Fungal Endophytes in Plant Health

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REVIEW

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Endophytes are the microorganisms, which colonize symptomless the living plant tissue without causing any immediate, overt, negative effect on the plant. Endophytes as the fungi that live internally and remain asymptomatic for at least part of their lifecycle. But they can also be aggressive saprophytes or opportunistic pathogens. There has been considerable interest in screening the endophyte for bioactive compound. A large number of compounds (secondary metabolites) have been extracted, isolated and characterized from endophytic microbes. Endophytes produce toxins that discourage insects and other grazing animals. Endosymbionts may escape all the deleterious infections that might occur on the surface of the plant. There are endophytes which are beneficial to their hosts, the best known in this group are the *Neotyphodium* and *Epichloë* species which can provide antiherbivore defense, as well as drought tolerance and improved nutrient use to their plant hosts. Fungal endophytes enhance plant immunity against chewing insects by promoting endogenous defense responses mediated by the jasmonic acid (JA) pathway. Some endophytic species may induce plant defense mechanisms which counteract pathogen attack, others produce antibiotic substances which inhibit pathogen growth, competition for plant space and resources may also occur between resident endophytes and incoming pathogens; finally, some parasites of plant pathogens are known to behave as endophytes. Endophytic fungi serve as a source of novel biologically active secondary metabolites. Endophytic fungi isolated from plants screened for biological activities. The structures of the biologically active compounds are determined. Liquid extracts from endophyte cultures have been found to inhibit the growth of several species of plant pathogenic fungi. The protection against a pathogen could be the result of direct competition among endophytes already present in leaves and the pathogen.

The outcome of some pathogen attacks may be dependent on the endophytic mycobiota associated to a host plant. Endophyte may promote plant growth by secreting different hormonal substances. Endosymbionts produce auxin, which enhanced the vegetative growth of the endophyte infected plants. The distribution of growth-promoting hormones produced by endophytic microorganisms towards plant tissues positively promotes plant growth. Endophytes possess vital ability to mobilize insoluble phosphate and provide nitrogen to their host plants. Microbial endophytes colonize plant tissues without symptomatic behaviour and consequently they compete with other microbial pathogens on the same ecological niches. Mechanism of endophyte mediated plant disease resistance induction of plant resistance is that fungal endophytes induced ISR associated with the expression of pathogenesis related genes.

Key words: Diversity, bioactive compounds, abiotic and biotic stress, defense and growth promotion

INTRODUCTION

Endophytes are the microorganisms, which colonize symptomless the living plant tissue without causing any immediate, overt, negative effect on the plant (Hirch and Broun, 1992). Anton de Bary (1866) first introduced the term "epiphytes" for fungi that live on the surface of their host and "endophytes" for those living inside the plant tissue (Azevedo, 1998). Later, this term was expanded as fungi and bacteria, including actinomycetes, which spend the whole or part of their life cycle colonizing inter- or intra- cellularly, inside the healthy living tissues of the host, typically causing no apparent symptom of disease. The word endophyte came from two Greek words, "endon" means within and "phyton" means plant.

1. Endophytes as the fungi that live internally and remain asymptomatic for at least part of their lifecycle. But they can also be aggressive saprophytes or opportunistic pathogens (Wilson, 1995).

2. Endophytes are plant-associated prokaryotes that form association with their host plants by colonizing the internal tissues, which has made them valuable for agriculture as a tool in improving crop performance (Azevedo *et al.* 2000).

There have been several studies on the plant endophyte relationship, especially for grasses such as tall fescue, where it has been shown that endophytes produce toxins that discourage insects and other grazing animals (Bultman and Murphy, 2000). Persian darnel, an annual grass, considered as a troublesome weed by wheat farmers, is probably the plant that has been studied most extensively as far as endophytes are concerned. Its endophyte content are often resistant to attack by certain insects (Clay, 1998; Bills, 1996). There has been considerable interest in screening the endophyte for bioactive compound (Kumar and Hyde, 2004). A large number of compounds (secondary metabolites) have been extracted, isolated and characterized from endophytic microbes (Tan and Zou, 2001; Strobel *et al.* 2004).

Kusari *et al.* 2014 demonstrated fungal endophytes can produce a plethora of bioactive natural products. The potential of endophytes, particularly endophytic fungi, capable of demonstrating desirable functional traits with worth exploitation using red biotechnology is well established. However, these discoveries have not yet translated into industrial bioprocesses for commercial production of biopharmaceuticals using fungal endophytes. The current challenges in transforming curiosity driven discoveries into industrial scale endophyte biotechnology is the need of the hour. The possible practical, feasible, and sustainable strategies that can lead to harnessing fungal endophyte-mediated pharmaceutical products are discussed. Hubbard *et al.* 2012 reported when colonized by the most effective fungal endophyte, the values of the parameters tested in wheat seeds exposed to heat stress resembled those of unstressed seeds.

Endophytes traditionally have been considered plant mutualists, mainly by reducing herbivory *via*

production of mycotoxins, such as alkaloids (Faeth *et al.* 2002). However, the vast majority of endophytes, especially horizontally-transmitted ones commonly found in woody plants, apparently have little or no effect on herbivores. For the systemic, vertically-transmitted endophytes of grasses, mutualistic interactions via increased resistance to herbivores and pathogens are more common. Nitrogen is a common currency in endophyte/host and plant/herbivore interactions in terms of limitations to host plant growth, enhanced uptake by endophytes, demand for synthesis of nitrogen-rich alkaloids, and herbivore preference and performance.

This article reviews the different endophytes associated with the crop plants, their mode of infection in the plants and how plants can be benefited with endophytisms.

Advantages of endophytism

Endophytism offers several advantages to the host plants. They are

(i) greater access to the nutrients,
(ii) protection from desiccation,
(iii) protection from the surface feeding insects, parasitic fungi, and
(iv) growth promotion.

Episymbiotic fungi modify the host tissue in order to acquire their nutrients. Epidermal cells in close association to stromata are hypertrophied and lack the waxy cuticle that normally prevents the escape of water and nutrients from the leaf (White and Glenn, 1994). Because the cuticle is absent on the leaf, the episymbiont may extract the nutrient from the leaf across the modified epidermal layer. In case of endosymbiosis, mycelium is distributed among the internal cells of the plant tissues (White and Owens, 1992). The extraction of nutrients from the host may be enhanced by modifying both internal and external tissues of the host plant to enhance the flow of nutrients to the stromal mycelium (White *et al.* 1997).

