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Influence of Sucrose and Pacloburtazol on Callus Growth and Somatic Embryogenesis in Date Palm cv. Bream

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A B S T R A C T

A study was conducted to examine different concentrations of paclobutrazol and sucrose for their effect on embryonic callus and subsequent embryogenesis in *Phoenix dactylifera* cultivar Bream. Shoot tips were excised from 2-3 years old offshoots, surface sterilized and inoculated onto Murashiege and Skoog, 1962 (MS) medium supplemented with 50 mg/L picloram and 3 mg/L N6-2-isopentyl adenine (2ip). Primary callus was transferred onto fresh MS medium containing 0.0, 0.5, 1.0, 1.5 or 2.0 mg/L of pacloburtazol or 30, 60, 90 and 120 g/L sucrose individually. Results were recorded after 12 weeks. A significant decrease in fresh weights while it increased dry weights and its percentage. The addition of 1.5 mg/L of pacloburtazol gave the highest dry weight (0.1 g) while addition of 2.0 mg/L gave highest dry weight percentage (18%). Increasing of sucrose concentration over 30g/L in the embryonic callus medium led to a significant increase in callus fresh and dry weights as well as dry weights percentage. Sucrose at concentration 90 g/L significantly increased callus fresh weights (2.54 g) while addition of 120 g/L gave highest dry weight (0.15 g) with percentage (8.43%). The number of formed embryos increased proportionally after the inclusion of pacloburtazol to the medium till reached to a significant level at the concentration 1.0 mg/L recording 12.8 embryos. However, mean fresh weights for these emerged embryos reached 1.81 g after inclusion of 1.5 mg/L of pacloburtazol. Number of embryos was increased at the concentration 60 g/L. It is concluded that both pacloburtazol and sucrose may play a positive role improving somatic embryogenesis in date palm var. Bream tissue cultures.

Introduction

Date palm (*Phoenix dactylifera* L.) (2n=2x=36) is a dioeciously, perennial, monocotyledon fruit trees belong to the family of Arecaceae (Barrow, 1998). Dates are the major fruit crop of arid climate region in Middle East and North Africa. It is

propagated traditionally by seeds or offshoots, but because of the heterozygous nature of the genetic makeup, the plantlets produced from seeds are not identical, are lesser in quality than the mother plant as well as being approximately 50 % male. Therefore, propagation by offshoots is better, but the numbers produced from the tree are limited, especially from superior and rare cultivars, so it cannot satisfy the need to establishing new groves. The use of plant tissue culture to supplement propagation by offshoots is necessary. Thus the propagation of date palm through offshoots is preferred over the seedlings. Since propagation through offshoots is slow and affected by their low survival rate, tissue culture of female plants has been preferred widely for mass production of true-to-type plants of elite varieties in demand.

Since the first attempts of date palm propagation via tissue culture that proposed by Schroeder (1970) and Reuveni et al. (1972) until now, two methods of propagation were developed, direct organogenesis (Al-Maari and Al Ghamdi, 1997; Al-Khateeb, 2002) and somatic embryogenesis which involve the production of somatic embryos from embryogenic callus was reported by many researchers (Reuveni, 1979; Mater, 1983; Omar, 1988 and Al Musawi, 2001). It presents a great potential for the rapid propagation and genetic resource preservation of this species. Therefore it becomes the most common micropropagation method in commercial plant tissue culture labs. Recently, Ibrahim (2012) initiated callus after the addition of 50 mg/L Picloram and 3.0 mg/L 2ip, re-cultured at 4 week intervals until transfer to embryogenic callus proliferation medium. Embryogenic calli are transferred to hormone free MS medium (Omar, 1992) or supplemented with 0.1 NAA (Jasim and Saad 2001). The low rate of asexual embryo formation and germination prompted many researchers to increase the yield and decrease the time of the processes.

It was found that supplementing the culture medium with 1 g/L apple seed powder (Saleh et al. 2006), 25 g/L corn seed powder (Jasim et al. 2008), 100 mg/L vitamin E (Al-Meer and Al-Ibresam, 2010) and 20% (v:v) coconut water or casein hydrolysate at 2.0 g/L (Khierallah and Hussein 2013). All these treatments increased somatic embryogenesis and germination percentages for several date palm cultivars.

