

Studies on physiological parameters in tissue culture and sucker plants of date palm (*Phoenix dactylifera* L.) cultivars

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(Received: 18.11.2014, Accepted: 28.01.2015)

Abstract

Production of planting material in date palm (*Phoenix dactylifera* L.) is the major constraint in commercial cultivation. This is on account of fact that rate of sucker production is limited and differ from variety to variety. Therefore, for production of planting material, recourse to tissue culture has been taken. Presently, at global level, few organizations are producing tissue culture plants but their performance is yet to be ascertained. Accordingly, the present study was planned to compare the photosynthetic parameters in 4 years old tissue culture/ sucker plants of date palm cv. Khalas and Khuneizi at vegetative growth stage. It was observed that in cv. Khuneizi the plants obtained from tissue culture/ sucker do not differ much in their photosynthetic parameters. However, in cv. Khalas the rate of photosynthesis at vegetative growth stage was higher as compared to plants obtained from sucker. Similar trend was observed for other parameters also such as transpiration rate, carboxylation efficiency and water use efficiency. Data on morphometry of leaflet also revealed that leaflets of cv. Khuneizi (Tissue culture and sucker) were at par but in cv. Khalas the leaflets of tissue culture plants were significantly larger than that of sucker plants. Therefore, our results demonstrate that plants obtained from tissue culture and sucker do not differ much in terms of photosynthetic parameters under arid conditions

Keywords: *Date palm, Photosynthesis, sucker, tissue culture, arid conditions*

Introduction

Date palm (*Phoenix dactylifera* L.) is an economically important fruit tree of arid region. Date cultivation has historical records dating back to 6000 BC along Tigris and Euphrates River in Iraq and to 2000 BC at Mohenjodaro along river Indus. It is one of the oldest plants cultivated by the man and its origin is either Mesopotamia or Gulf region. All parts of date plant is utilized in different ways since its cultivation (Chao and Krueger, 2007). In Indus valley, date palm is believed to have been introduced by the Soldiers of Alexander the Great in the fifth century B.C. and during the Muslim invasions at the beginning of the eight-century A.D. At present, some wild old grooves of seedling date palm are present on the coastal belt in Kachchh region of Gujarat which harbours a wide genetic variability in fruit characters (Murlidharan, 2008).

Despite the fact that western Rajasthan and part of Haryana, Punjab and Gujarat are suitable for commercial cultivation, now the farmers are coming forward to take this as commercial crop. This is on account of the fact that expansion of date palm is restricted due to lack of quality planting material. In order to develop planting material, recourse to tissue cultured has been adopted. At present some private and research very few organizations are producing tissue-cultured plants and are supplying to farmers and developmental agencies. However, their actual performance under field condition is yet to be ascertained. Accordingly, the present study was conducted to compare the morphometric and physiological parameters in 4 years old tissue culture and sucker plants of date palm cultivars, with a view to assess the variation between them. The results obtained constitute the text of the present communication.

Materials and Methods

Tissue culture and sucker raised plants of cvs.

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Khuneizi and Khalas constitute the material of the present study. The suckers of both the cultivars were collected from Regional Fruit Research Station, P.A.U., Abohar, Punjab and tissue culture plants of both the cultivars were obtained from M/s A.V. Thomas & Company, Cochin (Kerala). All the plants were planted in date palm block of ICAR-CIAH, Bikaner farm.

The studies were conducted during 2001-2002 on four-year-old plants maintained under normal cultural operations. For morphometric comparison, the data on plant height, length of leaf, length and width of leaflet (pinnae) were recorded. The observations on leaf/leaflet were taken on fully expanded leaf.

The data on photosynthetic parameters were recorded on fully expanded inner side middle pinnae using Infra Red Gas Analyser (Model LI-6200, LICOR, USA). The observations were recorded on 3 plants for each treatment and data was pooled to arrive at mean values. The carboxylation efficiency was calculated as ratio of photosynthetic rate and internal CO₂ concentration and water use efficiency as ratio of photosynthetic rate to transpiration as described by Das *et al.* (1999). The data thus obtained was subjected to statistical analyses using MSTAT Software.

