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Enhanced production of diosgenin through elicitation in micro-tubers of *Chlorophytum borivilianum* Sant et Fernand

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ABSTRACT

Diosgenin, one of the steroidal sapogenin, is a chief bioactive compound, commercially known for its pharmaceutical application to treat sexual dysfunction. The biosynthesis of plant secondary metabolites *in vitro* cultures is usually manipulated by different elicitors that lead to higher production than non-elicited cultures. In the present investigation, *in vitro* tubers of *Chlorophytum borivilianum* were produced on semisolid and stationary liquid Murashige and Skoog (MS) medium supplemented with various concentrations of sucrose. To elicit diosgenin contents, these micro-tubers were exposed *in vitro* to different concentrations of jasmonic acid (JA) and salicylic acid (SA) for 1-month. An optimum number of micro-tuber was obtained on MS semisolid medium containing 60 g/L of sucrose. However, the stationary liquid medium consisting of same sucrose level was found more suitable for increased production of micro-tubers than semisolid medium. Exposure to the lower doses of JA and SA individually induced the improved production of diosgenin in the micro-tubers of *C. borivilianum*. A 2.1-fold higher production of diosgenin was obtained after the 1-month exposure of micro-tubers to 25 μM of JA; while after the 25 μM of SA elicitation, 1.5-fold higher diosgenin in micro-tubers was obtained in comparison to diosgenin present in tubers of the mother plant. The results suggest that JA and SA have the considerable ability to stimulate the production of valuable diosgenin in the micro-tubers of *C. borivilianum*.

1. Introduction

The *in vitro* productions of secondary metabolites *via* plant tissue cultures are limited due to high water content, lower yield and lower accumulation of bioactive in cultured cells (Yukimune et al., 1996). To deal with such problems often the application of elicitors such as JA and SA has been attempted in a number of studies (Raomai et al., 2015; Verma et al., 2014; Zaheer et al., 2016). Moreover, the biosynthetic pathways of secondary metabolites are complex and require tissue specific architecture (Awad et al., 2014). Consequently, an organized tissue shows better stability in culture and produce tissue specific bioactive, in comparison to unorganized callus or suspension culture of the same species (Awad et al., 2014; Banerjee et al., 2012).

Chlorophytum borivilianum Sant et Fernand, an important medicinal herb native to India is a highly valuable herb having aphrodisiac potential and very useful to treat sexual disorders. The tubers of *C. borivilianum* are used in the Ayurvedic medicinal system in curing many diseases such as male impotency, arthritis and diabetes (Acharya et al., 2009; Chauhan et al., 2016a; Kaushik, 2005). Because of its important bioactive constituent, it is exploited rigorously as a very good alternative to 'Sildenafil'; a well-known drug to overcome sexual dysfunction. Moreover, diosgenin also exhibits anticancer property (Yan et al., 2015). Looking to its massive commercial exploitation and declining population, recently International Union for Conservation of Nature and Natural Resources (I.U.C.N., 2015) referred this species as "critically endangered".

The tuberous roots possess saponin and sapogenin (diosgenin) which has pharmaceutical application (Acharya et al., 2008; McAnuff et al., 2002) and was documented to constitute 2–17% of saponin and 0.18% of sapogenin of its dry weight (DW), depending upon the genotypes (Bordia et al., 1995; Kaushik, 2005). In addition, diosgenin is a precursor for the production of steroidal drugs and hormones such as testosterone, progesterone and glucocorticoids (Jasim et al., 2017). Thus, its enhanced production is of significant applications. Till now, there is a single available report about the production of diosgenin in the micro-tubers of *C. borivilianum* (Ashraf et al., 2013a). Hence, the present study was aimed to establish an effective micro-tuber production system for *C. borivilianum* to investigate the effects of JA and SA on the production of diosgenin in the *in vitro* developed tubers.

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2. Materials and methods

2.1. Plant material

For the study, the healthy non-infected tubers of *Chlorophytum borivilianum* were procured from the village Aakot (Akola, Maharashtra, India) with the geographical specifications 21.0973° N, 77.0536° E and 307 m above sea level. At the time of procuring, tubers were thoroughly screened following the keys developed by Shah (1978) in "Flora of Gujarat State, Part-II" for identification of the particular species.

2.2. Micro-shoot induction and culture conditions

Thin slices of crowns each having a single juvenile shoot bud, (≤1 cm in length) were considered as explants for micro-shoot induction on 'standard medium' as described in Chauhan et al. (2016b). The basal MS medium (Murashige and Skoog, 1962) supplemented with sucrose (30 g/L), and bacteriological agar (6 g/L) was used as culture media all through the study. The modifications made in MS medium for the different experiment was discussed in specific experimentation section. The pH of the culture media was adjusted to 5.6–5.8 using 1 N NaOH and/or 1 N HCl, prior to its autoclave at 103 kPa and 121 °C for 15 min. Cultures were incubated at 25 ± 2 °C with a 16/8 h photoperiod under 50 μmol m^{-2} s^{-1} light intensity provided by cool-white fluorescent tubes in culture room.

