# Chapter 6
# Enhancement of Medicinally Important Bioactive Compounds in Hairy Root Cultures of *Glycyrrhiza*, *Rauwolfia*, and *Solanum* Through In Vitro Stress Application

**Mrinalini Srivastava and Pratibha Misra**

**Abstract** Enhancement of secondary metabolites through elicitation in hairy root culture is a very effective method which is broadly used to simulate the stress responses in plants. Elicitors are compounds that induce plants to produce secondary metabolites at elevated levels and reduce the processing time required to achieve high product concentrations. Hairy root cultures are considered as an excellent alternative for the supply of pharmaceutically important secondary metabolites/bioactives, due to their inherent genetic and biochemical stability. Plant-based secondary metabolites are well accepted in India as well as other countries to cure even the serious medical problems. In this chapter, three medicinally important plants are discussed in which stress-based elicitation of secondary metabolites has been achieved in hairy root cultures. These three plants contain important secondary metabolites in their different parts. Glycyrrhizin found in *Glycyrrhiza glabra* plant is used as antiulcer, immunomodulatory, antiallergic, and anti-inflammatory. Glycyrrhizin is also effective against HIV and severe acute respiratory syndrome (SARS)-like viruses. In *Solanum* plant, steroidal glycoalkaloids contain pharmaceutically important secondary metabolites. Solasodine, a major alkaloid of *Solanum* plant, is used as a contraceptive in different parts of the world. Ajmaline and ajmalicine are important root-specific indole alkaloids of *Rauwolfia serpentina*. Ajmalicine is useful in circulatory disorders, while ajmaline is principally known for its antiarrhythmic and antihypertensive activities. The main objective of this chapter is to provide knowledge in these plants regarding elicitation-based enhancement of valuable secondary metabolites in the form of research studies conducted till date (as per author's knowledge).

**Keywords** Elicitors • Hairy root cultures • Medicinal plants • Secondary metabolites • Stress

M. Srivastava • P. Misra (✉)
CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India
e-mail: mrinalinisrivastava5@gmail.com; pratibhaflora@gmail.com



S. Malik (ed.), *Production of Plant Derived Natural Compounds through Hairy Root Culture*, https://doi.org/10.1007/978-3-319-69769-7_6

## Introduction

Plants synthesize a large range of natural products also known as secondary metabolites; some of them are incredibly important for the survival of mankind. Some secondary metabolites, such as essential oils and saponins, were produced by plants during long-term coevolution as a mechanism of defense response against pathogens (Du Fall and Solomon 2011; War et al. 2012). The productions of enormous varieties of secondary metabolites in plants have a direct impact on plant fitness. These secondary metabolites are being used for various purposes, such as food ingredients, medical drugs, and starting materials for chemical industry, etc. (Zhao et al. 2013). Consequently, pathogens and constituents of microbial cells, mainly the polysaccharide fractions or carbohydrates, can be used to stimulate the production of secondary metabolites in plant cell and tissue cultures (Zhao et al. 2005). Due to tremendous medicinal properties, the demand for medicinal plants is constantly increasing due to which some of them are under threatened category also (Muthukumar et al. 2004).

In recent years, all over the world, people are more aware and attentive toward natural medicines or plant extracts. According to a survey, 40% or more of the pharmaceuticals prescribed in Western countries are derived from natural resources. In Ayurveda, which was recognized as the original traditional system of Indian medicine, a remarkable and significant medical importance of a number of plant species has been well described (Gantait et al. 2014).

The majority of world's populations rely on plants for medicines including life-saving drugs since thousands of years (Tripathi and Tripathi 2003). Plants have been used in medicines either directly as traditional medicines or through formulations which are prepared and dispensed by traditional medical practitioners. About one-quarter of approved drugs contain plant extracts or active ingredients obtained from plant substances. Paclitaxel and vinblastine, most valuable anticancer agents, are derived solely from plant sources. Analgesic aspirin was also originally derived from species of *Salix* and *Spiraea* (Roberts 1988).

Plant secondary metabolite production was strongly influenced by various environmental factors such as humidity, light intensity, temperature, minerals, water, $CO_2$, etc. Climate change is causing an evident impact on plant vegetation including medicinal plants (Mishra 2016).

Plant cell culture techniques were used as an excellent alternative to study and produce plant secondary metabolites (Vanisree et al. 2004). It has emerged as a promising substitute to produce difficult-to-extract secondary metabolites. But the production of plant secondary metabolites through plant cell culture technology is suffering from many biotechnological and biological restrictions. Low yield of secondary metabolites in plant cell cultures is the major obstacle. In this context, the use of biotic and abiotic elicitors to improve the yield of bioactive compounds served as the best possible strategy (Tiwari and Rana 2015).

Techniques of plant tissue culture have been considered as a science mainly due to the requirement of specific environmental conditions for each medicinal plant.

Plant tissue culture is being used as a valuable tool to synthesize the same sort of chemicals as that from the natural plant. Besides this, some novel compounds are also synthesized via biotransformations (Moyo et al. 2011; Ahmad et al. 2013).

*Agrobacterium rhizogenes*-mediated transformations were broadly studied in medicinal plants. These modified root cultures are blessed with unique properties along with general advantages of in vitro cultures (Hussain et al. 2012). Hairy roots provide an excellent alternative due to the constant high production of secondary metabolites and maintenance of their stable nature for a longer period along with hormone-free growth over cell suspension cultures. Hairy roots synthesize bioactive compounds similar or even in higher amounts as compared to normal root cultures (Agostini et al. 2013). The hairy root cultures of many plant species have been established for the production of secondary metabolites (Zhao et al. 2014).

Accumulation of secondary metabolites in in vitro culture is directly or indirectly affected by elicitation. The mode of action of elicitation is simply based on the natural accumulation of secondary metabolites in plants as defense response, and elicitors are considered as substances or agents to induce plant defense responses (Zhou and Wu 2006). Elicitation can also be utilized to identify genes involved in the biosynthesis of bioactive secondary metabolites (Zhao et al. 2005). To increase the yield of effective compounds, various elicitors have been used with the hope of improving plant secondary metabolites (Li et al. 2011).

Elicitors are categorized under two categories, i.e., biotic (plant or pathogen origin) and abiotic (chemical, mineral, and physical agents). These elicitors are commonly used to increase the growth rate and production of secondary metabolites in hairy root systems (Soleimani et al. 2012). Elicitors are substances that cause the accumulation of phytoalexins in plants as well as induce the pathways related to defense response resulting in the synthesis of secondary metabolites in plants (Nourozi et al. 2014).

