

Influence of Sucrose on Growth and [6]-Gingerol Production of *In vitro*-Grown Ginger (*Zingiber officinale* Roscoe)

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ABSTRACT

The effect of sucrose on growth and [6]-gingerol production of *in vitro*-grown *Zingiber officinale* was investigated. Individual shoots from multiple shoot clumps were cultured in Murashige and Skoog (MS) basal medium supplemented with varying amounts of sucrose, *i.e.* 30, 60, 90 and 120 g/L. After three months of incubation, the growth and [6]-gingerol production of the cultures were compared. Results showed an increasing number of microrrhizomes formed in response to increasing concentration of sucrose from 30-90 g/L except at a higher concentration of 120 g/L which was found to be already inhibitory. Likewise, the highest [6]-gingerol production was observed in medium supplemented with 90 g/L sucrose suggesting a positive correlation of [6]-gingerol production with the number of microrrhizomes. These results suggest that sucrose concentration can be manipulated to improve [6]-gingerol production in ginger tissue culture.

Keywords: ginger, gingerol, microrrhizome, sucrose, *Zingiber officinale*.

INTRODUCTION

Zingiber officinale Roscoe, commonly known as 'ginger', belongs to family Zingiberaceae and is cultivated in many parts of the world¹. The rhizome of this plant is being utilized as a spice and diet supplement². It is very popular as an herbal medicine addressing a wide range of ailments such as arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis^{3,4}. It has antioxidant, anti-inflammatory and antimicrobial activities², and has also been recognized for its antitumor, anti-apoptotic, cytotoxic, anti-proliferative and anti-platelet activities⁵. These pharmacological properties are attributed to the presence of phenolic compounds.

The major phenolic compounds present in ginger rhizomes are the gingerols which exist as a series of homologues⁶. Among the gingerols, [6]-gingerol is the most pungent compound that is mainly present in fresh ginger rhizomes^{4,7,8}. Several studies demonstrated that formation of ginger rhizomes can be induced *in vitro*^{1,9-11}. These rhizomes termed 'microrrhizomes' were found to produce secondary metabolites with antioxidant activity equaling or even surpassing preparations obtained from commercial dried powdered rhizome of field-grown plants¹². *In vitro*-produced microrrhizomes could be very useful for the production of [6]-gingerol compared to field-produced rhizomes because the latter would require a large land area, is affected by seasonal and geographical variation, and is exposed to various pests and diseases, which adversely affect their medicinal qualities. But for microrrhizomes to become a feasible source of [6]-gingerol, it is necessary to

identify the factors that can improve [6]-gingerol accumulation under controlled condition.

Microrrhizomes are storage organs. Formation of various storage organs like bulbs, corms and tubers was found to be promoted by sucrose¹³⁻¹⁷. In *Curcuma longa*, increasing sucrose concentration from 30 g/L to 60 g/L resulted in increased microrrhizome formation¹⁸. This was also true with *Z. officinale* as has been observed previously¹. The possibility of enhancing [6]-gingerol production by increasing the number of microrrhizomes formed could be an effective strategy to come up with a feasible process of *in vitro* [6]-gingerol production. While sucrose has been found to increase microrrhizome formation in ginger, its effect on [6]-gingerol production has not been documented yet. This paper is the first report correlating [6]-gingerol production to microrrhizome formation as influenced by sucrose.

MATERIALS AND METHODS

Establishment of ginger shoot cultures

Mature rhizomes of *Zingiber officinale* cv. 'Imugan' were collected around the vicinity of BIOTECH, UPLB, College, Laguna, Philippines. They were planted in sterilized soil to induce sprouting. The sprouting buds were taken, washed thoroughly with detergent, and surface-sterilized using a 50% solution of a commercial bleach for 25 min followed by several rinses in sterile distilled water. The surface-sterilized buds were dissected to obtain the shoot apical meristems which were planted in solidified MS medium¹⁹ supplemented with 1 mg/L NAA + 1 mg/L BAP + 30 g/L sucrose. The resulting multiple shoot

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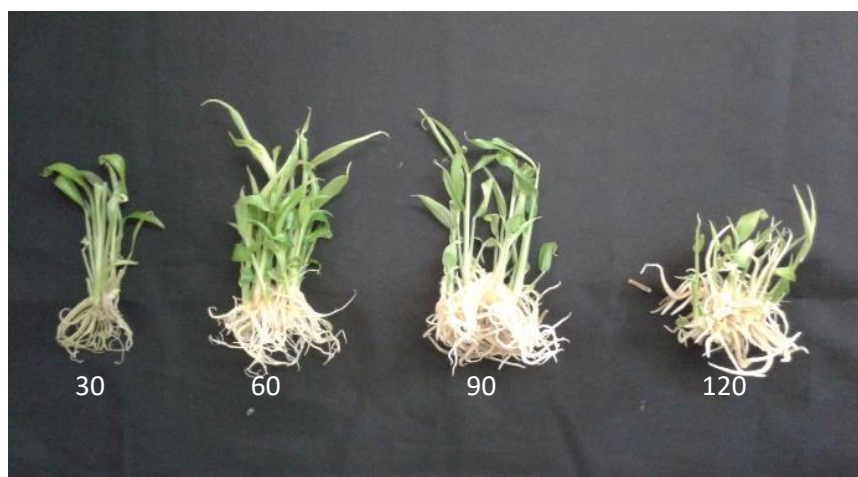


