Effects of Arbuscular Mycorrhizal Fungi on the Heat Resistance of *Dianthus hybridus* Seedlings

Yunxin Tang[1,#], Jia Huang[1,#], Yunjian Guo[2], Yongliang Luo[2], Chunwen Li[3], Zhongqun He[1,*], and Xiaoting Zhou[1,*]

[1]*College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China*
[2]*People's Government of Pidu District, Chengdu, Sichuan, 611730, China*
[3]*Chengdu Jinping Flower Seedling Limited Liability Company, Chengdu, Sichuan, 611730, China*
[#]*These authors contributed equally to this work*

Keywords: *Dianthus hybridus*, Arbuscular Mycorrhizal Fungi, High Temperature Stress.

Abstract: In order to explore whether AMF can inoculation enhance heat resistance of *Dianthus hybridus* seedlings under high temperature, growth indexes and gas exchange parameters chlorophyll fluorescence parameters as well as the main heat resistance evaluation indexs were tested. The results showed that the plant height and stem diameters of AMF-inoculated plants were significantly greater than those of non-inoculated plants. AMF treatment also increased the net photosynthetic rate. Under high temperature stress, AMF treatment plants decreased the MDA content, while it increased the activities of SOD, POD and CAT as well as proline content. Under high temperature stress, AMF treatment plants suppressed the decrease of Fv'/Fm', Y(II) and qP to the control level, and also reduced NPQ. Thus, application of AMF inoculation can promote both the plant growth and heat resistance in *Dianthus hybridus* seedlings.

1 INTRODUCTION

Dianthus hybridus is an important material for arranging flower beds and flower borders. To ensure the production of *Dianthus hybridus* around the year, seedling raising is often used. However, high temperature is one of the limited factors of seedling raising in *Dianthus hybridus* in summer, due to its sensitivity to heat. Under high temperature stress, the combination of strong sunlight and rapid transpiration will lead to plant protoplast dehydration and protein solidification, plant growth weakening, leaf color fading, wilting and yellow, and lead to the increase of deformed flowers. Therefore, it is important to carry out the research on the *Dianthus hybridus* of heat resistance. Therefore, it is important to carry out the research on the *Dianthus hybridus* of heat resistance. Arbuscular mycorrhizal fungi (AMF) is a kind of soil microorganism (Liu 2009), which can form a symbiotic relationship with more than 90% plant (Ma 2015). Studies have shown that AMF inoculation can promote growth of plant (Liu 2015, Ma 2014), improve the ability of plants to resist continuous cropping obstacles (Chen 2013), and enhance plant stress resistance (Liu 2011, Zhang 2016, Guo 2011, Sun 2012, Ye 2019). AMF can regulate the formation of secondary metabolites in the host plant by changing the root morphology of the plant, so as to improve the physical and chemical properties of the root environment. It also changes the microbial community structure around the root, activates and induces a variety of mechanisms in the process of infecting the host, increase the regulation of plant defensive enzyme activity and gene expression, as a result of improving plant abiotic stress tolerance. However, the effect of AMF inoculation on the heat resistance of *Dianthus hybridus* has not been reported.

This study was based on the inoculated AMF, measuring the growth indexes and gas exchange parameters of the leaves of *Dianthus hybridus*. The effect of AMF on the heat resistance of *Dianthus hybridus* seedlings under high temperature stress were also discussed. This authors tried to explore the effect of AMF inoculation on the growth of *Dianthus hybridus*, so as to provide a theoretical basis for the popularization and application of AMF in the production practice of *Dianthus hybridus.*

Tang, Y., Huang, J., Guo, Y., Luo, Y., Li, C., He, Z. and Zhou, X.
Effects of Arbuscular Mycorrhizal Fungi on the Heat Resistance of Dianthus hybridus Seedlings.
DOI: 10.5220/0011507800003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 1260-1263
ISBN: 978-989-758-595-1

2 MATERIALS AND METHODS

2.1 Materials

Dianthus hybridus cv. Fanxing was used in this work, the seeds were provided by Jinping flower seedling limited liability company. AMF products (including mixed bacterial agents such as Glomus mosseae, Acaulospora morrowiae and Gigaspora gigantean) were provided by Huai'an chaimihe agricultural science and technology development limited company. A commercial substrate was used for seedling raising, with pH5.5-6.5.

