Plant Cell Tiss Organ Cult (2016) 126:1–18
DOI 10.1007/s11240-016-0985-6

REVIEW

Chemical elicitors versus secondary metabolite production in vitro using plant cell, tissue and organ cultures: recent trends and a sky eye view appraisal

Charu Chandra Giri[1] · **Mohd Zaheer**[1]

Received: 23 September 2015 / Accepted: 28 March 2016 / Published online: 5 April 2016

Abstract Plant secondary biosynthetic pathways are exceedingly inducible by elicitors and facilitate enhanced metabolite production using plant cell tissue and organ cultures. Elicitors can regulate large number of control points and trigger the expression of key genes with increased cellular activities at biochemical and molecular level involving signal compounds. A large number of chemical elicitors viz: jasmonic acid (JA), methyl jasmonate (MeJA), salicylic acid (SA), acetyl salicylic acid (ASA), ethylene (ET) and ethrel (Ethe), heavy metals (HM), many types of chemical compounds (natural or synthetic) and their combinations are used for elicitation studies. Cell suspensions and hairy roots are commonly used culture systems followed by adventitious roots and multiple shoots for elicitation experiments. Amongst the elicitors and concentrations used 100 µM MeJA was found optimum for secondary metabolite enhancement in majority of experiments compared to SA and JA. Elicitor treatments promoted yield enhancements starting from 1.0 to maximum of 2230-fold across plant species studied. Elicitors singly with media additives, combination of elicitors and elicitors other than signal compounds along with hormones were found beneficial for enhanced secondary metabolite production. Further, a combination of target gene over expression and elicitor treatment also supported higher secondary product yield. The present communication presents information exclusively about the use of chemical elicitors for secondary metabolites production in vitro covering approximately more than a decade of research at one place in one review. Further, this extensive appraisal will be useful for the understanding and manipulation of secondary metabolites for enhanced production in vitro.

Keywords Culture systems · Elicitation · Mode of action · Overview · Yield enhancement

✉ Charu Chandra Giri
giriccin@yahoo.co.in

[1] Centre for Plant Molecular Biology (CPMB), Osmania University, Hyderabad, Telangana 500007, India

Introduction

Plants synthesize a diversity of low molecular weight, structurally complex chemical compounds commonly known as secondary metabolites. Secondary metabolites do have a very important role in plants interaction with its environment for their existence and sustenance (Pichersky and Gang 2000; Moore et al. 2014). Higher plants with inherent non-mobile status make own self-defense strategies (structural/chemical) for their survival. Thus, as a part of chemical defense strategy, plants synthesize enormous number of chemical compounds (secondary metabolites) to meet the pressing requirements of the plants. The estimates already revealed that, plants produce more than 100,000 secondary metabolites, while the total number may exceed over 500,000 once they are structurally characterized (Hadacek 2002).

Many secondary metabolites yet need characterization in plants for future health care and pharmaceutical bioprospecting (Lee 2010; Soejarto et al. 2012; Elkington 2014; Pan 2014). Scores of chemical compounds from plant origin are in current use for medicine preparations and the fact remains that plants continue to be the principal source for many important bioactive molecules/pharmacophores (Dias et al. 2012; Pan 2014; Raomai et al. 2015). Currently, one-fourth of all prescribed pharmaceuticals across the globe contain chemical compounds (secondary metabolites) that are directly, indirectly or via semi-synthetically derived solely from plants (Song et al. 2014).

Recently, research wing of British Broadcasting Corporation (BBC) has made a global ahead of its time forecast for world market of plant-derived drugs between 2013 extending up to 2020 stands at 35.4 billion US $ (http://www.bccresearch.com/market-research/biotechnology/botanical-plant-derived-drugs-report-io022g.html).

Plants grow in harsh surroundings and stay alive in a biotic and abiotic stressed environment. A wide array of defense strategies against biotic and abiotic stresses exhibited by plants through both preexisting innate (constitutive) and induced defense systems. These induced defense responses in plants generally triggered following recognition of chemical factors or molecules by cellular environment called elicitors. An elicitor probably defined as a factor, substance, molecule or an agent, which, when a minute quantity incorporated to an existing cellular background, it improves, the biosynthetic competence of specific compounds particularly secondary metabolites in the pathway (Bhalla et al. 2005; Zhao et al. 2005; Namdeo 2007; Onrubia et al. 2013; Murthy et al. 2014; Gorelick and Bernstein 2014).

In the recent past a number of reviews have been published for the production of secondary metabolites using in vitro plant tissue culture systems (Vanisree and Tsay 2004; Zhou and Wu 2006; Matkowski 2008; Karuppusamy 2009; Ramakrishna and Ravishankar 2011; Hussain et al. 2012; Murthy et al. 2014; Ramirez-Estrada et al. 2016). These reviews included collective citation and inclusion of all elicitor types (biological, chemical and physical) together in one review. Keeping in view the extensive use and potentials of chemical group of elicitors for secondary metabolite production in vitro, there is need to bring out a review dedicated exclusively to chemical elicitors. Considering the enormous existing and upcoming literature, only few selected representative examples depicting current trend of research developments covering more than one decade of approximate literature survey are included in this review.

Elicitors: mechanism of action

Elicitor cross talk with the receptors present in the plasma membrane of several plant cells by stimulating an array of defense responses. This multitasking ability of such elicitors is unique as well as multidimensional (Gorelick and Bernstein 2014). Research findings indicate that it can regulate large number of biochemical control points; trigger the expression of key genes and transcription factors too. Therefore, now it is a well-established fact that the elicitors have the ability to control array of cellular activities at biochemical and molecular level (Zhao et al. 2005; Baenas et al. 2014). Elicitor perception is able to increase the intensity of plant's response to biotic and abiotic stresses with the enhanced synthesis of signal compounds such as JA, SA, ET, NO etc. and its subsequent influence on secondary metabolite production.

Plant's response to wounding and in systemic resistance is an important function of JA in plant growth and development. In recent times, greater role of JA in plant defense strategy is discussed at length (Ballare 2011; Pirbalouti et al. 2014). Further, MeJA is a volatile unique molecule and has the knack to spread out interplant communication through airborne signals for defense responses (Wasternack 2014; Kazan 2015; Dar et al. 2015). JA involves signal transduction pathways with the participation of jasmonate zim domain (JAZ) proteins, which are further subjected to proteasomal degradation via Skp1/Cullin/F-box (SCF) complex. JAZ proteins degradation results in the release of MYB/MYC transcription factors, which ultimately stimulate JA-dependent gene expression in secondary metabolism (Wasternack 2014).

Endogenous signaling by salicylic acid in plants mediates defense against pathogens and initiates synthesis of both localized acquired resistance (LAR) and systemic acquired resistance (SAR). The signal can also move to nearby plants by the volatile ester, i.e. methyl salicylate a possible alert communication for pathogen attack that plants can understand (Santner and Estelle 2009; Vlot et al. 2009). In addition, the mode of action of SA and its complex interaction with other signal compounds such as JA, ET and NO has been studied in detail (Qiao and Fan 2008; Boatwright and Pajerowska-Mukhtar 2013; Caarls et al. 2015).

The fruit-ripening hormone ET is responsible for biochemical and morphological changes involved in fruit senescence. ET synthesis in plants is related to wounding, abiotic and biotic stresses (Kazan 2015). ET perception is accomplished by ET receptors. Five ET membrane-localized receptors have been identified in *Arabidopsis* (Wang et al. 2002; Yoo et al. 2009). Binding of ET inactivates the receptors e.g. CTR1 (Constitutive Triple Response) is inactivated which results in the suppression of the CTR1–MAPK pathway and the activation of the MKK9–MPK3 and -6 cascades, which phosphorylates Thr residues of EIN3 and EIL1 transcription factors in the nucleus. Phosphorylation of EIN3 and EIL1 probably increases stability, which finally decreases their interaction with EBF1 and -2 for subsequent gene expression (Yoo et al. 2009).

Nitric oxide (NO) a free radical identified as signaling molecule implicated in elicitor-mediated defense reactions of plants (Matsuura et al. 2014). Particularly, it was found that NO targets a number of proteins and enzymes in plants (Wilson et al. 2008). The elicitor molecule NO is also known to activate MAP kinase signaling pathways resulting in altered gene expression. Plants definitely perceive and respond to NO, but the mechanism is not clear. There are no specific plant NO receptor has been identified.

Abscisic acid (ABA) induces synthesis of both NO and H_2O_2. Thus, NO and Ca^{2+} are both strongly implicated in the signaling cascade that must operate. Thus, it would seem very likely that responses to NO are accomplished by signaling through cyclic guanosine monophosphate (cGMP), cyclic adenosine diphosphoribose (cADPR) and Ca^{2+}. NO alleviates the deleterious effect from reactive oxygen species (ROS) in establishing stress resistance responses. In addition to its signaling roles, NO may also function as a regulator for gene expression (Kopyra and Gwozdz 2004).

While discussing mode of action of elicitors, it is necessary to mention about the interaction of various signal compounds/elicitors and their role in gene regulation. As it is well-established fact that environmental stresses such as ozone, UV irradiation and wounding involve the generation of ROS and synthesis of ET. ROS related responses are influenced by the outcome of the interactions among JA, SA and ET. ASA and SA repress JA synthesis and following ET production. In the presence of UV-B stress, induction of PR-1 is dependent on ET, but not on JA suggesting that, ET is a signal required to activate the SA pathway (Wang et al. 2002; Qiao and Fan 2008). NO may directly or indirectly interact with other signaling molecules such as SA and ET and abiotic stress. A diagrammatic representation of elicitor mode of action is depicted in Fig. 1.

In vitro culture systems and elicitor treatment

Culture systems

Some of the culture systems and our own experiences such as somaclonal variation, nutrient media manipulation, precursor feeding, elicitation, hairy root culture, plant cell immobilization, biotransformation, large scale cultivation in bioreactor system and genetic manipulation are reported (Giri and Vijaya Laxmi 2000; Giri et al. 2001a, b, 2003; Vanisree and Tsay 2004; Abhyankar et al. 2005; Shyamkumar et al. 2007; Giri et al. 2009; Begum et al. 2009; Shimizu et al. 2010; Banerjee et al. 2012; Lee et al. 2015; Zaheer et al. 2016).

Callus culture system, has been used less frequently only to evaluate the effect of signal compounds for secondary metabolite production (Fig. 2). Generally, callus culture system used to detect the presence of bioactive compounds in cultured plant tissue in vitro. However, callus culture system are even used currently in elicitation studies (Pandey et al. 2015). Different experimental investigations on secondary metabolite production with few recent examples are mentioned in this review (Table 1). Another extensively used culture system for elicitor treatment and secondary metabolites production is cell suspensions compared to others for elicitation experiments (Fig. 2; Table 1). Obviously, the trend of literature on the use of cell suspension for elicitation studies is enormous and it becomes difficult to cite all of them in the present context (Table 1). However, few most recent findings are included in this assessment.

Hairy root cultures have proved to be a valuable culture system for elicitation studies due to its inherent characteristics of hormone autotrophy, uncontrolled growth, biosynthetic and genetic stability distinctiveness. Further, certain secondary metabolites are synthesized exclusively in the roots. Thus, transformed hairy root cultures have become important for secondary metabolite production in vitro (Giri et al. 2003; Guillon et al. 2006; Srivastava and Srivastava 2007; Murthy et al. 2014; Zaheer et al. 2016). Hairy root cultures are quite frequently used culture system compared to others except cell suspensions for elicitor treatment and secondary metabolite production (Fig. 2; Table 1). Literature on the use of hairy root cultures in elicitation studies for secondary metabolite production has accumulated considerably over years (Fig. 2; Table 1). Adventitious root is an appropriate culture system for production of secondary metabolites in vitro. Adventitious roots are induced with ease in vitro, incorporating low concentrations of auxin and can be cultured in large scale axenically analogous to hairy root cultures but comparatively with reduced growth rate. Being organized/differentiated culture system, adventitious roots also possess genetic and biosynthetic stability (Baque et al. 2012). Similar to other culture systems adventitious roots frequently used for elicitation but with reduced frequency (Fig. 2; Table 1).

Higher secondary metabolite content has been detected in leaves of specific plant species (Giri et al. 2009; Begum et al. 2009; Zaheer and Giri 2015). Multiple shoot culture is one such culture system, which can generate high biomass of leaves with cytokinin manipulation for secondary metabolite production and elicitor driven yield enhancement (Kikowska et al. 2015; Zaheer and Giri 2015). In this part of the review, few recent findings will be highlighted involving elicited multiple shoots for the production of secondary metabolites (Table 1).

