Mycorrhiza

The Oldest Association Between Plant and Fungi

Jagnaseni Barman, Aveek Samanta, Babita Saha and Siraj Datta

The symbiotic association between plant and fungi (mycorrhizal association) is an amazing phenomenon observed in nature. The mycorrhizal association is one of nature's boons for sustainable agriculture. In today's changing environment, indiscriminate use of pesticides and chemicals pose a great threat to the existence of mycorrhizal species. There is a need to spread awareness in order to save mycorrhizal fungi from extinction.

1

2

3

4

[1] Student of BTech Biotechnology at Haldia Institute of Technology.

[2] Presently working as an Assistant Professor of Botany at Prabhat Kumar College, West Bengal.

[3] Presently working as a Senior Secondary teacher (Biology) in Bhavans' N S C Bose Vidyaniketan, West Bengal.

[4] Presently serving at the Department of Biotechnology, Haldia Institute of Technology as a Professor and Head. He is fond of writing articles on various aspects of Science and Technology.

Introduction

Plants associate with other life forms (animals, bacteria or fungi) to complete their life cycle, to fight against pathogens or to thrive in adverse environments. The plant root and its associated living organisms are together called 'rhizosphere', the region of mycorrhizal association. Mycorrhiza is one of the best examples of symbiotic[1] association between plants and fungi. The term 'mycorrhiza' comes from Greek – *mycos* meaning fungus and *rhiza* meaning roots. In nature, more than eighty percent of angiosperms, and almost all gymnosperms are known to have mycorrhizal associations. There are mainly two types of mycorrhizal associations found in nature (*Box* 1) namely, endomycorrhizae or arbuscular-mycorrhizae (AM), eg., *Endogone*, *Rhizophagus*, etc. and ectomycorrhizae (EM), eg., *Laccaria bicolor*, *Amanita mascaria*, etc. Mycorrhizal associations help the host plants to thrive in adverse soil conditions and drought situations by increasing the root surface and mineral uptake efficiency. Environmental threats like increased temperature, changing climate and associated drought, soil infertility, etc., are some of the major challenges in agriculture and have to be mitigated to ensure global food security. In this context, mycorrhiza-based crop production is one

Keywords

Land pollution, mycorrhiza, plant defense, sustainable agriculture, symbiotic association, water stress.

Box 1. Types of Mycorrhiza

There are seven types of mycorrhizae (*Table* 1). These are endo (arbuscular), ecto, ectendo, arbutoid, monotropoid, ericoid, and orchidaceous mycorrhizae, as described by the scientists. Among them, endomycorrhizae and ectomycorrhizae are the most abundant and widespread (*Figure* A).

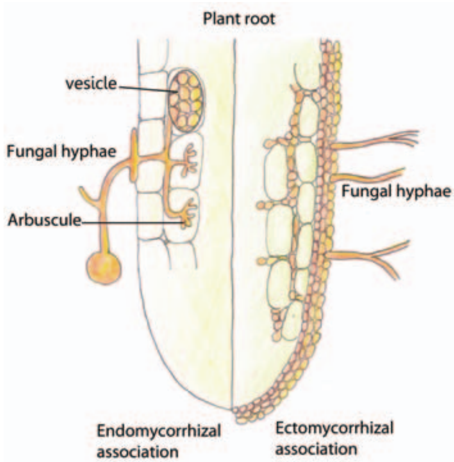


Figure A. Schematic representation of endo- and ectomycorrhizal association between plant and fungus. (Adapted from L Taiz and E Zeiger, *Plant Physiology*, 2002.)

In endomycorrhizal association of the vascular-rbuscular (VA) type, the fungi penetrate the cortical cells of roots and form clusters of finely divided hyphae which ultimately develops into arbuscules (small ellipsoidal structures). VA fungi forms a mutualistic association with around 80% of vascular plants. It belongs to the phylum Glomeromycota.

