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Effects of Plant Growth Regulators and Explant on Callus Induction in *Cuminum cymium* L.

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Abstract
Cumin (*Cuminum cyminum* L.) as a member of the Apiaceae family is one of the most important medicinal plants in Iran. The purpose of this study is to evaluate the effect of plant growth regulators and explant type on callus induction in cumin. For this purpose the cumin seeds (Kuhbanan accession) were disinfected with sodium hypochlorite and alcohol and cultured on MS basal medium. Leaf and hypocotyl explants were prepared from sterile seedlings and used to produce callus on MS medium containing 0.0, 0.5, 1.0 and 2.0 mg/l NAA with 0.0 and 0.5 mg/l BAP. The experimental was as completely randomized factorial design with three replications. The results of callus induction showed that the explants type, hormone, and their interactions have non-significant effects on the callus induction percentage. Also, explants showed significant effect on callus growth rate (CGR). However hormones and hormone- explant interactions did not have a significant effect on CGR. The results showed that the medium containing 1 mg/l NAA and 0.5 mg/l BAP was known as the best callus growth rate medium for cumin (0.238 mm/d). Comparing the mean interactions of the explants in hormone on CGR showed that 0.5 mg/l of NAA + 0.5 mg/l of BA in leaf explant has the highest effect (0.248 mm/d).

Key words: *Cuminum cyminum;* callus Induction; medicinal plants; explants

Introduction

Cumin (*Cuminum cyminum* L.) belongs to *Apiaceae* family is one of the most important medicinal plants and one of the oldest and economically species in the world. This plant family is one of the well-known families among flowering plants because of its worth properties (Masoumi *et al.*, 2012). Recent studies have indicated pharmaceutical, medicinal importance and antimicrobial effect of its oil extract (Tawfik & Noga, 2002).

Callus production was significantly influenced by the concentration of 2, 4-D and photoperiodic status. Callus induction was not observed on MS medium without any Plant Growth Regulator (PGR) in this study. This shows that 2,4-D is critical for callus induction of cumin(Soorni *et al.*, 2012)

Soorni *et al.* (2012) investigated the callus induction in *Cuminum cyminum* L. They reported that 0.1 mg/l NAA+ 0.4 mg/l BAP and 0.1 mg/l NAA +1 mg/l BAP combinations determined as the highest level for above character (25.83 % and 25 % respectively) (Soorni *et al.*, 2012).

Tawfik & Noga (2002) used leaflet and hypocotyl explant of Cumin. Calli were obtained on MS medium containing 4 µM 2, 4-D only or together with 4 µM Kin. Embryonic calli were developed on basal medium without hormones and plumules were observed on the treatment with 1 µM after 4 weeks.

Ebrahimi *et al.*, (2003) used embryo explants for cumin tissue culture yielding a large number of shoots within a short period of time without any subculturing. In this report, the best treatments were B5 medium containing 0.2 mg L^{-1} IAA and 1 mg L^{-1} BAP or 0.2 mg L^{-1} NAA and 0.2 mg L^{-1} BAP.

Sharifi (1995) used hypocotyl and cotyledon explants in *Cuminum cyminum* tissue culture. The callus growth was faster on B5 medium containing 2 mg L^{-1} NAA and 2 mg L^{-1} Kin. Plumule and shoot formation from hypocotyls and somatic embryogenesis were higher on

medium supplemented with 0.1 mg L^{-1} NAA and 2 mg L^{-1} Kin and MS medium containing 0.5 mg L^{-1} 2,4-D, respectively.

Martin (2004) used internode and leaf explants for regeneration of plantlet from *Eryngium foetidum* L. (Apiaceae). The callus formation occurred on MS medium supplemented with 5.37-10.74 µM NAA and 2.32 or 4.65 µM Kin. Then, somatic embryogenesis occurred on calli after transfer to half- strength liquid MS with 2.69 µM NAA and 1.16 µM Kin.

