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Enhanced Catecholamine Production in *Gomphrena globosa* Suspension Cultures

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Abstract

Secondary metabolites (SMs) are small organic molecules produced by plants that are mostly not essential for their growth, development and reproduction but are produced to confer a selective advantage to the plant. Since their concentration in the plant in a natural environment are low, the plants are many a times stimulated to enhance their production especially when they have significant commercial and medicinal applications. Elicitation is a technique used in tissue culture wherein external elicitors, either biotic or abiotic, induce stress in the plant thereby leading to stimulation of production of specific plant secondary metabolites. Catecholamines and their precursors have many medicinal applications and have been reported to occur in *Gomphrena* sp. The present study successfully identified elicitors (chitosan and copper sulphate) for the enhanced production of catecholamines and its precursors from suspension cultures of *G. globosa*. Both the biotic and abiotic elicitors were used in different concentration so as to compare the effect of each on catecholamine and/or precursor concentrations in the suspension cultures. Successful elicitation of these precursors as well as catecholamines was standardized using *G. globosa* callus as a model system.

Keywords: Catecholamines, *Gomphrena globosa,* LCMS/MS, elicitation, suspension culture

Introduction

Plant secondary metabolites include a wide class of chemicals that have commercial applications as antibiotics, flavoring agents, fragrances, insecticides, dyes etc. Many of them also have medicinal applications and are therefore economically desirable. These secondary metabolites are not necessary for the survival of individual cells of the plants but rather are involved in interaction of the cell with its surrounding thereby ensuring their survival. In the natural environment, production of these secondary metabolites amounts to less than 1 % of the dry weight of the plant and is highly dependent on the developmental stage of the plant (1, 2). Since they are production is on an on-demand basis, commercial production of these useful metabolites from plants grown in their natural habitats would not be economical.

In such a scenario, plant tissue culture becomes a valuable tool in mass production of these commercially valuable metabolites which could be enhanced by controlled additions of elicitors (both biotic and abiotic). Research also suggested that faster proliferation rate and biosynthetic cycle in cell and organ culture leads to higher metabolic rate and consequently increased synthesis of valuable secondary metabolites when compared with field grown plants (3). Stress in any form is a crucial factor in altering the phytochemical profile of the plant. Elici-

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tation using external elicitors will induce stress in the plant thereby leading to stimulation of production of specific plant secondary metabolites. Elicitors are chemically distinct from each other and various factors like type of elicitor, concentration, duration of exposure, treatment schedule, culture type, cell line, medium composition, presence or absence of growth regulator, and age or stage of the culture at the time of elicitor treatment are the major factors that can determine the effectiveness of the elicitation strategies on biomass and secondary metabolite production (4 -9).

Research suggested that abiotic elicitors like nitrogen source, sucrose and phosphate in the medium affect the concentration of L-DOPA in tissue culture of *Mucuna pruriens* (10). Scientists have also reported several fold increase in concentration of L- DOPA in presence of biotic elicitors like methyl jasmonate, pectin, chitin or precursor tyrosine in suspension cultures of *M. pruriens* and *M. prurita* (11). Production of dopamine has also been reported in the cell suspension cultures of *Celosia argentea* var. *plumosa* (12). Previous work has proved the presence of catecholamines and predecessors in the inflorescence of *Gomphrena globosa* (13). The present study attempted to induce callus production from *G. globosa*, quantify the catecholamines present in the callus and then the use of elicitors (chitosan as the biotic elicitor and copper sulphate as abiotic elicitor) to increase the production of catecholamines viz. dopamine and epinephrine as well as catecholamine precursors viz L-tyrosine and L-DOPA.

Materials and Methods

Callus induction

Plant material for callus induction

The plant *G. globosa* was grown in the garden of Ramnarain Ruia Autonomous College, Mumbai. Node, internode and leaf of *G. globosa* were selected as explants for induction of callus. These surface sterilized explants were then separately transferred under aseptic conditions within a laminar air flow to Murashiga and Skoog (MS) basal medium supplemented with BA (1 mg/L) + NA (0.1 mg/L) and MS basal medium with 2,4 D (1 mg/L) + Kn (0.5 mg/L). The tubes inoculated with the explants were kept in a culture room for callus initiation at 25 ± 2ºC, 9 hr photoperiod under white fluorescent light (3000 lux). The hormonal combination that showed the best callusing was selected for subculturing and elicitation studies.

Proliferation and Maintenance of the callus

For callus maintenance, one month old calli were transferred to MS + 2,4 D (1 mg/L) + Kn (0.5 mg/L). The cultures were maintained at 25 ± 2°C under a 9 hr photoperiod at 3000lux light intensity. After sufficient quantity of the callus was obtained, they were subjected to elicitation studies.

