SHAMS EL-DIN I. M., International Journal of Advances in Agricultural Science and Technology,
Vol.5 Issue.1, January- 2018, pg.9-30 **ISSN: 2348-1358**
Impact Factor: 6.057
NAAS Rating: 3.77

PROMOTING THE EMERGENCE ROOTS OF "SEWI" DATE PALM IN VITRO

SHAMS EL-DIN I. M. *
* CENTRAL LAB. FOR DATE PALM RESEARCHES AND DEVELOPMENT, ARC, GIZA, EGYPT

ABSTRACT: ***Plant tissue culture technique has proven as a quick and sustainable way to provide large-scale propagation of true-to-type date palm (Phoenix dactylifera L.) plants. Rooting is the final culture stage for transffered to greenhouse acclimatization which is the prime concept of micro propagation system. A good rooting system is the prerequisite for survival of ex vitro grown plantlets in the open field which responsible to absorb water and nutrients from the soil. The present work discussed the effectiveness of culture media components (¼ , ½ , ¾ and full strength of both Murashige & Skoog (MS) and Schenk & Hildebrandt (SH) medium) and some plant growth regulators as Indole butyric acid (0, 0.5, 1.0, 2.0 mg l^{-1}IBA), Triacontanol at 0, 5, 10, 20 µg l^{-1} TRIA and combination of the best concentration of TRIA with Gibberellic acid (GA_3) at 0, 0.5 and 1.0 mg l^{-1} and control treatment (free TRIA and GA_3) on rooting of date palm cv. Sewi in vitro. Finally, the rooted plantlets which produced from all treatments in the last experiment (TRIA and GA_3 combinations) were transferred separately to greenhouse and planted in plastic pots filled with peat moss+vermiculite+perlite 2:l:l (v/v/v) to determine the survival percentage. The results showed that the root number and length were significantly affected by MS and SH medium salts strength. Referring to MS medium, The highest number and length of root (3 roots and 8 cm) were obtained with quarter salts strength MS medium. In case of SH medium, The highest number and length of root (4.5 roots and 6 cm) were obtained with full strength SH medium. Also the results showed that the root number and length were significantly affected by IBA, TRIA and the combination between TRIA & GA_3. In case of IBA, the highest number and length of root (6 roots and 8 cm) were obtained at 1.0 mg l^{-1} IBA. Whereas in case of TRIA, the highest numbers and length of root (20 roots and 5 cm) were obtained at 5 µg l^{-1} TRIA. Finally, the highest number and length of root (27 roots and 6.4 cm) and the highest shoot length (17.3 cm) were obtained at 5µg l^{-1} TRIA+1.0 mg l^{-1} GA_3. In acclimatization stage, the best survival percentage (90%) after three months was obtained with the use of TRIA at 5***

µg l^{-1} in combination with GA_3 at 1.0 mg l^{-1} in compare with the control whereas survival percentage was (50%).

Keywords: Date palm, In vitro and Rooting.

Abbreviations: ***MS: Murashige and Skoog (1962) basal medium; SH: Schenk and Hildebrandt medium (1972); 2,4-D: 2,4-dichlorophenoxy acetic acid; 2-iP: N6-2-Isopentenyladenine; NAA: α-naphthalene acetic acid; BA: 6-benzylaminopurine; IBA: Indole butyric acid; TRIA: Triacontanol; GA_3: Gibberellic acid and AC: activated charcoal.***

INTRODUCTION

The date palm, *Phoenix dactylifera* L., is one of the most economically important perennial plants in arid areas of the Middle-East and the North Africa. Date palm cv. Sewi is an important variety, where it increases demand for food and many other commercial purposes. It is known that propagation of palm is very slow. Tissue culture is the most technology method to provide large-scale propagation of healthy true-to-type date palm plants. **AlKhateeb and Alturki (2014)** mentioned that the use of plant tissue culture technique for propagation of date palm is considered an alternative method to the conventional methods, whereas seed propagation is not common due to heterozygosis and dioeciously nature of the date palm which may result in producing off type plant and offshoot propagation is a slow method and its mortality is usually high **(Von-Arnold *et al*., 2002).**

Rooting is the final culture stage prior to acclimatization which is the prime concept of micro propagation system **(Ismail *et al*., 2011).** A good rooting system is the prerequisite for survival of *in vitro* grown plantlets in the field depends which might help to absorb water and nutrients from the soil **(Benková and Bielach, 2010)**.

The *in vitro* growth and development of a plant is determined by a number of complex factors: Physical growth factors (Light -Temperature - CO2 - pH); water, macro and micro elements, and sugar; and some organic substances (plant growth regulators, vitamins, amino acids). Whereas, other important variables, such as gelling agent, activated charcoal might be important factors for enhancement of rooting in tissue culture systems **(Arthur *et al*., 2006).**

Concentration of the medium salts play in some cases an important role in inducing roots. However, many workers preferred diluted strength of the MS medium salts for rooting of the *in vitro* produced shoots. **Das *et al.* (1996)** reported that regenerated shoots of *Echinochloa colona* were rooted on half-strength basal MS medium devoid of growth regulators.

