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Effects of vesicular-arbuscular mycorrhiza on the protective system in strawberry leaves under drought stress

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Abstract Vesicular-arbuscular mycorrhiza (VAM) plays a very important role in improving plant drought resistance abilities. A pot experiment was conducted during the 2008–2009 strawberry growing season in the greenhouse at Baoding, Hebei Province, China, aiming at exploring the influence of VAM on the drought tolerance of strawberry and elucidating the interaction mechanism. Three treatments were designed in our study, including routine water supply treatment (CK-WW), drought stress treatment with VAM fungi (GM-DS), and drought stress treatment without VAM fungi (CK-DS). Four indicators of each treatment were measured, respectively, including protective enzyme activity, pigment content, plasma membrane, and osmoregulation. The results showed that the treatment inoculated VAM fungi can increase the protective enzyme activity, osmoregulation, and antioxidant capacity membranaceous, especially the activity of catalase (CAT) and ascorbate peroxidase (APX). Inoculating strawberry plants with VAM fungi under drought stress could also lead to increasing the content of leaf pigment, accelerating the accumulation of free praline and soluble protein, improving the transport speed of soluble sugar, restraining the decomposition of chlorophyll, decreasing the malondialdehyde content and plasma membrane conductivity, and improving the H^+ -ATPase activity.

Keywords VA mycorrhiza, drought stress, strawberry, membrane protection system, osmotic stress, leaf pigment

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1 Introduction

Drought is one of the main adversities for crop growth and development in north China, which causes cellular dehydration, cell wall hardening, and programmed cell death acceleration. How to improve crop drought resistance effectively becomes a matter of concern in agricultural production and research (Su et al., 2003). The VAM is fungi-plant mutual symbiosis. It has been illuminated in previous study that mycorrhiza formation could increase root absorbing surface, reduce the leaf water potential and permanent wilting point under drought stress, increase the stomatal conductivity, improve the rate of transpiration and relative water content of leaves, and also promote hydrological cycle in plants. In addition, the mycorrhiza formation can induce a series of defense responses in plants when experienced adversity. The main responses are listed as follows: (1) the reinforcement of plant cell walls, (2) the accumulation of pathogenesis-related proteins, and (3) the strengthening of secondary metabolites (Li et al., 2002). Many studies have shown that drought resistance of crops could be improved by VAM (Sylvia and Williaxns, 1992; Lu et al., 2003). For example, Zhang and He (2007) reported that arbuscular mycorrhiza fungi can improve the protective system and increase the drought resistance of *Artemisia ordosica*. Wu and Xia (2005) reported that the ability of drought defense of *A. ordosica* was promoted by inoculation with the arbuscular mycorrhiza fungi.

Strawberry is a perennial herb and grows fast. The bearing ability of strawberry is strong, and its root system is shallow. Therefore, strawberry is more vulnerable to drought stress (Wang et al., 2008). In addition, with the development of greenhouse cropping, the yield and quality of strawberry is affected more and more seriously by drought stress, especially in the period of fruit enlargement due to the insulation and few watering in greenhouse (Xu and Chen, 2008). For the above reasons, strawberry was selected as the trial crop in our study and *Glomus mosseae* as the trial VAM. The objective of this study

was to illuminate the interaction mechanism between VAM fungus and host plant by testing the influences of the formation of VAM under drought stress on the protective system of strawberry leaves. The results may lay a foundation for further research.

2 Materials and methods

2.1 Materials

The trial strawberry variety is “Tudla,” which is multiplied by stolon. The trial VAM fungus *Glomus mosseae* was provided by the Beijing Academy of Agriculture and Forestry Sciences.

2.2 Methods

2.2.1 Preparation of plants with mycorrhiza

The strawberry seedlings with consistent growth were selected to be placed in nutritional bowls that contained sterilized matrix, which was the mixture of perlite and vermiculite in the proportion of 1:1. Each bowl was supplemented with 30 mL microbial inoculum (about 15000 inoculum potential units, IPU) (Li et al., 1999). Ten percent of plants were sampled periodically to analyze the infection rate (Phillip and Hayman, 1970). The plants were subjected to drought stress at a 90% infection rate. The treatment without microbial inoculum was set as control (CK).

