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STIMULATION OF GROWTH RATE OF DATE PALM PLANTLETS DERIVED FROM TISSUE CULTURE BY USING A MODIFIED FORMULATION OF GIBBERELLIC ACID

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ABSTRACT

Date palm plantlets derived from tissue culture suffer from slow rate of growth. Large sizes, of such plantlets, are also sold for higher price. Thus, producers and growers are demanding a method to stimulate the growth rate of these plantlets regenerated by tissue culture. In this study, gibberellic acid was applied alone at 100 ppm or informulations at 50 ppm. Phosphoric acid at 1%, v,v and urea at 1% w/v were used to increase the efficacy of sprayed GA_3.Various treatments were applied twice, one month apart using two cultivars, namely Nabtat seif and Sultana. Some morphological parameters were determined after nursing such plantlets in the plastic house for 7 months. This study revealed that the formulation containing GA_3 at 50 ppm plus phosphoric acid and urea, or Gibberellic acid at relatively higher concentration (100 ppm) were also able to significantly and consistently increase plantlet height, petiole length in Nabtat Seif and Sultana in addition to internade length in Sultana plantlets. Meanwhile, there was no significant difference between the efficacy of GA_3 (at 100 ppm) and the formulation containing GA_3 (at 50 ppm) plus phosphoric acid and urea. However, most treatments did not significantly increase the number of adult leaves as compared with the control. Thus, producers of date palm plantlets derived from tissue culture and growers might be able to stimulate their growth rate by adopting the procedure shown in this investigation.

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INTRODUCTION

There is an increasing demand on date palm offshoots in many dates production areas especially in the Gulf region. For example, in United Arab Emirates a minimum of 200 date palm trees must be planted in each farm granted by the government. Since the palm tree produces a limited number of offshoots in a certain time of its life span, and this number ranges from 1 to 33 offshoots depending on the cultivar
(Al- Jabbouri, 1993) or trees may not even produce any offshoots, research focused on the production of date palm derived from tissue culture. This technique results in true–to–type plants that are free of diseases and have the ability to produce great number of plants in a relatively short time as compared with conventional method of date palm reproduction. Date palm growers have been demanding much more vitro plants to fulfill their need.

However, there has been a general complaint about the slow rate of growth of such *in vitro* derived data palm plantlets. Companies that are selling micropropagated date palm plantlets have been raising prices of such plantlets in proportion to their size and if they had compound (adult) or simple (juvenile) leaves. More mature date palm plantlets also have more tolerance to stressfull conditions in the field especially heat and salt stresses, in addition to starting the fruiting phase earlier than smaller ones

Moreover, transplanted date palm plantlets must be shaded in the field to protect them against the heat stress injury. This shading results in reducing their photosynthetic efficiency and slowing down their vegetative growth. However, using organic or chemical fertilizers to feed transplanted offshoots is recommended to start after one year of transplanting to avoid chemical stress injury (Al- Shereiqi *et al.*, 1995). Thus, it is desired to stimulate the vegetative growth of such plants by safe and feasible means.

It is also important to invigorate the growth of such *in vitro* derived plantlets to enable them to tolerate harsh conditions in the field and to accelerate their maturity.

The objectives of this study were to stimulate the growth of tissue culture derived date palm plants and to accelerate leaf maturity by using two cultivars of high quality in the Gulf region, namely

Nabtat Seif and Sultana. It was also aimed at setting a regime that could be adopted by date palm growers by treating such plants in the plastic house so they reach to the desired plant size in a shorter time. Furthermore, date palm tissue culture laboratory can also benefit from such treatment after the acclimatization process in order to sell or distribute bigger plants.

MATERIALS AND METHODS

This study was conducted during the two successive seasons 1999 and 2000 using date palm plantlets derived from tissue culture through the organogenesis technique.

