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Preface to 15th International Symposium on Integrated Field Science “Biological Interactions for Sustainable Agriculture”

Masanori SAITO

Field Science Center, Graduate School of Agricultural Science, Tohoku University

The United Nation Sustainable Development Summit in 2015 adopted The Sustainable Development Goals (SDGs). The SDGs are a universal call to action to end poverty, protect the planet and ensure that all people enjoy peace and prosperity. SDGs will not be achieved unless agriculture and food production are sustainable. Sustainability of agriculture and food production will be achieved by careful management of agro-environment such as environmental friendly agricultural practices. These practices are largely based upon natural cycling function of agro-ecosystems, which is one of agro-ecosystem services. Recently, it is well recognized that biological interactions are basis of agro-ecosystem services. For example, plant-microorganisms symbioses, such as nitrogen fixation by root nodule of leguminous plants and phosphorus acquisition by mycorrhizal fungi in plant roots, are key processes to reduce chemical input to agro-ecosystem. Therefore, we set the present theme for 15th international symposium on integrated field science.

In this symposium, I would like to refer to “symbiosis” as a keyword. The term “symbiosis” is defined as a close and prolonged interaction between organisms of different species when they are at juxtaposition, by Heinrich Anton de Bary who is one of the pioneer scientists of plant pathology and mycology. Although the original definition of “symbiosis” is rather narrow, the term “symbiosis” is now extensively used with much broader meaning not only in biology but also in many different disciplines. Using with the broad sense of “symbiosis”, “symbiotic relationship” among different species, environmental factors should be a central role for sustainable agriculture.

In the first part of the symposium, we focused the specific symbiosis “arbuscular mycorrhiza” in agriculture. In the second part, we extended the meaning of symbiosis and focused rice production. In the symposium, 11 invited speakers from Czech, Australia, China, Spain and Japan presented their valuable research outcomes and 17 posters by young scientists were also presented. In this issue of JIFS, 10 symposium papers and abstracts of all presentation are included. The symposium was co-sponsored by International Education and Research Center for Food and Agricultural Immunology (CFAI), Applied Biodiversity Center, Graduate School of Agricultural Science, Tohoku University and Research Center for Fungal and Microbial Dynamism, Shinshu University. We are grateful for these centers.

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Symposium paper

Utilization of Organic Nitrogen by Arbuscular Mycorrhizal Hyphae in Soil - Zooming into the Hyphosphere Microbiome

Jan JANSA, Petra BUKOVSKÁ, Hana HRŠELOVÁ and David PÜSCHEL

Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, 14220 Praha 4, Czech Republic

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Corresponding Author
Jan JANSA, jansa@biomed.cas.cz

Abstract

Nitrogen (N) availability often limits growth and yields of crop plants. Utilization of synthetic N fertilizers resulted in great yield improvements but also brought with it negative consequences such as soil degradation and environmental pollution. Organic N sources are thus likely to play a greater role in future agriculture, as they did before the Green Revolution. Arbuscular mycorrhizal (AM) fungi form symbiosis with majority of world crops, providing their host plants with a multitude of nutritional and other benefits. Development of AM fungal hyphae is usually stimulated by organic N inputs into soil and the fungi can gain a significant share of N from the organic soil amendments and eventually transport the N to their host plants, in spite of the fact that they are unable to mineralize the organic materials themselves. It has been postulated that the AM fungi depend on activity of soil saprotrophs to gain access to organic N. Recent studies indicated that not only the primary degraders, but also their grazers such as soil protists, most likely play an important role in making the N once released from organic materials and then immobilized in microbial cells, available for uptake by the AM fungi. On the other hand, AM fungi are likely competing for free ammonium ions released by the protists with ammonia oxidizers, suppressing nitrification in consequence. In this short paper we provide new insights into organic N recycling from soil to plant and identify knowledge gaps to be filled by future research.

Nitrogen – importance, synthetic fertilizers, and future challenges

Nitrogen (N) availability is one of the most prevalent resource limitations of plant productivity in both natural and anthropogenic (e.g., agricultural) ecosystems (Gill and Finzi 2016; Hallin *et al.* 2009; Sochorová *et al.* 2016). High crop yields in current agriculture are, since the beginning of Green Revolution, maintained mainly due to massive inputs of synthetic N fertilizers, and this has led to global rearrangements of N cycling, reducing soil organic matter content and thus soil quality, increasing soil erosion, eutrophication of water bodies, and great societal dependency on fossil energy, above all the natural gas (Canfield *et al.* 2010; Lal *et al.* 2007; Pimentel and Pimentel 2008; Rockström *et al.* 2009; Steffen *et al.* 2015). With steadily increasing global human population and rocketing requirements for their food quality (e.g., meat or other products with large ecological footprint), largely stagnating crop yields also pose a serious uncertainty to global food security as well as political stability (Jansa *et al.* 2010; Lal 2009). This means that the synthetic N fertilizers that are important components of modern production agriculture for several decades, sustaining yields an order of magnitude higher than ever before, may bring along (particularly if used excessively) very dramatic negative consequences for soil quality and long-term ecosystem productivity, which may be difficult or impossible to reverse at decent temporal scales (Jones *et al.* 2013). Unwise and excessive applications of synthetic N fertilizers can also undermine other (unrelated) ecosystem services such as clean water supply even at distant places from agricultural fields – mainly because about half of the N applied to the fields is not taken up by the crops but is lost to the environment (Lassaletta *et al.* 2014; Somers and Savard 2015). If the fossil energy once becomes scarce, it is likely that organic N sources such as farmyard or green manure, compost or sewage sludge become as important as they were before the Green Revolution – yet it remains unclear if such organic agriculture could ever feed human population inhabiting the world today or projected to be there in the future (Cui *et al.* 2014; Muller *et al.* 2017; Razon 2015; Seufert *et al.* 2012)

Roots and mycorrhizas

Most plants take up N from soil with their underground organs, the roots. Legumes and few other plant taxa are a notable exception in this regards, because they derive a significant part or all of their N requirements from the biological dinitrogen fixation through tight association with diazotrophic prokaryotes (Sprent *et al.* 2017, and references therein). Plants take the N from the soil solution either as nitrate (NO_3^-), ammonium (NH_4^+) ions or small organic molecules such as aminoacids (Ganeteg *et al.* 2017; Klement *et al.* 2009; Miller and Cramer 2005; Warren 2013). Most plants, strictly speaking, do not have roots, however. They develop symbiotic relationship with specialized soil fungi called mycorrhizas, where some of the root functions (e.g., uptake of phosphorus) are taken over to smaller or larger extent (or almost completely) by the fungi (Smith *et al.* 2004). Since most herbaceous plants and particularly all mycotrophic (i.e., mycorrhiza-forming) crop species such as wheat, maize, rice, bean, potato and cassava develop only one, the primordial type of mycorrhizal association called arbuscular mycorrhizal (AM) symbiosis (Parniske 2008), we dedicate further text exclusively to this type of mycorrhizal symbiosis, intentionally leaving out the ecto-, ericoid- and other types of mycorrhizal symbioses (Smith and Read 2008) that do not play such an important role in production agriculture as the AM symbiosis.

The AM symbiosis is globally widespread, evolutionarily ancient and quantitatively important component of microbial communities in virtually all soils on Earth inhabited by suitable host plants - which is more than a half of extant plant species, actually (Brundrett 2002; Davison *et al.* 2015; Fitter 1990; Remy *et al.* 1994). Hyphae of AM fungi connect the soil environment with inner layer of root cortex, as well as they are providing functional interconnection between plant individuals of the same and/or of different species in a plant community (so called common mycorrhizal networks, CMN), transporting mineral nutrients towards plants while gaining carbon from their plant hosts, and also providing a highway for transport of signalling molecules and/or secondary metabolites (Babikova *et al.* 2013; Duhamel *et al.* 2013; Jakobsen *et al.* 1992; Lendenmann *et al.* 2011; Robinson and Fitter 1999; Simard and Durall 2004). This symbiosis plays important roles in plant acquisition from the soil of phosphate, micronutrients such as zinc and copper, in soil aggregate stabilization and conditioning soil hydraulic properties, shaping mycorrhizosphere microbiome, and interactions between plants themselves as well as interactions between plants and their pathogens (Bitterlich *et al.* 2018; Leifheit *et al.* 2015; Newsham *et al.* 1995; Philippot *et al.* 2013; Smith and Read 2008). Trading of resources in mycorrhizal symbiosis based on reciprocal reward concept such as phosphate for plant carbon has been documented between the plants and their fungal symbionts (Kiers *et al.* 2011), although patterns deviating from this concept such as asymmetric redistribution of symbiotic costs and benefits in multispecies plant communities interconnected by CMN have also been observed (Walder *et al.* 2012; Weremijewicz *et al.* 2016).

Arbuscular mycorrhiza and nitrogen

As every other living organism on Earth, known to science, the AM fungi also contain nucleic acids and proteins, compounds that require N for construction of the molecules. Therefore, the AM fungi require N for building up their biomass and maintaining their metabolic activity. Further on, as the cell walls of AM fungi contain N-rich biopolymer chitin (Bago *et al.* 1996; Balestrini and Bonfante 2014; Lanfranco *et al.* 1999; Shinya *et al.* 2015; Tisserant *et al.* 2012), this structural biomolecule represents another significant sink for N in AM fungal biomass. The AM fungi thus need lots of N for building up their biomass (see Kaschuk *et al.* (2009) and references therein) and thus it is not surprising that competition for N occurs between plants and the AM fungi at low N availabilities (Püschel *et al.* 2016). When the fungal needs are satisfied, additional N could be moved to plants and traded for plant carbon in a similar way as phosphorus, although the contribution of AM fungi to N uptake of plants is considered to be generally lower than the contribution to phosphorus uptake (Fellbaum *et al.* 2012; George *et al.* 1995; Hodge *et al.* 2010; Johansen *et al.* 1992; Mäder *et al.* 2000). The N could be taken up from the soil solution both as nitrate or ammonium ions, because ion channels for transport of both these ions have been characterized for the AM fungi (Garcia *et al.* 2016, and references therein). Interestingly, earlier research indicated that AM fungal hyphae can also take up and assimilate amino acids such as glycine and glutamate from the soil solution (Hawkins *et al.* 2000). Long-distance transport of N through the AM fungal hyphae occurs most likely in a form of arginine, possibly coupled with the polyphosphate granules (Cruz *et al.* 2007; Govindarajulu *et al.* 2005; Kikuchi *et al.* 2014). Release of N from the AM fungus to the plant is most likely in form of ammonium (Guether *et al.* 2009).

Organic N and mycorrhizas

Proliferation of AM fungal hyphae in soil is stimulated by organic N amendments – a phenomenon that has been described for several AM fungal species since a couple of decades ago. Originally, the research has been carried out with complex organic materials such as plant litter or baker´s yeasts applied in root-free zones (Gavito and Olsson 2003; Hodge *et al.* 2001; Hodge *et al.* 2000) and those results were confirmed recently for a range of pure N-containing polymers such as chitin, DNA and proteins (Bukovská *et al.* 2016, and references therein). The AM fungi were reported to speed up the decomposition of organic N, probably in concert with other (saprotrophic) microorganisms, acquire the N originally bound in the organic materials, and transport it towards the plant, where it could be (but not always was) transferred to the host plant (Bukovská *et al.* 2018; Hodge 2014; Hodge and Fitter 2010; Hodge *et al.* 2000; Thirkell *et al.* 2016). Although microbial communities in organic N patches accessible only to AM fungal hyphae differed between mycorrhizal and non-mycorrhizal pots (Herman *et al.* 2012; Nuccio *et al.* 2013), with Firmicutes responding positively to AM fungal presence and Actinobacteria and Comamonadaceae responding negatively to the same, it remains unclear which of the microbes were directly involved in the decomposition of the organic N, which of them could have been primed by AM hyphal exudates (Jansa *et al.* 2013, Kaiser *et al.* 2015) and which were just back-seat riders.

Several of the above studies have indicated that the AM fungal hyphae could transfer not less than 20% of the N supplied as soil organic amendment to the plants within just a few weeks (Bukovská *et al.* 2018; Hodge *et al.* 2001; Thirkell *et al.* 2016). This efficient "mining" of organic N has consequences for the soil microbes, too: recently, we showed that just a few weeks after placing the organic N in the AM fungal hyphosphere, both bacterial and fungal communities diminished in size due to presence of the AM fungal hyphae (Bukovská *et al.* 2018), a phenomenon strongly resembling so called Gadgil´s effect (Gadgil and Gadgil 1971; Verbruggen *et al.* 2016). The strongest reduction was recorded for ammonia-oxidizing bacteria, indicating that the AM fungi actually outcompeted ammonia oxidizers, responsible for the first step of nitrification (see **Fig. 1**), most likely through reducing NH_4^+ concentration in the soil solution (Bukovská *et al.* 2018; Veresoglou *et al.* 2012).

Another important phenomenon that we observed in our organic N patches was higher abundance of soil protists (Bukovská *et al.* 2018; Bukovská *et al.* 2016). Although the diversity of this heterogeneous group of organisms is still not easy to capture (Geisen 2016; Geisen *et al.* 2017), they seem to play a very important role in releasing N from the primary decomposers, via so called soil microbial loop (see also **Fig. 1**). This is because, for stoichiometric reasons, the protists release up to one third of N they ingest back to the soil solution as free ammonium ions (Bonkowski 2004; Trap *et al.* 2016). This is also why soil protists likely play a key role in increasing availability of the N supplied to soil originally as organic amendment to AM fungi, which virtually lack any potent exoenzymes and may not be able to compete successfully with the primary decomposers (Bukovská *et al.* 2018; Ekelund *et al.* 2009; Raynaud *et al.* 2006; Tisserant *et al.* 2012).

Open questions to be addressed in future

Above all, the "smoking gun" of the primary decomposition of soil organic N shall be carefully described in the subsequent studies, and linked to AM fungal activity, if any (e.g., priming of activity of the decomposers by AM hyphal exudates or cross-talking via signaling compounds, Kaiser *et al.* 2015). Ecological significance of soil protists (and soil microbial loop more generally) in making the organic N available to plant and AM fungi needs to be further studied, as this is an exciting and novel subject of soil ecology (Geisen *et al.* 2018). Consequences of AM fungal uptake and transport of N from the organic patches to their host plants should also be scrutinized as to the effects on other N transformation pathways (e.g., nitrification and denitrification). Previous research carried with just a handful of model plants and AM fungal genotypes should also be replicated for a broader selection of model organisms and soil properties to allow generalization of the observed effects. Particular care should then be dedicated to establishing realistic microbial communities in the model experiments, particularly with respect to including sufficient populations of slow-growing members such as ammonia oxidizers (Veresoglou *et al.* 2012, Bukovská *et al.* 2018).

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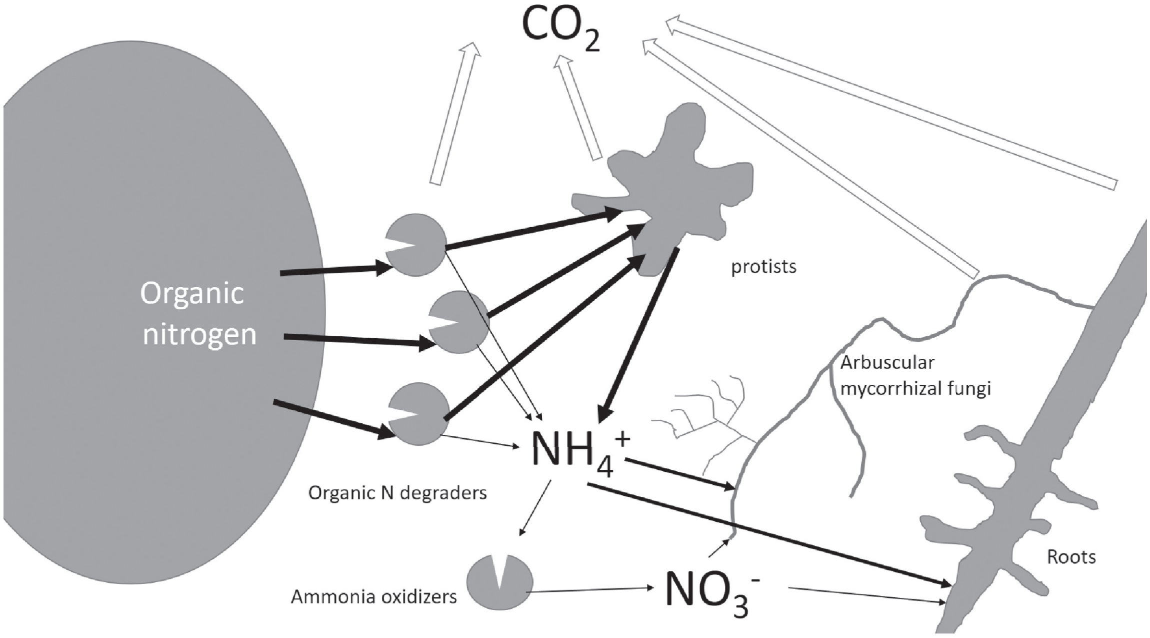

Fig. 1. A scheme showing trophic dependencies between and N transformations carried by different members of soil microbial community involved in decomposition of organic N and transport of the released N towards the plants. Thickness of black lines indicates assumed relative importance of the different pathways of recycling organic N to the plants in mycorrhizal systems.

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Use of Biochar for Sustainable Agriculture

Zakaria M. SOLAIMAN

SoilsWest, UWA School of Agriculture and Environment, and UWA Institute of Agriculture, The University of Western Australia,
Crawley, WA 6009, Australia

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Corresponding Author
Zakaria M. SOLAIMAN,
zakaria.solaiman@uwa.edu.au

Abstract

Biochar is the by-product of pyrolysis of organic biomass in an oxygen-free to the oxygen-limited environment. Biochar application to soil has been considered as a way to sequester carbon. Biochar research has become considerably innovative with important key findings on agronomic benefits, greenhouse gas emissions, soil remediation, soil fertility, soil health, *etc.* This review discusses the potential use of biochar in sustainable agriculture for improving crop yields, soil fertility, and nutrient cycling, along with potential risks involved with biochar application and strategies to avoid these risks. Biochar has the potential to improve crop productivity mainly by increasing nutrient and water use efficiency. However, improvements to crop production are often observed in nutrient-poor soils, while its application to fertile soils does not always improve crop yield. Production of biochar from good quality biomass sources at the proper temperature is a prerequisite for its use as a soil amendment to improve soil health, plant growth and crop production. The long-term effects of biochar on soil functions and its fate in different soil types require more research. Biochar may change the soil biological community composition and abundance, and the retention of pesticides applied. The key findings of biochar research have continually progressed, but more research is required before definitive recommendations can be made to end users regarding the effects of biochar application across a range of soil, climate and land management practices.

Introduction

Sustainable agriculture is the production of food, fibre, or other plant and animal products using farming practices that protect the environment, public health, human communities, animal welfare and economic development (**Fig. 1**). The aim of sustainable agriculture is to meet human's food and social needs without compromising the ability of future generations to meet their own needs. Sustainable agriculture is also the study of relationships between organisms and their environment, an understanding of ecosystem services. Food production has increased significantly in the world, but for meeting the increased demand of the growing population, it has to increase significantly by 2050. Higher crop production often arises from the use of improved crop varieties, fertilizers, pest control and irrigation, which have resulted in meeting food and nutritional demand. Despite high productivity, farmers face various constraints associated with sustainable agricultural systems. The present-day agriculture has challenged to fulfil various objectives of achieving food, fodder, fibre and fuel

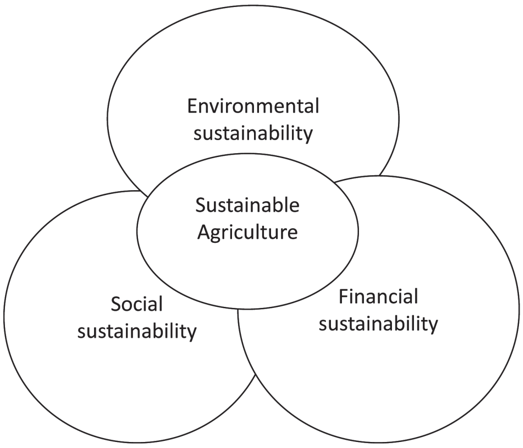

Fig. 1. Sustainable agriculture – interactions among environmental, social and financial sustainability

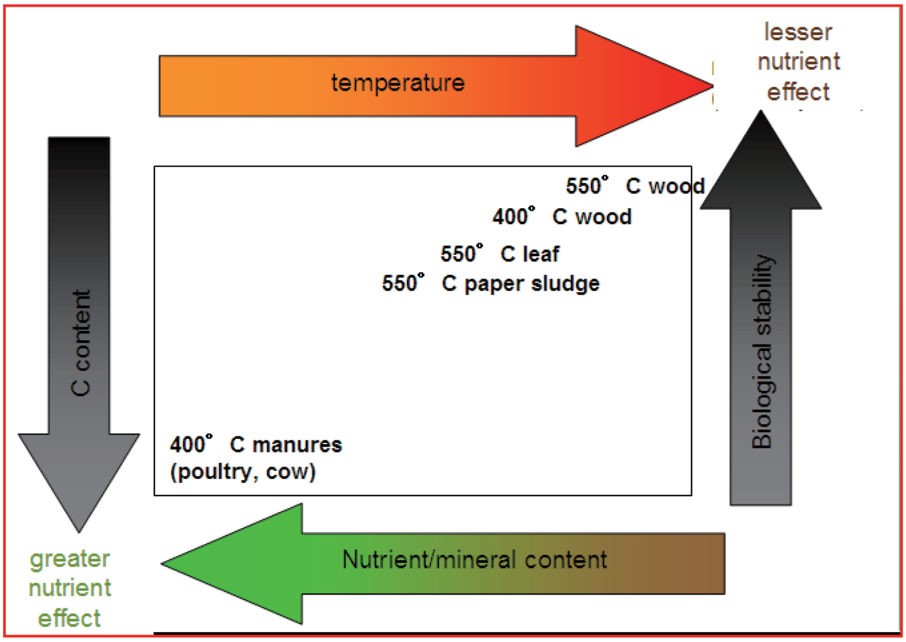

Fig. 2. Effect of temperature on the nutrient availability of plant and derived biochars (Source: Krull ES, CSIRO)

security as well as sustainability with emphasis on restoring soil resources, improving water quality, mitigating climate change and preserving natural resources for long-term use. With the new emphasis on sustainable agriculture comes a reviving of interest in soil health which emphasizes the integration of biological, chemical and physical measures of soil properties that affect farmers profit and the environment. The productive soil is an essential component of a sustainable environment which is the foundation upon which sustainable agriculture is built. For managing soil health certain organic amendments are need to be added. Among these amendments, the potential of biochar as a soil amendment in agriculture is recently recognized through the technology still remains underutilized in the farm level even though researches have progressed a lot in the past several years (Hussain *et al.*, 2017).

Recently, research on biochars has focused on enhancing plant growth, soil fertility, carbon sequestration, activities of microorganisms, mitigating climate change and remediation of contaminated soil (Anawar *et al.*, 2015; Biederman and Harpole, 2013; Chan *et al.*, 2007; Solaiman *et al.*, 2010). Because of the rapidly growing interest in biochar, this review articles exploring different aspects of the applications of biochar for crop production, improving soil fertility, microbial activities, C sequestration, greenhouse gas emission and alleviation of soil constraints. Furthermore, characterization of properties and functions of biochars, including improvement in soil biology and fertility, are elucidated.

Biochar characteristics

Biochar is the by-product of thermal combustion, called pyrolysis, of feedstock in the absence of or limited amount of oxygen at low temperatures (300-700°C). Commonly biochar has high carbon (C) content and varying C to nutrient ratio depending on the quality of feedstock used to produce biochar (**Fig. 2**). Commonly used feedstock for biochar production are either plant or animal biomass such as wood chips, crop residues, chicken manures and cow manures, and the suitability of these feedstocks for soil application is reliant on physical, chemical, economic and management factors. In addition, biochar is a highly porous materials and poses a large surface area (Downie *et al.*, 2009), and can develop dual surface charges (negative and positive charged) suggesting that biochar has anion and cation exchange capacities, thus decreasing leaching (Lehmann *et al.*, 2003), enhancing adsorption (Cheng *et al.*, 2008) and increase nutrient retention (Madiba *et al.*, 2016). Pyrolysis temperature and feedstock characteristics are largely controlling the physicochemical properties e.g. nutrients composition, particle and pore size distribution of the produced biochar (**Fig. 2**). For example, biochar properties reported having cation exchange capacities (CECs) ranged from 0 to 40 $\text{cmol}_c\ \text{kg}^{-1}$, C/N ratio from 7 to 500 or even higher and pH from neutral to alkaline (Joseph and Lehmann, 2015). Even though such inconsistency makes it challenging to identify the underlying mechanisms behind recognized effects but it also delivers a possibility to produce engineered biochar with required properties for a particular field site depending on soil type, hydrology, land use, soil contaminants and so on.

Effect of biochars on seed germination

Biochars may contain undesirable compounds such as crystalline silica, dioxin, polyaromatic hydrocarbons, phenolic compounds and heavy metals that are harmful to plants, microbes and even humans (Cao *et al.*, 2009; Thies and Rillig, 2009) as well as essential nutrients based on feedstocks used for biochar production (Gaskin *et al.*, 2008). Some of these compounds in biochar have the potential to either inhibit or stimulate seed germination and seedling growth. Biochar has been reported to both increase (Chan *et al.*, 2008; Yamato *et al.*, 2006) and decrease (Deenik *et al.*, 2010) plant growth and yield but some investigations reported the impact of biochar on early stages of plant growth such as on seed germination and seedling growth (Solaiman *et al*., 2012). Van Zwieten *et al*. (2010) showed that germination of wheat seed was increased with a 10 t/ha of paper mill biochar. In contrast, Free *et al*. (2010) stated that seed germination and early growth of maize were not influenced by biochars produced from a range of organic sources of feedstocks. The application of biochar to soil can alter organic matter mineralization (Steiner *et al*., 2008; Wardle *et al*., 1998) which is linked to the release of nutrients such as nitrogen (Manzoni *et al*., 2008). The resulting change in a nutrient status of the soil may affect both seed germination and seedling growth. Application of biochar to acidic soils can increase soil pH to alkaline levels, especially if higher rates of biochar are applied and changes occur to soil cation exchange capacity (Ogawa, 1994). The variable characteristics of biochar indicate that biochar responses will depend on the type and rate of biochar applied to soil as well as on soil characteristics such as soil C, pH, CEC and so on. As we tested, biochar can increase seed germination and initial root growth at a certain rate of application and then gradually decreases as the biochar rate increases (**Fig. 3**).

Biochar increases soil water holding capacity

The application of biochar at higher rates can increase soil water retention directly due to its high surface area (Lehmann, 2007) and indirectly via subsequent increases in the soil organic C (Blanco-Canqui and Lal, 2004). Several investigations of biochar application on crops reported that biochar may enhance soil water holding capacity (Sohi *et al*., 2009; Solaiman *et al*., 2012). This property of biochar may decrease the effects of drought on crop productivity in drought-prone areas like in Western Australia. Water holding capacity is largely correlated with the physical properties of biochar such as high surface area and high porosity. However, there is some debate because the water holding capacity is related to the type of feedstock that was used to produce the biochar, as well as the pyrolysis conditions of the biochar's production. These factors can influence the surface and pore structure of the biochar. However, if climate change leads to even higher drought conditions in many agricultural production areas of the world, biochar as a soil amendment from various feedstocks may still have a considerable positive impact on retaining soil water even though it is very variable (Lehmann and Joseph, 2009). A careful attention is needed to choose biochar for drought-prone conditions.

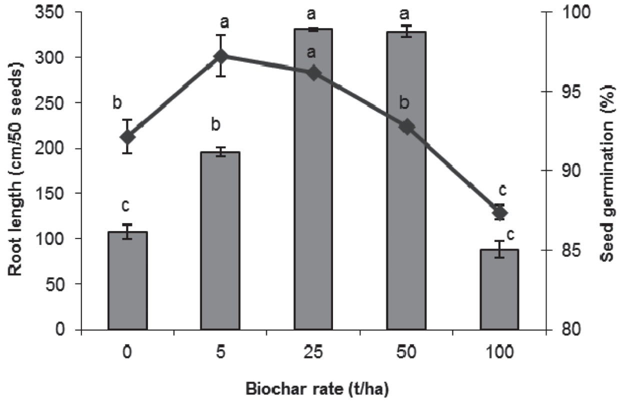

Fig. 3. Root length (histogram) and germination (line graph) of wheat var. Calingiri seeds with different rates of biochar

Role of biochar in soil carbon sequestration and climate change mitigation

The most encouraging aspect of biochar is that it could be an important renewable energy source with the potential to significantly mitigate greenhouse gas (GHG) emissions and slow climate change. The sequestration of biochar in soil, which makes soil darker in colour, is a robust way to store carbon to mitigate CO_2 emission (Lehmann, 2007). An illustration of this capacity of biochar that are estimates of potential atmospheric C offsets proposed by Lehmann, 2007 (showed in **Fig. 4**). The first part of the illustration shows the C sequestration process that represents the natural carbon cycle such as plants pull CO_2 from the atmosphere, part of that carbon is made into the plants' structures through the photosynthesis process. When plants die, they sequester C into the soil, but most of the C is rather quickly released back into the atmosphere as CO_2 through plant respiration and mineralization by soil microbiological activities. The relative amounts of CO_2 are more or less balanced and hence the process is called C neutral (Lehmann, 2007). Carbon neutral means that there is no net C added to the atmosphere other than what naturally occurs. Climate change is caused by net additions of C, called C positive, to the atmosphere. These additions are primarily due to burning C-based fossil fuel at an increasing rate over the past 500 years. Carbon negative refers to the actual net reduction of C in the atmosphere. The natural process is interrupted by capturing part of the biomass before it reaches the soil directly and using part (25% in the example above) for the production of bioenergy and part for the production of biochar (**Fig. 4**). The illustration shows that the biomass that is converted to energy (potentially in the forms of heat, gas or liquid fuels) releases part of the C in the form of CO_2 back into the atmosphere in an assumed C-neutral process. The other part of the biomass is converted into biochar and because of its stability sequesters all but 5% of the carbon (**Fig. 4**) in the soil and hence has the ability to provide a C negative source of energy. However, the capacity of biochar with energy production to offer C-negative renewable fuel is limited to critical points in the process of its production and consumption. Firstly, it is important that biochar added as a soil amendment remains stable and sequestered for a very long time. In climate change scenario, this refers to the issue of stability and in other words, it would be difficult to claim a permanent sequestration of C if the biochar C that was applied as a soil amendment was immediately released back into the atmosphere through possible soil microbiological mineralization processes. However, most research findings to date clearly demonstrate that biochar added to soil releases C back into the atmosphere at a very slow rate that is in excess of several hundred if not thousands of years (Lehmann and Joseph, 2015). It means a permanent sequestration, it is a much slower release compared with the soil C sequestration that occurs when agricultural practices such as conservation tillage are adopted as a means to mitigate climate change. It also offers safer and likely less expensive C sequestration than other methods related to the storage of CO_2 in the underground using C capture and sequestration technologies. Finally, the C-negative potential of biochar is either enhanced or limited

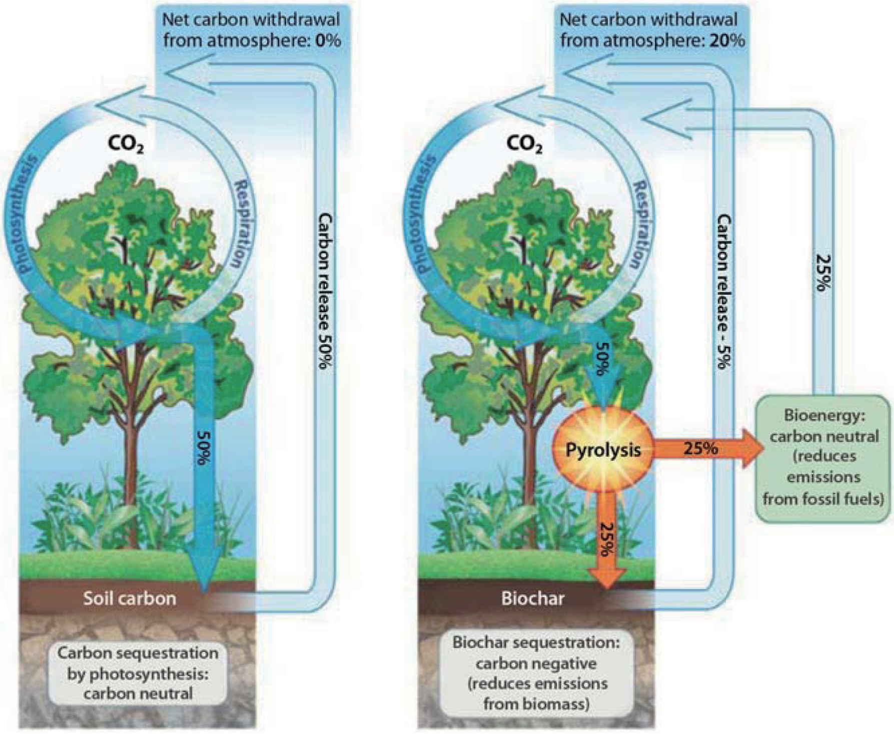

Fig. 4. Biochar can be a carbon-negative renewable fuel source (Lehmann, 2007). Source: International Biochar Initiative. Available at www.biochar-international.org/biochar/carbon and stated by (Schahczenski, 2010)

by the efficiency of energy production and the ability of the overall production process to limit CO_2 and other GHG emissions. In part, this is because of controversy over the research methodologies for measurement of biomass-based energy production (UNEP, 2009). Nonetheless, the life-cycle assessment of biochar needs to be examined for energy efficiency and GHG emissions. Only a few researches have employed this type of analysis, but to date it supports the conclusion that biochar results in a net reduction in GHG emissions (C-negative) and is an efficient use of biomass (Guant and Lehmann, 2008; Lehmann and Joseph, 2009; and Roberts *et al*., 2010).

