Omonrice 12: 33-37 (2004)

SELECTION OF SALT TOLERANCE GENOTYPES FROM DOUBLED HAPLOIDS IN RICE

Dang Minh Tam, Nguyen Thi Lang

INTRODUCTION

Salinity is the most widespread soil problem in rice-growing countries. Large areas of rice fields are in the sea border region, where salinity affects the crop. To breed a tolerant variety adapted to these soils, we have selected for new anther-culture-derived lines from the F_1 hybrids of six selected crosses among 27 crosses: C41/MR 159, Teqing/ Giza 159, Teqing/ Madhukar, Teqing/At 354, Teqing/Doc Phung, Teqing /Pokkali. Obtaining salt-tolerance lines from anther culture of F1 allow the rapid fixation of homozygosity concurrent with the transfer of salt tolerance of one to the other parent having a desirable plant type.

To develop a variety by conventional breeding, it takes long time with hard working. Anther culture techniques have been studied for their use in rice breeding since 1970's. Developing these lines from anther culture, which will be released as a new variety to farmers, needs about 3-4 years, nearly half as long as conventional cross-breeding methods.

This study aims at dealing with the performance and evaluation of anther-culture-derived lines from F_1 crosses in rice bred for salinity tolerance under saline conditions (0dS/m, 6dS/m, 15dS/m). The higher level of salt tolerance from traditional cultivars transfer into high-yielding lines with improved plant types was recorded.

MATERIALS AND METHODS

Single crosses were done to incorporate the salt tolerance of traditional varieties into high-yielding varieties.

Anther culture was made in F_1s of these crosses. The 156 anther-culture-derived lines obtained (Table 1) of six crosses (C41/MR 159, Teqing/ Giza 159, Teqing/ Madhukar, Teqing/At 354, Teqing/Doc Phung, Teqing /Pokkali in the lab of CLRRI for salt tolerance.

Table 1: Number of anther-culture-derived lines from selected crosses

No.	Parentage	Lines No. from anther culture for screening
1	C41/MR 159	9
2	Teqing/Giza 159	32
3	Teqing/Madhukar	43
4	Teqing/At 354	9
5	Teqing/Doc Phung	19
6	Teqing/Pokkali	44
	Total	156

156 dihaploid lines produced through the anther culture of F_1 crosses were evaluated for salt tolerance with Pokkali and IR29 were the tolerant and susceptible checks, respectively under saline conditions at EC of 0dS/m, 6dS/m, 15dS/m

Media

Medium for callus formation: N6 medium containing 0.5mg/l 2,4-D, 1mg/l NAA, 0.5mg/l BAP

Medium for regeneration: MS medium containing 1mg/l NAA, 1mg/l BAP, 0.2mg/l Kinetin

The working procedure and flow of materials in breeding rice by anther culture for salt tolerance are presented in the Figure 1:

