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Evaluation of stability and uniformity in tissue culture-date palm (*Phoenix dactylifera* L.) plants of cv. Berhee by using morphological characteristics

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Abstract

Date palm (*Phoenix dactylifera* . L) is a dioecious, monocot and tropical fruit that requires pollination for fruit set and economically production. It is propagated by offshoot routinely. The low number of offshoot and short time of offshoot production are considered as the most disadvantages for its propagation. Since 20th century, propagation of date palm through tissue culture techniques is conducted. Embryogenesis and organogenesis are the two main techniques for in vitro propagation of date palm. Somaclonal variation is the most problem in tissue culture of date palm that impose difficulties for growers. So, finding the easy and inexpensive method to control stability and uniformity are the most important factors in tissue culture propagation of date palm. In this experiment, we evaluated the qualities of tissue culture date palms c.v Berhee by analysis of some morphological characters. There was a lot of variation between propagated trees. It was noticed that some morphological factors can be considered as key factors to screen the type and off-type trees in this cultivar.

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Introduction

Date palm (*Phoenix dactylifera* . L) is one of the most important tropical fruits and it has a main role in food security and job creation in tropical reigns. It's a dioecious, perennial and monocot plant which requires pollination and fruit set to economically production. Over than 3000 cultivar of date palm are cultivated in the world. The most of these are in Middle East and North African countries. The number of Iranian native cultivar has been reported up to 400 cultivars (Dawson, 1982). Iran had the third position in date palm fruit production in the world (FAO, 2010). Date palm can be propagated by sexual (seed) and asexual (offshoot and tissue culture) methods (Nixon and Carpenter, 1978; Zaid and de Wet, 2002b). Seed propagation usually used in breeding program, however due to severe heterozygosis of date palm and increasing the rate of male palm by at least 50% is not recommended for mass propagation. The most common method of asexual reproduction of date palm is by using offshoot which produces plants similar to mother plant. Offshoots are raised from lateral buds on the base of stem near to the soil surface during juvenile phase. They grow for 3 to 5 years in connection to mother plant and they are separated thereafter for re planting. At this age, new palms usually start flowering.

A palm, produce a limited number of offshoots during the short period of growth and it is limited to the juvenile phase that is considered as the most disadvantage of asexual propagation by offshoot. Application of tissue culture techniques for date palm propagation is started from 1970 (Zaid and de Wet, 2002b). Embryogenesis and organogenesis are two main techniques in date palm in vitro propagation (Zaid.1999). In comparison, the multiplication rate of embryogenesis is higher than organogenesis (Alkhateeb *et al.,* 2006). Organogenesis technique involves the use of lateral or apical buds directly to produce new plant without any callus formation (Alkhateeb and Ali –Dinar, 2002). So, new plants were free from any variation and always they were true to type. While at embryogenesis pathway, the new plant will be produced indirectly from twigs, leaves and etc by callus formation and shows various diversity (Gurevich *et al.*, 2005). Using high concentration of auxin in order to stimulate callus induction is one of the main reasons of this phenomenon (Skirvin *et al.*, 1994; Al-Wasel, 2000 and Ramage *et al.*, 2004). Varieties show different reactions to tissue culture and each variety needs special conditions for tissue culture propagation. Basically tissue culture date palms begin to flower and fruit set from 6 to 8 years old.

Theoretically, tissue culture date palms have the same threats like mother plants. However, somaclonal variation is one of the major problems in tissue culture of date palm. The somaclonal variation includes differences in leaf structure, basically change of palm pattern, abnormal flower and also production of partenocarpic fruit (Aaouine, 2000; Alkhateeb and Ali-Dinar, 2002; Omer *et al.*, 1992).

In some cases somaclonal variation can be detected at early stages of date palm growth, but in most situations they are detected by flowering, fruit set and maturation. Sometimes, the frequency of somaclonal variation in tissue culture date palms is very high, though the reason is not clear and is under investigation (Gurevich *et al.*, 2005).

