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外源 H_2O_2 对小麦幼苗耐盐性的调节作用

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摘　要 :以 '高原 448 '小麦品种为材料 ,分别测定了 4 个处理(Hoagland 营养液、Hoagland 营养液 + 150 mmol/ L NaCl、Hoagland 营养液 + 150 mmol/ L NaCl + 10 μmol/ L H_2O_2 和 Hoagland 营养液 + 10 μmol/ L H_2O_2) 的小麦幼苗在第 2、4、6、8 天叶片叶绿素、丙二醛、可溶性糖和还原性谷胱甘肽含量. 结果显示 :外源 H_2O_2 提高了 NaCl 胁迫下 4 个时段小麦幼苗的叶绿素含量(8. 27 %、32. 57 %、10. 19 %、4. 86 %) 及还原性谷胱甘肽含量(3. 09 %、23. 97 %、5. 85 %、2. 11 %) ,显著提高了可溶性糖含量(14. 58 %、8. 43 %、16. 68 %、5. 8 % , $P < 0.05$) ,而显著降低了其丙二醛含量(17. 53 %、14. 04 %、4. 75 %、8. 47 % , $P < 0.05$) . 外源 H_2O_2 (10 μmol/ L) 使 NaCl 胁迫下叶绿素含量和还原性谷胱甘肽含量峰值提前 ,同时推迟了丙二醛峰值出现的时间. 研究表明 ,外源 H_2O_2 通过提高叶片叶绿素、可溶性糖和还原性谷胱甘肽含量以有效地增强小麦幼苗的耐盐性.

关键词 :过氧化氢 ;小麦幼苗 ; 耐盐性 ;调节作用

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Regulation of Exogenous Hydrogen Peroxide on Wheat Seedling Salinity Tolerance

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Abstract : The chlorophyll content ,MDA content ,soluble sugar content and GSH content were determined at the 2^{nd} ,4^{th} ,6^{th} and 8^{th} day ,respectively ,after treatments of Hoagland solution , Hoagland solution + 150 mmol/ L NaCl , Hoagland solution + 150 mmol/ L NaCl + 10 μmol/ L H_2O_2 and Hoagland solution + 10 μmol/ L H_2O_2 . The addition of exogenous H_2O_2 (10 μmol/ L) increased the wheat seedling chlorophyll content (by 8. 27 % ,32. 57 % ,10. 19 % and 4. 86 %) and GSH contents (by 3. 09 % ,23. 97 % ,5. 85 % and 2. 11 %) ,dramatically increased soluble sugar content (by 14. 58 % ,8. 43 % ,16. 68 % and 5. 8 % , $P < 0.05$) and dramatically decreased MDA content (by 17. 53 % ,14. 04 % ,4. 75 % and 8. 47 % , $P < 0.05$) compared to the salt stressed (Hoagland solution + NaCl) group on the 2^{nd} ,4^{th} ,6^{th} and 8^{th} day ,respectively ,after treatments. Exogenous H_2O_2 (10 μmol/ L) accelerated the maximum accumulation chlorophyll and GSH contents in NaCl stressed condition while decelerated the maximum accumulation content of MDA. Exogenous H_2O_2 enhanced wheat seedling salinity tolerance.

Key words : H_2O_2 ;wheat seedling ;salinity tolerance ;regulation

Soil salinity is one of the major abiotic stresses affecting plant growth and productivity globally.

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There are 380 million hectares of salinity soil in the world. One of third irrigable fields is affected by high salinity. In plants, hydrogen peroxide (H_2O_2) is thought to be increased under various abiotic stresses and to enhance gene expression of active oxygen scavenging (AOS) enzymes. In maize, H_2O_2 production was reported to increase in response to chilling stress, and exogenously applied H_2O_2 increased chilling tolerance[1]. Increased H_2O_2 production was also reported to occur gradually in response to salt stress in rice plants[2]. Pretreating rice seedlings with low level of H_2O_2 increased their salt tolerance[3]. Wheat, as a crop of great importance in the world, is one of the salt-sensitive crops. Accumulation of salts in irrigated soil is one of the primary factors limiting yield in wheat production. Research on salt stress tolerance in wheat is therefore of great importance for future practical advances. However, little research has been devoted to the role of exogenous H_2O_2 on salt tolerance of wheat plants. In the present study, we demonstrated that exogenously applied H_2O_2 caused protection against salt in young wheat seedlings.

