JEB®

Journal of Environmental Biology

p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP
Journal website : www.jeb.co.in ★ **E-mail :** editor@jeb.co.in

Triveni Enterprises
(Educational Services Set-up)

Original Research

DOI : http://doi.org/10.22438/jeb/44/2/MRN-4011

Elicitation based enhancement of solasodine production in *in-vitro* cultures of different *Solanum* species

V. Deshmukh[1,2], J. Deshpande[1] and M. Wani[1]*

[1]Plant and Environmental Biotechnology Research Lab, Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth University, Pimpri-411 033, India.
[2]MGM College of Agricultural Biotechnology, Aurangabad-431 007, India

***Corresponding Author Email :** minal.wani@dpu.edu.in ***ORCiD:** https://orcid.org/0000-0002-9695-0531

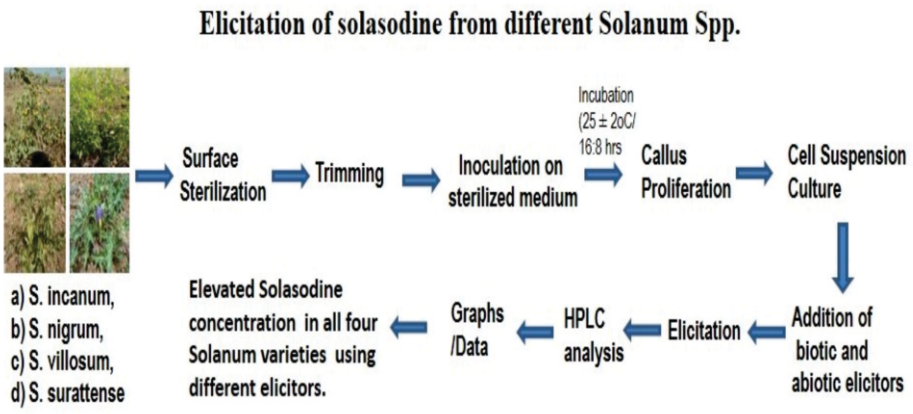
Received: 02.11.2021 *Revised*: 03.08.2022 *Accepted*: 10.11.2022

Abstract

Aim: To study the enhancement of solasodine content using elicitors such as NaCl, pectin, salicylic acid and yeast extract in cell suspension cultures of *Solanum incanum*, *Solanum nigrum, Solanum surattense* and *Solanum villosum*.

Methodology: *In-vitro* callus induction from leaf explants was carried out on MS media supplemented with auxin, 2, 4- Dichlorophenoxyacetic acid (2.0 mg l^{-1}). MS liquid medium supplemented with 2, 4-D (2.0 mg l^{-1}) and varied concentrations of different elicitors were used for cell suspension culture.

Results: The elicitor NaCl (150 mM) indicated maximum increment in solasodine production in three *Solanum* species studied. Salicylic acid with 75 µM resulted in considerable elevation in solasodine content in *Solanum* spp. Response to elicitation by pectin was high at different concentrations for different species of *Solanum*. Biotic elicitor yeast extract at 3.0 g l^{-1} concentration considerably increased solasodine production in *S. nigrum* and *S. villosum*. *Solanum villosum* exhibited best results in terms of solasodine concentration enhancement in response to pectin and yeast extract elicitors whereas *Solanum incanum* responded best to pectin followed by NaCl.



Interpretation: The current results indicated that NaCl, pectin, salicylic acid and yeast extract can be effectively applied as influential elicitors for the enhancing the production of solasodine in cell suspension culture of different *Solanum* species.

Key words: Callus, Cell suspension culture, Elicitors, *Solanum*, Solasodine, Steroidal alkaloids

How to cite : Deshmukh, V., J. Deshpande and M. Wani: Elicitation based enhancement of solasodine production in *in-vitro* cultures of different *Solanum* species. *J. Environ. Biol.*, **44**, 167-174 (2023).

Introduction

Plants are rich in various phytochemicals of different composition, which attribute to their medicinal properties. These plant secondary metabolites comprise alkaloids, glycosides, steroids, terpenes, essential oils, etc. Plant metabolites, alkaloids and polyphenols are identified as therapeutic agents. Steroidal alkaloids are organic molecules involved in the defence mechanism of plants and have no major role in plant growth, development or reproduction. Solanaceae family comprises about 80 genera including 3000 species, from which 15000 belong to the genus *Solanum*. *Solanumis* one of the important traditional medicinal plants with wide range of pharmacological properties like anticancer, antioxidant, hepatoprotective, antiulcerogenic and neuroprotective effects. It is extensively applied in various herbal and Ayurveda formulations in the form of syrup and capsules (Abdel-Rahim *et al.,* 2014; Chester *et al.,* 2017). Solanum species contain several free and glycosylated alkaloids which gradually gets converted into the precursor for the synthesis of steroids (Solouki *et al.,* 2011).

Solasodine, a steroidal alkaloid present in the genus *Solanum* is reported to have a huge demand in pharmaceutical industries because solasodine works as a key intermediate in the synthesis of progesterone and cortisone (Pawar *et al.,* 2008). Literature survey reveals that solasodine exhibits a wide spectrum of pharmacological activities like anticancer, hepatoprotective, immunomodulatory, antifungal, diuretic, antiandrogenic, antipyretic, cardiotonic, and activities related to central nervous system (Desai *et al.,* 2011). Positive effects of solasodine extracts from different *Solanum* species on HT-29 and MG-63 cancer cell lines have been noticed and can be used as an efficient anti-cancer agent which can inhibit cell growth (Deshmukh *et al*., 2022). Though solasodine exhibits such important therapeutic properties, its concentration in *in-vivo* plant is low which may not be sufficient to fulfil the increasing demand of the pharmaceutical industry. Glycoalkaloids like solasodine concentration may vary species to species as well as in the plant parts as their amount increases due to stress, injury and exposure to light or any elicitors.

