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Full Length Article

Assessment of Genetic Diversity and Relationships Among Diverse Rice Genotypes using Heading Date/QTLs Linked SSR Markers

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Abstract

Genetic diversity is the main source for plant breeder to develop new elite genotypes. The objective of this investigation is to study phenotypic and molecular genetic diversity among 150 rice (*Oryza sativa* L.) genotypes for their heading dates, due to the importance of this trait in avoiding some climate changes and minimize the water consumption. Results revealed a wide range of differences for heading dates ranged from 70 to 129 days. Genotypes were classified into four groups; very early, early, intermediate and late. Four SSR markers linked to heading date trait/QTLs were used to study 20 selected genotypes. Seventeen polymorphic alleles with molecular sizes ranged from 108 to 304 bp were amplified. Two out of the four primers (RM510 and RM585) produced the PCR expected product size (122 and 233 bp, respectively) in addition to other unexpected alleles. The RM585 primer generated an unexpected additional band with a molecular size of 257 bp. This band appeared only in five very early and early-dated genotypes and was completely absent from the intermediate and late genotypes. This primer also recorded the highest PIC value (0.765). RM7601 also produced an additional unexpected band with molecular size of 116 bp. This fragment only appeared in most very early and early heading date genotypes, not in intermediate and late genotypes. For the cluster analysis based on the SSR markers, the 20 rice genotypes were divided into two main clusters, which were respectively divided into three and two groups that matched in heading date.

Keywords: Rice; Heading date; Genetic variability; SSR markers

Introduction

Rice (*Oryza sativa* L., 2n = 24) is the most important staple food crop for more than 3.5 billion people (Xu *et al.* 2016; Saleh *et al.* 2020). It is cultivated in Egypt over an area of about 660 thousand hectares, with an annual production of about 4.6 million tons of paddy, with average productivity of 10 tons per hectare (EAS 2018; Elmoghazy and Elshenawy 2018; FAO 2018). Genetic variability is the basis of plant breeding as the success of any crop improvement program depends on the magnitude of genetic variability (Ganapathi *et al.* 2014; Sarker *et al.* 2015). The creation of variability in rice germplasm is one of the most effective methods that provides a wide range of genotypes that can be selected to develop new varieties with a desired combination of traits (Pandey *et al.* 2009; Sakran *et al.* 2022). Moreover, it accelerates the detection of promising genotypes without the need to evaluate all possible cross combinations in breeding programs (Palanga *et al.* 2016). Selection can be effectively practiced only in the presence of variability of desired traits. The development of early maturing rice genotypes is of great importance; it could be used to avoid certain climate changes such as high temperature during fertilization which represent a very serious problem affecting grain yield. The short duration rice varieties save time for planting other winter crops, save irrigation water, which represents the main constraint of rice cultivation and save efforts and expenses of rice cultivation. Heading date is determined by both genetic factors and environmental conditions (Andres and Coupland 2012). Cultivars with an appropriate heading date will be conductive to high grain yield by fully utilizing the light and temperature resources in their growing regions (Zhang *et al.* 2015). In rice, flowering time is regulated by the complex genetic mechanisms involving hundreds of quantitative trait loci; QTLs (Hori *et al.* 2016; Matsubara and Yano 2018; Liu *et al.* 2021).

DNA-based molecular markers have been used extensively for studying genetic diversity (Yadav *et al.* 2013; Sarif *et al.* 2020). Compared with agro-morphological markers, molecular markers are not influenced by environmental factors and are generally more

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sensitive to differences among genotypes at the DNA level, thus increasing their detection efficiency and fast (Ming *et al.* 2010). Currently, simple sequence repeats (SSRs) are the molecular tools used for diversity evaluation and detecting relationships among different crop species, populations, or individual rice accessions (Pachauri *et al.* 2013; Hoque *et al.* 2021). According to Allhgolipour *et al*. (2014), SSR markers are suitable for evaluating genetic diversity among closely related rice accessions. A study of Wang *et al*. (2014) has identified that microsatellite loci may be used to detect genetic variation and genetic relationships within rice through genome study as well as allelic diversity analysis.

The present investigation aimed to assess the genetic variability and heritability for heading date trait of 150 different rice genotypes under Egyptian conditions. Moreover, study of genetic diversity and phylogenetic relationships; among 20 selected genotypes, based on SSR markers related to heading date trait/QTLs.

