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Research Article

Gene Inheritance pattern of salinity tolerance identified through phenotypic scoring and marker data in mapping population of rice (Oryza sativa L.)

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Abstract

Soil salinity is one of the major constraints affecting rice production worldwide. Susceptibility or tolerance of rice plants to high salinity is a coordinated action of multiple stress responsive genes. Salt tolerant varieties can be produced through marker-assisted selection by introducing salt-tolerance genes. The current study aims to understand the inheritance pattern of salinity tolerance genes. The investigation was carried during 2014-2017, 25 days old seedlings were raised in nursery beds at Agricultural College and Research Institute, Madurai, Tamil Nadu and transplanted to main field at Vedapatti village in Thoothukudi district which is a hot spot area for saline condition in a Randomized Block Design under saline stress condition with two replications. After 21 days of transplanting, the phenotypic scoring was done for all the six generations. Ten plants each in P1, P2 and F1, 250 plants in F2 and 150 plants each in BC1 and BC2 for each cross in each replication were maintained for the study. The investigation indicated that the inheritance pattern of salinity tolerance gene was duplicate dominant genes as observed from the F2 ratio of 15:1 (susceptible: tolerant) in crosses 1 and 2 under salinity condition in both In-vivo and In-vitro.

Keywords: *Rice, Salinity, Inheritance pattern, Salt tolerance, Duplicate Genes*

Introduction

Rice is the staple food of more than 50% of the world's population (Aggarwal and Mall 2002). By the year 2025, 21% increase in rice production will be needed over that of year 2000 (Bhuiyan *et al.,* 2002). Salinity is one of the major obstacles in increasing production in rice growing areas worldwide. Therefore, development of salt tolerant varieties has been considered as one of the strategies to increase rice production in saline prone areas. The response of rice to salinity varies with growth stage. Several studies indicated that rice is tolerant during germination, vegetative growth stage and at maturity, and becomes very sensitive during early seedling stage (2-3 leaf stage), pollination and fertilization (Bhowmik *et al.,* 2009). At present time, to develop salinity tolerance rice varieties along with high-yield is a big challenge to the rice breeders. Breeding for salinity tolerance in rice requires reliable screening techniques. Screening of germplasm at seedling stage is readily acceptable as it is based on a simple criterion of selection; it provides rapid screening difficult at vegetative and reproductive stage (Gregorio *et al.,* 1997).

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The biotechnological tools offer an opportunity to the rice breeders along with existing breeding strategies to overcome the salt related tribulations. The ability of the plant to tolerate the salt stress up to an extent is of paramount importance to manage the resources optimally and this is the reason to develop the tailored crops with higher salt tolerance. Nowadays, major soil reclamation programmes involve both biological and hybrid approaches to combat the salt problem. These techniques must be rapid to keep pace with the large amount of breeding materials generated. Screening under field conditions is difficult due to stress heterogeneity presence of other soil related stresses and the significant influence of environmental factors such as temperature, relative humidity and solar radiation. These complexities, together with the degree of control of salinity and reproducibility, cause difficulties in developing and using reliable methods of screening voluminous materials. The conventional methods of plant selection for salt tolerance are not easy because of the large effects of the environment and low narrow sense heritability of salt tolerance (Gregorio, 1997). This hinders the development of an accurate, rapid and reliable screening technique. However, DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Recent progress and technical advances in DNA marker technology permit reduction of time and accuracy of breeding where pronounced effects of environment lead to poor selection efficiency (Bhowmik *et al.,* 2009).Keeping the above facts in view, the present investigation aimed to study and analyze inheritance pattern of salinity tolerance by using P_1, P_2, F_1, F_2, BC_1 and BC_2 generations derived from a cross between a salt-tolerant line, FL478, and a salt-susceptible varieties like ADT 43 and Improved White Ponni (IWP). The results will helpsto elucidate the inheritance pattern and number of genes controlling the salt-tolerance and will be useful in breeding programs for the development of salt tolerance coupled with high yielding rice varieties.

