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PRODUCTION OF RICE PLANTLETS ON NACL-STRESSED MEDIUM AND EVALUATION OF THEIR PROGENIES[1]

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Abstract

Improved ***in vitro*** methods were attempted in obtaining NaCl-tolerant plants in ***Oryza sativa*** cv. Tainung 67. Calli freshly initiated from germinating seeds were broken into minute aggregates and exposed for 30-40 days to MS medium containing 0.6, 1.0 or 1.5% NaCl. Tolerant calli were then isolated and regenerated into whole plants on differentiation medium also salinized with 0.6, 1.0 or 1.5% NaCl. Plantets were obtainable on 1.0% or lower NaCl-salinized differentiation medium, while on 1.5% NaCl, only roots were formed. The successfully regenerated plants were removed and maintained hydroponically until tillering. A single tiller was then excised from each regenerant and, after recovery, subjected to 0.6% NaCl-salinized nutrient solution for 30 days. The salt-treated plants were washed and thereafter maintained with non-salinized nutrient solution. At maturity, they were rated with respect to their salt tolerance. One half of the regenerants thus screened exhibited possible tolerance, while the rest were either sensitive or moderately sensitive. Based on seed quality and amount, 10 putative tolerant plant lines were chosen for further evaluation by an aseptic method, using R2 progeny seeds. The evaluation medium contained as much as 1.25% NaCl. All the ten plant lines tested demonstrated progress in mean seedling height (increasing from 41 to 91% of control), with four lines also associated with an increased biomass weight (19 to 37% of control). In addition, survival rates in all the ten lines were improved. Our results also showed that somaclonal plants regenerated from a single callus piece may respond differentially or the same under the specified stress conditions, depending upon the callus line number. The intra-callus variation may have originated from the culture procedure ***per se***.

Key words: Rice; NaCl; ***in vitro*** selection; aseptic germination; somaclonal variation; mutagenesis.

Introduction

Soils affected by high salinity are often unarable. To help alleviate the problem, special management practices such as leaching and irrigation with quality water

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are recommended (Carter, 1975). However, these measures are costly and may even negate the returns of production. The other approach lies in the development of salinity-resistant plants. NaCl is one of the major components in saline soils. Genotypes resistant to NaCl could, therefore, be of value either for direct commercial planting or as an alternative source of germplasm for incorporation in a breeding program.

Tissue or cell culture has been effectively used as a means to screen for desirable variants (see reviews of Maliga, 1978, 1984; Yoshida and Ogawa, 1983). Through this technique, cell lines resistant to deleterious levels of NaCl have been selected in *Nicotiana sylvestris* (Zenk, 1974; Dix and Street, 1975), *Nicotiana tabacum* (Nabors *et al.*, 1975), *Capsicum annus* (Dix and Street, 1975), *Medicago sativa* (Croughan *et al.*, 1978), *Saccharum spp.* (Liu and Yeh, 1982), rice (Croughan *et al.*, 1981), and more recently in *Cicer arietinum* (Pandey and Ganapathy, 1984). NaCl-tolerant plants have also been successfully regenerated or isolated in *Nicotiana tabacum* (Nabors *et al.*, 1980), haploid *Datura innoxia* (Tyagi *et al.*, 1981) *Kickxia ramosissima* (Mathur *et al.*, 1980), sugar cane (Liu and Yeh, 1984) and in flax (McHughen and Swartz, 1984).

This study reports our results of regenerating rice plantlets on NaCl-stressed medium with calli derived from germinating seeds, and the evaluation for possible tolerance in some of the regenerants, using R2 progenies.

Materials and Methods

Callus Induction

Seeds of *Oryza sativa* cv. Tainung 67 were dehulled, sterilized with 2.5% sodium hypochlorite for 10 minutes and soaked in water or in a phytohormone solution of 2 ppm each of 2,4-D and kinetin for 24 h at room temperature. Pretreatment with the hormone solution enhanced the number as well as the speed of seeds forming calli.

