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Salinity Tolerance and Traits Correlations of Selected Magic *Indica* Rice (*Oryza sativa* L.) Genotypes at Seedling Stage

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Salinity tolerance of MAGIC (Multiparent Advanced Generation Intercross) *indica* population of rice (*Oryza sativa* L.) was investigated at the seedling stage through phenotypic screening, morpho-physiological measurements and SSR analysis. Out of the original 970 MAGIC rice genotypes, 205 genotypes were initially selected based on salinity tolerance. After a second screening, 36 lines were subjected to a final replicated screening along with eight founder lines of MAGIC *indica*, and two checks (FL 478 and IR 29). Ten MAGIC *indica* genotypes (28%) were scored as tolerant and 26 (72%) moderately tolerant. Among the founder lines, IR 45427-2B-2-2B-1-1 scored tolerant, 4 lines scored moderately tolerant and 1 scored susceptible. Relatively, the percent reduction in fresh weight content for shoot biomass was observed to be higher among the genotypes than percent reduction in dry weight content. A reverse trend was observed for percent reduction in root biomass. Genotypes under normal treatment were observed to have longer roots and shoots than salt-stressed treatments. Comparatively, the Na^+-K^+ ratios of selected lines under salt stress were higher than the tolerant check, FL 478 except for IR 95044:4-B-5. A total of 32 SSR markers were used revealing 93 alleles. Twenty markers exhibited locus heterozygosity while five markers were monomorphic. RM 3843 exhibited the highest PIC (0.6489) while RM 315 exhibited the lowest (0.0830). No consistent clustering relationship among the genotypes based on the results of the phenotypic evaluation and Na^+-K^+ ratio values was observed except for IR 95044:4-B-5 and IR 95044:3-B-4 which were closely grouped with the tolerant check FL 478 and the founder line, IR 45427-2B-2-2B-1-1 which exhibits seedling stage salinity tolerance.

Keywords: MAGIC *indica*, morpho-physiological parameters, phenotypic screening, salinity tolerance, SSR analysis

Abbreviations: MAGIC — multiparent advanced generation intercross, SSRs — simple sequence repeats, SNAP — simple nutrient addition program, EC — electrical conductivity, dSm — deciSiemens per meter, SES — standard evaluation score, PIC — polymorphism information content

INTRODUCTION

Salinity, as a plant abiotic stress, ranks as the second most prevalent soil problem next to drought (Gregorio 1997). The deleterious effects of salinity arising through the impairment of plant productivity are often associated with (1) water deficit causing the low water potential of (Ψ) of plant cells; (2) specific ion toxicity, mainly related to excessive sodium (Na^+) and chloride (Cl^-) intake that may cause metabolic dysfunction as well as disturb membrane integrity and function; and (3) nutritional disorder mainly raised from the excessive sodium and chloride ions in the soil that results from the reduced uptake of the essential mineral nutrients (Arzani 2008; Arzani and Ashraf 2016; Munns and Tester 2008). In South and Southeast Asia, approximately 48 million ha can be potentially used for crop production. However, these failed to yield progressive outcomes while the other land portions are left unharnessed due to salinity problems (Phil Rice 2001). Salt stress is a serious problem in rice production since this species is the most sensitive of cereal crops (Munns and Tester 2008). It has been a major setback for rice production in coastal regions in the tropics and in inland areas wherein water irrigation is limited (Thomson et al. 2010). The Philippines has a relatively smaller number of saline-prone areas as compared with other South and Southeast Asia regions. Nevertheless, salinity stress is still one of the main problems encountered in crop production. In the Bicol and Cagayan Valley regions, 70,000 ha of land designated for rice production are distressed by saline water intrusion (Phil Rice 2001). Hence, it is important for

farmers and plant breeders to acquire knowledge on the response mechanisms of crops to salt tolerance, as well as on proper land management activities.

One of the latest innovations for improving rice is employing the multiparent advanced generation intercross (MAGIC) populations in rice breeding program. This involves the intercrossing of multiple founder lines that consist of traditional and modern varieties which are known to possess valuable agronomic traits such as tolerance to various biotic and abiotic stresses, wide adaptability, desired grain quality and high-yield potential. Moreover, salinity tolerance is one of the agronomic traits possessed by two of the founder lines of the MAGIC *Indica* rice population (IRRI 2010) wherein these lines are capable of growing in an environment prone to various salt concentrations such as NaCl, among others (Gregorio et al. 1997).

Nowadays, aside from only employing the morphological and physiological characteristics of rice in studying responses to salinity stress, the molecular basis of salinity tolerance and the genetics of rice can also be explored to further validate the results and to identify desirable alleles for breeding purposes (Mohammadi-Nejad et al. 2008; 2010). Microsatellites, or simple sequence repeats (SSRs) are commonly used DNA markers for genetic diversity and are powerful tools in quantitative loci analysis (Collard et al. 2005; Mohammadi-Nejad et al. 2008). In rice, microsatellites have been employed for classifying the genetic diversities among landraces (Zeng et al. 2004).

The results of this study may contribute in planning future breeding programs for salt tolerant rice genotypes which can be cultivated in saline environments. Moreover, the MAGIC population can be considered as a superior breed of lines of different agronomically significant traits that are valuable for the utilization of genes that may increase rice yield in the long run. Hence, this study was initiated to explore the MAGIC *indica* rice populations for salinity tolerance in screening genotypes at seedling stage based on morphological and physiological criteria; to calculate among these criteria; and to correlate the genotypes using SSR markers.

MATERIALS AND METHODS

Salinity Tolerance Screening at Seedling Stage

Seeds of 970 rice genotypes derived from the MAGIC *indica* rice populations, produced from a 35 eight-way intercross of elite rice founder lines (IRRI 2010) were randomly selected. These, along with two lines as checks (FL-478 as tolerant check and PSB Rc 82 as moderately tolerant check), were collected from the International Rice Research Institute (IRRI) Gene Bank and IRRI salinity tolerance breeding programs.

A sample screening method was adapted from the IRRI Discussion Paper Series No. 22 (Gregorio et al. 1997). For the initial screening, half strength SNAP (Simple Nutrient Addition Program) culture solution (0.5% SNAP A half strength, 0.5% SNAP B half strength, 99% H_2O) was used. Two one-day-old seedlings pre-germinated through soaking were planted in every hole of the seedling float. There were three replicates for each genotype. The set-ups were maintained at 29 °C/21 °C day/night temperature and 70% relative humidity.

Replacement of distilled water in the floats with salt solution [analytical NaCl (Merck KgaA, Germany) mixed into SNAP culture solution] was done 3 d after planting. The solution was set to an EC (electrical conductivity) of 12 dSm^{-1} (deciSiemens per meter) during the first week and EC = 18 dSm^{-1} by increasing the salt concentration after 2 wk. The pH level of the solution was constantly monitored and was maintained at 5.0–5.1 using 1 mole L^{-1} HNO_3 or 1 mole L^{-1} NaOH.

For both EC levels of salinization, the plant's visible symptoms of salt toxicity were scored according to the modified standard evaluation scores (SES) of visual salt injury at seedling stage (Gregorio et al. 1997).

