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DEVELOPING SALT TOLERANCE IN RICE BY MUTAGENESIS

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ABSTRACT

A series of experiments was conducted to determine the variability and the genetics of tolerance to salinity in indica rice. OMCS2000 and OM1490 and seeds were gamma-irradiated with the following dosages: 0, 10, 20, 30 Gy and the generated M_2 plants were screened for salt tolerance using a salinized hydroponic culture system.

Screenings were continued up the M_3 generation until putative salt tolerant mutants were identified. Survival rates of mutants were similar to those of tolerant checks Pokkali and IR29 under artificial salinization and commercial variety AS996 under the three level of EC = 0.6 and 15 dS/m. Screening techniques developed were depend upon agronomic traits of yield component characters. All experiments were conducted in a glasshouse with controlled relative humidity at CLRRI. A total of 500 mutant lines M_2 and M_3 indica were tested for tolerance to salinity at seedling stage. Large variation in salt tolerance among mutants lines was detected. Of the 500 line tested, 25 were found tolerant, 198 moderately, and 277 susceptible. The tolerant lines grown contitute to get M_4. Screening was conducted at both vegetative and reproductive stages. The parents, 20 lines of M_4 and Pokkali , IR29, AS996 were grown under salt stress. Of the 20 lines tested, 7 were found to be tolerant at salt stress of 15 dS/m; 13 susceptible. Salt stress reduced number of filled grains per panicle, 1000-grain weight and grain yield. Three lines OM1490-52 and OM1490-55, OMCS2000-451 exhibited better agronomic traits and higher yield. Two line OM2717 and OM2718 are recommended for saline areas, they can yield 4-5 t / ha under EC = 6.0 to 9.0 dS/m. Additive main effects and multiplicative interaction (AMMI) model with a predictive assessment of accuracy was carried out to explain GxE interaction. Grain yield was improved and it also developed seedling tolerance to salinity. The salt tolerant mutants could be atriibuted to use for further genetic studies, sources of salt tolerance in hybridization breeding program.

Key words: Mutant, Salt tolerance, Yoshida nutrient solution

INTRODUCTION

Saline soils are one of the major problems in agriculture, limiting crop growth and production in many parts of the world. The impact of salinity on agricultural productivity has been examined. Rice is moderately sensitive to salinity (Akbar et al 1972, Koreb and Abdel-Aal 174, Maas and Hoffman 1977). To overcome salinity problem, different strategies such as reclamation, drainage, water control, and varietal improvement can be used. Such as methods, however, have proven to be impractical due to the high costs and low returns to investments. The most practical approach is development of tolerant varieties. Several workers (Lang 2000, Buu et al 2003) have reviewed the concept of breeding for salt tolerance. The basic requirements are genetic variability, screening techniques and understanding of genetics and physiological mechanism of tolerance. Several rapid and reliable screening techniques are now available (Gregorio et al. 1993; Lang et al 2001a, b), but this information is limited to indica type development for salt tolerance in rice induced by mutagenesis. So, this experiment aimed to study the genetic for salt tolerance in rice induced by mutagenesis with specific objective were the following :

- To evaluate promising mutant lines under saline condition
- To find out selection criteria under saline condition
- To understand relationship between physiological characters and visual assessment for salt tolerance at seedling stage.

MATERIALS AND METHODS

Mutants lines of wild OM1490 and OMCS2000 were compared to tolerant check as Pokkali, and susceptible check as IR29 and local check AS996. This experiment was conducted in the experimental farm of Cuu Long Delta Rice Research Institute, Vietnam

1. Salinization of nutrient solution

Salinize the Yoshida nutrient solution by adding NaCl while stirring up to the desired EC (3 and 7.5g NaCl/ litre), nutrient solution gives an EC of 6 and 15 dS/m accordingly. Fill up the trays with this solution high enough to touch the nylon net bottom of the styrofoam. The effective culture solution needed per tray is 5-10 litres.

