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REGULAR ARTICLE

Effects of arbuscular mycorrhizal fungi on photosynthesis and chlorophyll fluorescence of maize seedlings under salt stress

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ABSTRACT

The impact of arbuscular mycorrhizal fungi (AMF) *Glomus. tortuosum* on morphology, photosynthetic pigments, chlorophyll (Chl) fluorescence, photosynthetic capacity and rubisco activity of maize under saline stress were detected under potted culture experiments. The experimental result indicated the saline stress notably reduced both dry mass and leaf area in contrast with the control treatment. Nevertheless, AMF remarkably ameliorated dry mass and leaf area under saline stress environment. Besides, maize plants appeared to have high dependency on AMF which improved physiological mechanisms by raising chlorophyll content, efficiency of light energy utilization, gas exchange and rubisco activity under salinity stress. In conclusion, AM could mitigate the growth limitations caused by salinity stress, and hence play a very important role in promoting photosynthetic capacity under salt stress in maize.

Keywords: Arbuscular mycorrhiza; Zea mays; Salinity; Photosynthesis; Chlorophyll fluorescence

INTRODUCTION

Plants grown naturally are faced with all kinds of environmental factors, bringing about adverse changes to the patterns of growth and development. Salinity is one of the major environmental factors which can impede natural growth, development and metabolism (Hashem et al., 2016; Alqarawi et al., 2014). Among all the cell functions restrained by salt stress, photosynthesis is the most sensitive physiological processes to salinity, increased salt concentrations in soil can inhibit photosynthetic enzymes activity, and cut down the content of photosynthetic pigments (Sheng et al., 2008), moreover, salt stress may bring much negative effect on stomatal conductance and intercellular CO_2 concentrations, then depressing photosynthesis (Elhindi et al., 2017). Additionally, photosystem II is sensitive to salinity, and salt stress will lead to occurrence of exceeding reduction when the plant is incapable of dissipating the excessive energy (Porcel et al., 2015; Baker, 2008). And the releasing ability to excessive energy may be estimated by measuring the yield chlorophyll fluorescence. Thus, chlorophyll fluorescence is widely studied to ascertain photosynthetic physiological mechanism in plants under stress environment (Xu et al., 2010; Waldhoff et al., 2002; Mallick and Mohn, 2003).

Arbuscular mycorrhizal fungi (AMF) can form mutualism with overwhelming majority land plants, and providing external safeguard to avoid being hurt under stress condition (Xu et al., 2016; Wu et al., 2015; Gong et al., 2013). It was verified that AMF was able to increase the resistance to salinity by accumulating various osmotic adjustment substances (Lin et al., 2017; Porcel et al., 2012), improving antioxidant enzyme activities (Huang et al., 2010), and facilitating the absorption and transfer of nutrients (Liu et al., 2013). Research of influential mechanism of salinity on photosynthesis was still at its preliminary stage, and the exploration of many key issues need to be urgently deepened. Thus far, the publications with regard to the effect of AMF on photosynthetic characteristics in saline environment were seldom seen.

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Maize is one of the most important food, feedstuff and economy crop around the world, and it is particularly sensitive to saline stress (Estrada et al., 2013). Soil salinity leads to a decrease in crop production, and the inhibition of photosynthetic processes (Pitman and Läuchli, 2002). Our main objective was to estimate the influence of *Glomus. tortuosum* on photosynthesis physiological mechanism of maize when confronted with salinity, and to bring insight into the role of AM in the mechanism study of plant resistance.

MATERIALS AND METHODS

Plant material and growth conditions

The experiments were conducted in soil, plant and water analysis laboratory from February to May 2017 at Huaiyin normal university, Jiangsu Province, China. The seeds of maize (ZD 958) were soaked with 70% ethanol for several minutes in order to complete disinfection process, after that, they were washed for number of times using purified water, and then were placed in culture dish covered with waterish filter paper, finally were laid up in artificial illumination incubator at 28 degrees for germination.

AM Fungi Inoculum

Glomus.tortuosum, was provided from the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, China, and there was 1,000 spores in per 10 mL microbial inoculum. 20 g microbial inoculums were applied at 5 cm depth underneath of maize seeds as strain treatment, equal amounts of inoculums after high-temperature sterilization were used for non-AMF treatment.

Experimental design

The experiment contained two mycorrhizal treatments (non-AM control and *Glomus.tortuosum*) at three kinds of salty environment. Four repetitions for any treatment totaling 24 pots (three seedlings per pot) were applied. And 2.0 kg sterilized mixture of yellow brown soil and sand (1:1.5, v/v) were put into each pot. A month and a half later, the maize plants were irrigated with 0, 150 and 300 mM NaCl for one week, afterwards, maize plants were used to detect the growing states and physiological characteristics.

Plant growth

The leaf area was recorded by AM350 Portable leaf area meter, and their dry mass was tested after oven-drying at 75°C for couple days.

Chlorophyll determination

Chlorophyll was extracted by means of the same volume ratio of ethanol and acetone, and Chl a and Chl b were decided with the method of absorbance measurement (Zhang and Zhang, 2006).

Chlorophyll fluorescence measurement

Chl fluorescence parameters were decided from a portable Chl fluorometer (Yaxin-1162, China). The primary fluorescence (Fo), maximal fluorescence (Fm) and maximum quantum efficiency of photosystem II (PSII) primary photochemistry (Fv/Fm) were recorded after a half-hour dark adaptation. Afterwards, both variable fluorescence (Fv) and potential photochemical efficiency (Fv/Fo) were ascertained by calculating with the help of corresponding formula.

