Sodium nitroprusside enhances biomass and gymnemic acids production in cell suspension of Gymnema sylvestre (Retz.) R.Br. ex. Sm.

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Received: 25 January 2021 / Accepted: 16 March 2021 © The Author(s), under exclusive licence to Springer Nature B.V. 2021, corrected publication 2021

Abstract
Gymnemic acids (a group of triterpenoid saponins) found in Gymnema sylvestre (Retz.) R.Br. ex Sm. works as the main hypoglycaemic active components. These can be the potential active pharmaceutical ingredient (API) to be used by pharmaceutical industries in modern medicines against diabetes. The present study aims to investigate the effectiveness of sodium nitroprusside (SNP) treatment for enhancement of cell suspension culture biomass and to quantify the production of deacylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII contents. Callus was obtained from in vitro derived leaves of G. sylvestre on MS medium fortified with 3.0 mg/L 2, 4-dichlorophenoxyacetic acid and 1.0 mg/L Kn (Kinetin), and the same were used further to produce cell suspension cultures. Cell suspensions were exposed to different concentrations of SNP (5, 10, 20 and 40 μM) and data were collected at 20, 30 and 40 days. Out of the tested concentrations, 20 μM SNP had the highest level of cell culture growth (398.94 ± 8.32 g/L Fresh cell weight (FCW) and 40.00 ± 0.75 g/L Dry cell weight (DCW) on 40-day as compared to control (MS with 3.0 mg/L 2, 4-dichlorophenoxyacetic acid and 1.0 mg/L Kn). High-performance liquid chromatography analysis showed that maximum accumulation of deacylgymnemic acid (5.51 mg/g DCW), gymnemagenin (2.80 mg/g DCW) and gymnemic acid XVII (2.08 mg/g DCW) in 20 μM SNP treatment which is (13.43, 13.86 and17.33 folds) higher than the respective control at 40 days exposure. This research suggests that Gymnema sylvestre cell suspension culture with optimal SNP elicitation treatment could be used as a good strategy for the large-scale production of these secondary metabolites at the industrial level.

Key message
This research suggests that Gymnema sylvestre cell suspension culture with SNP elicitation treatment could be used as a good strategy for the large-scale production of these secondary metabolites at the industrial level.

Keywords Suspension culture · Elicitation · Medicinal plant · Secondary metabolites

Communicated by Mohammad Faisal.
Introduction

Gymnema sylvestre (Retz.) R.Br. ex Sm. belongs to the family Apocynaceae, it is a slow growing perennial woody climber. It contains many phytomolecules among them gymnemic acids (GAs) and gymnemasonapins are major secondary metabolites classified as triterpenoid saponins having oleanane type structural conformations. These are glycosidic compounds having one or more glucose or its derivative unit directly attached to them through ester bond. These compounds are proved to block intestinal site hence decreasing overall glucose adsorption therefore used for clinical applications (Laha and Paul 2019; Khan et al. 2019). The plant takes more than two years to be fully mature and can be harvested only twice a year. The plant leaf is the part which majorly bears GAs that is only ≥ 2% w/w of dry leaves. G. sylvestre has been used in Ayurveda medicine since antiquity to treat diabetes, malaria and snake bite (Anjum and Hasan 2013; Khan et al. 2019). Ayurvedic practitioners prescribe this plant for the treatment of dyspepsia, jaundice, asthma, leucoderma and bronchitis (Anis et al. 2000; Laha and Paul 2019; Khan et al. 2019). Kanetkar et al. (2007) and Singh et al. (2008) found multiple potential applications mentioned in Ayurveda, Siddha and Unani system of medicine in India for treating diverse human complaints, but only a few achieved scientific information on its secondary metabolites production.

