

## Arbuscular mycorrhizal fungi promote photosynthesis in *Antirrhinum majus* L. under low-temperature and weak-light conditions

Wei LI<sup>1,2</sup>, Yan-Lin ZHAI<sup>1,2</sup>, Hong-Shuang XING<sup>1,2</sup>,  
Li-Jun XING<sup>1,2</sup>, Shao-Xia GUO<sup>1,2\*</sup>

<sup>1</sup>Qingdao Agricultural University, College of Landscape Architecture and Forestry, Qingdao, Shandong, 266109, P.R. China; [lwcsu\\_caf@163.com](mailto:lwcsu_caf@163.com); [151375641@qq.com](mailto:151375641@qq.com); [1639650304@qq.com](mailto:1639650304@qq.com); [xljdmn312@163.com](mailto:xljdmn312@163.com); [gsx2309@126.com](mailto:gsx2309@126.com) (\*corresponding author)  
<sup>2</sup>Institute of Mycorrhizal Biotechnology, Qingdao Agricultural University, Qingdao, Shandong, 266109, P.R. China

### Abstract

Low-temperature and weak-light conditions have major effects on the growth and flower quality of horticultural plants. A greenhouse pot experiment was performed to investigate the effects of arbuscular mycorrhizal fungi (AMF) (*Funneliformis mosseae* and *Glomus versiforme*) on the growth, photosynthesis, and chlorophyll fluorescence parameters of snapdragon (*Antirrhinum majus* L.) under low-temperature and weak-light stress. The growth and biomass of snapdragon were higher following inoculation with *F. mosseae* and *G. versiforme* compared with control plants. The percentage of mycorrhizal colonization and root activity were high in *A. majus* plants with AMF. AMF inoculation enhanced the net photosynthetic rate, transpiration rate, stomatal conductance, and water use efficiency of plants under low-temperature and weak-light conditions. Furthermore, the chlorophyll content, potential activity of photosystem II (PSII), effective photochemistry quantum efficiency of PSII, actual photochemical quantum efficiency of PSII, and photochemical quenching coefficient were higher in AMF-inoculated plants than in uninoculated plants. The application of AMF reduced the intercellular CO<sub>2</sub> concentration and non-photochemical quenching coefficient. Thus, snapdragon plants treated with *F. mosseae* and *G. versiforme* are more resistant to low-temperature and weak-light stress than untreated plants.

**Keywords:** arbuscular mycorrhizal fungi; *Antirrhinum majus* L.; chlorophyll fluorescence; low temperature and/or weak light; photosynthesis

### Introduction

Low-temperature and weak-light conditions affect the growth and flower quality of horticultural plants, and plants are often exposed to these abiotic stresses in facility cultivation systems. Low-temperature and weak-light conditions can inhibit the growth and development of plants and even lead to death (Sun *et al.*, 2015). Chlorophyll parameters play important roles in determining the resistance of plants to low-temperature and weak-light stress (Anamika *et al.*, 2014). Photosynthesis is highly sensitive to low-temperature and weak-light stress (Yu *et al.*, 2015); chlorophyll fluorescence parameters provide an objective reflection of the effects of

Received: 17 Nov 2022. Received in revised form: 11 Dec 2022. Accepted: 31 Jan 2023. Published online: 14 Feb 2023.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

external factors on photosynthesis (Murchie and Lawson, 2013). The net photosynthetic rate (Pn) and maximum photochemical efficiency of photosystem II (PSII) (Fv/Fm) of chrysanthemum decrease under low-temperature and weak-light conditions, and the intercellular CO<sub>2</sub> concentration (Ci) increases, which inhibits the growth and development of chrysanthemum and reduces its ornamental quality (Liang *et al.*, 2010). Similarly, the photosynthetic capacity of *Armeniaca vulgaris* is greatly reduced under low-temperature and low-light stress, and the functions of photosystem I (PSI) and PSII are disrupted (Sun *et al.*, 2015). Other studies have shown that the Pn, stomatal conductance (Gs), carboxylation efficiency, Fv/Fm, and actual photochemical efficiency of PSII of grafted *Capsicum frutescens* seedlings decrease significantly after low-temperature and weak-light stress treatment, and Ci first decreases and then increases (Zhang and Shang, 2010).

Arbuscular mycorrhizal fungi (AMF) are the most common symbiotic fungi (Harley and Smith, 2008), and they obtain nutrients and water by colonizing host plant roots; AMF can promote the growth of their plant symbionts (Zhang *et al.*, 2019). Some studies have shown that AMF can enhance the adaptability of plants to adverse environmental stresses (Chen *et al.*, 2013; Fakhech *et al.*, 2019; Fokom *et al.*, 2019; Wahid *et al.*, 2019; Ye *et al.*, 2019). *Glomus etunicatum* can alleviate low-temperature damage in maize plants by increasing their leaf chlorophyll content, photosynthesis, and chlorophyll fluorescence, which promotes host plant growth (Zhu *et al.*, 2010). The plant height, leaf number, leaf area, and ground weight of *Santalum album* are significantly higher when they are inoculated with *Glomus fasciculatum* and a mixture of *Glomus intraradices* and *G. fasciculatum* under low light conditions than when no AMF are inoculated (Binu *et al.*, 2015). A previous study has shown that inoculation with *Funneliformis mosseae* significantly increases the fresh weight and dry weight of cucumber seedlings, and this results in increases in their cold resistance (Chen *et al.*, 2013).

