Cellular and Molecular Biology

Kazemi *et al. Cell. Mol. Biol.*2016, 62 (6): 65-68
ISSN: 1165-158X
doi: 10.14715/cmb/2016.62.6.12

Original Research

Induction of linalool as a pharmaceutical and medicinal metabolite via cell suspension culture of cumin (*Cuminum cyminum* L.)

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Abstract: Cumin is an important medicinal plant in Iran. Plant cell suspension culture is a method for the production of medicinal and secondary metabolites. The linalool is a plant secondary metabolite that has been recognized as a neuroprotective agent. The purpose of this study was to evaluate the effects of salicylic acid elicitor on induction of linalool in cell suspension culture of cumin. For this purpose, the cumin seeds were prepared, to obtain sterile seedling, were disinfected with sodium hypochlorite and alcohol, and were cultured on MS basal medium. This research was conducted in two separate experiments including callus induction and suspension cultures. Leaf explants were prepared from sterile seedlings and used to produce callus on MS medium supplemented with 1 mg/l NAA and 0.5 mg/l BAP. In order to establish suspension culture, the appropriate calli were transferred to liquid medium. Then cell cultures were treated with elicitors. The effects of elicitor on the production of linalool secondary metabolite and cell viability were assessed by GC-Mass and tetrazolium test respectively. For this purpose, the salicylic acid (at concentrations of 0, 1, 2, 4 and 8 mg/l) was used. The experimental design was a completely randomized design with five treatments and three replications. The results of cell culture and GC-Mass analysis showed that salicylic acid had significant effects on the linalool production ($P<0.01$). At all concentrations of salicylic acid, viability of the cells in suspension culture experiments was lower than control. Increasing the elicitor concentrations lead to reduction in cell survival. In conclusion it is possible to produce linalool as a secondary metabolite and pharmaceutical agent in cell culture of cumin. It is necessary to determine the best combination of medium and elicitor.

Key words: *Cuminum cyminum,* Linalool Induction, Medicinal Plants, Cell suspension culture, Essential oil.

Introduction

Medicinal plants are known as an important source of secondary metabolites. In addition they are important commercially, economically and financially. Secondary metabolites have many applications in food industries, sanitary and pharmacy fields (1-7).

Some medicinal components are found in low concentrations in different plant species. Researchers have attempted to enhance these components. Plant biotechnology has suitable methods, such as cell culture, tissue culture, genetic engineering, molecular markers which can increase gene expression and gene production to produce drugs (4, 8-10).

In recent years, plant tissue culture techniques have been used as powerful tools to the micro-propagation and breeding of many plant species (11).

Plant tissue culture has numerous applications in the field of medicinal plants, including rapid and mass plant multiplication, pathogen-free plant production, enhancing performance and yield, protecting endangered species and the *in vitro* production of secondary metabolites (8, 11, 12).

In contrast to natural conditions, in the *in vitro* conditions, not only component production was increased but also new product produced. Secondary metabolites can be produced by tissue culture methods under sterile and controlled conditions and also pure and safer compounds are produced. Actually cell culture technique is the best and the most economical method to produce these metabolites (11).

Cumin (*Cuminum cyminum* L.) as a member of the Apiaceae family is one of the most important medicinal and pharmaceutical plants in Iran. This plant family is one of the well-known families among flowering plants because of its worth properties (13). Studies have shown pharmaceutical and medicinal importance of cumin, and antimicrobial effect of the cumin's oil extract (14).

Received January 10, 2016; **Accepted** May 13, 2016; **Published** May 30, 2016

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Linalool is the principal component of many essential oils that are known to possess several biological activities, which are attributable to these monoterpene compounds. This monoterpene compound is a major component of essential oils in various aromatic species. Several Linalool-producing species are used in traditional medical systems, including *Aeolanthus suaveolens* G. Psychopharmacological *in vivo* evaluation of Linalool showed that this monoterpene compound has dose-dependent sedative effects including hypnotic, anticonvulsant and hypothermic properties (15, 16).

Few investigators reported on *C. cyminum* cell culture. The aim of this research was to use cell culture for harvesting secondary metabolites of *C. cyminum* to produce linalool.

