

## Adverse Effect of H<sub>2</sub>O<sub>2</sub> Change on Morphology, Mitochondrial Membrane Permeability and Antioxidant Enzyme in Root of *Dianthus Chinensis* L. under Salt Stress

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**Abstract:** *Dianthus chinensis* L. is a salt-tolerant ornamental plant. Root is the first and critical part of plant to encounter soli salinity. In order to elucidate H<sub>2</sub>O<sub>2</sub> impact on root morphology and mitochondrial permeability transition as well as activities of antioxidant enzymes in root of *Dianthus chinensis* L., we treated seedling with H<sub>2</sub>O<sub>2</sub> and DMTU under NaCl. The results revealed that change of H<sub>2</sub>O<sub>2</sub> level under NaCl would negatively influence the root growth, as well as lower the value of mitochondrial membrane absorbance at 540 nm and the ratio of Cyt *c/a*. Meanwhile, SOD and POD under NaCl plus H<sub>2</sub>O<sub>2</sub> and NaCl plus DMTU were far lower than those under NaCl alone.

**Keywords:** *Dianthus chinensis* L., H<sub>2</sub>O<sub>2</sub>, mitochondrial membrane, root morphology, salt stress

### INTRODUCTION

Soil salinity, particularly due to NaCl, can be considered as the single most widespread soil toxicity problem that global plant growth faces at present. Because of sporadic precipitation during the growing season and its influence on soil water potential, inland saline environments tend to be more variable in soil salinity concentrations than coastal marshes which are regularly exposed to tidal action (Ungar, 1970, 1974).

*Dianthus chinensis* L. is an ornamental plant and can be tolerant to 150 mM NaCl (He *et al.*, 2009). Avsian-Kretchmer *et al.* (1999, 2004) had proved that salt or oxidative stress raised H<sub>2</sub>O<sub>2</sub> in citrus cell on transcriptional level. Root is the first and critical part of plant to encounter soil salinity. When seedling roots were treated directly with exogenous H<sub>2</sub>O<sub>2</sub>, programmed cell death was induced by H<sub>2</sub>O<sub>2</sub> which led to increasing mitochondrial permeability and decreasing  $\Delta\Psi_m$  in the root of *Malus hupehensis* Reh (Ma and Yang, 2006). Our previous results showed that salt stress and H<sub>2</sub>O<sub>2</sub> stress shortened seedling length, especially the root in *Dianthus chinensis* L. (He *et al.*, 2010). However, roles of H<sub>2</sub>O<sub>2</sub> and the link between H<sub>2</sub>O<sub>2</sub> and root reaction were still unclear in salt-tolerant plant under salt stresses.

In present study we treated seedlings with H<sub>2</sub>O<sub>2</sub> and DMTU under NaCl and tried to elucidate H<sub>2</sub>O<sub>2</sub> impact on root morphology and mitochondrial permeability transition as well as activities of antioxidant enzymes in root of *Dianthus chinensis* L.

### MATERIALS AND METHODS

**Plant materials and treatments:** Germinated seeds with more than 2 mm radicle were placed in Petri dish containing discs of filter paper soaked with different treatments. The treatments were as following: sterilized water, 2 mM DMTU, 10 mM H<sub>2</sub>O<sub>2</sub>, 100 mM NaCl, 100 mM NaCl+2 mM DMTU, 100 mM NaCl+10 mM H<sub>2</sub>O<sub>2</sub>. Each Petri dish contained 50 germinated seeds and each treatment was replicated 3 times. The seedlings grew in growth chamber at 22°C under light.

**Mitochondria extraction and assays:** Mitochondria were extracted in root after 6 and 8 days with 100 mM NaCl, 100 mM NaCl+2 mM DMTU, 100 mM NaCl+10 mM H<sub>2</sub>O<sub>2</sub>, respectively.