2. Management of nutrient solution

The maintenance of the nutrient solution is very important. Considerable attention has to be given in adjusting the pH daily.

Monitoring and maintaing the pH of the culture solution is very critical because this checks the balance of the available nutrients. Significant deviation of culture solution pH from 5.0 will make some nutrients toxic and others deficient, thus a reliable pH meter and its regular calibration are essential (Lang et al 2001b).

Due to add ammonium and nitrate are sources of nitrogen, the pH will decrease during the first few days because ammonium ion is favorably absorbed by the plant nitrate ion.

The pH will then increase when ammonium ion is depleted and more nitrate ion taken up by the plants. The increase of the pH in the nutrient solution could be used as an indicator that nitrogen source starts to be deficient. Change the nutrient solution every 7 day (Gregorio et al. 1997).

Sometime you can see algae during tested. However, you can adjust the pH twice a day. In the case of evaporation and transpiration, solution volume will be lossed in the trays. Make up the volume with distilled water every 3 days.

3. Handling of seedling and salinization

Tested seeds have to be heat treated for 5 days in an oven set at 40^{o}C to break seed dormancy. Proper breaking of the seed dormancy is very essential in this screening technique. Delay in germination of some entries will like make these entries more sensitive to salt. Seedling vigor has great advance at this point since salinization occur at very early seedling stage. After breaking the dormancy, surface sterilize seeds with fungicide and rinse well with distilled water. Place sterilized seeds in petri dishes with moistened filter papers and incubate at 30^{o}C for 48h to germinate. Sow two pregerminated seeds per hole on the styrofoam seedling float. The radicle should be inserted through the nylon mesh.

There are adequate nutrients in the endosperm for the seedlings to grow normally for 3-4 days. After 3 days, when seedlings are well established, replace the distilled water with salinized nutrient solution. Initial salinity is at EC = 6 dS/m. Three days later, increase salinity to 15 dS/m by adding NaCl to the nutrient solution.

Renew the solution every 8 days and maintain the pH at 5.0 daily.

Tested entries can be rated at 16 and 18 days after initial salinization.

4. Screenhouse

The plants were scored for salt injury symptom and general appearance based on the SES scale by IRRI.

5. Evaluation of salt tolerance through SD (survival day)

When seedlings were grown for 17 days, they were transferred to the solution containing NaCl under EC = 15 dS/m. Seedling survival days were recorded in days from seedling to death. The solution was changed by fresh one every weeks and pH was monitored at 5.0.

In the screening phase of the experiment, it is essential to include a susceptible and tolerant checks.

6. Performance test

Agronomic characters such as plant height, panicle length, panicle per hill, spikelets per panicle, filled grain (%), grain yield were investigated and compared with check varieties under saline field trials with 4 replications. Evaluations of these lines were done at maturity. Analysis of variance and mean comparisons of the data from saline field were carried out.

RESULT AND DISCUSSION

1. Generation and screening of mutants for salt tolerance

OM1490 and OMCS2000 seeds were gamma-irradiated with the following dosages: 20 GY and the generated M_2 plants were screened for salt tolerance using a salinized hydroponic culture system.

Screenings were continued up the M_3 generation until putative salt tolerance mutants were identified (Table 1).

Survival rates of mutants were compared to those of tolerant check Pokkali, susceptible check IR29 and commercial check AS996 under artificial salinization.

