Applied Biological Research 18 (2): 220-222; (2016) **SHORT NOTE**
DOI: 10.5958/0974-4517.2016.00035.5

INFLUENCE OF ELICITORS ON SAPONIN AND PROLINE PRODUCTION IN SUSPENSION CULTURES OF *Gymnema sylvestre* R Br.

Deepika Emmanuel[1]* and N.S. Shekhawat [2]

[1,2]*Plant Biotechnology Unit, Department of Botany, New Campus, Jai Narain Vyas University, Jodhpur – 342 033, Rajasthan (India)*
*e-mail: *mailde20@gmail.com*

(Received 13 January, 2016; Accepted 29 June, 2016)

Diabetes mellitus is a major disorder of key concern worldwide. So, the plants having anti-diabetic property are of significant interest. *Gymnema sylvestre* R.Br., a member of family *Asclepiadaceae*, is a potent anti-diabetic plant. It is a large, woody, much branched, slow growing perennial climber having pubescent young parts. This mesophytic to xerophytic plant is found in tropical to sub-tropical areas and generally grows wild in forests. Due to its sugar destroying property it is known as Gurmar in Hindi and Madhunashini in Sanskrit. Products of *G. sylvestre* are traditionally used in ayurvedic and homeopathic medicinal system for the treatment of diabetes mellitus due to its saponin content (Thakur *et al*., 2012). *G. sylvestre* also has anti-inflammatory, anti-cancer and antiviral activities (Arunachalam *et al*., 2015; Subashini and Rajendaran, 2015). The species holds large potential for bioprospecting as it yields the products for healthcare and medicine. The major phyto-constituents are gymnemasides, which are dammarane type of saponins and gymnemic acid and gymnemasaponins, which are oleanane type of saponins (Tiwari *et al.*, 2014). Secondary metabolites are produced in plant in defensive response to pathogenic attack and this response is triggered by various elicitors which act as signal molecules to plant defense mechanism (Zhao *et al.*, 2005) and ensure plant survival (Namdeo, 2007). The present study was aimed to evaluate the effect of elicitors like yeast extract and salicylic acid, in suspension cultures of *G. sylvestre*, at various concentrations and duration of treatment on *in vitro* accumulation of saponin.

A 9 year old twinning shrub, planted in the Experimental Nursery, AFRI, Jodhpur (India) was selected for the collection of explants. Young leaves were taken as explants for the formation of callus. The explants were treated with 0.1% (w/v) Bavistin (Hi Media, Mumbai) for 10-15 min., followed by 4-5 rinses with sterile distilled water. The surface sterilized explants were cut into small pieces of 1 x 1 cm size with the help of sterilized scissors and placed on semi-solid MS medium (Murashige and Skoog, 1962) supplemented with 2.26 µM 2,4-D and 0.46 µM kinetin. Suspension cultures were initiated from friable callus obtained on semi-solid MS medium after 4-5 subcultures. Fresh 500 mg callus was inoculated in 250 mL conical flask containing 40 mL liquid MS medium supplemented with 3% (w/v) sucrose, plant growth regulators like 2.26 µM 2,4-D and 0.46 µM kinetin. The pH of medium was adjusted to 5.8 (with 1N NaOH or 0.1N HCl) prior to autoclaving. These were maintained on a gyratory shaker at 100 rpm under cool fluorescent light at 25±2°C, 60% RH and 16 h light/8 h dark photoperiod. The cells were sub-cultured after every 20 days. Batch culture was established by sub-culturing actively growing cells in fresh liquid MS medium. Cell growth was observed after every 15 days under inverted microscope (Leica Microsystems, Germany).

For elicitation, **s**tock solution for each elicitor was prepared (strength 1 mg mL^{-1}) by using yeast extract and salicylic acid (Merck), which were autoclaved and introduced in suspension media in required volume. For these studies, biotic elicitation was caused by yeast extract (YE) while abiotic elicitation was carried out by using salicylic acid (SA) using concentrations of 0 (control), 50, 100 and 150 mg L^{-1}. For every sample there were three replicates which were analyzed over an incubation time of 24 and 48 h. The samples were prepared as per Makkar *et al.* (2007) and

spectrophotometric analysis of total saponin done through UV- Vis spectrophotometer at 544 nm using diosgenin caliberation curve. Proline was estimated spectrophotometrically at 520 nm using ninhydrin method (Bates *et al.,* 1973). The data was analyzed using one way ANOVA and significant difference between the means analyzed by Duncan's multiple range test.

One way ANOVA analysis revealed that elicitation with YE and SA had significant effect on total saponin production. Out of the three concentrations (50, 100, 150 mg L^{-1}) tested, 50 mg L^{-1} YE over 24 h was most effective for optimum saponin production, which gave a value of 5.17 mg g^{-1} DW callus of total saponin produced (Table 1). Best response was obtained on 100 mg L^{-1} SA treated for 24 h giving a value of 4.51 mg g^{-1} DW callus (Table 1). In present study, biotic elicitor (YE) gave better response than abiotic elicitor (SA). The results are in agreement with Goyal *et al.* (2008) in case of *Pueraria tuberosa* for the production of puerarin. The effect of any elicitor is dependent on a number of factors like elicitor's specificity, concentration, duration of treatment and growth stage of culture (Holden, 1988). In this study we obtained 6.7-fold increase over control in saponin content on YE treated over a concentration of 50 mg L^{-1} for 24 h. Similarly for SA, the optimum response was obtained on 100 mg SA L^{-1} treated for 24 h which showed 6.0-fold increase in saponin content.