Materials and Methods

The cumin seeds were supplied from Zagros Bioidea Co., Razi University Incubator. The seeds were cultured in *vitro* to obtain sterile seedling. The seeds were disinfected with sodium hypochlorite and alcohol and were cultured on MS basal medium. This research was conducted in two separate stages: callus induction and suspension cultures. The leaf explants were prepared from sterile seedlings and were used to induce callus on MS medium supplemented with 1 mg/l NAA and 0.5 mg/l BAP. In order to establish suspension culture the appropriate calli were transferred to liquid MS medium. Then cell cultures were treated with elicitors. The effects of elicitor on the production of linalool's secondary metabolites and cell viability were assessed by tetrazolium test. For this purpose, the salicylic acid (at concentrations of 0, 1, 2, 4 and 8 mg/l) was used. The experimental design was a completely randomized design with five treatments and three replications. The cell masses resulted from cell culture were analyzed by GC-Mass.

The data analysis and mean comparison were carried out by MSTAC-C software. The Duncan's Multiple Range Test was used for mean comparison. The regression analysis was done with the IBM SPSS software V16.

Results and Discussion

The results of cell culture, GC-Mass and variance analysis showed that salicylic acid had significant effects on linalool production (P<0.01) (Table 1).

Gharib (2007) investigated on the response of sweet basil (*Ocimum basilicum* L.) and marjoram (*Majorana hortensis*) plants to foliar application of salicylic acid in pot experiments. The GC/MS results of that experiment indicated that common components of *Ocimum basilicum* essential oil under all treatments were linalool (46.63 - 43.32%) (17).

Table 1. Mean squares for effects of salicylic acid on linalool production in cell suspension culture of cumin (*Cuminum cyminum* L.). Where S.O.V (source of variations), Df (degree of freedom), SS (sum of squares), MS (mean of squares) and C.V. (coefficient of variations).

S.O.V.	Df	SS	MS	P- value
Salicylic acid	4	33.024	8.256**	00.00
Error	10	0.430	0.043	
C.V. (%)			4.04	

Table 2. Mean comparison for effects of salicylic acid concentrations on linalool production in cell suspension culture of cumin (*Cuminum cyminum* L.) by Duncan's multiple range test.

Salicylic acid concentrations (mg/l)	Linalool percents
0	7.400 A
1	5.900 B
2	5.100 C
4	3.133 E
8	4.000 D

In a column, means followed by a same letter are not significantly different (P<0.01).

The results of Gharib (2007) experiment showed that compared to the controls, linalool percentage was reduced by increasing the concentration of salicylic acid. Our results, confirms the results of Gharib (2007) study (17).

The Duncan's Multiple Range Test showed that different concentrations of salicylic acid have different effects (P<0.001) on linalool production in cell suspension culture of cumin. This finding confirms the analysis of variance results (Table 2).

As can be seen in the mean comparison table, linalool production has decreased by increasing the salicylic acid concentration (Table 2).

The regression analysis showed that the relationship between these two variables (linalool production and salicylic acid concentration) was as Y = -0.4X + 6.0. The regression equation indicates that by increasing one salicylic acid unit, the linalool percentage will be reduced as much as 0.4 units (Fig. 1).

The viability of cells in the suspension culture experiments showed that this index was lower than control at all levels of salicylic acid. Also results showed that increasing the elicitor concentrations leads to reduction in cell survival.

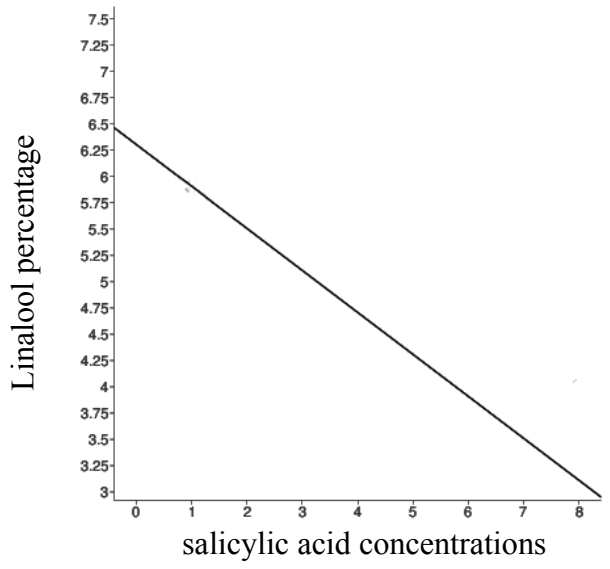

Figure 1. Regression relationship between salicylic acid concentrations and Linalool percentage in cumin (*Cuminum cyminum* L.) cell suspension culture.