Effect of biochars on soil health, soil fertility and crop production

Biochars can increase soil C storage and potential C sink, improve soil nutrient retention and nutrient availability, decrease nutrients leaching and maintain the balance of soil ecosystem by adding high aromatic structure into soil humus (Joseph and Lehmann, 2015; Madiba *et al*., 2016). Earlier research suggests that components of C in biochar are highly recalcitrant in soils, for example, the residence time for wood biochar being in the range of 100 to 1,000 years, i.e. around 10-1,000 times longer than residence times of the most soil organic matter (Verheijen *et al*., 2010). However, there is a paucity of data regarding the residence time of all biochars produced from various feedstocks will significantly be varied. Biochar increases the water holding capacity, pH, plant growth and root development as well as rhizobial and mycorrhizal colonization in roots (Solaiman *et al*., 2010; Rondon *et al*., 2007). The current global investigation is addressing the optimal application rates and processes, the effect of biochar additions on soil C sequestration and accumulation of nutrients over the long-term, the interaction of biochar with soil microbial communities and the long-term fate, stability and toxicity in soil needs further investigation.

Biochar is stable and the reactive biochar surface can strongly adsorb toxic substances, such as aluminium, manganese and H^+ in acid soils and arsenic, cadmium etc. in heavy metal contaminated soils (Berek *et al*., 2011). Biochar application into the soil is expected to enhance overall sorption capacity of soils towards anthropogenic organic contaminants e.g. pesticides and herbicides, in a mechanically unlike way than amorphous organic matter. It may greatly remediate toxicity and minimise transport of common pollutants in soils through reducing their bioavailability, increasing their localised accumulation, although the extent and rate of this need to be assessed. Biochar can reduce mobilities of some inorganic and organic pollutants in soil (Beesley *et al*., 2011). Feedstock properties and production temperature of biochar influence capacity of pollutant retention. Highly alkaline and water-soluble C can undesirably mobilise some elements and the large surface area may be toxic to soil fauna but may act as a microbial habitat (Jaafar *et al*., 2014).

Effect of biochars on soil constraints

Soil acidity is one of the major constraints for crop production throughout the world. Biochar can be played an important role in the management of acidic soils. The pH of biochar usually is influenced by the type of feedstock, production temperature and production duration (Liu and Zhang, 2012). For example, at production temperature of

300°C, the biochar produced from corn, peanut, and soybean straws were alkaline, but the pH of biochar produced from straws of canola and wheat were mild acidic (Yuan and Xu, 2011; Yuan *et al*., 2011c). Cheng *et al*. (2006) demonstrated a low pH at 5.4 measured in water when biochar was made at 350°C. The unlike pH values between biochar and soil may be the main cause of soil pH change. Acidic biochar could also increase soil pH when applied in soil with very low pH value (Cheng *et al*., 2006). There are a few studies focusing on the effect of biochar on the pH of alkaline soil. Van Zwieten *et al*. (2010) reported that the application of two biochars with pH values of 9.4 and 8.2 both increased the pH of Ferrosol having initial pH 4.2, but only one biochar increased the pH value of Calcarosol having initial pH 7.7. In mine tailing soil, the pH value of 8.1 was increased to 10.2 when biochar added at 10% application rate (Fellet *et al*., 2011). The research done by Yuan *et al*. (2011a) revealed that the pH value of biochar-amended acidic Ultisols decreased with increasing incubation time, even though the pH was still higher than that of the unamended control. This is because of the production of acidic functional groups from the oxidation of biochar during the incubation process (Cheng *et al*., 2006).

Biochar amendment increased soil pH and exchangeable cations and reduced Al saturation in soils. Association of H^+ ions with biochar and decarboxylation processes was likely to be the main factor neutralizing soil acidity (Wang *et al*., 2014). Therefore, the lack of change in soil pH at the higher biochar rate may be due to the displacement of exchangeable acidity and the high buffering capacity of biochar, thereby, retarding a further liming effect. Biochar type, application rate, and their interaction had significant effects on soil pH, EC, and CEC of the acidic soil of pH < 4.80 (Chintala *et al*., 2014). The addition of biochar in the highly weathered acidic soil recently showed the increase in seed emergence, above ground biomass, vegetation cover, N and P use efficiency and maize growth (Zhu *et al*., 2014). Xu *et al*. (2014) also showed that the effects of biochar application on P sorption were highly influenced by soil acidity. These variations suggest that the increase in P sorption with biochar addition is credited to Ca-induced P sorption and is less affected by Fe and Al oxides. Biochar application is found to have altered soil P availability was dependent on soil acidity, which has important contributions for improving soil productivity. The alkalinity of biochars was a key factor contributing to their liming potential (Yuan *et al*., 2011b) which made soil generally less acidic (Yuan *et al*., 2011c) and the ameliorating effects of biochar on soil pH increased with increasing biochar application rates (Yuan *et al*., 2011a). The increase of crop growth from biochar amendment of a typical Ultisol may result from an increased soil pH and CEC (Peng *et al*., 2011). Liang *et al*. (2014) suggested that biochar application to calcareous soils increased crop yield, soil pH and water holding capacity or could be used in calcareous soils without yield loss or significant impacts on nutrient availability.

Effect of biochars on greenhouse gas emissions from soil

The increase in GHG emission is the key factor of climate change for global warmings and the contribution of CO_2 emission alone is over 70% (IPCC, 2007a). The CO_2 emission through soil respiration is almost 10 times higher than that produced by fossil fuel burning (IPCC, 2007b). Therefore, the decrease of CO_2 emission from agricultural soil to mitigate the climate change is crucial. Biochar use has been suggested to improve soil C sequestration (Lehmann and Joseph, 2009), decrease N_2O emission (Van Zwieten *et al*., 2010; Spokas *et al*., 2009) as well as decrease CH_4 emission (Feng *et al*., 2012). Some studies have focused that biochar can reduce two GHGs responsible for global warmings such as N_2O and CH_4 emissions from the soil, as well as physically store in the soil and have significant impacts for climate change mitigation (Cayuela *et al*., 2014; Felber *et al*., 2014; Verheijen *et al*., 2010). For example, van Zwieten *et al*. (2010) stated that biochar produced from green waste decreased emissions from 1470 to 636 N_2O-N m^{-2} compare to the control (3165 N_2O-N m^{-2}) in a Ferrosol. However, some researcher reported that biochar did not reduce emissions in their experiments (Clough *et al*. 2010; Sheer *et al*. 2011). This displays different biochars affect GHG emissions from soils differently. It is obvious that soil water content (Sanchez-Martin *et al*., 2008), biochar feedstocks either plant or animal origin (van Zwieten *et al.,* 2010; Spokas *et al*., 2009; Yanai *et al*., 2007) and biochar pyrolysis temperature influence the potential to decrease GHG emissions (Singh *et al*., 2010). There are an enormous amount of researches done on the effect of biochar on soil CO_2 emission, however, the outcomes are not conclusive as a result of the diversity of the research materials and methods used.

The mechanisms involved in decreasing GHG emissions by biochar use are complex and speculative. However, basic mechanisms were provided by Yanai *et al*. (2007) who suggest that biochar application to soil increases the activity of microorganisms involved in the reduction of N_2O to gaseous nitrogen (N_2). Moreover, the activity of N_2O-reducing organisms is increased due to the alkalinity of biochar. Similarly, Yuan and Xu (2011) indicated that due to the increase of soil pH by liming effect is believed to decrease emissions and also amending soil acidity. Biochar also has a large surface area which is providing larger adsorption sites for NO_2, NO and N_2O formed and thus decreasing these gases to release from the soil ecosystem (Yanai *et al*., 2007).

Crop residues are either left or burnt in the paddock, returning most of the C to the atmosphere. The burning of these residues in the field releases a large number of pollutants to the atmosphere which causes serious local and regional environmental impacts (Li *et al*., 2007), and converts significant quantities of nutrients to gaseous form, which are then depleted from the site (Haider *et al*., 2013). Over the past several years, the application of biochar has been recommended as a soil amendment in agriculture (Lehmann and Joseph, 2015). Biochar can enhance soil C stock and nitrogen retention as well as improve soil functions (Depmster *et al*., 2012). Crop residues such as rice straw and husk accounted for 2.46 Gt C/yr, which is about 25% of the global CO_2 emissions from fossil fuels (Mattilia *et al*., 2012; Mohammadi *et al*., 2016). Several field trials with biochar application have shown high potential in decreasing GHG emissions and improving crop yield in paddy fields (Dong *et al*., 2013; Liu *et al*., 2014). Sui *et al*. (2016) reported a significant decrease in CH_4 emissions up to 87% following rice straw-derived biochar amendment in Chinese paddy soils. Most of these studies have used high rates (5-48 Mg/ha) straw biochar in rice cropping systems (Liu *et al*., 2014), and Mohammadi *et al*. (2016) considered that the maximum agronomic benefits occur at rates over 18 Mg/ha. However, some research has suggested that biochar can be beneficial at lower rates if treated with minerals (Josheph *et al*., 2015; Blackwell *et al*., 2015). The higher application rates of biochar increase input costs, to overcome this constraint, the development of minerals enriched biochar, having higher surface functionality, exchangeable cations, and higher water-extractable organic compounds have been proposed (Joseph *et al*., 2015; Chia *et al*., 2014). Chia *et al*. (2014) characterized a woody biochar enriched with manures, minerals and clays. Their chemical analyses of the enriched biochar revealed that it has high concentrations of exchangeable cations, available phosphorus and high acid neutralizing capacity. All these amendments were incorporated into the biochar structure and as a result higher concentration of dissolved organic carbon was released into the soil amended with enriched biochar (Lin *et al*., 2012). Joseph *et al*. (2015) observed that an acacia wood-derived biochar mixed with clay, chicken litter, and minerals improved growth of wheat at a low application rate (100 kg/ha). Sarkhot *et al*. (2012) reported that dairy manure effluent enriched woody biochar can promote C and nitrogen storage in soil and mitigate soil GHG emissions.

Life Cycle Assessment has been applied to investigate the carbon footprint of biochar production systems from a perspective of various feedstocks (Clare *et al*., 2014; Mohammadi *et al*., 2016), crop production (Mohammadi *et al*., 2016) or land treated (Peters *et al*., 2015) as a functional unit. These studies are limited to use of raw biochar and did not assess the application of enriched biochar. Further studies need to be carried out using biochar in combination with fertilisers in various farming systems.

Risks associated with biochar use

Several studies have estimated what level of carbon offsets income may be generated from biochar production, but these estimates of life-cycle assessment based on greenhouse gas emissions and price expectations of future unknown carbon prices. One of the important advantages of biochar is that it provides direct soil carbon sequestration compared to other ways of increasing soil carbon sequestration which is not easy to measure. But, the production of biochar has several potential regulatory issues to overcome before a biochar industry can be developed. Major issues include: (i) biochar is very light and easily broken into small particles that can become airborne. Difficult to apply to the soil and can cause potential carbon dust air pollution; (ii) air emission standards from biochar production have not been fully examined and may vary depending on the design of the pyrolysis equipment; (iii) water quality issues related to applied biochar on potentially erodible fields; (iv) potential heavy metal content of biochar and its effect on human and animal health. While these issues are not beyond solution, they will all have to be investigated and will likely add costs to the production and use of biochar as a soil amendment.

Economic potential of biochar for farmers

The economic potential of biochar production for farmers can come from several sources such as (i) a soil amendment that could partially replace fertiliser use; (ii) as a source of heat, bio-oil and gases for farm maintenance; and (iii) as potential income as a carbon credit in a carbon trade market. For example, it is feasible that a farm with significant renewable biomass sources available for harvest could convert biomass to heat and liquid or gas fuel for machinery operation and return the biochar back to the field to enhance fertility and collect a carbon credit payment (see **Fig. 5** for an illustration of possible income sources from biochar production). However, several economic, institutional and regulatory questions need to be answered before such a project could be fully optimized. First, the costs and values of on-farm biochar production need to be considered as well as how much will get back from carbon offsets trade market (Granatstein *et al*., 2009).

Conclusions and recommendations

Biochar potentially influences soil health and functions and interacts with many soil properties because of the wide range of effects from biochar addition to soil. The long-term effects of biochar application on soil health and functions including

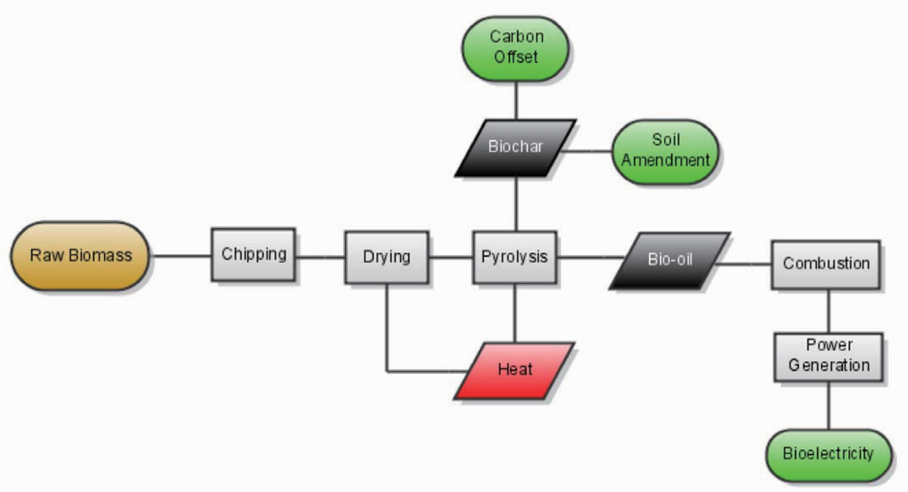

Fig. 5. Potential income sources from biochar production (Figure courtesy of Re-char, www.re-char.com/technology/mobile-pyrolysis) and Schahczenski, 2010).

Table 1. Role of biochar on soil, plant and environmental factors (reproduced from Solaiman and Anawar, 2015).

Statement	Description	Reference
Biochar increased crop production and nutrients uptake	Many studies recorded an increase in crop yield and nutrient uptake but in some cases the negative effects recorded	Blackwell *et al*. 2010 and 2015; Solaiman *et al*. 2010; Chan *et al*., 2007
Biochar increases arbuscular mycorrhizal colonisation in plant roots	Biochar increases soil physicochemical properties; indirect effects on mycorrhizae with interactions with indigenous soil microbes; plant-fungus signalling and detoxification of allelochemicals on biochar; and avoid from fungal grazers	Warnock *et al*., 2007; Solaiman *et al*., 2010
Biochars work as microbial habitats	Biochar increases or decreases soil microbial biomass and microbial activity depending on nutrient availability in soils	Steiner *et al*., 2008;Dempster *et al*., 2012; Jaafar *et al*., 2014
Increases earthworm abundance and activity	Earthworms have been shown to prefer some soils amended with biochar than those soils with no biochar addition	Topoliantz and Ponge, 2005; Van *et al*., 2006
Liming effect	Biochars have neutral to basic pH and several field experiments show an increase in soil pH after biochar application where the initial pH was low.	Cheng *et al*., 2006, 2008
Increases soil CEC	Biochar increases CEC of soil. The efficiency and duration of this CEC increase after addition to soils need to be examined.	Cheng *et al*., 2006, 2008
Effects on N cycle	N_2O emissions depended on the effects of biochar addition on soil hydrology and associated microbial processes. Mechanisms are largely remained to be explored	Yanai *et al*., 2007; Dempster *et al*., 2012
Biochar decreases soil microbial activity and N mineralisation	The activity of the microbial community decreased in the biochar-amended soil, through decreased soil organic matter decomposition and N mineralisation which may have been caused by the decreased microbial biomass carbon	Dempster *et al*., 2012
Biochars influence seed germination and early growth of seedlings	Biochar type and application rate influenced wheat seed germination and seedling growth. Germination and early root growth of mung bean and subterranean clover differed from that of wheat.	Solaiman *et al*., 2012
Biochar influences soil salinity	Biochars absorb salts and mitigate salt stress to plants demonstrating that biochar can ameliorate salt stress effects on plants and suggest uses of biochar to mitigate salinity in agricultural soils	Thomas *et al*., 2013; Lashari *et al*., 2015
Effect of biochars on soil pH dynamics	The pH of biochar is influenced by the type of feedstock, production temperature and production duration. Biochar type, application rate, and their interaction had significant effects on soil pH both in acidic and alkaline soils.	Liu and Zhang, 2012; Chintala *et al*., 2014, Madiba *et al*. 2015
Mobility and loss of biochar in the soil profile	Biochar mobility and loss through the soil profile and into the water resources have been rarely quantified and transport mechanisms remain poorly understood	Sohi *et al*., 2009
Biochar loss with soil by erosion	Spreading biochar to soil is likely to increase erosion of the biochar particles with soil both by wind and water	Jones *et al*., 2008
Effect biochar on soil organic matter dynamics	Effect biochar on soil organic matter dynamicsis influenced by combinations of soil-climate-management factors remains largely unknown	Marschner *et al*., 2008
Soil water holding capacity	Adding biochar to soil can have direct and indirect effects on soil water retention	Sohi *et al*., 2009; Solaiman *et al*. 2012
Priming effect	There is a possible priming effect exists and covers only the short term and a very small number of sample of biochar and soil types	Kuzyakov *et al*., 2000
Role of biochar pore size and connectivity	Although pore size distribution in biochar may alter key soil physical properties and processes (e.g. water retention, aeration, habitat)	Cheng *et al*., 2006
Biochar influences hydrophobicity	Influence of biochars on soil water repellency and hydrophobicity remains largely untested	Doerr *et al*., 2000
Agricultural management practices enhanced decomposition of biochar	Ploughing, sowing, planting, etc. with biochar-amendment influence the breakdown of biochar in the soil, thereby potentially reducing its carbon storage potential	Lehmann *et al*., 2003
Crop residue removal from the paddock for biochar production	Removal of crop residues from paddocks for use as a feedstock for biochar production impose multiple negative effects on soils	Lal and Pimentel, 2007
Biochar increases the sorption capacity of soils towards trace contaminants	Biochar increases the sorption capacity of soils to PAHs, pesticides and herbicides, and therefore influence toxicity, transport and fate of such contaminants	Yang and Sheng, 2003; Sheng *et al*., 2005; Hiller *et al*., 2007
Risk of contamination	Contaminants (e.g. PAHs, heavy metals, dioxins) that may be present in biochar having detrimental effects on soil properties, microbes and their functions.	Collison *et al*., 2009
Distribution and availability of contaminants within biochar	Very little experimental evidence is available on the short- and long-term occurrence and bioavailability of contaminants in biochar and biochar-amended soil.	Brown *et al*., 2009; McHenry, 2009
Reduces greenhouse gas emissions	Biochars decreases N_2O and methane emission from agricultural farming systems; Biochar can be locked in soils as C storage	van Zwieten *et al*., 2010; Feng *et al*., 2012
Occupational health and safety hazards	Dust exposure and fire hazards associated with the production, transport, application and storage of biochar need to be considered and occupational health and safety measures need to be taken to reduce such risks	Blackwell *et al*., 2009

its fate in different soil types and under diverse management practices still need to be explored. The characterization of biochar produced from a range of feedstocks is also vital. The insecurity of crop production gains at the accurate application rate of biochar and lack of information about additional benefits and few other concerns may have resulted in poor uptake of biochar technology in elsewhere in the world. Therefore, Singh *et al*. (2014) suggested exploring new opportunities to value-add to biochar beyond C sequestration by identifying emerging and novel applications of biochar. For example, Joseph *et al*. (2015) reported feeding cows with biochar has potential benefit to soil health and farm production.

Recent discoveries suggest that a proper selection of the feedstock materials and pyrolysis conditions might substantially reduce the emission levels of atmospheric pollutants and particulate matter associated with the biochar production. The implications of pollutants from pyrolysis to human health remain mostly an occupational risk, but a vigorous qualitative and quantitative assessment of such emissions from pyrolysis of biomass feedstock are lacking. While there is potential for reducing GHG emission by biochar addition to soil but careful selection of biochar type and rate of application in a range of soils is essential. In respect to a climate change mitigation perspective, biochar needs to be considered in parallel with other mitigation strategies as it may not be enough as an alternative to reducing GHG emission.

State-of-the-art biochar use strategies can help mitigate GHG emission, while farmers get benefits of improved soils and crop production. However, a risk assessment is necessary to protect the food web and human health. At present biochar research is often fragmented and repetitive. Therefore, national collaborative approaches are needed that will focus on (i) biochar production and characterization, (ii) potential for soil fertility improvement and crop production, (iii) economic analysis that includes life cycle assessment and (iv) environmental impact assessment. This approach should be used overcoming diverse soil issues in sustainable agriculture practices and recommendations for further research relating to biochar application to soil.

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Symposium paper

High-Resolution Functional Analysis of Arbuscular Mycorrhizal Symbiosis in Field Crops

Yoshihiro KOBAE

Department of Sustainable Agriculture, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, JAPAN

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life cycle, live imaging

Corresponding Author
Yoshihiro KOBAE, kobae@rakuno.ac.jp

Abstract

Generally, most field crops are colonized with a diverse range of species of arbuscular mycorrhizal fungi (AMF). Studies using a model AMF have shown that AMF colonization can improve plant nutrition and productivity. However, multiple AMF species may co-colonize plant roots, and the functionality of mycorrhizas in field conditions may depend on the combination of colonizing AMF species and their corresponding traits. Unfortunately, the colonization dynamics (i.e., lifecycle) of different AMF, their genetic structure, and their individual role under differing field conditions have not yet been elucidated. Despite significant advances in high-throughput sequencing techniques, accurate delimitation and identification of AMF species has been hampered owing to their hidden intraspecies or intraisolate genetic diversity. Similar to most of the microbes, most field AMF are considered to be unculturable, which indicates that most of these species contributing to mycorrhizal symbiosis have not yet been characterized. In order to reveal the potential and new functionality of field AMF and to utilize their biological value in agriculture, the species, genetics, and function of individual AMF should be clarified. Moreover, recent studies suggest that the colonization dynamics of AMF in roots relate to the functionality of the AMF at both the cellular and ecological level. However, there is inadequate data on this aspect of AM symbiosis in opaque soil. Live imaging of AM symbiosis has revealed the short yet highly dynamic aspect of the lifecycle of intracellular colonization, which is important for resource exchange between the two symbionts. In addition, we recently developed a novel, culture-independent technique that analyzes the nucleotide information in the roots of individual AMF species. In this article, I will focus on the future research direction of high-resolution functional analysis of AMF symbiosis with field crops.

Mosaic of AMF: heterogeneity of field mycorrhizas

Numerous AMF propagules (e.g., spores, hyphae, and root remnants) are usually present in soil. Accordingly, crops grown under field conditions almost always encounter diverse AMF species, and can simultaneously harbor some of them (Sanders *et al.* 1996; Kivlin *et al.* 2011). Strict host specificity, as observed between plants and their corresponding pathogenic fungi, has not been recognized for the colonization of AMF (Smith and Read 2008). Pot experiments inoculating plants with a single-AMF species often improves the nutrition and productivity of these plants under specific growth conditions (Tawaraya 2003; Smith and Read 2008). However, there are many cases in which nutrient uptake (e.g. phosphate) and biomass in inoculated plants does not increase compared with that of uninoculated plants (Smith and Read 2008). This is because of the drastically different level of mycorrhizal nutrient uptake among the AMF species (Munkvold *et al.* 2004). Supporting this, the inoculation of *Medicago sativa* with more than 30 different AMF species revealed that phosphate uptake performance differs markedly among the AMF species (Mensah *et al.* 2015). Given the absence of host specificity, the overlap of individual AMF colonization with other AMF species in the roots has been detected in only a 1-cm-long root fragment (van Tuinen *et al.* 1998). The ability of mycorrhizal roots to uptake phosphate under field conditions is assumed to be due to the combined abilities of multiple colonizing AMF species (Jansa *et al.* 2008); alternatively, only some of the AMF colonizing roots may temporarily contribute to phosphate uptake in response to specific environmental conditions (Compant *et al.* 2010).

Technical advances in high-throughput sequencing have enabled co-colonization characterization of genetically diverse AMF species in field crop roots (Öpik *et al.* 2009). The taxonomic resolution of at least the small subunit of nuclear ribosomal RNA (rRNA) gene, used in the best reference database for AMF (Öpik and Davison 2016), is thought to produce similar results as morphological delimitation (Davison *et al.* 2015). However, information regarding the AMF assemblage according to DNA-based approaches varies depending on the study (Öpik *et al.*, 2013; Hart *et al.*, 2015; Varela-Cervero *et al.*, 2015). Remarkably, the longer-read-length PacBio sequencing of the *Rhizophagus irregularis* genome suggested the presence of intraisolate variation in the rRNA genes (Maeda *et al.* 2017). It is still unclear whether this variation is commonly observed in AMF; however, this finding suggests the requirement to re-evaluate the resolving power of the commonly used DNA-based techniques for delimiting AMF species. Furthermore, most AMF in plant roots are thought to be unculturable or have not yet been cultured (Ohsowski *et al.* 2014). As a result, reference nucleotide sequences of most of the AMF species that colonize field roots have not yet been elucidated. Accordingly, we may have overlooked endemic, cryptic AMF species in roots under field conditions (Rosendahl 2008).

Despite the challenges in the taxonomic characterization of AMF occurring in field roots, high-throughput sequencing techniques can provide a comprehensive list of the AMF species that colonize roots in general. However, to accurately assess the functionality of AMF colonization, an evaluation of not only the genetic sequences of AMF but also the colonization dynamics of AMF may be necessary (van der Heijden and Scheublin 2007). Live imaging of rice seedlings that express symbiotic fluorescent marker proteins has revealed that the lifespan of arbuscules is short and limited (Kobae and Hata 2010; Kobae and Fujiwara 2014). Arbuscules are highly branched hyphal structures that are formed in root cortical cells and are responsible for the exchange of nutrients between AMFs and host cells. The development of arbuscule branches is influenced by nutritional conditions (Kobae *et al.* 2016). However, all arbuscules collapse within a few days after intracellular colonization, and roots are often colonized with intercellular hyphae without the formation of arbuscules (Smith and Read 2008). In addition, some AMF species tend to produce vesicles in roots (Graham *et al.* 1995). The vesicle protoplasm contains many nuclei, glycogen granules, small vacuoles, and lipid droplets (Bonfante-Fasolo 1984). Vesicles are thought to be the resting organs (Smith and Read 2008); therefore, vesicle-forming AMF should not be considered as AMF with an active role in mycorrhizas formation. Till date, the intracellular colonization cycle has rarely been taken into consideration when assessing the functionality of AMF; comparatively, the assessment has focused on the elucidation of genetic information of root-colonizing AMF species, which employ both the active state and the inactive state of colonization.

Improved analysis of symbiotic functional roles

Our current knowledge on mycorrhizal functionality has largely been obtained via laboratory studies conducted on culturable AMF isolates, and as only few AMF can be cultured, we have a limited understanding of how diverse AMF members cooperatively contribute to mycorrhizal functionality in field crops (Burleigh *et al.* 2002; van der Heijden *et al.* 2017). Some functionalities of mycorrhizal roots (e.g., phosphate uptake) can be determined using molecular markers or metabolic profiling of host–AMF interactions

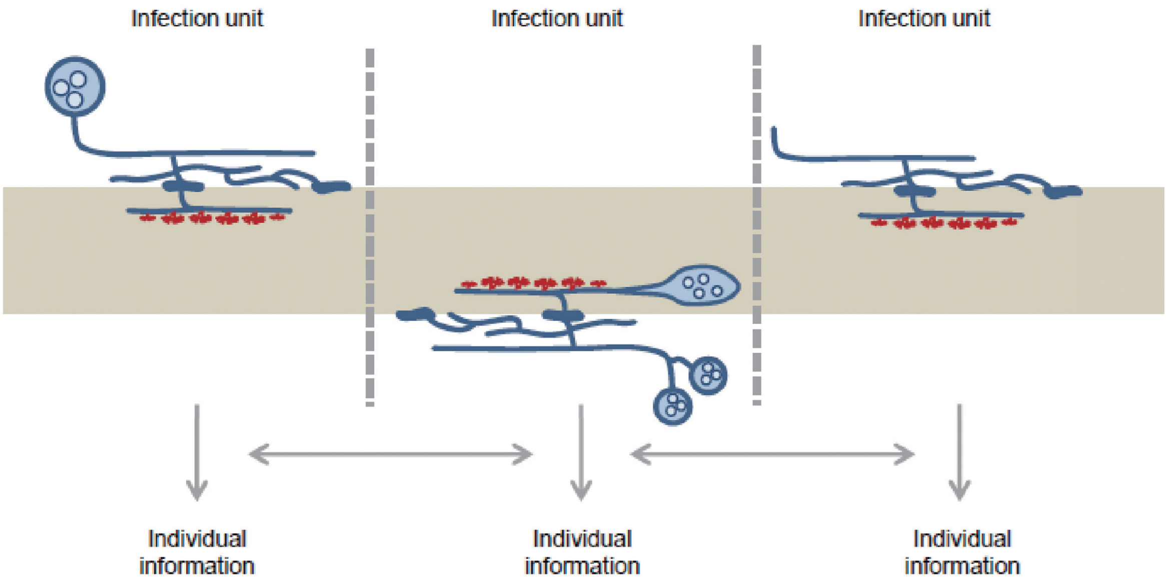

Fig. 1. Model diagram of high-resolution analysis for individual AMF species in field crops. The nutritional benefits provided by mycorrhiza in field crops are assumed to be a mosaic of the functions of diverse AMF species. An "infection unit" composed of an internal mycelium arising from a single entry point represents the individual AMF. The information from individual infection units can be integrated to identify the individual symbiotic role.

