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# Effect of elicitor on quercetin production in cell cultures of *Citrullus colocynthis* (Linn.) Schrad

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'Elicitors' for a plant refers to chemicals from various sources that can trigger physiological and morphological responses and phytoalexin accumulation. Elicitation is the induced or enhanced biosynthesis of metabolites due to addition of trace amounts of elicitors. As quercetin is considered as a bioactive ingredient of *Citrullus colocynthis*. Present study was carried out to examine the effect of phenylalanine on production of quercetin in liquid cultures. Exogenous incorporation of β-phenylalanine in liquid culture increased the yield of total quercetin about 2 to 3 fold in comparison to control (control = 3.05 mg/g dw; fed = 7.25 mg/g dw).

*Keyword:* Elicitors, Quercetin, β-phenylalanine, *Citrullus colocynthis*.

## 1. Introduction

Plants form an important part of our everyday diet, and plant constituents and their nutritional value have been intensively studied for decades. In addition to essential primary metabolites (e.g. carbohydrates, lipids and amino acids), higher plants are also able to synthesize a wide variety of low molecular weight compounds – the secondary metabolites. Plant secondary metabolites can be defined as compounds that have no recognized role in the maintenance of fundamental life processes in the plants that synthesize them, but they do have an important role in the interaction of the plant with its environment. The production of these compounds is often low (less than 1% dry weight) and depends greatly on the physiological and developmental stage of the plant [1, 2].

Higher plants are rich sources of bioactive constituents or phyto pharmaceuticals used in pharmaceutical industry. These plant derived natural products are used as drugs. Many of these pharmaceuticals are still in use today and often no useful synthetic substitutes have been found that possess the same efficacy and pharmacological specificity [3]. Currently one-fourth of all prescribed pharmaceuticals in industrialized countries contain compounds that are directly or indirectly, via semi-synthesis, derived from plants. Furthermore, 11% of the 252 drugs considered as basic and essential by WHO are exclusively derived from flowering plants [4]. Plant-derived drugs in western countries also represent a huge market value. In several cases, the natural product is more easily accepted by consumers than an artificially produced one. Many plants containing high-value compounds are difficult to cultivate or are becoming endangered because of over-harvesting. The

biotechnological production of valuable secondary metabolites in plant cell or organ culture is an attractive alternative to the extraction of whole plant material. However, the use of plant cell or organ cultures has had only limited commercial success. This is explained by the empirical nature of selecting high-yielding, stable cultures and the lack of understanding of how secondary metabolites are synthesized or how their synthesis is regulated [5, 6]. So there is a need to develop alternatives to the intact plant for the production and enhancement of plant secondary metabolites. Many biotechnological strategies have been hypothesized and experimented for enhanced production of secondary metabolites from medicinal plants. Some of these include screening of high yielding cell line, media modification, precursor feeding, elicitation, large scale cultivation in bioreactor system, hairy root culture, plant cell immobilization, biotransformation and others [7].

The enhanced production of the secondary metabolites from plant cell cultures through elicitation has opened up a new area of research which could have important economic benefits for pharmaceutical industry. Elicitation is the induced or enhanced biosynthesis of metabolites due to addition of trace amounts of elicitors [8]. An 'elicitor' may be defined as a substance which, when introduced in small concentrations to a living cell system, initiates or improves the biosynthesis of specific compounds.

Several parameters such as elicitor concentration and selectivity, duration of elicitor exposure, age of culture, cell line, growth regulation, nutrient composition, quality of cell wall materials, substrate enhancement of product accumulation etc. have been reported [9].

Exogenous supplementation of precursors and intermediates into the culture medium has been suggested as a means to increase the yield of various metabolites and is also a method to understand their biosynthetic pathways. Phenylalanine, an amino acid, is said to be the precursor of flavonoids [10]. Turnover of flavonoids and its association with growth has been reported by scientist [11] and phenylalanine ammonia lyase activity has also been reported [12].

Effect of dl-β-phenylalanine on flavonoids has been studied in tissue cultures of *Datura stramonium* [13], *Medicago sativa* [14], *Gossypium* cultivars [15] and *Balanites aegyptiaca* [16].