Al-wasel(2005) studding Somaclonal variation in different date palm cultivars (Medjool, Berhee, Sukari, Toury, Deglet Nuor, Khallas and Nabbat Saif) through morphological traits. He reported that the dwarfism, slow growth, structural abnormalities, curvature of the terminal bud, no fruit set (fruit Shesce) and multi carpel fruit as the most abnormalities. In addition the type and extent of variation were different among varieties.

By the way, so far, all of abnormalities in tissue culture date palm trees were recognized by visual inspection. So, we tried to survey stability and uniformity of tissue culture date palm plantlets by using morphological characteristics that are not affected by environmental conditions.

Material and methods

This research work was carried out on tissue culture date palm Berhee cultivar in tropical fruit research station in Booshehr province at the south of Iran. The station is at the distance of 75 km from the center of the province with 50 meters above sea level. Average annual rainfall is 260 mm. However, the annual evaporation is 3500-4000 mm. The climate of this location is arid and it has sandy soil.

Expremental Plants

Tissue culture materials have been planted in this station from 1999. Trees were propagated by embryogenesis method. Although plant materials were about 15 years old , but they were not able to produce commercial fruits. Tissue culture trees of this variety were planted at 6*6 m distances with the same management. In this experiment 14 tissue culture date palms were randomly selected as well as one offshoot palm of Berhee as control.

Statistical Analysis

The investigation was done as completely randomized design. Results were analyzed by using SPSS software. Test was done according to the Distinctness, Uniformity and Stability (DUS) guidelines of date palm (anonym).

Morphological Traits

Leaves are the main organelle of date palm trees. They determine the whole structure of a tree. Depending on variety, age of a palm and environmental conditions, leaves of a date palm are 3 to 6 m long and have a normal life of 3 to 7 years. At the tip of the leaf, there may be a single terminal leaflet or two leaflets forming. Leaf structure is variety and environment dependent. The leaf characters such as: leaf length, lamina length, lamina width, petiole with thorn length, petiole without thorn length and central leaflet size were analyzed in order to control the trueness to type of tissue culture tree. Observation of leaves were carried out in the middle of winter on four perfect leaves which distributed around date palm very well.(pic1)

Results

Variegation

The eight quantitative morphological variables were determined separately. Then ANOVA analyzes were done (Table 1). There were significant differences ($p<0.01$) between date palm tissue culture trees by morphological traits. In other words, there was a great deal of variability among the studied accessions.

Table 1. Variance analysis of morphological traits of Berhee cultivar.

S.O.V	DF	MS							
		Petiole length	Petiole without thorn section	Leaf length	Lamina length	Lamina wide	Length/wide lamina	Length of middle leaflet	Wide of middle leaflet
Tree	14	422.734**	21.626**	2629.154**	2012.758**	383.077**	0.929**	112.358**	0.915**
Error	60	1.483	0.177	3.090	1.475	0.545	0.002	0.935	0.073
C.V		1.6	5.9	0.5	0.5	1.1	2	2.2	7.5

** significant level at 1%.

By these eight morphological traits, Fourteen tissue culture plants and an offshoot, as a control from Berhee cultivar could be separated in different classes. Some traits such as petiole length, petiole without thorn section, leaf length ,lamina length, lamina wide, length to width lamina ratio could classified trees better than length and wide of middle leaflet (table 2).

The dissimilarities between tissue culture trees and offshoot plant (as control) were shown in table (3). Based on table 3, there were up to 100 % differences in tissue culture trees and also between them and offshoot tree (c).

Classification

According to dissimilarities matrix (table 3), there is some variation among tissue culture from the point of

morphological traits that separated them to different categories. Generally some of the trees are 100% distinct from others. In order to determine the similarities among experimental trees the cluster analysis was done based on morphological traits. In cluster analysis, tissue culture trees ranked as fallow (Diagram 1).

