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Effect of Different Concentrations of Calcium Silicate on the *in vitro* Rooting of Date Palm Shoots cv. Sewi

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Abstract: The current study focused on the effect of different concentrations of Calcium Silicate ($CaSiO_3$), Fe-EDTA and thiamine HCL added to culture medium at the appropriate subculture during rooting stage in order to get rapid and satisfied adventitious rooting and subsequently increase the survival percentage of the acclimatized plants in the greenhouse. This work describes the major advances of morphological and physiological differences in date palm plantlets Sewi cultivar with different concentrations of calcium silicate during the *in vitro* rooting stage. Four treatments of $CaSiO_3$ were tested through being added to MS medium at the concentrations 0, 2, 4, 8, and 16 mg/l with 0.1 mg/l NAA and 0.5 mg/l GA_3. The results showed that medium containing 2.0 mg/l and 4.0 mg/l $CaSiO_3$ promoted the plantlets development as well as the highest rooting. Supplementation of culture medium with 4.0 mg/l $CaSiO_3$, 0.1 mg/l NAA, 0.5 mg/l GA_3, 35 g/l sucrose, 0.15 g/l Fe-EDTA and 2.5 mg/l Thiamine HCL with ½ MS basal medium for 8 weeks of subculture significantly increased length of the plantlets and improvement of roots. There was an increase in levels of chlorophyll a, b and in addition to total protein content in the presence of calcium silicate.
Keywords: Calcium Silicate ($CaSiO_3$); rooting stage; Date palm; *in vitro*

1. Introduction

Date palm (*Phoenix dactylifera* L.) is a 'tree of life' which belongs to the family Arecaceae; it is one of the most economically important perennial plants in arid areas of the Middle-East and the North Africa. Date palm Sewi cv. is an important variety, with the increased food demand and many other commercial purposes. It is known that propagation of palm is very slow **(Chao and Krueger 2007).**

Tissue culture is the most technology method to provide large-scale propagation of healthy true-to-type date palm plants. **Al-Khateeb 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 **(Arnold *et al.*, 2002)**.

Rooting is an important *in vitro* stage of a micro propagation protocol of date palm and it 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 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 such as; light - temperature - CO2 – pH and physicochemical factors such as; water, macro and micro elements, sugar and some other organic substances such as; plant growth regulators, vitamins and amino acids. Composition of a culture medium has often been modified to stimulate the growth of particular plant material, the optimization of inorganic nutrients in the culture medium improves *in vitro* growth and morphogenesis of plant cells, tissue and organs.

Silicate (Si) is considered as a beneficial element for plants and according to **Epstein (1999)**, plants grown in silicate enriched environment differ from those grown in the absence of this element, especially in chemical composition, cell mechanical strength, leaf surface characteristics, tolerance to biotic and abiotic stresses and resistance to pests and diseases. It is the second most abundant element in the earth's crust and has been the subject of several studies that demonstrated its beneficial effects for agriculture contributing both to plant growth and crop yield **(Gunes *et al.*, 2007)**. The beneficial effects of Si on plants might be related to an increase in nutrient uptake as well as photosynthetic activity.

The inclusion of this element in the culture medium can also enhance the morphogenetic potential of plant cells, tissues and organs **(Sivanesan and Park, 2014).**

On the other hand, the addition of Gibberellic acid (GA_3) treatment promotes cell enlargement and cell division **(Buchanan *et al.*, 2000).** However, the stage of *in vitro* rooting is mainly affected by the chemical composition of the culture medium. Iron is one of the basic microelements used in micro propagation, since it is essential in many processes like chlorophyll and DNA synthesis **(Dunlap and Robacker, 1988)**. Furthermore, Fe is a part of peroxidases which mediates Indole Acetic Acid (IAA) catabolism and acts also a marker of the successive rooting phases **(Gaspar *et al*., 1992)**. Iron is always found in the nutrient medium in the chelated form Fe-EDTA (12%) which is suitable for micro propagation **(Dimassi-Theriou, 1989)**. The current study focused on the effect of different concentrations of $CaSiO_3$ and the effect of added Fe-EDTA with thiamine to the culture medium at the appropriate subculture during rooting stage in order to get rapid and satisfied adventitious rooting and subsequently increase the survival percentage of the acclimatized plants in the greenhouse.

2. Materials and Methods

This study was conducted in the Central Lab of Date Palm for Researches and Development - Agricultural Research Center, Egypt during the period from 2018 to 2019.

2.1 Culture medium

The culture medium used for *in vitro* cultures was the basal nutrient medium of MS **(Murashige and Skoog, 1962)** supplemented with (mg/l): 0.5 pyridoxine-HCL; 0.5 nicotinic acid; 0.1 thiamine-HCL; 2.0 glycine; 100.0 myo-inositol; 200.0 glutamine; 0.2 activated charcoal (AC); 35.0 g/l sucrose and solidified with 6.0 g/l agar. Adjust pH medium at 5.7 ± 0.1 by using KOH or HCl diluted solutions, the media was dispensed into big jars (300 ml) in aliquots of 50 ml per jar and capped

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with polypropylene closures. Subsequently the media were autoclaved for 20 minutes at 1.5 kg/cm^2 and 121 °C.

2.2 Explants and cultivation environment

The *in vitro* shoots of Sewi cv. used in the rooting experiment were collected from the proliferated clusters of shoots in the multiplication stage. Typical 10-12 cm long shoots were separated individually.

In the first experiment, the effects of medium texture on shoot elongation and rooting were evaluated. Shoots were cultured on ¾ MS basal medium composed of vitamins of MS, 0.1 mg/l NAA with 0.5 mg/l GA_3, 6.0 g/l agar; and supplemented with 4 different concentrations of $CaSiO_3$ at (2, 4, 8 and 16 mg/l). Medium without a silicate source was used as control. These shoots were re-cultured on fresh medium with different concentrations of $CaSiO_3$ for 6 weeks with 3 subcultures. All primary roots of the selected shoots were trimmed to the minimum length possible (0.5 cm) before starting the experiment. There were five treatments including the control; each treatment contained six replicates.

In the second experiment, the effects of added Fe-EDTA and Thiamine to culture medium were studied. Plantlets were cultured in test tubes on new medium supplemented with 4.0 mg/l $CaSiO_3$, 0.1 mg/l NAA, 0.5 mg/l GA_3, 20.0 g/l sucrose, 0.15 g/l Fe-EDTA and 2.5 mg/l Thiamine were added to ½ MS basal medium for 8 weeks. Six replicates were subjected to the experiment.

The cultures were incubated in a culture room at 27 + 1 °C under 3000 lux light intensity for 16 hours daily. After each subculture, the leaves length (cm), roots number and roots length (cm), adventitious roots number and length per plantlet were recorded after each subculture.

2.3 Estimation of total soluble proteins

Crude proteins were extracted according to the method described by **Lecouteux *et al.* (1993)**. The total proteins were measured by a spectrophotometer at 595 nm according to **Bradford (1976)**.

2.4 Estimation of chlorophyll content

The amount of chlorophyll was estimated by the method described by **(Abbas and Abbas, 1992)** using a spectrophotometer at 645 nm for chlorophyll a, 665 nm for chlorophyll b and then the total chlorophyll was calculated.

2.5 Plant acclimatization

There were several stages of acclimatization. Sterilize soil mixture (peat moss and perlite; 2:1 by volume), irrigate with water and fill plastic bags. Select well rooted plantlets with 18-22 cm in length, 2–3 leaves and thick base, the rooted shoots were taken out of the test tubes and the roots were washed with tap water to remove the agar residue and then soaked plantlets in a fungicide solution (Rizolex-T 50% 2.0 g/l) for 15 minutes then the plantlets were transferred to plastic bags containing a mixture soil. Immediately, cover the plantlets with plastic covers or polyethylene sheets in the greenhouse to maintain relative humidity at 90%, under 27± 2 °C, approximately, irrigate with ½ MS solution weekly or as needed. Moreover, reducing humidity can be done by making small cuts and bigger cuts in plastic covers through first couple of months and then removing it. After 24 weeks of *ex vitro* growth, transfer surviving plants and placed in pots with a 10 cm diameter which filled with the same previous planting medium and maintain in the shaded greenhouse **(Taha and Hassan, 2014).**

2.6 Statistical analysis

This experiment was designed as a randomized complete block design as described by **(Gomez and Gomez, 1984)**. The obtained data were statistically analyzed using MSTAT Computer Program **(MSTAT Development Team, 1989)**.

To verify differences among means of various treatments, means were compared using Duncan's Multiple Range Test as described by **(Duncan, 1955)**.