There are several important medicinal plants where elicitation strategy was used in hairy root cultures. *Momordica charantia* (Cucurbitaceae) is such plant where the role of jasmonic acid (JA) and salicylic acid (SA) was investigated on phenolic compound production and biomass accumulation in hairy root cultures. Hairy root cultures elicitated with JA and SA enhanced the production of phenolic compounds, flavonoid contents, and total phenolics significantly in comparison to non-elicited hairy root cultures (Chung et al. 2016), although only after SA treatment increase in biomass was reported in hairy root cultures.

Hairy root cultures of *Isatis tinctoria* were established for flavonoid (FL) production. Eight bioactive flavonoids, such as neohesperidin, rutin, buddleoside, quercetin, liquiritigenin, kaempferol, isoliquiritigenin, and isorhamnetin, were determined by LC-MS/MS (Gai et al. 2015). *A. rhizogenes* (ATCC15834)-mediated transformation in *Prunella vulgaris* produced 15–30 times more rosmarinic acid (RA) in comparison to intact plants. Further enhancement in RA content was achieved through elicitation by ethephon and SA (Ru et al. 2016). Hairy root cultures of *Valeriana officinalis* were treated with two to six times higher concentration of magnesium and calcium than in normal Murashige and Skoog's (MS) medium from 3 to 7 days. The uppermost amount of valerenic acid in hairy root culture was

7.9 times higher than the control (Torkamani et al. 2014). Vazquez-Flota et al. (2004) conducted an experiment in transformed root cultures of *Catharanthus roseus*. Many factors such as the concentration of medium, addition of biotic elicitors, and hydrolytic enzymes were used as strategies for enhancing the yield of alkaloids. Noteworthy results were obtained when sucrose concentration was enhanced from 3 to 4.5%. Treatment of *Aspergillus* and macerozyme increased the accumulation of ajmalicine although the addition of methyl jasmonate (MJ) was proved beneficial for increasing the yield of both ajmalicine and catharanthine (Vazquez-Flota et al. 1994).

This chapter is an effort to review all the studies on elicitation strategies for enhancing the content of secondary metabolites in hairy root cultures of three essential medicinal plants *Glycyrrhiza*, *Rauwolfia*, and *Solanum* spp.

## *Rauwolfia* spp.

*Rauwolfia* genus is primarily known for its bioactive terpenoid indole alkaloids (TIA), such as reserpine, ajmaline, ajmalicine, yohimbine, serpentine, vomiline, etc. (Joshi and Kumar 2000). These alkaloids are mainly present in root bark of the plant (Mehrotra et al. 2013). *R. serpentina* L. is an evergreen shrub having woody, glabrous, and perennial habit with a maximum height of 60 cm. The leaves of the plants are elliptic to lanceolate or obovate in shape in whorls of three; roots are tuberous with pale brown cork (Deshmukh et al. 2012). This plant is commonly known as "Sarpagandha," "Chandrabhaga," snakeroot plant, Chotachand, Chandrika, Harkaya, etc. (Mallick et al. 2012). It is a tropical plant belonging to family Apocynaceae. The roots and leaves of *R. serpentina* are of medicinal importance and catch the attention in the field of medicine because of the presence of secondary metabolites (Poonam and Mishra 2013). In India, it has been used as a part of the Ayurvedic system for curing various ailments (Pant and Joshi 2008). The roots of *R. serpentina* are used as a medication for treating insomnia, hypertension, mental agitation, gastrointestinal disorders, epilepsy, excitement, traumas, anxiety, excitement, sedative insomnia, schizophrenia, and insanity (Meena et al. 2009; Poonam and Mishra 2013). According to Rajendran and Agarwal (2007), fruits and seeds of this plant are used by ethnic tribes of Virudhunagar district of Tamil Nadu, India, for its medicinal or ethnobotanical properties. *R. serpentina* plant has been considered under endangered category by the International Union for the Conservation of Nature and Natural Resources (IUCN) (Shetty et al. 2014). This plant contains abundant medicinal properties and strongly suffered from habitat distortion (Mehrotra et al. 2015).

Hairy root cultures of *R. serpentina* plants proved to be a very advantageous alternate method among all conservational strategies. Ajmaline and ajmalicine are important root-specific indole alkaloids of *R. serpentina*. Ajmaline is principally known for its antiarrhythmic and antihypertensive activities, while ajmalicine is useful in circulatory disorders (Srivastava et al. 2006).

In a study, hypocotyl segments of *R. micrantha* were used for hairy root induction with the help of *A. rhizogenes* strain ATCC 15834. Half-strength MS medium in combination with 0.2 mg indole 3-butyric acid $L^{-1}$ and 0.1 mg α-naphthaleneacetic acid $L^{-1}$ showed enhancement in ajmaline and ajmalicine concentration in comparison to roots grown in an auxin-free medium. In this report, production of ajmaline and ajmalicine was reported for the first time in hairy root cultures of *R. micrantha* (Sudha et al. 2003).

A study was conducted to evaluate the effect of three elicitors, methyl salicylate, SA, and acetylsalicylic acid, on phenolic content of *R. serpentina.* The content of caftaric acid, caffeic acid, chlorogenic acids, and cichoric acid along with rutin (flavonoid) was investigated in shoots and roots of the plant. In the shoot of the plant, the content of the cichoric acid was significantly (0.05%) enhanced through the application of all elicitors. Salicylic acid was proved as the most effective elicitor at 10 M concentration. For chlorogenic acid, caftaric acid, and rutin, methyl salicylate at the concentration of 10 M proved to be best among all tested elicitors in shoots as well as in roots of the plants. All elicitors significantly increased the cichoric acid content, but best result was obtained for 1000 M salicylic acid (Nair et al. 2013).

Estimation of bioactive compounds is very necessary for the valuation of herbal drugs. Many natural factors such as climate, altitude, rainfall, etc. are responsible for the growth of plants, and these conditions also affect the contents of plant metabolites. Variation in geographical areas alters the level of secondary metabolites, and this information could be used for generating the special conditions to enhance the yield of bioactive compounds. Keeping this in mind, four different parts of Southern India were used to collect *Rauwolfia* samples for analyzing the reserpine level. Considerable variation in the concentration of reserpine has been recorded (Kumar et al. 2010).

Shetty et al. (2014) developed an efficient and reproducible method for the induction of callus and hairy roots from in vitro as well as in vivo explants of *R. serpentina.* Hairy roots were used for the large-scale production of secondary metabolites. Thin-layer chromatography results showed that the reserpine was present in in vivo and in vitro explants and callus and it can be enhanced through various methods. Further, hairy roots were induced from leaf explants, and these hairy roots would be utilized for large-scale production of secondary metabolites (Shetty et al. 2014).

