Effects of AMF infection on photosynthetic characteristics of tomato under salt stress

Yongdong Xie¹, Luxi Yang¹ and Zhongqun He¹*

¹College of Horticulture, Sichuan Agriculture University, Chengdu, Sichuan, 611130, China
*Corresponding author’s e-mail: hzqun328@163.com

Abstract. A pot experiment was conducted to study the effects of arbuscular mycorrhizal fungi (AMF) Glomus mosseae-2 on photosynthetic characteristics of tomato under different concentrations of NaCl (0.3%, 0.6% and 1%) stress. The results showed that salt stress reduced the net photosynthetic rate and light saturation point of tomato. Although AMF did not increase the light saturation point of tomato, it could increase the net photosynthetic rate, stomatal conductance, apparent quantum yield and CO₂ carboxylation efficiency of the leaves, and improve chloroplast photophosphorylation activity, which was conducive to maintaining the ability of chloroplast to absorb light energy and improving tomato light energy conversion efficiency and CO₂ utilization efficiency. When the inoculated and non-inoculated plants were treated with 0.3-0.6% NaCl, the photosynthesis decline was mainly affected by stomatal limitation, after 28 days of treatment with 1% NaCl, the decline of photosynthesis was mainly affected by non-stomatal factors, and inoculation with AMF could improve the photosynthesis of tomato under salt stress.

1. Introduction
Photosynthesis is the fundamental source for plant to synthesise organic matter and energy, and it is the basis of plant growth and development. Many studies have shown that salt stress inhibits photosynthesis in plants[1], but the reason has not yet formed a unified understanding. It is generally believed that osmotic stress may be one of the reasons why salt inhibits photosynthesis in plants[2]. AMF infecting host plants alters the carbon cycle and photosynthesis of plants[3]. Wu[4] found that AMF increased the transpiration rate (Tr), stomatal conductance (Gs) and net photosynthetic rate (Pn) of citrus under water stress. Syvertsen[5] reported that AMF had no effect on the gas exchange of citrus seedlings. The effects of AMF on the photosynthetic characteristics of plants, especially vegetable plants under salt stress, are rarely reported, and the effect of mycorrhizal on photosynthetic characteristics of tomato has not been reported. From the perspective of photosynthesis characteristics, this research studied the photosynthesis and chloroplast function of the inoculated and non-inoculated tomato plants under NaCl stress, aiming to provide a scientific basis for the mechanism of AMF improving salt tolerance of tomato and the popularization and application of AMF.

2. Materials and methods

2.1 Materials
The tomato for experimment was ‘Zhongza no. 9’, the strain was ‘Glomus mosseae-2’ (G.m, provided by Hungarian academy of agricultural sciences), and the soil was organic soil, its basic physical and
chemical properties are as follows: the pH value was 7.26, the organic matter was 11%, the rapidly available phosphorus was 150 mg·kg⁻¹, the rapidly available nitrogen was 451 mg·kg⁻¹, and the rapidly available potassium was 518 mg·kg⁻¹. After the soil was passed through 1 mm sieve, it was sterilized in a drying oven at 160 °C for 2 h.

2.2 Experiment design
The tomato seedlings was salt stressed after inoculated AMF for 45 days, and the plant height and stem diameter were determined before salt treatment. The test was not inoculated (control, NAM) and inoculated with G.m (AM). Each treatment was divided into four NaCl concentrations, which were 0%, 0.3%, 0.6%, 1%. Each treatment was repeated 8 times, completely randomly arranged, for a total of 64 pots. The salt-free treatment was watered with tap water (EC 0.8 d·m⁻¹). The brine was poured every 2-3 d, and the EC values after the last salt treatment reached 0.9, 3.4, 4.2, and 7.1 d·m⁻¹, respectively. In order to maintain the stabilization of salt concentration in the basin, a plate was put under the basin, and if there was liquid leakage, the exudate will be reversed.

2.3 Experiment methods
For estimation of photosynthetic pigments, fresh leaf tissue was macerated in 80% acetone for 24 h, the absorbance of the extracts was measured spectrophotometrically (640D, Beckman, USA) at 480, 645, and 663 nm[6]. The photophosphorylation activity was determined by the method of Avercheva[7]. Between 10 and 12 A.M. on a sunny day, a portable photosynthesis system (model LI-6400, LI-COR Inc., Lincoln, NE, USA) was used to determine response curve of net photosynthetic rate and net photosynthetic rate (Pn) on the second fully expanded leaves.

