Volatiles as priming agents that initiate plant growth and defence responses

Devendra Kumar Choudhary, Bhavdish N. Johri* and Anil Prakash

Department of Biotechnology and Bioinformatics Centre, Barkatullah University, Bhopal 462 026, India

Plants are exposed to attack by pathogens and insect pests. For protection against this stress, plants have evolved defensive strategies to counteract potential invaders. In the present review comparisons are drawn between herbivores-induced plant volatiles as an elicitor of plant defences and two other classes of signalling molecules, C6 green-leaf volatiles and C4 bacterial volatiles, which appear to prime plant defences thereby enhancing the capacity to mobilize cellular defence responses when plants are faced with herbivore/pathogen attacks. Several chemical changes in the soil are associated with plant growth-promoting rhizobacteria wherein particularly, the volatile components, 2,3-butanediol and acetoin are released extensively that trigger the maximum level of growth promotion.

Keywords: Defence response, plant growth, plant volatiles, primary agents.

PLANTS require a broad range of defence mechanisms to effectively combat invasion by microbial pathogens or attack by herbivorous insects. These mechanisms include pre-existing physical and chemical barriers and inducible defence responses that become activated upon attack by pathogens and insect pests. A concerted action of these defensive activities helps the plants minimize the damage caused by the attacker[1]. Plant resistance against herbivores and pathogens is subject to extensive phenotypic plasticity, which enables individuals to change their phenotype in response to the environmental conditions. Such phenotypic changes reflect upon interactions between members of a community. Plants are able to respond towards herbivore attack by defensive mechanisms which directly affect the herbivore, by maintenance or attraction of carnivores (predators and parasitoids). The phenotypic changes in plants caused by induced defences can vary with the type of attack. Symbiotic and pathogenic microorganisms that associate with the plant might play an important role in the induction process. The adaptiveness of phenotypic plasticity in terms of induced response depends on the balance of its physiological and ecological costs and benefits. Therefore, an integrative approach is necessary to consider ecological, molecular and chemical aspects for an indepth knowledge of induced defence[2].

Different attackers evoke varied plant responses because of specific elicitors or on account of wounding. Many resistance traits in plants are expressed in response to a first 'eliciting' attack[3]. Plant responses towards herbivory can be induced either by the feeding activity of the herbivore, or by deposition of egg of the herbivorous insect[4]. Interaction between plants and microorganisms results in changes in plant phenotype that can affect association between plants and microorganisms. Microorganisms living in the soil significantly affect plant quality that reflects on the aboveground community and thus mediate effects of the first trophic level on higher trophic levels, e.g. AM fungi determine the composition of animal communities on plants[5,6]. De novo synthesis of phytoalexins and pathogenesis-related (PR) proteins, and changes in cell-wall composition are associated with induced systemic resistance (ISR) or systemic acquired resistance (SAR) against the pathogen. Increased de novo production of secondary compounds is closely related with induced resistance (IR) against herbivores. IR can also be achieved indirectly by the attraction of enemies of the plants by volatile organic compounds (VOCs)[7–9].

Plants have a way to express themselves, but this is not a common perception. This is achieved mainly by emitting odours and chemicals; composition and intensity of these odours can carry information about the physiological status of the plants and on the stresses that they are being subjected to. It is well known that plants encounter multiple biotic and abiotic challenges simultaneously in their natural habitat. An effective spatial and temporal defence response is generated by plants against microbial pathogens and herbivore attack. Plants must be able to identify and prioritize each signalling pathway to exert the most appropriate defence strategy in order to minimize current and future damage, thereby preserving vegetative and reproductive growth. These complex biochemical and physiological responses result in tolerance or protection from further environmental challenges. Plants employ constitutive and induced defences to protect themselves from pathogen and herbivore attack[10].

Volatiles: General characteristics

Plants release a complex blend of volatiles in response to herbivore injury or microbial pathogens that provide

valuable cues for herbivores and their natural enemies (Table 1). Volatiles emitted by healthy or infested plants are used by herbivores to discriminate between host and non-host plants and assess the density of feeding insects on a plant. They can also serve as an attractant for herbivore predators and parasites. Healthy plants release volatiles into the atmosphere, but wounding and herbivore feeding change the volatile blend released by the plant. Various factors are responsible that influence constituents of volatile blends: (1) the herbivore species and its development stage; (2) plant species, genotype and age, and (3) environmental stress. Plants release volatiles in two phases in response to arthropods or mechanical damage, wherein several of them are released immediately (within 1 h) after injury.

Volatiles have diverse structures and arise from the activities of several biochemical pathways, wherein the most commonly released volatiles include C6 volatiles (lipoxygenase/hydroperoxide lyase-dependent pathways), indole and MeSA (the shikimic acid/tryptophane pathway), cyclic and acyclic terpenoids (isoprenoid pathway), and oximes and nitriles (derived from amino acids)[25]. The release of terpenoid and C6 volatiles is strongly influenced by the emission of linolenic and linoleic acids from the membrane. These C18 fatty acids provide substrates for the synthesis of jasmonic acid (JA), C6 green-leaf volatiles (GLVs) or insect-modified lipid elicitors for volatile production. Interestingly, JA treatment of plants induces the release of volatiles that are not emitted by healthy plants and also releases a complex blend of terpenoid volatiles in *Phaseolus lunatus*, maize and *Gerberaja mesonii*, which are not equivalent to those released after herbivore feeding, e.g. the carnivorous mite (*Phytoseiulus persimilis*) is attracted to volatiles from JA-treated plants relative to healthy plants and the mite plants infested with their prey, *Tetranychus urticae*[26]. The quantitative and qualitative differences in the volatiles released from mite-infested and JA-treated plants provide important cues to natural spider-mite enemies.

Table 1. Diversity and impact of volatiles on herbivore–plant interactions

Volatiles	Impact on herbivores and plant gene expression	Reference
trans-2-hexenal	Reduces aphid fecundity	65
	Reduces spider-mite fecundity	66
	Increase in *LOX* and *PAL* RNAs	67
	Reduces *Manduca sexta* feeding	68
cis-3-hexenol	Reduces aphid fecundity	65
Hexanol	Reduces aphid fecundity	65
	Increase in *LOX* RNAs	67
Hexanal	Reduces spider-mite fecundity	66
	Deters *M. sexta* feeding	68
cis-3-hexenal	Increase in *LOX* RNAs	67
trans-3-hexenyl acetate	Attractant for the cabbage aphid	69
C6 volatiles mixture	Attractant for Colorado potato beetle	70

