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# Physiological Evaluation of Drought Stress Tolerance and Recovery in Cauliflower (*Brassica oleracea* L.) Seedlings Treated with Methyl Jasmonate and Coronatine

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**Abstract** Coronatine (COR) is a chlorosis-inducing phytotoxin that mimics some biological activities of methyl jasmonate (MeJA). Although MeJA has been reported to alleviate drought stress, it is unclear if COR has the same ability. Our objective was to determine the influence of exogenously applied MeJA and COR on the growth and metabolism of cauliflower seedlings under drought stress and recovery. Both MeJA and COR enhanced the growth and accumulation of dry matter in cauliflower seedlings during drought-stressed and rewatering conditions. Treatment with MeJA or COR enhanced tolerance of drought stress through increased accumulation of chlorophyll and net photosynthetic rate. Enzymatic (superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, and glutathione reductase) and nonenzymatic antioxidant (proline and soluble sugar) systems were activated, and lipid peroxidant (malondialdehyde and hydrogen peroxide) was suppressed by MeJA and COR under drought stress. MeJA and COR also increased leaf relative water content and endogenous abscisic acid level under drought-stressed conditions. After rewatering, the contents of leaf water, chlorophyll, abscisic acid, and photosynthetic characteristics as well as enzymatic and nonenzymatic antioxidant systems showed nearly complete recovery. Both MeJA and COR can alleviate the adverse effects of drought stress and enhance the ability for water stress resistance through promotion of defense-related metabolism in cauliflower seedlings.

**Keywords** Cauliflower · Methyl jasmonate · Coronatine · Drought stress · Antioxidant defense · Photosynthesis

## Introduction

Drought and water stress are considered some of the main environmental factors limiting crop growth and yields in the world (Panozzo and Eagles 1999) and are major issues of future climate change (Loreto and Centritto 2008). Thus, crops must adapt and evolve survival strategies to increase their tolerance in their rapidly changing habitats. Crop developmental stages and drought stress severity and duration affect mainly crops resistant to water stress. In general, symptoms of drought stress are suppressed growth (Clua and others 2009; Galle and others 2010), reduced photosynthetic rate (Warren and Adams 2006; Flexas and others 2008), and accelerated leaf senescence (Rivero and others 2009). Moreover, drought stress can trigger an oxidative burst, accelerate the degradation of photosynthetic pigments and cell membrane damage, induce an array of antioxidant enzymes expression, and elicit membrane lipid accumulation (Zgallai and others 2005; Ai and others 2008).

Cauliflower (*Brassica oleracea* L. var. *botrytis*) is an important vegetable in China, which has the largest planting acreage and production of cauliflower in the world. According to a FAO estimate (http://www.fao.org/waicent/portal/statistics.en.asp), the annual production of cauliflower in China was 8.3 million tons, representing around

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46.3% of global production. Cauliflower is sensitive to drought stress at all growth stages, responding to drought with reduced growth and premature heading. Therefore, cauliflower growth is limited and its dry matter production and yield are more seriously depressed by water deficit (Cheruth and others 2009). Exposure of plants to drought stress induces numerous physiological and biochemical changes resulting in a disturbance of normal growth and development. Multiple factors activate the resistance response of plants to drought stress, and phytohormones are one of the most important endogenous substances involved in the mechanisms of susceptibility or tolerance of various plant species (Dodd and Davies 2004). Several studies have shown that the improvement of plant drought resistance is induced by phytohormones such as abscisic acid (Yang and others 2007), brassinosteroid (Behnamnia and others 2009), and jasmonates (Creelman and Mullet 1995; Pedranzani and others 2007). The physiological mechanisms and hormonal relationships that alleviate or enhance adverse effects of drought stress are unknown in cauliflower.

Methyl jasmonate (MeJA) is a naturally occurring plant growth regulator in higher plants. Exogenous MeJA can elicit a great variety of morphological and physiological responses to environmental stresses (Creelman and Mullet 1995). It has been reported that MeJA can alleviate damaging effects of drought stress by changing endogenous phytohormones, polyamines, and protein-banding patterns of soybean (Hassanein and others 2009). A role for MeJA in plant response to water deficit has been suggested because the stress induces the expression of jasmonate-responsive genes (Agrawal and others 2003). In addition, several reports have shown that jasmonates play an important role in signaling drought-induced antioxidant responses (Shan and Liang 2010). Exogenously supplied JA or MeJA can increase the transcript levels and activities of antioxidant enzymes in plants under water stress (Shan and Liang 2010). Coronatine (COR), a non-host-specific phytotoxin produced by several pathovars of *Pseudomonas syringae* (Bender and others 1999), is a structural and functional mimic of MeJA, but it is more active than MeJA with regard to production of secondary metabolites (Tamogami and Kodama 2000). However, COR and MeJA have similar but not identical activities in and effects on plants (Uppalapati and others 2005). Coronatine can induce a wide array of effects in plants, for example, inhibition of root elongation and hypertrophy, senescence, production of defense-related protease inhibitors, and resistance to abiotic stresses (Bender and others 1999; Schüler and others 2004; Uppalapati and others 2005; Ai and others 2008; Braun and others 2009). However, few studies have shown that exogenously applied MeJA and COR could alleviate or enhance adverse effects of drought stress in cauliflower.

The objective of this work, therefore, was to evaluate the role of exogenously applied MeJA and COR in counteracting drought stress in cauliflower. The present work was carried out in an attempt to understand the physiological mechanism of MeJA- and COR-induced tolerance to drought stress in cauliflower. For this, we investigated the changes of growth (expressed as dry weight) and photosynthesis (chlorophyll and net photosynthetic rate) in cauliflower treated with MeJA and COR under drought stress and water recovery. We also verified the hypothesis that MeJA- and COR-induced changes in growth and metabolism are possibly related to their influence on the oxidative response, that is, enzymatic (superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, and glutathione reductase) and nonenzymatic antioxidant (proline and soluble sugar) systems and lipid peroxidant (malondialdehyde and hydrogen peroxide). In addition, their regulatory role on abscisic acid was studied.

## Materials and Methods

### Plant Material, Growth Conditions, and Treatments

The cauliflower cultivar Xuefeng selected for this study is resistant to black rot disease; seeds were provided by Tianjin Vegetable Research Institute, China. Standard COR was provided by Professor Carol L. Bender of Oklahoma State University (Stillwater, OK, USA). The COR was prepared as described in Palmer and Bender (1993).

Cauliflower seeds were surface sterilized in 75% (v/v) ethanol solution for 10 min and rinsed three times with distilled water. The seeds were then sown in 12-cm-diameter × 12-cm-deep pots containing a mixture (1:1 by volume) of vermiculite and commercial garden soil (Jixiang Horticulture Company, Beijing, China) sterilized by heating to 120°C for 2 h. Pots were arranged in a greenhouse under 30/20°C and 10-h/14-h day/night conditions at China Agricultural University, Beijing. Seedlings were thinned to one per pot at the first true leaf stage.

