

Review

Regulation and accumulation of secondary metabolites in plant-fungus symbiotic system

Yuan Zhi-lin^{1, 2}, Dai Chuan-chao² and Chen Lian-qing^{1*}

¹Institute of Subtropical Forestry, Chinese Academy of Forestry, Fuyang, Zhejiang Province, 311400, China.

²Jiangsu Key Laboratory for Biological Diversity and Biotechnology, College of Life Science, Nanjing Normal University, Nanjing, Jiangsu Province, 210097, China.

Accepted 16 May, 2007

Plants have evolved adaptive strategies to mutualistic microbes penetration for both mycorrhizal fungi and endophytic fungi. Subsequently, an array of host plant defense responses and signal transduction is generated. A group of secondary metabolites are accumulated inducibly or enhanced constitutively in plant tissues during the process. Symbiotic fungi usually perform compatible and friendly interactions with host plants, which contribute to growth promotion and secondary metabolites accumulation simultaneously, such as alkaloids and terpenoid with pharmacological characteristics. Especially, some secondary metabolites derived from root exudation act as signal molecules, which induce the spore germination and hypha branching in mycorrhizal fungi. However, the precise mechanisms in some cases remain unclear so far and need to be further investigated. Above exciting and interesting results shed light on our understanding of the mystery of fungal elicitation of secondary metabolites accumulation in plant kingdom. Therefore a deeper insight in mutualistic symbiosis is of great importance for biological applications: (1) the plant/microbial co-culture system *in vitro* may be perfectly useful to guide the cultivation of medicinal plants for obtaining high level of bioactive compounds; (2) manipulating plant released signal molecules and isoprenoid metabolism will be effective to optimize and improve the function of mycorrhizae in forestry, agriculture and horticulture.

Key words: Secondary metabolites, mycorrhizal fungi, fungal endophytes and host plant.

INTRODUCTION

Plants may be considered as a famous chemical factory for biosynthesis of a huge array of secondary metabolites. Many of these chemicals are utilized as medicine, scent, dyes and pesticides and are of commercial importance.

Secondary metabolites are those compounds produced by plant which are not essential for plant growth and development. Nevertheless, their ecological roles have been extensively studied and have received more attentions in the past few years.

Environmental factors including biotic and abiotic stimuli, carbon-nutrition balance, genotype and ontogenesis usually control and regulate the biosynthesis of secondary metabolites in plants (Kliebenstein, 2004; Laitinen et al., 2005; Lerda, 2002; Mary Ann Lila, 2006). With regard to plant-microbe interactions, co-evolution betw-

een plants and their microbial partners are mediated via plant chemical defense (Bennett and Wallsgrove, 1994; Lu and Shen, 2004). All parts of plants are exposed to a heterogeneous or extreme environment. Synergism of plant secondary metabolites in response to a diversity of unfavorable environment factors including microbial invaders was revealed (Ryabushkina, 2005). On the other hand, the complexity of plant-microbe interaction may represent different adaptive mechanisms in plants.

For example, rhizosphere, phyllosphere and endosymbiotic microorganisms may establish neutral, beneficial and antagonistic relationships with plants (Gnanamanickam, 2006). Tremendous researches have revealed the molecular basis and principles of the plant-microbe interactive mechanism (Lugtenberg et al., 2002), which indicates that plant secondary products usually act as signal molecules or respond to pathogen and symbiont colonization. Although the roles of secondary metabolites in plant-pathogen interactions have been well-documented (Field et al., 2006; Hahlbrock et al., 2003;

*Corresponding author. E-mail: zhi_lin_yuan@hotmail.com.

