# MICROPROPAGATION STUDIES ON *ZAGHLOUL* AND *SEWI CULTIVARS* OF DATE PALM (PHOENIX DACTYLIFERA L.) 2 –SHOOT AND ROOT FORMATION

***Sherif F. El Sharabasy [1], Hussein A. Bosila [2], Said M. Mohamed [3] Khames A. Refay, [4] Ibrahim A. Ibrahim [5].**

**(1) Central Lab. Res. Development of date palms Agric. Res.Center. (2) Fac. Agric. Hort. Dept. Alazhar Univ. (3) Medicinal & Aromatic. Plants Dept. Hort. Res. Inst. Agric. Res.Center. (Fac. Agric. Hort. Dept. Alazhar Univ. (5) Gen. Eng. Biothec.Res. Inst. Menoufia Univ.**

**ABSTRACT**

The highest number of shoot of date palm ***Zaghloul cultivar*** was formed on Ms medium supplemented with 1 mg/l/ 2I, while the highest number of shoot of date palm ***Sewi cultivar*** was formed on Ms medium supplemented with 3.0 mg/l BA The highest rooting percentage (100%) was recorded for ***Zaghloul cultivar*** when MS basal medium supplemented with 0.5 mg/l IAA, 0.1, 0.5, 1.0 mg/l NAA + 3 g/l AC., 1.0 mg/l IAA+3mg/l IAC (3g/l was used, while the highest rooting percentage (100%) was recorded for ***Sewi cultivar*** when MS basal medium supplemented with for 1.0 mg/l NNA+3g/l IAC

Tissue culture micropropagtion has been employed to aid in the clonal propagation of numerous plant species. The inherent advantage of tissue culture over field propagation is the greater plant production potential from a single plant. Tissue culture techniques may offer a possible method to produce large numbers of genetically uniform palms. Several reports dealing with tissue culture in palms have appeared in the literature in the 1970's. Production of a sexual embryos and their subsequent development into free-living plants in oil palms was the first published report in the literature has obtained free-living plants from clonal date palm explant tissues derived from shoot tips, lateral buds, inflorescence ***(Tisserat, 1983).*** The prolific shoot growth was obtained from a variety of shoot tip explant types particularly the apical dome with two adjacent leaf primordial of date palm ***(Tisserat 1979)***. The actions of several auxins and cytokinins on development of *Phoenix dactylifera* L. seedling shoot tips and apical meristem were determined. Shoot tip explants consisted of the apical dome with two to four leaf primordial and varied in size from 0.5 to 1.0 cm ***(Zaid and Tisserat 1983)***. Tissues from leaf primordial, shoot apical cotyledons and roots of 2 to 6 month-old seedlings or from young leaves, meristem tips, epicotyls, hypocotyls or roots of 2 year old date palms planted as explants on MS medium (***Wangkaew et al.1991)***

## MATERIALS AND METHODS

Some trials were designed to study the effect of plant growth regulators on the growth and development of shoots derived from germinated embryos, which had grown on free growth regulator medium. The growth embryo explants (1-1.5 cm in height) were transferred to MS basal medium supplemented with different growth regulators as follows:

1. Control medium (MS basal medium without hormones).
2. Control medium + 3 g/L activated charcoal.
3. 1.0 mg/L BA 4. 2.0 mg/L BA
5. 3.0 mg/L BA. 6. 1.0 mg/L kin.
7. 2.0 mg/L Kin. 8. 3.0 mg/L Kin.
9. 1.0 mg/L 2ip. 10. 2.0 mg/L 2ip. 11. 3.0 mg/L 2ip.

**Culture environment:**

All cultures treatments were maintained at 27 $\pm$ 2ºC under 2000 Lux illumination by cool white fluorescent light for 16 hours photoperiod. The following parameters were recorded after 6 weeks:

- Survival percentage. - Shoot number. - Shoot length.

**Rooting stage:**

Shoots of Zaghloul and Sewi CVs., which were derived from shooting stage, were transferred to MS basal medium supplemented with different Auxins (Indole acetic acid (IAA), Indole butryic acid (IBA), and Naphthalene acetic acid (NAA) to study their role on root formation. Auxins concentrations were (0.0, 0.1, 0.5, 1.0 mg/L) in MS medium without or with 3.0 g/L activated charcoal (AC). Shoots of both CV were separated as individual shoot and cultured in the pervious media. The different treatments were incubated in a growth chamber at 27$\pm$2ºC under 4000 Lux illumination by cool white fluorescent light for 16 hours photoperiod.Each treatment consisted of 12 tubes (250x25 mm) and contained 50 ml of medium. The following parameters were recorded after 6 weeks:

- Survival percentage. - Shoot number.
- Shoot length (cm). - Root number.
- Root length (cm). - Root percentage.

