Chapter 3
Enhanced Efficiency of Medicinal and Aromatic Plants by PGPRs

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3.1 Introduction

Other than nutritional value for human and livestock, plants have gained significant attention in recent years due to their secondary metabolites, which are widely used in aromatic, therapeutic, or chemical industries. Higher plants use primary metabolites such as carbohydrates, lipids, and amino acids to synthesize various secondary metabolites that serve a variety of functions including plant defense against herbivores and microbes, protection against environmental stresses, and contribution to specific odors, tastes, and colors in plants (Seigler 1998). Plant secondary metabolites are unique sources for food additives, flavors, fragrances, and pharmaceuticals (Bennett and Wallsgrove 1994; Ravishankar and Rao 2000). Plants accumulate secondary metabolites mostly under stress conditions in response to various biotic and abiotic elicitors or signal molecules. Physiological traits and genetic diversity, environmental conditions, geographic variation, and evolution are among the main factors affecting the accumulation and composition of secondary metabolites (Figueiredo et al. 2008). Moreover, infection by microorganisms and abiotic factors such as osmotic stresses can induce particular secondary metabolite pathways in plants (Sanchez et al. 2004).

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Plant growth-promoting rhizobacteria (PGPRs) are a specific group of soil bacteria that aggressively colonize the rhizosphere and rhizoplane, and substantially improve plant growth and productivity. PGPRs function as plant growth promoters and biological control agents via direct or indirect mechanisms. Direct mechanisms by PGPRs include the provision of bioavailable phosphorus and nitrogen for plant uptake, sequestration of iron by siderophores, production of plant hormones like auxins, cytokinins, and gibberellins, and lowering ethylene levels inside plants using ACC deaminase that accumulate in plants subjected to biotic and abiotic stresses (Glick 1995; Glick et al. 1999; Mayak et al. 2004). The indirect mechanisms include the production of antibiotics, reducing iron availability for phytopathogens in the rhizosphere, enzymatic lysis of fungal cell wall and insect-gut membrane secreting chitinase enzyme for the hydrolysis of chitin layer of the eggshell of nematodes, competition with detrimental microorganisms for sites on plant roots, and induction of systemic resistance in plants against various pathogens and pests (Ramamoorthy et al. 2001). Bacterial strains showing PGPR activity have been reported for diverse bacterial taxa including *Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcus, Pseudomonas,* and *Serratia* (Gray and Smith 2005).

To date, PGPRs have been shown to promote the growth of cereals, ornamentals, vegetables, and food crops (Vessey 2003; Lugtenberg and Kamilova 2009; Mishra et al. 2010). However, a limited number of studies have been undertaken regarding the interactions between PGPRs and medicinal or aromatic plants. This chapter, therefore, aims to introduce proven or putative mechanisms by which PGPRs promote seed germination, growth, nutrient acquisition, and production of primary and secondary metabolites in aromatic and medicinal plants.

3.2 Seed Germination and Vigor Index in Medicinal Plants Under PGPRs Inoculation

Medicinal plants, native to the arid lands, often readily germinate within their native environment, while low germination rates have been observed under laboratory or field conditions (Gupta 2003). Recent advances in ex situ propagation methods, however, encourage the cultivation of these plants and reduce the pressure on their natural environment. Seeds and clones (produced by micro-propagation) are the most common means of propagation in medicinal plants. For the majority of species, seed is considered as the most effective and convenient propagation method.

Use of PGPRs as stimulants of seed germination in medicinal and aromatic species can provide more uniformity in germination, seedling emergence, and other growth stages in particular flowering, which is a critical time to achieve more bioactive secondary metabolites. PGPRs are able to increase the rate of seed

germination and seedling emergence and improve plant growth (Shaukat et al. 2006). The development stage of the plant organ (leaf, flower, and fruit ontogeny) can be a determinant factor for the composition of volatiles (Figueiredo et al. 1997; Badalamenti 2004). Several studies have reported increases in the yield of the volatiles from the flower bud to the mature flower. Concomitantly, the composition of secondary metabolites can undergo major changes, some components varying from traces to 10 % in the initial stages, and 50–70 % in the full flowering stage (Figueiredo et al. 2008). However, there are also reports indicating that the volatiles are largely accumulated before the organ is fully expanded (Figueiredo et al. 2008). Therefore, uneven or poor germination and subsequently inhomogeneous seedling growth can lead to the production of plants with variable content and composition of secondary metabolites.

Although PGPRs have been broadly used to improve seed germination and overall yield of many crops in different agro-ecosystems, there is a lack of literature on seed germination and vigor index in medicinal and aromatic species. Recently, the role of PGPRs on growth and phytochemical parameters, from seed germination to the mature flower stage, was evaluated in two types of medicinal plants containing different classes of secondary metabolites including alkaloids and essential oils (Ghorbanpour et al. 2013a, b, 2014). Inoculation of *Hyoscyamus niger* seedling radicles with 20 PGPR strains belonging to *Pseudomonas putida* (PP) and *P. fluorescens* (PF) on vigor index [seedling length (root length + shoot length) × germination %] under two conditions, in vitro (with agar media) or sand culture tubes, indicated that PGPRs can have contrasting effects on vigor index. The most efficient strains were shown to be those producing the optimum auxin level (PP-168 and PF-187). The PF-187 strain increased root and shoot elongation by 73 and 51 % compared with uninoculated controls, respectively. Moreover, two PP strains (PP-4 and PP-11) had negative effects on vigor index when compared with the uninoculated controls. Under both assay conditions, PF-187 and PP-168 strains were the most effective strains for early seedling development (Ghorbanpour and Hatami 2013). The fluorescent pseudomonads used had substantial effects on plant growth under various conditions particularly via auxin secretion. However, the production of this phytohormone at the amount beyond that is needed for plant produces additional levels of ACC, the immediate precursor of ethylene production, which significantly inhibits root elongation and decreases vigor index and plant growth (Fig. 3.1) (Glick et al. 1998).

Similar to *H. niger*, treatment of *Salvia officinalis* seeds by PGPRs including *P. fluorescens* (PF-23) and *P. putida* (PP-41, PP-108 and PP-159) differently affected the germination and vigor parameters (Ghorbanpour and Hatami 2014). The maximum (78.5 %) and minimum (16.75 %) germination percentages were recorded for PP-41 and PF-23 treatments, respectively. Also, the highest germination rate, root and shoot length, seedling vigor index, and the lowest mean germination time were recorded in seeds inoculated with PP-41, a strain with the ability to produce moderate auxin, when compared to other treatments (Fig. 3.2 and Table 3.1). According to the studies mentioned above, the net effect of plant–

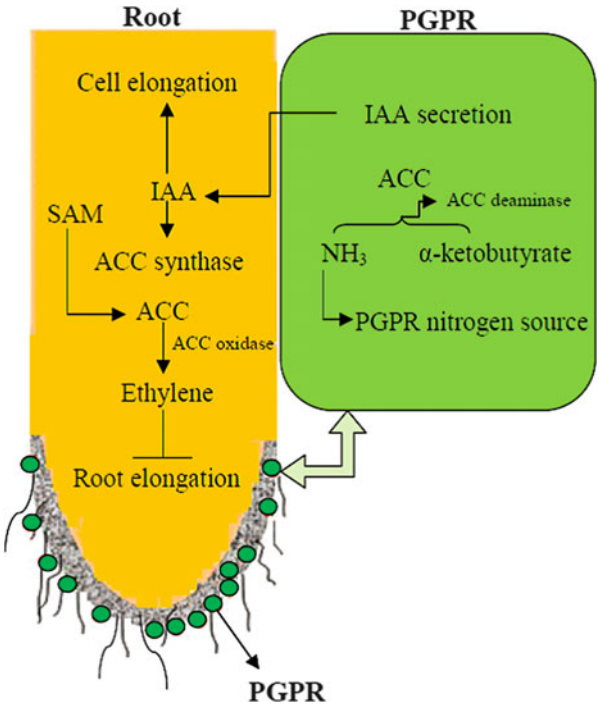

Fig. 3.1 Schematic model of PGPRs' effects on ethylene synthesis and inhibition of root elongation. *IAA* Indole acetic acid, *ACC* 1-aminocyclopropane 1-carboxylic acid, and *SAM* S-adenosylmethionine (Ghorbanpour and Hatami 2014)

PGPRs interactions on seed germination, root elongation, and subsequently vigor index could be positive, neutral, or negative.