Atta-Alla *et al* (1997) recorded that the proliferated *Yucca aloifolia* shoots rooted readily *in vitro* on ¼ strength MS medium. **Pereira *et al.* (1999)** indicated that the reduction in MS salts (from full strength to ¾ or ½ strength) increased the number of roots produced on explants of strawberry cv. Tangi. **Karhu (1997)** mentioned that reduced mineral nutrient concentration of modified MS medium allowed more root elongation. **Abul-Soad *et al*. (2007)** reported that ¾ MS medium salts strength was better than the full strength of MS in the rooting medium of date palm (*Phoenix dactylifera* L.) cv. Zaghloul. **Rasmia Darwesh *et al.* (2011)** they proved that greatest number of leaves and roots/plantlet resulted from 206.25 mg/l ammonium nitrate and 0.25 mg/l GA_3 and 412.5 mg/l ammonium nitrate (MS) and 0.15 mg/l GA_3 gave the best results for survival percentage of acclimatization of the date palm plantlets. **Shah *et al.* (2013)** reported that SH medium produced significantly high percent of *in vitro* rooting of *Aristolochia indica* L. in comparison to MS, B5, NN and WPM medium which produced minimum effect on rooting. Also SH medium produced maximum root number per explant in comparison with WPM, MS and NN medium.

Shoots regenerated *in vitro* may be produce roots spontaneously with no need for auxins. Auxins are a class of phytohormones which are involved in many aspects of tissue growth and development **(Davies, 1995)**. Exogenously applied natural or synthetic auxins could help for rooting **(Osterc and Štampar, 2011). Bekheet and Saker (1998)** reported that in date palm tissue culture, rooting of proliferated shoots was achieved upon supplementation of MS culture medium with 1.0 mg L^{-1} NAA. **Gadalla (2003)** reported that the highest significant value of rooting percentage of dry cultivars of date palm was observed when MS culture medium was supplemented with 3mg l^{-1} of either NAA or IBA. Gibberellic acid GA_3

treatment promotes cell enlargement and cell division **(Buchanan *et al*.2000)**

In the last years investigations were focused on natural plant substances and have proved their growth regulator effect. This property makes reasonable their application in the course of *in vitro* propagation in order to improve the efficiency of the process. 1-Triacontanol (TRIA), a long 30 carbon saturated primary alcohol (C_{30} H_{61} OH), was discovered in 1933 as a natural components of epicuticulary waxes of alfalfa (*Medicago sativa*) by **(Chibnall *et al*., 1933)**. Since then several experiments proved its growth promoting effect in greenhouse or field trials. The plant growth stimulating activities of TRIA (such as increase in dry weight, leaf area, and levels of reducing sugars, amino acids and soluble proteins) have been demonstrated by many researchers in many plants **(Ries, 1991)**. Some authors have witnessed the role of TRIA in micro-propagation of ornamental and other plants **(Reddy *et al*., 2002 and Tantos *et al*., 2001)**.

The objective of this work was to determine the effect of culture media components (¼ , ½ , ¾ and full strength of both Murashige & Skoog (MS) and Schenk & Hildebrandt (SH) medium**)** on rooting of date palm cv. Sewi *in vitro*. An additional objective of this study was to investigate the effect of some plant growth regulators **(**Indole butyric acid (IBA), Triacontanol (TRIA) and combination of TRIA & Gibberellic acid (GA_3) on rooting of "Sewi" date palm.

MATERIAL AND METHODS

This study was conducted at the Central Laboratory for Date Palm Researches and Development, Agriculture Research Center, Giza, Egypt during the period from 2014 to 2017.

I- **Plant material:**

The study was started with the selection of healthy - young - offshoots from mother date palm trees of semi dry cultivar Sewi, was obtained from a farm in Badrashin, Giza, Egypt. The young offshoots were of 3 - 4 years, ranging in weight from 5 - 7 kg and about 60 - 80 cm in length. The selected young offshoots were careful transferred to the laboratory after separation from mother tree and then prepared by removing the adventitious roots,

fibrous sheath and leaves by knife to appearing white soft leaves nearer the apical meristem had appeared. The apical meristem plus few leaves primordial was used as explant material. Explants were soaked under tap water for 1-2 hrs. explants sterilized with anti-oxidant solution (ascorbic and citric acid at 100 and 150 mg l^{-1}, respectively) for one hr. to avoid culture browning. Then explants were surface sterilized under aseptic conditions by use of ethyl alcohol (70%) for 30 sec followed by immersion in (0.5gm l^{-1}) mercuric chloride (HgCI2) for ten min and then rinsed two-times with sterile distilled water and transferred to double surface sterilization by commercial Clorox (5.25%) sodium hypochlorite (NaOCI) supplemented with two drops of Tween-20 per 100 ml solution, the first one by 40% Clorox for 15 min and thoroughly washed with sterilized distilled water for one time and the second one by 60% Clorox for 25 min and then washed with sterilized distilled water for three times. Under aseptic conditions, outer soft leaves were removed to obtain a shoot-tip. Shoot-tip 5 – 10 mm in length, composed of the apical meristem and (4-6) leaf primordial, cut longitudinally into four sections and inoculated individually onto sterilized **Murashige** and **Skoog** (1962) (MS) basal nutrient medium supplemented with 10 mg l^{-1} dichloro-phenoxy acetic acid (2,4-D), 3 mg l^{-1} N6-(2-iso-pentenyl adenine) (2-iP), 40 mg l^{-1} adenine sulfate, 200 mg l^{-1} KH2P04.2H20, 170 mg l^{-1} NaH2P04.2H20, 200 mg l^{-1} glutamine, 100 mg l^{-1} myo-inositol, 1.5 g l^{-1} activated charcoal, 40g l^{-1} sucrose and 6 g l^{-1} bacteriological agar **(Tisserat, 1982).** The medium pH was adjusted to 5.8. and dispensed on small jars 200 ml at rate of 30 ml per jar and autoclaved under 1.2 kg/cm2 pressure at 121°C for 20 minutes. Cultured jars were incubated at 25±2°C in total darkness and re-culture until formation of the embryogenic callus. After that cultures were transferred to fresh medium supplemented with 0.1 mg l^{-1} NAA for two months to obtain the somatic embryos. After that the somatic embryos were cultured on MS medium supplemented with 0.1 mg l^{-1} NAA and 0.05 mg l^{-1} BA for three months (one month interval) to obtain the shoots **(Ibrahim, 1999)**. After that the resulted shoots were transferred to MS medium free plant growth regulators for one month, and then the elongated shoots were harvested and used as explants in rooting experiments.