Elicitation is a process to enhance the synthesis of secondary metabolites by the plants in response to various physical, chemical or microbial or any other biological factors called elicitors. Biotic elicitors have biological origin, derived from microorganisms or plants, like pectin, chitin, yeast extract, chitosan, mannan-oligosaccarides (Gadzovska *et al.,* 2015), and abiotic elicitors are physical factors and chemical compounds that consist NaCl, salicylic acid, silver nitrate, copper sulphate, jasmonic acid, methyl jasmonate etc. (Singh and Dwivedi, 2018). Elicitation using *in-vitro* tissue culture techniques give promising and cost effective results for the production of commercially important secondary metabolites (Sharma *et al.,* 2011). Lu *et al.* (2001), reported that the culture of *Panax ginseng* treated with 3 g l^{-1} yeast extract resulted in 20-fold increase in the saponin content of suspension culture as compared to control. Alkaloid production in *Brugmansie candida* was enhanced considerably on using salicylic acid as elicitor (Pitta *et al.,* 2000). Yeast extract has been reported as an efficient elicitor to enhance the plumbagin production by 5.4 fold over the control plant (Thaweesak *et al*., 2011). Patel *et al.* (2013) stated that, elicitors can enhance the synthesis of plant secondary-metabolites by influencing the biosynthetic pathways for production of commercially important compounds like pharmaceuticals, food additives, flavours, and other industrial materials.

The interest in plant cell and organ cultures has increased due to plant biotechnology, as many of the scale up studies for the production of promising secondary metabolic products were successful (Pandey *et al.,* 2019; Szopa *et al.,* 2019; Ho *et al.,* 2020; Kokotkiewicz *et al.,* 2021). Elicitation strategies for the enhancement of bioactive components in edible plant sprouts has been reviewed in detail by Toro *et al.* (2021) and their benefits have been reported. The objective of the present investigation was to assess the effect of different elicitors- pectin, yeast extract, NaCl and salicylic acid on solasodine production in cell suspension cultures of *Solanum* spp. including *S. nigrum, S. surattense*, *S. villosum* and *S. incanum.*

Materials and Methods

S. nigrum, S. surattense, S. villosum and *S. incanum* plants were obtained from the hilly region of Aurangabad, Maharashtra, India and the experiments were performed at Department of Plant Biotechnology, MGM College of Agricultural Biotechnology and Dr. D.Y. Patil Biotechnology and Bioinformatics Institute. Collected young leaves were washed under running tap water and treated with tween twenty as a surfactant. Leaf explants were surface sterilized with 70% alcohol for 60 sec and with 0.01% $HgCl_2$ for 2 min. Surface sterilized explants were washed repeatedly with double distilled water to remove the traces of chemicals. Leaf explantsof different Solanum species under study were inoculated in Murashige and Skoog media supplemented with 2.0 mg l^{-1} 2, 4-D (Sridhar and Naidu, 2011). Cultures were maintained at 26 ± 2^0C with 60-70% humidity and 3000 lux with a photoperiod of 18 hr light and 6 hr dark. Induced calli were subcultured at 21 days interval to obtain friable callus. For the establishment of suspension culture, the resultant callus was crushed and transferred to 50 ml of MS liquid medium supplemented with 2, 4-D (2.0 mg l^{-1}) and incubated at 100 rpm on rotary shaker at 25 ± 2^0C and sub-cultured weekly.

For the enhanced production of solasodine content in *Solanum* cell suspension cultures, biotic and abiotic elicitors were introduced in the suspension medium. For elicitation, the MS medium (pH 6) containing 2, 4-D (2.0 mg l^{-1}) were supplemented with different elicitors- pectin, yeast extract, NaCl and salicylic acid, at four different concentrations. Pectin was added @ 0.75%, 1.0%, 1.25% and 1.5% in different culture flasks whereas Yeast extract were added separately at 2.5, 3.0, 3.5 and 4.0 g l^{-1} concentrations. NaCl @ 50 mM, 100 mM, 150mM and 200 mM and Salicylic acid @ 25µM, 50 µM, 75 µM and 100 µM

concentrations were used separately. Suspension cultures consisting elicitors NaCl and salicylic acid were incubated for 7 to 14 days, while pectin and yeast extract for 21 days, respectively (Maurya *et al.,* 2019). Cell suspensions were filtered through a sterile nylon mesh (100mm) after incubation and filtrate containing solasodine contents were collected into a sterile flask and the cell biomass was separated. Filtrate was concentrated by heating at 37^0C and compound obtained in powdered form. One gram powder from suspension cultures of each variety was dried at 60^0C and extracted with 25 ml of methanol separately and hydrolysed with 1M HCl to remove the sugar. The residue was dissolved in 10 ml ethanol and filtered through 0.2 µM membrane filter. The crude extracts were analysed by HPLC for qualitative and quantitative estimation of solasodine following the method of Desai *et al.* (2014) with some modifications.

A Dionex Ultimate 3000 HPLC system with quaternary pumps, auto sampler and diode array detector with Chromeleon software (version 6.80) was used for analysis of solasodine content. The column used was C18 Chromegabond MC18 60 A (150 mm X 4.6 mm I.D., 5um) and the injection volume was 20 µl. Mobile phase used was acetonitrile (10 mM) and ammonium acetate buffer (pH 7.7) 65:35 ratio with flow rate of 2.0 ml min^{-1}. Detection was carried out at 205 nm and column temperature was set at 40^0C. A standard curve was plotted using solasodine obtained from Sigma Aldrich. The concentration of solasodine in the test samples was estimated with reference to the standard curve. The experiments were set in completely randomized design. Four concentrations each of elicitors were used and each experiment was performed in triplicates. Statistical analysis was carried by Duncan's Multiple Range Test ($p<0.05$) using SAS program.