Jahanian et al. (2012) studied the effects of seed inoculation of artichoke (*Cynara scolymus*) with different PGPR strains (*Azotobacter, Azospirillum, P. putida* PP-41, and PP-168) on seed germination and plant early growth characteristics. The combination of PP-168, *Azotobacter,* and *Azospirillum* strains was the most effective treatment in increasing the germination percentage, number of normal plants, radicle and shoot weight, shoot length, and vigority and in decreasing the mean time of germination. Moreover, either sole or the integrated application of phosphate-solubilizing bacteria along with nitrogen-fixing ones led to significant increases in radicle and shoot length, shoot weight, coefficient of velocity of germination, seedling vigor index, and significant decrease in mean germination time compared to uninoculated controls.

The PGPR strain P-35 with multiple PGPR activities (like IAA, ammonia, HCN, and catalase) was subjected to seed germination test for *Withania somnifera* plants. The results established a significant enhancement in seed germination rate as well as root and shoot growth of this valuable medicinal plant (Rathaur et al. 2012).

A commercial soil amendment containing a mixture of four PGPR strains (*Azospirillum lipoferum, Azotobacter chroococcum, P. fluorescens, and Bacillus megaterium*) was evaluated for impact on germination and initial growth of *Catharanthus roseus* (Lenin and Jayanthi 2012). The results indicated that

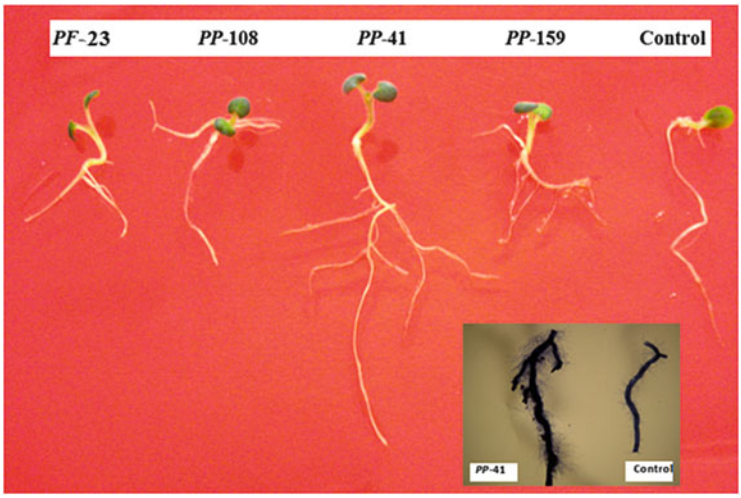

Fig. 3.2 Effects of *Pseudomonas putida* (PP-41, PP-108 and PP-159 strains) and *P. fluorescens* (PF-23 strain) on root morphology, root hair formation, and seedling vigor index in *Salvia officinalis* L. (Ghorbanpour and Hatami 2014)

Table 3.1 Seed germination behaviors and seedling vigor index in *Salvia officinalis* plants inoculated with *Pseudomonas putida* (PP-41 and PP-159) and *P. fluorescens* (PF-23) strains (Ghorbanpour and Hatami 2014)

PGPR strain treatment	Germination characteristics					
	Germination percentage (%)	Mean germination time (day)	Germination rate (seed/day)	Root length (cm)	Shoot length (cm)	Vigor index
Control	41.25^{c}	$7.75^{a, b}$	0.66^{c}	3.92^{c}	2.32^{c}	257.03^{c}
PP-41	78.50^{a}	4.25^{c}	1.05^{a}	8.45^{a}	4.20^{a}	992.13^{a}
PP-159	57.75^{b}	7.5^{b}	0.83^{b}	6.47^{b}	3.37^{b}	570.40^{b}
PP-23	16.75^{d}	9.75^{a}	0.17^{d}	2.25^{d}	1.87^{c}	69.63^{d}

In each column, values followed by different letters differ significantly ($P < 0.01$) according to Duncan's multiple range test

inoculation by PGPR strains significantly increased germination rate and vigor index. Harish Kumar and Maheshwari (2011) studied five bacterial strains (TR1–TR5) isolated from the root nodules of fenugreek (*Trigonella foenum-graecum*) for their plant growth promontory traits. The TR2 isolate was identified as *Rhizobium leguminosarum,* and the other four strains were *Ensifer meliloti*. The maximum increments in vigor index, nodule number, and root and shoot biomass were recorded for seeds inoculated with consortium (TR1 + TR2) followed by single inoculation as compared to uninoculated fenugreek plants. In addition, seed treatment of two *Acacia senegal* genotypes with *B. licheniformis* or *Sinorhizobium saheli,* either individually or in combination, had positive effects on the phenotypic traits of germination (Singh et al. 2011). However, inhibition of seed germination

has also been recorded when *Ambrosia artemisiifolia* seeds were inoculated with *P. fluorescens* (Vrbnicanin et al. 2011). Moreover, *P. fluorescens* has been classified as either deleterious rhizobacteria (DRB) (Zdor et al. 2005) or PGPR (Jaleel et al. 2007), depending on the experimental conditions in which bacterial cultures develop.

Growth promotion and beneficial effects conferred by PGPRs may involve various mechanisms of action. Direct growth promotion by PGPRs is regarded as one of the most important mechanisms of action, which include the production of plant growth regulators such as indole acetic acid (IAA) (Mishra et al. 2010), gibberellic acid (Narula et al. 2006), cytokinins (Castro et al. 2008), and ethylene (Saleem et al. 2007). The improved germination rate in plants inoculated by PGPRs (Nelson 2004) may be due to the increased synthesis of hormones like gibberellins, which would have triggered the activity of specific enzymes such as a-amylase that promote early germination by facilitating starch assimilation (Bharathi 2004). Moreover, significant increases in seedling vigor have been attributed to better synthesis of auxins (Bharathi 2004).

3.3 PGPRs Stimulate Plant Growth and Modify Enzyme Activities in Medicinal and Aromatic Plants Under Normal or Stress Conditions

Plant growth in the field is a reflection of diverse interactions with physiochemical and biological components that exist in the soil and modulated by environmental conditions. Microorganisms are a driving force for fundamental metabolic processes in soil. The genetic and functional diversities of the extensive microbial populations have a critical impact on soil function and plant growth (Nannipieri et al. 2003). Plant production and health are negatively affected by a large number of both biotic and abiotic stresses through the formation of reactive oxygen species (ROS). The induction of ROS-scavenging enzymes including superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT) is the most common mechanism for detoxifying ROS synthesized under stress conditions (Munns and Termaat 1986). To deal with these biotic and abiotic stresses, chemical inputs have been extensively used during the past few decades to achieve high yields, causing harmful effects on the environment. Sustainable agriculture needs to be further promoted as a key strategy to counteract the rapid decline in environmental quality via the gradual reduction in the use of chemical fertilizers and pesticides accompanied by greater use of the biological and genetic potential of plant species and microbial communities in order to gain sustainable high yields. The plant's ability to modify its physiology and metabolism to either avoid or partially overcome the environmental stresses can be improved by the aid of certain microorganisms existing in the rhizosphere (Govindasamy et al. 2008). Here, we introduce several detailed

mechanistic studies exploring the positive effects of PGPRs on medicinal plants under normal or stress conditions.

