Chapter 12
Endophytic Bacteria-Mediated Regulation of Secondary Metabolites for the Growth Induction in *Hyptis suaveolens* Under Stress

Yachana Jha

Abstract Two endophytic bacteria *Pseudomonas pseudoalcaligenes* and *Pseudomonas aeruginosa* have been isolated from the root of paddy and *Suaeda nudiflora* wild mosque plant to evaluate their growth promoting ability in one of the important medicinal plant *Hyptis suaveolens* under stress. The endophytic isolates have been inoculated either alone or in combination in *Hyptis suaveolens* and have enhanced the growth and essential oil content as well as yield of the *Hyptis suaveolens* under normal condition and under stress. It reduces the negative effects of stress and shows enhanced plant growth compared to control, while the essential oil content and yield decrease due to inoculation of endophytic bacteria compared to control. But due to enhanced vegetative growth, overall yield increases. Inoculation with endophytic bacteria increases the phenolic content under normal condition and decreases under stress. Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing of different diseases. So inoculation with such beneficial bacteria serves as a better option for utilization of degraded wastelands for cultivation of medicinal and aromatic plants.

Keywords Endophytic bacteria · *Hyptis suaveolens* · Secondary metabolite · Essential oil · Stress

---

Y. Jha (✉)
N. V. Patel College of Pure and Applied Sciences, S. P. University, Anand, Gujarat, India

D. Egamberdieva, A. Tiezzi (eds.), *Medically Important Plant Biomes: Source of Secondary Metabolites*, Microorganisms for Sustainability 15,
https://doi.org/10.1007/978-981-13-9566-6_12

12.1 Introduction

Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources. The cultivation of medicinal and aromatic plants has gained more importance, and the trade in products of these plants is estimated to be over US $ 3000 million per annum. *Hyptis suaveolens* (L.) Poit plant commonly known as Wilayati tulsi belongs to the family *Lamiaceae* and is an ethnobotanically important medicinal plant. It is a coarse, erect, branched, and hairy annual herb commonly called wild spikenard. The plant is bitter, minty, and aromatic. The plant has been considered as an obnoxious weed, distributed throughout the tropics and subtropics. Almost all parts of this plant are being used in traditional medicine to treat various diseases, as its medicinal constituents include the strong essential oils, tannin, flavonoids, etc. The phytochemical screening of leaf extracts shows the presence of secondary metabolites and essential oils having antibacterial activity. Essential oils and secondary metabolites are the major reason for medicinal properties of *Hyptis suaveolens* (L.) Poit plant (Moreira et al. 2010). The leaves have been utilized as a stimulant, carminative, sudorific, galatogogue, and to cure parasitic cutaneous diseases (Mandal et al. 2007). It has also been used as analgesic, decongestant, and antipyretic and stimulates blood circulation. Decoction of the herb as tea is effective for fever associated with cold and flatulence. *Hyptis suaveolens* (L.) Poit is a medicinal plant used in traditional medicines. In higher plants a wide variety of secondary metabolites are synthesized from primary metabolites and are needed in plant defense against stress like protection against pathogens or environmental stresses. Plant secondary metabolites are unique sources for food additives, flavors, and pharmaceuticals, and such chemicals include calcium, abscisic acid (ABA), salicylic acid (SA), polyamines, jasmonates (JA), and nitric oxide which are involved in stress responses in plants (Tuteja and Sopory 2008). The accumulation of such natural products strongly depends on the growing conditions, such as the temperature, the light regime, and the nutrient supply (Ballhorn et al. 2011). The levels and composition of secondary metabolites in plants vary according to genotype, climate factors such as seasonal variation, light intensity, relative humidity and temperature, environment stimuli, and agronomical practices. Cultivation factors such as soil type, compost, mulching, and fertilization can also affect the plant secondary metabolites. In addition, more severe environmental influences, such as various stress conditions, will also impact on the metabolic pathways responsible for the accumulation of secondary plant products. Plant secondary metabolites are often referred to as compounds that have no fundamental role in the maintenance of life processes in the plants, but they are important for the plant to interact with its environment for adaptation and defense. The generation of ROS in plants is triggered by different kinds of environmental stresses, such as high light, high or low temperature, salinity, drought, nutrient deficiency, and pathogen attack. ROS also act as signaling molecules involved in growth and developmental processes, pathogen defense responses such as hypersensitive reaction and systemic acquired resistance, stress hormone production, acclimation, and programmed cell death (Apel and Hirt 2004). It is well-known that free radical and other reactive oxygen species formed in the living cells play an important role in metabolism. Natural products from medicinal plants are known to be chemically balanced, effective, and least injurious with none or much reduced side effects as compared to synthetic medicines. The objective of this study is to investigate the effect of endophytic bacteria, inoculated on the growth, secondary metabolite production, and oil yield of *Hyptis suaveolens* plants. The collaboration of endophytic bacteria and their effect on the biological growth response of plants under stress is complex. The inoculation of endophytic bacteria in plant, alone or in groups, can confer tolerance to plant against adverse environmental condition, improves nutrient availability, and helps the plant to overcome stress by regulating secondary metabolite production.

12.2 Secondary Metabolites of Plant

Secondary metabolites are organic molecules that are not involved in the normal growth and development of an organism. These compounds are an extremely diverse group of natural products synthesized by plants, fungi, bacteria, algae, and animals. Most of secondary metabolites, such as terpenes, phenolic, and alkaloids, are classified based on their biosynthetic origin. Different classes of these compounds are often associated to a narrow set of species within a phylogenetic group and constitute the bioactive compound in several medicinal, aromatic, colorant, and spice plants.

