

Effects of Inoculation of Arbuscular Mycorrhizal Fungus and *Apophysomyces spartina* on P-uptake of Castor Oil Plant (*Ricinus communis* L.) and Rhizosphere Soil Enzyme Activities under Salt Stress

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Abstract [Objective] In order to explore the mechanism of combined inoculation microorganisms in improving coastal saline soil property and plant growth. [Method] The pot experiment was used to assess the effects of different inoculated proportion of arbuscular mycorrhizal fungi (AMF) and Phosphate-solubilizing fungus, *Apophysomyces spartina*, on growth, chlorophyll contents, P-uptake of castor bean (*Ricinus communis* L.) and rhizosphere soil pH values, available P concentrations, enzyme activities. [Result] The mixed inoculation of AMF and *A. spartina* significantly reduced soil pH value, increased soil available phosphorous contents, improved the activities of soil invertase, urease, neutral phosphatase, and alkaline phosphatase. Chlorophyll contents, P-uptake, and plant dry weight of castor bean were also increased. The optimal proportion of the number of AMF spores to *A. spartina* colonies was 28.56:11.5×10⁵, which had positive effects on saline soil and could stimulate plant growth under greenhouse condition. [Conclusion] Appropriate proportion of AMF and *A. spartina* had the potential to enhance coastal saline soil property and promote castor bean growth.

Key words AM fungus; *Apophysomyces spartina*; Castor bean; Soil enzyme; Coastal saline soil

Castor oil plant (*Ricinus communis* L.) is a species of flowering plant in the spurge family, *Euphorbiaceae*, and its seed is the castor bean, which contains 40% – 60% of oil^[1]. Castor oil has a wide range of uses, such as for pharmaceuticals, paints, aviation lubricants, inks, etc., which lead to a steady increase in market demand for castor oil. However, the current world production of castor oil is difficult to meet industrial demand. With the continuous shrink of arable land area and increase of population, the use of large areas of saline coastal areas to grow castor gains more and more concerns^[2]. However, soil salinization is one of the major limiting factors for crop produc-

tion in coastal area^[3]. One important reason is the salt stress which reduces the absorption of plants to mineral nutrients, especially phosphorus. Because phosphate ions and Ca²⁺, Mg²⁺, Zn²⁺ are easy to convert into the forms which are difficult to absorb by the plants in the saline-alkali soil^[4–5]. Currently in addition to the application of chemical fertilizer and genetic engineering measures, the use of microorganisms to mitigate the adverse effects of phosphorus deficiency and salt stress has become an effective way to promote crop growth, which is an economical and environmentally friendly^[6–7].

Arbuscular mycorrhizal (AM) fungus has been proved to reduce yield

盐胁迫下 AM 真菌和盐沼磷质霉菌对蓖麻磷吸收和根际土壤酶活性的影响

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摘要 [目的]为了探索复合接种微生物对海滨盐土改良以及促进生长植物的作用机理。[方法]利用盆栽试验考察在海滨盐土中接种不同比例的 AM 真菌和盐沼磷质霉菌 (*Apophysomyces spartina*)对蓖麻生长、叶绿素含量、磷吸收和土壤速效磷含量、pH 值、土壤酶活性的影响。[结果]AM 真菌和盐沼磷质霉菌混合接种可显著降低土壤 pH 值, 增加土壤速效磷含量, 提高土壤脲酶、转化酶、中性和酸性磷酸酶活性, 同时提高了叶片叶绿素含量并增加了蓖麻对磷营养的吸收, 在盐胁迫下有效促进了蓖麻幼苗生长。温室条件下在海滨盐土中接种 AM 真菌和盐沼磷质霉菌促进蓖麻生长最有效的混合比例是 28.56:11.5×10⁵ (AM 真菌孢子数: 盐沼磷质霉菌落数)。[结论]适当比例的 AM 真菌与解磷真菌具有提高海滨盐土质量并促进蓖麻生长的潜力。

