Effects of Coronatine on Growth, Gas Exchange Traits, Chlorophyll Content, Antioxidant Enzymes and Lipid Peroxidation in Maize (Zea mays L.) Seedlings under Simulated Drought Stress

Baoqing Wang, Zhaohu Li, A. Egrinya Eneji, Xiaoli Tian, Zhixi Zhai, Jianmin Li and Liusheng Duan*

(State key Laboratory of Plant Physiology and Biochemistry and College of Agronomy and Biotechnology, China Agricultural University, Beijing 100094, P.R. China)

Abstract: Coronatine is a phytotoxin that affects the accumulation of defence-related metabolites in plants but information on how its effects may be mediated by environmental stress is scanty. An experiment was carried out to determine the changes in growth, gas exchange, relative water content, chlorophyll (Chl) content, antioxidant enzymes and lipid peroxidation in maize (Zea mays L., var. ‘Nongda 3138’) seedlings treated with coronatine under simulated drought stress. Seedlings raised hydroponically in a growth chamber with simulated drought for 8d (long-period drought) or 3d (short-period drought) were treated with or without coronatine at the three-leaf stage. Under the drought condition, treated with coronatine significantly increased the fresh weight and relative water content in leaves of seedling leaves. The increase was accompanied by increased rates of photosynthesis and transpiration, and the maintenance of Chl pigments. Coronatine had no effects on catalase (CAT), guaiacol peroxidase (POD) and glutathione reductase (GR) under normal condition, but it significantly enhanced activities of CAT, POD and GR in stressed seedlings under the long-period drought treatment. Under the short-period drought treatment, the POD and GR activity in the seedlings treated with coronatine were much higher than in those not treated. Malondialdehyde (MDA) increased sharply under drought condition, but treatment with coronatine significantly reduced it by 15%. The total Chl content of leaves under the drought condition was markedly increased by the treatment with coronatine. Seedlings subjected to a short-period drought had reduced water content, but recovered fairly well by the treatment with coronatine with negligible effects on most physiological and biochemical processes. The application of coronatine alleviated the drought stress in maize seedlings and enhanced their tolerance of water stress through changes in physiological and anti-oxidant enzyme activities.

Key words: Antioxidant enzymes, Coronatine, Drought, Gas exchange, Maize seedlings.

Coronatine is a phytotoxin produced by several pathovars of Pseudomonas syringae including pv. atropurpurea, glycinea, maculicola, morsprunorum and tomato (Bender et al., 1999). The most prominent effect of coronatine is an intense spreading of chlorosis in leaf tissues of plants. Previous research results indicated that coronatine mimics a precursor of jasmonic acid (JA) which is involved in the biological processes of plant growth and development (Sakai, 1980, 1981; Sakai et al., 1984; Ferguson and Mitchell, 1985; Mino et al., 1987; Kenyon and Turner, 1990; Weiler et al., 1993, 1994). Coronatine affects the accumulation of defense-related secondary metabolites such as pterocarpan-type glyceollin, sakuranetin, momilactone A, alkaloid, volatile materials and taxol (Ichihara and Toshima, 1998; Tamogami and Kodama, 2000; Fliegmann et al., 2003; Lauchli and Boland, 2003). It can also induce the accumulation of proteinase inhibitors associated with herbivory (Ryan, 1992) and the expression of a special gene JIP (JA-induced protein gene) under osmotic stress in barley (Hause et al., 1996). Investigations have revealed that coronatine showed different physiological effects on different tissues (Kenyon and Turner, 1990), and it is plausible that it acts differently in monocotyledon and dicotyledon plants (Uppalapati et al., 2005). Many reports have shown that coronatine mediates the stress response associated with pathogens and herbivory (Lauchli and Boland, 2003; Schuler et al., 2004), and it was suggested to mediate plant responses to environment stresses. Our previous study showed that coronatine alleviated salinity stress in cotton by improving the antioxidative defense system and DPPH-radical scavenging activity (Xie et al., 2008), but its effects on drought and osmotic stress is still unknown.
Drought is one of the most limiting factors of crop production, and one of the most important drought-related injuries is from osmotic stress, which leads to dehydration of plant cells and stunted growth. Furthermore, it disturbs the process of photosynthesis and respiration through the accumulation of activated oxygen species (AOS). Osmotic stress impairs the photosynthetic electron transport systems (Sharma and Dubey, 2005). Plants have both enzymatic and non-enzymatic antioxidant systems to prevent or alleviate the damage caused by AOS. Several enzymes can efficiently detoxify AOS, whereas superoxide radicals which are the first AOS produced in plant cells are detoxified by superoxide dismutase (SOD). Hydrogen peroxide is reduced to $\text{H}_2\text{O}$ and destroyed by catalase (CAT) and different peroxidase such as guaiacol peroxidase (POD) and ascorbate peroxidase (APX). The decreased formation of glutathione reduces the ascorbate oxidized by APX and the glutathione is again reduced by the glutathione reductase (GR) (Alscher et al., 1997). Therefore, exogenous application of some free radical scavengers could decrease the membrane damage and increase the enzymatic defence systems against AOS.

