International Journal of Agricultural Science Research Vol. 4(2), pp. 031-034, February 2015
Available online at http://academeresearchjournals.org/journal/ijasr
ISSN 2327-3321 ©2015 Academe Research Journals

Full Length Research Paper

Effect of abscisic acid (ABA) on somatic embryogenesis in date palm (*Phoenix dactylifera* L)

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Accepted 26 January, 2015

***In vitro* propagation of date palm ensures large scale multiplication of genetically uniform planting material. Abscisic acid (ABA) plays an important role in the accumulation of nutritive products (proteins, lipids and glycides) during the development and maturation of asexual embryo formation. This study investigates the impact of abscisic acid on asexual embryo formation in date palm, their maturation, problem of vitrification and oxidation. Embryogenic callus was developed on MS media using the following hormone concentrations (ABA- 2.5, 1.0, 0.5, 0.25, 0.1 and 0.0 mg/l) in a culture at room temperature of ± 25°C and light for 16 h/day. Results indicated that maturation of asexual embryo of the date palm variety, Sheshi, after six months of culture and addition of ABA (1.0 mg/l in media containing NAA 2 mg/l, BAP 0.5 mg/l, and Kinetin 0.5 mg/l) resulted in a high proportion of asexual embryo formation. The study also revealed that increase in the hormone concentration resulted in decrease in the number of embryos obtained.**

Key words: Abscisic acid, callus, embryos, date palm.

INTRODUCTION

Date palm, *Phoenix dactylifera* L. (Arecaceae), is a heterozygous and dioecious crop of the arid regions of the world predominant in the Middle East and North Africa. According to the Food and Agriculture Organization of the United Nations, the global date production has increased from just 1.8 million tons in 1962 to 7 million tons in 2010 (Anonymous, 2006; FAO stat, 2010). The Kingdom of Saudi Arabia is estimated to have 23 million date palms and is among the top three date producing countries with a wide genetic diversity of 400 cultivars of which 10 are important and produce 1.3 million tons of dates annually, accounting for 17% of the global date production.

Besides abiotic stress, date palm cultivation suffers from serious biotic stress namely: Bayoud disease (*Fusarium oxyporium* f.sp. *albedinis*) in North Africa (Sedra, 2011) and red palm weevil (*Rhynchophorus ferrugineus* Olivier) in the Middle East and North Africa (Faleiro, 2006). The crop is usually propagated through offshoots. However, with the increased demand for quality offshoots that is becoming increasingly difficult to meet and in order to propagate pest free material, micropropagation techniques involving tissue cultured palms that are true to type is becoming popular. Tissue cultured date palms are also easy to transport and plant.

Micropropagation of date palm on the commonly used MS media (Murashige and Skoog, 1962) faces problems such as browning and vitrification of embryo tissue, recalcitrance of explants, blockage of growth due to non availability of the appropriate type and quantity of the nutrient (proteins, sugars and lipids) resulting in the production of weak embryos. Finding the appropriate media is vital for the efficient production of embryos for the successful micropropagation of date palm using tissue culture techniques. It is therefore necessary to suitably modify the media to ensure improved embryo

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production.

Auxins, cytokinins, gibberellins, ABA and ethylene are the naturally occurring plant hormones of which auxins and cytokinins regulate cell division and differentiation of explants in tissue culture systems. Previous work has shown that regulating abscisic acid (ABA) levels in the tissue culture media promotes somatic embryo quality by increasing desiccation tolerance and preventing precocious germination. ABA is also employed to induce somatic embryos to enter a quiescent state and synchronize maturation of somatic zygotes in plant tissue culture systems. ABA can also act as anti-transpiration agent during acclimatization of tissue cultured plantlets (Rai et al., 2011).

ABA when used alone in plant tissue culture was found to suppress shoot regeneration in tomato (Sharp et al., 2000). However, with other plant growth regulators, ABA was seen to promote growth (Ficcadenti and Rotino, 1995; Maggon and Singh, 1995; Zhang et al., 2010). ABA has also been reported for the regulation of seed maturation in maize (White et al., 2000).