Plant cell and tissue cultures ensured that cell suspension cultures have been explored to serve as an alternative source for large scale production of useful secondary metabolites (Thorat et al. 2017). Also, accumulation of secondary metabolite in in vitro cultures outperformed those seen in plants cultivated in the field (Das and Bandyopadhyay 2020). Accumulation of secondary phyto-constituents in cell suspension or callus culture is highly dependent on different types of elicitors which lead to activate biosynthetic pathway route key enzymes (Sharan et al. 2019; Pandey et al. 2020).

The recent studies showed that signalling molecules involved in the regulation of plant metabolite synthesis include reactive oxygen species, nitric oxide (NO), calcium ion, polyamines (PAs) salicylic acid and jasmonic acid (Ma et al. 2013). Sodium nitroprusside (SNP), highly reactive bioactive molecule that plays an important role in plant tissue and organ culture such as growth and development of plant (Kolbert et al. 2008), stimulates callogenesis regeneration response in Albizzia lebbeck (Kalra and Babbar 2010), enhances callus and multiple shoot induction (Sarropoulou and Maloupa 2017; Subiramani et al. 2019; Pandey et al. 2020), promote root formation and callus induction (Hesami et al. 2020) and enhanced shoot regeneration and improved salinity stress in soybean (Karthik et al. 2019). Ötvös et al. (2005) reported that SNP in combination with 2,4-d significantly stimulated cell division and embryogenic cell growth in Medicago sativa. In Hyoscyamus niger, supplementation of SNP at 50 μM increased callus fresh weight (Samsampour et al. 2018). As a result, this led us to study the effect of sodium nitroprusside (SNP) in secondary metabolites production from suspension culture of G. sylvestre for enhancement of deacylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII metabolites.

Material and methods

Resource of plant material and culture condition

In this study, Gymnema sylvestre immature follicles were obtained from the field of CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow. The immature follicles were sterilized according to our previous experiment (Mahendran et al. 2021). The immature embryo was inoculated on MS (Murashige and Skoog 1962) basal medium with sucrose (3.0%) and agar (0.8%) to initiate aseptic seedlings. The seedlings were maintained in cooling white fluorescent light (60 μmol m–2 s–1) with light/dark (16/8 h) of photoperiod at 25 ± 2 °C. In vitro derived young leaves were utilized as explants for the establishment of callus and cell suspension culture of G. sylvestre.

Chemicals

Murashige and Skoog (1962) medium, 2,4-Dichlorophenoxyacetic, (2,4-d), Kinetin (Kn), and Sodium nitroprusside (SNP) were purchased plant tissue culture grade from Hi-media (Mumbai, India). HPLC grade methanol and acetonitrile obtained from Merck (Mumbai, India) and ultra-pure water was prepared using a Milli-Q (Millipore, France) was used. Deacylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII standards were supplied by Sigma-Aldrich (Münich, Germany).

Cell suspension culture conditions

In the present study, cell suspension cultures initiated from 30 days old whitish friable callus (1 g) that was inoculated into 50 mL of MS liquid medium supplemented with 2,4-d (3 mg/L) and Kn (1.0 mg/L) combination served as a control. The cultures were incubated on a shaker (100 rpm) at 25 ± 2 °C for 16/8 h (light/dark).
**Sodium nitroprusside (SNP) elicitation**

In the present investigation, SNP was dissolved in distilled sterile water and sterilized through syringe filters then used at different concentrations (5, 10, 20 and 40 μM). SNP supplemented on the same day of cell suspension initiated and the cells were harvested at 20, 30 and 40 days. Cell suspension culture was collected from the medium at 20, 30 and 40 days for determination of cell growth biomass accumulation (fresh and dry cell weight) and yield of GAs contents.

At the end of each culture intervals, the cell suspension was harvested from the culture medium for fresh cell weight and dry cell weight measurement. Harvested cells passed through a two-layer muslin cloth to remove the water from the cell and measured the fresh cell biomass weight. Further, cells biomass was placed on a Petri dish and dried at 50 °C in a hot air oven for 24 h and cell dry biomass measured.

