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Screening of Arbuscular Mycorrhizal Fungi for Symbiotic Efficiency with Sweet Potato

J. P. Gai,¹ G. Feng,¹ P. Christie,^{1,2} and X. L. Li¹

¹Department of Plant Nutrition, China Agricultural University, Beijing, P. R. China

²Agricultural and Environmental Science Department, Queen's University Belfast, Belfast, UK

ABSTRACT

A greenhouse study was conducted to study the efficiency of 14 isolates of arbuscular mycorrhizal (AM) fungi isolated from a local agricultural soil on the productivity of sweet potato (*Ipomoea batatas*). The different AM fungi enhanced the biomass and nutritional status of sweet potato seedlings to different extents. The genus *Glomus* was more effective than *Acaulospora* or *Scutellospora*. Efficiency also varied among isolates of *Glomus* irrespective of individual host plant or location of origin. Intraspecific differences were sometimes greater than interspecific differences. Benefits deriving from fungal isolates were positively correlated with the root-colonization rate and the abundance of extraradical propagules of the AM fungi. Taking plant yield parameters, nutritional status of the plants, and fungal attributes into consideration, GEGM (*Glomus etunicatum* together with *Glomus mosseae*) and GE6 (*Glomus etunicatum*) were the most effective AM symbionts for sweet potato under the experimental conditions.

Keywords: mycorrhizal efficiency, arbuscular mycorrhizal, sweet potato

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are important soil microorganisms in agroecosystems (Smith and Read, 1997). They are active in increasing the availability and uptake of soil phosphate and trace elements by many plants, thereby enhancing host plant growth (Hamel, 1996; Dodd, 2000). Numerous

Address correspondence to X. L. Li, Department of Plant Nutrition, College of Agricultural Resources and Environmental Sciences, China Agricultural University, 2 Yuan Ming Yuan West Road, Beijing 100094, P. R. China. E-mail: lixl@cau.edu.cn

studies have shown that individual species of AM fungi, and even fungal isolates in one species, differ in their ability to promote plant growth, and that promotion of plant growth can depend on the particular matching of plant and fungal species (Johnson, 1993; Johnson and Pfleger, 1992; Johnson et al., 1992, 1997; Jansa et al., 2002). When tested on a single plant species, the fungal isolates can increase, decrease, or have little effect on plant growth (Burgess et al., 1994; Feng et al., 2001; van der Heijden and Kuiper, 2001; O'Keefe and Sylvia, 1993). This inter- and intraspecific variation makes it essential to screen for efficient AM fungi for particular host-plant species.

Sweet potato (*Ipomea batatas*) is a food crop widely cultivated in the north of China, ranking third in importance after wheat (*triticum aestivum* L.) and maize (*zea mays* L.). In practice, the growth and yield of sweet potato are severely limited because of the wide-spread calcareous soils that have high phosphorus (P)-fixing capacities and consequently low levels of plant-available phosphate. It is traditional practice to prepare vegetative seedlings of sweet potato in seedling beds and then transfer stem cuttings by hand to the field, which facilitates direct low-cost inoculation with AM fungi to increase sweet-potato yield.

In the present study, AM fungi were isolated from the rhizosphere soil of staple crops in Hebei province, North China. An attempt was made to investigate the biodiversity of various isolates and species of AM fungi, examine the relationship between plant yield response and the ecological attributes of the fungal isolates, and then select isolates according to their effectiveness in promoting nutrient acquisition by the host sweet potato plants.

MATERIALS AND METHODS

The greenhouse investigation was conducted at China Agricultural University in Beijing. The soil used was collected from a depth of 0–15 cm in a cultivated field in Hebei province. Selected properties [dry-matter (DM) basis] of the soil were as follows: pH (in water) 7.1, 0.83% organic matter, 0.076% total nitrogen (N), 9.6 mg kg⁻¹ 0.5 M NaHCO₃-extractable P, and 108 mg kg⁻¹ 1 M NH₄OAc-extractable potassium (K). Soil was passed through a 1 mm sieve, sterilized by autoclaving at 120°C for 2 h, and then air-dried. Before planting, the soil was fertilized with 150 mg N (as NH₄NO₃), 400 mg K (as K₂SO₄) and 20 mg P (as KH₂PO₄) kg⁻¹. Three kilograms fertilized soil mixed with 1 kg sterilized sand were used in each pot.

Sweet potato [*Ipomoea batatas* (L.) Lam.] was used as the host plant. Stem cuttings (20 cm) were pre-cultured in sterilized sand to develop their roots before the experiment. One week later, uniform seedlings were selected and one seedling was transplanted into each pot.