The effects of inoculation with PGPR strains *P. putida* (PP-168) and *P. fluorescens* (PF-187) on growth parameters, proline and chlorophyll content, leaf relative water content (RWC), as well as antioxidant enzymes activity (SOD, POX, and CAT) were investigated in *Hyoscyamus niger* under three water-deficit stress (WDS) levels of 30 % (W1), 60 % (W2), and 90 % (W3) water depletion of field capacity (Ghorbanpour et al. 2013a). Inoculation with PP and PF strains minimized the deleterious effects of WDS on growth parameters, whereas uninoculated plants had a grave reduction in plant growth. The number of leaves, leaf area, and leaf greenness decreased with the increase in water stress levels, but PP- and PF-inoculated plants had lower reduction percentages compared to uninoculated control plants. The greatest accumulation of proline was found in PF-inoculated plants against severe WDS. In contrast, proline accumulation in PP-inoculated plants and in uninoculated control plants was observed only up to the W2 treatment level, and it later started to decline, particularly in the uninoculated control plants. Furthermore, inoculation with PP and PF strains significantly improved the chlorophyll content of plants. The results also unearthed that the RWC was significantly higher in plants subjected to PP and PF strains under all WDS conditions than their respective controls. This effect may be associated with the hydraulic nature of branch root junctions, which facilitate the radial flow of water (Kothari et al. 1990). The advantageous effects of PGPRs and common adaptation mechanisms of plants exposed to WDS are always mutually related to exceptional changes in root morphological and anatomical traits such as root branching networks and biomass (Fig. 3.3). The PF strain had higher efficiency than the PP strain in plants growing under moderate (W2) and severe (W3) WDS conditions. This outstanding capacity might be linked with the geographical origin of the PF strain as it was isolated from the wheat rhizosphere in rainfed wheat fields (dry land farming), where water is restricted and dry periods often take place. In contrast, the PP strain was isolated from wheat rhizosphere in irrigated wheat fields showing no phosphate-solubilizing activity and inferior performance under limited water supply compared to the PF strain (Ghorbanpour et al. 2013a).

Thus, it can be concluded that selection of PGPR strains should be based on multiple plant growth-promoting characteristics and their ecological adaptation with respect to the potential abiotic stresses of the host plant.

Inoculation with PGPRs can stimulate the activities of antioxidant enzymes and increase proline accumulation under drought stress conditions (Kohler et al. 2008) and induce systemic resistance against fungal plant diseases through the activation of various defense-related enzymes (Bharathi 2004). The activities of SOD and POX in root and leaf tissues of *Hyoscyamus niger* plant increased to a significant extent after inoculation with PP and PF strains (and with WDS treatment as well), while CAT activity decreased with increasing WDS, except in PF-inoculated plants (Ghorbanpour et al. 2013a). This is in keeping with a report by Kohler et al. (2008), suggesting that the overexpression of SOD, if accompanied by enhanced

Fig. 3.3 Effects of seed inoculation with *Pseudomonas putida* (PP-168) and *P. fluorescens* (PF-187) on root and shoot growth in *Hyoscyamus niger* (Ghorbanpour et al. 2013a)

H_2O_2-scavenging mechanisms like CAT and POX activities, is an important antidrought mechanism to cope with oxidative stress during WDS conditions. The effects of water stress and PGPR strains *Pseudomonades* sp., *B. lentus,* and *Azospirillum brasilense* were assessed on proline, soluble carbohydrates, chlorophyll, and mineral content in basil (*Ocimum basilicum* L.) plants (Heidari et al. 2011). The proline and soluble carbohydrate content increased significantly with increasing water stress in plants inoculated with *Pseudomonas* sp. and *B. lentus*, respectively. The chlorophyll content was also increased in all plants inoculated with the PGPR strains.

PGPR-mediated resistance has been documented against certain biotic stresses. Bacterization of seeds of cucumber plants with different PGPR strains resulted in enhanced growth and efficiency, and also induced resistance against bacterial wilt, which is caused by *Erwinia tracheiphila* and transmitted by a beetle vector (Zehnder et al. 2001). In this case, the induced systemic resistance was attributed to a reduction in cucurbitacin (a secondary metabolite stimulating the beetle feeding) and induction of other plant defense mechanisms.

The general mechanisms by which PGPRs enhance plant growth and productivity are given in Fig. 3.4 which are as follows: (1) producing plant growth regulators including IAA (Mishra et al. 2010), gibberellic acid (Narula et al. 2006), cytokinins and analogs (Castro et al. 2008), jasmonate, salicylate, and volatile growth stimulants such as ethylene and 2,3-butanediol (Saleem et al. 2007; Vessey 2003); (2) producing ACC deaminase (1-amino-cyclopropyl-carboxylic acid) to reduce the ethylene levels in the roots of developing plants (Dey et al. 2004); (3) asymbiotic nitrogen fixation (Ardakani et al. 2010); (4) exhibition of antagonistic activity against plant pathogens by producing iron-chelating

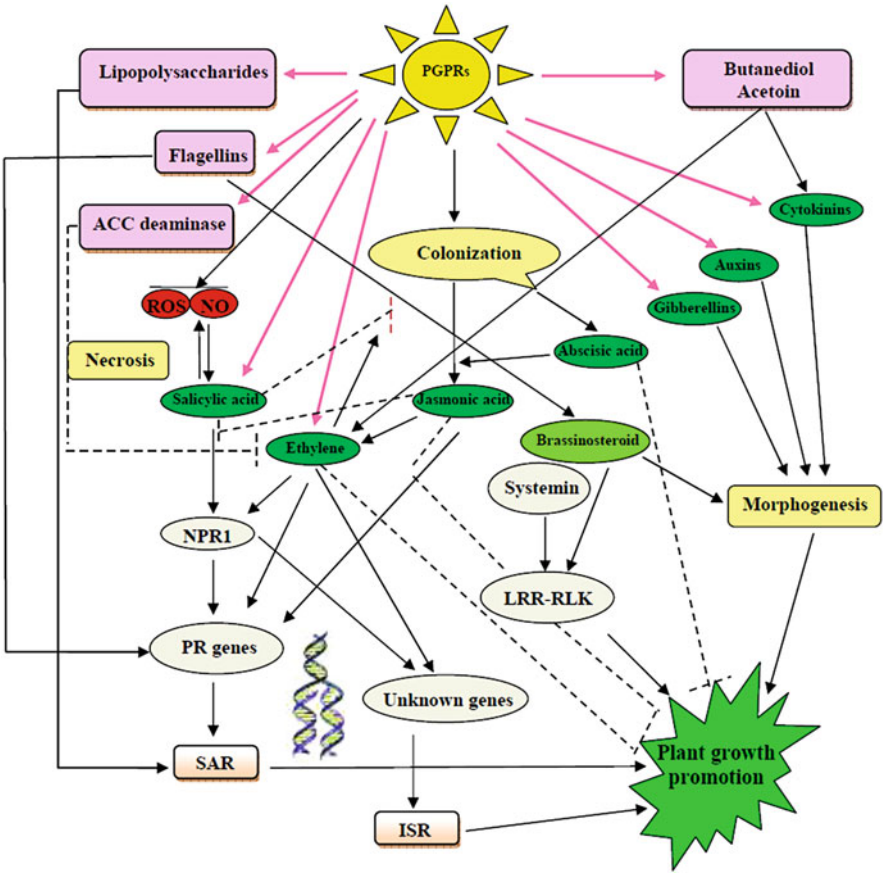

Fig. 3.4 A diagram of signaling cascades involved in plant growth promotion by PGPRs. *Thick pink arrows* indicate secretions or bioactive components from the PGPRs. *Dark green ovals* are phytohormones; *pink boxes* show the exudate elicitors in the signaling cascades; *solid black arrows* represent active signaling pathways; *broken lines* indicate inhibitory effects [modified from Ping and Boland (2004)]. *Abbreviations*: *ISR* induced systemic resistance, *SAR* systemic acquired resistance, *NO* nitric oxide, *NPR1* nonexpressor of PR genes, *PR* pathogenesis-related proteins, *ROS* reactive oxygen species, *LRR-RLK* leucine-rich repeat receptor-like kinases, *ACC* 1-Aminocyclopropane-1-carboxylate deaminase

siderophores, ß-1,3-glucanase and chitinase enzymes, antibiotics, fluorescent pigments, and cyanide (Pathma et al. 2011); and (5) solubilization of phosphate and other nutrients (Hayat et al. 2010). PGPRs may simultaneously apply a combination of these mechanisms to improve the plant's performance (van Loon 2007; Martinez-Viveros et al. 2010). However, regardless of the type of mechanism involved, PGPRs must colonize the rhizosphere or root itself (Glick 1995).

