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REVIEW

Biotic elicitors: a boon for the in-vitro production of plant secondary metabolites

Rakesh Bhaskar[1] · Louretta Spandhana Eruva Xavier[1] · Giriprasad Udayakumaran[1] · Dharani Shree Kumar[1] · Rashmitha Venkatesh[1] · Praveen Nagella[1]

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Abstract

Plant secondary metabolites are produced naturally in the plant system as a defense mechanism to combat environmental stress factors. These metabolites are extensively used in food, cosmetics, agrochemicals and pharmaceutical sectors. With the applications of plant tissue culture, any particular organ which is the major site for secondary metabolite production can be targeted and cultured. Recently, a new strategy to increase the metabolite production in plants has been employed with the use of elicitors. These elicitors are the chemical substances that trigger the biosynthetic pathways by activating certain transcriptional factors and upregulating the genes. Hence the secondary metabolite production increases in the plant system due to the stress developed by the introduction of the elicitors. Generally, elicitors may be abiotically derived from non-living sources or biotically derived from the living sources. In the present review, the mechanism of biotic elicitation and the applications of biotic elicitors like bacterial, fungal, algal elicitors and other polysaccharides extracted from them has been discussed extensively. It has been noted that the addition of bacterial elicitors like *Rhizobium rhizogenes* showed a 94% increase in genistein production while *Escherichia coli* showed a 9.1-fold increase in diosgenin production. Similarly, fungal elicitors like *Aspergillus niger* increased thiophene production by 85% and a 26-fold increase in sanguinarine production was seen when the cultures were treated with *Botrytis* sps. Algal extracts like *Haematococcus pluvialis* increased the betalain production by 2.28 folds while *Botryococcus braunii* elicited Vanillin, Vanillylamine and Capsaicin by 3-fold, 6-fold and 2.3-fold respectively.

Keywords Secondary metabolites · In-vitro production · Elicitation · Biotic elicitors

Introduction

From centuries plants are playing a major role in fulfilling the needs of all living organisms especially human beings for their food, shelter and medicine due to the presence of various compounds in plants which are referred as metabolites or phytochemicals. Metabolites like carbohydrates, amino acids and lipids are produced in high quantity and make up the ultrastructure of the plant hence referred to as primary metabolites. Apart from these, plants also synthesize a wide range of chemical compounds in trace amounts known as secondary metabolites to fulfill their physiological roles by facilitating the plants to withstand and interact with the environment. These secondary metabolites possess biological activity against pathogens and play a major role in the defense and signaling of the plant system (Bourgaud et al. 2001; Guerriero et al. 2018). The concept of secondary metabolites was first described by Albrecht Kossel who won the noble prize for physiology or medicine in 1910. The secondary metabolites are produced in plants as a defense response and the metabolites are often induced due to stress signals, such as pathogen invasion, environmental factors and nutrient deficits. Some of these metabolites are important as they are used as preventive or curative drugs for certain diseases (Ramachandra Rao and Ravishankar 2002).

The secondary metabolites of the plants are broadly classified based on their structure as phenolics, alkaloids, saponins and terpenes (Hussein and El-Anssary 2019). Some of the major pathways in biosynthesis of these metabolites

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✉ Praveen Nagella
praveen.n@christuniversity.in

[1] Department of Life Sciences, CHRIST (Deemed To Be University), Hosur Road, Bangalore 560 029, India

are shikimic acid pathway (tannins, phenols and aromatic alkaloids), malonic acid pathway (terpenes, alkaloids and steroids), acetate-malonate pathway (alkaloids, fatty acids and phenols) and pentose phosphate pathway (glycosides) (Kabera et al. 2014). General representation of the biosynthetic pathway of secondary metabolite production is illustrated in Fig. 1.

These wide ranges of secondary metabolites are used in biopesticides, agrochemicals, flavouring agents, essential oils, food additives and most importantly in pharmaceutical industries for medicine and cosmetics (Ramakrishna and Ravishankar 2011). In the global market, many biotic compounds like quinine, vincristine, papervine, ephedrine, caffeine etc. are in high demand and these compounds are often restricted to a particular genus or species of plant (Verpoorte et al. 2002). Naturally, plants produce less than 1% dry weight of secondary metabolites and the production depends on the developmental and physiological stage of the plant along with the environmental conditions (Dixon 2001; Oksman-Caldentey and Inzé 2004). The production of secondary metabolite by plants do not satisfy the need of mankind and the entire plant is utilized for the phytochemical extraction, therefore, the shortage and overuse of the plants are a major limitation. To meet the existing demand, the plants are grown in-vitro using biotechnological techniques as they eliminate the climatic, geographical and edaphic barriers and also promote the plant growth in all seasons and provide a favorable environment for the continuous supply of phytochemicals (Yukimune et al. 1996; Karuppusamy 2009). Depending on the metabolite and its source, different plant tissue culture techniques such as cell suspension, cellular extracts and their culture filtrates (Biswas et al. 2016, 2018), root and shoot multiplication, callus cultures (Awad et al. 2014), adventitious root, hairy root cultures, autoclaved cells etc. are developed for production of useful secondary metabolites (Buitelaar et al. 1992). Production of pure compounds like taxol, morphine, L-DOPA, capsaicin, vinblastine, vincristine and berberine by tissue culture showed only limited success (Vanisree et al. 2004; Vijaya Sree N et al. 2010). To overcome the limitation of the quantity of the bioactive compounds several biotechnological strategies like optimization of the medium, providing suitable culture environments, permeabilization, immobilization, elicitation, precursor feeding, metabolic engineering, biotransformation methods, use of bioreactors, and micropropagation are employed (Naik and Al-Khayri 2016). This review article presents most recent developments in the field of biotic elicitation with equal importance to all the types of biotic elicitors used till date. Apart from this, a simplified tabular column with the plant used, target metabolite, type of biotic elicitor used and results has been tabulated for a quick review for the readers.

Elicitors and their classification

Elicitation is one of the important strategies of biotechnology to enhance the production of secondary metabolites by the addition of certain substances called elicitors (Ramakrishna and Ravishankar 2011; Halder et al. 2019). Elicitor is defined as a substance that is applied in small quantity to a living system to trigger the biosynthesis of a specific compound which plays an important role in the adaptation of plant system and to overcome their stressful conditions (Thakur et al. 2019). Elicitors are considered as signaling molecules that activate transcriptional factors which regulate

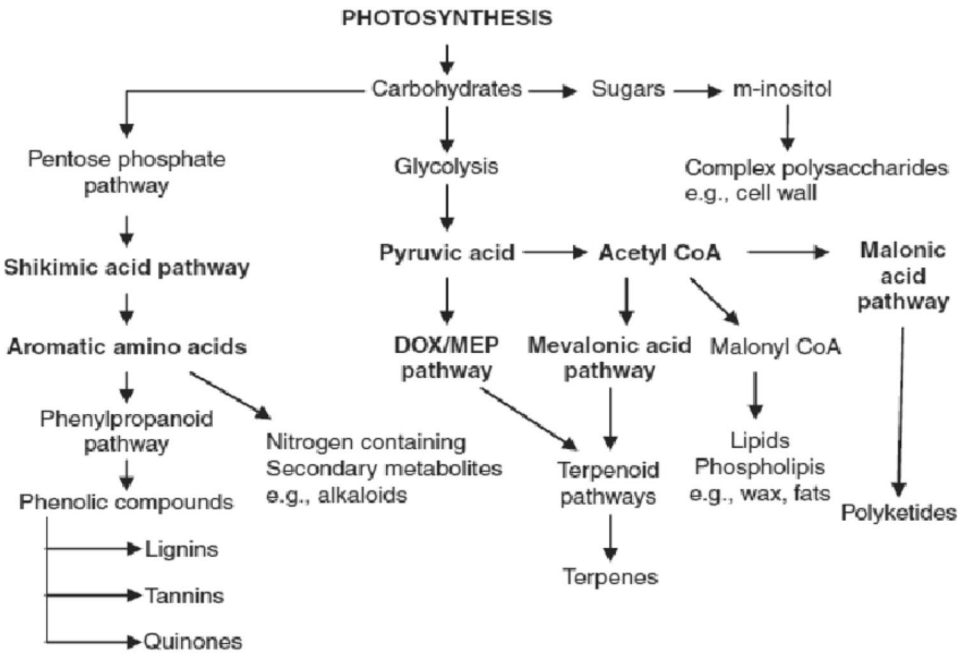

Fig. 1 Schematic representation showing various biosynthetic pathways for secondary metabolite production (Reproduced from Ghasemzadeh and Jaafar, 2011)

