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The Effect Of Salicylic Acid And Mannitol On The Propagation Of The Adventitious Shoot Buds Of The Date Palm Owidicv. In Vitro

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Abstract

This study was conducted in the tissue culture laboratory of the date palm Research Center, University of Basrah, during the period 2/1/2018 to 31/12/2019. It investigates four concentrations of salicylic acid (SA) and mannitol (MA) (0, 1, 2 and 3) mmoleL^{-1}. It also investigates the effects of all these concentrations on the development of vegetative buds of date palm Owidi cv. In vitro.

The results of this study revealed that buds cultured on a medium supplemented with SA at 2 mmolL^{-1} had the highest fresh and dry weights, which were 12.60 and 1.23 g, respectively, and had significant difference when compared to other treatments, with the exception of SA at 3 mmol$L^{-1}$for the fresh weight.

Furthermore, culture medium supplemented with 2 mmolL^{-1} SA or 1 mmolL^{-1} MA showed significantly higher buds number, with 20.28 and 19.5, respectively,when compared to the control.

The results indicated that when media was supplemented with MA at all concentrations, the content of proline and phenolic increased significantly, whereas when media was supplemented with SA at all concentrations, the content of proline decreased significantly.

In the elongation stage, SA and MA at all levels significantly increase the length of adventitious shoot buds and the content of chlorophyll and carotene compared to the control., with the highest length observed in SA at 3 mmolL^{-1} with 8.8 cm, while with 6.70 and 0.024 µgg^{-1} FW, respectively for chlorophyll and carotene in MA at 2 mmolL^{-1}.

Keywords: Apical bud, chlorophyll, fresh weight, phenol, proline.

Introduction

The use of plant tissue culture technologies for large-scale plant multiplication has become more common. Plant tissue culture techniques have recently gained industrial relevance in the areas of plant propagation, disease removal, plant enhancement, and secondary metabolite synthesis, in addition to its use as a research tool (Thorpe, 2007).

Several technical problems may be encountered during the different stages of date palm tissue culture at the laboratory, such as Browning of cultured tissues (Muhsen et al., 2015 ;Al-Mayahi et al.,2020), Vitrification of tissues (Muhsen 2007a ;Muhsen et al., 2020) and Bacterial and fungal contaminations (Muhsen et al., 2014).

Salicylic acid(SA) is a beta hydroxy acid and a monohydroxybenzoic acid. It serves as a plant hormone and is commonly utilized in organic synthesis. It is able to activate multiple pathogen resistance proteins and appears to have a role in systemic acquired resistance to infections (Georgeet al., 2008).

Mannitol(MA) is one of the alcoholic sugars that, due to their small size, have an easy movement within plant tissues and play an important role in plant vital processes such as respiration, energy release, and ATP production, also involved in plant growth through increased cell division and elongation, as well as carbohydrate metabolism (Taiz and Zeiger, 2006; Meena et al., 2015).In date palm tissue cultures.

Ibrahim et al (2014) found that SA may serve a beneficial effect in enhancing callus development and regulating somatic embryogenesis of bream cultivar.Al-Mayahi (2016) found that in medium enriched with 50 mgL^{-1} salicylic acid, maximal growth and chlorophyll content of shoots were recorded after 75 days of cultured date palm Nersy cv. Also, Muhsen et al (2020) reported that the addition of SA at 1 mgL^{-1} increases the diameter of the buds of and the leaf content of the chlorophyll, carotene and carbohydrate of Barhi cv., meanwhile reducing deformed buds number and phenols content.

MA significantly affects shoot bud proliferation of date palm cv. Mejhoul but with lower effect than sucrose (Mazri et al., 2016). Also, El-Bahr et al. (2016) found that adding 40 or 60 gL^{-1}MA to date palm Bartamoda cultivar resulted in the best percentage of survival under osmotic stress.

The aim of this work was to establish a viable approach for date palm Owidi cultivar in vitro conservation by introducing SA and MA into the culture medium to increase adventitious shoot buds features.

Materials and methods

The current study was carried out in the plant tissue culture laboratory of the Date Palm Research Center-University of Basrah for the period 2/1/2018 to 31/12/2019.