2.2.2 Experiment treatments

Three treatments were designed as (1) drought stress treatment with VAM fungal inoculation (GM-DS), (2) drought stress treatment without VAM fungal inoculation (CK-DS), and (3) routine water supply without VAM fungal inoculation (CK-WW). The content of water in the treatment of routine water supply was 20% (percentage of weight). The water content in the treatment of drought stress was 10% (percentage of weight). The water content was adjusted at 8:00 am everyday. Each treatment was repeated three times, with five bowls per treatment. After conducting the experiment for 5 days, 10 days, and 15 days, all the indicators were tested including the perme-

ability of leaf cell membrane, the content of soluble protein (SP), soluble sugar (SG), dissociation proline and MDA, the activity of superoxide dismutase (SOD), peroxidase (POD), CAT and APX, and the content of photosynthetic pigment of leaves. The tests were conducted according to Zhang (2001). The membrane stability index (MSI) was tested according to He et al. (1999). The H⁺-ATPase was tested according to Ohnishi and Kobert (1975).

2.2.3 Data analyses

All the data were analyzed by DPS (Data processing system) software version 7.05. Differences among treatments were identified with Duncan’s multiple comparison test.

3 Results and analysis

3.1 Effects of VAM on the protective system in strawberry leaves under drought stress

3.1.1 Test of protective enzyme activity

The activity of protective enzymes in strawberry leaves after the formation of VAM under drought stress was shown in Table 1. The results showed that VAM fungus inoculation could slow down the decline of the protective enzyme activity caused by drought stress significantly. We can see in Table 1 that the protective enzyme activity of the treatments under drought stress was significantly lower than that of routine water supply treatment, which indicated that the drought stress decreased the activity of the four enzymes in strawberry leaves. Five days after the treatment, there was no significant difference for protective enzyme activity under drought stress between the treatments with or without VAM fungus inoculation. With the extension of processing time the four enzyme activity of the treatment without VAM fungus inoculation under drought stress appeared a downtrend, while the treatment with the inoculation kept comparatively stable. Ten days after the inoculation, the activities of SOD, CAT, and APX of the treatment with VAM fungus inoculation were significantly higher than those without the inoculation. The activity of POD had no significant difference between the treatments with or without the inoculation.

Table 1 Effects of VAM on the protection enzyme activity in strawberry leaves under drought stress

item (U·g ⁻¹ ·s ⁻¹ FW)	SOD			POD			CAT			APX		
	5 d	10 d	15 d	5 d	10 d	15 d	5 d	10 d	15 d	5 d	10 d	15 d
CK-WW	23.32a	22.13a	24.32a	223.22a	233.15a	231.24a	236.51a	241.63a	237.62a	1.18a	1.23a	1.22a
GM-DS	18.24b	19.22b	18.24b	199.53b	193.53b	181.84b	209.52b	212.33b	223.41b	1.01b	1.16b	1.14b
CK-DS	17.39b	16.27c	14.39c	194.22b	190.42b	166.74c	207.42b	205.24c	173.78c	1.07b	0.96c	0.64c

Note: Different letters in the same column indicate significance at 0.05 probability level.

Fifteen days after the inoculation, the four enzyme activities of the treatment with inoculation were significantly higher than those without the inoculation.

3.1.2 Assay of H⁺-ATPase activity, the MDA content, the plasmalemma stability, and relative electrical conductivity

In Fig. 1, the results indicated that the VAM fungus inoculation had a great effect on the content of MDA, the activity of H⁺-ATPase of plasmalemma, the stability of plasmalemma, and relative conductivity of plasmalemma under drought stress. Compared with the plant without VAM fungus inoculation, the stability of plasmalemma and the activity of H⁺-ATPase were increased; the content of MDA and the conductivity of plasmalemma were reduced in the inoculated plants. The VAM fungus inoculation had an obvious protective function on the plasmalemma. All trial physiological indicators of plasmalemma in the treatment without VAM fungus inoculation showed a declining trend with the extension of treated time under drought stress; however, the VAM fungus inoculation could obvious delay the downtrend. It was most obvious that VAM fungus inoculation could inhibit the increase of the MDA content caused by drought stress, and this suppression is closely related to the time of drought stress.