Results and Discussion

i) Plant morphometry

a) Cv. Khalas

Plant morphometric parameters of sucker and tissue-cultured plants of cv. Khalas are presented in Table 1. Perusal of data reveals that plant height in sucker-raised plant was 266.66 ± 57.73 cm whereas the plants raised through tissue culture were shorter with magnitude of 120.0 ± 50.0 cm. The comparison of means reveals that the plants raised from tissue culture were significantly shorter than that of plants raised through sucker/offshoots.

Perusal of data on length of leaf reveals that the fully expanded leaf of sucker raised plant was 140.2 ± 59.56 cm long whereas that from tissue culture plants was 110.2 ± 30.09 cm. Comparison of means reveals that the two are at par. Data on leaflet length and width also follow identical trend. The length of leaflet of sucker-raised plant was 23.6 ± 2.5 cm and 2.56 ± 0.18 cm wide. The values show that there is no major difference between the two groups.

B) Cv. Khuneizi

The data collected on plant morphometric parameters of cv. Khuneizi is presented in Table 2.

Perusal of data reveals that plants raised through tissue culture are shorter (126.0 ± 13.87) as compared to those obtained through sucker (180.0 ± 12.24 cm). The comparison of means reveals that the difference in plant height is statistically significant.

The data on length of leaf reveals that the leaves of sucker-raised plants are longer (170.6 ± 16.3 cm) as compared to those obtained from tissue culture raised plants (107.8 ± 9.03 cm). The comparison further shows that the difference is statistically significant. Identical trend is also demonstrated by data on length of leaflet. Perusal of data reveals that leaflet is longer in sucker-raised plants (31.2 ± 1.09 cm) as compared to plants raised through tissue culture (27.2 ± 0.83 cm).

ii) Photosynthetic parameters

a) Cv. Khalas

The data on photosynthetic parameters of cv. Khalas are presented in Table 3. During the present study, the data on net photosynthesis rate, stomatal resistance, transpiration rate, carboxylation efficiency and water use efficiency was recorded. It was observed that net photosynthesis rate was marginally higher in plants raised through tissue culture ($15.34 \pm 1.05 \mu\text{mol m}^{-2}\text{s}^{-1}$) as compared to plants raised through suckers ($13.41 \pm 0.16 \mu\text{mol m}^{-2}\text{s}^{-1}$). Comparison of mean reveals that the two values are at par and do not show any difference.

Stomatal resistance does not show any difference in magnitude in two groups under study. Its value was $0.077 \pm 0.011 \text{ cm s}^{-1}$ in sucker-raised plants and $0.102 \pm 0.003 \text{ cm s}^{-1}$ in tissue culture raised plants.

Similar trend was also observed in transpiration rate which was $6.84 \pm 0.2 \text{ mmol m}^{-2}\text{s}^{-1}$ in sucker raised plants and $6.5 \pm 0.2 \text{ mmol m}^{-2}\text{s}^{-1}$ in tissue culture raised plants. The values were non-significant.

Both the groups demonstrated similar carboxylation efficiency too. It was $0.048 \pm 0.002 \mu\text{mol CO}_2 \text{ fixed m}^{-2}\text{s}^{-1} \text{ ppm}^{-1}$ of internal CO₂ in sucker plants and $0.058 \pm 0.0003 \mu\text{mol CO}_2 \text{ fixed m}^{-2}\text{s}^{-1} \text{ ppm}^{-1}$ of internal CO₂ in tissue culture raised plants, which was statistically significant. The difference between sucker and tissue culture plants with respect to water use efficiency was non-significant.

b) Cv. Khuneizi

The data pertaining to photosynthetic parameters are presented in Table 4. The perusal of data

reveals that net photosynthesis rate was higher in tissue culture plants ($12.06 \pm 2.46 \mu\text{mol m}^{-2} \text{s}^{-1}$) as compared to sucker raised plants. However, comparison of means show that the difference was non-significant.

Data on stomatal resistance revealed that the magnitude was relatively high in sucker-raised plants as compared to tissue culture raised plants. However, this

difference was also statistically non significant on mean comparison.

