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RESEARCH ARTICLE

Phenotypic assessment of RILs of an interspecific biparental population (IR29/*Oryza glaberrima* Steud.) for seedling salinity tolerance and exploration QTL/haplotype in African rice (*O. glaberrima* S.) genome

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Abstract

Salinity is one of the most predominant abiotic stress factors affecting crop production across the planet earth. Rice is canonically a glycophytic species that becomes sensitive to salinity stress, especially at the seedling and early reproductive growth stage. Several studies have explored the potential of African rice (*Oryza glaberrima* Steud.) and its interspecific progenies to be salinity tolerant at the seedling stage of growth. In this context, we tested an interspecific biparental (IR29/*O. glaberrima* Steud.) recombinant inbred lines (RIL) population in the present study for seedling salinity tolerance and identified a few extreme tolerant and sensitive RILs in the population and assigned (SES = 3; highly tolerant) and (SES = 9; highly sensitive) scores to the contrasting RILs in salinity stress. A few relevant parameters had been scored and a majority had shown very sharp contrast in phenotype in parents and extreme RILs. A higher ratio of stress/control phenotypic data had been observed in extreme tolerant RILs, whereas the ratio was much lower in the opposite extreme lines indicating the probable regulation of seedling salinity-related parameters by QTL region(s). Also, an assessment of the parental genotypes with *Saltol* QTL-specific SSR markers revealed the possibility of the existence of a novel QTL region in our African rice accession. Hence, this study opens an avenue for the discovery and mapping of novel QTL/candidate genes for seedling salinity tolerance that can be further utilized in rice breeding programs for improvement in stress tolerance in high-yielding cultivars.

Keywords: *Oryza glaberrima* Steud., phenotyping, salt tolerance, *Saltol* QTL.

Introduction

Globally, there are more than 833 Mha of soil (8.7% of the planet) affected with salinity. The majority of these soils are present in Asia, Africa and South America. In addition, 20 to 50% of irrigated soils in all continents contain excess salts, indicating that soil degradation leads to major challenges in food production for more than 1.5 billion people across the world. Altogether, salt-affected soils pose a threat to the global fight against hunger and poverty (FAO 2021). In coastal districts of Bangladesh, the production of high-yielding rice varieties will reduce by 15.6% where salinity level in the soil is predicted to surpass 4 dS^{m-1} by 2050 (Dasgupta et al. 2014). Therefore, reducing the spread of salinization and increasing the salinity tolerance level of important food crops, including paddy are major agricultural concerns to ensure global food security.

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Rice is the staple food for more than half of the population worldwide (Zhang et al. 2023). In Africa, *Oryza glaberrima* Steud., commonly known as African rice, is an indigenous staple food and one of the two independently domesticated rice species (Portères 1956; Linares 2002). Previously, African rice accessions were reported to be tolerant to salinity stress (Linares 2002; Platten et al. 2013). Several studies have shown that different accessions of *O. glaberrima* S. show different levels of salinity tolerance in hydroponics solution (Awala et al. 2010; Prodjinoto et al. 2017). Hydroponics screening experiments have shown that interspecific progenies, including New Rice for Africa (NERICA) varieties are more tolerant to salinity stress than *O. glaberrima* S. at the seedling stage of growth (Awala et al. 2010). Since the whole genome sequence of *O. glaberrima* S. is available (Wang et al. 2014), the genetic potential of African rice can be efficiently utilized for rice improvement under salinity stress.

On the basis of growth and developmental stages, the sensitivity of rice to salinity stress varies, i.e., relatively tolerant at germination, more sensitive at early seedling and reproductive stages and attaining higher tolerance at the time of active tillering and grain filling stage (Ismail et al. 2007; Walia et al. 2007). A prominent enhancement in sodium (Na^+) concentration in root, stem and shoot tissue accompanied by a marked reduction in dry matter and quantum yield of PSII has been observed during salinity stress at the early seedling stage (García Morales et al. 2012). The majority of the rice varieties are salt-sensitive in nature with the exception of a number of traditional landraces which are highly salt-tolerant viz., 'Pokkali', 'Nona Bokra' (Gregorio et al. 2002) and wild halophyte *Oryza coarctata* Roxb. harbouring exclusive salt-tolerant genes in comparison with cultivated rice (Mondal et al. 2018).

