

Genetic Diversity for Cold Tolerance at Seedling Stage in Rice (*Oryza sativa* L.) under Egyptian Conditions

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BREEDING rice for cold tolerance is one of the important ways for climate change facing, which attacks a large amount of the cultivated areas all over the world countries. This study was performed at Sakha Agricultural Research Station during two rice growing seasons 2015 and 2016 to evaluate some rice accessions which belong to different rice types for the ability of cold tolerance in seedling stage at early growing season under Egyptian conditions. The 40 rice accessions used in this study were evaluated at two early dates of sowing 1st March and 1st April as a cold condition, in addition to control sowing date at 1st May. Twenty six SSR markers related to cold tolerance at seedling stage were used in order to determine the genetic diversity and polymorphism among the studied genotypes. Low temperature degree condition significantly decreased the mean values of the all studied traits for all accessions compared with the control condition. Significant differences were observed among the studied genotypes under both different growing conditions. Japonica varieties are found to be more tolerant than Indica varieties. SSR markers RM265, RM324, RM163 and RM270 showed highly polymorphism among the studied rice accessions and more over differentiate them to different groups based on their ability for cold tolerance. Giza 177, Sakha 102 and Sakha 104 out of the Egyptian rice genotypes showed a good performance at low temperature degrees condition in early growing season. The genotypes CT6749-36-7-2-M-M, Milyang 80, PSRM1-17-4B-13, Ryong Sung 25 can be used as a donor for cold tolerance in rice breeding program.

Keywords: Rice, Evaluation, Cold tolerance, Seedling stage, SSR markers

Introduction

Rice (*Oryza sativa* L.) is one of the major staple food crops and critical to food security for billions of people around the world. Egypt is the largest rice producer in the Near East region. The rice cultivated area in Egypt is about 0.6 million ha annually, all is under irrigation eco- system (Kim et al., 2011). Rice cultivation takes place at Egyptian Nile delta especially in the northern part. Constantly changing climatic conditions in Egypt and around the world demand constant efforts to understand and adapt to environmental challenges and developing new varieties for sustainable crop production. The challenge is even greater for crops such as rice (*Oryza sativa* L.), which is the staple of more than half the world's population and grown under diverse environmental conditions. So, rice breeders considered to be the most pragmatic approach for better yield against environmental challenge and

population increase. One important strategy for increasing crop productivity is to minimize losses due to biotic and abiotic stresses by developing more stress-tolerant varieties (Boyer, 1982 and Khush, 1999).

Intraspecific genetic variability for cold tolerance has been reported in rice and according to reports Japonica cultivars are more tolerant to low temperatures than Indica cultivars (Andaya and Mackill, 2003). Genetic variability for cold tolerance has been evaluated by researchers at germination, seedling and reproductive stages in rice breeding programs. Cold stress in rice leads to poor germination and stunted growth of seedlings, delays flowering, lengthens the growth cycle, reduces tillering, increases plant mortality (Mackill and lei, 1997) and causes significant yield loss in rice production (Murai et al., 1991 and Nagasawa et al., 1994), thus enhancing cold tolerance through the use of a diverse germplasm

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of Japonica rice is a very important objective in rice breeding. To enhance cold tolerance in rice, it is therefore imperative to exchange and evaluate various rice germplasm from temperate rice-growing countries for cold stress at various growth stages in key Japonica rice cultivation sites. Elite cold-tolerant rice germplasm also need to be crossed with a diverse set of elite cultivars from each country. However, there are limited genetic resources for the improvement of cold tolerance in temperate Japonica rice cultivars. Some tropical Japonica cultivars have been reported to exhibit cold tolerance (Glaszmann *et al.*, 1990 and Saito *et al.*, 2001).

Various methods can be used to create cold injury environments to evaluate cold tolerance at the booting and reproductive stages in rice. However, screening several materials for cold tolerance is difficult, more so in producing reliable results. Therefore, it is necessary to use a reproducible phenotyping method to measure tolerant and sensitive traits under cold stress and apply improved QTL analysis methods to identify effective QTLs for cold tolerance at the reproductive stage. SSR markers produce a high frequency of polymorphic alleles in rice and are suitable for evaluating genetic diversity among closely related rice cultivars, including Japonica rice (Suh *et al.*, 2004). Several studies aim to identify QTLs conferring cold tolerance at early growth stage by using SSR molecular markers including cold tolerance at the seedling, tillering stage and the growth ability of seedling under low temperatures in rice. The detected QTLs associated with the cold tolerance in early growth period will provide the effective genetics evidences for the molecular marker assisted breeding (Qia *et al.*, 2004, Xu *et al.*, 2008 and Ye *et al.*, 2010).

Problems for farmers leading to loss of production as well as reduced milling quality caused by water deficit at late June and high temperature at August. Thus, development of rice varieties tolerant to low temperatures at the early stages will allow farmers to plant their own season crop in April beginning at least. Hence, they able to avoid the water deficit at tillering, and panicle initiation stages and high temperature at ripening stage exhibited high negative effect on grain quality. This study aims to evaluate some introduced genotypes and Egyptian commercial varieties in addition to promising lines using phenotyping and genotyping characterization to identify the best genetic sources for cold tolerance breeding program.

Materials and Methods

Plant materials and phenotyping

A total of 40 rice accessions belong to the three rice subspecies; Japonica, Indica and Indica/Japonica used at this study to evaluate its ability for cold tolerance at seedling stage under early growing season. The experiment was conducted at the green house of Rice Research & Training Center (RRTC), Sakha, Egypt. The rice germplasm accessions were originally procured from the International Rice Research Institute (IRRI), Philippines and Rice Research & Training Center (RRTC), Egypt (Table 1). The grains of the 40 rice accessions were sown in plastic seedling plates with a size of 60 cm × 30 cm × 5 cm, supplied with nutrient soil and managed by the recommended agricultural practices, at two early dates of sowing 1st March and 1st April as a cold stress, in addition to control sowing date at 1st May. Randomized complete block design (RCBD) with three replicates was the experimental design and repeated in two rice growing seasons of 2015 and 2016. During the two sowing dates the degrees of temperature were lower than normal condition for rice germination and growing, the temperature degrees in Table 2 were recorded by Rice Research and Training Center meteorological station, Sakha, Kafr Elsheikh, Egypt. When the seedlings were at 30 days age, five seedling observations were recorded: germination %, germination index, seedling height, root length and number of leaves/seedling. The cold reduction index used to determine the accessions ability to tolerate the cold effects and calculate as follow: [(the value under normal conditions – the value under stress)/the value under normal conditions]*100.