Plantlets were regenerated and acclimatized by the Date Palm Research and Development Unit that follows United Arab Emirates University and located at Al- Ain city in United Arab Emirates. The two used cultivars were Nabtat Seif and Sultana. Each plantlet had 4-5 leaflets. Each plantlet was transplanted to a pot (capacity 18 liters) where each one was grown independently in its own pot under the plastic house conditions. The pot soil composed of sand: peat moss as 1: 1 v/ v. The experiment was completely randomized with six replications per treatment. Each separate plantlet represented one replication. Plantlets were treated by using a hand sprayer and treatment solutions were sprayed to the run off. All treatments had the surfactant (S. B. Super) at 0.2% (v/v) to reduce the surface tension of sprayed solution and to increase the contant angle of sprayed droplets. The treatments were: control (water spray), GA_3 at 50 ppm, phosphoric acid (1%, v/v), urea (1%, w/v), GA_3 at 100 ppm, GA_3 (50 ppm) plus urea (1%), GA_3 (50 ppm) plus phosphoric acid (1%), and finally GA_3 (50 ppm) plus phosphoric acid (1%) and urea (1%). Plantlets received the treatments twice one month apart. All plants received nutrition by spraying with calcium nitrate (2%. w/v) plus the surfactant S.B super (at 0.2% v/v) two months after the second treatment. Measurements were taken after seven months of nursing the plantlets in the plastic house. These measurements, were plant height (cm), stem length (cm), stem girth (cm), leaf petiole length (cm), internode length at both sides of the adult leaf (pinae) expressed in the tables as sides one (right side) and two (left side) and the number of adult (partially and fully segmented) and juvenile leaves (lanceolate

and folded). The analysis of variance was conducted by using the Costat computer software. Comparison of means was done by the Duncan's Multiple Range at 0.05% level.

RESULTS AND DISCUSSION

Change in plant height in response to used treatments are shown in Table 1. The data indicated that both GA_3 at 50 and 100 ppm were able to increase "Nabtat Seif" plant height significantly as compared with the control in the first season. However, GA_3 at 50 ppm tended to increase plant height but the increase was not statistically significant in the second season.

Moreover, GA_3 (at 50 ppm) plus either urea or phosphoric acid resulted in significant increase in plant height in the first season. Even when the increase was not significant, all GA_3 treatments resulted in a general trend of increased plant height (Fig. A and B). Furthermore, the formulation containing GA_3 at 50 ppm plus phosphoric acid and urea resulted in a significant and consistent increase in plant height as compared with the control in both seasons. This later formulation did not also vary significantly from GA_3 at 100 ppm in terms of their effect on plant height. In addition, the use of either phosphoric acid or urea alone did not result in a significant increase in plant height when compared with the control.

With regard to the changes in stem length of "Nabtat Seif" it was found that GA_3 spray alone either at 50 or 100 ppm did not result in a consistent increase in stem length. However, the formulation GA_3 at
50 ppm plus urea caused a significant increase in stem length in both seasons. Again, phosphoric acid or urea spray alone did not result in a significant increase in stem length as compared with the control. Moreover, the formulation containing GA_3 (50 ppm) plus phosphoric acid and urea tended to increase stem length especially in the second season (Table 1).

Stem girth of "Nabtat Seif" plantlets did not significantly change in response to various used treatments in the first season. In a similar way, GA_3 at 50 ppm in the formulation containing phosphoric acid and urea was not able to significantly increase stem girth in both seasons. Even the treatment that increased stem length did not

adversely affect stem girth significantly when compared with the control.

Petiole length was significantly influenced by many treatments in “Nabtat Seif”. The results in Table 1 indicated that GA_3 whether at 50 or 100 ppm was able to significantly increase petiole length in both seasons when compared with the control. Meanwhile, both concentrations of GA_3 did not vary significantly in terms of their effect on petiole length. The addition of either urea or phosphoric acid to GA_3 (50 ppm) did not result in any added advantage in petiole length when compared with GA_3 (50 ppm) spray alone (Fig. 1 C, D). Furthermore, the formulation containing GA_3 at 50 ppm plus phosphoric acid and urea had a consistent increase in petiole length when compared with the control or spraying phosphoric acid or urea alone. Use of GA_3 at 100 ppm did not result in a significant difference when compared with using GA_3 at 50 ppm but in the formulation containing phosphoric acid and urea.