The resulting mitochondria was used to detect mitochondrial permeability, mitochondrial membrane potential and the ratio of Cyt *c/a* following the protocols of Jin *et al.* (2002), Braidot *et al.* (1998) and Tonshin *et al.* (2003), respectively.

**Extraction and assay of enzymes:** Roots after treatments for 6 and 8 days with 100 mM NaCl, 100 mM NaCl+2 mM DMTU and 100 mM NaCl+10 mM H<sub>2</sub>O<sub>2</sub> were used to extract enzymes. SOD was conducted according to Beyer and Fridovich (1987). POD was determined following Kuroda *et al.* (1990).

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

**Root morphology:** Typan Blue staining was conducted according to Zhang *et al.* (2010) and observed under stereo microscope.

## RESULTS

**H<sub>2</sub>O<sub>2</sub> served as a critical signal during root growth in *Dianthus chinensis* L. under normal condition and salt stress:** NaCl stress inhibited root length of *Dianthus chinensis* L. (He *et al.*, 2009). Dimethylthiourea (DMTU) used as a hydrogen peroxide trap (Agzio and Zacchini, 2001). Increasing H<sub>2</sub>O<sub>2</sub> level by H<sub>2</sub>O<sub>2</sub> or decreasing H<sub>2</sub>O<sub>2</sub> level by DMTU under normal condition depressed taproot growth but induced lateral root in *Dianthus chinensis* L. (Fig. 1a). Taproots with 100 mM NaCl+2 mM DMTU or 100 mM NaCl+10 mM H<sub>2</sub>O<sub>2</sub> were significantly shorter than that with 100 mM NaCl alone and no lateral root was visible (Fig. 1b).

Typan Blue Staining is a good way to show the level of cell death. In our study the dead cell seemed be in root tips and no obvious difference in staining was observed under treatments (Fig. 1a, b).

**H<sub>2</sub>O<sub>2</sub> impact on mitochondrial membrane permeability in root of *Dianthus chinensis* L. under long-term salt stress:** Mitochondrial membrane absorbance under 100 mM NaCl+10 mM H<sub>2</sub>O<sub>2</sub> or 100 mM NaCl+2 mM DMTU was higher than that under NaCl after 6 days. With time all of the absorbance at 540 nm under different treatments increased, but the absorbance under NaCl increased significantly and was higher than the others (Fig. 2).

As far as mitochondrial membrane potential was concerned, no difference was among the treatments after 6 days. Although the values increased after 8 days, there was no significant difference among treatments (Fig. 3).

The ratio of Cyt c/a with 100 mM NaCl+10 mM H<sub>2</sub>O<sub>2</sub> was a little bit higher than the others after 6 days (Fig. 4). With time all of the ratios lowered. Ratio with 100 mM NaCl+10 mM H<sub>2</sub>O<sub>2</sub> sharply decreased and became the lowest among the treatments. However, no obvious difference was observed after 8 days.

The above results suggested that mitochondrial membrane in root of salt-resistant plant has the ability to restore itself under salt stress with time, but such ability seemed be weakened due to the change of H<sub>2</sub>O<sub>2</sub> level.

**H<sub>2</sub>O<sub>2</sub> role on activity of SOD and POD in root of *Dianthus chinensis* L. under long-term salt stress:** Compared with the situation after 6 days, activities of SOD under all of treatments sharply decreased after 8 days, especially those with 100 mM NaCl+10 mM



Fig. 1: Root morphology of *Dianthus chinensis* L. after being treated for 8 days a) from left to right: CK, 2 mM DMTU, 10 mM H<sub>2</sub>O<sub>2</sub>, b) from left to right: 100 mM NaCl, 100 mM NaCl + 2 mM DMTU, 100 mM NaCl + 10 mM H<sub>2</sub>O<sub>2</sub>

