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ELICITATION OF FORSKOLIN IN SUSPENSION CULTURES OF *COLEUS FORSKOHLII* (WILLD.) BRIQ. USING BACTERIAL ELICITORS

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MS liquid medium supplemented with 3 mg/l NAA and 1 mg/l BAP with pH 5.7 was used for suspension cultures. Suspension cultures subjected to biotic elicitors *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Proteus aureus* induced increase in forskolin content. Among all the elicitors tried *Bacillus subtilis* proved to be the best elicitor at 20% concentration which was 9 times higher than the control.

Key words: *Coleus forskohlii*, suspension cultures, callus cultures, secondary metabolites.

Elicitor-induced accumulation of secondary metabolites received wide acceptance because of its ability to improve the productivity of the plant cell systems significantly (Eliert 1987). Kang *et al*. (2009) reported higher accumulation of bilobalide and ginkgolides in *Ginkgo biloba* cell cultures. The effects of bacterial elicitors on the growth and forskolin content were studied in the suspension cultures of *Coleus forskohlii*. *In vitro* production of bioactive forskolin with increased focus towards yield enhancement can provide new means of obtaining large quantity of forskolin in shorter period of time. In the present study an attempt to enhance the yield of forskolin in *in vitro* using bacterial elicitors has been carried out.

MATERIALS AND METHODS

Different explants from *in vitro* grown plantlets such as root, leaf, petiole, internode and node were excised, surface sterilized and inoculated on MS medium fortified with 2 mgl^{-1} 2,4-D. pH of the media was adjusted to 5.7-5.8 prior to the addition of agar. The media was autoclaved at 15 psi, 121^{0}C for 20 mins. The cultures were incubated at 25$\pm$2^{0}C temperature under dark for callus proliferation.

Initiation and Maintenance of Suspension cultures: MS liquid medium supplemented with 3 mgl^{-1} NAA and 1 mgl^{-1} BAP with pH 5.7 was used for suspension cultures. About 50 ml of liquid medium was dispensed into 250 ml Erlenmeyer flasks and friable callus weighing approximately 5 gms was transferred to the flasks under aseptic conditions. Cultures were incubated on a rotary shaker at 120 rpm in darkness and temperature was maintained at 25$\pm$2^{0}C. After 15 days of incubation cell suspensions were filtered through appropriate sterile sieves to obtain single cells and a few celled aggregates which were used as inoculums for subculture. Fine cell suspensions were obtained by repeated subculturing of callus and removing small clumps at every stage. The cell suspension cultures were maintained on the same medium by subculturing at 2-week interval.*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Proteus aureus* cultures were procured from Padmashree Institute of Management Studies, Bangalore.

Elicitor Preparation: Bacteria were grown in 250 ml Erlenmeyer flasks containing 50 ml nutrient broth at 30^{0}C. When the cultures reached stationary phase, the medium with the cells was autoclaved for 20 minutes and the cells were separated from the medium by centrifugation. The filtrate was used as elicitor and referred as culture filtrate (CF).The cells were washed four times with distilled water and were resuspended in an equal amount of distilled water to that of the filtrate and

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homogenized. The homogenate was autoclaved again and used as elicitor and referred as cell extract (CE).

RESULTS AND DISCUSSION

Effect of biotic (bacterial) elicitors

Influence of *Staphylococcus aureus*

Many a times elicitors though used to yield enhancement, there are reports which expressed negative impact on secondary metabolite synthesis. For instance decrease in thiophene production was reported after using 20% *Staphylococcus aureus* extract in *Tagetes patula* by Buitelaar *et al.* (1992). In the present study effect of different concentrations of cell and filtrates of *Staphylococcus aureus* were tested on elicitation of forskolin production in suspension cultures of *Coleus forskohlii* (Table 1). As the concentration of both cell extract and filtrate of *Staphylococcus aureus* increased there was a steady increase in forskolin levels from 1.4 to 3.5 fold in cell suspension cultures after 24 hours. However, there were negative effects of all the treatments after 48 hours exposure. The data subjected to statistical F-test indicated a significant difference at 5% level in response to various concentrations of cell extract and filtrate in terms of forskolin content at 24 hours (F=1400*) and 48 hours (F=3332*). Suvarnalatha *et al.* (1994) conducted a similar study with *Staphylococcus aureus*. This group attempted to increase anthocyanin production in cell cultures of carrot and they successfully enhanced the yield of anthocyanin 72% which is higher than the control. But for anthocyanin production in cell cultures of carrot (*Daucus carota*) the optimal elicitor concentration for maximal elicitation was at 5% (v/v) (Suvarnalatha *et al*. 1994) and the elicitation levels with *Staphylococcus aureus* was 72% higher than the control.

