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Effects of plant hormones on callus induction of different explant types of Mountain celery (*Kelussia Odoratissima* Mozaff.)

E. Abdollahi Sahlabadi*, G.A. Ranjbar, N.A. Babaeyan Jelodar, N.A. Bagheri

Agricultural Sciences and Natural Resources University, Sari, Iran

Key words: Mountain Celery, *Kelussia Odoratissma* Mozaffarian, callus induction, NAA, BAP, explants.

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Abstract

Mountain Celery (*Kelussia Odoratissima* Mozaff.) from Umbelliferae family is native to central Zagros mountains of Iran. It is a valuable medicinal plant that popularly used in Iran. Because of severe exploitation it is recently experiencing the danger of extinction. In order to examine its micro propagation possibility, the effects of two types of hormones including Naphtalin Acetic Acid (NAA) and 6-Benzylamino Purine (BAP) on callus induction have been tested using MS culture medium. Explants of stem, leaf and root were used in four levels of hormones in a factorial experiment based on Completely Randomized Design (CRD) with three replications. Results indicated that application of hormone combination containing (5.0 and 10 $mg.l^{-1}$ NAA with 1.0, 2.0 with 3.0 $mg.l^{-1}$ BAP), hormonal treatments (10 and 15 $mg.l^{-1}$ NAA) and (15 $mg.l^{-1}$ NAA with 3.0 $mg.l^{-1}$ BAP) have determined as the best treatments for callus induction from root explant ($\overline{X}$ = 100 %) and ($\overline{X}$ = 95.55 %). Combinations containing (5.0, 10 and 15 $mg.l^{-1}$ NAA with 1.0, 2.0 and 3.0 $mg.l^{-1}$ BAP) have determined as the best treatments for callus induction from leaf explant ($\overline{X}$ = 100 %). Hormonal combination containing (5.0 $mg.l^{-1}$ NAA with 1.0, 2.0 and 3.0 $mg.l^{-1}$ BAP) and (10 $mg.l^{-1}$ NAA with 0.0, 1.0 and 3.0 $mg.l^{-1}$ BAP) have determined as the best treatments for callus induction from stem explant ($\overline{X}$ = 100 %).

* **Corresponding Author:** E. Abdollahi Sahlabadi ✉ enayatolla.abdollahi@gmail.com

Introduction

Mountain Celery (*Kelussia Odoratissima* Mozaff.) is a perennial plant with aromatic stems and leaves. It is from Apiaceae (Umbelliferae) family and growing in early spring in some areas of Esfahan and ChaharMahal and Bakhtiari (Gandomkar, 1999). The plant makes a very pleasant smell when collecting in beginning of the growing season, while its leaves are still yellow. The temperature in native dispersion of Mountain Celery is usually lower than 20˚C with 127 days of frosting duration, reaching to lower than zero in late autumn and winter. Mountain Celery is a sweet-smelling plant which has anti-inflammatory, sedative, and anti-tussive properties, suitable for using in garnish food (Omidbeigi *et al*, 2008).

Mountain Celery with medicinal and food usages is special for some rangelands in Iran that has not been reported from other parts of the world. Its stem height varies between 120 to 200 cm and it has right and fusiform root with a big lump full of nutrition in upper portion. It has leaves with claw-shaped notch and long petioles but without sheath at the bottom. The inflorescence of Mountainous Celery in terminal umbels include fertile yellow flowers but most flowers of the lateral umbels with radius of 2 to 8 cm are male and non-fertile. Seeds are Plate-shaped and large with yellowish-brown in color and have distinctly three vein in its surface (Mozaffarian, 2003).