In *R. serpentina*, the presence of two nitrogen-containing compounds, vomilenine and reserpine, was first time examined through DART technique. For identification the intact hairy roots were analyzed by holding them in the gap between the DART source and the mass spectrometer. The confirmation of the structures of the identified compounds was made through their accurate molecular formula determinations (Madhusudanan et al. 2008).

Harisaranraj et al. (2009) concentrated on the production of reserpine by elicitating embryogenic suspension cultures of *R. serpentina* through different concentrations (50–500 μM) of MJ. Reserpine content significantly increased by the effect of MJ, although the fresh weight, dry weight, and growth ratio of embryos were

significantly decreased by increasing MJ concentrations. The highest yield was 7.3-fold enhancements.

Nurcahyani and Anggarwulan (2008) studied the effect of $Cu^{2+}$ on callus growth and reserpine synthesis of in vitro cultures of *R. serpentina*. The increase in reserpine production was observed at 5 and 10 μM concentration of $Cu^{2+}$ after 15 days of elicitation, although decrease in concentration was reported at higher concentration of $Cu^{2+}$.

An efficient transformation system for *R. serpentina* was established with *A. rhizogenes* strain LBA 9402. The transformed root lines showed significant differences in their reserpine content (Ray et al. 2014).

There is the constant global demand of terpenoid indole alkaloids as the natural sources of these alkaloids were unable to fulfill such a huge demand. In this regard, hairy root cultures of *R. serpentina* provide an ultimate alternative source for the production of these alkaloids (Goel et al. 2010). Benjamin et al. (1993) also reported hairy roots induction with the use of *A. rhizogenes* strain ATCC 15834 from the leaf explants of *R. serpentina* (Goel et al. 2010; Liu et al. 2012; Sudha et al. 2003). Different *A. rhizogenes* strains, i.e., SV4, LBA9402, and SV2, were evaluated for their transformation ability. Among them SV2 strain was found more competent for the induction of hairy roots in leaf explants of *R. serpentina* (Sarma et al. 1997). ATCC 15834 strain of *Agrobacterium* was also exploited for their transformation ability regarding induction of hairy roots in *R. micrantha* (Sudha et al. 2003). Madhusudanan et al. (2008) reported induction of hairy roots in leaf explants of *R. serpentina* using A4 strain. A novel compound, 3-epi-a-yohimbine, was reported from these hairy roots. Plantlet formation from high reserpine yielding hairy root lines of *R. serpentina* has also been reported (Mehrotra et al. 2013). The effect of biotic and abiotic elicitors on the production of important metabolites in hairy root cultures of *R. serpentina* was analyzed. Ajmalicine production could be stimulated up to 14.8-fold at 100 mM concentration of NaCl after 1 week of treatment. However, ajmaline concentration could only be increased up to 2.9-fold at 100 mg $L^{-1}$ dose of mannan after 1 week of treatment (Srivastava et al. 2016).

## *Solanum* spp.

*Solanum khasianum* was originated from India; stem and leaves of this bushy annual or short-lived perennial plant are packed with spines. Steroidal glycoalkaloids are abundant in *Solanum* genus. These alkaloids are used as the precursor for the synthesis of steroidal drugs. Solasodine is the aglycone moiety of glycoalkaloids. Solasodine can be easily converted to 16-dehydropregnenolone, which is a key intermediate in the synthesis of steroidal drugs, such as progesterone and cortisone. Due to the presence of these valuable alkaloids, in the traditional system of medicine, *Solanum* species are used for the treatment of several diseases as in liver diseases, in asthma, and in different kinds of inflammation (Patel and Krishnamurthy 2013).

Shilpha et al. (2015) used leaves of *S. trilobatum* L. for the establishment of hairy root cultures of the infection of *A. rhizogenes.* Various strains, like MTCC 2364, MTCC 532, and ATCC along with A4, were used in this study. An elicitation strategy was performed for enhancing the solasodine level in *S. trilobatum*. A hairy root line elicited with methyl jasmonate (4 M) for 2 weeks, a 1.9-fold and 6.5-fold enhanced production of solasodine (9.33 ± 0.04 mg $g^{-1}$ DW) was obtained in comparison to unelicited and nontransformed normal roots, respectively. Real-time PCR analysis was also performed for monitoring the expression level of HMG-CoA reductase during the first 2 weeks of elicitation. A significant enhancement was also observed in the total flavonoids (521.09 mg $g^{-1}$ DE), total phenolics (150.42 mg $g^{-1}$ DE), and radical scavenging activity (83.3%) at 4 M MJ concentration in comparison with control (Shilpha et al. 2015).

Jain and Singh (2015) evaluated *S. melongena* (L.), a medicinally important plant of Solanaceae family, for its efficiency for induction of hairy roots using various strains of *A. rhizogenes*, such as LBA 9402, NCIM 5140, MTCC 532, A4, and R1022. Hypocotyl explants infected with NCIM 5140 strain proved as most effective regarding hairy root induction. Acetosyringone with 100 μM in cocultivation media enhanced the incidence of hairy root induction within a short period. A study was performed for demonstrating the effect of different elicitors, viz., SA, yeast extract (YE), and pectin, on solasodine production in *S. melongena* (L.) hairy root cultures. Pectin with 1% concentration was observed to be the most efficient elicitor to enhance the solasodine production from 6.5 to 151.23 μg $g^{-1}$ DW, i.e., 23-fold over hairy root control (μg $g^{-1}$ DW) and 88-fold in comparison to field control (1.71 μg $g^{-1}$ DW) (Jain and Singh 2015).

Hairy root cultures of *S. aculeatissimum* were developed through *A. rhizogenes* strain 15834. Production of steroidal saponin along with root growth was investigated under various culture conditions. Gamborg's B5 medium was found suitable for growth and steroidal saponin production. Growth and steroidal saponin production were enhanced when 100 μg $L^{-1}$ auxin was added to the medium. The addition of 2,4-D inhibited growth, although production of steroidal saponin was highest with NAA (Ikenaga et al. 1995).

The effect of various elicitors was examined on solasodine production in hairy root cultures of *S. elaeagnifolium* Cav. Chitosan, hemicellulase, $H_2O_2$, Ag-$NO_3$, *Hormonema* sp., and *Pythium* sp.'s homogenates were used as elicitors, but no effect on solasodine production was observed. Homogenates of *Sclerotinia sclerotiorum* reduced the solasodine production about 30% in comparison to the control. This activity could be attributed to the fact that *S. sclerotiorum* elicitation induced the sesquiterpene biosynthesis instead of alkaloid production (Parsons et al. 2006).

