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RESEARCH ARTICLE

Introgression of dual abiotic stress tolerance QTLs (*Saltol* QTL and *Sub1* gene) into Rice (*Oryza sativa* L.) variety Aiswarya through marker assisted backcross breeding

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Abstract Salinity and submergence are two very prominent abiotic stress conditions affecting rice yield adversely in the coastal agro ecosystem. Marker Assisted Backcross Breeding (MABB) is an efficient and fast track molecular tool to incorporate a desired stress tolerant QTL/gene into an improved cultivar. The present study was carried out for the introgression of *Saltol* QTL responsible for salinity tolerance and *Sub1* gene responsible for submergence tolerance into the high yielding rice variety Aiswarya independently through MABB. Final objective of the study is to develop dual stress tolerant (tolerance to salinity and submergence) Aiswarya rice variety by pyramiding the both target QTLs introgressed BC_2F_2 progenies having maximum background homozygosity. The donors of *Saltol* QTL and *Sub1* gene used in the present study were FL478 and Swarna *Sub1*, respectively. Based on the background genome analysis of the introgressed plants, the plants with > 85–90% background similarity were selected for pyramiding of *Saltol* QTL and *Sub1* gene into the elite background of rice variety Aiswarya. Those selected introgressed lines with *Saltol* QTL and *Sub1* gene will be again crossed to pyramid both *Saltol* QTL and *Sub1* gene into the rice variety Aiswarya. Such a mega rice variety pyramided with dual stress tolerant QTLs is the expected outcome of this study and can be recommended for cultivation in the flood prone saline coastal agroecosystem.

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Introduction

Rice, (*Oryza sativa* L.) is the most important food crop in India and is the staple food for half of the world's population and providing 30–80% of the daily calories required in Asian population (Hossain and Narciso 2002). In developing countries, rice is the principal source of calories and nutrients. Various abiotic stresses such as drought, salinity, submergence, and extreme temperature cause a threatening impact on the growth and productivity of rice (Gregorio et al. 2013). Among these, salinity and submergence are the major issues faced by farmers of coastal agroecosystem. Farmers suffer from crop losses caused by the intrusion of saline water into rice fields and occasional flash floods during the monsoon season. Submergence stress due to unexpected heavy rains adversely affected rice crops and reduced the grain yield drastically (Sarkar and Bhattacharjee 2011). Rice can be susceptible to salinity at the seedling and reproductive stages (Lutts et al. 1995). The flowering stage is also a highly sensitive stage that is affected by salinity stress (Singh et al. 2007) and yield potential at the mature stage was affected due to salinity (Todaka et al. 2012). Salinity affects yield parameters such as panicle emergence, flowering, panicle length, spikelet number per panicle, and grain yield (Thomson et al. 2010). Submergence affects many plant physiological processes such as water absorption, respiration, photosynthetic activity and substantially reduce crop productivity (Fukao et al. 2011).

Thus, to tackle this abiotic stress induced yield reductions in stress fields; a permanent and sustainable approach

is required. The most viable solution is to introgress the genes for abiotic stress tolerance into already developed improved high yielding rice cultivar through MABB. It has been identified that salinity tolerance is controlled by quantitative trait locus (QTL) on chromosome 1 which is responsible for low Na^+ high K^+ uptake (Mohammadi et al. 2008) and submergence tolerance is controlled by quantitative trait locus (QTL) on chromosome 9 (Neeraja et al. 2007). MABB is a precise and effective method to introgress the trait of interest without altering the essential characters of elite varieties. The objective of the present study was to improve the rice cultivar Aiswarya for salinity and submergence tolerance using the MABB strategy. In this paper, the parallel introgression of *Saltol* QTL and *Sub1* gene through MABB strategy are discussed in detail which is a pre-requisite for pyramiding of these two abiotic stress tolerance QTLs, into the rice variety Aiswarya.

Materials and methods

Rice varieties – recurrent and donor parents

The saline tolerant rice breeding line FL-478, developed at IRRI (The International Rice Research Institute 1996) was used as the donor of *Saltol QTL.* The donor of *Sub1* gene used was the Swarna *Sub1,* submergence tolerant rice variety developed at International Rice Research Institute, manila, Philippines. The recurrent parent used for improvement in this study is a high yielding rice cultivar, Aiswarya, developed at the Regional Agricultural Research Station, Pattambi, which is sensitive to salinity and submergence with an average yield of 6.3—6.5 tons/ha.

Breeding scheme for *Saltol QTL* introgression

The recurrent parent (RP) Aiswarya and donor parent (DP) FL-478 were crossed and F1 generation was raised. In case of *Saltol QTL* introgression, in each generation both phenotypic screening and genotypic screening were done, and the QTL introgressed plants were advanced into next generation. In the F_1 generation genotypic screening was done in the phenotypically survived plants under saline condition and plants with heterozygous loci for the target trait alone were selected and backcrossed with the respective recurrent parent and raised BC_1F_1 generation. The heterozygous plants with the target locus in the BC_1F_1 generation were again selected for backcrossing with the respective recurrent parent to raise BC_2F_1 progenies. The heterozygous BC_2F_1 plants were selected to raise BC_2F_2 progenies by selfing and the homozygous plants in the target locus alone were selected from the selfed BC_2F_2 progenies (Fig. 1). After the Phenotyping of the BC_2F_2 progenies the plants with Score-3 (SES, IRRI) alone were selected for genotyping. The SES is a plant score of 1–9 spectrum based on morphological manifestation/stress symptoms under stress conditions where a lower SES score shows resistance and a higher score shows a sensitive response (Table 1).

Breeding scheme for *Sub 1* gene introgression

The recurrent parent (RP) Aiswarya and donor parent (DP) Swarna *Sub1* were crossed and F1 generation was raised. In case of *Sub1*gene introgression the screening in each generation was done by genotyping and phenotypic screening was done in the final stage. In the F_1 generation genotypic screening was done and plants with heterozygous loci for the target trait alone were selected and backcrossed with the respective recurrent parent and raised BC_1F_1 generation. The heterozygous plants with the target locus in the BC_1F_1 generation were again selected for backcrossing with the respective recurrent parent to raise BC_2F_1 progenies. The heterozygous BC_2F_1 plants were selected to raise BC_2F_2 progenies by selfing and the homozygous plants in the target locus alone were selected from the selfed BC_2F_2 progenies. After the Phenotyping of the BC_2F_2 progenies the plants with Score-3 (SES, IRRI) alone were selected (Fig. 2).

DNA isolation and PCR assay

The young leaf samples were collected, and genomic DNA was extracted using CTAB method (Doyle and Doyle 1987) The concentration of DNA was measured using Nano Drop 2000c (Thermo Scientific). Target gene sequence was amplified by PCR assay, in which the total volume of reaction is 20 µl containing 2µlDNA template, 10µlPCR Master Mix (Thermo Scientific) consist of 1XTaq Buffer, Taq DNA polymerase, $MgCl_2$, 1 µl each of reverse and forward primer along with 6 µl water. To begin the PCR program the initial denaturation temperature was raised to 95°C then annealing temperature lowered to 55–65°C and extension temperature at 72°C. The cycle of changing temperature was then repeated up to 30–35 cycles.

Quality control with electrophoresis and DNA visualization

The DNA quality was tested by 8% Polyacrylamide gel electrophoresis (PAGE). Silver stain method were used for staining gels and were visualized using Gel Doc (Bio-Rad Gel Doc XR +). Selected SSR markers were used for foreground, recombinant and background selection.