Fully soaked seeds were then mutagenized with 0.025 M EMS (ethylmethane sulfonate) for 4 hours at 26±1°C. Upon completion of treatment, the seeds were subjected to another cycle of sterilization and rinsing before being placed on basal MS medium (Murashige and Skoog, 1962) for callus induction at 26°C in the dark. The medium was supplemented with 2 ppm each of 2,4-D and kinetin. Calli appeared from the scutellar tissue of the seed embryos 5–6 days after the inoculation, with formation of calli reaching the peak by 10–12th day, at that time vigorous-growing calli were excised and subcultured on fresh identical medium for further growth.

Selection of NaCl-Tolerant Mutant Cells or Crumbs

Two weeks after the subculture, individual callus had attained ca. 5 mm in

diameter. They were then collected and cut up into crumbs of sizes predominantly no larger than 0.5 mm in length. Cutting up of calli was achieved inside the test tube with a small pair of surgical scissors. The 'minced' calli were then exposed to a selection medium for 30–40 days for the selection and proliferation of possible NaCl-tolerant cells or crumbs. The selection medium was identical to the medium used for callus induction except that NaCl was incorported at the level of 0.6, 1.0 or 1.5%.

Differentiation

Callus crumbs that survived and showed vigorous growth on the selection medium were allowed to proliferate undisturbed to sizes ca. 3–4 mm in diameter. They were then isolated and transferred onto modified MS medium for regeneration into plantlets. The medium was supplemented with 0.2 ppm NAA and 2 ppm kinetin. NaCl at 0.6, 1.0 or 1.5% was also incorporated in the regeneration medium. The cultures were incubated under a regime of 16 h photoperiod (5000 luxes) at 26±1°C. Plantlet differentiation was observable 4 to 6 weeks after transfer.

Plantlets successfully regenerated were transferred to a larger vial for continued growth. When plantlets reached the height of 10–15 cm, they were removed and hardened for 2 weeks on nutrient solution (Yoshida *et al.*, 1976), which was lightly salinized with 0.3% NaCl, to minimize possible 'shock' to the plantlets. After hardening, the plantlets were transferred to vermiculite in a 4-liter Wagner's pot and thereafter maintained with nutrient solution.

Screening of Regenerated Plants for Possible NaCl Tolerance

The plants that had been maintained on the non-salinized solution were allowed to grow to the stage where 2 to 3 tillers were separable. A single, vigorous tiller with some roots attached was carefully excised from the mother plant. After it was labelled, the tiller was returned to the nutrient solution for continued growth, while the mother plant was transplanted in the field for later seed-harvesting and observation of morpho-agronomic characters. Ten days later, when the excised tiller had recovered from the separation disturbances and showed signs of new growth, NaCl at 0.6% was introduced to the nutrient solution. The solution was changed once a week. Salinization continued for 30 days or until most of the control plants (Tainung 67) were near death. Plant responses to the added NaCl were observed every week and tentatively graded with respect to salt tolerance. Upon completion of salt treatment, the plants were washed with tap water and thereafter maintained with nonsalinized solution until maturity. Finally, at the end of the experiment, plants evaluated were classified as highly tolerant (HT), tolerant (T), moderately sensitive (MS) and sensitive (S) on such additional basis as growth depression, chlorosis, inward rolling of leaves, leaf burn, inhibition of

tiller initiation, and seed-setting. HT plants were defined as those exhibiting growth comparable to control plants (CK1) maintained on non-salinized solution under the same conditions; whereas S plants were those showing NaCl toxicities similar to control plants (CK2) cultured on 0.6% salinized solution. T and MS categories were plants in between.