Five hundred forty seedlings were randomly divided into nine treatments comprising three regulator treatments (deionized water as control, MeJA and COR × three water status: well watered, water deficit stress, and post-stress rewatering). There were 60 seedlings per treatment, and each treatment had three replications (20 seedlings per replication).

Deionized water, 10 μM MeJA, and 0.1 μM COR were separately applied by foliar spray when the seedlings had a fully expanded fourth leaf. After 24 h, water deficit stress treatments commenced by not watering seedlings for 8 days; seedlings for the rewatering treatment were returned

to the well-watered condition after 5 days of stress, whereas the seedlings for well-watered treatments remained under normal water conditions. For the physiological measurements, the fourth leaf was sampled on the 8th day after treatments. The leaf samples were frozen in liquid nitrogen and stored at −40°C.

### Biomass Determination

For dry matter determination, seedlings were collected on the 8th day after treatment to determine the fresh and dry weights. Seedlings were separated into roots and shoots, oven-dried at 105°C for 30 min, and then at 80°C for 24 h.

### Relative Water Content (RWC)

The fourth true leaf was sampled on the 8th day after treatment to determine RWC. After determining the fresh weight, the samples were soaked in distilled water for 24 h and cleansed of surface water with absorbent paper before taking the saturation fresh weight. Samples were then dried at 105°C for 15 min and then at 80°C to constant weight, which was recorded. Calculation of RWC of leaf tissue was based on Munné-Bosch and others (2003).

### Photosynthesis and Chlorophyll Content

Net photosynthetic rate ($P_n$), stomatal conductance ($G_s$), transpiration rate ($Tr_{mmol}$), and intercellular $CO_2$ ($C_i$) of the fourth leaf were analyzed with a portable photosynthetic system (LI-6400; LI-COR, Lincoln, NE, USA). The relative chlorophyll contents of leaves were obtained with the portable chlorophyll meter SPAD-502 (Minolta, Japan).

### Chlorophyll Fluorescence Parameter

The chlorophyll fluorescence parameter $F_v/F_m$ of the fourth leaf was measured using a portable chlorophyll fluorescence detector (PAM2100; Walz, Germany).

### Determination of Proline and Soluble Sugar

Proline content was determined according to Monreal and others (2007) with some modification. Fresh samples (0.5 g) were ground with 3% sulfosalicylic acid (5 ml) and clarified by centrifugation. The supernatant (2 ml) was mixed with the same volume of acetic acid and acid ninhydrin (3 ml); the mixture was oven-incubated at 100°C for 40 min, and the reaction was finished in an ice bath. The reaction mixture was extracted with toluene (5 ml) and the absorbance was read at 520 nm, using toluene as a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis.

Contents of total soluble sugars were extracted and analyzed according to Ci and others (2009). The leaf samples (0.5 g) were homogenized in 2 ml of 80% (v/v) alcohol, and the mortar was washed three times with 3 ml of 80% alcohol. The homogenates were placed at room temperature for 30 min and then centrifuged at 4,000*g* for 20 min. The supernatant was stored at 4°C. The supernatant (0.5 ml) was mixed with 3 ml of anthrone and the mixtures were incubated at 95°C for 10 min. The absorbance at 620 nm was then recorded.

### $H_2O_2$ and Malondialdehyde (MDA) Contents

Leaf samples (0.5 g) were homogenized at 4°C in 8 ml of 50 mM ice-cold phosphate buffer (pH 7.0) containing 1% (w/v) polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 15,000*g* for 20 min at 4°C and supernatants were stored at −80°C until assayed. The $H_2O_2$ content was measured using the method of Xie and others (2008).

The MDA content was determined according to the method of Kuk and others (2003) with slight modification. The supernatant (1 ml) was added to 2 ml of 20% (v/v) trichloroacetic acid containing 0.5% (m/v) thiobarbituric acid. The mixture was incubated in boiling water for 20 min and cooled immediately. The solution was centrifuged at 10,000*g* for 10 min and the MDA content was calculated using the extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$ by measuring the absorbance at 532 and 600 nm.

### Antioxidant Enzyme Activity Assay

Superoxide dismutase (SOD) activity was determined following the method of Kuk and others (2003). Activity was expressed in enzyme units per mg protein. One unit of SOD is defined as the amount of enzyme that inhibits the reduction rate of NBT by 50%.

Peroxidase activity (POD) was determined by the guaiacol oxidation method (Nakno and Asada 1981). The reaction mixture consisted of 50 ml of 0.2 M phosphate buffer (pH 6.0), 19 µl guaiacol, and 28 µl of 30% $H_2O_2$. The reaction started after adding 0.5 ml of the supernatant to 1.5 ml of the reaction mixture, and changes in $A_{470}$ were recorded for 3 min. Enzyme activity was expressed as an increase in absorbance $min^{-1}$ $mg^{-1}$ protein.

Catalase (CAT) activity was estimated by monitoring the disappearance of $H_2O_2$ by recording the decline in absorbance at 240 nm according to Ai and others (2008), in a reaction mixture containing 50 mM sodium phosphate buffer (pH 7.8), 100 mM 30% $H_2O_2$, and crude enzyme extract.

Ascorbate peroxidase (APX) activity was determined according to the method of Nakno and Asada (1981). Glutathione reductase (GR) activity was assayed by

monitoring the glutathione-dependent oxidation of NADPH at 340 nm (Kuk and others 2003; Ai and others 2008).

### Extraction, Purification, and Determination of ABA

Extraction and purification of endogenous ABA were carried out as previously described in Yang and others (2001). Leaf samples (0.5 g) were homogenized at 4°C in 5 ml of 80% (v/v) methanol extraction medium containing 1 ml butylated hydroxytoluene as an antioxidant. The extracts were incubated at 4°C overnight and centrifuged at 4,000 rpm for 15 min at the same temperature. Then, the supernatant was applied to preconditioned Chromosep $C_{18}$ columns ($C_{18}$ Sep-Park Cartridge, Waters, Milford, MA, USA). The hormone fractions were eluted with 10 ml of 100% (v/v) methanol, and 10 ml ether from the columns were dried under $N_2$ and dissolved in 4 ml phosphate buffer containing 0.1% (v/v) Tween 20 and 0.1% (w/v) gelatin (pH 7.5). The endogenous levels of ABA were determined by an indirect ELISA technique (Yang and others 2001).

### Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) and the means were separated using Duncan's multiple-range tests at the 5% level of significance.

## Results

### Changes in Dry Matter Accumulation During Drought Stress and Recovery

The dry weight of cauliflower seedlings was reduced by 37.3% after 8 days of drought stress and by 33.1% after rewatering (Table 1). However, MeJA and coronatine (COR) markedly alleviated biomass reduction under drought stress by increasing the dry weight by 10.2 and 9.4% relative to control plants. After rewatering, the dry weights of MeJA- and COR-treated plants were 9.9 and 7.4% higher than control plants. Dry weight of seedlings under well-watered conditions was slightly higher in MeJA- or COR-treated plants than in control plants.