Internode length on both sides of the compound leaf was not, generally, influenced by various treatments. In side one (right side) of the leaf, only the formulation of GA_3 plus phosphoric acid and urea tended to increase the internode length especially in the first season as compared with the control in “Nabtat Seif” compound leaf. Similar trend was obtained for internode length in side 2 (the left side) of the compound leaf. The number of adult and juvenile leaves was not generally influenced by various treatments. Even with the use of the higher concentration of GA_3 (100 ppm) was not able to accelerate the formation of compound leaves from such simple leaflets that were generated by tissue culture (Table 1).

With regard to the morphological features of "Sultana" date palm plant as influenced by the treatments, it was found that using GA_3 alone either at 50 ppm or 100 ppm resulted in greater plant height than that of the control in both seasons. In a similar manner, the addition of phosphoric acid or urea to GA_3 at 50 ppm resulted in a significant increase in plant height in both seasons as compared with the control but did not significantly vary from just using GA_3 at 50 ppm alone. The formulation containing GA_3 plus phosphoric acid and urea also increased the plant height significantly in both seasons relative to the control. Moreove, the use of either phosphoric acid or

urea alone did not result in a significant stimulation to the plant height of "Sultana" (Table 2).

Stem length was not generally influenced by various treatments. Even at the relatively higher concentration of GA_3 (100 ppm), no significant increase was found in stem length when compared with the control. Similarly, the formulation of GA_3 plus phosphoric acid and urea tended to increase stem length but not enough to result in a significant change.

Responses of stem girth to various treatments were similar to that obtained with stem length. The use of GA_3 at 50 ppm plus phosphoric acid or urea or even the formulation of GA_3 plus phosphoric acid and urea did not result in a significant increase in stem girth when compared with the control in both seasons. The relatively higher GA_3 concentration (100 ppm) was not consistent in its effect on stem girth in both seasons.

Petiole length, however, was significantly affected by several treatments. GA_3 whether at 50 or 100 ppm resulted in a significant increase in petiole length in both seasons when compared with the control. The combination of GA_3 (50 ppm) plus phosphoric acid caused a significant increase in petiole length when compared with the control or phosphoric acid alone.

Meanwhile, the combination of GA_3 (50 ppm) plus urea tended to give greater petiole length than the control but this effect was significant only in the second season. The formulation of GA_3 plus phosphoric acid and urea was also significantly effective in increasing petiole length and did not significantly vary from that efficacy of GA_3 at 100 ppm (Table 2).

The response of the internode length in side one (right side of the compound leaf) was not significant due to spraying phosphoric acid or urea alone relative to the control.

However, GA_3 (at 100 ppm) and the formulation containing GA_3 (at 50 ppm) plus phosphoric acid and urea caused a significant increase in internode length when compared to the control. In a similar way, internode length in side two (the left side of the compound leaf) increased by the above two treatments consistently in both seasons.

The number of adult and Juvenile leaves was not generally affected by various treatments. Almost all treatments in both season

caused a similar conversion from simple to compound leaves. As stated by Al- Ghamdi (1993) vitro plants foliage can be classified into two major classes, namely: juvenile and adult leaves. Each class has two types of leaves as follow: the Juvenile leaves could be lanceolate type (the first appeared leaves that are simple, lanceolate in shape with three sub- parallel ribs). The second juvenile leaves are called folded (they are unsegmented, they are shorted and wider than the lanceolate type and had more than three folds).