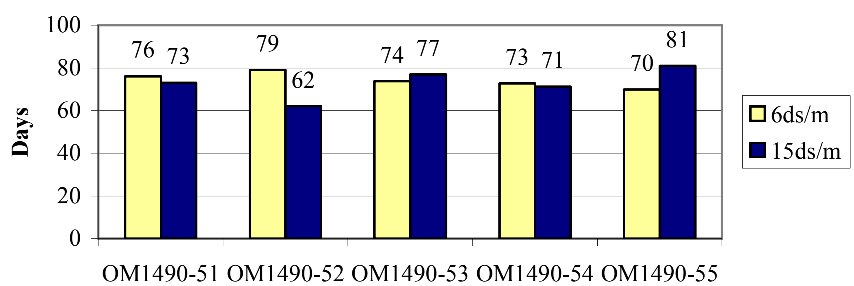
Table 1: Screening under salt stress of mutants

Dosage GY	M_2 plants screened	M_2 plants survived	M_3 plants survived	M_4 plant survived	M_5 plants survived	M_6 plants survived
5	2560	102	17	12	5	1
10	3030	200	60	12	3	1
20	1100	18	8	3	2	1
30	210	5	0	0	0	0

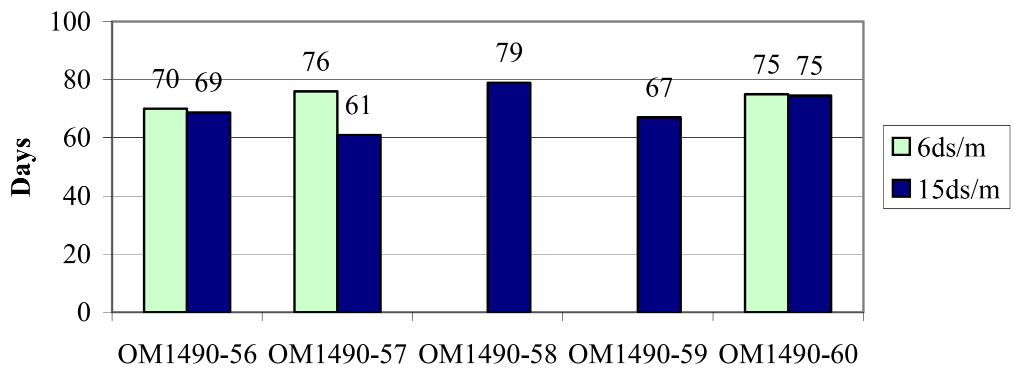
Survival days of OM1490 mutant lines ranged from 70 days to maturity at EC = 6 dS/m. Of these tested lines OM1490-58 and 59 could available days to maturity. Under the EC 15 dS/m, survival days of OM1490 mutant lines ranged from 61 to 81 days. Of these tested mutant lines OM1490-53, 55 and 58 exhibited the longest survival days.

Survival days of OMCS2000 mutant lines ranged from 58 to days to maturity at EC 6 dS/m. Among those lines OMCS2000-454, 456, 457 and 458 were survived up to maturity. Under 15 dS/m, survival days of these mutant lines ranged from 62 to days to maturity. Of these lines OMCS2000-459 and 460 were survived up to maturity. The survival days of OMCS2000-453, 454 and 455 under salt stress were noticed to be the same of tolerant check.