2.2 Experimental Design

The experiment was conducted in the laboratory of college of horticulture, Sichuan Agricultural University from January 2021 to September 2021. Before inoculation, the flowerpots were disinfected with 75% ethanol and ventilated to dry. Two treatments were set up: (1) CK: without adding AMF in the substrate; (2) AMF: adding AMF in the substrate. The Arbuscular mycorrhizal fungal agent (spore concentration of 120 per gram) was mixed with the seedling substrate and loaded them into 50-well plates, with an average dosage of 50 g per plate. Then the plates were placed in artificial climate chamber with the temperature of 25℃/18℃ (day/night), and photoperiod 12h/12h (day/dark) simultaneously, with Hogland nutrition solution every three days. As for heat stress experiment, the *Dianthus hybridus* seedlings were moved into the artificial climate box for pretreatment (25℃/ 18℃ (day/night)) after 50 days of inoculation, with photoperiod of 12h/12h (light/dark), and the relative humidity was 70%. After 3 days, the temperature of the artificial climate box was changed into 35℃/30℃ (day/night), the light intensity and air humidity remained unchanged. The mature leaves of the same growth part of each plant were sampled on the fifth day after heat stress.

Plant height and stem diameter were measured by using vernier caliper. The methods described by Lu et al (Lu 2009) were used for the determination of free proline, and the methods of measurement of antioxidant enzyme activities and malondialdehyde (MDA) content were described by Wang et al (Wang 2021). Gas exchange parameters were measured by LI-6400XT Portable Photosynthesis System. Photosynthetic fluorescence parameters were measured by portable fluorescence parameter meter (PAM2500).

2.3 Statistical Analyses

Statistical analysis was carried out by using SPSS 18.0 statistical software. The data were analyzed by one-way ANOVA, with the least significant difference at the 5% confidence level.

3 RESULTS AND DISCUSSION

3.1 Effects of AMF on Growth Indexes and Gas Exchange Parameters in *Dianthus hybridus*

After 50 days of AMF inoculation, compared with no inoculation (CK), the AMF treatment significantly increased the plant height of *Dianthus hybridus*. At the same time, after AMF inoculation, the net photosynthetic rate, stomatal conductance, intercellular carbon dioxide concentration and transpiration rate of *Dianthus hybridus* leaves were significantly improved. Previous studies have shown that arbuscular mycorrhizal can improve the strong seedling index of wheat (Ma 2014). This study found that AMF treatment also promoted the growth of *Dianthus hybridus*, which was consistent with the research results in Poncirus trifoliata by Zuo et al (Zuo 2014).

Table 1: Effects of AMF on growth indexes and gas exchange parameters in *Dianthus hybridus*.

Treatments	Plant height (cm)	Stem diameter (cm)	Pn ($\mu mol \cdot co_2 m^{-2} \cdot s^{-1}$)	Gs ($mol\ H_2O \cdot m^{-2} s^{-1}$)	Ci ($\mu mol \cdot co_2 \cdot mol^{-1}$)	Tr ($mmol\ H_2O \cdot m^{-2} s^{-1}$)
CK	15.60±1.09b	0.06±0.01b	2.03±0.38b	0.08±0.02b	336.19±1.61b	1.44±0.38b
AMF	20.83±2.86a	0.12±0.02a	6.12±1.07a	0.16±0.02a	355.63±7.93a	3.83±0.78a

Value are means ± standard errors. Means with the same letter within each column are not significantly different at $p < 0.05$

.

