



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

K. Vijaya Durga ^{a*}, P. Venkata Ramana Rao ^{b++},
P. V. Satyanarayana ^{c++} and K. Jayalalitha ^a

^a Department of Genetics and Plant Breeding, Agricultural College, Bapatla, ANGRAU, 522101, India.

^b Division of Genetics and Plant Breeding, Regional Agricultural Research Station, Maruteru, Andhra Pradesh, 534 122, India.

^c ANGRAU, Andhra Pradesh, 532484, India.

Authors' contributions

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

Article Information

DOI: <https://doi.org/10.9734/ijpss/2025/v37i125909>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/150224>

Original Research Article

Received: 10/10/2025

Accepted: 23/12/2025

Published: 29/12/2025

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 F₂ 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 F₂ 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

⁺⁺ Director of Research;

*Corresponding author: E-mail: kunjavijayadurga@gmail.com;

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 was 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 $20\ ng/\mu L$ for PCR amplification. Genotyping of the F_2 population was performed using polymorphic SSR markers. The PCR reactions were performed with $1\ \mu L$ each of forward and reverse primer, $0.5\ \mu L$ of $2.5\ mM$ dNTPs mix, $1\ \mu L$ of $10\ X$ buffer containing $25\ mM\ MgCl_2$, $1\ \mu L$ of $1\ U/\mu L$ Taq polymerase and $3\ \mu L$ of sterile millipore water was added to make up the volume to $10\ \mu L$. The PCR amplification profile was plasty with foremost denaturation at $94\ ^\circ C$ for 7 min, 35 cycles of $94\ ^\circ C$ for 45 s, $55\ ^\circ C$ for 45 s, $72\ ^\circ C$ for 1 min, and a final extension at $72\ ^\circ C$ 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.

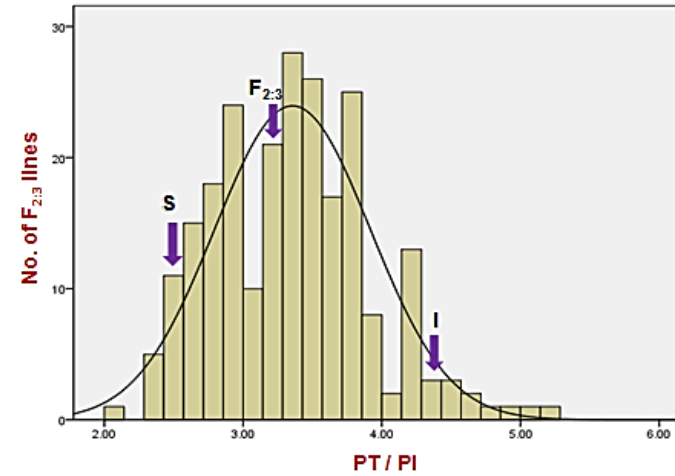
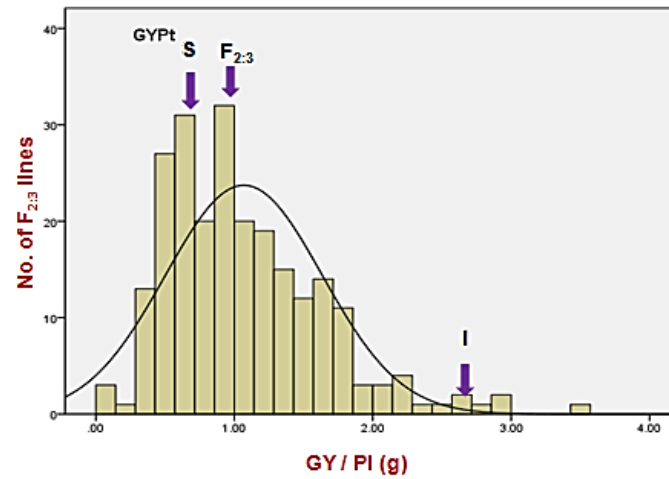
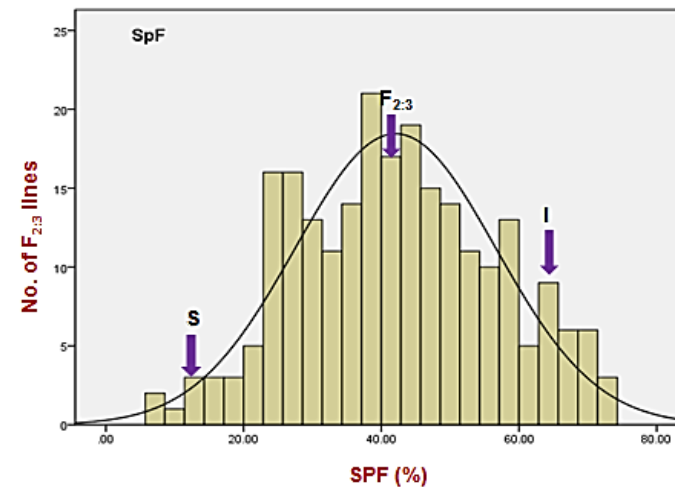
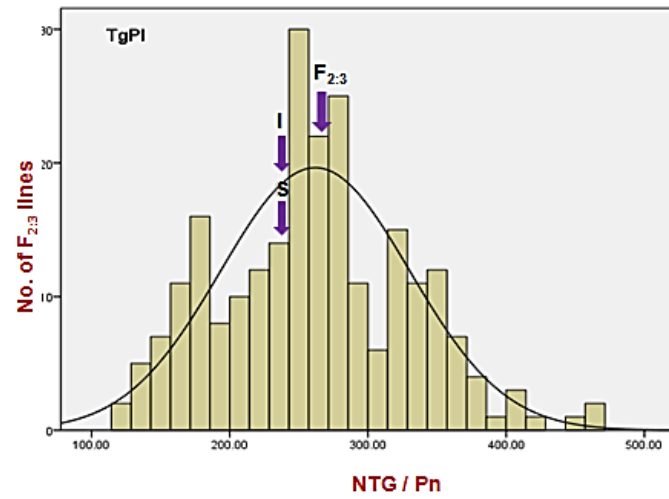
3.2 Linkage Map Construction Using SSR Markers