the expression of genes that are concerned with secondary metabolite production (Yamaner et al. 2013; Zhao 2015). For the enhanced activity of elicitors and maximum production of secondary metabolites in-vitro, elicitor specificity, their concentration, the time course of elicitation with host growth stage and nutrient stage of culture should be optimized (Patel and Krishnamurthy 2013). Elicitors are classified based on their nature as abiotic (non-biological) and biotic (biological) (Radman et al. 2003). Abiotic elicitors are further grouped under three categories such as physical, chemical and hormonal (Naik and Al-Khayri 2016). Biotic elicitors are substances derived from the biological origin and they are further divided into polysaccharides and microorganism based. Polysaccharide elicitors are extracted, isolated and purified from the cell wall of biological organisms like plant-derived or animal-derived which are chitin, lignin, pectin and cellulose. Microorganism-based elicitor includes cell extracts of yeast, bacteria and fungi (Namdeo A. G. 2007).

Mechanism of biotic elicitors

The mechanism is based on elicitor-receptor interaction which leads to a cascade of biochemical events. Though all elicitors start with receptor interaction on the plasma membrane, inside the cell, elicitors trigger various pathways altering biochemical and physiological processes and this leads to the production of different secondary metabolites or defense responsive compounds (Ferrari 2010; Shasmita et al. 2018). The mechanism starts with the binding of the elicitor to the receptor present on the plasma membrane. Elicitor-receptor interaction leads to alteration of ions present across the cell membrane such as the influx of calcium ions (Ca^{2+}) and efflux of the cation (K^{+}) and anions (Cl^{-}) (Jabs et al. 1997; Shabala and Pottosin 2014). In plants, as a response to the environmental variations and pathogen signals, calcium acts as a secondary messenger and it is found that calcium channels are activated within few minutes of elicitor addition into the system. This ion flux leads to cytoplasmic acidification and increases extracellular pH leading to depolarization of plasma membrane (Mathieu et al. 1996; Sakano 2001; Zhao et al. 2005). Few elicitors induce alkalization of apoplast which leads to an influx of protons and some elicitors induce acidification of apoplast which leads to efflux of vacuolar protons (Bolwell et al. 2002; Angelova et al. 2006). As a result of elicitor interaction, there is increased activity of the plant phospholipases and protein phosphorylation (Zhao 2015). This leads to the synthesis of secondary messengers such as D-myo-inositol 1,4,5-triphosphate ($InsP_3$) Diacylglycerol (DAG) and activation of ca^{2+} mediated secondary messengers (Gillaspy 2011; Aldon et al. 2018). This is followed by activation of the mitogenic protein kinase (MAPK) pathway which leads to a series of events like phosphorylation of MAP kinase etc. These molecules are transported to the nucleus through a nuclear pore complex leading to phosphorylation of specific transcriptional factors (Pitzschke et al. 2009; Sinha et al. 2011; Colcombet et al. 2016). In some plants, as a response to elicitor and G -protein-coupled receptor (GPCR) interaction, NADPH oxidase is activated which is responsible for the generation of Active Oxygen Species (AOS) (Mishra et al. 2012), which leads to acidification of cytoplasm and activation of proteins like chitinases, glucanases, hydroxy proline-rich glycoproteins and protease inhibitors and this leads to activation of transcriptional factors leading to the expression of corresponding defense genes like jasmonates, ethylene, salicylates and later as a defense response plant produces phytoalexins which are secondary metabolites (Zhao et al. 2005; Angelova et al. 2006). The overall mechanism of biotic eliciatation is illustrated in Fig. 2.

Bacterial elicitation

Bacteria are single-celled microorganisms found in the environment (Dzhavakhiya and Shcherbakova 2007). The use of bacterial cells, bacterial cellular components and bacterial cellular extracts to elicit a response in plants is called bacterial elicitation. They are also used to induce secondary metabolite production from plants in the in-vitro conditions. Each bacterial species can elicit different secondary metabolites in different quantities. The difference is because of the elicitor-membrane receptor binding, G protein activation, cytoplasmic acidification and reactive oxygen species generation (Zhao et al. 2005; Biswas et al. 2016). Different types of bacterial components such as live cells (Park et al. 2006; Awad et al. 2014), cell homogenate (Buitelaar et al. 1992; Jung et al. 2003), cellular extracts (Gandi et al. 2012; Chodisetti et al. 2013) and culture filtrates (Biswas et al. 2016, 2018) are used to elicit secondary metabolite production. In the environment, plants face huge challenges due to the presence of bacteria in the soil, this stimulates the production of secondary metabolites as a defense mechanism in plants. Similarly, live bacterial cultures can also induce secondary metabolite production in the in-vitro conditions, but very few studies have reported using live bacteria as elicitors (Mañero et al. 2012).

The bacterial cultures of *Bacillus aminovorans, Bacillus cereus, Agrobacterium rhizogenes, Agrobacterium tumefaciens and Rhizobium leguminosarum* (0.5 ml of culture) were used to elicit glycyrrhizic acid in the root cultures of *Taverniera cuneifolia* (Roth) Arn. The bacterium *R. leguminosarum* has shown the highest elicitation (6.37 mg/g) when compared to untreated root cultures (1.48 mg/g). Other bacteria have shown a significant increase in the production whereas *A. tumefaciens* has shown no significant increase in

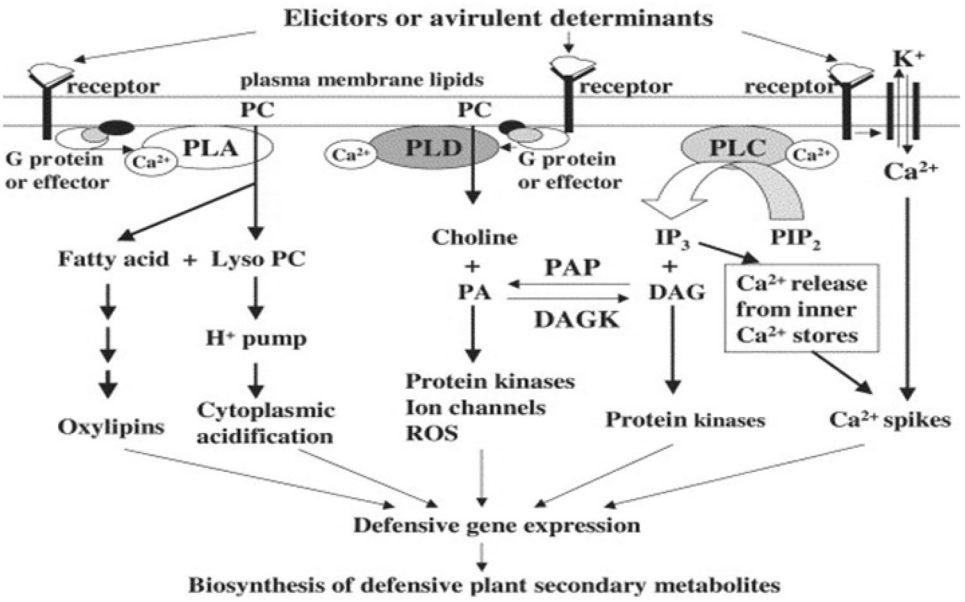

Fig. 2 Schematic representation of the general mechanism of biotic elicitors (Reproduced from Zhao et al. 2005)

