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Role of Exogenous Carbohydrate and Amino Acid Sources on Biomass and Colchicine Production in Non-transformed Root Cultures of *Gloriosa superba*

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Key words: Root culture, Colchicine, *Gloriosa superba*, Carbohydrates, Amino acids

Abstract

Elicitation strategies were studied for yield enhancement of colchicine, produced by root cultures of *Gloriosa superba*. Adventitious root cultures were established and grown in media containing 3 mg/l NAA and 1 mg/l BA. Root cultures showed variations in biomass as well as colchicine production in the presence of different exogenous carbohydrates and amino acid. Among the different sources of carbohydrates used - fructose, sucrose and dextrose gave a substantially higher biomass yield than the control. Maximum biomass was obtained in the presence of fructose. Root cultures growing in mannitol supplemented medium resulted in maximum accumulation of colchicine (0.32%). Among the amino acids, serine and phenylalanine significantly enhanced colchicine accumulation in root cultures. 0.02 mM glutamine supplemented media showed maximum (ten-fold) increase of root biomass.

Introduction

In recent years, various strategies have been developed to assess biomass accumulation and synthesis of secondary compounds in cultures (Yu et al. 2005; Fazal et al. 2008, Murthy et al. 2014). As compared to other culture techniques, root culture is widely exploited for production of bioactive compounds due to high efficiency and similarities with those from mother plants. Adventitious roots showed higher stability in their growing environment and synthesize sufficient amounts of secondary metabolites into their intercellular spaces, which

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can be more easily extracted, and can be grown in a phytohormone manipulated medium with low inoculums but a high growth rate (Sivakumar 2006, Cui et al. 2010, Baque et al. 2012, 2013).

Elicitation can be used as one of the important strategies in order to get better productivity of the bioactive secondary products and reducing production costs (Ghosh et al. 2002, Hussain et al. 2012). The influence of the carbohydrate source and concentration on plant cell/organ cultures are significantly important because cultures are usually grown by using carbohydrate as a single, simple sugar or a combination of simple sugars such as glucose, fructose, maltose, and sucrose. Recently, sugars have been recognized as signaling molecules that affect the growth, development, and metabolism of cultured cells; therefore, a suitable carbohydrate source and concentration should be identified for the production of secondary metabolites in cell and organ cultures (Wang and Weathers 2007, Praveen and Murthy 2012, Murthy et al 2014). Another significant nutrient in plant cell and tissue culture is amino acid. The addition of amino acids to the media is important for stimulating cell growth. Also the amino acids are often the key compounds in the biosynthetic pathway of most of the secondary metabolites including colchicine (Ghosh et al. 2002).

Gloriosa superba (Colchiciaceae) is one of the very important medicinal plants due to the presence of colchicine in roots and biotechnological approaches for improvement in propagation have been attempted (Jha et al. 2005). Colchicine, commonly classified as an anti-inflammatory agent, is a mainstay for the treatment of gout and a second-line therapy for other conditions, including pericarditis, familial Mediterranean fever and Behcet's disease (Paul et al. 2013). Since the roots of *G. superba* accumulate colchicine, the present paper reports. The *in vitro* mass cultivation of roots for the production of colchicine as influenced by carbohydrates and amino acids.

Materials and Methods

Root cultures of *Gloriosa superba* were initiated from 1 cm long root tip explants derived from *in vitro* regenerated plantlets on solid MS supplemented with 3 mg/l NAA and 1 mg/l BA (NB medium) at 24 ± 1ºC under complete darkness (Ghosh et al. 2006, 2007). These cultures were maintained successfully in the same medium for six months with regular subculture after six weeks.

To study the effect of different carbon sources (*viz.* sucrose, dextrose, fructose and mannitol) and amino acid sources (*viz.* glycine, phenylalanine, arginine, serine and glutamine) on growth and colchicine accumulation, root segments (inoculum FW 1.0 - 1.5 g) were cultured in liquid NB medium. The carbohydrate solutions(1-5%) or amino acids (0.01 - 0.1 mM) were added to the medium at

day zero and the cultures were incubated under complete darkness at 24 ± 1°C with continuous shaking on a gyratory shaker at 50 rpm. The root tissues were harvested after 6 weeks and the FW was recorded. The roots were then dried at 50°C in an incubator to determine the DW and colchicine content. The volume of residual liquid media in every set was recorded and the colchicine content of this medium was also analysed. Qualitative and quantitative analysis of colchicne in dried root tissues and liquid medium was done following method published earlier (Hayashi et al. 1988, Ghosh et al. 2002).

