

## Short Communication

# Cold stratification, but not stratification in salinity, enhances seedling growth of wheat under salt treatment

Lei Wang<sup>1</sup>, Hong-Ling Wang<sup>2</sup>, Chuan-Hua Yin<sup>1</sup> and Chang-Yan Tian<sup>1\*</sup>

<sup>1</sup>State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China.

<sup>2</sup>Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, 830011, China.

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**Cold stratification was reported to release seed dormancy and enhance plant tolerance to salt stress. Experiments were conducted to test the hypothesis that cold stratification and cold stratification in salinity could enhance seedling growth of wheat under salt treatment. The results clearly demonstrated that cold stratification significantly improved emergence and shoot length of wheat seedlings under salt stress as compared to non-soaked control and distilled water soaked pre-treatment. However, cold stratification in salinity did not have a positive effect. Cold stratification, but not stratification in salinity, seems to be an effective technology for improving wheat growth in saline soil.**

**Key words:** Cold stratification, salt stress, seedling emergence, wheat.

## INTRODUCTION

Salt stress is one of the major growth-limiting factors in irrigated agriculture in north China. About 8% of arable land is affected by salinity in China (Yang and Bi, 1996). Soil salinity adversely affects seedling emergence, crop growth and agricultural production. Among different methods used to cope with salinity, seed pre-sowing treatment (seed priming) is an easy and cheap technique (Iqbal and Ashraf, 2006). Many seed pre-sowing treatments have been used to increase salt tolerance of wheat. These pre-treatment techniques include salt solution priming (NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O, Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, KNO<sub>3</sub>) (Chaudhuri and Wiebe, 1968), hydropriming (water soaked pre-treatment) (Chaudhuri and Wiebe, 1968), biopriming (*Trichoderma harzianum*) (Rawat et al., 2011), vitamin and hormonal priming (abscisic acid, ascorbic acid, benzylaminopurine, indoleacetic acid, indolebutyric acid, kinetin, salicylic acid, sodium salicylate, thiamin and tryptophane) (Al-Hakimi and Hamada, 2001; Afzal, 2006; Iqbal and Ashraf, 2006, 2007; Iqbal et

al., 2006; Khan et al., 2011) and pre-sowing chilling (cold stratification) (Iqbal and Ashraf, 2010).

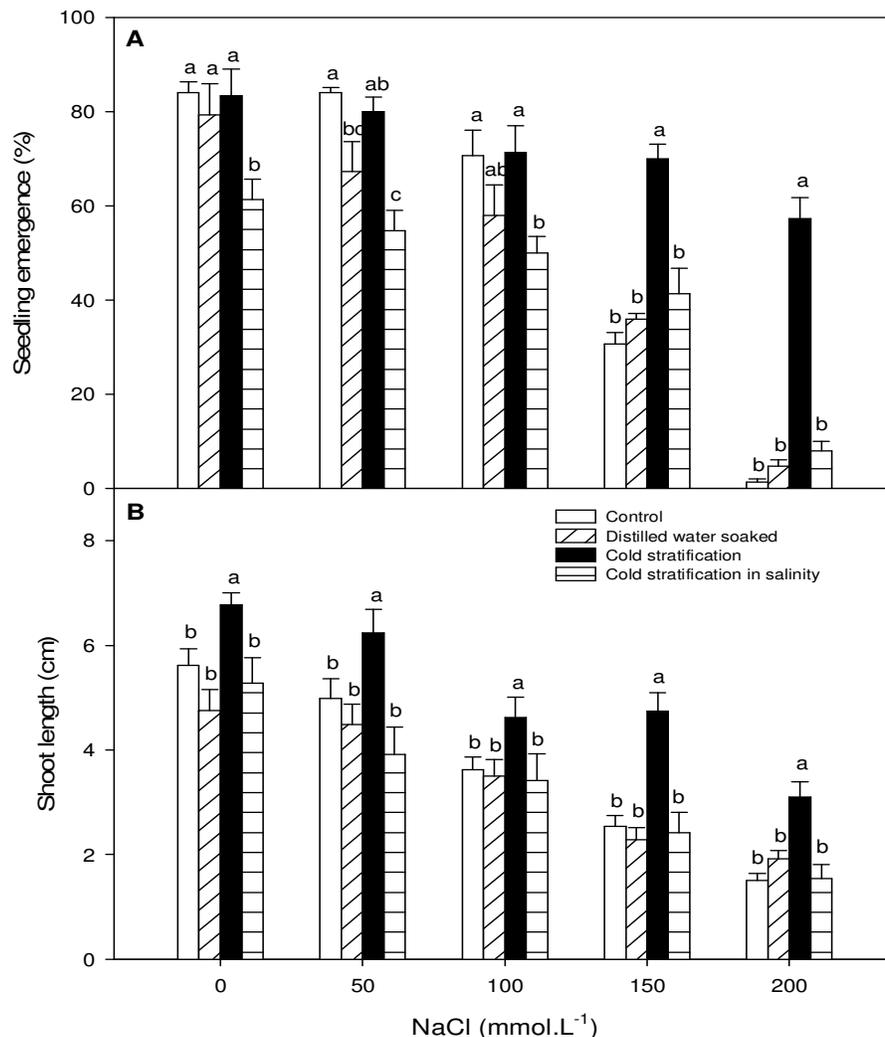
Cold stratification has been applied to break seed dormancy, increase the germination percentage and rate, and enhance growth and yield in a range of plant species (including crops) (Singh and Banerji, 1983; Bungard et al., 1997; Olmez et al., 2009; Olmez, 2011; Okay et al., 2011). Several research studies have been done on the use of cold stratification as priming method applied in the laboratory and on a farm scale to overcome salinity problem (Sharma and Kumar, 1999; Qu et al., 2008; Wang et al., 2008; Iqbal and Ashraf, 2010). However, little research has been done on the cold stratification in salinity pre-treatment (Redondo-Gómez et al., 2011).

