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Morphological and biochemical impact of different decontamination agents on date palm (*Phoenix dactylifera* L.) procallus

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Abstract

This study was conducted to evaluate the phytotoxic effects of different antibiotics and fungicides on the procallus of date palm (*Phoenix dactylifera* L.). The fungicides Switch® and Beltanol and the antibiotics chloramphenicol and gentamycin were used in this study. Results confirmed the lethal effects of Switch®, gentamycin, and chloramphenicol (at 100 mg/l in dicamba treatment) on date palm tissues. The treated shoot tips did not develop into procallus, and their growth was completely inhibited. Beltanol treatments significantly decreased the biochemical parameters of the procallus treated with 2, 4-D and dicamba. In the Beltanol-treated tissues, the initial period of callus induction was prolonged from 63.33 and 69.66 days to 97.66 and 117.66 days. The callus induction percentage of the plants treated with the decontamination agents was also decreased. The fresh and dry weights of the procallus and the browning percentage and intensity of the cultured tissues in Beltanol and chloramphenicol treatments (100 mg/l) increased compared with those in the control treatments. Total carbohydrates, free proline content, total phenolic compounds, free amino acid, total soluble protein, and peroxidase activity were significantly affected by Beltanol and chloramphenicol (100 mg/l). By contrast, low chloramphenicol concentrations resulted in acceptable levels of biochemical components. Therefore, we recommend the use of low chloramphenicol and Beltanol concentrations instead of Switch and gentamycin as decontamination agents to prevent or treat microbial contamination in date palm tissue cultures.

Keywords: Antibiotics; contamination; date palm; fungicides; micropropagation; phytotoxicity.

Introduction

Date palm (*Phoenix dactylifera* L.) is an important member of Arecaceae. This species is grown mainly in the Middle East and in other family regions worldwide (Al-Khalifah et al., 2012, Abass, 2013a). The production of date palm is approximately 7.4 million tons and worth 3.6 billion US dollars per annum. Most of this amount comes from the Middle East (FAOSTAT, 2011; Abass et al., 2015).
Dates are a well-known energy source and are rich in nutrients. Date fruits are composed of 70% carbohydrates, mostly sugars, and 15%–30% water. Dates are also a good source of different minerals, including iron, potassium, and calcium, as well as low amounts of sodium and fat (Al-Shahib and Marshall, 2003; Thabet et al., 2010; Dayani et al., 2012, Abass, 2013a). Date palm is produced through different processes, such as micropropagation. Micropropagation is defined as asexual plant multiplication starting from a single individual (Aaouine, 2003; Al-Khayri 2005, 2007). In micropropagation, selected superior cultivars can be rapidly propagated for commercial and environmental interests. This method can be considered as an acceptable approach to satisfy the world's demand of approximately 1–2 million superior date palm cultivars per year (Jain, 2007, 2012, Abass, 2016). Date palm micropropagation can be applied in two different procedures, namely, organogenesis and somatic embryogenesis. Somatic embryogenesis consists of several steps, including explant, procallus, embryogenic callus, somatic embryos and plantlets (Al-Khalifah and Shanavaskhan, 2012). However, date palm micropropagation is confronted by numerous limitations, including microbial contamination, tissue browning and media, vitrification, shoot tip necrosis, and somaclonal variation (Abass, 2013 a, b). Microbial contamination, which can occur in all of the stages of tissue culturing, is considered as the most common problem. Date palm explants are exposed to microbial infections in all stages of tissue culturing (Leifert and Waites, 1992), contamination coming with the explants themselves, or occurring during the propagation procedures (Al-Mussawi, 2010, Abass, 2015). Several consequences have been associated with microbial contamination or infection of cultured tissues. For example, infected tissues become degraded or experience browning because various substances, such as degrading enzymes (cellulase, phenol oxidase, and others) and toxins, are released into the medium (Hameed and Abass, 2006). Two different microbial groups, which are fungi and bacteria, have been associated with date palm tissue cultures (Odutayo et al., 2007, Abass, 2013b); Fungi represent a major group of contaminants in Iraqi laboratories. Several fungal genera, including *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Epicoccum*, *Eurotium*, *Penicillium*, and *Fusarium*, have been isolated and identified from cultured tissues (Hameed and Abass, 2006, Abass et al., 2007, Al-Mayahi et al., 2010, Abass, 2013b). Bacteria are important sources of contamination, and their elimination requires complex procedures, such as surface sterilization and antibiotic utilization (Leifert and Waits, 1992), The most common bacterial genera isolated from cultured date palm

