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Genetic Dissection of Reproductive Stage Salinity Tolerance in Rice Using Salt Tolerant Cultivar MTU 1061

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Enhancement in salt tolerance at reproductive stage is very essential for increase in the grain yield and extremely desirable to sustain production in salinity affected areas. In the current study, 234 F_2 population were derived from MTU 1061, which is salinity tolerant high yielding variety and MTU 1121, saline sensitive rice variety. We applied 1,001 rice SSR markers spanning all 12 chromosomes of rice to study parental polymorphism. The linkage map has been generated with 104 polymorphic markers and 234 F_2 mapping population. We identified 25 QTLs which explained 0.2-7.9% phenotypic variance at LOD score 2.10-6.55 using Kosambi mapping function by IciMapping. The two parental lines contributed QTLs for the yield traits. In our study, majority of the

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QTLs were mapped for the first time and these QTLs controlling reproductive stage salinity tolerance associated traits, will be useful in marker-assisted breeding programs to develop salinity tolerant rice varieties.

Keywords: Salinity; rice; QTL; reproductive stage; salt tolerance; Mapping.

1. INTRODUCTION

"Rice (*Oryza sativa* L.) is the world's most essential staple crop and is a main source of energy and nutrition for about fifty per cent of the world's population" (Chakraborty *et al*. 2023). "However, the productivity of rice is enormously impacted due to soil salinity which is the second most global soil problem next to drought in rice cultivating areas of the world" (Waziri *et al*. 2016). "Soil salinization is a widespread environmental barricade due to natural weathering, irrigation-related factors, improper drainage and climate change. About 1125 million hectares (ha) of the world's land, including 20% of the irrigated farmlands, are affected by salinization. Among the salt-affected irrigated farmlands, 20 million ha belong to the India, the first highest, followed by China (7 million ha) and United States (5.2 million ha)" by Pruthi *et al*. (2022a).

"Rice has large-scale area that is negatively impacted by salinity worldwide. There are numerous ways by which rice repel induced salinity such as osmotic adjustment, cell-level partitioning (tissue tolerance), tissue-level partitioning and recirculation. However, excessive absorption of salt in the rhizosphere forces a decrease in the osmotic potential of the soil, as a result it limits the water availability as well as influence water potential regulation of sensitive species. In addition, high concentration of Na^+ is extremely toxic for the crop, reduce plant growth and metabolism, and negatively affects the enzymes activities" (Ahmadizadeh *et al*. 2021).

"In rice salinity tolerance is a quantitative attribute that is polygenic in nature. Salinity has an effect on rice growth at all growth stages with fluctuating degree, but it is very sensitive during seedling and reproductive stages. Seedling and reproductive stage tolerances are feeble correlated, as separate sets of genes control the tolerance against of salt stress" (Razzaque *et al*. 2017). "Tolerance to salinity stress at the seedling stage is crucial for crop initiation, while grain yield is finally determined by reproductive stage tolerance. Moreover, pollination and fertilization periods are extremely sensitive to salinity. The salinization has an effect on spikelet formation, germination of the pollen which eventually lessen the number of filled grains/panicle and augment unfilled grains and thereby decrease the spikelet fertility at the flowering stage. Salinity also reduces 1000 grain weight and panicle length, ensuing of decrease in productivity" (Sen *et al.* 2017). "At the reproductive stage, the effect of salinity is considerably more than the vegetative stage" (Mondal *et al*. 2019).

"A number of agronomic practices were employed to minimize the effect of the salt stress on crop plants in general and rice in particular. Water and soil management practices are salient, but these are not efficient in terms of gaining additional yield gain. While, for salinity tolerance using conventional breeding methods, have been found unsuccessful due to the firm environmental effects on genotypic expression and the low narrow sense heritability of salt tolerance. Additionally, deficit of an accurate rapid and reliable screening technique under natural conditions gives finite success in developing salt tolerant varieties" (Aliyu *et al*. 2011). All the methods have their own advantages and disadvantages and hence depending upon the situation, necessary methods are to be followed for reduction in yield.

"An additional factor to be estimated for evolving salt tolerant varieties in rice genotypes with varying tolerance levels depends on particular growth stages of the crop. Earlier reports found that salt tolerance at the seedling stage is either poorly correlated with salinity tolerance at the reproductive stage. Since the level of salinity tolerance is dependent on particular stage of the rice plant, screening for salinity tolerance in breeding programs was recommend in 2 steps: 1) evaluating salt tolerance of huge segregating populations under controlled environment conditions at seedling stage and 2) at reproductive stage" (Venkata Ramana Rao *et al*. 2017).

"Mapping of vital quantitative trait loci (QTL) accounting for salinity tolerance in rice is desirable for making rapid progress in the breeding programs. *"Saltol"* a major QTL for salt tolerance at seedling stage was identified in a

population derived from the cross between a tolerant landrace Pokkali and a susceptible variety IR29" (Thomson *et al.* 2010). "But, still findings in reproductive stage salt tolerance is finite and there is seldom any report of robust QTLs at the reproductive stage salt tolerance. The major reasons for such insufficiency of published reports are the time-consuming and onerous phenotyping protocols required for the reproductive stage as compared with the relatively simple screening protocols for the seedling stage" (Hossain *et al*. 2015).

Recognizing the above challenges, the present study was undertaken to identify QTLs for several traits associated with salt tolerance in rice at reproductive stage using F_2 population derived from the cross between MTU 1121 (salinity susceptible) and MTU 1061 (salinity tolerant). The tolerant F_2 lines identified in this study will facilitate the development of adaptable salt tolerant varieties using marker assisted selection in future.

2. MATERIALS AND METHODS

2.1 Choice of Parents and Generation of F_2 Mapping Population

MTU 1121 was used as recurrent parent while MTU 1061, a salinity tolerant rice cultivar was used as donor. MTU 1121 is a high yielding, early maturing, leaf blast and BPH tolerant variety with low grain shattering, released from RARS, Maruteru. It is extensively cultivated in all over the state but it is susceptible to salinity. Crossing way taken up using MTU 1121 as female and MTU 1061 as male and after one generation of selfing F_2 population was generated.

2.2 Phenotyping for Reproductive Stage Salinity

"The phenotyping of $F_{2:3}$ population for reproductive stage salinity tolerance was taken up in *Rabi* 2019-20 at RARS, Maruteru. A total of 234 $F_{2:3}$ plants were screened for salinity tolerance at reproductive stage in greenhouse following the standard protocol of IRRI with some modifications" (Gregorio *et al*. 1997) (Supplementary Fig. 1). The screening experiment was conducted in a randomized complete block design with 2 replications. All lines were germinated in the laboratory and were placed on the soil surface of each plastic pot filled with fertilized soil (50N, 25P and 25K mg kg^{-1}) and these pots were kept in each plastic tray filled with ordinary tap water. When the seedlings attained advance boot stage, they were subjected to initial salt stress of EC 6 dSm^{-1} and the stress was increased to 12 dSm^{-1} at flowering stage. At time of maturity plant height, days to flowering, number of productive tillers were taken on three plants in each replication. At time of harvesting, individual plants were harvested and data on number of total grains per panicle, panicle length, number of filled grains per panicle, spikelet fertility (%), grain yield per plant were recorded.

