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ORIGINAL ARTICLE

Evaluation the effect of different growth hormones on callus and regeneration of *Matthiola incana L* through *in vitro* culture

Vahid Abdoosi, Elham Sadat Mousavi *, Aida Abdali,
Department of Horticulture, Faculty of Agriculture and Natural Resource, Science and Research Branch, Islamic Azad University, Tehran, Iran
Corresponding Author*: elisadatmoosavi@yahoo.com

ABSTRACT
Matthiola incana is an annual plant of Brassicaceae species. The flower of this plant has two types of double flower and low flower, double flower varieties which are more concerned for economical situations and markets; they are sterile and no seed generated. Moreover the Matthiola incana L is concerned less for tissue culture techniques. In this study it is tried to use the thin layer cultivation system in order to obtain different aims such as the definition of the optimum condition for callus making, regeneration and also gene transfer and making difference and changing in the plant. In this study the effect of different growth hormones on callus and regeneration of Matthiola incana L. in different MS mediums were investigated. In order to determine the best callus situation various levels of NAA ,BAP, and ABA hormones were separately evaluated based on completely randomized design with three replications. Regeneration of obtained callus of mentioned MS mediums were carried out in the MS medium including NAA ,BAP, and ABA. Results indicated that different explants and different hormones combines had significant difference in callus. The callus of cotyledons explants (in hormonal treatment of 2mg/L BAP+ 0.5 mg/L NAA) with 98.66% had the biggest amount of regeneration and t he minimum was obtained in media containing 0.5 mg/L NAA + 0.5 mg/L ABA in abaxial explants.
***Key words**: Matthiola incana L., explants, hormone, callus, regeneration*

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INTRODUCTION

Tissue culture technology, a branch of plant science in recent decades has been developing an important tool for basic and applied research and commercial applications. Conventional breeding methods are extremely time consuming because they depend on cross pollination, seed germination and selection as well as vegetative regeneration. Modern plant biotechnology and genetic engineering has the potential to reduce the time needed for traditional breeding (1). Brassicaceae family is a big family with great economical importance which has been less studied from the point of view of tissue culture. The present study was designed to identify the ideal conditions for micro propagation of this plant.

Matthiola incana (Brassicaceae) is an important ornamental plant. (2). Most important of these parameters are the plant growth regulators content in the culture media (3). Plant growth regulators act like signals to stimulate, inhibit or regulate growth in the developmental programs of plants (4). Cytokinins are usually used on the micropropagation media to stimulate axillaries shoot proliferation (5, 6). However, the ideal concentrations differ from species to species and need to be established accurately to obtain the effective rates of multiplication. Rooting is a crucial step to the success of micropropagation. Auxins enhance the germination, root induction and seedling growth of many species (7, 8, and 9). The number of papers dealing with the *in vitro* cloning of *Matthiola incana* is scarce. Multiple shoot buds were differentiated from cotyledon explants of *Matthiola incana*, cultured on medium containing BAP and NAA (10, 11). Plantlets were regenerated from protoplast culture of *Matthiola incana* in medium supplemented with BAP, 2,4-D and NAA (12). Different organs of *Matthiola incana* exhibit differential morphogenic potential. Probably, the change in response depends on the exogenous and endogenous plant growth regulators (10). Nowadays, studies generally analyze the effect that a plant growth regulator exercises on the explants after a short period of time, and not its influence on later development (13).

This study was conducted to identify the best type of explants from *in vitro* grown seedling and growth regulator concentration and combination for callus induction by using *in vitro* techniques.

METHODS AND MATERIALES

Plant material:

Explants of (*Matthiola incana*) were collected from 2 months old seedling in the green house at Isfahan University of Iran. These explants were surface sterilized with 70% (v/v) ethanol for 1 min, followed by 10% (v/v) solution of commercial bleach (Clorox, 5.25% (w/v) of sodium hypochlorite) added with one drop of poly ethylene sorbitan monoleate for 15 min, then rinsed 3 times with sterile distilled water. The explants were aseptically cut into small pieces. Young leaves with the size 0.5× 0.5 cm, stem segments with the size 1 cm, cotyledon, hypocotyls, abaxial, peduncle, Calyx and petal were placed on different mediums supplemented with different combinations of hormones.

