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Effect of precursor feeding on quercetin production in callus cultures of *Merremia aegyptia* and *Merremia dissecta*.

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ABSTRACT

L-Phenylalanine is an aromatic amino acid and a substrate of phenylalanine ammonia lyase (MW 270-330kDa.) (EC 4.3.1.24) that catalyzes L-phenylalanine into ammonia and trans-cinnamic acid in the biosynthesis of plant phenolic compounds as it is involved in phenylpropanoid pathway of plants producing various flavonoids, phenylpropanoids, and lignin in plants. Optimisation of suitable concentrations of plant growth regulator and harvest time was accomplished with the aim of enhancing the production of quercetin in callus cultures of *Merremiaaegyptia* and *Merremiadissecta* through precursor feeding. Merremiadissecta callus cultures showed 1.2 fold increase in quercetin content in precursor fed cultures and stem cultures of *Merremiaaegyptia* showed two fold increase in quercetin content supplemented with 50 mg/100ml PA analysed through high performance thin layer chromatography.

KEYWORDS: Phenyl alanine, *Merremiadissecta*, *Merremiaaegyptia*, HPTLC

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INTRODUCTION:

Plant cell and organ culture has emerged as potential area of secondary metabolites production 1 that are used as pharmaceuticals, agrochemicals, flavors, fragrances, coloring agents, bio pesticides, and food additives. The selection of high-producing cells and optimization of medium parameters such as suitable medium, salt, sugar, nitrogen, phosphate, and plant growth regulator levels; and physical factors such as temperature, illumination, light quality, medium pH, agitation, aeration, and environmental gas (e.g., oxygen, carbon dioxide, and ethylene) is required for their production. Several chemical and physical factors have been recognized, which could influence biomass accumulation and synthesis of secondary metabolites in plant cell and organ cultures such as type of culture medium, suitable salt strength of the medium, types and levels of carbohydrates, nitrate levels, phosphate levels and growth regulator levels[2] .

The production of secondary metabolites by cell suspension culture is not always rewarding; thus, organ culture methods (e.g., root, embryo, and shoot culture methods) have been developed for various plant species as alternatives for production of secondary metabolites [3,4]. Many plant cell cultures have also been used to convert precursors into products by utilizing pre-existing enzyme systems such as the addition of loganin, tryptophan, and tryptamine enhanced the production of secologan in [5] and in dole alkaloids [6] in Catharanthus roseus suspension cultures. Effect of phenylalanine on flavonoids has been studied in tissue cultures of *Hydrocotyle bonariensis* [7] and *Silybum marianum* [8] . However for better results, factors such as the timing and concentration of the precursor should be considered prior addition to the cell culture medium. Sophisticated modern quantification techniques such as UV–Vis spectrophotometry, TLC, HPTLC, HPLC, and NMR provide quantitative information on cells and organs under culture conditions. For example, TLC, UV, HPLC and HPTLC analyses have been used for quantification of metabolites in cell suspensions and *in vitro* regenerated organs of *Hypericum perforatum* [9] Callus cultures were fed with phenylalanine and given stress conditions for a period of 3-7 weeks. The level of enhancement in the augmented callus cultures were determined and compared to controlled callus cultures.

MATERIALS AND METHODS

Unorganised static callus cultures were established from young leaf and stem explants on Murashige and Skoog's (MS) basal medium supplemented with NAA (1.0 mg/l) in combination with BAP (0.5 mg/l) in *M. aegyptia* and NAA (2.0 mg/l) in combination with BAP (0.5 mg/l) in *M. dissecta.* Obtained callus tissues were then transferred to fresh MS medium with additives singly supplemented with concentrations of phenylalanine (25 mg/100 ml, 50 mg/100 ml, 75 mg/100 ml and 100 mg/100 ml). These cultures were allowed to grow upto their maximum growth age. 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. The dried samples were separately soxhlet extracted in 80% methanol (100 ml/gm dry weight) on a water bath for 24 hrs. Each of the extracts was concentrated and reconcentrated in petroleum ether (40°-60°C) (fraction-I), ethyl ether (fraction-II) and ethyl acetate (fraction-III) in succession. Each of the steps was repeated thrice to ensure omplete extraction in each case. Fraction I was rejected since it was rich in fatty substances whereas fraction II and fraction III of each of the test samples was hydrolysed by refluxing with 7% H_2SO_4 (10 ml/gm residue) for 5 hours. The mixture was filtered and the filtrate was extracted with ethyl acetate in a separating funnel. The ethyl acetate layer was washed with distilled water till neutrality and dried. The residues Were taken up in small volumes of methanol separately and then subjected to various tests for quercetin.

RESULTS:

Identification and quantification of bioactive secondary metabolite quercetin was carried out using HPTLC techniques. Results indicated a gradual increase in growth index upto five weeks; after that it declined. Highest GI was reported in callus cultures obtained when fed with phenylalanine (50 mg/100 ml) at fifth week of growth. Further quantification of quercetin was confirmed by high performance thin layer chromatography (HPTLC) and peak identification was carried out on the basis of an authentic sample of quercetin. Rf value (0.51) of quercetin isolated from the plant samples coincided with the Rf value of standard quercetin (0.51) in the chromatogram.

DISCUSSION AND CONCLUSION

Flavonoids are one of the major secondary metabolites imparting medicinal values for human beings. Of these, quercetin is considered as an active ingredient which has many biological roles. In the enhancement study, It has been observed that combination as well as concentration of different plant growth regulators in combination affects the synthesis of secondary metabolites and hence, optimization of medium could enhance the production of secondary metabolites 10,11 . Similarly effect of phenylalanine on quercetin content has been studied in tissue cultures of *Gossypium* cultivars [12] , *Balanites aegyptica* [13] and *Cassia angustifolia* [14] . Enhancement of quercetin via precursor feeding technique in *Pluchea lanceolata* has also been done [15] . According to this study, Phenylalanine [16] and Cinnamic acid [17] are reported to be the precursors of quercetin. Addition of precursors (Phenylalanine and Cinnamic acid) in *in vitro* callus cultures of *Pluchea lanceolata* resulted in multifold increase in quantity of quercetin. In our investigation, callus cultures of *M. aegyptia* and *M. dissecta* were fed with phenyl alanine (25-100 mg/ml) and were given stress

conditions for obtaining better results. Five weeks of feeding and experimental condition resulted in higher GI and produced about 1 to 2 fold higher quantity of quercetin when tested as compared to unelicited controls. Merremia dissecta callus cultures showed 1.2 fold increase in quercetin content and stem cultures of Merremia aegyptia showed two fold increase in quercetin content in five weeks old callus cultures. 4.79mg/g and 7.44 mg/g dry weight quercetin were observed in callus tissue fed with 50 mg PA/100 ml medium as compared to 3.75mg/g and 3.71 mg/g dry weight of quercetin content observed in control cultures of both the samples. Therefore, the protocol described in this report is expected to contribute to the future studies for large scale production of certain biologically active plant metabolites in various species from their in vitro propagated elite materials. Accumulation of quercetin in callus cultures can be exploited for its large-scale production in a possible array of health promoting benefits as anti-oxidant, anti-inflammatory and anti-cancerous agents in future.

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