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RESEARCH ARTICLE

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Effect of $CaCl_2$ treatment on the changing of drought related physiological and biochemical indexes of *Brassica napus*

Abstract Some experiments revealed that the Ca^{2+} has a relationship with drought resistance. In this paper, some physiological and biochemical indices were studied in order to analyze the effect of Ca^{2+} treatment on the drought resistance of *Brassica napus*. The physiological and biochemical experiments revealed that the proline content and the soluble sugar contents in the Ca^{2+}-treated *B. napus* were much higher than those of water-treated *B. napus*. However, the content of malonaldehyde in the Ca^{2+} treated *B. napus* was lower than that of control. The transpiration and stomatal conductance analysis show that the transpiration and stomatal conductance in the Ca^{2+}-treated materials were lower than those of water-treated materials. Further research revealed that the stomata hatch of Ca^{2+}-treated materials was markedly less than that of water-treated materials, which indicated that the closure of the stomata was the main reason for the decrease of transpiration and stomatal conductance.

Keywords *Brassica napus*, physiological and biochemical index, stomatic observation

1 Introduction

Rapeseed is one of the most important oil seed crops in China. The planting areas and the production account for 1/3 of the world's total (Fu, 2000). Poor water management, which intensifies the competition for limited water resources, and global warming all highlight the looming water crisis that threatens agricultural productivity worldwide (Hu et al., 2006), and has shown an increase in threatening rapeseed production. The total yield of rapeseed decreased by 10% on the average and the decreased yield in Hunan, Hubei, Anhui and Jiangxi provinces reached as high as 20% under the drought environment in 2003. Understanding plant tolerance to drought and studying the dry farming are therefore of fundamental importance. Lots of researches have revealed that the drought resistance can be greatly increased if the seeds are forged by drought treatment or reasonably applied with mineral elements. Most reports that document the enhancement of drought tolerance by means of overexpression of selected genes done in some crops, *SNAC1*-overexpressing transgenic plants show significantly improved drought resistance under field conditions and strong tolerance to salt stress (Hu et al., 2006).

Calcium is the second signal messenger in cells which has an important regulative function in plant development and has a close relationship with some plant resistances to environment. The Ca^{2+} signals are produced through the transient influx of plant cells in low temperature, drought and salinity stress. The Ca^{2+} is again transferred into internal calcium stores from the cytosol by the transfer systems of H^+/Ca^{2+} enzyme and Ca^{2+}-ATP enzyme, etc. (Braam and Davis, 1990). The active oxygen molecules can be produced rapidly which starts up other signal pathways and series of protective physiological reactions in order to decrease the damage produced by the multiplicity of stress signals (Knight, 2000; Xu and Heath, 1998). Proline and glycine betaine are probably the most widely distributed osmolyte and are thought to protect plants by maintaining the water balance between plant cells and the environment and increase the content of proline and glycine betaine in plants so as to enhance their drought resistance, which has aroused the attention

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of researchers (Hong et al., 2000; Rontein et al., 2002). Few researches are involved in the effects of calcium in *B. napus* in drought induced environment at present. This paper reports the changes of some of the physiological characters of *B. napus* in the drought-treated process in Ca^{2+}- and water-treated conditions.

2 Methods

2.1 Experimental materials

B. napus cultivar L078 was used in this study.

2.2 Treatment of experimental materials

According to the previous studies in 2005, the concentration of $CaCl_2$ used in the experiment in 2006 was 40 $mmol\cdot L^{-1}$. 100 *B. napus*, which were tidily growing in bolting time, transplanted to flowerpots and cultivated for one week. Those plants were divided into two groups: one group was insufflated with 50 mL $CaCl_2$ solution on the leaves per plant for seven successive days; the other group was treated with the same volume of distilled water in the same way. Thereafter, continual drought treatment was performed. In the process of drought treatment, soil water content was measured by using the TDR (MP-160) soil water system. The water content of 70% was considered as drought conditions. The measurement of the water content of the soil shows that the water content was reduced with the drought treatment with figures of 95.8%, 83.5%, 67.4%, 45.6% and 31.2% in the experimental period.

2.3 Measurement of proline, soluble sugar and malonaldehyde

Old and functional leaves were distinguished according to the method of Qiu and Hu (2002). That is, counting backwards, the 4th and the 1st–2nd leaves are considered as functional leaves and old leaves, respectively. The content of proline, soluble sugar and malonaldehyde was measured using method of Li et al. (2000).

