Effects of Water Stress on the Protective Enzyme Activities and Lipid Peroxidation in Roots and Leaves of Summer Maize

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Abstract

A systematic study was conducted to determine the effects of water stress on the activities of protective enzymes and lipid peroxidation in maize. The results showed that: under water stress, the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) in leaves and roots increased sharply at prophase and metaphase growth stages, such as, male tetrad stage, but then declined towards the physiological maturity. The protective enzyme activities in roots were lower than those in leaves. The content of malondialdehyde (MDA) increased according to the severity of water stress. The content of MDA in roots was lower than that in leaves. The activities of protective enzymes and lipid peroxidation in roots were positively related to that in leaves with most of the correlation coefficients being significant. The content of soluble proteins in roots and leaves decreased with increasing drought stress. The ear characteristics deteriorated and the economic yields of maize decreased significantly under water stress. The main factors that caused reduction of yields were the decrease in the number of ear kernels and 100-kernel weight.

Key words: maize, water stress, protective enzyme activities, lipid peroxidation

INTRODUCTION

Maize (Zea mays), as the main food, is an economical and forage crop. It is one of the most important crops throughout the world. With the population expansion in China, the demand for crop supply has increased correspondingly, so it is urgent to improve maize yields even under the unfavorable conditions. Depending on different soil water status, biochemical changes to various extents, occurred in maize grains. Moreover, unfavourable dry weather may have potentially deleterious consequence on crop quality and agricultural production. Reasons may be high temperature and changes in precipitation patterns, especially reduction in precipitation. These may possibly be the results of the projected global environmental changes during the crop growth period. Furthermore, in the semiarid zone in northern China, maize production suffers during severe drought, which often occurs from mid-April to September, leading to direct economic loss. Therefore, it has become a hot issue to elucidate the possible responses and adaptation of plants to drought.

Numerous studies have demonstrated that adverse conditions (water deficiency) can induce membrane damage, increase membrane permeability and the accumulation of free radicals in plants. As a reaction to the adverse conditions, anti-oxidative enzymes and small molecule substances can be produced to remove those active oxygen radicals (Sun et al. 2003; Wang et al. 2002). In many plants, it was found that the free radicals could not be removed thoroughly because of too...
high an amount of free radicals or the weakening of the anti-oxidative enzyme system. Therefore damage to the plant cells may occur when the plant suffers from water deficiency (Iturbe-Ormatexe et al. 1998). The response mechanisms of reactive oxygen scavenging system and lipid peroxidation to drought stress are very complex, as they are not only dependent on plant genotype and stress intensity, but also there are different modes of enzyme reactions. Therefore this problem has not yet been understood clearly. Other studies about water stress reactions of maize mainly reported about experiments where maize was exposed to short-time and sudden water stress (Smirnoff 1998), and about the crops in fields where drought stress has occurred gradually, during the whole development stages of maize. In the case of sudden or staggered stress (Wang et al. 1995), it may lead to the irreversible damage to membrane and components in cells, whereas in the case of gradual stress it led the plants to endure more severe stress probably because it did not lead to serious damage to the components in cells.

In the experiment described here the drought conditions were therefore applied for the whole growing season, to broaden the experimental conditions as described in the literature. The effects of water stress on the protective enzyme activities and on lipid peroxidation in maize roots and leaves at different soil drought stress levels, from third leaf stage to maturity by controlling irrigation. During rain the trial area was covered with a mobile rain shelter. The water was supplied before maize emergence was identical in each plot. Plants were thinned to 21 plants per plot about three weeks after emergence. The date of fertilizer applications and field management were the same for all treatments. The trial design was a completely randomized plot design with three replications.