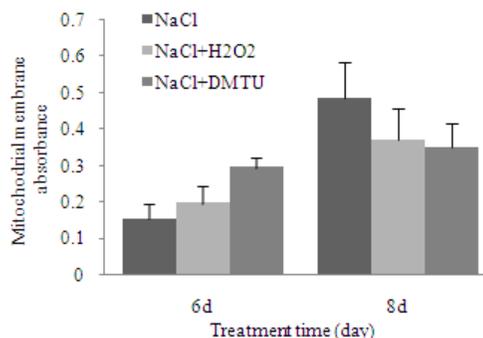


Fig. 2: Mitochondrial membrane absorbance in root of *Dianthus chinensis* L. under treatment

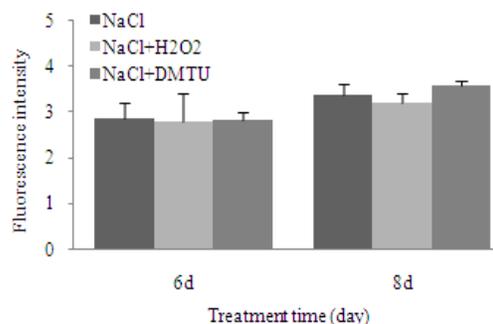


Fig. 3: Mitochondrial fluorescence intensity in root of *Dianthus chinensis* L. under treatment

H<sub>2</sub>O<sub>2</sub> and 100 mM NaCl+2 mM DMTU. SOD under NaCl was the highest among the treatments (Fig. 5).

The highest activity of POD was found in root after 6 days under NaCl and then followed by those under 100 mM NaCl+10 mM H<sub>2</sub>O<sub>2</sub> and 100 mM NaCl+2 mM DMTU. Two days later, POD activities significantly drop under all of treatments. However, POD under NaCl was still significantly higher than the others (Fig. 6).

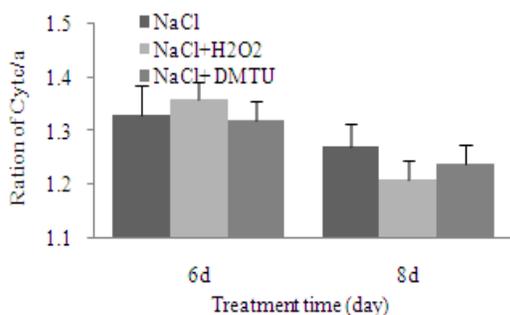


Fig. 4: Mitochondrial Cyt c/a in root of *Dianthus chinensis* L. under treatment

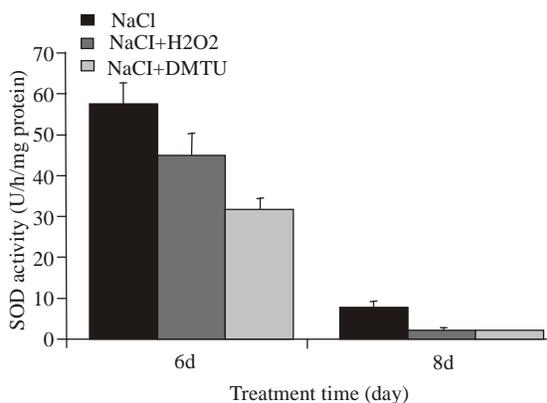


Fig. 5: SOD activity in root of *Dianthus chinensis* L. under treatment

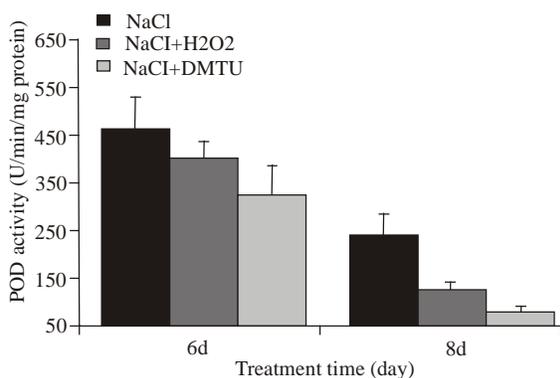


Fig. 6: POD activity in root of *Dianthus chinensis* L. under treatment

The above results showed that activity of SOD and POD was inhibited by NaCl with time. However, they would be heavily damaged if H<sub>2</sub>O<sub>2</sub> level was changed under NaCl in salt-tolerant plant.