Elicitation study

Establishment of callus suspension culture

The callus of *G. globosa* was maintained on MS+ 2,4 D (1 mg/L) + Kn (0.5 mg/L). The cell suspension culture was established in liquid MS medium supplemented with the same hormones. This suspension culture was used for elicitation studies using chitosan (100 mg/L and 200 mg/L) as the biotic and copper sulphate (0.1mg/L and 0.5 mg/L) as the abiotic elicitors.

0.5 gm of *G. globosa* callus was transferred in 40 ml of the liquid MS medium containing 2,4 D (1 mg/L) and Kn (0.5 mg/L).

The flasks were kept on shaker incubator (at temperature between 25 ± 2°C at an rpm between 70-80) for a period of 5 days followed by addition of the elicitors and then subsequently removing the culture bottles on Day 5, Day 10 and Day 15 respectively.

Sample preparation

The suspended cells (after incubation) were weighed and then subjected to methanolic extraction for 18 hrs. The filtrate was also subjected to extraction of catecholamines and

precursors in methanol in the same manner. The callus extracts and the filtrate extracts were then used for estimation of catecholamines by LCMS/MS.

Standard preparation

10 ml of the 1000 ppm stock solution of the standards; L-Tyrosine, L-DOPA, Dopamine and Epinephrine; were prepared. The standards were dissolved in minimum quantity 0.1 N HCl and the volume made upto 10 ml with methanol.

LC parameters

HPLC (Shimadzu Prominence Binary Gradient System, Shimadzu Corporation, Japan) equipped with a binary pump (20AD), degasser, an autosampler (SIL-20AC), a temperature-controlled column compartment CTO-20AC and photodiode array detector (SPD-M20A) was used. Chromatographic data was acquired using Labsolutions software. The analysis was done using Shim-pack MAqC-ODS I (150 mm x 4.6mm I.D.,5µm) column. The mobile phase comprised of-(A) 0.1% formic in water (B) 0.1% Formic acid in acetonitrile in a gradient mode.

Results and Discussion

Of the hormonal combinations used best callusing was observed for leaf explant of *G. globosa* on MS+2,4-D (1 mg/L) + Kn (0.5 mg/L). Previous work has reported the presence of catecholamines and predecessors in the inflorescence of *G. globosa* (13). Light dependent synthesis of epinephrine, norepinephrine and dopamine in *Portulaca grandiflora* callus has been reported by researchers (14). An attempt was therefore made in the current study to detect the catecholamines and precursors by stimulating their synthesis through elicitation using biotic and abiotic elicitors.

LCMS/MS quantitation of L-tyrosine, L-DOPA, dopamine and epinephrine from both callus and filtrate was carried out. On considering the total concentration (from callus and filtrate) of L-tyrosine was highest on day 10 (3.898±0.191 ppb) with 200 mg/L chitosan while the total concentration of L-DOPA was highest on day 15 (0.785±0.075 ppb) when 0.5% $CuSO_4$ was used (Fig 1 and 4). The total concentration of dopamine was highest on day 15 (0.689±0.053 ppb) when 0.5% copper sulphate was used as the elicitor whereas, the total concentration of epinephrine was highest on day 10 (3.258±0.630 ppb) with 200 mg/L chitosan (Fig 2 and 3). The current study presented that 200mg/L chitosan led to a four-fold increase of L-tyrosine(precursor) on day 10 from callus and a 3.3-fold increase in epinephrine on day 10. Similar high concentration of epinephrine was reported in callus of *P. grandiflora* (14). The current study also observed that with 0.5% $CuSO_4$ a seventy-fold increase in L-DOPA concentration on day 15 and a fifty-two-fold increase in dopamine concentration on day 10 was observed. A 9.25-fold increase in L-DOPA content in the callus of *H. enneaspermus* and presence of dopamine (not more than 42.08 mg/ gm dry weight) in suspension cultures of *Celosia argentea* var. *plumosa* has been reported by researchers (12, 15). In general, significant accumulation of catecholamine precursor L-tyrosine was observed in callus when chitosan was used as elicitor while L-DOPA accumulated when copper sulphate was the elicitor. Dopamine and epinephrine concentration were lower than control callus concentrations. Quantitation of the catecholamines and their precursors from filtrate showed high concentration of L- tyrosine and epinephrine by day 10 when chitosan was the elicitor while L-DOPA and dopamine production enhanced by day 15 with copper sulphate as elicitor. The present work also concluded that of all the components quantitated, the content of L-tyrosine was the highest followed by epinephrine, L-DOPA and dopamine respectively. The study also noted that while L- tyrosine was maximally retained in the cell, L-DOPA, dopamine and epinephrine were maximally released into the medium. L-tyrosine, a precursor of L-DOPA and catecholamines (dopamine and epinephrine), is an amino acid required for protein synthesis as well as for synthesis of other commercially important secondary metabolites

like betalains, capsaicin, benzophenanthridine, rosmarinic acid, coumaric acid, caffeic acid, cinnamic acid, securinine, phenylethanoid glycosides, other alkaloids and phenolics to name a few (16- 24). The maximal retention of L-tyrosine within the cell and its high content can therefore be attributed to it being an important precursor of protein synthesis and betalains which are known to be produced in *G.globosa*.