Results and Discussion

The growth of the root tissues was measured as the DW yield per litre medium (initial dry wt = 1 g) after six weeks in culture (Fig. 1). Media supplemented with 1, 3 and 5 % dextrose showed three-four-fold increase in biomass. Addition of 5% mannitol showed nearly three-fold increase in biomass. Sucrose, showed more than three-fold increase in biomass at 3%, but higher concentration caused a decrease in biomass. Among the four carbohydrate sources used in the present study, supplementation of 3% fructose to the medium resulted in maximum biomass yield of the root cultures (Fig. 1). In all the cases, however, increase in concentrations of the carbohydrates resulted in an increase in DW/FW ratio of the root tissues (Fig. 2). The higher values of DW/FW ratio may be probably as a consequence of more storage material, or a hydric stress of the tissues.

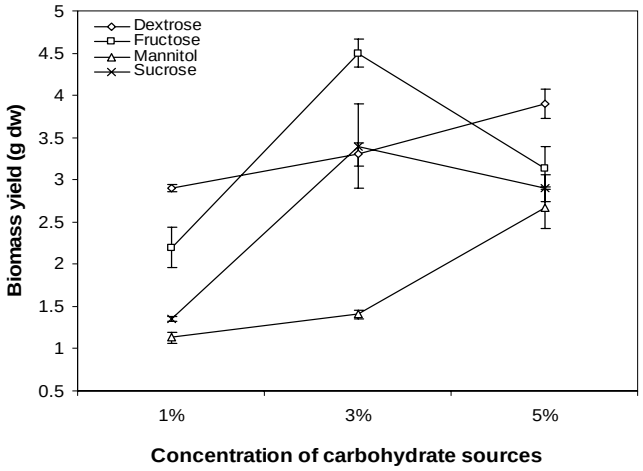

Fig. 1. Effect of different carbohydrate sources on growth of the root organ cultures on liquid NB medium after six weeks in culture in dark at 24 ± 1°C. Biomass yield represent dry wt in g/l medium (initial dry wt = 1 g/l). Values represent mean ± standard error of three experiments with 10 replicates.

Analysis of effect of carbohadrtae sources on colchicine content of the root cultures (Fig. 3) revealed that sucrose, at lower concentration (1%) enhanced accumulation of colchicine (0.14%) in root cultures; however the biomass yield of the root cultures were too low (1.3-fold) at this concentration. Low concentration of dextrose (1%) also favoured colchicine accumulation (0.11%) by the cultures. In presence of 1% dextrose 0.11% colchicine was observed in the root cultures. Colchicine could not be detected in the cultures containing 5% dextrose. However, root cultures growing in presence of mannitol at the lowest concentration showed the maximum accumulation of colchicine (0.32%).

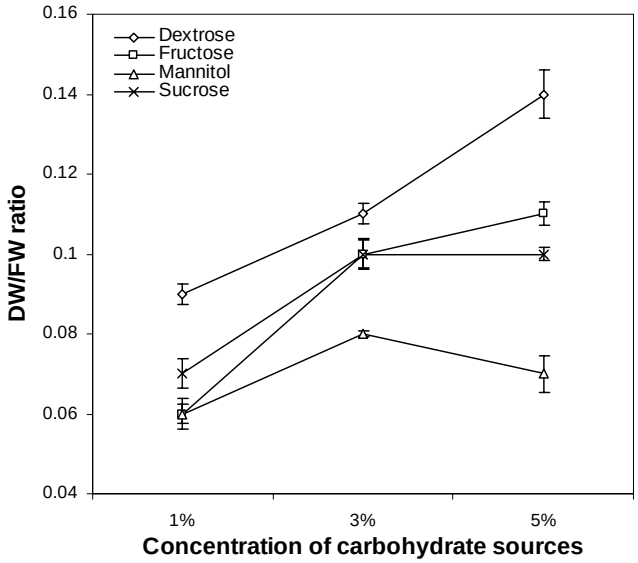

Fig. 2: Effect of different carbohydrate sources on dry wt/fr wt ratio (DW/FW) of the root organ cultures on liquid NB medium after six weeks in culture in dark at 24 ± 1°C. Values represent mean ± standard error of three experiments with 10 replicates.

Growth patterns in terms of biomass yield (g dw) of the cultures in presence of the five different amino acids (Fig. 4) revealed that, growth was not much affected in presence of different concentrations arginine. Higher concentration (0.05 mM) of glycine resulted in seven-fold increase in biomass. Phenylalanine at all concentrations enhanced growth of root cultures, maximum growth (five - six fold) being in presence of 0.02 mM phenylalanine. Glutamine at all concentrations significantly enhanced growth of the cultures, 0.02 mM resulted in ten-fold increase in biomass. Serine also altered the biomass yield, maximum biomass was noted in presence of 0.1 mM serine.

Unlike the carbohydrates studied, the DW/FW ratio was comparatively consistent in presence of the different amino acids, rather the ratio decreased with increasing concentrations of amino acids (Fig. 5), except serine which showed a steep hike in DW/FW ratio at the highest concentration used in the present study (0.1 mM).

