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Field performance of anther-culture-derived lines from F_1 crosses of Indica rices under saline and nonsaline conditions

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Summary. Field experiments were conducted to evaluate and compare the yield and agronomic characteristics of anther-culture-derived (AC) lines in rice and the parents under saline and nonsaline soil conditions. The yield stability of two entries was also evaluated by comparing their performance under two distinct nonsaline soil environments. The test entries were planted in randomized complete block design with four replications at each test location. Data were collected on grain yield, yield components, and agronomic characteristics. This study demonstrates that through anther culture, it is possible to produce homozygous diploid lines in a short time. The possibility of regenerating recombinants with desirable characteristics such as good plant type and salinity tolerance, higher yield, and increased resistance to pests and diseases from both the parents is high.

Key words: *Oryza sativa* L. – Anther culture – Yield – Salt tolerance

Introduction

Salinity is the most widespread soil problem in rice-growing countries (Senadhira 1987). There are approximately 400 million hectares of salt-affected land in the world (Flowers et al. 1977), of which 54 million are found in South and Southeast Asia (Akbar and Ponnamperuma 1982).

Breeding for salinity tolerance in rice is difficult. This may be due to the involvement of several genes controlling the character as well as the still insufficient knowledge of the mechanisms controlling rice salt tolerance (Akita and Cabuslay 1988; Yeo et al. 1990). It is aggravated by the fact that in areas where such stresses are found, only one cropping season per year is common, which implies that generation of stable lines takes much longer.

To complement conventional breeding for rice improvement, various tissue culture techniques, especially somatic cell culture, have been utilized (Sun et al. 1983; Oono 1983; Schaeffer et al. 1984). However, the use of somatic cell culture technique to improve salinity tolerance in rice has not been satisfactory. Either the heritability of salinity tolerance among regenerants was inconclusive (Oono and Sakaguchi 1978, 1980) or the regenerated plants produced from NaCl-enriched medium were 100% sterile and the plant type recovered was poor (Zapata and Abrigo 1986). In two separate studies using seed-derived calli, salt-tolerant variants were regenerated from calli that were grown in salt-stressed or salt-free media (Vajrabhaya et al. 1989; Subhashini and Reddy 1989). However, field tests of these materials were not conducted.

One approach to overcome the difficulty of obtaining salt-tolerant lines through exposure of cultured somatic cells to high levels of salinity is to use anther culture. Anther culture of F_1s allows the rapid fixation of homozygosity concurrent with the transfer of salt tolerance of one to the other parent having a desirable plant type.

This study is the first to deal with the field performance and evaluation of the yield potential of anther-culture-derived lines in rice bred for salinity tolerance under saline and nonsaline conditions. It also confirms the possibility of transferring through anther culture the high level of salt tolerance from traditional cultivars into high-yielding lines with improved plant type and pest and disease resistance, without losing the desirable characteristics of the latter in the process.

Table 1. Number of AC-derived lines from crosses involving salt-tolerant parents at different field evaluation stages

Cross no.	Parentage (female/male)	Los Banos (nonsaline)			Lanjagan (saline)	
		ON[a] 1986WS	OYT[b] 1987DS	RYT[c] 1989DS	1-row 1987WS	RYT 1988WS
IR51485	IR42/IR4630-22-2-5-1-3	9	1	1	1	1
IR51491	IR4630-22-2-5-1-3/Pokkali	21	3	2	10	2
IR51500	IR5657-33-2/IR4630-22-2-5-1-3	79	9	3	9	3
Total		109	13	6	20	6

[a] ON – Observational nursery; three-row plots
[b] OYT – Observational yield trial; ten-row plots (no replication)
[c] RYT – Replicated yield trial; ten-row plots (four replications)

Materials and methods

Test materials

Single crosses of IR51485, IR51491, and IR51500 were done in the 1985 dry season (DS, January to June) and IR53649 in the 1985 wet season (WS, July to December). These crosses involved six Indica parents. In general, these crosses were made to incorporate the salt tolerance of traditional varieties into the semidwarf, insect-tolerant, and high-yielding modern varieties.

Anther culture of these crosses was done in the season following hybridization, using the standard method established at the IRRI Tissue Culture Laboratory (Zapata 1985). The 109 anther-culture-derived lines obtained (Table 1) were initially screened at IRRI in the 1986WS. Selections based on agronomic characteristics resulted in the identification of six promising lines. Promising lines have excellent seedling vigor with short, erect leaves and sturdy culms. These lines together with the parents were planted in replicated yield trials (RYT) under saline and nonsaline conditions.

