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EFFECTS OF ARBUSCULAR MYCORRHIZAL INOCULATION ON OSMOREGULATION AND ANTIOXIDANT RESPONSES OF BLUEBERRY PLANTS

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Abstract

Effects of the arbuscular mycorrhizal (AM) fungi, *Glomus mosseae*, *G. intraradices*, and *G. etunicatum*, on plant growth, antioxidant content, osmoregulation, and nutrition were investigated in 'Premier' blueberry (*Vaccinium ashei*) plants exposed to low-temperature stress. Low temperature decreased mycorrhizal colonization, growth, levels of leaf soluble sugar, ascorbic acid (ASA) and root viability. However, at low temperatures, levels of leaf superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were found to increase, accompanied by increases in levels of ASA, malondialdehyde (MDA), and proline. *G. mosseae* especially, significantly increased levels of SOD, POD, CAT and ASA, but decreased levels of MDA in plants. AM-inoculated plants had higher contents of proline, soluble sugar, phosphorus, potassium, calcium and magnesium than non-AM-inoculated plants, especially in the *G. mosseae*-inoculated plants. These results indicate that *G. mosseae* has the potential to enhance resistance of 'Premier' blueberry plants against low-temperature stress through improving antioxidant content, osmotic adjustment and mineral nutrition.

Introduction

Blueberry (*Vaccinium* spp.) is one of the most important horticultural crops in the world. Over the past decade, many areas of China have invested in blueberry culture, thus making blueberry the fastest-growing species in fruit production (Li *et al*. 2018). However, late spring cold events have severely affected blueberry production in areas of Yangtze River in China (Liu *et al*. 2017). Thus, understanding the mechanism by which blueberry plants resist against low-temperature stress is a high priority.

In nature, *Vaccinium* plants grow in acidic soils and spontaneously form mutualistic symbiotic associations with certain soil fungi belonging mainly to the phylum Ascomycota, called 'ericoid mycorrhizae' (Smith and Read 2008). Ericoid mycorrhizae are specific to species of the families Ericaceae and Epacridaceae, due to their co-evolution with the native host plants growing in limiting edaphic conditions, allowing the plant to complete its life cycle. However, some reports indicated that the absence of ericoid mycorrhizal fungi inoculum may allow *Vaccinium* plants to associate with arbuscular mycorrhizal (AM) fungi (Koske *et al*. 1990). Vega *et al*. (2009) found high levels of AM colonization in *Vaccinium* plants cropped with the native populations of AM endophytes. AM inoculation significantly enhanced growth of *Vaccinium* plants (Arriagada *et al*. 2012). AM symbiosis is the most extended mutualistic association in nature, and, unlike the ericoid mycorrhizae, it is not host specific. Many workers have observed that AM symbiosis could increase plant resistance against low-temperature stress, not only through improving water conservation capacity, chlorophyll concentrations, and photosynthetic capacity (Zhu *et al*. 2010a), but also via attenuating membrane lipid peroxidation and plasma membrane permeability, and increasing

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osmolyte accumulation, antioxidant enzyme activities, and secondary metabolism (Chen *et al*. 2013, Liu *et al*. 2017). However, on citrus, Wu and Zou (2010) reported that the beneficial effects of AM inoculation were almost lost at low temperature. Nevertheless, under low- temperature conditions, the effects of different AM fungi on chilling tolerance of blueberry plants are not well understood.

Thus, the aim of this study is to evaluate the effect of colonization by *Glomus mosseae*, *G. intraradices* and *G. etunicatum* on 'Premier' Rabbit-eye blueberry (*Vaccinium ashei* Reade) plants under low-temperature stress. Comparisons of plant osmoregulation, antioxidant content, and mineral nutrition were investigated.

Materials and Methods

Two-year-old blueberry seedlings growing in sterile soil with uniform height were selected for these experiments. Lateral shoots were cut, and the main shoots were defoliated and decapitated at 5 - 7 cm above the ground. The roots of the seedlings were severely pruned (the major part of the root system was cut) to maintain highly similar root systems before the treatments and to induce new root development. Subsequently, these seedlings were transplanted into plastic pots (19 cm in depth and 21 cm in mouth diameter, one plant per pot) containing 3.0 kg of autoclaved experiment mixture (0.11 MPa, 121°C, 2 hrs) of clay loam, quartz sand and vermiculite (3:1:1, v/v/v), with the pH value 5.3, organic matter 19.26 g/kg, total nitrogen (N) 1.16 g/kg, and available phosphorus (P) 70.36 mg/kg, potassium (K) 36.87 mg/kg, calcium (Ca) 371. 06 mg/kg, magnesium (Mg) 41.91 mg/kg, zinc (Zn) 1.43 mg/kg, and copper (Cu) 0.188 mg/kg.