The ectomycorrhizal fungi invade a partial region of the host root without penetrating the cortical cells and form a thick mantle around the roots. Ectomycorrhizal associations are present in 3% of vascular plants and the fungi belong to the phylum Ascomycota and Basidiomycota.

of the key components of sustainable agriculture practices.

[1] The beneficial association of two different species.

How Did Mycorrhiza Evolve?

Fossil records and DNA-based phylogenetic[2] analysis suggest that around 450–500 million years ago, mycorrhiza evolved with the

[2] The evolutionary relationship among organisms.

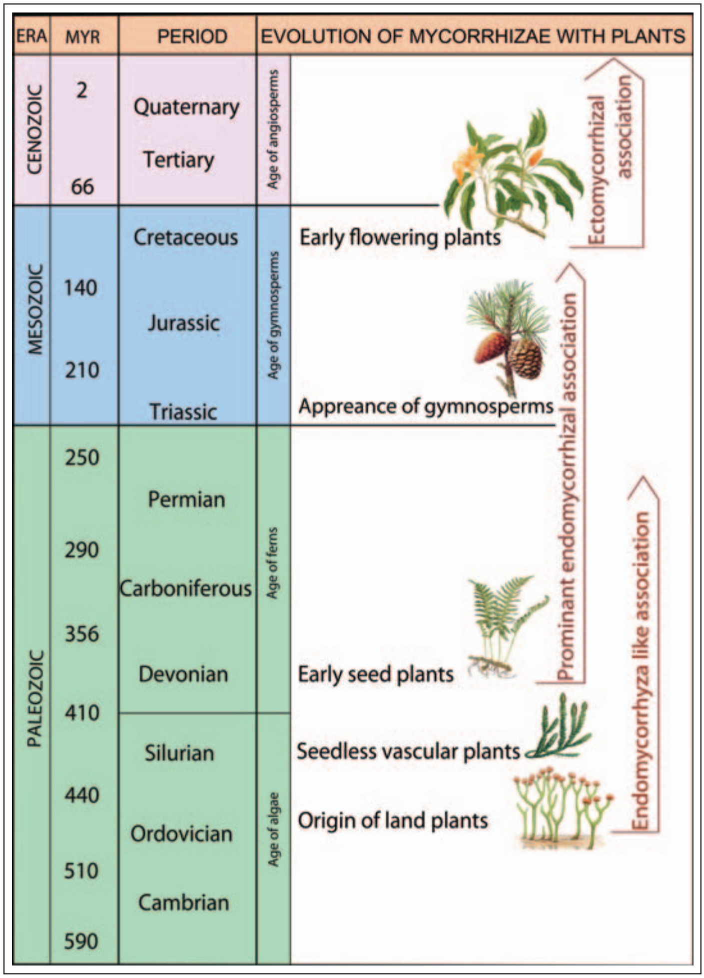
Mycorrhizal Type	Fungal Taxa	Plant Taxa	Intracellular Colonization	Fungal Sheath	Vesicle
Arbuscular	Glomeromycota	Bryophyta Pteridophyta Gymnosperms Angiosperms	Present	Absent	Present or Absent
Ecto	Basidiomycota Ascomycota Zygomycota	Gymnosperms Angiosperms	Absent	Present	Absent
Ectendo	Basidiomycota Ascomycota	Gymnosperms Angiosperms	Present	Present or Absent	Absent
Arbutoid	Basidiomycota	Ericales	Present	Present or Absent	Absent
Monotropoid	Basidiomycota	Monotropoideae	Present	Present	Absent
Ericoid	Ascomycota	Ericales Gymnosperms	Present	Absent	Absent
Orchidaceous	Basidiomycota	Orchids	Present	Absent	Absent

Table 1. Major categories of mycorrhizae and their attributes.

first land plants as an endomycorrhizal association. Subsequently, about 200–150 million years ago, with increasing organic content of the soil, ectomycorrhiza evolved. Mycorrhizal associations appears to be a co-evolutionary[3] process (*Figure* 1). Early events of mycorrhizal symbiosis may have involved reciprocal genetic exchanges among ancestral plants and free-living fungi. In endo- and ectophytic association, both the partners may have evolved in parallel in response to environmental changes, and gradually became interdependent for sharing limited resources. Thus, most of the present-day plants have a mutualistic symbiosis with mycorrhizal fungi.

