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REGENERATION OF RICE PLANTLETS ON NACL-STRESSED MEDIUM BY ANTHER CULTURE*

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Abstract

This study aimed to develop rice lines tolerant to salt stress. Seeds of Tainan 5 were treated with ethly methanesulfonate and grown to adult plants. Anthers excised from the plants during bolting stage were inoculated in defined medium containing 0.6 and 1.0% NaCl. Anther calli induced from these two media were rather few. Approximately, 2.6% anthers inoculated on 0.6% NaCl-stressed medium developed calli, while only 0.25% anthers on 1.0% NaCl-stressed medium grew onto callus.

Calli cultured on NaCl-stressed medium, 31% of them regenerated plantlets. A total of 44 plantlets was obtained. However, only 5 of them were normal greens, and the rest were albinos. The 5 normal green plantlets were regenerated from a single callus. One of the five died after they were transplanted onto pots outdoor. The other 4 fully grown plants have large awned florets and highly sterile kernels. A total of 8-10 seeds was collected from each plant. Therefore, it seems reasonable to assume that polyploids rather than diploids were involved in these plants.

Introduction

Anther culture is, in essence, microspore culture, even though the role of the involved anther tissue is important and cannot be disregarded. The number of microspores or pollens contained in each anther varies with species, e.g., 450-900 pollen grains in rice. If genetic changes have been previously induced on the anther donor plants, the vast number of microspores developed in the anthers will, surviving severe diplontic and haplontic cell competition, provide a pool of variability upon which screening could be carried out with a suitable system. Since the introduction of anther culture, much effort has been directed to the intrinsic and extrinsic factors influencing the rate of callus formation and subsequent plantlet regeneration

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(Keller and Stringham, 1978; Dunwell, 1978, and Hu *et al.*, 1978). Little has been utilized of its *in vitro* potentials in the selection of desirable mutants. This communication reports our attempts in recovering plantlets on NaCl-stressed medium by planting rice anthers from donor plants which were raised from seeds previously treated with ethyl methanesulfonate (EMS).

Materials and Methods

Production of donor plants

Seeds of *Oryza sativa* cv. Tainan 5 (*japonica*) were dehulled, sterilized with 2.5% sodium hypochloride for 10 minutes and soaked in water for 24 hours at room temperature. Fully soaked seeds were then mutagenized with 0.025 M EMS for 4 hours at 26±1 C. Upon completion of treatment, seeds were again throughoutly rinsed before being placed on basal MS medium (Murashige and Skoog, 1962) for germination. Germination conditions were also at 26+1 C with 16 hours light photoperiod of 8,000 luxes. When seedlings attained the height of 10–15 cm, they were removed from culture vials and hardened on nutrient soluton for 10–14 days before being transplanted in outdoor concrete beds.

Culture of anthers

Followed the standard procedures adopted in the laboratory. 1) Anthers with microspores predominantly in the uninucleate stage were used for culture. 2) Basal MS medium supplemented with 4 mg/l α-naphthalene acetic acid (NAA) and 2 mg/l kinetin. 3) Two levels of NaCl concentrations 0.6 and 1.0% were used. 4) Culture conditions were in dark at 26±1 C.

Differentiation of plantlets

Calli successfully initiated from the plated anthers were allowed to proliferate to about 2 mm in diameter and then transferred onto medium SR (salt-regeneration) for regeneration into plantlets. The medium was salinized with 0.6 or 1.0% NaCl containing 0.2 mg/l NAA and 2 mg/l kinetin. Cultures were maintained under the same conditions as for seed germination, except that light intensity was reduced to 4,000 luxes. Organogenesis was observable 4–6 weeks after the transfer.

Result

The number of anthers developed calli on the NaCl-stressed medium are given in Tables 1 and 2. The rate of callus induction was extremely low, i.e., 2.61% and 0.25% on the 0.6% and 1.0% NaCl media respectively. To compare with the controls which were not treated with EMS, about 15% of

Table 1. *Anther callus developed from EMS-treated seeds of Tainan 5 on 0.6% (w/v) NaCl-stressed medium*

Plant No.	Treated hr.	Number of anther cultured	% anther forming calli
1	4	109	3.66 (4)*
2	4	87	2.29 (2)
3	4	47	2.12 (1)
4	4	89	2.24 (2)
5	5	464	2.58 (12)
6	5	276	1.44 (4)
7	5	591	0.84 (5)
8	5	24	8.33 (2)
9	8	42	0 (0)
Total		1,729	2.61±2.38

* Actual number of calli formed

Table 2. *Anther callus developed from EMS-treated seeds of Tainan 5 on 1% (w/v) NaCl-stressed medium*

Plant No.	Treated hr.	Number of anther cultured	% anther forming calli
1	4	99	0 (0)
2	4	96	0 (0)
3	4	28	0 (0)
4	4	71	0 (0)
5	4	72	0 (0)
6	4	77	0 (0)
7	5	427	0.46 (2)
8	5	257	2.33 (6)
9	8	23	0 (0)
10	8	158	0 (0)
11	8	32	0 (0)
Total		1,340	0.25
Control** 1	4	326	14.72 (48)

** Anther on non-salted medium

anther cultured on standard medium without salt would proliferate calli. The data indicated that the level of NaCl added was inhibitory to the initiation of calli. The inhibition was more severe on 1.0% NaCl medium than that on 0.6% NaCl.