**Morphology of suspension cells**

100 mg cells from suspension cell cultures were checked in 1 mL 0.005% (w/v) neutral red and acridine orange solution to analysis the morphological variations during growth phases. 30 μL of stained cells were transferred to the slide and observed under the light microscope (Olympus, India).

**High-performance liquid chromatography (HPLC) analysis**

**Sample and standards stock preparation**

Exactly weighed 1 g of dried biomass obtained from cell culture and wild plant leaves were grounded into a relatively homogenous powder using a grinder. Powder was then defatted using 10 mL of petroleum ether at 50 °C for 6 h and dried. Sample was then extracted with a mixture of 70:30 (Water: Methanol) at 50 °C for 6 h for complete extraction of gymnemic acids. The extract was dried using rotatory evaporator and 10 mg of each of prepared extract was weighed accurately and dissolved in methanol (1 mL) for making final concentration of 10 mg/mL and kept 15 min for sonication and filtered on a 4 mm membrane filter and kept at 4 °C until further use.

Deacylgymnemic acid, gymmemagenin, gymnemic acid IV and gymnemic acid XVII standards were supplied by Sigma-Aldrich (Münich, Germany). Standard stocks were prepared by diluting each of them separately at a concentration of 1 mg/mL in methanol. 20 μL of injection volume of sample was injected into the HPLC system.

**Quantification of deacylgymnemic acid, gymmemagenin, gymnemic acid IV and gymnemic acid XVII**

Our previously reported gradient method was used for the quantification of GAs in samples Mahendran et al. (2021). The chromatographic conditions were Waters HPLC system built with a binary pump, photodiode array detector (PDA) and Rheodyne injector. SunFire C18 column (5 µm, 250×4.6 mm) and Empower Pro software (Waters, USA) was used for analysis. The detection was achieved at 205 nm, column temperature kept at 30 °C and the flow rate was 1 mL/min. The deacylgymnemic acid, gymmemagenin, gymnemic acid IV and gymnemic acid XVII contents were quantified using peak area obtained through HPLC.

**Statistical analysis**

Each experiment was repeated thrice containing 5 replicates each and data expressed in terms of mean ± standard deviation (SD). The significant difference between control and SNP treatment was analysed by Tukey’s HSD (honestly significant difference) test using one-way ANOVA with IBM SPSS statistics (version 20.0, USA). Significant was considered when P < 0.05.

**Results and discussion**

**Initiation and growth of cell suspension cultures**

Nitric oxide (NO) is a bioactive molecule which can be generated through the breakdown of NO donor sodium nitroprusside molecules (SNP). Recently, SNP has been reported as an elicitor involved in regulating several signalling responses (Siddiqui et al. 2017; Khezerluo et al. 2018; Hao et al. 2020; Kara et al. 2020). In previous studies, many researchers have reported that SNP treatments are very effective in shoot regeneration of Chrysanthemum cultivars (Arun et al. 2017), Canscora diffusa (Subiramani et al. 2019), *Antirrhinum majus* (Rezaei Zafarghandi and Rahmati Joneidabad 2020) *Valeriana jatamansi* (Pandey et al. 2020) and callus induction in Dioscorea opposita (Xu et al. 2018), *Hyoscyamus niger* (Samsampour et al. 2018), *Canscora diffusa* (Subiramani et al. 2019), *Ficus religiosa* (Hesami et al. 2020) and *Valeriana jatamansi* (Pandey et al. 2020). Furthermore, SNP play important roles in increasing metabolites accumulation (Khezerluo et al. 2018; Pradhan et al. 2020; Tsolmon et al. 2020).