the production (1.46 mg/g) (Awad et al. 2014). The bacterial cultures of *Rhizobium radiobacter* and *Rhizobium rhizogenes* are co-cultured with adventitious root culture of *Albizzia kalkora* (Roxb.) to elicit the production of isoflavone (daidzein and genistein) in them. Addition of *R. rhizogenes* showed a 35% increase in daidzein and a 94% increase in genistein production while *R. radiobacter* showed a 12% increase in daidzein and 89% increase in genistein production on the 6th day of treatment (Park et al. 2006). Atropine production has been significantly reduced to 0.017% from 0.116% (control) and 0.095% from 0.116% when *B. cereus* and *Staphylococcus aureus* cultures were used respectively in the hairy root culture of *Datura metel* L. (Shakeran et al. 2015). Co-culturing of *B. cereus* with hairy root culture of *D. metel L.* showed a 13.5-fold increase, the addition of cellular extract showed a 2-fold increase and addition of culture supernatant showed a 4-fold increase of tanshinone production respectively (Wu et al. 2007). *Pseudomonas sp.* has shown to enhance the production of rosmarinic acid in the shoot-culture of *Rosmarinus officinalis* L. (Yang et al. 1997). Cell homogenization is a process by which the cell membranes are ruptured and the cellular components are released into the solution which is referred to as cell homogenate. This cell homogenate contains the pathogenic metabolite which triggers the plant defense responses and as a result, the secondary metabolites are produced in the plants. The cell homogenates of *Bacillus subtilis, E. coli, Pseudomonas aeruginosa* and *S. aureus* showed a 50, 35, 35 and 20% increase in thiophene production in the hairy root cultures of *Tagetes patula* L. (Buitelaar et al. 1992). Raw and autoclaved homogenates of *P. aeruginosa, B. cereus* and *S. aureus* were used to elicit scopolamine production in the hairy root cultures of *Scopolia parviflora* (Dunn) Nakai. Raw homogenates enhanced scopolamine production though no significant difference was seen between each of the elicitor used. However, browning of roots was observed after 24 hour of incubation while autoclaved homogenate showed similar scopolamine production as the control (Jung et al. 2003).

In the bacterial cultures, bacteria produce various compounds which are released to the culture media and they are also capable of eliciting defense responses in plants. So, the bacterial cultures are centrifuged and the cells are pelleted, the supernatant is filter sterilized and it is considered as the culture filtrates. The culture filtrates of *Pseudomonas monteilii* and *Bacillus circulans* were used to elicit ginsenoside production in cell suspension culture of *Panax quinquefolius* L., *P. monteilii* showed a 2.5-fold increase while *B. circulans* showed a slight decline in total ginsenoside production after five days of treatment. Prolonged exposure of elicitors has shown a 20–30% decline in total ginsenoside production (Biswas et al. 2016). Similarly, *Serratia marcescens* at 1.25 and 2.5% concentration showed a 1.6 and 2.5-fold increase respectively while *B. subtilis* at 2.5% concentration showed a 1.8-fold increase in ginsenoside production in cell suspension cultures of *Panax sikkimensis* R. N. Banerjee., (Biswas et al. 2018). The culture filtrates of gram-negative bacteria like *Mesorhizobium huakuii, Mesorhizobium amorphae, Bradyrhizobium ganzhouense, Azotobacter beijerinckii* and gram-positive bacteria like *Lactobacillus plantarum, Leuconostoc sp.,* and *Bacillus sp.* have been used for ginsenoside production in root cultures of *Panax ginseng* C.A. Meyer. All gram-positive and gram-negative bacteria showed an increase in ginsenoside production but the highest production was seen in gram-negative bacteria (Le et al. 2018). *B. subtilis* and *E. coli* culture filtrates were used to elicit

diosgenin production in the cell suspension culture of the *Helicteres isora* L., *E. coli* (1.5%) showed a 9.1-fold increase while *B. subtilis* (2%) showed a 6.1-fold increase in diosgenin production (Shaikh et al. 2020). The culture filtrate of *E. coli, S. aureus, B. cereus* and *P. aeruginosa* enhanced anthocyanin by 22% in the callus culture of *Daucus carota* L. (Suvarnalatha et al. 1994). The culture filtrate of *Stenotrophomonas maltophilia* has shown a 3-fold increase in hypericin production in the shoot culture of *Hypericum perforatum* L. (Mañero et al. 2012). The cellular extracts of *A. rhizogenes* resulted in high accumulation (66.12 mg/g) of gymnemic acid and the cellular extracts of non-pathogenic bacteria *B. subtilis* and *E. coli* resulted in low accumulation (47.97 mg/g and 33.25 mg/g respectively) of gymnemic acid in the cell suspension cultures of *Gymnema sylvestre* (Retz.) schult. (Chodisetti et al. 2013). The cell-free cellular extracts of *E. coli, B. subtilis, A. rhizogenes* and *A. tumefaciens* have been used to elicit andrographolide production in cell suspension cultures of *Andrographis paniculata* (Burm.f.) Nees. Out of which *E. coli* elicited highest (8.3 mg/g) andrographolide production whereas *A. tumefaciens* showed no significant increase in the production (Gandi et al. 2012). The cellular extracts of *Pseudomonas sp.* and *Enterobacter sp.* are used to elicit the alkaloids production in the protocorm-like body cultures of *Pinellia ternate* (Thumb) breit., *Pseudomonas* sp. showed 69–166% increase in guanosine, 45–1143% increase in trigonelline and 26% increase in inosine production. While *Enterobacter* sp. showed 371–1143% increase in guanosine, 114–500% increase in trigonelline and 5–17% increase in inosine production when compared to control (Liu et al. 2010). The cellular extract of *Cronobacter sakazakii* were used to elicit antioxidants and bactericidal phenolic compounds in the plant tissues of *Dionaea muscipula* (Makowski et al. 2020). The cellular extract of *P. aeruginosa* was studied as the elicitor to produce rosmarinic acid, caffeic acid, carnosic acid, carnosol, and rosmanol in the callus culture of *R. officinalis.* Rosmarinic acid accumulation increased from 3.7 to 3.9 µg/mL, caffeic acid from 0.5 to 2.3 µg/mL, carnosic acid from 2.6 to 3.1 µg/mL and carnosol from 1.7 to 2.4 µg/mL (Rashid et al. 2011). In the callus culture of *Ammi majus*, *Enterobacter sakazakii* cellular extract showed a 12% increase in scopoletin, in cell suspension culture, a 9.6% increase in umbelliferone production and in the hairy root culture 2.3 and 0.3% increase in umbelliferone and bergapten production respectively (Staniszewska et al. 2003). The *A. tumefaciens* and *A. rhizogenes* cellular extracts significantly increased the production of xanthone to 6.95 and 5.04 mg/g respectively from 3.7 mg/g (untreated) in the cell suspension cultures of *H. perforatum* L. (Tusevski et al. 2015). Celluar extracts of *A. rhizogenes, Pectobacterium carotovorum* and *E. sakazakii* has enhanced the production of acteoside, baicalin, wogonin, scutellarin, and wogonoside while *C. sakazakii* and *Klebsiella pneumonia* enhanced wogonin and *K. pneumoniae* alone enhanced chrysin production in the hairy root cultures of *Scutellaria lateriflora* L. (Wilczańska-Barska et al. 2012). Common bacterial elicitors used for the production of secondary metabolites have been listed in Table1.

Fungal elicitation

Among the biotic elicitors, fungal elicitors (both free-living and endophytic) are the most important and widely used for the synthesis of commercial compounds. The interaction between the fungi and the plant results in the induction of hypersensitive responses which activates defence pathways in the plant thereby increasing the phytoalexins (Baldi et al. 2009) and inducing secondary metabolite production more effectively (Zhai et al. 2017). The pure fungal cultures are usually obtained from the hyphal tip culture (Salehi et al. 2019).

