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Coronatine enhances drought tolerance via improving antioxidative capacity to maintaining higher photosynthetic performance in soybean

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ABSTRACT

Coronatine (COR), a structural and functional mimic of jasmonates, is involved in a wide array of effects on plant development and defence responses. This study was conducted to explore the role of exogenously applied COR in alleviating the adversities of drought stress in soybean. COR treatment markedly increased the activities of antioxidant enzymes and proline content, and reduced the accumulation of malondialdehyde and hydrogen peroxide under drought stress. Thus, COR-treated plants had higher leaf relative water content and lower electrolye leakage, which led to higher chlorophyll content, activities of RuBPCase and PEPCase, and net photosynthetic rate compared to control plants exposed to drought. COR also increased maximal efficiency of PS II photochemical reaction and photochemical quenching coefficient, but decreased non-photochemical quenching coefficient. These beneficial effects led to enhanced photosynthetic performance and the translocation of assimilated ^{14}C which promoted growth and accumulation of dry biomass in COR-treated soybean plants subjected to drought. Interestingly, COR application did not affect the growth and biomass accumulation under well-watered condition. These results suggested the involvement of COR on improving drought tolerance in soybean by modulating antioxidant systems and membrane stability to maintain higher photosynthetic performance.

1. Introduction

Soybean (*Glycine max*) is one the world's major staple crops and provides the largest source of vegetable oil and protein for human and animal. Soybean is mostly planted in rainfed agricultural regions, and drought is recognized as the most devastating environmental stress, which can inhibit soybean growth and reduce yield by about 40% [1]. Also, Soybean is considered sensitive to drought stress, especially during critical periods of soybean ontogeny [2]. Water stresses at the seedling stage decreased soybean yield by 20% and at the flowering stage decreased yield by 46%, owing to decreased photosynthetic rate, stomatal conductance, and transpiration rate of soybean [3].

Drought stress is widely considered to be one of major issues of future climate change, and also the main environmental factor limiting crop growth and yields [4]. Exposure of plants to drought stress induces numerous physiological and biochemical changes resulting in a disturbance of normal growth and development [5]. In general, symptoms of drought stress are reduced photosynthetic rate [6], suppressed growth [7], and accelerated leaf senescence [8]. By contrary, drought stress also activates defense mechanisms that function to increase drought tolerance in plants [9]. Plant resistance to drought stress involves perception of stress signals and subsequent signal transduction, resulting in activation of various physiological and metabolic responses [10]. Phytohormone such as abscisic acid or jasmonates is one of the most important stress signals involved in the mechanisms of susceptibility or tolerance of various plant species [11]. Several studies have shown that the improvement of plant drought resistance is induced by exogenous ABA [12], salicylic acids [13], and jasmonates [14].

Phytohormone such as jasmonate, may act as modulator involved in the mechanisms of tolerance or susceptibility of various plant species [15]. Exogenous application of jasmonates modulates several physiological responses leading to improved tolerance against abiotic stress [16]. Methyl jasmonate improves the drought tolerance of soybean by modulating the membrane lipid peroxidation and antioxidant activities [17]. Under salt stress, jasmonates recover salt inhibition on dry mass production in rice [18] and

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; Ci, intercellular CO_2; COR, coronatine; F_v/F_m, maximal efficiency of PS II photochemical reaction; G_s, stomatal conductance; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; NPQ, non-photochemical quenching coefficient; PEPCase, phosphoenolpyruvate carboxylase; P_n, net photosynthetic rate; POD, peroxidase activity; q_p, photochemical quenching coefficient; RuBPCase, ribulose-1,5-bisphosphate carboxylase; RWC, relative water content; SOD, superoxide dismutase; Tr, transpiration rate.

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diminish the inhibitory effect of NaCl on the rate of $^{14}CO_2$ fixation and protein content in *Pisum sativum* [15].

Coronatine (COR), a chlorosis-inducing nonhost-specific phytotoxin produced by several pathovars of *Pseudomonas syringae*, is a structural and functional mimic of jasmonates, but is more active than jasmonates [19,20]. COR can induce a wide array of effects in plants, including root retardation and hypertrophy, senescence, accumulation of defense-related protease inhibitors and secondary metabolites, and resistance to abiotic stresses [20,21]. COR has been validated to improve the stress tolerance or resistance of plants, such as salinity in cotton [22], chilling in cucumber [23], and drought in cauliflower and rice [24,25]. Several reports have shown that COR plays an important role in signaling drought-induced antioxidant responses which increase the transcript levels and activities of antioxidant enzymes in plants under environmental stress [24,25]. However, few studies focused on COR how to modulate photosynthetic performance enhancing plant growth, and identify that exogenously applied COR could alleviate or enhance adverse effects of drought stress in soybean.

The objective of the work, therefore, was to evaluate the role of exogenously applied COR in counteracting drought stress in soybean. It was hypothesized that COR improved the drought tolerance in soybean by modulating antioxidant systems and membrane stability leading to higher photosynthetic performance and translocation of assimilated ^{14}C. For these, we investigated the changes of growth, antioxidant systems and membrane stability, photosynthesis and chlorophyll fluorescence, and partitioning of assimilated ^{14}C in COR-treated soybean under drought stress.

