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Role of arbuscular mycorrhiza in alleviating the effect of cold on the photosynthesis of cucumber seedlings

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Abstract

Arbuscular mycorrhiza (AM) is known to enhance the rate of photosynthesis in plants, but there is little information on whether this effect is maintained at low temperature when the development of AM fungi is restrained. We therefore investigated the influence of AM on gas exchange, PSII fluorescence, and some photosynthesis-related biochemical parameters in cucumber seedlings under cold stress. Cold stress decreased, as expected, the chlorophyll content, net photosynthetic rate, and parameters related to photochemical quenching, while increasing nonphotochemical quenching and sugar contents in leaves. In contrast, AM had opposite effects on most of the determined parameters; it improved the efficiency of photosynthesis in the cucumber seedlings both at cold stress and at control ambient temperature. In addition, we recorded significant alleviation of the cold stress effect on sugar contents in leaves, which indicated that higher carbon-sink strength was an important factor maintaining higher efficiency of photosynthesis in mycorrhizal cucumber seedlings under cold stress.

Additional key words: arbuscular mycorrhizal fungi; carbohydrate; chlorophyll *a* fluorescence; cold stress; *Cucumis sativus* L.

Introduction

Low temperature is one of the most severe abiotic stresses affecting crop growth and yield (Bunn *et al*. 2009, Zhang *et al*. 2009), and causes considerable economic losses. Many biochemical and physiological processes occur during cold acclimation, accompanied by molecular changes (Su *et al*. 2010). These include increasing accumulation of osmolytes, cryoprotectants, and hydrogen peroxide in the protoplasm (Xin and Browse 2000, Zhang *et al*. 2009) as well as alteration of plasma membranes (Janicka-Russak *et al*. 2012). The interaction of stress with sugar signaling also regulates plant metabolism pathways under abiotic stress conditions (Gupta and Kaur 2005). During cold stress, sucrose accumulation in leaves leads to feedback inhibition of photosynthesis (Ruelland *et al*. 2009). Low temperature reduces photosynthesis and electron transport activity in plants, which generates conditions of excess light (Schöner and Heinrich Krause 1990). When PSII is exposed to excess light, reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), hydroxyl radical ($^{\bullet}OH$), and H_2O_2, are formed by the interaction of molecular oxygen and triplet chlorophyll (Chl) (Asada 2006, Yadav 2010). ROS directly affect chloroplasts and inhibit the repair of photodamaged PSII (Asada 2006), thereby causing problems arising from photoinhibition and photodamage of the photosynthetic apparatus (Oquist and Huner 1991, Murata *et al*. 2007).

Arbuscular mycorrhiza fungi (AMF) are ubiquitous soil fungi that form symbioses with over 80% of terrestrial plant species (Smith and Read 2008, Schüßler *et al*. 2001). The extensive hyphal network of AMF promotes plant uptake of nutrients (especially phosphate) and water (Finlay 2008) from the soil. In return, AMF obtain

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Abbreviations: AMF – arbuscular mycorrhizal fungi; C_a – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; Chl – chlorophyll; DM – dry mass; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximal quantum yield of PSII photochemistry; F_v'/F_m' – maximum efficiency of PSII; FM – fresh mass; g_s – stomatal conductance; L_s – stomatal limitation; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; ROS – reactive oxygen species; q_P – photochemical quenching coefficient; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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photosynthetic products from their host plants (Solaiman and Saito 1997, Bago *et al*. 2003, Selosse *et al*. 2006). The carbon budget of plants is tightly linked to the efficiency of photosynthesis. Stimulation of the rate of photosynthesis has been regularly described in mycorrhizal plants and attributed mainly to their increased carbon demand due to the carbon cost of the symbiosis (Wright *et al*. 1998, Kaschuk *et al*. 2009, Bulgarelli *et al*. 2017). As the AM-induced increase in photosynthetic rate is usually greater than the carbon costs, mycorrhizal symbiosis can result in sink stimulation of photosynthesis and improve the efficiency of photosynthetic product use in relation to total plant biomass (Kaschuk *et al*. 2009). This represents an adaptation mechanism that allows plants to take advantage of nutrient supply from their symbionts without compromising the total amount of photosynthates available for plant growth.

Symbiosis with AMF is known to generally increase the tolerance of plants to abiotic stresses (Smith and Read 2008). Stress alleviation has been also repeatedly shown at the level of photosynthesis, mainly for plants exposed to drought stress (Pinior *et al*. 2005) or salt stress (Sheng *et al*. 2008, Hajiboland *et al*. 2010, Porcel *et al*. 2015). This has been mostly attributed to improved water status of mycorrhizal plants, which enabled them to maintain higher gas-exchange capacity (Drüge and Schonbeck 1993, Wu and Xia 2006, Sheng *et al*. 2008). Arbuscular mycorrhiza is also documented to positively affect plant performance under temperature stress: plant growth was enhanced with AMF at high temperature (Martin and Stutz 2004) and low temperature (Wu and Zou 2010, Chen *et al*. 2013), though the benefits provided by AMF were lower under lower temperature (8–15°C) (Ruotsalainen and Kytöviita 2004). In relation to the photosynthetic apparatus, the symbiotic association may alleviate the damage of cold stress *via* attenuating membrane lipid peroxidation, increasing the contents of photosynthetic pigments, osmotic accumulation, enhancing the activities of antioxidant enzymes and inducing the production of secondary phytomedicinal metabolites (Latef and He 2011, Bunn *et al*. 2009, Chen *et al*. 2013, Liu *et al*. 2014). As low temperature also strongly inhibits assimilation of photosynthetic sucrose (Stitt and Hurry 2002), which ultimately leads to the inactivation of Rubisco, mycorrhiza also could enhance photosynthesis by constituting an additional carbon sink (Kaschuk *et al*. 2009).

