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ORIGINAL ARTICLE

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Castor bean growth and rhizosphere soil property response to different proportions of arbuscular mycorrhizal and phosphate-solubilizing fungi

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Abstract A pot culture experiment was performed to study the effects of infection with different proportions of arbuscular mycorrhizal fungus (AMF) and phosphate-solubilizing fungus (PSF) on the rhizosphere soil property of castor bean (*Ricinus communis* L.). One AMF, *Glomus mosseae*, and one PSF, *Mortierella* sp. (Ms), were applied to non-sterilized coastal saline soil. The plant dry mass, leaf chlorophyll content, and P-uptake of castor bean were assessed. In coastal saline soil, the different proportions of both fungi-inoculated seedlings showed significantly greater shoot and root dry weight than the controls, which had lower root-to-shoot ratios than the inoculated seedlings. An increase in phosphorus (P) and chlorophyll contents was also observed in the inoculated seedlings compared with the controls. The appropriate Ms proportion seemed to be advantageous for AMF colonization. However, available P content of fungi-treated soil increased in proportion to the increase in Ms population and AMF colonization. By contrast, the pH of inoculated soil decreased because of the increased proportion of *Mortierella*, and electrical conductivity values showed a negative correlation with AMF colonization. Soil enzyme activities (i.e., urease, invertase, neutral phosphatase, and alkaline phosphatase) and soil organic matter were also stimulated by inoculation with different proportions of both fungi. However, the catalase activities of inoculated soil were inhibited compared with those of the control soil. Results from this study prove that castor bean planting associated with an appropriate proportion of AMF and PSF will benefit the amelioration of coastal saline soils of eastern China.

Keywords Arbuscular mycorrhizal fungus (AMF) · Phosphate-solubilizing fungus (PSF) · Proportion · Soil enzyme activity · Castor bean · Coastal saline soil

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Introduction

High soil salinity causes hyperionic and hyperosmotic stress and may result in plant demise (Evelin et al. 2009), such that the salinization of soil has become a serious problem in many parts of the world. However, the development of salt-tolerant crops and the desalination of soil by leaching excess salt to mitigate high soil salinity and to minimize crop loss are expensive processes (Munns 2005). Some microorganisms, particularly beneficial bacteria and fungi, can improve plant performance under stressful environments. Arbuscular mycorrhizal fungi (AMF) have been found to promote plant growth and salinity tolerance by increasing the acquisition of nutrients (especially phosphorus, P) (Ruiz-Lozano et al. 1996; Farzaneh et al. 2011), decreasing Na uptake (Al-Karaki 2006; Estrada et al. 2013), and alleviating water stress (Sheng et al. 2008). Meanwhile, some P-solubilizing microorganisms (PSM) can solubilize insoluble forms of P under salt stress (Srividya et al. 2010). This property is important for plant growth because P can precipitate with Ca^{2+}, Mg^{2+}, and Zn^{2+} ions in salt-stressed soil and may

consequently become unavailable to plants (Grattan and Grieve 1999).

The positive effects of combined inoculation with AMF and PSM on plant growth have been demonstrated in numerous studies (Zaidi et al. 2003; Osorio and Habte 2001, 2013). The extensive mycorrhizal hyphae network formed around roots can efficiently take up P released by PSM and translocate P to roots, thereby providing a sustainable nutrient supply for higher crop yield (Azcón and Barea 1996; Sabannavar and Lakshman 2009), especially under salt stress (Zhang et al. 2011). Zaidi et al. (2003) reported that a negative effect was observed on all considered chickpea plant parameters when *Glomus fasciculatum* was added to the combination of *Rhizobium* sp. and *Penicillium variable*, although the available P status of the soil is improved by the addition of *Pseudomonas striata* with *Rhizobium* sp. and AMF. An increase in the population of PSM and spore density of AMF in the combined inoculated soil was also observed by Zaidi et al. (2003), but Chandanie et al. (2009) reported that *Glomus mosseae* decreased the population development of *Trichoderma harzianum*. However, a negative correlation was found between levels of soil P concentration and the colonization of plant roots by AMF (Eom et al. 1999; Machineski et al. 2011). Considering that the available P level increased with increasing PSM population inoculated in soil, we study the interaction between the increased or decreased population of PSM and AMF and their effects on saline soil property.

