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Characterisation of salinity tolerant varieties using SSR markers in rice (*Oryza sativa* L.)

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Abstract
As soil salinity is a major abiotic stress limiting the crop production in rice, there is a necessity to develop a new salt tolerant rice cultivar through breeding methods. Here is a method consisting of BC_3F_2 generation with an objective to intro gress "Saltol" QTL for salinity tolerance in a rice variety of cotton Dora sannalu (MTU 1010), with FL478 (Pokkali) as donor, by the SSR markers RM10793 and AP3206 in recombinant selection.

After screening in foreground selection using RM10694 marker, further recombinant selection with RM10793 and AP3206 markers has been proceeded. The resultants selected from RM10793 and AP3206 markers are Double Recombinants. Selection of Double recombinants is necessary for selecting back cross progeny with the target gene and recombination events between the target locus and linked flanking markers on both the sides. After recombinant selection, the double recombinants were further subjected to phenol typing *i.e.,* screening for salinity tolerance under hydroponics.

Keywords: *saltol*, selection, backcross, recombinant selection, salinity tolerance, productivity, salinity, SSRs markers, families, population, plants

Introduction
Rice (*Oryza sativa* L.) is world's second most important cereal after wheat and is staple food for more than half of the world's population. It is a major source of dietary component for more than 2.7 billion people on a daily basis and is cultivated in one-tenth of the earth's arable land. It is grown in 165 million ha worldwide with a production of 746 million tonnes and an average productivity of 4.5 t/ ha. In India, it is cultivated in an area of 434 lakh ha with an annual production of 104 million tonnes and an average productivity of 2.4 t/ ha (Annual report, 2016-17). Andhra Pradesh is one of the important state contributing to the production and productivity of rice in the country with an area of about 24 lakh ha with an annual production of 8.5 mt and an average productivity of 3.5 t/ha (Agricultural statistics 2015).

Various abiotic stresses including high or low temperature, water scarcity, high salinity and heavy metals exert drastic antagonistic efforts on crop metabolism and thereby reducing the plant growth, development and ultimately crop productivity. Among these, soil salinity is a major factor limiting the crop production globally (Kumar *et al.,* 2010) [4]. Soil salinity affects large areas of the world cultivated land causing significant reductions in crop yield. Out of the total rice cultivated area in the world, 30% is affected by salinity (Prasad *et al.,* 2000) [7]. More than 90% of world's rice production comes from Asia, especially South and South East Asia that has about 21.5 million ha salt affected area, of which 12 million ha are saline and 9.5 million ha are alkaline/sodic (Munns and Tester, 2008). Of the two salinity situations, inland salinity (mainly sodicity) is alarmingly on the increase in the irrigation commands due to defective irrigation management and lack of drainage.

Rice is a salt sensitive crop and the salinity has pronounced effects on different developmental growth stages (Arpad *et al.,* 2017) [3]. Many studies have documented that rice is resistant to salinity at the germination stage but becomes very sensitive during seedling stage (2-3 leaves). Rice tolerates salinity throughout the vegetative growth, but it is highly sensitive to salinity during reproductive stage especially during pollination and fertilizing period. At the flowering stage, the salinization affects spikelet formation, germination of the pollen which ultimately reduce the number of filled grains/panicle and increases unfilled grains and thereby reduces the spikelet fertility. Salinity also reduces panicle length and 1,000 grain weight, resulting in reduced productivity (Tran *et al.,* 2017) [8].

Materials and Methods

Experimental material consisting of BC_3F_2 generation of the cross MTU 1010 x FL 478 (80 families - each comprising 108 plants) was used with an objective to introgress '*Saltol*' a major QTL quantitative trait loci for salinity tolerance which was located on rice chromosome 1 into mega rice variety MTU 1010. The donor, FL 478, a RIL developed at IRRI, Philippines using IR 29 as recipient and traditional Indian cultivar Pokkali as donor.

Genotyping of selected plants of BC_3F_2 (80 families - each comprising 108 plants) was performed during *Kharif* 2014. The SSR simple sequence repeats (microsatellites) marker, RM 10694 which is tightly linked to *Saltol* QTL was used as foreground marker, while the SSR marker RM 10793, on distal end and AP 3206 on proximal end were used as recombinant markers for precise transfer of the QTL (Fig 1).

In the present study, a large population of 8,640 plants from 80 families were studied. Genotyping (foreground, recombinant and background selection) of such huge population is very difficult. Hence some plants were selected from each family which were similar to the recurrent parent based on the plant height and number of ear bearing tillers plant^{-1}. These plants which were similar to the recurrent parent further helps in the large recovery of the recurrent parent genome.