2.3 Statistical Analysis

The analysis of variance (ANOVA) for every trait was computed with $F_{2:3}$ lines and replication as a fixed effect and random effect, respectively. The relationship among different morphological and physiological traits was determined by computing Pearson correlation coefficients. The data analysis was carried out using Social Sciences software version 2007 (www.spss.com). The distribution pattern of the $F_{2:3}$ lines for salinity tolerance traits were visualized from the histograms were constructed in Microsoft Excel 2013.

2.4 Genotyping of F_2 population Using SSR Markers

Leaf tissue was collected from all the 234 F_2 lines along with parents grown in control condition with no salt stress. The genomic DNA was extracted using CTAB method by Chen and Ronald (1999). The concentration of DNA in each sample was evaluated by eight channel spectrophotometers (Nanodrop Technologies, U.S.A). The DNA concentration of every sample was adjusted to a final concentration of 20 ng/µL for PCR amplification. Genotyping of the F_2 population was performed using polymorphic SSR markers. The PCR reactions were performed with 1µl each of forward and reverse primer, 0.5 µl of 2.5 mM dNTPs mix, 1 µL of 10 X buffer containing 25 mM $MgCl_2$, 1 µl of 1 U/µl Taq polymerase and 3 µl of sterile millipore water was added to make up the volume to 10 µl. The PCR amplification profile was plasty with foremost denaturation at 94 ^{0}C for 7 min, 35 cycles of 94 ^{0}C for 45 s, 55 ^{0}C for 45 s, 72 ^{0}C for 1 min, and a final extension at 72 ^{0}C for 7 min. The PCR products were electrophoresed in 3% polyacrylamide gels. The resolved bands were documented using gel documentation system (SYNGENE Gene flash U.K.). The scoring of the bands in F_2 population was done based on the banding pattern of the parents.

2.5 Construction of Linkage Map and QTL Mapping

The genotypic data of the F_2 lines were used to estimate the genomic composition of each line. The physical positions of the SSR markers were obtained from Gramene (www.gramene.org). The genotypic data were used for generation of linkage map using QTL IciMapping software v. 4.1 (www.isbreeding.net/software). The mapping method i.e., Inclusive Composite Interval Mapping for Additive QTL (ICIM-ADD) was used to identify QTLs at LOD threshold of 2.0. The position of the QTL and its effects were estimated. F_2 lines with high salt tolerance were selected based on the morphological, physiological traits, stress indices and presence of QTLs.

3. RESULTS AND DISCUSSION

3.1 Phenotypic Variation for Salinity Tolerance Traits at Reproductive Stage

The parents and $F_{2:3}$ population exhibited a wide range of variation for different evaluated yield traits studied under salt stress (EC 12 dSm^{-1}). Significant differences were observed for all the traits between the parents in Table 1. The donor parent MTU 1061 recorded higher values for plant height, days to flowering, panicle length, number of filled grains $panicle^{-1}$, spikelet fertility, grain yield / plant and productive tillers $plant^{-1}$ and the recurrent parent 'MTU 1121' registered significantly higher value for number of total grains $panicle^{-1}$ compared to MTU 1061. The mean values of the $F_{2:3}$ lines were between the parents 'MTU 1121' and 'MTU 1061' except for plant height which was lower and panicle length, number of total grains $panicle^{-1}$ which were higher than both the parents. The plant height of 'MTU 1061' was high (102 cm) compared to MTU 1121 (95 cm) while the $F_{2:3}$ population had a mean plant height of 66.12 cm. The $F_{2:3}$ population had a mean days to flowering of 98 days, which was in between both the parents. The mean panicle length of MTU 1061 was 17.11 cm while it was 12.57 cm in MTU 1121 and 17.65 cm in the $F_{2:3}$ lines. Recurrent parent had low number of filled grains $panicle^{-1}$ (30) compared to MTU 1061 & $F_{2:3}$ lines which had number of filled grains $panicle^{-1}$of 159 and 111 respectively. The $F_{2:3}$ population recorded mean number of total grains $panicle^{-1}$ (262) which was higher than both the parents. Donor parent had high spikelet fertility (64.21%) compared to MTU 1121 (12.09%) and $F_{2:3}$ lines (42.10%). The mean of grain yield /plant of $F_{2:3}$ lines (1.06 g) while it was 0.72 g for MTU 1121 and 2.83 g for MTU 1061. The $F_{2:3}$ population recorded mean productive tillers per plant (3.36) which was closer to the MTU 1061 (4.42) and was higher than that of MTU 1121 (2.41). Most of the phenotypic traits under reproductive stage salinity followed normal distribution which indicated sufficient variability present in F_2 population (Fig. 1). Similar findings were reported by Mohammadi *et al*. (2013) who studied 232 F_2 plants from Sadri and FL478 at reproductive stage.

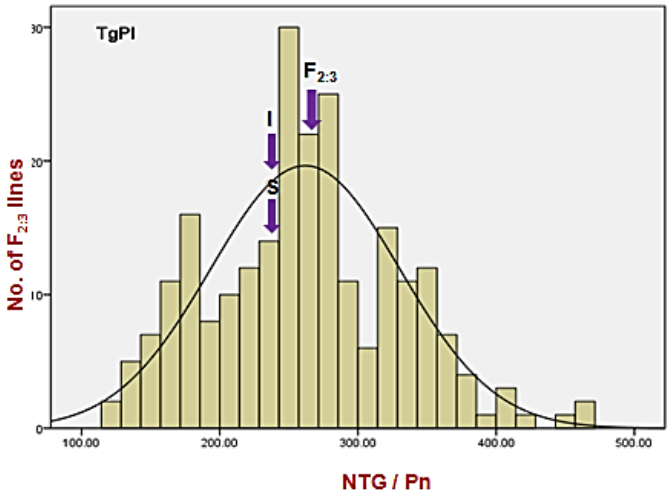
3.2 Linkage Map Construction Using SSR Markers