Table 1: Effect of salicylic acid and yeast extract on total saponin production in *Gymnema sylvestre*

Concentration	Yeast extract		Salicylic acid	
(mg L^{-1})	For 24 h	For 48 h	For 24 h	For48 h
0	0.77 ± 0.02^{f}	0.68 ± 0.02^{f}	0.75 ± 0.03^{f}	0.63 ± 0.03f
50	5.17 ± 0.05^{a}	4.87 ± 0.02^{b}	4.26 ± 0.02^{b}	3.69 ± 0.05c
100	4.27 ± 0.04^{a}	3.51 ± 0.03^{d}	4.51 ± 0.06^{a}	4.44 ± 0.03a
150	3.49 ± 0.05^{d}	1.05 ± 0.05^{e}	3.49 ± 0.04^{d}	1.17 ± 0.06e

The cells of suspension culture accumulated proline under the influence of elicitation. For YE, the highest amount of proline was 0.947 mg g^{-1} FW callus at 50 mg L^{-1} concentration over 24 h (Table 2). For SA, the highest proline content of 0.527 mg g^{-1} FW callus was produced by the cell suspension under the influence of 100 mg L^{-1} SA treated for 24 h (Table 2). The cells showing highest total saponin production was also found correlated with maximum proline production. Similar observations have been reported in the suspension cultures of *Rubia tinctorum* (Perassolo *et al.,* 2011). The proline content was higher for 24 h duration of treatment for both the elicitors as compared to 48 h treatment. A maximum increase of 8.3-folds in proline content was noticed in YE as compared to control. For SA, maximum production was 4.87-folds higher than control.

Table 2: Proline production as affected by salicylic acid and yeast extract in *Gymnema sylvestre*

Concentration	Yeast extract		Salicylic acid	
(mg L^{-1})	For 24 h	For 48 h		For 24 h
0	0.113 ± 0.145^{d}	0.109 ± 0.367^{d}	0.108 ± 0.115^{d}	0.013 ± 0.450^{e}
50	0.947 ± 0.228^{a}	0.732 ± 0.047^{a}	0.454 ± 0.023^{b}	0.368 ± 0.172^{c}
100	0.453 ± 0.544^{b}	0.367 ± 0.429^{c}	0.527 ± 0.245^{a}	0.466 ± 0.046^{b}
150	0.361 ± 0.117^{c}	0.077 ± 0.238^{e}	0.361 ± 0.033^{c}	0.087 ± 0.023^{e}

Arunachalam, K.D., Arun, L.B., Annamalai, S.K. and Arunachalam, A.M. 2015. Potential anticancer properties of bioactive compounds of *Gymnema sylvestre* and its biofunctionalized silver nanoparticles. *International Journal of Nanomedicine,* **10**: 31-41.

Bates, L.S., Waldern, R.P. and Teare, F.D. 1973. Rapid determination of free proline from water stress studies. *Plant and Soil*, **39**: 205-207.

Goyal, S. and Ramawat, K.G. 2008. Increased isoflavanoid accumulation in cell suspension cultures of *Pueraria tuberose* by elicitors. *Indian Journal of Biotechnology,* **7**: 378-382.

Holden, M.A., Holden, P.R. and Yeoman, M.M. 1988. Elicitation of cell cultures. pp. 97-98. **In**: *Manipulating Secondary Metabolism in Culture* (eds. R.J. Robins, M.J.C. Rhodes *et al.*). Cambridge University Press, Cambridge, UK.

Makkar, H.P.S., Siddhuraju, P. and Becker, K. 2007. *Plant Secondary Metabolites*. Humana Press Inc., Totowa, New Jersey, USA.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, **15**: 473-497.

Namdeo, A.G. 2007. Plant cell elicitation for production of secondary metabolites: A review. *Pharmacognosy Reviews*. **1**: 69-79.

Perassolo, M., Quevedo, C.V., Giulietti, A.M. and Talou, J.R .2011. Stimulation of the proline cycle and anthraquinone accumulation in *Rubia tinctorum* cell suspension cultures in presence of glutamate and two proline analogs. *Plant Cell Tissue and Organ Culture*, **106**: 153-159.

Subashini, M.S. and Rajendran, P. 2015. *In vitro* screening of anti HBV and anti HIV properties of *Gymnema sylvestre R.Br*. leaves from Kolli Hills, Tamil Nadu, India. *International Journal of Current Microbiology and Applied Sciences*, **4**: 542-547.

Thakur, G.S., Sharma, R., Sanodiya, B.S., Pandey, M., Prasad, G. and Bisen, P.S. 2012. *Gymnema sylvestre*: An alternative therapeutic agent for management of diabetes. *Journal of Applied Pharmaceutical Sciences*, **2(12)**: 1-6.

Tiwari P., Mishra, B.N. and Sangwan, N.S. 2014. Phytochemical and pharmacological properties of *Gymnema sylvestre*: An important medicinal plant. *BioMed Research International*, Article ID 830285, 18 pages, *http://dx.doi.org/10.1155/2014/830285*.