MICROPROPAGATION STUDIES ON *ZAGHLOUL* AND *SEWI CULTIVARS* OF DATE PALM (PHOENIX DACTYLIFERA L.) 3 – PLANTLET ACCLIMATIZATION

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ABSTRACT

The obtained results showed clearly, that peatmoss: sand: vermiculite (1:1:1 vv.) was the most suitable growing media for hardening date palm ***Phoenix dactylifera*** **L** Zaghloul cultivar seedling during the acclimatization period in this study during 18 months .This medium served 80% from 3 months even 18 months.

These media also resulted in the highest increase in shoot length, leaf number comparing with the other growing media after 18 month.

INTRODUCTION

Plantlets of date palm can be successfully transferred to 1:1 peat: vermiculite mixture when they reach about 12 cm in length and have distinct taproots and 2-. 3 leave ***Tisserat (1981a). T***he best survival (100% after 8 weeks) was recorded for 10-12 cm plantlets of date palm transferred to a peat: vermiculite mixture and covered with transparent plastic ***(Tisserat 1981b)***. Plantlets of date palm can be transplanted to growing medium consisting of peatmoss and vermiculite in 1:1 v/ ratio ***(Tisserat 1982)***. ***Tisserat (1984)*** described in detail a procedure for establishment of *in vitro* developed date palm plantlets in soil. The initial size of the plantlets was a critical factor in their survival. A minimum height averaging between 10 and 12 cm appeared to be necessary for maximum survival on transplantation. Plantlets were transplanted into a 1:1 mixture of peatmoss and vermiculite. ***Bhansali and Kaul (1991)*** reported that plantlets are hardened off under high light and low nutrient levels before transfer to sterilized soil after 8 weeks at high humidity and under a low temperature, followed by a few weeks under net-house benches, the plants are transferred to the open. So far, 15 palms (70% success rate) have been established outdoors. ***Sharma et al. (1990)*** indicated that regenerated plants were transplanted to sterilized soil containing fem and a clay-sand mixture. Under conditions of high

humidity, regular fungicide application and 2-3 of sunlight day, new leaves and roots appeared in 60% of plants after 1-2 months. ***Shakib et al. (1994)*** mentioned that plantlets of date palm were transferred to soil in the greenhouse when they were 10-15 cm tall.

MATERIALS AND METHODS

Seedlings, which produced from rooting stage (about 12-14 cm in length), were transferred from the test tubes under tap water to free the root from agar. The Seedlings were planted in plastic pots (tyrpido) (5-x 18 cm) and plastic bag filled after 6 months with the following growing media:

Treatment	Peatmoss	Sand	Vermiculite
1	1	0	0 (v/)
2	1	1	0
3	2	1	0
4	3	1	0
5	0	1	0
6	1	2	0
7	1	3	0
8	1	1	1
9	2	1	1
10	3	1	1
11	1	2	1
12	1	3	1

Plastic pots (Tyrpido) and plastic bag incubated under 4000-5000 Lux intensity derived from green house after enveloped in polyethylene bags, which were tightly closed to maintain high humidity. After 4 weeks polyethylene bags were completely opened. Nutrient solution contained 1.0 g/l of crystalline fertilizer was added to pots two weeks after transplanting. The following parameters were recorded.

1. Survival percentage. 2. Number of leaves. 3. Shoot length.
After one, three, six, nine, twelve and eighteen months.

RESULTS AND DISCUSSION

The most important stage in tissue culture is transferring the aseptic cultured from controlled to the free-living environment and ultimately to the final location.

Data presented in tables (1and2) show the effect of planting medium on survival percentage, number of leaves and shoot length after 1, 3, 6, 9, 12 and 18 months.

Survival percentage ranged from 70 to 90% after three months, 30-80% after 6 months and from 20 to 80% after 12 months. The best result was obtained with planting medium containing the equal ratio from peat, sand and vermiculite where the survival percentage was 80% after 6, 9, 12 and 18 months.

Generally, the results showed clearly that peatmoss: sand; vermiculite (1:1:1:V/V) mixture medium was the most suitable growing media for date palm (*Phoenix dactylifera L.*) Zaghloul cultivar seedling in

Table (1): Effect of transplanting media (Peat, sand and vermiculite) on survival % of date palm (*Phoenix dactylifera* L.) Zaghloul cultivar grown *in-vitro* (acclimatization stage) after 18 months.

No.	Transplanting media			Survival %					
				In pots (tyrpido)			Plastic bag		
	Peat	Sand	Verm.	After one month	After3 months	After 6 months	After 9 months	After 12 months	After 18 months
1	1	0	0	70	40	30	30	20	20
2	1	1	0	70	50	50	40	40	40
3	2	1	0	90	90	80	80	70	70
4	3	1	0	70	60	50	50	40	40
5	0	1	0	80	50	30	30	20	20
6	1	2	0	80	70	70	60	60	60
7	1	3	0	70	50	40	30	30	30
8	1	1	1	90	90	80	80	80	80
9	2	1	1	90	80	70	70	60	60
10	3	1	1	80	70	60	50	50	50
11	1	2	1	80	70	60	60	60	60
12	1	3	1	70	70	50	50	40	40

Table (2): Effect of transplanting media (peat, sand and vermiculite) on growth and development of date palm (*Phoenix dactylifera* L.) Zaghloul cultivar grown in ex vitro (acclimatization stage) after 18 months.