Medium:

Medias consisting MS (Murashig – Skoog, 1962) and 2% sucrose were provided (14). Different hormones added to media. A volume of 20 ml of nutrient medium was dispensed into 3 glass jar that was capped with a polystyrene screw cap. The pH of media was adjusted to 5.7 with 0.1N NaoH or 0.1N Hcl prior to autoclaving at 1.05 kg/cm^2, 121°c for
20 min.

Callus induction:

For callus induction, sterile explants were aseptically placed on B5 medium supplemented with different combination hormons containing 25 g/L (w/v) of sucrose and 7 g/L (w/v) agar. The explants were incubated at 25±1 °c under dark and light (1200 lux) condition. After 8 weeks of culture, the percentage of explants producing callus was recorded. The observation was made on the morphology of callus formed in different phytohormones tested.

Experimental design and statistical analysis

The experiments were done in factorial based on completely randomized design (CRD). Data were analyzed by using MSTATC. In order to determine significances, Duncan Multiple Test will be done (5%, 1% level).

RESULTS AND DISCUTION

Experiment 1: Effect of different concentrations of BAP and NAA on the size of callus, callus induction and regeneration

- Cotyledon explants

The results of the analysis of variance show that different hormonal treatments have significant effect at the 1% level (Table 1), which means the hormonal treatments were significantly different. Well as various hormone treatments had no significant effect on callus size (Table 1). Table 4.2 shows size the callus in various hormonal treatments. According to this table, we can see that the 2 mg/L BAP+ 0.5 mg /L NAA had greatest amount of callus size (based on code 5). The lowest callus size was observed in control (based on code 1). Considering mean comparison hormonal treatments are not significantly different. Just 1 mg/L NAA+ 1 mg/L BAP is significantly different to others and the lowest has observed (67%), (Table 2). It should be noted that callus production were unable to regenerated of cotyledon explants. Different combinations of hormones BAP, NAA and IAA were studied for callus production of cotyledon and hypocotyls explants of pepper plants. The results showed the highest rate of callus was relieved on MS medium containing 5 mg/L BAP and 1/0 mg /L NAA, respectively. In this study found that 3 mg /L AgNO3 + 5 mg /L BAP produced a larger and clearer callus and callus production time is reduced (15). Barsby (1986) reported leaf explants regenerated of three species of the family Wallflower (*B.oleracea, B.naus, B.campestris*) in a medium containing NAA and BAP hormones (16). The results in this study showed that regeneration was not done in any of the hormonal treatments which was not agree with of other researchers on other plant species (16,17).

Hypocotyls explants

The results of the analysis variance indicated that different hormonal treatments had a significant effect on callus size and percentage at the 1%. Which means that treatment were significantly different (Table 3). Maximum of the callus size was found at 2 mg/L BAP +0.5 mg/L NAA and hormonal treatments 1.5mg/L BAP+ 0.5mg/L NAA. Minimum callus size (based on Code 1) observed in control, 1mg /L BAP+0.5 mg/L NAA and 1 mg/L BAP+1.5 mg/L NAA (Table 4-4). Comparison of the mean percentage considered that the various treatments had not differences significant. There are significantly different in medium containing 1 mg/L NAA + 1 mg /L BAP. The minimum was observed at (69/66%), (Table 4). The callus did not produce from hypocotyls explants. Zhang et al (2011) studied different concentrations of BAP and NAA to direct regeneration in cotyledon and hypocotyls explants of pepper. They observed the

highest percentage of plant regeneration on MS medium containing 6 mg /L BAP and 1 mg /L NAA (18). But in this study, similar results were not obtained. It may be due to different tissues and organs of the plant, growth regulators and species which affected on callus induction and plant regeneration from calluses (19). In other studies on the Brassicaceae family were observed that hormonal combinations of NAA and BA did not affect the regeneration of cotyledon and hypocotyls explants. A low percentage of regeneration was obtained by Zee and Hui, 1977 (17).