1 Materials and methods

1.1 Plant materials and treatments

Seeds used in the experiment were from spring wheat (*Triticum aestivum* L.) variety "Plateau 448". The variety was developed by Northwest Institute of Plateau Biology, the Chinese Academy of Sciences (CAS) and released in 1999. It has become one of the major cultivars in the Qinghai-Tibet Plateau and Northwest Spring Wheat Area. The seeds were surface sterilized with 70 % alcohol and thoroughly rinsed by tap water and then transferred to two sheets of sterilized moist filter paper. Seeds were placed in plastic trays for germination at 28 ℃ for 48 h in the dark. Uniform germinated seeds were selected and sown into the culture dishes, and grown hydroponically in a growth chamber. The temperature of the growth chamber was maintained at (27 ± 2) ℃ and light intensity was 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The humidity of the growth chamber was between 60 %～70 %. The Hoagland nutrition was aerated continuously and replaced every two days. When the third leaf sprouted, treatments were made as follows: (1) Hoagland solution; (2) Hoagland solution + 150 mmol/L NaCl; (3) Hoagland solution + 150 mmol/L NaCl + 10 μmol/L H_2O_2; (4) Hoagland solution + 10 μmol/L H_2O_2. Leaves at the same leaf order were harvested at 9 O'clock on the 2^{nd}, 4^{th}, 6^{th} and 8^{th} day after treatment, respectively and then stored at - 70 ℃ for determination of chlorophyll content, MDA content, soluble sugar content and GSH content. All the treatments were performed in triplicates.

1.2 Methods

Chlorophyll content was determined as described in Yang's method[4]. MDA and soluble sugar content were determined following Ellman's method[5]. Reduced glutathione (GSH) content was determined following Ellman's method[6]. All the determinations were accomplished by 751-UV spectrophotometer and performed in triplicates and the average values were presented. Excel and SPSS15.0 were used for data statistic analysis.

2 Results

2.1 Effects of exogenous H_2O_2 on chlorophyll content in wheat seedling

As we all know, photosynthesis is one of the key metabolic pathways that are responsible for growth and development of plants[7] and chlorophyll is the most important photosynthetic pigment in plants. Therefore chlorophyll content could be used as an index of salt tolerance of plants. As shown in Fig. 1, after wheat seedlings were grown in 150 mmol/L NaCl, the leaf chlorophyll content was decreased by 13.58 %, 12.82 %, 3.27 % and 1.82 % on the 2^{nd}, 4^{th}, 6^{th}, 8^{th} day, respectively, compared to the control (Hoagland solution treated only). While the chlorophyll content of those groups with Hoagland + NaCl + H_2O_2 treated was much higher than that of the groups with salt stressed (150 mol/L NaCl). The addition of H_2O_2 (10 μmol/L) to salt stressed wheat seedlings increased their chlorophyll contents by 8.27 %, 32.57 %, 10.19 % and 4.86 % on the 2^{nd}, 4^{th}, 6^{th} and 8^{th} day, respectively. Especially, on the 4^{th}, 6^{th}, and 8^{th}

day after treatments, the chlorophyll content of Hoagland + NaCl + H_2O_2 treated group was even higher than that of the Hoagland treated group. On the 2nd and 8th day after treatment of Hoagland + H_2O_2, the chlorophyll content in wheat leaves was a little higher than control. Furthermore, the maximum chlorophyll content appeared on the 6th day in Hoagland + NaCl treated group while on the 4th day in Hoagland + NaCl + H_2O_2 treated group.

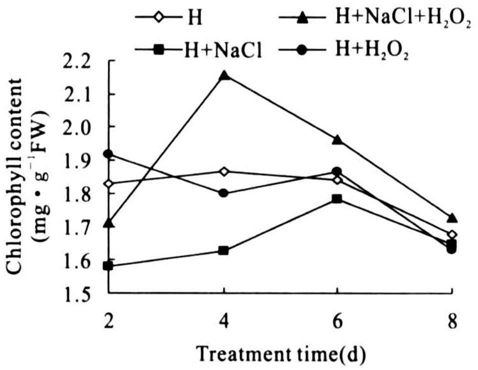

Fig. 1　Chlorophyll content in wheat seedling with different treatments and time

H represents Hoagland solution; Results are presented as means and standard errors from three replications. The same as below

2.2　Effects of exogenous H_2O_2 on lipid peroxidation in wheat seedling

Lipid peroxidation of wheat seedlings after treatments, measured as MDA content, is shown in Fig. 2. After salt stressed treatment (150 mol/L NaCl), the MDA content was increased by 13.89 %, 27.63 %, 29.35 % and 19.67 % on the 2nd, 4th, 6th and 8th day, respectively, compared with the control. While the MDA content was decreased by 17.53 %, 14.04 %, 4.75 %, 8.47 % in the Hoagland + NaCl + H_2O_2 on the 2nd, 4th, 6th and 8th day after treatments ($P < 0.05$), respectively, compared with the salt stressed group. In addition, on the 2nd, 4th, 8th day after treatments, the MDA content of the Hoagland + H_2O_2 treated group was decreased a little compared to the control. MDA content was increased before 4th and decreased during the 4th～8th day after treatment of Hoagland + NaCl. While the MDA content was increased until the 6th day and then decreased. Exogenously added H_2O_2 not only decreased MDA content but decelerated the appearance of maximum MDA content in NaCl stressed condition.