The mycorrhizal inocula, provided by the Institute of Plant Nutrition and Resources at the Beijing Academy of Agriculture and Forestry Sciences, consisted of spores, soil, hyphae and infected root fragments from a stock culture of *G. mosseae* (No. BGC HUN01A), *G. intraradices* (No. BGC AH01), *G. etunicatum* (No. BGC HEB04). The inoculation dose was ~600 spores per pot. The inocula were placed 5 cm below roots at the time of transplantation. In the non-AM treatment, the same weight of autoclaved mixture was added to the medium.

The experiment was a randomized block design with *G. mosseae*, *G. intraradices*, and *G. etunicatum* inoculation or remained non-inoculation at two temperature treatments (25 and 10°C). Three replicates (six plants in each replicate) were designated for each treatment. Before being exposed to the two temperature treatments, all plants were grown in a greenhouse at 20 - 25°C with 12 hrs day light and 75 - 85% relative humidity from 22 March to 17 October. The plants were supplied with half strength Hoagland's No.2 nutrient solution (half-strength macronutrients and full-strength micronutrients) every 20 days (approximately 500 ml per plant). The modified full-strength nutrient solution contained 4 mmol/l $Ca(NO_3)_2 \cdot 4H_2O$, 6 mmol/l KNO_3, 1 mmol/l $NH_4H_2PO_4$, 2 mmol/l $MgSO_4 \cdot 7H_2O$, 46 μmol/l H_3BO_3, 9 μmol/l $MnCl_2 \cdot 4H_2O$, 0.3 μmol/l $CuSO_4 \cdot 5H_2O$, 0.1 μmol/l H_2MoO_4, and 50 μmol/l Fe-EDTA. Different temperature treatments were given to the plants for one week by placing them into the two climate chambers with a 12 hrs photoperiod and 75 - 85% relative humidity.

At harvest time, a portion of fresh roots was carefully washed and cut into 1 cm pieces to fix in formalin-acetic acid-alcohol solution. These root pieces were cleaned with 10% (w/v) KOH and stained with 0.05% (w/v) trypan blue in lactophenol (Phillips and Hayman 1970), and microscopically detected for root mycorrhizal colonization. The mycorrhizal colonization was counted by the following formula: Mycorrhizal colonization (%) =100 × root length infected/root length detected. When samples were collected, shoot and root, were separately sampled to record plant fresh weight.

Leaf soluble sugar and proline were determined by the anthrone, and ninhydrin methods, respectively (Wang 2006). Ascorbic acid (ASA) was extracted with ice-cold 6% (v/v) $HClO_4$ and

measured according to Wang (2006). Root viability was determined using chlorinated triphenyl tetrazolium method (Wang 2006).

Leaf superoxide dismutase (SOD) (EC 1.15.1.1), catalase (CAT, EC 1.11.1.16) and peroxidase (POD, EC 1.11.1.7) were extracted according to Chen *et al.* (2008) and determined according to Giannopolitis and Ries (1977). Malondialdehyde (MDA) was extracted with 80% (v/v) ethanol and determined according to Hodges *et al.* (1999).

The dried leaves, stems and roots were ground to a fine powder and wet-digested in HNO_3-$HClO_4$ (4:1 v/v) before P, K, Ca and Mg analysis by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer Optimal 2100 DV, Perkin Elmer, USA).

The data were subjected to analysis of variance (ANOVA) using Statistical Product and Service Solutions software (SPSS Institute Inc. Chicago, Illinois, USA) and differences were compared by DMRT with a significance level of $p < 0.05$.

Results and Discussion

No colonization occurred in the roots of non-AM-inoculated blueberry plants under normal and low temperature conditions. In inoculated plants, low temperature decreased mycorrhizal colonization. However, the mycorrhizal colonization was higher in the plants inoculated with *G. mosseae* than with *G. etunicatum* or *G. intraradices* under the normal and low temperature regimes. Regardless of the temperature treatment, *G. mosseae* inoculation significantly enhanced the fresh weight of plants (Table 1).

