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HORMONAL SIGNALING IN SHAPING ROOT MICROBIOME : A REVIEW

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ABSTRACT

Plant development and productivity rely upon microorganisms related to plant roots. These rhizospheric microbes are involved in many processes that help in improvement of agricultural soil productivity, including preservation of soil structure, nutrient cycling, disease control and degradation of pollutants. Understandably, any information about possible strategies for manipulation of soil microbial communities to increase crop productivity is highly relevant. Application of microbial strains to extend benefits to plants has clearly demonstrated their advantage in agriculture. Rhizosphere is an important focus of studies for molecular biologists, ecologists and plant biologists to further their understanding of plant microbe interactions occurring in this complex zone. In this context, the current research sector is focused on areas of 'omics' such as proteomics, metabolomics, transcriptomes and secretomics. The information obtained from these 'omics' studies can assist in visualization of a complete picture of complex plant microbe interactions. Direct outcome of this interaction is application of beneficial microbial communities in the rhizosphere to enhance crop yields and disease suppression. In this review, the brief overview of underground interactions of plant-microbe communications and the role plant hormones is aimed. Many rhizospheric microorganisms beneficial for plant growth are widely reported. They include bacteria like *Pseudomonas*, *Burkholderia* and fungi like *Trichoderma* and *Gliocladium.* Chemotacit signaling enables soil bacteria to sense and react towards chemical compounds released by plant roots. It provides a competitive advantage to bacteria in colonization of plant root surfaces which is a precondition for the organization of beneficial associations. Chemical signals include Salicylic acid (SA), Jasmonic acid (JA), Ethylene (ET) and Flavonoids among others. SA plays a role as stress messenger to counter the invading organisms by initiating hypersensitive response in the plant. It also fashions the development of endophytic microflora. It has a role in the regulation of root colonization, by Arbuscular Mycorrhizal fungi (*Glomus mosseae or Glomus intraradices*) and also in identifying compatible bacterial strain for colonization, as evidenced in various studies. Both JA and SA activate defense system against pathogen`s attack in plants whereas JA and ET are important regulators of Induced Systemic Resistance (ISR) and they trigger defense response in association with each other. Flavonoids also play a role in shaping of rhizospheric communities, cause chemoattraction of rhizobia towards the root, inhibit root pathogens and mediate allelopathic interactions between plants. The genetic determinants for nodulation in *Rhizobium meliloti* have been found to be activated by flavonoids. The article also outlines the importance of 'omics' approach to conceptualize a complete picture of below ground communication between microbes and plants. This information can be exploited for enhanced plant nutrient acquisition, yield, better stress tolerance, etc as is elucidated in the last section.

Keywords **:** Plant-microbe interaction, root endophytic microbes, salicylic acid, jasmonic acid, flavonoids, ethylene.

Introduction

The demographic figures show that 83 million people are added to the world population every year. It has led to increase in the demand for agricultural productivity which is expected to rise from current 59% to 98% by 2050 (Elferink *et al.*, 2016). Attention must be directed towards issues faced by agriculture sector and improving the technologies which help in

making the world food secure. There are many obstacles in achieving food security such as poverty, undernourishment, expected population growth, economic growth, changing consumption patterns, the required boost in agricultural production, climate change and water scarcity etc. Mainly, cereals crops are consumed in different forms as food all over the world. Wheat, rice and maize, the main cereal crops of world, meet more than 50% calorie intake of man. Wheat is grown and harvested from 214 million hectares' area followed by rice and maize which are grown on 154 million hectares area and 140 million hectares area, respectively (Brar and Khush, 2013).

In conventional agriculture there is enormous dependence on chemical fertilizers and pesticides (Bhardwaj *et al.,* 2014). Chemical fertilizers are industrially controlled products which contain known amount of nitrogen, phosphorous and potassium. They are vital for productivity in agriculture but their misuse causes air and ground water contamination by eutrophication of water bodies (Youssef and Eissa, 2014). Environmental hazards caused by chemical fertilizers have fueled the demand for biological based organic fertilizers. Organic farming is a practice that uses organic inputs in cultivation. It adds to the biodiversity of soil (Megali *et al.,* 2013). One important tool of organic farming is biofertilizers which contain microbes and/or plant nutrients or even particular organic components which help in stimulation of microbial activity and thereby increase mobilization of nutrients from soil directly or indirectly. A variety of miroorganisms are used as biofertilizers, *Azotobacter* and *Rhizobium help in nitrogen fixation process, Pseudomonas sp., Bacillus subtilis* and *Bacillus megaterium play role in phosphorous solubilization.* Sulphur is solubilized by bacterial genus *Thiobacillus* (Mohammadi and Sohrabi, 2012).

Microbes in soil

Soil ecosystem and its functions are important for soil sustainability and crop production. The knowledge of the soil functions plays an important role in improving agricultural practices and conservation methods. Moreover, the change in the relative diversity and functionality of soil microbial communities affect nutrient cycling processes and the organization and efficiency of natural plant communities (Morgan *et al.,* 2005). These microbial communities influence mineral solubilisation and mobilization processes. Different groups have been reported from various natural and extreme environments worldwide as plant microbiomes (epiphytic, endophytic and rhizospheric). These beneficial microbiomes enhance plant growth and improve plant nutrition uptake through solubilisation of phosphate, potassium and zinc, nitrogen fixation and other mechanisms including siderophore production, plant growth promotion, etc.

Rhizospheric bacteria

Rhizosphere is area around roots in the soil where maximum level of bacterial activity is found. Some rhizospheric microbiomes of particular crops are given in Table I. The beneficial microorganisms promote plant development and protect plant against pathogens through induction of many mechanisms (Mendes *et al.,* 2013). Several genera found in rhizosphere having plant growth promoting (PGPR) traits are *Agrobacterium, Alcaligenes, Arthrobacter, Actinoplanes, Azotobacter, Azospirillium, Bacillus, Pseudomonas* sp., *Rhizobium, Bradyrhzobium, Erwinia, Enterobacter, Amorphosporangium, Cellulomonas, Flavobacterium, Streptomyces* and *Xanthomonas* (Mohammadi and Sohrabi 2012). Rhizospheric fungi from Deuteromycetes include *Trichoderma* and *Gliocladium.* These microbes help plant in stimulation of root expansion, disease prevention and control of abiotic stress (Mendes *et al.,* 2013). Proteobacteria phylum has been detected in the rhizosphere of many plant species- rice and wheat (Breidenbach *et al.,* 2016); Maize (Peiffer *et al.,* 2013); potato and sugar beet (Mendes *et al.,* 2011); oat (Mendes *et al.,* 2011). Both α and γ –proteobacteria and actinomycetes are associated with rhizospheric soils of pea and faba beans (Sharma *et al.,* 2005).

