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Molecular characterization and sugar content of some date palm (*Phoenix dactylifera L.)* Cultivars in Saudi Arabia

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Date palms are now grown extensively around the world. There are many cultivars such as Ajwa, Nabtet Ali, Rothanah, Barhi, and Sokkari dates, known for their superior quality. The Ajwa cultivar, however, has particular religious and medicinal significance. The aim of the study was to determine the sugar content of dates from five cultivars in Saudi Arabia. Also, genetic relationship of Ajwa cultivar collected from different locations to Ajwa cultivar from Madinah location. The results of sugar content proved that the dates of five cultivars were rich in sugar (56.60 - 83.75 % dry weight). Cultivar "Barhi" showed only monosaccharides. However, cultivars "Sukkari and Nabat Ali" contained the highest sucrose, a disaccharide, (47.7 and 33.3%). Cultivars "Ajwa and Rothans" showed lower content of disaccharide (22.94 and 10.5%) but higher level of monosaccharides (51.69 and 53.6%), respectively. Analysis of genetic relationships among the samples of Ajwa cultivar collected from different locations was studied using ISSR markers. On the basis of UPGMA analysis the samples were clustered into three clusters. The sample of cultivar Ajwa collected from location 'Al-meznib' did not markedly diverge from Ajwa cultivar (AL-madinah). Therefore, the polymorphism detected suggested that ISSR markers are reliable for date palm germplasm characterization.

Keywords: date palm, sugar content, ISSR markers, genetic relationship

INTRODUCTION

Date palm (*Phoenix dactylifera* L.), the tree of life, is the major fruit tree in most Arabian countries and it is widely grown in the Middle East countries, and especially in the Kingdom of Saudi Arabia. According to a recent economics survey in the Kingdom of Saudi Arabia, in 2016 there were 20 million date palms produce annually almost 1.1 million tons of dates fruits, which represents more than15% of the world total production (FAO, 2017). Today, Saudi is the second largest producer of dates in the world, with more than 300 types of date, each with its own taste and texture (Assirey, 2015). Madinah location is considered the first producer of dates in Saudi Arabia (Assirey, 2015). Fruits of date palm are rich in mineral salts and vitamins and are an excellent material for producing refined sugar, concentrated juice, confectionery pastes and fermentation products (Al-Farsi et al. 2005). The study of Khan et al. (2016), also found that dates contain many flavonoid glycosides, which have anti-oxidant properties. The inhibition rate in Ajwa dates is equal to commercial anti-oxidant supplements. Another benefit is that the sugars in Ajwa dates are monosaccharides, which makes them suitable for people with Type 2 diabetes (Khan et al. 2016).

The future strategy for producers for exporting dates to foreign market would necessitate the

need for modern technology to identify the date palms cultivars genetically to be in a position to meet the international standards of the required cultivars at a competitive price. Information about genetic variability within and between genotypes of date palm is an important factor to study and to classify genotypes into different heterotric groups. The advent of DNA-based genetic markers, such as restriction fragment length polymorphisms (RFLP) and random amplified polymorphic DNA (RAPD) have become more efficient, reliable and useful (Al-Moshileh et al. 1994). Al-Moshileh et al. (2004) have applied RAPD for the discrimination among date palm cultivars at the genomic level. Also, inter simple sequence repeat Inter Simple Sequence Repeat (ISSR) are a type of DNA markers which involve the use of microsatellite sequences directly in the polymerase chain reaction (PCR) for DNA amplification (Sanchez de la Hoz et al. 1996). ISSRs have been used successfully in genome mapping a variety of crop species including jojoba and turfgrass (Al-Soqeer et al. 2012 and Al-Humaid and Motawei, 2004). ISSR and SSR-DNA markers were found to be the most suitable techniques in molecular characterization of date palm (Elshibli and Korpelainen, 2011; Cullis, 2011). Ajwa variety is belonging to holy city of Madinah however, the same variety is harvested in Al-Qassim location also brought into Madinah and selling it with the same name. Therefore, the aim of this study is to investigate the sugar content in Ajwa cultivar and other date palm cultivars. In addition, genetic relationship of Ajwa cultivar collected from different locations to Ajwa cultivar from Madinah location.

MATERIALS AND METHODS

Plant materials

Mature date trees of five cultivars (Ajwa, Nabtet Ali, Rothanah, Barhi, and Sokkari) genetically different from each other, represent the most economical value for the date cultivars in Saudi Arabia, were selected for this study. All the selected trees were almost of the same age and uniform in growth. The trees should be in a good physical condition, and were subjected to the same horticultural management practices. Date fruits of the selected cultivars were collected and transferred immediately to the laboratory, plant production and protection department, College of Agric. & Vet. Med., Qassim Univ. Samples similar in shape, color, and degree of development were divided into groups and wiped free of dirt, weighed, and stored till performing the required analysis.