Snapdragon (*Antirrhinum majus* L.) is a perennial herbal flower in the family Scrophulariaceae; it has been widely used for its rich color and unique appearance (Fan and Yan, 2015). Snapdragon originated in the Mediterranean coastal region, and it grows optimally between 15 and 16 °C. It is susceptible to freezing damage when temperatures are below 5 °C, and such temperatures can lead to blossom failure or even death (Inaba and Ohshiro, 2010). Snapdragon has long been cultivated under low-temperature and weak-light conditions during the winter months in northern China. Artificial measures such as heat and light supplementation are needed to promote the growth of snapdragons at high latitudes. Few studies have examined the physiological mechanisms underlying the adaptability of snapdragon with AMF to low-temperature and weak-light stress. The aim of this study was to explore approaches that could be used to mitigate the damage caused by low-temperature and weak-light stress on snapdragon plants via biological means to promote their growth and development. Thus, we examined the effects of AMF (*F. mosseae* + *Glomus versiforme*) inoculation on the growth, chlorophyll content, photosynthetic gas exchange parameters, and chlorophyll fluorescence parameters of snapdragon under low-temperature and weak-light conditions by measuring eco-physiological and morphometric variables. The results of our study provide new insights into the physiological mechanism underlying the resistance of snapdragons with AMF to low-temperature and low-light stress.

## Materials and Methods

### *Plants and AMF material*

Snapdragon seeds were purchased from Takii Seed Co., Ltd. Qingdao Branch, China (36.269304°N, 120.43027°E). They were surface sterilized by soaking in 10% (v/v) solution of hydrogen peroxide for 10 min and rinsed with sterile distilled water. The soil was collected from the campus of Qingdao Agricultural University; it was then sieved and mixed with turf (1:1). The mixture was sterilized by high-pressure steam at 120 °C for 2 h to eliminate the effect of indigenous AMF.

The AMF inoculated in this study were *F. mosseae* and *G. versiforme*, and the inoculum was provided by the Institute of Mycorrhizal Biotechnology of Qingdao Agricultural University. After propagation for four months using *Trifolium repens* as a host plant, soil containing fungal spores, hyphae, and the infected root fragments of the host plant was used as inoculum.

#### *Experimental design and treatments*

The experiment was carried out in the controlled climate laboratory of Qingdao Agricultural University. Various light intensity and temperature conditions were tested, including low temperature and weak light intensity (4 °C, 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , LW), low temperature and normal light intensity (4 °C, 500  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , LN), normal temperature and weak light intensity (20 °C, 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , NW), and normal temperature and normal light intensity (20 °C, 500  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , NN). Under each light intensity and temperature condition, two treatments, one in which plants were inoculated with AMF (+AMF) and one in which plants were not inoculated with AMF (CK), were conducted. Thus, there were a total of eight treatments, with five replicates for each treatment.

Surface-sterilized seeds were sown in plastic pots (24 cm in diameter×18 cm high) containing 2 kg of air-dried substrates. After germination, the seedlings were thinned to one per pot. In the AMF treatment, a total of 30 g of soil containing *F. mosseae* and *G. versiforme* was added to the culture matrix, and the AMF were applied at an inoculation potential of 12,000 [IP=N×W×K+S, where IP is the inoculation potential, N is the number of vesicles contained in the root segment per unit length, W is the root weight (g), K is the root length per unit mass (cm), and S is the number of spores in the inoculum per unit mass or volume] (Liu and Chen, 2007); the CK was inoculated with the same amount of sterile inoculants.

Pots were cultured in a controlled climate chamber with a 12-h photoperiod at 20 °C and humidity of 65%. Half of the Hoagland solution was supplied once a week, and the pots were weighed every 3 days to adjust the water content. After 75 days of cultivation, seedlings of AMF-inoculated and uninoculated snapdragons were randomly subjected to LW, LN, NW, and NN conditions; after 10 days of treatment, each index was determined, and this process was repeated five times.

#### *Mycorrhizal colonization rate and root activity*

The Trypan blue staining method was used to measure mycorrhizal colonization after dyeing (Biermann and Linderman 1981); the mycorrhizal colonization rate was measured under a microscope using the modified cross-bonded method with the following formula:  $C = R_c/R_t \times 100\%$ , where C (%) is the colonization rate,  $R_c$  is the total number of root segments colonized, and  $R_t$  is the total number of root segments. The methods used to determine the arbuscule rate, as well as the numbers of entry points and vesicles, were based on those described in Liu and Chen (2007). Root activity was measured using the triphenyl tetrazolium chloride (TTC) method (Wang *et al.*, 2018). Briefly, 0.5 g of fresh roots were immersed in 10 mL of an equally mixed solution of 0.4% TTC and phosphate buffer; it was then kept in the dark at 37 °C for 2 h. Subsequently, 2 mL of 1 mol/L  $\text{H}_2\text{SO}_4$  was added to stop the reaction with the roots. The roots were dried with filter paper and then extracted with ethyl acetate. The red extractant was transferred to the volumetric flask, and a volume of 10 mL was achieved by adding ethyl acetate. The absorbance of the extract at 485 nm was recorded. Root activity was expressed as TTC reduction intensity: root activity = amount of TTC reduction ( $\mu\text{g}$ )/fresh root weight (g) × time (h).

#### *Growth and physiological indexes*

Plant height was measured using a graduated meter. Basal diameter, leaf width, and leaf length were measured using digital calipers. All plants were harvested in the morning 30 d after sowing; they were first washed with tap water, followed by distilled water. The aboveground and underground parts of the seedlings were separated, and the fresh weight was determined per pot. The samples were oven-dried at 105 °C for 15

min and then at 80 °C to a constant weight; the dry weight was recorded. The total chlorophyll content was measured using a hand-held chlorophyll meter (SPAD-502, Konica Minolta Co., Tokyo, Japan).