[3]Simultaneous evolution of two closely associated species.

Figure 1. The evolution of mycorrhiza through different geological time periods. In endo- and ectomycorrhizal associations, both the partners parallelly evolved in response to environmental changes and gradually became interdependent on each other. Thus, most of the present-day plants have a mutualistic symbiosis with mycorrhizal fungi.



Most of the present-day plants have a mutualistic symbiosis with micorrhizal fungi

Molecular Mechanisms Behind Mycorrhizal Symbiosis

AM fungi are obligate biotrophs, solely dependent on the host plants for their survival. The symbiotic mechanism comprise many steps (*Figure* 2). The first step is the search for the host root which is an important step in fungal-root-colonization process. The second step is penetration of fungi into the host root for colonization and final establishment of mycorrhizal symbiosis. These steps are described in detail as follows.

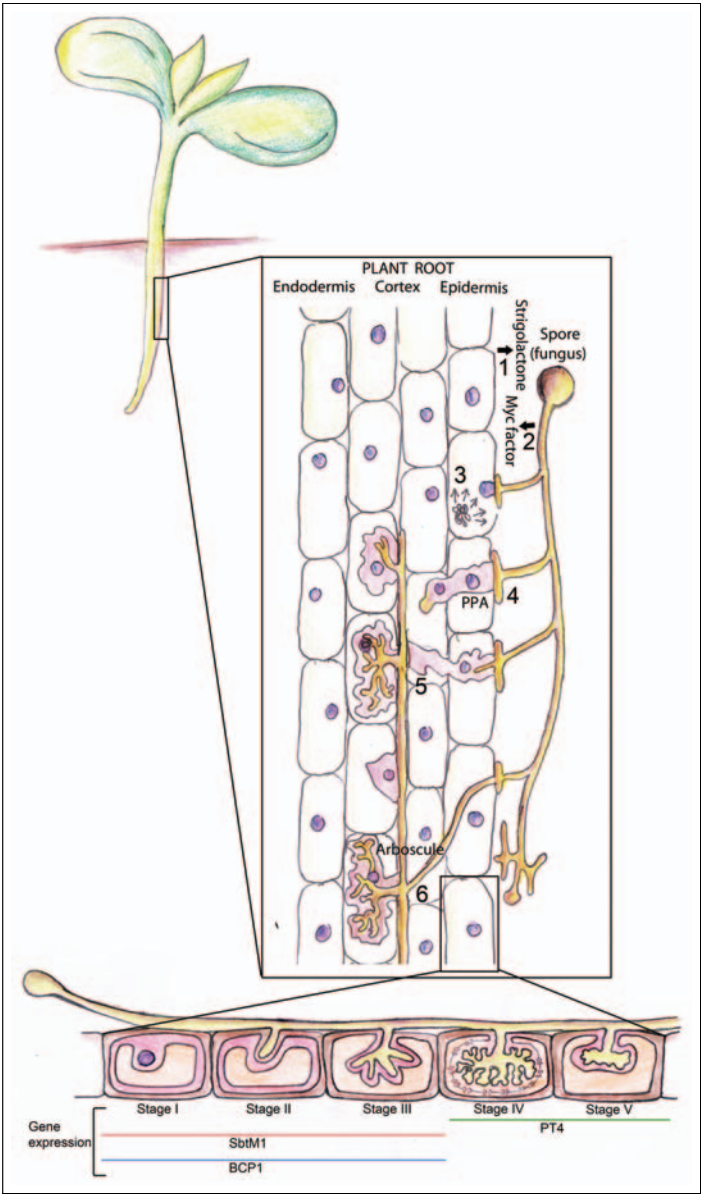


Figure 2. Diagrammatic representation of developmental stages of arbuscular development. **Stage 1:** formation of PPA; **Stage 2:** entry of fungal hyphae; **Stage 3:** formation of bird's foot; **Stage 4:** formation of mature arbuscule; **Stage 5:** collapse of arbuscule.