The adult leaves could be partially segmented (partial segmentation of the lamina starting from the base of the leaf but still have at the leaf apex several folded leaves), or the adult leaves could be fully segmented (has the characteristic morphology of the normal compound adult leaf with a complete segmentation)

The present study provided evidences that date palm plantlets positively responded to GA_3 spray especially through the increase in plant height and the elongation of leaf petioles. Although the use of GA_3 at 100 ppm enhanced the plant growth, but some features of this growth could be stimulated by the use of GA_3 at 50 ppm in the presence of small amount of phosphoric acid and urea (Fig. 1).

Urea was found to increase the partitioning of sprayed material into the cuticle (Farag, 1989) while phosphorus is an important element for enhancing the plant vigor. Most GA applications on monocotyledons were on herbaceous plants. To the best of our knowledge, no previous studies have been done on date palm offshoots or tissue culture plants.

Gibberellins were found to stimulate the rate of photosynthesis when sprayed on barley plants (Wareing *et al*., 1968). In another study, Livine and Vaadia, (1985) found that gibberellins spray on barley increased the rate of photosynthesis, increased stomatal opening and the rate of transpiration. Similarly, Harber and Toblert (1957) found an increase in the rate of photosynthesis in oats. Pholem loading with carbohydrates was also found to increase as a result of GA spray as well as the activity of the most important enzyme in the photosynthetic pathways (Wareing et al., 1968, Arteca and Dong, 1981, Baker, 1985) namely, ribulose biphosphate carboxylase oxygenase (Rubisco).

The output of this research would be important for marketing stronger and more vigorous date palm plantlets derived from tissue culture. Growers of these in vitro plantlets might also adopt this formulation to obtain larger plants that resist harsh conditions in the orchard.

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الملخص العربي

تنشيط معدل نمو نباتات نخيل التمر النسيجية باستخدام تركيبة محوره من حمض الجبريلليك

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تعانى النباتات الصغيرة النسيجية من نخيل التمر من بطء معدل النمو، وتباع الأحجام الأكبر من تلك النباتات بسعر أعلى ولذلك يتطلب كل من المنتجين والمزارعين وجود طريقة لتنشيط نمو هذه الشتلات الناتجة من زراعة الأنسجة. استخدم فى هذه الدراسة حمض الجبريلليك وحدة بتركيز 100 جزء فى المليون أو فى تركيبه بتركيز 50 جزء فى المليون، وقد استخدم حمض الفوسفوريك بتركيز 1% (حجم/ حجم) واليوريا بتركيز 1% (وزن/ حجم) لزيادة فعالية حمض الجبريلليك مع التركيز الأقل. تمت المعاملة بالرش مرتين بينهما فترة شهر باستخدام الصنفين نبته سيف وسلطانة خلال موسمى 1999، 2000 وقد أخذت بعض القياسات المورفولوجية بعد فترة رعاية للنباتات المعاملة فى الصوبة لمدة سبعة شهور. أوضحت هذه الدراسة أن التركيبة المحتوية على حمض الجبريلليك بتركيز 50 جزء فى المليون وحمض الفوسفوريك واليوريا أو حمض الجبريلليك وحدة عند التركيز الأعلى (100جزء فى المليون) كانت قادرة على إحداث زيادة معنوية وبطريقة ثابتة فى ارتفاع النبات وطول عنق الورقة فى الصنفين نبته سيف وسلطانة بالإضافة إلى طول السلامية فى نباتات الصنف سلطانة. ولم يوجد اختلاف معنوى بين فعالية حمض الجبريلليك (عند 100جزء فى المليون) وبين التركيبة المحتوية على حمض الجبريلليك (عند 50جزء فى المليون) مضافا له حمض الفوسفوريك واليوريا. مع ذلك، لم تؤثر معظم المعاملات معنويات على عدد الأوراق البالغة (المركبة) بالمقارنة مع الكنترول.

وهكذا فإن يمكن لمنتجى شتلات نخيل التمر النسيجية والمزارعين أن يقوموا بتنشيط معدل نمو تلك الشتلات باستخدام المعاملات والنظام الموضح فى هذه الدراسة.

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