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Alternative oxidase pathway is involved in the exogenous SNP-elevated tolerance of *Medicago truncatula* to salt stress

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ABSTRACT

Exogenous application of sodium nitroprusside (SNP) would enhance the tolerance of plants to stress conditions. Some evidences suggested that nitric oxide (NO) could induce the expression of alternative oxidase (AOX). In this study, *Medicago truncatula* (Medicago) was chosen to study the role of AOX in the SNP-elevated resistance to salt stress. Our results showed that the expression of *AOX* genes (especially *AOX1* and *AOX2b1*) and cyanide-resistant respiration rate (V_{alt}) could be significantly induced by salt stress. Exogenous application of SNP could further enhance the expression of *AOX* genes and V_{alt}. Exogenous application of SNP could alleviate the oxidative damage and photosynthetic damage caused by salt stress. However, the stress resistance was significantly decreased in the plants which were pretreated with *n*-propyl gallate (*n*PG). More importantly, the damage in *n*PG-pretreated plants could not be alleviated by application of SNP. Further study showed that effects of *n*PG on the activities of antioxidant enzymes were minor. These results showed that AOX pathway played an important role in the SNP-elevated resistance of Medicago to salt stress. AOX could contribute to regulating the accumulation of reactive oxygen (ROS) and protect of photosystem, and we proposed that all these were depend on the ability of maintaining the homeostasis of redox state.

1. Introduction

Numerous studies suggest that nitric oxide (NO) participate in the process of plant growth and development, as well as the response to various stress conditions (Bellin et al., 2013; He et al., 2004; Siddiqui et al., 2011; Zhao et al., 2009). It has been demonstrated that NO could function as a signal molecule to activate the downstream components in NO-based signaling cascades leading to changes of gene expression (Grün et al., 2006; Lamattina et al., 2003). NO could also directly modify the target proteins by *S*-nitrosylation (Tada et al., 2008). However, the biological roles of NO in plants are far more than these, and the relatively limited knowledge of NO is mainly attributed to the lack of NO-related mutants. In view of this situation, NO donors are naturally chosen to study the diverse biological roles of NO in plants.

NO donors are compounds that could produce NO when applied to the biological systems, and they are able to either mimic an endogenous NO-related response or substitute for an endogenous NO deficiency (Floryszak-Wieczorek et al., 2006). To our knowledge, almost three different kinds of donors are used in the current studies, they are sodium nitroprusside (SNP), *S*-nitroso-Nacetylpenicillamine (SNAP) and nitrosoglutathione (GSNO), respectively (Arasimowicz-Jelonek et al., 2011). Among these donors, SNP is the most widely used one (Filippou et al., 2012; Floryszak-Wieczorek et al., 2006). There are many studies suggest that exogenous SNP could alleviate the damage caused by various abiotic stress. Several studies suggest that application of low concentration of SNP could alleviate salinity induced oxidative damage in roots of cucumber (Shi et al., 2007), seed of alfalfa (Wang et al., 2012), seedlings of cotton (Liu et al., 2014). Similarly, the studies carried out in different plants under various stresses, such as cadmium stress (Singh et al., 2008; Xu et al., 2010), herbicide stress (Qian et al., 2009), chilling stress (Yang et al., 2011) and aluminum stress (Aftab et al., 2012), also demonstrated that SNP could

Abbreviations: AOX, alternative oxidase; NO, nitric oxide; SNP, sodium nitroprusside; *n*PG, *n*-propyl gallate; Medicago, *Medicago truncatula*; qRT-PCR, quantitative real-time PCR; V_{alt}, cyanide-resistant respiration rate; V_{cyt}, cytochrome respiration rate; V_t, total respiration rate; ROS, reactive oxygen; RWC, relative water content; EL, electrolyte leakage; MDA, malonaldehyde.