Table 1. Mycorrhizal colonization and fresh weight of 'Premier' blueberry plants under normal temperature (NT) and low temperature (LT) conditions with *G. etunicatum*, *G. intraradices* and *G. mosseae*, or without (Non-AMF).

Temperature condition	AMF status	Mycorrihizal colonization (%)	Shoot fresh weight (g/plant)	Root fresh weight (g/plant)	Fresh weight (g/plant)
NT	Non-AMF	0.00g	1.55 ± 0.04c	2.41 ± 1.23c	3.96 ± 1.59c
	G. etunicatum	29.33 ±1.53e	2.51 ± 0.11bc	3.94 ± 1.34abc	6.45 ± 2.30bc
	G. intraradices	39.00 ± 2.00c	2.81 ± 0.22bc	4.21 ± 0.76abc	7.02 ± 0.78abc
	G. mosseae	59.00 ± 2.00a	4.27 ± 1.77a	5.91 ± 2.61a	10.18 ± 4.32a
LT	Non-AMF	0.00g	1.42 ± 0.07c	2.12 ± 0.59c	3.54 ± 0.60c
	G. etunicatum	25.67 ± 3.06f	2.55 ± 0.18bc	3.19 ± 0.79bc	5.74 ± 0.61bc
	G. intraradices	34.00 ± 2.65d	2.35 ± 0.11bc	2.99 ± 1.19bc	5.34 ± 1.14bc
	G. mosseae	52.33 ± 1.53b	3.15 ± 0.66ab	5.00 ± 0.97ab	8.15 ± 1.60ab
Significance					
LT		**	NS	NS	NS
AMF		**	**	**	**
LT×AMF		*	NS	NS	NS

Values represent the mean ± SE of three replicates (n = 3), samples from six plants were collected for each replicate. Different letters indicate significant differences at $p < 0.05$ in each column. NS: Not significant. *$p < 0.05$; **$p < 0.01$.

Low temperature decreased leaf soluble sugar and ASA concentrations, and root viability of blueberry plants, while increasing leaf proline concentration. Regardless of the temperature

treatment, AM fungi, especially *G. mosseae*, significantly enhanced leaf soluble sugar and proline concentrations, and root viability (Table 2).

Table 2. Leaf proline, soluble sugar and ascorbic acid (ASA) contents, and root viability of 'Premier' blueberry plants under normal temperature (NT) and low temperature (LT) conditions with *G. etunicatum*, *G. intraradices* and *G. mosseae*, or without (Non-AMF).

Temperature condition	AMF status	Proline (μg/g)	Soluble sugar (mg/g)	Ascorbic acid (mg/g)	Root viability (mg/g/h)
NT	Non-AMF	88.60 ± 4.57g	0.97 ± 0.04bc	102.06 ± 4.76c	9.25 ± 0.34b
	G. etunicatum	94.32 ± 4.17g	1.01 ± 0.06b	119.01 ± 4.06b	12.21 ± 1.46a
	G. intraradices	125.78 ± 4.78e	1.09 ± 0.03b	121.99 ± 1.46b	12.18 ± 1.20a
	G. mosseae	149.98 ± 2.92d	1.33 ± 0.07a	141.40 ± 1.88a	13.31 ± 0.39a
LT	Non-AMF	115.12 ± 2.52f	0.73 ± 0.06d	84.21 ± 4.53d	7.44 ± 0.36c
	G. etunicatum	172.99 ± 5.60c	0.83 ± 0.13cd	101.73 ± 3.66c	9.32 ± 0.77b
	G. intraradices	216.22 ± 6.11b	1.02 ± 0.06b	117.08 ± 1.23b	9.33 ± 1.17b
	G. mosseae	238.54 ± 7.04a	1.10 ± 0.14b	123.06 ± 1.75b	10.46 ± 0.99b
Significance					
LT		**	**	**	**
AMF		**	**	**	**
LT × AMF		**	NS	**	NS

Values represent the mean ± SE of three replicates (n = 3), samples from six plants were collected for each replicate. Different letters indicate significant differences at $p < 0.05$ in each column. NS: Not significant. *$p < 0.05$; **$p < 0.01$.