Biological characteristics of endophytic species
Tissue colonization and specificity

Many endophytes locally infect plant parts, being restricted to a small tissue area. This is supported

by the fact that often, several endophytic species are recovered from different fragments of the same plant.

In contrast, *Neotyphodium* and *Epichloë* species systemically infect the intercellular space of leaves, reproductive stems, and seeds of their hosts. These systemic endophytes can be isolated from multiple fragments of the same plant. Tissue and organ specificity also occurs, and some endophytes may be found in specific plant parts such as roots, leaves, or twigs, while others may infect several of these parts (Stone *et al.* 2004). Regarding the specificity shown by endophytes for their host plants, specialists as well as generalists do occur in this group. For example, *Neotyphodium* endophytes have a narrow host range, being confined to one or two plant species. Other endophytic fungi such as *Alternaria*, *Penicillium*, or *Piriformosfora* have wide host ranges, encompassing species within different genera or plant families (Stone *et al.*, 2004; Waller *et al.* 2005).

Out of the multiple species that can penetrate and infect a plant, only a relatively small subset, that of the pathogens, produces disease. This shows that part of the plant disease cycle is shared by pathogens of endophytes. Once a fungus enters a plant it can behave as an endophyte or as a pathogen, and it seems that a majority of plant associated fungi act as endophytes. What is the difference between infection processes caused by endophytes and pathogens, is a good question for plant pathologists. Some studies directed to answer such question point out to fungal as well as to host characteristics. For instance, a mutation in a single locus can convert a pathogen such as *Colletotrichum magna* Jenkins and Winstead in a mutualistic endophyte (Freeman and Rodriguez, 1993). However, some isolates of the above species may behave as a pathogen in cucurbits or as an endophyte in some species of other plant families (Redman *et al.* 2001).

Fungal endophytes have the ability to colonize inter- or intra-cellularly. The colonization process involves several steps, including host recognition, spore germination, penetration of the epidermis and tissue multiplication. Once the endophytes are successfully colonized in the host tissue, the endophytic niche becomes established. In the endophytic niche, endophytes will obtain a reliable source of nutrition from the plant fragment, exudates and leachates and protect the host against other microorganisms (Gao *et al.* 2010).

Symbiotic lifestyles

Different symbiotic lifestyles occur among endophytes. Depending on the species involved, the outcome of a plant-endophyte interaction can range from antagonism to mutualism. This is why the distinct range of plant-endophyte interactions has been referred as a continuum (Saikkonen *et al.* 1998; Schulz and Boyle, 2005). Apparently healthy plants are the raw material used for endophyte surveys. Therefore, latent pathogens may be isolated from such plants if sampling is done before symptoms appear (Mostert *et al.*, 2000; Photita *et al.* 2004). However, latent pathogens do not seem to constitute an important fraction of endophyte assemblages, most endophytes do not cause symptoms on plants. For example, out of 109 different fungal species identified in *Dactylis glomerata* L, only 5 corresponded to known pathogens of that grass (Sánchez Márquez *et al.* 2007). Some saprophytic fungi commonly found in senescent plant parts have been isolated as endophytes from healthy tissues (Promputtha *et al.*, 2007). Such endophytic species behave as latent saprophytes, these fungi may be asymptomatic and spatially restricted while their hosts grow, but will grow unrestricted and reproduce when the infected host tissue senesces or dies. At the other end of the continuum there are endophytes which are beneficial to their hosts, the best known in this group are the *Neotyphodium* and *Epichloë* species which can provide antiherbivore defense, as well as drought tolerance and improved nutrient use to their plant hosts (Schardl *et al.* 2004). In addition to other mutualistic species known to benefit plants by protecting them against pathogens, endophytes such as *Piriformospora indica* Sav. Verma, Aj. Varma, Rexer, G. Kost and P. Franken (Waller *et al.* 2005), *Acremonium strictum* W. Gams (Hol *et al.* 2007), and some *Stagonospora* species (Ernst *et al.*, 2003) can enhance the growth of their hosts.

Transmission and acquisition of endophytes

Endophytes may infect plants by means of horizontal transmission, when their inoculum is transported to another plant, or vertically when they infect the seed progeny of an infected plant. Horizontal transmission seems to be the predominant mechanism of dispersion among

endophytic species. Some studies have shown that seeds and seedlings are virtually endophyte-free, and the incidence of fungal endophytes increases as leaves or seeds grow older (Arnold *et al*. 2003; Gallery *et al*. 2007). This type of dynamics must be driven by horizontal transmission. There is a paradox related to the understanding of the mechanisms of horizontal transmission of endophytes: for horizontal transmission to occur, endophytic inoculum has to be produced and dispersed. But in an asymptomatic host, where and when is the inoculum produced?. In the case of latent saprophytes, the inoculum which infects new hosts could be produced when infected host tissue dies. Some saprophytes which produce fructifications in dead plant parts have been identified as endophytes in healthy tissues (Sánchez Márquez *et al*. 2007). From this point of view, leaf litter may be an important source of endophytic inoculum (Bills and Polishook, 1994). In other situations, inoculum may be produced in an inconspicuous way in infected hosts. For example, some grasses infected by *Epichloë* endophytes have a microscopic layer of hyphae and conidia in the surface of their leaves. It is thought that this inoculum might horizontally infect new hosts (Tadych *et al*. 2007). Phytophagous insects may also take part in the spread of endophytes, since spores of some fungal species are resistant to gut digestion, and are present in their fecal pellets (Devarajan and Suryanarayanan, 2006).

Vertically transmitted endophytes are discovered by means of studying seed transmitted fungi, and such studies are scarce (Gallery *et al*. 2007).

