaves was higher than that in drought, with an initial sharp decrease and then a slow decrease. Over the same time in the drought treatment, the CAT value in the lower fruit branch leaves showed a gentle descending trend (Fig. 8). The changing trend of both was similar to that of the soluble protein contents, Fig. 5, of lower fruit branch leaves under two treatments, suggesting possibly a close relationship between CAT activity and soluble protein contents. However, there were slowly decreasing trends in the middle fruit branch leaves under two treatments with no significant difference.

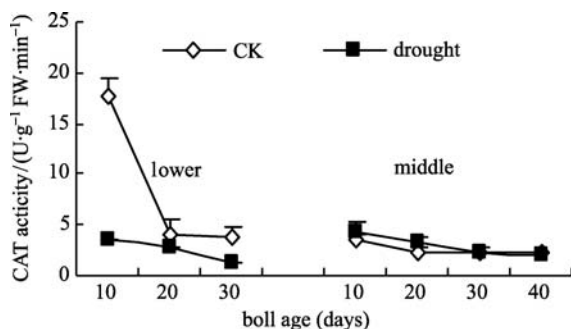


Fig. 8 Changes of activities of CAT in the bottom and middle interior branch

3.2.5 Correlation analysis between the soluble protein content and CAT activity of lower fruit branch leaves

A correlation analysis between the soluble protein content and the CAT activity of the lower fruit branch leaves in the CK treatment showed a highly significant correlation ($r = 0.934^{**}$), indicating that a certain relationship may exist between these leaf proteins (Table 1). However, under the drought treatment, there was no such correlation found, which might be for lots of new stress proteins' production and/or protein macromolecules' degradation.

4 Discussion

When plants suffer drought or other stress, we might expect that the synthesis rate of total protein would

decrease, but the synthesis of some original proteins (namely stress-induced proteins) may be induced or up-regulated to adjust osmotic potential of cells in order to keep a certain turgor and thus to ensure the normal proceeding of physiological processes such as cell growth, stomatal opening, photosynthesis, and so on (Chen and Wang, 2003; Zhu and Zhang, 2003; Xie et al., 2005). The stem leaves and fruit branch leaves are the main sources to provide bolls with organic substances. According to the photosynthetic supply principle near developing organs, the fruit branch leaves on the same node provide most of the organic nutrients to the developing fruit at the node (Ashley, 1972; Benedilt and Kohel, 1975; Zou, 2000a). In our study, it was found that the soluble proteins of stem leaves and fruit branch leaves increased when encountering certain drought stress. With the stress strengthening, the soluble protein content of leaves decreased but ascended somewhat in the late growth stage. However, the soluble protein content of the lower fruit branch leaves under drought was less than that of CK without an obvious decrease. This resulted, possibly, because the cotton leaf proteins showed different reactions at different times or/and positions under a drought stress. Also, it showed that the sensitivity to drought on the lower branches was weak in facilitating the maintenance of basic physiological functions.

The plants generate more reactive oxygen species once suffering a drought stress. The protective enzyme system (SOD, POD, and CAT), as the main enzymes in scavenging of reactive oxygen species, could effectively inhibit the peroxidation of cellular membrane lipid. Under the drought stress, the higher SOD activity plays a role in clearing away $O_2 \cdot$ and generating H_2O_2 , which could be further changed into water by CAT and POD (Liu, 2006). Currently, the relationship between the cellular protection enzyme activities and the stress tolerance in cotton plants is unclear. It was reported that a positive correlation was observed between the activity of protective enzyme system and the antioxidation protection under drought (Li et al., 1994; Wang et al., 2008a). However, a study showed that the activities of SOD and CAT in wheat under drought were decreased, and the POD activity rose to some extent (Wu et al., 2007). In our study, we showed that the times of antioxidant enzymes as a role in clearing reactive oxygen species were different. The activity of CAT was high in prophase, and the activities of SOD and POD rose later, which was in accordance with the conclusion of Mao et al.

Table 1 Pearson correlation between the soluble protein content and CAT activity of lower fruit branch leaves

item	CK-Pr.	CK-CAT	D-Pr.	D-CAT
CK-Pr.	1.000	–	–	–
CK-CAT	0.934**	1.000	–	–
D-Pr.	0.419	0.164	1.000	–
D-CAT	0.831	0.632	0.490	1.000

Note: ** indicate significance at 0.01 level.

(2001) and Weng et al. (2007), indicating that the latter two enzymes played a cooperative effect in clearing reactive oxygen radicals. However, the view differs from that of Wang et al. who held that the POD activity of forages decreased but the activities of SOD and CAT rose under drought (Wang et al., 2008b), which may be related to the different materials of experiments and the drought treatment ways. We also observed that the POD activities in main stem leaves of cotton and the lower and middle fruit branch leaves under drought all showed a phenomenon of an initial fall and then a rise, which is possibly related to the action characteristics of POD in the drought field.

The high CAT activity and soluble protein content before July 22 (full-boll stage) showed that the soluble protein of stem leaves provided a better protection, and simultaneously, the CAT mainly cleared the H_2O_2 . With the continuous stress, the oxidative damage degree of plant was aggravating, and the SOD and POD took effect gradually. Also, the lower and middle fruit branch leaves showed a decreasing trend in the CAT activity and soluble protein content while the activities of SOD and POD were enhanced. Among these, under CK treatment, the soluble protein content and the CAT activity of the lower fruit branches leaves had a highly significant correlation ($n = 7$, $r = 0.934^{**}$). However, under drought treatment, there was no significant correlation between the soluble protein content and the CAT activity of cotton. The soluble protein content of the middle fruit branches had an ascending trend at the 40-day boll age, which was similar to that of the stem leaves at end without clear reasons. Moreover, the changing trend of the activities of POD and CAT in the middle fruit branch leaves in the two treatments were similar, indicating that the middle fruit branch leaves make a greater physiological impact than the main stem leaves.

This experiment mainly discussed the variation of the soluble protein amount and the activities of protective enzyme system but was not focused on the species and function of specific proteins nor on the definition of the action mechanism research of protective enzymes in cotton leaves. Therefore, further experiments are needed.

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