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Research article

Coumarin pretreatment alleviates salinity stress in wheat seedlings

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ABSTRACT

The potentiality of COU to improve plant tolerance to salinity was investigated. Wheat grains were primed with COU (50 ppm) and then grown under different levels of NaCl (50, 100, 150 mM) for two weeks. COU pretreatment improved the growth of wheat seedling under salinity, relative to COU-untreated seedlings, due to the accumulation of osmolytes such as soluble sugars and proline. Moreover, COU treatment significantly improved K^+/Na^+ ratio in the shoots of both salt stressed and unstressed seedlings. However, in the roots, this ratio increased only under non-salinity. In consistent with phenylalanine ammonia lyase (PAL), phenolics and flavonoids were accumulated in COU-pretreated seedlings under the higher doses of salinity, relative to COU-untreated seedlings. COU primed seedlings showed higher content of the coumarin derivative, scopoletin, and salicylic, chlorogenic, syringic, vanillic, gallic and ferulic acids, under both salinity and non-salinity conditions. Salinity stress significantly improved the activity of peroxidase (POD) in COU-pretreated seedlings. However, the effect of COU on the total antioxidant capacity (TAC) was only obtained at the highest dose of NaCl (150 mM). The present results suggest that COU pretreatment could alleviate the adverse effect of salinity on the growth of wheat seedlings through enhancing, at least partly, the osmoregulation process and antioxidant defense system.

1. Introduction

Crop productivity is greatly threatened by a plethora of abiotic stresses. Among these, salinity is one of the major factors responsible for limiting seed germination, plant growth and development as well as yield production. Unfortunately most of crop plants cannot grow in high salt concentrations (Läuchli and Grattan, 2007). Because of the developing area of salt-affected land, salinity becomes an everlasting challenge to agriculture and food supply (Flowers, 2004). Accordingly, improving salinity-tolerance of crop plants is a strategic goal for governments all over the world. Physiologically, salinity can be considered as a complex case of stress, comprising osmotic and salt-specific stresses (Flowers, 2004; Munns et al., 2006).

To cope with salinity, tolerant plant species have some general responses and mechanisms to maintain proper water uptake, scavenge free radicals and alleviate the adverse effect of excess ions (Chen et al., 2013). One of these mechanisms is the osmoregulation, by the synthesis and accumulation of compatible solutes or osmolytes (Rhodes et al., 2002). Osmolytes are low molecular weight molecules accumulated in the cytosol at high molar concentrations without harming the cell, such as sugars and sugar alcohols, proline, arginine, polyamines, and glycine betaine (Shao et al., 2009). These osmolytes not only increase cellular osmotic pressure and maintain cell turgidity but also protect cell compartments from oxidative damage, acts as signaling molecules and stimulate the activity of antioxidant enzymes (Rolland et al., 2002; Sekmen et al., 2014).

Accumulation of phenolic compounds is a common plant response to different types of biotic and abiotic stresses such as pathogen attack, wounding, nutrient deficiency, radiation, high temperature and chilling (Abu El-Soud et al., 2013; Al-Wakeel et al., 2013; Dixon and Paiva, 1995). Phenolic compounds represent a diverse group of plant secondary metabolites comprises simple phenylpropanoids, coumarins, benzoic acid derivatives, lignin and its precursors, flavonoids and tannins (Wilfred and Nicholson, 2006). Phenolic compounds, in concentration and structure dependent manner, can act as scavengers of free radical, lipid peroxidation inhibitors and membrane stabilizers (Arora et al., 2000; Catherine, 1997; Michalak, 2006; Milić et al., 1998; Verstraeten et al., 2003).

Plant phenolics could be utilized in agriculture as eco-friendly

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alternatives for weeds and pests management (Zeng et al., 2008). Some phenolic compounds such as cinnamic, ferulic and ellagic acids were shown to increase the plant tolerance to abiotic stresses like chilling, salinity and osmotic stress (Abu El-Soud et al., 2013; Li et al., 2011; Singh et al., 2013). A potent class of plant phenolics is coumarin (2H-chromen-2-one; COU) and its derivatives (Razavi, 2011). They have attracted much attention for its plant growth regulatory, antioxidant, cytotoxic and antimicrobial properties (Al-Amiery et al., 2012; Saleh et al., 2014). COU was reported to affect many plant physiological processes at the different stages of growth and development, however its effect is dose and species-dependent (Abenavoli et al., 2004; Pergo et al., 2008).

Recently, we have investigated the potentiality of COU, as seed priming or foliar application treatments, to improve the accumulation of soluble sugars, proteins and phenolic compounds in sunflower and faba bean (Al-Wakeel et al., 2013; Saleh et al., 2014). We have demonstrated that coumarin treatment has improved the level of chlorogenic, ferulic and salicylic acids as well as the coumarin derivatives scopolin and ayapin under normal and to more extent under phytopathogen challenge conditions (Al-Wakeel et al., 2013). Based on the aforementioned observations, COU can be a candidate for improving plant tolerance to salinity stress. Accordingly, the objective of this study is to assess the impact of COU on growth, as well as biosynthesis and accumulation of osmolytes and phenolic compounds in seedling of wheat (*Triticum aestivum* L.), one of the most economic crops in the world, under both normal and salt stressed conditions; and to evaluate the potentiality of COU to ameliorate the salt stress on wheat seedlings.