The species diversity of fungal endophytes

The procedures most commonly used for endophyte surveys are based on the surface disinfection of apparently healthy plant tissue samples to kill epiphytic fungi (Bills, 1996). The surface-disinfected plant samples are subsequently placed on a synthetic growth media and, when endophytic hyphae emerge from the plant tissue and start growing in the agar medium, isolations can be made. With this technique obligate biotrophs or fungi not growing well in the selected medium will not be isolated. As a result, the real number of endophytic species in a sample can be underestimated. Non culturable endophytes do exist, and fortunately some techniques allowing

their detection in plant tissues have been developed (Neubert *et al*. 2006; Duong *et al*.; 2006; Gallery *et al*. 2007). An average of about 50 endophytic species per plant species was found in surveys done before the year 2000 (Stone *et al.*, 2004).

➢ When molecular methods for the identification of fungi began to be applied to endophyte research (i.e. rDNA sequencing; Arnold *et al*. 2000; Guo *et al*. 2000), the number of fungal species identified per host plant species increased substantially. The reason for this is that an important proportion of fungal isolates may be sterile in laboratory cultures.

➢ Kwasna *et al*. 2008 described the pure-culture isolation from roots was compared with transformation of total DNA from roots followed by sequence analysis of ITS 1/2 rDNA of representative clones as methods for determining the abundance and composition of microbiota in roots of *Betula pendula, Fagus sylvatica*, *Larix decidua, Prunus serotina* and *Quercus petraea*. The results from the two methods differed greatly, with no overlap between the taxa identified. Pure-culture isolation revealed greater species diversity (47 taxa), the most frequent fungi being Ascomycota, including *Penicillium* spp., *Phialoce-phala fortinii*, *Pochonia bulbillosa*, *Sesquicillium candelabrum* and *Trichoderma* spp.

➢ Genotypic identification methods have allowed to identify, or at least to distinguish, among sterile cultures. When the results of endophyte surveys are analyzed in species accumulation curves it is often found that these curves are non-asymptotic, suggesting that if more plant samples would have been analyzed, more endophytic species would have been discovered. These results imply that most surveys of endophytic mycobiota underestimate the real number of species associated to a given plant species. In fact, when mathematical estimators of the total species abundance are applied to field data (Chazdon *et al.*, 1998), the number of endophytic species potentially associated to a plant species is often estimated in several hundreds (Sánchez Márquez *et al*. 2007).

Endophyte assemblages are composed by rare or singleton species which are isolated only once or very few times, and by dominant or plural species which are frequently isolated from a given

host species (e.g. Neubert *et al*. 2006). Singleton species are the main factor driving non-asymptotically species accumulation curves. When the singleton species found in a survey are excluded from the data used to plot a species accumulation curve, the resulting lines may be asymptotic. This suggests that the number of plural taxa commonly associated to a given plant species is much more limited than the group of singleton species which occasionally infect a plant. Another factor contributing to the large diversity observed in endophytic assemblages is geographical variation, the taxa isolated from the same host species tends to change from one location to another (Collado *et al*. 1999). In a geoclimatic context, endophytic assemblages appear to be richer in tropical than in temperate or cold zones of the world. (Fisher *et al.* 1995; Arnold and Lutzoni, 2007). Plant age also has an effect upon endophyte diversity. As time of exposure to endophyte inoculum increases, plants seem to accumulate an increasing number of endophytes in their tissues. Because of this, older plant parts may harbour more endophytes than younger ones (Arnold *et al*. 2003). It is thought that only a small proportion, about 5 % of the existing fungal species are known (Hawksworth, 2001). As new environments are explored, new species will be found and classified. Endophytic fungi represent a very diverse group, and as new studies on this kind of fungi are made, it is very likely that progress in the discovery of new fungal species will be made.

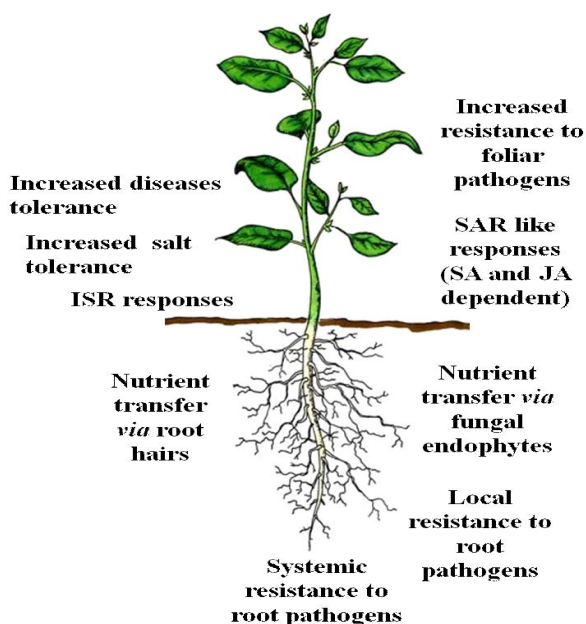### Endophyte mediated host plant disease resistance

Endophyte infection may alter plant biochemistry in a way that defense mechanisms against pathogens are induced. *Piriformospora indica* Sav. (Verma, Aj. Varma, Rexer, G. Kost and P. Franken) is a root endophyte with a wide host range, including several species of cereals and *Arabidopsis*. Barley plants inoculated with this endophyte have shown resistance to a vascular pathogen [*Fusarium culmorum* (W.G. Sm.) Sacc.] and a leaf pathogen [*Blumeria graminis* (DC.) Speer], in addition to an increase in yield and salt stress tolerance (Waller *et al*. 2005). The protection against the leaf pathogen appears to be mediated by a mechanism of induced resistance, because in the pathogen-inoculated plants there is a defense response involving the localized death of host.

A significant increase in resistance to dollar spot disease, caused by *Sclerotinia homoeocarpa* F.T. Benn., has been observed in *Festuca rubra* L. cultivars infected by *Epichloë festucae* Leuchtm., Schardl and M.R. Siegel. (Clarke *et al*. 2006). Cultivars of several turfgrass species infected by *Epichloë* and *Neotyphodium* endophytes are commercially available at the present time. The efficient vertical transmission of these endophytes has allowed the production of infected seed at a commercial scale. Since *Neotyphodium* and *Epichloë* infected cultivars have shown increased resistance to herbivores, plant pathogens, and some conditions of abiotic stress, the use of such symbiotic cultivars can result in a reduction in the use of insecticides and fungicides in lawns (Brilman, 2005). Similar applications of other species of endophytes may be seen in the future. The above studies suggest that the outcome of some pathogen attacks may be dependent on the endophytic mycobiota associated to a host plant. Therefore, the endophytic assemblage of a given species may represent a source of organisms with potential applications for disease control in the same plant species.

