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Research article

Nitric oxide, as a downstream signal, plays vital role in auxin induced cucumber tolerance to sodic alkaline stress

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ABSTRACT

Nitric oxide (NO) and auxin (indole-3-acetic acid; IAA) play vital roles in regulating plants tolerance to abiotic stresses. This study showed that both NO and IAA could induce cucumber plants tolerance to sodic alkaline stress, which depended on their roles in regulating reactive oxygen species (ROS) scavenging, antioxidative enzymes activities, Na^+ accumulation and protecting photosystems II (PSII) from damage. In addition, IAA has significant effect on NO accumulation in cucumber root, which could be responsible for IAA-induced sodic alkaline stress tolerance. Further investigation indicated that the function of IAA could be abolished by NO scavenger (cPTIO). On the contrary, IAA transport inhibitor (NPA) showed no significant effects on abolishing the function of NO. Based on these results, it could be concluded that NO is an essential downstream signal for IAA-induced cucumber tolerance to sodic alkaline stress.

1. Introduction

The salinization of soil is one of the most challenging environmental stresses limiting crop productivity globally. Sodic and saline-sodic soils occur within the boundaries of at least 75 countries, and their extent has increased steadily in several major irrigation schemes throughout the world (Shi and Wang, 2005). Some reports have clearly demonstrated alkaline salts ($NaHCO_3$ and Na_2CO_3) are more destructive to plants than neutral salts (NaCl and Na_2SO_4) with more area all over the world (Shi and Wang, 2005). Worldwide, 831 million hectares are affected by salt stress, including 434 million hectares suffering from alkalinity (Gong et al., 2014a). It is well known that plants exposing to neutral salt stress generally suffer from osmotic stress and Na^+ injury (Yang et al., 2007). Besides, high pH of sodic alkaline stress is another inhibitor for plants growth and development. The high pH of rhizosphere directly cause Ca^{2+}, Mg^{2+} and $H_2PO_4^-$ to precipitate (Gong et al., 2014b), and may inhibit ion uptake as well as disrupt the ion homeostasis in plant cells (Yang et al., 2007), resulting more serious oxidative stress at the subcellular scale. Thus, plants in alkaline soil showed more complex physiological reaction compared with salt stress. Mechanism and regulation of higher plants tolerance to neutral salinity stress have been extensively studied; unfortunately, the adaption mechanism to alkalinity in plants is short of deep investigation (Shi and Wang, 2005). Recently, increasing attention has been paid to the effect of sodic alkaline stress on plants, especially in the research of redox homeostasis and signal transduction factors (Gong et al., 2014a).

Auxin, mainly for indole-3-acetic acid (IAA), has long been recognized as a major regulator of plant growth and development, especially for the regeneration of root system. Recent studies indicated that IAA was also involved in plant tolerance to stress conditions (Pujari and Chanda, 2002; Chakrabarti and Mukherji, 2003). *IAA-Ala Resistant3* (*IAR3*) stimulated lateral root development under drought stress by releasing bioactive IAA from an inactive precursor in *Arabidopsis* (Kinoshita et al., 2012). There is also a report that an increase in IAA contents promotes the formation of an attraction signal in the leaf growth zone in response to salt stress, and foliar application of IAA could improve the chlorophyll level and membranes stability in salinity-stressed maize plants (Kaya et al., 2010).

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Nitric oxide (NO) is another important signal molecular to play crucial roles in plant tolerance to abiotic stress by regulating ROS metabolism and stress-induced genes expression (Khana et al., 2012). It has been demonstrated that NO act as a downstream signal of IAA in regulating some physiological processes including the formation of adventitious root in sunflower hypocotyls (Yadav et al., 2013), the enhancement of ferric-chelate reductase (FCR) activity in response to iron deficiency in *Arabidopsis* (Chen et al., 2010), and regulation of stomatal closure against osmotic stress in *Pisum sativum* (Kolbert et al., 2008). Our previous studies indicated that application of NO had significant effect on improving sodic alkaline stress tolerance in cucumber and tomato plants (Gao et al., 2013; Gong et al., 2014a). However, little was known about if IAA could induce NO signal under sodic alkaline stress conditions, as well as IAA-induced sodic alkaline stress tolerance depends on NO signal. Although further confirmation of IAA-induced NO production is necessary for root development (Terrile et al., 2012), the observations previously reported could imply the presence of an unbeknown responsible for sodic alkaline stress tolerance regulated by IAA-induced NO signal.

Cucumber (*Cucumis sativus* L.) is worldwide vegetable that has shallow root system and is sensitive to sodic alkaline stress. Economic loss to cucumber growers under sodic alkaline stress has been evidenced due to reduction in yield and dietetic values in recent years. Based on previous study, we are interested in whether IAA alleviated sodic alkaline stress on cucumber, and whether NO plays as downstream of auxin signal in relieving damage by $NaHCO_3$ in cucumber. We were inclined to investigate whether IAA and NO might have an interactive effect on cucumber tolerance to sodic alkaline stress. We hypothesized that IAA could stimulate and induce NO accumulation. Interestingly, IAA's mitigative effect on sodic alkaline stress could be inhibited by the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO). On the contrary, the mitigative effect of NO could not be removed by IAA transport inhibitor 1-naphthylphthalamic acid (NPA). These results indicate that NO may act as a downstream signal of IAA in cucumber seedlings under sodic alkaline stress. In this study, the roles of IAA and NO in regulating $NaHCO_3$-induced oxidative stress were assessed in cucumber seedlings. Thus, we firstly provide evidence that IAA is involved in the induction of cucumber tolerance to sodic alkaline stress and NO acts as a downstream signal of IAA in this pathway.