Elicitor treatment

In general, exogenous supply of signal compounds (chemical elicitors) such as JA, SA, ET, NO and HM, synthetic chemicals etc. to different culture systems creates a plant under stress like cellular environment. The elicitors not only bring change quantitatively (yield enhancement of existing biomolecules) but also found to alter/synthesize secondary metabolites qualitatively (discovery of entirely new/novel compounds). The prime objective of incorporating elicitors artificially onto in vitro cultures is to misguide the cells or tissues for a possible biotic/abiotic attack,

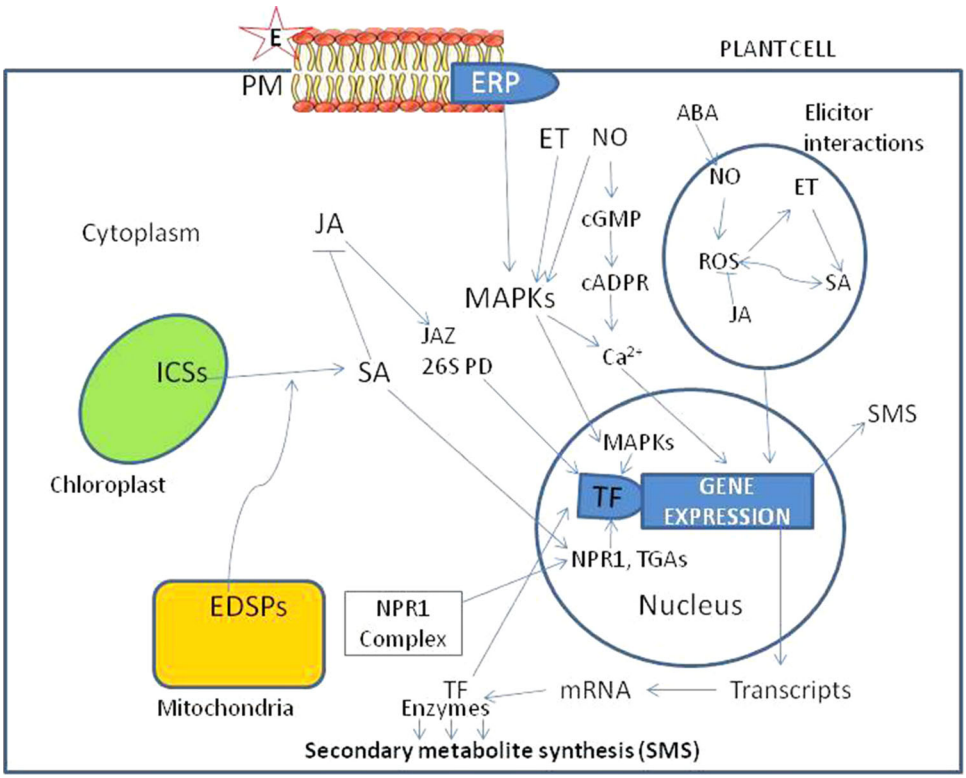

Fig. 1 Diagrammatic depiction of elicitors and their mode of action mimicking possible elicitation mechanism using elicited plant cell, tissue and organ cultures in vitro. *ABA* abscisic acid, Ca^{2+} calcium ion, *cADPR* cyclic adenosine diphosphoribose, *cGMP* cyclic guanosine monophosphate, *E* elicitor, *EDSPs* enchanced disease susceptibility proteins, *ERP* elicitor receptor perception, *ET* ethylene, *ICSs* isochorismate synthases, *JA* jasmonic acid, *JAZ* jasmonate zim domain, *MAPKs* mitogen activated protein kinases, *NO* nitric oxide, *NPRI* non-expressor of pothogenesis-related genes 1, *PM* plasma membarane, *ROS* reactive oxygen species, *SA* salicylic acid, *TF* transcription factors, *TGAs* leucine zipper transcription factors, *26S PD* 26S proteasomal degradation

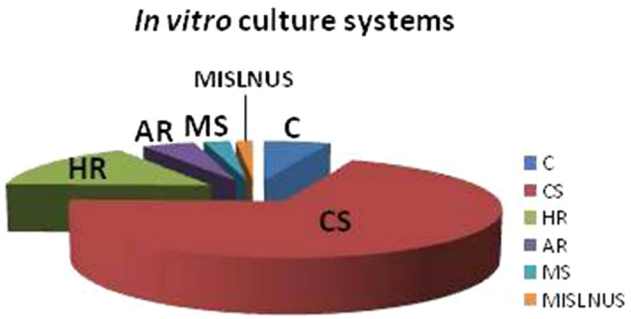

Fig. 2 Overall sample frequency of different in vitro culture systems used in chemical elicitation experiments for secondary metabolite production. *C* callus, *CS* cell suspension, *HR* hairy roots, *AR* adventitious roots, *MS* multiple shoots, *MISLNUS* miscellaneous

mimicking the external environment similar to natural conditions under stress related damage in plants. The manifestations of elicitor treatment with altered/modified/elevated genetic and biochemical activities in the cellular background is observed as enhanced yield of target chemicals, higher gene expression and discovery of entirely new/novel biomolecules (Caretto et al. 2011; Murthy et al. 2014; Ramirez-Estrada et al. 2016). The qualitative and quantitative variations in the content of bioactive natural products (secondary metabolites) are in expected direction following elicitor treatment.

Chemical elicitors such as jasmonic acid (JA), methyl jasmonate (MeJA), 2-hydroxyl ethyl jasmonate, salicylic acid (SA), acetyl salicylic acid (ASA), trifluro ethyl salicylic acid, ethylene (ET), nitric oxide (NO), sodium nitropruside (SNP), ethrel or ethephon (Ethe) are used for elicitation studies. In addition, di-methyl sulfo-oxide (DMSO), pyrazine 2-carboxylic acid, benzo (1, 2, 3) thiadiazole-7-carboxylic acid-s methyl ester (BION), eugenol, polyamines, morphactin, sodium acetate, sorbitol, benzothiadiazole, saccharin, hydrogen peroxide, silver nitrate, triton-x, copper sulphate, ancymidol, cyclodextrins, methylated cyclodextrins, cantharidin, putrescine, glutathione, heavy metal ions (HM), Ca^{+2} cAMP, catharidin, and many more have been employed for secondary metabolite manipulation in vitro. Different chemical elicitors used for elicitation, gene isolation and study of secondary metabolic pathway in post genomic era are carried out (Oksman-Caldentey and Inzé 2004). Let us discuss some of the recent findings on

Table 1 Selected examples depicting studies on chemical elicitors and production of secondary metabolites in vitro using plant cell, tissue and organ cultures

Plant species	Culture system	Elicitor concentration (μM)[a]	Product	Maximum yield enhancement in folds[b]	References
Capsicum chinense	CS	SA (1000 μM), CaI	Capsaicin	3.7	Kehie et al. (2016)
Salvia castanea	HR	MeJA (200 μM), Ag^{+}	Tanshinone	1.99	Li et al. (2016)
Papavar orientale	HR	MeJA (100 μM), SA	Morphine	6.18	Hashemi and Naghavi (2016)
Astragalus membranaceus	HR	MeJA (283 μM), SA, ASA	Isoflvonoid	9.7	Gai et al. (2016)
Psoralea corylifolia	HR	JA (10 μM), ASA	Daidzin	7.3	Zaheer et al. (2016)
Bacopa monnieri	MS	JA (5 μM), SA, $CuSO_4$	Bacoside	3.08	Sharma et al. (2015)
Perovskia abrotanoides	AR	$AgNO_3$ (25 μM), MeJA	Tanshinone	1.9	Zaker et al. (2015)
Artemisia absinthium	CS	MeJA (5 μM), JA	[a]Flavonoids, phenolics	1.39	Ali et al. (2015)
Andrographis paniculata	MS	SA (20 μM), JA	Andrographolide	3.4	Zaheer and Giri (2015)
Ocimum basilicum, Ocimum kilimandscharicum, Ocimum sanctum, Ocimum grattisimum	C	MeJA (200 μM)	Betulinic acid	1.9	Pandey et al. (2015)
Gymnema sylvestre	CS	MeJA (150 μM), SA	Gymnemic acid	15.4	Bhuvaneswari et al. (2015)
Paris polyphylla	MRs	SA (363 μM)	Steroidal saponins	3.6	Raomai et al. (2015)
Tripterygium wilfordii	AR	MeJA (100 μM), SA	[a]Triptolide, wilforgine, wilforine	10.40	Miao et al. (2014)
Papaver somniferum	CS	SA (250 μM), H_2O_2, CO_2	Sanguinarine	2.6	Verma et al. (2014)
Cathranthus roseus	CS	Artemisinic acid (129 μM)	[a]Vindoline, vinblastin	6.0	Liu et al. (2014)
Papaver bracteatum	CS	MeJA (100 μM), US	Thebaine	44.50	Zare et al. (2014)
Anisodus luridus	HR	ASA (1000 μM), UV-B	Tropane alkaloids	6.2	Qin et al. (2014)
Arnebia euchroma	CS	MeJA (5 μM)	Shikonin derivatives, shikonofuran derivatives, rosmarinic acid	12.0	Wang et al. (2014)
Plumbago rosea	CS	JA (100 μM)	Plumbagin	4.95	Silja et al. (2014)
Digitalis purpurea	MS	KCl (200,000 μM) SA, Mannitol sorbitol, PEG-6000, NaCl	Cardiotonic glycolides	8.7	Patil et al. (2013)
Rosa hybrida	C	MeJA (0.5 μM), SA	Anthocyanin	1.3	Ram et al. (2013)
Panax ginseng	HR	MeJA (100 μM)	Ginsenoside Rg3	5.0	Kim et al. (2013)
Panax ginseng	CS	DCCD (10 μM)	Ginsenoside	3.0	Huang et al. (2013)
Achyranthes bidentata	CS	MeJA (600 μM)	20-HE	2.6	Wang et al. (2013)
Withania somnifera	MS	SA (100 μM) MeJA	Withanolide A	16.0	Sivanandhan et al. (2013)
Valeriana amurensis	AR	MeJA (446 μM) JA, SA	Valtrate	3.5	Cui et al. (2012)
Genista tinctoria	CS	MeJA (100 μM), DMSO	Isoflavones	5.0	Luczkiewicz and Kokotkiewicz (2012)
Eryngium planum L	[a]C, CS	MeJA (100 μM)	[a]Rosmarinic acid CGA, CA	3.0	Kikowska et al. (2012)
Salvia miltiorrhiza	HR	MeJA (100 μM) SNP	Tanshinone	3.9	Liang et al. (2012)

Table 1 continued

Plant species	Culture system	Elicitor concentration (μM)[a]	Product	Maximum yield enhancement in folds[b]	References
Vitis vinifera	CS	MeJA (25 μM), CD	Trans resveratrol	713.0	Belchi-Navarro et al. (2012)
Salvia miltiorrhiza	HR	MeJA (100 μM), Ag^+	Tanshinone	5.7	Kai et al. (2012)
Mitragyna speciosa	MS	MeJA (10 μM)	Mitragynine	3.0	Wungsintaweekul et al. (2012)
Fagopyrum esculentum	CS	MeJA (200 μM), SA (600 μM)	D-chiro-inositol	3.7	Hu et al. (2011)
Artemisia annua	CS	MeJA (22 μM), miconazole	Artemisinin	3.0	Caretto et al. (2011)
Pueraria candollei	HR	MeJA (200 μM), SA	Isoflavonoid	3.0	Udomsuk et al. (2011)
Artemisia annua	CS	MeJA (100 μM), CD (50,000 μM)	Artemisinin	300.0	Durante et al. (2011)
Centella asiatica	WPC	MeJA (100 μM)	Centellosides	2.44	Yoo et al. (2011)
[a]*Hypericum hirsutum, H. maculatum*	MS	SA (50 μM), JA	Hyperforin, hypericin, [a]pseudohypericin	13.58	Coste et al. (2011)
Calophyllum inophyllum	CS	HM, Cr (1 mM)	Inophyllum D	2230.0	Pawar and Thengane (2011)
Ruta graveolens	MS	SA (200 μM), MeJA	Furanocoumarin	5.9	Diwan and Malpathak (2011)
Allium sativum	C	Sulphur (1000 μM), $CaSO_4$ (93 μM)	Alliin	2.3	Nasim et al. (2010)
Capsicum chinense	CS	MeJA (500 μM), SA	[a]Vanillin, capsaicinoid	5.0	Gutierrez-Carbajal et al. (2010)
Drosera burmanii	WPC	MeJA (50 μM)	Plumbagin	3.0	Putalun et al. (2010)
Linum album	CS	SA (10 μM)	Podophyllotoxin	3.0	Yousefzadi et al. (2010)
Camptotheca acuminata	C	SA (100 μM), HM, MeJA	HCPT	25.0	Pi et al. (2010)
Leucojum aestivum	C, [a]SE	ET (10 μM)	Galanthamine	6.0	Ptak et al. (2010)
Artemisia annua	MS	DMSO (0.25 v/v)	Artemisinin	2.26	Mannan et al. (2010)
Datura metel L	AR	SA (500 μM), $CaCl_2$, $AlCl_3$, NaCl, Na_2SO_4	Hyoscyamine, scopolamine	3.1, 4.0	Ajungla et al. (2009)
Artemisia annua	HR	SNP (10 μM), CB, NO	Artemisinin	2.3	Wang et al. (2009)
Psoralea corylifolia	HR	SA (1000 μM) PA	Isoflavones	1.3	Shinde et al. (2009)
Catharanthus roseus	[a]MS, HR, CS	MeJA (10 μM) SA, ET	Vindoline	6.5	Vazquez-Flota et al. (2009)
Cayratia trifolia	CS	SA (500 μM), MeJA, Ethe	Stilbenes	6.2	Roat and Ramawat (2009)
Catharanthus roseus	HR	JA (238 μM) + Dexamethasone (3 μM)	Vinblastine, vincristine	170.0	Peebles et al. (2009)
Panax notoginseng	CS	HEJ (200 μM)	Ginsenoside	4.4	Hu and Zhong (2008)
Vitis vinifera	CS	MeJA (20 μM)	Stilbene, anthocyanin	28.0	Belhadj et al. (2008)
Panax ginseng, Echinacea purpurea	AR	MeJA (200 μM)	Ginsenosides	8.0	Wu et al. (2008)
Artemisia annua	HR	SNP (50 μM), NO, OSE	Artemisinin	2.0	Zheng et al. (2008)