Materials and Methods

The six generations of the two cross combinations (cross 1 (ADT 43 x FL 478) and cross 2 (IWP x F L478)) were raised in nursery beds at Agricultural College and Research Institute, Madurai, Tamil Nadu, India. 25 days old seedlings were transplanted to mainfield at Vedapatti village, Thoothukudi district which is a hot spot area for saline condition in a Randomized Block Design under saline stress condition with two replications, adopting a spacing of 20 cm between rows and 15 cm between plants. The details of EC and pH of soil as well as water of the experimental plot is 4.82 dSm^{-1}, 8.09 as well as 4.78 dSm^{-1} , 8.19 respectively. Ten plants each in P_1, P_2 and F_1, 250 plants in F_2 and 150 plants each in BC_1 and BC_2 for each cross in each replication were maintained for the study. After 21 days of transplanting, the phenotypic scoring for all the six generations was evaluated for their tolerance level to salinity using IRRI standard protocol (Gregorio *et al.,* 1997) (Table 1). Observations were recorded in all the available plants in P_1, P_2, F_1, F_2, BC_1 and BC_2 generations for the biometrical traits (Plate 1 to 6).

Table1. Modified Standard Evaluation Score (SES) of visual salt injury at seedling stage

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	HT- Highly tolerant

3	Nearly normal growth, but leaf tips of few leaves whitish and rolled.	T - Tolerant
5	Growth severely retarded, most leaves rolled, only a few are elongating.	MT- Moderately tolerant
7	Complete cessation of growth, most leaves dry some plants dying	S - Susceptible
9	Almost all plants dead or dying	HS – Highly susceptible

Regarding phenotypic scoring for salinity, the scales 1 and 3 are grouped as tolerant and scores 5, 7 and 9 are grouped under susceptible category.

SSR marker studies for salinity tolerance

Genomic DNA was isolated from young leaves of the parents, ADT43, IWP and FL478 after transplanting to the experimental field using the modified CTAB protocol as described by Ausubel *et al.* (1994). A total of 36 SSR primers were used for parental survey. Out of 36 primers, only thirteen primers showed polymorphism among the parents *viz.,* ADT43, IWP and FL478. The remaining primers showed no amplification, hazy bands and some gave amplified products, but these were monomorphic. The respective polymorphic markers alone were used for assessment of salinity inheritance pattern on F_1, F_2, BC_1 and BC_2 generations.Among the thirteen polymorphic markers, only four Simple Sequence Repeats (SSR) primers *viz.,*RM 23, RM 3412, RM 493 and RM 8094 markers were used for parental survey under salinity condition. (Fig. 1).

Primer	Sequence (5'⟶3')	AT (°C)	Type of marker	Expected size (bp)
RM 23F	GGAAAGAATGATCTTTTCATGG	55	SSR	146
RM 23R	CTACCATCAAAACCAATGTTC	55	SSR	211
RM 3412F	AAAGCAGGTTTTCCTCCTCC	55	SSR	211
RM 3412R	CCCATGTGCAATGTGTCTTC	55	SSR	209
RM 493F	TAGCTCCAACAGGATCGACC	55	SSR	261
RM 493R	GTACGTAAACGCGGAAGGTG	55	SSR	162
RM 8094F	AAGTTTGTACACATCGTATACA	55	SSR	146
RM 8094R	CGCGACCAGTACTACTACTA	55	SSR	211

(Mc Couch *et al.* 2002)

The plants in all the generations *viz.,* P_1, P_2, F_1, F_2, BC_1 and BC_2 in both replications were used for maker scoring. The scoring was done for the marker data by means of visualizing the band appearance. If the band of individual plant matches with the donor parent, the plant is designated as score '1' and the final scores is added upto the total population in each generation. Conversely, if the band of the individual plant matches with the female parent (susceptible),

the plant is designated as score '0' and the final score is added upto the total population in each generation. The plant falls under the category of score '0' means it is designated as susceptible and score'1' means it is tolerant.

Data Analysis

Chi-square test

The goodness of fit to Mendelian segregation ratio for Salinity and submergence tolerance (tolerance: susceptible) in the F_2 and backcrosses for selected four cross combinations both for marker data and phenotypic scoring were subjected to Chi-square test.

$$\chi^2 = \Sigma (O-E)^2 / E$$

Where,
O = Observed frequency of plants
E = Expected frequency of plants

The significance of Chi square value was tested against the table value with (n-1) degrees of freedom, where 'n' is the total number of segregating classes (Stansfield, 1991).