First experiment: Effect of different concentration of MS and SH media on rooting of "Sewi" date palm:

Healthy formed shoots of "Sewi" date palm (about 5-6 cm long) were cultured in aseptic conditions on four strengths of both MS and SH medium salts (¼ , ½ , ¾ and full strength), supplemented with 1 mg l^{-1} NAA, 30 g l^{-1} sucrose, 2mg l^{-1} glycine, 4 g l^{-1} bacteriological agar and 0.25g l^{-1} AC. Each treatment comprised three replicates. Each replicate contained four mason glass jars of 750 ml capacity with perforated covers, each jar contained 60 ml medium and one shoot. Jars were kept in the incubation room under 3000 Lux light intensity for 16 hr. light / day and 25 ± 2 ℃. Data were recorded as root number and root length after eight weeks.

Second experiment: Effect of different concentration of IBA on rooting of "Sewi" date palm:

In this experiment, the shoots were cultured on the best medium from the previous experiment supplemented with 30 g l^{-1} sucrose, 2mg l^{-1} glycine, 4 g l^{-1} bacteriological agar, 0.25g l^{-1} AC and four concentrations (treatments) of 4-(3-Indolyl) butyric acid (IBA, $C_{12}H_{13}NO_2$) (0, 0.5, 1.0, 2.0mg l^{-1}). The cultures were kept in the incubation room at the same conditions in previous experiment. Data were recorded as root number and root length after eight weeks.

Third experiment: Effect of different concentration of Triacontanol on rooting of "Sewi" date palm:

In this experiment, the shoots were cultured on the best medium from the first experiment (full strength of SH medium) supplemented with 30 g l^{-1} sucrose, 2mg l^{-1} glycine, 4 g l^{-1} bacteriological agar, 0.25g l^{-1} AC and four concentrations 0, 5, 10, 20 µg l^{-1} of 1-Triacontanol $CH_3(CH_2)_{28}CH_2OH$) TRIA. The cultures were kept in the incubation room at the same conditions in previous experiment. Data were recorded as root number and root length after eight weeks.

Fourth experiment: Effect of combination treatments of Triacontanol and GA_3 on rooting of "Sewi" date palm:

In this experiment, the best treatment of triacontanol in the previous experiment were used in combination with three concentrations of Gibberellin A_3 ($C_{19}H_{22}O_6$) at 0, 0.5, and 1 mg l^{-1} GA_3 and control which

free TRIA and GA_3 at the same conditions in previous experiment. Efficiency of rooting, number of roots per plant, root length and shoot length were measured after eight weeks from the onset incubation.

Fifth experiment: Effect of combination treatments of Triacontanol and GA_3 on acclimatization of "Sewi" date palm:

The rooted plantlets were produced from all treatments in experiment four were transferred to greenhouse, and planted in plastic pots filled with peat moss + vermiculite + perlite 2:l:l (v/v/v) and covered with transparent plastic bags for 2 weeks in the greenhouse with temperature at 28/20ºC (day/night), light intensity 4000-5000 Lux almost, 12 hours daily photoperiod and relative humidity of 85%, then the plastic bags were punched up 2 cm from two sides after one week and then another punch for one week and then the plastic bags were removed and irrigated, and then they were put under the plastic tunnels. The survival percentage was recorded after every month from transplanting for three months. Each treatment comprised 3 replicates, with 4 pots in each replicate.

Statistical analyses

All experiments were arranged in a Randomized Complete Block Design. Each treatment comprised three replicates, each replicate contained four mason glass jars of 750 ml capacity with perforated covers, each jar contained 60 ml medium and one shoot (about 5-6 cm long). The experiments were repeated three times. The mean values were compared using LSD method at 5% level according to **Snedecor and Cochran, (1980)**

RESULTS AND DISCUSSION

First experiment: Effect of different concentration of MS and SH media on rooting of "Sewi" date palm:

Data presented in Table (1) and figures (1&2) revealed that the root number and length of date palm cv. Sewi after eight weeks from the onset of culture on rooting medium were significantly affected by MS and SH medium salts strength.