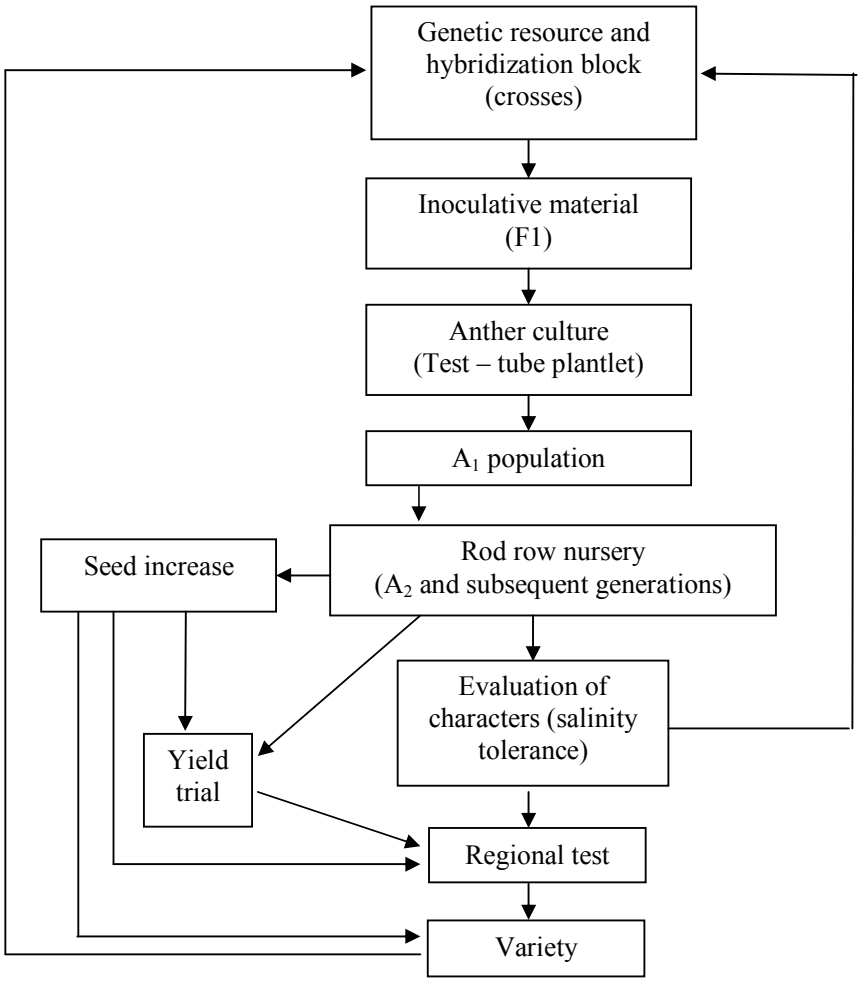

Fig 1. Working procedure and flow of materials in breeding rice by anther culture for

RESULTS

1. Callus formation and regeneration

Table 2: The percentage of callus formation from the anthers

Designation	Anther inoculated (No.)	Callus produced		Callus transferred (no.)	Green plantlets	
		No.	%		No.	%
C41/MR 159	360	2	0.56	2	1	50.0
Teqing/Giza 159	480	8	1.67	8	1	12.5
Teqing/Madhukar	360	5	1.39	5	1	20.0
Teqing/At 354	240	3	1.25	3	1	33.3
Teqing/Doc Phung	480	6	1.25	6	3	50.0
Teqing/Pokkali	360	5	1.38	5	2	40.0

In the medium for callus formation, the percentage of callus products is varied from 0.56 to 1.67, but the percentage of green plantlets is higher in the medium for regeneration. Anther culture of these crosses was done in the season after receiving F1s in the greenhouse. The 156 anther-culture-derived lines obtained have different characters in major agronomic characters (Table 3).

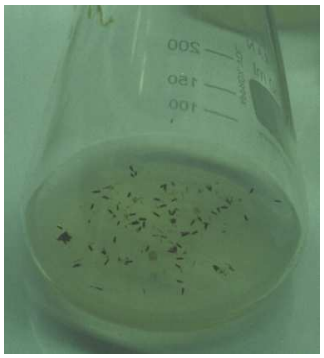

Fig 2: Anthers were cultured in MS medium

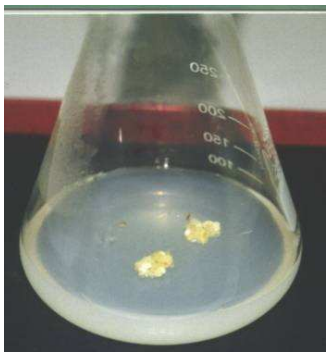

Fig 3: Callus formation from anthers culture

Fig 4: Regenerated plants in root formation medium

2. A_2 generation transplanting

Anther-culture-derived lines from test tube plantlets were cultivated in cluster in pots and divided into single seedlings for transplanting in the greenhouse.

Table 3: Comparison of major agronomic characters of anther culture lines in A_1 generation

Designation	Growth duration (days)	Plant height (cm)	Length of ear (cm)	Ears (No.) per plant	Grains (No.) per ear
C41/MR 159	70-95	67-98	16.0-23.5	10.0-28.0	30.0-125.0
Teqing/Giza 159	67-117	67-117	16.6-23.4	9.0-42.0	32.0-145.0
Teqing/Madhukar	83-97	68-108	18.0-22.6	6.0-30.0	68.6-123.8
Teqing/At 354	95	57-107	21.6-24.4	11.0-21.0	91.0-175.6
Teqing/Doc Phung	85-98	72-127	15.6-23.0	5.0-34.0	46.6-169.2
Teqing/Pokkali	92-102	100-210	18.5-31.8	3.0-16.0	23.4-188.4

Diploid pollen plants in the A_1 generation usually are many types as a result of segregation and recombination different characters of the parents.

In anther culture breeding, the characters of two parents can be made complementary at an early generation and a plant can immediately be made homozygous. Because the characters in anther-cultured rice are controlled by dominant and recessive genes and interallelic complementation, selecting moderate parents according to a breeding target is important (Din et al 1980, Li et al 1981, Zhang et al 1980). Because the range of variation within pollen plants correlates closely with the genetic bases and heterogeneity of inoculative materials. To spread the genetic bases, it is necessary to use distant parents. F_1 plants is used as inoculative materials in our experiments. A_2 plants were used to evaluate such qualitative characters as salt tolerance in both the laboratory and the field.