Endophytic bacteria

Microorganisms that reside in plant tissues without any negative effects to their host are known as endophytes (Gaiero *et al.,* 2013). The endophytic microbiomes present within the plant are different from the rhizospheric microbiomes of plant which suggest that plants have great influence on composition of the endophytic microflora. Endophytes are classified into three categories on the basis of plant inhabiting life strategies (Hardoim *et al.,* 2008). Obligate endophytes are unable to live outside the host and are transferred through seed not via rhizosphere. Facultative endophytes are those which are capable of living outside the host but will colonize the plant root when opportunity comes and third ones are passive endophytes that do not vigorously look for colonizing the plant, but it enters the plant on stochastic events like open wounds near root vicinity. Four system essential for driving endophytic community structure are: (1) soil aspects that decide survival (2) plant factors that influence compatibility and colonization (3) ability of microbes to compete for entry and

survival within the root. Based on these factors different crops have their own endophytic microbiome composition. *Azorhizobium* is a well-known endophyte of both rice and wheat. Rice is also colonized by *Pseudomonas, Azospirillum, Bacillus, Pantoea* and *Herbaspirillum*. *Bacillus, Micrococcus, Flavobacterium* have been detected in the internal tissue of wheat. *Enterobacter* is associated with maize crop which is inhabited by other Gram positives as *Arthrobacter* and *Corynebacterium*. Potato endophytic microbiome is dominated by Gram negatives as *Pseudomonas, Flavobacterium, Xanthomonas* and *Agrobacterium* (Table II).

Endophytes facilitate plant growth through three main processes: phytostimulation, biofertilization and biocontrol (Bloemberg and Lugtenberg, 2001). Their potential as microbial inoculants reduce the need for chemicals such as pesticides and fertilizers which is very important step in development of sustainable agricultural practices. Additionally, the distribution of endophytes within the plant depends upon capability of microbe to colonize and also availability of the plant resources (Gaiero *et al.*, 2013).

Pathogenic bacteria

Plant pathogens present in soil are the main cause in the reduction of productivity of food, feed, fiber and fuel crops. These plant pathogens depend upon host for reproduction, development and for establishment in the rhizosphere. Pathogens can survive successfully in soil by formation of protective structures. The harmful microorganisms present in soil include fungi such as *Fusarium oxysporum*, *Verticillium* spp. and *Rhizoctonia solani* and oomycetes include *Phythuim* spp. and *Phytophthora* spp. and nematodes. (Coninck *et al.*, 2014). These pathogens cause damping off in seedlings which restrict root development, stunted growth, wilting and plant death.

The pathogenic fungi, oomycetes and also nematodes are agronomically more important than plant pathogenic bacteria in temperate climates. *Aphanomyces euteiches* is a root pathogenic oomycete which infects many legumes and cause major loss of economy in temperate regions (Mathesius, 2009). It causes browning of roots along with decrease in root weight. Oospores appear from infected roots which afterwards re-infect other plants. Another group of destructive oomycete which infect many plants is *Phytophthora* sp (Tyler *et al.*, 2006). The bacterial genera such as *Pectoacterium*, *Ralstonia* cause substantial economic damage in some crops. *Agrobacterium tumefaciens*, *Ralstonia solanacearum*, *Dickeya dadanthi* and *Dickeya solani* and *Pectobacterium carotovorum* and *Pectobacterium atrosepticum*, all attack plant via roots (Mansfield *et al.*, 2012). *Agrobacterium* sp. and protist *Plasmodiophora brassicae* are root infecting microbes that induce gall formation in roots (Coninck *et al.*, 2014). Viruses also infect plants with the help of vectors like nematodes or zoosporic fungi for invasion in roots (Macfarlane, 2003). Various crops and infecting plant pathogens in rhizosphere are listed in Table III.

Importance of microbes in soil for fulfillment of needs of plant

The soil supports upper half of the plant through its root system as it grows. It provides all the necessary minerals and elements which are important for plant development. Plant needs maximum of 17 nutrients for its growth, development and reproduction (Morgan *et al.*, 2005). Out of these, 14 are sourced from the soils which includes six macronutrients such as Nitrogen, Potassium, Phosphorous, Sulphur, Magnesium and Calcium and eight microutrients namely Boron, Chlorine, Copper, Iron, Manganese, Molybedenum, Nickel and Zinc. These nutrients are taken up by the plant from soil in their ionic form. The microbes present in soil interact with plant and help them in acquiring essential mineral nutrients and prevent the accumulation of toxic elements (Morgan *et al.*, 2005).

In soil ecosystem, biological nitrogen fixation (BNF) is considered to be an important process which maintains nitrogen balance. In this process, nitrogen fixing bacteria convert elemental nitrogen (N_2) to plant usable forms (NH_3). Some free-living nitrogen fixing bacteria are *Azotobacter*, *Azospirillium* and blue-green algae. Symbiotic nitrogen fixers include *Rhizobium*, *Frankia* and *Azolla* (Gupta, 2004). In agronomic practices, *Rhizobium* inoculants are used for nitrogen fixation in legumes. For rice cultivation biofertilizer having *Azolla* strains are used in many countries such as Vietnam, China, Thailand and Philippines. It is shown that co-inoculation of *Pseudomonas* and *Bacillus* strains along with *Rhizobium* spp. stimulates development, nodulation and nitrogen fixation in Chickpea (Mohammadi and Sohrabi, 2012). Phosphate Solubilising Bacteria (PSB) solubilizes fixed phosphorous in soil, which converts inorganic phosphorous (unavailable) to soluble forms HPO_4^{2-} and $H_2PO_4^-$ (available) with the help of processes such as organic acid production, chelation and ion exchange reactions. The most powerful phosphate solubilizers are *Pseudomonas*, *Bacillus*, *Rhizobium*, *Enterobacter* and some fungal strains *Penicillium* and *Aspergillus* (Mohammadi and Sohrabi, 2012). Potassium is another vital nutrient which play key role in soil

fertility and plant growth. It is present in very small concentration in majority of soils, ranging between 0.04%-3.0%. Moreover, 98% of potassium is bound within phyllosilicates structure which is the layer of silicate minerals found in slit and clay fractions of the soil. The other 2% exists in soil solution or on exchange sites to become available for plants (Shelobolina *et al.*, 2014). The ability to oxidize Fe^{2+} from primary phyllosilicates mineral, in such a way that they release iron and potassium from these minerals, is present in only some of the microbial strains (Shelobolina *et al*., 2014). The strains which help in potassium mobilization are *Bradyrhizobium japonicum, Cupriavidus necator, Ralstonia solanacearum, Dechloromonas agitate,* and *Nocardioides species.* These microbes release acids which lead to potassium mobilization from potassium-containing minerals (e.g. mica, biotite, kaolinite and smectite) and chelation of silicon ions (Sheng and He, 2006).

