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Research article

Androsterone-induced molecular and physiological changes in maize seedlings in response to chilling stress

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ABSTRACT

The aim of study was to elucidate the influence of foliar sprays of androsterone in alleviating detrimental effects of chilling stress in maize seedlings. Eleven-days-old maize seedlings were treated with 10^{-9} mol L^{-1} androsterone and then transferred to a chamber with temperature of 10/7 °C (day/night) for 3 days. The stress injury was measured in terms of increase in electrolyte leakage, superoxide production and hydrogen peroxide level, and decrease in chlorophyll content. Androsterone application mitigated significantly the chilling-induced stress injury. Under chilling stress, the oxidative damage which was measured as malondialdehyde content was lesser in androsterone-applied seedlings that were associated with greater activities of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POX), ascorbate peroxidase (APX) and glutathione reductase (GR). Moreover, SOD, POX and APX isozymes exhibited a strong correlation with changes in their activities. Androsterone application enhanced the level of antioxidant compounds like ascorbic acid, glutathione, proline and carotenoid as well as activities of antioxidant enzymes. Similarly, while androsterone treatment increased total antioxidant status, it reduced total oxidant status relative to chilling-stressed seedlings alone. Soluble protein profile was significantly changed by only chilling stress and chilling stress plus androsterone treatment, as well. According to these findings, it is possible to say that androsterone could be used to alleviate the damaging effects of chilling stress by improving antioxidative system in maize seedlings. This is the first study elucidating the effects of androsterone on resistance to chilling stress of plants.

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1. Introduction

Environmental stresses, such as salinity, drought, extreme temperatures and chemical toxicity, lead to a series of morphological, physiological and molecular changes that negatively affect plant growth and productivity [1]. Among these abiotic stresses, chilling is a major stress that limits the productivity and geographical distribution of many important crops such as maize and chickpea [2,3].

Chilling stress is known to induce many abnormalities like damage to membranes, generation of reactive oxygen species (ROS), protein denaturation and accumulation of toxic compounds etc. at various organizational levels of the cells [4,5]. Enhanced level of ROS such as superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) radical contribute to chilling damage [6]. Plant's responses to chilling stress differ from each other. To protect cellular membranes and organelles from the harmful effects of ROS, plants have evolved very efficient antioxidant systems comprised of antioxidant enzymes and compounds [7]. The former class includes enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT). The latter class includes a wide variety of chemical compounds such as ascorbate (AsA), glutathione (GSH), proline and carotenoids [8]. Antioxidative system scavenges the ROS up to water [9]. When production of ROS exceeds the capacity of the antioxidative system, oxidative damage occurs [10]. Plants should maintain the antioxidant activities to counteract with oxidative stress. Induction of plant resistance is, therefore, vital and necessary under stress conditions.

It is well known that the potential yield and geographical extension of crops could be increased if tolerance of stress conditions is improved [3,11]. One of the ways used in improving stress tolerance is exogenous treatment of the plants with chemicals or growth regulators having proven role in improvement the plant resistance [12–14]. Recently, some researchers reported that mammalian sex hormones (MSH) also have remarkable effects in the improvement tolerance of plants growing under salinity stress [13–15]. MSH naturally present in plants are now isolated from various plant species. Many studies have made to elucidate

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presence, quantities, receptors, specific binding sites and roles of these compounds in plants. In addition, many researchers reported that when exogenously applied, MSH has positive effects on various stages from seed germination to flowering as well [16–22]. Due to presence as natural and in very low concentrations in structure of plants, these compounds are plant hormone candidates and can be considered as plant regulators.

A perusal literature indicates that no reports are available about the involvement of androsterone, one of the MSH, in response of maize to chilling stress at its seedling stage. Considering the previous reports, we hypothesized that androsterone might have a vital role in improvement the chilling-sensitivity of maize. In order to examine whether this mammalian hormone is involved in the induction of defense responses to low temperature stress, this study was undertaken therefore to determine the physiological and biochemical changes in pre-treated with androsterone maize seedlings exposed to cold stress.

2. Materials and methods

2.1. Plant material, growth conditions and hormone treatment

Uniform maize (*Zea mays* L.) seeds were surface-sterilized for 10 min with 10% commercial NaOCl solution and then washed few times with distilled water. The sterilized-seeds were sowed in sand-filled pots and grown for 11 d in a growth chamber at a temperature of 25–27/20–22 °C (day/night), relative humidity of 70% and a 16 h photoperiod. On the eleventh day, 50 ml of 10^{-9} mol L^{-1} androsterone solution along with 0.01% Tween-20 was sprayed on 1 pot. Control (unstressed-seedlings) and chilling-stressed seedlings were only treated with distilled water along with 0.01% Tween-20. All of the seedlings were placed in growth chamber for 12 h for full absorption of applied-solutions. Then the seedlings (except for control group) were transferred to a different growth chamber with temperature of 10/7 °C (day/night). Control group were kept at normal conditions (25–27/20–22 °C). On the third day of low temperature stress, leaves were sampled for analyses. Both temperature of treatment (10/7 °C) and androsterone concentration (10^{-9} mol L^{-1}) were chosen based on preliminary experiments.

