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REGULAR ARTICLE

Salt resistance of interspecific crosses of domesticated and wild rice species

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Abstract

Background: Salt stress negatively affects rice growth and yield in many parts of the world. Cultivated rice (*Oryza sativa* L.) is very sensitive to salt stress. Breeding attempts to develop salinity-adapted rice varieties have been hampered by the quantitative nature of adaptation and limited genetic variability in cultivated rice.

Aims: We aimed to explore the potential of wild rice species for improving adaptation to salinity. We screened two populations of introgression lines (ILs) derived from crosses between *O. sativa* (cv. Curinga) × *O. meridionalis* (CM population) and between *O. sativa* (cv. Curinga) × *O. rufipogon* (CR population) to identify quantitative trait loci (QTLs) and associated resistance mechanisms to salt stress.

Methods: We used previously developed ILs and screened them for adaptation to salt stress. In addition, we performed physiological, biochemical, and mineral analysis with the most resistant ILs identified for each population.

Results: Three and 19 QTLs for different vegetation indices were identified for the CM and CR population, respectively. We identified two ILs with superior resistance to salinity. These ILs showed enhanced vegetation indexes and maintained relatively high gas exchange under salt stress. In addition, these ILs showed less damage to cell membranes and reduced formation of H_2O_2, when compared with the recurrent parent, *O. sativa*.

Conclusion: Our study demonstrated that rice wild relatives are promising sources of salinity resistance. Introgressions of *O. meridionalis* and *O. rufipogon* into the *O. sativa* genome can confer increased resistance to salinity excess.

KEYWORDS
Oryza meridionalis, Oryza rufipogon, Oryza sativa, QTL analysis, rice wild relative, salinity resistance

1 | INTRODUCTION

Salinity stress is a major limiting factor affecting crop production, especially in arid and semi-arid environments, coastal areas, and lands where crop production is based on irrigation with poor drainage systems (Munns and Tester, 2008; De Leon et al., 2016). This scenario affects approximately 1 billion hectares in more than 100 countries, which accounts for more than 20% of irrigated and 8% of rainfed

agricultural land (Qadir et al., 2014; Asif et al., 2018). According to FAO, the annual loss in agricultural productivity caused by salinization is estimated to be US$ 31 million (FAO, 2018).

Rice (*Oryza sativa* L.) is a major cereal crop and a primary source of food for more than half of the world's population (Elert, 2014). However, rice is the most salt-sensitive species among all cereals (Munns & Tester, 2008) and shows significant growth reduction due to soil salinity at a threshold of 4 dSm^{-1} for most cultivated varieties (Munns, 2005). In general, salinity reduces growth rate, cell expansion, leaf area, number of tillers, and photosynthetic rate. Besides, salinity causes senescence of the older leaves and can lead to premature death of the plant in extreme cases (Munns & Tester, 2008; Sirault et al., 2009). Generally, root growth is less affected than shoot development (Munns & Tester, 2008). Notably, seedling and reproductive stages are the most critical growth stages (Hoang et al., 2016). Yield components such as panicle length, spikelet number per panicle, grain yield, and panicle emergence and flowering are also affected by salinity stress (Puram et al., 2017). Therefore, rice breeding programs to improve salt adaptation have become a pressing need (Flowers, 2004), especially in saline and coastal areas such as Asia and the Pacific, Australia, and some regions in Africa (FAO, 2018).

Adaptation to salinity in plants can be associated with three distinct mechanisms: (1) osmotic stress tolerance, in which tolerant plants maintain stomatal conductance and growth, while sensitive plants show rapid stomatal closure and reduced growth; (2) Na^+ or Cl^- exclusion from leaf blades (LB), in which resistant plants prevent the accumulation of Na^+ in shoot tissues by exclusion mechanisms in the xylem and decreasing root-to-shoot translocation; (3) tissue tolerance to accumulated Na^+ or Cl^-, in which the ions are compartmentalized in mesophyll cell vacuoles, preventing toxicity caused by ion presence in the cytoplasm. The occurrence and the relative importance of each mechanism depends on the species and genotype (Munns & Tester 2008, Roy et al., 2014; Munns et al., 2020a, 2020b). Additional mechanisms to cope with salinity stress rely on biosynthesis and accumulation of osmolytes, efficient reactive oxygen species (ROS) detoxification, and programmed cell death (Hoang et al., 2016; Puram et al., 2017).

Salinity adaptation in rice is a genetically and physiologically complex trait, controlled by multiple *loci* and genes, since salt influences various plant processes at all levels of organization (Koyama et al., 2001). The genetic basis of salt adaptation in rice is widely documented by quantitative trait loci (QTL) analysis. A large number of QTLs, associated with different mechanisms of salinity adaptation, have been identified during the seedling stage, using recombinant introgression lines (RILs) and backcross lines derived from salt tolerant *O. sativa* Pokkali (Alam et al., 2011; Dahanayaka et al., 2017; De Leon et al., 2016, 2017; Chen et al., 2020). Other studies employed progeny from several crosses (*Koyama et al*., 2001; *Wang et al*., 2012; *Tiwari et al*., 2016; *Puram et al*., 2017; *Jahan et al*., 2020). A major QTL was identified on the short arm of chromosome 1 using a population of RILs derived from a cross between IR29 (susceptible) × Pokkali (tolerant). This QTL, designated as *Saltol*, explained up to 43% of Na^+/K^+ of phenotypic variation for shoot (Gregorio et al., 1997, 2002). In the same region, another important QTL was also detected for shoot K^+ concentration (qSKC1) in a F3 population derived from a cross between salt-tolerant Nona Bokra and a susceptible variety Koshihikari (Lin et al., 2004).

The well-characterized and widely employed donors for salinity adaptation in rice belong to the *indica* subspecies, including Pokkali and Nona Bokra (Thomson et al., 2010; Platten et al., 2013; Reddy et al., 2014; Waziri et al., 2016). However, these genotypes present several undesirable traits such as low yield, photosensitivity, shattering, red pericarp, awn, susceptibility to lodging, and are unadapted to most of the rice growing regions (Gregorio et al., 2002; Puram et al., 2017). Moreover, most of the world's elite rice varieties are produced from inbred varieties developed exclusively from crosses between accessions from the same or related subpopulations (Lu et al., 2005; Ali et al., 2010). This resulted in a significant reduction in genetic diversity for salinity adaptation in cultivated rice, impairing significant improvements and the development of new varieties capable of tolerating soil salinity (Solis et al., 2020).

Developing adapted varieties requires novel genetic diversity, which can be obtained from rice wild relatives (RWR). These species represent a source of unexplored genetic and allelic variation, with great potential for improvement of traits of economic interest, such as tolerance to biotic and abiotic stresses (Wing et al., 2005). Only 10%–20% of the genetic diversity present in wild species is represented in cultivated rice (Stein et al., 2018). Among the RWR, the six species from *O. sativa* complex with AA genomes are commonly employed in the improvement of traits due to the ease of crossing and gene transfer to cultivated rice (Brar & Khush, 2018; Solis et al., 2020).

An efficient approach to introgress qualitative and quantitative traits from landraces and RWR to elite adapted varieties is based on backcrossing. In rice, the use of advanced backcross populations or introgression lines (ILs) has been widely employed in genetic studies. Previously, two populations of interspecific ILs in a common recurrent parent were developed (Arbelaez et al., 2015). The 32 and 48 ILs were derived from crosses between *O. sativa* cv. Curinga and *O. meridionalis* Ng. accession (W2112) (here denominated CM population) and between *O. sativa* cv. Curinga and *O. rufipogon* Griff. accession (IRGC 105491) (CR population), respectively, were employed in this work.

In this study, these two populations were screened under salinity stress aiming at understanding the interspecific genetic variation in adaptation to salt stress. Our specific hypotheses were: (1) novel tolerance or resistance QTLs for salinity excess can be identified in RWR as donors of the traits; (2) *O. meridionalis* and *O. rufipogon* introgressions can confer resistance to salt stress, and (3) RWR introgressions can improve the physiological performance of rice plants under salt stress.

2 | MATERIALS AND METHODS

2.1 | Plant material and salt stress screening

Two populations with a common recurrent parent (*O. sativa* L., cv. Curinga.) were used in this study. The CM population consisting of 32 ILs was generated from crosses of *O. sativa* × *O. meridionalis* (Ng. accession W2112). The CR population with 48 lines was developed from

crosses of *O. sativa* × *O. rufipogon* (Griff accession IRGC 105491) (Arbelaez et al., 2015). Seeds of both populations were originally obtained from Dr. Susan McCouch.

