

Effect of Different Silicate Concentrations and Sources on the *in vitro* Multiplication of Date Palm Shoots cv. Maktoum

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Abstract: This study was conducted in the Central Lab of Date Palm for Researches and Development - Agricultural Research Center, Egypt during the period from 2016 to 2017. The objective of the present study was to evaluate the effects of different silicate concentrations, combinations and their sources on the growth characteristics of date palm shoots cultivated *in vitro* during multiplication phase. Three sources of silicate were tested: potassium silicate and calcium silicate each at concentrations of 0.0, 2.0, 5.0, 10.0 and 15.0 mg/l, plus sodium silicate at concentrations of 1.0 and 2.0 mg/l in all possible combinations. The 1 mg/l sodium silicate supplement with different combinations of K₂SiO₃ and CaSiO₃ gave the best results in the plant growth development.

Key words: Date palm • *In vitro* • Silicates • Multiplication • Rooting stage • Acclimatization • Chlorophylls • Cellulose and hemicellulose

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a member of the Areaceae (Palmae) family. It is one of the oldest fruit crop mainly cultivated in North Africa, Middle East, Near East of Asia and some dispersed areas of Europe and America [1]. In addition, date palm contributes significantly to the income of the rural population, since almost all parts of this plant are used [2]. Date palm with 2n = 36 is a dioecious perennial monocotyledon fruit tree. It is believed to be a native of the Arabian Gulf region, possibly in Southern Iraq [3]. The growing demand for date palms requires massive multiplication of selected plants. This could be achieved through tissue culture.

Tissue culture techniques, such as micro-propagation through shoot tips, is an alternatives to be used instead of traditional propagation methods. *In vitro* techniques have been successfully used because they provide a large amount of plantlets from mother plants with high genetic and phytosanitary patterns. In addition, the multiplication occurs in a short period, took small spaces and without

seasonal interruption. However, the acclimatization process is an important stage that deserves special care because during it the plants will become able to grow in the field [4].

Date palm was propagated from offshoots, which were produced by the date palm trees in the early part of their life. However, this traditional method is relatively slow to establish new date palm plantations, since a limited number of offshoots are produced by the tree during its life. In addition, seedy propagated plants do not obtain true-to-type plants due to heterozygous and require up to seven years to reach adulthood fruiting stage [5]. Propagation of date palm through *in vitro* techniques presents an efficient alternative method for the conventional methods [6]. Since 1970, intensive efforts have been undertaken into large-scale micro-propagation of date palm using some techniques such as somatic embryogenesis and organogenesis [7].

The composition of the culture medium plays an important role in the cell and tissue growth responses. Silicate seems to be beneficial element for plants

according to Epstein [8], who found that plants grown in silicate enriched environment differ from those grown in their absence; especially in chemical composition, cell mechanical strength, leaf surface characteristics, tolerance to biotic and abiotic stresses and resistance to pests and diseases.

Silicate (Si), as a macro-element, has a vital role in plants cycles. It is considered as one of the important beneficial nutrients for plant growth [9]. Its physical accumulation in the plant cell walls, resulted in the reduction of water loss; improvement of plant architecture and prevention of the penetration of pathogens and insects [10]. Another reported benefits on plants from Si applications had the improvement of leaf structure, greater photosynthetic activity and induction of 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 [11, 12]. So many studies have reported that the use of Si in an *in vitro* condition can be beneficial effect for vegetative propagation dependent plants due to good effects that they provide.

Several studies proved that adding of Si to the tissue culture medium enhances callus growth, shoot regeneration, root induction, stimulates somatic embryogenesis and improves morphological, anatomical and physiological criteria of plantlets, as well as, the longevity of calli and organs with a potential for plant regeneration. *In vitro* culture is a useful system for studying physiological and biochemical functions of Si in plants at a molecular level [13].

The objectives of this work were evaluating the effect of silicate sources, concentrations and combinations on the *in vitro* growth, acclimatization, contents of chlorophylls, cellulose and hemicellulose of Date palm shoots cv. Maktoum.

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.

Explants Preparation and Sterilization: The micropropagation process was started with the selection of healthy offshoots from mother date palm trees of Maktoum cv., were removed from their mother plants having a weight of 5-7 kg and height of 50-80 cm. Shoot tips and leaves primordial used as an explants of the

offshoots excised, after removing all outer leaves, fibers and roots; then surface-sterilized. The explants were surface sterilized under aseptic conditions by using ethyl alcohol (70%) for 1 min followed by immersion in (0.5 g/l) mercuric chloride (HgCl_2) for 5 min and then rinsed one-time with sterile distilled water and transferred to double surface sterilization by commercial Clorox (5.25%) sodium hypochlorite (NaOCl) 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.

Culture Medium: The culture medium used for *in vitro* cultures was the basal nutrient medium of MS Murashige and Skoog [14] supplemented with (mg/l): 0.5 pyridoxine-HCL; 0.5 nicotinic acid; 0.1 thiamine-HCL; 100 myo-inositol; 2 glycine; 200 glutamine; 0.2 activated charcoal (AC); 35 g/l sucrose and after solidified with 6 g/l agar. The media was dispensed into small jars (150 ml) in aliquots of 40 ml per jar and capped with polypropylene closures. Subsequently the media were autoclaved for 20 minutes at 1.5 kg/cm² and 121°C.

Multiplication Stage: The proliferated clusters of shoots in the multiplication stage were cultured for three subcultures each of four weeks. The MS basal medium was supplemented with 0.05 mg/l BA (Benzyl adenine) and 0.1 mg/l NAA (Naphthalene acetic acid). Three sources of Si were tested: sodium silicate (Na_2SiO_3), potassium silicate (K_2SiO_3) and calcium silicate (CaSiO_3), at a dose of 0, 1, 2, 5, 10 and 15 mg/l. Medium without any source of Si was used as control. Shoots number, leaves number and leaves length were recorded after one and three months of culture. There were nine replicates of each treatment.