tissues are *Bacillus*, *Proteus*, and *Staphylococcus* (Al-Hadithi et al., 2007; Al-Dosary et al., 2011).

Several decontaminant agents have also been used to control or reduce microbial contamination in date palm tissue cultures. Different antibiotics, including amoxicillin, chloramphenicol, gentamycin, griseofulvin, nystatin, and streptomycin at concentrations of 25–100 mg/l, have been incorporated into culture media during initiation stages (Al-Kaby, 2004, Al-Mussawi, 2010, Al-Dosary et al., 2011), Several fungicides, such as Beltanol, Benlate, Carbendazim, and Score, have been used to eradicate fungal contaminants (Al-Kaby, 2004, Abass et al., 2007, Al-Mayahi et al., 2010).

The present study aimed to determine the most suitable antibiotics and fungicides and their optimum concentrations beneficial for the growth and physiology of Hillawii cv. date palm procallus.

Results and Discussion

Phytotoxic effect of decontamination agents on date palm procallus growth

-The effect of decontamination agents on date palm procallus morphological parameters

Statistical analysis revealed that decontamination agents, namely, Switch, Beltanol, chloramphenicol, and gentamycin, significantly affected the morphological parameters of date palm (Fig. 2). Most of the examined parameters decreased significantly after date palm was treated with these decontamination agents. Switch (1 g/l), gentamycin (50 mg/l), and chloramphenicol (100 mg/l in dicamba treatment) completely inhibited the growth of date palm shoot tip segments at 2, 4-D and dicamba auxins on the callus induction medium. These exposed segments did not develop into swelling or procallus during incubation.

The initial periods of callus induction were 63.33±.05 and 69.66±2.51 days at 2, 4-D and dicamba control treatments, respectively. These periods were prolonged significantly to 97.66±2.51 and 117.66±2.51 days at Beltanol treatment. Chloramphenicol treatments also prolonged the required time to induce procallus compared with the control treatments (Fig. 2 A). The initiation percentage of callus in 2, 4-D and dicamba treatments showed that the employment of decontamination agents except chloramphenicol at low concentration 50 mg/l in dicamba treatment reduced the callus induction percentage, which was 51%±1.73 and 41.66%±2.08 in the control treatment.

The percentages of callus initiation in the callus induction medium supplemented with dicamba and chloramphenicol (50 mg/l) did not significantly differ from those of the control treatment (Fig. 2B).

The fresh and dry weights of procallus were markedly reduced after the date palm cultures were treated with the decontamination agents in 2, 4-D and dicamba treatments except chloramphenicol at a low concentration of 50 mg/l in dicamba treatment. Thus, the fresh and dry weights were significantly decreased up to twofold in Beltanol and chloramphenicol (100 mg/l) treatments in 2, 4-D and dicamba. No evident effect was observed in the fresh and dry weight of date palm procallus treated with chloramphenicol at a low concentration (50 mg/l) in dicamba (Figs. 2C and 2D). The date palm cultures treated with Beltanol and chloramphenicol (100 mg/l) in 2, 4-D exhibited a severe browning phenomenon. Their browning percentages were 30%±2.00 and 50.66%±2.08, respectively. The browning percentage of the control cultures was 0%. The lowest browning percentage was observed in the cultured tissues treated with 50 mg/l chloramphenicol (Fig. 2E). The highest browning intensity, which was (+++) according to the scale of Abul-Soad et al. (2012), was observed in the plants treated with Beltanol and chloramphenicol (100 mg/l). Our experiments revealed the acute toxic effects of Switch, gentamycin, and chloramphenicol at a high concentration of 100 mg/l in dicamba treatment, as evidenced by the growth and development of shoot tip segments, which completely failed to produce any procallus during incubation in the callus induction medium supplemented with either 2, 4-D or dicamba.