3.2 Effects of VA M on the osmoregulation matter content in strawberry leaves under drought stress

It was shown in Table 2 that there were some differences in the osmoregulation capacity between the plants with VAM fungus inoculation under drought stress and the plants with

routine water supply; although, the increase of cell osmotic potential caused by drought stress had been apparently slowed down by VAM fungi inoculation. After inoculation for 5 days under drought stress, there was no significant difference in proline content between the plants without inoculation and with inoculation. After inoculation for 10 days, the proline content of plants with inoculation was significant lower than that without inoculation, and the VAM fungal inoculation could significantly not only slow down the accumulation of SG, which accumulated substantially under drought stress, but also improve SG transport ability *in vivo*. The content of SP in the treatment with VAM fungus inoculation under drought stress was lower than that of the treatment with routine water supply, but the substantial decomposition of SP had been alleviated significantly under drought stress compared with the treatment without the VAM inoculation.

3.3 Effects of VA mycorrhiza on the photosynthetic pigments in strawberry leaves under drought stress

The influence of VAM mycorrhiza on the content of photosynthetic pigments in strawberry leaves was shown in Table 3. VAM fungi inoculation could slow down the reduction of Chla + b caused by drought stress effectively. The difference was significantly between the treatments with or without the inoculation, and the VAM fungus inoculation could improve Chla/b under drought stress. On the tenth day of inoculation, there was a significant difference, and the VAM fungus inoculation had an effect on the change of carotenoid (Car) content under drought stress that can inhibit the decomposition of Car signifi-

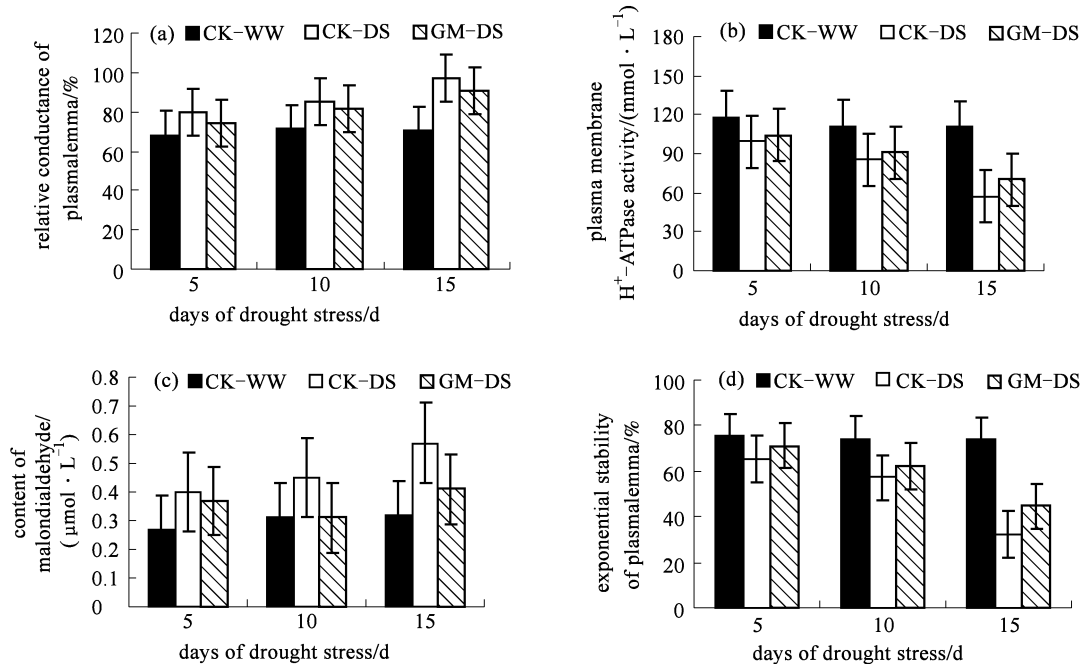


Fig. 1 Effect of VA mycorrhiza on the material in leaves of strawberry under drought stress

Table 2 Effects of VAM on the osmosis accommodation content in strawberry leaves under drought stress

item	Pro/($\mu\text{g}\cdot\text{g}^{-1}$ FW)			SG/($\text{mg}\cdot\text{g}^{-1}$ FW)			SP/($\mu\text{g}\cdot\text{g}^{-1}$ FW)		
	5 d	10 d	15 d	5 d	10 d	15 d	5 d	10 d	15 d
CK-WW	101.33b	99.32c	101.42c	0.36b	0.49c	0.42c	532.17a	521.24a	514.33a
GM-DS	128.62a	143.42b	159.32b	0.40b	0.66b	0.72b	525.26a	452.18b	324.28b
CK-DS	129.24a	167.18a	203.71a	0.55a	0.87a	0.95a	513.24b	421.24c	204.22c

Note: Different letters in the same column indicate significance at 0.05 probability level.