Lenin and Jayanthi (2012) observed that root inoculation of *Catharanthus roseus* with different PGPR strains (*Azospirillum lipoferum, Azotobacter chroococcum, P. fluorescens,* and *B. megaterium*) increased chlorophyll and nutrient (N, P, and K) content. They concluded that PGPRs in combination have a greater potential to increase plant growth, nutrient uptake, and yield. Similarly, vegetative growth and chemical composition in *Catharanthus roseus* were promoted by the combined treatment of *Azotobacter* and phosphate-solubilizing bacteria (Attia and Saad 2001). Together, inoculation with diverse PGPR strains can contribute to maintaining good soil health and fertility in order to achieve sustainable high yields and high-quality products.

Arbuscular mycorrhizal (AM) fungi interact synergistically with other soil microorganisms such as nitrogen-fixing bacteria (Barea and Azcon-Aguilar 1983), phosphate-solubilizing bacteria (Villegas and Fortin 2002), and biocontrol agents (Abdel-Fattah and Mohamedin 2000) to favor plant growth. This interaction could be direct by providing niche and/or habitat or indirect by modifying physiology of the host plant (Bianciotto et al. 2000; Walley and Germida 1997). PGPRs in combination with other beneficial microorganisms such as AM fungi can induce plants to produce certain metabolites making the rhizosphere a more suitable environment for their stay (Dutta and Podile 2010). Certain PGPRs have been reported to enhance the activity of AM fungi and plant growth, consequently (Jayanthi et al. 2003). It appears that PGPRs and AM fungi establish mutually beneficial relationships in rhizosphere to support their co-existence and promote the plant's performance.

Investigations into the interaction between the AM fungus *G. aggregatum* and the PGPR strain *B. coagulans* and *T. harzianum* in soil and their consequent effects on growth, nutrition, and enzyme activity of *Solanum viarum* seedlings demonstrated that plant biomass and nutrient (P, Fe, Zn, Cu, K, and Mn) content were maximum in the plants co-inoculated with all three microorganisms (Hemashenpagam and Selvaraj 2011). The positive effects were probably due to the enhanced mycorrhizal colonization resulting in efficient nutrient uptake. The results also showed that acid phosphatase, alkaline phosphatase, and dehydrogenase activities in the root zone soil of all the inoculated seedlings were significantly higher compared to those in uninoculated control plants. Moreover, root zone soil of plants co-inoculated with all three microbes had higher *B. coagulans* population suggesting the stimulatory effect and synergistic activity between the organisms involved. Here, the mycorrhiza helper bacteria enhanced the activity of *G. aggregatum* presumably by producing organic acids which serve as a carbon source to the fungus or by producing hydrolytic enzymes enabling the AM fungus to penetrate and ramify in the root system of the host plant (Lakshmipathy

et al. 2002; Selvaraj et al. 2008). In another study, inoculation of pot marigold (*Calendula officinalis* L.) seeds with PGPR strains (*Azotobacter, Pseudomonas,* and *Azospirillum*) and the AM inocula substantially increased growth parameters, root and shoot dry weight, photosynthetic pigments (chlorophyll a and b, carotene, and xanthophylls), and the content of N, P, and K in leaves and roots (Hosseinzadah et al. 2011).

Kohler et al. (2008) investigated the effects of inoculation with the PGPR strain *P. mendocina* Palleroni, alone or in combination with an AM fungus (*G. intraradices* or *G. mosseae*), on activities of antioxidant enzymes (SOD, CAT, and POX), phosphatase and nitrate reductase, and solute accumulation in leaves of *Lactuca sativa* L. cv. Tafalla under different levels of water stress. At moderate drought, PGPR and AM inoculation with *G. intraradices,* alone or in combination, significantly stimulated the nitrate reductase activity. At severe drought, inorganic fertilization and *P. mendocina* inoculation, alone or in combination with either of the selected AM fungi, significantly increased phosphatase activity in lettuce roots and proline accumulation in leaves. Inorganic fertilization and combined treatment of PGPR with either AM fungus showed the highest values of leaf POX activity under severe drought conditions. The highest CAT activity was recorded in the fertilized plants inoculated by *P. mendocina* grown under severe stress conditions. These results highlight the potential capacity of PGPRs to alleviate the oxidative damage produced under WDS (Kohler et al. 2008). Similarly, Liddycoat et al. (2009) demonstrated the remarkable effects of PGPRs on plant vigor and productivity under stress conditions. The effects of PGPRs (*Pseudomonas* sp.) treatment on 3-week-old seedlings and seeds of two asparagus (*Asparagus officinalis* L.) cultivars (Guelph Millennium and Jersey Giant) were studied. According to the results, single inoculation of seedlings resulted in positive growth response under optimal and drought stress for both cultivars tested. Seed inoculation led to enhanced growth for Guelph Millennium under optimal conditions, while no positive response was observed for the Jersey Giant cultivar under either normal or stress treatments.

The literature noted above highlights the key role of PGPRs to improve nutrition and productivity in various plants with therapeutic and industrial significance. The potential biofertilizer activities of PGPRs could be divided into five distinct areas including biological N_2 fixation, increasing the nutrient availability in rhizosphere, increasing the root surface area, enhancing beneficial symbioses of the host plant, and finally the combinations of all these mechanisms (Bhattacharyya and Jha 2012). However, the effectiveness of PGPRs–plant interactions depends on soil biological components, the genetic and physiological properties of the organisms involved, and their adaptation to the existing ecosystem-related constraints.

3.4 PGPRs Function as Biotic Elicitors in the Biosynthesis of Plant Secondary Metabolites

Plant secondary metabolites are unique sources for pharmaceuticals, fragrances, flavors, food additives, and other industrially important compounds. The major roles of plant secondary metabolites are to protect plants from attack by insect pests, herbivores, and phytopathogens or to help plants survive other biotic and abiotic stresses. The environmental stresses (microbial, physical, or chemical factors) can function as biotic/abiotic elicitors leading to an increase in the production of secondary metabolites (Radman et al. 2003; Ghorbanpour et al. 2013a, b, 2014). The biotic elicitors have biological origin and are derived from microorganisms such as fungi, bacteria, viruses, or plant cell wall components and chemicals that are released by plants against phytopathogens or herbivore attack. Thus, elicitors could be employed for improving the production of plant valuable secondary metabolites (Namdeo 2007).

Rhizosphere microbes such as PGPRs are best known as biotic elicitors, which have the potential to induce the synthesis of secondary metabolites in plants. Below, we summarize some of the recent studies dealing with the major role of PGPRs to improve the production of secondary metabolites in plants. The effects of PGPR strains *P. putida* (PP-168) and *P. fluorescens* (PF-187) were studied on the root and shoot content and yield of two tropane alkaloids hyoscyamine (HYO) and scopolamine (SCO) in black henbane (*Hyoscyamus niger*) under different WDS levels (30, 60, and 90 % water depletion of field capacity; W1, W2, and W3, respectively) at vegetative, full flowering, and seed-ripening stages (Ghorbanpour et al. 2013a, b). The SCO content of roots in PGPR-inoculated and uninoculated control plants increased significantly with increasing WDS up to W2 treatment, and later it started to decline, except for PF-inoculated plants, which kept an upward trend continuously. The highest root SCO content was observed in the PF-inoculated plants under W3 conditions. Also, the maximum root HYO content was observed in W3 treatment, where both PP and PF strains had identical effects in this regard. In shoots, however, HYO content significantly increased with increasing WDS in both PGPR treatments. The SCO content of shoots in all employed treatments had same changes as root, and was mildly increased with increasing WDS only under PF treatment. Inoculation of *H. niger* plants with the PF strain promoted HYO and SCO accumulation in both root and shoot organs. Almost the same trend was observed for alkaloid yield in both tissues under all employed treatments. Although shoot HYO yield decreased with increasing WDS in both PGPR-inoculated and uninoculated control plants, the reduction percentage in PGPR treatments was lower than uninoculated controls. Shoot SCO yield also decreased with increasing WDS in PP-inoculated and uninoculated control plants, but in plants inoculated with the PF strain showed unchanged. The largest total alkaloids (HYO + SCO) yield belonged to the PP treatment under W1 conditions. Accordingly, it can be concluded that an integrative use of effective PGPRs (biotic elicitor) and WDS (abiotic elicitor) could be an encouraging and eco-friendly strategy for increasing