关键词 AM 真菌; 盐沼磷质霉菌; 蓖麻; 土壤酶; 海滨盐土

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losses of plants in saline alkali soil, which probably because it can increase the absorption of nutrients^[8-9], reduce sodium intake^[9], and improve Rhizobium content and soil conditions^[10]. In addition, some soil microbes can dissolve insoluble phosphate^[11], which are called P-solubilizing microorganisms (PSM), including bacteria (such as *Pseudomonas*, *Enterobacter*, *Bacillus subtilis*), fungi (such as *Penicillium* and *Aspergillus*, etc.) and actinomycetes^[12]. The P-solubilizing mechanism of PSM might be relevant to the organic acid metabolism, ions chelation and exchange reactions of microorganisms growth environment^[13]. However, Gyaneshwar *et al.*^[14] pointed out that 70% of the PSM screened under laboratory conditions did not increased the crop production or not promote the phosphorus absorption of plants.

Studies have shown that mycorrhizal plants can release the carbohydrates into the rhizosphere soil, and PSM can use these carbohydrates to carry out the metabolism and secrete organic acids or release protons^[10]. In addition, the large mycelia network around the plant root system formed by AM fungi could effectively absorb the phosphate released by the PSM, which is transferred to the roots. Some studies also have proved the effectiveness of the mixed application of AM fungi and PSM^[15-17]. Previously, we had isolated a phosphate solubilizing strain from the coastal saline soil, which was identified as the *Apophysomyces spartina* (preserved in China General Microbiological Culture Collection Center, Accession No. CGMCCNo.7717), and we had proved that the mixed inoculation of this PSM strain with the AM fungus under salt stress had effectively improved the soil phosphorus release capacity and promoted plant growth^[7]. However, it remains a challenge to find the appropriate mixing ratio of AM fungus and PSM fungus under laboratory and field conditions. Therefore, in this study, we measured the indicators like the biomass of castor oil plant, phosphorus content in the roots and leaves, soil enzyme activities (such as converting enzyme, catalase, urease, neutral and alkaline phosphatase), soil

available phosphorus content to examine the effects of different inoculating proportions of AM fungus and PSM fungus on castor oil plant growth and coastal saline soil quality, with the aim to provide technical support and theoretical basis for the application of bio-fertilizers in the sustainable agricultural development of coastal saline soil in China.

Materials and Methods

Test soil

The test soil for pot culture was the coastal saline soil from Sheyang Salt Field, Sheyang County, Yancheng City, Jiangsu Province, and its physical and chemical properties were

Table 1 The physical and chemical properties of test soil

pH	Conductivity//dS/m	Total nitrogen g/kg	Hydrolysable nitrogen mg/kg	Total potassium g/kg	Total phosphorus//g/kg	Available phosphorus//mg/kg	Organic matter g/kg
8.47	1.05	0.29	31.65	15.27	0.25	19.37	10.56

shown in Table 1.

Test strains and microbial inoculum

The strains of AM fungi (AMF) were *Glomus mossea*, *Glomus diaphanum*, *Glomus etunicatum* and *Glomus caledonium*. The inoculant was the sandy soil containing spores (952 spores/100 g soil), hyphae, infected root segments obtained by expanding and propagating with sorghum as the host. The culture method of *A. spartina* (AS) was in accordance with that of ZHANG *et al.*^[7].

Test design

There were 5 treatments set according to the different proportions of AM and AS agents: AM agent of 30 g, AS agent of 10 ml (number of AMF spores:AS colonies = $71.42:4.6 \times 10^5$) (T1); AM agent of 25 g, AS agent of 15 ml (number of AMF spores:AS colonies = $52.61:6.9 \times 10^5$) (T2); AM agent of 20 g, AS agent of 20 ml (AMF spore number:AS colonies = $35.63:9.2 \times 10^5$) (T3); AM agent of 15 g, AS agent of 25 ml (number of AMF spores:AS colonies = $28.56:11.5 \times 10^5$) (T4); AM agent of 10 g, AS agent of 30 ml (number of AMF spores:AS colonies = $15.45:13.8 \times 10^5$) (T5).