Table 1 continued

Plant species	Culture system	Elicitor concentration (μM)[a]	Product	Maximum yield enhancement in folds[b]	References
Pueraria tuberose	CS	Ethe (100 μM)	Isoflavonoids	14.0	Goyal and Ramawat (2008)
Hypericum sampsonii, [a]*Hypericum perforatum*	MS	MeJA (50 μM), DHPJ	[a]Hypericin, hyperforin, pseudohypericin	2.0	Liu et al. (2007)
Erythrina Americana	CS	JA (100 μM)	Erysodine	4.3	San Miguel-Chavez et al. (2007)
Panax ginseng	AR	SNP (100 μM)	Saponin	3.7	Tewari et al. (2007)
Centella asiatica, *Ruscus aculeatus*, [a]*Galphimia glauca*	WPC	MeJA (100 μM)	Triterpene, sterols	152.0	Managas et al. (2006)
Taxus yunnanensis	CS	US + SNP (10 μM)	Baccatin	8.0	Wang et al. (2006)
Taxus canadensis	CS	MeJA (200 μM) + ET (7.4 ppm)	Taxanes	4.2	Senger et al. (2006)
Panax ginseng	AR	MeJA (100 μM), Ethe (50 μM)	Ginsenoside	6.3	Bae et al. (2006)
Tropaeolum majus	HR	ASA (200 μM) SA, MeJA, BABA	Glucotropaeolin	3.0	Wielanek and Urbanek (2006)
Panax ginseng	HR	SA (500 μM), ASA (500 μM)	Ginseng saponin	1.1	Jeong et al. (2005)
Hypericum perforatum	CS	NO (2 and 6 μM)	Hypericins	3.2	Xu et al. (2005)
Panax ginseng, Panax quinquefolium L	AR	MeJA (200 μM)	Saponins	4.0	Ali et al. (2005)
Taxus chinensis	CS	PFPJA (100 μM), MeJA, TFEJA	Tax-C	2.9	Qian et al. (2005)
Coleus forskohlii	HR	MeJA (100 μM), SA	Rosmarinic acid	3.4	Li et al. (2005)
Centella asiatica	WPC	MeJA (100 μM)	Asiaticoside	5.0	Kim et al. (2004)
Cupressus lusitanica	CS	MeJA (100 μM), ET	β-thujaplicin	3.0	Zhao et al. (2004)
Scopolia parviflora	AR	MeJA (10 μM), SA	[a]Hyoscyamine, scopolamine	2.4	Kang et al. (2004)
Trigonella foenumgraecum	CS	Ethe (5 ppm)	Diosgenin	2.2	Gomez et al. (2004)
Catharanthus roseus	MS	MeJA (100 μM)	Vindoline	10.0	Hernández-Domínguez et al. (2004)

20HE 20-hydroxyecdysone, *ACC* 1-aminocyclopropane-1-carboxylic acid, Ag^{+} silver ion, $AgNO_3$ silver nitrate, $AlCl_3$ aluminium chloride, *AR* adventitious root, *ASA* acetylsalicylic acid, *BABA* beta aminobutyric acid, *C* callus, *CA* caffeic acid, $CaCl_2$ calcium chloride, *CaI* calcium ionophore, $CaSO_4$ calcium sulphate, $CdCl_2$ cadmium chloride, *CB* cerebroside, *CD* cyclodextrins, *CGA* chlorogenic acid, *ChP* chitoplant, *CI* cell immobilization, CO_2 carbon dioxide, *Coro* coronatine, *Cr* chromium, *CS* cell suspension, $CuCl_2$ copper chloride, $CuSO_4$ copper sulfate, *DCCD N,N′*-dicyclohexylcarbodiimide, *DHPJ* 2,3-dihydroxypropyl jasmonate, *DMSO* dimethyl sulfoxide, *EC* embryo culture, *ET* ethylene, *Ethe* ethrel/ethephon, *GA* gum arabic, *HCPT* 10-hydroxy-camptothecin, H_2O_2 hydrogen peroxide, *HEJ* 2-hydroxyethyl jasmonate, $HgCl_2$ mercuric chloride, *HM* heavy metals, *HR* hairy root, *JA* jasmonic acid, *KCl* potassium chloride, *MeJA* methyl jasmonate, *MG* mesquite gum, *MRs* mini rhizomes, *MS* multiple shoot, Na_2SO_4 sodium sulphate, *NaCl* sodium chloride, *NO* nitric oxide *OSE* oligosaccharide elicitor, *PA* polyamines, *PABA* p-amino benzoic acid, *PEG* polyethylene glycol, *PFPJA* pentafluoropropyl jasmonate, *SA* salicylic acid, *SE* somatic embryo, *SiP* silioplant, *SNP* sodium nitroprusside, *SPCA* substituted pyrazinecarboxamides, *SS* sodium salicylate, *TFEJA* trifluoroethyl jasmonte, *US* ultrasound, *UV-B* ultraviolet B, *WPC* whole plant culture

[a] Optimum concentration of elicitor promoting highest yield of secondary metabolites

[b] Maximum yield enhancement (in folds) obtained with specific elicitor amongst different elicitors tested

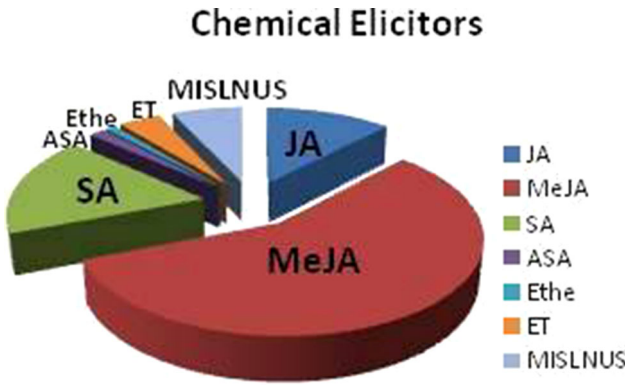

Fig. 3 Real time analysis of different chemical elicitors used across in vitro culture systems for the production of secondary metabolites from plants. *JA* jasmonic acid, *MeJA* methyl jasmonic acid, *SA* salicylic acid, *ASA* acetyl salicylic acid, *Ethe* ethrel, *ET* ethylene, *MISLNUS* miscellaneous

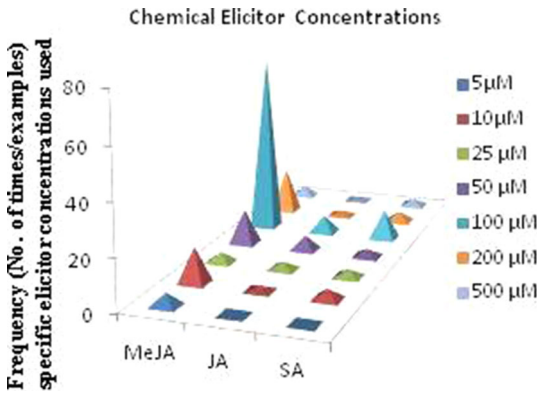

Fig. 4 Real time analysis of different chemical elicitors concentrations using in vitro culture systems for the production of secondary metabolites from plants. *MeJA* methyl jasmonic acid, *JA* jasmonic acid, *SA* salicylic acid

frequently used chemical elicitors and their applications using various plant in vitro culture systems.

JA and related compounds

JA and its related signal molecules in particular MeJA used extensively for elicitation studies involving in vitro culture systems (Fig. 3; Table 1). Keeping in view the enormous literature on the use of JA and its related molecules in elicitation studies, a few latest examples cited herein involving different culture systems such as callus, suspension cells, hairy roots, adventitious roots and multiple shoots. Elicitation with 0.5 µM MeJA influenced betalain pigment production in callus culture of *Bougainvillea* (Lakhotia et al. 2014). Recently, betulinic acid with anti-cancer property was reported involving elicitation of callus cultures of different *Ocimum* species (Pandey et al. 2015). MeJA at 200 µM resulted in 2-fold (5.10 ± 0.18 % DW) increase of betulinic acid compared to (2.61 ± 0.19 % DW) from untreated control callus cultures of *O. basilicum*. Peruvoside production at the tune of 8.93 mg/l in cell suspension cultures of *Thevetia peruviana* with the incorporation of 100 mg/l MeJA was achieved (Zabala et al. 2010). Elicitation and permeabilization influenced accumulation and storage profile of phytoestrogens in suspension cultures of *Genista tinctoria*. Maximum levels (198.3 mg/100 g DW genistein: 3.0 fold); 15.21 mg/100 g DW daidzein: 5.0-fold increase were obtained (Luczkiewicz and Kokotkiewicz 2012).

Irrespective of different plant species and culture systems used for elicitation expriments, the concentration of elicitors was vital. An analysis indicated that the concentration of JA or MeJA ranged from as low as 5.0 to 500 µM across different plant species and in vitro culture systems was studied (Fig. 4). In a real time assessment of collected literature on the concentration of elicitors revealed that 100 µM MeJA used in maximum number of experiments involving several plants and culture systems (Fig. 4; Table 1). In another report, a 2.6-fold enhancement in phytoecdysteroids (20-hydroxyecdysone); was obtained from MeJA (0.6 mM) elicited cell suspension cultures of *Achyranthes bidentata* (Wang et al. 2013). Enhanced shikonofuran derivatives production (12-fold) in response to 5.0 µM MeJA treatment in shikonin proficient and shikonin-deficient cell lines from suspension cultures of *Arnebia euchroma* was also studied (Wang et al. 2014). Elicitation of antioxidant secondary metabolites with jasmonates and gibberellic acid reported in cell suspension cultures of *Artemisia absinthium* (Ali et al. 2015). Cell suspension cultures of *A. absinthium* when treated with 1.0 mg/l MeJA resulted total phenolic content 6.7 mg GAE (Gallic Acid Equivalent)/g DW and 1.0 mg/l JA it produced 6.7 mg GAE/g DW compared to control 5.32 mg GAE/g DW. Whereas, 1.0 mg/l MeJA resulted 2.19 mg QE (Quercetin Equivalent)/g DW in total flavonoid content and 1.0 mg/l JA produced 1.89 mg QE/g compared to 1.57 mg QE/g DW in control. Recently, 150 µM MeJA elicitation promoted 15.4-fold increased gymnemic acid production in cell suspension cultures of *G. sylvestre* (Bhuvaneswari et al. 2015).

Addition of 200 µM MeJA promoted 11.22 mg/g DW total phenolics and 3.60 mg/g DW total flavonoids, 60.28 and 32.93 mg/g DW in hairy roots of Ginseng and *Echinacea*, respectively (Wu et al. 2008). Hairy root cultures are also used for enhanced Rg3 ginsenoside biosynthesis in *Panax* elicited by MeJA. In this study, a 7-day treatment of hairy roots with 0.1 mM MeJA leads to the detection of Rg3 by HPLC analysis contrast to its absence in untreated control (Kim et al. 2013). Recently, Matsuura et al. (2014) have reviewed the biotechnological benefits of oxidative stress and elicitation on indole alkaloid production. Daidzin content enhancement up to 7.3-fold (3.43 % DW) was

obtained with 10 μM JA treatment in hairy root cultures of *Psoralea corylifolia* compared to 0.478 % DW in control (Zaheer et al. 2016).