**Table 1** Effect of MeJA and COR on dry weight of cauliflower seedlings under different water conditions

| Treatment | Dry matter accumulation (g plant$^{-1}$) | | |
|---|---|---|---|
| | WW | DS | RW |
| Control | 0.324 a[a] | 0.234 b | 0.243 b |
| MeJA | 0.334 a | 0.258 a | 0.267 a |
| Coronatine | 0.328 a | 0.256 a | 0.261 a |

*WW* well-water conditions, *DS* drought stress, *RW* rewatering

[a] Data are means of three independent replicates. Within each column, means followed by same letters are not significantly different according to LSD test at $P \leq 0.05$

### Changes in Photosynthetic Parameters During Drought Stress and Recovery

Under well-watered conditions, MeJA- and COR-treated plants showed higher net photosynthetic rate ($P_n$), stomatal conductance ($G_s$), intercellular $CO_2$ concentration ($C_i$), and transpiration rate ($Tr_{mmol}$) than control plants (Fig. 1a–d). Drought stress had adverse effects on $P_n$, $G_s$, $C_i$, and $_{Trmmol}$ across treatments, although the pattern and intensity of these effects varied. However, the photosynthetic parameters in MeJA- and COR-treated plants were remarkably higher than control plants. Rewatering after drought stress resulted in the recovery of $P_n$, $G_s$, $C_i$, and $Tr_{mmol}$ to varying degrees across treatments. Whereas the $P_n$ and $Tr_{mmol}$ in MeJA- and COR-treated plants recovered fully to levels observed for well-watered plants, the $G_s$ and $C_i$ recovered to only 93 and 83% of well-watered plants.

### Changes in Chlorophyll (*Chl*) Content and $F_v/F_m$ During Drought Stress and Recovery

Under well-watered conditions, the chlorophyll contents of MeJA- and COR-treated plants were higher than those of control plants. For MeJA- and COR-treated plants, similar changes were observed during drought stress, as *Chl* content determined from SPAD values decreased from 40.9 to 38.6, but *Chl* values were 12.0-12.9% higher in MeJA- and COR-treated plants than in control plants (Fig. 2a, b). During rewatering, the *Chl* content was restored to values observed under well-watered conditions.

The $F_v/F_m$ values declined considerably by greater than 13.9% with progression of drought stress, and for MeJA- and COR-treated plants they were markedly increased by 6.0 and 8.3%, respectively, under drought stress. However, this reduction was completely reversed after rewatering.

### Changes in Membrane Leakage and Lipid Peroxidation During Drought Stress and Recovery

Nonsignificant changes in the relative water content (RWC) of leaves were observed in all treated plants under well-watered conditions. In contrast, the RWC declined from 95 to 77% under drought stress (Fig. 3c). The RWC of MeJA-treated leaves was the highest, and the RWC of COR-treated plants was higher than that of control plants

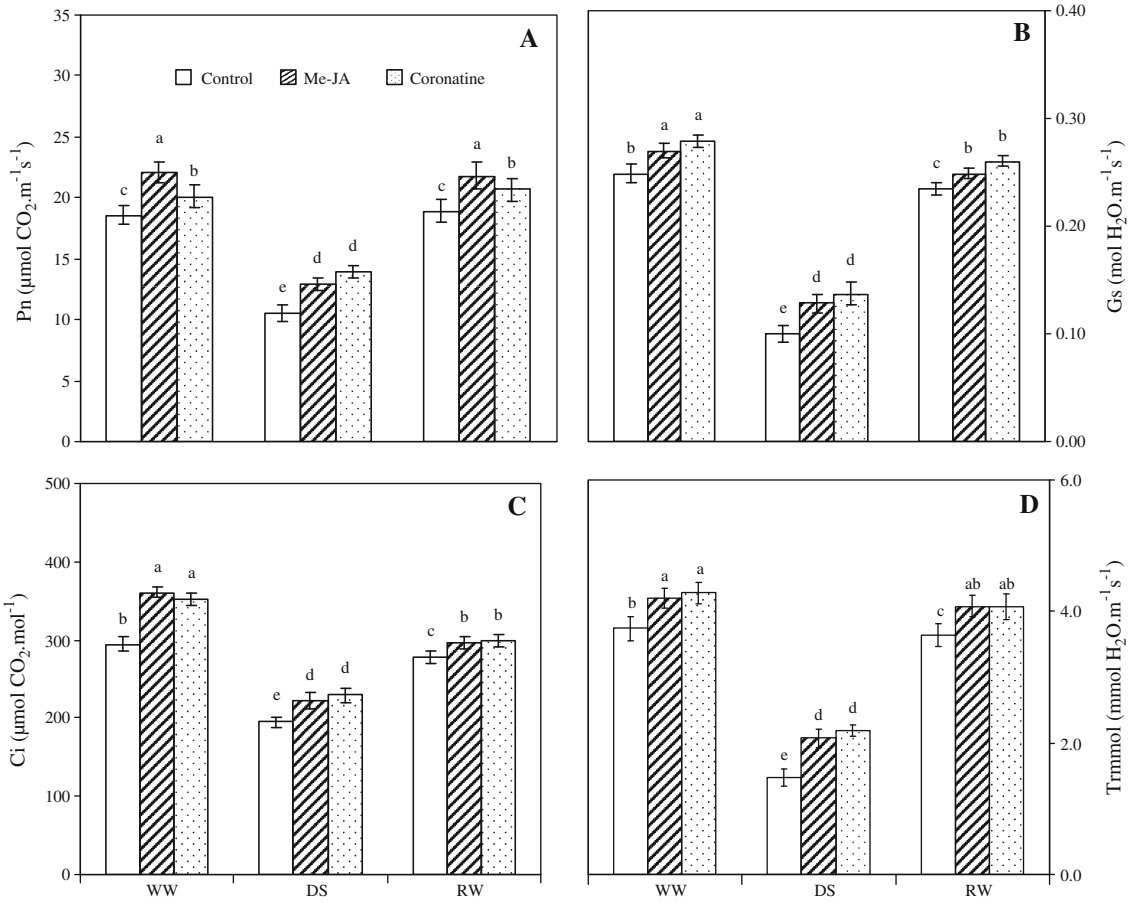


**Fig. 1** Effect of MeJA and COR on net photosynthetic rate ($P_n$) (**a**), stomatal conductance ($G_s$) (**b**), intercellular $CO_2$ concentration ($C_i$) (**c**), and transpiration rate ($Tr_{mmol}$) (**d**) in leaves of cauliflower seedlings grown under different water conditions. *WW* well-water conditions; *DS* drought stress; *RW* rewatering. Data are means of three determinations ± standard deviation (*error bars*). Bars with the same letters are not significantly different according to LSD test at $P \leq 0.05$

under drought stress. However, the RWC was immediately restored in differently treated plants after rewatering.

