*J. King Saud Univ.*, Vol. 13, *Agric. Sci.* (1), pp. 65-73, Riyadh (A.H. 1421/2001)

# Phenotypic Comparison of Tissue Culture-derived and Conventionally Propagated Date Palm (*Phoenix dactylifera* L.) cv. Barhi Trees. I. Vegetative Characteristics

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(Received 17/12/1419; accepted for publication 12/8/1420 )

Abstract. The study was conducted to assess the uniformity of tissue culture-derived trees to the conventionally propagated trees of date palm cv Barhi. Tissue culture-derived plants were more vigorous, uniform and produced significantly much more primary, secondary and aerial off-shoots in comparison to the normal plants. The other vegetative characteristics, such as leaf length, leaflet zone, leaflet length, leaflet angle, thorn area, thorn length, thorn angle, were not reliable traits to assess the uniformity of tissue culture-derived plants to conventionally propagated plants since there were significant differences not only between the tree type (either tissue culture derived or conventionally propagated plants) but also among the trees of the same tree type. Therefore, further study is needed to confirm whether tissue culture technique produces identical propagules of the elite date palm cultivars or would result in somatic variation among *in vitro* produced plants.

## Introduction

Date palm (*Phoenix dactylifera* L.) is the most important fruit crop grown in Saudi Arabia. There are more than 18.2 million trees. Mature trees produce about 649.00 metric tones of fruits [1, pp. 52-53]. The nutritional value of date palm fruit is high. It is considered an excellent source of energy (80% sugar), minerals and vitamins [2, 3, pp. 161-175].

Vegetative propagation of date palm by offshoots is the common method, whereas sexual propagation (by seeds) is not widely practiced due to heterozygosity since most of seed-derived female progenies are not true to type. Nevertheless, asexual propagation is not efficient because only a limited number of offshoots per tree is produced, which remain attached to the parents for a long period (2-3 years) until they reach an appropriate size and adequate root system develops. Moreover, the separation of offshoots is laborious and a large number may not survive after transplantation if

they are poorly detached [4, pp. 535; 5, pp. 289-323]. *In vitro* propagation provides a practical means to clone desired palm trees and to obtain a large number of high quality and disease-free propagules. Many reports have shown the potential utilization of tissue culture to propagate palm trees [6-12; 13, pp. 471-492]. Micropropagation through somatic embryogenesis has been the most successful means to propagate date palm trees *in vitro*. Nevertheless, Somaclonal variation has been associated with tissue culture [14-16].

This phenomenon has been observed by many researchers working with different crops, which makes it possible to anticipate in all tissue culture experiments [17]. Date palm growers in Saudi Arabia are reluctant to purchase tissue culture-derived date palm propagules since they are not sure of their uniformity to the desired cultivars. Therefore, This study was conducted to draw some phenotypic information on the behavior of tissue cultured-date palm trees in comparison to offshoots-derived trees.

## Materials and methods

Two private farms (Al-Abdaleh and Al-Mansoriah farms located in Al-Qassim-Onaizah, Saudi Arabia) in which both tissue culture-derived and conventionally propagated (by offshoots) date palm cv Barhi trees were selected for this study. The tissue culture-derived palm trees were imported from DPD (Date Palm Developments Ltd., Baltonsborough Glastonbury Somerset. BA6 8QG, England) via Al-Soany company (P. O. Box 21012, Al-Safat, Kuwait).

The DPD used mainly the apical meristem and some surrounding tissues to initially induce callus formation and then the somatic embryoes which were allowed to elongate to develop a complete plantlet *in vitro*. The tissue culture-derived palm trees in both sites were transplanted in the field after they produced at least two unfully developed leaves (this was the stage when they were received from the AL-Soany company) (Fig. 1). The offshoot palm trees were detached from the parents at the age of 2-3 years and transplanted directly in the same field of tissue cultured trees but in different lines (All this work has been done by the farmers themselves). In both farms, offshoots and tissue cultured-derived palm trees were transplanted in the fields on 15/03/1992. Old enough tissue culture-derived palm trees were chosen to ensure their stability in the field environment and to eliminate any carry-over effects of any factor(s) that may be imposed by the medium components *in vitro*, especially growth regulators, which might cause epigenetic variation, unstable variation.