SHAMS EL-DIN I. M., International Journal of Advances in Agricultural Science and Technology,
Vol.5 Issue.1, January- 2018, pg.9-30 ISSN: 2348-1358
Impact Factor: 6.057
NAAS Rating: 3.77

Table (1): Effect of different concentration of MS and SH media on rooting of "Sewi" date palm

Treatment		Root number	Root length (cm)
No. of media	Type and strength of media		
M1	1/4 MS	3	8
M2	1/2 MS	2.5	4
M3	3/4 MS	2.4	2
M4	Full MS	1.7	0.9
M5	1/4 SH	2	2
M6	1/2 SH	3	3
M7	3/4 SH	3.5	5
M8	Full SH	4.5	6
L S D at 0.05		**0.2**	**0.3**

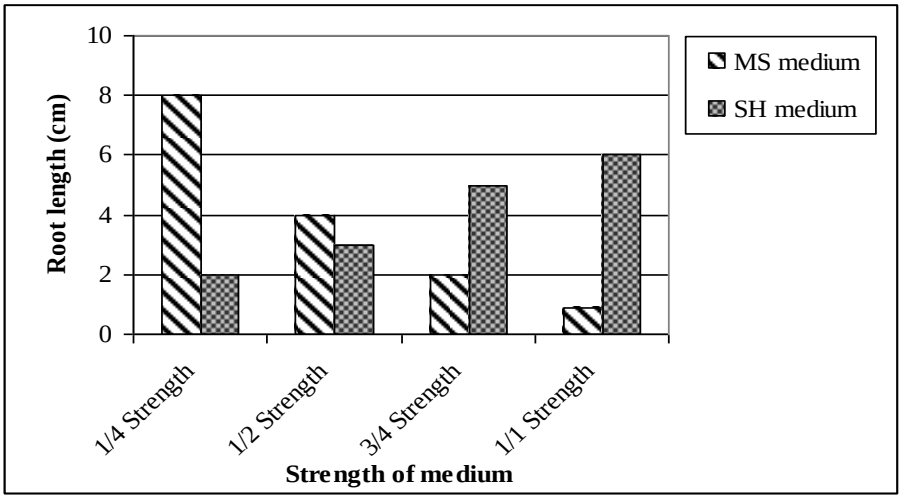

Fig. (2): Effect of different concentration of MS and SH media on root length (cm) of "Sewi" date palm

Referring to MS medium, the highest number and length of root (3 roots/plantlet and 8 cm) was obtained with MS medium salts strength at quarter (M1), but the lowest number and length of root (1.7 roots/plantlet and 0.9 cm) was obtained with MS medium at full strength (M4). In case of SH medium, it have been noticed the opposite whereas, the highest number and length of root (4.5 roots/plantlet and 6 cm) was obtained with SH medium at full strength (M8), but the lowest number and length of root (2 roots/plantlet and 2 cm) was obtained with SH medium salts at quarter (M5). Also, it is clear from data presented in Table (1) that SH medium superior than MS medium in root number as 4.5 roots/plantlet by M8 and 3 roots/plantlet by M1, but MS medium superior than SH medium in root length as 8 cm by M8 and 6 cm by M1.

These data are in accordance with **Karhu (1997)** who mentioned that reduced mineral nutrient concentration of modified MS medium allowed more root elongation. **Abul-Soad *et al*. (2007)** reported that ¾ MS medium salts strength was better than the full strength of MS in the rooting medium of date palm (*Phoenix dactylifera* L.) cv. Zaghloul.

Also, **Bidarigh and Azarpour (2013)** were found that the highest root length and root number in micro cuttings of tea were obtained with application redundancy MS medium and 1mg/L IBA. Whereas the lowest root length and root number were obtained with full MS medium and 1mg/L IBA.

Whereas **Shah *et al.* (2013)** reported that SH medium produced significantly high percent of *in vitro* rooting of *Aristolochia indica* L. , a medicinal woody perennial climber plant of immense pharmaceutical value, in comparison to MS, B5, NN and WPM medium which produced minimum effect on rooting. Also SH medium produced maximum root number per explant in comparison with WPM, MS and NN medium.

 ISSN: 2348-1358
Impact Factor: 6.057
NAAS Rating: 3.77

Second experiment: Effect of different concentration of IBA on rooting of "Sewi" date palm:

Table (2): Effect of different concentration of IBA on rooting of "Sewi" date palm

Treatment		Root number	Root length (cm)
No. of Treatment	**Concentration of IBA mg l^{-1}**		
1	0.0	1	1.3
2	0.5	4	6
3	1.0	6	8
4	2.0	5.3	5.9
L S D at 0.05		**0.2**	**0.3**

Data in table (2) and figures (3&4) clearly show that IBA concentrations had a significant effect on root number and length of date palm cv. Sewi.

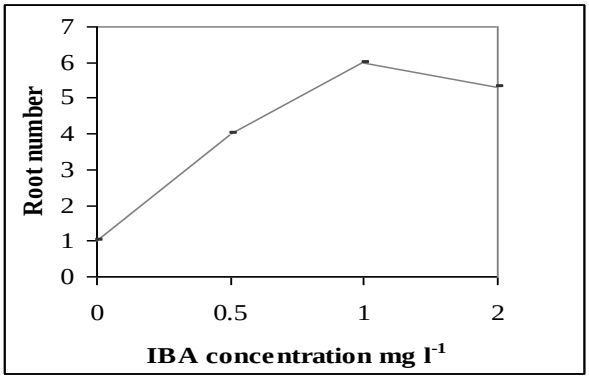

Fig. (3): Effect of different concentration of IBA on root number of "Sewi" date palm