However, root colonization by AMF is often restrained at temperatures below 15°C (Hetrick and Bloom 1984, Liu *et al*. 2004, Zhu *et al*. 2010b) and has been repeatedly reported to confer less growth promotion to the host plant at low temperature (Ruotsalainen and Kytöviita 2004, Wu and Zou 2010, Ma *et al*. 2015). This is attributed to changes in the plant's carbon-nutrient balance, when carbon and nutrient acquisition, carbon consumption and nutrient demand are differentially affected by cold (Ruotsalainen and Kytöviita 2004). It is therefore important to understand how mycorrhiza influences different aspects of plant physiology at low temperature. To evaluate the interactive effects of mycorrhiza and cold stress on cucumber photosynthesis, we conducted an experiment in which mycorrhizal and non-mycorrhizal plants were exposed to cold stress in a full-factorial design. We determined a range of parameters associated with gas exchanges and Chl fluorescence measurements, in terms of CO_2 assimilation and the functionality of the photosynthetic apparatus, respectively. Moreover, sucrose and soluble sugar concentrations were simultaneously measured in order to detect carbohydrate metabolism in cucumber leaves. The main question of our experiment was whether beneficial effects of arbuscular mycorrhiza on plant photosynthesis are maintained at low temperature.

Materials and methods

Plant, fungal material, and treatments: Inoculation was performed with the AMF *Rhizophagus irregularis* (Blaszk, Wubet, Renker & Buscot) Schüßler and Walker (2010), isolate PH5 (Malcová and Gryndler 2003). The inoculum was prepared in the Institute of Botany (*ASCR*, Průhonice, Czech Republic) and consisted of a dried cultivation substrate (1:1 mixture of zeolite and sand) containing spores (~65 g^{-1}), extraradical hyphae, and chopped roots of the host plant, *Zea mays* L.

Cucumber (*Cucumis sativus* L. cv. Zhongnong No. 26) seeds were germinated at 28°C on Petri dishes lined with two layers of filter paper moistened with sterile distilled water. The germinated seeds were then transferred into plastic pots (one seed per pot). Half of the seeds were inoculated with 10 g of the *R. irregularis* inoculum per pot (mycorrhizal treatment); the other half received the same amount of inoculum, sterilized by autoclaving (non-mycorrhizal treatment). The size of the pots was 13 × 13 cm (height × diameter) and each pot contained 480 g of a mixture of peat and vermiculite (1:1, v/v) as the cultivation substrate. Prior to use, the substrate was sieved through a 4-mm mesh and sterilized for 4 h at 160°C. Its chemical properties were as follows: pH 4.67, organic matter of 25.8%, 520 mg(available N) kg^{-1} (Kachurina *et al*. 2000), 305 mg(available K) kg^{-1} (Hanway and Heidel 1952), and 96.8 mg(available P) kg^{-1} (Olsen *et al*. 1954). The plants were cultivated for 40 d in a greenhouse, with average temperatures of 26/20°C (day/night) and photon flux density ranging from 700 to 1,200 μmol(photon) m^{-2} s^{-2}. During this experimental period, the plants were supplied weekly with 100 mL of 1/4 strength Hoagland solution without P. After 40 d of cultivation, 25 seedlings per treatment were transferred to two growth chambers and subjected to two different temperature conditions for a further 14 d: ambient temperatures 25/15°C (day/night) and cold-stress conditions (C) 15/10°C (day/night). In this

experimental system, P limitation of the experimental plants promoted the development of arbuscular mycorrhiza; the plants did not display any visible symptoms of P deficiency but were P-deficient at the end of the cultivation period according to P contents in shoots (Ma *et al*. 2015).

Thus, the experiment had a two by two factorial design and consisted of four treatments: (*1)* noninoculated cucumber plants (NI); (*2*) inoculated cucumber plants (AMF); (*3*) noninoculated cucumber plants exposed to cold stress (NI+C); (*4*) inoculated cucumber plants exposed to cold stress (AMF+C).

Plant growth and root colonization: Plant growth parameters and percentages of mycorrhizal root colonization were determined before the start of the temperature treatments (0 week) and after two weeks of temperature treatments (2 weeks). To determine the dry mass of the cucumber plants, the shoots and roots of ten replicate plants per treatment were dried in a hot-air oven at 75°C for 72 h to constant mass. To determine the percentage of mycorrhizal root colonization, the root systems of three randomly selected plants per treatment were carefully washed, cut into 1-cm-long segments, cleared in 10% KOH at 90°C for 40 min, acidified in 2% lactic acid at 90°C for 20 min, and stained with 0.05% trypan blue at 90°C for 30 min (Phillips and Hayman 1970). Mycorrhizal colonization was determined according to the magnified (100×) intersections method (McGonigle *et al*. 1990) using light microscopy (*Olympus BX41*, *Olympus Corp.*, Tokyo, Japan).