And control group was set to each treatment, which was as follows: 30 g of sterilized AM fungi, 30 g of AM agent filtrate, 10 ml of AS medium (CK1); 25 g of sterilized AM fungi, 25 g

of AM filtrate, 15 ml of AS medium (CK2); 20 g of sterilized AM fungi, 20 ml of AM agent filtrate, 20 ml of AS medium (CK3); 15 g of sterilized AM fungi, 15 g ml of AM agent filtrate, 25 ml of AS medium (CK4); 10 g of sterilized AM fungi, 10 ml of AM agent filtrate, 30 ml of AS medium (CK5). Each treatment was repeated three times and arranged in random block.

The test was carried out in the greenhouse of the Halophyte Research Lab of Nanjing University. On May 10, 2009, the castor seeds were soaked in the 5% sodium hypochlorite solution for 10 min, and then rinsed with sterile distilled water. The unsterilized saline soil was mixed with the

vermiculite according to the quality ratio of 3:1, and each pot was added with 600 g of the mixture of saline soil and vermiculite as the substrate; each pot was sowed with 3-4 seeds, and after shooting, the castor oil plant seedlings with similar growth vigor were selected from each pot. According to the test design, appropriate doses of AMF agent and AS agent were inoculated into the soil, which were mixed evenly with the soil after adding. The test was begun on May 10, 2009, during the test, all plants were timely watered and managed regularly. In the greenhouse, the daily temperature was controlled at 28 and 18 °C at night, and the relative humidity was 65%-85%.

Soil samples collection

After 60 d, according to the method of Riley and Barber^[18], the whole plant was pulled out from the plastic pot to collect soil samples through softly shaking the roots, and the soil samples were collected by the a sterile petri dish. The soil samples were divided into 2 parts that one was preserved at 4 °C for biochemical analysis, and the other part was air-dried at room temperature for chemical analysis.

Analysis of plants and microorganisms

The biomass of the plant tissue was determined after baked in the oven at 80 °C for 72 h. The mycor-

rhizal infection rate was measured using the method of Phillips and Hayman^[19], and the number of AS colonies was determined using the method of Pikovskaya^[20].

Soil analysis

Chlorophyll content was measured by using the spectrophotometer method^[21], and with the digestion solution of Kjeldahl determination adding with vanadium molybdate as the chromogenic reagent, the phosphorus contents in the leaves and roots were measured by spectrophotometer method^[22]. Soil available phosphorus was measured using sodium bicarbonate extraction-Mo-Sb colorimetry^[23]. The activities of soil invertase, catalase, urease and phosphatase were determined according to the method of GUAN^[24].

Statistical analysis

SPSS 18.0 was used to carry out the Duncan multiple range analysis of the test data.

Results and Analysis

AM infection rate and number of AS colonies

As shown in Table 1, the castor oil plants with no inoculation showed low AMF infection rate, while the roots of the plants inoculated with AMF and AS at any proportion showed significantly higher infection rate than the control ($P < 0.05$). Mycorrhizal infection rate increased with the decrease of AMF proportion, and the infection rate of the inoculated plants reached the highest in treatment T4. However, the infection rates of treatments T5, T3, T2 and T1 were all significantly lower than that of treatment T4. The number of AS colonies increased with the increase of inoculation proportion of AM to AS in the soil (Table 1).

Plant biomass

In addition to treatment T2, the plant dry weights of all other inoculated treatments increased significantly ($P < 0.05$, Table 2). And the dry weights of treatments T3, T4 and T5 were significantly higher than those of treatments T1 and T2, and among all the treatments, the plant dry weight of treatment T4 was the highest. However, there was no significant difference in plant dry weight between treatment T3 and treatment T5.

Chlorophyll content

Compared with the control, the leaf chlorophyll contents in the plants inoculated with any proportion of AMF to MS increased significantly ($P < 0.05$, Table 2). The chlorophyll content of treatment T4 was significantly higher than that of treatment the T3 and T5, while the leaf chlorophyll contents of treatment T1 and T2 were relatively lower. There was no significant difference between treatment T3 and T5, which was similar to that between T1 and T2.

