Plant Cell Reports (1995) 14:385–388

Plant Cell Reports
© Springer-Verlag 1995

Water loss and polyethylene glycol-mediated acclimatization of *in vitro*-grown seedlings of 5 cultivars of date palm (*Phoenix dactylifera* L.) plantlets

Abdelouahhab Zaid [1] **and Harrison Hughes** [2]

[1] Department of Biology, Faculty of Sciences – Semlalia, P.O. Box S15, Marrackech, Morocco
[2] Department of Horticulture, Colorado State University, Fort Collins, CO 80523, USA

Received 9 September 1993/Revised version received 12 September 1994 – Communicated by W. Parrott

Abstract. Plantlets derived from shoot-tips of seedlings from five cultivars of date palm, *Phoenix dactylifera* L., were subjected to polyethylene glycol in liquid medium. Comparisons of water loss of detached leaves among *in vitro*-grown, polyethylene glycol-treated and greenhouse-grown plants showed significant differences with treatment for all cultivars studied. For each treatment, significant differences were also found among cultivars. The common result was that the percent of moisture loss of non-treated *in vitro*-grown plantlets was almost twice that of greenhouse-grown plants. Polyethylene glycol-treated plantlets showed a water loss of approximately 27%, similar to that of greenhouse plants as compared to an average of 40% in control plants. This demonstrates the possibility of using polyethylene glycol as an osmoticum for *in vitro* acclimatization of plantlets prior to transfer to soil.

Abbreviations : EW, epicuticular wax; MS, Murashige and Skoog (1962) medium; NAA, α naphthalene acetic acid; PEG, polyethylene glycol; PPFD, photosynthetic photon flux density.

Introduction

One of the major obstacles to the practical application of plant tissue culture to mass propagation has been the difficulty of successful transfer of plantlets from *in vitro* conditions to a soil medium (Boxus and Quoirin, 1977; Preece and Sutter, 1991). Even when gradual acclimatization has been used, poor survival and slow growth of plantlets have been commonly reported (Lee *et al.*, 1985; Pierik, 1987; Crane and Hughes, 1990). This is unfortunate because the ultimate success of plant tissue culture as a commercial means of plant propagation depends on the ability to transfer plantlets out of culture on a large scale, at low cost, and with a high survival rate.

Acclimatization presents challenges at least equal to those posed by the initiation of cultures because it marks the end of artificial control and the beginning of autonomous plant growth. Approximately 20 years ago, Murashige (1974) stated that research concerning the preparation of *in vitro* plantlets for transfer to soil had been neglected. Since that time many scientists have become interested in the effects that the transfer process has on tissue cultured plantlets (Grout and Crisp, 1977; Sutter and Langhans, 1982; Ziv, 1986; Zaid, 1990; Debergh, 1991; Preece and Sutter, 1991). PEG has only recently been used *in vitro* to reduce desiccation and death *ex vitro*. (Short *et al.*, 1987; Dami and Hughes, 1991; Safadi, 1992). Hence, the need for expensive and time-consuming acclimatization procedures in the greenhouse could be eliminated or at least reduced. The objective of the present study was to determine the effect of PEG on moisture loss of *in vitro*-grown date palm plantlets.

Materials and Methods

Seedling shoot tips from five date palm cultivars (Majhool, Deglet Nour, Khadraoui, Zahidi and Sayer) were cultured *in vitro* as described by Zaid and Tisserat (1983). Six-month old tissue cultured plantlets with one pair of expanded leaves and three roots which where approximately five cm in length were used for this study as controls. Shoot-tip-derived plantlets were cultured on 50 ml liquid MS medium with 0.1 mg/l of NAA and 20% (w/v) of PEG 8000 (Sigma Co.) with a molecular weight of 7000-9000. The water potential provided by the PEG augmented media was -1.1614 MPa. Culture conditions were 26 ± 3°C with 16 hours light supplied by cool fluorescent lights at 80μmol m^{-2} s^{-1} PPFD. The plantlets were supported by filter paper bridges and grown for one week before transfer to soil. Each treatment consisted of 10

Correspondence to: H. Hughes

replicates of one plant per jar (200 x 50 mm). Greenhouse-grown seedlings were six-months-old and were grown under ambient temperature (25° ± 5°C) and natural light.

