Influence of Calcium on Antioxidant System and Nitrogen Metabolism of Muskmelon Seedlings under Nutrient Solution Hypoxia

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Abstract

Two varieties of muskmelon were cultivated in hydroponics culture using the following [Ca\(^{2+}\)] in nutrient solution under hypoxia with 4mmol·L\(^{-1}\) Ca\(^{2+}\) (hypoxia), hypoxia with 10mmol·L\(^{-1}\) Ca\(^{2+}\) (hypoxia+Ca\(^{2+}\)), hypoxia with 0mmol·L\(^{-1}\) Ca\(^{2+}\) (hypoxia-Ca\(^{2+}\)), and normal dissolved oxygen with 4mmol·L\(^{-1}\) Ca\(^{2+}\) was the control. Leaf samples were collected at 0, 2, 4, 6 and 8d from all treatments for fresh weight, reactive oxygen species (ROS) and nitrogen metabolism analyses. The results showed that compared to control treatment, the fresh weights of both varieties were decreased and activities of antioxidant enzymes, productive rate of ROS, contents of MDA and NH\(_4^+\)-N, NO\(_3^--\)N were increased under hypoxia. Calcium application treatment alleviated the harmful effects of hypoxia to seedlings, as manifested by the higher levels of fresh weights, activities of antioxidant enzyme, contents of NH\(_4^+\)-N, NO\(_3^--\)N under hypoxia, while the ROS productive rate and MDA content were lower. The calcium-deficient treatment aggravated the harmful effects of hypoxia, which were reversed by calcium application. The different levels of response by the two varieties show differences in tolerance to hypoxia.

INTRODUCTION

Hypoxia in the root-zone is thought to be a major determinant in the adverse effects of nutrient solution culture (Gao et al., 2004b). Oxygen deficiency might first be sensed by the mitochondrial electron transport chain, caused by NADP(H) oxidative blockage and the reduced adenylate energy charge of the cell (Subbaiah and Sachs, 2003). As a second messenger, [Ca\(^{2+}\)] in cytoplasm rapidly increases under external stimuli, which is involved in perception and regulation of response of plants to environmental stress (Pandey et al., 2000). There is ample evidence for the role of Ca\(^{2+}\) in plant growth and development, maintenance and modulation of various cell functions under environmental stress, such as cold (Gao et al., 2004a), drought (Sučoň and Šmata, 2003), anoxia (Chen et al., 2002). Subbaiah et al (2003) demonstrated that Ca\(^{2+}\) is a transducer of low O\(_2\) signal, but a detailed mechanism of events and the relationship that exists between exogenous application Ca\(^{2+}\) and plant growth, reactive oxygen species (ROS) and nitrogen metabolism under hypoxia are not clear. In the present study, muskmelon seedlings were used to examine the effects of exogenous application Ca\(^{2+}\) and exclusion Ca\(^{2+}\) in nutrient solution under hypoxia on growth, ROS and nitrogen metabolism and to elucidate the relationship between Ca\(^{2+}\) enhancement of hypoxia tolerance and metabolism of ROS and nitrogen metabolism.

MATERIALS AND METHODS

Two varieties of muskmelon (Cucumis melo L. var. raticulatus Naud) ‘Xiyu No.1’ and ‘Dongfangxingguang’ were cultivated in autumn in 2003. At the 3 true-leaf stage, seedlings were removed from the nutrient cubes, the roots were rinsed with distilled water, and uniform seedlings were divided into four groups then transferred into Hoagland nutrient solution (pH 6.3±0.1, EC 2.0–2.2) in plastic containers (65L). After 2d culture, the first set of seedlings was transferred into normal Hoagland nutrient solution (4 mmol L\(^{-1}\) Ca\(^{2+}\)) and exposed to normal dissolved oxygen (DO) by using vigorous aeration (30min/h)
to keep DO at 8±0.2mg L⁻¹ (Control). The second set of seedlings were cultured in normal Hoagland nutrient solution containing 4mmol L⁻¹ Ca²⁺ but exposed to reduced levels of dissolved oxygen (hypoxia). The O₂ was balanced with N₂ by using a DO analyzer (Quantum-25, Quantum Analytical Instruments Inc, American) to keep DO at 2±0.2mg L⁻¹. The third and fourth groups of seedlings were cultured in DO 2±0.2mg L⁻¹ with exogenous application of Ca²⁺ 10mmol L⁻¹Ca²⁺ (Hypoxia+Ca²⁺) and without Ca²⁺ (Hypoxia-Ca²⁺) in nutrient solution respectively.