Proline and soluble sugar play an important role in osmotic regulation. Proline values for MeJA- and COR-treated plants were similar to that of control plants under well-watered conditions. The values increased from 42 to 183 nmol $g^{-1}$ under drought stress (Fig. 3a) and were highest for COR-treated leaves. These increases were slightly reversed upon rewatering, but proline values in MeJA- and COR-treated plants were higher than values observed at well-watered conditions.

Soluble sugar values changed little under well-watered conditions and followed a similar trend during drought stress and rewatering (Fig. 3d). During drought stress, soluble sugar was significantly increased in MeJA- and COR-treated plants and was completely restored to well-watered values within 4 days of rewatering.

There was no difference in MDA values among treatments under well-watered conditions (Fig. 3e). Under drought stress conditions, the MDA values in MeJA- and COR-treated plants were 32.8 and 16.5% lower than those of control plants. After rewatering, the values of MeJA-treated plants were restored to well-watered levels, but those of COR-treated and control plants were about 17% higher than that of well-watered plants.

Hydrogen peroxide ($H_2O_2$) as a strong oxidant, together with MDA, has toxic effects on the enzymes and membrane. The $H_2O_2$ values of MeJA- and COR-treated plants were slightly lower than that of control plants under well-watered conditions (Fig. 3b). During drought stress, the value for COR-treated plants was the lowest across treatments, and the value for MeJA-treated plants was lower than that of control plants. $H_2O_2$ was completely restored to well-watered levels after rewatering.

## Changes in Antioxidant Enzyme Activities During Drought Stress and Recovery

Cell membrane lipid peroxidation is an important index of plant injury due to drought stress. This arises from

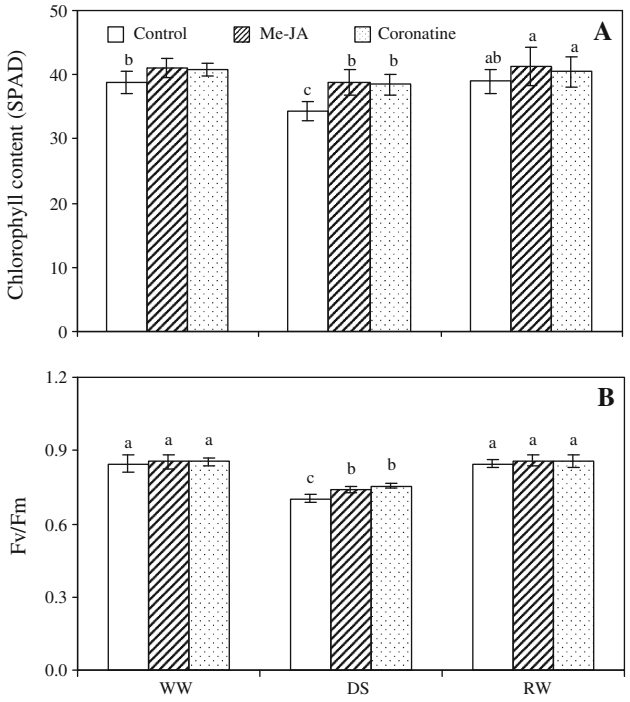


**Fig. 2** Effect of MeJA and COR on chlorophyll content (**a**) and chlorophyll fluorescence parameter, $F_v/F_m$ (**b**) in cauliflower seedlings under different water conditions. *WW* well-water conditions; *DS* drought stress; *RW* rewatering. Data are means of three determinations ± standard deviation (*error bars*). Bars with the same letters are not significantly different according to LSD test at $P \leq 0.05$

accumulation of free radicals when the balance between free radical generation and removal in plant is disturbed. Catalase, APX, SOD, POD, and GR are important defensive enzymes for scavenging the enzyme system of active oxygen free radicals. Slightly different levels of CAT and APX were observed in different treatments under well-watered conditions. Under drought stress, CAT values in MeJA and COR-treated plants were 40.0 and 47.3% higher than in control plants (Fig. 4a). The APX values of MeJA- and COR-treated plants were 33.3 and 50.0% higher than that of control plants. After rewatering, APX values were restored to slightly above well-watered levels (Fig. 4c). The CAT values were about 32% higher than well-watered values.

There were no marked changes in SOD and POD values under well-watered conditions (Fig. 4b, d). Under drought stress, the SOD and POD increased across treatments to varying degrees; the SOD values in MeJA- and COR-treated plants were 19.1 and 24.5% higher than that in control plants, and POD values in MeJA- and COR-treated plants were 22.0 and 41.2% higher than that in control plants. However, this increase was reversed after rewatering.

The GR values in MeJA- and COR-treated plants were 27.3 and 18.2% higher than that in control plants under well-watered conditions (Fig. 4e). Under drought stress, the values in MeJA- and COR-treated plants were 15.0 and 20.0% higher than that in control plants. During rewatering, GR values decreased across treatments but values in MeJA- and COR-treated plants remained slightly higher than that in control plants.

### Changes in Abscisic Acid (ABA) During Drought Stress and Recovery

There was no significant change in ABA values under well-watered conditions (Fig. 5). Under drought stress, the ABA values in MeJA- and COR-treated plants were 21.1 and 23.6% higher than that in control plants. After rewatering, the values in MeJA- and COR-treated plants were restored to well-watered levels, resulting in somewhat higher ABA values in MeJA- and COR-treated plants than that in the corresponding controls.

## Discussion

### Well-watered Conditions

Exogenously applied JA and MeJA inhibited or promoted morphological and physiological changes in plants. The pleiotropic action of JA and MeJA was in a concentration-dependent manner. For example, MeJA at a concentration of 100 μM or 1.0 mM arrested the germination, growth, and leaf expansion in several plants (Jubany-Marí and others 2010; Zalewski and others 2010). Application of JA and MeJA to leaves decreased expression of photosynthesis-related genes, reduced translation and increased degradation of Rubisco, reduced the net photosynthesis rate, and caused a loss of chlorophyll in barley or soybean (Wierstra and Kloppstech 2000; Scandalios 1993). In contrast, MeJA at sub-micromolar concentrations could promote lateral root initiation and growth (Tung and others 1996). Mabood and others (2006) showed that treatment with MeJA at 50 μM stimulated growth, dry matter accumulation, and grain yield under short soybean season field conditions. Enhanced biomass production and high levels of photosynthetic pigments, monosaccharides, and proteins were observed in *Wolffia arrhiza* exposed to 1.0 μM JA (Piotrowska and others 2010). In the present study, our results indicated that MeJA at 1.0 μM stimulated dry matter accumulation in cauliflower seedlings by promoting accumulation of chlorophyll and increasing the net photosynthetic rate, $G_s$, $C_i$, and transpiration rate (Figs. 1, 2). Although COR-treated plants had higher chlorophyll content and photosynthetic rates, COR treatment did not affect total seedling biomass as reported earlier for rice or cotton (Ai and others 2008; Xie and others 2008).