In the final screening for the salt-tolerant lines, the most salt-tolerant lines (lowest SES scores) based on the results of salinity stress reactions at EC = 12 dSm^{-1} and 18 dSm^{-1} were selected. The lines selected from the original 970 genotypes, together with the checks FL 478 (tolerant) and IR 29 (sensitive), and the eight founder lines of MAGIC *indica* population, namely: Fedearroz 50, Sanhuangzhan-2. IRRI 123 (PsB Rc 82), IR-77186-122-2-2-3 (NSIC Rc 158), IR77298-14-1-2-10, IR4630-22-2-5-1-3, IR45427-2B-2-2B-1-1 and Sambha Mahsuri + *Sub1* were grown in Peter's nutrient solution (1g L^{-1} Peter's solution, 200 mg L^{-1} Fe) under the same conditions. Final screening and selection were done in two replicates: 2 salinized solution set-ups; and 2 normal solution set-ups. The first normal solution set-up was the control set-up and was used for physiological Na^+-K^+ ratio analysis. The second normal solution set-up was used for DNA extraction and molecular analysis. Eight founder lines of the MAGIC *indica* population were included in the final screening. FL 478 and IR 29 were used as the tolerant

and susceptible checks, respectively.

Plant Growth and K^+-Na^+ Measurements

For morphological measurements, leaf and root samples from the salinized replicates and the normal solution set-ups were collected, weighed for fresh weights (g) and measured for leaf and root lengths (cm). After incubation, the samples were weighed for their dry weight and the percentage reduction (%) of the samples in fresh weight and dry weight content were computed. Shoot Na^+-K^+ concentrations were measured using the procedure of Gregorio et al. (1997).

Data of SES scores, morphological parameters and the Na^+-K^+ ratio were statistically analyzed using one-way analysis of variance (ANOVA) procedure in the Statistical Analysis Systems (SAS 2000) software.

Molecular Analysis

DNA Extraction and Quantification

Lyophilized leaf samples were finely cut and placed in eppendorf tubes with 1–2 steel balls. Using the GenoGrinder (CentriPrep, USA) to pulverize the leaf tissues, the samples were shaken for 45 min at 500 rpm. The resulting powder was added with 800 µL 2X CTAB preheated at 65 °C. Then, the products were incubated at 65 °C for 30–60 min followed by addition of 800 µL of 24:1 chloroform:isoamyl alcohol. The tubes were then centrifuged at full speed (12,000 rpm) for 10 sec. The resulting upper (aqueous) phase was decanted into 1.1 µL deep well plates followed by addition of 600 µL isopropanol. The plate was then stored for 12 h at -20 °C followed by centrifugation at 2,500 rpm for 20 min. The resulting pellets were washed with 70% EtOH, then dried and eventually re-suspended in 200 µL 1X TE buffer.

Quantification of DNA of the selected genotypes along with the standard lambda (λ) DNA and 1 kb ladder was done using 1% agarose gel run at 150 V for 1 h. The gel was stained using silver nitrate and was viewed using the Alpha Imager (Alpha Innotech Inc.). Quantified DNA samples were diluted to 50 ng μL^{-1} and were stored in 96-well plates that were maintained at -20 °C.

DNA Amplification through Polymerase Chain Reaction (PCR)

Saltol markers, including 32 SSR markers, were selected from chromosomes 1, 2, 3, 4, 5, 7, 8, 9, 10, 11 and 12 of rice (Table 1). Reactions were conducted in a 20 µL reaction mixture containing 2 µL 10X PCR buffer, 1 µL dNTPs, 1 µL each of the forward and reverse primers, 10.4 µL nanopure sterile water and 0.6 µL $MgCl_2$, 1 µL Taq polymerase and 3 µL of template DNA. Each of the DNA samples was dispensed separately in a Costar 96-microwell PCR plate with a drop of sterile mineral oil.

PCR amplification was performed in G-Storm PCR thermocyclers with an initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55–65 °C for 45 sec and extension at 72 °C for 1 min, followed by final extension for 5 min at 72 °C, wherein the annealing temperature depends on the SSR marker used.

Gel Electrophoresis and Microsatellite Scoring

The amplified DNA fragments were run in 8% polyacrylamide gel electrophoresis for primers that have a ≤250 bp product size and 6% polyacrylamide gel was used for products in which primers have a ≥250 bp product size, along with a 1-Kb ladder. The gels were run in 1X TBE buffer at 120 V for 1–2.5 h depending on the product size.

Allelic bands that were detected for each marker were scored as: 1 – presence of band; 0 – absence of band; NC – negative check. Power Marker version 3.25 (Liu and Muse 2005) was used for the computations of allele frequencies, allele per locus, observed heterozygosity for each locus and polymorphism information content (PIC), as well as in performing the cluster analysis using Nei and Takezaki (1983) similarity index feature and in constructing the phylogenetic map. The assembled phylogenetic map was visualized using the Tree View software. Haplotype analysis was done according to McCartney et al. (2004) and Anderson (2003) as cited by (Mohammadi-Nejad et al. 2008).

RESULTS

Phenotypic Evaluation of Salinity Tolerance

Salinity stress response at seedling stage of 970 rice genotypes of the MAGIC indica population was assessed. The phenotypic responses among the genotypes in the final screening for salinity tolerance at EC = 12 dSm^{-1} and 18 dSm^{-1} varied. The genotypes were clustered into five groups as: highly tolerant (score 1), tolerant (score 3), moderately tolerant (score 5), sensitive (score 7) and highly sensitive (score 9) based on the SES for visual injury. Out of the 970 rice genotypes, 205 genotypes were observed as tolerant, with reference to the tolerant FL 478. After the final

screening of the 205 genotypes, 36 lines showed moderate to high tolerance to salinity stress at EC = 12 dSm^{-1} and EC = 18 dSm^{-1} and were selected together with the two checks and six founder lines for morphological, physiological and genotype analysis using 32 SSR markers, comprising a total of 44 genotypes.

Based on the frequency distribution of the response of the 44 lines to salinity stress in the replicate screening (Fig. 1), 13 genotypes (30%) were scored 3.0 to 3.5 and were noted as tolerant with reference to FL 478. For moderately tolerant, 29 lines (64%) were scored 4 to 5, while one line (2%) was scored 6 and another line (2%), IR 29, was highly sensitive to stress with a score of 9. This indicates that at least two means within the population according to their SES had a significant difference and more than 25% of the population was significantly similar with the mean trait SES of FL 478/IR 29. This was based on the mean SES of the salinized treatments at EC = 18 dSm^{-1} (Table 3).

Comparison of Growth Parameters

Variation was observed among the 44 genotypes for the percent reduction in fresh weight and dry weight content

Table 1. Sequence of 32 microsatellite markers in rice (*Oryza sativa* L.) used in the study and their sequence (adapted from Gramene).