VOCs play an important role in attracting parasitic and predatory insects that are natural enemies of herbivores and this mechanism is called 'indirect induced defence'. Besides, VOCs can serve as airborne signals that induce resistance in the neighbouring, unharmed plants. Considerable research has been done to identify key volatiles in VOC-induced resistance. Apart from methyl jasmonate itself, particularly the C6 GLVs were found to trigger jasmonate-dependent defence reaction. The VOC-induced defence activity remained rather moderate when compared to insect attack or elicitors from insect regurgitant[24]. Recently, Engelberth *et al.*[20] have provided a more plausible mechanism of VOC-induced resistance. They discovered that the activity of three GLVs, (Z)-3-hexenal, (Z)-3-hexen-1-ol, and (Z)-3-hexenyl acetate was not so much reflected in their ability to activate defence directly, but rather by a priming effect that leads to an augmented defence expression upon stress treatment. Maize plants pretreated with these C6 volatiles accumulated higher levels of endogenous JA upon elicitation and produced enhanced amounts of VOC upon treatment with caterpillar regurgitant, whereas other typical VOCs from maize failed to trigger priming in the plant. Available information shows that priming by VOC is a common defence strategy in plants, although the identity of VOC triggering the priming may vary among plant species.

C6 GLVs prime plant defence response

C6-volatiles produced by the catalytic activity of hydroperoxide lyase (HPL), have been reported in all green tissues and are considered as the earliest component released from the damaged leaves. The biosynthesis of GLVs from an 18-C fatty acid precursor involves two enzymatic steps, catalysed by lipoxygenase (LOX) and HPL; depending on the degree of saturation of the substrate, HPL produces either (Z)-3-hexenal or (Z)-3-hexanal. Alcohol dehydrogenase (ADH) which is an isomerization factor (IF), and/or acetylation leads to the production of other C6 volatiles, including (E)-2-hexenal, (E)-2-hexenol, (Z)-3-hexenol, hexenol, and (Z)-3-hexenyl acetate. The C12-component is processed to the plant-wounding signal, traumatin (12-oxo-E-10-dodecanoic acid) via 12-oxo-Z-9-dodecanoid acid intermediate[23].

Emission of GLVs from leaf tissue triggers responses in neighbouring plants[23]. C6 volatiles influence plant–herbivore and plant–pathogen interactions at several levels. First, C6 volatiles stimulate expression of wound-response gene. Second, they reduce aphid fecundity, spider-mite fecundity and caterpillar feeding. Third, C6 volatiles are used as attractants for beetle and aphids, and finally, they have antimicrobial and antifungal activity at biologically relevant concentration[26]. Exogenous application of (E)-2-hexanal to *Arabidopsis* seedling induces a group of genes that closely mimic methyl jasmonate (MeJA) induction as

well as trigger the upregulation of LOX and phenylalanine ammonia lyase (PAL) genes. Moderate level of VOC emission and gene induction on exposure of plants to herbivore damage presents the possibility of an indirect role for C6 components in triggering plant defences. The signalling role and metabolic turnover of (E)-2-hexenal as well as other C6 volatile components in triggering plant VOC emission has come into focus primarily based on: (i) the ability of C6 volatile component to prime neighbouring plants against impending herbivory, and (ii) the deactivation and exportation of C6 components from the leaf tissue by acetylation of C6 component in planta[27].

What role do GLVs play in priming the chemical defence of a plant? JA, an endogenous signalling molecule which reflects direct and indirect defence responses in plants has been monitored in insect-treated corn plants with or without pretreatment with C6 VOCs[23]. The elevation of JA in GLV-pretreated plants remained higher over a period of 3 h. The GLV-pretreated plants released ca. 4 µg of total VOCs at 4–6 h post-induction compared to 2.4 µg from non-primed control plants[20].

Emission of volatiles by plants in response to herbivory

Several eliciting chemicals from plant pathogens are known for a long time, whereas only few elicitors released by herbivores have been identified. New researches have shown that indirect defences of plants are mediated by the release of plant volatiles. In fact, plants emit an enormous spectrum of VOCs that are highly specific for the species and age of both the plant and the herbivore[11]. Several of these VOCs are constitutively emitted by undamaged healthy plants, but herbivore damage commonly induces plants to emit larger amounts, which also leads to *de novo* synthesis of several VOCs. Such herbivore-induced plant volatiles (HIPVs) can be emitted by various parts of the plant, including leaves, flower buds and roots. Not only does feeding by herbivorous insects induce the release of HIPVs, even deposition of eggs by such herbivorous insects can induce the plant to emit HIPVs[12]. Herbivores induce several well-characterized plant defences and wound-response pathways, including novel pathways, to alter plant gene expression. They produce signals (elicitors) to activate plant gene expression and volatile synthesis[13].

Characteristics of HIPVs and the induction process

HIPV blends are highly complex and consist of a variety of divergent VOCs, including alkanes, alkenes, aldehydes, alcohols, ketones, ethers, esters and carboxylic acids. HIPVs play a central role in mediating interactions between plants and herbivores, herbivores and their natural enemies, plants and microorganisms and also among

themselves. *In vitro* studies have shown that HIPVs deter oviposition by arthropod herbivores, attract natural enemies of these herbivores and even induce defence genes and VOC emission in neighbouring plants and prime such plants to respond faster to future herbivore attack[12]. Induction and emission of HIPVs depends on the interaction between biotic factors, viz. plant hormones, herbivore-derived elicitors, associated microorganisms and abiotic factors, e.g. wounding, O_3 and CO_2 concentration, temperature, light and UV.

Investigations of herbivore-induced plant volatiles have made considerable progress during the last one decade. Analytical chemical investigations have helped in elucidating the plant as the producer of volatiles which have been characterized after initial studies at the behavioural level, demonstrating that herbivore-induced volatiles attract carnivorous arthropods. At the mechanistic level, analytical, chemical and biochemical approaches have provided information on signal-transduction pathways involved in the biosynthesis of the induced volatiles[8]. Behavioural studies have demonstrated that the volatiles affect several other interactions in the food web, viz. plant–plant and plant–herbivore interactions[36]. Several research groups have initiated these studies employing molecular approaches, wherein chemical ecology of induced plant volatiles has entered a new and exciting phase. The issue of HIPVs has been subject to several recurring questions in the past 10 years[37].

The increasing interest in molecular genetics, which also extends to the field of chemo-ecology, is likely to provide new tools to address evolutionary questions accurately. Several major questions are in focus for plant volatiles[38]: (i) What is the nature of the chemical blend that attracts a predator and parasitoid, and how does variation in blend composition affect responses by these attackers? (ii) What is the impact of HIPVs on the composition of the animal community? (iii) Can plants change the emission of induced volatiles affected by environmental conditions influenced by carnivores? (iv) What is the effect of carnivore attraction on plant fitness? (v) To what extent do HIPVs affect interactions among competing plant? (vi) How important are the HIPVs in the natural ecosystem?