Establishment of an efficient cell culture system is one of the essential prerequisite for genetic improvement studies (Kahrizi *et al.*, 2011). So far, several studies have been published about cumin callus induction (Tawfik and Noga, 2001; Tawfik and Noga, 2002; Ebrahimie *et al*., 2003; Ebrahimie *et al*., 2007; Valizadeh *et al*., 2007; Soorni *et al*., 2012) but there is no study or only limited studies have been performed on the effect of explant types and different concentrations of NAA and BA on cumin callus induction. For this reason, this experiment was conducted.

Materials and Methods

Plant Materials

Medicinal plant cumin (Kuhbanan accession) was used as plant material in current research. The seeds were prepared from Bisetoon Shafah Co., Kermanshah Science and Technology Park.

Seed sterilization and germination

Cumin seeds were surface disinfected with 70% ethanol for 1 min and surface sterilized with 1.5% sodium hypochlorite for 7 min. The seeds were then thoroughly washed with sterile distilled water four times and were blot-dried briefly in the laminar flow hood. The sterilized seeds were then placed on MS medium (Murashige & Skoog, 1962) containing 0.7% agar and 3% sucrose without any plant growth regulator (PGR) and incubated at 25°C for 16-h light/8-h dark photoperiod for germination and plant elongation.

Callus induction

The MS medium containing 0.7% agar and 3% sucrose was used as basal medium for callus induction experiments. To compare the callus production indices, three different concentrations of BAP (0.0, 0.5 and 1 mg/l), four different concentrations of NAA (0.0, 0.5, 1.0 and 2 mg/l) and two explant type (leaf and hypocotyl) were used based on completely randomized block design in three replications. The explants were cultured on medium with different hormone balances including twelve compositions [(0 mg/l BAP + 0 mg/l NAA), (0 mg/l BAP + 0.5 mg/l NAA), (0 mg/l BAP + 1mg/l NAA), (0 mg/l BAP + 2 mg/l NAA), (0.5 mg/LBAP +0 mg/l NAA), (0.5mg/L BAP +0.5 mg/l NAA),(0.5 mg/l BAP + 1 mg/l NAA) and (0.5 mg/l BAP + 2mg/l NAA),(1 mg/l BAP+0 mg/l NAA),(1 mg/l BAP+ 0.5 mg/l NAA),(1 mg/l BAP+1 mg/l NAA), (1 mg/l BAP+2mg/l NAA)] The cultures were incubated in dark at 25°C conditions. The callus diameter (in mm) was measured four times (20, 25, 30 and 35 days after explant culture) and growth rate (in mm/d) was recorded in 28 days after explant culture. Analysis of variance and mean comparison (Duncan's test) for above traits was performed by MSTATC software.

Results

After recording of data related to callus indices, the statistical analysis of data including analysis of variance and mean comparison were carried out. The callus induction was occurred in both leaf and hypocotyl explants (Fig. 1). The effects of explant type, hormone concentration and their interaction have been analyzed, which explained separately in the following section.

The characters for callus induction including the callus induction percentage (%) and growth rate (in mm/d) were analyzed.

Explant type

Analysis of variance results (Table 1) showed that there were no significant differences among explants types for callus induction percentage but there are significant differences ($p<0.01$) among explants types for callus growth rate. So that the leaf explant showed more CGR (0.172 mm/d) than hypocotyl explant (0.167 mm/d).

Hormone concentration

Analysis of variance results (Table 1) indicated that there were no significant differences among hormone levels for callus induction percentage and callus growth rate. The mean comparison (Table 2) showed that the concentration of 1 mg/l of NAA and 0.5 mg/l BA had the highest effects (0.238 mm/d) on callus growth rate.