Influence of *Bacillus subtilis*

In suspension cultures of *Plumbago rosea*, *Bacillus subtilis* exhibited maximum effect at 1.0% (v/v) and the plumbagin yield was 6.34 mg/g DCW (Komaraiah *et al.* 2002). The optimum concentration of *Bacillus subtilis* was 2% and the thiophene yield was 50% high compared to the control in *Tagetes patula* hairy root cultures (Buitelaar *et al*. 1992). In the present study different doses of *Bacillus subtilis* were tested, (Table 2) and the highest forskolin yield above 4-fold was achieved when cultures were exposed to 20% concentration of elicitor for 24 hours. There was a 3.3-fold enhancement of forskolin when 15% CF was used. *Bacillus subtilis* at high concentration increased forskolin content, however, there was a decrease in biomass yield. The data subjected to statistical F-test indicates a significant difference at 5% level in terms of forskolin content at 24 hours

Table 1. Effect of *Staphylococcus aureus* on Elicitation of Forskolin in Suspension Cultures of *Coleus forskohlii*

Elicitor	Concentration (%)	Forskolin content (mg/kg DCW)	
		24 hours	48 hours
		Mean – SD	Mean – SD
Cell Extract	5 %	250.0 – 1.0	70.0 – 0.6
	10 %	370.3 – 1.0	44.8 – 0.6
	15 %	410.0 – 1.0	19.9 – 0.7
	20 %	564.8 – 0.6	12.0 – 0.3
Cell Filtrate	5 %	269.8 – 0.4	22.1 – 0.7
	10 %	479.9 – 0.7	36.0 – 0.2
	15 %	559.9 – 0.5	55.1 – 0.7
	20 %	350.0 –0.7	40.0 – 0.7
Control	5 %	174.0 – 1.0	77.1 – 0.1
	10 %	120.1 – 1.1	42.1 – 0.6
	15 %	94.9 – 0.7	33.0 – 0.8
	20 %	83.9 – 0.2	24.0 – 0.6
F-Value		1400*	3332*
SE		0.447	0.346
CD at 5 %		1.845	1.010

* Significant at 5 % Level, Number of Replication=3

Table 2. Effect of *Bacillus subtilis* on Elicitation of Forskolin in Suspension Cultures of *Coleus forskohlii*

Elicitor	Concentration (%)	Forskolin content (mg/kg DCW)	
		24 hours	48 hours
		Mean – SD	Mean – SD
Cell Extract	5 %	35.3 – 2.2	94.2 – 3.0
	10 %	70.4 – 2.3	40.1 – 2.9
	15 %	60.3 – 1.9	25.3 – 3.1
	20 %	48.3 – 2.4	9.0 – 0.8
Cell Filtrate	5 %	148.3 – 4.2	105.5 – 3.2
	10 %	370.0 – 4.9	56.1 – 3.0
	15 %	580.1 – 6.0	42.2 – 2.8
	20 %	760.3 – 7.3	18.0 – 0.8
Control	5 %	174.0 – 1.0	77.1 – 0.1
	10 %	120.1 – 1.1	42.1 – 0.6
	15 %	94.9 – 0.7	33.0 – 0.8
	20 %	83.9 – 0.2	24.0 – 0.6
F-Value		1300*	577*
SE		2.082	1.269
CD at 5 %		6.077	3.704

* Significant at 5 % Level, Number of Replication=3

(F=1300*) and 48 hours (F=577*). There was no literature evidenced with the usage of *Bacillus subtilis* as an elicitor to affect secondary metabolism.

