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Effect of Jasmonic Acid (JA) and Glutamine on Callus Induction of Madagascar Periwinkle Plant (*Catharanthus roseus* L. cv. Nirvana Pink Blush) by *in vitro* Culture

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A B S T R A C T

The current study was conducted to examine the role of Jasmonic acid and Glutamine in callus growth and multiplication. Seeds were germinated after disinfect by NaOCl at 4.5% for 20 min and cultured on full strength Murashige and Skoog (1962)(MS) medium. callus was initiated from Cotyledons explants taken from seeding were cultured growth on MS medium supplemented with different concentration of growth hormone include the Cytokinins Benzyl Amino Purine (BAP) at concentration (0.0,2.0) mg/l. and Auxin Naphthalene Acetic Acid (NAA) at concentration (0.0,1.0,2.0,4.0) mg/l for both growth regulators. Depending upon the result of chemical analysis, the combination of BAP at 2.0 Mg/L with NAA at1.0 Mg/L choised and used Jasmonic acid and Glutamine with different concentration of Jasmonic acid at concentration (0.0, 4.0) mg/l and Glutamine at concentration (250,300,350,400) Mg/L for 8 week culture period in callus induction. The results showed the presence of significant differences between the treatments in the fresh and dry weight of callus after five weeks from culture. 2.0 mg/l BAP treatment was significantly superior on control treatment in fresh and dry weight of callus, which reached 0.335 and 0.160 mg, respectively. Also, the two concentrations of NAA (1.0 mg/l) were significantly superior on control treatment in the same of two characteristics (0.332 and 0.150 mg fresh weight, and dry weight, respectively). The treatment of interaction between BAP and NAA (0.2+1.0 mg.L^{-1}) has given the highest significant difference in fresh and dry weight reached 0.483 and 0.215 mg, respectively. While less fresh and dry weight when treatment was control treatment, which reached 0.0 mg. The 4.0 mg/l Glutamine treatment was significantly superior on control treatment in fresh and dry weight of callus, which reached 290.42 and 40.855 mg, respectively. Also, the 350 mg/l concentration of Glutamine was significantly superior on control treatment in the same of two characteristics (286.38 and 54.664 mg fresh and dry weight). The treatment of interaction between Jasmonic acid and Glutamine (4.0 mg/l + 300 mg/l) has given the highest significant difference in fresh and dry weight reached 345.35 and 54.664 mg, respectively. While less fresh and dry weight when treatment 0 Jasmonic acid + 250 Glutamine mg/l concentrations, which reached 184.47 and 10.18 mg, respectively.

Introduction

Callus is an undifferentiated parenchyma cells producing from the cutting and wound areas of explants. Its induction depends on source of explant used and medium components. Callus

may be friable or solid textures and its color is yellow, white or green depending on source of explant and type of plant (George *et al*., 2000; Trigiano *et al*., 2008). The hormonal balance between auxins and cytokinins is necessary in organogenesis by *in vitro* culture. It was found that the high percentage of auxin/cytokinin lead to the formation of roots. While, increasing the level of cytokinin/auxin leads to the formation of shoots. The balanced levels of plant growth regulators lead to the continued formation of callus tissue (Skoog and Miller, 1957). Taiz and Zeiger (2002) noted that maturity cells are stimulated to divide when cultured in medium containing plant growth regulators, especially auxins and cytokinins. Staba (2000) explained that callus is usually incubated in the dark to avoid organ formation. Jasmonic acid is considered to be one of the growth hormones leading to aging, which reduces the level of gene expression (Edris, 2010).

Glutamine is an important amino acid that enters the process of cell protein synthesis and thus builds enzymes that play an important role in the plant's biosynthesis. It also regulates the acid and base balance of the cell to produce ammonia, an important source of cellular energy or a source of carbon. Glutamine also gives nitrogen in many bio-processes, including the synthesis of purines that are involved in building nucleic acids, an important carrier of ammonia (Brosnan, 2003; Aledo, 2004; Guyton, 2006; Yuneva *et al*., 2007).

Taha *et al*., (2009) found when their culturing callus of periwinkle plant on liquid MS medium supplemented with five types of amino acids and 300 mg.L-1 glutamine leads to increased quantities of Vincristin and Vinblastin formed. The aim of this study is to know the effect of acid Jasmonic and Glutamine in the induction and differentiation of callus of cotyledon leaves of periwinkle seedlings.