Result and Discussion

Inheritance studies for salinity tolerance using Marker analysis

To study the inheritance of salinity tolerance, the chi-square test for the deviation from the expected genetic ratios of the different generations *viz.,* F_1, F_2, BC_1 and BC_2 of the cross 1 (ADT43 x FL478) and cross 2 (IWP x FL478) were made by marker analysis under Marker Assisted Selection and the results are presented in Tables 2 and 3. The target QTL was monitored by markers linked to the *Saltol* QTL *viz.,* RM 23, RM 3412, RM 493, and RM 8094 were used for foreground selection of salinity tolerance based on the parental survey(Fig. 2 and 3).

Cross 1 (ADT 43 x FL 478)

In F_2 generation, foreground selection was performed on 250 plants in cross 1(ADT43 x FL478). Out of 250 plants, 12 plants showed fixed donor allele (tolerant allele (T)) and 238 plants showed fixed recipient allele (susceptible allele (S)) for RM 3412 and 19 plants exhibited donor allele and 231 plants exhibited recipient allele for RM 23. Moreover, out of 250 plants studied 17 plants showed fixed donor allele (tolerant allele (T)) and 233 plants showed fixed recipient allele (susceptible allele (S)) for the marker RM 493, whereas 13 plants evidenced fixed donor allele (tolerant allele (T)) and 237 plants showed fixed recipient allele (susceptible allele (S)) for the marker RM 8094 in cross 1(ADT43 x FL478) under salinity. The results exhibited for all the four markers were fitted to the expected 1:15 ratio of this generation for the homozygous donor allele and homozygous recipient allele groups with a non-significant chi-square value of 0.838, 0.780, 0.130 and 0.469 at a probability level of 0.05. (Table 2 and Fig. 2). The marker study revealed that the F_1s of cross 1 are susceptible to salinity. The F_2 of cross 1 (ADT43 × FL 478) segregated into 12 tolerant and 238 susceptible for RM 3412, 19 tolerant and 231 susceptible for RM 23, 17 tolerant and 233 susceptible for RM 493 and 13 tolerant and 237 susceptible for the marker RM 8094. The results of the cross fit into 1:15 ratio

of tolerant and susceptible indicated by the chi-square goodness of fit. Based on the present study the inheritance of salinity tolerance was found to be controlled by digenic duplicate interaction (duplicate dominant genes) as observed from the F_2 ratio of 15:1 (susceptible: tolerant) in crosses 1. The salinity tolerance trait is polygenic in nature and lacks maternal influence (Mishra *et al.,* 1998). (Fig. 3)

Regarding BC_1 generation of cross 1, the foreground selection by all the four markers revealed that 1 plant exhibited tolerant allele (donor allele (T)) and 149 plants showed susceptible allele (recipient allele (S)) for marker RM 3412 and 3 plant exhibited tolerant allele (donor allele (T)) and 147 plants showed susceptible allele (recipient allele (S)) for marker RM 23. The marker RM 493 evidence that 2 plant exhibited tolerant allele (donor allele (T)) and 148 plants showed susceptible allele (recipient allele (S)) and 5 plant exhibited tolerant allele (donor allele (T)) and 145 plants showed susceptible allele (recipient allele (S)) for marker RM 8094 under salinity condition. Foreground selection was carried out over 150 plants in BC_2 generation of cross 1 using RM 23, RM 3412, RM 493, and RM 8094. Out of 150 plants, 36 plants showed homozygous donor allele (T) and 114 plants showed homozygous recipient allele (S) for the marker RM 3412. Besides this, 37 plants exhibited tolerant allele (donor allele (T)) and 113 plants showed susceptible allele (recipient allele (S)) for two markers *viz.,* RM 23 and RM 493. The maker RM 8094 evidenced that 39 plants exhibited tolerant allele (donor allele (T)) and 111 plants showed susceptible allele (recipient allele (S)) for marker RM 3412 (Fig. 3). The results fitted to the expected 1:3 (T: S) ratio in B_2 generation with a non-significant chi-square value of 0.080, 0.009, 0.009 and 0.080 respectively. In rice, important traits such as salt-tolerance, yield and quality are controlled by polygene's or gene complexes that are described as quantitative trait loci (QTLs) (Seung Yeob Lee *et al.,* 2006). Salinity stress tolerance is a mutagenic trait and involves activity of many genes working in co-ordination (Waziri *et al.,* 2016).The marker analysis revealed that all the BC_1 progenies derived from cross 1 were susceptible.