Pacloburtazol, or (2*S*,3*S*)-1-(4-chlorophenyl) -4,4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-o is a heterocyclic nitrogen-containing compound is a plant growth retardant and triazole fungicide. It is a known antagonist of the plant hormone gibberellin. It acts by inhibiting gibberellin biosynthesis, reducing internodial growth to give stouter stems, increasing root growth, causing early fruit set and increasing seed set in plants such as tomato and pepper. It is found that pacloburtazol enhances somatic embryogenesis in plant tissue culture (Goerge et al., 2008). Sidky et al. (2007) reported that plantlets transferred onto MS medium at half strength supplemented with 0.1 mg/L of NAA, 1 g/L of AC, 40 or 50 g/L of sucrose and 4 mg/L of paclobutrazol, increased thickness of plantlets, accelerated root formation and promoted secondary root formation. Sucrose is the main carbohydrate in culture media which plays an important role in *in vitro* cultures as an energy and carbon source, as well as an osmotic agent. In addition, carbohydrate-modulated gene expression. Sucrose is almost universally used for micropropagation purposes as it is so generally utilizable by tissue cultures (Goerge et al., 2008). Therefore the aim of this study is to examine various concentrations of pacloburtazol and sucrose

on enhancing embryonic callus and subsequent embryogenesis in *Phoenix dactylifera* Bream cv.

Materials and Methods

Offshoots (2-3 years old) of Bream cultivar were chosen and detached from mother palm. Leaves were dissected acropetaly. Shoot tips of 2 cm in length (apical meristem with soft inner leaves), were excised along with immature fiber of 1.5 cm in diameter. Explants were dipped in antioxidant solution consisted of 150 mg/L citric acid plus 100 mg/L ascorbic acid (Tisserat, 1991). Explants were surface sterilized with 2.0% sodium hypochlorite solution containing few drops of Tween-20 for 20 minutes under vacuum, and rinsed three times with sterile distilled water. They transferred to Petri dishes where leaf primordia were removed except the two pairs surrounding the apical meristem which then divided longitudinally into four equal segments and cultured in jars aseptically. The medium of initiation stage was composed of Murashige and Skoog (1962) (MS) salts plus the following (in mg/L); thiamine-HC1 1.0; pyridoxine-HCl 1.0; adenine sulfate.2H2O 40; myo-inositol 100; NaH2PO4.2H2O 170; sucrose 30000 activated charcoal 2000 and agar-agar 7000. The pH of the medium was adjusted to 5.7 with 0.1N NaOH or HC1, before the addition of agar. The medium was dispensed into culture jars with aliquots of 25 ml in each, and then covered with polypropylene caps and autoclaved under 1.04 kg/cm² at 121 °C for 15 minutes. Callus initiation medium was supplemented with 50 mg/L picloram and 3 mg/L N6-2-isopentyl adenine (2ip). Primary callus was obtained after 24 weeks of growth in full darkness. Calli were then transferred onto fresh MS medium containing 0.0, 0.5, 1.0, 1.5 or 2.0 mg/L of pacloburtazol or 30, 60, 90 and 120 g/L sucrose individually (250 mg/jar). Cultures were incubated in a growth room under low light intensity of 1000 lux for 16 hours daily at 27±1 °C for four weeks. Results of callus fresh and dry weights, number of germinated embryos and mean fresh weight of ten embryos were recorded after 12 weeks. Experiments were conducted as factorial using Complete Randomized Design (CRD), with ten replicates. Least significant differences (LSD) were used to compare means at 5% level probability.

Result and Discussion

Incorporation of the callus initiation medium with pacloburtazol exhibited a significant decrease in fresh weights while it increased dry weights and its percentage (Table 1). The addition of 1.5 mg/L of pacloburtazol gave the highest dry weight (0.1 g) while addition of 2.0 mg/L gave highest dry weight percentage (18%).

Table.1 Callus fresh and dry weights initiated on MS medium supplemented with 50 mg/L picloram and 3 g/L 2iP at different concentrations of pacloburtazol for 12 weeks.

Pacloburtazol (mg)	Callus fresh wt (g)	Callus dry wt (g)	% Dry wt
0.0	1.16	0.05	4.31
0.5	1.04	0.07	6.73
1.0	0.85	0.08	9.41
1.5	0.65	0.10	15.38
2.0	0.50	0 .09	18.00
LSD 0.05	0.759	0.094	

Increasing of sucrose concentration over 30g/L in the embryonic callus medium led to a significant increase in callus fresh and dry weights as well as dry weights percentage (Table 2). Sucrose at concentration 90 g/L significantly increased

callus fresh weights (2.54 g) while addition of 120 g/L gave highest dry weight (0.15 g) with percentage (8.43%).

Table.2 Callus fresh and dry weights initiated on MS medium supplemented with 50 mg/L of picloram and 3 g/L of 2iP at different concentrations of sucrose for 24 weeks.

Sucrose (g)	Callus fwt (g)	Callus dwt (g)	% dry wt
30	1.67	0.09	5.39
60	1.92	0.07	3.65
90	2.54	0.12	4.72
120	1.78	0.15	8.43
LSD 0.05	0.65	0. 05	

The number of formed embryos increased proportionally after the inclusion of pacloburtazol to the medium till reached to a significant level at the concentration 1.0 mg/L recording 12.8 embryos (Table 3). However, mean fresh weights for these emerged embryos fluctuated with a maximum weight reached 1.81 g after inclusion of 1.5 mg/L of pacloburtazol. All concentrations of pacloburtazol caused no significant differences in the mean of 10 embryos fresh weights compared with those grown on pacloburtazol free medium.