Tissue culture-derived trees and conventionally propagated trees of date palm in both regions were selected randomly. The number of tissue culture-derived trees was 42 and 40 in region one and two respectively, while for conventionally propagated trees was 16 and 12 in region one and two respectively. The tree height was determined with a flexible tape, attached to long stick, from the soil level up to the end point of the leaf in the shoot tip of the tree. Three fully expanded leaves were chosen randomly around

the trunk of each tree. The length of leaf was measured with rigid tape. The leaf was assessed for various vegetative characteristics such as leaflet area, leaflet length, leaflet angle, thorn area, thorn length, and thorn angle. Eight leaflets and eight thorns were randomly chosen along each leaf to assess the vegetative characteristics. The leaflet and thorn length was determined with the rigid tape while a protractor was used to determine their angles. The number of primary off-shoots, secondary off-shoots (shoots developed from the primary off-shoots), and aerial shoots were determined by counting.

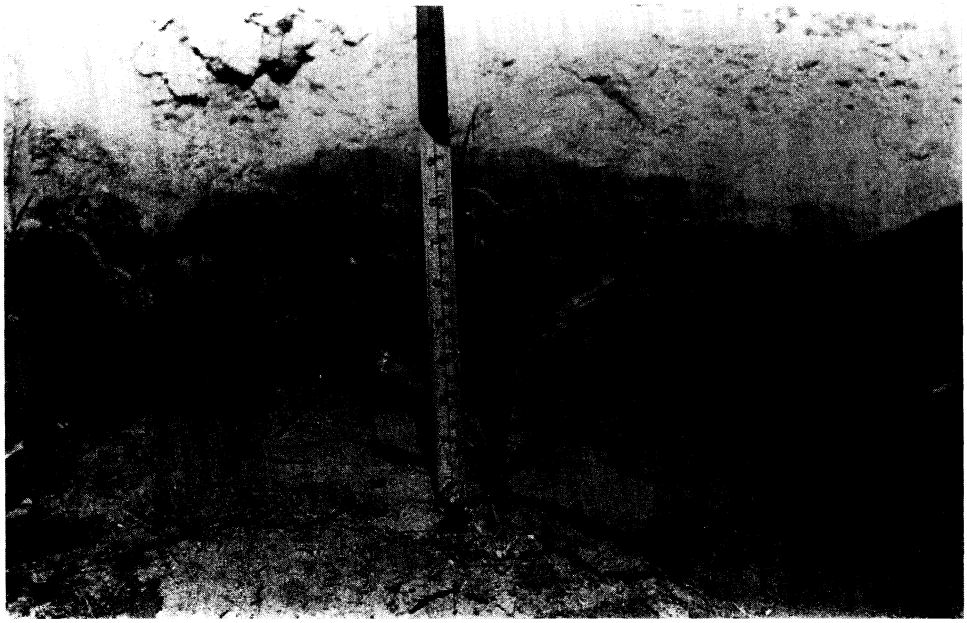

**Fig. 1. The stage of tissue cultured-derived plant of date palm cv Barhi when transplanted in the permanent site.**

All data were subjected to analysis of variance using general linear model procedure (GLM) [18] and means were separated by the least significant differences (LSD) for unequal replications.

## Results

Significant differences were apparent between tissue culture-derived trees and offshoots trees. The region did not have considerable effects on morphological traits. Tissue culture-derived trees produced more primary, secondary offshoots and aerial shoots than conventionally propagated trees (Table 1, Fig. 2). Although off-shoot trees

were taller than tissue culture-derived trees, the difference was slight. Furthermore, tissue culture plants were more uniform in height and trunk size (Fig 3).

**Table 1. Assessment of some vegetative traits among tissue culture-derived Barhi trees vs conventionally propagated trees (planted in 1992)**

| Region | Tree type[1] | No of trees | Tree height[2] (cm) | No of primary off-shoot s | No of Secondary off-shoots | No of arial shoots |
|---|---|---|---|---|---|---|
| 1 | TC | 42 | 497.98b | 7.86a | 7.69a | 1.86a |
| | OS | 16 | 545.00a | 2.81c | 0.75c | 0.19b |
| 2 | TC | 40 | 397.33c | 5.13b | 4.90b | 2.15a |
| | OS | 12 | 424.67c | 2.58c | 0.10c | 0.67b |
| Significance: | | | * | * | * | * |

[1] TC= tissue culture-derived trees; OS=off-shoot, common vegetaive propagation method of date palm.
[2] Height of tree from the soil level to tallest point of the tree.
* LSD multiple comparison for unequal replication at 0.05 significance level. Means within a column followed by different letters are significantly different.