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



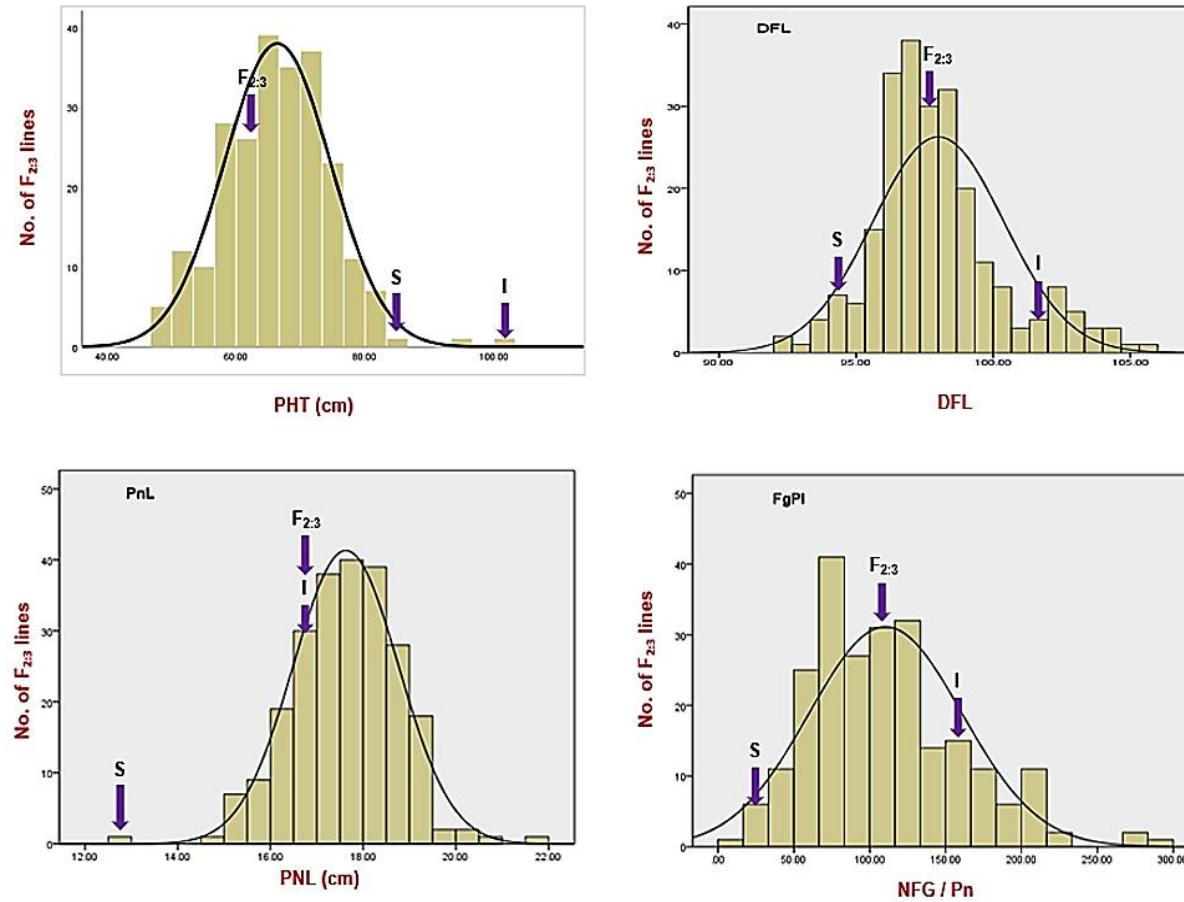


Fig. 1. Frequency distribution of $F_{2:3}$ lines for 8 yield traits under salt stress ($EC = 12 \text{ dSm}^{-1}$) at reproductive stage. I, S and $F_{2:3}$ show the pinpoint of the mean phenotypic values of Indra, Sri Druthi and the $F_{2:3}$ population. PHT, plant height; DFL, days to flowering; PNL,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