## RESULTS AND DISCUSSION

### Shoot formation

Data presented in tables (1 and 2) show clearly that survival percentage was 100% for both examined CVs. (Zaghloul and Sewi) under the effect of the different used treatments in this study. 2ip were superior to kinitin and BA for shoot formation. Statistical analysis of variance indicated that shoot growth was of significant value affected by cytokinin sources and its tested concentrations. In this concern, shoot number and length were of negative correlation responses with increasing kin and 2ip levels, however, these parameters were responded differently to increasing BA concentration. Increasing BA concentration increased shoot number without significant differences, while, shoot length had the opposite trend and the differences between its levels were of significant value.

The variations in shoot growth of Sewi CV. were responded differently to cytokinins source and their examined concentrations in this

Table (1) Effect of different concentrations of cytokinins (BA or Kin or 2ip) on shoot formation of date palm *(Phoenix dactylifera L.)* Zaghloul cultivar derived from somatic embryogenesis after 6 weeks.

| Treatments | Concentrations (mg/l) | Survival % | Shoot Growth | |
|---|---|---|---|---|
| | | | No. of shoots | Shoot length |
| Control | 0.0 | 100 | 1.333 | 1.750 |
| Control + AC. | 0.0+ 3 g/l | 100 | 1.167 | 3.500 |
| BA | 1.0 | 100 | 2.583 | 4.833 |
| BA | 2.0 | 100 | 3.000 | 3.708 |
| BA | 3.0 | 100 | 3.250 | 3.667 |
| Means | | | 2.946 | 4.070 |
| Kin | 1.0 | 100 | 3.250 | 3.417 |
| Kin | 2.0 | 100 | 3.083 | 3.208 |
| Kin | 3.0 | 100 | 3.083 | 2.875 |
| Means | | | 3.138 | 3.170 |
| 2ip | 1.0 | 100 | 4.417 | 4.000 |
| 2ip | 2.0 | 100 | 3.217 | 3.417 |
| 2ip | 3.0 | 100 | 3.833 | 3.042 |
| Mean | | | 3.822 | 3.490 |
| LSD. 0.05 | | | 0.867 | 0.999 |

AC = Activated charcoal

Table (2) Effect of different concentrations of cytokinins (BA or Kin or 2ip) on shoot formation of date palm *(Phoenix dactylifera L.) Sewi* cultivar derived from somatic embryogenesis after 6 weeks.

| Treatment | Concentrations (mg/l) | Survival % | Shoot Growth | |
|---|---|---|---|---|
| | | | No. of shoots | Shoot length |
| Control | 0.0 | 100 | 4.917 | 2.333 |
| Control AC | 0.0+ 30 | 100 | 3.167 | 3.042 |
| BA | 1.0 | 100 | 3.417 | 2.750 |
| BA | 2.0 | 100 | 3.667 | 1.667 |
| BA | 3.0 | 100 | 5.917 | 1.875 |
| Means | | | 4.336 | 2.097 |
| Kin | 1.0 | 100 | 3.333 | 2.417 |
| Kin | 2.0 | 100 | 2.338 | 2.875 |
| Kin | 3.0 | 100 | 2.417 | 2.542 |
| Means | | | 2.696 | 2.611 |
| 2ip | 1.0 | 100 | 2.583 | 3.125 |
| 2ip | 2.0 | 100 | 5.750 | 2.000 |
| 2ip | 3.0 | 100 | 3.500 | 2.417 |
| Means | | | 3.944 | 2.514 |
| LSD 0.05 | | | 0.830 | 0.865 |

AC = Activated charcoal

study. In this concern, number of shoots which were formed from explants treated with BA were of positive correlation response with increasing BA levels from 1 mg/l to 3 mg/l. Increasing kinitin level negatively correlated with number of shoots/explant. Increasing 2ip level increased shoot number from 2.696 shoot/explant for 1 mg/l 2ip to 5.750 shoot/explant for 2 mg/l 2ip, while it decreased to 3.5 shoot/explant when 3 mg/l 2ip was used. However, control medium without activated charcoal was more of significant value suitable for shoot number formation (4.917 shoots/explant) than the most of cytokinins sources levels used except 3 mg/l BA and 2 mg/l 2ip.