The *Saltol* QTL, derived from a cross between IR29 and Pokkali and mapped on the short arm of chromosome 1, is a large effect QTL (> 40% phenotypic variability for shoot Na^+/K^+ ratio at seedling stage) (Bonilla et al. 2002). A hotspot of QTL for shoot Na^+ content and Na^+/K^+ ratio exists in rice chromosome 1 (Negrao et al. 2011; Jing et al. 2017) where *Saltol* QTL localizes. Alongwith this, related QTL has been identified from several salt-tolerant landraces such as Nona Bokra (Lin et al. 2004), CSR27 (Pandit et al. 2010) and Pokkali (Alam et al. 2011). A comprehensive study surveyed diverse salt-tolerant genomic resources and reported the presence of a major fraction of salt-tolerant QTLs/genes on chromosome 1 through chromosome 6 in the rice genome (Ganie et al., 2019). However, QTL mapping from new donors is essential for the breeders to develop robust salt-tolerant rice cultivars. With this background, in the present investigation, we have made an attempt to develop a recombinant inbred line (RIL) population by an interspecific crossing between two contrasting genotypes, viz. IR29 (salt-sensitive, seedling stage) and *O. glaberrima* S. (salt-tolerant, seedling stage) and screened the population for seedling stage salinity tolerance. Based on the observation in the naked eye, we have selected a few extreme RILs having prominent contrast in tolerance and sensitive phenotypes. These RILs had been evaluated for different relevant phenotypic parameters for seedling salinity stress. Interestingly, we have observed that *Saltol* QTL is absent in *O. glaberrima* S., which raises the possibility that the salt-tolerant parental line harbors either a different *Saltol*-specific haplotype or novel QTL(s) associated with seedling salinity tolerance traits. Our study, therefore, provides a roadmap for mapping novel QTL and candidate genes for seedling salinity, which may be utilizable in rice improvement for salinity tolerance.

Materials and methods

Plant materials and development of mapping population

One pair of contrasting rice genotypes, such as IR 29 (*O. sativa* L., EC605403) and *O. glaberrima* Steud. (accession no. TKM239, EC1182038) were utilized as pollen recipient and donor parent, respectively, to develop the RIL mapping population. TKM239 was earlier found to be salt-tolerant at the seedling stage in a hydroponics screening experiment (unpublished data). IR29 (*Oryza sativa* L.) is a high-yielding (dry season- 4.464 t/ha, wet season- 3.02 t/ha), early maturing (days to maturity- 116 days after sowing) rice cultivar. However, sensitivity to salinity stress limits its productivity in problem soil (Khush and Virk, 2005). An interspecific cross-pollination was attempted at the Indian Agricultural Research Institute (ICAR-IARI), New Delhi, India, in *Kharif*, 2017. The resulting F_1 hybrid was self-pollinated to develop F_2 population, which was advanced up to $F_{6:7}$ generation to obtain biparental, recombinant inbred lines (RILs) mapping population by single seed descent (SSD) method. A total of 197 $F_{6:7}$ lines and the parents were used for the initial screening experiment.

Plant growth and evaluation of morpho-physiological parameters

The two parents IR29, TKM239 and 197 $F_{6:7}$ RILs were subjected to salinity treatment at the seedling stage in hydroponics Yoshida nutrient solution (Yoshida et al. 1976). A couple of check varieties, such as IR36 (salt-sensitive) and FL478 (salt-tolerant), were screened alongside. The experiment was conducted in a controlled environment with 16 hours of light and 8 hours of dark photoperiod at 25 to 26°C with a relative humidity of 60 to 70% for the growth of the seedlings. Seeds were germinated in moist germination paper in petri plates at 30°C for four days before transferring to hydroponics (pH = 5.0) in perforated PCR plates in plastic trays for the growth of the seedlings for the next two weeks and salinity stress was imposed thereafter. The seedlings