Optimization of elicitors with different concentrations of JA, MeJA and SA were used to enhance the valtrate production. However, optimal production (10.58 mg/g DW) was obtained only with 100 mg/l MeJA compared to JA and SA in adventitious roots of *Valeriana amurensis* (Cui et al. 2012). Elicitor treatment at 50 μM MeJA and XAD-7 resulted in 3.55-fold triptolide production. Whereas, XAD-7 alone was resulted in 49.11-fold increase in wilforgine, MeJA at 100 μM promoted 10.40-fold wilforine in adventitious root fragment liquid cultures of *Tripterygium wilfordii*. However, there was no significant increase in alkaloid content with SA treatment alone or combined with XAD-7 when compared to control (Miao et al. 2014). Further, an exceptionally high percentage of enhancements of bioactive compound production in adventitious root cultures of *Eleutherococcus koreanum* Nakai by elicitation with MeJA and SA was studied (Lee et al. 2015). A detailed study in *E. koreanum*, optimal concentration of elicitor MeJA at 50 μM increased biomass and bioactive compounds production of five target bioactive molecules such as eleutherosides B and E, chlorogenic acid, and total phenolics and flavonoids which were on an average 37.77 % higher than the untreated control root cultures.

Shoot cultures of *Eryngium planum* grown in 1.0 mg/l BAP + 0.1 mg/l indole acetic acid containing medium and treated with 100 μM MeJA for 48 h resulted in 19.795 mg/g DW enhanced phenolic compounds, which was 4.5-fold higher than 4.36 mg/g DW in control (Kikowska et al. 2015). Production of increased plumbagin (3.0-fold) was also reported following 50 μM MeJA elicitation of *Drosera burmanii* whole plant cultures (Putalun et al. 2010). A 3.08-fold enhancement in bacoside (8.46 mg/g DW) was obtained when shoot cultures of *Bacopa monnieri* treated with 1.0 mg/l JA compared to 2.74 mg/g DW bacoside in control shoots (Sharma et al. 2015). JA at 25 and 50 μM promoted 3.3 and 3.0-fold increase in andrographolide content in multiple shoot cultures of *Andrographis paniculata* after eighth week, respectively when compared to control (Zaheer and Giri 2015).

SA and related compounds

Similar to other chemical elicitors, SA and its related compounds are used comparatively lesser than MeJA for elicitation studies (Fig. 3; Table 1). Keeping in view many research findings and the importance of salicylic as a potent elicitor, we will restrict ourselves to few recent examples on elicitation involving in vitro plant cell and tissue culture systems. Five-fold increase of capsaicinoid synthesis following SA or MeJA (500 μM) elicitation in Habanero pepper (*Capsicum chinense* Jacq.) cell suspension cultures was obtained (Gutierrez-Carbajal et al. 2010). Elicitation with SA promoted improved podophyllotoxin production and its correlation with lignan biosynthesis in cell cultures of *Linum album* with increased expression of genes (Yousefzadi et al. 2010). Treatment of *L. album* cell cultures with 10 μM SA for 3 days resulted in 3.0-fold increase in podophyllotoxin content compared to non-elicited control cultures. A detailed study on the influence of SA on the production of phenolic compounds e.g. salvianolic acid B and caffeic acid was reported in cell suspension cultures of *Salvia miltiorrhiza*. SA concentrations ranging between 6.25 and 22.5 mg/l found to enhance the phenolic compounds. Further, the biosynthetic enzymes such as phenylalanine ammonia-lyase (PAL), tyrosine aminotransferase (TAT), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) enzymes were found upregulated by the application of SA (Dong et al. 2010). SA and MeJA promoted increased production of D-chiro-inositol compared to untreated control in cell suspension cultures of *Fagopyrum esculentum* (Hu et al. 2011). Observations indicated that MeJA at 0.4 mM resulted in 6.141 mg/g D-chiro-inositol, whereas, 0.6 mM SA accumulated 5.521 mg/g. However, combination of 0.2 mM MeJA and 0.6 mM SA resulted in 7.575 mg/g D-chiro-inositol production, which was 3.72-fold higher than the untreated control cultures.

Like other elicitors, it revealed that, the concentration of SA and its related compounds used in the elicitation ranged from as low as 5.0 μM to as high as 500 μM. In a realistic appraisal of literature on the concentrations of elicitors revealed that, 100 μM SA also used in majority of studies (Fig. 4). Significant increase in 10-hydroxycamptothecin (HCPT) content was obtained when callus cultures of *Camptotheca acuminata* were treated with 100 μM SA (Pi et al. 2010). *Pueraria candollei* hairy root culture treatment with SA at 100 μM resulted in 20 ± 1 mg/g dry wt isoflavonoid compared to control (14 mg/g dry wt) after 3 days of elicitation (Udomsuk et al. 2011). SA at 50 mg/l elicited saponin production up to 87.66 ± 1.66 mg/gm DW in transverse thin cell layer cultures of *Paris polyphylla* which was 3.6-fold higher than in vivo rhizome (Raomai et al. 2015). Maximum sanguinarine content (0.058 % DW) following 250 μM SA elicitation in cell suspension cultures of *Papaver somniferum* was detected (Verma et al. 2014).

Influence of 500 μM SA elicitations on accumulation of hyoscyamine (4.35 mg/g DW) and scopolamine is (0.28 mg/g DW) in adventitious root cultures of *Datura metel* was studied (Ajungla et al. 2009). ASA at 25 μM increased 2.3-fold (1.44 % DW) daidzin content when compared to untreated control (0.62 % DW) roots of *P. corylifolia* (Zaheer et al. 2016). SA at 50 μM resulted in enhanced hypericin (7.98-fold) and pseudohypericin

(13.58-fold) production in *H. hirsutum* (Coste et al. 2011). Effect of elicitors MeJA (10 μM) and SA (200 μM) on furanocoumarin production up to 4.7 and 5.9-fold, respectively; observed and in situ product removal was studied using shoot cultures of *Ruta graveolens* (Diwan and Malpathak 2011). Elicitation with SA at 20 and 50 μM to multiple shoot cultures of *A. paniculata* accumulated 3.4 (1.654 % DW) and 3.1-fold (1.483 % DW) andrographolide content, respectively; when compared to 0.478 % DW in control (Zaheer and Giri 2015).

ET and ethrel/ethephon

A close assessment of existing literature has revealed that ET is not a widespread chemical elicitor used for secondary metabolite yield enhancement keeping in view its positive as well as negative effects. Therefore, ET: a less frequently used compared to other elicitors for elicitation studies and limited too few sporadic findings (Fig. 3; Table 1).

Further, general trend has been that the application of ET alone for secondary metabolite accumulation was not encouraging. However, combined treatment of ET along with any other signal molecule found beneficial for enhanced production of secondary metabolites indicating its interactive role. It revealed that the concentration of ET is a vital determinant for the altered biosynthesis of secondary metabolites as higher concentrations slow down and on the other hand, lower one enhances the biosynthetic ability of tissue under treatment.

Few reports are documented on ET treatments and is included here starting from recent to earlier findings. Combined application and interaction of JA and ET in *Cupressus lusitanica* cell cultures for the production of β-thujaplicin were investigated (Zhao et al. 2004). ET gas at 200 ppm increased β-thujaplicin production (22 mg/l) in cell suspension cultures of *C. lusitanica* compared to untreated control (11 mg/l). The culture systems such as cell suspension, hairy roots and rootless shoot cultures of *Catharanthus roseus* were used to evaluate the influence of MeJA, SA and ET on indole alkaloid accumulation (Vazquez-Flota et al. 2009). In a study it was found that, irrespective of the use of signal compounds alone or in combinations, the type of culture system was also critical in rendering helpful effect on accumulation of alkaloids. MeJA at 10 μM and 5 ppm ET was effective for enhanced vindoline production in rootless shoot cultures, which was about 6.5 times higher than the untreated control cultures. ET alone reduced catharanthine accumulation in these cultures. In *Leucojum aestivum*, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase induced ET production and shown positive effect on galanthamine production (Ptak et al. 2010). ACC (10 μM) increased ET production up to 26.4 nl/g fresh weight, FW in callus and 2.1 nl/g FW in somatic embryos. The galanthamine content was (2 % DW) which was six times higher than control (0.34 % DW) in somatic embryos cultured on medium supplemented with ACC. In the recent times, ET is not that frequently used elicitor to be experimented for the enhancement of secondary metabolites. Use of ET as elicitor is much more of academic than practical importance. A possible involvement of ET in shikonin biosynthesis in *Lithospermum erythrorhizon* shoot cultures examined and it found that, ET acted for a regulatory role in shikonin biosynthesis involving *L. erythrorhizon* shoot cultures (Touno et al. 2005). Enhanced shikonin production up to threefold (0.23 % DW) was achieved when *L. erythrorhizon* shoot cultures were treated with 10 μl ET per petridish containing 25 ml of media compared to untreated control.

Ethrel or 2-chloroethylphosphonic acid commonly known as ethephon is solely marketed by Bayer Crop Science Group. Ethrel (Ethe) used not that frequently in elicitation studies compared to other leading elicitors (Fig. 3; Table 1). Elicitor treatment of SA (500 μM), MeJA (100 μM) and Ethe (500 μM) to cell suspension cultures of *Cayratia trifolia* after 1 week resulted in 3–6-fold [5–11 mg/l DM (dry mass)] increase in stilbenes content within 15 day of culture compared to untreated control (Roat and Ramawat 2009). Elicitation of *Bupleurum falcatum* transgenic adventitious roots with MeJA (5 and 20 μM), SA (1 and 5 μM) and Ethe (1 and 10 μM) induced squalene synthase gene activity upregulation and higher production of phytosterol and saikosaponins (Kim et al. 2011).

NO and related compounds

The study on the role of NO in UV-B for flavonoid production in *Gingko biloba* callus was reported (Hao et al. 2009). UV-B along with 0.2 mM SNP influenced PAL regulation and resulted in enhanced flavonoid production. Enhanced taxol production (11 % more than control cultures) was obtained with 20 μM SNP treatment using immobilized cell culturs of *Taxus cupsidata* (Xiao et al. 2009). A 2.3-fold (22.4 mg/l) increase of artemisinin was obtained with combined treatment of SNP (10 μM) and cerebroside (30 μg/ml) compared to untreated control (9.6 mg/l) in hairy root cultures of *A. annua* (Wang et al. 2009). Application of 0.1 mM SNP resulted in increased terpenoid catharanthine production in hairy root cultures of *C. roseus* (Li et al. 2011). Studies on evolution of ROS with 100 μM NO-induced tanshinone production (tanshinone I, cryptotanshinone, dihydrotanshinone I and tanshinone IIA, with increased yield by 80, 170, 60 and 180 %; respectively; compared to control) in *S. miltiorrhiza* hairy roots has also been carried out (Liang et al. 2012).

Combined treatments of elicitors and media additives

Research findings indicate that combined treatments of elicitors and media additives have shown positive effect on enhancement of secondary metabolite production in vitro. Influence of single elicitor no doubt has promoting effect on yield enhancement, however; elicitor combinations have shown improved results. In *Boswellia serrata* callus cultures four major boswellic acid isomers were found manipulated by biotic and abiotic elicitors (Ghorpade et al. 2011). Na_2SO_4 at 10 and 15 mg/l resulted in enhanced production of acetyl-11-keto-β-boswellic acid (AKBBA) and 11-keto-β-boswellic acid (KBBA), respectively; which was 10.0-fold compared to control callus cultures. Combined effect of increased sucrose concentration and/or elicitation using MeJA generated positive response. The rosmarinic acid (RA), chlorogenic acid content and caffeic acid was found to be increased in the callus and other culture systems compared to the leaves and roots of intact plants (Kikowska et al. 2012). In this study, a 3.0-fold (16.24 mg/g) increase of RA content in callus and 2.0-fold (3.91 mg/g) in cell suspension cultures was achieved post elicitation with 100 μM MeJA.

