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Coronatine alleviates salinity stress in cotton by improving the antioxidative defense system and radical-scavenging activity

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Summary

Coronatine (COR) is a chlorosis-inducing phytotoxin that mimics some biological activities of methyl jasmonate. This study investigated whether COR confers salinity tolerance to cotton and whether such tolerance is correlated with changes in the activity of antioxidant enzymes. COR at 0.01 μM was applied hydroponically to cotton seedlings at the two-leaf stage for 24 h. A salinity stress of 150 mM NaCl was imposed after completion of COR treatment for 15 d. Salinity stress reduced biomass of seedlings and increased leaf superoxide radicals, hydrogen peroxide, lipid peroxidation, and electrolyte leakage. Activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione reductase (GR), and of the stable free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), scavenging activity were altered by salinity to varying degrees. Pretreatment with COR increased the activities of CAT, POD, GR, and DPPH scavenging activity in leaf tissues of salinity-stressed seedlings. Thus, COR might reduce the production of reactive oxygen species by activating antioxidant enzymes and DPPH-radical scavenging, thereby preventing membrane peroxidation and denaturation of biomolecules.

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; COR, coronatine; DAB, 3, 3-diaminobenzidine; DPPH, 1, 1-diphenyl-2-picrylhydrazyl; GR, glutathione reductase; JAs, jasmonates; MDA, malondialdehyde; POD, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase

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Introduction

Coronatine (COR) is a chlorosis-inducing non-host-specific phytotoxin produced by several members of the *Pseudomonas syringae* group of pathovars (Bender et al., 1999; Cintas et al., 2002) and induces a wide array of effects in plants. It leads to diffuse chlorosis of leaves, anthocyanin production, tendril coiling, and root retardation (Feys et al., 1994; Uppalapati et al., 2005), and promotes senescence in tobacco (Kenyon and Turner, 1990). COR stimulates ethylene production in tobacco leaves (Kenyon and Turner, 1992) and enhances auxin synthesis in tomato leaves (Uppalapati et al., 2005). Moreover, COR increases defense-related protease inhibitors and secondary metabolites, such as volatiles, nicotine, and alkaloid, and may play an important role in resistance to abiotic stress, such as salinity stress (Schüler et al., 2004).

Salinity is a major factor that limits plant development and crop productivity. Cotton (*Gossypium hirsutum*) is categorized as moderately salt-tolerant with a salinity threshold level of 7.7 dS m^{-1}, but its growth is severely reduced at high salinity levels, especially at the seedling stage (Ashraf and Ahmad, 2000). The decline in growth due to salinity is associated with damage caused by the superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) (Parida et al., 2004; Jebara et al., 2005). Free radicals disrupt normal metabolism through peroxidating lipids, which causes degradation and impairment of structural components and leads to membrane leakage and changes in activities of enzymes bound to membranes (Bor et al., 2003).

Cell membrane stability has been widely used to characterize stress tolerance, and high membrane stability is correlated with abiotic stress tolerance (Ashraf, 2002; Meloni et al., 2003). Increased production of $O_2^{\cdot-}$ and H_2O_2 in plants under salinity stress was associated with malondialdehyde (MDA) accumulation (Gossett et al., 1996; Parida et al., 2004; Verma and Mishra, 2005). MDA, a decomposition product of polyunsaturated fatty acids, is an indicator of membrane damage (Fadzilla et al., 1997).

Plants possess several antioxidants that protect against these potentially cytotoxic species of activated oxygen (Parida et al., 2004). Superoxide dismutase (SOD; EC 1.15.1.1) converts $O_2^{\cdot-}$ to H_2O_2. Catalase (CAT; EC 1.11.1.6) and a variety of peroxidases (POD; EC 1.11.1.7) catalyze the breakdown of H_2O_2. Although CAT is apparently absent in chloroplasts, H_2O_2 could be detoxified through the ascorbate–glutathione cycle with ascorbate peroxidase (APX; EC 1.11.1.11) and glutathione reductase (GR; EC 1.6.4.2). Hence, the potential of antioxidant enzymes to quench $O_2^{\cdot-}$ and H_2O_2 is related to stress tolerance of plants (Ashraf, 2002; Sairam and Srivastava, 2002). Recently, the use of 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a stable free radical to measure radical-scavenging activity has been widely employed (Benard and Runner, 2004). Higher DPPH-radical scavenging activity corresponded with the level of stress tolerance of cucumber seedlings (Kang and Saltveit, 2002).

COR has induced expression of *ATHCOR1*, also described as *AtCLH1*, a chlorophyllase (chlorophyll-chlorophyllido hydrolase; EC 3.1.1.14)-coding gene (Benedetti et al., 1998; Benedetti and Arruda, 2002; Kariola et al., 2005). Chlorophyllase is the first enzyme in the chlorophyll degradation pathway (Takamiya et al., 2000). Certain defects in chlorophyll degradation have resulted in increased oxidative stress and lesion development in plants (Matile and Hörtensteiner, 1999; Mach et al., 2001). *AtCLH1* is involved in tolerance to high-light-intensity stress (Kariola et al., 2005). Increased levels of reactive oxygen species (ROS) and induction of antioxidant defense systems have been observed in *AtCLH1*-silenced plants.

Feys et al. (1994) indicated that COR acts as a mimic of jasmonates (JAs), recognized as a new class of plant growth regulator, and it is more active than JAs for inducing production of secondary metabolites (Tamogami and Kodama, 2000). JAs are involved in stress responses by regulating oxidative reactions and inducing antioxidant defenses (Jung, 2004).

Uppalapati et al. (2005) showed that COR and JAs share similar, but not identical, activities and affect multiple phytohormone pathways. We are not aware of studies identifying the effect of COR on free radicals and the antioxidative defense system in plants under salt stress. Exploring the potential of plant-growth-regulating chemicals for minimizing generation of free radicals and alleviating membrane damage is a stepping-stone to salt stress management in plants (Verma and Mishra, 2005). Thus, our objective was to assess the potential of COR for scavenging free radicals through changes in the antioxidative system of cotton seedlings under salinity stress.

Materials and methods

Plant material and growth conditions

The research was conducted in a growth chamber under 28 °C/20 °C and with a 12 h photoperiod at 400 μmol m^{-2} s^{-1} photosynthetically active radiation. The relative humidity was 30 ± 2%. Cotton (*G. hirsutum*