Figure 1: Survival Days of OM1490 Mutant Lines



Fugure 2 : Survival Days of OM1490 Mutant lines



Fugure 3 : Survival Days of OMCS2000 Mutant Lines

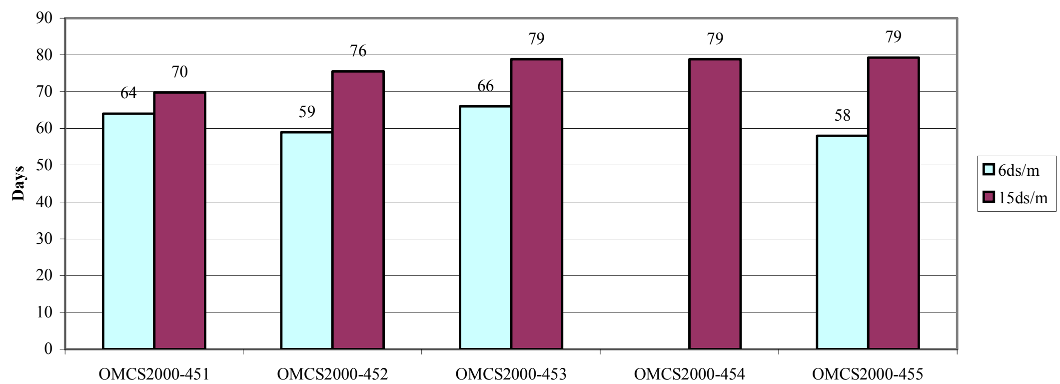
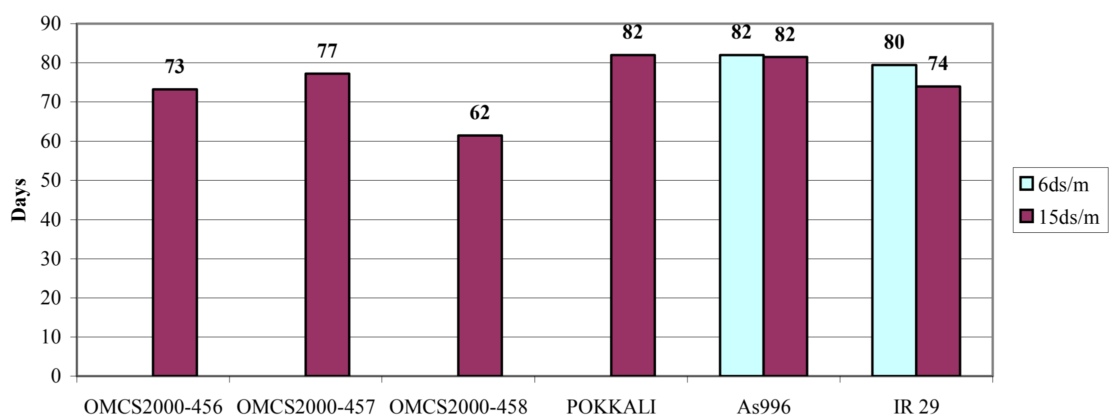


Figure 4:Survival Days of OMCS2000 Mutant Lines



2.Agronomic characters of OM1490 mutants and OMCS2000 mutants

Table 2 : Agronomic traits of OM1490 mutants

Designation	HD (days)	Plant height (cm)	Pan. No. /hill	Pan. length	Total grain	Filled grain (%)	1000-grain wgt.	HI	Yield (g/plt)
OM1490-51	63	62.73	**5**	18.37	51	37.75	14.83	0.250	2.22
OM1490-52	66	65.26	4	17.11	78	32.33	**15.45**	0.301	**2.85**
OM1490-53	64	64.02	4	16.11	52	43.27	14.66	0.299	**2.81**
OM1490-54	65	60.17	4	11.95	48	25.96	13.79	0.192	1.44
OM1490-55	67	64.01	4	17.64	**74**	42.51	**15.09**	0.332	2.67
OM1490-56	66	53.89	4	16.02	57	**53.55**	14.91	0.337	2.36
OM1490-57	63	61.68	**5**	15.79	60	43.97	**15.20**	0.328	2.17
OM1490-58	62	62.29	**5**	15.78	46	33.88	**15.70**	0.161	1.92
OM1490-59	66	62.58	4	15.83	61	**64.74**	14.90	0.395	2.35
OM1490-60	64	66.13	4	16.49	65	43.54	13.77	0.336	1.73
POKKALI	72	114.5	4	20.70	63	47.58	18.16	0.185	3.18
AS996	63	67.33	4	20.56	63	25.76	12.88	0.116	1.26
IR29	71	61.93	3	20.37	52	54.36	15.80	0.177	1.87
SE	0.67	2.06	0.67	0.65	4.21	4.815	8.35	0.043	0.62
LSD (5%)	1.87 **	5.76 **	1.89 ns	1.83 **	11.8 **	13.49 **	4.5 ns	0.121 **	1.75 ns