were grown with a standard protocol as per the Standard Evaluation System (SES) of IRRI for salinity screening and the salt injury scoring was performed in a 1 to 9 scale (Redona 2013). Initially, the parents were evaluated in 100, 150 and 200 mM salt (NaCl) stress for 7 days to standardize the concentration of salinity stress for screening the entire population. One set of controls was maintained alongside. The pH of the solution was maintained with HCl (0.1 N) and NaOH (1 M) in every 2-day interval till the scoring of the phenotypes. Based on the observation of the contrasting phenotype of the parents, individual lines/families of the RIL population were similarly subjected to salinity treatment. Based on the visual appearance of the RILs in salinity stress, five very green, healthy lines and five nearly dried-up RILs were selected randomly (extreme RILs) and an SES score was given to all 10 RILs. Phenotypic parameters such as root and shoot length, fresh weight (FW) and dry weight (DW) of root and shoot, length of the large (L) type lateral root, small (S) type on L-type root, proline and trehalose content from leaves in salinity stress were recorded from parents and compared with the control. Lateral root lengths were measured with ImageJ software (Schneider et al. 2012). On the basis of parental contrast, RILs were evaluated for the same contrasting parameters. All seedlings were dried in a hot-air oven (Sanco Industries Ltd.) at 65℃ for 5 days before measurement of the dry weight. Five biological replicates were scored for the initial six phenotypic parameters and three replicates for the rest of the parameters.

Measurement of chlorophyll content

The leaf chlorophyll content was estimated in a UV-vis spectrophotometer (Varioscan Flash, Thermo Scientific, USA). Leaf samples were immersed in 80% (v/v) acetone for 2 hours at 50℃ (Arnon, 1954) and 200 µL of leaf extract was analyzed for absorbance at 645 nm (A_{645}) and 663 nm (A_{663}) in a microplate reader. The concentration of pigment was measured according to the method described by Lichtenthaler (1987).

Total chlorophyll (mg/g FW) = $\{(20.2\times A_{645}) + (8.02\times A_{663})\}$ V/1000×W

Where A – absorbance at specific wavelengths, i.e., 645 nm (A_{645}) and 663 nm (A_{663}).

V – final volume of chlorophyll extract in 80% acetone.

W – fresh weight of the leaf tissue extracted (mg).

Fingerprinting F_1 hybrid and assessment of the presence or absence of Saltol QTL in parental lines

Genomic DNA was isolated and purified from the leaf samples collected from the irrigated plot (kharif, 2018) from a single fully grown healthy plant from each of the parental lines, F_1 hybrid plant and FL478 (harbors *Saltol* QTL) at vegetative growth stage using CTAB method (Doyle and Doyle 1987). The diluted gDNA samples were PCR amplified with four SSR markers viz. RM493, RM10793, RM3412 and RM10772 (tightly linked to *Saltol* QTL region in FL478) and run on 1.5% agarose gel electrophoresis to confirm the true hybrid status of F_1 plant alongwith two parental lines. Alongwith this, the DNA amplicon size of TKM239, IR29 and FL478 were compared for an initial assessment of the presence/absence of a *Saltol*-specific haplotype or the existence of novel QTL (genomic region) associated with seedling salinity tolerance.

Results

Phenotypic evaluation for seedling salinity tolerance in the parents and extreme RILs

A total of 197 RILs ($F_{6:7}$) were developed by crossing two interspecific parental lines (Fig. 1). Two contrasting parental lines and two check varieties were evaluated for seedling stage salinity tolerance in hydroponics solution. At 25 days after sowing (DAS), the parental salinity screening experiment revealed that at extremely high salinity levels (200 mM), both the parental lines dried up after 1 week of stress, whereas at exposure to 150 mM (very high) NaCl, TKM239 survived, IR29 dried up although symptoms were clearly visible on the leaf surface of the tolerant parent. A clearcut phenotypic difference was observed, i.e., IR 29, highly salt-sensitive (SES = 9) and TKM239, highly salt-tolerant (SES = 3) at 100 mM NaCl (EC = 11.65 dS/m) stress (Fig. 2A, Supplementary Fig. 1, Supplementary Table S1). Hence, the subsequent salinity screening of extreme RILs was executed at 100 mM NaCl (Fig. 2B).

L-type lateral root length was prominently enhanced in TKM239 and reduced in IR29 in salinity whereas, S-type on L-type root almost disappeared in IR29 in stress, although in TKM239, slight reduction in stressed S-type root had been observed (Fig. 3).