MARKER	CHR. NO.	PRIMER		PRODUCT SIZE (bp)
		Forward	Reverse	
RM 315	1	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG	133
RM 490	1	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG	101
RM 1287	1	GTGAAGAAAGCATGGTAAATG	CTCAGCTTGCTTGTGGTTAG	162
RM 10694	1	TTTCCCTGGTTTCAAGCTTACG	AGTACGGTACCTTGATGG-TAGAAAGG	194
AP 3206f	1	GCAAGAATTAATCCATGTGAAA-GA	ATGCTCTGGCTCCCTCAAG	167
RM 3412b	1	TCATGATGGATCTCTGAGGTG	GGGAGGATGCACTAATCTTTC	110
RM 10748	1	CATCGGTGACCACCTTCTCC	CCTGTCATCTATCTCCCTCAAGC	95
RM 493	1	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG	211
RM 10793	1	GACTT-GCCAACTCCTTCAATTCG	TCGTCGAGTAGCTTCCCTCTCTACC	123
RM 562	1	CACAACCCACAAACAGCAAG	CTTCCCCCAAAGTTTTAGCC	243
RM 7075	1	TATGGACTGGAGCAAACCTC	GGCACAGCACCAATGTCTC	155
RM 10745	1	TGACGAATTGACACACCGAG-TACG	ACTTCACCGTCGGCAACATGG	188
RM 10764	1	AGATGTCGCCTGATCTT-GCATCG	GATCGACCAGGTTGCATTAACAGC	237
RM 140	1	TGCCTCTTCCCTGGCTCCCCTG	GGCATGCCGAATGAAATGCATG	261
RM 495	1	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	159
RM 13197	2	AAACCTCCGGCTCATTCTTGC	ACTCGAATCGTATCGGCTTGAGG	183
RM 127	4	GTGGGA-TAGCTGCGTCGCGTCG	AGGCCAGGGTGTTGGCATGCTG	223
RM 3843	4	ACCCTACTCCCAACAGTCCC	GGGGTCGTACGCTCATGTC	172
RM 178	5	TCGCGTGAAAGATAA-GCGGCGC	GATCACCGTTCCCTCCGCCTGC	131
RM 125	7	ATCAGCAGCCATGG-CAGCGACC	AGGGGATCATGTGCCGAAGGCC	147
RM455	7	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC	144
RM 152	8	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG	157
RM 44	8	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	132
RM 433	8	TGCGCTGAACTAAACACAGC	AGACAAACCTGGCCATTCAC	248
RM 105	9	GTCGTCGACCCATCG-GAGCCAC	TGGTCGAGGTGGGGATCGGGTC	141
RM 24330	9	AATCCGCGGGAGCAATCAACC	CGATGACCAATGACGAGGTGAGG	86
RM 171	10	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG	347
RM 536	11	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC	247
RM 144	11	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTG-CATG	295
R M 19	12	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	250
RM 28102	12	CACTAATTCTTCGGCTCCAC-TTTAGG	GTGGAAGCTCCGAGAAAGTGC	169

Retrieved February 2012, from Gramene Marker View or http://www.gramene.org/

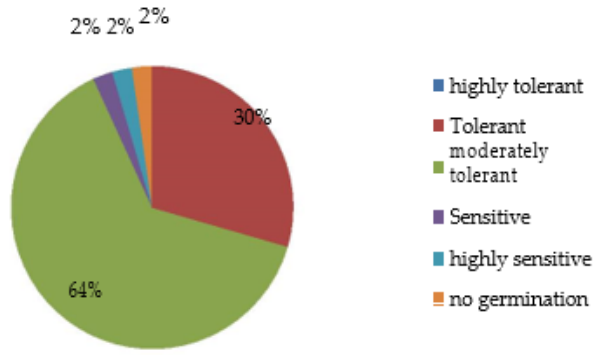

Fig. 1. Frequency distribution of the response to salinity stress of 46 rice (*Oryza sativa* L.) genotypes in the replicate screening.

for shoot biomass. It was noted that IR 29 had high percent reduction in biomass (76.82%) as compared to the tolerant FL 478 (42.35%) (Fig. 2). There was no consistent relationship between the SES of the genotypes and percent reduction in shoot biomass for fresh weight and dry weight content. Percent reduction in fresh weight content was observed to be higher among the genotypes than percent reduction in dry weight content.

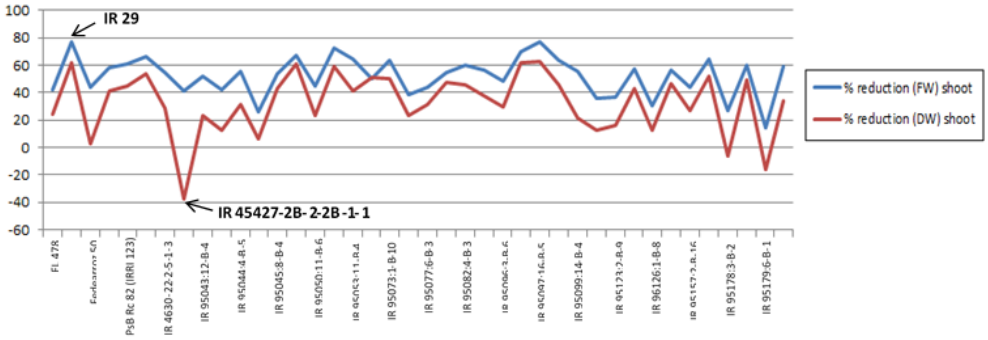

Fig. 2. Percent reduction in shoot biomass based on fresh weight and dry weight content of 44 rice (*Oryza sativa* L.) genotypes.

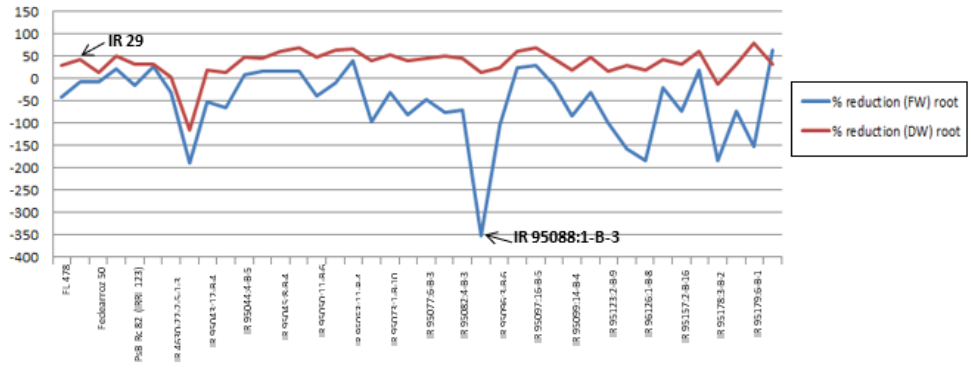

Fig. 3. Percent reduction in root biomass based on fresh weight and dry weight content of 44 rice (*Oryza sativa* L.) genotypes.

For the root biomass, percent reduction among the genotypes in dry weight content was higher than fresh weight content. The magnitude of reduction also varied. There was also no correlation between the SES and the percent reduction. However, IR 29 also had a high percent reduction for root biomass based on dry weight content (42%). The lowest percent reduction obtained was -351.95% in IR 95088:1-B-3 for fresh weight content, with SES of 4.0 (Fig. 3).

Genotypes under the normal treatment were observed to have relatively longer shoots (ranging from 41.0 to 63.0) and root lengths (ranging from 14.250 to 35.0) compared to lines under salinized treatments for shoot (ranging from 22.750 to 40.750) and root (ranging from 13.750 to 23.250) lengths. Under salinized treatment, at least two means within the population based on shoot and root lengths had significant difference, and with reference to the mean trait of FL 478 or IR 29, almost 75% of the population was significantly similar (Table 2). For the normal set-up, at least two means within the population in terms of shoot and root lengths varied significantly. Almost 75% of the population was significantly similar within the mean shoot and root length of FL 478 or IR 29 (Table 3).