2.2. Measurement of contents of total chlorophyll and carotenoid

Total chlorophyll and carotenoid content in fresh leaves were estimated using the method of Lichtenthaler and Buschmann (2001) [23]. About 0.5 g fresh leaf tissue were ground in a mortar and pestle containing 5 ml acetone (80%). The Optical Density of the solution was read at 662 and 645 nm (chlorophyll) and 470 nm (carotenoids). Photosynthetic pigments were expressed as mg g^{-1} FW.

2.3. Measurement of electrolyte leakage

Electrolyte leakage (EL) was measured according to Lutts et al. (1995) [24]. Fresh leaf samples were washed three times with double-distilled water (ddH_2O) to remove surface contamination. Twenty leaf-discs, approximately 5 mm^2, were placed in a closed vial containing 5 ml of ddH_2O and incubated on a rotatory shaker for 24 h. Subsequently, the first electrical conductivity of the solution (EC_1) was determined. Samples were then autoclaved at 120 °C for 20 min and then the last electrical conductivity (EC_2) was recorded after cooling the solution at room temperature. The EL was calculated as:

$$EL(\%) = (EC_1/EC_2) \times 100$$

2.4. Enzyme extraction and assays

The leaves (500 mg) were homogenized in 5 ml of 10 mmol L^{-1} potassium phosphate buffer (pH 7.0) containing 4% (w/v) polyvinyl pyrolidone and 1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA) in order to determine the activities of superoxide dismutase (SOD), guaiacol peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR). The homogenate was centrifuged at 12 000× *g* for 15 min at 4 °C and the supernatant obtained was used as an enzyme source. Leaf soluble protein content was measured by method of Bradford (1976) [25] in which bovine serum albumin was used as standard.

SOD activity was estimated according to the method of Giannopolities and Ries (1977) [26]. The assay medium contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM *p*-nitro blue tetrazolium chloride (NBT), 2 mM riboflavin, 0.1 mM EDTA, and 5 ml enzyme extract. One unit of enzyme activity was determined as the amount of the enzyme to reach an inhibition of 50% NBT reduction rate by monitoring the absorbance at 560 nm.

POX activity was measured using guaiacol as a substrate [27]. The increase in absorbance at 470 nm due to the guaiacol oxidation was recorded for 5 min. The reaction mixture contained 25 mM phosphate buffer (pH7.0), 0.05% guaiacol, 1.0 mM H_2O_2 and 0.1 ml enzyme extract. One unit of enzyme activity was defined as the amount that causes a change of 0.01 in absorbance per mg protein and per minute. The activity was expressed as U mg^{-1} protein.

CAT activity was determined by measuring the rate of decrease in absorbency at 240 nm of a solution of 12.5 mM H_2O_2 in 50 mM KH_2PO_4 (pH 7.0) at 30 °C [28]. The amount of enzyme per assay was adjusted so that the rate of the reaction was linear for at least 2 min. One unit is defined as the amount of enzyme catalyzing the decomposition of 1 μmol H_2O_2 per minute calculated from the extinction coefficient for H_2O_2 at 240 nm of 0.036 cm^2 μmol^{-1}.

APX activity was assayed according to Nakano and Asada (1981) [29]. The assay mixture consisted of 0.5 mM ascorbic acid, 0.1 mM H_2O_2, 0.1 mM EDTA, 50 mM sodium phosphate buffer (pH 7.0), and 0.15 ml enzyme extract. Enzyme activity was quantified using the molar extinction coefficient for ascorbate (2.8 (mmol $L^{-1})^{-1}$ cm^{-1}).

GR activity was measured according to Foyer and Halliwell (1976) [30]. The oxidized glutathione (GSSG)-dependent oxidation of NADPH was followed at 340 nm in a 1 ml reaction mixture containing 100 mM sodium phosphate buffer (pH 7.8), 0.5 mM GSSG, 50 μl extract, and 0.1 mM NADPH. One unit GR was defined as 1 mmol ml^{-1} GSSG reduced min^{-1}.

2.5. Measurement of contents of superoxide anion, hydrogen peroxide and malondialdehyde

Superoxide ($O_2^{\bullet -}$) production was measured as described by Elstner and Heupel (1976) [31]. Fresh leaves (0.2 g) were homogenized in 1 ml of 50 mM phosphate buffer (pH 7.8), and the homogenate was centrifuged at 10 000*g* for 10 min. Then, 0.5 ml of the supernatant was added to 0.5 ml 50 mM phosphate buffer (pH 7.8) and 0.1 ml of 10 mM hydroxylamine hydrochloride. After 1 h reaction at 25 °C, the mixture was added to 1 ml of 17 mM sulfanilamide and 1 ml of 7 mM α-naphthylamine at 25 °C for 20 min. The specific absorbance at 530 nm was determined. Sodium nitrite was used as a standard solution to calculate the production rate of $O_2^{\bullet -}$.

Hydrogen peroxide (H_2O_2) content was measured according to Sergiev et al. (1997). Leaf tissues (500 mg) were homogenized in ice bath with 5 ml 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 12 000 *g* for 15 min and 0.5 ml of the supernatant was added to 0.5 ml 10 mM potassium phosphate buffer (pH 7.0) and 1 ml 1 M KI. The absorbance of supernatant was read at 390 nm. The content of H_2O_2 was measured using standard curve.

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