Statistical analysis

The obtained data were statistically analyzed for analysis of variance according to Gomez and Gomez (1984) by using computer statistical software of MSTAT-C. Treatment means were compared by LSD. Cluster analysis was analyzed by Paleontological statistics PAST software, version 2.17 (Saitou and Nei, 1987).

SSR markers analysis

The fresh leaves of 40 rice accessions were sampled for DNA extraction according to modified CTAB (Cetyl Try Methyl Ammonium Bromide) method (Rogers and Bendich, 1988). A total of twenty six SSR markers associated with cold tolerance from previous studies were used in the polymorphism survey. PCR was performed as follow: The 10-μL PCR reaction mixture contained

1 x buffer, 0.2 μ M of each primer, 50 μ M of dntps, 0.5 unit of Taq polymerase (Tiangen Company, Beijing, china), and 10 ng of genomic DNA as a template. The thermal cycler was programmed for a first denaturation step of 4 min at 94°C, followed by 30 cycles, each of 94°C for 30 s, 55° for 30 s and 72°C for 30 s and final extension step of 5 min at 72°C. The PCR products were separated on 8.0% non-denaturing polyacrylamide gel and detected using the silver staining method. Generated DNA bands were analyzed and scored 1 for the presence- or 0 for the absence of allele. Four SSR markers (RM 265, RM324, RM163 and RM270) were highly polymorphic among the studied genotypes which used for genotyping are listed in table 3 (Jiang et al., 2011 and Yang et al., 2013) and these markers located on chromosomes 1, 2, 5 and 12, respectively.

Resultes and Discussion

Analysis of variance

Analysis of variance showed that the mean square values due to years (Y) were significant and highly significant for all studied traits except germination index. Genotypes (G) and treatments (T) were highly significant for all studied traits, while all possible interactions (Y x G, Y x T, G x T and Y x G x T) showed highly significant for all studied traits except number of leaves /seedling (Table 4). These results indicating for high variances between genotypes in addition to effective treatments by sowing date as a different temperature under Egyptian conditions. On other hand, a little effect of years under Egyptian conditions. Abdelmaksoud et al., 2007; Elgamal, 2013 and Elgamal et al., 2015 reported that the effect of years is not highly significant on rice growing under Egypt conditions.

TABLE 1. Origin and type of the 40 rice accessions used in this study

No.	Accession name	Origin	type	No.	Accession name	Origin	Type
1	CT6749-36-7-2-M-M	Chile	Japonica	21	Giza 181	Egypt	Indica
2	H270-30-2-1	Argentina	Indica	22	Giza 182	Egypt	Indica
3	Barakat (K787-13)	India	Indica	23	Sakha 101	Egypt	Japonica
4	Hexi 25	China	Japonica	24	Sakha 102	Egypt	Japonica
5	HSC16	Hungary	Indica	25	Sakha 103	Egypt	Japonica
6	IR63347-AC201-1	Philippines	Indica	26	Sakha 104	Egypt	Japonica
7	L10573(ACC68106)	USA	Japonica	27	Sakha 105	Egypt	Japonica
8	LOTO	Italy	Indica	28	Sakha 106	Egypt	Japonica
9	Milyang 80	Korea	Indica /Japonica	29	E. Yasmine	Egypt	Indica
10	PEGASO	Italy	Indica	30	GZ4328-1-2-1-3	Egypt	Indica/ Japonica
11	PSRM1-17-4B-13	Philippines	Indica	31	GZ4362-34-2-1-3	Egypt	Indica/ Japonica
12	Ryong Sung 25	Korea	Japonica	32	GZ4461-4-2-3-1	Egypt	Indica/ Japonica
13	SOHAECHAL	Korea	Japonica	33	GZ4514-3-1-3-1	Egypt	Indica/ Japonica
14	ZHONG JIA	China	Indica /Japonica	34	GZ9057-34-6-1-3-2(Giza179)	Egypt	Indica/ Japonica
15	ZHONG JU3	China	Japonica	35	GZ9057-34-6-1-3-3	Egypt	Indica/ Japonica
16	Giza 171	Egypt	Japonica	36	GZ9523-2-1-1-1	Egypt	Indica/ Japonica
17	Riho	Egypt	Japonica	37	GZ9577-4-1-1	Egypt	Indica/ Japonica
18	Giza 175	Egypt	Japonica	38	CT9506-44-2-1-1-M-3-4p-M-1	Colombia	Indica
19	Giza 177	Egypt	Japonica	39	CT9882-16-4-2-3-2-p-M	Colombia	Indica
20	Giza 178	Egypt	Indica/ Japonica	40	CT9505-17-3-1-1-M-1-3p-M	Colombia	Indica

TABLE 2. Average of temperature degrees between day and night for growing dates

Month	2015			2016		
	1 st – 10 th	11 th – 20 th	21 th – 30 th	1 st – 10 th	11 th – 20 th	21 th – 30 th
March	17.35 °C	16.23 °C	18.38 °C	17.55 °C	16.31 °C	18.39 °C
April	19.80 °C	20.98 °C	23.77 °C	19.30 °C	18.60 °C	21.46 °C
May	25.67 °C	23.67 °C	25.73 °C	23.21 °C	24.35 °C	25.91 °C

