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In vitro culture and callus induction of chamomile (*Matricaria chamomilla* L.) explants under different concentrations of plant growth regulators

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Abstract

Chamomile (*Matricaria chamomilla* L.) is one of the most important herbal medicine plants. The aim of this research was to study effect of different concentrations of growth regulators of Auxin on callus induction and growth in Chamomile's various explants; factors were: NAA at four levels (0, 1, 2, 4 mgl^{-1}) Kinetin at four levels (0, 0.5, 1, 2 mgl^{-1}), explants cultured on basal MS medium containing growth regulators. We found that callus initiation in explants was faster at 1 mgl^{-1} NAA and 1 mgl^{-1} Kinetin. Leaf explants on a medium containing 1 mgl^{-1} NAA and 1 mgl^{-1} Kinetin has been highest percentage of callus induction with an average of 93.26 percent. Axillary bud and stem discs explants have been the highest percentage of callus induction with an average of 89.68 and 80.75 %, respectively, is obtained in same treatment combination. The effect of different concentrations of NAA and Kinetin on callus volume that derived from different explants is significant and stem explants in 1 mgl^{-1} NAA and 1 mgl^{-1} Kinetin have been produced maximum callus volume with an average 17.49.

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Introduction

Chamomile (*Matricaria chamomilla* L.) is one of the most important herbal medicine plants (Salamon, 1992). This plants have pharmaceutical properties and used for the treatment of many diseases, Chamomile plant has been used for anti-inflammatory (Haghi *et al.* 2004), Anti-allergy, Relaxing, And to treatmeant of skin rashes or acne and skin infections, German chamomile (*Matricaria chamomilla* L.) and Roman chamomile (*Chamaemelum nobile*) are the two major types of chamomile used for health conditions (Pourohit *et al.,* 2004; Nirr, 2002; McKay & Blumberg, 2006). A number of researchers believe that MC is able to do everything (Salamon 1990). Cultured plant cells and tissues are widely recognized as promising alternatives for the production of valuable secondary metabolites (Lipsky, 1992). Currently, MC propagated by seed, But due to the low content of secondary metabolites; its production is inefficient and costly (Azizi, 2006) and needs a lot of manpower and time. Therefore, the study of tissue culture for micro propagation systems, breeding and also Production and extraction of secondary metabolites from the culture suspension is necessary. There are several reports of MC's tissue culture and micro propagation, which different parts of the plant have been used as explants. In most of these reports explants from leaves, stems and flowers have been used for callus culture. Reichling *et al* (1984) optimized callus cultures from sterilized stem segments of BK2 variety chamomile in MS medium containing 27.7 Mμ NAA and 11.9 Mμ Kinetin levels. Kintzios and Michaelakis (1999) Studied late flower's callus induction and embryogenesis In MS medium with 26.8 Mμ NAA and 11.5 Mμ Kinetin, also suggested that Desired results are obtained when All tissue culture process be performed in 16 hours dark and 8 hours light. The aim of this research was to study the effect of different concentrations of growth regulators of Auxin on callus induction and growth in MC's various explants.

Materials and methods

Plant material and disinfection

This research project was carried out in the faculty of agriculture, Ilam University, Iran. In this study, we used German chamomile (*Matricaria Chamomilla* L.) to get Axillary bud and leaf and stem's explants. All explants were washed in current tape water for 30 min, then surface sterilized in a 70% ethanol for 60 seconds and followed by sodium hypochlorite solution (NaCl with 1% active chlorine) for 10 minutes.

Culture Condition and Traits evaluated

After thorough washing in sterile water, the sterile explants of Axillary buds, leaves and stems, were cut into 0.5 cm × 0.5 cm segments, and cultured on basal MS medium (Murashige & Skoog, 1962) supplemented with 0.7% (w/v) agar, 3% (w/v) sucrose. Sucrose was used as the carbon source and Agar as solidifying agent. The PH was adjusted to 5.8 before autoclaving at 121 °C for 15 min. The cultures were incubated under controlled light regime (16:8 h light/dark photoperiod) at 25.1 °C, with subsequent sub-culturing for 2 months, after which, aggregating callus morphology was obtained. In experiments evaluating the effect of NAA and Kinetin on callus induction, factors were: NAA at four levels (0, 1, 2 and 4 mgl^{-1}) Kinetin at four levels (0, 0.5, 1 and 2 mgl^{-1}), explants in three levels: Stem, Axillary buds, and leaves. Traits of the day of beginning callus induction, callus volume average, percentage of callus induction were analyzed. Callosum volume assessment was performed using Hookeroni ordinal scale. In Hooker's method, callus volume is determined based on the size of 23 different circlesin which the smallest circle with a diameter of 1 mm indicates the minimum volume and the largest circle with adiameter of 23 mm represents the maximum volume of callus (Bilia *et al.,* 2002).