There is a current boom in interest among ecologists to include the belowground interactions in studies of plant and associated organisms; VOCs have also been found to be involved in tritrophic signalling belowground. A root-specific *Arabidopsis* terpene synthase[42] has been characterized that was responsible for the formation of the volatile monoterpene 1,8-cineole, with possible involvement in belowground interaction. One more VOC has been identified, the so called 'sesquiterpene' and (E)-β-caryophyllene, as a belowground herbivore-induced volatile signal that attracts entomopathogenic nematodes which infect and kill larvae of the corn root worm, *Diabrotica virgifera*, a voracious pest[12].

HIPVs induce indirect defence in neighbouring plants

A large number of plants have the ability to respond to leaf damage with the induction of a variety of defences. Plants may also respond to herbivory with the emission of volatile chemicals in addition to leaf toxins[14]. From a phytocentric viewpoint, the attraction of carnivores (predators and parasitoids) to plants under attack by virtue of the release of HIPVs, has been classified as a form of indirect plant defence[22]. HIPVs decrease the negative consequences of plant fitness caused by herbivorous attack. Attraction of insects to plants and other host organisms involves detection of specific semiochemicals, i.e. natural signal chemicals mediating changes in behaviour and development of plants[15]. Such chemicals provide information about the status of attack of the emitting plant and its use not only at higher trophic levels, but also of neighbouring plants of the same or another species[16,17]. An increasing number of investigations of different plant species suggests that plants can perceive volatile signals which are reflected in changes in transcription of defence-related genes[18]. Further studies have revealed that exposure to herbivore-induced (HI) VOCs results in changes in the abundance of phytohormones and increased production of defence-related metabolites, e.g. terpenoids, proteinase inhibitors and phenolic compounds[19,20,21].

HIPVs and plant–plant interaction

Kost and Heil[22] have reported a new mechanism of communication between herbivore-damaged and undamaged plants growing under natural conditions, wherein they have employed Lima bean plant (*Phaseolus lunatus* L., Fabaceae) to investigate whether HI VOC induced another indirect defence strategy, i.e. secretion of extrafloral nectar (EFN) in conspecific plant neighbours and whether this enhanced the defence status of the receiving plant under natural conditions. EFN secretion was induced by VOCs released from herbivore-damaged bean tendrils as well as by a synthetic VOC blend that resembled the natural one, wherein the GLV, i.e. (3Z)-hex-3 enyl acetate, one of the constituents of the HI blend, was sufficient to elicit the defence reaction. The reduction in herbivore damage is obvious in case of predators that kill their prey instantly, leaving little room for doubt about the benefit to the plant; but this is not true in all cases. No studies have actually been undertaken that quantify the effect of carnivore attraction on plant fitness. In case of plant–spider mite predation, mite system benefit seems obvious as without predatory mites decimating the spider-mite population, the plant is overexploited and dies before producing seeds. A recent study that quantified plant fitness in terms of the number of seeds produced showed

that parasitization of the caterpillar *Pieris rapae* (Lepidoptera: Pieridae), which is a specialized herbivore of Brassicaceae, by the specialized solitary braconid parasitoid *Cotesia rubecula* (Hymenoptera: Braconidae) resulted in considerable fitness benefit for *Arabidopsis thaliana* plant[8].

Functional diversity of HIPVs

It is stated that plants release secondary metabolites against herbivore attack and other parasites that are considered being associated with defence. VOCs that are emitted by plants show a puzzling diversity, wherein VOCs formed as a result of by-products of plant processes are emitted to the atmosphere by virtue of their volatility, with no apparent function[32]. Yet several volatile compounds act as signals for plant protection and communication and the so-called HIPVs described or synonymously called 'inducible volatile organic compounds' (IVOCs) are emitted from plant foliage after herbivore damage[33,34]. It has been investigated that these novel compounds can act as an indirect plant defence by repelling nonspecific herbivores or by attracting predators and parasitoids of herbivores[35,36]. In addition, HIPV/IVOC emissions are induced by ozone (O_3) and fungal pathogens wherein mould infection can enhance the attraction of parasitoids to herbivore-damaged plant[36,37,38].

Functional role of IVOCs

Knowledge of constitutive and inducible VOCs is ambiguous, wherein constitutive VOCs released from healthy, intact plants can become inducible volatiles after foliar damage[34,39]. The advantage of novel IVOCs is that they are *de novo* synthesized only when needed and therefore they are more economical in the context of carbon usage that does not reduce plant fitness[8]. There is a broad diversity of known IVOCs that include alkenes, alkanes, carboxylic acids and alcohols, but among them dominating compounds tend to be terpenes and C6 GLVs[39].

In response to herbivore damage GLVs, viz. (Z)-3-hexenyl acetate are released from younger intact leaves, indicating that the LOX pathway that oxidizes catabolic products of fatty acids, has been activated[41]. Monoterpenes constitute about 100% of the total VOC emissions from intact cabbage plants, which is below 60% in herbivore-damaged plants wherein emission of novel inducible compounds, viz. homoterpenes and sesquiterpenes takes place[34]. Sesquiterpenes are emitted from flowers[42], but considerable amounts can also be emitted from the foliage of herbivore-damaged plants[34], although the concentration is much lower in intact plants[43]. It has been reported that sesquiterpenes are produced at high levels in O_3-resistant tobacco immediately after O_3 exposure[44].

Functional levels of IVOCs

There are distinctive functions between plant physiology and the atmosphere for all biogenic VOCs that are derived from IVOCs, which are highly reactive with O_3 and OH^- radicals that have several functions in intra- and interspecific communication[45,46]. Four functional levels of IVOCs have been reported (Figure 1).

(i) *Plant tissue level:* Herbivore[19] and ozone damage[48] in plants induces production of volatile plant hormone ethylene; if its perception is chemically halted, then O_3-induced cellular damage in the leaves increases[49]. Ethylene acts as a switching molecule that stimulates the production of JA and IVOCs by reducing the production of constitutive defence compounds such as nicotine after herbivore damage[46]. Herbivore-damaged wild-type *Arabidopsis* plants induce greater production of IVOCs, e.g. the sesquiterpene β-caryophyllene and the monoterpenes, than do damaged jasmonate-deficient plants[50].

(ii) *Plant surface level:* Plant pathogens induce the production of IVOCs that inhibit the spread of pathogen into plant tissues because of their antimicrobial activities[36,51]. Leaf boundary-layer thickness is dependent on laminar and turbulent air flow as well as on leaf size and structure. Low volatile compounds, viz. monoterpenes and sesquiterpenes are more reactive with O_3 than with iso-

prene in the atmosphere[45,52]. In several plant species[37,44], induced production of monoterpenes, sesquiterpenes and homoterpenes starts after exposure to high concentration of O_3.

