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Research Article

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The Enhancement of Kaempferol by Yeast Elicitor in Callus Cultures of *Oxystelma Esculentum* (L.F)

K Devi Maliga[1] and N Yogananth[2*]

[1]Research and Development Centre, Bharathiar University, Coimbatore, Tamilnadu, India
[2]PG & Research Department of Biotechnology, Mohamed Sathak College of Arts & Science, Chennai, Tamilnadu, India

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ABSTRACT

The present investigation is the comparative analysis of kaemferol from field grown plant parts and in vitro callus and also the enhancement of kaempferol by the influence of yeast elicitor were determined. The stem explants showed highest percentage of callus induction (74.34 ± 0.67) and stem explants showed the highest callus growth in terms of fresh weight (3.90 ± 0.05 g) and dry weight (0.41 ± 0.01g). The maximum content of kaempferol in three type of samples was observed in this order; yeast elicitor treated samples> in vitro raised callus samples> field grown samples. The highest amount of kampferol was found in 2 ml yeast elicitor treated with stem callus sample (0.958 mg/g. dw). Therefore, from the current findings, it was proved that the highest amount of kaempferol in elicitor treated in vitro callus is provides resource for valuable drugs preparation against various diseases.

Key words: *Oxystelma esculentum*; Asclepiadaceae; Kaempferol; Yeast elicitor

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INTRODUCTION

Many pharmaceutics are produced from the secondary metabolites of plants at present. Secondary metabolites are very less or not noticeable in native plant parts and sometimes also very low level in dedifferentiated cells such as callus tissues or suspension cultured cells. For plant cell culture to be economically feasible, certain methods have been discovered and developed that would allow for consistent generation of high yields of secondary metabolites from cultured cells [1]. Other methods employed are selection of high-producing strains, addition of precursors, biotransformation, elicitor treatment, application of immobilized cells and product secretion into the culture media [1]. *Oxystelma esculentum* (L.F) is an important medicinal plant belonging to the family Asclepiadaceae used in the traditional systems of medicine for various infections viz., gleet, gonorrhea, pain in the muscles, cough, antiseptic, depurative, diabetes and jaundice [2]. The plant contains calogenin [3], lupeol [4], stigmasterol [5], kaempferol [6] and aesculin [7]. Kaempferol is one of the important polyphenolic flavonoids of *Oxystelma esculentum*. It is a natural plant product with potentially useful pharmacological and nutraceutical activities. It is common in vegetable, fruits, plant and herbal medicines. Over the years, Studies have shown that can help treatment of cancers, cardiovascular disease, neuron disorder, cholesterol and serve as antioxidant and anti-inflammatory [8].
The aim of the present work, to induce the callus formation from leaf and stem explants of *Oxystelma esculentum* and the comparative analysis of kaemferol from field grown plant parts and *in vitro* callus and also the enhancement of kaempferol by the influence of yeast elicitor were determined.

MATERIALS AND METHODS

In vitro Culture

Explants of leaf and stem were collected from *Oxystelma esculentum* and washed thoroughly under running tap water and then treated with a few drops of Tween-80 for 10 minutes with constant shaking. This followed by successive three washing with distilled water and the explants were washed with 70% ethyl alcohol for few seconds and washed with distilled water for 3-4 times. After that, the explants were transferred to laminar air flow chamber and disinfected with 0.1% HgCl2 for 2 minutes and washed with sterile distilled water for 5-7 times. Then, the explants were placed in sterile Petri plates before inoculation. The sterilized leaves were injured all over the surface and used for callus induction. The excised explants were cultured on MS medium supplemented with different concentrations of NAA (0.50, 1.00, 1.5, and 2.0 mg/l) along with cytokinins of BAP (0.25 mg/l). Each experiment/treatments was repeated thrice. Analysis of variance was carried out and the differences between the treatments were determined by Duncan's Multiple Range Test.

Kaempferol Anaysis

One gram of each of the air-dried stem, leaf and 1 gm of 1.5 mg/l NAA + 0.25 mg/l BAP callus of the stem and leaf of *Oxystelma esculentum* was accurately weighed and extracted with methanol. Each methanol extract was filtered through 0.2 µm PTFE membrane filter before injection into HPLC column.

