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Research article

THE IDENTIFICATION OF RAPD AND ISSR INFORMATIVE MARKERS WITH SOME QUALITY TRAITS OF FRUIT IN SOME OF IRANIAN DATE PALM

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ABSTRACT: The association of 11 morphological traits with molecular markers was investigated by 24 molecular markers based on PCR (14 ISSR primers and 10 RAPD primers) in 15 cultivars of date palm collected from the south and south west of Iran. Using simple linear and multiple regressions, each of the 11 traits was regressed on all 284 available polymorphic markers (162 ISSR markers and 122 RAPD markers). A total of 89 ISSR and 84 RAPD markers gave significant associations with at least one of the 11 traits, either with linier or with multiple regression. Considerable amount of morphological changes were justified by UBC 886 primers (of ISSR primers) and Oligo 42 (RAPD marker) by polymorphism information content (PIC), 0.239 and 0.230, respectively. These results showed that based on good distribution of polymorphism inter-simple sequence repeat amplification (ISSR) and random amplification polymorphic (RAPD) in genome of date palm and the markers with high association with morphological traits, they can be used in identification of informative markers association with important morphological traits.

Keywords: Linear regression, Molecular markers, Morphological traits, Multiple regression, Polymorphic markers

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is the second cultivation product in Iran and its discovery dates back to more than 4000 years ago and more than 400 cultivars exist in the country [6]. The molecular markers are important tools to the management of germplasm samples in gen bank, the evaluation of genetic relationships, the selection of the best plants and the investigation of the similarity and difference between various samples, that use several molecular markers for achieve this aim [4]. One of the important applications of molecular markers is Marker Assistance Selection that has great importance in breeding of plants. As in plants breeding the aim is selection of plants with ideal morphological traits; any technique helping the early selection of the attribute can made the breeding period shorter and increases the efficiency of the selection. This is more effective when we need a long time for appearing the selective trait or the plant is cross-pollinated and thus trait appears after pollination after cross. In Marker Assistance Selection, based on the presence of Linked marker with the gene, we can find about its presence [2]. The linkage based association analysis studies, tracing of various genes related to cultivation traits is possible but mostly due to the high distance between the marker and cultivation attribute, in addition to making difficult marker assistance selection, separation and similarity of the required gene, a few numbers of genotypes are applied as parent in mapping of the population. In order to overcome these limitations of linkage- based analysis, in the recent past, association studies have been conducted, which not only allow mapping of genes/QTL with higher level of confidence, but also allow detection of gene/QTL, which would otherwise escape detection in linkage-based studies [13, 17]. In plant systems also, a few association studies have been conducted [14, 18, 19]. SSR markers were applied to find associated markers with 16 agronomic characters of cultivation types and wild types of soybean and 27and 34 associated markers with these traits were identified in cultivation and wild types respectively [21].

To study the relation of 30 polymorph RAPD primers (of OPM primers series) with 30 morphological traits in field condition and hydroponic cultivation of cane were applied stepwise regression. The results showed that each of OPM 15, OPM 20 had close relation with 4 traits and determination coefficient of these two primers was in 0.100 to 0.970. Other traits with at least 1 and at most 3 primers of OPM and determination coefficient of about 0.100 to 0.990 had close relation [3]. The relation of 13 quantity traits and 140 molecular markers SSR in 70 local Aegilops in Iran were investigated and 87 informative markers at least related to one of the traits were introduced. Their results showed that Xgwm271-5D at size 140 base pair had the highest R^2 values (37.7, 29.5 and 11% respectively) justified to flower length, the number of spikelet in spike and the length of nodes of spike traits [15]. Although Iran is rich in the terms of date palm germplasm, there is no study about the relation of molecular markers with important morphological traits in date palm. The current study aimed to identify informative markers with quality traits of fruit in some data palm cultivars of Iran by ISSR and RAPD.