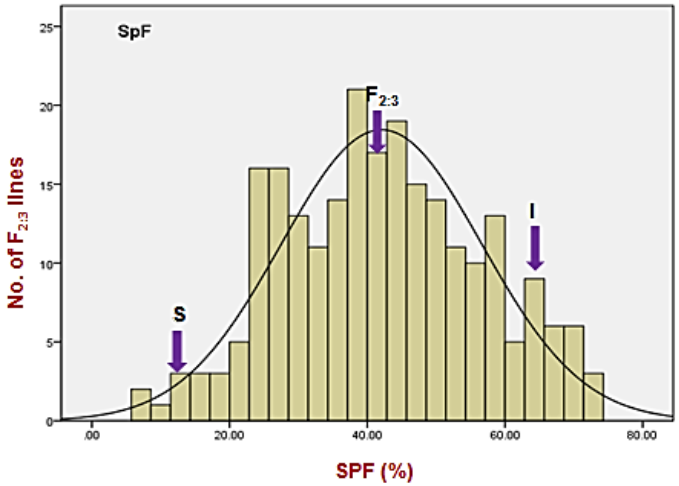
Both the parents, MTU 1121 and MTU 1061 were screened for polymorphism using SSR markers. A total of 1,001 SSR markers covering whole genome were used for detecting polymorphism between the parents. A total of one hundred and four polymorphic markers were used for genotyping (Supplementary Table 1). Using Kosambi mapping function by IciMapping V.4.1 software for construction of the linkage map and it covered 2956 cM from genotypic data of 234 F_2 plants with 104 SSR polymorphic markers and phenotypic data of eight different traits (Supplementary Fig. 2). Ahmadizadeh *et al*. (2021) generated a linkage map using 461 SNP markers which exposed a entire map length of 1154.48 cM at reproductive stage.

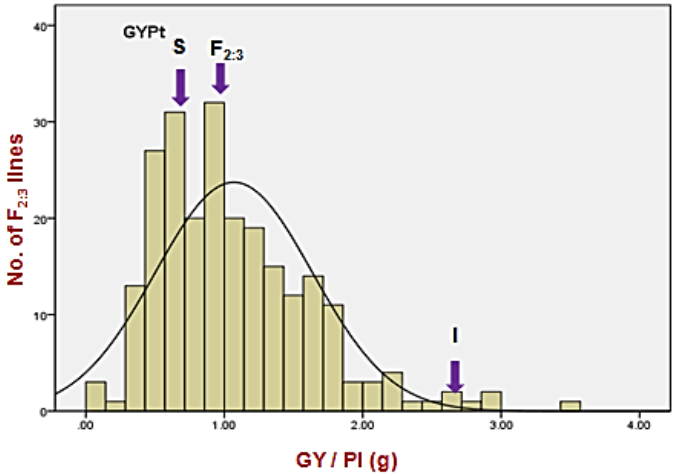
ICIM-ADD mapping was used to identify additive QTLs which revealed a total of 25 QTLs (Table 2; Fig. 2). The 25 QTLs for yield component traits were spanning across all chromosomes, with the exception of chromosomes 2, 5, 11 and 12 with LOD values ranging from 2.10 to 6.55 and an accounted for PVE ranging from 0.2% to 7.9%.

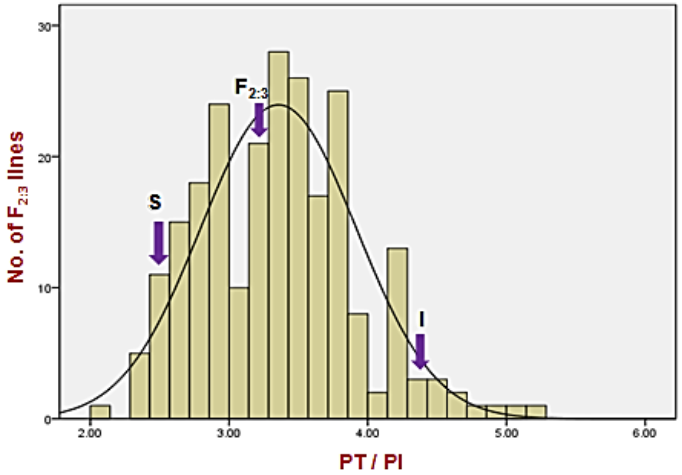
3.3 QTLs for Traits Related with Salinity Tolerance at the Reproductive Stage

Plant height: Plant height was controlled by two QTLs mapped on chromosomes 4 and 8 designated as *qPHT-4-1, qPHT-8-1,* respectively. *qPHT-4-1* detected to the interval RM6089-RM3474 with the small effect explaining 7.1% of the total phenotypic variance. MTU 1061 contributed the height increasing allele for *qPHT-4-1*. The other QTL, *qPHT-8-1*, mapped to the interval RM5485-RM149 with exhibited 0.8% of the total phenotypic variance, while allele from MTU 1121 increased plant height at *qPHT-8-1*. These results are in accordance with the findings of Pruthi *et al*. (2022b), who identified one QTL


TgPI
$F_{2:3}$
I
S
No. of $F_{2:3}$ lines
NTG / Pn


SpF
$F_{2:3}$
S
I
No. of $F_{2:3}$ lines
SPF (%)


GYPt
S
$F_{2:3}$
I
No. of $F_{2:3}$ lines
GY / PI (g)


