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ORIGINAL PAPER

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In vitro CULTURE OF BIG-SAGE (*Lantana camara* L.) PLANT

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ABSTRACT

The study was conducted to mass micropropagation of big sage (*Lantana camara* L.) plant by shoot multiplication technique. The treatments 2.22 and 2.66 µmol·L^{-1} BA gave the highest significant increase in the percentage of response to shoot multiplication and number of shoots per explant compared to the other treatments as reached 96.70% and 100.00% and 4.33 and 6.00 shoots, respectively. The results showed that these two treatments did not differ significantly between them. While the 1.33 µmol·L^{-1} BA gave the lowest values in the percentage of response to shoot multiplication and number of shoots per explant were 80.00% and 2.00 shoots per explant, respectively. The MS medium supplemented with 4.30 or 5.37 µmol·L^{-1} NAA gave a high response to root formation, number of roots per shoot and root length. While the MS medium supplemented with 6.44 or 7.52 µmol·L^{-1} NAA gave low values in these characteristics. The MS medium with 2.22 or 2.66 µmol·L^{-1} concentration of BA or 7.52 µmol·L^{-1} concentration of NAA recorded the highest significant increase in the percentage of response to callus formation. While the MS medium supplemented with 1.33 µmol·L^{-1} BA or 4.30 µmol·L^{-1} NAA gave less response to the callus formation.

Key words: benzyl adenine, callus induction, explant, micropropagation rooting, shoot multiplication

INTRODUCTION

Lantana camara L. belongs to plant family Verbenaceae, commonly known as big-sage. Big-sage is a low, erect, vigorous, aromatic and evergreen shrub which can grow to 2–4 meters in height. It is also considered as an ornamental garden plant [Kalita et al. 2012]. The leaf is ovate or ovate oblong, arranged in opposite pairs. Leaves are bright green, rough, finely hairy. The stem is woody, square in cross section, hairy when young, cylindrical and up to 15 cm thick as it grows older [Gorai et al. 2016]. Flower heads contain 20–40 flowers, usually 2.5 cm across, the color varies from white, cream or yellow to orange pink, purple and red. Flowering occurs between August and March, or all year round if adequate moisture and light are available [Lonare et al. 2012]. Big-sage is a medicinal plant which is mainly used as traditional medicine [Kalita et al. 2012]. Leaf extracts of big-sage exhibit antimicrobial, fungicidal, insecticidal and nematicidal properties [Reddy 2013, Veraplakorn 2016]. Verbascoside, which possesses antimicrobial, immunosuppressive and antitumour activities, has been extracted from big-sage [Kalita et al. 2012]. Big-sage plant propagates by seeds but their germination rate is low. This plant also proliferates by traditional vegetative propagation methods such as cuttings and layering to obtain a few plants [Samani et al. 2014]. As a result of the above mentioned reasons, many researchers have conducted numerous studies on the tissue culture of this plant. Affonso et al. [2007] found that the study of the micropropagation of big-sage plant that the addition of N6-benzyladenine (BA) at a concentration of 0.99 mg·L^{-1} to the MS medium [Murashige and Skoog 1962] led to an increase in the number of shoots and nodes of each explant. Waoo et al. [2013]

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also indicated that the concentration of 0.5 mg·L^{-1} of the BA gave the highest percentage of response to the shoot proliferation and the maximum number of shoots when the shoot tips or nodes were cultured as explants by *in vitro* culture. While Samani et al. [2014] found that adding 8 mg·L^{-1} concentration of BA to the proliferation medium gave the maximum number of shoots per nodal segment. The study of Veraplakorn [2016] indicated a high shoot multiplication rate on MS medium supplemented with 3.6 mg·L^{-1} concentration of BA. Shoots gave good root regeneration with healthy plantlets on MS medium supplemented with 1.5 mg·L^{-1} of indole-3-butyric acid (IBA). While Charan and Kamlesh [2015] found high response to shoot multiplication of big-sage plant when added low concentrations of BA (1.5 mg·L^{-1}) and IBA or 1-naphthaleneacetic acid (NAA – 0.2 mg·L^{-1}) to MS medium. Veraplakorn [2016] refers to a large callus size formation at the basal end of big-sage shoots on the MS media added with 3.6 mg·L^{-1} or 7.5 mg·L^{-1} of NAA combined with 9.0 mg·L^{-1} BA. Leaf explants of this plant were more suitable for callus initiation and formation. This study was carried out with the aim of propagating a big-sage plant in a micropropagation technique to obtain large numbers similar to the mother plant and free of pathogens.