Rooting Stage: Resultant shoots were transferred to big jars (1 - 2 shoot / jar) containing 40 cm³ of rooting medium consisting of MS salts and the following (in mg/l): Thiamine-HCl 0.4, myo-inositol 100, sucrose 40 gm/l and agar 6 gm/l. The auxin of NAA was added in concentration 1.0 mg/l in combination with IBA (Indole buteric acid) at 0.5 mg/l. There were nine replicates for each treatment and cultures were incubated in a culture room at 27 + 1°C and 1000 lux light intensity for 16 hours daily. Leaves number, leaves length, rooting percentage, roots number and root length were recorded after four and five months of culture.

Acclimatization Stage: The plantlets were washed with distilled water and treated with fungicide (Benlet 2 g/l) for 10 minutes, then transplanted into peat moss and perlite as a mixture (2:1). Plants were placed in pots with a 10 cm diameter that were filled with a peat/perlite mixture and placed in a greenhouse or under plastic tunnels. Pots were irrigated with 1/2 strength MS salts and plastic covers were removed gradually after eight weeks.

Determination of Chlorophylls Content: Contents of chlorophyll a, b and total were quantified according to the method of Arnon [15] Five leaves of each treatment were collected and 0.5 g of leaf tissues were macerated in liquid nitrogen and solubilized in 80% acetone. The material was centrifuged at 8000 xg for 15 minutes. The supernatant was collected for determination of the contents of pigments using a spectrophotometer (663 nm and 645 nm).

Determination of Contents of Cellulose and Hemicellulose: The contents of cellulose and hemicellulose were determined using the method of Soest and Wine [16].

Statistical Analysis: This experiment was designed as a randomized complete block design (RCBD) as described by Gomez and Gomez [17]. The obtained data were statistically analyzed using MSTAT Computer Program [18]. To verify differences among means of various treatments, means were compared using Duncan's Multiple Range Test as described by Duncan [19].

RESULTS AND DISCUSSION

The effect of 8 different combinations of sodium, potassium and calcium silicate on the growth of date palm cv. Maktoum explants were studied. Similar explants (each explant with 2 shoots and 4 leaves) obtained after

three subcultures on the multiplication medium supplemented with 0.1 mg/l NAA and 0.05 mg/l BA had been selected to begin the experiment. The explants were transferred onto another medium supplemented with 0.1 mg/l NAA and 0.05 mg/l BA in addition to various concentrations of Na₂SiO₃, K₂SiO₃ and CaSiO₃.

Number of Shoots: There was a significant interaction between the three sources of silicate for the number of shoots, leaves and length of leaves. After one month of culturing on the multiplication medium (0.1 mg/l NAA + 0.05 mg/l BA) with the different concentrations of silicate combination; a good growth response was noticed with the level of 2 mg/l Na₂SiO₃ combined with different concentrations of K₂SiO₃ and CaSiO₃ compared to the level of 1 mg/l Na₂SiO₃ with the same concentrations of K₂SiO₃ and CaSiO₃. The number of shoots with 2 mg/l Na₂SiO₃ started with 4.33 decreased to 2.67 which increased again to 4.00 reached 5.00 with increasing the concentrations of K₂SiO₃ and CaSiO₃ in the order of 5, 10 and 15 mg/l. Whereas with the level of 1 mg/l Na₂SiO₃ and the same concentrations of K₂SiO₃ and CaSiO₃, the number of shoots started from 3.33 decreased to 3.00 and continued to decrease to 2.33 then increased again to 3.67 while the control (with no silicates) recorded the lowest number of shoots (2.67), as shown in Table 1. It can be concluded that the highest number of shoots (5.00) was recorded with the highest concentration of each of Na₂SiO₃, K₂SiO₃ and CaSiO₃ (2, 15, 15 mg/l, respectively).

Number of Leaves: The same trend was detected with the number of leaves which decreased from 8.67 to 7.00 then increased to 9.33 and continued increasing to 14.33 with constant concentration of Na₂SiO₃ (2 mg/l) and various concentrations of K₂SiO₃ and CaSiO₃ combination (2, 5, 10 and 15 mg/l, respectively). These results were compared to the corresponding results recorded with the

Table 1: Effect of various concentrations of sodium, potassium and calcium silicate on the number of shoots and leaves as well as the length of leaves (cm) after one month of subculture

Silicate (mg/l)						
Treatments	Na ₂ SiO ₃	K ₂ SiO ₃	CaSiO ₃	No. of Shoots	No. of Leaves	Length of Leaves (cm)
N	0	0	0	2.67 cd	6.00 f	6.00 b-d
N1	2	2	2	4.33 ab	8.67 b-d	4.83 d
N2	2	5	5	2.67 cd	7.00 ef	9.20 a
N3	2	10	10	4.00 a-c	9.33 bc	6.67 bc
N4	2	15	15	5.00 a	14.33 a	5.17 cd
N5	1	2	2	3.33 b-d	8.00 c-e	7.50 b
N6	1	5	5	3.00 b-d	7.67 de	6.67 bc
N7	1	10	10	2.33 d	5.67 f	5.00 cd
N8	1	15	15	3.67 a-d	9.67 b	7.50 b