Plant phosphorus content

The phosphorus contents in the leaves and roots of the plants inoculated with AMF and AS increased sig-

nificantly ($P < 0.05$, Table 2). The effects of inoculated proportions of AMF and AS on leaf phosphorus contents in all treatments were in the order of T4 > T3 > T5 > T1 > T2. The phosphorous content in the root was the highest in treatment T3 among all treatments. There was no significant difference in root phosphorus content in treatment T1, T2 and T5.

Soil pH value and available phosphorus content

All inoculated soils showed lower pH values than that of the control soil (Table 3). Meanwhile, the pH value of the inoculated soil decreased with the increase of the inoculated proportion of AS, which reached the minimum in

Table 2 The AM infection rate and number of AS colonies in the treatments inoculated with different proportions of AM and AS

		Treatment				
		1	2	3	4	5
AM infection rate//%	T	23.33 aC	27.67 aC	50.46 aB	62.67 aA	53.58 aB
	CK	4.43 bA	5.13 bA	4.46 bA	5.57 bA	4.43 bA
AS colony number CFU×10 ³ /g of dry soil	T	3.06 aC	7.65 aB	8.62 aB	10.11 aAB	14.23 aA
	CK	0 bA	0 bA	0 bA	0 bA	0 bA

The different lowercase letters of the same indicator represent significant difference with the control, and the different uppercase letters represent significant difference with the treatments inoculated with different proportions of AM and AS.

Table 2 Effects of different inoculated proportions of AM and AS on the dry weight, chlorophyll content, leaf and root phosphorus contents of castor bean

		Treatment				
		1	2	3	4	5
Plant dry weight g/plant	T	0.37 aC	0.25 aC	0.77 aB	1.16 aA	0.75 aB
	CK	0.27 bA	0.27 aA	0.25 bA	0.28 bA	0.28 bA
Total chlorophyll content//mg/g	T	34.4 aC	33.46 aC	37.41 aB	42.24 aA	37.23 aB
	CK	27.13 bA	25.26 bA	26.65 bA	28.43 bA	29.36 bA
Leaf phosphorus content//mg/g	T	8.88 aD	7.80 aE	12.77 aB	15.55 aA	11.55 aC
	CK	5.08 bA	4.97 bA	5.11 bA	5.34 bA	5.07 bA
Root phosphorus content//mg/g	T	4.43 aC	4.41 aC	5.14 aA	4.77 aB	4.33 aC
	CK	2.75 bA	2.55 bA	2.74 bA	2.55 bA	2.62 bA

The different lowercase letters of the same indicator represent significant difference with the control, and the different uppercase letters represent significant difference with the treatments inoculated with different proportions of AM and AS.

Table 3 Effects of different inoculated proportions of AM and AS on the pH and available phosphorus contents of the coastal saline soil

		Treatment				
		1	2	3	4	5
pH	T	7.08 bA	7.23 bA	6.70 bB	6.68 bB	6.56 bB
	CK	7.72 aA	7.52 aA	7.02 aA	7.38 aA	7.24 aA
Available phosphorus content//mg/Kg	T	22.49 aD	26.01 aC	28.47 aB	33.43 aA	26.80 aC
	CK	15.97 bA	16.57 bA	15.73 bA	16.13 bA	16.21 bA

The different lowercase letters of the same indicator represent significant difference with the control, and the different uppercase letters represent significant difference with the treatments inoculated with different proportions of AM and AS.

treatment T5. The pH values in treatments T3, T4 and T5 were significantly lower than the pH values of treatment T1 and T2 ($P < 0.05$). Soil available phosphorus contents of all inoculated treatments were significantly higher than the control ($P < 0.05$, Table 3). Available P content gradually increased with the increase of the inoculated proportion of AS, but the available P content was the highest in treatment T4, while decreased significantly in treatment T5.

