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Changes of arbuscular mycorrhizal traits and community structure with respect to soil salinity in a coastal reclamation land



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ABSTRACT

A comprehensive knowledge on the relationship between soil salinity and arbuscular mycorrhizal fungi (AMF) is vital for a deeper understanding of ecosystem functioning under salt stress conditions. The objective of this study was to determine the effects of soil salinity on AMF root colonization, spore count, glomalin related soil protein (GRSP) and community structure in Saemangeum reclaimed land, South Korea. Soil samples were collected and grouped into five distinct salt classes based on the electrical conductivity of soil saturation extracts (ECse). Mycorrhizal root colonization, spore count and GRSP were measured under different salinity levels. AMF community structure was studied through three complementary methods; spore morphology, terminal restriction fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis (DGGE). Results revealed that root colonization (P < 0.01), spore count (P < 0.01) and GRSP (P < 0.01) were affected negatively by soil salinity. Spore morphology and T-RFLP data showed predominance of AMF genus Glomus in Saemangeum reclaimed land. T-RFLP and DGGE analysis revealed significant changes in diversity indices between non ($EC_{se} < 2 \text{ dS/m}$) and extremely (ECse > 16 dS/m) saline soil and confirmed dominance of Glomus caledonium only in soils with ECse < 8 dS/m. However, ribotypes of Glomus mosseae and Glomus proliferum were ubiquitous in all salt classes. Combining spore morphology, T-RFLP and DGGE analysis, we could show a pronounced effect in AMF community across salt classes. The result of this study improve our understanding on AMF activity and dominant species present in different salt classes and will substantially expand our knowledge on AMF diversity in reclaimed lands.

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1. Introduction

Saemangeum is one of the world's largest reclamation sites which added 283 square kilometers of land and 118 square kilometers of lake to South Korea's total geographical area. The reclamation site is located at the estuaries of Mangyeonggang and Dongjingang rivers in the west coast of South Korea. Saemangeum reclamation project was initiated in 1991 with the first step of this project being the construction of the world largest sea dike on the Yellow Sea for a stretch of 33.9 km and the removal of seawater, which were completed in 2010. The reclaimed land was planned to be used for agriculture, industry, tourism, global business, scientific research, new and renewable energy, ecological environment and residential districts. A large percentage of the reclaimed land is allotted for agricultural purposes (30.3%), and land preparation for crop cultivation was initiated during 2011 and expected to be completed by 2020. Despite initial plans to use these lands for organic agriculture and horticultural crop production, initial crop establishment studies failed due to high salt content in the soil (Ryu et al., 2010).

High salt in soil has several detrimental effects such as physiological drought to plants, cell toxicity by accumulating sodium and chloride ions in cell and nutrient imbalance due to reduced uptake of nutrients from soil (Evelin et al., 2009; Ruiz-Lozano et al., 2012). Mechanical removal of salt from soils or development of salt tolerant crops for reclaimed site is not economically feasible under current scenario. A viable alternative is the use of microorganisms that mitigate salinity stress to improve plant growth. Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs which form symbiotic associations with more than 80% of the land plants (Smith



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Fig. 1. Location of Saemangeum reclaimed land and sampling site (obtained from Google Earth).

and Read, 2008). AMF mitigate salt stress through improving nutrient uptake (Wilson et al., 2012), water uptake (Ruiz-Lozano et al., 2012), photosynthetic activity (Aroca et al., 2013) and production of antioxidant molecules (Hajiboland et al., 2010) in plants. However, high salinity appears to be detrimental on AMF fitness as there have been considerable evidence with regards to their spore germination, root colonization and community structure (Juniper and Abbott, 2006; Campanelli et al., 2013). These issues can be addressed by identifying native salt tolerant AMF species and their use as a bioinoculant for crop growth. Native salt tolerant AMF species can be more effective in root colonization and plant growth improvement compared to non native AMF species (Caravaca et al., 2003).