Quercetin is the most important flavonoid in experimental studies. It act as antihistamines (which is useful in reducing allergy symptoms and help in reducing inflammation associated with various forms of arthritis). Quercetin also work as anti-inflammatory, antioxidant and anticancer substances [17]. It also helps in solving problems of cellular regeneration, hemorrhoids, menopausal symptoms and non-healing ulcers. Biosynthetic pathway of flavonoids is shown in Chart-1.

The present study is related to the effect of precursor phenylalanine on growth and production of quercetin in cell cultures of *Citrullus colocynthis.*

## 2. Materials and Methods

To examine the effect of incorporation of precursor/s on biosynthesis of quercetin and growth of the tissues, different concentrations of phenylalanine were incorporated on liquid MS medium.

Eighteen months old unorganized callus cultures from nodal stem explant were established on MS medium supplemented with BAP (2.0 mg/l), NAA (2.0 mg/l) and 3.0% sucrose. The callus tissue was transferred to fresh MS medium with above mentioned additives and MS medium singly supplemented with 25 mg/100 ml, 50 mg/100 ml, 75 mg/100 ml and 100 mg/100 ml of phenylalanine. The liquid cultures were maintained on rotatory shaker (100 rpm) and maintained for six weeks after frequent subculturing at 4-6 weeks interval in fresh liquid medium under uniform conditions of light and temperature. The callus tissues were dried, till a constant weight was achieved, powdered, weighed and subjected to quercetin analysis. Five replicates were used in each case and their mean values were taken into consideration.