Results and Discussion

The present study includes the enhancement of solasodine production by using biotic and abiotic elicitors in cell suspension cultures of different Solanum species viz., *S. incanum*, *S. nigrum*, *S. surattense* and *S. villosum*. Results indicated a positive influence of the elicitors on the production of solasodine. Results indicated a positive influence of elicitors on the production of solasodine from in vitro cell cultures of different *Solanum* species. Initiation of leaf callus on MS medium supplemented with constant concentration of 2.0 mg l^{-1} 2,4-D was observed and recorded 12-15 days after inoculation in *S. incanum, S. nigrum, S. surattense* and *S. villosum,* respectively. After sub culturing, fully grown friable callus were taken for suspension culture studies. *In-vitro* generated calli were used for the initiation and establishment of cell suspension cultures and studies were carried out using different elicitors.

Suspension cultures with elicitors were observed at 7, 14, 21, 28 days of inoculation for the cell culture growth and was studied for solasodine content. The effect of two elicitors each, biotic and abiotic, *i.e.,* pectin, yeast extract and NaCl and salicylic acid were studied on solasodine production in suspension cultures of four species, *S. incanum, S. nigrum, S. surattense* and *S. villosum*. Control cultures were maintained without the addition of elicitors. The results revealed that, different elicitors and their variable concentrations exhibited different effect on solasodine production. Solasodine content also showed variations based on the species. HPLC chromatogram of solasodine was obtained using solasodine (1mg ml^{-1}) from Sigma Aldrich and was compared and calculated for solasodine sample. Standard (Sigma compound) and extracted samples were run separately and retention time was compared for the analysis of solasodine. The results indicated that the retention time exhibited by the standard solasodine and the extractions were very close.

Various biotic and abiotic elicitors are reported to influence the yield of secondary metabolites. In the present study, the effect of four different concentrations of NaCl (50, 100, 150 and 200 mM) on the production of solasodine in cell suspension cultures of four species of *Solanum* (Fig. 1). Increased up to 150mM in *S. incanum, S. nigrum* and *S. surattense*, followed by a decline. The maximum solasodine content was observed at 150 mM NaCl in *S. surattense* (0.181 mg g^{-1}), *S. nigrum* (0.178mg g^{-1}) and *S. incanum* (0.120 mg g^{-1}). *S. villosum* showed higher concentration of 0.171 mg g^{-1} at 100 mM NaCl and decreased thereafter. NaCl as an elicitor indicated best response for solasodine content elevation in *S. surattense* followed by *S. nigrum, S. villousum* and *S. incanum*, respectively. Similar increase in solasodine content was reported at 150 mM NaCl in *S. nigrum* (Sutcovic *et al.,* 2011) and in regenerating callus (Bhat *et al.,* 2008; Rocha *et al.,* 2005) where as in *S. khasianuma* 100mM NaCl showed best response (Srivastava *et al.,* 2016). Application of NaCl may impart an osmotic imbalance in cultured cells and thereby salt stress which enhance the production of secondary metabolites like solasodine. Na^+ and Cl− cause ionic disproportion in formation of reactive oxygen species, may lead to disturbance in the cell homoeostasis and activation of defence-related genes (Timperio *et al.,* 2008; Du *et al.,* 2010), which enhances the secondary metabolite production in plant cells. The effect of abiotic elicitor salicylic acid at different concentrations (25, 50, 75 and 100 µM) was observed for the production of solasodine in cultures of four species of *Solanum*. Salicylic acid can be used as an elicitor in *in-vitro* cultures to enhance synthesis of secondary metabolites by gene regulation related to secondary metabolite biosynthesis in plants (Taguchi *et al.,* 2001). The results (Fig. 2) revealed that all the Solanum species under study showed maximum solasodine production at 75µM salicylic acid concentration. *S. surattense* and *S. nigrum produced* highest solasodine content (0.175 mg g^{-1}) followed by *S. villosum* (0.169 mg g^{-1}) and *S. incanum* (0.110 mg g^{-1}). Overall, the effect of salicylic acid in solasodine production was highest in *S. surattense* followed by *S. villosum* and *S. incanum*.

The increment in solasodine concentration was less prominent on using this elicitor as compared to other elicitors under study. Salicylic acid induced stress in plant cells may lead to cell damage and the secondary metabolite production may occur for activation of defence mechanism. A 50-200 % increase in anthocyanin and phenol contents was reported in *W. coagulans*