- Stem explants

The results of the analysis of variance show that different hormonal treatments had significant effect on callus size and percentage at 1% level. This means that there is meaningful difference in hormonal treatment (Table 5). Comparison of size of callus in different hormonal treatments hormonal treatments showed that maximum callus was formed and grew well in 1.5 mg/L BAP + 1.5 mg/L NAA (based on code 4). The minimum were observed in medium supplemented with 0.5 mg/L NAA + 0.5 mg/L BAP. According to the comparison of mean there was no significant effect of hormonal treatment on callus induction . Among the different hormones, callus was minimally derived from explants in treatments containing 1mg/L NAA+ 0.25 mg/L BAP (67/66%) (Table 6). It should be mentioned that the callus from stem explants unable to regenerate. The positive effect of BAP with NAA on callus induction is reported by Sarabadani and *et al* (2008) and Odutayo(2005) in *Vigna unguiculata* (20,21).

Experiment 2: Effect of different concentrations of hormones ABA and NAA on callus size, callus induction and regeneration

Abaxial explants

The results of the analysis of variance show significant effects on callus size and percentage at the 1% level (Table 7). There was significant effect of various hormonal treatments. Comparison of size of callus in different hormonal treatments showed that maximum callus was induced at 0.5 mg/L ABA + 2 mg /L (based on code 5). The minimum callus induction were observed in medium supplemented with 1mg/l ABA + 2 mg/L NAA and 0.5 mg/l ABA++0.5 mg/L NAA(Table 4-8). According to the comparison of the mean maximum callus induction percentage observed in treatments with 1.5 mg /L ABA + 1.5 mg/L NAA (98.33%) and hormonal treatments 0.5 mg /L ABA + 0.5 mg /L NAA showed the lowest level (22.33%) (Table 8). It should be noted that callus derived from Abaxial have no ability to regeneration. According to the results can be seen NAA as an auxin had an effective role in increasing the volume of callus. Best result was observed at intermediate concentrations ABA and high concentrations of NAA. Reports show that auxin and cytokinin induced callus in ornamental plants (22, 23).

- Calyx explants

The results of the analysis of variance showed that significant effects are observed at different hormonal treatments at 5% level. The results also indicated that the different hormonal treatments have no significant effect on callus size (Table 9). According to comparison of various maximum callus size observed in treatments with 2 mg /L ABA + 2 mg/L (based on code 5). Minimum callus size was observed in medium containing2 mg/L ABA + 0.5 mg/L NAA (based on Code 1) (Table 10). According to the mean comparison of different hormonal treatments the highest rate can be observed in 2 mg /L ABA +0.5 mg/L NAA (67.97%) and the lowest level (66.69%) treated with 1 mg /L ABA+ 2 mg /LNAA (Table 10). It should be noted the callus produced from calyx explants were not able to regenerate. In this study we have found that optimal callus induced in low concentrations of ABA along with high concentrations of NAA in the calyx.

- Peduncle explants

The results of the analysis of variance showed that different hormonal treatments have significant effects on callus size and percentage at the 1% level (Table 11). Comparison of size of callus in different treatments revealed that greatest amount of callus size belonged to the 1.5 mg/L ABA +2mg/L NAA (based on code 4). Minimum callus size was observed at treatment with 1 mg /L ABA +0.5 mg /L NAA (based on code 1) (Table 12). According to the mean comparison highest rate of callus induction was shown in hormone-treated 0.5 mg /L ABA +1.5 mg/L NAA (99.29%). And the lowest rates callus induction (31.67) treated with 1 mg /L ABA + 2 mg /L NAA (Table 12). It should be mentioned that the callus produced from peduncle explants were not able to regenerate. Callus induction has been reported by application of auxin and cytokine (24, 25).

- Petal explants

The results of the analysis of variance showed that different hormonal treatments have significant effects on callus size and percentage at the 1% level (Table 13). Comparison of size of callus in different treatments revealed that greatest amount of callus size belonged to the 2 mg/L ABA +2mg/L NAA (based on code 3). Minimum callus size was observed at treatment with 1 mg /L ABA +1 mg /L NAA , 1.5 mg /L ABA +1 mg /L NAA and 2 mg /L ABA +1 mg /L NAA. According to the mean comparison highest rate of callus induction was shown in hormone-treated 2 mg /L ABA +2 mg/L NAA (98%). And the lowest rates

callus induction (26%) treated with 1 mg /L ABA + 1 mg /L NAA (Table 12). It should be mentioned that the callus produced from petal explants were not able to regenerate. Optimal callus induced in high concentrations of NAA and ABA.