Gas-exchange measurements and Chl fluorescence were measured after two weeks of temperature treatments, in three randomly selected plants per treatment. Measurements were taken on the same position of the third leaf from the apex using a portable photosynthesis system (*LI-6400; LI-COR Inc*, Lincoln, NE, USA). The net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentrations (C_i) were measured at 380 ± 10 μmol(CO_2) mol^{-1}, 25°C, 70% relative humidity, and light intensity of 800 μmol(photon) m^{-2} s^{-1}. Stomatal limitation (L_s) was calculated as $L_s = 1 - C_i/C_a$ (where C_i and C_a represent the intercellular and ambient CO_2 concentrations, respectively) according to Farquhar *et al*. (1982). The minimum (F_0) and maximum (F_m) fluorescence yield of the dark-adapted state were determined in leaves after 12 h of dark adaptation; the minimal (F_0') and the maximal (F_m') fluorescence yield in the light-adapted state as well as the steady-state fluorescence yield (F_s) were measured after light adaption at 600 μmol(photon) m^{-2} s^{-1}. Using the fluorescence parameters in both dark- and light-adapted leaves, the following parameters were calculated: (*1*) maximum quantum yield of PSII photochemistry: $F_v/F_m = (F_m - F_0)/F_m$; (*2*) maximum efficiency of PSII: $F_v'/F_m' = (F_m' - F_0')/F_m'$; (*3*) effective quantum yield of PSII photochemistry: $\Phi_{PSII} = (F_m' - F_s)/F_m'$; (*4*) photochemical quenching coefficient: $q_P = (F_m' - F_s)/(F_m' - F_0')$; (*5*) nonphotochemical quenching: $NPQ = (F_m - F_m')/F_m'$ (Baker 2008).

Determination of biochemical parameters: All the parameters described below were determined in three replicates per treatment in plants harvested two weeks after exposure to the temperature treatments.

Chl was extracted according to the method described by Han (1996). The third fully expanded leaf (0.5 g) was immediately ground to homogenate with small amounts of quartz sands, calcium carbonate powders, and 2 mL of 95% ethanol, and then 10 mL of ethanol was added. After 3–5 min, the extracting solution was filtered into brown volumetric flask and added to a constant volume of 50 ml by 95% ethanol. Chl content was measured at 665 nm and 649 nm by a spectrophotometer (*SP-1900UV*, *Shimadzu*, Japan).

For determination of the other parameters, shoots and roots were washed under running water and random subsamples of 0.5 g fresh mass (FM) were immediately frozen in liquid nitrogen.

H_2O_2 in leaves was extracted according to Doulis *et al*. (1997). Briefly, 0.5 g fresh mass (FM) of leaf tissue was ground in liquid nitrogen, then 2 mL of 0.2 M HClO4 added. The mixture was transferred to a 10-mL plastic tube, and 2 mL of 0.2M $HClO_4$ was then added. The homogenate was centrifuged at 2,700 × g for 30 min at 4°C, the supernatant was then collected, adjusted to pH 6.0 using 4M KOH, and centrifuged at 110 × *g* for 1 min at 4°C. The supernatant was put onto a *AG1 × 8* prepacked column (*Bio-Rad*, Hercules, CA, USA) and H_2O_2 was eluted with 4 mL of double-distilled H_2O. The sample (800 μL) was mixed with 400 μL of reaction buffer, containing 4 mM 2,2'azinodi (3-ethylbenzthiazoline-6-sulfonic acid) and 100 mM potassium acetate at pH 4.4, 400 μL of deionized water, and 0.25 U of horseradish peroxidase. H_2O_2 was determined by a spectrophotometric assay (Willekens *et al*. 1997). H_2O_2 was expressed in mmol g^{-1} (FM).

Carbohydrates were analyzed as described by Hu *et al*. (2009). Samples of 0.5 g(FM) of leaf tissue were homogenized and extracted three times, in 5 mL of 80% ethanol for 30 min each time at 80°C. The samples were then centrifuged for 10 min at 3,000 × *g*. Supernatants were treated as leaf samples to analyze the content. An *Agilent 1200* HPLC system (*Agilent Technologies*, Palo Alto, CA, USA) was used to determine the total concentration of carbohydrates in the extracts. Carbohydrate compounds were separated on a *Waters Sugar Pak* column (*Agilent Technologies*, 6.5 × 9 × 300 mm) at 50°C, with water as the mobile phase at a flow rate of 0.5 mL min^{-1}. Sucrose was identified by comparison of retention times of known standards purchased from *Sigma-Aldrich Trade Co*, *Ltd.*

(Shanghai, China), and quantified by a refractive index detector (*G1362A RID*, *Agilent Technologies*).

Statistical analysis: Plant parameters were statistically analyzed using two-way analyses of variance (*ANOVA*) with the fixed factors 'inoculation' and 'cold stress'. Root colonization was evaluated by one-way *ANOVA* with the factor 'cold stress'. Comparisons among means were carried out using *Duncan*'s multiple range test at a significance level of $P<0.05$ using the *SPSS* (*IBM*, Chicago, IL, USA) *19.0* software. *GraphPad Prism ver. 5* (*GraphPad Software Inc*, La Jolla, CA, USA) was used to prepare graphs.