Materials and Methods

The present study was conducted at Genetics Department, Faculty of Agriculture, Kafr Elsheikh University, Egypt and Rice Research and Training Center (RRTC), Sakha, (31°05'36.4"N 30°55'45.6"E and 4m elevation) Kafr Elsheikh, Egypt during the two summer seasons; 2017 and 2018. The experimental soil is silty clay and the temperature ranged from 22 to 38℃ (for maximum) and 15 to 28℃ (for minimum) during the growing season.

Plant material and experimental design

One hundred fifty rice (*Oryza sativa* L.) genotypes; six local genotypes (Giza 177, Giza 178, Sakha 102, Giza 171, NABATAT ASMAR and Egyptian Yasmin) and 144 exotic genotypes obtained from Egyptian Rice Gene Bank (ERGB) of RRTC, were used in this study. Names and origins of the 150 studied rice genotypes are listed in Table 1. All the 150 genotypes were grown in a randomized complete block design (RCBD) in three replications; each consisting of one row/genotype. Each row was 5 m long and contained 25 seedlings with 20×20 cm spacing among rows and hills. All standard crop management were applied as recommended by RRTC (RRTC 2013) and by Elmoghazy and Elshenawy (2018).

Heading date trait

The heading date trait was scored according to the International Rice Research Institute (IRRI) Standard Evaluation System (IRRI 2014). The 150 rice genotypes were classified into four groups as follows: Genotypes with heading date ranged from 70 to 92 days are very early, from 93 to 110 days are early, from 111 to 120 days are intermediate and from 121 to 130 days are late.

Molecular analysis

Based on heading date scoring, 20 genotypes (five genotypes/group) were selected for molecular characterization using four SSR molecular markers. Genomic DNA was isolated from 100 mg healthy leaves (three weeks old) of the 20 selected rice genotypes using CTAB method (Murray and Thompson 1980).

SSR markers and PCR amplification

Four SSR DNA markers (introduced from Eurofins Genomics Co., Germany); related to heading date trait/QTLs, were screened on DNA templates. All primer sequences were directly downloaded from Gramene database (www.gramene.com). Details of primer sequences, chromosomal locations, repeat motifs and expected product sizes are given in Table 2. The PCR amplification reaction was performed in 10 μL reaction mixture, containing 1 μL of DNA template (15 ng/μL), 1 μL of each of the forward and reverse primers (10 nmol/μL), 5 μL of 2X PCR master mix (amaROnePCR^{TM} - GeneDireX, Inc) and 2 μL of double distilled water (ddH_2O).

A thermocycler (TECHNE TC-412) was used to carry out the PCR amplification programme as follows: an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 50–55ºC for 30 sec and primer extension at 72°C for 1 min. By the end of the 35th cycle, a final extension step at 72°C for 5 min was performed. The PCR amplified products were stored at -20°C until use. The PCR amplified fragments were separated by electrophoresis on 3% agarose gel stained with ethidium bromide, visualized under a UV transillumination and then photographed using Biometra gel documentation unit (BioDoc, Biometra, Germany). Molecular size of the separated fragments was determined against a known DNA ladder (HyperLadder^{TM} 100 bp, Bioline) using Gel Analyzer 10 Program.

Data analysis

Data of heading date trait was subjected to analysis of variance (ANOVA) for randomized complete block design (RCBD), by using the statistical analysis software program MSTAT-C; version 2.10 (MSTAT-C 1991). Based on the combined analysis of the two studied seasons, the genotypic (GV) and phenotypic (PV) variances, genotypic (GCV%) and phenotypic (PCV%) coefficient of variations and heritability in broad sense (h_b^2) were calculated according to Falconer (1981). Least significant difference (LSD) method was used to compare means at 5% level of probability. For molecular analysis, data of SSR markers were introduced as binary values 1 and 0 for the presence and absence of an amplified band, respectively. Number of alleles was determining and polymorphic information content of the locus *i* (*PICi)* was calculated according to Roldan-Ruiz *et al.*

Table 1: The 150 studied rice genotypes, their country of origins and the mean of combined phenotypic data for heading date trait over the two seasons, 2017 and 2018 under Egyptian conditions. These genotypes were obtained from Egyptian Rice Gene Bank, located at RRTC, Sakha, Egypt. Each genotype was carefully examined and purified