Soil enzymes catalyze all biochemical reactions and are an integral part of nutrient cycling in soil. Therefore, soil enzyme activities have been taken as indicators of changes in soil ecology or soil fertility resulting from the interactions between inoculants and indigenous microbial populations in soil (Nannipieri et al. 2003). Among the soil enzymes involved in N cycling, urease is the most prominent in terms of mediating the conversion of organic nitrogen to inorganic nitrogen through the hydrolysis of urea to ammonia (Byrnes and Freney 1995). Phosphatase serves an essential function in the release of inorganic P from organically bound P returned to soil (García-Gil et al. 2000). Invertase catalyzes the hydrolysis of sucrose to glucose and fructose and is linked to soil microbial biomass (Frankenberger and Johanson 1983). Catalase, an intracellular oxidoreductase, can promote the decomposition of hydrogen peroxide and protects cells from damage caused by reactive oxygen species (Yao et al. 2006). However, increasing soil salinity has an adverse effect on catalase (Frankenberger and Bingham 1982), alkaline phosphatase (García and Hernandez 1996), invertase, and urease (Omar et al. 1994). To our knowledge, no information is available regarding the ecological influence of AMF and PSM inoculation on soil enzyme activities in coastal saline soil.

Castor bean (*Ricinus communis* L.), from the family *Euphorbiaceae*, is an oilseed plant with commercial varieties having an oil concentration between 40 % and 60 %. Castor oil is used widely as lubricating oil, medicine, paint, and superior feedstock for biorefinery (King 2010). Castor bean showed dependence on AMF in soil with low levels of P (Machineski et al. 2011) and was reported to be a promising candidate for the amelioration of saline soils (Wu et al. 2012). After two castor bean growth seasons in saline soil, significant reductions in soil salinity and electrical conductivity (EC) as well as increases in P, organic matter, microbial activity, and diversity were observed (Wu et al. 2012). However, castor bean seedling growth was inhibited drastically by salt stress and P deficiency (Jeschke et al. 1997; Pinheiro et al. 2008). Meanwhile, because the impact of AMF colonization can differ widely among host plants as well as soil conditions, many ecological hypotheses about the effects of AMF and/or PSM on plant growth or soil property were tested in experimental soil under strict controlling conditions to eliminate the influence of some environmental factors (Sasaki et al. 2001; Fujiyoshi et al. 2006; Seres et al. 2006; Chandanie et al. 2009; Machineski et al. 2011). Prior to the present study, no report has been made on the effect of PSM and AMF on castor bean growth and rhizosphere soil properties, especially on the soil enzyme activities of seashore saline soil under controlling conditions. This study aims to clarify this influence.

Materials and methods

Plant culture

Chinese castor bean (var. "Zi Bi") was used as the host plant. The experiment was conducted from 10 May 2012 to 10 July 2012 at the Halophyte Research Laboratory of Nanjing University, Nanjing, China. The substrate used was a mixture of coastal saline soil and vermiculite (3:1 mass ratio) that was sieved (2 mm). Saline soil was collected from the seashore farm of "Jinhai" in Dafeng City, Jiangsu Province, and had the following characteristics: pH 8.47; EC 1.05 dS m^{-1}, salinity 4.31 ‰, total N 0.29 g kg^{-1}, hydrolyzable N 31.65 mg kg^{-1}, total K 15.27 g kg^{-1}, total P 250.3 mg kg^{-1}, available P 19.37 mg kg^{-1}, and organic matter 10.56 g kg^{-1}. The field moisture capacity of the soil was 38.6 %. AMF spores were isolated from saline soil using the wet sieving and decanting method (Gerdemann and Nicolson 1963). The spore number was 153.7 per 100 g dry soil, and the species were identified as *Glomus mosseae*, *G. spurcum*, *Acaulospora scrobiculata*, and *G. rubiforme*. PSM population (bacteria and fungus) was not found. Plants were grown in a greenhouse under controlled conditions: light intensity of 220 μEm^{-2} s^{-1} at 28 °C for 16 h at daytime and at 18 °C at night. The relative humidity in the greenhouse during the experiment was 65–85 %.

Fungal inocula

Glomus mosseae (Nicol. and Gerd.) is capable of mitigating saline stress (Porras-Soriano et al. 2009) and is

dominant in the experimental site soil. *G. mosseae* (Gm) was thus chosen for this study. Gm, which was isolated from saline soil in Hebei Province, China, was obtained from the Bank of Glomales. The soil inoculum was collected from a 6-month-old pot culture of the fungus grown on sorghum in sterile sandy soil and consisted of spores (9,520 spores per 100 g soil), hyphae, and colonized root fragments. *Mortierella* sp. (Ms) was isolated from salt-affected coastal soil samples collected from the seashore of Jiangsu Province. Ms has been identified as a phosphate-solubilizing fungus that could significantly enhance available soil P concentrations under salt stress (Zhang et al. 2011). The liquid inoculum of Ms was prepared as follows: 3 mL sterile water was added to an Ms test tube slant, and the 3 mL mixture was then poured into 50 mL modified Martin culture medium (MMCM) (1 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 11.5 g NaCl, 5 g peptone, 10 g glucose, 10 g gelose, 100 mL 1/30,000 Bengal red water solution, and 900 mL demineralized water) that was autoclaved for 30 min at 121 °C. Ms was grown on a rotating shaker at 180 rpm and 28 °C for 48 h, after which it was added (5 % of volume) to the MMCM and then cultured on a shaker for 96 h at 180 rpm and 28 °C. The final concentration was 2.3×10^5 colony forming units (CFU) mL^{-1}, and the solution was stored at 4 °C until use. In addition, a portion of Gm and Ms inocula was autoclaved at 121 °C for 90 min three times for control treatments.

