Original: *Cotton Science*, 2006, 18(6): 342~346

Effects of the Salinity Priming on the NaCl Tolerance of Transgenic Insect Resistant Cotton (*Gossypium hirsutum* L.)

LIN Jun, SUN Yu-Jiang, LÜ You-Jun, ZHU Shui-Jin*

(*Agronomy Department, College of Biotechnology and Agriculture, Zhejiang University, Hangzhou, Zhejiang* 310029, *China*)

Abstract: The effect of seed priming with salts on germination and emergence under NaCl tolerance was studied with two commercial transgenic insect resistant cotton cultivars, CCRI 41 and Z-905, as the materials, and their genetic background cultivar, CCRI 23, as check. The results indicated that it was inhibitive for seed germination and field emergence of the upland cotton when treated by 0.5% NaCl, especially for the transgenic insect resistant cotton cultivars which the germination percentage and field emergence stressed by 0.5% NaCl was much lower than that of their genetic background check. However, priming treatment with a certain concentration salts could alleviate the harmful of the NaCl stress significantly. After the treatment of seed priming, the germination percentage and seedling emergence on the condition of 0.5% NaCl stress increased significantly, and the seed tolerance to the salt stress enhanced greatly. There were some differences in the effect of the seed priming treatments on the seed germination and field emergence, stressed by NaCl, between the transgenic insect resistant cotton and their genetic background one, and the formers, with weak tolerance against NaCl stress, had a better effect than that of their genetic background one without exogenous insect resistant genes. There were some differences among the priming agents, of which Na_2SO_4 was the best. In addition, there existed interactions between the concentration of the priming agents and the process time. The process time should be longer for the priming agents with lower concentration than that with higher concentration.

Key words: salinity stress; transgenic insect resistant cotton; seed priming; seed germination

Soil Salinization is one of the main obstacles in agricultural production. There are about 2700 $\times 10^4$ hm^2 saline lands in China and extended in a speed of $2.0\times10^3 \sim 4.0 \times 10^3$ hm^2 annually. Soil salinization has led to a huge economic loss in agricultural production in China[1]. Cotton (*Gossypium hirsutum* L.) is one of the salt-tolerant plants, sometimes it is called a pioneer crop in the saline land[2], but it is still harmful to cotton production with high salinity[3]. The capability of cotton tolerant to saline varies with growth stages. During the cotton growth season, it is the weakest stage for the salt tolerant ability in seed germination and emergence, as well as the seedling period[4]. It is difficult for seedling emergence when the salt content in the soil is higher than 0.2%. If it is higher than 0.4%, cotton seedling cannot emerge, and if the salt content in the soil is higher than 0.6%, the cottonseeds cannot germinate anymore [5-7].

With the fast extending of the transgenic insect resistant cotton in major cotton producing regions of China, the problem of the saline tolerance in transgenic insect resistant cotton has been concerned intensively, and how to improve the salt tolerant capacity of transgenic insect resistant cotton has become an important subject for the cotton production in China. The most e-

Received: 2006-02-20
*: Corresponding author, E-mail: shjzhu@zju.edu.cn

Sponsors: National 973 Program (No: 2004CB117305), and the National Natural Science Foundation of China (No: 30471108, 30671325).

conomic and effective method is to breed and utilize the salinity tolerant germplasm and cultivars, using the conventional breeding method, as well as the modern biotechnological methods. However, due to the lack of high salt tolerant germplasm resources and other reasons, there are few practical salt tolerant cultivars have been derived, and salt tolerant cotton breeding can not meet the demand of the development of cotton production, although considerable progress has been made in cotton breeding for salt tolerant cultivars [8]. Therefore, agronomic cultivation is still a major method to improve the salt tolerant capability of plants up to now. Seed priming which was firstly proposed by Heydecker et al[15] is a technique to control hydration and drying afterward for the seeds. Seeds treated with priming agents can not only increase the seed germination percentage which lead to the uniformity of the seeding especially under unfavorable environmental conditions, but also enhance the capability of seedling adapted to the saline stress[9-11]. However, there were few reports on the seed priming which affected the salt-tolerant ability of transgenic insect resistant cotton. In this paper, the priming treatments on transgenic insect resistant cotton for saline stress response were carried out under controlled conditions in order to determine an effective method to improve the capability of transgenic cotton on saline stress.