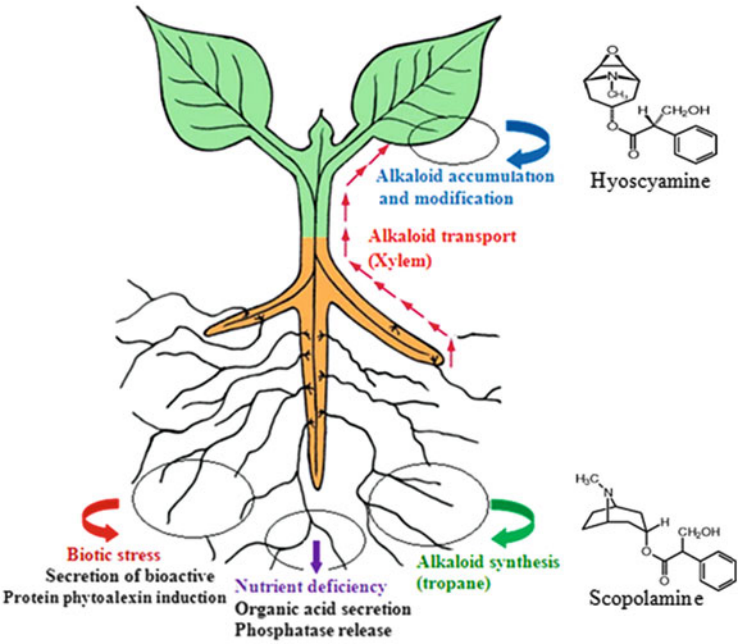

Fig. 3.5 Diagram of a generic tropane alkaloid-producing plant like *Hyoscyamus niger*. The diagram highlights the key role of plant roots in determining the spatial and temporal patterns of bioactive secondary metabolites such as hyoscyamine and scopolamine synthesis in response to biotic and abiotic (nutrient deficiency) stresses

the yield and content of these two alkaloids in *H. niger* organs (Ghorbanpour et al. 2013a). Furthermore, PP-inoculated plants under W1 conditions had higher proportion of fine roots compared to other treatments. Plant fine roots without secondary growth have been found to be the principal site for production of tropane alkaloids and the location for main enzymes involved in their biosynthesis pathway (Suzuki et al. 1999). Although root is known to be the location for the biosynthesis of tropane alkaloids in Solanaceae, these alkaloids may be transported through the xylem to the aboveground parts of the plant (Figs. 3.5 and 3.6).

Higher plants activate various defense mechanisms when attacked by microbial pathogens such as fungi, bacteria, or viruses. These defense responses include suicide of the attacked host cell (hypersensitive response); the production of antimicrobial secondary metabolites (phytoalexins); the production of pathogenesis-related (PR) proteins with potential antimicrobial properties; and the production and oxidative cross-linking of cell wall polymers (Penninckx et al. 1998). The effective defense system is a result of a synchronized expression of a series of these defense responses (Ayers et al. 1976). Ghorbanpour et al. (2013a, b) observed that *Hyoscyamus niger* plants inoculated with PGPRs had higher values of HYO than SCO, which could be due to the high antimicrobial activity of HYO. Abdel-Motal et al. (2009) investigated the antifungal activity of HYO and SCO against

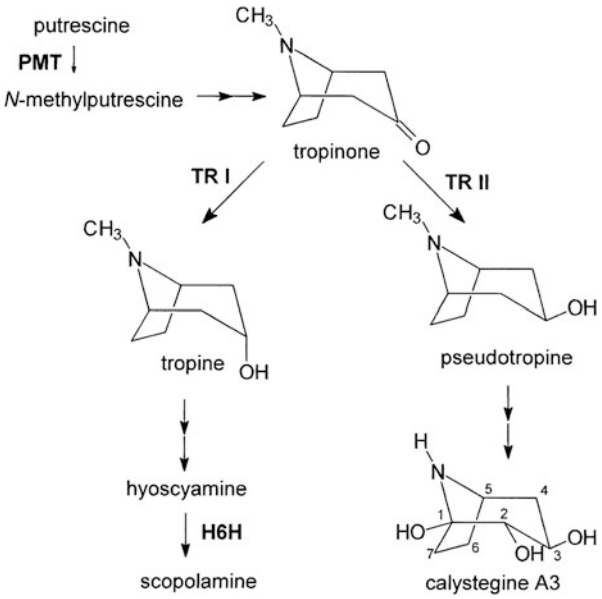

Fig. 3.6 Biosynthetic steps for tropane alkaloids. *PMT* putrescine N-methyltransferase; *TR I* tropine-forming tropinone reductase; *TR II* pseudotropine-forming tropinone reductase; *H6H* hyoscyamine-6-Hydroxylase

40 fungal strains associated with *Hyoscyamus muticus* and found that all fungal strains were tolerant to SCO but sensitive to HYO.

The essential oils (EOs) production can also be positively affected by PGPRs. Treatment of cuttings and foliage of *Salvia officinalis* plants with PGPR strains *P. fluorescens* (PF-23) and *P. putida* (PP-41 and PP-159) significantly affected the EOs content, yield, and composition (Ghorbanpour et al. 2014). The highest (3.95 g/plant) and lowest (1.22 g/plant) EOs yields were observed for PP-159 and uninoculated plants, respectively. Plants inoculated with PP-159 or PP-41 showed significant increases in total EOs yield of 69.1 and 68.5 % compared to uninoculated controls, respectively. The increases in total essential oil yield in response to PGPRs inoculation were due to both increased plant dry weight and the biosynthesis of terpenes. The increased EOs yield was associated with a significantly larger density of trichomes, the main structure for EOs secretion (Fig. 3.7). Totally, 27 different compounds were identified in the EOs of *Saliva officinalis* plants under all employed treatments. Inoculation with PGPRs (PP-159 in particular) stimulated the production of certain monoterpenes such as *cis*-thujone, camphor, and 1,8-cineol.

Essential oils serve important ecological roles. The reported increases in the synthesis of EOs can be considered as a defensive response to colonization by harmful microorganisms, since several EOs exhibit antimicrobial properties (Sangwan et al. 2001). The EOs compounds rich in *cis*-thujone, camphor, and 1,8-cineole (eucalyptol) are well-known chemicals with strong antimicrobial activity against different pathogenic bacteria (Tzakou et al. 2001; Cha et al. 2005). Ghorbanpour et al. (2014) proved that the EOs of *Saliva officinalis* plants under