Statistical methods

The experiment was arranged as factorial based on randomized complete block design in 3 replications; Data analysis was carried out using SAS, Minitab and Excel. Mean comparisons were conducted using Duncan multiple ranges test (α = 5 %).

Results and discussion

Response of cultures is dependent to the Composition of used PGR and the explants types. Explants gave callus when cultured on MS medium supplemented with different concentrations of Kinetin and NAA. The effect of different concentrations of Kinetin and NAA on callus induction of MC are presented in Table 1. Although the same in vitro conditions were provided to all explants, there was a marked difference between days to beginning callus induction, callus volume average, percentage of callus induction (Table 1). Experimental results show that callus was formed at different times among three kinds of explants (leaves, stem segment and Axillary buds). Growth regulator NAA and Kinetin were found to be equally good for callus induction and NAA, Kinetin were both determined to have a significant effect on callus production (Table 1). The main effects, NAA and Kinetin, as well as the interaction between them, NAA* Kinetin, were found to have a significant effect on callus production (day to beginning callus, volume and percentage of callus induction) (Table 1).

Table 1. Analysis of variance evaluated in effect of MS basal media supplemented with different levels of NAA and Kinetin on induction of callus.

Sorce of variation	Degree of freedom	Time to beginning callus initiation (day)	Callus volume average	Percentage of callus induction
Replication	2	17.93	212.3	14.8
NAA	3	166.34***	6345.91***	137.30***
Kinetin	3	151.14***	4557.73***	103.59***
Explant	2	133.53**	569.73	4.17
NAA × Kinetin	9	53.05**	1455.31***	14.51**
NAA × Explant	6	54. 5*	924.52**	19.93***
Kinetin × Explant	6	15.86	138.53	8. 5
NAA×Kinetin×Explant	18	21.85	449.14*	9.76**
Experimental Error	94	18.51	258.97	5. 8

*, **, *** shows significant difference at 5, 1 and 0.1 percent, respectively.

Time to callus initiation

Time to begin callus induction was found to be explants dependent and analysis of variance showed that the effect of NAA at different levels of Kinetin on callus initiation and explant types was significant (table 1). We found that callus formation in explants was faster at 1mgl^{-1} NAA and 1 mgl^{-1} Kinetin as shown in Figure 1. Callus was initiated in the 12 days from leaves explants, in the range of 12-15 days; in the 15 days from stem segment explants, the range was between 15-22 days; in the 11 days from buds explants, the range was between 11-22 days. We observed in control treatment (without growth regulators), Leaves explants Has been Lowest time for callus induction; This explant for callus induction requires less time to initiate at Growth regulators (NAA) levels compared to stem and Axillary bud explants. For Axillary bud and stem explants, we obtained lowest time to begin callus induction at 1 mgl^{-1} NAA as shown in Figure 2.

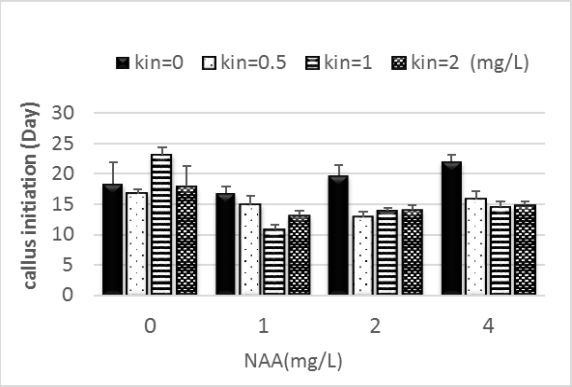

Fig. 1. The effect of different concentrations of growth regulators NAA and Kinetin on day to beginning callus.