(iii) *Ecosystem level:* The most thoroughly investigated aspect of IVOCs is their induction by herbivore damage and their role as semiochemicals for parasitoids of herbivores[40]. Volicitin reported in the saliva and regurgitate of insects is the inductor of IVOCs, whereas ethylene is a plant hormone volatile reported in herbivore-damaged plants that stimulates the production of IVOCs[47,53].

IVOCs released under laboratory conditions after herbivore damage induce the production of IVOCs in nearby healthy plant or activate genes that are responsible for the production of these compounds[54,55]. Plant-to-plant signalling in the field has failed over distances >10 cm, yet signals have the capacity to attract predators and parasitoids[56] over distances greater than 10 cm, e.g. *Nicotiana attenuate* plants are able to reduce herbivore damage by 90% by attracting predators when the plants are grown 3–5 cm apart, but herbivore damage is reduced by only 20% when the plants are grown >20 m apart[33].

(iv) *Atmospheric level:* More than 80% of plant-emitted VOCs are chemically reactive and can regulate the oxidative capacity of the troposphere, including CO, O_3, and aerosol[57]. Total VOC emission from herbivore-damaged plants could be 2.5-fold higher than that from intact plants[34]. Several IVOCs can participate in the formation of phytotoxic O_3 in the presence of sunlight together with high concentration of nitrogen oxides from urban sources. The precipitation of nitrogen during O_3 formation might compensate for the cost of O_3-induced tissue damage after O_3-phytotoxicity to the plant[58]. The equilibrium between OH radicals, O_3 and plant-emitted terpenes varies in atmospheric processes. Bonn and Moortgat[52] reported that the low volatile sesquiterpenes emitted from plants are mainly responsible for the reaction between VOCs and O_3 and for fine-particle aerosol formation in remote areas. Sufficient data are not available in the context of aerosol formation processes that relate to VOC emission in nature[59].

4. Atmosphere level
Control of temperature, humidity and irradiation levels with aerosols and enhanced cloudiness

3. Ecosystem level
Communication with conspecifics and other trophic levels

2. Leaf surface level
Protection against harmful gases and biotic organisms

1. Tissue level
Protection against cellular damage

$+O_3 + NO_3 + OH$

(E)-Ocimene   DMNT   (E)-β-Farnesene
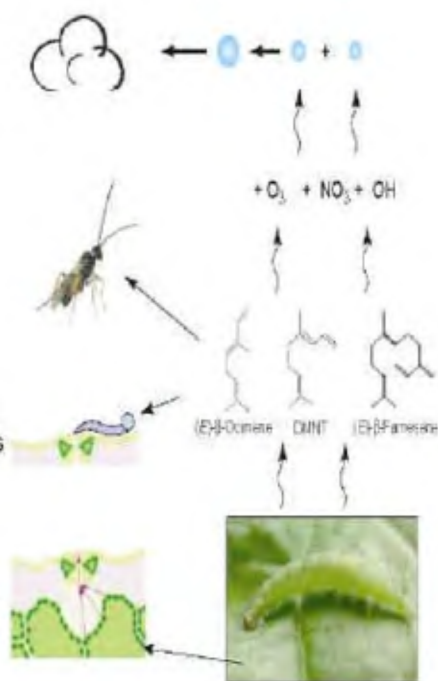
Figure 1. A model of four functional levels of phytogenic inducible volatile organic compounds. From Holopainen[64]. Copyright Elsevier. Reproduced with permission.

C4 bacterial volatiles trigger plant defences

The rhizosphere supports diverse microorganisms that stimulate the growth of plants; in this category are placed the so-called plant growth-promoting rhizobacteria (PGPR) that maintain soil health by employing a wide variety of mechanisms including nitrogen fixation, enhanced solubilization of P, and phytohormone production[72]. Among bacteria, pseudomonads have been considered to be important rhizosphere organisms, wherein considerable research is underway globally to exploit the potential of

one such group of bacteria that belongs to fluorescent pseudomonads (FLPs). FLPs help in maintenance of soil health and protect the crops from pathogens. Gaur et al.[73] reported that 50–60% of FLPs recovered from the rhizosphere and endorhizosphere of wheat grown in the Indo-Gangetic Plains were growth promontory. Field trial of pseudomonad strain GRP$_3$ in particular, has been investigated[74] extensively, in which significant increase in yield varied from 5.6 to 18%. Strain GRP$_3$ and associated pseudomonads have provided interesting information concerning iron acquisition and control of plant disease in a variety of crops. For example, Sharma et al.[75] have reported that Pseudomonas sp. could alleviate the problem of iron non-availability, particularly in calcareous soils by incorporation of siderophore-producing strains such as GRP$_3$. In a study of iron nutrition in mung bean (Vigna radiata L. Wilzeck), bacterization of seeds resulted in increased peroxidase and lowered catalase activity in the roots. Co-treatment of plants with 10 μM Fe-citrate along with GRP$_3$ resulted in improved chlorophyll content as well suggesting the operation of heterologous siderophore uptake system in mung bean. Under in vitro assay conditions, this pseudomonad has been found antagonistic against zoosporic pathogens, Pythium aphanidermatum and Phytophthora nicotianae that cause occurrence of pre- and post-emergence damping-off in chile and tomato[76]. Several of these pseudomonads were found to produce exocellular rhamnolipids that could intercalate the zoosporic membrane, thus hindering disease development. A series of rhamnolipids of the strain GRP$_3$ have now been characterized employing NMR and mass spectrometric analysis[77].

While the microbiological processes occurring in the top few centimetres of the earth's surface determine the existence of life, the opacity of the soil has traditionally precluded knowing the functional attributes of many resident communities that make up the belowground biodiversity (BGBD). This scenario has recently changed, since one can now see the opaque material through lasers and can study the communities in situ utilizing the tools of 'new biology'. Johri et al.[71] elucidated the means of employing soil microorganisms to optimize the productivity of the soil, through an understanding of the microbial processes related to BGBD.