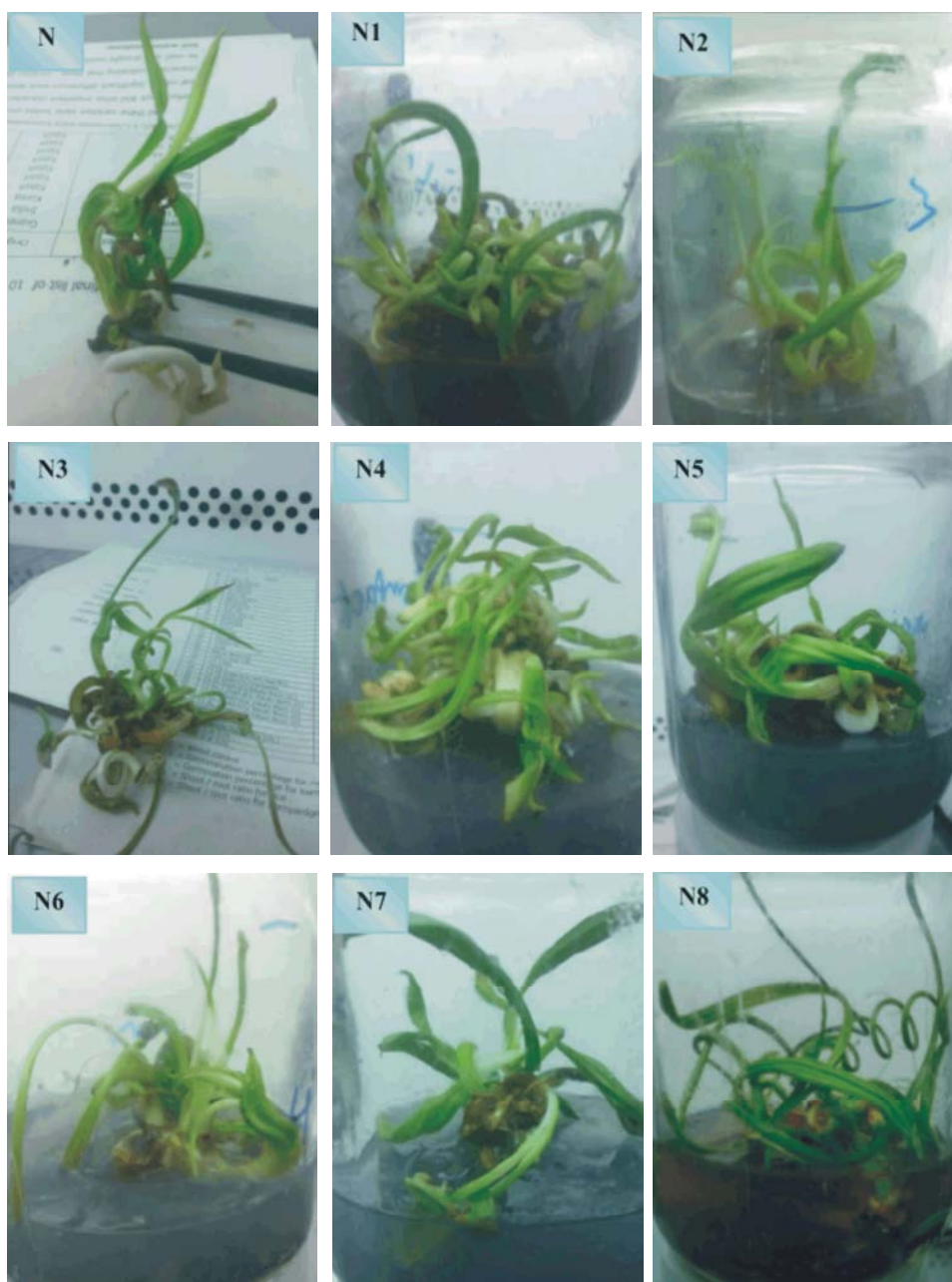


Fig. 1: Effects of different silicate sources and concentrations on the *in vitro* shootlets development of date palm cv. Maktoum, after one month of subculture

level of 1 mg/l Na_2SiO_3 , where, the number of leaves started from 8.00 decreased to 7.67 more decreased to 5.67 and increased again to 9.67 with the highest concentration of K_2SiO_3 and CaSiO_3 combination (15 mg/l), while the control recorded the lowest number of leaves (6.00). It can be concluded that the highest number of leaves (14.33) was recorded with the highest concentration of each of Na_2SiO_3 , K_2SiO_3 and CaSiO_3 (2, 15, 15 mg/l, respectively).

Length of Leaves: The highest leaf length (9.20 cm) was recorded with 2, 5, 5 mg/l Na_2SiO_3 , K_2SiO_3 and CaSiO_3 , respectively followed by 7.50 cm with 1, 2, 2 mg/l and 1, 15, 15 mg/l and then 6.67 cm with 2, 10, 10 mg/l and 1, 5, 5 mg/l. The other concentrations of the silicate combination (N1, N4 and N7) recorded the worst leaf lengths (4.83, 5.17 and 5.00 cm, respectively) compared to the control, which recorded 6.00 cm, as shown in Fig. 1.

Table 2: Effect of various concentrations of sodium, potassium and calcium silicate combinations on the number of shoots and leaves as well as the length of leaves (cm) after three months of subcultures

Silicate (mg/l)						
Treatments	Na ₂ SiO ₃	K ₂ SiO ₃	CaSiO ₃	No. of Shoots	No. of Leaves	Length of Leaves (cm)
N	0	0	0	2.67 cd	6.00 e	5.50 cd
N1	2	2	2	2.33 d	6.67 de	4.33 d
N2	2	5	5	2.67 cd	6.67 de	8.83 a
N3	2	10	10	2.67 cd	8.00 cd	5.33 cd
N4	2	15	15	5.00 a	12.67 a	4.83 d
N5	1	2	2	4.33 ab	9.00 bc	6.83 bc
N6	1	5	5	2.67 cd	6.33 de	4.00 d
N7	1	10	10	5.00 a	10.33 b	7.67 ab
N8	1	15	15	4.33 ab	9.00 bc	6.83 bc

From the previous data, it can be concluded that, after one month of subculture the highest number of shoots (5.00) and the highest number of leaves (14.33) were obtained on using the silicate combination with the concentration 2, 15, 15 mg/l which recorded a shorter leaf length (5.17 cm). On the other hand, the longest leaf length (9.20 cm) was recorded with the silicate combination of concentration 2, 5, 5 mg/l.