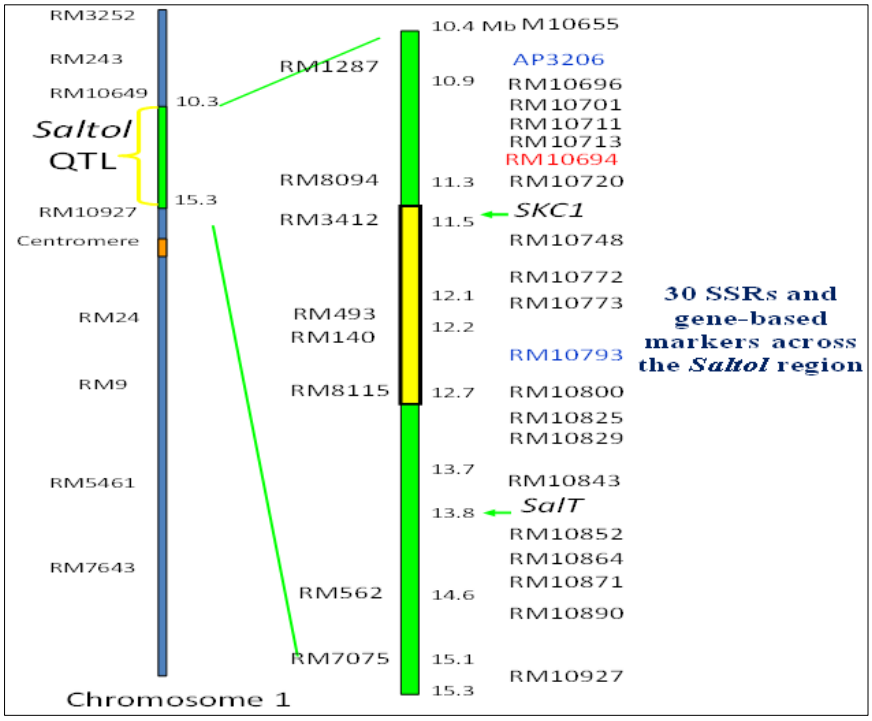

Fig 1: 30 SSRs and gene-based markers across the *saltol* region

Results and Discussion

Recombinant selection

The important purpose of recombinant selection was to reduce the size of donor chromosome segment containing the *Saltol* QTL. In the present study, two markers RM 10793 and AP 3206 that flank a target *Saltol* QTL were used for recombinant selection. All plants which were selected in foreground selection were screened in recombinant selection. In recombinant selection, plants with A type allele of MTU 1010 were selected and plants with B type allele of FL 478 and plants with both alleles were rejected (Fig.2). After recombinant selection the double recombinants were further subjected to phenotyping *i.e.*, screening for salinity tolerance under hydroponics.

Fig 2: Recombinant selection of DST-37 family using AP 3206 M- 100 bp DNA Ladder, P_1- MTU 1010, P_2- FL 478

Recombinant selection for identifying plants with recombinant events

The recombinant selection involves selecting backcross progeny with the target gene and recombination events between the target locus and linked flanking markers. The purpose of recombinant selection was to reduce the size of the donor chromosome segment containing the target locus (*i.e.*, size of the introgression). This is important because the rate of decrease of this donor fragment is slower than for unlinked regions and many undesirable genes that negatively affect crop performance may be linked to the target gene from the donor parent (*i.e.*, linkage drag).

Recombinant selection on proximal end with AP 3206 marker

The recombinant selection at the proximal side of *Saltol* QTL

was performed using the marker AP 3206 for selecting backcross progeny with the target gene and recombination events between the target locus and linked flanking markers.