In BC_2 generation of the cross 1 were segregated into 36 tolerant and
114 susceptible for RM 3412, 37 tolerant and 113 susceptible (RM 23 and RM 493) and
39 tolerant and 111 susceptible (RM 8094) in cross 1. In cross 2 there was segregation of 32: 118 (RM 3412), 39:111 (RM 23), 43:107 (RM 493) and 42:108 (RM 8094) for tolerant: susceptible respectively in cross 2. In cross 1, the chi-square test was non-significant showing the fitness of the expected ratios of 1:3 (tolerant: susceptible) in BC_2 generation (Table 2 and 3). Hence the segregation and inheritance of BC_1 and BC_2 generations confirm the corresponding F_1 and F_2 generation segregation for salinity based on marker study.

Cross 2 (IWP x FL 478)

The marker analysis using RM 3412 revealed that
17 plants with homozygous donor allele (T) for saline tolerance and 233plants showed homozygous recipient allele (S) which were susceptible. The marker RM 23 evidence that 14 plants with homozygous donor allele (T) for saline tolerance and 236plants showed homozygous

recipient allele (S). Whereas, 10 plants with homozygous donor allele (T) for saline tolerance and 240plants showed homozygous recipient allele (S) for RM 493. Moreover, out of 250 plants studied for salinity tolerance, 12 plants with homozygous donor allele (T) for saline tolerance and 238plants showed homozygous recipient allele (S) for the marker RM 8094. The results were fitted with 1:15(T:S) ratio of expected ratio as indicated by the chi-square goodness of fit analysis. (Table 3). The results were fit into 1:15 ratio of tolerant and susceptible indicated by the chi-square goodness of fit. Based on the present study the inheritance of salinity tolerance was found to be controlled by digenic duplicate interaction (duplicate dominant genes) as observed from the F_2 ratio of 15:1 (susceptible: tolerant) in cross 2. (Fig. 4)

In BC_1, foreground selection using RM 3412 exhibited on 150 plants in that
2 plants showed homozygous donor allele (T) and 148 plants showed homozygous recipient allele (S) for salinity tolerance, whereas the marker RM 23 showed 3 plants with homozygous donor allele (T) for saline tolerance and 147plants showed homozygous recipient allele (S). Moreover the markers RM 493 and RM 8094 evidence that 1 and 4 plants with homozygous donor allele (T) for saline tolerance and 149 and 146plants showed homozygous recipient allele (S) (Table 3). In BC_1 generation of cross 2, the marker RM 3412 showed 2 tolerant and 148 susceptible, RM 23 evidenced 3 tolerant and 147 susceptible, RM 493 exhibited 1 tolerant and 149 susceptible and RM 8094 marker revealed 4 tolerant and 146 susceptible under salinity. The marker analysis revealed that all the BC_1 progenies derived from cross 1 and cross 2 were susceptible.

Whereas in 150 BC_2 progenies of cross 2, 32 tolerant and 118 were susceptible by the presence of homozygous donor allele (T) and homozygous recipient allele (S) respectively by molecular marker analysis using RM 3412.The markers RM 23 and RM 493 showed 39 and 43 plants with homozygous donor allele (T) for saline tolerance and 111 and 107plants had homozygous recipient allele (S), while RM 8094 exhibited 42 plants with donor allele (T) and 108 plants with recipient allele (S) for salinity tolerance in cross 2. These results are fitted to the expected 1:3 (T: S) ratio in B_2 generation with a non-significant chi-square value of 1.068. In cross 2 there was segregation of 32: 118 (RM 3412), 39:111 (RM 23), 43:107 (RM 493) and 42:108 (RM 8094) for tolerant: susceptible respectively. In cross 2, the chi-square test was non-significant showing the fitness of the expected ratios of 1:3 (tolerant: susceptible) in BC_2 generation. (Fig. 6)