For Na^+-K^+ ratio, FL 478 and IR 29 had comparable values of 0.1972 and 0.1812, respectively, under the normal set-up, while for the salinized treatment, Na^+-K^+ ratio values of FL 478 (0.6917) and IR 29 (3.007) varied significantly. The Na^+-K^+ ratio values of the genotypes under normal treatment were lower than the Na^+-K^+ ratio under salinized treatment. Among the 43 rice genotypes, two accessions (Sanhuangzhan-2 and IR 95044:4-B-5) had comparable Na^+-K^+ ratio values with the tolerant FL 478. With reference to FL 478 as the tolerant check, almost 75% of the

Table 2. Mean comparisons of genotypes of rice (*Oryza sativa* L.) across all shoot and root growth traits analyzed for the salinized treatment at α = 0.05.

Geno-type No.	Designation	SES Score	Shoot Length Mean	Root Length Mean	Shoot K^+-Na^+ Ratio Value Mean
1	FL 478	3.5^{cd}	40.750^{ab}	17.250^{e-k}	0.6917^{k-l}
2	IR 29	9.0^{a}	23.750^{no}	13.750^{l}	3.0070^{a-c}
3	Fedearroz 50	6.0^{b}	31.000^{e-m}	14.250^{j-l}	2.8806^{a-f}
4	Sanhuangzhan-2	5.0^{cb}	36.500^{a-f}	15.500^{h-l}	0.6828^{k-l}
5	PSB Rc 82 (IRRI 123)	5.0^{cb}	29.250^{i-n}	17.750^{d-j}	1.5284^{c-l}
6	IR 77298-14-1-2-10	5.0^{cb}	31.250^{d-l}	15.750^{g-l}	-
7	IR 4630-22-2-5-1-3	5.0^{cb}	25.000^{m-o}	14.000^{k-l}	1.2328^{f-l}
8	IR 45427-2B-2-2B-1-1	3.0^{d}	37.250^{a-d}	16.250^{g-l}	1.2170^{f-l}
9	IR 95043:12-B-4	5.0^{b}	30.000^{g-m}	13.750^{l}	0.8449^{j-l}
10	IR 95044:3-B-4	3.0^{d}	28.250^{k-o}	21.250^{a-c}	0.8902^{h-l}
11	IR 95044:4-B-5	4.0^{cd}	34.000^{c-k}	14.000^{k-l}	0.5367^{l}
12	IR 95045:8-B-2	4.0^{cd}	41.750^{a}	21.250^{a-c}	1.1933^{b-l}
13	IR 95045:8-B-4	3.0^{d}	37.250^{a-d}	21.750^{ab}	1.5648^{c-l}
14	IR 95049:2-B-2	3.0^{d}	32.500^{c-l}	23.250^{a}	1.2664^{e-l}
15	IR 95050:11-B-6	5.0^{cb}	35.000^{b-i}	17.000^{e-l}	2.3325^{a-k}
16	IR 95052:3-B-2	5.0^{cb}	29.750^{h-n}	21.750^{ab}	2.3187^{a-k}
17	IR 95053:11-B-4	3.0^{d}	37.250^{a-d}	19.000^{b-g}	1.0586^{h-l}
18	IR 95073:1-B-6	3.0^{d}	35.000^{b-i}	16.250^{g-l}	1.2713^{e-l}
19	IR 95073:1-B-10	3.0^{d}	36.250^{a-f}	17.000^{e-l}	0.7556^{j-l}
20	IR 95076:12-B-4	4.0^{cd}	35.500^{b-h}	16.750^{f-l}	2.9789^{a-d}
21	IR 95077:6-B-3	4.0^{cd}	27.500^{l-o}	18.500^{b-h}	2.8044^{a-f}
22	IR 95078:8-B-1	5.0^{cb}	34.500^{c-j}	20.250^{a-e}	3.6274^{a}
23	IR 95082:4-B-3	5.0^{cb}	34.750^{b-j}	19.000^{b-g}	2.4880^{a-j}
24	IR 95088:1-B-3	4.0^{cd}	38.250^{a-c}	17.000^{e-l}	3.2061^{a-c}
25	IR 95096:3-B-6	5.0^{cb}	30.750^{f-m}	17.500^{e-j}	2.0840^{a-l}
26	IR 95056:2-B-5	4.0^{cd}	32.250^{c-l}	20.000^{a-f}	3.0726^{a-c}
27	IR 95097:16-B-5	5.0^{cb}	28.250^{k-o}	19.750^{b-f}	1.5577^{c-l}
28	IR 95099:9-B-4	4.0^{cd}	30.000^{g-m}	18.250^{c-h}	2.1723^{a-l}
29	IR 95099:14-B-4	5.0^{cb}	30.750^{f-m}	21.250^{a-c}	1.8493^{b-l}
30	IR 95106:6-B-5	4.0^{cd}	28.750^{j-o}	14.500^{h-l}	1.8493^{c-l}

Table 2. Mean comparisons of genotypes of rice (*Oryza sativa* L.) across all shoot and root growth traits analyzed for the salinized treatment at α = 0.05.

Genotype No.	Designation	SES Score	Shoot Length Mean	Root Length Mean	Shoot K^+-Na^+ Ratio Value Mean
31	IR 95123:2-B-9	4.0^{cd}	41.750^{a}	18.000^{c-h}	1.2163^{f-l}
32	IR 95126:1-B-4	4.0^{cd}	37.000^{a-e}	19.000^{b-g}	2.3004^{a-k}
33	IR 95126:1-B-8	3.0^{d}	36.500^{a-f}	19.000^{b-g}	2.5296^{a-i}
34	IR 95126:1-B-10	5.0^{cb}	36.000^{a-g}	16.750^{f-l}	3.4450^{ab}
35	IR 95157:2-B-16	5.0^{cb}	32.000^{d-l}	16.000^{g-l}	2.1321^{a-l}
36	IR 95160:3-B-16	5.0^{cb}	34.000^{c-k}	18.750^{b-h}	1.1936^{f-l}
37	IR 95178:3-B-2	5.0^{cb}	30.000^{g-m}	17.750^{d-i}	2.5605^{a-h}
38	IR 95179:2-B-3	3.0^{d}	34.750^{b-j}	17.000^{e-l}	2.7597^{a-g}
39	IR 95179:6-B-1	3.0^{d}	38.250^{a-c}	18.000^{c-h}	2.1535^{a-l}
40	IR 95182:3-B-1	4.0^{cd}	29.500^{h-n}	20.000^{a-f}	1.2708^{e-f}
41	IR 95182:7-B-1	4.0^{cd}	34.500^{c-j}	19.750^{b-f}	1.3018^{d-l}
42	IR 95182:11-B-4	4.0^{cd}	22.750^{o}	17.000^{e-l}	1.6054^{c-l}
43	IR 95183:7-B-7	3.0^{d}	33.750^{c-k}	21.000^{a-d}	1.0697^{g-l}
44	IR 95182:7-B-1	4.0^{cd}	34.500^{c-j}	19.750^{b-f}	1.3018^{d-l}
		LSD = 1.6017	LSD = 6.2047	LSD = 3.4607	LSD = 1.6972

Means with the same superscript letter are not significantly different.

population was significantly different with the mean Na^+-K^+ ratio of FL 478 or IR 29.