The inhibitory effect of gentamycin on the cultured date palm tissues is consistent with many other observations regarding the lethal effects of gentamycin at recommended concentrations (50 and 100 mg/l) for plant tissue cultures. For instance, Dodds and Roberts (1981) demonstrated the inhibitory effect of gentamycin on callus division and cytodifferentiation of Romaine lettuce (*Lactuca sativa* L.) and Jerusalem artichoke cactus (*Helianthus tuberosus* L.). Gentamycin was also very toxic and lethal at dose of 100 mg/l for shoot initiation and shoot growth in tobacco and tansy (*Tanacetum vulgare* L.) (Eicholtzet al., 1982; Keskitalo et al., 1998; Thomas, 2004). Thus, it's possible to test less concentration of Gentamycin 10 mg/l; such concentration did not affect the growth and development of two species of *Allium* (Fellner et al., 1991).

Gentamycin, which is widely employed in animal and plant tissue cultures, is a broad-spectrum anti-bactericidal agent that suppresses the growth of Gram-positive and Gram-negative bacteria by inhibiting protein synthesis in bacterial cells (Habiba et al., 2002). Chloramphenicol is a broad-spectrum bacteriostatic agent that inhibits protein synthesis. This antibiotic is also widely used as an effective agent against Gram-positive and Gram-negative bacteria (Gholameraz et al., 2008). Its lethal effects at high concentrations (100 mg/l) can be attributed to their inhibitory effects on solute and water uptake (Peaud-Lenoel and de GournayMargerie, 1962). High chloramphenicol concentrations can inhibit the callus growth, decrease their growth rate, and prevent their development (Bhau and Wakhlu, 2001), the toxic effect of chloramphenicol on cultured tissues is consistent with that described by Biasi (1995), who observed that 100 mg/l chloramphenicol completely prevents the growth of avocado tissue cultures and 50 mg/l chloramphenicol induces a 50% decrease in the percentage of callus formation. The fresh weight of avocado tissue cultures decreases from 530 mg in the control treatment to 43 mg in the 50 mg/l chloramphenicol treatment. By comparison, low chloramphenicol concentrations do not elicit a toxic effect on the growth of the callus of two *Allium* species, as observed in our experiments (Fellner et al., 1996). The phytotoxic and lethal effects of Switch on the cultured date palm tissues are consistent with those described by Saladin et al. (2003). They found that Switch causes several consequences, such as growth reduction and alteration of carbohydrate and nitrogen metabolism in treated plants. These phenomena could be accounted for the growth retardation of the date palms treated with Switch. Beltanol also caused an increase in time requirement to induce the procallus of Hillawii cv. and decrease the percentage of callus induction regardless of the type of auxin. This inhibition was accompanied with a significant reduction in the fresh and dry weights of procallus up to twofold. Our results revealed that Beltanol and chloramphenicol at high

Table 1. Fungicides, recommended application, and concentrations of active ingredients used.

Fungicide	Active ingredient and concentration %	Chemical group	Common name	Company	Tested Conc.
Switch	Cyprodinil 37.5% Fludioxonil 25%	Anilinopyrimidine Phenylpyrole	Swicth	Syngenta	1 gm/l
Beltanol	Chinosol 50%	Quinolone	Chinosol	Stahler	1 ml/l

Fig 1. Date palm offshoot dissecting. A-B: Date palm shoot tip before excision form Hillawii cv. C- Shoot tip after excision, D. Four segments of shoot tips.

concentrations severely affected the produced procallus and increased the browning. This parameter increased by threefold to fivefold, as observed in the control treatment. Browning is induced by oxidation on a plant tissue surface. The oxidation of phenolic compounds produces quinones that are highly reactive and toxic to cultured tissues (Zaid, 1984, Abohatem et al., 2011, Al-Samir et al., 2015, Abass, 2016). The browning of cultured date palm tissues is probably one of the causes of growth retardation. As a result, the morphological parameters examined in this study significantly decreased. These phenomena are widely known decisive limitations in culture establishment and development (Zaid, 1984, Dobranszki and Teixeira de Silva, 2010, Abass, 2013b).