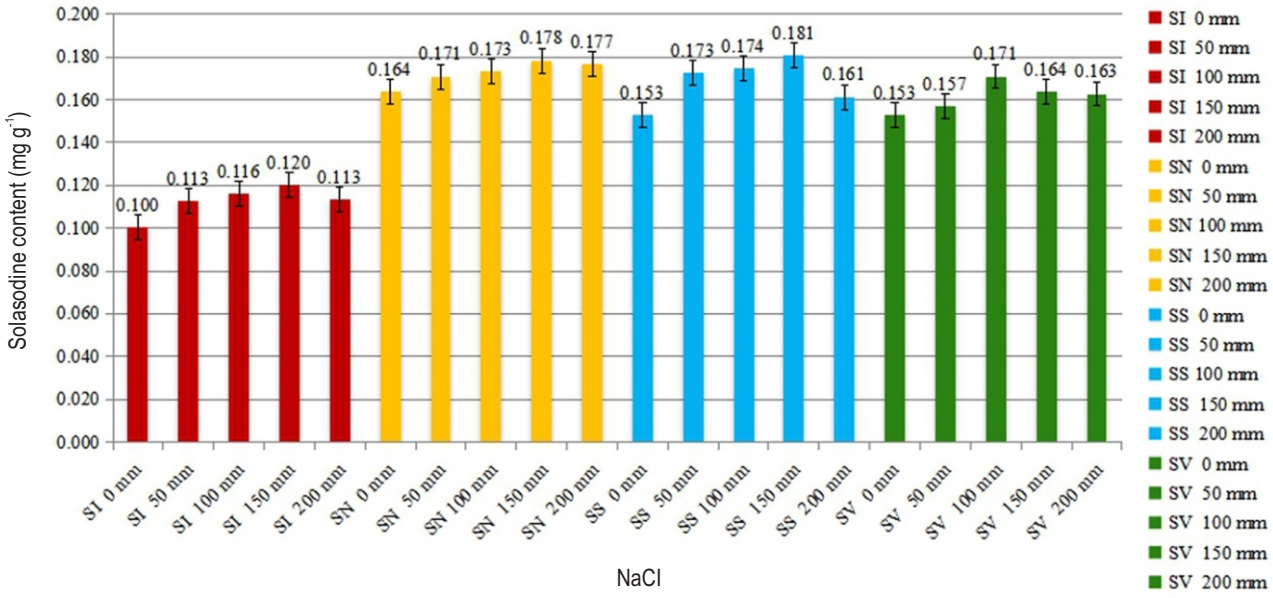


Fig. 1: Effect of different concentrations of NaCl on solasodine content in cell suspension cultures of (a) *S. incanum* (b) *S. nigrum* (c) *S. surattense* and (d) *S. villosum*.

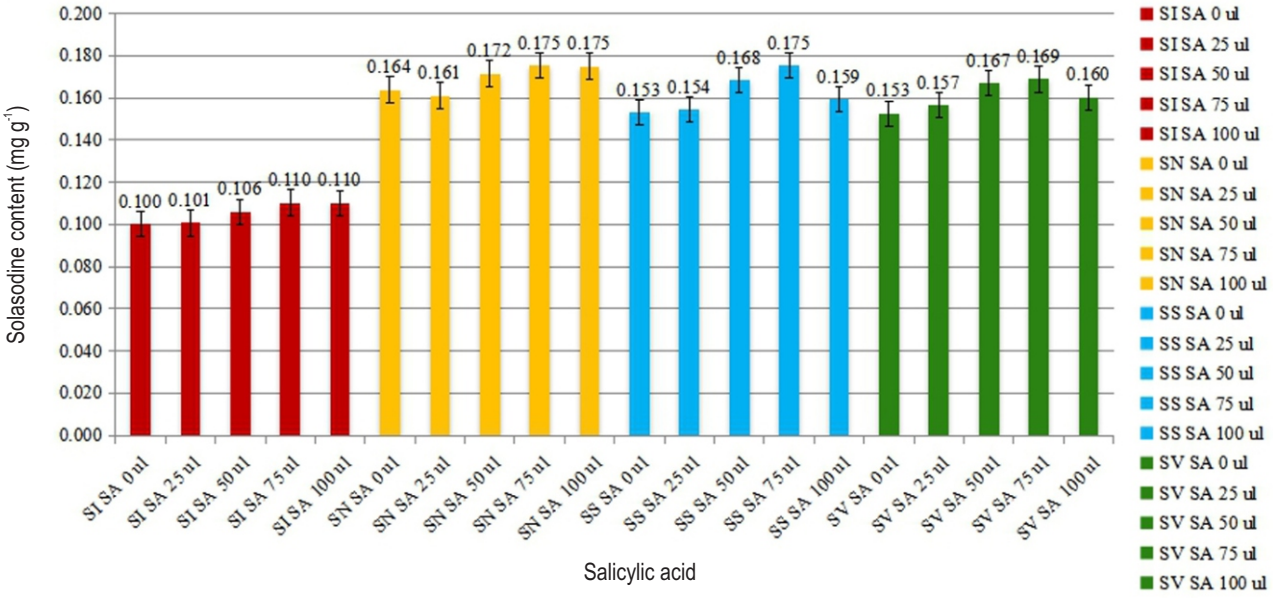


Fig. 2: Effect of different concentrations of salicylic acid on solasodine content in cell suspension culture of (a) *S. incanum* (b) *S. nigrum* (c) *S. surattense* and (d) *S. villosum*.

when 150 and 200 µM salicylic acid was applied (Maurya *et al.,* 2019). Similar to the present results, Acikgoz *et al.* (2019) observed camphor accumulation in cells when treated with salicylic acid in cell suspension cultures of *Achillea gypsicola.* Salicylic acid leaded accumulation of phenolic and flavonoid contents were reported in Fagonia indica (Khan *et al*., 2019). Exogenous application of salicylic acid may induce synthesis of secondary metabolites in plant cells in culture. Pectin, a biotic elicitor has been reported for its effective use in numerous plant cell cultures for enhancing commercially important plant secondary metabolites (Chakraborty and Chattopadhyay, 2008). Best response in solasodine content using pectin as an elicitor was recorded by *S. incanum* cell cultures with an increment of 40% when compared to control and the lowest by *S. nigrum* (9.15%) (Fig. 3). Highest solasodine content during the study was exhibited by *S. nigrum* 0.179 mg g^{-1} (9.15 %) at 1% pectin followed