(Adapted from C Gutjahr and M Parniske, *Annual Review of Cell and Developmental Biology*, Vol.29, pp.593–617, 2013.)

Initial Recognition of Host Plant Roots by Fungi

Some bioactive[4] molecules like strigolactones secreted by the roots help fungi identify their host plants. Strigolactones also stimulate AM fungal growth and its branching. The fungi recipro-

[4]These are chemicals released from some specialized cells or tissues to induce functions in nearby cells or tissues of the same or different organism.

cate to this signal by secreting a set of hypothetical factors known as mycorrhizal factors (Myc). These factors also play a major role in communication between AM fungi and nitrogen-fixing bacteria. The AM interactions are established further with the induction of seven genes (*SYM* genes) (*Figure* 3). When the host Myc Factor Receptor(s) (MFR) perceive Myc signals, cytosolic calcium secretion is induced in root cells. A second membrane protein (SYMPK) is activated, which codes for a receptor-like kinase with the potential to recognise AM fungal signals directly or indirectly. SYMPK transduce these signals from the cytoplasm to the nucleus by phosphorylating an unknown substrate through its kinase domain.

The localization of all downstream elements present in the cytoplasm, activates rapid signal transduction[5] into the nucleus. Thus, a repeated oscillation of Ca^{2+} concentration occurs in the nucleus and cytoplasm, through the alternate activity of Ca^{2+} channels and transporters. These calcium oscillations are decoded by a calmodulin-dependent protein kinase (CCaMK). CCaMK phosphorylates the product of one of the *SYM* genes (CYCLOPS).

[5] Transmission of chemical signals from one cell to another.

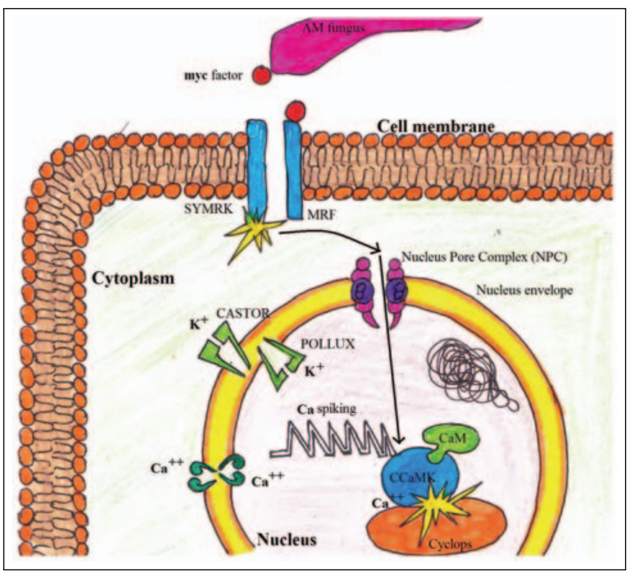


Figure 3. Pictorial representation of different gene(s) and signal protein(s) interaction and molecular mechanism of host recognition by AM fungi.

(Adapted from M Parniske, *Nature Reviews Microbiology*, Vol.6, pp.763–775, 2008.)

This eventually leads to the regulation of other genes and finally root colonization.

Penetration and Establishment of Mycorrhizae

After chemical acquaintance, the fungal hyphae[6] and the host root interacts with each other, and the hyphae gradually start its propagation into the host root by forming the 'hyphopodium'[7]. Many genes get activated subsequently, owing to hyphopodium formation. This is the primary step of colonization. Then a pre-penetration apparatus (PPA), which is indispensable for fungal penetration is developed. This structure allows the fungi to grow inside the plant without breaking the integrity of the cells.