**Biosynthetic pathway for production of flavonoids**

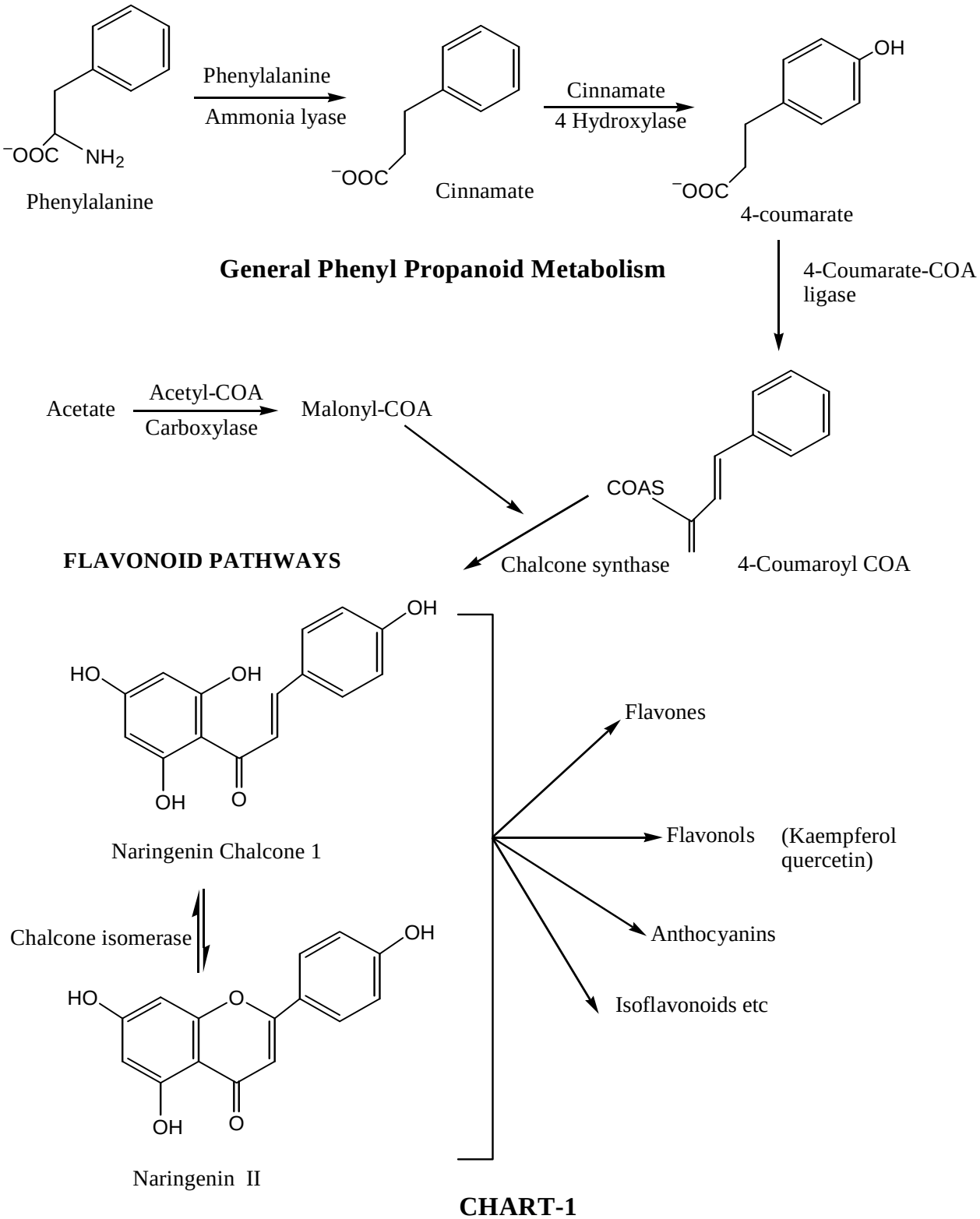


**CHART-1**

### 2.1 Quantitative estimation

The method of quantitative estimation of the identified flavonoids was carried out colorimetrically [18]. Stock solutions of quercetin were separately prepared by dissolving the authentic samples in methanol. Six concentrations (25 µg/ml to 150 µg/ml) of each of the standard samples were spotted on silica gel 'G' coated and activated plates. Separated plates of each of the concentrations of quercetin were used and these chromatograms were developed in the same solvent system as used in quantitative method (n-butanol, acetic acid and water, 4:1:5, upper layer). Such developed chromatograms were air-dried and visualized under UV light. The fluorescent spots were marked and collected along with adsorbent in separate tubes, the mixture was shaken vigorously, centrifuged and the supernatants were collected separately.

The volume of the elutes was made upto 10 ml by adding spectroscopic methanol. To each of the samples 3 ml of 0.1 ml of aluminium chloride was added, stoppered tightly and the mixture was shaken vigorously. Such tubes were kept at room temperature for 20 minutes. Five such replicates were prepared in each case and their optical densities (O.D.) were measured using spectrophotometer (systronic UV-Vis-118) set at 440 nm against a blank (10 ml spectronic methanol + 3 ml 0.1 m $AlCl_3$) for quercetin. Regression curves for quercetin were separately plotted in between their respective concentrations and optical densities, which follows Beer's law.

Each of the ethyl ether and ethyl acetate extracts were dissolved in 1 ml of spectroscopic methanol and applied (0.1 ml) on silica gel 'G' coated glass plates along with the authentic quercetin as marker and developed as above. Fluorescent spots coinciding with those of the reference compounds were marked, scrapped, eluted with methanol separately and the samples were prepared as detailed above. The optical density in each case was colorimetrically recorded as above. The amount of quercetin in the various test samples was then determined (mg/g dw) by comparing with those of their respective standard regression curves.

### 3. Results

Results indicate a gradual increase in growth index upto six weeks; after that it declined. GI increased when suspension culture was fed with phenylalanine (25-50 mg/100 ml). Further increase in phenylalanine concentration (75-100 mg/100 ml) decreased GI. Maximum GI (4.85 mg/g dw) as observed in 6 weeks old tissue fed with 50 mg/100 ml of phenylalanine and minimum GI (0.95 mg/g dw) was observed in 2 weeks old tissue fed with 100 mg/100 ml of phenylalanine [Fig. 1].