3.2 Effects of AMF on Proline, Malondialdehyde (MDA) Contents and Antioxidant Enzyme Activities in *Dianthus hybridus* at High Temperature

It can be seen from the Table 2 that the activities of antioxidant enzymes including SOD, POD and CAT were increased on the 5th day of high temperature stress. And AMF treatment also increased the activities of antioxidant enzymes. Compared with the heat stress treatment without AMF inoculation (HT), SOD, POD and CAT were increased by 18.09%, 7.22% and 29.86% under HT+AMF treatment, respectively. On the other hand, compared with the control, the content of proline were increased by 43.98% and 141.43% in HT and HT+AMF, respectively. HT+AMF treatment significantly increased the activity of SOD compared with HT. Protective enzyme systems such as SOD, POD and CAT and ascorbic acid glutathione (AsA-GSH) cycle system play an extremely important role in the process of scavenging reactive oxygen species free radicals and peroxides. They work synergistically with peroxidase isozymes and other non enzymatic systems, so as to regulate membrane permeability, prevent membrane lipid peroxidation and reduce the damage of membrane system. In this study, there was higher antioxidant enzyme activities with the AMF treatment.

On the other hand, compared with the control, the content of MDA were increased by 46.88% in HT and were increased by 31.25% in HT+AMF. The improvement of antioxidant enzyme activity can promote the scavenging ability of reactive oxygen species and improve the stress adaptability. Proline is an osmotic protective agent, and its accumulation is positively correlated with the stress resistance of plants. The results of this study showed that AMF inoculation could significantly improve the content of proline in *Dianthus hybridus* in responce to heat stress, improving the ability of heat adaptation. The results were consistent with the results in *Lactuca satica L.* by Ma et al (Ma 2015).

Table 2: Effects of AMF on proline, MDA contents and antioxidant enzyme activities of *Dianthus hybridus* at high temperature.

Treatments	SOD activity ($U \cdot g^{-1}FW$)	POD activity ($U \cdot g^{-1}FW$)	CAT activity ($U \cdot g^{-1}FW$)	MDA content ($\mu g \cdot g^{-1}FW$)	Pro content ($\mu g \cdot g^{-1}FW$)
CK	8.83±0.66b	11.98±0.90b	3.50±1.04b	0.32±0.03b	15.71±4.34c
HT	10.61±0.75b	14.81±1.04a	5.29±0.47ab	0.47±0.05a	22.62±3.01b
HT+AMF	12.53±1.25a	15.88±1.40a	6.87±1.12a	0.22±0.08b	37.93±2.00a

Value are means ± standard errors. Means with the same letter within each column are not significantly different at $p < 0.05$.

3.3 Effects of AMF on Chlorophyll Fluorescence Parameters of *Dianthus hybridus* Under Heat Stress

High temperature stress significantly decreased Fv'/Fm', Y(II), qP in chlorophyll fluorescence parameters of *Dianthus hybridus* leaves, but significantly increased NPQ, indicating that high temperature inhibited the potential activity of photosynthetic reaction center II and hindered cyclic photosynthetic electron transfer of *Dianthus hybridus* leaves. Compared with HT, HT+AMF could inhibit the decreases of Fv'/ Fm', Y (II) and qP under high temperature, indicating that the inhibitory effect of high temperature on photosynthetic electron transfer was reduced to a certain extent. In addition, Fv/Fm did not decrease after 5d of high temperature stress, indicating that the photosynthetic structure of *Dianthus hybridus* leaves were not damaged.

Table 3: Effect of AMF on chlorophyll fluorescence parameters of *Dianthus hybridus* at high temperature.

Treatment	Fv/Fm	Fv'/Fm'	Y(II)	qP	NPQ
CK	0.79±0.01a	0.64±0.04a	0.49±0.02a	0.79±0.01a	0.61±0.15b
HT	0.79±0.01a	0.56±0.03b	0.27±0.04b	0.55±0.12b	0.86±0.07a
HT+AMF	0.78±0.01a	0.69±0.02a	0.54±0.02a	0.84±0.03a	0.48±0.02b

Value are means ± standard errors. Means with the same letter within each column are not significantly different at $p < 0.05$.