Superoxide dismutase (SOD) activity was determined by assaying its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). One unit of SOD activity was defined as the amount of the enzyme to bring about 50% inhibition of the photochemical reduction of NBT (Giannopolitis and Ries 1977). Peroxidase enzyme (POD) activity was measured by measuring the increase in absorbance at 470nm as Guaiacol was oxidized according to the method of Kwak (1996). Catalase (CAT) activity was determined according to the method of Dhindsa (1981) by measuring the decrease in absorbance at 240nm for 1min following the decomposition of H₂O₂. One unit of CAT activity was defined as decrease of 0.1A value for required enzyme quantity.

Hydrogen peroxide (H₂O₂) content determination was performed by monitoring the increase in absorbance at 550nm according to the method of Matsumura (2002). Superoxide radical ion (O₂⁻) productive rate was determined according to the method of Wang (1990) and sodium nitrite was used as standard. Malondialdehyde (MDA) content was determined by reaction of trichloroacetic acid (TCA) and thiobarbituric acid (TBA) according to the method of Dhindsa (1981).

Nitrate-nitrogen (NO₃⁻-N) and ammonium-nitrogen (NH₄⁺-N) content was determined according to the method of Gao (2004a), potassium nitrate and leucine was used as the standards respectively.

Statistical analysis of the data was performed by Excel statistical software. Duncan Multiple Range Test (SAS) was used for mean separation.

RESULTS

Effect of Ca²⁺ on Fresh Weight of Muskmelon Seedlings under Hypoxia

Table 1 indicates that after 8d of culture under hypoxia conditions, the fresh weights of seedlings in hypoxia+Ca²⁺, hypoxia alone and hypoxia-Ca²⁺ decreased compared with those of the control treatment. The decrease in fresh weight under hypoxia-Ca²⁺ was greater than that of hypoxia alone, and under hypoxia+Ca²⁺ was the least. The fresh weight of ‘Dongfangxingguang’ was higher than that of ‘Xiyu No.1’ seedlings under all treatments.

Effect of Ca²⁺ on the Activity of Antioxidant Enzymes under Hypoxia

SOD activity in ‘Xiyu No.1’ and ‘Dongfangxingguang’ seedlings under hypoxia alone and hypoxia+Ca²⁺ increased significantly, reaching a peak 2d after hypoxia treatment, however, the SOD activity of hypoxia+Ca²⁺ treatment was higher than those of hypoxia alone (Fig.1). SOD activity with hypoxia-Ca²⁺ treatment increased slightly but was significantly lower than that control during the hypoxia at 6-8d. SOD activity of hypoxia-Ca²⁺ and control treatments was lower than those of hypoxia alone and hypoxia+Ca²⁺ treatment.

POD activity of seedlings of both varieties with hypoxia+Ca²⁺ treatment increased significantly compared to the other three treatments, peaking at 6d of hypoxia treatment (Fig.1). POD activity of hypoxia alone and hypoxia-Ca²⁺ treatments increased initially then decreased, but they were both higher than that of the control at peak activity.

Similar to SOD and POD activities, CAT activity of both varieties with hypoxia+Ca²⁺, hypoxia alone and hypoxia-Ca²⁺ treatments increased, peaking at 4d after hypoxia treatment (Fig.1). CAT activity with hypoxia+Ca²⁺ treatment was higher than that of hypoxia alone. In ‘Xiyu No.1’ seedlings, the Ca²⁺ deficient treatment (hypoxia-Ca²⁺) was not different from the control.

Generally, antioxidant enzyme activity was consistently higher for ‘Dongfangxingguang’ than for ‘Xiyu No.1’ seedlings for any one treatment and time of determination.
Effect of Ca\(^{2+}\) on ROS and MDA Levels in Seedlings under Hypoxia

H\(_2\)O\(_2\) levels of ‘Xiyu No.1’ and ‘Dongfangxingguang’ seedlings with hypoxia alone increased at initially, peaking at 2d after treatment and was higher than those of the control during the whole time course (Fig. 2). H\(_2\)O\(_2\) levels were higher with hypoxia-Ca\(^{2+}\) than with hypoxia+Ca\(^{2+}\).

