

The Combined Role of Sucrose with IBA and NAA in Rooting of Date Palm Somatic Embryos cv. Khanaizi

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Key words: Auxins, Rooting, Combined effect, Date palm, Somatic embryos

Abstract

The combined role of carbohydrate and auxin is effective in root formation of date palm somatic embryos. The best combination resulted a mean of 2.5 roots per embryo and reached up to ten good and healthy roots when the rooting medium containing 60 g/l sucrose supplemented with NAA was used. 60 g/l sucrose combined with 0.4, 0.6 and 0.8 mg/l of either NAA or IBA gave about 74% root thickness. IBA with a low sucrose concentration of 30 g/l promoted root length, while NAA was found to be superior over IBA, when a high concentration of sucrose of 60 g/l was used which may be due to the effect of NAA is on the main root while IBA on the lateral root.

Introduction

Date palm (*Phoenix dactylifera* L.) is a very important plantation crop and for millennia has been of immense value in maintaining and enhancing the quality of human life of Middle East inhabitants (Abo El-Nil et al. 1987).

Propagation of palms *in vitro* has been achieved through the initiation of embryogenic callus which subsequently gave rise to asexual plantlets (Gabr and Tisserat 1985)

Date palm plantlets from callus have poor root system due to the lack of adventitious roots (Hassan et al. 2008), the establishment of an effectual root system on *in vitro* is vital for subsequent success throughout acclimatization to autotrophic condition, In date palm however, embryos cultured on a hormone free medium often produce shoots only and require another step for rooting and shoot elongation, usually on medium enriched with NAA (Al-Khayri 2003)

The maturation of somatic embryo, germination, *in vitro* rooting and plant establishment can be influenced by various *in vitro* factors, such as sugar and auxin concentrations (Al-Khayri 2003, Al-Khateeb 2008a).

Plant cell, tissue or organ culture normally requires a carbohydrate supply in order to satisfy energy demands. It is well-known in plant tissue culture a continuous supply of carbohydrates is essential as the photosynthetic activity of *in vitro* plant tissues are reduced due to low light intensity, high relative humidity and limited gas exchange.

Limited studies, however, addressed factors affecting the formation and germination of somatic embryos in date palm such as temporary sucrose starvation (Al-Khayri 2003).

Therefore, this study was carried out to determine optimum conditions for the rooting of date palm somatic embryos in response to various concentrations of sucrose and auxin to minimize the period of culture system, shoot development and rooting.

Materials and Methods

Long somatic embryos (1 to 2 cm) of date palm cv. Khanezi Fig. 6(A) were selected from MS hormone free medium and inoculated vertically on the MS rooting media with either 30 or 60 g/l sucrose supplemented with 0, 0.2, 0.4, 0.6, and 0.8 mg/l of either NAA or IBA.

Other additives of the rooting medium were 100 mg/l myo-inositol, 0.1 mg/l thiamine-HCl, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine-HCl, 2 mg/l glycine. The medium was solidified with 7 g/l agar (purified agar-agar/gum agar; Sigma), adjusted to pH 5.7 dispensed in 60 ml culture tube (15 ml per tube), capped with closer and autoclaved for 20 min at 121°C and 1×10^5 Pa (1.1 kg/cm). The cultures were incubated at $26 \pm 2^\circ\text{C}$ in 16 h photoperiods (50 mmol/m²/s) and subcultured after six weeks to a fresh (MS) hormone-free medium.

The experiment consisted of factorial arrangements of treatments (sucrose at two levels, auxin type at two levels and auxin concentration at five levels) in a completely random design. Twelve replicates (culture tube) were assigned per treatment with one embryo per tube. Data were analyzed using the statistical analysis system, general linear model (GLM procedure, SAS Institute Inc., 2004) and means were evaluated by LSD.

The response was assessed 12 weeks later, on the number, length, density and thickness of embryo's roots.