Experiments were conducted in a climate-controlled greenhouse at the Institute of Crop Science and Resource Conservation (INRES) of Bonn University, Germany, from May to July 2018 (first experiment) and from January to March 2019 (second experiment). Natural light was supplemented with artificial light, between 7 am and 9 pm to ensure a minimum photosynthetic photon flux density of 400 μmol m^{-2} s^{-1}. The day/night temperature was set to 28/22°C. Seeds were germinated and seedlings were grown in a mesh floating on solutions containing 0.5 mM $CaCl_2$ and 10 μM $FeCl_3$ for 2 weeks at 28°C. For the salt stress screening, six seedlings of each genotype were transplanted into 60-L containers (n = 8 containers for each treatment) filled with half-strength nutrient solution (Yoshida et al., 1976). After 12 days, nutrient solutions were changed to full strength with the following composition: 2.86 mM NH_4NO_3, 0.32 mM NaH_2PO_4, 1.12 mM K_2SO_4, 1.0 mM $CaCl_2$, 1.65 mM $MgSO_4$•7 H_2O, 9.10 mM $MnCl_2$•4 H_2O, 0.52 mM $(NH_4)_6Mo_7O_{24}$•4 H_2O, 18.5 mM H_3BO_3, 0.150 mM $ZnSO_4$•7 H_2O, 0.150 mM $CuSO_4$•5 H_2O, 35.7 mM Fe-EDTA. The pH was adjusted to 5.5 every 2 days and the nutrient solutions were refreshed every week.

2.2 | First screening experiment for QTL mapping

After 6 days, half of the rice plants (n = 3 per genotype, 4 weeks old plants having four to five leaves) were exposed to 60 mM of sodium chloride (+NaCl). The spectral reflectance between 380 and 1050 nm on the second fully expanded leaf was evaluated at 5 and 16 days after the onset of the treatment, with a PolyPen RP 410 PSI (Photon Systems Instruments, Drasov, Czech Republic). Leaf injury scores as measures of salt stress were evaluated 10 and 16 days after the onset of the treatment in all fully expanded leaves of the main tiller, based on Gregorio et al. (1997). Scores ranged from 0 to 10, where 0 means leaves without salinity injury and 10 means completely dead leaves. Plant materials were harvested at 18 days after the onset of the treatment. Shoot and root length, dry weight, and tiller number were measured. The reduction of shoot and root growth were calculated as relative values = (measurement in stress treatment)/(measurement in control). Shoot samples of the same genotype in stress treatment were pooled for and sodium (Na^+) and potassium (K^+) concentrations were determined, as described below.

2.3 | QTL mapping for salt stress resistance

Chromosome segment substitution lines (CSSL) physical maps were constructed using simple sequence repeat markers, single nucleotide polymorphisms markers from 6K Infinium, and genotype by sequencing platforms (Arbelaez et al., 2015). QTL mapping was carried out using the CSL program in IciMapping v4.1 software (www.isbreeding.net/software/?type = detail&id = 18). Maximum likelihood ratio test based on stepwise regression for additive QTL (RSTEP-LRT-ADD) was calculated with the following parameters: multicollinearity control (−1) and, probability required for entering variables to stepwise regression of residual phenotype on marker variables 0.0001. The threshold [−log $p(F)$] to declare a significant association between the trait and chromosome segment was set based on a permutation test (1000 permutations, $p = 0.01$) for each trait, according to Balakrishnan et al. (2020).

2.4 | In-depth characterization of the most resistant lines (second experiment)

After the screening for salt stress resistance and QTL analysis, the most resistant ILs (lower salinity injury score [SIS] and lower Na shoot concentration) from CR population (named as CR47) and from CM population (CM6) and *O. sativa* cv. Curinga and *O. rufipogon* were selected for further physiological and biochemical analyses. The plants of these genotypes were germinated, cultivated, and submitted to the same conditions as described before. Four replicates were used, each containing eight plants of each genotype. At that time, we were unable to germinate the seeds of *O. meridionalis* to perform the experiment, and could therefore not confirm its phenotype seen in the first experiment.

2.5 | Leaf spectral reflectance measurements

Leaf spectral reflectance was measured on the youngest and second youngest fully expanded leaves with a PolyPen RP410 equipment (n = 8 plants per genotype and n = 4 containers) 4, 11, and 18 days after the onset of the treatment. Each index was calculated as provided below: normalized difference vegetation index:

$$NDVI = (R_{NIR} - R_{RED}) / (R_{NIR} + R_{RED}), \quad (1)$$

where R represents the reflectance at a given wavelength (Huang et al., 2013); normalized pigment chlorophyll index (Peñuelas et al., 1994):

$$NPCI = (R_{680} - R_{430})/(R_{680} + R_{430}); \quad (2)$$

renormalized difference vegetation index (Roujean & Breon, 1995):

$$RDVI = (NIR - RED) / (NIR + RED)^{1/2}; \quad (3)$$

modified chlorophyll absorption in reflectance index (MCARI) (Daughtry et al., 2000) and MCARI1 (Haboudane et al., 2004):

$$MCARI = [(R_{700} - R_{670}) - 0.2 \times (R_{700} - R_{550})] \times (R_{700}/R_{670}), \quad (4)$$

$$MCARI1 = 1.2 \times [2.5 \times (R_{790} - R_{670}) - 1.3 \times (R_{790} - R_{550}); \quad (5)$$

optimized soil-adjusted vegetation index (Rondeaux et al., 1996):

$$OSAVI = (1 + 0.16) \times (R_{790} - R_{670})/(R_{790} - R_{670} + 0.16); \quad (6)$$

triangular vegetation index (Broge & Leblanc, 2001):

$$TVI = 0.5 \times [120 \times (R_{750} - R_{550}) - 200 \times (R_{670} - R_{550}); \quad (7)$$

carotenoid reflectance index I (Gitelson et al., 2002):

$$CRII = \frac{1}{R510} - \frac{1}{R550} \quad (8)$$

photochemical reflectance index:

$$PRI = \frac{R_{531} - R_{570}}{R_{531} + R_{570}}; \quad (9)$$

structure intensive pigment index (Penuelas et al., 1995):

$$SIPI = (R_{790} - R_{450})/(R_{790} + R_{650}); \quad (10)$$

anthocyanin reflectance index 1 (Gitelson et al., 2001):

$$ARI1 = \frac{1}{R_{550}} - \frac{1}{R_{700}}; \quad (11)$$

Carter index 1 (Carter, 1994):

$$Ctr1 = \frac{R695}{R420}; \quad (12)$$

Lichtenthaler index 1 and 2 (Lichtenthaler et al., 1996):

$$Lic1 = \frac{R_{790} - R_{680}}{R_{790} + R_{680}}, \quad (13)$$

$$Lic2 = \frac{R440}{R690}. \quad (14)$$

2.6 | Gas exchange measurements

Gas exchange was measured with a portable photosynthetic gas exchange system Li-Cor 6400-XT (LI-COR, Inc., Lincoln, NE, USA) at 15 days after the onset of the treatment. The first and second fully expanded leaf on the main tiller of each plant were measured between 10 am to 4 pm, according to Wairich et al. (2021). One plant of each genotype per replicate was measured ($n = 4$) in each treatment.

2.7 | Measurements of morphological traits

Lines were harvested 22 days after the onset of the treatment. For each plant, the root and shoot length and dry weight were determined.

2.8 | H_2O_2 staining in leaves

In situ detection of H_2O_2 in leaves was conducted with the upper part of the youngest and second youngest fully expanded leaf according to Wu et al. (2019) after 22 days of treatment. H_2O_2 formation was visualized as brown precipitation, documented by a digital camera Canon 550D (Canon Deutschland GmbH, Krefeld, Germany).

2.9 | Electrolyte leakage measurement

Cell membrane damage by salt stress was evaluated on half of the youngest fully expanded leaves ($n = 4$ per genotype). Briefly, the leaf was cut into small pieces (≈ 4 cm), washed three times with distilled water, and stored in centrifuge tubes filled with 30 mL of milli-Q water. Initial electrical conductivity (EC) was measured at the moment of the harvest (ECi) and at 24 h (ECt) after harvest (ECf). Then, EC was measured after the samples being autoclaved (ECf). The electrolyte leakage was calculated as (Bajji et al., 2002):

$$(ECf - ECi) / (ECt - ECi) \times 100. \quad (15)$$

2.10 | Malondialdehyde assay

Malondialdehyde (MDA) was measured according to Hodges et al. (1999), as modified by Höller et al. (2014) and described in detail in Wairich et al. (2021).

2.11 | Determination of sodium and potassium ion concentrations

Na and K concentrations in LB, culm and sheath (CS), and in roots were determined. Different tissues from *O. sativa* cv. Curinga, CM6 and CR47 were dried at 60°C for 7 d. Dry samples were ground to fine powder with stainless steel spheres followed by digestion as described in Wairich et al. (2021). Na and K concentrations were measured by atomic emission spectrometry (Na: 589 nm; K: 767 nm) (Eppendorf ELEX 6361; Eppendorf, Hamburg, Germany).