Test locations

Comparative performance under saline and nonsaline conditions. Replicated yield trials were conducted under nonsaline conditions at IRRI, Los Banos, The Philippines in the 1989DS, while RYT were conducted under saline conditions at Lanjagan, Iloilo, The Philippines in the 1988WS. Iloilo is classified as having coastal saline soil with electrical conductivity (Ece) ranging from 4 to 34 mmho/cm. The soil is high in organic matter (2.74%) and has a relatively high cation exchange capacity (11.5 meq/100 g).

Comparative performance under two nonsaline conditions. Bilocational RYT were conducted to evaluate the yield stability of AC lines under different nonsaline environments. Two anther culture lines from the cross IR53649 (AC7 and AC8) and the parents were concurrently grown for two seasons – 1988DS and 1988WS – at IRRI, Los Banos, The Philippines and in the Maligaya Rice Research Training Center (MRRTC) in Munoz, Nueva Ecija, The Philippines.

The rainy season starts late at MRRTC, so that the dry season extends up to July or August. Its annual rainfall total for 1988 was 1,816.7 mm.

The climate in Los Banos is characterized by early onset of rain in May, and the wet season extends up to December. Its 1988 annual rainfall total was 2,458.1 mm.

Field layout

The AC lines and parents were planted in 5.8 m × 2.0 m plots in randomized complete block design with four replications. Plant spacing was 20 cm each between hills and rows, with one seedling transplanted per hill. Soil fertilization rate (kg/ha) was 30:30:30 (N:P:K) in saline conditions and 100:50:50 and 80:50:50 during the dry and wet seasons, respectively, in nonsaline conditions. Eighty percent of all the fertilizer requirement was applied at planting and the rest at the panicle initiation stage. Complete insect and disease protection was provided throughout the growing season.

Data collection

Plant height and culm and panicle lengths were measured from 12 plants selected randomly from the center row of each plot. Seedling vigor, panicle exsertion, days to flower, panicle type, leaf senescence, and phenotypic acceptability were also recorded according to the standard evaluation system for rice (International Rice Research Institute 1988). The number of days to flowering was recorded when approximately 75% of the plants in a plot showed completely emerged panicles.

Data on yield components were taken from a 1 m^2 area (a total of 25 plants) of each test plot. Data on total tiller and panicle number of each plant were recorded prior to harvesting. All the harvested plants were dried and the individual panicles were hand-threshed. The filled grains were separated from the unfilled and incompletely filled grains by specific gravity (Zapata and Ella 1988).

Percent spikelet fertility was computed as:

$$\text{Fertility (\%)} = \frac{\text{Filled grains (no.)}}{\text{Total grains (no.)}} \times 100.$$

Data on 1,000-grain weight were taken randomly from three samples of each 25-plant sampling unit. Seed samples were oven-dried at 130 °C for 4 h to reach 0% moisture content. The 1,000-grain weight was determined per sample using the formula:

$$\text{1,000-grain wt (g)} = \frac{\text{Wt of oven-dried filled grains (g)}}{\text{No. of filled grains}} \times 1{,}000.$$

Grain yield was determined using the formula (Yoshida 1981):

$Y = N \times W \times F \times 10^{-5}$, where:

Y = grain yield (tons/ha)
N = spikelet number/m^2
W = 1,000-grain weight (g), and
F = filled spikelets (%).

Statistical analysis

Analysis of variance (using the single factor RCB) was performed and Duncan's Multiple Range Test (DMRT) was used to compare the yield and yield components of the entries.

Results

Field evaluation of agronomic characteristics

Under saline conditions, all AC-derived lines exhibited better seedling vigor and showed better panicle exsertion than the parents (Table 2). AC_1 and AC_4 were rated excellent for phenotypic acceptability and panicle type. In addition, AC_1 showed late and slow senescence compared to the other test lines. The AC lines were either earlier to flower than both parents (as in the case of AC_2 and AC_3) or intermediate between the parents.

Under nonsaline conditions, however, most of the lines had intermediate flowering dates relative to the parents, except for AC_1, which had longer days to flower than both parents (Table 3). As in saline conditions, the phenotypic acceptability scores of AC_1 and AC_4 were high.

In both environments, all AC-derived lines had short, erect leaves with sturdy culms.

Replicated yield trials under saline and nonsaline conditions

AC_1 had a significantly higher grain yield than IR4630 and the salt-tolerant check, Pokkali, under both conditions (Table 4). Although AC_1 had a relatively low 1,000-grain weight, this was compensated for by its high number of filled grains.

