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Genetic Identification of Unknown Date Palm Genotypes and their Relation to Saudi Date Palm Cultivars

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Abstract

In Saudi Arabia, date palm is the most important fruit tree, and little is known about its germplasm. The aim of the present, study was to analyze genetic diversity among 27 date palms using ISSR markers and also to study genetic relationship between unknown date palm genotypes and commercial Saudi date palm cultivars. Among the fourteen ISSR primers used, eight of them showed a net amplification of DNA fragments. The repeats $(AG)_8$ were the most abundant in date palm. The molecular variance showed variability of 60% among genotypes. The date palm genotypes were clustered to four groups. The dendrogram showed that the following genotypes are in the same subgroup: unknown genotype (UN1) from Al-Bosr; and cultivar Barhi; cultivar "Khlas" and unknown genotype (SL) "Slaga". Cultivar "Nabtit Ali" and unknown genotype "Sahimia". Moreover, two individuals (YS and BS) of "Sukary" were found in the same subgroup and in the same level. This was the case of cultivar "Barhi" individuals and cultivar "Khlas" individuals. However, yellow Sukary (YS) was not in the same group of red Sukary (RS). It is the first time to establish a relation between male palm and date palm cultivars, and also to report that male palm and cultivar yellow Sukary were in the same group. Results showed that some unknown date palm genotypes are closely grouped with Saudi cultivars. Results also revealed the existence of genetic variation in the *invGE/GF* locus for sugar content among date palm genotypes. Further work is needed to investigate the association between phenotype of sugar components and invertase genes.

Keywords: Date palm genotypes; Male palm; Genetic diversity; ISSR markers; Invertase genes

Introduction

Date palm (*Phoenix dactylifera*) is of social and economic importance and a strict dioecious evergreen tree capable of living over 100 productive years. The origin of the date palm is southern part of Iraq. The date palm trees were transferred by humans to many other countries around the world including Saudi Arabia and are the major economic tree in the arid zone. The production of date palm in Saudi Arabia is more than 10% of the world's production and the varieties in Saudi Arabia is around 340 out of approximately 2,000 varieties reported in different countries [1].

Molecular characterization and genetic diversity of date palm genotypes and identification of genes controlling traits will improve the prospects of date palm breeding for yield and other agronomic traits. It also provides a means to answer long-standing questions about date palm diversity and the history of domestication [2].

ISSR and SSR-DNA markers were found to be the most suitable techniques in molecular characterization of date palm [3,4]. It was also found to be very powerful for genotyping [5] and marker-assisted selection in breeding programs [6,7]. The analysis of genetic diversity of date palms by using SSR-markers is very important to develop DNA fingerprints The SSR markers are used to analyze the genetic diversity among date palm cultivars to develop a DNA fingerprints [8].

Sugar contents of date palm fruit are important to the consumers. Researchers are investigating how to control sugar composition in food crops especially in date palm. The enzyme invertases (*β-D-fructofuranosidase EC3.2.1.26)* hydrolyzes disaccharide sucrose into glucose and fructose [9]. Invertase alleles associated with the sweetening trait in breeding populations can provide a diagnostic marker for the selection of cultivars. Diagnostic markers derived from invertase gene can be used to screen date palm genotypes. The transcription of invertase showed higher levels at the late fruiting stage of date palm [10]. Hazzouri et al. [2] reported candidate mutations for trait variation in date palm including nonsense polymorphisms and presence/absence variation in gene content.

The objectives of this work were to identify date palm cultivars using ISSR markers and examine the molecular diversity of the *invGE* and *invGF* genes for sugar content in date palm genotypes. In addition, genetic relationship of unknown date palm genotypes to commercial Saudi date palm cultivars was studied.

Material and Methods

Plant materials

A set of 27 date palm samples were used in this study (Table 1). Among them, 26 were females, belonging to 9 Saudi cultivars and 11 unknown genotypes and one palm was male. Six cultivars contained two samples from different locations and three cultivars contained one sample.

DNA extraction

Total genomic DNA of genotypes of *P. dactylifera* was extracted using the method described by Sedra et al. [11].

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ISSR assay

The ISSR analysis was carried out according to Negaoka and Ogihara [12]. PCR amplification was conducted in a thermal cycler (Thermolyne Amplitron) according to Negaoka and Ogihara [12].