Results and Discussion

The role of NAA as an effective auxin in root formation has been established in many plants moreover in date palm micropropagation, sucrose plays an important role in the initiation of roots. The addition of sucrose to culture

medium caused an increase in the number and length of adventitious roots per shoot as compared with either glucose or fructose (Hassan et al. 2008). The type of concentration of auxin and sucrose effected significantly on the root number of date palm somatic embryos, the best combination resulted in a mean of 2.5 roots per embryo (Fig. 1) and reached up to 10 good and healthy roots when the rooting medium with 60 g/l sucrose, supplemented with NAA was used. Al-Khateeb (2008a) noticed that the root formation was enhanced as the sugar concentration increased (60 g/l and above). This rooting of buds generally reduced their ability to multiply during multiplication stage and occasionally inhibited bud formation especially in higher concentration of sugar.

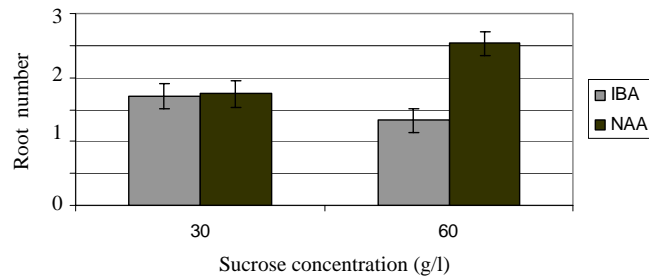


Fig. 1. Effect of IBA and NAA and sucrose concentrations in root number.

The rooting medium with 60 g/l sucrose combined with IBA gave the lowest root number with a mean of 1.3 root per embryo (Fig. 1), which contradicted with the findings (Yasodha et al. 2008), the auxin type IBA is essential for in vitro root formation in the cultures raised from mature tissues, where the carbon source is glucose instead of sucrose. Other findings recorded were rooting percentage and the number of roots enhanced with increasing date syrup concentration. This raise the possibility that root formation required more energy than bud or shoot formation since shoot formation was enhanced under low date syrup concentration (Al-Khateeb 2008b).

Data illustrated in (Fig. 1) show in the rooting medium containing 30 g/l sucrose with the auxins (NAA or IBA) did not exert any influence on the number of roots, where both of them gave mean of 1.7 roots per embryo.

The relation between different auxin and sucrose concentration apparently effected embryos root length of date palm cv. Khanaizi where 60 g/l sucrose with NAA gave a mean length of 2.6 cm (Fig. 2) and reached up to 7 cm (Fig. 6 B), while IBA with 30 g/l sucrose gave a mean of 2.4 cm length (Fig. 2). The combination of 60 g/l sucrose with NAA was found to best as against NAA with 30 g/l sucrose. Asemota et al. (2007) proposed to incorporate high sucrose and

moderate levels of NAA that will satisfactorily support the growth of roots in date palm plantlets cultured *in vitro*.

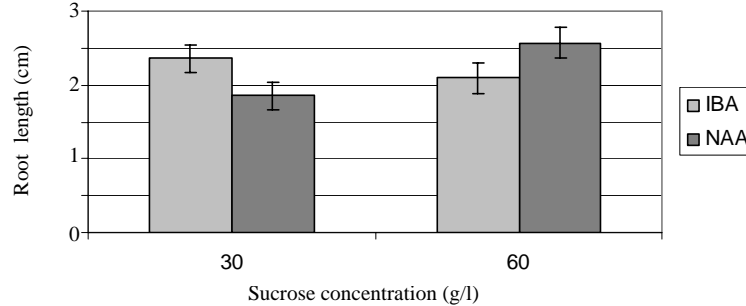


Fig. 2. Effect of IBA and NAA and sucrose concentrations in root length.

There was no significant difference in the root density of the main and lateral roots with NAA and IBA when the embryos were cultured in 30 g/l sucrose was found to be about 30% (Fig. 3).

