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REVIEW PAPER

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ARBUSCULAR MYCORRHIZA – PARTNER IN COMMUNICATION

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ABSTRACT

Arbuscular mycorrhiza is one of the most common type of mycorrhiza in plant kingdom. Process of plant root colonization by arbuscular mycorrhizal fungi is consisted of four phases: presymbiotic phase, phase of contact and hyphae penetration to the roots, growth phase of hyphae inside the roots and phase of mycorrhizal intracellular structure development. The formation of symbiosis between fungi and host plant requires the exchange of molecular signals between these organisms. Plant signal molecules are described as strigolactones and cutin monomers whereas fungal signal molecules are lipo-chito-oligo-saccharides and short chito-oligosaccharides. During the contact with plant roots fungal hyphae form appresorium on the surface of epidermis. After appresorium creation, the pre-penetration apparatus (PPA) is formed in plant cell, which is a structure defining a route of the hyphae overgrowing across the plant cell. Afterwards the fungus penetrates the epidermal cell and the cell of root cortex, where hyphae leave the cell and enter into appoplast, growing and branching along the root axis.

Key words: arbuscular mycorrhizal fungi, root colonization, strigolactones, signaling

INTRODUCTION

Arbuscular mycorrhiza (AM) is the oldest and the most commonly spread type of mycorrhiza in plant kingdom. This form of symbiosis is created by arbuscular mycorrhizal fungi, which live in symbiosis with at least 70–90% of plant species [Smith and Read 2008]. The presence of arbuscular mycorrhiza in the majority of plants suggests that its origin was probably connected with the colonization of land by plants [Wang and Qiu 2006]. When the first plants colonized land, an essential problem was probably the uptake of phosphate ions, having limited mobility in soil as a result of creation insoluble compounds with dominant cations in soil i.e. Al^{3+}, Fe^{3+}, Ca^{2+}. Poorly branched rhizomes of the earliest land plants e.g. *Agalophyton* were probably not able to uptake of P from soil. Therefore it is assumed that *Glomeromycota* fungi played an important role in the colonization of land by plants helping them in nutrient uptake in the presence of poor root development and deficiency of available forms of nutrients [Fitter 2005].

Arbuscular mycorrhizal fungi increase the absorption surface of plant roots and the bioavailability of some nutrients, what contribute to enhancing nutrient and water uptake by plants [Karagiannidis et al. 2007]. Moreover, these fungi stimulate the secretion of plant hormones [Toussaint et al. 2007], increase the intensity of photosynthesis [Zhu et al. 2014] and plant tolerance to abiotic and biotic stress factors including heavy metals, drought, salinity, pathogenic fungi and nematodes [Li and Christie 2001, Vincente-Sánchez et al. 2014, Ruiz-Lozano et al. 2016].

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STRUCTURES OF ARBUSCULAR MYCORRHIZA

Arbuscural mycorrhizal fungi include structures localized outside and inside the root. There are external structures such as external hyphae and spores and the internal structures like internal hyphae, arbuscules and vesicles [Smith and Read 2008]. The structures formed by arbuscular mycorrhizal fungi were described at the beginning of twentieth century by Gaullaud, who classified them into two anatomic groups: *Arum* and *Paris.* In *Arum* type intercellular hyphae of fungi grow rapidly in the root cortex along well developed intercellular air spaces. Short branches of hyphae penetrate the cortical cell wall and grow dichotomously in the cell lumen and finally form highly branched arbuscules. Vesicles may develop both inside and between cells of plant root. Hyphal coils may be formed during the penetration but they are not the major component of colonization. By contrast, in *Paris* type cortical colonization is characterized by extensive development of intracellular coiled hyphae, which spread directly from cell to cell. Arbuscule-like branches are sometimes formed from this coil, however intercellular development of fungal structures is marginal. Vesicles are only formed between cells of plant root [Smith and Read 2008].

ROOT COLONIZATION BY ARBUSCULAR MYCORRHIZAL FUNGI

There are three main sources of inoculum in the soil: spores, infected root fragments and hyphae, all named as propagules. Spores are the best described sources of inoculum and identification of fungal species is based on their morphology. Nuclear division, utilization of carbohydrates and lipids and consequently creation of branched hyphae are observed during germination of spores [Smith and Read 2008]. This process does not require the presence of host plant roots [Akiyama et al. 2005, Besserer et al. 2006]. Created hyphae penetrate the soil in search of host plant and if they do not establish the contact to three weeks, their growth is inhibited. The inhibition of growth of hyphae is followed by removal of cytoplasm, cell nuclei and other organelles as well as the formation of compartments, what allows the spores and hyphae maintain long vitality and ability to re-germination and colonization of plant roots [Logi et al. 1998].