Secondary metabolites are frequently produced at highest levels during transition from active growth to stationary phase. The producer organism can grow in the absence of their synthesis, suggesting that secondary metabolism is not essential, at least for short-term survival. The genes involved in secondary metabolism provide a “genetic playing field” that allows mutation and natural selection to fix new beneficial traits via evolution. The secondary metabolism as an integral part of cellular metabolism relies on primary metabolism to supply the required enzymes, energy, substrates, and cellular machinery and contributes to the long-term survival of the plant (Roze et al. 2011). A simple classification of secondary metabolites includes three main groups: terpenes (such as plant volatiles, cardiac glycosides, carotenoids, and sterols), phenolics (such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins, and lignin), and nitrogen-containing compounds (such as alkaloids and glucosinolates). Nowadays, medicinal and aromatic plants have undergone a transition from unknown or minor agricultural plantings to major crops that farmers may consider as alternatives to traditional food or feed crops. The steadily increasing agricultural role is driven by consumer interest in these plants for culinary, medicinal, and other anthropogenic applications. The use of plants, foods, and herbal products is increasing due to consumer awareness of their various health benefits. Due to the ever-increasing population, the pressure on arable lands for cultivation of food crops has amplified; therefore, utilization of degraded wastelands is a viable option for cultivation of medicinal and aromatic plants. So, for the enhanced production of secondary metabolite, endophytic bacteria-inoculated *Hyptis suaveolens* can be cultivated at normal as well as stressed condition for human welfare.

12.3 Isolation and Inoculation of Endophytic Bacteria

Plant-bacterial associations have been studied for many decades. However, a complete understanding of the mechanisms utilized by plant endophytic bacteria had remained somewhat indefinable, often making it difficult to take full advantage of these complex relationships to reproducibly improve the growth of plants. The bacterial endophytes can offer several benefits to the host plant, particularly growth promotion and protection from pathogens and diverse environmental conditions. Under adverse condition bacterial endophytes are able to communicate and interact with the plant more efficiently (Coutinho et al. 2015).

The autotrophic plants need minerals for life cycle, and an adequate supply of mineral nutrients is necessary for optimum plant growth. However, when adequate amounts of essential nutrients are present in soil, plants may still show deficiencies due to the nonavailability of these mineral nutrients. Microorganisms can help plants to grow by providing soluble mineral nutrients converted by acidification from insoluble mineral or via mobilization of essential nutrients that can also help in plants' growth improvement. To increase plant nutrient status by the endophytic bacteria by associative nitrogen fixation, phosphorus and potassium solubilization, and siderophores production, changing the absorptivity is a good option for sustainable plant growth. Bacterial genera such as *Bacillus*, *Pseudomonas*, and *Brevibacillus* are well-known to promote growth and yield in different nonleguminous plant (Karlidag et al. 2007). Two bacterial strains are isolated from the root of paddy and *Suaeda nudiflora* wild mosque plant from Khambhat near the seashore of Gujarat as a previously published method (Jha et al. 2011). And the soil sample is tested in SICART (Sophisticated Instrumentation Centre for Applied Research and Testing) laboratory by extracted water sample method. The soil possesses the following physiochemical properties: pH 6.58, electrical conductivity 1480 μS/cm, salinity 8.6%, nitrate 112.5 mg kg^{-1}, chloride 128 mg kg^{-1}, sulfate 155 mg kg^{-1}, ammonia nitrogen 23.3 mg $kg^{-}1$, CEC 3 cmol, and organic carbon 5500 mg kg^{-1}. Molecular identification of bacterial isolates has been done by isolation of total genomic DNA and PCR amplification with 16S rDNA specific primers 16S F: 5′AGAGTTTGATCCTGGCTCAG3′ and 16S R: 5′AGGTTACCTTGTTACGACTT3′ followed by sequencing as our published method (Jha and Subramanian 2012). PCR amplicons of 16S rDNA of about 1500 bp are obtained for both the isolates as discrete bands in agarose gel (Fig. 12.1). The phylogenetic trees have been constructed using BLAST software by the comparison of the 16S rDNA sequence of isolates and related genera from a database using the neighbor-joining (NJ) algorithm and maximum likelihood (ML) method. The two isolates are identified by molecular analysis by nucleotides homology and phylogenetic analysis as *Pseudomonas pseudoalcaligenes* (GenBank Accession Number: EU921258) and *Pseudomonas aeruginosa* (GenBank Accession Number: JQ790515).

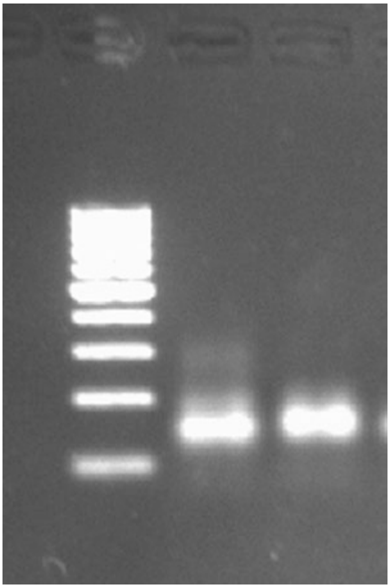

Fig. 12.1 Agarose gel showing the amplified 16S rDNA of isolates, where M = 1 KB marker, L1 = *Pseudomonas aeruginosa*, and L2 = *Pseudomonas pseudoalcaligenes*