$F_{2:3}$
S
I
No. of $F_{2:3}$ lines
PT / PI

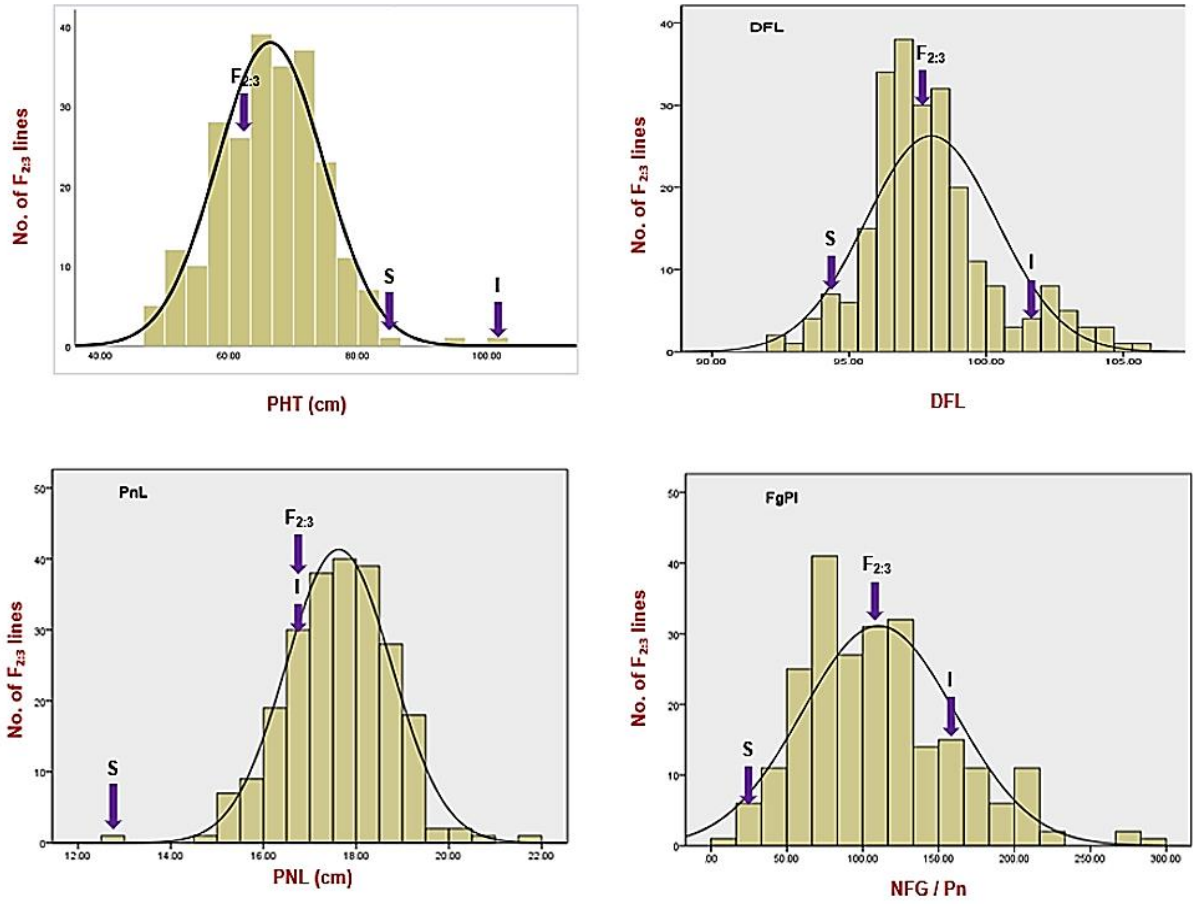


Fig. 1. Frequency distribution of $F_{2:3}$ lines for 8 yield traits under salt stress (EC= 12 dSm^{-1}) at reproductive stage. I, S and $F_{2:3}$ show the pinpoint of the mean phenotypic values of Indra, Sri Druthi and the $F_{2:3}$ population. PHT, plant height; DFL, days to flowering; PNL, panicle length; NFG/Pn, number of filled grains $panicle^{-1}$; NTG/Pn, number of total grains $panicle^{-1}$; SPF, spikelet fertility, GY/Pl, grain yield/plant; PT/PL, productive tillers $plant^{-1}$

Table 1. Mean values of yield components of MTU 1061/MTU 1121 $F_{2:3}$ population and their parents grown under salt stress (EC @ 12 dSm^{-1}) at the reproductive stage

			$F_{2:3}$ progenies		
Trait	**MTU 1061**	**MTU 1121**	**Range**	**Mean**	**Skewness**
Plant height (cm)	102.00	95.00	46.75-102.00	66.12	-0.19
Days to flowering	102.19	94.66	92.33-106.00	97.98	0.82
Panicle length (cm)	17.11	12.57	14.95-21.59	17.65	0.07
Number of filled grains $panicle^{-1}$	158.95	30.10	10.25-296.16	110.59	0.83
Number of total grains $panicle^{-1}$	247.51	248.77	116.75-458.83	261.92	0.23
Spikelet fertility (%)	64.21	12.09	6.92-73.05	42.10	0.08
Grain yield/plant (g)	2.83	0.72	0.02-3.45	1.06	1.09
Productive tillers $plant^{-1}$	4.42	2.41	2.00-5.16	3.36	0.46

(*qPH1.42*) on chromosome 1 for plant height with phenotypic variation of 13%.

Days to flowering: Two QTLs were detected for days to flowering. *qDFL-8-1* was mapped near marker RM5485 located on chromosome 8 and accounted for 1% of the phenotypic variation with a 2.63 LOD score. The other QTL, *qDFL-9-1*, located on chromosome 9 near RM24011, ascribed for 7.6% of the total phenotypic variation and LOD value was 2.18. Earlier, Mohammadi *et al*. (2013) using Sadri/FL478 F_2 progenies have mapped three QTLs *viz*., *qDTF4.1s*, *qDTF6.1s* and *qDTF10.1s*, for days to flowering and Pruthi *et al*. (2022a) have detected two QTLs *viz*., *qDFF1.1CN* and *qDFF8.1CN* using ILs population explained a total phenotypic variance of 11% and 16%, respectively.

MTU 1061 had an additive effect of increased days to flowering of both the alleles (*qDFL-8-1* and *qDFL-9-1*).

Panicle length: ICIM mapping detected to no chromosome region mapped for panicle length. These results were in contradictory with Hossain *et al*. (2015), who reported two QTLs (*qPL-1.2* and *qPL-7.4*) on chromosomes 1 and 7 for panicle length and total phenotypic variance ranging between 4.2 to 35.1 and LOD value from 3.2 to 11.4.

Number of filled grains / panicle: The number of filled grains is major yield component for the salinity tolerance at the reproductive stage. Seven QTLs were revealed to have a significant LOD score for number of filled grains per panicle. One QTL each was detected on chromosomes 1, 3, 4, 7, 8, 9 and 10 with 1.3 %, 1.4 %, 1.5 %, 1.6 %, 1.3 %, 1.6 % and 1.5 % of the phenotypic variation explained by these alleles, respectively. MTU 1121 alleles had a positive effect, increasing number of filled grains per panicle for three QTLs *viz., qNFG-1-1, qNFG-7-1* and *qNFG-10-1*, while MTU 1061 allele increased number of filled grains per panicle for four QTLs (*qNFG-3-1, qNFG-4-1*, *qNFG-8-1* and *qNFG-9-1*). Similar findings were reported by Ahmadizadeh *et al*. (2021), who mapped one QTL (*qFG_S-3-1*) for number of filled grains on chromosome 3 enacted 7.77% of the total phenotypic variance. Three QTLs, MTU 1121 allele had an increasing effect and rest of the QTLs from MTU 1061 allele.

Number of total grains /panicles: A total of two significant QTLs were found for number of total grains per panicle on chromosome 8 namely *qNTG-8-1* and *qNTG-8-2*. The positions of these QTLs were 155 cM (between RM3395-RM404) and 286 cM (between RM5485-RM149) on the genetic map of chromosome 8 respectively. These two additive QTLs were significant LOD value of 2.66 and 2.35, while minor with a phenotypic variance of 7.0% and 0.7% respectively. These results are in conformity with the findings of Mondal *et al*. (2019), who detected one QTL on chromosome 11 (*qTS11.1*) with a 3.5 LOD value and explaining 15.8% of the phenotypic variation. One QTL from the MTU 1061 allele and other QTL from the MTU 1121 allele.

