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SHORT REPORTS

Elicitation and enhancement of bacoside production using suspension cultures of *Bacopa monnieri* (L.) Wettst

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Abstract
The effect of different elicitors [jasmonic acid, salicylic acid] and precursors [calcium pantothenate, cholesterol, sodium nitroprusside] on the stimulation of biomass and secondary metabolite production in suspension cultures of *Bacopa monnieri* was studied. Induction of primary callus cultures was successfully carried out on the Gamborg's B_5 (B_5) medium fortified with 2, 4-D (1.0 mg l^{-1}) using *Bacopa monnieri* leaves as explants. The friable fine suspension cell culture was raised on parent B_5 media without agar. The elicitation using different elicitors and precursors at varying concentrations was carried out over a period of 3, 6, 9, 15 days. Elicitor treated cultures showed marked increase in biomass and bacoside production around 6th–9th day (0.98 GI DW). In the present study, salicylic acid at 1.0 mg l^{-1} induced a maximum elicitation in bacoside content (6.58 mg g^{-1} DW). The present study provides favorable evidence on the potential of bacoside production using suspension cultures of *B. monnieri*. The study results also indicate the beneficial effects of elicitation on metabolite production in in vitro suspension cultures of *B. monnieri* plant known for its cognitive improving properties.

Keywords Bacosides · Elicitation · Cell suspension cultures · HPLC

Introduction

Plant cell culture (PCC) is a well-reported platform for secondary metabolite production in pharmaceutical industries. This approach provides several advantages in comparison to other potential strategies, especially for the production of NPs with complex structures (Ochoa-Villarreal et al. 2016). Cell suspension cultures provide a continuous, reliable source of natural products and thus, could be used for large-scale metabolite production. Plant cells in suspension cultures provide a unique combination of physical and chemical environments that must be accommodated in large-scale bioreactor process (Hussain et al. 2012). Elicitation is currently the most promising technique and used widely for increasing biomass and secondary metabolite production in plant cell cultures. These molecules induce a signal across plant cells which, in turn, stimulate secondary metabolic pathways and downstream transcription factors in response to the external stimuli thus resulting in production of secondary metabolites (Ahuja et al. 2016). *Bacopa monnieri* is a medicinal plant species highly acclaimed in Ayurvedic literatures for its cognition improving abilities owing to triterpenoid saponin bacosides (Koul et al. 2014; Kharde et al. 2018). Earlier reported findings attempted elicitation process using whole plant shoot cultures to increase the bacoside production (Sharma et al. 2015, 2019); thus, there is a need to find an alternative approach keeping plant conservation in mind. Plant cell suspension cultures have shown tremendous benefits over shoot cultures towards large scale plant metabolite production and conservation (Ochoa-Villarreal et al. 2016). However, there are inadequate uses of studies on the use of cell suspension culture systems in Bacopa. Salicylic acid has been widely reported as a successful effective signal molecule amongst all other types of abiotic elicitors (Ramirez-Estrada et al. 2016). In the present study an attempt was conducted to regenerate cultures on Gamborg's B_5 media supplemented with 2, 4-D (1.0 mg l^{-1}) and also to evaluate the effect of different precursors and elicitors on bacoside production.

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Methodology

B. monnieri plantlets procured from CSIR-IIIM Jammu (Plant Accession no: BM001) were vegetatively habituated at Herbal garden SMVDU, Katra. Healthy leaf explants were used for friable callus induction on Gamborg's B_5 plant medium with 2, 4-D (1.0 mg l^{-1}). Friable cell suspension cultures for bacoside production were raised on parent medium without agar (Fig. 1). Different signal molecules at concentration of 1 mg l^{-1}, such as elicitors: jasmonic acid, salicylic acid, and precursors: calcium pantothenate, cholesterol, sodium nitroprusside were used to evaluate their influence on biomass production and bacoside content at regular intervals of 3, 6, 9, 15 days. Ethanol and sterile distilled water at similar concentrations as test solutions were used as controls for the experiments. The elicitated cultures were placed on an incubated shaker at 80 rpm speed fitted with a photoperiodic timer (16/8 h). These cultures were then harvested and extracted for further quantitative HPLC analysis as already standardized process by Sharma et al. (2019).

Statistical calculation

The data collected from different treatments for 10 replicates were analyzed using statistical tool ANOVA (analysis