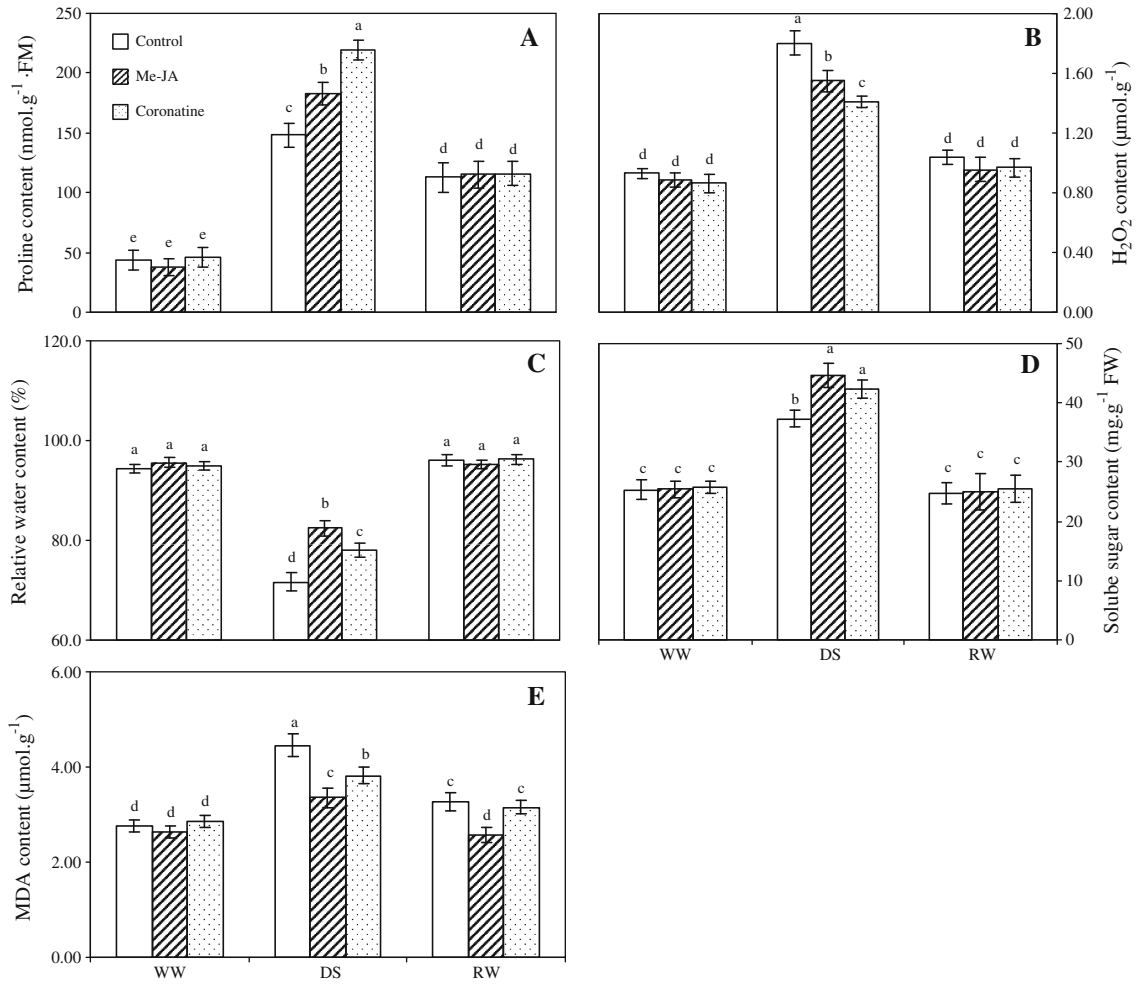


**Fig. 3** Effect of MeJA and COR on the contents of free proline (**a**), $H_2O_2$ (**b**), RWC (**c**), soluble sugar (**d**), and MDA (**e**) in leaves of cauliflower seedlings under different water conditions. *WW* well-water conditions; *DS* drought stress; *RW* rewatering. Data are means of three determinations ± standard deviation (*error bars*). Bars with the same letters are not significantly different according to LSD test at $P \leq 0.05$

Whereas most previous studies were based on external MeJA applications through plant roots (Ali and others 2006; Parra-Lobatoa and others 2009; Maksymiec and Krupa 2002), this study focused on changes in nonenzymatic and enzymatic antioxidant systems based on foliar application of MeJA at lower concentrations. Our results showed slight decreases in $H_2O_2$ and MDA content and increases in the activities of SOD, POD, CAT, APX, and GR in cauliflower seedling leaves treated with MeJA (Figs. 3, 4). These agreed with the results reported in Jung (2004) and Kumari and others (2006), that is, total activities of antioxidative enzymes increased greatly in response to MeJA in *Arabidopsis* and peanut seedlings.

The accumulation of soluble sugar and proline in MeJA-treated plants was less than that in control plants, whereas the relative water and ABA contents and $F_v/F_m$ showed no difference between MeJA-treated and control plants, suggesting that MeJA treatment did not induce osmotic stress in the cauliflower seedlings. Application of COR also enhanced the activities of SOD, POD, CAT, APX, and GR and decreased the accumulation of $H_2O_2$ and MDA. Our results were in agreement with those of Ai and others (2008) and Xie and others (2008), who examined the effect of COR on enzyme antioxidant defense systems in rice and cotton.

## Drought Stress Conditions

In our study drought stress seriously decreased chlorophyll content (Fig. 2), net photosynthetic rate, $G_s$, $C_i$, and transpiration rate (Fig. 1), which led to inhibition of dry matter production in cauliflower seedlings (Table 1). A similar inhibition of dry matter production and yield reduction by water deficit were reported in Cheruth and others (2009). Exogenous application of MeJA could improve plant

