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CELL MUTATIONS FOR SALT TOLERANCE SCREENING IN TISSUE CULTURE[1,2]

SHIU-CHO WOO, CHING-KIT WONG and SU-WAN KO

Institute of Botany, Academia Sinica
Nankang, Taipei, Taiwan, 11529 Republic of China

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Abstract

A number of regenerated rice plants were previously derived from a cultivated variety Tainung 67 by raising its level of salt tolerance. Seeds of 13 selections were germinated and treated with 1% salt for two weeks to evaluate their tolerance to salt stress. Studies on seedling fresh weight and seedling height were used to evaluate the response of salt stress. A total of 3 out of 13 lines tested showed positive response in fresh weight and less reduction on seedling height during salt stress. Seedlings were cutbacked to 3 height levels, i. e., 25, 20 and 15 cm tall. Most of the regenerated lines showed similar response in cutback tests. The study confirmed salt tolerance developed in regenerated plants.

Key words: Salt tolerance; tissue culture; rice

Introduction

Rice is a highly staple crop in Taiwan. Most of the present cultivars have been improved for high yielding and photo-insensitivity. However, those grown along the coastal paddy yield low as compared with the inland rice. This is considered to be the effect of saline soil and salty mist from the ocean. This research thus aims to raise the level of salt tolerance of rice cultivar without drastically interrupting its major genetic structure.

Materials and Methods

A batch of rice *Oryza sativa* L. var. Tainung 67 seeds was treated with 0.025 M ethyl methanesulfonate (EMS) for 4 hours in 1981. Seeds were dehusked

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and inoculated on MS medium (Murashige and Skoog, 1962) for callus induction and plant regeneration. All media were supplemented with 0.6–1.0% sodium chloride. Plantlets regenerated were secured and grown to maturity with 0.6% salted nutrient solution. A total of 158 plants was harvested and the seeds derived from a single regenerated plant (RP) were grown onto plant lines. The well developed seeds of 12 selected plant lines were used for the second passage of screening. Approximately 15–20 good seeds from a single line were germinated, and 10 good seedlings were selected for screening. Seedlings were not treated with salt until 15-cm tall. The salt treatment was continued for two weeks and the solution changed every other day in an unheated glasshouse. Seedling fresh weight, seedling height and seedling survival were studied to investigate the eflect of salt stress.

Seedling cutbacks were applied at 3 height levels, i.e., seedlings of 25–30 cm tall cutbacked to 25 cm, those of 20–25 cm cutbacked to 20 cm, and those of 15–20 cm cutbacked to 15 cm. Survivals were rated in a walk-in growth chamber at 25±1°C. Seedlings were raised on stainless steel screen suspended over plastic plastic pots with Kimura's liquid solution (Yoshida *et al.*, 1976). The salted solution was applied 24 h later after the cuback, 4 pots per treatment and 100 seedlings per pot.

Results

Results of seedling height and seedling weight of the tested lines with salt treatment are shown in Table 1. The RPs did not gain much in these two mentioned characteristics with salt stress of 14 days. However, there were 3 lines, No. 3,7, and 13 overgrew the control. Generally, symptoms of necrosis appeared at the leaf tip 3 days after the treatment, and gradually enlarged downward. Those seedlings' leaf tips dried up slowly were considered of having certain level of tolerance. A total of 7 plant lines, No. 3,5,7,8,10,11, and 12 developed less necrosis than the controls. The plant height and fresh weight of line 3 were taller and heavier than the control however. The increased fresh weight of regenerated lines ranged from 14.7 to 54.3% wider than 37.2-49.0% of the control's. The variation in seedling-height reduction of the regenerated lines was -23 to -36% which was also larger than the controls of 0 to -19%

Results of seedlings cutbacked to 25 cm are given in Table 2. The fresh weight growth of regenerated lines ranged 17 to 45%; it was somewhat better than the control of 12.4%. For the seedling height, all seedlings have more or less carried some necrosis and dried up from the tip to the basal portion of leaves. The control seedlings were found highly sensitive to stress. Seedling height was reduced by 45.6% because of fast expanding necrosis during stress. For the regenerated lines, they showed certain degree of tolerance. The leaves of seedlings dried up rather slowly. Thus, the seedling height reduction varied from 32.4 to 43.2% which was

Table 1. *Growth of seedling fresh weight and height with 1% salt for two weeks*
Average fresh weight and height of 10 seedlings.