(Sawers *et al.* 2017; Beaudet *et al.* 2017). However, it is important to understand which AMF species are responsible for the predicted functionalities.

To fully elucidate the functionalities of mycorrhiza-forming AMF in field crops, the mosaic population of these organisms must be taken into account, considering each individual species as a piece of the functionality puzzle. A need for exploring the cellular functions of AMF *in situ* and at the single-cell level has been pointed out by many studies (Limpens and Geurts 2014; Öpik and Davison 2016; Taylor *et al.* 2016; van der Heijden *et al.* 2017). In particular, genetic and functional analyses of individual AMF species in roots grown under field conditions can substantially contribute to understand the biology of AMF-field crop symbiosis. Recently, a new technique for elucidating the rRNA gene information of metabolically active, single-AMF colonization (infection unit) has been reported (Kobae *et al.* 2016). Combining this method with comparative omics using the latest single-cell-level micro-transcriptome analysis coupled with the reconstruction of specific AMF genomes will allow us to expand our basic knowledge of AMF genetics, mycorrhization processes, and functionality expression. In this approach, plants expressing fluorescent markers (e.g., phosphate transporter-GFP rice) (Kobae and Hata 2010) can be utilized to efficiently detect functional infection unit (**Fig. 1**). The combined investigation of macro (fields) and functional micro mycorrhizal samples determines the next logical step that helps deeper understanding of the function of crop mycorrhizas. This understanding of AMF colonization of field crops will enable more efficient utilization of mycorrhizas for crop production.

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Symposium paper

The Role of AM Symbiosis in Plant Adaptation to Drought Stress

Baodong CHEN[1,2], Yanbo HU[1], Tao LI[1], Lijiao XU[1,2], Xin ZHANG[1] and Zhipeng HAO[1]

[1]State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences,
Chinese Academy of Sciences, Beijing 100085, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China

Keywords
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fungal biodiversity

Corresponding Author
Baodong CHEN, bdchen@rcees.ac.cn

Abstract

Drought is a worldwide eco-environmental problem and becomes more and more serious in (semi-) arid areas under global climate changes. Drought stress poses detrimental effects on plant growth and development, while plants have evolved a series of mechanisms at the cellular, tissue, and whole-plant level to resist the adverse impacts of drought stress. In this symposium paper, we summarized our recent work on the significance of arbuscular mycorrhizal (AM) symbiosis in plant drought tolerance. Large-scale field investigations in the farming-pastoral ecotone of northern China revealed strong dependency of AM fungal biodiversity and community structure on precipitation and soil properties. By using the bald root barley (*brb*) mutant, we provided direct evidence to demonstrate similar growth dependency of drought-stressed barley on AM fungus as on root hairs, and AM fungus could compensate for the absence of root hairs under drought stress. Moreover, we cloned two functional aquaporin genes (*GintAQPF1* and *GintAQPF2*) from AMF and provided evidence for potential water transport via AMF to host plants. Our recent results proved that AM fungi enhanced the plant drought tolerance through regulating the key functional genes (14-3-3 protein gene and the PIP genes) in ABA signaling pathway. These original work have identified the ecological and physiological significance of AM symbiosis in plant adaption to drought stress, and also laid foundations for further research into the mechanisms underlying the synergetic drought tolerance of AM associations.

Introduction

Arbuscular mycorrhizal (AM) fungi are ubiquitous symbiotic fungi for terrestrial plants. Root colonization to obtain carbohydrates is the foremost strategy for the survival and growth of AM fungi. As reciprocal rewards, mycorrhizal fungal mycelia facilitate root uptake of soil water and mineral nutrients from soil to root (Kiers *et al.* 2011). Mycorrhizal symbiosis substantially improves plant growth and development, particularly under abiotic (e.g., drought) stress conditions (**Fig. 1**). AM fungal mycelium can extend and cross-link via roots of congeneric species or different plants in a plant community to form common mycelial networks (CMNs). CMNs provide opportunities for material exchange and energy transfer from plant to plant (He *et al.* 2010). Therefore, mycorrhizal symbiosis has the ecological significance in nutrient transfer and carbon sequestration at global scale, and also has profound impacts on structure and productivity of terrestrial ecosysem, as well as its adaptation and resilience in responses to environmental changes.

In recent years, studies on ecophysiology of AM fungi, partiularly against abiotic (e.g., drought) stresses, have become research hotspots in the field of soil biology. Remarkable progresses have been achieved in the fields such as physiological bases of mycorrhizal symbiosis, genetic and metabolic regulations of stressed plants by AM fungi. Simultaneously, applications of mycorrhizal technology show tremendous potential in improvements of agricultural production and eco-environmental protections. In this symposium paper, based on the obtained research findings, we described AM fungi diversity in responses to environmental changes and anthropogenic disturbance in the farming-pastoral ecotone of northern China, then provided molecular and physiological evidence for AM symbiosis improving plant drought tolerance.

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Fig. 1. Drought tolerance of tobacco plants was improved by AM symbiosis in a pot experiment. Pots in the left, non-inoculation control; pots in the right, tobacco plants inoculated with AM fungus *Rhizophagus irregularis*.

AM fungal diversity in the farming-pastoral ecotone of northern China

The farming–pastoral ecotone of northern China is the most severely degraded zone in China (Hu *et al.* 2014). The fragile ecosystem is sufferring from natural (such as drought and sandstorms) and anthropogenic disturbances (such as overgrazing and unsustainable arable farming). Through large-scale field investigations in the ecotone, we found high AM fungal diversity across different steppe types and strong dependency of AM fungal communities on precipitation. Our results showed that increased precipitation decreased AM fungal biomass and altered the fungal community structure; while MAT, MAP, and soil properties significantly influenced AM fungal community composition (Xiang *et al.*, 2016). Moreover, land use conversion from grassland to farmland significantly reduced AMF richness and also significantly altered AMF community composition through mediations by available phosphorus (P), soil physical and chemical properties (soil texture, soil carbon, nitrogen and soil pH). Land use has a partly predictable effect on AMF communities across this ecologically relevant area of China; high soil P concentrations and poor soil structure are particularly unfavorable to AMF in this fragile ecosystem (Xiang *et al.* 2014).

Relative importance of AM fungus and root hairs in plant drought tolerance

AM fungi and root hairs are major contributors to root uptake of soil water and mineral nutrients; so they are closely related with plant water relations and protection of plants against drought stress. However, a relative contribution of AM fungus and root hairs to plant drought tolerance is difficult to distinguish due to unacquirability of bald-root plant mutant. A recent study by Brown *et al.* (2013) demonstrated, by using barley mutants exhibiting different root hair lengths, the importance of root hair length in shoot P accumulation and plant growth under drought stress. Using a bald root barley (*brb*) mutant and its wild type, we provided first and direct evidence to demonstrate that drought-stressed barley shared almost the same growth dependency on AM fungus (*R. intraradices*) as on root hairs; AM fungus could almost compensate for the absence of root hairs under drought-stressed conditions (Li *et al.* 2014). We outlined the difference in strategies between AM fungi and root hairs to improve plant drought tolerance: *R. intraradices* mainly through an improved uptake of soil water and P, while root hairs probably through an improvement of shoot P nutrition status.

Molecular evidence for potential water transport via AMF to host plants

Water movement in plant roots involves in two main pathways (Steudle 2000): (1) apoplastic water-transport pathway (cell walls); (2) cell-to-cell water-transport pathway from cell to cell via plasmodesmata or cross cell membranes. Under drought stress, water transport via apoplastic pathway is significantly decreased due to stomatal closure-caused decline in leaf transpiration; cell-to-cell transport pathway becomes more important for roots in water uptake and transport. Plants aquaporins (AQPs) are molecular proteinaceous membrane channels that facilitate the membrane transport of water and other small, uncharged molecules such as ammonia and glycerol (Martínez-Ballesta and Carvajal 2016). Maurel *et al.* (2008) previously studied mycorrhizal effects on plant drought tolerance and provided indirect evidence to show that AM fungi can improve plant water status by regulating plant aquaporin activities. However, the molecular basis for direct involvement of AM fungi in plant water relations still has not been established. In a recent study (Li *et al.* 2013), we cloned two full-length aquaporin genes, namely *GintAQPF1* and *GintAQPF2*, by rapid amplification of cDNA 5′- and 3′-ends from an AM fungus, *Glomus intraradices*. The *GintAQPF1* and *GintAQPF2* are the first two functional aquaporin genes from AMF reported to date. The expression of the two genes in arbuscule-enriched cortical cells and extraradical mycelia of maize roots was enhanced significantly under drought stress. Our data strongly support potential water transport via AMF to host plants.

Molecular mechanisms underlying the synergetic drought tolerance of the AM symbiosis

Abscisic acid (ABA) is known as a key hormone in mediating plant response to drought stress (Sah *et al.* 2016).

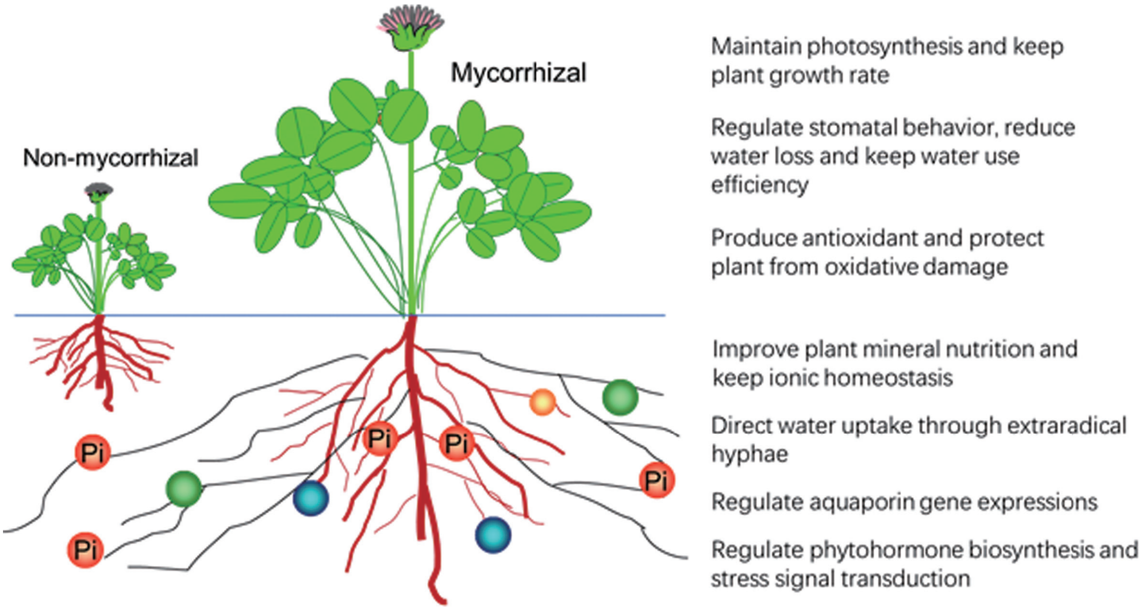

Fig. 2. Physiological mechanisms underlying the improved drought tolerance of host plants by AM symbiosis.

Drought stress can first induce ABA synthesis in roots; then through ABA-dependent signaling pathway or converging with ABA-independent pathways, the stress signal is quickly transducted and triggers plant genetic and subsequently physiological responses (Knight and Knight 2001). AM fungi have been postulated to regulate expression of drought-responsive genes in the ABA-dependent signaling pathway (Ruiz-Lozano 2003). Our recent results supported the postulation and proved that AM symbiosis could improve plant water relations and plant drought tolerance through regulation of the 14-3-3 genes in the ABA signaling pathway. In a split-root experiment, we found that AM fungi simultaneously up-regulated the expression of plant genes encoding D-myo-inositol-3-phosphate synthase (IPS) and 14-3-3-like protein GF14 (14-3GF) (Li *et al.* 2016), which were responsible for ABA signal transduction, leading to activation of 14-3-3 protein and aquaporins in *R. intraradices*, and finally improved plant water relations. Co-expression of IPS and 14-3GF is potentially responsible for the crosstalk between maize plant and *R. intraradices* under drought stress. Based on transcriptome and metabolome analysis, our results proved that AM fungi can promote the synthesis and transport of lignin and strengthen the cell wall through regulating the phenylpropane synthesis. The genes involved in glycolysis were up-regulated by AM fungi to promote the decomposition of carbohydrates and fatty acids metabolism, finally leading to improved plant drought tolerance. Meanwhile, the AM fungi themselves can potentially achieve enhanced drought tolerance through stimulating glycolysis and P450 metabolism (unpublished data).

In summary, together with previous studies, our work clearly demonstrated that AM symbiosis can effectively improve plant performance under drought stress. Mycorrhizal fungi may modulate plant physiological responses to water deficiency, e.g. regulate stomatal behavior (possibly through regulating phytohormone biosynthesis and ionic homeostasis), reduce water loss and keep water use efficiency; produce antioxidant and protect plant from oxidative damages, and consequently protect photosynthetic system and maintain plant growth rate. On the other hand, extraradical hyphae of AM fungi can access water resources unavailable for roots and directly take up and transport water to plants, which involves regulation of AM fungal aquaporin genes and directly contributes to improved plant water relations under drought stress (**Fig. 2**). The beneficial effects AM symbiosis on plant drought tolerance strongly supported the potential use of AM fungi for sustainable agriculture and also ecological restoration of degraded ecosystem in the drought stressed area. However, further research is still needed to deep into the molecular mechanisms of synergetic drought tolerance of AM fungi and their host plants. There is also a need of field trials to demonstrate AM functions under natural conditions.

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

Katsuharu SAITO

Faculty of Agriculture, Shinshu University, Japan

Keywords
arbuscular mycorrhizal fungi, arbuscule, fertilizer, phosphate, polyphosphate, P translocation

Corresponding Author
Katsuharu SAITO, saitok@shinshu-u.ac.jp

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

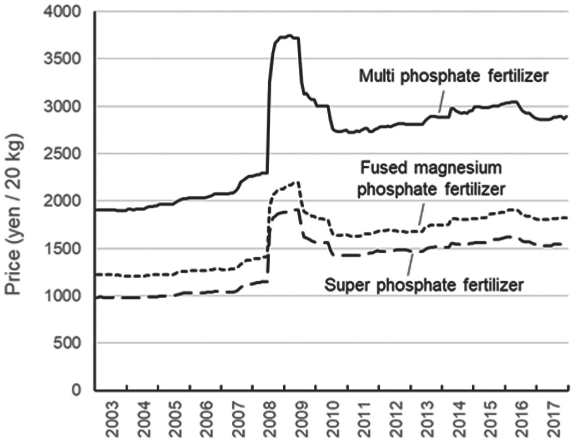

Fig. 1. Change in prices of phosphate fertilizers for the last 15 years based on the statistical survey on prices in agriculture by Ministry of Agriculture, Forestry and Fisheries of Japan (http://www.maff.go.jp/j/tokei/kouhyou/noubukka/).

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 *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).

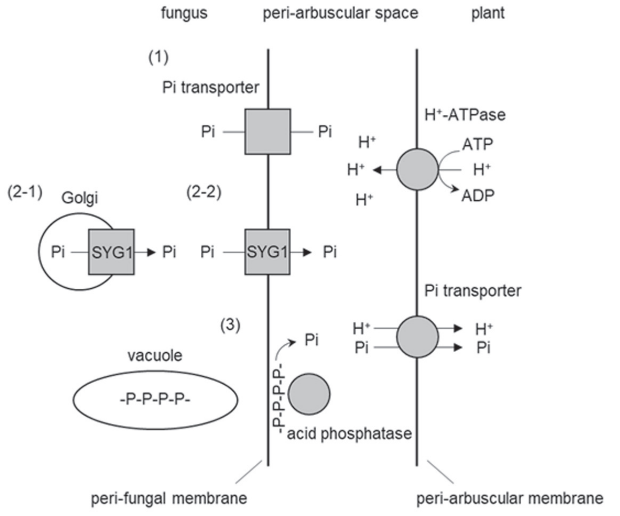

Fig. 2. Phosphate transfer between arbuscular mycorrhizal fungi and plants. Three hypothetical models of phosphate transfer based on Saito and Ezawa (2016) and Ezawa and Saito (2018) are shown. Gray circles and triangles show proteins derived from plants and arbuscular mycorrhizal fungi, respectively.

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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Application of the Ultimate Arbuscular Mycorrhizal Inoculant MYCOGEL® in Japan: Results and Prospects

María MARTÍN[1], Aitor RUBIO[1], Efrén REMESAL[1], Custodia CANO[2,3] and Alberto BAGO[2]

[1]Agrocode Bioscience S.L., Almería (Spain)
[2]*In vitro* Mycorrhizas Lab, Dept. Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín (CSIC) Granada (Spain)
[3]Cusal INGENIO S.L., Granada (Spain)

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Corresponding Author
Alberto BAGO, alberto.bago@eez.csic.es

Abstract

Arbuscular mycorrhizas (AM) are mutualistic symbioses occurring between the vast majority of land plant roots and a reduced group of soilborne fungi, the arbuscular mycorrhizal fungi (AMF). The fungus provides the plant of water and mineral and organic nutrients acquired very efficiently from the soil via fungal hyphae, which enhances plant nutritional status and physiological equilibrium, and results in higher yield and a healthier and more sustainable crop production. However, the obligate biotrophic status of AMF has hampered up to recently the large-scale production and application of AMF as inoculants. Conventional AMF inoculants consist of solid grain or powder substrates mainly containing dormant fungal spores, usually in a too-low percentage and often difficult to detect and verify for their vitality. These inoculants are difficult to apply homogeneously via watering systems, slow to establish symbiosis and, what is worst, usually contain non-desired microorganisms, due to their non-*in vitro* production and formulation. This situation gave a U-turn ten years ago with the presentation of the first ultrapure, gel-type mycorrhizal inoculant in the world, MYCOGEL®, produced and commercialized *in vitro* to preserve all its quality and traceability from the lab to the field. MYCOGEL® promotes a very quick and specific AM response to the plant, thus exerting all AM benefits from the beginning of its lifespan, to finally enhance fruit production in terms of amount and quality. In this paper we present the first results obtained in Japan on MYCOGEL® application to different crops in agronomic conditions. Rice, green onion, lettuce, tomato, onion, green pepper, celery and grape were the crops tested, with important increases in crop productivity, quality and yield value. The relative importance of the percentage of mycorrhizal colonization of the roots *vs*. the agronomic effects observed is also discussed.

Introduction

For many years, arbuscular mycorrhizas (AM) have been referred to as one of the most promising solutions to the increasing use (and abuse) of chemical fertilizers, phytochemicals and pesticides in plant production. The ability of AM fungi (AMF) for nutrient (especially phosphorous) and water uptake, transport and release to the host root via their extraradical hyphae has been early described and largely documented (Smith and Read, 2010). More recently, an important role of AMF in alleviating both biotic and abiotic stresses in plants has also been reported, making certainly this group of symbiotic fungi a target for research and technology transfer. All this considering, it could appear surprising that, after more than five decades of AM research, AM inoculation is not yet a 'must' in all plant growers' notebooks and protocols.

The main reason for this is one of the most intriguing and complex characteristics of these groups of soilborne fungi: their obligated biotrophic nature, i.e., the fact that they are unable to complete their life cycle in the absence of a host root to establish symbiosis with. Although some advances have been made recently on the clues of this obligated biotrophy (Bago and Bécard, 2002; Jiang *et al*., 2017; Keymer *et al*., 2017; Luginbuehl *et al*., 2017), AMF axenic culture and mass production seems to be far from reality nowadays. In fact, the closest we are to this possibility is the so-called monoxenic culture of AM (*in vitro* co-culture of AMF and root organ cultures, Declerck *et al*. 2005).

Until 1988, the sole way to culture AMF was to prepare solid substrates, to grow plants (either from seeds or seedlings) on it, and to add a "starter" (usually consisting of AMF dormant spores or mycorrhizospheric soil) containing propagules of one or more species/isolates of the fungi. After 6 to 12 months these would have colonized the plant and their extraradical mycelium would have extended within the substrate, producing new spores and thus propagating. It is easy to understand that such conventional technique was not suitable to carry out fine studies on AMF biology (such as biochemical or genetic studies), and absolutely inadequate to produce traceable, pure inoculum with minimal standards of quality. Although some researchers tried to establish *in vitro* cultures of AMF, firstly axenically (Mosse, 1962), then using root organ cultures (Mosse and Hepper, 1975), it was not until Bécard and Fortin (1988) adjusted the culture medium to make it compatible for both root and fungus, that the first complete successful monoxenic culture of AMF was established. Eight years later, St Arnaud *et al*. (1996) reported successful growth of extraradical mycelium and extensive sporulation in an independent compartment from the root. These two papers opened wide the door towards progress in mycorrhizal research, and consequently towards mass production of AMF propagules under *in vitro* conditions.

In 2005, Cano and Bago went a step forward, and designed a protocol for mass production of AMF by using, as a basis, monoxenic cultures of special characteristics. These cultures, after being processed, render a semisolid, gel-type inoculant containing not only dormant spores, but also infective hyphae and active mycorrhizal root pieces whose vitality and infectivity is preserved by the gel formulation. Initially (2005) presenting a concentration of 2×10^3 propagules/ml, it has reached nowadays a total concentration of 5×10^4 propagules/ml, a rate never seen before in AM technology. After being tested both in lab and agronomic conditions, this brand new mycorrhizal inoculant, named MYCOGEL®, was released to the market in 2007, firstly in Spain, then internationally.

MYCOGEL® does not contain any other microorganism besides AMF, nor any additive other than certain compounds issued naturally during the plant-fungal symbiotic association, which are retained by the gel and which may act as signals to the plant roots, thus preparing them for the imminent colonization while activating plant metabolism even prior to AMF contact (Cano *et al*., unpublished). MYCOGEL® is presented in a ready-to-use format, easily soluble in tap water to be applied either via irrigation systems, root immersion, injection to soil or spray-application to the substrate of seedlings boxes. This ensures an easy application, compatible with all usual agricultural practices, thus avoiding special care or restriction imposed by conventional mycorrhizal products.

Materials and methods

The experiments here described were carried out at different agronomic sites in Japan, by using as main testing product MYCOGEL® (Agrocode Bioscience™, Roquetas de Mar, Almería, Spain), an ultrapure AM-inoculant produced *in vitro* by means of patented technology and protocols (Cano and Bago, 2005). Conversely to conventional AM inoculants, which consist of solid pellets or thin, unsoluble powder, MYCOGEL® is a semi-solid gel containing at least 5×10^4 AMF propagules/ml.

In some of the experiments, two additional products besides MYCOGEL® were added: Rhyzo® (Kimitec Group™, Roquetas de Mar, Almería, Spain) is a root enhancing additive and a bio-nutrient whose power is based on its specific aminogram, B-vitamins and high phosphorous concentration. It is particularly indicated for encouraging rooting, and activating the root system, thus increasing growth potential for all type of crops during the initial stages of the vegetative cycle. Dosis used for this product was 1g/L when applied to seedling boxes, and 3 x 0,3g/L when applied directly to the soil. The second product used in some of the experiments was Bombardier® (Kimitec Group™, Roquetas de Mar, Almería, Spain), a liquid fertilizer obtained from the concentration of selected vegetables, with high concentration of nitrogen, amino acids, organic matter, polysaccharides, and fulvic acid. Dosis used for this product was 2cc/L every 15 days.

Detailed protocols for the different experiments carried out are summarized in **Table 1**. Protocols vary depending on the crop studied, since MYCOGEL® doses should be adjusted to the type of root (morphology and growth speed), to be applied as close as the growing roots and as early as possible after planting, in order to obtain the effects as soon as possible. Usually, one application of MYCOGEL® is enough to obtain the desired effects in annual crops; sometimes reinforce doses are recommended in particular situations, such as pluriannual crops, however this was not the case of the experiments described here.

Table 1 also describes the type of chemical fertilization used in each experiment, which, in general, should never be applied together with MYCOGEL®, but at least two weeks before or after, not to interfere with AMF germination and/or root colonization. The duration of each experiment was correlated to the lifespan of each crop, and it is also shown for each experiment in **Table 1**. Finally, parameters measured for each experiment are specified in the last column of the Table.

All numerical results presented were statistically analyzed by Tukey's test at a significance level of $p=0.05$. Where appropriated, different letters show statistical significant differences between treatments.

To test mycorrhizal colonization of MYCOGEL®-treated plants, trypan blue staining was carried out on samples of the different roots for each experiment, according to standard protocols (Phillips and Hayman, 1970). Percentage of colonization was measured according to Giovanetti and Mosse (1980).

Results and Discussion

Results obtained for each of the experiments described in this paper are summarized in **Table 2**. Detailed aspects for each of the experiment are shown in **Figs. 1 to 8**.

Table 1. Detailed protocols for the different experiments carried out in Japan with MYCOGEL® as AMF inoculant.

Exp. No.	Plant (variety)/ site of culture / trial surface/ plant density	Treatments	MYCOGEL® application and doses	Fertilization used	Experiment duration	Parameters measured
1	Rice (Yukiwakamaru) Yamagata 2000m^2 150 plants/m^2	- Rhyzo - MYCOGEL® +Rhyzo	- To seedlings in seedling box - 1 week before planting - 1ml/L	Basal 150Kg NPK/Ha; Top dressed with fertilizer (10-10-10)	4 months	- Total productivity (Kg/Ha) - Yield value (€/Ha)
2	Green onion (Morinokanade) Yamagata 300m^2 30 plants/m^2	- Untreated - MYCOGEL® +Rhyzo	- 1 ½ month after planting 1L/Ha	Basal 40Kg NPK/Ha; Top dressed with fertilizer (10-10-10)	4 months	- Root development - Total productivity (product size)
3	Lettuce (Raptor) Ibaraki 500m^2 7,5 plants/m^2	- Untreated - MYCOGEL®	- 1 day before plug seedling planting - 1ml/L	100Kg N/Ha	40-50 days	- Total productivity - Yield value -Resistance
4	4.1 Tomato (Rinka) Ibaraki 300m^2	- Untreated - MYCOGEL®	- 2 weeks after planting - 1L/Ha	Chemical fertilizer as appropriated	5 months	Root development
	4.2 Tomato (Rinka) Ibaraki 300m^2	- Untreated - MYCOGEL® +Rhyzo	- 2 weeks after planting - 1L/Ha	Chemical fertilizer as appropriated	5 months	- Average yield/plant -Root weight/plant - Average stem diameter/ plant
	4.3 Tomato (Rinka) Ibaraki 300m^2	- Untreated - MYCOGEL® +Rhyzo +Bombardier	- 2 weeks after planting - 1L/Ha	Chemical fertilizer as appropriated	5 months	- No. of damaged fruits - Average yield/plant
5	Onion (Lucky) Tokyo 300m^2 30 plants/m^2	- Untreated - MYCOGEL® at planting - MYCOGEL® 2 weeks after planting	- 1 or 2 week after planting - 1L/Ha	Basal 20Kg NPK/Ha	4 months	- Total productivity - Yield value
6	Green pepper (Ace) Ibaraki 300m^2 13 plants/m^2	- Untreated - MYCOGEL® + Bombardier	- 2 weeks after planting - 1L/Ha	Basal 300Kg N/Ha, chemical fertilizer as required	8 months	- Total productivity - Yield value
7	Celery Nagano 300m^2	- Untreated - MYCOGEL®	- To seedlings in seedling box - 0.5ml/L - 2 ~ 3 days before transplanting	Coated fertilizer	2 ½ months	- Plant vigour - Stem uniformity - Root volume - Size / plant
8	Grape (Mascot Zipangu) Okayama 300m^2	- Untreated - MYCOGEL® + Bombardier	- 1L/Ha	Basal, with compost	4 months after product application	- Plant vigour - Post-yield

Experiment 1. Effect of MYCOGEL® on rice (var. Yukiwakamaru)

The application of MYCOGEL® + Rhizo® on rice (var. Yukiwakamaru) rendered better, healthier-looking plants compared to the just Rhizo®-applied treatment (**Fig.1**). This was translated into a higher productivity (+10.6%) and higher yield economic value (+179.80 €/Ha) at the end of the experiment (**Fig. 1, Table 2**). It is important to note here that in a previous experiment, the application of Rhizo®-only to the plants rendered a 19,3% extra productivity when compared to an untreated plot; this suggests that the MYCOGEL® + Rhizo® combination could induce up to a 30% increase in crop productivity, and over 500€/Ha extra earnings for the grower when compared to untreated plants.

It was known that arbuscular mycorrhizal inoculation has a positive effect on rice when applied prior to flooding conditions, resulting in greater grain production and a better nutritional status of rice plants (Solaiman and Hirata, 1995, 1998). These experiments were conducted under greenhouse conditions, but, could they be translated to agronomic conditions? The results shown here confirm the utility of rice inoculation with arbuscular mycorrhizas in the "real world" when using the appropriated product, and open new prospect to extensive use of MYCOGEL® on this crop, of key importance for social economy in different Countries around the world.

Experiment 2. Effect of MYCOGEL® on green onion (var. Morinokanade)

The application of MYCOGEL® on green onion (var.

Table 2. Results obtained in the different experiments carried out in Japan with MYCOGEL® as AMF inoculant.

Exp. No.	Crop	Product	Parameter measured	Control	Treated	Difference
1	Rice (var. Yukiwakamaru)	MYCOGEL® +Rhyzo®	Total productivity (Kg/Ha)	5450	6030	+10.6% (+580 Kg/Ha)
			Yield value (€/Ha) (0.31€/Kg)	1689.50	1869.30	+179.80€
2	Green onion, (var. Morinokanade)	MYCOGEL® +Rhyzo®	Total productivity (2L category)	51%	60%	+9%
3	Lettuce (var. Raptor)	MYCOGEL®	Total productivity (Kg/Ha)	22200	24000	+8.10% (+1800 Kg/Ha)
			Yield value (€/Ha) (0,18€/Kg)	3996	4320	+324€
			Increased resistance to Spot Bacterial Disease	--	Yes	--
4	Tomato (var. Rinka)	MYCOGEL® (4.1)	Root development	--	Higher	+10% to 30%
		MYCOGEL® +Rhyzo® (4.2)	Average yield per plant (Kg)	2.4	3.0	+24.5%
			Root weight per plant (g)	175.1	176.9	+1%
			Average stem diameter per plant (cm)	1.859	1,954	+5.1%
		MYCOGEL® +Rhyzo® +Bombardier® (4.3)	Number of damaged fruits	21	13	-40%
			Average yield per plant (Kg)	1.46	1.99	+36%
5	Onion (var. Lucky)	MYCOGEL®	Total productivity (Kg/Ha)	54000	59000	+9.26% (+5000 Kg/ha)
			Yield value (€/Ha) (0,25€/Kg)	13500	14750	+1250€
6	Green pepper (var, Ace)	MYCOGEL® +Bombardier®	Total productivity (Kg/Ha)	70000	87500	+25% (+17500 Kg/Ha)
			Yield value (€/Ha) (0,63€/Kg)	44100	55125	+11025€
7	Celery	MYCOGEL®	Plant vigour, stem uniformity, root volume, size per plant	--	Higher	--
8	Grape (var. Mascot Zipangu)	MYCOGEL® +Bombardier®	Plant vigour, post-yield	--	Higher	--

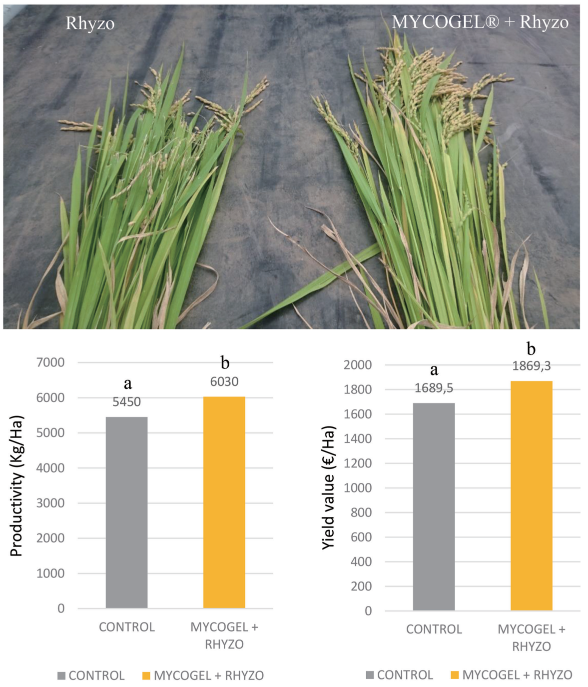


Fig. 1. Results obtained after application of MYCOGEL® + Rhyzo® on rice (var. Yukiwakamaru). Different letters show significant differences (p=0.05)

Morinokanade) resulted in a very important increase in product size (**Fig. 2**): a 9% increase in plants belonging to the "2L category" was noted when treated with MYCOGEL®, compared to untreated control plants (**Fig. 2, Table 2**). Since the fruit of the biggest size gets the highest value at the market, this translates into important extra earnings for the grower. Besides this, an important increase in root volume of MYCOGEL®-amended plants compared to control plants was obtained, which indicates a better nutritional status and physiological development of inoculated plants.