3. Results and Discussion

Successful transplanting of the *in vitro* date palm plantlets depends mainly on the adventitious roots. One of the main objectives of this study was to initiate ideally the adventitious roots of the young shoots to benefit the development of more resistant plantlets with better performance during acclimatization.

In the first experiment, the effects of medium different concentrations of $CaSiO_3$ on shoot elongation and rooting.

In the first subculture (after 6 weeks), as shown in **Table (1)** was resulted the following data, **in leaves length,** all $CaSiO_3$ concentrations had a significant effect on leaves length. The concentrations of $CaSiO_3$ at 4.0 and 2.0 mg/l yielded the longest leaves (16.00 and 14.67 cm). Resulted also showed that with increasing the concentration of $CaSiO_3$ to 8.0 and 16.0 mg/l caused decreased in length of leaves (13.0 and 12.83 cm, respectively) compared with the others treatments.

Roots number, there was a significant difference in the number of roots across the different silicate concentrations. The highest number of roots (5.00 roots/plantlet) was obtained with MS medium supplemented with $CaSiO_3$ at 4.0 mg/l. On the other hand, the lowest number of roots recorded in the control treatment and with the highest concentration of $CaSiO_3$ at 16.0 mg/l (1.00 and 1.33 roots/plantlet, respectively).

Roots length, there was a significant difference in root length among different concentrations of $CaSiO_3$. The longest roots length was (3.50 cm) in MS medium supplemented with 4.0 mg/l $CaSiO_3$ followed by treatments containing $CaSiO_3$ at 2.0 and 8.0 mg/l which gave roots length (2.17 and 2.00 cm, respectively). The lowest result (1.0 cm and 1.08 cm, respectively) recorded with the control treatment and the highest concentration of $CaSiO_3$ at 16.0 mg/l.

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Adventitious roots, the concentrations of $CaSiO_3$ applied to the MS culture medium affected date palm plantlets growth *in vitro*. There was a significant difference in number and length of adventitious roots of the date palm plantlets among different concentrations of $CaSiO_3$. As demonstrated in (**Fig. 1, C**) no initiation for any adventitious roots in the first subculture with the control treatment or while using $CaSiO_3$ at 2.0 mg/l, the concentration of $CaSiO_3$ at 4.0 mg/l leads to the appearance of adventitious roots (3.0 adventitious roots with length 1.13 cm). However, increase the concentrations of $CaSiO_3$ to 8.0 or 16.0 mg/l resulted in no adventitious roots.

Table (1). Response of date palm leaves, roots and adventitious roots with different $CaSiO_3$ concentrations after 6 weeks in culture (first subculture)

Calcium silicate (mg/l)	Leaves number	Leaves length	Roots number	Roots length	Adventitious roots number	Adventitious roots length
0.0	2.00 a	12.67 b	1.00 c	1.00 c	0.00 b	0.00 b
2.0	2.00 a	14.67 ab	3.33 b	2.17 b	0.00 b	0.00 b
4.0	2.00 a	16.00 a	5.00 a	3.50 a	3.00 a	1.13 a
8.0	2.00 a	13.00 b	2.67 b	2.00 b	0.00 b	0.00 b
16.0	2.00 a	12.83 b	1.33 c	1.08 c	0.00 b	0.00 b

Numbers carrying different letters a, b, c or d are significantly different.

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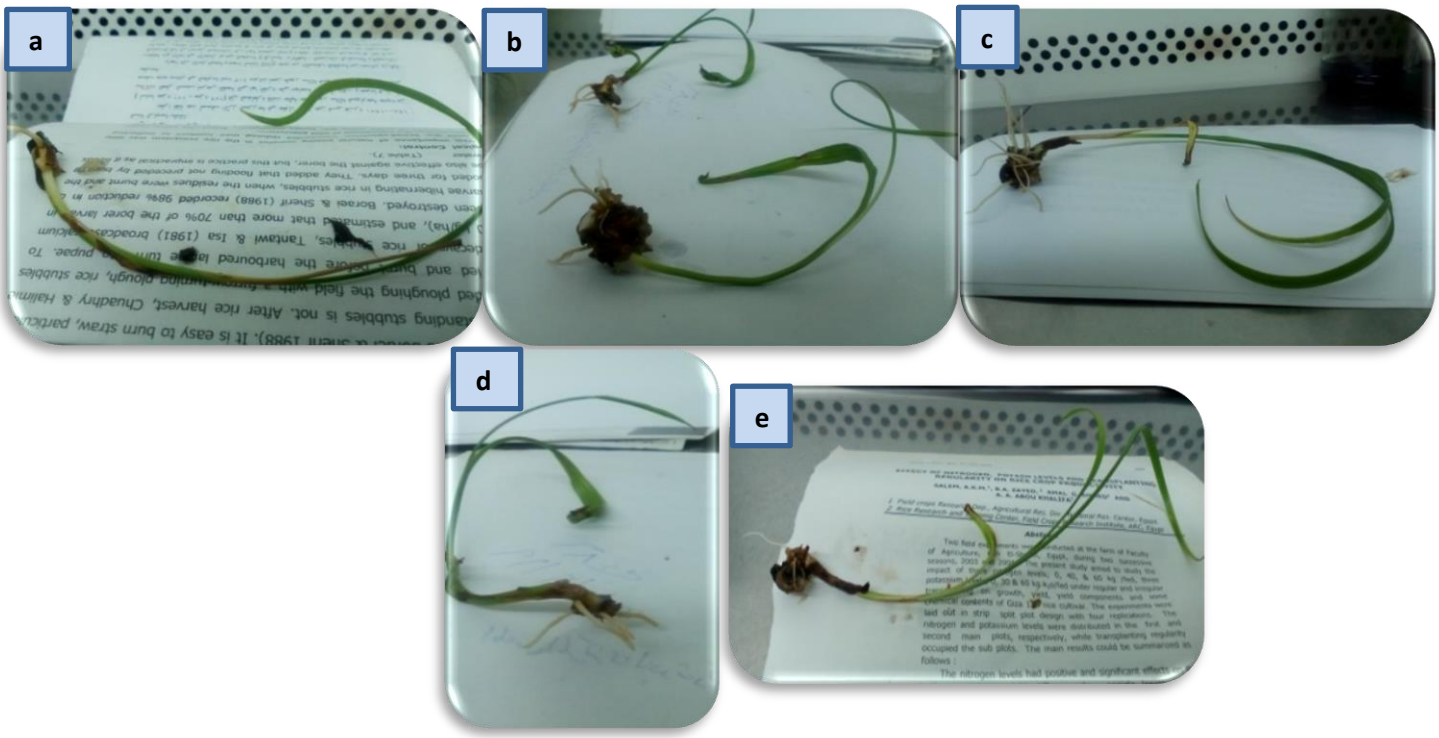

Fig. (1). Effect of silicate treatments (a) Control, (b) 2 mg/l, (c) 4 mg/l, (d) 8 mg/l and (e) 16 mg/l on shoot elongation and rooting of date palm cv. Sewi after the first subculture

In the second subculture (after 12 weeks), the data in **Table (2)** showed that in **leaves length,** the highest leaves length (22.00 cm) was resulted with treatment 4.0 mg/l $CaSiO_3$ followed by the treatment 2.0 mg/l $CaSiO_3$ which recorded leaves length (18.0 cm). While the lowest leaves length (15.00 and 16.67 cm, respectively) was found with the high concentration of $CaSiO_3$ at 16.0 mg/l and the control treatment, respectively.

Roots number, there was significant difference in the number of roots across the different silicate concentrations. The highest number of roots (8.33 roots/plantlet) was obtained with MS medium supplemented with $CaSiO_3$ at 4.0 mg/l. Increased the concentration of $CaSiO_3$ to 8.0 mg/l caused decreased the number of roots (4.67 roots/plantlet). On the other hand, the lowest roots number

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(3.33 and 3.00 roots/plantlet, respectively) was recorded with the control treatment and with $CaSiO_3$ treatment at 16.0 mg/l.

Roots length, there was significant difference in roots length for the different treatments of $CaSiO_3$, where the best roots length was found (8.0 cm) with the addition of 4.0 mg/l of $CaSiO_3$ in MS medium. The treatment with 2.0 mg/l $CaSiO_3$ resulted in a good roots length (5.0 cm) compared with the other treatments which recorded the worst root length.

Adventitious roots, there was significant interaction among the different concentrations of $CaSiO_3$ for number and length of adventitious roots of the date palm plantlets as shown in (**Fig. 2,C)** the highest number and length of adventitious roots (13.33 adventitious roots/plantlet and 1.92 cm) was obtained with MS medium supplemented with 4.0 mg/l $CaSiO_3$, followed by the treatment $CaSiO_3$ at 2.0 mg/l which recorded (9.67 adventitious roots/plantlet and 1.0 cm). The lowest number and length of adventitious roots (1.0 adventitious root/plantlet with 0.5 cm and 3.67 adventitious roots/plantlet with 0.83 cm, respectively) obtained with the control treatment and $CaSiO_3$ at 8.0 mg/l, respectively. However, by increasing the concentration of $CaSiO_3$ to 16.0 mg/l the growth of adventitious roots was inhibited.