In hairy root cultures of *S. khasianum*, the effects of biotic and abiotic elicitors were observed on the production of important metabolites. Solasodine content could be enhanced up to 4.0-fold and 3.6-fold at 100 mM and 200 mM NaCl, respectively, after 6 days of treatments (Srivastava et al. 2016).

In *S. khasianum*, increased proportion of $CO_2$ in the medium enhanced the growth and secondary metabolite production in hairy root cultures. Light and temperature play a role in the determination of growth and secondary metabolite

production in these hairy root cultures. These factors also control the appearance of green color in hairy roots (Jacob and Malpathak 2004). Transgenic hairy root cultures were also established for *S. khasianum* plant. Single-chain variable fragment (scFv) protein against solamargine was expressed in transgenic hairy roots. Results showed that the concentration of solasodine glycoside could be increased to 2.3-fold in transgenic hairy roots than nontransgenic hairy roots. It may be concluded that scFv expression in transgenic lines might have stimulated biosynthesis pathways. Plantlet regenerated from these hairy roots and fruits obtained from these transgenic plants also contained twofold higher solasodine glycoside content in comparison to plants generated by nontransgenic hairy roots (Putalun 2011).

For the enhancement of solasodine concentration, Quadri and Giulietti (1993) observed the effect of a fungal elicitor obtained from *Alternaria* sp. on suspension culture and entrapped cells of *Solanum elaeagnifolium* Cav. Fourteen-day-old cultures were used for elicitation of 1% FW/V autoclaved homogenates. In suspension culture, 0.9–1.5 mg $g^{-1}$ DW (65%) increase and in entrapped cells 0.75–1.4 mg $g^{-1}$ DW (about 95%) enhancement were observed. The maximum accumulation was obtained after 72 h of elicitation.

In this study, the combination of water stress and infection of plant-parasitic nematodes was studied on the nutritional quality of tomatoes (Atkinson et al. 2011). The level of phenolic compounds, carotenoids, and sugar in fruits was estimated along with physiological responses after plant encountered with one or both the stresses. The amount of carotene and carotenoid lycopene was found lesser in water-stressed tomatoes but showed diverse responses after combined stress. Nematode stress was responsible for enhancement in flavonoid level, albeit with reduced yield, although the level of chlorogenic acid was positively affected by water stress, nematodes, and combined stress. Combined stress also enhanced sugar level. These results enlightened the utility of the combination of stress.

An (2014) observed the effects of yeast extract (YE) and MeJA on the growth and solasodine production of *S. hainanense* cells. The results showed that various concentrations of MeJA (50–250 μM) and YE (1–4 g $L^{-1}$) have different eliciting influences. The increase in solasodine concentration was observed through elicitation of 3 g $L^{-1}$ of YE and 50 μM of MeJA at the initial stage of cell culture where the increase was 1.9- and 1.3-fold, respectively, over the non-elicited cells. According to this study, YE (biotic elicitor) was found more successful in enhancing solasodine production than MeJA (abiotic elicitor).

## *Glycyrrhiza* spp.

*G. glabra* L. plant belongs to Fabaceae family and is the inhabitant of Central and Southwest Asia. Cultivation of this plant occurred in Northern India, Italy, France, the UK, the USA, Russia, Spain, Germany, and China (Parvaiz et al. 2014). *G. glabra* is commonly known as Jothi-madh, Mulhatti (Hindi), Yashtimadhu, Madhuka (Sanskrit), licorice, liquorice, sweetwood (English), Jashtimadhu, Jaishbomodhu

(Bengali), Yastimadhuka, atimaddhura (Kannada), Jethimadhu (Gujarat), Iratimadhuram (Malayalam), Jatimadhu (Oriya), Jeshtamadha (Marathi), Athimaduram (Tamil) and Atimadhuranu, and Yashtimadhukam (Telugu) (Jatav et al. 2011). It is a perennial shrub, with hardy habit and height up to 2.5 m. The leaves are alternate, compound, and imparipinnate. Leaves have four to seven pairs of leaflets oblong, lanceolate, and elliptical in shape. The flowers are narrow lavender to violet in color. The fruit is up to 1.5-cm-long compressed legume or pod. Seeds are brown in color and reniform in shape (Jatav et al. 2011).

*G. glabra* plant is blessed with many medicinal properties. This plant is used in the treatment of dyspepsia, gastric ulcers, fevers, liver ailments, asthma, bronchitis, sore throats, Addison's disease, and rheumatoid arthritis. It is also useful as an antitussive, expectorant, and laxative. In ancient times, this plant was also suggested in cases of women sterility. Licorice root is considered under top five herbs, which are recommended for the treatment of fatigue. This herb decreases temptation for sugars and increases cortisol activity in the human body. Glycyrrhizin is present in a very high amount in licorice roots. Besides glycyrrhizin, some other triterpene saponins are also present. Saponins are used for various purposes as foaming and detergent and also as emulsifying and sweetening agents (Nasrollahi et al. 2014). Although licorice roots contain many beneficial properties, some side effects are also associated, due to high doses and prolonged use of this, such as hypokalemia, hypertension, mineralocorticoid effects, myoglobinuria, lethargy, quadriplegia, etc. (Nasrollahi et al. 2014).

The roots of licorice contain a large amount of glycyrrhizin (up to 15%) and oleanane-type triterpene saponins. These saponins are used in various foods and industrial, cosmetic, and pharmaceutical applications. Saponins are commercially used in food industry as foaming, detergent, emulsifying, wetting, and sweetening agents (Hostettmann and Marston 2005; Shibata 2000). These compounds are also utilized in cleansing and personal care sectors and also as ingredients in the cosmetics such as shampoos, shower gels, hair conditioners, liquid soaps, lotions, baby care products, toothpastes, and mouthwashes. The pharmacological properties of triterpenes have been broadly studied which showed that these compounds have significant medicinal properties. Besides this, they also showed involvement in plant defense responses. Glycyrrhizin is also efficient against several viruses, such as HIV (Ito et al. 1987, 1988) and severe acute respiratory syndrome (SARS caused by coronavirus-like viruses) (Cinatl et al. 2003).

The use of licorice is more than 4000 years old. It is considered under important medicinal plants mentioned in Assyrian herbal (2000 BC). Licorice is used as laxative, demulcent, antitussive, expectorant, and sweetener from traditional Siddha system of medicine. It is used for curing acute respiratory problems, gastritis, gastric ulcers, inflammatory conditions in general, and adrenal exhaustion. Compounds found in licorice roots possess both estrogenic and antiestrogenic activity, and due to these properties, this important herb is used for treating the female hormonal problems (Jatav et al. 2011).

