RESEARCH

Screening of some rice genotypes for salinity tolerance using agro-morphological and SSR markers

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ABSTRACT

Salinity is a main obstacle of rice (*Oryza sativa* L.) cultivation. Selecting rice genotypes for salinity tolerance based on phenotypic characteristics alone is inefficient and less reliable, finally will delay progress in rice breeding program. The recent advantages of molecular markers such as simple sequence repeat (SSR) could be used to detect associated salt tolerance markers in rice. This study was conducted to detect genetic variation within some rice genotypes using SSR markers and to identify salt tolerance in the studied genotypes. Forty-five rice genotypes were evaluated for their agro-morphological characteristics under non-stress and saline conditions for two growing seasons in 2018 and 2019. Using 21 SSR primers located on chromosome 8, only 18 SSR primer generated polymorphic patterns with a total of 76 alleles, whereas the other 3 primers were monomorphic. The number of alleles per locus ranged from 2 to 6 alleles with an average of 4.22 alleles per locus. The polymorphic information content (PIC) values varied from 0.30 (RM342) to 0.71 (RM6976) with an average PIC of 0.49. Out of the 18 polymorphic markers only 5 primers (RM 6976, RM7631, RM 5556, RM152 and RM342) perfectly distinguished rice genotypes. The best preforming genotypes under salinity stress were N22, IR 63731-1-1-4-3-2, GZ 7112-1-2-1-4, FL 478, TCCP 266-1-3B-10-2-1, IR 65600-127-6-2, IR 68011-15-1-1 and IR 66160-5-2-3-2. Thus, SSR markers are effective to detect high polymorphisms and variations among the rice genotypes, which could facilitate improving salt tolerance of commercial Egyptian rice varieties exhibiting high yield potential. In addition, the selected genotypes might be integrated into breeding programs for salinity tolerance.

Key words: *Oryza sativa*, rice genetic diversity, salinity, simple sequence repeats (SSRs).

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops in the world and serves as the staple food for over a third of the world's population (Mohammadi-Nejad et al., 2010). One of the most significant factors in the vegetative and reproductive stages that hinder the growth and production of rice is arable soil salinity (Mojakkir et al., 2015). Salinization has a detrimental effect on agricultural production, farmers' living conditions, the economy at various levels, and ecosystem balance, including the quality of natural resources (FAO, 2019). Salinity is projected to have catastrophic global impacts, resulting in land losses of 30% over the next 25 years, rising to 50% by 2050 (Bannari and Al-Ali, 2020). Salinity decreases the ability of plants to absorb water and results growth reduction. Mukta et al. (2017) and Yichie et al. (2018) mentioned that modern rice varieties are highly sensitive to salinity, thereby reducing the production of rice. Technologies that minimize the spread of salinization, decrease salinity levels in crop fields or increase the salt tolerance of crops must therefore be established and employed. Progress in salinity tolerance breeding is slow due to the

following factors: limited knowledge of genetics of tolerance, complexity of the different tolerance mechanisms involved, inadequate screening techniques, low performance of selection, and weak understanding of salinity and environmental interactions (Bhowmik et al., 2009). Breakthrough in salinity tolerance breeding was possible after identify of qualitative trait loci (QTLs) that allowed fast integration of traits into modern high yielding and common varieties through marker-assisted backcrossing (Huyen et al., 2012). According to Mason (2015), simple sequence repeats (SSRs) are co-dominant, highly informative, multi-allele genetic markers which are experimentally reproducible and transferable among their related species. The difference in the number of repeated tandem units results in highly polymorphic banding patterns observed by PCR, using locus-specific flanking region primers where they are identified. With the recent advancement in the field of molecular marker, it is now possible to determine both simple inherited and quantitative characteristics and then identify the individual salinity tolerance control genes which could promote rice selection for this low inherited trait (Aliyu et al., 2011).

Recently, Karimah et al. (2021) analyzed genetic diversity of rice genotypes based on the agro-morphological and SSR. Genetic diversity evaluation could provide useful information for the genetic improvement of salt-tolerant rice. It has long been understood that salinity can lead to sterility in rice, particularly if imposed during pollen development and fertilization; hence, high-yielding salt-tolerant rice varieties must have tolerance at the reproductive stage. Screening for salinity tolerance in rice genotypes is therefore important to evaluate the true value of plant genetic resources for the development of salinity tolerant varieties (Barrera et al., 2019). Measurements of morphological and physiological variations in quantitative and economically significant characteristics typically estimate genetic diversity among the parental genotypes. Diversity ensures a large gene pool from which characteristics of economic benefits can be exploited. In order to increase salt tolerance and test salinity tolerance in rice germplasm, access to effective screening techniques to identify salt-tolerant genotypes is necessary. Salinity screening has been well recorded in various plants, especially in rice. A species finds it difficult to adapt to the ever evolving environmental and biotic stresses without diversity (El-Refaee et al., 2011). With a wide range of crop varieties, a breeder can screen and choose materials for different purposes (El-Refaee et al., 2018). Therefore, the current study was conducted to screen 45 rice genotypes under salinity using SSR marker to identify salt tolerant genotypes and to assess genetic diversity among them for future use in breeding programs.

MATERIALS AND METHODS

Genetic materials and field procedures

Forty-five genotypes of rice (*Oryza sativa* L.) were obtained from Egyptian Rice Germplasm Unit (ERGU), at Rice Research Department, Field Crops Research Institute, Agricultural Research Center Sakha, Egypt (Table 1), then tested under non-stress (NS) and saline (S) conditions during 2018 and 2019 rice growing seasons at Sakha (non-saline condition) and El-Sirw locations (saline condition), respectively. In each location, after 30 d from sowing, seedlings of each 45 genotypes of rice were individually transplanted in the permanent field in three rows. Each row was 5 m long and contained 25 hills adopting spacing of 20 cm. Randomized complete block design (RCBD) was used with three replicates with the same set of genotypes. All recommended agricultural practices were applied for the permanent rice field in each location. The genotypes were assessed under non-stress (NS) and saline (S) conditions (EC 3.14 and 10.7 dS m^{-1}, respectively) measured at the beginning of seasons (Table 2). The water used for irrigation during growing seasons had salinity level of 1.45 dS m^{-1} under saline condition and 0.54 dS m^{-1} under non-stress condition.

Measured traits

Nine field traits were measured in the end of season except days to heading after 50% flowering; including plant height (cm), days to heading (d), number of tillers $plant^{-1}$, number of productive tillers $plant^{-1}$, 1000 grains weight (g), number of filled grains $panicle^{-1}$, fertility percentage (%), harvest index and yield $plant^{-1}$ (g). The data were recorded according to the standard evaluation system (SES) for rice (IRRI, 1996) and the statistical analysis was conducted according to the statistical model of Gomez and Gomez (1984).

Table 1. Forty-five genotypes of rice used in this study for salinity tolerance assessment.