Table (2). Effect of different concentrations of $CaSiO_3$ on leaves and roots development of the *in vitro* date palm plantlets after 12 weeks in culture (two subcultures).

Calcium silicate (mg/l)	Leaves number	Leaves length	Roots number	Roots length	Adventitious roots number	Adventitious roots length
0.0	2.33 a	16.17 c	3.33 d	1.33 d	1.00 d	0.50 c
2.0	2.33 a	18.00 b	5.67 b	5.00 b	9.67 b	1.00 b
4.0	2.67 a	22.00 a	8.33 a	8.00 a	13.33 a	1.92 a
8.0	2.00 a	16.67 c	4.67 bc	3.33 bc	3.67 c	0.83 bc
16.0	2.00 a	15.00 d	3.00 d	2.17 cd	0.00 d	0.00 d

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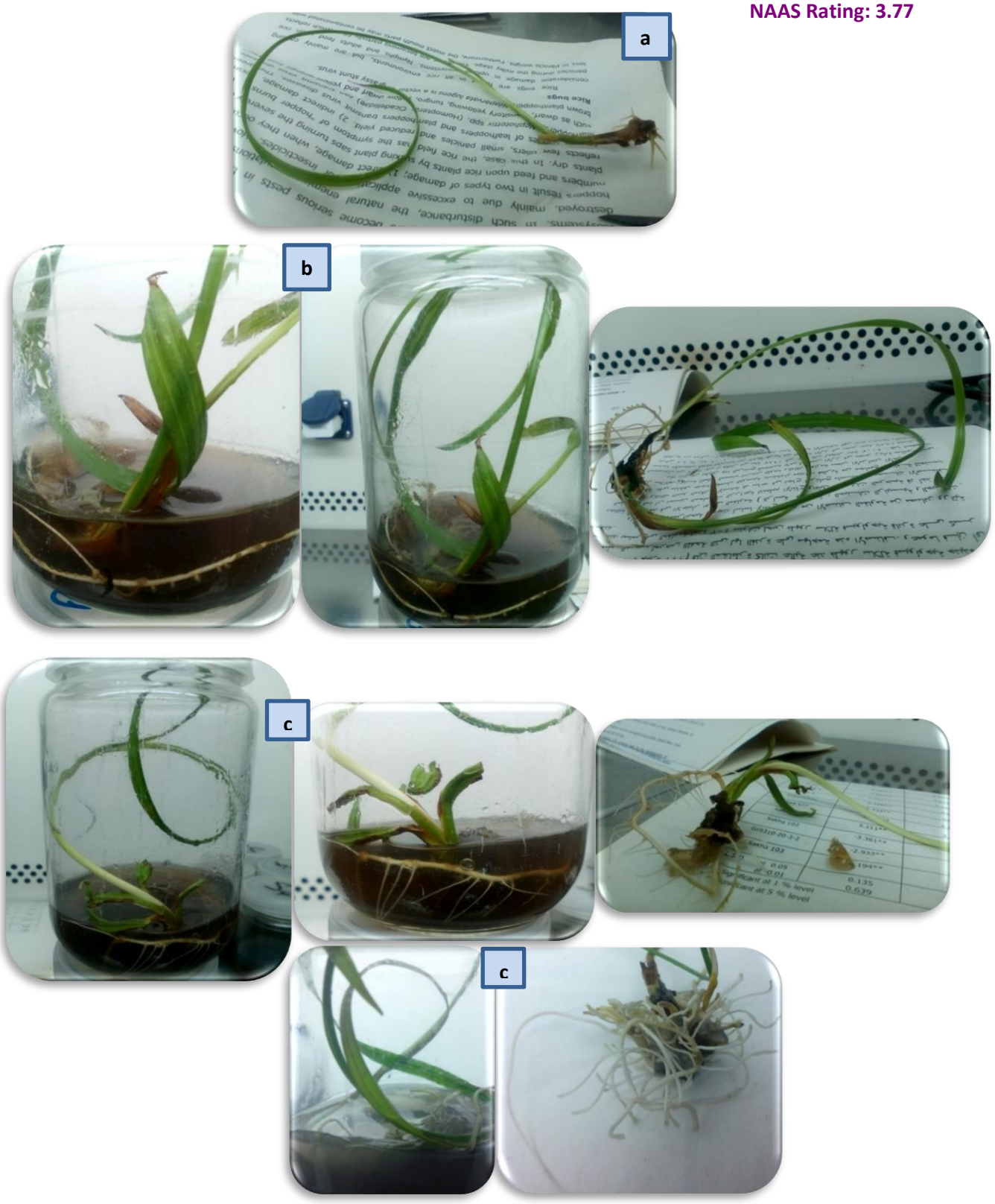

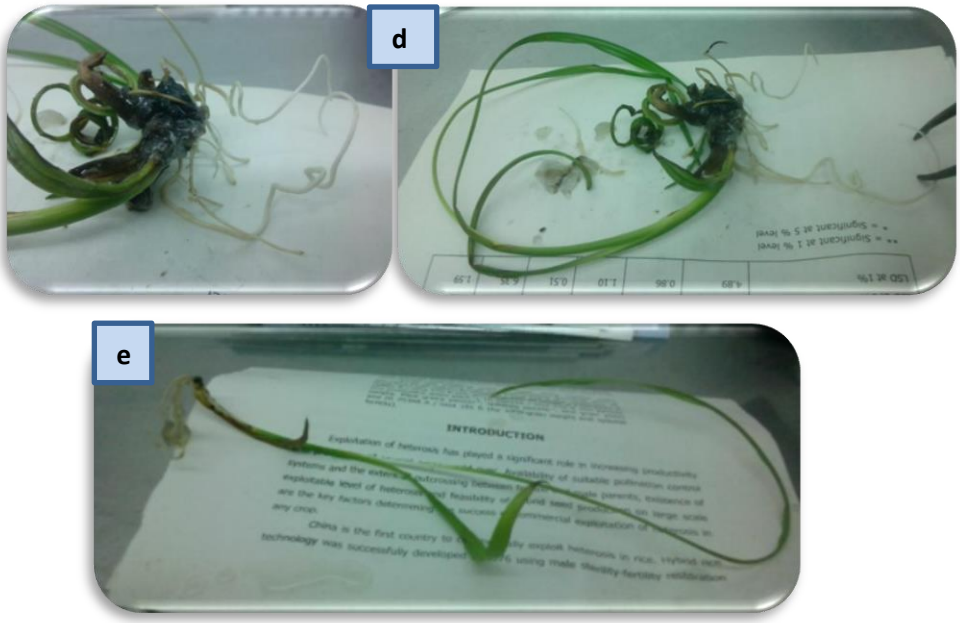

Fig. (2). Effect of different concentrations of $CaSiO_3$ (a) Control, (b) 2 mg/l, (c) 4 mg/l, (d) 8 mg/l and (e) 16 mg/l on *in vitro* rooting of date palm plantlets after 2 subcultures in Sewi cv.

In the third subculture (after 18 weeks), the data in **Table (3)** showed that in **leaves length,** the shoots of date palm were re-cultured on media containing different concentrations of $CaSiO_3$ for three subcultures. All $CaSiO_3$ concentrations had a significant effect on leaves length. $CaSiO_3$ at 2.0 and 4.0 mg/l yielded the longest leaves as (23.00 and 23.67 cm, respectively). The high concentrations of $CaSiO_3$ at 8.0 and 16.0 mg/l caused decreased in the leaves length (18.67 and 16.67 cm, respectively). Resulted also showed that the lowest leaves length was recorded with the control medium (18.42 cm).

Roots number, rooting is an important *in vitro* stage of a micro propagation protocol of date palm. As the high survival percentage of acclimatized plants is concerned, the success of entire *in vitro* cycle of date palm depends mainly on the adventitious roots quality before acclimatization. The roots number of date palm Sewi cv. after 3 months from the onset of culture of rooting medium were

significantly affected by different concentrations of $CaSiO_3$. There was significant difference in the number of roots across the different silicate concentrations. The highest number of roots (10.67 roots/plantlet) was obtained with MS medium supplemented with $CaSiO_3$ at 4.0 mg/l. The treatment 2.0 of $CaSiO_3$ also resulted a good number of roots (7.33 roots/plantlet) compared with the control treatment which recorded (5.67 roots/plantlet). The increase in the number of roots formed *in vitro* is accompanied with the increase in the area of root/substrate contact, reflecting higher absorption of nutrients. On the other hand, the lowest number of roots (3.67 roots/plantlet) was recorded in the treatment with 16.0 mg/l $CaSiO_3$.