Percentage of callus induction

The effect of different concentrations of NAA and Kinetin on callus induction of explants was significant (table 1). The study of factors evaluated (NAA, Kinetin and explant) also showed leaf explants on a medium containing 1 mgl^{-1} NAA and 1 mgl^{-1} Kinetin has been

the highest percentage of callus induction with an average 93.26 percent. Axillary bud and stem's explants has been the highest percentage of callus induction with an average of 89.68 and 80.75 %, respectively, Is obtained In same treatment combination as shown in Figure 3, the highest callus induction In all three explants can be obtained by the use of the same level of growth regulators.

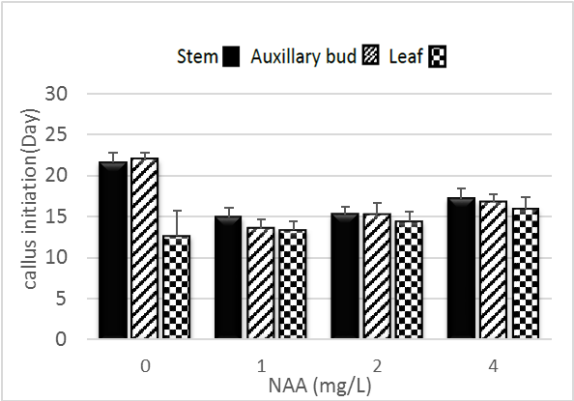

Fig. 2. Effect of different concentrations NAA on day to beginning callus induction in explants (stem, Axillary bud, leaf).

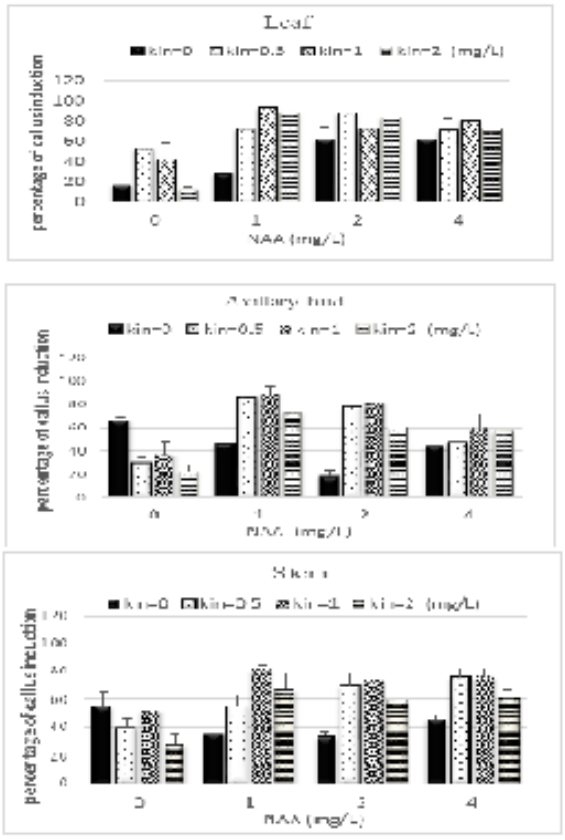

Fig. 3. Effect of different concentrations NAA and Kinetin on percentage of callus induction in explants (stem, Axillary bud, leaf).

Callus volume average

The results obtained show that the effect of different concentrations of NAA and Kinetin on callus volume derived from different explants is significant (table 1). Also we observed that stem explant in 1 mgl^{-1} NAA and 1 mgl^{-1} Kinetin levels have been produced maximum callus volume with an average 17.49. Also Axillary bud and leaf explants have maximum callus volume in same treatment combination with average 16.20 and 15.52, respectively. Maximum callus volume in all three explants can be obtained by the use of the same level of growth regulators as shown in Figure 4.