MATERIALS AND METHODS

Plant material

In this study, 15 date palm cultivars of Iran (Table 1) that for each cultivars 5 single plant randomly was selected (75 genotype were studied) and this experiment were investigated in population form. The plant materials were collected from the date palm gardens of the south and south west of Iran. The tree performance (Kg), the weight of ten stones (gr), type of fruit tissue (dry, semi-dry, soft), fruit weight (gr), fruit length and diameter (cm), form of fruit (circular, egg-shaped, ellipse, pear-shaped), stone length and diameter (mm), the ratio of fruit weight to stone (gr) of date palm and the ratio of length to the width of stone (mm). To measure the performance of the tree, of each cultivar, 10 trees were selected randomly and after harvesting fruits, the performance of each tree was measured. Fruit and stone data were collected on 25 fruits that were selected randomly of each tree and middle of clusters. Fruit form, tissue, length and diameter and stone were measured in accordance with the national guidelines for the conduct of tests for distinctness, uniformity and stability in "Date Palm" of seed and plant certification and registration institute of Iran [8]. For all measurements, the scale with accuracy was 0.001 g and vernier caliper with 0.01 mm accuracy was used.

RAPD and ISSR amplification

Maroof method [9] with a little change for each genotype separately. 24 ISSR primers of UBC primers were applied and only 14 primers of them (Table 3) were amplified and showed polymorphism.

Table 1- Name, abbreviations and origin of 15 date palm cultivars evaluated (for each cultivars randomly was selected 5 genotypes and this study was performed in population form)

Number	Name	Collection location	Code	Geographical characteristics of collection location
				Longitude (N), Latitude (E)
1	Barhi	Abadan	A	30º 12´- 48º 24´
2	Barim	Ahwaz	B	31º 15´- 48º 37´
3	Khazravi	Ahwaz	C	31º 15´- 48º 37
4	Deiry	Ahwaz	D	31º 15´- 48º 37
5	Zahedi	Ahwaz	E	31º 15´- 48º 37
6	Estemeran	Ahwaz	F	31º 15´- 48º 37
7	Ovidi	Ahwaz	G	31º 15´- 48º 37
8	Farsi	Abadan	H	30º 12´- 48º 24´
9	Kabkab	Behbahan	I	30º 35´- 50º 14´
10	Gantar	Ahwaz	J	31º 15´- 48º 37
11	Degeltnor	Ahwaz	K	31º 15´- 48º 37
12	Majul	Ahwaz	L	31º 15´- 48º 37
13	Piaram	Jahrom	M	28º 29´- 53º 33´
14	Shahani	Ahwaz	N	31º 15´- 48º 37
15	Mazafati	Bam	P	29º 06´- 58º 21´

Also, 20 RAPD primers were applied; only 10 primers (Table 3) were amplified and showed polymorphism. Both ISSR and RAPD primers were designed by Metabion company, Germany [10] and were used in genetic diversity studies. RAPD and ISSR DNA reactions were carried on a thermo cycler (Bio-Rad). The profile used consisted of an initial denaturation for 4 min at 94° C, followed by 35 cycles was as Touchdown. Amplification products were visualized by running on 1.5% agarose gel in 1 X TBE buffer system, followed by ethidium bromide (0.5 µg mL^{-1}) staining. Fragment size was estimated by using a 100 base pairs (bp) molecular size ladder.

Data analysis

Clear and distinct amplification products were scored as '1' for presence and '0' for absence of bands in Excel software. PIC was calculated for each primer by formula

$$PIC = \sum\left(1 - P_i^2\right) / n \qquad (1)$$

p_i was frequency of ith allele, n the total number of genotypes [20]. The marker index was calculated of the product of the number of multiple bands in multiple content indexes [7]. The bands on all the gels were scored in 1-0 binding format and used in regression analysis with morphological data of 11 different traits recorded on 75 genotypes. Simple linear and multiple regression analysis by stepwise method were done for 11 morphological traits in 75 genotypes of date palm by SPSS and in each analysis, one of the morphological traits entered as dependent variable (Y) and all markers were as independent variables (X). By identification of the markers that justify high percentage of variation, their location was determined on gel.