Trait	MTU 1061	MTU 1121	Range	F _{2:3} progenies	
				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 F₂ 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	<i>qPHT-4-1</i>	4	94	RM6089-RM3474	2.61	7.1	-5.30	I
2	PHT	<i>qPHT-8-1</i>	8	286	RM5485-RM149	2.10	0.8	1.74	S
3	DFL	<i>qDFL-8-1</i>	8	286	RM5485-RM149	2.63	1.0	-0.52	I
4	DFL	<i>qDFL-9-1</i>	9	108	RM24011-RM24087	2.18	7.6	-0.20	I
5	NFG	<i>qNFG-1-1</i>	1	109	RM10793-RM11125	2.10	1.3	2.66	S
6	NFG	<i>qNFG-3-1</i>	3	179	RM15488-RM483	2.10	1.4	-32.97	I
7	NFG	<i>qNFG-4-1</i>	4	97	RM6089-RM3474	2.76	1.5	-42.05	I
8	NFG	<i>qNFG-7-1</i>	7	184	RM22168-RM22171	3.08	1.6	41.92	S
9	NFG	<i>qNFG-8-1</i>	8	146	RM3395-RM404	2.52	1.3	-34.54	I
10	NFG	<i>qNFG-9-1</i>	9	69	RM23679-RM23865	3.23	1.6	-1.21	I
11	NFG	<i>qNFG-10-1</i>	10	38	RM25519-RM6100	2.70	1.5	2.30	S
12	NTG	<i>qNTG-8-1</i>	8	155	RM3395-RM404	2.66	7.0	-30.87	I
13	NTG	<i>qNTG-8-2</i>	8	286	RM5485-RM149	2.35	0.7	16.95	S
14	SPF	<i>qSPF-1-1</i>	1	123	RM10793-RM11125	2.32	3.7	2.06	S
15	SPF	<i>qSPF-3-1</i>	3	286	RM15838-RM5924	2.16	3.6	2.09	S
16	SPF	<i>qSPF-6-1</i>	6	101	RM3431-RM5957	3.13	1.4	5.76	S
17	GY	<i>qGY-3-1</i>	3	176	RM15488-RM483	3.53	2.5	-0.40	I
18	GY	<i>qGY-4-1</i>	4	98	RM6089-RM3474	3.03	2.8	-0.45	I
19	GY	<i>qGY-6-1</i>	6	115	RM3431-RM5957	2.93	2.1	0.35	S
20	GY	<i>qGY-6-2</i>	6	201	RM5957-RM20661	4.42	2.8	0.43	S
21	GY	<i>qGY-7-1</i>	7	186	RM22168-RM22171	4.23	2.9	0.46	S
22	GY	<i>qGY-8-1</i>	8	286	RM5485-RM149	2.42	0.2	0.14	S
23	GY	<i>qGY-10-1</i>	10	35	RM25519-RM6100	6.55	2.9	0.05	S
24	PT	<i>qPT-6-1</i>	6	216	RM5957-RM20661	2.22	7.9	-0.33	I
25	PT	<i>qPT-10-1</i>	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/PI, grain yield/plant; PT/PI, Productive tillers plant⁻¹