A perusal of literature revealed that cell suspension culture has to be the most capable method used to increase the cell growth, biomass and secondary metabolite production in vitro cultures (Mahendran et al. 2018; Hu et al. 2019; Açıkgóz, 2020). In this study, white friable callus obtained
The accumulation of FCW and DCW were 71.21 ± 1.56 and 40.00 ± 0.75 g/L DCW) and was nearly 1.17 folds higher at 40th day with 20 μM SNP (398.94 ± 8.32 g/L FCW and 7.05 ± 0.04 g/L observed on 20th day with 20 μM SNP that were 1.43 and 1.41 times higher compared with control. The maximum amount of biomass accumulation was achieved from 3.0 mg/L (2, 4-D) + 1.0 mg/L (Kn) (Fig. 1A, B), and same combination was used to establish a culture of cell suspension in which there were no aggregations or clumps of cells noted. In the present study, suspension cell culture growth kinetic was monitored in terms of fresh cell weight (FCW) and dry cell weight (DCW) and results are shown in Fig. 2. Cell suspension growth was shown to be increased greatly with the increasing concentration of SNP. Among the different concentrations of SNP tested, the highest accumulation of FCW and DCW were 71.21 ± 1.56 and 7.05 ± 0.04 g/L observed on 20th day with 20 μM SNP that were 1.43 and 1.41 times higher compared with control. The biomass accumulation in cell culture increased with increasing concentration of SNP up to an optimal level of 20 μM (Fig. 1C). SNP has increased the growth of cell culture biomass at 5–20 μM concentrations tested and the entire exposure period of the study compared with the control. The maximum amount of biomass accumulation was achieved at 40th day with 20 μM SNP (398.94 ± 8.32 g/L FCW and 40.00 ± 0.75 g/L DCW) and was nearly 1.17 folds higher than control. Earlier studies demonstrated that elicitors are very effective for the enhancement of cell growth and increase metabolites accumulation. Furthermore, the type of elicitors, concentration and exposure period favours the biomass accumulation in cell suspension culture (Salma et al. 2020; Zare-Hassani et al. 2019; Açıkçıgöz, 2020).

Our study confirms the efficiency of SNP treatments, promoted cell growth and biomass accumulation. Similar effectiveness of SNP on cell growth has been reported in Hypericum perforatum (Xu et al. 2005). Furthermore, SNP in combination with 2,4-D significantly promoted embryogenic cell in Medicago sativa (Ötvös et al. 2005). Some studies have shown that SNP either alone or in combination treatments are very effective for callus induction (Samsampour et al. 2018; Subiramani et al. 2019; Pandey et al. 2020). Results of the present investigation in G. sylvestre cell suspension cultures suggested that SNP at 40 μM drastically diminished cell growth and biomass accumulation (225.39 ± 5.13 g/L FCW) compared with control (339.08 ± 1.76 g/L FCW) at 40 days (Fig. 2). Similar inhibitory effects have been reported at higher concentrations of elicitors in Psoralea corylifolia (Gajula et al. 2018), Leucaena aspera (Vijendra et al. 2020) and Ocimum basilicum (Açıkçıgöz, 2020).

**Morphology of G. sylvestre cell suspension**

The morphological differences at different growth times in the suspension cultures are shown in Fig. 1C, F. The cells were rapidly growing and individually or in cell masses of small groups of cells aggregated and settled at the bottom of the flask were detected in the suspension culture (Fig. 1C, D). The shape of the cells varied in size with round, oval shape or elongated shaped and health of cell visible clearly nuclei and cell components (Fig. 1G, H). In contrast, death cells showed cytoplasm and cell wall shrinkage (Fig. 1E, F, I).