Experimental treatment and design

The experimental design was full factorial, with five inoculation treatments and corresponding five non-inoculation treatments: the five inoculation treatments (M_1–M_5) were conducted with five different proportions of Gm and Ms; the five non-inoculation treatments (CK_1–CK_5) were conducted with five different proportions of sterilized Gm, filtrate Gm, and sterilized Ms. The specific details of these treatments are listed in Table 1. The filtrate Gm used in the non-inoculation treatments was a filtrate (< 20 μm) of Gm inocula to provide the microbial populations accompanying the Gm inocula. Each treatment was replicated three times in a randomized block design with one plant for each replicate. On 10 May 2012, three castor bean seeds were surface-sterilized by soaking in a 5 % NaClO solution for 10 min and then rinsed with sterile distilled water. The seeds were then transferred to pots (diameter 25 cm, height 30 cm) and filled with 600 g unsterilized substrate. Seeding depth was 1.5 cm. Each pot had three seedlings. Before semination, Gm, Ms, sterilized Gm, filtrate Gm, and sterilized Ms were placed in the soil below the castor bean seeds according to the design. Plants were irrigated with water at 3-day intervals for 60 days.

Harvest and measurement

Whole plants were harvested from pots after 60 days, and the soil that adhered to the roots was collected in a sterile culture dish as ''rhizosphere soil''. Soil samples were divided into two parts: one part was stored at 4 °C for biological and biochemical analyses, whereas the other was air-dried at room temperature for chemical analysis.

All shoot and root samples were dried in a forced-air oven at 80 °C for 72 h for biomass determination. The AMF colonization was determined according to McGonigle et al. (1990). The chlorophyll concentrations of leaf as well as the P content of shoots and roots were calculated according to Gao (2000).

Analysis of soil property

The available P concentration of the soil was determined using the sodium bicarbonate-extractable P colorimetric method (Olsen et al. 1954). Soil pH was measured using a glass electrode and a soil-to-water ratio of 1:2.5 (Dick et al. 2000). EC of the soil was measured using a conductivity meter (Model DDS-11A; Leizi, Shanghai, China). Soil organic matter (SOM) in soil samples was estimated according to standard methods (Kalra and Maynard 1991). The most probable number technique

Table 1 Treatments in the experiment

Treatment	Gm (g)	Ms (mL)	Sterilized Gm (g)[a]	Filtrate Gm (mL)[b]	Sterilized Ms (mL)[c]
M_1	30	10	0	0	0
CK_1	0	0	30	30	10
M_2	25	15	0	0	0
CK_2	0	0	25	25	15
M_3	20	20	0	0	0
CK_3	0	0	20	20	20
M_4	15	25	0	0	0
CK_4	0	0	15	15	25
M_5	10	30	0	0	0
CK_5	0	0	10	10	30

Gm Glomus mosseae (Nicol. and Gerd.), *Ms Mortierella* sp.
[a]Soil inocula were autoclaved at 121 °C for 90 min three times
[b]A filtrate (< 20 μm) of soil inocula to provide the microbial populations accompanying the Gm inocula
[c]Ms inocula were autoclaved at 121 °C for 90 min three times

was applied for the estimation of Ms populations in the soil (Li et al. 2008). The activities of soil invertase (E.C. 3.2.1.26), catalase (E.C. 1.11.1.6), urease (E.C. 3.5.1.5), and phosphatase (E.C. 3.1.3.2) were determined according to Ohshima et al. (2007), Trasar-Cepeda et al. (1999), Nannipieri et al. (1980), and Kandeler et al. (1999), respectively. The activities of soil enzymes were assayed 1 week after sampling.

Statistical analysis

All data were analyzed statistically by analysis of variance using the SPSS software package (SPSS 15.0 for Windows) with the means separated using the Duncan test at P values <0.05.

Results

Plant growth

Except for the M_2 treatment, inoculation treatments increased the root and shoot dry weights of plants significantly compared with the control plants ($P < 0.05$, Fig. 1a, b). Treatments of M_3, M_4, and M_5 showed significantly higher root and shoot dry weights than M_1 and M_2. M_4 had the highest dry weight (root and shoot). No significant difference was found between M_3 and M_5. Treatments of M_2, M_4, and M_5 had significantly higher root-to-shoot dry weight ratio than the control. M_4 had the highest ratio ($P < 0.05$, Fig. 1c).