Genetic Diversity Using SSR Markers

A total of 32 markers for chromosomes 1, 2, 3, 4, 5, 7, 8, 9, 10, 11 and 12 of rice were used to amplify the 44 selected genotypes. These SSR markers employed are established *Saltol* markers which can be significant for molecular analysis and characterization of the genotypes in the *Saltol* region (Sabouri et al. 2009).

Five markers (RM 3867, RM 315, RM 433 and RM 178) showed monomorphism in banding pattern while the remaining 27 markers were polymorphic. Using the Power Marker version 3.25 (Liu and Muse 2005), a total of 93 alleles were detected which ranged from 1 to 4, with an average of 2.906. Among these, RM 3843, RM 490 and RM 10748 (Fig. 4) manifested the highest number of alleles (4) while RM 178 had the least (Table 4).

The SSR marker, RM 178 was the highest on the frequency of the major allele. Relatively, it was observed that the frequency of the major allele was inversely related to PIC value. PIC values ranged from 0.0830 to 0.6489 with a mean of 0.3611. RM 3843 which had the highest PIC value among the markers can be proven to be superior for genetic diversity analysis of the trait being studied. In addition, RM 490, RM 3412b and RM 493 also yielded high PIC values (PIC ≥ 0.5511) whereas RM 315 showed the lowest PIC value (PIC = 0.0830).

Locus heterozygosity, an indicator of genetic variability, was also observed in the primers. Among the 32 SSR markers, 20 exhibited locus heterozygosity, with a mean of 0.1072 (Table 4).

Cluster Analysis

Genetic diversity among the selected genotypes as evaluated by the primers was established through cluster analysis using the software program Tree View (Eisen 1999). Based on the constructed dendrogram (Fig. 5), genotype FL 478, the tolerant check, was grouped together with IR 45427-2B-2-2B-1-1, which is a MAGIC founder line exhibiting tolerance to seedling stage salt stress. It was also examined that the susceptible IR 29 was closely clustered with FL 478, which may be accounted to FL 478 being a recombinant inbred line from the cross between Pokkali (tolerant) and IR 29 (susceptible)

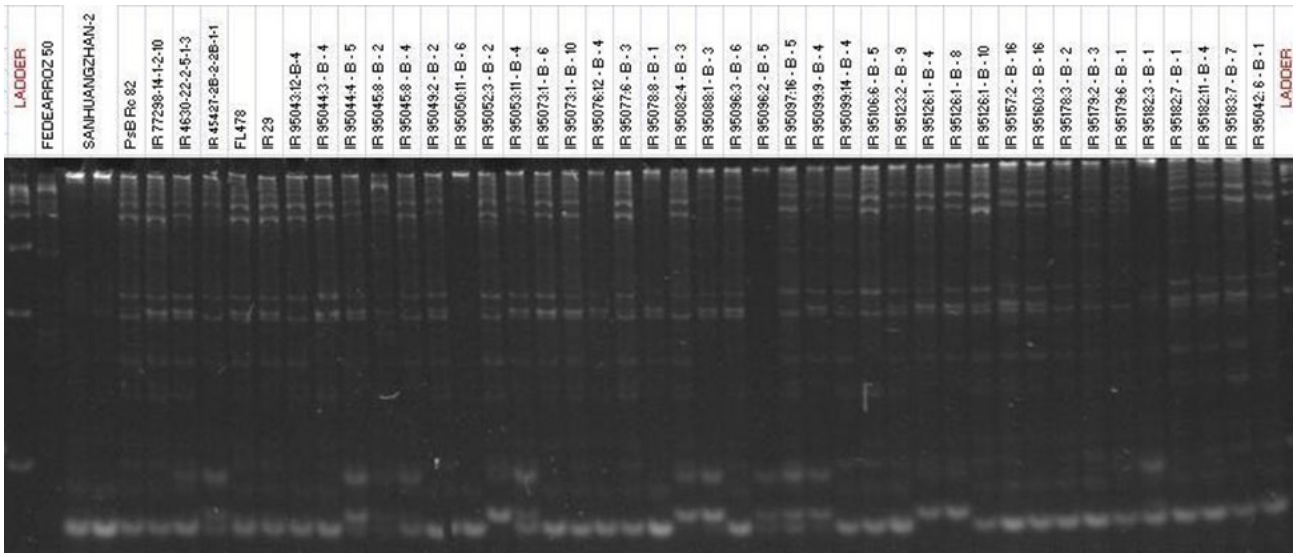

Fig. 4. DNA bands amplified from leaves of 44 genotypes of rice (*Oryza sativa* L.) using SSR marker RM 10748 and electrophoresed in 8% polyacrylamide gel. Ladder = 100 bp.

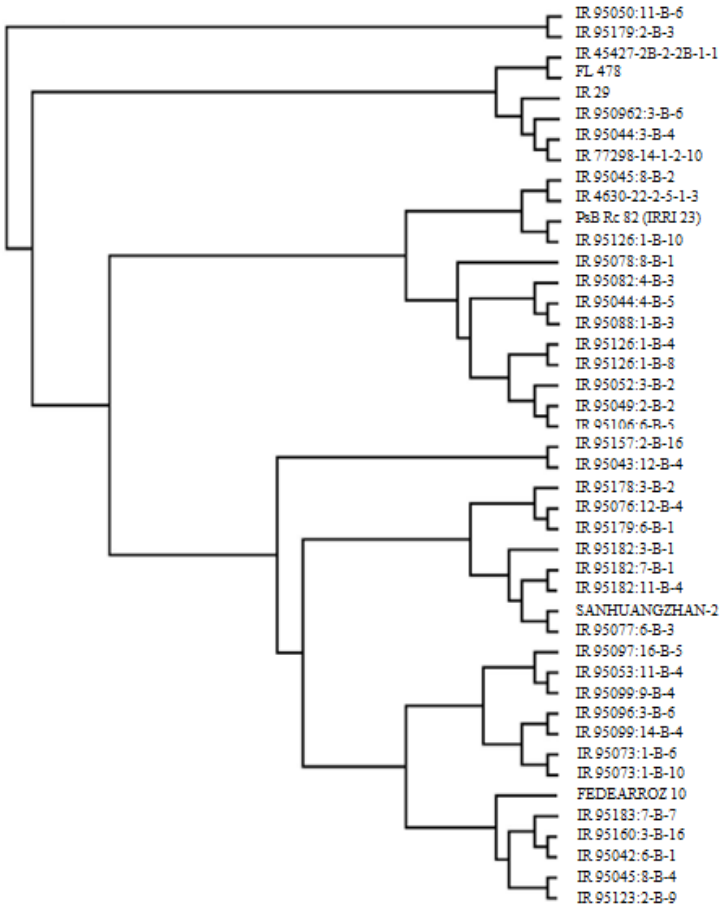

Fig. 5. Dendrogram constructed showing the genetic relatedness of 36 selected genotypes of rice (*Oryza sativa* L.) from the MAGIC *indica* population with reference to FL 478 and salt tolerant founder lines: IR 45427-2B-2-2B-1-1 and IR 4630-22-2-5-1-3 based on the 32 markers on chromosomes 1, 2, 3, 4, 5, 7, 8, 9, 10, 11 and 12 according to the unweighted pair group mean algorithm (UPGMA) with the Nei's similarity index.