The obtained results show also clearly that the shoot length of explants received 2 mg/l BA (1.667 cm/explant) was shorter than the different treatments used and control (with or without activated charcoal) followed by 3 mg/l BA (1.875 cm/explant). The tallest shoots were recorded for 1 mg/l 2ip (3.125 cm/explant) without significant differences with control + activated charcoal treatment (3.042 cm/explant).

Results under discussion are in agreement El-Hennawy and Wally (1980), Zaid and Tisserat (1983), Gabr and Tisserat (1985), Nasir *et al.*, (1994), Al-Kharyi and Al-Maarri (1997), and El-Hamadi *et al.* (1999).To propagate plants *in vitro* by adventitious organ or embryo formation, in principle, it is necessary that they are capable of regeneration. The ability to regenerate is determined by genotype, the environmental conditions (nutrient supply, regulators and physical conditions), and the developmental stage of the plant. It is well known that some family and genera have high regeneration ability. Juvenile plants have a greater regeneration capacity than adult plants. Since adult plants are generally used for vegetative propagation, this means that especially in the woody species, an attempt should be made to rejuvenate them before use.

Rejuvenation by means of meristem culture, despite the difficulties associated with this method, is still the most favored techniques since it maintains genetic stability; eliminates fungi and bacteria, and can sometimes result in the additional advantage of obtaining virus-free material (Pierik 1987). Somatic embryogenesis has been carried with a high degree of success with a number of plants. For example, the date palm (Tisserat, 1979; Mater, 1986; Dass *et al.*, 1989; Shakib *et al.*, 1994 and Ibrahim, 1999) and the oil palm (Jones, 1983; Blake, 1983; Litz *et al.*, 1985) are cloned at the moment on a large scale by callus, embryo and shoot formation.

**Rooting Stage:**

From the presentations in tables (3 and 4) it appears that, 100 % survival was recorded for the different examined treatments in this study, regardless of tested CVs. Statistical analysis of variance for the obtained results show that, shoot growth of Zaghloul and Sewi date palm CVs. was responded differently to the different auxins used and its concentration as well as their combinations with activated charcoal. In this concern, no significant differences were recorded between shoot number/explant and the different treatments used in this study regardless of the tested date palm CVs.

Table (3) Effect of different concentrations of (NAA, IBA or IAA) and activated charcoal (A.C) on shoot and roots formation of date palm (*Phoenix dactylifera* L.) Zaghloul CV *in vitro* after 6 weeks.

| Treatments | Concentration (mg/l) | Survival % | Shoot growth | | Roots information | | |
|---|---|---|---|---|---|---|---|
| | | | No of shoots | Shoot length | No. of roots | Root length | Rooting % |
| Control | 0.0 | 100 | 1.00 | 10.417 | 1.167 | 0.750 | 33.33 |
| NAA | 0.1 | 100 | 1.333 | 14.917 | 0.00 | 0.00 | 0.00 |
| | 0.5 | 100 | 1.333 | 10.083 | 0.00 | 0.00 | 0.00 |
| | 1.0 | 100 | 1.333 | 11.333 | 1.333 | 1.250 | 50.00 |
| Means | | | 1.333 | 12.111 | 0.444 | 0.416 | 16.666 |
| IBA | 0.1 | 100 | 1.167 | 5.750 | 1.333 | 1.750 | 66.66 |
| | 0.5 | 100 | 1.167 | 11.000 | 1.500 | 2.00 | 66.66 |
| | 1.0 | 100 | 1.167 | 14.083 | 1.833 | 2.157 | 66.66 |
| Means | | | 1.167 | 11.277 | 1.555 | 1.969 | 66.66 |
| IAA | 0.1 | 100 | 1.333 | 11.417 | 1.500 | 0.667 | 33.33 |
| | 0.5 | 100 | 1.167 | 8.917 | 3.000 | 3.167 | 10000 |
| | 1.0 | 100 | 1.00 | 9.230 | 0.167 | 0.950 | 16.66 |
| Means | | | 1.165 | 9.860 | 1.555 | 1.595 | 49.99 |
| Control | 0.0+3 g/l | 100 | 1.00 | 14.167 | 2.833 | 5.917 | 83.33 |
| NAA+ AC | 0.1+ 3 g/l | 100 | 1.333 | 14.833 | 4.000 | 10.833 | 100.00 |
| | 0.5+3 g/l | 100 | 1.167 | 14.167 | 3.500 | 6.333 | 100.00 |
| | 1.0+3 g/l | 100 | 1.333 | 14.000 | 3.500 | 4.417 | 100.00 |
| Means | | | 1.276 | 14.300 | 3.600 | 7.194 | 100.00 |
| IBA+A.C. | 0.1+ 3 g/l | 100 | 1.333 | 15.000 | 2.667 | 2.417 | 66.66 |
| | 0.5+3 g/l | 100 | 1.333 | 13.167 | 2.500 | 2.833 | 66.66 |
| | 1.0+3 g/l | 100 | 1.333 | 10.583 | 1.667 | 5.750 | 83.33 |
| Means | | | 1.333 | 12.250 | 2.278 | 3.666 | 72.216 |
| IAA+A.C. | 0.1+ 3 g/l | 100 | 1.00 | 10.083 | 0.333 | 1.083 | 33.333 |
| | 0.5+3 g/l | 100 | 1.00 | 10.417 | 0.663 | 1.417 | 33.333 |
| | 1.0+3 g/l | 100 | 1.167 | 9.750 | 2.667 | 4.833 | 100.00 |
| Means | | | 1.055 | 10.083 | 1.221 | 2.444 | 55.553 |
| LSD 5% | | | NS | 2.370 | 2.083 | 3.037 | |