The concentration of iron on earth surface is very high and it is mostly found in ferric (Fe^{3+}) form. It is essential micronutrient which plays role in soil fertility and is required by all the living organisms. The Ferrous (Fe^{2+}) form is oxidized to ferric (Fe^{3+}) form in soil thereby forming insoluble compounds and leaving little amount of iron for microbial or plant assimilation (Ma, 2005). In the rhizosphere, tough competition exists among bacteria, fungi and plant for acquiring iron. Because of this, few strains of bacteria produce low-molecular mass proteins known as siderophores (Vansuyt *et al.*, 2007). These molecules have high affinity to chelate (Miethke and Marahiel, 2007) and solubilize iron from mineral or organic compounds. Siderophores showed high affinity with Fe^{3+} to form complexes. The Gram-positive and Gram-negative bacteria reduce ferric ions (Fe^{3+}) to ferrous (Fe^{2+}) by transport complexes through cell membrane. By this process siderophores solubilize iron from unavailable minerals or organic compounds to available form (Rashid *et al.,* 2016).

Phytohormones such as auxins, cytokinins, gibberellins and ethylene are produced by plant growth promoting rhizobacteria (PGPR). Among plant growth regulators most common is indole acetic acid (IAA) (Sharma *et al.,* 2003). The production of indole acetic acid by plant growth promoting rhizobacteria involves indermediate formation such as indole-3-pyruvic acid and indole-3-acetic aldehyde. This pathway is the most common in bacteria like *Pseudomonas, Rhizobium, Bradyrhizobium, Agrobacterium, Enterobacter and Klebsiella* (Shilev, 2013). The biosynthesis of other plant hormones (cytokinins or gibberellins or both) are carried out by several plant growth promoting rhizobacteria as *Azotobacter sp., Rhizobium sp., Pantoea agglomerans, Rhodospirillum rubrum, Pseudomonas fluorescens, Bacillus subtilis* and *Paenibacillus polymyxa* (Etesami *et al.,* 2009). PGPR also produce antibiotics through a process called as antibiosis. It is considered to be one of the most powerful biocontrol mechanisms against phytopathogens (Shilev, 2013). *Pseudomonas* species produce samphisin, 2,4-diacetylphloroglucinol (DAPG), oomycin A, phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone, and cyclic lipopeptides (Lugtenberg and Kamilova, 2009) *Bacillus*, *Streptomyces*, and *Stenotrophomonas* sp also produce antibiotics such as oligomycin A, kanosamine, zwittermicin A, and xanthobaccin to inhibit the proliferation of plant pathogens (Gupta *et al*., 2015).

Plant-microbe associated interactions

Plants release high amount of carbon compounds which act as chemo-attractants for microbes and help in directing the microbe's movement in the stream of chemical gradients. This movement is commonly referred to as chemotaxis and plays an essential part in initiation of communication between plant roots and microbes. The components released by plant roots interact with microbes in positive, negative and neutral ways (Morgan *et al.,* 2005). *Pseudomonas*, *Rhizobium* and *Agrobacterium* species are known to increase their root colonization efficiency in the rhizosphere due to chemo-tactic response (Haichar *et al.*, 2014).

Bacterial chemotaxis provides a competitive advantage to bacteria in colonization of plant root surfaces which is a precondition for the organization of beneficial associations. Motile soil bacteria sense and act in response to different chemical compounds released by plant roots. The root exudates and rhizo-deposits released by plant roots form nutrient gradient in the soil which chemo-tactically attract bacteria (Badri *et al.,* 2009). The root hair zones, the root tips and the points of emergence of secondary roots are the sites where root colonization is initiated due to plentiful amount of root exudates released in these regions (Scharf *et al.,* 2016). The composition of root exudates varies with plant species, stage of development, or environmental conditions (Lakshmanan *et al.,* 2014).

Root exudates constitute flaked cells of root cap, mucilage, and soluble and non-soluble exudates, which may contain free amino acids, proteins, carbohydrates, alcohols, vitamins, hormones (Bacilio-Jimenez *et al.,* 2003) sugars, aromatic acids, aliphatic acids, fatty acids, sterols, phenolics, enzymes, proteins, plant

growth regulators and secondary metabolites (Badri *et al.,* 2009). The mechanism of secretion is mainly of two types: passive or active. The passive method has been followed by low molecular weight organic compounds. Secondary metabolites, polysaccharides, proteins, phenolic compounds are released by active transport (Huang *et al.,* 2014). These compounds attract beneficial microbes towards them and prevent invasion by pathogens. Root exudation composition is also influenced by bacteria and fungi colonizing the plants (Matilla *et al.,* 2010). It is reported that Arbuscular Mycorrhizal Fungi (AMF) change plant root exudation qualitatively e.g. increasing secretions of gibberellins, phenolics and nitrogen compounds and reduction in potassium ions concentration, phosphorous and total sugars concentration (Jones *et al.,* 2004). In *Arabidopsis thaliana* it was observed that the plant which were inoculated with *Pseudomonas putida* KT2440 had different root exudation composition in comparison to plant in which *P. putida* was not present (Matilla *et al.,* 2010).

Compounds present in root exudates are instrumental in selective recruitment of rhizospheric bacteria (Rudrappa *et al.,* 2008). Flavonoids represent one of the largest classes of phenylpropanoid derived secondary metabolites in plants and constitute a large proportion of root exudates. Isoflavones present in soyabean roots draw the symbiont *B. japonicum.* Mycorrhizal spore germination and hyphal branching is stimulated by flavonoids (Hassan and Mathesius, 2012). In nodulation, species-specificity is due to structural variations in the rhizobia-derived Nod factors, together with variations in the plant-derived-signal flavonoids. *Rhizobium* lives in specialized organs in legumes and helps the plant in uptake of nitrogen in the form of ammonia (Oldroyd and Downie, 2008). Recognition of AM fungi and rhizobia by plant roots is essential for colonization. When Nod factors are produced, the flavonoids are released by plant roots to attract rhizobia which upon perception stimulate nodulation in plant (Radutoiu *et al.,* 2017). Phenylpropanoids plays role in defense and prevention of plant from pathogen invasion. In Barley, fungal infection with *Fusarium graminearum* is resisted by rapid accumulation and secretion of phenylpropanoids.