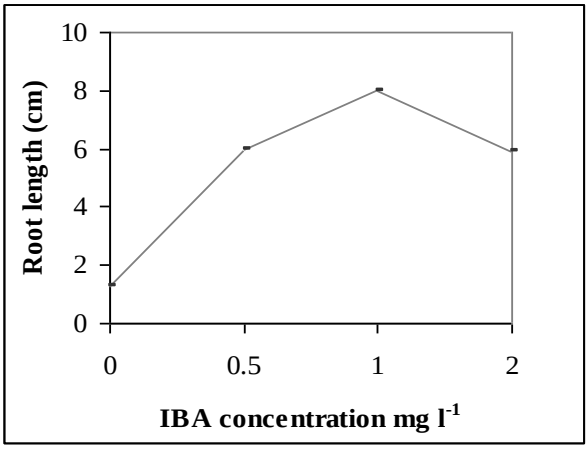

Fig. (4): Effect of different concentration of IBA on root length (cm) of "Sewi" date palm

The root number significantly increased from 1 to 4 and further to 6 after two months as IBA concentration increased from 0.0 to 0.5 and 1.0 mg l^{-1} respectively. At the highest IBA concentration i.e. 2mg l^{-1} the root numbers were decreased to 5.3 roots/plantlet. In respect of the root length, it is clear that there are significant differences between all treatments. The root length increased from 1.3 cm to 6 cm and further to 8 cm as IBA concentration increased from 0.0 to 0.5 and 1.0 mg l^{-1} respectively. However, at the highest IBA concentration (2mg l^{-1}) the root length was significantly decreased to 5.3 cm.

Many workers were reported favoring the addition of IBA at 1.0 mg l^{-1} in the culture media to induce rooting. Examples include **Ibrahim *et al.* (2017)** reported that root formation of date palm "Medjool and Khalas" shoots cultured in MS medium supplemented with 1mg l^{-1} IBA *in vitro*. **Kim *et al.* (1998)** they mentioned that the most effective treatment for root

number root length and shoot height of green ash (*Fraxinus pennsylvanica*), was 1.0 mg l^{-1}IBA. **Niccol *et al.* (1994)** recorded that the use of indole-3-butyric acid (IBA) was successful in initiating roots from *Eucalyptus microcorys* at an optimum concentration (1.0 mg l^{-1}IBA).

Third experiment: Effect of different concentration of Triacontanol on rooting of "Sewi" date palm:

Table (3): Effect of different concentration of Triacontanol on rooting of "Sewi" date palm

Treatment		Root number	Root length (cm)
No. of Treatment	Concentration of Triacontanol µg l^{-1}		
1	0.0	1	1.3
2	5	20	5
3	10	10	4
4	20	7	3
L S D at 0.05		**2**	**0.5**

Data in Table (3) and figures (5&6) show that a significant increase of both root number and root length was obtained with increasing triaconanol concentration from 0.0 to 5 μg l^{-1} where 20 roots with 5 cm in length.

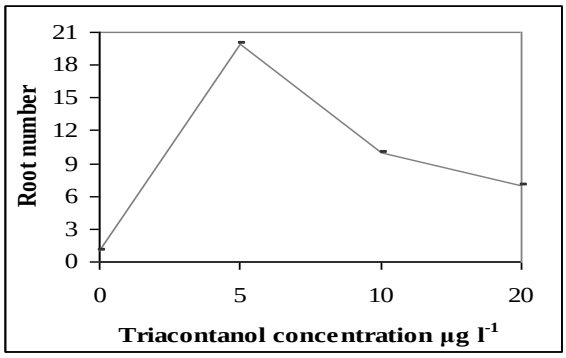

Fig. (5): Effect of different concentration of Triacontanol on root number of "Sewi" date palm

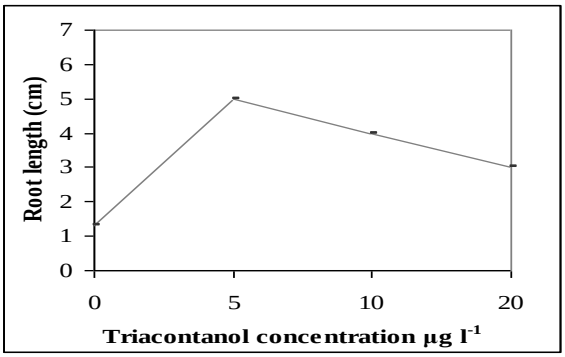

Fig. (6): Effect of different concentration of Triacontanol on root length (cm) of "Sewi" date palm

In contrast, increasing Triaconanol to 10 and 20 μg l^{-1} had a negative effect. These results are in accordance with the findings of **Lebedev and Schestibratov (2013)** they reported that application of growth stimulators such as triacontanol can be especially effective on plantlets *in vitro* of tree species. These chemicals act on the most plant physiological and biochemical processes at very low concentrations. Their use can be particularly effective during rooting and acclimatization, which are the most critical stages of clonal micro-propagation.

Also Tantos *et al.* (2001) Mentioned that the application of triacontanol in the rooting stage of two woody, economically important fruit plant species (apple (*Malus domestica* cv. JTE-E4) and sour cherry (*Cerasus fruticosa* cv. Probocskai) rootstocks) caused a significant increase in the number of roots per plant.

Reddy *et al.* (2002) mentioned that TRIA can be used as an effective growth regulator in the micro-propagation of *Capsicum frutescens* and *Decalepis hamiltonii,* and they reported that TRIA enhanced shoot growth and chlorophyll content of leaves and also influenced root induction and supported growth of the roots.