The aim of this study was to explore the role of cold stratification pre-treatment and particularly cold stratification in salinity in improving emergence and growth in wheat seedlings under salt treatment.

## MATERIALS AND METHODS

Caryopses of the spring wheat cultivar Xindong No. 26 used in all treatments were healthy and obtained from the same batch. Fifty caryopses with different pre-treatments were placed on two layers

\*Corresponding author. E-mail: [halophyte@gmail.com](mailto:halophyte@gmail.com), [tianchy@ms.xjb.ac.cn](mailto:tianchy@ms.xjb.ac.cn). Tel: +86-991-7885301. Fax: +86-991-7885320.



**Figure 1.** Effects of different pre-treatments (control, distilled water soaked, cold stratification and cold stratification in salinity) on seedling emergence percentages (A) and shoot length (B) of wheat at 25°C in 12 h light : 12 h dark photoperiod. Different lower-case letters at each NaCl concentration indicate significant differences ( $P < 0.05$ ) between pre-treatments (Bonferroni test). Control: non-soaked; distilled water soaked: primed in distilled water at room temperature (18 to 24°C) for 8 h; cold stratification: caryopses were placed on moist (distilled water) Whatman No.1 filter paper in 5-cm-diameter Petri dishes and chilled at 1°C for 4 weeks; cold stratification in salinity: caryopses were placed on moist (80 mmol L<sup>-1</sup> NaCl) filter paper and chilled at 1°C for 4 weeks.

of Whatman No. 1 filter paper moistened with 9 ml of distilled water or NaCl solution (50, 100, 150 and 200 mmol L<sup>-1</sup>) in 5-cm-diameter Petri dishes; sealed with parafilm and placed in a light incubator at 25°C with three replicates for 5 days. Caryopsis was considered to have emerged when the shoot grew 1.5 cm long. Twenty seedlings of each treatment were randomly selected to measure shoot length at the end of incubation.

## RESULTS AND DISCUSSION

A two way ANOVA showed significant effects for salinity

and pre-treatments on both seedling emergence percentages and shoot length of spring wheat cultivar Xindong No. 26 ( $P < 0.001$ ).

Seedling emergence percentages decreased with the increase in salinity concentrations (Figure 1A). Distilled water soaked pre-treatment showed little effect on seedling emergence. Cold stratification in salinity significantly inhibited seedling emergence at the lower salinities (0 to 100 mmol L<sup>-1</sup> NaCl). Cold stratification improved seedling emergence in higher salinities (150 to 200 mmol L<sup>-1</sup> NaCl) (Figure 1A).

Shoot length decreased with the increase in salinity concentrations (Figure 1B). Shoot length after distilled water soaked pre-treatment and cold stratification in salinity was similar to un-soaked control. However, the shoot length after cold stratification was the longest in each salinity concentration (Figure 1B).

In the present study, salinity caused significant decrease in emergence percentages and growth of wheat seedlings. Caryopses primed with cold stratification proved to be effective in inducing salt tolerance at seedling emergence stage in wheat. These results are in consistent with those of Iqbal and Ashraf (2010), who observed that germination percentage and rate, shoot dry mass of seedlings and grain yield decreased with increasing salinity, while caryopses treated with cold stratification (pre-sowing chilling) decreased the negative effect of salinity. The beneficial effects of cold stratification could be attributed to ionic homeostasis and hormonal balance (Sharma and Kumar, 1999; Iqbal and Ashraf, 2007, 2010). Caryopses primed with cold stratification in salinity proved to be non-effective. Though cold stratification or salt solution pre-treatment alone is an effective method to improve salt tolerance of wheat caryopses, the combination of these two methods fail to improve seedling emergence and growth. These results are not completely in agreement with those of Redondo-Gómez et al. (2011), who reported that negative effect of cold stratification on salinity was restricted to the intermediate saline concentration. The reason for inducing salt tolerance by salt pretreatment has been discussed in detail (Chaudhuri and Wiebe, 1968; Tajdoost et al., 2007). However, there is no proper explanation for negative effects of cold stratification in salinity. A possible explanation of this phenomenon is that toxicity of NaCl counteracts the positive effects of cold stratification.