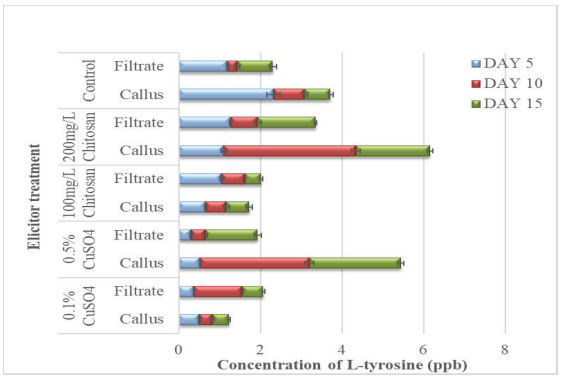

Fig.1: Quantitation of L-tyrosine(ppb) from suspension culture of *G.globosa* in presence of biotic and abiotic elicitors.

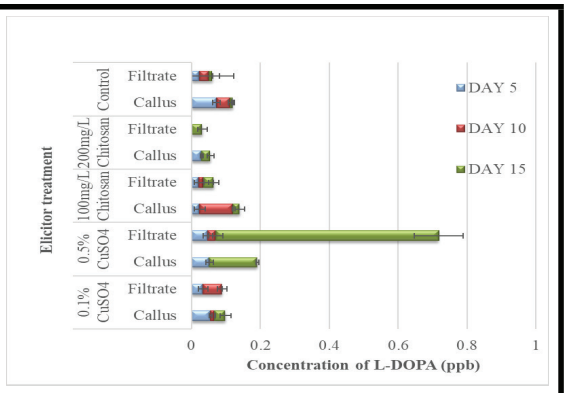

Fig.2: Quantitation of L-DOPA (ppb) from suspension culture of *G.globosa* in presence of biotic and abiotic elicitors.

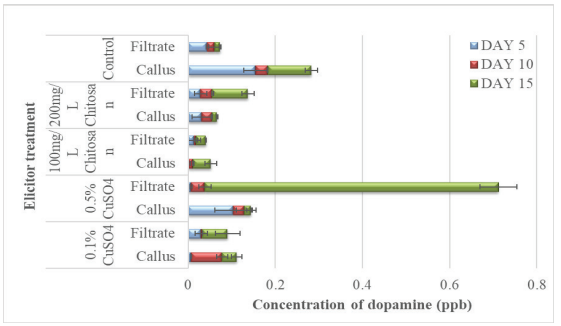

Fig.3: Quantitation of dopamine (ppb) from suspension culture of *G.globosa* in presence of biotic and abiotic elicitors.

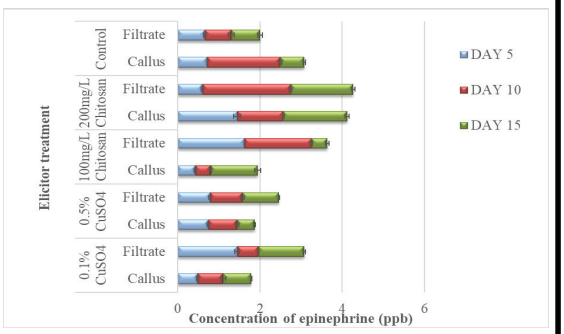

Fig.4: Quantitation of epinephrine (ppb) from suspension culture of *G.globosa* in presence of biotic and abiotic elicitors.

Conclusion

The LCMS/MS quantitation showed that chitosan was the best elicitor for enhancing L-tyrosine and epinephrine content while copper sulphate was best suited for enhancing L-DOPA and dopamine concentrations. The present study can be used as a model system to increase production these metabolites from other plant sources via tissue culturing.

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References

1. Dixon, R.A. (2001). Natural products and plant disease resistance. *Nature,* 411**:** 843–847.

2. Oksman-Caldentey, K. M., and Inzé, D. (2004). Plant cell factories in the post-genomic era: new ways to produce designer secondary metabolites. *Trends in Plant Science*, **9(9):** 433-440.

3. Rao, R.S. and Ravishankar, G.A. (2002). Plant cell cultures: chemical factories of secondary metabolites. *Biotechnology Advances*, 20**:**101–153.

4. Namdeo A. (2007). Plant cell elicitation for production of secondary metabolites: a review. *Pharmacognosy Reviews,* 1**:** 69–79.

5. Ramakrishna, A. and Ravishankar, G. A.(2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling and Behavior*, 6**:** 1720–1731.