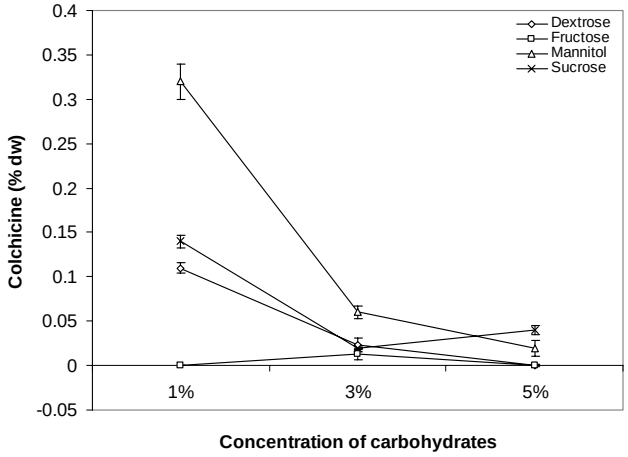

Fig. 3. Effect of different carbohydrate sources on colchicine content of the root organ cultures on liquid NB medium after six weeks in culture in dark at 24 ± 1°C. Colchicine content of the root organ cultures are represented as percentage i.e. g/100 g dry wt. Values represent mean ± standard error of three experiments with 10 replicates.

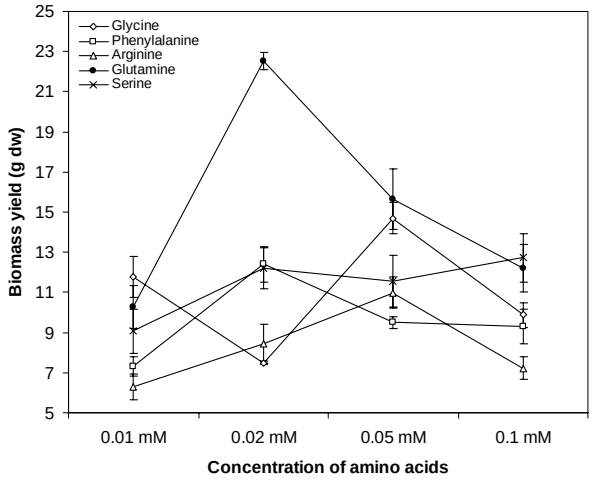

Fig. 4. Effect of different amino acids on growth of the root organ cultures on liquid NB medium after six weeks in culture in dark at 24 ± 1°C. Biomass yield represent dry wt in g/l medium (initial dry wt = 1 g/l). Values represent mean ± standard error of three experiments with 10 replicates.

Analysis of the effects of amino acid on colchicine content revealed that (Fig. 6) among the four concentrations of glycine used, optimum accumulation of colchicine (0.03%) occurred in presence of 0.02 mM glycine. Arginine and glutamine were not favourable for colchicine accumulation. Phenylalanine at 0.01 mM enhanced colchicine accumulation (0.1%) in the root cultures. However, serine, at the lowest concentration used in the present study, was the most favourable for colchicine formation by the root cultures, i.e. in presence of 0.01 mM serine the cultures accumulated 0.16% colchicine (Fig. 6).

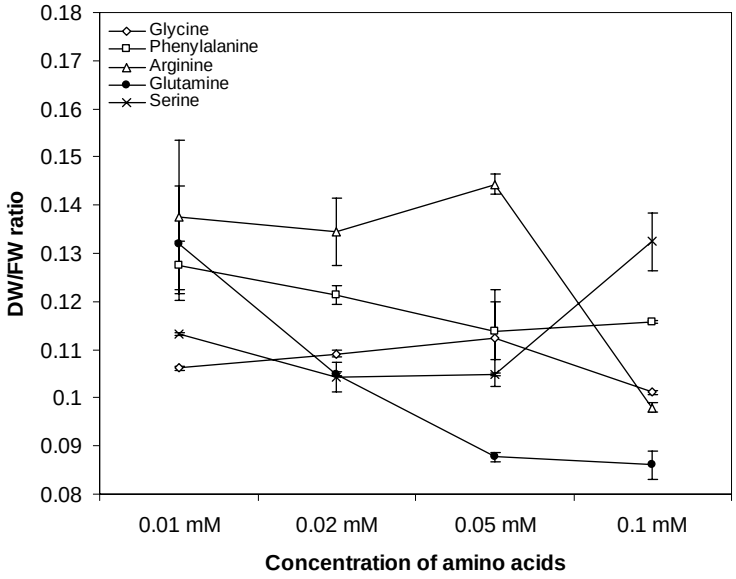

Fig. 5. Effect of different amino acids on dry wt/fr wt ratio (DW/FW) of the root organ cultures on liquid NB medium after six weeks in culture in dark at 24 ± 1°C. Values represent mean ± standard error of three experiments with 10 replicates.