2. Materials and methods

2.1. Plant materials and treatments

Soybean seeds (*G. max* L. cv. Zhonghuang 13) were surface sterilized in 75% (v/v) ethanol solution for 1.0 min, and rinsed three times with distilled water. The seeds were then sown in the pots (15 cm × 15 cm × 20 cm deep) containing a mixture of vermicompost and sand (1:1, v/v) and grown in a greenhouse under 30 °C/20 °C and 10 h/14 h day/night conditions. Four seeds were sown per pot. After the seedlings reached the first true leaf stage, they were thinned to two plants per pot. Hoagland nutrient solution [26] and water were supplied sufficiently throughout and thus potential nutrients and drought stress were avoided.

Two hundred and forty seedlings were randomly divided into 4 treatments comprising two regulator treatments including deionized water as control and COR (0.1 μM, based at our preliminary experiment and the research of Wu et al. [25]) and two water treatments including well-watered and drought stress. COR was dissolved in the 10-fold (m/v) methanol and then diluted with water to 0.1 μM. Deionized water and 0.1 μM COR were separately applied by foliar spray at the V_3 stage in soybean seedlings. After 24 h, drought stress treatments commenced by subjecting seedlings to be held on without watering for 7 days, and then seedlings were re-watered for 5 days, while the seedlings for well-watered treatments remained under normal water conditions. There were 60 seedlings per treatment, and each treatment had three replications (20 seedlings per replication).

The uppermost expanded leaf was labeled to sample for physiological and biochemical measurements after COR treatments. The samples were measured and collected at 7th day after drought stress and 5th day after re-watering. The corresponding well-watered samples were measured and collected at 8th day after COR treatments. At harvest, the plant height was measured, and then the plant was separated into shoots and roots. Then all samples were kept at 105 °C for 30 min and dried at 70 °C to determine the shoot and root dry weight. Fresh samples of all treatments were used for immediate assays or frozen in liquid nitrogen and stored at −80 °C for physiological and biochemical analysis.

2.2. Analysis of CO_2 gas exchange, chlorophyll content and chlorophyll fluorescence

Net photosynthetic rate (P_n), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Trmmol) of labeled leaves were measured with a portable photosynthesis system (LI-6400, LI-COR, Lincoln, USA). Leaf discs were removed from the labeled leaves and extracted with 95% ethanol in dark for 48 h until they were blanched. The concentrations of chlorophyll were determined spectrophotometrically according to Porra et al. [27].

Chlorophyll fluorescence was measured by the PAM-2000 chlorophyll fluorescence system (Heinz Walz, Effieltrich, Germany) according to Yang and Lu [28]. After a dark adaptation period of 30 min, minimum fluorescence (*Fo*) was determined by a weak red light (0.06 mmol m^{-2} s^{-1}), and maximum fluorescence of dark-adapted leaf (*Fm*) was measured during a subsequent saturated light pulse (3000 mmol mmol m^{-2} s^{-1} for 0.8 s). The steady state fluorescence (*Fs*) reaching within 7–8 min was thereafter recorded and a second saturating pulse of white light (3000 mmol m^{-2} s^{-1}) was imposed to determine the maximum fluorescence level in the light-adapted state (*Fm′*). To determine the minimal fluorescence level in the light-adapted state (*Fo′*), the actinic light was removed while a 3 s far-red light (5 mmol m^{-2} s^{-1}) was used to illuminate the leaf in order to re-oxidize the PSII center. The fluorescence parameters were calculated according to Xu et al. [29].

2.3. Determination of activities of RuBPCase and PEPCase

The extraction procedure of ribulose-1,5-bisphosphate carboxylase (RuBPCase, EC 4.1.1.39) followed those of Makino et al. [30]. RuBPCase in the supernatant was assayed at 25 °C in a medium that contained 100 mM Bicine at pH 8.2, 5 mM $MgCl_2$, 10 mM $NaHCO_3$, 5 mM creatine phosphate, 1 mM ATP-2Na, 0.1 mM NADH, 0.3 mM RuBP, 10 units of phosphocreatine kinase, 10 units of glyceraldehydes 3-phosphate dehydrogenase and 10 units of phosphoglycerate kinase, as described previously Sawada et al. [31]. Enzymatic activity was corrected for the decrease in absorbance at 340 nm in a control assay medium prepared without ribulose bisphosphate. RuBPCase was activated for 20 min at 0 °C after the preparation of the supernatant in an activation medium that contained 75 mM HEPES-KOH at pH 7.5, 10 mM $MgCl_2$ and 10 mM $NaHCO_3$, and its activity was also measured for determining total activity.

Phosphoenolpyruvate carboxylase (PEPCase, EC 4.1.1.31) was assayed spectrophotometrically at 25 °C as described by Gonzalez et al. [32]. Leaves of plants were rinsed in distilled water and homogenized in a buffer containing 50 mM TRIS–HCl (pH 7.5), 10 mM $MgCl_2$, 10% (v/v) glycerol, 1 mM EDTA, and 14 mM β-mercapto-ethanol. 1 mM PMSF and 10 μg ml^{-1} leupeptin were added to avoid or minimize proteolysis. The homogenate was centrifuged at 13,000 × *g* for 15 min and the supernatant was again centrifuged at 100,000 × *g* for 30 min. The soluble extracted protein was dialysed against the same homogenization buffer and used for PEPCase activity assay directly. PEPCase was determined by coupling its activity to malate dehydrogenase-catalysed NADH oxidation in 1.5 ml final volume of a standard buffer containing 100 mM TRIS–HCl (pH 8.0), 5 mM $MgCl_2$, 2.5 mM PEP, 0.2 mM NADH, 10 mm $NaHCO_3$, and 15 μg ml^{-1} MDH (Boehringer-Mannheim). NADH oxidation was determined at 340 nm in a model V550 spectrophotometer (Jasco) at 25 °C.