The aqueous extract of *Aspergillus niger, A. flavus, Penicillium notatum* and *Fusarium oxysporum* were used to elicit anthocyanin production in *D. carota* L. mycelial extracts of *A. flavus* gave maximum elicitation with a two-fold increase in the anthocyanin production whereas *P. notatum* and *F. oxysporum* treatments were not much effective (Rajendran et al. 1994). Similarly, in another study *A. niger* increased thiophene production by 85% when compared to control in *T. patula* L. (Buitelaar et al. 1992). *A. niger*, when used as an elicitor in *Mentha piperita* L. cell cultures showed enhanced production of menthol (140.8 mg/L) which was higher when compared to other non-biological elicitation (Chakraborty and Chattopadhyay 2008). *A. niger* showed a 9-fold increase in gymnemic acid production in the cultures of *G. sylvestre* (Retz.) schult. (Devi and Srinivasan 2011). *A. niger and Rhizopus stolonifera* showed a 4.9 and 3.8-fold increase respectively in glycyrrhizin production in the cultures of *Abrus precatorius* L. (Karwasara et al. 2011). *Oldenlandia umbellate* L. is a commercially important dye yielding plant. Elicitors like *A. niger*, *Mucor prayagensis* and *Trichoderma viride* were used to enhance its growth. Maximum response were observed in *A. niger* treated cultures which showed 79 shoots and 47 roots (Saranya and Velayutham 2019). The culture filtrates of *Trichoderma atroviride* and *T. harzianum* were used to elicit ginsenoside from *P. quinquefolius* L. cell suspension cultures. *T. atroviride* produced the highest ginsenoside (3.2 times higher than control) after 5-day treatment (Biswas et al. 2016). They were also used to elicit dual metabolite ginsenoside and anthocyanin in cell suspension cultures of *P. sikkimensis* R. N. Banerjee (Biswas et al. 2018). The effect of three fungal elicitors was tested on *Centella asiatica* L. cultures, the addition of *T. harzianum* filtrate showed a 2.53-fold increase in asiaticoside production while *Colletotrichum*

Table 1 Bacterial elicitors used in the in-vitro secondary metabolite production

Sl. no	Bacterial Elicitor used	Target plant	Target Metabolite	Type of culture	References
01	*Agrobacterium rhizogenes*	*Gymnema sylvestre* (Retz.) schult	Gymnemic acid	Cell suspension culture	Chodisetti et al. (2013)
02		*Andrographis paniculata* (Burm.f.) Nees	Andrographolide		Gandi et al. (2012)
03		*Hypericum perforatum* L	Xanthone		Tusevski et al. (2015)
04		*Scutellaria lateriflora* L	Acteoside, Baicalin, Wogonin, Scutellarin, & Wogonoside	Hairy Root Culture	Wilczańska-Barska et al. (2012)
05		*Taverniera cuneifolia* (Roth) Arn	Glycyrrhizic acid	Root Culture	Awad et al. (2015)
06	*Agrobacterium tumefaciens*	*Andrographis paniculata* (Burm.f.) Nees	Andrographolide	Cell suspension culture	Gandi et al. (2012)
07		*Hypericum perforatum* L	Xanthone		Tusevski et al. (2015)
08		*Taverniera cuneifolia* (Roth) Arn	Glycyrrhizic acid	Root Culture	Awad et al. (2016)
09	*Bacillus aminovorans*	*Taverniera cuneifolia* (Roth) Arn	Glycyrrhizic acid	Root Culture	Awad et al. (2014)
10	*Bacillus cereus*	*Scopolia parviflora* (Dunn) Nakai	Scopolamine	Hairy Root Culture	Jung et al. (2003)
11		*Salvia miltiorrhiza* Bunge	Tanshinone	Hairy Root Culture	Wu et al. (2007)
12		*Salvia miltiorrhiza* Bunge	Tanshinone	Hairy Root Culture	Wu et al. (2007)
13		*Daucus carota* L	Anthocyanin	Callus Culture	Suvarnalatha et al. (1994)
14		*Taverniera cuneifolia* (Roth) Arn	Glycyrrhizic acid	Root Culture	Awad et al. (2016)
15		*Datura metel* L	Atropine	Hairy Root Culture	Shakeran et al. (2015)
16		*Salvia miltiorrhiza* Bunge	Tanshinone		Wu et al. (2007)
17	*Bacillus circulans*	*Panax quinquefolius L*	Ginsenoside	Cell suspension culture	Biswas et al. (2016)
18	*Bacillus sp.* CWJ-1	*Panax ginseng* C.A. Meyer	Ginsenoside	Adventious Root Culture	Le et al. (2018)
19	*Bacillus sp.* LHW-1				
20	*Bacillus subtilis*	*Tagetes patula* L	Thiophene	Hairy Root Culture	Buitelaar et al. (1992)
21		*Gymnema sylvestre* (Retz.) schult	Gymnemic acid	Cell suspension culture	Chodisetti et al. (2013)
22		*Andrographis paniculata* (Burm.f.) Nees	Andrographolide		Gandi et al. (2012)
23		*Panax quinquefolius* L	Ginsenoside		Biswas et al. (2018)
24		*Helicteres isora* L	Diosgenin		Shaikh et al. (2020)
25	*Bradyrhizobium ganzhouense*	*Panax ginseng* C.A. Meyer	Ginsenoside	Adventious Root Culture	Le et al. (2018)
26	*Cronobacter sakazakii*	*Dionaea muscipula* J. Ellis	Phenolic Compounds	Shoot culture	Makowski et al. (2020)
27		*Scutellaria lateriflora* L	Wogonin	Hairy Root Culture	Wilczańska-Barska et al. (2012)
28	*Enterobacter sakazakii*	*Ammi majus* L	Umbelliferone, Scopoletin, Bergapten, and Dehydrogeijerin	Callus, Cell suspension culture and Hairy Root Culture	Staniszewska et al. (2003)
29		*Scutellaria lateriflora* L	Acteoside, Baicalin, Wogonin, Scutellarin, & Wogonoside	Hairy Root Culture	Wilczańska-Barska et al. (2012)
30	*Enterobacter sp.*	*Pinellia ternate* (Thunb.) Makino	Alkaloids (Guanosine, Inosine &Trigoneline)	Protocorm-like Body culture	Liu et al. (2010)

Table 1 (continued)

Sl. no	Bacterial Elicitor used	Target plant	Target Metabolite	Type of culture	References
31	*Escherichia coli*	*Tagetes patula* L	Thiophene	Hairy Root Culture	Buitelaar et al. (1992)
32		*Gymnema sylvestre* (Retz.) schult	Gymnemic acid	Cell suspension culture	Chodisetti et al. (2013)
33		*Andrographis paniculata* (Burm.f.) Nees	Andrographolide		Gandi et al. (2012)
34		*Helicteres isora* L	Diosgenin		Shaikh et al. (2020)
35		*Daucus carota L*	Anthocyanin	Callus Culture	Suvarnalatha et al. (1994)
36	*Klebsiella pneumoniae*	*Scutellaria lateriflora* (Dunn) Nakai	Wogonin & Chrysin	Hairy Root Culture	Wilczańska-Barska et al. (2012)
37	*Lactobacillus plantarum*	*Panax ginseng* C.A. Meyer	Ginsenoside	Adventious Root Culture	Le et al. (2018)
38	*Leuconostoc sp.*				
39	*Mesorhizobium amorphae* GS3037				
40	*Mesorhizobium amorphae* GS336				
41	*Mesorhizobium huakuii*				
42	*Pectobacterium carotovorum*	*Scutellaria lateriflora* L	Acteoside, Baicalin, Wogonin, Scutellarin, & Wogonoside	Hairy Root Culture	Wilczańska-Barska et al. (2012)
43	*Pseudomonas aeruginosa*	*Tagetes patula* L	Thiophene	Hairy Root Culture	Buitelaar et al. (1992)
44		*Scopolia parviflora* (Dunn) Nakai	Scopolamine		Jung et al. (2003)
45		*Rosmarinus officinalis* L	Rosmarinic acid, Caffeic acid, Carnosic acid, Carnosol, and Rosmanol	Callus Culture	Rashid et al. (2011)
46		*Daucus carota* L	Anthocyanin		Suvarnalatha et al. (1994)
47	*Pseudomonas fluorescens*	*Hypericum perforatum* L	Hypericin and Pseudohypericin	Shoot culture	Mañero et al. (2012)
48	*Pseudomonas monteilii*	*Panax quinquefolius* L	Ginsenoside	Cell suspension culture	Biswas et al. (2016)
49	*Pseudomonas sp.*	*Pinellia ternate* (thunb.) Makino	Alkaloids (Guanosine, Inosine &Trigoneline)	Protocorm-like Body culture	Liu et al. (2010)
50		*Rosmarinus officinalis L*	Rosmarinic acid	Shoot culture	Yang et al. (1997)
51	*Rhizobium leguminosarum*	*Taverniera cuneifolia* (Roth) Arn	Glycyrrhizic acid	Root Culture	Awad et al. (2017)
52	*Rhizobium radiobacter*	*Albizia kalkora* (Roxb.) Prain	Isoflavone	Adventious Root Culture	Park et al. (2006)
53	*Rhizobium rhizogenes*				
54	*Serratia marcescens*	*Panax quinquefolius* L	Ginsenoside	Cell suspension culture	Biswas et al. (2018)
55	*Staphylococcus aureus*	*Tagetes patula* L	Thiophene	Hairy Root Culture	Buitelaar et al. (1992)
56		*Scopolia parviflora* (Dunn) Nakai	Scopolamine		Jung et al. (2003)
57		*Daucus carota L*	Anthocyanin	Callus Culture	Suvarnalatha et al. (1994)
58		*Datura metel* L	Atropine	Hairy Root Culture	Shakeran et al. (2015)
59	*Stenotrophomonas maltophilia*	*Hypericum perforatum* L	Hypericin and Pseudohypericin	Shoot culture	Mañero et al. (2012)