[6]Hyphae are long thread-like fungal filaments and mycelium is the intertwined mass of hyphae.

[7]These are special type of hyphal branch composed of lobed cells with which the fungi attach to the cell wall of the plant partner.

The final step of this symbiotic process is the formation of arbuscules[8]. These arbuscules accommodate the fungi into the host cell cytoplasm. The arbuscular cells function as machines for nutrient transport and acquisition. Numerous genes and proteins are involved in the process of nutrient uptake which finally help in the accomplishment of symbiosis.

[8]Small tree-like structures.

The molecular mechanism adopted by EM fungi is almost similar but not identical to that of AM fungi. Further research is awaited to unravel the details of the processes underlying EM fungal associations.

How the Partners Benefit!

Plants provide carbon to their fungal partners. The photosynthetic product hexose is transported to the arbuscular part of fungal cytoplasm, and gets converted into glycogen and TAG (triacylglycerol) (*Figure* 4). These are suitable forms of carbohydrates that are easily transported to long distances within the fungal network. In the case of plants, mycorrhiza increases the surface area of roots for improved uptake of water and nutrients. Immobile nutrients are absorbed by the plants through diffusion. In nutrient-depleted, tropical regions with excessive rainfall where essential nutrients are leached from soil surfaces, mycorrhizal fungi can

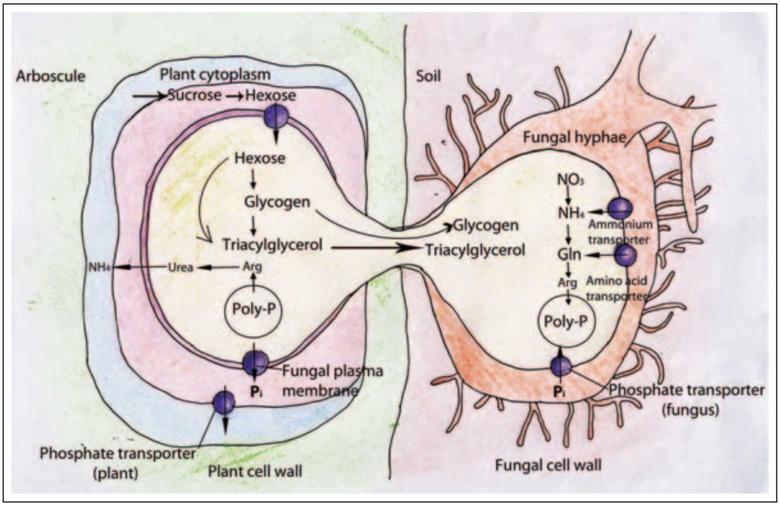


Figure 4. The process of nutrient transportation across the plant and fungus. Phosphate acquisition takes place through the fungal part and is transported to the plant through the phosphate transporter Pht1. Nitrogen is transported through the nitrogen transporter AMT1 to the plant partner. In return, the plant provides carbon sources to its mycorrhizal fungi partners for their nutrition.

(Adapted from M Parniske, *Nature Reviews Microbiology*, Vol.6, pp.763–775, 2008.)

extend their external hyphae beyond the depleted zones. As a result, more volume of soil becomes accessible to plant roots. Therefore, plants with mycorrhizal associations are more efficient in the absorption of nutrients like nitrogen, phosphorus, potassium, and calcium.

Mycorrhiza increases the surface area of plant roots for improved uptake of water and nutrients.