Plant materials and sterilization

Date palm cv. Owidi offshoots around four years old were separated from mature females growing in the north of Basrah governorate and utilized as plant materials. The offshoots were washed up by water, then cut in an ascending order by a sharp knife. Internal leaves were gradually removed, and apical buds measuring 10 cm in length and 8 cm in diameter were collected (Figure 1) and placed in an antioxidant solution (150 mg/l ascorbic acid + 100 mg/l citric acid) to avoid browning of the explants, then preserved at 4 ° C.Surface sterilization of the explants was performed in the laminar air flow cabinet using a 20% (v:v) sodium hypochlorite solution with one drop of tween20 per 100 cm^3 of solution, shaking and stirring for 20 minutes, and then sterile distilled water was used to rinse the explants three timesaccording to (Muhsen et al., 2016 ; Muhsen 2007b).

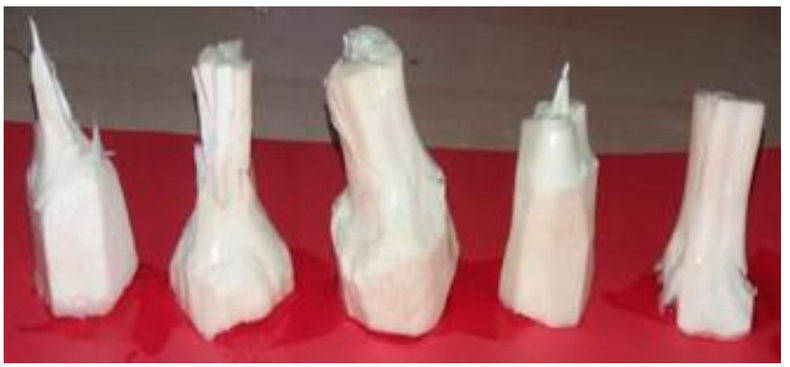

Figure (1). Apical buds used in the study

Nutrient Media

Murashige and Skoog (1962) medium (MS) with Gamborg vitamins as described by Gamborg et al. (1968) manufactured by Phytotechnology lab. 4.4 g of MS with the substances inserted in Table (1) was added to one liter of distilled water. After correcting the acidity of the medium to 5.8, solidification agent (agar) was added and the medium was heated to 95 °C, The medium was then split into tubes (25 x 2.5 cm) with 20 ml and into 350 ml jars with 50 mltubes and jars were tightly sealed with cotton and aluminum foil and autoclaved for 20 minutes at 121°C and 1.05 bar steam pressure, then vigorously shook to homogenize the medium and left until cultured.

Table (1) additives to the culture medium

Additive	Concentration (mgL^{-1})
Sucrose	30000
Sodium dihydrogen orthophosphates	170
Myoinositol	100
Adenine sulphate	30
Hcl Thiamine-Hcl	0.5
Glycine	2
Riboflavin	1
NAA (Naphthalene acetic acid)	0.5
NOA (Nophthyacitic acid)	0.5
2-ip (Isopentenyl adenine)	0.5
BA (Benzyl adenine)	0.5
Kn (Kinetin)	0.5
Activated charcoal	1000
Agar	8000

Explants culture

Apical shoot buds were cultivated on pre-prepared medium in a laminar airflow cabinet sterilized with ethanol (70%) and then incubated in darkness at 27±2 °C for six weeks with reculture on a similar medium. The adventitious shoot buds appeared after five recultures (Figure 2), and the plants were exposed to 1000 lux illumination for three months to develop enough buds for the current study trials.

Figure (2): Adventitious shoot buds produced in this study

Multiplication experiment

Four concentrations of SA(0,1,2,3)mmolL^{-1}were used together with four concentrations of MA(0,1,2,3) mmol$L^{-1}$to study the development of adventious budsof date palm Owedi cv.,0.5 g of adventitious shoot buds mass was cultured in jars containing the same medium used to induce apical buds with some modifications, such as the addition of: sucrose with 4000 mgL^{-1}, Adenine sulphate with 40mgL^{-1} and glycine 5 mg$L^{-1}$and not adding NOA and 2ip,the cultures wer incubated at 27±2 and 1000 lux light intensity. The subculture was performed once every 30 days, and the experiment continued for four months.10 replications per concentration were used to study the following traits:

1. Fresh weight (f.w) of adventitious shoot buds was measured after 120 days of culture.

2. Dry weight (d.w): tubes were randomly selected, the buds were removed from tubes and cleaned, and the samples were then placed in ovens at 65 °C for 72 hours before being weighed.