Local people by many years consumtion of Mountain Celery in different ways believe that it has analgesic, anti-inflammatory, sedative and anti-cough effects. New scientific results also confirmed that flavonoids, as the important part of plant composition mainly commulated in its seeds, stem and inflorescence, have anti-inflammatory, anti-viral, anti-diabetic, anti-cancer and anti-toxins effects (Asghari, *et al*, 2004). Mountain Celery has two essential oil compounds and flavonoids. The main compounds of its essential oil is 2- Hydro butyldiene phthalide and butyldiene phthalide. This phthalide constructed about 70% of the essential oil plant. The phlavonid use as anti-inflammatory, anti-allergic, vascular protection, anti-thrombosis and gastrointestinal protective and has inhibitor properties of the arachidonic acid pathway and 5 – Lypvaksynazh anti-diabetic, anti-lipid peroxidation and anti-cancer (DadkhahTehrani, 1999 and Evans, 1989). Sarabadani Tafreshi *et al.* (2007) introduced 10 $mg.L^{-1}$NAA and 2 $mg.L^{-1}$ BAP hormonal treatment as the best combination for callus induction from root explant and cut embryos in Ferula Gummosa. Hadi *et al.* (2011) reported that petiole explants on medium culture containing BA and NAA demonstrated better response of quantity and quality in callus production in Ferula Gummosa. Also, they emphasized that callus produced with the help of 1 $mg.L^{-1}$ BAP2 and 1 $mg.L^{-1}$ NAA10 hormonal treatment were more compacted with good appearance. In discriminate and non-normative harvesting on many cases because of damaging roots or tuberson roots puts Mountain Celery on endangered exposal (Asghari *et al*, 2004).

Tissue culture, genetic engineering biotechnology are the most important tools for rescuing, propagating this valuable native plant to increase the production of secondary metabolites. In present work effects of different plant hormones concentrations (BAP and NAA) and various types of explants (roots, stems and leaves) have been studied on invitro callus induction of Mountain Celery.

Materials and methods

Study area

The study has undertaken in laboratory in Sari Agricultural Sciences and Natural Resources University in 2012 using a factorial experiment based on completely randomized design with three replications. Seeds were Prepared from Department of Natural Resources of Chaharmahal and Bakhtyari (seed region Sraqaseyd). For removing inhibitors seeds have firstly soaked in one liter distilled water for 48 hours and then the water were renewed every six hours until inhibitors completely exit from seeds then washed with water and liquid soap. Seeds then were surface sterilized using 70% (v/v) ethanol for 1 min followed by 50 % (v/v) sodium hypochlorite for 25 minutes all under the Laminair flow hood. After each step of sterilizing by ethanol and hypochlorite,

seeds were thoroughly washed three times with sterile distilled water (Sarabadani Tafreshi *et al.* 2007). The utensil and materials used in all stages of the study were autoclaved for 20 minutesat121 Celsius degrees.

Methods

The sterilized seeds were transferred onto petridishes containing MS culture media. The petridishes completely sealed with para film and transferred into a refrigerator with a temperature of4-6Celsius degrees. After obtaining seedlings with proper vigor root, leaf and stem explants were prepared for using in callus induction phase (Fig 4). MS culture medium containing different concentrations of hormones, including four levels of NAA (0, 5, 10 and 15 mgL^{-1}) and four levels of BAP (0.0, 1.0, 1.5 and 2 $mg.L^{-1}$. To facilitate callus induction, surface of leaves was scratched by scalpel before planting. Cultured explants for callus induction were maintained in incubator at 17 ± 2°C under a photoperiod of 16/8 h light/dark and 75% relative humidity. First signs of callus formation were observed after 10 day. Subcultural was performed with two weeks interval time and data were recorded after two weeks from starting the experiment. Experiment was performed with three replicates and 6 petri dishes in each treatment and 5 explants per petri dish were cultured.

In addition to hormones, all media were supplemented with $30gl^{-1}$ sucrose and $6gl^{-1}$ agar and pH was tuned in range of 5.8.

Normalize the data for callus induction percentage analysis was performed using Arcsin$\sqrt{X + 0.01}$ formula. Data analysis using statistical software SPSS18 and MSTSTC and comparison of means were performed using Duncan test.

Result

Root explant

Analysis of variance for callus induction (table 1) indicated that simple and interaction effects of all treatments were different in highly significant level ($p < 0.01$). Means comparisons tests showed that hormonal concentrations 10 $mg.l^{-1}$ NAA and 2.0 $mg.l^{-1}$ BAP was the best treatments for callus induction from root explant. Hormonal combinations containing (5.0 and 10 $mg.l^{-1}$ NAA with 1.0, 2.0 with 3.0 $mg.l^{-1}$ BAP), hormonal treatments (10 and 15 $mg.l^{-1}$ NAA) and (15 $mg.l^{-1}$ NAA with 3.0 $mg.l^{-1}$ BAP) have determined as the best treatments for callus induction ($\overline{X}$ = 100 %) and ($\overline{X}$ = 95.55 %). Also control treatment has the lowest callus induction ($\overline{X}$ = 11.11 %) using root explant (fig 1).

Table 1. Variance analysis of induction callus and fresh weight treats in different hormones concentration (BAP and NAA) of root, stem and leaf explants.