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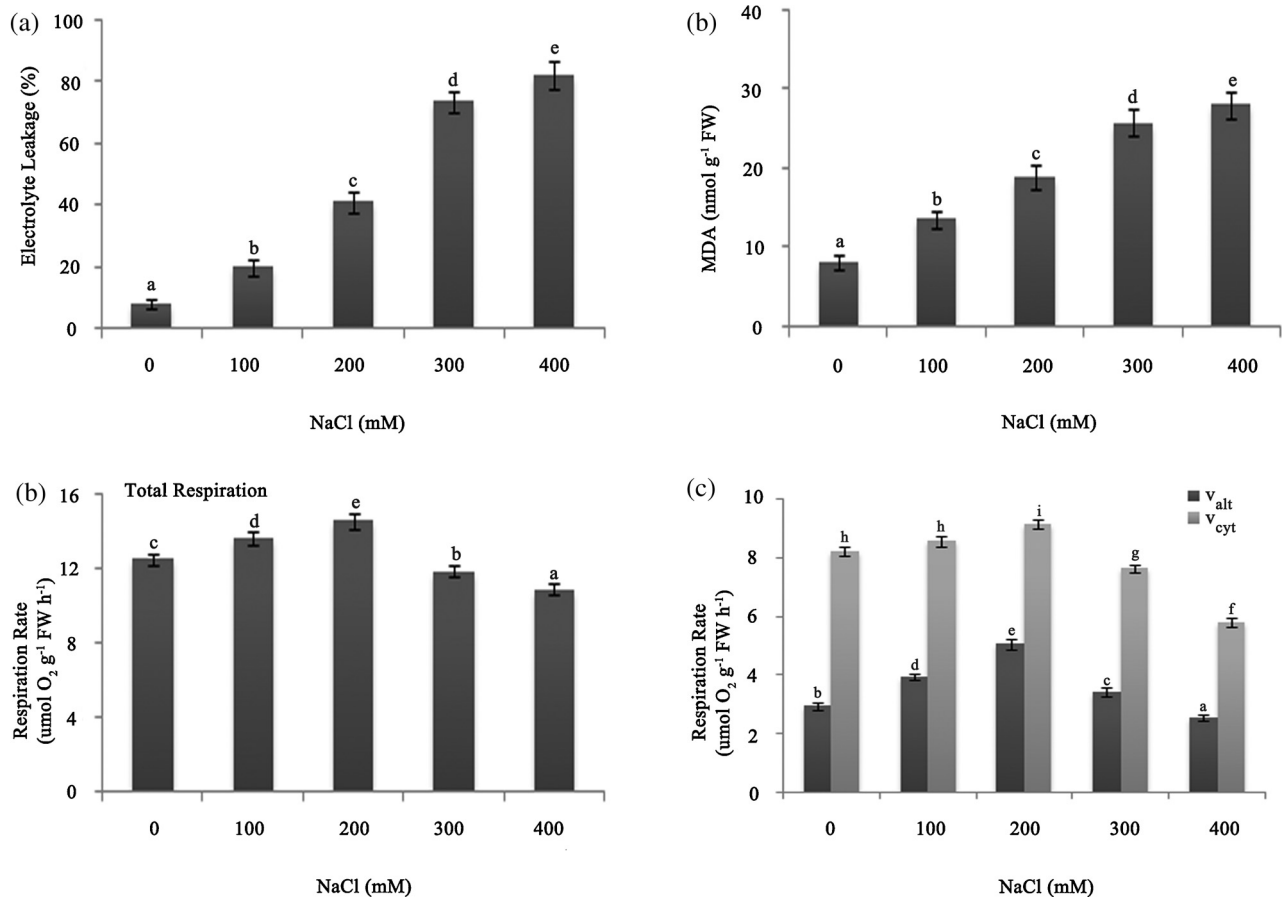

Fig. 1. Effect of salt stress on the oxidative damage and respiration rate in Medicago. Electrolyte leakage (EL) (a), malonaldehyde (MDA) content (b), total respiration rate (V_t) (c), Cyanide-resistant respiration rate (V_{alt}) and cytochrome respiration rate (V_{cyt}) (d) in the Medicago leaves were measured. All the leaf sample was collected after the plants were treated with NaCl for 72 h. Different letters indicate statistically significant differences between treatments ($P < 0.05$). Data are the mean values ± SD of three biological repeats. Error bars show standard deviations ($n = 3$).

significant enhance the activity of an enzymes to response to correspond stress condition. Recent studies showed that exogenous application of SNP could affect the content of polyamine and proline in plants under normal growth conditions (Filippou et al., 2013) and stress conditions (Fan et al., 2012, 2013; Farooq et al., 2009). Furthermore, SNP could increase chlorophyll content and maintain the stability of photosynthetic complexes in thylakoid membranes, which was beneficial to plants, especially under stresses conditions (Chen et al., 2013, 2014; Gong et al., 2014; Liu and Guo, 2013; Procházková et al., 2013).

In higher plants, there is a unique component in the mitochondrial electron transport chain, alternative oxidase (AOX), which could catalyze the alternative respiratory pathway. The difference between cytochrome pathway and alternative pathway is that the latter could bypass two of the three sites of energy conservation to produce heat instead of ATP (Finnegan et al., 2004). Although the alternative respiration is low in unstressed plants, it could be enhanced after exposure to pathogens, low temperature, drought, and other stresses (Dahal et al., 2014; Király et al., 2008; Watanabe et al., 2008). A large number of evidences have suggested that the enhanced alternative pathway could improve the stress tolerance through maintaining the redox homeostasis in the plants (Umbach et al., 2005; Vanlerberghe, 2013). Several recent researches indicated that the alternative pathway could be induced by the elevated NO and possess the plants a higher stress tolerance. Exogenous application of NO donors could enhance the transcription of *AOX1a* gene and the activity of AOX in Arabidopsis suspension cells (Huang et al., 2002). Infection of plants with virus could induce NO, which could act as an upstream signal to activate the AOX pathway to respond to the invasion of virus (Fu et al., 2010; Jian et al., 2015). However, there is still lacking related evidences to verify whether the AOX pathway is involved in the resistance to abiotic stress mediated by NO.

Medicago truncatula (Medicago) is a model plant to study the physiological characters of Legumes, and there are some differences between medicago and other plants. To our knowledge, there are four members (*AOX1*, *AOX2a*, *AOX2b1* and *AOX2b2* genes) in AOX family which have been identified in Medicago (Cavalcanti et al., 2013; Mhadhbi et al., 2012), and there are very few studies to analyze the role of AOX in Medicago. In view of the strong stress resistance, Medicago was chosen to study the role of AOX in the SNP-elevated tolerance to salt stress in the present study. Our results demonstrated that the AOX pathway played an important role in the SNP-elevated stress resistance. SNP could significantly alleviate the oxidative damage in Medicago, but not in the Medicago pretreated with *n*-propyl gallate (*n*PG). Furthermore, the possible mechanism involved in this process was also investigated.

2. Materials and methods

2.1. Plant materials, growth conditions and chemical treatments

The Medicago ecotype R108 was used in the present study, all the seeds were kindly provided by Dr. Jiangqi Wen (The Samuel Roberts Noble Foundation, USA). Seeds of R108 were dipped in sulfuric acid for 5 min to degrade the seed coat, and rinsed thoroughly