TABLE 3. List and sequences of polymorphic primers

No	Name	Forward sequence	Reverse sequence
1	RM265	5'-CGAGTTCGTCCAAGTGAGC-3'	5'-CATCCACCATTCCACCAATC-3'
2	RM324	5'-CTGATTCCACACACTTGTGC-3'	5'-GATTCCACGTCAGGATCTTC-3'
3	RM163	5'-ATCCATGTGCGCCTTTATGAGGA-3'	5'-CGCTACCTCCTTCACTTACTAGT-3'
4	RM270	5'-GGCCGTTGGTTCTAAAATC-3'	5'-TGCGCAGTATCATCGGCGAG-3'

TABLE 4 . Analysis of variance and the mean squares of years, treatments and genotypes, and the inter actions for all studied traits

S. O. V.	D.F.	G %	G. I	S.H	R. L	N.L/S
Reps (R)	2	3.890	0.029	0.094	1.106	4.213
Years (Y)	1	764.0**	0.001	0.350**	3.199**	3.627*
Treatments (T)	2	7637**	9.474**	1212**	623.3**	188.6**
Genotypes (G)	39	2088 **	0.121**	8.525**	6.205**	5.680**
Y.T	2	734.2**	0.002*	0.022**	0.575**	0.520
Y.G	39	89.97**	0.004**	0.265**	0.162**	1.088
T.G	78	842.1**	0.038**	2.636**	2.504**	1.004
Y.T.G	78	95.18**	0.003**	0.262**	0.168**	0.770
Residual	478	7.663	0.001	0.020	0.045	0.784

Note: *, ** significant at 0.05 and 0.01 levels of probability, respectively.

G %: germination percentage, G.I: germination index, S.H: seedling height, R.L: root length and N.L/S: number of leaves / seedling. Treatments are different temperature degrees as a three different dates of sowing.

Phenotyping characterization

Low temperature effects on rice seedling growth such as; poor and delayed germination, retarded seedling growth, leaves discoloration and inhibited rooting (Lee 2001; Shu and Jayab 2001). Results presented in Tables 5 - 7 showed that there is no particular accession recorded the highest values for all studied traits at the different levels of low temperature along with the control during the two growing seasons. On the other hand, some of the studied genotypes, *i.e.* CT6749-36-7-2-M-M (1), Milyang 80 (9), Ryong Sung 25 (12), CT9505-17-3-1-1-M-1-3p-M (40), Giza 177 (19), Sakha 102 (24), Sakha 104 (26) showed good results for most studied traits.

For Germination % the accession Giza 177(19) followed by Sakha 104 (26), Giza 171(16), Sakha 101(23), Sakha 102 (24) and Riho (17) scored the highest values under the lowest cold degree for the two growing seasons with range from 83.67% to 69.00 %. Regarding to germination index the Accessions Milyang 80 (9), Giza 177 (19), Sakha 104 (26), Sakha 102 (24), Ryong Sung 25 (12) and Sakha 103(25) showed the best performance after germination under the lowest degree of temperature for the two growing seasons and the values ranged from 0.80 to 0.70.

With respect to, seedling height the accessions CT9505-17-3-1-1-M-1-3p-M (40), Zhong Jia(14),

CT6749-36-7-2-M-M (1), Giza 177(19), Giza 171(16) and Sakha 102 (24) had the highest seedling under the hardest stress for the two grown seasons with values 5, 4.5, 4, 3.83, 3.83 and 3.67cm respectively. While the accessions CT9505-17-3-1-1-M-1-3p-M (40), CT9882-16-4-2-3-2-p-M (39), CT6749-36-7-2-M-M (1), Giza 177 (19), PSRM1-17-4B-13(11) and Riho(17) showed the deepest root length under the coldest growing date for the two growing seasons with values ranged from 5 to 3.17cm. In relation to number of leaves per seedling the accessions Milyang 80 (9), Sakha 104 (26), Ryong Sung 25 (12), CT6749-36-7-2-M-M (1), Giza 177 (19) and Sakha 102 (24) recorded the highest number of leaves under cold stress with values ranged from 3.66 to 2.66. These results indicated that these accessions have a good ability for growing at early growing dates when the temperature degrees are under the normal levels, and it could be conducted that these accessions have a cold tolerance trait. In order to emphasizes the cold tolerant accessions by using cold reduction index, the accessions CT6749-36-7-2-M-M, H270-30-2-1, Milyang 80, PSRM1-17-4B-13, Ryong Sung 25, Giza 177, Sakha102, Sakha 104, CT9882-16-4-2-3-2-p-M (Table 8) gave the lowest reduction index that means these accessions haven't been affected by cold stress.

TABLE 5. The mean performance of accessions for three sowing dates at two years of 2015 and 2016 for germination percentage and germination index

