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Effect of BA, NAA, Methyl Jasmonate and Mannitol on Callus Induction of Periwinkle Plant (*Catharanthus roseus* L. cv. Heatwave Mix) by In Vitro Culture

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ABSTRACT

The study was conducted to examine the role of BA, NAA, Methyl jasmonate and Mannitol 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. The 0.25 mg.L^{-1} BA treatment was significantly superior on control treatment in fresh and dry weight of callus, which reached 0.293 and 0.152 mg, respectively. Also, the two concentrations of NAA (0.5 and 1.0 mg.L^{-1}) were significantly superior on control treatment in the same of two characteristics (0.218 and 0.272 mg fresh weight, and 0.099 and 0.152 mg dry weight, respectively). The treatment of interaction between BA and NAA (0.25+1.0 mg.L^{-1}) has given the highest significant difference in fresh and dry weight reached 0.444 and 0.269 mg, respectively. While less fresh and dry weight when treatment was control treatment, which reached 0.0 mg. The 8000 mg.L^{-1} Mannitol treatment was significantly superior on control treatment in fresh and dry weight of callus, which reached 332.42 and 53.81 mg, respectively. Also, the 75 mg.L^{-1} concentration of Methyl jasmonate was significantly superior on control treatment in the same of two characteristics (347.19 and 55.67 mg fresh and dry weight). The treatment of interaction between Mannitol and Methyl jasmonate(8000 mg.L^{-1}+ 75 mg.L^{-1}) has given the highest significant difference in fresh and dry weight reached 451.25 and 69.17 mg, respectively. While less fresh and dry weight when treatment 0 mannitol + 25 mg.L^{-1} concentrations, which reached 211.99 and 25.38 mg, respectively.

تأثير BA و NAA ومثيل الجاسمونيت والمانيتول على استحداث كالس نبات عين البزون *Catharanthus roseus* L. cv.Heatwave Mix خارج الجسم الحي

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الخلاصة

أجريت دراسة لاختبار دور BA و NAA و الجاسمونيت مثيل والمانيتول في نمو وتضاعف الكالس. توضح النتائج وجود فروقات معنويه بين المعاملات المدروسة في الوزن الطري والجاف للكالس المستحث بعد خمسة أسابيع من الزراعة. أن تركيز 0.25 ملغم.لتر$^{-1}$ بنزيل الأدنين قد تفوق معنويا على معاملة المقارنة في الوزن الطري والجاف للكالس بلغ 0.293 و 0.152 غم على التوالي. كما تفوق تركيزي نفثالين حامض الخليك (0.5 و 1.0 ملغم.لتر$^{-1}$) معنويا على معاملة المقارنة في الوزن الطري والجاف للكالس (0.218 و 0.272 ملغم وزن طري و 0.099 و 0.152 ملغم وزن جاف على التوالي). أن معاملة التداخل بين البنزيل أدنين ونفثالين حامض الخليك (0.25+1.0 ملغم.لتر$^{-1}$) أعطت فرقا عالي المعنوية في الوزن الطري والجاف للكالس بلغ 0.444 و 0.269 ملغم على التوالي. بينما أعطت معاملة المقارنة أقل معدل في الوزن الطري والجاف والذي بلغ 0.0 ملغم. أن المعاملة بالمانيتول تركيز 8000 ملغم.لتر$^{-1}$ قد تفوقت معنويا على معاملة المقارنة في الوزن الطري والجاف للكالس الذي بلغ 332.42 و 53.81 ملغم على التوالي. كذلك وجد أن المعاملة بالجاسمونيت مثيل بتركيز 75 ملغم.لتر$^{-1}$ أدى الى تفوق معنوي على معاملة المقارنة في نفس الصفتين المدروسة (347.19 و

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55.67 ملغم على التوالي). أما معاملة التداخل بين المانيتول والجاسمونيت مثيل (75+8000 ملغم.لتر$^{-1}$) فقد أعطت أعلى تفوق معنوي في الوزن الطري والجاف للكالس بلغ 451.25 و 69.17 ملغم على التوالي. بينما أقل وزن طري وجاف للكالس كان عند المعاملة (25+0.0) ملغم.لتر$^{-1}$ مانيتول + مثيل جاسمونيت على التوالي والذي بلغ 211.99 و 25.38 ملغم على التوالي.

Introduction:

The periwinkle plant is an important source of many pharmaceutical compounds, pesticide, flavors, perfume and colors (Taiz and Zeiger, 2002). This plant is considered important economic, medical, as contains many active compounds such as Serpentine and Ajmalicine which are used in the treatment of hypertension (Jennifer, 2004). This plant also contains alkaloids important in the treatment of many dangerous diseases (Ferreres *et al*., 2008). It belongs to Apocynaceae Family, which includes many of the evergreen herbaceous perennial plants. The periwinkle plant has a height of 40-80 cm, which is propagating seeds and stem cuttings (Gilman and Howe, 1999). Moreover, being a fast-growing and wonderful beauty ornamental plant (Ferreres *et al*, 2008). The plant tissue culture technique used for the purpose of obtaining effective medical compounds (Mulabagal and Tsay, 2004; Karuppussamy, 2009). Methyl jasmonate is a plant hormone plays an important role in some physiological processes such as photosynthesis and flowering and senescence (Choing and Choi, 2003). In one study found when add the methyl jasmonate to the suspension cell culture resulted in increased production of a gallic acid compound from callus of *Lactuca sativa* plant by an amount equivalent of 35 times the comparison to the control treatment (Kim *et al*., 2007). This hormone is also used as a compound for the defense of the plant in the environmental changes and biotic and abiotic stress conditions (Zhou *et al*., 2013). Mannitol is an alcoholic sugar is cyclic, which is produced naturally in many plants (Burger *et al*., 2000). Whereas Zulkepli and Samad,(2011) demonstrated that the addition of BA and NAA in a concentration of (0.2 ,1.25)mg/L^{-1} respectively gave the highest increment in the fresh weight of Periwinkle plant that reached 1.54 g. Zhao *et al*. (2000) found increase in Ajmalicine alkaloid in callus of periwinkle plant when Mannitol added to MS medium, comparison with control treatment. The present study aims to examine the role of Methyl jasmonate and Mannitol in callus induction and multiplication.

Materials and Methods:

The study was conducted in the Plant Tissue Culture laboratory in the Department of Horticulture and Landscape Design, College of Agriculture, University of Diyala. The periwinkle seeds obtained from the American seed production company "Pan.American".

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, 0.5, 1.0 and 1.5 mg.L^{-1}). Benzyl adenine (BA) added at two concentrations (0.0 and 0.25 mg.L^{-1}). The different concentration of NAA and BA were used to determine the optimal concentration for callus induction.

Explants sterilization: Periwinkle seeds 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 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 4 weeks from seed culture.

Callus induction:

1. The cotyledons cultured in MS medium (10ml) supplemented with 0.0 or 0.25 mg.L^{-1} (BA) and 0.0, 0.5, 1.0 or 1.5 mg.L^{-1} (NAA). Each treatment represented ten replications.
2. Callus was multiplied through the cultivation of the best medium (MS salts + 0.25 mg.L^{-1} BA + 1.0 mg.L^{-1}NAA).

Effect of BA and NAA on callus induction: Has been taking the weight of 100 mg of callus was grown on MS medium containing: 0.0 or 0.25 mg.L^{-1} BA + 0.0, 0.5, 1.0 or 1.5 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 5 weeks from culture (Plate 1, A and B).

Effect of Methyl jasmonate and Mannitol on callus induction: Has been taking the weight of 150 mg of callus was grown on MS medium containing: 0.25 mg.L^{-1} BA + 1.0 mg.L^{-1} NAA + 25, 50, 75 or 100 mg.L^{-1}Methyl jasmonate + 0.0, 8000 mg.L^{-1}Mannitol. 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 5 weeks from culture (Plate 1, C and D).

Statistical analysis: The factorial experiments were carried out using Completely Randomized Design (CRD). The data were analyzed using SAS (2002). The means of treatments were measured by Duncan Multiple Range Test under the 5% probability level. Each treatment included 10 replicates, each containing one explant (Al-Sahuki and Wahib, 1990).

Results and Discussion:

1. Effect of BA and NAA on callus induction: Results from the two Tables 1 and 2 showed the presence of significant differences between the treatments in the fresh and dry weight of callus after five weeks from culture. The 0.25 mg.L^{-1} BA treatment was significantly superior on control treatment in fresh and dry weight of callus, which reached 0.293 and 0.152 mg, respectively. Also, the two concentrations of NAA (0.5 and 1.0 mg.L^{-1}) were significantly superior on control treatment in the same of two characteristics (0.218 and 0.272 mg fresh weight, and 0.099 and 0.152 mg dry weight, respectively). The treatment of interaction between BA and NAA (0.25+1.0 mg.L^{-1}) has given the highest significant difference in fresh and dry weight reached 0.444 and 0.269 mg, respectively. While less fresh and dry weight of callus induction (Plate 1, B), 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 (BA) and auxin (NAA), which are also a growth promoter that have a significant and important role in cell division, leading to increased size and weight.

Table (1) Effect of NAA and BA on fresh weight of callus (mg) induced from cotyledonary leaf of the periwinkle plant by *In vitro*

BA concentration (mg.l^{-1})	*NAA concentration (mg.l^{-1})*				*Mean of BA*
	0.0	0.5	1.0	1.5	
0.0	0.000 c	0.099 bc	0.099 bc	0.045 c	0.060 b
0.25	0.101 bc	0.336 a	0.444 a	0.290 ab	0.293 a
Mean of NAA	0.050 b	0.218 a	0.272 a	0.168 ab	

*mean in each column followed by the same letter are not significantly different (P ≤ 5%)

Table (2)Effect of NAA and BA on dry weight of callus (mg) induced from cotyledonary leaf of the periwinkle plant by *In vitro*