3. Result

3.1 Effects of AMF on chlorophyll content in tomato leaves under NaCl stress
As can be seen from table 1, chlorophyll a (Chla), chlorophyll b (Chlb) and total chlorophyll (Chl) content decreased with the extension of salt concentration and stress time. At 5 d of salt stress, there was no significant difference in Chla and Chlb treated by 0% and 0.3% between AM and NAM, and AM treated with 0.6% and 1% NaCl were significantly higher than the NAM. At 15 and 30 d of salt stress, the Chla and Chlb in AM and NAM treated with same salt concentration were significantly higher than the NAM. Table 1 also showed that AMF could significantly slow down Chla and Chlb degradation under high concentration salt stress.

| Chlorophyll (mg·g⁻¹) | Stress days (d) | NaCl concentration (%) | AM | NAM |
|----------------------|-----------------|------------------------|----|-----|
|                      | 0   | 0.3  | 0.6  | 1   | 0   | 0.3  | 0.6  | 1   |
| Chla                 | 5   | 30.73abc | 30.83b | 30.47a | 30.29bc | 29.71abc | 29.65ab | 29.16b | 29.15c |
| 15                   | 30.53ab | 29.11bc | 28.60b | 26.85c | 28.95b | 27.08c | 26.49c | 23.92d |
| 30                   | 29.82ab | 27.05b | 23.53d | 21.28c | 28.86b | 25.24c | 21.53c | 18.17c |
| Chlb                 | 5   | 19.98ab | 18.64bc | 16.69c | 15.66e | 18.97ab | 18.02b | 15.62c | 13.91d |
| 15                   | 19.37ab | 16.19bc | 13.41c | 11.97d | 18.80b | 12.145d | 12.65ed | 9.47e |
| 30                   | 18.98ab | 11.09c | 8.73d | 7.09f | 16.52b | 10.28c | 6.97f | 5.07f |
| Chl                  | 5   | 50.71ab | 49.47bc | 47.19d | 45.95e | 48.69bc | 47.67ed | 44.78f | 42.06f |
| 15                   | 49.90ab | 45.30b | 42.00c | 38.81c | 47.76b | 39.22d | 40.14f | 33.38e |
| 30                   | 41.21ab | 38.14c | 32.25e | 28.38f | 45.38b | 35.52d | 28.50f | 23.24f |

Note: Little letters in the row indicate significant differences at P<0.05 level.
3.2 Effects of AMF on chloroplast Photophosphorylase activity in tomato leaves under NaCl stress

Chloroplast photophosphorylase activity was measured under NaCl stress for 30 days. Photophosphorylase activity decreased with the increase of salt concentration, and AM were significantly larger than NAM at the same salt concentration (table 2). Treated with 0, 0.3%, 0.6% and 1% NaCl, the AM were 5.6%, 10.6%, 24.3% and 32.7% higher than the NAM, respectively. 1% NaCl stress resulted in 52.7% and 62.4% reduction of photophosphorylase activity in AM and NAM, respectively compared with treatment without salt stress.

Table 2. Effect of NaCl stress on photophosphorylation activity of chlorophyll in leaves of tomato

| NaCl concentration (%) | Photophosphorylase activity (μmol ATP·mg⁻¹ Chl·h⁻¹) |
|------------------------|---------------------------------------------------|
|                        | AM | NAM  |
| 0                      | 79.737<sup>a</sup> | 75.521<sup>b</sup> |
| 0.3                    | 72.122<sup>c</sup> | 65.215<sup>d</sup> |
| 0.6                    | 58.079<sup>e</sup> | 46.726<sup>f</sup> |
| 1                      | 37.662<sup>g</sup> | 28.385<sup>h</sup> |

3.3 Response curve of net photosynthetic rate under NaCl stress

Fig. 1 showed that the light response curve of net photosynthetic rate (Pn) at 16<sup>th</sup> day of salt stress. Under the conditions of leaf temperature, atmospheric relative humidity and atmospheric CO<sub>2</sub> concentration were relatively stable, the Pn of AM and NAM before salt stress and 1% salt stress were all increased with the increase of photosynthetically active radiation (PAR). When the Pn of AM and NAM treated with 1% NaCl increased to the maximum, and PAR exceeded 1200 μmol CO<sub>2</sub>·m<sup>2</sup>·s<sup>-1</sup>. Although the saturated PAR of AM and NAM with same salt-treated were substantially the same, the photosynthesis rate of AM under saturated light intensity was higher than that of NAM. During linear phase (PAR was about 20-200 μmol CO<sub>2</sub>·m<sup>-2</sup>·s<sup>-1</sup>), the slope of PAR-Pn was the apparent quantum yield (AQY, which represented the conversion efficiency of light energy), and the linear equation could be obtained separately. The AQY of AM and NAM before salt stress were calculated, which were 0.0427 and 0.0226 respectively. The AQY of the 1% NaCl treatment was 0.0291 and 0.0136, indicating that the salt stress reduced the light energy conversion efficiency of tomato leaves, while AMF could improve the photosynthesis of tomato.