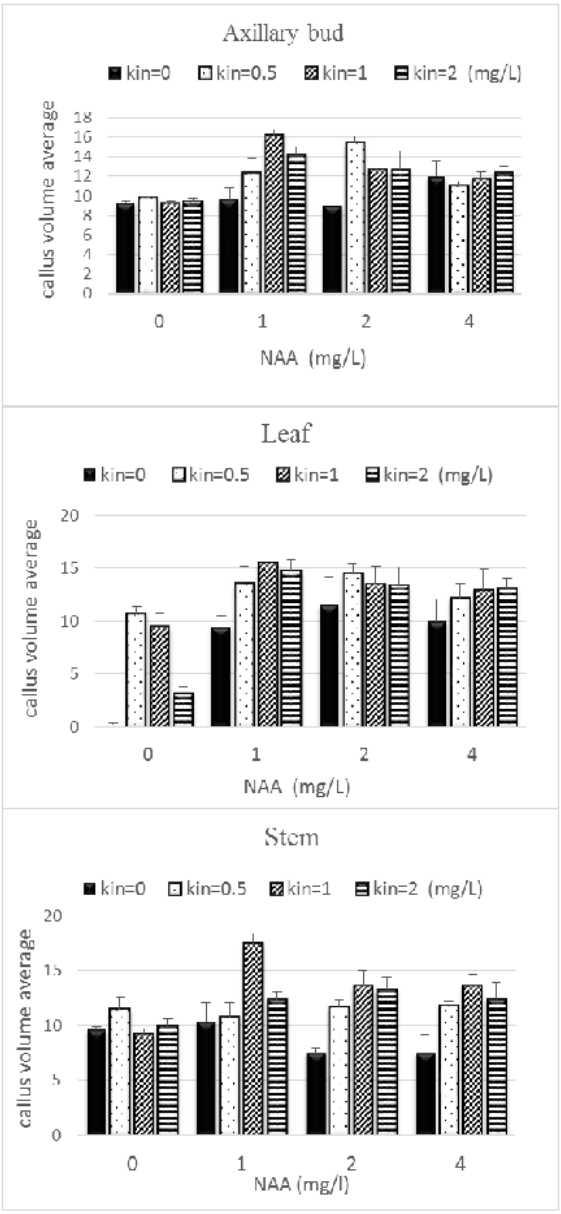

Fig. 4. Effect of different concentrations NAA and Kinetin on callus volume average in explants (stem, Axillary bud, leaf).

Discussion

In this experiment, the effect of explants and different concentrations of NAA and Kinetin on callus induction in German chamomile were studied. Optimization of callus induction and multiplication of cells in the cell suspension culture is the first step to produce of high amounts of active compounds. The first step in plant tissue culture is to develop a callus culture from the whole plant. Callus is a proliferated mass of cells without any significant differentiation. A callus can be obtained from any portion of the whole plant containing dividing cells (Chattopadhyay *et al.,* 2002). Phytohormones or growth regulators are required to induce callus tissues and to promote the growth of many cell lines. Since each plant species requires different kinds and levels of phytohormones for callus induction, its growth, and metabolites production, it is important to select the most appropriate growth regulators and to determine their optimal concentrations. One of the aims of establishing the mass of undifferentiated cells in vitro was as support for plant propagation and maintain it for a long time in vitro to establish suspension cultures. Callus which could have maintain the power of division in a long time Are suitable for gene transfer and genetic engineering techniques and also for germplasm conservation (Chan *et al.,* 2008). Successful results by other researchers was obtained from Explants culture (stem, leaf and Axillary bud) in medium containing NAA and Kinetin that The results of our experiments are correspond with the results of these reports (Reichling *et al.,* 1976; Reichling *et al.,* 1979; Reichling *et al.,* 1984.). The results showed that with increasing concentrations of NAA and Kinetin To 1 mgl^{-1}, Growth of explants and callus induction was increased and then was not performed positive changes in explants callus induction but also callus production decreased at high levels. Leaf explants have been higher percentage of callus induction In comparison with Axillary buds and stem explants. Effect of Auxin on callus induction in many plants such as several species of medicinal plants as *Holarrhena antidgsenterica wall* and *Allium Chinensis* and *Hemidesmus indicus* L. have been confirmed (Raha *et al.,* 2003; Yan *et al.,* 2009). In previous research on Chamomile has been observed when used of NAA or 2,4 –D at 1 or 0.5 mgl^{-1} concentration for callus induction adding Kinetin at less or equal concentrations is necessary for callus growth that have been correspond with results obtained in this study.

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