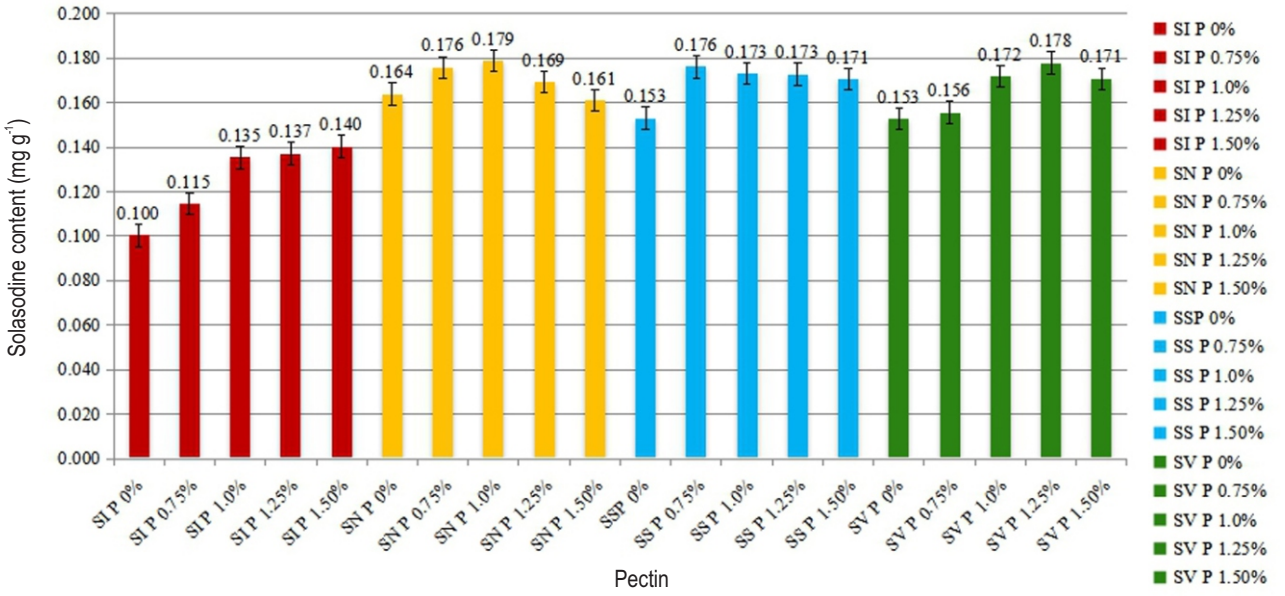


Fig. 3: Effect of different concentrations of Pectin on solasodine content in cell suspension culture of (a) *S. incanum* (b) *S. nigrum* (c) *S. surattense* and (d) *S. villosum*.

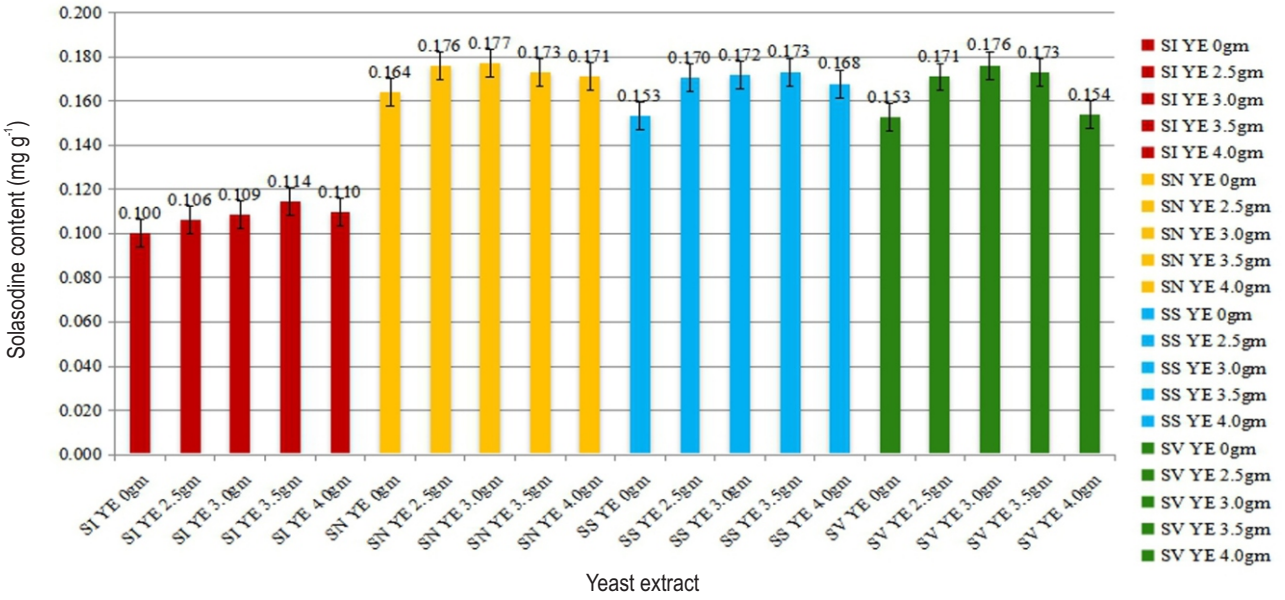


Fig. 4: Effect of different concentrations of yeast extract on solasodine content in cell suspension culture of (a) *S. incanum* (b) *S. nigrum* (c) *S. surattense* and (d) *S. villosum*.