Table1. Analysis of variance the effect of different concentrations of BAP and NAA on some traits cotyledon in *Matthiola incana*

callus induction percentage	callus size	degree of freedom	source of variatoin
(MS)mean of square		df	SOV
265.45**	2.14	12	(Treat)
56.23	1.15	26	(Error)
7.85	38.08		(C.V)

Table2. Effect of different concentrations of BAP +NAA on some traits of cotyledon in *Matthiola incana*.

	treatment	explants	callus size	callus induction percentage
1	0 BAP + 0 NAA	cotyledon	1	98.66[a]
2	0.5 BAP + 0.5 NAA	cotyledon	4	100[a]
3	0.5 BAP + 1 NAA	cotyledon	4	100[a]
4	0.5 BAP + 2 NAA	cotyledon	3	98.66[a]
5	1 BAP + 0.5 NAA	cotyledon	2	100[a]
6	1 BAP + 1 NAA	cotyledon	2	67[b]
7	1 BAP + 1.5 NAA	cotyledon	3	99.33[a]
8	1 BAP + 2 NAA	cotyledon	3	100[a]
9	1.5 BAP + 0.5 NAA	cotyledon	3	99.33[a]
10	1.5 BAP + 1 NAA	cotyledon	3	99.66[a]
11	1.5 BAP + 1.5 NAA	cotyledon	3	89[a]
12	2 BAP + 0.5 NAA	cotyledon	5	99.66[a]
13	2 BAP + 1 NAA	cotyledon	3	89[a]

Means with the similar letters are not significantly different at 5% level of probability using Duncan test

Table3. Analysis of variance for the effect of different concentrations of BAP and NAA on some traits hypocotyls in *Matthiola incana*

callus induction percentage	callus size	degree of freedom	source of variation
(MS)		df	SOV
227.8**	2.93**	10	(Treat)
3.36	0.45	22	(Error)
1.91	28.89		(C.V)

Table4. Effect of different concentrations of BAP +NAA on some traits of hypocotyles in *Matthiola incana*.

	treatment	explants	callus size	callus induction percentage
1	0 BAP + 0 NAA	hypocotyls	1	98.33[a]
2	0.5 BAP + 0.5 NAA	hypocotyls	2	98.66[a]
3	0.5 BAP + 1 NAA	hypocotyls	4	98.66[a]
4	1 BAP + 0.5 NAA	hypocotyls	1	98.33[a]
5	1 BAP + 1 NAA	hypocotyls	2	69.66[b]
6	1 BAP + 1.5 NAA	hypocotyls	1	99 [a]
7	1.5 BAP + 0.5 NAA	hypocotyls	5	98[a]
8	1.5 BAP + 1 NAA	hypocotyls	3	98.33[a]
9	1.5 BAP + 2 NAA	hypocotyls	2	98.66[a]
10	2 BAP + 1 NAA	hypocotyls	5	98.66[a]
11	2 BAP + 2 NAA	hypocotyls	3	97.66[a]

Means with the similar letters are not significantly different at 5% level of probability using Duncan test

Table5. Analysis of variance for the effect of different concentrations of BAP and NAA on some traits stem in *Matthiola incana*

callus induction percentage	callus size		source of variation
(MS) mean of squar		df	SOV
390.53**	2.98**	6	(Treat)
5.71	0.33	14	(Error)
2.55	26.94		(C.V)

Table6. Analysis of variance for the effect of different concentrations of BAP and NAA on some traits stem in *Matthiola incana*

	treatment	explants	callus size	callus induction percentage
1	0 BAP + 0 NAA	stem	1	98 [a]
2	0.5 BAP + 0.5 NAA	stem	1	98.33[a]
3	1 BAP + 0.5 NAA	stem	2	98.33[a]
4	1 BAP + 1 NAA	stem	2	67.66[b]
5	1 BAP + 1.5 NAA	stem	2	97.33 [a]
6	1.5 BAP + 1.5 NAA	stem	4	97.66[a]
7	2 BAP + 0.5 NAA	stem	3	97.33[a]