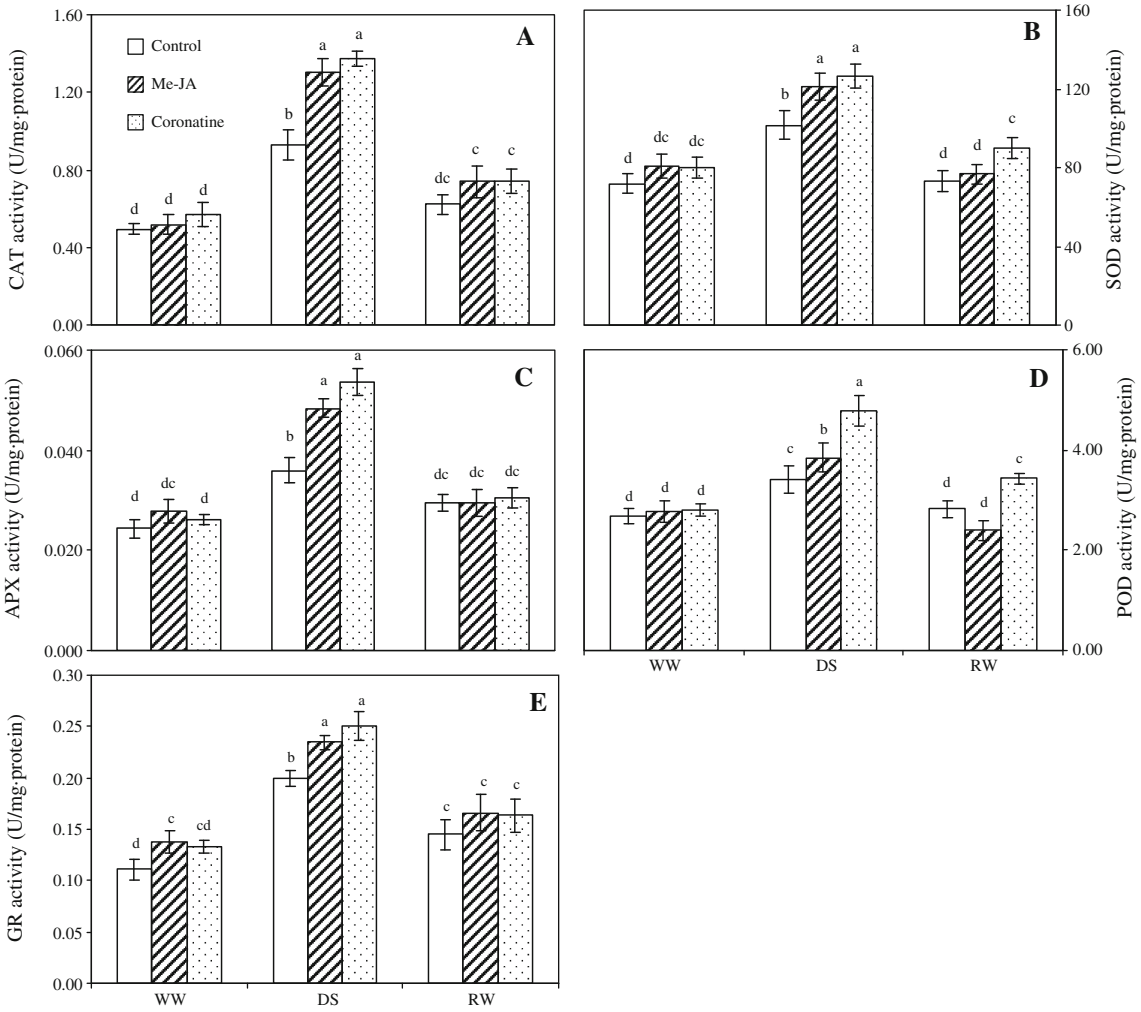


**Fig. 4** Effect of MeJA and COR on enzyme activities of CAT (**a**), SOD (**b**), APX (**c**), POD (**d**), and GR (**e**) in leaves of cauliflower seedlings under different water conditions. WW, well-water conditions; DS, drought stress; *RW* rewatering. Data are means of three determinations ± standard deviation (*error bars*). Bars with the same letters are not significantly different according to LSD test at $P \leq 0.05$

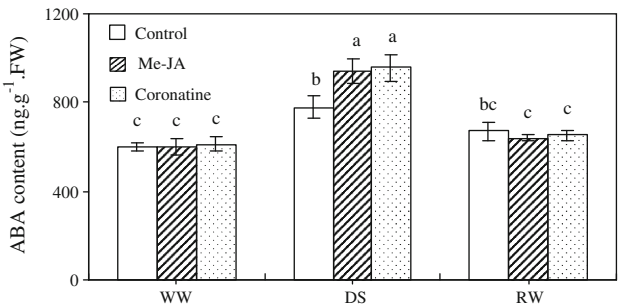


**Fig. 5** Effect of MeJA and COR on the ABA content in leaves of cauliflower seedlings under different water conditions. *WW* well-water conditions; *DS* drought stress, *RW* rewatering. Data are means of three determinations ± standard deviation (*error bars*). Bars with the same letters are not significantly different according to LSD test at $P \leq 0.05$

tolerance to environmental stresses, including drought and low temperature (Gao and others 2004). MeJA application greatly mitigated the adverse effects of NaCl on soybean growth, chlorophyll content, leaf photosynthetic rate, and proline content (Yoon and others 2009). In our study, MeJA application to cauliflower seedlings significantly increased photosynthesis and chlorophyll content and promoted biomass production under water deficit stress. Like MeJA, COR application increased dry matter production, chlorophyll content, and leaf photosynthetic rate under drought stresses. Our results were in agreement with observations for maize and rice seedlings treated with COR under drought stress (Wang and others 2008; Ai and others 2008).

Drought stress could damage the chlorophyll pigments and photosynthetic electron transport system, which led to production of reactive oxygen species (Zgallai and others 2005). To counter the adverse effects of reactive oxygen species, plants evolved numerous nonenzymatic and enzymatic antioxidant defenses. JA or MeJA were involved mainly in response to water stress by regulating oxidative reactions and inducing antioxidant defenses (Shan and Liang 2010).

Exogenously supplied COR also alleviated drought stress by activating antioxidant enzymes and inducing resistant protein expression(Ai and others 2008). In this experiment, in response to exogenous MeJA or COR treatment under drought stress, the $H_2O_2$ and MDA contents were significantly reduced and accumulation of soluble sugar and proline and the $F_v/F_m$ ratio were markedly promoted in cauliflower seedlings, suggesting that MeJA or COR application could alleviate drought-induced injury in cauliflower. It was widely recognized that accumulation of soluble sugar, proline, and betaine led to a decline in cell osmotic potential, and plants had to absorb water from the outside to maintain cell turgor and adjust osmotic balance (Dong and others 2002).

The accumulation of proline, MDA, and $H_2O_2$ also was regarded as a sign of drought stress (Ai and others 2008). Apart from the positive effect of MeJA and COR on nonenzymatic antioxidants, they stimulated the activity of enzymatic antioxidants and reduced the production of lipid peroxide in cauliflower seedlings. The activities of functionally interrelated antioxidant enzymes such as SOD, POD, CAT, APX, and GR were significantly increased by MeJA or COR treatment. Thus, enhanced activities of these enzymes were generally associated with an adaptation to high levels of active oxygen species and environmental changes in cauliflower seedling.

In response to water stress, the leaf water status as measured by the relative water content gradually decreased. MeJA or COR application significantly inhibited drastic reductions in leaf water content and maintained reasonable leaf water status in cauliflower seedlings under drought stress. The results confirmed the findings of Wang and others (2008) for COR effects on maize or of Pan and Gu (1995) for MeJA effects on peanut. It had been reported that ABA accumulation could be induced by JA in plant cells (Creelman and Mullet 1995), and ABA was involved in cell signaling systems for drought in plants (Zhang and others 2001).