Bastias *et al*. 2017 proposed that, *Epichloë* fungal endophytes enhance plant immunity against chewing insects by promoting endogenous defense responses mediated by the jasmonic acid (JA) pathway. They also discussed about the ecological scenarios where this effect of endophytes on plant defenses would be most beneficial for increasing plant fitness. *Neotyphodium* endophytes and some *Epichloë* species (e.g. *E. festucae* Leuchtm., Schardl and M.R. Siegel, *E. sylvatica* Leuchtm. and Schardl) are vertically transmitted to host progeny by means of seed infection. Close to 100% of the seeds produced by an infected plant contain fungal mycelium near the embryo and in the aleurone layer. These seeds will give rise to asymptomatic infected plants. Therefore, these endophytic species are vertically transmitted in a fashion similar to a maternally inherited character (Schardl *et al*. 2004). Because of this, the incidence of these endophytes is very high in natural populations of their hosts (Arroyo García *et al*. 2002).

Phytohormones are present in trace amounts in plant tissues, and not only regulate plant developmental processes, but also play important roles in plant responses to biotic stress. Salicylic

acid (SA) and jasmonic acid (JA), in particular, have been shown to play a central role in mediating stress responses in plants (Halim *et al*. 2006; Bari and Jones, 2009). Exogenous SA induces pathogenesis-related (PR) genes and thus enhances resistance to a broad range of pathogens (Grant and Lamb, 2006). Pathogenic infections often increase SA levels in both pathogen-challenged and unaffected tissues (Park *et al*. 2007). JAs play an important role in protection against wounding, herbivory and pathogen attack (Staswick & Tiryaki, 2004). JAs are synthesized from á-linolenic acid by the enzymes of the lipoxygenase pathway. According to Smith *et al*. 2009 the expression of induced *plant* defenses is mediated by complex signaling networks in which

pathogenesis-related (PR) proteins. ISR, induced by some non-pathogenic rhizobacteria, is mediated by jasmonic acid or ethylene and is not associated with the accumulation of PR proteins (Vallad and Goodman, 2004; Tripathi *et al*. 2008). These PR proteins comprise a variety of enzymes, some of which may act directly to lyse the invading cells, including chitinases and â-1,3-glucanases (Fukuda and Shinshi, 1994), reinforce cell wall boundaries to resist infections, or induce the localized cell death. Fungal endophytes induced ISR may also associate with the expression of pathogenesis related genes. *F. solani,* isolated from the root tissues of tomato elicited induced systemic resistance against the tomato foliar pathogen, (*Septoria lycopersici)* and triggered PR genes expression in the roots (Kavroulakis *et al*. 2007).

Indirect effect on enhanced plant resistance

All plants in natural ecosystems appear to be symbiotic with fungal endophytes. This highly diverse group of fungi can have profound impacts on plant communities through increasing fitness by conferring abiotic and biotic stress tolerance, increasing biomass and decreasing water consumption, or decreasing fitness by altering resource allocation. Despite more than 100 yr of research resulting in thousands of journal articles, the ecological significance of these fungi remains poorly characterized (Rodriguez, 2009). Plants develop several mechanisms against unfavorable environment such as drought, cold, salt stress or pathogens. Morphological and biochemical changes, including cellular necrosis, hypersensitive response and phytoalexin production respond to the various stresses rapidly. During the long term evolution, two types of innate resistance, non-specific (general) resistance and specific resistance are formed to resist the pathogens infestation (Kiraly *et al*. 2007). Since fungal endophytes may evolve from the plant pathogenic fungi, plant defense could be triggered by fungal endophytes such as pathogens. Actually, the defense of plant associated with endophytes is increased through resistance enhancement and secondary metabolites production.

the *plant* hormones jasmonic acid (*JA*) and salicylic acid (*SA*) play key *roles*. *JA*-mediated signaling pathways are implicated in the regulation of antiherbivore defenses, while the *SA* pathway is associated with defense responses against pathogens.

Induction of plant resistance

Over the past two decades, many researchers have focused on plant resistance responses to pathogens and parasites of various scales. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are the two forms of induced resistances. SAR, induced by the pathogen infection, is mediated by salicylic acid and associated with the accumulation of

Evidence showing that endophytes have a role in the outcome of plant – pathogen interactions leading to disease has been increasing in recent years. Diverse mechanisms by which they may

counteract pathogen development have been observed. For example, some endophytic species may induce plant defense mechanisms which counteract pathogen attack, others produce antibiotic substances which inhibit pathogen growth, competition for plant space and resources may also occur between resident endophytes and incoming pathogens; finally, some parasites of plant pathogens are known to behave as endophytes.

The infection process mainly depends on the host pathogen interaction. Competition of ecological niche and nutrition, hyperparasites and predation are between diverse micro-organism that live in endophytic niche, especially between the endophytes and pathogens. Endophytic colonization occupy ecological niche and leave no space for the pathogens, which could be the common reason for the fungal endophytes inhibiting the pathogen infection in the plant.

A mechanism of induced plant resistance did not seem to be involved, because differences in disease severity were observed between endophyte-inoculated and non-inoculated leaves of the same plant. In this case, the protection against a pathogen could be the result of direct competition among endophytes already present in leaves and the pathogen (Arnold *et al*. 2003). For instance, most tissue available for infection may be already occupied, or endophytes may produce zones of inhibition restricting the entry of other fungi.

Some endophytes may be mycoparasites. *Acremonium strictum* W. Gams is an endophyte which has been frequently isolated from *Dactylis glomerata* L. and other grasses (Sánchez Márquez *et al*. 2007); recently it has been shown that this fungus is a mycoparasite of *Helminthosporium solani* Durieu and Mont., a potato pathogen (Rivera Varas *et al*. 2007).

Fungal endophytes are generally thought to protect the plant by rapid colonization and thereby exhausting the limited available substrates so that none would be available for pathogens to grow (Pal and Gardener, 2006). Furthermore, the plants produce lignin and other cell-wall deposits to limit the growth of endophytes and cause it to be a virulent (Harman *et al*. 2004). As a result, the cell wall becomes re-reinforced after endophytic colonization, thus it becomes difficult for pathogens to infest.