Table 1
Origin and taxa of AM fungal isolates

Code	AM fungal species	Sampling site	Host plant	Soil Olsen-P	Soil pH
GE1	<i>G. etunicatum</i>	Baoding	<i>Setaria</i> Beauv.	10.4	8.28
GE2	<i>G. etunicatum</i>	Baoding	<i>Zea mays</i> L.	7.1	8.33
GE3**	<i>G. etunicatum</i>	Cangzhou	<i>Ipomoea batatas</i> Lam.	7.1	8.35
GE4	<i>G. etunicatum</i>	Cangzhou	<i>Urtica fissa</i> Pritz.	10.0	8.16
GE5*	<i>G. etunicatum</i>	Xinglong	<i>Setaria</i> Beauv.	3.8	6.51
GE6	<i>G. etunicatum</i>	Zhangjiakou	<i>Ipomoea batatas</i> Lam.	4.3	8.60
GM1	<i>G. mosseae</i>	Xingtai	<i>Sorghum vulgare</i> Pers	12.8	8.47
GM2**	<i>G. mosseae</i>	Cangzhou	<i>Ipomoea batatas</i> Lam.	7.1	8.35
GM3	<i>G. mosseae</i>	Beijing	<i>Zea mays</i> L.	13.8	8.16
GM4	<i>G. mosseae</i>	Xinglong	<i>Phaseolus vulgaris</i> Linn.	105.1	7.64
GEGM	<i>G. etunicatum</i> / <i>G. mosseae</i>	Shijiazhuang	<i>Sorghum vulgare</i> Pers	2.4	8.27
GI	<i>G. intraradices</i>	Xinglong	<i>Cucumis sativus</i> L.	21.0	8.16
AM	<i>A. morrowea</i>	Baoding	<i>Sorghum vulgare</i> Pers	1.6	8.32
SG*	<i>S. gilmorei</i>	Xinglong	<i>Setaria</i> Beauv.	3.8	6.51

*Isolates GE5 and SG were derived from the same soil sample; and **isolates GE3 and GM2 were obtained from the same soil sample.

A total of 14 isolates representing five species of AM fungi isolated from the agricultural soil in Hebei province were tested. Their origin and taxa are shown in Table 1. The isolate coded as GEGM was originally considered to be *Glomus etunicatum* but was later found to include spores of *G. mosseae*. The fungi were further maintained in pot culture. Identification was made with the help of Dr. John Dodd of the International Institute of Biotechnology, UK. Classification was based on morphological features and molecular techniques. At the same time the seedlings were transplanted, 200 spores of each isolate were placed near the roots of sweet potato. Fifteen treatments, an uninoculated control plus inoculation with each of the 14 isolates were established, and there were four replicates per treatment.

The plants were harvested after four months' growth. Shoots and roots were separated and the oven dry weights (70°C for 48 h) were determined. The oven-dried plant shoots and roots were milled and dry ashed. Tissue P was determined colorimetrically according to Murphy and Riley (Murphy and Riley, 1962).

The proportion of root length colonized by AM fungi was determined by the method of Trouvelot et al. (Trouvelot et al., 1986) after staining sub-samples of roots with Trypan blue (Phillips and Hayman, 1970). Thirty 1 cm-long root segments were examined from each pot. Hyphal density was determined by the

method of Jakobsen et al. (Jakobsen et al., 1992) and calculated according to the method of Zhao et al. (Zhao et al., 1997). Mycorrhizal effectiveness and hyphal P contribution were calculated as shown below (van der Heijden and Kuiper, 2001).

Mycorrhizal effectiveness (%) = [(Dry weight of mycorrhizal plant – Dry weight of non-mycorrhizal plant)/Dry weight of mycorrhizal plant] × 100

Hyphal P contribution (%) = [(P uptake of mycorrhizal plant – P uptake of non-mycorrhizal plant)/P uptake of mycorrhizal plant] × 100

Data were tested by analysis of variance and means were compared by the least significant difference (LSD) test at the 5% level.

RESULTS

Effect of Different Isolates on the Biomass of Sweet Potato

In general, mycorrhizal inoculation resulted in an increase in plant biomass. However, the 14 isolates had different efficiencies, with isolates GEGM, GE5, and GE6 showing the most mycorrhizal effectiveness in promoting plant biomass and isolates GEGM, GE5, and GM2 leading to the largest quantity of tuber production (Table 2).