This study aims to assess the impact of ABA on *in-vitro* production of date palm embryos in terms of the number of embryos produced and effects on embryos browning and vitrification in shoot tip explants in micropropagation of date palm (Sheshi Cv). Protocols adopted for this study are described subsequently.

MATERIALS AND METHODS

Experimental protocols involved the following sequence:

Preparation of offshoot

For offshoot preparation, 2-4 year old offshoots of the date palm cultivar Sheshi (5-7 Kg) were selected for the trial from the gene bank of the Date Palm Research Centre (DPRC), Al Hassa, Saudi Arabia. The leaves and leaf sheath were gradually removed from the selected offshoot until the shoot tip region was exposed.

Sterilization of shoot tip

- The shoot tip was detached and sterilized in ascorbic acid (150 ml/L) and citric acid (150 ml/l).
- The shoot tip was washed with distilled water several times under the lamina flow hood and dipped in 70% ethanol for 1-2 min.
- Subsequently the shoot tip was sterilized for 20 min in 1.5% epochloride solution (30% chlorox) containing a few drops of tween 20.
- This was followed by washing of the sterilized shoot tip with distilled water several times to neutralize effect of chlorox.

Explants culture

- The shoot tip was further dissected to arrive at the apical bud that was prepared into several explants which were inserted into tubes containing tissue culture media.
- Explants were cultured on a basic MS media (Murashige and Skoog, 1962) enriched with the hormones 24D (5 mg/L) and BAP (5 mg/L) so as to obtain the first callus.
- The explants inoculated culture tubes were stored in a dark room of 25°C ± 2 for 3-6 months.
- The callus produced was transferred to MS media fortified with hormones (NAA - 2 mg/L), (BAP and Kinetin - 0.5 mg/L each).

Abscisic acid (ABA) treatments

- The embryogenic callus was transferred to tubes containing MS media treated with the following concentrations of abscisic acid namely, 2.5, 1.0. 0.5, 0.25, 0.1 and 0.0 mg/L.
- Each treatment had four tubes that were replicated 5 times.

Observations

- Monthly observations were recorded for a period of four months for the following parameters: (i) embryos produced, (ii) browning of embryos and (iii) vitrification of embryos.
- Each parameter in every tube was assigned a grade during every observation on a scale of 1-5 where 1 represents 20% while a score of 5 denotes 100%.
- Cumulative mean grade was calculated at the end of the experiment for each of the above three parameters and data were subjected to statistical analysis (ANOVA p=0.05). Results obtained are presented and discussed subsequently.

RESULTS AND DISCUSSION

Results presented in Table 1 reveal that ABA treatments had a significant impact on the number of embryos produced with the highest cumulative grade index being recorded at 0.1 ABA concentration. The number of embryos produced however reduced with the increase in the concentration of ABA (Table 1 and Figure 1). Our results are in agreement with the findings of Zhang et al. (2010) who reported that promotion of somatic embryos is ABA dependent in Japanese larch. Reports from the Philippines showed that ABA increased green plant regeneration in a zeatin free medium in rice (Ella and Zapata, 1991). Similar results confirming the role of ABA promoting growth of embryogenic callus in tissue culture systems for cassava have been reported (Danso et al., 2008). In micropropagation studies of tea, the concentration of ABA applied was reported to inhibit the conversion of globular and heart embryos to shoot (Ghanati and Ishka, 2009). In our results, higher levels of ABA reduced embryo production of the date palm cultivar studied.

With regard to browning and vitrification, Table 1 and

Table 1. Influence of abscisic acid (ABA) levels (0 - 2.5 mg/L) on embryo production, browning and vitrification in tissue culture embryos of date palm (Sehshi cultivar).

Concentration of ABA (mg/L)	Mean cumulative grade index (Scale 1-5)		
	Embryos produced	Embryo browning	Embryo vitrification
2.50	0.303[c]	0.472	1.249
1.00	0.384[bc]	0.311	1.383
0.50	0.391[bc]	0.304	0.959
0.25	0.342[c]	0.704	0.989
0.10	0.661[a]	0.288	1.048
0.00 (Control)	0.617[ab]	0.617	1.116
CD (p=0.05)	0.265	NS	NS

CD: Critical Difference. NS: Treatment means not significantly different. Figures followed by same letters within the column are statistically similar. Scale (1-5, where 1 is 20% and 5 is 100%).