1 Material and Methods

1.1 Plant materials

There were three entries named CCRI 41, Z-905, and CCRI 23. Among them, CCRI 41 was the transgenic insect-resistant cotton cultivar with two genes combination (*Bt*+*CpTi*), which was transformed by the Pollen-Tube-Pathway (PTP) method, provided by Cotton Research Institute of Chinese Academy of Agricultural Sciences. Z-905 was derived by backcross of the transgenic insect resistant cotton (Bt), provided by Zhejiang University. CCRI 23 was a normal commercial cultivar derived from a mixed crossing and selection method from a complex crossing combination (5658×5254×4067×Jimian 8), provided by Cotton Research Institute of Chinese Academy of Agricultural Sciences. It was the genetic background parent of CCRI 41 and Z-905, used as check in this experiment.

1.2 Methods

1.2.1 *Treatment of seed priming.* Delinted seeds were treated as follows: (1) treatments with different concentrations of salt priming agents ($CaCl_2$, K_2SO_4 and Na_2SO_4), were 0.1%, 0.5% and 1.0%, respectively; (2) treatment in different priming time (6 hours, 12 hours and 24 hours). After priming treatment, the seeds were dried at room temperature.

1.2.2 *Seed germination test.* The seeds after priming were put into the sand germination utensils containing 0.5% NaCl, then placed in a constant temperature germination incubator (30℃) for germination. The germination experiment was carried out using orthogonal experimental design with three repetitions, and 50 seeds for each treatment. Using the non-NaCl treatment as check, and the germination energy was statistical analysis after three days of germination and the data of germination percentage were collected after 12 days of germination.

1.2.3 *Emergence experiment in field.* Primed seeds were sowed in an artificial saline field on the experimental farm of Zhejiang University, Hangzhou, China, on May 10, 2005. Before planting, the soil salinity was analyzed and adjusted to 0.5% NaCl with edible salt uniformly, and the check field was less than 0.02% in salts. The experiment was carried by random block design with three replications, and 200 seeds were sowed each block. The emergence percentage and field emergence were investigated after 5 days of planting.

1.2.4 *Data analysis.* Using DPS statistics software for statistical analysis, which was provided by Tang Qi-yi, Zhejiang University.

2 Results and Analysis

2.1 Effect of NaCl on the germination and emergence of the transgenic insect resistant cotton

Although cotton is one of the most salt-tolerant crops, inhibiting effect of the 0.5% NaCl treatment on the seed germination was significant(Table 1). Compared with the CK, germination on the water, the germination energy after NaCl treatment reduced by 20.3% (CCRI 23) ~ 33.8% (CCRI 41), and the germination percentage reduced from 17.6% of CCRI 23 to 31.2% of CCRI 41. The difference between the treatment and CK was significant at 1% level. The NaCl treatment had a significant effect in field experiment as well. Compared with their related cultivar in CK, the emergence percentage of CCRI 41, Z-905 and CCRI 23 decreased by 23.5%, 38.3% and 39.8 %, respectively, in 0.5% NaCl treatment field, and all the differences were significantly at 1% level. There were significant differences in salt tolerance among the cultivars, and the two transgenic insect resistant cultivars were less tolerant than their genetic background one (CCRI 23). Because the entry cultivars had the same genetic background, the difference of salt-tolerance among them might relate to exogenous insect resistant gene, although it need more experiment evidences.

Table 1 Effect of 0.5% NaCl on germination and emergence of cotton seeds

	GE/%			GP/%			FE/%		
Cultivars	CK	Treated	±CK	CK	Treated	±CK	CK	Treated	±CK
CCRI 41	89.3	55.5	-33.8**	96.7	65.5	-31.2**	95.3	45.5	-49.8**
Z-905	87.3	60.3	-27.0**	95.5	70.0	-25.5**	93.0	54.7	-38.3**
CCRI 23	86.0	65.7	-20.3**	93.3	75.7	-17.6**	92.5	59.0	-33.5**

Note: * and ** as the significant at 5% and 1%, respectively; GE as germination energy; GP as germination percentage; and FE as field emergence; the same as below.

2.2 Effect of salinity priming on the salt tolerance of the transgenic insect resistant cotton

Cotton seeds after the treatment of three different priming agents were used in germination test and field experiment under the tress of 0.5% NaCl, and the results were shown in Table 2 and Table 3.