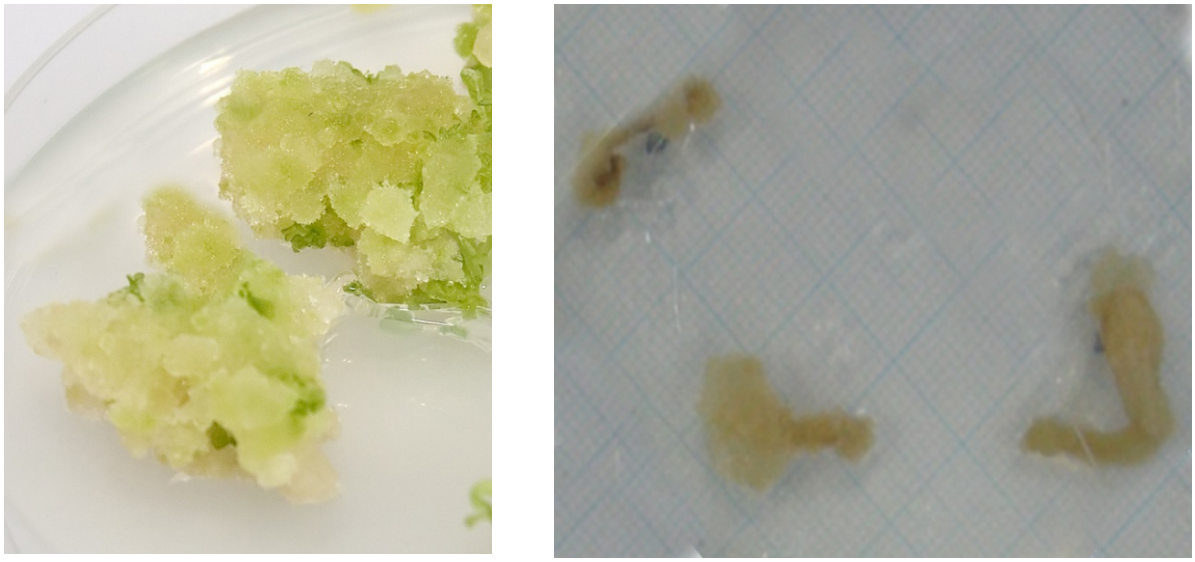

Fig. 1. The inducted callus in leaf (left) and hypocotyl (right) explants in *Cuminum cyminum* L.

Table 1. Mean squares for explant and hormone effects on callus induction percentage and callus growth rate in *Cuminum cyminum* L.

Source of variation	CIP	CGR
Explant (E)	750.000^{ns}	0.004**
Hormone (H)	560.990^{ns}	0.012^{ns}
E × H	524.545^{ns}	0.005^{ns}
Error	485.000	0.002
CV	23.81	28.38

** Significant differences in 0.01 level, ns: Non- significant, CGR: callus growth rate, CIP: callus induction percentage and CV: coefficient of variations.

Interaction effects of explant type × hormone concentration

Analysis of variance (Table 1) showed that there is a no significant difference between interaction effects of explant type × hormone concentration on callus induction percentage and callus growth rate.

Mean comparison for interaction effects of explant × hormone concentrations on callus growth rate in *C. cyminum* L. (Table 3) showed that NAA (0.5 mg/L) + BA (0.5 mg/L) in leaf explant had the highest effect (0.248 mm/d).

Table 2. Mean comparison for effect of hormonal level on callus growth rate in *Cuminum cyminum* L. GR: growth rate. Similar letters in each column didn't have any significant statistical difference in 0.05 levels.

Hormonal level (mg/l)	CGR (mm/d)
NAA (0) + BA (0)	0.1380 E
NAA (0) + BA (0.5)	0.1170 E
NAA (0) + BA (1)	0.1540 C-E
NAA (0.5) + BA (0)	0.1570 C-E
NAA (1) + BA (0)	0.1830 B-D
NAA (2) + BA (0)	0.1410 DE
NAA (0.5) + BA (0.5)	0.1880 BC
NAA (0.5) + BA (1)	0.1990 A-C
NAA (1) + BA (0.5)	0.2380 A
NAA (1) + BA (1)	0.2020 AB
NAA (2) + BA (0.5)	0.1970 A-C
NAA (2) + BA (1)	0.1550 C-E

Discussion

Cumin (*Cuminum cyminum* L.) is an annual medicinal plant that is cultivated for the production of dry ripe fruits and has economical importance (Lawrence, 1995).