Many microorganisms show symbiotic or competitive behavior to other microbe groups for their establishment in plant roots by perception and translation of signals (Lareen *et al.,* 2016). They produce secondary metabolites to compete with other microbes present in the rhizosphere for the survival or for invasion in plant roots (Lareen *et al.,* 2016). These include antibiotics, toxins, lytic enzymes and siderophores (Bais *et al.,* 2006). Microorganisms influence plant to alter the composition of root exudates and to secrete their favourable root exudates for selective enrichment of specific microorganisms in the rhizosphere (Lareen *et al.,* 2016). It is evident that antimicrobial compounds and secondary metabolites also have an essential role in development of microbiome in the rhizosphere.

Mechanism of plant-microbe interactions

Interaction between the induced strain and host plant trigger successful induction of plant defense (Van Wees *et al.,* 2008). Different rhizospheric bacteria are involved in initiation of Induced Systemic Resistance (ISR) in variety of plants species when invading microbes are either beneficial or pathogenic (Bakker *et al.,* 2007). Mechanisms used by the plant to find a connection between defense and root microbiome development share some general features (Gutjahr *et al.,* 2009). SAR and ISR are two different types of defense signalling pathway. Plant hormones as Salicylic acid (SA) and Jasmonic acid (JA) play role in both beneficial and pathogenic communication. SA is a signal molecule which has important role in regulation of pathogen-induced Systemic Acquired Resistance (SAR) (Van Wees *et al.,* 2008). SA dependent defense is effective against biotropic pathogens which inhabit live plant (Glazebrook 2005). JA /ET (Ethylene) are signal molecules which play important role in Induced Systemic Resistance (ISR) which give resistance against necrotrophic pathogens which kills plant cell. *Alternaria brassicicola* is a necrotrophic fungus which is resisted by ISR (Induced Systemic Resistance) defense signaling but not by SAR (Systemic Acquired Resistance) signaling pathway. Turnip crinkle virus is biotropic and is resisted by SAR and not by ISR (Ton *et al.,* 2002).

It has been revealed that SA level increases in roots of wild-type rice and pea at early stages of Arbruscular Myccorhiza (AM) interaction due to formation of hyphopodia on the root surface (Blilou *et al.,* 2000). The increase in SA seen at early stages of AM colonization in *Arabdopsis* is due to presence of fungal strains in roots which trigger defense-gene (Liu *et al.,* 2003). Similar results were observed in wild type pea roots and alfa alfa roots when they were inoculated with *Rhizobium* strain. Inoculation with mutant strain defective in Nod factor production abolished symbiont recognition by this plant. The documented literature statuses that when symbiont invades the roots then the plant defense system involving SA is triggered. Later, production of SA ceases to facilitate colonization. However, the defense system remains active if the

symbiont is perceived as a pathogenic at early stages of interaction (Gutjahr *et al.*, 2009).

JA has been reported to be secreted by roots, as in soyabean and wheat in the rhizosphere, showing that microorganism present in soil near roots might be exposed to JA molecules. The direct effect of JA on AM fungi is not known. *Rhizobia* induced expression of Nod genes and production of Nod factors has been observed upon exogenous application of JA and methyl jasmonate (MeJA). The combination of JA and flavonoids showed enhanced Nod factor production (Mabood *et al.*, 2006).

Plant Hormones in signaling

Flavonoids

Flavonoids are polyaromatic compounds having 15 carbon-skeleton structures. They are low molecular weight phenyl propanoid secondary metabolites which are product of central phenylpropanoid pathway. They are synthesized from p-coumaroyl-CoA and malonyl-CoA (Hassan and Mathesius, 2012). Cinnamic acid or dihydro-coumaric acids which are substrates for CoA esters are involved in synthesis of some rare flavonoids. There are more than 10,000 flavonoids known to be present in plant kingdom. They are mostly present in higher plants but some specific structures are unusual for particular plant families, like isoflavonoid structure is odd for Papilionoidae sub-family of leguminosae (Shaw *et al.*, 2006). When formed, flavonoids are present in the plant vacuoles in sugar conjugated form (glycosides, e.g. Genistin) (Shaw *et al.*, 2006). Chalcones, flavones, flavonols, anthocyanins, proanthocyanidins (condensed tannins) and aurones are the main subgroups of flavonoids found in higher plants (Abdel-Lateif *et al.*, 2012).

Flavonoids have important role in many beneficial functions of plant like enhanced spore germination to increase hyphal growth, hyphal branching and in formation of secondary spores (Abdel- Lateif *et al.*, 2012). In soyabean roots, the apoplastic β-glucosidases have been observed to secrete isoflavones. It is essential for root-microbe interactions to release flavonoid aglycones (Hassan and Mathesius, 2012). Flavonoids are secreted by passive ways from decomposing root cap and border cells (Shaw *et al.*, 2006). Sometimes the end products of flavonoids get concentrated to specific locations and perform regulatory development functions (Hassan and Mathesius *et al.*, 2012).

The accumulation of particular flavonoids in *Medicago truncatula* roots begins at the start of mycorrhization and continue at the different stages involved in symbiosis, showing mycorrhizal-fungal specificity (Abdel-Lateif *et al.*, 2012). They are accumulated along the length of root, root cap cells, root tip, in nodule primordial of subterranean clover *Trifolium subterraneum* (Abdel- Lateif *et al.*, 2012) and lateral root primodia of *Arabdopsis* (Abdel-Lateif *et al.*, 2012). The expansion in AM colonization in roots depends upon particular flavonoids. It has been reported that the composition of flavonoids differs in root tissue and shoot tissue which were grown with and without AM fungus in *Trifolium repens*. It evidently indicates that the colonization of AM fungus influences the metabolism of flavonoids (Abdel-Lateif *et al.*, 2012).