Inheritance studies through Phenotypic Scoring

All the six generation *viz.,* P_1, P_2, F_1, F_2, BC_1 and BC_2 of cross 1 and cross 2 were evaluated in field condition at farmer field and phenotypic scoring was done at panicle initiation stage. The F_1 progenies derived from all the crosses (cross 1 and 2) were susceptible to salinity. Regarding the segregating generations based on phenotypic scoring under field condition, out of 250 plants, 21 plants with homozygous donor allele (T) for saline tolerance and 229plants had homozygous recipient allele (S) and in cross 2, the 250 plants were segregated as 17 plants with homozygous donor allele (T) for saline tolerance and 223plants had homozygous recipient allele (S), the chi-square test for the expected ratio was 1:15 (tolerant: susceptible) in F_2. All the

plants in BC_1 were susceptible to salinity. In cross 1 (37 tolerant: 113 susceptible) and cross 2(41 tolerant: 109 susceptible), the chi-square test was non-significant showing the fitness of the expected ratios of 1:3 (tolerant: susceptible) in BC_2 generation (Table 4 and 5).

The results revealed that F_1 progenies derived from all the crosses (cross 1 and 2) were susceptible to salinity. Regarding F_2 generation under field condition, in crosses 1 and 2, the chi-square test for the expected ratio was also found to be 1:15 (tolerant : susceptible) with the segregation of 21:229 and 17:233 in cross 1 and cross 2 respectively. So the inheritance of salinity tolerance was found to be controlled by duplicate dominant genes as observed from the F_2 ratio of 15:1 (susceptible: tolerant) in crosses 1 and 2. All the plants in BC_1 were susceptible to salinity. In BC_2 of the cross 1 and cross 2, the chi-square test was non-significant showing the fitness of the expected ratios of 1:3 (tolerant: susceptible). The back cross ratio 1:3 (tolerant: susceptible) found to confirm the salinity tolerance in field condition also controlled by duplicate dominant genes with digenic duplicate dominant epistasis.

Conclusion:

Based on the SSR marker and field study, the inheritance pattern for salinity tolerance revealed that the tolerance was recessive to susceptibility. The segregation pattern observed in F_2 indicated a ratio of 15:1 for susceptible and tolerant in both the crosses. The above inheritance pattern for salinity tolerance was confirmed by the test cross ratio of 3:1 in BC_2 generation. The 15:1 genetic ratio for susceptible: tolerant indicated the difference between parents in both the crosses was based on the two pairs of genes, each one was duplicate of other gene. The obtained results indicated that, the salt tolerance was governed by duplicate dominant epistasis, the dominant allele masking the expression of recessive alleles at two loci. The segregation pattern were confirmed by the test cross ratio exposed through both marker and phenotypic scoring. Based on the inheritance pattern, the fact embedded on the salinity tolerance identified and it was controlled by two pairs of genes, each one was duplicate of other gene. Hence the gene action for salinity tolerance was found to be controlled by digenic with duplicate interaction.

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Table 2. Inheritance for salinity tolerance byRM 23, RM 3412, RM 493, and RM 8094markers data - Cross 1 (ADT43 x FL478)

Generations	Marker	Marker Score			Susceptible : Tolerant (Ratio)		Chi - square value	Probability between
		Total	1(T)	0 (S)	Observed	Expected		
P_1		20	0	20	All susceptible	All susceptible	-	-
P_2		20	20	0	All tolerant	All tolerant	-	-
F_1		20	0	20	All susceptible	All susceptible	-	-
F_2	RM 3412	250	12	238	15.20:0.76	15:1	0.838	0.50-0.30
	RM 23	250	19	231	14.78:1.22	15:1	0.780	0.50-0.30
	RM 493	250	17	233	14.91:1.09	15:1	0.130	0.50-0.30
	RM 8094	250	13	237	15.17:0.83	15:1	0.469	0.50-0.30
BC_1	RM 3412	150	1	149	1 tolerant	All susceptible	-	-
	RM 23	150	3	147	3 tolerant	All susceptible	-	-
	RM 493	150	2	148	2 tolerant	All susceptible	-	-
	RM 8094	150	5	145	5 tolerant	All susceptible	-	-
BC_2	RM 3412	150	36	114	3.04:0.96	3:1	0.080	0.95-0.50
	RM 23	150	37	113	3.01:0.98	3:1	0.009	0.95-0.50
	RM 493	150	37	113	3.01:0.98	3:1	0.009	0.95-0.50
	RM 8094	150	39	111	2.96:1.04	3:1	0.080	0.95-0.50