Sources of Variation	df	MS					
		Root		Leaf		stem	
		Callus induction	Callus fresh weight	Callus induction	Callus fresh weight	Callus induction	Callus fresh weight
NAA	3	0.288**	5.492**	0.601**	2.312**	1.348**	5.56**
BAP	3	0.321**	1/018**	1.148**	0.745**	0.195**	1.803**
NAA×BAP	9	0.299**	0.176**	0.101**	0.024**	0.081**	0.075**
Error	32	0.016	0.002	0.005	0.002	0.008	0.003
CV (%)	-	1.5	3.5	0.84	3.5	1.19	3.8

Callus fresh weight were measured and in hormonal combination containing (2.0 $mg.L^{-1}$ BAP with 10 $mg.L^{-1}$ NAA) were observed the most callus fresh weight ($\overline{X}$ = 2.4 gr) and control treatment has the lowest callus fresh weight ($\overline{X}$ = 0.120 gr).

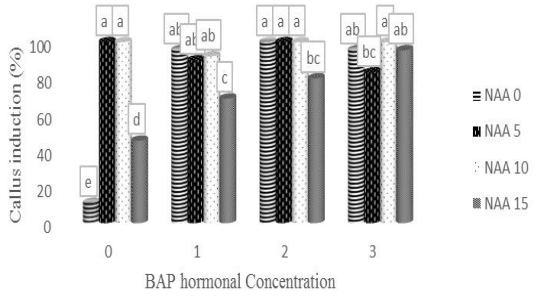

Fig. 1. Interaction of different concentrations of BAP and NAA on callus induction using root explants.

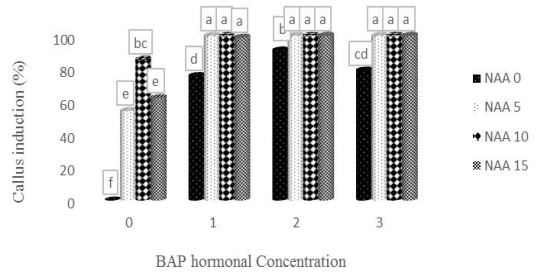

Fig. 2. Interaction of different concentrations of BAP and NAA on callus induction using leaf explants.

Leaf explant

Analysis of variance for leaf callus induction (table 1) indicated that simple and interaction effects of BAP and NAA hormones were different in highly significant level ($p < 0.01$). Means comparisons tests showed that hormonal concentrations 10 mg.l^{-1} NAA and 1.0 mg.l^{-1} BAP was the best treatments for callus induction from leaf explant. Hormonal combinations containing (5.0, 10 and 15 mg.l^{-1} NAA with 1.0, 2.0 and 3.0 mg.l^{-1} BAP) have determined as the best treatments for callus induction ($\overline{X}$ = 100 %). Also control treatment has not observed the callus induction using leaf explant (fig 2).

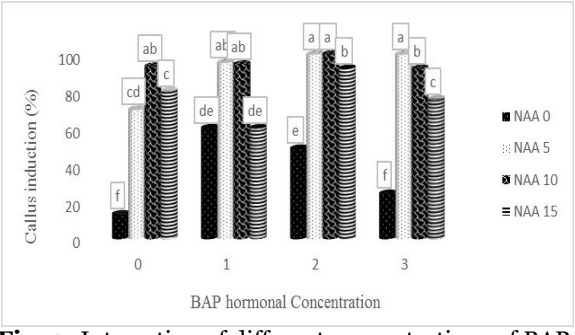

Fig. 3. Interaction of different concentrations of BAP and NAA on callus induction using stem explants.

Callus fresh weight were measured and in hormonal combination containing (2.0 mg.L^{-1} BAP with 5.0 mg.L^{-1} NAA) were observed the most callus fresh weight ($\overline{X}$ = 1.73 gr).

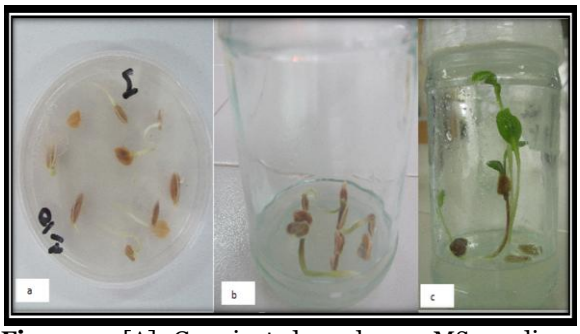

Fig. 4. [A] Germinated seeds on MS medium without hormones and [B and c] seedling from seeds planted on MS medium without hormones.

