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RESEARCH ARTICLE

In vitro propagation method of *Ficus carica* at Taif governorate using tissue culture technique

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Abstract

The experiment has been conducted to achieve a method for multiple-shoot induction with apical buds collected from mature tree of *Ficus carica* L., family Moraceae using Murashige and Skoog's (MS) medium supplemented with different growth regulators giberellic acid (GA3), benzyladenine (BA), kinetin (Kin). Data indicated that (BA) at the rate of 5 mg/L and (GA3) at 1 mg/L enhanced shoot multiplication. The best number of leaves was obtained from medium supplemented with 3 mg/L (BA) combined with (GA3) at 1 mg/L. In addition, plant length increased when (GA3) was added alone at 1 mg/L, followed by (GA3) combined with either (Kin) at 5 mg/L or (BA) at 3 mg/L compared with the control and the difference was significant.

INTRODUCTION

Ficus carica L. (Moraceae), commonly known as Fig plant which considered as one of the traditional Mediterranean crops. Taif governorate is the main fig producer in Saudi Arabian Kingdom. Fig is deciduous tree or large shrub, growing to a height of 7–10 meters (23–33 ft), with smooth white bark. Its fragrant leaves are 12–25 centimeters (4.7–9.8 in) long and 10–18 centimeters (3.9–7.1 in) across, and deeply lobed with three or five lobes Fig can be propagated through several vegetative methods as cutting, grafting and layering. However difficults controlling nematodes (*Meloidogyne*), mites (*Eriophyes fici*), and mosaic virus have been problematic thus, improvement of propagation and planting techniques is needed to face these challenges (Chrystiane et al., 2004). Only 20-30% of the cuttings obtained from the traditional methods survive. Besides, for large–scale propagation, several cuttings will be needed (equate for mass production) to increase cultivated area of fig and that will not be available thus tissue culture has become an important technique for propagation and breeding of fig and other woody plants and, an alternative method to overcome such problems. The application of tissue culture methods offers new prospects for rapid multiplication of many plants (Mustafa and Taha, 2012).

Fig has wide variety of chemical constituents, its use in traditional medicine as remedies for many health problems, and its biological activities. The plant has been used traditionally to treat various ailments such as gastric problems, inflammation, and cancer. Phytochemical studies on the leaves and fruits of the plant have shown that they are rich in phenolics, organic acids, and volatile compounds. However, there is little information on the phytochemicals present in the stem and root. Reports on the biological activities of the plant are mainly on its crude extracts which have been proven to possess many biological activities. Some of the most interesting therapeutic effects include anticancer, hepatoprotective, hypoglycemic, hypolipidemic, and antimicrobial activities (Shukranul et al., 2013).

MATERIALS AND METHODS

This study was carried out in the Plant Tissue Culture Laboratory, Faculty of Science, Taif University, Saudi Arabia.

Sterilization method:

Young shoot-tips of *Ficus carica* L. were subjected to surface sterilization by washing in tap water containing soap and small drops of Tween 20, 1 min. in ethanol 70%, washed with sterile distilled water and immersed in 30% commercial Clorox solution (1% Sodium hypochlorite) for 15 min. Shoot-tips were washed three times with sterile distilled water in laminar air flow hood to remove the residuals.

Culture media:

Sterilized shoot-tips were cultured in full strength of Murashige and Skoog (1962) (MS) medium supplemented with benzyladenin (BA) at 3.0, 4.0, and 5.0 mg/l and kinetin (Kin) at 3.0, 4.0, and 5.0 mg/l combined with 1.0 mg/l giberellic acid (GA3). Agar was used at 8g/L, and sucrose at 30 g/L. The media were distributed into clean jars. Each of which contained 30 ml of nutrient media. The media was adjusted to pH 6.2 before autoclaving for 15 min. at 121 ºC, 1.5 kg/cm^3. All treatments were incubated in the growth chamber at 26±2 ºC and exposed to 16 hr. light/day photoperiod under constant fluorescent light of 1500 Lux.

Experimental treatments:

The experiment consisted of eight treatments; each treatment included five jars (each contained three explants). The following combination treatments were carried out:

1- 0.0 mg/l (BA) or (Kin) + 0.0 mg/l (GA3) (control).
2- 1.0 mg/l (GA3)
3- 3.0 mg/l (BA) + 1.0 mg/l (GA3).
4- 4.0 mg/l (BA) + 1.0 mg/l (GA3).
5- 5.0 mg/l (BA) + 1.0 mg/l (GA3).
6- 3.0 mg/l (Kin) + 1.0 mg/l (GA3).
7- 4.0 mg/l (Kin) + 1.0 mg/l (GA3).
8- 5.0 mg/l (Kin) + 1.0 mg/l (GA3).

Experimental design and statistical analysis:

A complete randomize design was used throughout the research. The obtained data were subjected to analysis of variance and the treatment means were compared using L.S.D. test as outlined by Snedecor and Cochran (1975).

Data recorded:

The following data were recorded after 3 weeks from culturing on media for:

1- Average number of shoots per explant.
2- Average length of shoots (cm).
3 Average number of leaves.