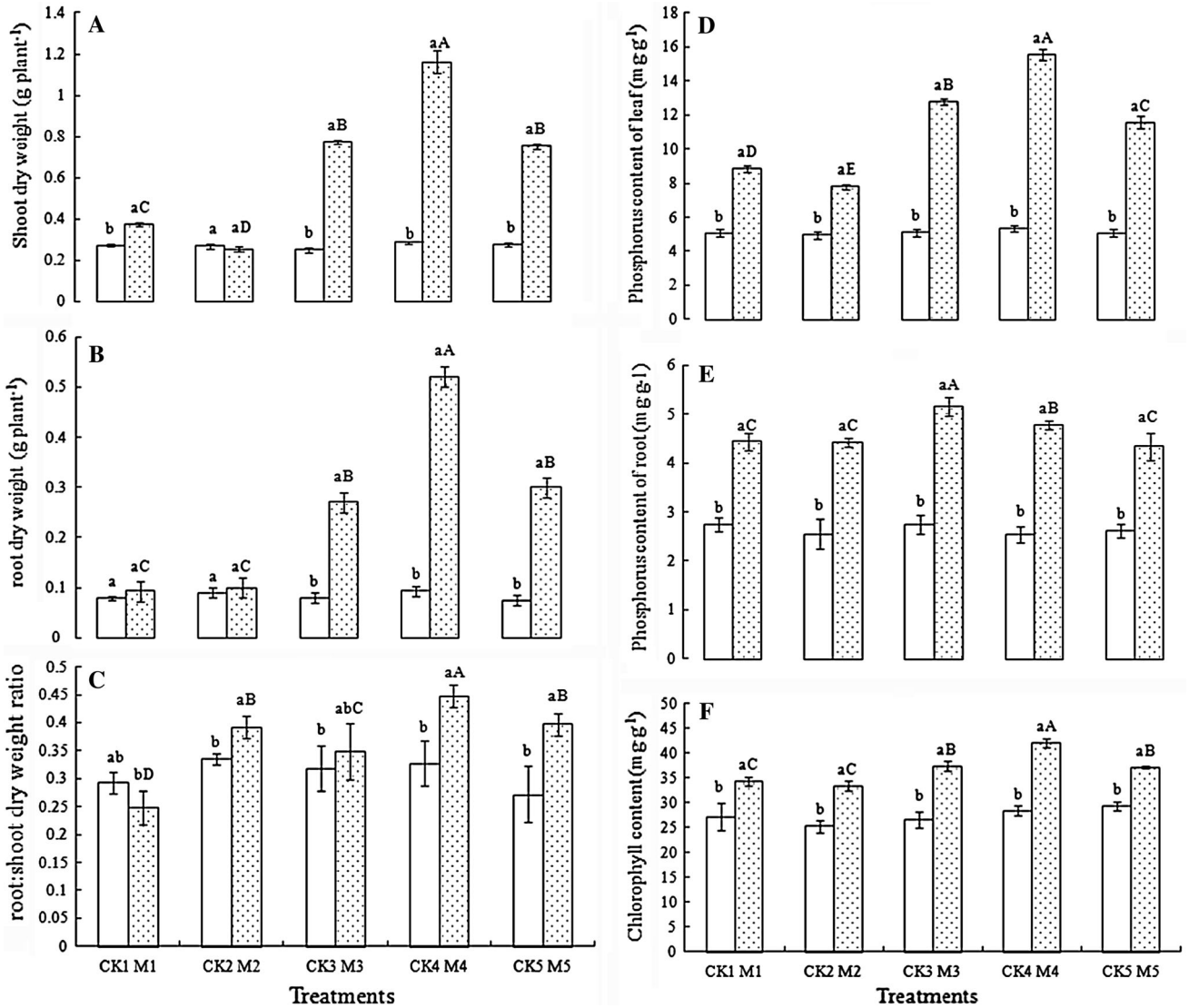

Fig. 1 **a** Shoot dry weight; **b** root dry weight; **c** root-to-shoot dry weight ratio; **d**, **e** P contents of the leaf (**d**) and root (**e**); and **f** chlorophyll contents of leaves of the castor bean with different proportions of *Glomus mosseae* (Gm) and *Mortierella* sp. (Ms) treatments. Data are expressed as mean ± SE of three replicates. Means followed by the same *lowercase letters* (between the same proportion of inoculation and control treatments) or the same *capital letters* (within five inoculation treatments) are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test

P and chlorophyll concentrations of plant

P concentrations of leaves and roots were enhanced significantly by inoculation treatments compared with control plants ($P < 0.05$, Fig. 1d, e). According to the positive effects of inoculation on P concentrations of leaves, the order is $M_4 > M_3 > M_5 > M_1 > M_2$. However, significant differences were observed among treatments. P concentration in the roots of M_3 had the highest value among all inoculation treatments by a significantly higher margin ($P < 0.05$, Fig. 1e). No significant difference was found among the P contents in the roots of M_1, M_2, and M_5 ($P > 0.05$, Fig. 1e).