Accession nr	Name	Source
1	Giza 177	ERGU-Egypt
2	Giza 178	ERGU-Egypt
3	Giza 179	ERGU-Egypt
4	Giza 182	ERGU-Egypt
5	E. Jasmine	ERGU-Egypt
6	Sakha 101	ERGU-Egypt
7	Sakha 104	ERGU-Egypt
8	Sakha 105	ERGU-Egypt
9	Sakha 106	ERGU-Egypt
10	GZ 1368-S-5-4	ERGU-Egypt
11	GZ 10241-19-11-1-4	ERGU-Egypt
12	Sakha 108	ERGU-Egypt
13	SKC2015	ERGU-Egypt
14	IM16	IRRI-Philippines
15	IR 88628-B-B-45	IRRI-Philippines
16	IR 83106-B-B-9-31	IRRI-Philippines
17	GZ 9730-1-1-1-2	ERGU-Egypt
18	Sakha 107	ERGU-Egypt
19	IR 60080-46 A	IRRI-Philippines
20	IRAT 170	IRRI-Philippines
21	IR 11L465	IRRI-Philippines
22	N22	IRRI-Philippines
23	IR 100634-96-AJY-22	IRRI-Philippines
24	SPBG	IRRI-Philippines
25	IR 63731-1-1-4-3-2	IRRI-Philippines
26	HHZ 8-SAL6-SAL3-Y1	IRRI-Philippines
27	IRGC 78936	IRRI-Philippines
28	A22	IRRI-Philippines
29	IR 88628-B-B-10	IRRI-Philippines
30	IR 88628-B-B-16	IRRI-Philippines
31	IR 88628-B-B-21	IRRI-Philippines
32	IR 88628-B-B-31	IRRI-Philippines
33	IR 88628-B-B-36	IRRI-Philippines
34	IR 45427-2B-2-2B-1-1	IRRI-Philippines
35	GZ 7112-1-2-1-4	ERGU-Egypt
36	IRAT161	IRRI-Philippines
37	FL 478	IRRI-Philippines
38	TCCP 266-1-3B-10-2-1	IRRI-Philippines
39	IR 65600-127-6-2	IRRI-Philippines
40	IR 69853-70-3-1-1	IRRI-Philippines
41	IR 68011-15-1-1	IRRI-Philippines
42	IR 65597-29-3-2-3	IRRI-Philippines
43	IR 66158-38-3-2	IRRI-Philippines
44	IR 66160-5-2-3-2	IRRI-Philippines
45	IRRI 148	IRRI-Philippines

ERGU: Egyptian Rice Germplasm Unit; IRRI: International Rice Research Institute.

Table 2. Chemical characteristics of experimental soil and irrigation water at two different locations over two seasons.

			Cation				Anion				
Site	pH 1:2.5	EC	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	SO_4	Cl^{-}	HCO^{-3}	CO^{-3}	Soil texture
		dS m^{-1}	meq L^{-1}				meq L^{-1}				
Normal soil	8.10	3.14	15.50	28.50	35.00	0.57	64.00	15.50	3.85	-	Clay
Saline soil	7.90	10.70	28.00	70.50	150.00	1.25	151.00	105.00	3.00	-	Clay
Irrigation water of normal soil	-	0.50	4.50	4.85	5.02	0.22	7.40	2.10	18.00	2.00	
Irrigation water of saline soil	-	1.35	4.30	10.20	18.10	0.43	20.30	8.80	3.25	1.25	

Microsatellite marker analysis

Total genomic DNA was extracted from fresh leaves of studied genotypes using cetyltrimethylammonium bromide (CTAB) method according to Murray and Thompson (1980). Purity and concentration of DNA was monitored spectrophotometrically (Nano-Drop 1000 spectrophotometer; ThermoFisher, Wilmington, Delaware, USA). Based on the published rice microsatellite framework map, 21 primers were chosen for the genetic diversity study (Table 3). All simple sequence repeat (SSR) primers are located on chromosome 8. The original source, repeat motifs, primer sequences and chromosomal positions for these markers are available at the rice genome database (http://www.gramene.org).

Following the protocol of Ravi et al. (2003), SSR analysis was performed. PCR amplification reactions were carried out in a total volume of 20 µL containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM each dNTPs, 0.2 µM each forward and reverse primers, 1 unit Taq polymerase and 20 ng template DNA. Thermal cycler was programmed to 1 cycle of 5 min at 94 °C as an initial hot start and strand separation step. This was followed by 35 cycles of 1 min at 94 °C for denaturation, 1 min for annealing temperature for each primer used and 30 s at 72 °C for primer elongation. Finally, 1 cycle of 7 min at 72 °C was used for final extension. Amplified products were stored at -20 °C until further use. The bands were separated by running the PCR products on 3% agarose gel at 80 V for 1 h in 0.5% TBE along with 50 bp DNA ladder and stained with ethidium bromide. The gel was viewed using gel documentation.

Allele scoring and data analysis

For each of the microsatellite primer pairs in each genotype, the polymorphic bands were graded on the basis of presence "1" or absence "0" for bands producing a matrix of "1" and "0". Compared with a known molecular weight marker (50 bp DNA ladder), the size (number of nucleotide base pairs) of the amplified bands for each SSR marker was determined based on its migration distance. The summary statistics including major allele frequency and gene diversity were determined using Power-Marker version 3.25 genetic analysis software (Liu and Muse, 2005). The polymorphic information content (PIC) value of a marker was calculated according to the following formula: $PIC = 1 - \sum P_i^2$, where P_i is the frequency of its allele (Anderson et al., 1993). Mean allele numbers, PIC values, and genetic similarities were calculated on the basis of different rice genotypes and microsatellite classes. Based on these distances, a cluster diagram was constructed by the unweighted pair group method with arithmetic mean (UPGMA) (average linkage) method to create a dendrogram. The genetic distances and dendrogram analysis were computed using Numerical Taxonomy and Multivariate Analysis System, version 2.1 (NTSYSpc) (Rohlf, 2000).

Table 3. SSR markers used in the current study and some of their basic features.

Nr	Marker	Chromosome	Expected product size	Motif	Annealing temperature	F primer sequence	R primer sequence
1	RM408	8	128	(CT)13	63	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC
2	RM152	8	202	(GGC)10	60	ACGCCCTTCTGGATGTAGTC	GAAGAGAGCAGCGTGACATG
3	RM8018	8	120	(AT)21	59	AATTCATACACACTTGTGCC	ATTTGCTTGAGCAAGCTTAT
4	RM5556	8	102	(TG)15	62	ATCTCCCTCCCTCTCCTCAC	TCCACACCTTCACAGTTGAC
5	RM544	8	248	(TC)9	62	TGTGAGCCTGAGCAATAACG	GAAGCGTGTGATATCGCATG
6	RM547	8	240	(ATT)19	63	TAGGTTGGCAGACCTTTTCG	GTCAAGATCATCCTCGTAGCG
7	RM6008	8	165	(CCG)8	60	AGAGAAGAGAGAAGCGCACG	CATACATCACACGAGGACGG
8	RM8243	8	207	(CA)11	57	CTCGTGCAACCATTATATTC	ACCTTAGCTGTCCTGAATTG
9	RM3395	8	110	(CT)17	63	ACCTCATGTCCAGGTGGAAG	AGATTAGTGCCATGGCAAGG
10	RM1384	8	175	(AG)36	59	TTAATCCATCCTGTAGCTGG	TCGCTATCAACACTACCTGC
11	RM6471	8	250	(GCC)9	63	TCTCCCATCTCCCATCTCAC	TGGTGATTGTGACAGATCGC
12	RM6990	8	130	(TTG)8	61	GGTGTGATCCTTTCTGATGC	ACGGGTGTGATCCCAGATAC
13	RM7027	8	110	(AAAT)6	64	AGGACCTGGACTTTATGGGC	CCTGCACTGCTCCACAGTAC
14	RM6193	8	150	(CGG)8	63	CAAGAAGCTCTGGGCTAACG	GTTCTTGTGCCGTATCCTCC
15	RM3153	8	155	(CA)25	62	CGGTTCTTTTCACATGGTCG	ATCACAACAAGCTCGACGTG
16	RM284	8	145	(GA)8	61	ATCTCTGATACTCCATCCATCC	CCTGTACGTTGATCCGAAGC
17	RM342	8	190	(CAT)12	61	CCATCCTCCTACTTCAATGAAG	ACTATGCAGTGGTGTCACCC
18	RM6976	8	150	(TTC)15	60	CTCATGGGGCTTCTTCCC	CCCATTGGATAGAATCCCAG
19	RM419	8	190	(AG)12	63	TCTCCTTTGGTATGCGTGTG	GCTGCTGCTCCACTTTTCTC
20	RM7631	8	120	(TTCT)6	63	GGTCACTCATGGTGCATGTC	CACACTCACTCACTCACTTGAC
21	RM5545	8	155	(TG)14	61	CAGCACTCCTCCCCTACCAG	GGCTAAGTCAGCGTGAGACC