These results are in agreement with Soares *et al.* [20], whose used silicate sources in the shoot multiplication of *Cattleya loddigesii* and found that K₂SiO₃ was more effective than Na₂SiO₃. On the other hand, increasing the concentration of Si in the shoot induction medium significantly increased the average number of shoots per explant. Among the various concentrations tested, 7.2 mM Si was found to be the best for adventitious shoot induction [21].

The effect of silicate on the formation of bulblets from bulb scale explants of *Lilium longiflorum* was studied and the results suggested that bulblet formation can be significantly enhanced by Si as the number of bulblets increased with increasing the concentration of Si in the culture medium containing BA [22]. Also, Silicate supplementation to the culture medium increases the shoot induction frequency and average number of shoots per explant [23]. Furthermore, it has been shown that Si can be effective for shoot regeneration in rice and reed [24, 25].

After three subcultures of different silicate concentrations, it was found that the repeat planting on the media containing high Na₂SiO₃ concentration (2 mg/l) had the growth of the explants remained constant and decreased with some concentrations.

Number of Shoots: From the data in Table 2 it can be concluded that the highest number of shoots (5.00) was obtained in the third subculture using the silicate

combination with the concentration 2, 15, 15 mg/l however; with other combinations (2, 5 and 10 mg/l K₂SiO₃ and CaSiO₃) the number of shoots decreased and ranged between 2.33 and 2.67, respectively.

However, the continuous re-culture on the multiplication medium containing 1 mg/l Na₂SiO₃ with different concentrations of K₂SiO₃ and CaSiO₃, the results was very good.

The highest number of shoots (5.00) was obtained in the third subculture using the highest concentration of K₂SiO₃ and CaSiO₃ at (15 mg/l) with 1 mg/l Na₂SiO₃. The number of shoots with 1 mg/l Na₂SiO₃ started with 3.67 increased to 4.33, then decreased to 2.67 which increased again and reached to 5.00 with increasing the concentrations of K₂SiO₃ and CaSiO₃ in the order of 2, 5, 10 and 15 mg/l, as shown in Fig. 2.

Number of Leaves: With the continuous re-culture on a medium containing 2 mg/l Na₂SiO₃ and different concentrations of K₂SiO₃ and CaSiO₃ decreased the number of leaves, where the lowest number was 6.67 with N1 and N2 treatment, increased to 8 and 12.67 leaves, with N3 and N4 treatments, respectively.

However, after 3 subcultures with Na₂SiO₃ at 1 mg/l and different combinations of K₂SiO₃ and CaSiO₃ was found that the number of leaves relatively increased to 8.67 and 9.0 leaves/explant then decreased to 6.33 leaves/explant which increased again and reached to 10.33 leaves/explant with increasing the concentrations of K₂SiO₃ and CaSiO₃, in the order of 2, 5, 10 and 15 mg/l, respectively.

Length of Leaves: From the data in Table 2, it can be concluded that regarding the leaf length, there was no noticeable change in all treatments but it was noticed decreased length of leaves with 2 mg/l Na₂SiO₃ and different concentrations of K₂SiO₃ and CaSiO₃. The lowest