Roots length, there was significant difference in roots length when using different concentrations of $CaSiO_3$. They indicated that the addition of $CaSiO_3$ leads to an increasing rooting, root length ranged from (2.50 to 13.33 cm) with different treatments of $CaSiO_3$. The concentration 4.0 mg/l of $CaSiO_3$ resulted in the best roots length (13.33 cm), which were significantly different from other treatments, followed by the roots length of (7.50 cm) with treatment at 2.0 mg/l. There was reduction to (7.0 and 2.5 cm) in roots length observed with increasing $CaSiO_3$ concentration to 8.0 and 16.0 mg/l, respectively. Also, the control treatment reported the worst result (2.58 cm). Thus, it is evident that the addition of $CaSiO_3$ in the medium MS is beneficial to promote both number and length of roots.

Adventitious roots, the concentrations of $CaSiO_3$ applied to the MS culture medium affected date palm plantlets growth *in vitro*. There was significant interaction among the different concentrations of $CaSiO_3$ for number and length of adventitious roots of the date palm plantlets during three subcultures. The best result was the treatment 4.0 mg/l $CaSiO_3$ (**Fig 3, C**) which recorded the highest number and length of adventitious roots (18.0 adventitious roots/plantlet and 3.0 cm) also using $CaSiO_3$ at 2.0 mg/l produce (14.0 adventitious roots/plantlet with 2.33 cm). However, by increasing the concentration to 8.0 mg/l resulted in the decrease in the number and length of adventitious roots (8.17 adventitious roots/plantlet with 1.0 cm), continuous increasing the concentration of $CaSiO_3$ to

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16.0 mg/l gave the worst result (3.0 adventitious roots/plantlet with 0.50 cm) compared with the control treatment which recorded (2.0 adventitious roots/plantlet with 1.67 cm).

Table (3). Plantlets growth and adventitious roots development of date palm Sewi cv. on different concentrations of $CaSiO_3$ after 18 weeks in culture (three subcultures).

Calcium silicate (mg/l)	Leaves number	Leaves length	Roots number	Roots length	Adventitious roots number	Adventitious roots length
0.0	2.67 ab	18.42 b	5.67 c	2.58 c	2.00 e	1.67 bc
2.0	2.67 ab	23.00 a	7.33 b	7.50 b	14.00 b	2.33 ab
4.0	3.33 a	23.67 a	10.67 a	13.33 a	18.00 a	3.00 a
8.0	2.00 b	18.67 b	6.33 bc	7.00 b	8.17 c	1.00 cd
16.0	2.00 b	16.67 c	3.67 d	2.50 c	3.00 d	0.50 d

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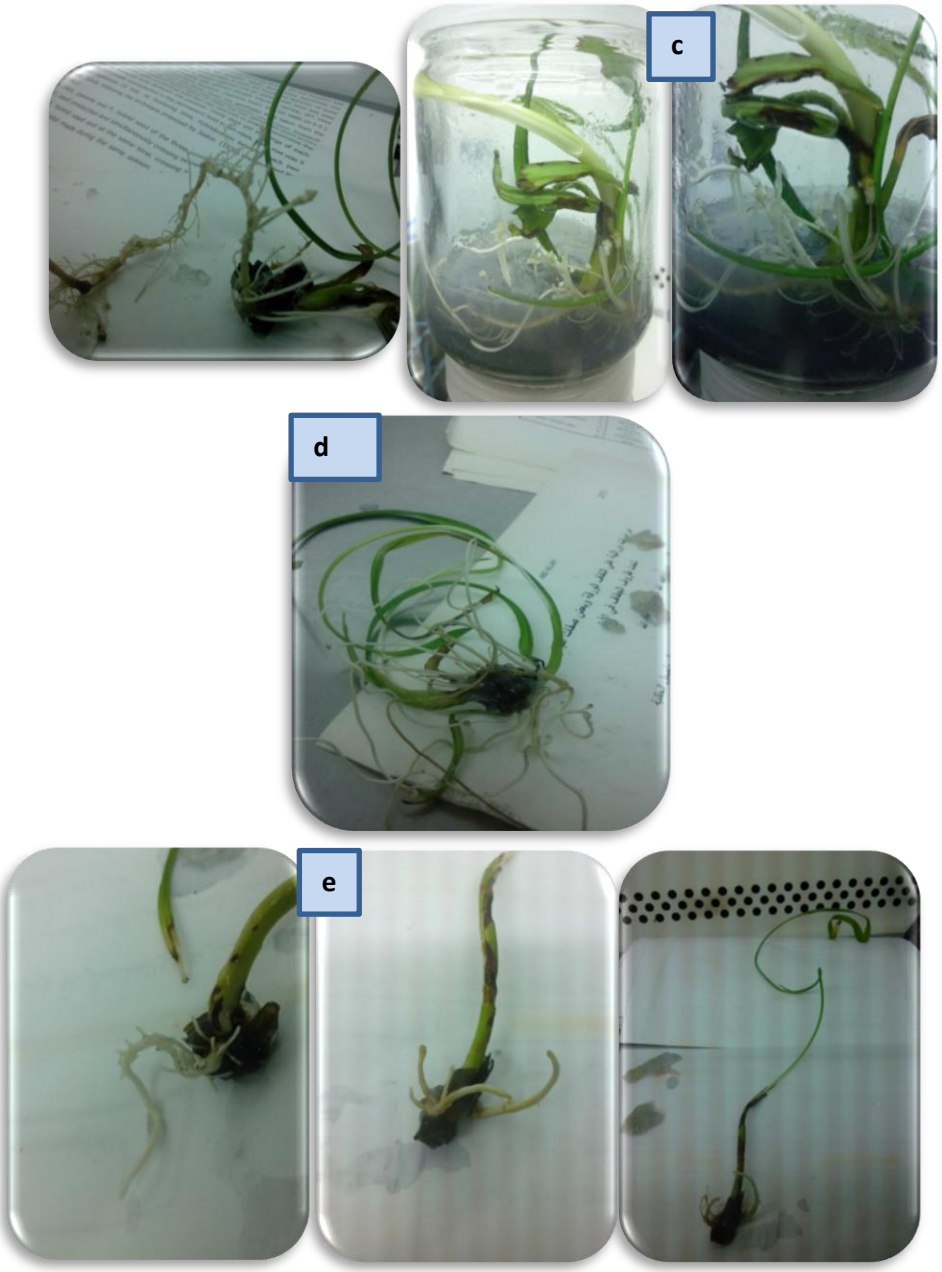

Fig. (3). Leaves, roots and adventitious roots development of the *in vitro* date palm plantlets for different treatments of $CaSiO_3$ (a) Control, (b) 2 mg/l, (c) 4 mg/l, (d) 8 mg/l and (e) 16 mg/l after 3 subcultures.

On increasing the number of roots formed *in vitro*, the area of root/substrate contact also increased reflecting higher absorption of nutrients **(Soares *et al.*, 2011)**. Thus, $CaSiO_3$ contributed to the increase in the number and length of main roots and adventitious roots. As concluded from this experiment during three subcultures that MS medium supplemented with 0.1 mg/l of NAA, 0.5 mg/l GA_3, 35 g/l sucrose and 4.0 mg/l $CaSiO_3$ is the most effective for shoot elongation, rooting and plantlet acclimatization.

This difference in the results could be explained by the fact that the *in vitro* plant growth, organs, tissues and cells depends on the development of optimized culture media for each species and the perfect interaction of essential components as sources of carbon and mineral nutrients **(Pasqual, 2001)**. This finding is consistent with the results of **Pasqual *et al.* (2011)** obtained higher length of the aerial part for *in vitro* orchids using 2.0 mg/l calcium silicate. In the same direction, **Torabinejad *et al.* (1995)** found that a calcium silicate material was developed and recommended for root end filling because of its good physical and chemical properties.

This study was carried out to investigate the effects of silicate (Si) (as calcium silicate), in addition to the control treatment on growth and some biochemical constituents of date palm Sewi cv. cultured *in vitro*. Addition of Si could improve the growth of shoots and increase the protein content and leaf chlorophyll content of stressed plants **(Al Mayahi, 2016)**.

Sivanesan and Jeong (2014) reported that addition of 3.6 µM Si significantly increased *Ajuga multiflora* plant height, chlorophyll content, root length, fresh and dry weights of shoot and root as compared with the control and in line with **Soares *et al.* (2012)** who found that MS medium supplemented with 0.5 and 2.0 mg/l of $CaSiO_3$ promotes the growth of orchid plant where the greatest shoots length was observed at the 2.0 mg/l of calcium silicate. On the other hand, **Asmar *et al.* (2015)** observed that as the concentration of Si increased in the culture media, the number of roots and the average length of the roots system decreased.