Table 2 Effect of salinity priming on the germination of the transgenic cotton stressed by NaCl

	CK		$CaCl_2$		K_2SO_4		Na_2SO_4	
Cultivars	GE/%	GP/%	GE/%	GP/%	GE/%	GP/%	GE/%	GP/%
CCRI 41	55.5	65.5	77.7	83.7	80.7	90.0	75.5	81.7
Z905	60.3	70.0	76.5	84.5	80.0	89.5	74.3	80.3
CCRI 23	65.7	75.7	72.3	84.0	78.7	88.3	71.7	77.7
Average	60.5	70.4	75.5	84.1	79.8	89.3	73.8	79.9

In general, there were remarked catabatic effects of the salinity treatment of tree priming agents on the stress of NaCl, the results and trend were similar among the three cultivars. For the priming agent of $CaCl_2$, the average germination energy and germination percentage under 0.5% NaCl stress were 75.5% and 84.1%, respectively, which were higher than without the priming treatment by 15.0% and 13.7%, respectively. For the priming agent of K_2SO_4, the average germination energy and germination percentage under the 0.5% NaCl stress were 78.8% and 89.3%, respectively, which were 15.0% and 13.7% higher than that of CK, respectively. For the Na_2SO_4, the results were 73.8% for the germination energy and 79.9% for germination percentage, which were higher than those of CK by 13.3% and 9.5%, respectively. According to the statistical analysis, all the three priming agents, except the average germination percentage of Na_2SO_4 priming treatment, were effective significantly at 1% level in increase of the seed germination. The results of field emergence experiment showed that the average seedling emergence rate under the 0.5% NaCl stress were 74.9%, 84.6% and 75.3% for the priming treatments by $CaCl_2$, Na_2SO_4 and Na_2SO_4, which were significantly higher than that of their untreated ones by 21.9%, 31.5% and 22.3%, respectively. The results showed that there was a marked improvement of salinity tolerance for the transgenic insect resistant cotton through salt priming.

Table 3 Effect of salinity priming on the seed field emergence of the transgenic cotton stressed by NaCl (field emergence rate)

Cultivars	$CaCl_2$			K_2SO_4			Na_2SO_4		
	CK	Treated	Difference	CK	Treated	Difference	CK	Treated	Difference
CCRI 41	45.5	74.3	28.8**	45.5	80.6	35.1**	45.5	73.4	27.9**
Z-905	54.7	76.4	21.7**	54.7	88.1	33.4**	54.7	77.1	22.4**
CCRI 23	59.0	74.1	15.1*	59.0	85.1	26.1**	59.0	75.5	16.5*
Average	53.1	74.9	21.9**	53.1	84.6	31.5**	53.1	75.3	22.3**

Among the different types of upland cotton cultivars, CCRI 23, the non-transgenic cotton cultivar as the genetic background one of two transgenic insect resistant cultivars, was most tolerant to NaCl stress. Its germination energy and germination percentage were 65.7% and 75.7%, respectively, 10.2% and 10.2% higher than CCRI 41, the double genes isogenic line, and 5.4% and 5.7% higher than Z-905, the single gene isogenic line, respectively, although the difference between CCRI 23 and Z-905 was insignificant at 5% level. However, after the treatment of seed priming by different chemicals, the germination energy and germination percentage of transgenic insect resistant cotton cultivars increased significantly. Although this increase took place in non-transgenic cotton (CCRI 23), the increase extent was much less than that of transgenic ones, shown in Table 3 and Table 4. Thus it can be seen that the result of seed priming on the improvement of salt-tolerant ability for the transgenic insect-resistant cotton was much better.

2.3 Effect of priming agents and method on the salt tolerance of transgenic insect-resistant cotton

According to the effects of three salt priming agents, it can be found that all the three priming agents had improved germination energy, germination percentage and field emergence under the stress of 0.5% NaCl, and Na_2SO_4 was the best one. The results of statistical analysis showed that there were insignificantly differences in germination energy and germination percentage among the three priming agents, and emergence in the field after the treatment with Na_2SO_4 priming agent was significantly better than the others.

For the concentration and the treatment time of priming agents, all the three concentrations of the priming agents in this experiment could improve the seed germination energy, germination percentage, and emergence in the field, and those of the treated seeds were higher than that of the non-treated ones significantly. However, there were some differences among the treatment time and the treatment concentration of the priming agents on the salinity tolerance of cottonseeds. In general, the effective of lower concentration (0.1%) of the priming agents treated for relative longer treatment (24 hours) was the best one, followed by the lower concentration with medium treatment time (12 hours). Regarding medium concentration (0.5%) of the priming agents, the best treatment time was 12 hours, followed by the long treatment (24 hours), and for the high concentration of the priming agents, short treatment (6 hours) was the best. There were some interactions among the different priming agents, different concentration and different treatment time, and the trend among the three different salt priming agents was same, although those interactions were not significant at 5% level.