Super oxide responded similar to H\(_2\)O\(_2\) with the hypoxia alone and hypoxia+Ca\(^{2+}\) treatments peaking at 4d after treatment while that with hypoxia-Ca\(^{2+}\) increased throughout the treatment time for both seedling varieties (Fig. 2). O\(_2\)\(^{−}\) productive rate of hypoxia+Ca\(^{2+}\) treatment was intermediate between hypoxia alone and the control.

The response of MDA to the four treatments is shown in figure 2. MDA was highest with a peak at 6d for in both varieties. No differences were observed between hypoxia alone and the control throughout the entire time course in ‘Dongfangxingguang’ seedlings. The hypoxia-Ca\(^{2+}\) treatment showed the lowest MDA levels and these levels were significantly lower than those of the control; the differences were larger at 6d and 8d.

Although the response of H\(_2\)O\(_2\), O\(_2\)\(^{−}\), and MDA to the various treatments followed a similar trend in both varieties, levels of H\(_2\)O\(_2\), O\(_2\)\(^{−}\), and MDA were significantly lower in ‘Dongfangxingguang’.

Effect of Ca\(^{2+}\) on NO\(_3\)\(^−\)-N and NH\(_4\)\(^+\)-N Contents under Hypoxia

The peak response of NO\(_3\)\(^−\)-N varied with the treatment and variety: for hypoxia alone, the peak was at 6 and 2d, for hypoxia+Ca\(^{2+}\) at 4 and 2d, with hypoxia-Ca\(^{2+}\) at 4 and 2d while it was 2 and 4d for the control in ‘Xiyu No.1’ and ‘Dongfangxingguang’ seedlings respectively (Fig. 3). The highest NO\(_3\)\(^−\)-N content was observed with hypoxia-Ca\(^{2+}\) and the lowest with the control.

Similarly, NH\(_4\)\(^+\)-N contents varied with the varieties and increased significantly under hypoxia and hypoxia+Ca\(^{2+}\). However, NH\(_4\)\(^+\)-N contents of the hypoxia-Ca\(^{2+}\) treatment were lower than those of hypoxia alone, and for the control on 4-8d for ‘Xiyu No.1’ seedlings (Fig. 3).

NO\(_3\)\(^−\)-N and NH\(_4\)\(^+\)-N contents of ‘Dongfangxingguang’ seedlings were higher than those of ‘Xiyu No.1’ for similar treatments.

DISCUSSION

Roots mainly contribute to the ability of plant survival against environmental stress. Under nutrient solution hypoxia, the roots of seedlings were in an O\(_2\) deficient environment that blocked the mitochondrial and chloroplast electron transport systems. The disabled electron transport system created an environmental of lower cell energy charge, higher deleterious metal ion and deoxidative force (Guo et al., 1999). These factors accelerated the production of ROS, the accumulation of lipid peroxidation products, which could have destroyed the construction, fluidity and function of cell membrane and damaged proteins, chlorophylls and nucleic acids. As a result, the growth and development of seedlings was severely inhibited. Hypoxia is brought on by an overproduction of ROS (Scandalios, 1993). The level of lipid peroxidation in seedlings was indicated by MDA content (Dhindsa, 1981). To prevent or alleviate these disruptions, plants have evolved sophisticated mechanisms of scavenging ROS, such as antioxidant enzyme (POD, SOD, CAT) and other antioxidant materials. The variation in contents of NO\(_3\)\(^−\)-N and NH\(_4\)\(^+\)-N are sensitive indices of reflecting the plant metabolism under hypoxia (Gao et al, 2004a). Nitrate reductase (NR) plays the important role of O\(_2\) in accepting electrons, thus alleviating the harmful effects of hypoxia (Guo et al., 1998). The results of present paper indicated that the fresh weight of both varieties under hypoxia were lower than those of with normal O\(_2\) concentration (control). ROS productive rate, MDA content, antioxidant enzyme activities, NO\(_3\)\(^−\)-N and NH\(_4\)\(^+\)-N levels were higher than those of the control indicating that seedlings had the ability to tolerate hypoxia in the short term but this ability broke down in the long run.