RESULTS AND DISCUSSION

ANOVA

The data was independently analyzed to validate the variations among the genotypes tested over the two seasons studied (Table 4). The combined ANOVA during non-stress and saline conditions showed that all studied traits were significantly influenced by seasons and genotypes at 1% ($p < 0.01$) and 5% ($p < 0.05$) probability levels, respectively. These results ascertain the fact of the assumption for distinct genetic background of the genotypes used in this study. In general, ANOVA which showed differences among the genotypes (significant or highly significant) referred to the items of experimental design is differed and the comparison between them is valid. In addition, the existence of adequate genetic diversity among the studied genotypes was reflected.

Mean performances

The mean performance of studied genotypes for measured traits showed the highest values under non-stress followed by saline conditions (Table 5). The mean values of studied traits were reduced under salinity conditions for most of genotypes under study, suggesting genetic variability among these genotypes for their salinity tolerance. Concerning plant height, the genotype TCCP 266-1-3B-10-2-1 showed highest value under non-stress (127.53 cm), followed by IR 65600-127-6-2 (125.57 cm). However, genotypes N22, FL 478, TCCP 266-1-3B-10-2-1, IR 65600-127-6-2, IRRI 148, Giza 178, Giza 179, and GZ 1368-S-5-4, the smallest decrease in plant height was shown to be, it thus confirms that it is tolerant to stress of salinity. Giza 177 was (62.91 cm) the shortest genotype under saline conditions, moreover; the genotypes SKC2015 and IR 68011-15-1-1 showed shortest value under non-stress and saline conditions (92.76, 93.64, 65.94 and 70.86 cm, respectively). In this study, the sensitive genotype Giza 177 was strongly reduced in plant height, reduced plant height stature was also reported by Dhar et al. (2012). Inhibition of cell expansion in the leaf growth zone caused by salinity may have caused a reduction in plant height for sensitive varieties (Adak et al., 2019).

For days to heading, it is clear that the earlier plants were observed from IR 88628-B-B-31 followed by Giza 179 they had 86.26 and 86.85 d respectively under non-stress condition. Meanwhile, under stress condition the same genotype IR 88628-B-B-36 followed by genotype IRAT 170 gave the lowest values (72.56 and 74.05 d) respectively. Obviously, saline conditions caused earliness in heading which increased in the most of all genotypes. While, it was interesting to note that the tested genotypes were affected differently by saline conditions. The results showed that the most affected genotypes were IR 88628-B-B-31 and IR 88628-B-B-16 that recording about 22-day earliness under saline conditions.

Table 4. Mean squares from the analysis of variance for the studied traits.

		Mean squares					
Trait	Condition	Year (Y)	Replicates within year	Genotypes (G)	GxY interaction	Pooled error	CV%
Degrees of freedom		1	4	44	44	176	
Plant height, cm	NS	64.17**	13.22	952.91**	0.95	7.74	2.96
	S	34.27**	69.16**	595.76**	9.02**	4.79	2.81
Days to heading, d	NS	1189.72**	3.10	423.54**	1.99	3.69	2.21
	S	410.95**	237.32**	554.27**	1.70	1.95	1.44
Number of total tillers plant^{-1}	NS	9.03	6.21	180.38**	0.55	4.25	10.35
	S	17.08*	19.03**	140.79**	2.54	3.10	11.86
Number of productive tillers plant^{-1}	NS	17.97*	2.40	172.69**	1.22	3.27	10.69
	S	0.64	13.17**	117.91**	2.63	2.85	13.66
1000 grains weight, g	NS	16.43*	9.80**	107.64**	1.47	2.76	5.22
	S	4.73	29.51**	75.50**	4.83**	2.77	7.08
Number of filled grains	NS	0.02	16.04*	8968.65**	3.50	5.52	1.67
	S	8.07	431.45**	7785.66**	4.87	3.98	1.87
Fertility percentage, %	NS	17.40*	13.00*	11.59**	2.47	4.04	2.13
	S	61.89**	292.92**	14.32**	6.24*	4.27	2.54
Yield harvest index, %	NS	584.36**	32.41**	91.21**	4.21	3.56	4.61
	S	228.83**	137.78**	107.34**	7.67**	3.23	5.42
Grain yield plant^{-1}, g	NS	7.86	1.33	718.04**	49.89**	6.12	5.89
	S	42.86**	18.77**	236.26**	10.44**	3.94	7.86

NS: Non-stress; S: salt stress.

Table 5. Mean performances of the studied genotypes for nine traits in both environments during 2018 and 2019 growing seasons.