Experiment 3. Effect of MYCOGEL® on lettuce (var. Raptor)

In this experiment a Spot Bacterial Disease Resistant lettuce variety (Raptor) was used. This disease is a newly-described one, caused by the bacteria *Xanthomonas campestris pv. vitians*, which affects mainly lettuce and causes devastating losses which have been reported to be up to 80-100%. Therefore, it was quite important to assess the compatibility of MYCOGEL® to resistant varieties and test its potential to even ameliorate crop production under these conditions.

Although at a first glance control lettuce produced in the experiment could appear bigger in size (**Fig. 3**), total productivity, measured as Kg/Ha, showed otherwise (**Fig. 3, Table 2**): an increase of 8.1% in production of MYCOGEL®-

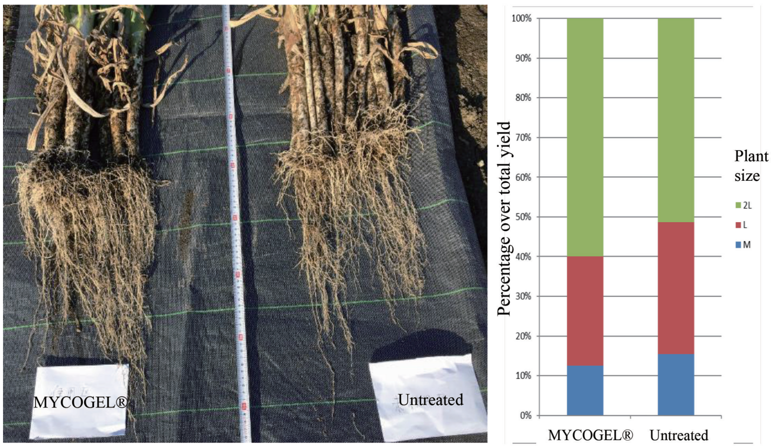


Fig. 2. Results obtained after application of MYCOGEL® on green onion (var. Morinokanade).

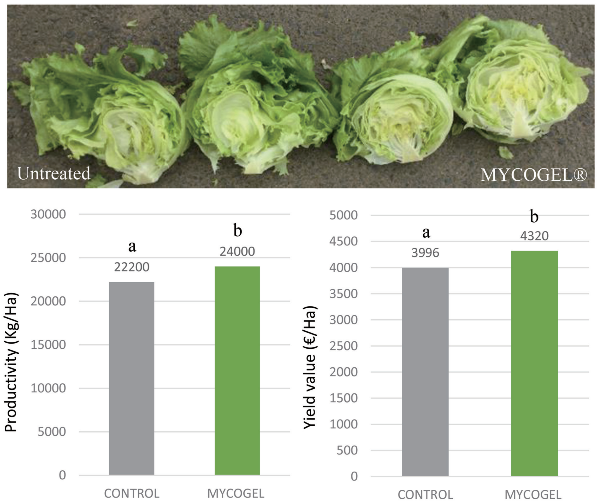


Fig. 3. Results obtained after application of MYCOGEL® on lettuce (var. Raptor). Different letters show significant differences (p=0.05)

amended plants was obtained. This would translate into +324€/Ha extra earnings for the grower. MYCOGEL®-treated lettuces wrapped firmly and beautifully, and no signs of the disease could be seen on them.

Therefore, we can conclude that the application of MYCOGEL® for lettuce production resulted not only in better yield and increased earnings, but also in healthier plants and a decrease losses caused by Spot Bacterial Disease.

Experiment 4. Effect of MYCOGEL® on tomato (var. Rinka)

Three different experiments were carried out with tomatoes as a testing crop. On the first of them, in which application of MYCOGEL®-only was compared to untreated control plants, inoculated plants showed a higher root development (**Table 2**), ranging between +10 up to +30%, as well as a larger root hair volume, which indicated a better nutritional status of the plant. It was not possible to finish this experiment up to fruit production.

In the second experiment with tomatoes, inoculated plants were amended with a combination of MYCOGEL® +Rhyzo®. In this case crop production analysis rendered an increase of 1% in root weight per plant and a 5.1% increase in average stem diameter per plant, which finally brought to an increase of +24.5% in average yield per plant (**Table 2, Fig. 4**). This should translate on important extra earnings for the grower. Finally, on the third experiment carried out with tomatoes, untreated plants were tested against MYCOGEL® + Rhyzo®, and MYCOGEL® + Rhyzo® + Bombardier® -inoculated plants respectively. Results rendered an extra crop production (measured as average yield/plant) of +18% in the case of MYCOGEL® +Rhyzo® plants), which raised to a +36% in the case of MYCOGEL®+Rhyzo®+Bombardier® -treated plants (**Table 2, Fig. 4**). As shown in **Fig. 4**, treating plants with the combination MYCOGEL®+Rhyzo® +Bombardier® also resulted in a lower number of damaged roots at the end of the experiment compared to untreated plants; namely a 40% decrease in negative symptoms was noted.

Treated tomatoes appeared healthier-looking and more reddish compared to untreated ones, which is in agreement with certain results in which lycopene content was increased by MYCOGEL® inoculation in different tomato varieties (Reva *et al.*, 2018). Lycopene is a recognized anti-oxidant and anti-cancer agent (Ono *et al.*, 2018), therefore inoculation of tomato plants with the combination of MYCOGEL® + Rhyzo® + Bombardier® may not only be an economic question, but also a healthy question, in agreement with social demand and government directives.

Experiment 5. Effect of MYCOGEL® on onion (var. Lucky)

In this experiment two inoculation timing were tested: first, applying MYCOGEL® at planting; and second, applying it 2 weeks after planting. The results obtained show (**Fig. 5, Table 2**) a 10% increase in average weight per plant compared to untreated plants in the first case (at planting) and a 5% increase in average weight per plant over untreated plants in the second case (2 weeks after planting). It is well-known that application of arbuscular mycorrhizas to plants should be carried out as early as possible along the plant lifespan, since young plants are more receptive to AM symbiosis, but also because of the interest of obtaining the mycorrhizal-induced beneficial effects as soon as possible. Our results confirm

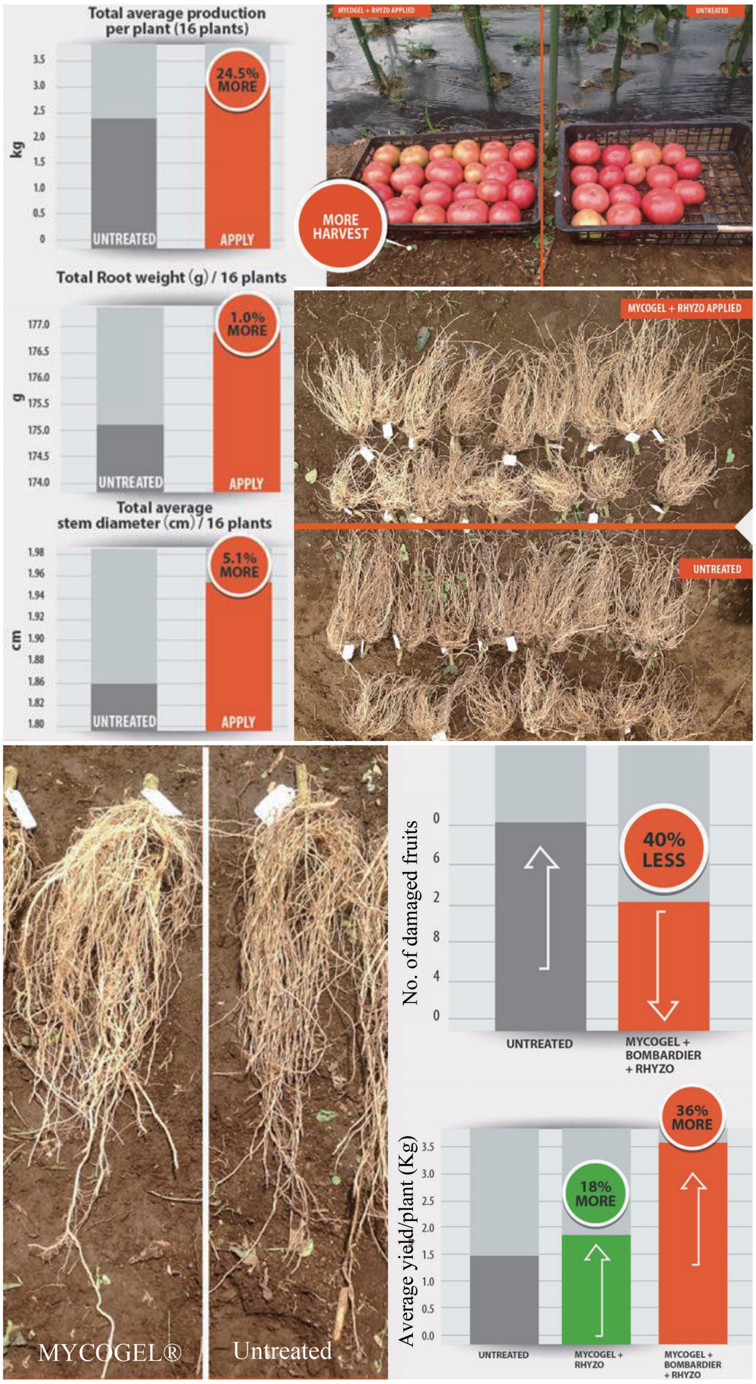


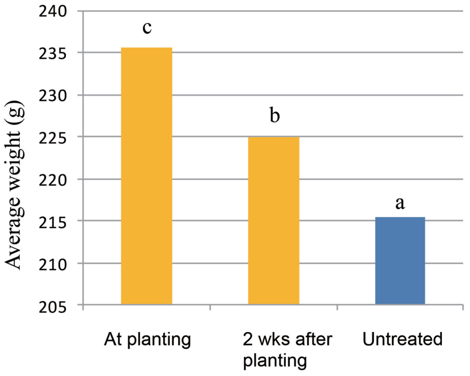
Fig. 4. Results obtained after application of MYCOGEL® + Rhyzo® or MYCOGEL® + Rhyzo® + Bombardier® on tomato (var. Rinka).

these facts and stress the importance of treating plants under agronomical conditions with MYCOGEL® at the earliest convenience, always following the technical instructions provided on each particular case and for each particular crop.

A 9.26% increase in total productivity, measured as Kg/Ha, was obtained when treating plants with MYCOGEL® compared to untreated controls (**Table 2**). Considering an average price in the market of 0.25€/Kg, the economic benefits of applying MYCOGEL® would increase in 1250€/Ha extra income, which is a non-negligible figure. These important economic benefits are even more convincing when considering the extra beneficial properties conferred to the soil by arbuscular mycorrhizas, such as a better aeration, improvement in soil structure, enhancement of soil microbial diversity and C recycling. More than 450 million years of co-evolution between arbuscular mycorrhizas and land plants have certainly result in a natural, environmental-friendly way for agronomic production that humans should take advantage of, moreover now that biotechnological tools such as MYCOGEL® are available.

Experiment 6. Effect of MYCOGEL® on green pepper (var. Ace)

Results for green pepper (var. Ace) with MYCOGEL® combined with Bombardier® are the most spectacular ones among those reported on this paper (**Fig. 6, Table 2**). Up



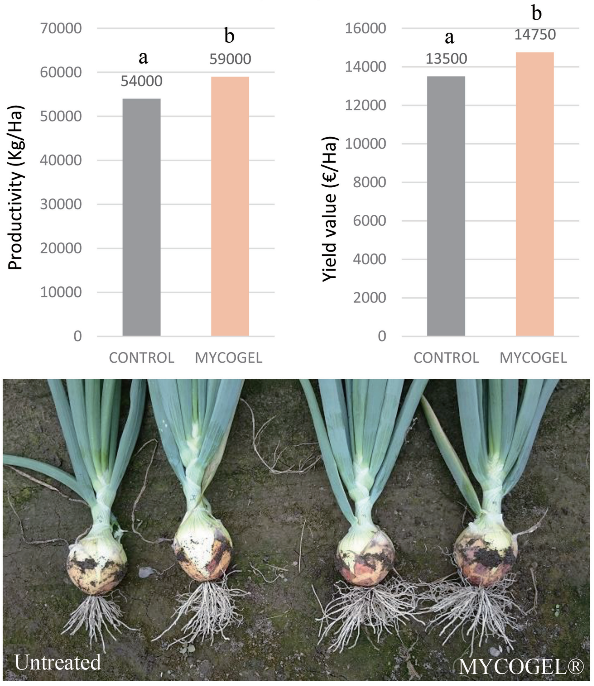


Fig. 5. Results obtained after application of MYCOGEL® on onion (var. Lucky). Different letters show significant differences (p=0.05)

to a 25% extra productivity was obtained in terms of Kg/Ha, meaning earnings increase of somewhat 11000€/Ha. As discussed before, this result should encourage growers to make confidence on mycorrhizal technology and on mycorrhizal products which demonstrate high standards of quality. In this sense, it would be desirable that the scientific community, together with governmental rulers and environmental agents would establish clear mycorrhizal product should meet in order to be eligible as market products. Also, it would be interesting that Governments would encourage the growers to use of such products by making them easily available and/or even subsidized. This could promote a cleaner, low-input agronomy while increasing circular economy, which is nowadays one of the main targets across Countries.

Experiments 7 and 8. Qualitative effect of MYCOGEL® on celery and grape (var. Mascot Zipangu)

In these two last experiments just qualitative measurements were carried out, however they pointed once more to the interesting benefits conferred by MYCOGEL® to the crops.

In the case of celery (**Fig. 7**), higher amount of active, white roots, together with higher vigor, uniformity and growth rate was obtained compared to untreated plants. Inoculated plants also presented less steam bend and disease loses, which translated in higher productivity and greater profitability.

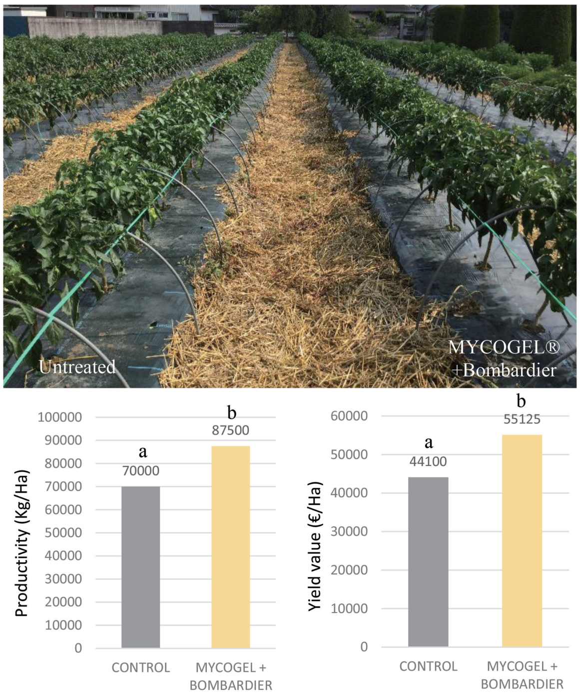
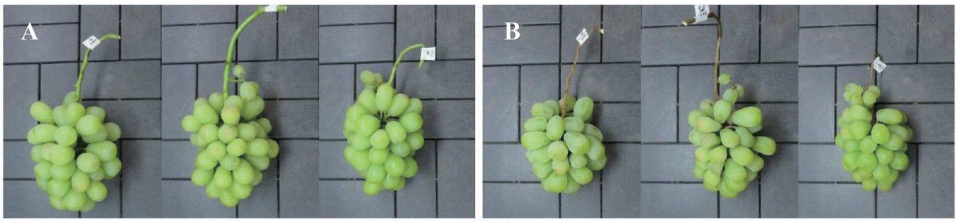


Fig. 6. Results obtained after application of MYCOGEL® + Bombardier® on green pepper (var. Ace). Different letters show significant differences (p=0.05)

In the case of grape, treatment of plants with MYCOGEL® +Bombardier® render an increase in plant vigor, fruit quality and, importantly, fruit longer of post-harvest life (**Fig. 8**): while treated bunches of grapes collected on July, 21st showed little changes after 3weeks (August, 10th), untreated bunches of grapes collected the same day showed rotten units after that time. This effect of extended post-harvest life has been also observed in other fragile cultures, such as strawberries (Huelva, Spain) and tomatoes (Granada, Spain), and probably has to do with the anti-oxidant abilities conferred by mycorrhizal symbioses to the plant. Post-harvest loss is an important issue, especially in export crops which should maintain good appearance and properties as much as possible until arriving to destination. Mycorrhizal extra-endurance properties in this sense would be certainly welcomed by producers.

In these two cultures fertilization was amended by means of coated fertilizer (celery) or basal fertilization just with compost (grape). It is important to stress here that applying the adequate fertilization type and level in crops amended with MYCOGEL® is crucial: in the past (a view is still retained by certain researchers and technical advisors) there existed the idea that applying mycorrhizas was not at all compatible with applying chemical fertilization, and particularly that addition of P was strictly forbidden. This was based on the well-known fact that high P concentrations inhibits AMF spore germination and even root colonization (Hepper, 1980; Same

Fig. 7. Results obtained after application of MYCOGEL® on celery. A, C, E, untreated plants. B, D, F, treated plants.



MYCOGEL® + Bombardier, July, 21st MYCOGEL® + Bombardier, August, 10th

Untreated, July, 21st Untreated, August, 10th

Fig. 8. Results obtained after application of MYCOGEL® + Bombardier® on grape. A, treated plants on July, 21st; B, treated plants on August, 10th; C, untreated plants on July, 21st; B, untreated plants on August, 10th.

et al., 1978). However, P is an essential nutrient for plant grow and crop production and that it should never be avoided, but make it compatible, when inoculating crops with mycorrhiza (Grant *et al*., 2005). To do this, it is important to never apply MYCOGEL® and fertilization at the same time, but waiting for the fungus to have colonized the root (i.e., about 2 weeks), then apply fertilization at convenience. A good alternative to this is to use slow nutrient-release fertilizers, which consist of granules of fertilizer coated with a special resin providing the exact fertilized dosage on each moment of the crop. Also, natural manure as a basal fertilization is a highly compatible practice, exactly the two techniques used in these two experiments.

AMF colonization results

Analyses for mycorrhizal colonization of roots sampled from the different crops tested rendered very low rates of root colonization (<5%). Furthermore, in some of the crops, mycorrhizal colonization could even not be detected. Although these results might be initially surprising, the fact is that low rates of host root colonization are the rule rather than the exception when using *in vitro*-produced AMF inoculum. However, the important effects obtained for treated plants (such as those presented in this paper) are consistent, and have been reported in many trials both under lab or agronomic conditions. How is this possible?

There are three possible explanations for this fact: first, although generally assumed that there is a direct correlation between percentage of root colonization and plant benefits (i.e., the higher the % colonization is, the better the plant nutritional status is), this has been shown not to be true in different situations (Treseder, 2013). For instance, Ávila-Peralta *et al*. (2015) found that, from a given level of colonization on, increase in root colonization by AMF resulted in a decreased symbiotic effectiveness and host benefits, indicating that, in those situations, the AMF behave somehow parasitically rather than symbiotically. Already in 1988 Douds *et al*. shown that there is an optimal level of mycorrhizal colonization above which the plant receives no enhanced nutrient uptake (benefit) yet continues to support mycorrhizal metabolism (cost). This colonization extent depends on many factors, among other: the nutritional status of the plant, water and nutrient availability in soil, light intensity and plant and fungal genotypes (Treseder, 2013). In fact, the final extent of root colonization is a combination of *both*, plant and fungal control of intraradical colonization. It is easy to understand that, from the point of view of the plant, acquisition of the most nutrient and water influx possible with the least cortical fungal "invasion" would be the best situation; on the other hand, from the point of view of the AM fungus, the least energy, resources and C (in terms of fungal structures) it should allocate to its intraradical mycelium to get the most C compounds out from the root, the better. In other words, if just one, very efficient, restricted infection unit would be sufficient to support satisfying bidirectional nutrient transport between both symbionts, that should be good enough to render the observed plant (and fungal) extra growth and benefits. Further research is needed to confirm this hypothesis and to understand why *in vitro*-issued inoculants seem to behave more efficiently at lower colonization rates compared to conventionally-produced inoculants.

A second possible explanation for the reduced AM colonization observed is the difficulty of collecting all root pieces from agricultural soils, in which roots have grown extensively and not confined, as it is the case of pot cultures. It is well known that mycorrhizal colonization is most active at subapical zones of secondary and higher-order roots. These zones are very fragile and are frequently discarded when collecting roots out of the soil. If restricted infection units were established precisely at these zones, the total, real root colonization would be strongly underestimated.

A third possible explanation for the low colonization observed is a more paradigmatic-challenging one: that physical AM colonization of roots is not as necessary as thought for the plant to obtain benefits from the presence of AMF, but the presence of certain signal molecules emitted by the AMF as a response of symbiosis (or pre-symbiosis), which could act as potent biostimulants profoundly affecting plant physiology. This hypothesis is being tested actually with surprising results (Cano *et al*., unpublished) and could make a turning point in our understanding of AM mutualistic symbiosis.

In conclusion, the application of the ultrapure AM inoculant MYCOGEL® to different crops in Japan resulted in important benefits, not only from an economic point of view, but also in terms of healthier fruits and a more sustainable management of agricultural soils. Plant growers can benefit from this new technological tool which copes with the highest standards of quality and efficiency, and is in agreement with the Governmental directives in terms of circular economy.

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Research Progress and the Limiting Factors of Direct Seeding Rice in Central China

Weiqin WANG[1] and Lixiao NIE[1,2]

[1]National Key Laboratory of Crop Genetic Improvement, MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China
[2]Hubei Collaborative Innovation Center for Grain Industry, Yangtze University, Jingzhou, Hubei 434023, China

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Corresponding Author
Lixiao NIE, nielixiao@mail.hzau.edu.cn

Abstract

Replacement of puddled transplanted rice (PTR) by direct-seeded rice (DSR) can potentially reduce consumption of resources and decrease emissions of greenhouse gases while maintaining grain yields in central China. However, direct seeding has not been widely adopted in this region. This review was undertaken to better understand the problems and opportunities for replacing PTR with DSR in central China. The seeding rate, crop growth, grain yield, water productivity, nitrogen use efficiency, greenhouse gas emissions and root development were compared between DSR and PTR. With good water management, grain yield of DSR is similar to or higher than that of PTR while increasing irrigation water productivity and nitrogen use efficiency, reducing greenhouse gas emissions, and decreasing labor requirement. However, problems that include lodging, weak root development, weed infestations and poor crop establishment under drought, waterlogging, or chilling stresses might limit wide-scale adoption of DSR in central China. Varieties bred and selected for direct seeding, guidelines for improved nutrition, water, and weed management practices, and the development of suitable planting machines and sowing management for DSR are needed. In addition, incorporating DSR into the double season rice systems could be promising strategies to increase rice production in central China.

1. Introduction

Rice (Oryza sativa L.) is the staple food for more than half of the world's population, especially in tropical Latin America and East, South and Southeast Asia (Seck *et al.*, 2012). Transplanting is the major rice establishment method and 77% of rice is transplanted globally (Rao *et al.*, 2007). In China, 95% of the rice is produced under puddled transplanted conditions with prolonged periods of flooding (Peng *et al.*, 2009). However, several problems such as labor shortage, water scarcity and climate change, have severely limited the development of puddled transplanted rice (PTR) in China. Under PTR, large quantities of water are consumed during land preparation and farming process (Bouman, 2009), and most of the water is wasted through surface evaporation and percolation, thus resulting in low water use efficiency (Farooq *et al.*, 2011). PTR is considered to be one of the major sources of greenhouse gas emissions in agricultural production systems. It has been reported that rice paddies contribute 11% of global total anthropogenic CH4 emissions (Smith *et al.*, 2007). Furthermore, the process of conventional transplanting has a large labor cost, with the labor shortage in China. Taking the disadvantages of high demands of resources and high greenhouse gas emission, PTR is no longer suitable for the sustainable development, and there is a trend that PTR needs to be replaced by mechanized and simplified intensive rice production methods.

Direct seeding rice (DSR), which refers to the process of directly sown the seeds into soil rather than transplanting seedlings in a puddled PTR field (Liu *et al.*, 2015), has emerged as an alternative option to PTR. DSR is becoming popular nowadays because of less water consumption, reduced labor intensity, facilitating to mechanization during crop establishment, and less methane emission (Pathak *et al.*, 2011). Besides, DSR can be sown under zero tillage (Rao *et al.*, 2007), and incorporating DSR into zero tillage planting would largely increase resource use efficiency while simultaneously reduce soil erosion, improve soil properties, and conserve soil moisture (Chauhan *et al*., 2006). The following direct-seeding methods have been suggested for rice in China: (1) dry direct-seeded rice (DDSR), in which dry rice seeds are drilled or broadcasted on non-puddled soil after dry tillage, zero tillage, or on a raised bed, and (2) wet direct-seeded rice (WDSR), in which dry seeds or sprouted rice seeds are broadcast or sown in lines on wet and puddled soil. Another principal method of direct-seeded rice is water seeding, in which sprouted rice seeds are broadcast in soil withstanding water (Kumar and Ladha 2011). Previous studies have compared the variances between different types of direct-seeded rice and traditional transplanted rice for yield, water use efficiency, and establishment methods. Generally, the yield performance of dry direct-seeded rice and wet direct-seeded rice was close to the yield of traditional transplanted rice (Mitchell *et al*. 2004; Rickman *et al*. 2001). Meanwhile, direct-seeded rice required lower irrigation water due to fewer continuous flooded days in the main field and less water use during land preparation compared with traditional transplanted rice. Zhao *et al*. (2007) documented higher grain yields and lower water use for dry direct-seeded rice compared with transplanted rice. In order to evaluate the possibility of replacing PTR with DSR in Central China, present study reviewed the research progress and the limiting factors of direct seeding rice in central China.

2. Effects of pre-sowing seed treatments on seed germination and early seedling growth of direct-seeded rice under drought, water-logging, and chilling stresses.

In central China, poor and uneven crop establishment severely limited the large scale adoption of DSR. After direct sowing, the rice seeds were easily suffered from abiotic stresses, such as chilling, drought, and waterlogging in Central China (Ma *et al*., 2011; Miro and Ismail, 2013). Abiotic stresses delayed or reduced the process of rice seed germination, inhibit the root development and shoot elongation, result in poor and uneven establishment, and finally cause yield decline (Sipaseuth *et al*., 2007; Guan *et al*., 2009). Nevertheless, pre-sowing seed treatments, such as seed coating, seed priming and seed pelleting could improve the seed germination and seedling vigor particularly under unfavorable environmental conditions (Farooq *et al*., 2006). In order to improve crop establishment of DSR, the effects of different pre-sowing seed treatments on seed emergence, seedling growth were examined under various stress condition (**Fig. 1**). The metabolic events associated with seed germination and stress resistance were also incorporated (Zheng *et al*., 2016; Hussain *et al*., 2016a; Hussain *et al*., 2016b; Wang *et al*., 2016). The results revealed that seed priming were effective in promoting seed germination, enhancing seedling growth of DSR under chilling (Hussain *et al*., 2016a; Wang *et al*., 2016), drought (Zheng *et al*., 2016), and waterlogging stresses (Hussain *et al*., 2016b). The positive effects of seed priming treatments on

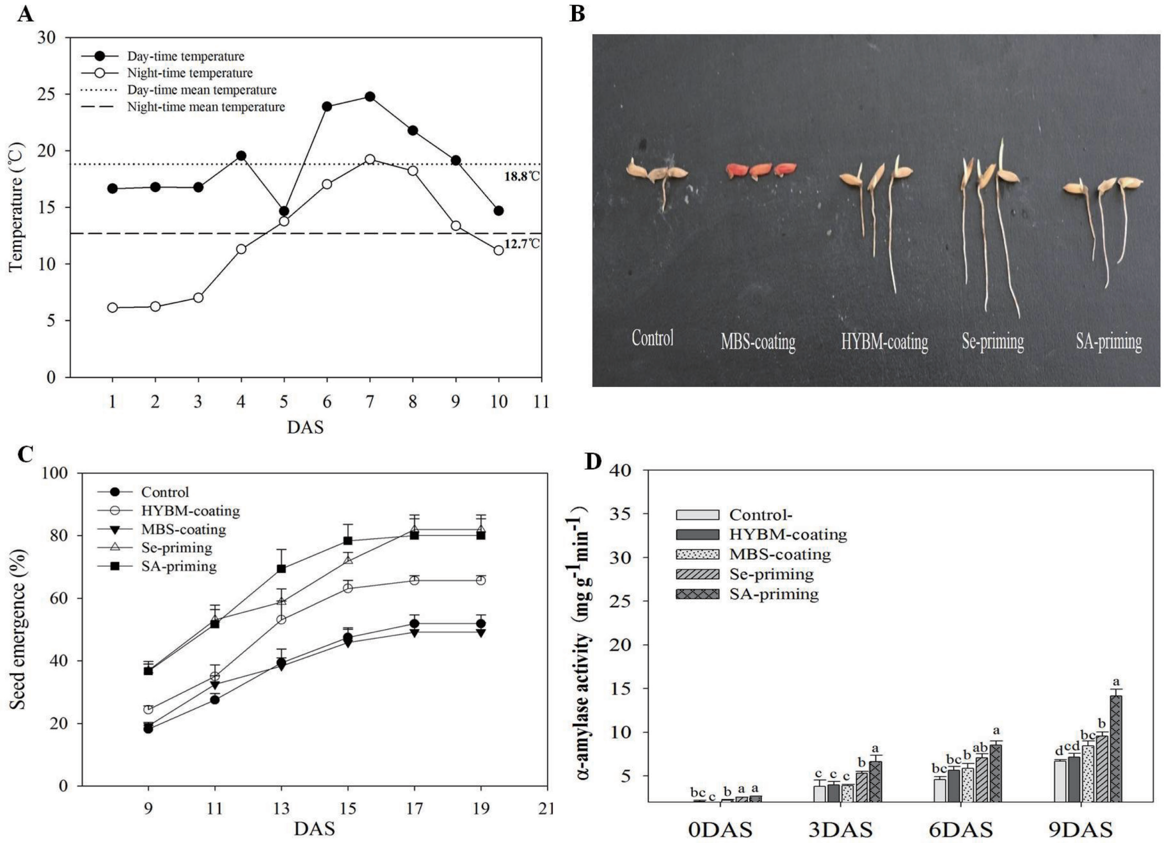


Fig. 1. Effect of pre-sowing seed treatments on seed germination and seedling growth of direct-seeded rice under abiotic stress. A: Air temperatures during seed germination. B: Pictorial illustration of seed germination with pre-sowing seed treatments under chilling stress. C: Germination dynamics of treated and non-treated seeds of rice. D: Variations in α-amylase activity of rice seeds and seedlings with different pre-sowing seed treatments at 0, 3, 6, and 9 DAS.

enhancement of stress tolerance were associated with vigorous starch metabolism, increased respiration rate, better membrane integrity, and the enhanced antioxidant system in the primed seeds and seedlings.