2.4 Measurement of transpiration and stomatal conductance

Single functional and old leaf photosynthetic rate (*Pn*) and stomatal conductance to CO_2 (*Gs*) were measured with a portable type apparatus for photosynthesis and transpiration measurements (LI-6400, LICOR). The measurement was conducted with a one-hour interval from 8:00 to 17:00.

2.5 Observation of stomata of leaves

The functional leaves of *B. napus* in different treatments were selected, with the dust wiped off, coated with the nail polish and covered with the lucid rubberized fabric after the nail polish air-dried naturally on the surface of leaves. The lucid rubberized fabric were later ripped off, observed and photographed under an Olympus AH_2 microscope (Wei et al., 2002).

3 Results

3.1 Variation of proline content in the process of drought treatment

Generally speaking, the changing tendency of the proline content in the leaves of *B. napus* increased at first and then decreased no matter when the leaves are treated with Ca^{2+} or distilled water in the process of drought treatment. However, the proline content in the Ca^{2+}-treated old and functional leaves was higher than that of the distilled water treated leaves (Fig. 1), with obvious variance between different treatments ($P < 0.05$). This result revealed that the Ca^{2+} treatment has a certain effect on proline production. Further researches exhibited that the content of proline in Ca^{2+}-treated functional leaves was markedly higher than that of Ca^{2+}-treated old leaves in the later drought process, which indicated that the functional leaves had more obvious responses to Ca^{2+} treatment.

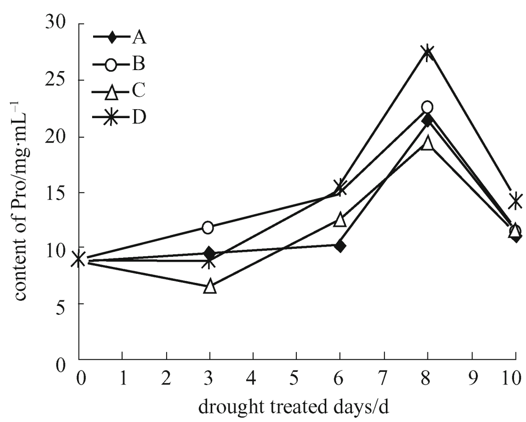

Fig. 1 Variation of proline (Pro) content in drought induced process
Note: A, B, C and D represent water-treated old leaves, $CaCl_2$-treated old leaves, water-treated functional leaves and $CaCl_2$-treated functional leaves, respectively. The same for Figs. 2–5.

3.2 Variation of soluble sugar content in the process of drought treatment

The changing of soluble sugar content was similar to that of proline, increasing at first and decreasing in the later drought process (Fig. 2). The content of soluble sugar in the Ca^{2+}-treated functional leaves were a little higher than that of old leaves. However, no obvious difference was observed in the distilled water-treated old and functional leaves ($P > 0.05$). The increase of soluble sugar in the process of drought treatment was in favor of maintaining the osmotic potential and had a certain effect for the normal growth of plant.

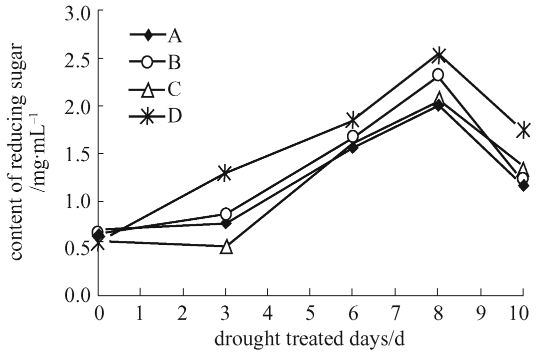

Fig. 2 Variation of sugar content induced in drought process

3.3 Variation of malonaldehyde content in the process of drought treatment

Drought can stimulate the generation of active oxygen species by perturbing the metabolism involved in electron transfer, which can lead to damage of cellular membranes and accumulation of thiobarbituric acid-reactive substances associated with lipid peroxidation. Malonaldehyde (MDA) is the final decomposed product of lipoperoxidation and the content of MDA can reflect the level of damage. The present study revealed that the content of MDA in Ca^{2+}- and distilled water-treated leaves all increased in the process of drought treatment, but the MDA content in the Ca^{2+}-treated leaves was obviously lower than that of distilled water-treated leaves (Fig. 3), which indicated that the Ca^{2+} could protect the stability of membranes and might have a certain effect on drought resistance. Further research also revealed that the MDA content in Ca^{2+}-treated old and functional leaves show a significant difference ($P < 0.05$). It was indicated that the functional leaves was more sensitive to Ca^{2+} than old leaves.