Soil water content was measured between 0-100 cm soil depth at 20 cm intervals by a neutron probe (503DR, ICT, USA). Additionally, soil samples were taken between 0-20 cm soil depth and oven dried at 65°C for water content determination. The probe was calibrated with volumetric soil samples. One aluminum access tube was installed to a depth of 1.5 m in the central row of each plot. A water meter was used to control the irrigation water supply. To maintain the aimed soil water content the plots were irrigated about every three to seven days (Fig. 1). The amount of irrigation water (W) was calculated according to the following equation:

\[ W = \gamma H A (W_u - W_0) \]

\( \gamma \) = bulk density, \( H \) = soil depth, \( A \) = area of each

**MATERIALS AND METHODS**

Plant materials and treatments

Field experiments of summer maize in concreted plots (plot area 4 m², depth 1.5 m, concrete isolation around each plot) were carried out in the summer seasons of 2002 and 2003 (both from June to October) at Laiyang Agricultural College, Shandong Province in northern China. The basic properties of the fluvo-aquic medium loam soil were: soil water-holding capacity (gravimetric): 25 %, bulk density: 1.34 g cm⁻³, organic matter: 1.04 %, available N: 72.54 mg kg⁻¹, available P: 24.58 mg kg⁻¹, and available K: 65.72 mg kg⁻¹.

The maize cultivar Nongda 108, a main type of high yield cultivar in northern China was subjected to different soil water treatments of 80±5% (full water supply, treatment F), 60±5% (light water stress, treatment L) and 40±5% (serious water stress, treatment S) relative water content from third leaf stage to maturity by controlling irrigation. During rain the trial area was covered with a mobile rain shelter. The water was supplied before maize emergence was identical in each plot. Plants were thinned to 21 plants per plot about three weeks after emergence. The date of fertilizer applications and field management were the same for all treatments. The trial design was a completely randomized plot design with three replications.

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![Fig. 1 Soil relative water content during maize development under different drought treatments (average of two growth periods).](https://example.com/fig1.png)
plot, \( W_u \) = upper limit of aimed soil water content, \( W_0 \) = actual soil weight water content before irrigation.

Indices assays

Two almost identically grown plants per plot were sampled at the following five growth stages: vegetative growth; early tasseling stage; end of tassel emergence; early milk; physiological maturity (40, 47, 58, 65, and 89 days after seeding). The first outspread leaf from top was cut and wiped with a wet pledget. Plant roots were sampled with a shovel in 0 to 80 cm soil depth below the maize stem and separated from the soil by washing over a sieve (2 mm mesh size). All samples were immediately frozen under liquid \( N_2 \) and then stored at super-low refrigerated conditions (-80°C) for further analysis.

Enzyme assays

Frozen leaf and root segments (0.5 g) were crushed into fine powder in a mortar in an ice-bath. 5.0 mL of 0.05 mol L\(^{-1}\) pH 7.8 phosphate buffer with 1% polyvinylpyrrolidone (PVP) was used as an extraction buffer. The homogenate was centrifuged at 15000xg for 15 min at 4°C. 10 mL phosphate buffer with pH 7.8 was added to the supernatant, which was used for enzyme (SOD, POD, CAT), MDA, and soluble protein content analysis.

SOD activity was assayed according to the method of Wang et al. (1983): One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction, measured with a UV/Vis spectrophotometer (DU-640, Beckman Company, USA) at 560 nm. CAT activity was determined by potassium permanganate titration (Gisnnopolitis and Nries 1977). The reaction mixture contained 2.9 mL 50 mM phosphate buffer (pH 7.0), 1.0 mL 10 mM \( H_2O_2 \), and 100 µL enzyme extract in a 3 mL volume. POD activity was analyzed in 2.9 mL 0.05 mol L\(^{-1}\) phosphate buffer containing 1.0 mL 0.05 mol L\(^{-1}\) guaiacol and 1.0 mL 2% \( H_2O_2 \) (AmaloK et al. 1994). The increase in absorbance at 470 nm was recorded after adding 2.0 mL 20% chloroacetic acid. Measurement of MDA was according to the method of Zhang (1992). Soluble protein content was determined by the method of Coomassie brilliant blue G-250 coloration with BSA as standard (Gisnnopolitis and Nries 1977).

Statistical analysis

Each determination was carried out on three separate samples and analyzed in triplicate and results were then averaged. Data were assessed by analysis of variance (ANOVA) and by Duncan’s multiple range test (\( P < 0.05 \)). Values followed by the same letters are not significantly different and different letters within treatments indicate significant differences at the 0.05 probability level (Table 2?). The \( t \)-test was used to test the significance of the coefficient of correlation according to Pearson. (These sentences are the same to that in Table 2 notes. Are they necessary here?)