Table 1: Recombinant selection using AP 3206 as marker

S. No	Family	Total plants studied	Plants (MTU 1010 type)	Plants (FL478 type)	Plants (Heterozygous)
1	DST-31	31	6	17	8
2	DST-32	9	0	5	4
3	DST-33	25	3	18	4
4	DST-34	15	14	1	0
5	DST-35	11	10	1	0
6	DST-36	5	5	0	0
7	DST-37	15	15	0	0
8	DST-38	13	11	2	0
9	DST-39	11	11	0	0
10	DST-40	3	3	0	0
11	DST-41	17	9	5	3
12	DST-42	22	17	3	2
13	DST-43	17	17	0	0
14	DST-44	19	19	0	0
15	DST-45	18	18	0	0
16	DST-46	21	19	2	0
17	DST-47	15	12	0	3
18	DST-48	13	11	0	2
19	DST-50	21	3	14	4
20	DST-51	19	7	8	4
21	DST-52	19	3	10	6
22	DST-53	23	7	9	7
23	DST-54	16	0	9	7
24	DST-55	12	4	5	3
25	DST-56	6	2	3	1
26	DST-57	6	1	3	2
27	DST-58	11	2	5	4
28	DST-60	13	1	8	4
29	DST-61	17	1	10	6
30	DST-62	7	0	5	2
31	DST-63	15	3	7	5
32	DST-64	10	1	5	4
33	DST-65	13	3	6	4
34	DST-67	14	0	9	5
35	DST-68	11	0	7	4
36	DST-69	4	0	3	1
37	DST-70	9	5	2	2
38	DST-74	2	0	2	0
39	DST-75	5	0	3	2
40	DST-76	4	0	3	1
41	DST-77	5	0	3	2
42	DST-78	5	0	4	1
43	DST-79	4	0	3	1
44	DST-80	9	2	4	3
45	DST-81	13	1	7	5
46	DST-82	3	0	2	1
47	DST-83	8	0	5	3
48	DST-84	6	0	4	2
49	DST-85	2	0	2	0
50	DST-89	4	1	1	2
51	DST-90	7	0	7	0
52	DST-91	13	2	8	3
53	DST-92	5	3	2	0
54	DST-93	5	2	1	2
55	DST-94	6	0	6	0
56	DST-96	9	9	0	0
57	DST-97	2	0	2	0
58	DST-98	1	1	0	0
59	DST-99	7	4	2	1
60	DST-100	8	5	3	0
61	DST-101	12	10	2	0
62	DST-102	6	4	2	0
63	DST-103	10	9	0	1

64	DST-104	11	8	3	0
65	DST-105	4	1	1	2
66	DST-107	5	1	2	2
67	DST-108	7	4	3	0
68	DST-109	7	4	3	0
69	DST-110	7	7	0	0
	Total	728	321	272	135

Out of the total 728 plants (obtained from foreground selection) screened in recombinant selection using the marker AP 3206, 321 plants were MTU 1010 type which were selected and 272 plants were FL 478 type and 135 plants were heterozygous which were rejected. The per cent selected plants in recombinant selection using AP 3206 was 44.09% (Table 1).

Out of the total 728 plants screened in recombinant selection using the marker AP 3206, 321 plants of MTU 1010 type were selected. Out of total 80 families, 11 families were discarded in foreground selection. The remaining 69 families were studied in recombinant selection. In recombinant selection, 100% plants were selected from 10 families *viz.*, DST-36, DST-37, DST-39, DST-40, DST-43, DST-44, DST-45, DST-96, DST-98 and DST-110 while none of the plants were selected from 19 families *viz.*, DST -32, DST-54, DST-62, DST-67, DST-68, DST-69, DST-74, DST-75, DST-76, DST-77, DST-78, DST-79, DST-82, DST-83, DST-84, DST-85, DST-90, DST-94, and DST-97.

Highest number of plants (19 each) were selected in the families DST-44 and DST-46 while lowest number of plants (one each) were selected in the families DST-57, DST-60, DST-61, DST-64, DST-81, DST-89, DST-105 and DST-107.

The results in the present study were in agreement with Vu *et al.* (2012) [9] who introgressed *Saltol* from FL 478 to BT 7. They used the marker RM 1287 for recombinant selection at proximal end. Further, the results were in agreement with Linh *et al.* (2012) [5] who used RM 1287 and RM 10694 as recombinant markers at proximal end. In the present study also a total of 321 plants were identified to be similar with recurrent parent when AP 3206 was used for recombinant selection.

Recombinant selection on distal end with RM 10793 marker

The recombinant selection at the distal side of *Saltol* QTL was performed using the marker RM 10793 for selecting backcross progeny with the target gene and recombination events between the target locus and linked flanking markers (Fig.3).

Fig 3: Recombinant selection of DST-46 family using RM 10793 M-100 bp DNA Ladder, P_1- MTU 1010, P_2- FL 478

Recombinant Selection for Identifying Plants with Recombinant Events

Table 2: Recombinant selection using RM 10793 marker

S. No	Family	Total plants studied	Plants (MTU 1010 type)	Plants (FL 478 type)	Plants (Heterozygous)
1	DST-31	31	6	17	8
2	DST-32	9	0	5	4
3	DST-33	25	3	19	3
4	DST-34	15	14	1	0
5	DST-35	11	10	1	0
6	DST-36	5	5	0	0
7	DST-37	15	15	0	0
8	DST-38	13	11	2	0
9	DST-39	11	11	0	0
10	DST-40	3	3	0	0
11	DST-41	17	9	4	4
12	DST-42	22	17	3	2
13	DST-43	17	17	0	0
14	DST-44	19	19	0	0
15	DST-45	18	18	0	0
16	DST-46	21	19	2	0
17	DST-47	15	12	1	2
18	DST-48	13	11	2	0
19	DST-50	21	19	2	0
20	DST-51	19	19	0	0
21	DST-52	19	18	1	0
22	DST-53	23	23	0	0
23	DST-54	16	16	0	0
24	DST-55	12	12	0	0
25	DST-56	6	6	0	0
26	DST-57	6	6	0	0
27	DST-58	11	11	0	0
28	DST-60	13	13	0	0
29	DST-61	17	16	1	0