There is evidence that plant responses to drought can be modulated by various plant growth regulators such as abscisic acid (ABA), salicylic acid (Agarwal et al., 2005), JA, betain (Gao et al., 2004) and spermidine (Blamowski et al., 2001). Yan et al. (1999) reported that coronatine could enhance the resistance of millet seedlings to water stress. Low concentrations of coronatine may increase the relative water content (RWC) in leaves of maize seedlings, but the physiological and biochemical effects of coronatine on maize under drought are not well known.

Therefore, our objective was to determine the effects of coronatine on the drought tolerance of maize seedlings, and explore its potential application to field crop production. We compared the effects of coronatine on the growth, leaf RWC, gas exchange traits, chlorophyll pigments, lipid peroxidation and antioxidant enzyme defence system of maize seedlings under normal, long-period and short-period drought conditions.

### Materials and Methods

#### 1. Plant materials and treatments

Sterilized maize (*Zea mays* L., var. ‘Nongda 3138’) seeds were germinated for 4 days at room temperature (25°C). The resulting seedlings were grown in Hoagland’s nutrient solution (Hoagland and Arnon, 1950) in a phytotron at 25/20°C day/night temperature with a 16-light and 8-h dark period. The relative humidity inside the phytotron was 65% and an irradiance of 400 $\mu$mol m$^{-2}$s$^{-1}$ at the leaf level was provided from metal halide lamps. Coronatine was applied to the nutrient solution at a concentration of $2 \times 10^{-8}$ mol L$^{-1}$, which was considered adequate for improving salinity resistance when applied to the seedlings developed three unfolded leaves. Our preliminary evaluation also showed this concentration to have the most measurable effects on maize seedlings. Drought (8d or 3d) stress was simulated by adding 15% polyethylene glycol (PEG-6000) to the nutrient solution 24 hours after coronatine application. The water potential in PEG-6000 treated solutions was -0.5 MPa. Three days after drought, half of the stressed plants were transferred to nutrient solution for the next 5 days (short-period drought treatment), and half remained in drought stress (long-period drought treatment). Thus we had coronatine-treated and water-treated plants, grown at normal, long-period and short-period drought conditions. The six treatments described in Table 1 were studied in a completely randomised design with four replications.