Table 2. Classification of tissue culture trees based on mean of morphological characteristics.

Tree	Treats							
	Petiole length	Petiole without thorn section	Leaf length	Lamina length	Lamina wide	Length/wide lamina	Length of middle leaflet	Wide of middle leaflet
T1	80.2 d	7.0 d	329.7 f	256.5 i	59.9 j	4.35 b	40.9 g	3.56 cd
T2	76.8 e	5.6 fg	333.6 e	260.1 h	72.0.e	3.61 f	39.5 gh	3.10 e
T3	81.1 d	7.0 d	307.3 i	228.5 l	62.0 i	3.69 e	50.3 b	4.20 a
T4	61.2 g	10.0 a	322.6 g	260.0 h	78.1 d	3.33 j	38.4 h	3.30.de
T5	74.8 f	4.0 h	351.5 c	277.1 c	67.3 g	4.12 c	45.0 ef	3.90 bc
T6	91.6 a	6.6 de	357.0 b	274.6 d	69.4 f	3.96 d	45.8 de	3.20 e
T7	65.0 i	6.0 ef	297.5 k	231.7 k	68.5 f	3.38 ij	38.8 h	3.30 e
T8	71.0 g	5.0 g	304.3 j	232.0 k	66.1 gh	3.51 g	43.5 f	3.20e
T9	87.1 c	7.3 d	358.4 b	271.0 e	56.0 k	4.84 a	44.1 ef	3.60 cd
T10	71.0 g	9.7 ab	319.2 h	247.5 j	66.0 gh	3.75 e	50.5 b	4.28 a
T11	75.4 ef	4.0 h	378.3 a	295.0 a	86.5 a	3.41 hi	44.4 ef	4.10 ab
T12	71.2 g	9.9 ab	351.6 c	277.8 c	80.1 c	3.47 gh	53.3 a	4.0 ab
T13	67.3 h	5.7 fg	333.8 e	268.4.g	65.5 h	4.09 c	47.0d	3.60 cd
T14	86.6 c	9.0 c	352.2 c	269.1 ef	66.0 gh	4.07 c	38.8 h	3.10 e
Control (offshoot)	89.3 b	9.2 bc	343.8 d	285.5 b	83.1 b	3.44 gh	48.6 c	4.02 ab

T1- T14 represents the tissue culture tress.

Different letter in each column indicate significant differences at 5% level according to LSD TEST.

Based on diagram 1, tissue culture trees divided to four groups. Tree with number 11 (T11) was completely distinct from others, the leave characters of this palm were different. Other trees were classified in three different classes. T7, T8, and T3 Palms were located in a separate class. Also T1, T2, T13, T4 and T10 were putted in the same class. The T5, T6, T9, T12, T14 were the nearest classes to control based on morphological characteristics.

Table 3. Dissimilarity based on morphological characteristics among accession.

Tree	Rescaled Squared Euclidean Distance														
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	C
T1	.00														
T2	.01	.00													
T3	.12	.17	.00												
T4	.06	.03	.18	.00											
T5	.08	.05	.40	.13	.00										
T6	.11	.08	.43	.22	.02	.00									
T7	.17	.20	.04	.13	.46	.55.	.00								
T8	.12	.15	.01	.12	.38	.45	.00	.00							
T9	.09	.09	.41	.23	.02	.01	.53	.43	.00						
T10	.03	.04	.05	.04	.17	.23	.07	.04	.22	.00					
T11	.42	.31	.92	.42	.12	.12	1.00	.90	.18	.56	.00				
T12	.14	.08	.43	.13	.02	.05	.49	.42	.08	.19	.10	.00			
T13	.03	.01	.22	.03	.03	.10	.24	.19	.09	.05	.28	.05	.00		
T14	.06	.04	.34	.15	.01	.00	.44	.35	.01	.17	.17	.06	.06	.00	
C	.15	.09	.46	.18	.05	.04	.54	.46	.10	.24	.13	.03	.10	.06	.00

T1- T14 represents the tissue culture tress.