In addition to the combination of elicitors, a systematic treatment of *Argemone mexicana* cell cultures with MeJA and SA promoted sanguinarine production (Trujillo-Villanueva et al. 2012). A 9.0-fold increase in sanguinarine synthesis compared to untreated control was obtained following the sequential treatment. *Vitis vinifera* cell suspension cultures produced improved trans-resveratrol extracellularly using cyclodextrins and MeJA (Belchi-Navarro et al. 2012). The combination of MeJA (100 μM) and L-tyrosine (2 mM) increased the thebaine yield up to 84.62 mg l^{-1} at 6 days after treatment in cell suspension cultures of *Papaver bracteatum* (Zare et al. 2014). About 3.4–4.6 fold enhancements in anthocyanin production was achieved following combined treatment of elicitor and precursor in cell suspension cultures of *V. vinifera* (Qu et al. 2011). Combined application of JA and gibberellic acid favoured for enhanced antioxidant secondary metabolites production in cell suspension cultures of *A. absinthium* L. (Ali et al. 2015). Elicitation of *A. annua* cell suspension cultures with 22 μM MeJA and 200 μM miconazole promoted 2.5–3.0-fold increase in artemisinin production. Further, MeJA treatment influenced expression of genes such as CYP71AV1, CPR and DBR2 related to artemisinin biosynthesis (Caretto et al. 2011). Enhanced artemisinin production (27 μmol g/l i.e. 300-fold higher compared to untreated control) in *A. annua* cell suspension cultures was also obtained by combined elicitation of 50 mM [2,6-di-*O*-methyl)-β-cyclodextrin (DIMEB)] and 100 μM MeJA with media additive β-Cyclodextrins (Durante et al. 2011).

In hairy root cultures of *P. corylifolia*, increased isoflavone daidzein production up to 2.2 and 0.228 % DW genistein were obtained after 48 h of elicitation treatment. This study exploited combined application of elicitation and precursor feeding (1.0 mM SA and 2.0 mM phenylalanine), respectively resulting in 1.3-fold higher daidzein and genistein production (Shinde et al. 2009). Combined elicitation of cerebroside and NO, promoted increased artemisinin production (22.4 mg/l: 2.3-fold increase) in *A. annua* hairy roots (Wang et al. 2009). MeJA elicitation at 100 μM combined with sucrose and L-phenylalanine feeding to root cultures of *Taxus x media var. Hicksii* promoted improved taxane production (Sykłowska-Baranek et al. 2015). Citronellal at 50 mg/l was found critical and promoted 3.35 mg/g DW valtrate content compared to 2.95 mg/g DW in control without precursor in *V. amurensis* (Cui et al. 2012). Recently, adventitious root culture system of *Echinacea purpurea* was exploited for enhanced production of secondary metabolites such as caffeic acid derivatives (CADs), phenols and flavonoids (Mobin et al. 2015).

A combination of plant growth regulators and elicitors (JA, SA) on production of secondary metabolites (hypericin and pseudohypericin) involving shoot cultures of *H. hirsutum* and *H. maculatum* was studied. SA was more effective in stimulating the accumulation of hypericins. JA at 250 μM promoted 1.55-fold of hypericin in *H. maculatum* and 3.0-fold in *H. hirsutum*. SA (50 μM) resulted in increased plant growth and production of hypericins (hypericin: 7.98-fold, pseudohypericin: 13.58-fold) in shoot cultures of *H. hirsutum* and *H. maculatum* (Coste et al. 2011). Multiple shoots of *D. purpurea* have proved to be an ideal in vitro culture system for production of cardiotonic glycosides following elicitation and other media manipulation (Patil et al. 2013). BAP at 7.5 μM was suitable for shoot biomass and addition of 50 μM SA resulted in 2.17-fold digitoxin enhancement. Increased concentration of SA (200 μM) resulted in 7.46-fold production of digoxin over control shoot cultures (g/g DW). An addition, of media additive progesterone (200 mg/l), promoted ninefold digoxin and with 300 mg/l a 12-fold increase in digitoxin was obtained. L-tryptophan at 0.5 mM enhanced 2.0-fold cichoric acid, 1.26-fold chlorogenic acid, 39.5 mg/g DW ± 1.7 phenols and 34.2 mg/g DW ± 1.4 of flavonoids, which was 56 and 24 % more than the untreated control. SA at 100 μM along with 0.6 mg/l BAP and 20 mg/l spermidine resulted in enhanced production of withanolide A (16-fold), withanolide B (13-fold), withaferin A (15-fold) and withanone (14-fold) in multiple shoot cultures of *Withania somnifera* (Sivanandhan et al.

2013). In addition increase in centellosides production (2.40 and 2.44-fold increase of madecassoside and asiaticoside, respectively) resulted from 0.1 mM MeJA elicitation in *Centella asiatica*. However, combined treatment of thidiazuron and MeJA, a 1.8-fold increase recorded compared to only MeJA treatment (Yoo et al. 2011).

Chemical elicitors other than regular signal compounds and hormones

It is evident that signal molecules and their related compounds and hormones have been used quite frequently for elicitation studies. In this section, we will enlist and discuss the exploitation of few chemical compounds as elicitors other than regular signal compounds and hormones. Plant gum driven elicitation promoted guggulsterone synthesis in cell suspension cultures of *Commiphora wightii* (Dass and Ramawat 2009). Gum arabic (100 mg/l) elicited 2.4-fold (198.1 µg/l) whereas mesquite gum (50 mg/l) increased twofold (163.1 µg/l) guggulsterone production, respectively when compared to control (84.2 µg/l) in cell suspension cultures of *C. wightii*. Treatment of elicitor pyrazinecarboxamides revealed noticeable influence on flavonolignans synthesis in *Silybum marianum* callus and cell suspension cultures. Application of *N*-(3-iodo-4-methylphenyl) pyrazine-2-carboxamide at 2.95×10^{-4} mol/l increased production of silychristin after 6-hours of elicitation, which was 2-times higher than control sample. Enhance production of silychristin was reached after 72 h with 2.53×10^{-3} mol/l *N*-(3-iodo-4-methylphenyl)-5-*tert*-butyl-pyrazine-2-carboxamide which was 12 times more than the control in callus culture of *S. marianum* (Tumová et al. 2010).

Higher dipyranocoumarin production (243-fold increase in inophyllum A and 284-fold inophyllum B) was obtained in stem callus derived cell suspension cultures of *Calophyllum inophyllum* with 1.0 mM HM chromium treatment. Further, highest enhancement up to 2230-fold inophyllum D (dipyranocoumarin) was observed with the same (1.0 mM HM chromium) treatment (Pawar and Thengane 2011). An increase in genistin (11.60 mg/g DW) and daidzein (8.31 mg/g DW) were recorded with new synthetic substance, 2-(2-fluoro-6-nitrobenzylsulfanyl) pyridine-4-carbothioamide (1 µmol/l) treatment after 48 h in cell suspension cultures of *Trifolium pratense*. This enhancement was at the tune of 152 and 151 %, respectively when compared with control. In addition, a maximum content of flavonoids (5.78 mg/g DW) an increase in production by 142 % was obtained when compared to untreated control with 6-hour 100 µmol/l elicitor application (Kasparova et al. 2012). In a different finding, the effect of 10 µM *N,N′*-dicyclohexylcarbodiimide (DCCD) on ginsenoside biosynthesis using cell suspension cultures of *Panax ginseng* was investigated and the maximal content of total ginsenosides increased to 3.0-fold compared to untreated control (Huang et al. 2013). Treatment of *Perovskia abrotanoides* adventitious root cultures with 25 µM $AgNO_3$ enhanced tanshinone production (12.98 µg/g DW) compared to untreated control (6.79 µg/g DW) roots (Zaker et al. 2015).

Treatment of gypsum (16 mg/l) to in vitro tissues of *Allium sativum* enhanced alliin production in garlic leaves and plantlets (3.74 and 3.69 µg/g dry wt) compared to control cultures (2.22 and 2.18 µg/g dry wt), respectively (Nasim et al. 2010). Increase in artemisinin and dihydroartemisinic acid in *A. annua* shoot cultures was obtained following dimethyl sulfoxide (DMSO) treatment and is attributed to ROS generation (Mannan et al. 2010). A treatment of 0.25 and 2.0 % (v/v) DMSO resulted in 2.26-fold enhancement in artimisinin and its precursor dihydroartimisinin synthesis, respectively in *A. annua* multiple shoot cultures. *Bacopa monnieri* shoot cultures treated with 45 mg/l of $CuSO_4$ promoted 1.42-fold increase in bacoside content (8.73 mg/g DW) compared to untreated (6.14 mg/g DW) controls (Sharma et al. 2015).

Influence of enhanced gene expression and elicitor treatment

It is quite common in elicitation studies that the elicitors promote yield increase and simultaneous enhanced gene expression for the target secondary metabolite in the background. These studies are helpful for the isolation of unknown genes and their exploitation in engineering of secondary metabolic pathways (Oksman-Caldentey and Inzé 2004). Overexpression of isolated target gene is a common strategy for securing increased yield of secondary metabolites (Thethewey 2004). However, in the recent past, it was found that a combined strategy of gene overexpression for key (rate limiting) enzyme in a secondary biosynthetic pathway and simultaneous elicitor application is exceedingly favourable for increased product yield. MeJA and 2-hydroxyethyl jasmonate (HEJ) elicitation at 200 µM evaluated singly to study gene expression profile and ginsenoside content in *Panax notoginseng* cell cultures (Hu and Zhong 2008). MeJA and HEJ mediated elicitation; influenced transcription of several ginsenoside biosynthetic genes e.g. squalene synthase, squalene epoxidase and cycloartenol synthase. Amongst two elicitors, HEJ at 200 µM promoted highest ginsenoside production (2.89 ± 0.15/100 mg DW) compared to 0.65 ± 0.06/100 mg DW in untreated control cultures. Elicitation of cell cultures of *V. vinifera* with MeJA application alongside media additive sucrose influenced pathogenesis related protein genes (phenylalanine ammonia lyase, chalcone synthase, stilbene synthase etc.) expression and

anthocyanin accumulation was quite promising (Belhadj et al. 2008). Sucrose (2 %) and MeJA at 20 μM promoted 23 and 28-fold increase in piceids (*trans* and *cis*-piceids) a major resveratrol derivative after 12 h, respectively. Combination of MeJA and sucrose produced anthocyanin of about 3.9 μmol/g FW more than sevenfold increase after 120 h compared to control.

In a significant finding, elicitation of hyoscyamine 6 β-hydroxylase (*h6h*) overexpressing *Atropa baetica* transgenic hairy roots resulted in 25-fold increase in *h6h*, 125-fold putrescine methyl transferase (*pmt*) with ASA and MeJA, respectively (el Jaber-Vazdekis et al. 2008). JA at 50 mg/l, elicitation stimulated ORCA3 transcriptional regulator up to a maximum of 170-fold increase in ORCA3 transcripts in *C. roseus* hairy roots. This JA driven elicitation triggered terpenoid indole alkaloid metabolites pathway genes in *C. roseus* (Peebles et al. 2009). The effect of constitutive (octadecanoid-responsive *Catharanthus* AP2/ERF domain) and application of MeJA and SNP to *C. roseus* hairy roots activated overexpression of CrORCA3 and resulted the decrease in catharanthine accumulation. However, MeJA (50 mg/l) treatment not only enhanced mRNA transcripts of pathway genes but also an increase in catharanthine content was obtained (Zhou et al. 2010).

In another report the squalene synthase (BfSS1) gene showed its overall regulatory influence on triterpene saponins biosynthesis following MeJA treatment of *Bupleurum falcatum* roots and enhanced saikosaponin and phytosterol production (Kim et al. 2011). In a molecular study, a number of genes for key enzymes (3-hydroxy-3-methylglutaryl-CoA reductase, 1-deoxy-D-xylulose-5-phosphate synthase, farnesyl diphosphate synthase, geranylgeranyl diphosphate synthase and copalyl diphosphate synthase) in tanshinone biosynthesis pathway were characterized in *S. miltiorrhiza* hairy root cultures following elicitor treatment (Kai et al. 2012). Up-regulation of several genes following 100 μM MeJA treatment of hairy roots promoted highest tanshinone production (0.931 mg/g DW) on day 9 which was 5.78-fold higher compared to 0.161 mg/g DW in control. Recently, an increase in taxane production was also reported involving 100 μM MeJA and coronatine driven elicitation of *Taxus x media* transgenic hairy root cultures (Sykłowska-Baranek et al. 2015).

Conclusion and future perspectives

Keeping in view the enormous nature of existing literature on chemical elicitation studies for secondary metabolite production using plant cell and tissue culture systems is uncatchable. It is in actuality becomes a huge task to document the entire progress in real time sense in one token review. However, in the present exercise, attempts are made to bestow a sky eye view selected representative projection of current trend on the influence of exclusively chemical elicitors and their profound effect on secondary metabolite production using different plant cell and tissue culture systems.

Now it is an established fact that chemical elicitors are having immense multitasking ability to drive a number of cellular functions at both biochemical and genetic level (Boatwright and Pajerowska-Mukhtar 2013; Caarls et al. 2015). Elicitor receptor perception in the plasma membrane, contribution of signals from organs such as chloroplast and mitochondria and participation of 26S proteasomal degradation pathway facilitated by JA are key control points in the mechanism of elicitor action. Further, the involvement of MAPKs in cytosol by ET, NO and Ca^{++} dependent regulation lead to direct gene expression or via transcription factor mediation. In addition, interaction of signal compounds (NO, JA, ET and SA) generates ROS which contribute towards gene expression and production of requisite transcripts for enzymatic synthesis of enhanced secondary metabolites.