Compared with the control plants, inoculations with any proportion of Gm and Ms exhibited significantly increased chlorophyll concentrations in leaves ($P < 0.05$, Fig. 1f). The chlorophyll concentration of M_4 was significantly higher than that of M_3 and M_5, and inoculations with M_1 and M_2 showed significantly lower concentrations of chlorophyll in leaves ($P < 0.05$, Fig. 1f). However, no significant difference between M_3 and M_5 was observed, and the same phenomenon was observed between M_1 and M_2 ($P > 0.05$, Fig. 1f).

AM colonization and Ms populations

All plants inoculated with whatever proportions of Gm and Ms showed significantly higher root colonization than the control ($P < 0.05$, Fig. 2a). AM colonization increased along with the increasing proportion of Ms, except for M_5. Plants inoculated with M_4 showed the highest root colonization. Ms populations were not found in the control soil samples, whereas Ms populations increased because of the increasing proportion of Ms in inoculation treatments (Fig. 2b).

Physical and chemical properties of soil

The pH values of all inoculated soils were lower than those of the control soils (Fig. 3a). Meanwhile, the pH values of inoculated soil declined with increasing proportion of Ms and reached the minimum value at M_5 treatment. Colonization with any proportion of Gm and Ms showed significantly higher available P content in soil compared with controls ($P < 0.05$, Fig. 3b). Available P concentrations of inoculated soils increased as AMF colonization increased. Treatments of M_3, M_4, and M_5 had significantly lower EC values than those of controls ($P < 0.05$, Fig. 3c). SOM contents of M_2, M_4, and M_5 were significantly higher than those of controls ($P < 0.05$, Fig. 3d).

Soil enzyme activities

The radar graphs show the ratios of soil enzyme activities in inoculated soil compared with those in the control soil ($T_i = M_i/CK_i$; e.g. for urease activity, T_1 is the ratio of the urease activities of M_1 to those of CK_1). The increase in urease activities in T_4 was significantly higher than that in other treatments ($P < 0.05$, Fig. 4), and other inoculation treatments showed no significant difference in urease activities when compared with the control soil sample ($P > 0.05$, Fig. 4). Inoculation with Gm and Ms could improve invertase activities in soil, and significant differences were found for M_3, M_4, and M_5 compared with the control soil samples ($P < 0.05$, Fig. 4). The increase in neutral phosphatase and alkaline phosphatase activities in T_3 and T_4 were significantly higher than those in other treatments ($P < 0.05$, Fig. 4), and M_4 treatment had the highest phosphatase activity. Data on T_2, T_3, and T_4 showed that catalase activities of CK_2, CK_3, and CK_4 were significantly higher than those of the inoculated treatments ($P < 0.05$, Fig. 4).

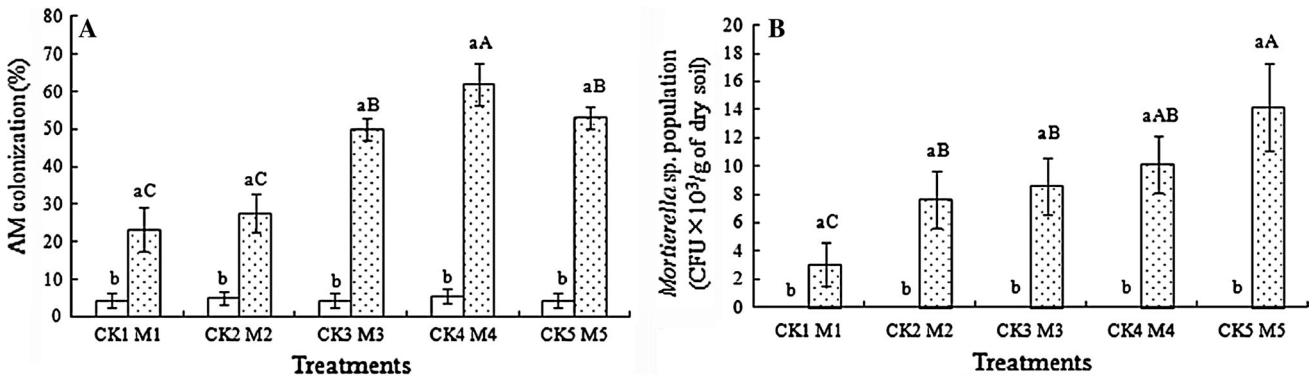

Fig. 2 **a** Arbuscular mycorrhizal colonization and **b** MS populations of different proportions of Gm and Ms treatments. Data are expressed as mean ± SE of three replicates. Means followed by the same *lowercase letters* (between the same proportion of inoculation and control treatments) or the same *capital letters* (within five inoculation treatments) are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test

