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Photosynthetic capacity, ion homeostasis and reactive oxygen metabolism were involved in exogenous salicylic acid increasing cucumber seedlings tolerance to alkaline stress

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ABSTRACT

Although various works have been done in alleviating effects of exogenous salicylic acid (SA) on abiotic stress in plants, it was not reported whether exogenous SA had effects on alkaline-tolerance of plants. In the present study, we found that the effect of exogenous SA depended on the concentration and 75 μM SA showed the best remission effect on cucumber plants under alkaline stress. The effect of 75 μM SA on detailed change of photosynthetic capacity, reactive oxygen metabolism and ionic homeostasis in alkaline-treated cucumber plants were further investigated. Alkaline stress significantly caused ion imbalance, decreased photosynthetic pigment content, inhibited PSII activity and net photosynthetic rate. With prolonging stress time, electrolyte leakage, MDA and H_2O_2 contents were significantly increased in cucumber leaves, while antioxidative enzymes activity and ascorbate-glutathione cycle increased first and then decreased. Application of 75 μM SA reduced Na^+ accumulation, maintained ionic homeostasis and normal operation of photosystem, enhanced the reactive oxygen species (ROS) scavenging system, reduced oxidative damage, and alleviated lipid peroxidation, thereby improved the alkaline-tolerance of cucumber plants.

1. Introduction

The soil salt-alkalization has already become a worldwide environmental problem limiting the agricultural productivity (Gao et al., 2013; Gong et al., 2014). There are over 800 million hectares of salt-alkali soil across the world, which spans more than 100 countries and accounts for 60 percent of the world's total arable land (Shi and Sheng, 2005). Salt-alkali soil can be classified into two types: saline soil (with neutral salt as the main ingredient such as NaCl and Na_2SO_4) and alkali soil (with basic salt as the main ingredient such as Na_2CO_3 and $NaHCO_3$). There is now a general consensus that salt-alkaline stress inhibits plant growth and development through osmotic stress, ion toxic and oxidative stress (Chaves et al., 2009; Gong et al., 2013, 2014). Compared with neutral salt stress, alkaline stress causes much more damage and metabolic disorder in plants due to its additional high pH, which directly causes precipitation of Ca^{2+}, Mg^{2+} and $H_2PO_4^-$, disturbs ion absorption and mineral nutrient, leading to metabolism disorder more seriously (Gong et al., 2013, 2014; Liu et al., 2015). In recent years, some progresses have been done to improve the crop tolerance to salt-alkaline stress, which include conventional breeding and transgenic technique application as well as some plant growth regulator application (Kasukabe et al., 2004, 2006; Roy and Wu, 2001). Salicylic acid (SA) is a phenolic phytohormone with natural activity in higher plants. There are two distinct pathways for SA biosynthetic in plants, the isochorismate (IC) pathway and the phenylalanine ammonia-lyase (PAL) pathway (Yusuf et al., 2013). Moreover, SA plays exclusive role in various physiological and biochemical process such as plant growth, thermogenesis, flower induction and uptake of ions (Klessig and Malamy, 1994; Yusuf et al., 2013).Therefore, some researchers argue that SA may be regarded as a new kind of plant endogenous hormone (Raskin, 1992; Vlot et al., 2008), and the study of SA has been a research hotspot and received conspicuous achievements. Now, it is well accepted that SA is critical for the induction of the hypersensitive response (HR) and systemic acquired resistance (SAR) as well as the stress-tolerance gene expression of plants (Courtois and Courtois, 2012; Gaffney et al., 1993; Hayat et al., 2010; Mortimer and Dupree., 2013). Exogenous SA could induce pathogen-related proteins (PRs) in tobacco, cucumber, potato, bean, cowpea and rice, and improved resistance to variety pathogens including fungi and bacteria (Song et al., 2015; Guo et al., 2012). In addition, it was also found that SA could improve the disease resistance of plants via promoting the production of lignin and phytoalexin (Mohr and Cahill, 2007). In the

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study of abiotic stress tolerance, it was observed that SA and its analogues could induce the plant to produce salt and drought resistant characters, such as stomatal closure, reduced leaf transpiration, increased membrane lipid unsaturation, reduced cell electrolyte osmosis, involvement of plant cell mitochondria cyanide-resistant and non-phosphorylation pathways and jasmine metabolism (Eraslan et al., 2008; Lei et al., 2010; Singh and Usha, 2003). Later research proved that SA could significantly improve the selectivity of Na^+ transport and the selectivity of K^+ transport upwards, resulting in the strength of salt tolerance in plants (Jayakannan et al., 2013). According to Kováčik et al. (2009), under salt stress, SA could increase the activity of antioxidant enzymes and reduced the accumulation of malondialdehyde in chamomile, which is the product of membrane lipid peroxidation. Ghassemi-Golezani and Lotfi (2015) demonstrated that exogenous SA counteracted the NaCl deleterious of mung bean via activating the photosynthetic process. Although extensive works have been done in alleviating effects of exogenous SA on salt stress, it has not been reported whether exogenous SA could decrease the alkaline stress on plants.