Materials and Methods

This project was conducted in the plant tissue culture laboratory, in the Department of Horticulture and Landscape Design, College of Agriculture, University of Diyala. During 21 May – 25 October / 2015. The major objective of this study was to increase the production of callus to the tissue culture medium *Catharanthus roseus* L. The periwinkle seeds cv. Nirvana Pink Blush obtained from the American seed production company "Pan. American"

Explants sterilization

Periwinkle seeds cv. Nirvana Pink Blush of current study equipped by the American company for seed production. This seeds were isolated and washed thoroughly under tap water to remove dust on the seed coat. Then the seeds were sterilized with 4.5 % sodium hypochlorite solution with 3-4 drops of tween20 for 20 minutes (Al-Zuhairi, 2016) and washed 3-4 times with distilled water inside the laminar air-flow cabinet. The sterilized seeds cultured on MS medium without hormones. They placed in a growth room under controlled conditions (temperature 25±2°C, 16/8 h photoperiod). Cotyledons were excised from cultures after 6 weeks from seed culture.

The media preparation

MS salts (Murashige and Skoog, 1962), vitamins (1.0 $mg.L^{-1}$), plant growth regulators and sucrose (30 $gm.L^{-1}$) are using in the medium of callus induction. The pH of medium is adjusted to 5.7 by sodium hydroxide and hydrochloric acid solution concentration of 0.1 N for each of them. Naphthalene acetic acid (NAA) added to MS medium in different concentrations (0.0, 1.0, 2.0 and 4.0 $mg.L^{-1}$). Benzyl Amino Purine (BAP) added at two concentrations (0.0 and

0.2 mg.L^{-1}). The different concentration of NAA and BAP were used to determine the optimal concentration for callus induction.

Callus induction

The cotyledons cultured in MS medium (10ml) supplemented with 0.0 or 2.0 mg.L^{-1} (BAP) and 0.0, 1.0, 2.0, 3.0 or 4.0 mg.L^{-1} (NAA). Each treatment represented ten replications.

Callus was multiplied through the cultivation of the best medium (MS salts + 2.0mg.L^{-1} BAP + 1.0mg.L^{-1}NAA).

Callus was multiplied through the cultivation of the best medium (MS salts +Jasmonic acid at 0.0, 4.0 mg.L^{-1}Glutamine + 250, 300, 350, 400mg.L^{-1}).

Effect of NAA and BAP on callus multiplication

Has been taking the weight of 100 mg of callus was grown on MS medium containing: 0.0 or 2.0 mg.L^{-1} BAP + 0.0, 1.0, 2.0, 3.0 or 4.0 mg.L^{-1} NAA. Each treatment represented ten replications. They placed in a growth room under controlled conditions (temperature 25±2°C and darkness). The fresh and dry weights of callus were calculated after 8 weeks from culture.

Effect of jasmonic acid and glutamine on callus multiplication

Has been taking the weight of 150 mg of callus was grown on MS medium containing: 4.0 mg.L^{-1} BAP + 1.0 mg.L^{-1} NAA + 0.0, 4.0 mg.L^{-1}Jasmonic acid+ 250, 300, 350 or 400 mg.L^{-1}. Glutamine Each treatment represented ten replications. They placed in a growth room under controlled conditions (temperature 25±2°C and darkness). The fresh and dry weights of callus were calculated after 8 weeks from culture.

Statistical analysis

Completely randomized design was used with 10 replicates. The data were subjected to the analysis of variance and mean values were compared using revised-LSD as described by Snedicor and Cochran (1980).

Results and Discussion

Effect of BAP and NAA on callus multiplication

Results from the two tables (Tables 1 and 2) showed the presence of significant differences between the treatments in the fresh and dry weight of callus after 8 weeks from culture. The 2.0 mg.L^{-1} BAP treatment was significantly superior on control treatment in fresh and dry weight of callus, which reached 0.335 and 0.160 mg, respectively. Also, the concentrations of NAA (1.0 mg.L^{-1}) were significantly superior on control treatment in the same of two characteristics (0.332 and 0.272 mg fresh weight, and 0.150 mg dry weight, respectively). The treatment of interaction between BAP and NAA (2.0+1.0 mg.L^{-1}) has given the highest significant difference in fresh and dry weight reached 0.483 and 0.215 mg, respectively. While less fresh and dry weight when of callus induction (Plate 1, A) when treatment was without growth regulators (control treatment), which reached 0.0 mg. The reason for the fresh and dry weight increase of callus are cytokinin (BAP) and auxin (NAA), which are also a growth promoters that have a significant and important role in cell division, leading to increased size and weight. The reason for the increase in the fresh and dry weight of callus is cytokinin (benzyl amino purine) and auxin (naphthalene acetic acid) by encouraging growth and their role in the division and enlargement of cells that led to increased callus in size and weight. This may be due to

the physiological balance between auxin and cytokinin. The addition of both growth regulators to the medium of culture is necessary for the induction of callus. Cytokinin works with auxin as a key to initiating cell division. Adenine, the cytokinin molecule, may be the optimal balance. The difference between explants may be due to the anatomical structure and its physiological development (Goodwin, 1985; Mineo, 1990).