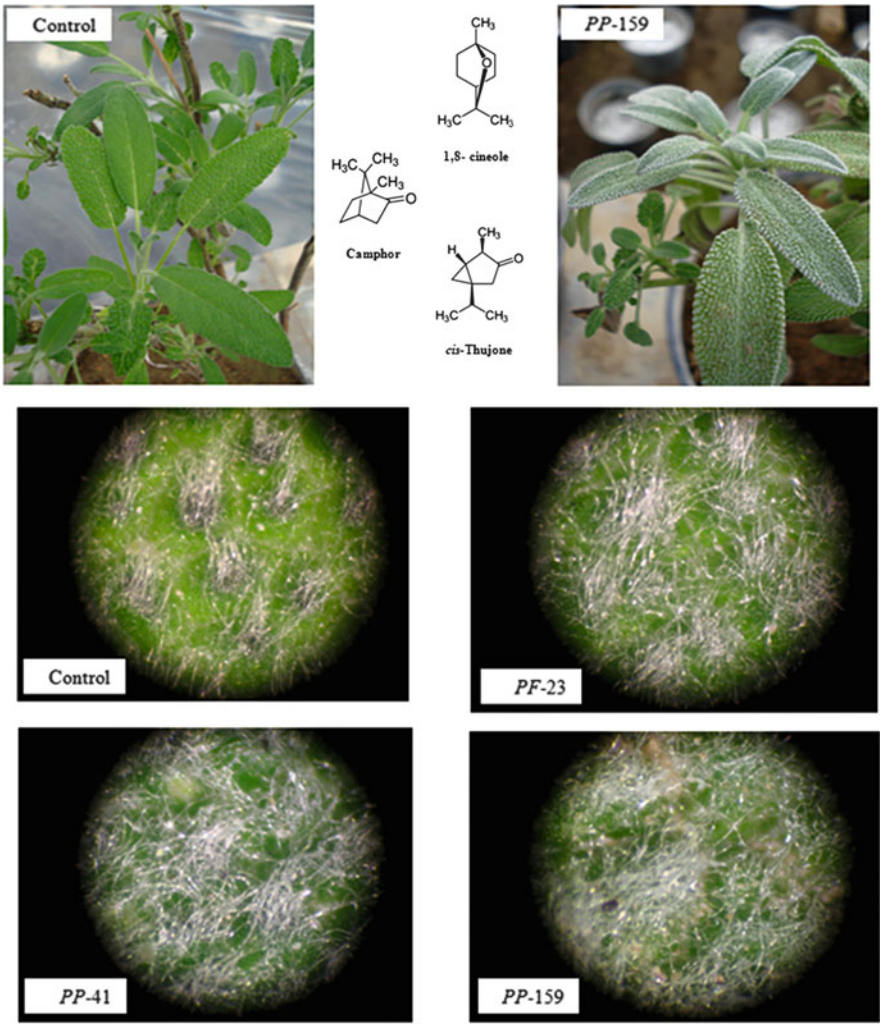

Fig. 3.7 Effect of foliar application of *Pseudomonas putida* (PP-41 and PP-159 strains) and *P. fluorescens* (PF-23 strain) on density of trichomes in *Salvia officinalis* plants grown in pot cultures under greenhouse conditions (Ghorbanpour et al. 2014)

PGPRs treatment (PP-159) have strong antibacterial activity (disc diffusion) against the test pathogenic microorganisms including gram-positive (*Staphylococcus aureus, S. epidermidis, Enterococcus faecalis,* and *Streptococcus agalactiae)* and gram-negative (*Escherichia coli*) bacteria (Tables 3.2 and 3.3). The EOs obtained from PP-inoculated plants showed the maximum antibacterial activity with 23.44-mm inhibition zone against *Staphylococcus aureus*. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values for *Escherichia coli* were 5 and 10 µl for EOs obtained from control plants, while

Table 3.2 Antibacterial activity of *Salvia officinalis* essential oils against test microorganisms in plants inoculated with a PGPR strain (PP-159) and uninoculated control plants (Ghorbanpour et al. 2014)

Pathogenic bacteria	Inhibition zone (mm)		EOs (μL) in 5 ml of pathogen suspension							
			EOs of control plants				EOs of PP-159-inoculated plants			
	EOs of control plants	EOs of PP-159 treated plants	5	10	20	30	5	10	20	30
Staphylococcus aureus	19.54 ± 1.61	23.44 ± 1.74	−	+	+ +	+ + +	+	+ +	++ +	++ ++
Staphylococcus epidermidis	14.32 ± 1.42	18.36 ± 1.14	−	+	+ +	+ + +	−	+	++	++ +
Escherichia coli	8.65 ± 0.76	11.78 ± 0.65	−	−	−	+	−	−	+	++
Enterococcus faecalis	12.62 ± 0.98	13.54 ± 1.12	−	+	+ +	+ + +	−	+	++	++ +
Streptococcus agalactiae	11.23 ± 1.01	10.42 ± 0.93	−	+	+ +	+ + +	−	+	++	++ +

Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm), − (negative) = 0 mm; + (weak) = 1–4 mm; ++ (moderate) = 5–10 mm; +++ (strong) = 10–15 mm and ++++ (very strong) ≥16 mm

Table 3.3 Antibacterial activity of *Salvia officinalis* essential oils (MIC and MBC, μg/ml) against test microorganisms in plants inoculated with a PGPR strain (PP-159) and uninoculated plants using the disc diffusion method (Ghorbanpour et al. 2014)

Pathogenic bacteria	EOs of control plants		EOs of PP-159-inoculated plants	
	MIC	MBC	MIC	MBC
Staphylococcus aureus	2	4	1	2
Staphylococcus epidermidis	3	5	3	4
Escherichia coli	5	10<	3	6
Enterococcus faecalis	3	4	2	4
Streptococcus agalactiae	3	6	4	6

MIC minimal inhibitory concentration; *MBC* minimal bactericidal concentration

these values were 3 and 6 μl for plants inoculated with PP-159, respectively (Table 3.3).

Banchio et al. (2008) studied the effects of PGPR strains *P. fluorescens, B. subtilis, Sinorhizobium meliloti, and Bradyrhizobium* sp. on qualitative and quantitative composition of EOs in *Origanum majorana*. The results demonstrated that inoculation with PGPRs can increase the production of certain terpenes. Plants

inoculated with *Bradyrhizobium* sp. or *P. fluorescens* showed significant increases in total EOs yield by 10- and 24-fold, respectively. Based on the results, they suggested that increases in total EOs yield in response to inoculation were not merely due to increased biomass, and might have resulted from increased biosynthesis of terpenes. The main compounds affected by inoculation with *P. fluorescens* were terpinene-4-ol, *cis*-sabinene hydrate, *trans*-sabinene hydrate, and a-terpineol as the concentrations of these compounds in inoculated plants were >1,000-fold higher than uninoculated controls. Furthermore, the lack of effect of *B. subtilis* and *S. meliloti* strains tested was attributed to their poor adaptation to root exudates and/or insufficient root colonization.

The synergistic effects of combined inoculation of PGPRs with AM fungi have been reported on the production of EOs in plants. According to Prasad et al. (2012), the chemical composition of geranium oil was significantly affected by inoculation with phosphate-solubilizing bacteria (PSB) and/or AM fungi and phosphate fertilization. The content of linalool, geranial, 10-epi-γ-eudesmol, and citronellol in geranium oil increased and that of *cis*- and *trans*-rose oxide decreased by inoculation with PSB alone or in combination with AM fungi as compared to uninoculated controls. The changes in various constituents in the EOs of all inoculated and fertilized geranium plants could be related to the enhanced uptake of P and divalent metallic cations in plant tissues (Prasad et al. 2012).

In a study by Vafadar et al. (2013), tissue culture-regenerated plantlets of *Stevia rebaudiana* Bertoni were transferred to pots and subsequently inoculated with PGPR strains (*B. polymyxa, P. putida,* and *Azotobacter chroococcum*) and an AM fungus (*G. intraradices*). Although inoculation with a single microorganism significantly increased the stevioside content, the highest stevioside value was obtained in plants dually inoculated with *G. intraradices* + *Azotobacter chroococcum,* followed by *G. intraradices* + *B. polymyxa* and *Azotobacter chroococcum* + *P. putida*. Triple inoculations had less positive effects compared to dual inoculations, probably due to higher competition between microorganisms (Vafadar et al. 2013).

The root system of Italian oregano (Origanum × majoricum) was subjected to inoculation with three PGPR strains (*P. fluorescens, B. subtilis,* and *Azospirillum brasilense*), and the EOs content was measured (Banchio et al. 2010). The total EOs yield for plants inoculated with *P. fluorescens* or *Azospirillum brasilense* was approximately 2.5-fold higher than controls, without change of quantitative oil composition. The major EOs compounds *cis*- and *trans*-sabinene hydrate, γ-terpinene, carvacrol, and thymol showed increased biosynthesis.

Nonpathogenic PGPRs have been shown to stimulate the biosynthesis of secondary metabolites in plants through a mechanism termed ISR (induced systemic resistance) (Van Loon and Glick 2004). It is well established that biological agents can act as effective elicitors of key enzymes involved in biosynthetic pathways of secondary metabolites (Chen et al. 2000), which are clearly related to plants' defense responses against pathogenic agents despite being induced by nonpathogenic bacteria (Kloepper 1993).

Biosynthesis of terpenoids depends on primary metabolism, e.g., photosynthesis, and oxidative pathways for carbon and energy supply (Singh et al. 1990).