Figure 1: Ginger plantlets cultured in a medium containing sucrose at varying levels from 30 g/L to 120 g/L. Note the profuse rooting with the corresponding inhibition of shoot growth in response to increasing sucrose concentration.

cultures were maintained at $25\pm 2^{\circ}\text{C}$ under a photoperiod of 16 h light and 8 h darkness.

Culture conditions

Individual shoots from *in vitro*-grown multiple shoot cultures were taken and planted in solidified MS medium containing 1 mg/L NAA + 1 mg/L BAP. Sucrose was added to the culture medium at various levels: 30, 60, 90 and 120 g/L. The cultures were incubated at $25\pm 2^{\circ}\text{C}$ under a photoperiod of 16 h light and 8 h darkness.

Growth comparison

The cultures were harvested after three months of incubation. Growth of the cultures was compared by determining the following parameters: plant biomass, number of shoots, length of shoots, and number of microrrhizomes. Plant biomass was taken as the fresh weight of cultured plantlets immediately after harvest.

Preparation of extracts

Cultured plantlets were individually macerated, soaked in methanol overnight and filtered. The filtrate was

Statistical analysis

The experiment was laid out using a Completely Randomized Design. There were four treatments, with each treatment replicated 10 times. Data were analyzed for significant differences using a one-way ANOVA. Comparison among treatment means was done by LSD at 5% level of significance.

RESULTS AND DISCUSSION

To determine the effect of sucrose on growth of ginger cultured *in vitro*, an experiment was done wherein ginger was cultured in a medium containing varying levels of sucrose. Fig. 1 shows the plantlets cultured for three months in a medium containing sucrose at varying levels from 30 g/L to 120 g/L. As shown in Fig. 1, increasing sucrose concentration resulted in profuse rooting and root thickening with the corresponding inhibition of shoot growth. There were also numerous shoot-like structures present in ginger cultured in a medium with 90 g/L and 120 g/L sucrose but they did not develop the green leafy structures peculiar to shoots and therefore were not considered as true shoots.

concentrated under vacuum using a rotary evaporator set at $\leq 40^{\circ}\text{C}$. The residue was resuspended in water and partitioned with ethyl acetate. The ethyl acetate layer was collected and dried over anhydrous sodium sulfate overnight. The ethyl acetate layer was then evaporated to dryness and the residue resuspended in 1 mL methanol. This was stored at 4°C prior to [6]-gingerol analysis.

[6]-Gingerol analysis

Quantitative determination of [6]-gingerol was performed by reversed-phase HPLC²⁰. Chromatographic separation was achieved using an Inertsil ODS-3 column (250 x 4.6 mm) maintained at 25°C . The mobile phase consisted of 60:40 (v/v) acetonitrile: water ran isocratically at a flow rate of 1 mL/min for 5 minutes. The detection wavelength was set at 282 nm^{21} . [6]-Gingerol content per culture bottle was determined after comparison with a calibration curve using authentic [6]-gingerol (Sigma-Aldrich, St. Louis, Missouri) as standard.

Table 1 shows the different growth parameters which were also taken to compare the influence of sucrose on tissue cultured ginger. As shown in Table 1, the plant biomass of ginger cultured in a medium with 30 g/L sucrose was only 6.05 g. This increased to 12.42 g and 17.51 g when sucrose concentration was increased to 60 g/L and 90 g/L, respectively. However, further increasing sucrose concentration to 120 g/L resulted in a lower plant biomass of 8.52 g.

There was no microrrhizome formed when ginger was cultured in a medium containing 30 g/L sucrose. Increasing the concentration of sucrose to 60 g/L resulted in the induction of only two microrrhizomes. But when sucrose concentration was increased to 90 g/L, there was a dramatic increase in the number of induced microrrhizomes (16.60). Further increasing sucrose concentration to 120 g/L resulted in a lower number of induced microrrhizome (10.20). A similar observation was reported on *Curcuma longa* wherein induction of microrrhizome was inhibited by high sucrose concentration²².

The highest number of shoots formed was observed when ginger was cultured in a medium containing 60 g/L (8.1 shoots), and there were fewer shoots formed when sucrose

Table 1: Effect of varying amounts of sucrose on some growth parameters of ginger tissue cultures.