MATERIALS AND METHODS

The study was carried out in the plant tissue laboratory at the Faculty of Agriculture at the University of Basrah in southern Iraq. The young plants of the big-sage (*Lantana camara* L.) plant sterilized their surface in a solution containing 1.05% sodium hypochlorite with 2–3 drops of the Tween20 material for 20 min. It was then washed with sterilized distil water at three times in the laminar flow air cabinet. The shoot tips of the big-sage were then excised by a sterile blade and used as explants.

Shoot multiplication stage. The MS medium used to experiment of shoot multiplication stage. Glycine, thiamine-HCl, pyridoxine, nicotinic acid were added at a concentration of 1 mg·L^{-1} for each of them. In addition, 40 mg·L^{-1} adenine sulfate, 170 mg·L^{-1} sodium hydrogen orthophosphate, 30 g·L^{-1} sucrose and 0.54 µmol·L^{-1} NAA were added to the MS medium. BA was added at different concentrations (1.33, 1.78, 2.22 and 2.66 µmol·L^{-1}) to the MS medium. The pH of the medium was adjusted at 5.7 by using hydrochloric acid (HCl) and sodium hydroxide (NaOH) at one normality concentration. The MS medium was then heated to 90°C with continuous stirring using the hot-plate magnetic stirrer and the addition of 6 g·L^{-1} agar to facilitate its melting and to obtain a homogeneous mixture of the MS medium. The 20 ml medium was poured into glass culture tubes with a dimension of 2.5 × 18 cm. The pipe nozzle was closed using medical cotton and aluminium foil. The MS medium was sterilized by autoclave at 121°C and 1.05 kg·cm^{-2} for 20 min. The shoot tips were cultured on these sterile MS media. The cultures incubated at a temperature of 25 ±2, light density at 1000 lux and photoperiod at 16 h light and 8 h darkness. The percentage of the response of explant to shoot multiplication, the number of shoot per explant, shoot length (cm) and response to callus initiation were recorded after eight weeks of culturing.

The rooting stage. The produced shoots from the multiplication stage were cultured on the MS medium prepared for rooting under the same incubation conditions of the previous experiment. The same concentrations of the previous medium components were used for rooting experiment except for growth regulators. NAA was added with different concentrations at 1.61, 2.15, 2.69, 3.22 µmol·L^{-1} or 4.30, 5.37, 6.44 and 7.52 µmol·L^{-1}. The percentage of shoot response to rooting, number of primary root per shoot, root length (cm) and response to callus initiation were calculated after eight weeks of culturing.

Experimental design and statistical analysis. The simple experiments are designed according to completely randomized design (RCD). Each experimental treatment was repeated ten times. The data were subjected to the analysis of variance and mean values were compared using revised LSD at 5% [Snedecor and Cochran 1986].

RESULTS AND DISCUSSION

Shoot multiplication experiment. The results from Table 1 show significant differences between the mean of the treatments in the percentage of response to the shoot multiplication, the number of shoots per explant and shoot length after eight weeks of *in vitro* culture. The treatments 2.22 and 2.66 µmol·L^{-1} BA

gave the highest significant increase in the percentage of response to shoot multiplication and number of shoots per explant compared to the other treatment as reached 96.70% and 100.00% and 4.33 and 6.00 shoots, respectively (Tab. 1, Fig. 1 A, B and C). The results showed that these two treatments did not differ significantly between them. While the 1.33 µmol·L^{-1} BA gave the lowest values in the percentage of response to shoot multiplication and number of shoots per explant were 80.00% and 2.00 shoots per explant, noticed from the same figure that there are no significant differences between the two superior treatments. The MS medium with BA at 2.66 µmol·L^{-1} gave the highest response reached 93.33% (Fig. 1E). While the MS medium supplemented with 1.33 µmol·L^{-1} BA gave less response to the callus formation of 56.67%. Cytokinins are organic compounds produced by the plant that encourages cell division, tissue differentiation, and breaking apical dominance and shoot formation of callus tissue [Hopkins and Hüner 2008]. The

Table 1. Effect of different concentrations of N6-benzyladenine (BA) on shoot multiplication of big-sage (*Lantana camara* L.) after eight weeks

BA concentration (µmol·L^{-1})	Response to shoot multiplication (%)	No. of shoots per explant	Shoot length (cm)
1.33	80.00	2.00	4.77
1.78	83.30	2.33	3.23
2.22	96.70	4.33	2.83
2.66	100.00	6.00	2.33
R-LSD ($P \geq 0.05$)	7.69	2.03	0.99

respectively. This treatment did not significantly difference from the 1.78 µmol·L^{-1}. These results of the current study had been agreed with the study which reported on micropropagation of big-sage plant [Waoo et al. 2013]. Veraplakorn [2016] did not agree with the present study. The high response to the shoot multiplication due to BA which is one of the important cytokinins that stimulates cell division in meristematic tissues and the growth of lateral buds and morphogenesis of plant as well as it works to break the apical dominance that leads to the growth and elongation of lateral shoots [Hopkins and Hüner 2008, Taiz and Zeiger 2010]. Results of the present study have been agreed with other studies in the effect of benzyl adenine in the shoot multiplication [Ibrahim et al. 2013, Abbas et al. 2014].