Salicylic acid

The importance of SA arises from its role in the mediation of resistance (R)-gene resistance and basal immune responses, and from the positive link between SA-mediated defence and the small interfering RNA (siRNA) antiviral machinery (Alamillo *et al*. 2006; Baebler *et al*. 2014; Hunter *et al*. 2013). SA biosynthesis and signalling are activated on recognition of viral effectors by R gene products, which conditions incompatible interaction. Activation of the incompatible interaction results in several responses to limit viral propagation at the infection site, including the accumulation of reactive oxygen species (ROS) and pathogenesis-related (PR) proteins, induction of the hypersensitive response (HR), callose deposition, tissue disorganization, changes in the size and shape of chloroplasts, nuclear and nucleolar degradation, and programmed cell death (PCD) (Baebler *et al*. 2014; Dinesh-Kumar *et al*. 2000). SA is also responsible for the activation of systemic acquired resistance (SAR) in distal tissues, which lessens the effects of secondary attacks.

Jasmonic acid

The term *jasmonate* to include the biologically active intermediates in the pathway for jasmonic acid biosynthesis, as well as the biologically active derivatives of jasmonic acid. These compounds are widely distributed in plants and affect a variety of processes (Creelman and Mullet, 1997), including fruit ripening, production of viable pollen, root growth, tendril coiling, plant response to wounding and abiotic stress, and defenses against insects and pathogens.

JA is an oxygenated fatty acid (oxylipin) involved in resistance to necrotrophic pathogens and insect infestation (Thaler *et al*. 2004). Together with ethylene, JA regulates induced systemic resistance (ISR), which is invoked by non-pathogenic microbes, such as rhizobacteria. A study has shown that rhizobacterium-mediated induction of JA reduces the symptoms of CMV infection in Col-0 (Ryu *et al*. 2004). Furthermore, unravelling the effects of JA on compatible and incompatible interactions requires further kinetic analyses involving other antagonistic/synergistic

defence hormones, which may be involved in such regulation at the initial phase of infection.

Systemic Acquired Resistance

Systemic acquired resistance (SAR) is a form of induced resistance that is activated throughout a plant after being exposed to elicitors from virulent, avirulent, or nonpathogenic microbes, or artificial chemical stimuli such as chitosan or salicylic acid (SA) (Vallad and Goodman, 2004; Gozzo and Faoro, 2013). Induction of SAR is characterized by the accumulation of salicylic acid to stimulate defense mechanisms, often resulting in a localized hypersensitive response. SA is not a mobile signal agent, but is proposed to be the first chemical in the induction of pathogenesis-related (*PR*) genes, especially *PR-1*(Sticher *et al*. 1997; Fu and Dong, 2013). *PR* genes code for proteins such as chitinases and â-1,3-glucanases among others, and play a role in either preventing or slowing colonization of pathogens in the host (Sudisha *et al.* 2012).

Interactions with nematodes

Inhibitory effects against some species of migratory and sedentary endoparasites occur in grasses infected by *Neotyphodium* endophytes (West *et al.* 1988; Kimmons *et al.* 1990). *Neotyphodium* species infect aerial tissues, not roots. Therefore, the inhibitory effects observed in infected plants were interpreted as the result of fungal alkaloids being translocated to roots. This was supported by the fact that some naturally occuring *Neotyphodium* strains deficient in the production of ergot alkaloids do not show protective effects as good against *Pratylenchus* sp. as those observed in ergot alkaloid producing strains (Timper *et al.* 2005). In contrast, other experiments showed that the amount of ergot alkaloids translocated to roots is very small, and experiments with *Neotyphodium* knockout mutants having their pathway of ergot alkaloid synthesis disrupted and suggested that these alkaloids are not responsible for the inhibition of nematode populations in endophyte infected plants (Panaccione *et al.* 2006). Nevertheless, other types of alkaloids with antiherbivore activity are produced by *Neotyphodium* species, and chemical changes such as the production of phenolic compounds do occur in *Neotyphodium*-infected roots (Malinowski and Belesky, 2000) the nematicidal effects are observed. In conclusion, *Neotyphodium* endophytes provide host plants with

protection against several nematode species, but the mechanism of action underlying this process is still unknown.

Non pathogenic strains of *Fusarium oxysporum* E.F. Sm. and Single isolated from plant roots are other group of endophytes known to be implicated in antinematode activity. Culture filtrates of *F. oxysporum* have an inhibitory effect on *Meloidogyne incognita* Kofoid and White, suggesting that fungal toxins could be the mechanism of interaction (Hallmann and Sikora, 1996). In conclusion, it is very likely that fungal endophytes affect the outcome of nematode attacks in plants, and certain endophytes could be used for nematode protection in agriculture.

Interactions with bacteria and viruses

Tests of the influence of endophytes upon bacterial and viral pathogens are not as numerous as those made with other plant pathogens. Bactericidal effects of endophyte culture extracts have been demonstrated and do not seem to differ from those observed for fungi or nematodes (Wang *et al.* 2007). In the case of viruses, the incidence of barley yellow dwarf virus (BYDV) was lower in *Lolium pratense* infected by *Neotyphodium* than in endophyte free plants. Since BYDV is transmitted by means of aphid vectors, toxic fungal alkaloids may be the reason for this effect, in fact, aphid reproduction was lower in endophyte infected plants than in those free of endophyte (Lehtonen *et al.* 2006). A very interesting connection of a different kind exists among endophytes and viruses. A *Curvularia* endophyte of the plant *Dichantelium lanuginosum* (Elliott) Gould was found to confer tolerance to high soil temperatures to the plant. Further observation of this system led to the discovery that a virus infecting the endophyte was an important factor contributing to the heat tolerance observed in the plants. Further observation of this system led to the discovery that a virus infecting the endophyte was an important factor contributing to the heat tolerance observed in the plants. Furthermore, the virus-infected endophyte could be used to confer heat tolerance to tomato plants (Marquez *et al.* 2007).