#### 2. Measurement of relative water content and gas exchange

The RWC was measured on the youngest fully expanded leaves following the method of Turner (1981). Five plants were examined in each replication. Fresh weight (FW) of leaves was determined immediately after harvest, and then leaf discs were allowed to float on distilled water until fully rehydrated. Leaf discs were weighed for turgid weight (TW). The turgid leaves were dried in a hot air oven at 80°C to a constant weight and dry weight (DW) was recorded. The RWC of the leaves was calculated as:

### Table 1. Description of treatments used in the study.

| Treatment and their acronyms | Description |
|-----------------------------|-------------|
| Normal Control              | No drought stress, no application of coronatine (control) |
| COR                         | CK + application of coronatine (20 nmol L$^{-1}$) at the 3-leaf stage. |
| Long-period drought         | Drought (8 days) condition simulated by adding 15% PEG-6000 to culture solution |
| PEG 8                       | Application of coronatine + PEG8 |
| COR + PEG 8                 | Growing under drought condition as above for 3 days; seedlings were transferred to continue growth in normal culture solution for the remaining days of observation. |
| Short-period drought        | Application of coronatine + (PEG 3 + RW) |

CK, control; COR, coronatine; PEG, polyethylene glycol 6000; PEG 3, PEG treatment for 3 days; PEG 8, PEG treatment for 8 days.
was 75 under two 15 W fluorescent lamps (detected irradiance containing the mixture were shaken and incubated for the assay. Riboflavin was then added and tubes were homogenised in liquid N2 using a mortar and pestle and then re-suspended in 5 mL of 100 mM potassium phosphate buffer (pH 7.0) containing 2 mM ethylenediaminetetraacetic acid (EDTA) and 1% (w/v) polyvinylpyrrolidone (PVP) (Xu and Huang, 2004). The extraction buffer used for the APX assay contained 0.2 mM ascorbate. The homogenate was centrifuged (15,000 g, for 20 min), and the supernatant was used for the assays.

The SOD activity was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT) using the method of Dhindsa et al. (1981). A 3 mL reaction mixture containing 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM NBT, 2 mM riboflavin, 0.1 mM EDTA, and 0 or 50 μL enzyme extract was prepared for the assay. Riboflavin was then added and tubes containing the mixture were shaken and incubated under two 15 W fluorescent lamps (detected irradiance was 75 μmol m−2 s−1) for 10 min. The absorbance of the reaction mixture was read at 560 nm.

The CAT activity was determined by measuring the rate of disappearance of hydrogen peroxide using the method of Kang and Saltveit (2002). The reaction mixture for the determination contained 2.5 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1% hydrogen peroxide, and 50 μL enzyme extract diluted to keep measurements within the linear range of the analysis. The decrease in hydrogen peroxide was inferred from the decline in absorbance at 240 nm.

The POD activity was determined according to Upadhyaya et al. (1985) in a reaction mixture containing 2.5 mL of 50 mM phosphate buffer (pH 7.0), 1 mL of 1% hydrogen peroxide, 1 mL of 1% guaiacol and 50 μL enzyme extract. The increase in absorbance at 420 nm was followed for 1 min.

The APX activity was determined according to the method of Chen and Asada (1989) in a 1 mL reaction mixture of 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 0.5 mM ascorbate, 1.54 mM hydrogen peroxide, and 50 μL enzyme extract. The oxidation of ascorbate was followed by the decrease in the absorbance at 240 nm.

The GR activity was assayed by measuring the decrease in absorbance at 334 nm due to the oxidation of NADPH (Klapheck et al., 1990) in a 1 mL reaction mixture of 0.1 M Tris-HCl, pH 8.0, 1 mM EDTA, 0.1 mM NADPH, 1 mM GSSG and 50 μL enzyme extract at 30°C.

Protein content was determined using bovine serum albumin (BSA) as a standard, according to the method of Bradford (1976). Five plants were examined in each replication. Each of the reaction system was at 25-30°C in enzyme and protein assay. All enzymes activities were calculated per milligram of protein per minute and expressed as a percentage of the control.