The effect of decontamination agents on date palm procallus Biochemical parameters

The effects of decontamination agents on the biochemical parameters of date palm procallus were also investigated. Statistical analysis revealed that all of the examined biochemical features were significantly affected by the type and concentrations of decontamination agents (at the significance level of 0.01; Tables 2 and 3). Compared with the values obtained in the control treatment, the total carbohydrates of the plants treated with Beltanol were high, particularly reaching 34.25±1.25 and 40.0±1.08 mg/g FW in 2, 4-D and dicamba, respectively. The total carbohydrates of the plants treated with 100 mg/l chloramphenicol in 2, 4-D and 50 mg/l chloramphenicol in dicamba also increased. Similar results were observed in the proline content of procallus exposed to chloramphenicol and Beltanol. The highest proline contents were 3.78±0.16 and 3.51±0.22 µg/l at 2, 4-D treatment (Table 2).

Beltanol and chloramphenicol treatments induced a significantly high accumulation of phenolic compounds. The level of these compounds were 0.56±0.06 and 1.1±0.1 mg/l at 2,4-D and dicamba, respectively, and these values reached the maximum levels of 0.99±0.1 and 1.5±0.2 mg/l in Beltanol treatment with 2, 4-D and dicamba, respectively. The second-highest values were obtained in the procallus treated with chloramphenicol. Chloramphenicol treatments at 50 mg/l in 2, 4-D and dicamba did not elicit any toxic effect on the free amino acid content of the procallus treated with the examined decontamination agents. Beltanol treatments reduced the free amino acid contents by up to twofold compared with the free amino acid contents obtained in the control treatments of 1.46±0.07 and 1.02±0.06 mg/l 2, 4-D and dicamba, respectively (Tables 2 and 3).

Chloramphenicol at 50 mg/l did not also cause a toxic effect on the total soluble protein content of the procallus treated with auxin 2, 4-D 1.45±0.05 mg/l and dicamba 0.85±0.05 mg/l compared with those in the control treatment 1.13±0.05 and 0.79±0.05 mg/l. By contrast, the total soluble protein contents were decreased up to twofold in the tissue cultures treated with high concentrations of Beltanol or chloramphenicol. The significantly highest peroxidase activity was detected in the plants treated with Beltanol. The peroxidase activity increased from 10.98±0.42 and 20.5±0.50 unit/g/min in the control treatment to 23.91±1.18 and 25.75±1.63 unit/g/min in 2, 4-D and dicamba treatments, respectively. Compared with the control treatment, low chloramphenicol concentrations (50 mg/l) did not elicit any toxic effect (Tables 2 and 3).

The total carbohydrates also increased by twofold to threefold in the plants treated with Beltanol and chloramphenicol compared with the control treatments. This finding indicated that date palm procallus responded to the stress caused by these decontamination agents. This finding is also consistent with that described by Rhodes and Wooltron (1978) and El-Shafey et al. (1999). They demonstrated that carbohydrates can be considered as a good indicator of plant responses to stress. Similar responses were also observed in free proline content, total phenolic compounds, and peroxidase activity. This result suggested that the cultured date palm tissues could adapt to biochemical compound accumulation and antioxidant activity caused by fungicide and antibiotic treatments. Proline content, phenol content, and peroxidase activity are positively correlated with plant

Table 2. The effect of some decontamination agents on biochemical features of date palm procallus Hillawii cv. cultured on 2, 4-D auxin.