by 0.178 mg g^{-1} (16.3 %) in *S. villosum* at 1.25% pectin concentration. In *S. surattense* at 0.75 % pectin recorded 0.176 mg g^{-1} (15.03%) solasodine whereas *S. incanum* 0.140 mg g^{-1} (40%) with 1.5 % pectin concentration. The results indicate an increased elicitation effect of pectin as compared to control culture. Similar increment in solasodine content was reported in *S. melongena* with an addition of 1% pectin as elicitor (Jain and Singh, 2015). Enhancement of hypericin biosynthesis by elicitation using pectin has been reported in different *Hypericum* species (Yamaner *et al.*, 2013; Gadzovska *et al*., 2015). The present results recommend the beneficial of pectin as an elicitor for *S. incanum* cell cultures for solasodine production. Biotic elicitation in suspension cultures can be an alternative with a potential, and can be applied in bioreactor systems. Recently, Bhaskar *et al.* (2022)

reported that inductors including algal, fungal, bacterial elicitors and their extracts can promote phytocompound production in cultures. Yeast extract is an important biotic elicitor rich in Vitamin B-complex. It is one of the most commonly used elicitor for elevating secondary metabolite production in cell suspension cultures. Elicitation effect of yeast extract was found more prominent in *S. villosum* followed by *S. incanum. S. nigrum* cell cultures were non-responsive in terms of enhancement of solasodine content towards yeast extract as an elicitor, Whereas *S. nigrum* showed 0.177 mg g^{-1} (1.72%) solasodine content with 3 g l^{-1} yeast extract treatment which was highest in concentration, followed by 0.176 mg g^{-1}(15.03%) in *S. villosum* with same elicitor. *S. surattense* indicated 0.173 mg g^{-1}(13.07%) solasodine while in *S. incanum* it was only 0.114 mg g^{-1} (14%)with 3.5 g l^{-1} yeast extract (Fig. 4). This is in agreement with solasodine production enhancement reported by Loc *et al.* (2014) where 3 g l^{-1} of yeast extract produced 220.5 mg g^{-1} of solasodine in *S. hainanense* which was higher than the control.

Yeast extract induced alkaloid production is reported to be efficient in *C. roseus* cell cultures (Maqsood and Abdul, 2017). Biotic elicitation by *E coli* and *B. subtilis* filtrates were used to elevate diosgenin production in *Helicteres isora* L. cell suspension cultures (Shaikh *et al.,* 2020). The results revealed that usage of bioticagents as elicitors can enhance the formation of product in terms of concentration and can save the process time for commercial level production of pharmacologically important secondary metabolites. Several limitations like higher cost, low amount of active phytochemicals produced by the plants grown in field are affecting the supply of medicinally important secondary metabolite to the pharmaceutical industries. *In-vitro* culture techniques are assisting for the conservation of natural sources and increasing the yield of these phytochemicals. Wide applications of abiotic and biotic elicitors are reported for the enhancement of secondary metabolite production in plant cell cultures and hairy root cultures (Halder *et al.,* 2019). Application of elicitors activate defence metabolic responses, and thus enhances secondary metabolite production.

Addition of elicitors can be a method for enhancing secondary metabolite yield *in-vitro*. Rini and Raghu (2020) demonstrated that the treatment of elicitors in plant cell culture may misguide the cultured cells by mimicking the external environmental conditions under stress, which will activate secondary pathways. In the present study, *Solanum villosum* exhibited best results in terms of solasodine concentration enhancement in response to pectin and yeast extract elicitors whereas *Solanum incanum* responded best to pectin followed by NaCl. Solasodine content increment was highest with NaCl elicitor addition in the media for *S. surratence* when compared to other elicitors. *S. nigrum* cell cultures responded best for solasodine content increase with application of pectin followed by NaCl as an elicitor. Success of an elicitation process for secondary metabolite production is affected by different factors like type of culture, type of elicitor and their concentration, and also on incubation period (Shakya *et al.,* 2019). The present results for assessing the efficacy of different elicitors indicated biotic elicitor pectin as one of the promising constituent for solasodine production from different Solanum cell cultures studied. Recently, Rakesh and Praveen (2022) Reproted significance of an optimized protocol to obtain higher biomass and high L-DOPA yield from the *in-vitro* cell suspension cultures of *Mucuna pruriens* (L.). Modifications in the suspension culture media can lead to higher production rate of secondary metabolites and addition of elicitors are noticed to be favourable by inducing stress in the cells. Plant species with higher solasodine content and enhanced properties can be used for the commercial production of phyto-constituent. Of the different Solanum species studied, it was indicated that in *in-vitro* cultured cells, solasodine concentration was higher in S. nigrum followed by *S. surattense* and *S. villosum*. The Elicitation effect, was more prominent in *S. surattense* and *S. incanum* cell cultures and was higher in response to pectin for solasodine production. Present study indicates that the response of plants cells to elicitors may vary from species to species and also plant cells respond variably to different elicitors. Optimizing the elicitor type for a particular species may enable the increased synthesis of biologically active components of medicinal value.

Several unique pharmacologically significant molecules like steroidal alkaloid- solasodine are obtained from different *Solanum* species. These glycoalkaloids are potential alternative to diosgenin for the commercial synthesis of steroidal drugs. The results obtained from this study revealed that potential elicitors likeNaCl, salicylic acid, pectin and yeast extract can be applied to elevate the production of solasodine from *Solanum* species cultivated *in vitro*. Elicitation effect was more prominent in *S. surattennse. S. incanum* cell cultures. Current research work can provide a baseline for cost effective method, by added biotic and abiotic elicitors, for large scale production of medicinally important phyto-constituent solasodine, using plant tissue culture system.

Acknowledgments

Authors are grateful to the Director, Dr. D.Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth, Pune for the research facilities. Also thanks to Secretary, Mahatma Gandhi Mission, Aurangabad and Principal, MGM College of Agricultural Biotechnology, Aurangabad for providing the laboratory facilities.

Authors' contribution: V. Deshmukh: Data collection, methodology, review; **J. Deshpande:** Review, manuscript draft preparation; **M. Wani:** Conceptualization, idea, data interpretation, editing and supervision.