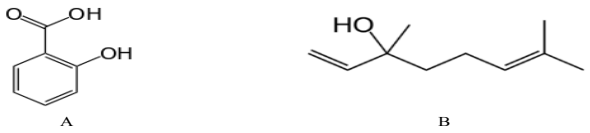

Figure 1. The chemical structure of salicylic acid (A) andlinalool (B).

The salicylic acid is known as a monohydroxybenzoic acid, a kind of phenolic acid and a beta hydroxy acid. Its chemical formula is C7H6O3 (Fig. 2A). This colorless crystalline organic acid is widely applied in organic synthesis and functions as a plant hormone. Salicylic acid results from the metabolism of salicin. Besides being an important active metabolite of aspirin (acetylsalicylic acid), which acts in part as a prodrug to salicylic acid, it is probably best known for its use as a key component in topical anti-acne products. The salts and esters of salicylic acid are known as salicylates (18-21).

The salicylic acid has been known to exert its effects through several different pathways. It produces its anti-inflammatory effects through suppressing the activity of cyclooxygenase (COX), an enzyme that is responsible for the production of pro-inflammatory mediators such as the prostaglandins. Unlike most other nonsteroidal anti-inflammatory drugs (NSAIDs), salicylic acid suppresses COX not via direct inhibition of COX like but by suppressing the expression of the enzyme (by a yet-unelucidated mechanism). Salicylic acid has also been shown to activate adenosine monophosphate-activated protein kinase (AMPK), and it is thought that this action may be important in the anticancer effects of the salicylic acidand its prodrugs aspirin and salsalate. In addition, the antidiabetic effects of salicylic acid are probably mediated by AMPK activation primarily through an allosteric conformational change that increases levels of phosphorylation. The salicylic acid also uncouples oxidative phosphorylation, which leads to increased ADP: ATP ratio and AMP:ATP ratio in the cell. Finally, salicylic acid may change the AMPK activity and subsequently exert its anti-diabetic effects through altered energy status of the cell. Even in AMPK knock-out mice, however, there is an anti-diabetic effect, and this implies at least one additional, yet-unidentified action of the compound. The salicylic acid has known to regulate c-Myc level at both transcriptional and post-transcription levels. Inhibition of c-Myc may represent an important pathway by which aspirin exerts its anti-cancer effect and reduce the occurrence of cancer in epithelial tissues (22, 23).

The Linalool has been known as a naturally occurring terpene alcohol chemical (Fig. 2B). It is found in many flowers and spice plants with many commercial applications, the majority of which are based on its pleasant scent (floral, with a touch of spiciness). Its other names are β-linalool, linalyl alcohol, linaloyl oxide, p-linalool, allo-ocimenol, and 3,7-dimethyl-1,6-octadien-3-ol.

In higher plants linalool is biosynthesized from isopentenyl pyrophosphate via the universal isoprenoid intermediate geranyl pyrophosphate, throughout a class of membrane-bound enzymes named monoterpene synthases. One of these, linalool synthase (LIS), has been showed to produce (S)-linalool in several floral tissues (24, 25).

Linalool is used as a scent in 60–80% of perfumed hygiene products and cleaning agents such as soaps, detergents, shampoos, and lotions. It is also applied as a chemical intermediate. Vitamin E is one common downstream product of linalool. Also, the linalool is applied by pest professionals as a flea, fruit fly and cockroach insecticide (5, 26, 27).

The linalool is applied in some mosquito-repellent products; however, the EPA notes that "a preliminary screen of labels for products containing linalool (as the sole active ingredient) indicates that efficacy data on file with the Agency may not support certain claims to repel mosquitos." (5, 26, 27).

Acknowledgements

The authors wish to thank Ms. Matin Ghaheri (Razi University of Kermanshah) for manuscript editing. Thanks to Zagros Bioidea Co., Razi University Incubator for all supports.

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