As shown in Table 3, about 31% of the calli incubated on the salinized medium developed into plantlets, and a total of 44 plantlets was obtained. However, only five plantlets were normal greens. They were all derived from a single callus, and regenerated on 0.6% NaCl medium. One plant died after being transplanted to the field. The other four plants were successfully grown to maturity. All four plants have normal stature and large spikelets with awns. These traits did not show in the donor variety of Tainan 5. Most of the spikelets were sterile. However, 8–10 normal seeds were secured from each plant. Based on the poor fertility and the largeness of the seeds obtained, the ploidy levels of the plants probably varied other than diploid.

Discussion

Salt resistance has always be considered to be a very important trait in crop improvement. Since the incorporation of the trait can widen the adaptability, it may make the crop to be even grown in area with salt accumulation. The salt resistance could be bred with various means. However, the genotypic structure with good agronomic performance should be retained and not be disrupted because of resistance incorporation. Under these conditions, the induction of salt resistance becomes the most feasible means to suit the purpose. The method of induced mutations through anther culture appears to be a most likely approach which may directly reach the goal. Dix and Street (1975) reported cell lines of *Nicotiana sylvestis* were able to grow on liquid media containing 1 and 2% sodium chloride. Chen *et al.* (1980) studied the growth of tobacco calli on seawater, NaCl, and mannitol stresses. The stress inhibited cell growth. Nabor *et al.* (1980) discovered that NaCl-tolerant cell lines of *Nicotiana tobaccum* survived in 8.8 gr/l NaCl were the maximum. Normal lines were tolerant to 1.6 gr/l NaCl level. Their work revealed the attempts to induce mutants on cell basis instead of plant level. Thus, the basis of induced mutation would put the

Table 3. *Regeneration of normal green and albino plantlets on NaCl-stressed medium*

% of NaCl	Number of callus inoculated	No. of callus forming plantlet	
		green	albino
0.6	25	1	7
1.0	4	0	1
Total	29	1	8

vast population in laboratory, and could be handled with less manpower. Our work of inducing NaCl-tolerant lines, in addition to callus tissue, mutagen EMS was applied. The application of EMS intends to enhance the frequency of mutations on cell populations. Nevertheless, the direct use of EMS on anther callus and plated on stressed medium would cause deterious effects and reduce the survival of cell population. Similar findings on alfafa was found by Croughan *et al.* (1978). Within two months of exposure to the stress medium, 99% of their plated cells exhibited arrested growth and discoloration. This fact elucidates that the NaCl-stressed medium would give a severe cell selection. And only those with tolerance may survive on stressed medium. Therefore, for increasing the number of variants, the plated cells or calli would carry a varied genetic background. To fit the stated assumption, we treated the seeds with mutagen EMS then grow them to be donor plants, and cultured the anthers of the donors on stressed medium. Under this system, the vast number of microspores produced in the culture would serve as a pool of genetic variation. The mutated cells have to pass a series of diplontic and haplontic cell selections to carry the mutated genotype from seed cells to microspores however.

In this study though the number of calli and plantlets regenerated were rather limited, the approach was considered to be encouraging. The low culturability may be due to the high sensitivity of anthers to NaCl, and the anthers may reduce the transport of nutrient from medium to microspores. In order to bypass the difficulty, prospective anthers were subjected to culture on non-salted medium for a short period of five weeks before being subjected to NaCl-stressed medium. This would allow potential microspores to adapt, divide and to proliferate to a certain extent. They would render somewhat more culturable in the NaCl-stressed medium. A process more or less similar to ours was also suggested by Chaleff and Stolarz (1981).

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花藥之培養與耐鹽植株之分化

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本報告之目的在利用細胞遺傳誘變劑，配合花藥組織培養，探討耐鹽或抗鹽水稻之品系。臺南五號爲供試材料，其種子用乙基甲基烷磺酸處理後，在長成植株取其花藥培養之，花藥在含食鹽之培養基上所發展之癒合組織數目很少，在含食鹽 0.6% 時，約 2.61% 之花藥可產生癒合組織，在含食鹽1.0%時祇有 0.25% 花藥生長癒合組織，在無食鹽時可獲得14.72% 之癒合組織癒合組織。

培育於含食鹽培養基時，有31%可分化小株，共獲得44小株，其中祇有 5 株爲綠色，皆得自同一之癒合組織，其餘均爲白株，僅 4 株成長成熟，其小花較親系之小花大些，並且有芒，而高度不孕，每株祇收到810粒種子，諒爲多倍體。