3.4 Effects of AMF on net photosynthetic rate (Pn) of tomato leaves under NaCl stress

With the increase of NaCl concentration and stress time, the Pn of AM was significantly higher than NAM (Fig. 2). There was little effect of 0.3% NaCl stress on tomato. However, the tomato plants
without inoculated G.m were slightly sensitive to salt stress, at 7th day, 0.6% NaCl treatment showed large decrease, and 1% NaCl treatment decreased most. The falling range of NAM was larger than that of AM, and Pn of NAM was lower than that of the NAM. At 14th and 28th day, Pn of AM was 1.167 and 1.916 times of the NAM respectively. Therefore, salt stress for a long time severely inhibited the photosynthesis of tomato leaves, and AMF alleviated the inhibitory effect of salt on photosynthesis of tomato leaves.

Fig. 2 Net photosynthetic rate of AM (a) and NAM (b) under NaCl stress

4. Discussion
The thylakoid membrane is the structural basis for the absorption, transmission and transformation of chloroplast light energy[8]. Various pigment protein complexes for plant energy absorption, transmission and conversion are distributed on the thylakoid membrane. Pigment is one of important components of the thylakoid membrane and is the receptor for light energy. Therefore, under salt stress, the destruction of the pigment protein complex on the thylakoid membrane will inevitably affect the absorption and conversion of light energy. The pigment content of plant leaves under salt stress is related to the salt tolerance of plants and the type and concentration of salt. The results indicated that NaCl stress reduced Chl a and Chlb content in tomato leaves, which accelerated the degradation of chlorophyll, especially Chlb. Chlb is an important component of PSII light harvesting pigment protein complexe. Its reduction would directly damage the structure and function of this complex, and the ability of chloroplast to absorb light energy would also be reduced, thereby photophosphorylase which reflects the light energy conversion of the chloroplast activity was inhibited. Inoculation with AMF could significantly increase the content of photosynthetic pigments, and slowed down the degradation of Chl under salt stress, and increased the photophosphorylase activity. Therefore, the plants inoculated AMF with advantage in the capture of light energy, especially at higher concentrations (0.6%, 1%) and longer time of NaCl stress.

Some studies have shown that salt-tolerant plants had less change in pigment content than salt-sensitive plants under salt stress. The decrease in chlorophyll content was mainly due to the degradation of Chl b (chlorophyll b) by Chlase, but there was little effect on Chl a (chlorophyll a) and carotenoid content (Car)[9]. Han[10] showed that Chl a, Chlb and Car decreased in watermelon seedlings treated with 70 mmol·L-1 NaCl. Inoculation with AMF affected the content of Chl in host plants. Borie and Rubio (1999) studied the effects of different phosphorus (P) level and other nutrient elements on cucumber plants. The results showed that the Chl content of plants increased, and the Chl content of lower P level was significantly higher than that of untreated. There were similarities and differences between this study and previous researches, which may be related to the different materials and test conditions.
References

[1] Sudhir P., Murthy S. D. S. (2004) Effects of salt stress on basic processes of photosynthesis. Photosynthetica, 42(2): 481-486.

[2] Mandhania S., Madan S., Sawhney V. (2006) Antioxidant defense mechanism under salt stress in wheat seedlings. Biol. Plantarum, 50(2): 227-231.

[3] Goicoechea N., Baslam M., Erice G., et al. (2014) Increased photosynthetic acclimation in alfalfa associated with arbuscular mycorrhizal fungi (AMF) and cultivated in greenhouse under elevated CO2. J. Plant Physiol., 171(18): 1774-1781.

[4] Wu Q. S., Xia R. X. (2006) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J. Plant Physiol., 163(4): 0-425.

[5] Syvertsen J. P., Graham J. H. (1990) Influence of vesicular arbuscular mycorrhizae and leaf age on net gas exchange of Citrus leaves. Plant Physiol., 94(3): 1424-1428.

[6] Arnon, D. I. (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol., 24, 1-15.

[7] Avercheva O. V., Bassarskaya E. M., Zhigalova T. V., et al. (2010) Photochemical and photophosphorylation activities of chloroplasts and leaf mesostructure in Chinese cabbage plants grown under illumination with light-emitting diodes. Russ. J. Plant Physiol., 57(3): 382-391.

[8] Dekker J P, Boekema E J. (2005) Supramolecular organization of thylakoid membrane proteins in green plants. BBA - Bioenergetics, 1706(1): 12-39.

[9] Zhu X. G., Zhang Q. D. (1999) Advances in the research on the effects of NaCl on photosynthesis [J]. Chin. Bull. Bot., 16(4), 332-338. (in Chinese)

[10] Han Z. P., Guo S. R., Feng J. Q., et al. (2008) Effect of salinity on plant growth, photosynthetic pigments and proline content in leaves of watermelon seedlings. J. Nanjing Agric. Univ., 31(2), 32-36. (in Chinese)