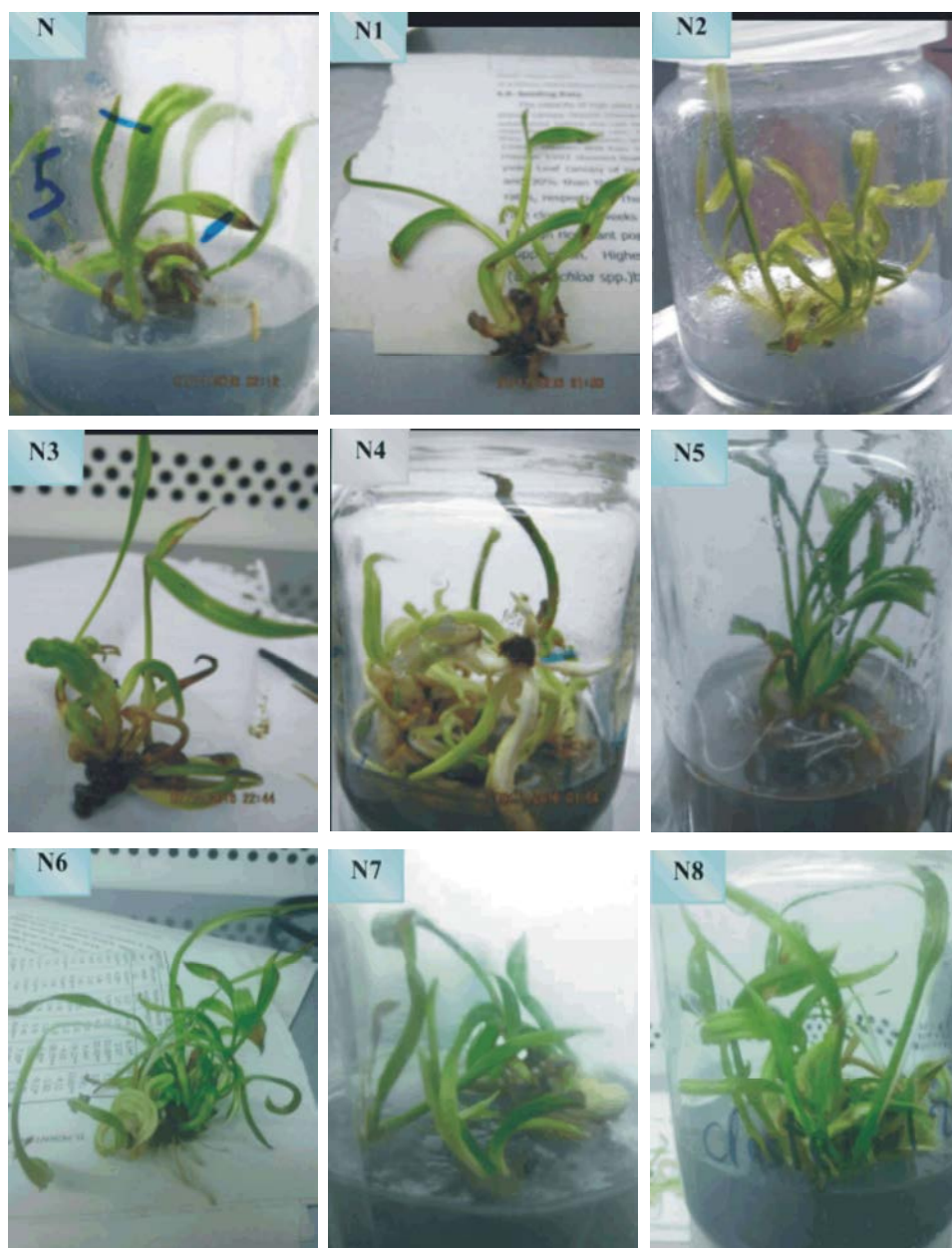


Fig. 2: Effects of different silicate on the shootlets growth of date palm cv. Maktoum after three months of subcultures

length of leaves (4.33, 5.33 and 4.83 cm) was obtained in the third subculture on using the silicate combination with the concentration 2, 2, 2 mg/l; 2, 10, 10 mg/l and 2, 15, 15 mg/l. whereas the longest length of leaves (8.83 cm) was recorded with the silicate combination of concentration 2, 5, 5 mg/l.

Using Na_2SiO_3 at 1 mg/l, the length of leaves was good where it recorded the high length of leaves (7.44 cm) was recorded with 1, 2, 2 mg/l Na_2SiO_3 , K_2SiO_3 and CaSiO_3 , respectively followed by 6.83 cm with 1, 5, 5 mg/l and then

4.0 cm with 1, 10, 10 mg/l then increased again to 7.67 cm with 1, 15, 15 mg/l compared to the control which recorded 5.50 cm.

These results are in agreement with Soares *et al.* [20] who found that MS medium has the highest concentrations of K_2SiO_3 and low Na_2SiO_3 increased the number of leaves in the seedlings Orchid. Similar results were observed by Villa *et al.* [26] on black mulberry leaves cultivated *in vitro*, where leaves number increased with the increase in K_2SiO_3 concentrations. In addition, Silva

Table 3: Effect of various concentrations of sodium, potassium and calcium silicate combinations on the number of shoots and leaves, as well as, the length of leaves (cm), number of roots and length after four months of subcultures

Silicate (mg/l)								
Treatments	Na ₂ SiO ₃	K ₂ SiO ₃	CaSiO ₃	No. of shoots	No. of leaves	Length of leaves (cm)	No. of roots	Length of roots (cm)
N	0	0	0	6.67 b	1.00 f	0.50 f	1.00 f	0.50 f
N1	2	2	2	4.83 cd	2.00 de	0.50 f	2.00 de	0.50 f
N2	2	5	5	10.23 a	5.00 c	2.00 e	4.00 c	2.00 e
N3	2	10	10	6.17 bc	1.00 f	0.50 f	1.00 f	0.50 f
N4	2	15	15	7.44 b	3.00 d	3.50 cd	3.00 d	3.50 cd
N5	1	2	2	10.44 a	6.00 ab	5.50 b	6.00 a	5.50 b
N6	1	5	5	7.17 b	7.00 a	4.00 cd	5.00 ab	5.00 c
N7	1	10	10	4.50 d	2.00 de	6.50 a	2.00 de	6.50 a
N8	1	15	15	6.78 b	2.00 de	5.00 c	2.00 de	5.00 c

[27] who worked with gerbera (*Gerbera jamesonii*), found a larger number of sheets using CaSiO₃ compared to the other silicon sources which indicated that this element is absorbed by the roots and translocated to the shoot, where it plays physiological roles and structures in leaf anatomy. Another study reported that the growth and development of plants are affected by Si application. For instance, *Phalaenopsis hybrid* plantlets cultured in Went and Vacin medium containing CaSiO₃ showed increased leaf growth [28].

The results of another study investigating the effects of diverse Si sources, such as Na₂SiO₃, K₂SiO₃ and CaSiO₃ on the anatomical characteristics and growth of strawberry seedlings showed that the seedling's fresh and dry weight were increased in MS medium supplemented with 1.0 g/l Na₂SiO₃ [29].

Number of shoot

From the data in Table 3, it can be reported that the growth and development of plants are affected by Si application. However, after four subcultures from using high concentration of Na₂SiO₃ at 2 mg/l with different concentrations of K₂SiO₃ and CaSiO₃, it was found that continuous the number of shoots in decreasing and ranged between 2.67, 2.33 and 4.00 shoots/explant. Also, when using Na₂SiO₃ at 1 mg/l with different combinations of K₂SiO₃ and CaSiO₃ led to decreased in shoots number (2 - 4.33 shoots/explant).

Number of Leaves: From the data in Table 3, it can be reported that the number of leaves ranged between (6.67 to 9.0 leaves/explant) with constant concentration of Na₂SiO₃ (2 mg/l) and various concentrations of K₂SiO₃ and CaSiO₃ combination (2, 5, 10 and 15 mg/l, respectively). These results were compared to the corresponding results recorded with the use a lower concentration of Na₂SiO₃ at (1 mg/l) where the treatment N6 (1, 5, 5 mg/l) recorded the highest number of leaves

(10 leaves) followed by the treatment N5 (1, 2, 2 mg/l) which recorded 9 leaves then decreased to 7.0 more decreased to 6.67 with the highest concentration of K₂SiO₃ and CaSiO₃ combination (15 mg/l), while the control recorded the lowest number of leaves (6.00). It can be concluded that the highest number of leaves (10.0 leaves/explant) was recorded with the concentration of each of Na₂SiO₃, K₂SiO₃ and CaSiO₃ (1, 5, 5 mg/l, respectively).