In rhizobia association, flavonoids also play role as chemoattractant. The secretion of flavonoids at various stages of nodulation in legume roots has been detected (Abdel- Lateif *et al.*, 2012). Large amounts of flavonoids are secreted near the location of *Rhizobium* infection i.e. root tips. Moderate amount of it is present near the root hair zone (Zuanazzi *et al.*, 1998). The molecular dialogue is required by flavonoids to initiate symbiosis. In studies of *Sinorhizobium meliloti* it was revealed that those flavonoids which play role in chemotaxis of rhizobia association deal in a different manner to attract bacteria towards root for developing connections for colonization and also infection for nodulation (Abdel-Lateif *et al.*, 2012). In the early stages of infection, particular flavonoids released in the rhizosphere by the plant roots help in production of Nod factors by *S meliloti*. The flavonoids are involved in Nod factors production due to its identification by the bacterial nodD protein (Buer *et al.*, 2010). These Nod factors promote the nodulation in the plant. The evidence of involvement of flavonoids in actinorrhizal associations' regulation has also been found. Actinorrhizal association involves actinomycete *Frankia* for formation of nitrogen fixing nodules (Shaw *et al.*, 2006).

Flavonoids also play role as antioxidants and metal chelators to change soil environment. In *Medicago sativa* (alfa-alfa) root exudates have potential to dissolve ferric phosphate for making iron and phosphate available to the plant. Flavonoids also help in inhibition of pathogens and pests (bacteria, fungi and insects) (Makoi and Ndakidemi 2007).

Salicylic acid

Salicylic acid (SA) is widely known plant phenolic compound. It is present in forms such as acetyl SA and methyl salicylate in the plants (Ghazijahani *et al.*, 2014). The biosynthesis of SA has been elucidated in tobacco, it was found that SA is

formed from cinnamic acid using benzoic acid. Among all other crops tested for SA content rice seedlings (*Oryza sativa* L.) had the highest levels of SA in between 0.01 and 37.19 µg/g fresh weight. The lower leaves of rice plant had low amount of SA than upper (younger) leaves. In healthier leaves of rice SA was found to be two times higher in concentration in comparison to which was observed in tobacco (Silverman *et al.,* 1995).

SA plays essential role in signaling pathway that controls local and systemic defense responses. These are stimulated by beneficial and parasitic organisms (Van Wees *et al.,* 2008). The stimulation of SA is essential during the early stages of root colonization (Alonso-Ramirez *et al.,* 2014). Upon infection or invasion plant initiate Systemic Acquired Resistance (SAR) in which SA plays role as signal molecule (Van Wees *et al.,* 2008). For SAR functions, the presence and accumulation of SA is very important. SAR is defense signaling against biotropic pathogen which dwell internally in the plant and is stimulated by accumulation of SA inside the plant tissue. The SA proceeds by means of local and systemic induction of pathogen related (PR) proteins which have antipathogen activity. The coordinate expression of PR genes and SA signal molecule trigger SAR. However, lower levels of SA have been reported to be beneficial for plant development (Ghazijahani *et al.,* 2014).

In a study, two transgenic plants NahG and CSA were inoculated with Arbruscular Mycorrrhizal Fungi (*Glomus mosseae* or *Glomus intraradices*). NahG had low levels of SA and CSA had high levels of SA before inoculation. After inoculation it was observed that NahG had increased level of colonization and CSA had reduced mycorrhization. But in the end of the experiment the wild type and both transgenic plants had similar level of colonization. This indicated that the high level of SA affects the delay of root colonization by AMF in intial stages, but it does not affect the final root colonization (Herrera-Medina *et al.,* 2003). Some members of family *Cornybacterineae, Pseudonocardineae* and *Streptomycineae* have exhibited positive relationship with SA for root colonization in plant (Badri *et al.,* 2013).

The increase in SA acts as endogenous marker for disease resistance and also helps plant in developing resistance against other pathogenic microorganisms (Herrera-Medina *et al.,* 2003). It played a major role in disease resistance in interaction between pathogenic fungus *Magnaporthe grisea* and rice plant (Silverman *et al.,* 1995). In dicotyledonous plants, it acts as natural inducer of disease resistance. SA is a stress messenger in the plant and protects the plant from pathogenic invasion (Ghazijahani *et al.,* 2014) by inducing hypersensitivity response. It is reported that SA could induce the alternative oxidase enzyme activity in mitochondria that involve in stress alleviation mechanism (Ghazijahani *et al.,* 2014). Another study with tobacco and cucumber plant showed that when pathogen gains entry then SAR is triggered in the tissues where pathogen infection has not reached so as to protect these parts of plant.

SA-dependent defense response is suppressed in symbionts for intiation of symbiotic relationship with mycorrhiza (Lopez-Raez *et al.,* 2010). The suppression of SA related defense pathway was observed in the case of interaction between plant beneficial basidiomycete *Piriformospora indica* and *Arabdopsis* (Jacobs *et al.,* 2011) and also in the organization of *Rhizobium*-legume association (Peleg- Grossman *et al.,* 2009).

SA, JA and ET pathways are related allowing the plant to be at vigilance against any microbial invasion (Van Wees *et al.,* 2008). Failure of all three defense hormones affects the microflora and results in abnormal root microbiome. It has been reported that SA signalling helps plant in recruiting specific bacterial families for colonization in root tissues. The ability of plant to select specific bacterial families is distrupted if any disturbance encountered in SA signalling pathway (Lebeis *et al.,* 2015).

Jasmonic acid

Jasmonic acid (JA) and methyl jasmonate (MeJA) are common regulators of development of higher plants They also elicit response against environmental changes and defense gene expression. JA was first isolated from *Lasiodiplodia theobrome* fungus cultures. Its concentration was found to be more than 10 µM in higher plants. JA/MeJA was found to be maximum in flowers and reproductive tissues, whereas in mature leaves and roots its concentration was very low. Jasmonates are synthesized in lipoxygenase-dependent process from linolenic acid (Creelman and Mullet, 1995).

Jasmonates have an important role in plant regulation towards necrotrophic pathogens. Salicylic acid (SA) and Jasmonic acid (JA) are related to each other either antagonistically or synergistically (Gutjahr *et al.,* 2009). Both SA and JA interact to influence the biotropic interactions and sometimes their negative crosstalk impacts plant susceptibility to exploitation by pathogens (Gutjahr *et al.,* 2009).

JA signalling plays role in chemical communication between plant roots and soil microbes

by releasing different phenolic compounds as plant derived signals. SA and *Et al.*,so induced release of different phenolic compounds. *Arabdopsis* has received maximum consideration for the JA signalling pathway than any other plant species. The root flora development of *Arabdopsis* is greatly impacted by JA (Carvalhais *et al.,* 2017). Application of methyljasmonate (MeJA) or SA in the roots of *Arabidopsis* resulted in change in root exudation composition. MeJA treated roots exclusively secrete kaempferol-3-O-β-D-glucopyranoside-7-O-α-1-rhamnoside. MeJA also leads to increase in exudation concentration in plant (Carvalhais *et al.,* 2017).