Figure 2 indicates that the MS medium with BA at 2.22 and 2.66 µmol·L^{-1} recorded the highest significant increase in the percentage of response to callus formation compared with the other two treatments, reached 86.67% and 93.33%, respectively. It is also difference in the growth of callus is due to an increase in the concentration of BA in MS medium, which has become the supra-optimal cause to cell division [Ibrahim 2012, Ibrahim and Daraj 2015].

The results of the study showed that the addition of NAA to the MS medium in concentrations at 1.61, 2.15, 2.69 and 3.22 µmol·L^{-1} did not lead to the root formation. However, when NAA was added to the MS medium at high concentrations (4.30, 5.37, 6.44 and 7.52 µmol·L^{-1}), the shoots of big-sage plant responded to root formation (Tab. 2, Fig. 1D). The results in Table 2 show no significant differences between the treatments in the shoot response to root formation. The MS medium supplemented with 4.30 or 5.37 µmol·L^{-1} NAA gave a high response to root formation reached 100% for each of them. While the MS medium supplemented with 6.44 or 7.52 µmol·L^{-1} NAA gave a low response to root formation reached 90% for each of them. The reason for the low response to root formation at high concentration is resulting from the interaction between the exogenous and endogenous auxins

that led to the inhibition in root initiation [Ibrahim and Daraj 2015]. Results of the present study have been agreed with other studies in the effect of NAA in the root initiation [Ibrahim 2012, Abbas et al. 2014].

Table 2 shows that the MS medium supplemented with 5.37 µmol·L^{-1} NAA gave a significant increase in the number of roots per shoot. The results showed no significant differences among the other treatments (4.30, 6.44 and 7.52 µmol·L^{-1} NAA) in the number of roots per shoot. The MS medium supplemented with 7.52 µmol·L^{-1} concentration of NAA gave the lowest value in the number of roots per shoot reached 4.00 roots. That the increase in the number of roots in the shoots cultured in the MS medium with 5.37 µmol·L^{-1} concentration of NAA is due to the optimal concentration.