Allele-specific marker assays for sugar content in date palm

Allele-specific marker assays for sugar content in date palm genotypes were conducted using invertase alleles *invGF* and *invGF*. Amplification was carried out in 25 µL reaction volumes using the primer sequences shown in Tables 2 and 3. Amplification was performed in a thermal cycler (Thermolyne Amplitron) programmed

No.	Code	Name	Number of samples	Location
DSH1	MS	Male	1	Al-Qassim
Date palm cultivars				
DSH2	YS	Yellow Sukary	1	Al-Qassim
DSH3	BS	Yellow Sukary	1	Al-Bosr
DSH4	RS	Red Sukary	1	Al-Qassim
DSH5	BA	Barhi	1	Al-Qassim
DSH6	BB	Barhi	1	Al-Bosr
DSH7	Kh	Khlas	1	Al-Qassim
DSH8	Bkh	Khlas	1	Al-Bosr
DSH9	Sh	Shakra	1	Al-Qassim
DSH10	NB	Nabtit Ali	1	Al-Qassim
DSH11	BNB	Nabtit Ali	1	Al-Bosr
DSH12	Wn1	Wnana	1	Al-Qassim
DSH13	Wn2	Wnana	1	Al-Bosr
DSH14	ROM	Rothana	1	Al-Madina
DSH15	ROQ	Rothana	1	Al-Qassim
DSH16	RH	Rshodia	1	Al-Qassim
Unknown genotypes				
DSH17	NI	Nifia	1	Al-Qassim
DSH18	Q	Qatara	1	Al-Qassim
DSH19	SAL	Salma	1	Al-Qassim
DSH20	KO	Kodia	1	Al-Qassim
DSH21	UN1	Unknown genotype	1	Al-Qassim
DSH22	UN2	Unknown genotype	1	Al-Bosr
DSH23	SL	Slaga	1	Al-Qassim
DSH24	HI	Hilalia	1	Al-Qassim
DSH25	QR1	Qarawia	1	Al-Bosr
DSH26	DA	Dahisia	1	Al-Bosr
DSH27	Sa	Sahimia	1	Al-Qassim

Table 1: List of collected date palm genotypes.

Primers	Sequence	Amplified products	Fraction polymorphic fragments*
UBC807	$(AG)_8T$	9	9/9
UBC810	$(GA)_8T$	6	6/6
UBC811	$GA)_8C$	3	2/3
UBC22	$(TC)_8A$	4	4/4
UBC23	$(TC)_8C$	3	3/3
UBC824	$(TC)_8G$	3	3/3
UBC25	$(AC)_8T$	4	3/4
UBC826	$(AC)_8C$	4	2/4
*Determined empirically.			

Table 2: ISSR primers used in this study and a summary of ISSR markers.

Primer name	Forward primer sequence 5'–3'	Reverse primer sequence 5'–3'
InvGE-1	CTC AGC ATC ACA GGT TTT AAC	TCA TTA CAA CTA ATT CAA TTG
InvGE-2	GAA AAG CTC TTC TCT TTG GGG T	CCG GAC CAA GCA CCA TAT TT
InvGE-3	GGG TTC GAC TAT CCA AGG TG	CAG CAC CAA AAC TCT CCA CTA C
InvGF-4	GTT GGG CTT TGC CAG TTA TC	GCC CCA TAC TGA CCC ATT TG
InvGF-5	GTT ACA GGA ATC ACA CCT GCA C	CTT TCC ACC AGC ACC AAA AC
InvGE-6	GAG CAA GGG AGA AAT GTT TGA	AAA CAT CTT GGG CAT AAA GGT C

Table 3: PCR primers for specific amplification of fragments of invertase genes *invGE* and *invGF*.

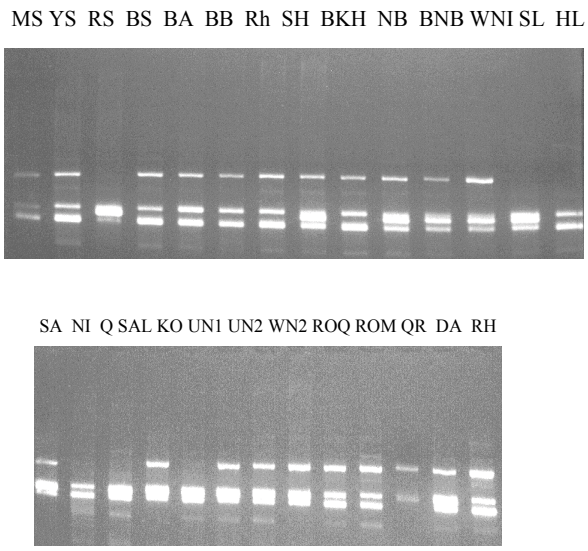

Figure 1: Agarose gels showing fragments of PCR-amplified ISSR primer UBC822 with date palm genotypes (27 genotypes abbreviations referred to Table 1).