No.	Genotype	Origin	Heading Date (days)
1	E B Gopher	Texas, United States	105
2	PR 325	United States	108
3	OwariMochi	Japan	120
4	WC 3777	Francisco Morazán	114
5	Tehran	Iran	71
6	Lang ShweiKeng	China	104
7	BG 79	Guyana	70
8	D. Sancho	Lisboa, Portugal	72
9	Barbado	Lisboa, Portugal	70
10	Sanakevelle	Liberia	124
11	MARATELLI	Italy	73
12	Chin	Panama	115
13	Italica Carolina	Lubelskie, Poland	70
14	Italica M1	Lubelskie, Poland	70
15	Alvario	Portugal	75
16	TAICHUNG 33	Taiwan	114
17	Romanica	Pest, Hungary	70
18	Rexora	Mozambique	124
19	IR 334-17-1-3-1	IRRI Philippines	124
20	Amber 33	Iraq	119
21	Giza 177	Egypt	93
22	Giza 178	Egypt	100
23	Blue Rose Sela	Argentina	113
24	Sakha 102	Egypt	101
25	Bungara	Rwanda	115
26	KhaoHao	Laos	120
27	J.P. 5	Pakistan	108
28	Nauta	Loreto, Peru	112
29	P 761-40-2-1	Colombia	113
30	NEANG MEAS	Cambodia	114
31	Fa Yiu Tsai	Hong Kong	116
32	JambaramVermelho	Guinea-Bissau	116
33	Kathmandu Valley No.1	Nepal	76
34	Daudzai	Pakistan	129
35	Thangone	Laos	111
36	PratoaPrecoce	Brazil	109
37	Precocinho	Brazil	109
38	Imbolo II	Congo	110
39	Onu B	Congo	115
40	Shima	Iraq	116
41	Giza 171	Egypt	113
42	P 1289	Turkey	112
43	CH 242-32	Biobío, Chile	109
44	HalwaGose Red	Iraq	113
45	MIYANG	China	107
46	Zira	Kenya	113
47	MEDUSA	Lombardia, Italy	119
48	HD14	Australia	112
49	Gidej	Azerbaijan	115
50	Grassy	Haiti	113
51	B459A1-49-1-2-1	Texas, United States	112
52	Dular	Dhaka, Bangladesh	115
53	Cesariot	Occitanie, France	113
54	CIGALON	France	113
55	IR 2061-214-2-3	IRRI Philippines	120
56	Precoz de Machiques	Aragua, Venezuela	71
57	Sesilla	Bulgaria	122
58	WC 3398	Nayarit, Mexico	108
59	B441B-24-4-5-1	Indonesia	123
60	Quinimpol	Philippines	119
61	WC 3395	Jamaica	117
62	Sadri Type	Iraq	119
63	Criollo	Mexico	119
64	PuangNigern	Thailand	120
65	Daido	Mongolia	73
66	Natapasume	Taiwan	119
67	Rz No. 111	Congo	114
68	Apure	Aragua, Venezuela	119
69	WC 2810	Pohnpei, Micronesia	120
70	Ao Chiu 2 Hao	Sichuan Sheng, China	119
71	AgulhaBranco	El Salvador	117
72	MataoLizo	El Salvador	117
73	Secano do Brazil	El Salvador	115
74	ItalicaAlef	Former, Soviet Union	73
75	NABATAT ASMAR	Giza, Egypt	121
76	LOMELLO	Italy	73
77	I KUNG PAO 5-3-4	Taiwan	115
78	AP 439	Venezuela	115
79	Aguja	Bolivia	115
80	Cola de Burro	Bolivia	112
81	Mojito Colorado	Bolivia	116
82	Campino	Portugal	114
83	Laat	Suriname	114
84	MONTICELLI	Lazio, Italy	76
85	STIRPE 82 CHIAPPELLI	Piemonte, Italy	76
86	KamBauNgan	Hong Kong	76
87	IguapeCateto	São Paulo, Brazil	115
88	MUTSU HIKARI	Aomori, Japan	76
89	JUMA 1	Dominican Republic	123
90	KhaoKhao	Thailand	121
91	NAYIMA 45	Iraq	117
92	IARI 7449	Assam, India	113
93	Agbede	Nigeria	116
94	Sika	Cameroon	123
95	KhaoPhoi	Laos	113
96	KhaoLuang	Laos	115
97	NIQUEN	Biobío, Chile	73
98	IR 1614-168-2-2	Philippines	122
99	Basmati Sufaid	Punjab, Pakistan	109
100	Chak 48	Punjab, Pakistan	117
101	B35	Punjab, Pakistan	122
102	DhanSufaid	Punjab, Pakistan	118
103	Jhona	Punjab, India	112
104	P 79	Colombia	110
105	Ratua Red Nehri	Punjab, Pakistan	118
106	GPNO 19314	Brazil	118
107	Choei-ine	Japan	72
108	GPNO 16379	Pohnpei, Micronesia	72
109	Chao Hay b	Laos	109
110	Glutinous	Hong Kong	111
111	TJ	Guyana	74
112	Ambalalava 1283	Madagascar	74
113	PD 46	Sri Lanka	74
114	IacaEscuro	Guinea	119
115	PATNAI 6	Yangon, Myanmar	119
116	BIRIBRA	Ghana	76
117	WW 3/200	Netherlands	115
118	Subdesvauxii Vase	Portugal	115
119	E. Yasmine	Egypt	120
120	HON CHIM	Hong Kong	76
121	MAKALIOKA 752	Madagascar	116
122	COLOMBIA 1	Colombia	112
123	Batatais	Brazil	115
124	R 29/1	Congo	115
125	R 98	Congo	116
126	R 98/1	Congo	110
127	R 99/3	Congo	110