Effect of jasmonic acid and glutamine on callus multiplication

The tables 3 and 4 showed the presence of significant differences between the treatments in the fresh and dry weight of callus after five weeks from culture. The 4.0 mg.L^{-1} Jasmonic acid treatment was significantly superior on control treatment in fresh and dry weigh of callus, which reached 290.42 and 40.855 mg, respectively. Also the 300 and 350 mg.L^{-1} concentration of Jasmonic acid was significantly superior on control treatment in the same of two characteristics (46.947 and 286.38 mg fresh and dry weight). The treatment of interaction between Jasmonic acid and Glutamine (300 mg.L^{-1}+ 4.0 mg.L^{-1}) has given the highest significant difference in fresh and dry weight of callus induction (Plate 1C) reached 345.35and 54.664 mg, respectively.

Table.1 The chemical material composition additives to MS medium used for callus induction

Seq.	Chemical material	Quantity (mg l-1)
1	Salt	full strength
2	Pyrodoxine –Hcl	0.5
3	Glycine	2.0
4	Nicotine acid	0.5
5	Thiamine–Hcl	0.1
6	Myo-inositol	0.1
7	Agar	7000
8	Sucrose	30000
9	Benzyl Amino Purine (BAP)	(0.0,2.0)
10	Naphthalene acetic acid(NAA)	0.0,1.0,2.0,4.0)(

Table.2 Effect of NAA and BAP on fresh weight of callus (mg) induced from cotyledonary leaf of the periwinkle plant by *in vitro*

BAP concentration (mg.l^{-1})	NAA concentration (mg.l^{-1})				Mean of BAP
	0.0	1.0	2.0	4.0	
0.0	0.000 D	0.182BDC	0.167BDC	0.093DC	0.110B
2.0	0.245BC	0.483A	0.311AB	0.302ABC	0.335A
Mean of NAA	0.123B	0.332A	0.239AB	0.198AB	

Table.3 Effect of NAA and BAP on dry weight of callus (mg) induced from cotyledonary leaf of the periwinkle plant by *in vitro*

BAP concentration ($mg.l^{-1}$)	NAA concentration ($mg.l^{-1}$)				Mean of BAP
	0.0	1.0	2.0	4.0	
0.0	0.000 B	0.087AB	0.090AB	0.096AB	0.068B
2.0	0.108AB	0.215A	0.150AB	0.172AB	0.160A
Mean of NAA	0.054A	0.150A	0.120A	0.134A	

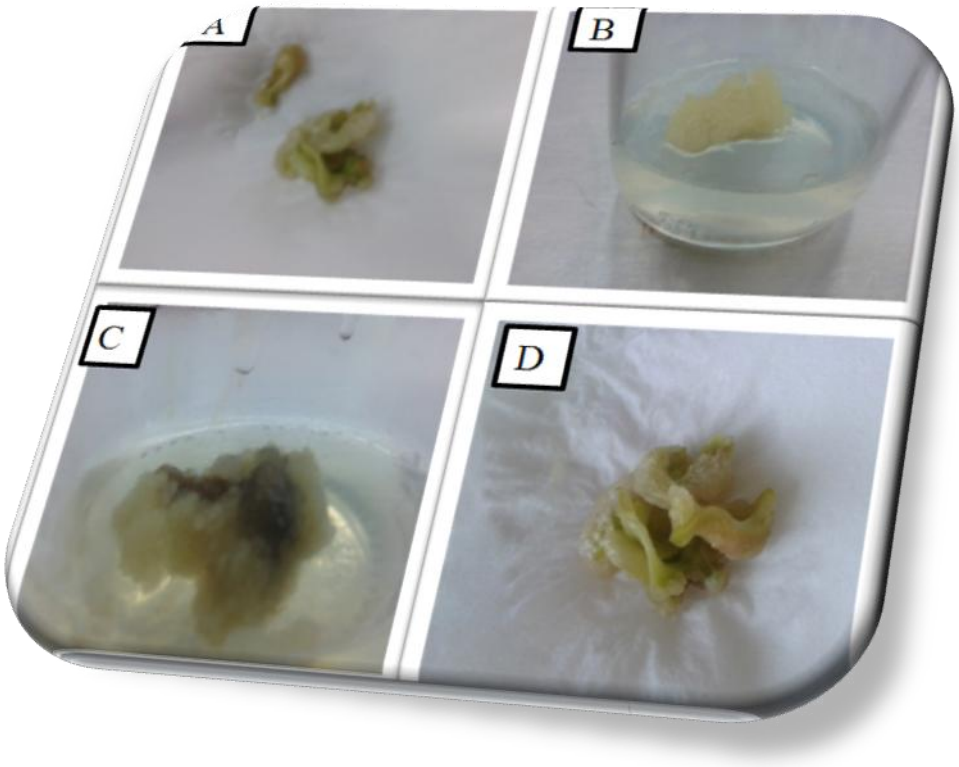
Table.4 Effect of Jasmonic acid (JA)and Glutamine on fresh weight of callus (mg) induced from cotyledonary leaf of the periwinkle plant by *in vitro.*