### DISCUSSION

Reactive oxygen species universally forms in growth and developmental processes. However, when plant are exposed to biotic or abiotic stresses, including salinity, an enhanced production of ROS can cause

membrane peroxidation, protein denaturation and DNA damage (Savouré *et al.*, 1999; Fidalgo *et al.*, 2004). In our study H<sub>2</sub>O<sub>2</sub> play an important role under salt stress in root growth of *Dianthus chinensis* L. Changing its level under NaCl further inhibited the root growth. The result of Zhang *et al.* (2010) also showed that Cd inhibits root elongation by accumulating hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in root tips of Arabidopsis (2010).

Mitochondrial Permeability Transition (MPT) is mainly based on the of level Mitochondrial Permeability Transition Pore (MPTP). High level of MPTP means decrease of mitochondrial membrane potential and initiation of cell death (Li and Wu, 2002). Mitochondrial membrane absorbance at 540 nm can reflect the level of MPTP. High value meant high level of integrity on mitochondrial membrane. In our study mitochondrial membrane absorbance at 540 nm obviously increased and the value of mitochondrial membrane potential kept stable under NaCl with time.

The ration of Cyt c/a revealed the amount of Cyt c on membrane of mitochondrial (Jin *et al.*, 2002). Contrary to the result of high absorbance at 540 nm and stable mitochondrial membrane potential, decreasing in ration of Cyt c/a suggested that more Cyt c released in mitochondria under NaCl with time. Over-opening of MPTP and decrease of mitochondrial membrane potential led Cyt c to release which induced programmed cell death with H<sub>2</sub>O<sub>2</sub> treatment (Ma and Yang, 2006). In our study the reaction of MPTP and mitochondrial membrane potential was not consistent with ration of Cyt c/a under NaCl. Meanwhile, H<sub>2</sub>O<sub>2</sub> change under NaCl sharply lowered the mitochondrial membrane absorbance at 540 nm and the ration of Cyt c/a. It suggested that mitochondrial membrane in root of salt-resistant plant, *Dianthus chinensis* L. has the ability to restore itself under salt stress with time, but such ability seemed be weakened if the level of H<sub>2</sub>O<sub>2</sub> changed.

NaCl inhibited the activities of SOD and POD with time. However, Changing H<sub>2</sub>O<sub>2</sub> level under NaCl enhanced the inhibition, which might influenced the root growth in *Dianthus chinensis* L. To prevent accumulation of ROS, plant will induce defence system to balance it (Fidalgo *et al.*, 2004). *Dianthus chinensis* L. seedling can survive under salt stress which suggested that it might be some kind of defense system involved in protecting stability of mitochondrial membrane under salt stress via H<sub>2</sub>O<sub>2</sub> level in *Dianthus chinensis* L. Hence, changing H<sub>2</sub>O<sub>2</sub> level would lead to lethal damage in root of *Dianthus chinensis* L. under NaCl. So mechanism of salt-tolerance should be conducted further in *Dianthus chinensis* L.

### CONCLUSION

H<sub>2</sub>O<sub>2</sub> is an important signal under salt stress in *Dianthus chinensis* L. Changing its level under NaCl would negatively influence the root growth as well as damage mitochondrial membrane and lower activity of SOD and POD in root of *Dianthus chinensis* L.

## ACKNOWLEDGMENT

This research was supported financially by National Natural Science Foundation of China (31060264, 31260486), China Scholarship Council and Cooperation Project between Inner Mongolia Agricultural University and China Agricultural University, respectively.

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