4 CONCLUSION

Comprehensive analysis showed that high temperature stress had significant effects on the physiological parameters and chlorophyll fluorescence parameters of *Dianthus hybridus*. Inoculation with AMF can alleviate the damage caused by high temperature stress to the activity of antioxidant enzymes, osmotic regulators and photosynthetic electron transport system, and improve the heat resistance of *Dianthus hybridus* to a certain extent.

ACKNOWLEDGEMENTS

This work was financially supported by Introduction and breeding of new varieties of European flowers (NO.2022349001).

REFERENCES

Chen, K., Sun, J.Q., Liu, R.J., Li, M. (2013) Effects of arbuscular mycorrhizal fungi on the growth of grafted watermelon seedlings and the activity of defensive enzymes in roots. Chinese Journal of Applied Ecology., 24(1): 135-141. In Chinese.

Guo, S.X., Wang, Z.J., Zhao, Y.W. (2011) Effects of Arbuscular Mycorrhizal Fungi on Leaf Anatomical Structure and Transpiration characteristics of Paeonia suffruticosa under High Temperature Stress. Editorial Dept.of Journal of QAU., 28(3): 165-167. In Chinese.

Lu, W.J., Zhang, S.H., Guo, C.J., Duan, W.W., Xiao, K. (2009) Studies on the physiological parameters related to nitrogen use efficiency in wheat cultivars with different nitrogen utilization. Journal of Plant Nutrition and Fertilizers., 15(5): 985-991. In Chinese.

Liu, R.J., Jiao, H., Li, Y., Li, M., Zhu, X.C. (2009) Research advance in species diversity of Arbuscular Mycorrhizal Fungi. Chinese Journal of Applied Ecology., 20(9): 2301-2307. In Chinese.

Liu, A.R., Chen, S.C., Liu, Y.Y., Li, Y.N., He, C.X. (2011) Effects of AM fungi on leaf photosynthetic and antioxidant enzyme activities under low temperature. Acta Eologica Sinica., 31(12): 3497-3503. In Chinese.

Liu, H., Chen, W., Zhou, Y., Li, X., Ren, A.Z., Gao, Y.B. (2015) Effects of Endophytic Fungi and Arbuscular Mycorrhizal Fungi on the growth of Leymus chinensis. Plant Physiology Journal., 39(5): 477-485. In Chinese.

Ma, F., Su, M., Wang, L., Zhang, X., Li, S.Y. (2014) Effects of Arbuscular mycorrhizal fungi on the growth of wheat. Acta Eologica Sinica., 34(21): 6107-6114. In Chinese.

Ma, T., Liu, R.J., Li, M. (2015) Effects of Arbuscular Mycorrhizal Fungi on Heat-tolerance of *Lactuca satica L.* Plant Physiology Journal., 51(11): 1919-1926. In Chinese.

Sun, J.Q., Liu, R.J., Li, M. (2012) Advances in the Study of Increasing Plant stress Resistance and Mechanisms by Arbuscular Mycorrhizal Fungi. Plant Physiology Journal., 48(9): 845-852. In Chinese.

Wang, B.Q., Zhao, Y., Zhu, X.L., Wang, W.T., Wei, X.H. (2021) Effects of Saline-alkali Stress on Photosynthetic Characteristics and Antioxidant System in Quinoa Seedlings Leaf. Acta Agrestia Sinica., 29(8): 1689-1696. In Chinese.

Ye, L., Zhao, X., Bao, E.C., Cao, K., Zou, Z.R. (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:e00863.

Zou, Y.M., Wu, Q.S., Li, Y., Huang, Y.M. (2014) Effects of arbuscular mycorrhizal fungi on root system morphology and sucrose and glucose contents of Poncirus trifoliata. Chinese Journal of Applied Ecology., 25(4): 1125-1129. In Chinese.

Zhang, S.S., Kang, H.M., Yang, W.Z., Xiang, Z.Y. (2016) Effects of Arbuscular mycorrhizal fungi on growth and photosynthetic characteristics of Nyssa yunnanensis seedlings under drought stress. Acta Eologica Sinica., 36(21): 6850-6862. In Chinese.