The process of plant root colonization by arbuscular mycorrhizal fungi is consisted of four phases, which are defined as: presymbiotic phase, contact phase and hyphae penetration to the roots, phase of hyphae spreading inside the roots and phase of development of mycorrhizal intracellular structures [Smith and Read 2008].

The symbiosis between fungi and host plant requires the exchange of molecular signals between these organisms. As it was mentioned above, in the absence of host plants, fungal spores are able to germinate, however growth of hyphae is inhibited. This phenomenon is probably caused by the lack of molecular signals secreted by plant roots which stimulate growth of fungal hyphae [Bécard et al. 2004]. Recognition of plant signal changes the direction of hyphae extension and initiates the searching of the appropriate contact point of hyphae with the root, and creation of appresorium [Vierheilig et al. 1995]. Until recently it was thought that flavonoids might have been the signal molecules in establishing of symbiosis [Vierheilig et al. 1998]. However, Buee and co-authors [2000] proved that despite of the stimulating effect on the growth of mycelium, flavonoids are not essential for formation of mycorrhiza. Authors proved that root exudates of carrot stimulated the growth of mycelium and formation of symbiosis between the fungus and host plant. They demonstrated the presence of these compounds in all tested host plants i.e. carrot, sorgo, corn, pea whereas they did not find them in plants not creating the symbiosis with AMF i.e. arabidopsis, rape and cabbage. These exudates have recently been identified as strigolactones, which are chemical compounds belonging to the group of sesquiterpens, derivatives of carotenoids [Akiyama et al. 2005, Besserer et al. 2006]. They are secreted at low concentration, which also may suggest their role as the signaling molecules establishing and maintaining the symbiosis with arbuscular mycorrhizal fungi. Strigolactones stimulate the seed

germination of parasitic plants of the *Orobanche* family as well as negatively regulate growth of plants [Marzec and Muszyńska 2012]. Increase of strigolactone secretion is observed under condition of phosphorus and sometimes nitrogen deficiency in the root zone whereas the increase in the concentration of these macronutrients has a limited effect on strigolactone exudation [Yoneyama et al. 2012]. Perception of strigolactones by fungal cells increases their physiological activity leading to an increase of ATP production and division of mitochondria [Besserer et al. 2006, 2008]. These processes induce the first phase of the symbiosis between fungus and host plant, called presymbiotic phase, in which the spore germination, growth and branching of the hyphae are observed [Parniske 2008]. Bonfante and Genre [2015] suggest that fungi receiving a signal in the form of strigolactones, activate the response in their cells, involving metabolic processes of cell wall, what leads to a growth of hyphae and increase of the strigolactone production. The latest studies [Wang et al. 2012, Murray et al. 2013] indicate a role of cutin monomers in establishing symbiosis between fungus and host plant. Cutin monomers are hydroxylated aliphatic acids which may be secreted on the surface of roots as required signal differentiating appresorium form fungi hyphae [Murray et al. 2013]. From the fungus "Myc factor" (diffusible fungal molecule), which induces specific for the symbiosis response in the roots of host plant, participate in presymbiotic phase [Parniske 2008]. These molecules ("Myc factor") have recently been identified as lipo-chito-oligo-saccharides and short chito-oligosaccharides, which sequestration is increased as a result of strigolactone reception by germinating fungal spores [Maillet et al. 2011, Genre et al. 2013]. Maillet and co-authors [2011] proved that *Rhizophagus intraradices* (*Glomus intraradcices*) secreted molecules containing a mixture of sulphate and non-sulphate lipo-chito-oligo-saccharides, stimulation the formation of mycorrhiza in plant species of the families *Fabaceae, Asteraceae, Umbelifereae*. Secreted by fungus, lipooligosacharides and chitooligosacharides are received by root epidermal cells of host plants and activate the mechanism associated with early stages of root colonization including changes in the concentration of calcium (Ca^{2+} spiking) in the cells of epidermis and root hairs [Genre et al. 2013, Sun et al. 2015]. The influx of Ca^{2+} into the cytoplasm turns into a regular oscillatory changes in the concentration of this ion [Sun et al. 2015]. The repeated model of changes in the concentration of calcium ions is called the signature of calcium. Information encoded in the calcium signature may be decoded by a corresponding sensor proteins, that activate signaling pathways and regulate processes of fungal infection as well as arbuscule organogenesis.