SOD, POD, and CAT activities, and MDA concentration in the leaves of blueberry plants were increased by the low temperature. However, regardless of the temperature treatment, AM fungi, especially *G. mosseae*, significantly enhanced leaf SOD, POD and CAT activities, but decreased leaf MDA concentration (Fig. 1).

G. mosseae especially, significantly increased P, K, Ca and Mg concentrations in the shoots, and K concentrations in the roots of blueberry plants. Additionally, Ca concentrations in *G. intraradices* and *G. mosseae*-inoculated roots, and Mg concentrations in *G. mosseae*-inoculated roots were higher than in non-AM-inoculated roots, however, when compared with non-AM-inoculated plants, *G. etunicatum* and *G. intraradices*-inoculated plants had lower root P concentrations, while in *G. mosseae*-inoculated plants, root P concentrations were not affected (Fig. 2).

The positive effect of AM fungi, especially *G. mosseae*, on growth under normal and low temperature conditions was in agreement with many studies on other plant species (Gavito *et al*. 2003 and Zhu *et al*. 2010b). Under low-temperature stress, AM-inoculated blueberry plants had higher levels of leaf SOD, POD, CAT, and ASA, but lower levels of leaf MDA than non-AM-inoculated plants, implying that the higher levels of antioxidant compounds in mycorrhizal plants could remove reactive oxygen species (ROS) in time, and alleviate cell lipid peroxidation, protecting the organism against oxidative damage. These results were consistent with previous reports obtained from other blueberry cultivars (Liu *et al*. 2017), and maize plants (Zhu *et al*. 2010b). On the other hand, *G. mosseae*-inoculated plants had higher SOD, POD, and CAT activities but a lower MDA content compared with *G. etunicatum* and *G. intraradices*-inoculated plants. This is in accordance with the result of mycorrhzial colonization, implying that the

blueberry plants displayed different responses to the different AM fungi; the higher activities of antioxidase enzymes in *G. mosseae*-inoculated plants, can effectively remove ROS caused by low temperature, and alleviate cell lipid peroxidation, protecting the organism against oxidative damage.

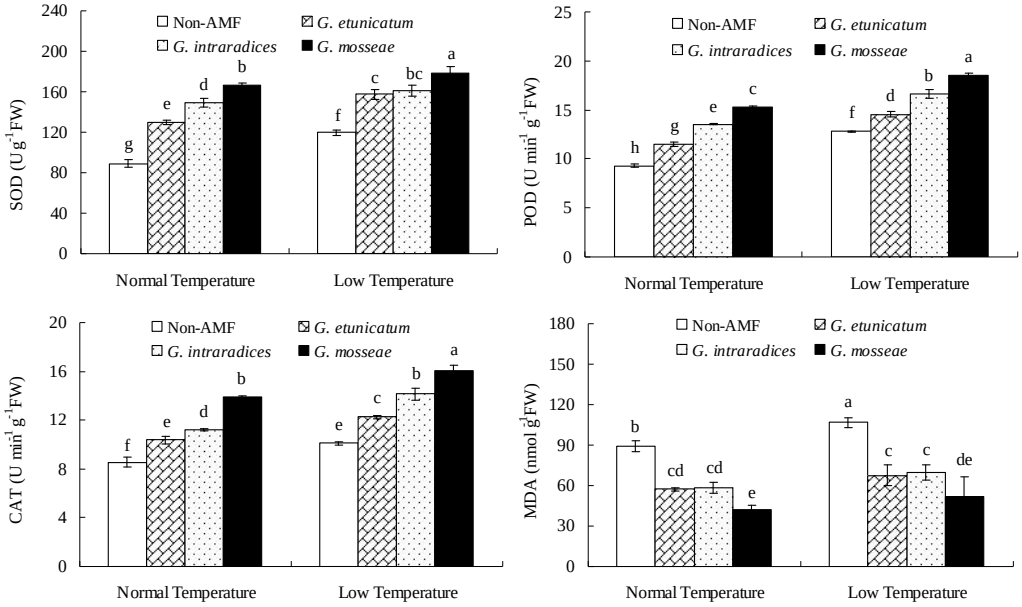

Fig. 1. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities**,** and malondialdehyde (MDA) concentration in the leaves of 'Premier' blueberry plants under normal temperature (NT) and low-temperature (LT) conditions with *G. etunicatum*, *G. intraradices*, and *G. mosseae*, or without (Non-AMF). Bars with the same letter are not significantly different at $p < 0.05$.