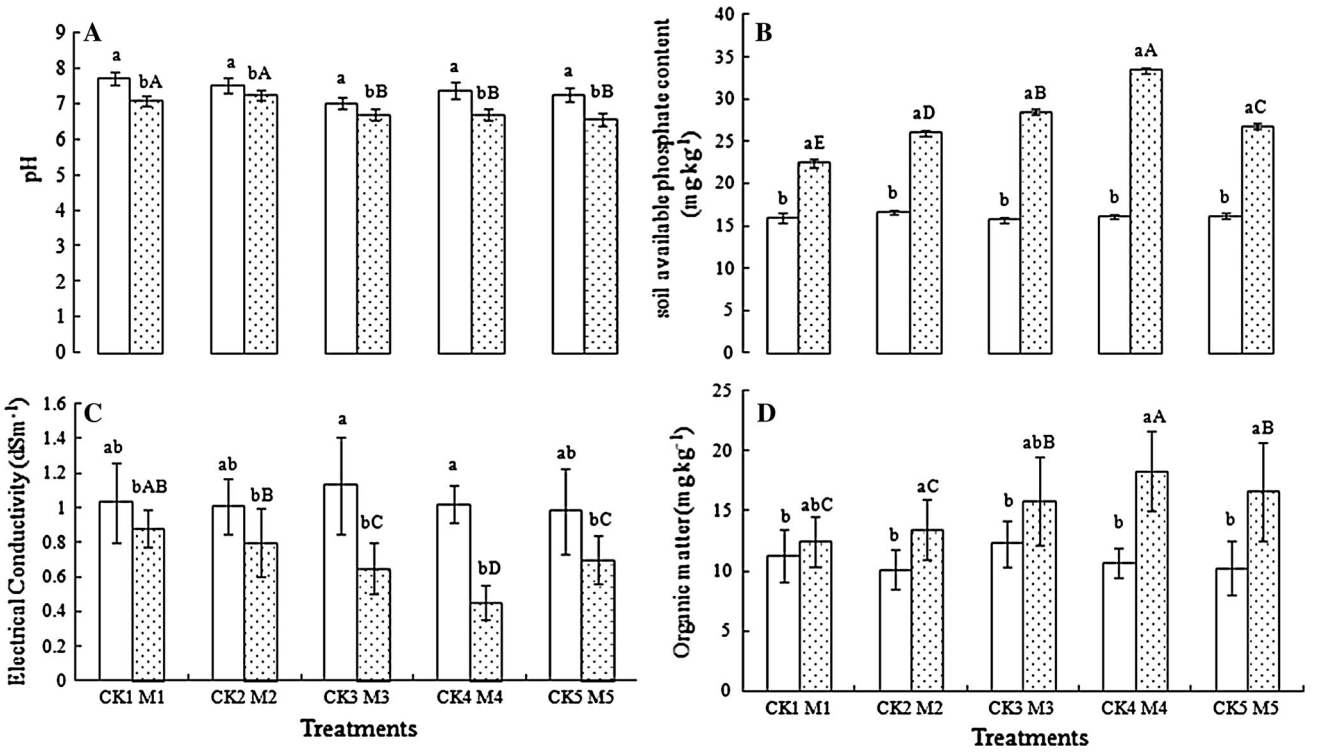

Fig. 3 **a** pH, **b** available P content, **c** electrical conductivity, and **d** organic matter content of different proportions of Gm and Ms soil treatments. Data are expressed as mean ± SE of three replicates. Means followed by the same *lowercase letters* (between the same proportion of inoculation and control treatments) or the same *capital letters* (within five inoculation treatments) are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test

Discussion

Interaction among Gm, Ms, and indigenous microorganisms

Although salinity suppresses the formation of AM, AMF has been known to occur naturally in saline environments (Yamato et al. 2008). Mycorrhizal roots colonized by indigenous AMF of non-inoculated soils were observed in the present study, and inoculation treatments could significantly enhance AM colonization rate. Nevertheless, the results of this study also showed that mycorrhizal root colonization increased with the increasing proportion of Ms, along with decreasing proportion of Gm. This finding is probably attributable to the fact that the Ms population and AMF in the inocula can promote the release of available P needed for the growth of both the indigenous and the inoculated AMF. Simultaneously, Clark (1997) indicated that a high pH (> 7.5) could limit root colonization by AMF. Therefore, the decreased soil pH and EC (Fig. 3a, c) observed in this study, which induced by inoculation of Gm and Ms, may conversely provide an appropriate environment for AM colonization and other microbial populations in the rhizosphere, such as mycorrhiza helper bacteria, which are known to stimulate mycelial growth of mycorrhizal fungi and/or enhance mycorrhizal formation (Gryndler et al. 2000). Furthermore, the presence of soil microorganisms cause the application of Ms to produce compounds that increase root cell permeability and the rates of root exudation, which would then stimulate mycorrhizal fungal mycelia in the rhizosphere or facilitate root penetration by the fungus (Khan et al. 2009). Moreover, the Ms population increases with the increasing proportion of Ms in inoculated treatments. This result confirms that PSM interacts well with AMF in P-deficient soils or saline soil (Poi et al. 1989; Zhang et al. 2011). Meanwhile, the AM colonization rate reached a peak value under M_4 treatment and then declined (Fig. 1) probably because of the minimal amount of AMF inocula in M_5, which generally decreased the colonization of plant roots by AMF. Simultaneously, Osorio and Habte (2013) found that both Ms and *G. fistulosum* were established in the root cortex of leucaena as endophytes, where they appeared to interact competitively for root colonization sites. However, Bolan (1991) reported that P application to P-deficient soils can increase AM colonization. All these results may indicate that AM colonization can be stimulated by PSM in coastal saline soil, such that an appropriate proportion of AMF/PSF should be established.