Cucumber (*Cucumis sativus* L.) is an important cash crop, which is edible widely in the world due to its palatable flavor and rich nutrient. As a glycophyte, cucumber is vulnerable to alkaline stress no matter in protected or open-field cultivation, restricting it from sustainable production. However, few research has been focused on alkaline tolerance of cucumber. Therefore, how to improve alkali-tolerance is critical for cucumber production in saline-alkaline soil area. In the present study, the purpose was to determine whether exogenous SA can improve the alkaline-tolerance of cucumber seedlings and define the potential physiological mechanisms of alkaline tolerance regulated by SA. For this purpose, we investigated the effect of SA with different concentrations on cucumber seedlings under $NaHCO_3$ stress and further characterized the mitigation effect of 75 μM SA, which showed the best mitigation effect. And 75 μM SA was used to further investigate the mechanism of SA alleviating alkaline stress, and some physiologial parameters including photosynthetic capacity, ion homeostasis and reactive oxygen metabolism were analyzed. The expected results will provide a theoretial basis for SA application increasing cucumber tolerance to alkaline stress.

2. Materials and methods

2.1. Plant materials and experimental design

The experiments were organized into two series. In the first series, we investigated the effects of exogenous SA with various concentrations on net photosynthetic rate (Pn) and biomass accumulation of cucumber seedlings under $NaHCO_3$ treatment; in the second series, we further characterized the alleviated alkaline stress effect of 75 μM SA, which showed the best effect on alkaline stress mitigation.

2.1.1. Selection of appropriate exogenous SA concentration on the alleviation of cucumber seedlings under alkaline stress

Cucumber (*Cucumis sativus* L. cv. Jinyan-4) seeds were pregerminated at 28 ℃ for 24 h after soaking with warm water for 3 h, then seeds were sowed into plug trays filled with vermiculite. When the cotyledons flattened, seedlings with consistent growth were selected and transplanted into hydroponic pots containing 5 L Hoagland nutrient solution, the formula was according to previous descriptions (Shi et al., 2007). With 5 seedlings per pot, renewed the solution every 2 days and aerated pumping over 30 min a day. $NaHCO_3$ treatments started after 8 days pre-culture, 2 days after SA spraying pretreatment. There are eight concentrations (0, 50, 75, 100, 125, 150, 175 and 200 μM) of exogenous SA was applied in this experiment, respectively. This experiment contains the control (with Hoagland nutrient solution and spraying 0 μM SA) and 8 treatments (added 30 mM $NaHCO_3$ on the basis of Hoagland nutrient solution and spraying exogenous SA with different concentrations from 50 to 200 μM as above mentioned). Spraying exogenous SA at 4 PM the 2 days before and 3 days after $NaHCO_3$ treatment, and drops uniformly attachment to the leaves as the standard of SA application. The seedlings were randomly allocated, 3 pots for each treatment and 5 plants for per pot. Physiological parameters were measurement after 8 days of $NaHCO_3$ treatments.

2.1.2. Experiment design for the effect of optimum salicylic acid concentration on cucumber tolerance to alkaline stress

Based on the above study, the optimum concentration of 75 μM SA was used to further investigate its effect on cucumber tolerance to alkaline stress. Plant materials preparation and precultivation was the same with 2.1.1. In this part, the experiment design consisted of 3 treatments: Control (with Hoagland nutrient solution, spraying 0 μM SA); Stress (30 μM $NaHCO_3$ was added to Hoagland nutrient solution, spraying 0 μM SA); Stress + SA (30 mM $NaHCO_3$ was added to Hoagland nutrient solution and spraying 75 μM exogenous SA). Spraying exogenous SA was operated at 4 PM of 2 days before $NaHCO_3$ treatment and the 3, 8, 13 days after $NaHCO_3$ treatment, drops uniformly attachment to the leaves was as the standard for SA application. The seedlings were randomly allocated with 10 pots each treatment and 5 plants per pot. Physiological parameters were measured in the 0, 3, 6, 9, 12, 15 days after $NaHCO_3$ treatment.

2.2. Determination of fresh weight

At the end of treatment, plants were cleaned by deionized water, then separated into shoots and roots and weighed, respectively. The shoots and roots were dried in an oven at 105 ℃ for 20 min and 75 ℃ for 72 h, then weighted.

2.3. Determination of net photosynthetic rate

The second fully expanded leaf was chosen for net photosynthetic rate (Pn) determination using photosynthesis equipment (*LI-6400, Lincoln, USA*) at a photon flux density of 800 $\mu M\ m^{-2}\ s^{-1}$ and circumambient CO_2 concentration of 340 μM.

2.4. Determination of photosynthetic pigments

Chlorophyll and carotenoid were extracted with 95% ethanol and measured the absorbance at 663.3, 646.8 and 470 nm by spectrophotometer for chlorophyll a, chlorophyll b and carotenoid, respectively (Arnon, 1949).

2.5. Determination of chlorophyll a fluorescence parameters

Chlorophyll fluorescence parameters *Fo, Fm, Fs, Fo' and Fm'* were determined by chlorophyll fluorometer (*Fluor*Cam7; Photon Systems Instruments; U.S.A.) equipped with fluorescence chambers on the second fully expanded leaf after 30 min dark treatment. The related indicators were measured and calculated according to the following formulas (Maxwell and Johnson, 2000):

The maximum PSII quantum yield : $F_v/F_m = (F_m\text{-}F_o)/F_m$.

The actual photochemical efficiency of PSII : $\Phi PSII = (Fm'\text{-}\ Fs)/Fm'$.

The photochemical efficiency of open PSII centers : $Fv'/Fm' = (Fm'\ \text{-}\ Fo')/Fm'$.

The photochemical quenching coefficient : $q_P = (Fm'\ \text{-}Fs)/(Fm'\ \text{-}\ Fo')$.

The non-photochemical quenching : $NPQ = Fm/Fm'\ \text{-}\ 1$.

The fraction of light absorbed in PSII antennae that was dissipated via