The Pn, Gs, Ci, and transpiration rate (Tr) of leaves were determined during 8:30–10:30 on fully expanded first blades using a portable photosynthetic CIRAS-3 instrument (PP Systems, USA) 30 d after sowing. Measurements were repeated three times for each blade for three blades per pot, and the averages were recorded. The water use efficiency (WUE) was calculated as the ratio of Pn/Gs. Chlorophyll fluorescence parameters were measured using Pocket PEA (Hansatech Instruments Ltd., UK). Seedlings were kept in the dark for 30 min before measuring the fluorescence of blades; the blades used for fluorescence measurements were the same ones used for measurements of photosynthetic indices. The minimal fluorescence level ( $F_0$ ), maximal fluorescence level ( $F_m$ ),  $F_v/F_m$ , potential activity of PSII ( $F_v/F_0$ ), effective photochemistry quantum efficiency of PSII ( $F_v/F_m$ ), actual photochemical quantum efficiency of PSII ( $\phi$ PSII), photochemical quenching coefficient (qP), and non-photochemical quenching coefficient (NPQ) were also determined.

#### *Data analysis and statistics*

All statistical analyses were conducted using Excel 2010 and SPSS16.0. Analysis of variance (ANOVA) was used to evaluate the effects of treatments on variables ( $p < 0.05$ ). Significant differences between individual means were determined using Duncan's multiple range tests ( $p < 0.05$ ). Sampling analyses were repeated at least three times under the same conditions to minimize experimental error. Data were shown as mean  $\pm$  standard deviation of three replicates.

## **Results**

#### *AMF colonization and root activity*

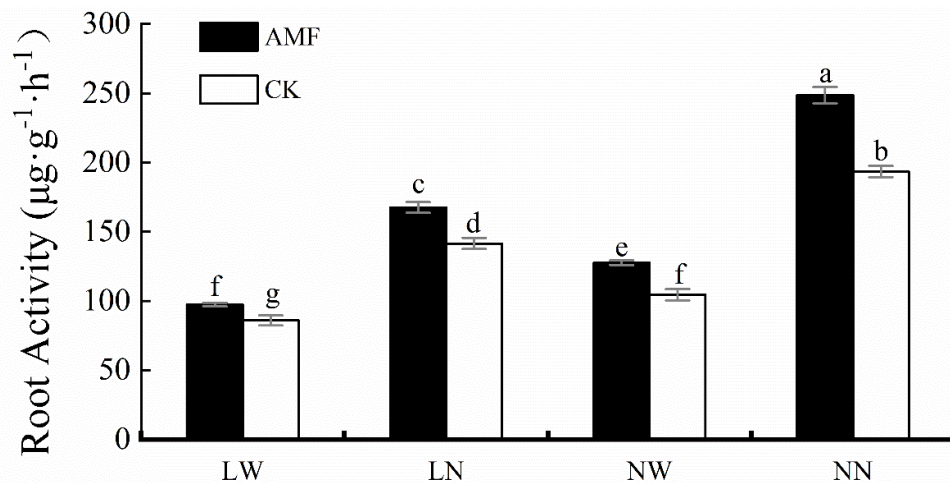
Root colonization by AMF was significantly affected by LW, LN, and NW treatments, as sharp decreases were observed in all indicators of colonization (Table 1); the interactions between these indicators were significant (Table 2). NN+AMF snapdragon roots were largely colonized by AMF, as indicated by the high percentage of mycorrhizal colonization and arbuscule rate (Table 1). Microscopic observations confirmed that the numbers of entry points and vesicles were high in all AMF-inoculated plants (Table 1).

The root activity of snapdragon was low in the LW, LN, and NW treatments. The root activity of snapdragon was higher in the LW, LN, and NW treatments under inoculation with AMF than in the control treatment, and root activity was 17.4%, 18.6%, 19.2%, and 28.5% higher in snapdragon in the LW, LN, and NW, and NN treatments, respectively, under AMF inoculation than in these same treatments without AMF inoculation (Figure 1).

**Table 1.** The effect of low temperature, weak light, and AMF colonization on the root colonization of *Antirrhinum majus* L

Treatments	Mycorrhizal colonization (%)	Arbuscule rate (%)	Numbers of entry point root (cm)	Vesicles root (cm)
LW+AMF	50.2 ± 0.1 c	29.0 ± 1.1 b	8.3 ± 0.7 b	14.0 ± 0.6 b
LN+AMF	57.6 ± 0.7 b	31.3 ± 0.9 ab	9.3 ± 1.2 ab	15.3 ± 0.3 ab
NW+AMF	54.4 ± 0.6 bc	32.3 ± 0.8 ab	9.6 ± 0.3 ab	15.7 ± 0.3 ab
NN+AMF	66.6 ± 0.9 a	34.0 ± 1.7 a	11.0 ± 0.6 a	16.7 ± 0.3 a
LW	-	-	-	-
LN	-	-	-	-
NW	-	-	-	-
NN	-	-	-	-
Test of significance				
low temperature	*	**	*	*
weak light	**	**	*	*
low temperature × weak light	***	***	***	***

Note: Data are mean ± standard deviation of three replicates. Different lowercase letters (a, b, c, and d) indicate significant differences. The threshold for statistical significance was  $p < 0.05$  according to Duncan's multiple range test. LW: low temperature and weak light intensity (4 °C, 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ); LN: low temperature and normal light intensity (4 °C, 500  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ); NW: normal temperature and weak light intensity (20 °C, 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ); NN: normal temperature and normal light intensity (20 °C, 500  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ); AMF: snapdragon inoculated with *F. mosseae* and *G. versiforme*. "NS" indicates that the differences are not significant; \*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ .

**Figure 1.** Effects of AMF on root activity of snapdragon under different treatments

Means followed by different letters in the same group are significantly different at  $p < 0.05$ , according to Duncan's multiple range test.