RESULTS

Effects of water stress on protective enzyme activities in roots and leaves

The effects of water stress on the protective enzymes SOD, CAT, and POD in maize roots and leaves are shown in Fig.2. Under three different water treatments, the activities of SOD, CAT, and POD in leaves and roots first increased drastically and then declined during the period of maize development. The protective enzyme activities in roots were lower than those in leaves.

The activities of SOD in roots and CAT in roots and leaves were obviously increased at the vegetative growth period (40 days after seeding) and early period of tasseling (47 days after seeding), whereas the activities of SOD in leaves were only enhanced at the vegetative growth period (Fig.2). Compared to full water supply (treatment F), the activities of SOD in roots and leaves in treatment S increased significantly by 17 and 12%, 40 days after seeding. In treatment L the activities of SOD in roots and leaves increased drastically by 39 and 45%. The CAT activity in treatment S increased significantly by 77% (roots) and 67% (leaves) compared to treatment F, it increased by 35% (roots) and 137% (leaves) compared to treatment L at early tasseling stage (47 days after seeding). The activities of POD, both in roots and leaves, were enhanced at the vegetative growth period (40 days after seeding), early period
of tasseling (47 days after seeding), and end of tassel emergence (58 days after seeding). Compared to treatment F, POD activity in roots and leaves increased significantly by 63 and 52% in treatment S and in treatment L it increased by 34 and 29%, 58 days after seeding. In the later growing season the activities of SOD, CAT, and POD then decreased to the level of treatment F or even gradually lower. At physiological maturity stage (89 days after seeding) the activities of SOD, CAT, and POD in roots were significantly decreased by about 36, 14, and 41% in treatment S compared to the levels of treatment F, while they were decreased by 2, 14, and 23% in treatment L in comparison with the levels of treatment F. In leaves, the activities of SOD, CAT, and POD in treatment S were decreased by 32, 33, and 42% when compared with the levels of treatment F, and in treatment L they were decreased by 11, 20, and 14%, 89 days after seeding.

Effects of water stress on lipid peroxidation (MDA content) in roots and leaves

MDA is one of the ultimate products as a result of lipid peroxidation damage by free radicals. During maize growth, MDA content in roots and leaves increased from vegetative growth to the early tasseling period.

Fig. 2 SOD, CAT, and POD activity during summer maize development under different water stress treatments. The data in the figure is the average of the two years. The same as below.
then decreased during the flowering period and increased again in the fruit development period. The content of MDA in roots was lower than that in leaves (Fig. 3).

Under drought stress, the MDA content in roots and leaves both increased in dependence of drought intensity, development stage, and plant organ (Fig. 3). The MDA content in roots and leaves increased drastically in treatments L and S from 16 to 64% when compared with the level of treatment F. Over the whole experiment period increase of MDA content in treatment S was between 35 and 401% when compared with the levels of treatment F. Significant differences were observed among the different water treatments. The highest increase of MDA in the roots was observed at vegetative growth period (40 days after seeding) with 401% (treatment S compared with treatment F). MDA content reached 5.36 µmol g⁻¹ FW in treatment S. MDA content in leaves was highest in treatment S at physiological maturity period (89 days after seeding) with 39.70 µmol g⁻¹ FW, being nearly 180%, of the value of treatment F. Although the MDA content in maize leaves was generally higher than in roots, the relative increase in MDA content because of drought was higher in the roots than in the leaves.

Effects of water stress on soluble protein content in roots and leaves

Contents of soluble proteins in roots and leaves increased gradually in the developmental stages. The content of soluble proteins in the roots was maximal 47 days after seeding (early tasseling stage), whereas the maximum in the leaves was reached 58 days after seeding (end of tassel emergence period). In the later vegetation period the content of soluble proteins decreased both in roots and leaves (Fig. 4). The content of soluble proteins in roots was lower than in leaves.

Under drought stress, the content of soluble protein was reduced both in roots and leaves. The amount of reduction was related to drought intensity, drought duration, and growth stage (Fig. 4). During the vegeta-

![Fig. 3](image1.png) Effects of different drought treatments on MDA content in roots and leaves of summer maize.