lindemuthianum decreased asiaticoside production but showed higher biomass and *F. oxysporum* had an inhibitory effect on shoot growth and poor yield of asiaticosides (Prasad et al. 2013). The *H. perforatum* L. cultures were treated with the extracts of *F. oxysporum, Phomaexigua* and *Botrytis cinerea* resulting in reduced biomass and rapid increase of phenylpropanoid and naptho-dianthrones. Hypericin and pseudohypericin were significantly increased in the early growth phase and later gradually declined (Gadzovska et al. 2015). The culture filtrates of *Micromucoris abellina* in *Catharanthus roseus* (L.) G. Don caused a drastic and rapid increase of indole alkaloid biosynthesis leading

to the production of 400 µg/L ajmalicine and 600 µg/L catharanthine while only trace amounts of ajmalicine and 5.8 µg/L catharanthine were detected in control (Dicosmo et al. 1987). The cultures of *Papaver somniferum* L. were incubated with 1 ml of *Botrytis* sps. which showed a 26-fold increase in sanguinarine production when compared to control while 5 ml of *Colletotrichum gloeosporoides* elicited 43% of total sanguinarine produced. Other elicitors like *Rhodotorula rubra, Helminthosporium gramineum* and *Sclerotinia sclerotiorum* showed a relatively weaker response in sanguinarine production (Eilert et al. 1985). The cell suspension cultures of *Corylus avellane* L. were treated with endophytic fungi like *Chaetomium globosum* and *Paraconiothyrium brasiliense* isolated from *Taxus baccata* L. and *C. avellane* L. *respectively*. Maximum elicitation was seen when 10% (v/v) *C. globosum* was added which showed 4.1 times higher production of paclitaxel on 17th day (Salehi et al. 2019). Common fungal elicitors used to trigger the metabolite production is shown in Table 2.

Algal Elicitation

Algae and cyanobacteria constitute the major phytoplankton over the earth. The entire plant body or its components can be utilized in various fields of research. One of their most promising applications is in the elicitation studies. Marine algae is an under-utilized bioresource, out of which

Table 2 Fungal elicitors used in the in-vitro secondary metabolite production

SI. No	Fungal elicitor used	Target plant	Target secondary metabolite/pigment	Type of culture	References
1	*Aspergillus niger* and *Rhizopus stolonifer*	*Abrus precatorius* L	Glycyrrhizin	Cell Suspension Culture	Karwasara et al. (2011)
2	*Penicillium oxalicum*	*Artemisia annua* L	Artemisinin	Callus Culture	Zheng et al. (2016)
3	*Chrysosporium palmorum, Eurotium rubrum, Micromucorisabellina*	*Catharanthus roseus* (L.) G. Don	Ajmalicine and Catharanthine	Cell Suspension Culture	Dicosmo et al. (1987)
4	*Colletotrichum lindemuthianum*	*Centella asiatica* (L.) Urb	Asiaticoside	Axillary Shoot Culture	Prasad et al. (2013)
5	*Chaetomium globosum* and *Paraconiothyrium brasiliense*	*Corylus avellana* L	Paclitaxel	Cell Suspension Culture	Salehi et al. (2019)
6	*Aspergillus niger, A. flavus, Penicillium notatum, Fusarium oxysporum*	*Daucus carota* L	Anthocyanin	Callus Culture	Rajendran et al. (1994)
7	*Aspergillus niger*	*Gymnema sylvestre* (Retz.) schult	Gymnemic acids	Cell Suspension Culture	Devi et al. (2011)
8	*Fusarium oxysporum, Phoma exigua* and *Botrytis cinerea*	*Hypericum perforatum* L	Phenylpropanoid and Naphtodianthrone	Cell Suspension Culture	Gadzovska et al. (2015)
9	*Aspergillus niger*	*Mentha piperita* L	Methanol	Cell Suspension Culture	Chakraborty et al. (2008)
10	*Rhizophagus irregularis*	*Ocimum basilicum* L	Rosmarinic acid	Hairy Root Culture	Srivastava et al. (2016)
111	*Alternaria panax*	*Panax ginseng* C.A. Meyer	Ginsenoside	Adventitious Root Culture	Hao et al. (2020)
112	*Trichoderma atroviride* and	*Panax quinquefolius* L	Ginsenoside	Cell Suspension Culture	Biswas et al. (2016)
113	*Trichoderma harzianum*	*Panax sikkimensis* R. N. Banerjee	Ginsenoside and Anthocyanin	Cell Suspension Culture	Biswas et al. (2018)
114	*Rhodotorula rubra, Alternaria zinnae, Helminthosporium gramineum, Sclerotinia sclerotiorum, Verticillium dahliae*	*Papaver somniferum* L	Sanguinarine	Cell Suspension Culture	Eilert et al. (1985)
115	*Phytophthora megasperma*	Soybeans	Glyceollin	Cell Suspension Culture	Ebel et al. (1976)
116	*Fusarium conglutinans,*	*Tagetes patula* L	Thiophene	Hairy-Root Culture	Mukundan and Hjortso (1990)
17	*Aspergillus niger, Fusarium oxysporum, Penicillium expansum*	*Tagetes patula* L	Thiophene	Hairy Root culture	Buitelaar et al. (1992)

only a few are used as a source of food, for therapeutic and other biological applications (Vinoth et al. 2012). Seaweed extracts and their cell wall components can also act as a prominent elicitor providing enormous beneficial aspects such as plant protectants, improved yield and increased tolerance to various stresses (Sbaihat et al. 2015). Seaweeds majorly comprises of carbohydrates made up of oligosaccharides and polysaccharides (Arman and Qader 2012). Introduction of these algal polysaccharides such as carrageenans, fucans, laminarans and ulvans into the plant system activates a series of defense cascade leading to the resistance against pathogens and triggers the secondary metabolite production (Stadnik and de Freitas 2014). Chemical analysis of algal extracts reveals the presence of various active compounds, macro and micronutrients, growth regulators which also support the in-vitro growth of the plants apart from acting as an elicitor (Satish et al. 2015). Hence the entire algal mass or their individual components help in in-vitro growth, mass propagation, callus culture etc.