2.12 | Statistical analysis

Mean values were compared by the Student's *t*-test ($p \leq 0.05$) using the GraphPad Prism 8 (GraphPad Software) for Windows. Three-way analysis of variance (ANOVA) was used to analyze the effects of treatment, genotype, time, and the interaction on different traits with the program R, packages *nlme* (Cayuela, 2010), and *emmeans* (Russell et al., 2020).

3 | RESULTS

3.1 | ILs showed variable resistance under salt stress

To identify possible genomic introgressions from wild relatives that confer salt stress resistance, 32 ILs from CM population and 48 ILs

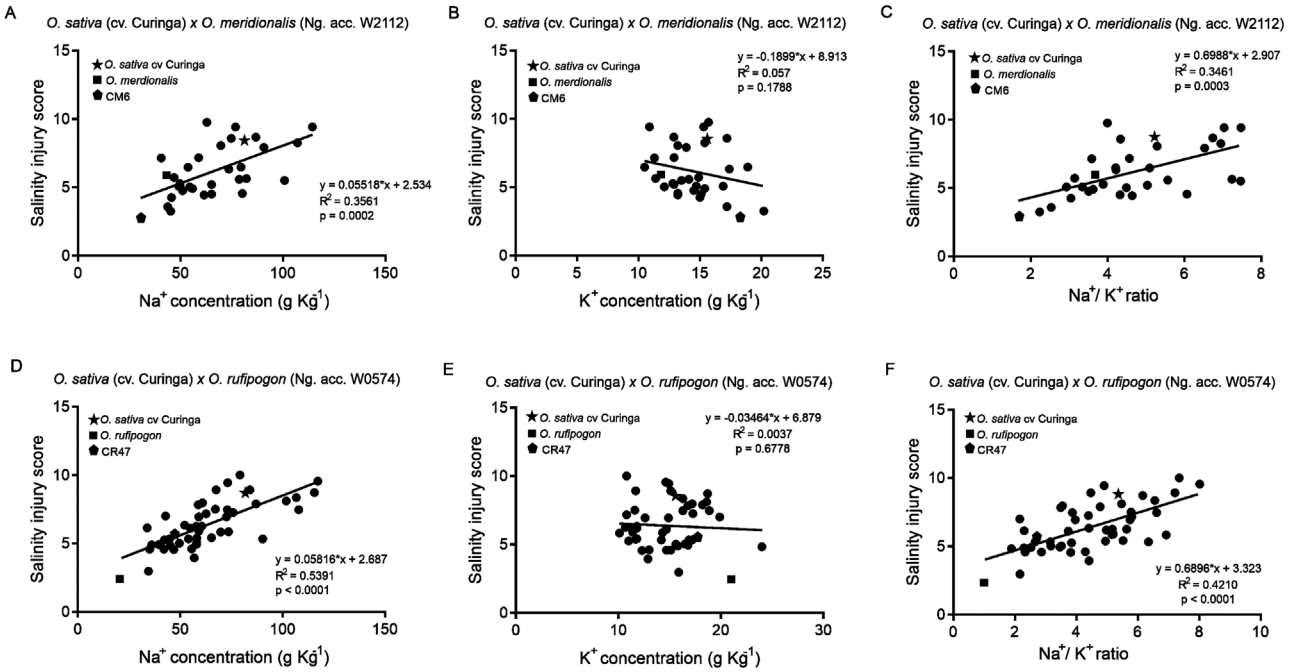

FIGURE 1 Linear regression of salinity injury scores (SIS) and shoot Na^+ and K^+ concentrations in *O. sativa* cv. Curinga × *O. meridionalis* (Ng. acc. W2112) (CM population) and *O. sativa* cv. Curinga × *O. rufipogon* Griff (Ng. acc. W0574) (CR population) exposed to salt stress (60 mM) for 18 days. (A) Linear regression of shoot Na^+ concentration × SIS in CM population. (B) Linear regression of shoot K^+ concentration × SIS in CM population. (C) Linear regression of shoot Na^+/K^+ ratio × SIS in CM population. (D) Linear regression of shoot Na^+ concentration × SIS in CR population. (E) Linear regression of shoot K^+ concentration × SIS in CR population. (F) Linear regression of shoot Na^+/K^+ concentration × SIS in CR population. The star data points represent the parent, *O. sativa* cv. Curinga (A–F). The square data points represent the parent *O. meridionalis* (Ng. acc. W2112) (A–C) and the parent *O. rufipogon* (D–F). The pentagon data points represent tolerant lines with low symptom formation and low Na concentration in shoots CM6 (A–C) and CR47 (D–F). The black data points represent the 32 and 48 ILs, with the exception of CM6 (A–C) and CR47

from CR population were screened under 60 mM NaCl (+NaCl) stress for 18 days (Tables S1, S2, and S3).

In general, lines from the CM population showed significant reduction in biomass and growth-related parameters in the salt stress treatment. The reduction reached up to 90% in shoot dry weight for the wild parent *O. meridionalis* (Figures S1A–S1F). However, the IL CM6 was the only line showing an increase in shoot dry weight under +NaCl (Figure S1D). Eight ILs exhibited significantly lower occurrence of symptoms compared to the recurrent parent, *O. sativa* cv. Curinga, after 18 days of treatment (Figure S2F). Among these lines, CM6 presented lower concentrations of Na^+ and higher concentrations of K^+ in shoot tissue when compared to *O. sativa* (Figures 1A and 1B), showing the lowest Na^+/K^+ ratio observed (Figure 1C).

Similarly, some ILs from the CR population showed high resistance to salt stress in terms of biomass production and growth-related parameters (relative value > 1.0), especially when compared with the phenotypes observed in the CM population (Table S1 and Figures S2A–S2F). This higher resistance was possibly conferred by the wild parent, *O. rufipogon*, which exhibited lower SIS and Na^+ concentration (Figure S2F and Figure 1D), as well high K^+ concentrations in shoot tissue (Figure 1E) compared with *O. sativa* and the 48 ILs from the CR population.

The correlation of SIS with shoot Na^+ and K^+ concentration, and Na^+/K^+ ratio was evaluated in both populations (Figure 1). For the CM population a positive correlation was observed between SIS and Na^+ concentration and between SIS and Na^+/K^+ ratio. Shoot Na^+ concentration and Na^+/K^+ ratio both explained approximately 35% of the observed variation in SIS ($p = 0.0002$ and $p = 0.0003$, respectively) (Figures 1A and 1C). In the CR population, similar results were observed, as shoot Na^+ concentration and Na^+/K^+ ratio explained approximately 54% and 42% of the observed variation in SIS, respectively ($p < 0.0001$ and $p < 0.0001$, respectively) (Figures 1D and 1F). However, no correlation was observed for shoot K^+ concentration and SIS in both populations (Figures 1B and 1E). Taken together, these results indicate that more resistant genotypes, such as CM6 from CM population and *O. rufipogon* and CR47 from CR population, accumulated less Na^+ in shoot tissue, pointing toward a Na exclusion mechanism conferring resistance.

3.2 | QTL analysis revealed regions associated with salt stress resistance in both populations

For the CM population, QTL analysis identified three putative QTLs on chromosome 1, 3, and 5 (Table 1) associated with two reflectance indices at the seedling stage. A major QTL *qNPCId5_3* was identified on chromosome 3, segment 3-3, for measurements performed 5 days after the onset of the treatment, which explained 77.5% of phenotypic variation (PVE) with LOD score of 11. The IL CM6 contained an

TABLE 1 QTLs identified associated with traits evaluated during the screening of the population *O. sativa* cv. Curinga × *O. meridionalis* (Ng. acc. W2112) (LOD > 3.5)

QTL	Trait	Chr	Peak marker position (bp)	Chr segment and interval (bp)	LOD	PVE (%)	Add	Introgression line
qNPCId5_3	Normalized Pigment Chlorophyll Ratio Index	3	12628439	3_3 (11694464–15872706)	11.0	77.5	−5.1	CM6
qPRId16_1	Photochemical Reflectance Index (PRI)	1	22254822	1_5 (20803521–26235041)	9.7	24.0	1.6	CM14
qPRId16_5	Photochemical Reflectance Index (PRI)	5	4877897	5_1 (2378143–6506422)	14.2	51.1	−1.7	CM12 and CM15

QTL: Quantitative trait loci; Chr: chromosome number, LOD: logarithm of the odds; PVE: phenotypic variation explained by QTL; Add: additive effect—denotes the effect of replacing the *O. sativa* allele with the wild allele on the phenotypic value.