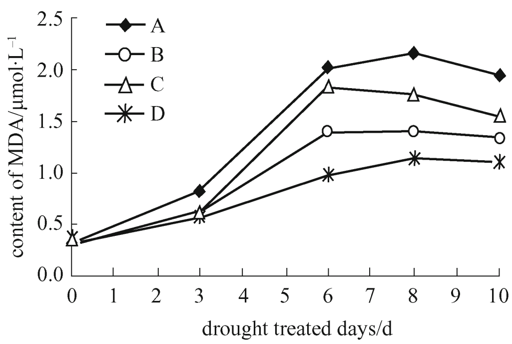

Fig. 3 Variation of MDA content in the drought induced process

3.4 Variation of transpiration and stomatal conductance of *B. napus* in the process of drought treatment

Only the six-day drought treated plants were used for studying the variation of transpiration in a day. The results revealed that the daily changing patterns of transpiration between functional and old leaves in different treatments were similar and the transpiration pattern of distilled water-treated leaves show a double apex curve and that the peak values appeared at 11:00 and 14:00. Little difference was found in the transpiration pattern of Ca^{2+}-treated leaves, whose transpiration rate was lower than that of water-treated leaves, and decreased as a whole though the old leaves show a peak value at 11:00 (Fig. 4). On the other hand, the transpiration rate of functional leaves was obviously higher than that of old leaves in the distilled water-treated leaves ($P < 0.01$), which indicated that the water was mainly transported out through functional leaves. The transpiration rate of the Ca^{2+}-treated leaves was markedly decreased, which indicated that Ca^{2+} could increase the drought resistance of *B. napus* through decreasing the water consumption.

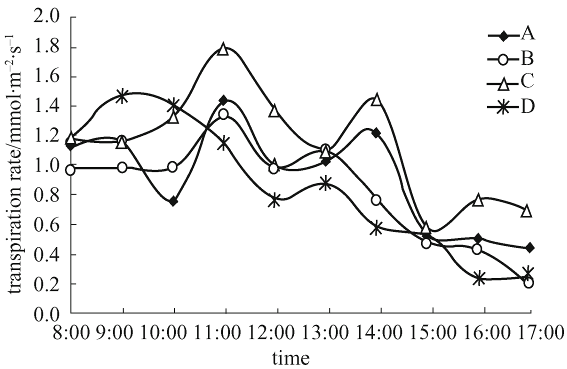

Fig. 4 Variation of transpiration rate in a day of different types of treatment in *B. napus*

Our experimental result shows that the changing pattern of stomatal conductance tendency in the distilled water-treated leaves in a day was similar to that in the Ca^{2+}-treated leaves without an obvious peak value (Fig. 5). The stomatal conductance in both treatments decreased in a day, but the average value of stomatal conductance of the distilled water-treated leaves was higher than that of Ca^{2+}-treated leaves. Further analysis revealed that the difference between the distilled water and Ca^{2+}-treated functional leaves reached a significant level ($P < 0.01$), which indicated that the Ca^{2+} treatment could decrease the stomatal conductance significantly. The observation on the stomas shows that the placket of stoma in the distilled water-treated leaves was larger than that of Ca^{2+}-treated leaves on the average (Fig. 6). About 57% of the stomas tended to close in the Ca^{2+}-treated three-day functional leaves in the time of high temperature in a day (especially from 10:00 to 15:00), Additionally, the degree of placket of stomas in the distilled water treatment was much larger than that in the Ca^{2+} treatment, which indicated that the placket of stoma can be affected by the Ca^{2+}, and the changing of placket of stoma will further affect the transpiration of leaves.