**HPLC analysis of gymnemic acids**

To enhance the deacetylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII content in suspension culture of G. sylvestre, different concentrations of SNP (5, 10, 20 and 40 μM) and days (20, 30 and 40) were investigated. The results displayed that SNP had significantly boosted the production of deacetylgymnemic acid, gymnemagenin, gymnemic acid IV and gymnemic acid XVII at all tested concentration over control. However, SNP at 20 μM dose produced the highest accumulation of deacetylgymnemic acid (1.80 mg/g DCW), gymnemagenin (1.91 mg/g DCW), gymnemic acid IV (0.74 mg/g DCW) and gymnemic acid XVII (1.14 mg/g DCW) contents for 20 days of exposure in cell suspension culture, which was represented 6.09, 17.36, 1.01 and 9.5 folds higher compared with the control cultures (Fig. 3A–D). Similarly, Xu et al. (2005) demonstrated that cell cultures of Catharanthus roseus treated with 10 and 20 mM SNP enhanced formation of catharanthine, ajmalicine and total alkaloids. Khezerluo et al. (2018) found that maximum accumulation of hyoscyamine and scopolamine production (1.2-fold and 1.5-fold) in hairy root culture of Hyscynamus reticulatus was detected at 50 and 100 μM SNP at 48 and 24 h.

Interestingly, higher amount of gymmegenin (2.86 mg/g DCW) and gymnemic acid IV (1.24 mg/g DCW) was also observed at 5 μM of SNP treated suspension cell culture at 30 and 40 days of culture respectively (Figs. 4B and 5C). Likewise, the maximum (3.25 mg/g) gymnemic acid XVII production was observed at 10 μM SNP at 40 days (Fig. 5D). Similarly, Wang et al. (2009) reported that SNP (10 μM) and cerebrosides (30 μg/mL) combination in hairy root callus of Artemisia annua exhibited more effective for improving artemisinin production up to 2.3-fold over the control. In another study, researchers reported that lower concentrations of SNP (15 μM) have been more effective in enhancing catharthine production in suspension cells culture of C. roseus (Xu and Dong 2005). Likewise, the maximum 3.2-fold hypericin production in suspension
culture was observed at 15.0 mmol/L SNP at 14 days (Xu et al. 2005).

In the present study, SNP at 20 μM treatment at 30 days resulted in the highest deacylgymnemic acid (2.32 mg/g DCW) Fig. 4A, whereas 40 μM SNP treatment at 30 days cell suspension culture period produced maximum of gymnemagenin (2.53 mg/g DCW), gymnemic acid IV (1.24 mg/g DCW) and gymnemic acid XVII (1.90 mg/g DCW) contents over control and 20 μM SNP (Fig. 4B–D). Nevertheless 20 μM SNP treatment yielded higher 13.43

Fig. 2 Growth index of G. sylvestre cell suspension culture indicating the fresh and dry biomass of cells and time intervals (20th, 30th and 40th day)

Fig. 3 Effect of different concentrations of SNP on deacylgymnemic acid (A), gymnemagenin (B), gymnemic acid IV (C) and Gymnemic acid XVII mg/g (D) content in cell suspension cultures of G. sylvestre at 20 days
Fig. 4 Effect of different concentrations of SNP on deacylgymnemic acid (A), gymnemagenin (B), gymnemic acid IV (C) and Gymnemic acid XVII mg/g (D) content in cell suspension cultures of *G. sylvestre* at 30 days.

Fig. 5 Effect of different concentrations of SNP on deacylgymnemic acid (A), gymnemagenin (B), gymnemic acid IV (C) and Gymnemic acid XVII mg/g (D) content in cell suspension cultures of *G. sylvestre* at 40 days.
Fig. 6  HPLC chromatogram of *G. sylvestre* suspension cells. A HPLC chromatogram of 4 standard mixed solutions. B 20 μM SNP treatment at 30 days. C 40 μM SNP treatment at 30 days
(5.51 mg/g DCW) fold deacylgymnemic acid (Fig. 5A), 13.86 fold (2.80 mg/g DCW) gymnemagenin (Fig. 5B) and 17.33 fold (2.08 mg/g DCW) gymnemic acid XVII production in G. sylvestre cell suspension cultures compared with respective control, however, this deacylgymnemic acid, gymnemagenin and gymnemic acid XVII yield was found to be 3.11, 1.78 and 1.18 fold lesser at SNP (40 μM) treated cultures compared with respective SNP 20 μM. In contrast to this study, Tsołmon et al. (2020) in their investigation of Sophora alopecuroides cell suspension culture, reported that accumulation of oxymatrine content was 18.6 μg/g higher in suspension culture treated at higher 200 μM Jasmonic acid (JA) and 50 μM SNP combination. The ginsenoside accumulation was recorded highest at 200 μM of SNP treatment (Rahimi et al. 2016).