Table 3. Inheritance for salinity tolerance byRM 23, RM 3412, RM 493, and RM 8094markers data - Cross 2 (IWP x FL478)

Generations	Marker	Marker Score			Susceptible : Tolerant		Chi - square value	Probability between
		Total	1(T)	0 (S)	Observed	Expected		
P_1		20	0	20	All susceptible	All susceptible	-	-
P_2		20	20	0	All tolerant	All tolerant	-	-
F_1		20	0	20	All susceptible	All susceptible	-	-
F_2	RM 3412	250	17	233	14.90:1.00	15:1	0.129	0.30-0.20
	RM 23	250	14	236	15.10:0.90	15:1	0.179	0.30-0.20
	RM 493	250	10	240	15.36:0.64	15:1	2.157	0.20-0.10
	RM 8094	250	12	238	15.23:0.77	15:1	0.895	0.50-0.30
BC_1	RM 3412	150	2	148	2 tolerant	All susceptible	-	-
	RM 23	150	3	147	3 tolerant	All susceptible	-	-
	RM 493	150	1	149	1 tolerant	All susceptible	-	-
	RM 8094	150	4	146	4 tolerant	All susceptible	-	-
BC_2	RM 3412	150	32	118	3.14:0.85	3:1	1.076	0.50-0.30
	RM 23	150	39	111	2.96:1.04	3:1	0.080	0.95-0.50
	RM 493	150	43	107	2.85:1.14	3:1	1.076	0.50-0.30
	RM 8094	150	42	108	2.88:1.12	3:1	0.720	0.50-0.30

Table 4. Inheritance of salinity tolerance by phenotypic scoring in Cross 1 (ADT43 x FL478)

Generation	Observed values		Expected ratio	χ^2values	Probability between
	Resistant	Susceptible			
P_1	-	20	-	-	-
P_2	20	-	-	-	-
F_1	1	19	-	-	-
F_2	21	229	1: 15	1.970	0.10-0.20
BC_1	-	147	-		
BC_2	37	113	1 : 3	0.008	0.95-0.50

Table 5. Inheritance of salinity tolerance by phenotypic scoring in Cross 2 (IWP x FL478)

Generation	Observed values		Expected ratio	χ^2values	Probability between
	Resistant	Susceptible			
P_1	-	20	-	-	-
P_2	20	-	-	-	-
F_1	2	18	-	-	-
F_2	17	233	1 : 15	0.127	0.30-0.20
BC_1	-	141	-		
BC_2	41	109	1 : 3	0.435	0.50-0.30