Genotypes	Germination (%)						Germination Index					
	2015			2016			2015			2016		
	1 st March	1 st April	1 st May	1 st March	1 st April	1 st May	1 st March	1 st April	1 st May	1 st March	1 st April	1 st May
1	36.00	73.00	91.67	41.33	72.00	92.00	0.66	0.80	0.96	0.66	0.69	0.98
2	11.03	78.00	91.33	20.30	75.00	91.33	0.60	0.82	0.94	0.60	0.80	0.94
3	37.33	73.00	83.00	38.33	71.00	85.33	0.20	0.40	0.90	0.40	0.60	0.90
4	24.33	83.33	90.67	31.00	73.00	92.00	0.30	0.40	0.94	0.30	0.60	0.94
5	24.67	75.33	81.33	29.33	72.00	81.33	0.20	0.50	0.84	0.33	0.50	0.84
6	15.00	91.33	93.33	43.33	88.30	93.33	0.40	0.60	0.90	0.40	0.60	0.90
7	70.00	81.33	91.00	66.66	80.00	91.00	0.30	0.40	0.92	0.33	0.40	0.92
8	17.30	60.33	90.33	31.33	60.00	90.33	0.30	0.30	0.88	0.30	0.40	0.88
9	42.33	63.67	81.33	42.33	61.00	83.33	0.80	0.80	0.93	0.80	0.80	0.96
10	20.30	57.67	63.67	35.33	60.00	65.66	0.40	0.60	0.68	0.40	0.60	0.76
11	33.67	86.67	88.33	36.67	85.30	88.33	0.60	0.60	0.90	0.60	0.66	0.90
12	48.00	75.67	93.33	49.33	75.00	96.67	0.71	0.88	0.94	0.70	0.90	0.95
13	37.67	77.33	78.33	41.67	75.33	79.33	0.46	0.70	0.84	0.46	0.70	0.84
14	22.00	80.00	82.33	43.66	78.00	82.33	0.36	0.80	0.88	0.36	0.80	0.88
15	49.27	75.33	74.00	50.67	75.33	77.33	0.44	0.80	0.84	0.44	0.80	0.84
16	79.67	81.33	86.33	78.33	82.00	86.33	0.50	0.84	0.90	0.50	0.84	0.90
17	72.00	95.33	97.67	69.00	92.30	97.67	0.60	0.80	0.90	0.60	0.80	0.90
18	33.00	93.33	100.00	55.33	85.70	97.66	0.40	0.68	0.88	0.40	0.68	0.88
19	83.67	91.33	94.33	80.33	90.00	94.33	0.74	0.90	0.97	0.75	0.80	0.97
20	39.67	86.00	74.00	40.00	85.00	74.00	0.65	0.80	0.93	0.65	0.80	0.93
21	13.33	78.00	85.33	25.00	79.00	85.33	0.18	0.60	0.98	0.41	0.66	0.98
22	35.00	80.00	75.33	54.66	77.66	75.33	0.21	0.70	0.96	0.33	0.70	0.96
23	78.00	65.00	82.67	78.33	66.00	82.67	0.18	0.66	0.97	0.33	0.66	0.97
24	74.33	90.67	95.00	73.33	90.00	95.00	0.72	0.88	0.96	0.72	0.88	0.96
25	67.33	90.67	100.00	70.00	85.00	98.66	0.70	0.80	0.98	0.70	0.76	0.98
26	81.67	83.33	91.67	80.00	84.00	91.67	0.72	0.78	0.98	0.74	0.81	0.98
27	64.33	84.67	96.67	61.00	82.00	96.67	0.73	0.60	0.94	0.60	0.66	0.94
28	49.67	61.67	62.00	48.66	60.00	62.00	0.70	0.80	0.97	0.66	0.80	0.97
29	15.30	60.33	58.00	32.33	62.00	66.66	0.12	0.60	0.90	0.33	0.60	0.90
30	59.30	57.67	67.33	52.31	55.70	67.33	0.44	0.70	0.94	0.44	0.70	0.94
31	59.33	46.00	70.67	51.33	48.30	70.67	0.44	0.60	0.92	0.44	0.60	0.92
32	24.33	52.67	56.33	38.70	55.00	62.33	0.50	0.80	0.94	0.50	0.80	0.94
33	59.33	68.67	69.33	57.66	68.67	69.33	0.60	0.80	0.90	0.60	0.80	0.90
34	30.33	70.33	60.67	45.00	68.66	60.67	0.44	0.70	0.92	0.33	0.70	0.92
35	48.00	59.33	56.00	56.66	63.00	69.66	0.40	0.80	0.94	0.40	0.80	0.94
36	32.33	31.67	52.33	44.66	45.68	55.33	0.40	0.80	0.92	0.40	0.80	0.92
37	54.00	79.33	89.33	55.66	75.70	89.33	0.30	0.70	0.80	0.40	0.70	0.80
38	50.00	60.00	92.00	50.00	60.66	92.00	0.50	0.80	0.80	0.50	0.80	0.83
39	40.00	60.00	90.00	42.00	57.70	90.00	0.60	0.80	0.88	0.60	0.80	0.88
40	60.00	70.00	98.00	62.00	70.00	95.33	0.65	0.90	0.96	0.75	0.86	0.98
LSD 5%	2.412	3.368	2.941	2.607	3.166	3.079	0.043	0.041	0.043	0.038	0.041	0.041
LSD 1%	3.469	4.846	4.231	3.752	4.556	4.430	0.062	0.059	0.062	0.055	0.059	0.059

TABLE 6. The mean performance of the studied rice accessions for three sowing dates at two years of 2015 and 2016 for seedling height and root length