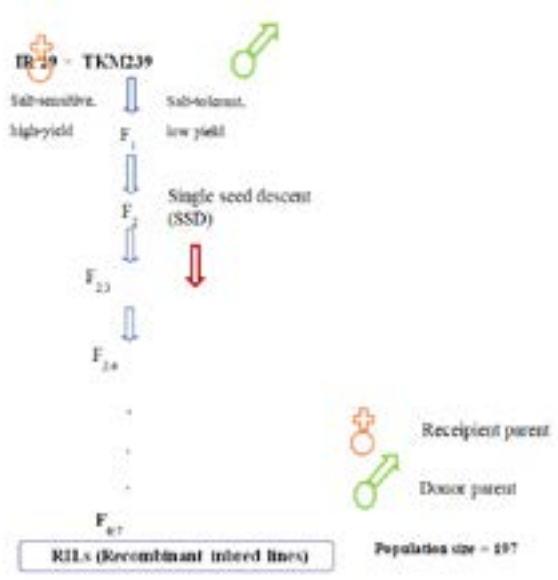

Fig. 1. Development of biparental RIL mapping population

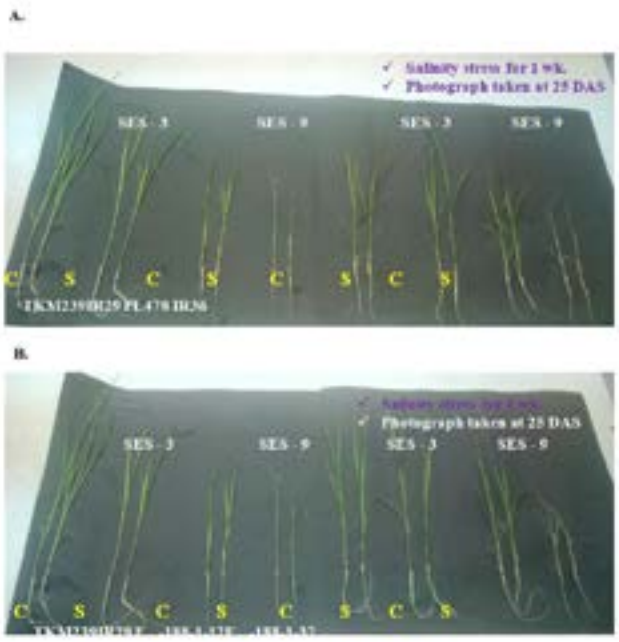

Fig. 2. Seedling salinity screening at hydroponics solution. (A) Parents and checks, (B) Parents and two representative RILs showing extreme contrast in phenotype in salinity, C = Control, S = stress

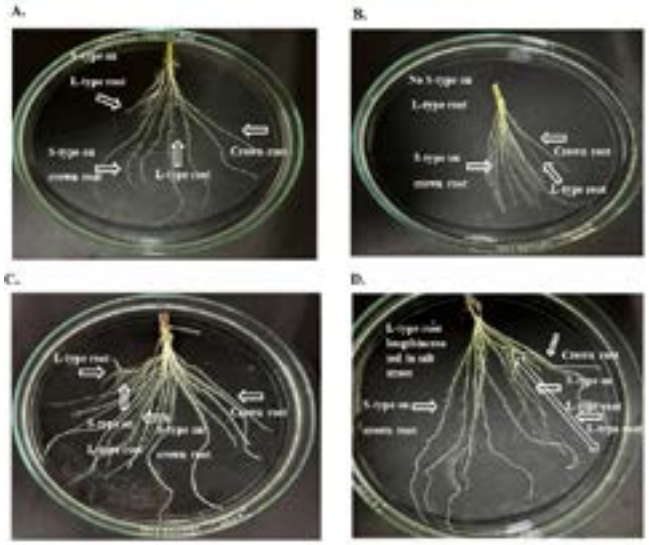

Fig. 3. Parental contrast in seedling salinity for L-type and S on L-type root length. (A) IR29_control, (B) IR29_stress, (C) TKM239_control, (D) TKM239_stress

Based on the phenotypic data obtained from different parameters associated with seedling salinity, both TKM239 and FL478 showed less reduction in root and shoot length, root and shoot fresh weight, root and shoot dry weight in stress in comparison with control. Whereas, reduction in IR29 and IR36 were much higher in stress than control, indicating the sensitivity of parent and negative check variety used in the present study (Fig. 4).

However, no difference in proline and trehalose content was found between the parental lines; hence, these were not scored in the extreme RILs. A total of 5 pairs of RILs were randomly chosen as extremely salt-tolerant and salt-sensitive based on their visual phenotypic appearance in salinity stress. Phenotypically, extreme RILs followed the pattern of their corresponding parents and displayed similar or higher contrast among tolerant and sensitive lines under salinity vs. control conditions when compared with the performance of the parental lines (Table 1).