#### Plant growth

LW, LN, and NW treatment inhibited the growth and development of snapdragon, and inoculation with AMF alleviated the inhibitory effects of low temperature and weak light on snapdragon growth. The plant height, stem diameter, and dry weight were higher in NN+AMF plants than in plants in the other treatments, especially LW+AMF and LW+CK plants (Table 2). The leaf width and leaf length were greater in NW+AMF plants than in plants in the other treatments. The plant height, stem diameter, and dry weight were lowest in the LW+CK plants. Leaf width and leaf length were lowest in LN+CK plants (Table 2). AMF inoculation

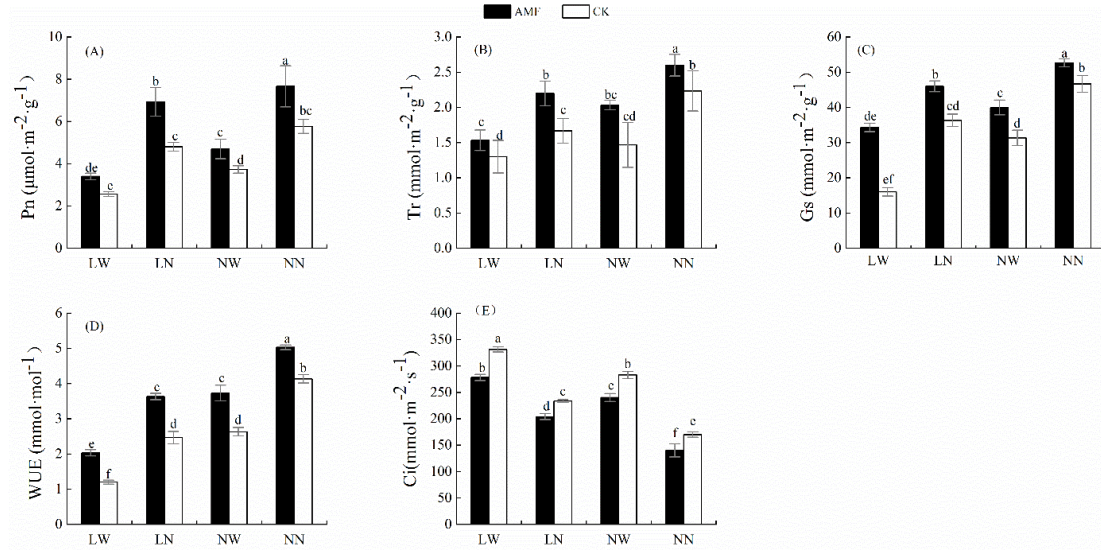
significantly promoted the growth of snapdragon, and interactions between LW, LN, and NW were significant (Table 2).

**Table 2.** Morphometric parameters of *Antirrhinum majus* L. under different treatments

Treatments	Plant height (cm)	Stem diameter (mm)	Leaf width (cm)	Leaf length (cm)	Dry weight (g)	
					Aboveground part	Underground part
LW+AMF	15.88 ± 0.21 abcd	5.13 ± 0.04 abc	2.12 ± 0.03 ab	6.25 ± 0.09 ab	3.14 ± 0.06 cd	0.48 ± 0.01 bcd
LN+AMF	16.24 ± 0.13 ab	5.22 ± 0.07 ab	2.07 ± 0.07 ab	6.08 ± 0.03 bcd	3.61 ± 0.16 ab	0.53 ± 0.01 ab
NW+AMF	16.33 ± 0.36 ab	5.18 ± 0.03 abc	2.22 ± 0.06 a	6.39 ± 0.19 a	3.28 ± 0.16 bc	0.51 ± 0.01 bc
NN+AMF	16.37 ± 0.31 a	5.31 ± 0.09 a	2.09 ± 0.03 ab	6.23 ± 0.05 abc	3.73 ± 0.11 a	0.55 ± 0.01 a
LW	15.15 ± 0.19 d	4.78 ± 0.12 d	1.83 ± 0.03 cd	6.01 ± 0.06 cde	2.86 ± 0.07 d	0.37 ± 0.02 e
LN	15.36 ± 0.37 cd	4.85 ± 0.12 d	1.74 ± 0.05 d	5.81 ± 0.04 e	3.23 ± 0.14 bcd	0.45 ± 0.01 d
NW	15.49 ± 0.19 bcd	4.94 ± 0.12 cd	1.99 ± 0.04 bc	6.17 ± 0.04 abcd	3.17 ± 0.15 cd	0.47 ± 0.01 d
NN	16.06 ± 0.17 abc	5.03 ± 0.07bcd	1.77 ± 0.09 d	5.99 ± 0.06 de	3.31 ± 0.15 bc	0.47 ± 0.03 cd
Test of significance						
AMF	*	**	**	**	**	***
low temperature	**	*	NS	NS	*	NS
weak light	**	*	**	**	**	NS
low temperature × weak light	***	**	**	**	**	*
AMF × low temperature	NS	*	**	**	*	**
AMF × weak light	NS	*	**	**	NS	*
AMF × low temperature × weak light	NS	NS	**	**	*	*

### Gas exchange

There was a significant effect of AMF inoculation on the total chlorophyll content, Pn, Tr, Gs, WUE, and Ci of snapdragon ( $p < 0.05$ , Table 3). The LW, LN, and NW treatments had a significant negative effect on the Pn, Tr, Gs, and WUE of both AMF and CK plants (Figure 2A, B, C, D); Ci was significantly higher in the LW, LN, and NW treatments than in the NN treatment (Figure 2e). In the LW treatment, AMF significantly increased the Pn, Tr, Gs, and WUE by 32.7%, 42.1%, 72.4%, and 67.8%, respectively, and decreased Ci by 15.2%. In the LN treatment, AMF significantly increased the Pn, Tr, Gs, and WUE by 31.1%, 32.3%, 26.6%, and 46.9%, respectively, and decreased Ci by 12.6%. In the NW treatment, AMF significantly increased the Pn, Tr, Gs, and WUE by 29.2%, 38.8%, 28%, and 41.3%, respectively, and decreased Ci by 15.2% (Figure 2, Table 3).