Nr	Genotype	Plant height		Days to heading		Tillers plant^{-1}		Productive tillers plant^{-1}		1000-grain weight	
		NS	S	NS	S	NS	S	NS	S	NS	S
		cm		d		Nr		Nr		g	
1	Giza 177	104.31	62.91	90.24	72.08	18.92	12.37	16.75	10.87	28.68	20.02
2	Giza 178	94.28	84.94	98.66	90.16	22.62	19.98	21.62	17.98	26.36	21.29
3	Giza 179	96.20	86.52	86.85	80.57	23.59	20.34	21.59	18.51	27.99	22.76
4	Giza 182	99.72	75.61	95.07	85.92	21.86	14.38	18.49	12.05	29.82	23.69
5	E. Jasmine	101.87	79.72	95.55	97.95	24.55	17.11	21.83	15.00	23.52	19.72
6	Sakha 101	96.39	71.63	93.55	81.90	24.81	19.71	21.81	17.05	30.63	25.02
7	Sakha 104	103.42	92.17	91.37	87.73	24.36	21.55	21.91	18.05	28.35	23.36
8	Sakha 105	105.31	78.35	92.65	80.83	25.29	16.01	22.09	13.01	30.06	20.76
9	Sakha 106	112.53	83.17	91.68	81.55	25.41	20.49	21.81	17.49	31.77	24.33
10	GZ 1368-S-5-4	100.31	90.17	98.48	90.31	28.43	23.81	26.97	21.81	27.23	23.99
11	GZ 10241-19-11-1-4	97.98	83.33	87.75	82.16	22.48	18.42	21.48	17.42	31.91	25.13
12	Sakha108	101.31	78.72	93.33	84.16	20.82	12.99	17.68	11.27	26.59	23.03
13	SKC2015	92.76	65.94	87.98	74.85	21.10	19.46	18.10	16.46	30.73	22.52
14	IM16	107.09	82.61	98.57	84.83	13.97	9.43	11.28	6.88	35.93	27.53
15	IR 88628-B-B-45	101.42	78.02	97.43	108.19	18.16	17.22	15.17	13.55	33.56	29.25
16	IR 83106-B-B-9-31	108.09	92.17	91.91	92.47	17.04	12.55	14.26	10.99	33.57	20.13
17	GZ 9730-1-1-1-2	106.42	85.50	87.65	78.25	19.61	18.46	15.87	10.22	34.34	24.00
18	Sakha 107	104.20	83.06	93.50	77.64	16.16	11.87	13.33	9.93	26.40	23.78
19	IR 60080-46 A	102.53	90.78	94.00	84.09	14.58	9.68	11.74	8.10	36.08	31.84
20	IRAT 170	99.78	88.67	90.43	74.05	13.78	10.21	11.78	8.57	34.07	23.63
21	IR 11L465	100.87	80.26	88.87	80.95	18.79	15.81	16.01	13.76	35.12	21.78
22	N22	106.98	102.15	91.94	86.61	28.40	25.14	28.40	23.48	26.10	19.98
23	IR 100634-96-AJY-22	110.87	90.72	97.76	87.23	24.57	20.85	21.57	17.85	33.14	27.23
24	SPBG	98.53	74.17	93.89	91.57	15.41	12.66	12.80	10.60	24.14	22.04
25	IR 63731-1-1-4-3-2	101.42	78.28	110.61	102.00	27.70	25.20	27.25	22.20	27.48	22.41
26	HHZ 8-SAL6-SAL3-Y1	100.31	83.72	95.02	89.40	19.06	13.45	16.22	11.40	33.59	20.10
27	IRGC 78936	118.31	103.50	130.12	103.51	16.11	10.83	13.11	8.02	32.84	22.44
28	A22	109.76	94.83	87.69	79.36	18.80	14.63	15.97	12.63	35.47	22.90
29	IR 88628-B-B-10	99.98	76.96	87.73	79.46	16.72	12.91	13.88	10.91	34.22	22.73
30	IR 88628-B-B-16	102.53	74.61	114.71	92.97	14.38	11.21	11.60	9.16	33.08	23.86
31	IR 88628-B-B-21	100.42	76.78	112.91	111.71	13.63	9.22	11.25	7.22	33.42	21.27
32	IR 88628-B-B-31	98.42	75.72	111.84	84.82	15.07	10.04	11.94	8.43	31.99	27.51
33	IR 88628-B-B-36	104.09	82.39	86.26	72.56	18.60	12.94	15.57	10.94	33.73	24.07
34	IR 45427-2B-2-2B-1-1	99.09	87.06	91.62	86.78	11.62	8.51	9.67	6.47	33.68	23.69
35	GZ 7112-1-2-1-4	98.64	79.39	93.32	89.76	15.73	12.28	12.40	9.80	23.47	16.83
36	IRAT 161	116.09	97.22	93.05	82.44	20.93	12.77	17.43	10.85	27.19	23.14
37	FL 478	97.81	91.50	110.81	103.95	17.40	13.51	14.52	10.46	36.79	31.63
38	TCCP 266-1-3B-10-2-1	127.53	118.50	90.35	86.50	14.87	12.41	11.20	8.48	35.26	27.13
39	IR 65600-127-6-2	125.57	115.94	96.28	90.86	20.3	13.90	17.30	10.23	36.49	30.01
40	IR 69853-70-3-1-1	103.64	82.22	97.61	81.17	19.57	15.10	16.57	12.10	33.30	28.43
41	IR 68011-15-1-1	93.64	70.86	112.89	92.98	15.34	9.75	12.01	8.92	33.64	28.26
42	IR 65597-29-3-2-3	96.42	73.06	113.74	98.56	16.27	13.07	13.27	11.11	32.57	18.56
43	IR 66158-38-3-2	101.42	89.22	103.13	93.25	16.42	11.63	13.22	9.80	33.19	20.32
44	IR 66160-5-2-3-2	102.53	82.94	105.25	92.14	14.19	9.35	11.37	7.35	33.34	28.41
45	IRRI 148	110.87	101.33	89.28	81.40	16.47	10.11	13.19	7.56	25.77	21.57

NS: Non-stress; S: salt stress.

Meanwhile, 8 d or less differences were observed for Giza 178, Giza 179, GZ 1368-S-5-4, N22, IR 63731-1-1-4-3-2, HHZ 8-SAL6-SAL3-Y1, IR 45427-2B-2-2B-1-1, GZ 7112-1-2-1-4, FL 478, TCCP 266-1-3B-10-2-1, IR 65600-127-6-2 and IRRI 148 under the same conditions. The genotypes displaying the lowest reduction in the heading date indicate that they are tolerant to saline conditions. The results in Table 5 revealed that the highest number of productive tillers plant^{-1} under both conditions were evident for genotypes N22, IR 63731-1-1-4-3-2 and GZ 1368-S-5-4, which estimated values were 28.40, 27.25 and 26.97 panicles at non-stress, and 23.48, 22.20 and 21.81 panicles at saline conditions respectively. Such rice genotypes with more panicles under both stress conditions are expected to have increased grain yield. With respect to 1000 grains weight, the heaviest grains detected in the genotypes FL 478 followed by genotype IR 65600-

127-6-2 which their estimated values were 36.79 and 36.49 g at non-stress and 31.65 and 30.01 g at saline conditions, respectively. Therefore, because plant tolerance at the reproductive stage is directly linked to grain yield, it is essential for rice varieties to have tolerance at the reproductive stage in order to obtain good rice yields (Hossain et al., 2015).

Concerning number of filled grains per panicle in Table 6, the most desirable mean values were obtained from the genotypes IR 88628-B-B-21, IR 88628-B-B-45 and IR 88628-B-B-31 under both conditions. Rice genotypes with a greater number of filled grains panicle^{-1} are expected to have increased grain yield. For spikelet fertility percentage and harvest index (Table 6), the most desirable mean values were recorded from genotypes GZ 7112-1-2-1-4, IR 69853-70-3-1-1, IR 88628-B-B-31 and IR 68011-15-1-1 under both conditions. Reproductive stage is one of the most sensitive growth stages under the saline conditions (Ahmed et al., 2019). As far as grain yield is concerned, this is the most critical stage,

Table 6. Mean performances of the studied genotypes for nine characters in both environments during 2018 and 2019 growing seasons.