A study in *G. inflata* hairy roots was performed using different elicitors, such as chitosan and MeJA, for enhancing the glycyrrhizin contents. Chitosan did not

significantly alter the content; however, on increasing the duration, the content of glycyrrhizin decreased. 100 μM MeJA after 5 days of treatment enhanced glycyrrhizin content 5.7 times higher than the control. Further increase in duration decreases glycyrrhizin content (Vasconsuelo and Boland 2007). Yeast extract treatment proved as effective for enhancing the glycyrrhizin yield but lesser effective in comparison to MeJA (Wongwicha et al. 2011).

The effect of MeJA, chitosan, and yeast extract on *G. inflata* (Batal) hairy root cultures was monitored. MeJA at 100 μM concentration was the most effective for increasing the glycyrrhizin production up to 108.9 ± 1.15 μg $g^{-1}$ DW after 5 days of elicitation (Putalun et al. 2011).

An investigation was performed for induction of hairy roots in *G. glabra* using strain K599 in leaf explants, and a comparative study was also done to analyze its growth kinetics at shake flask and bioreactor level. Four different basal media were used in this study, MS basal semisolid, NB, B5, and WP medium. Among them, maximum TF frequency was observed on MS basal semisolid medium, although WP medium could not induce hairy root formation. NB modified medium supported best hairy root growth. Approximately 20 times enhancement in root biomass was reported after 45 days of culture, after increasing this culture period browning of roots started. Under the same set of conditions, normal roots exhibited only twofold increase in biomass in shake flask cultures (Mehrotra et al. 2008).

Licoagrochalcone A and licoagrocarpin, two new prenylated flavonoids, were isolated from the hairy root cultures of *G. glabra*. By spectroscopic evidence, the structures of the new compounds were elucidated as 3-prenyl-2′,4,4′-trihydroxychalcone and (6a*R*, 11a*R*)-4-prenyl-3-hydroxy-9-methoxypterocarpan, respectively (Asada et al. 1998).

Flavonoids are economically important compounds, but its amounts are insufficient in hairy roots. To conquer this difficulty, the combination of transgenic approach and elicitation techniques was used to increase the flavonoid production. cDNA encoding chalcone isomerase (chi) gene was overexpressed in hairy roots of *G. uralensis* Fisch. Subsequently, transgenic and wild cultures were elicited with PEG 8000 (2%) and yeast extract (YE) (0.1%), and the combination of these two elicitors is also used. Total flavonoids were extracted and measured. The obtained results demonstrated that the highest flavonoid was obtained in double-treated transgenic hairy roots (2.838 g 100 $g^{-1}$ DW). The amount of flavonoid in wild-type hairy roots and the untreated transgenic hairy roots was 0.842 and 1.394 (g 100 $g^{-1}$ DW), respectively. The enhanced accumulation of flavonoids was also correlated with the elevated level of chi transcripts and CHI activity; it confirmed the key role of chi in the flavonoid biosynthesis. This research verified that the combination of PEG8000-YE elicitation with metabolic engineering was an effective strategy to enhance the flavonoid production in hairy roots of *G. uralensis* Fisch (Zhang et al. 2009).

In transformed *A. precatorius* cell suspension cultures, twofold increase in glycyrrhizin yield was obtained against untransformed cultures. To improve the yield of glycyrrhizin, some fungal elicitors prepared from *Aspergillus niger* and *Rhizopus stolonifer* were tested at different concentrations in transformed cell suspension

cultures of *A. precatorius*. The maximum enhancement of 4.9- and 3.8-fold in glycyrrhizin contents was obtained with *A. niger* (7.5% v/v) and *R. stolonifer* (5.0% v/v), respectively, on the fifth day after elicitor treatment (Karwasara et al. 2011). In seedlings of *G. uralensis* growth, lipid peroxidation, osmolyte concentration, antioxidant metabolism, and Si content were examined under control conditions as well as salt and drought stress conditions [100 mM NaCl with 0, 10, and 20% of PEG-6000 (polyethylene glycol-6000)] with or without 1 mM Si. The addition of Si markedly affected the *G. uralensis* growth in a combination of NaCl and PEG treatment. The addition of Si improved germination index, germination rate, seedling vitality index, and biomass under control and NaCl treatment. Si also increased radicle length under control, NaCl, and combination of NaCl and PEG treatment (NaCl-10% PEG), while under NaCl-20% PEG combination, it decreased some parameters such as radicle length, seedling vitality index, and germination parameters. The salt and drought stress-induced oxidative stress were modulated by Si application (Zhang et al. 2017).

Two *A. rhizogenes* strains MTCC 2364 and MTCC 532 were evaluated in terms of a number of hairy roots, transformation frequency, and glycyrrhizin production in *Abrus precatorius*. After elicitation with methyl jasmonate, maximum glycyrrhizin production (2.5-fold) was found in hairy roots transformed with strain 532 (Sajjalaguddam and Paladugu 2016).

A study was performed by Li et al. (2016) on a 1-year-old *G. uralensis* Fisch. ex DC (Fabaceae), treating it with three different exogenous phytohormones, like auxin (indole-3-acetic acid), gibberellins, and MeJA in the months of June and July. Control plants were treated with water. The glycyrrhizic acid content of roots was significantly increased in the plants which were treated in the month of June. The increase also occurred in the plants which were treated in July, but the effect was lesser in comparison to the plants treated in June. Auxin at 40 mg $L^{-1}$ and gibberellin at 40 mg $L^{-1}$ concentration significantly enhanced the accumulation of glycyrrhizic acid in *G. uralensis* roots. Methyl jasmonate at 100 and 25 mg $L^{-1}$ in June and July, respectively, also significantly promoted glycyrrhizic acid content. Major active compositions, such as isoliquiritin, liquiritin, liquiritinapioside, and isoliquiritinapioside, were found positively correlated with glycyrrhizic acid content (Li et al. 2016).

Shabani et al. (2009) conducted an experiment in *G. glabra* and reported the maximum yield of glycyrrhizin which occurred at 2 mM MeJA concentration after 24-h treatment, in the form of 3.8-fold increase as compared to the control.