Seeds of *Hyptis suaveolens* have been washed thoroughly with distilled water followed by surface sterilization with 0.1% $HgCl_2$ solution for 4 min and 70% ethanol for 10 min. The washed seeds are kept in a shaker for 1 h in autoclaved distilled water on a rotary shaker and then transferred to Petri dishes containing tryptone glucose yeast extract agar medium to test for possible contamination at 30 °C. The germinated seedlings devoid of any contamination are used for inoculation experiments. To study the effect of the isolated bacteria on the physiological and biochemical parameters, 4 days old germinated seedlings devoid of any contamination are transferred to culture tubes containing 400 μl Hoagland's nutrient medium, 400 μl micronutrients, and 1% agar in 40 ml distilled water. Before the transfer, bacterial inoculums of the isolated bacteria *Pseudomonas pseudoalcaligenes* and *Pseudomonas aeruginosa* have been added with the medium at a concentration of 6×10^8 cfu ml^{-1}. To obtain a mixture of both bacterial cultures, an equal volume of both the cultures is mixed in the medium to give a concentration of 6×10^8 cfu ml^{-1}. The tubes are incubated at 27 °C in a 12 h light–dark cycle in a growth chamber. Seven days old plants are carefully removed from different test tubes inoculated with the strain of bacterium and planted in a pot. Similarly the control plants (uninoculated) are also transferred to a fresh pot. Seedlings are planted at the rate of four plants per pot and watered at the time of transplantation of the seedlings. The present study indicates that inoculation with endophytic bacteria, viz., *P. pseudoalcaligenes* and *P. aeruginosa*, both alone or in combination significantly enhanced all the growth parameter and led to recovery of the plants from the saline stress (Table 12.1). The results obtained clearly demonstrated that the stress adversely affects the growth of the plants. However, when the plants were inoculated with the endophytic bacteria, the extent of growth suppression decreased suggesting participation of the endophytic bacteria in alleviation of some of the debilitating effects of stress.

Table 12.1 Effect of endophytic bacteria on growth parameters of *Hyptis suaveolens* under stress

Treatments	Germination %	Survival %	Plant height (m)	Dry weight (kg)	RGR relative growth rate
Normal					
Control	71.1^{d}	85.7^{d}	1.223^{fg}	0.423^{cde}	31.28^{fg}
Control + *P. aeruginosa*	75.4^{bc}	89.1^{bc}	1.431^{c}	0.398^{fg}	33.63^{c}
Control + *P. pseudoalcaligenes*	76.8^{b}	91.3^{b}	1.489^{a}	0.479^{ab}	34.32^{b}
Control + *P. aeruginosa* + *P. pseudoalcaligenes*	82.2^{a}	93.2^{a}	1.470^{ab}	0.497^{a}	35.97^{a}
Stressed					
Control	22.3^{d}	32.2^{cd}	1.021^{h}	0.378^{gh}	29.26^{h}
Control + *P. aeruginosa*	24.1^{bc}	32.6^{bc}	1.276^{def}	0.414^{def}	31.92^{ef}
Control + *P. pseudoalcaligenes*	25.7^{b}	33.1^{ab}	1.312^{cd}	0.442^{bc}	32.54^{de}
Control + *P. aeruginosa* + *P. pseudoalcaligenes*	27.2^{a}	33.8^{a}	1.297^{de}	0.431^{bcd}	33.32^{cd}

Values are the means of replicates. Values with different letters are significantly different at $P < 0.05$ (Duncan's Test). Values in columns followed by the same letter are not significantly different at ($P \leq 0.05$)

12.4 Endophytic Bacteria-Mediated Regulation of Nutrients for Secondary Metabolites Production

When plants are stressed, secondary metabolite production may increase, and growth is often inhibited, because the carbon fixed is predominantly allocated to secondary metabolites. According to the theory of functional balance (Hendrik et al. 2012), plants increase the allocation of biomass to shoots if carbon gain is affected by limited resources above ground, such as light and CO_2. Similarly, plants increase biomass allocation to roots in the presence of low levels of below-ground resources, such as water and nutrients. Under prolonged stress, the nutrients allocated to secondary metabolites are predominantly reverted back to maintain cell osmotic balance to maintain metabolic activity of plant. Stress adversely affects plant nutrient acquisition, especially in the root, resulting in a significant decrease in shoots dry biomass.

The collaboration of endophytic bacteria and their effect on the biological growth response of plants under stress is complex. In our study, the foliar contents of N, P, K, Na, and Ca in endophytic bacteria-inoculated plant are estimated by taking 1 g of plant material digested in tri-acid mixture in the ratio of 9:3:1 by using specific filter on digital flame photometry. The foliar Na concentration is higher in the non-inoculated control plants, while P concentration is higher in the plants inoculated with the endophytic bacteria under stress. The plants inoculated with endophytic bacteria alone and in combination show higher levels of foliar K. Potassium is an osmotically active solute that contributes to water absorption at the cell and whole

Table 12.2 Effect of endophytic bacteria on nutrient minerals concentration of *Hyptis suaveolens* under stress

Treatments	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Na (mg kg^{-1})	Ca (mg kg^{-1})
Normal					
Control	0.926^{d}	0.671^{d}	0.647^{cd}	0.823^{ef}	0.924^{cd}
Control + *P. aeruginosa*	1.093^{c}	0.845^{c}	0.754^{bc}	0.718^{cd}	0.835^{ab}
Control + *P. pseudoalcaligenes*	1.321^{b}	0.894^{ab}	0.837^{ab}	0.644^{bc}	0.746^{bc}
Control + *P. aeruginosa* + *P. pseudoalcaligenes*	1.471^{a}	0.973^{a}	0.915^{a}	0.528^{b}	0.651^{a}
Stressed					
Control	0.782^{h}	0.516^{h}	0.516^{gh}	0.953^{a}	0.998^{de}
Control + *P. aeruginosa*	0.897^{fg}	0.677^{f}	0.672^{ef}	0.825^{de}	0.839^{f}
Control + *P. pseudoalcaligenes*	0.928^{ef}	0.766^{fg}	0.716^{fg}	0.723^{gf}	0.771^{fg}
Control + *P. aeruginosa* + *P. pseudoalcaligenes*	1.021^{e}	0.811^{e}	0.854^{e}	0.642^{h}	0.732^{h}

Values are the means of replicates. Values with different letters are significantly different at $P < 0.05$ (Duncan's Test). Values in columns followed by the same letter are not significantly different at ($P \leq 0.05$)

plant level (Table 12.2) and helps stressed plant in maintaining central metabolic activity for its survival. In our study, inoculations of plants with endophytic bacteria always have higher N_2 and carbon concentration under normal and stress conditions. Deficiencies of important nutrient like N, P, K, and S usually cause a greater concentration of secondary metabolite like phenolic compounds, and abundant N generally reduces phenolic accumulation in plant (Gershenzon 1983), which is easily regulated and maintained by endophytic bacteria. The levels of phenolic compounds are directly related to secondary metabolism and show the sensitivity of plant response to nutrient deficiency. The inoculation of endophytic bacteria in plant, alone or in groups, can confer tolerance to plant against adverse environmental condition and also improves other nutrient availability and helps the plant to overcome stress by regulating secondary metabolite production.