### 4. Determination of contents of malondialdehyde and chlorophyll

Lipid peroxidation was estimated from the level of malondialdehyde (MDA) production following the thiobarbituric acid (TBA) method as described in Buege and Aust (1978). Maize leaves (0.5 g) were harvested and homogenized with a mortar and pestle in 5 mL of 0.5% (v/v) TBA solution in 20% (v/v) trichloroacetic acid. The homogenate was centrifuged at 20,000 g for 15 min and the supernatant was heated in a boiling water bath for 25 min and allowed to cool in an ice bath. The supernatant was centrifuged at 20,000 g for 15 min, and used for spectrophotometric determination of MDA. Absorbance at 532 nm was recorded and corrected for non-specific absorbance at 600 nm. The MDA concentrations were calculated using an extinction coefficient of 156 mM−1 cm−1 from the formula: MDA (mol g−1 fresh wt.) = [(A532−A600)/156] × 105 × dilution factor (Du and Bramlage, 1992).

Chl was extracted and assayed according to Hiscox and Israelstam (1979). Leaves of five seedlings (0.5 g) from each replication were soaked in 10 mL dimethyl sulfoxide for 48 h in darkness at room temperature. Total Chl content of extracts was determined spectrophotometrically. Chl content (mg g−1 fresh weight) was calculated according to the formula: (20.2 × A665 + 8.02 × A645) × dilution factor.

### 5. Statistical analyses

The data were analyzed by ANOVA, using SAS (version 8.0) software. Treatment means were compared using Duncan’s multiple range test (P<0.05). Values presented are means of three independent experiments.

### Results

#### 1. Changes in biomass of seedlings

Both the fresh and dry weights of seedlings were significantly reduced by simulated drought treatments...
Table 2. Influence of coronatine on fresh and dry weight (g/shoot) of maize seedlings under normal, long- and short-period drought.

| Treatments         | Fresh weight (g shoot\(^{-1}\)) | Dry weight (g shoot\(^{-1}\)) |
|--------------------|----------------------------------|--------------------------------|
|                    | Days after application of coronatine | Days after application of coronatine | Days after application of coronatine |
|                    | 4d  | 6d  | 8d  | 4d  | 6d  | 8d  | 4d  | 6d  | 8d  |
| Control            | 15.0 a | 21.0 a | 27.0 a | 1.40 a | 1.90 a | 2.10 a |
| COR                | 15.0 a | 21.0 a | 27.0 a | 1.40 a | 1.80 a | 2.00 a |
| PEG8               | 6.0 c | 7.0 d | 8.0 d | 0.80 b | 0.90 d | 0.90 b |
| COR + PEG8         | 8.0 b | 9.0 c | 10.0 c | 0.90 b | 1.00 cd | 1.00 b |
| PEG3 + RW          | 8.0 b | 13.0 bc | 22.0 b | 0.70 b | 1.30 bcd | 1.80 a |
| COR + PEG3 + RW    | 9.0 b | 15.0 bc | 26.0 ab | 0.70 b | 1.40 bc | 1.80 a |

Values are means of three independent experiments. Means followed by different letters within a column are statistically different (\(P<0.05\)) according to Duncan’s multiple range test. The abbreviations for treatments are as shown in Table 1.

Table 3. Influence of coronatine on the relative water content (%) of maize seedlings under normal, long- and short-period drought.

| Treatments         | Relative water content (%) | Days after application of coronatine |
|--------------------|----------------------------|-----------------------------------|
|                    |                            | 4d  | 6d  | 8d  |
| Control            | 98.0 a                     | 98.0 a | 95.0 a |
| COR                | 97.0 a                     | 97.0 a | 95.0 a |
| PEG 8              | 79.0 c                     | 77.0 c | 72.0 c |
| COR + PEG 8        | 85.0 b                     | 84.0 b | 80.0 b |
| PEG 3 + RW         | 90.0 ab                    | 94.0 a | 94.0 a |
| COR + PEG 3 + RW   | 95.0 a                     | 97.0 a | 97.0 a |

Values are means of three independent experiments. Means followed by different letters within a column are statistically different (\(P<0.05\)) according to Duncan’s multiple range test. The abbreviations for treatments are as shown in Table 1.