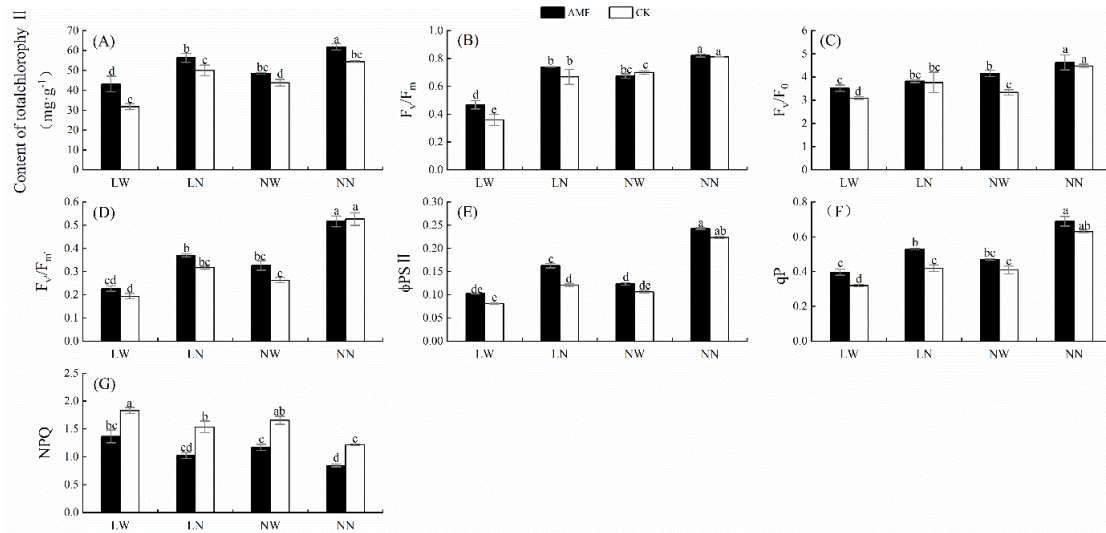
**Figure 2.** Effects of AMF on A: Pn, B: Tr, C: Gs, D: WUE, and E: Ci under different treatments. Bars topped by different letters indicate that values differed significantly at  $p < 0.05$ , according to Duncan’s multiple range test.

*Chlorophyll fluorescence*

The total chlorophyll content and NPQ of snapdragon leaves were significantly affected by AMF inoculation ( $p < 0.05$ , Table 3). The total chlorophyll content was higher in AMF-inoculated plants than in control plants (Figure 3, Table 3).

**Table 3.** Results of three-way ANOVA of the effects of low temperature, weak light, arbuscular mycorrhizal (AM) fungi, and their interactions on the root activity, Pn, Tr, Gs, Ci, WUE, total chlorophyll content,  $F_v/F_m$ ,  $F_v/F_0$ ,  $F_v/F_m'$ ,  $\phi$ PSII, qP, and NPQ

Source of variation	Root activity	Pn	Tr	Gs	Ci	WUE	Total chlorophyll	$F_v/F_m$	$F_v/F_0$	$F_v/F_m'$	$\phi$ PSII	qP	NPQ
AMF	*	*	*	*	*	*	*	NS	NS	NS	NS	NS	*
low temperature	*	*	**	*	**	**	NS	*	*	*	***	**	*
weak light	**	***	***	**	***	**	**	**	**	**	***	**	**
low temperature × weak light	***	***	***	***	***	***	***	***	***	***	***	***	***
AMF × low temperature	NS	NS	NS	NS	*	*	NS	NS	NS	NS	**	NS	NS
AMF × weak light	*	*	*	*	**	*	NS	*	*	*	***	*	NS
AMF × low temperature × weak light	**	**	**	**	***	**	*	**	**	**	***	**	NS



**Figure 3.** Effects of AMF on A: chlorophyll content, B:  $F_v/F_m$ , C:  $F_v/F_0$ , D:  $F_v/F_m'$ , and E:  $\phi$ PSII, F: qP and G: NPQ under different treatments

Bars topped by different letters indicate that values differed significantly at  $p < 0.05$ , according to Duncan's multiple range test.

LW, LN, and NW treatments had a significant negative effect on the total chlorophyll,  $F_v/F_m$ ,  $F_v/F_0$ ,  $F_v/F_m'$ ,  $\phi$ PSII, and qP of both AMF-inoculated and uninoculated plants compared with plants in the NN treatment, and NPQ was significantly higher in plants in the LW, LN, and NW treatments than in the NN treatment (Figure 3A, B, C, D, E, F, and G). In the LW treatment, AMF significantly increased the mean content of total chlorophyll,  $F_v/F_m$ ,  $F_v/F_0$ ,  $F_v/F_m'$ ,  $\phi$ PSII, and qP by 35.7%, 12.8%, 13.9%, 21.1%, 25.9%, and 25%, respectively, and decreased NPQ by 25.1%. In the LN treatment, AMF significantly increased the mean content of total chlorophyll,  $F_v/F_m$ ,  $F_v/F_0$ ,  $F_v/F_m'$ ,  $\phi$ PSII, and qP by 12.6%, 1.7%, 1.9%, 15.6%, 4.2%, and 26.2%, respectively, and decreased NPQ by 33.8%. In the NW treatment, AMF significantly increased the mean content of total chlorophyll,  $F_v/F_m$ ,  $F_v/F_0$ ,  $F_v/F_m'$ ,  $\phi$ PSII, and qP by 21.8%, 2.5%, 23.5%, 26.9%, 17%, and 14.6%, respectively, and decreased NPQ by 30.5% (Figure 3, Table 3).