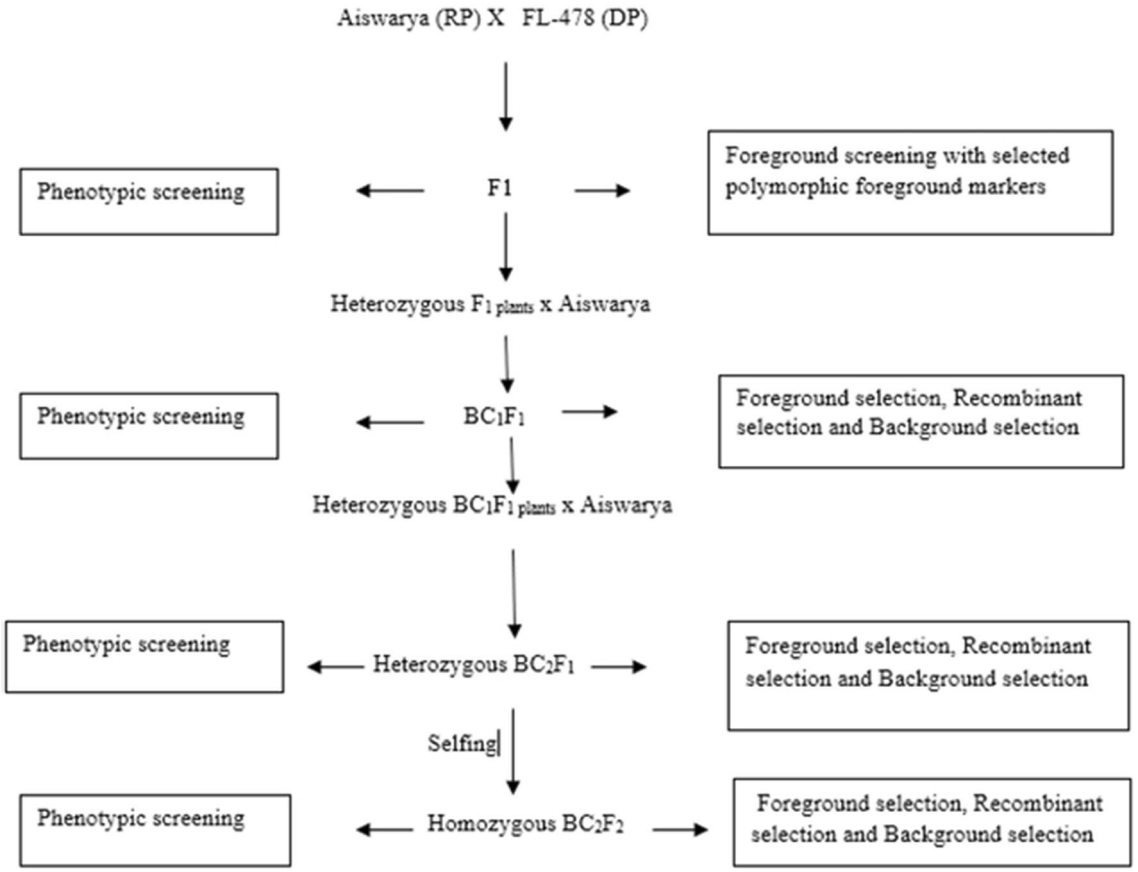

Fig. 1 Breeding scheme of *Saltol* QTL Introgression into mega rice variety Aiswarya, as a Recurrent Parent (RP) and *Saltol*- FL-478, as a Donor Parent (DP)

Table 1 Standard evaluation system (SES) score for salinity tolerance (IRRI 1996)

Score	Observation	Tolerance
1	Nearly normal growth, no leaf symptoms	Highly Tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately Tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly Susceptible

Polymorphism assay and selection of SSR markers

Simple sequence repeats are co-dominant markers which occur at high frequency and they prominent throughout the genome. SSR markers shows a high level of polymorphism between the plant varieties. A total of 545 SSR markers were screened for polymorphism between the recurrent parent and donor parents, which is narrated in Table 10.

Foreground, recombinant and background selection for Saltol QTL

For the screening of *Saltol* QTL introgressed progenies of all generations, 7 foreground markers AP3206, SKC10, RM3412, RM8094, RM10713, RM10711, RM10772, 6 recombinant markers RM10793, RM493, RM10696, RM10701, RM1287, RM10694 and 545 genome wide SSR markers were screened between parents and then polymorphic markers were selected for genotypic screening of progenies of Aiswarya X FL-478.

Foreground, recombinant and background selection for Sub1 gene

For the screening of *Sub1* gene introgression and background genome recovery 11 foreground markers which are IYT1, IYT3, AEX, Sub1A203, ART3, ART5, Sub1C173, Sub1AB1, Sub1BC1, Sub1BC2 and Sub1BC3, 16

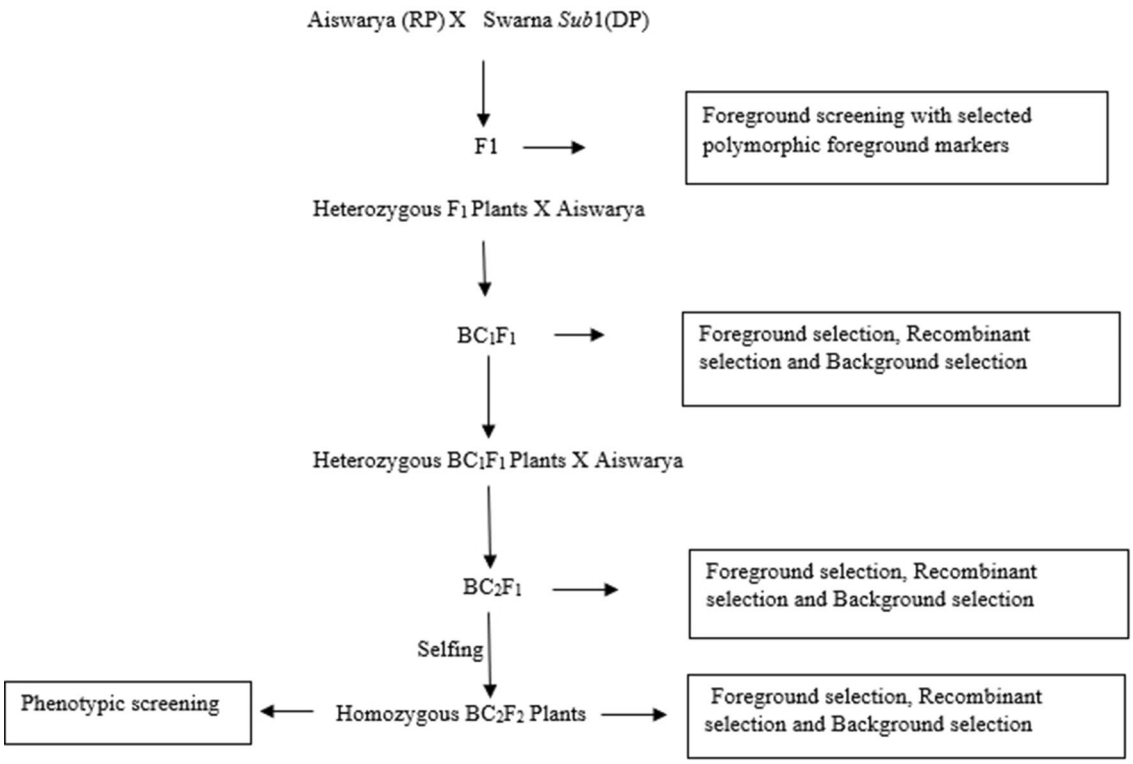

Fig. 2 Breeding scheme of *Sub1*gene Introgression into mega rice variety Aiswarya, as a Recurrent Parent (RP) and Swarna *Sub1,* as a Donor Parent (DP)

recombinant markers RM316, RM219, RM464, RM23668, RM23679, RM8303, RM23770, RM23788, RM23778, RM23805, RM23887, RM23917, RM23922, RM23958, RM23928 and RM24005, and 545 genome wide markers were screened and evaluated. The markers which showed polymorphism between parents were selected for genotypic screening of all generations.

Analysis of molecular data

The Microsoft Excel sheet containing Saltol marker and Sub1 marker data was used and analysed in the Graphical Tools for Genotyper (GGT 2.0) (Ralph, 2008). GGT analysis software was used to measure recovery of parent genome in selected Introgressed progenies of Saltol X Sub1 lines. The alleles were scored for homozygous recipient allele, homozygous donor allele and heterozygous allele as 'A', 'B', 'C', respectively. So, the percentage of background genomic recovery were calculated.

Screening for salinity tolerance

Phenotypic screening at seedling stage for salinity and submergence was done. The salinity screening was done using the standard protocol SES developed at IRRI. The pre germinated seeds were sown in the holes on the float in distilled water for 3 days. FL-478 plants were used as the check and RP Aiswarya was used as susceptible check. On fourth day, distilled water was replaced with Yoshida nutrient solution having EC of 6 dS m^{-1} and on 7th day the Yoshida nutrient solution was replaced with fresh nutrient solution having EC of 12 dS m^{-1}.The pH of the solution was maintained at 5.5 daily by adding either 1 N NaOH or 1 N HCL. The nutrient solution was replaced after every 7 days up to 21 days. The tolerance score was done using SES score developed at IRRI, 1996. Table 1 describes the SES score for salinity tolerance.

Screening for submergence tolerance

In screening for submergence tolerance, germinated seedlings of the selected progenies along with donor and recurrent parents were sown in pots. The experimental pots with fourteen-day old seedlings were completely submerged for 21 days in 1.5 m height tanks filled with water. The average depth of water was 90 cm and pH was 7.2. Control sets were also maintained for each of the varieties without any submergence treatment. After the completion of submergence treatment the plants were removed and 14 days after the completion of submergence treatment tolerance as per SES developed at IRRI. Table 2 describes the SES score for submergence tolerance.