RESULTS AND DISCUSSION

a- Number of *F. carica* shoots:

Data represented in Table (1) and Figures (1, 2 and 3) illustrate the significant enhancing effect of different concentrations of (GA3), kinetin and (BA) on shoot production of *F. carica*. Most of the treatments significantly increased number of shoots per explant than the control. The highest number of shoots (7.25) was obtained as a result of modifying MS medium with 5.0 mg/l (BA) + 1.0 mg/l (GA3) comparing with absence of (BA). Replacing (BA) with the same concentration of (Kin) significantly decreased number of shoot per explant to about 50%. Cytokinins have been shown to activate RNA synthesis and to stimulate protein synthesis and enzyme activity. On the other hand, the results indicated that, in *F. carica*, the presence of kinetin was not effective as BA to induce shoot formation. Our results were supported by Sen and Sharma (1991) who reported that the most effective cytokinin in promoting *W. somnifera* shoot proliferation from shoot tips was BA, shoot multiplication rates were higher at greater BA concentration; kinetin was less effective than BA in inducing shoot multiplication.

It is also appeared from the table that (BA) has a variable effect according to the level used**.** In this concern, the results obtained by Vaario et al. (1999) suggested that the addition of BAP at concentrations of 0.5 and 1.0 mg/l stimulated the development of *Abies firma* new needles and the number of newly developed needles (NDN) was maximized at the concentration of 1.0mg/l. However, there were no significant differences among average number

of NDN at concentrations from 0 to 1.0 mg/l. Concentration of 2.0 mg/l BAP suppressed needle development. Combination of 3.0 mg/l (Kin) and 1.0mg/l (GA3) showed little effect on shoot formation relative to other combinations shown in the table.

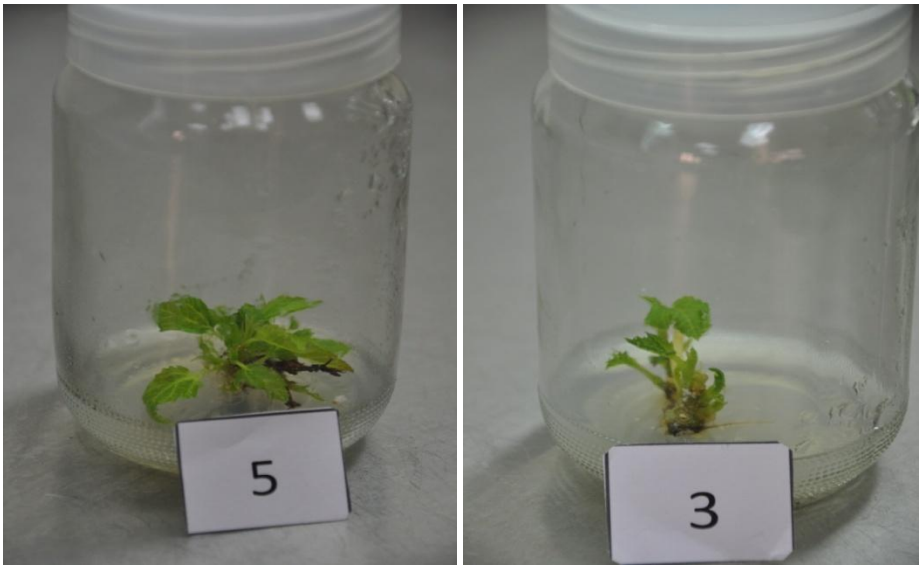

Figure 1: Treatment (5) consists of 5.0 mg/l (BA) and 1.0 mg/l (GA3) gave a significant increment of shoot number/explants absolutely.

Figure 2: Treatment (3) gave highly increment in leaf number with compact appears.

Table (1): Effect of growth regulators on growth performance of Fig shoot.

Treatments	Number of shoots/ explants	Shoot length/ explant (cm)	Number of leaves/ explant
0.0 mg/l (GA3) + 0.0 mg/l (BA) + 0.0 mg/l (Kin) (control)	1.00 d	2.33 c	2.68 c
1.0 mg/l (GA3)	2.25 bcd	3.60 a	3.50 c
3.0 mg/l (BA) + 1.0 mg/l (GA3)	7.00 a	3.53 ab	15.25 a
4.0 mg/l (BA) + 1.0 mg/l (GA3)	4.75 ab	3.15 ab	8.25 b
5.0 mg/l (BA) + 1.0 mg/l (GA3)	7.25 a	3.13 ab	8.25 b
3.0 mg/l (Kin) + 0.1 mg/l (GA3)	1.75 cd	2.80 bc	5.50 bc
4.0 mg/l (Kin) + 1.0 mg/l (GA3)	3.75 bc	2.83 bc	6.00 bc
5.0 mg/l (Kin) + 1.0 mg/l (GA3)	3.25 bcd	3.53 ab	5.00 bc
L.S.D at 5%	2.73	0.74	3.98

b- Shoot length (cm) of *F. carica*:

Data of shoot-tips grown on MS medium including different concentrations of growth regulators shown in the same table and figure (4). It is clear that the absence of growth regulators (control treatment) significantly produced the shortest shoots of *F. carica* (2.33 cm). However, inclusion of 1.0 mg/l (GA3) in the medium significantly elongated the shoots but less than the control, while inclusion of 3.0 mg/l BA and 1.0 mg/l (GA3) showed the same equal significant with 5.0 mg/l Kin and 1.0 mg/l (GA3) of elongation (3.53), shown in the table 1.