Genotypes	Seedling height (cm)						Root length (cm)					
	2015			2016			2015			2016		
	1 st March	1 st April	1 st May	1 st March	1 st April	1 st May	1 st March	1 st April	1 st May	1 st March	1 st April	1 st May
1	4.00	5.00	7.67	4.00	4.87	7.33	3.50	4.17	4.67	3.50	4.66	5.66
2	2.83	4.00	5.50	4.83	4.10	5.57	2.67	4.33	4.33	3.50	4.33	4.66
3	2.83	3.83	5.83	3.17	3.83	5.83	2.33	4.83	5.33	3.00	4.66	5.33
4	2.83	5.17	7.00	3.33	5.23	7.00	2.50	5.00	5.50	2.33	5.00	5.50
5	3.17	4.67	6.83	2.67	4.50	6.83	2.83	4.67	6.00	2.00	4.67	6.00
6	2.83	4.33	7.00	2.67	4.33	7.50	2.33	3.83	6.33	2.00	3.66	6.33
7	1.83	4.67	7.67	3.17	4.50	7.67	2.50	3.67	5.83	2.67	3.67	5.83
8	3.00	4.67	6.50	3.33	4.67	6.57	3.00	3.83	5.00	3.00	3.66	5.00
9	3.67	4.33	6.83	2.67	4.30	6.83	3.17	4.17	5.17	1.83	4.33	5.33
10	3.67	5.33	6.83	2.50	5.40	6.83	2.83	3.50	5.67	2.00	3.50	5.67
11	3.50	4.17	5.50	2.67	4.00	5.67	3.33	3.83	4.83	2.83	3.83	4.83
12	3.67	5.33	7.67	3.83	5.50	7.67	3.17	3.83	6.00	3.33	3.60	6.00
13	3.00	4.33	7.67	3.17	4.50	7.67	3.00	3.50	5.50	3.17	3.50	5.50
14	4.50	4.50	9.00	3.50	4.67	8.50	3.00	4.33	6.17	2.67	4.33	6.33
15	2.83	5.50	9.67	2.50	5.33	8.67	2.83	3.33	6.00	2.33	3.33	6.00
16	3.67	4.17	7.67	3.67	4.33	7.67	3.17	3.17	5.00	3.17	3.00	5.00
17	3.33	5.33	9.67	3.67	5.33	8.67	3.17	3.67	7.30	3.33	3.67	7.20
18	2.83	4.00	7.67	3.67	4.00	7.67	2.67	3.33	4.50	3.00	3.33	4.50
19	3.67	5.83	8.33	3.83	5.67	8.33	3.33	4.33	6.83	3.50	4.33	6.83
20	3.67	4.33	9.33	2.50	4.67	8.33	3.00	3.67	6.67	2.50	3.67	6.33
21	2.00	4.33	8.50	2.67	4.33	8.50	2.17	2.33	7.00	2.33	3.00	7.00
22	2.50	4.50	7.00	2.67	4.33	7.00	2.50	2.50	6.67	2.17	2.66	6.67
23	2.73	4.33	7.33	2.83	4.33	7.33	2.17	2.50	5.50	2.33	2.33	5.50
24	3.50	5.00	9.00	3.83	5.33	8.00	2.67	3.17	7.00	2.33	3.17	7.33
25	3.00	5.33	7.00	2.83	4.67	6.83	2.83	3.33	6.67	2.17	3.67	6.17
26	3.33	4.50	8.33	2.67	4.67	8.33	2.50	3.83	7.17	2.33	3.67	7.00
27	3.33	5.33	7.83	2.33	5.33	7.67	3.33	4.17	5.83	2.17	4.00	5.83
28	3.00	4.83	8.67	2.33	5.33	8.33	2.67	3.27	6.50	2.17	3.00	6.33
29	1.83	4.67	8.00	2.17	4.67	8.00	1.83	2.83	6.33	2.00	3.00	6.33
30	2.50	4.33	6.00	2.33	4.33	6.00	2.67	3.33	5.00	2.50	3.33	5.00
31	2.50	3.83	6.00	2.33	3.67	6.00	2.50	2.50	4.33	2.50	2.50	4.33
32	2.50	3.33	6.33	1.67	3.67	6.33	2.67	3.50	5.17	2.66	3.50	5.33
33	2.50	5.17	7.00	2.17	5.33	7.00	2.67	3.17	6.17	2.33	3.17	6.17
34	2.50	5.50	7.33	2.50	5.50	7.33	2.67	3.83	5.17	1.83	3.83	5.33
35	2.33	4.50	6.67	2.67	4.50	6.67	2.17	2.33	5.17	2.33	2.66	5.17
36	2.17	3.00	5.50	2.33	3.00	5.50	2.00	2.00	3.83	2.00	2.66	4.00
37	2.83	4.17	7.00	2.83	4.17	7.00	2.50	2.17	4.67	2.50	2.33	4.67
38	2.33	3.83	6.00	3.33	4.00	6.00	2.67	4.00	5.00	3.00	3.00	5.00
39	3.00	4.00	6.00	3.33	4.00	6.33	4.00	4.00	6.33	3.00	4.00	6.00
40	5.00	8.00	9.00	4.00	7.67	8.67	5.00	6.00	8.00	4.33	6.66	8.33
LSD 5%	0.234	0.307	0.445	0.242	0.355	0.417	0.304	0.350	0.407	0.339	0.330	0.393
LSD 1%	0.336	0.442	0.639	0.346	0.468	0.598	0.436	0.504	0.586	0.488	0.476	0.566

TABLE 7. The mean performance of the studied rice accessions for three sowing dates at two years of 2015 and 2016 for number of leaves/seedling

Genotypes	No. of leaves/seedling					
	2015			2016		
	1 st March	1 st April	1 st May	1 st March	1 st April	1 st May
1	2.66	3.00	3.66	3.00	3.00	3.66
2	2.66	2.00	4.66	2.66	2.00	4.00
3	1.33	1.66	3.00	1.33	1.66	2.66
4	1.33	2.00	2.00	1.66	2.00	2.00
5	2.00	2.33	3.66	2.00	2.33	3.66
6	2.00	2.00	2.66	2.00	2.00	2.66
7	1.66	2.00	2.66	1.66	2.00	2.66
8	2.00	2.00	3.66	2.00	2.00	3.66
9	3.66	3.66	4.00	3.66	3.66	4.33
10	1.33	1.00	2.66	1.33	1.00	3.00
11	1.33	2.66	3.00	1.66	2.33	3.00
12	3.00	3.66	4.00	3.00	3.66	4.00
13	1.00	1.00	2.33	1.00	1.33	2.66
14	1.00	1.00	2.66	1.00	1.00	2.66
15	2.66	1.00	4.00	2.66	1.00	4.00
16	1.33	1.66	3.00	1.00	1.66	2.33
17	1.66	1.00	2.33	1.66	1.66	3.00
18	1.00	1.66	3.00	1.00	1.66	2.66
19	2.66	3.00	4.00	2.66	3.33	4.00
20	1.66	2.00	3.00	1.66	2.00	3.00
21	1.66	1.00	2.66	1.66	1.66	3.00
22	1.00	1.33	2.00	1.00	1.00	2.33
23	1.00	1.00	2.33	1.00	1.00	2.66
24	2.66	3.00	4.00	2.66	3.33	3.66
25	1.66	2.33	3.66	1.66	2.25	4.00
26	3.00	3.33	4.00	3.33	3.00	4.33
27	1.33	2.66	3.66	1.33	2.33	3.66
28	1.33	2.66	4.00	1.33	2.00	4.00
29	1.33	2.00	4.33	1.33	2.33	4.33
30	1.00	1.00	2.00	1.00	1.00	2.33
31	1.00	1.33	3.33	1.00	1.00	3.33
32	1.00	1.00	2.00	1.00	1.00	2.00
33	1.00	1.66	3.66	1.00	1.66	3.66
34	1.00	1.66	2.00	1.00	1.66	2.33
35	1.00	1.66	2.00	1.00	1.66	2.33
36	1.00	1.00	1.66	1.00	1.00	1.66
37	1.00	1.00	1.66	1.00	1.00	1.66
38	2.00	2.00	3.00	2.00	2.00	3.00
39	2.33	2.00	4.00	2.33	2.00	4.00
40	2.33	3.33	4.00	2.66	3.33	4.33
LSD 5%	0.339	0.381	0.345	0.313	0.361	0.371
LSD 1%	0.488	0.548	0.496	0.450	0.518	0.534