Table 2 Standard evaluation system (SES) score for submergence tolerance (IRRI 1996)

Score	Percentage survival	Tolerance
1	100%	Minor visible symptoms
3	95–99%	Some visible symptoms
5	75–94%	Moderately injury
7	50–54%	Severe injury
9	0–94%	Complete death

Results and discussion

The MABB approach was used to introgress the *Saltol* QTL and *Sub 1* genes into Aiswarya rice variety and is an effective and appropriate method for introgressing our gene of interest and recovering the maximum genome background of recurrent parents in progenies (Collard and Mackill 2007). The highly tolerant F1 plants were selected based on phenotyping and genotyped with selected foreground markers for confirmation, and the tolerant lines with heterozygous locus were alone selected. The selected tolerant lines in F1 and BC1F1 generations were backcrossed with recurrent parent and genotyped with foreground, recombinant and background primers. At last from the selfed BC_2F_2 lines having the salinity and submergence tolerance score similar to FL-478 and Swarna *Sub1* with maximum recipient genotype recovery could be identified.

Parental Polymorphism Assay of Foreground, Recombinant and Background Markers

Total 545 SSR markers covering 12 chromosomes were used for the parental polymorphic screening between recurrent parent and donor parent. For salinity screening 88 polymorphic markers and for submergence screening 90 polymorphic markers were selected and used for screening of every F1, BC_1F_1, BC2F1 and BC2F2 generation. Foreground markers are used for efficient selection of target locus and these markers are tightly linked with the target gene (Hospital and Charcosset 1997). The recombinant markers that flank a target gene are used for eliminating the undesirable gene as quick as possible and linkage drag can be minimized (Hasan et al. 2015). Background selection involves selecting progenies with the maximum proportion of RP genome, and these markers are unlinked to the target locus. Genetic analysis of the salt tolerant rice varieties using SSR markers through molecular characterization is done (John and Shylaraj.2017). The result was verified with some other previous reports of the polymorphic markers between the parents in target region of *Saltol* (Niones 2004). Within the Sub1 cluster the diagnostic markers developed and microsatellite markers along the Sub1 region has been reported (Neeraja et al. 2007). Sub1 QTL was introgressed into the most popular rice variety, Jyothi, from the donor parent Swarna-Sub1 for submergence tolerance (John and Shylaraj 2017). Number of Polymorphic markers screened and used for parental screening are narrated in Table 3 below. Tables 4,5,6,7,8,9 describes the name of selected foreground, recombinant and background SSR markers. Total 545 genomewide SSR markers screened for parental polymorphism in both salinity and submergence were narrated in Table 10.

Genotyping of F_1 generation

Saltol F1 generation

For the foreground selection of *Saltol* F1 generation, 35 / 55 plants were heterozygous for AP3206, SKC-10 and RM3412 foreground markers. After that recombinant selection was done with RM493 and RM10696. Recently the highly salt tolerant rice variety FL478 has been used to transfer *Saltol* QTL into the high yielding grown cultivars ASS996 using MABC strategy in Vietnam (Huyen et al. 2012). Figure 3 shows the genotypic screening with SKC10 marker.

Sub1 F1 generation

For the selection of *Sub1* F1 generation of the 48 plants, 26 plants were heterozygous for ART5, SUIBC2 And SUB1C173 foreground markers and recombinant selection carried out with RM8303, RM23770 and RM24005. The successful introgression of *sub1* from donor rice variety IR64 into popular rice variety AS996 through MABC using ART5 and SC3 has been done in Vietnam (Cuc et al. 2012). After selection of heterozygous plants, they were backcrossed with the respective recurrent parents to develop BC_1F_1 plants. Figure 4 shows the Genotypic screening of *Sub1* F1 progenies with foreground marker ART5.

Genotyping of BC_1F_1 generation

Saltol *BC_1F1 generation*

In 90 plants of BC_1F_1 the 58 plants were *Saltol* introgressed plants, confirmed with AP3206, SKC-10 and RM3412 foreground markers and Recombinant selection was carried out on 58 *Saltol* introgressed plants and 66 *Sub1*

Table 3 Polymorphic markers screened and markers used for parental screening

SSR markers screened for polymorphism				Polymorphic markers selected	
		Salinity	Submergence	Salinity	Submergence
1	Foreground markers	**7**	11	3	3
2	Recombinant markers	6	16	2	3
3	Background markers	545	545	88	90

Table 4 *Saltol* SSR markers used for foreground selection

Primer	Sequence	Distance (mb)
AP206 F	TTCTCATCGCACCATCTCTG	11.2
AP206R	GGAGGAGGAGAGGAAGAAG	
SKC10F	ATAGGGGATATTGGCTGCAC	11.2
SKC10R	CAACCAAGCGTGACTAAAAAGA	
RM3412F	TGATGGATCTCTGAGGTGTAAAGAGC	11.6
RM3412R	TGCACTAATCTTTCTGCCACAGC	

Table 5 Saltol Polymorphic SSR markers used for Recombinant selection

Primer	Sequence	Distance (mb)
RM493 F	GTACGTAAACGCGGAAGGTGACG	12.3
RM493R	CGACGTACGAGATGCCGATCC	
RM10696F	CCTTCGACTCCATGAAACAAACG	10.6
RM10696R	TCTCTTTGCCCTAACCCTATGTCC	

introgressed plants. Background analysis was done of *Saltol* introgressed plants with selected SSR markers and indicated a recovery of 64%. The Introgression of Saltol QTL into an elite rice variety Jyothi is done using marker assisted selection (Rohini and Shylaraj 2017). Seventeen *Saltol* BC_1F_1 recombinant plants were further used for backcross breeding. Figure 5 shows the Genotypic screening of *Saltol* BC1F1 progenies with foreground marker SKC-10 and Fig. 6 shows the Genotypic screening of *Saltol* BC1F1 progenies with recombinant marker RM10696.

Sub1 BC_1F1 generation

In 94 BC1F1 plants, 66 plants were *Sub1* introgressed plants, confirmed with ART5, SUIBC2 And SUB1C173 and Recombinant selection was carried out on 58 *Saltol* introgressed plants and 66 *Sub1* introgressed plants. Background analysis was done in Sub1 introgressed plants with selected SSR markers and indicated a recovery of 67%. Nineteen *Sub1* BC_1F_1 recombinant plants were further used for backcross breeding. Figure 7 shows the Genotypic screening of *Sub1* BC1F1 progenies with foreground marker ART5 and Fig. 8 shows the Genotypic screening of *Sub1* BC1F1 progenies with recombinant marker RM8303.

Genotyping of BC_2F_1 and BC_2F_2 generation

Saltol BC_2F_1 and BC_2F_2 generation

In BC_2F_1 generation 10 plants with *Saltol* introgressed were phenotypically and genotypically analyzed and indicated a recovery of 75% to 82%. After that, all the selected BC_2F_1 plants were selfed and BC_2F_2 generation were obtained. On the basis of phenotypic and genotypic screening, the best four plants with maximum genome recovery of 89% to 92% were selected. Figure 9 shows the Genotypic screening of *Saltol* BC2F2 progenies with foreground marker SKC-10. Figure 10 shows the Genotypic screening of *Saltol trait specific* BC2F2 progenies with recombinant marker RM10696. The Fig. 11 shows the graphical representation of selected best plant with 92% background genomic recovery.