12.5 Endophytic Bacteria-Mediated Regulation of Concentration of Photosynthetic Pigments for Secondary Metabolites Production

The plant growth is controlled by a multitude of physiological, biochemical, and molecular processes. However, stressful environments considerably hamper the plant growth by altering the ultrastructure of the organelles and concentration of various photosynthetic pigments (Wu and Kubota 2008).Chlorophyll is vital for photosynthesis, which allows plants to absorb energy from light. Fresh leaves are used for chlorophyll measurements, by placing fresh leaf samples (0.5 g) in a shaker

Table 12.3 Effect of endophytic bacteria on the photosynthetic pigment and oil yield of *Hyptis suaveolens* under stress

Treatments	Chl a (mg g-1FW)	Chl b (mg g-1FW)	Carotenoid (mg g-1FW)	Oil Content %	Oil yield (ml Pot^{-1})
Normal					
Control	0.876^{d}	0.481^{d}	0.427^{cd}	0.763^{ef}	0.832^{cd}
Control + *P. aeruginosa*	1.075^{c}	0.672^{c}	0.564^{bc}	0.828^{cd}	0.975^{ab}
Control + *P. pseudoalcaligenes*	1.082^{b}	0.612^{ab}	0.517^{ab}	0.784^{bc}	0.924^{bc}
Control + *P. aeruginosa* + *P. pseudoalcaligenes*	1.541^{a}	0.873^{a}	0.725^{a}	0.958^{b}	1.141^{a}
Stressed					
Control	0.562^{h}	0.356^{h}	0.296^{gh}	0.853^{a}	0.868^{de}
Control + *P. aeruginosa*	0.683^{g}	0.437^{f}	0.412^{ef}	0.815^{de}	0.779^{f}
Control + *P. pseudoalcaligenes*	0.678^{ef}	0.376^{fg}	0.366^{fg}	0.762^{gf}	0.751^{fg}
Control + *P. aeruginosa* + *P. pseudoalcaligenes*	0.861^{e}	0.541^{e}	0.484^{e}	0.744^{h}	0.732^{h}

Values are the means of replicates. Values with different letters are significantly different at $P < 0.05$ (Duncan's Test). Values in columns followed by the same letter are not significantly different at ($P \leq 0.05$)

with 80% acetone until the leaves are completely bleached. The extract is centrifuged at 13,000 rpm for 10 min, and the supernatant is used to measure chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid by taking absorbance at 663, 645, and 470 nm, respectively, using spectrophotometer. In the present study, endophytic bacteria-inoculated plants show increased chlorophyll a, b, and carotenoids in comparison to control plants (Table 12.3).Chlorophyll content is influenced by the nitrogen concentration; as the levels of N_2 increased, chlorophyll a, b, and total chlorophyll are also enhanced. Endophytic bacteria having nitrogen fixing ability must enhance concentration of N_2 in plant and result in high level of chlorophylls and carotenoids also. The increase in chlorophyll content with increasing nitrogen has been reported by Suza and Valio (2003).

Carotenoids are necessary for photoprotection of photosynthesis, and they play an important role as a signaling precursor during the plant development under stress. They have significant potential to enhance nutritional quality and plant yield (Jha and Subramanian 2013). The chlorophyll a, b, and total chlorophyll are negatively related with secondary metabolites. Competition between secondary metabolites and chlorophyll contents fits well with the prediction of protein competition model, where secondary metabolites content is controlled by the competition between protein and secondary metabolites biosynthesis pathway and its regulation. The negative relationship between secondary metabolites and chlorophyll content is a sign of gradual switch of investment from protein to polyphenolics production (Meyer et al. 2006). Results of present study indicate that the production of chlorophyll content competes with the production of secondary metabolites due to endophytic bacteria inoculation under stress. Affendy et al. (2010) have reported an increase in the production of secondary metabolites of *O. stimaneus* under low irradiance due to increase in availability of phenylalanine, a precursor for secondary metabolites and protein production. The production of secondary metabolites is more prioritized under low nitrogen levels due to the restriction of protein production as exhibited by reduced chlorophyll production.

12.6 Endophytic Bacteria-Mediated Regulation of Essential Oil

Essential oils are secondary metabolites whose production is associated with primary metabolism and with availability of soil nutrients (Shulka et al. 1992). Plants synthesize essential oils for a variety of purposes, including protection of the plant against fungi and bacteria, allelopathic activity, defense against insects (terpenoids), attraction of pollinators, and dispersal agents to favor the dispersion of seeds and pollens. The major activities of essential oils are antimicrobial, sedative, anti-inflammatory, bactericidal, antiviral, antifungal (fungicidal), and preservative for foods. The essential oils found in the genus *Hyptis* have a great importance as a source of bioactive constituents, especially due to their biological properties such as antimicrobial, cytotoxic, and insecticide (Kuhnt et al. 1995).