RESULTS

Morphological results

Table 2 shows Pearson correlation matrix between different traits. The results showed that tree performance with the weight of ten stones (r=-0.506**), the ratio of length to fruit diameter (r=-0.260*), fruit tissue (r=-0.425**), stone length (r=-0.597**) had significant and negative correlation and had positive and significant correlation with fruit weight to stone (r=0.580**) and fruit diameter (r=0.306**). The weight of ten stones with fruit weight to stone (r=-0.453**) had negative and significant correlation and with fruit length (r=0.435**), the ratio of length to fruit diameter (r=0.284*), fruit form (r=0.411**), fruit tissue (r=0.318**), stone length (r=0.541**), stone diameter (r=0.376**) had positive and significant correlation. There was a positive and significant correlation between fruit weight to stone (r=0.781**), fruit length (r=0.306**), fruit diameter (r=0.303**), fruit form (r=0.360**) with fruit weight. The fruit weight with fruit tissue (r=-0.290*) had significant and negative correlation. The ratio of fruit weight to stone had significant and positive correlation with fruit diameter (r=0.282*). While it showed negative and significant correlation with the ratio of length to fruit diameter (r=-0.274*), fruit tissue (r=-0.393**) and stone length (r=-0.248*). The results showed that fruit length with fruit diameter (r=0.247*), the ratio of length to fruit diameter (r=0.340**), fruit form (r=0.468**) had positive and significant correlation. Fruit diameter with length to fruit diameter (r=-0.779**) and stone length (r=-0.264*) had significant and negative correlation and had positive correlation with fruit form (r=0.274*). The results showed that the ratio of length to fruit diameter with stone length (r=0.310**) and fruit tissue with stone length (r=0.348**) had positive and significant correlation.

The results of ISSR and RAPD markers in 75 genotypes

By 14 ISSR primers, 162 fragments were amplified and all bands were polymorph and polymorphism percentage was calculated 100%. The size of the fragments of ISSR primers was between 100 to 2250 base pairs. The distribution of PIC value for ISSR primers was ranging. 0.064 for UBC 853 to 0.319 for UBC 888 with average value 0.193. The marker index in ISSR primers was ranging 0.257 for UBC 853 to 4.507 for ISSR 06 with average 2.441 (Table 3). Of 10 RAPD primers, 132 bands were produced, 122 of which were polymorph and 10 were monomorphs. The size of the bands was ranging between 100 to 2500 base pairs. PIC distribution for RAPD primers was ranging 0.144 (Oligo 342) to 0.304 (Oligo 211) with average 0.288. The marker index was ranging from 0.864 for Oligo 342 to 6.992 for Oligo 211 with average 2.915 (Table 3).

The results of molecular and morphological data regression

Simple regression: Data on each of 11 morphological traits were separately regressed on each of the polymorphic markers, including 162 ISSR markers and 132 RAPD markers. In each marker class, while there was marker, on each of which more than one trait regressed significantly, there were also individual traits which regressed significantly on more than one marker.

Table 2- Pearson correlation matrix between 11 morphological traits in75 genotypes of 15 cultivars of Iranian date palm

Traits	Abbreviations	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
Tree performance (Kg)	X1	1										
Weight of ten stones (g)	X2	-0.506**	1									
Fruit weight (g)	X3	0.210	0.157	1								
fruit weight to stone (g)	X4	0.580**	-0.453**	0.781**	1							
Fruit length (cm)	X5	0.015	0.435**	0.306**	0.011	1						
Fruit diameter (cm)	X6	0.306**	0.005	0.303**	0.282*	0.247*	1					
fruit length to the diameter	X7	-0.260*	0.284*	-0.109	-0.274*	0.340**	-0.779**	1				
Fruit form(circular, ellipse, egg- and pear-shaped)	X8	-0.036	0.411**	0.360**	-0.006	0.468**	0.274*	0.044	1			
Fruit tissue (dry, semi-dry, soft)	X9	-0.425**	0.318**	-0.290*	-0.393**	0.207	-0.047	0.152	-0.052	1		
stone length (mm)	X10	-0.597**	0.541**	0.089	-0.248*	0.112	-0.264*	0.310**	0.185	0.348**	1	
stone diameter (mm)	X11	0.094	0.376**	0.166	-0.069	0.172	0.093	0.002	0.111	0.022	-0.058	1

** significant correlation at 1% (P < 0.01) * significant correlation at 5 % (P < 0.05)

Table 3- Details of 14 ISSR and 10 RAPD primers used for PCR amplification of 75 genotypes of 15 date palm cultivar, their primer sequence, polymorphic information content (PIC) values and marker index