Sub1 BC_2F_1 and BC_2F_2 generation

In BC_2F_1 generation 12 plants with *Sub1* introgressed were phenotypically and genotypically analyzed and indicated a recovery of 70% to 80%. After that, all the selected BC_2F_1 plants were selfed and BC_2F_2 generation produced. On the basis of phenotypic and genotypic screening, the best four plants with maximum genome recovery of 85% to 93%

Table 6 Saltol Polymorphic SSR markers used for Background selection

Chr. No	SSR markers	Position (cM)	Chr.No	SSR markers	Position (cM)	Chr.No	SSR markers	Position (cM)
1	1196	0	**4**	RM 537	0	**6**	RM 439	117
1	RM 10209	18.8	**4**	RM 3471	16.7	**7**	RM 295	0
1	RM 600	61.30	**4**	RM 3317	32.1	**7**	RM 3859	42.4
1	RM595	78.40	**4**	RM471	53.8	**7**	RM 214	61.5
1	RM 246	115.2	**4**	RM 401	63.4	**7**	RM 11	93.8
1	RM 212	148.7	**4**	RM 142	100.2	**7**	RM 118	130.5
1	RM 472	171.6	**4**	RM 273	116.8	**7**	RM 420	144.6
2	RM 174	33.8	**4**	RM 153	0.5	**8**	RM 408	0
2	RM 1347	26.6	**4**	RM17960	18.8	**8**	RM1235	12.8
2	RM 71	49.8	**4**	RM 1089	37.2	**8**	RM 1376	15.2
2	RM 1352	48.1	**5**	RM 169	57.9	**8**	RM547	27.3
2	RM 5430	11.5	**5**	RM 6229	63.6	**8**	RM25	59.6
2	RM 1303	81.7	**5**	RM 1187	96.1	**8**	RM149	122.1
2	RM 526	136.3	**5**	RM249	122.1	**8**	RM 447	131.3
2	RM240	158	**5**	RM5770	103.9	**9**	RM 1328	0
2	RM 498	194.6	**5**	RM334	141.8	**9**	RM 316	1.5
3	RM 175	18.9	**6**	RM508	0	**9**	RM 3912	46.3
3	RM545	35.3	**6**	RM469	2.8	**9**	RM 1553	68.2
3	RM 227	120.1	**6**	RM 197	15.1	**9**	RM 189	90.7
3	RM 168	138.6	**6**	RM 276	30.8	**9**	RM 205	114.7
3	RM6135	158.2	**6**	RM 527	59.9	**10**	RM 214	27.8
3	RM 85	179.7	**6**	RM 454	86.5	**10**	RM 2504	35.9
3	RM7324	166.4	**6**	RM 1340	82.9	**10**	RM 311	46.4
3	RM520	191.6	**6**	RM 30	115.7	**10**	RM184	79.3
10	RM 294A	109.4	**11**	RM 6965	101.9	**12**	RM 1105	91.4
11	RM 332	12.3	**11**	RM 206	104.2	**12**	RM 1296	108.2
11	RM 536	42	**11**	RM 144	121.4	**12**	RM 17	109.1
11	RM 7120	56.2	**12**	RM1328	0	**12**	RM 1227	130.8
11	RM 229	66.5	**12**	RM 19	20.9			
11	RM 209	84.7	**12**	RM 27973	57.6			

Table 7 Submergence SSR markers used for foreground selection

Primer	Sequence	Distance (mb)
SUB1C173F	AACGCCAAGACCAACTTCC	Exon of Sub*1C*
SUB1CI73R	AGGAGGCTGTCCATCAGGT	
ART5F	CAGGGAAAGAGATGGTGGA	Sub1C promoter
ART5R	TTGGCCCTAGGTTGTTTCAG	
Sub1BC2F	AAAACAATGGTTCCATACGAGAC	Between *Sub1B* and *C*
Sub1BC2R	GCCTATCAATGCGTGCTCTT	

were selected. Figure 12 shows the Genotypic screening of *Sub1* BC2F2 progenies with foreground marker ART5. Figure 13 shows the Genotypic screening of *Sub1* BC2F2 progenies with recombinant marker RM8303. The Fig. 14 shows the graphical presentation of best selected plant with 93% background genomic recovery.

Phenotypic Screening for Salinity tolerance and submergence tolerance

The selected *Saltol* BC_2F_2 lines showed salinity tolerance at seedling stage under salt stress. The salinity tolerance score of these lines were similar to the donor variety FL-

Table 8 Submergence SSR markers used for recombinant selection

Primer	Sequence	Distance (mb)
RM8303F	AGGGGAGAGGACACACACAC	2.3
RM8303R	GGATCCTCCTGCAAAATCAA	
RM23770F	GACCTTGTCCAGAGTGATTTTG	3.7
RM23770R	ATTTGAGAATAACTTTTCCTACTTCG	
RM23958F	GAGACAGATGTGTACGGTTTGGTG	7.9
RM23958R	TTGACAAGGGAATTGAAGGAGAAG	

Table 9 Submergence Polymorphic SSR markers used for Background selection

Chr. No	SSR Marker	Position (cM)	Chr. No	SSR Marker	Position (cM)	Chr. No	SSR Marker	Position (cM)
1	RM 3252	0.0	4	RM 261	31.1	6	RM 340	137.5
1	RM 595	78.4	4	RM 401	63.4	7	RM 295	0.0
1	RM1196	107.5	4	RM 273	116.8	7	RM 3859	42.4
1	RM246	115.2	4	RM 142	100.2	7	RM 125	44.4
1	RM443	122.7	4	RM 559	173.3	7	RM 214	61.5
2	RM7382	0.0	4	RM 1155	58.9	7	RM 11	93.8
2	RM 1347	26.6	5	RM 153	0.0	7	RM 1362	116.1
2	RM324	66.0	5	RM 169	57.9	7	RM 420	144.6
2	RM 5430	111.5	5	RM 188	108.7	8	RM 337	0.1
2	RM7485	145.7	5	RM 334	11.7	8	RM 1235	12.8
2	RM 240	158.0	5	RM 6467	143.8	8	RM 1376	15.2
3	RM 175	18.9	5	RM 30	174.6	8	RM 547	27.3
3	RM 545	35.3	6	RM 469	2.8	8	RM 3481	44.7
3	RM 282	61.5	6	RM 402	3.2	8	RM 310	57.0
3	RM5626	122.4	6	RM 197	15.1	8	RM 25	59.6
3	RM 118	138.6	6	RM 276	30.8	8	RM 433	87.2
3	RM 227	229.7	6	RM 454	86.5	8	RM 149	122.1
4	RM 551	0.0	6	RM 1340	82.9	8	RM 447	131.3
8	RM 264	138.2	10	RM 1374	72.8	11	RM 206	104.2
9	RM 1328	0.0	10	RM 184	79.3	11	RM 144	121.4
9	RM 316	1.5	10	RM 6016	83.0	11	RM 27172	123.3
9	RM 3912	46.3	10	RM 294A	109.4	11	RM 2136	136.8
9	RM 3164	72.1	11	RM 332	12.3	12	RM 247	12.0
9	RM 328	82.4	11	RM 287	57.4	12	RM 19	20.9
9	RM 245	112.3	11	RM 7303	64.2	12	RM 3246	48.2
10	RM 216	27.8	11	RM 229	66.5	12	RM 27973	57.6
10	RM 2504	35.9	11	RM 1341	80.2	12	RM 235	82.0
10	RM 596	34.8	11	RM 5961	79.9	12	RM 1103	91.4
10	RM 311	46.4	11	RM 209	84.7	12	RM 17	109.1
10	RM 1108	55.3	11	RM 6965	101.9	12	RM 1227	130.8

478. A salinity tolerant Q5DB was developed using marker assisted selection, which was controlled by a major Saltol QTL (Huyen et al. 2013) and the selected *Sub1* introgressed BC_2F_2 lines showed submergence tolerance and recovery after submergence of 21 days so the tolerance score of these lines were similar to the donor variety Swarna *Sub1*. A single major quantitative trait locus (QTL) *SUBMERGENCE 1 (SUB1)* near the centromere of chromosome 9 in the rice genome along with a number of minor QTLs linked to submergence tolerant had been identified (Xu et al. 2006). Based on these results the introgression of *Saltol* and *Sub1* QTL in these lines was