**Fig 2. Off-shoots arose from tissue culture-derived plant of date palm cv Barhi.**

There were significant differences between tree type in the leaf length, leaflet length and angle, and thorn length and angle, however, there was no clear trend showing the effects of tree type on these morphological traits (Table 2). There was a significant difference in single trait among tissue culture-derived trees or conventionally propagated trees (Table 3).

**Fig. 3. Orchard of tissue culture-derived trees of date palm cv Barhi showing the uniformity of the trees.**

**Table 2. Some morphological differences among tissue culture-derived Barhi trees vs conventionally propagated trees (planted in 1992)**

| Region | No of trees | Leaf length (cm) | Leaflet zone length (cm) | Thorn zone length (cm) | Leaflet angle | Leaflet length (cm) | Thorn length | Thorn angle (cm) |
|---|---|---|---|---|---|---|---|---|
| 1 | 42 | 335.87b | 227.41b | 74.65c | 30.52c | 47.08b | 22.88c | 8.60bc |
| | 16 | 374.73a | 254.40a | 89.50a | 26.80d | 53.13a | 28.53b | 8.87b |
| 2 | 40 | 309.69c | 215.50c | 74.26c | 38.86b | 42.26d | 32.84a | 8.45c |
| | 12 | 332.17b | 227.36b | 79.94b | 41.90a | 45.98c | 33.56a | 10.14a |
| Significance | | * | * | * | * | * | * | * |

[1] TC= tissue culture-derived trees; OS= off-shoot, common vegetaive propagation method of date alm.
*LSD multiple comparison for unequal replication at 0.05 significance level. Means within column followed by different letters are significantly different.

## Discussion

The obvious differences between tree type were observed in numbers of primary, secondary and aerial shoots produced. Tissue culture-derived trees produced significantly more primary, secondary and aerial shoots. This could be attributed to that tissue culture plants were transplanted to their permanent place when they were about 20 cm long (Fig.1) while the off-shoots of the same cultivars were about more than two meter long. Therefore, the tissue culture plants had very active axillary buds near the

soil level, which had the chance to develope into off-shoots. In addition, this ability mighty be related to that tissue culture plants were more vigorous and juvenile than off-shoot-derived plants, whereas off-shoot derived trees remained attached to the mother plants for more than two years. During this time most of axillary buds became dormant and lost their chance to form off-shoots before they were transplanted to the permanent site. AL-Ghamdi [18, pp. 139-200] found that *in vitro* propagated plants were more juvenile.

There were obvious differences between tissue culture plants and off-shoot derived plants in tree height at transplantation, off-shoot-derived trees were more than 2.0 meter long. Tissue culture plants grew slowly at the first two years and after they developed numerous and strong root system they grew faster and became about as high as off-shoot derived trees; the differences were 47.02 and 27.34 cm in region 1 and 2, respectively. Al-Ghamdi [19] reported that faster height increment was more evident when *in vitro* propagated plants transferred to the field. Similarly, Booij *et al.*, [20] observed no significant difference between in vitro plants and off-shoots.

With respect to the other morphological characteristics, such as leaflet area, leaflet length, leaflet angle, thorn area, thorn length, and thorn angle, no clear or consistent differences were observed between tissue culture and off-shoot-derived trees. Significant differences were noticed within the trees of the same tree type (Table 3). Therefore, these characteristics would not be reliable to compare the trees of the same tree type or between different tree type. However, the vegetative characteristics have been used with other plant species to screen for somatic variants among tissue culture ants [21, 22]. Thus a further investigation is needed to assess the flowering and fruiting of tissue culture-derived plants and to make a solid information about the use of tissue culture technique to clonally propagate elite date palm cultivars or the percentage of variation that tissue culture may induce.

Overall, it could be concluded from this study that tissue culture-derived plants had better growth habit and resulted in more uniform date palm orchard. They also produced much more primary and secondary off-shoots.

***Acknowledgment***. Grateful acknowledgment goes to Dr. Sami M. Al-Shazely, Essam Sharaan, Abdulrahman Al-Hussianan, and Abdullah Al-Dekheel allah for all their efforts in the course of doing this study. The author is very grateful to Dr. Maher H. Khalel and Abd El-Sattar El-Daoushy for statistical assistance and Dr. M. O. A. Warrag and Dr. F. Al-Al-Saad for revising the manuscript. A special thanks goes to Mohammed A. El-Kobbia for his cooperation in making and developing the photographs.

