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Effects of nitric oxide on some physiological characteristics of maize seedlings under waterlogging

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Nitric oxide (NO), as a new signal molecular, is now proved to be involved in plant development and resistances to many biotic and abiotic stresses. In this study, an experiment was conducted to assess the effects of sodium nitroprusside (SNP), a NO donor, on both morphological and physiological characteristics of maize under waterlogging stress. Results showed that SNP could increase height, dry weight and activities of catalase, peroxidase and superoxide dismutase, and decrease MDA content and ion leakage ratio under waterlogging stress. This finding shed light on the fact that NO can improve the resistance to waterlogging. To our knowledge, it is the first report of NO function in waterlogging.

Key words: Nitric oxide, maize, waterlogging, antioxidant enzyme.

INTRODUCTION

In animals, nitric oxide is considered as a diffusible multifunctional secondary messenger playing pivotal roles in synaptic transmission, vasodilatation, erection, egg fertilization, defense against pathogenic microorganisms, and apoptosis (Schmidt and Walter, 1994; Anbar, 1995). Recently, increasing evidence showed that NO also plays important roles in diverse physiological processes in plants. NO can mediate stomatal movement (Bright et al., 2006; Garcia-Mata and Lamattina, 2007), modulate the expression of cell cycle genes (Correa-Aragunde et al., 2006), stimulate seed germination (Llibourel et al., 2006), be involved in the recognition of pollen and stigma during pollination (Hiscock et al., 2007), suppress floral transitions (He et al., 2004) and regulate plant maturation and senescence (Mishina et al., 2007).

Besides plant developmental processes, NO can also be involved in the regulation of plant defense responses to biotic and abiotic stresses. Not only biotic and abiotic stresses can promote or suppress NO production, but also applications of NO donors (sodium nitroprusside (SNP)) enhance plant tolerance to specific stresses (Delledonne et al., 1998; Zhao et al., 2007). For example, NO is associated with induced plant tolerance to salinity (Molassiotis et al., 2010); promotes the germination of wheat seeds under osmotic stress by improving antioxidant capacity (Zhang et al., 2005); decreases heavy metal induced oxidative damage in many plants (Yu et al., 2005; Singh et al., 2008; Rodríguez-Serrano et al., 2009), moreover, it has been proved that NO can ameliorate UV-induced damage by lowering H2O2 content and ion leakage and enhancing the activities of scavenging enzymes (Shi et al., 2005).

Waterlogging is an environmental stress factor for plants in wetland areas. It is thought that waterlogging limits gas diffusion, and thus reduce O2 concentration and redox potential in soil, which further leads to some physical and chemical changes (Ruiz-Sánchez et al., 1996). As a result, the accumulation of acetaldehyde and other compounds derived from anaerobic metabolism occurs (Blokchina et al., 2003). However, compared to other abiotic stresses (such as salt, drought etc.), the information of waterlogging is relative less because of
the considerable diversity and complexity in waterlogging-prone environments. NO is now regarded as a new signal molecular, but no reports are found about the response of exogenous applications of NO on plants under waterlogging stress. Our objective in this study is to analyze whether and how exogenous NO is involved in waterlogging tolerance.

MATERIALS AND METHODS

Seeds of maize (Zea mays L.) Zhongdan 5384 were surface sterilized for 5 min in 1% (w/v) sodium hypochlorite, and washed in distilled water. The sterilized seeds were soaked in distilled water for 12 h. Seeds fully imbibed were germinated at 28°C in the dark. The germinating seeds were then sown on two layers of hydrophilic gauze in trays and covered with two layers of wet hydrophilic gauze. When the primary roots were about 0.5 cm, the seedlings were then transferred to a chamber with 70% relative air humidity, 30/28°C day/night temperature, a day/night cycle of 16/8 h and a 300 µmol m⁻² s⁻¹ photon flux density. The seedlings were watered with Hoagland nutrition solution. When the ligules of the third leaf were visible, waterlogging stress treatments were performed. For the control (CK), plants grew under the above condition unchanged. Plants treated with different concentrations of SNP result in different effects, 50 and 500 µmol L⁻¹ SNP can significantly alleviate waterlogging stress, while 500 µmol L⁻¹ SNP significantly alleviated the decrease of chlorophyll content than S0 plants. This showed that SNP can keep to a relative higher level. This can explain why the heights and dry weights of these plants are higher than that of S0 plants under waterlogging treatment. Plants treated with 50 and 500 µmol L⁻¹ SNP significantly alleviated the decrease of both height and dry weight of maize seedlings under waterlogging stress (Table 1). Compared to CK plants, a marked decrease in chlorophyll content was also observed in S0 plants. In contrast, chlorophyll content in leaves treated with 50 and 500 µmol L⁻¹ SNP can keep to a relative higher level. This can explain why the heights and dry weights of these plants are higher than that of S0 plants under waterlogging treatment. Plants treated with 2000 µmol L⁻¹ SNP grew more poor and kept a lower chlorophyll content than S0 plants. This showed that treatments with different concentrations of SNP result in different effects, 50 and 500 µmol L⁻¹ SNP treatments could significantly alleviate waterlogging stress, while 2000 µmol L⁻¹ SNP treatment could aggravate the stress injury.