Table 10 Genome wide SSR markers used for parental polymorphism assay

Sl. No	Primer name	Chr. No	Sl. No	Primer name	Chr. No	Sl. No	Primer name	Chr. No
1	RM499	1	27	RM140	1	53	RM5536	1
2	RM3252	1	28	RM595	1	54	RM315	1
3	RM323	1	29	RM129	1	55	RM472	1
4	RM476A	1	30	RM113	1	56	RM431	1
5	RM10209	1	31	RM562	1	57	RM3362	1
6	RM1	1	32	RM24	1	58	RM6840	1
7	RM220	1	33	RM9	1	59	RM165	1
8	RM6289	1	34	RM5	1	60	OSR23	1
9	RM283	1	35	RM306	1	61	RM104	1
10	RM151	1	36	RM488	1	62	RM529	1
11	RM576	1	37	RM1196	1	63	RM414	1
12	RM490	1	38	RM246	1	64	RM14	1
13	RM575	1	39	RM237	1	65	RM568	1
14	RM259	1	40	RM443	1	66	RM3340	2
15	RM35	1	41	RM403	1	67	OSR17	2
16	RM243	1	42	RM128	1	68	RM154	2
17	RM583	1	43	RM543	1	69	OSR14	2
18	RM600	1	44	RM302	1	70	RM279	2
19	RM578	1	45	RM212	1	71	RM279	2
20	RM292	1	46	RM102	1	72	RM1075	2
21	RM572	1	47	RM476B	1	73	RM1347	2
22	RM580	1	48	RM486	1	74	RM8	2
23	RM312	1	49	RM226	1	75	RM555	2
24	RM23	1	50	RM265	1	76	RM174	2
25	RM81A	1	51	RM297	1	77	RM1352	2
26	RM238A	1	52	RM8236	1	78	RM71	2
79	RM1081	2	120	RM406	2	161	RM5959	3
80	RM492	2	121	RM213	2	162	RM503	3
81	RM5812	2	122	RM482	2	163	RM135	3
82	RM452	2	123	RM207	2	164	RM426	3
83	RM550	2	124	RM266	2	165	RM6135	3
84	RM438	2	125	RM498	2	166	RM532	3
85	RM5101	2	126	RM535	2	167	RM504	3
86	RM4499	2	127	RM7382	2	168	RM1221	3
87	RM465A	2	128	RM81B	3	169	RM203	3
88	RM324	2	129	RM60	3	170	RM7324	3
89	RM262	2	130	RM132	3	171	RM186	3
90	RM561	2	131	RM22	3	172	RM55	3
91	RM1303	2	132	RM523	3	173	RM168	3
92	RM341	2	133	RM569	3	174	RM448	3
93	RM5430	2	134	RM231	3	175	RM3583	3
94	RM475	2	135	RM175	3	176	RM416	3
95	RM3352	2	136	RM489	3	177	RM520	3
96	RM1385	2	137	RM545	3	178	RM293	3
97	RM1367	2	138	RM36	3	179	RM227	3
98	RM3220	2	139	OSR16	3	180	RM468	3
99	RM106	2	140	RM517	3	181	RM571	3
100	RM263	2	141	RM546	3	182	RM422	3

Table 10 continued

Sl. No	Primer name	Chr. No	Sl. No	Primer name	Chr. No	Sl. No	Primer name	Chr. No
101	RM3275	2	142	RM218	3	183	RM422	3
102	RM526	2	143	RM3204	3	184	RM143	3
103	RM599	2	144	RM232	3	185	RM130	3
104	RM5607	2	145	RM251	3	186	RM114	3
105	RM221	2	146	RM563	3	187	RM514	3
106	RM525	2	147	RM5626	3	188	RM570	3
107	RM573	2	148	RM554	3	189	RM148	3
108	RM1092	2	149	RM282	3	190	RM442	3
109	RM5421	2	150	RM338	3	191	RM85	3
110	RM318	2	151	RM473D	3	192	RM307	4
111	RM6	2	152	RM2614	3	193	RM2146	4
112	RM3650	2	153	RM156	3	194	RM5414	4
113	RM240	2	154	RM411	3	195	RM551	4
114	RM7485	2	155	RM487	3	196	RM401	4
115	RM425	2	156	RM16	3	197	RM537	4
116	RM250	2	157	RM347	3	198	RM6770	4
117	RM1063	2	158	RM5172	3	199	RM3471	4
118	RM166	2	159	RM6213	3	200	RM5953	4
119	RM208	2	160	RM319	3	201	RM335	4
202	RM518	4	243	RM413	5	284	RM190	6
203	RM3317	4	244	RM194	5	285	RM510	6
204	RM261	4	245	RM1089	5	286	RM111	6
205	RM456B	4	246	RM5994	5	287	RM276	6
206	RM185	4	247	RM6229	5	288	RM402	6
207	RM177	4	248	RM169	5	289	RM6467	6
208	RM471	4	249	RM509	5	290	RM527	6
209	RM417	4	250	RM1237	5	291	RM5745	6
210	RM1155	4	251	RM598	5	292	RM1161	6
211	RM142	4	252	RM465C	5	293	RM6818	6
212	RM119	4	253	RM249	5	294	RM5753	6
213	RM1136	4	254	RM146	5	295	RM541	6
214	RM5320	4	255	RM39	5	296	RM1925	6
215	RM273	4	256	RM291	5	297	RM454	6
216	RM1703	4	257	RM430	5	298	RM162	6
217	RM252	4	258	RM473B	5	299	RM275	6
218	RM456A	4	259	RM1090	5	300	RM343	6
219	RM3217	4	260	RM440	5	301	RM1340	6
220	RM241	4	261	RM459	5	302	RM5814	6
221	RM1100	4	262	RM1187	5	303	RM528	6
222	RM451	4	263	RM161	5	304	RM1161	6
223	RM470	4	264	RM305	5	305	RM1150	6
224	RM303	4	265	RM173	5	306	RM30	6
225	RM317	4	266	RM534	5	307	RM340	6
226	RM5047	4	267	RM188	5	308	RM439	6
227	RM1018	4	268	RM538	5	309	RM461	6
228	RM255	4	269	RM5770	5	310	RM103	6
229	RM348	4	270	RM233B	5	311	RM494	6
230	RM349	4	271	RM6200	5	312	RM295	7

Table 10 continued

Sl. No	Primer name	Chr. No	Sl. No	Primer name	Chr. No	Sl. No	Primer name	Chr. No
231	RM131	4	272	RM421	5	313	RM192	7
232	RM124	4	273	RM5968	5	314	RM436	7
233	RM127	4	274	RM178	5	315	RM427	7
234	RM280	4	275	RM26	5	316	RM1132	7
235	RM567	4	276	RM274	5	317	RM3555	7
236	RM559	4	277	RM87	5	318	RM125	7
237	RM153	5	278	RM480	5	319	RM1135	7
238	RM507	5	279	RM334	5	320	RM180	7
239	RM122	5	280	RM133	6	321	RM3484	7
240	RM5693	5	281	RM508	6	322	RM6111	7
241	RM5796	5	282	RM197	6	323	RM214	7
242	RM17960	5	283	RM469	6	324	RM500	7
325	RM445	7	366	RM44	8	407	RM219	9
326	RM6776	7	367	RM32	8	408	RM524	9
327	RM1362	7	368	RM72	8	409	RM342B	9
328	RM11	7	369	RM88	8	410	RM5526	9
329	RM3859	7	370	RM195	8	411	RM105	9
330	RM560	7	371	RM330B	8	412	RM321	9
331	RM182	7	372	RM350	8	413	RM1817	9
332	RM336	7	373	RM404	8	414	RM2144	9
333	RM351	7	374	RM483	8	415	RM409	9
334	RM70	7	375	RM1345	8	416	RM460	9
335	RM455	7	376	RM137	8	417	RM3912	9
336	RM1085	7	377	RM331	8	418	RM566	9
337	RM505	7	378	RM1109	8	419	RM4692	9
338	RM1134	7	379	RM1235	8	420	RM434	9
339	RM234	7	380	RM223	8	421	RM410	9
340	RM18	7	381	RM515	8	422	RM2482	9
341	RM47	7	382	RM284	8	423	RM257	9
342	RM478	7	383	RM6193	8	424	RM1896	9
343	RM118	7	384	RM556	8	425	RM3700	9
344	RM134	7	385	RM210	8	426	RM242	9
345	RM429	7	386	RM531	8	427	RM108	9
346	RM1253	7	387	RM419	8	428	RM288	9
347	RM5426	7	388	RM256	8	429	RM553	9
348	RM172	7	389	RM149	8	430	RM278	9
349	RM420	7	390	RM308	8	431	RM201	9
350	RM2381	7	391	RM230	8	432	RM160	9
351	RM248	7	392	RM433	8	433	RM107	9
352	RM408	8	393	RM1309	8	434	RM328	9
353	RM337	8	394	RM502	8	435	OSR28	9
354	RM152	8	395	RM458	8	436	RM189	9
355	RM1308	8	396	RM477	8	437	RM3164	9
356	RM1376	8	397	RM447	8	438	RM2855	9
357	RM3481	8	398	RM281	8	439	RM215	9
358	RM3262	8	399	RM264	8	440	RM1026	9
359	RM25	8	400	RM41	9	441	RM1328	9
360	RM6471	8	401	RM296	9	442	RM 245	9