		Filled grains		Fertility percentage		Harvest index		Grain yield plant^{-1}	
Nr	Genotype	NS	S	NS	S	NS	S	NS	S
		Nr		%		%		g	
1	Giza 177	112.18	80.63	94.67	70.07	37.37	25.70	33.68	20.02
2	Giza 178	127.24	99.73	92.16	79.08	42.20	34.59	35.36	27.29
3	Giza 179	136.47	118.23	93.89	79.69	36.62	31.84	32.99	26.76
4	Giza 182	126.94	95.43	94.35	81.11	41.19	36.58	32.82	24.69
5	E. Jasmine	149.58	122.84	94.06	81.53	38.41	34.56	27.52	19.72
6	Sakha 101	116.33	87.27	92.52	78.68	38.24	31.44	37.63	23.02
7	Sakha 104	112.63	87.77	93.48	80.37	39.69	35.23	34.35	27.36
8	Sakha 105	109.91	77.58	94.38	71.95	39.94	34.18	33.06	20.76
9	Sakha 106	100.41	79.69	92.94	80.15	34.66	28.90	35.77	23.33
10	GZ 1368-S-5-4	161.81	110.68	92.77	78.82	35.79	31.99	31.23	24.99
11	GZ 10241-19-11-1-4	123.86	95.75	94.19	81.55	35.02	30.92	31.91	21.13
12	Sakha108	151.24	95.03	95.53	81.21	41.57	37.46	36.59	27.03
13	SKC2015	97.60	75.31	93.52	80.68	38.21	30.36	34.73	20.52
14	IM16	178.53	90.17	93.43	80.36	35.86	31.90	35.93	27.53
15	IR 88628-B-B-45	234.78	209.39	94.73	83.35	39.56	34.50	33.56	27.25
16	IR 83106-B-B-9-31	199.30	173.17	93.58	81.09	33.35	25.82	33.57	20.13
17	GZ 9730-1-1-1-2	117.49	96.46	94.78	82.45	38.43	32.87	34.34	24.00
18	Sakha 107	117.44	76.96	94.30	81.80	41.94	36.88	33.40	27.78
19	IR 60080-46 A	176.02	125.65	94.67	82.58	37.52	32.36	36.08	28.84
20	IRAT 170	115.63	112.46	95.24	81.67	40.99	33.92	34.07	23.63
21	IR 11L465	96.58	89.47	93.80	80.18	35.93	30.65	35.12	21.78
22	N22	166.60	150.43	93.44	80.23	30.16	25.37	26.10	23.98
23	IR 100634-96-AJY-22	149.06	106.42	93.16	80.97	37.83	33.91	33.14	27.23
24	SPBG	121.36	62.26	94.14	80.80	36.10	30.45	24.14	22.04
25	IR 63731-1-1-4-3-2	128.10	107.70	95.03	82.99	37.84	33.88	27.48	23.41
26	HHZ 8-SAL6-SAL3-Y1	120.51	120.09	93.01	81.15	32.89	27.31	33.59	20.10
27	IRGC 78936	152.56	108.74	93.18	79.23	23.88	19.00	32.84	22.44
28	A22	117.69	89.43	94.24	81.20	40.33	24.74	35.47	22.90
29	IR 88628-B-B-10	93.22	80.13	94.10	81.42	43.88	35.90	34.22	22.73
30	IR 88628-B-B-16	136.86	135.74	93.93	81.60	41.10	36.26	33.08	23.86
31	IR 88628-B-B-21	253.50	185.42	96.41	82.28	41.78	36.16	33.42	21.27
32	IR 88628-B-B-31	233.85	193.64	97.44	82.86	37.44	31.40	31.99	27.51
33	IR 88628-B-B-36	97.08	78.30	93.84	80.39	35.70	29.86	37.73	24.07
34	IR 45427-2B-2-2B-1-1	104.29	81.54	95.99	81.51	42.13	24.99	33.68	23.69
35	GZ 7112-1-2-1-4	138.67	103.90	97.83	82.66	45.94	30.12	23.47	16.83
36	IRAT 161	110.81	88.87	91.74	80.32	38.73	31.14	27.19	23.14
37	FL 478	130.86	125.21	94.52	81.81	40.15	36.44	36.79	31.63
38	TCCP 266-1-3B-10-2-1	180.60	142.62	95.85	81.82	38.82	33.36	35.26	27.13
39	IR 65600-127-6-2	148.63	140.51	94.58	81.94	36.25	31.52	36.49	30.01
40	IR 69853-70-3-1-1	154.67	150.97	97.75	83.03	44.27	21.34	33.30	28.43
41	IR 68011-15-1-1	173.19	158.36	96.81	85.46	34.13	27.42	33.64	28.26
42	IR 65597-29-3-2-3	137.70	113.01	96.36	81.16	38.57	30.54	32.57	18.56
43	IR 66158-38-3-2	102.58	89.53	95.26	83.13	36.21	32.02	33.19	20.32
44	IR 66160-5-2-3-2	230.89	109.38	96.35	82.19	32.56	25.43	33.34	28.41
45	IRRI 148	127.14	69.08	95.95	81.47	38.41	32.72	25.77	21.57

NS: Non-stress; S: salt stress.

since successful fertilization at this stage is eventually converted into grain yield. As the rice genotypes in this study were grown after transplantation under saline conditions, their pollen viability was dramatically affected. This led to inadequate fertilization and poor seed setting as a consequence. Almost all genotypes reduced their percentage of spikelet fertility under stress, but those that dramatically decreased the percentage of fertility in conjunction with a very high reduction in grain yield were regarded as the sensitive genotypes for the reproductive stage (Hossain et al., 2015).

There are highly significant differences among most of the studied genotypes. Distinctly, grain yield per plant, the most desirable mean values were recorded from genotypes IR 65600-127-6-2, FL 478, IR 69853-70-3-1-1 and IR 66160-5-2-3-2 under both conditions. It is clear that each year's ranking of genotypes according to grain yield was different, suggesting different genotype responses to saline conditions. Those findings indicate that there is a genetic basis for variations in the expression of yield potential under salinity.

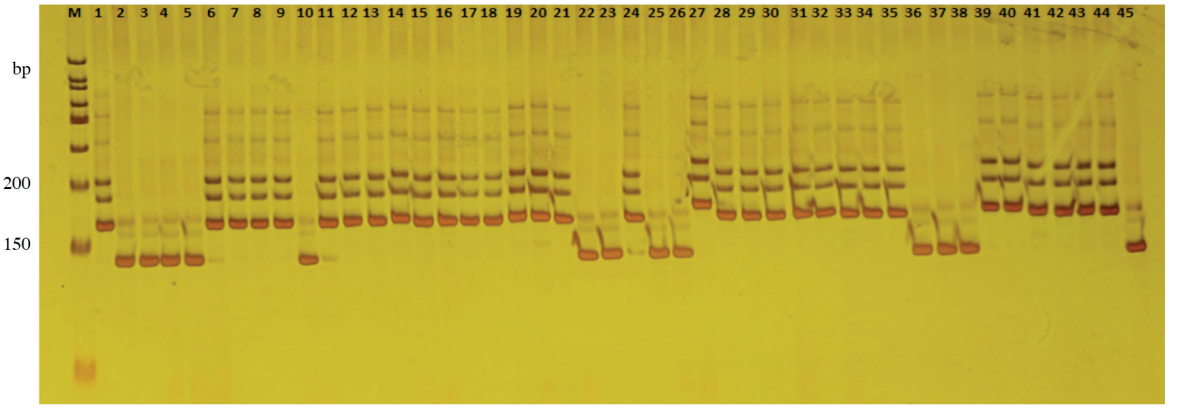
The decrease in overall vigor was caused by saline conditions, especially in the number of filled grains and the grain yield per plant. This may possibly be attributed to a high decrease in pollen viability under stress as a result. In sensitive genotypes, this is more pronounced than in tolerant rice genotypes like IR 63731-1-1-4-3-2, FL 478 and TCCP 266-1-3B-10-2-1. Adak et al. (2019) hypothesized that slow plant growth significantly reduced grain yields due to osmotic stress forced by a high concentration of salts in the root zone.

The response of genotypes to each condition was different based on the findings of each trait. It has been found that the studied traits of all studied genotypes are significantly influenced by salinity stress. The best values of the studied characteristics were provided by these genotypes during non-stress, but some genotypes could perform well under saline conditions. Using mean performance as an indicator of adaptation, the genotypes N22, IR 63731-1-1-4-3-2 (25), GZ 7112-1-2-1-4 (35), FL478 (37), TCCP 266-1-3B-10-2-1 (38), IR 65600-127-6-2, IR 68011-15-1-1 and IR 66160-5-2-3-2 appears to be widely adapted and relatively salt tolerant under salinity conditions, although their yield potential may be lower than that of genotypes adapted to the non-stress. Selection based on just yield may not be accurate, but selection via yield and its components is more efficient (El-Refaee et al., 2018). It is obviously of great interest to the plant breeder to choose genetically distinct individual from the mean of a segregating population.