Table 3 Effects of VAM on the pigment content in strawberry leaves under drought stress

item	Chla + b/($\text{mg}\cdot\text{g}^{-1}$ FW)			Chla/b			Car/($\text{mg}\cdot\text{g}^{-1}$ FW)		
	5 d	10 d	15 d	5 d	10 d	15 d	5 d	10 d	15 d
CK-WW	27.30a	24.75a	26.54a	2.56ab	3.45b	2.37b	75.78a	74.47b	75.82a
GM-DS	26.98a	23.05b	17.80b	3.11a	4.26a	4.18a	75.35a	85.36a	65.06b
CK-DS	24.12b	18.85c	10.20c	3.10a	3.20b	4.02a	74.02a	71.21b	41.36c

Note: Different letters in the same column indicate significance at 0.05 probability level.

cantly under drought stress. Ten days after the inoculation the content of Car in the treatment with the inoculation was even higher than that of the routine water supply treatment.

4 Discussion

The formation of VA mycorrhiza can improve the drought resistance of plants significantly, which has been proven in the previous research on wheat, poplar, wild jujube, bean, and many other crops (Al-karaki, 1998; Li et al., 1999; Liang et al., 2003; Lu et al., 2003), but it is still not clear about the mechanism of VAM to enhance the drought resistance of plants. Some researches showed that the formation of VAM could enhance the activity of antioxidant enzymes of SOD, POD, CAT, and H^+ -ATPase in host plants and reduce the content of MDA and electrical conductivity of plasma membrane significantly (Wu and Xia, 2003; Wu et al., 2004a; Wu et al., 2007). However, some reported that the formation of VAM could reduce the activity of POD, and the activity of CAT and SOD reached a peak in the middle period of drought stress (Liu et al., 2007; Zhao and He, 2007). In our study, it showed that the formation of VAM could alleviate the reduction of the protective enzyme activity in plants under the drought stress in some extent and enhance the conductivity of membranaceous. Besides, it played a great role in promoting the activity of SOD, CAT, H^+ -ATPase, and APX. It also could improve the membranaceous stability. These results indicated that the formation of VAM increased the drought resistance of plants by promoting the defense response of protective enzyme system in host plants, which then reduced the membranaceous peroxidation of plants.

Some researches showed that the formation of VAM could improve the osmoregulation ability of host plants by

accelerating the accumulation of osmolyte and then improve the drought resistance of host plants (Wu et al., 2004a). In some research, it was shown that the inoculation of VAM fungi could reduce the accumulation of soluble sugar and soluble proline and accelerate the accumulation of soluble proteins (Wu et al., 2007). However, some researches showed that VAM fungi accelerated the accumulation of soluble proteins and soluble sugar and reduced the content of free proline under drought stress (Zhang and He, 2007). In our study, it showed that VAM could delay the decomposition of soluble proteins, reduce the accumulation of soluble sugar, which indicated that the formation of VAM could improve the osmoregulation ability of plants, and enhance the drought resistance. The accumulation rate of soluble proline could be accelerated in host plants according to our research, which is different from the results in previous researches. The reason may be the extent and time of drought stress that could affect the accumulation of soluble proline significantly. This is not a common phenomenon in plants (Wen et al., 1998). The mechanism of the accumulation of soluble proline caused by mycorrhiza formation should be further studied in the future.

In our research, it was shown that the formation of VAM fungi could improve the content of pigment in leaves under drought stress significantly, which is consistent with the results in previous researches (Wu et al., 2004b; Zhao and He, 2007). The reason may be because the formation of mycorrhiza could increase the rate of leaf transpiration, reduce the temperature of leaves, and then restrain the decomposition of chlorophyll.

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