Table 10 continued

Sl. No	Primer name	Chr. No	Sl. No	Primer name	Chr. No	Sl. No	Primer name	Chr. No
361	RM5999	8	402	RM1553	9	443	RM205	9
362	RM6032	8	403	RM5799	9	444	RM6364	10
363	RM6008	8	404	RM316	9	445	RM474	10
364	RM310	8	405	RM464	9	446	RM474	10
365	RM547	8	406	RM464	9	447	RM330A	10
448	RM2504	10	489	RM552	11	530	RM277	12
449	RM216	10	490	RM116	11	531	RM27973	12
450	RM239	10	491	RM120	11	532	RM519	12
451	RM311	10	492	RM6115	11	533	RM1246	12
452	RM5689	10	493	RM479	11	534	RM313	12
453	RM1375	10	494	RM5731	11	535	RM83	12
454	RM467	10	495	RM536	11	536	RM463	12
455	RM5629	10	496	RM7303	11	537	RM1986	12
456	RM6100	10	497	RM7120	11	538	RM235	12
457	RM596	10	498	RM287	11	539	RM270	12
458	RM1108	10	499	RM209	11	540	RM1103	12
459	RM184	10	500	RM229	11	541	RM2854	12
460	RM271	10	501	RM5961	11	542	RM6396	12
461	RM6128	10	502	RM1341	11	543	RM1296	12
462	RM269	10	503	RM457	11	544	RM17	12
463	RM258	10	504	RM473E	11	545	RM1227	12
464	RM5666	10	505	RM6965	11			
465	RM1374	10	506	RM206	11			
466	RM171	10	507	RM254	11			
467	RM304	10	508	RM456C	11			
468	RM3123	10	509	RM2136	11			
469	RM6016	10	510	RM6094	11			
470	RM294A	10	511	RM224	11			
471	RM228	10	512	RM139	11			
472	RM484	10	513	RM144	11			
473	RM147	10	514	RM27172	11			
474	RM333	10	515	RM1880	12			
475	RM496	10	516	RM2851	12			
476	RM590	10	517	RM3483	12			
477	RM591	10	518	RM19	12			
478	RM4B	11	519	RM453	12			
479	RM20B	11	520	RM247	12			
480	RM286	11	521	RM117	12			
481	RM26652	11	522	RM491	12			
482	RM7163	11	523	RM512	12			
483	RM7173	11	524	RM179	12			
484	RM1240	11	525	RM415	12			
485	RM1124	11	526	RM2529	12			
486	RM332	11	527	RM1036	12			
487	RM5704	11	528	RM3246	12			
488	RM167	11	529	RM101	12			

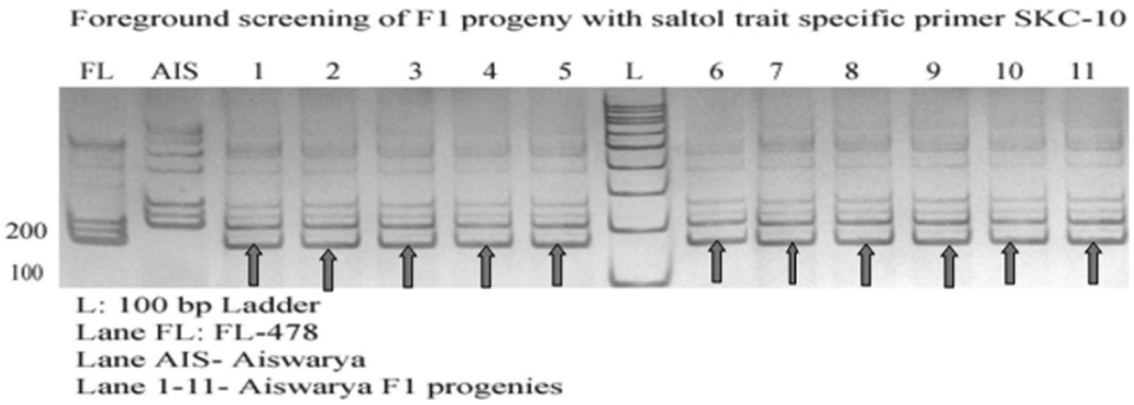

Fig. 3 Genotypic screening of *Saltol trait specific* F1 progenies with foreground marker SKC-10 (Aiswarya X FL 478)

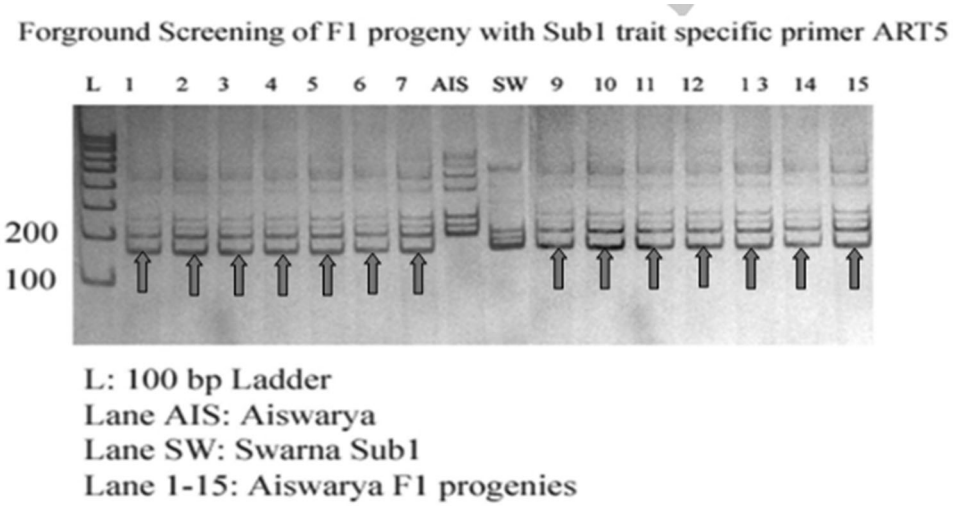

Fig. 4 Genotypic screening of *Sub1 trait specific* F1 progenies with foreground marker ART5 (Aiswarya X Swarna Sub1)

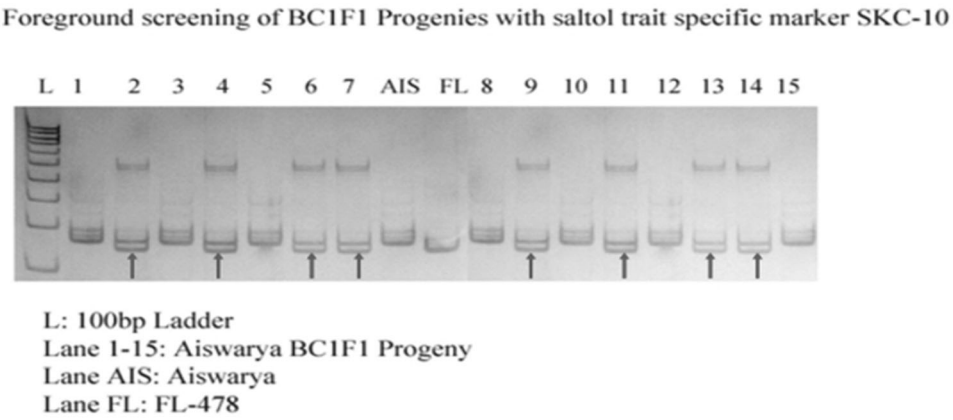

Fig. 5 Genotypic screening of *Saltol trait specific* BC1F1 progenies with foreground marker SKC-10

confirmed. Figure 15 shows the phenotypic screening of selected progenies for salinity tolerance and Fig. 16 shows the phenotypic screening of submergence tolerance.

Outcome of the study

The present study could develop 5 best lines of Saltol QTL introgressed Aiswarya with 85%–92% of background homozygosity of Aiswarya and 4 best lines of Sub1 gene

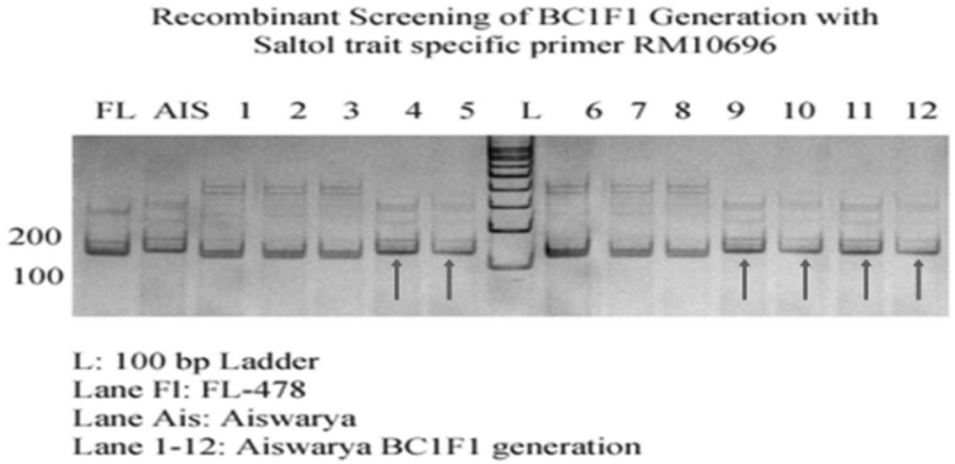

Fig. 6 Genotypic screening of *Saltol trait specific* BC1F1 progenies with recombinant marker RM10696