Role of bacterial volatiles in ISR

The role of volatiles of microbial origin as signal molecules for plant defence has come to light recently. Plants have the ability to acquire enhanced level of resistance to pathogens after exposure to biotic stimuli provided by different PGPR. These in association with plant roots elicit a steady state of defence or ISR in plants. This is often referred to as rhizobacteria-mediated ISR. PGPR-elicited ISR was initially observed in carnation, common

bean and cucumber with reduced susceptibility to Fusarium wilt, halo blight, and Colletotrichum orbiculare respectively. PGPR[28] that colonize root systems with seed applications protect plants against foliar disease include Pseudomonas fluorescens, P. putida, Bacillus pumilus, and Serratia marcescens. A network of interconnected signalling pathways regulates induced defences of plants against pathogens. The primary components of the network are plant signal molecules – salicylic acid (SA), JA, ethylene, and probably nitric oxide. Exogenous application of these often results in higher level of plant resistance to pathogens. Signal transduction leading to ISR has been seen to be triggered by several low-molecular weight volatile compounds of microbial origin in the rhizosphere that may even include any of the above molecules. It has been suggested that signal transduction leading to ISR requires responsiveness to both JA and ethylene. Methyl jasmonate (MeJA) and the ethylene precursor, i.e. 1-aminocyclopropane-1-carboxylate (ACC) are effective in inducing resistance against phytopathogenic microflora. It is postulated that ethylene signalling is required at the site of application of inducers which are involved in the generation or translocation of the systematically transported ISR signals. Mutation in plant signalling pathways points to an active role by SA or JA and/or ethylene in activating ISR. Mediation of ethylene levels by microbial ACC plays a key role in signal transduction, viz. the ISR-inducing P. fluorescens bacterium enhances bacterial ACC-converting capacity and leads to potentiated level of ethylene emission in Arabidopsis, infected by a phytopathogen. Volatile signals generated by certain non-pathogenic bacteria have also been shown to trigger defence responses in Arabidopsis[29,30]. JA and ethylene act in concert in activating the defence responses. JA and derivatives induce the expression of genes encoding defence-related proteins, e.g. thionins and proteinase inhibitors, whereas ethylene activates several members of the pathogenesis-related (PR) gene superfamily. They also act synergistically in stimulating elicitor-induced PR gene expression and systematically induce defence responses.

ISR by airborne bacterial signal(s)

Rhizospheric emissions of VOCs by PGPR present the complication of de-adsorbing low-molecular weight compounds from the soil matrix, instead of airborne VOCs, viz. C6 GLVs that can be easily sampled by head-space collection of the living plants. Ryu et al.[31] examined the role of airborne bacterial metabolites in triggering ISR by growing PGPR and Arabidopsis seedlings on separate sides of divided petri dishes. ISR was activated by exposure of Arabidopsis seedlings to VOCs from the Bacillus sp., on continuous exposure for as short as 4 days, by a significant reduction in symptomatic leaves inoculated

with the soft rot-causing pathogen, *Erwinia carotovora*. VOCs collected from growth-promoting bacteria, *B. subtilis* (strain GB03) and *B. amyloliquefaciens* (strain IN937a) showed consistent difference in the composition of volatile blends compared to VOCs that were recovered from non growth-promoting bacterial strain DH5α. Strains GB03 and IN937a consistently released[30] two most abundant compounds (Figure 2): 2,3-butanediol and 3-hydroxy-2-butanone (acetoin), while these metabolites were not released from DH5α or water-treated MS media. Several other VOCs were also observed, including dodecane, 2-undecanone, 2-tridecanone, 2-tridecanol and tetramethyl pyrazine from a complex bacterial bouquet that did not exhibit ISR priming activity[31]. Bacteria employ different mechanisms to produce VOCs, for example, in *Bacillus* sp., 2,3-butanediol and acetoin were produced under low atmospheric O_2 partial pressure to provide an alternative electron sink for the regeneration of NAD^+, when usual respiration was not possible.

No disease protection was observed when *Bacillus* strains were genetically blocked for the production of 2,3-butanediol. This confirmed the priming activity of the compound to induce resistance against disease. The involvement of known signalling pathways in *Arabidopsis* was screened by exposing defined mutants and transgenic plant lines to bacterial emissions containing VOCs, especially 2,3-butanediol. ISR triggered by GB03 VOC was independent of SA, NPR1, and JA signalling pathways, but was more or less mediated by ethylene. Interestingly, ISR activation

by strain IN937a was independent of all the signalling pathways and this opens up the possibility of involvement of additional VOCs that utilize alternative pathways to trigger ISR.

Ryu *et al.*[31] conducted petri-dish assays, wherein they exposed the whole plant to the plume of bacterial VOCs, which reflects as to whether the site of plant VOC perception is above or belowground for soil-grown plants. The sphere of microbial VOCs for rhizobacteria could be within the soil or above ground and the possibility existed that VOCs are produced at sufficient levels for aerial tissues to perceive and respond to bacterial volatiles. Alternatively, an endogenous signal or signal transports information from the root zone to the aerial portion of the plant and this necessitates the presence of some mobile messenger within the plant because of the systemic nature of induced resistance.

The transcriptional responses of over 8000 genes have been surveyed for rhizobacteria-mediated ISR to study the mechanism of systemic defence responses that are triggered by PGPR[22]. A substantial change was observed in the expression of almost 100 genes which were associated locally in the roots, and none of the genes tested showed consistent change in expression in response to effective colonization of the roots. Onset of ISR in the leaves is not associated with detectable change in gene expression because of invariant pattern in transcript profiles. When PGPR-treated plants were challenged with a bacterial leaf pathogen, an augmented expression pattern in ISR-expressive leaves was observed in over 80 genes. This suggests a priming mechanism triggered by plant exposure to PGPR, which allowed the plant to respond faster and more strongly upon pathogen attack.

Conclusion

It is interesting to analyse whether VOCs are by-products of various plant processes or they are actively produced and used as a sophisticated 'language' by plants to pursue communication with other organisms[32,55]. Plants are capable of disseminating information to their environment by employing VOCs and they have the capacity to change the growth condition employing reactive VOCs. No doubt plants have evolved the capacity to release and detect VOCs in their environment; the emission of plant odours transmits signals to other organisms and members of its own species. VOCs released from flowers are well-known attractants of insect pollinators and C6 volatiles induced with tissue damage are potent infochemicals for aphids and other herbivore insects[60]. Plant VOCs convey signals between neighbours, whereby defence mechanisms are induced in undamaged plants because of volatiles produced by nearby infested plants. These infochemicals comprise of specific plant volatiles, MeSA, MeJA, and cis-jasmonate[61]. It has been reported that plant components
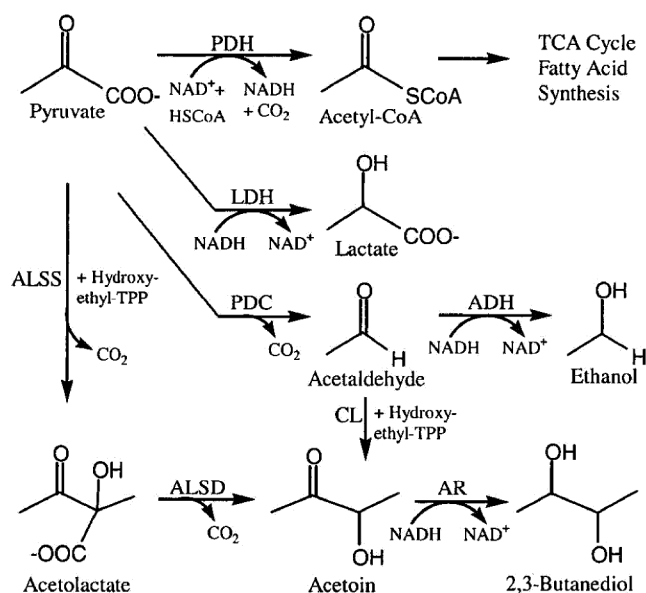
Figure 2. Proposed pathways for anaerobic fermentation in *Bacillus subtilis* (modified from Meiners *et al.*[11]). Enzymes with known coding genes include pyruvate dehydrogenase (PDH), lactate dehydrogenase (LDH), pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), acetolactate synthase (ALSS), acetolactate decarboxylase (ALSD), and acetoin reductase (AR). From Ryu *et al.*[30]. Copyright *Proc. Natl. Acad. Sci. USA*. Reproduced with permission.