Statistical analysis

For all the measurements, three replicates per treatment were used. Statistical differences were determined using Student’s two-tailed t test.

RESULTS AND DISCUSSION

Effects of NO on maize seedlings growth under waterlogging

Morphological characteristics are more valuable to evaluate the plant resistance. Waterlogging decreased both height and dry weight of maize seedlings. After 7 days of waterlogging treatment, the growths of all the maize seedlings under stress conditions were inhibited. Compared to the S0 plants, plants treated with 50 and 500 µmol L⁻¹ SNP significantly alleviated the decrease of both height and dry weight of maize seedlings under waterlogging stress (Table 1). Compared to CK plants, a marked decrease in chlorophyll content was also observed in S0 plants. In contrast, chlorophyll content in leaves treated with 50 and 500 µmol L⁻¹ SNP can keep to a relative higher level. This can explain why the heights and dry weights of these plants are higher than that of S0 plants under waterlogging treatment. Plants treated with 2000 µmol L⁻¹ SNP grew more poor and kept a lower chlorophyll content than S0 plants. This showed that treatments with different concentrations of SNP result in different effects, 50 and 500 µmol L⁻¹ SNP treatments could significantly alleviate waterlogging stress, while 2000 µmol L⁻¹ SNP treatment could aggravate the stress injury.

### Table 1. Effects of NO on maize seedlings growth under waterlogging. Height and dry weight were measured a 7 days after treatments, total chlorophyll contents were measured at 1, 3, 5, 7 days, respectively, after treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Height (cm)</th>
<th>Dry weight (g)</th>
<th>Total chlorophyll content (mg g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>3 days</td>
<td>5 days</td>
</tr>
<tr>
<td>CK</td>
<td>25.2ᵃ</td>
<td>0.1512ᵃ</td>
<td>9.6ᵇ</td>
</tr>
<tr>
<td>S0</td>
<td>19.6ᶜ</td>
<td>0.1159ᶜ</td>
<td>8.4ᵇ</td>
</tr>
<tr>
<td>S50</td>
<td>23.1ᵇ</td>
<td>0.1367ᵇ</td>
<td>8.1ᵇ</td>
</tr>
<tr>
<td>S500</td>
<td>21.9ᵇ</td>
<td>0.1370ᵇ</td>
<td>9.3ᵇ</td>
</tr>
<tr>
<td>S2000</td>
<td>19.1ᶜ</td>
<td>0.1160ᶜ</td>
<td>9.7ᵇ</td>
</tr>
</tbody>
</table>

Data represent the means ±SE of three experimental replicates; values with different letters in the same row are significantly different (P < 0.05).
Effects of NO on antioxidant activities in maize seedling leaves under waterlogging

Studies have shown that waterlogging could induce the production of active oxygen such as \( \text{O}_2^* \) and \( \text{H}_2\text{O}_2 \) which can cause oxidative stress (Yiu et al., 2009). Some antioxidants can inhibit or delay the oxidation stress by scavenging free radicals. For example, SOD is a key enzyme to alleviate the oxidative damage by removing \( \text{O}_2^* \), POD and CAT can further remove \( \text{H}_2\text{O}_2 \) (Sairam et al., 2005). In this study, SOD, POD and CAT activities were measured under water stress. Compared to CK plants, activities of SOD kept decreasing in S0 plants during the stress treatment period. While activities of SOD in S50 and S500 plants increased at the beginning and kept steady to a higher level, and at last decreased. Activities of SOD in S2000 plants were higher than that in S0 plants, but lower than that in S50 and S500 plants at the most of stages under study. Similarly, activities of CAT in S0 plants decreased throughout the period of stress treatment. Treatment with 50 and 500 µmol L\(^{-1}\) SNP could keep the enzyme activities to higher levels. Treatments with 2000 µmol L\(^{-1}\) SNP had less effect than that of 50 and 500 µmol L\(^{-1}\) SNP. On the contrary, compared to CK plants, activities of POD increased in all water stress treated plants including SNP treaded and non-SNP treated plants. Compared to S0 plants, activities of POD in SNP treated plants increased more higher, especially in S500 plants. This result shows that treatment with 50 and 500 µmol L\(^{-1}\) SNP treatments could keep activities of antioxidant enzymes to higher levels under waterlogging thus reduce the oxidative damages in a certain degree (Table 2).