Nutritive factors like the carbohydrates and nitrogen (in the form of amino acids) supply are important parameters influencing growth and alkaloid production (Berlin et al. 1988) in plant tissue culture. There are indications that the nature and level of carbon and nitrogen supplied as substrate may influence secondary metabolism in different cultures (Zenk et al. 1977). In the present study, among the four carbohydrates tested, fructose, sucrose and dextrose gave substantially higher biomass yield than mannitol. Mannitol has been designated as an inactive carbon source. Maximum biomass was noted in presence of fructose in the medium. Only a limited number of studies have been reported where fructose exhibited similar or improved growth rates compared to glucose

or sucrose in cultures (Zwayyed et al. 1991). Increasing concentrations of the carbohydrates also increased the dry matter content (DW/FW ratio). These results are in agreement with those of Sellés et al. (1997). Reduction of osmotic pressure in the medium due to low concentration of carbohydrates might have enhanced water and nutrient uptake from the substrate. As a consequence, the DW/FW ratio was lower in treatments with low concentrations of carbohydrates and increased with their increasing concentrations. Carbon pool plays an important role in the accumulation of secondary metabolites in the plant tissues in culture. Mannitol showing least accumulation of biomass resulted in maximum accumulation of colchicine in the root cultures.

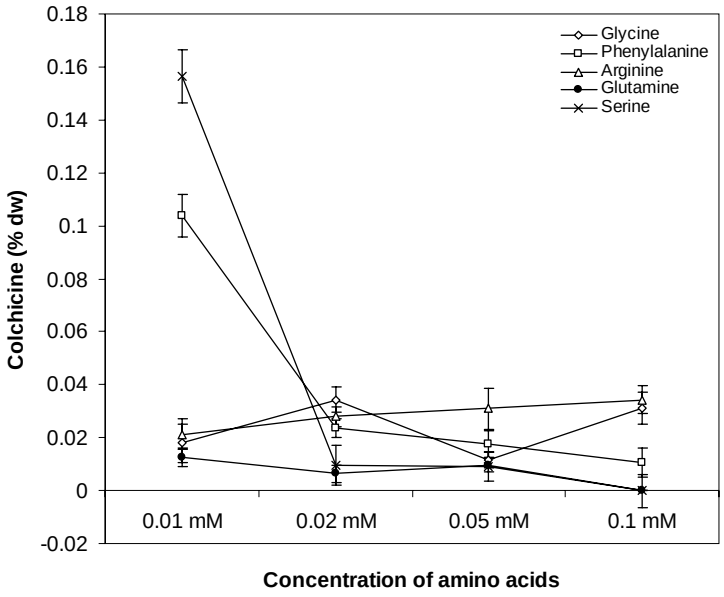

Fig. 6. Effect of different amino acids on colchicine content of the root organ cultures on liquid NB medium after six weeks in culture in dark at 24 ± 1°C. Colchicine content of the root organ cultures are represented as percentage i.e. g/100 g dry wt. Values represent mean ± standard error of three experiments with 10 replicates.

The amino acids are often the key compounds in the biosynthetic pathway of most of the secondary metabolites. In our present study, serine and phenylalanine significantly enhanced colchicine accumulation in root cultures of *G. superba*. Similar trends were observed in *Taxus* cell suspension cultures by Fett-Neto et al. (1994). The promotion of colchicine in root cultures by phenylalanine is related to its involvement as a precursor of ring A and carbons 5, 6 and 7 of Ring B of colchicine (Battersby et al. 1972, Hill and Unrau 1965). Serine is derived from Calvin cycle products. The increased colchicine

accumulation in root cultures in presence of serine is probably related to its catabolism (Fett-Neto et al. 1994). Once metabolized the amino acid could enter the shikimic acid pathway, leading to phenylalanine synthesis. Thus, it is possible that serine is catabolised prior to entering the colchicine biosynthetic pathway; catabolites from this compound could provide the building blocks for phenylalanine, which could be utilized in the formation of Ring A and carbons 5, 6 and 7 of Ring B of colchicine. However, these amino acids did not promote release of colchicine in the medium related to the control (0.02 mM glycine).

The experiments confirmed the inverse relationship observed between growth and colchicine yield in *G. superba* root cultures. Fructose and glutamine were separately effective in promoting growth of the cultures in comparison to cultures grown in presence of sucrose and glycine, but these components did not promote colchicine accumulation by the cultures.

Compared to cell growth kinetics, which is usually an exponential curve, most secondary metabolites are produced during the plateau phase. This lack of production during the early stages can be explained by carbon allocation mainly distributed for primary metabolism (building of cell structures and respiration) when growth is very active. On the other hand, when growth stops, carbon is no longer needed in large quantities for primary metabolism and secondary compounds are more actively synthesized (Bourgaud *et al.* 2001).

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