3. Estimation of optimum seeding rate for hybrid rice varieties in direct-seeded rice.

Hybrid rice varieties possess the potential to grow under dry direct seeded rice (DDSR) system and can perform well even at low sowing rate. Present study investigated the yield responses of three hybrid rice varieties to different sowing rates and explored the physiological basis for grain yield formation under DDSR system. An inbred rice variety was grown as control. Results showed that reducing sowing rates of hybrid rice varieties from 240 seeds m^{-2} to 90 seeds m^{-2} did not reduce grain yield, while that of inbred rice varieties declined with decreasing sowing rates. Decreased sowing rates of inbred rice varieties recorded insufficient tillers, and lower panicle number that decreased their yield (**Table 1**). Contrarily, for hybrid rice varieties grain yield was maintained even at reduced sowing rate because of their enhanced tillering capacity, higher specific leaf weight (SLW), and increased spikelet number per panicle (SPP), which might have compensated the reduced sowing rate. Our results suggested that the sowing rate of hybrid rice varieties can be reduced to 90 seeds m^{-2} without compensating yield of DDSR. However, adversities of low sowing rate in DDSR like weeds and poor stand establishment should be overcome to achieve maximum crop yield.

4. Comparisons of rice yields and resource use efficiencies among different rice planting methods.

In central China, the rice yields and resource use efficiencies among different rice planting methods were compared (**Fig. 2**). The grain yield in DSR was significantly influenced by water management. In WDSR, the grain yields were in the range of 9.50-11.59 t ha^{-1}, which was comparable to the grain yields in PTR. However, significant yield reductions were observed in DDSR compared with PTR. When averaged across years, the grain yield in DDSR was 9.47 t ha^{-1}, which was 2.9% lower than that in PTR (**Table 2**).

Table 1. Yield and its components of four rice varieties under three sowing rates.

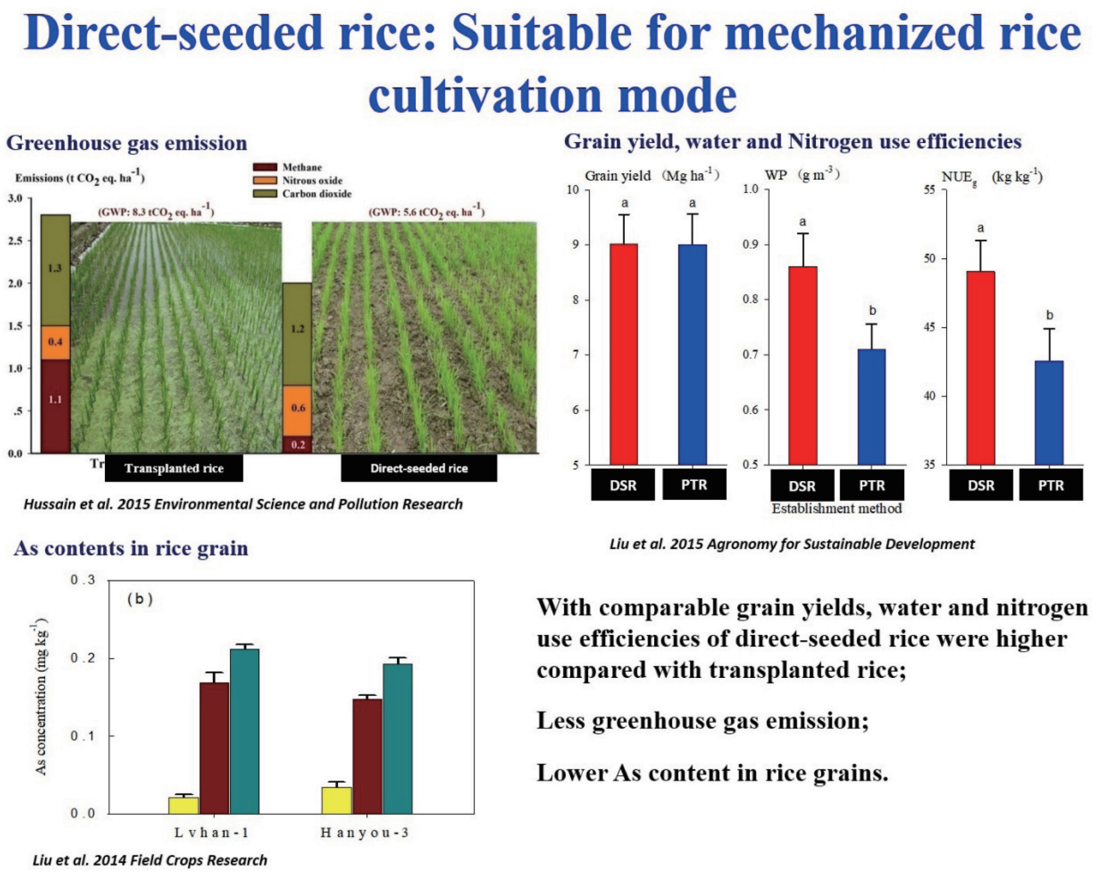
Variety	Sowing rate (seeds m^{-2})	Yield (t ha^{-1})	Panicles (m^{-2})	Spikelets panicle^{-1}	Spikelets m^{-2} (×10^{3})	Grain filling (%)	1000-GW (g)
HHZ	90	8.64 c	272 b	173.8 a	47.0 b	85.5 a	18.4 c
	150	9.07 b	316 ab	161.4 ab	50.5 ab	87.3 a	18.8 b
	240	9.48 a	333 a	154.8 b	51.6 a	86.8 a	19.2 a
FLYX1	90	8.81 a	247 a	167.8 a	38.6 a	86.0 b	22.7 c
	150	8.77 a	261 a	147.9 ab	38.4 a	89.5 a	23.5 b
	240	8.58 a	269 a	133.0 b	34.3 a	88.5 ab	24.2 a
YLY6	90	9.93 a	253 a	174.4 a	43.3 a	83.8 a	26.0 b
	150	10.23 a	254 a	158.3 b	40.1 ab	82.4 a	26.2 b
	240	10.29 a	279 a	134.7 c	38.8 b	80.9 a	27.0 a
YLY1	90	10.21 a	271 b	176.3 a	47.8 a	83.8 a	23.2 b
	150	10.44 a	281 ab	163.4 ab	46.1 a	83.1 a	23.5 ab
	240	10.33 a	298 a	153.4 b	45.4 a	83.7 a	23.8 a

Within a column for each cultivar, means followed by the different letters are significantly different from each other according to LSD (0.05). Inbred rice variety: HHZ (Huanghuazhan); hybrid rice varieties: FLYX1 (Fengliangyouxiang1), YLY6 (Yangliangyou6), YLY1 (Y-liangyou1). ns denotes non-significance based on analysis of variance.

Table 2. Grain yield and yield components of the four rice cultivars under dry direct-seeded rice (DDSR), wet direct-seeded rice (WDSR), and puddled transplanted rice (PTR).

Variety	Establishment methods	Yield (t ha^{-1})	Spikelets (m^{-2}×10^{3})	Panicles (m^{-2})	Spikelets panicle^{-1}	Grain filling (%)	1000-GW (g)
HHZ	DDSR	8.59 b	50.48 a	390 b	129.4 a	77.5 b	20.1 b
	WDSR	9.50 a	53.63 a	485 a	110.6 b	81.6 a	20.6 a
	PTR	8.63 b	41.36 b	309 c	133.9 a	83.8 b	20.8 a
LDQ7	DDSR	8.99 b	40.09 a	289 b	138.8 a	84.4 a	24.8 a
	WDSR	10.09 a	43.23 a	355 a	122.9 a	86.6 a	24.0 a
	PTR	9.07 b	35.07 b	260 b	135.5 a	86.4 a	24.6 a
YLY6	DDSR	10.60 b	38.30 b	291 ab	132.8 a	88.5 a	28.3 a
	WDSR	11.59 a	42.63 a	344 a	124.4 a	86.4 a	27.7 a
	PTR	9.54 c	35.29 c	256 b	138.2 a	87.6 a	28.1 a
YLY1	DDSR	9.69 b	39.77 b	345 a	150.2 b	83.0 a	25.8 a
	WDSR	10.65 a	46.72 a	397 a	149.1 b	83.8 a	24.1 b
	PTR	9.55 b	35.92 b	291 b	183.1 a	86.6 a	25.9 a

Note:Means followed by the different lowercase letters are significantly different from each other within the group according to LSD (0.05). Inbred rice varieties: HHZ (Huanghuazhan), LDQ7 (LvdaoQ7); hybrid rice varieties: YLY6 (Yangliangyou6), YLY1 (Yliangyou1).



Direct-seeded rice: Suitable for mechanized rice cultivation mode

Greenhouse gas emission

Hussain et al. 2015 Environmental Science and Pollution Research

Grain yield, water and Nitrogen use efficiencies

Liu et al. 2015 Agronomy for Sustainable Development

As contents in rice grain

Liu et al. 2014 Field Crops Research

With comparable grain yields, water and nitrogen use efficiencies of direct-seeded rice were higher compared with transplanted rice;

Less greenhouse gas emission;

Lower As content in rice grains.

Fig. 2. Comparisons of rice yields and resource use efficiencies among different rice planting methods.

DSR had lower water consumption and higher water productivity than PTR. The minimum water input and irrigation time were observed in DDSR (Liu *et al*., 2015). Compared with PTR and WDSR the irrigation water consumption in DDSR was reduced by 68.3% and 62.3%, respectively. The total irrigation in WDSR was decreased by 15.8% as compared with PTR (Liu *et al*., 2015; Tao *et al*., 2016). Delaying the first flood irrigation time in DDSR decreased the number of irrigation procedures and conserved irrigation water (Jiang *et al*., 2016). When the first flood irrigation was postponed, the number of irrigations was reduced from eight to four in 2014 and from twelve to seven in 2015, respectively. Consequently, the amount of irrigation water was also decreased from 376 to 185 mm in 2014 and from 477 to 283 mm in 2015, respectively.

In order to estimate the future potential impact of greenhouse gas emissions on the ecosystem, the global warming potential (GWP) was calculated in mass of CO_2 equivalents (kg CO_2 equivalent ha^{-1}) over a 100-year time horizon (GWP=CH4×25+N2O×298; Forster *et al*., 2007). The emissions of CH_4 and N_2O varied with different establishment methods and water managements. Recent research revealed that CH_4 emission in DSR was significantly decreased by 77.6% while the N_2O emission was significantly enhanced by 285.7% as compared with those in PTR. Consequently, GWP value was significantly lower in DSR than in PTR. The CH_4 emission from DSR system was even lower than that from PTR systems, while the N_2O emission was drastically increased to 2-3 folds compared with the N_2O emission from PTR and WDSR systems. The lowest GWP was observed in DDSR among different establishment methods and water managements (**Fig. 3**).

5. Problems and Prospects.

Lodging, which could result in sizeable reduction in grain yield due to decreased photosynthesis by self shading, and hamper grain quality due to increased colouring and decreased taste, is one of the most serious problems that influence the stability of DSR (Kano, 1995; Kashiwagi *et al*., 2005). Severe lodging near maturity in DSR was observed in our study (**Fig. 4**). Tao *et al*. (2016) reported that the lodging area in DSR accounted for 45% of the total planting area, which was caused by the decreased breaking resistance of the rice node and increased lodging index as compared with PTR. While in PTR, no lodging was occurred during the course of the study. Previous studies documented that root lodging is very common in DSR because of the rather shallow root system (Terashima, 1997). High seeding rate is another important factor that increases the risk of lodging in DSR. The high planting density would lead to the elongation of the stems, which consequently results in smaller stem diameter and thinner stem walls (Liu *et al*., 2014). On the other hand, the disease and pests at high plant density are frequently occurred due to the high humidity environment under DSR (Balasubramanian and Hill, 2002). In order to reduce lodging risk in DSR, a range of approaches has been suggested. Adjusting seeding rate is one of the most effective practices to reduce lodging risk (Kim *et al*., 1993). Other cultural practices may also minimize lodging risk in DSR include using subsurface or anaerobic seeding, adjusting

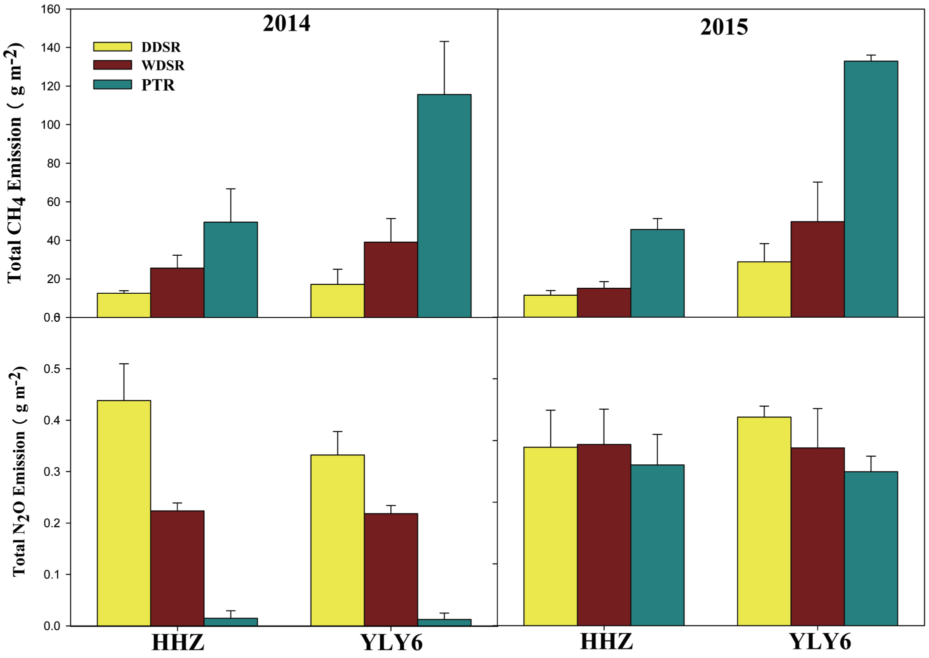


Fig. 3. Total CH_4 and N_2O emissions from dry direct-seeded rice (DDSR); wet direct-seeded rice (WDSR) and puddled transplanted rice (PTR) conditions.

Fig. 4. Photographs of lodging in direct-seeded rice (DSR) during the grain filling stage. DSR: Direct-seeded rice; PTR: Puddled transplanted rice.

the rate and time of N application and midseason drainage (Kim *et al*., 1995; Kim *et al*., 1999).

Weed is one of the major obstacles for developing DSR in central China. DSR are more likely to suffer from weeds infection than PTR (Singh *et al*., 2016), as the transplanted seedlings are more competitive to the emerged weeds (Rao *et al*., 2007). High rate of weed infection could decrease seedling emergence, increase pests and diseases infection, and lead to serious yield losses. Ramzan (2003) suggested that weeds were responsible for 74% yield losses in DSR and sometimes it may result in total crop failure. Many researches have reported that herbicides could successfully control weeds in DSR system (Chauhan *et al*., 2011; Singh *et al*., 2015). Nevertheless, intensive herbicide use may cause environmental contamination and enhance herbicide resistance. Meanwhile, several non-chemical methods have been recommended to successfully control weed without environment pollution. One of effective strategies is to use weed-competitive cultivars. Chauhan (2012) suggested that early seedling vigor and rapid canopy cover ability are the important traits for weed-competitive cultivars in DSR systems. Water management significantly inhibited weed growth. Chauhan and Johnson (2008) suggested that early and continuous, but shallow (<2 cm) flooding suppressed emergence and growth of weeds. Other non-chemical strategies include adjusting the row spacing, mulch covering and sterilizing seedbed before sowing.

Weedy rice (Oryza sativa f. spontanea) is another problem that causes the yield losses in DSR in central China. Weedy rice can take up 60% of the applied N fertilizer (Burgos *et al*., 2006). Li (2012) reported that weedy rice occurred in 80% of the DSR field, which lead to 15%-25% yield losses in Anhui province. One latest research reported that the weedy rice is de-domesticated from the cultivated rice, which suggested that the control of weedy rice is much more difficult than that of other weeds (Li *et al*., 2017). The strategies to prevent weedy rice need to be established based on the homology between weedy rice and cultivated rice. Although most herbicides that are selective for rice are not effective in controlling weedy rice. Shen *et al*. (2013) reported that integrate application of pretilachlor and fenclorim as pre-emergence herbicide could efficiently prevent the spread of weedy rice. Besides, sterilizing the field by non-selective herbicide prior to sowing and the use of weedy rice-free seeds is one of the best preventive measures to control weedy rice (Chauhan 2013; Singh *et al*., 2013).

The double season rice is the important rice cropping system in central China due to increase multiple crop index and thus contributing substantially to rice supply (Ray and Foley, 2013). The dominant rice establishment method in double season rice system is transplanting, which consumes large amount of labors and resources. As the results of continuous migration of labor from rural areas to cities and the crisis of water shortage, the planting area of double season rice is decreasing rapidly (Cai and Chen, 2000). To ensure food safety in central China, it is crucial to replace double season transplanting with more simplified cropping system. Incorporating DSR into double season rice system (Double-dry-seeded-rice) could be a promising strategy. "Double dry seeded rice" refers to the process of crop establishment by dry-direct-sowing the seeds in the field in both early and late seasons. Although double dry seeded rice could significantly increase resource use efficiency and maintain multiple cropping index, some limiting factors constrained the further development of this system. Compared with PTR, DSR lacks the nursery stage, which means rice has a longer field growth stage than transplanted rice. If the sowing date of direct-seeded early rice were postponed, the direct-seeded late rice would not be able mature due to the low temperature during grain filling stage in October to November in China (Gong *et al*., 2013). There are mainly two options to solve this problem, one is the selection of short growth duration varieties. However, very few varieties could achieve relatively high grain yield with short growth durations. The other option is advancing the sowing date of early direct-seeded rice to early or mid-April. However, seeds and seedlings of direct-seeded early rice may easily suffer from chilling stress which could result in poor crop establishment.

6. Conclusion

With good water management, grain yield of DSR is similar to or higher than yield of PTR while increasing irrigation water productivity and nitrogen use efficiency, reducing greenhouse gas emissions, and decreasing labor requirement. However, problems that include lodging, weak root development, weed infestations and poor crop establishment under drought, waterlogging, or chilling stresses might limit wide-scale adoption of DSR in central China. Varieties bred and selected for direct seeding, guidelines for improved nutrition, water, and weed management practices, and the development of suitable planting machines and sowing management for DSR are needed. In addition, incorporating DSR into the double season rice or ratoon rice systems could be promising strategies to increase rice production in central China.

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Symposium paper

Root Phenotyping with Root Modeling: Towards Sustainable Rice Production

Ryosuke TAJIMA

Field Science Center, Graduate School of Agricultural Science, Tohoku University, Yomogida, Osaki, Miyagi, 989-6711, Japan

Keywords
modeling, phenolyping, rice, root system

Corresponding Author
Ryosuke TAJIMA, tazy@tohoku.ac.jp

Abstract

Improving root system architecture (RSA) has a huge potential both on crop production and environmental impacts. To improve RSA, we need to deeply understand RSA but it is extremely difficult to determine a few root phenotypes. In this review, we proposed to analyze the phenotype of RSA using root model: one is with datasets from lab-based experiments and the other is with limited datasets from field observations. First, we could predict RSA in the field even at the end of the growth season with data in the paper culture at the early growth stage. Second, we could predict the parameters which can't be measurable in the field from field observations. These approaches could be potentially powerful techniques for identifying the root phenotypes.

Introduction

Root system architecture (RSA) in rice production system has been shown to be important both for production and environmental impacts. Improving RSA increases resource acquisition, especially in low-input conditions. For example, Uga *et al*. (2013) found a *DEEPER ROOTING1* (*DRO1*) gene making the growth angle of nodal root larger. Introducing *DRO1* gene to IR 64 which has a shallow root system, the introduced plant relatively had a deeper root system and higher yield performance under drought condition than IR 64. Increasing resource acquisition also leads to decrease the resource loss to groundwater and reduce irrigation and fertilizers, which links to mitigate environmental impacts. In addition, improving RSA could have a potential to increase carbon stocks in the soil. The output from root (root exudates and root decompositions) in the growth season and the remains of root after yielding are major carbon sources. Increasing carbon from root could enhance soil organic carbon. As a result, it would reduce atmospheric CO_2 concentration. In Paustian *et al*. (2016), the mitigation effects per hectare of improving RSA estimates larger than that of biochar application and the applicable area of improving RSA estimates as large as that of management practices.

To improve RSA, we need to deeply understand RSA: the performance of root phenotypes and their interactions in the whole root system from seeding to yielding under the various field conditions. However, it is extremely difficult even to determine some root phenotypes. Even to estimate partial root length density at only one growth stage, the process is back-breaking, time-consuming and often to need many workers.

Here, for getting over the process with less effort, we propose to analyze the phenotype of RSA using root model which simulates RSA to understand it in the field.

Root model description

Root models have been developing from the 1970s with developing and generalizing computers and the associated equipment. In Dunbabin *et al*. (2013), six current root models were introduced but almost all models could not be used without the collaboration with the developers. Currently, a few root models are becoming open-source, but it has been difficult to say that the models are easy to use for the potential users yet. Therefore, I'm developing another root architectural model named Seurat (Simulator for evaluating and understanding root architecture). In rice, root system consists of one seminal root and numerous nodal roots which are generated from the stem. One nodal root consists of parent root and numerous lateral roots which are generated from the parent root. Each seminal, nodal and lateral root consists of many cells but, in Seurat, a mass of cells sets as a dot and each root presents as a troop of dots. The dots have various parameters, such as elongation rate, diameter, gravitropism and position, which are updatable depending on endogenous and ectogenous factors such as age and environment.

Running Seurat needs the basic information of nodal roots and the parameters of individual roots. The basic information of nodal roots is easily measurable in both laboratory and field because the information is estimated with observation of basal part of the rice plant. The basic information of nodal

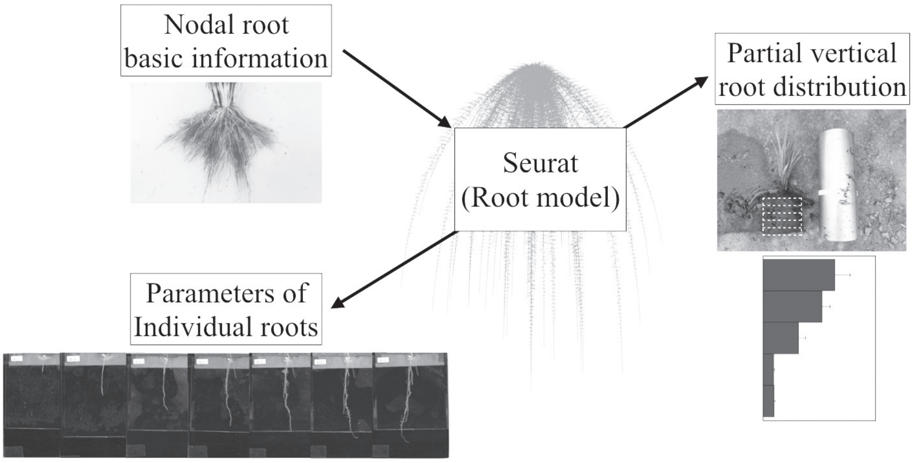

Fig. 1. The outline of root phenolyping with root model.

roots consists of the time of nodal root emergence, vertical and horizontal growth angle of each nodal root. The nodal root emergences could be controlled by several rules, therefore, if the rules become clearer than now, it could be easier to set nodal root information. On the other hands, it is difficult to measure the parameters of individual roots directly. Therefore, we should get the parameters in lab-based experiments or predict the parameters from field observations using Seurat.

Phenotyping with root model

In this review, I introduce two ways for phenotyping root system using root model: one is with datasets from lab-based experiments and the other is with limited datasets from field observations.

1) Using laboratory-based experiments

I predicted RSA with the datasets of the parameters of RSA at the early growth stage of rice plants in the laboratory-based experiment. Some lab-based experiments such as paper or soil cultures against transparent plates allow for non-destructive and time-lapse observations on individual plants. These experiments could take a large amount of photo with less work. According to my experiment, the root grew between paper and transparent plates. I took the images at several times with an image scanner. This paper culture provided various data by analyzing time-lapse two-dimensional images. In my results, running root model with the datasets of this laboratory-based experiment, I could predict RSA in the field even at the end of the growth season. And I suggested a little bit difference of traits at very early growth stage might become a large difference of root system at the end of the growth stage in the field. However, there is concern that the conditions with laboratory-based experiments don't simulate field conditions, e.g. the observed roots that grow against plates in the paper culture could not be similar to that in the soil. On the other hands, in laboratory-based experiments, root box is more similar to field conditions than paper culture because of using soil, but it is harder to measure root traits than paper culture.

2) Using field observations

I predicted the parameters of RSA which can't be measurable in the field with the field observation. I used the basic information of nodal roots and the data of nodal root partial vertical distribution in the soil at a one growth stage. Nodal root number and growth angle which easily identified from the observations around the basal part were used as the information of nodal roots. In nodal root distribution, we took the cylinder soil monolith (10cm diameter) located just under the rice plant in less than 10cm soil depth. This is monolith method which is one of the old but standard methods. This soil monolith with roots was divided into five layers. These parts were carefully washed and nodal root length was measured with image analysis (Tajima and Kato 2011, 2013). Using these datasets, we attempted to predict the parameters of individual nodal roots, the elongation rate and gravitropism, with Nelder-Mead method which is a commonly-used nonlinear optimization method. As a result, we could find the parameters through the procedure. However, it's very preliminary result because, in this trial, taking root distribution at only one time was too limited to predict the accurate parameters of RSA. If we can collect more data which has already existed such as vertical root distribution taken in old but standard methods like the monolith method, more realistic RSA could be predicted.

Summary and Outlook

I proposed the model-based approach for phenotyping in root system with datasets both from laboratory-based experiments and field observations (**Fig. 1**). Using root model with laboratory-based datasets at early growth stage could predict the RSA at later growth stage in the field. In addition, using root model with the limited datasets of partial root distribution at one growth stage in the field might estimate the several root phenotypes which have been extremely difficult to measure in the field. These approaches could be potentially powerful techniques for identifying the root phenotypes. The data of phenotype identified using root model can be relatively easily acquired. These big datasets could develop root modeling. Thus, there is a mutually complementary relationship between root phenotyping and modeling. Developing this relationship should accelerate to propose a groundbreaking RSA ideotype and contribute RSA improvement for boosting up rice production and reducing environmental impacts in the future.

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Symposium paper

Analysis of the Disease-Suppressing Effects of Microorganisms Included in Nursery Soils for Organic Farming of Rice

Sugihiro ANDO and Hideki TAKAHASHI

Graduate School of Agricultural Science, Tohoku University, 468-1 Aramaki Aza Aoba, Aoba-ku, Sendai 980-0845, Japan

Keywords
Oryza sativa, *Burkholderia glumae*, *Burkholderia plantarii*, seedling rot, microbiome, microbial diversity, robustness

Corresponding Author
Sugihiro ANDO,
sugihiro.ando.a2@tohoku.ac.jp

Abstract

In general, microorganisms in soil are important for crop cultivation. Therefore, we analyzed the disease-suppressing effect of soils from organic farms and characterized the microorganisms present in the soils. We found that nursery soils from organic rice farms in Japan had a disease-suppressing effect on rice seedling rot caused by *Burkholderia glumae*, *B. plantarii* and seedling blast. We isolated disease-suppressive bacteria from the soils and investigated whether the bacteria activated the ethylene-dependent plant immune system. We also performed a microbiome analysis to compare nursery soils from organic farms with those from conventional farms, and found that richness and diversity were higher in organic farm soils. The bacterial populations in organic farm soils were robust to irrigation and seedling growth. These findings imply that a robust bacterial population contributes to suppression of proliferation of bacterial pathogens. Finally, we attempted to develop a new disease suppression technique using bacterial communities cultured from organic nursery soils. The bacterial mixtures cultured from some organic farm soils exhibited disease-suppressing activity. We also monitored changes in soil bacterial populations after adding the bacterial mixture to conventional nursery soil, and found that a stable bacterial community was related to disease suppression. These findings imply that a robust bacterial community is related to disease-suppression activity. Therefore, it is possible that a disease suppression system similar to the nursery soil of organic farms would be effective when a cultured bacterial mixture is applied to conventional nursery soil.

Introduction

The development of agricultural chemicals has contributed to efficient disease control and a stable food supply. However, agricultural systems that depend on excessive agricultural chemicals are problems in terms of the environmental load they impose. The importance of environmentally friendly agriculture has been recognized in recent years. In organic farming systems, diseases are controlled without using agricultural chemicals. Since serious loss of yield occurs in conventional farming without agrochemicals, we postulated that there are several unknown mechanisms underlying disease suppression in organic agriculture. In general, microorganisms in soil have important roles in disease suppression in naturally occurring disease-suppressing soil (Weller *et al.*, 2002; Haas and Defago, 2005). We found that nursery soil for rice from organic farms had a disease-suppressing effect. In this study, we characterized the disease-suppressing effect of the organic farm soils. We also compared the microbial diversity of organic farm nursery soils using 16S and 18S rRNA gene analysis. Based on the results, we attempted to develop a new technique for disease suppression using the bacterial community from organic farm soils.

1. Disease-suppressing effect of organically farmed nursery soil

In Japan, standard rice nursery management procedures involve growing healthy seedlings in nursery beds. Such practices are also used in organic rice farming. However, seedling disease often emerges during this step and can sometimes become a major barrier to rice cultivation. To analyze the disease-suppressing effects of organic farming, we collected nursery soils used for rice from 12 independent organic farmers in Japan. These nursery soils were made by the organic farmers using their own original methods.

Rice seeds inoculated with *Burkholderia glumae* were sown in the 12 organic farm nursery soils and in three commercial conventional nursery soils. After 9 days of cultivation, we confirmed that all of the organic farm soils suppressed the seedling rot symptoms caused by *B. glumae* to a greater extent than the commercial soils (**Fig. 1**). A disease-suppressing effect of the tested organic farm soils was also confirmed against the seedling rot caused by *B. plantarii* and seedling blast (**Table 1**). This effect of organic farm soils was attenuated by heat treatment with an autoclave (121°C for 15 min.), similar to other disease-suppressive soils (Mendes *et al.*, 2011). These results suggested that soil microorganisms are involved in the disease-suppressing effects of the organic farm soils. (Ando *et al.*, 2014). A disease-suppressing effect seems to be a common feature of organic farm soils.