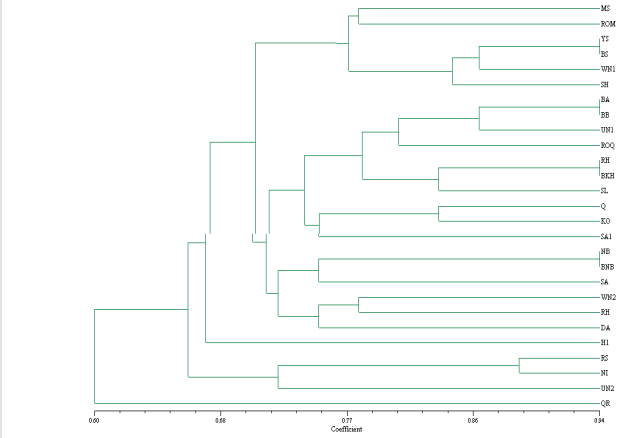

Figure 2: Dendrogram constructed from ISSR markers and showing the clustering of the tested date palm genotypes (27 genotypes abbreviations referred to Table 1).

for 1 cycle of 30 s at 94°C; and 40 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C; followed by 5 min at 72°C.

Cluster analysis

Data of molecular markers was scored for computer analysis on the basis of the presence or absence of the amplified products for each primer. Basically, if a product is present in a genotype, it was designated "1", but if absent it was designated "0" after excluding irreproducible bands. Pair-wise comparisons of cultivars, based on the presence or absence of unique and shared polymorphic products, were used to generate similarity coefficients. The similarity coefficients were used to construct a dendogram by UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) using NTSYS-PC [13].

Results and Discussion

ISSR analysis

Among the fourteen ISSR primers used, eight showed a net amplification of DNA fragments (Table 2). The most informative primer, considering percentage of polymorphism (%P=100), were UBC 807, UBC 810, UBC 822, UBC 823, and UBC 823. The highest number of polymorphic bands was UBC 807. Figure 1 represents an example of agarose gel showing the pattern of DNA fragments amplified with UBC 822. The repeats $(AG)_8$ was the most abundant in date palm. Zhao et al. [14] stated that the AG-SSR repeat is the most abundant and polymorphic among di-nucleotide and comprises 85.7% of palm genome. The motif AG is the most abundant and highly polymorphic in both annual and perennial plants [15]. The role of the AG motif in the function of plant genes needs further investigations. The different sizes of the DNA fragments amplified showed the polymorphism of the ISSR markers. This polymorphism is used for the determination of the differences between genotypes and also to calculate the genetic parameters. A total of 36 alleles were detected for 8 selected ISSR loci. The number of alleles per locus varied from 3 (UBC 823 and UBC 824) to 9 (UBC 807) (Table 2). The overall analysis indicated that the genome of all genotypes exhibited abundant microsatellite repetitive sequence [16].

Genetic diversity among date palm genotypes

The similarity dendrogram showed 4 main groups and the molecular variance showed 60% of variability among genotypes (Figure 2). The first group was divided into 2 subgroups. The first subgroup constituted by 2 individuals (YS and BS) of "Sukary", 1 individual (WN1) of "Wnana" from Al-Qassim, cultivar (SH) "Shakra", male (MS) and 1 individual (ROM) of cultivar "Rothana" from Al-Madina. The second subgroup was composed of the rest of the commercial Saudi date palm cultivars and unknown date palm genotypes. This subgroup was divided into 5 sub-subgroups. The first contained 2 individuals of cultivar "Barhi", unknown genotype (UN1) from Al-Bosr and cultivar "Rothana". The second contained 2 individuals of cultivar "Khlas" and unknown genotype (SL) "Slaga". The third contained three unknown genotypes (Qatara, Salma and Kodia). The fourth contained 2 individuals of cultivar "Nabtit Ali" and unknown genotype "Sahimia". The fifth contained 1 individual (WN2) of Saudi cultivar "Wnana" from Al-Bosr, Saudi cultivar "Rshodia", and unknown genotype "Dahisia". The second and fourth groups were constituted by unknown genotypes "Hilalia" and "Qarawia". The third group was constituted by cultivar "Red Sukary" and unknown genotype "Nifia" and unknown genotype (UN1) from Al-Qassim. It noteworthy that these unknown date palm genotypes have no previous relation to date palm cultivars. The dendrrogram showed that unknown genotype (UN1) from Al-Bosr and cultivar Barhi were in the same subgroup. Also, cultivar "Khlas" and unknown genotype (SL) "Slaga" were in the same subgroup. Cultivar "Nabtit Ali" and unknown genotype "Sahimia" were in the same subgroup. However, the other unknown genotypes were separated in different groups. Moreover, the dendrogram showed that 2 individuals (YS and BS) of "Sukary" were in the same subgroup and in the same level. The individuals of this cultivar did not show any genetic variability. This was the case of cultivar "Barhi" individuals and cultivar "Khlas" individuals. However, yellow Sukary (YS) was not in the same group with red Sukary (RS). They were two cultivars which had the same name but were genetically different. It should be noted that male palm and cultivar yellow Sukary were in the same group (Figure 2). It is the first time to establish a relation between male palm and date palm cultivars.