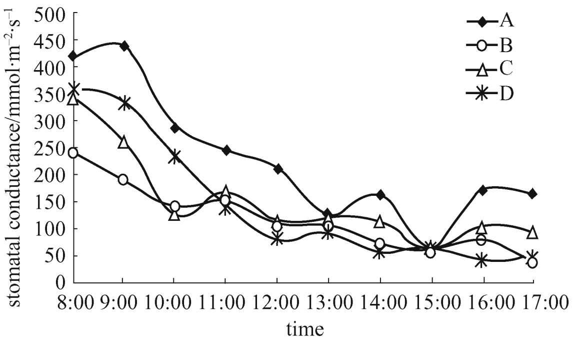

Fig. 5 Daily variation of stomata conductance of *B. napus* in different treatments

4 Discussion

Drought stress is one of the major stress factors limiting plant growth. Plant response to water stress involves changes in carbon assimilation and metabolism (Souza et al., 2004; Miyashita et al., 2005; Cai et al., 2007). Water stress significantly decreases starch content, but increases the contents of total soluble sugars, MDA and proline in *B. napus*, similarly as reported for other species in previous studies (Souza et al., 2004; Cechin et al., 2006; Cai et al., 2007). The drought stress can lead to cellular dehydration which causes osmotic stress and water removal from the cytoplasm into the extracellular space resulting in a reduction of the cytosolic and vacuolar volumes. Meanwhile, the drought stress also restrains the uptake of calcium. The calcium is one of the necessary elements for the plant development, which has a close relationship with the drought resistance regulation (Joel, 2002). Cheng et al. (1997) revealed that the $CaCl_2$ treatment of seeds and leaves of cotton can improve their resistance to drought and it is proved that the Ca^{2+} can improve the drought resistance by protecting the membrane from damage. Ca^{2+} also can increase the seed germination and seeding development under salt stress (Bonilla et al., 2004). In our present studies, the contents of soluble sugar and proline in the $CaCl_2$-treated leaves were improved significantly, which confirms that the Ca^{2+} can increase the accumulation of soluble sugar and proline. Higher accumulation of soluble sugars in *B. napus* may result from higher metabolic impairment affecting sugar composition in the leaf or its translocation, contributing to the inhibition of photosynthetic efficiency during water stress (Campos et al., 1999; Cai et al., 2007).

In plant cells, calcium functions as a second messenger coupling a wide range from extracellular stimuli to intracellular responses (Bartels and Sunkar, 2005). The involvement of Ca^{2+} signaling in response to osmotic and ionic stress is well documented. NaCl causes a rapid and transient increase in cytosolic calcium, which in turn triggers many signal transduction pathways, including the regulation of enzymatic activity, ion channel activity and gene expression, which results in diverse cellular responses (Bartels and Sunkar, 2005). Stoma is the main port for plant transpiration. The degree of opening and closing of the stoma has a close relationship with the transpiration. The present study revealed that the transpiration and stomatal conductance of Ca^{2+}-treated leaves can be markedly decreased in the drought treatment compared with the control. Further observation on the degree of opening and closing of the stoma confirmed that the Ca^{2+} treatment can lead to the closing of leaf stomas, which might be the reason for the decrease of transpiration and stomatal conductance of Ca^{2+}-treated leaves. Cell-type specific changes in cytosolic calcium levels were observed in *Arabidopsis* root cells in response to drought (Kiegle et al., 2000). The transient increases of Ca^{2+} in the cytosol of the guard cells of stomas inhibited the K^+ inter-channel of cell membrane and enhanced the K^+ out-channel of

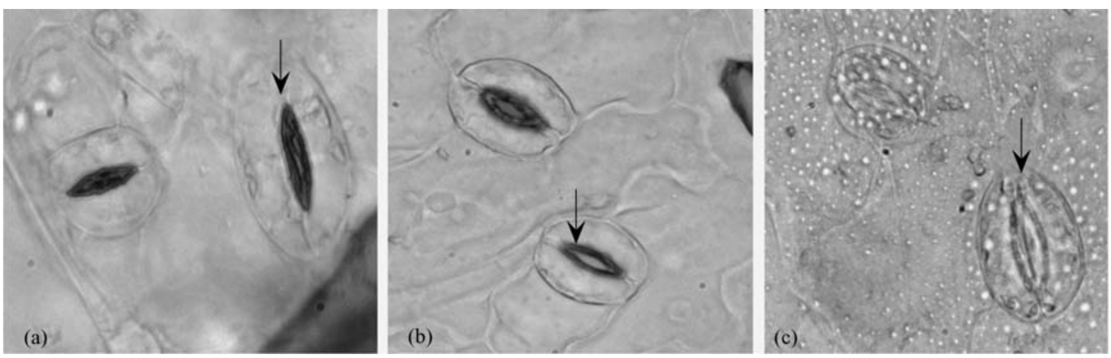

Fig. 6 Effect of different treatments on the stomata of *B. napus*
Note: (a) and (b) were the stomas in drought induced 3 days by being treated with Ca^{2+} and no Ca^{2+}, respectively, (c) was the stoma treated with normal water.