Table 1: Continue

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128	Nema	Iraq	118
131	GPNO 22236	Kankan, Guinea	123
132	IM 16	Nigeria	111
133	ECIA76-S89-1	Cuba	111
134	TAKAO 11	Taiwan	117
135	WC 1006	Iraq	117
136	Rubra	Former, Soviet Union	116
137	LATE CALORO	Australia	122
138	TrionfoFassone	Piemonte, Italy	114
139	YabaniMontakhab 57	Odisha, India	116
140	Precosur	Entre Ríos, Argentina	111
141	IR 773A1-36-2-1-3	Philippines	121
142	IARI 5753B	Assam, India	115
143	Mack Khoune	Laos	120
144	H64-9-1	Argentina	119
145	Mabla	Punjab, Pakistan	110
146	GPNO 29157	Jiangsu Sheng, China	119
147	Hae Zo	Korea, South	119
148	Vary TarvaOsla	Portugal	75
149	CHONTALPA 437	Mexico	115
150	Sathi Basmati	Punjab, Pakistan	122
	Average		107
	Max		129
	Min		70
	Stander Deviation		17.02
	Stander Error		1.39

(2000) as follows: *PICi = 2fi(*1-*fi)*; Where *fi* represents the frequency of the present bands and (1-*fi*) is the frequency of the absent bands. The PIC value of each primer was calculated using the average PIC values for all primer.

To determine the genetic relationships among the 20 selected rice genotypes, a dendrogram was constructed based on Jaccard's similarity coefficient (Jaccard 1901) using the Unweighted Pair-Group Method with Arithmetic mean (UPGMA) analysis (Nei 1973).

Results

Analysis of variance for heading date trait

Based on the combined data, analysis of variance represented in Table 3 showed highly significant differences among the 150 studied rice genotypes and between 2017 and 2018 seasons for heading date trait. Highly significant differences were also found for the interaction between genotypes and years. Significance of genotype mean squares revealed that there are genetic differences among genotypes in case of heading date trait. The existence of genetic variability is the key component of breeding programs for broadening the gene pool to develop high yielding varieties (Aditya and Bhartiya 2013; North 2013).

Mean performance and heading date scoring

The mean performance of heading date trait was scored for the 150 studied genotypes (Table 1). A wide range of differences was observed among genotypes with mean performance values ranged from 70 days (BG79, Barbado, Italica Carolina, Italica M1 and Romanica genotypes) to 128 days (Daudzai genotype). Results of heading date scoring indicated that the majority of studied genotypes heading date was belonging to the intermediate group including 87 genotypes of the 150 studied genotypes, while the minority was to late group of heading date by 16 genotypes. Mean performance of heading date for the 20 rice genotypes (five genotypes/group) which were selected for molecular characterization on the basis of their heading date estimates are listed in Table 4.