The bacterial populations which are capable of suppressing plant pathogens and insects are influenced by JA signalling in plant root (Schlaeppi *et al.,* 2015). In this way when under attack by pathogen, plants evolve mechanism to recurit symbiont to tolerate biotic stress. JA signalling inhibits colonization of incompatible strains of nitrogen-fixing *Azocarpus* bacteria in rice (*Oryza sativa*) and it also plays a role in suppression of nodulation in *Lotus japonicus* (Carvalhais *et al.,* 2017).

JA signaling has displayed fluctuations in a study on root-biotroph interaction. JA systemically suppresses or promotes root colonization depending on the plant species, concentration and possibly temporal and spatial distribution within the tissue (Gutjahr *et al.,* 2009). Fourteen-fold concentration of JA in roots (Hauese *et al.,* 2007) and nine-fold increase in shoots were observed (Kapoor, 2008) in the tissue where AM had colonized. This result presents positive finding about role of JA signaling in colonization of organism in plant. On the contrary, the transgenic or mutants impaired in biosynthesis or signalling of JA or SA showed reduction in mycorrhization (Riedel *et al.,* 2008; Foo *et al.,* 2013; Nair *et al.,* 2015).

In another study on tomato plant, there was restriction in AMF establishment when pathogens such as *Fusarium oxysporum* and *Botyris cinerea* were encountered (Jung *et al.,* 2012). The concentration of MeJA increased four times in the leaves of AM colonized in comparison to non-colonized tomato plant (Nair *et al.,* 2015). It was reported that there was no increase in JA concentration in roots of *Nicotiana attenuata* plant colonized with AM (Riedel *et al.,* 2008) but there was increase in JA levels in roots of AM colonized soyabean plants (Nair *et al.,* 2015). JA signalling pathway gives new insights into the mechanism involved in recruitment of microbes and the responses elicited in plant-microbe interaction.

Ethylene

Ethylene (ET) is a gaseous hormone known for many physiological and developmental processes in plants like flower and leaf senescence, ripening, seedling emergence, organ abscission and is involved in tolerance of abiotic and biotic stress. Ethylene reacts to pathogen attack and stimulates defense system by increase in its concentration or production rate. During stress conditions the ethylene production increases leading to stress alleviation. Various types of pathogenesis related proteins are produced by ethylene. It is done by inducing the phenylpropanoid pathway for making the cell walls of several plant species rigid. However, in some studies it was found that ethylene is not very important part of plant defense system. ET may different roles to play in different plant species which is influenced by cross talk with SA and JA. (van Loon *et al.,* 2006).

SA, JA and ET exhibit contrasting roles in modifying the mutualistic colonization in plant tissues. They also play role in restricting pathogenic invasion in plant (Herrera- Medina *et al.,* 2008). The invasion of *Rhizophgus irregularis* (formerly *Glomus intraradices*) for mutualistic colonization in plant tissue is inhibited by ET and JA. SA acts differently from ET and JA and leads to delayed colonization. Consequently, *R. irregularis* has evolved an advanced mechanism where they produce SP7 protein that interact with ET signaling pathway (ERF19) to suppress ET signalling for successful colonization. *R. irregularis* has not found such mechanism to counter SA and JA signalling pathway for colonization (Kloppholz *et al.,* 2011). *Et al.*,so plays a role in intiation of nodulation process by *Piriformodpora indica* as in *Sesbania rostrata* (Khatabi *et al.,* 2012). It restricts the hyphal development of this endophytic fungus to protect plant root tissues from infection and also to prevent any impact on plant productivity (Camehl *et al.,* 2010).

Ethylene regulates disease resistance as Salicylic acid (SA) and Jasmonic acid (JA) (van Loon *et al.,* 2006). It acts as a virulence factor for the establishment of bacteria and pathogenic fungi in plant root (Chague *et al.,* 2006). The ability of bacterial leaf pathogen *Pseudomonas glycinea* to colonize in Soyabean plant is weakened in a mutant lacking ability to produce ethylene (Weingart *et al.,* 2001). It is reported that during infection the increase in ethylene levels promote disease rather than eradicating it. It is shown that the chlorosis and stunting growth of cucumber caused by cucumber mosaic virus and epinasty and defoliation caused by soilborne fungus *Verticillium dahliae* in cotton is due to ethylene. It is reported that in plant-pathogen interactions ethylene sometimes act as

virulence factor of pathogen. Contrarily, it also acts negatively against action of pathogens. When applied before development of infection ethylene works against pathogen but if produced during infection or applied after occurrence of symptoms it induces progress of the disease. At confined infections sites ethylene is formed and it is sensed by adjoining cells which then stimulate defense system in response to increase level of the signaling compounds (van Loon *et al.,* 2006). Evidence suggests that spatial communications of various signals interacting in network type manner induce ethylene regulated resistance.

Exploitation for Human Benefit

Root exudates are key player in the below ground 'plant-microorganisms' interactions. Lack of understanding of plant-microbe interactions in terms of exudates chemistry is inadequate. The metabolic profiling of root secretions is limited to targeted analysis of some compounds such as organic acids, flavonoids and fatty acids. Molecules involved in these interactions have not been fully characterized. Tools such as 'Metabolomics', 'Secretomics', 'Metagenomics', 'Proteomics' can be used for the determination of exude molecules released by plant at different stages of development and also in different soils. Multi-disciplinary research involving many different fields as microbiology, plant physiology, soil ecology, soil physio-chemistry is warranted to understand this biochemical dialogue between the plant and microbes (Haichar *et al.,* 2014).

Exogenous application of natural compounds related to root exudates on plants have been found to influence microflora of soil. Manipulating the composition of root microbial communities is needed for benefits of plant, such as increased nutrient acquisition, yield and for tolerance of abiotic and biotic stress, (Quiza *et al.,* 2015). The recent advances in these approaches include 1. changes in plant exudates through genetic engineering or breeding 2. application of microbial inoculants 3. land management (like tillage and pH change) (Quiza *et al.,* 2015; Ryan *et al.,* 2009). There are some problems regarding these approaches such as when inoculants are added they have to compete with native organisms (Martinez-Viveros *et al.,* 2010). There are also complications of maneuvering the root exudation as it is not clear how to increase the quantity of particular compound in root exudates without affecting other compounds in the exudate (Ryan *et al.,* 2009). The root exudates are secreted near root apices but most microbes reside at basal regions of roots (Dennis *et al.,* 2010). Therefore, the land management approach is extremely challenging to accomplish. These new strategies for selecting beneficial microbiomes by targeting mechanisms of plant that have naturally evolved to select for mutualistic symbioses enhance holobiont appropriateness.