Both types of plants were at par in their transpiration rates. The transpiration rate of sucker-raised plant was $5.46 \text{ mmol m}^{-2} \text{s}^{-1}$ whereas in tissue culture raised plant it was $5.37 \text{ mmol m}^{-2} \text{s}^{-1}$. Data on water use efficiency also revealed a non-significant

Table 1. Plant morphometric parameters of sucker and tissue culture raised plants of Khalas

	Plant parameters in cm			
	Plant height	Length of leaf	Length of leaflet	Width of leaflet
Khalas (Sucker)	266.66 ± 57.73	140.2 ± 59.66	25.2 ± 3.83	3.16 ± 0.35
Khalas (Tissue culture)	120.00 ± 50.00	110.0 ± 30.09	23.6 ± 2.5	2.56 ± 0.18
CD at 5%	0.048	NS	NS	NS

Table 2. Plant morphometric parameters of sucker and tissue culture raised plants of Khuneizi

	Plant parameters in cm			
	Plant height	Length of leaf	Length of Leaflet	Width of leaflet
Khuneizi (Sucker)	180.0 ± 12.24	170.6 ± 16.3	31.2 ± 1.09	2.93 ± 0.115
Khuneizi (Tissue Culture)	126.00 ± 13.87	107.8 ± 9.03	27.2 ± 0.83	2.63 ± 0.115
CD at 5%	0.023	NS	NS	NS

Table 3. Photosynthetic parameters of sucker and tissue culture raised plants of Khalas

	Photosynthetic parameters				
	$P_N (\mu\text{mol/m}^2 \text{s}^{-1})$	Stomatal Resistance (cm s^{-1})	Transpiration ($\text{mmol m}^{-2} \text{s}^{-1}$)	Carboxylation efficiency	Water use efficiency ($\mu\text{mol/mmole}$)
Khalas (Suckers)	13.41 ± 0.16	0.077 ± 0.011	6.84 ± 0.2	0.048 ± 0.002	1.946 ± 0.04
Khalas (Tissue culture)	15.34 ± 1.05	0.1022 ± 0.003	6.5 ± 0.2	0.058 ± 0.0003	2.30 ± 0.20
CD at 5%	NS	NS	NS	0.014	NS

Table 4. Photosynthetic parameters of sucker and tissue culture raised plants of Khuneizi

	Photosynthetic parameters				
	$P_N (\mu\text{mol/m}^2 \text{s}^{-1})$	Stomatal Resistance (cm s^{-1})	Transpiration ($\text{mmol m}^{-2} \text{s}^{-1}$)	Carboxylation efficiency	Water use efficiency ($\mu\text{mol/mmole}$)
Khuneizi (Suckers)	10.69 ± 0.020	0.221 ± 0.02	5.46 ± 0.03	0.037	1.95 ± 0.15
Khuneizi (Tissue culture)	12.06 ± 2.46	0.134 ± 0.002	5.37 ± 0.01	0.027	2.24 ± 0.105
CD at 5%	NS	0.02	NS	NS	NS

difference between the mean value obtained for sucker raised (1.95) and tissue culture raised (2.24) plants. Similarly, observation was also recorded in their carboxylation efficiency 0.037 for sucker and 0.027 for tissue cultured plants and the differences was non significant.

Use of tissue culture plants has become a common practice to develop commercial orchards. This is on account of fact that such plants provide uniformity in genetic material. In date palm too, numerous attempts have been made to raise tissue culture plants (Reuveni, 1979; Tissert, 1979; Meter, 1983; Shakib *et al.*, 1994). At present, a large number organizations are engaged in production of tissue cultured plants (Zaid and de Wet, 1999). The evaluation of tissue cultured and sucker raised plants of Barhee was conducted in Kachchh, Gujarat where it has been demonstrated that initially for 1-3 years tissue culture plants have better growth than sucker plants but later they have same magnitude (Murlidharan *et al.*, 2010).

Despite, this very little has been done to compare the plants raised from different techniques for their morphological and physiological parameters. It has been demonstrated that in vitro raised plants show morphological, structural, physiological and biochemical differences from these produced conventionally. These include reduced epicuticular wax, altered leaf anatomy, excessive water loss and stomatal abnormalities (Zaid and Hughes, 1995a,b,c). In another study conducted using tissue culture and offshoot raised plants of Barhee, it was shown that there were no difference in 81 out of 97 morphological and biochemical parameters investigated (Hajian, 2007).

