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Symbiotic Nutrient Exchange in Arbuscular Mycorrhiza

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Summary

Arbuscular mycorrhizal (AM) fungi are obligate symbionts that cannot be cultured by conventional means. Culturing them accordingly requires inoculation to host plants and subsequent propagation. Culture collection of AM fungi was developed. Based upon a series of isolation, we found a new group of AM fungi. AM fungi supply host plants with phosphorus and in turn obtain photosynthates from those plants. It was also developed a method to isolate physiologically active intrradical hypahe which is responsible for P supply to plant and fungal C acquisition from plant. For the first time, the functions shown by intraradical hyphae of AM fungi within plant roots were reproduced *in vitro*. Furthermore, it was also found extensive tubular vacuole system in AM fungal hyphae which are not been found in any other taxa of fungi.

Adverse impacts from excessive use of chemical fertilizers or inappropriate use of agrochemicals have prompted research and development of cultivation techniques that use fewer synthetic chemicals and instead leverage inherent features of soil ecosystems. In the 1980s, researchers focused on mycorrhizal fungi that colonize the roots of plants as symbionts and promote the growth of host plants by supplying phosphorus to them. Private companies in particular conducted applied research to develop microbial inoculums of arbuscular mycorrhizal fungi (AM fungi) that colonize the roots of crops. However, very little research had been done on identifying the AM fungal flora of cropland soils of Japan or on elucidating the physiological functions of AM fungi. Accordingly, Saito and his co-workers started by classifying the species that would comprise the foundation of research on AM fungi, and have since conducted research on the ecology and physiology of AM fungi species to develop a body of knowledge as a foundation for the exploitation of AM fungi. In this paper, we reviewed some topics on their taxonomy and physiology.

Discovery of a New Phylogenetically Distinct Group of AM Fungi

AM fungi are obligate symbionts that cannot be cultured by conventional means. Culturing them accordingly requires inoculation to host plants and subsequent propagation. Identification of AM fungi also requires detailed observation and recording of the morphology of their spores. The AM fungal flora of Japanese cropland and grassland have been investigated by using a number of methods including directly extracting spores from soil and identifying them from their morphology, and by inoculating plants with rhizosphere soil itself to propagate the species present. Strains that have been subcultured have been registered with the National Institute of Agrobiological Sciences' gene bank and are available for research.

Identification of species requires the isolation of single spores. It was discovered that a single strain forms spores of two different morphologies that under the existing classification system would belong to two different taxonomic groups. The rDNA base sequence revealed that this strain belongs to a new taxonomic group differing from known groups (Sawaki *et al.*, 1997). This discovery prompted an extensive revision of the system used to classify AM fungi (Walker *et al.*, 2007).

The Mechanism Governing Phosphorus and Carbon Exchange between AM Fungi and Plants

AM fungi supply host plants with phosphorus and in turn obtain photosynthates from those plants. This exchange of nutrients has long been known, but very little information exists on the mechanisms underlying the transfer of substances between fungus and host plant. Saito (1995) accordingly developed a method for separating from the roots the intraradical hyphae that serve as the interface for substance exchange between the AM fungi and plants within plant roots, without affecting their physiological functions, and studied their physiology. It was discovered that the functions of AM fungi differ greatly according to location with respect to the host plant, with intraradical hyphae showing very different enzyme activity profiles from those of extraradical hyphae. Saito and his co-workers found that (1) glucose is the main form of photosynthate transferred from plants to AM fungi; (2) the intake of sugars by AM fungi appears to be linked to their supply of phosphorus to the host plant; (3) an alkaline phosphatase specific to intraradical hyphae plays a role in this phosphorus supply process (Solaiman and Saito, 1997; Solaiman *et al.*, 1999; Solaiman and Saito, 2001; Kojima and Saito; 2004; Aono *et al.*, 2004). This is the first ever report of the functions shown by intraradical hyphae of AM fungi within plant roots being reproduced *in vitro*. Furthermore, they found the significance of polyphosphate in phosphorus

storage and translocation by very sensitive enzymatic assay (Ohtomo and Saito, 2005 ; Takanishi *et al.*, 2009).

Using a fluorescent probe and laser scanning confocal microscopy to investigate the vacuoles responsible for transporting substances within the hyphae of *Gigaspora margarita*, an AM fungus, it was found them to be tubular in shape and arranged in extensive bundles that also move as one (Uetake *et al.*, 2002). This was the first report of this kind of system, which appears to be unique to AM fungi, and is assumed to play a role in the brisk transport of substances along the hyphae of AM fungi. Small acidic vesicles and other organelles unique to AM fungi were also found (Saito *et al.*, 2004). Furthermore, they developed an enzyme-liked method to show localization of polyphosphate and succeeded in showing the localization of polyphosphate in tubular vacuoles (Saito *et al.*, 2005 ; Kuga *et al.*, 2008).

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