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Research Article

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Growth of apical and lateral buds of *Conadria fig* cultivar as affected by growth regulators

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Abstract Current study aimed to maximize multiplication rate, growth parameters and producing superior plantlets which can tolerate acclimatization conditions of *Conadria fig* cultivar through utilizing of Thidiazuron (TDZ), isopentenyladenine (2iP) separately. Shoots- that passed establishment stage- were cultured on MS medium that contained different concentrations of TDZ and 2iP (0.5,1 and 1.5 mg/l). In addition, activated charcoal and auxin type indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) were applied in rooting stage to evaluate their influences on root formation. Data indicated that number of shoots was produced on lower concentration of 0.5 mg/l 2iP while, 0.5 mg/l TDZ improved shoot thickness. Also, supplementation of the culture medium with 1.5 mg/l TDZ raised chlorophyll content. However, the optimal combination for number of roots and root length was 1.0 mg/l IBA+0.1mg/l IAA. Meanwhile, using of 1.0 mg/l IBA+0.1 mg/l IAA or 0.1 mg/l IAA increased root percentage (100%) as compared with auxin free medium plus activated charcoal, On another hand no beneficial effect was obtained from adding 0.1 mg/l IAA and1.0mg/l IBA+0.1mg/l IAA to the culture medium when root percentage was concerned. Conclusion of current study was gathering both of 2iP and TDZ since each hormone had a positive impact on proliferation rate, plantlet growth performance and chlorophyll content of fig (Conadria cv.), thereby gathering both of them may lead to promising results. Also, more studies should be carried out to state the optimum combination of TDZ and 2iP to produce ideal proliferation rate with vigor plantlets.

Keywords *In vitro* proliferation, *Conadria fig* cultivar, Thidiazuron (TDZ), isopentenyladenine (2iP)

Introduction

Ficuscarica L. a member of the family *Moraceae* was one of the earliest cultivated for its figs. Through micropropagation of fig tree it is possible to obtain pathogen free plantlets and this is one of the basic requirements for a successful commercial orchard. The most important technique of micropropagation of fig tree had the advantage of large scale production, providing plantlets whenever needed and using cultures of apical meristems and axillary buds to re-general proliferation shoots [1-5]. Efficient of TDZ in stimulating adventitious shoots in several fig cultivars had been reported by [6] and the best shoot regeneration of fig was achieved on the MS medium supplemented with 2.0 mg/l TDZ [4]. Development of an efficient regeneration protocol is essential for successful *in vitro* propagation in fig [7]. Also, many studies indicated to simulative effects of active charcoal on morphogenesis as a result of its irreversible absorption of inhibitory compounds in the culture medium and decreasing phenolic compound, the toxic metabolites and brown exudates accumulation. On other hand, few studies reported that, the

effect of active charcoal on growth regulator uptake is still unclear but some workers believe that active charcoal may gradually release certain absorbed products such as growth regulators and nutrients which become available to plants [8-10]. This study aimed to investigate the effect of different concentrations of TDZ and 2iP on proliferation stage of *Conadria fig* cultivar and impact of adding auxin and active charcoal to MS on root formation.

Material and Methods

This study was carried out in the tissue culture laboratory of Pomology Dept. National Research Center during 2016 to 2017. Explants (apical and lateral buds) of *Conadria fig* cultivar that produced from the establishment stage were cultured individually on basal Murashige and Skoog (1962) [11] supplemented with different concentrations of TDZ and 2iP , 30 g sucrose and 6 g/L Di-fico Bacto agar during the proliferation stage. The pH of the medium was adjusted at (5.6-5.8) then autoclaved at 121 °C and 15 Ib/inc for 15 minutes. All cultures of explants had been incubated under 16 hours of artificial light (fluorescent light at 30 um/sec) and 8 hours of darkness average temperature at 23 ±2°C.

Effect of different concentrations of TDZ and 2iP

Different concentrations of TDZand 2iPi.e. 0.5, 1.0 and 1.5 mg/l were investigated to determine the most suitable concentration that inducedthe highest proliferation of *Conadria fig* cultivar.

Effect of active charcoal, IAA and IBA

The proliferated shoots of *Conadria fig* cultivar were isolated and cultured on one-half strength MS medium supplemented with active charcoal (2gm), IAA (0.1mg/l) and IBA at the rate of 1.0 mg /l + 0.1mg/l IAA were studied to find out the best treatments encourage the highest root formation. At the end of experiments average number of shoots, shoot length, shoot thickness, root length, number of root and root percentage were recorded after 6 weeks of culturing.