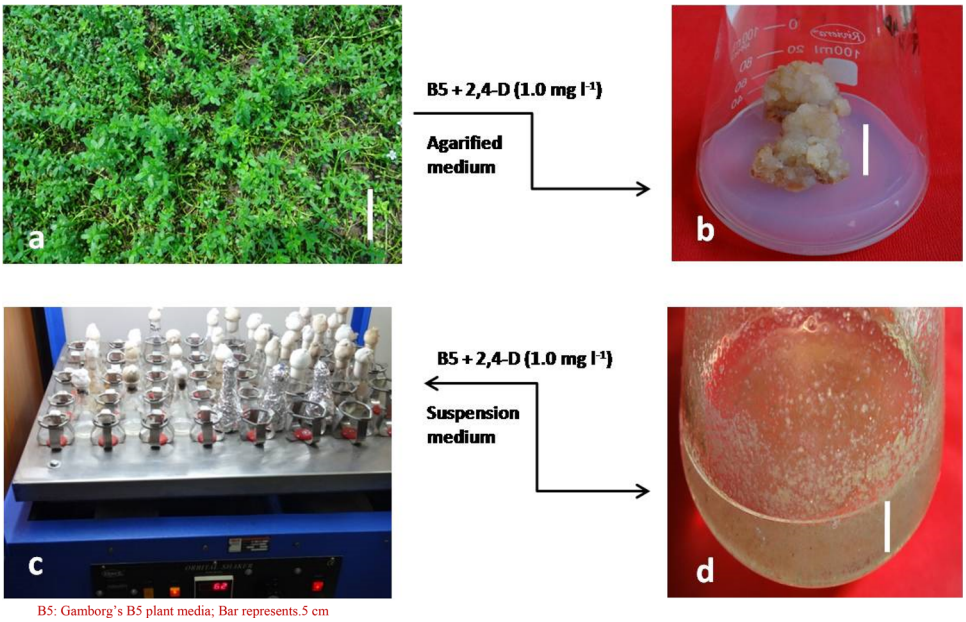

Fig. 1 Initiation and scaling up process of callus suspension cultures of *B. monnieri* plant on Gamborg's B5 plant medium. **a** Field grown plants of *B. monnieri*; **b** Friable callus induced on agarified Gamborg's media supplemented with 2,4- D (1.0 mg l-1); **c** Callus suspension cultures incubated under shaking light conditions on incubator shaker at 80 rpm; **d** Fine suspension bacopa culture after 6th day of growth under shaking light conditions

Table 1 Effect on total bacoside content (mg g^{-1}) at different day intervals in suspension cultures under shaking light conditions

Sample type	Additive (1 mg l^{-1})	Total bacoside content (mg g^{-1})*			
		3rd Day ± SE	6th Day ± SE	9th Day ± SE	15th Day ± SE
Control	Mother culture (without additive)	3.11 ± 0.17	3.19 ± 0.22	3.23 ± 0.26	0.7 ± 0.07
Control (Solvents used for preparation of additive)	Water	0.33 ± 0.06	0.12 ± 0.05	0.006 ± 0.004	0.003 ± 0.002
	Ethanol	0.13 ± 0.03	0.23 ± 0.08	0.03 ± 0.01	0.005 ± 0.001
Abiotic elicitors	Jasmonic acid	4.55 ± 0.74	5.23 ± 0.08	5.3 ± 0.27	2.4 ± 0.24
	Salicylic acid	4.9 ± 0.25	6.36 ± 0.48	**6.58 ± 0.39**	2.85 ± 0.09
Precursors	Cholesterol	3.7 ± 0.05	3.82 ± 0.44	4.29 ± 0.48	2.38 ± 0.18
	Sodium nitroprusside	4.08 ± 0.52	4.12 ± 0.13	4.25 ± 0.40	1.2 ± 0.15
	Calcium pantothenate	0.43 ± 0.08	0.66 ± 0.05	0.9 ± 0.04	1.51 ± 0.15

Bold value represents statistically significant maximum bacoside content (mg gm^{-1}) in the series of experiments conducted for the study

[Initial start culture (6 day old): 3.33 ± 0.81]

*Values represent mean ± SE of 10 replicates in each culture condition and SE is the Standard Error calculated in SPSS 17 where $P \leq 0.005$