Leaves from the *in vitro*-control, *in vitro* acclimatized (PEG treated) and greenhouse-grown plants were examined for rate of moisture loss when detached and allowed to air-dry (approximately 10% relative humidity) with time. All expanded leaves were six-months-old. Twenty leaves for each treatment were weighed immediately after excision from plants and then every 30 minutes thereafter for 4 hours. Dry weights were obtained after oven drying (70° ± 2°C) for 24 hours. The percentage moisture loss was calculated using the following formula:

$$\frac{(FW_{to} - DW_t) - (FW_t - DW_t)}{(FW_{to} - DW_t)} \times 100$$

Where FW_{to} = fresh weight at time zero, FW_t = fresh weight after time, DW_t = dry weight.

The moisture loss data were analyzed by SAS (1985) using analysis of variance for repeated measurements and student-Neuman-Keuls test for mean separation at the 5% level of significance.

Results and Discussion

The Percent Moisture Loss Study

Six-month old greenhouse-grown seedlings from the five date palm cultivars lost an average of 20 to 30% water after the first hour and 50 to 60% after 4 hrs (Table 1). 'Majhool' seedlings had the lowest percent moisture loss (22 and 52 after 1 and 4 hrs, respectively) while 'Zahidi' seedlings had the highest percent moisture loss (30 and 62 after 1 and 4 hrs, respectively). A significant difference was found between these two cultivars (Majhool and Zahidi) at both 1 and 4 hours. This reflects the typical cultivar habit in that 'Zahidi' is grown under irrigation while 'Majhool' is grown in the drier areas of cultivation. There were minimal differences in water loss among the other three cultivars. In plantations, these three have similar water requirements and are often grown in the same area.

The percent moisture loss of *in vitro*-grown leaves was considerably greater than that of greenhouse-grown plants of similar age (Tables 1 and 2). This is true at each of the 30, 60, 120, 180 and 240-minute intervals of air-drying. These tables also show that after 60-minutes, an average difference of 100% greater water loss was found for all cultivars. It clearly demonstrates the inability of *in vitro*-grown nonacclimatized plants to resist desiccation. Similar results were reported for tobacco (Safadi, 1992), 'Silvan' blackberry (Donnelly *et al.*, 1987), *Solanum laciniatum* (Conner and Thomas, 1981), 'Valiant' grape (Dami, 1991), *Brassica oleracea* var. *botrytis*

Table 1. Percent moisture loss from detached leaves of six-month-old greenhouse-grown seedlings of five date palm cultivars

Cultivar	% Moisture loss with time in minutes[a]					
	30	60	90	120	180	240
Majhool	10.7B	21.9B	30.7B	36.1B	42.9B	51.9B
Deglet Nour	14.2B	22.5B	29.4B	36.7B	45.9B	55.2AB
Sayer	18.2A	26.7A	34.3AB	41.2AB	50.2AB	57.3AB
Khadraoui	17.0A	25.7AB	32.2AB	39.4AB	49.6AB	56.2AB
Zahidi	19.1A	29.7A	37.2A	44.1A	53.7A	62.1A

[a] Each treatment combination is an average of 20 leaves with percent moisture loss calculated as follows:

$$\% \text{ moisture loss} = \frac{(FW_{to} - DW_t) - (FW_t - DW_t)}{(FW_{to} - DW_t)} \times 100$$

For each time, means not followed by a common letter differ significantly from one another at 0.05 level of significance

Table 2. Percent moisture loss from detached leaves of six-month-old *in vitro*-grown seedlings of five date palm cultivars

Cultivar	% Moisture loss with time in minutes[a]					
	30	60	90	120	180	240
Majhool	22.9B	40.2A	46.8B	52.9C	66.1AB	72.3BC
Deglet Nour	31.5A	58.3B	66.8A	72.6A	81.4C	85.5A
Sayer	26.7A	44.7A	52.2A	57.5AB	64.2B	69.9C
Khadraoui	20.7B	42.1A	50.1AB	59.7A	68.7A	77.1A
Zahidi	23.4AB	41.3A	48.6AB	55.1BC	68.8A	76.5AB

[a] Each treatment combination is an average of 20 leaves with percent moisture loss calculated as follows:

$$\% \text{ moisture loss} = \frac{(FW_{to} - DW_t) - (FW_t - DW_t)}{(FW_{to} - DW_t)} \times 100$$

For each time, means not followed by a common letter differ significantly from one another at 0.05 level of significance

(Grout and Crisp, 1977; Wardle *et al.*, 1983), *Malus domestica* (Brainerd and Fuchigami, 1981; Sutter, 1988) and *Pseudotsuga menziesii* (Mohammed and Vidaver, 1990).