It was also reported that Si was able to increase the rooting of the *Phalaenopsis hybrid* **(Zhuo, 1995)**. Another study examining the development of *Phragmites australis* also showed that the effect of Si on plant growth and roots formation is dependent on the genotype. Si has a significant effect on development of plant and root morphogenesis of common reed **(Mathe *et al*., 2012).** It was also reported by **Colombo *et al*. (2016)** that root fresh and dry masses decreased in response to increased concentrations of Si, accompanied by a decrease in the number of roots and average length of the root system. In the 2.0 g/l Si concentration treatment, a reduction of approximately 50% in roots fresh and dry masses was observed when compared to the treatment without Si.

In addition to the morphological effects, Si is an element that provides some benefits to the plant, such as its physical accumulation in the plant cell walls, resulting in the reduction of water loss, improvement of plant architecture and prevention of the penetration of pathogens and insects **(Santos *et al*., 2011)**. Another reported benefit on plants from Si application is the improvement on leaf structure, greater photosynthetic activity **(Asmar *et al*, 2013a)** demonstrated that the addition of $CaSiO_3$ in culture medium increased photosynthesis in the banana cv. 'Maçã' *in vitro*.

Other results showed that leaves of plants grown in medium containing silicate especially $CaSiO_3$, develop leaf structures with greater potential for photosynthesis, giving to these plants better conditions to adapt to the heterotrophic *ex vitro* environment **(Costa *et al*., 2009)**. Silicate also induces a number of metabolic reactions that affect the natural defense of plants, resulting in the formation of phenolic compounds and other chemicals such as phytoalexins and lignins **(Pozza *et al*., 2004)**. The addition of calcium silicate provided greater thickness of upper and lower epidermis, mesophyll, palisade parenchyma and increased the photosynthesis rate.

Thus, the increase in the photosynthetic rate observed in seedlings grown in the presence of calcium silicate may be related to changes in the anatomical structure that allowed a more efficient capture of CO_2 and higher production of

chlorenchyma rich in chlorophyll that accumulates mainly in the palisade parenchyma and are the major molecules involved in photosynthesis **(Pasqual *et al.*, 2011).** This study also indicated that silicate is able to enhance the stability of cell walls through elongation and subsequent division, leading to the maintenance of the cell shape that may be vital for its function and survival.

In this context, the translocation of mineral elements from the medium, which are loaded by the root xylem may have been enhanced in the plants grown *in vitro* with $CaSiO_3$ due to the higher root pressure induced by transpiration of the leaves, in turn caused by their improved stomatal conductance because of the bigger stomata. If the element distribution follows the transpiration rate, more elements will be delivered to the developed organs of the aerial part **(Yamaji and Ma, 2014)** and the photosynthetic rate will be higher **(Costa *et al.*, 2018)**. Therefore, *A. blanchetiana* plants with higher stomatal conductance and consequently increased CO_2 absorption, as well as higher nutrient uptake from the medium are desirable because they have greater photosynthetic capacity and *in vitro* growth rate.

Some plant hormones, such as auxins and gibberellins have been identified effects on *in vitro* plant cell and tissue cultures **(Molnár *et al.*, 2011).** It is known that auxins play an active role in root formation by the induction of root initials **(IPGSA, 1998)**. These results are consistent with those by **El-Hammady (1999)** using NAA who noticed that the average root length decreased with the increasing auxin concentration. Many researchers have mentioned the importance of NAA in the rooting of date palm shoots *in vitro* **(Al-Maari and Al-Gamdi, 1997)**.

Mazri and Meziani (2013) found that in the cv. Najda shoot elongation was faster in the medium supplemented with hormones when compared to hormone free medium, which also adds high frequency of root formation. **Bekheet (2013)** suggested 1.0 mg/l NAA induces better and optimum rooting at the same concentration IAA or IBA. **Meziani *et al.* (2015)** reported that the cv. Mejhoul shoots grew an average of 13.4 cm with an average 4.6 roots number per shoot with wide and green leaves from 3 months old hormone-free half MS medium.

Also, gibberellins are plant growth hormones that play critical roles in diverse developmental processes, such as dormancy, germination, stem elongation, flowering, enzyme induction, leaf senescence and fruit senescence **(Sahebi *et al.*, 2016)**. It has been reported that added Si can increase the levels of gibberellins in rice cultivars **(Hwang *et al.*, 2007)**. The gibberellin plays a pivotal role in the growth and development of plants. The stimulating effect of GA_3 on shoot induction has been reported in apple by **Isogai *et al.* (2008)** in *Cephaelis ipecacuanha* by **Kaushal *et al.* (2005)** and in citrus by **Pérez-Tornero *et al.* (2010)**.

These results do not agree with **Colombo *et al.* (2016)** evaluate the *in vitro* growth in modified culture medium containing different doses of silicate, the calcium silicate salt was used at doses 0.0, 0.5, 1.0, 1.5 and 2.0 g/l of a 1 molar solution added to the MS culture medium for good seedling development and *ex vitro* quality of *Cattleya labiata*. A decrease in the growth of seedlings similar to the dose of calcium silicate was observed. The doses of calcium silicate did not favor the *in vitro* growth of *Cattleya labiata* Lindley.

Different concentrations of $CaSiO_3$ did not interfere on the roots number and root length; this source also resulted in high roots number and lower root length especially at 1.0 and 2.0 mg/l. The roots number was significantly lower than the control at 2.0 mg/l $CaSiO_3$ **(Martins *et al.*, 2011)** and **Asmar *et al.* (2013b)** found no significant difference in roots number for any of the used silicate sources and did not affect the *in vitro* development of various plant species, mainly of ornamentals, such as the *C. forbesii* Lindley **(*Colombo et al.*, 2016)** orchids. Hence, it can be concluded that rooting quality of the *in vitro* plantlets of date palm was the vital factor for the increased survival percentage in the greenhouse.

In the second experiment, the effects of adding Fe-EDTA and thiamine to culture medium on date palm rooting *in vitro* after 8 weeks of subculture were studied, as shown in **Fig. 4 and 5**.

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The plantlets from last subculture from experiment of different concentrations of $CaSiO_3$ were cultured on new medium supplemented with the best concentration of $CaSiO_3$ at 4.0 mg/l and added 0.15 g/l Fe-EDTA with 2.5 mg/l Thiamine. The leaves length of plantlets recorded 25.50 cm with 11.33 root/plantlet of length 15.83 cm. There were 20.00 adventitious roots with length 4.17 cm appear on the primary root which develop directly from the plantlets. These adventitious roots produce lateral roots of the same type with approximately the same diameter throughout their length.

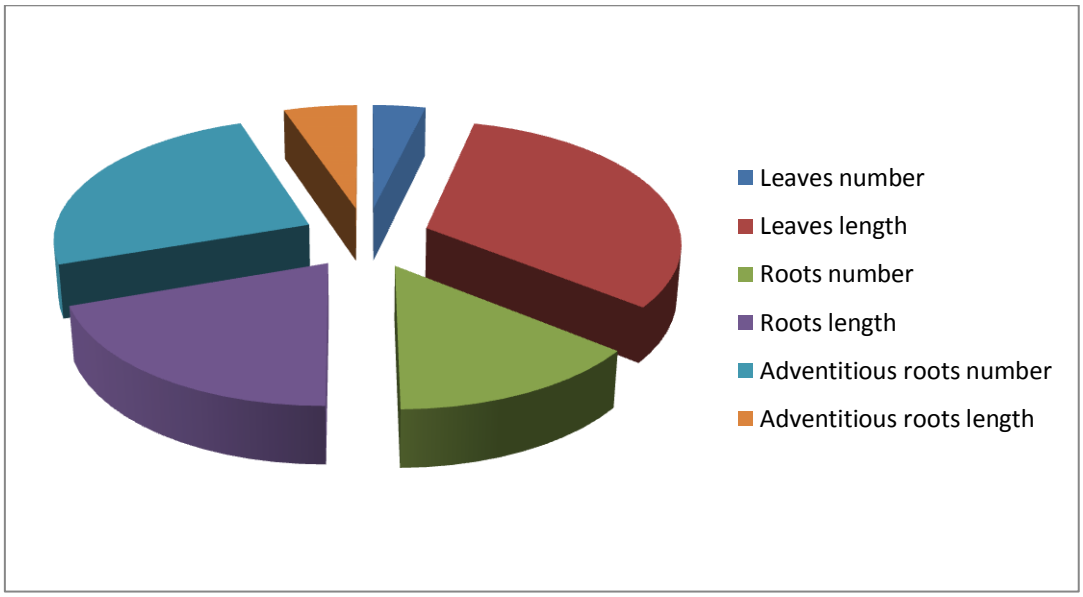

Fig. (4). Response of date palm leaves, roots and adventitious roots to one subculture on a medium supplemented with 4.0 mg/l $CaSiO_3$, Fe-EDTA and thiamine

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Fig. (5). Adventitious roots development on ½ MS medium contain $CaSiO_3$ at 4.0 mg/l, 0.15 g/l Fe-EDTA and 2.5 mg/l Thiamine after 8 weeks in culture.