Spikelet fertility: Three QTLs were identified for spikelet fertility in which the MTU 1121 allele increased spikelet fertility at all three loci under stress. The QTLs *qSPF-1-1*, *qSPF-3-1*and *qSPF-6-1*, explained 3.7 %, 3.6 % and 1.4 % of the phenotypic variance, with LOD value of 2.32, 2.16 and 3.13 respectively. On chromosome 5, Ahmadizadeh *et al*. (2021), has reported one QTL (*qSpkF_S-5-1*) explained as high as 24.86% of the phenotypic variation for spikelet fertility. Similarly, Chen *et al*. (2021), has mapped two

Table 2. Additive QTLs detected for yield traits under reproductive stage salinity in the $F_{2:3}$ lines by inclusive composite interval mapping (ICIM)

S. No.	Trait	QTL	CHR	Position (cM)	Marker interval	LOD	PVE (%)	Additive effect	Parental source of increasing allele
1	PHT	*qPHT-4-1*	4	94	RM6089-RM3474	2.61	7.1	-5.30	I
2	PHT	*qPHT-8-1*	8	286	RM5485-RM149	2.10	0.8	1.74	S
3	DFL	*qDFL-8-1*	8	286	RM5485-RM149	2.63	1.0	-0.52	I
4	DFL	*qDFL-9-1*	9	108	RM24011-RM24087	2.18	7.6	-0.20	I
5	NFG	*qNFG-1-1*	1	109	RM10793-RM11125	2.10	1.3	2.66	S
6	NFG	*qNFG-3-1*	3	179	RM15488-RM483	2.10	1.4	-32.97	I
7	NFG	*qNFG-4-1*	4	97	RM6089-RM3474	2.76	1.5	-42.05	I
8	NFG	*qNFG-7-1*	7	184	RM22168-RM22171	3.08	1.6	41.92	S
9	NFG	*qNFG-8-1*	8	146	RM3395-RM404	2.52	1.3	-34.54	I
10	NFG	*qNFG-9-1*	9	69	RM23679-RM23865	3.23	1.6	-1.21	I
11	NFG	*qNFG-10-1*	10	38	RM25519-RM6100	2.70	1.5	2.30	S
12	NTG	*qNTG-8-1*	8	155	RM3395-RM404	2.66	7.0	-30.87	I
13	NTG	*qNTG-8-2*	8	286	RM5485-RM149	2.35	0.7	16.95	S
14	SPF	*qSPF-1-1*	1	123	RM10793-RM11125	2.32	3.7	2.06	S
15	SPF	*qSPF-3-1*	3	286	RM15838-RM5924	2.16	3.6	2.09	S
16	SPF	*qSPF-6-1*	6	101	RM3431-RM5957	3.13	1.4	5.76	S
17	GY	*qGY-3-1*	3	176	RM15488-RM483	3.53	2.5	-0.40	I
18	GY	*qGY-4-1*	4	98	RM6089-RM3474	3.03	2.8	-0.45	I
19	GY	*qGY-6-1*	6	115	RM3431-RM5957	2.93	2.1	0.35	S
20	GY	*qGY-6-2*	6	201	RM5957-RM20661	4.42	2.8	0.43	S
21	GY	*qGY-7-1*	7	186	RM22168-RM22171	4.23	2.9	0.46	S
22	GY	*qGY-8-1*	8	286	RM5485-RM149	2.42	0.2	0.14	S
23	GY	*qGY-10-1*	10	35	RM25519-RM6100	6.55	2.9	0.05	S
24	PT	*qPT-6-1*	6	216	RM5957-RM20661	2.22	7.9	-0.33	I
25	PT	*qPT-10-1*	10	7	RM25092-RM25255	2.56	1.4	0.02	S

Parental source of increasing allele: I, Indra (MTU 1061); S, Sri Druthi (MTU 1121);

CHR, chromosome; LOD, logarithm of odds; PVE, phenotypic variation explained by each QTL; PHT, plant height; DFL, days to flowering; NFG/Pn, number of filled grains / panicle; NTG/Pn, number of total grains/panicles; SPF, spikelet fertility; GY/Pl, grain yield/plant; PT/Pl, Productive tillers plant^{-1}

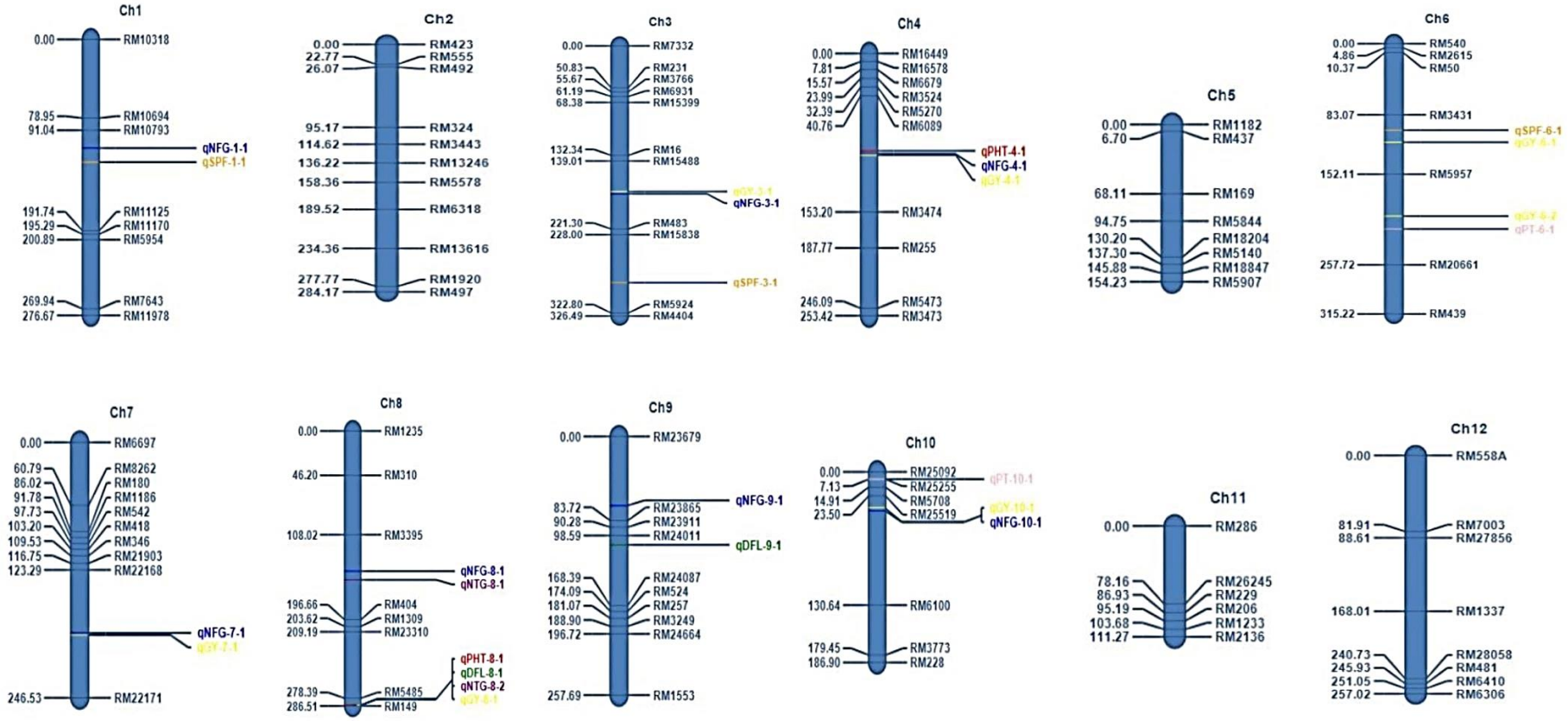


Fig. 2. Genetic linkage map showing the positions of QTLs for eight yield traits under reproductive stage salinity in the $F_{2:3}$ population of Indra developed in Sri Druthi background. QTLs were detected by inclusive composite interval mapping (ICIM) using QTL IciMapping software V. 4.1

QTLs (*qSPP1.1* and *qSPP11.1*) with significant LOD values 3.44 and 3.46, respectively.