TABLE 8. Selected accessions for cold tolerance based on cold reduction index (%) using combined data over all two growing seasons

NO	Genotypes	Germination percentage	Germination index	Seedling height	Root length	no. of leaves /seedling
1	CT6749-36-7-2-M-M (1)	57.97	32.65	45.43	38.16	22.68
2	H270-30-2-1 (2)	82.85	36.17	31.24	33.80	33.50
3	Milyang 80 (9)	49.20	16.67	53.59	53.10	15.47
4	PSRM1-17-4B-13 (11)	60.18	33.33	45.59	36.23	44.67
5	Ryong Sung 25 (12)	49.66	25.79	51.11	45.83	25.00
6	Giza 177 (19)	13.07	23.20	54.98	50.00	33.50
7	Sakha102 (24)	22.28	25.00	59.28	65.89	27.32
8	Sakha 104 (26)	11.82	25.51	63.99	65.50	26.91
9	CT9882-16-4-2-3-2-p-M (39)	54.44	31.82	50.00	41.67	41.75

Cluster based on morphological dendrogram

Cluster Analysis was used to arrange a set of variables into clusters. Its objective was to sort cases (variables) into groups, or clusters, so the degree of association was strong within members of the same cluster and weak between members of different clusters. This description maybe abstracted through use of the particular to the general class or type (Anderberg, 1973).

The cluster analysis for the data obtained from phenotyping, (Figure 5) showed that the phylogenetic analysis divided the studied accessions into two main groups (A and B) in addition to some sub groups belong to each main group. Group A including 13 rice accessions with the majority of Indica and Indica/ Japonica types and a few numbers of Japonica type accessions only three. In general, the group (A) including the rice accessions that showed poor or moderate performance under low temperature conditions. On other hand the group B including 27 rice accessions divided into two sub groups, the majority of these sub groups for Japonica accessions with 13 accessions, six Indica/ Japonica accessions and eight Indica type. Evidently, the group B including the most superior rice accessions under low temperature conditions. these results are in agreement with the previous results which reported that Japonica type accessions are more cold tolerance than Indica type (Renata *et al.*, 2010; Sanghera *et al.*, 2011 and Suh *et al.*, 2013). Due to the useful breeding programs are depending on modern and adapted knowledge of genetic diversity among varieties to utilize the available genetic resources to create new genotypes, morphological markers reflect not only the genetic contribution of the genotypes but also the interaction of the genotype with environment in which it is revealed (Thenmozhi and Rajasekaran, 2013).

Genotyping

SSR polymorphism

The objective of this investigation to evaluate the ability of cold tolerance for the 40 rice accessions based on their genetic diversity using molecular markers related with cold tolerance at seedling stage. Out of 36 SSR markers used in this study, four markers showed highly polymorphic pattern among the studied genotypes, the electrophotogram for the amplified DNA fragments for these markers are shown in Fig. 1- 4. The number of polymorphic alleles ranged from five by RM324 to nine by RM265 markers. The RM265 marker amplified nine different alleles for the studied rice accessions (Figure 1), the molecular weight of these alleles ranged between 460 bp and 160 bp, while the RM324 marker amplified five different alleles, (figure 2), the molecular weight of these alleles ranged between 290 bp and 120 bp. The RM163 marker amplified nine different alleles with molecular weight ranged between 490 bp and 230 bp (Fig. 3). The RM270 marker amplified eight different alleles with molecular weight ranged between 250 bp and 100 bp (Fig. 4).

Phylogenetic analysis

Regarding the cluster analysis for the data obtained from the four SSR markers (Figure 6) the results showed that the phylogenetic analysis divided the studied accessions into two main groups (A and B) in addition to some sub groups belong each main group. Group A included 18 rice accessions with the majority of Indica and Indica/ Japonica types and a few number of Japonica type accessions only four accessions, in general the results Indicated that this

group (A) including accessions with poor or moderate ability for cold tolerance under low temperature conditions. On other hand the group B included 22 accessions divided into two sub groups, the majority of these sub groups for Japonica accessions with 11 accessions, five Indica/ Japonica accessions and six Indica type. Apparently, the group B including the most of superior accessions under low temperature conditions such as CT6749-36-7-2-M-M, Milyang 80, PSRM1-17-4B-13, Ryong Sung 25, Giza 177, Sakha102, Sakha 104, CT9882-16-4-2-3-2-p-M, these results confirming the phenotypic results. These results are in a harmony with those reported by Suh et al., 2013.

Molecular markers have the potential to determine genetic diversity and to help the management of plant genetic resources. In contrary of morphological traits, molecular markers can demonstrate the differences among genotypes at the DNA level, providing a more direct, accurate and efficient tool for germplasm characterization and management. This emphasizes the urgent need of molecular mapping for cold tolerance to detect linked DNA marker(s). Doing so will greatly help in improving selection efficiency for cold tolerance.