Results

Development of AMF and plant growth: No AMF colonization was found in the roots of the noninoculated treatments. Roots of the inoculated cucumber plants were well colonized by AMF. Before the start of the temperature treatments, root colonization by the AMF hyphae, arbuscules, and vesicles were 48, 48, and 27%, respectively (Table 1). During the subsequent two-week period of temperature treatments, all root colonization parameters increased both at ambient temperature and under cold stress. Under cold stress, however, the increase was significantly lower than that at an ambient temperature (Table 2).

At the beginning of the temperature treatments, inoculated plants had significantly higher root biomass than that of noninoculated plants, but they did not differ in shoot biomass. After two weeks at ambient temperature, the dry mass (DM) of both shoots and roots were significantly higher in the inoculated than that in the noninoculated plants. In the cold-stressed plants, however, only root biomass was higher in the inoculated than in the noninoculated plants, while shoot biomass did not differ between the inoculation treatments.

Table 1. Root colonization by arbuscular mycorrhizal fungal hyphae, vesicles, and arbuscules, shoot and root dry mass (DM) of the experimental plants before the start of the temperature treatments (0 week) and after two weeks of temperature treatments. The seedlings were either inoculated (AMF) or noninoculated (NI) with *Rhizophagus irregularis* and cultivated under an ambient temperature or exposed to cold stress (C) in the temperature treatments. Values are means ± SE. *n* (hyphae, arbuscular, vesicles) = 3; *n* (shoot and root DM) = 10. Significances are given according to *ANOVA* performed on the '2-week' data: one-way *ANOVA* with the factor 'Cold stress' for the root colonization parameters; two-way *ANOVA* with the factors 'Inoculation' and 'Cold stress' for the dry mass. Significant differences are indicated (with *F*-values in parentheses): *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$, n.s. – not significant, n.d. – not determined. Values marked with *different letters* are significantly different at $P<0.05$, according to *Tukey*'s multiple comparison test performed on all data shown for each parameter.

	Treatment	Hyphae [%]	Arbuscular [%]	Vesicles [%]	Shoot DM [g per plant]	Root DM [g per plant]
0-week	NI	0 ± 0	0 ± 0	0 ± 0	1.03 ± 0.13^{d}	0.22 ± 0.06^{e}
	AMF	48 ± 4^{c}	47 ± 1^{c}	27 ± 1^{c}	1.32 ± 0.07^{d}	0.26 ± 0.04^{d}
2-week	NI	0 ± 0	0 ± 0	0 ± 0	3.17 ± 0.33^{b}	0.61 ± 0.08^{b}
	AMF	82 ± 0^{a}	83 ± 2^{a}	57 ± 4^{a}	3.24 ± 0.23^{a}	0.81 ± 0.07^{a}
	NI+C	0 ± 0	0 ± 0	0 ± 0	1.99 ± 0.04^{c}	0.45 ± 0.03^{c}
	AMF+C	73 ± 2^{b}	77 ± 4^{b}	39 ± 4^{b}	2.14 ± 0.19^{c}	0.60 ± 0.02^{b}
Significance (*F* value)						
Inoculation (AMF)		n.d.	n.d.	n.d.	n.s.	** (18.5)
Cold stress (C)		** (31.1)	** (95.9)	* (19.8)	***(56.3)	** (18.6)
AMF × C		n.d.	n.d.	n.d.	n.s.	n.s.

Gas-exchange parameters: Cold stress significantly decreased P_N as compared to ambient temperature. In both temperature treatments, P_N was significantly higher in the inoculated plants than in the noninoculated plants (Table 2, Fig. 1*A*). Cold stress also significantly decreased g_s; the inoculation significantly enhanced this parameter at ambient temperature, but had no effect under cold stress (Table 2, Fig. 1*B*). Cold stress significantly increased C_i in noninoculated plants, while inoculation significantly alleviated this effect of cold stress (Table 2, Fig. 1*C*). L_s increased by cold stress in both noninoculated and inoculated plants. However, under cold stress, L_s remained lower in the inoculated plants than that in the noninoculated plants (Table 2, Fig. 1*D*).

Chl fluorescence: Cold stress significantly decreased all Chl fluorescence parameters, except NPQ, which significantly increased (Table 2, Fig. 2). There was no significant interaction between cold stress and AMF inoculation, except for in the efficiency of F_v'/F_m'. This means that the effect of cold stress on most Chl fluorescence parameters was similar, regardless of inoculation. F_v'/F_m' was reduced by cold stress to a lesser degree in the inoculated plants than that in the noninoculated plants (Fig. 2*D*). Inoculation significantly increased Φ_{PSII} and q_P, and decreased NPQ. Inoculation had no effect on maximal PSII efficiency.

Chl and H_2O_2: The effects of cold stress on Chl and H_2O_2 content in plant leaves were significantly affected by

Table 2. Summary of the effects of the experimental factors, 'Inoculation with *Rhizophagus irregularis'* and 'Cold stress' and their interaction on gas-exchange and chlorophyll fluorescence parameters, chlorophyll, H_2O_2, sucrose, and total sugar concentrations in leaves and roots of 54-d-old cucumber plants. Significant differences are indicated (with *F*-values in parentheses): *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$, n.s. – not significant.