Hybridity test of F_1 and assessment of the presence or absence of Saltol QTL in parental lines

The DNA amplicon size difference between TKM239 and IR29 indicated the polymorphic status of all 4 SSR markers. The presence of two DNA bands (heterozygote) in F_1 hybrid DNA amplicon was similar to the positions of the two contrasting parental DNA bands, indicating that this was the true hybrid. Alongwith this, a size difference in the PCR amplicons of two parental lines in comparison with the FL478 amplicon was observed (Fig. 5). The variation in the amplicon size between TKM239 and FL478 (Fig. 5) indicates that the genome of African rice (TKM239) contains either a different *Saltol*-specific haplotype or novel QTL region(s) governing seedling salinity tolerance traits. The PCR primer details have been listed in Supplementary Table S2.

Discussion

Global climate change has threatened human civilization by promoting rapid soil degradation in agricultural land worldwide. Soil salinization is one of the serious soil degradations, which can arise from natural and human-mediated activity such as irrigation in arid and semi-arid regions (Kader and Lindberg 2008). The term 'salinity' represents all the problems of the soil accumulating excess salts, which can be categorized into saline-sodic (alkaline) and saline-neutral soils (IRRI, 2011). Furthermore, the sea level along the Indian coast has been rising at an average rate of about 1.3 mm per year and the trend is likely to continue in the future. There is a possibility of an increase in flooding by about 10 to 30% over the existing magnitudes (World Bank Report 2008). The majority of the salt-affected lands in coastal regions, monocropped with rice during the wet season, produce very low yields due to the intrusion of seawater inside lowland paddy fields because of tidal waves and uprise of water resulting in saltwater flooding (Singh and Sarkar 2014). Therefore, salt-tolerant rice varieties need to be developed in order to increase productivity and maintain a balance between the demand and supply of rice (Ganie et al. 2017).

Africa is the only continent in the world where *O. glaberrima* S. and *O. sativa* L., the two species (genome AA, 2n = 24) are cultivated (Vaughan et al. 2003). In farmers' fields in Africa, *O. glaberrima* S. is constantly substituted by *O.sativa* L. because of its low yield potential, shattering

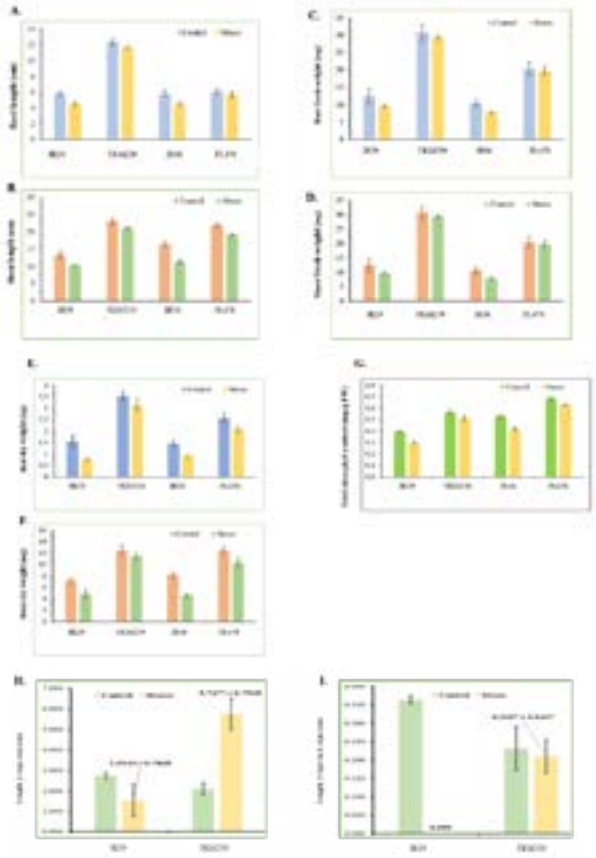

Fig. 4. Phenotypic difference between parents and checks for several parameters associated with seedling salinity tolerance. (A) Root length (cm), (B) Shoot length (cm), (C) Root fresh weight (mg), (D) Shoot fresh weight (mg), (E) Root dry weight (mg), (F) Shoot dry weight (mg), (G) Total chlorophyll content (mg/g FW), (H) Length_L-type root (cm), (I) Length_S on L-type root (cm). FW = Fresh weight, Data – Mean ± SEM, SEM = Standard error of mean