On other hand shoot length significantly responded with these treatments. Data concerning the effect of different auxin sources and concentrations on shoot length of Zaghloul CV. show that, NAA with or without activated charcoal combinations was more suitable auxin in order to increase shoot length. In this concern applying 0.1 mg/l NAA resulted in the tallest shoot (14.917-cm) comparing with its other levels used and the different concentrations auxins. While, the shortest shoot (5.750 cm/plant) was formed by Zaghloul CV. explants treated with 0.1 mg/l IBA.

Combined auxin concentrations with activated charcoal significantly increased shoot length of Zaghloul CV. In this concern, NAA treatments combined with activated charcoal had relatively the same result regardless of NAA levels used. The values recorded in this case were 14.833, 14.167 and 14.00 cm/plant, for 0.1, 0.5 and 1.0 mg/l NAA + 3 mg/l activated charcoal, respectively.

Table (4) Effect of different concentrations of (NAA, IBA or IAA) and activated charcoal (A.C) on shoot and roots formation of date palm (*Phoenix dactylifera* L.) Sewi cultivar culture in vitro after 6 weeks.

| Treatments | Concentration (mg/l) | Survival % | Shoot growth | | Roots information | | |
|---|---|---|---|---|---|---|---|
| | | | No of shoots | Shoot length | No. of roots | Root length | Rooting % |
| Control | 0.0 | 100 | 1.00 | 10.833 | 0.500 | 0.667 | 16.66 |
| NAA | 0.1 | 100 | 1.00 | 14.417 | 0.167 | 0.417 | 16.66 |
| | 0.5 | 100 | 1.16 | 12.583 | 0.833 | 0.750 | 33.33 |
| | 1.0 | 100 | 1.33 | 12.583 | 1.333 | 1.583 | 50.00 |
| Means | | | 1.16 | 13.194 | 0.777 | 0.916 | 33.33 |
| IBA | 0.1 | 100 | 1.00 | 10.750 | 0.667 | 1.083 | 33.33 |
| | 0.5 | 100 | 1.16 | 10.833 | 1.500 | 1.667 | 66.66 |
| | 1.0 | 100 | 1.50 | 10.750 | 1.667 | 1.500 | 50.00 |
| Means | | | 1.22 | 10.777 | 1.278 | 1.416 | 49.99 |
| IAA | 0.1 | 100 | 1.00 | 11.500 | 0.00 | 0.000 | 0.00 |
| | 0.5 | 100 | 1.00 | 10.500 | 0.833 | 1.083 | 33.33 |
| | 1.0 | 100 | 1.00 | 10.417 | 1.167 | 1.000 | 33.33 |
| Means | | | 1.00 | 10.805 | 0.666 | 0.694 | 22.22 |
| Control | 0.0+3g/l | 100 | 1.00 | 14.250 | 1.833 | 2.750 | 50.00 |
| NAA+ AC | 0.1+3g/l | 100 | 1.00 | 14.417 | 2.833 | 4.917 | 83.33 |
| | 0.5+3g/l | 100 | 1.16 | 13.417 | 2.833 | 6.667 | 100.00 |
| | 1.0+3g/l | 100 | 1.00 | 13.500 | 4.000 | 6.917 | 100.00 |
| Means | | | 1.05 | 13.778 | 3.222 | 6.167 | 94.44 |
| IBA+A.C. | 0.1+3g/l | 100 | 1.33 | 14.417 | 2.333 | 2.750 | 66.66 |
| | 0.5+3g/l | 100 | 1.16 | 13.917 | 2.833 | 4.250 | 66.66 |
| | 1.0+3g/l | 100 | 1.33 | 11.583 | 2.500 | 3.167 | 83.33 |
| Means | | | 1.273 | 13.305 | 2.555 | 3.389 | 72.22 |
| IAA+A.C. | 0.1+ 3 g/l | 100 | 1.00 | 13.167 | 0.833 | 1.000 | 33.33 |
| | 0.5+3 g/l | 100 | 1.00 | 11.667 | 1.167 | 6.833 | 50.00 |
| | 1.0+3 g/l | 100 | 1.16 | 12.167 | 1.667 | 3.667 | 66.66 |
| Means | | | 1.05 | 12.333 | 1.222 | 2.166 | 49.99 |
| LSD | | | NS | 2.778 | 1.834 | 2.518 | |