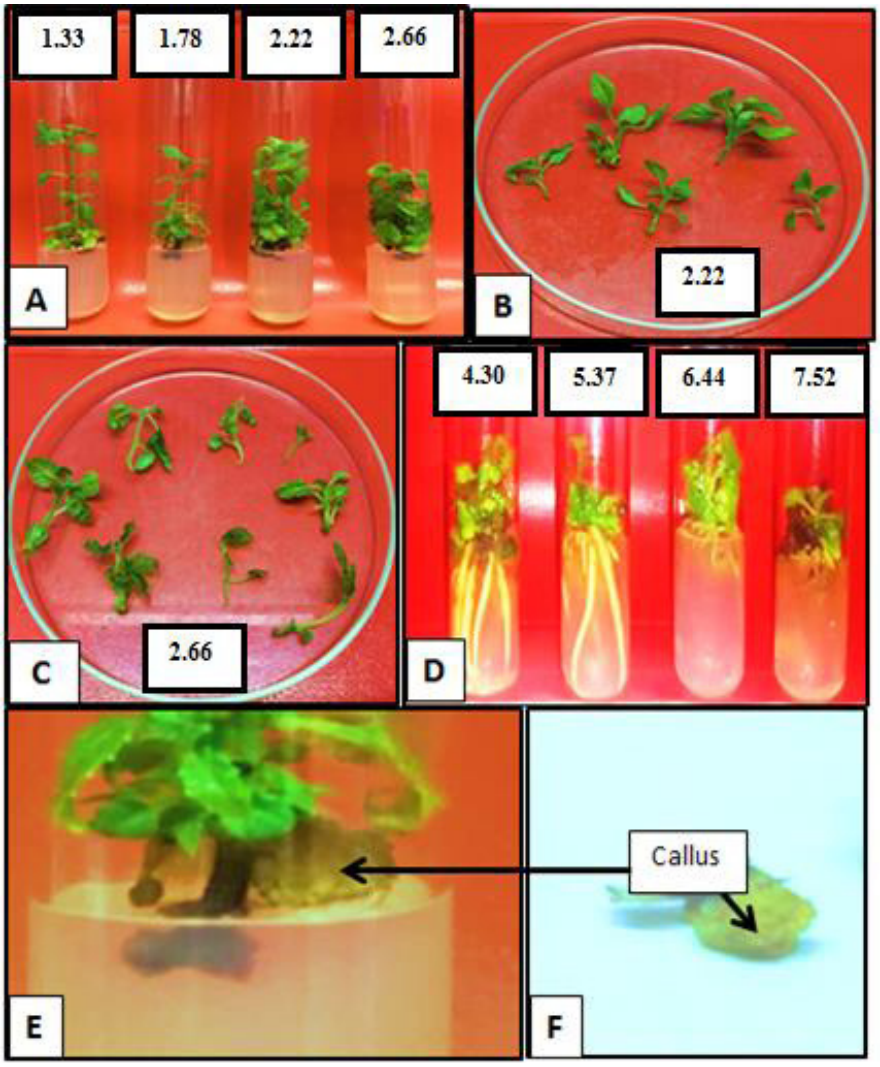

Fig. 1. Micropropagation of big-sage (*Lantana camara* L.) plant

A – Shoot multiplication on MS medium supplemented with different concentrations of BA
B – Shoot formation on MS medium supplemented with 2.22 µmol·L^{-1} concentration of BA
C – Shoot formation on MS medium supplemented with 2.66 µmol·L^{-1} concentration of BA
D – Root formation on MS medium supplemented with different concentrations of NAA
E – Callus initiation on MS medium supplemented with 2.66 µmol·L^{-1} concentration of BA
F – Callus initiation on MS medium supplemented with 7.52 µmol·L^{-1} concentration of NAA

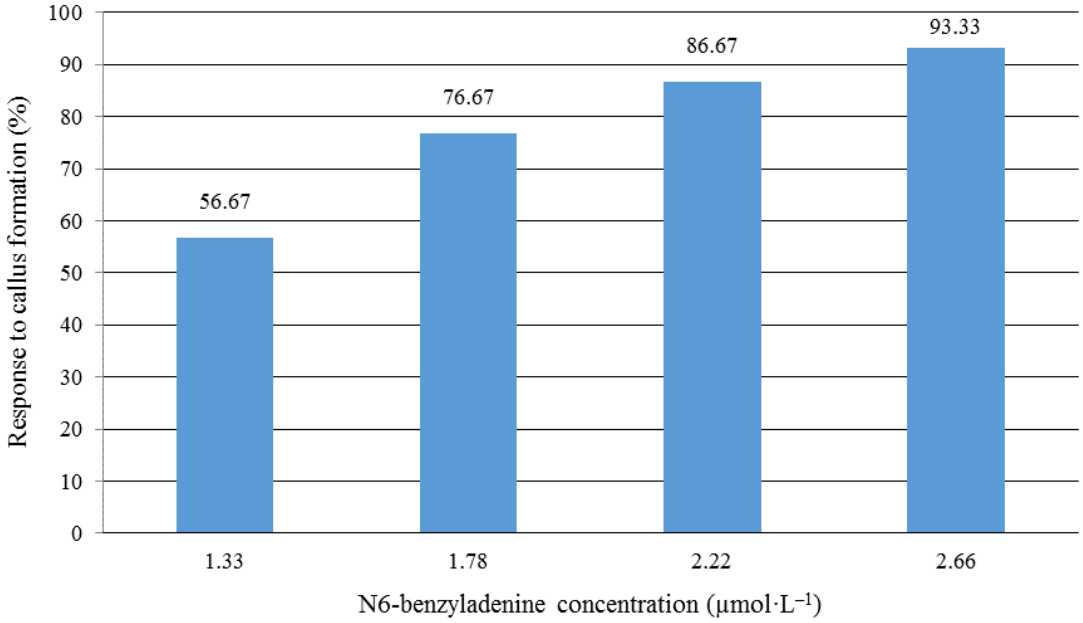

Fig. 2. Effect of different concentrations of N6-benzyladenine on the response of explant to callus formation after eight weeks from culture. RLSD ($P \geq 0.05$) = 9.47

Table 2. Effect of different concentrations of 1-naphthaleneacetic acid (NAA) on the rooting of big-sage (*L. camara* L.) shoots after eight weeks

NAA concentration (µmol·L^{-1})	Response to rooting (%)	No. of primary roots per shoot	Root length (cm)
4.30	100.00	6.33	7.43
5.37	100.00	11.00	7.73
6.44	90.00	6.00	2.07
7.52	90.00	4.00	1.17
R-LSD ($P \geq 0.05$)	NS*	5.44	0.97