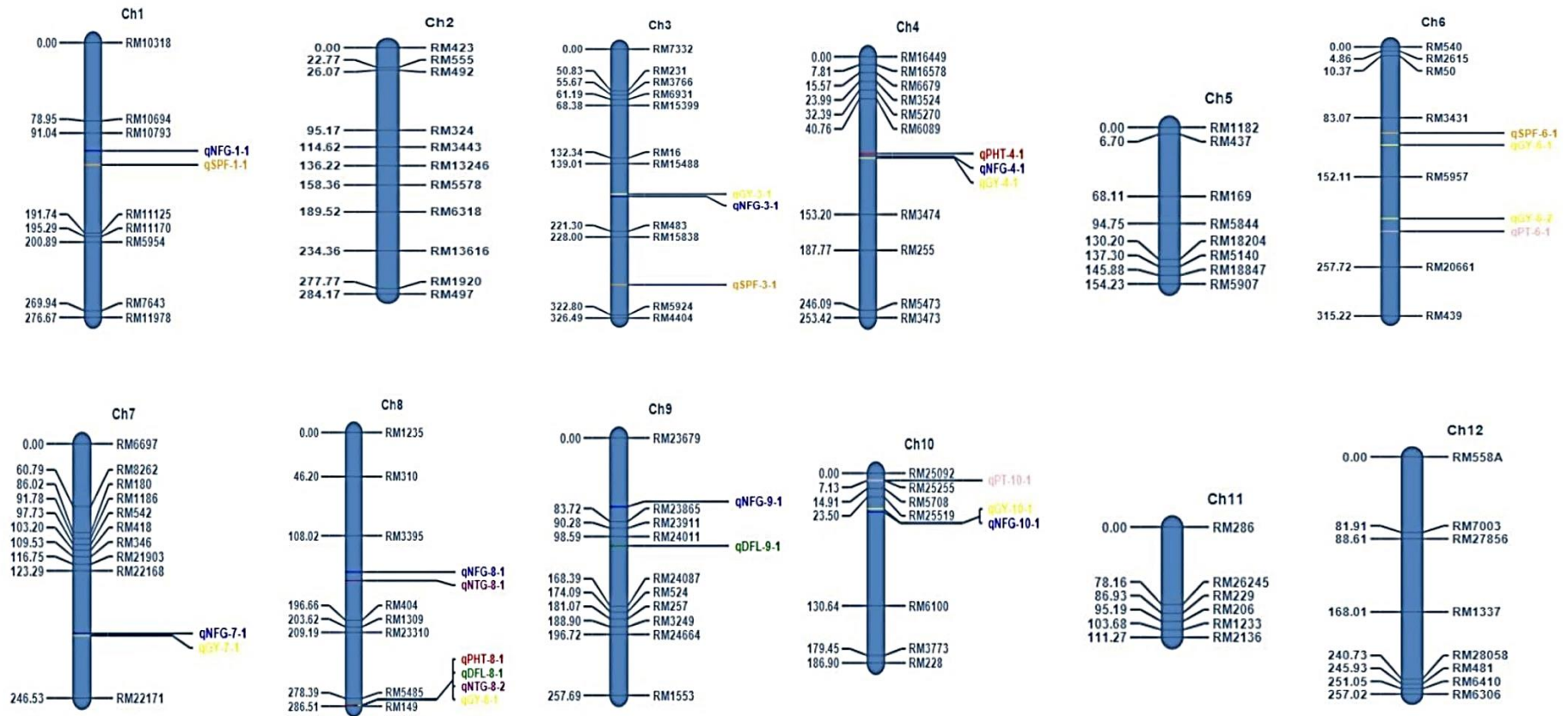


Fig. 2. Genetic linkage map showing the positions of QTLs for eight yield traits under reproductive stage salinity in the F_{2:3} population of Indra developed in Sri Druthi background. QTLs were detected by inclusive composite interval mapping (ICIM) using QTL IciMapping software V. 4.1

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 F₂ 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 F₂ 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 F₂ 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.

REFERENCES

- Ahmadizadeh, M., Jelodar, N. B., Nejad, M. G., Bagheri, N., & Singh, R. K. (2021). High-density linkage mapping for agronomic and physiological traits of rice (*Oryza sativa* L.) under reproductive-stage salt stress. *Journal of Genetics*, 100(51), 1–12. <https://doi.org/10.1007/s12041-021-01301-6>
- Aliyu, R., Adamu, A. K., Muazu, S., & Alonge, S. O. (2011). Tagging and validation of SSR markers to salinity tolerance QTLs in rice (*Oryza* spp.). *International Conference on Biology, Environment and Chemistry*, 328–332.
- Chakraborty, K., Roy, S., Jena, P., Dash, G. K., Barik, M., Behera, M., Sar, P., Senapaty, J., Baig, M. J., & Swain, P. (2023). Identification of multiple abiotic stress tolerant donors for climate-resilient rice (*Oryza sativa*) development. *The Indian Journal of Agricultural Sciences*, 93(3), 258–262. <https://doi.org/10.56093/ijas.v93i3.132345>
- Chen, D. H., & Ronald, P. C. (1999). A rapid DNA miniprep method suitable for AFLP and other PCR applications. *Plant Molecular Biology Reporter*, 17, 53–57. <https://doi.org/10.1023/A:1007585532036>
- Chen, J., Tai, L., Luo, L., Xiang, J., & Zhao, Z. (2021). Mapping QTLs for yield component traits using overwintering cultivated rice. *Journal of Genetics*, 100(31), 1–11. <https://doi.org/10.1007/s12041-021-01279-1>
- Gregorio, G. B., Senadhira, D., & Mendoza, R. D. (1997). *Screening rice for salinity tolerance* (IRRI Discussion Paper Series No. 22). International Rice Research Institute. https://ageconsearch.umn.edu/record/19700/files/irri_discussion_paper_22.pdf
- Hossain, H., Rahman, M. A., Alam, M. S., & Singh, R. K. (2015). Mapping of quantitative trait loci associated with reproductive-stage salt tolerance in rice.

- Journal of Agronomy and Crop Science*, 201(1), 17–31.
<https://doi.org/10.1111/jac.12086>
- Mohammadi, R., Mendiolo, M. S., Diaz, G. Q., Gregorio, G. B., & Singh, R. K. (2013). Mapping quantitative trait loci associated with yield and yield components under reproductive stage salinity stress in rice (*Oryza sativa* L.). *Journal of Genetics*, 92(3), 433–443.