Mészáros (2006) reported that significant increase root number of Gerbera was evident at the lowest TRIA concentration. Also they reported that the number of roots of Asparagus was significantly enhanced by 2µg/l triacontanol too, and photosynthetic system of the plants was positively influenced by the triacontanol treatments. The earlier researchers suggested that TRIA directly activates the genes that control photosynthesis. These genes in turn activate the enzymes controlling the chemistry of photosynthesis.

Fourth experiment: Effect of combination treatments of Triacontanol and GA_3 on rooting of "Sewi" date palm:

Table (4): Effect of combination treatments of Triacontanol and GA3 on rooting of "Sewi" date palm

Treatment			Root number	Root length (cm)	Shoot height (cm)
No. of Treatment	Concentration mg l^{-1} Triacontanol µg l^{-1} + GA_3 mg l^{-1}				
T0	0.0	0.0	1	1.3	9
T1	5	0.0	20	5	13

T2	5	0.5	23	6	15
T3	5	1.0	27	6.4	17.3
L S D at 0.05			**2.8**	**0.3**	**0.5**

It is clear from data in Table (4) and Fig. (7-9) that there are significant gradual increases of root number, length and shoot height were obtained with increasing GA_3 concentration in the presence of TRIA.

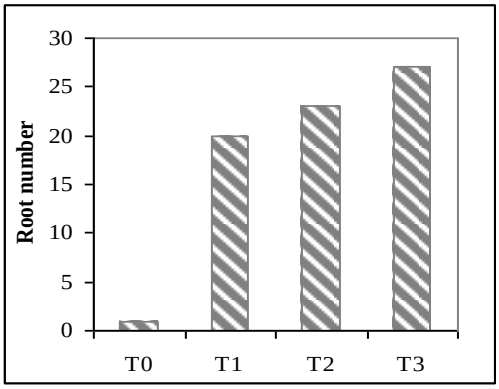

Fig. (7): Effect of combination treatments of Triacontanol and GA_3 on roots number of Date Palm cv. Sewi after two months from culture. (T0= 0 µg l^{-1}Triacontanol+0 mg l^{-1} GA_3, T1= 5.0 µg l^{-1} Triacontanol + 0.0 mg l^{-1} GA_3, T2= 5.0 µg l^{-1} Triacontanol + 0.5 mg l^{-1} GA_3 and T3= 5.0 µg l^{-1} Triacontanol + 1.0 mg l^{-1} GA_3)

ISSN: 2348-1358
Impact Factor: 6.057
NAAS Rating: 3.77

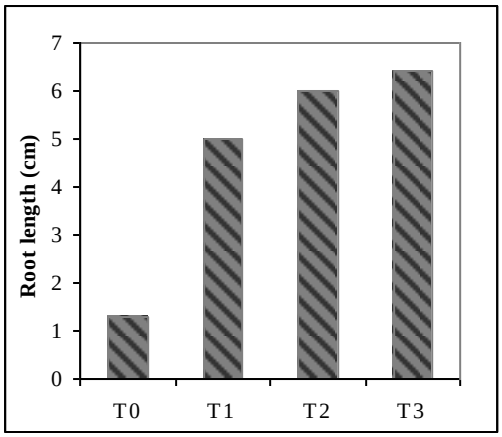

Fig. (8): Effect of combination treatments of Triacontanol and GA_3 on root length (cm) of Date Palm cv. Sewi after two months from culture. (T0= 0 µg l^{-1}Triacontanol+0 mg l^{-1} GA_3, T1= 5.0 µg l^{-1} Triacontanol + 0.0 mg l^{-1} GA_3, T2= 5.0 µg l^{-1} Triacontanol + 0.5 mg l^{-1} GA_3 and T3= 5.0 µg l^{-1} Triacontanol + 1.0 mg l^{-1} GA_3)

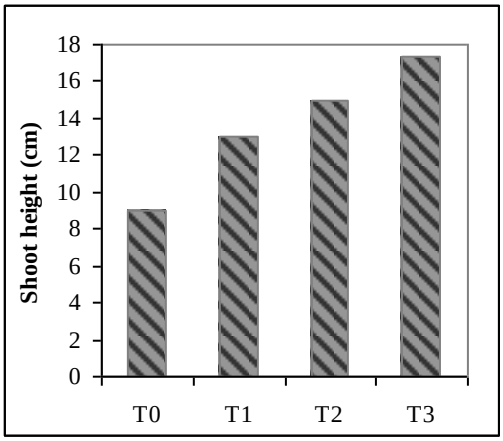

Fig. (9): Effect of combination treatments of Triacontanol and GA_3 on shoot height (cm) of Date Palm cv. Sewi after two months from culture. (T0 = 0 µg l^{-1}Triacontanol + 0 mg l^{-1} GA_3, T1= 5.0 µg l^{-1} Triacontanol + 0.0 mg l^{-1} GA_3, T2= 5.0 µg l^{-1} Triacontanol + 0.5 mg l^{-1} GA_3 and T3= 5.0 µg l^{-1} Triacontanol + 1.0 mg l^{-1} GA_3)

These results are in accordance with the findings of some researchers. **Idrees *et al.* (2010)** mentioned that the effects of triacontanol alone and its combined application with GA_3 on growth of *Coriandrum sativun* L. was found to be significant for increasing in shoot and root lengths. GA_3 treatment promotes cell enlargement and cell division (**Buchanan *et al.* 2000**), while TRIA rapidly elicits a secondary messenger which moves rapidly throughout the plant resulting in stimulation of growth (dry weight increase) and water uptake (**Ries and Wert 1988**). **Moore 1989 and Khan *et al.* 2006** reported that an increase in growth parameters like shoot and root lengths, fresh and dry weights in plants treated with combined application of 10^{-6} TRAI + 10^{-6} GA_3 is in accordance with the well known fact that exogenous application of plant growth regulators evokes the intrinsic genetic potential of the plant causing increase in elongation of internodes as a consequence of cell division and cell wall extensibility.