![Fig. 4](image2.png) Soluble protein content in roots and leaves of summer maize under different drought treatments during the vegetation period.
tive growth period (40 days after seeding) the soluble protein content in roots and leaves of treatment F were 2.07 mg g⁻¹ FW and 11.84 mg g⁻¹ FW, that of treatment L were 79 and 91% of those in treatment F, and in treatment S they were 73 and 72% of those in treatment F. The peak contents of soluble proteins in roots (47 days after seeding) were 3.16 mg g⁻¹ FW (treatment F), 3.03 mg g⁻¹ FW (treatment L), and 2.74 mg g⁻¹ FW (treatment S). Peak contents in leaves (58 days after seeding) were 18.47 mg g⁻¹ FW (treatment F), 17.21 mg g⁻¹ FW (treatment L), and 14.50 mg g⁻¹ FW (treatment S).

Relationship between protective enzyme activities and lipid peroxidation in roots and leaves

The activities of protective enzymes and lipid peroxidation in the roots were positively related to those in the leaves, most of the correlation coefficients being significant (Table 1).

### Table 1 Correlation coefficients of protective enzyme activities and lipid peroxidation (MDA content) between roots and leaves of maize under water stress

<table>
<thead>
<tr>
<th></th>
<th>CAT</th>
<th>POD</th>
<th>SOD</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light water stress</td>
<td>0.96*</td>
<td>0.83*</td>
<td>0.58</td>
<td>0.72*</td>
</tr>
<tr>
<td>Severe water stress</td>
<td>0.84*</td>
<td>0.84*</td>
<td>0.74*</td>
<td>0.77*</td>
</tr>
</tbody>
</table>

CAT, catalase; POD, peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde.

*,** Significance of the correlation coefficient on the P < 0.05 and P < 0.01 level.

### Table 2 Effect of different drought treatments on yield and its components of maize

<table>
<thead>
<tr>
<th></th>
<th>Treatment F</th>
<th>Treatment L</th>
<th>Treatment S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear length (cm)</td>
<td>24.1 ± 0.2a</td>
<td>18.6 ± 0.1b</td>
<td>7.8 ± 0.7c</td>
</tr>
<tr>
<td>Ear diameter (mm)</td>
<td>29.4 ± 0.5a</td>
<td>26.1 ± 0.9b</td>
<td>16.5 ± 1.3c</td>
</tr>
<tr>
<td>Ear kernels number (n)</td>
<td>488 ± 25a</td>
<td>352 ± 9b</td>
<td>30 ± 10c</td>
</tr>
<tr>
<td>100-kernel weight (g)</td>
<td>27.27 ± 0.60a</td>
<td>22.31 ± 1.35 a</td>
<td>16.37 ± 0.62 b</td>
</tr>
<tr>
<td>Grain yield (kg m⁻²) or Grain yield in 4 m²(kg)?</td>
<td>2.82 ± 0.28a</td>
<td>2.08 ± 0.08a</td>
<td>0.53 ± 0.10b</td>
</tr>
</tbody>
</table>

F, full water supply (80 % of soil water capacity); L, light water stress (60 % of soil water capacity); S, severe water stress (40 % of soil water capacity). Each value represents the mean of three replications with at least three plants sampled in each replication in two growing seasons (2002, 2003). Results are the means ± standard errors. Data were assessed by analysis of variance (ANOVA) and by Duncan’s multiple range test (P < 0.05). Values followed by the same letters are not significantly different and different letters within treatments indicate significant differences at the 0.05 probability level.

## DISCUSSION

Active oxygen was accumulated in plants under water stress. There is a defensive system in plants, that is to say, plants have an internal protective enzyme-catalyzed cleanup system, which is fine and elaborate enough to avoid injuries of active oxygen, thus guaranteeing normal cellular function (Wang 2002). When the plants suffered from water stress, the whole defensive system needs to be activated in order to resist the active oxygen injury. Single antioxidant enzymes or antioxidants cannot resist the injuries. It was found that the activities of SOD, POD, and CAT of many plants were affected by drought (Jiang and Ren 2004). Numerous studies have shown that the degree of injury caused by drought was negatively correlated to the increase of activities of SOD, POD, and CAT (Dhindsa and Matow 1981; Chowdhury and Choudhuri 1985). But some re-

**Effects of water stress on yield and yield components of maize**

Maize yield was highly related to drought stress, leading to high yield reduction with decreasing soil water content (Table 2). No economic yield was formed in treatment S as it decreased to only 19% compared with treatment F. The main factors that caused yield reduction were the decrease of the grain number per ear and the drop of 100-kernel weight.