(Gregorio 1997; Bonilla et al. 2002). In general, some of the selected genotypes which scored as tolerant based on SES, along with the founder line PSB-Rc 82, were clustered together with FL 478 and IR 45427-2B-2-2B-1-1. However, no constant pattern of clustering among the genotypes which scored tolerant, moderately tolerant and susceptible was observed. The only genotypes which were almost closely clustered to FL 478 and IR 45427-2B-2-2B-1-1 on the constructed dendrogram, which were tolerant based on the SES and had comparable Na^+-K^+ ratio values relative to the tolerant FL 478 were: IR 95044:3:B-4 and IR 95044:4-B-5, which both scored tolerant and had Na^+-K^+ ratios of 0.8902 and 0.5367, respectively.

DISCUSSION

The MAGIC *indica* rice population was formulated from breeding different lines with desirable agronomic traits including salinity tolerance that might have been contributed by two founder lines, namely: (1) IR 45427-2B-2-2B-1-1, which exhibits salinity tolerance at the seedling stage and (2) IR 4630-22-2-5-1-3, which manifests moderate tolerance to salt stress for both seedling and reproductive stages. Hence, some of the rice accessions from the population could have inherited the salinity tolerance mechanism.

In this study, out of the original 970 genotypes that were screened, 36 lines were selected as

Table 3. Mean comparisons of genotypes of rice (*Oryza sativa* L.) across all shoot and root growth traits analyzed for the normal treatment at α = 0.05.

Genotype No.	Designation	Shoot Length Mean	Root Length Mean	Shoot K^+/Na^+ Ratio Value Mean
1	FL 478	53.500^{c-o}	20.250^{f-m}	0.1972^{bc}
2	IR 29	45.000^{m-r}	20.500^{f-l}	0.1812^{bc}
3	Fedearroz 50	46.000^{l-q}	23.750^{b-j}	0.2399^{bc}
4	Sanhuangzhan-2	54.000^{b-m}	24.350b-j	0.1927^{bc}
5	PSB Rc 82 (IRRI 123)	56.750^{a-j}	23.000^{c-j}	0.2123^{bc}
6	IR 77298-14-1-2-10	59.500^{a-e}	20.500^{f-l}	0.4999^{bc}
7	IR 4630-22-2-5-1-3	48.500^{l-q}	16.750^{k-m}	0.1565^{c}
8	IR 45427-2B-2-2B-1-1	48.750^{h-q}	16.000^{l,m}	0.1485c
9	IR 95043:12-B-4	45.250^{l-q}	21.500^{c-l}	0.1980^{bc}
10	IR 95044:3-B-4	44.450^{o-r}	22.000^{d-l}	0.3594^{bc}
11	IR 95044:4-B-5	60.750^{a-c}	24.000^{b-j}	0.3669^{bc}
12	IR 95045:8-B-2	53.500^{c-o}	35.000^{a}	1.2050^{ab}
13	IR 95045:8-B-4	56.000^{a-k}	27.750^{b-d}	1.0710^{a-c}
14	IR 95049:2-B-2	57.500^{a-i}	27.500^{b-e}	0.8286^{a-c}
15	IR 95050:11-B-6	54.500^{b-l}	24.500^{b-j}	0.7587^{bc}
16	IR 95052:3-B-2	58.000^{a-h}	28.500^{bc}	0.7394^{bc}
17	IR 95053:11-B-4	63.000^{ab}	21.500^{e-l}	0.6888^{bc}
18	IR 95073:1-B-6	58.000^{a-h}	24.000^{b-j}	0.3751^{bc}
19	IR 95073:1-B-10	60.500^{a-d}	24.500^{b-j}	0.4158^{bc}
20	IR 95076:12-B-4	56.500^{a-j}	21.500^{e-l}	0.6666^{bc}
21	IR 95077:6-B-3	44.500^{n-r}	20.000^{g-m}	0.7926^{bc}
22	IR 95078:8-B-1	56.500^{a-j}	23.000^{c-j}	0.8251^{a-c}
23	IR 95082:4-B-3	53.500^{c-o}	19.000^{i-m}	1.0505^{a-c}
24	IR 95088:1-B-3	58.500^{a-g}	24.500^{b-j}	1.0374^{a-c}
25	IR 95096:3-B-6	49.500^{g-q}	22.000^{d-l}	0.5953^{bc}
26	IR 95056:2-B-5	56.000^{a-k}	24.000^{b-j}	0.6360^{bc}
27	IR 95097:16-B-5	56.250^{a-k}	28.000^{b-d}	0.5226^{bc}
28	IR 95099:9-B-4	47.000^{k-q}	26.000^{b-g}	0.6678^{b,c}
29	IR 95099:14-B-4	42.000^{p-r}	20.000^{g-m}	0.1474^{c}
30	IR 95106:6-B-5	45.750^{l-q}	16.750^{k-m}	0.2168^{bc}

Table 3. Mean comparisons of genotypes of rice (*Oryza sativa* L.) across all shoot and root growth traits analyzed for the normal treatment at α = 0.05.

Genotype No.	Designation	Shoot Length Mean	Root Length Mean	Shoot K^+/Na^+ Ratio Value Mean
31	IR 95123:2-B-9	54.000^{b-m}	24.750^{b-f}	0.7824^{bc}
32	IR 95126:1-B-4	59.000^{a-f}	26.250^{b-f}	0.8002^{bc}
33	IR 95126:1-B-8	62.000^{a-c}	27.250^{b-e}	1.8576^{a}
34	IR 95126:1-B-10	51.000^{e-p}	23.000^{c-j}	0.2711^{bc}
35	IR 95157:2-B-16	51.250^{d-p}	16.250^{lm}	0.1821^{bc}
36	IR 95160:3-B-16	55.500^{a-k}	29.500^{ab}	0.2063^{bc}
37	IR 95178:3-B-2	47.750^{j-q}	22.500^{c-k}	0.2439^{bc}
38	IR 95179:2-B-3	56.000^{a-k}	25.500b-h	0.1707^{bc}
39	IR 95179:6-B-1	50.500^{e-p}	23.750^{b-j}	1.8576^{a}
40	IR 95182:3-B-1	37.250^{r}	14.250^{m}	0.2394^{bc}
41	IR 95182:7-B-1	64.000^{a}	24.500^{b-j}	0.8535^{a-c}
42	IR 95182:11-B-4	41.000^{q-r}	19.500^{h-m}	0.2093^{bc}
43	IR 95183:7-B-7	53.750^{b-n}	19.750^{h-m}	0.3799^{bc}
44	IR 95182:7-B-1	50.000^{f-q}	18.500^{j-m}	0.3699^{bc}
		LSD = 9.262	LSD = 6.0082	LSD = 1.0431

Means with the same superscript letter are not significantly different.

tolerant to salt stress and were used in the final replicate screening, along with the 8 MAGIC *indica* Plus founder lines and the checks, FL 478 and IR 29. The selection was based on the intensity of salt injury manifested by the susceptible lines with reference to the moderately tolerant PSB Rc 82 such as cessation of growth, complete chlorosis, leaf rolling and leaf elongation, against the tolerant lines which had minimal phenotypic damages at EC = 12 dSm^{-1} and EC = 18 dSm^{-1}.