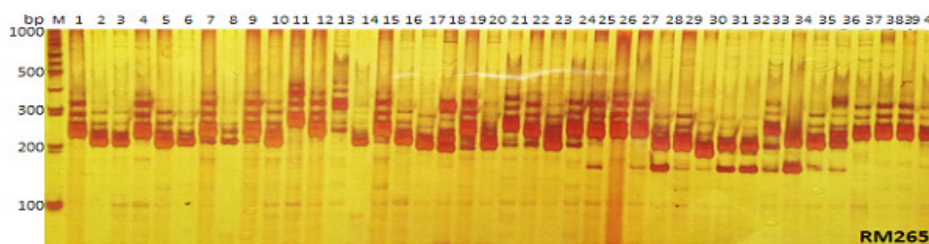


Fig. 1. Genetic Polymorphism among the 40 genotypes using RM 265

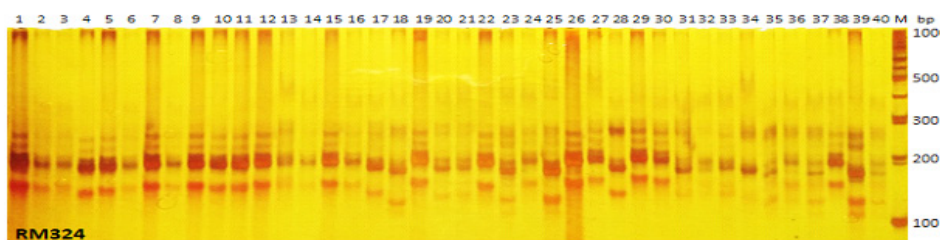


Fig. 2. Genetic Polymorphism among the 40 genotypes using RM 324

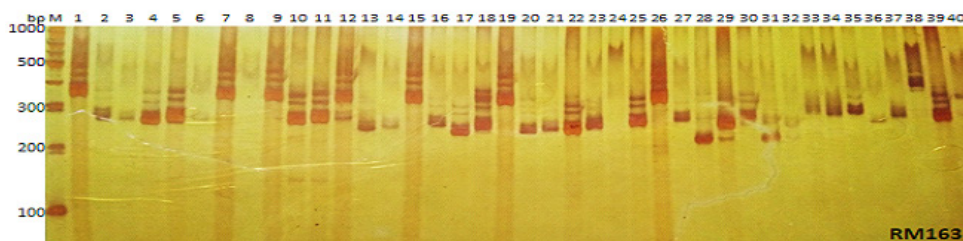


Fig. 3. Genetic Polymorphism among the 40 genotypes using RM 163

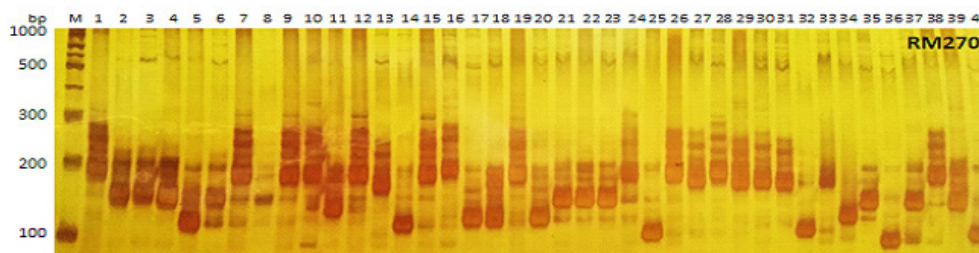


Fig. 4. Genetic Polymorphism among the 40 genotypes using RM 270

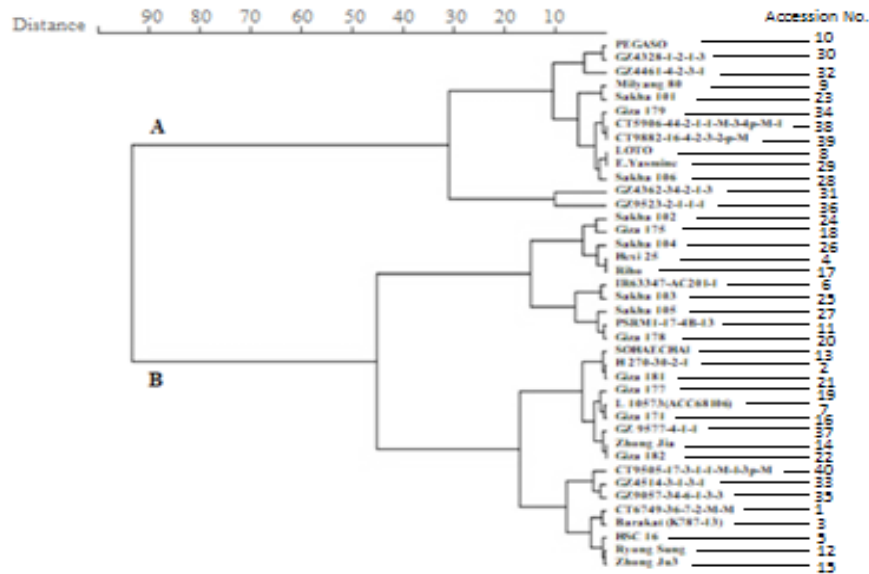


Fig. 5. Dendrogram of 40 rice accessions based on phenotyping data using hierarchical cluster analysis (wards method)

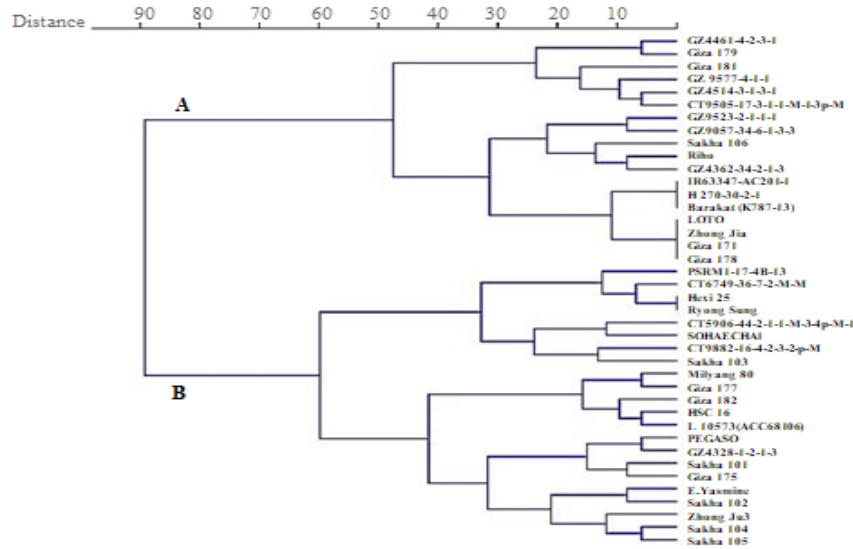


Fig. 6. Dendrogram of 40 rice accessions for cold tolerance, clustered using four SSR markers related with cold tolerance at seedling stage