Table.3 Mean number of germinating embryos and mean weight of ten embryos initiated from calli after supplementation with different concentrations of pacloburtazol.

Pacloburtazol (mg)	No. of embryos	Mean wt 10 embryos (g)
0.0	8.0	0.98
0.5	11.5	1.44
1.0	12.8	1.63
1.5	10.5	1.81
2.0	9.6	1.45
LSD 0.05	3.663	0.429

Table 4 shows that all levels of sucrose resulted in a significant increase in number of formed embryos compared with those initiated on a medium with 30 g/L of sucrose. Number of embryos was increased at the concentration 60 g/L. These increments in number of embryos although led to increasing the mean fresh weight of 10 emerging embryos, but the highest was recorded at 120 g/L of sucrose recording 2.40g.

Table.4 Mean number of germinating embryos and mean fresh weight of ten embryos initiated from calli after supplementation with different concentrations of sucrose.

Sucrose (g)	No. of embryos	Mean wt 10 embryos (g)
30	8.0	1.44
60	10.2	1.79
90	8.6	1.33
120	7.5	2.40
LSD 0.05	2.719	0.702

Micropropagation via somatic embryogenesis has become a vital mean for propagating many plant species. Optimization of the culture medium may require some supplements other than plant growth regulators which are normally added to the nutrient medium. It is clear from the reported data (Tables 1 & 2) that pacloburtazol and sucrose has increased callus dry weights percentage when supplemented to the callus maintenance medium separately. This study has proved (tables 3&4) that pacloburtazol and sucrose have improved embryogenesis represented by almost increasing the number of embryos when both were supplemented individually. They may trigger competent cells to form cell aggregates which then developed to embryos.

It is found that pacloburtazol enhanced date palm somatic embryo number while at 0.25 or 0.5 mg/L stimulated embryos germination. Length of embryos was in the highest average with using 0.5 mg/L Pacloburtazol (Ibrahim et al., 2011). Previously, pacloburtazol has been shown to be beneficial in some tissue culture system including somatic embryo, but the results often depend on the step or stage of process (Hutchinson *et al*., 1997). A brief survey of literature showed plant response activity in the range of 0.1-3.0 mg/L. The addition of pacloburtazol, at all tested levels (0.33, 1.0 or 3.0 mg/L), increased somatic embryo growth of Loblolly pine (*Pinus tadea* L.) genotypes except one genotypes at 0.33 mg/L Pacloburtazol that was similar to the control (Pullman *et al*., 2005).

Li and Wolyn (1997) found that inclusion of ancymidol, ABA, uniconazol or pacloburtazol in the embryo induction medium significantly improved somatic embryo development in Asparagus. The beneficial effect was seen mainly in an improvement of embryo quality, resulting in more embryos capable of germination into plants. In Orange citrus, pacloburtazol at 0.1-1.0 mg/L induced 2.5-5.0% of abaxil leaf regions to form embryos but this stimulation was not significant when compared with control (Chen and Chang, 2003). Chen *et al*. (2005) reported that the continuous application of pacloburtazol enhanced the formation and proliferation of meristematic clusters of Daylily (*Hemerocallis* spp.). Treated clusters significantly accumulated higher starch levels compared to non treated clusters. High level of starch accumulation presumably serve as an extra energy source for proliferation of meristematic cluster. Chen and Ziv (2004) stated that plant growth retardants affected cell division and cell enlargement, probably by interfering with gibberellin biosynthesis.

As sucrose is the main energy and carbon source in culture medium, increasing its concentration can be useful to enhance cell division and subsequent callus weight increment. On the other hand, increasing of osmotic potential of a medium by adding more sucrose can influence whether somatic embryogenesis can occur and can regulate the proper development of embryos. As shown above, a low osmotic potential (60 g sucrose) is often favorable, but the highest level is not beneficial.

Hassan and Taha (2012) found that the highest percentages of explants formed callus were achieved using 0.15 M sucrose or sorbitol in induction medium while in proliferation stage, the highest callus fresh weight was observed using 0.1 M sucrose+0.05 M sorbitol. During multiplication and germination stage the highest number of germinated embryos was resulted from 0.05 M sucrose+0.1 M sorbitol, while 0.2 M sucrose enhanced secondary embryo formation. Placing tissues in solutions with high osmotic potential will cause cells to become plasmolysed, leading to the breaking of cytoplasmic interconnections between adjacent cells (plasmodesmata).

The finding can be exploited commercially by date palm micro-propagators to almost increase their production. Searching for other media supplements with the aim of increasing embryo numbers and weights is a vital aspect and requires intensive investigation.

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