Presence of AMF has been confirmed in soils under different environmental conditions (Yang et al., 2010; Babu and Reddy, 2011; Guo and Gong, 2014), and they play an important role in crop establishment and improving soil health in reclaimed sites (Louis, 1990). One of the several factors used by AMF to improve soil health is the synthesis of glomalin related soil protein (GRSP), which helps in soil aggregation and acts as a long term sink for carbon and nitrogen storage (Dai et al., 2013).

Even though there is a high potential for retrieving quantitative estimates through spore morphological studies, the results are sometimes misleading due to the limited morphological differences between AMF species. Molecular techniques have been widely used to circumvent the problems associated with species level AMF diversity analysis (Kruger et al., 2012). Terminal restriction fragment length polymorphism (T-RFLP) is one of the most widely used high throughput fingerprinting techniques in mycorrhizal diversity analysis, which is fast and highly reproducible. However, the issues related to retrieving sequence information directly from terminal restriction fragments are difficult and T-RFLP needs to construct clone libraries to obtain such information. Denaturing gradient gel electrophoresis (DGGE) provides rapid information for comparing microbial community between samples, and it is possible to retrieve the phylogenetic information of the community from excised bands (Ma et al., 2005). In this study, we used three complementary techniques such as spore morphotyping, T-RFLP and DGGE to obtain a clear reflection on mycorrhizal diversity. Several investigators in the recent past have analyzed AMF diversity in salt marshes (Wilde et al., 2009; Guo and Gong, 2014) and coastal area (Lusite and Levinsh, 2010) using morphological and molecular techniques. Results of the above mentioned studies revealed a shift in AMF species dominance and decrease in mycorrhizal traits as soil salinity increased. However, knowledge on AMF diversity and mycorrhizal traits in other salt affected sites such as Saemangeum reclaimed land remains to be studied and can contribute to better understanding of the nature and roles of AMF under such conditions. Hence, the present investigation was aimed to study the impact of soil salinity on AMF community structure and mycorrhizal traits in Saemangeum reclaimed land.

2. Materials and methods

2.1. Study area and sample collection

Saemangeum reclaimed land is located in Jeollabuk-do province, South Korea. Latitude and longitude of the sampling location are N 35°46'13.28 and E126°37'21.80, respectively. Location of the study area and sampling site is shown in Fig. 1. Our preliminary work in Samangeum reclaimed land revealed unequal spatial salt gradients (data not shown). Thirty five soil samples (10 cm radius and 15 cm depth; approximately, one kg for each sample) were randomly collected along with plant roots and placed in polyvinyl chloride bags before transporting to the laboratory using an ice box. All collected samples were at least 5 m apart from each other, so that the chances of sharing the same AMF community between samples are reduced. Salinity levels of all thirty five soil samples were determined by measuring the electrical conductivity of the soil saturation extracts (ECse). Based on the ECse values, samples were grouped into five salt classes as follows: non saline $(EC_{se} < 2 \text{ dS/m})$, slightly saline $(EC_{se} 2-4 \text{ dS/m})$, moderately saline (EC_{se} 4–8 dS/m), very saline (EC_{se} 8–16 dS/m) and extremely saline $(EC_{se} > 16 \text{ dS/m})$ as per United States Department of Agriculture (USDA) soil salinity classification (Scianna, 2002). To maintain equal number of samples in each salt class, three samples with similar ECse values were selected per salt class. The resulting fifteen samples were taken for further experiments. For molecular analysis, spore count and chemical analysis, each of the fifteen independent samples were divided into three parts. One part of the sample used for molecular study was stored at -20 °C, and the other two parts used for spore isolation and chemical analysis were stored at room temperature. Soil texture (sandy loam) and chemical properties such as pH, organic matter content (OM), available phosphorus (Av.P2O5) and exchangeable cations were measured using standard laboratory protocols and the results are presented in Table 1.

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