Parameter	Inoculation (AMF)	Cold stress (C)	AMF × C
Net photosynthetic rate, P_N [μmol m^{-2} s^{-1}]	*** (60.6)	*** (83.1)	n.s.
Stomatal conductance, g_s [mol(H_2O) m^{-2} s^{-1}]	n.s.	** (18.0)	*** (54.5)
Intercellular CO_2 content, C_i [μmol(CO_2) m^{-2} s^{-1}]	** (17.6)	* (7.6)	** (26.0)
Stomatal limitation, L_s	* (7.3)	*** (63.2)	n.s.
Maximal quantum yield of PSII photochemistry, F_v/F_m	n.s.	*** (161.4)	n.s.
Effective quantum yield of PSII photochemistry, Φ_{PSII}	*** (39.9)	*** (302.0)	n.s.
Maximum efficiency of PSII, F_v'/F_m'	*** (33.4)	*** (285.3)	** (29.0)
Photochemical quenching coefficient, q_P	*(11.2)	*** (77.1)	n.s.
Nonphotochemical quenching, NPQ	** (15.5)	*** (114.3)	n.s.
Leaf chlorophyll content [μg g^{-1}(FM)]	*** (370.4)	* (9.4)	*** (91.3)
Leaf H_2O_2 content [mmol g^{-1}(FM)]	*** (178.0)	** (16.5)	** (22.1)
Leaf sucrose concentration [mg g^{-1}(FM)]	* (7.5)	*** (124.4)	*** (47.5)
Leaf total sugar concentration [mg g^{-1}(FM)]	n.s.	*** (39.3)	* (8.7)

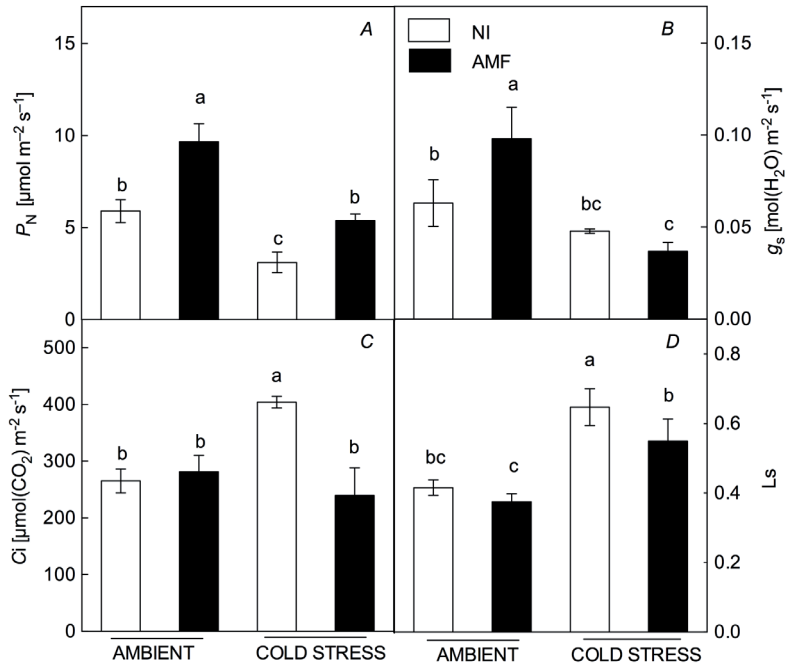

Fig. 1. Net photosynthetic rate (P_N, *A*), stomatal conductance (g_s, *B*), intercellular CO_2 content (C_i, *C*), and stomatal limitation (L_s, *D*) in the leaves of 54-d-old cucumber plants. The seedlings were either noninoculated (NI, *white bars*) or inoculated with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (AMF, *black bars*), and kept at an ambient temperature or exposed to cold stress for the last two weeks of the cultivation period. Values marked by *different letters* are significantly different at $P<0.05$ according to *Duncan*'s test ($n = 3$).

both experimental factors as well as by their interaction, meaning that noninoculated and inoculated plants responded differently to cold stress (Table 2). The Chl content increased by inoculation under both temperature conditions, and significantly decreased by cold stress more in the plants after the inoculation treatment (Fig. 3*A*). The H_2O_2 content was reduced by inoculation in both temperature treatments, but the difference between the inoculation treatments was larger under cold stress than that under at ambient temperature (Fig. 3*B*).