Prime		Primer sequence		Annealing temperature		PIC value		Marker index	
ISSR	RAPD	ISSR	RAPD	ISSR	RAPD	ISSR	RAPD	ISSR	RAPD
UBC888	oligo203	5´-BDB CAC ACA CAC ACA CA-3´	5´-CAC GGC GAG T-3´	52	34	0.292	0.204	3.796	2.448
UBC887	oligo33	5´-DVD TCT CTC TCT CTC TC-3´	5´-CCG GCT GGA A-3´	51	34	0.294	0.195	3.528	2.925
UBC886	oligo29	5´-VDV CTC TCT CTC TCT CT-3´	5´-CCG GCC TTA C-3´	52	34	0.222	0.233	3.996	1.398
UBC823	oligo345	5´-TCT CTC TCT CTC TCT CC-3´	5´-GCG TGA CCC G-3´	52	36	0.307	0.281	1.535	3.653
UBC826	oligo349	5´-ACA CAC ACA CAC ACA CC-3´	5´-GGA GCC CCC T-3´	52	36	0.246	0.224	2.706	2.464
ISSR06	oligo213	5´-GAG AGA GAG AGA GAG AC-3´	5´-CAG CGA ACT A-3´	52	30	0.261	0.209	3.654	2.09
UBC841	oligo214	5´-GAG AGA GAG AGA GAG AYC-3´	5´-CAT GTG CTT G-3´	55	30	0.275	0.256	3.575	3.328
UBC853	oligo42	5´-TCT CTC TCT CTC TCT CRT-3´	5´-TTA ACC CGG C-3´	53	32	0.222	0.230	0.888	2.999
UBC824	oligo342	5´-TCT CTC TCT CTC TCT CG-3´	5´-GAG ATC CCT C-3´	52	32	0.306	0.144	1.53	0.864
UBC842	oligo211	5´-GAG AGA GAG AGA GAG AYG-3´	5´-GAA GCG CGA T-3´	55	32	0.274	0.304	3.014	6.992
UBC889		5´-DBD ACA CAC ACA CAC AC-3´	-	51		0.263		3.156	
UBC835		5´-AGA GAG AGA GAG AGA CYA-3´	-	55		0.282		3.948	
UBC884		5´-HBH AGA GAG AGA GAG AG-3´	-	51		0.309		2.163	
UBC840		5´-GAG AGA GAG AGA GAG AYT-3´		53		0.296		4.736	

ISSR marker: The results variance of regression analysis showed significant association of 89 ISSR markers with 11 traits. In figure 1 (above figure) showed a sample of informative ISSR markers with regression relation with fruit form trait. The highest number of marker was identified in ratio of fruit weight to stone (16 markers) and lowest marker number was identified in stone diameter trait (3 markers). Associated markers explained up to 25.1 % (for seed diameter) to 14.7% (for fruit weight ratio to stone) of the total variation available for different individual traits (Table 4). In fruit weight to stone and tree performance, ISSR06 as head group marker with the highest determination coefficient (16.8%, 14.5%, respectively) were defined.

RAPD marker: The results variance of regression analysis of RAPD marker showed significant relation between 84 primers with the traits. Figure 1 (below figure) shows a sample of informative RAPD marker related to fruit form trait. The lowest and highest numbers of RAPD marker were related to fruit form trait (12 markers) and fruit diameter trait (4 markers). The associated markers each explained up to 32.0% (for seed diameter) to 75.6% (for fruit form) of the morphological variation for 11 different traits (Table 4). Oligo 214 primers in seed diameter trait (with 26%) and Oligo 211 in fruit form (24.1%) had the highest determination coefficient as head group marker.

Table 4- Details of simple linear regression analysis using ISSR and RAPD markers involving 11 different morphological traits

Traits	ISSR		RAPD	
	T [a]	R^2_T [b]	T	R^2_T
Weight of ten stones (g)	7	0.376	9	0.573
Tree performance(Kg)	11	0.747	5	0.373
Fruit tissue (dry, semi-dry, soft)	7	0.407	9	0.506
Fruit weight (g)	6	0.366	9	0.529
fruit weight to stone (g)	16	0.847	5	0.407
Fruit length (cm)	8	0.521	11	0.671
Fruit diameter (cm)	12	0.678	4	0.348
fruit length to the diameter	7	0.376	9	0.573
Fruit form (circular, ellipse, egg- and pear-shaped)	5	0.417	12	0.756
Seed length (mm)	7	0.379	6	0.409
Seed diameter (mm)	3	0.251	5	0.320