These results were in agreement with **Trejgell *et al.* (2012)** who reported that supplementation of the medium with Fe-EDTA as Fe source significantly increased the level of chlorophyll in the leaves. After shoots growing on the MS medium supplemented with Fe-EDTA, the number of roots per shoot was significantly higher. The essential micronutrients (minor elements) for plant cell and tissue growth include iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu) and molybdenum (Mo). Iron is usually the most critical of all the micronutrients. There have been trials to solve difficulty to dissolve and precipitate after media preparation by using ethylene diaminetetraacetic acid (EDTA)-iron chelate (Fe-EDTA) **(Murashige and Skoog, 1962)**. The iron source commonly used in the media is Fe-EDTA, it is unstable in light and hence iron rapidly becomes unavailable to plants. In addition, more than 75% of available iron is consumed by the explants during the first week of culture **(Ramage and Williams, 2003)**. Also, **Sadeghi *et al.*, (2015)** reported that 100% *in vitro* rooting of *Prunus empyrean*) rootstock was achieved on ½ strength MS medium with 0.5 mg/l IBA, 1.6 mg/l thiamine and 150 mg/l iron.

On the other hand, thiamine when associated with cytokinin plays an important role in inducing roots and callus growth. Addition of thiamine to pea embryos affects roots and shoots growth concurrently. A study showed that tomato roots have capability to exhibit prolonged thiamine dependency **(Bonner, 1937)**. Thiamine is a vitamin shown to have significant rooting on pacific yew, an evergreen, *Taxus brevifolia* Peattie. Upon adding thiamine, **Chee *et al.* (1995)** obtained 61.5% of adventitious rooting in *T. brevifolia* compared to 30% without thiamine. Furthermore, thiamine and IBA had significant effects on rooting percentage in such a way that even lower concentrations of thiamine caused increment of rooting, in which the lowest rooting percent occurred in medium without thiamine. While, the highest percent was obtained in medium with thiamine at 1.6 mg/l, IBA at 1, 1.3 and 1.6 mg/l or thiamine at 2.8 mg/l, IBA at 1.3 and 1.6 mg/l. Length of roots was also affected by thiamine in which application of thiamine at 1.6 mg/l produced the longest root and control had the shortest roots **(Sepahvand *et al.*, 2012)**.

However, these results recorded by **Hasan *et al.* (2010)** indicated that the number of roots per explant varied significantly with the different concentrations of Fe-EDTA, and produced maximum number of roots (1.82) at (100 mg/l) but can be described as small and weak roots.

Estimation of total soluble proteins

The addition of silicate (Si) caused an increase in the total soluble protein content of shoots (0.68 mg/g^{-1}) compared to the control (0.34 mg/g^{-1}), that may be due to the role of Si in binding amino acids to form certain proteins (**Soundararajan *et al.*, 2014)**, additionally Si is actively involved in the formation of DNA and functioning of mRNA **(Abbas *et al.*, 2015)**.

Estimation of chlorophyll

Results in **Fig. 6.** showed that addition of 4.0 mg/l $CaSiO_3$ caused an increase in contents of chlorophyll a, b and total chlorophyll compared to the control treatment.

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Chlorophyll plays an important role in the photosynthesis and is responsible for light assembling. (**Gong *et al.*, 2005)** observed that application of Si increased the chlorophyll contents in wheat, which may be attributed to the effect of Si in preserving the percentage of water in leaves thus preventing destruction of photosynthetic process and chlorophyll (**Mali and Aery, 2008)**. Furthermore, the deposition of silicate in the cell wall also increases tissue resistance and promotes better performing plants due to leaf situation and their interception of light (**Lana, *et al.*, 2003)**. From another point of view, silicate treatments were shown to cause changes to nitrogen metabolism (**Watanabe *et al.*, 2002)** it caused the decrease in nitrogen absorption which is an essential element necessary for the produce of chlorophyll.

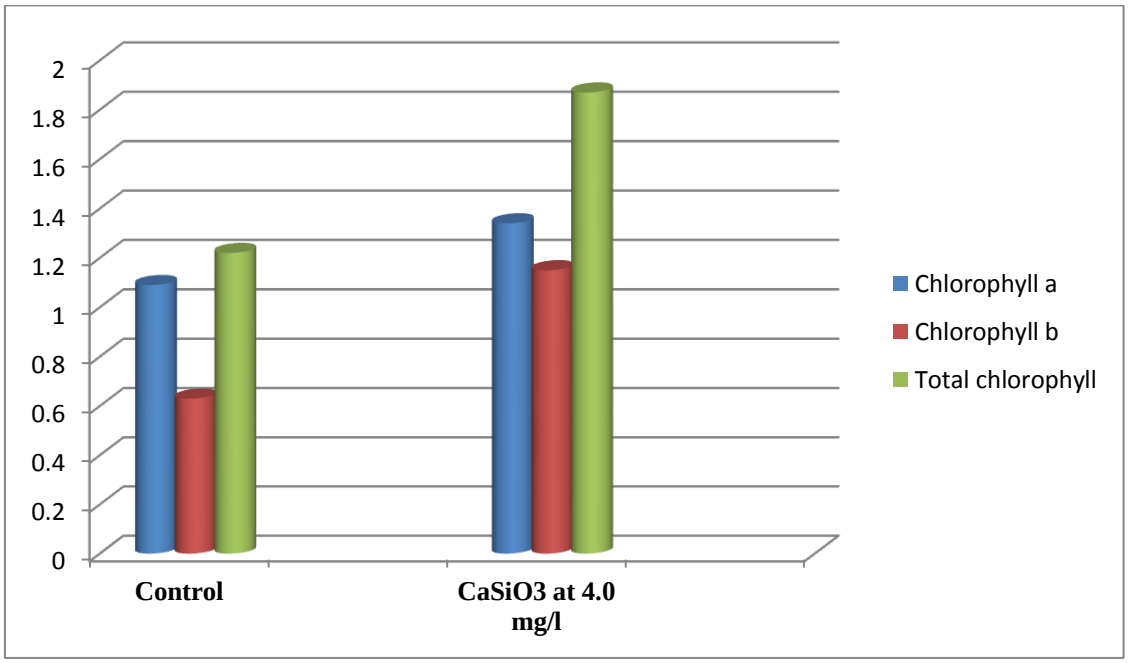

Fig. (6). Effect of adding 4.0 mg/l $CaSiO_3$ on chlorophyll content (µg ml^{-1})

The positive effects of the added Si in the culture medium on chlorophyll content have been reported by several researches **(Asmar *et al.*, 2015; Soundararajan *et al.*, 2013; Manivannan *et al.*, 2018; Rezende *et al.*, 2018; Martins *et al.*, 2018).** However, it seems to be dependent on the Si source. *A. blanchetiana* plants cultured *in vitro* with $CaSiO_3$ had higher chlorophyll content, as also verified by **Manivannan *et al.* (2018) and Martins *et al.* (2018)**.

From the results of the present study, it can be concluded that, there was advancement in leaf combination with enhanced contents of chlorophyll due to $CaSiO_3$ supplementation. These results confirm the results obtained from previous reports that the addition of Si could maintain the level of chlorophyll in capsicum (*Capsicum annuum*), suggesting that Si could alleviate drought stress which induced destroy in the photosynthetic systems (**Lobato *et al.*, 2009)**. Also, the results are in agreement with those reported by **(Li *et al.*, 2007)** who found the significant Si induced enhancement of chlorophyll concentrations in sorghum and maize plants.

Acclimatization Stage

The acclimatization is the last step of *in vitro* culture. This step needs special attention to be completed plantlets will be taken to grown in field conditions, therefore, they need to be healthy and uniform. In addition, acclimatization of plantlets derived from tissue culture confirmed the efficiency of the method used. The gradual lifting of plastic covers in the greenhouse assisted in formation of the cuticle layer and regulation of stomatal action. The soil mixture containing (peat moss and perlite; 2:1 by volume) was the best and gave 70% survival of acclimatized plants, as shown in **Fig. 7.**

The accumulation of silicate in the leaves leads to the formation of a protective barrier and regulates the water loss by transpiration. This helps acclimatization of micro propagated plants because the main cause of mortality during this stage is the loss of water by the low functionality of the stomata and slender epicuticular wax layer **(Silva, 2007 and Pasqual *et al.*, 2011)**. Moreover, **Martins *et al.* (2018)** concluded that the supplementation of the culture medium with calcium silicate led

to improved growth, anatomical and physiological characteristics; resulting in the development of more resistant plantlets with better performance during acclimatization.