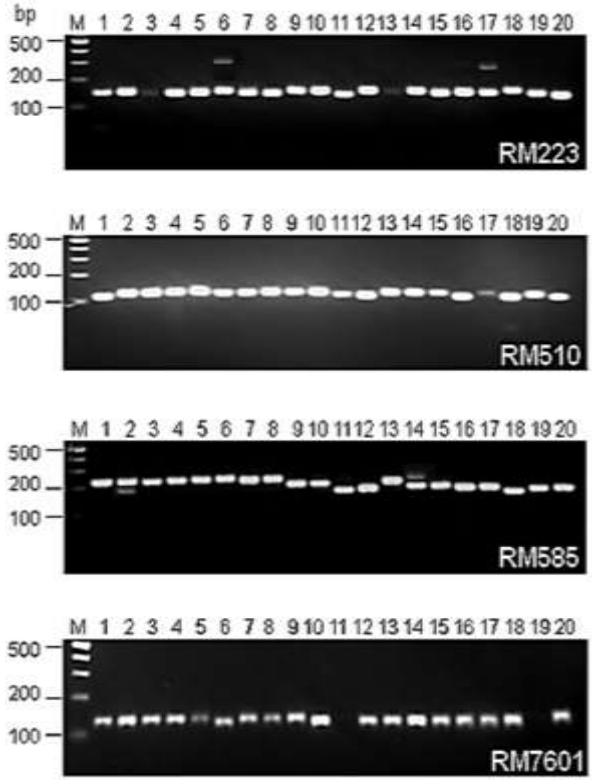

Fig. 1: Profiles of DNA amplified fragments generated by RM223, RM510, RM585 and RM7601 markers for the 20 rice genotypes selected based on heading dates. M: 100 bp DNA ladder. Genotypes 1-5 (very early): BG 79, Barbado, Italica Carolina, Italica M1 and Romanica. Genotypes 6-10 (early): Giza 177, Giza 178, Sakha 102, Lang ShweiKeng and E B Gopher. Genotypes 11-15 (intermediate): KhaoHao, IR 2061-214-2-3, PuangNigern, WC 2810 and Mack Khoune. Genotypes 16-20 (late): GPNO 22236, Sanakevelle, Rexora, IR 334-17-1-3-1 and Daudzai

Molecular characterization

Polymerase chain reactions for RM223, RM510, RM585 and RM7601 SSR markers; which were reported to be linked to heading date trait/QTLs, were carried out with DNA templates of the 20 selected genotypes. Genotypic screening of the 20 genotypes with the four SSR markers are presented in Fig. 1 and Table 5.

Table 2: Forward (F) and reverse (R) primer sequences, chromosomal locations (CL), repeat motifs, annealing temperature and expected PCR product sizes of the used four SSR markers

Primer	F/R Primer 5'→3'	CL	Repeat motif	Annealing temperature (ºC)	Expected product size (bp)	References
RM223	F -GAGTGAGCTTGGGCTGAAAC R- GAAGGCAAGTCTTGGCACTG	8	(CT)25	55	165	Khatab *et al.* (2016)
RM510	F- AACCGGATTAGTTTCTCGCC R- TGAGGACGACGAGCAGATTC	6	(GA)15	55	122	Khatab *et al.* (2016)
RM585	F- CAGTCTTGCTCCGTTTGTTG R- CTGTGACTGACTTGGTCATAGG	6	(TC)45	55	233	Khatab *et al.* (2016)
RM7601	F -GCCTCGCTGTCGCTAATATC R-CAGCCTCTCCTTGTGTTGTG	7	(TGGA)7	50	133	Fatimah *et al.* (2014)

-groups, IIa and IIb, which contain the most late genotypes

Table 3: Mean squares of heading date trait based on the combined data of the two studied seasons; 2017 and 2018

Source of variation	Degree of freedom	Mean square
Years	1	327.610**
Rep/Year	4	0.262
Genotypes	149	1738.428**
Genotypes ×Years	149	21.760**
Error	596	6.125

** Significant differences at 1% level of probability

Table 4: Mean, genotypic and phenotypic variances, genotypic and phenotypic coefficients of variation, and heritability in broad sense for heading date trait for the 150 studied genotypes

Parameter	Heading date
Mean (days)	107.22
Genotypic variance (GV)	288.72
Phenotypic variance (PV)	294.82
Genotypic coefficient of variation (GCV %)	15.85
Phenotypic coefficient of variation (PCV %)	16.01
Heritability (%)	97.93

Table 5: Mean performance of heading date trait for the 20 selected rice genotypes