3 Conclusions and Discussion

Cotton is one of the most tolerant economic crops to the salinity, but there were still some serious effects of the salt tress on the cotton production[9-11]. Our experiment showed that the foreign insect resistant genes that transformed to the upland cotton led to the decrease of the salt tolerance in cotton. The difference of the salt tolerance between Z-905 transformed the *Bt* insect resistant gene and its background cultivar was not significant, but that between CCRI 41, transformed *Bt* + *CpTi* insect resistant genes, and CK was significant at 5% level. As the two transgenic cultivars were developed from the same genetic background cultivar CCRI 23, their genetic background was almost same as the isogenic lines each other. So theoretically, their salt-tolerant differences among the three tested cultivars were related to the foreign insect resistant genes. However, the reasons of the foreign insect resistant genes that lead to salt-tolerant decline of the transgenic insect resistant cotton need to be further studied.

There were many reports about the effect of seeds priming on the salt-tolerance of plants, and most of them found that seeds priming could increase the salt-tolerance[12-14]. Our experiment indicated that the salt priming could effectively alleviate the effect of salt stress on germination and field emergence of cotton seed by increasing seed germination percentage and field emergence under salt stress conditions greatly. Seed germination and field emergence on the salt stress condition were better for the three different priming agents with different treatment concentrations and time than those without priming. For the selection of the priming agents, all the three priming agents designed in this experiment were effective, and Na_2SO_4 was the best one. However, it should be noted that salt-tolerance improvement by salt priming in transgenic insect resistant cotton was greater than that of their genetic background parent in this experiment. Further study is need to determine if it is a universal effect.

References:

[1] Jiangsu Agronomic Academy. Cotton tecnology in Jiangsu[M]. Nanjing: Jiangsu Science and Technology Press, 1992.

[2] Cotton Research Institute of Chinese Academy of Agricultural Sciences. Cotton cultivation in China[M]. Beijing: Chinese Agricultural Press, 1983.

[3] LIU Guo-qiang, Lu Li-ming, Liu Jin-ding. The study on identification of salt tolerance of cotton germplasm resources [J]. Crop Germplasm Resources, 1993 (2): 21-22.

[4] SUN Xiao-fang, Liu You-liang, Chen Qin. Advance in studies of salinity tolerance in cotton [J]. Cotton Science, 1998, 10(30): 18-24.

[5] JIA Yu-zhen, Zhu Xi-yue, Tang Yu-di, et al. Studies on the index of salt tolerance in cotton emergence and seedling stage [J]. Journal of Henan Agricultural University, 1987, 21(1): 30-41.

[6] YE Wu-wei, Liu Jin-ding. The effect of NaCl and edible salt on cotton seeds germination [J]. China Cotton, 1994, 21(3): 14-15.

[7] ZHOU Tao-hua. Effects of NaCl stress on cotton seeds germination and seedling growth[J]. China Cotton, 1995, 22(4): 11-12.

[8] LI Fu-guang, Li You-lan, Li Feng-lian, et al. The effect of salinity stress on somatic cell of cotton(*G. hirsutum* L.) [J]. Cotton Science, 1994, 6(1): 37-40.

[9] WANG Pei-zheng, Yan Shan, Gao Xiang. Genetical correlation and principal component analysis on quantity character in upland cotton with the drought/salt resistance and dwarf characters [J]. Cotton Science, 2002, 14(3): 154-157.

[10]XIN Cheng-song, Dong He-zhong, Tang Wei, et al. Advance in studies of salt damage and the physiological and molecular mechanism of salt tolerance in cotton [J]. Cotton Science, 2005, 17(5): 309-313.

[11] LÜ You-jun, Ye Wu-wei, Zhu Shui-jin. Effects of additional salinity treatment on germination of upland cotton stressed by NaCl [J]. Cotton Science, 2005, 17(4): 256.

[12]CAYUELA E, Perez AF, Caro M, et al. Priming of seeds with NaCl induces physiological changes in tomato plants grown under salt stress[J]. *Physiologia Plantarum*, 1996, 96: 231-236.

[13]SIVRITEPE H O, Eris A, Sivritepe N. The effect of NaCl priming on salt tolerance in melon seedlings[J]. *Acta Horticulturae*, 1999, 492: 77-84.

[14] AMZALLAG G N, Lerner H R, Poljakoff M A. Induction of increased salt tolerance in Sorghum bicolor by NaCl pretreatment[J]. Journal of Experimental Botany, 1990, 41: 29-34.