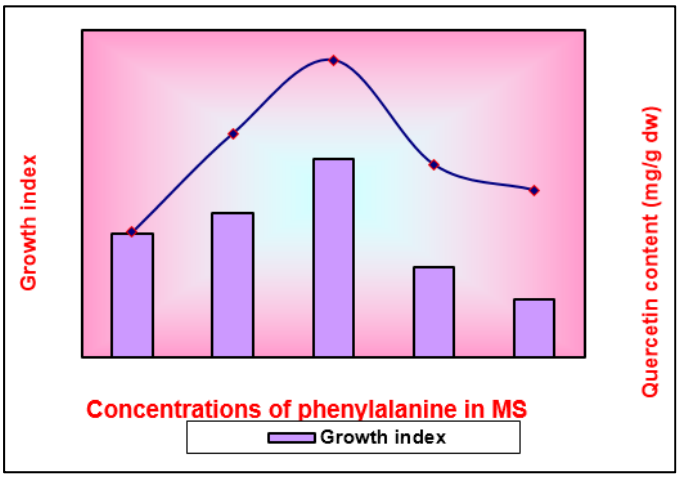


**Fig 1:** Effect of phenylalanine on growth indices and production of quercetin in 6 weeks old tissue.

Total quercetin content (free and bound quercetin) showed a marked increase in tissue fed with phenylalanine upto 50 mg PA/100 ml of medium thereafter it declined gradually and was rather low in tissues grown on the medium fed with 100 mg PA/100 ml of medium but this amount was still higher as compared to the amount obtained from tissues grown on control medium (Table 1).

Maximum quercetin content was found in six weeks old callus cultures. Maximum (7.25 mg/g dw) quercetin was observed in callus tissue fed with 50 mg PA/100 ml medium and minimum (4.08 mg/g dw) in tissues grown on media fed with 100 mg PA/100 ml of medium (as compared to control 3.05 mg/g dw) [Fig. 1].

## 4. Discussion

The present study showed that incorporation of phenylalanine increased the amount of total quercetin content in liquid cultures. Maximum GI (4.85 mg/g dw) was observed in 6 weeks old tissue fed with 50 mg/100 ml of phenylalanine and minimum GI (0.95 mg/g dw) was observed in 2 weeks old tissue fed with 100 mg/100 ml of phenylalanine. The maximum (7.25 mg/g dw) quercetin content was observed in six weeks old callus tissue fed with 50 mg PA/100 ml medium and minimum (4.08 mg/g dw) in tissues grown on media fed with 100 mg PA/100 ml of medium (as compared to control 3.05 mg/g dw). Similarly effect of β-phenylalanine on quercetin content has been studied in tissue cultures of *Gossypium* cultivars [15], *Balanites aegyptiaca* [16] and *Cassia angustifolia* [19]. Cinnamic acid to be a more effective precursor than phenylalanine for quercetin biosynthesis in *Pluchea lanceolata* [20].

**Table 1:** Effect of β–phenylalanine on growth of tissue and production of quercetin on liquid culture of *Citrullus colocynthis*

| Age of cultures in weeks | | GI | Quercetin | | Total quercetin |
|---|---|---|---|---|---|
| | | | Free | Bound | |
| **Control : MS medium** | | | | | |
| 2 | | 2.4 | 0.375 | 0.684 | 1.059 |
| 4 | | 2.7 | 0.885 | 1.090 | 1.975 |
| **6** | | **3.0** | **1.130** | **1.920** | **3.050** |
| 8 | | 2.5 | 0.980 | 1.210 | 2.190 |
| **MS + Phenylalanine 25 mg/100 ml** | | | | | |
| 2 | | 2.15 | 1.89 | 2.00 | 3.89 |
| 4 | | 3.10 | 2.22 | 2.35 | 4.57 |
| **6** | | **3.52** | **2.60** | **2.86** | **5.46** |
| 8 | | 3.26 | 2.42 | 2.50 | 4.92 |
| **MS + Phenylalanine 50 mg/100 ml** | | | | | |
| 2 | | 3.82 | 2.42 | 2.86 | 5.28 |
| 4 | | 4.16 | 2.93 | 3.42 | 6.35 |
| **6** | | **4.85** | **3.40** | **3.85** | **7.25** |
| 8 | | 4.32 | 3.11 | 3.25 | 6.36 |
| **MS + Phenylalanine 75 mg/100 ml** | | | | | |
| 2 | | 1.40 | 1.32 | 1.52 | 2.84 |
| 4 | | 1.76 | 1.86 | 1.90 | 3.76 |
| **6** | | **2.21** | **2.48** | **2.22** | **4.70** |
| 8 | | 1.92 | 1.92 | 2.98 | 3.90 |
| **MS + Phenylalanine 100 mg/100 ml** | | | | | |
| 2 | | 0.95 | 1.12 | 1.32 | 2.44 |
| 4 | | 1.05 | 1.43 | 1.76 | 3.19 |
| **6** | | **1.40** | **1.98** | **2.10** | **4.08** |
| 8 | | 1.21 | 1.50 | 1.83 | 3.38 |

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