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Effects of NaCl Stress on Cotton Tissue Culture and Plant Regeneration

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Abstract

Hypocotyl and callus of cotton (*Gossypium hirsutum* L. cv. Coker 201) were cultured on MS basal medium supplemented with various concentrations of NaCl in order to study the effects of salt stress on callus induction, growth, proliferation, embryogenesis and plant regeneration of cotton. Experiment results indicated that along with the increase of NaCl level, the percentage of induction, the growth rate, the survival rate, the moisture content, embryogenesis rate of cotton callus and the plant regeneration rate and normal seedling rate of cotton tissue culture decreased.

Introduction

Cotton is an important economic and fiber crop. To study the effects of salt stress on cotton have profound significance for breeding of salt resistant varieties. The study of the effect of salt stress on cotton growth and development with the methods of irrigation cotton field using sea water or salt water or using salt pond have been reported. But these methods have many abuses, such as longer study cycle, higher study cost, more work time and unsteady because that the results were influenced easily by around environment. So, we must investigate new methods to evaluate exactly the effect of salt stress on cotton growth and development.

With the development of modern biotechnology, people are attempting to study the influence of salt stress on plant growth and development with plant tissue culture technique and certain progress (Zhou *et al*., 1989) have been obtained.

Recently, great progress has been made in cotton tissue culture (Zhang and Feng, 1992a, b) and the procedure of cotton somatic embryogenesis and plant regeneration has been establishment (Trolinder and Goodin, 1987; Voo *et al*., 1991; Zhang and Li, 1992). These would lay a foundation for the use of plant tissue and cell culture technique on cotton breeding, production, preservation and appraisal of germplasm (Zhang and Feng, 1992b). The study of the effects of salt stress on cotton tissue culture was the direct application on the study of cotton salt resistance. Only by clearing the effects of salt stress on the induction, growth, proliferation, differentiation of callus and plant regeneration of cotton tissue culture, can we go deep into studying the screening of salt-tolerant variation and rapidly appraisal *in vitro* of cotton. So some articles about the effect of salt stress on cotton tissue culture have been reported (Li *et al*., 1992; Wang *et al*., 1991), but only the effect of salt stress on the induction of cotton callus were discussed. The other questions, such as the effect of salt stress on the proliferation, differentiation, embryogenesis of callus and plant recovery have not been reported. The purpose of this experiment is to look for a method of evaluating cotton salt resistance rapidly and exactly.

Materials and Methods

Gossypium hirsutum L. cv. Coker 201 was chosen for this study because it could be induced somatic embryos and plant regeneration easily. Seeds that were delinted with 95 percent H_2SO_4, were steriled in 0.1 percent $HgCl_2$ for 10 minutes, followed by three rinses in sterile distilled water and than soaked in sterile water for 3 to 7 hours. Softened seed coats were removed and the kernels were germinated for 3 days on MS basal medium at 282 under darkness. Transversal hypocotyl sections ("discs") ranged from 3- to 5-mm thickness were take from the lower half of 3-day seedling and were inoculated on MS basal medium supplemented with 0, 3, 5, 10, 15, 20, 25, 30 g/L NaCl respectively to study the effects of various amount of salt stress on the induction of callus. The callus that were induced on MS medium without NaCl were proliferated by subculture every one month on MS basal medium supplemented with 0.1 mg/L KT, 0.2 mg/L IAA and 0.1 mg/L 2, 4-D, which were prepared to use in late experiments.

After 30 days, callus that were subcultured on MS medium without NaCl were transferred to the MS medium supplemented with 0, 1, 3, 5, 7, 10, 15, 20, 30, 40 g/L NaCl respectively to study the effects of salt stress on the growth and proliferation of callus, somatic embryogenesis and plant regeneration in cotton tissue culture. The growth rate of callus that explant produced was expressed by the follow methods: + + +, + +, + stand for respectively good, moderate, poor. The growth rate of callus was used to compare callus response to salt stress, it was expressed by the follow formula:

$$\text{The growth rate} = \frac{\text{Callus weight after subculture (g)}}{\text{Inoculated callus weight (g)}}$$

All experiments were carried out in a controlled environment growth room at 291, irradiance of 2000Lux or so, 14 hours light/10 hours dark photo periods.