Fungi on contact with plant roots form on the surface of epidermis the structure called hypopodium (appresorium). Hypopodium, formed on the surface of plant root, is a site of hyphae penetration into the root, followed by the overgrowth of rhizodermal cell layer [Giovannetti et al. 1993]. Establishing and stabilization of symbiosis between fungus and host plant contribute to molecular changes involving gene activation or silencing. Molecular activity is associated with both structural and physiological changes in the root epidermal cells, what creates a favorable environment for penetrating hyphae [Sedas et al. 2009]. After these changes, about 4–5 hours after appresorium formation, the prepenetrating apparatus (PPA) is formed in plant cell. PPA is a structure which defines a passage route of growing hyphae across plant cell. Formation of PPA is preceded by the migration of the nucleus in the direction of hypopodium, which is anticipated point of penetration of fungus. The migration of nucleus is accompanied by the reorganization of microtubule and radiation of thin actin bunches from the nucleus to the point of contact of the fungus with the plant cell. Then the nucleus moves at a speed of 15–20 $\mu m \cdot h^{-1}$ along the developing PPA pointing the direction of its formation. Composed of microtubules, microfilaments and endoplasmic reticulum PPA is a thin cytoplasmic bridge, in the shape of a tube, extending across the cell. After formation of PPA, the fungus penetrates the epidermal cell and reaches to the cortical cells of the root, where the hyphae leave the cell and penetrate into the appoplast, increasing and branching along the axis of the root [Genre et al. 2013].

Hyphae developing inside the root stimulate the formation of structures similar to PPA in the cortical cells. Through these structures hyphae penetrate the cell and develop to form the arbuscule. During penetration of cell wall and formation of the arbuscule trunk, hyphae do not penetrate through cell membrane but stretch it, causing separation of arbuscule from the cytoplasm of plant cell. This membrane, called PAM (periarbuscular membrane), despite the different specialization still retains some characteristic similar to membrane surrounding the plant cell [Parniske 2008], and its specialization is connected with activity of enzymes, nutrient transporters and aquaporins [Porcel et al. 2006, Aroca et al. 2007]. The cell membrane surrounding arbuscule together with plant cell membrane and cell wall residues form a kind of space (interface) which is involved in the transport of the nutrients from the fungal cell into plant cells. The formation and development of arbuscule is accompanied by changes in the structure of plant cells. Their physiological activity is increased, nucleus and nuclei becomes larger and moves from lateral to central part of the cell and then is surrounded by the arbuscule. The organization of cytoskeleton is changed, the volume of the cytoplasm and the number of mitochondria and plastid is increased and vacuoles are broken down [Timonen and Peterson 2002]. The development of arbuscules may be stimulated by lysophosphatidylcholine (LPC), which is key signal molecule that activates expression of the genes transporters of phosphorus [Drissner et al. 2007]. According to Javot and co-authors [2007] the activity of phosphorus transporters is critical for AM symbiosis and the loss of function of phosphorus transporters leads to premature death of arbuscules.

Alexander and co-authors [1988] proved that the development of arbuscule is similar in wheat, oat and maize colonized by *Rhizophagus fasciculatum* (*Glomus fasciculatum*) and takes about 8.5 days. Arbuscules develop in the cells of plant roots until they reach the maximum growth and fill the entire cell. Afterwards they are separated from the structures of the fungus, decay and disappear. The development and dying of arbuscules in the cells occur at the same time, therefore arbuscule at different stages of their development are simultaneously observed in plant cells [Javot et al. 2007].

The above-mentioned description of colonization is related to the *Arum* type of mycorrhiza. Slightly less is known about the development of the intracellular structures of the fungus in the *Paris* type of mycorrhiza. They are more compact and homogeneous, what results from growth of fungus directly from cell to cell, rather than growing in the intercellular spaces. Furthermore, time for the development of mycorrhizal structures in the *Paris* type is longer than *Arum* [Cavagnaro et al. 2001a, b].

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