As a major and necessary element of plant growth, Ca\(^{2+}\) in cytoplasm increases rapidly under environmental stress and plays an important role in transduction stress signal and stabilizing the structure of cell membrane (Gao et al., 2004b). The increase of calcium...
concentration in cytoplasm is closely related with enhancement of antioxidant activities in plants. Ca²⁺ and calmodulin stimulated SOD activity in purified plasma membrane of tobacco seedlings under heat stress (Gong et al., 1998). Maize germ that were treated with CaCl₂ maintained relatively higher SOD activities and lower levels of MDA contents than those with distilled water treatment under heat stress (Gong et al. 1998). In the present study, exogenous application of calcium alleviated growth inhibition under hypoxia, enhanced antioxidant enzyme activities and contents of NO₃⁻-N and NH₄⁺-N were higher than under hypoxia, but ROS and MDA contents were lower than those under hypoxia, which indicated that Ca²⁺ treatment influenced ROS and nitrogen metabolism of muskmelon seedlings. On the other hand, calcium deficiency (hypoxia-Ca²⁺) aggravated the harmful effects of hypoxia, which were reversed by Ca²⁺ application.

Plants have evolved the ability to adapt to unfavorable environmental conditions (Gao et al., 2004b), but different species/varieties of plants have different mechanisms for tolerating environment stress (Drew, 1997). The present study indicated that the fresh weight, the contents of NO₃⁻-N and NH₄⁺-N, the activities of antioxidant enzymes of ‘Dongfangxingguang’ seedlings were higher than those of ‘Xiyu No.1’ under hypoxia with or without exogenous application calcium. However, the contents of ROS and MDA of ‘Dongfangxingguang’ were lower than those of ‘Xiyu No.1’, which may point to the fact that ‘Dongfangxingguang’ is better adapted to hypoxia conditions than ‘Xiyu No.1’.

In conclusion, hypoxia influenced growth, the antioxidant systems and nitrogen metabolism of muskmelon seedlings. Application exogenous calcium enhanced hypoxia tolerance, indicating that calcium played a vital role in muskmelon seedlings tolerance to hypoxia conditions. Moreover, the effect of exogenous calcium was greater with the hypoxia-tolerant variety than with the hypoxia-sensitive variety.

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Literature Cited

Dhindsa, R.S., Plumb-Dhindsa, P. and Thorpe, T.A. 1981. Leaf senescence correlated with increase levels of membrane permeability and lipid peroxidation and decrease levels dismutase and catalase. J. Exp. Bot. 32: 93-101

Tables

Table 1. Effect of Ca$^{2+}$ on fresh weight of muskmelon seedlings at 8d of hypoxia stress

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Whole plant (g)</th>
<th>Roots (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>17.87b</td>
<td>3.99b</td>
</tr>
<tr>
<td>Xiyu No.1</td>
<td>Hypoxia stress</td>
<td>10.87e</td>
<td>2.84d</td>
</tr>
<tr>
<td></td>
<td>Hypoxia+Ca$^{2+}$</td>
<td>13.41d</td>
<td>3.66c</td>
</tr>
<tr>
<td></td>
<td>Hypoxia-Ca$^{2+}$</td>
<td>7.18g</td>
<td>1.45e</td>
</tr>
<tr>
<td>Dongfangxingguang</td>
<td>Control</td>
<td>22.80a</td>
<td>5.03a</td>
</tr>
<tr>
<td></td>
<td>Hypoxia stress</td>
<td>15.24c</td>
<td>3.09c</td>
</tr>
<tr>
<td></td>
<td>Hypoxia+Ca$^{2+}$</td>
<td>18.50b</td>
<td>4.08b</td>
</tr>
<tr>
<td></td>
<td>Hypoxia-Ca$^{2+}$</td>
<td>9.98f</td>
<td>1.55d</td>
</tr>
</tbody>
</table>

The letter in difference are significantly different at $P=0.05$. 

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Fig. 1. Effect of Ca\textsuperscript{2+} on the activities of CAT, SOD, and POD in leaves of ‘Xiyu No.1’ and ‘Dongfangxingguang’ seedlings under hypoxia stress for 0, 2, 4, 6, 8d.
Fig. 2. Effect of Ca\(^{2+}\) on H\(_2\)O\(_2\) content, O\(_2\) productive rate and MDA content in leaves of ‘Xiyu No.1’ and ‘Dongfangxingguang’ seedlings under hypoxia stress for 0,2,4,6,8d.

Fig. 3. Effect of Ca\(^{2+}\) on the contents of NO\(_3\)-N and NH\(_4\)+-N in leaves of ‘Xiyu No.1’ and ‘Dongfangxingguang’ seedlings under hypoxia stress for 0,2,4,6,8d.