Hot water extracts of 25 strains of cyanobacterium members like *Nostoc sp*., *Anabaena sp*., *Synechococcus sp*. and *Xenococcus sp*. along with their dialysates and non-dialysates were added to the cell suspension cultures of *D. carota* L. to study its effect on the embryogenesis. It was observed that the plantlet formation increased on an average by 3.7-fold when these marine cyanobacterial extracts were added. A 4.2 and 3.0-fold increase was seen when dialysates and non-dialysates of the filamentous form were added respectively whereas a 3.2 and 5.2-fold increase was seen when dialysates and non-dialysates of unicellular strains were added respectively. Overall, the maximum number of plantlets was seen when non-dialysate of *Synechococcus sp.* (240) and *Anabaena sp*. (211) were added while minimum (32) was seen in control (Wake et al. 1991). Torpedo shaped somatic embryos obtained from the cell suspension cultures of *D. carota* L. was encapsulated to form artificial seeds with the addition of hot water extract of *Synechococcus sp.* (50, 200, 400 and 800 mg/L) and their non-dialysates (10, 50, 100, 200 mg/L). High frequency of germination (94%) was seen each in 100 and 200 mg/L of non-dialysate when added followed by 91, 90 and 81% in 400, 800 and 200 mg/L of extract respectively. While 50 and 10 mg/L of non-dialysate showed 77 and 58% respectively. The least of 57 and 35% seed germination were observed in 50 mg/L of extract and control respectively (Wake et al. 1992). Pure cultures of *Anabaena sps*. and *Nostoc carneum* belonging to Nostocaceae members cultured in Bold's Basal medium was used as an elicitor treatment for the neem cell suspension cultures. Two concentrations of *Anabaena sps.* were taken (265 cells/mL and 530 cells/mL), of which higher concentration of algal cells resulted in a 5-fold increase in biomass in callus line-1 and decreased the biomass in callus line-2 and vice versa was seen in lower concentration. Similarly, addition of *N. carneum* (1670 cells/mL) initially increased the biomass by 2-fold later its efficiency decreased. *N. carneum* and *Anabaena sp*. increased the protein content by a twofold and threefold respectively. Addition of *Anabaena sp.* (265 cells/mL) in the cell suspension triggered the synthesis of a triterpenoid, azadirachtin (0.32 g/µL) detected through HPLC. Other concentrations of elicitors failed to induce the production of azadirachtin (Poornasri. et al. 2008). Various microalgae were used to elicit the red pigment formation in the cultures of *Carthamus tinctorius* L. out of which *Nostoc linckia*, *Anabaena cylindrica* and *A. variabilis* showed 8-fold increase in the red pigment formation (5.21 mg/L) while the least was seen in *Chlorococcum sp.*, *Chlorella sp.* and *Scenedesmus sp*. (0.61 mg/L) (Hanagata et al. 1994). Phycocyanin, a biliprotein present in *Spirulina platensis*, a blue-green alga was extracted and introduced into *Capsicum frutescens* L. and *D. carota* L. cell cultures in five different concentrations (1.5, 3.0, 6.0, 12 and 24 µg/ml). The addition of phycocyanin in *C. frutescens* L. cultures showed no significant growth but capsaicin production increased in all the concentrations of phycocyanin, but the highest production was seen in 3.0 – 6.0 µg/ml (190 µg capsaicin/g of fresh weight). There was a 2-fold increase in capsaicin on 3rd day later it reduced to 1.5-fold on the 6th day when compared to control. While the application of phycocyanin in *D. carota* L. in lower concentrations (3.0 and 6.0 µg/ml) slightly increased the callus growth but at higher concentrations callus growth decreased. Initially, all concentrations of phycocyanin elicited anthocyanin production and the highest was seen in 3.0 µg/ml (24.8 mg anthocyanin /0.1 g of fresh weight) on 3rd day but later on 12th day they declined. Thus, phycocyanin shows early elicitation in both cultures (Ramachandra Rao et al. 1996). Similarly, three-week-old callus of *C. frutescens* L. developed from leaf and hypocotyl were transferred to the media containing the filter-sterilized acetone extracts of *Botryococcus braunii*, a colonial Chlorophyceae microalga was taken in four different concentrations (1, 2, 4 and 8 mg/L). 8 mg/L showed a threefold increase in total chlorophyll content, a 2-fold increase in the seed germination rate, a 1.5-fold increase in shoot and leaf length and a one-fold increase in root length. After 15 days maximum carotenoid content (0.18 mg/g) was seen in 8 mg/L followed by 4 mg/L (0.156 mg/g) and 2 mg/L (0.134 mg/g). Also, 8 mg/L showed a 3-fold, 6-fold and 2.3-fold increase while 4 mg/L showed 2-fold, 3-fold and a fold increase in vanillin, vanillylamine and capsaicin respectively (Sharma et al. 2010). Aqueous extracts of *Haematococcus pluvialis* and *Spirulina platensis* were used to elicit food color, betalain production from *Beta vulgaris* L. and an insecticide, thiophene from *T. patula* L. Addition of *H. pluvialis* extract to the hairy root cultures of *B. vulgaris* L. increased the biomass by 1.25-fold and the betalain production by 2.28-fold initially and later its content decreased after 15th day while

S. platensis extract showed a 1.13-fold increase in biomass and 1.16-fold increase in betalain synthesis. When the hairy root cultures of *T. patula* L. were treated with *H. pluvialis* extract it showed a 1.40-fold increase in the biomass and 1.2 times increase in thiophenes while extract of *S. platensis* neither showed any significant effect on biomass nor thiophene accumulation (Ramachandra Rao et al. 2001). Aqueous extracts of a red seaweed *Kappaphycus alvarezii* were added to the cultures of *Picrorhiza kurroa* Royle ex Benth, an endangered medicinal plant in four different concentrations (0.1, 1.0, 2.0 and 3.0 g/L) to elicit the picroside-I production. Out of which 2.0 g/L was found to be best for the *in-vitro* growth as there were 3.23, 1.55, 2.41 and 2.42-fold increase in biomass, plant length, number of roots and shoots respectively on 30th day. Hence this concentration remains best for shoot multiplication and root induction. Also, it was noted that with the addition of seaweed extracts picroside-I content increased 3–4 folds at 25 °C and 2–3 folds at 15 °C respectively (Sharma et al. 2015). The various algal elicitors used for the biotic elicitation of secondary metabolites is summarized in Table 3.

Elicitation using polysaccharides

Polysaccharides are biotic elicitors as they are found in all living organisms like plants, animals and microbes. Polysaccharides are biopolymers formed by the bonding of monosaccharides (Fukui et al. 1990). The physical and chemical properties of polysaccharides are characterized by their structural orientation (Nartop 2018). Polysaccharides according to their biological functions in living organisms are categorized into storage polysaccharides (reservoirs of energy like glycogen and starch) and structural polysaccharides (providing structure and support) (Fukui et al. 1990). Polysaccharides play an important role in the cellular communication, shielding the plants from stress conditions etc. Polysaccharide elicitors are categorized into two groups: endogenous (cellulose, pectin etc.) and exogenous (chitin, chitosan etc.) (Nartop 2018). Polysaccharides can be extracted, isolated and purified from living organisms or they can be chemically synthesized. Polysaccharides are very commonly used to elicit antimicrobial metabolites (Paulert et al. 2009; Lu et al. 2019). Yeast extract is one of the elicitors majorly used for the secondary metabolite synthesis as well as to study the plant defense responses (Putalun et al. 2007). Yeast extract stimulated synthesis of several important metabolites in various plants (Funk et al. 1987; Jeong et al. 2005).