Conclusion

In conclusion, this work suggested that the effects of SNP treatment approach improved cell culture growth (biomass) and yield of gymnemic acids (triterpenoid saponins) content in cell suspension cultures of G. sylvestre. 20 μM SNP treatment and exposure time of 40 days showed the highest rate of cell culture biomass accumulation (398.94 ± 8.32 g/L FCW and 40.00 ± 0.75 g/L DCW) and the maximum production of deacylgymnemic acid (5.51 mg/g DCW), gymnemagenin (2.80 mg/g DCW) and gymnemic acid XVII (2.08 mg/g DCW) compared with the control culture. The present study has demonstrated that establishment of efficient cell suspension cultures of G. sylvestre for biomass and enhanced production of triterpenoid saponins (gymnemic acids) at industrial scale.

Acknowledgements

First author gratefully acknowledges the financial support by SERB, New Delhi, India for awarding DST-SERB-National Post-Doctoral Fellowship (PDF/2017/000368). The authors are also thankful to the Director, CSIR-CIMAP for providing necessary facilities.

Author contributions

GM: designed the experiment, performed tissue culture experiments, contributed to writing and edited manuscript. DK: designed the experiment, performed HPLC experiments and analysed data. SKV: performed tissue culture experiments, analysed data., SF, AC, ZIW and ZH: performed tissue culture experiments. PKR: conceptualization, methodology, formal analysis and editing, LR: conceptualization, supervision, funding acquisition, review, editing and improved the manuscript.

Declarations

Conflict of interest

All authors declare that there is no conflict of interest.

References

Açıkgoz MA (2020) Establishment of cell suspension cultures of Gymnema sylvestre (Gurmar) L., and enhanced production of pharmaceutical active ingredients. Ind Crop Prod 148:112278

Anis M, Sharma MP, Iqbal M (2000) Herbal ethnomedicine of the Gwalior forest division in Madhya Pradesh. India Pharm Biol 38(4):241–253

Anjum T, Hasan Z (2013) Gymnema Sylvestre plant used by peoples of Vidisha district for the treatment of diabetes. Int J Eng Sci Invent 2(6):98–102

Arun M, Naing AH, Jeon SM, Ai TN, Aye T, Kim CK (2017) Sodium nitroprusside stimulates growth and shoot regeneration in Chrysanthemum. Hortic Environ Biotechnol 58:78–84

Das D, Bandyopadhyay M (2020) Novel approaches towards overproduction of andrographolide in vitro seedling cultures of Andrographis paniculata. S Afr J Bot 128:77–86

Gajula H, Kumar V, Vijendra PD, Rashajekar J, Sannabommaji T, Basappa G (2018) A combination of elicitor and precursor enhances psoralen production in Psoralea corylifolia Linn. suspension cultures. Ind Crop Prod 124:685–691

Hao YJ, An XL, Sun HD, Piao XC, Gao R, Lian ML (2020) Ginsenoside synthesis of adventitious roots in Panax ginseng promoted by fungal suspension homogenate of Alternaria panax and regulated by several signalling molecules. Ind Crops Prod 150:112414

Hesami M, Tohidfar M, Alizadeh D, Daneshvar MH (2020) Effects of sodium nitroprusside on callus browning of Ficus religiosa: an important medicinal plant. J For Res 31(3):789–796

Hu M, Hu Z, Du L, Du J, Luo Q, Xiong J (2019) Establishment of cell suspension culture of Lonicera japonica Thunb. and analysis of its major secondary metabolites. Ind Crops Prod 137:98–104

Kalra C, Babbar SB (2010) Nitric oxide promotes in vitro organogenesis in Linum usitatissimum L. Plant Cell Tiss Organ Cult 103:353–359