3. Evaluation of salinity tolerance

A total of 720 plants out of 1200 green plants derived from six three way crossed F_1 hybrids were spontaneous DHs. Mature seeds (A_2 generation) were harvested, and their salinity tolerance were estimate at seedling stage using 6dS/m and 15dS/m. The frequencies of high salt tolerant DH s were 30.00-33.33% (Table 4).

Table 4: Relative response of anther culture lines and the tolerance checks at salinity of 6dS/m and 15dS/m.

Parentage	6dS/m		15dS/m	
	Survival lines No.	Percentage survival (%)	Survival lines No.	Percentage survival (%)
C41/MR159	3	46.66	3	30.00
Teqing/Giza 159	12	37.50	5	15.63
Teqing/Madhukar	15	34.88	5	11.63
Teqing/At 354	6	66.67	3	33.33
Teqing/Doc Phung	14	73.68	2	10.53
Teqing/Pokkali	20	45.45	4	11.00
Pokkali	7	70.00	4	40.00
IR 29	3	30.00	0	0.00

Pregerminated seeds were salinized at EC of 6dS/m and 15dS/m. Survivals ranged from 37.5%, 73.68% for Teqing / Giza 159, and Teqing /Doc phung, respectively as compared to 70.0% for Pokkali (check) at salt stress of 6dS/m.

Twenty six anther-culture-derived lines that tolerated to salt medium at 15dS/m are observing for early seedling stage and flowering stage in the net-house of CLRRI.

Fig 5: Screening in salt medium at EC =15 dS/m

CONCLUSION

Anther culture techniques shortened the time necessary for selection, produced original information on a given genotype rapidly, and created new genotypes *in vitro* for salt stress tolerance.

Twenty six anther-culture-derived lines that tolerated to salt stress at 15dS/m have been selected and continuously screened in the net-house of CLRRI. Investigations on morphological and physiological traits will be implemented to select good lines for yield trials to understand GxE interaction.

Anther-culture-derived lines from test tube plantlets were cultivated in cluster in pots and divided into single seedlings for transplanting in the field. The aneuploid is eliminated through selection.

REFERENCES

Chu CC, CC Wang, CS Sun, KC Yin, CY Chu and FY Bi. 1975. Establishment of an efficient medium for anther culture of rice through comparative experiments on nitrogen sources. Sci Sinica 18:659-668

Ding Hui-jing, Jin Lian-ying, and Gao Yue-ying. 1981. The induction of Keng type pollen plant and the observation and utilization of the progenies. In Shen Jin-hua et al, eds. Studies on anther culture rice breeding.

Li Mei-fang, Chen Yin-quan, and Sen Jin-hua. 1981. Preliminary studies on the inheritance of characters of rice progenies derived from anther culture. In Shen Jin-hua et al, eds. Studies on anther culture rice breeding.

Murashige T, F Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15:473-497

Nguyen Thi Lang 2002. Protocol for basic of biotechnology. Agricutural Publishing House. Ho Chi Minh City, Vietnam

Zapata FJ, M Akabar, D Senadhira, and DV Seshu,IRRI. 1989. Salt tolerance of anther culture-derived rice lines. International Rice Research Institute Newsletter 14(1):6-7

Zhang Zhen-hua, Zheng Zu-ling, Sun wei-gen, and Zhang Lingnan. 1981. Breeding of new rice varieties through multiple recombination between lines of pollen plant.. In Shen Jing-hua et al, eds. Studies on anther culture rice breeding.

SUMMARY IN VIETNAMESE

Chọn dòng chống chịu mặn thông qua nuôi cấy túi phấn

Sáu tổ hợp lai được chọn để nuôi cấy túi phấn là C41/MR 159, Teqing/ Giza 159, Teqing/ Madhukar, Teqing/At 354, Teqing/Doc Phung, Teqing /Pokkali. Vật liệu được xử lý mặn với EC = 0, 6 và 15 dS/m.

Môi trường tạo callus: N6 bao gồm 0.5mg/l 2,4-D, 1mg/l NAA, 0.5mg/l BAP

Môi trường tái sinh: MS bao gồm 1mg/l NAA, 1mg/l BAP, 0.2mg/l Kinetin

Tỉ lệ cây sống sót trong điều kiện xử lý mặn là 37.5% trong tổ hợp Teqing/Giza 159, và 73.68% trong Teqing/Doc phung so với đối chứng Pokkali là 70.0%, ở độ mặn 6dS/m. Các dòng nuôi cấy túi phấn của tổ hợp lai Teqing/At 354, và C41/MR159 có tỉ lệ cây sống sót sau khi xử lý mặn ở EC= 15dS/m cao nhất (33,3 và 30,0%, theo thứ tự)