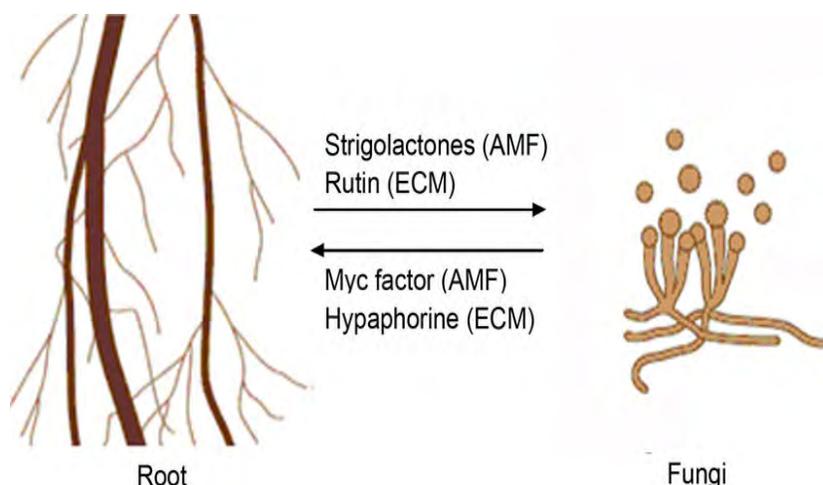


Figure 1. Signals exchange and dialogue in soil between plant roots and mycorrhizal fungus. AMF, arbuscular mycorrhizal fungus; ECM, ectomycorrhizal fungus. Modified from Bais et al. (2006).

Saunders and O'Neill, 2004; Grayer and Kokubun, 2001), only limited information is available from published studies about the significance of host secondary products involved with plant-mutualistic fungi associations, especially in plant-endophyte interaction. In this review, we attempt to comment on the accumulation and regulation of secondary metabolites in plants in the process of compatible and friendly interaction with symbiotic fungi from the cellular physiological point of view.

Plant secondary metabolism changes during the establishment of symbionts with mycorrhizal fungi

Mycorrhizal associations are the most important and prevalent mutualistic symbiosis which involve 3-way interactions between host plants, mutualistic fungi and soil factors. In contrast to plant-pathogen interactions, mycorrhizal associations shape compatible and friendly relationships. Morphologically, some specialized infection structures such as haustoria invaginating inside host cells are formed in biotrophic fungal pathogens. Nevertheless, nutrients and signals bi-directional exchange is performed through diverse functional structures in mycorrhizal symbionts including arbuscules (arbuscular mycorrhiza), Hartig net (ectomycorrhiza) and hyphal coils (orchid mycorrhiza).

A large numbers of literature have verified that multiplicity of signals and diversity of signaling pathways exist during the establishment of mycorrhizal associations with regulation of symbiosis-specific genes expression (Harrison, 2005; Hause and Fester, 2005). In pre-symbiotic phase, plant and their fungal partner secrete signals into soil, mostly secondary metabolites, subsequently perceived by roots and mycelium inducing morphological and physiological changes (Figure 1). Admittedly,

identification and characterization of chemical nature of these signals will probably play an important role in agricultural and horticultural applications, since they are considered as “green molecules” to mediate and enhance the symbiotic interaction in the field (Akiyama et al., 2005; Akiyama and Hayashi, 2006; Bécard et al., 2004; Martin et al., 2001). Just like the well-studied plant-pathogen interaction, phytoalexins accumulation in mycorrhizal-infected roots was also investigated, but to a much lower level than in plant pathogen interaction. This raises the possibility that signal perception and transduction proceed via similar pathways between the symbiosis and pathogenesis of plants (Garcia-Garrido and Ocampo, 2002; Baron and Zambryski, 1995). However, the defense response in plant-mycorrhizal association is probably weak and transient once the symbiosis becomes established. Several mechanisms participating in the regulatory events have been hypothesized in detail (Hause and Fester, 2005). In summary, fine-tune regulatory mechanisms and compatibility are involved with the plant-mycorrhizal fungus interactions (Figure 1).

In the past few years, many studies focus on mycorrhizal fungus-mediated regulation of secondary metabolites biosynthesis in plants, which may participate in the chemical dialogue between two organisms. To our knowledge, there has been extensive research devoted to studying the terpenoids metabolism in mycorrhizal-infected plants. Recently, the significances of isoprenoid metabolism in arbuscular mycorrhizal roots have been reviewed (Strack and Fester, 2006). It was shown that some gramineous plant roots accumulated mycorradicin, so-called “yellow pigment” compounds upon mycorrhization. Another category of mycorrhiza-induced secondary metabolite is blumenin. Chemical analysis have identified that they are carotenoid-origin of cyclohexenone derivatives (Strack et al., 2003). Arbuscular mycor-