Phosphorus is an extremely immobile element present in the soil. The major role of vascular-arbuscular (VA) fungi is to supply phosphorus to plant roots via phosphate transporters present in the hyphal membrane. The networks of filamentous, extraradical[9] hyphae of AM fungi help in the uptake of freely available phosphates. Extension of fungal hyphae generally begins beyond the host root so that greater soil volume can be used for phosphate acquisition. AM fungi can hydrolyze organic phosphates present in the soil and provide soluble phosphates to their host plant. Phosphate transporter of the Pht1 family of fungi helps in the uptake of inorganic phosphate into the cytosol. Then the phosphate gets transferred to the fungal vacuole where polymerization occurs to form polyphosphate chains (poly-P). The poly-P is transferred to the intraradical hyphae, where hydrolyzation takes place by liberating free phosphate for transfer to the interfacial apoplast[10] of the AM fungi. Fungi provide phosphorus as poly-P pool to the plants. In soil with low phosphate content, mycorrhizae also help

[9]The mycorrhizal hyphae which are present outside the root of the host plant.

[10]The space outside a plant cell which forms a common boundary.

plants absorb copper and zinc by similar mechanisms.

The networks of mycorrhizal hyphae help plants absorb freely available phoshpates.

Nitrogen uptake is also very important for plant growth. Nitrogen is available in the soil as ammonium and nitrate. Ammonium, nitrate, and amino acids are absorbed by the extraradical mycelium of fungi. Nitrogen is generally taken up in the form of ammonium through a protein transporter named AMT1 (fungal origin). Among amino acids, arginine is typically involved in the translocation of nitrogen. Within the extraradical mycelium, ammonium combines with glutamate to form glutamine due to the activity of glutamine synthetase. After glutamine synthesis, arginine synthesis takes place with help of the enzyme arginosuccinate synthetase. Arginine is the final product utilized by plants.

There are several ways by which AM fungi help plants to absorb water from the soil. AM fungal hyphae grow into the soil matrix, and create a skeletal structure to hold primary soil particles together by physical enlargement. Soil structure and its porosity are important factors for water retention, especially during the dry season. AM fungi can also change the hormonal flow of information from plant roots to shoots, and affect stomatal responses when soil water potential is lowered. It has been reported that mycorrhizal associations help plants increase nutrient uptake during water-stressed conditions by increasing hydraulic conductivity in roots.

The rhizosphere is the site where microorganisms interact with both plant roots and soil constituents. The higher carbon demand of AM fungi competitively inhibits the growth of plant pathogens. Furthermore, the mycorrhizal fungal partner can also improve the nutrient status of the host plant by compensating the loss of root biomass due to pathogen attack by increasing its tolerance. With AM formation, production of plant defense chemicals like phenolic substances, phytoalexins, and chitinases are increased. Symbiotic processes are not affected by these chemicals but systemic plant defense mechanisms are turned on which in turn defend host plants from other harmful pathogens. Competitive inhibition of pathogens by endo- and ectomycorrhizal fungi is demonstrated to protect host plants from diseases like root rot, collar

Mycorrhizal associations also protect plants against heavy metal toxcity.

disease, etc.

Mycorrhizal associations also protect plants against heavy metal toxicity. Ectomycorrhizal fungi protect trees from high concentration of toxic heavy metals like copper, zinc, iron, manganese, cadmium, nickel, etc., by accumulating and immobilizing them in the mycorrhizal mantle. The plants associated with mycorrhizal fungi also benefit from fungal detoxification systems. The detoxification mechanisms include extracellular heavy metal chelation by root exudates (eg., glycoprotein glomalin), binding of heavy metals to rhizodermal cell walls, and avoidance of heavy metal uptake. The large surface area of fungal hyphae is an important sink point for heavy metals. Fungal vesicles are also sites for storage of toxic compounds. Thus, mycorrhizal fungi help in improving soil health by phytoremediation[11].

[11]The efficient use of plants for removal, degradation or detoxification of chemicals present in the soil surface or ground water.

Threats to Mycorrhizal Associations!

Mycorrhizae are major components of soil ecosystems and thus are essential for the survival of plant species. They also act as indicators of plant health and soil toxicity. Agricultural practices largely impact the activity of mycorrhiza. Soil tillage breaks up AM hyphal networks leading to a significant reduction in colonization of roots and phosphorus absorption from the soil. Crop breeding[12] may expedite the loss of AM fungal diversity if the selected hybrid plant genotype is unable to associate with the previous fungal partner. Indiscriminate use of fertilizers and pesticides can inhibit the formation and growth of both endo- and ectomycorrhiza.