Shoot length was of negative correlation responses with increasing IBA levels combined with activated charcoal, while this response was of positive value when IBA concentrations were used without activated charcoal combination. Moreover the tallest shoots (15.0 cm/plant) was recorded for Zaghloul CV. explants treated with 0.1 mg/l IBA + 3.0 mg/l activated charcoal. The shortest shoots (9.75 cm/plant) was formed by Zaghloul explants treated with 0.5 mg/l IAA + 3.0 mg/l activated charcoal.

Data illustrate the effect of auxin treatments and its combinations with activated charcoal on Sewi CV. shoot length show that NAA has the pronounced and significant effect on this parameter comparing with IBA or/and IAA treatments as well as control. However, no significant differences were found within each auxin levels used in this study.

From the recorded data in Tables (5 and 6) it appears that root formation on date palm explants was differently responded to the

different examined auxins and their combinations with activated charcoal. Rooting of date palm, Zaghloul CV. show relatively more vialability to format roots (33%) than Sewi CV. (16%). However, using NAA in the rate of 0.1 and 0.5 mg/l retarded Zaghloul CV. explants ability to format any roots (0.0%) comparing with Sewi CV. explants which were stimulated to format it under these treatments. Moreover, IBA stimulate Zaghloul CV. explants to format roots, regardless of its levels. While, Sewi CV. explants responded differently to. The highest rooting (100%) was recorded for Zaghloul CV. explants, which were treated with 0.5 mg/l IAA comparing with 33.33% for those treated with 0.1 mg/l IAA or 16.66% for 1.0 mg/l IAA treated explants.

On the other hand combining auxin treatments with 3.0 mg/l activated charcoal, generally stimulated date palm explants to format roots resulting in 100% rooting for Zaghloul CV. explants treated with the different levels of NAA + 3.0 mg/l activated charcoal comparing with 83.33%, 100% and 100% for Sewi CV. explants treated with 0.1, 0.5 and 1.0 mg/l NAA + 3.0 mg/l activated charcoal. Combining the different IBA levels with 3.0 mg/l activated charcoal had the same results for both date palm CVs. (66.66%, 66.66% and 83.33%). The lowest rooting % was recorded for both date palm c.v. Treated with IAA levels + 3.0 mg/l activated charcoal (55.33 and 49.99 for Zaghloul and Sewi CVs., respectively).

Statistical analysis of variance show clearly that root number as well as root length differently responded to auxin treatments and their combinations with activated charcoal. The highest number of roots/plant (3.00) with the tallest roots (3.167 and 23.157) were recorded for Zaghloul explants treated with 0.5 mg/l IAA and 0.5 mg/l IBA, respectively. While, the lowest root number and length (0.0) were recorded for Zaghloul CV. explants treated with 0.1 and 0.5 mg/l NAA, respectively.

Combining auxin treatments with 3.0-mg/l activated charcoal significantly stimulated both date palms CVs. (Zaghloul and Sewi) to format more number of roots/plant and increasing their length. The highest root number (4 roots/ plant) with the tallest roots (10.833 cm/plant) was formed by Zaghloul CV. explants treated with 0.1 mg/l NAA + 3.0 mg/l activated charcoal. Comparing with 4 root/plant and 6.917 cm/plant for Sewi CV. explants treated with 1.0 mg/l NAA + 3.0 mg/l activated charcoal while, the lowest number of roots/plant (0.333) with the shortest roots (1.083 cm/plant) was formed by Zaghloul CV.

explants treated with 0.1 mg/l IAA + 3.0 mg/l activated charcoal, compared with 0.833 root/plant and 1.0 cm/plant for Sewi CV. explants.