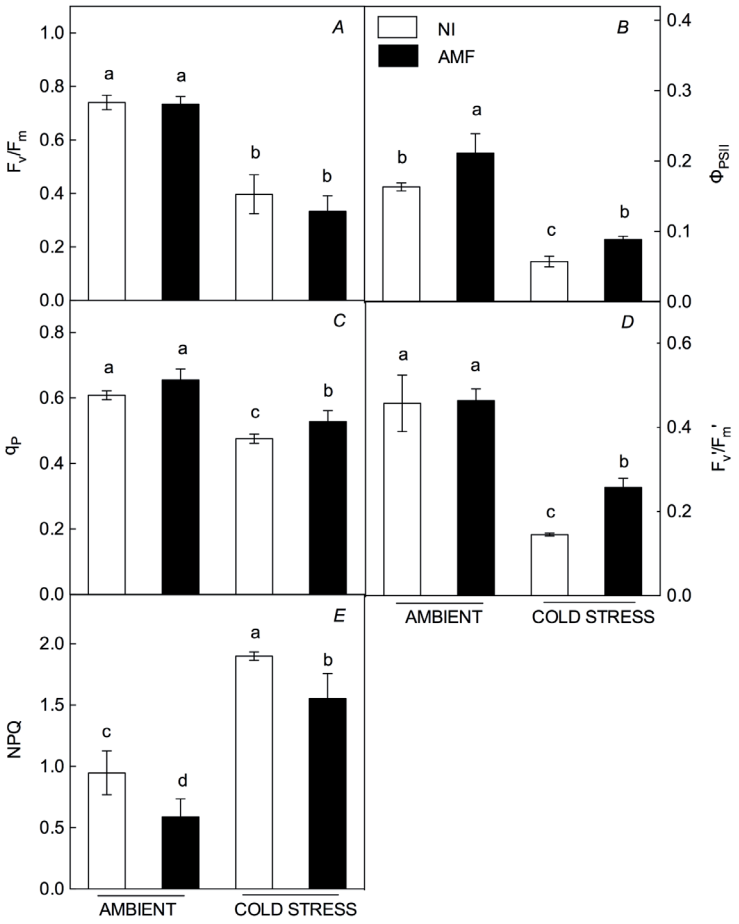

Fig. 2. Maximal photochemical efficiency of PSII (F_v/F_m, *A*), the effective quantum yield of PSII photochemistry (Φ_{PSII}, *B*), photochemical quenching coefficient (q_P, *C*), the maximum efficiency of PSII (F_v'/F_m', *D*), and nonphotochemical quenching (NPQ, *E*) of 54-d-old cucumber plants. The seedlings were either noninoculated (NI, *white bars*) or inoculated with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (AMF, *black bars*) and kept at an ambient temperature or exposed to cold stress for the last two weeks of the cultivation period. Values marked by *different letters* are significantly different at $P<0.05$ according to *Duncan*'s test ($n = 3$).

Sucrose and total sugar: Cold stress generally increased the sucrose and total sugar contents in cucumber leaves, but the extent of the effect depended on the inoculation treatment (Table 2, Fig. 4). Sucrose content was increased by cold stress, much more in the noninoculated plants than that in the inoculated plants. Noninoculated plants had higher sucrose content than that of the inoculated plants under cold stress, while the difference was reversed at ambient temperature. A similar trend was evident for the total sugar contents, though inoculated and noninoculated plants did not significantly differ at either temperature treatment.

Discussion

As expected, cold stress reduced the efficiency of photosynthesis in cucumber, which was evident on a range of effects on the gas-exchange parameters and Chl fluorescence. Enhancement of photosynthesis by mycorrhiza has been previously reported for a range of plants (Boldt *et al*. 2011, Bulgarelli *et al.* 2017) and is attributed to improved nutrition and increased carbon sink of mycorrhizal plants (Wright *et al*. 1998, Kaschuk *et al*. 2009). At low temperature, however, the development of AMF may be restrained (Hetrick and Bloom 1984, Liu *et al*. 2004, Zhu *et al*. 2010b) and nutrient supply to the host plant reduced (Wang *et al*. 2002). This was also indicated in our experimental system with cucumber, where we recorded a slight, but significant reduction of the intraradical development of the AMF reduced by low temperature. In a previous study, we also showed that the mycorrhizal pathway of P uptake was down-regulated by cold stress in cucumber (Ma *et al*. 2015). Despite this, mycorrhiza improved the photosynthetic performance of cucumber also under cold stress.

The P_N clearly decreased at low temperature but was enhanced by mycorrhiza both at ambient and at low temperature similarly. Decreased CO_2 assimilation can be caused by stomatal limitation or by nonstomatal factors (Begcy *et al*. 2012, Liu *et al*. 2012, Shu *et al*. 2014, Zhang *et al*. 2009): CO_2 assimilation is primarily limited by g_s if accompanied by a decrease in g_s and C_i. On the other hand, if g_s decreases but C_i does not change or increases, the decrease in CO_2 assimilation should be ascribed to nonstomatal factors. According to this, CO_2 assimilation of the cold-stressed plants, both non-mycorrhizal and mycorrhizal, was primarily reduced by nonstomatal factors in our experiment, which is in accordance with previously