Soil enzyme activity

Soil urease activity of treatment T4 was significantly higher than that in CK4, and also higher than all other treatments ($P < 0.05$, Table 4). There was no significant difference in urease activity among treatments T1, T2 and T3. The mixed inoculation of AMF and AS could improve the activity of soil invertase, and the invertase activities of treatments T3, T4 and T5 were significantly higher than that of the control ($P < 0.05$). The invertase activities of treatments T1 and T3 were significantly higher than those in treatment T2, T4 and T5, while there was no significant difference in invertase activities between treatments T2, T4 and T5. The neutral phosphatase activities of treatments T3 and T4 were significantly higher than that in all other treatments, which reached the highest in treatment T4 ($P < 0.05$). Compared with the control, there was no significant effect on neutral phosphatase activity in treatment T1, T2 and T5. The changes of alkaline phosphatase activities were similar to those of neutral

phosphatase activities, but the alkaline phosphatase activity was the highest in treatment T3 ($P < 0.05$).

Conclusions and Discussion

Although salinity could inhibit the formation of AM, numerous studies have shown that AMF could survive in saline environment^[25-26]. In this test, there were mycorrhizal structures in the treatments with no inoculation; while the treatments inoculated with AM fungi significantly improved the mycorrhizal infection rate. The results also showed that the mycorrhizal infection rate of castor oil plant increased with the decrease of AMF inoculation proportion, which might probably because the increase of AS proportion increased the content of soil available phosphorus that was needed for the growth of AM fungi. The results proved our precious speculation that AS could promote the secretion of plant hormones in saline soil which could increase the content of available phosphorus and improve mycorrhizal infection rate^[7]. Meanwhile, the mycorrhizal infection rate reached the peak when the proportion of number of AMF spores to number of AS colonies was $28.56 : 11.5 \times 10^5$, and then declined. This might because the increase of soil available phosphorus content restricted the AM fungi infection of plant root system^[27]. All of these results indicated that there was a critical phosphorus concentration in the coastal saline soil planted with AMF. In addition, the in-

crease of other nutrients contents in the liquid fungi agent could also affect the mycorrhizal infection rate. Valentin and Michele^[28] observed that the application of nitrogen could promote the mycorrhizal colonization, but Corkidi *et al.*^[29] found that high nitrogen level could decrease or inhibit mycorrhizal colonization.

In this study, the results showed that the mixed inoculation of PSM and AMF could significantly improve the dry weight of plants. The similar synergistic effect has also been found in plants like cotton^[29], soybean^[30]. The reason might that the mixed inoculation of PSM and AMF could form a broad mycorrhizal hyphae network to effectively absorb the dissolved phosphorus, which could stimulate plant fixation process^[11]. In addition, AMF could promote the adsorption to phosphorus of plants growing under saline conditions, which could reduce the negative effects of Na^+ and Cl^- , thereby maintaining the integrity of vacuolar membrane^[31]. These conclusions had been proved by the results of this study that inoculating AMF and AF could increase the phosphorous contents in leaves and roots. Many studies have observed a higher chlorophyll content in mycorrhizal plants under salt stress^[32], suggesting that under salt stress mycorrhizal plants have stronger chlorophyll synthesis capacity than the non-mycorrhizal plants^[33]. In this study, the results also showed that the mixed inoculation of AMF and AS significantly increased plant chlorophyll content. This might because the

Table 4 Effects of different inoculated proportions of AM and AS on the urease, invertase, neutral phosphatase, alkaline phosphatase and catalase in coastal saline soil

		Treatment				
		1	2	3	4	5
Urease// $\text{NH}_3\text{-N}/(\text{g soil})24\text{ h}$	T	1.77 bD	2.14 abCD	1.98 abD	3.14 aA	2.54 abB
	CK	1.87 abB	1.99 bB	1.88 bB	2.00 bB	2.37 bA
Invertase// $\text{mg Glu}/(\text{g soil})24\text{ h}$	T	19.22 abA	14.73 abBC	19.29 aA	12.89 aC	13.03 aC
	CK	17.39 bA	12.88 bB	11.87 bB	8.03 bC	7.61 bC
Neutral phosphatase// $\text{mgP}_2\text{O}_5/(\text{g soil})24\text{ h}$	T	2.14 abCD	1.91 bD	2.66 aB	3.03 aA	2.20 aCD
	CK	1.87 bC	2.12 abB	2.23 bB	2.39 bA	2.28 aB
Alkaline phosphatase// $\text{mgP}_2\text{O}_5/(\text{g soil})24\text{ h}$	T	1.42 abC	0.94 abD	2.25 aAB	1.85 aB	0.88 aD
	CK	0.94 bA	0.74 bB	0.75 bB	0.59 bC	0.77 bB
Catalase// $\Delta A/(\text{g soil})\text{min}$	T	2.38 bB	2.64 bB	2.66 bB	2.90 bB	3.27 abAB
	CK	4.46 aA	3.71 aB	3.53 aB	3.87 aB	2.76 bC