Giri et al. (2003) found that net photosynthesis of PGPRs hosting plants increases as a result of improved nutritional status. Factors linked with increased dry matter production may influence the interrelationship between primary and secondary metabolism, leading to increased biosynthesis of secondary metabolites (Shulka et al. 1992). The increased plant biomass appears to be correlated with a greater availability of substrate for monoterpene biosynthesis (Harrewijn et al. 2001). The increased concentration of monoterpenes in PGPR-inoculated plants may be due to growth-promoting substances produced by the microorganisms, which affect metabolic pathways in plants.

The effect of combined inoculation of *Begonia malabarica* Lam. (Begoniaceae) plants by an AM fungus (*G. mosseae*), a PGPR strain (*B. coagulans*), and *T. viride* was studied on the production of secondary metabolites (Selvaraj et al. 2008). Plants inoculated with microbial consortium consisting of *G. mosseae* + *B. coagulans* + *T. viride* showed the highest increase in leaf secondary metabolites (total phenols, ortho dihydroxy phenols, flavonoids, alkaloids, and tannins) followed by the plants dually inoculated with *G. mosseae* + *B. coagulans*.

In a similar study, the effects of the AM fungus *G. aggregatum,* the PGPR strain *B. coagulans,* and *T. harzianum* were evaluated on secondary metabolites content of *Solanum viarum* seedlings (Hemashenpagam and Selvaraj 2011). Triple inoculation of *G. aggregatum* + *B. coagulans* + *T. harzainum* resulted in maximum secondary metabolites including total phenols, orthodihydroxy phenols, flavonoids, alkaloids, saponins, and tannins. Here, the higher secondary metabolites values in inoculated plants were attributed to the enhanced mycorrhizal colonization and improved nutrient status of the host plants.

Cappellari et al. (2013) investigated the effects of single inoculation and co-inoculation with two PGPR strains (*P. fluorescens* and *Azospirillum brasilense*) on EOs composition and phenolic content in Mexican marigold (*Tagetes minuta*) and observed that EOs yield increased by 70 % in *P. fluorescens*-inoculated and co-inoculated plants in comparison with uninoculated controls, without altering the EOs composition. The biosynthesis of major EOs components increased in inoculated plants. The total phenolic content was two-fold higher in singly inoculated or co-inoculated treatments than in uninoculated control plants. Accordingly, they suggested that considering the economic importance of monoterpenes and phenolic compounds for a variety of applications in food and cosmetic industries, *P. fluorescens* and other suitable PGPRs have clear potential for improving EOs yield and productivity of cultivated medicinal plants.

Employing microorganisms as coculture in biotization has been another important area of research in recent decades. Biotization is a metabolic response of in vitro-grown plant materials to microbial inoculants leading to developmental and physiological changes of the derived propagules, which enhances resistance against biotic and abiotic stresses in plants. Here, plantlets are usually cocultured with PGPRs to achieve higher biomass and secondary metabolites. For instance, *Origanum vulgare* L. plantlets cocultured with *Pseudomonas* spp. produced more phenolics and chlorophyll than nonbacterized control plants (Nowak 1998).

The use of biotic elicitors is one of the effective strategies to increase the production of important secondary metabolites in plants. Secretions or bioactive

components from PGPRs (Fig. 3.4), besides being involved in plant growth promotion, are the components that were found to work in an elicitor signal transduction network. On the other hand, indole acetic acids (IAAs), cytokinins (CTKs), gibberellins (GAs), brassinosteroids (BRs), salicylic acid (SA), jasmonic acid (JA or analogs), ethylene, abscisic acid (ABA), nitric oxide (NO), and ROS which increase plant immunity by activating defense pathways, have long been observed to be transducers of elicitor signals in the production of plant secondary metabolites. Multiple signaling pathways and important mechanisms of action of elicitors in the biosynthesis of plant secondary metabolites are shown in Fig. 3.8. Signal perception is regarded as the first committed step of the elicitor signal transduction pathways in plants. Following perception, plant receptors are activated initially, and then in turn they activate their effectors such as ion channels, GTP-binding proteins (G-proteins), and protein kinases. The activated effectors transfer the elicitor signals to secondary messengers, which further amplify the elicitor signal to other downstream reactions (Ebel and Mithoefer 1998; Blume et al. 2000). The sequentially occurring events and reactions in elicitor-induced defense pathways can be organized as follows: perception of elicitor by a receptor, reversible phosphorylation and dephosphorylation of plasma membrane proteins and cytosolic proteins, cytosolic [Ca^{2+}] cyt spiking, plasma membrane depolarization, Cl^- and K^+ efflux/H^+ influx, extracellular alkalinization and cytoplasmic acidification, mitogen-activated protein kinase (MAPK) activation, NADPH oxidase activation and ROS production, early defense gene expression, ethylene and jasmonate production, late defense response gene expression, and accumulation of secondary metabolites (Zhao et al. 2005).

Different molecules produced by PGPRs play a crucial role in pathways linked to the biosynthesis of secondary metabolites. Salicylic acid (SA) is a well-known inducer of plant's systematic acquired resistance (SAR) in plant–microbe interactions through inducing expression of genes related to the biosynthesis of certain classes of secondary metabolites in plants (Taguchi et al. 2001). For example, indole alkaloids can be induced in *C. roseus* cell cultures by acetylsalicylic acid, an analog of SA (Zhao et al. 2000). Nitric oxide (NO), besides its effects on root branching and architecture, serves as a signal molecule for plant growth, development, and defense (Neill et al. 2002). Transcriptional profiling studies showed that NO treatment induces some stress- and disease-related signal transduction component genes along with defense genes, implying that the NO signal pathway(s) could be involved in secondary metabolism (Aziz et al. 2003). In addition, fungal elicitors were shown to stimulate saponin production in ginseng cell cultures, and this is partially mediated by NO, with NO biosynthesis also being induced by the fungal elicitor (Hu et al. 2003).

Exposure of plant cell culture or intact plant to jasmonic acid (JA), methyl jasmonate, as well as their conjugated compounds can stimulate the biosynthesis of secondary metabolites (Tamogami et al. 1997). The JA signaling pathway is generally regarded as an integral signal for the biosynthesis of many plant secondary products including terpenoids, flavonoids, alkaloids, and phenylpropanoids. Many elicitors (like pathogens and PGPRs) stimulate endogenous JA biosynthesis in plants, so the JA signaling pathway functions as a transducer or mediator for