Length of Leaves: The worst treatments regarding the leaf length were N1 and N7 (4.5 and 4.83 cm, respectively). Regarding the leaf length, there was no noticeable change in all treatments but it was noticed the leaves length decreased with 2 mg/l Na₂SiO₃ and different concentrations of K₂SiO₃ and CaSiO₃. The high length of leaves was 10.23 cm recorded in the treatment N2 with Na₂SiO₃ at concentration 2 mg/l and 5, 5 mg/l of K₂SiO₃ and CaSiO₃.

The use of Na₂SiO₃ with concentration of 1 mg/l led to the length of leaves was good, where it recorded the highest length of leaves 10.44 cm with N5 and with increasing the K₂SiO₃ and CaSiO₃ concentration to 5 and 10 mg/l, the length decreased to 7.17 cm and 4.50 cm, respectively. The increase in the concentration of K₂SiO₃ and CaSiO₃ to 15 mg/l increased the leaf length again to 6.78 cm compared to the control, which recorded the worst results in all of the studied characters, as shown in Fig. 3.

Number of Roots and Length of Roots: All plantlets resulting from previous subcultures were then transferred to a pre-rooting medium containing 1 mg/l NAA and 0.5 mg/l IBA as a control medium and then different combinations of silicate salts were used.

Regarding the effect of different media on the number and length of roots, it was found that the control medium recorded the lowest roots number (1 root) and the

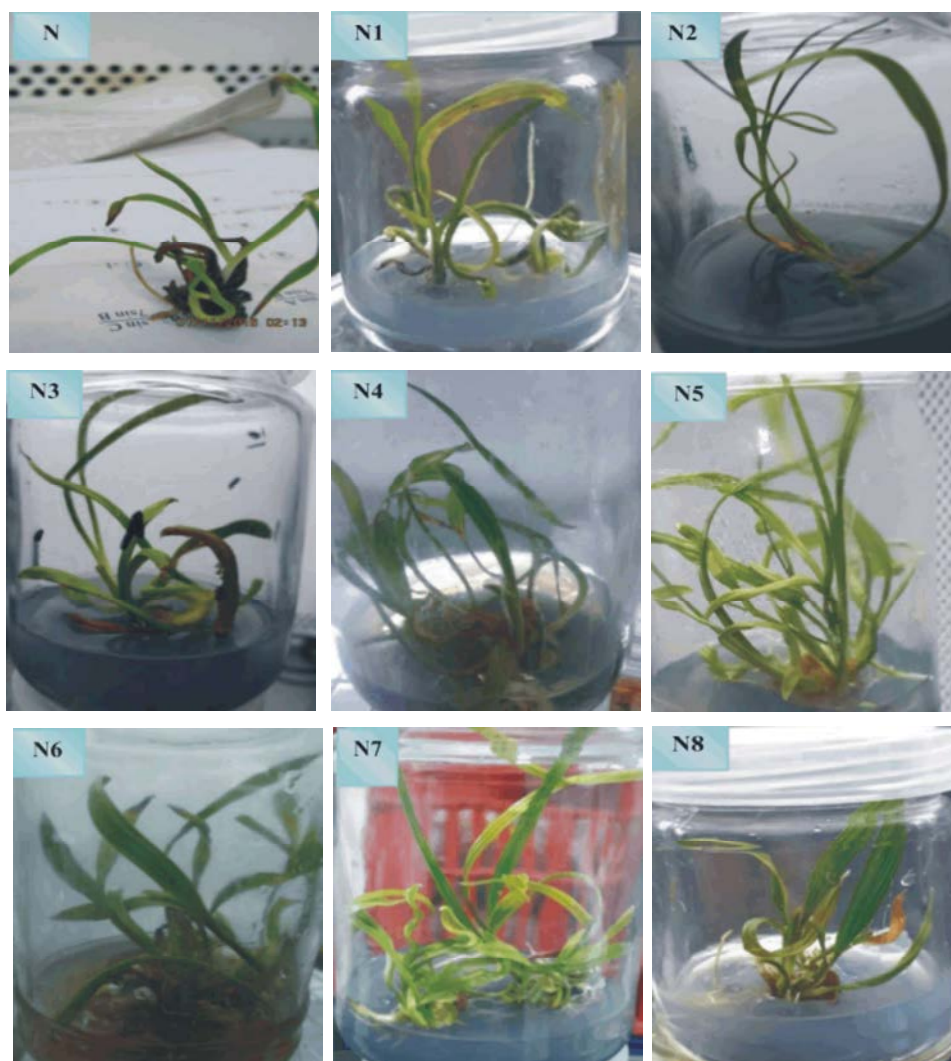


Fig. 3: Effect of different silicates on the shoots growth and rooting of date palm cv. Maktoum, after 4 months in culture

shortest length (0.5 cm). The number of roots started from 2 root which increased to 4.0 root then decreased to 1.0 root and increasing again to 3.0 root with constant concentration of Na_2SiO_3 (2 mg/l) and various concentrations of K_2SiO_3 and CaSiO_3 combination (2, 5, 10 and 15 mg/l, respectively).

These results were compared to the results recorded with 1 mg/l Na_2SiO_3 . The treatment of N5 recorded the highest number of roots (6.0) with length 5.5 cm, followed by 5.0 roots of length 5.0 cm with the treatment N6. However, the increase in K_2SiO_3 and CaSiO_3 in the treatment N7 decreased the number of roots to (2.0) with the highest length (6.5 cm).