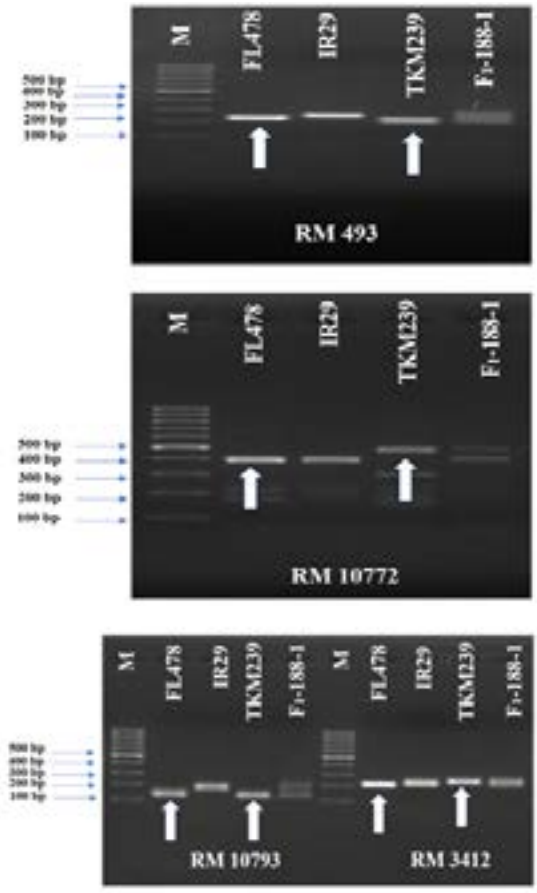

Fig. 5. Fingerprinting of F_1 hybrid and parents with four polymorphic *Saltol*QTL linked SSR markers and assessment of presence/absence of novel QTL/haplotype in TKM239 (*O.glaberrima* S.) rice genome. M = 100 bp DNA ladder. F_1-188-1 – F_1 hybrid derived from IR29/TKM239

Table 1. Relative phenotypic data of extreme RILs were evaluated in this study. Relative data = $Stress_{(mean)}/Control_{(mean)}$, Chl = Chlorophyll, FW = Fresh weight (mg)

		Relative phenotypic data {$stress_{(mean)}/control_{(mean)}$}								
Extreme RILs (IR29/TKM239; $F_{6:7}$)	SES (stress)	Root length (cm)	Shoot length (cm)	Root fresh wt. (mg)	Shoot fresh wt. (mg)	Root dry wt. (mg)	Shoot dry wt. (mg)	Total Chl content (mg/g FW)	L-type root length (cm)	S-type root length (cm)
188-1-12	3	1.024	0.956	0.933	0.929	0.415	0.983	0.826	2.062	0.751
188-1-26	3	1.056	0.798	0.894	0.689	0.359	0.862	0.918	3.683	0.480
188-1-42	3	0.928	1.102	1.090	0.991	0.420	0.950	0.919	1.627	0.477
188-1-46	3	0.917	1.092	1.226	1.020	0.398	0.946	0.820	3.596	0.425
188-1-52	3	1.039	1.026	0.914	0.827	0.447	0.927	0.822	3.546	0.764
188-1-131	9	0.783	0.522	0.616	0.532	0.367	0.736	0.552	0.555	0.000
188-1-152	9	0.621	0.516	0.571	0.602	0.206	0.636	0.559	0.441	0.000
188-1-159	9	0.777	0.414	0.755	0.525	0.279	0.550	0.444	0.520	0.231
188-8-37	9	0.786	0.434	0.805	0.351	0.270	0.568	0.468	0.325	0.156
188-8-86	9	0.580	0.475	0.707	0.263	0.248	0.697	0.546	0.109	0.136

SES 3 = Extreme tolerant and SES 9 = Extreme sensitive RILs at seedling salinity stress
A comparatively higher ratio of relative phenotypic data had been observed among extreme RILs ($F_{6:7}$) within the population (IR29/TKM239).

and susceptibility to lodging and the massive introduction of improved, high-yielding rice cultivars (Ghesquière et al. 1997). Although, smallholder farmers in many African countries, viz. Nigeria, Mali, Senegal, Sierra Leone, and Togo still cultivate African rice in different environments (Teeken 2015). The resilience of African rice in the farmers' field is mainly due to its hardiness, pest and disease resistance and good response to low-input farming systems (Wang et al. 2014). The genetic potential of this indigenous rice as a rich reservoir of genes conferring resistance/tolerance to various biotic and abiotic stress factors makes this an important material for research in abiotic and biotic stress tolerance (WARDA 1997; Sarla and Swamy 2005).