Stem explant

Analysis of variance for stem callus induction (table 1) indicated that simple and interaction effects of BAP and NAA hormones were different in highly significant level ($p < 0.01$). Means comparisons tests showed that hormonal concentrations 10 mg.l^{-1} NAA and 3.0 mg.l^{-1} BAP was the best treatments for callus induction from stem explant. Hormonal combination containing (5.0 mg.l^{-1} NAA with 1.0, 2.0 and 3.0 mg.l^{-1} BAP) and (10 mg.l^{-1} NAA with 0.0, 1.0 and 3.0 mg.l^{-1} BAP) have determined as the best treatments for callus induction ($\overline{X}$ = 100 %). Also control treatment has the lowest callus induction ($\overline{X}$ = 13 %) using stem explant (fig 3).

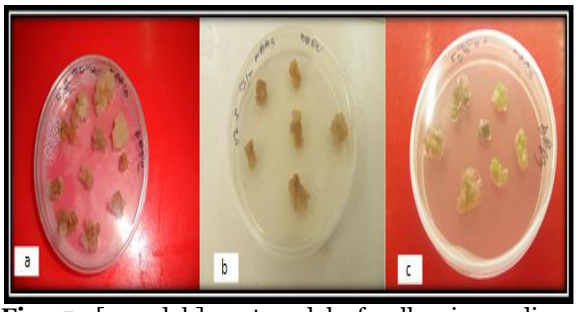

Fig. 5. [a and b] root and leaf callus in medium containing 2.0 mg.L^{-1} BAP and 5.0 mg.L^{-1} NAA, [c] leaf callus in medium containing 3.0 mg.L^{-1} BAP and 5.0 mg.L^{-1} NAA.

Callus fresh weight were measured and in hormonal combination containing (3.0 mg.L^{-1} BAP with 15

mg.L^{-1} NAA) were observed the most callus fresh weight ($\overline{X}$ = 2.12 gr) and control treatment has the lowest callus fresh weight ($\overline{X}$ = 0.16 gr).

Discussion

Result showed that hormonal treatments (5 and 10 mg.L^{-1} NAA) and (2 mg.L^{-1} BAP) determined as the best treatments for callus induction in root explant, also these combinations had callus induction in all root explants, which consistent with results obtained by Hadi *et al.* (2011). Those reported that hormonal treatments (10 mg.L^{-1} NAA) and (2 mg.L^{-1} BAP) as the best combination for callus induction in root explant in Ferula Gummosa. The hormonal combinations containing NAA (5.0, 10 and 15 mg.L^{-1}) with BAP (1.0, 2.0 and 3.0 mg.L^{-1}) determined as the best treatments for callus induction in leaf explant, which had been inconsistent with the results obtained by Monokesh *et al.* (2014). They stated that maximum induction of callus obtained from combination of 2 mg.L-1 2, 4-D and 0.5 mg.L^{-1} NAA from leaf explant in Achyrathes Aspera L.

In leaf explant, all hormonal combination had high callus induction percent that showed the better respond in callus induction than using of only hormonal NAA and BAP.

Sarabadani Tafreshi *et al.* (2007) and Sharafi *et al.* (2007) reported that different levels of the NAA and BAP hormonal combinations had high callus induction percent in Ferula Gummosa, which had been consistent with the results of this experiment The hormonal combinations containing NAA (5.0 mg.L^{-1}) with BAP (1.0, 2.0 and 3.0 mg.L^{-1}) and NAA (10 mg.L^{-1}) with BAP (0.0, 1.0 and 2.0 mg.L^{-1}) determined as the best treatments for callus induction in stem explant. However application of higher than NAA 10 mg.L^{-1} and BAP 2.0 mg.L^{-1} of hormonal concentrations caused reduction in callus induction rate in stem explant, which strongly confirmed the results by Monokesh *et al.* (2014). The initial callus induction was constituted at the cutting edges that have more direct contact with culture media which had similarity been stayed in results obtained by Aza and Noga (2002). Results of fresh weight showed that inducted callus with root explant has higher weight than leaf and stem explant.

Generally, in medicinal plant, one way of secondary metabolite production (that has many usage in medical industrial) is from callus samples so in this study appropriate hormonal treatments and explant (root explant) that show high percent in induction and callus formation was introduced.

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