Fig. (7). Successfully acclimatized of date palm plantlets cv. Sewi

However, using silicate in the *in vitro* cultivation is beneficial for providing better photosynthetic apparatus, higher contents of cellulose and hemicellulose during acclimatization of banana plants. **Asmar *et al.*, (2013b)** found that the acclimatization process is fundamental because it provides an increase in the epicuticular wax layer on 'Grande Naine' banana leaves. The epicuticular wax is a polymer complex with important functions in the cells epidermal. Among them, protection against water loss and it acts also in the protection against the excess of lightness **(Alkini *et al.*, 2006)**. In addition, silicate offers a possible tool to obtain bromeliads of greater quality that are resistant to acclimatization. This element contributes to the final quality of the plant, since its accumulation in the leaves confers plant protection, increases its photosynthetic capacity, reduces water loss and promotes its growth and in general minimizes the biotic and abiotic stress factors that can affect the young plant **(Epstein, 1999).**

Accordingly, the protocol suggested in this study is practically successful in the micro propagation of date palm, Sewi cv.

References

Abbas, M. F. and Abbas, M. G. (1992). Care and Storage of Fruits and Vegetables in Practice. University of Basrah, Basrah.

Abbas, T.; Balal, R. M.; Shahid, M. A.; Pervez, M. A.; Ayyub, C. M.; Aqueel, M. A. and Javaid, M. M. (2015). Silicate-Induced Alleviation of NaCl Toxicity in Okra (*Abelmoschus esculentus*). Is Associated with Enhanced Photosynthesis, Osmoprotectants and Antioxidant Metabolism. Acta Physiologia Plantarum, 37 (6): 1-15.

Al-Khateeb, A. A. and Alturki, S. M. (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.

Alkini, Y.; Bona, Ç.; Boerger, M. R. T.; Costthe, CG; Barros, C.F. (2006). Epidermis. In: Appezzato-DA-Glory, B.; Carmello-Warrior, SM. Plant Anatomy. Viçosa, MG: UFV, p. 87-108.

Al–Maari, K. W. and Al – Ghamdi, A. S. (1997). Micro propagation of five date palm cultivars through *in vitro* axillary buds proliferation. Du. J. Agri. Sci. 13: 55 – 71.

Al-Mayahi, A. M. (2016). Effect of Silicon (Si) Application on *Phoenix dactylifera* L. Growth under Drought Stress Induced by Polyethylene Glycol (PEG) *in Vitro*. American Journal of Plant Sciences, 7 (13): 1711-1728.

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

Asmar, S. A.; Castro, E. M.; Pasqual, M.; Pereira, F. J. and Soares, J. D. R. (2013a). Changes in leaf anatomy and photosynthesis of micro propagated banana plantlets under different silicon sources. Science Horticulture, 161 (1): 328-332.

Asmar, S. A.; Pasqual, M.; Araujo, A. G. ; Silva, R. A. L.; Rodrigues, F. A.; Pio, L. A. S. (2013b). Morphophysiological characteristics of acclimatized 'Grande Naine' banana plants in response to *in vitro* use of silicate, Semina: Agricultural Sciences, 34 (1): 73-82.

Asmar, A. S.; Soares, J. D. R.; Silva, R. A. L.; Pasqual, M.; Pio, L. A. S. and Castro, E. M. (2015). Anatomical and structural changes in response to application of silicon (Si) *in vitro* during the acclimatization of banana cv. 'Grand Naine'. Australian Journal of Crop Science, 9: 1236–1241.

Bekheet, S. A. (2013). Direct organogenesis of date palm (*Phoenix dactylifera* L.) for propagation of true-to-type plants. Sci. Agri., 4: 85-92.

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

Bonner, J. (1937). "The Role of Vitamins in Plant Development," Botanical Review, 3 (12): 616 – 640.

Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry, 72: 248 – 254.

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.

Chao, C. C. T. and Krueger, R. R. (2007). The date palm (*Phoenix dactylifera* L.): Overview of biology, uses and cultivation. Horticultural Science, 42 (5): 1077-1082.

Chee, P. P. (1995). "Stimulation of Adventitious Rooting of Taxus Species by Thiamine," Plant Cell Reports, 14 (12): 753-757.

Colombo, R. C.; Favetta, V.; Faria, R. T.; Andrade, F. A. and Melem, V. M. (2016). Response of *Cattleya forbesii* orchid to increasing silicon concentrations *in vitro*. Rev. Caatinga., 29: 18–24.

Costa, B. N. S.; Costa, I. J. S.; Dias, G. M. G.; Assis, F. A.; Pio, L. A. S.; Soares, J. D. R. and Pasqual, M. (2018). Morpho-anatomical and physiological alterations of passion fruit fertilized with silicon. Pesq. Agropec. Bras., 53: 163–171.

Costa, F. H. S.; Castro, E. M.; Pasqual, M.; Pereira, J. E. S. and Oliveira, C. (2009). Anatomical alterations of micro propagated banana trees in response to *ex vitro* acclimatization. Rural Science, 39: 386–392.

Dimassi-Theriou, K. (1989). Factors Affecting Shoot Proliferation and Rooting *in vitro* of the Peach Rootstock GF-677 (*Prunus amygdalus* x *P. persica*) and Petunia (*Petunia hybrida*). Ph.D. Thesis. Aristotle University of Thessaloniki, Scientific Annals of Agriculture. Suppl. **6**. Vol. KZ.

Duncan, D. B. (1955). Multiple range and multiple F test. Journal of Biometrics, 11: 1 - 42.

Dunlap, J. R. and Robacker, K. M. (1988). Nutrient salts promote light-induced degradation of indole-3- acetic acid in tissue culture. Plant Physiology, 88: 379–382.

El–Hammady, A. A. (1999). Regeneration of date palm "Sewi" cv. plantlets by somatic embryogenesis through callus with reference to the genetic stability. in The Inter. Conf. Date Palm. Assiut Univ. Egypt. 117 – 131.

Epstein, E. 1999. Silicon. Annual Review of Plant Physiology and Plant Biology, 50: 641-664.

Gaspar, T.; Kevers, C.; Hausman, J. F.; Berthon, J. Y. and Ripetti, V. (1992). Practical uses of peroxidase activity as a predictive marker of rooting performance of micro propagated shoots. Agronomie, 12: 757-765.

Gomez, K. A. and Gomez, A. A. (1984). Statistical Procedures for Agricultural Research. John Wiley and Sons, New York, USA, 680 p.

Gong, H.; Zhu, X.; Chen, K.; Wang, S. and Zhang, C. (2005). Silicate Alleviates Oxidative Damage of Wheat Plants in Pots under Drought. Plant Science, 169: 313-321.

Gunes, A.; Inal, A.; Bagci, E. G. and Coban, S. (2007). Silicon mediated changes on some physiological and enzymatic parameters symptomatic of oxidative stress in barley grown in sodic-B toxic soil. J. Plant Physiol., 164: 807–811.

Hasan, S. Z. U.; Ahmad, T.; Hafiz, I. A. and Hussain, A. (2010). Direct plant regeneration from leaves of Prunus Rootstock GF-677 (*Prunus amygdalus* X *P. persica*). Pak. J. BOT., 42 (6): 3817-3830.

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

Hwang, S. J.; Hamayun, M.; Kim, H. Y.; Na, C. I.; Kim, K. U.; Shin, D. H.; Kim, S. Y. and Lee, I. J. (2007). Effect of nitrogen and silicon nutrition on bioactive gibberellin and growth of rice under field conditions. J. Crop Sci. Biotechnol., 10: 281–286.

IPGSA. (1998). International Conference on Plant Growth Substances. 13 - 17. Makuhari Masse, Chiba Japan.

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

Isogai, S.; Touno, K. and Shimomura, K. (2008). Gibberellic acid improved shoot multiplication in *Cephaelis ipecacuanha*. *In Vitro* Cell Dev. Biol. Plant, 44: 216–220.

Kaushal, N.; Modgil, M.; Thakur, M. and Sharma, D. (2005). *In vitro* clonal multiplication of an apple rootstock by culture of shoot apices and axillary buds. Indian J. Exp. Biol., 43: 561 - 565.

