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# Molecular monitoring of field-inoculated AMF to evaluate persistence in sweet potato crops in China

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**Abstract**

The effect of inoculation with arbuscular mycorrhizal fungi (AMF) on crop productivity under small-scale farming conditions and the persistence of the fungal inoculum in the field were investigated over 2 years. Sweet potato plants were inoculated with various combinations of AMF and grown under traditional Chinese farming procedures. Plantlets from germinated tubers were inoculated in a soil/sand mixture at the time of hand transplanting into the field. A technique for long-term preservation of the root samples and a fast, reliable DNA extraction method were developed to track and evaluate the persistence of the selected AMF in two field trials in China. The AMF rDNA was specifically amplified by nested PCR from colonized sweet potato roots collected from the field trials, and polymorphism of the 5′-end of the large ribosomal subunit was used to monitor fungi at the species level. AMF varied in their ability to establish after inoculation, and in their effect on yield and quality of sweet potato tubers.


*Keywords:* Arbuscular mycorrhiza; Sweet potato; Inoculum monitoring; LSU rDNA; Nested PCR; Field trail

## 1. Introduction

Soil and rhizosphere microbial activities are major factors that determine the availability of nutrients to plants and consequently have significant influences on plant health and productivity (Jeffries et al., 2003). Maintenance of sustainable soil fertility depends greatly on the ability to harness the benefits of rhizosphere microorganisms such as arbuscular mycorrhizal fungi (AMF), which form a symbiotic association with the roots of most plant families. This ubiquitous and ancient symbiosis promotes plant acquisition of inorganic nutrients, notably phosphate, in exchange for carbon compounds and protection against diverse biotic or abiotic stresses (Smith and Read, 1997). Use of the arbuscular mycorrhiza symbioses may provide an alternative to high inputs of fertilizers and pesticides in sustainable crop production systems (Gianinazzi and Schüepp, 1994). Within this context, we investigated the possibility of using selected AMF to improve sweet potato crop production in small-scale sustainable agriculture in China. Plant-available phosphorus is generally low in many agricultural soils in China (Davies et al., 2002) due to phosphorus deficiency and/or high phosphate-fixing capacities. Heavy inputs of

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fertilizers and pesticides are necessary to restore an acceptable level of plant productivity. Such management practices are expensive and commonly aggravate the problem because they are a source of pollution, which further affects soil structure and activity, having deleterious effects on the environment and food quality.

Sweet potato (*Ipomoea batatas* L.) is a staple food crop, which is widely cultivated in North China, ranking only after wheat and maize. In practice, sweet potato yields are limited because of the wide occurrence in production areas of calcareous soils, which have high phosphate-fixing capacities and consequently induce a low level of plant-available phosphate. Traditionally, vegetative seedlings of sweet potato are prepared by germinating tubers in sand beds and stem cuttings are then hand transplanted to the field. This procedure facilitates the early inoculation of plantlets with AMF fungi as the inoculum can be placed in the planting hole at the same time as the plantlet during planting out into the field.

Correct identification of individual AMF is essential for tracking the persistence of introduced inoculum in the field. AMF structures within the plant root cortex generally lack distinctive traits so that their identity cannot be distinguished using morphological characters ribosomal gene polymorphism has been targeted to evaluate AMF fungal diversity in plant roots (van Tuinen et al., 1998a,b; Kjøller and Rosendahl, 2000; Jansa et al., 2002; Renker et al., 2003; Vandenkoornhuyse et al., 2003; Gollotte et al., 2004) and characterization of the large subunit region (LSU) of ribosomal RNA genes, in combination with nested PCR, has proven suitable for analyzing phylogenetic relationships and developing molecular probes to detect AMF species colonizing plant roots in microcosm experiments (van Tuinen et al., 1998a; Jacquot et al., 2000; Kjøller and Rosendahl, 2000), in the field (Kjøller and Rosendahl, 2001; Turnau et al., 2001; Gollotte et al., 2004) or even in aquatic plants (Nielsen et al., 2004).

In this paper, we report the impact of AMF inoculation on the yield and quality of sweet potato under traditional Chinese farming conditions. PCR probes were designed from the D1-D2 region of the LSU RNA gene to evaluate the quality of AMF inoculum produced on a large-scale and to monitor the fate of selected AMF after inoculation into the field.

## 2. Materials and methods

### 2.1. Fungal material

Spores of *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe BEG 12, and *G. intraradices* Schenck and Smith BEG 141 were obtained from the reference culture collection of the International Bank for the Glomeromycota (IBG, http://www.kent.ac.uk/bio/beg). A mixed AMF commercial inoculum Endol® was provided by Biorize Sarl (France). Three isolates of AMF, originally isolated from soil samples collected in the Hebei province (PRC) and identified at the morphological and molecular level, were also used in this study: a *Glomus mosseae* isolate (Gm93), registered in the IBG as BEG 167, and two *G. etunicatum* isolates HB-XH08-Gsp5 (registered as BEG 168) and HB-Bd45-Gsp4 (not registered in the IBG).

AMF inocula were produced for field trials by the Kaifa company (Wuchang, Hubei Province, PRC) on a large-scale (80 kg) under controlled glasshouse conditions. Endol® or fungi from pure pot cultures were inoculated into trays of disinfected (twice autoclaved) batches of their soil of origin. Maize, *Astragalus chinensis* and sorghum were used as host plants and harvested 6 months after inoculation. Root samples of individual plants were taken 8 weeks after contact with the inoculum, and either dried (50 °C) and stored at room temperature for molecular analysis, or stained with trypan blue after partial digestion in KOH (Philipps and Hayman, 1970) to confirm AMF colonization. At harvest, mycorrhizal colonization levels were checked and most probable number of propagules (method described by Gianinazzi-Pearson et al., 1985) was estimated to guarantee a minimum of $10^4$ propagules/kg soil-based inoculum. Roots and soil were air-dried, thoroughly mixed and transported to the field sites (Daxing, Hebei Province, PRC).

### 2.2. Plant propagation and inoculation with *G. intraradices* BEG 141

Tubers of the sweet potato variety Hongxin (Daxing, PRC) were germinated in sand. Approximately, 4 weeks after germination, plantlets were broken off from the tuber and transplanted into 400 ml pots containing a disinfected (180 °C, twice at 24 h intervals), neutral soil/sand (3:1) mix to which 50 g of a soil-based inoculum of *G. intraradices* BEG 141 (spores, mycelium and colonized roots) had been added into the planting hole. Plants were grown under constant conditions (photoperiod 16 h, temperature 19–22 °C, relative humidity 60–70%, light intensity 320 μmol $m^{-2}$ $s^{-1}$ and received weekly, 16 ml of Long Ashton nutrient solution (Hewitt, 1966) without phosphate.