Means with the similar letters are not significantly different at 5% level of probability using Duncan test

Table7. Analysis of variance for the effect of different concentrations of ABA and NAA on some traits abaxial in *Matthiola incana*

callus induction percentage	callus size	degree of freedom	source of variation
(MS)mean square		df	SOV
3649.63**	5.77**	6	(Treat)
66.23	0.42	14	(Error)
12.80	28.05		(C.V)

Table8. Analysis of variance for the effect of different concentrations of ABA and NAA on some traits abaxial in *Matthiola incana*

	treatment	explants	callus size	callus induction percentage
1	0.5 ABA + 0.5 NAA	Abaxial	1	22.33[d]
2	0.5 ABA + 1 NAA	Abaxial	4	96.67[a]
3	0.5 ABA + 2 NAA	Abaxial	5	97.33[a]
4	1 ABA + 2 NAA	Abaxial	1	24.33[d]
5	1.5 ABA + 1.5 NAA	Abaxial	4	98.33[a]
6	1.5 ABA + 2 NAA	Abaxial	2	39.00[c]
7	2 ABA + 1.5 NAA	Abaxial	2	67.00[b]

Means with the similar letters are not significantly different at 5% level of probability using Duncan test

Table9.. Analysis of variance for the effect of different concentrations of ABA and NAA on some traits calyx in *Matthiola incana*

callus induction percentage	callus size	degree of freedom	source of variation
(MS)mean square		df	SOV
11846.5*	2.48[n.s]	5	(Treat)
573.16	1.11	12	(Error)
24.37	39.93		(C.V)

Table10.. Analysis of variance for the effect of different concentrations of ABA and NAA on some traits calyx in *Matthiola incana*

	treatment	explants	callus size	callus induction percentage
1	0.5 ABA + 1.5 NAA	calyx	2	96.33[a]
2	1 ABA + 0.5 NAA	calyx	2	60.33[ab]
3	1 ABA + 2 NAA	calyx	3	40.67[b]
4	2 ABA + 0.5 NAA	calyx	1	97.67[a]
5	2 ABA + 1 NAA	calyx	3	97.33[a]
6	2 ABA + 2 NAA	calyx	5	96.67[a]

Means with the similar letters are not significantly different at 5% level of probability using Duncan test

Table11. Analysis of variance for the effect of different concentrations of ABA and NAA on some traits peduncle in *Matthiola incana*

callus induction percentage	callus size	degree of freedom	source of variation
(MS)mean square		df	SOV
2638.04**	4.06**	4	(Treat)
535.6	0.93	10	(Error)
24	25.25		(C.V)

Table12. Analysis of variance for the effect of different concentrations of ABA and NAA on some traits peduncle in *Matthiola incana*

	treatment	explants	callus size	callus induction percentage
1	0.5 ABA + 1.5 NAA	peduncle	3	99.29[a]
2	1 ABA + 0.5 NAA	peduncle	1	53[b]
3	1 ABA + 2 NAA	peduncle	2	31.67[b]
4	1.5 ABA + 2 NAA	peduncle	4	58.33[ab]
5	2 ABA + 2 NAA	peduncle	3	98[a]

Means with the similar letters are not significantly different at 5% level of probability using Duncan test

Table13. Analysis of variance for the effect of different concentrations of ABA and NAA on some traits petal in *Matthiola incan*

callus induction percentage	callus size	degree of freedom	source of variation
(MS)mean square		df	SOV
3682.9**	2.26**	4	(Treat)
196.6	0.33	10	(Error)
19.31	30.07		(C.V)

Table14. Analysis of variance for the effect of different concentrations of ABA and NAA on some traits petal in *Matthiola incana*

	treatment	explants	callus size	callus induction percentage
1	0.5 ABA + 1.5 NAA	petal	2	97.67[a]
2	1 ABA + 1 NAA	petal	1	26[b]
3	1.5 ABA + 1 NAA	petal	1	43.67[b]
4	2 ABA + 1 NAA	petal	1	97.67[a]
5	2 ABA + 2 NAA	petal	3	98[a]

Means with the similar letters are not significantly different at 5% level of probability using Duncan test

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