It can be concluded that the best root number was recorded with the use of 1 mg/l Na_2SiO_3 with 2, 5 mg/l for each of K_2SiO_3 and CaSiO_3 to 6, 5 roots of length 5.5, 5 cm

and the formation of 4 secondary roots of 0.5 cm length was noticed.

It was also noticed that the lowest bacterial contamination was detected in the treatment N4 supplemented with the highest concentration of silicate sources.

These results are in line with the results of Soares *et al.* [20] who found that there was significant root formation in the treatment containing 5 mg/l K_2SiO_3 compared to the other treatments. Furthermore, Silva [27] reported that silicon deposition on the wall of cells makes the plant more resistant to the action of fungi and insects and prevents loss excessive water, decreasing the rate of perspiration. This study also showed that an increase in the Si concentration in the MS media leads to a decrease in the number of leaves grown per shoot. In addition, it

Table 4: Effect of various combinations of sodium, potassium and calcium silicate on the number of leaves, length of leaves, number of roots and length of roots (cm) after five months of subcultures.

Treatment	No. of leaves	Length of leaves (cm)	No. of roots	Length of roots (cm)
N5	7.33 bc	8.03 a	9.00 a	8.00 b
N6	10.67 a	6.67 ab	7.00 b	10.00 a

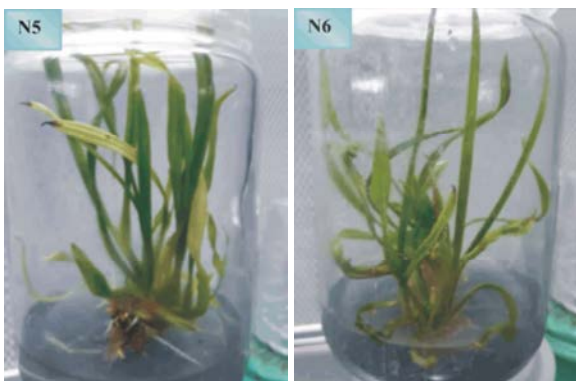


Fig. 4: Effect of various combinations of silicate on the number of leaves length of leaves, number of roots and length of roots (cm) of date palm cv. Maktoum after five months of subcultures.

has been reported that Si treatment of the culture medium causes an increase in the growth traits of plants under salinity stress [30]. The results are in conformity with Mali and Arey [31] who observed that Si additions increased the root and shoot lengths as well as leaf area of *Vigna unguiculata*. Also, Si sources have different influences on *in vitro* plants. Most of them have shown that Na_2SiO_3 caused lower growth rate and chlorophyll content compared to CaSiO_3 [11].

It was cleared from Table 4 and Fig. 4 that there was significant difference between the two treatments (N5 and N6) regarding number of leaves, number and length of roots after five months of subcultures. The highest number of leaves (10.67) with length (6.67 cm) recorded with treatment N6 (1 mg/l Na_2SiO_3 + 5 mg/l K_2SiO_3 + 5 mg/l CaSiO_3). The treatment N5 (1 mg/l Na_2SiO_3 + 2 mg/l K_2SiO_3 + 2 mg/l CaSiO_3) resulted in the highest leaf length (8.03 cm). On the other hand, the largest number of roots (9.0) obtained with the treatment N5 while the longest root length (10 cm) was recorded with the treatment N6.

These results are in agreement with; the benefits of silicate conferred on plants are his contribution to the wall structure roots and leaves. So this element has no definite metabolic role in plants and their action, according to Malavolta [32], causes which, together contribute to higher productivity. Root length significantly increased with increasing concentration of Si in the culture medium. The greatest root length (15.4 cm) was obtained on the

MS medium containing 7.2 mM Si [21]. Silicate tends to accumulate in the leaves forming a protective barrier and regulating plant water loss by perspiration, assisting the process acclimatization of micropropagation. When transferred to the *ex vitro* environment, the main cause mortality during this process is due to the loss of water, the low stomata and layer functionality epicuticular wax [33]. It has been reported that the inclusion of Si in the rooting medium causes an increase in the thickness of the leaf tissue and a deposition of epicuticular wax in strawberry Braga *et al.* [29] and banana (*Grande Naine*) Asmar *et al.* [11, 34] plantlets. The modified rooting medium supplemented with K_2SiO_3 , Na_2SiO_3 and CaSiO_3 improved the leaf tissue anatomy of banana plantlets [35]. It was also reported that Si was able to increase the rooting of the *Phalaenopsis hybrid* [28]. In study with strawberry, Braga *et al.* [29] checked that the deposition of wax was observed with the use of silicate (Na_2SiO_3) thus avoiding the water loss through the epidermis. Seedlings grown in sodium silicate displayed significant differences, with increased photosynthetic and transpiration rates, stomatal conductance and internal CO_2 concentrations. Under *in vitro* conditions, the addition of sodium silicate to the culture medium affected the photosynthesis and leaf anatomy of *A. andraeanum*, developing anatomical and physiological characteristics that contributed to the survival *ex vitro* [36]. The action of silicate has been associated with several indirect effects such as increased efficiency of photosynthetic capacity, reduction of perspiration and increased mechanical resistance of cells [37].