Effects of NO on membrane stability in maize seedling under waterlogging

MDA is the product of biomembrane lipid peroxidation, its content in plants reflects the degree of membrane injury. Ion leakage ratio also reflects the degree of plant cell membrane injury under stress treatment (Pérez-Tornero et al., 2009). As shown in Table 3, MDA content in CK plants kept stable during the measurement stages. MDA content in all the plants subjected to waterlogging stress kept increasing during all the stages under study, while the MDA content followed the order: S0 plants > S2000 plants > S 50 plants > S 500 plants. There was a statistically significant difference between S50 or S500 plants and S0 plants. Compared to CK plants, ion leakage ratio in both SNP treated plants and non-SNP treated plants under waterlogging stress treatment increased rapidly to higher levels. There was no obvious difference between SNP treated plants and non-SNP treated plants at the early stress treatment stage, but after 5 days of treatment, there was a statistically significant difference between SNP treated plants and non-SNP treated plants. The ion leakage ratio in SNP treated plants was significantly lower than that of non-SNP plants. This result indicates that SNP can reduce

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**Table 2.** Effects of NO on activities of antioxidant enzymes in maize seedlings under waterlogging. Activities of antioxidant enzymes were measured at 1, 3, 5, 7 days, respectively, after treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SOD activity (U mg(^{-1}) protein)</th>
<th>POD activity (U mg(^{-1}) protein)</th>
<th>CAT activity (U mg(^{-1}) protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>3 days</td>
<td>5 days</td>
</tr>
<tr>
<td>CK</td>
<td>6.55( ^a )</td>
<td>6.62( ^a )</td>
<td>6.81( ^a )</td>
</tr>
<tr>
<td>S0</td>
<td>8.03( ^b )</td>
<td>5.92( ^b )</td>
<td>5.87( ^b )</td>
</tr>
<tr>
<td>S50</td>
<td>8.94( ^b )</td>
<td>6.82( ^a )</td>
<td>6.88( ^a )</td>
</tr>
<tr>
<td>S500</td>
<td>11.14( ^c )</td>
<td>6.62( ^a )</td>
<td>7.15( ^a )</td>
</tr>
<tr>
<td>S2000</td>
<td>7.41( ^d )</td>
<td>6.58( ^a )</td>
<td>6.13( ^b )</td>
</tr>
</tbody>
</table>

Data represent the means ±SE of three experimental replicates; values with different letters in the same row are significantly different (P < 0.05).

**Table 3.** Effects of NO on MDA content and ion leakage ratio in maize seedlings under waterlogging. MDA content and ion leakage ratio were measured at 1, 3, 5, 7 days, respectively, after treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDA content (n mol g(^{-1}) FW)</th>
<th>Relative electric conductivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>3 days</td>
</tr>
<tr>
<td>CK</td>
<td>25.0( ^c )</td>
<td>26.2( ^c )</td>
</tr>
<tr>
<td>S0</td>
<td>29.8( ^c )</td>
<td>37.4( ^c )</td>
</tr>
<tr>
<td>S50</td>
<td>24.8( ^c )</td>
<td>31.6( ^c )</td>
</tr>
<tr>
<td>S500</td>
<td>24.6( ^c )</td>
<td>31.3( ^c )</td>
</tr>
<tr>
<td>S2000</td>
<td>28.1( ^c )</td>
<td>36.6( ^c )</td>
</tr>
</tbody>
</table>

Data represent the means ±SE of three experimental replicates; values with different letters in the same row are significantly different (P < 0.05).
the injury to cell membrane and maintain the stability of cell membrane under stress. Also the effect of 50 and 500 μmol L\(^{-1}\) SNP treatments was better than that of 2000 μmol L\(^{-1}\) SNP treatment.

Conclusions

Growth inhibition and oxidative stress are common responses to environmental stress and also the most important indices of plant tolerance. SOD, POD and CAT are redox antioxidants involved in cell defense against oxidative stress (Passardi et al., 2005). In this study, the enzymes activities in SNP-treated plants, especially in S500 plants kept to higher levels than that in S0 plants, while the MDA content and ion leakage ratio, the important indices of oxidative stress, in S500 plants were lower than that in S0 plants. The increased protective enzyme activities are in response to the decreased MDA content and ion leakage ration. This shows that exogenous NO might reduce the oxidative stress by increasing protective enzyme activities, and then enhancing the tolerance under waterlogging. On the other hand, total chlorophyll content in S500 plants were higher than that in S0 plants, this could explain why the growth inhibition in S0 plants was more serious than that in S500 plants. In conclusion, our findings shows that application of exogenous NO can mitigate oxidative damage, improve activities of antioxidant enzymes, increase chlorophyll content under waterlogging treatment, and thus enhance the tolerance to waterlogging.

ACKNOWLEDGEMENTS

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