Microsatellite variations of the screened rice genotypes

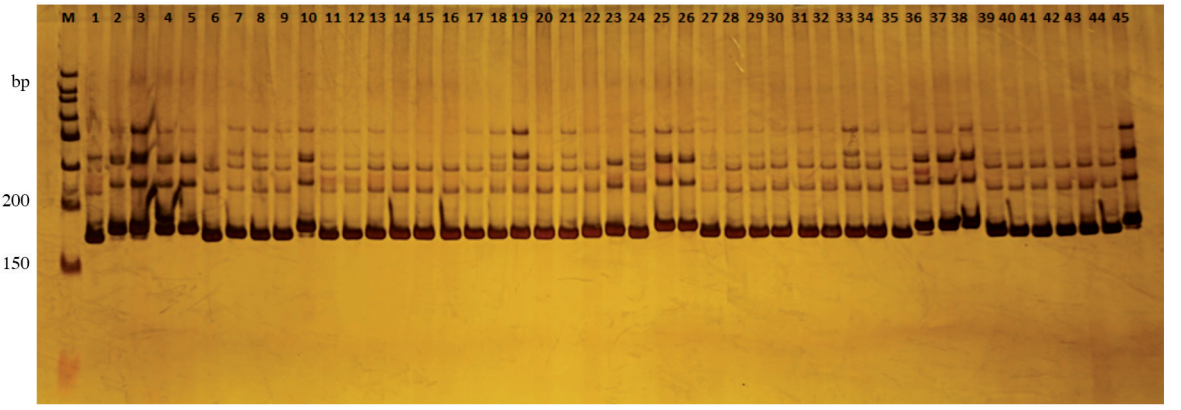
Twenty-one SSR markers were screened, three primers were monomorphic (RM1384, RM6990 and RM6193), hence the rest 18 primers were polymorphic and have informative bands. Primers RM6976, RM7631 and RM5556 showed bands that separated the tolerant and sensitive genotypes. Primers RM6976 (Figure 1), RM7631 (Figure 2) and RM5556 (Figure 3) were able to distinguish five highly moderate salinity tolerant Egyptian genotypes. It also distinguished another six exotic genotypes that consider as saline conditions including FL 478, the salt tolerant control. The Egyptian genotypes

Figure 1. A gel image of the banding pattern of the 45 studied rice genotypes with primer RM6976.



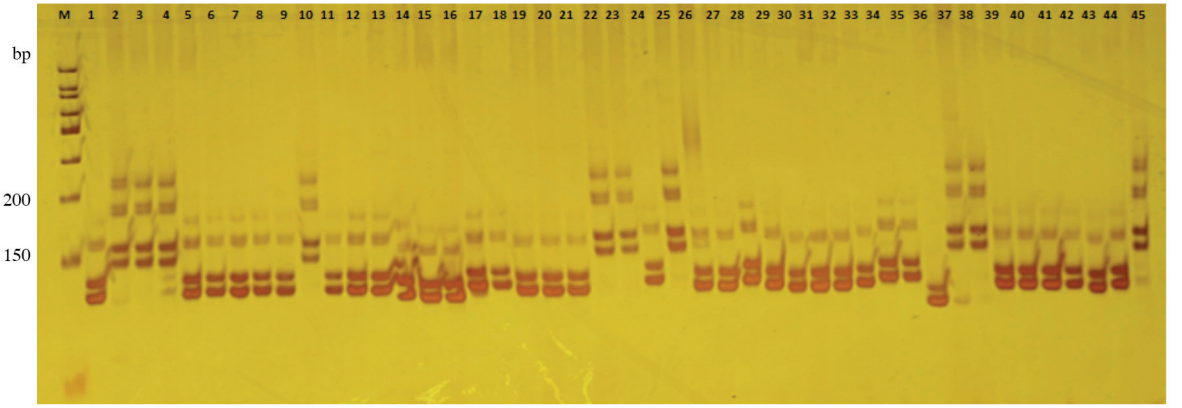
M is 50 bp ladder, rice genotypes from left to right are: (1) Giza 177, (2) Giza 178, (3) Giza 179, (4) Giza 182, (5) E. Jasmine, (6) Sakha101, (7) Sakha104, (8) Sakha105, (9) Sakha106, (10) GZ1368, (11) GZ10241, (12) Sakha108, (13) SKC2015, (14) IM16, (15) IR88628, (16) IR83106, (17) GZ9730, (18) Sakha107, (19) IR60080, (20) IRAT170, (21) IR11L465, (22) N22, (23) IR100634, (24) SPBG, (25) IR63731, (26) HHZ8, (27) IRGC78936, (28) A22, (29) IR 88628-B-B-10, (30) IR 88628-B-B-16, (31) IR 88628-B-B-21, (32) IR 88628-B-B-31, (33) IR 88628-B-B-36, (34) IR 45427, (35) GZ 7112, (36) IRAT161, (37) FL 478, (38) TCCP 266, (39) IR 65600, (40) IR 69853, (41) IR 68011, (42) IR 65597, (43) IR 66158, (44) IR 66160, (45) IRRI 148.

Figure 2. A gel image of the banding pattern of the 45 studied rice genotypes with primer RM7631.



M is 50 bp ladder, rice genotypes from left to right are: (1) Giza 177, (2) Giza 178, (3) Giza 179, (4) Giza 182, (5) E. Jasmine, (6) Sakha101, (7) Sakha104, (8) Sakha105, (9) Sakha106, (10) GZ1368, (11) GZ10241, (12) Sakha108, (13) SKC2015, (14) IM16, (15) IR88628, (16) IR83106, (17) GZ9730, (18) Sakha107, (19) IR60080, (20) IRAT170, (21) IR11L465, (22) N22, (23) IR100634, (24) SPBG, (25) IR63731, (26) HHZ8, (27) IRGC78936, (28) A22, (29) IR 88628-B-B-10, (30) IR 88628-B-B-16, (31) IR 88628-B-B-21, (32) IR 88628-B-B-31, (33) IR 88628-B-B-36, (34) IR 45427, (35) GZ 7112, (36) IRAT161, (37) FL 478, (38) TCCP 266, (39) IR 65600, (40) IR 69853, (41) IR 68011, (42) IR 65597, (43) IR 66158, (44) IR 66160, (45) IRRI 148.

Figure 3. A gel image of the banding pattern of the 45 studied rice genotypes with primer RM5556.



M is 50 bp ladder, rice genotypes from left to right are: (1) Giza 177, (2) Giza 178, (3) Giza 179, (4) Giza 182, (5) E. Jasmine, (6) Sakha101, (7) Sakha104, (8) Sakha105, (9) Sakha106, (10) GZ1368, (11) GZ10241, (12) Sakha108, (13) SKC2015, (14) IM16, (15) IR88628, (16) IR83106, (17) GZ9730, (18) Sakha107, (19) IR60080, (20) IRAT170, (21) IR11L465, (22) N22, (23) IR100634, (24) SPBG, (25) IR63731, (26) HHZ8, (27) IRGC78936, (28) A22, (29) IR 88628-B-B-10, (30) IR 88628-B-B-16, (31) IR 88628-B-B-21, (32) IR 88628-B-B-31, (33) IR 88628-B-B-36, (34) IR 45427, (35) GZ 7112, (36) IRAT161, (37) FL 478, (38) TCCP 266, (39) IR 65600, (40) IR 69853, (41) IR 68011, (42) IR 65597, (43) IR 66158, (44) IR 66160, (45) IRRI 148.

were namely Giza 178, Giza 179, Giza 182, E. Jasmine and GZ 1368-S-5-4. In the same text, the exotic genotypes are namely N22, IR 100634-96-AJY-22, IR 63731-1-1-4-3-2, FL 478 and TCCP 266-1-3B-10-2-1. All the rice genotypes generate similar bands to FL 478 and IRRI 148 the salt tolerant control genotypes. Related to the markers RM6976, RM7631, and RM5556, these bands had a molecular weight of 140, 170, and 150 bp, respectively, that were found in the salt tolerant varieties and could be considered as markers associated with salt tolerance.