Line	Seedling fresh weight (g)			Seedling height (cm)		
	Dec. 1	Dec. 25	Increase (%)	Dec. 1	Dec. 25	Reduced (%)
1	2.195	2.853	30.0	19.8	18.9±6.6	− 4.5
3	2.448	3.776	54.3	23.0	21.5±5.9	− 6.5
4	2.485	3.571	43.7	25.8	20.1±4.2	−22.1
5	2.222	2.932	32.0	21.5	17.6±5.2	− 3.9
6	2.362	3.319	40.5	24.2	17.6±5.4	−15.1
7	2.584	3.841	48.6	25.8	21.9±5.6	− 3.9
8	2.489	3.273	31.5	24.3	21.2±2.9	− 3.1
10	2.453	3.182	30.0	24.7	21.6±4.4	− 3.1
11	2.704	3.864	42.9	24.7	22.4±3.8	− 2.3
12	2.387	2.755	14.7	24.5	18.0±3.4	− 6.5
13	2.733	3.801	49.7	25.7	21.2±3.7	−17.5
14	2.133	2.438	30.0	23.1	14.7±2.6	−36.4
Control	2.150	3.169	47.4	23.5	20.7±6.1	−11.7

Table 2. *Growth of seedling fresh weight and height after being cutbacked to 25 cm tall*

	Fresh weight (g)			Height (cm)	
	Jan. 1	Jan. 14	Increase (%)	Jan. 14	Reduced (%)
Regenerated line 4	2.03	2.39	17.6	14.2	−43.2
〃 〃 6	1.85	2.34	26.7	15.9	−36.4
〃 〃 7	2.49	2.92	17.0	15.5	−38.0
〃 〃 10	2.54	3.16	25.0	14.8	−40.8
〃 〃 13	1.80	2.61	45.3	16.9	−32.4
Control	1.68	1.89	12.4	13.6	−45.6

less than the controls.

Results of seedlings cutbacked to 20 cm are given in Table 3. The fresh weight of 8 out of 9 regenerated lines was larger than the control, and 7 regenerated lines suffered less necrosis. This result somewhat agreed with that of Table 2.

Results of seedlings cutbacked to 15 cm are given in Table 4. The fresh weight of 4 out of 5 regenerated lines tested was higher than the control, and less in the height reduction. Overall, the cutback to 3 height levels responded similarly to salt stress. The characteristic did not vary much with seedling condition.

For the survival test, a total of 10 regenerated lines was rated and the

Table 3. *Growth of 20-25 cm seedlings being cutbacked to 20 cm*

		Fresh weight (g)			Height (cm)	
		Jan. 14	Jan. 30	Increase (%)	Jan. 14	Reduced (%)
Regenerated line	3	1.71	2.02	+18.6	15.2	−20
〃	〃 4	1.83	2.18	+19.1	13.6	−32
〃	〃 6	1.82	2.60	+44.0	17.2	−14
〃	〃 7	1.90	2.28	+20.0	17.6	−12
〃	〃 8	2.11	2.87	+36.0	16.9	−16
〃	〃 10	1.78	2.03	+14.1	13.1	−34
〃	〃 12	1.96	2.06	+ 5.1	13.2	− 5.2
〃	〃 13	2.03	2.59	+27.6	17.0	−17.4
〃	〃 14	1.78	2.26	+27.0	16.4	−17.9
Control		1.89	2.03	+ 7.2	15.5	−22.5

Table 4. *Growth of 15-20 cm seedlings being cutbacked to 15 cm*

		Fresh weight (g)			Height (cm)	
		Jan. 14	Jan. 30	Increase (%)	Jan. 14	Reduced (%)
Regenerated line	6	1.21	1.48	+ 2.23	14.3	− 4.6
〃	〃 8	1.50	1.77	+17.9	13.5	−10.0
〃	〃 12	1.74	1.89	+ 8.3	13.7	− 8.7
〃	〃 13	1.51	1.55	+ 2.8	12.8	−14.7
〃	〃 14	1.38	1.42	+ 2.6	15.0	0
Control		1.60	1.64	+ 2.4	12.9	−14.7

results were as follows: SS19, 21%; SS35, 46%; SS40, 66%; SS41, 20%; SS65, 14.6%; SS105, 31.1%; SS118, 36.5%; SS115, 20.7%; SS128, 20.7% and SS120, 30.7% which was respectively higher than the two controls of 10.1 and 7.4%. The aforementioned lines survived in 1% salt for two weeks. The evidence supported the results of seedling fresh weight and seedling height that the regenerated lines have carried certain level of salt tolerance.