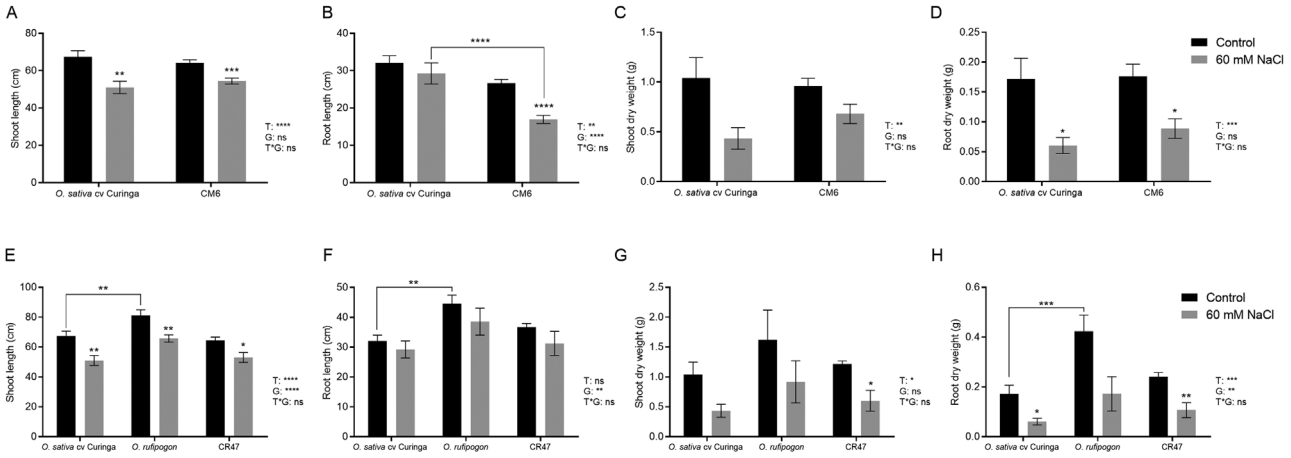

FIGURE 2 Responses of recurrent parent, *O. sativa*, and selected introgression lines exposed to salt stress (60 mM) for 21 days. (A and E) Shoot length (cm); (B and F) root length (cm); (C and G) shoot dry weight (g); (D and H) root dry weight (g). Values are the averages ± SE ($n = 8$). Asterisks indicate statistical difference when comparing control *versus* +NaCl treatment (Student's *t*-test, **p* value < 0.05; ***p* value < 0.01; ****p* value < 0.001; *****p* value < 0.0001). Asterisks above the horizontal bars indicate statistical difference when comparing with *O. sativa* cv. Curinga (Student's *t*-test, ***p* value < 0.01; ****p* value < 0.001; *****p* value < 0.0001)

introgression from *O. meridionalis* in this segment. In addition, two QTLs *qPRId16_1* and *qPRId16_5* were identified for PRI of 16 days posttreatment. These QTLs are located on chromosome 1, segment 1-5, and on chromosome 5 on segment 5-1, and explained 24% and 51% of PVE, respectively (Table 1).

For the CR population, 19 QTLs were identified on chromosomes 1, 2, 3, 4, and 7 (Table 2). For measurements taken 5 days after the onset of the treatment four QTLs were identified. Two QTLs are present on chromosome 1, segment 1-1, for MCARI and PRI, which explained 21.5% and 34.75% of PVE, respectively. Another two QTLs on chromosome 2, *qMCARId5_2* and *qPRId5_2* explained 44.5% and 57% of the phenotypic variation. For the QTLs localized on chromosome 1, CR30 and CR47 carried introgressions from the wild parent, *O. rufipogon*, whereas the ILs CR8, CR36, and CR41 had introgressions from the wild parent in the segment 2-2. For measurements taken 16 days after the onset of the treatment, six QTLs associated with six different traits were identified on chromosome 1. All these QTLs were located on segment 1-1. The same traits that were associated with QTLs on chromosome 1 at 16 days after the onset of the treatment were also associated with QTLs located on chromosome 3, with exception of *qSIPId16_3*, which was exclusively identified on chromosome 3. In addition, one QTL on chromosome 4 and one in chromosome 7 were also identified 16 days after the onset of the treatment.

Based on these results we selected lines CM6 and CR47 for further characterization as they contained at least one QTL (Tables 1 and 2), and as they scored favorably in terms of leaf injury (Figure 1).

3.3 | ILs CM6 and CR47 showed higher resistance to salinity

Salt treatment had effects on biomass traits. Shoot length was significantly reduced by salt treatment in all genotypes (Figures 2A and 2E).

TABLE 2 QTLs identified associated with physiological, morphological and mineral traits evaluated during the screening of the population *O. sativa* cv. Curinga × *O. rufipogon* Griff accession (IRGC 105491) (LOD > 3.5)

QTL	Trait	Chr	Peak marker position (bp)	Chr segment and interval (bp)	LOD	PVE (%)	Add	Introgression line
qMCARId5_1	Modified Chlorophyll Absorption in Reflectance Index	1	616367	1_1 (315225–5026962)	11.4	21.5	3.0	CR30 and CR47
qPRId5_1	Photochemical Reflectance Index	1	616367	1_1 (315225–5026962)	32.8	34.8	5.6	CR30 and CR47
qMACRI1d16_1	Modified Chlorophyll Absorption in Reflectance Index 1	1	616367	1_1 (315225–5026962)	24.6	35.8	1.8	CR30 and CR47
qOSAVId16_1	Optimized Soil-Adjusted Vegetation Index	1	616367	1_1 (315225–5026962)	20.9	33.6	1.0	CR30 and CR47
qMCARId16_1	Modified Chlorophyll Absorption in Reflectance Index	1	616367	1_1 (315225–5026962)	39.6	39.2	5.0	CR30 and CR47
qTVId16_1	Triangular Vegetation Index	1	616367	1_1 (315225–5026962)	24.5	35.8	1.8	CR30 and CR47
qLic1d16_1	Lichtenthaler Index 1	1	616367	1_1 (315225–5026962)	12.2	23.2	0.6	CR30 and CR47
qRDVId16_1	Renormalized Difference Vegetation Index	1	616367	1_1 (315225–5026962)	13.0	24.0	0.8	CR30 and CR47
qMCARId5_2	Modified Chlorophyll Absorption in Reflectance Index	2	6752292	2_2 (5837031–10169097)	17.1	44.6	−3.1	CR8, CR36 and CR41
qPRId5_2	Photochemical Reflectance Index	2	6752292	2_2 (5837031–10169097)	38.0	57.2	−5.0	CR8, CR36 and CR41
qMACRI1d16_3	Modified Chlorophyll Absorption in Reflectance Index 1	3	5329436	3_2 (5124568–10010232)	26.2	42.2	−1.4	CR12 and CR13
qOSAVId16_3	Optimized Soil-Adjusted Vegetation Index	3	5329436	3_2 (5124568–10010232)	21.3	35.2	−0.7	CR12 and CR13
qMCARId16_3	Modified Chlorophyll Absorption in Reflectance Index	3	5329436	3_2 (5124568–10010232)	42.3	50.4	−4.0	CR12 and CR13
qTVId16_3	Triangular Vegetation Index	3	5329436	3_2 (5124568–10010232)	26.2	42.4	−1.4	CR12 and CR13
qLic1d16_3	Lichtenthaler Index 1	3	5329436	3_2 (5124568–10010232)	17.1	43.1	−0.5	CR12 and CR13
qSIPId16_3	Structure Intensive Pigment Index	3	5329436	3_2 (5124568–10010232)	16.5	47.3	−0.4	CR12 and CR13
qRDVId16_3	Renormalized Difference Vegetation Index	3	5329436	3_2 (5124568–10010232)	18.2	45.0	−0.8	CR12 and CR13
qSIPId16_4	Structure Intensive Pigment Index	4	31162642	4_7 (30119566–35824355)	7.6	13.5	0.2	CR4 and CR34
qMCARId16_7	Modified Chlorophyll Absorption in Reflectance Index	7	12945	7_1 (12955–3973986)	13.0	2.4	−0.9	CR35 and CR44

Abbreviations: QTL: Quantitative trait loci; Chr: chromosome number, LOD: logarithm of the odds; PVE: phenotypic variation explained by QTL; Add: additive effect—denotes the effect of replacing the *O. sativa* allele with the wild allele on the phenotypic value.