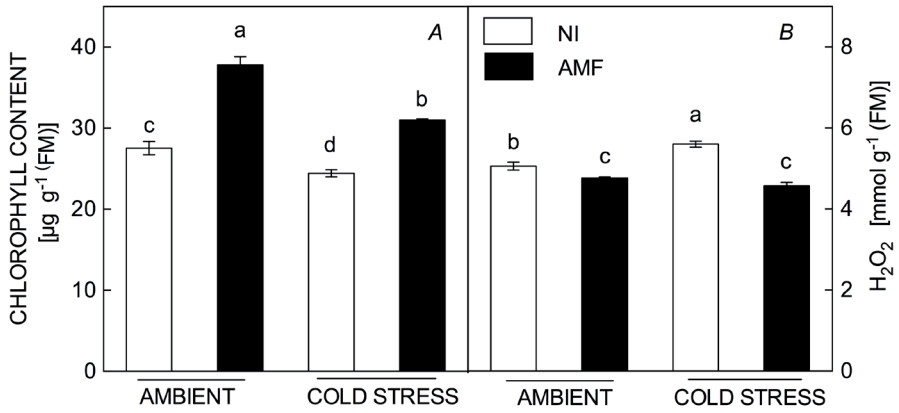

Fig. 3. Concentration of chlorophyll (*A*) and H_2O_2 (*B*) in the leaves of 54-d-old cucumber plants. The plants were either noninoculated (NI, *white bars*) or inoculated with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (AMF, *black bars*), and exposed to cold stress or kept at an ambient temperature for the last two weeks of the cultivation period. Values marked by *different letters* are significantly different at $P<0.05$ according to *Duncan*'s test ($n = 3$).

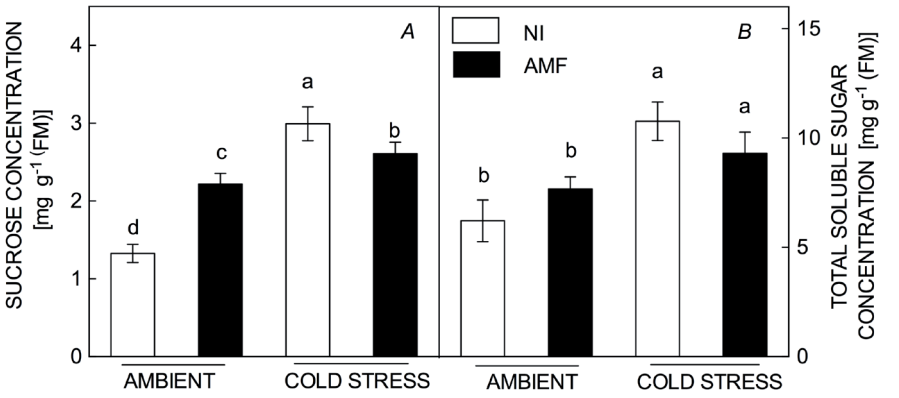

Fig. 4. The concentrations of sucrose (*A*) and total sugar (*B*) in the leaves of 54-d-old cucumber plants. The plants were either noninoculated (NI, *white bars*) or inoculated with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (AMF, *black bars*), and exposed to cold stress or kept at an ambient temperature for the last two weeks of the cultivation period. Values marked by *different letters* are significantly different at $P<0.05$ according to *Duncan*'s test ($n = 3$).

reported results for cold-stressed maize (Zhu *et al*. 2010b) and for drought-stressed plants (Wu and Xia 2006, Sheng *et al*. 2008).

Interestingly, and in contradiction to the above mentioned studies, g_s was enhanced by mycorrhiza in our experiment only in plants growing at ambient temperature, while it was unaffected in the cold-stressed plants. Higher g_s is often coupled with higher photosynthetic efficiency in mycorrhizal plants (Fay *et al*. 1996, Nowak 2004, Boldt *et al*. 2011). In a recent meta-analysis, however, Augé *et al*. (2015) showed that mycorrhizal effects on g_s are related to a wide range of factors. Among others, g_s of mycorrhizal plants is positively related to their mycorrhizal growth response and the extent of root colonization by AMF (Augé *et al*. 2015). As mycorrhizal root colonization and mycorrhizal growth response were reduced by cold stress, these two factors may explain why g_s of mycorrhizal plants decreased under cold stress to the level encountered in non-mycorrhizal plants.

The rate of photosynthesis is also influenced by the contents of important photosynthetic pigments (especially Chl) and changes in cell pigments are widely related to cold-induced oxidative stress (Saltveit and Morris 1990, Badiani *et al*. 1993), impairment in pigment biosynthetic pathways or degradation (Ashraf and Harris 2013). In our experiment, AM symbiosis enhanced the Chl content (Chl *a*+*b*) under cold stress, though the effect was smaller than at ambient temperature. Hence, higher Chl content may have contributed to the enhanced P_N of the mycorrhizal plants at both temperature treatments as previously reported by Zhu *et al*. (2010b).

Parameters of Chl fluorescence are powerful indicators for the functioning of the photosynthetic apparatus under stress conditions (Vonshak *et al*. 2001). The F_v/F_m ratio (maximal quantum yield of PSII photochemistry) indicates the overall photosynthetic capacity of leaves (Jamil *et al*. 2007, Tang *et al*. 2007, Balouchi 2010). It is close to 0.8 in the leaves of unstressed plants, and decreases when

plants suffer stress resulting in injury or inactivation in a proportion of PSII reaction centers and, consequently, chronic photoinhibition (Baker and Rosenqvist 2004, Zlatev 2009, Vaz and Sharma 2011). In our experiment, F_v/F_m was substantially decreased by the cold treatment in accordance with previous reports (Fracheboud *et al*. 1999, Oliveira and Peñuelas 2005, Zhu *et al*. 2010b), while it was unaffected by mycorrhiza. Mycorrhizal plants were frequently found to have higher F_v/F_m than non-mycorrhizal plants (Porcel *et al*. 2015, Sheng *et al*. 2008), but absence of mycorrhizal effect on F_v/F_m has also been reported (Fay *et al*. 1996). F_v/F_m may be, however, less important for assessing the tolerance of plants to cold stress than parameters related to electron transport, such as the maximum efficiency of PSII (F_v'/F_m') and effective quantum yield of PSII photochemistry (Φ_{PSII}) (Fracheboud *et al*. 1999). These parameters decreased by cold treatment in our experiment, similarly as F_v/F_m, but also increased by mycorrhiza, which indicates that mycorrhizal plants used the captured excitation energy more efficiently than non-mycorrhizal plants.