6. Wang, J. W. and Wu, J. Y.(2013). Effective elicitors and process strategies for enhancement of secondary metabolite production in hairy root cultures. *Biotechnology of Hairy Root Systems,* 55–89.

7. Naik, P. M. and Al–Khayri, J. M.(2016). Abiotic and biotic elicitors–role in secondary metabolites production through *in vitro* culture of medicinal plant. *Abiotic and Biotic Stress in Plants-Recent Advances and Future Perspectives*, 247–277.

8. Dhiman, N., Patial, V., and Bhattacharya, A. (2018). The current status and future applications of hairy root cultures. In *Biotechnological Approaches for Medicinal and Aromatic Plants* (pp. 87-155). Springer, Singapore.

9. Kaur.K. and Pati. P. K. (2018). Stress-induced metabolite production utilizing plant hairy roots. *Hairy Roots- An Effective Tool of Plant Biotechnology*, 123–145.

10. Wichers, H.J., Visser, J.F., Huizing, H.J. and Pras, N. (1993). Occurrence of L-DOPA and dopamine in plants and cell cultures of *Mucuna pruriens* and effects of 2, 4-D and NaCl on these compounds. *Plant Cell, Tissue and Organ Culture*, **33:**259–264.

11. Raghavendra, S., Kumar, V., Ramesh, C.K. and Moinuddin Khan, M.H. (2012). Enhanced production of L-DOPA in cell cultures of *Mucuna pruriens* L. and *Mucuna prurita* H. *Natural Product Research*, 26(9)**:** 792-801.

12. Guadarrama-Flores, B., Rodriguez-Monroy, M., Cruz-Sosa, F., Garcia-Carmona, F., and Gandia-Herrero, F. (2015). Production of dihydroxylated betalains and dopamine in cell suspension cultures of *Celosia argentea* var. *plumosa*. *Journal of Agricultural and Food Chemistry*, 63(10)**:** 2741-2749.

13. Sandhya M, Jessy P and Shailesh D: LCMS/MS Quantitation of catecholamines and their predecessors from *Portulaca oleracea* and *Gomphrena globosa International Journal of Pharmaceutical Sciences and Research,* 12(11): 1000-06.

14. Endress, R., Jager, A. and Kreis, W.,

(1984). Catecholamine Biosynthesis Dependent on the Dark in Betacyanin-forming *Portulaca* callus. *Journal of Plant Physiology,* **115:** 291- 295.

15. Sathish, S., Vasudevan, V., Karthik, S. , Pavan, G. and Manickavasagam, M. (2019). Enhanced L-DOPA production from elicited cell suspension culture of *Hybanthus enneaspermus* (L.) F. Muell. *Plant Biotechnology Reports,* **13:** 613–621.

16. Parry, R.J. (1978). Biosynthesis of the Securinega alkaloids. Investigations of the early and late stages of the pathway. *Bioorganic Chemistry,* **7(3):** 277-288.

17. Zenk, M.H. (1994). The formation of benzophenanthridine alkaloids. *Pure and Applied Chemistry*, **66(10-11):** 2023-2028.

18. Veeresham,C., Kokate, C.K., Venkateswarlu, V. and Ramesh Babu, B. (1994). Influence of DLDOPA and L- tyrosine on biproduction of capsaicin in callus culture of *Capsicum annum* Linn. *Indian Journal of Experimental Biology*, 32: 223-224.

19. Croteau, R., Kutchan, T. M., and Lewis, N. G. (2000). Natural products (secondary metabolites). *Biochemistry and Molecular Biology of Plants*, 24: 1250-1319.

20. Ouyang,J., Wang,X., Zhao, B. and Wang, Y.(2005). Enhanced production of phenylethanoid glycosides by precursor feeding to cell culture of *Cistanche deserticola*. *Process Biochemistry,* 40(11)**:** 3480-3484.

21. Namdeo, A. G., Jadhav, T. S., Rai, P. K., Gavali, S., and Mahadik, K. R. (2007). Phcog Rev.: Review Article Precursor feeding for enhanced production of Secondary metabolites: A review. *Pharmacognosy reviews*, 1(2):227-231.

22. Roy, D., and Mukhopadhyay, S. (2012). Enhanced rosmarinic acid production in cultured plants of two species of *Mentha*. *Indian Journal of Experimental Biology*, **50:** 817-825.

23. Martins, N., Roriz, C. L., Morales, P., Barros, L., and Ferreira, I. C. F. R. (2017). Coloring attributes of betalains: A key emphasis on stability and future applications. *Food and Function*, 8(4)**:** 19–23.

24. Wang, M., Lopez-Nieves, S., Goldman, I. L., and Maeda, H. A. (2017). Limited tyrosine utilization explains lower betalain contents in yellow than in red table beet genotypes. *Journal of Agricultural and Food Chemistry*, 65(21)**:** 4305-4313.