Available P, pH, EC, and SOM response to different proportions of Gm and Ms

The results of this study show that the available P contents of inoculated soils increased with increasing Ms proportion, except in M_5. This trend may be explained by the fact that PSM can solubilize inorganic P compounds, which is known to reverse the process of P-fixation. Simultaneously, the increasing AM colonization could also result in increased available P content. Because Lapeyrie (1988) suggested that organic acid production by AM could solubilize the insoluble mineral P. Therefore, the highest proportion of Ms (M_5) only showed a lower concentration of available P compared with M_4 and M_3 (Fig. 3b) when M_5 exhibited lower AM colonization. Moreover, soil phosphatase mediates the release of inorganic P from organically bound P and returns it to the soil (García-Gil et al. 2000). Accordingly, the enhanced neutral phosphatase and alkaline phosphatase activities in T_3 and T_4 (Fig. 4) are possibly key reasons for the increase in available P content in inoculated soils. However, the highest soil available P concentration and the highest P concentration in the leaf were observed in plants under the M_4 treatment (Fig. 1d). Owing to M_4 treatment, this set-up had the highest AM colonization (Fig. 2a), and the extensive mycorrhizal hyphae network that formed around roots can efficiently take up P released by the Ms. Thus, a higher plant P uptake was stimulated by the combined inoculation of Ms and Gm. This observation may indicate that available P concentration in saline soil had positive correlations with Ms population and AM colonization, and AMF can absorb P from the soil solution (Bolan 1991).

In this study, pH values of the inoculated soil declined because of the increasing proportion of Ms. This result may confirm that the major mechanism in the solubilization of rock phosphate by Ms is related to the reduction in pH attributed to organic acid production (Osorio and Habte 2001). However, the function of AMF in the reduction of pH attributed to organic acid production should also be considered. The result of EC values also showed a negative correlation with AM colonization but not with Ms population. This result may be attributed to the capability of AMF to promote castor bean growth significantly (Fig. 1a, b), consequently enhancing the uptake of salt ions. Similarly, Giri et al. (2003) reported that mycorrhiza inoculation significantly reduced soil EC. By contrast, our former study found that AMF and PSM inoculation treatments significantly enhanced soil EC under greenhouse conditions (Zhang et al. 2011). Therefore, further research is required to explain the underlying mechanism behind this phenomenon.

Glomalin is a glycoprotein produced by AMF, the majority of which is contained in hyphae and spores of AMF (Driver et al. 2005). Glomalin was previously reported to be a critical portion of SOM (Haddad and Sarkar 2003). In this study, increasing AMF colonization rates suggested an increase in glomalin production. Furthermore, more glomalin is secreted by AMF under saline conditions than under normal conditions (Hammer and Rillig 2011). In addition, glomalin acts as an insoluble glue to stabilize aggregates, which decreases organic matter degradation significantly by protecting labile compounds within soil aggregates (Wright and Anderson 2000). Thus, SOM contents would be enhanced. Results showing that SOM contents of M_2, M_4, and M_5 treatments were enhanced significantly by Gm and Ms confirm the aforementioned hypothesis. M_4 treatment had the highest values in SOM content. Moreover, the highest AM colonization can probably be attributed to M_4 treatment.

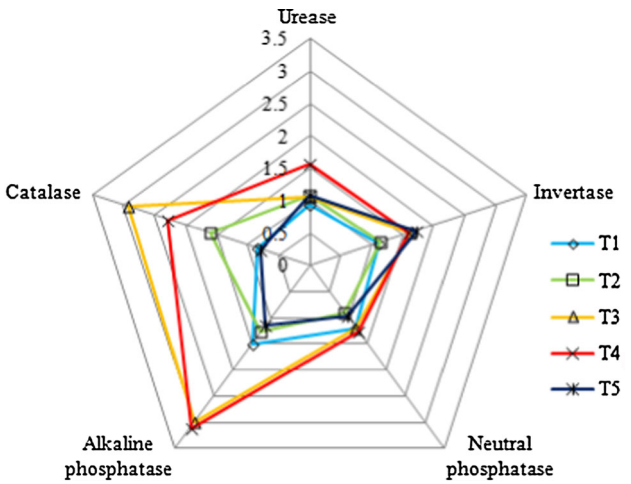

Fig. 4 Radar graphs illustrating the increases in soil enzyme activities after addition of a different proportion of Gm and Ms in saline soil under the greenhouse condition. $T_1 = M_1/CK_1$; $T_2 = M_2/CK_2$; $T_3 = M_3/CK_3$; $T_4 = M_4/CK_4$; $T_5 = M_5/CK_5$. Exceptional catalase activity of $T_i = CK_i/M_i$