Genetic diversity and identification of most informative markers

Out of 21 SSR primers used, three SSR primers were monomorphic and 18 SSR primers produced polymorphic bands. All the SSR primers were located on and covered chromosome 8. The chromosome 8 was the best choice in order to identify possibly new QTLs with significant phenotypic effect for salt tolerance, since the *Saltol* gene located on chromosome 1 (Davla et al., 2013). The 18 primers produced a total of 76 alleles, with an average of 4.22 alleles per locus (Table 7).

Table 7. SSR primers used with their parameters major allele frequency, number of alleles, gene diversity and polymorphic information content (PIC).

Nr	Primers	Major allele frequency	Number of alleles	Gene diversity	PIC
1	RM408	0.40	5	0.70	0.66
2	RM152	0.81	4	0.43	0.38
3	RM8018	0.70	5	0.51	0.46
4	RM5556	0.50	4	0.68	0.64
5	RM544	0.50	5	0.67	0.58
6	RM547	0.70	4	0.53	0.51
7	RM6008	0.50	2	0.47	0.41
8	RM8243	0.70	5	0.52	0.47
9	RM3395	0.50	4	0.62	0.55
10	RM6471	0.60	4	0.44	0.41
11	RM7027	0.85	4	0.34	0.31
12	RM3153	0.60	3	0.54	0.44
13	RM284	0.70	4	0.41	0.41
14	RM342	0.60	5	0.33	0.30
15	RM6976	0.40	6	0.73	0.71
16	RM419	0.82	3	0.40	0.36
17	RM7631	0.50	4	0.71	0.68
18	RM5545	0.70	5	0.57	0.53
	Total	11.08	76	9.60	8.81
	Mean	0.62	4.22	0.53	0.49

PIC: Polymorphic information content.

The highest polymorphic allele frequency was 85% produced by RM7027 primer and the lowest allele frequency was 40% produced by RM408 and RM6976 primers. For all 18 SSR primers, the general average allele frequency was 62%. Among the 45 rice genotypes screened, PIC of the 18 SSR polymorphic primers ranged from 0.30 to 0.71, with an average of 0.49. The genetic diversity within the population was 53% but RM6976 generated the highest diversity of 73.0% and RM342 the lowest diversity with value 33.0%. Primer RM6976 generated the highest PIC of 0.71 followed by RM7631 and RM408 respectively, with RM342 having the least PIC of 0.30. Primer RM6976 produces the highest diversity discrimination of 73.0% followed by RM7631 with 71.0% and RM408 with 70.0% respectively; while RM342 produces the lowest genetic diversity of 33.0%.

These findings are partially in line with those obtained by Anyomi et al. (2018) with 31 SSR primers, 36 rice genotypes were screened. The 28 polymorphic primers generated a total of 116 alleles with an average of 4.14 alleles per locus. The average allele frequency was 0.60. The PIC ranged from 0.053 to 0.829, with an average of 0.471. The genetic diversity within the population was 51.6%. Davla et al. (2013), who recorded a higher average PIC of 0.67 for 26 SSR markers within the range of 0.50 to 0.89, are also in line with the current findings. The number of alleles obtained per locus was 7.1, which was higher than the values that found in this study. Singh et al. (2011) noted marginally higher alleles (83) with a lower average of 2.76 alleles per marker in their genetic diversity analysis of rice genotypes using 30 SSR markers, but they had a high PIC value ranging from 0.54 to 0.96. All the parameters of their diversity were higher than those obtained in this investigation. A total of 168 alleles were found by Islam et al. (2012); the number of alleles per locus ranged from 2 to 6, which was similar to that obtained in this study (2 to 6); and the value obtained in this study was similar to an average of 4.2 alleles per locus. The value of PIC ranged from 0.21 to 0.76, with an average of 0.57 higher than this study's value of 0.49. Ganie et al. (2016) recorded higher parameters than those obtained in this study, they had a total of 176 alleles. Their number of alleles per locus was high, ranging from 6 to 22, with 14.6 alleles per locus on average. In order to differentiate the germplasm used, their primers were thus very useful. Roychowdhury et al. (2013) also found a total of 122 alleles higher than that obtained in this study, although compared to this study, the primer used had a significantly lower allele range of 2 to 5 alleles (2 to 6). A lower average of 3.21 alleles per locus was also recorded, but the PIC value was 0.524, which was higher than the results of this research. The number of alleles per locus ranged from 3 to 8, with an average number of alleles per locus of 4.86, as stated by Aliyu et al. (2011). With relation to the markers used in this analysis, this suggests almost the same degree of diversity.

A marker's high PIC value suggests a high probability of detecting the number of alleles between cultivars. A PIC value above 0.50 reveals a high degree of polymorphism. On this basis, the very good primers for this diversity analysis were RM408, RM5556, RM544, RM547, RM3395, RM6976, RM7631 and RM5545. The findings of this research indicate that the markers used are revealing and good for studies on genetic diversity. It is effective and cost-efficient to use microsatellites. They are abundant, co-dominant, highly reproducible and interspersed in the genome as compared with other markers. In particular, in rice genetic studies, microsatellite markers have been widely applied because they are capable of detecting high levels of allelic variation. The SSR markers play an important role in identifying salt tolerance genes that can be useful in developing new cultivars for plant breeders. In order to accelerate genetic advancement in rice, molecular markers could be used to tag QTL and determine their contributions to the phenotype by selecting desirable alleles at certain loci in the marker assisted selection (MAS) method. This is faster, more reliable and more cost-effective under saline field conditions than traditional screening (Aliyu et al., 2011). The results in this study indicate a great genetic resource for improving the salinity of rice in Egypt. In breeding programs to enhance rice materials for farmers, SSRs found here can be integrated.

Selection of salt tolerant genotypes

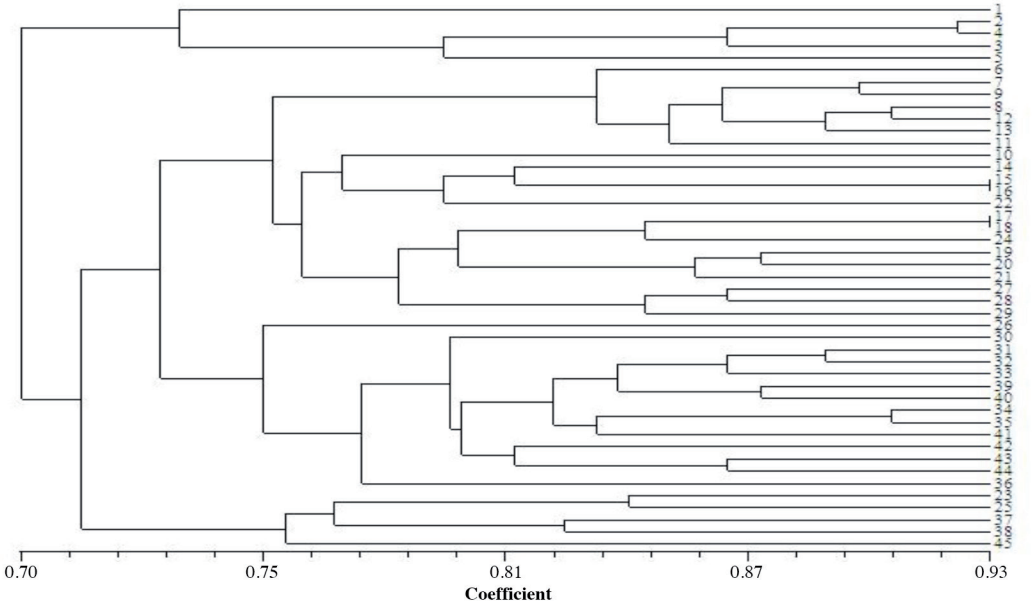
Progress in salt tolerance rice breeding requires determining the main locus at various growth stages with salt tolerance. Of the 18 polymorphic primers screened, only RM6976, RM7631, RM5556, RM152 and RM342 primers were able to differentiate between tolerant genotypes, namely the Egyptian genotypes Giza 178, Giza 179, Giza 182, E. Jasmine and GZ 1368-S-5-4. In the same text, the exotic genotypes are namely N22, IR 100634-96-AJY-22, IR 63731-1-1-4-3-2, FL 478, TCCP 266-1-3B-10-2-1 and IRRI 148 from sensitive ones such as Giza 177. Based on primer RM10711, Giza 178, Giza 179, Giza 182, E. Jasmine and GZ 1368-S-5-4 as local Egyptian genotypes were tolerant to salinity stress. In the same text, the exotic genotypes N22, IR 100634-96-AJY-22 and IR 63731-1-1-4-3-2 were tolerant to saline conditions. This indicates that selected SSR markers could be used to evaluate rice genotypes for salt tolerance then used as marker assisted selection in rice breeding.