2. Materials and methods

2.1. Plant materials, growth conditions and stress treatments

Cucumber (*C. sativus* L. cv. Jinyan 4) seeds were germinated on moisture filter paper in the dark at 28 °C for 2 days, and germinated seedlings were transferred to the growth chamber filled with vermiculite and grown in light incubators maintained at 28 °C: 15 °C (day: night) with a photoperiod of 14–10 h for 10 days. Then they were transplanted into 5 L black plastic containers containing full aerated Hoagland nutrient solution, with four seedlings per container. After 15 days of pre-culture, the treatments were started. 1 μM Indole-3-acetic acid (IAA), 0.1 mM sodium nitroprusside (SNP; an NO donor), 0.1 mM 1-naphthylphthalamic acid (NPA; an auxin efflux blocker) and an 1 mM 2,4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO; a specific scavenger of NO) were used in different treatments as 0 mM and 30 $NaHCO_3$ were separately applied for control and sodic alkaline stress. Nutrient solution including all substances in each treatment was renewed every two days. The experiment was arranged in a randomized complete block design with five replicates. Each replicate include three containers. Physiological indices were assessed 10 d after treatment.

2.2. Determination of biomass and Na^+ content

Plants were divided into shoots and roots, which were rinsed with deionized water. The samples were placed in an oven run at 105 °C for 20 min, and then dried at 75 °C to constant. These dried plants were weighed to record the plant dry mass. For determination of Na^+ concentrations, the mixed powders of dried samples were digested in a mixture of H_2SO_4–H_2O_2, and the concentration of Na^+ was determined with an atomic absorption spectrophotometer (*TAS-990*, *Purkinje General*, *Beijing*, *China*) (Shi and Wang, 2005).

2.3. Determination of root activity

Root activity was determined by triphenyltetrazolium chloride (TTC) reduction method (Gong et al., 2014b). 0.5 g of roots was put in to 10 ml 0.5 mM PBS buffer (pH 7.0) containing 0.4% (w/v) TTC at 37 °C for 1 h, and then stopped the reaction with 2 ml 1 M H_2SO_4. Resultant of reaction (triphenyl formazan) was extracted by acetic ether and root activity was analyzed by the reduction of TTC at 485 nm.

2.4. Photosynthetic apparatus analysis

Photosynthetic parameters were determined on the third fully expanded leaves by a photosynthesis system (*LI-6400, Lincoln, USA*). Chlorophyll was extracted in 80% acetone and measured according to previous descriptions (Yang et al., 2007). Chlorophyll fluorescence parameters were measured on the third fully expanded leaves after 30 min in the dark using chlorophyll fluorescence imaging (*FluorCam7; Photon Systems Instruments; U.S.A.*). The maximum PSII quantum yield [$F_v/F_m = (F_m - F_o)/F_m$]; the photochemical efficiency of open PSII centers [$F_v'/F_m' = (F_m' - F_o')/F_m'$]; actual photochemical efficiency of PSII [$\Phi_{PSII} = (F_m' - F_s)/F_m'$]; photochemical quenching coefficient [$q_P = (Fm' - Fs)/(F_m' - F_o')$]; non-photochemical quenching [$NPQ = (F_m - F_m')/F_m'$]. unbalance of excitation energy distribution between PSII and PSI [$\beta/\alpha - 1 = (F_m' - F_o')/(F_m' - F_s) - 1$]; fraction of light absorbed in PSII antennae that was utilized in photosynthetic electron transport [$P = F_v'/F_m' \times q_P$]; fraction of light absorbed in PSII antennae that was dissipated via thermal energy dissipation in the antennae [$D = 1 - F_v'/F_m'$]; and fraction of light absorbed in PSII antennae that was in excess [$E_x = F_v'/F_m' \times (1 - q_P)$] were determined (Maxwell and Johnson, 2000).

2.5. Analysis of ROS generation

The histochemical staining of O_2^- and H_2O_2 was performed as follows. In the case of O_2^-, leaves were vacuum infiltrated directly with 0.1 mg ml^{-1} NBT in 25 mM K-HEPES buffer (pH 7.8) and incubated at 25 °C in the dark for 2 h. In the case of H_2O_2, leaves were vacuum infiltrated with 1 mg ml^{-1} DAB in 50 mM Tris-acetate (pH 3.8) and incubated at 25 °C in dark for 24 h. In both cases, leaves were rinsed in 80% ethanol for 5 times at 70 °C, and photographed (Xia et al., 2009).

O_2^- was quantified using the method of hydroxylamine oxidation. 0.5 ml extraction was mixed with 1 ml hydroxylamine and incubated at 25 °C for 1 h. Then 1 ml 17 mM of *p*-aminobenzene sulfonic acid and 7 mM of α-naphthylamine solution were added and the solution was incubated for an additional 20 min. The reaction was measured spectrophotometrically at 530 nm and O_2^-