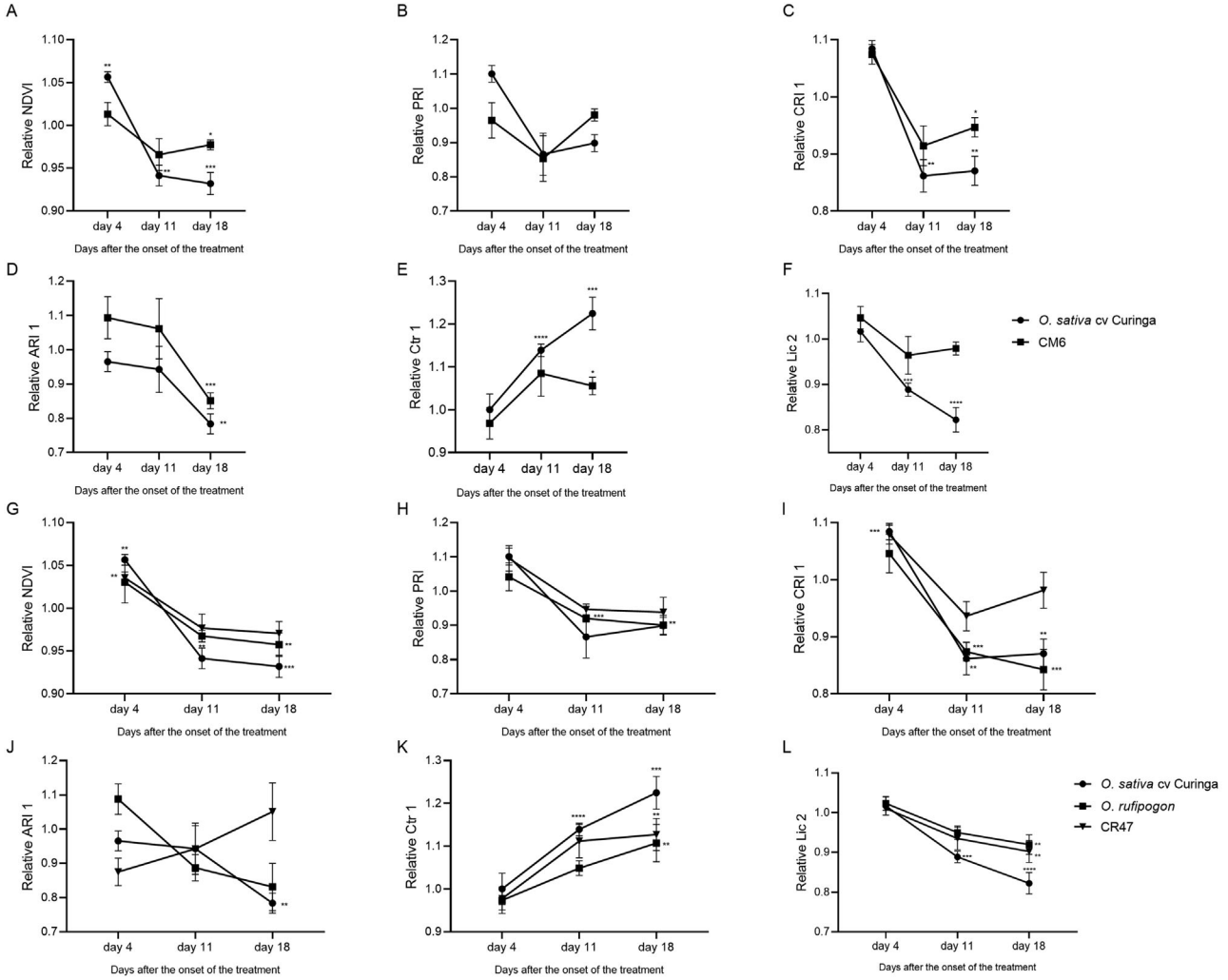

FIGURE 3 Leaf spectral reflectance indices of introgression lines under control and salt stress. (A and G) Relative normalized difference vegetation index (NDVI); (B and H) relative photochemical reflectance index (PRI); (C and I) relative carotenoid reflectance index 1 (CRI 1); (D and J) relative anthocyanin reflectance index 1 (ARI 1); (E and K) relative Carter index 1 (Ctr 1); (F and L) relative Lichtenthaler index 2 (Lic 2). (A–F) Circle and square represent *O. sativa* cv. Curinga and CM6, respectively. (G–L) Circle, square and triangle represent *O. sativa* cv. Curinga, *O. rufipogon* and CR47, respectively. Values are the averages ± SE ($n = 8$). Asterisks indicate statistical difference when comparing control *versus* +NaCl treatment (Student's *t*-test, **p* value < 0.05; ***p* value < 0.01; ****p* value < 0.001; *****p* value < 0.0001)

However, despite the significant reduction in shoot length, shoot dry weight was not affected by salt treatment, with exception of CR47 (Figures 2C and 2G). In the CM population root tissue was more affected by treatment than shoots, since root dry weights were reduced by salinity in all genotypes evaluated (Figure 2D). However, for the CR population, a similar reduction between shoot and root tissue was observed (Figures 2E–2H). Interestingly, the recurrent parent, *O. sativa* cv. Curinga, only presented reduction of shoot length and root dry weight under salt treatment, and the wild parent *O. rufipogon* only reduced shoot length, reinforcing the salt resistance of this wild species (Figure 2).

3.4 | Selected ILs showed enhanced vegetation indices under salt stress

Leaf spectral reflectance indices were employed to quantify the symptoms of the ILs when submitted to salt stress. In both populations, the majority of indices showed a significant treatment and time effect, while genotypic effects were observed only for a smaller number of indices (Table S4). For both populations, salt treatment significantly reduced the NDVI at the end of the treatment period, with the exception of the IL CR47, indicating a reduction in chlorophyll content in plants under salt treatment when compared with plants under control conditions (Figures 3A and 3G). Changes in xanthophyll pigments, as an indicator of photosynthetic light use efficiency, are represented by PRI, which was only significantly affected by salt treatment in the wild parent, *O. rufipogon* (Figures 3B and 3H). Both indices associated with the leaf pigments carotenoids (CRI1) and anthocyanin (ARI1), showed a significant reduction by salt treatment, starting from 11 days after the onset of treatment for CRI1 and in 18 days for ARI1 (Figures 3C and 3D). On the other hand, the IL CR47 did not show differences for CRI1 and ARI1 (Figures 3I and 3J). For Ctr1 index, in general all lines presented an increase under salt treatment (Figures 3E and 3K). The

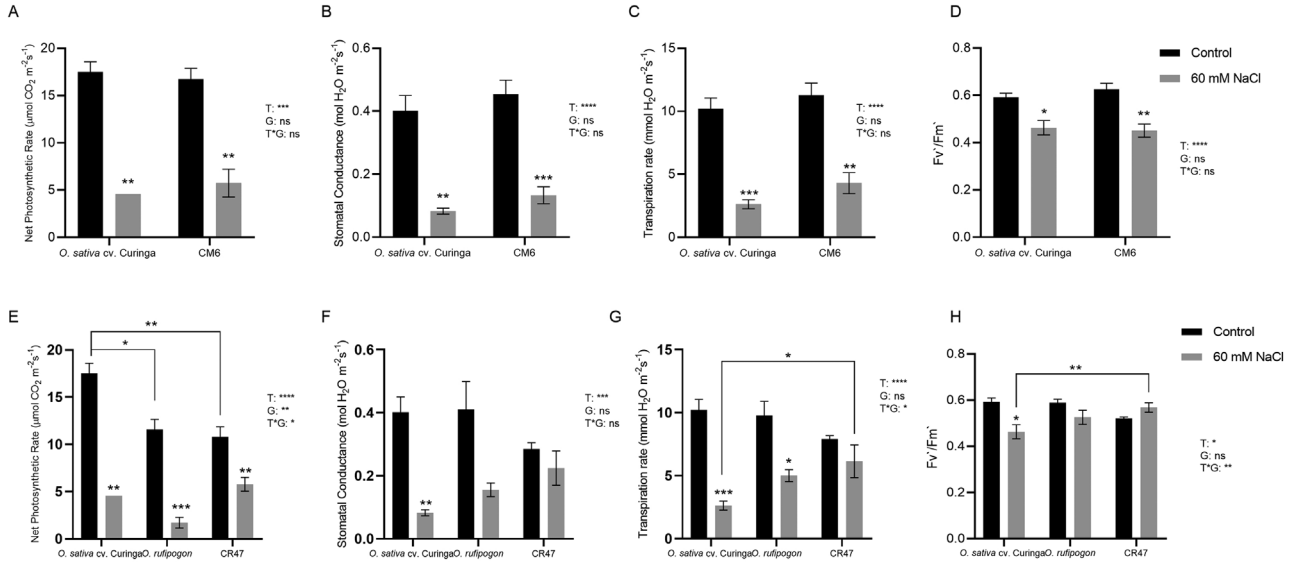

FIGURE 4 Responses of gas exchange, photosynthetic activity, and chlorophyll fluorescence in recurrent and wild parent and introgression lines from both populations exposed to salt stress (60 mM) for 15 days. (A and E) net photosynthetic rate; (B and F) stomatal conductance; (C and G) transpiration rate; (D and H) photosystem II efficiency. Values are the averages ± SE ($n = 4$). Asterisks indicate statistical difference when comparing control *versus* +NaCl treatment (Student's *t*-test, **p* value < 0.05; ***p* value < 0.01; ****p* value < 0.001). Asterisks above the horizontal bars indicate statistical difference when compared with *O. sativa* cv. Curinga (Student's *t*-test, **p* value < 0.05; ***p* value < 0.01)

opposite pattern was observed when evaluating the reflectance ratio of blue/red light with the exception of CM6, all genotypes showed a significant decrease of relative values of Lic2 under salt treatment (Figures 3F and 3L). In summary, resistance of the two selected lines was demonstrated by the fact that CM6 from CM population showed the highest relative values for NDVI, PRI, CRI1, and Lic2, and CR47 from CR population did not differ from control and salt treatment in the majority of the indices evaluated.