The different lowercase letters of the same indicator represent significant difference with the control, and the different uppercase letters represent significant difference with the treatments inoculated with different proportions of AM and AS.

inoculation of AS increased the content of soil available phosphorus in saline soil, which could stimulate the production of plant hormone^[34].

PSM could dissolve soil inorganic phosphorus compounds, reversing the process of phosphorus fixation^[35]. However, Bolan^[36] pointed out that AM fungi only absorbed phosphorus from soil solution, while there was no evidence that it can dissolve insoluble soil phosphate substances. In this test, we proved that the inoculation of AMF and PSM of different proportions could significantly increase the available phosphorus content in soil, which was consistent with the results of our previous study on the effects of inoculating *G. mosseae* and AM on *Kosteletzkya virginica* growth and enzyme activities of rhizosphere and bulk soils^[7]. Meanwhile, the content of available phosphorus was highest in T4 which also had the highest mycorrhizal infection rate, rather than in T5 which had the largest number of AM colonies. This may because AMF could stimulate the P-solubilizing capacity of AS^[37]. Currently, the theory of organic acid has been widely accepted as the phosphate solubilization mechanism of microorganism. Maliha *et al.*^[38] believed that the organic acid released from soil fungi or any other P-solubilizing microbial resulted in the decrease of pH value. In this test, the synergistic effects between AMF and AS reached the peak at the proportion of number of AMF spores to number of AS colonies of 28.56:11.5×10⁵. It suggested that the infection rate of AMF reached the maximum at this proportion, which could secrete organic acid or release protons to make the soil pH reach the minimum value, and finally the concentration of soil available phosphorus reached the highest.

The measurement of soil enzyme activities has been proposed as an indicator of the soil ecological changes resulted from the interactions between inoculated microbial and indigenous microbial populations in the soil^[39–41]. Phosphatases mediates the process in which inorganic phosphorus releases from organic phosphorus compound and returns to the soil^[42]. Urease, which catalyzes the hydrolysis of urea, plays an important role in the nitrogen cycle

of releasing inorganic nitrogen^[43]. Catalase has been proved to be part of the antioxidant defense system. Satefani *et al.*^[44] suggested that the activity of catalase activity was an important indicator of soil fertility. Converting enzyme plays a key role in releasing carbohydrates of low molecular weight as the energy of microorganisms. Although the activities of alkaline phosphatase, catalase, invertase and urease are inhibited by soil salinity^[45], in this study, the inoculation of AMF and AS enhanced the activities of soil enzymes (*i.e.*, urease, invertase, neutral and alkaline phosphatase) under salt stress. This might because the inoculation of AMF and AS increased the biomass of soil microbial, which could mitigate the negative effects of salt stress on enzyme activities^[7]. In this study, the highest value of soil enzyme appeared at different proportions of AMF to AS, and due to the proportions of AMF to AS and the changes of nutrients in the microbial inoculums, it was difficult to explain the different responses of soil enzymes to the inoculation of AMF and AS. In addition, in the determination of enzyme activities, the activities of extracellular enzymes and intracellular enzymes^[40], as well as soil colloid and environment, which could restrict the microbial activities in cells, should also be taken into account^[41]. However, the inoculation of AMF and AS significantly inhibited the activity of catalase, which was contrary to the results of Frankenberger and Bingham^[46]. The reason might be that the inoculation of AMF and AS mitigated the salt stress, which had negative effect on plant growth, thus, it only needed catalase of low activity to eliminate salt stress.

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