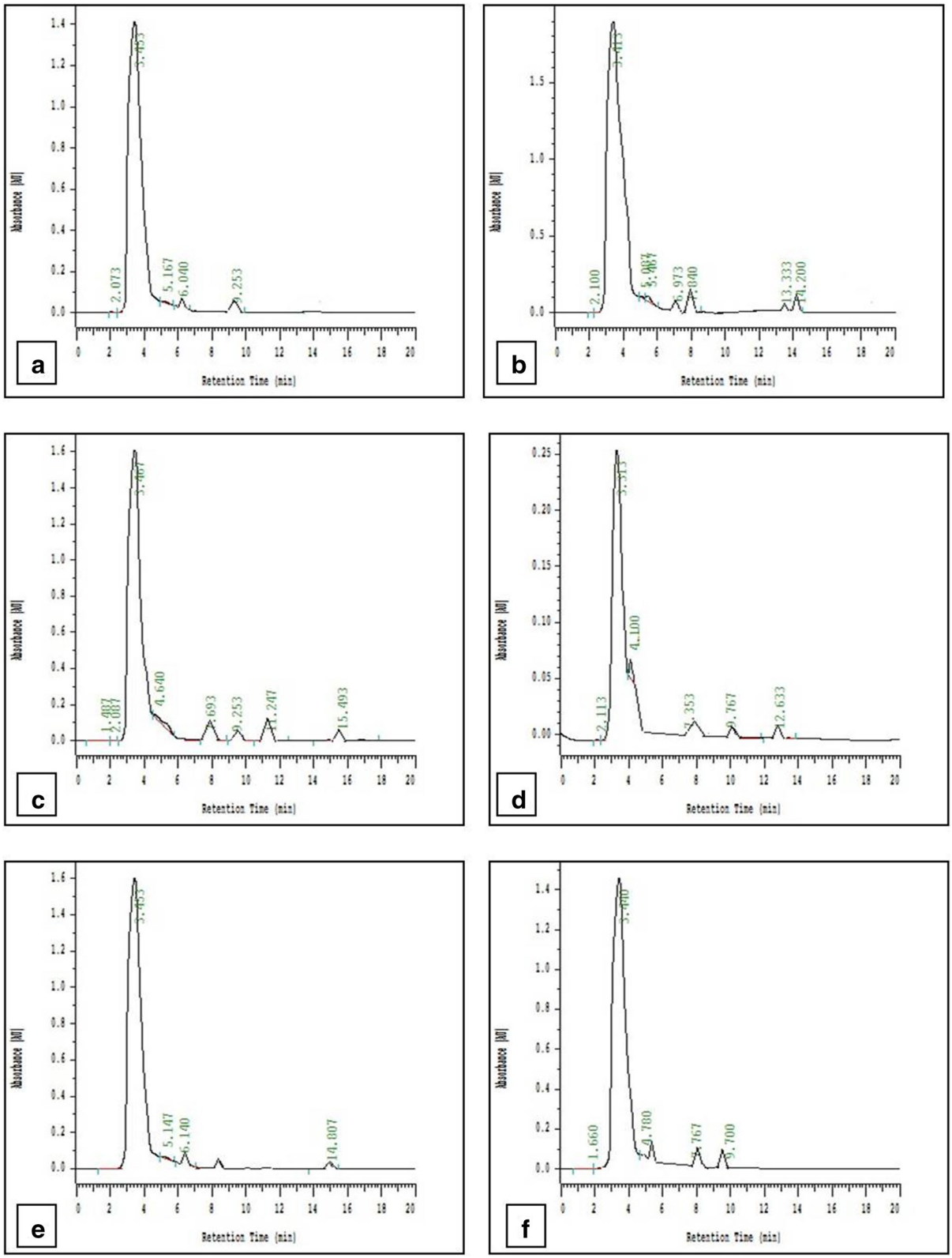

Fig. 2 Effect of different elicitors and precursors on total bacoside content in suspension cultures supplemented with respective additives at 9th day of growth during kinetic study. HPLC chromatograms of **a** Mother culture, **b** Cholesterol, **c** Salicylic acid, **d** Calcium pantothenate, **e** Sodium nitroprusside, and **f** Jasmonic acid

of variance) using SPSS version 17 (SPSS Inc., Chicago, USA). The intention of using this tool was to assess the differences in means (for 10 replicates) and major differences between means were analyzed at $P \leq 0.05$.

Results and discussion

Elicitor treated cultures show a marked increase in biomass in accordance with the exponential curve exhibiting maximum biomass i.e., 3.2 mg gm^{-1} DW around 6th to 9th day period which decreases generally around 15th day (0.09 mg gm^{-1}). This fact confirms that bacoside production could be enhanced around this period. Earlier reports also confirm that an exposure of 6–9 days incubation period increased the bacoside content in shoot cultures (Sharma et al. 2015). Maximum biomass was achieved using elicitor calcium pantothenate followed by jasmonic acid, cholesterol, salicylic acid, and sodium nitroprusside. It has been reported that calcium and pentothenic acid gets elevated in cellular levels in response to light, salinity, drought, and cold stresses during in vitro growth conditions thereby influencing in vitro morphogenesis (Isah 2019). The bacoside content analyzed through HPLC analysis were found higher in cultures elicitated with salicylic acid (6.58 ± 0.39) followed subsequently by jasmonic acid (5.3 ± 0.27), sodium nitroprusside (4.25 ± 0.40), cholesterol (4.29 ± 0.48), and least in calcium pantothenate (0.9 ± 0.04), respectively (Table 1; Fig. 2). The results are in correlation with previous findings which state that salicylic acid increases the production of sesquiterpenes and triterpenoids in suspension cultures of other important plant species (Lu et al. 2016). In addition to this, previous study reports demonstrated elicitation and advanced in vitro techniques for enhancing bacoside production using shoot cultures of *Bacopa monnieri* (Sharma et al. 2015, 2019). The present study demonstrated that a protocol for enhancing bacoside production in suspension cultures of this medicinally important herb is a value addition to the previously reported data. Using suspension cultures over shoot cultures may also provide an alternative towards plant conservation. Thus it was possible to increase the bacoside content through elicitation experiment and the methodology could be used for large scale production of bacosides.

The present study is the first of its kind, enabling enhanced bacoside production in cell suspension cultures using suitable elicitors and precursors. The protocol optimized for increment of bacoside content in suspension cultures could be used successfully for large-scale prospective and commercial production of valuable metabolites of *Bacopa monnieri*.

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Author contributions Both authors contributed equally for the research work.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

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