Essential oils are volatile liquids that can be synthesized by all plant organs and are stored in secretory cells, cavities, canals, and epidermic cells. In the present study, essential oil (EO) extraction from the fresh herbage is performed by hydrodistillation in Clevenger's apparatus for 1 h and 30 min. Oil content (w/v) and total oil yield (ml) are estimated. The essential oil has been analyzed on an Agilent 4890D gas chromatograph fitted with a column (30 m × 0.25 mm, film thickness 0.25 μm, Supelco Wax-10).The leaves being the source of the essential oil are thus the most economically viable parts of the *Hyptis suaveolens*, and the oil yield is hence directly proportional to the number of leaves. In the present study, the leaf-stem ratio and thus the oil yield in endophytic bacteria-inoculated plants are higher at normal condition. But under stress inoculation with endophytic bacteria shows no significant effect on oil yield as shown in Table 12.3. Inoculation with endophytic bacteria under stress reduces the oil yield and has also been reported by Arvini et al. (2012), but due to enhanced plant growth, the overall yield per plant increased. The decreased oil yield due to inoculation with endophytic bacteria may be due to reduced stress which ultimately reduces the oil content. In the present study, the gas chromatographic (GC) analysis of the *Hyptis suaveolens* essential oil enabled us to compare 11 major compounds, viz., α-pinene, β-pinene, sabinene, myrcene, limonene, 1, 8-cineole, menthone, isomenthone, menthyl acetate, neomenthol, and menthol (Table 12.4). The percentage concentration of menthol, the characteristic constituent of the *Hyptis suaveolens* essential oil, varied with the treatments and stress. The effect of stress is more profound on menthol content as it decreased under stress irrespective of the microbiological application. The oil content in a plant is largely dependent on the physiological state of the plant. Enhanced production of secondary metabolites including essential oil may depend directly on improved nutritional status and on primary metabolism of

Table 12.4 Effect of endophytic bacteria on the essential oil contents of *Hyptis suaveolens*

Treatments	α-pinene	β-pinene	Sabinene	Myrcene	Limonene	1,8-Cineole	Menthone	Iso-menthone	Menthyl acetate	Neo-menthol	Menthol
	1.478^{a}	2.214^{a}	2.572^{a}	3.312^{a}	3.752^{a}	4.211^{a}	12.232^{a}	12.891^{a}	13.543^{a}	14.342^{a}	16.348^{a}
Normal											
Control	0.54^{gh}	0.55^{gh}	0.13^{fg}	0.23^{fg}	0.71^{ef}	0.113^{ef}	0.895^{f}	2.436^{ef}	3.423^{e}	1.641^{hi}	81.21^{ef}
Control + *P. aeruginosa*	0.74^{e}	0.68^{d}	0.317^{d}	0.33^{cd}	0.846^{d}	0.119^{cd}	0.964^{d}	2.655^{d}	2.861^{h}	1.786^{cd}	83.11^{cd}
Control + *P. pseudoalcaligenes*	0.831^{c}	0.876^{c}	0.336^{c}	0.388^{c}	0.984^{c}	0.129^{c}	1.237^{c}	2.982^{bc}	3.142^{f}	1.987^{c}	81.87^{e}
Control + *P. aeruginosa* + *P. pseudoalcaligenes*	0.954^{b}	1.129^{b}	0.386^{b}	0.473^{b}	1.181^{b}	0.191^{b}	1.541^{b}	3.028^{b}	3.342^{c}	2.182^{b}	83.19^{c}
Stressed											
Control	0.723^{d}	0.614^{e}	0.17^{e}	0.28^{de}	0.753^{e}	0.121^{de}	0.904^{de}	2.541^{de}	3.553^{d}	1.845^{de}	84.36^{b}
Control + *P. aeruginosa*	0.53^{ef}	064^{fg}	0.14^{ef}	0.19^{ef}	0.665^{g}	0.118^{fg}	0.791^{fg}	2.347^{fg}	3.424^{b}	1.744^{fg}	76.82^{g}
Control + *P. pseudoalcaligenes*	0.54^{fg}	0.59^{ef}	0.13^{gh}	0.17^{gh}	0.572^{h}	0.089^{gh}	0.725^{h}	1.981^{gh}	3.123^{fg}	1.792^{ef}	71.37^{gh}
Control + *P. aeruginosa* + *P. pseudoalcaligenes*	0.511^{i}	0.489^{i}	0.08^{i}	0.143^{i}	0.391^{i}	0.067^{i}	0.611^{i}	1.649^{i}	2.971^{hi}	1.659^{gh}	68.21^{i}

Values are the means of replicates. Values with different letters are significantly different at $P < 0.05$ (Duncan's Test)

[a] Retention time (min)

plants following endophytic bacterial inoculation (Farag et al. 2006; Jha and Subramanian 2016). Plants may be considered as a famous chemical factory for biosynthesis of a huge array of secondary metabolites. Many of these chemicals are utilized as medicine, scent, dyes, and pesticides and are of commercial importance. Secondary metabolites are those compounds produced by plant which are not essential for plant growth and development. Environmental factors including biotic and abiotic stimuli, carbon-nutrition balance, genotype, and ontogenesis usually control and regulate the biosynthesis of secondary metabolites in plants (Mary Ann Lila 2006). With regard to plant-microbe interactions, coevolution between plants and their microbial partners are mediated via plant chemical defense.