Effects of optimized inoculation on rhizosphere enzyme activities

Soil enzyme activity is often used as an index of total microbial activity and fertility of a soil sample. Dhruva et al. (1992) also reported that soil enzyme activity is an indicator of ecosystem function rather than just a measurement of perturbation. Although soil salinity inhibits some soil enzyme activities (Frankenberger and Bingham 1982; García and Hernandez 1996), coastal saline soil enzyme activities were enhanced by different combined proportions of Gm and Ms, apart from catalase, in this study. This observation can be attributed to two reasons. First, both Gm and Ms inoculations increased soil microbial biomass, which is believed to be the primary origin of the soil enzyme (Nannipieri et al. 2003). Second, the decreased soil pH and EC induced by the co-inoculation of Gm and Ms may have mitigated the negative effects of salt stress on enzyme activities. In particular, the result of M_4 treatment had the most po-

sitive effect on soil enzyme activities, indicating that an appropriate proportion or amount of exotic microbes is needed to collaborate with indigenous microorganisms.

However, catalase activities were inhibited significantly by the inoculation of Gm and Ms, which is contrary to the results of Frankenberger and Bingham (1982). This difference may be attributed to the inoculation of Gm and Ms, which had mitigated the negative effects of salt stress on plant growth excellently compared with non-inoculated plants, as well as to the lower catalase activities needed to eliminate salt stress. Moreover, soil enzyme activities can be increased, decreased, or unaffected by soil acidity (Kang and Freeman 1999; Acosta-Martinez and Tabatabai 2000). In this study, lower soil pH of inoculation treatments was a probable cause of the decreased catalase activities. In addition, the changing proportion of Gm and Ms, presence of soil colloids, and even harsh environments limit extracellular and intracellular enzyme activities (Nannipieri et al. 2003). Therefore, further research is necessary to explain the different responses of enzyme activities of coastal saline soils inoculated with different proportion of PSF and AMF.

Effects of inoculation on plant growth

The different proportions of Gm- and Ms-inoculated treatments improved the dry matter yield of castor bean significantly. This improvement may be attributed to the amelioration of the features of saline soil as well as to the promotion of plant P absorption by microbe inoculation, as previously described. The plant root is the key structure in contact with saline soil, such that greater inhibitory effects of salt stress on shoot growth than on root growth and larger plant root-to-shoot ratios under saline conditions have been reported by some researchers (Berta et al. 1995; Zandavalli et al. 2004; Sheng et al. 2009). However, the current experiment showed that inoculated plants exhibited larger root-to-shoot dry weight ratios compared with non-inoculated plants. This finding suggests that greater plant root-to-shoot ratios may be a protective reaction against salt injury, and the inoculation of Gm and Ms is likely to stimulate the protective reaction by transporting more photosynthates to roots than non-inoculated plants. In contrast to our results, Zandavalli et al. (2004) and Sheng et al. (2009) reported that AM colonization decreased plant root-to-shoot ratio under saline conditions. This observation can probably be attributed to the different salt resistance mechanisms of plants. Moreover, microbe inoculation significantly improved chlorophyll concentrations in leaves, which may be attributed to phytohormone production resulting from the increase in soil soluble P promoted by Ms in saline soil (Toro et al. 1996) or to the reduced interference of salt owing to chlorophyll synthesis in mycorrhizal compared with non-mycorrhizal plants (Giri and Mukerji 2004; Wu et al. 2010).

In conclusion, mycorrhizal root colonization and Ms population were stimulated by the increasing proportion of Ms in fungal inocula, along with decreasing proportion of Gm in coastal saline soil under greenhouse conditions. The interaction between different proportions of Gm and Ms may be beneficial to the reclamation of coastal saline soil property owing to the increase in available soil P and organic matter contents, decreasing soil pH and electrical conductivity values, and stimulation of soil enzyme activities (i.e., urease, invertase, neutral phosphatase, and alkaline phosphatase). However, the appropriate proportions of Gm and Ms were crucial in the collaboration with indigenous microbes and in the stimulation of P uptake and chlorophyll concentrations in leaves of castor bean. The results from this study prove that the application of an appropriate proportion of arbuscular mycorrhizal fungi and P-solubilizing microorganisms will benefit the amelioration of saline soils in the coastal region of eastern China and promote the growth of the oilseed plant castor bean.

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