Statistical Design

Statistical analysis of data, analysis of variance (ANOVA) and mean separation were carried out using Duncan's multiple range test and significances were determined at the ($p \leq 0.01$) level. Data analysis was performed using ASSISATAT version 7.7beta (2015).

Results and Discussion

Proliferation Stage

Table (1) and Figure(1) showed the effect of different concentrations of TDZ and 2iP on number of shoots, shoot length and shoot thickness parameters of *Conadria fig* cultivar. It is clear that lower concentration of 2iP (0.5 mg/l) significantly increased number of shoots followed by TDZ at rates (1 and 0.5 mg/l respectively) as well as higher concentration of 2iP (1.5mg/l) in comparison with the other treatments. Meanwhile, 2iP was more effective compared with TDZ treatments in regard to shoot length increment, with poor differences among 2ip concentrations. Moreover, the lowest concentration of TDZ (0.5mg/l) recorded a significantly increasing for shoot thickness. A glance in table (2) and figure (1) showed that number of leaves was significantly increased when 1.0 mg/l2iP was used followed by higher concentration of TDZ (1.5mg/l) in comparison with other treatments. Hormone (2iP) surpassed TDZ in its effect on leaf area. Moreover, 2iP at low level (0.5 mg/l) produced the highest leaf area (6.54 cm^2) followed with other two concentrations of 2iP (1 & 1.5 mg/l respectively without any remarkable differences among these concentrations). Moreover, higher concentration of TDZ (1.5 mg/l) resulted in significantly increasing chlorophyll content.

In general, the previous results indicate that 2iPat low concentration (0.5mg/l) enhanced number of shoots. While, TDZ improved shoot thickness. However, 2iP at different concentrations inducing the best shoot length and leaf area. Also, supplementation of the medium with (1.5 mg/l)TDZ encouraged chlorophyll. These results are in harmony with the finding of [4 and 6]. They found that the highest shoots of *Ficuscarica* L. were regenerated with high concentration of TDZ (2.0 mg /l) in addition, optimal shoot regeneration (100%) was obtained on MS medium supplemented with (2.0 mg/l) TDZ and (1.0mg/l)2iP [5].

Table 1: Effect of different concentrations of TDZ and 2iP on number of shoot, shoot length and thickness of *Conadria fig* cultivar

Parameter / Treatment	Number of shoot (number)	Shoot length (cm)	Shoot thickness (mm)
TDZ (0.5 mg/l)	26.33b	2.50 b	0.77 a
TDZ (1 mg/l)	22.50b	1.05 c	0.50bc
TDZ (1.5 mg/l)	13.67c	2.18 b	0.65ab
2iP (0.5 mg/l)	32.00a	5.67 a	0.50bc
2iP (1 mg/l)	8.67c	6.50a	0.40c
2iP (1.5 mg/l)	21.33b	5.83a	0.35c

Means in columns with different letter are significantly different according to LSD test (P < 0.05).

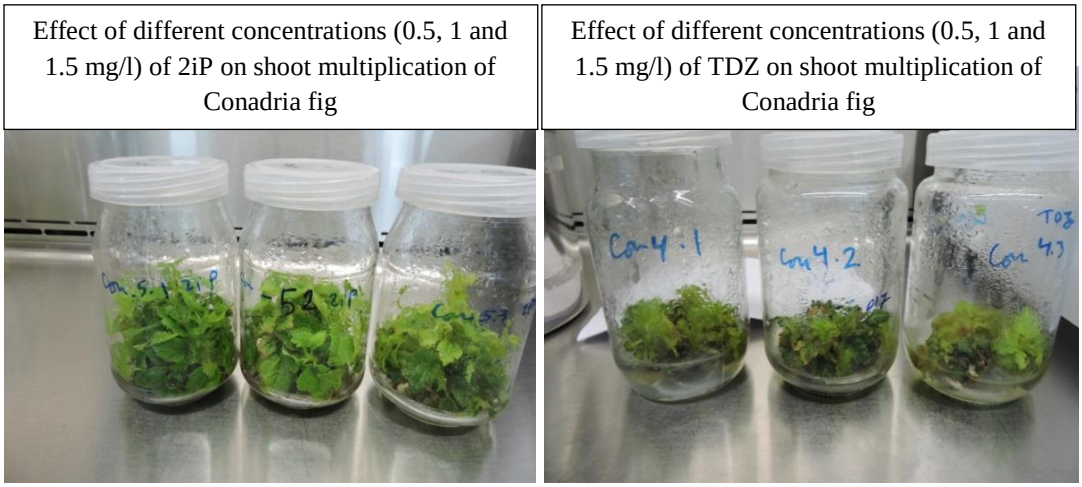

Figure 1: Effect of different hormones on proliferation rate of Conadria fig cultivar (right figure for 2iP and left on for TDZ)