<https://doi.org/10.1007/s12041-013-0285-4>
- Mondal, S., Borromeo, T. H., Diaz, M. G. Q., Amas, J., Rahman, M. A., Thomson, M. J., & Gregorio, G. B. (2019). Dissecting QTLs for reproductive stage salinity tolerance in rice from BRRI dhan 47. *Plant Breeding and Biotechnology*, 7(4), 302–312.
<https://doi.org/10.9787/PBB.2019.7.4.302>
- Pruthi, R., Chapagain, S., Coronejo, S., Singh, L. L., & Subudhi, P. K. (2022a). Quantitative trait loci, candidate genes, and breeding lines to improve salt tolerance at the flowering and seedling stages in rice. *Food and Energy Security*.
<https://doi.org/10.1002/fes3.433>
- Pruthi, R., Puram, V. R. R., Ontoy, J., & Subudhi, P. K. (2022b). Genetics of yield component traits under salt stress at flowering stage and selection of salt tolerant pre-breeding lines for rice improvement. *Genetica*.
<https://doi.org/10.1007/s10709-022-00160-y>
- Pundir, P., Devi, A., Krishnamurthy, S. L., Sharma, P. C., & Vinaykumar, N. M. (2021). QTLs in salt rice variety CSR10 reveals salinity tolerance at reproductive stage. *Acta Physiologiae Plantarum*.
<https://doi.org/10.1007/s11738-020-03183-0>
- Razzaque, S., Haque, T., Elias, S. M., Rahman, M. S., Biswas, S., Schwartz, S., Ismail, A. M., Walia, H., Juenger, T. E., & Seraj, Z. (2017). Reproductive stage physiological and transcriptional responses to salinity stress in reciprocal populations derived from tolerant (Horkuch) and susceptible (IR29) rice. *Scientific Reports*, 7, 46138.
<https://doi.org/10.1038/srep46138>
- Sen, T. T. H., Nhi, P. T. P., & Sen, T. T. (2017). Salinity effect at seedling and flowering stages of some rice lines and varieties (*Oryza sativa* L.). *Journal of Agricultural Science and Technology A and B*, 7, 32–39.
<https://doi.org/10.17265/2161-6256/2017.10.005S>
- Thomson, M. J., de Ocampo, M., Egdane, J., Rahman, M. A., Sajise, A. G., Adorada, D. L., Tumimbang-Raiz, E., Blumwald, E., Seraj, Z. I., Singh, R. K., Gregorio, G. B., & Ismail, A. M. (2010). Characterizing the Saltol quantitative trait locus for salinity tolerance in rice. *Rice*, 3(2–3), 148–160.
<https://doi.org/10.1007/s12284-010-9053-8>
- Venkata Ramana Rao, P., Ontoy, J., Linscombe, S., & Subudhi, P. K. (2017). Genetic dissection of seedling stage salinity tolerance in rice using introgression lines of a salt tolerant landrace Nona Bokra. *Journal of Heredity*.
<https://doi.org/10.1093/jhered/esx067>
- Waziri, A., Kumar, P., & Purty, R. S. (2016). Saltol QTL and their role in salinity tolerance in rice. *Austin Journal of Biotechnology & Bioengineering*, 3(3), 1–5.
<https://www.austinpublishinggroup.com/bio-technology-bioengineering/fulltext/ajbb-v3-id1067.php>