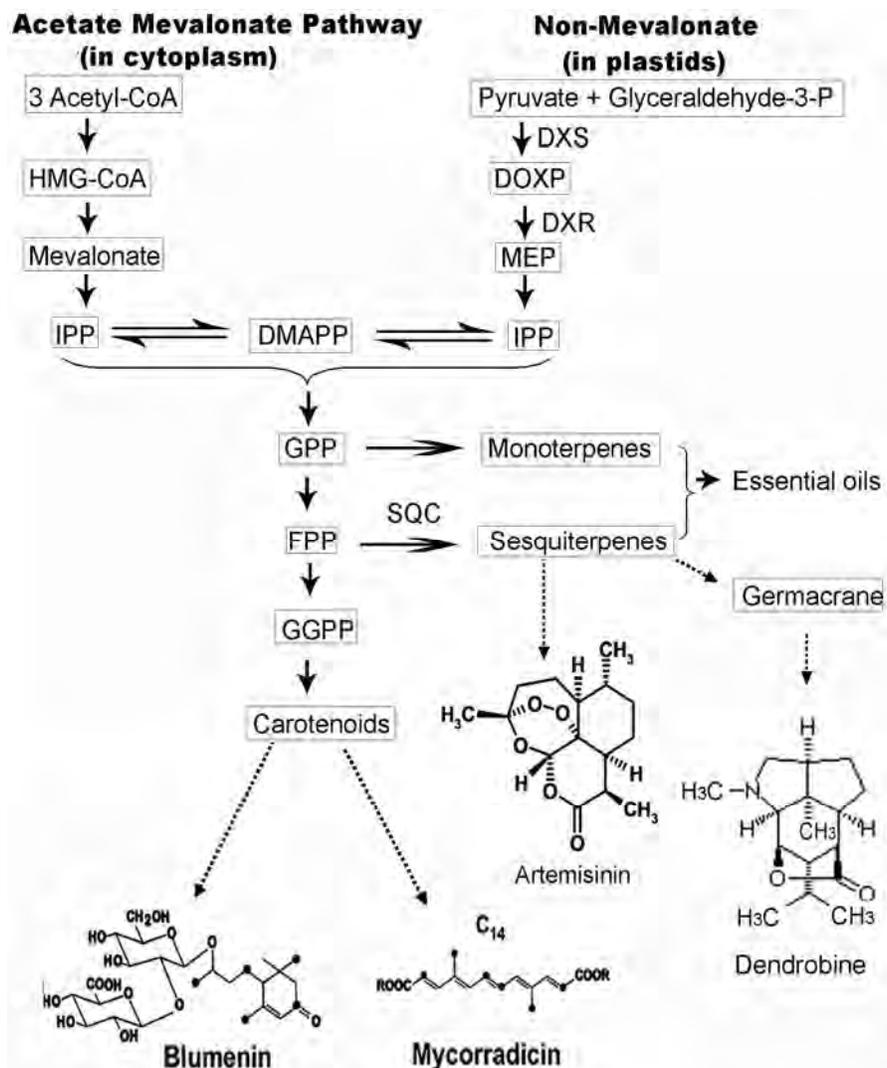


Figure 2. Isopentenyl diphosphate biosynthesis pathway (MVA and MEP) probably induced or promoted by mycorrhizal fungus and non-mycorrhizal fungal endophytes in plants. DOXP, 1-deoxy-D-xylulose 5-phosphate; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; GAP, glyceraldehydes 3-phosphate; HMG-CoA, β -hydroxy- β -methylglutaryl-CoA; IPP, isopentenyl diphosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; DMAPP, dimethylallyl diphosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; SQC, sesquiterpene cyclase (Strack et al., 2003; Edwards et al., 1970; Weathers et al., 2006).

rhizal fungus induced the accumulation of mycorradicin via non-mevalonate methylerythritol phosphate pathway (MEP pathway). cDNA encoding two enzymes central to this pathway, 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) have been cloned from plants (Figure 2). Strong induction of transcript levels of DXS and DXR in mycorrhizal-plants has been investigated (Walter et al., 2000). Detailed knowledge of the roles of mycorradicin and blumenin in mycorrhizal symbiosis will open up new perspectives for further research. These intriguing findings will probably lead to novel strategies and insights to

develop and maintain AM symbiosis such that symbiotic interaction between roots and mycorrhizal fungus could be promoted along with apocarotenoids accumulation. Furthermore, arbuscular mycorrhizal fungus may be exploited as a bioinoculant to improve the essential oil concentration of some medicinal plants. Essential oils are also terpenoids based on C5 subunits (isoprenoid). Application of these bioactive compounds in the pharmaceutical industry is prevalent around the world. Some essential oil-rich plants are officially in pharmacopoeias of most of the countries (Copetta et al., 2006; Kapoor et al., 2002) (Figure 2).