30	DST-62	7	6	1	0
31	DST-63	15	15	0	0
32	DST-64	10	10	0	0
33	DST-65	13	13	0	0
34	DST-67	14	14	0	0
35	DST-68	11	11	0	0
36	DST-69	4	4	0	0
37	DST-70	9	9	0	0
38	DST-74	2	2	0	0
39	DST-75	5	5	0	0
40	DST-76	4	4	0	0
41	DST-77	5	5	0	0
42	DST-78	5	5	0	0
43	DST-79	4	4	0	0
44	DST-80	9	9	0	0
45	DST-81	13	13	0	0
46	DST-82	3	3	0	0
47	DST-83	8	8	0	0
48	DST-84	6	6	0	0
49	DST-85	2	2	0	0
50	DST-89	4	4	0	0
51	DST-90	7	7	0	0
52	DST-91	13	13	0	0
53	DST-92	5	5	0	0
54	DST-93	5	4	1	0
55	DST-94	6	6	0	0
56	DST-96	9	0	9	0
57	DST-97	2	0	0	2
58	DST-98	1	0	1	0
59	DST-99	7	0	3	4
60	DST-100	8	0	5	3
61	DST-101	12	0	10	2
62	DST-102	6	0	4	2
63	DST-103	10	0	9	1
64	DST-104	11	0	8	3
65	DST-105	4	0	1	3
66	DST-107	5	5	0	0
67	DST-108	7	0	7	0
68	DST-109	7	6	0	1
69	DST-110	7	7	0	0
		728	564	120	44

Out of the total 728 plants (obtained from foreground selection) screened in recombinant selection using the marker RM 10793, 564 plants were MTU 1010 type which were selected and 120 plants were FL 478 type and 44 plants were heterozygous which were rejected. The per cent selected plants in recombinant selection using RM 10793 was 77.33% (Table 2). When compared with AP 3206 marker at proximal end, more number of plants were selected in recombinant selection at distal end.

Out of the total 728 plants screened in recombinant selection using the marker RM 10793, 564 plants were MTU 1010 type and were selected. Out of 69 families studied in recombinant selection using RM 10793, 100% plants were selected in 41 families *viz.*, DST-36, DST-37, DST-39, DST-40, DST-43, DST-44, DST-45, DST-51, DST-53, DST-54, DST-55, DST-56, DST-57, DST-58, DST-60, DST-63, DST-64, DST-65, DST-67, DST-68, DST-69, DST-70, DST-74, DST-75, DST-76, DST-77, DST-78, DST-79, DST-80, DST-81, DST-82, DST-83, DST-84, DST-85, DST-89, DST-90, DST-91, DST-92, DST- 94, DST-107 and DST-110. Not a even a single plant was selected in 12 families *viz.*, DST-32, DST-96, DST-97, DST-98, DST-99, DST-100, DST-101, DST-102, DST-103, DST-104, DST-105 and DST-108.

Highest number of plants (23 no.) were selected in the family DST-53 followed by the families DST-44, DST-46, DST-50 and DST-51 with 19 no. of plants in each family. A total of 18 plants each were selected in the families DST-45 and DST-52. Lowest number of plants (two each) were selected in the families DST-74 and DST-85 followed by the family DST-33 with three selected plants.

The results in the present study were in agreement with Vu *et al.* (2012) [9] who introgressed *Saltol* from FL 478 to BT 7. They used the markers RM 562, RM 7075, RM 10843 and RM 10852 for recombinant selection at distal end. Further, the results were in agreement with Linh *et al.* (2012) [5] who used RM 562 and RM 7075 as recombinant markers at distal end. In the present study also a total of 563 plants were identified to be similar with recurrent parent when RM 10793 was used for recombinant selection.

Selection of double recombinants

Selection of double recombinants is necessary for selecting backcross progeny with the target gene and recombination events between the target locus and linked flanking markers on both the sides. Selection of double recombinants ensures that target locus of the donor segment was introgressed exactly with no unlinked regions and undesirable effects.