3.Number of shoot buds: calculated using the following equation:

$$\text{Number of shoot buds} = \text{number of buds at begin experiment} - \text{number of buds at end of experiment}$$

4. Estimate proline content. The plant tissue content of free Proline was estimated according to Bates et al (1973) method.

5. Determination of total phenols content

Phenolic content were estimated by the Folin-Denis method (Singleton and Rossi, 1965).

Elongation experiment

Five buds from the previous stage were cultured in jars with the same medium of the multiplication experiment, with the exception that the adenine sulphate concentration to 50 mgL^{-1} and no growth regulators were added. Cultures were incubated at 27±2 °C with 16 hours of light at 3000 lux. Subculture was done once every 30 days, and the experiment continued for 4 months. 10 replications per concentration were used to study the following traits:

1. Bud length: measured by using a ruler metric.
2. Chlorophyll and carotene content: determined according to Porra (2002).

Statistical analysis

The current study experiments were designed as simple experiments using the completely Randomized design (CRD). The results were analyzed using the statistical software Genestat version (8.1) of 2007 and the means were compared according to the least significant differences (LSD) test at a probability level of 0.05 (Al- Sahwki and Wehayeb, 1990) .

Results and discussion

Multiplication stage

At all levels investigated and as shown in table (2), SA and MA significantly increased fresh and dry weight, as well as the number of buds, as compared to the control. Except for SA at 3 $mmoleL^{-1}$ in fresh weight and MA at 1 $mmolL^{-1}$in number of buds, SA at 2 $mmoleL^{-1}$ significantly increased both fresh and dry weight, and also the number of buds, when compared to the other treatments, which were 12.60 g, 1.23 g, and 12.19, respectively, which were respectively in the control, 5.40 g, 0.49 g and 9.10. In contrast, as compared to the control, SA significantly reduced proline and total phenol content at all tested levels, but MA significantly increased them at all tested levels. The control had a proline content of 25.08 $\mu g\ g^{-1}$ FW, whereas SA at 3 $mmolL^{-1}$ lowered it to 10.33 $\mu g\ g^{-1}$ FW and MA at 3 $mmolL^{-1}$ increased it to 36.70 $\mu g\ g^{-1}$ FW. Furthermore, SA at 3 $mmolL^{-1}$ significantly decreased total phenol content from 100.54 $\mu.gg^{-1}$d.w in control to 79.90 $\mu.gg^{-1}$ d.w, whereas MA at 3 $mmolL^{-1}$ significantly raised it to 138.20 $\mu.gg^{-1}$ d.w.

The role of SA in regulating growth, phylogenetic processes, nutrient absorption, protein synthesis, ethylene inhibition, respiration, and protection of the functions of certain cellular organelles may explain the increase in fresh and dry weight and number of shoot buds, which in turn encouraged this increase (Hayat and Ahmed,2007; Muhsen et al., 2020).Increased SA concentrations induced a drop in proline and total phenol content because SA plays an important role in regulating plant responses to environmental stress and increasing plant tolerance to severe conditions (Gunes et al, 2007).Also, accumulation of proline and phenol is one of SA's adaptation mechanisms, since it supplies the plant with N and C elements, which are important energy sources (El-Khallal et al., 2009 ; Ebed and Mohsen, 2020).

