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Exogenous Nitric Oxide Enhances Root Activity, Decreases H_2O_2 Accumulation by Increasing Activities of CAT and GR in Root of Potato cv. Desiree under Water-stress

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Abstract: We studied water content, H_2O_2 content, antioxidant enzymes activities of different organs and root activity in potato cv. Desiree pretreated with NO under water-stress. It was found that the bound water content in root and root activity was significantly increased by treating NO before water-stress. Pretreatment with NO decreased the H_2O_2 content and raised activities of CAT and GR significantly in roots under water-stress. The results suggested that NO might enhance the resistance to water-stress in potato.

Keywords: Antioxidant enzymes, H_2O_2 content, NO, potato, water-stress

INTRODUCTION

Potato is the third most important food crop in the world after rice and wheat in terms of human consumption (http://cipotato.org/potato). China, the world's biggest consumer of potatoes, expects that fully 50% of the increased food production it will need to meet demand in the next 20 years will come from potatoes (http://cipotato.org/potato). Drought is a major threat to productivity and food security in many regions in China. Minimizing the adverse influence of drought is very important to potato yield and quality.

Nitric Oxide (NO) is proposed to be one of the important second messengers in plant cells (Beligni *et al*., 2002) and increasing evident showed that it exerts a protective effect in response to drought stress (Garcia-Mata and Lamattina, 2002; Lei *et al*., 2007; Zhao *et al*., 2008; Nasibi and Kalantari, 2009). Externally applied NO donors (Sodium Nitroprusside (SNP)) enhance plant tolerance to specific stresses (Delledonne *et al*., 1998; Garcia-Mata and Lamattina, 2002; Uchida *et al*., 2002; Zhao *et al*., 2007).

Plants under water-stress may be exposed to oxidative stress through the production of Reactive Oxygen Species (ROS) (Iturbe-Ormaetxe *et al*., 1998; Munné-Bosch and Peñuelas, 2003). Among different ROS, only hydrogen peroxide (H_2O_2) can cross plant membranes and it has been increasingly proved to be one of the most important signals in plant cell signaling, especially in elicitor-induced defenses responses (Vanderauwera *et al*., 2005; Gadjev *et al*., 2006). Several studies have shown that the protective effect of NO against a biotic stresses is closely related to the NO-mediated reduction of ROS in plants (Kopyra and Gŵozdz, 2003; Zhang *et al*., 2003; Hsu and Kao, 2004). It was found that NO appeared to serve as an antioxidant agent able to scavenge H_2O_2 to protect plant cells from oxidative damage (Laxalt *et al*., 1997; Sang *et al*., 2008). ROS-scavenging enzymes include Superoxide Dismutase (SOD), Peroxidase (POD), Ascorbate Peroxidase (APX), Glutathione Reductase (GR) and Catalase (CAT) (Mittler *et al*., 2004). The emerging main challenges are to obtain an understanding of how these processes are coordinated within the plant.

In our previous study we found exogenous nitric oxide can inhibit the water loss in leaves in potato cv. Desiree (He *et al*., 2011). The objective of present study was to investigate the influence of exogenous NO on oxidative damage and antioxidant system in potato seedlings under water-stress.

MATERIALS AND METHODS

Plant material and culture conditions: Uniform sprouts with 4-5 cm length were cut from sprouted seed potatoes of Desiree in dark and sown in trays with vermiculite to produce roots and leaves in growth chambers under 16 h light/8 h dark cycle with consistent temperature of 22°C. When the plant produced 4-5 leaves, uniform plants were picked up from trays, washed with top water and used in experiment. All of the experiments were conducted in 2011.

Plant roots were initially immersed in 0 and 0.01 mM SNP solutions for 2 h, respectively. And then the roots being washed 5 times with tap water were immersed in 20% PEG-6000 solution at 25°C in light. After 4 h of treatment the plants were divided into root, stem and leave and used for further analysis. Sodium Nitroprusside (SNP) is NO donor.

The experiment was based on a randomized complete block design with three replications and eight plants were used in each replicate.

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Water content analysis: The water content and bound water content of root, stem and leave were tested and calculated according to Zhang (1980).

Root activity analysis: Root activity was tested hourly after water-stress onset according to Zhou and Li (2005).

H_2O_2 content: H_2O_2 content of root, stem and leaf were measured after water-stress according to Lin *et al*. (1988).