Fifth experiment: Effect of combination treatments of Triacontanol and GA_3 on survival percentage of "Swei" date palm after three months:

Treatment			Survival percentage		
No. of Treatment	**Concentration mg l^{-1} Triacontanol µg l^{-1} + GA_3 mg l^{-1}**		**after one month**	**after two months**	**after three months**
1	0.0	0.0	70	60	50
2	5	0.0	90	80	80
3	5	0.5	90	80	80
4	5	1.0	100	90	90
L S D at 0.05			**1.5**	**2**	**2.1**

Table (5): Effect of combination treatments of Triacontanol and GA3 on survival percentage of "Swei" date palm after three months *

* Data have been transformed according to Snedecor and Cochran (1980)

Data presented in Table (5) revealed that the survival percentage of "Swei"date palm through adaptation stage was affected significantly pay combination treatments of Triacontanol and GA3. The highest record of survival percentage was 90% at 5 µg l^{-1} TRAI+ 1 mg l^{-1} GA_3 compared with (50%) at control after three months. These results are in accordance with the findings of **Lebedev and Schestibratov (2013)** they reported that triacontanol can be especially effective on plantlets of tree species particularly during rooting and acclimatization. **Idrees *et al.* (2010)** mentioned that the effects of triacontanol alone and its combined application with GA_3 on growth of *Coriandrum sativun* L. was found to be significant.

Conclusion

The present work represented an efficient protocol for *in vitro* rooting using SH medium supplemented with Triacontanol in combination with GA_3 of the most economically importance date palm cultivar, Sewi.

The best treatment in rooting stage, cultures were grown on full strength of SH medium contained 5.0 µg l^{-1} Triacontanol in combination with GA_3 at 1.0 mg l^{-1} for two months.

Adaptation stage after three months

Plate (1): "Sewi"date palm in rooting and adaptation stage

REFERENCES

[1]. **Abul-Soad, A. A., N. R. El-Sherbeny and S. I. Baker (2007).** Effect of basal salts and sucrose concentrations on morphogenesis in test tubes of female inflorescence of date palm (*Phoenix dactylifera* L.) cv. Zaghloul. Egy. J. Agri. Res. 85(1B): 385- 394.

[2]. **AlKhateeb, A. A. and S. M. Alturki (2014).** A comparison of liquid and semi-solid cultures on shoot multiplication and rooting of three date palm cultivars (*Phoenix dactylifera* L.) *in vitro*. Adv. Environ. Biol., 8(16), 263-269.

[3]. **Arteca, R. N. (1996).** Plant Growth Substances: Principles and Applications, Chapman and Hall Ink, New York, pp 61-64.

[4]. **Arthur, G. D.; W. A. Stirk and S. J. Van (2006).** Effects of autoclaving and charcoal on root-promoting substances present in water extracts made from gelling agents. Bioresour. Technol., 97: 1942-1950.

[5]. **Atta-Alla, H.; Staden, J-Van and Van–Staden, J. (1997).** Micropropagation and establishment of *Yucca aloifolia*. Plant Cell, Tissue and Organ Culture, 48(3):209-212.

[6]. **Bekheet, S. A. and H. S. Saker (1998).** *In vitro* propagation of Egyptian date palm: Direct and indirect shoot proliferation from shoot tip explants of *Phoenix dactylifera* L.cv. Zaghloul. The First International Conference on Date Palm, In Egypt, 150-155.

[7]. **Benková, E. and A. Bielach (2010).** Lateral root organogenesis-from cell to organ. Curr. Opin. Plant Biol., 13: 677-83.

[8]. **Bidarigh, S. and E. Azarpour (2013).** Evaluation of the effect of ms medium levels on rooting in micro cuttings of Tea (*Camellia sinensis* L.) under *in vitro* culture condition. 8(1): 24-28.

[9]. **Buchanan, B. B.; Gruissem W.; Jones R. L. (2000).** Biochemistry and molecular Biology of plants, American Society of Plant Physiologists, Rockville, Maryland, pp 852-854.

[10]. **Chibnall, A. C.; Williams; A. L. Latner and A. H. Pipes (1933)** The isolation of n-triacontanol from lucerne wax. Biochem J. 27: 1885-1888.

[11]. **Das, P.; S. Samantaray and G. R. Rout (1996).** Organogenesis and *in vitro* flowering of *Echinochloa colona*. Effect of growth regulators and explant types. Biologia Plantarum. 38(3):335-342.

[12]. **Davies, P. J. (1995).** Plant Hormones. Dordrecht, The Netherlands: Kluwer Academic Publishers, p. 833.

[13]. **GadAlla, E. G. (2003).** Propagation of dry varieties of date palm. PhD thesis, Faculty of Agriculture, Cairo University, Egypt.

[14]. **Houtz, R. L.; S. K. Ries and N. E. Tolbert (1985).** Effect of triacontanol on Chlamydomonas. I- Stimulation of growth and photosynthetic CO2 assimilation. Plant Physiology 79, 357-364.