2. Relative water content

The RWC of leaves in non-stressed seedlings (Control) was significantly higher than that in long-period drought treatment (PEG8) and was not affected by coronatine in normal treatments (Table 3). However, seedlings treated with coronatine (COR + PEG8) under long-period drought maintained a much higher RWC than those untreated (PEG8). Seedlings under short-period drought recovered quickly as evidenced from the high RWC values in PEG3 and COR + PEG3 which were similar to those in non-stressed seedlings.

3. Gas exchange parameters

The rate of photosynthesis (Pn) decreased significantly in the seedlings under long-period (8d) drought compared with other treatments (Fig. 1A). Although coronatine had little effect on Pn in non-stressed seedlings, it either increased Pn significantly or maintained its levels during long- and short-period drought.

In non-stressed seedlings under normal condition, the rate of transpiration (T) did not vary significantly. The transpiration rate decreased substantially under long- and short-period drought, but coronatine significantly increased it under long-period drought on the 8th day of observation (Fig. 1B).

4. Antioxidant enzymes assay

The soluble protein content of leaves and activities of antioxidant enzymes were analyzed on the 8th day after coronatine application (Fig. 2A). Coronatine treatment caused a marked increase in soluble protein content in both drought conditions, but seedlings treated with coronatine under short-period drought (COR + PEG3) contained it (soluble protein) at the highest levels.

Under normal conditions, coronatine had negligible effects on the activities of SOD, CAT, POD, APX and GR (Fig. 2B-F) in maize seedlings but under drought, it significantly increased the activities of POD and CAT under long-period drought and that of POD under short-period drought. The activities of POD and CAT were highest under long-period drought, following coronatine application. The GR activity in coronatine applied seedlings increased in both drought treatments (COR + PEG8 and COR + PEG3 + RW).

5. Lipid peroxidation and chlorophyll content

In the absence of drought stress, there were no effects of coronatine on the MDA content, which is an indicator of leaf membrane damage (Fig. 3).
In contrast, the MDA content increased sharply under long-period drought, which is an evidence of membrane damage, but this was significantly reduced (15%) by the treatment with coronatine (COR + PEG8). The MDA content under short-period drought (PEG3) was not affected by coronatine (COR + PEG3) and was much lower than that under long-period drought.

Contents of Chl $a$, Chl $b$, and Chl $a+b$ were affected by drought and coronatine treatment (Table 4). Under non-stress, coronatine had little effect on Chl $a$, but increased Chl $b$. However, seedlings treated with coronatine under short-period drought stress (COR + PEG3) showed higher values of Chl $a$ than those not treated. Chl $b$ was significantly greater in coronatine-treated seedlings under the drought treatments (COR + PEG8 and COR + PEG3) than those not treated. Although drought reduced significantly the total Chl content (Chl $a+b$) in PEG8 treatment, the Chl $a+b$ in coronatine applied seedlings (COR + PEG8) remained similar to that under normal conditions. The Chl $a+b$ in coronatine-applied seedlings under both drought conditions (COR + PEG8 and COR + PEG3) were much greater than the seedlings not treated with coronatine (PEG8 and PEG3). The coronatine treatment decreased the Chl $a$/Chl $b$ ratio, although it was not significant in the normal condition (Table 4).

**Discussion**

Coronatine increased the fresh biomass (FW) of the seedlings under long-period drought possibly through its favourable effect on the maintenance of high leaf water content. Such an increase in FW was similar to that induced by ABA (Young et al., 1992). It is therefore conceivable that coronatine influences FW and RWC of leaves through a mechanism which is similar to ABA. Conversely, coronatine failed to
improve the biomass under normal and short-period drought as reported previously by Yan et al. (1999).