Rhizosphere biology is affected by changes in plant hormone synthesis pathways including flavonoids Alternate synthesis pathways give information concerning mechanism of flavonoid transport and exudation. Increased amount of flavonoid in the rhizosphere comprises 1. changes in the biosynthesis process that results in overexpress, newly express, or inhibit synthesis or alter glycosylation 2. up-regulation of flavonoid exudation and 3. induction of desired pathways by coupling of changed expression to rhizosphere signals. The information about the enhancers and factors of transcription that are specific for flavonoid induction is necessarily required. A pioneering strategy to elucidate genetic makeup of the production pathway would help in production of different flavonoids from various plant species for their useful exploitation in agriculture.

SA has a role in initiation and stimulation of Systemic Acquired Resistance (SAR) in response to presence of pathogen and during the initiation of AM symbioses. The exogenous addition of SA to rice inoculated with AM fungi decreased the extent of colonization in the initial stages but did not affect formation of appressoria. This demonstrates the inhibitory effects of salicylic acid on the establishment of AM fungi (Hauese *et al.,* 2007). In this way the information regarding all defense hormones of a particular crop and their interaction with specific microbe communities can be developed.

JA modulates colonization by affecting carbohydrate portioning. It restricts excessive fungal invasion in mutualistic associations. The current availability of the annotated genome sequence for *Pochonia chlamydosporia* provides understanding of the molecular basis of plant development and growth promotion by this fungus in *Arabidopsis thaliana*. The further investigations of *A. thaliana* and *P. chlamydosopria* system can pave the way for its future applications in economically important crops (Zavala-Gonzalez *et al.,* 2016). It is likely to involve the processes that are induced by jasmonates during mycorrhization. The genes responsible for different jasmonates functioning have been recognized by transcriptional analysis of mycorrhizal and non-mycorrhizal roots. These genes help in the production of enzymes catalyzing production of secondary metabolites or proteins related to systemic acquired resistance (SAR). These norms have similarity with the

effect of plant growth promoting bacteria on enhancing plant defense system (Miransari *et al.,* 2012).

The understanding of execution of SA, JA and ET related networks is now available through transcriptome analyses as well (van Loon *et al.,* 2006). The transcriptome analysis provides data regarding environment cues and the various regulators involved in functioning of multiple genes. The elucidation of the underlying mechanisms and the involvement of phytohormones in plant defense responses have been discussed with respect to temporal and spatial analyses (van Loon *et al.,* 2006).

The emphasis on elucidation of molecular aspects involved on the role of signaling networks for establishment of symbiosis between host and symbiont may have great impact on symbiosis efficiency. Under different conditions and for higher yield, the establishment of symbiont can be improved by biotechnological interventions. This can be ecologically and agriculturally significant (Miransari *et al.,* 2012).

Conclusion

Microbes present in soil which are involved in many processes can help in enhancement of agricultural soil productivity, maintenance of soil structure, nutrient cycling, disease control and degradation of pollutants. Also, modern farming ways that sustain enrollment and preservation of beneficial microbial communities in the soil also contribute to plant by increasing its yield and protecting it from pathogens. Rhizospheric region is an important focus of studies for molecular biologists, ecologists and plant biologists for furthering understanding of plant-microbe interactions. The current research sector focuses on areas of 'omics' such as proteomics, metabolomics, transcriptomes and secretomics which further knowledge about these interactions for agricultural benefit. The information obtained from these 'omics' studies can strengthen capability to visualize a complete picture of these complex multi-species interactions. Since pathogens are a major concern, it is necessary to recognize how successful root pathogens overcome root defenses. The genomes of root pathogens will reveal new insights into various strategies used by these pathogens including genes involved in penetration and adhesion as well as genes encoding effectors by next generation sequencing and comparative analyses of different genomes. Large numbers of potential effectors encoding genes are revealed from the nucleotide sequences of these micro-organisms. Currently, only a few microbes are sequenced and only few effectors from root pathogens have been functionally characterized and future research in this area needs to be strengthened to gain new insights into root infection. Finally, we have presented an overview of underground interactions crucial in plant-microbe communications and the role of root exudates or plant hormones. Studies on root exudates as signaling agents have been cited. There are many other unresolved phenomena yet to be revealed for better understanding of interactions at an ecological level. The inability to extract and analyze trace amounts of potentially liable natural products could be the problems faced in the biochemical characterization of exudates. There are many other compounds that help in initiation of plant-microbe interactions but are still remain unidentified.

Table 1: Presence of bacterial and archael taxa in rhizospheric microbiome of some plant species

Plant species	Rhizosphere microbiome composition	References
Rice (*Orzya sativa* L.)	*Gemmatimonadetes, Proteobacteria, Verrucomicrobia*	Prasanna *et al.,* 2011; Breidenbach *et al.,* 2016.
Wheat (*Triticum aestivum*)	*Gemmatimonadetes, Proteobacteria, Verrucomicrobia*	Prasanna *et al.,* 2011; Breidenbach *et al.,* 2016.
Maize (*Zea mays*)	*Burkholderiales*, *Oceanospirillales*, *Sphingobacteriales* orders of the *Proteobacteria*	Aira *et al.,* 2010; Chauhan *et al.,* 2011; Salles *et al.,* 2004; Peiffer *et al.,* 2013.
Potato (*Solanum tuberosum*)	Mainly species belonged to *Proteobacteria, Firmicutes* (*Bacillus sp.* and *Pseudomonas sp.*) and *Actinobacteria*, *Bacteriodes* and *Acidobacteria.* (families *Streptomycetaceae, Micromonosporaceae,* and *Pseudomonadacea*)	Weinert *et al.,* 2011; Mendes *et al.,* 2011.
Sugar beet (*Beta vulgaris*)	*Proteobacteria, Firmicutes and Actinobacteria*	Mendes *et al.,* 2011.
Oat (*Avena sativa*)	Most dominated *Proteobacteria* and *Firmicutes* and some less expected phyla include *Actinobacteria*, *Verrucomicrobia* and *Nitrospora.*	DeAngelis *et al.,* 2009; Mendes *et al.,* 2011.
Pea (*Pisum sativum* L. cv. Duel)	*Actinomycetes, α* and *γ –proteobacteria, Actinobacteridae Sphaerobacteridae* and *Rubrobacteridae*	Sharma *et al.,* 2005; Gathumbi *et al.,* 2002.
Faba beans (*Vicia faba* L., cv. Scirocco)	*Actinomycetes, α* and *β-proteobacteria, Verrucomicrobia, Fibrobacter/Acidobacter, Green Nonsulfur bacteria* and *Nitrospira*	Sharma *et al.,* 2005; Gathumbi *et al.,* 2002.