Ahmed and Baig (2014) observed the effect of diverse biotic elicitors such as fungal extract prepared from *Aspergillus niger* and *Penicillium notatum* on cell cultures of *Psoralea corylifolia* L. Besides this, yeast extract and chitosan were also studied. Ninefold enhancements were reported in psoralen concentration in treated cells over control one after *A. niger* elicitation. Elicitation with *P. notatum* yeast extract and chitosan caused four- to sevenfold higher psoralen productions in contrast to control cells. Above all, extract of *A. niger* at 1.0% v/v increased the highest accumulation of psoralen in the cultured cells, i.e., 9850 μg $g^{-1}$ DCW.

The effect of various arbuscular mycorrhizal (AM) fungi was observed on the growth and development of *G. glabra* (licorice). Several species of AM, such as *Glomus intraradices* and *Glomus mosseae*, and a mixture of fungi (*G. intraradices*, *G. cladoideum*, *G. mosseae*, *G. caledonium*, *G. microagregatum*, and *G. etunicatum*) were used in the study. Licorice growth rates were determined by measuring the colonization rate of the plants by the fungi, dry plant biomass, phosphorus concentration, and concentration of secondary metabolites. The results of this study showed that the AM fungi enhanced the leaf and root biomass, phosphorus content, and secondary metabolite content of plants (Liu et al. 2014).

For addressing the difference between secondary metabolite content of closely related plant species and their hybrids, a study was conducted between three *Glycyrrhiza* species (*G. uralensis*, *G. glabra*, and *G. inflata*). The *Glycyrrhiza* species (genotypes) for 95 batches of samples were identified by DNA bar codes of the internal transcribed spacer and trnV-ndhC regions. The chemotypes were revealed by LC/UV- or LC/MS/MS-based quantitative analysis of 151 bioactive secondary metabolites, including 17 flavonoid glycosides, 24 saponins, and 110 free phenolic compounds. For the 76 homozygous samples, the three *Glycyrrhiza* species showed significant biosynthetic preferences, especially in coumarins, chalcones, isoflavones, and flavonols. In total, 27 species-specific chemical markers were discovered. The 19 hybrid samples indicated that hybridization could remarkably alter the chemical composition and that the male parent contributed more to the offspring than the female parent did. This is hitherto the largest-scale targeted secondary metabolomics study of medicinal plants and the first report on uniparental inheritance in plant secondary metabolism. The results are valuable for biosynthesis, inheritance, and quality control studies of licorice and other medicinal plants (Song et al. 2017).

## Conclusions

The combination of hairy root culture and elicitation in medicinal plants is the very promising strategy for enhancing the yield of secondary metabolites, due to the high demand of bioactive compounds and their poor yield in natural sources. Significant enhancement (up to commercial level) was obtained by implementation of these techniques. Study of these fruitful topics increases the knowledge of elicitation or stress-related parameters. Readers could utilize these techniques to improve the yield of other commercially important secondary metabolites in different plants.

## References

Agostini E, Talano MA, Gonzalez PS, Oller AL, Medina MI (2013) Application of hairy roots for phytoremediation: what makes them an interesting tool for this purpose? Appl Microbiol Biotechnol 97:1017–1030

Ahmed SA, Baig MMV (2014) Biotic elicitor enhanced production of psoralen in suspension cultures of *Psoralea corylifolia* L. Saudi J Biol Sci 21:499–504

Ahmad S, Garg M, Tamboli ET, Abdin MZ, Ansari SH (2013) In vitro production of alkaloids: Factors, approaches, challenges and prospects. Pharmacogn Rev 7(13):27–33

An TNT (2014) Effects of yeast extract and methyl jasmonate on the enhancement of solasodine biosynthesis in cell cultures of *Solanum hainanense* Hance. J BioSci Biotechnol 3(1):1–6

AsadaY, Li W, Yoshikawa T(1998) Isoprenylated flavonoids from hairy root cultures of *Glycyrrhiza glabra*. Phytochemistry 47(3):389–392

Atkinson NJ, Dew TP, Orfila C, Urwin PE (2011) Influence of combined biotic and abiotic stress on nutritional quality parameters in Tomato (*Solanum lycopersicum*). J Agric Food Chem 59(17):9673–9682

Benjamin B, Roja G, Heble M (1993) *Agrobacterium rhizogens* mediated transformation of *Rauvolfia serpentina*: regeneration and alkaloid synthesis. Plant Cell Tiss Org Cult 35:253–257

Chung IM, Thiruvengadam M, Rekha K, Rajakumar G (2016) Elicitation enhanced the production of phenolic compounds and biological activities in hairy root cultures of Bitter melon (*Momordica charantia* L.) Braz Arch Biol Technol 59:1–10

Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr H (2003) Glycyrrhizin, an active component of licoriceroots, and replication of SARS-associated coronavirus. Lancet 361:2045–2046

Deshmukh SR, Ashrit DS, Patil BA (2012) Extraction and evaluation of indole alkaloids from *Rauwolfia serpentina* for their antimicrobial and antiproliferative activities. Int J Pharm Pharm Sci 4:329–334

Du Fall LA, Solomon PS (2011) Role of cereal secondary metabolites involved in mediating the outcome of plant-pathogen interactions. Metabolites 1:64–78

Gai QY, Jiao J, Luo M, Wei ZF, Zu YG, Ma W, Fu YJ (2015) Establishment of hairy root cultures by *Agrobacterium rhizogenes* mediated transformation of *Isatis tinctoria* L. for the efficient production of flavonoids and evaluation of antioxidant activities. PLoS One 10:e0119022

Gantait S, Debnath S, Nasim Ali M (2014) Genomic profile of the plants with pharmaceutical value. Biotech 4(6):563–578

Goel MK, Goel S, Banerjee S, Shanker K, Kukreja AK (2010) *Agrobacterium rhizogenes*-mediated transformed roots of *Rauwolfia serpentina* for reserpine biosynthesis. Med Aromat Plant Sci Biotechnol 4:8–14

Harisaranraj R, Suresh K, Babu SS (2009) Production of reserpine in somatic embryos of *Rauwolfia serpentina* cultured in bioreactors by the induction of elicitor (Methyl Jasmonate). Glob J Biotechnol Biochem 4:143–147

Hostettmann K, Marston A (2005) Saponins. Cambridge University Press, Cambridge

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

Ikenaga T, Oyama T, Muranaka T (1995) Growth and steroidal saponin production in hairy root cultures of *Solanum aculeatissimum*. Plant Cell Rep14(7):413–417

Ito M, Nakashima H, Baba M, Pauwels R, De Clercq E, Shigeta S, Yamamoto N (1987) Inhibitory effect of glycyrrhizin on the in vitro infectivity and cytopathic activity of the human immunodeficiency virus [HIV (HTLV-III/LAV)]. Antivir Res 7:127–137