2.3. Micro-tuberization using semisolid medium

Meristematic clumps bearing micro-shoots of 1–1.5 cm in lengths and an initial mean weight of 0.6 g were carefully excised from the *in vitro* cultures grown on semisolid medium and were used as explants for micro-tuberization. Excised micro-shoots were placed onto 250 ml Erlenmeyer flasks containing 50 ml of semisolid medium supplemented with 30, 60 or 90 g/L of sucrose. Now, the cultures were incubated at 25 ± 2 °C and under 16/8 h photoperiod provided by cool-white fluorescent tubes. The micro-tuber production rate was evaluated by counting the number of micro-tubers produced on each explant and measuring the lengths of *in vitro* produced tubers along with growth index of the whole plant, after eight weeks of micro-shoots inoculation. Growth index was calculated following the below formula of Silja and Satheeshkumar (2015);

$$\text{Growth index} = \frac{\text{Final biomass weight} - \text{Initial biomass weight}}{\text{Initial biomass weight}}$$

2.4. Micro-tuberization using stationary liquid medium

For micro-tuberization over stationary liquid medium, meristematic clumps bearing micro-shoots of 1.5–2.0 cm lengths and with an initial mean weight of 0.9 g, were excised and placed over nylon mesh of Growtek container (Tarson Ltd., Kolkata, India), of 1000 ml capacity, containing 100 ml stationary liquid medium supplemented with different concentrations of sucrose *viz.*; 30, 60 or 90 g/L. The cultures were incubated at above-described culture conditions, and for eight weeks of time without any subculture. Micro-tuber formation rate was recorded after eight weeks by evaluating the number of micro-tubers produced per explant, measuring lengths of the micro-tubers along with the growth index of the whole plant.

2.5. Preparation of elicitors and elicitor treatments

SA and JA were evaluated for their potency to induce production of diosgenin in micro-tubers of *C. borivilianum*. Stock solutions of both the elicitors were prepared by dissolving 250 mg of SA in 0.5 ml of absolute ethanol and 250 mg of JA in 0.5 ml of dimethyl sulfoxide, separately

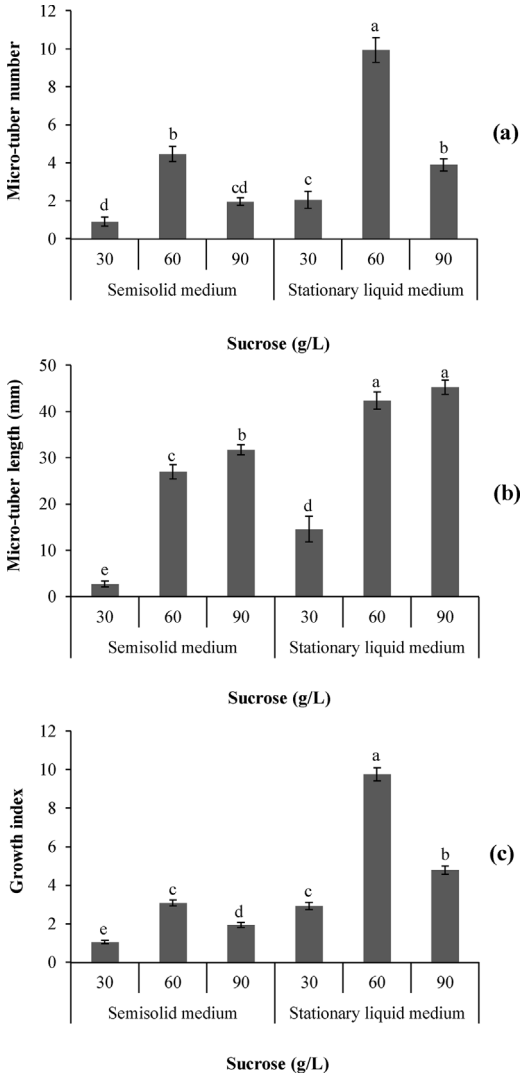

Fig. 1. *In vitro* tuberization potential of *Chlorophytum borivilianum* in MS semisolid and stationary liquid medium with different sucrose level after 8 weeks of culture (a) micro-tuber number, (b) micro-tuber length and (c) growth index. Mean bars that are followed by the same letters do not differ ($p = 0.05$).

ANOVA	*df*	*F*	*p*
Micro-tuber number	5	66.649	< 0.0001
Micro-tuber length	5	108.887	< 0.0001
Growth index	5	228.151	< 0.0001

(Zaheer and Giri, 2015). These stock solutions were then diluted up to 25 mg/ml, by adding sterile double-distilled water and were filtered using sterilized 0.22 μ bacterial filtration unit. Now, two different doses, *i.e.* 25 and 50 μM of both SA and JA were aseptically added into the micro-tuberization medium, *i.e.* the best-performed medium in terms of micro-tuberization of *C. borivilianum*. The one-month-old micro-tubers bearing cultures of initial weight 2.1 g and lengths of 2.5–3.0 cm were used as explants and were placed over nylon mesh of Growtek containers containing micro-tuberization medium consisting of above-mentioned concentrations of SA and JA, individually. Now, these cultures were incubated under the normal culture conditions. The micro-tubers were harvested after 4 weeks of elicitor treatments. Thereafter, the harvested micro-tubers were dried at room temperature for 2 weeks of time for further extraction of diosgenin.