**Table 3. Maximum and minimum means ± standard error of some morphological traits of tissue culture-derived trees and conventionally propagated trees (planted in 1992) in each region**

| Region | Tree type[1] | Leaf length (cm) | Leaflet zone length (cm) | Thorn area length (cm) | Leaflet angle | Leaflet length (cm) | Thorn length | Thorn angle(cm) |
|---|---|---|---|---|---|---|---|---|
| 1 | TC | 386.67 ±6.67[2] | 256.67 ± 33.91 | 104.00 ± 5.77 | 38.33 ± 1.23 | 52.72 ± 0.97 | 10.46 ± 0.61 | 47.08± 5.33 |
| | | 254.00 ± 3.46 | 194.67 ± 0.88 | 28.33 ± 4.41 | 21.25 ± 1.21 | 42.25 ± 0.92 | 6.63 ± 0.38 | 12.50± 1.70 |
| | OS | 420.00 ± 0.0 | 285.00 ± 2.89 | 103.33 ± 1.67 | 45.42 ± 2.62 | 57.17 ± 1.28 | 10.83 ± 0.50 | 54.58± 3.01 |
| | | 298.33 ± 6.01 | 190.00 ± 5.77 | 76.67 ± 6.01 | 19.58 ± 1.12 | 48.25 ± 0.88 | 5.54 ± 0.36 | 18.75± 1.93 |
| 2 | TC | 348.33 ± 3.28 | 241.67 ± 4.41 | 94.33 ± 8.09 | 59.17 ± 1.64 | 46.25 ± 0.57 | 10.25 ± 0.43 | 45.83± 2.27 |
| | | 270.00 ± 5.77 | 186.67 ± 38.44 | 56.67 ± 1.67 | 25.83 ± 1.55 | 36.88 ± 0.58 | 5.96 ± 0.41 | 17.08± 1.20 |
| | OS | 393.33 ± 6.67 | 270.00 ± 5.77 | 103.33 ± 4.41 | 61.04 ± 2.33 | 51.04 ± 0.94 | 14.25 ± 2.29 | 46.25± 2.91 |
| | | 278.33 ±15.90 | 183.33 ± 8.82 | 63.33 ± 1.67 | 33.75 ± 1.05 | 39.17 ± 0.67 | 8.79 ± 0.35 | 25.00± 1.93 |

**[1] TC= tissue culture-derived trees; OS= off-shoot, common vegetaive propagation method of date palm.**

**[2] Maximum and minimum means± standard error within the tree type.**

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# مقارنة للصفات المورفولوجية للنخيل صنف البرحي المكاثر نسيجياً وبالطريقة التقليدية (عن طريق الفسائل). ١. الصفات الخضرية

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(قدم للنشر في ١٤١٩/١٢/١٧هـ؛وقبل للنشر في ١٢/ ٨/ ١٤٢٠ هـ)

**ملخص البحث.** أجريت هذه الدراسة لتقييم مدى التشابه بين النخيل صنف البرحي المكاثر نسيجياً والنخيل المكاثر بالطريقة التقليدية. بينت الدراسة أن النمو الخضري للنخيل النسيجي أكثر قوة وتجانساً في الحقل بالأضافة إلي كثرة إنتاجها للفسائل الأولية والثانوية والرواكيب عند مقارنتها بالنخيل العادي. أما من ناحية الصفات الخضرية الأخرى مثل طول الورقة، طول منطقة الوريقات، طول وزاوية خروج الوريقات، طول منطقة الأشواك، طول وزاوية خروج الأشواك، فانه لا يمكن الاعتماد عليها لمقارنة النخيل النسيجي مع النخيل المكاثر تقليدياً حيث أوضحت الدراسة أن هناك تباينات ليس فقط بين طريقتي التكاثر (طريقة زراعة الأنسجة وطريقة الفسائل) بل كانت هناك اختلافات معنوية بين أشجار الطريقة الواحدة. لذا فأنه ينصح بإجراء المزيد من الأبحاث للحصول علي معلومات والتأكد من سلامة استخدام طريقة زراعة الأنسجة في إكثار الأصناف الجيدة من نخيل البلح (التمر) أو أن هذه الطريقة الحديثة يمكن أن تؤدي إلي إنتاج طفرات جسمية بين النباتات النسيجية.