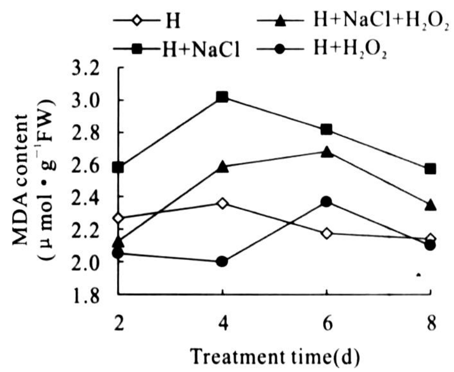

Fig. 2　MDA content in wheat seedling with different treatments and time

2.3　Effects of exogenous H_2O_2 on soluble sugar content in wheat seedling

Soluble sugar content is shown in Fig. 3. Both salt stressed treatment (Hoagland + NaCl) and Hoagland + NaCl + H_2O_2 treatment increased the wheat seedling soluble sugar content to different degrees on the 2nd, 4th, 6th and 8th day after treatments. The stressed treatment (Hoagland + NaCl) increased soluble sugar content by 22.38 %, 11.66 %, 8.41 %, 7.07 % on the 2nd, 4th, 6th and 8th day after treatments, respectively, compared with the control. While the soluble sugar contents of Hoagland + NaCl + H_2O_2 treated group were increased by 36.96 %, 20.09 %, 25.09 %, 12.87 % on the 2nd, 4th, 6th and 8th day, respectively, compared with the control. Hoagland + NaCl + H_2O_2 treatment further increased the soluble sugar by 14.58 %, 8.43 %, 16.68 % and 5.8 %, respectively, compared to the salt stressed group ($P < 0.05$). Soluble sugar contents were increased from 2nd to 4th day and then decreased during the next 4 days in the all treatments except for the Hoagland + H_2O_2 treated group.

2.4　Effects of exogenous H_2O_2 to GSH content of wheat seedling

GSH content is displayed in Fig. 4. Salt stress treatment (Hoagland + NaCl) decreased the GSH content by 8.68 %, 20.27 %, 10.06 % and 8.62 % on the 2nd, 4th, 6th and 8th day, respectively, compared with the control. The addition of H_2O_2 (10 μmol/L) to Hoagland + NaCl increased the GSH content by 3.09 %, 23.97 %, 5.85 % and 2.11 %, respectively, compared with the salt stressed group at the 2nd, 4th, 6th, 8th day after treatments. GSH content was increased more dramatically in Hoagland + NaCl + H_2O_2 treated group than in Hoagland + NaCl treated group from 2^{th} to 4^{th} day after treatment. The appearance of maximum GSH content was also accelerated by exogenously added H_2O_2 in NaCl stressed condition.

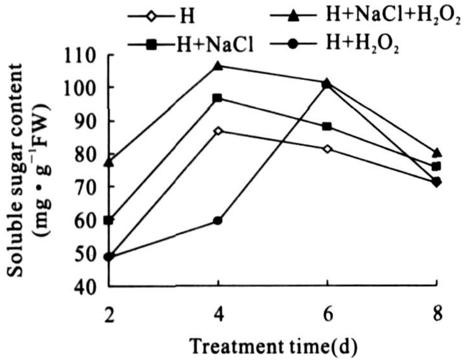

Fig. 3 Soluble sugar content in wheat seedling with different treatments and time