Treatment	Total Carbohydrates mg/g FW	Free Proline content µg/g	Total Phenols mg/g	Free Amino Acid mg/g	Total Soluble Protein mg/g	Peroxidase Activity Unit/g/min
Control	$11.83^{c}\pm0.76$	$1.43^{b}\pm0.12$	$0.56^{b}\pm0.06$	$1.46^{a}\pm0.07$	$1.13^{b}\pm0.05$	$10.98^{c}\pm0.42$
Belt	$34.25^{a}\pm1.25$	$3.51^{b}\pm0.22$	$0.99^{a}\pm0.1$	$0.60^{c}\pm0.05$	$0.43^{d}\pm0.02$	$23.91^{a}\pm1.18$
Chlora. 1	$10.53^{c}\pm0.45$	$1.23^{b}\pm0.07$	$0.43^{b}\pm0.02$	$1.63^{a}\pm0.07$	$1.45^{a}\pm0.05$	$10.16^{c}\pm0.76$
Chlora. 2	$21.25^{b}\pm1.14$	$3.78^{a}\pm0.16$	$0.86^{a}\pm0.04$	$0.85^{b}\pm0.05$	$0.73^{c}\pm0.02$	$20.25^{b}\pm1.14$

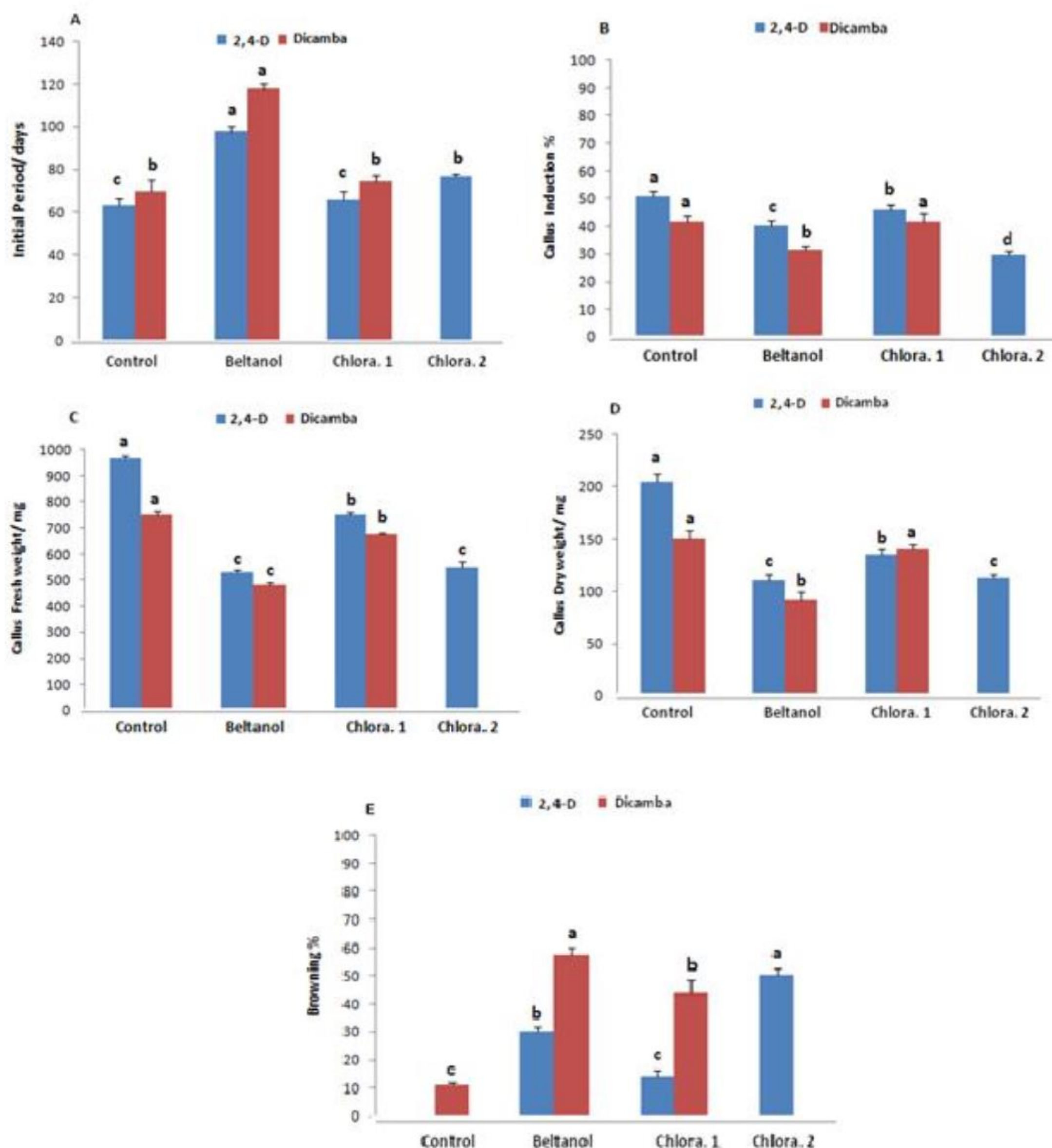

Fig 2. The effect of some decontamination agents on morphological features of date palm procallus Hillawii cv. A. Initial period/days. B. % Callus induction. C. Callus fresh weight. D. Callus dry weight. E. Browning (%).

responses to a wide range of stressors, such as low temperature, water deficiency, salinity, heavy metal exposure, radiation, and high 2,4-D concentrations (Naidu et al., 1991, Hare et al., 1998, Al-Khateb et al., 2002, Munns, 2005, Sharma and Dietz, 2006, Abohatem et al., 2011, El-Samir et al., 2015). An increase in phenol content and peroxidase activity could be one of the causes of browning severity of procallus treated with Beltanol and chloramphenicol (100 mg/l). Free amino acid and total soluble protein contents of the plants treated with Beltanol and chloramphenicol at a high concentration of 100 mg/l decreased by up to twofold lower than those observed in the