Progeny Testing of Presumptive Tolerant Plants

Regenerated plants that had been identified by the salinized water culture method as possessing possible tolerance were further evaluated, using R2 progeny seeds (the second generation of the regenerants) harvested from their respective mother plants in the field. Based on seed quality and amount, 10 putative tolerant lines were chosen for the testing. The 10 lines were 001-2, 004-1, 004-2, 004-5, 006-1, 007-2, 008-1, 008-2, 009-1 and 009-4. Also included in the experiment were two normal unselected lines 028 and 030, which were derived from different callus crumbs maintained and regenerated on non-NaCl stressed medium. Aseptic evaluation methods developed in our laboratory were employed. The detailed procedures and the medium used have been previously reported (Wong *et al.*, 1985). Basically, dehulled seeds from each line were thoroughly sterilized, fully imbibed in water and then placed in a test-tube containing 15 ml of 1.25% (w/v) NaCl-salinized medium. Each culture tube contained exactly 10 seeds and, except otherwise stated, at least 10 uncontaminated cultures were successfully established for each line. Culture conditions were at 26°C with 16 h photoperiod of 6,000 luxes. After 30 days in culture in the NaCl-stressed medium, the total biomass produced in each culture tube was removed, weighed and its mean seedling height determined. Survival rate was also scored for each line tested. Analysis of variance was then conducted and Duncan's Multiple Range Test used to test the significance of difference among the means.

Results

Responses of Calli Crumbs on NaCl-Stressed Medium

Minced calli subcultured on standard medium were yellowish and vigorous-growing, but when exposed to NaCl-stressed medium, they turned brownish as soon as 48 hours after exposure and became necrotic within 2 weeks. However, areas of new growth could occur on and around the necrotic crumbs 30-40 days later. These areas of new growth were probably NaCl-tolerant. No attempts were made to subculture these new callus sectors. Instead, they were allowed to grow undisturbed untill their sizes reached ca. 3-4 mm in diameter. They were then isolated and transferred onto salinized differentiation medium for regeneration into plantlets.

Because of the great variations in the initial size of the callus crumbs used in

the selection experiment, frequency of crumbs producing NaCl-resistant areas was almost impossible to determine. However, based on obsevation, it seemed that more new areas of growth could appear on lower than higher NaCl-selection medium. This might imply that tolerance to higher NaCl at the callus level may probably be controlled by more than one gene. Moeljopawiro and Ikehashi (1981) reported that NaCl tolerance in rice at the whole plant level is polygenic in nature.

Differentiation

As shown in Table 1, the calli initiated from the scutellar tissue of germinating T67 seeds were very rhizogenic (root-forming), even in the presence of NaCl. The situation is true for both preselected and non-preselected calli. The formation of whole plants in non-preselected calli was, however, greatly inhibited by the addition of NaCl, but less so in those calli isolated on NaCl (see specifically responses of calli in the selection-and-regeneration categoris of 0.0–0.0, 0.0–1.0 and 1.0–1.0; the first number in each category refers to %NaCl used in selection, while the second number indicates the %NaCl employed for plantlet regeneration). For calli in category 1.5-1.0% NaCl, 4.76% was regenerable into whole plants. Some few plants were also obtained from the selection-and-regneration category of 1.5–0.6% NaCl. Whether these regenerated plants are due to adaptation or as a result of the imposed selective pressure can be determined by progeny testing, once seeds are secured from the regenerants.

Table 1. *Differentiation responses of preselected and non-preselected T67 seed calli on NaCl-stressed medium*

Callus origin (% NaCl used)		No. of calli incubated	Undifferentiated	Necrotic	Rooting	No. of calli producing plantlets
Select.	Reg.					
0.0	0.0	107	11	9	46	41(38.3)*
0.0	1.0	153	8	32	110	3(1.96)
0.0	1.5	206	3	53	150	0(0.00)
0.6	0.6	24	3	0	16	5(20.8)
1.0	1.0	152	63	1	74	14(9.20)
1.5	0.6	17	2	0	13	2(11.8)
1.5	1.0	105	32	4	63	5(4.76)
1.5	1.5	95	28	14	53	0(0.00)

* The figure in parentheses indicates the actual percentage.

On differentiation medium containing as much as 1.5% NaCl, no whole plant could be obtained, even for those calli preselected with NaCl at the same level. This indicated that the upper limit of salinization for regeneration has been

Table 2. *Responses of regenerated lines (R1 generation) in 0.6% NaCl-salinized nutrient solution*

Each line was represented by one plant initiated from an excised tiller.