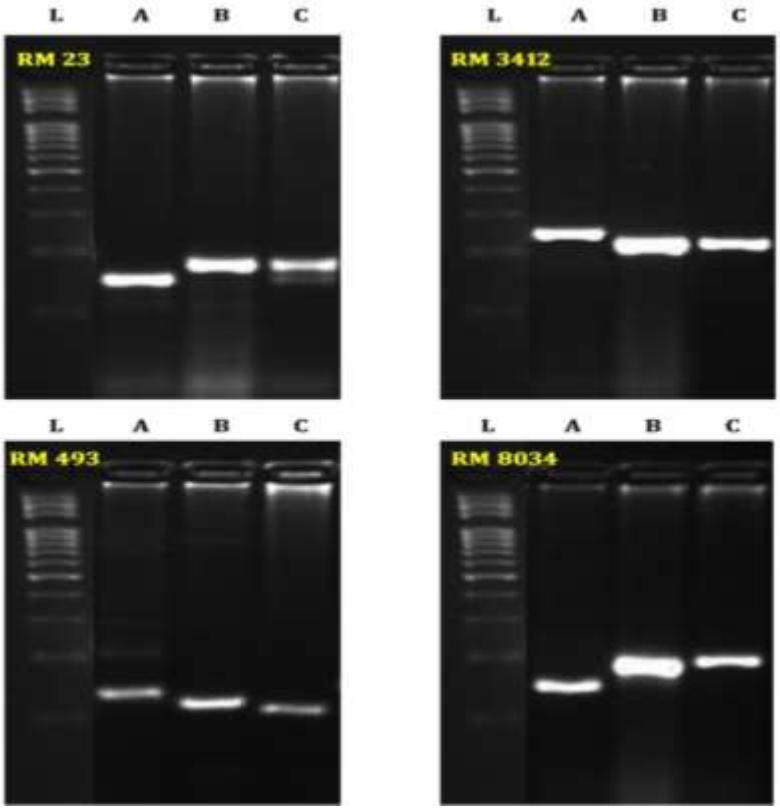


Fig. 1 SSR profile showing polymorphism between the parents by using RM 23, RM 3412, RM 493 and RM 8094 (L-100bp ladder, A-ADT43, B-FL478 and C-IWP)

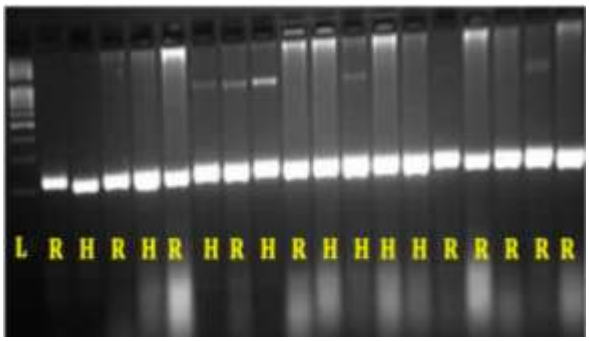


Fig. 2 Foreground selection of F_2 population by using tightly linked *saltol* marker RM 3412
(L-100bp ladder, R-Recipient Allele, D-Donor Allele and H-Hybrid Allele)

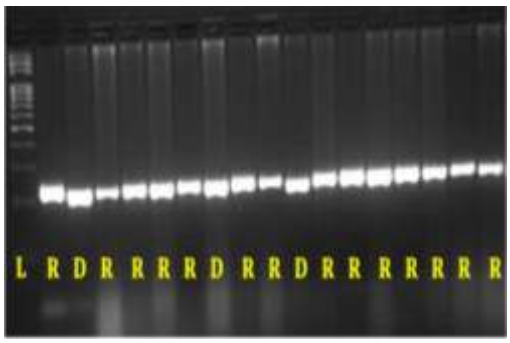


Fig. 3 Foreground selection of B_2 population by using tightly linked *saltol* marker RM 3412
(L-100bp ladder, R-Recipient Allele, D-Donor Allele and H-Hybrid Allele)

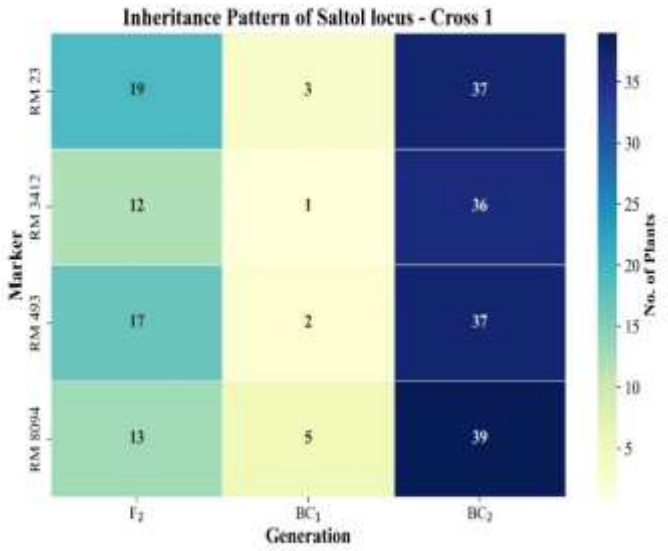


Fig. 4 Heatmap depicted the inheritance pattern of *Saltol* locus in Cross 1 (ADT43 x FL478)