[a] T: Total number of markers showing significant association with the traits

[b] R^2_T : Maximum variation of trait explained by a marker out of total significantly associated markers

Multiple regressions*:* The significant relation of 89 markers of 162 polymorph ISSR markers and 84 markers of 122 polymorph RAPD markers are shown with the separation of different traits and different number of ISSR marker (3-16) and RAPD (4-12) and significance of their regression model for first marker (first independent variable) and total associated markers with desirable cultivation trait (total independent variables entering regression model) are shown in table 5. In ISSR marker, stone diameter showed significance linear regression with 3 markers, fruit form with 5 markers, fruit weight with 6 markers, each of the traits of the weight of 10 stones, fruit tissue, the ratio of length to stone diameter and stone length with 7 markers, fruit length with 8 markers, tree performance with 11 markers, fruit diameter with 12 markers and the ratio of flesh to the stone was shown by 16 markers. In RAPD marker, significance regression model for fruit diameter with 4 markers, tree performance, the ratio of fruit weight to stone and seed diameter with 5 markers, the stone length with 6 markers, the weight of ten stones, fruit tissue, fruit weight and the ratio of length to fruit diameter with 9 markers, the fruit length with 11 markers and fruit form with 12 markers are shown. All regression models were significant for ISSR and RAPD marker and for all traits at level 1% (Table 5). Of 294 DNA markers (162 ISSR markers and 132 RAPD markers), 173 markers (89 ISSR markers and 84 RAPD markers) with at least one of 11 traits of fruit, stone and tree performance characteristics in both marker systems showed association. UBC 886 primer of ISSR marker except the traits of fruit form, stone length and stone diameter with other traits had association; Oligo 42 primer of RAPD marker had association with all the traits except stone length (Table 6).

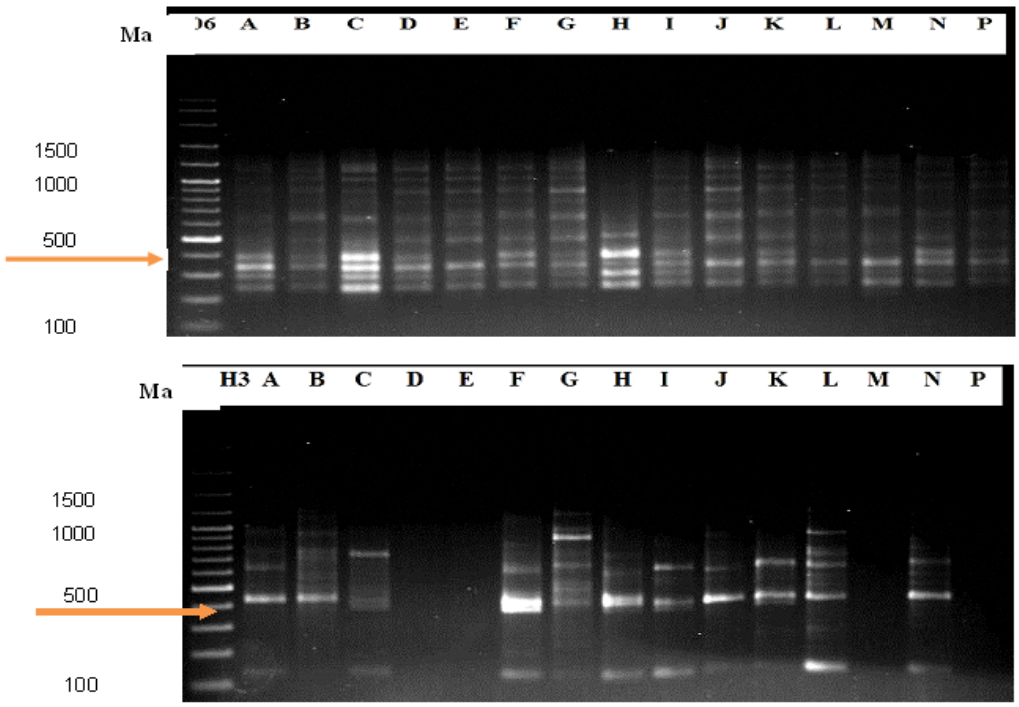

Figure 1- polymorphism in a representative set of 15 cultivars detected using ISSR primer ISSR06 (above) and RAPD primer Oligo 211 (bellow). The arrows shown informative markers with fruit form by ISSR 06 and Oligo 211 primers (Ma is size marker)