Funding: The funding for the project is obtained from Dr. D. Y. Patil Vidyapeeth, Pune and MGM College of Agricultural Biotechnology, Aurangabad.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: The authors declare that there is no conflict of interest.

Data availability: Not applicable.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology*.

References

Abdel-Rahim, E.A., Y.E. Abdel-Mobdy, R.F. Ali and H.A. Mahmoud: Hepatoprotective effects of *Solanum nigrum* Linn fruits against cadmium chloride toxicity in Albino rats. *Biol. Trace. Elem. Res.,* **160**, 400-408 (2014).

Acikgoz, M.A., S.M. Kara, A. Aygun, M.M. Ozcan and A.Y.E. Bati: Effects of methyl jasmonate and salicylic acid on the production of camphor and phenolic compounds in cell suspension culture of endemic Turkish yarrow (*Achillea gypsicola*) species. *Turk. J. Agricul. Fore.,* **43**, 351-359 (2019).

Bhaskar, R., L.S.E. Xavier and G. Udayakumaran: Biotic elicitors: A boon for the *in-vitro* production of plant secondary metabolites. *Plant Cell Tissue Organ Cul.,* **149**, 7-24 (2022).

Bhat, M.A., S. Ahmad, J. Aslam, A. Mujib and Mahmooduzzfar: Salinity stress enhances production of solasodine in *Solanum nigrum* L. *Chem. Pharm. Bull. (Tokyo)*, **56**, 17-21 (2008).

Chakraborty, A. and S. Chattopadhyay: Stimulation of menthol production in *Mentha piperita* cell culture. *In-Vitro Cell. Dev. Biol. Plant,* **44**, 518-524 (2008).

Chester, K., S. Paliwal, W. Khan and S. Ahmad: UPLC-ESI-MS/MS and HPTLC Method for quantitative estimation of cytotoxic glycosides and aglycone in bioactivity guided fractions of *Solanum nigrum* L. *Front. Pharmacol.,* **8**, 434 (2017).

Desai, A.B., V.G. Kagathara, H. Joshi, A.T. Rangani and H. Mungra: Evaluation of antiamnesic effect of solasodine in mice. *Int. J. Pharm. Tech. Res.,* **3**, 732-740 (2011).

Desai, S., P. Tatke and S. Gabhe: A new validated HPLC-DAD method for estimation of solasodine from *Solanum nigrum* Linn. Extracts and formulations. *Nat. Prod. J.,* **4**, 1-5 (2014).

Deshmukh, V., S. Ballav, S. Basu, S. Mishra, J. Deshpande and M. Wani: Anti-proliferative activities of solasodine extracts from different Solanum spp. cell cultures on colon and bone carcinoma cell lines. *J. Appl. Biol. Biotechnol.,* **10**, 114-119 (2022).

Du, C.X., H.F. Fan, S.R. Guo, T. Tezuka and J. Li: Proteomic analysis of cucumber seedling roots subjected to salt stress. *Phytochemistry*, **71**, 1450-1459 (2010).

Gadzovska Simic, S., O. Tusevski, S. Maury, D. Alain, L. Eric, J. Claude and H. Daniel: Polysaccharide elicitors enhance phenylpropanoid and naphtodianthrone production in cell suspension cultures of *Hypericum perforatum*. *Plant Cell. Tissue. Organ Cult.,* **122**, 649–663 (2015).

Halder, M., S. Sarkar and S. Jha: Elicitation: A biotechnological tool for enhanced production of secondary metabolites in hairy root cultures. *Eng. Life Sci.,* **19**, 880–895 (2019).

Ho, T.T., K.C. Le, S.W. Kim and S.Y. Park: Culture condition optimization and FTIR analysis of *Polygonum multiflorum* Thunb. adventitious root cultures grown in an air-lift bioreactor system. *Plant Cell. Tissue. Organ Cult.,* **144**, 371–381. (2020).

Jain, A. and S. Singh: Effect of growth regulators and elicitors for the enhanced production of solasodine in hairy root culture of *Solanum melongena* (L.). *J. Indian Bot. Soc.,* **94**, 23-39 (2015).

Khan, T., T. Khan, C. Hano and B.H. Abbasi: Effects of chitosan and salicylic acid on the production of pharmacologically attractive secondary metabolites in callus cultures of *Fagonia indica*. *Indus. Crops Prod.,* **129**, 525–535 (2019).

Kokotkiewicz, A., B. Zabiegala, P. Kubica, A. Szopa, A. Bucinski, H. Ekiert and M. Luczkiewicz: Accumulation of volatile constituents in agar and bioreactor shoot cultures of *Verbena officinalis* L. *Plant Cell Tissue, Organ Cult.,* **144**, 671–679 (2021).

Loc, N.H., N.T. Huu, T.P. Anh, L.T. Thi, M. Khuyen and T. Nu: Effects of yeast extract and methyl jasmonate on the enhancement of solasodine biosynthesis in cell cultures of *Solanum hainanense* Hance. *J. BioSci. Biotech.,* **3**, 1-6 (2014).

Lu, M.B., H.L. Wong and A. Teng: Effects of elicitation on the production of saponin in cell culture of *Panax ginseng*. *Plant Cell Rep.,* **20**, 674-677 (2001).

Maqsood, M. and M. Abdul: Yeast extract elicitation increases vinblastine and vincristine yield in protoplast derived tissues and plantlets in *Catharanthus roseus*. *Rev. Bras. Farmacogn.,* **27**, 549–556 (2017).

Maurya, B., K.K. Rai, N. Pandey, L. Sharma, N.K. Goswami and S.P. Rai: Influence of salicylic acid elicitation on secondary metabolites and biomass production in *in-vitro* cultured *Withania coagulans* (L.). *Dunal. Plant Archi.,* **19**, 1308-1045 (2019).