Reg. Line	Callus origin (% NaCl used)		Plant height (cm)		Tiller initiation at harvest	Seed-set at harvest	Tolerance level
	Select	Reg.	Initial	20 days after salinization			
001- 1	1.5	0.6	57	70	−	−	MS*
2	1.5	0.6	64	65	−	+	T
3	1.5	0.6	75	82	−	−	MS
002- 1	1.0	1.0	65	60	−	−	S
2	1.0	1.0	65	50	−	−	S
3	1.0	1.0	60	45	−	−	S
4	1.0	1.0	64	64	−	−	S
5	1.0	1.0	65	53	+	−	MS
6	1.0	1.0	74	65	−	+	MS
004- 1	?	1.0	65	75	+	+	T
2	?	1.0	65	70	+	+	T
3	?	1.0	62	55	−	−	S
4	?	1.0	56	50	−	−	S
5	?	1.0	80	80	+	+	T
005- 1	1.5	0.6	69	70	−	−	MS
2	1.5	0.6	73	65	−	−	S
3	1.5	0.6	70	68	−	−	S
4	1.5	0.6	67	65	−	−	S
5	1.5	0.6	50	45	−	−	S
6	1.5	0.6	73	75	−	−	MS
7	1.5	0.6	74	70	−	+	T
8	1.5	0.6	65	65	−	−	MS
10	1.5	0.6	73	65	−	−	MS
006- 1	1.0	1.0	74	80	+	+	T
007- 1	1.0	1.0	65	55	−	−	S
2	1.0	1.0	70	65	+	+	T
008- 1	1.0	1.0	78	70	+	+	T
2	1.0	1.0	72	60	+	+	T
009- 1	?	1.0	76	70	+	+	T
2	?	1.0	55	45	−	−	S
3	?	1.0	68	80	−	+	T
4	?	1.0	75	80	+	+	T
5	?	1.0	60	55	−	−	S
010- 1	1.0	1.0	55	70	−	+	T
2	1.0	1.0	55	65	+	+	T
3	1.0	1.0	70	75	+	+	T
011- 1	1.0	1.0	74	70	+	−	MS
2	1.0	1.0	53	65	−	−	S
3	1.0	1.0	70	70	+	+	T
4	1.0	1.0	70	70	+	−	MS
5	1.0	1.0	65	70	−	+	T
012- 1	1.0	1.0	48	60	+	+	T
013- 1	0.6	0.6	58	55	−	+	T
2	0.6	0.6	58	70	+	+	T
015- 1	0.6	0.6	70	70	−	+	T
016- 1	1.0	1.0	55	50	−	−	S
017- 1	0.6	0.6	60	50	−	−	S
CK1- 1	**		52	Dead	−	−	S
2			55	Dead	−	−	S
3			55	50	−	−	S
4			55	60	−	−	S
5			45	55	−	−	S
CK2- 1	**		65	90	+++	+++	not rated
2			54	80	+++	+++	not rated
3			58	85	+++	+++	not rated
4			58	90	+++	+++	not rated
5			54	80	+++	+++	not rated

* T=tolerant; MS=moderately sensitive; S=sentive

** CK1 refers to control plants (T67) cultured on 0.6% NaCl-salinized nutrient solution. CK2 refers to control plants (T67) cultured on standard nutrient solution.

reached or slightly exceeded for T67 seed calli. Future *in vitro* improvement efforts should, therefore, be focused on overcoming this threshold by isolating calli that are capable of differentiating whole plants at or around 1.5% NaCl, as the developed plants will probably carry a much higher level of tolerance.

Screening

Caution should be exercised in interpretating the data frem Table 2, since all the observations or measurements were based on a single plant, except CK1 and CK2.

Ten days after NaCl (0.6%) was introduced to the nutrient solution, plants began to show growth depression. Leaves were droopy, chlorotic, and burned at the tips. Tiller initiation was severly inhibited. As shown in Table 2, of the 47 regenerants evaluated, none showed tolerance of the HT (highly tolerant) category. According to criteria listed in Materials & Methods, about one half of the plants screened exhibited possible tolerance, while the rest were either sensitive (S) or moderately sensitive (MS). Whether the tolerance observed is heritable or only as a result of environmental induction (epigenetic) awaits further experiments with progenies (R2) harvested from their respective mother plants in the field.