[15]. **Ibrahim I. A.; A. M. Badr-Elden; H. A. Emara; M. F. Gabr; A. A. Nower and T. M. Abd Elaziem (2017).** Improving In Vitro Somatic Embryos Production of Medjool and Khalas Date Palm Cultivars via Modification of Ammonium and Potassium Nitrate. Global Journal of Botanical Science, 5:16-24.

[16]. **Ibrahim, A. I. (1999).** Somaclonal variation during micropropagation of date palm via embryogensis. The First International Conference, in Egypt, on Plant Tissue Culture and its Application, pp. 189-199.

[17]. **Idrees, M.; M. Mastoor; A. Khan; T. Aftab and M. Naeem (2010).** Synergistic effects of Gibberellic acid and Triacontanol on growth, physiology, enzyme activities and essential oil content of Coriandrum sativum L. The Asian and Australasian Journal of Plant Science and Biotechnology, 4(1): 24-29.

[18]. **Ismail, R. M., H. E. Elazab; G. M. Hussein and E. A. Metry (2011).** In vitro root induction of faba bean (Vicia faba L.). GM Crops. 2: 176-181.

[19]. **Karhu, S. T. (1997).** Rooting of Blue honeysuckle microshoots. Plant Cell, Tissue and Organ Culture, 48(3):153-159.

[20]. **Khan, M. M. A.; C. Gautam; F. Mohammad; M. H. Siddiqui; M. Naeem and M. N. Khan (2006).** Effect of gibberellic acid spray on performance of tomato. Turkish Journal of Biology 30, 11-16.

[21]. **Kim M. S.; N. B. Klopfenstein; B. M. Cregg and M. S. Kim (1998).** *In vitro* and *ex vitro* rooting of micropropagated shoots using

three green ash (*Fraxinus pennsylvanica*) clones. New Forests.6(1):43-57.

[22]. **Lebedev, V. and K. Schestibratov (2013).** Effect of natural and synthetic growth stimulators on *in vitro* rooting and acclimatization of common ash (*Fraxinus excelsior* L.) microplants. Natural Science, 5(10): 1095-1101.

[23]. **Liu, P. B. W. and B. Loy (1976).** Action of gibberellic acid on cell proliferation in the subapical shoot meristem of watermelon seedlings. American Journal of Botany 63, 700-704.

[24]. **Mészáros, A. (2006).** Application of methods suitable for improving the efficiency of in vitro propagation on horticultural plants. Department of Biological Science. Budapest University, Hungary.

[25]. **Moore, T. C. (1989).** *Biochemistry and Physiology of Plant Hormones*, Springer-Verlag Inc, New York, pp 113-121.

[26]. **Murashige, T. and F. Skoog (1962).** A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.

[27]. **Niccol, R. J.; P. A. Regan and L. F. Filippis (1994).** Simplified protocol for the micropropagation of selected *Eucalyptus* and *Banksia* species. Australian-Forestry. 57(4):143-147.

[28]. **Osterc, G. and F. Štampar (2011).** Differences in endo/exogenous auxin profile in cuttings of different physiological ages. J. Plant Physiol., 168: 2088-2092.

[29]. **Pereira, J. E. S.; V. J. Bianchi; L. F. Dutra and L. Fortes (1999).** *In vitro* strawberry *(Fragaria X Ananassa duchesne)* rooting in different MS medium concentrations. Ciencia-Rural., 29(1):17-20.

[30]. **Rasmia, S.S. Darwesh, Zeinab, E. Zaid and Rehab, A. Sidky (2011).** Effect of Ammonium Nitrate and GA_3 on Growth and Development of Date Palm Plantlets *in Vitro* and Acclimatization Stage. Research Journal of Agriculture and Biological Sciences, 7(1): 17-22

[31]. **Reddy, B. O.; P. Giridhar and G. A. Ravishankar (2002).** The effect of triacontanol on micropropagation of *Capsicum frutescens* and *Decalepis hamiltonii* W & A. Plant Cell, Tissue and Organ Culture, 71(3): 253-258.

[32].Ries, S. (1991). Triacontanol and its second messenger 9-β-L(+)-adenosine as plant growth substances. *Plant Physiology* 95, 986-989.

[33].Ries, S. and V. F. Wert (1988). Rapid elicitation of second messengers by nanomolar doses of triacontanol and octacosanol. Planta 173, 79-87.

[34].Schenk, R. U. and A. C. Hildebrandt (1972). Medium and Techniques for Induction and Growth of Monocotyledonous and Dicotyledonous Plant Cell Cultures. Can. J. Bot., 50: 199 – 204.

[35].Shah, S. N.; A. M. Husaini and F. Shirin (2013). Micropropagation of the Indian Birthwort *Arsitolochia indica* L. International Journal for Biotechnology and Molecular Biology Research, 4(6): 86-92.

[36].Snedocor, G. W. and W. C. Cochran (1980). Statistical Methods. 7th ed. Iowa State University, Press, Iowa, USA. pp.507.

[37].Tantos, Á.; A. Mészáros; T. Farkas; J. Szalai and G. Horváth (2001). Triacontanol supported micropropagation of woody plants. Plant Cell Reports, 20:16–21.

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

[39].Von Arnold, S.; I. Sabala; P. Bozhkov; J. Dyachok and L. Filonova (2002). Developmental pathways of somatic embryogenesis. Plant Cell Tissue Organ Culture, 69: 233- 249.