Out of all the biotic and abiotic elicitors used in the hairy root cultures of *Pueraria candollei*, wall.ex Benth., yeast extract (0.5 mg/ml) efficiently induced isoflavonoid 4.5-fold higher than the control (60 mg/g) (Udomsuk et al. 2011). Similar results were also observed in *Salvia miltiorrhiza* Bunge cell cultures where yeast extract showed 10-fold higher tanshinone production (2.3 mg/g) which was more efficient compared to other biotic and abiotic elicited cell cultures (Zhao et al. 2012). Addition of yeast extract (1.5 mg/L) in germinating embryos of *C. roseus* (L.) G. Don showed highest yield of vinblastine (22.74%), vincristine (48.49%), and high levels of alkaloids (Maqsood and Abdul 2017). Yeast, as an elicitor also showed an efficient increase on biomass along with metabolite production (Funk et al. 1987; Putalun et al. 2007).

There are several other elicitors sharing origin with yeast extract such as chitosan, chitin, mannan used in the in-vitro induction of pharmaceutically useful metabolites (Baque et al. 2012). Withaferin-A production was stimulated 4.03-fold higher than the control when chitosan was added (100 mg/L) to the hairy root cultures of *Withania somnifera* (L.) Dunal. (Thilip et al. 2019). Similarly, chitosan (150 mg/L) drastically improved an active antimalarial compound, artemisinin accumulation in the hairy root cultures of *Artemisia annua* L. (Putalun et al. 2007). Addition of chitosan (200 mg/L) to the immobilized cells of *Plumbago rose*a L. resulted in 8.2-fold higher accumulation of plumbagin over control (Komaraiah et al. 2003). Chitosan (50 mg/L) along with salicylic acid and jasmonic acid in cell suspension cultures of *Azadirachta indica* A. Juss induced a 5-fold increase of azadirachtin, an active natural bio pesticide (Prakash and Srivastava 2008). Chitosan along with chitin induces phytoalexin, phenylpropanoid and naphtodianthrone production in plants (Orlita et al. 2008; Gadzovska et al. 2015). Chitin (200 mg/L) induced phenylpropanoid and naphtodianthrone production in cell suspension cultures of *H. perforatum* L. Pectin and dextran stimulated pseudohypericin 1.7 and 1.5-fold respectively and also increased the phenylalanine ammonia lyase activity in *H. perforatum* L., cultures (Gadzovska et al. 2015). While 0.01% chitin and 0.1% chitosan induced all the phytoalexins and showed a higher biomass than control in the shoots of *Ruta graveolens* L. (Orlita et al. 2008).

Certain endophytic fungal derived polysaccharides play an important role in the elicitation process (Cheng et al. 2006; Wiktorowska et al. 2010). These endogenous organisms produce polysaccharides like mannan which are highly active (Fukui et al. 1990). Mannan is a primary polysaccharide majorly extracted from the yeast cell wall. Mannan stimulated pseudohypericin and hypericin 2.8 and 1.7-fold higher respectively in the cultures of *H. adenotrichum* Spach. This study also reported enhanced production of secondary metabolites in the presence of mannan and pectin (Yamaner et al. 2013). A 3.83-fold increased production of diosgenin was seen when water extracted mycelial polysaccharides (20 mg/L) of *F. oxysporum* isolated from the rhizome of *Dioscorea zingiberensis* C.H Wright. was

Table 3 Algal elicitors used in the *in-vitro* secondary metabolite production

Sl. No	Algal elicitor used	Target plant	Target metabolite/pigment	Type of culture	References
1	*Anabaena sp.*	*Azardirachta indica* A. Juss	Azadirachtin	Cell suspension	Poornasri et al. (2008)
2	*Anabaena cylindrica*	*Carthamus tinctorius* L	Kinobeon A (Red pigment)	Cell suspension	Hanagata et al. (1994)
3	*Anabaena variabilis*				
4	*Botryococcus braunii*	*Capsicum frutescens* L	Vanillin, Vanillylamine Capsaicin	Shoot multiplication	Sharma et al. (2010)
5	*Chlorococcum sp.*	*Carthamus tinctorius* L	Kinobeon A (Red pigment)	Cell suspension	Hanagata et al. (1994)
6	*Chlorella sp.*				
7	*Haematococcus pluvialis*	*Beta vulgaris* L	Betalain	Hairy root cultures	Ramachandra Rao et al. (2001)
8		*Tagetes patula* L	Thiophenes		
9	*Kappaphycus alvarezii*	*Picrorhiza kurroa* Royle ex Benth	Picroside-I	Shoot multiplication	Sharma et al. (2015)
10	*Nostoc carneum*	*Azardirachta indica* A. Juss	Azadirachtin	Cell suspension	Poornasri et al. (2008)
11		*Carthamus tinctorius* L	Kinobeon A (Red pigment)	Cell suspension	Hanagata et al. (1994)
12	*Nostoc linckia*	*Carthamus tinctorius* L	Kinobeon A (Red pigment)	Cell suspension	Hanagata et al. (1994)
13	*Spirulina platensis*	*Capsicum frutescens* L	Capsaicin	Cell suspension	Ramachandra Rao et al. (1996)
14		*Daucus carota* L	Anthocyanin		
15		*Beta vulgaris* L	Betalain	Hairy root cultures	Ramachandra Rao et al. (2001)
16		*Tagetes patula* L	Thiophenes		

introduced into the hairy root cultures of *D. zingiberensis* C.H Wright (Li et al. 2011).

Polysaccharides like pectin derived from plants are extensively used for elicitation studies (Veerashree et al. 2012). When agro-pectin was introduced into the cell suspension culture of *Lithospermum erythrorhizon* Siebold & Zucc., it induced naphthoquinone shikonin metabolite content (Fukui et al. 1983). Different elicitors like pectin, sodium alginate, gum arabic, chitosan and yeast extract were used to elicit anthocyanin and phenolic compounds in cell suspension cultures of *Vitis vinifera* L. Highest anthocyanin production (1.8-fold times higher than control) was observed in pectin elicited cultures on the 9th day (Cai et al. 2012). An extract from yeast cell wall when added to the hairy root cultures of *P. ginseng* C.A. Meyer it remarkably increased the saponin content (66.9 mg/g) which was 1.17-fold higher than the control (Jeong et al. 2005).

Dextran, a polysaccharide present in the bacterial cells obtained from sucrose by the action of an enzyme dextran sucrase can be used as an potent elicitor (Nagella and Murthy 2010; Rahpeyma et al. 2015). The wounds on *Solanum lycopersicum* L. caused by *B. cinerea* infection when treated with dextran and laminarin induced high phenylpropanoid and flavonoids in them (Lu et al. 2019).

Alginate, a frequently and commonly used elicitor is a polysaccharide extracted from the seaweeds (Paulert et al. 2009). There are several other seaweed polysaccharides such as ulvan, carrageenan, laminarin, etc., which are efficiently involved in secondary metabolite pathway activation (Salah et al. 2018). In recent research, several seaweeds are under study due to their significant ability to enhance the secondary metabolite production (Thilip et al. 2019). Sodium alginate induced a 1.7-fold increase in total phenolics in the cell suspension cultures of *V. vinifera* L. (Cai et al. 2012). Ulvan when added (2000 mg/L) to the shoot cultures of *Olea europae* L. stimulated active phenolic compounds and declined the wilt disease (Salah et al. 2018). Similarly, laminarin when added (0.05 mg/L) to the cell suspension cultures of *Pueraria candollei* Wall.ex Benth induced high accumulation of isoflavonoids (Korsangruang et al. 2010). Polysaccharides of various origin which are used to trigger the metabolite production in the in-vitro cultures is presented in Table 4.

Conclusion

From the above discussion it is very much evident that biotic elicitors play a major role in elicitation of secondary metabolites. The production of secondary metabolites like guanosine and trigonelline is found to be increased to a greater extent by introducing bacterial elicitors like *Pseudomonas sp.* and *Enterobacter sp*. Fungal elicitor *A. niger* has shown 9-fold increase in gymnemic acid production. An 8-fold increase was observed in the formation of red pigment in *C. tinctorius* L. by the use of microalgal elicitors. Yeast extract showed a significant increase in production of metabolites such as vinblastine and vincristine. From the above works it can be noted that when fungal elicitors were used there was a higher, significant increase in the metabolite produced in certain plants followed by the bacterial elicitation. However, this is not universal as the elicitation depends primarily on the plant species, type of culture, target metabolites and the type of elicitor used. From all the studies that has been reported it is well understood that any form or type of biotic elicitor introduced into the plant system will definitely have its effect over the secondary metabolite production. However, in most cases there has been a positive effect shown. There is a significant increase in the phytochemicals produced due to the introduction of these elicitors which triggers various biochemical pathways.