12.7 Endophytic Bacteria-Mediated Regulation of Secondary Metabolites Production Under Biotic Stress

Plants are facing numerous biotic stress and adverse environmental conditions. They respond to such stress through several morphological, biochemical, molecular mechanisms and by their interactions among respective signaling pathways (Nejat and Mantri 2017). Biotic stresses in plants are caused by pests, parasites, and pathogens, primarily responsible for plant diseases. Prone to attacks by pathogens and pests, plants utilize complicated chemical defense mechanisms consisting of metabolic adaptations. However, many plant pathogens can manipulate the host metabolism to induce favorable nutritional condition and to counteract defense responses. The plants employ a highly intricate defense system that is capable of protecting themselves from the majority of attackers. Plant immunity is multilayered and consists of preformed, constitutive as well as inducible defense mechanisms (Pieterse et al. 2009). Besides physical preformed barriers such as the cell wall, plants also possess highly effective preformed chemical defenses called secondary metabolite (González-Lamothe et al. 2009). These are constitutively present products of secondary plant metabolism and represent first-line defense, which are released and activated as antimicrobial compounds upon pathogen entry. Biocontrol using endophytic bacteria may be an alternative method for controlling plant diseases to maintain plant growth (Jha 2018). Systemic plant resistance induced by endophytic bacteria as elicitor represents ISR and can protect plants against a wide range of pathogens through the activation of secondary plant metabolites, i.e., PAL, β-1, 3-glucanases, and phenolic (Jha et al. 2011). The result of the present study showed that plant inoculated with endophytic bacteria has better induction of PR proteins like PAL, β-1, 3-glucanases, and phenolic compared to non-inoculated *Hyptis suaveolens* plant, which is due to its elicitation effect prior to infection (Table 12.5). Elicitation is the induced or enhanced biosynthesis of metabolites due to addition of trace amounts of elicitors. In the same way, metabolic profiling of two rice cultivars inoculated with two different endophytic bacteria under stress conditions showed modified profiles of secondary metabolites with phenolic compounds such as flavonoids and hydroxyl cinnamic derivatives (Chamam et al. 2013). However inoculation with

Table 12.5 Effect of endophytic bacteria PAL, β-1, 3-glucanases, phenolic of maize under stress ($n = 5$)

Treatment	Proline mMol min^{-1} g^{-1}	Glycine betaine mMol min^{-1} g^{-1}	PAL (nmol of trans cinnamic acid min^{-1} g^{-1})	β-1, 3-glucanases (nmol of Glucose min^{-1} g^{-1})	Phenolic (mgg^{-1} of the gallic acid equivalent)
Normal					
Control	1.32^{cd}	0.8^{d}	0.25^{d}	0.221^{d}	0.74^{cd}
Control + *P. aeruginosa*	1.49^{c}	1.02^{c}	0.29b^{c}	0.229^{c}	0.82^{bc}
Control + *P. pseudoalcaligenes*	1.53^{b}	1.23^{b}	0.32^{b}	0.237^{b}	0.87^{ab}
Control + *P. pseudoalcaligenes* + *P. aeruginosa*	1.59^{a}	1.32^{a}	0.38^{a}	0.244a	0.99^{a}
Stressed					
Control	1.41^{d}	1.09^{cd}	0.23^{d}	0.219^{d}	0.95^{cd}
Control + *P. aeruginosa*	1.56^{bc}	1.11^{c}	0.28^{ab}	0.235^{b}	1.08^{c}
Control + *P. pseudoalcaligenes*	1.58^{b}	1.29^{ab}	0.26^{a}	0.241^{a}	1.13^{b}
Control + *P. pseudoalcaligenes* + *P. aeruginosa*	1.61^{a}	1.31^{a}	0.31^{c}	0.2547^{bc}	1.24^{a}

For each parameter, values in columns followed by the same letter are not significantly different at ($P \leq 0.05$)

endophytic bacteria affects the composition of secondary metabolites in shoots, pointing toward systemic effects, and helps plant to survive under stress. Therefore, understanding the relationship between growth and the production of secondary metabolites under different stress environments is significant in managing plants' growth conditions to acquire the maximal yield of biomass and phyto-medicinal compounds (Jha et al. 2014a, b). Plant secondary metabolites are often referred to as compounds that have no fundamental role in the maintenance of life processes in the plants, but they are important for the plant to interact with its environment for adaptation and defense. Secondary metabolites have significant practical applications in medicinal, nutritive, and cosmetic purposes, besides, importance in plant stress physiology for adaptation.

12.8 Endophytic Bacteria-Mediated Regulation of Secondary Metabolites Production Under Abiotic Stress

The vast metabolic diversity observed in plants is the direct result of continuous evolutionary processes. Environmental factors significantly affect plant growth and biosynthesis of secondary metabolites. Plant growth and productivity are negatively affected by abiotic stress. Plant secondary metabolites are compounds that play an essential part in the interaction of plants with abiotic stress. Abiotic stress causes reduction in plant growth with a resulting increase in production of secondary metabolites like phenolic compounds as a defense mechanism (Arbona et al. 2013). Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants. In the present study, the leaf extract is used for the determination of the total phenol content using gallic acid for standard by spectrophotometer (absorbance 735 nm). This study showed that inoculation of endophytic bacteria alone is sufficient to increase the phenolic content in *Hyptis suaveolens* plant in normal state and stress don't caused any further increase in it (Table 12.5). The synthesis of phenolics in ginger can be increased and affected under abiotic stress which has also been reported by Ghasemzadeh et al. (2010). Plants produce diverse secondary plant products that are triggered by a wide range of abiotic factors, to cope with environmental changes, but when inoculated with endophytic bacteria, it will regulate and modulate both primary and secondary metabolites of the plant for the better survival of plant, especially under stress (Jha 2018). Global changes in environmental conditions due to human activities appear to influence endogenous plant metabolites for adaptation. Moreover, plants have adapted to produce several metabolites that are species-specific and dependent on environmental factors. Various plant metabolites, such as polyamines, flavonoids, jasmonic acid, methyl jasmonate, glycine betaine, and so on, have a protective role during abiotic stress. These phytochemical derivatives of secondary metabolism confer a multitude of adaptive and evolutionary advantages to the producing plants (Bilgin et al. 2010).