MA induced osmotic stress and water deficit has been widely investigated in many plant species (Cha-Um et al., 2009). When exposed to stressful conditions, plants accumulate an array of metabolites, such as proline and phenol, which play an important role in plant metabolism and development. A large body of data suggests a positive correlation between proline accumulation and plant stress (Ashraf et al., 2004). Under stress, proline aids in the stabilization of subcellular

structures (such as membranes and proteins), the scavenging of free radicals, and the buffering of cellular redox potential (Tonon et al., 2004).Phenolic chemicals can be employed as hydrogen donors, causing free radicals to be quenched. Strong antioxidant activities have been observed for phenolic acids and flavonoids. They have the ability to react to radicals and catalyze oxidative processes, which allows them to scavenge free radicals and stabilize them in plants (Saed-Moucheshi etal., 2014), that could explain the buildup of proline and phenols in tissues exposed to MA.

Table (2) Effect of SA and MA in f.w and d.w, number of shoot buds, proline and total phenols

Treatment ($mmolL^{-1}$)*	F.w (g)	D.w (g)	Number of buds	Proline($\mu g\ g^{-1}$f.w)	Total phenols ($\mu g g^{-1}$ d.w)
Control	5.400	0.490	9.10	25.08	100.54
SA 1	10.320	0.990	14.80	14.46	91.87
SA 2	12.160	1.230	20.28	12.19	86.76
SA 3	11.960	1.150	18.76	10.33	79.90
MA 1	10.840	1.010	19.50	27.25	112.48
MA 2	9.770	0.860	15.48	32.80	129.65
MA 3	6.180	0.550	11.90	36.70	138.20
LSD	0.520	0.071	1.08	1.95	3.33

*mmol(milli mol);L(Liter); g (gram); mg(milligram); µg,(microgram);f.w(fresh weight);d.w(dry weight)

Elongation stage

Table 3 shows that SA and MA significantly enhanced the length of buds as compared to the control. The significantly longer bud length was 8.8 cm under SA at 3 $mmolL^{-1}$, while the significantly shorter length was 4 cm in MA at 3 $mmolL^{-1}$.In addition, as compared to the control, SA and MA considerably enhanced chlorophyll and carotene content. MA exceeded SA in terms of increasing chlorophyll and carotene levels. Significantly higher chlorophyll content was 6.70 mgg^{-1}, which was found in MA at 2 $mmolL^{-1}$, whereas significantly lower content was found in control at 1.7 mgg^{-1}. In regard of carotene content, treatment with MA at 1 mmol.L resulted in 0.027 mgg^{-1}, which was significantly higher than other treatments, while the lowest content was 0.006 mgg^{-1} in the control.

Table (3) Effect of SA and MA in length of buds and content of chlorophyll(chl.) and carotene(car.)

Treatment ($mmolL^{-1}$)	Length of bud (cm)*	Chl. (mgg^{-1} f.w)	Car. ($mg.g^{-1}$ f.w)
Control	4.5	1.7	0.006
SA 1	6.1	2.40	0.009
SA 2	7.4	2.98	0.013
SA 3	8.8	3.80	0.014

MA 1	6.6	4.90	0.027
MA 2	7.9	6.70	0.024
MA 3	4.0	5.8O	0.023
LSD (0.05)	0.36	0.06	0.0002

Cm (Centimeter)

SA's superiority over the control is explained by its physiological role, which is to accelerate the synthesis of chlorophyll and carotene pigments, which then accelerates photosynthesis and increases the activity of several enzymes involved in the growth process (Ramawat, 2004; Hayat and Ahmed, 2007). Plants subjected to moderate levels of stress enhance the synthesis of photosynthetic pigments and improve the ability of cells division and expansion, positively affecting growth (Gill et al., 2001;Valenzuela et al., 2005). According to Islam et al. (2009), exposing sugar cane somatic callus to osmatic shock with PEG 5% resulted in the development of plantlets from somatic callus that were characterized by the strength of their growth and increased the content of chlorophyll and carotene when compared to the control treatment.

Conclusions

The current study was found that adding salicylic acid and mannitol sugar to the culture media used to produce date palms in vitro had a good effect. In the phase of multiplication, salicylic acid at 2 $mmolL^{-1}$ stimulated the growth of shoots and increased their number, while at 3 $mmolL^{-1}$ it enhanced the length of shoots in the elongation stage. Adding mannitol to the culture medium stresses plant tissues, resulting in an increase in proline and total phenol buildup despite improved pigment photosynthetic.

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