Lana, R. M. Q.; Korndorfer, G. H.; ZanãoJúnior, L. A.; Silva, A. F. and Lana, A. M. Q. (2003). Effect of Calcium Silicate on the Productivity and Silicate Accumulation in the Tomato Plant. Bioscience Journal, 19: 15-20.

Lecouteux, C. G.; Lai, F. M.; Bryan, D. and Mckesie, B. D. (1993). Maturation of Alfalfa (*Medicago sativa* L.) Somatic Embryos by Abscisic Acid, Sucrose and Chilling Stress. Plant Science, 94: 207-213.

Li, Q. F.; Ma, C. C. and Shang, Q. L. (2007). Effects of Silicate on Photosynthesis and Antioxidative Enzymes of Maize under Drought Stress. Chinese Journal of Applied Ecology, 18: 531-536.

Lobato, A. K. S.; Coimbra, G. K.; Neto, M. A. M.; Costa, R. C. L.; Filho, B. G. S.; Neto, C. F. O.; Luz, L. M.; Barreto, A. G. T.; Pereira, B. W. F.; Alves, G. A. R.; Monteiro, B. S. and Marochio, C. A. (2009). Protective Action of Silicate on Water Relations and Photosynthetic Pigments in Pepper Plants Induced to Water Deficit. Research Journal of Biological Sciences, 4: 617- 623.

Mali, M. and Aery, N. C. (2008). Silicate Effects on Nodule Growth, Dry-Matter Production and Mineral Nutrition of Cowpea (*Vigna unguiculata*). Plant Nutrition and Soil Science, 171: 835-840.

Manivannan, A.; Soundararajan, P.; Cho, Y. S.; Park, J. E. and Jeong, B. R. (2018). Sources of silicon influence photosystem and redox homeostasis-related proteins during the axillary shoot multiplication of *Dianthus caryophyllus*. Plant Biosyst., 152: 704–710.

Martins, A. D.; Martins, J. P. R.; Batista, L. A.; Dias, G. M. G; Almeida, M. O.; Pasqual, M. and Santos, H. D. O. (2018). Morpho-physiological changes in *Billbergia zebrina* due to the use of silicates *in vitro*. Anais da Academia Brasileira de Ciências, 90 (4): 3449 - 3462.

Mathe, C.; Mosolygo, A.; Suranyi, G.; Beke, A.; Demeter, Z. and Toth, V. R. (2012). Genotype and Explants Type Dependent Morphogenesis and Silicon Response of Common Reed (*Phragmites australis*) Tissue Cultures. Aquatic Botany, 97: 57-63.

Mazri, M. A. and Meziani, R. (2013). An improved method for micro propagation and regeneration of date palm (*Phoenix dactylifera* L.). J. Plant Biochem. Biotechnol., 22: 176-184.

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

Meziani, R.; Jaiti, F.; Mazri, M. A.; Anjarne, M.; Chitt, M. A.; El-Fadile, J. and Alem, C. (2015). Effects of plant growth regulators and light intensity on the micro propagation of date palm (*Phoenix dactylifera* L) cv. Mejhoul. Journal of Crop Science and Biotechnology, 18: 325 - 331.

Molnár, Z.; Virág, E. and Ördög, V. (2011). Natural substances in tissue culture media of higher plants. Acta Biologica Szegediensis, 55 (1): 123-127.

MSTAT Development Team, (1989). MSTAT user's guide: a microcomputer program for the design management and analysis of agronomic research experiments. Michigan State University, East Lansing, USA.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-479.

Pasqual, M. (2001). Specialization course to distance plant tissue culture (CTV). Lavras: UFLA / FAEPE, 97 p.

Pasqual, M.; Soares, J. D. R.; Rodrigues, F. A.; Araújo, A. G. and Santos, R. R. (2011). Light quality and silicon on growth *in vitro* of native and hybrid orchid species. Horticultura Brasileira, 29: 324–329.

Pérez-Tornero, O.; Tallon, C. and Porras, I. (2010). An efficient protocol for micro propagation of lemon (*Citrus limon*) from mature nodal segments. Plant Cell Tissue and Organ Culture, 100: 263–271.

Pozza, A. A. A.; Alves, E,; Pozza, E. A.; Carvalho, J. G.; Montanari, M.; Guimarães, P. T. G. and Santos, D. M. (2004). Effect of Silicon on Cercosporiosis Control on Three Vseriousness of Coffee maker. Phytopathol. Bras., 29 (2): 185-18.

Ramage, C. M. and Williams, R. R. (2003). Mineral uptake in tobacco leaf discs during different developmental stages of shoot organogenesis. Plant Cell Rep. 21: 1047–1053.

Rezende, R. A. L. S.; Rodrigues, F. A.; Soares, J. D. R.; Silveira, H. R. O.; Pasqual, M. and Dias, G. M. G. (2018). Salt stress and exogenous silicon influence physiological and anatomical features of *in vitro* grown cape gooseberry. Ciência Rural, 48 (1): 1-9.

Sadeghi, F.; Yadollahi, A.; Kermani, A. J. and Eftekhari, M. (2015). Optimizing culture media for *in vitro* proliferation and rooting of Tetra (*Prunus empyrean*) rootstock. J. Genet. Eng. Biotechnol., 13 (1): 19–23.

Sahebi, M.; Hanafi, M. M. and Azizi, P. (2016). Application of silicon in plant tissue culture. *In Vitro* Cellular and Developmental Biology - Plant, 52 (3): 226-232.

Santos, E. S.; Filho, J. C.; Lacerda, J. T. and Oak, R. A. (2011). Yam (Dioscorea) technologies of production and preservation environment. Agricultural Technology and Science, 1 (1): 31-36.

Sepahvand, S.; Ebadi, A.; Kamali, K. and Ghaemmaghami, S. A. (2012). Effects of Myo-Inositol and Thiamine on micro propagation of GF677 (Peach × *Almond Hybrid*). Journal of Agricultural Science, 4 (2): 275-280.

Silva, D. P., (2007). Culture media and sources of silicate in *in vitro* gerbera development. Lavras: UFLA. 84 p.

Sivanesan, I. and Jeong, B. R. (2014). Silicon Promotes Adventitious Shoot Regeneration and Enhances Salinity Tolerance of *Ajuga multiflora* Bunge by Altering Activity of Antioxidant Enzyme. Scientific World Journal, 1-10.

Sivanesan, I. and Park, S. W. (2014). The role of silicon in plant tissue culture. Front Plant Sci., 5: 1–4.

Soares, J. D. R.; Pasqual, M.; Araujo, A. G.; Castro, E. M.; Pereira, F. J. and Braga, F. T. (2012). Leaf anatomy of orchids micro propagated with different silicon concentrations. Acta Scientiarum Agronomy, 34 (4): 413–421.

Soares, J. D. R.; Pasqual, M.; Rodrigues, F. A.; Villa, F. and Araujo, A. G. (2011). Silicon sources in the micro propagation of the Cattleya group orchid. Acta Scientiarum Agronomy, 33 (3): 503–507.

Soundararajan, P.; Sivanesan, I.; Jana, S. and Jeong, B. R. (2014) Influence of Silicate Supplementation on the Growth and Tolerance to High Temperature in *Salvia splendens*. Horticulture Environment and Biotechnology, 55: 271-279.

Soundararajan, P.; Sivanesan, I.; Jo, E. H. and Jeong, B. R. (2013). Silicon promotes shoot proliferation and shoot growth of *Salvia splendens* under salt stress *in vitro*. Hort. Environ. Biotechnol., 54: 311–318.

Taha, R. A. and Hassan, M. M. (2014). Using Low Levels of Seawater to Enhance Growth and Development of Date Palm Embryogenic Cultures. Asian Journal of Agricultural Sciences, 6 (2): 69-74.

Torabinejad, M.; Hong, C. U.; McDonald, F. and Ford, T. R. P. (1995). Physical and chemical properties of a new root-end filling material, Journal of Endodontics, 21 (7): 349-353.

Trejgell, A.; Libront, I. and Tretyn, A. (2012). The effect of Fe-EDDHA on shoot multiplicationand *in vitro* rooting of *Carlina onopordifolia* Besser. Acta Physiol. Plant, 34: 2051–2055.

Watanabe, S.; Fujiwara, T.; Yoneyama, T. and Hayashi, H. (2002). Effects of Silicate Nutrition on Metabolism and Translocation of Nutrients in Rice Plants. Developments in Plant and Soil Sciences, 92: 174-175.

Yamaji, N. and Ma, J. F. (2014). The node, a hub for mineral nutrient distribution in graminaceous plants. Trends Plant Sci., 19: 556–563.

Zhuo, T. S. (1995). The detection of the accumulation of silicon in *Phalaenopsis* (Orchidaceae). Annals of Botany, 75 (3): 605–607.