2. Analysis of the microbial diversity of organic farm soils

Since microbial diversity is high in disease-suppressing soils (Garbeva *et al.*, 2004), we examined the microbial diversity of organic farm soils by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) and next-generation sequencing. We extracted total DNA from 12 organic farm soils and two commercial soils, and then amplified bacterial 16S and fungal 18S rDNA by PCR. The PCR products were subject to PCR-DGGE analysis and the richness and evenness were analyzed based on the patterns of the PCR bands. The bacterial richness and evenness, and the richness of fungi, were higher in organic farm soils than in the two commercial soils. We also examined the robustness of the bacterial diversity of the organic farm soils to environmental change. After irrigation with distilled water for 7 days and cultivation of rice seedlings, we extracted total DNA from two commercial soils and two organic farm soils. Fragments of 16S rDNA were amplified from the extracted DNA and subject to next-generation sequencing. In the commercial soils, the distribution of the bacterial populations was largely affected by irrigation and cultivation of rice seedlings. By contrast, the bacterial populations of the organic farm soils were relatively stable against environmental change, indicating that robustness to environmental change is high in organic farm soils (Takahashi *et al.*, 2018). We postulated that a robust bacterial population contributes to suppression of the invasion and proliferation of external pathogens.

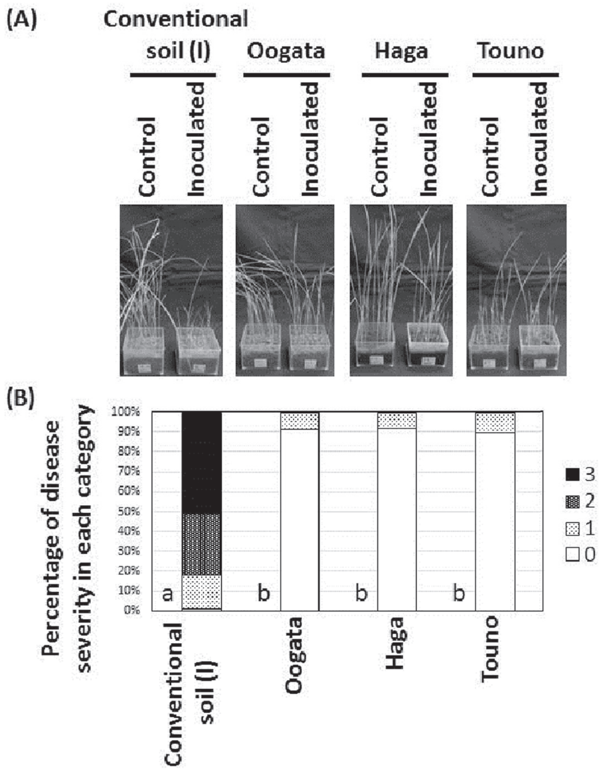

Fig. 1. Suppression of seedling rot caused by *B. glumae* in organic farm soils. Rice seeds inoculated with *B. glumae* were sown on commercial or organic farm soils collected from three locations (Oogata, Haga and Touno). Disease symptoms were assessed after 9 days of cultivation at 30°C with a 14 h photoperiod. (A) Photographs of symptom development. The uninoculated control is also shown. (B) Disease severity was assessed on a scale ranging from 0 to 3 (0, healthy; 1, growth suppression and chlorosis; 2, partially dead; 3, dead). The disease severity (%) is shown. Different letters significant differences in disease severity (Steel-Dwass test, n = 25, *P*<0.001).

3. Screening bacteria with disease-suppressing activity from organic farm soil

We also attempted to isolate disease-suppressing bacteria from the organic farm soils. A suspension of organic farm soil was streaked on solid nutrient agar (NA) and incubated at 25°C for 1 day. Formed colonies were randomly isolated, and the 16S rDNA sequences determined. We classified these bacteria based on the 16S rDNA sequences and assessed their disease-suppressing activity. We found that application of *Pseudomonas* sp. W6 and Y3 strains suppressed the seedling rot caused by *B. glumae* (Ando *et al.*, 2014). Since some *Pseudomonas* strains activate plant immune responses (Pieterse *et al.*, 2014), we examined the expression of defense-related genes after the application of strains W6 and Y3, and found that the expression of *OsACS2*, an ethylene biosynthetic gene, was stimulated by the bacteria at 2 days post-inoculation (**Fig. 2**). We also confirmed that ethylene production was enhanced by the application of these bacteria, suggesting that the bacteria activated the ethylene-dependent plant immune system. Furthermore, we discovered some bacteria with antimicrobial activity against *B. glumae*, suggesting that several disease-suppressing mechanisms work together in organic farm soil, including a robust microbiota, and immune and antimicrobial activities.

4. Development of a new disease-suppressing technique using culturable bacteria from organic farm soil

Soil microorganisms have important roles in the disease-suppressing activity of organic farm soils, as described above. We aimed to develop a new disease-suppression strategy using the microbial populations of organic farm soils. However, this was difficult because most soil microorganisms are unculturable with artificial medium (McCaig *et al.*, 2001). Therefore, we first attempted to develop a disease-suppression

Table 1. Summary of disease suppresive activity of organic-farmed soils.

Location of organic rice farm in Japan	Seedling rot caused by *B. glumae*	Seedling rot caused by *B. plantarii*	Rice seedling blast
Ishikawa, Fukushima	Effective	Effective	Not tested
Wakuya, Miyagi	Effective	Effective	Not tested
Naruko, Miyagi	Effective	Effective	Not tested
Nogi, Tochigi	Effective	Not tested	Not tested
Haga, Tochigi	Effective	Effective	Effective
Oogat, Akita	Effective	Effective	Not tested
Shimoda, Shizuoka	Effective	Not tested	Not tested
Touno, Iwate	Effective	Not tested	Not tested
Kaminokawa, Tochigi	Effective	Not tested	Not tested
Saitama, Saitama	Effective	Not tested	Not tested
Niigata, Niigata	Effective	Not tested	Effective
Higashimatsushima, Miyagi	Effective	Not tested	Not tested
Conventional (L)	No effect	No effect	Not tested
Conventional (K)	No effect	No effect	Not tested
Conventional (I)	No effect	No effect	No effect

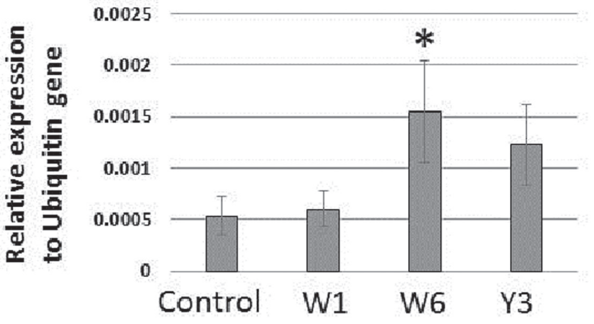

Fig. 2. The expression of *OsACS2* after treatment with *Pseudomonas* sp. isolated from organic farm soil. Rice seeds (cv. Koshihikari) were disinfected by immersion in hot water (60°C for 10 min.) and incubated for 2 days at 28°C in tap water. The germinated seeds were sown in commercial soil and treated with a suspension of bacterial isolates (*Pseudomonas* sp. isolate W1, W6, or Y3). The optical density unit at 600 nm (OD_{600}) of each bacterial suspension was adjusted to 0.5, and 10 mL of the suspension was added to the soil in each pot. Total RNA was extracted from the rice seedlings 2 days after the bacterial treatment. Quantitative RT-PCR was performed with specific primers for *OsACS2*. The expression levels are given relative to that of the ubiquitin gene. The asterisk indicates a significant difference compared with the untreated control (Dunnett's test, n = 3, $P<0.05$).

technique using bacterial communities cultured from organic nursery soils. We cultured bacterial communities from five organic soils on NA and examined their disease-suppressing effects in rice with bacterial panicle rice blight grown in conventional nursery soil. The bacterial populations of two organic farm soils (Haga and Touno) showed a disease-suppressing effect at 9 days post inoculation (dpi), which was not seen with the bacterial populations of Nogi, Oogata, and Kaminokawa. We also examined changes in the bacterial populations of the soils using 16S rDNA of PCR-DGGE at 9 dpi. Although major applied bacteria could not increase in the soil, the bacterial population formed in the soil was not affected by *B. glumae* inoculation during disease suppression. However, the bacterial population in soil was largely affected by inoculation with the pathogen when severe seedling rot was observed. These results suggest that a culturable bacterial mixture used for disease suppression can yield a bacterial population that is robust against invasion of the soil by bacterial pathogens. This could serve to suppress disease after application of a culturable bacterial mixture to the soil.

We also attempted to improve culture conditions to enhance the disease-suppressing activity of the bacterial community cultured from organic farm soils. In general, more microorganism species were cultured under nutrient-poor conditions compared with nutrient-rich conditions in a previous study (Tamaki *et al.*, 2005). Therefore, we used diluted NA medium (1/1,000) to culture the soil bacteria and the disease-suppressing effect tended to be stronger when diluted medium was used. For example, the bacterial community from the Nogi organic farm soil showed disease-suppressing activity only when diluted NA medium was used. PCR-DGGE analysis of 16S rDNA indicated that the cultured bacterial population differed markedly depending on the culture conditions, and a stable bacterial community in the applied soil was related to disease suppression.

Finally, we examined whether repeatedly cultured bacterial mixtures retain disease-suppressing activity. We streaked a bacterial mixture from the Haga organic farm soil on the same medium again to yield a secondary bacterial mixture. When NA medium was used, the disease-suppressing activity of the bacterial mixture disappeared with repeated culture, in association with the unstable bacterial population that formed in the soil. However, we confirmed the disease-suppressing activity in the secondary bacterial mixture cultured on diluted NA medium (1/1,000). PCR-DGGE analysis showed that the bacterial population formed in the soil after adding the secondary bacterial mixture remained robust against invasion by bacterial pathogen. These results suggest that using low-nutrient medium is important for conferring strong and stable disease-suppressing activity in a cultured bacterial mixture. This finding is also important for the development of disease-suppressing techniques, where methods for amplification of a bacterial community via repeated culture could be developed based on these data.

Conclusion

In this study, we showed that the diversity of microorganisms in organic farm soils used for growing rice seedlings is greater than that in commercial soils. Furthermore, a wide microbial distribution is associated with disease-suppressing effects. During an attempt to develop a disease-suppressing technique using bacterial communities from organic farm soils, we found that a mixture of culturable bacteria on low-nutrient medium showed high disease-suppressing activity on addition to commercial soil. This disease-suppressing effect was related to the robust bacterial community that forms in the soil after adding the bacterial mixture, suggesting that a culturable bacterial community has disease-suppressing effects similar to those of organic farm soils. Our findings could lead to new strategies for the biological control of plant diseases using culturable microbe communities from disease-suppressing soils. However, the bacterial mixtures of some organic farm soils did not show any disease-suppressing effects under the conditions tested. Further analyses to determine the optimal conditions for the culture, application, and storage of bacteria are necessary to establish crop-protection technologies. Identifying the bacterial species that play key roles in disease suppression should be a target for future studies.

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Symposium paper

Recovery of Tsunami-Affected Paddy Soil Using Calcium Materials for Sustainable Agriculture

Toyoaki ITO, Hisashi NASUKAWA, Toru UNO, Ryosuke TAJIMA and Masanori SAITO

Graduate School of Agricultural Science, Tohoku University

Keywords
exchangeable sodium percentage,
sodium disorder,
potassium and calcium uptake inhibition,
steel-making slag fertilizer

Corresponding Author
Toyoaki ITO,
toyoaki-ito@nafu.ac.jp

Abstract

The tsunami attacked the regions along Pacific coast and caused severe damage to the lowland farmlands with topsoil outflow and salt disorder. In most of the tsunami-affected farmland, desalinization work was carried out by irrigation, resulting in sufficient removal of water-soluble salt of the tsunami-affected soils. However, according to the monitoring surveys of the soil where the farmland recovery projects had been completed, some fields had a poor nutrient balance. To solve the problem, we examined the effectiveness of applying calcium-silicate materials (steel-making slag fertilizers) in alleviating Na disorders using model desalinated tsunami-affected soil in 2013. We found that the fertilizer made of steel-making slag was effective in restoring the productivity of desalted tsunami-affected soils containing high amount of Na.

Introduction

The 2011 Japan earthquake triggered a great tsunami. Many lives were lost and tremendous damage was caused by the Great East Japan Earthquake and tsunami disaster of March 11, 2011. The tsunami attacked the regions along Pacific coast and caused severe damage of topsoil outflow and salt disorder to the lowland farmlands. About 15,000 ha of paddy fields had been damaged in Miyagi Prefecture alone. Of the damaged agricultural lands, about eighty-five % was paddy field. Most of the tsunami-affected farmland were desalinized by irrigation and water-soluble salt was effectively removed from the tsunami-affected soils.

However, according to the monitoring survey of the soil where the farmland recovery projects had been completed, some fields had a poor basic cation balance (Ito, T., 2015). Some of the exchangeable calcium (Ca) ion had been displaced by sodium (Na) ion derived from seawater during the desalinization process. As a result of the exchange reactions between Na and Ca in the soil, some of Na ion had remained in the exchange sites of soils and exchangeable Ca had been reduced. In soils with high concentration of exchangeable Na, crops sometimes show poor growth due to the excess uptake of Na, Na disorder (Anil *et al*., 2005; Gong *et al*., 2006; Matoh *et al*., 1986). In order to mitigate Na disorders and restore soil productivity, it is essential to optimize the basic cation balance in the soils where desalinization has been implemented. According to IRRI report (Dobermann and Fairhurst 2000), in soils where exchangeable sodium percentage exceeds 20%, rice yield may begin to decrease. According to Gupta and Sharma (1990), rice yield decreased to 50% in the soil with ESP of more than 80%. When rice absorbed sodium excessively, uptake of potassium (K) and Ca was inhibited and rice growth was limited (Kinraide, 1999).

To solve the problem, we examined the effectiveness of applying calcium materials (steel-making slag fertilizers) in alleviating Na disorders. We conducted the cultivation experiment using model desalinated tsunami-affected soil in 2013.

Materials and methods

In order to clarify the effectiveness of calcium materials such as a steel-making slag fertilizer and gypsum in alleviating Na disorders of rice plant, the cultivation experiment was conducted using a model desalinated tsunami-damaged soil in 2013.

Preparation of desalinated tsunami-affected soil

An alluvial soil (clay content: 18%, total carbon content: 1.8%) was collected from normal paddy field without tsunami attack. The soil was submerged by seawater and was then desalinated by flooding and draining repeatedly with fresh water. The prepared soil (desalinated soil) showed low electric

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conductivity of soil suspension (soil:deionized water =1:5) of 0.20 dS m^{-1}, exchangeable Ca of 3.5 cmol $_{(c)}$ kg^{-1} and exchangeable Na percentage of 46%. The EC value is lower than the critical value causing rice salinity injury (Nakada, 2011). Exchangeable Ca contents decreased and exchangeable Na contents drastically increased with sea water immersion and washing by fresh water.

Cultivation experiment

Rice cultivation experiment was conducted in the paddy field of Field Science Center of Tohoku University in 2013. We packed the desalinated soil with a plastic frame of 0.076 m^2 without bottom in the paddy fields after applying Ca materials. We set five treatments; no application of Ca material, steel-making slag fertilizer application of 200, 400 g m^{-2}, gypsum application (corresponding to slag 200 g m^{-2}), and original soil without sea water immersion and desalination treatments. Application rate of gypsum was determined so as to be similar to the slag fertilizer of 200 g m^{-2} for the applied amount of Ca. The steel-making slag fertilizer used in this study contains 114 g kg^{-1} total silicate, 426 g kg^{-1} total CaO and 190 g kg^{-1} total iron (Gao *et al.*, 2016). We transplanted two seedlings with six leaves of rice (*Oryza sativa* L., Hitomebore) to each experimental plot made by a plastic frame after applying chemical fertilizers with application rates of N-P_2O_5-K_2O of 4-6-6 g m^{-2} to packed soils. Cultivation experiments were conducted with three replications.

Potassium, Ca, Na and silicon (Si) of rice straw, and brown rice yields were measured at the harvest time. The harvested rice grain was hulled and the brown rice of 1.7 mm or larger was collected using a multi-stage sieve apparatus. The brown rice yield was measured by correcting the water content to 15%. The Ca, Na and Si concentrations was measured using the harvested samples of rice plant dried at 70°C for 48 h. The dried plant samples were crushed and digested with sulfuric acid and oxygen peroxide, and the cation content was analyzed by atomic absorption spectrophotometry. The silicate content was analyzed by colorimetric method after being decomposed by HCl and HF.

During the cultivation period, Ca, Na and Si concentrations in soil solution water were measured. Soil solution water was sampled from a depth of 7 cm in the plow layer through a porous cup that was buried before planting. Exchangeable cations of soils in all treatments were determined after rice harvest to investigate the effect of applied Ca materials on cation balance of the soils. Statistical analysis was conducted by Tukey-Kramer test (0.05>p).

Results and discussions

Figure 1 and 2 show the changes of Ca and Na concentrations of soil solutions collected from plow layers in four treatments. Sodium concentrations showed about 3 to 10 times Ca concentrations in the control treatment. Calcium concentrations in soil solutions increased with applications of slag or gypsum and it was the highest in the gypsum treatment. Calcium concentration in the steel-making slag treatment with application rate of 200 g m^{-2} was lower than the gypsum treatment in spite of same total Ca input in the two treatments. It indicates that the steel-making slag dissolves more slowly than gypsum. On the other hand, there was no difference in Na concentrations among all the treatments. Increasing of Na concentration in the soil solution from 12, June to 3, July is considered to be due to progression of ferrous iron formation under submerged condition and ion exchange between ferrous iron in solution and Na ion in the exchange site of soil. These data show that the desalted soil supplies significant amount of Na to rice plant, and slag and gypsum can increase plant available Ca in soils.

Slag treatments with 200 and 400 g m^{-2} increased brown rice yields by 8 and 17% than the control treatment with significant difference for slag 400, respectively (**Fig. 3**). Gypsum treatment did not increase the rice yield in spite of increasing Ca and K contents and decreasing Na content in rice straw, as described below. This is considered to be due to injury of rice roots caused by hydrogen sulfide derived from sulfate reduction.

Calcium and K concentrations in rice straws increased with 10 to 18% and 3% by slag and gypsum applications, respectively (**Figs. 4 and 5**). On the other hand, Na concentrations in rice straws were reduced with 8 to 14% by slag and gypsum treatments compared with the control plot, respectively (**Fig. 6**). Slag treatments enriched silicate contents of rice straws at maturity stage with significant difference (**Fig. 7**). It suggests that the Ca-containing materials such

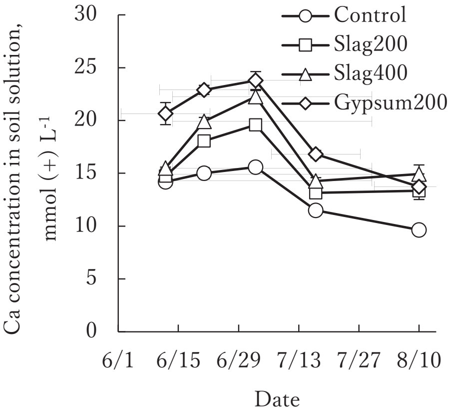

Fig. 1. Temporal pattern of calcium concentration in the soil solution.

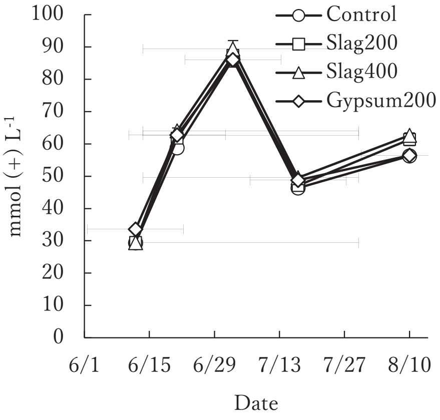

Fig. 2. Temporal pattern of sodium concentration in the soil solution.

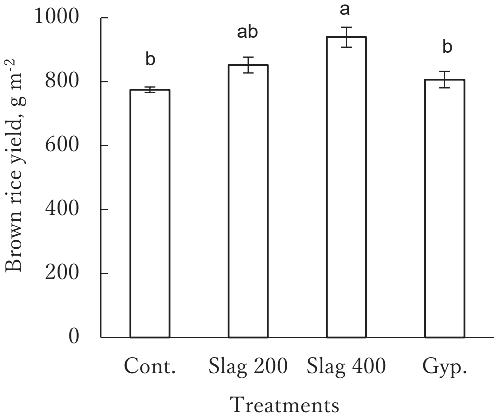

Fig. 3. Mean (±SE) brown rice yield in each treatment. Bars with the same letters are not significantly different ($P < 0.05$) according to the Tukey– Kramer test.

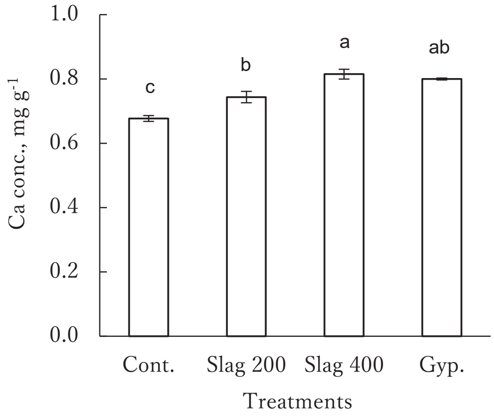

Fig. 4. Mean (±SE) calcium concentration in rice straw in each treatment. Bars with the same letters are not significantly different ($P < 0.05$) according to the Tukey– Kramer test.

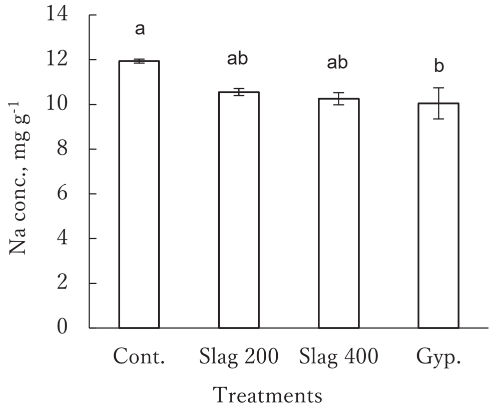

Fig. 5. Mean (±SE) sodium concentration in rice straw in each treatment. Bars with the same letters are not significantly different ($P < 0.05$) according to the Tukey– Kramer test.

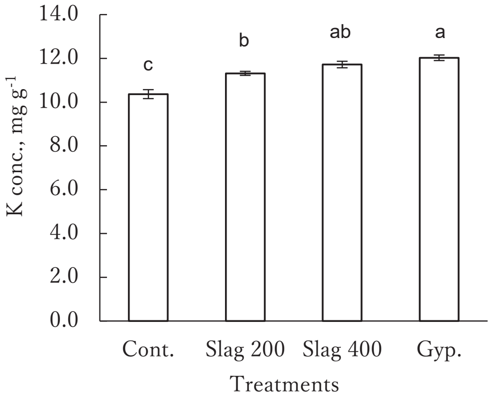

Fig. 6. Mean (±SE) potassium concentration in rice straw in each treatment. Bars with the same letters are not significantly different ($P < 0.05$) according to the Tukey– Kramer test.

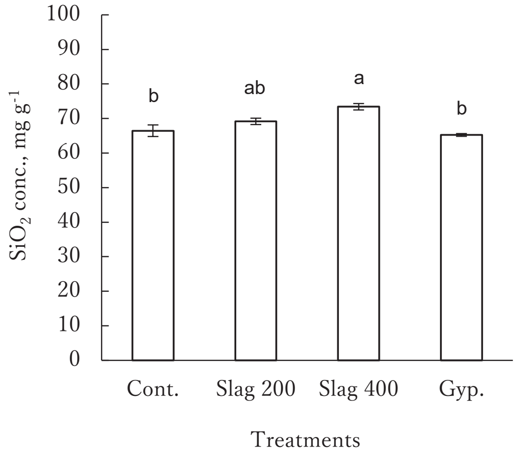

Fig. 7. Mean (±SE) silicate concentration in rice straw in each treatment. Bars with the same letters are not significantly different ($P < 0.05$) according to the Tukey– Kramer test.

as steel-making slag and gypsum suppress Na uptake and improve K nutrient condition of rice plant with accelerating Ca absorption.

It is known that Ca or Si applications can mitigate Na disorder of rice. Supplementation of Ca decreases Na absorption and increases K absorption (Khan *et al*., 1992; Song *et al*., 2006). Also, it is well known that silicate is positively taken up by rice and increase photosynthetic capacity and resistance to insects and salt resistance (Ma, 2004). Silicate is deposited on the rice leaves and suppresses unnecessary transpiration from the cuticle, resulting in improvement of water utilization efficiency and photosynthetic capacity. Furthermore, the improvement of the physical strength of leaves and stems by absorption of silicate is known to be effective to increase rice standing uprightness and the light receiving posture. That can result in improving photosynthetic capacity of plant. Moreover silicate application reduces Na uptake in rice and alleviates Na injury (Matoh *et al*, 1986; Gong *et al*., 2006). Steel-making slag and gypsum applications did not accelerate Na leaching from plow layer soils but increased the contents of plant available Ca (exchangeable Ca) in soils (**Table 1**).

It is concluded that the fertilizer made by steel-making slag is more effective in restoring the productivity of desalted tsunami-affected soils containing high amount of Na, compared with gypsum. Slag can supply Ca and Si effective in alleviating Na disorder of rice and accelerating rice growth, and does not have a risk of increasing hydrogen sulfide injury to rice roots other than gypsum.

Table 1. Apparent exchangeable cations in the soils at the beginning and end of the cultivation experiment

Timing/Treatment	Ca	Mg	K	Na	ESP[1]
Beginning	6.7	7.3	1.0	12	43.6
End					
Control	7.0	5.3	0.72	3.2	19.5
Slag 200	8.5	5.7	0.66	3.4	18.7
Slag 400	9.9	6.2	0.70	3.5	17.1
Gypsum	8.8	5.0	0.68	3.0	17.2

[1] Exchangeable sodium percentage (ESP) = ex. Na / (ex. Ca + ex. Mg + ex. K + ex. Na) × 100

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15th International Symposium on Integrated Field Science

“Biological Interactions for Sustainable Agriculture”

Date: March 13-15, 2018

Venue: Graduate School of Agricultural Science, Tohoku University (Aobayama campus), Sendai city, Miyagi, Japan

Sponsor and Organizer: Field Science Center, Graduate School of Agricultural Science, Tohoku University

Co-sponsors: International Education and Research Center for Food and Agricultural Immunology (CFAI), Applied Biodiversity Center, Graduate School of Agricultural Science, Tohoku University
Research Center for Fungal and Microbial Dynamism, Shinshu University

Corresponding organizer: Prof. Masarnori Saito
Laboratory of Environmental Crop Science
Field Science Center

Cover Photo: Spores of arbuscular mycorrhizal fungi
isolated from Lahar soil, Philippines (M. Saito)

Program

March 13 (Tue) (13:00 – 17:40)

11:00～		Registration
13:00	Makoto OSADA (Director of Field Science Center, Tohoku University)	Welcome address
13:10	Masanori SAITO (Field Science Center, Tohoku University)	Perspectives of the symposium

Part 1: Function and management of soil microorganisms in agro-ecosystems with special reference to arbuscular mycorrhizal fungi

13:20	Jan JANSA (Institute of Microbiology, Academy of Sciences of the Czech Republic)	Utilization of organic nitrogen by arbuscular mycorrhizal hyphae in soil - zooming into the hyphosphere microbiome
13:50	Zakaria M. SOLAIMAN (School of Agriculture and Environment, University of Western Australia)	Use of biochar for sustainable agriculture
14:20	Yoshihiro KOBAE (NARO Hokkaido Agric. Res. Cent.)	Toward the high-resolution functional analysis of arbuscular mycorrhizal symbiosis in field crops
14:50		Coffee break & Poster preview
15:20		Lightning talk for poster presentation
15:40	Baodong CHEN (Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences)	The role of arbuscular mycorrhizal symbiosis in plant adaptation to drought stress
16:10	Katsuharu SAITO (Shinshu University)	Molecular mechanisms underlying P translocation and metabolism in arbuscular mycorrhizal fungi
16:40	Turgut Yigit AKYOL (Graduate School of Life Science, Tohoku University)	Effect of Arbuscular Mycorrhizal Fungi Inoculation on the Root Fungal and Bacterial Communities of Bunching Onion
17:10	Daniel PÉREZ (Agrocode Bioscience S.L., Spain)	Application of the ultimate arbuscular mycorrhizal inoculant MYCOGEL® in Japan: results and prospects

*Welcome reception will be held in the evening on March 13.

March 14 (Wed) (9:30 – 13:00)

9:30		Poster presentation

Part 2: Frontiers of sustainable rice production system

10:30	Lixiao NIE (Huazhong Agricultural University)	The Possibility of Replacing Puddled Transplanted Flooded Rice with Direct-seeded rice in Central China: A review
11:00	Ryosuke TAJIMA (Field Science Center, Tohoku University)	Root phenotyping with root modeling: towards sustainable rice production
11:30	Sugihiro ANDO (Graduate School of Agricultural Science, Tohoku University)	Analysis of disease-suppression effect of microorganisms included in nursery soils for organic farming of rice
12:00	Toyoaki ITO (Field Science Center, Tohoku University)	Recovery of tsunami-affected paddy soil using calcium materials for sustainable agriculture
12:30	Masanori SAITO	Closing remark

March 15 (Thu)

Field trip (Nature and agriculture around Sendai city, Kawatabi Field Science Center)

Poster session

P1	Mengjia FENG, Chika TADA and Ryo SUGAWARA Methane fermentation of organic waste with different C/N ratios
P2	Ayako FUKUNAGA, Maki NISHIKAWA and Mari SASANUMA Effect of arbuscular mycorrhizal fungi application on Welsh Onion growth and yield
P3	Soh FURUYA, Chinatsu YONEZAWA, Naoki ISITSUKA and Shoichiro KOJIMA An Experimental Study of Crop Discrimination Using Pi-SAR2 Data
P4	Shintaro HARA and Masanori SAITO Release of inorganic phosphate from Ferric phytate by bacteria isolated from arbuscular mycorrhizal fungal hyphosphere
P5	Issei KAWAMURA, Daiki SAITO, Ayumi SADAIKE, Takayuki NAKAJIMA, Toyoaki ITO, Yoshihisa SUYAMA, Koki HOMMA Effect of mix cropping of determinate and indeterminate soybean lines on canopy structure in Kawatabi Field center in 2017
P6	Wataru MATSUZAKI, Toru UNO, Ryosuke TAJIMA, Masanori SAITO and Toyoaki ITO Environment-friendly Rice Cultivation with Reduction of Pesticide and Chemical Fertilizer Usage in Katsurao Village in Fukushima Prefecture, Japan
P7	Yuto NAKANO, Wataru MATSUZAKI, Toru UNO, Ryosuke TAJIMA, Masanori SAITO and Toyoaki ITO The effect of three major insecticides applied in nursery boxes on terrestrial arthropods in paddy fields of Miyagi Prefedture, Jpapan
P8	Kensuke OHSHIMA, Toru UNO, Ryosuke TAJIMA, Toyoaki ITO and Masanori SAITO Growth medium for seedling production of arbuscular mycorrhizal fungi-based cultivation of Welsh onion
P9	Cristiano Dela PICCOLLA, Etelvino Henrique NOVOTNY, Ryosuke TAJIMA and Masanori SAITO Effect of biochar pyrolysed at different temperatures on plant-AM fungi symbiosis in a soil with low phosphorus content
P10	Daiki SAITO, Momoko OGAWA, Issei KAWAMURA, Ayumi SADAIKE, Koki HOMMA, Takayuki NAKAJIMA, Toyoaki ITO and Yoshihisa SUYAMA Effect of Mix Cropping of Determinate and Indeterminate Lines on Sink-Source Balance in Soybean Grown in Kawatabi Field Center
P11	Risa SUEKI, Toru UNO, Ryosuke TAJIMA, Toyoaki ITO and Masanori SAITO The relationship between seedling quality and root system of rice seedling in organic farming analyzing with root modeling
P12	Kyoko SUGA, Toru UNO, Ryosuke TAJIMA, Toyoaki ITO and Masanori SAITO Analysis of differences in rice panicle structure between organic and conventional farmings using image analysis technique
P13	Takae SUZUKI, Toru UNO, Ryosuke TAJIMA, Toyoaki ITO and Masanori SAITO Optimum level of soil available phosphorus for AMF inoculation to Welsh onion in non-allophanic Andosol
P14	Toru UNO, Ryosuke TAJIMA, Toyoaki ITO and Masanori SAITO Effectiveness of winter-flooding in organic rice farming and some relating management practices
P15	Weiqin WANG, Ye TAO and Lixiao NIE Lower global warming potential and higher yield of wet direct-seeded rice in Central China
P16	Tomohiro WATANABE, Toru UNO, Ryosuke TAJIMA, Toyoaki ITO and Masanori SAITO The relationship between deep rooting and nitrate leaching of wheat in subsoil acidity
P17	Yumi MIURA and Chinatsu YONEZAWA Classification of polarimetric SAR imagery for forest extraction with airborne LiDAR data

1-1. Utilization of Organic Nitrogen by Arbuscular Mycorrhizal Hyphae in Soil - Zooming into the Hyphosphere Microbiome

Jan JANSA, Petra BUKOVSKÁ, Hana HRŠELOVÁ, and David PÜSCHEL

Institute of Microbiology, Czech Academy of Sciences, Videnska 1083, 14220 Praha 4, Czech Republic

Nitrogen (N) availability often limits growth and yield of crop plants. This is also why the utilization of synthetic N fertilizers (among other measures) during 20th century lead to unprecedented increases (multiplications) in yields, but also to great dependency of global agricultural production on fossil energy, and to widespread degradation of soil quality. To sustain primary production of agricultural systems in the future, it is important to re-design many aspects of agricultural land management, aiming at improving soil quality and fostering recycling of resources, particularly organic N in forms of green and farmyard manure, sewage sludge and various kinds of compost.