Table 2: Endophytic bacteria present in agricultural crops

Plant species	Endophytes	References
Rice (*Orzya sativa* L.)	*Azorhizobium* sp, *Pseudomonas* sp, *Agrobacterium* sp., *Azospirillium* sp, *Bacillus* sp, *Azocarpus* sp, *Herbaspirillium* sp, *Methylobacterium* sp, *Klebsiella* sp, *Flavobacterium* sp, *Pantoea* sp, *Rhodopseudomonas* sp	Stoltzfous *et al.*, 1997; Reddy *et al.*, 2007; Reinhold-Hurek and Hurek 1998; Elbeltagy *et al.*, 2001; Kaga *et al.*, 2009; Okunishi *et al.*, 2005.
Wheat (*Triticum aestivum*)	*Azorhizobium* sp, *Flavobacterium* sp, *Micrococcus* sp, *Bacillus* sp, *Streptomyces* sp	Webster *et al.*, 1998; Coombs and Franco 2003; Rosenblueth and Martinez-Romero 2006.
Maize (*Zea mays*)	*Burkholderia* sp, *Enterobacter* sp, *Bacillus* sp, *Arthrobacter* sp, *Microbacterium* sp, *Pseudomonas* sp, *Corynebacterium* sp, *Vibrio* sp	McInroy and Kloepper 1995; Surette *et al.*, 2003; Rosenblueth and Martinez-Romero 2006.
Potato (*Solanum tuberosum*)	*Bacillus* sp, *Micrococcus* sp, *Pseudomonas* sp, *Flavobacterium* sp, *Xanthomonas* sp, *Agrobacterium* sp	Lodewyckc *et al.*, 2002; Rosenblueth and Martinez-Romero 2006.
Cotton (*Gossypium hirsutum*)	*Pseudomonas* sp, *Bacillus* sp, *Corynebacterium* sp, *Enterobacter* sp, *Klebsiella* sp, *Vibrio* sp, *Burkholderia* sp	McInroy and Kloepper 1995; Misaghi and Donndelinger 1990; Musson *et al.*, 1995; Lodewyckc *et al.*, 2002.
Sugarcane (*Saccharum officinarum*)	*Herbaspirillium seropedicae, H. rubribalbicans, Acetobacter diazotrophicus*	Dong *et al.*, 1994; Lodewyckc *et al.*, 2002; Rosenblueth and Martinez-Romero 2006.
Peas (*Pisum sativum* L. cv. Duel)	*Bradyrhizobium* sp, *Rhizobium* sp, *Bacillus* sp, *Pseudomonas* sp, *Corynebacterium* sp, *Enterobacter* sp.	Sharma *et al.*, 2005; Gathumbi *et al.*, 2002.
Faba beans (*Vicia faba* L., cv. Scirocco)	*Bradyrhizobium sp, Rhizobium sp, Bacillus* sp, *Pseudomonas* sp, *Corynebacterium* sp, *Enterobacter* sp	Sharma *et al.*, 2005; Gathumbi *et al.*, 2002.

Table 3: Plant pathogens present in the rhizosphere

Plant species	Pathogens	References
Rice (*Orzya sativa L.*)	*Xanthomonas oryzae pv. oryzicola, Burkholderia glumae, Xanthomonas oryzae pv. oryzae, Phythium* sp., *Magnaporthe oryzae, Rhizoctonia solani, Ustilaginoidia virens, Cochliobolus miyabeanus, Fusarium fujikuroi,*	Coninck *et al.*, 2014; Dean *et al.*, 2005; Condon *et al.*, 2013; Wiemann *et al.*, 2013; Lee *et al.*, 2005; Bogdanove *et al.*, 2011; Lim *et al.*, 2009; Liu and Wang 2016.
Wheat (*Triticum aestivum*)	*Pseudomonas syringae subsp Syringae, Gaumannomyces graminis var tritici, Xanthomonas campestris pv. Translucens, Erwinia rhapontici, Claviceps purpurea, Ustaligo tritici, Phythium sp, Magnaporthe grisea*	Zhang *et al.*, 2008; Pechanova and Pechan 2015.
Maize (*Zea mays*)	*Colletotrichum graminicola, Aspergillus flavus, Fusarium verticillioides, F. graminearum, Curvularia lunata*	Coninck *et al.*, 2014; Pechanova and Pechan 2015.
Potato (*Solanum tuberosum*)	*Streptomyces scabies, Pectobacterium carotovorum, Dickeyasolani, Clavibacter michiganensis, Ralstonia solanacearum, Alternaria solani, Phytophtora infestans, Sclerotinia sclerotiorum*	Gardan *et al.*, 2003; Samson *et al.*, 2005; Aittamaa *et al.*, 2008.
Cotton (*Gossypium hirsutum*)	*Xanthomonas axonopodis, Rhizoctonia solani, Fusarium* sp, *Pythium* sp, *Verticilium dahlia,Colletotrichum gossypii*	Zhang *et al.*, 2008.
Tomato (*Solanum lycopersicum*)	*Phytophthora, Fusarium oxysporum* f.sp. *lycopersici (Fol)*	Coninck *et al.*,2014.
Peas (*Pisum sativum* L. cv. Duel)	*Aphanomyces euteiches, Ascochyta sp., Phytophthora clandestine, Rhizoctonia solani, Phoma mediaginis*	Tinivella *et al.*, 2009, Barbetti *et al.*, 2007.
Bean (*Phaseolus vulgaris*)	*Colletotrichum lindemuthianum, Aphanomyces euteiches, Phytophthora clandestine, Phoma mediaginis*	Tinivella *et al.*, 2009, Barbetti *et al.*, 2007.
Citrus	*Pseudomonas syringae, Xylella fastidiosa, Xanthomonas campestris* pv. *citri, Candidatus liberibacter asisticus, Botrytis cinerea, Phytophthora palmivora, Ashbya gossypii, Colletotrichum gloeosporioides, Trichoderma viride, Penicillium digitatum, Alternaria citri*	Berendsen *et al.*, 2012.

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