containing six C atom, e.g. (E)-2-hexenal induce the expression of defence-related genes in intact plants, which can be rapidly emitted from damaged plant tissues[62]. In addition, a defensive role for terpenes as volatile elicitors has been discussed in excised Lima bean (*Phaseolus lunatus*)[62].

Several reports have discussed the role of microorganisms interacting to trigger salubrious plant responses, e.g. nitrogen fixation, SAR and growth promotion beside several deleterious outcomes, viz. soft rot and chlorosis. Other reports have probed the role of microbial VOCs where they trigger biochemical changes of either primary or secondary plant metabolism. It has been envisaged that selected *Bacillus* PGPR strains emit VOCs that can elicit plant defences. VOCs and the subset of collected VOCs have been reapplied as airborne chemicals to *Arabidopsis* seedlings which contain sufficient chemical information to trigger ISR, as measured by the ability of the seedlings to resist infection.

Biotic and abiotic elicitors have been reported to induce elevated VOC emission in many plant species. This discussion brings out the following moot questions: (i) Can bacteria be induced to trigger elevated levels of VOCs? (ii) Was the medium used to optimize for VOCs emission from the bacterial strains tested? (iii) How do changes in bacterial growth conditions influence emission profiles?

The medium conditions (0.5% (w/v) agar, 1.5% (w/v) sucrose, and 0.4% (w/v) TSA) have been tested so far to optimize for low-medium VOCs emissions. This medium was abundant in sugar, where oxygen did not act as limiting factor initially. It became limiting with microbial growth when the petri dishes were covered and sealed with parafilm. The environment wherein these bacteria reside in the soil is influenced by low amount of oxygen; thereby leading to pyruvate metabolism and the production of 2,3-butanediol and acetoin. Reduction of acetoin to 2,3-butanedion regenerates NAD^+ in its oxidized form. It is interesting to note that the enzymes for generating 2,3-butanediol and acetoin have been identified in tobacco (*Nicotiana tabacum*), corn (*Zea mays*), carrot (*Daucus carota*) and rice (*Oryza sativa*) cultures[31].

Piterse et al.[28] discussed a model for signal transduction in PGPR-mediated ISR using mutant lines of *Arabidopsis* and *P. fluorescens* strain WCS417r, wherein ISR triggered by PGPR was dependent on JA, ethylene, and *Npr1* (a regulatory gene which encodes salicylate dehydrogenase), and it was independent of SA. It appears that VOCs from strain IN937a triggered ISR through an ethylene-independent signalling pathway, whereas VOCs from strain GB03 appear to operate through an ethylene-dependent pathway. Characterization by Ton et al.[63] of an *ISr1* locus involved in ISR signalling in *Arabidopsis* by genetic and inhibitor studies found that rhizobacteria-mediated ISR does not require JA signalling, although this locus does encode a component of the ethylene res-ponse which is required for the expression of rhizobacteria-activated ISR.

1. Pieterse, C. M. J., Ton, J. and van Loon, L. C., Cross-talk between plant defence signalling pathways: Boost or burden? *Agric. Biotechnol. Network*, 2001, **3**, 1–8.
2. Dicke, M. and Hilker, M., Induced plant defences: From molecular biology to evolutionary ecology. *Basic Appl. Ecol.*, 2003, **4**, 3–14.
3. Heil, M., Ecological costs of induced resistance. *Curr. Opin. Plant Biol.*, 2002, **5**, 1–6.
4. Hilker, M., Rohfritsch, O. and Meiners, T., The plant's response towards insect egg deposition. In *Chemoecology of Insect Eggs and Egg Deposition* (eds Hilker, M. and Meiners, T.), Blackwell Publishers, Berlin, 2002, pp. 205–233.
5. Paul, N. D., Hatcher, P. E. and Taylor, J. E., Coping with multiple enemies: An integration of molecular and ecological perspectives. *Trends Plant Sci.*, 2000, **5**, 220–225.
6. Gange, A. C., Stagg, P. G. and Ward, L. K., Arbuscular mycorrhizal fungi affect phytophagous insect specialism. *Ecol. Lett.*, 2002, **5**, 11–15.
7. Dixon, R. A., Natural products and plant disease resistance. *Nature*, 2001, **411**, 843–847.
8. Dicke, M. and van Loon, J. J. A, Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomol. Exp. Appl.*, 2000, **97**, 237–249.
9. Heil, M., Koch, T., Hilpert, A., Fiala, B., Boland, W. and Linsenmair, K. E., Extrafloral nectar production of the ant-associated plant, *Macaranga tenarius*, is an induced, indirect, defensive response elicited by jasmonic acid. *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 1083–1088.
10. Ryan, C. A., The systemic signalling pathway: Differential activation of defensive genes. *Biochim. Biophys. Acta*, 2000, **1477**, 112–122.
11. Meiners, T., Westerhaus, C. and Hilker, M., Specificity of chemical cues used by a specialist egg parasitoid during host location. *Entomol. Exp. Appl.*, 2000, **95**, 151–159.
12. Alessandra, M. D. and Turlings, T. C. J., Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. *Analysis*, 2006, **131**, 24–32.
13. van de Ven, W. T. G., Le Vesque, C. S., Perring, T. M. and Walling, L. L., Local and systemic changes in squash gene expression in response to silverleaf whitefly feeding. *Plant Cell*, 2000, **12**, 1409–1423.
14. Arimura, G. I., Kost, C. and Boland, W., Herbivore-induced, indirect plant defences. *Biochim. Biophys. Acta*, 2005, **1734**, 91–111.
15. Pickett, J. A. et al., Plant volatiles yielding new ways to exploit plant defence. In *Chemical Ecology: From Gene to Ecosystem* (eds Dicke, M. and Takken, W.), Springer, The Netherlands, 2006, pp. 161–173.
16. Karban, R. and Maron, J., The fitness consequences of interspecific eavesdropping between plants. *Ecology*, 2002, **83**, 1209–1213.
17. van Poecke, R. M. P. and Dicke, M., Indirect defence of plants against herbivores: Using *Arabidopsis thaliana* as a model plant. *Plant Biol.*, 2004, **6**, 387–401.
18. Paschold, A., Halitschke, R. and Baldwin, I. T., Using 'mute' plants to translate volatile signals. *Plant J.*, 2006, **45**, 275–291.
19. Tscharntke, T., Theissen, S., Dolch, R. and Boland, W., Herbivore induced resistance and interplant signal transfer in *Alnus glutinosa*. *Biochem. Syst. Ecol.*, 2001, **29**, 1025–1047.
20. Engelberth, J., Alborn, H. T., Schmelz, E. A. and Tumlinson, J. H., Airborne signals prime plants against insect herbivore attack. *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 1781–1785.
21. Ruther, J. and Kleier, S., Plant–plant signalling: Ethylene synergizes volatile emission in *Zea mays* induced by exposure to (z)-3-hexen-1-ol. *J. Chem. Ecol.*, 2005, **31**, 2217–2222.