## Discussion

In this experiment, low-temperature and weak-light stress inhibited the growth of snapdragon, and LW treatment had a more substantial negative effect on snapdragon than the LN and NW treatment. We found that mycorrhizal symbiosis had a positive effect on the growth and biomass of snapdragon seedlings in the LW, LN, and NW treatments; this might stem from interactions with AMF, which can enhance plant root activity and promote plant growth and development (Mathur *et al.*, 2018; Pavithra and Yapa, 2018). A previous study has shown that AMF obtains photosynthates by infecting host plant root systems, which enhances the absorption of mineral nutrients by host plants and reduces the effects of abiotic stress (Porcel *et al.*, 2012; Dhanushi and Neelamanie, 2018; Pollastri *et al.*, 2018). Our findings indicate that AMF symbiosis plays a key role in alleviating the deleterious effects of low-temperature and weak-light stress on snapdragon.

AMF colonization was lower in LW+AMF, LN+AMF, and NW+AMF plants than in NN+AMF plants. Our findings are consistent with the results of previous studies showing that root colonization decreases in *Chrysanthemum morifolium* in response to cadmium stress (Wang *et al.*, 2018) and salt stress (Lin *et al.*, 2018), indicating that the LW and NW treatments both affected the activity of photosynthesis-related enzymes and that LN and NW reduced the light energy absorption of snapdragon. LW, LN, and NW inhibited



the Pn, which affects the supply of carbon to AMF from plants, and this leads to a reduction in the colonization rate of AMF. These findings indicate that the mycelium elongation and branching of AMF are inhibited under abiotic stress (Gao *et al.*, 1998).

Photosynthesis is one of the most important indicators of physiological sensitivity to environmental stress (Chaves *et al.*, 2009). Previous studies have shown that Pn is a good indicator of the degree of physiological sensitivity to abiotic stress (Xu *et al.*, 2008). Previous research has shown that decreases in Pn indicate the magnitude of plant stress (Xu *et al.*, 2016, Zou *et al.*, 2015), which is consistent with our finding that the Pn of snapdragon is markedly lower in the LW, LN, and NW treatments than in the NN treatment. The reduction in Pn in the LW, LN, and NW treatments was not only related to the loss of photosynthetic capacity but also to decreases in Gs (Figure 2 A, C). The Gs of snapdragon in our study decreased in the LW, LN, and NW treatments. AmayaCarpio *et al.* (2009) found that the Pn and photosynthetic capacity of buttercup were significantly higher when plants were inoculated with *Glomus intraradices* than when they were not inoculated with AMF. Inoculating snapdragon with AMF increased the Gs, Tr, and Pn of leaves, which promoted the accumulation of assimilates. In the AMF treatment, increases in WUE were pronounced in the presence of AMF. Increases in WUE allow plants to tolerate conditions in the LW, LN, and NW treatments (Figure 2 D), and increases in Gs in snapdragon leaves might be related to changes in the concentrations of endogenous hormones (Cosme and Wurst, 2013). AMF not only improve plant growth but also promote plant hormone-mediated defense responses (He *et al.*, 2017). Our findings indicate that LN and NW conditions reduced the Gs of snapdragon leaves, which led to decreases in CO<sub>2</sub> absorption and Pn. The Tr of plants decreased under LW conditions. AM symbiosis enhances the gas exchange capacity, which promotes photosynthesis, reduces stomatal resistance, and enhances CO<sub>2</sub> assimilation and transpiration flux (Mathur *et al.*, 2018). AM symbiosis also alleviates declines in the transpiration rate and promotes the intake of H<sub>2</sub>O, which maintains the Pn high.

The chlorophyll concentration is a key factor affecting plant photosynthesis (Parvin *et al.*, 2020). Increases in the chlorophyll content due to mycorrhizal colonization in the LW, LN, and NW treatments have also been observed in previous studies (Mathur, Sharma and Jajoo, 2018). The chlorophyll content was higher in AMF plants than in CK plants in our experiment. Chlorophyll fluorescence parameters are strong predictors of the photosynthetic ability and energy conversion efficiency of PSII (Chen *et al.*, 2017). Plants maintain the balance between photosynthetic electron transfer and carbon metabolism through non-photochemical processes and improve the electron transfer activity of PSII (Roháček, 2002). Here, we found large differences in Fv/Fm, Fv/Fo, Fv'/Fm',  $\phi$ PSII, qP, and NPQ between CK plants and AMF-inoculated plants. In the LW, LN, and NW treatments, decreases in Fv/Fm, Fv/Fo, Fv'/Fm',  $\phi$ PSII, and qP were accompanied by increases in NPQ, and increases in NPQ are thought to be a mechanism of energy dissipation that protects the photosynthetic apparatus against excess light (Demmig-Adams and Adams Iii 1992). Inoculation with AMF can increase the photochemical activity of the PSII reaction center, enhance the heat dissipation rate, reduce damage to the PSII photosynthetic center induced by abiotic stress, and decrease the inhibition of photosynthetic electron transfer (Borkowska, 2006). Specifically, the LW, LN, and NW treatments inhibited the synthesis of chlorophyll and destroyed the structure of chloroplasts. AMF inoculation alleviated the degradation of chlorophyll and the disintegration of chloroplasts to some extent, which helped enhance the photosynthetic rate and chlorophyll fluorescence parameters.

## Conclusions

In conclusion, low-temperature stress and weak-light stress result in decreases in root activity and root biomass accumulation, as well as declines in the chlorophyll content and photosynthetic capacity of leaves. However, inoculation with AMF significantly increased the root activity and chlorophyll content of

snapdragon in the LW, LN, and NW treatments. The photosynthetic carbon assimilation capacity and chlorophyll fluorescence parameters of snapdragon were also increased to varying degrees. Therefore, inoculation of snapdragon with AMF can enhance their tolerance to low-temperature and weak-light stress via changes in plant morphology and photosynthetic functions.

### Authors' Contributions

W.L. and S.G. designed the experiment. H.X. and L.X. performed the experiment. W.L. and H.X. participated in writing the manuscript. Y.Z., and W.L. participated in revising the manuscript. All authors read and approved the final manuscript.

### Ethical approval (for researches involving animals or humans)

Not applicable.