Table 3. The effect of some decontamination agents on biochemical features of date palm procallus Hillawii cv. cultured on Dicamba auxin.

Treatment	Total Carbohydrates mg/g FW	Free Proline content µg/g	Total Phenols mg/g	Free Amino Acid mg/g	Total Soluble Protein mg/g	Peroxidase Activity Unit/g/min
Control	$25.33^{b}\pm1.04$	$2.05^{b}\pm0.12$	$1.10^{ab}\pm0.10$	$1.02^{b}\pm0.06$	$0.79^{a}\pm0.05$	$20.50^{b}\pm0.50$
Belt	$40.00^{a}\pm1.80$	$4.30^{a}\pm0.30$	$1.50^{a}\pm0.20$	$0.53^{c}\pm0.02$	$0.41^{b}\pm0.05$	$25.75^{a}\pm1.63$
Chlora. 1	$22.91^{b}\pm0.80$	$2.19^{b}\pm0.21$	$0.93^{b}\pm0.05$	$1.25^{a}\pm0.04$	$0.85^{a}\pm0.05$	$19.92^{b}\pm0.32$

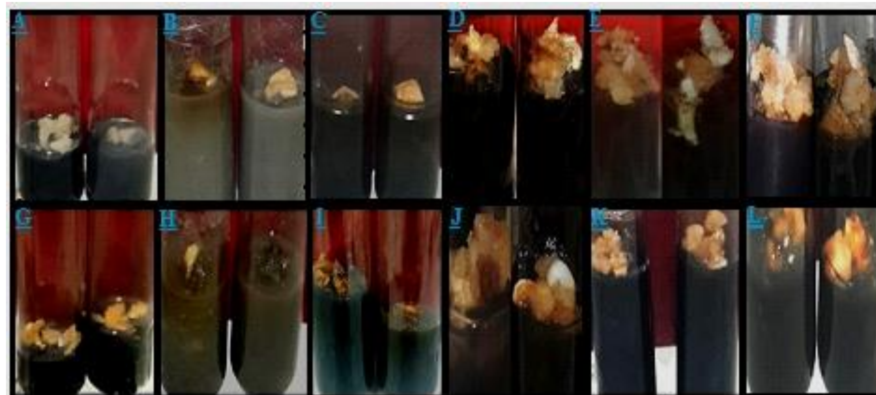

Fig 3. Browning intensity of date palm procallus of Hillawii cv. treated with different decontamination agents**.** (A) 2, 4-D as control treatment. (B) 2, 4-D with Switch treatment. (C) 2, 4-D with Gentamycin treatment. (D) 2, 4-D with Beltanol treatment. (E) 2, 4-D with Chloramphenicol (50 mg/l) treatment. (F) 2, 4-D with Chloramphenicol (100 mg/l) treatment. (G) Dicamba as control treatment. (H) Dicamba with Switch treatment. (I) Dicamba with Gentamycin treatment. (J) Dicamba with Beltanol treatment. (K) Dicamba with Chloramphenicol (50 mg/l) treatment. (L) D with Chloramphenicol (100 mg/l) treatment.