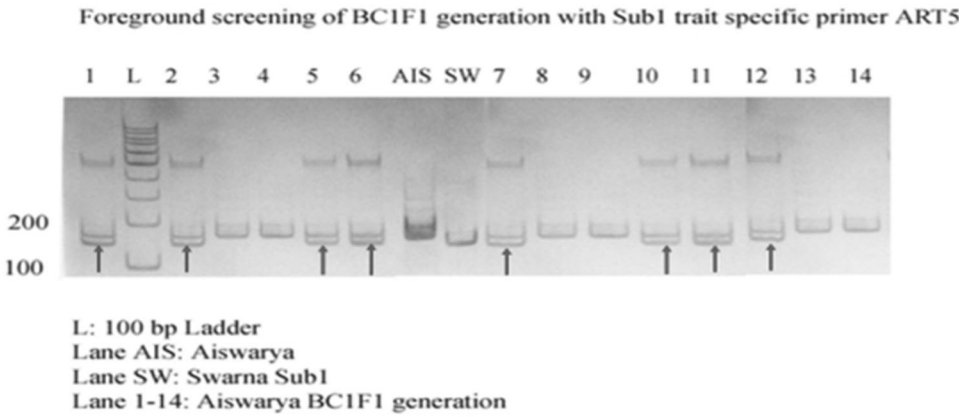

Fig. 7 Genotypic screening of *Sub1 trait specific* BC1F1 progenies with foreground marker ART5

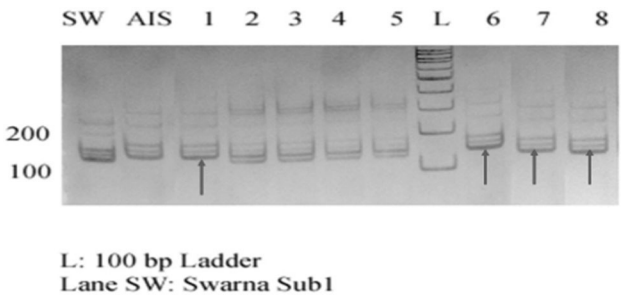

Fig. 8 Genotypic screening of *Sub1 trait specific* BC1F1 progenies with recombinant marker RM8303

introgressed lines of Aiswarya with 88%–93% of background homozygosity of Aiswarya. These lines can be released for cultivation in the saline prone coastal areas as well as in submergence prone coastal areas. Further these lines can be used to pyramid both the abiotic stresses into the variety Aiswarya to develop a dual stress tolerant rice variety.

Conclusion

This work aimed to develop a *Saltol* QTL and *Sub1* QTL introgressed Aiswarya variety by using Marker Assisted Backcross Breeding. This study can make a good impact on rice breeding through the development of a salinity and submergence tolerant variety Aiswarya which can be cultivated throughout Kerala. Climatic changes and environmental issues harming rice production, which is the most important food crop in India (Kurokawa et al. 2018). Abiotic stresses include drought, salinity, submergence, heat, cold etc. disapprovingly impends crop production and causes yield loss in low land areas and coastal areas (Pareek et al. 2010). Due to unexpected linkage drag it is difficult to use conventional breeding method to introgress these tolerant genes into high yielding varieties (Jeung et al. 2005). Hence using molecular breeding is a good option which did not affect grain quality and yield. A rice cultivar OMCS2000 developed with improved salt tolerant genes using maker assisted selection (Lang et al. 2008).

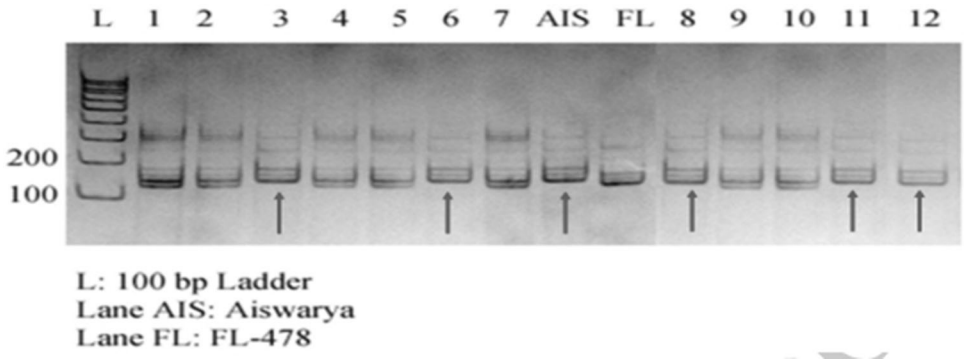

Fig. 9 Genotypic screening of *Saltol trait specific* BC2F2 progenies with foreground marker SKC-10

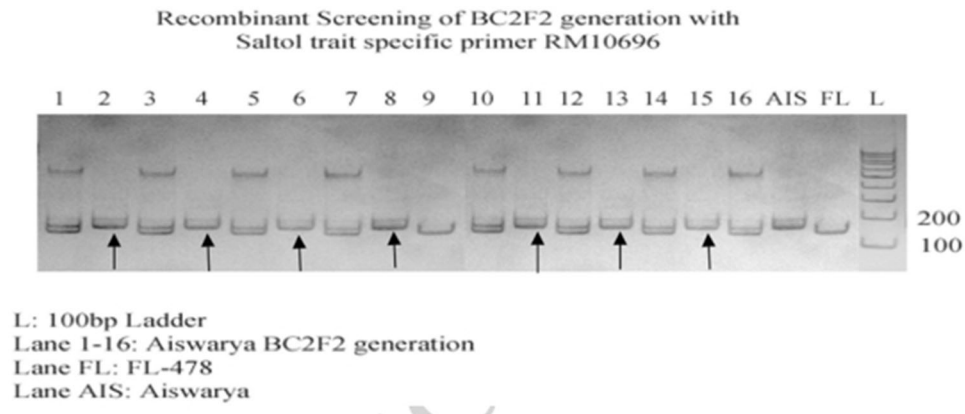

Fig. 10 Genotypic screening of *Saltol trait specific* BC2F2 progenies with recombinant marker RM10696

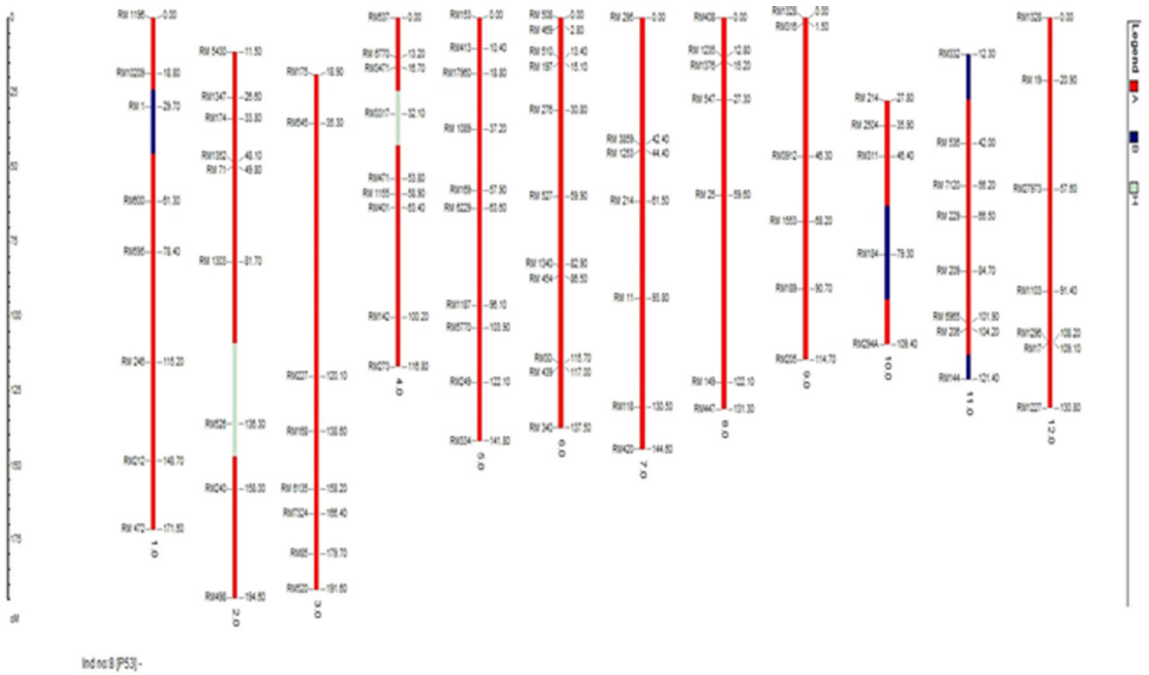

Fig. 11 Graphical presentation of the best BC_2F_2 plant with *Saltol QTL* introgression (A: Homozygous to the recipient genome B: Homozygous to the donor genome H: Heterozygous region)