** = highly significantly different at 1%, ns = non significantly different at 5%

Table 3 : Agronomic traits of OMCS2000 mutants

Designation	HD (days)	PH	PN	PL	TG	FG (%)	1000 GW	HI	Yield (g/plt)
OMCS2000-451	62	69.53	6	18.87	65	34.57	15.48	0.176	**3.06**
OMCS2000-452	64	71.17	4	18.81	65	34.52	14.94	0.172	1.71
OMCS2000-453	62	75.26	5	18.10	60	33.22	15.90	0.281	1.98
OMCS2000-454	62	72.25	4	18.05	66	37.55	16.75	0.192	1.09
OMCS2000-455	63	72.80	5	20.03	73	30.28	16.47	0.373	2.01
OMCS2000-456	62	71.28	4	20.91	60	**36.63**	16.30	0.171	1.87
OMCS2000-457	61	70.81	5	18.25	63	18.93	16.11	0.136	1.69
OMCS2000-458	63	73.23	5	18.22	62	**36.69**	**17.72**	0.241	1.95
OMCS2000-459	64	72.96	5	18.75	66	26.72	16.78	0.160	2.3
OMCS2000-460	64	71.42	5	19.64	68	27.44	17.12	0.185	1.94
POKKALI	73	114.5	4	20.70	63	47.58	18.16	0.185	3.18
AS996	63	67.33	4	20.56	63	25.76	12.88	0.116	1.26
IR29	71	61.93	3	20.37	52	54.36	15.80	0.177	1.87
SEm	0.57	2.198	0.60	0.978	3.65	3.701	8.14	0.326	0.44
LSD (5%)	1.59 **	6.158 **	1.69 ns	2.739 ns	10.2 ns	10.37 **	4.72 ns	0.914 **	1.23 ns

** = highly significantly different at 1%, ns = non significantly different at 5%

HD : heading date 1000GW : 1000-grain weight (gm) PH : Plant height (cm)
HI : Harvest Index PN : Panicle number per hill Y : Yield (g / plant)
PL : Panicle length (cm) TG : Total grains per panicle FG% : Filled grain %

Among OM1490 mutants, the highest yield was obtained in OM1490-52 and 53. All of these mutants showed higher yield performance than commercial check AS996. However, F value for grain yield was not significantly different at 5% level, and the ones for other traits significantly (table 2).

Among OMCS2000 mutants, the highest yield was obtained in OMCS2000-451. All of these mutants showed higher yield performance than commercial check AS996. However, F value for grain yield was not significantly different at 5% level, and the ones for other traits significantly (table 2).

3. Phenotyping

Table 4 : Salt tolerance score and yield of mutants under salt stress

Designation	1 dS/m	6 dS/m	15 dS/m	Yield (g/plant)
OM1490-51	1	7	7	2.22
OM1490-52	1	5	**5**	**2.85**
OM1490-53	1	7	7	**2.81**
OM1490-54	1	7	7	1.44
OM1490-55	1	5	**5**	**2.67**
OM1490-56	1	5	7	2.36
OM1490-57	1	5	**5**	2.17
OM1490-58	1	5	7	1.92
OM1490-59	1	**3**	7	2.35
OM1490-60	1	**3**	9	1.73
OMCS2000-451	1	**3**	**5**	**3.06**
OMCS2000-452	1	5	7	1.71
OMCS2000-453	1	5	7	1.98
OMCS2000-454	1	5	9	1.09
OMCS2000-455	1	**3**	7	2.01
OMCS2000-456	1	5	7	1.87
OMCS2000-457	1	5	7	1.69
OMCS2000-458	1	5	**5**	1.95
OMCS2000-459	1	**3**	**5**	2.3
OMCS2000-460	1	**3**	**5**	1.94
Pokkali (T check)	1	3	5	3.18
AS996(local check)	1	7	7	1.26
IR29 (S check)	1	9	9	1.87

The tolerant mutants at salt stress of 6dS/m were recorded: OM1490-59, OM1490-60, OMCS2000-451, OMCS2000-455, OMCS2000-459 and OMCS2000-460.