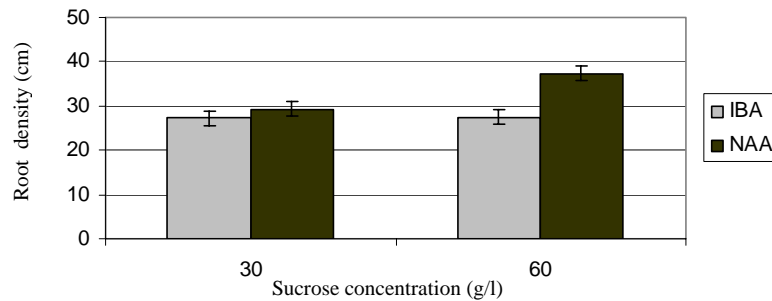


Fig. 3. Effect of IBA and NAA and sucrose concentrations in root density.

Comparing the root number in Fig. 1 and root density in Fig. 3, NAA was found to be superior over IBA when sucrose was 60 g/l, which may be due to the effect of NAA on the main root while IBA on lateral root. MacGregor et al. (2008) demonstrate that direct contact between the aerial tissues and sucrose in the growth media is necessary and sufficient to promote emergence of lateral root primordia from the parent root. Mild osmotic stress is perceived by the root, which then sends an abscisic acid-dependent signal that causes a decrease in the permeability of aerial tissues; reducing the uptake of sucrose from the culture media, which leads to a repression of lateral root formation

Root thickness was highly effected by sucrose and auxin concentration regardless the type of auxin used.

The concentrations of 0.4, 0.6 and 0.8 mg/l of auxin with 60 g/l sucrose gave about 74% of root thickness (Fig. 6C) compared to other concentration (Fig. 4). Low concentration of sucrose 30 g/l had no effect and all the auxin concentration

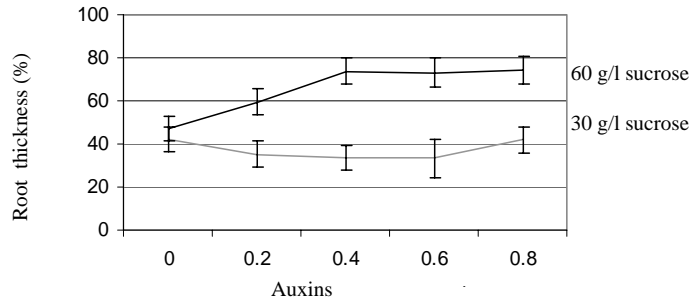


Fig. 4. Effect of auxin and sucrose concentration on root thickness.

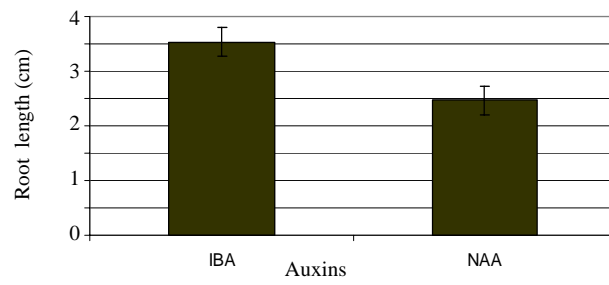


Fig. 5. Effect of IBA and NAA on the length of leaves.