Sugar content

Samples were spiked with various combinations of standard sugars (1–5 ppm) to monitor recovery, and the sugar concentration was calculated from peak area measurements (Langemeier and Rogers, 1995).

The statistical analysis of the obtained data was performed using JMP Ver. 11 (SAS Institute 2013) to compare means of date palm cultivars for sugar content. Significant differences among cultivars means were calculated based on Tukey's HSD at $P < 0.05$.

DNA Extraction

Total genomic DNA of cultivar "Ajwa" samples which collected from different locations (Madinah (2 samples), Al-meznib, Bakriah, Ryadh, and Sadeer) were extracted using the method described by Sedra et al. (1998). The quality and quantity of the DNA were determined by using UV-Spectrophotometer at wavelengths of 260 and 280nm.

ISSR assay

The ISSR-PCR method was carried out according to Negaoka and Ogihara, (1997). Amplification was carried out in 25 µl reaction volumes, containing 1X Taq polymerase buffer (50 mM KCl, 10mM Tris, pH 7.5, 1.5 mM Mg Cl2) and 1 unit of Taq polymerase (Pharmacia Biotech, Germany) supplemented with 0.01% gelatin, 0.2 mM of each dNTPs (Pharmacia Biotech, Germany), 50 pmol of ISSR primers and 50 ng of total genomic DNA. Amplification was performed in a thermal cycler (Thermolyne Amplitron).

Data Handling and Cluster Analysis

Data were scored for computer analysis on the basis of the presence or absence of the amplified products for each ISSR 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 based on a simple matching. The similarity coefficients were used to construct a dendogram by UPGMA (Unweighted Pair-Group

Method with Arithmetical Averages) using NTSYS-PC (Rohlf, 2000).

RESULTS AND DISCUSSION

Sugar content of date palm cultivars

The data of sugars revealed that total sugar content in the date palm cultivars ranged from 56.60 to 83.75 percent on dry weight basis of the fruit (Table 1). Among them, cultivar "Barhi" showed only monosaccharides. However, cultivars "Sukkari and Nabat Ali" contained the highest sucrose, a disaccharide, (47.7 and 33.3%) in addition to monosaccharides (36.05 and 23.03%), respectively. Unlike cultivars "Ajwa and Rothans" showed lower content of disaccharide (22.94 and 10.5%) but higher level of monosaccharides (51.69 and 53.6%), respectively. The most important commercial characteristics of dates are based on the sugar content which is significant for both consumption and for fruit processing (Fadel, 2008). Because dates contain relatively high sugar content, they may also have an important agro-industrial future as a potential source of refined sugar (Samarawira, 1983; Bacha et al., 1987; Ahmed et al., 1995). In general, dates can be grouped into two types depending on the types of sugars contained in the dates. There are sucrose (a disaccharide) containing dates or reducing sugar (monosaccharide) containing dates (Sawaya et la., 1983). It is important to note that the sugar in Ajwa dates is almost monosaccharaides, making the date beneficial for people who suffer from Type 2 diabetes (Khan et al., 2016).

Molecular characterization of cultivar 'Ajwa' collections

Ten ISSR primers were used to amplify DNA segments from six samples of date palm cultivar 'Ajwa' collected from different locations (Madinah (2 samples), Al-meznib, Bakriah, Ryadh, and Sadeer). All six samples of Ajwa were distinguished by their band patterns. Example of polymorphism is shown in figure 1. Unweighted pair group method arithmetic average ordered the samples of cultivar Ajwa into three main clusters (Fig. 2). Cluster I contained the samples (D1, D2 from Madinah and D3 from Al-meznib). Cluster II contained the two samples (D5 from Ryadh and D6 from Sadeer). Cluster III contained the sample of cultivar Ajwa collected from location 'Bakriah' (D4). The dendrogram constructed from simple matching with UPGMA analysis revealed that the samples of cultivar 'Ajwa' are not monophelic. This confirms findings of Haider et al. (2012). Chaluvadi et al. (2019) reported that differences were observed in all accessions with the same name of date palm cultivar, confirming that mutation is ongoing especially for highly polymorphic markers. The fact that the sample of cultivar Ajwa collected from location 'Al-meznib' did not markedly diverge from Ajwa cultivar (AL-madinah) suggests a narrow genetic diversity of populations from which these samples have been derived. On the other hand, the last samples were less similar to the sample of cultivar 'Ajwa' collected from location 'Bakriah'. Therefore, ISSR-markers should be of high value for date palm germplasm characterization and genetic maintenance (Chaluvadi et al., 2019; Jaradat, 2014).