For the morphological parameters, the shoot dry weight content of the selected genotypes was relatively higher when subjected under salinized conditions since there was a higher % reduction in shoot biomass among the genotypes in fresh weight content than the dry weight content.

The higher values of dry weight content of the salt-treated genotypes can be attributed to the decreased translocation of the stored food materials in the cotyledons at highly saline conditions since osmotic adjustments play an important role in the salt tolerance mechanism of plants when subjected to salt stress. Lower water content may be attributed to an increased Na^+ concentration in the plant tissues (Misra and Dwivedi 2004).

Misra and Dwivedi (2004) found that the salt-sensitive cultivar SML-32 had elevated levels of intracellular Na^+ but decreased levels of K^+, whereas the reverse was observed in the tolerant cultivar T-44. Conversely, opposite findings were reported in other studies. As salt stress and the concentration of NaCl to which the plants are exposed increases, the measured shoot dry weight contents decrease in rice (Puvanitha and Mahendran 2017; Ologundudu et al. 2014; Morales et al. 2012) and in maize (Turan et al. 2010). Puvanitha and Mahendran (2017) indicated that the decrease in shoot dry weight was related to decreased leaf production, eventually leading to lesser rate of photosynthesis and less dry matter accretion.

Likewise, the lower values of root dry weight content among the selected genotypes as salinity increases can be attributed to the combined effects of osmotic adjustments and of ions Na^+ and Cl^-. Since the roots are the first plant parts affected and injured from exposure to abiotic stress, even the minimal amounts of root damage can allow ion influx to the shoot. Root damage and eventually death, caused by ion toxicity, can inhibit further water uptake leading to higher water deficits and lower photosynthetic rates (Puvanitha and Mahendran 2017; Misra and

Table 4. Summary statistics for the amplified 32 SSR markers for assessing genetic diversity for salt tolerance in chromosome 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12 of MAGIC indica population of rice (*Oryza sativa* L.).

Marker	Frequency of Major Allele	Allele Number	Gene Diversity	Locus Heterozygosity	PIC*
RM 127	0.8182	3	0.3130	0.0000	0.2894
RM 140	0.6818	3	0.4822	0.3864	0.4318
RM 3843	0.4091	4	0.7014	0.0000	0.6489
RM 13197	0.8182	3	0.3048	0.3182	0.2701
RM 28102	0.5455	3	0.5155	0.0000	0.4040
AP 3206f	0.8295	3	0.2895	0.0682	0.2587
RM 7075	0.5909	3	0.5462	0.2955	0.4712
RM 490	0.4545	4	0.6397	0.0682	0.5689
RM 1287	0.7500	2	0.3750	0.0000	0.3047
RM 32412b	0.4773	3	0.6356	0.0227	0.5640
RM 10748	0.7045	4	0.4700	0.2273	0.4361
RM 24330	0.7727	3	0.3747	0.1591	0.3408
RM 10793	0.7159	3	0.4285	0.1136	0.3677
RM 44	0.7500	3	0.3853	0.0000	0.3266
RM 493	0.4545	3	0.6281	0.0909	0.5511
RM 495	0.6591	3	0.5052	0.3636	0.4511
RM 455	0.6136	3	0.5052	0.0909	0.4156
RM 562	0.5227	3	0.6064	0.2727	0.5349
RM 3867	0.9545	3	0.0878	0.0000	0.0859
RM 315	0.9545	2	0.0868	0.0909	0.0830
RM 105	0.5227	3	0.5548	0.0000	0.4592
RM 152	0.4886	3	0.6304	0.3409	0.5594
RM 125	0.8523	3	0.2621	0.1136	0.2454
RM 10745	0.7273	3	0.4329	0.0455	0.3918
RM 19	0.7045	3	0.4597	0.0000	0.4152
RM 536	0.8182	3	0.3099	0.2727	0.2822
RM 144	0.8750	3	0.2260	0.0227	0.2131
RM 10764	0.8977	3	0.1883	0.0682	0.1789
RM 10694	0.4773	3	0.5610	0.0000	0.4628
RM 171	0.6136	2	0.4742	0.0000	0.3618
RM 433	0.8864	2	0.2014	0.0000	0.1812
RM 178	1.0000	1	0.0000	0.0000	0.0000

*PIC = polymorphism information content

Dwivedi 2004). In the case of the root and shoot lengths, growth reduction observed among the genotypes when exposed to salinity stress can be attributed to dehydration, toxic results of high salt concentrations and interference on absorption and translocation of nutrients (Misra and Dwivedi 2004). Moreover, based on the

findings of Hu et al. (2012) in Bermuda grass genotypes, salt stress had negative effect on the root and shoot lengths wherein they cited that inhibition in growth parameters can be potentially caused by nutritional deficiencies or ionic imbalance as a result of increased Na^+ concentrations.

The salinity tolerance mechanism based on Na/K balance is characterized as having minimal Na^+ concentration, greater K^+ concentration and increased K^+/Na^+ ratio values which is indicative of the ability to sustain ion balance and high biomass when exposed to salinity stress (Reddy et al. 2017). However, in this study, based on the Na^+-K^+ ratios, the obtained higher values of the selected MAGIC *indica* rice genotypes compared to the tolerant FL 478 under salinized conditions may be due to the inherent genetic diversity of the population wherein a probability of the fine recombination of genes in the population resulted in a new mechanism against salinity stress which is different from the Na/K balance regulation or tissue tolerance.

In the molecular analysis of the selected MAGIC *indica* rice genotypes, the 20 markers which exhibited locus heterozygosity may indicate that the population was genetically diverse. For the PIC, RM 3843 exhibited the highest value, indicating that this marker is useful in differentiating genotypes based on salinity tolerance. When the marker-assisted backcross scheme for *Saltol* was developed, RM 3412b was among the best markers within the *Saltol* region based on polymorphism (Thomson et al. 2010), and is consistent with the high PIC value of the marker in this study. Furthermore, in the same study, RM 493, which is telomeric to *Saltol*, was effective in flanking the region, and is in line with the high PIC value obtained for this marker. Moreover, the markers RM 3412 and RM 493, which are both established within the *Saltol* QTL on chromosome 1, were able to show high PIC values (0.81) with 10 alleles and were proven useful for haplotype analysis of salt-tolerant genotypes (Islam et al. 2012). In another study involving the *HKT1;5* gene of the *HKT* ion family transporter genes, the association of this gene to high salinity tolerance in 95 Indian wild rice genotypes was confirmed. Additionally, the *HKT1;5* gene is located within 1.2 Mb upstream away of RM 3412 in the *Saltol* region (Mishra et al. 2016). The genetic diversity among the genotypes, as analyzed by these molecular markers, can be useful in solving agronomic problems such as salinity stress in crop production (Zeng et al. 2004).

The genetic correlation among the rice genotypes was constructed in a dendrogram based on informative microsatellite alleles. The absence of a constant clustering pattern may be attributed to the diverse genetic composition inherent in the genotypes within the population. The genotypes IR 95044:4-B-5 and IR 95044:3-B-4 were found to be the most tolerant to salt stress among the tested MAGIC *indica* genotypes based on the criteria of phenotypic screening results, Na^+-K^+ ratio and genetic relatedness with FL 478 and the founder line IR 45427-2B-2-2B-1-1, which implies the probability that these genotypes might have inherited the salt tolerance trait among others.