With this background, in the present study, we have made a salinity screening of a RIL population derived from an interspecific cross between an Asian (salt-sensitive) and African (salt-tolerant) rice genotypes for seedling stage salinity tolerance and identified a few RILs with extreme phenotypic differentiation. The different *Oryza* species were selected for distant hybridization in order to obtain new sources of gene(s)/allele(s)/haplotype, which may provide superior tolerance to seedling salinity stress. The extreme RILs represented all possible QTL/gene combinations present in the parental lines and a few new combinations arising due to genetic recombination of alleles from both the parental lines in the population conferring higher degree of trait variation among a few, restricted number of RILs in the population. Likewise, a comparatively lower ratio of relative phenotypic data (stress/control) had been observed in all the seedling salinity-related parameters evaluated in contrasting RILs indicating the presence of probable transgressive segregants in the population. The remaining RILs will probably display a variable degree of salt tolerance/sensitivity for different seedling salinity-related parameters once tested in the future. This pattern will be indicative of a continuous variation among various trait-related parameters and the probable regulation of seedling salinity tolerance by polygenic mode of inheritance from parent to RIL progenies.

Moreover, the data obtained from the fingerprinting of DNA amplicons revealed that the F_1 hybrid plant was the immediate progeny of IR29 and TKM239 accession of *O.glaberrima* S. in the present study. In addition, the size difference of DNA amplicons obtained from the two parental lines in comparison with FL478 (*Saltol* QTL) conveys the information that TKM239 has either novel QTL(s) for seedling salinity traits or the salt-tolerant parent contains a different *Saltol* specific haplotype. Identifying superior allelic combinations from a wide range of salt-tolerant donors is important as the genetic base of the source of salt-tolerance has been narrowed down due to the massive utilization of Pokkali and Nona Bokra rice genotypes (salt-tolerant) in a major proportion of the rice breeding programs for enhancement of salt-tolerance capacity in high-yielding varieties. The new genetic resource for seedling salinity tolerance provides ample scope to identify undiscovered candidate gene(s) and therefore, information can be obtained on unknown cellular and molecular mechanisms associated with the trait in question.

Moreover, a few studies have been executed on the discovery of quantitative trait loci (QTL) associated with salinity tolerance in rice at the seedling stage (Gregorio et al. 1997; Leon et al. 2016; Mazumder et al. 2020). However, more intense efforts are necessary. Also, in African rice, at the tillering and flag leaf stage, an AP2 DNA-binding domain containing transcription factor *OglDREB2A* was seen to be upregulated and the expression pattern was seen to be conserved among several rice genotypes having variable salt tolerance (Gumi et al. 2018). Furthermore, a few novel and conserved microRNAs (miRNA) related to salinity tolerance had been identified in the halophyte *O. coarctata* Roxb. (Mondal et al. 2015) and target modules specific to salt-responsive miRNA had also been reported in *O. glaberrima* Steud. (Mondal et al. 2018). Across the world, there is no report of any QTL(s) associated with salinity tolerance in the African rice genome at any plant growth stage to date. Therefore, our long-term objective is to identify and map novel seedling salinity-linked QTL(s) by statistically correlating the phenotypic and genotypic (marker) data. As African and Asian rice are far apart from each other on an evolutionary scale, certainly, there is a possibility of the existence of novel, unexplored genomic region (QTL) governing different parameters linked to seedling salinity tolerance. *Saltol* QTL is associated with seedling salinity tolerance. It shows very high phenotypic variability in the RIL population based on IR29 and Pokkali parental backgrounds (Gregorio et al. 1997). Therefore, if we land up on any *Saltol* QTL-specific haplotype identified in the studied population, still, there will be a road to detect superior haplotype conferring a high degree of seedling salinity tolerance. In the future, the putative QTL(s)/candidate gene(s)/haplotype identified from our study can be further introgressed into the high-yielding varieties (HYVs) preferred by the farmers through marker-assisted backcross breeding (MABB) with an aim to develop improved cultivars of rice having enhanced climate resilience for cultivation in saline farmlands. If multiple QTLs are identified, a strategy can be designed to pyramid those QTLs/genes in HYVs of rice to develop superior crops (climate-smart crops) that will be able to endure optimum tolerance to salinity stress in the salt-affected arable lands for a longer duration, especially in the coastal regions across the globe with an aim to obtain a reasonable grain yield.