Table 5- Details of analysis of variances (ANOVA) involving multiple regressions for 11 morphological traits using 162 ISSR and 122 RAPD polymorphic bands (only those having significant regression are included)

Traits	Source of variance	Mean squares			
		df	$ISSR_{complete}$	df	$RAPD_{complete}$
Weight of ten stones (g)	x	7	8.299**	9	9.833**
	y	67	1.440	65	1.016
Tree performance (Kg)	x	11	2495.417**	5	2741.029**
	y	63	147.216	69	333.609
Fruit tissue (dry, semi-dry, soft)	x	7	8.642**	9	8.366**
	y	67	1.316	65	1.129
Fruit weight (g)	x	6	28.945**	9	27.862**
	y	68	4.419	65	3.437
fruit weight to stone (g)	x	16	116.891**	5	168.478**
	y	58	5.349	69	17.777
Fruit length (cm)	x	8	1.167**	11	1.093**
	y	66	0.130	63	0.093
Fruit diameter (cm)	x	12	0.697**	4	1.074**
	y	62	0.064	70	9.115
fruit length to the diameter	x	7	8.299**	9	9.833**
	y	67	1.440	65	1.016
Fruit form (circular, ellipse, egg- and pear-shaped)	x	5	8.227**	12	6.217**
	y	69	0.834	62	0.388
Seed length (mm)	x	7	29.393**	6	37.021**
	y	67	5.040	68	4.725
Seed diameter (mm)	x	3	2.413**	5	1.841**
	y	71	0.304	69	0.284

** Significance at 1% ($P < 0.01$) df: Degree of freedom.
$ISSR_{complete}$: A percent of the changes are justified by all independent variables of ISSR entering regression.
$RAPD_{complete}$: A percent of the changes are justified by all independent variables of RAPD entering regression.
x: regression y: residual

Table 6- Markers common in both simple linear and multiple regression analysis (for example see figure 1. for associated of molecular marker and morphological traits)

Traits	Marker type	Markers designation
Weight of ten stones (g)	ISSR	UBC835, UBC889, UBC824, UBC886
	RAPD	Oligo349, Oligo211, Oligo213, Oligo42, Oligo342
Tree performance (Kg)	ISSR	ISSR06, UBC853, UBC889, UBC888, UBC886, UBC823, UBC835
	RAPD	Oligo213, Oligo345, Oligo33, Oligo214, Oligo42
Fruit tissue (dry, semi-dry, soft)	ISSR	UBC841, UBC886, UBC823, UBC826, UBC835
	RAPD	Oligo42, Oligo33, Oligo342, Oligo211, Oligo349, Oligo345
Fruit weight (g)	ISSR	UBC823, UBC853, UBC886, UBC889, ISSR06
	RAPD	Oligo213, Oligo345, Oligo33, Oligo203, Oligo42
fruit weight to stone (g)	ISSR	UBC824, UBC835, UBC889, UBC840, UBC888, ISSR06, UBC853, UBC886
	RAPD	Oligo213, Oligo342, Oligo211, Oligo349, Oligo42
Fruit length (cm)	ISSR	UBC823, UBC826, UBC884, UBC842, UBC886, UBC889, UBC835
	RAPD	Oligo42, Oligo211, Oligo349, Oligo203, Oligo33, Oligo214, Oligo213
Fruit diameter (cm)	ISSR	UBC889, UBC840, UBC886, UBC842, UBC841, UBC887, UBC888, UBC884, UBC826, ISSR06
	RAPD	Oligo29, Oligo42, Oligo33, Oligo203
fruit length to the diameter	ISSR	UBC853, UBC889, UBC884, UBC886, UBC824, UBC835
	RAPD	Oligo213, Oligo349, Oligo33, Oligo203, Oligo42
Fruit form (circular, ellipse, egg- and pear-shaped)	ISSR	UBC835, UBC826, ISSR06, UBC824, UBC842
	RAPD	Oligo211, Oligo33, Oligo203, Oligo349, Oligo42, Oligo213
Seed length (mm)	ISSR	UBC824, UBC823, UBC840, UBC888, ISSR06, UBC853, UBC887
	RAPD	Oligo29, Oligo214, Oligo345, Oligo213
Seed diameter (mm)	ISSR	UBC888, UBC840, UBC823
	RAPD	Oligo29, Oligo349, Oligo33, Oligo214, Oligo42