Extraction and assay of enzymes: After treatment with water-stress different parts of the plant were homogenized with 0.1 M sodium phosphate buffer (pH 6.8) in a chilled pestle and mortar. The homogenate was centrifuged at 12000 g for 20 min and the resulting supernatant was used for determination of enzymes activity. The whole extraction procedure was carried out at 4°C. APX was determined according to Nakano and Asada (1981). POD was determined following Kuroda *et al*. (1990). The assays of CAT, SOD and GR were conducted according to Aebi (1983), Beyer and Fridovich (1987) and Rao *et al*. (1996), respectively.

Protein contents were determined according to the Bradford (1976) method using Bovine Serum Albumin (BSA) as standard.

Statistical analysis: All data presented are the mean values. Means were compared by one-way analysis of variance and Duncan's multiple range tests at 5% level of significance.

RESULTS

NO increases bound water content in root under water-stress: When plant root pretreated with NO the water content lowered in root and rose in leave, but no obvious difference was observed under water-stress (Fig. 1). By analyzing component of the water content, we found the bound water content significantly increased in root pretreated with NO (Fig. 2). High level of bound water content meant the resistance to water stress was enhanced (Xiao *et al*., 2005). Consequently, NO might increase the resistance to water stress by increasing bound water content in root.

NO enhances root activity under water-stress: The root activity was recorded with time after water-stress onset. Compared with no pretreatment root pretreated with NO had high activity and the root activity in the first 2 h was significantly higher than that without pretreatment under water-stress (Fig. 3). There was line-specific increase in the root activity without pretreatment of NO in first 3 h and then kept stable under water-stress. However, root activity was very different when it pretreated with NO (Fig. 3). It

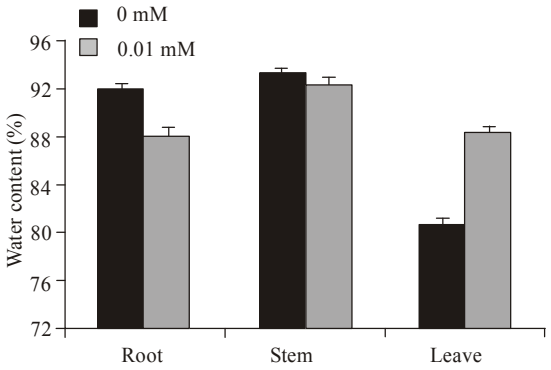

Fig. 1: Water content in different organs of potato pretreated with NO under water-stress

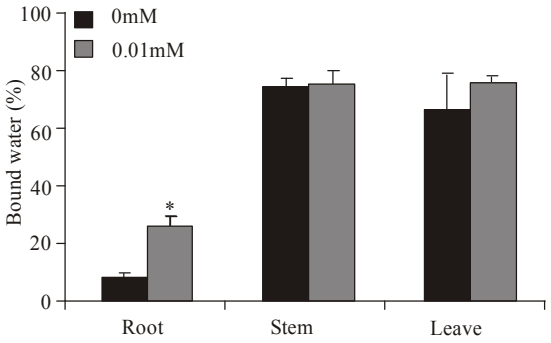

Fig. 2: Bound water content in different organs of potato pretreated with NO under water-stress
*: Significant difference ($p \leq 0.05$) between treatment and untreatment (Fisher's protected LSD test)

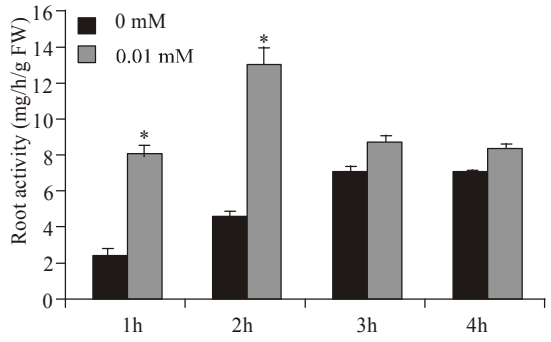

Fig. 3: NO affects on root activity of potato under water-stress
*: Significant difference ($p \leq 0.05$) between treatment and untreatment (Fisher's protected LSD test)

increased at 1 h after water-stress onset and reached the highest at 2 h and then declined at 3 h following keeping stability. The result suggested that NO had the effect on improving root activity.