Influence of *Pseudomonas aeruginosa*

The effect of different concentrations of cell and filtrates of *Pseudomonas aeruginosa* were tested on elicitation of forskolin production in suspension cultures of *Coleus forskohlii*. The treatment where in 5% of *Pseudomonas aeruginosa* CE was administered to the culture, resulted in the higher level of total forskolin (635 gm/kg DCW) compared to the control (174± mg/kg DCW) after 24-hour administration of the elicitor. There was an increase in forskolin production by 173 and 118% in comparison to the control after 24-hour administration at concentrations 10 and 15% of CE, respectively (Table 3). There was no reduction in biomass production at lower concentrations but at higher concentrations there was slight decrease in biomass yield. However there was significant difference in forskolin content at 24 hours (F=3600*) and 48 hours (F=1372*). The effect of the biotic elicitor *Pseudomonas aeruginosa* in the form of a homogenate and an aqueous suspension of dead cells on the production of anthracene derivatives by the tissue culture of *Rheum palmatum* L. of different age and origin was examined by Kasparova and Siatka (2002). The production increased by 1.027% after 6-hour elicitation. *Pseudomonas aeruginosa* at 0.5% (w/v) resulted in 8.76 mg/gm DCW of plumbagin in suspension cultures of *Plumbago rosea* (Komaraiah *et al*. 2002). Enhanced accumulation of anthracene derivatives in *R. palmatum* callus cultures was achieved using *P. aeruginosa* (Tumova *et al*. 1995).

Influence of *Escherichia coli*

Elicitation of thiophene production by using *Escherichia coli* in *Tagetes patula* resulted in 35% increase compared to control at 1.5% concentration (Buitelaar *et al*. 1992). The influence of *E. coli* on the production of secondary metabolites was investigated in *Ononis arvensis*, *Arctostaphylos uva-ursi*, *Rheum palmatum*, *Rhaponticum carthamoides*, *Datura meteloides* and *Bergenia crassifolia* callus cultures. *Escherichia coli* homogenates added to *A. uva-ursi* increased the production of phenolic glycosides. Small amounts of bergenin were produced by *B. crassifolia*. The increased accumulation of flavonoid glycosides on *O. arvensis* was observed with 24 hours of culture independent of elicitor concentration (Tumova *et al*. 1995). Different concentrations of *E. coli* elicitor were added to suspension cultures of *Coleus forskohlii* at day 6 of the cultivation period, for elicitation of forskolin accumulation. Elicitor concentrations were varied between 5 and 20%. After addition of elicitor to cell cultures the cells were harvested and assayed for dry weight and forskolin during 24 and 48 hours. The addition of 15% CF was found to result in higher forskolin levels (547.2 ± 2.3 mg/kg DW) in the cells which were comparable to the control (174.0 ± 1.0 mg/kg DW) at 24 hours after addition of elicitor. CF at 10% also resulted in higher forskolin content (703.7 ± 7.5 mg/kg DW) which was comparable to the control (Table 4). At 5 and 20%, the forskolin levels were low compared to 10 and 15% CF of *E. coli*. There was a significant difference at 5% level in forskolin content when compared at 24 hours (F=315*) and 48 hours (F=4535*).

Table 3. Effect of *Pseudomonas aeruginosa* on Elicitation of Forskolin in Suspension Cultures of *Coleus forskohlii*

Elicitor	Concentration (%)	Forskolin content (mg/kg DCW)	
		24 hours	48 hours
		Mean – SD	Mean – SD
Cell Extract	5 %	635.3 – 2.4	18.0 – 0.4
	10 %	676.4 – 0.8	38.5 – 1.0
	15 %	380.2 – 3.1	49.9 – 0.7
	20 %	200.3 – 2.2	32.4 – 1.3
Cell Filtrate	5 %	288.2 – 3.3	37.9 – 0.7
	10 %	54.2 – 1.3	31.2 – 0.9
	15 %	32.5 – 1.1	56.0 – 1.1
	20 %	20.4 – 1.1	77.7 – 1.4
Control	5 %	174.0 – 1.0	77.1 – 0.1
	10 %	120.1 – 1.1	42.1 – 0.6
	15 %	94.9 – 0.7	33.0 – 0.8
	20 %	83.9 – 0.2	24.0 – 0.6
F-Value		3600*	1372*
SE		1.000	0.513
CD at 5 %		2.919	1.497

* Significant at 5 % Level, Number of Replication=3

Table 4. Effect of *Escherichia coli* on Elicitation of Forskolin in Suspension Cultures of *Coleus forskohlii*