DISCUSSION

Molecular markers linked with QTL/major genes for traits of interest are being routinely developed in several crops using materials derived from planned crosses such as F2, RIL, DH populations, etc. Hopefully, some of these markers will be used for MAS in future wheat breeding programs. However, non-availability of mapping populations and substantial time needed to develop such populations are sometimes major limitations in the identification of molecular markers for specific traits. Another limitation is the absence of tight linkage observed in these studies. To overcome these limitations, and as an alternative to planned populations, molecular markers for traits of interest have been identified through association studies conducted using germplasm collections [5]. This study involved a set of 15 population of date palm, which combined an important population of Iranian date palm, exhibition to high genetic diversity with RAPD and ISSR markers for the 11 morphological traits examined during the present study. To identify some of varieties of date palm of Saudi Arabia were applied RAPD fingerprinting in 5 varieties. This research was reported that of 12 RAPD primers, 64 bands were produced [1]. In another study applied RAPD and ISSR markers to evaluate genetic diversity and gender determination of 6 male and female genotypes of date palm and polymorphs of RAPD and ISSR was 60.2 and 73 respectively [11]. Correlation of 11 traits was performed by pearson method and result showed that two by two correlation of most of the traits was significant. In this study reported that there is a significant and positive correlation between the weight of 30 stone and the ratio of fruit weight to stone with stone weight had positive and significant correlation. Also, there is a negative and high correlation between tree height, weight of 30 stone, the ratio of the weight of 30 stones to fruit and stone weight [16].

The results showed that some of the markers were associated with more than one trait. Based on significant correlation between some of morphological traits, we can say that correlation of some of traits with each other is more than the others. The results of this study are useful for providing initial data for indirect selection of the traits via related markers. Thus, in breeding purposes, these primers can be useful for identification of informative markers with high correlation with important cultivation traits.

Some of the markers as UBC 886 and Oligo 42 were correlated with more than one traits. The stepwise regression results showed that considerable amount of the changes were justified by these two markers and location of genes of these traits were close on chromosome and can be useful for providing initial data about indirect selection of the traits via linked markers. In the same research, association analysis was performed between 14 agronomically traits and 519 SSR (221 markers), SAMPL (43 markers) and AFLP (255 markers) markers in wheat. In SSR markers, the highest value of R^2 for harvest index trait with 28% and 291 bp length in Xwmc44 primer, in SAMPL markers, the highest value of R^2 for harvest index with 20% and 250bp length in primer compound XCCSS6MCAG and in AFLP markers, the highest value of R^2 for floret number in cluster was defined by 29% and 160bp in primer compound XCCSEAACMCTC. The study showed that above markers had uniform distribution around cultivation traits and by sequence of the markers with high R^2; we can hope to find controlling genes of cultivation traits and markers with considerable relation with the traits for saturation of linkage maps [17]. The relation between bacterial blight resistance traits was studied in bean with genome of this plant by RAPD molecular marker. By stepwise regression analysis, eAAEmCAG183 and eAAEmCAG333 justified 51.1% of phenotype variety and there was a high correlation between plant genome and resistance to bacteria disease with this marker [22]. By investigating association analysis between 11 morphological traits in Medicago sativa L. and SSR markers by stepwise regression method was identified that 17 SSR markers have associating with at least one of the traits. The study showed that considerable numbers of morphological changes (wet and dry weight of leaf, stem dry weight, total dry weight and total wet weight) were justified by B14B03 marker. They found that these markers can be applied in selection of favorite plants [12]. To provide association maps, the marker data of the current study is useful for selection of good primers. Although some of the markers were used to do breeding purposes in other types of plant but the lack of dispersed population for cultivation traits and molecular primers were the most important limitations in identification of the markers associated with cultivation traits [5]. By informative markers of cultivation traits including performance components, especially about the markers with definite chromosome location, by producing the lines with chromosome replace, the desirable cultivation traits are transferred via crossing in a line. By sequencing of informative bands and their comparison with the existing sequences in data bank increase the reliability of selection by Marker and its simulation [12].

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