Table 3. *Mean seedling height and biomass production of regenerated lines (R2 progenies)*

Reg Line	callus origin (% NaCl used)		*n**	Height			Biomass		
	Select.	Reg.		cm	% Control	CV (%)	gm	% Control	CV (%)
CK(T67)	—	—	12	3.68±1.15a**	100	31.3	0.89±0.14 ab**	100	15.7
001-2	1.5	0.6	12	6.14±0.68cde	166.8	11.1	0.90±0.09ab	101	10.0
004-1	?	1.0	12	5.22±0.57bc	141.8	10.7	0.92±0.05abc	103	5.4
004-2	?	1.0	12	6.18±0.87cde	168.9	14.1	1.03±0.13bcde	115.7	12.6
004-5	?	1.0	12	5.82±1.02cde	158.2	17.5	1.08±0.15de	121.3	13.9
006-1	1.0	1.0	12	5.83±0.85cde	158.4	14.6	0.91±0.10abc	102.2	11.0
007-2	1.0	1.0	11	5.49±1.10bcd	149.2	20.0	0.94±0.11abcd	105.6	11.7
008-1	1.0	1.0	11	6.09±1.65cde	165.5	27.1	0.94±0.19abcd	104.5	20.4
008-2	1.0	1.0	12	7.03±0.99e	191.0	14.1	1.14±0.09e	128.1	7.9
009-1	?	1.0	10	5.90±0.68cde	160.0	11.5	1.06±0.09cde	119.1	8.5
009-4	?	1.0	12	6.72±0.68de	182.6	10.1	1.16±0.15e	130.3	12.9
028	0.0	0.0	10	6.59±0.71de	179.1	10.8	1.16±0.07e	130.3	6.0
030	0.0	0.0	8	4.22±1.28ab	114.7	30.3	0.87±0.09a	97.7	10.3

* '*n*' represents the number of uncontaminated cultures successfully established, with each culture containing 10 seeds aseptically germinated and grown for 30 days at 1.25% NaCl-salinized medium.

** Different alphabets represent significance at 5% as tested by Duncan's Multiple Range Test.

As about their performance in the field, all the mother plants were normal in stature and, except some plants, had fertility of not less than 75%.

Progeny Testing

Compared to their donor parent, variety T67, all the regenerated lines, except line 030, exhibited a positive improvement in terms of mean seedling height, increasing from 41 to 91% of control (Table 3). Four selected lines viz., 004-5, 008-2, 009-1 and 009-4 were also associated with an increase in biomass. Additionally, the number of seeds and/or seedlings not succumbing under the specified stress conditions was also improved (Table 4). For example, the survival rates of lines 004-2, 004-5, 004-5, 008-2, 009-1 and 009-4 were respectively 90.8%, 92.5%, 86.7% and 90.0%, while in control it was only 73.3% (Table 4). All these data indicate that cellular selections and subsequent plantlet regeneration are effective in obtaining salt-tolerant plants.

Table 4. *Survival rates of regenerated lines (R2 progenies)*

The figure in parentheses indicates the actual number of seeds and/or seedlings scored; seeds were aseptically germinated and grown for 30 days at 1.25% NaCl-salinized medium.

Line	T67	001-2	004-1	004-2	004-5	006-1	007-2
%	73.3	89.2	87.5	90.8	92.5	85.0	81.8
	(120)	(120)	(120)	(120)	(120)	(120)	(110)
Line	008-1	008-2	009-1	009-4	028	030	
%	83.6	86.7	87.0	90.0	97.0	88.8	
	(110)	(120)	(100)	(120)	(100)	(80)	