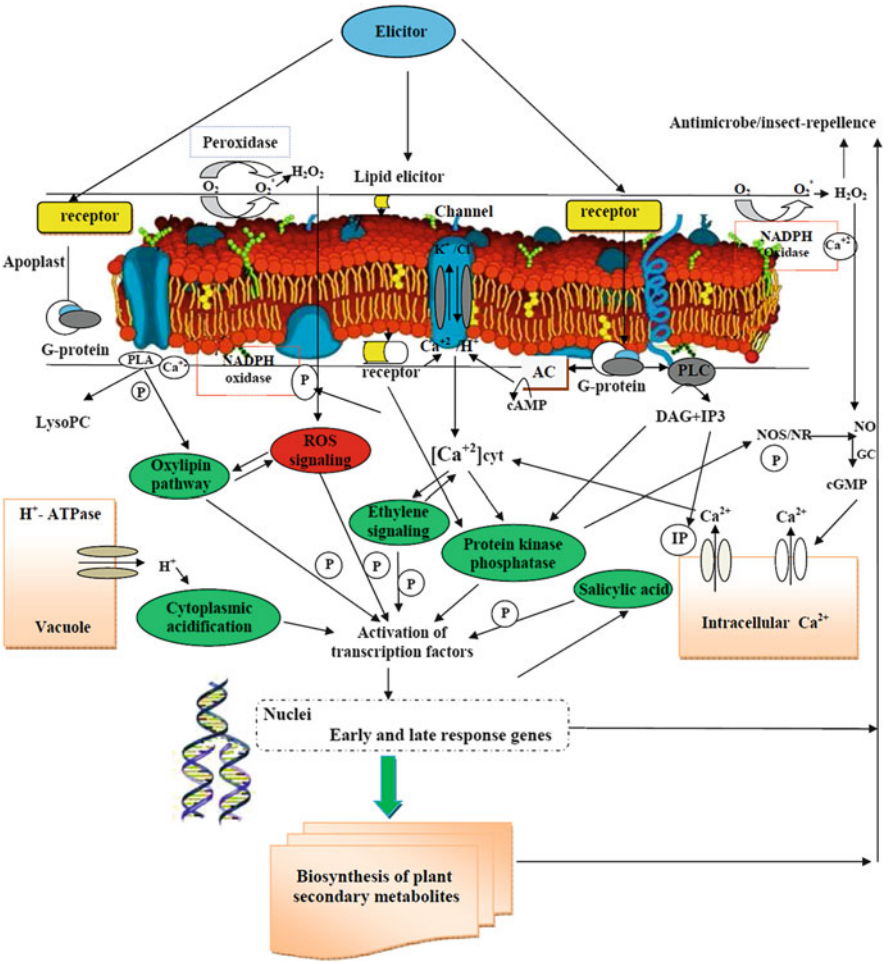

Fig. 3.8 A schematic model of signal transduction events by elicitors, leading to the expression of genes encoding enzymes involved in the biosynthesis of secondary metabolites in plants. Different elicitors are perceived by distinct membrane receptors, though they may activate the same signaling pathways. The activated receptors may then activate ion channels and G-proteins and subsequently activate phospholipases (such as PLA_2), through Ca^{2+} signaling or by G-protein coupling. Phospholipases hydrolyze phospholipids such as PC, into fatty acid and lysoPC; the former can function as a precursor for biosynthesis of JA and related oxylipins via the octadecanoid pathway, or peroxidized by reactive oxygen species (ROS) to produce another class of pentacyclic oxylipin phytoprostanes. Elicitors also activate mitogen-activated PK (MAPK) cascades that phosphorylate transcription factors regulating the expression of early and late response genes. All these pentacyclic oxylipins can activate biosynthesis of secondary metabolites in plants (modified from Zhao et al. 2005)

elicitor signaling pathways, leading to the accumulation of secondary metabolites in plants (Mueller et al. 1993). Application of methyl-jasmonate (0.5 mM) significantly increased the quantity of monoterpenes in basil (*Ocimum basilicum*) via increasing the number of transcripts of the enzymes linked to metabolic pathways of monoterpenes (Kim et al. 2003).

Ethylene is another phytohormone that regulates a wide range of plant processes including growth, development, and defense responses, and its production can be induced by various stresses and microbial infections like PGPRs. However, the concentration of ethylene in the culture is critical for acquiring desirable effects, i.e., low concentrations can promote the elicitor-induced production of secondary metabolites, whereas high concentrations may have inhibitory effects (Zhao et al. 2004).

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Growth regulators and plant hormones stimulate plant growth and terpene biosynthesis in a broad number of aromatic plant species, which result in beneficial changes in terpene quality and quantity (Farooqi and Sharma 1988).

Secretion of volatile organic compounds (VOCs) by PGPRs can be another possible mechanism for enhancing the production of plant secondary metabolites. All organisms produce VOCs, which are characterized by low molecular weight and high vapor pressure, and play important roles in communication within and between organisms. Bacterial VOCs have been reported as a rich source for new natural compounds that may increase crop productivity and EOs yield in medicinal and aromatic plants. Santoro et al. (2011) studied the effects of VOCs released by three PGPR strains (*P. fluorescens*, *B. subtilis,* and *Azospirillum brasilense*) on EOs composition of *Mentha piperita* (peppermint). The results showed that the production of monoterpenes increased two-fold in plants inoculated with *P. fluorescens,* which also increased biosynthesis of the two major EOs, (+) pulegone and (−) menthone. Menthol in *Azospirillum brasilense*-inoculated plants was the only major EOs constituent that showed a significant decrease. It has also been reported that the PGPR strain *B. subtilis* GB03 releases VOCs that elevate EOs accumulation in *Ocimum basilicum* (Banchio et al. 2009). Two major EOs components, R-terpineol and eugenol, increased by two- and ten-fold, respectively. This was seen in plants exposed to GB03 VOCs or with root inoculation, as compared to uninoculated controls. Some of the PGPRs proven to be biotic elicitors for the production of secondary metabolites in medicinal and aromatic plants are presented in Table 3.4.

Table 3.4 Efficient biotic elicitors used for the production of secondary metabolites in different plant species

PGPRs as elicitors	Plant species	Elicitation of secondary metabolites	Reference
Pseudomonas putida and *fluorescens*	*Hyoscyamus niger* L.	Hyoscyamine and scopolamine	Ghorbanpour et al. (2013a, b)
Pseudomonas putida and *fluorescens*	*Salvia officinalis* L.	*Cis*-thujone, camphor, 1,8-cineole	Ghorbanpour et al. (2014)
Glomus aggregatum, Bacillus coagulans, and *Trichoderma harzianum*	*Solanum viarum*	Total phenols, ortho-dihydroxy phenols, tannins, flavonoids, saponins, and alkaloids	Hemashenpagam and Selvaraj (2011)
Pseudomonas fluorescens	*Catharanthus roseus*	Ajmalicine	Jaleel et al. (2007)
Pseudomonas fluorescens	*Catharanthus roseus*	Serpentine	Jaleel et al. (2009)
Pseudomonas fluorescens, Bacillus subtilis, and *Azospirillum brasilense*	*Mentha piperita*	(+) pulegone and (−) menthone	Santoro et al. (2011)
Bacillus cereus	*Salvia miltiorrhiza* Bunge	Tanshinone	Zhao et al. (2010)
Trichoderma viride	*Catharanthus roseus*	Ajmalicine	Namdeo et al. (2002)
Glomus mosseae, Bacillus coagulans, and *Trichoderma viride*	*Begonia malabarica* Lam	Total phenols, ortho-dihydroxy phenols, tannins, flavonoids, and alkaloids	Selvaraj et al. (2008)
Pseudomonas fluorescens and *Bradyrhizobium* sp.	*Origanum majorana* L.	Terpinene- 4-o1, *cis*-sabinene hydrate, *trans*-sabinene hydrate, and α-terpineol	Banchio et al. (2008)
Pseudomonas fluorescens, Bacillus subtilis, and *Azospirillum brasilense*	*Origanum* × *majoricum*	*Cis*- and *trans*-sabinene hydrate, gamma-terpinene, carvacrol, and thymol	Banchio et al. (2010)
Hormonema ssp. homogenates	*Brugmansia candida*	Hyoscyamine and scopolamine	Pitta-Alvarez et al. (2000)
Bacillus polymyxa, Pseudomonas putida, Azotobacter chroococcum, and *Glomus intraradices*	*Stevia rebaudiana*	Stevioside	Vafadar et al. (2013)
Arbuscular mycorrhizal and phosphate-solubilizing bacteria	Rose-scented geranium (*Pelargonium* sp.)	Citronellol, geraniol, geraniol, and 10-epi-γ eudesmol	Prasad et al. (2012)

(continued)

Table 3.4 (continued)

PGPRs as elicitors	Plant species	Elicitation of secondary metabolites	Reference
Pseudomonas aeruginosa and *Pseudomonas fluorescens*	*Pisum sativum*	Phenolic compounds (gallic, cinnamic, and ferulic acid)	Bahadur et al. (2007)
Bacillus subtilis and *Pseudomonas fluorescens*	*Pelargonium graveolens*	Essential oil yield	Mishra et al. (2010)
Bacillus subtilis GB03	*Ocimum basilicum*	α-terpineol and eugenol	Banchio et al. (2009)
Pseudomonas fluorescens and *Azospirillum brasilense*	*Tagetes minuta*	Monoterpenes and phenolic compounds	Cappellari et al. (2013)

3.5 Conclusions

Infection by microorganisms as well as physiological and genetic factors and environmental conditions are the main agents affecting the accumulation and composition of secondary metabolites in plants. Among these, PGPRs seem to be a promising candidate considering their well-established role in plant nutrition, tolerance against biotic and abiotic stresses and enhancing the yield of different classes of secondary metabolites. As an environmentally friendly strategy, PGPRs should be considered to achieve sustainable high yields of industrially important secondary metabolites in plants using minimum chemical inputs.

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