Kanetkar P, Singhal R, Kamat M (2007) Gymnema sylvestre : a memoir. J Clin Biochem Nutr 41(2):77–81

Kara Z, Yazar K, Doğan O, Vergili E (2020) Sodium nitroprusside and gibberellin effects on seed germination and seedling development of grapevine (Vitis vinifera L.). Cvs. Eksî Kara and Gök Üzüm Erwerbs-Obstbau 62:61–68

Karthik S, Pavan G, Krishnan V, Satish S, Manickavasagam M (2019) Sodium nitroprusside enhances regeneration and alleviates salinity stress in soybean [Glycine max (L.) Merrill]. Biocat Agric Biotechnol 19:101173

Khan F, Sarkar MMR, Ming LC, Mohamed IN, Zhao C, Sheikh BY, Tsong HF, Rashid MA (2019) Comprehensive review on phytochemicals, pharmacological and clinical potentials of Gymnema sylvestre. Front Pharmacol 10:1223

Khezerloo M, Hosseini B, Amiri J (2018) Sodium nitroprusside stimulated production of tropane alkaloids and antioxidant enzymes activity in hairy root culture of Hyoscyamus reticulatus l. Acta Biol Hung 69(4):437–448

Kolbert Z, Bartha B, Erdei L (2008) Exogenous auxin-induced NO synthesis is nitrate reductase-associated in Arabidopsis thaliana root primordia. J Plant Physiol 165:967–975

Laha S, Paul S (2019) Gymnema sylvestre (Gurmar): a potent herb with anti-diabetic and antioxidant potential. Pharmacog J 11:201–206

Ma MM, Zhan YG, Wang XD, Sun ML, Fan GZ (2013) Interaction between putrescine and hydrogen peroxide on regulating triterpenoid production in Betula platyphylla. Chinese Tradit Herb Drugs 44:2916–2922