Supplementary material

Supplementary Supplementary Table S1 and S2 and Supplementary Fig. 1 are provided and can be accessed at

www.isgpb.org

Authors' contribution

Conceptualization of research (TKM, SGK); Designing of the experiments (TKM, PCK, SGK, AM); Contribution of experimental materials (SGK, TKM); Execution of field/lab experiments and data collection (AM, MR); Analysis of data (AM) and interpretation (AM, MR, TKM, SGK); Preparation of the manuscript (AM) improvement of the manuscript (AM, PCK, TKM).

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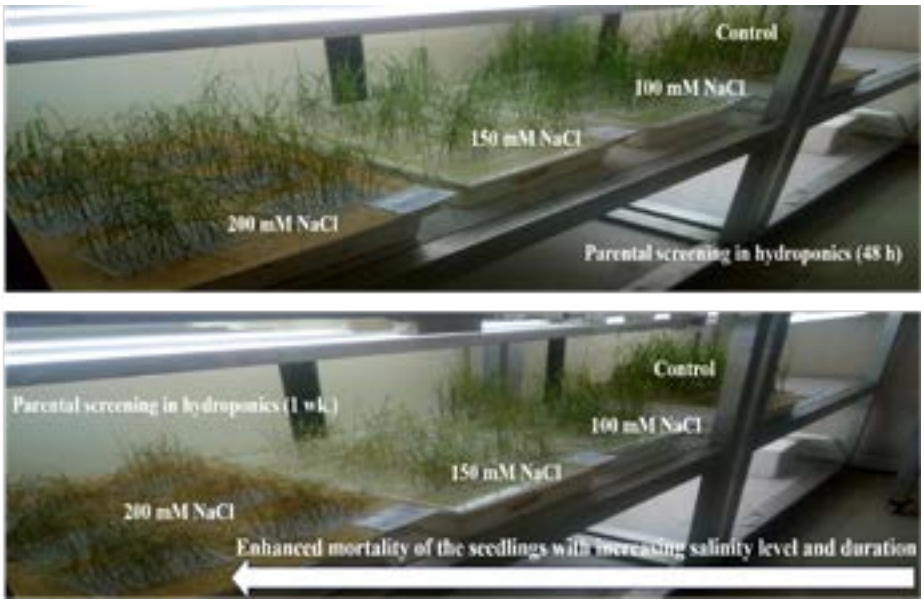
Supplementary Table S1. Standard evaluation system for rice and SES scores of parents and check varieties in salinity stress.

Standard evaluation system for rice (IRRI)					
Salt injury score (SES) in salinity stress	Phenotype	Category	Genotype	Salt injury score (SES) in salinity stress	Remarks
Scale					
1	Growth and tillering nearly normal	Control plants	IR29	9	Highly sensitive
3	Growth nearly normal but there is some reduction in tillering and some leaves are whitish and rolled	Highly tolerant	TKM239 (*O.glaberrima* S.)	3	Highly tolerant
5	Growth and tillering reduced; most leaves are whitish and rolled; only a few elongating	Moderately tolerant	IR36	9	Highly sensitive
7	Growth completely ceases; most leaves dry; some plants dying	Moderately sensitive	FL478	3	Highly tolerant
9	Almost all plants dead or dying	Highly sensitive			

Supplementary Table S2. Summary of the SSR markers utilized in this study

S. No.	SSR	Primer Sequence	Tm(°C)	Length(bp)	Amplicon size (bp)
1	RM493	Forward- TAGCTCCAACAGGATCGACC	53.8	20	211
		Reverse - GTACGTAAACGCGGAAGGTG	53.8	20	
2	RM10793	Forward- GACTTGCCAACTCCTTCAATTCG	55.3	23	123
		Reverse- TCGTCGAGTAGCTTCCCTCTCTACC	61	25	
3	RM3412	Forward- AAAGCAGGTTTTCCTCCTCC	51.8	20	211
		Reverse- CCCATGTGCAATGTGTCTTC	51.8	20	
4	RM10772	Forward- GCACACCATGCAAATCAATGC	52.4	21	395
		Reverse- CAGAAACCTCATCTCCACCTTCC	57.1	23	



Supplementary Fig. 1. Parental screening for seedling salinity tolerance