Exogenous application of jasmonic acid rapidly increased the endogenous level of ABA in rice (Rakwal and Komatsu 2000). Our results clearly showed that drought-stressed cauliflower treated with MeJA or COR showed a marked increase in endogenous ABA contents (Fig. 5). Therefore, the increase in ABA content was an adaptive response and safeguard mechanism of cauliflower plants to cope with drought stress.

### Recovery During Rewatering

Leaf water status recovered gradually after rewatering of cauliflower seedlings, as observed in *Cistus albidus* (Jubany-Marí and others 2010). As $P_n$ and chlorophyll content were restored to normal values after rewatering, values of $G_s$, $C_i$, and $Tr_{mmol}$ at recovery were slightly lower than those under well-watered conditions. Our results agreed with previous reports that photosynthetic characteristics after rewatering were lower than those of well-watered values but were greatly higher than those of water-stressed values (Anyia and Herzog 2004). Similarly, MeJA or COR application ensured near complete recovery in leaf water content, $P_n$, chlorophyll content, and other photosynthetic characteristics after rewatering. Cauliflower seedlings recovered upon rewatering and resumed growth, indicating that the plants had mechanisms of adaptation to water stress.

An increase in $H_2O_2$ was observed during recovery from water stress in lichens (Weissman and others 2005), bluegrass (Bian and Jiang 2009), and *Cistus albidus* (Jubany-Marí and others 2010). However, this was not the case in *Cistus albidus* plants exposed to drought when the release of water stress was gradual (Jubany-Marí and others 2010). Our results showed that the $H_2O_2$ level declined to that in well-watered conditions, and MeJA or COR application did not affect the declining trend, as reported in Sofo and others (2005). During the rewatering phase, the activities of SOD, POD, CAT, APX, and GR decreased, and levels of proline and soluble sugar were reduced in cauliflower leaves. Our results indicated that the downregulation of the enzymatic antioxidant system observed in rewatered plants, possibly owing to a reduced need for removal of activated oxygen species, was consistent with the recovery in leaf water potential and gas exchange of cauliflower seedlings.

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## References

Agrawal GK, Tamogamib S, Iwahashic H, Agrawala VP, Rakwal R (2003) Transient regulation of jasmonic acid-inducible rice MAP kinase gene (OsBWMK1) by diverse biotic and abiotic stresses. Plant Physiol Biochem 41:355–361

Ai L, Li ZH, Xie ZX, Tian XL, Eneji AE, Duan LS (2008) Coronatine alleviates polyethylene glycol-induced water stress in two rice (*Oryza sativa* L.) cultivars. J Agron Crop Sci 194:360–368

Ali MB, Yu KW, Hahn EJ, Paek KY (2006) Methyl jasmonate and salicylic acid elicitation induces ginsenosides accumulation, enzymatic and non-enzymatic antioxidant in suspension culture *Panax ginseng* roots in bioreactors. Plant Cell Rep 25:613–620

Anyia AO, Herzog H (2004) Genotypic variability in drought performance and recovery in cowpea under controlled environment. J Agron Crop Sci 190:151–159

Behnamnia M, Kalantari KhM, Rezanejad F (2009) Exogenous application of brassinosteroid alleviates drought-induced oxidative stress in *Lycopersicon esculentum* L. General Appl Plant Physiol 35:22–34

Bender CL, Alarcon CF, Gross DC (1999) *Pseudomonas syringae* phytotoxins: mode of action, regulation and biosynthesis by peptide and polypeptide synthetases. Microbiol Mol Biol Rev 63:266–292

Bian SM, Jiang YW (2009) Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of *Kentucky bluegrass* in response to drought stress and recovery. Sci Hortic 120:264–270

Braun Y, Smirnova AV, Weingart H, Schenk A, Ullrich MS (2009) Coronatine gene expression in vitro and in planta, and protein accumulation during temperature downshift in *Pseudomonas syringae*. Sensors 9:4272–4285

Cheruth AJ, Paramasivam M, Abdul W, Muhammad F, Hameed JA, Ramamurthy S, Rajaram P (2009) Drought stress in plants: a review on morphological characteristics and pigments composition. Int J Agric Biol 11:100–105

Ci DW, Jiang D, Dai TB, Jing Q, Cao WX (2009) Effects of cadmium on plant growth and physiological traits in contrast wheat recombinant inbred lines differing in cadmium tolerance. Chemosphere 77:1620–1625

Clua A, Paez M, Orsini H, Beltrano J, Newsletter L (2009) Incidence of drought stress and rewatering on *Lotus tenuis.* Effects on cell membrane stability. Lotus Newslett 39:21–27

Creelman RA, Mullet JE (1995) Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. Proc Natl Acad Sci USA 92:4114–4119

Dodd IC, Davies WJ (2004) Hormones and the regulation of water balance. In: Davis PJ (ed) Plant hormones: biosynthesis, signal transduction, action! Dordrecht. Kluwer Academic Publishers, The Netherlands, pp 493–512

Dong DF, Jiang LG, Nie CR, Zhang PG, Chen NP (2002) Effects of long-lasting brassinosteroid ts303 and propyl dihydroj asmonate on peanut resistance to drought. J Peanut Sci 31:29–33

Flexas J, Ribas-Carbó M, Diaz-Espejo A, Galmés J, Medrano H (2008) Mesophyll conductance to $CO_2$: current knowledge and future prospects (2008). Plant Cell Environ 31:602–621

Galle A, Florez-Sarasa I, Thameur A, Paepe R, de Flexas J, Ribas-Carbo M (2010) Effects of drought stress and subsequent rewatering on photosynthetic and respiratory pathways in *Nicotiana sylvestris* wild type and the mitochondrial complex I-deficient CMSII mutant. J Exp Bot 61:765–775

Gao XP, Wang XF, Lu YF, Zhang LY, Shen YY, Liang Z, Zhang DP (2004) Jasmonic acid is involved in the water-stress-induced betaine accumulation in pear leaves. Plant Cell Environ 27: 497–507

Hassanein RA, Hassanein AA, El-Din AB, Salama M, Hashem HA (2009) Role of jasmonic acid and abscisic acid treatments in alleviating the adverse effects of drought stress and regulating trypsin inhibitor production in soybean plant. Aust J Basic Appl Sci 3:904–919

Jubany-Marí T, Prinsen E, Munné-Bosch S, Alegre L (2010) The timing of methyl jasmonate, hydrogen peroxide and ascorbate accumulation during water deficit and subsequent recovery in the Mediterranean shrub *Cistus albidus* L. Environ Exp Bot 69:47–55

Jung S (2004) Effect of chlorophyll reduction in *Arabidopsis thaliana* by methyl jasmonate or norflurazon on antioxidant systems. Plant Physiol Biochem 42:225–231