Hyperparasitism and predation

Hyperparasitism is another ecological strategy that endophytes provide to protect the host plant. In

hyperparasitism, the pathogen is directly attacked by a specific endophyte that kills it or its propagules. Fungal endophytes parasitize around the hyphae of pathogens by various means such as coiling, twisting, penetrating the hyphae of pathogens and secreting lyase to decompose the cell wall of pathogens. Microbial predations are the mode of action of endophyte to suppress the plant pathogens. *Trichoderma* produces a range of enzymes that are directly used against the cell walls of fungi to utilize the fragment of pathogens (Gao *et al.* 2010).

Secondary metabolites synthesized by endophytes

Many fungal endophytes produce secondary metabolites and some of these compounds are antibiotics having antifungal, antibacterial and insecticidal properties, which strongly inhibit the growth of other microorganisms, including plant pathogens (Gunatilaka, 2006).

Kusari *et al.*, 2013 investigated interactions that endophytes have with host plants, insect herbivores and with each other, and the diverse molecular mechanisms that might trigger similar chemical responses in both plants and endophytes. Elucidating such networks can enhance discovery of desirable endophytes and further sustain production of host plant compounds using the isolated endophytes.

Antimicrobials

The crude fungal extract inhibited *E. coli* and *Klebsiella* by producing bioactive compounds such as alkaloids, flavonoids, saponins, steroids and terpenoids (Senthilmurugan *et al.*, 2013). Kusari *et al.,* (2009) isolated, identified and characterized an endophytic fungus, A*spergillus fumigatus* from *Juniperus communis* and *L. horstmann*, as a novel producer of deoxypodophyllotoxin and performed *in vitro* antimicrobial assay against a panel of pathogenic bacteria. The study concluded the production of deoxypodophyllotoxin (found in the host) by the cultured endophyte which is an enigmatic observation. This demonstrates the horizontal transmission of genes from the host plant to its endophytic counterpart. It would be interesting to further study the deoxypodophyllotoxin production and regulation by the cultured endophyte as well as their scale up process for consistent and dependable production.

Antifungal

Cryptosporiopsis quercina is the imperfect stage of *Pezicula cinamomea*, a fungus commonly associated with the hardwood species in Europe. It was isolated by Strobel *et al.* (1999) as an endophyte from *Tripterigeum wilfordii*, a medicinal plant native to Eurasia. On Petri plates, *C. quercina* exhibited excellent antifungal activity against *C. albicans*. A peptide antimycotic "cryptocandin" was isolated and characterized form *C. quercina,* which was the chemical compound secreted by *C. quercina* in Petri plates having antifungal activity. "Cryptocin", a unique tetramic acid, is also produced by *C. quercina*. This compound possesess potent activity against *Pyricularia oryzae* as well as numbers of plant pathogenic fungi. The minimum inhibitory concentration (MIC) of this compound against *P. oryzae* being 0.39 ìg/mL was examined as a natural chemical control agent for rice blast and used as a base model to synthesize other antifungal compounds (Li *et al.*, 2000). Doley and Jha (2010) isolated 253 isolates belonging to 12 genera of fungal endophytes from *Rauwolfia serpentine*, an important medicinal plant of Assam. They found that 58% of isolated fungi were anamorphic, followed by Ascomycotina (42%). The recovery of Ascomycotina (68%) was more than anamorphic fungi (32%) during summer season. During winter season, however, anamorphic fungi (55%) were more frequent than the Ascomycotina (45%). Species of *Trichoderma, Nigrospora* and *Curvularia* were the most frequently isolated endophytes from *R. serpentine.* The crude extract of *Nigrospora* spp. also showed antipathogenic activity against *F. oxysporum* and *Phytophthora* spp. Coombs *et al.* (1963) isolated 38 strains of endophytic actinomycetes from surface-sterilized wheat and barley root and tested their antagonistic activity for pathogens to wheat roots such as *Gaeumannomyces graminis, R. solani* and *Pythium* spp. They demonstrated that 17 of these isolates displayed statistically significant activity in the plant against *G. graminis* and some of them were able to control the development of disease symptoms in the treated wheat plants exposed to the former two pathogens in field soil. Li *et al.* (2011) isolated an important endophyte *Talaromyces flavus* from a mangrove plant *Sonnertia apetala. T. flavus* is responsible to produce a new nonsesquiterpene peroxides (talaperoxides AD (1-4). This chemical may be

exploited in the management of certain plant diseases.

Many endophytic species produce antibiotic substances (Schulz and Boyle, 2005; Strobel, 2002; Wang *et al*., 2007). Liquid extracts from endophyte cultures have been found to inhibit the growth of several species of plant pathogenic fungi (Liu *et al*., 2001; Park *et al*., 2005; Inácio *et al*., 2006; Kim *et al*., 2007). If such compounds where produced by endophytes *in planta*, this could constitute a defense mechanism against fungal pathogens. Experiments where plant protection against pathogenic fungi is observed after the inoculation of plants with endophytes, as well as after the application of endophytic culture filtrates, suggest that the endophyte may produce an antifungal compound or a substance that induces plant defense mechanisms in the plant. This is the case with *Chaetomium* and *Phoma* endophytes of wheat, when these fungi were previously inoculated in plants, reduced severity of foliar disease caused by *Puccinia* and *Pyrenophora* spp. was observed and, the same protective effect was observed when only endophytic culture filtrates were applied to the plants (Dingle and McGee, 2003; Istifadah and McGee, 2006). In these experiments the effects of culture filtrates upon plant pathogens were not tested. When a mixture of six species of endophytes frequently isolated from cacao (*Theobroma cacao* L.) trees was used to inoculate leaves of endophyte-free seedlings of this plant species, the severity of a leaf disease caused by a *Phytophthora* sp. was significantly reduced in endophyte-inoculated leaves. Sadrati *et al*. (2013) isolated 20 endophytic fungi and 23 endophytic actinomycetes from wheat (*Triticum durum*). All the fungal extract showed antimicrobial activity. *Penicillium* sp. showed highest inhibition against *C. albicans*.