control treatment. Amino acids play a pivotal role in plant life processes, such as growth and development. Amino acids are also precursors and components of proteins (Hayat et al., 2012). Therefore, the decrease in the free amino acid and total soluble protein contents could be accounted for the delay of procallus emergence and the reduction of fresh and dry weights of procallus (Mustafa et al., 2013, Shehata et al., 2014). Biochemical analysis revealed that 50 mg/l chloramphenicol did not stimulate any negative effect of procallus on 2, 4-D and dicamba auxin. This finding did not significantly differ from that obtained in the control treatments.

Materials and Methods

Plant materials

2-3 years old offshoots of date palm Hillawii cultivars were selected and detached from their mother plants. Offshoots were dissected acropetaly until the shoot tips appeared. Shoot tips of 3 cm (apical meristems with leaf primordia) were excised with immature fiber 2 cm in diameter and then applied into antioxidant solution consists of 150 mg/L citric acid and 100 mg/L ascorbic acid to prevent browning (Tisserat, 1991). Explants were sterilized in commercial bleach (sodium hypochlorite) 20% containing one-two drops of Tween-20 as emulsifier for 20 min with vacuum and rinsed three times with sterile distilled water. Subsequently transferred to Petri dishes and all leaf primordia were removed except two pairs surrounding the apical meristems (Fig 1 A-C).

Initiation stage

The apical meristems were divided longitudinally into four segments (Figure 1D), and cultured on medium composed of basal salts (MS) (Murashige and Skoog, 1962), with additional 3 mg/l 2ip and 3 g/l activated charcoal. 2, 4-D was used at the concentrations of 50 mg/l, while Dicamba was used at the concentrations of 10 mg/l (Al-Samir et al., 2015). The pH of the medium was adjusted to 5.7 with 0.1 N NaOH, before the addition of agar. Media were dispensed into culture test tube with 25 ml in each, subsequently covered with cotton and aluminum foil. Autoclaving at 121 ° C and 1.04 kg/ cm^2 for 15 min was followed. All cultures were incubated in a culture room under darkness at 27±2 ° C until initiation of callus. Subcultures were performed on the same medium and growth conditions every 4 weeks (Al-Khayri, 2011).

Effect of fungicides and antibiotics on morphological parameters of date palm tissue cultures at initiation stage

The most common and used fungicides (Switch and Beltanol) and antibiotics (Chloramphenicol and Gentamycin) (Table 1) in control of date palm tissue cultures microbial contamination were selected to evaluate their toxic effect on the growth of date palm callus at initiation stage. All of tested concentrations were incorporated into the callus induction medium after autoclaving with an exception for Chloramphenicol which added before autoclaving. All of treatments (in addition to control treatments for both 2, 4-D and Dicamba) were observed for their growth, and once the procallus emerged on each explants, the following parameters were measured:

Initial Period (Day) For Callus Induction

Percentage of Callus Initiation

The percentage of callus initiation was calculated following the formula:

$$\% \text{ Callus initiation } = \frac{\text{Number of explants produced callus}}{\text{Total number of explants}} \text{ X } 100$$

-*Procallus fresh and dry weight /mg.*

Browning percentage and intensity

All date palm cultures were observed for browning response, and the indicator of Abul-Soad (2012) was followed as expression of: -, +, ++ and +++ with the interpretation of no response, poor, moderate and high, respectively.