Results and Discussion

Effect of NaCl stress on the induction of cotton callus: NaCl deferred the appearance and reduced the induction rate of

Table 1: Effects of NaCl stress on the induction of cotton callus

NaCl (g/L)	Number of explant	Date of callus	Number of callus	Callus induction	Growth rate	Colour of callus	Lethal rate of
0	50	6	50	100.0	+ + +	greyish brown	0.0
3	40	6	40	100.0	+ + +	tawny	0.0
5	30	7	28	93.3	+ +	light yellow	0.0
10	30	11	27	90.0	+	yellow	3.3
15	29	20	13	44.8	+	yellow	54.5
20	29	30	5	17.2	+	yellow	75.9
25	30	35	1	3.3	+	dark yellow	96.7
30	30	-	0	0.0	-	-	100.0

Table 2: Effects of NaCl stress on the proliferation of cotton callus

NaCl (g/L)	Number of explant survival	Number of callus	Survival rate(%)	Growth rate	Moisture content(%)	The ratio of dry and fresh weight
0	33	33	100.0	13.6	97.6	2.4
5	19	15	78.9	5.5	95.5	4.5
10	26	9	34.6	2.0	93.3	6.7
15	29	5	17.2	1.9	92.5	7.5
20	36	6	16.6	1.2	92.0	8.0
30	25	4	16.0	1.3	-	-
40	13	0	0.0	-	-	-

Table 3: Effects of NaCl stress on the differentiation and development of cotton somatic embryos

NaCl	Number of embryos	Ratio of somatic embryos (%)			Development course of embryos
		Immature embryo (I)	Mature embryo (M)	M:1	
0	228	77.5	24.5	1:3.0	cotyledonary stage
5	129	77.8	22.2	1:3.2	cotyledonary stage
10	90	85.0	15.0	1:5.7	cotyledonary stage
15	67	84.6	15.4	1:5.5	torpedo stage
20	31	87.5	12.5	1:7.0	heart stage
30	0	-	-	-	-

Table 4: Effects of NaCl stress on the germination of cotton somatic embryos and plant regeneration

NaCl (g/L)	Number of embryos	Rooted rate (%)	Shooted rate (%)	Germination regeneration	Plant seedling rate (%)	Normal seedling rate (%)	Abnormal seedling rate (%)
0	26	38.5	46.2	53.8	30.8	37.5	62.5
5	30	30.0	43.3	46.7	26.7	25.0	75.0
10	29	10.3	17.2	17.2	10.3	16.7	83.3
15	13	7.7	7.7	7.7	7.7	0.0	100.0
20	9	0.0	0.0	0.0	0.0	-	-

cotton callus (Table 1). Callus appeared on MS medium without NaCl in 6 days and the percentages of callus was 100 percent after 40 days of culture. The percentage of inducted callus decreased with the increasing of NaCl concentrations. When the NaCl level increased to 30 g/L, the explant were not able to produce callus. High NaCl levels resulted in the death of hypocotyl explant, which the semilethal concentration was 15 g/L and the lethal concentration was 30 g/L. The colour and growth rate of callus produced on MS medium with various NaCl levels were different. The colour transformed from greyish brown to deep yellow, the growth speed declined along with the increasing of NaCl levels.

Effect of NaCl stress on the proliferation of cotton callus: NaCl had a great effects on the growth and proliferation of cotton callus (Table 2). The growth was reduced with the increasing of NaCl levels, but the effects were different among various NaCl level. Low concentration (5 g/L) of NaCl affected little survival of callus, but the growth of the treatment with O g/L of NaCl is almost 2.5 times then that of the treatment with 5 g/L of NaCl; moderate concentration

(10-20 g/L) of NaCl inhabited the growth of callus and high concentration (20 g/L) of NaCl stopped the growth of callus. Callus or cell morphology differed with the various concentration of NaCl. With the increasing of NaCl level, the moisture content of callus decreased and the ratio of dry weight to fresh weight increased. Cytology observation indicated that the cells lessened and cytoplasm densened with the increasing of NaCl concentrations.