BA concentration ($mg.l^{-1}$)	*NAA concentration ($mg.l^{-1}$)*				*Mean of BA*
	0.0	0.5	1.0	1.5	
0.0	0.000 c	0.042 bc	0.036 bc	0.035 bc	0.028 b
0.25	0.067 bc	0.155 ab	0.269 a	0.156 bc	0.152 a
Mean of NAA	0.034 b	0.099 ab	0.152 a	0.076 ab	

*mean in each column followed by the same letter are not significantly different (P ≤ 5%)

2. Effect of Methyl jasmonate and Mannitol on callus induction: 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 8000 $mg.L^{-1}$ Mannitol treatment was significantly superior on control treatment in fresh and dry weigh of callus, which reached 332.42 and 53.81 mg, respectively. Also, the 75 $mg.L^{-1}$ concentration of Methyl jasmonate was significantly superior on control treatment in the same of two characteristics (347.19 and 55.67 mg fresh and dry weight). The treatment of interaction between Mannitol and Methyl jasmonate (8000 $mg.L^{-1}$+ 75 $mg.L^{-1}$) has given the highest significant difference in fresh and dry weight of callus induction (Plate 1, D), reached 451.25 and 69.17 mg, respectively. While less fresh and dry weight when treatment 0 mannitol + 25 $mg.L^{-1}$ concentrations, which reached 211.99 and 25.38 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.

Table (3)Effect of Methyl jasmonate and Mannitol on fresh weight of callus (mg) induced from cotyledonary leaf of the periwinkle plant by *In vitro*

Mannitol concentration ($mg.l^{-1}$)	Methyl jasmonate concentration ($mg.l^{-1}$)				Mean of Methyl jasmonate
	25	50	75	100	
0.0	211.99 d	214.87 d	243.13 dc	239.61 dc	227.40 b
8000	254.93 dc	344.33 b	451.25 a	279.17 c	332.42 a
Mean of Methyl jasmonate	0.050 b	279.60 b	347.19 a	259.39 b	

*mean in each column followed by the same letter are not significantly different (P ≤ 5%)

Table (4)Effect of Methyl jasmonate and Mannitol on dry weight of callus (mg) induced from cotyledonary leaf of the periwinkle plant by *In vitro*

Mannitol concentration ($mg.l^{-1}$)	*Methyl jasmonate concentration ($mg.l^{-1}$)*				*Mean of Methyl jasmonate*
	25	50	75	100	
0.0	25.38 c	26.09 c	42.17 bc	38.50 bc	33.03 b
8000	44.91 bc	51.88 ab	69.17 a	49.26 ab	53.81 a
Mean of Methyl jasmonate	35.44 b	38.99 b	55.67 a	43.88 ab	

*mean in each column followed by the same letter are not significantly different (P ≤ 5%)

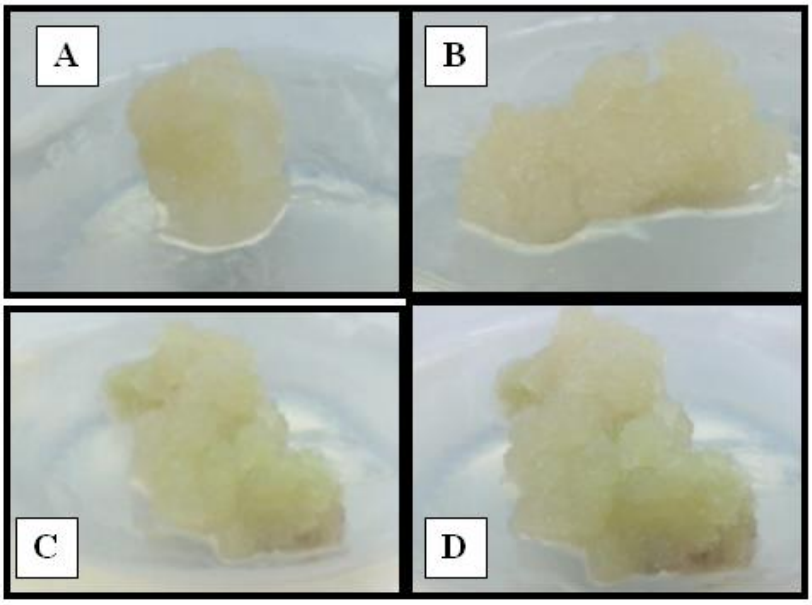

Plate (1): Effect of BA and Naphthalene acetic acid (A, B), and Mannitol and Methyl jasmonate (C, D) on callus multiplication of plant.

A: MS medium + 0.25 mg.L^{-1} BA + 0.5 mg.L^{-1} NAA.
B: MS medium + 0.25 mg.L^{-1} BA + 1.0 mg.L^{-1} NAA.
C: MS medium + 8000 mg.L^{-1} Mannitol + 50 mg.L^{-1} Methyl jasmonate.
C: MS medium + 8000 mg.L^{-1} Mannitol + 75 mg.L^{-1} Methyl jasmonate.

Conclusions: We are concluded from the present study 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 BA, NAA, Methyl jasmonate and Mannitol, according to the nature of growth**.**

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