Effect of fungicides and antibiotics on biochemical parameters of date palm tissue cultures at initiation stage

Total carbohydrates

The total carbohydrates were quantified as glucose by anthrone technique adopted from Watanabe et al. (2000). briefly, 0.5 g of fresh weight procallus were homogenized with 80% of ethanol, followed by a centrifugation at 5000 rpm for 10 min, the supernatants were collected into fresh new tube (1 ml for each tube) and 3 ml of Anthrone reagent (50 ml of 95% H_2SO_4 + 50 mg Anthrone) was added and left for 10 min at water bath (100 ° C), then cooled on ice. total carbohydrates were measured at 620 nm, using glucose as a standard.

Free proline content

Free proline content was measured spectrophotometrically according to Bates et al. (1973), by utilizing sulphosalicylic acid and ninhydrin reagent. Briefly, 0.5 g of fresh exposed callus was homogenized in 10 ml of 30% (w/v) sulphosalicylic acid and filtered through Whatman No. 1 paper and 2 ml of the filtrate was added to 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent. The mixture was heated at 100 º C for 1 h, and then cooled on ice. The reaction was extracted with 4 ml of toluene and the absorbance at 520 nm was measured against a toluene blank.

Total phenolic content

The total phenolic content was determined with the Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1965). Gallic acid was used as a reference standard; Folin–Ciocalteu reagent was prepared (prediluted 10-fold with distilled water) and left at room temperature for 5 min, followed by addition of sodium bicarbonate (1.2 ml: 7.5%, w/v) to the mixture. After standing for 60 min at room temperature, absorbance was measured at 765 nm. Results were expressed as milligram of Gallic acid equivalent (mg GAE/ g).

Free amino acids

The free amino acids were determined according to the method of Lee and Takahashi (1966) by utilizing ninhydrine reagent. In brief, 0.5 g tissue was incubated in 70% ethanol overnight, and then washed with double distilled water. A 1.5 ml of 55% glycerol and 0.5 ml ninhydrin solution were added, boiled at 100 °C for 20 min and cooled down. The final volume was made up to 6 ml with double distilled water, followed by the measuring of the optical density at 570 nm.

Total soluble proteins

Total soluble protein extraction and estimation were carried out accordingly. In brief, 0.5 g tissue was homogenized in a mortar and pestle with 1.0 ml (0.1 M) phosphate buffer (pH 7.0), placed on ice and centrifuged at 5000 rpm for 10 min. another centrifugation with 0.5 ml TCA, then the supernatant was discarded, and the pellet was dissolved in 1.0 ml of 0.1 N NaOH after washing with double distilled water. Total soluble protein content of homogenates was determined according to Bradford (1976) with Coomasie Brilliant Blue using bovine serum albumin as standard at 595 nm.

Peroxidase activity

Peroxidase activity was detected according to the method of Kim and Yoo (1996). Each one unit of peroxidise catalyzing the oxidation of guaiacol in one minute U/min/g. The increase in the absorption as a result of tetraguaiacol formation was measured at 470 nm.

Statistical analysis

A complete randomized design was employed in all experiments with four replicates, the results presented here were analysed by using the software SPSS for windows (version 10.0). Statistical significant was confirmed by ANOVA (Analysis of variance) and with revised least significant difference (RLSD) test at the probability level of 0.01, with four replicates for each parameter. All results were expressed as mean and standard deviation of the mean.

Conclusions

This study revealed the lethal effects of Switch, gentamycin, and chloramphenicol at a high concentration of 100 mg/l in dicamba treatment on the growth of cultured tissues of date palm Hillawii cv. Beltanol and chloramphenicol concentrations remarkably reduced the fresh and dry weights of callus treated with 2, 4-D and dicamba by up to twofold lower than the control treatments did. The toxic effects of Beltanol and chloramphenicol at high concentrations were accompanied by an increase in the levels of biochemical parameters, such as total carbohydrates, total phenolic compounds, proline content, and peroxidase activity. No to mild effects were elicited by low chloramphenicol concentrations (50 mg/l). On the basis of our results, we recommend the use of low Beltanol and chloramphenicol concentrations rather than Switch and gentamycin as decontamination agents to prevent microbial growth or to treat microbial contaminants in date palm tissue cultures.

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