No.	Transplanting of media			Growth of plants											
				In Pots (Tyrpido)						Plastic bag					
	Peat	Sand	Verm.	After one month		After 3 months		After 6 months		After 9 Months		After 12 months		After 18 months	
				No. of leaves	shoot length	No. of Leaves	shoot Length	No. of leaves	Shoot length	No. of leaves	Shoot Length	No. leaves	Shoot length	No. of leaves	Shoot length
1	1	0	0	1.90	19.00	1.20	11.60	1.20	10.90	1.20	12.90	1.00	10.10	1.40	12.50
2	1	1	0	1.90	19.70	1.50	15.40	2.00	18.90	1.60	17.90	2.00	21.80	2.70	25.80
3	2	1	0	2.60	24.50	2.70	28.20	5.00	29.10	8.20	35.90	3.30	38.20	4.50	45.50
4	3	1	0	1.90	18.90	1.80	18.60	2.00	18.80	2.00	22.40	2.00	21.90	2.80	26.60
5	0	1	0	2.20	21.60	1.50	15.60	1.20	10.90	1.20	13.50	1.00	11.00	1.40	13.50
6	1	2	0	2.20	22.00	2.10	22.00	2.70	26.90	2.40	27.30	2.90	33.80	4.00	46.60
7	1	3	0	1.90	19.40	1.50	16.00	1.60	15.40	1.20	14.00	1.50	16.66	2.10	20.00
8	1	1	1	2.50	24.50	2.60	28.50	3.00	31.40	3.10	38.00	3.80	45.50	5.10	53.90
9	2	1	1	2.40	24.80	2.30	25.70	2.70	27.10	2.80	32.40	3.00	33.30	4.10	39.90
10	3	1	1	2.20	22.10	2.00	23.10	2.40	25.00	2.00	25.00	2.50	29.30	3.50	34.40
11	1	2	1	2.30	22.80	2.10	29.90	2.30	25.30	2.40	30.10	2.90	35.20	4.10	41.60
12	1	3	1	2.00	19.70	2.10	23.80	2.00	20.50	2.00	23.90	2.00	23.10	2.80	27.10

Acclimatization stage. In this medium, seedling grows well, with 80% survival after 18 months. Also, the same media was the best, while, the tallest shoots (53.90 cm/ plant) and the highest leaves number (5.10 leaves / plan after 18 months.

From the obtained data, we found that peatmoss alone was not suitable for date palm hardening as well as peatmoss + sand at 3:1 ratio. The addition of vermiculite to peatmoss and sand improved survival percentage and growth parameters estimated as number of leave and shoot length. **Tisserat (1981)** found that date palm plantlets could be successfully transferred to 1:1 peatmoss: vermiculite mixture when they reach about 12 cm in length and have distinct taproots and 2-3 leaves. **Tisserat (1984)** mentioned that the initial size of the plantlets was a critical factor in their survival. A minimum height averaging between 10 and 12 cm appeared to be necessary for maximum survival on transplantation. Plantlets were transplanted into a 1:1 mixture of peatmoss and vermiculite. **Madhuri and Shankar (1998)** reported that date palm plantlets were successfully transferred to pots containing a mixture (1:1) of vermiculite and peatmoss. A plant which has obtained in vitro differs in many respects from one produced *in vivo* **(Pierik, 1987)**. Date palm plantlets survival percentage was low for many reasons, with plants grown in test tubes, the cuticle (wax layer) is often poorly developed because of the relative humidity, which his often 90-100% *in vitro*. This results in extra water loss through cuticular evaporation, when the plant is transferred to soil, since the humidity of the air *in vivo* is much lower. Leaves of an *in vitro* plant, often thin, soft, and photosynthetically not very active, are not well adapted for in vivo climate. Test tube plants have smaller and fewer palisade cells to use light effectively, and have larger mesophyll air space. Stomata do not operate properly in tissue culture plants; open stomata in tissue culture plants cause the most significant water stress during the first few hours of acclimatization in tissue culture plants poor vascular connections, between the shoots and roots may reduce water conduction. It must also be realized that the in vitro plant has been raised has as a heterotrophic while it must be autotrophic *in vivo,* sugar must be replaced through photosynthesis.

It can be seen from the observations above that *in vitro* plants should be given time to get used to the *in vivo* climate and/or allowed to acclimatize (already *in vitro*), and become hardened off. Acclimatization can take place by allowing the *in vitro* plants to be gradually get used to the *in vivo* climate and/or allowed to acclimatize (already *in vitro*), and become hardened off.

Acclimatization can take place by allowing the in vitro plants to gradually get used to a lower relative humidity, which is the case in vivo. Development of a stomata closure mechanism is a very important component of acclimatization **(Pierik, 1987)**. Roots that have originated *in vitro* appear to be vulnerable and not to function properly *in vivo* (Few or no root hairs); they quickly die off and must be replaced by newly formed subterranean roots. The poorly developed root system makes *in vivo* growth for such a plant very difficult, especially when there is high evaporation. It is vital that the *in vitro* plant losses as little water as possible *in vivo* **(Sutler and Hutzell, 1984)**.

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