Toward Assessing the Contribution of Arbuscular Mycorrhizal Symbiosis to Plant P Nutrition

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Toward Assessing the Contribution of Arbuscular Mycorrhizal Symbiosis to Plant P Nutrition

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Abstract

Phosphorus is one of the essential elements for plant growth and survival. However, phosphate concentration in soil solution is generally low due to the formation of its precipitates or its fixation to soil particles. One of the strategies plants employ to increase phosphate uptake from soil is the formation of symbiotic associations with fungi. Many land plants form symbiotic associations with arbuscular mycorrhizal fungi belonging to the subphylum Glomeromycotina. Host plants can absorb phosphate via hyphal networks of arbuscular mycorrhizal fungi via the mycorrhizal pathway. In laboratory experiments with well controlled growth conditions, we typically observe an increase in plant biomass resulting from improved plant phosphorus nutrition by the fungal colonization. However, the mycorrhizal effect is not always obvious in the field, possibly due to variable environmental factors and ineffective combinations of plant and fungal species. An evaluation of the mycorrhizal functions in the field is needed in order to utilize the symbiotic associations in agriculture. However, no diagnostic assessment for the mycorrhizal effect has been developed because the mechanism underlying phosphate translocation via the mycorrhizal pathway remains unclear. This article summarizes current knowledge of phosphate translocation mechanisms in arbuscular mycorrhizal symbiosis and discusses the development of methods for assessing the contribution of the mycorrhizal pathway to plant phosphorus nutrition in the field.

Phosphate in crop production

Phosphorus (P) is an essential microelement for plant growth and survival. P is used as a vital component of nucleic acids and biological membranes, bioenergetic molecules including ATP and signal transduction molecules. In crop production, phosphate (Pi) fertilizers are crucially important agricultural materials for supplying P to plants.

Pi fertilizers are produced from mined Pi rock that contains a relatively high amount of P. Pi rock is a finite resource that is unevenly distributed around the world. The main producers of Pi rock are China, the western Sahara (especially Morocco), the U.S.A, and Russia (Jasinski 2018). The longevity of remaining Pi rock reserves is still under debate as assumptions about demand and supply of P and the depletion model employed differ greatly among studies (Cordell and White 2014). For example, reported estimates of Pi rock reserve vary year to year. Although a huge amount of P resources remain in the world, Pi rock that does not come from the dwindling high-grade reserves is of questionable P concentration, purity, and accessibility (Cordell and White 2014). Recently, circumstances surrounding P resources are rapidly changing. The U.S.A. has shifted export of P materials from Pi rock to the wet-process phosphoric acids of high value that are produced from Pi rock. In 2008, the price of Pi fertilizers transiently increased 1.5-fold in Japan primarily due to a 135% export tariff placed on Pi by China (**Fig. 1**). The Japanese government took urgent action worth approximately 300 million yen to address the problem (Ministry of Agriculture, Forestry and Fisheries). In the last fifteen years, the prices of Pi fertilizers have gradually increased by approximately 150%. The increase in fertilizer prices is serious for farm management because the cost of fertilizers is approximately 10% of the total cost of crop production.

The consumption of Pi in agriculture has dramatically increased over the last one hundred years and will continue to increase in the future (Cordell *et al.* 2009, Cordell and White 2014, 2015). The production of Pi rock has also increased

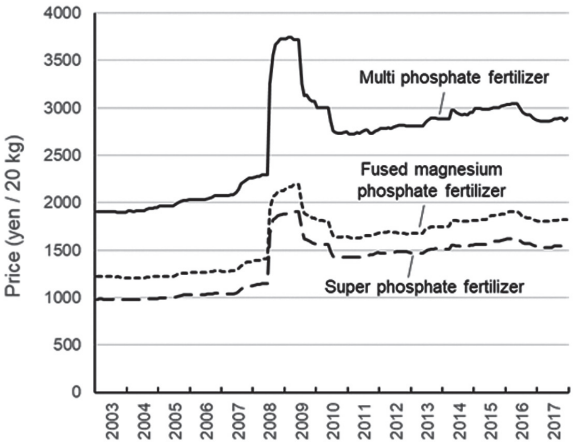

Fig. 1. Change in prices of phosphate fertilizers for the last 15 years based on the statistical survey on prices in agriculture by Ministry of Agriculture, Forestry and Fisheries of Japan (http://www.maff.go.jp/j/tokei/kouhyou/noubukka/).