S. No.	Genotype	Heading date (days)	Group
1	BG 79	70	Vary early
2	Barbado	70	Vary early
3	Italica Carolina	70	Vary early
4	Italica M1	70	Vary early
5	Romanica	70	Vary early
6	Giza 177	93	Early
7	Giza 178	99	Early
8	Sakha 102	100	Early
9	Lang ShweiKeng	103	Early
10	E B Gopher	105	Early
11	KhaoHao	119	Intermediate
12	IR 2061-214-2-3	119	Intermediate
13	PuangNigern	119	Intermediate
14	WC 2810	119	Intermediate
15	Mack Khoune	120	Intermediate
16	GPNO 22236	123	Late
17	Sanakevelle	123	Late
18	Rexora	124	Late
19	IR 334-17-1-3-1	124	Late
20	Daudzai	129	Late

All SSR fragments were determined and a high level of polymorphism (100%) was observed suggesting a high level of diversity among the used genotypes. The PIC (Polymorphic Information Content) values ranged from 0.418 (RM510) to 0.765 (RM585) with an average of 0.559 per marker (Table 6). Accordingly, primer RM585 was the most polymorphic primer while it produced a total of six alleles with different molecular sizes and also recorded the highest PIC value (0.765).

A total of 17 fragments with an average of 4.25 alleles/marker were amplified in the present study. Alleles molecular size varied from 108 to 304 bp among the 20 genotypes. Multiple PCR amplicons ranged from 3 for RM510 and RM7601 to 6 for RM585 were yielded. The amplified number of alleles were varied among genotypes from 3 to 5 alleles. The three SSR primers; RM223, RM510 and RM585, produced PCR bands in all the studied genotypes, while RM7601 primer did not amplify any bands in Khao Hao (intermediate genotype) and IR 334-17-1-3-1 (late genotype) (Fig. 1 and Table 6).

Out of the four SSR markers, two primers (RM510 and RM585) produced the PCR expected product sizes, 122 and 233 bp, respectively, in addition to other unexpected alleles. On the other hand, the other two primers; RM223 and RM7601, had several unexpected alleles ranging from 3 alleles of molecular size 108, 116 and 125 bp for RM7601 to 5 alleles of molecular size ranging from 136 to 304 bp for RM223 but did not produce an allele of the expected size.

Cluster analysis

Based on molecular data of SSR markers linked to heading date trait/QTLs, Jaccard's similarity coefficients were calculated and a dendrogram was constructed using the UPGMA method to determine the genetic relationships among the 20 rice genotypes (Fig. 2). The dendrogram showed a clear separation of the 20 rice genotypes into two main clusters (I and II) at a genetic similarity of 9.0%. The first cluster (I) included 15 genotypes which were divided into three groups (Ia, Ib and Ic). Group Ia had two late heading date genotypes (Sanakevelle and IR 334-17-1-3-1). The seven genotypes in group Ib were divided into two sub-groups at a genetic similarity of 47.8%. The first one (Ib-1) contained four genotypes; with 100% genetic similarity, which were very early (Italica M1 and Romanica) and early (Giza 178 and Sakha 102) for heading date trait. However, the other three very early genotypes (BG 79, Barbado and Italica Carolina) were separated in the second sub-group

Table 6: Genotypic screening of the 20 selected rice genotypes (based on heading dates) for the used four SSR markers; RM223, RM510, RM585 and RM7601. + and -, means presence and absence of respected allele, respectively. Some alleles were amplified only at very early and early heading genotypes like 304 bp of RM223 and 116 bp of RM 7601 while other alleles were found only at intermediate and late genotypes like 266 bp and 136 bp of RM223, 122 bp of RM510, 261 bp, 223 bp and 208 bp of RM585 and 125 bp of RM7601. R^2 values were highly significant, which mean high association of the used markers to the trait

Primer	Expected size (bp)	Presented alleles (bp)	Range of size (bp)	Number of alleles	Genotypes																					PIC value and R^2
					Very early genotypes					Early genotypes					Intermediate genotypes					Late genotypes						
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
					BG 79	Barbado	Italica Carolina	Italica M1	Romanica	Giza 177	Giza 178	Sakha 102	Lang ShweiKeng	E B Gopher	KhaoHao	IR 2061-214-2-3	PuangNigern	WC 2810	Mack Khoune	GPNO 22236	Sanakevelle	Rexora	IR 334-17-1-3-1	Daudzai		
RM223	165	304	168	5	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		266			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-		
		154			-	-	-	-	-	+	-	-	+	+	-	+	+	+	-	-	-	+	-	-	0.503	
		148			+	+	+	+	+	-	+	+	-	-	-	-	-	-	+	+	+	-	+	-	0.002	
		136			-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+		
RM510	122	130	14	3	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	-	+	-	0.418	
		122			-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	+	-	+	0.002	
		116			+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		261			-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-		
		257			-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-		
RM585	233	233	61	6	+	+	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	0.765	
		223			-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	0.0003	
		208			-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	+	+		
		200			-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-		
		125			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+		
RM7601	133	116	17	3	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	0.549	
		108			-	-	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+	+	-	-	0.0004	