Discussion

Regenerated plants developed from tissue culture usually have passed through cellular selection on defined medium. Regenerated rice plants derived from salt stress medium are presumed to have carried salt tolerance, since only cells with tolerance would survive on salt stress and then develop onto intact plants. This approach is rather simple and seems to be highly feasible. However, a number of mechanisms as reviewed by Rains (1972) may be responsible. Dix and Street (1975)

studied salt tolerance in tobacco cells. Several cell lines were found to be much more tolerant than their parental lines. Later Croughan (1981) found several rice cell lines had become salt dependent. These researches encouraged the attempts that the salt tolerance could be obtained through tissue culture on stressed medium. And, only those regenerated planted with the tolerance would be practical.

Since the regenerated plants were developed from salt stressed medium and solution, it is reasonable to assume that they carry certain level of tolerance. However, their seedlings segregated widely with respect to the induced salt stress. It is highly likely that regeneated plants are heterozygous and do not breed true. Another possibility would be the structure of chimera existing in the regenerated plants. Thus any regenerated plants with root tolerance to salt may survive. Therefore, progenies from regenerated plants may not carry any tolerance and even become more sensitive than their parental variety, Tainung 67. The variety has never been screened for salt tolerance during it breeding process, however. The tolerant seedlings secured from the subsequent generations are expected of carrying higher level of tolerance. In fact, it seems likely that the tolerance would be fixed in a few cycles of screening. The inheritance of tolerance seems to be rather complex; it seems to be conditioned by a number of factors in addition to nuclear genes. This would lead to speculating that the physiological growth of plants and even the seed quality would play some role in salt tolerance.

Since no field plot was salted, the screening tests were done at seedling in pots. The traits of seedling fresh weight, seedling height and survival rate responded were actually the result of stress at the initial stage of plant growth. So far, no segregating population has been grown in salted condition from seed germination to maturity; the stage of plant growth revealing the tolerance has not yet been studied. Nevertheless, seedlings are the juvenile of plants. We are reasonable to assume that seedlings are most sensitive to stress than any other growth stage of a plant. Therefore, the tolerance identified at seedlings shall be reliable to stress throughout the complete growth.

On the other hands, the plant growth in the field conditions may differ with pots in nutrient condition. Significant difference would be the content of soil organic matter and the component of culture medium. Chen *et al.* (1980) reported that tobacco cells absorbing more K and Ca ions became more tolerant to salt than those without them. Their findings may extend the idea that the minor elements in organic matter of soil would make plants less sensitive to salt comparing with those depending on nutrient solutions only. Our use of crude salt would expect to bringing results closer to natural habitats.

Results of salt stress vary with temperature of glasshouse. Usually, seedlings respond to salt rapidly under high temperature, and the level of mild tolerance could not be identified owing to the rapid enlargement of necrosis. The reaction

of mild tolerance was overrun by necrosis. Therefore, mild or moderate tolerant symptoms cannot be seen, since the high temperature in the glasshouse increase plant transpiration and enhance necrosis development. The rapid enlargement of necrosis may be caused by the less functioning of plant roots in stressed solution. Therefore, the amount of water absorbed by the less functioning roots of the tolerant plants does not fit the consumption of plant transpiration under high temperature. This finding suggests that the screening can better be conducted at optimum temperature, 25±1°C. Then different levels of tolerance can be readily identifled.

The seedling growth of rice is usually rather uniform. However, minor variation in seedling height still exists due to the variation in seed physiology and micro-environments. Thus, taller seedlings are presumed healthier and more vigrous than shorter ones. Three levels of cutbacks investigated in this study gave rather similar results, indicating that the regenerated lines were or less carrying salt tolerance though the tolerant levels varied. The physiology of seedlings did not likely interfere much with salt stress.

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耐鹽細胞之檢定與組織培養

吳旭初 黃楨傑 柯淑婉

中央研究院植物研究所

水稻品種，臺農67號爲試驗材料，經組織培養後獲得許多再分化系統。其目的在提高該水稻之耐鹽能力。在所得之再分化系統中，選擇種子發育優良者十三系統，經在鹽液（1%）中，檢定其耐鹽能力。秧苗之鮮重及秧苗高度用以測定鹽之效應。供試十三系統中，祇有三系統，其鮮重增加率超過對照，而秧苗枯萎程度又能少於對照。剪苗試驗，先將秧苗剪成三種高度，25、20及15公分。這三種秧苗對鹽之反應相似，即耐鹽之秧苗經剪苗後，無論留秧多長都耐鹽。反之亦然。這試驗進一步肯定發展中耐鹽之結果。