Our experiments indicated that cold stratification increased the ability of wheat to grow successfully under saline conditions, whereas cold stratification in salinity was not effective in inducing salt tolerance. Cold stratification for several weeks might be used to induce resistance to salt stress of wheat. In addition, this pre-treatment is simple, and does not need expensive chemicals and special equipments.

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## REFERENCES

- Afzal I, Basra SMA, Farooq M, Nawaz A (2006). Alleviation of salinity stress in spring wheat by hormonal priming with ABA, salicylic acid and ascorbic acid. *Int. J. Agric. Biol.* 8: 23-28.
- Al-Hakimi AMA, Hamada AM (2001). Counteraction of salinity stress on wheat plants by grain soaking in ascorbic Acid, thiamin or sodium salicylate. *Biol. Plant.* 44: 253-261.
- Bungard RA, Mcneil D, Morton JD (1997). Effects of chilling, light and nitrogen-containing compounds on germination, rate of germination and seed imbibition of *Clematis vitalba* L. *Ann. Bot.* 79: 643-650.
- Chaudhuri II, Wiebe HH (1968). Influence of calcium pretreatment on wheat germination on saline media. *Plant Soil.* 28: 208-216.
- Iqbal M, Ashraf M (2006). Wheat seed priming in relation to salt tolerance: growth, yield and levels of free salicylic acid and polyamines. *Ann. Bot. Fenn.* 43: 250-259.
- Iqbal M, Ashraf M, Jamil A (2006). Seed enhancement with cytokinins: changes in growth and grain yield in salt stressed wheat plants. *Plant Growth Regul.* 50: 29-39.
- Iqbal M, Ashraf M (2007). Seed treatment with auxins modulates growth and ion partitioning in salt-stressed wheat plants. *J. Integr. Plant Biol.* 49: 1003-1015.
- Iqbal M, Ashraf M (2010). Changes in hormonal balance: a possible mechanism of pre-sowing chilling-induced salt tolerance in spring wheat. *J. Agron. Crop Sci.* 196: 440-454.
- Khan MB, Gurchani MA, Hussain M, Freed S, Mahmood K (2011). Wheat seed enhancement by vitamin and hormonal priming. *Pak. J. Bot.* 43: 1495-1499.
- Okay Y, Günöz A, Khawar KM (2011). Effects of cold stratification pretreatment and pH level on germination of *Centaurea tchihatcheffii* Fisch. et Mey. seeds. *Afr. J. Biotechnol.* 10: 1545-1549.
- Olmez Z (2011). Effects of cold stratification and H<sub>2</sub>SO<sub>4</sub> on seed germination of sea buckthorn (*Hippophae rhamnoides* L.). *Afr. J. Biotechnol.* 10: 4586-4590.
- Olmez Z, Gokturk A, Karasah B, Yilmaz H (2009). Effects of cold stratification and sulphuric acid pre-treatments on germination of three provenances of smoke-tree (*Cotinus coggygia* Scop.) seeds in greenhouse and laboratory conditions. *Afr. J. Biotechnol.* 8: 4964-4968.
- Qu XX, Baskin JM, Wang L, Huang ZY (2008). Effects of cold stratification, temperature, light and salinity on seed germination and radicle growth of the desert halophyte shrub, *Kalidium caspicum* (Chenopodiaceae). *Plant Growth Regul.* 54: 241-248.
- Rawat L, Singh Y, Shukla N, Kumar J (2011). Alleviation of the adverse effects of salinity stress in wheat (*Triticum aestivum* L.) by seed biopriming with salinity tolerant isolates of *Trichoderma harzianum*. *Plant soil*, DOI: 10.1007/s11104-011-0858-z.
- Redondo-Gómez S, Andrades-Moreno L, Parra R, Mateos-Naranjo E, Sánchez-Lafuente (2011). Factors influencing seed germination of *Cyperus capitatus*, inhabiting the moving sand dunes in southern Europe. *J. Arid. Environ.* 75: 309-312.
- Sharma PC, Kumar P (1999). Alleviation of salinity stress during germination in *Brassica juncea* by pre-sowing chilling treatments to seeds. *Biol. Plant.* 42: 451-455.
- Singh A, Banerji D (1983). Enhancement in growth and yield of wheat (*Triticum aestivum* L.) by pre-sowing chill treatment to grains. *Ann. Bot.* 51: 585-589.
- Tajdoost S, Farboodnia T, Heidari R (2007). Salt pretreatment enhance salt tolerance in *Zea mays* L. seedlings. *Pak. J. Biol. Sci.* 10: 2086-2090.
- Wang L, Huang ZY, Baskin CC, Baskin JM, Dong M (2008). Germination of dimorphic seeds of the desert annual halophyte *Suaeda aralocaspica* (Chenopodiaceae), a C<sub>4</sub> plant without Kranz anatomy. *Ann. Bot.* 102: 757-769.
- Yang RZ, Bi YY (1996). Transformation of salinized farmlands in China. *J. Arid Land Resour. Environ.* 10: 22-30 (in Chinese with English abstract).