At genus/species level, the isolates showed different responses. *A. morrowea* and *S. gilmorei* showed little effect, and isolates in *Glomus* showed much larger effects than other genera. The most efficient isolates in improving plant biomass were all in *Glomus*.

Intraspecific differences also occurred, even with the same host plant or original soil. Isolate GE6 greatly enhanced shoot and tuber dry weight and isolates GE5, GE4, and GE2 showed positive effects on tuber productivity, while other isolates showed little effect on plant production. The effectiveness of isolates in *Glomus mosseae* showed a similar trend. The isolate of *G. etunicatum* that was contaminated with *G. mosseae* showed the highest efficiency in promoting plant biomass.

Phosphorus Uptake of Sweet Potato under Different Inoculation Treatments

Inoculation with 14 AM isolates enhanced P nutrition at different levels (Table 3). GEGM and GE6 were most effective in host-plant P nutrition but isolates SG, AM, GE1, GE1, and GM1 showed no significant effects.

Table 2
Effect of inoculation with different isolates on the biomass of sweet potato

AM fungal isolate	Dry weight (g pot ⁻¹)			Mycorrhizal effectiveness (%)
	Shoots	Roots	Tubers	
Control	8.3bc	3.8a	7.1efg	0
GE1	10.0ab	3.3a	6.3g	4
GE2	10.1ab	3.0ab	10.5cd	19
GE3	9.8abc	3.6a	9.3cdef	15
GE4	7.6bc	2.6ab	10.9cd	9
GE5	9.3abc	2.0b	14.5ab	26
GE6	12.3a	3.5a	10.1cd	26
GM1	9.7abc	3.6a	6.9fg	5
GM2	7.5bc	3.1ab	11.9bc	15
GM3	10.5ab	3.5a	10.0cde	20
GM4	8.6bc	3.8a	8.8defg	10
GEGM	12.2a	3.2ab	14.9a	37
GI	9.9abc	3.6a	10.7cd	20
AM	7.5bc	3.4a	8.8defg	2
SG	6.7c	3.0ab	10.1cd	3

Within each column, means followed by the same letter are not significantly different by LSD at the 5% level.

Tuber phosphorus seems to be particularly sensitive to inoculation with AM fungi. Most isolates (10 isolates, 71% of the total number) greatly elevated the P concentration in the tubers, while only GE1, GE3, GE6, GEGM, and GI showed positive effects on shoot P concentration. Hyphal P contribution to the plant P varied among the different inoculation treatments.

The interspecific and intraspecific differences among the isolates in improving P absorption were similar to their effect on plant biomass.

Colonization and Propagule Numbers of AM Fungi

All the inoculation treatments formed mycorrhizal structures within the roots of sweet potato. Colonization rates of the 14 inoculation treatments varied widely (Table 4). GEGM, GE6, GM1, and GE1 showed greater root colonization than other isolates.

Spore density of different isolates varied from nine to 436 spores in 10 g soil. Isolate GEGM formed the most spores in the rhizosphere soil. Isolates GE1 and GE6 were intermediate, and SG, GM2, and AM formed the fewest spores.

Table 3
Phosphorus uptake of sweet potato inoculated with different AM fungal isolates and the hyphal contribution to plant P uptake

AM fungal isolate	P uptake (mg pot ⁻¹)			Hyphal P contribution (%)
	Shoots	Roots	Tubers	
Control	4.7def	3.0abcd	4.7g	0
GE1	13.0abc	3.9abc	5.6fg	46
GE2	10.3bcde	3.3abcd	9.6cd	47
GE3	15.5ab	4.4ab	7.4def	55
GE4	4.5def	2.4cd	7.3def	13
GE5	7.2cdef	1.9d	12.5ab	43
GE6	17.1a	4.1abc	12.0bc	63
GM1	10.5bcd	3.4abcd	6.5efg	40
GM2	3.9ef	2.9bcd	7.6def	14
GM3	7.0cdef	3.8abc	8.6de	37
GM4	1.9f	3.7abcd	7.8def	8
GEGM	15.5ab	3.5abcd	14.7a	64
GI	11.4abc	4.8a	7.8de	49
AM	7.7cdef	3.8abc	6.4efg	31
SG	3.7f	3.1abcd	7.3efg	12

Within each column, means followed by the same letter are not significantly different by LSD at the 5% level.

There were large differences in hyphal density among the 14 isolates, with GEGM, GE1, and GE6 showing the highest values (Table 4).

Correlation Analysis of Yield Parameters and Fungal Propagules

The correlation analysis of mycorrhizal colonization, spore density, and hyphal density with yield parameters showed that the three indices were all positively correlated with shoot dry weight, shoot P content, and tuber P content, but were not correlated with root dry weight or root P content (Table 5).