However, in this study, the plants were of 4 years of age and did not show any morphological variations. The only difference observed was in the height of plant that was more in plants raised through sucker as compared to tissue cultured plants. Thus, the results reveal that in morphometric parameters, both the types of plants were at par.

Comparison of photosynthetic parameters has not been compared in date palm. However, in banana attempts were made and it has been recorded that plants raised from suckers were at par with those raised from tissue culture (Shivashankara *et al.*, 2001). The results are also in line with those obtained in banana. In both the cultivars under study all the parameters such as net photosynthesis rate, stomatal resistance, carboxylation

efficiency and water use efficiency do not vary with type of planting material used. Although, Zaid and Hughes (1995c) reported that in vitro plants have more water loss as compared to these produce conventionally. But in this study the transpiration rate remained at par in both the cultivars when compared in field condition.

Thus, from the above findings it can be inferred that irrespective of source of planting material, the field established plants do not differ much in terms of morphological and physiological parameters.

References

- Chao, C. C. T. and Krueger, R. R. 2007. The date palm (*Phoenix dactylifera* L.): overview of biology, uses, and cultivation. *Hort. Science*, 42 (5): 1077-1082
- Das, C., Sengupta, T., Salim, P.K., Mishra, A.K., Sen, S.K. and Saratchandra, B. 1999. Quantitative analysis of photosynthetic parameters in mulberry leaf. *Indian Journal of Plant Physiol.*, 4(3): 171-174.
- Hajian, S. 2007. Quantity and quality comparison of offshoot and tissue cultured Barhee date palm trees. Proc. III International Date Palm Conference, *Acta Hort* 736, ISHS, 2007, pp 293-300.
- Mater A. A. 1983. Plant regeneration from callus cultures of *Phoenix dactylifera* L. *Date Palm Journal*, 2: 1, 157-77.
- Muralidharan, C.M., Tikka, S.B.S. and Verma, P. 2008. Date palm cultivation in Kachchh. Technical bulletin No. 02/ 2008. Date Palm Research Station, S.D. Agricultural University, Mundra- Kachchh, pp 36.
- Muralidharan, C.M., Verma, P., Badiyavedra, D. A. 2010. Field performance of tissue culture date palm cv. Barhee in arid ecosystem of Gujarat. In. Souvenir, National Workshop on Date palm, CIAH, Bikaner, 4th Oct, 2010, pp 144-150.
- Reuveni, O. 1979. Embryogenesis and plantlet growth of date palm (*P. dactylifera* L.) derived from callus tissues. *Plant Physiol.*, 63: 138.
- Shakib, A.M., Khoshkam, S., Majidi, E. 1994. Plant regeneration of datepalm variety Estamaran by tissue culture. *Seed and Plant*, 9: 3-4, 8-11
- Shivashankara, K.S., Kumar, V. and Reddy, B.M.C.

2001. Physiology of growth differences between tissue culture plants and sucker in dwarf Cavendish and Robusta banana. *Indian J. Hort.*, 58(3): 244-245
- Tisserat, B. 1979. Propagation of datepalm (*Phoenix dactylifera* L.) in vitro. *J. Expt. Bot.*, 30: 1275-83.
- Zaid, A and de Wet, P.F. 1999. Date palm propagation. In Date palm cultivation. (Ed. Zaid, A.) FAO Plant production and protection paper 156.
- Zaid, A. and Hughes 1995a. A comparison of stomatal functions and frequency of in vitro, in vitro polyethylene glycol treated and greenhouse grown plants of date palm (*Phoenix dactylifera* L.). *Tropical Agriculture* (Trinidad), 72: N2: 130-134.
- Zaid, A. and Hughes 1995b. In vitro acclimatization of date palm (*Phoenix dactylifera* L.) plantlets: a quantitative comparison of epicuticular leaf wax as a function of polyethylene glycol treatment. *Plant Cell Reports*, 15: 111-114
- Zaid, A. and Hughes 1995c. Water loss and polyethylene glycol-mediated acclimatization of in vitro grown seedlings of 5 cultivars of date palm (*Phoenix dactylifera* L.) Plantlets. *Plant and Cell Reports*, 14: 385-388.