In this study, we induced callus in both leaf and hypocotyl explants. In this study, the callus induction percentage (%) and growth rate (in mm/d) were analyzed.

Table 3. Mean comparison for interaction effects of explant × hormone concentrations on callus growth rate in *Cuminum cyminum* L. GR: growth rate. Similar letters in the tables showed that they didn't have any significant statistical difference in 0.05 levels.

Hormonal level (mg/l)	GR(mm/d) in leaf explant	GR(mm/d) in hypocotyl explant
NAA (0) + BA (0)	0.1240 F-H	0.1520 E-H
NAA (0) + BA (0.5)	0.1280 F-H	0.1060 H
NAA (0) + BA (1)	0.1620 D-H	0.1460 E-H
NAA (0.5) + BA (0)	0.1460 E-H	0.1680 C-H
NAA (1) + BA (0)	0.1760 C-G	0.1900 A-F
NAA (2) + BA (0)	0.1640 C-H	0.1180 GH
NAA (0.5) + BA (0.5)	0.2480 A	0.1280 F-H
NAA (0.5) + BA (1)	0.1980 A-E	0.2000 A-E
NAA (1) + BA (0.5)	0.2300 A-C	0.2460 AB
NAA (1) + BA (1)	0.1800 C-G	0.2240 A-D
NAA (2) + BA (0.5)	0.2120 A-E	0.1820 B-G
NAA (2) +BA (1)	0.1660 C-H	0.1440 E-H

Our results showed that the explant type has no significant effect on the percentage of callus induction, but the growth rate was affected significantly. According to statistical analysis, it seems that leaf explant has the highest capacity for callus production in *C. cyminum* L.

Tawik & Noga (2002) induced calli in *C. cyminum* from hypocotyl and leaf explants. They showed that the hypocotyl segment respond better than leaf explants.

In this study, there was no significant difference between hormone concentrations in callus induction percentage and callus growth rate. Also there was no significant difference between interaction effects of explant type × hormone concentration for callus induction percentage and callus growth rate.

The mean comparison indicated that at 1 mg/l of NAA and 0.5 mg/l of BA the hormone had the highest effects on callus growth rate.

Moon & Stomp (1997) reported the effect of 2, 4-D concentrations on callus induction. In addition, they reported that 2, 4-D has the highest effect on callus induction rather than IAA and NAA plant growth regulators. In this study, we just applied the NAA and BA plant growth regulators.

Tawik & Noda (2002) induced callus in *C. cyminum* by using 2, 4-D and kinetin. The companionship of auxin and a cytokinin can improve the callus induction. The interactions of auxin and cytokinin control many aspects of growth and differentiation via a complicated manner. Recent advances in understanding of the metabolism and cell cycle help us to elucidate the mechanism of actions of these hormones in controlling the various physiological and developmental responses.

Molecular and genetic studies reveal the interactions of these hormones at several levels including post-translational modification, hormone availability and activity, their participation in signaling pathways, and gene expression regulation (Kahrizi *et al.,* 2011).

Our results showed that leaf explant has more CGR levels copared to hypocotyl explant. One mg/l of NAA and 0.5 mg/l of BA had the highest effect on callus growth rate. Comparing the mean interactions of the hormone concentrations on callus growth rate in *C. cyminum* L. showed that 0.5 mg/l of NAA + 0.5 mg/l of BA in leaf explant has the highest effect.

Conclusion

Our results demonstrated that the explant type, hormone and their interactions have non-significant effects on the percentage of callus induction. The explants showed a significant effect on callus growth rate. The medium containing 1 mg/l NAA and 0.5 mg/l BAP showed the highest callus growth rate. The NAA (0.5 mg/l) + BA (0.5 mg/l) in leaf explant demonstrated the highest effect (0.248 mm/d). Finally, our results confirmed the explant type

and plant growth regulators effects on callus induction and growth.

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