The secondary metabolite compound is measured as glycine betaine (GB) equivalents and proline as a major osmoprotectant. In our study, accumulation of glycine betaine-like quaternary compounds and proline is significantly higher in the plants' leaves inoculated with both *P. pseudoalcaligenes* and *P. aeruginosa* (Table 12.5). Accumulation of QACs enhanced in the *Hyptis suaveolens* leaves inoculated with both *P. pseudoalcaligenes* and *P. aeruginosa* compared to *Hyptis suaveolens* plant treated with either of the *P. pseudoalcaligenes* and *P. aeruginosa* alone under stress. Many plant species naturally accumulate QACs and proline as major organic osmolytes when subjected to different abiotic stresses. These compounds are thought to play an adaptive role in mediating osmotic adjustment and protecting subcellular structures in stressed plants (Asharf and Foolad 2007). When the environment is adverse and plant growth is affected, metabolism is profoundly involved in signaling, physiological regulation, and defense responses. At the same time, in feedback, abiotic stresses affect the biosynthesis, concentration, transport, and storage of primary and secondary metabolites.

12.9 Endophytic Bacteria-Mediated Regulation of Gene Expression for Secondary Metabolites Production

During evolution, plants have developed a wide variety of highly complicated and competent mechanisms to sense, respond, and adapt to a wide range of environmental changes. Under adverse or limiting growth conditions, plants respond by activating tolerance mechanisms at multiple levels of organization (molecular, tissue, anatomical, and morphological), by adjusting the membrane system and the cell wall architecture, by altering the cell cycle and rate of cell division, and by metabolic tuning. Plants perceive stress signals through receptors that trigger molecular cascades to transmit the signals to regulatory systems via ion channels, signaling proteins, and secondary messengers (Choudhary et al. 2012). At a molecular level, many genes are induced or repressed by stress, involving a precise regulation of extensive stress-gene networks (Müller and Stelling 2009). Plants have several mechanisms to overcome such stress by stimulating production of secondary metabolite or induction of defensive gene. The regulatory system is composed of various components, including phytohormones, transcription factors (TFs), mitogen-activated protein kinases, and phosphatases that regulate the expression of various stress-responsive genes (Osakabe et al. 2014). In order to establish a favorable energy balance for defense, the upregulation of defense-related pathways is compensated by the downregulation of genes involved in other metabolic pathways. Plant secondary metabolites are often referred to as compounds that play important role for the plant to interact with its environment for adaptation and defense. The biochemical change involved in plant stress responses is by the synthesis of new proteins and hormone that have direct or indirect action on the course of stress. It is therefore surprising that induction of stress-related gene can be induced prior to stress, i.e., merely by inoculation with endophytic bacteria (Jha et al. 2014a, b), but till date there is no report on the induction or expression of gene in *Hyptis suaveolens* under stress. Endophytic bacteria induced different small protein molecules in plant under stress as well as control conditions to establish itself in the host plant and to protect the plant under stress. Mechanisms of endophytic bacteria-mediated phytostimulation would help us to find more capable strains having the ability to function efficiently for sustainable production of important secondary metabolites under different agro-ecological conditions (Jha and Subramanian 2018a, b).

12.10 Conclusion

Secondary metabolism comprises a coordinate series of coupled enzymatic conversions that utilizes limited products of primary metabolism as substrates. Secondary metabolism uses highly organized systematic mechanisms that integrate into developmental, morphological, and biochemical regulatory patterns of the entire plant metabolic network. To improve the production of secondary metabolites, one of the main problems is the lack of basic knowledge of the biosynthetic routes and mechanisms responsible for the production of plant metabolites. The productivity of the desired metabolites is limited by the lack of particular precursors, and biotransformation using an exogenous supply of biosynthetic precursors may improve the accumulation of such compounds. Elicitation or compounds produced by the endophytic bacteria or stress also responsible for triggering the formation of secondary metabolites. The interdisciplinary intensive research efforts are required for identification of genes and enzymes involved in plant secondary metabolism of metabolic pathways leading to the biosynthesis of secondary metabolites. While coordinated induction of all genes is not easy, endophytic bacteria have the ability to modulate the primary product for the production of desired secondary metabolite for the survival of the host plant in normal as well as under stress condition.

References

Affendy H, Aminuddin M, Azmy M, Amini MA, Assis K, Tamir AT (2010) Effects of light intensity on *Orthosiphon stamineus* Benth treated with different organic fertilizers. Int J Agric Res 5:201–207

Ann Lila M (2006) The nature-versus-nurture debate on bioactive phytochemicals: the genome versus terroir. J Sci Food Agric 86:2510–2515

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399

Arbona V, Manzi M, Ollas CD, Gómez-Cadenas A (2013) Metabolomics as a tool to investigate abiotic stress tolerance in plants. Int J Mol Sci 14:4885–4911

Arvini P, Vafabakhsh J, Mazaheri D, Noormohamadi G, Azizi M (2012) Study of drought stress and plant growth promoting rhizobacteria (PGPR) on yield, yield components and seed oil content of different cultivars and species of brassica oilseed rape. Ann Biol Res 3(9):4444–4451

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216

Ballhorn DJ, Kautz S, Jensen M, Schmitt S, Heil M, Hegeman AD (2011) Genetic and environmental interactions determine plant defences against herbivores. J Ecol 99:313–326

Bilgin DD, Zavala JA, Zhu J, Clough SJ, Ort DR, De Lucia EH (2010) Biotic stress globally downregulates photosynthesis genes. Plant Cell Environ 33:1597–1613

Chamam A, Sanguin H, Bellvert F, Meiffren G, Comte G, Wisniewski-Dyé F (2013) Plant secondary metabolite profiling evidences strain-dependent effect in the *Azospirillum Oryza sativa* association. Phytochem Lett 87:65–77

Choudhary P, Yu JQ, Yamaguchi-Shinozaki K, Shinozaki K, Tran LS (2012) Benefits of brassino steroid crosstalk. Trends Plant Sci 17:594–605

Coutinho BG, Licastro D, Mendonça-Previato L, Cámara M, Venturi V (2015) Plant-influenced gene expression in the rice endophyte *Burkholderia kururiensis* M130. Mole Plant-Microbe Interact 28:10–21