Arbuscular mycorrhizal (AM) fungi (recruiting from Glomeromycotina and Mucoromycotina) accompany plants since the beginning of their colonization of terrestrial environments, establishing intimate relationship with their roots, rhizoids and/or thalli, and being heavily implicated in plant acquisition of phosphorus (P) and micronutrients, and in plant carbon economy. They colonize roots of nearly three quarters of all plant species currently on Earth, including most of the agricultural crop plants (particularly cereals and most legumes) By extending their hyphae far away from the P depletion zone established around the roots, the AM hyphae greatly increase the volume of soil from which the plant acquire soil nutrients. The AM fungal hyphae also interact with soil particles, promoting soil aggregate stability and soil hydraulic properties, and with other soil microorganisms, while foraging for resources they require.

Particularly, the AM fungi need lots of mineral N to build their own biomass – for which purpose their genome contains genes coding for both ammonia and nitrate transporters, facilitating uptake of these N forms to the hyphae from the soil solution. Multiple studies also reported transport of N from the soil via AM fungal hyphae to their host plants, which may be important for plant N acquisition under some situations. Yet the AM fungi are apparently incapable of efficient biodegradation of biopolymers on their own. To utilize organic N, the AM fungi obviously have to build alliance with other saprotrophic microbes in soil, which are capable of organic N degradation (or at least cleaving the ammonia groups off from the organic moiety). Exact identities of such microbes and/or food chain members involved in release of N from organic sources and increasing mineral N availability to AM hyphae remains unclear as yet, however.

Our recent research showed consistent stimulation of AM hyphal proliferation in soil patches enriched with various organic N sources (plant litter, chitin, proteins) and efficient transport of N originating from such sources back to the host plant (*Andropogon gerardii*). Heterogeneous microbial communities are likely involved in primary degradation of the biopolymers, but there are strong indications that soil protists are the key functional group to liberate the N (originally supplied as soil organic amendment) from the microbial biomass and make it available as free ammonia ions to the AM fungal hyphae. Through efficient uptake of ammonia ions from the soil solution, the AM fungal hyphae indirectly suppress growth/activity of soil ammonia oxidizers and thus the entire nitrification pathway. The AM fungi could thus become an important component of future agricultural production systems, not only with respect to efficient P uptake by the plants, but also as facilitators of organic N recycling.

1-2. Use of Biochar for Sustainable Agriculture

Zakaria M. SOLAIMAN[1,2]

[1]SoilsWest, UWA School of Agriculture and Environment
[2]UWA Institute of Agriculture, The University of Western Australia
Email: zakaria.solaiman@uwa.edu.au

Biochar, the by-product of pyrolisis of organic biomass in an oxygen-free to oxygen-limited environment, is increasingly being investigated due to its potential benefits for soil health, crop yield, carbon (C) sequestration, and greenhouse gas (GHG) mitigation. Biochar research has progressed considerably with important key findings on agronomic benefits, C sequestration, greenhouse gas emissions, soil acidity, soil fertility, soil health, soil salinity, *etc.* However, more consideration is required before definitive recommendations can be made to farmers regarding the effects of biochar application across a range of soils, climates and land management practices. This review discusses the potential use of biochar in sustaianble agriculture for improving crop yields, soil fertility, and nutrient cycling, along with potential risks involved with biochar application and strategies to avoid these risks.

Biochar has potential to improve crop productivity mainly by increasing nutrient use efficiency and water holding capacity. However, improvements to crop production are often recorded in highly degraded and nutrient-poor soils, while its application to fertile and healthy soils does not always increase crop yield. Furthermore, as biochars are produced from a variety of feedstocks, certain contaminants can be present. Heavy metals in biochar may affect plant growth as well as rhizosphere microbial communities and functions.

Plant growth responses to biochar range from positive to negative according to biochar P concentration, available soil P and arbuscular mycorrhizal (AM) root colonisation. At higher the N concentration in biochar, lower levels of AM colonisation have been found. Growth responses of wheat and clover to application of the different biochars can be correlated, and biochar contributions to soil fertility can vary with biochar properties. We shown that the higher the nutrient (P and N) concentrations in biochar corresponded with lower AM colonisation. Production of biochar from good biomass sources at appropriate temperature is a prerequisite for its use as a soil amendment to improve soil health and plant growth.

The long-term effects of biochar on soil functions and its fate in different soil types require immediate attention. Biochar may change the soil biological community composition and abundance and retention of pesticides applied. As a consequence, weed control in biochar-amended soils may be difficult as pre-emergence herbicides may become less effective.

Innovative biochar use strategies can help mitigate greenhouse gases, while farmers get benefits of improved soils and crop production. However, a risk assessment is necessary to protect the food web and human health. At present biochar research is often fragmented and repetitive. New scientific evidence is not connected or implemented, due to the lack of interaction and knowledge exchange. National collaborative approches are needed that will focus on (i) biochar production and characterisation, (ii) potential for soil fertility improvement and crop production, (iii) economic analysis that includes life cycle assessment and (iv) environmental impact assessment. This approach should be used overcoming diverse soil constraints and recommendations for further research relating to biochar application to soil.

1-3. Toward the High-Resolution Functional Analysis of Arbuscular Mycorrhizal Symbiosis in Field Crops

Yoshihiro KOBAE

NARO Hokkaido Agric. Res. Cent.

Most field crops are generally colonized with a diverse species of arbuscular mycorrhizal fungi (AMF). Studies using a model AMF have shown that AMF colonization can improve plant nutrition and productivity. However, the functionality of mycorrhizas in the field likely depends on the combination of traits of the multiple AMF species that co-colonize the roots. Unfortunately, the colonization dynamics (lifecycle) of AMF, their genetic structure, and the role of individual AMF under field conditions have not been elucidated.

Despite significant advances in high-throughput sequencing techniques, the hidden intra-species or intra-isolate genetic diversity present in AMF has hampered the accurate delimitation and identification of AMF species. As is the case for many microbes, most field AMF are thought to be unculturable, indicating that most AMF species contributing to mycorrhizal functioning have not been characterized. To reveal the potential, new functionality of field AMF and utilize their biological value in agriculture, the species, genetics, and function of AMF should be clarified. Moreover, recent discoveries suggest that the colonization dynamics (lifecycle) of AMF in roots are related to their functionality from the cellular level to the ecological level. However, there is little data on the dynamic aspect of AM symbiosis in opaque soil.

The biology of AMF has yet to be elucidated. To advance our understanding of AMF functionality in the field, it is necessary to clarify the biological basis of AM symbiosis. We developed a live imaging technique for AM symbiosis that reveals the highly dynamic aspect of the short lifecycle of intracellular colonization (Kobae and Hata 2010; Kobae and Fujiwara 2014), which is an important step for resource exchange between the two symbionts. In addition, we recently developed a novel technique for analyzing the nucleotide information of individual AMF in roots in a culture-independent manner (Kobae et al. 2017). In this presentation, I will focus on the future direction of research on high-resolution functional analysis of this symbiosis in field crops.

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1-4. The Role of Arbuscular Mycorrhizal Symbiosis in Plant Adaptation to Drought Stress

Baodong CHEN[1,2], Tao LI[1], Lijiao XU[1,2], Xin ZHANG[1] and Zhipeng HAO[1]

[1]State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences,
Chinese Academy of Sciences, Beijing 100085, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China

It has been well demonstrated that arbuscular mycorrhizal (AM) symbiosis can improve plant drought tolerance. To reveal the relative importance of mycorrhizal fungi and root hairs in plant water relations, a bald root barley (brb) and its wild type (wt), were grown with or without inoculation of the AM fungus, *Rhizophagus intraradices* under well-watered or drought conditions, and plant physiological traits relevant to stress resistance were recorded. The experimental results indicated that AM fungi could almost compensate for the absence of root hairs under drought-stressed conditions. Moreover, our results confirmed that AM fungi could enhance plant drought tolerance by improving P uptake and plant-water relations, which subsequently promoted plant photosynthetic performance and growth, while root hairs presumably contributed to the improvement of plant growth and photosynthetic capacity through an increase of shoot P concentration.

In the following study, we cloned two full-length aquaporin genes, namely *GintAQPF1* and *GintAQPF2*, by rapid amplification of cDNA 5'- and 3'-ends from an AM fungus, *Glomus intraradices*. The expression of the two genes in arbuscule-enriched cortical cells and extraradical mycelia of maize roots was enhanced significantly under drought stress. Aquaporin localization, activities and water permeability were examined by heterologous expression in yeast. *GintAQPF1* and *GintAQPF2* are the first two functional aquaporin genes from AM fungus. Our data strongly support potential water transport via AM fungus to host plants.

In a split-root experiment, we further investigated the interactions between host plant and AM fungus under drought stress. Although mycorrhizal inoculation in either one or both compartments systemically decreased abscisic acid (ABA) content in the whole root system subjected to systemic or local drought stress, we observed local and/or systemic AM effects on root physiological traits and the expression of the functional genes in both roots and AM fungus *R. intraradices*. Interestingly, the simultaneous increase in the expression of plant genes encoding D-myo-inositol-3-phosphate synthase (IPS) and 14-3-3-like protein GF14 (14-3GF), which were responsible for ABA signal transduction, was found to be involved in the activation of 14-3-3 protein and aquaporins (GintAQPF1 and GintAQPF2) in *R. intraradices*. These findings suggest that co-expression of *IPS* and *14-3GF* is responsible for the crosstalk between maize plant and *R. intraradices* under drought stress.

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1-5. Molecular Mechanisms Underlying P Translocation and Metabolism in Arbuscular Mycorrhizal Fungi

Katsuharu SAITO

Faculty of Agriculture, Shinshu University

In soil, mineral concentrations are generally low and some minerals are unavailable for plants due to the formation of their precipitates or the fixation to soil particles. Plants have developed several strategies for mineral uptake from soil. For example, Plants secrete organic acids and enzymes to liberate available minerals from the precipitates and organic forms, and uptake soluble minerals via various transporters on plasma membrane. Other plant strategy for mineral uptake is the formation of symbiotic associations with fungi. Many land plants form symbiotic associations with arbuscular mycorrhizal (AM) fungi belonging to subphylum Glomeromycotina. Host plants can absorb minerals such as phosphate from soil via hyphal networks of AM fungi, which results in the improvement of plant nutrition and the increase of plant yield (Saito and Ezawa, 2016). The pathway of mineral uptake via AM symbiosis is called mycorrhizal pathway. On the other hand, the mineral uptake by plant roots from soil is known as the direct pathway.

The mycorrhizal effects on plant yield and P nutrition are obvious in the laboratory where growth conditions are well controlled. However, the mycorrhizal functions are not always effective in fields due to fluctuating environmental factors and the combinations of plant and fungal species. It is important to evaluate the mycorrhizal function in fields for utilizing AM symbiosis in agriculture using a diagnostic assessment for the activity of the mycorrhizal pathway. However, any procedure of the diagnostic assessment has not been developed because the mechanism underlying P uptake via mycorrhizal pathway remains unclear.

AM fungi uptake soil phosphate by phosphate transporters on plasma membrane in extraradical hyphae. Phosphate in hyphae is rapidly converted into polyphosphate, which then accumulates in fungal vacuoles and cell wall. Polyphosphate is translocated to intraradical hyphae and converted into short chain of polyphosphate. Phosphate liberated from polyphosphate is thought to be released from arbusculated hyphae into periarbuscular space.

We have investigated polyphosphate metabolism in AM fungi to elucidate molecular mechanisms underlying P transfer from AM fungi to the host plants. Several key genes in polyphosphate metabolism have been identified from both AM fungi and plant based on reverse genetics. Further, we analyzed gene expression profiles during AM fungal colonization using a next generation sequencing technology, and detected some candidate genes possibly involved in AM development and P metabolism (Handa et al., 2015). In this symposium, I will present our recent studies on P transfer between AM fungi and plants and discuss the development of a diagnostic assessment of mycorrhizal pathway in fields.

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1-6. Effect of Arbuscular Mycorrhizal Fungi Inoculation on the Root Fungal and Bacterial Communities of Bunching Onion

Turgut Yigit AKYOLI[1], Rieko NIWA[2], Hideki HIRAKAWA[3], Shigenobu YOSHIDA[2],
Tatsuhiro EZAWA[4] and Shusei SATO[1,3]

[1]Graduate School of Life Sciences, Tohoku University
[2]Central Region Agricultural Research Center, National Agriculture and Food Research Organization (NARO)
[3]Kazusa DNA Research Institute
[4]Graduate School of Agriculture, Hokkaido University

Root microbiota is crucial for plant health. Arbuscular mycorrhizal fungi (AMF) are important members of root microbial community as they provide several soil nutrients to the plant, especially phosphorus. AMF can be used as biofertilizers for sustainable agriculture. Bacteria are the third component of arbuscular mycorrhizal associations since it was shown that development and performance of AMF can be mediated by them. The interactions between AMF and bacteria can have important consequences in agriculture. Up to now, several reports have been published showing the effect of AMF inoculation on the bacterial and fungal communities. However, these studies were generally performed under highly controlled conditions and the techniques used in them have significant resolution limitations. Additionally, the majority of field inoculation efforts have been carried out in severely degraded soils or soils with very low inoculum potential. In this study, we used high-throughput sequencing to perform a combined investigation of root fungi and bacteria of bunching onion (*Allium fistulosum* L.) inoculated with a commercial AMF inoculum and grown under field conditions with native AMF communities. We found that the total abundance of the inoculated AMF varied among the fields and reduced with time. Plant age and inoculation had significant impacts on the root fungal and bacterial assemblages. The effect of inoculation was more pronounced on the early stages of plant growth. To our knowledge, this is the first study performed in large scale to reveal the effects of AMF inoculation on the root fungal and bacterial assemblages in soils with high inoculum potential.

1-7. Application of the Ultimate Arbuscular Mycorrhizal Inoculant MYCOGEL® in Japan: Results and Prospects

Daniel PÉREZ[1], María MARTÍN[1], Efrén REMESAL[1], Custodia CANO[2,3] and Alberto BAGO[2]*

[1]Agrocode Bioscience S.L., Almería (Spain)
[2]In vitro Mycorrhizas Lab, Dept. Soil Microbiology and Symbiotic Systems,
Estación Experimental del Zaidín (CSIC)Granada (Spain)
[3]MYCOVITRO S.L., Granada (Spain)

Arbuscular mycorrhizas (AM) are mutualistic symbioses occurring between the vast majority of land plant roots and a reduced group of soilborne fungi, the arbuscular mycorrhizal fungi (AMF). While colonizing the root, the fungus provides the plant of water and mineral and organic nutrients it acquires very efficiently from the soil, thus enhancing plant nutritional status and physiological equilibrium. Mycorrhizal colonization also promotes plant self-defence and protection against pests which, all in all, translates into a higher yield and healthier crop production.

However, the obligate symbiotic status of the fungus has hampered up to recently the large-scale production and application of AMF as inoculants. Conventional AMF inoculants consist of solid grain or powder substrates in which dormant spores of the fungus are mixed, usually in a low percentage and quite difficult to detect and verify for their vitality. These inoculants are difficult to apply homogeneously via watering systems, they are quite slow to induce mycorrhization and, what is most important, they usually contain non-desired microorganisms due to their non-in vitro production and formulation.

Here we present the first ultrapure, gel mycorrhizal inoculant in the world, MYCOGEL®, which is produced and commercialized in vitro to preserve all its quality and traceability from the lab to the field. The patented technology behind MYCOGEL®, as a result of more than 40 years of scientific research in Spain, allows it to contain not only dormant spores, but also infective hyphae and active mycorrhizal root pieces at a total amount of 50 x 10^6 propagules/L, a rate never seen before in AM technology. MYCOGEL® promotes a very quick and specific AM colonization of the plant, thus exerting all AM benefits from the beginning of the plant lifespan, to an enhanced fruit production in terms of amount and quality.

In a time where sustainability and biodiversity/environmental protection take an important place in any agricultural politics and practices, the use of MYCOGEL® is mandatory to cope these issues while maintaining and even increasing production with the highest standards of quality and efficacy. First results of the application of MYCOGEL® in production of rice, cherry-tree and tomato in Japan will be presented, and the results obtained, discussed.

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2-1. The Possibility of Replacing Puddled Transplanted Flooded Rice with Direct-Seeded Rice in Central China: A review

Lixiao NIE[1,2] and Weiqin WANG[1]

[1]MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River,
Huazhong Agricultural University
[2]Hubei Collaborative Innovation Center for Grain Industry, Yangtze University

Replacement of puddled transplanted rice (PTR) by direct-seeded rice (DSR) can potentially reduce consumption of resources and decrease emissions of greenhouse gases while maintaining grain yields in central China. However, direct seeding has not been widely adopted in this region. This review was undertaken to better understand the problems and opportunities for replacing PTR with DSR in central China. The seeding rate, crop growth, grain yield, water productivity, nitrogen use efficiency, greenhouse gas emissions and root development was compared between DSR and PTR. With good water management, grain yield of DSR is similar to or higher than yield of PTR while increasing irrigation water productivity and nitrogen use efficiency, reducing greenhouse gas emissions, and decreasing labor requirement. However, problems that include lodging, weak root development, weed infestations and poor crop establishment under drought, waterlogging, or chilling stresses might limit wide-scale adoption of DSR in central China. Varieties bred and selected for direct seeding, guidelines for improved nutrition, water, and weed management practices, and the development of suitable planting machines and sowing management for DSR are needed. In addition, incorporating DSR into the double season rice or ratoon rice systems could be promising strategies to increase rice production in central China.

Keywords: direct-seeded rice, grain yield, water productivity, nitrogen use efficiency, greenhouse gas emissions

2-2.

Root Phenotyping with Root Modeling: Towards Sustainable Rice Production

Ryosuke TAJIMA

Field Science Center, Tohoku University

Root system architecture (RSA) in rice production system has been shown to be important for resource acquisition, especially in the low-input conditions. Furthermore, improving RSA would have a potential to increase carbon stocks in the soil and, as a result, it would reduce atmospheric CO2 concentration without decreasing the yield. To design and develop RSA for the sustainable rice production, we need to understand the performance of RSA, i.e. the performance of individual root trait and their interactions in the actual field. However, it is extremely difficult even to determine root traits such as the root elongation rate of RSA in the field. In this presentation, two possibilities using the root model which simulates RSA on a computer are represented to understand the performance of RSA in the field.

First, it is to predict RSA in the field with the datasets of RSA in the early growth stage of rice plants in the lab-based experiments. Some lab-based experiments, such as paper or soil cultures against transparent plates, allow for non-destructive and time-lapse observations on individual plants. These experiments provide the datasets with analyzing two-dimensional images that are taken with the camera or the scanner. Running root model with the datasets from the lab-based experiments, RSA in the field could be predicted even at the end of the growth season, though there are concerns that the conditions with lab-based experiments don't simulate the field, e.g. the observed roots that grow against plates could not be same to that in the soil.

Second, it is to use several easy-measurable root traits in the field to predict the whole of RSA. Several root traits, such as the number of nodal roots in rice plants, could be easily identified from the observations around the root base. In addition, with the partial data of vertical root distribution that is taken in old but standard methods such as trench excavations and soil cores from the soil for the validation, more realistic RSA could be predicted. The vertical root distribution could exist as the legacy data since they have been taken for decades. We also would be able to use them for the validation.

Developing a mutually complementary relationship between root phenotyping and modeling should accelerate to propose a groundbreaking RSA ideotype and contribute RSA improvement for boosting up rice production and reducing environmental impacts in the future.

2-3. Analysis of Disease-Suppression Effect of Microorganisms Included in Nursery Soils for Organic Farming of Rice

Sugihiro ANDO and Hideki TAKAHASHI

Graduate School of Agricultural Science, Tohoku University

In organic farming systems, diseases are controlled without using agricultural chemicals. Since serious losses of yield occur in conventional farming without agrochemicals, we postulated that there are several unknown mechanisms underlying disease suppression in organic agriculture. In general, microorganisms in soil are important for crop cultivation. Therefore, we analyzed the disease-suppressive effect of soils from organic farms and characterized the microorganisms present in the soils. We found that nursery soils from organic rice farms in Japan had a disease-suppressive effect on rice seedling rot caused by *Burkholderia glumae*, *B. plantarii* and seedling blast (Ando et al., 2014). We isolated disease-suppressive bacteria and fungi from the soils. Application of the *Pseudomonas* sp. W6 and Y3 strains isolated from nursery soil suppressed the seedling rot caused by *B. glumae* and enhanced ethylene production, suggesting that the bacteria activated the ethylene-dependent plant immune system.

We also performed a microbiome analysis to compare organic farming nursery soils with those from conventional farms, and found that richness and diversity were higher in organic farm soils. The bacterial populations in organic farm soils were robust to irrigation and seedling growth. These findings suggest that a robust bacterial population contributes to suppression of the proliferation of bacterial pathogens (Takahashi et al., 2018).

Finally, we attempted to develop a new disease suppression technique using bacterial communities cultured from organic nursery soils. We cultured bacterial mixtures from organic soils on nutrient agar and potato peptone glucose agar and examined their disease-suppressive effects on bacterial panicle rice blight in rice grown in conventional nursery soil. The bacterial mixtures from some organic farm soils exhibited disease-suppressive activity. The disease-suppressive effect tended to be strong when diluted medium (1/1,000) was used for bacterial culture. We also monitored the changes in soil bacterial populations using polymerase chain reaction denaturing gradient gel electrophoresis analysis of 16S rDNA after adding the bacterial mixture to conventional nursery soil, and found that a stable bacterial community was related to disease suppression. These findings suggest that a robust bacterial community was related to the disease-suppression activity. Therefore, it is possible that a disease suppression system similar to the nursery soil of organic farm will be effective when a cultured bacterial mixture is applied to conventional nursery soil. Our findings could inform new strategies for the biological control of plant diseases using culturable microbe communities from disease-suppressive soils.

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2-4. Recovery of Tsunami-Affected Paddy Soil Using Calcium Materials for Sustainable Agriculture

Toyoaki ITO, Hisashi NASUKAWA, Toru UNO, Ryousuke TAJIMA and Masanori SAITO

Graduate School of Agricultural Science, Tohoku University

Many lives were lost and tremendous damage was caused by the Great East Japan Earthquake and tsunami disaster of March 11, 2011. The tsunami attacked the regions on Pacific coast and caused severe damage to the lowland farmlands by topsoil washout and salt injury. About 15,000 ha of paddy fields had been damaged in Miyagi Prefecture alone. It was found that water soluble salt content in the topsoil decreased to less than the acceptable limits by around 1000 mm of natural rainfall. In most of the tsunami-affected farmland, desalinization work was carried out by irrigation, resulting in sufficient removal of water-soluble salt content of the tsunami-affected soils. However, according to the monitoring surveys of the soil where the land consolidation projects had been completed, some fields had a poor nutrient balance. Some of the calcium (Ca) ion absorbed by negative charge sites of soils had been displaced by sodium (Na) ion derived from seawater and washed out by the desalinization process. As a result of the reactions in the soil, some of Na ion had remained in the exchange sites of soils and exchangeable Ca had been reduced. In soils with high concentration of exchangeable Na, crops sometimes show poor growth due to the excess uptake of Na (Na injury). In order to lower the risk of Na disorders and restore soil productivity, it is necessary to optimize the basic cation balance in the soils where desalinization has been implemented.

To solve the problem, we examined the effectiveness of applying calcium-silicate materials (steel-making slag fertilizers) in alleviating Na disorders using model desalinated tsunami-affected soil in 2013. An alluvial soil collected from normal paddy field was submerged by seawater and was then desalinated by flooding and draining repeatedly fresh water. The prepared soil showed EC of 0.42 dS m^{-1}, exchangeable Ca of 6.7 cmol(+) kg^{-1} and exchangeable Na percentage of 44 %. Rice cultivation experiment using *Hitomebore* was conducted in the paddy field with three replications. We stuffed a plastic frame of 0.076 m^2 with the prepared soil after four treatments (no application of material, steel-making slag fertilizer application of 200, 400 g m^{-2}, gypsum application corresponding to slag 200 g m^{-2} as application amount of Ca).

Gypsum and two slag treatments increased brown rice yields by 4, 10, 12 % than the control treatment, respectively, with significant difference for slag treatment of 400 g m^{-2}. steel-making slag fertilizers enriched concentrations of Ca, K and Si in the rice plants and reduced Na concentration at the maturity stage. It is known that Ca or Si applications can improve Na injury of rice. Supplementation of Ca decreases Na absorption and increases K adsorption (Khan et al., 1992; Song et al.) It is known that silicon application reduces Na uptake and improves rice growth (Yeo et al., 1999). It is concluded that the fertilizer made of steelmaking slag is effective in restoring the productivity of desalted tsunami-affected soils containing high amount of Na.

p1. Methane Fermentation of Organic Waste with Different C/N Ratios

Mengjia FENG [1], Chika TADA[1] and Ryo SUGAWARA[2*]

[1]Graduate School of Agricultural Science, Tohoku University
[2]Japan Organics Recycling Association
*Corresponding author

There is interest in using methane fermentation to convert the non-edible parts of agricultural crops and the waste discharged from food processing into biogas. However, organic waste derived from agricultural crops is obtained seasonally and regionally, making it difficult to obtain raw materials consistently throughout the year. In addition, using the organic waste discharged from a single establishment may result in an inappropriate C/N ratio depending on the waste composition. Therefore, this study examined the methane gasification efficiency of organic waste with different C/N ratios.

For Methane fermentation of mixed organic waste with C/N ratio of 25, (1) the methane production was about 1.3 times higher than with a single organic waste with a C/N ratio of 52 and (2) the pH was stable at 6.5-7.3 throughout the experiment, which is within the optimum range of methane fermentation. Using the waste with a C/N ratio of 52, the pH fell to 5.9 and frequent pH adjustment by adding alkali was necessary to maintain continuous methane fermentation. (3) The concentration of volatile fatty acids decreased in the mixed organic waste with the C/N ratio of 25, while organic acid accumulated in the waste with a C/N ratio of 52.

Therefore, for stable, efficient methane fermentation of organic waste, it is desirable to mix organic waste and adjust the C/N ratio to 25. Instead of fermenting methane at one business site, it is better to ferment methane in a joint facility using waste from several businesses that produce different kinds of organic waste to be able to adjust the organic waste to an appropriate C/N ratio.

p2. Effect of Arbuscular Mycorrhizal Fungi Application on Welsh Onion Growth and Yield

Ayako FUKUNAGA, Maki NISHIKAWA and Mari SASANUMA

INARO Western Region Agricultural Research Center

Arbuscular mycorrhizal fungi are expected to reduce the need for phosphate fertilizers. Our previous study on the effects of arbuscular mycorrhizal fungi inoculation on soybean and Welsh onion (Allium fistulosum L. ‘Motokura’) showed that there were no growth promoting effects of the inoculation on soybean in the field; however, the inoculation increased Welsh onion growth and yield. Therefore, in the present study, we conducted further field inoculation experiments in Welsh onion. Welsh onion was sown with the inoculum R10 (Idemitsu Kosan Co., Japan) in nursery beds without indigenous arbuscular mycorrhizal fungi. Phosphate fertilizer was used only in the non-inoculated nursery beds. Six-week-old seedlings were transplanted to fields and cultivated for 4 months. We used two fields located in Kyoto and Shiga, in Japan. Soil textures in both fields are Andosols. In the field, the test plants were grown using an experimental design with two treatments (inoculation and non-inoculation) and two phosphate fertilizer levels (0 and 20 kg per 10a) with 4 repetitions (blocks).

The inoculated seedlings showed poor growth before transplantation because of the lack of phosphate fertilizer. However, after transplantation, in the Kyoto field, the inoculated seedlings showed rapid growth and their fresh weight was higher than that of the non-inoculated seedlings on the 35th day of transplantation and at the time of harvest under the lower phosphate fertilization level. In the Shiga field, the difference in seedling growth tended to decrease as cultivation progressed, but the yield did not differ between the inoculation and non-inoculation treatments under either fertilization level.

These results suggest that arbuscular mycorrhizal fungi inoculation improves the growth of Welsh onion under field conditions. However, some issues hinder the practical application of arbuscular mycorrhizal fungi inoculation in the field. For example, the optimal method for inoculation in nursery beds, which would allow for mechanical transplantation of seedlings, needs to be determined.

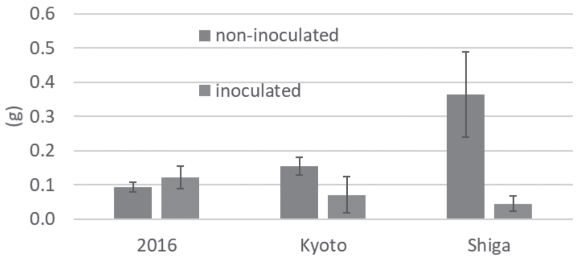

Fig.1 fresh weight of seedling at 6weeks

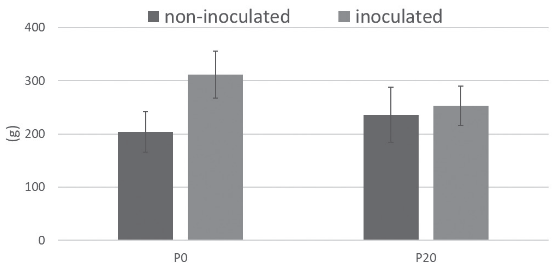

Fig.2 Fresh weight of shoots(25weeks after transplanting) in Kyoto field

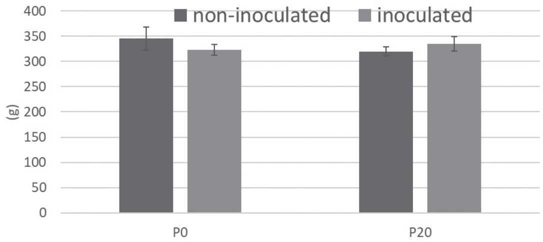

Fig.3 Fresh weight of shoots(26weeks after transplanting) in Shiga field

p3. An Experimental Study of Crop Discrimination Using Pi-SAR2 Data

Soh FURUYA[1], Chinatsu YONEZAWA[1], Naoki ISHITSUKA[2] and Shoichiro KOJIMA[3]

[1]Graduate School of Agricultural Science, Tohoku University, Japan
[2]National Agricultural and Food Research Organization, Japan
[3]National Institute of Information and Communications Technology, Japan

Crop discrimination in agricultural field using remote sensing data is expected for food security management. Synthetic Aperture Radar (SAR) would play a powerful role of remote sensing instrument, because it enables us to obtain high-resolution imagery under all weather conditions. Polarimeric SAR has potential to detect differences of surface characteristics and properties. In this study, the possibility of crop discrimination using air-borne polarimetric X-band SAR was examined.