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	ggcctaaccaacacatgtcc
2	RM10694	1	(AC)18	55	194	ttccctggtttcaagcttacg	agtacggtacctgatgtagaaagg
3	RM10793	1	(AGAT)7	55	124	gacttgccaactcctcaattcg	tcgtcgagtagcttccctcttacc
4	RM11125	1	(CT)22	55	211	ccaagaaccctagctccctctcc	tcgacgagatcctcctcgtaaacc
5	RM11170	1	(AAAG)6	55	267	cctgcaacttaagctgtgtgcc	gaaagcgaaacacgacatagtc
6	RM5954	1	(ACC)11	55	163	gacctcgactcctactccgactcc	cctcgctcctaagtcctctcc
7	RM7643	1	(AAAT)7	61	178	aaaccgctgctctctattcg	cttgagcgaccaacgaaatacc
8	RM11978	1	(GTGG)5	55	297	ggcctttagtagagaagacatgg	gaatgattatgccctaggttgc
9	RM423	2	(TTC)9	55	273	agcaccatgaccttatgttg	ccttttcagtagccctccc
10	RM555	2	(AG)11	55	223	ttgacatgcgaaatggagatgg	ttggatcagccaaaggagacc
11	RM492	2	(GA)11	55	224	gaaagcgaggtgaaacgaagc	ctaggcccaaggtttaccacacg
12	RM324	2	(CAT)21	55	175	gattccacgtcaggatctctgg	gctcaccagttgagattgaaagg
13	RM3443	2	(CT)19	50	118	ccgcatctgcacctctaaatcc	gtacacgcctgtagctgttgc
14	RM13246	2	(AAT)21	55	208	gcaccagctcaagagaagtgagg	agagatgcctgtttcactgacg
15	RM5578	2	(TG)21	50	150	agaatacggatgggcacaatgg	tattctctggtgtgtgcttgg
16	RM6318	2	(CTT)12	55	199	aagtgcctcgaattacacatctcc	gctgcttctgtccagtgtgacc
17	RM13616	2	(TTA)22	55	452	gatctaaaccctcttccacaagc	cggccaataataatgcactcc
18	RM1920	2	(AT)18	55	127	gcctggtaagtgtgaatgaatgg	gtgaattcctccttggtcttg
19	RM497	2	(CAC)11	55	213	gctgctgtgtgtgtgtgtcg	cacaggctcctctcacctatgg
20	RM7332	3	(ACAT)11	50	205	acactgtacaccacacttcagc	cacaccaaagggaattagg
21	RM231	3	(TCG)5(GA)16	55	182	ccagattatttctgaggtc	gcatggttgcagttaagatagg
22	RM3766	3	(GA)18	55	152	cgcggtcgatcgatctctctcc	ggccagagtacgtgccagatgc
23	RM6931	3	(TTA)32	50	209	gcatttgctgtaccttctgttg	aaccacacacatccacgagtc
24	RM15399	3	(AT)28	55	174	aggctcaggcgtagtctgtatcg	acagggatgccatagttgttagg
25	RM16	3	(GA)16	55	181	gtgcccaggagtagttgtctcc	gacgtgtacacatagccaaatcatcc
26	RM15488	3	(TG)24	55	282	cgtaacctcactgtgcttatcagg	atagctcctgcccctacctacg
27	RM483	3	(AT)26	55	325	cttcaccataaaacccggag	acaccggtgatctgttagcc
28	RM15838	3	(TTG)14	55	262	cgatgtcattcggtagaacaagc	cctagtcaaggcatggtcaatcc
29	RM5924	3	(ATT)29	55	209	gctcaactgctgttagaggattacc	agctctcccaagaaactgaacc

Table 1 cont...