CONCLUSION

The MAGIC *indica* population is a genetically diverse breed of rice genotypes which are known to possess relevant agronomic traits. Screening of the initial 970 rice genotypes for seedling stage salinity tolerance resulted in 36 rice lines which showed moderate to high tolerance to salinity stress based on phenotypic evaluation. This result may indicate that the selected genotypes may potentially possess the salinity tolerance trait. Nonetheless, further evaluation should be done such as assessment of the MAGIC lines in field trials, screening for salinity tolerance at different growth stages and further genetic characterization of the population to validate the results of this study. Correlation with other traits such as the Na^+-K^+ ratio and genetic diversity using SSR markers, however, did not show a constant pattern of association among the 36 selected genotypes, except for the genotypes IR 95044:4-B-5 and IR 95944:3-B-4 which may be attributed to the complex genetic background of the population. Hence, the MAGIC *indica* population can be a feasible source of novel QTLs which can be vital in studying the genetic control of traits and as source of recombined lines with various agronomic traits for breeding superior rice varieties.

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REFERENCES CITED

ARZANI A. 2008. Improving salinity tolerance in crop plants: A biotechnological review. In Vitro Cell Dev Biol Plant 44: 373–383.

ARZANI A, ASHRAF M. 2016. Smart engineering of

genetic resources for enhanced salinity tolerance in crop plants. Crit Rev Plant Sci 35: 146–189.

BONILLA P, DVORAK J, MACKILL D, DEAL K, GREGORIO GB. 2002. RFLP and SSLP mapping of Salinity tolerance genes in chromosome 1 of rice (Oryza sativa L) using recombinant inbred lines. Philipp Agric Scientist 85: 68–76.

COLLARD BCY, JAHUFER MZZ, BROUWER JB, PANG ECK. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142: 169–196.

EISEN M. 1999. Cluster and Tree View Manual. Stanford University. Retrieved 10 February 2012 from the World Wide Web. http://rana.lbl.gov/manuals/ClusterTreeView.pdf

GREGORIO GB. 1997. Tagging salinity tolerance genes in rice using amplified fragment length polymorphism (AFLP). [PhD dissertation]. College, Laguna, Philippines: University of the Philippines Los Baños. (Available at the IRRI Library).

GREGORIO GB, SENADHIRA D, MENDOZA RD. 1997. Screening rice for salinity tolerance. IRRI Discussion Paper Series No. 22. International Rice Research Institute, Manila, Philippines. p. 1–30.

HU L, HUANG Z, LIU S, FU J. 2012. Growth response and gene expression in antioxidant-related enzymes in two Bermuda grass genotypes differing in salt tolerance. J Am Soc Hort Sci 137(3): 134–143.

[IRRI] International Rice Research Institute. 2010. Population development through multi-parent advanced generation inter-crosses (MAGIC) among diverse genotypes to facilitate gene discovery for various traits in rice. International Rice Research Institute, Manila, Philippines. p. 1–5.

ISLAM MR, GREGORIO GB, SALAM MA, COLLARD BCY, SINGH RK, HASSAN L. 2012. Validation of Saltol linked markers and haplotype diversity on chromosome 1 of rice. Mol Plant Breed 3(10): 103–114.

LIU K, MUSE SV. 2005. Powermarker: Integrated analysis environment for genetic marker data. Bioinformatics 21(9): 2128–2129.

MCCARTNEY CA, SOMMERS DJ, FEDAK G, CAO W. 2004. Haplotype diversity at Fusarium head blight resistance QTLs in wheat. Theor Appl Genet 109: 261–271.

MISHRA S, SINGH B, PANDA K, SINGH BP, SINGH N, MISRA P, RAI V, SINGH NK. 2016. Association of SNP haplotypes of HKT family genes with salt tolerance in Indian wild rice germplasm. Rice 9: 15.

MISRA N, DWIVEDI UN. 2004. Genotypic difference in salinity tolerance of green gram cultivars. Plant Sci 166: 1135–1142.

MOHAMMADI-NEJAD G, ARZANI A, REZAI AM, SINGH RK, GREGORIO GB. 2008. Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the *saltol* QTL. Afr J Biotechnol 7(6): 730–736.

MOHAMMADI-NEJAD G, SINGH RK, ARZANI A, REZAI AM, SABOURI H, GREGORIO GB. 2010. Evaluation of salinity tolerance in rice genotypes. Int J Plant Prod 4(3): 199–208.

MORALES SG, TREJO-TELLEZ LI, MERINO FC, CALDANA C, ESPINOSA-VICTORIA D, CABRERA BEH. 2012. Growth, photosynthetic activity and potassium and sodium concentration in rice plants under salt stress. Acta Sci-Agron 34(3): 317–324.

MUNNS R, TESTER M. 2008. Mechanisms of salinity tolerance. Ann Rev Plant Biol 59: 651–681.

NEI M, TAKEZAKI N. 1983. Estimation of genetic distances and phylogenetic trees from DNA analysis. Proc 5th World Cong Genet Appl Livestock Prod 21: 405–412.

OLOGUNDUDU AF, DELUSI AA, AKINWALE RO. 2014. Effect of salt stress on germination and growth parameters of rice (*Oryza sativa* L.). Not Sci Biol 6(2): 237–243.

[PHILRICE] Philippine Rice Research Institute. 2001. Management of salt affected soils for rice production. Rice Technology Bulletin No. 40. Department of Agriculture, Philippine Rice Research Institute, Philippines. p. 1–22.

PUVANITHA S, MAHENDRAN S. 2017. Effect of salinity on plant height, shoot and dry weight of selected cultivars. Sch J Agric Vet Sci 4(4): 126–131.

[SAS] Statistical Analysis System. 2000. SAS/STAT Users Guide, Version 7. Cary, NC: Statistical Analysis System Institute, Electronic Version.

SABOURI A, SAJISE AG, DE OCAMPO M, VISPO AN, REFUERZO L, ARCETA M, MAMIIT A, THOMSON MJ, GREGORIO GB, SINGH RK. 2009. Diversity within the widely-studied salt-tolerant landrace Pokkali. SABRAO J Breed Genet 41, Special Supplement, August 2009.

THOMSON MJ, DE OCAMPO M, EGDANE J, AKHLASUR RAHMAN M, GODWIN SAJISE A, ADORADA DL, TUMIMBANG-RAIZ E, BLUMWALD E, SERAJ ZI, SINGH RK, GREGORIO GB, ISMAIL AM. 2010. Characterizing the *Saltol* quantitative trait locus for salinity tolerance in rice. Rice 3: 148–160.

TURAN MA, ELKARIM AHA, TABAN N, TABAN S. 2010. Effect of salt stress on growth and ion distribution and accumulation in shoot and root of

maize plant. Afr J Agric Res 5(7): 684–588.

ZENG L, KWON TR, LIU X, WILSON C, GRIEVE CM, GREGORIO GB. 2004. Genetic diversity analyzed by microsatellite markers among rice (*Oryza sativa* L.) genotypes with different adaptations to saline soils. Plant Sci 166: 1275–1285.