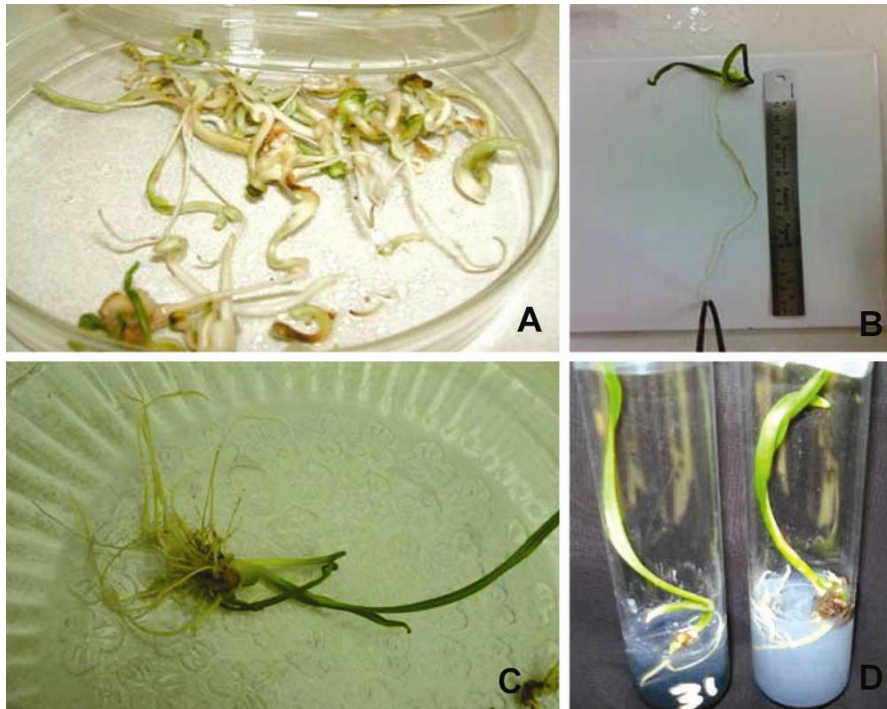


Fig. 6: (A). Somatic embryos (1-2 cm long of date palm). (B) Root length up to 7 cm. (C) Root density of main and lateral roots. (D) Plantlets with leaves and roots.

with 30 g/l of sucrose has the same effect with the control treatment. (Fig. 4), Al-Khateeb (2001) finds that the sugar concentrations and their interactions had significant effects on root thickness also. The root initiation and growth were high energy requiring processes that could only occur at the expense of available metabolic substrates, which were mainly carbohydrates. The data of Hassan et al. (2008) showed that the highest significant mean of root thickness was observed when 45 g/l sucrose was added to culture medium.

Generally NAA influences number of roots (Fig. 1) as against IBA in length of leaf (Figs. 5, 6D).

References

- Abo El-Nil M, Al-Ghamdi AS and Turjoman A** (1987). Role of tissue culture in propagation and genetic tree improvement of date palm. A perspective review. Proceedings of second symposium on the date palm, in Saudi Arabia, **1**: 1-27.
- Al-Khateeb AA** (2008a) Regulation of in vitro bud formation of date palm (*Phoenix dactylifera* L.) cv. Khanezi by different carbon sources. Bioresource Technology **99** (14): 6550-6555.
- Al-Khateeb AA** (2008b). Enhancing the Growth of Date Palm (*Phoenix Dactylifera*) in vitro Tissue by Adding Date Syrup to the Culture Medium. Scientific Journal of King Faisal University (Basic and Applied Sciences) **9**(1): 71-85.
- Al-Khateeb AA** (2001) Influence of different carbon sources on in-vitro root formation of date palm (*Phoenix dactylifera* L.) cv khanezi. Proceeding of the second international conference on date palm (Al-Ain UAE): 559-566.
- Al-Khayri JM** (2003) *In vitro* germination of somatic embryos in date palm: Effect of auxin concentration and strength of MS salts. Current Sci. **84**(5): 680-683.
- Asemota O, Eke CR and Odewale JO** (2007) Date palm (*Phoenix dactylifera* L.) in vitro morphogenesis in response to growth regulators, sucrose and nitrogen African Journal of Biotech. **6**(20): 2353-2357.
- Gabr MF and Tisserat B** (1985) Propagating palms in vitro with special emphasis on the date palm (*Phoenix dactylifera* L.). Scientia horticulturae **25**: 255-262.
- Hassan MM, Gadalla EG and Abd-El Kareim AH** (2008) Effect of sucrose and abscisic acid on *in vitro* growth and development of date palm during rooting stage. Arab J. Biotech. **11**(2): 281-292.
- MacGregor DR, Deak KI, Ingram PA and Malamy JE** (2008) Root System Architecture in Arabidopsis Grown in Culture Is Regulated by Sucrose Uptake in the Aerial Tissues. The Plant Cell **20**: 2643-2660.
- Yasodha R, Kamala S, Kumar SPA, Kumar DP and Kalaiarasi K** (2008) Effect of glucose on *in vitro* rooting of mature plants of *Bambusa nutans*. Scientia Horticult. **116**(1): 113-116.