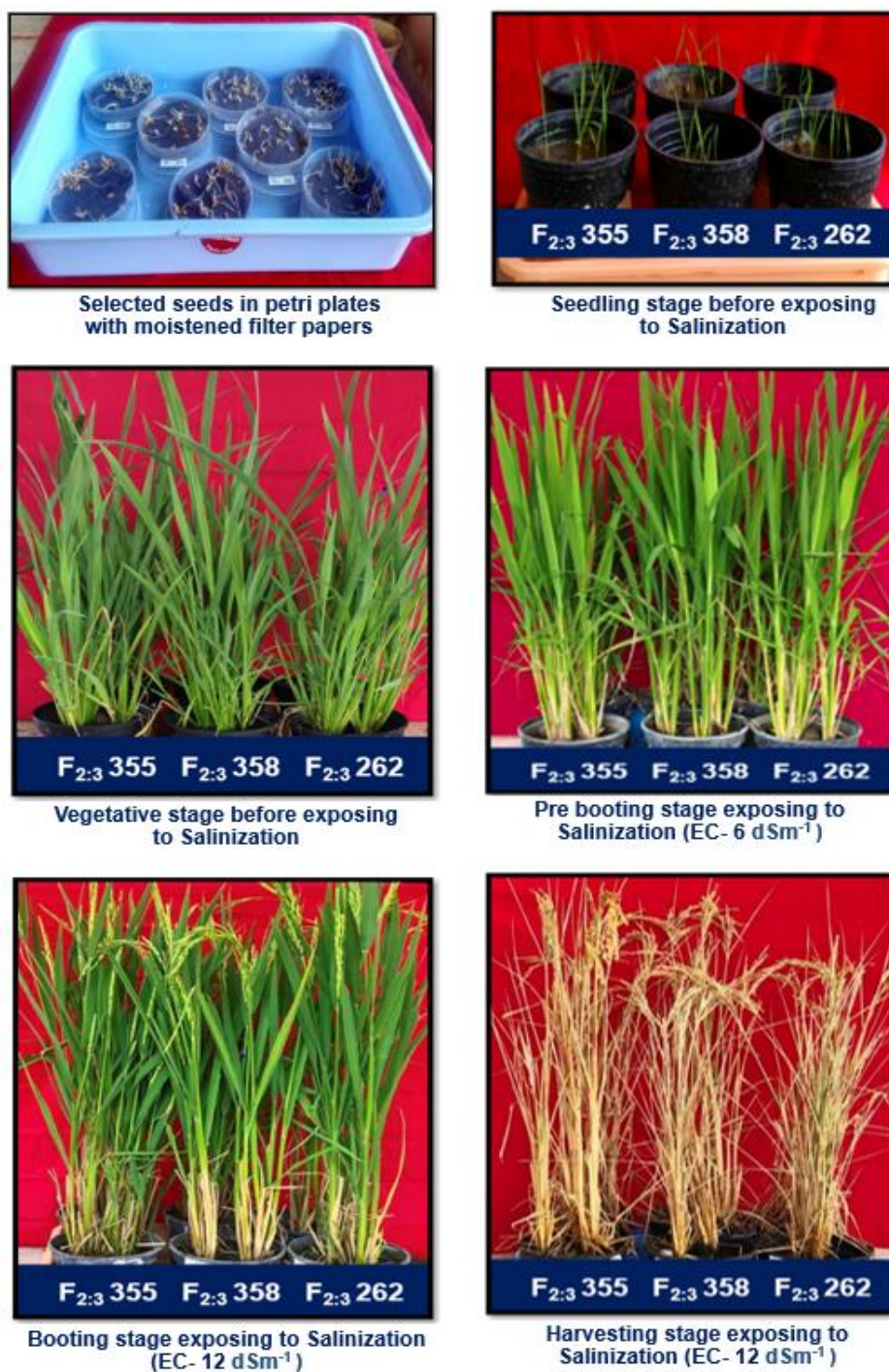
S. No.	Primer name	Chromosome number	SSR motif	Annealing temperature (°C)	Expected product size (bp)	Forward primer	Reverse primer
30	RM4404	3	(TA)19	55	105	gagatggcagtgtaagctaaacagg	tgaggacgccaatatggcaagg
31	RM16449	4	(AT)29	55	202	tcgcatgctaacttgagacg	caggaacatgtgtgcaatcg
32	RM16578	4	(AT)20	55	490	ggtgaattctactaagcggatcg	agccttattagtctacacctgtaacc
33	RM6679	4	(TAA)18	61	141	tttaggccgtaagagcgacatgg	atatgccgatgcagaacaagatcg
34	RM3524	4	(CT)31	50	129	ctgtctccgtcttctcactcg	tggagaaatctcccttctgagc
35	RM5270	4	(TA)45	55	162	tggcattgattacacctcagc	ttcagatgagaagcaagcactcg
36	RM6089	4	(CCT)10	61	170	cgatggccagcgatctcc	ccaccgaatcgaataaccacaagc
37	RM3474	4	(CT)21	50	174	acctcaccttccctcgattgg	gttggtgtcttctcccatagc
38	RM255	4	(AGG)5(AG)2-(GA)16	55	144	gaggaggaggaggagatcagg	aacgaaaccgctcagttaacc
39	RM5473	4	(TC)20	55	105	ggagataagacacgaggggaattatgc	agattaactacgcgcgtcatcc
40	RM3473	4	(AG)23	55	144	gcataccgtaatgttggtgaagc	aatagcaactgggaggaggttaagg
41	RM1182	5	(AG)14	50	164	cttctccgcttccctctcc	tgtaccagtgcaccgagagttgg
42	RM437	5	(AG)13	55	275	atccctcctctgtcaatgttg	tcaggagggttctagctactgg
43	RM169	5	(GA)12	67	167	cacctctccaagatccttatgc	ctctctgtctgtctgtgtgc
44	RM5844	5	(ATA)22	50	195	aacgtggcatccatgttagtacc	agctaggagccattgtcgaagg
45	RM18204	5	(ATAC)15	55	441	gaaactagagatgcacacatcc	atggtaagtactccctcatcc
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	tccacttcccttctctgtatcc	gccgacataacaagctagcaagg
49	RM540	6	(AG)16	55	172	gcctaaggctcatttatg	ctaggcctgccagattgaac
50	RM2615	6	(AT)30	55	164	atctcgttcatactgcttgacc	gactggttccttcatgttacc
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	ccaacggtgggtgtgtatagc
54	RM20661	6	(AACA)5	55	151	gaacacatgacaccactttgc	gcgtttctcattctgttcttgc
55	RM439	6	(AAT)13	55	269	ctgggtctaatactcgtcctaattgc	cgctctcataacagtccactcc
56	RM6697	7	(TAA)36	50	229	tattcccgggagatccaacagc	aagatccagtcgatttggtcagg
57	RM8262	7	(AGG)9	55	200	aacagatatactcgggcagcattagc	tgactctccgtggttaaacacc