AC_2 and AC_3 did not significantly differ in yield from the parents. The yields of these lines were adversely affected by low spikelet fertility.

On the other hand, AC_5 had a significantly higher yield than both parents under saline conditions. It also outyielded the parents under nonstressed conditions, although the yield advantage over the higher-yielding parent, IR5657, was not significant. AC_4 and AC_6 were also promising, with respect to yield. The higher yields of these lines were due to the higher values of their yield components.

Yield evaluation under two nonsaline environments

AC_7 and AC_8 had significantly higher grain yields than Nona Bokra in both seasons and locations, but were comparable in yield and filled grain to the other parent (Table 5). In general, the spikelet fertility of the entries were low. This could be due to the typhoons during the flowering and grain-filling stages.

Table 2. Morphological characteristics of AC-derived lines from crosses involving salt-tolerant parents under saline conditions

AC line or parent	Seedling[a] vigor	Panicle[b] exsertion	Panicle[c] type	Senescence[d]	Phenotypic[e] acceptability	Days to flower
IR42	1	–[f]	–	–	–	–
IR4630	3–5	5	1–5	5	3	110
AC1	1	3	1	1	1	115
IR4630	3–5	5	1–5	5	3	110
Pokkali	5	3	5–9	5	3	96
AC2	3–5	5	5	5	3	90
AC3	3	5	5	5	3	92
IR5657	1	5	5	5	5	92
IR4630	3–5	5	1–5	5	3	110
AC4	1	3	1	5	1	94
AC5	1	5	5	5	3	94
AC6	3	3	5	5	3	96

[a] Seedling vigor (scored in seed bed without salt stress): 1 = extra vigorous, 3 = vigorous, 5 = intermediate or normal
[b] Panicle exsertion: 1 = well exserted, 3 = moderately exserted, 5 = just exserted
[c] Panicle type: 1 = compact, 5 = intermediate, 9 = open
[d] Senescence: 1 = late and slow, 5 = intermediate, 9 = early and fast
[e] Phenotypic acceptability: 1 = excellent, 3 = good, 5 = fair
[f] Died 2 weeks after transplanting

Table 3. Agronomic characteristics of AC-derived lines and their parents under nonsaline conditions. IRRI, 1988DS

AC line or parent	Plant height[a] (cm)	Panicle length[a] (cm)	Tiller no.[a]	Days to flower	Exsertion	Phenotypic acceptability
IR42	126	27	19	102	3	3
IR4630	128	28	11	102	3	3
AC1	130	29	20	106	3	1
IR4630	128	28	11	102	3	3
Pokkali	133	29	6	74	3	3
AC2	124	31	10	83	3	3
AC3	122	32	11	86	3	3
IR5657	95	26	21	85	3	3
IR4630	128	28	11	102	3	3
AC4	116	24	18	88	3	1
AC5	104	23	17	88	3	3
AC6	108	24	15	90	3	3

[a] Average of 12 plants per replicate

Table 4. Yield component * of promising anther-culture-derived lines and their parents under saline (S) and nonsaline (NS) conditions

AC line or parent	Filled grain/m²		Fertility (%)		1,000-grain wt (g)		Grain yield (tons/ha)	
	S[a]	NS[b]	S	NS	S	NS	S	NS
IR42	–[c]	26,874 A	–	76.3 CD	–	17.2 E	–	4.6 AB
IR4630	9,128 CDE	15,265 C	79.5 ABC	72.8 DE	20.9 A	20.8 BC	1.9 EF	3.2 C
AC1	17,061 A	27,397 A	72.0 BC	78.8 BC	18.2 A	18.2 D	3.1 A	5.0 A
IR4630	9,128 CDE	15,265 C	79.5 ABC	72.8 DE	20.9 A	20.8 BC	1.9 EF	3.2 C
Pokkali	7,922 DE	14,491 C	76.8 ABC	84.8 A	22.7 A	22.3 A	1.8 EF	3.2 C
AC2	8,264 DE	15,294 C	73.0 BC	69.9 E	21.3 A	20.8 BC	1.8 EF	3.2 C
AC3	8,682 DE	14,146 C	70.3 C	70.3 E	22.5 A	22.5 A	2.0 DEF	3.2 C
IR5657	10,604 BCD	21,370 B	75.0 BC	83.1 AB	19.6 A	20.1 C	2.1 CDEF	4.3 AB
IR4630	9,128 CDE	15,265 C	79.4 ABC	72.8 DE	20.9 A	20.8 BC	1.9 EF	3.2 C
AC4	11,782 BC	19,391 B	79.9 AB	78.8 BC	21.8 A	22.2 A	2.6 ABCD	4.3 AB
AC5	13,280 B	21,072 B	81.0 AB	85.1 A	20.8 A	21.5 AB	2.8 AB	4.5 AB
AC6	10,630 BCD	19,870 B	74.4 BC	83.2 AB	20.7 A	20.8 BC	2.2 BCDE	4.1 B