Though the biotic elicitors enhance the metabolite production there are few disadvantages which needs to be addressed. All biotic elicitors do not impact the secondary metabolite production in a significant manner. Hence, the experiment needs to be designed widely to optimize the concentration and the type of biotic elicitors to be used. Also, the chemical constituents and its concentration in the crude extracts are unknown hence it is very much uncertain to conclude which component from the extract is actually responsible for the elicitation. Apart from these, the biotic elicitors affect the physiological parameters as they induce a stress environment for the plant growth. Nevertheless, though many studies have been reported we are yet to understand the working of biotic elicitors at their molecular level. There are many biological organisms with potent role in elicitation which has not been explored till date. Hence, when such microbes or plant extracts (like weeds) are explored they can be used in elicitation studies which will also help us to curb their unwanted growth and utilize them in a larger scale in an eco-friendly manner. Combination of two or more biotic elicitors for the metabolite production has not been reported in any studies so far. Owing to this as a major lacuna in the field of elicitation, biotic elicitors have opened a plethora of gateways to improve the in-vitro secondary metabolite production for the upcoming researchers.

Table 4 Polysaccharides used in the in-vitro secondary metabolite production

SL. No	Elicitor used	Target plant	Target secondary Metabolite/ Pigment	Type of culture	References
1	Alginate	*Vitis vinifera L*	Anthocyanin, Phenolic acid	Cell suspension	(Cai et al. 2012)
2		*Stevia rebaudiana* (Bertoni) Bertoni	Steviosides	Shoot culture	(Bayraktar et al. 2016)
3	Agropectin	*Lithospermum erythrorhizon* Siebold & Zucc	Naphthoquinone (shikonin)	Cell suspension	(Fukui et al. 1983)
5	Carrageenan	*Withania somnifera* (L.) Dunal	Withaferin-A	Hairy root culture	(Thilip et al. (2019)
7	Chitin	*Agelica gigas* Nakai	Decursinol angelate	Hairy root culture	(Cho et al. 2003)
8		*Ruta graveolens* L	Coumarins	Shoot culture	(Orlitan et al. 2008)
9		*Gymnema sylvestre* (Retz.) schult	Gymnemic acid	Cell suspension	(Veerashree et al. 2012)
10		*Hypericum perforatum* L	Phenylpropanoid (phenolics, flavonoids, flavanols and anthocyanins) and Naphtodianthrones(hypericin, pseudohypericin)	Cell suspension	(Gadzovska et al. 2015)
11		*Stevia rebaudiana* (Bertoni) Bertoni	Steviosides	Shoot culture	(Bayraktar et al. 2016)
13	Chitosan	*Nicotiana tabacum* L	Phytoalexins	Cell suspension	(Brodelius et al. 1989)
14		*Morinda citrifolia* L	Anthraquinones	Cell suspension	(Doernenburg and Dietrich 1994)
15		*Plumbago rosea* L	Plumbagin	Cell suspension	(Komaraiah et al. 2003)
16		*Tinospora cordifolia* (Willd.) Miers	Arabinogalactan	Callus culture	(Roja et al. 2005)
18		*Cistanche deserticola* Y. C. Ma	Phenylethanoid glycosides	Cell suspension	(Cheng et al. 2006)
20		*Artemisia annua* L	Artemisinin	Hairy root culture	(Putalun et al. 2007)
21		*Azadirachta indica* A. Juss	Azadirachtin	Cell suspension	(Prakash and Srivastava 2008)
22		*Pueraria candollei* wall.ex Benth	Isoflavonoids	Cell suspension	(Korsangruang et al. 2010)
23		*Morinda citrifolia* L	Phenolics and Flavonoids	Leaf culture	(Baque et al. 2012)
24		*Brassica oleracea* L	Phenolic compounds	Shoot culture	(Barrientos et al. 2014)
25		*Fagonia indica* Burm. F	Phenolics and Flavonoids	Shoot culture	(Khan et al. 2019)
27		*Withania somnifera* (L.) Dunal	Withaferin-A	Hairy root	(Thilip et al. (2019)
28	Dextran	*Hypericum perforatum* L	Phenylpropanoid and Naphtodianthrones	Cell suspension	(Gadzovska et al. 2015)
29		*Solanum lycopersicum* L	Phenylpropanoid and Flavonoids	Cell suspension	(Lu et al. 2019)
30	Laminarin	*Pueraria candollei* wall.ex Benth	Isoflavonoids	Cell suspension	(Korsangruang et al. 2010)
31	Mannan	*Hypericum adenotrichum* Spach	Hypericins	Shoot culture	(Yamaner et al. 2013)
32		*Atractylodes lancea* (Thunb.) DC	Volatile Oils	Shoot culture	(Chen et al. 2016)

Table 4 (continued)

SL. No	Elicitor used	Target plant	Target secondary Metabolite/ Pigment	Type of culture	References
33	Pectin	*Calendula officinalis* L	Oleanolic acid	Cell suspension	(Wiktorowska et al.2010)
34		*Vitis vinifera* L	Anthocyanin	Cell suspension	(Cai et al. 2012)
35		*Gymnema sylvestre* (Retz.) schult	Gymnemic acid	Cell suspension	(Veerashree et al. 2012)
36		*Hypericum adenotrichum* Spach	Hypericins	Shoot culture	(Yamaner et al. 2013)
37		*Morindaci citrifolia* L	Flavonoids	Leaf culture	(Baque et al. 2012)
38		*Hypericum perforatum* L	Phenylpropanoid and Naphthodianthrones	Cell suspension	(Gadzovska et al. 2015)
39		*Stevia rebaudiana* (Bertoni) Bertoni	Stevioside	Shoot culture	(Bayraktar et al. 2016)
40	Ulvan	*Phaseolus vulgaris* L	Glucuronic acid	Shoot culture	(Paulert et al. 2009)
41		*Olea europaea* L	Phenolics	Shoot culture	(Bensalah et al. 2018)
42	Water extracted mycelial polysaccharides	*Dioscorea zingiberensis* C.H.Wright	Diosgenin	Hairy Root culture	(Li et al. 2011)
43	Yeast Extract	*Glycine max* (L.) Merr	phytoalexins	Cell Suspension	(Funk et al. 1987)
44		*Panax ginseng* C.A. Meyer	Saponin	Hairy Root culture	(Jeong et al. 2005)
45		*Artemisia annua* L	Arteminisin	Hairy Root culture	(Putalun et al. 2007)
46		*Pueraria candollei* Benth	Isoflavonoids	Hairy Root culture	(Udomsuk et al. 2011)
47		*Salvia miltiorrhiza* Bunge	Tanshinone	Cell Suspension	(Zhao et al. 2010)
48		*Gymnema sylvestre* (Retz.) schult	Gymnemic acid	Cell Suspension	(Veerashree et al. 2012)
49		*Fagopyrum tataricum* (L.) Gaertn	Flavonoids	Shoot culture	(Zhao et al. 2012)
50		*Hemidesmus indicus* (L.) R.Br	2-hydroxy-4-methoxybenzaldehyde (MBALD)	Hairy Root culture	(Kundu et al. 2012)
51		*Stevia rebaudiana* (Bertoni) Bertoni	Stevioside	Shoot culture	(Bayraktar et al. 2016)
52		*Morus alba* L	Betulin and Betulinic acid	Hairy Root culture	(Park et al. 2017)
53		*Catharanthus roseus* (L.) G. Don	Vinblastine and Vincristine	Callus culture	(Maqsood et al. 2017)

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Data availability All data generated or analysed during this study are included in this published article.

Declarations

Conflict of interest All the authors declare that there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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