Sucrose concentration (g/L)	Plant biomass (g)	Number of microrhizomes	Number of shoots	Shoot length (cm)
30	6.1 ± 2.9 ^c	0 ± 0 ^d	4.4 ± 2.3 ^b	11.1 ± 0.8 ^b
60	12.4 ± 2.5 ^b	2.0 ± 1.2 ^c	8.1 ± 2.6 ^a	14.7 ± 1.4 ^a
90	17.5 ± 3.3 ^a	16.6 ± 3.6 ^a	5.1 ± 2.1 ^b	11.8 ± 0.8 ^b
120	8.5 ± 4.7 ^{bc}	10.2 ± 1.9 ^b	5.3 ± 1.3 ^b	6.7 ± 2.1 ^c

^aTreatment means with different letters within a column are significantly different by LSD at 5% level of significance.

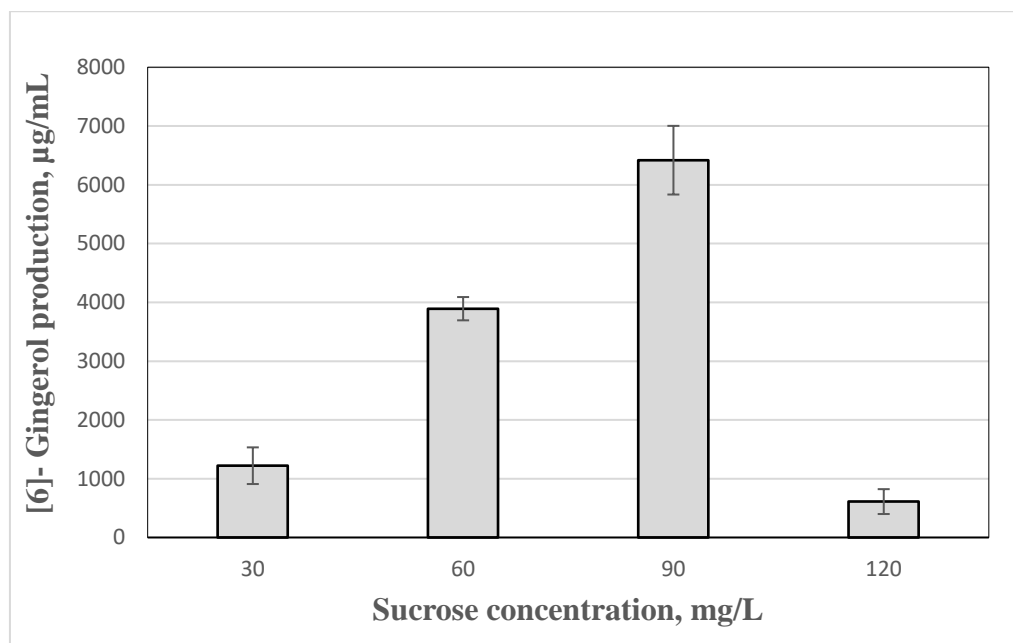


Figure 2: [6]-Gingerol production by ginger grown in MS medium with varying levels of sucrose.

concentration was increased to 90 g/L and 120 g/L, with 5.1 and 5.3 shoots, respectively. Shoot length was also highest when ginger was cultured in a medium containing 60 g/L (14.7 cm). It was inhibited when sucrose concentration was increased to 90 g/L and 120 g/L, with shoot lengths of 11.8 cm and 6.7 cm, respectively.

The data on different growth parameters demonstrated the positive correlation between sucrose concentration and growth of cultured ginger as can be seen in the increasing plant biomass with increasing sucrose concentration. Initially, an increase of sucrose concentration to 60 g/L resulted in increasing the number and length of multiple shoots formed. But at 90 g/L, sucrose was already inhibitory to shoot formation resulting in fewer and shorter shoots. However, even with fewer and shorter shoots, the highest plant biomass was still obtained in ginger cultured in medium with 90 g/L sucrose. This increase in plant biomass can be attributed to the increase in the number of microrhizomes.

Analysis of [6]-gingerol production by ginger grown in MS medium with varying levels of sucrose revealed that its production increases as the sucrose concentration increases (Fig. 2). At 30 g/L sucrose, [6]-gingerol production was 1221.15 µg/mL. Increasing sucrose concentration to 60 g/L and 90 g/L resulted in increased [6]-gingerol production of 3892.02 µg/mL and 6418.61 µg/mL, respectively. However, further increasing sucrose

concentration to 120 g/L resulted in inhibition of [6]-gingerol production (610.05 µg/mL). These results suggest that the optimum sucrose concentration which yielded the highest [6]-gingerol production is 90 g/L. This is also the sucrose concentration which yielded the highest plant biomass and the most number of microrhizomes.

CONCLUSION

Sucrose is a carbohydrate source commonly used in plant tissue culture medium. As a carbohydrate source, it normally affects the growth and metabolism of the culture. In ginger tissue culture, increasing sucrose concentration increases microrhizome formation. This increase in microrhizome formation also increases [6]-gingerol production. These results suggest that sucrose concentration can be manipulated to improve [6]-gingerol production in ginger tissue culture.

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