### Acknowledgements

This research was supported by The High-level Science Foundation of Qingdao Agricultural University (663/1115006). We thank Christopher A. from EditorBar ([www.editorbar.com](http://www.editorbar.com)) for English language editing.

### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

### References

- Abdessamad F, Najat M, Lahcen O, Hafidi M (2019). Contributions of indigenous arbuscular mycorrhizal fungi to growth of *Retama monosperma* and *Acacia gummifera* under water stress (case study: Essaouira sand dunes forest). *Journal of Sustainable Forestry* 38(7):686-696. <https://doi.org/10.1080/10549811.2019.1602057>
- Amaya-Carpio L, Davies JFT, Fox T, He C (2009). Arbuscular mycorrhizal fungi and organic fertilizer influence photosynthesis, root phosphatase activity, nutrition, and growth of *Ipomoea carnea* ssp. *fistulosa*. *Photosynthetica* 47(1):1-10. <https://doi.org/10.1007/s11099-009-0003-x>
- Anamika M, Arnd GH, Kumud BM (2014). Chlorophyll fluorescence emission can screen cold tolerance of cold acclimated *Arabidopsis thaliana* accessions. *Plant Methods* 10(1):38. <https://doi.org/10.1186/1746-4811-10-38>
- Biermann B, Linderman RG (1981). Quantifying vesicular-arbuscular mycorrhizae: A proposed method towards standardization. *New Phytologist* 87(1):63-67. <https://doi.org/10.1111/j.1469-8137.1981.tb01690.x>
- Binu NK, Ashokan PK, Balasundaran M (2015). Influence of different arbuscular mycorrhizal fungi and shade on growth of sandal (*Santalum album*) seedlings. *Journal of Tropical Forest Science* 27(2):158-165.
- Demmig-Adams B, Adams III WW (1992). Photoprotection and other responses of plants to high light stress. *Annual Review of Plant Biology* 43:599-626. <https://doi.org/10.1146/annurev.pp.43.060192.003123>
- Borkowska B. (2006). Chlorophyll a fluorescence method as a physiological marker of plant response to light stress and endo-mycorrhiza (AMF). *Acta Horticulturae* 711:177-182. <https://doi.org/10.17660/ActaHortic.2006.711.21>
- Chaves MM, Flexas J, Pinheiro C (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany* 103:551-560. <https://doi.org/10.1093/aob/mcn125>

- Chen ML, Yang G, Sheng Y, Li PY, Qiu HY, Zhou XT, ... Chao Z (2017). *Glomus mosseae* inoculation improves the root system architecture, photosynthetic efficiency and flavonoids accumulation of liquorice under nutrient stress. *Frontiers in Plant Science* 8:931. <https://doi.org/10.3389/fpls.2017.00931>
- Chen SC, Jin WJ, Liu AR, Zhang SJ, Liu DL, Wang FH, ... He CX (2013). Arbuscular mycorrhizal fungi (AMF) increase growth and secondary metabolism in cucumber subjected to low temperature stress. *Scientia Horticulturae* 160:222-229. <https://doi.org/10.1016/j.scienta.2013.05.039>
- Cosme M, Wurst S (2013). Interactions between arbuscular mycorrhizal fungi, rhizobacteria, soil phosphorus and plant cytokinin deficiency change the root morphology, yield and quality of tobacco. *Soil Biology & Biochemistry* 57:436-443. <https://doi.org/10.1016/j.soilbio.2012.09.024>
- Dhanushi P, Neelamane Y (2018). Arbuscular mycorrhizal fungi inoculation enhances drought stress tolerance of plants. *Groundwater for Sustainable Development* 7:490-494. <https://doi.org/10.1016/j.gsd.2018.03.005>
- Fokom R, Adamou S, Essono D, Ngwasiri DP, Eke P, Mofor TC, ...Sharma AK (2019). Growth, essential oil content, chemical composition and antioxidant properties of lemongrass as affected by harvest period and arbuscular mycorrhizal fungi in field conditions. *Industrial Crops and Products* 138:111477. <https://doi.org/10.1016/j.indcrop.2019.111477>
- Gao SY, William L. P, Richard T. K (1998). Integrated root system age in relation to plant nutrient uptake activity. *Agronomy Journal* 90(4):505-510. <https://doi.org/10.2134/agronj1998.00021962009000040011x>
- He L, Li CY, Liu RJ (2017). Interactions between arbuscular mycorrhizal fungi and beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera:Noctuidae). *Mycorrhiza* 27:525-535. <https://doi.org/10.1007/s00572-017-0771-2>
- Harley JL, Smith SE (2008). *Mycorrhizal symbiosis*. Elsevier Ltd:2008-01-01. <https://doi.org/10.1097/00010694-198403000-00011>
- Inaba Z, Ohshiro M (2010). Effects of planting density and methods of raising seedlings on flowering, yield and quality of cut flowers in snapdragons (*Antirrhinum majus* L.). *Environment Control in Biology* 43(3):201-210. <https://doi.org/10.1016/j.fusengdes.2008.06.053>
- Lin JX, Peng XY, Hua XY, Sun SN, Wang YN, Yan XF (2018). Effects of arbuscular mycorrhizal fungi on *Leymus chinensis* seedlings under salt-alkali stress and nitrogen deposition conditions: from osmotic adjustment and ion balance. *RSC Advances* (26):14500-14509. <https://doi.org/10.1039/c8ra00721g>
- Lin Y, Zhao X, Zhao EC, Cao K, Cao ZR (2019). Effects of arbuscular mycorrhizal fungi on watermelon growth, elemental uptake, antioxidant, and photosystem II activities and stress-response gene expressions under salinity-alkalinity stresses. *Frontiers in Plant Science* 10:863. <https://doi.org/10.3389/fpls.2019.00863>
- Liu RJ, Chen YL (2007). *Mycorrhizology*. Science press, Beijing.
- Mathur S, Sharma MP, Anjana J (2018). Improved photosynthetic efficacy of maize (*Zea mays*) plants with arbuscular mycorrhizal fungi (AMF) under high temperature stress. *Journal of Photochemistry and Photobiology B: Biology*. 180:149-154. <https://doi.org/10.1016/j.jphotobiol.2018.02.002>
- Murchie EH, Lawson T (2013). Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany* 64(13):3983-3998. <https://doi.org/10.1093/jxb/ert208>
- Pollastri S, Savvides A, Pesando M, Lumini E, Volpe MG, Ozudogru EA, ...Balestrini R (2018). Impact of two arbuscular mycorrhizal fungi on *Arundo donax* L. response to salt stress. *Planta* 247:573-585. <https://doi.org/10.1007/s00425-017-2808-3>
- Porcel R, Ricardo A, Juan MR (2012). Salinity stress alleviation using arbuscular mycorrhizal fungi. *Agronomy for Sustainable Development* 32:181-200. <http://dx.doi.org/10.1007/s13593-011-0029-x>
- Roháček K (2002). Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica* 40(1):13-29. <https://doi.org/10.1023/A:1020125719386>
- Parvin S, Maarten VG, Tanzima Y, Erik V, Olivier H (2020). Effects of single and multiple species inocula of arbuscular mycorrhizal fungi on the salinity tolerance of a Bangladeshi rice (*Oryza sativa* L.) cultivar. *Mycorrhiza* 30:431-444. <https://doi.org/10.1007/s00572-020-00957-9>
- Shuang F, Yan GY (2015). The analysis and prospect of development of fresh cut flower industry based on the patent analysis. *Agricultural Science & Technology* 16(9):2064-2069. [in Chinese]. <https://doi.org/10.16175/j.cnki.1009-4229.2015.09.050>