Table 3: Selection of double recombinants using AP 3206 and RM 10793 markers

S. No	Family	Total plants studied	Plants selected (AP 3206)	Plants selected (RM 10793)	Total double recombinant selection
1	DST-31	31	6	6	6
2	DST-32	9	0	0	0
3	DST-33	25	3	3	3
4	DST-34	15	14	14	14
5	DST-35	11	10	10	10
6	DST-36	5	5	5	4
7	DST-37	15	15	15	15
8	DST-38	13	11	11	11
9	DST-39	11	11	11	9
10	DST-40	3	3	3	3
11	DST-41	17	9	9	9
12	DST-42	22	17	17	16
13	DST-43	17	17	17	14
14	DST-44	19	19	19	18
15	DST-45	18	18	18	18
16	DST-46	21	19	19	18
17	DST-47	15	12	12	12
18	DST-48	13	11	11	11
19	DST-50	21	3	19	2
20	DST-51	19	7	19	7
21	DST-52	19	3	18	2
22	DST-53	23	7	23	7
23	DST-54	16	0	16	0
24	DST-55	12	4	12	2
25	DST-56	6	2	6	2
26	DST-57	6	1	6	1
27	DST-58	11	2	11	2
28	DST-60	13	1	13	1
29	DST-61	17	1	16	1
30	DST-62	7	0	6	0
31	DST-63	15	3	15	3
32	DST-64	10	1	10	1
33	DST-65	13	3	13	1
34	DST-67	14	0	14	0
35	DST-68	11	0	11	0
36	DST-69	4	0	4	0
37	DST-70	9	5	9	4
38	DST-74	2	0	2	0
39	DST-75	5	0	5	0
40	DST-76	4	0	4	0
41	DST-77	5	0	5	0
42	DST-78	5	0	5	0
43	DST-79	4	0	4	0
44	DST-80	9	2	9	1
45	DST-81	13	1	13	1
46	DST-82	3	0	3	0
47	DST-83	8	0	8	0
48	DST-84	6	0	6	0
49	DST-85	2	0	2	0
50	DST-89	4	1	4	1
51	DST-90	7	0	7	0
52	DST-91	13	2	13	2
53	DST-92	5	3	5	1
54	DST-93	5	2	4	1
55	DST-94	6	0	6	0
56	DST-96	9	9	0	0
57	DST-97	2	0	0	0
58	DST-98	1	1	0	0
59	DST-99	7	4	0	0
60	DST-100	8	5	0	0
61	DST-101	12	10	0	0
62	DST-102	6	4	0	0
63	DST-103	10	9	0	0
64	DST-104	11	8	0	0
65	DST-105	4	1	0	0
66	DST-107	5	1	5	1

67	DST-108	7	4	0	0
68	DST-109	7	4	6	2
69	DST-110	7	7	7	7
	Total	728	321	564	244

The above table represents the total number of double recombinant lines selected from 728 plants (obtained from foreground selection) using two markers RM 10793 and AP 3206. Out of the total 728 plants, 244 plants were double recombinants from 69 families. (Table 3).

Out of 69 families studied in recombinant selection using AP 3206 and RM 10793, 100% of the studied plants were double recombinants in four families *viz.*, DST-37, DST-40, DST-45 and DST-110 while none of the plants were double recombinants in 29 families *viz.*, DST- 32, DST-54, DST-62, DST-67, DST-68, DST-69, DST-74, DST-75, DST-76, DST-77, DST-78, DST-79, DST-82, DST-83, DST-84, DST-85, DST-90, DST-94, DST-96, DST-97, DST-98, DST-99, DST-100, DST-101, DST-102, DST-103, DST-104, DST-105 and DST-108.

Highest number of double recombinant plants (18 no.) were selected in the families DST-44, DST-45 and DST-46 followed by the family DST-42 which has 16 double recombinants. A total of 15 double recombinants were selected in the family DST-37. Lowest number of plants (one each) were selected in the families DST-57, DST-60, DST-61, DST-64, DST-65, DST-80, DST-81, DST-89, DST-92, DST-93 and DST-107.

The results were in agreement with Linh *et al.* (2012) [5] who used two markers each on the proximal and distal ends for selection of double recombinants. Also the results were in agreement with Vu *et al.* (2012) [9] who used on marker RM 1287 at proximal end and RM 562, RM 7075, RM 10843 and RM 10852 at distal end for selection of the double recombinants. When they screened 264 heterozygous plants (BC_2F_1) with these five recombinant markers, only 19 plants were identified to be recurrent parent type. Hence by using recombinant selection the size of the donor chromosome segment containing the target locus can be minimized. These selected plants were further studied in hydroponics method.

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