Moreover, alkaloids are also constitutive defense-related secondary metabolites in plants. These including trigonelline, castanospermine and camptothecin will also be enhanced by an arbuscular mycorrhizal fungus inoculum (Abu-Zeyad et al., 1999; Wei and Wang, 1989; Rojas-Andrade et al., 2003). Some of medicinal plants have been used as traditional Chinese medicine (TCM) with anti-tumour activity. For example, *Dendrobium nobile* and *Dendrobium candidum* (Orchidaceae) are two famous herbs in China, which establish symbiotic relationship with orchid mycorrhizal fungus. Using co-culture system *in vitro*, plant growth effect and dendrobine (pseudo-alkaloid or sesquiterpene alkaloid) production were promoted to a certain extent (Chen and Guo, 2005).

Secondary metabolites accumulation in plants challenged with endophytes

Plant-endophyte association is another type of symbiotic relationship with similar or higher degrees of complexity with respect to mycorrhizae (Scannerini et al., 2001). Endophytes usually occur in above-ground plant tissues, but also occasionally in roots (for example, dark septate endophytic fungi have been isolated from various plants), and are different from mycorrhizae by lacking external hyphae (Mandyam and Jumpponen, 2005; Faeth and Fagan, 2002). Prevailing views contend that fungal endophytes are presumably thought to have evolved from plant pathogenic fungi (Freeman and Rodriguez, 1993; Kogel et al., 2006; Saikkonen et al., 1998). Although some root endophytic fungus requires host cell death for proliferation during forming mutualistic symbiosis with plant (Deshmukh et al., 2006), it is universally hypothesized that endophyte-host interactions involve a balance of antagonism and exhibit great phenotypic plasticity compared to plant pathogens (Schulz and Boyle, 2005). However, signals released from two partners and their roles remain largely unknown.

Researchers have endeavored to elucidate the molecular mechanisms during the establishment of plant-endophytic association (Sheremeti et al., 2005; Bailey et al., 2006). However, only few documents refer to the plant secondary metabolism mediated by the fungal endophytes. Peppermint growth and terpene production of *in vitro* generated plants (*Mentha piperita*) in response to inoculation with a leaf fungal endophyte indicate variation of the essential oil profile by fungal infection. However, no significant differences were appreciable in total essential oil productivity between infected plants and control plants (Mucciarelli et al., 2003). Our previous research showed that the weight of roots, seedlings and terpenoid production of *Euphorbia pekinensis* increased after they were inoculated with an extensive host range endophytic *Phomopsis* sp. Cytochemical analysis showed that the enzymatic activities of PAL (phenylalanine ammonia-lyase) and DXR in plant tissues were

promoted upon the endophytic fungus colonization (data have not yet been published). Meanwhile, microbial elicitor derived from some fungal endophytes also promotes biomass and induces the terpenoids (artemisinin) biosynthesis and production in plant suspension cells (Wang et al., 2006). It seems likely that both mycorrhizal fungi and fungal endophytes infection might result in specific-enhancement of the MEP pathway metabolic flux in plants.

The red resin of *Dracaena cochinchinensis* is commonly used in traditional Chinese medicine for the treatment of traumatic and visceral hemorrhages. Chemical studies have revealed that the resin contains various flavonoids (Zheng et al., 2004). An endophytic *Fusarium* sp. was isolated from the roots of *D. cochinchinensis*. Co-culture system *in vitro* found that red resin emerged and accumulated in the inoculated sites (Jiang et al., 2003). In addition, endophytic actinomycetes may also affect plant growth either by nutrient assimilation or enhanced secondary metabolites (anthocyanin) synthesis (Hasegawa et al., 2006). The precise mechanisms, however, need to be further demonstrated. We speculate that recognition of endophytes by host plants trigger a cascade of signal transduction, which give rise to a series of plant defense responses similar to plant-pathogen interaction, thus leading to a noticeable change in plant metabolic state.