Jasmonic acid(JA)concentration ($mg.l^{-1}$)	Glutamine concentration ($mg.l^{-1}$)				Mean of Jasmonic acid(JA)
	250	300	350	400	
0.0	184.47D	202.55DC	269.52B	251.79BC	227.08B
4	254.76BC	345.35A	303.25AB	258.33BC	290.42A
Mean of Glutamine	219.61B	273.95A	286.38A	255.06AB	

Table.5 Effect of Jasmonic acid (JA) and Glutamine on dry weight of callus (mg) induced from cotyledonary leaf of the periwinkle plant by *in vitro*

Jasmonic acid(JA)concentration ($mg.l^{-1}$)	Glutamineconcentration ($mg.l^{-1}$)				Mean of Jasmonic acid(JA)
	250	300	350	400	
0.0	10.18C	19.473BC	42.001A	19.714BC	22.843B
4	20.077BC	54.664A	51.893A	36.787AB	40.855A
Mean of Glutamine	15.131C	37.069AB	46.947A	28.250B	

Plate.1 Effect of Naphthalene Acetic Acid(NAA) and Benzyl Amino Purine (BAP)(A,B)and Jasmonic acid(JA) and Glutamine(C,D) on callus multiplication of Plant



A:MS medium + 1.0 mg.L^{-1} NAA+ 2.0 mg.L^{-1} BAP
B:MS medium+ 2.0 mg.L^{-1} NAA+ 2.0 mg.L^{-1} BAP
C:MS medium+ 300 mg.L^{-1}Jasmonic acid(JA) + 4.0 mg.L^{-1} Glutamine
D:MS medium+ 350 mg.L^{-1}Jasmonic acid(JA) + 4.0 mg.L^{-1} Glutamine

While, less fresh and dry weight when treatment 0 Jasmonic acid + 250 mg.L^{-1} Glutamine concentrations, which reached 184.47 and 10.18 mg, respectively. Study results agreed with what he found Ueda and Kato (1982) on the soybean plant. As noted callus growth was significantly affected when Jasmonic acid at low concentration (0.45-4.50 µmol) added to medium of callus induction. Li *et al*., (2014), also pointed out that the Methyl jasmonate significantly effect on the induction and growth of callus, especially the concentration of 125 µmol. These results revealed this might be due to the rapid uptake of reduced nitrogen which provided by this amino acid (Al- Khayri, 2001). Glutamine and glutamic acid are directly involved in the assimilation of NH4+. A direct supply of these amino acids should therefore enhance the utilization of both nitrate and ammonium nitrogen and its conversion into amino acids (George, 1993). The addition of glutamine in date palm tissue culture media increased callus quality and somatic embryos formation (Jasim, 2001), add structure RNA and DNA. The results of the study agreed with the results of the Al-Memary (2014) to obtain the highest fresh weight of the callus (0.697 gm) from culturing explants of cotyledonary leaves on the MS medium supplying 0.4mg.L^{-1} Glutamine. These results may be explained by the fact that Glutamine is one of the amino

acids involved in building proteins that work on enzymes that play a role in most bio-processes as well as the building of both RNA and DNA (Dalaly, 1994). This is consistent with EL-Sharabasy *et al*., (2012) that the addition of Glutamine to the farming community with the presence of growth regulators led to a doubling of callus growth. This, according to EL-Sharabasy *et al*., (2012), suggests that the addition of Glutamine to the culture medium, with the presence of plant growth regulators, has callus growth and multiplication (Table 5).

In conclusion, the positive role of Jasmonic acid and glutamine in this study leads to the recommendation to include them in the c micropropagation program (callus production) and concluded from the present study also that cotyledon leaf of periwinkle plants have ability of growth and induction of indirect callus when they are cultured in the right medium and concentration of BAP, NAA.

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