(Ib-2). Group Ic contained six genotypes which differed in their heading dates, including three early genotypes (Giza 177, E B Gopher and Lang Shwei Keng) and three intermediate genotypes (Puang Nigern, WC 2810 and Mack Khoune). These six genotypes in group Ic were separated from the other nine genotypes (groups Ia and Ib) at similarity percentage of 26.2%. Cluster II included five genotypes; represented the intermediate and late heading date genotypes, which were classified into two groups; IIa and IIb, with 23.4% similarity. The first group (IIa) consisted of the two genotypes; KhaoHao (intermediate) and Daudzai (late), while the second group (IIb) included three rice genotypes; the intermediate genotype (IR 2061-214-2-3) and the two late genotypes (GPNO 22236 and Rexora).

Discussion

Understanding of genetic variability nature of a trait is very important for plant breeder to know the role of environment in the expression of this trait. Thus, genetic variability of heading date trait was determined on the basis of the results of combined analysis of the two seasons; 2017 and 2018 indicating real differences between the studied genotypes and wide genetic base to select the superior genotypes for crossing and selection. Similar findings were reported by (Elgamal 2019; Al-daej *et al.* 2023). The genetic parameters: such as variance components (GV and PV), coefficients of variation (GCV and PCV) has a narrow difference and the estimate of PV was slightly higher than GV. Regarding GCV and PCV parameters, the GCV value was close to PCV value, as well as high estimates of heritability in broad sense (h_b^2) indicating that, the heading date trait has less affected by environment. Heading date trait is under genetic control, selection would be successful based on phenotypic performance. These results agree with those of (Ahmadikhah 2010; Mallimar *et al.* 2015; Rashid *et al.* 2017; Gyawali *et al.* 2018).

From molecular genetics point of view, it was of great interest to notice that RM223 primer did not produce the expected size band of 165 bp in any of the used genotypes, but was able to produce two distinct unexpected bands with molecular sizes of 148 and 154 bp. The two unexpected bands were appeared in both early and late genotypes under study. Therefore, this primer does not play any role for heading date trait in the used rice genotypes.

For RM510 primer, it also generated an important unexpected band with molecular sizes of 130 bp, but it does not play any role for heading date trait while it was present in all the four heading date groups. On the other hand, this primer generated the expected size band of 122 bp which was appeared in five genotypes belonging to the intermediate (KhaoHao and IR 2061-214-2-3) and late (GPNO 22236, Rexora and Daudzai) genotypes. Therefore,

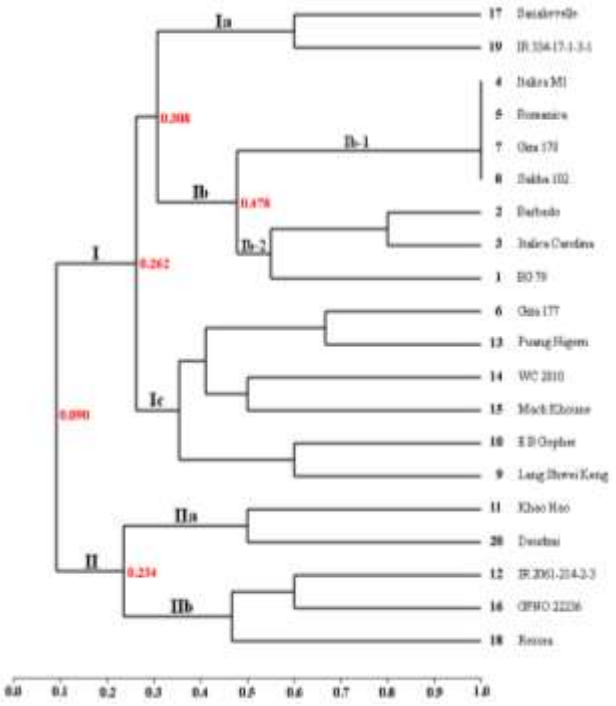

Fig. 2: The UPGMA dendrogram derived from four SSR markers; linked to heading date, showing genetic relationships among the selected 20 rice genotypes based on similarity indices. The studied rice genotypes were divided into two main groups I and II with genetic similarity of 9%. Group I contained 15 genotypes which divided into two sup-groups Ia and Ib, this group contain most very early, early and intermediate genotypes. The group II was divided also to two sup-groups, IIa and IIb, which contain the most-late genotypes

this fragment could be considered as a good marker for the intermediate and late rice genotypes for heading date trait.