It is well known that osmotic adjustment is considered to be an important constituent of chilling tolerance mechanisms. In this study, AM-inoculated plants, especially *G. mosseae*-inoculated plants, had higher levels of leaf proline and soluble sugar than non-AM-inoculated plants, suggesting that AM fungi can adjust cell osmotic potential to protect the activities of enzyme systems by promoting the accumulation of osmoregulation compounds, reducing the damage caused by membrane lipid peroxidation, in return enhancing resistance of blueberry plants against chilling stress. In contrast, Abdel Latef and He (2011) reported that proline concentration in mycorrhizal tomato plants was lower than in non-mycorrhizal plants under salinity stress. The different changes may be associated with the types and degrees of stress, and tolerance of plant species.

In the present investigation, AM fungi, especially *G. mosseae*, increased P concentrations in the shoots, K, Ca, and Mg concentrations in the shoots and roots of 'Premier' blueberry plants. These results were in agreement with those found by many investigators, such as Abdel Latef and He (2011), who found that the concentrations of P, K and Mg were higher in mycorrhizal plants compared with non-mycorrhizal tomato plants under nonsaline and saline conditions. Similarly, other blueberry (Liu *et al.* 2017) or citrus (Khalil *et al*. 2011 and Chen *et al.* 2017) plants inoculated with AM fungi tended to increase nutrient acquisition under adverse conditions. Such increases in nutrient contents in response to the mycorrhizal effects were highly associated, respectively, with

the level of each mycorrhizal colonization, among the three fungi, *G. mosseae* was the most efficient for its ability to increase mineral contents of 'Premier' blueberry plants.

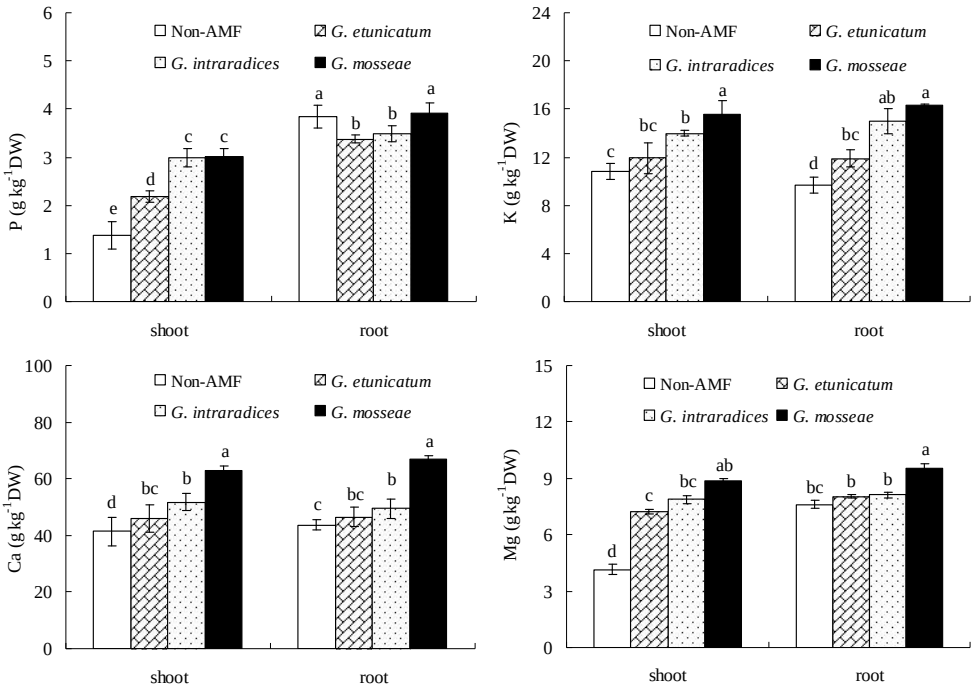

Fig. 2. Phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) concentrations in the shoots and roots of 'Premier' blueberry plants with *G. etunicatum*, *G. intraradices* and *G. mosseae*, or without (Non-AMF). Bars with the same letter are not significantly different at $p < 0.05$.

Present results suggested that AM fungi, especially *G. mosseae* could increase resistance of blueberry plants against low-temperature stress through improving antioxidant content, osmotic adjustment and mineral nutrition. However, it is necessary to conduct further studies to better understand the role of AM fungi in blueberry production.

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