2. Materials and methods

2.1. Plant material and growth conditions

Grains of wheat, cultivar Misr 1, were obtained from the Agricultural Research Center (ARC), Giza, Egypt. Grains were surface sterilized for 20 min in 5.0% sodium hypochlorite and then washed several times with distilled water. The disinfected grains were divided into two groups; one of them was soaked in 50 ppm COU, dissolved in 0.1% ethanol in distilled water, for 4 h at 22 °C while the other group was immersed in the same amount of ethanol and water for the same time (unprimed). Afterthought, the grains were left to dry in an air cabinet. The COU-primed or unprimed grains were sown in plastic pots filled with peat moss. Pots were irrigated with half strength Hoagland's nutrient solution containing 0, 50, 100 or 150 mM NaCl and incubated in growth chamber at 22 ± 2 °C with 12 h photoperiod and 75% relative humidity. Two weeks after emergence, seedlings were harvested, the roots rinsed with distilled water thoroughly then gently blotted between paper towels, and their growth parameters (length, fresh, dry weights and moisture content) were recorded. Some fresh seedlings from each group were ground immediately under liquid nitrogen and stored at −20 °C until used for biochemical analysis.

2.2. Determination of Na^+ and K^+

For extraction of Na^+ and K^+ ions 100 mg of oven dried tissues were digested with 10 ml 30% HNO_3 for 48 h, and then diluted using deionized water. The concentrations of Na^+ and K^+ were determined using an atomic absorption spectrometer (Perkin–Elmer, Model 3300).

2.3. Determination of soluble sugars

Soluble sugars were extracted by boiling a known weight of liquid nitrogen-powdered tissues in distilled water for 1 h in a water bath (El-Tayeb et al., 2006). The extract was cooled and centrifuged at 5000 g for 10 min, after that the supernatant was made up to a known volume. The total soluble sugars in the extract were determined with anthrone sulfuric acid reagent (Yemm and Willis, 1954). Briefly, 0.5 mL of the extract was mixed with 4.5 mL of anthrone-sulfuric acid reagent and boiled in a water bath for 7 min. After cooling, the developed blue green color was measured at 620 nm against water-reagent blank. Concentration of sugars was determined using glucose as a standard and calculated as mg glucose g^{-1} fresh weight.

2.4. Determination of proline content

Free proline content was determined according to Bates et al. (1973). A known weight of liquid nitrogen -powdered tissue was homogenized in10 ml of 3% aqueous sulfosalicylic acid for 10 min, followed by centrifugation at 5000 g for 10 min. A total of 2 ml of the clear supernatant was mixed with 2 ml of freshly prepared acid ninhydrin reagent and 2 ml glacial acetic acid, then boiled in water bath for 1 h. After cooling in an ice bath, the mixture was mixed with 4 ml toluene and vigorously stirred for 20 s. Thereafter, the chromophore-containing toluene was aspirated from the aqueous phase, and its absorbance measured at 520 nm against toluene. The proline content was determined from a standard curve of proline and calculated as μmol g^{-1} fresh weight.

2.5. Determination of total phenolic and flavonoids content

Total soluble Phenolic compounds were extracted with 70% ethanol, according to the method outlined by Sauvesty et al. (1992). The Folin-Ciocalteu phenol method (Lowe, 1993) was used for phenolics determination. One ml of the phenolic extract was mixed with 1 ml of 10% Folin-Ciocalteu phenol reagent and 1 ml of 20% anhydrous sodium carbonate, then completed up to a known volume with distilled water. The absorbance of the blue color was measured after 30 min at 650 nm against a water-reagent blank. The phenolic content was obtained from the standard curve of catechol, then expressed as mg catechol g^{-1} fresh weight.

Total flavonoids were extracted and determined as described by Sakanaka et al. (2005). A known weight of liquid nitrogen-powdered tissues was homogenized in methanol using a pestle and mortar, then the slurry centrifuged at 3000 g for 10 min. The clear supernatant was completed up to known volume with methanol. 0.25 ml of the extract was mixed with 1.25 ml of distilled water in a test tube, followed by addition of 75 μl of 5% (w/v) sodium nitrite solution. After 6 min of incubation, 150 μl of 10% (w/v) aluminum chloride solution was added and the mixture was allowed to stand for a further 5 min before addition of 0.5 ml of 1 M sodium hydroxide. The mixture was completed up to 2.5 ml with distilled water and mixed well. The absorbance was measured immediately at 510 nm. The concentration of total flavonoids was calculated using a standard curve of catechin and expressed as mg catechin g^{-1} fresh weight.

2.6. Determination of individual phenolics by HPLC

For Extraction of Phenolic compounds, 0.5 g oven dried shoot tissues was ground in a mortar with a mixture of acetone/methanol (1:1, v/v) at room temperature. After centrifugation at 3000 g for 10 min, the clear extract was evaporated under vacuum (40 °C) and the pellet was dissolved in 1 ml of HPLC spectral grade methanol.

The procedure outlined by Weisz et al. (2009)with some modifications was used for separation of individual phenolic compounds of each sample. Analyses were carried out using a Perkin–Elmer HPLC system (USA) equipped with a binary LC-250