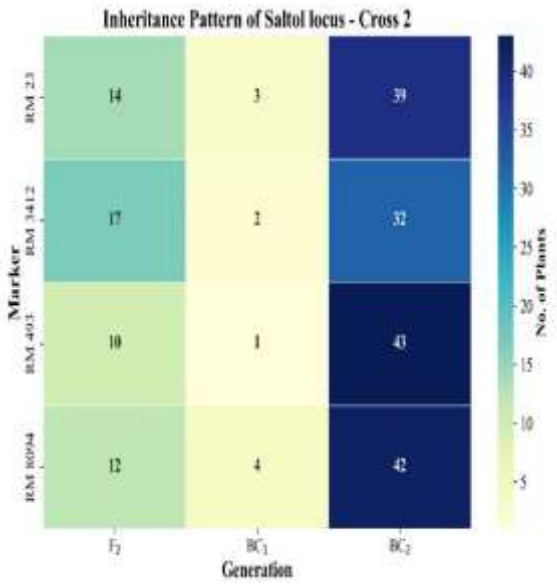


Fig. 5 Heatmap depicted the inheritance pattern of *Saltol* locus in Cross 2 (IWP x FL478)

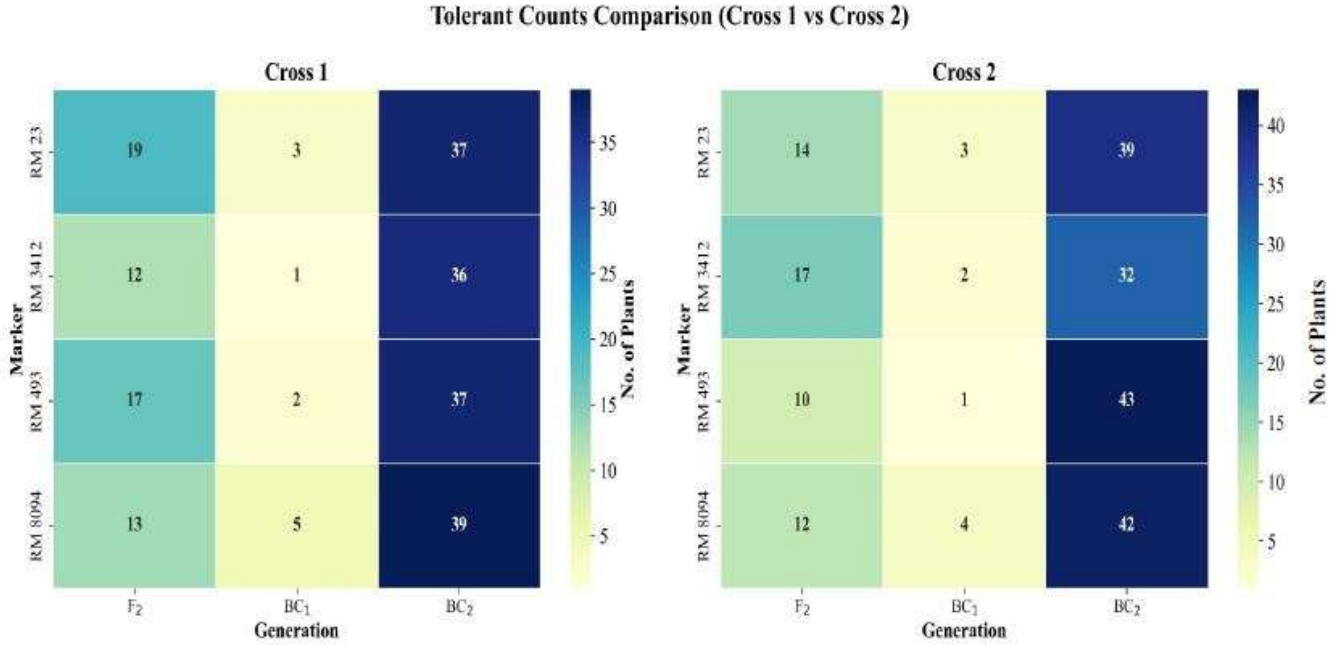


Fig. 6 Comparing the inheritance pattern of *Saltol* locus in Cross 1 (ADT43 x FL478) and Cross 2 (IWP x FL4



Plate 1. Segregants for salinity tolerance



Plate 2. Plants with recipient allele



Plate 3. Segregants with donor allele



Plate 5. Field view of Backcross generation plants



Plate 6. Plants with recipient allele shows spikelet sterility under field condition

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