Ito M, Sato A, Hirabayashi K, Tanabe F, Shigeta S, Baba M, De Clercq E, Nakashima H, Yamamoto N (1988) Mechanism of inhibitory effect of glycyrrhizin on replication of human immunodeficiency virus (HIV). Antivir Res 10:289–298

Jacob A, Malpathak N (2004) Green hairy root cultures of *Solanum khasianum* Clarke-a new route to in vitro solasodine production. Curr Sci 87:1442–1447

Jain A, Singh S (2015) Effect of growth regulators and elicitors for the enhanced production of solasodine in hairy root culture of *Solanum melongena* (L.) J Indian Bot Soc 94(1 and 2):3–39

Jatav VS, Singh SK, Khatri P, Sharma AK (2011) Recent pharmacological trends of *Glycyrrhiza glabra* Linn. Unani Res 1:1–11

Joshi N, Kumar N (2000) Rauwolfia. In: Kumar N (ed) Aromatic and medicinal plants in Central Himalayas. Pithoragarh, Uttarakhand

Karwasara VS, Tomar P, Dixit VK (2011) Influence of fungal elicitation on glycyrrhizin production in transformed cell cultures of *Abrus precatorius* Linn. Pharmacogn Mag 7:307–313

Kumar VH, Shashidhara S, Rajendra C (2010) Reserpine content of *Rauwolfia serpentina* in response to geographical variation. Int J Pharma BioSci 1(4):429–434

Li WD, Hou J, WangW, Tang X, Liu C, Xing D (2011) Effect of water deficit on biomass production and accumulation of secondary metabolites in roots of *Glycyrrhiza uralensis*. Russ J Plant Physl 58:538–542

Li YP, Yu CX, Qiao J, Zang YM, Xiang Y, Ren GX, Wang L, Zhang XY, Liu CS (2016) Effect of exogenous phytohormones treatment on glycyrrhizic acid accumulation and preliminary exploration of the chemical control network based on glycyrrhizic acid in root of *Glycyrrhiza uralensis*. Rev Bras Farm 26:490–496

Liu W, Chen R, Chen M, Zhang H, Peng M, Yang C, Ming X, Lan X, Liao Z (2012) Tryptophan decarboxylase plays an important role in ajmalicine biosynthesis in *Rauvolfia verticillata*. Planta 236:239–250

Liu H, Tan Y, Nell M, Zitter-Eglseer K, Wawscrah C, Kopp B, Wang S, Novak J (2014) Arbuscular mycorrhizal fungal colonization of *Glycyrrhiza glabra* roots enhances plant biomass, phosphorus uptake and concentration of root secondary metabolites. J Arid Land 6:186–194

Madhusudanan K, Banerjee S, Khanuja SP, Chattopadhyay SK (2008) Analysis of hairy root culture of *Rauvolfia serpentina* using direct analysis in real time mass spectrometric technique. Biomed Chromatogr 22:596–600

Mallick SR, Jena RC, Samal KC (2012) Rapid in vitro multiplication of an endangered medicinal plant sarpgandha (*Rauwolfia serpentina*). Am J Plant Sci 3:437

Meena AK, Bansal P, Kumar S (2009) Plants-herbal wealth as a potential source of ayurvedic drugs. Asian J Tradit Med 4:152–170

Mehrotra S, Kukreja AK, Khanuja SPS, Mishra BN (2008) Genetic transformation studies and scale up of hairy root culture of *Glycyrrhiza glabra* in bioreactor. Electron J Biotechnol 11:69–75

Mehrotra S, Goel MK, Rahman LU, Kukreja A (2013) Molecular and chemical characterization of plants regenerated from Ri-mediated hairy root cultures of *Rauwolfia serpentina*. Plant Cell Tiss Org Cult 114:31–38

Mehrotra S, Goel MK, Srivastava V, Rahman LU (2015) Hairy root biotechnology of *Rauwolfia serpentina*: a potent approach for the production of pharmaceutically important terpenoid indole alkaloids. Biotechnol Lett 37:253–263

Mishra T (2016) Climate change and production of secondary metabolites in medicinal plants: a review. Int J Herb Med 4(4):27–30

Moyo M, Bairu MW, Amoo SO, Staden JV (2011) Plant biotechnology in South Africa: Micropropagation research endeavours, prospects and challenges. S Afr J Bot 77(4):996–1011

Muthukumar T, Udaiyan K, Shanmughavel P (2004) Mycorrhiza in sedges?an overview. Mycorrhiza 14(2):65–77

Nair VD, Panneerselvam R, Gopi R, Hong-Bo S (2013) Elicitation of pharmacologically active phenolic compounds from *Rauvolfia serpentina* Benth. Ex. Kurtz. Ind Crop Prod 45:406–415

Nasrollahi V, Mirzaie-asl A, Piri K, Nazeri S, Mehrabi R (2014) The effect of drought stress on the expression of key genes involved in the biosynthesis of triterpenoid saponins in licorice (*Glycyrrhiza glabra*). Phytochemistry 103:32–37

Nourozi E, Hosseini B, Hassani A (2014) A reliable and efficient protocol for induction of hairy roots in *Agastache foeniculum*. Biologia 69(7):870–879

Nurcahyani N, Anggarwulan E (2008) The reserpine production and callus growth of Indian snake root (*Rauvolfia serpentina* (L.) benth. Ex Kurz) culture by addition of Cu2+. Int J Herb Med 4(4):27–30

Pant KK, Joshi SD (2008) Rapid multiplication of *Rauvolfia serpentina* Benth. ex. kurz through tissue culture. Sci World 6:58–62

Parsons J, Giulietti AM, Talou JR (2006) Effects of elicitation on solasodine production by *Solanum eleagnifolium* Cav. hairy root cultures. Acta Fam Bonaerense 25(3):414–420

Parvaiz M, Hussain K, Khalid S, Hussnain N, Iram N, Hussain Z, Ali MA (2014) A review: medicinal importance of *Glycyrrhiza glabra* L.(Fabaceae family). Global J Pharmacol 8:8–13

Patel H, Krishnamurthy R (2013) Elicitors in plant tissue culture. J Pharmacogn Phytochem 2(2):60–65

Poonam AS, Mishra S (2013) Physiological, biochemical and modern biotechnological approach to improvement of *Rauwolfia serpentina*. J Pharm Biol Sci 6:73–78

Putalun W (2011) Technology of compact MAb and its application for medicinal plant breeding named as missile type molecular breeding. Curr Drug Discov Technol 8:24–31