In vitro-grown (control) plantlets of 'Deglet Nour' were the most sensitive to desiccation with the highest percent moisture loss of 58.3 and 85.5 after 1 and 4 hrs, respectively (Table 2). Seedlings from the other cultivars had an average water loss of approximately 40 and 70% after 1 and 4 hrs, respectively.

Effect of Polyethylene Glycol (PEG)

In vitro-grown PEG-treated (acclimatized) plantlets showed a lower water loss than *in vitro*-grown, non PEG-treated plantlets (Tables 2 and 3). This was observed in all cultivars. 'Deglet Nour' control plantlets lost 58% and 85% water after 1 and 4 hrs, respectively, while the PEG-acclimatized plantlets lost 27% and 49% water after 1 and 4 hrs, respectively (Tables 2 and 3).

Table 3. Percent moisture loss from detached leaves of six-month-old *in vitro* PEG-treated seedlings of five date palm cultivars

	%Moisture loss with time in minutes[a]					
Cultivar	30	60	90	120	180	240
Majhool	14.2C	27.3A	32.8A	36.9A	43.5A	48.2A
Deglet Nour	16.6BC	27.1A	32.1A	36.4A	44.3A	49.1A
Sayer	23.0A	29.0A	35.8A	43.0A	49.2A	56.0A
Khadraoui	21.3AB	32.8A	38.7A	44.3A	49.6A	53.9A
Zahidi	16.1BC	26.3A	30.7A	35.6A	41.7A	45.1A

[a] Each treatment combination is an average of 20 leaves with percent moisture loss calculated as follows:

$$\% \text{ moisture loss} = \frac{(FW_{to} - DW_t) - (FW_t - DW_t)}{(FW_{to} - DW_t)} \times 100$$

For each time, means not followed by a common letter differ significantly from one another at 0.05 level of significance

Greenhouse-grown plants (Table 1) and PEG-acclimatized plantlets (Table 3) showed little difference in water loss over time for all cultivars. Table 4 shows that there is a significant difference in percent water loss between greenhouse-grown plants and *in vitro*-grown plantlets of cv. Sayer. However, no significant difference was found between greenhouse-grown and PEG-treated *in vitro* plantlets of this cultivar. Other cultivars behaved similarly (data not shown), hence it is apparent that PEG has a potential use in the acclimatization of *in vitro*-grown plantlets. *In vitro*-grown leaves were obviously shriveled as compared to those of PEG-acclimatized and greenhouse-grown plants. Furthermore, observations of relative performance (data not included) showed a greater survival of transferred PEG-acclimatized plantlets.

Table 4. Percent moisture loss from detached leaves of seedlings of date palm 'Sayer' grown under different conditions

	% Moisture loss with time in minutes[a]					
Cultivar	30	60	90	120	180	240
GH 6 MO	18.23A	26.70A	34.25A	41.20A	50.23A	57.27A
IV 6 MO	26.75B	44.75B	52.23B	57.52B	64.20B	69.88B
IV 6 MO + PEG	22.99B	29.99A	35.78A	42.96A	49.21A	56.00A

[a] Each treatment combination is an average of 20 leaves with percent moisture loss calculated as follows:

$$\% \text{ moisture loss} = \frac{(FW_{to} - DW_t) - (FW_t - DW_t)}{(FW_{to} - DW_t)} \times 100$$

For each time, means not followed by a common letter differ significantly from one another at 0.05 level of significance

Acclimatized plantlets thus should be able to minimize desiccation caused by transplantation to greenhouse or field conditions. These results confirm previous studies where PEG has been used as an osmoticum to decrease the water potential of culture solutions (Heyser and Nabors, 1981; Kawasaki *et al.*, 1983; Bhaskaran *et al.*, 1985).