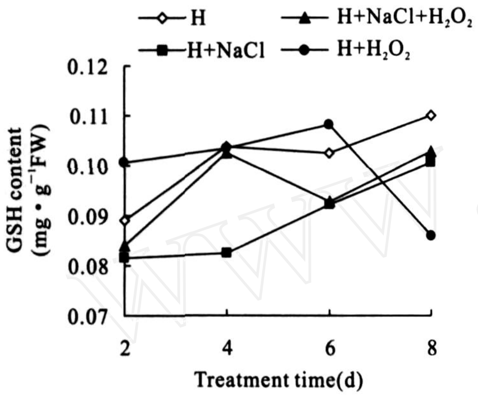

Fig. 4 GSH content in wheat seedling with different treatments and time

3 Discussion

Compounds that are able to reduce damaging effects of various stresses should be of great importance from both the theoretical and application points of view. The ROS hydrogen peroxide (H_2O_2) has generally been viewed as a toxic cellular metabolite. However, it is clear that it may also function as a signal molecule in both plant and animal cells[8]. The generation of H_2O_2 is increased in response to a wide variety of abiotic and biotic stresses, and some authors have suggested that H_2O_2 plays a dual role in plants: at low concentrations, it acts as a messenger molecule involved in acclimatory signaling, triggering tolerance against various abiotic stresses, and at high concentrations it orchestrates programmed cell death[1,9]. Thus, it appears likely that H_2O_2 accumulation in specific tissues, and in the appropriate quantities, may benefit plants by mediating plant acclimation and cross-tolerance to both biotic and abiotic stresses[9].

In the present study we showed that exogenous H_2O_2 caused protection against salt stress in wheat young seedlings (Fig. 1～4). Chlorophyll content could be used as an index of salt tolerance of plants. Decreasing of wheat seedling chlorophyll content caused by salt stress (150 mmol/L NaCl) was alleviated by the H_2O_2 (10 μmol/L) to different degrees. Hoagland + H_2O_2 (10 μmol/L) treatment also increased the wheat seedling chlorophyll content of Hoagland + H_2O_2 group to the level of being beyond the control on the 4^{th}, 6^{th} and 8^{th} day after treatment. At the same time, the appearance of maximum chlorophyll content was accelerated by exogenously added H_2O_2 (Fig. 1). That indicated the exogenous H_2O_2 could play some protective role in salt stress condition.

Salt stress is known to result in extensive lipid peroxidation, which has often been used as indicator of salt-induced oxidative membrane damages[8]. MDA is an end product of lipid peroxidation. In the present study (Fig. 2), we found that MDA content was decreased by the exogenously added H_2O_2 (10 μmol/L) compared with salt stressed group. We can see clearly from the data that the protection caused by H_2O_2 against salt stress occurred mainly in the early stage after treatment of H_2O_2 (10 μmol/L). It also showed that H_2O_2 had little effect on unstressed wheat seedlings. Exogenously added hydrogen peroxide not only decreased MDA content but decelerated the appearance of maximum MDA content in the NaCl stressed condition.

Water deficiency caused by salt stress is a common phenomenon in plants. Water deficiency often leads to metabolic disorders. In order to keep a relatively higher osmotic potential (ψ_s), plants often positively synthesize and accumulate some compatible solutes which could increase the osmotic potential of plant cells. Soluble sugar is one of compatible solutes. In order to protect plant cells from losing water excessively, soluble sugar content was increased under salt stressed condition, while the addition of H_2O_2 (10 μmol/L) further increased the accumulation of soluble sugar in wheat seedlings (Fig. 3), hence protected wheat seedling cells by keeping a relatively higher osmotic potential (ψ_s) and improving their salt tolerance. It was showed that at the 2^{nd} and 6^{th} day after treatment of Hoaglan + H_2O_2 (10 μmol/L), the soluble sugar content was also higher than control (Fig. 3). So we could speculate it is the "Pie-up Effect" of salt stress and H_2O_2.

GSH is an important metabolite as it is involved in the protection of plants from oxidative stress buildup due to various environmental stresses[10-12]. GSH is one of the non-enzymatic antioxidants like ascorbate (ASA), tocopherols and carotenoids for scavenging reactive oxygen species (ROS) produced in the stressed condition. One of the damages caused by the salt stress is the oxidative stress. Our work showed that salt stress decreased the GSH content while the addition of H_2O_2 (10 μmol/L) increased the GSH content compared to the salt stressed treatment, hence increased the salt tolerance of wheat seedlings. The appearance of maximum GSH content was also accelerated by exogenously added H_2O_2 in NaCl stressed condition. In addition, H_2O_2 (10 μmol/L) also increased the GSH content of unstressed group, but its influences on unstressed group was much less than that of salt stressed group.

We found that H_2O_2 as a newly discovered signal molecule was much more effective in salt stressed condition than in unstressed condition. It could be hypothesized that H_2O_2 may induce the expression of series of related defense genes immediately when salt stress occurs. It is reported that endogenous H_2O_2 accumulation occurs at the early stage, providing evidence that H_2O_2 might be an inducer of antioxidation mechanism[1]. In our experiment, exogenous H_2O_2 was added and the 2^{nd}, 4^{th}, 6^{th} and 8^{th} day after treatments were selected for the determination of physiological indexes. We also hypothesize that H_2O_2 regulate the physiology and biochemical metabolism mainly during the few hours after treatment. Therefore, it is worth understanding the effects of H_2O_2 on salt tolerance during the few hours after treatment in the future.

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