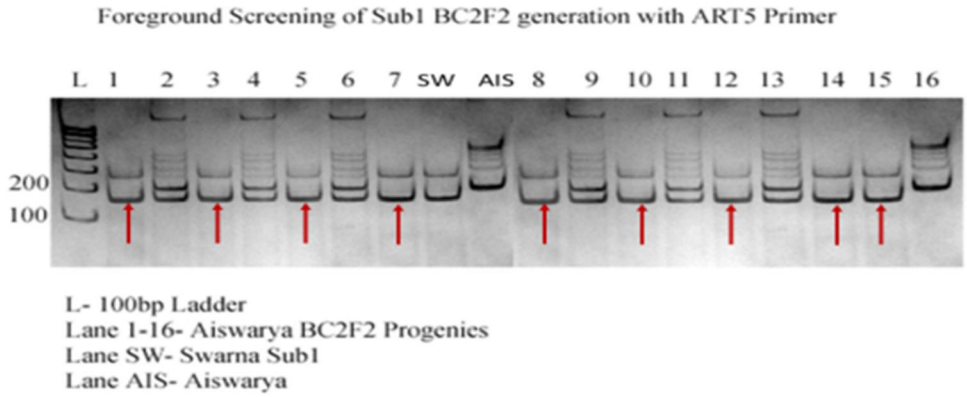

Fig. 12 Genotypic screening of *Sub1 trait specific* BC2F2 progenies with foreground marker ART5

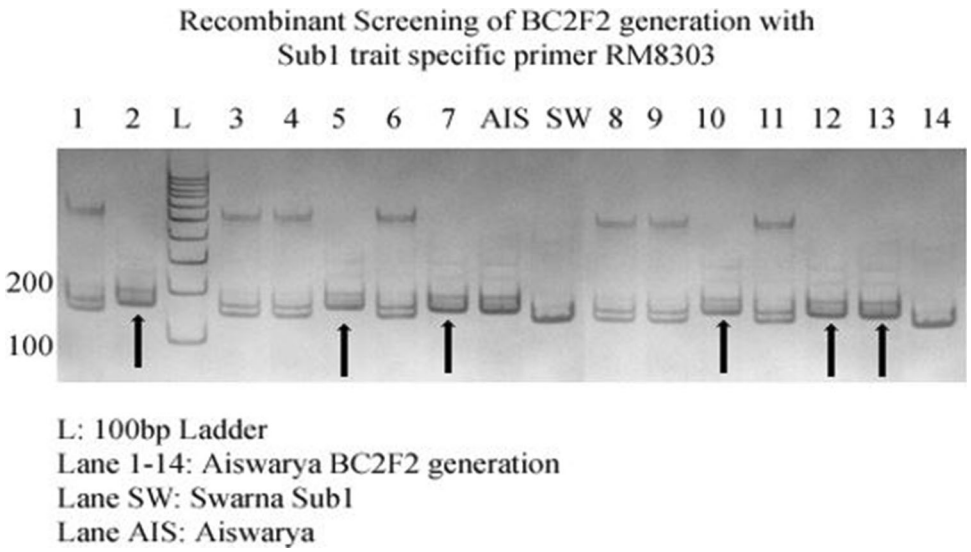

Fig. 13 Genotypic screening of *Sub1 trait specific* BC2F2 progenies with recombinant marker RM8303

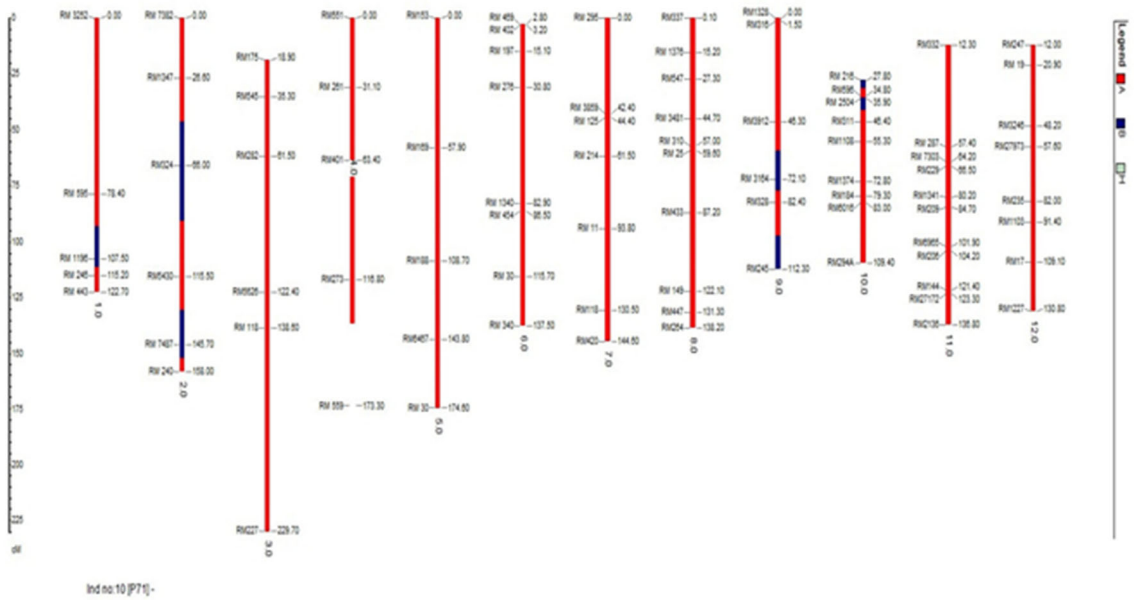

Fig. 14 Graphical presentation of the best BC2F2 plant with Sub 1 gene introgression (A: Homozygous to the recipient genome B: Homozygous to the donor genome H: Heterozygous region)

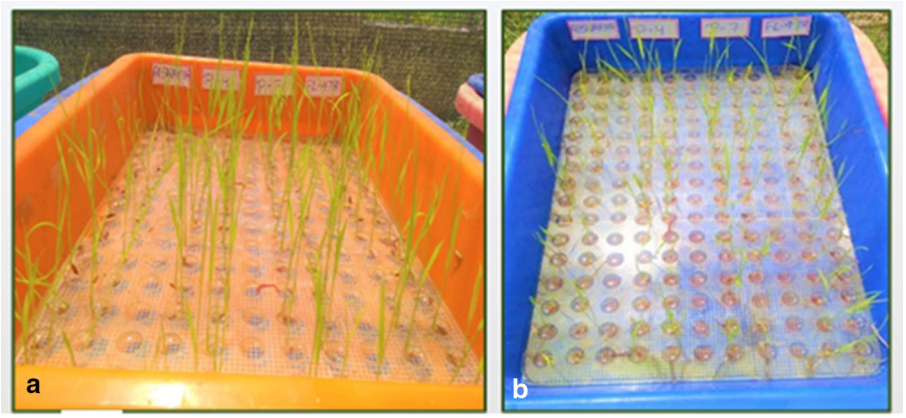

Fig. 15 Phenotypic screening for salinity screening. **a** Control set of progenies without salinity stress **b** progenies with salinity stress

Fig. 16 Phenotypic screening of submergence tolerance

Salt tolerance is controlled by many eleven QTL which are located on 5 chromosomes from the population of Nona Bokra and Koshihikari (Ren et al., 2005). 10.000 hectares of rice were submerged by 2011 floods, due to which faced a severe economic loss and loss of rice productivity (MARD 2011). Approximately 40.000 km^2, a huge amount of area will be disappeared if sea level rise is by 1 m (Khanh et al. 2013).

In this study we used the MABB breeding method to introgress the Saltol gene and Sub1 gene into a popular rice variety Aiswarya by phenotypic and genotypic selection. Using SSR markers the Saltol gene and Sub1 gene introgression was confirmed. Our results accomplish that a salinity tolerant gene (Saltol) from the donor parent FL478 was successfully transferred into Aiswarya and submergence tolerant gene Sub1 from the donor parent Swarna Sub1 was successfully transferred into Aiswarya independently. The future line of work of the study is to pyramid both these abiotic stresses into the rice variety Aiswarya to make it dual stress tolerant variety. Better profitability can be achieved directly benefiting the farmers by increasing their harvest in saline and flood affected lands. Due to this sole reason this work will have a significant impact on the socio-economic factors of low-lying areas and will lead to upliftment of farmers of these regions.

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