Table 1: Sugar content of dates from five cultivars (g/100g dry weight

Cultivars	Monosaccharide sugar (%)	Disaccharide sugar (%)	Total sugar (%)
Barhi	83.19 a*	0.0 e	83.19 a
Ajwa	51.69 b	22.94 c	74.63 b
Sukkari	36.05 c	47.70 a	83.75 a
Rothana	53.60 b	10.50 d	64.10 bc
Nabtat Ali	23.03 d	33.30 b	56.60 c

* Means in the same column followed by the same letter are not significantly different at $p = 0.01$ while those with different letters are significantly different.

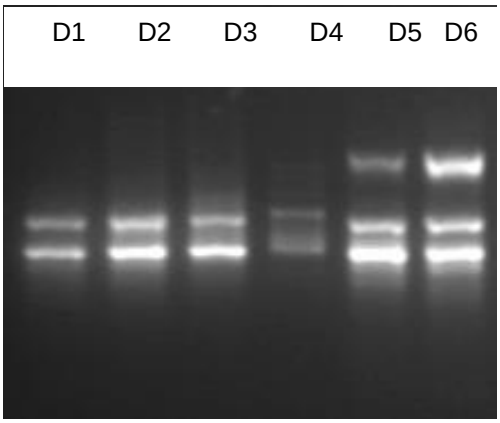

Figure 1: Polymorphism revealed using primer UBC 426 to amplify genomic DNA purified from six samples of date palm cultivar Ajwa (*left to right*) collected from Madinah "D1, D2", Al-meznib"D3", Bakariah "D4", Ryadh "D5" and Sadeer "D6"

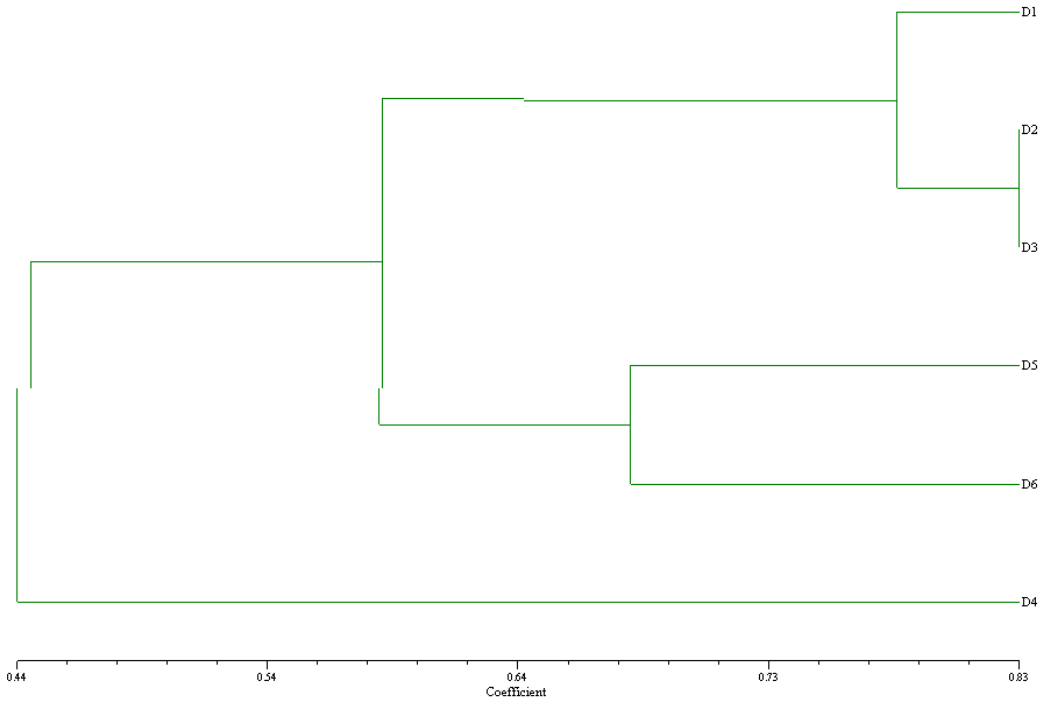

Figure 2: Dendrogram constructed from similarity coefficients showing the clustering of six samples of date palm cultivar 'Ajwa' collected from Madinah "D1, D2", Al-meznib"D3", Bakariah "D4", Ryadh "D5" and Sadeer "D6".

CONCLUSION

The results show that date palm cultivars have a high content of sugar (56.60 - 83.75%). The sugar in Ajwa dates is almost monosaccharaides, making the date beneficial for human health. In addition, the results showed that the samples of cultivar 'Ajwa' collected from different locations are not monophelic. Therefore, ISSR markers could be used to evaluate the genetic diversity of date palm.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

SMA designed and performleed the experiments and also wrote the manuscript.

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