[12]The classical genetic approach to improve crop plants in terms of quality or quantity.

Land and air pollution, mining, deforestation, etc., are some of the non-agricultural activities that have severe impacts on mycorrhizal survivability. The most common industrial air pollutants (SO_2, NO_2, O_3, etc.) emitted into the atmosphere cause severe loss of viability of mycorrhizal propagules resulting in a significant reduction of mycorrhizal colonization in roots. O_3 pose an indirect threat to mycorrhizal activity by degrading the photosynthetic pigments which lead to lower photosynthesis rate and hence lower the carbon sources channeled to the fungal partner. Decomposition of excessive ammonia present in the atmosphere cause physiological alterations such as cellular acidosis in plants and mycorrhizal species. Terrestrial pollutants such as polyaromatic hydrocarbons also have an adverse impact on mycorrhizal species.

Conclusion

Mycorrhizal symbiosis is one of the crucial factors that determine plant and soil health. In addition, mycorrhiza enhances mineral uptake ability and tolerance to drought stress. It also induces resistance against soil pathogens, and reduces sensitivity to toxic substances in their host plants. But present day practices of agriculture may lead to the destruction of these beneficial associations. Anthropogenic activities like slash and burn cultivation, mining, waste disposal, and clear-cutting of forests are also detrimental to mycorrhizae. The fundamental importance of mycorrhizal associations is evident in restoration and revegetation of unfertilized/fallow lands. However, use of mycorrhizal biotechnology (engineered establishment of mycorrhizal associations) in land reclamation and revegetation is not well-practiced in many parts of the world. It is crucial to recognize and understand the molecular and ecological roles of mycorrhiza for agriculture, horticulture, forestry, and soil remediation. Thus, development of mycorrhizal biotechnologies may be a better, nature-friendly alternative for agricultural practices like addition of inorganic fertilizers, and can go a long way in maintaining a sustainable environment for our future generations.

Indiscriminate use of fertilizers and pesticides can inhibit the growth of mycorrhiza.

Acknowledgement

Authors acknowledge the contribution of Poulomi Saha, final year BTech student of Haldia Institute of Technology for making the initial diagrams.

Suggested Reading

[1] M A Turk, T A Assaf, K M Hameed, A M Al-Tawaha, Significance of Mycorrhizae, *World Journal of Agricultural Sciences*, Vol.2, No.1, pp.16–20, 2006.

[2] M Parniske, Arbuscular Mycorrhiza: The Mother of Plant Root Endosymbiosis, *Nature Reviews Microbiology*, Vol.6, pp.763–775, 2008.

[3] M Brundrett, Diversity and Classification of Mycorrhizal Associations, *Biological Review*, Vol.79, No.3, pp.473–495, 2004.

[4] C Gutjahr, M Parniske, Cell and Developmental Biology of Arbuscular Mycorrhiza Symbiosis, *Annual Review of Cell and Developmental Biology*, Vol.29, pp.593–617, 2013.

[5] Z A Siddiqui, J Pichtel, *Mycorrhizae: Sustainable agriculture and forestry*. Eds. Z A Siddiqui, M S Akhtar, K Futai, Springer Science, Netherlands, pp.1–36, 2008.

[6] P Bonfante, A Genre, Mechanism Underlying Beneficial Plant-fungus Interactions in Mycorrhizal Symbiosis, *Nature Communications*, Vol.1, No.8, 2010.

Address for Correspondence

Jagnaseni Barman[1]

Aveek Samanta[2],

Babita Saha[3]

Siraj Datta[4]

Department of Biotechnology

Haldia Institute of Technology

ICARE Complex

Haldia, West Bengal 721 657

India.

Emails:

[1] titly179@gmail.com

[2] aveekbot@gmail.com

[3] babitasaha@gmail.com

[4] dattasiraj@gmail.com