vacuole. The outflow of K^+ leads to the closing of stomas (Zhang, 2005). The research on the gene expression of Ca^{2+} treated leaves is now in progress.

References

Bartels D, Sunkar R (2005). Drought and salt tolerance in plants. Critical Reviews in Plant Sciences, 24: 23–58

Bonilla I, El-Hamdaoui A, Bolaños L (2004). Boron and calcium increase *Pisum sativum* seed germination and seedling development under salt stress. Plant and Soil, 267: 97–107

Braam J, Davis R W (1990). Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in *Arabidopsis*. Cell, 60: 357–364

Cai H, Biswas D K, Shang A Q, Zhao L J, Li W D (2007). Photosynthetic response to water stress and changes in metabolites in *Jasminum sambac*. Photosynthetica, 45(4): 503–509

Cechin I, Rossi S C, Oliveira V C, Fumis T F (2006). Photosynthetic responses and proline content of mature and young leaves of sunflower plants under water deficit. Photosynthetica, 44: 143–146

Cheng H L, Tang L S, Zhang Y G, Yan J Y, Zhang H (1997). The effect of $CaCl_2$ on drought resistance of cotton seedlings. China Cotton, 24(12): 17–18 (in Chinese)

Fu T D (2000). The Utilization of Hybrid Rapeseed Breeding. Wuhan: Hubei Scientific Technology Press (in Chinese)

Hong Z, Lakkineni K, Zhang Z, Verma D P S (2000). Removal of feedback inhibition of 1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. Plant Physiology, 122: 1129–1136

Hu H, Dai M, Yao J, Xaio B, Li X, Zhang Q, Xiong L (2006). Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proceeding of the National Academy of Science, 103(35): 12987–12992

Joel A, Wu Y, Chang H (2002). Transcriptome changes for *Arabidopsis* in response to salt, osmotic, and cold stress. Plant Physiology, 130: 2129–2141

Kiegle E, Moore C A, Haseloff J, Tester M A, Knight M R (2000). Celltype-specific calcium responses to drought, salt and cold in the *Arabidopsis* root. Plant Journal, 23: 267–278

Knight H (2000). Calcium signaling during abiotic stress in plants. Int Rev Cytol, 195: 269–325

Li H S (2000). The Principle and Technology of Plant Physiology and Biochemistry. Beijing: Higher Education Press (in Chinese)

Miyashita K, Tanakamaru S, Maitani T, Kimura K (2005). Recovery responses of photosynthesis, transpiration, and stomata conductance in kidney bean following drought stress. Environmental and Experimental Botany, 53: 205–214

Qiu D Y, Hu L Y (2002). Effect of nitrogen levels on endogenous hormones content of rapeseed (*Brassica napus* L.). Journal of Huazhong Agricultural University, 21(3): 213–216 (in Chinese)

Rontein D, Basset G, Hanson A D (2002). Metabolic engineering of osmoprotectant accumulation in plants. Metabolic Eng, 4: 49–56

Souza R P, Machado E C, Silva J A B, Lagôa A M M A, Silveira J A G (2004). Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. Environmental and Experimental Botany, 51: 45–56

Wei A L, Chang Z J, Xing Y, Li J (2006). Comparison of stomata features of octoploid *Tritielytrigia* varieties and *Triticum aestivum* varieties with different water-requirements. Acta Botanica Boreali-Occidentalia Sinica, 26 (8): 1727–1731

Xu H, Heath M C (1998). Role of calcium in signal transduction during the hypersensitive response caused by basidiospore-derived infection of the cowpea rust fungus. Plant Cell, 10: 585–597

Zhang M Q (2005). The Molecular Physiology and Genetic Improvement. Beijing: Science Press (in Chinese)