On the other hand, the concentration of 3.0 mg/l (Kin) +1.0 mg/ l (GA3) and the concentration of 4.0 mg/l (Kin) +1.0 mg/ l (GA3) gave the shortest shoots (2.83 cm) compared with the other treatments and the difference was not significant. Supporting results were obtained by Bouza et al. (1994) who reported that the cytokinin used in shoot multiplication of *Paeonia suffriticosa* was associated with further shootlet elongation and rooting stages, and that both were related to the accumulation of endogenous levels of indole acetic acid and the subsequent decrease in the cytokinin levels at the end of multiplication cycle.

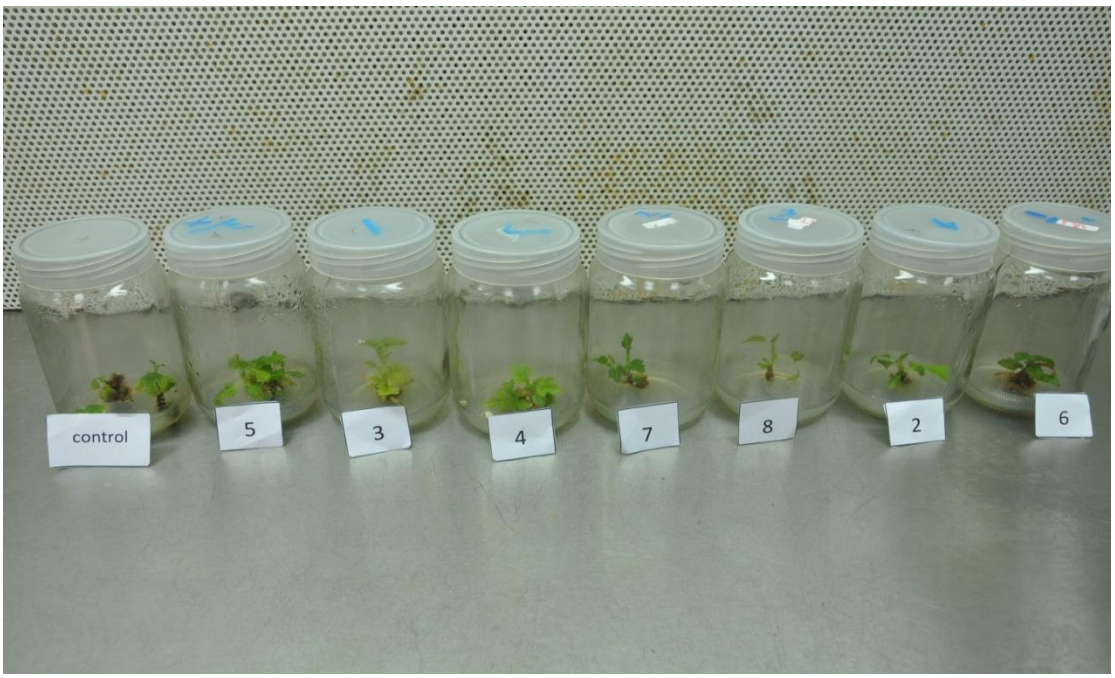

Figure 3: All treatments with its numbers showed that the gradually increment occurred in number of shoots/explants.

Figure 4: Treatment (2) contained 1mg/l GA3 only gives the highly significant increment in plant length compared with control and their treatments.

c- Number of *F. carica* leaves:

High increment was found from leaves (15.25) cultured on a medium with 3.0 mg/l BA and 1.0 mg/l (GA3) followed by significant increment (8.25) which derived from the concentration of 4.0 mg/l BA compared with the previous one. Data obtained from 5.0 mg/l BA and 1.0 mg/l (GA3) also gave the same result of significant increment. On the other hand, remarkable significant decrement of leaf number (3.5) obtained when (GA3) was applied alone at 1.0 mg/l. Number of compound leaves were more in treated plants as compared to control. Application of GA3 showed remarkable increase in the number of compound leaves. Furthermore, leaves treated with GA3 were light green, however, BA and Kin treatments showed healthy lush green leaves with increase in the number of compound leaves. Bhatti and Ahmed (2004) reported similar results on *Lens culinaris* Medik, since mixed dose of GA3 + IAA and GA3 + kinetin increased the number of compound leaves by 17.9 and 16.2 after 30 days in comparison to control.

CONCLUSION

According to the previous data we concluded that the level of 5.0 mg/l (BA) and 1.0 mg/l (GA3) gave a significant increment of shoot number/explants absolutely. On the other hand, the tallest shoot/explants obtained when (GA3) was 1.0 mg/l**,** whereas the optimum number of leaves/explants recorded when BA was 3.0 mg/l and (GA3) 1.0 mg/l.

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