Elicitor	Concentration (%)	Forskolin content (mg/kg DCW)	
		24 hours	48 hours
		Mean – SD	Mean – SD
Cell Extract	5 %	172.7 – 5.2	93.0 – 2.0
	10 %	77.1 – 1.7	159.4 – 3.6
	15 %	40.0 – 1.8	300.2 – 2.1
	20 %	10.9 – 0.5	89.4 – 0.8
Cell Filtrate	5 %	213.0 – 2.0	136.2 – 2.3
	10 %	547.2 – 2.3	161.2 – 2.9
	15 %	703.7 – 7.5	140.3 – 2.4
	20 %	400.2 – 2.4	99.4 – 1.4
Control	5 %	174.0 – 1.0	77.1 – 0.1
	10 %	120.1 – 1.1	42.1 – 0.6
	15 %	94.9 – 0.7	33.0 – 0.8
	20 %	83.9 – 0.2	24.0 – 0.6
F-Value		315*	4535*
SE		10.255	1.125
CD at 5 %		26.758	3.284

* Significant at 5 % Level, Number of Replication=3

Table 5. Effect of *Proteus aureus* on Elicitation of Forskolin in Suspension Cultures of *Coleus forskohlii*

Elicitor	Concentration (%)	Forskolin content (mg/kg DCW)	
		24 hours	48 hours
		Mean – SD	Mean – SD
Cell Extract	5 %	602.2 – 4.9	43.2 – 2.0
	10 %	610.1 – 5.0	112.4 – 2.1
	15 %	580.3 – 4.9	113.6 – 2.2
	20 %	510.2 – 5.4	250.6 – 2.1
Cell Filtrate	5 %	40.1 – 2.0	307.5 – 1.8
	10 %	63.3 – 1.9	64.2 – 2.1
	15 %	75.4 – 2.4	50.5 – 2.0
	20 %	50.2 – 1.9	38.3 – 1.8
Control	5 %	174.0 – 1.0	77.1 – 0.1
	10 %	120.1 – 1.1	42.1 – 0.6
	15 %	94.9 – 0.7	33.0 – 0.8
	20 %	83.9 – 0.2	24.0 – 0.6
F-Value		1800*	8747*
SE		1.826	0.983
CD at 5 %		5.330	2.869

* Significant at 5 % Level, Number of Replication=3

Influence of *Proteus aureus*

The effect of *Proteus aureus* was tested by adding the CE and CF at concentrations of 5, 10, 15 and 20% and comparing the results with a control without elicitor. Forskolin contents with 610 mg/kg DCW of the cell dry weight were measured 24 hours after elicitation in comparison to the control 174 mg/kg DCW of the cell dry weight levels at 10% of CE. The data subjected for statistical analysis indicates the significant difference in forskolin content at 24 hours (F=1800*) and 48 hours (F=8747*) (Table 5). There was no literature evidenced with the usage of *Proteus aureus* as an elicitor to affect secondary metabolism.

REFERENCES

Buitelaar RM, Cesario MT & Tramper J 1992 Elicitation of thiophene production by hairy roots of *Tagetes patula*. *Enzyme Microb Technol* **14** 2-7.

Eilert U 1987 Methodology and aspect of application. In: *Cell culture and somatic cell genetics of plants.* Constabel F & Vasil IK (eds.), San Diego. 153-196.

Kang SM, Min JY, Kim YD, Karigar CS, Kim SW, Goo GH & Choi MS 2009 Effect of biotic elicitors on the accumulation of bilobalide and ginkgolides in *Ginkgo biloba* cell cultures. *J Biotechnol* **139(1)** 84-88.

Kasparova M & Siatka T 2002 Effect of salicylic acid on production of anthracene derivatives in a culture of *Rheum palmatum* L. *in vitro*. *Ceska Slov Farm* **51(4)** 177-181.

Komaraiah P, Naga Amrutha R, Kavi kishore PB & Ramakrishna SV 2002 Elicitor enhanced production of plumbagin in suspension cultures of *Plumbago rosea* L. *Enzyme Microbial Technol.* **31** 634-639.

Suvarnalatha G, Rajendran L, Ravishankar GA & Venkataraman LV 1994 Elicitation of anthocyanin production in cell cultures of carrot (*Daucus carota* L.) by using elicitors and abiotic stress. *Biotechnology letters* **16** 1275-1280.

Tumova L, Dusek J & Duskova J 1995 The influence of biotic elicitor on the production of therapeutically important secondary metabolites. *Meded Fac Land bouwwet Rijksuniv Gent* **60(4a)** 2101-2104.