Effects of NaCl stress on the differentiation and development of cotton somatic embryos: Cotton somatic embryogenesis was inhibited by NaCl (Table 3) and a clear relation between the levels of NaCl added to the medium and somatic embryogenesis event was observed. 1 g callus that were cultured on MS medium without NaCl could produce 228 somatic embryos, but that were cultured on MS medium supplemented with 10 g/L NaCl could only produce 90 somatic embryos. When the NaCl concentration was more than 30 g/L in the MS medium, embryogenesis could not take place from the callus.

Salt stress affected not only the total number of the embryos produced, but also the developing course of somatic embryos and shus it affected the ratio of immature to mature embryos. With the increasing of NaCl concentrations the percentage of the mature embryos decreased and that the immature embryos increased The callus could not produce somatic embryos at 30 g/L NaCl. The somatic embryos that were produced on MS medium supplemented with 0-10 g/L NaCl could develop to cotyledonary stage, but we could only observe torpedo embryos on the MS medium supplemented with 15-20 g/L NaCl. On other medium with higher concentration of NaCl, we could not observed somatic embryos at any development stage.

Effects of NaCl stress on the germination of cotton somatic embryos and plant regeneration: Somatic embryos that were produced from the MS medium supplemented with various concentration NaCl stress were transferred to the germinated medium (Zhang *et al*., 1993a) without NaCl and germinated soon. Somatic embryos germination begun on the medium with either root initiation or shoot growth within 10 days.

The effects of stress caused by various NaCl concentration on germination of cotton somatic embryos and plant regeneration were various (Table 4). The percentages of embryos that developed shoots, roots and both shoots and roots were inhibited by adding NaCl to MS basal medium. There after, the percentage of germination or plant regeneration of cotton somatic embryos decreased with increasing NaCl concentrations.

Regenerative plants were also affected by NaCl that was added to MS basal medium. The change of the percentage of normal seedling with different NaCl concentrations was the main expression. Along with the increasing of NaCl level, the percentage of normal seedling decreased, the percentage of abnormal seedling increased. When the concentration of NaCl was up to 15 g/L, there were not any normal plants in regenerative plants.

On this basis, we have been studying to screen salt-tolerant variation and we have obtained salt-tolerant embryogenic cell lines and we have obtained somatic embryos and regenerative plants from these cell lines (Zhang *et al*., 1993b, 1995).

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References

Li, F., X. Li and F.L. Li, 1992. Study on property and selection of salt tolerance of cotton cell. Acta Gossypii Sinica, 4: 92-93.

Trolinder, N.L. and J.R. Goodin, 1987. Somatic embryogenesis and plant regeneration in cotton (*Gossypium hirsutum* L.). Plant Cell Rep., 6: 231-234.

Voo, K.S., C.L. Rugh and J.C. Kamalay, 1991. Indirect somatic embryogenesis and plant recovery from cotton (*Gossypium hirsutum* L.). *In Vitro* Cell. Dev. Biol. Plant, 27: 117-124.

Wang, Z., Z. Shen and D. Yi, 1991. First reports on selection of salt-tolerance of cotton. China Cotton, 18: 16-16.

Zhang, B., X. Li and F. Li, 1995. Selection of cotton NaCl-tolerant embryogenic cell lines and plant regeneration. Sci. Agric. Sinica, 28: 33-38.

Zhang, B.H. and R. Feng, 1992a. List of cotton tissue and cell culture. Plant Physiol. Commun., 28: 308-314.

Zhang, B.H. and R. Feng, 1992b. Status of research work for cotton biotechnology. Prog. Biotechnol., 12: 18-21.

Zhang, B.H. and X. Li, 1992. Somatic embryogenesis of cotton and it's production of artificial seeds. Acta Gossypii Sinica, 4: 1-8.

Zhang, B.H., X. Li, F. Li, W. Wang and F. Li, 1993a. Plant recovery from cotton somatic embryos. Acta Agric. Boreali-Occidentalis, 2: 24-28.

Zhang, B.H., X.L. Li and F.L. Li, 1993b. Selection and plant regeneration of NaCl-tolerant variant of cotton callus *in vitro*. Plant Physiol. Commun., 29: 423-425.

Zhou, R.R., Y. Yang and S. Yu, 1989. Progress of selection and plant salt-tolerance variant using plant tissue culture technology. Plant Physiol. Commun., 5: 11-16.