22. Kost, C. and Heil, M., Herbivore-induced plant volatile induce an indirect defence in neighbouring plants. *J. Ecol.*, 2006, **94**, 619–628.

23. Paré, P. W., Farag, M. A., Krishnamachari, V., Zhang, H., Ryu, C. M. and Kloepper, J. W., Elicitors and priming agents initiate plant defence responses. *Photosyn. Res.*, 2005, **85**, 149–159.

24. Conrath, U. *et al.*, Priming: Getting ready for battle. *Mol. Plant–Microbe Int.*, 2006, **19**, 1062–1071.

25. Dicke, M., Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? *Entomol. Exp. Appl.*, 1999, **91**, 131–142.

26. Walling, L. L., The myriad plant response to herbivores. *J. Plant Growth Reg.*, 2000, **19**, 195–216.

27. Farag, M. A., Fokar, M., Abol, H., Zhang, H., Allen, R. D. and Paré, P. W., (Z)-3-Hexenol induces defence genes and downstream metabolites in maize. *Planta*, 2005, **220**, 900–909.

28. Pieterse, C. M. J., van Wees, S. C. M., Ton, J., Van Pelt, J. A. and van Loon, L. C., Signaling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. *Plant Biol.*, 2002, **4**, 535–544.

29. Hase, S., Van Pelt, J. A., van Loon, L. C. and Pieterse, C. M. J., Colonization of *Arabidopsis* roots by *Pseudomonas fluorescens* primes the plant to produce higher levels of ethylene upon pathogen infection. *Physiol. Mol. Plant Pathol.*, 2003, **62**, 219–226.

30. Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Wei, H. X., Paré, P. W. and Kloepper, J. W., Bacterial volatiles promote growth in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 4927–4932.

31. Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Wei, H. X., Kloepper, J. W. and Paré, P. W., Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol.*, 2004, **134**, 1017–1026.

32. Peñuelas, J. and Llusiá, J., Plant VOC emissions: Making use of the unavoidable. *Trends Ecol. Evol.*, 2004, **19**, 402–404.

33. Kessler, A. and Baldwin, I. T., Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, 2001, **291**, 2141–2144.

34. Vourinen, T., Reddy, G. V. P., Nerg, A-M. and Holopainen, J. K., Monoterpene and herbivore-induced emissions from cabbage plants grown at elevated atmospheric CO_2 concentration. *Atmos. Environ.*, 2004, **38**, 675–682.

35. Heil, M., Direct defence or ecological costs: Responses of herbivorous beetles to volatiles released by wild Lima bean (*Phaseolus lunatus*). *J. Chem. Ecol.*, 2004, **30**, 1289–1295.

36. Cardoza, Y. J., Teal Peter, E. A. and Tumlinson, J. H., Effect of peanut plant fungal infection on oviposition preference by *Spodoptera exigua* and on host-searching behaviour by *Cotesia marigiventris*. *Environ. Entomol.*, 2003, **32**, 970–976.

37. Vourinen, T., Nerg, A-M. and Holopainen, J. K., Ozone exposure triggers the emission of herbivore-induced plant volatiles, but does not disturb tritrophic signalling. *Environ. Pollut.*, 2004, **131**, 305–311.

38. Piel, J., Atzorn, R., Gäbler, R., Kühnemann, F. and Boland, W., Cellulysin from the plant parasitic fungus *Trichoderme viridae* elicits volatile biosynthesis in higher plants via the octadecanoid signalling cascade. *FEBS Lett.*, 1997, **416**, 143–148.

39. Van Den Boom, C. E. M., Van Been, T. A., Posthumus, M. A., De Grout, A. and Dicke, M., Qualitative and quantitative variation among volatile profiles induced by *Tetranychus urticae* feeding on plants from various families. *J. Chem. Ecol.*, 2004, **3**, 69–89.

40. Dudareva, N., Pichersky, E. and Gershenzon, J., Biochemistry of plant volatiles. *Plant Physiol.*, 2004, **135**, 1893–1902.

41. Chen, F, Tholl, D., D'Auria, J. C., Farooq, A., Pichersky, E. and Gershenzon, J., Biosynthesis and emission of terpenoid volatiles from *Arabidopsis* flowers. *Plant Cell*, 2003, **15**, 481–494.

42. Weins, J. A., Cates, R. G., Rottenberry, J. T., Cobb, N., Van Horne, B. and Redak, R. A., Arthropod dynamics on Sagebrush (*Artemisia tridentate*) – Effect of plant chemistry and avian predation. *Ecol. Monogr.*, 1991, **61**, 299–321.

43. Heiden, A. C. *et al.*, Emission of volatile organic compounds from ozone-exposed plants. *Ecol. Appl.*, 1999, **9**, 1160–1167.

44. Atkinson, R. and Arey, J., Gas-phase tropospheric chemistry of biogenic volatile organic compounds: A review. *Atmos. Environ.*, 2003, **37**, 197–219.

45. Kahl, J., Siemens, D. H., Aerts, R. J., Gäbler, R., Kühnemann, F., Preston, C. A. and Baldwin, I. T., Herbivore-induced ethylene suppresses a direct defence but not a putative indirect defence against an adapted herbivore. *Planta*, 2000, **210**, 336–342.

46. Vahala, J., Keinänen, M., Schützendübel, A., Polle, A. and Kangasjärvi, J., Differential effects of elevated ozone on two hybrid aspen genotypes predisposed to chronic ozone fumigation. Role of ethylene and salicylic acid. *Plant Physiol.*, 2003, **132**, 196–205.