The tolerant mutants at salt stress of 15dS/m were OM1490-52, OM1490-55, OM1490-57, OMCS2000-451, OMCS2000-458, OMCS2000-459 and OMCS2000-460 as Pokkali check.

Of these tested lines, OMCS2000-451 offered the highest yield under both salt stress of 6 dS/m and 15 dS/m.

4. Correlation among agronomic parameters under salt stress

Table 5 :Correlation coefficient of agronomic traits and yield

	HD	PH	PN	PL	TG	FG%	1000 GW	HI	EC	Yield
HD	1									0.06ns
PH	0.17	1								0.36ns
PN	-0.10	0.31	1							**0.65****
PL	-0.07	0.34	0.16	1						0.21ns
TG	-0.16	0.30	-0.01	**0.52**	1					0.13ns
FG%	0.26	0.23	0.23	0.05	-0.10	1				**0.54***
GW	0.20	**0.64**	0.26	0.37	0.05	-0.17	1			0.15ns
HI	0.09	0.16	0.32	0.08	0.17	**0.61**	-0.25	1		**0.57***
EC	-0.03	-0.31	**-0.66**	-0.08	0.16	-0.47	**-0.63**	-0.39	1	**-0.62****

* = significantly correlated, ** = highly significantly correlated, ns = non significantly correlated, - = negatively correlated

HD: heading date PH: Plant height PN: Panicle number per plant
EC: Electrical Conductivity PL: Panicle length Y: Yield
TG: Total grains per panicle FG%: Filled grain % 1000GW: 1000 grain weight
HI: Harvest Index

Table 5 indicated that:

Positive and significant correlation between panicle number per plant, harvest index, filled grain percentage with yield were recognized.

Negative and significant correlation between EC and yield was noticed.

5. Grain yield

Table 6: Grain yield of OM1490 mutants under different EC levels (g/plant)

Designation	0 dS/m	6 dS/m	15 dS/m	Average
OM1490-51	5.45	**0.78**	0.42	**2.22**
OM1490-52	**6.97**	**1.23**	0.34	**2.85**
OM1490-53	**7.46**	0.30	0.66	**2.81**
OM1490-54	3.62	**0.71**	0.00	1.44
OM1490-55	**6.85**	**0.80**	0.38	**2.67**
OM1490-56	4.73	0.09	**2.27**	**2.36**
OM1490-57	4.73	**0.78**	**1.01**	**2.17**
OM1490-58	4.19	0.13	**1.43**	1.92
OM1490-59	4.80	0.27	**1.98**	**2.35**
OM1490-60	2.84	0.25	**2.09**	1.73
POKKALI	6.66	0.12	2.76	3.18
AS996	3.10	0.05	0.64	1.26
IR29	4.68	0.18	0.75	1.87
Average	5.08	0.44	1.13	2.22

SE for EC effect = 0.30, and $LSD_{0.05}$ = 0.84**
SE for variety effect = 0.62, and $LSD_{0.05}$ = 1.75ns
SE for ECx variety effect = 1.08, and $LSD_{0.05}$ = 3.03ns
** = highly significantly different at 1%, * = significantly different at 5%, ns = non significantly different at 5%

Table 7: Grain yield of OMCS2000 mutants under different EC levels (g/plant)

Designation	0 dS/m	6 dS/m	15 dS/m	Average
OMCS2000-451	**4.47**	0.09	**4.63**	**3.06**
OMCS2000-452	3.14	0.13	**1.86**	1.71
OMCS2000-453	2.93	**0.40**	**2.63**	1.98
OMCS2000-454	0.99	**0.61**	1.68	1.09
OMCS2000-455	2.22	**0.56**	**3.25**	**2.01**
OMCS2000-456	**4.45**	0.06	1.11	1.87
OMCS2000-457	**4.63**	0.09	0.36	1.69
OMCS2000-458	**4.06**	**0.89**	0.89	1.95
OMCS2000-459	**5.45**	0.18	1.27	**2.30**
OMCS2000-460	**4.40**	**0.30**	1.13	1.94
POKKALI	6.66	0.12	2.76	3.18
AS996	3.10	0.05	0.64	1.26
IR29	4.68	0.18	0.75	1.87
Average	3.94	0.28	1.76	1.99