Table 1 cont...

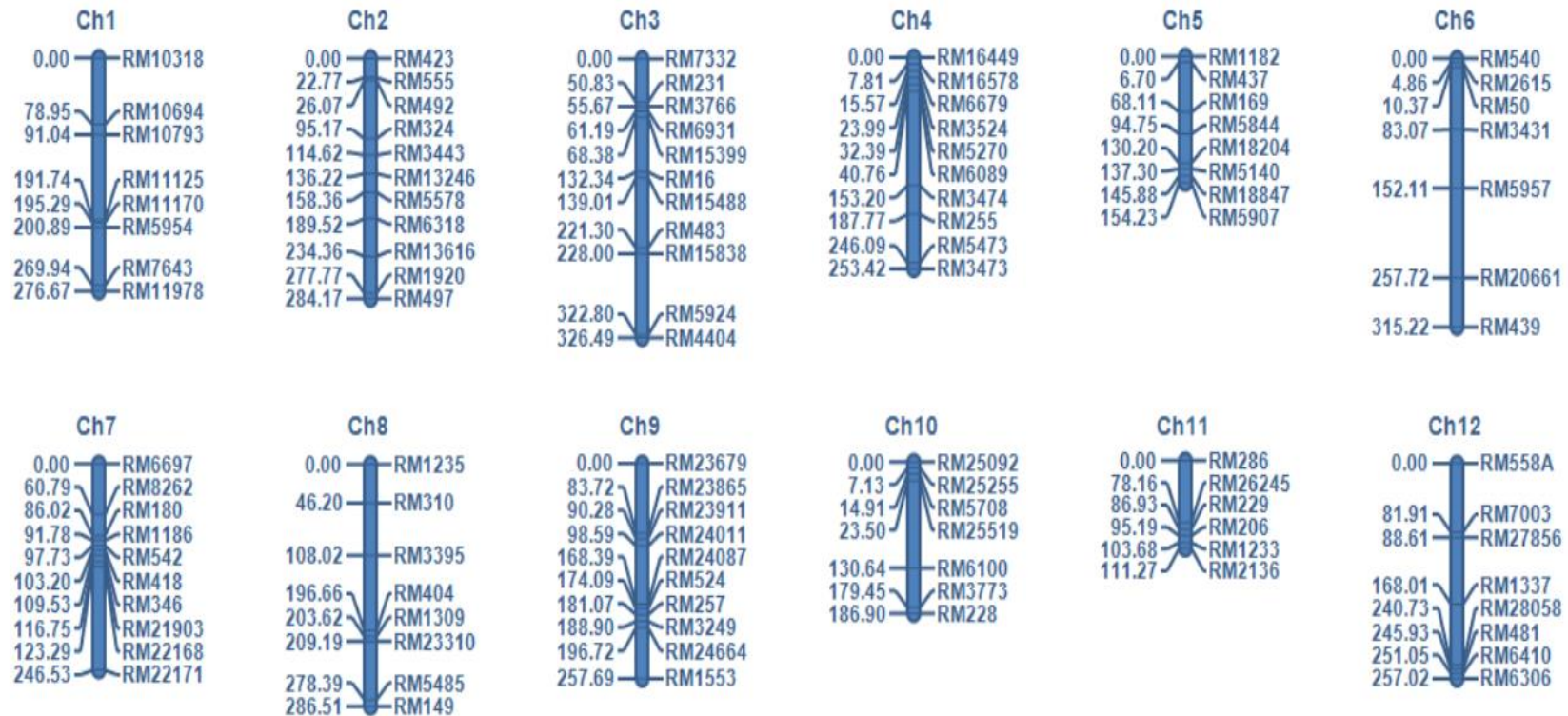
S. No.	Primer name	Chromosome number	SSR motif	Annealing temperature (°C)	Expected Product size (bp)	Forward primer	Reverse primer
58	RM180	7	(ATT)10	55	110	ccttcctccttcttcagctctgc	caacttgctctactgtggtgagg
59	RM1186	7	(AG)14	50	108	atatggtcattggctgggaaagagg	caccattatcctgcgggtaggc
60	RM542	7	(CT)22	55	113	cgctccttctagctccatctcc	aactgcaacgagtaaggcagagg
61	RM418	7	(ATT)21	55	283	cgatcgagcatcaacacaacg	gacgtatcgctatcgctatgc
62	RM346	7	(CTT)18	55	175	cgagagagcccataactacg	acaagacgacgagggga
63	RM21903	7	(AT)30	55	434	tggaacgacagagggtggtgagg	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	gagaaacacaatcagtgcacacc	ctgaaattgcacttcactgg
67	RM310	8	(GT)19	55	105	gacttgtgtgtgtgtgtgtgg	actgccatagcatttccctagc
68	RM3395	8	(CT)17	55	97	cttgggaacttcacctcatgg	ctgagagaagccacaggattaatgg
69	RM404	8	(GA)33	55	236	ggagcagctaaggcagataagagg	gccttcattgctcagaagacagc
70	RM1309	8	(AG)19	55	152	gaggacactgacgacagcttgg	cgcgcaaatcattaagtgcagg
71	RM23310	8	(TA)47	55	362	gatgaggcccagatttacattcc	ctaagacttcctgacgatgtacatgc
72	RM5485	8	(TC)22	55	140	atgattgcactctgcactcactgc	atacctgtttccaatgcgtagcc
73	RM149	8	(AT)10	55	253	ggaagcctttcctcgtaacacg	gaacctaggccgtgttcttgc
74	RM23679	9	(AGAA)10	55	189	tcacagcttagtgcatgttgagc	gattcacctggcaatgagaacg
75	RM23865	9	(CT)10	55	148	tcacccattctctctcacc	catacgccatacaaatgaacc
76	RM23911	9	(TG)13	55	269	tgccctgcacttatctctgatgc	gatgaacctaaaggcagtttcc
77	RM24011	9	(AG)25	55	424	aagatcgtagccgaagagtgacc	ggctaagagtgccaagacgagagg
78	RM24087	9	(AC)22	55	263	cactcagatttgccgatcc	gctgatccagatctacctgacacc
79	RM524	9	(AT)11	55	198	atcatagcccagaccaagaatgc	agatgaagagcaggaaccgtagg
80	RM257	9	(CT)24	55	147	ccgtgcaactaaatccaaacagg	ggaatcctatatgagccagtgtgg
81	RM3249	9	(CT)13	55	151	ggatcatgtcaaatcggaagagg	gctcaagcttcacagatcacagg
82	RM24664	9	(AT)35	55	362	atctaaacacagccctagtgc	gagccataactttaccatgtgc
83	RM1553	9	(AT)13	55	161	ttattgtccatgcggtacaacg	caaccacctcatcacaactcc
84	RM25092	10	(TG)15	55	479	ctatctccctgatgcgtacatgc	aatcagcgcgtgacaattcg
85	RM25255	10	(TGCG)5	55	190	ccactactcgtgtccggtacg	gttgggcgtcttgacgaacg
86	RM5708	10	(AAT)14	55	186	tggtatccataaccttgacagc	gtcacgtatcgaaacagtctcc
87	RM25519	10	(TA)42	55	260	gggtgattaattactggtcggaagg	gctggttgatcggaattacagg
88	RM6100	10	(CGA)8	50	144	ttccctgcaagattctagctacacc	gttcgtcgaccaagaactcagg
89	RM3773	10	(GA)18	55	150	ttgtgcctaagaatcatctctcc	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	tctaactctggccattagtccttg	aagtagacgaggacgacgacagg
91	RM286	11	(GA)16	55	110	ctggcctctagctacaaccttg	aaactctcgctggattcgatagg
92	RM26245	11	(ATCT)12	55	142	gctactaggccaccacgtgatcc	agcgtttataggaaacccacacaacg
93	RM229	11	(TC)11(CT)5C3(CT)5	55	116	atatgagttgctgtcgtg	caacttgcacccctcc
94	RM206	11	(CT)21	55	147	cccatgcgtttaactattct	cgttccatcgatccgatgg
95	RM1233	11	(AG)15	55	175	atgggcacgtgtaattcattcg	atcctcgaaagtaggagtaggaaagc
96	RM2136	11	(AT)22	55	136	cgggtgtgtaaaactccgaagcacc	tgccgtggctcattagtggc
97	RM558A	12	(ATTG)5	55	246	gatcatcgccaagaacacatgc	cacctgacaaaggagcagatcg
98	RM7003	12	(AAAC)6	50	101	ctctagctctcatggatgg	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	ccttctgctcgtgtcactctacc	cccggaagtattccaaggatcg
102	RM481	12	(CAA)12	55	169	agagagcccctaatttccg	aggtacgctcacctgtggac
103	RM6410	12	(GAG)8	55	177	ggaagaagcatctgcagtagtagacg	gaattagccgacagcgtcttg
104	RM6306	12	(CTT)9	50	88	gtattggtcaccgggtctaagtcg	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

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of the publisher and/or the editor(s). This publisher and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© Copyright (2025): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:

<https://pr.sdiarticle5.com/review-history/150224>