Another interesting point that emerged from this experiment is that somaclonal plants regenerated from a common callus piece may respond differentially or the same to the imposed stress of 1.25% NaCl, depending upon the callus line number (Table 3). For example, tolerant plant lines 009-1 and 009-4 were both regenerated from a common callus (Line #009). These two lines did not differ significantly from each other in biomass production and mean seedling height, indicating that they may have originated from a single tolerant cell. However, with regard to 008-1 and 008-2 (both derived from callus number 008), significant difference was observed in biomass, but not in mean seedling height, implying that the two lines may have derived from different cells, or variation may have occurred during the process of cell growth and/or plantlet differentiation. The same is also true of lines 004-1 and 004-5 in terms of biomass production, but no significant difference can be detected between 004-1 and 004-2, or 004-2 and 004-5, in either

parameter (Table 3). The implication of this type of somaclonal variation will be discussed later.

With regard to the two normal unselected lines (028 and 030), contrasting results were obtained. These two lines were both originated from different callus crumbs, maintained and regenerated on non-NaCl stressed medium. Line 028 surpassed control by 79.1% in mean seedling height and 30.3% in biomass. However, for line 030, it was as sensitive as T67 to NaCl stress (Table 3).

Discussion

In the selection for variants with the method of tissue culture, suspended cultured cells are often employed or advocated. This is primarily because of the possibility of obtaining highly dispersed and homogeneous cell populations, which are more ideal as unit size for cellular screening experiments (Chaleff, 1983). However, in cereals, especially in rice, the production of finely divided cells is not easily feasible and may require several months of repeated sieving and selection of rapidly growing single cells. Moreover, not all genotypes are responsive to the manipulations. Another serious limitation is that prolonged periods of culture often result in loss of regenerative ability and the accumulation of unwanted genetic abnormalities (Oono, 1981; D'Amto, 1978; Chen and Chen 1980).

In the present system, we employed calli newly derived from germinating seeds. It is unique in that callus is young (age not over 25 days after seed inoculation), vigorous and has good regenerative ability. In addition, donor seeds are treated with EMS prior to the processes of callus initiation and proliferation. This manuvering provides additional variability in the ensuing cell population and, at the same time, eliminates or minimizes the direct adverse effects of the mutagen, if is applied directly on the callus level. By manipulating such conditions as soaking donor seeds in 2,4-D solution before inoculation, addition of yeast extract or casein hyhrolysate in the induction medium, increased incubation temperature (32°C) and light intensity (10,000 luxes), we are now able to initiate and grow callus to ca. 5 mm in diameter from each individual seed in as short as 10–14 days after seed inoculation (unpublished results). The exact number of cells in each induced callus is not yet determined but three calli of the above size, when finely broken into aggregates, will fully cover an area of 1.5×3 cm.

It is estimated that by using the current improved method, 200 to 300 calli will be sufficient for any modest *in vitro* selection program. The ease of obtaining large amount of young and genetically variable calli and the simplicity of the system can, therefore, be advantageously extended to the selection for other desirable variants such as tolerance to herbicides, pathogen toxins or heavy metal toxicities, etc.

In the evaluation of the regenerated plant lines for salt tolerance, we observed

that lines derived from a single callus crumb may respond differentially or similarly under the specified stress conditions, depending upon the callus line. Since EMS was applied previously in the donor seeds, this could be explained that mutations were induced in different cells of the same callus piece. Another likely possibility could be that the observed variation was induced by the culture procedure itself. The phenonmenon of altered characters among regenerated plants is referred to as somaclonal variation (Larkin and Scowcroft, 1981).

Wide morpho-agronomic variation has been reported in regenerated rice plants (Oono, 1981; Suenaga *et al.*, 1982) and in other species such as sugar cane, tobacco and wheat (See reviews of Maliga, 1984; Meins, 1983). Somaclonal variation used to be a nuisance, but recent advances show that it is an unexpectedly rich source of genetic variability and is currently being exploited for crop improvement (Larkin and Scowcroft, 1981; Reisch, 1983). In the regeneration experiment, we also observed that when a differentiating callus was loosely separated and transferred to a larger culture vessel, more plants than usual could be produced. Since the regenerated plants may be genetically variable, as indicated in this study and in others (Maliga, 1984; Meins 1983), there exists an unique opportunity of obtaining more tolerant plants, and possibly plants with more tolerance from a preselected callus piece, if NaCl of similar or higher strength is also imposed throughout the regeneration stage, or shortly after the putative resistant callus begins to differentiate. This manuvering provides additional selection not only at the cellular level, but also at the whole plant level. A more or less similar approach was attempted by Liu and Yeh (1984) in obtaining NaCl tolerant sugar-cane plants.