Grain yield/plant: Yield is the most significant and ultimate factor for determining salinity tolerance at reproductive stage. The findings of the present study mapped seven QTLs namely *qGY-3-1, qGY-4-1, qGY-6-1, qGY-6-2, qGY-7-1, qGY-8-1* and *qGY-10-1* on chromosomes 3, 4, 6, 7, 8 and 10 respectively. With minor phenotypic variance ranging from 0.2% to 2.9% and LOD score from 2.42 to 6.55. Initial, Mohammadi *et al*. (2013), using Sadri/FL478 F_2 populations have mapped four QTLs on chromosomes 2, 4, 6 and 8, designated as *qGY2.1s*, *qGY4.1s*, *qGY6.1s* and *qGY8.1s*, respectively. Hossain *et al*. (2015), reported three QTLs namely *qGY-2.1, qGY-3.1* and *qGY-12.1* and explained 3.8 %, 8.1 % and 12.4 % of the variance, respectively. Two QTLs, MTU 1061 allele had an increasing effect and rest of the QTLs from MTU 1121 allele.

Productive tillers / plant: Two loci significantly associated with productive tillers per plant were found on chromosomes 6 and 10 using ICIM mapping and designated as *qPT-6-1* and *qPT-10-1*, and accounted for 7.9% and 1.4% of the phenotypic variation respectively. On chromosome 2, Pundir *et al*. (2021), has detected QTL for no. of productive tillers bearing fertile spikelets, between the marker interval HvSSR02-54 and RM263 and explained 5% of phenotypic variation with LOD score 4.1 using F_2 population. The allele from MTU 1061 increased productive tillers per plant at *qPT-6-1*, while allele from MTU 1121 increased productive tillers per plant at *qPT-10-1*.

4. CONCLUSION

As a conclusion, in reproductive stage, salinity tolerant traits detected in the present study using F_2 mapping population of the cross, MTU 1061 and MTU 1121, should be confirmed with more number of SSR/SNP markers to obtain the saturated map in the stable population like RILs across the different environments to confirm the regions or genes associated with salinity tolerant traits. This could help in the development of variety with reproductive stage salinity tolerance in rice.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SUPPLEMENTARY

Supplementary Table 1. Polymorphic markers between MTU 1061 and MTU 1121

S. No.	Primer name	Chromosome number	SSR motif	Annealing temperature (°C)	Expected product size (bp)	Forward primer	Reverse primer
1	RM10318	1	(ACAT)5	55	187	tgtctcacacattgcacacttacc	ggcctaacccaacacatgtcc
2	RM10694	1	(AC)18	55	194	tttccctggtttcaagcttacg	agtacggtaccttgatggtagaaagg
3	RM10793	1	(AGAT)7	55	124	gacttgccaactccttcaattcg	tcgtcgagtagcttccctctctacc
4	RM11125	1	(CT)22	55	211	ccaagaaccctagctccctctcc	tcgacgagatcctcctcgtaaacc
5	RM11170	1	(AAAG)6	55	267	cctgcaacttaagcttgttgtcc	gaaagcgaaacacgacatagtcc
6	RM5954	1	(ACC)11	55	163	gacctcgactcctactccgactcc	cctcgtcctcaagtgcctctcc
7	RM7643	1	(AAAT)7	61	178	aaaccgccgtcctcctattcg	cttgagcgcaccaacgaaatacc
8	RM11978	1	(GTGG)5	55	297	ggcctttgttagagaagacatgg	gaatgattatgccctaggttgc
9	RM423	2	(TTC)9	55	273	agcacccatgccttatgttg	ccttttttcagtagccctccc
10	RM555	2	(AG)11	55	223	ttgacatgcgaaatggagatgg	ttggatcagccaaaggagacc
11	RM492	2	(GA)11	55	224	gaaagcgaggtgaaacgaagc	ctaggcccaaggtttaccaaacg
12	RM324	2	(CAT)21	55	175	gattccacgtcaggatcttctgg	gctcaccagttgagattgaaagg
13	RM3443	2	(CT)19	50	118	ccgcatctgcacctctaaatcc	gtacacgcctgtagctcgttgc
14	RM13246	2	(AAT)21	55	208	gcaccagctcaagagaagtgagg	agagatgcctggtttcactgacg
15	RM5578	2	(TG)21	50	150	agaatacggatgggcacaatgg	tattcttctggtgtgtgccttgg
16	RM6318	2	(CTT)12	55	199	aagtgcctcgaattacacatctcc	gctgcttctgtccagtgagacc
17	RM13616	2	(TTA)22	55	452	gatctaaaccctctttccacaagc	cggccaatatataatgcactcc
18	RM1920	2	(AT)18	55	127	gcctggtaagtggtaatgtaatgg	gtgaattcctccttggtcttgg
19	RM497	2	(CAC)11	55	213	gctgcttgtgttgttgttgtcg	cacaggctcctcttcacctatgg
20	RM7332	3	(ACAT)11	50	205	acactgtacaccacacttcagc	cacaccaaagggaaattagg
21	RM231	3	(TCG)5(GA)16	55	182	ccagattatttcctgaggtc	gcatggtttgcagttaagataggg
22	RM3766	3	(GA)18	55	152	cgcgttcgatcgatctctctcc	ggccagagtacgtgccagatgc
23	RM6931	3	(TTA)32	50	209	gcatttgcttgtaccttctgttgg	aaccacacacatccacgagtcc
24	RM15399	3	(AT)28	55	174	aggctcaggcgtagtctgtatcg	acagggatgccatagttgctagg
25	RM16	3	(GA)16	55	181	gtgcgccaggagtagttgtctcc	gacgtgtacacatagccaaatcatcc
26	RM15488	3	(TG)24	55	282	cgctaacctcactgtgcttatcagg	atagctcctgcccttacctcacg
27	RM483	3	(AT)26	55	325	cttccaccataaaaccggag	acaccggtgatcttgtagcc
28	RM15838	3	(TTG)14	55	262	cgatgtcattcggtagaaacaagc	cctagtcaaggcatggtcaatcc
29	RM5924	3	(ATT)29	55	209	gctcaactgctgtttagaggattacc	agctctcccaagaaactgaacc