Aliyu et al. (2011) used RM5556 on a collection of 150 different genotypes of rice with a salt-tolerant var. Pokkali and found the marker to be very informative. In their research on salt tolerance in some rice accessions, Davla et al. (2013) also considered the primer very insightful. In introgressive salinity tolerance QTLs Saltol in rice var. AS996 with 500 BC2F1 individuals, Huyen et al. (2012) used RM10793, RM10711. Rana et al. (2018) used 12 parental survey SSR markers, including three polymorphic SSR markers, OSR34, RM443 RM408 and RM169 RM152, which were selected to test 26 F_3 salt tolerance rice lines. Fifteen lines were classified as salt tolerant with respect to marker OSR34, nine lines were sensitive and two lines were heterozygous Sajib et al. (2012) selected various SSR primers and identified 15 rice lines using RM231 and RM544 primers as salt tolerant.

Genetic divergence of rice population as revealed by the dendrogram

The similarity values obtained for each pair wise comparison of SSR markers of the genotypes studied were used to create the dendrogram using the neighbor-joining (NJ) method based on UPGMA. The dendrogram constructs the genotypes into clusters, showing the genotypes' diversity. The 45 genotypes have been divided into two main groups (Figure 4). Group I consisted of the most highly moderate tolerant Egyptian rice var. Giza 178, Giza 179, Giza 182 and E. Jasmine with 0.80 similarities. Moreover, the moderate sensitive var. Giza 177 came separately but mixing with the previous tolerant ones in the same cluster. The grouping shown in cluster does not explicitly reflect saline conditions levels. This result was comparable to the result obtained by Kanawapee et al. (2011), which grouped the moderately tolerant cv. IR64 with the extremely sensitive rice 'Khao Kaset' and 'IR34'. Anyomi et al. (2018) reported that it was not surprising to see some sensitive cultivars in the same cluster mixing with tolerance. Group II consisted of the rest 40 rice genotypes, which are further subdivided into two clusters with 0.73 similarities. The most five tolerant exotic genotypes come separately alone in cluster-I with 0.75. The genotypes are namely IR 100634-96-AJY-22 and IR 63731-1-1-4-3-2, FL 478 and TCCP 266-1-3B-10-2-1. In the same text, IRRI 148 came alone separately but mixing with the previous tolerant ones in the same cluster. Cluster-II containing 35 genotypes, further divided into two sub clusters. Sub Cluster-I is further divided into three sub-groups (21 genotypes) with 0.76 similarities. The seven moderate saline conditions Egyptian genotypes came together in sub-group-I. These genotypes are namely Sakha 101, Sakha 104, Sakha 105, Sakha 106, Sakha 108,

Figure 4. Dendrogram of the clustering for rice genotypes to salt tolerant with the SSR markers.



SKC2015, GZ 10241-19-11-1-4. These genotypes are closely related in their genetic background, this probably explains why they were in the first cluster together, even though at different sub clusters. Subgroup-II is containing six genotypes, GZ 1368-S-5-4 and N22 came alone separately from each other but involved in the same subgroup. This possibly indicates that in saline environments they have the same behavior (tolerant). Subgroup-III is containing the rest nine rice genotypes. Regarding sub cluster-II is further divided into three sub-groups (14 genotypes). HHZ 8-SAL6-SAL3-Y1 and IRAT 161 came alone separately from each other in different subgroups but involved in the same sub cluster-II, this probably explains that they have the same behavior for saline conditions (tolerant).

Group I have highly moderate tolerance Egyptian rice vars. Giza 178, Giza 179, Giza 182 and E. Jasmine. Giza 177 came out separately from this group because of it sensitivity behavior for salinity tolerance, hence it is considering the most Egyptian rice variety sensitive for salinity under this study based on the agro-morphological performance. Group II consisted of the rest 40 rice genotypes, the five most tolerant exotic genotypes are namely IR 100634-96-AJY-22, IR 63731-1-1-4-3-2, FL 478, TCCP 266-1-3B-10-2-1 and IRRI 148. Moreover, the seven moderate saline tolerant conditions Egyptian genotypes are namely Sakha 101, Sakha 104, Sakha 105, Sakha 106, Sakha 108, SKC2015 and GZ 10241-19-11-1-4. In addition to genotypes, GZ 1368-S-5-4, N22, HHZ 8-SAL6-SAL3-Y1 and IRAT 161 have the same behavior for saline stress conditions (tolerant).

The genotype did not cluster with any of the others, demonstrating how different and varied it was from the rest. The cluster analysis developed may have been grouped according to their location and genetic origin for the 45 rice genotypes studied. As these cultivars have different genetic backgrounds, Kanawapee et al. (2011) have proposed intercrossing cultivars from various clusters. Kanawapee et al. (2011) suggested intercrossing KDML 105 with salinity tolerant SPR90 out of 30 different rice cultivars examined, since both have different genetic background and physiological tolerance levels for salinity and also have different characteristics and physiology. They indicated that the progeny derived from KDML 105 would have better characteristics than both parents. The IRRI germplasm showed a high degree of heterogeneity that shows how genetically diverse they are and how rich the germplasm is. For rice breeding, this is good as it suggests a rich array of genes that could be beneficial for improving the crop. These findings further demonstrated the divergence of the studied population (El-Refaee et al., 2018). In breeding to enhance local rice cultivars, this diversity can be explored. It was possible for microsatellite markers to differentiate between salt tolerant and susceptible entries.

CONCLUSIONS

Genotypes N22, IR 63731-1-1-4-3-2, GZ 7112-1-2-1-4 FL478, TCCP 266-1-3B-10-2-1, IR 65600-127-6-2, IR 68011-15-1-1 and IR 66160-5-2-3-2 were selected based on the morphological performance under salt stress as well as by SSR markers to be tolerant to salinity stress. The selected SSR markers (RM6976, RM7631, RM408, RM5556 and RM544) demonstrated polymorphism in 45 rice genotypes. These markers could be able to discriminate against tolerant genotypes from sensitive genotypes; their polymorphic information content (PIC) values were high supporting their ability and usefulness to separate the rice genotypes in this study under salt stress. Consequently, they would be candidate markers and genotypes for the further production of improved varieties and would be used to breed salt-tolerant cultivars with higher potential yields.

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