DISCUSSION

In general, the benefits of arbuscular mycorrhizal fungi are related to the rate and extent of mycorrhizal formation (Kahiluoto et al., 2000). When field application is considered, fungal propagules including extraradical mycelium and spores also need to be considered because they are important for the persistence of AM fungi in roots and soils and their competition with other fungi (Abbott et al., 1992; Bever et al., 2001). Therefore, root colonization, hyphal density,

Table 4

Colonization and spore density of different isolates after inoculation of sweet potato

Isolate code	Colonization rate (%)	Hyphal density (mg ⁻¹)	Spore density (spores 10 g)
Control	0.0i	0.0f	0f
GE1	67.3ab	4.4a	213b
GE2	34.9ef	0.5def	99de
GE3	39.6e	1.3cde	73def
GE4	20.6gh	0.4ef	29def
GE5	54.0cd	1.4bcd	111cd
GE6	71.8a	3.5a	205bc
GM1	57.4bc	2.3bc	51def
GM2	25.2fg	0.9def	12ef
GM3	44.1de	2.3b	112cd
GM4	17.2gh	0.7def	63def
GEGM	74.8a	3.7a	436a
GI	37.1ef	0.7def	61def
AM	15.9ef	0.5de	12ef
SG	11.3hi	0.4ef	9ef

Within each column, means followed by the same letter are not significantly different by LSD at the 5% level.

and spore density, along with plant P uptake and biomass, were chosen as the parameters for the selection of efficient isolates. In the present study, GEGM and GE6 were the most efficient isolates as they affected the yield of sweet potato.

There was wide variation in the degree of functional compatibility between *Ipomoea batatas* and the fungal isolates tested. At the genus level, isolates in *Glomus* enhanced the biomass and P nutrition more than did *Acaulospora* or *Scutellospora*. *Glomus* isolates also displayed much greater mycorrhizal

Table 5
Results of Pearson correlation analysis

Fungal parameter		Plant dry weight			Plant P content		
		Shoots	Roots	Tubers	Shoots	Roots	Tubers
M%	Correlation	0.8594	-0.0974	0.2531	0.7798	0.3732	0.7677
	P values	0.0001	0.7300	0.3627	0.0006	0.1707	0.0008
Hyphal density	Correlation	0.7882	-0.0156	0.0725	0.6471	0.3197	0.7233
	P values	0.0005	0.4231	0.7974	0.0091	0.2453	0.0023
Spore density	Correlation	0.8150	-0.0469	0.4164	0.5972	0.3563	0.6661
	P values	0.0002	0.8682	0.1226	0.0187	0.1924	0.0067

colonization and extraradical mycelium than the other two genera (Table 4). This result indicates that more efficient fungi formed a more effective association with the host plant (Brundrett, 2004). In our experiment, the effectiveness of isolates in *Glomus* varied with host or location of origin, results that are similar to those of Monzon and Azcón (1996) and van der Heijden and Kuyper (2001). Intraspecific differences were sometimes larger than interspecific differences. Such variation in efficiency of AM fungi may be attributable to their intrinsic ability to explore a larger volume of area for nutrients, compatibility between the plant and the fungi, and the interactions between the endophytes and their environment (Bagyaraj, 1992; Dodd et al., 2000).

It is often assumed that all species of AM fungi have the same function, due to the ubiquity of the association and the fact that AM fungi occupy a similar plant/soil niche. However, there is increasing evidence that the mechanisms for establishing functional mycorrhiza may differ among species and genera (Boddington and Dodd, 1998, 1999). Our study indicated that the effects of two fungal species of the same origin were not at all the same. The mycorrhizal effectiveness of isolates GE3, GM2, GE5, and SG were different, whether in terms of in-plant yield or P absorption. Van der Heijden and Kuyper (2001) obtained similar results when they investigated whether the plant and fungal origins could influence the effectiveness of the mycorrhizal association, which may reflect the possibly different roles of members of a single fungal community. In our study, GEGM (which comprised two AM species of the same origin) provided the greatest benefit to the host plant. One explanation may be the complementary functions of ecological niches or functions of different species. Alternatively, inoculation with two fungal taxa might improve the overall compatibility of plant and fungi. Commercial inocula often consist of two or more fungi. Further research is warranted on combined inoculation with two or more fungi rather than individual taxa. Under natural conditions, plants will usually be colonized by a range of fungi, so we need more information on guilds of fungi as they affect host growth and nutrition under field conditions.

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