Elicit the Kaempferol by using Yeast Elicitor

10 grams of the yeast extract was dissolved in 50 ml to distilled water and after addition of ethanol (80% v/v) it was incubated at 6°C for 4 days and then supernatant was decanted. This process was repeated and the final precipitate was dissolved in 75 ml of distilled water and the solution of final concentrations of 0.25 and 0.8 mg/ml were added to the cultures [9]. The stem and leaf derived callus was sub cultured on MS media supplemented with NAA 1.5 mg/l and 0.25 mg/l BAP. The media were impregnated with yeast extract (1,2 and 3 ml/L) as an elicitor was tested on the same MS media. The calli were left to grow for a period of 2 weeks. On 14 days of elicitation, callus cultures were harvested and 200 mg of fresh wet tissues were extracted in 2 ml of absolute methanol for 24 hrs. The extracts were centrifuged at 10.000 rpm for 15 min and collect the supernatant.

The HPLC analysis of the various powedered samples of the field grown plant culture, callus culture and elucidated culture were carried out with JASCO HPLC, using a C-18µ Bondapak column and acetonitrile: water (50:50) + 0.1% phosphoric acid as solvent system at a flow rate 0.8 ml/min and detector set at 350 nm. The kaempferol were identified by comparison of their retention times with those of authentic kaempferol samples (received from Sigma Aldrich, Bangalore, India) and quantified using a standard calibration curve. Kaempferol yields were calculated using the total dry weight of tissue and expressed in terms of percentage of kaempferol content.

RESULTS AND DISCUSSION

Callus induction was observed in MS media containing different concentrations and combination of NAA with BAP. There was a notable range of variation in percentage of callus formation and average fresh weight of callus. The stem explants showed highest percentage of callus induction (74.34 ± 0.67^{d}) in MS medium containing 1.50 mg/L NAA and 0.25 mg/L BAP (Table 1). The maximum fresh weight and dry weight were observed in same concentrations of plant growth regulators. Like that of our study Yogananth *et al*., (2012) reported the callus induction in leaf explant of *Dregea volubilis* [10]. Among the plant parts, the leaf powder showed the maximum content of kaempferol (0.392 mg/g. dw) and the minimum was observed in stem (0.096 mg/g. dw) in stem powder (Table 2 and Figure 1)

Table 1: Effect of NAA with BAP on callus induction, callus growth of young stem and leaf explants of *Oxystelma esculentum*

Conc. of NAA & BAP (mg/l)	Stem Explants			Leaf Explants		
	% of response	Fresh weight	Dry weight	% of response	Fresh weight	Dry weight
0.50 + 0.25	55.02 ± 1.21^{a}	2.48 ± 0.09^{a}	0.25 ± 0.02^{a}	53.10 ± 0.16^{a}	2.16 ± 0.07^{a}	0.21 ± 0.04^{a}
1.00 + 0.25	65.35 ± 1.34^{b}	3.23 ± 0.03^{b}	0.32 ± 0.03^{b}	62.60 ± 0.67^{c}	2.95 ± 0.03^{b}	0.26 ± 0.01^{b}
1.50 + 0.25	74.34 ± 0.67^{d}	3.90 ± 0.05^{d}	0.41 ± 0.01^{d}	70.35 ± 0.48^{d}	3.10 ± 0.02^{c}	0.36 ± 0.03^{d}
2.00 + 0.25	69.99 ± 0.78^{c}	3.60 ± 0.08^{c}	0.37 ± 0.02^{c}	58.10 ± 0.27^{b}	3.04 ± 0.03^{c}	0.29 ± 0.02^{c}

± - standard error. Means followed by the same letter not significantly different by the DMRT at P<0.05 level of significance.

Table 2 and Figure 2 show the effect of NAA+ BAP combination on the content of kaempferol compounds. The maximum kaempferol content of leaf derived callus extracts was 0.646 mg/g. dw whereas the kaempferol content in the stem derived callus extracts were 0.512 mg/g. dw. In the same way, Yogananth *et al*., found that solasodine content was higher in callus cultures than field grown leaves of *Solanum nigrum* [11]. The plant growth regulators (especially auxin) supplemented to medium for inducing specific morphogenetic response, also cause and sometimes add stress to the cultivated cells [12].

Table 2: Kaempferol production from native plant, callus and elicitor treated callus samples

S. No	Samples	Kaempferol content (mg/g. dw)
1	Stem	0.096
2	Leaf	0.392
3	Stem callus	0.512
4	Leaf callus	0.646
5	Stem callus+ 1ml elicitor	0.888
6	Stem callus+ 2 ml elicitor	0.724
7	Stem callus+ 3 ml elicitor	0.543
8	leaf callus + 1 ml elicitor	0.947
9	leaf callus + 2 ml elicitor	0.958
10	leaf callus + 3 ml elicitor	0.812