C: control.

Discussion

Variation

One of the major weaknesses of mass tissue culture palm propagation is the appearance of undesirable off-type plants caused by somaclonal variation. Based on the results of analysis variance, the existence of variation among tissue culture trees were shown obviously. Not only there was variation between tissue culture trees and those reproduce by offshoot but also there was significant differences in tissue culture derived trees.

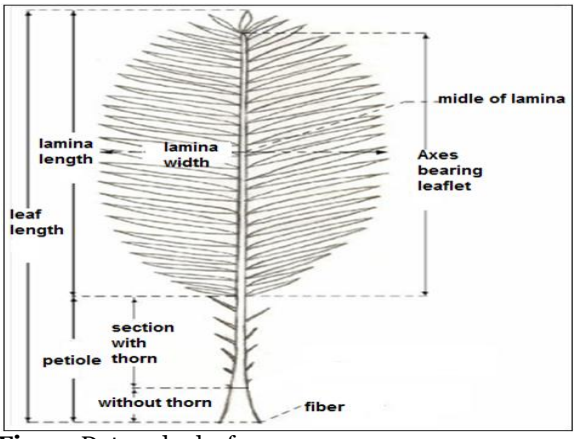

Fig. 1. Date palm leaf.

Morphological Key Factors

Some morphological characteristics such as leaf length, lamina length, petiole length, section of petiole without turns, lamina width, length/width lamina and Length of middle leaflet as asterisked traits were useful to classify tissue culture trees based on DUS date palm guideline. These were not affected by environmental conditions and would be able to distinguish differences among tissue culture derived trees.

Stability and Uniformity

Although in tissue culture as an asexual propagation method, we expected to reproduce true to type and uniform materials, but a wide range of dissimilarities among tissue culture trees was observed in a way that they divided to four completely distinctive groups. So, it can be said that, tissue culture trees didn't have sufficient stability which impose difficulties to growers.

Therefore, the theory of uniformity in date palm tissue culture plants c.v Berhee was rejected. This could caused difficulties for growers used them. Because, these kinds of variations among tissue culture plants might be resulted higher risk for investment.

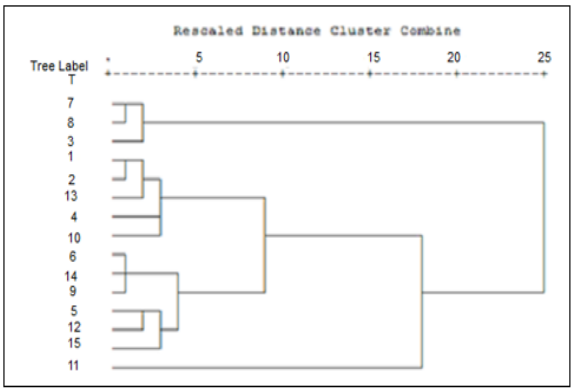

Fig. 2. Cluster analysis of Berhee trees.

In this survey, we found that some morphological traits that determine the size of tree were key factors to show somaclonal variation among tissue culture trees. Our finding are in line with Al- Wasel 2001, 2005; Ramage *et al.*, 2004; Skirvin *et al.*, 1994; Alkhateeb *et al* ., 2006 that reported somaclonal variation in tissue culture plants of date palm c.vs Medjool, Berhee, Sukari, Toury, Deglet Nuor, Khallas and Nabbat Saif date palm cultivars. Those works showed variability like dwarfism, variegation, apical bending, abnormal leaves and pseudo offshoot based on visual assessment, but we analyzed factors that determine the size of tree. So in this case, our finding was completely different with their results.

Conclusion

Our finding can be used to control the uniformity and stability of tissue culture trees propagated by somatic embryogenesis in all laboratories that propagate tissue culture date palm, in order to its lower cost compared to other quality control systems.

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