Putalun W, Wongwicha W, Tanaka H, Shoyama Y (2011) Enhanced glycyrrhizin production in *Glycyrrhiza inflata* hairy roots cultures using elicitation. Planta Med 77:PB14

Quadri LE, Giulietti AM (1993) Effect of elicitation on the accumulation of solasodine by immobilized cells of *Solanum eleagnifolium* Cav. Enzym Microb Technol 15:1074–1077

Rajendran S, Agarwal S (2007) Medicinal plants conservation through sacred forests by ethnic tribals of Virudhunagar district, Tamil Nadu. Indian J Tradit Knowl 6:328–333

Ray S, Majumder A, Bandyopadhyay M, Jha S (2014) Genetic transformation of sarpagandha (*Rauvolfia serpentina*) with *Agrobacterium rhizogenes* for identification of high alkaloid yielding lines. Acta Physiol Plant 36:1599–1605

Roberts MF (1988) Medicinal products through plant biotechnology. In: Robins RJ, Rhodes MJC (eds) Manipulating Secondary Metabolism in Culture. Cambridge: University Press, pp 201–216

Ru M, An Y, Wang K, Peng L, Li B, Bai Z, Wang B, Liang Z (2016) *Prunella vulgaris* L. hairy roots: culture, growth, and elicitation by ethephon and salicylic acid. Eng Life Sci 16:494–502

Sajjalaguddam RR, Paladugu A (2016) Influence of *Agrobacterium rhizogenes* strains and elicitation on hairy root induction and Glycyrrhizin production from *Abrus precatorius*. J Pharm Sci Res 8(12):1353–1357

Sarma D, Kukreja AK, Baruah A (1997) Transforming ability of two *Agrobacterium rhizogenes* strains in *Rauvolfia serpentina* (L.) leaves. Indian J Plant Physiol 2:166–168

Shabani L, Ehsanpour A, Asghari G, Emami J (2009) Glycyrrhizin production by in vitro cultured *Glycyrrhiza glabra* elicited by methyl jasmonate and salicylic acid. Russ J Plant Physiol 56:621–626

Shetty M, Harisha G, Jayanth Y, Kumar H (2014) Production of secondary metabolites from invitro cultures of *Rauwolfia serpentina* (L.) Benth. Int J Sci Res Eng Technol 2:844–852

Shibata S (2000) A drug over the millennia: pharmacognosy, chemistry, and pharmacology of licorice. J Pharm Soc Japan 120:849–862

Shilpha J, Satish L, Kavikkuil M, Largia MJV, Ramesh M (2015) Methyl jasmonate elicits the solasodine production and anti-oxidant activity in hairy root cultures of *Solanum trilobatum* L. Ind Crop Prod 71:54–64

Soleimani T, Keyhanfer M, Piri KH, Hsanloo T (2012) Morphological evaluation of hairy roots induced in *Artemisia annua* L. and investigating elicitation effects on hairy roots biomass production. Intl J Agric Res Rev 2:1005–1013

Song W, Qiao X, Chen K, Wang Y, Ji S, Feng J, Li K, Lin Y, Ye M (2017) Biosynthesis-based quantitative analysis of 151 secondary metabolites of Licorice to differentiate medicinal *Glycyrrhiza* species and their hybrids. Anal Chem 89:3146–3153

Srivastava A, Tripathi A, Pandey R, Verma R, Gupta M (2006) Quantitative determination of reserpine, ajmaline, and ajmalicine in *Rauvolfia serpentina* by reversed-phase high-performance liquid chromatography. J Chromatogr Sci 44:557–560

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

Sudha CG, Reddy BO, Ravishankar GA, Seeni S (2003) Production of ajmalicine and ajmaline in hairy root cultures of *Rauvolfia micrantha* Hook f., a rare and endemic medicinal plant. Biotechnol Lett 25:631–636

Tiwari R, Rana C (2015) Plant secondary metabolites: a review. Int J Eng Res Gen Sci 3(5):661–670

Torkamani MRD, Abbaspour N, Jafari M, Samadi A (2014) Elicitation of valerenic acid in the hairy root cultures of *Valeriana officinalis* L (Valerianaceae). Trop J Pharm Res 13:943–949

Tripathi L, Tripathi JN (2003) Role of biotechnology in medicinal plants. Trop J Pharm Res 2(2):243–253

Vanisree M, Chen YL, Shu-Fung L, Satish MN, Chien YL, HsinSheng T (2004) Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures. Bott Bull Acad Sin 45:1–22

Vasconsuelo A, Boland R (2007) Molecular aspects of the early stages of elicitation of secondary metabolites in plants. Plant Sci 172:861–875

Vazquez-Flota F, Moreno-Valenzuela O, Miranda-Ham MDL, Coello-Coello J, Loyola-Vargas V (1994) Catharanthine and ajmalicine synthesis in *Catharanthus roseus* hairy root cultures. Plant Cell Tiss Org Cult 38:273–279

Vazquez-Flota F, Carrillo-Pech M, Minero-Garcia Y, and De Lourdes Miranda-Ham M (2004) Alkaloid metabolism in wounded *Catharanthus roseus* seedlings. Plant Physiol Biochem 42:623–628

War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S, Sharma HC (2012) Mechanisms of plant defense against insect herbivores. Plant Signal Behav 7:1306–1320

Wongwicha W, Tanaka H, Shoyama Y, Putalun W (2011) Methyl jasmonate elicitation enhances glycyrrhizin production in *Glycyrrhiza inflata* hairy roots cultures. Z Naturforsch C66:423–428

Zhang HC, Liu JM, Lu HY, Gao SL (2009) Enhanced flavonoid production in hairy root cultures of *Glycyrrhiza uralensis* Fisch by combining the over-expression of chalcone isomerase gene with the elicitation treatment. Plant Cell Rep 28(8):1205–1213

Zhang W, Xie Z, Wang L, Li M, Lang D, Zhang X (2017) Silicon alleviates salt and drought stress of *Glycyrrhiza uralensis* seedling by altering antioxidant metabolism and osmotic adjustment. J Plant Res 130(3):611–624

Zhao J, Davis LC, Verpoorte R (2005) Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnology Adv 23(4):283–333

Zhao N, Wang G, Norris A, Chen X, Chen F (2013) Studying plant secondary metabolism in the age of genomics. Crit Rev Plant Sci 32:369–382

Zhao JL, Zou L, Zhang CQ, Li YY, Peng LX, Xiang DB, Zhao G (2014) Efficient production of flavonoids in *Fagopyrum tataricum* hairy root cultures with yeast polysaccharide elicitation and medium renewal process. Pharmacogn Mag 10:234–240

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(5):789