in response to the demand for Pi fertilizers. However, the production is predicted to decrease due to the reduction of high quality Pi rock deposits (Cordell *et al.* 2009). In the future, there is a concern about a large difference between the demand and supply of P resources. In order to overcome this issue, there is a need to use P resources efficiently through P recycling or other methods (Cordell and White 2014).

It is known that the efficiency of Pi fertilizer use is extremely low (Syers *et al.* 2008). In vegetable crop production, the usage of Pi fertilizers is very large due to the low use efficiency. The remaining Pi not taken up by plants is adsorbed by soil particles. It is very important to increase use efficiency to reduce production costs and conserve agricultural environments. A promising strategy for increasing Pi use efficiency is to utilize arbuscular mycorrhizal (AM) symbiosis in agriculture.

Challenges in utilizing arbuscular mycorrhiza for crop production

AM is a symbiotic association between many land plants and soil-borne fungi belonging to the subphylum Glomeromycotina (Smith and Read 2008). This association facilitates Pi uptake from soil, which results in the increase of plant biomass. AM fungi obtain carbohydrates derived from plant photosynthesis for their growth and reproduction. AM fungal hyphae generated from spores in the soil colonize roots. Then, AM fungi extend intraradical hyphae in roots and form arbuscules that are highly branched structures in host cortical cells. Arbuscules are thought to be the site of nutrient exchange between AM fungi and the host. After obtaining carbohydrates from the host, AM fungi spread extraradical mycelia into the soil, which promotes Pi uptake beyond the depletion zone of Pi around roots. The route of Pi translocation from AM fungi to plant roots is called the mycorrhizal pathway. AM symbiosis also provides other benefits to the host plant such as the facilitation of mineral uptake including N, copper and zinc, tolerance to pathogen attack and drought, and the stabilization of soil structure.

The use of AM fungi in agriculture is likely to reduce chemical fertilizer application. In a conventional agricultural system, a large amount of Pi fertilizer is applied to the field because most of the Pi is adsorbed by the soil, and only a small amount of available Pi is taken up by plant roots. In a new agricultural system using AM symbiosis, plants potentially uptake more Pi through the mycorrhizal pathway even if a reduced amount of Pi is applied to the soil. At present, AM fungi are used for crop production in two primary ways. The first is the inoculation of AM fungal materials to fields or nursery beds. AM fungal inoculums are produced by several companies for some crops such as Welsh leek and strawberry. The second use of AM fungi is the utilization of indigenous AM fungi in the field. In a crop rotation system in the Hokkaido district, the propagule density of indigenous AM fungi in soil increased after the cultivation of a mycorrhizal host crop (sunflower), leading to the increased yield of a succeeding crop (maize) in the following season (Karasawa *et al.* 2000, Karasawa *et al.* 2001, Karasawa *et al.* 2002).

However, the effects of inoculated and indigenous AM fungi are not always positive. Variation in environmental conditions and differential community structures of indigenous AM fungi can affect the expression of mycorrhizal functions. For example, AM fungal colonization decreases under high Pi conditions, such as in the arable lands in Japan, resulting in a low AM effect. Even if an excellent AM fungal inoculum for improving plant P nutrition is developed, the colonization in host roots is often decreased by competition with indigenous AM fungi and other soil microorganisms. The AM effect also depends on the combination of plant species or cultivar and AM fungal species. For example, mycorrhizal dependencies [(inoculated plant–non-inoculated plant) / non-inoculated plant] in cultivars of Welsh onion (*Allium fistulosum*) inoculated with *Glomus fascieculatum* range from positive to negative (Tawaraya *et al.* 1999, Tawaraya *et al.* 2001). It has also been noted that the domestication of crops is linked to a reduction in mycorrhizal responsiveness (Martín-Robles *et al.* 2018).