Farag MA, Ryu CM, Sumner LW (2006) GC-MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants. Phytochemistry 67:2262–2268

Gershenzon J (1983) Changes in levels of plant secondary metabolites under water and nutrient stress. Recent Adv Phytochem 18:273–320

Ghasemzadeh A, Jaafar HZE, Rahmat A (2010) Elevated carbon dioxide increases contents of flavonoids and phenolic compounds, and antioxidant activities in Malaysian young ginger (*Zingiber officinale* Roscoe.) varieties. Molecules 15:7907–7922

González-Lamothe R, Gabriel M, Mariza G, Moussa SD, Malouin F, Bouarab K (2009) Plant Antimicrobial Agents and Their Effects on Plant and Human Pathogens. IJMS https://doi.org/10.3390/ijms10083400

Hendrik P, Karl JN, Peter B, Reich J, Oleksyn P, Poot Liesje M (2012) Biomass allocation to leaves, stems and roots: meta-analyses of inter specific variation and environmental control. New Phytol 193:30–50

Jha Y (2018) Induction of anatomical, enzymatic, and molecular events in maize by PGPR under biotic stress. In: Meena V (ed) Role of rhizospheric microbes in soil. Springer, Singapore

Jha Y, Subramanian RB (2012) Isolation of root associated bacteria from the local variety of rice GJ-17. World Res J Geoinformatics 1:21–26

Jha Y, Subramanian RB (2013) Paddy physiology and enzymes level is regulated by rhizobacteria under saline stress. J Appl Bot Food Qual 85:168–173

Jha Y, Subramanian RB (2016) Rhizobacteria enhance oil content and physiological status of *Hyptis suaveolens* under salinity stress. Rhizosphere 1:33–35

Jha Y, Subramanian RB (2018a) From interaction to gene induction: an eco-friendly mechanism of PGPR-mediated stress management in the plant. In: Egamberdieva D, Ahmad P (eds) Plant microbiome: stress response. Microorganisms for sustainability, vol 5. Springer, Singapore

Jha Y, Subramanian RB (2018b) Chapter 12: Effect of root-associated bacteria on soluble sugar metabolism in plant under environmental stress. In: Ahmad P, Ahanger MA, Singh VP, Tripathi DK, Alam P, Alyemeni MN (eds) Plant metabolites and regulation under environmental stress. Academic, pp 231–240

Jha Y, Subramanian RB, Patel S (2011) Endophytic bacteria induced enzymes against *M. grisea* in *O. sativa* under biotic stress. Afr J Basic Appl Sci 3:136–146

Jha Y, Sablok G, Subbarao N, Sudhakar R, Fazil MHU, Subramanian RB, Squartini A, Kumar S (2014a) Bacterial-induced expression of RAB18 protein in Oryza sativa salinity stress and insights into molecular interaction with GTP ligand. J Mol Recognit 27:521–527

Jha Y, Subramanian RB, Sahoo S (2014b) Antifungal potential of fenugreek coriander, mint, spinach herbs extracts against Aspergillus niger and *Pseudomonas aeruginosa* phyto-pathogenic fungi. Allelopath J 34:325–334

Karlidag H, Esitken A, Turan M, Sahin F (2007) Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of leaves of apple. Sci Hortic 114:16–20

Kuhnt M, Probstle A, Rimpler H, Bauer R, Heinrich M (1995) Biological and pharmacological activity and further constituents of *Hyptis verticillata*, planta. Medica 61:227–232

Mandal SM, Mandal KC, Day S, Pati BR (2007) Antimicrobial activity of the leaf extracts of *Hyptis suaveolens*. Indian J Pharm Sc 69:568–569

Meyer S, Cerovic ZG, Goulas Y, Montpied P, Demotes-Mainard S, Bidel LPR, Moya I, Dreyer E (2006) Relationships between optically assessed polyphenols and chlorophyll contents, and leaf mass per area ratio in woody plants: a signature of the carbon–nitrogen balance within leaves? Plant Cell Envirn 29:1338–1348

Moreira ACP, Lima EO, Wanderley PA, Carmo ES, Souza EL (2010) Chemical composition and antifungal activity of *Hyptis suaveolens* (L.) Poit leaves essential oil against *Aspergillus* species. Braz J Microbiol 41:28–33

Müller D, Stelling J (2009) Precise regulation of gene expression dynamics favors complex promoter architectures. PLoS Comput Biol 5(1):e1000279

Nejat N, Mantri N (2017) Plant immune system: crosstalk between responses to biotic and abiotic stresses the missing link in understanding plant defense. Curr Issues Mol Biol 23:1–16

Osakabe Y, Yamaguchi-Shinozaki K, Shinozaki K, Tran LS (2014) ABA control of plant macroelement membrane transport systems in response to water deficit and high salinity. New Phytol 202:35–49

Pieterse CMJ, León-Reyes A, Van der Ent S, Van Wees SCM (2009) Networking by small-molecule hormones in plant immunity. Nat Chem Biol 5:308–316

Roze LV, Chanda A, Linz JE (2011) Compartmentalization and molecular traffic in secondary metabolism: a new understanding of established cellular processes. Fungal Genet Biol 48:35–48

Shulka A, Abad Farooqi AH, Shukla YN, Sharma S (1992) Effect of triacontanol and chlormequat on growth, plant hormones and artemisinin yield in Artemisia annuaL. Plant Growth Regul 11:165–171

Suza R, Valio IFM (2003) Leaf optical properties as affected by shade in samplings of six tropical tree species differing in successional status. Braz J Plant Physiol 15:49–54

Tuteja N, Sopory SK (2008) Chemical signaling under abiotic stress environment in plants. Plant Signal Behav 3:525–536

Wu M, Kubota C (2008) Effects of high electrical conductivity of nutrient solution and its application timing on lycopene, chlorophyll and sugar concentrations of hydroponic tomatoes during ripening. Sci Hortic 116:122–129