3.5 | CR47 maintained relatively high gas exchange under +NaCl

For all tested genotypes, salt treatment had significant effects on photosynthetic parameters (Figure 4). *O. sativa* cv. Curinga and CM6 showed a strong inhibition of net photosynthetic rate, stomatal conductance, leaf transpiration rate, and photosystem II efficiency under salt stress (Figures 4A–4D). On the other hand, the wild parent, *O. rufipogon*, and the IL CR47 did not show a decrease in stomatal conductance, and photosystem II efficiency under +NaCl (Figures 4E–4H). Taken together, these results suggest that the IL CR47 was less affected by salt treatment in terms of gas exchange measurements.

3.6 | CM6 showed less damage to cell membrane and reduced oxidative stress under +NaCl treatment

Measurements of electrolyte leakage of leaves harvested from plants cultivated in control and salt stress conditions were taken after 21 days of treatment to evaluate injuries to the membranes by electrolyte leakage. CM6 showed lower damage to the membranes when plants were cultivated under salt stress, although there were no differences to the recurrent parent under control conditions (Figure 5A). *O. sativa* cv. Curinga and CR47 showed a significant increase of electrolyte leakage after 24 h when plants were cultivated under salt stress, but CM6 did not (Figure 5A).

Lipid peroxidation caused by salinity was evaluated by shoot MDA concentration measurement. Significant treatment and genotypic effects were observed (Figure 5B). Although CM6 showed a significant increase of MDA in the treatment, this value was significantly lower than that of the recurrent parent.

The results from DAB staining further confirmed the resistance of the two selected ILs. In the control, no differences for the H_2O_2 generation between all genotypes were observed (Figure 5C). More abundant brown precipitates, as an indicator of H_2O_2 production, were observed in the leaves of plants cultivated under +NaCl treatment, especially the IL CR47 (Figure 5C). Taken together, these results reinforce enhanced resistance to salt stress presented by CM6.

3.7 | Mineral analyses

Na^+ and K^+ concentrations were analyzed in three different tissues, LB, CS, and roots to determine the compartmentalization of these elements. ANOVA showed significant treatment and genotypic effects on the Na^+ and K^+ concentrations in shoot tissues (Figure 6). However, for root tissue, only the treatment effect was highly significant, with the exception of K^+ concentration. The salt stress treatment significantly increased Na^+ concentration in both shoot organs, that is, LB and CS (Figures 6A and 6B). Both CM6 and CR47 stored significantly

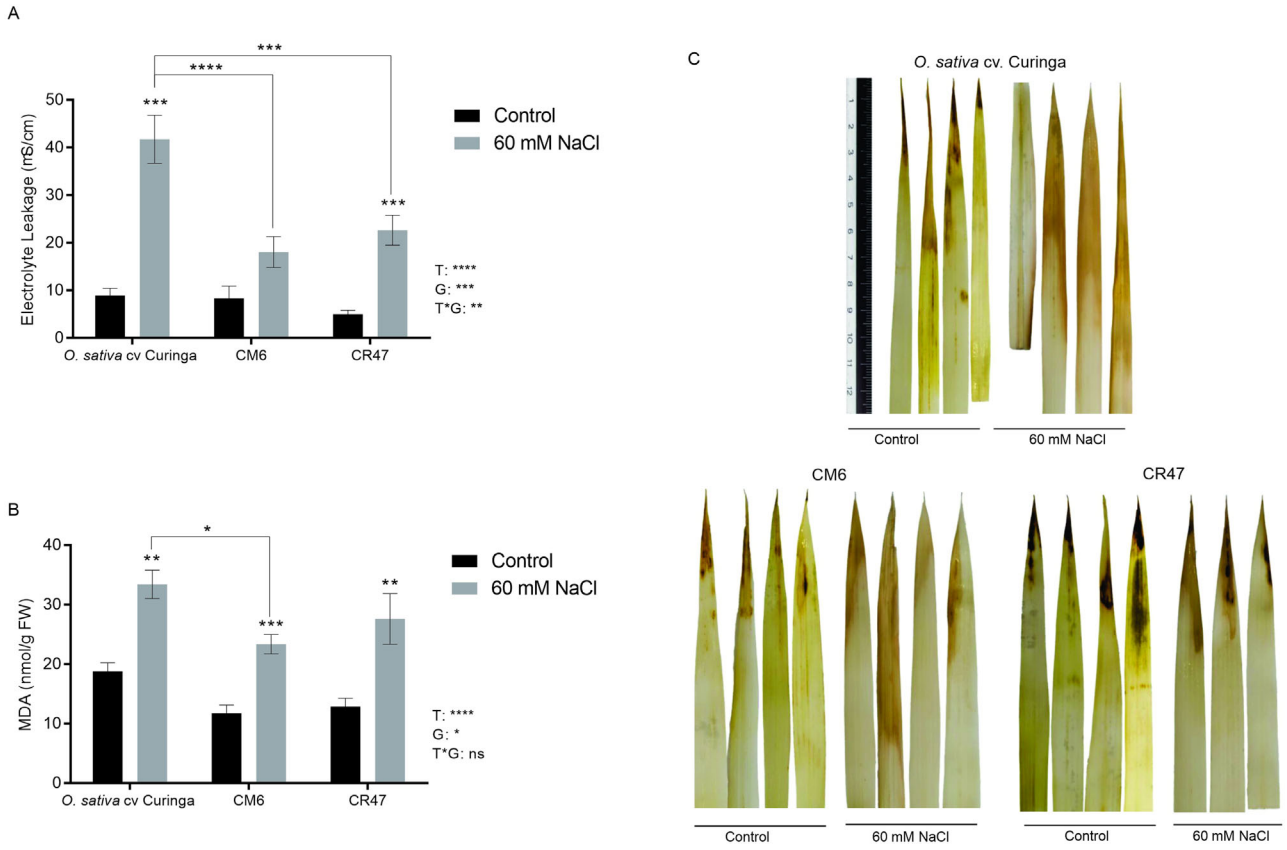

FIGURE 5 Physiological analysis of the tolerance to salt stress. (A) Electrolyte leakage in leaves; (B) shoot MDA concentration; (C) hydrogen peroxide staining using 3,3′−5 diaminobenzidine of recurrent parent and selected introgression lines CM6 and CR47 submitted to control and NaCl toxicity for 21 days ($n = 4$). (A and B) Bars represent the averages ± SE ($n = 4$). Asterisks indicate statistical difference when comparing control *versus* +NaCl treatment (Student's *t*-test, $^{**}p$ value < 0.01; $^{***}p$ value < 0.001). Asterisks above the horizontal bars indicate statistical difference when compared with *O. sativa* cv. Curinga (Student's *t*-test, $^{*}p$ value < 0.05; $^{***}p$ value < 0.001; $^{****}p$ value < 0.0001)

less Na^+ in LB when compared with the recurrent parent (Figure 6A). In addition, despite the decrease of K^+ concentration in the LB, CM6 maintained a significantly higher value than *O. sativa* (Figure 6D). These results are in agreement with the Na^+/K^+ ratio in LB (Figure 6G). However, when evaluating the Na^+ concentration in CS, CM6 accumulated almost twice as much as in the LB, in contrast to *O. sativa* and CR47 that accumulated similar concentrations in both tissues (Figures 6A and 6B). The K^+ concentration in CS was significantly lower in all genotypes under salt stress, however, both ILs presented significantly higher concentrations when compared to *O. sativa* (Figure 6E). Na^+ and K^+ concentrations in roots significantly increased and decreased, respectively, in all genotypes evaluated under salt stress (Figures 6C and 6F). No genotypic differences were observed for measurements in this tissue, indicating that resistance was associated with shoot tissue.