One of the problems with utilizing AM symbiosis in agriculture is that we do not have a useful method for assessing AM function in the field (e.g., the quantification of Pi uptake through hyphal networks of AM fungi). Under well controlled laboratory conditions, the AM effect is almost always apparent when plants inoculated with AM fungi in Pi-limited soil are compared to non-inoculated plants. In the field, AM fungi are ubiquitous, making it difficult to set up control plots for examining AM effects on crop P nutrition and yield. Fungicide application is one frequently used method to reduce AM fungal density in soil. However, the variation in population of soil microorganisms by the application is not negligible. P content in plants is one measure of plant P nutritional status, but it is difficult to discern whether P derives from soil Pi directly taken up by plant roots or AM fungal hyphae. Root length colonized with AM fungi is frequently measured to assess the population density of AM fungi in the field. The colonization level is not always related to the activity of the mycorrhizal pathway. Recently, Sawers *et al.* (2017) demonstrated that P translocated via the mycorrhizal pathway is correlated with the total length of extraradical hyphae of *Rhizophagus irregularis* in soil, although functional diversity among fungal isolates is well known (Smith *et al.* 2011). In order to develop useful methods for assessing AM function in field, there is a need to understand mechanisms of Pi translocation through the mycorrhizal pathway.

Mechanism of P translocation in AM

Plants take up Pi via the mycorrhizal pathway when they are colonized with AM fungi. Overall, soil Pi is absorbed by extraradical hyphae of AM fungi, translocated to the host roots through the fungal hyphae and transferred from arbuscules to the host cells. AM fungi can uptake the inorganic form of phosphate (orthophosphate) from soil solution through Pi transporters localized in the plasma membrane of extraradical mycelium (Harrison and van Buuren 1995). AM fungi are also able to utilize organic phosphates by secreting acid phosphatases into soil (Sato *et al.* 2015), yet the quantitative contribution of organic phosphates to total absorbed Pi is unknown. Pi taken up by the hyphae is rapidly converted into polyphosphate (polyP), which is a linear chain of three to thousands of Pi residues (Ezawa *et al.* 2004). PolyP is thought to be synthesized by the vacuolar transporter chaperone (VTC) complex. In yeast, the VTC complex mainly localizes in tonoplast and synthesizes polyP using ATP as a substrate (Hothorn *et al.* 2009). PolyP is accumulated in yeast vacuoles (Saito *et al.* 2005). AM fungi also store a large amount of polyP in vacuoles (Kuga *et al.* 2008) that are bundles of the tubular form (Uetake *et al.* 2002). PolyP in tubular vacuoles is translocated to host roots possibly by water flow mediated by a fungal aquaporin (Kikuchi *et al.* 2016).

PolyP translocated to intraradical hyphae and arbuscules is shorter than that in extraradical hyphae, indicating that Pi is liberated from polyP in the fungal cells within roots (Solaiman *et al.* 1999, Ohtomo and Saito 2005, Takanishi *et al.* 2009). Although mechanisms of Pi release from AM fungi to the host cells are largely unknown, several hypotheses have been proposed (Saito and Ezawa 2016, Ezawa and Saito 2018) (**Fig. 2**). The first model is that Pi is exported from fungal hyphae to the peri-arbuscular space by fungal Pi transporters. The peri-arbuscular space is an apoplastic space between fungal hypha and the peri-arbuscular membrane derived from host plants. Several Pi transporters have been identified in AM fungi, but those Pi transporters are possibly responsible for Pi uptake not export (Balestrini *et al.* 2007). At present, no fungal Pi exporter gene is identified. Recently, the AM fungal SYG1 protein, which is a SPX domain-containing protein, has attracted attention (Ezawa and Saito 2018). The SPX domain acts as a binding site of inositol polyphosphates, of which the level in cells is related to cytosolic Pi concentration (Wild *et al.* 2016). The interaction between the SPX domain and inositol polyphosphates regulates the enzymatic activity of SPX-containing proteins depending on cellular Pi levels. SYG genes are widely distributed in eukaryotes. Homologs of the AM fungal SYG1 in plants and animals are PHO1 and XPR1, respectively. Plant PHO1 is involved in Pi export via the Golgi/trans-Golgi network (Arpat *et al.* 2012). Animal XPR1 localizes in the plasma membrane and act as a Pi exporter (Giovannini *et al.* 2013). Based on this knowledge of PHO1 and XPR1, the involvement of AM fungal SYG1 in Pi release from arbuscules is suggested (Ezawa and Saito 2018). The third model of Pi release is the secretion of polyP from arbuscules and the liberation of Pi from polyP by plant acid phosphatases in the peri-arbuscular space (Saito and Ezawa 2016, Ezawa and Saito 2018). Ezawa *et al.* (2005) showed that a secreted purple acid phosphatase of marigolds is involved in Pi metabolism and transport in arbuscular mycorrhiza (Ezawa