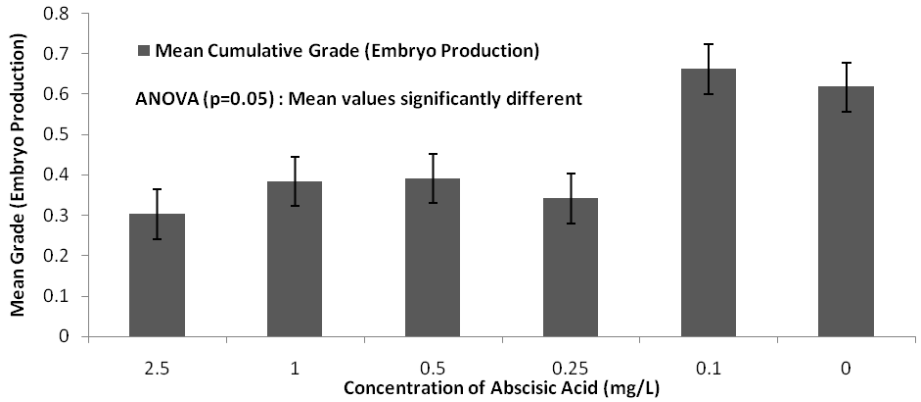

Figure 1. Influence of abscisic acid on embryo production in tissue culture date palm (SheshiCv).

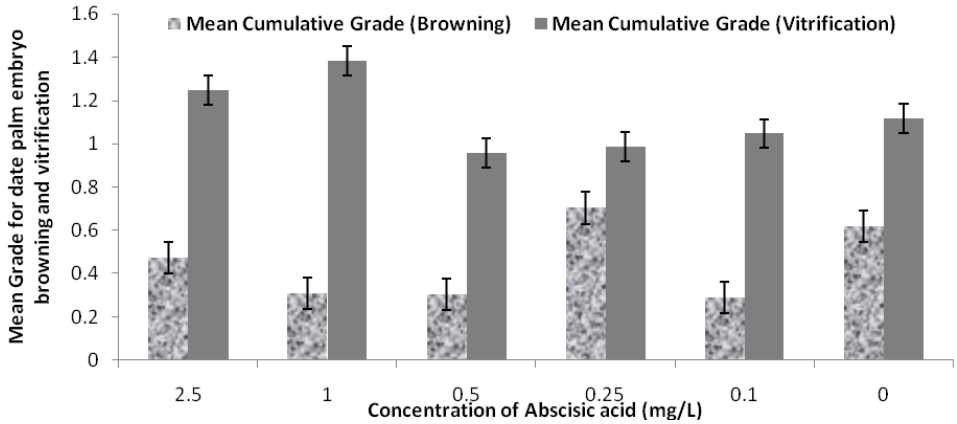

Figure 2. Influence of abscisic acid on tissue culture date palm embryo browning and vitrification (Sheshi Cv). ANOVA (P=0.05): Mean values not significantly different.

Figure 2 indicate that the ABA concentrations tested were not significantly different for these parameters and were statistically similar to control where no ABA was added to the media. El Bellaj and Hadrami (2004) attributed browning in tissue culture date palm callus to the production of phenolic compounds. In the case of our

study, it can be inferred that ABA had no role to play in both cases of browning and vitrification of embryos and could be related to the production of phenols.

We conclude that ABA at a low concentration of 0.1 mg/L when incorporated in MS media containing NAA 2 mg/L, BAP 0.5 mg/L and Kinetin 0.5 mg/L could enhance embryo production in date palm tissue culture systems.

ACKNOWLEDGEMENT

The authors acknowledge the support provided by the Date Palm Research Centre, Al Hassa, Saudi Arabia, to carry out this work through the FAO project (UTF/SAU/043/SAU).

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