- Sun S, Zhang LT, Wang JX, Wang SM, Gao HJ, Gao HY (2008). Effects of low temperature and weak light on the functions of photosystem in *Prunus armeniaca* L. leaves in solar greenhouse. Journal of Applied Ecology 19(3):512-516. [in Chinese]. <http://dx.doi.org/10.13287/j.1001-9332.2008.0139>
- Sun ZY, Chen YQ, Valentin S, Liang HM, Li WH, Huang SQ, Peng CL (2015). Responses of the hybrid between *Sphagneticola trilobata* and *Sphagneticola calendulacea* to low temperature and weak light characteristic in south China. Scientific Reports 5(1):16906. <https://doi.org/10.1038/srep16906>
- Wang YH, Wang MQ, Li Y, Wu AP, Huang JY (2018). Effects of arbuscular mycorrhizal fungi on growth and nitrogen uptake of *Chrysanthemum morifolium* under salt stress. PloS One 13(4):e0196408. <https://doi.org/10.1371/journal.pone.0196408>
- Wahid F, Muhammad S, Shah F, Muhammad A, Imtiaz AK, Emre A, ... Nisar AK (2019). Arbuscular mycorrhizal fungi improve the growth and phosphorus uptake of mung bean plants fertilized with composted rock phosphate fed dung in alkaline soil environment. Journal of Plant Nutrition 42(15):1760-1769. <https://doi.org/10.1080/01904167.2019.1643371>
- Xu X, Yang F, Xiao XW, Zhang S, Korpelainen H, Li CY (2008). Sex-specific responses of *Populus cathayana* to drought and elevated temperatures. Plant, Cell & Environment 31:850-860. <https://doi.org/10.1111/j.1365-3040.2008.01799.x>
- Xu XY, Yu JH, Xie JM, Hu LL, Li H (2016). Effects of exogenous salicylic acid and brassinolide on photosynthesis of cucumber seedlings under low temperature stress. The Journal of Applied Ecology 27(9):3009-3015. [in Chinese]. <https://doi.org/10.13287/j.1001-9332.201609.020>
- Yu XN, He J, Li HX (2015). Effects of exogenous melatonin on photosynthetic characteristics of eggplant seedlings under low temperature and weak light stress. Proceedings of the 2015 6th International Conference on Manufacturing Science and Engineering. <https://doi.org/10.2991/ICMSE-15.2015.130>
- Zhang ZG, Shang QM (2010). Photosynthetic characteristics of pepper leaves under low temperature, weak light and salt stress. Scientia Agricultura Sinica 43(1):123-131. <https://doi.org/10.3864/j.issn.0578-1752.2010.01.015>
- Zhang ZF, Azim M, Zhang JC, Huang YQ, Zhou LW (2019). Effects of arbuscular mycorrhizal fungi on inoculated seedling growth and rhizosphere soil aggregates. Soil and Tillage Research 194:104340. <https://doi.org/10.1016/j.still.2019.104340>
- Zhou ZZ, Ma HM, Liang KN, Huang GH, Khongsak P (2012). Improved tolerance of teak (*Tectona grandis* L.f.) seedlings to low-temperature stress by the combined effect of arbuscular mycorrhiza and paclobutrazol. Journal of Plant Growth Regulation 31(3):427-435. <https://doi.org/10.1007/s00344-011-9252-6>
- Zhu XC, Song FB, Xu HW (2010). Effects of arbuscular mycorrhizal fungi on photosynthetic characteristics of maize under low temperature stress. Chinese Journal of Applied Ecology 21(2):470-475. [in Chinese]. <https://doi.org/10.13287/j.1001-9332.2010.0017>
- Zou CM, Wang YQ, Cao WD, Liu C, Zhang XH, Zhang S (2015). Response of photosynthesis and growth to weak light regime in different Adzuki bean (*Vigna angularis*) varieties. The Journal of Applied Ecology 26(12):3687-3692. <https://doi.org/10.13287/j.1001-9332.20151016.014>



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



**License** - Articles published in *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.

© Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

**Notes:**

- **Material disclaimer:** The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.

- Maps and affiliations: The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- Responsibilities: The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.