The dendrogram constructed here revealed that date palm cultivars examined are not monophelic and provide evidence of divergence among all tested genotypes. This confirms findings of [5,16]. Also, Bodian et al. revealed the existence of genetic variation among Moroccan date palm cultivars. Results also indicated that some unknown date palm genotypes are closely grouped with Saudi cultivars. This can be explained by the presence of a common genetic origin among the tested genotypes in spite of their great diversity [16].

Molecular diversity at the *invGE/GF* locus

As presented in Table 3 six pairs were used to amplify by PCR specific parts of the *invGE/GF* locus [9]. The amplification products of the *invGE/GF* primers presented different fragment patterns in date palm genotypes (Figure 3). The *invGE* gene is orthologous to the region of the tomato *Lin5* gene, which is responsible for fruit-sugar-yield QTL [17].

The similarity dendrogram (Figure 4) showed 3 main groups. The first group included male genotype, eight date palm cultivars and two unknown genotypes (Hilalia and Slaga), and was divided into two sub-groups. The second group included the other genotypes except one unknown genotype "Qarawia" which clustered in the third group. The results revealed the existence of genetic variation in the *invGE/GF* locus

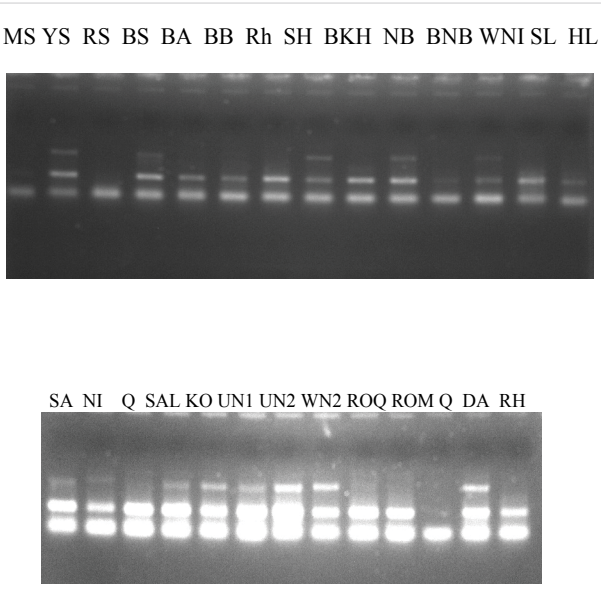

Figure 3: Agarose gels showing fragments of PCR-amplified *invGE/GF* primer GF4 with date palm genotypes (27 genotypes abbreviations referred to Table 1).

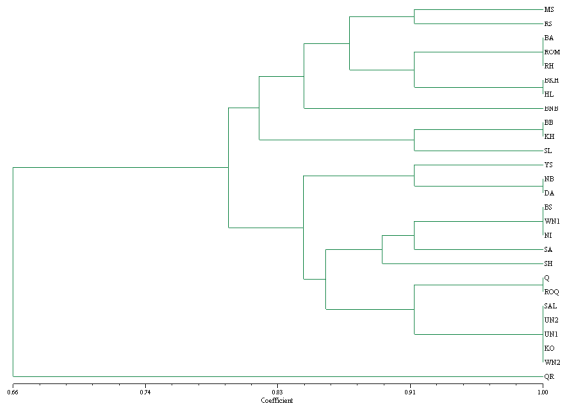

Figure 4: Dendrogram constructed from the *invGE/GF* primers and showing the clustering of the tested date palm genotypes (27 genotypes abbreviations referred to Table 1).

among date palm genotypes. Invertase genes play a fundamental role in sugar accumulation in plants [18]. Li et al. [9] suggested that variation of sugar yield in fruits is controlled by functional variants of invertase genes. The present study can lead to further investigation of phenotype associated with the invertase genes.

Conclusion

The repeats $(AG)_8$ was the most abundant in date palm. The dendrogram constructed revealed that date palm cultivars examined are not monophelic and provide evidence of divergence among all tested genotypes. It is the first time to establish a relation between male palm and date palm cultivars, and also to report that male palm and cultivar yellow Sukary were in the same group. Some unknown date palm genotypes are closely grouped with Saudi cultivars. Results also revealed the existence of genetic variation in the *invGE/GF* locus for sugar content among date palm genotypes. Further work is needed to investigate the association between phenotype of sugar components and invertase genes.

Ethics Approval

This study was approved by College of Agriculture & Veterinary Medicine, Ethics Committee, Qassim University of Saudi Arabia.

Consent to Participate

This research was part of a research program in the Department of Plant production & Production, Kingdom of Saudi Arabia and is approved.

Availability of Data and Materials

The datasets analyzed during the current study are available from the corresponding author on a reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Conflicts of Interest

There are no conflicts of interest.

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