NO decreases H_2O_2 content in root under water-stress: The highest H_2O_2 content was in leave, following by stem and root under water-stress without NO treatment (Fig. 4). Application NO before water-

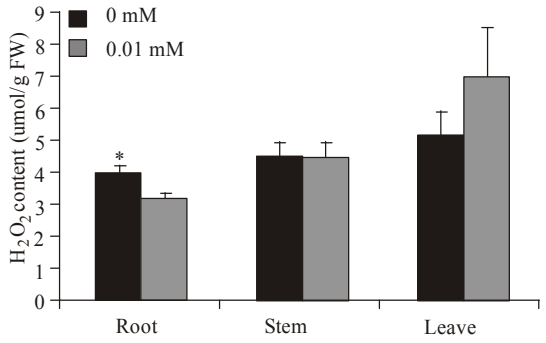

Fig. 4: NO affects on H_2O_2 content of potato under water-stress
*: Significant difference ($p \leq 0.05$) between treatment and untreatment (Fisher's protected LSD test)

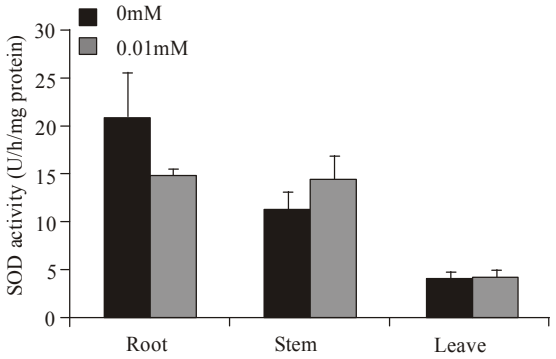

Fig. 5: NO on SOD activity in potato under water-stress

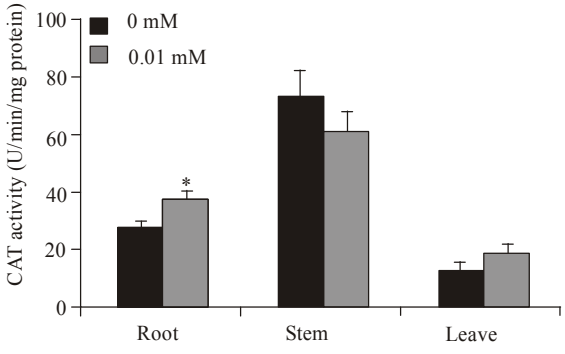

Fig. 6: NO on CAT activity in potato under water-stress
*: Significant difference ($p \leq 0.05$) between treatment and untreatment (Fisher's protected LSD test)

stress had the same situation as that without application that H_2O_2 content was higher in leave than in stem and root. However, pretreatment significantly lowered the level of H_2O_2 content in root and no obvious difference was in stem and leave. The data showed that NO might not promote the H_2O_2 transfer from root to other part of the plant, but might scavenge H_2O_2 in root by inducing scavenging system.

Relation between NO and antioxidant enzyme activities under water-stress: The generation of ROS

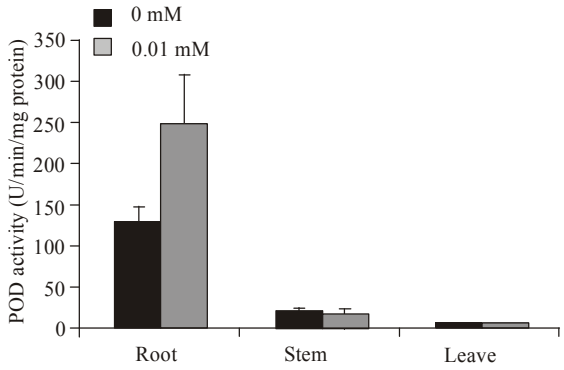

Fig. 7: NO on POD activity in potato under water-stress

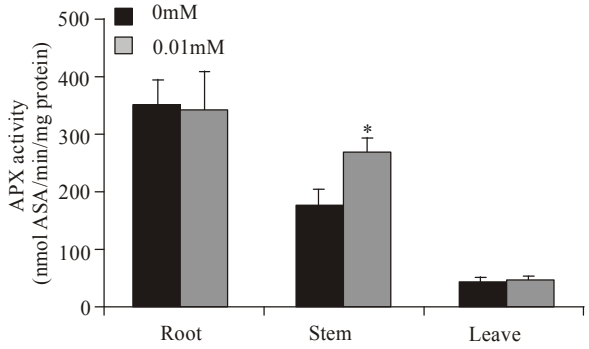

Fig. 8: NO on APX activity in potato under water-stress
*: Significant difference ($p \leq 0.05$) between treatment and untreatment (Fisher's protected LSD test)

is limited or scavenged by a series of antioxidant enzymes. SOD is an H_2O_2-producer enzyme. In our study the highest level of SOD was in root and then in stem and leave whether with or without NO pretreatment under water-stress (Fig. 5). Meanwhile no significant difference was observed.