SE for EC effect = 0.21; and $LSD_{0.05}$ = 0.59**
SE for variety effect = 0.44, and $LSD_{0.05}$ = 1.23ns
SE for ECx variety effect = 0.76, and $LSD_{0.05}$ = 2.13**
** = highly significantly different at 1%, * = significantly different at 5%, **ns** = non significantly different at 5%

OM1490 mutants

Under salt stress of 6 dS/m; OM1490-51, OM1490-52, OM1490-54, OM1490-55 and OM1490-57 performed high yield potential.

Under salt stress of 15 dS/m; OM1490-56, OM1490-57, OM1490-58, OM1490-59 and OM1490-60 offered high yield potential.

At the control EC = 0 dS/m, OM1490-52, OM1490-53 and OM1490-55 offered high yield potential.

OMCS2000 mutants

Under salt stress of 0dS/m; high yield potential was recognized as OMCS2000-451, OMCS2000-456, OMCS2000-457, OMCS2000-458, OMCS2000-459 and OMCS2000-460.

Under salt stress of 6 dS/m, high yield potential was OMCS2000-453, OMCS2000-454, OMCS2000-455, OMCS2000-458 and OMCS2000-460

Under salt stress of 15 dS/m, high yield potential was OMCS2000-451, OMCS2000-452, OMCS2000-453 and OMCS2000-455.

CONCLUSION

Promising lines under salt stress of 6dS/m were recognized on the mutant lines OM1490-59, OM1490-60, OMCS2000-451, OMCS2000-455, OMCS2000-459 and OMCS2000-460. Under salt stress of 15 dS/m OM1490-52, OM1490-55, OM1490-57, OMCS2000-451, OMCS2000-458, OMCS2000-459 and OMCS2000-460 were described as moderately tolerance as tolerant score of Pokkali check.

High yield potential was noticed in the following genotypes as OM1490-52, OM1490-53, OM1490-55 and OMCS2000-451.

Genotypic coefficient variance was much smaller than phenotypic coefficient variance with respect to panicle number per hill, panicle length and yield indicating the influence by environment. Plant height, panicle lenght, total grains per panicle and 1000-grain weight were also highly influenced by environmental factors.

Panicle number per plant, filled grain percentage (%) and harvest index were considered as direct effects to grain yield under saline condition. Therefore the most important selection criteria under saline conditions were recommended as these three traits.

These mutants can now be used by breeders for further genetic studies such as rice functional genomics, molecular marker assisted selection at CLRRI.

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Chọn tạo giống lúa chống chịu mặn bằng đột biến gen

Thí nghiệm được thực hiện nhằm xác định biến di di truyền tính trạng chống chịu mặn của kiểu gen indica, đặc trưng là hai giống lúa OM1490 và OMCS2000, được xử lý bằng tia gamma với liều lượng chiếu xạ 0, 10, 30, 30 Gy. Thí nghiệm được quan sát ở hai thời kỳ: giai đoạn mạ và giai đoạn phát dục

Giống lúa có triển vọng ở điều kiện EC= 6dS/m là: OM1490-59, OM1490-60, OMCS2000-451, OMCS2000-455, OMCS2000-459 and OMCS2000-460.

Giống lúa có khả năng chống chịu mặn tốt ở EC = 15dS/m là OM1490-52, OM1490-55, OM1490-57, OMCS2000-451, OMCS2000-458, OMCS2000-459, OMCS2000-460, tương đương với giông1ng chuẩn kháng Pokkali