The increase in q_P and decrease in NPQ in mycorrhizal plants, as compared to non-mycorrhizal plants, is in line with this assumption. Sheng *et al*. (2008) observed, at salt stress, that mycorrhizal plants had both higher q_P and higher NPQ values than non-mycorrhizal plants, and suggested that higher NPQ values are indicative for more efficient protection of leaves from light-induced damage. Other authors, however, reported, in accordance with our results, lower NPQ values in mycorrhizal plants alongside with higher values of parameters related to photochemical quenching (Boldt *et al*. 2011, de Andrade *et al*. 2015, Porcel *et al*. 2015). NPQ can increase as a result of processes that protect the leaf against light-induced damage (in accordance with the assumption of Sheng *et al*. 2008), but also as a consequence of the damage itself (Maxwell and Johnson 2000). In line with the latter explanation, the pronounced increase of NPQ in the cold-stressed cucumber plants resulted from light-induced damage to PSII, and significantly lower NPQ of mycorrhizal plants indicated that their leaves suffered less damage.

However, it is important to stress that the observed higher efficiency of solar energy use in mycorrhizal cucumber cannot be directly related to alleviation of cold-induced damage to PSII, as it was recorded not only at cold stress, but also at ambient temperature. A similar pattern was reported in an earlier study for cold-stressed maize plants (Zhu *et al*. 2010b). In contrast, clear alleviation of stress-induced damage to the photosynthetic apparatus by mycorrhiza was observed in plants exposed to arsenic (de Andrade *et al*. 2015), salt stress (Sheng *et al*. 2008, Hajiboland *et al*. 2010, Porcel *et al*. 2015) or drought stress (Pinior *et al*. 2005). It seems therefore that cold-stressed mycorrhizal plants rely on similar mechanisms enhancing their photosynthetic efficiency as plants growing at ambient temperature, while direct interaction of mycorrhizal effects with the stress factor may occur when plants are exposed to other types of abiotic stresses, such as improvement of the plant water status at salinity stress (Sheng *et al*. 2008).

It has been suggested that carbon transport to AMF constitutes an important mechanism of increasing the rate of photosynthesis in mycorrhizal plants (Kaschuk *et al*. 2009, Bulgarelli *et al.* 2017). Mycorrhiza can act as a further carbon sink in plants, thus stimulating the depletion of sucrose in the photosynthesizing tissues (Wright *et al*. 1998 – photosynthesis and biomass production). As triose-P-utilization is one of the biochemical processes that limit photosynthesis, this may stimulate the photosynthetic rate of mycorrhizal plants generally, and especially under conditions where the carbon sink of the plants is decreased by an abiotic stress such as cold (Ruelland *et al*. 2009). In our experiment, mycorrhizal plants had higher sucrose contents in their leaves than non-mycorrhizal plants at ambient temperature, while cold stress pronouncedly increased the leaf sucrose concentration in non-mycorrhizal plants to higher level than that in mycorrhizal plants. Higher amounts of hexoses in the leaves of mycorrhizal than that of non-mycorrhizal plants, as recorded at ambient temperature, have been previously reported (Wright *et al*. 1998, Boldt *et al*. 2011) and attributed to the enhanced carbohydrate metabolism of mycorrhizal plants. Under the conditions of stress, however, sucrose accumulation in leaves is indicative for reduced sink strength, and this effect of cold was partly alleviated by mycorrhiza. We may therefore hypothesize that the mycorrhizal contribution to carbon-sink strength becomes relatively more important under cold stress than under ambient temperature, even if the development of the fungus is restrained at cold. In addition to direct carbon consumption by the fungus, mycorrhiza may have affected the carbon-sink strength indirectly, by increasing the root biomass (but not the shoot biomass) of the host plant at cold stress. Enhancement of root growth but not shoot growth under cold stress has been previously reported in maize (Zhu *et al*. 2010a) and may be attributed to partial protection of the belowground organ against the cold.

Conclusion: Our results show that mycorrhiza increases the photosynthetic efficiency of cold-stressed cucumber seedlings by protecting their photosynthetic apparatus against light-induced damage and increasing their carbon sink. While the effect of mycorrhiza on gas exchange and chlorophyll fluorescence was comparable at ambient and low temperature, the higher carbon-sink strength of mycorrhizal plants became relatively more important at low temperature. The results of our study thus constitute an important contribution to the discussion of the role of arbuscular mycorrhiza at low temperature. We showed that beneficial effects of arbuscular mycorrhiza on plant

photosynthesis were maintained at cold, even if the importance of the symbiosis for plant growth may decrease (Ruotsalainen and Kytöviita 2004, Wu and Zou 2010, Ma *et al*. 2015).

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