In the present context, cell suspensions are maximum used compared to other culture systems in elicitor treatment experiments. The hairy roots have proved to be a valuable culture system for elicitation studies. Amongst the chemical elicitors, JA, SA and its related compounds; are used extensively for elicitation. Overall survey reveals that concentrations of chemical elicitors do matter for enhanced production of secondary metabolites. Quite interestingly 100 μM concentration across different chemical elicitors are used for elicitation experiments using culture systems of diverse plants. In particular, 100 μM MeJA has been used in majority of findings surveyed. It is difficult to comprehend the basis for the selection of desired elicitor concentrations. However, the choice of concentrations look arbitrary based on logistic standardization experiments and influenced by previous published literature. Adequate understanding and knowledge on the mechanism of action of eliciting signal compounds may show the way to decide on the requisite concentration of elicitors for proper experimentation and promising results.

Chemical elicitors generally used singly or in combinations with other agents for better results. The combinations can be of different permutations such as between two, between three or sometimes-single elicitor or more than one elicitor combine with essential macro or micronutrients, precursors and media additives. Further, at times it is also observed that elicitors combined with physical factors such as UV light, temperature regime and pulsed electric field yielded better results for secondary metabolite production (Hao et al. 2009; Saw et al. 2012). In addition, some unique combination strategies such as over expression of a rate limiting enzyme (key gene) combined with an

elicitor e.g. MeJA can bring about multifold increase in secondary metabolite production in vitro compared to the application of elicitor alone (Peebles et al. 2009). The literature survey and overall review of data indicated that a maximum of more than 2000-fold yield enhancement could be achieved through elicitation. This is a positive indication for exploitation of elicitation driven enhanced plant secondary metabolite production in vitro using different biotechnological approaches.

The initial pre-genomic research initiatives included knowhow to understand natural product biosynthesis using biochemical and genetic approaches. Simple concept of elicitation and the use of chemical elicitors in particular have changed the basic strategy of understanding genes involved in secondary metabolite synthesis. In the post-genomic era use of chemical elicitors, in particular MeJA to study plant metabolic network demonstrated for the first time for secondary metabolism in tobacco cells (Goossens et al. 2003). The basic knowledge of elicitation mechanism at biochemical, genetic and molecular level will be helpful to manipulate secondary metabolic pathways to develop quality foods and pharmaceuticals in health care for practical applications (Baenas et al. 2014). Therefore, the chemical elicitors compared to other biotic (biological elicitors) has become unique for its multitasking role in triggering array of cellular activities which ultimately help to understand biosynthesis of secondary metabolites using molecular and genetic engineering techniques for commercial exploitation.

Acknowledgments Authors would like to thank sponsors University Grants Commission (UGC), Department of Science and Technology (DST), New Delhi OU-DST-PURSE Project and thank OU-UGC-CPEPA Programme UGC, New Delhi for financial support. Mr. Mohd Zaheer thanks UGC and DST, New Delhi for the Research Fellowships.

Complaince with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abhyankar G, Reddy VD, Giri CC, Rao KV, Lakshmi VV, Prabhakar S, Vairamani M, Thippeswamy BS, Bhattacharya PS (2005) Amplified fragment length polymorphism and metabolomic profiles of hairy roots of *Psoralea corylifolia* L. Phytochemistry 66:2441–2457

Ajungla L, Patil PP, Barmukh RB, Nikam TD (2009) Influence of biotic and abiotic elicitors on accumulation of hyoscyamine and scopolamine in root cultures of *Datura metal* L. Indian J Biotechnol 8:317–322

Ali MB, Yu KW, Hahn EJ, Paek KY (2005) Differential responses of anti-oxidants enzymes, lipoxygenase activity, ascorbate content and the production of saponins in tissue cultured root of mountain *Panax ginseng* C.A. Mayer and *Panax quinquefolium* L. in bioreactor subjected to methyl jasmonate stress. Plant Sci 169:83–92

Ali M, Abbasi BH, Ali GS (2015) Elicitation of antioxidant secondary metabolites with jasmonates and gibberellic acid in cell suspension cultures of *Artemisia absinthium* L. Plant Cell, Tissue Organ Cult 120:1099–1106

Bae KH, Choi YE, Shin CG, Kim YY, Kim YS (2006) Enhanced ginsenoside productivity by combination of ethephon and methyl jasmoante in ginseng (*Panax ginseng* C.A. Meyer) adventitious root cultures. Biotechnol Lett 28:1163–1166

Baenas N, García-Viguera C, Moreno DA (2014) Elicitation: a tool for enriching the bioactive composition of foods. Molecules 19:13541–13563

Ballare CL (2011) Jasmonate-induced defenses: a tale of intelligence, collaborators and rascals. Trends Plant Sci 16:249–257

Banerjee S, Singh S, Rahman LU (2012) Biotransformation studies using hairy root cultures—a review. Biotechnol Adv 30:461–468

Baque MA, Moh SH, Lee EJ, Zhong JJ, Paek KY (2012) Production of biomass and useful compounds from adventitious roots of high-value added medicinal plants using bioreactor. Biotechnol Adv 30:1255–1267

BBC research report: botanical and plant-derived drugs in global markets. http://www.bccresearch.com/market-research/biotechnology/botanical-plant-derived-drugs-report-bio022g.html

Begum F, Nageswara Rao SS, Rao K, Prameela Devi Y, Giri A, Giri CC (2009) Increased vincristine production from *Agrobacterium tumefaciens* C-58 induced shooty teratomas of *Catharanthus roseus*. Nat Prod Res 23:973–981

Belchi-Navarro S, Almagro L, Lijavetzky D, Bru R, Pedreño MA (2012) Enhanced extracellular production of trans-resveratrol in *Vitis vinifera* suspension cultured cells by using cyclodextrins and methyljasmonate. Plant Cell Rep 31:81–89

Belhadj A, Telef N, Saigne C, Cluzet S, Barrieu F, Hamdi S, Mérillon JM (2008) Effect of methyl jasmonate in combination with carbohydrates on gene expression of PR proteins, stilbene and anthocyanin accumulation in grapevine cell cultures. Plant Physiol Biochem 46:493–499

Bhalla R, Narasimhan K, Swarup S (2005) Metabolomics and its role in understanding cellular responses in plants. Plant Cell Rep 24:562–571

Bhuvaneswari CH, Rao K, Gandi S, Giri A (2015) Gymnemic acid enhancement in the suspension cultures of *Gymnema sylvestre* by using the signaling molecules—methyl jasmonate and salicylic acid. In Vitro Cell Dev Biol-Plant 51:88–92

Boatwright JL, Pajerowska-Mukhtar K (2013) Salicylic acid: an old hormone up to new tricks. Mol Plant Pathol 14:623–634

Caarls L, Pieterse CM, Van Wees SC (2015) How salicylic acid takes transcriptional control over jasmonic acid signaling. Front Plant Sci 6:170 **(online first)**

Caretto S, Quarta A, Durante M, Nisi R, De Paolis A, Blando F, Mita G (2011) Methyl jasmonate and miconazole differently affect arteminisin production and gene expression in *Artemisia annua* suspension cultures. Plant Biol 13:51–58

Coste A, Vlase L, Halmagyi A, Deliu C, Coldea G (2011) Effects of plant growth regulators and elicitors on production of secondary metabolites in shoot cultures of *Hypericum hirsutum* and *Hypericum maculatum*. Plant Cell, Tissue Organ Cult 106:279–288

Cui L, Wang Z-Y, Zhou X-H (2012) Optimization of elicitors and precursors to enhance valtrate production in adventitious roots of *Valeriana amurensis* Smir. ex Kom. Plant Cell, Tissue Organ Cult 108:411–420

Dar TA, Uddin M, Khan MMA, Hakeem KR, Jaleel H (2015) Jasmonates counter plant stress: a Review. Environ Exp Bot 115:49–57

Dass S, Ramawat KG (2009) Elicitation of guggulsterone production in cell cultures of *Commiphora wightii* by plant gums. Plant Cell, Tissue Organ Cult 96:349–353

Dias DA, Urban S, Roessner U (2012) A historical overview of natural products in drug discovery. Metabolites 2:303–336

Diwan R, Malpathak N (2011) Bioprocess optimization of furanocoumarin elicitation by medium renewal and re-elicitation: a perfusion-based approach. Appl Biochem Biotechnol 163:756–764

Dong J, Wan G, Liang Z (2010) Accumulation of salicylic acid-induced phenolic compounds and raised activities of secondary metabolic and antioxidative enzymes in *Salvia miltiorrhiza* cell culture. J Biotechnol 148:99–104

Durante M, Caretto S, Quarta A, De Paolis A, Nisi R, Mita G (2011) β-Cyclodextrins enhance artemisinin production in *Artemisia annua* suspension cell cultures. Appl Microbiol Biotechnol 90:1905–1913

el Jaber-Vazdekis N, Barres ML, Ravelo AG, Zarate R (2008) Effects of elicitors on tropane alkaloids and gene expression in *Atropa baetica* transgenic hairy roots. J Nat Prod 71:2026–2031

Elkington BG (2014) New finding of an anti-TB compound in the genus Marsypopetalum (Annonaceae) from a traditional herbal remedy of Laos. J Ethnopharmacol 151:903–911

Gai QY, Jiao J, Luo M, Wang W, Gu CB, Fu YJ, Ma W (2016) Tremendous enhancements of isoflavonoid biosynthesis, associatedgene expression and antioxidant capacity in *Astragalus membranaceus* hairy root cultures elicited by methyl jasmonate. Process Biochem. doi:10.1016/j.procbio.2016.01.012

Ghorpade RP, Chopra A, Nikam TD (2011) Influence of biotic and abiotic elicitors on four major isomers of boswellic acid in callus culture of *Boswellia serrata* Roxb. Plant Omics J 4:169–176

Giri CC, Vijaya Laxmi G (2000) Production of transgenic rice with agronomically useful genes. Biotechnol Adv 18:653–683

Giri A, Dhingra V, Giri CC, Singh A, Ward OP, Narasu ML (2001a) Biotransformation using cell, tissue, organ cultures and enzyme systems: current trend and future prospects. Biotechnol Adv 19:175–199

Giri A, Giri CC, Dhingra V, Narasu ML (2001b) Enhanced production of podophyllotoxin from *Agrobacterium rhizogenes* transformed cultures of *Podophyllum hexandrum*. Nat Prod Lett 15:229–235

Giri A, Giri CC, Narasu ML (2003) Recent advances in applications of transgenic hairy roots. In: Jaiwal PK, Singh RP (eds) Plant genetic engineering: improvement of commercial plants—II. Sci Tech Publisher LLC, Houston, pp 23–81

Giri CC, Giri A, Narasu ML (2009) *Catharanthus roseus* (Periwinckle) a potential drug source for cancer chemotherapy and biotechnological interventions: an overview. In: Trivedi PC (ed) Medicinal plants: utilization and conservation. Aviskar Publishers, Jaipur, pp 475–504

Gomez P, Ortuno A, Del Rio JA (2004) Ultrastructural changes and diosgenin content in cell suspensions of *Trigonella foenum-graecum* L. by ethylene treatment. Plant Growth Regul 44:93–99

Goossens A, Hakkinen ST, Laakso I, Seppänen-Laakso T, Biondi S, De Sutter V, Lammertyn F, Nuutila AM, Söderlund H, Zabeau M, Inzé D, Oksman-Caldentey KM (2003) A functional genomics approach toward the understanding of secondary metabolism in plant cells. Proc Natl Acad Sci USA 100:8595–8600

Gorelick J, Bernstein N (2014) Elicitation: an underutilized tool in the development of medicinal plants as a source of therapeutic secondary metabolites. In: Sparks DL (ed) Advances in agronomy, Book Series, vol 124. Elsevier, UK, pp 201–230

Goyal S, Ramawat KG (2008) Ethrel treatment enhanced isoflavonoids accumulation in cell suspension cultures of *Pueraria tuberosa*, a woody legume. Acta Physiol Plant 30:849–853

Guillon S, Tremouillaux-Guiller J, Pati PK, Rideau M, Gantet P (2006) Hairy root research: recent scenario and exciting prospects. Curr Opin Plant Biol 11:341–346

Gutierrez-Carbajal MG, Monforte-Gonzalez M, de Miranda-Ham ML, Godoy-Hernandez G, Vazquez-Flota F (2010) Induction of capsaicinoid synthesis in *Capsicum chinense* cell cultures by salicylic acid or methyl jasmonate. Biol Plant 54:430–434

Hadacek F (2002) Secondary metabolites as plant traits: current assessment and future perspectives. Crit Rev Plant Sci 21:273–322