47. Overmeyer, K., Brosché, M. and Kangasjärvi, J., Reactive oxygen species and hormonal control of cell death. *Trends Plant Sci.*, 2003, **8**, 335–342.

48. Thaler, J. S., Farag, M. A., Paré, P. W. and Dicke, M., Jasmonate deficient plants have reduced direct and indirect defence against herbivores. *Ecol. Lett.*, 2002, **5**, 764–774.

49. Huang, J., Cardosa, Y. J., Schmelz., E. A., Raina, R., Engelberth, J. and Tumlinson, J. H., Differential volatile emissions and salicylic acid levels from tobacco plants in response to different strains of *Pseudomonas syringae*. *Planta*, 2003, **217**, 767–775.

50. Bonn, B. and Moortgat, G. K., Sesquiterpene ozonolysis: Origin of atmospheric new particle formation from biogenic hydrocarbons. *Geophys. Res. Lett.*, 2003, **30**, 1585.

51. Gouinguené, S. P. and Turlings, T. C. J., The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiol.*, 2002, **129**, 1296–1307.

52. Arimura, G. I., Ozawa, R., Shimoda, T., Nishioka, T., Boland, W. and Takabayashi, J., Herbivore-induced volatiles elicit defence genes in Lima bean leaves. *Nature*, 2000, **406**, 512–515.

53. Dicke, M., Agrawal, A. A. and Bruin, J., Plants talk but are they deaf? *Trends Plant Sci.*, 2003, **8**, 403–405.

54. Karban, R., Marun, J., Felton, G. W., Ervin, G. and Eichenseer, H., Herbivore damage to sagebrush induces resistance in wild tobacco: Evidence for eavesdropping between plants. *Oikos*, 2003, **100**, 325–332.

55. Peñuelas, J. and Llusiá, J., BVOCs: Plant defence against climate warning? *Trends Plant Sci.*, 2003, **8**, 105–109.

56. Lerdau, M. and Slobodkin, L., Trace gas emissions and species dependent ecosystem services. *Trends Ecol. Evol.*, 2002, **17**, 309–312.

57. Kulmala, M., How particles nucleate and grow. *Science*, 2003, **302**, 1000–1001.

58. Berkitt, M. A. *et al.*, New roles for cis-jasmone as an insect semiochemical and in plant defence. *Proc. Natl. Acad. Sci., USA*, 2000, **97**, 9329–9334.

59. Weber, H., Fatty-acid derived signals in plants. *Trends Plant Sci.*, 2002, **7**, 217–224.

60. Gatehouse, J. A., Plant resistance towards insect herbivores: A dynamic interaction. *New Phytol.*, 2002, **156**, 145–169.

61. Ton, J., Davison, S., van Wees, S. C. M., van Loon, L. C. and Pieterse, C. M. J., The *Arabidopsis* ISR1 locus controlling rhizobacteria-mediated ISR is involved in ethylene signalling. *Plant Physiol.*, 2001, **125**, 652–661.

62. Holopainen, J. K., Multiple functions of inducible plant volatiles. *Trends Plant Sci.*, 2004, **9**, 529–533.

63. Hildebrand, D. K., Brown, G. C., Jackson, D. M. and Hemilton-Kemp, T. R., Effects of some leaf-emitted volatile compounds on aphid population increase. *J. Chem. Ecol.*, 1993, **19**, 1875–1887.

64. Kasu, T., Brown, G. C. and Hildebrand, D. F., Formation of lipoxygenase products in *Phaseolus vulgaris* L. leaves as a response to two-spotted spider mite (Acari: Tetranychidae) feeding and

their effect on spider mite populations. *J. Kans. Entomol. Soc.*, 1995, **68**, 27–34.

65. Bate, N. J. and Rothstein, S. J., C6-volatiles derived from the lipoxygenase pathway induce a subset of defence-related genes. *Plant J.*, 1998, **16**, 561–569.

66. Avdiushko, S. A., Brown, G. C., Dahlman, D. L. and Hildebrand, D. F., Methyl jasmonate exposure induces insect resistance in cabbage and tobacco. *Environ. Entomol.*, 1997, **26**, 642–654.

67. Visser, J. H., Piron, P. G. M. and Hardie, J., The aphid's peripheral perception of plant volatiles. *Entomol. Exp. Appl.*, 1996, **80**, 35–38.

68. Bolter, C. J., Dicke, M., van Loon, J. J. J., Visser, J. H. and Posthumus, M. A., Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *J. Chem. Ecol.*, 1997, **23**, 1003–1023.

69. Johri, B. N., Choudhary, D. K. and Chaudhuri, S., How to use soil microorganisms to optimize soil productivity. In *Food and Water Security* (ed. Aswathanarayana, U.), Taylor & Francis/Balkema, The Netherlands, 2007, pp. 51–62.

70. Barea, J. M., Pozo, M. J., Azćon, R. and Azćon-Aguilar, C., Microbial co-operation in the rhizosphere. *J. Exp. Bot.*, 2005, **56**, 1761–1778.

71. Gaur, R., Shani, N., Kawaljeet, Johri, B. N., Rossi, P. and Aragno, M., Diacetyl phloroglucinol-producing *Pseudomonas* do not influence AM fungi in wheat rhizosphere. *Curr. Sci.*, 2004, **86**, 453–457.

72. Johri, B. N., Technology development and demonstration of a new bacterial inoculant (GRP3) for improved legume production, Project Report, Government of Uttar Pradesh, 2001.

73. Sharma, A., Johri, B. N., Sharma, A. K. and Glick, B. R., Plant growth-promoting bacterium *Pseudomonas* sp. strain GRP_3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzek). *Soil Biol. Biochem.*, 2003, **35**, 887–894.

74. Sharma, A., Wray, V. and Johri, B. N., Rhizosphere *Pseudomonas* sp. strains reduce occurrence of pre- and post-emergence damping-off in chile and tomato in Central Himalayan Region. *Arch. Microbiol.*, 2007, **187**, 321–335.

75. Sharma, A., Jansen, R., Nimtez, M., Johri, B. N. and Wray, V., Rhamnolipids from the rhizosphere bacterium *Pseudomonas* sp. GRP_3 that reduces damping-off disease in chile and tomato nurseries. *J. Nat. Prod.*, 2007, **70**, 941–947.

MEETINGS/SYMPOSIA/SEMINARS

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Manipal College of Pharmaceutical Sciences
Madav Nagar
Manipal 576 104
Ph: 0820-2922482
E-mail: cpe.mcops@manipal.edu

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Contact: Dr K. Anbalagan
Director
The Electrophoresis Institute (Biotech)
Yercaud 636 601
Ph: 04281-222626
Fax: 04281-222256
Mobile: 9443222480
E-mail: phoresis@hotmail.com