CAT is the predominant enzyme controlling H_2O_2 level. POD scavenged H_2O_2 produced by SOD. APX and GR, the enzymes of the ascorbate-glutathione cycle scavenge H_2O_2 and convert it to H_2O and O_2. The highest level of CAT in plant organs existed in stem, following by in root and leave (Fig. 6). NO strengthened CAT activity in root and leave, especially in root, which was significant at 0.05 levels. POD exhibited the highest activity in root and then in stem and leave (Fig. 7). Although NO enhanced the POD activity no obvious difference was observed between NO and no-NO. The activity of APX in plant organs was completely different with GR. In terms of APX the highest was in root and then in stem and leave whether with or without NO (Fig. 8). Except in stem no obvious difference was observed in root and leave with NO pretreatment. NO improved the activity of GR in root and stem, but decreased it in leave (Fig. 9). Meanwhile the increasing activity of GR showed statistically significant in root. According to H_2O_2 content in root

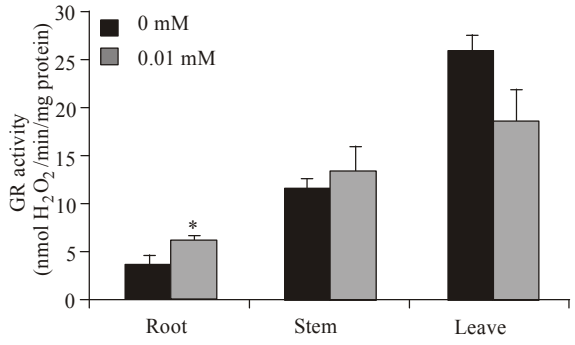

Fig. 9: NO on GR activity in potato under water-stress
*: Significant difference ($p \leq 0.05$) between treatment and untreatment (Fisher's protected LSD test)

we proposed that the antioxidant enzymes, CAT and GR would play an important role on scavenging H_2O_2 in potato under water-stress.

DISCUSSION AND CONCLUSION

Nitric oxide acts as a signaling molecule with different kinds of physiological functions. Application of exogenous NO can mediate various physiological processes to abiotic stresses. NO often functions together with ROS in various ways and plays an important role in environmental stresses (Delledonne *et al*., 2001). Studies have shown that the protective effect of NO against abiotic stresses is closely related to the NO-mediated reduction of ROS in plants (Kopyra and Gŵozdz, 2003; Zhang *et al*., 2003; Hsu and Kao, 2004; Sang *et al*., 2008). It is known that H_2O_2 accumulates under water-stress (Dat *et al*., 1998; Jiang and Zhang, 2002). Application of exogenous NO before water-stress significantly improved bound water content in root and root activity. Meanwhile the H_2O_2 content in root was obviously lowered.

NO appeared to serve as an anti-oxidant agent able to scavenge H_2O_2 to protect plant cells from oxidative damage (Laxalt *et al*., 1997). As we know CAT, POD, APX and GR are major anti-oxidant enzymes in plant cells and have multiple molecular forms of is enzymes and are located in different cellular compartments (Mittler *et al*., 2004). So the next we wanted to know was the relation among NO, H_2O_2 and the antioxidant enzymes.

There was no obvious influence on SOD and POD activity in root when exogenous NO was applied before water-stress. CAT is the predominant enzyme controlling H_2O_2 level and CAT1 expression was induced by applied H_2O_2 in *Arabidopsis* (Xing *et al*., 2007). APX and CAT were also found to be induced by NO in maize leaves (Zhang *et al*., 2007). Application NO on root before water-stress significantly increased CAT activity in root. Although APX and GR are enzymes of the ascorbate-glutathione cycle scavenge H_2O_2, the activity level in different organs in potato was completely different. In terms of APX the highest was in root and then in stem and root. GR was completely opposite. Meanwhile they reacted differently to NO pretreatment under water-stress. Exogenous NO had little effect on APX but obviously increased GR in root under water-stress.

Based on the root physiological reaction we deduced that application of exogenous NO before water-stress could decrease H_2O_2 content by inducing activity of CAT and GR in root. Consequently root activity was enhanced. In all resistance of root to water-stress was enhanced by exogenous NO in root of potato.

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