These results are non-agreement with; results were observed in the leaves of blackberry (*Rubus spp.*) cultivated *in vitro*; the number of leaves increased as incremental concentrations of K_2SiO_3 were added to the modified MS culture medium and the highest number of leaves was observed at a concentration of 1 g/l K_2SiO_3 [26]. The maximum length of leaves (tomato) was found with concentrations of 0.1-1.0 mg/l of potassium silicate in nutrient solution from Hoagland [38]. No statistical differences in the number of leaves of seedlings of the banana variety 'Maçã' grown in MS medium as compared to MS medium containing sodium, potassium and calcium silicates and when studying the effect of sodium, potassium and calcium silicates added to an MS culture

medium for the micropropagation of banana variety ‘Maçã’, observed no statistical differences in the length of the roots or their fresh and dry masses when compared to the control medium (MS medium with no silicate addition) [39]. The lowest number of shoots (8.37) was obtained with the use of 20 mg/l of silicate sodium and 20 m/l of potassium silicate and the root number and the growth of *C. loddigesii* seedlings grown in modified Knudson medium supplemented by Na₂SiO₃ (20.0 mg/l) and K₂SiO₃ (5.0 mg/l) were significantly increased [20].

Acclimatization: The acclimatization is the most important stage in the protocol of date palm micropropagation. Factors affecting the successful production of free-living date palm, including length of plantlets, strength of root system, humidity conditions and number of leaves and composition of the soil. In the present study, successful adaptation of *in vitro* plantlets of date palm cv. Maktoum was obtained by transplanting well rooted plantlets into pots contained peat moss and perlite into a mixture (2:1) under high humidity conditions, as shown in Fig. 5. The highest survival (70%) may be due to the healthy and well developed root system and the composition of transplanting medium. Similar procedure has been reported by Tisserat [40] who elucidated that high survival rate was obtained when date palm plantlets with 2-3 foliar leaves and of shoot length greater than 10 cm (with a well-developed adventitious root system) were transplanted in pots containing a mixture of peat moss and vermiculite. However, Othmani *et al.* [5] reported that rooted plantlets of date palm cv. Deglet Nour were hardened through growing in liquid medium containing half the strength of MS, coupled with incubation under high intensity illumination prior to transfer to the soil mixture.

Determination of Chlorophylls Content: The physiological observations in leaves of date palm cv. ‘Maktoum’ micropropagated using silicate sources on culture medium showed that Si provided significant effects on the organization of tissues, the chlorophylls and biocompounds formed by the cells, such as cellulose, hemicellulose.

Leaves of micropropagated plants with Si sources showed higher levels of chlorophyll a, b and total. Treatment N6 with (Na₂SiO₃ 1 mg/l + K₂SiO₃ 5 mg/l + CaSiO₃ 5 mg/l) added to the medium resulted in leaves more chlorophyll a (2.8 mg ml⁻¹) compared to other treatments. Also, treatments N6 showed plants with higher levels of chlorophyll b (1.7 mg ml⁻¹) and total chlorophyll (3.3) compared to the control Table 5.



Fig. 5: Acclimatization stage

Table 5: Chlorophyll a, b, total and a/b ratio of leaves of date palm ‘Maktoum’ cultivated *in vitro* with different silicate sources after 5 months.

Treatments	Chlorophyll (µg ml ⁻¹)		
	a	b	Total
Control	1.2	0.7	1.15
N5	2.2	1.2	2.2
N6	2.8	1.7	3.4

Asmar *et al.* [21] reported that K₂SiO₃ and CaSiO₃ treatments showed a significant increase in the production of chlorophyll b (40.59%) and total chlorophyll (26.24%) compared with the other treatments. Overall increase in the levels of chlorophyll a, b and total in the presence of CaSiO₃. Supplementation of culture medium with Na₂SiO₃ promoted increase in length, fresh and dry weight of shoots. The increase in chlorophyll production in plants grown with the addition of Si is in agreement with the results reported by Yao *et al.* [41] and Asmar *et al.* [34].

Determination of Contents of Cellulose and Hemicellulose: There was no significant difference between the two treatments of silicates. The biocompounds cellulose and hemicellulose had no significantly influenced by application of different combinations of Si. Higher contents of cellulose (1.4% and 1.6%) were obtained with the use of N5 and N6, respectively. There was a higher hemicellulose content (1.7 and 2.0%), when we used the same treatments compared to the control medium, as shown in Table 6.

Table 6: Levels of cellulose and hemicellulose of date palm leaves (Maktoum) cultivated *in vitro* with different silicate sources after five months

Treatments	Cellulose (%)	Hemicellulose (%)
Control	0.8 b	0.6 b
N5	1.4 a	1.7 a
N6	1.6 a	2.0 a

It is known that Si acts in the natural defense of plants, resulting in the formation of tannins and other chemicals such as lignins [42]. Silicate added to the culture medium can benefit the plants by increasing the hemicellulose and lignin content, thereby increasing the hardness of the cell wall. These changes increase the plants survival rate during acclimatization. The direct effects of silicate are accompanied by several indirect effects including an increase in photosynthetic capacity, reduction of transpiratory rates, greater plant growth and increased mechanical resistance of the cells [43]. Si participated directly or indirectly in the synthesis of cellulose and hemicellulose because its presence in the medium led to higher levels of these biomolecules in leaves of banana 'Grande Naine'. These plants had chemical substances on plant resistance to pathogens, once the limitation to action of them was generated by forming a physical barrier [21]. Modifications in the middle composition such as the addition of sources of silicate promote effects beneficial in plants by increasing the hemicellulose and lignin; thus increasing the stiffness in the cell wall, causing high rates are achieved of plant survival in acclimatization [8].

CONCLUSION

Date palm shootlets present higher growth (number of shoots, number of leaves and length of leaves) in culture medium MS plus 1 mg/l of Na_2SiO_3 with different combinations of K_2SiO_3 and CaSiO_3 after three subcultures. Continuous reculture on medium supplemented with 2.0 mg/l Na_2SiO_3 with different concentrations of K_2SiO_3 and CaSiO_3 decreasing the growth of shootlets. Higher number of roots is verified with addition of 1 mg/l Na_2SiO_3 to the culture medium plus 2 and 5 mg/l for K_2SiO_3 and CaSiO_3 .

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