Primer RM585 was of great interest in this study, it was able to generate the expected size band of 233 bp in three very early genotypes (BG 79, Barbado and Italica Carolina), two early genotypes (Lang ShweiKeng and E B Gopher) and one intermediate genotype (Puang Nigern). On the other hand, this primer generated an additional unexpected band with molecular size of 257 bp which was appeared only in five genotypes belonging to the very early (Italica M1 and Romanica) and early (Giza 177, Giza 178 and Sakha 102) genotypes and did not appear in the intermediate and late rice genotypes. Also, another additional unexpected band with molecular size of 208 bp was amplified only in the intermediate (IR 2061-214-2-3) and late (GPNO 22236, Sanakevelle, IR 334-17-1-3-1 and Daudzai) genotypes for heading date trait. These results proved that this primer is considered very important for studying heading date earliness in rice. Moreover, this primer is considered the best one for evaluation of heading date trait in rice breeding programs (Khatab *et al.* 2016; Weerasinghe *et al.* 2022), while it was able to produce the highest number of polymorphic alleles (6 alleles) and also recorded the highest PIC value (0.765).

For RM7601 primer, it proved to be very important for heading date studies in the used rice genotypes. It failed to produce the expected size fragment of 133 bp, but was able to produce another additional unexpected band with molecular size of 116 bp. It was surprised that this fragment was appeared only in all the five genotypes of very early group (BG 79, Barbado, Italica Carolina, Italica M1 and Romanica) in addition to three early genotypes (Giza 178, Sakha 102 and Lang ShweiKeng), but was completely absent in the intermediate and late genotypes under study. This fragment may play an important role in the very early and early heading date genotypes under study. Producing more alleles than the expected; using SSR primers, was previously reported by (Galal *et al.* 2014; Aboulila and Galal 2019).

Finally, we recommend in further study that both unexpected DNA fragments with molecular sizes of 257 and 116 bp which were only generated in the very early and early genotypes by RM585 and RM7601 SSR primers, respectively, should be isolated, sequenced and compared with the heading date responsible genes in rice gene banks.

Interestingly, the intermediate and late genotypes were closely related and placed in the same cluster (cluster II) suggesting that these genotypes were grouped according to their gene pools. The same trend was observed for the other two groups of genotypes, which separated in group Ia, and also group Ib which included seven genotypes (early or very early) in two sub-groups. Thus, there was a close relationship among the seven genotypes which clustered in group Ib with a similarity percentage of 47.8%. These results were supported by (Dutta *et al.* 2011; Khatab *et al.* 2016; Galal and Aboulila 2018; Elgamal 2019). They showed that the phylogenetic analysis; based on SSR markers, grouped the genotypes belonging to the same gene pool in the same clusters. Thus, SSR markers were useful for studying the genetic diversity and defining the genetic relationships among rice genotypes. In this respect, SSR molecular markers have been extensively used for identifying and characterizing of gene(s) linked to important traits (Wang *et al.* 2012; Salah *et al.* 2021) and phylogenetic relationship and genetic diversity analysis among rice genotypes (Das *et al.* 2013; Babu *et al.* 2014; Filiz *et al.* 2018). This allows fast screening at an early stage of growth; independent of environmental conditions, that consequently speed up breeding (Tester and Langridge 2010; Weerasinghe *et al.* 2022). Thus, these markers have proven to be the choice for marker-assisted selection (MAS) in rice breeding programs.

Conclusion

Heading date is one of the serious aspects determining regional and seasonal adaptation for climate changes and has been a main target of selection in rice breeding programs. Some rice genotypes were used to study phenotypic and

molecular diversity. High amount of diversity was detected among genotypes by both morphology and molecular markers. The SSR markers produced some alleles which were specific to heading date in most screened genotypes; those markers could be used as MAS for rice cultivar identification and associated with heading date.

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Author Contributions

The authors were contributed equally in this research and preparation of the paper

Conflicts of Interest

No conflict of interest concerning this research

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