In any breeding or *in vitro* selection program for the improvement of crop plants, testing and retesting of the products are indispensable. Early identification of promising plants will certainly help reduce the immense time and resources subsequently involved. The salinized water culture method, as used in this study, is helpful in identifying immediate regenerated plants (R1) as possessing possible tolerance; all the ten plant lines, as previously screened by the method, were later shown, through progeny testing, to surpass control in mean seedling height, with four lines also associated with an increase in biomass weight. However, when a large number of regenerated plants are produced, the described method will become too resources and time-consuming. A more efficient system should therefore be devised. Stem cuttings of rice, when planted on suitable rooting media will form roots and shoots, and later develop into mature plants (Kumar, 1981 and unpublished results of this lab.). A salt-tolerant plant will be expected to do the same (have this regenerative ability) even under saline conditions, while sensitive ones may not. We are currently exploring its feasibility as a rapid means of characterizing regenerants for possible salt tolerance.

Various strategies are known to be involved in imparting salt tolerance in

plants such as avoidance, exclusion, and physiological tolerance. (Flowers *et al*,, 1977; Greenway and Munns 1980). The successfully regenerated tolerant lines, if later proved stable in succeeding advanced generations, will provide good materials for elucidating the underlying cellular or anatomical basis for tolerance. If novel or more than one mechanism(s) are uncovered, plants with different modes of tolerance can be combined together or hybridized with existing tolerant genotypes through a conventional breeding program to produce line(s) capable of tolerating a much higher level of NaCl.

Finally, our study demonstrated that it is possible through *in vitro* selection to obtain NaCl-tolerant plants in rice cutivar T67. A recent study showed that tolerant plants can also be isolated from within the cultivar by practicing pure-line selection (Wong *et al.*, 1985). Therefore, we do not exclude the finite possibility that cellular selection may not be operating on the variability occurring naturally and/or induced by EMS, but simply acting as a retention mechanism for salt-tolerant cells incidentally derived from a tolerant seed line. Further studies should, therefore, involve using a sensitve and genetically pure line, and subjecting it to the described protocol of callus initiation, cellular selections and plantlet regeneration. If progress can be made, then the validity of our system will be more convincingly confirmed.

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水稻幼株分化自含鹽培養基及其後代耐鹽性之評估

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粳型品種臺農67號，用組織培養方法，希望能够獲得耐鹽系統。種子癒合組織分割成小顆粒狀後，便培養於含鹽之 MS 培養基。培養基之含鹽量爲：0.6, 1.0 及 1.5%共三種。癒合組織能够在含鹽 0.6 及 1.0%之培養基上再分化成小株； 而 1.5%鹽者癒合組織祇能分化若干小根。再分化成之小株繼續用水耕液方式培養，至分蘗出現時爲止。每一再分化小株，割取分蘗一支，待恢復正常生長勢後在含鹽 0.6%之水耕液中培養 30天，然後將稻株用清水洗淨，繼續用不含鹽之水耕液培養稻株至成熟。種子收穫後再做秧苗耐鹽試驗，約有半數系統具有若干程度之耐鹽性，而另一半對鹽具有若干不同之敏感反應。

耐鹽十系統，種子發育良好數量充足者選作第二代（R_2）耐鹽檢定，檢定在試管中進行，培養基含 NaCl 達1.25%，所選用之十系統秧苗高度超過對照系統41–91%，其中系統鮮重超過對照19–37%不等，選系之生存率亦較高。再分化稻株，雖然得自相同一塊之癒合組織，各稻株對鹽之反應亦不一致；亦有對鹽之反應相同者。這癒合組織內對鹽反應之差異也許由於培養過程中所造成的。