When compared to the control levels of treatment F (Table 2), grain yields and the numbers of ear kernels were decreased by 26 and 28% for treatment L, and 81 and 94% for treatment S. Moreover, ear length, ear diameter, and 100-kernel weight were decreased in treatment S by 6.3 cm, 30.3 mm, and 9.9 g (32, 56, and 60%), compared with treatment F.
results were different from this, for example, Sun et al. found that SOD and POD activities increased and CAT activity decreased under water stress (Wang et al. 2002). Some others got the similar results (Inkai Iturbe-Ormatexe et al. 1998). In this study, it was found that SOD, POD, and CAT activities in roots and leaves increased at prophase and metaphase, but decreased at anaphase (Fig. 2). These differences may result in different researchers using different experimental methods, i.e., different materials and different treatment methods. In this study the soil drought stress was applied during the whole growth period. The increase of activities of the three enzymes at the prophase and metaphase was probably one of the response mechanisms, alleviating injury and increasing resistance to drought at anaphase. SOD, POD, and CAT may be the first protective system becoming active to resist drought. When crops encountered drought at an early phase, the system protecting the plant from oxidant injuries played a very important role. During anaphase, plants were rapidly detoxified by various cellular enzymatic and nonenzymatic mechanisms and DNA injury repair systems. Thus they got used to drought and completed their life cycle.

Protective enzyme activities in roots were lower and also more sensitive to water stress than those in leaves. Soil drought probably acted directly on roots, whereas leaves could reduce water transpiration by curling and stoma regulation. Furthermore, the degree of leaf injury caused by drought could be lessened through morphological adaptation and regulation of stem and sheath water content, stem diameter or plant height. It was obvious that root cells suffered more severe injury because of soil drought as cell membranes broke and endogenetic protective enzyme activities decreased and MDA (the product of lipid peroxidation) accumulated. In conclusion, it was noted that roots suffered more severe injury than leaves under drought stress. More attention should be paid to this.

The decrease of the activities of the protective enzymes SOD, POD, and CAT were closely correlated to the accumulation of MDA, interacting as both cause and effect. On the one hand, because of the reduction of the activities of the enzymes, free radicals accumulated and even exceeded the injury threshold. On account of that, the content of MDA increased and the plasma membrane suffered from injury through direct and indirect initiation of lipid peroxidation. On the other hand, accumulation of MDA inhibited the activities of the enzymes so their protective function was lost and the membrane injury further worsened. This showed that the ability of crops to resist drought was connected to the activities of protective enzymes and their defensive function. These mechanisms may be the main physiological action and defense from injury of crops under drought stress. Furthermore, the content of soluble protein in roots and leaves decreased under drought stress, and the more severe the water stress was, the more it decreased.

CONCLUSION

In conclusion, the results clearly suggest that in maize, under drought stress, during different growth periods, the activities of SOD, POD, and CAT in roots and leaves basically decrease and membrane lipid peroxidation increase. Direct injury to the cell membrane system is then induced, maybe indicating one of the physiological response mechanisms to drought conditions. This conclusion is the same as previous ones. However, the change of activities of the three protective enzymes and the change of MDA content are different at different growth periods because of the different intensity and duration of drought stress. The activities of SOD and POD increased at the vegetative growth period and then greatly declined. The longer and more severe the drought stress is, the lower is the activity of the protective enzymes and the higher the content of MDA. The early tasseling to milky stages are the most important stages during the whole growth period with respect to drought stress. The more severe the stress are in these stages, the more seriously the mechanisms of physiological protection and the membrane system are hurt. This emphasizes the importance of critical growth stages for water supply.

Under drought stress during the whole vegetation period the accumulation of assimilates are reduced and the translocation and redistribution of nutrients are affected. This may have been one of the main reasons that the capacity of the sink organ descended, yield properties deteriorated significantly, and economic yield dropped sharply.
Acknowledgments
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