TABLE 9. Matrix for SSR amplified fragments for the studied genotypes at the locus RM265

MW	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40		
400	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
340	1	0	0	0	0	0	1	0	1	0	1	1	1	0	1	0	0	1	1	0	1	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	0
300	1	1	1	1	1	0	1	0	1	1	1	1	1	0	0	1	0	0	0	1	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0	1	1	1	0
270	0	1	1	1	1	0	0	0	1	1	1	1	1	0	0	1	0	0	0	0	1	1	0	1	1	1	1	1	0	1	0	0	0	1	0	1	0	1	1	1	0	
240	1	0	0	1	0	1	1	1	1	1	0	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	1	1	1	1	1	1	
215	0	1	1	0	1	1	1	1	0	1	0	0	0	1	0	1	1	1	1	1	0	0	1	1	0	0	0	0	1	0	1	1	1	1	1	1	1	0	0	0	0	
200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1	0	1	1	0	0	1	0	0	0	0	0	0	
190	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	1	1	0	0	0	0	0	0	0

TABLE 10. Matrix for SSR amplified fragments for the studied genotypes at the locus RM324

MW	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40		
275	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
200	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	1	0	0	1	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	
190	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
165	1	1	0		1		1	1	1	1	1	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0
150	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
140	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

TABLE 11. Matrix for SSR amplified fragments for the studied genotypes at the locus RM163

MW	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40			
410	1	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	
380	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	
330	1	0	0	1	1	1	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	
280	0	0	0	1	1	0	0	0	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	
250	0	1	1	1	1	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	
230	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
220	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	

TABLE 12. Matrix for SSR amplified fragments for the studied genotypes at the locus RM270

MW	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40		
260	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
230	1	0	0	0	0	0	1	0	1	1	0	1	1	0	1	1	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
200	1	1	1	1	0	0	1	0	1	1	0	1	0	0	1	1	0	0	1	0	1	1	1	1	0	0	1	0	1	1	1	1	0	1	0	0	0	1	1	1	1	
185	1	0	0	0	1	0	1	0	1	1	1	0	1	0	1	1	0	1	0	0	0	0	0	1	0	0	1	1	1	1	1	1	0	1	0	0	0	0	1	1	0	
160	0	1	1	1	0	1	0	1	1	1	1	0	0	0		1	1	1	0	1	0	1	1	0	0	0	0	1	1	1	1	0	0	0	1	0	1	0	1	1		
125	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	1	0	1	0	0	0	1	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	1	0	0	
100	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	

Conclusion

In conclusion, the present study showed reliable amount of genetic variation which exists among rice accessions can be utilized efficiently to select parent for improving cold tolerance. The rice genotypes Giza 177, Sakha 102, Sakha 104, CT6749-36-7-2-M-M, Milyang 80, PSRM1-17-4B-13 and Ryong Sung 25 showed good performance in their ability to cold tolerance, so they could be used in initiated the breeding program for growing at low temperature levels under Egyptian conditions. Furthermore, due to most of morphological traits are strongly associated with the four SSR markers namely RM 265, RM324, RM163 and RM270, so it could be use these markers as simple tools for selection in the early generation during the applied breeding programme.

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التنوع الوراثي لتحمل البرودة على الأرز في مرحلة البادرة تحت الظروف المصرية

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من المعروف أن تربية الأرز لتحمل درجات الحرارة المنخفضة (البرودة) تعتبر واحدة من أهم الطرق لمواجهة التغيرات المناخية والتي تؤثر بشكل كبير و فعال على مساحات شاسعة على مستوى العالم. أجريت هذه الدراسة في محطة البحوث الزراعية بسخا خلال موسمى 2015 م و 2016 م لتقييم بعض التراكيب الوراثية من الأرز لمعرفة مدى قدرتها على تحمل البرودة في مرحلة البادرة في مواعيد زراعة مبكرة خلال الأول من مارس و الأول من أبريل بالإضافة الى الميعاد المثالي وهو الأول من مايو. وتم استخدام بعض المعلمات الجزيئية SSR لمعرفة الاختلافات الوراثية على المستوى الجزيئي بين التراكيب الوراثية المستخدمة. وقد أشارت قيم المتوسطات الى انخفاض معنوي على كل الصفات التي تم دراستها إذا ما قورنت بامواعيد الزراعة المثالية. وتم ملاحظة اختلافات معنوية بين التراكيب الوراثية حيث أعطت التراكيب الوراثية ذات الطراز الياباني قدرة أعلى على تحمل البرودة من التراكيب الوراثية ذات الطراز الهندي. وقد أعطت المعلمات الوراثية الجزيئية RM265 ، RM324 ، RM163 ، RM270 أعلى نسبة اختلافات على المستوى الجزيئي في مدى تحملها لدرجات الحرارة المنخفضة. وقد سجلت الأصناف المحلية جيزة 177 و سخا 102 و سخا 104 أعلى قيم متوسطات تحت درجات الحرارة المنخفضة في مواعيد الزراعة المبكرة ، بينما التراكيب الوراثية من الممكن أن تستخدم كمعطي عام لجينات تحمل البرودة وأستخدامها في برامج التربية.