[a] Iloilo, RYT 1988WS
[b] IRRI, RYT 1989DS
[c] Died 2 weeks after transplanting
* In each column, numbers followed by a common letter are not significantly different at 1% by DMRT

Table 5. Comparison of filled grain and grain yield[a] of AC-derived lines from IR53649 (IR8192/Nona Bokra) and their parents in two nonsaline environments

AC line or parent	Filled grain (per m²)				Grain yield (t/ha)			
	IRRI		MRRTC		IRRI		MRRTC	
	1988DS	1988WS	1988DS	1988WS	1988DS	1988WS	1988DS	1988WS
IR8192	26,010 A	10,895 A	29,910 A	11,658 B	5.39 A	2.40 A	6.44 A	2.51 A
Nona Bokra	19,493 B	6,293 B	13,337 B	5,942 C	3.58 B	1.30 B	2.88 B	1.20 B
AC7	23,993 AB	11,538 A	24,122 A	12,629 AB	5.42 A	2.55 A	5.46 A	2.80 A
AC8	26,724 A	13,002 A	29,896 A	16,186 A	5.24 A	2.42 A	5.95 A	3.09 A

[a] Average of four replications. Within each column, means followed by a common letter are not significantly different at 1% level by DMRT

Discussion

The plant vigor and yield of the anther-culture-derived lines were either better than both parents or as good as the better parent in both saline and nonsaline field conditions. The better performance of the anther culture lines was more likely due to the possible screening of recombinants in vitro, such that only those microspores that could tolerate the stress of being subjected to artificial growth conditions survived and eventually regenerated into plants. As a consequence, anther culture hastened the fixation of vigor not only as a result of fixation of homozygosity but also by discriminating the less-fit recombinants. Similarly, in barley anther culture, lines exhibiting elevated levels of tolerance to salt probably resulted from recombination of genes rather than from spontaneous mutation (Ye et al. 1987).

In our study, two of the six AC lines tested yielded higher than the better parent under normal conditions, while four were superior to the better parent under saline conditions (Table 4). This indicates that most of the anther culture lines tested are superior to the parents, since these were able to give higher yields especially in the saline environment. The ability of the anther culture plants to produce well in conditions with or without stress is critical, as the amount of salinity can vary across a field and during the growing season.

The significant increase in the number of filled grains contributed primarily to the high yield of the most outstanding line, AC_1. As mentioned earlier, yield is a direct function of the spikelet number per square meter, 1,000-grain weight, and fertility. Although this line exhibited a lower 1,000-grain weight than the better parent, its significantly higher filled grain compensated for this negative factor, to give a significantly high yield. This result is similar to that observed in protoplast-derived rice plants, which yielded higher due to increased panicle number, despite the fact that they had lower seed fertility and 1,000-grain weight than the control (Ogura et al. 1987). Furthermore, all the AC lines had short, erect leaves with sturdy culms, traits inherited from the Indica parents, making them more resistant to lodging than Pokkali and Nona Bokra, which had long, broad, drooping leaves and tall, weak stems.

Stability of yield of the AC-derived lines and their parents is evident in the bilocational test under nonstressed conditions (Table 5). This indicates that the characteristics of the doubled haploid lines, especially vigor, have been fixed through anther culture.

Previous studies on stressing somatic cells in salt-enriched media were not always successful, simply because the regenerated plants were not fertile and aberrations were common due to the prolonged exposure of cells to toxic salt levels (Zapata and Abrigo 1986). However, in the present study using anther culture, 6 out of 109 lines (5.5%) were promising. This percentage producing a salt-tolerant recombinant through anther culture is apparently higher than conventional breeding since, in the latter, thousands of plants generally need to be screened to identify a desirable recombinant.

It is evident that the advantages of anther culture, such as significantly shortening the breeding cycle and increasing the selection efficiency (as shown by the recovery of more recombinants with desirable characteristics from fewer number of lines available for selection), have been recognized.

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