Application of coronatine also enhanced the levels of Chl and photosynthesis under long-period drought. Drought-induced reduction in photosynthesis was previously ascribed to the reduction in the conductivity of the stomata (Németh et al., 2002). In our study, the transpiration rate did not reduce in leaves of coronatine treated plants during stress period, probably because of the improved water balance. The rate of transpiration depends not only on the influence of the stomata, but also on leaf water content and other factors (Ghannoum et al., 2003). Todorov et al. (1998) also found a higher rate of transpiration in maize plants treated with ABA under drought condition. Coronatine might have regulated the stomatal conductivity to reduce leaf water consumption under drought stress as reported for ABA (Schroeder et al., 2001; Luan, 2002) and JA or methyl jasmonate (MeJA) (Lan et al., 2004), but this remains to be examined further.

Although coronatine significantly increased the content of Chl b under drought conditions, it retarded the Chl a content of seedlings. Our observation on the effect of coronatine on Chl contents was similar to that in ABA-treated wheat (Agarwal et al., 2005). However, the wavelength of Chl b harvesting light energy is not efficiently absorbed by Chl a. In addition, Chl b plays an important role in regulation of photosynthetic antenna size (Yamasato et al., 2005). That is to say,
seedlings treated with coronatine may capture more light energy under stress. A close relationship exists between lipid peroxidation and content of Chl (Dhindsa et al., 1981). Malondialdehyde is a product of lipid peroxidation and is used to scale the degree of damage in cell membranes under drought stress. Thus, the observed decrease in the level of MDA in coronatine-treated treatments under drought could explain the increased level of Chl content in our experiment. The action of coronatine under drought played a key role in protecting the structure and function of cell membranes and Chl pigments.

Coronatine significantly enhanced the activity of antioxidant enzymes (CAT, POD, GR) under long-period drought stress, while the POD and GR remained at higher levels after short-period drought. Drought not only damages the Chl pigment but also destroys normal photosynthetic electron transport system, which leads to production of AOS (Zgallai et al., 2005). In general, plants are protected from oxidative stress by a complex of antioxidant enzyme systems. Our data suggest that coronatine might have induced the decrease in oxidative stress, which was responsible for increasing membrane stability and Chl content. This effect was manifested by an increase in RWC and seedling growth. Our results are in conformity with those of Jiang and Zhang (2001; 2002) who examined the effect of low concentration (0.5 mM) of ABA on maize seedlings.

In this study, application of coronatine supported a considerably higher water content of leaves especially when seedlings were subjected to drought stress and it improved the gas exchange traits in leaves of maize seedlings (rates of photosynthesis and transpiration). It also promoted the activities of antioxidant enzymes, especially CAT, POD as well as GR and reduced cell membrane damage due to drought. Thus, we conclude that the application of 20 nmol L\(^{-1}\) coronatine may alleviate drought in maize seedlings. We also suggest that crop chemical regulation is one of the ways to induce tolerance and mitigate the effects of drought stress on maize seedlings.

Table 4. Influence of coronatine on the chlorophyll (Chl) content (mg g\(^{-1}\) FW) in the youngest fully developed leaves of maize seedlings at the end of all treatment programs.

| Treatments            | Chl \(a\) | Chl \(b\) | Chl \(a + b\) | Chl \(a / Chl b\) |
|-----------------------|-----------|-----------|---------------|-------------------|
| Control               | 1.240 ab  | 0.300 c   | 1.540 ab      | 4.130 b           |
| COR                   | 1.210 ab  | 0.350 ab  | 1.560 ab      | 3.460 b           |
| PEG 8                 | 1.100 b   | 0.200 d   | 1.300 c       | 5.500 a           |
| COR + PEG 8           | 1.140 b   | 0.350 ab  | 1.490 ab      | 3.260 c           |
| PEG 3 + RW            | 1.130 b   | 0.300 c   | 1.430 b       | 3.770 b           |
| COR + PEG 3 + RW      | 1.270 a   | 0.400 a   | 1.670 a       | 3.180 c           |

Values are means of three independent experiments. Means followed by different letters within a column are statistically different (\(P<0.05\)) according to Duncan’s multiple range test. The abbreviations for treatments are as shown in Table 1.

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