Mahendran D, Kavi Kishor PB, Sreeramanan S, Venkatachalam P (2018) Enhanced biosynthesis of colchicine and...
thiocolchicoside contents in cell suspension cultures of *Glo-
riosa superba* L. exposed to ethylene inhibitor and elicitors. Ind Crops Prod 120:123–130
Mahendran G, Iqbal Z, Kumar D, Verma SK, Rout PK, Rahaman L (2021) Enhanced gymnemic acids production in cell suspension cultures of *Gymnema sylvestre* (Retz.) R.Br. ex Sm. through elicitation. Ind Crop Prod 162:113234
Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:173–197
Ötvos K, Pasternak TP, Miskolezi P, Domoki M, Dorjgotov D, Szucs A, Bottka S, Dudits D, Feher A (2005) Nitric oxide is required for, and promotes auxin-mediated activation of, cell division and embryogenic cell formation but does not influence cell cycle progression in *Alfalfa* cell cultures. Plant J 43:849–860
Pandey S, Sundararajan S, Ramalingam S, Pant B (2020) Effects of sodium nitroprusside and growth regulators on callus, multiple shoot induction and tissue browning in commercially important *Valeriana jatamansi* Jones. Plant Cell Tiss Organ Cult 142:653–660
Pradhan N, Singh P, Dwivedi P, Pandey DK (2020) Evaluation of sodium nitroprusside and putrescine on polyethylene glycol induced drought stress in *Stevia rebaudiana* Bertoni under in vitro condition. Ind Crops Prod 154:112754
Rahimi S, Kim YJ, Renuka Devi BS, Oh JY, Kim SY, Kwon WS, Yang DC (2016) Sodium nitroprusside enhances the elicitation power of methyl jasmonate for ginsenoside production in Panax ginseng roots. Res Chem Intermed 42:2937–2951
Rezaei Zafarghandi MS, Rahmati-Joneidabad M (2020) Indirect shoot organogenesis and in vitro root formation of *Antirrhinum majus* L. by using sodium nitroprusside. Adv Hortic Sci 34(1):105–111
Salma U, Kundu S, Ali MdN, Nirma Mandal N (2018) Elicitor-mediated enhancement of wedelolactone in cell suspension culture of *Eclipta alba* (L.) Hassk. Plant Cell Tiss Org Cult 134:409–421
Samsampour D, Sadeghi F, Asadi M, Ebrahimzadeh A (2018) Effect of nitric oxide (NO) on the induction of callus and antioxidant capacity of *Hyoscyamus niger* under in vitro salt stress. J Appl Bot Food Qual 91:24–32
Sarropoulou V, Maloupa E (2017) Effect of the NO donor sodium nitroprusside (SNP), the ethylene inhibitor cobalt chloride (CoCl2) and the antioxidant vitamin E “α-tocopherol” on *in vitro* shoot proliferation of *Sideritis raeseri* Boiss & Heldr subsp *Raeseri*. Plant Cell Tiss Organ Cult 128:619–629
Sharan S, Sarin NB, Mukhopadhyay K (2019) Elicitor-mediated enhanced accumulation of ursolic acid and eugenol in hairy root cultures of *Ocimum tenuiflorum* L. is age, dose, and duration dependent. S Afr J Bot 124:199–210
Siddiqui MH, Alamri SA, Al-Khaisany MYY, Al-Qutami MA, Ali HA, Khan MN (2017) Sodium nitroprusside and indole acetic acid improve the tolerance of tomato plants to heat stress by protecting against DNA damage. J Plant Interact 12(1):177–186
Singh VK, Umar S, Ansari SA, Iqbal M (2008) *Gymnema sylvestre* for diabetics. J Herbs Spices Med Plants 14(1–2):88–106
Subiramanii S, Sundararajan S, Sivakumar HP, Rajendran V, Ramal-
ingam S (2019) Sodium nitroprusside enhances callus induction and shoot regeneration in high value medicinal plant *Canscora diffusa*. Plant Cell Tiss Org Cult 139:65–75
Thorat AS, Sonone NA, Choudhuri VV, Devarumath RM, Babu KH (2017) Plant regeneration from cell suspension culture in *Sacharum officinarum* L. and ascertaining of genetic fidelity throughRAPD and ISSR markers. 3 Biotech 7:16
Tsolmon M, Oyundari G, Yu O, Kalaisev S (2020) Establishment of callus and cell suspension culture of *Sophora alopecuroides* Linn. for the production of oxymatrine. J Physiol 12:035–039
Vijendra PD, Jayanna SG, Kumar V, Torankumar Sannabommaiji T, Rajashekar J, Hari Gajula H (2020) Product enhancement of triterpenoid saponins in cell suspension cultures of *Leucas aspera* Spreng. Ind Crops Prod 156:112857
Wang JW, Zheng LP, Zhang B, Zou T (2009) Stimulation of artemisinin synthesis by combined cerebroside and nitric oxide elicitation in *Artemisia annua* hairy roots. Appl Microbiol Biotechnol 85:285–292
Xu M, Dong J (2005) Elicitor-induced nitric oxide burst is essential for triggering catharanthine synthesis in *Catharanthus roseus* suspension cells. Appl Microbiol Biotechnol 67:40–44
Xu M, Dong J, Zhang G (2005) Enhancement of hypericin production and cell growth of *Hypericum perforatum* L. suspension culture by nitric oxide, Chin J Biotechnol 21:66–70
Xu J, Yin H, Wang W, Mi Q, Liu X (2009) Effects of sodium nitroprus-
side on callus induction and shoot regeneration in micropropagated *Dioscorea opposita* opposita. Plant Growth Regul 59:279–285
Zare-Hassani E, Motafakkerazad R, Razeghi J, Kosari-Nasab M (2019) The effects of methyl jasmonate and salicylic acid on the production of secondary metabolites in organ culture of *Ziziphora persica*. Plant Cell Tiss Org Cult 138:437–444

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