Based on this research and similar results with cauliflower and chrysanthemum (Short *et al.*, 1987), tobacco (Safadi, 1992) and grape (Dami, 1991), it is possible to use PEG as a means to acclimatize *in vitro*-grown plantlets and to reduce their subsequent desiccation and improve survival after transfer. If true, the need for expensive and time consuming acclimatization procedures in the greenhouse could be eliminated or at least reduced.

However, further research is necessary to more precisely quantify the percentages of water lost through the stomates and through the cuticle. The absence or low level of EW from *in vitro*-grown date palm likely contributed to the rapid loss of water during transplantation (Zaid and Hughes, 1989).

In conclusion, the *in vitro*-grown PEG-treated plantlets were not significantly different in percent moisture loss from greenhouse-grown plants, and significantly less than untreated, *in vitro*-grown date palm plantlets.

Acknowledgments. This study was supported by the Colorado Agricultural Experiment Station, Project 604. A. Zaid gratefully acknowledges funding from the Food and Agriculture Organization of the United Nations (Project RAB/88/024) and the U.S. Council for International Exchange of Scholars (Fulbright Program).

References

Bhaskaran S, Smith RH, Newton RJ (1985) Plant Physiol. 79:266-269

Boxus P, Quoirin M (1977) Acta Horticulturae 78:373-378

Brainerd KE, Fuchigami LH (1981) J. Amer. Soc. Hort. Sci. 106:515-518. Conner AJ, Thomas MB (1981) The Inter. Plant Prop. Soc. Combined Proceedings 31:342-357

Conner LN, Thomas MB (1981) Inter. Plant Prop. Soc. Comb. Proc. 31:342-357

Crane J, Hughes HG (1990) HortScience 25(7):794-795

Dami I (1991) M.Sci. Thesis. Colorado State University, Fort Collins, CO, 115 pp

Dami I, Hughes HG (1991) HortScience 26:725 (Abstr.)

Debergh PC (1991) Acta Horticulturae 289-291-300

Donnelly DJ, Skelton FE, Nelles JE (1987) J. Amer. Soc. Hort.Sci. 112(3):566-569

Grout BWW, Crisp P (1977) Acta Horticulturae 78:289-296

Heyser JW, Nabors MW (1981) Plant Physiol. 68:1454-1459

Kawasaki J, Akiba T, Moritsugu M (1983) Plant and Soil 75:75-85

Lee NI, Wetzstein H, Sommer HE (1985) Plant Physiol. 78:637-641

Mohammed GH, Vidaver WE (1990) Plant Cell, Tissue and Organ Culture 21:111-117

Murashige T (1974) Ann. Rev. Plant Physiol. 25:135-166

Murashige T, Skoog F (1962) Physiol. Plant. 15:473-497

Pierik RLM (1987) *In vitro* culture of higher plants. Martinus Nijhoff Publishers, Dodrecht, Netherlands

Preece JE, Sutter EG (1991) In: P.C. Debergh and R.H. Zimmerman (eds.), Micropropagation: Technology and Applications. Kluwer Academic Publishers, Dordrecht, pp. 71-93

SAS (1985) SAS User's Guide: Statistics, version 5 (ed.) SAS Institute, Inc. Cary, N.C.

Safadi F (1992) Ph.D. Dissertation. Colorado State University, Fort Collins, CO

Short KC, Warburton J, Roberts AV (1987) Acta Horticulturae 212:329-334

Sutter EG (1988) J. Amer. Soc. Hort. Sci. 113(2):234-238

Sutter EG, Langhans RW (1982) Can. J. Bot. 60:2896-2902

Wardle K, Dobbs EB, Short KC (1983) J. Amer. Soc. Hort. Sci.-389

Zaid A (1990) Ph.d. Dissertation. Colorado State University, Fort Collins, CO (Diss. Abstr. 91 DAI 51/068), 147 pp

Zaid A, Tisserat BH (1983) Date Palm Jour. 2(2):163-182

Zaid A, Hughes HG (1989) HortScience 24:122 (Abstr.)

Ziv M (1986) In: L.A. Withers and P.G. Alderson (eds.). Plant tissue culture and its agricultural applications. Butterworths, London, pp. 187-196