Results under discussion are in harmony with that reported before by **Tisserat (1981), and Sharma** *et al.* **(1984)** .In order to improve in vitro adventitious rooting, the isolated plantlets were cultured on media containing 0.1, 1.0 and 10.0 mg/l IAA or NAA in various physical conditions. Optimum adventitious rooting and subsequent plant survival was obtained by culturing plantlets in medium containing 0.1 mg/l NAA for 8-16 weeks prior to transplanting to soil **(Tisserat, 1982)**. Date palm plants may be obtained by transferring individual young plants to MS medium supplemented with 0.1 mg/l NAA to enhance rooting and 0.01 mg/l BA to improve shoot system **(Omar, 1988)**.

## REFERENCES


Al-Kharyi, J.M. and K.W. Al-Maarri (1997). Effect of seasonal variation on the regeneration capacity of date palm. *In vitro*, 33: 3, 22

Dass, H.C.; R.K. Kaul; S.P. Joshi and R.R. Bhansali (1989) *In vitro* regeneration of date palm plantlets. Current Science, 58: 1, 22-24.

El-Hennawy, H.M. and Y.A. Wally (1980) Vegetative propagation of date palm is explant culture *in vitro*. Egypt. J. Hort., 7 (2): 211-220.

Gabr, M.F. and B. Tisserat (1985), "Propagating palms *in vitro* with special emphasis on the date palm *(Phoenix dactylifera* L.)". Scientia Horticulture, 25, 255-262.

Ibrahim, A.I (1999) Somaclonal variation during micropropagtion of date palms via embryogenesis. The First International Conference, a Plant Tissue Culture and it's Application, Cairo, Egypt. P. 189.

Jones, L.H. (1983). The oil palm and its clonal propagation by tissue culture. Biologist, 30 (4): 181-184.

Litz, R. E. (1984). *In vitro* somatic embryogenesis from nuclear callus of monoembryonic Mango. Hort. Science, 19(5): 715-717.

Mater, A.A. (1986). "*In vitro* propagation of *Phoenix dactylifera* L." Date Palm J. 4 (2): 137-152.

Nasir, I.A.; M.A. Khan and S.J. Butt (1994), "*In vitro* culture of date palm (*Phoenix dactylifera* L.) Through excised embryo". Sarhad Journal of Agriculture, 10:6, 633-637.

Omar, M.S. (1988), "Callus initiation, asexual embryogenesis and plant regeneration in *Phoenix dactylifera* L.". Date Palm Journal, 6:265-275.

Pierik, R.L.M. (1987). *In vitro* culture of Higher Plants. Martinus Nizhoff Publishers, Boston.

Shakib, A.M.; S. Khoshkam and E. Hervan (1994), "Plant regeneration of date palm variety Estamaran by tissue culture". Seed and Plant, 9: 3-4, 8-11.

Sharma, D.R.; S. Dawrara and J.B. Chowdury (1984), "Somatic embryogenesis and plant regeneration in date palm (*Phoenix dactylifera* L.) CV. Khadravi through tissue culture". Indian Journal of Experimental Biology, 22, 596-598.

Tisserat, B. (1979), "Propagation of date palm (*Phoenix dactylifera* L.) *In vitro*" Journal of Exp. Bot, 30: 1275-1283.

Tisserat, B. (1981) Date palm tissue cultures. USDA/A RS Advances in Agricultural Technology, Western series, No.17, Agricultural Res. Ser, Oakland, California.

Tisserat, B. (1981), Production of free-living palms through tissue culture". Date Palm J., 1:43-54.

Tisserat, B. (1982) Factors involved in the production of plantlets from date palm callus cultures". Euphytica, 31:1, 201-214.

Wangkaew, P.; Pienngarm, B. and T., Polthampitak. (1991) Tissue culture of date palm. Kaenkaset Khon Kaen Agric. J., 19 (4): 191-200.

Zaid, A. and B. Tisserat (1983a), "*In vitro* shoot tip differentiation in *Phoenix dactylifera* L. ". Date Palm J., 2(2): 163-182.