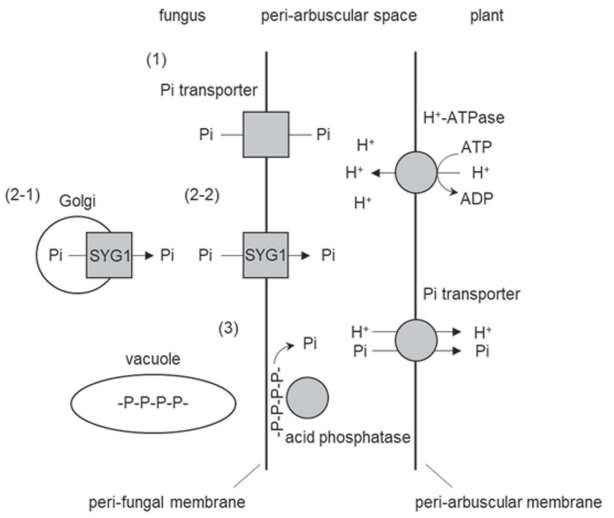

Fig. 2. Phosphate transfer between arbuscular mycorrhizal fungi and plants. Three hypothetical models of phosphate transfer based on Saito and Ezawa (2016) and Ezawa and Saito (2018) are shown. Gray circles and triangles show proteins derived from plants and arbuscular mycorrhizal fungi, respectively.

et al. 2005). Acid phosphatase activity is very active in the peri-arbuscular space according to enzyme histochemical studies (Dreyer *et al.* 2008). We observed that polyP localizes to the cell wall as well as vacuoles in AM fungi, indicating that AM fungi secret polyP as a component of the cell wall (Kuga *et al.* 2008). Based on these findings, we have proposed that polyP is released into the cell wall of arbuscules and then hydrolyzed into Pi by plant acid phosphatases secreted into the peri-arbuscular space (Saito and Ezawa 2016).

Released Pi in the peri-arbuscular space is then taken up by the plant Pi transporter localized on the peri-arbuscular membrane (Harrison *et al.* 2002, Javot *et al.* 2007). The symbiotic Pi transporter is driven by the gradient of electrochemical potential between the peri-arbuscular space and the host cells that is established by the plant H^+-ATPase (Krajinski *et al.* 2014, Wang *et al.* 2014). Pi acquired via the mycorrhizal pathway is loaded into the xylem and translocated to the shoot.

Toward the development of methods for assessing AM functions in field

In root nodule symbiosis between legume plants and rhizobia, the acetylene reduction assay has been used to estimate the activity of nitrogen fixation. In contrast, a method for assessing Pi transfer via the mycorrhizal pathway has not been developed. We are now developing methods for assessing the AM effect on the improvement of plant P nutrition by analyzing molecular mechanisms underlying P translocation and metabolism in AM symbiosis. Potential targets for the analysis are genes and proteins related to polyP metabolism including fungal VTC and plant acid phosphatases, as polyP is the main storage and translocation form in AM fungi. If enzymatic activities of those target proteins are related to the amount of Pi transfer via the mycorrhizal pathway, the contribution of AM to plant P nutrition in the field may be estimated. At present, soil diagnosis is mainly based on physical and chemical properties of soil. This is because the

function of soil microorganisms in crop production has not been quantitatively evaluated. In the future, we will build a strategy for reducing fertilizer application by fully developing a method for evaluating AM effect.

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