Co-culture system for the biotic elicitation of secondary metabolite production in plants with symbiotic fungi

It has been universally accepted that plant secondary metabolites actively participate in plant-microbe interaction, not exclusive to plant-symbiotic fungi associations. Enhancement of secondary products accumulation in plant is of great importance in medicinal plants cultivation industry. Therefore, co-culture system is assumed to be a meaningful and effective tool to biotic elicitation of secondary metabolite production in plants upon symbiotic fungi infection. Moreover, mostly large groups of terrestrial plants ubiquitously harbor both endophytic fungi and mycorrhizal fungi in their inner tissues. Therefore, we presume that plants will achieve superior outcomes through dual inoculation with mycorrhizal fungi and endophytic fungi; probably above-ground and below-ground plant parts establish two types of symbiotic associations and result in increasing microbial genetic diversity in plant tissues. Moreover, metabolic profiling of secondary metabolites of symbiotic fungi-infected plants should be performed using HPLC fingerprint to determine relevant changes in the metabolites pattern in comparison to non-infected plants due to the complex chemical structures of bioactive molecules (Figure 3), which are substantial base of the special functions and activities for "Chinese medicine".

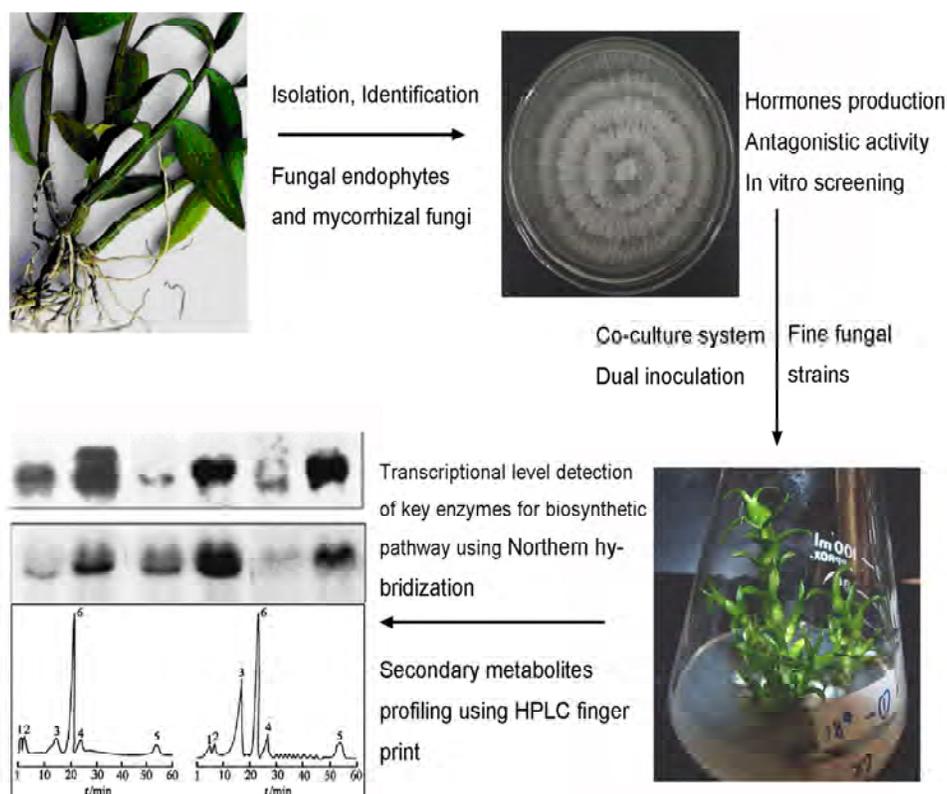


Figure 3. Brief procedures for co-culture system to improve secondary metabolites production in plants with symbiotic fungi. The northern hybridization is from Walter et al. (2000).

Conclusions

The roles played by mutualistic symbioses in plant secondary metabolism have been underestimated. One of the outcomes of plant-mutualistic fungi interactions results in reprogramming the host cell's metabolic state. Secondary metabolites accumulation in plants in the course of plant-symbiotic fungi interaction definitely impels the development of attractive strategies to bring medicinal plants cultivation into new era for pharmaceutical purpose. Symbiotic fungi may be cultivated *in vitro* and applied to host under controlled environmental conditions to analyze their potential effects on plant morphogenesis and secondary metabolism. Therefore, symbiotic fungal inoculum will be exploited into commercial microbial agents for the sustainable development of traditional Chinese medicine. Otherwise, manipulating the release of plant-derived signals (secondary metabolite) and isoprenoid metabolism through metabolic engineering offers the promising opportunity to mediate the formation and functions of mycorrhizae in nature.

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