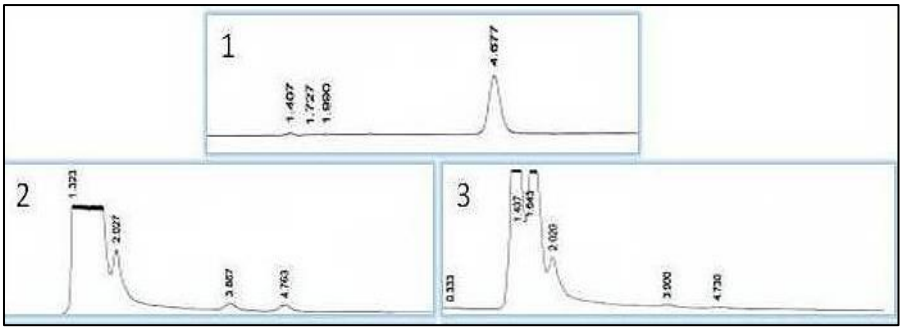

Figure 1: HPLC Chromatogram of Kaempferol Standard and field grown plant parts

1.Standard – Kaempferol 2. Leaf powder 3. Stem powder

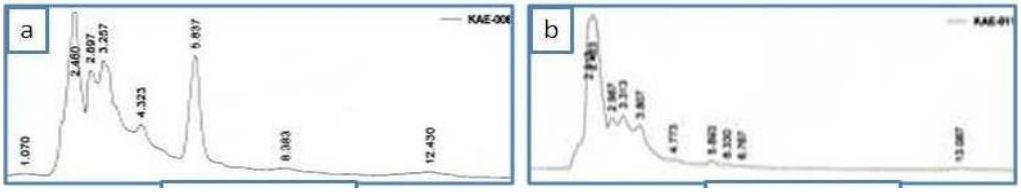

Figure 2: HPLC chromatogram of stem callus and leaf callus

a) Stem callus b) Leaf callus

For elicitor treatment, the leaf callus produced the highest quantity of kaempferol (0.958 mg/g. dw) in 2 ml yeast elicitor treated sample and the stem callus, the maximum quantity (0.888 mg/g. dw) was recorded in 1 ml yeast elicitor treated sample (Table 2 and Figure 3). Yeast elicitor is one of the most commonly used elicitors for the induction or enhancement of secondary metabolite formation. Angeles and Jorge reported the yeast extract possesses a powerful elicitor of silymarin accumulation in *Silybum marianum* [13].

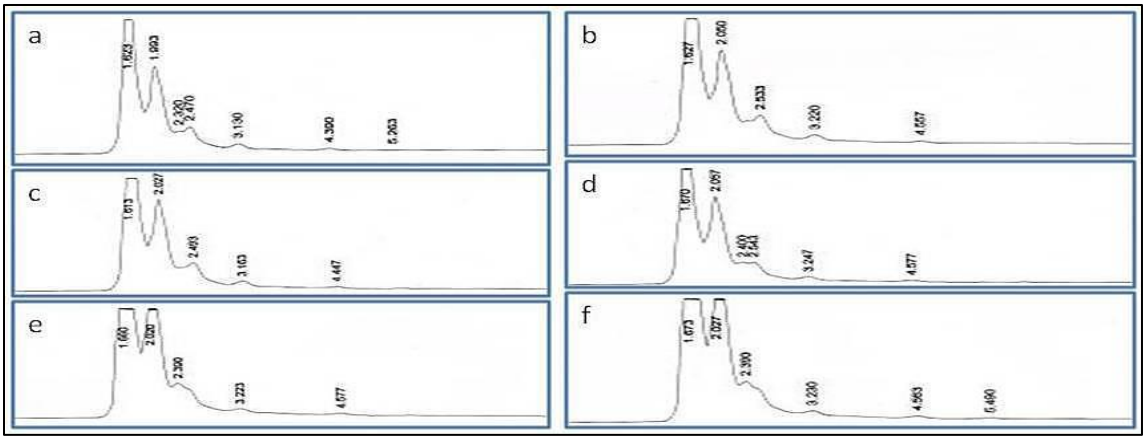

Figure 3: HPLC chromatogram of elicitor treated stem and leaf callus

a) Stem callus + 1 ml elicitor b) Stem callus + 2 ml elicitor c) Stem callus + 3 ml elicitor d) Stem callus + 1 ml elicitor e) Stem callus + 2 ml elicitor f) Stem callus + 3 ml elicitor

CONCLUSION

In conclusion, the kaempferol analysis in three different ways, the elicitor treated callus culture gave maximum amount of kaempferol when compared to *in vitro* raised callus and field grown plant parts. This indicates that yeast can be used as a permeabilizing agent to obtain kaempferol on a large scale without sacrificing the cells.

Conflict of Interest

The authors declare that there is no conflict of interests.

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