Taken together, these results suggest that the ILs CM6 and CR47 and the wild rice, *O. rufipogon*, are resistant to salinity stress. In addition, the mechanism underlying resistance of the ILs is possibly different, since CR47 showed the lowest reduction in photosynthesis but more damage associated to cellular membranes and higher production of ROS than CM6. On other hand, CM6 showed the lowest Na^+/K^+ ratio in shoot tissue, especially in LB.

4 | DISCUSSION

4.1 | Introgressions from wild rice as source of resistance to salt stress in *O. sativa*

Using two populations of interspecific ILs derived from crosses between *O. sativa* × *O. meridionalis* and *O. sativa* × *O. rufipogon* (Arbelaez et al., 2015) was justified by the fact that both wild species are part of the primary gene pool, in which all species have the AA genome compatible for crosses with *O. sativa* (Brar and Khush, 2018). In addition, the natural occurrence and previously reported adaptation to abiotic stress of these RWR also supported the use of these populations. *O. rufipogon* is distributed in South and Southeast Asia and is commonly grown in swamps, marshes, swampy grasslands, and in deep water rice fields (Brar & Khush, 2018). This species has been used for breeding salinity-tolerant lines (Tian et al., 2011; Ganeshan et al., 2016; Wang et al., 2017; Prusty et al., 2018; Quan et al., 2018). Similarly, *O. meridionalis* is found across Northern Australia from the Kimberley region in Western Australia to Queensland, and New Guinea and is known to tolerate heat and drought in extreme temperature conditions (Scafaro et al., 2010; Brar & Khush, 2018; Yichie et al., 2018).

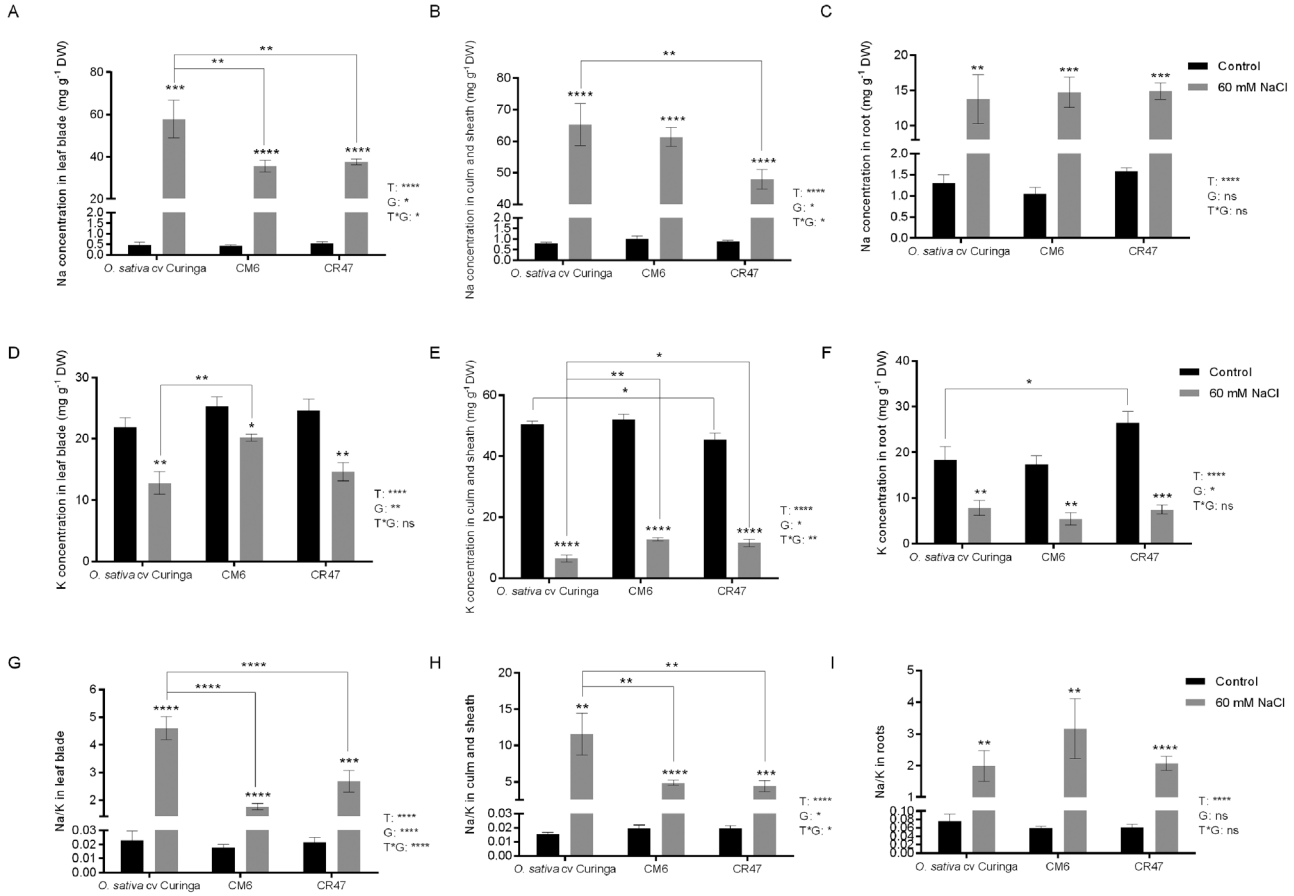

FIGURE 6 Na^+ and K^+ concentrations and Na^+/K^+ ratio in three different organs of *O. sativa* cv. Curinga, CM6 and CR47 exposed to control conditions and to salt stress (60 mM) for 21 days ($n = 4$). (A) Na^+ concentration in leaf blade (mg g^{-1} DW); (B) Na^+ concentration in sheath and culm (mg g^{-1} DW); (C) Na^+ concentration in roots (mg g^{-1} DW). (D) K^+ concentration in leaf blade (mg g^{-1} DW); (E) K^+ concentration in sheath and culm (mg g^{-1} DW); (F) K^+ concentration in roots (mg g^{-1} DW). (G) Na^+/K^+ concentration in leaf blade; (H) Na^+/K^+ ratio in sheath and culm; (I) Na^+/K^+ concentration in roots. Asterisks indicate statistical difference when comparing control *versus* Na^+ treatment (Student's *t*-test, **p* value < 0.05; ***p* value < 0.01; ****p* value < 0.001; *****p* value < 0.0001). Asterisks above the horizontal bars indicate statistical difference when compared with *O. sativa* cv. Curinga (Student's *t*-test, **p* value < 0.05; ***p* value < 0.01; *****p* value < 0.0001)

Rice seedlings were screened under salinity stress for a variety of physiological, morphological, and mineral traits since salt stress influences several processes in plants (Koyama et al., 2001). Resistance to salt stress in the seedling stage based on Na exclusion often relies on the capacity to maintain a low Na^+ concentration or a high K^+ in shoot tissue, and in consequence a low Na^+/K^+ ratio (Yeo & Flowers, 1982; Tester & Davenport, 2003; Munns & Tester, 2008), which was observed for CM6 and CR47. In addition, the correlations between SIS and Na^+ concentrations and SIS and Na^+/K^+ ratio supported these results for both populations (Figure 1). Therefore, in both the populations, resistance seems to be based on Na exclusion mechanisms.

4.2 | Comparison between QTLs detected in this study and those previously reported

We analyzed colocalization of QTLs identified in our study with genes and QTLs that were previously identified (http://qtaro.abr.affrc.go.jp/). For the CM population, the chromosomal segment 3-3 colocalized with genes *OsLEA3-2* and *OsDIS1*. Rice seedlings overexpressing *OsLEA3-2* showed a stronger growth performance under salinity and a better recovery post drought stress (Duan & Cai, 2012). On the other hand, *OsDIS1* negatively regulated drought response by modulating the expression of stress-related genes in rice (Ning et al., 2011). A previous study based on a population of 118 recombinant inbred lines from the cross IR4630 × IR15324 (Koyama et al., 2001) reported three significant QTLs associated with Na^+ uptake, Na^+/K^+ ratio, and K^+ concentration that colocalized with segment 1-5. In this same segment, we found the *OsHsfA7* gene, that, when overexpressed, confers tolerance to salt and drought stresses in rice seedlings (Liu et al., 2012). Also, the segment 5-1 contained the gene *OsTZF1*, which can confer salt and drought tolerance in rice plants (Jan et al., 2013).