Table 1 cont…

S. No.	Primer name	Chromosome number	SSR motif	Annealing temperature (°C)	Expected product size (bp)	Forward primer	Reverse primer
30	RM4404	3	(TA)19	55	105	gagatggcagtgtcaagctaaacagg	tgaggacgccaatatggcaagg
31	RM16449	4	(AT)29	55	202	tcgcatgctaacttgagacg	caggaacatgtgtgcaatcg
32	RM16578	4	(AT)20	55	490	ggtgaattctactaagacggatcg	agccttattagtctcacctcgtaacc
33	RM6679	4	(TAA)18	61	141	tttaggccgtaagagcgaacatgg	atatgccgatgcagaacaagatcg
34	RM3524	4	(CT)31	50	129	ctgtctccgtcttcctcactcg	tggagaaatctcccttcctgagc
35	RM5270	4	(TA)45	55	162	tggtcattgattacaccctcagc	ttcagatgagaagcaagcactcg
36	RM6089	4	(CCT)10	61	170	cgatggccagcgtgatctcc	ccaccgaatcgaataaccacaagc
37	RM3474	4	(CT)21	50	174	acctcacctttccctcgattgg	gttggttgcttcctcccatacg
38	RM255	4	(AGG)5(AG)2-(GA)16	55	144	gaggaggaggaggagagatcagg	aacgaaaccgctcagttcaacc
39	RM5473	4	(TC)20	55	105	ggagataagacacgagggaattatgc	agattaactacgcgcgctcatcc
40	RM3473	4	(AG)23	55	144	gcataccgtaatgttggtgaagc	aatagcaactgggaggaggtaagg
41	RM1182	5	(AG)14	50	164	cttcttccgcttcctcccttcc	tgtaccagtgcaccgagagttgg
42	RM437	5	(AG)13	55	275	atccctcctctgctcaatgttgg	tcagggagggtcctagctactgg
43	RM169	5	(GA)12	67	167	cacctcctccaagatccttatgc	ctctctgtctcgctgtctgttgc
44	RM5844	5	(ATA)22	50	195	aacgtggcatccatgttagtacc	agctaggagccattgtcgaagg
45	RM18204	5	(ATAC)15	55	441	gaaactagagatgcacacatcc	atggtaagtactccctccatcc
46	RM5140	5	(TA)37	55	190	ggcactcgtatttctcaacttctcc	gggtgtatcaggagtacaggttgc
47	RM18847	5	(CT)18	55	292	gctggctaagctaaggctataccg	atctagctagcaaggaggcaagg
48	RM5907	5	(ATT)19	55	187	tccacttcccttctcttgtaccc	gccgacataacaagctagcaagg
49	RM540	6	(AG)16	55	172	gcctaaggctcatttatg	ctaggcctgccagattgaac
50	RM2615	6	(AT)30	55	164	atctcgttcatactgcttgacc	gactggtttccttcatgttacc
51	RM50	6	(CTAT)4(CT)15	55	201	actgtaccggtcgaagacg	aaattccacgtcagcctcc
52	RM3431	6	(CT)18	55	161	aagggaacattctggaagacacg	acacattgcgtgtagtgtgaagc
53	RM5957	6	(CAG)8	55	90	acaagacgttgccaaccatcc	ccaacggtggtggtagtgatagc
54	RM20661	6	(AACA)5	55	151	gaacacatgacaccacctttgc	gcgtttctcattctgttcttgc
55	RM439	6	(AAT)13	55	269	ctgggtctaatctcgtcctaaattgc	cgcctctcataacagtccactcc
56	RM6697	7	(TAA)36	50	229	tattcccgggagatccaacagc	aagatccagtcgatttggttcagg
57	RM8262	7	(AGG)9	55	200	aacagatatactcgggcagcattagc	tgactcctccgtggtaaacacc