Kuk YI, Shin JS, Burgos NR, Hwang TE, Han O, Cho BH, Jung S, Guh JO (2003) Antioxidative enzymes offer protection from chilling damage in rice plants. Crop Sci 43:2109–2117

Kumari GJ, Reddy AM, Naik ST, Kumar SG, Prasanthi J, Sriranganayakulu G, Reddy PC, Sudhakar C (2006) Jasmonic acid-induced changes in protein pattern, antioxidative enzyme activities and peroxidase isozymes in peanut seedlings. Biol Plant 50:219–226

Loreto F, Centritto M (2008) Leaf carbon assimilation in a water-limited world. Plant Biosyst 142:154–161

Mabood F, Zhou XM, Lee KD, Smith DL (2006) Methyl jasmonate, alone or in combination with genistein, and Bradyrhizobium japonicum increases soybean (*Glycine max* L.) plant dry matter production and grain yield under short season conditions. Field Crop Res 95:412–419

Maksymiec W, Krupa Z (2002) The in vivo and in vitro influence of methyl jasmonate on oxidative processes in *Arabidopsis thaliana* leaves. Acta Physiol Plant 24:351–357

Monreal JA, Jiménez ET, Remesal E, Morillo-Velarde R, García-Mauriño S, Echevarría C (2007) Proline content of sugar beet storage roots: response to water deficit and nitrogen fertilization at field conditions. Environ Exp Bot 60:257–267

Munné-Bosch S, Jubany-Marí T, Alegre L (2003) Enhanced photo- and antioxidative protection, and hydrogen peroxide accumulation in drought-stressed *Cistus clusii* and *Cistus albidus* plants. Tree Physiol 23:1–12

Nakno Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–871

Palmer DA, Bender CL (1993) Effects of environmental and nutritional factors on production of the polyketide phytotoxin coronatine by *Pseudomonas syringae* pv. *glycinea*. App Environ Microbiol 59:1619–1626

Pan RC, Gu H (1995) Effect of methyl jasmonate in the growth and drought resistance in peanut seedlings. Acta Phytophysiol Sin 21:215–220

Panozzo J, Eagles H (1999) Rate and duration of grain filling and grain nitrogen accumulation of wheat cultivars grown in different environments. Aust J Agr Res 50:1007–1015

Parra-Lobatoa MC, Fernandez-Garciab N, Olmosb E, Alvarez-Tinaut CM, Gómez-Jiménez MC (2009) Methyl jasmonate-induced antioxidant defence in root apoplast from sunflower seedlings. Environ Exp Bot 66:9–17

Pedranzani H, Sierra-de-Grado R, Vigliocco A, Miersch O, Abdala G (2007) Cold and water stresses produce changes in endogenous jasmonates in two populations of *Pinus pinaster Ait*. Plant Growth Regul 52:111–116

Piotrowska A, Bajguz A, Czerpak R, Kot K (2010) Changes in the growth, chemical composition, and antioxidant activity in the aquatic plant *Wolffia arrhiza* (L.) *Wimm*. (*Lemnaceae*) exposed to jasmonic acid. J Plant Growth Regul 29:53–62

Rakwal R, Komatsu S (2000) Role of jasmonate in the rice (*Oryza sativa* L.) self-defense mechanism using proteome analysis. Electrophoresis 21:2492–2500

Rivero RM, Shulaev V, Blumwald E (2009) Cytokinin-dependent photorespiration and the protection of photosynthesis during water deficit. Plant Physiol 150:1530–1540

Scandalios JG (1993) Oxygen stress and superoxide dismutases. Plant Physiol 101:7–12

Schüler G, Mithöfer A, Baldwin T, Berger S, Ebel J, Santos JG et al (2004) Coronalon: a powerful, tool in plant stress physiology. FEBS Lett 563:17–22

Shan CJ, Liang ZS (2010) Jasmonic acid regulates ascorbate and glutathione metabolism in *Agropyron cristatum* leaves under water stress. Plant Sci 178:130–139

Sofo A, Tuzio AC, Dichio B, Xiloyannis C (2005) Influence of water deficit and rewatering on the components of the ascorbate–glutathione cycle in four interspecific *Prunus* hybrids. Plant Sci 169:403–412

Tamogami S, Kodama O (2000) Coronatine elicits phytoalexin production in rice leaves (*Oryza sativa* L.) in the same manner as jasmonic acid. Phytochemistry 54:689–694

Tung P, Hooker TS, Tampe PA, Reid DM, Thorpe TA (1996) Jasmonic acid: effects on growth and development of isolated tomato roots cultured in vitro. Int J Plant Sci 157:713–721

Uppalapati SR, Ayoubi P, Weng H, Palmer DA, Mitchell RE, Jones W et al (2005) The phytotoxin coronatine and methyl jasmonate impact multiple phytohormone pathways in tomato. Plant J 42:201–217

Wang BQ, Li ZH, Eneji E, Tian XL, Zhai ZX, Li JM, Duan LS (2008) 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. Plant Prod Sci 11:283–290

Warren CR, Adams MA (2006) Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use in photosynthesis. Plant Cell Environ 29:192–201

Weissman L, Garty J, Hochman A (2005) Characterization of enzymatic antioxidants in the *Lichen Ramalina lacera* and their response to rehydration. Appl Environ Microbiol 71:6508–6514

Wierstra I, Kloppstech K (2000) Differential effects of methyl jasmonate on the expression of the early light-inducible proteins and other light-regulated genes in barley. Plant Physiol 124:833–844

Xie Z, Duan L, Tian X, Wang B, Eneji AE, Li Z (2008) Coronatine alleviates salinity stress in cotton by improving the antioxidative defense system and radical scavenging activity. J Plant Physiol 165:375–384

Yang J, Zhang J, Wang Z, Zhu Q, Wang W (2001) Hormonal changes in the grains of rice subjected to water stress during grain filling. Plant Physiol 127:315–323

Yang J, Zhang J, Liu K, Wang Z, Liu L (2007) Abscisic acid and ethylene interact in rice spikelets in response to water stress during meiosis. J Plant Growth Regul 26:318–328

Yoon JY, Hamayun M, Lee SK, Lee IJ (2009) Methyl jasmonate alleviated salinity stress in soybean. J Crop Sci Biotech 12:63–68

Zalewski K, Nitkiewicz B, Lahuta LB, Głowacka K, Socha A, Amarowicz R (2010) Effect of jasmonic acid-methyl ester on the composition of carbohydrates and germination of yellow lupine (*Lupinus luteus* L.) seeds. J Plant Physiol 167:967–973

Zgallai H, Steppe K, Lemeur R (2005) Photosynthetic, physiological and biochemical responses of tomato plants to polyethylene glycol-induced water deficit. J Integr Plant Biol 47:1470–1478

Zhang DP, He FL, Jia WS (2001) Protein phosphorylation is involved in the water stress-induced ABA accumulation in the roots of *Malus hupehensis Rehd*. Chin Sci Bull 46:855–858