Table 2: Effect of different concentrations of TDZ and 2iP on number of leaves, leaf area and chlorophyll of *Conadria fig* cultivar

Parameter / Treatment	Number of leaves (number)	Leaf area (cm^2)	Chlorophyll (SPAD)
TDZ (0.5 mg/l)	2.53 cd	1.58 d	36.27ab
TDZ (1 mg/l)	3.52 bc	2.49 c	34.77 ab
TDZ (1.5 mg/l)	4.20 b	1.23 d	48.97 a
2iP (0.5 mg/l)	1.75 d	6.54 a	36.57ab
2iP (1 mg/l)	6.11 a	4.72 b	26.07bc
2iP (1.5 mg/l)	2.95 bcd	4.35 b	18.66 c

Means in columns with different letter are significantly different according to LSD test (P < 0.05).

Rooting stage

Data presented in table (3) and figure (2) shows that supplementing the medium with (1.0mg/l IBA + 0.1mg/l IAA) or (0.1 mg/l IAA) recorded high rooting percentage (100%) in comparison with active charcoal (74.4%). However, number of roots and root length were significantly increased by culturing on MS medium supplemented with (1.0mg/l IBA + 0.1 mg/l IAA). Finally, Figure (3) shows plantlets that survived in acclimatization stage with 78% rate when cultured in pots contained mixture of sand: peatmoss (1:1).

In general, the aforementioned results indicated that supplementation of the medium with auxin encouraged root percentage as compared with active charcoal. However, using (1.0 mg/l IBA +0.1mg/l IAA) enhanced number of roots and root lengths. These results are in harmony with the finding of (5, 12 and 13]. They found that the best

medium for rooting of fig cultivar was MS medium in the presence of 1.2 and 2.5µM IBA or NAA. Also, active charcoal not only increased the root percentage response but also reduced the callusing of the explants [14]. Activated charcoal (AC) is composed of carbon, arranged in a quasigraphitic form of small particle size. . It is an essential compound of several plant tissue culture media, which decreases browning of cultured tissues and media by adsorption of toxic and other compounds like polyphenols released by cultured tissues [15]. Also, Priya Dharishini *et al*.,[16] showed that addition of active charcoal, at concentration ranging from 0.5 to 2 g/l, promotes more roots formation. However, a decrease in roots formation was observed with active charcoal concentration of more than 2 g/l. In addition, Pan *et al*.,[17] reported that root number per hypocotyl segment of *Daucus carota* decreased in the NAA or IAA-containing media in the presence of Active Charcoal. Besides, In *Thapsia garganica*, rooting of the stock culture kept on MS medium supplemented with BA and NAA were rooted on ½ MS medium fortified with IBA prior to AC (0.5 g/l) treatment and 50% of the plantlets rooted while an average number of 6 roots per shoot were observed in each shoot [18]. All aforementioned above can be interpreted by what stated by Gunnar ad Tage [19] who thought that activated charcoal removes substances from the medium, one of which might be auxin.

Table 3: Effect of different concentrations of active charcoal, IBA and IAA on root percentage, root number and root length of *Conadria fig* cultivar

Parameter / Treatment	Root percentage (%)	Root number (number)	Root length (cm)
Active charcoal	74.40 b	4.29 b	0.95 b
IBA (1.0 mg/l) + IAA (0.1 mg/l)	100.00 a	5.67 a	3.06 a
IAA (0.1 mg /l)	100.00 a	3.10 c	2.11 ab

Means in columns with different letter are significantly different according to LSD test ($P < 0.05$).

Figure 2: Effect of active charcoal , IBA (1 mg/l)+ IAA (0.1 mg/l) and IAA (0.1 mg/l) on rooting parameters of regenerated shoots of Conadria fig cultivar

Figure 3: Plantlets after acclimation of Conadria fig cultivar

Conclusion

Current study, highlighted on the importance of TDZ as hormone-like compound and its efficiency in producing healthy and ideal plantlets and recommend to gathering 2iP with TDZ to produce plantlets with good quality. Moreover, emphasis on value of qualitative more than quantitative for produced plantlets by tissue culture application. Finally, adding auxin in rooting medium was recommended to induce root formation with high quality.

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