For the CR population, the QTL associated with segment 1-1 colocalized with *OsamiR393*, a microRNA that negatively regulates salt, alkali, and drought tolerance in rice (Gao et al., 2011; Xia et al., 2012). In addition, in this segment contains *OsHsp17.0* (Zou et al., 2012), *OsPLDα1* (Shen et al., 2011), and *OsDREB2A* (Mallikarjuna et al., 2011), which were involved in salt tolerance. Some of the QTLs identified by Koyama

et al. (2001), associated with Na^+ uptake, Na^+/K^+ ratio, and K^+ concentration, were also identified in CM population, and colocalized with segment 1-1 in CR population. A monosaccharide transporter, *OsGMST1*, induced by salt stress in rice plants (Cao et al., 2011) colocalized with QTLs associated with segment 2-2. In addition, QTLs associated with drought tolerance were identified in a recombinant inbred population (Lafitte et al., 2004; Cui et al., 2008). In a similar way, the QTLs associated with segment 3-2 also colocalized with genes associated with salt and drought response. Lines overexpressing *OsDSM3*, which encodes an inositol 1,3,4-trisphosphate 5/6-kinase, showed significantly increased sensitivity to drought and salt stress (Du et al., 2011). In addition, an isoform of glutamine synthetase, OsGS1;2, a small heat-shock protein, OsHSP17.7, and *OsSDIR1*, associated with drought tolerance was also present in this genomic region (Cai et al., 2009; T. Gao et al., 2011; Sato & Yokoya, 2008). Furthermore, *OsMAPK5*, a positive regulator of drought and cold tolerance, and *RCN1/OsABCG5*, a salt tolerance factor play a role reducing the Na^+/K^+ ratio colocalizing with this segment (*Xiong & Yang*, *2003*; Matsuda et al., 2014). For the QTL associated with segment 4-7, a huge number of QTLs (Courtois et al., 2000; Zhang et al., 2001; Babu et al., 2003; *Zou et al*., *2005*; Qu et al., 2008) and genes (Chou et al., 2014) related to drought tolerance were previously reported, indicating that this is a conserved genomic region for response to this stress. In addition, the same region contained *OsAM1*, a putative potassium efflux antiporter. This gene is induced by salt and the mutant *am1* showed enhanced sensitivity to salinity stress (Sheng et al., 2014). Finally, the segment 7-1 colocalized with *OsCIPK23*, which is induced by drought, ABA and NaCl (Yang et al., 2008). This segment also colocalizes with five QTLs for salt tolerance in a F2 population derived from Nona Bokra × Koshihikari (Lin et al., 2004).

The large extent of colocalization of wild rice introgressions with previously identified regulators of salinity and drought response in *O. sativa* suggests that substitution of genes from cultivated rice for the wild ones, and the possible change of the expression/regulation of these genes could be a potential strategy in breeding salt-resistant rice.

4.3 | The resistance presented by the ILs is possibly conferred by multiple mechanisms

In interpreting our results, it should be considered that symptoms caused by salt stress could be alleviated by silicon (Si), which we did not add to the nutrient solution in this current experiment. Si can alleviate the effects of salinity by blocking apoplastic, transpirational bypass flow of Na^+ from root to shoot, decreasing shoot Na^+ concentration and Na^+/K^+ ratio (Gong et al., 2006; Flam-Shepherd et al., 2018). This response to Si occurs in an organ-specific pattern (Yan et al., 2020). Since Si is a beneficial element but not an essential nutrient for plants, we opted not to add Si to the nutrient solutions in order not to mask subtle genotypic differences in salinity response.

All genotypes included in our second experiment showed a reduction of shoot length under salt stress at the final of the treatment, but no decrease in shoot dry weight was observed (Figure 2).

Only the recurrent parent, *O. sativa* cv. Curinga, and CM6 showed a lower stomatal conductance, suggesting that CR47 experienced less osmotic stress. The osmotic phase of salt stress inhibits growth of young leaves and reduces stomatal conductance of mature leaves (Munns & Tester, 2008).

Vegetation indices represented another line of evidence for increased resistance of CR47 as it showed no significant treatment response for the majority of vegetation indices such as NDVI, estimated at the youngest and second youngest fully-expanded leaves (Figure 3). The retention of high chlorophyll content in the leaves under salt stress is an indication of tissue tolerance (Yeo & Flowers, 1986; Munns et al., 2016;).

Another important mechanism to adapt to salinity stress is Na^+ exclusion from LB and retention in less photosynthetically active tissue, such as CS, while maintaining a low Na^+/K^+ ratio in the cytoplasm (Flowers & Colmer, 2008; Munns & Tester, 2008; Reddy et al., 2017; Munns et al., 2020a). Both ILs accumulated less Na^+ in LB. In addition, the Na^+/K^+ ratio in LB was lower in CM6 than in CR47. A possible explanation for this could be the fact that CM6 did not show decreased shoot dry weight after growth in saline solution, and the decrease in transpiration rate of this IL under salt stress (Figure 4). In addition, CM6, maintained higher Na^+/K^+ ratio in the roots, possibly contributing to avoidance of excess accumulation of Na^+ ions in the leaves.

As a cellular salt stress response, ROS accumulate under salt stress (Apel & Hirt, 2004), which causes damage and oxidation of polyunsaturated fatty acids of cell membranes (Hodges et al., 1999). The lower level of lipid peroxidation observed in CM6 (Figure 5) thus provides another piece of evidence for salt resistance. Taken together, our results indicate that both ILs possess more than one mechanism of salt resistance at the seedling stage.

4.4 | RWR are a promising source of resistance to salt stress

The rich genetic diversity of RWR, in addition to the different natural growth environments and morphological characteristics present in these species, suggests that unexplored salinity tolerance mechanisms may occur (Vaughan et al., 2003; Atwell et al., 2014; Menguer et al., 2017; Brar & Khush, 2018). In this context, the development of ILs from crosses between *O. sativa* and a RWR is an approach for the potential breeding of elite lines.

To date, only a few RWR have been identified as sources of salt adaptation and were utilized in rice improvement (Tian et al., 2011; Ganeshan et al., 2016; Wang et al., 2017; Prusty et al., 2018; Quan et al., 2018). However, a recent screening experiment of one representative of each 24 *Oryza* species reported variable tolerance under 240 mM NaCl, and accessions from *O. rufipogon* and *O. meridionalis* were classified as sensitive (Prusty et al., 2018) probably due to use of distinct accessions of these wild species and a different screening protocol. However, the resistance presented by *O. rufipogon* in our study is in accordance with other previous studies (Tian et al., 2011; Zhou et al., 2016; Solis et al., 2020), making this species an attractive source of

germplasm for salinity resistance breeding. In addition, the use of wild relatives conferring salinity stress adaption to cultivated species has been successfully employed in other species such as for bread wheat (*Triticum aestivum*) from *T. monococcum* (James et al., 2011), and from wild tomato (*Lycopersicon cheesmanii*) for the cultivated tomato *L. esculentum* (Rick & Chetelat, 1995).

Several previous mapping studies employing interspecific crosses of *O. sativa* and *O. rufipogon* reported QTLs for salt tolerance (Tian et al., 2011; Wang et al., 2017; Quan et al., 2018). However, these QTLs did not colocalize with the ones reported in the present study and they were not further characterized regarding their physiological basis.

Besides the fact that information regarding the improvement of salt tolerance in cultivated rice using genes from RWR is limited, only few cultivars developed by the interspecific hybridization of cultivated rice and *O. rufipogon* and *O. nivara* have been released (Ganeshan et al., 2016; Solis et al., 2020).

O. meridionalis is considered a candidate species for tolerance to heat and drought stresses, based on the distribution of its natural habitats (Atwell et al., 2014; Menguer et al., 2017; Brar & Khush, 2018). Tolerance to salinity stress varies widely in this species (Yichie et al., 2018), but no genes or tolerance mechanisms from *O. meridionalis* were further described. Under our growth conditions, the wild parent *O. meridionalis* was moderately resistant. However, one IL, CM6, outperformed both parents, indicating that the introgression from *O. meridionalis* conferred the resistance to salinity to *O. sativa*.

5 | CONCLUSION

Our study indicates that RWR are a novel source of salt resistance traits for rice breeding. As the adaptation to salinity is controlled by multiple genes, the development and use of ILs carrying favorable alleles or chromosome segments from resistant wild relatives is a promising approach toward improving salinity resistance in cultivated rice by traditional breeding methods or biotechnology approaches. In this work, we identified two ILs that outperformed their sensitive parent Curinga. We demonstrated that these ILs employed more than one mechanism of salt resistance at the seedling stage, which is attractive since salt stress impacts on various plant processes at all levels of organization. The use of these favorable traits from *O. meridionalis* and *O. rufipogon* would contribute greatly to the improvement of salt resistant rice cultivars.

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DATA AVAILABILITY STATEMENT

The data that support the findings associated with the QTL analysis of this study are openly available in Rice Diversity at http://www.ricediversity.org/.

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