The airborne X-band SAR, Pi-SAR2 data acquired in full polarimetric mode in August 2013 and 2014 were analyzed in order to discriminate agricultural crop types. The target area was an agricultural field in Field Science Center, Tohoku University located in Osaki-shi, Miyagi-ken. Yamaguchi four-component decomposition method was applied to the Pi-SAR2 data to decompose the data into scattering mechanisms. We also computed polarimetric parameters as scattering entropy, anisotropy and alpha angle using Cloude-Pottier eigenvalue/eigenvector decomposition. An imagery obtained from high resolution satellite, Worldview-2, was used to make a vector data set of agricultural percels. This vector data was overlaid on the decomposition images of Pi-SAR2 data, and the mean value for each agricutural parcel was computed.

By visual interpretation of four-component decomposition images, agricultural parcels in cultivated dent corn and rye were discriminated from other parcels. It is difficult to distinguish parcels of paddy rice from that of grass plant. There are several grass plant parcels, and some of them showed different scattering characteristics from others. These results suggest the posibility of the crop discrimination using Pi-SAR2 data.

p4. Release of Inorganic Phosphate from Ferric Phytate by Bacteria Isolated from Arbuscular Mycorrhizal Fungal Hyphosphere

Shintaro HARA[1#] and Masanori SAITO[1]

[1] Graduate School of Agricultural Science, Tohoku University
[#]present address: Graduate School of Life Sciences, Tohoku University

Phytic acid, *myo*-Inositol hexakisphosphate (IHP), is one of the predominant forms of organic P in soil, and most IHP in soil is derived from plant residues. However, IHP in acidic soil forms insoluble mineral complexes with Fe, and Al. Thus, it is not yet well understood how Fe-, and Al-IHP is mineralized and becomes available to plants. Recently, we confirmed P transfer from Fe-IHP via arbuscular mycorrhizal fungi to host plant, suggesting that Fe-IHP may be degraded by microbes (Hara et al., 2016). Furthermore, we isolated IHP utilizing bacteria around alginate beads containing Fe-IHP buried in arbusuclar mycorrhizal hyphosphere and found that major isolates were *Sphingomonas* spp. and *Arthrobacter* spp. In this study, we evaluated ability of these isolated IHP utilizing strains to liberate inorganic P (Pi) from Fe-IHP .

Total 8 strains of *Arthrobacter* spp., *Caulobacter* spp. and *Sphingomonas* spp., which were isolated from alginate beads containing Fe-, or Ca-IHP buried in soil in previous study (Hara et al., 2016), were used. The Fe-IHP degrading activity was estimated by release of Pi in a phytate-specific limiting medium (Richardson et al., 1997) which contained 1 mM Fe-IHP as the sole P source. Test strains were grown in 10 mL of the phytate-specific limiting medium with horizontal shaking (150 rpm) at 25°C for 1 week. Release of Pi to the medium was examined. The concentration of Pi was measured by the malachite green method.

Four strains, 2 strains of *Arthrobacter* spp. and 2 strains of *Sphingomona*s spp., which were isolated from the alginate beads containg Fe-IHP showed significantly higher Pi concentration in the medium than that without inoculation, indicating release of Pi from Fe-IHP. Amount of released Pi was 2.9 mg Pi L^{-1} in *Arthrobacter* sp. FeAH19, and that in *Sphingomonas* sp. FeGH1 was 1.4 mg Pi L^{-1}. On the other hand, other 4 strains, 2 strains of *Caulobacter* sp. and 2 strains of *Sphingomonas* spp., which were isolated from the alginated beads containing Ca-IHP did not show release of Pi from Fe-IHP. Because these strains can release inorganic P from soluble IHP, such as Ca-IHP and Na-IHP, removal of Fe from Fe-IHP may be a key step of Fe-IHP degradation.

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p5. Effect of Mix Cropping of Determinate and Indeterminate Soybean Lines on Canopy Structure in Kawatabi Field Center in 2017

Issei KAWAMURA[1], Daiki SAITO[1], Ayumi SADAIKE[1], Takayuki NAKAJIMA[1],Toyoaki ITO[1],
Yoshihisa SUYAMA[1,2] and Koki HOMMA[1,2]

[1]Graduate School of Agricultural Science, Tohoku University, Japan
[2]Applied Biodiversity Center, Graduate Tohoku University, Japan

Soybean (*Glycine max* (L.) Merr.) is generally divided into two types by stem growth habit: determinate type (DET) and indeterminate type (IND). IND has longer stem growth period, which ordinary produce more nodes and foliage, and increase yield potential. IND cultivars are recognized as one of the reasons to attain higher yield in the Midwest in USA. However, the higher yield by IND is rarely achieved in Japan because of excess foliage and lodging. The authors have tested mix cropping of IND with DET to incorporate higher yield potential of IND into soybean production in japan where DET cultivars are commonly used.

Sadaike et al. (2017) reported that mix cropping of IND and DET from recombinant hetero lines, Kariko1222, had a slightly positive effect on land equivalent ratio, but its effect on yield was negligible. Ogawa et al. (2017) showed that IND had larger leaf area index (LAI) in mix cropping, suppressing LAI of DET. The change in LAI might imply that IND has better environment in terms of solar radiation interception, but the productivity per LAI in IND was not improved by mix cropping. The fact suggest that evaluation of solar radiation interception and its use efficiency is the key to incorporate higher yield potential of IND to DET canopy.

The authors continued the experimental trial of mix cropping of DET and IND in 2017 and measured vertical distribution of LAI. The measurement was conducted in 10 cm-vertical interval by plant canopy analyzer (LI2200C, Li-Cor) together with 1 m-long sensor of solar radiation at three times (August 3, 14 and 30, 2017).

The measurement showed that difference between mix cropping and solo cropping of IND was obvious at 60-100 cm-height from soil surface: LAI in solo cropping of IND was higher than that in mix cropping. The effect of difference on canopy structure, solar radiation interception and productivity will be analyzed further.

p6. Environment-Friendly Rice Cultivation with Reduction of Pesticide and Chemical Fertilizer Usage in Katsurao Village in Fukushima Prefecture, Japan

Wataru MATSUZAKI, Toru UNO, Ryosuke TAJIMA, Masanori SAITO and Toyoaki ITO

Graduate School of Agricultural Science, Tohoku University

Agriculture of Fukushima prefecture was seriously damaged by the Fukushima first nuclear power plant accident in 2011. It was equal Paddy rice cultivation was forbidden for five years after the accident also in Katsurao village in Fukushima. However, in 2017, the prohibition of rice production was withdrawn in a wide area of Katsurao village. To introduce environmentally conscious rice cultivation will be effective in restoring rice agriculture in Katsurao Village because environmental conservation may be valuable for raising agricultural products value and farmer's income. Then, in this study, we conducted environmentally conscious rice cultivation with reduction of pesticide and chemical fertilizer (ECRC) in Katsurao village and compared the rice yields of ECRC and conventional rice cultivation (CRC).

We investigated four pairs of paddy fields including ECRC and CRC fields (A, B, C, D sites). Rice varieties were 'Hitomebore' at three sites and 'Satoyamanotubu' at D site. For fertilization, mixed fertilizer containing 50% chemical fertilizer and 50% organic fertilizer and chemical fertilizer were used in ECRC and CRC fields, respectively with nitrogen application rate of 7 kg/10a. Pesticides were applied according to local conventional system in CRC fields. On the other hand, one (active ingredient: thiamethoxam) of the insecticides used in the conventional treatment was not applied in ECRC fields. During cultivation period, we surveyed rates of leaves damaged by rice water weevils (*Lissorhoptrus oryzophilus*) and rice leaf beetles (*Oulema oryzae*), that occurred in the early rice growth season. We investigated brown rice yield and yield components. The leaf rates damaged by the two insects did not differ significantly between different cultivation systems (n = 4). Reduction of insecticide did not enlarge plant damage suffered by insect pests. Brown rice yields of ECRC increased by 9% and 3% compared with CRC in A and B sites, respectively. In contrast, they decreased by 7% and 31% in C and D sites, respectively. For yield components, panicle numbers per unit area decreased remarkably in C site (12.4%) and D site (19.1%). In D site, lower amounts of soil organic matter (low nitrogen fertility) may result in decreasing yield for ECRC field. In ECRC system, half of applied nitrogen fertilizer was organic fertilizer and its mineralization proceeded slowly and imperfectly. However, brown rice yields and yield components were not significantly different between two cultivation systems (one-way AOV, n = 4). Consequently, in semi-mountainous areas in Fukushima prefecture (Katsurao village), environmental conscious rice cultivation system, including reduction of chemical fertilizer and insecticide, may be able to produce rice yields comparable to conventional system.

p7. The Effect of Three Major Insecticides Applied in Nursery Boxes on Terrestrial Arthropods in Paddy Fields of Miyagi Prefecture, Japan

Yuto NAKANO, Wataru MATSUZAKI, Toru UNO, Ryosuke TAJIMA,
Masanori SAITO and Toyoaki ITO

The Kawatabi Field Science Center, Graduate School of Agricultural Science, Tohoku University

It is obvious that environmental conservation is also essential for agriculture because degradation of global environment and ecology is in progress. Reduction of agrochemicals is essential for environment-friendly agriculture which emphasizes the conservation of biodiversity. Nowadays, it is a general method to add insecticides to a nursery box soil in order to control harmful insects occurring in the early growth stage of rice in Japan. However, the effects of insecticides applied in spring on harmful insect and the other insects or arthropods are not fully clarified in paddy fields through rice growing period. The objective of this study is to investigate the influence of major insecticides added to nursery boxes on the densities of arthropods including herbivore insects in paddy fields through rice growing season.

Research was conducted in the paddy fields of Field Science Center, Tohoku University (Osaki city, Miyagi Prefecture, Japan) from June to August in 2017. We arranged two sets of paddy fields (about 3000 m^2 per each) with different insecticide treatments with two replications. One set had three treatments including application of insecticides (chlorantraniliprole or thiamethoxam) and no insecticide (control), another set included the fields with and without cyantraniliprole. We researched the ratio of leaves injured by the rice water weevil (*Lissorhoptrus oryzophilus*), which is one of the representative pests in the rice fields, and the abundance of organisms were investigated by the methods of sweeping using a sweep net and visual observation.

The percentage of leaves injured by rice water weevils in June was 0.22 % and 0.04 % in the fields treated with chlorantraniliprole and thiamethoxam, respectively, in contrast to 4.3 % in the control, and 0.07 % in the field treated with cyantraniliprole (0.74 % in the control). Furthermore, according to the survey conducted at August 1st and 28th no rice weevils were found in the fields applied with every insecticide. On the contrary, we found many rice water weevils in the control field. The result indicates the effectiveness of the insecticides applied in nursery boxes in controlling the pest. There were the tendencies for greater abundance of Araneae, Chironomidae, and Hymenoptera at August 1st and 28th and Zygoptera at July 21st in the control field than in the field treated with cyantraniliprole.

p8. Growth Medium for Seedling Production of Arbuscular Mycorrhizal Fungi-Based Cultivation of Welsh Onion

Kensuke OHSHIMA[1], Toru UNO[2], Ryosuke TAJIMA[2], Toyoaki ITO[2] and Masanori SAITO[2,3]

[1]Faculty of Agriculture, Tohoku University
[2]Graduate School of Agricultural Science, Tohoku University
[3]Japan Science and Technology Agency

It has been demonstrated that pre-inoculation of Welsh onion (*Allium fistulosum* L.) seedlings with arbuscular mycorrhizal fungi (AMF) is effective to improve its growth in field after transplantation, to increase P uptake, and to reduce P fertilizer (Tawaraya et al. 2012; Suzuki et al. 2015, 2017).

Growth medium containing organic material like peat moss is widely used for seedling production of Welsh onion. However, such organic-based medium has been considered to suppress AMF colonization (Saito, personal communication). In the above literatures, therefore, andosol-based medium was used for seedling production, and its physicochemical properties was not so optimum in terms of workability and seedling growth. In this study, we aimed at development of organic-based growth medium for AMF-based cultivation of Welsh onion.

Basic andosol-based medium was composed of an equal volume of non-allophonic Andosol, zeolite, and “Akadama” soil. It was blended with organic amendments such as peat moss, coconut fiber, composted bark, and chaff charcoal at 0%, 10%, 30%, 50%, or 100%, respectively, and some physiochemical properties of them were examined. Based on these analyses, 9 kinds of medium were selected for further experiments. 4 kinds of organic amendments were added to the basic andosol-based medium at 0%, 30%, and 50%, respectively. Commercial AMF inoculum, *Glomus* sp. R-10, was added to the mixed medium at rate of 5%. Welsh onion was grown in these medium in a growth chamber for 60 days. Non-inoculated plants ware also grown as control. Growth parameters of Welsh onion, AMF colonization, N and P concentration in plant tissues were examined.

As a result, there were no significant differences between basic andosol-based medium and almost all organic medium in terms of AMF colonization and growth promotion effects. Mycorrhizal responses of some organic medium were better than inorganic medium. Under high P conditions (1,000 mg P_2O_5/L dry soil), positive mycorrhizal responses were found. Thus, the organic medium presented in this study is effective for seedling production of AMF-based cultivation of Welsh onion.

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p9. Effect of Biochar Pyrolysed at Different Temperatures on Plant-AM Fungi Symbiosis in a Soil with Low Phosphorus Content

Cristiano Dela PICCOLLA[1], Etelvino Henrique NOVOTNY[2], Ryosuke TAJIMA[3] and Masanori SAITO[3]

[1]Graduate school of Soil and Plant Nutrition, Center of Nuclear Energy in Agriculture, University of Sao Paulo
[2]EMBRAPA Soils, Brazil
[3]Field Science Center, Graduate School of Agricultural Science, Tohoku University

Phosphorus low content in soils located in the tropics limits crop yield, principally when below optimum fertilizer doses are applied. One way for plants to overcome phosphorus deficiency is through the establishment of a symbiotic interaction with arbuscular mycorrhizal fungi (AMF), which are organisms ubiquitous in soils. However, plant colonization with AMF is not abundant and beneficial for plant growth at all times, but certainly could be enhanced through soil management practices such as addition of organic material and small amounts of nutrients. Biochar, a product obtained by pyrolysis of organic materials has higher stability in soils than vegetal wastes and contains soluble nutrients in its ash fraction. Therefore, biochar could be employed to assist AMF symbiosis, plant nutrient uptake and growth.

Our objective was to evaluate the effect of addition of two contrasting biochars to soil on plant-AMF symbiosis, plant growth and nutrient uptake. Biochars produced from *Eucalyptus* wood chips pyrolysis at 300 and 700 °C were applied to a red soil from Okinawa, Japan. The soil was autoclaved and inoculated with AM fungi, *Glomus* sp. R10. A dwarf cultivar of sorghum was cultivated for 4 and 8 weeks in growth chamber. Additionally, spore germination of AMF fungus, *Gigaspora margarita*, was evaluated in two germination media (soil and sand) in order to verify the effect of biochar in media with contrasting buffer capacity.

Biochar produced at 700 °C increased soil pH and diminished toxic aluminum in the pot experiment, which augmented plant root growth through formation of longer and thinner roots. Ameliorated root system increased plant colonization by AMF (symbiosis) and consequently phosphorus uptake. We attributed lack of effect of biochar 300 °C on plant symbiosis and growth to spore germination inhibition verified for this biochar. Conversely, 700 °C biochar increased hyphae length in germinated spores.

p10. Effect of Mix Cropping of Determinate and Indeterminate Lines on Sink-Source Balance in Soybean Grown in Kawatabi Field Center

Daiki SAITO[1], Momoko OGAWA[1], Issei KAWAMURA[1], Ayumi SADAIKE[1], Koki HOMMA[1,2],
Takayuki NAKAJIMA[1], Toyoaki ITO[1,2] and Yoshihisa SUYAMA[1,2]

[1]Graduate School of Agricultural Science, Tohoku University, Japan
[2]Applied Biodiversity Center, Graduate School of Agricultural Science, Tohoku University

Stem growth habit of soybeans (*Glycine max* (L.) Merr.) are generally classified into determinate type (DET) and indeterminate type (IND). DET is commonly planted in Japan, while IND is planted in Midwest U.S. and contributes to attain higher yield. IND has longer vegetative growth period than DET, generally producing larger amount of leaves which associate higher productivity. They also associate stronger stability to compensate damages by insects and weather.

The introduction of IND to soybean cultivation in Japan has been tested in many studies, but they often showed no yield advantage because of lodging or excess foliage (e.g. Chonan et al., 2016). To regulate such disadvantages the authors tried mix cropping of DET and IND in Kawatabi Field Center since 2016. Sadaike et al. (2017) reported that the mix cropping showed a positive effect in terms of land equivalent ratio, but the effect on yield was negligible. To improve the productivity, ana;ysis for effect of mix cropping on crop growth and yield formation would be necessary.

This study focussed on sink-source balance in soybean to evaluate the balance of yield formation and crop growth. For the purpose, grain weight was measured as sink capacity and leaf area was measured as source capacity. The balance was evaluated at each node, and the effect of mix cropping on the balance was analyzed.

DET and IND derived from ‘Kariko 1222’ which are ‘Ohsuzu’×’Athow’ recombinant hetero lines were used. They planted each in sole cropping, and both in mix cropping (replacement arrangement) in a randomized block design with three replications. The dates of sowing were 13 June 2017. Measurements of leaf area were made at full bloom period (R2) and beginning seed period (R5). Harvested at full maturity period (R8) and measured grain weight. Some of results will be shown in the presentation.

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p11. The Relationship Between Seedling Quality and Root System of Rice Seedling in Organic Farming Analyzing with Root Modeling

Risa SUEKI, Toru UNO, Ryosuke TAJIMA, Toyoaki ITO and Masanori SAITO

Faculty of Agriculture, Tohoku University

Organic farming, one of the leading sustainable agricultural systems, has recently been focused. In organic rice farming, it is crucial to enhance seedling quality since the seedling quality affects the grain yield through plant growth in paddy field. In the rice nursery, root system could be the important part in relation to nutrient uptake until transplanting and the seedling establishment after transplanting. However, there are few studies about root system in organic nursery. In this study, we investigated the rice seedling including root system of organic rice seedling with root model compared to the conventional seedling.

At first, we investigated the various shoot and root traits of organic rice (var. Hitomebore) seedling compared to the conventional seedling in the actual nursery on Kawatabi Field Science Center. Next, we set up the experiment that rice plants were grown with four different plant densities (10,14,19,23 plants/pot simulating 35, 50, 65, 80 g grain/nursery box, respectively) in the small pots witch simulated organic nursery. We also measured the shoot and root traits of seedling in this experiment. Using the root model with the datasets of these experiments for the validation, we predicted the root length density and nitrogen uptake throughout rice growth in the nursery.

The shoot dry weight per plant height, which is the index of seedling quality, is significantly higher in the organic nursery than in the conventional nursery. Both total root length and nodal root number are also significantly greater in the organic nursery than in the conventional nursery. These results suggested that root system could relate to seedling quality. In the pot experiments with the different plant densities, the shoot and root traits were no significant differences. The low seedling density could have minor effects in seedling on the organic nursery. Using root model, we predicted that nitrogen uptake per plant and root length density throughout plant growth in the organic nursery were greater than in the conventional nursery because of the condition of fertilizer and plant density. The rice seedling of organic farming could have better nitrogen status and more apposite root length density than that of conventional farming. In sensitivity analysis which changed plant density in root model, seeding 40-60 g grain per nursery box was most appropriate in organic farming. These estimates could lead to the capacity development of organic rice farming.

p12. Analysis of Differences in Rice Panicle Structure Between Organic and Conventional Farmings Using Image Analysis Technique

Kyoko SUGA, Toru UNO, Ryosuke TAJIMA, Toyoaki ITO and Masanori SAITO

Faculty of Agriculture, Tohoku University

In organic farming, the panicle number is usually smaller than in conventional farming. Therefore, in order to heighten yield in organic farming, it is important to increase the spikelet number per panicle and the ripening rate. For analyzing these two yield components in detail, we focused on analyzing the panicle structure that is how each spikelet on the panicle is formed and ripe. In this study, we developed the panicle structure analysis using the image analysis technique and applied the technique to analyze differences between organic and conventional rice production.

We cultivated rice plants in organic and conventional paddy fields, Kawatabi Field Science Center, Tohoku University. We investigated the tiller number and plant height as crop growth and, at harvest, yield and four yield components: the panicle number, the spikelet number per panicle, the ripening rate and grain weight. At harvest, we also investigated the panicle structure using the image analysis technique. In this technique, after collecting the main panicle of each rice plant, we set the grains on a scanner as the position of each grain on the panicle can be known and got the images with the scanner. We evaluated the thickness of grains, which is the important index of the grain quality in Japan, using image analysis. We pre-analyzed the relationship between the grain images and their thickness.

The panicle number and the plant height were smaller and lower in the organic farming than in the conventional farming throughout crop growth. As a result, The panicle number in conventional farming was almost twice that in organic farming and the yield in conventional farming was 1.6 times higher than in conventional farming. On the other hand, the spikelet number was significantly higher in organic farming than in conventional farming and the ripening rate also tended to be higher in organic farming than in conventional farming. In the panicle structure analysis, the primary and secondary branches of the panicle in organic farming are greater than in conventional farming but the grains in organic farming tended to have small grain thickness. From these results, for increasing yield in organic farming, it could be possible to control the number of branches of the panicle and to increase the fertility of the grain.

p13. Optimum Level of Soil Available Phosphorus for AMF Inoculation to Welsh Onion in Non-Allophanic Andosol

Takae SUZUKI[1], Toru UNO[1], Ryosuke TAJIMA[1], Toyoaki ITO[1] and Masanori SAITO[1],[2]

[1]Graduate School of Agricultural Science, Tohoku University
[2]Japan Science and Technology Agency

It was demonstrated that inoculation of arbuscular mycorrhizal fungi (AMF) to Welsh onion (*Allium fistulosum* L.) was effective to improve its growth and to reduce phosphorus (P) fertilizer (Tawaraya *et al*., 2012). Non-allophanic Andosol is a volcanic soil with high P fixing capacity, so application of AMF is expected for efficient use of P fertilizer. However, it is not yet clarified what level of soil available P is suitable for effective AMF inoculation in non-allophanic Andosols.

To clarify the optimum level of soil available P in a non-allophanic Andosol, we conducted field experiments of Welsh onion for 4 years in Field Science Center of Tohoku University, Japan. Welsh Onion was inoculated with a commercial inoculum containing *Rhizophagus* sp. R10 and grown for about 8 weeks in nursery bed in greenhouse. The seedlings were transplanted to the plots with different levels of soil available P due to previous fertilization. Soil available P of the plots were Very high (VH), High (H), Medium (M), and Low (L).

The AMF inoculation significantly increased the yields in the M and H plots, while no effect was found in the VH plot. In the L plot, plant growth was so poor that the inoculation did not clearly improve yields. Additional P fertilizer was applied upon transplanting, but this P fertilization did not much affect the yield of Welsh onion. These results indicate that a medium to high level of soil available P is needed to increase effectiveness of AMF inoculation to Welsh onion in the non-allophanic Andosol.

p14. Effectiveness of Winter-Flooding in Organic Rice Farming and Some Relating Management Practices

Toru UNO, Ryosuke TAJIMA, Toyoaki ITO and Masanori SAITO

Graduate school of Agricultural science, Tohoku University

Rice farming combined with winter-flooding method has recently been recognized as one of the important environment-preservative farming methods, and its area has been increasing greatly in Japan. However, it is not well reported about yearly change of productivity of this farming method and management practices, especially in comparison with organic farming.

Therefore, we conducted field experiments in Field Science Center, Tohoku University, to investigate productivity of organic rice farming combined with winter-flooding (WOF) in comparison with organic farming without winter-flooding (OF) and conventional farming (CF) over 4 years. Yields of WOF were generally lower than those of CF throughout 4 years but were equal or higher than those of OF, indicating that winter-flooding is one of promising techniques in organic rice farming.

We also examined some management practices in WOF. i) Application of rice bran (0.8 t/ha) just after transplanting was effective for weed control. ii) Repeated mechanical weeding (3 times) in a month after transplanting was also effective to decrease weeds and sometimes increased rice yield. iii) Repeated puddling during 1 month before transplanting did not clearly increase rice yield but significantly increased shoot dry weight and tiller number depending on year. These effects may be caused by enhancement of soil nitrogen mineralization with repeated puddling.

These results indicated that winter-flooding is potentially effective to increase yield in organic rice farming and that yield may be further improved by adopting proper management practices such as repeated puddling.

p15. Lower Global Warming Potential and Higher Yield of Wet Direct-Seeded Rice in Central China

Weiqin WANG[1], Ye TAO[1] and Lixiao NIE[1,2]

[1]MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River,
Huazhong Agricultural University
[2]Hubei Collaborative Innovation Center for Grain Industry, Yangtze University

Direct-seeded rice is a promising option because it saves water and labor, and it increases productivity. Nonetheless, few studies have evaluated the transition from traditionally transplanted rice to direct-seeded rice. Here we compared yield, water productivity and greenhouse gas emissions of dry direct-seeded rice, wet direct-seeded rice and transplanted rice in Central China in two consecutive years. We grew four rice cultivars: Huanghuazhan, LvdaoQ7, Yangliangyou6 and Yliangyou1. We measured grain yield, yield components, water consumption, water productivity, and greenhouse gas emissions. Our results show that the grain yield of wet direct-seeded rice was 10.8 % higher than that of transplanted rice, when averaged across cultivars and both years. Grain yield of dry direct-seeded rice and transplanted rice was similar. Water productivity of dry direct-seeded rice was 11.6% higher than that in transplanted rice. Water productivity of wet direct-seeded rice was 13.4% higher than that in transplanted rice. Global warming potential was 76.2 % lower for dry direct-seeded rice and 60.4 % lower for wet direct-seeded rice, than for transplanted rice. Wet direct-seeded rice was found to be more susceptible to lodging than dry direct-seeded rice and transplanted rice. Overall, wet direct-seeded rice is the best system for Central China due to higher grain yield and water productivity, and lower global warming potential. Dry direct-seeded rice may also be suitable for some regions where water is scarce for soil puddling during land preparation.

keywords: dry direct-seeded rice, wet direct-seeded rice, transplanted rice, grain yield, water productivity, greenhouse gas emission

p16. The Relationship Between Deep Rooting and Nitrate Leaching of Wheat in Subsoil Acidity

Tomohiro WATANABE, Toru UNO, Ryosuke TAJIMA, Toyoaki ITO and Masanori SAITO

Graduate School of Agricultural Science, Tohoku University, Japan

Nitrogen, the most important nutrient in crop production, exists as mainly nitrate (NO_3-N) in the arable field. NO_3-N easily moves to the topsoil and, when it leaches into groundwater, has a negative impact on water quality and increases indirect N_2O emissions. Wheat, which is one of the staple crops in the world and increasing the production in Japan can use NO_3-N not only in the topsoil but also in the subsoil. Therefore, wheat should enhance crop production and reduce NO_3-N leaching if wheat could use more NO_3-N in the subsoil. However, rooting to the subsoil in the wheat could be severely suppressed under acidic soil, especially in Andosols, which is one of the major soil types in Japan. In this study, we tested the relationship between rooting to the subsoil and NO_3-N leaching of wheat in the Andosols.

We cultivated four common (AU49, Harukirari, Haruyokoi, Shirogane) and one spelt wheat genotypes in the pot filled with thick high humic nonallophanic Andosols. The pot consisted of the topsoil and the subsoil parts. All the topsoil parts were limed for neutralizing soil acidity and the subsoil parts were set up in the two treatments: liming and no liming. We irrigated and collected the leaching water from the bottom of the pot every two weeks. At harvesting (80 days after seeding), we harvested aboveground parts of wheat and carefully washed the roots from the soil for the estimation after the soil was divided into two layers. In addition, NO_3-N concentration in the soil was measured.

The grain yield, shoot and root dry weights were more surpassed in the liming treatment than that in the no liming treatment in all the tested wheat genotypes. With liming, NO_3-N concentration in soil represented lower than that with no liming in the subsoil. NO_3-N concentration in the leaching water also tended to be low in the liming treatment though it was different in five wheat genotypes. Spelt wheat showed the highest root length density and the lowest NO_3-N concentration with no liming in the subsoil while the plant growth of AU49 and Harukirari were limited and NO_3-N concentration tended to be high in the subsoil acidity. These results suggested the subsoil acidity could be one of the important factors in limiting crop production increasing NO_3-N leaching. The trait of deep rooting ability of wheat under subsoil acidity could be an effective solution to take up NO_3-N linking to both lower the crop production and NO_3-N leaching.

p17. Classification of Polarimetric SAR Imagery for Forest Extraction with Airborne LiDAR Data

Yumi MIURA and Chinatsu YONEZAWA

Graduate School of Agricultural Science, Tohoku University

Forest resource assessment is important because it plays a significant role for absorbing carbon dioxide and storing them in their trunks. In recent years, the progress of analysis method with optical imagery and Synthetic Aperture Radar (SAR) imagery using object-oriented classification for vegetation maps is accelerating. SAR transmits a micro wave from an antenna and receives its backscattering. SAR has all-weather capability and it enables us to obtain data frequently. Obtained data provides physical characteristics of the Earth’s surface. To analyze or decompose multiple polarized micro wave helps us to observe the condition of land surface. Volume scattering component is dominated in forestry. Furthermore, light detection and ranging called LiDAR is used to detect height of tree crowns or buildings and reconstruct 3D models. We can get height information from LiDAR data with two-dimensionally. In this study, we developed a method to extract forest area by integration of polarimetric ALOS-2 PALSAR-2 imagery and LiDAR data. We also assessed the usefulness of our method. ALOS-2 PALSAR-2 is a unique L-band SAR sensor and it has potential of full polarimetric observation. The result of this study shows a possibility to update local-land-use map.

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Information to Authors from Journal of Integrated Field Science (JIFS)

The Journal publishes articles in all areas of field science in Agricultural science. The journal is an English magazine started in 2003 fiscal year when Integrative Field Science Center, Graduate School of Agricultural Science, Tohoku University, has started. Our journal places the edit committee. Under the committee, an original paper including short paper, proceedings, a review, description, and data are published. An original paper will be peer reviewed by two referees. Our journal has published one volume in principle every year and we will start to publish it as an on-line journal from volume 15 (JIFS: http://www.agri.tohoku.ac.jp/jp/about/field/jifs/index.html). We will also publish all the manuscripts on the web site as e-journal (Tohoku University Repository: https://tohoku.repo.nii.ac.jp/).

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If a literature is non-English language is cited, note the language at the end of the literature. Examples for literature citation are given below.

Lauenroth, W. K. and R. Gill (2003) Turnover of root systems, H. de Kroon and E.J.W. Visser (Eds.), Root Ecology, Springer, Berlin, pp. 61-89.

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The case articles from journals, give the name of the author, the year of publication in parentheses, the title of the paper, the name of the journal, volume and the page, in that order. The case from books, give the name of the author, the year of publication in parentheses, the title of the articles, the name of the book, the name of the editors, the page (inclusive), and the name and city of the publisher, in that order.

Tables

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