Pandey, P., S. Singh and S Banerjee: *Ocimum basilicum* suspension culture as resource for bioactive triterpenoids: yield enrichment by elicitation and bioreactor cultivation. *Plant Cell Tissue Organ Cult.,* **137**, 65–75 (2019).

Patel, K., R.B. Singh and D.K. Patel: Medicinal significance, pharmacological activities, and analytical aspects of solasodine: A concise report of current scientific literature. *J. Acute. Dise.,* **2**, 92-98 (2013).

Pawar, P.K., T.P. Borse, R.Z. Pinjari and V.L. Maheshwari: A simple technique for rapid quantitative determination of solasodine from cultured hairy roots of *Solanum surattense*. *J. Herb. Med. Toxicol.,* **2**, 7-10 (2008).

Pitta-Alvarez, S.I., T.C. Spollansky and A.M. Giulietti: The influence of different biotic and abiotic elicitors on the production and profile of tropane alkaloids in hairy root cultures of *Brugmansia candida*. *Enzy. Microb. Technol.,* **26**, 252-258 (2000).

Rakesh, B. and N. Praveen: Establishment of *Mucuna pruriens* (L.) DC. callus and optimization of cell suspension culture conditions for the production of anti-Parkinson's drug: L-DOPA. *J. App. Biol. Biotech.,* **10**, 125-135 (2022).

RiniVijayan, K.P. and A.V. Raghu: Methods for enhanced production of metabolites under *in-vitro* conditions. In: Plant Metabolites: Methods, Applications and Prospects (Eds.: S. T. Sukumaran, S. Sugathan and S. Abdulhameed). Springer Nature Singapore Pvt. Ltd., 111-140 (2020).

Rocha, L.K., A.J. Oliveira, C.A. Mangolin and M.F.P.S. Machado: Effect of different culture medium components on the production of alkaloid in callus tissue of *Cereus peruvianus* (Cactaceae). *Acta. Scientiarum. Biol. Sci.,* **27**, 37-41 (2005).

Shaikh, S., V. Shriram, T. Khare and V. Kumar: Biotic elicitors enhance diosgenin production in *Helicteres isora* L. suspension cultures via up-regulation of CAS and HMGR genes. *Physiol. Mol. Biol. Plants*, **26**, 593–604 (2020).

Shakya, P., G. Marslin, K. Siram, L. Beerhues and G. Franklin: Elicitation as a tool to improve the profiles of high-value secondary metabolites and pharmacological properties of *Hypericum perforatum*. *J. Pharm. Pharmacol.,* **71**, 70-82 (2019).

Sharma, M., A. Sharma, A. Kumar and S.K. Basu: Enhancement of secondary metabolites in cultured plant cells through stress stimulus. *American J. Plant Physiol.,* **6**, 50-71 (2011).

Singh, A. and P. Dwivedi: Methyl-jasmonate and salicylic acid as potent

elicitors for secondary metabolite production in medicinal plants: A review. *J. Pharma. Phyto.,* **7**, 750-757 (2018).

Solouki, M., H. Ramroudi and A. Tavassoli: Comparison and evaluation of steroid alkaloid solasodine on *in vivo* and *in vitro* cultures of *Solanum surattense* Burm L. *Afr. J. Microbiol. Res.,* **5**, 3810-3814 (2011).

Sridhar, T.M. and C.V. Naidu: An efficient callus induction and plant regeneration of *Solanum nigrum* (L.) - An important antiulcer medicinal plant. *J. Phytol.,* **3**, 23-28 (2011).

Srivastava, M., S. Sharma and P. Misra: Elicitation based enhancement of secondary metabolites in *Rauwolfia serpentina* and *Solanum khasianum* hairy root cultures. *Pharmaco. Maga.,* **12**, 315-320 (2016).

Sutkovic, J., D. Ler and M.R.A. Gawwad: *In vitro* production of solasodine alkaloid in *Solanum nigrum* under salinity stress. *J. Phytol.,* **3**, 43-49 (2011).

Szopa, A., A. Kokotkiewicz, M. Bednarz, K. Jafernik, M. Luczkiewicz and H. Ekiert: Bioreactor type affects the accumulation of phenolic acids and flavonoids in microshoot cultures of *Schisandra chinensis (Turcz.)* Baill. *Plant Cell Tissue Organ. Cult.,* **139**, 199–206 (2019).

Taguchi, G., T. Yazawa, N. Hayashida and M. Okazaki: Molecular cloning and heterologous expression of novel glucosyltransferases from tobacco cultured cells that have broad substrate specificity and are induced by salicylic acid and auxin. *Eur. J. Biochem.,* **268**, 4086-4094 (2001).

Thaweesak, J., S. Seiichi, T. Hiroyuki and P. Waraporn: Elicitation effect on production of plumbagin in *in-vitro* culture of *Drosera indica* L. *J. Med. Plant. Res.,* **5**, 4949- 4953 (2011).

Timperio, A.M., M.G. Egidi and L. Zolla: Proteomics applied on plant abiotic stresses: role of heat shock proteins (HSP). *J. Proteomics,* **71**, 391-411 (2008).

Toro, M.T., J. Ortiz, J. Becerra, N. Zapata, P. Fierro, M. Illanes and M.D. López: Strategies of elicitation to enhance bioactive compound content in edible plant sprouts: A bibliometric study. *Plants (Basel, Switzerland*), **10**, 2759 (2021).

Yamaner, O., B. Erdag and C. Gokbult: Stimulation of the production of hypericins in *in vitro* seedlings of *Hypericum adenotrichum* by some biotic elicitors. *Turkish J. Bot.,* **37**,153-159 (2013).

On Line Copy