* NS – Non-significant

The results in Table 2 showed that the treatments with a concentration of 4.30 and 5.37 µmol·L^{-1} concentrations of NAA led to the formation of long and thick roots. While treatments 6.44 and 7.52 µmol·L^{-1} NAA gave short and thin roots (Fig. 1D). The two treatments 4.30 and 5.37 µmol·L^{-1} NAA gave the highest significant increase in root length of 7.43 and 7.73 cm, respectively. The results showed no significant differences between the two treatments. The optimal concentration of NAA led to the stimulation of cell division and elongation at the bottom of the shoot causing the initiation, growth and enlargement of roots. While the treatments 6.44 and 7.52 µmol·L^{-1} gave a lower means of root length of 2.07 and 1.17 cm, respectively. These results of the current study did not agreed with the study which reported on *Lantana camara* plant [Veraplakorn 2016].

The results in Figure 3 showed that MS medium which added 7.52 µmol·L^{-1} NAA gave a significant increase in response to callus formation compared

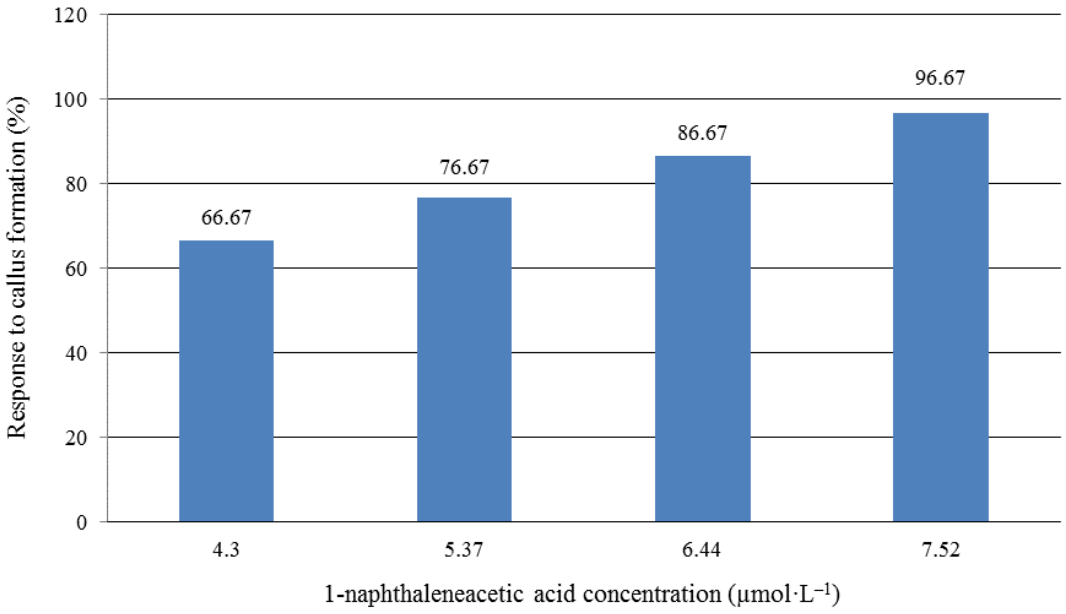

Fig. 3. Effect of different concentrations of NAA on the response of explant to callus formation after eight weeks from culture. RLSD ($P \geq 0.05$) = 9.47

with other treatments (Fig. 1F). The response to callus formation for this treatment reached 96.67%. While 4.30 µmol·L^{-1} NAA treatment gave the lowest percentage for response to callus formation reached 66.67%. Figure 3 shows that the callus formation increased with increasing auxin concentration that added to MS medium of root formation stage. Similar results were obtained by another worker, using the auxin naphthalene acetic acid with *Lantana camara* plant [Veraplakorn 2016]. The auxins play an important role in cell induction to division and growth because they are increasing respiration rate to provide energy for cell division [Al-Maari 1995]. The results obtained in the present work were similar to those reported for jujube (*Ziziphus* spp. Lam.) plant, regarding the importance of auxins for callus induction [Assareh and Sardabi 2005, Ibrahim et al. 2011].

CONCLUSION

The shoot tips that are culturing on the MS medium, with 2.22 or 2.66 µmol·L^{-1} BA, give the highest response to the shoot multiplication. The shoots culturing on the MS medium, which is supplemented with 4.30 or 5.37 µmol·L^{-1} NAA, give the highest response to root formation. The shoots culturing on the MS medium, which is supplemented with 2.22 or 2.66 µmol·L^{-1} BA or 7.52 µmol·L^{-1} NAA, give the highest response to callus initiation.

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