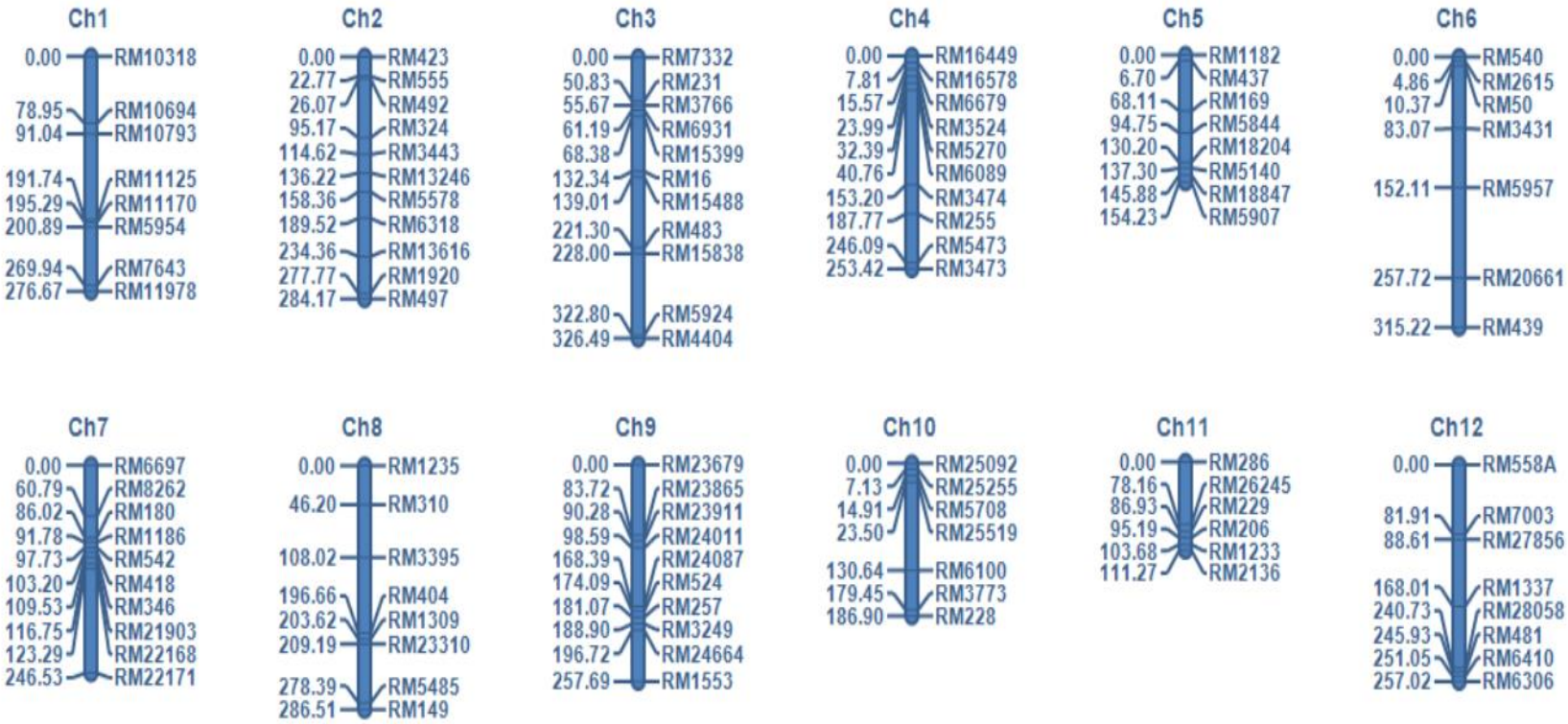
Table 1 cont…

S. No.	Primer name	Chromosome number	SSR motif	Annealing temperature (°C)	Expected Product size (bp)	Forward primer	Reverse primer
58	RM180	7	(ATT)10	55	110	ccttctccttctttcagcttctgc	caacttgctctacttgtggtgagg
59	RM1186	7	(AG)14	50	108	atatggtcattggctgggaaagagg	caccattatcctgcgggtaggc
60	RM542	7	(CT)22	55	113	cgctcctttctagctccatctcc	aactgcaacgagtaaggcagagg
61	RM418	7	(ATT)21	55	283	cgatcgagcatcaacacaacg	gacgtatcgcgtatcgtcatgc
62	RM346	7	(CTT)18	55	175	cgagagagcccataactacg	acaagacgacgaggga
63	RM21903	7	(AT)30	55	434	tggaacgacagaggtggtgagg	ggaatagctcccgcgtaatctcg
64	RM22168	7	(TAA)18	55	190	ttggctgtgagcctgtgattcc	gcgaccgcacagcttagtagtacc
65	RM22171	7	(TTA)29	55	391	tagtaccgccattaccattcatcc	gacggtgggactcctaattacagc
66	RM1235	8	(AG)15	55	118	gagaaacacaatcagtgacacc	ctgaaattgcacttcactgg
67	RM310	8	(GT)19	55	105	gacttgtggttgttgcttgttgg	actgccatatgcatttccctagc
68	RM3395	8	(CT)17	55	97	cttgggaaacttcacctcatgg	ctgagagaagccacaggattaatgg
69	RM404	8	(GA)33	55	236	ggagcagctaaggcagataagagg	gccttcatgcttcagaagacagc
70	RM1309	8	(AG)19	55	152	gaggacactgacgacagcttgg	cgcgcaaatcattaagttcagg
71	RM23310	8	(TA)47	55	362	gatgaggcccagatttacatttcc	ctaagacttcctgacgatgtcaatgc
72	RM5485	8	(TC)22	55	140	atgattgcatctgcatcactgc	atacctgtttccaatgcgtagcc
73	RM149	8	(AT)10	55	253	ggaagcctttcctcgtaacacg	gaacctaggccgtgttctttgc
74	RM23679	9	(AGAA)10	55	189	tcacagcttagtgcatgttgagc	gattcacctggcaatgagaacg
75	RM23865	9	(CT)10	55	148	tcatcccattctcttcctcacc	catacggccatacaaatgaacc
76	RM23911	9	(TG)13	55	269	tgcctgcacttatctcttgatgc	gatgaacctaaagggcagtttcc
77	RM24011	9	(AG)25	55	424	aagatcgtagccgaagagtgacc	ggctaaagagtccaagacgagagg
78	RM24087	9	(AC)22	55	263	cactcagatttggccgatcc	gctgatccagatctacctgacacc
79	RM524	9	(AT)11	55	198	atcatagcccagaccaagaatgc	agatgaagagcaggaaccgtagg
80	RM257	9	(CT)24	55	147	ccgtgcaacttaaatccaaacagg	ggaatcctatatgagccagtgatgg
81	RM3249	9	(CT)13	55	151	ggatcatgtcaaatcggagaagg	gctcaagcttcacagatcacagg
82	RM24664	9	(AT)35	55	362	atctaaacacagccctagttgc	gagccataactttaccatgtgc
83	RM1553	9	(AT)13	55	161	ttattgtccatgcggtacaacg	caaccacctcatcaccaactcc
84	RM25092	10	(TG)15	55	479	ctatctcccttgatgcgtacatgc	aaatcagcgcgtgacaattcg
85	RM25255	10	(TGCG)5	55	190	ccactactcgctgtccggtacg	gttgggcgtcttgacgaacg
86	RM5708	10	(AAT)14	55	186	tggtatccataaccttgacagc	gtcacgtatcgaacagtctcc
87	RM25519	10	(TA)42	55	260	ggtgattaattactggtcggaagg	gctggtttgatcggaattacagg
88	RM6100	10	(CGA)8	50	144	ttccctgcaagattctagctacacc	tgttcgtcgaccaagaactcagg
89	RM3773	10	(GA)18	55	150	ttgtgccctaagaatcatctctcc	gactggatgaaaggatacaacagc

Table 1 cont…

S. No.	Primer name	Chromosome number	SSR motif	Annealing temperature (°C)	Expected product size (bp)	Forward primer	Reverse primer
90	RM228	10	(CA)6(GA)36	55	154	tctaactctggccattagtccttgg	aagtagacgaggacgacgacagg
91	RM286	11	(GA)16	55	110	ctggcctctagctacaaccttgc	aaactctcgctggattcgatagg
92	RM26245	11	(ATCT)12	55	142	gctactaggccaccacgtgatcc	agcgtttataggaacaccacacaacg
93	RM229	11	(TC)11(CT)5C3(CT)5	55	116	atatgagttgctgtcgtgcg	caacttgcatcctcccctcc
94	RM206	11	(CT)21	55	147	cccatgcgtttaactattct	cgttccatcgatccgtatgg
95	RM1233	11	(AG)15	55	175	atgggcacgtgtaattcattcg	atcctcgaaagtaggagtaggaaagc
96	RM2136	11	(AT)22	55	136	cggtgtgtaaaactccgaagcacc	tgccgtggctcattagtggtc
97	RM558A	12	(ATTG)5	55	246	gatcatcgtccaagaacacatgc	cacctgacaaaggagcagatcg
98	RM7003	12	(AAAC)6	50	101	ctctagctctctcatggatgg	aatcatagggcagacatacagc
99	RM27856	12	(AT)21	55	384	gcagcgactactacttcgaaagc	accaaccgggactaaagatcg
100	RM1337	12	(AG)21	55	210	agtggcccgaacctgtataacc	gagcaggtgcaatgctgagg
101	RM28058	12	(AT)24	55	193	ccttctgcttcgtgtcactctacc	cccggaagtattccaaggtatcg
102	RM481	12	(CAA)12	55	169	agagagcccctaaatttccg	aggtacgctcacctgtggac
103	RM6410	12	(GAG)8	55	177	ggaagaagcatctgcagtagtagacg	gaattagccgacagcgtcttgg
104	RM6306	12	(CTT)9	50	88	gtattggtcaccggtctaagtcg	attattggccctaggtcaacacc

Supplementary Fig 1. Phenotyping of $F_{2:3}$ population for salinity tolerance at Reproductive stage



Supplementary Fig 2. Linkage map constructed by IciMapping software V. 4.1 using 104 SSR markers

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