Antioxidants

Harper *et al*. (2003) isolated two compounds, "pestacin" and "isopestacin" from the culture broth of *Pestalotiopsis microspora*, an endophyte isolated from *Terminalia* spp. in New Guinea. Both the compounds had antimicrobial as well as antioxidant properties. Electron spin resonance spectroscopy measurement confirmed its antioxidant properties because of their structural similarities with flavonoides. Saraswati *et al*. (2013) reported that fermented medium of endophytic

fungus *Fennelia nivea* NRRL 5504 showed antioxidant property against DPPH through free radical scavenging effect. Terpenes, triterpenes, phenolic compounds, tannins, flavonoids and saponins were the antioxidant compounds isolated from the fermentation of liquid potato dextrose broth, which showed 86.51% free radical. Sadrati *et al*. (2013) isolated 20 endophytic fungi and 23 endophytic actinomycetes from wheat (*Triticum durum*). The crude extract of *Penicillium* and *Aspergillus* had antioxidation activity with an inhibition percentage of 78.96 and 73.97, respectively.

Insecticidal products

Several endophytes are known to have anti-insect properties. Webber (1981) was probably the first to report an example of plant protection given by an endophytic fungus, *Phomopsis oblonga* on elm tree against the beetle *Physocnemum brevilinenu*. It was suggested that the endophytic fungus *P. oblonga* was responsible for reducing the spread of the Dutch elm disease causing agent *Ceratocystis ulmi* by controlling its vector *P. brevilineum.* It is believed that ergot alkaloids and mycotoxin are mainly responsible for their anti-insecticide properties. Nodulisporic acids, novel indole diterpines, isolated from *Bontia daphnoides* that exhibited potent insecticidal properties against the larvae of bowl fly, worked by activating insect glutamate-gated chloride channel (Demain, 2000). Daisy *et al*. (2002) isolated another endophytic fungus, *Muscodor vitigenus*, from liana plant (*Paullina paullinioides*), which yielded naphthalene as major product. It could be used as active ingredient against common mothballs. It also showed a promising preliminary result as an insect deterrent and exhibited potent insect repellent against wheat stem sawfly (*Cephus cinctus*).

Nematicidal products

Hallman and Sikora (1994) reported that *F.oxysporum* reduced the population of plant parasitic root knot nematode in tomato roots in Kenya. They suggested that culture filtrate of endophytic *F. oxysporum* were toxic to these nematode causing inactivation and death. Shahasi *et al*. (2006) isolated nine endophytic *F. oxysporum* and screened for the production of secondary metabolites antagonistic to *Radopholus similis* in the culture. Undiluted and diluted culture

filtrates were tested against the motile stages and eggs of *R. similis*. All the isolates tested *in vitro* showed antagonistic activity, causing paralysis of *R. similis* motile stages. The percentage of paralyzed nematodes increased with increase in the length of exposure time to the culture filtrates. After 24 h exposure in culture filtrates, up to 100% of the treated nematodes were paralyzed compared to 26.5% in the control treatments. Nematode mortality rates after 24 h exposure in the culture filtrates ranged from 76.4 to 100%. Paralysis was reversible at lower concentrations of the filtrates. Males of *R. similis* were more sensitive to the culture filtrates than the females. Culture filtrates of all the isolates exhibited inhibitory effects on hatching of *R. similis* eggs. The results confirmed the potentialilty of using endophytic *F. oxysporum* as biological control agents against *R. similis* and for toxic derivatives from their secondary metabolism to be used as potential nematicides.

Biologically active compounds

Stierle *et al.,* (1993) researched on taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew, *Taxus brevifolia*. The hyphomyceteous fungus when grown in a semi-synthetic liquid medium produced taxol and related compounds. Taxol was identified by mass spectrometry, chromatography and reactivity with monoclonal antibodies specific for taxol. Both [1-14C] acetic acid and L-[U-14C] phenylalanine served as precursors of [14C] taxol in fungal cultures. Schulz *et al.,* (2002) reviewed on how endophytic fungi serve as a source of novel biologically active secondary metabolites. Accordingly in course of the last 12 years, 6500 endophytic fungi were isolated from herbaceous plants and trees screened them for biological activities and have isolated and determined the structures of the biologically active compounds. The substances isolated were originated from different biosynthetic pathways belonging to diverse structural groups. The potential role of the endophyte and its biologically active metabolites in its association with its host has been investigated. Correlations were found between biological activity and biotope *e.g.* a higher proportion of the fungal endophytes in contrast to the soil isolates inhibited at least one of the test organisms for antialgal and herbicidal activities. It was seen that the fungal endophytes possess the exoenzymes necessary to colonize their hosts. Certain endophytic interactions associated with roots of the host may be mutualistic improving growth of the host and supplying the mycobiont with enough nourishment to extensively colonize the host's roots. Further plant defense metabolites are higher in plants infected with endophytes. Hence the interaction fungal endophyte–plant host is characterized by a finely tuned equilibrium between fungal virulence and plant defence. Not only the endophyte has to compete with epiphytes and pathogens but presumably also has to regulate metabolism of the host in their delicately balanced association. The utilization of a biotope such as that of the fungal endophyte is one aspect of intelligent screening and that fungi in different biotopes are still need to be exploited.

Anticancer activity

Hundley (2005) did structure elucidation of bioactive compounds isolated from endophytes of *Alstonia Scholaris* and *Acmena graveolens*. In the present study, an endophyte of the genus *Xylaria* was isolated from a stem of *A. scholaris* its mycelia and exudate extracted and the extract assayed for growth inhibition of HeLa cancer cells *in vitro*. Several known compounds were isolated and identified based on NMR, infrared and mass spectral data. The compounds identified are 19, 20-epoxycytochalasin C; 19, 20-epoxycytochalasin D and xylobovide. Two other compounds, fusaric acid and dehydrofusaric acid were discovered in an endophyte of the Hypocreales family inhabiting the plant *A. graveolens.*

Kumala *et al.,* (2007) experimentally produced cytotoxic secondary metabolites from the fermentation broth of the endophytic fungus isolated from the fruits of *Brucea javanica. In vitro* cytotoxic assays were performed using leukemia cell line L1210. LC-MS analysis of the F4 fraction of n-butanol extracts of secondary metabolites revealed bruceocin and canthin–6 compounds as cytotoxic constituents. These compounds were previously reported in the same host plant. Hence the present study could demonstrate the possibility of the endophytic fungi living symbiotically within the host plant producing cytotoxic secondary metabolites.