Hao G, Du X, Zhao F, Shi R, Wang J (2009) Role of nitric oxide in UV-B-induced activation of PAL and stimulation of flavonoid biosynthesis in *Ginkgo biloba* callus. Plant Cell, Tissue Organ Cult 97:175–185

Hashemi SM, Naghavi MR (2016) Production and gene expression of morphinan alkaloids in hairy root culture of *Papaver orientale* L. using abiotic elicitors. Plant Cell, Tissue Organ Cult 125:31–41

Hernández-Domínguez E, Campos-Tamayo F, Vázquez-Flota F (2004) Vindoline synthesis in in vitro shoot cultures of *Catharanthus roseus*. Biotechnol Lett 26:671–674

Hu FX, Zhong JJ (2008) Jasmonic acid mediates gene transcription of ginsenoside biosynthesis in cell cultures of *Panax notoginseng* treated with chemically synthesized 2-hydroxyethyl jasmonate. Process Biochem 43:113–118

Hu Y, Yu Y, Piao C, Liu J, Yu H (2011) Methyl jasmonate and salicylic acid induced D-chiro-inositol production in suspension cultures of buckwheat (*Fagopyrum esculentum*). Plant Cell, Tissue Organ Cult 106:419–424

Huang C, Qian ZG, Zhong JJ (2013) Enhancement of ginsenoside biosynthesis in cell cultures of *Panax ginseng* by *N,N′*-dicyclohexylcarbodiimide elicitation. J Biotechnol 165:30–36

Hussain MS, Fareed S, Ansari S, Rahman MA, Ahmad IZ, Saeed M (2012) Current approaches towards production of secondary plant metabolites. J Pharm Bioallied Sci 4:10–20

Jeong GT, Park DH, Ryu HW, Hwang B, Woo JC, Kim D, Kim SW (2005) Production of antioxidant compounds by culture of *Panax ginseng* C.A. meyer hairy roots. Appl Biochem Biotechnol 121–124:1147–1158

Kai G, Liao P, Xu H, Wang J, Zhou C, Zhou W, Qi Y, Xiao J, Wang Y, Zhang L (2012) Molecular mechanism of elicitor-induced tanshinone accumulation in *Salvia miltiorrhiza* hairy root cultures. Acta Physiol Plant 34:1421–1433

Kang SM, Jung HY, Kang YM, Yun DJ, Bahk JD, Jk Yang, Choi MS (2004) Effects of methyl jasmonate and salicylic acid on the production of tropane alkaloids and the expression of PMT and H6H in adventitious root cultures of *Scopolia parviflora*. Plant Sci 166:745–751

Karuppusamy S (2009) A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures. J Med Plants Res 3:1222–1239

Kasparova M, Siatka T, Klimesova V, Dusek J (2012) New Synthetic Pyridine Derivate as potential elicitor in production of isoflavonoids and flavonoids in *Trifolium pretense* L. suspension culture. Sci World J 2012:1–5

Kazan K (2015) Diverse role of jasmonates and ethylene in abiotic stress tolerance. Trends Plant Sci 20:219–229

Kehie M, Kumaria S, Tandon P (2016) Biotechnological enhancement of capsaicin biosynthesis in cell suspension cultures of Naga King Chili (*Capsicum chinense* Jacq.). Bioprocess Biosyst Eng 39:205–210

Kikowska M, Budzianowski J, Krawczyk A, Thiem B (2012) Accumulation of rosmarinic, chlorogenic and caffeic acids in in vitro cultures of *Eryngium planum* L. Acta Physiol Plant 34:2425–2433

Kikowska M, Kędziora I, Krawczyk A, Thiem B (2015) Methyl jasmonate, yeast extract and sucrose stimulate phenolic acids

accumulation in *Eryngium planum* L. shoot cultures. Acta Biochim Pol 62:197–200

Kim OT, Kim MY, Hong MH, Ahn JC, Hwang B (2004) Stimulation of asiaticoside accumulation in the whole plant cultures of *Centella asiatica* (L.) urban by elicitors. Plant Cell Rep 23:339–344

Kim YS, Cho JH, Park S, Han JY, Back K, Choi YE (2011) Gene regulation patterns in triterpene biosynthetic pathway driven by overexpression of squalene synthase and methyl jasmonate elicitation in *Bupleurum falcatum*. Planta 233:343–355

Kim OT, Yoo NH, Kim GS, Kim YC, Bang KH, Hyun DY, Kim SH, Kim MY (2013) Stimulation of Rg3 ginsenoside biosynthesis in ginseng hairy roots elicited by methyl jasmonate. Plant Cell, Tissue Organ Cult 112:87–93

Kopyra M, Gwozdz EA (2004) The role of nitric oxide in plant growth regulation and responses to abiotic stress. Acta Physiol Plant 26:459–472

Lakhotia P, Pal SK, Singh SK, Singh MC, Prasad KV, Kishan S (2014) Influence of biotic and abiotic elicitors on production of betalain pigments in bougainvillea callus cultures. Indian J Hortic 71:373–378

Lee KH (2010) Discovery and development of natural product-derived chemotherapeutic agents based on a medicinal chemistry approach. J Nat Prod 73:500–516

Lee EJ, Park SY, Paek KY (2015) Enhancement strategies of bioactive compound production in adventitious root cultures of *Eleutherococcus koreanum* Nakai subjected to methyl jasmonate and salicylic acid elicitation through airlift bioreactors. Plant Cell, Tissue Organ Cult 120:1–10

Li W, Koike K, Asada Y, Yoshikawa T, Nikaido T (2005) Rosmarinic acid production by *Coleus forskohlii* hairy root cultures. Plant Cell, Tissue Organ Cult 80:151–155

Li M, Peebles CA, Shanks JV, San KY (2011) Effect of sodium nitroprusside on growth and terpenoid indole alkaloid production in *Catharanthus roseus* hairy root cultures. Biotechnol Prog 27:625–630

Li B, Wang B, Li H, Peng L, Ru M, Liang Z, Yan X, Zhu Y (2016) Establishment of *Salvia castanea* Diels f. tomentosa Stib. hairy root cultures and the promotion of tanshinone accumulation and gene expression with Ag^{+}, methyl jasmonate, and yeast extract elicitation. Protoplasma 253:87–100

Liang ZS, Yang DF, Liang X, Zhang YJ, Liu Y, Liu FH (2012) Roles of reactive oxygen species in methyl jasmonate and nitric oxide-induced tanshinone production in *Salvia miltiorrhiza* hairy roots. Plant Cell Rep 31:873–883

Liu XN, Zhang XQ, Sun JS (2007) Effects of cytokinins and elicitors on the production of hypericins and hyperforin metabolites in *Hypericum sampsonii* and *Hypericum perforatum*. Plant Growth Regul 53:207–214

Liu J, Zhu J, Tang L, Wen W, Lv S, Yu R (2014) Enhancement of vindoline and vinblastine production in suspension-cultured cells of *Catharanthus roseus* by artemisinic acid elicitation. World J Microbiol Biotechnol 30:175–180

Luczkiewicz M, Kokotkiewicz A (2012) Elicitation and permeabilisation affect the accumulation and storage profile of phytoestrogens in high productive suspension cultures of *Genista tinctoria*. Acta Physiol Plant 31:1–16

Mangas S, Bonfill M, Osuna L, Moyano E, Tortoriello J, Cusido RM, Piñol MT, Palazón J (2006) The effect of methyl jasmonate on triterpene and sterol metabolisms of *Centella asiatica*, *Ruscus aculeatus* and *Galphimia glauca*. Phytochemistry 67:2041–2049

Mannan A, Liu C, Arsenault PR, Towler MJ, Vail DR, Lorence A, Weathers PJ (2010) DMSO triggers the generation of ROS leading to an increase in artemisinin and dihydroartemisinic acid in *Artemisia annua* shoot cultures. Plant Cell Rep 29:143–152

Matkowski A (2008) Plant in vitro culture for the production of antioxidants—a review. Biotechnol Adv 6:548–560

Matsuura HN, Rau MR, Fett-Neto AG (2014) Oxidative stress and production of bioactive monoterpene indole alkaloids: biotechnological implications. Biotechnol Lett 36:191–200

Miao GP, Zhu CS, Yang YQ, Feng MX, Ma ZQ, Feng JT, Zhang X (2014) Elicitation and in situ adsorption enhanced secondary metabolites production of *Tripterygium wilfordii* Hook. f. adventitious root fragment liquid cultures in shake flask and a modified bubble column bioreactor. Bioprocess Biosyst Eng 37:641–650

Mobin M, Wu CH, Tewari RK, Paek KY (2015) Studies on the glyphosate-induced amino acid starvation and addition of precursors on caffeic acid accumulation and profiles in adventitious roots of *Echinacea purpurea* (L.) Moench. Plant Cell, Tissue Organ Cult 120:291–301

Moore BD, Andrew RL, Kulheim C, Foley WJ (2014) Explaining intraspecific diversity in plant secondary metabolites in an ecological context. New Phytol 201:733–750

Murthy HN, Lee EJ, Paek KY (2014) Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. Plant Cell, Tissue Organ Cult 118:1–16

Namdeo AG (2007) Plant cell elicitation for production of secondary metabolites: a review. Pharmacogn Rev 1:69–79

Nasim SA, Dhir B, Kapoor R, Fatima S, Mahmooduzzafar Mujib A (2010) Alliin production in various tissues and organs of *Allium sativum* grown under normal and sulphur-supplemented in vitro conditions. Plant Cell, Tissue Organ Cult 101:59–63

Oksman-Caldentey KM, Inzé D (2004) Plant cell factories in the postgenome era: new ways to produce designer secondary metabolites. Trends Plant Sci 9:433–440

Onrubia M, Cusido RM, Ramirez K, Hernandez-Vazquez L, Moyano E, Bonfill M, Palazon J (2013) Bioprocessing of plant in vitro systems for the mass production of pharmaceutically important metabolites: paclitaxel and its derivatives. Curr Med Chem 20:880–891

Pan W (2014) Bioactive compounds from *Vitex leptobotrys*. J Nat Prod 77:663–667

Pandey H, Pandey P, Singh S, Gupta R, Banerjee S (2015) Production of anti-cancer triterpene (betulinic acid) from callus cultures of different *Ocimum* species and its elicitation. Protoplasma 252:647–655

Patil JG, Ahire ML, Nitnaware KM, Panda S, Bhatt VP, Kishor PB, Nikam TD (2013) In vitro propagation and production of cardiotonic glycosides in shoot cultures of *Digitalis purpurea* L. by elicitation and precursor feeding. Appl Microbiol Biotechnol 97:2379–2393

Pawar KD, Thengane SR (2011) Influence of abiotic elicitation on production of dipyranocoumarins in suspension cultures of *Calophyllum inophyllum* L. Acta Physiol Plant 33:2149–2158

Peebles CA, Hughes EH, Shanks JV, San KY (2009) Transcriptional response of the terpenoid indole alkaloid pathway to the overexpression of ORCA3 along with jasmonic acid elicitation of *Catharanthus roseus* hairy roots over time. Metab Eng 11:76–86

Pi Y, Jiang K, Hou R, Gong Y, Lin J, Sun X, Tang K (2010) Examination of camptothecin and 10-hydroxycamptothecin in *Camptotheca acuminata* plant and cell culture, and the affected yields under several cell culture treatments. Biocell 34:139–143

Pichersky E, Gang DR (2000) Genetics and biochemistry of secondary metabolites: an evolutionary perspective. Trends Plant Sci 5:439–445

Pirbalouti AG, Sajjadi SE, Parang K (2014) A review (research and patents) on jasmonic acid and its derivatives. Arch Pharm Chem Life Sci 347:229–239