Wijeratne *et al.,* (2008) isolated sesquiterpene, quinones and related metabolites from *Phyllosticta*

spinarum a fungal strain endophytic in *Platycladus orientalis* of the Sonoran Desert. Five new metabolites (+)-(5*S*,10*S*)-42 -hydroxymethylcyclozonarone (**1**), tauranin (**2**) 3-ketotauranin (**3**), 3R-hydroxytauranin (**4**),12-hydroxytauranin (**5**), and phyllospinarone (**6**), were isolated and the structures of these new compounds were determined on the basis of their 1D and 2D NMR spectroscopic data and chemical interconversions. Tauranin showed activity when evaluated for inhibition of cell proliferation assay in a panel of five cancer cell lines that also induced apoptosis in PC-3M and NIH 3T3 cell lines during flow cytometry.

Antiparasitic activity

Lactones of endophytic origin have antiparasitic activity against *Plasmodium falciparum*. Three lactones were isolated from the culture medium of the endophytic fungus *Xylaria* sp. One was identified as (+)-phomalactone (**1**). The others were 6-(1-propenyl)-3, 4, 5, 6-tetrahydro-5-hydroxy-4Hpyran- 2-one (**2**) and 5-hydroxymellein (**3**). Compounds 1 and 2 were reported for the first time as constituents of *Xylaria*. Also this study was the first report showing the activity of these lactone compounds against a chloroquine-resistant *Plasmodium falciparum* strain, Romero *et al*. (2008).

Different fungal species have been exploited as an alternative source of plant secondary metabolites. Endophytic fungi colonize plants internally without apparent adverse effects and do occur ubiquitously in plants. They are known to produce a number of important secondary metabolites including anticancer, antifungal, antidiabetic and immunosuppressant compounds *e.g.* paclitaxel, torreyanic acid, cytochalasins etc. have been isolated from endophytic sources. The discovery of Stierle and his co-workers, studies carried out by Strobel and Daisy had raised scope of using the endophytic fungus as a sustainable alternative source of important plant secondary metabolites. However our poor understanding of the evolutionary significance of these organisms and their dynamic interaction with their respective hosts results in failure of exploiting endophytic fungi in diverse arenas Priti *et al*. (2009).

Volatile compounds

Atractylodes lancea, also known as CangZhu in China, belongs to the *Asteraceae* family of plants

and have found the main medicinal active ingredients of *A. lancea* were volatile oils, whose principal components include sesquiterpenes and polyacetylene secondary metabolites, such as ² -caryophyllene, zingiberene, ² -sesquiphellandrene, caryophyllene oxide, hinesol, ² -eudesmol, atractylone, and atractylodin (Yuan *et al*., 2016). Chen *et al*., 2016 identified the active structural material of the endophytic fungus *Gilmaniella* sp. AL12. These volatile oils and eight main components (² -caryophyllene, zingiberene, ² -sesquiphellandrene, caryophyllene oxide, hinesol, ² -eudesmol, atractylone, and atractylodin) were observed in *Atractylodes lancea* plantlets inassociation with the endophytic fungi. *Muscodor albus* is a recently described endophytic fungus obtained from small limbs of *Cinnamomum zeylanicum* (cinnamon tree). Strobel *et al*.,(1999) described that this xylariaceaous fungus effectively inhibits and kills certain other fungi, and bacteria, by virtue of a mixture of volatile compounds that it produces. Each of the five classes of volatile compounds produced by the fungus (alcohols, esters, ketones, acids and lipids) had some inhibitory effect against the test fungi and bacteria, but none was lethal. However, collectively they acted synergistically to kill a broad range of plant- and human-pathogenic fungi and bacteria. The most effective class of inhibitory compounds was the esters, of which 1-butanol, 3-methyl-, acetate was the most active biologically.

Enzymes

When endophyte colonizes on the plant surface, they produce enzymes such as â-1,3- lucanases, chitinases and cellulases to hydrolyze the plant cell wall. In addition, these enzymes also have a function to suppress the plant pathogen activities directly and have the capability of degrading the cell wall of fungi and Oomycetes (Gao *et al*. 2004). Senthilmurugan *et al*. (2013) isolated *Botrytis* sp. as endophyte from the aerial roots of *Ficus benghalensis* in India. The culture of this fungus produced amylase and laccase enzyme.

Fungal endophytes as plant growth promoters

Endophyte may promote plant growth by secreting different hormonal substances. Porter *et al*. (1979) reported that endosymbionts produced auxin, which enhanced the vegetative growth of the endophyte infected plants. They also reported the

mechanism of increased drought tolerance in the plants due to higher ability of meristem to recover after drought condition. Waqas *et al.*, 2015 determined for the first time, the efficacy and capacity of the endophytic fungi *Penicillium citrinum* LWL4 and *Aspergillus terreus* LWL5 to trigger SAR and thereby regulate hormone signaling networks involved in the defense against Sclerotium rolfsii stem rot caused in sunflower plants. The sunflower (*Helianthus annuus* L.) and its resident fungal endophytes were used to understand these interactions between pathogenic infections and endophytic priming.

Plant growth-promoting endophytes (PGPE) inhabit plant tissues and the close linkage of endophytes inside plant tissues facilitates nutrients exchange and enzymes activity (Khan *et al.* 2015; Murphy *et al.* 2014). The distribution of growth-promoting hormones produced by endophytic microorganisms towards plant tissues positively promotes plant growth (Lin and Xu, 2013). Endophytes possess vital ability to mobilize insoluble phosphate and provide nitrogen to their host plants (Matsuoka, *et al.* 2013; Shi *et al.* 2011). Microbial endophytes colonize plant tissues without symptomatic behaviour and consequently they compete with other microbial pathogens on the same ecological niches. Therefore, the established plant-endophyte association improves plant health via different mechanisms displayed by endophytes and potentially contributes the protection of plant host against microbial pathogenesis (Malhadas *et al.* 2017). PGPE produce various bioactive compounds with several biological activities which can be directly or indirectly described as plant growth-promoting (PGP) agents. Approximately most of the plants harbour endophytes interior their tissues; however, available information on PGPE and their biological activities is not equivalent to the high distribution of endophytes. A superior comprehension of the native endophytes of plants may help clarify their capacities and potential in enhancing plant growth and establishing a sustainable system for crop production (Hassan, 2017).

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