Ptak A, Tahchy AEI, Wyzgolik G, Henry M, Laurain-Mattar D (2010) Effects of ethylene on somatic embryogenesis and galanthamine

content in *Leucojum aestivum* L. cultures. Plant Cell, Tissue Organ Cult 102:61–67
Putalun W, Udomsin O, Yusakul G, Juengwatanatrakul T, Sakamoto S, Tanaka H (2010) Enhanced plumbagin production from in vitro cultures of *Drosera burmanii* using elicitation. Biotechnol Lett 32:721–724
Qian ZG, Zhao ZJ, Xu Y, Qian X, Zhong JJ (2005) A novel synthetic fluoro-containing jasmonate derivative acts as a chemical inducing signal for plant secondary metabolism. Appl Microbiol Biotechnol 68:98–103
Qiao W, Fan LM (2008) Nitric oxide signaling in plant responses to abiotic stresses. J Integr Plant Biol 50:1238–1246
Qin B, Ma L, Wang Y, Chen M, Lan X, Wu N, Liao Z (2014) Effects of acetylsalicylic acid and UV-B on gene expression and tropane alkaloid biosynthesis in hairy root cultures of *Anisodus luridus*. Plant Cell, Tissue Organ Cult 117:483–490
Qu J, Zhang W, Yu X (2011) A combination of elicitation and precursor feeding leads to increased anthocyanin synthesis in cell suspension cultures of *Vitis vinifera*. Plant Cell, Tissue Organ Cult 107:261–269
Ram M, Prasad KV, Singh SK, Hada BS, Kumar S (2013) Influence of salicylic acid and methyl jasmonate elicitation on anthocyanin production in callus cultures of *Rosa hybrida* L. Plant Cell, Tissue Organ Cult 113:459–467
Ramakrishna A, Ravishankar GA (2011) Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal Behav 6:1720–1731
Ramirez-Estrada K, Vidal-Limon H, Hidalgo D, Moyano E, Golenioswki M, Cusidó RM, Palazon J (2016) Elicitation, an effective strategy for the biotechnological production of bioactive high-added value compounds in plant cell factories. Molecules 21:182
Raomai S, Kumaria S, Kehie M, Tandon P (2015) Plantlet regeneration of *Paris polyphylla* Sm. via thin cell layer culture and enhancement of steroidal saponins in mini-rhizome cultures using elicitors. Plant Growth Regul 75:341–353
Roat C, Ramawat KG (2009) Elicitor-induced accumulation of stilbenes in cell suspension cultures of *Cayratia trifolia* (L.) Domin. Plant Biotechnol Rep 3:135–138
San Miguel-Chavez R, Soto-Hernandez M, Ramos-Valdivia AC, Kite G (2007) Alkaloid production in elicited cell suspension cultures of *Erythrina americana* Miller. Phytochem Rev 6:167–173
Santner A, Estelle M (2009) Recent advances and emerging trends in plant hormone signaling. Nature 459:1071–1078
Saw NMMT, Riedel H, Cai Z, Kutuk O, Smetanska I (2012) Stimulation of anthocyanin synthesis in grape (*Vitis vinifera*) cell cultures by pulsed electric fields and ethephon. Plant Cell, Tissue Organ Cult 108:47–54
Senger RS, Phisalaphong M, Karim MN, Linden JC (2006) Development of a culture sub-population induction model: signaling pathways synergy and taxanes production by *Taxus canadensis*. Biotechnol Prog 22:1671–1682
Sharma M, Ahuja A, Gupta R, Mallubhotla S (2015) Enhanced bacoside production in shoot cultures of *Bacopa monnieri* under the influence of abiotic elicitors. Nat Prod Res 29:745–749
Shimizu Y, Maeda K, Kato M, Shimomura K (2010) Methyl jasmonate induces anthocyanin accumulation in *Gynura bicolor* cultured roots. In Vitro Cell Dev Biol-Plant 46:460–465
Shinde AN, Malpathak N, Fulzele DP (2009) Enhanced production of phytoestrogenic isoflavones from hairy root cultures of *Psoralea corylifolia* L. using elicitation and precursor feeding. Biotechnol Bioprocess Eng 14:288–294
Shyamkumar B, Anjaneyulu C, Giri CC (2007) Genetic transformation of *Terminalia chebula* Ritz. and detection of tannin in transformed tissue. Current Sci 92:361–367
Silja PK, Gisha GP, Satheeshkumar K (2014) Enhanced plumbagin accumulation in embryogenic cell suspension cultures of *Plumbago rosea* L. following elicitation. Plant Cell, Tissue Organ Cult 119:469–477
Sivanandhan G, Rajesh M, Arun M, Jeyaraj M, Kapil Dev G, Arjunan A, Manickavasagam M, Muthuselvam M, Selvaraj N, Ganapathi A (2013) Effect of culture conditions, cytokinins, methyl jasmonate and salicylic acid on the biomass accumulation and production of withanolides in multiple shoot culture of *Withania somnifera* (L.) Dunal using liquid culture. Acta Physiol Plant 35:715–728
Soejarto DD, Gyllenhaal C, Kadushin MR, Southavong B, Sydara K, Bouamanivong S, Xaiveu M, Zhang HJ, Franzblau SG, Tan GT, Pezzuto JM, Riley MC, Elkington BG, Waller DP (2012) An ethnobotanical survey of medicinal plants of Laos toward the discovery of bioactive compounds as potential candidates for pharmaceutical development. Pharm Biol 50:42–60
Song MC, Kim EJ, Kim E, Rathwell K, Nama SJ, Yoon YJ (2014) Microbial biosynthesis of medicinally important plant secondary metabolites. Nat Prod Rep 31:1497–1509
Srivastava S, Srivastava AK (2007) Hairy root culture for mass-production of high-value secondary metabolites. Crit Rev Biotechnol 27:29–43
Sykłowska-Baranek K, Pilarek M, Bonfill M, Kafel K, Pietrosiuk A (2015) Perfluorodecalin-supported system enhances taxane production in hairy root cultures of *Taxus x media var. Hicksii* carrying a taxadiene synthase transgene. Plant Cell, Tissue Organ Cult 120:1051–1059
Tewari RK, Lee SY, Hahn EJ, Paek KY (2007) Temporal changes in the growth, saponin content and antioxidant defense in the adventitious roots of *Panax ginseng* subjected to nitric oxide elicitation. Plant Biotechnol Rep 1:227–235
Thethewey RN (2004) Metabolite profiling as an aid to metabolic engineering in plants. Curr Opin Plant Biol 7:196–201
Touno K, Tamaoka J, Ohashi Y, Shimomura K (2005) Ethylene induced shikonin biosynthesis in shoot culture of *Lithospermum erythrorhizon*. Plant Physiol Biochem 43:101–105
Trujillo-Villanueva K, Rubio-Pina J, Monforte-Gonzalez M, Ramírez-Benítez E, Vázquez-Flota F (2012) The sequential exposure to jasmonate, salicylic acid and yeast extract promotes sanguinarine accumulation in *Argemone mexicana* cell cultures. Biotechnol Lett 34:379–385
Tůmová L, Tůma J, Megusar K, Dolezal M (2010) Substituted pyrazinecarboxamides as abiotic elicitors of flavolignan production in *Silybum marianum* (L.) gaertn cultures in vitro. Molecules 15:331–340
Udomsuk L, Jarukamjorn K, Tanaka H, Putalun W (2011) Improved isoflavonoid production in *Pueraria candollei* hairy root cultures using elicitation. Biotechnol Lett 33:369–374
Vanisree M, Tsay HS (2004) Plant cell cultures—an alternative and efficient source for the production of biologically important secondary metabolites. Int J App Sci Eng 1:29–48
Vazquez-Flota F, Hernandez-Domınguez E, de Lourdes Miranda-Ham M, Monforte-González M (2009) A differential response to chemical elicitors in *Catharanthus roseus* in vitro cultures. Biotechnol Lett 31:591–595
Verma P, Khan SA, Mathur AK, Ghosh S, Shanker K, Kalra A (2014) Improved sanguinarine production via biotic and abiotic elicitations and precursor feeding in cell suspensions of latex-less variety of *Papaver somniferum* with their gene expression studies and upscaling in bioreactor. Protoplasma 251:1359–1371
Vlot AC, Dempsey DA, Klessig DF (2009) Salicylic acid, a multifaceted hormone to combat disease. Ann Rev Phytopathol 47:177–206
Wang KL, Li H, Ecker JR (2002) Ethylene biosynthesis and signaling networks. Plant Cell 14:S131–S151
Wang JW, Zheng LP, Wu JY, Tan RX (2006) Involvement of nitric oxide in oxidative burst, phenylalanine ammonia-lyase

activation and taxol production induced by low-energy ultrasound in *Taxus yunnanensis* cell suspension cultures. Nitric Oxide 15:351–358

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

Wang QJ, Zheng LP, Sima YH, Yuan HY, Wang JW (2013) Methyl jasmonate stimulates 20-hydroxyecdysone production in cell suspension cultures of *Achyranthes bidentata*. Plant Omics J 6:116–120

Wang S, Guo LP, Xie T, Yang J, Tang JF, Li X, Wang X, Huang LQ (2014) Different secondary metabolic responses to MeJA treatment in shikonin proficient and shikonin-deficient cell lines from *Arnebia euchroma* (Royle) Johnst. Plant Cell, Tissue Organ Cult 119:587–598

Wasternack C (2014) Action of jasmonates in plant stress responses and development–applied aspects. Biotechnol Adv 32:31–39

Wielanek M, Urbanek H (2006) Enhanced glucotropaeolin production in hairy root cultures of *Tropaeolum majus* L. by combining elicitation and precursor feeding. Plant Cell, Tissue Organ Cult 86:177–186

Wilson ID, Neill SJ, Hancock JT (2008) Nitric oxide synthesis and signaling in plants. Plant, Cell Environ 31:622–631

Wu CH, Murthy HN, Hahn EJ, Paek KY (2008) Establishment of adventitious root co-culture of Ginseng and *Echinacea* for the production of secondary metabolites. Acta Physiol Plant 30:891–896

Wungsintaweekul J, Choo-malee J, Charoonratana T, Keawpradub N (2012) Methyl jasmonate and yeast extract stimulate mitragynine production in *Mitragyna speciosa* (Roxb.) Korth. shoot culture. Biotechnol Lett 34:1945–1950

Xiao WH, Cheng JS, Yuan YJ (2009) Spatial–temporal distribution of nitric oxide involved in regulation of phenylalanine ammonialyase activation and taxol production in immobilized *Taxus cuspidata* cells. J Biotechnol 139:222–228

Xu MJ, Dong JF, Zhang G (2005) Enhancement of hypericin production and cell growth of *Hypericum perforatum L.* suspension cells by nitric oxide. Chin J Biotechnol 21:66–70

Yoo SD, Cho Y, Sheen J (2009) Emerging connections in the ethylene signaling network. Trends Plant Sci 14:270–279

Yoo NH, Kim OT, Kim JB, Kim SH, Kim YC, Bang KH, Hyun DY, Cha SW, Kim MY, Hwang B (2011) Enhancement of centelloside production from cultured plants of *Centella asiatica* by combination of thidiazuron and methyl jasmonate. Plant Biotechnol Rep 5:283–287

Yousefzadi M, Sharifi M, Behmanesh M, Ghasempour A, Moyano E, Palazon J (2010) Salicylic acid improves podophyllotoxin production in cell cultures of *Linum album* by increasing the expression of genes related with its biosynthesis. Biotechnol Lett 32:1739–1743

Zabala MA, Angarita M, Restrepo JM, Caicedo LA, Perea M (2010) Elicitation with methyl-jasmonate stimulates peruvoside production in cell suspension cultures of *Thevetia peruviana*. In Vitro Cell Dev Biol-Plant 46:233–238

Zaheer M, Giri CC (2015) Multiple shoot induction and jasmonic versus salicylic acid driven elicitation for enhanced andrographolide production in *Andrographis paniculata*. Plant Cell, Tissue Organ Cult 122:553–563

Zaheer M, Reddy VD, Giri CC (2016) Enhanced daidzin production from jasmonic and acetyl salicylic acid elicited hairy root cultures of *Psoralea corylifolia* L. (Fabaceae). Nat Prod Res. doi:10.1080/14786419.2015.1054823

Zaker A, Sykora C, Gossnitzer F, Abrishamchi P, Asili J, Mousavic SH, Wawrosch C (2015) Effects of some elicitors on tanshinone production in adventitious root cultures of *Perovskia abrotanoides* Karel. Ind Crops Prod 67:97–102

Zare N, Farjaminezhad R, Asghari-Zakaria R, Farjaminezhad M (2014) Enhanced thebaine production in *Papaver bracteatum* cell suspension culture by combination of elicitation and precursor feeding. Nat Prod Res 28:711–717

Zhao J, Zheng SH, Fujita K, Sakai K (2004) Jasmonate and ethylene signalling and their interaction are integral parts of the elicitor-signalling pathway leading to β-thujaplicin biosynthesis in *Cupressus lusitanica* cell cultures. J Exp Bot 55:1003–1012

Zhao J, Davis LC, Verpoorte R (2005) Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol Adv 3:283–333

Zheng LP, Guo YT, Wang JW, Tan RX (2008) Nitric oxide potentiates oligosaccharide-induced artemisinin production in *Artemisia annua* hairy roots. J Integr Plant Biol 50:49–55

Zhou LG, Wu JY (2006) Development and application of medicinal plant tissue cultures for production of drugs and herbal medicinals in China. Nat Prod Rep 23:789–810

Zhou ML, Zhu XM, Shao JR, Wu YM, Tang YX (2010) Transcriptional response of the catharanthine biosynthesis pathway to methyl jasmonate/nitric oxide elicitation in *Catharanthus roseus* hairy root culture. Appl Biochem Biotechnol 88:737–750