Photosynthetic parameters measurement

Net photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (gs), and intercellular CO_2 concentration (Ci) were detected by means of domestic portable photosynthetic measure system (Yaxin-1101, China) from 09:00 to 11:00 am.

Rubisco activity

Rubisco activities were measured following the method described by Usuda (1985).

Statistical analysis

Data analysis process were fulfilled by the least significant differences test (LSD) using the SPSS software (version. 16.0, Inc., 107 Chicago, IL, USA). Statistical significance was set at $p < 0.05$.

RESULTS

Plant growth

Mycorrhizal symbiosis could favorably impact the growth of maize seedlings under different salinity levels. From morphological features, salt stress could make maize leaves weak and withered, and these symptom were more obvious in non-AM plants. Two growth parameters studied in present experiment all revealed a significant reduction with the increase of salinity, such decrease in salt treated plants was alleviated after inoculating with AMF (Fig. 1).

Chlorophyll

AM maize plants had a high chlorophyll content disregard for any salinity treatment (Table 1). Compared with non-AM seedlings, Chl a in AM plants was 31.03%, 29.59%, and 75.00% higher, Chl b was 38.71%, 32.56%, and 85.71% greater, while Chl a+b was 33.71%, 30.50%, and 78.08% higher under 0, 150, 300 mM NaCl stress, respectively. And all chlorophyll component contents depressed significantly with a rise in salinity, these reductions caused by salinity were more apparent in non-AM than in AM plants. Whereas, chlorophyll a/b ratio significantly increased,

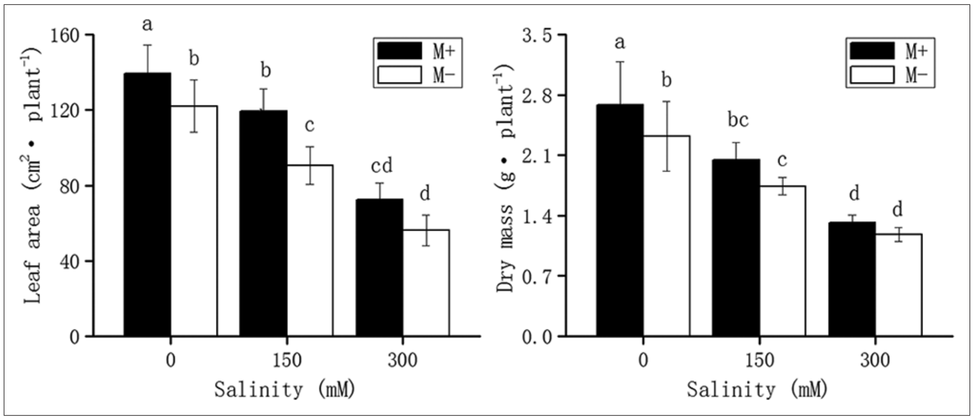

Fig 1. The effect of salinity and *Glomus. tortuosum* on leaf area and dry mass of maize plants.

Table 1: The effect of *Glomus. tortuosum* on chlorophyll content of maize plants under salt stress

Salinity (mM)	Inoculation	Chlorophyll content			
		a	b	a+b	a/b
0	M+	1.52±0.01a	0.86±0.09a	2.38±0.03a	1.77±0.01c
	M-	1.16±0.02b	0.62±0.07b	1.78±0.01b	1.87±0.01c
150	M+	1.27±0.02b	0.57±0.06b	1.84±0.02b	2.23±0.02b
	M-	0.98±0.01c	0.43±0.02c	1.41±0.02c	2.28±0.02b
300	M+	0.91±0.02c	0.39±0.04c	1.30±0.01c	2.33±0.03b
	M-	0.52±0.01d	0.21±0.03d	0.73±0.00d	2.48±0.04a

which was caused mainly by a sharper decrease in Chl b concentration than Chl a.

Chlorophyll fluorescence

With the enhancement of salt stress, the Fm, Fv/Fm, and Fv/Fo were significantly reduced (Fig. 2). However, Fo got promoted dramatically for all maize seedlings with the intension of salinity. The Fo in non-AM plant was 4.82%, 6.67%, and 4.85% higher at 0, 150, and 300 mM NaCl compared with AM plants respectively. By comparison, under different NaCl levels, Fm in mycorrhizal plants was 21.02%, 5.28%, and 5.70% higher, Fv/Fm was 8.33%, 6.06%, and 6.78% greater, while Fv/Fo was 37.40%, 17.86%, and 18.18% higher respectively.

Gas exchange

All the photosynthetic parameters except Ci were of a high level in AM maize seedlings grown in all treatments (Table 2). As the salinity intensified, the Pn, E, and gs were all showing a continuous decrease trend for all maize plants, but AM seedlings exhibited relatively lower decreasing range. In addition, the reverse trend was observed in case of Ci, the Ci in AM plants was 13.9%, 18.2%, and 29.6% lower at 0, 150, and 300 mM NaCl contrasted with non-AM plants respectively. The higher salinity, the more significant it decrease.

RuBPcase

In comparison with control, the application of salt stress treatments can reduce rubisco activity in maize leaves, and the degree of decline was reinforced according to the intensity of salt stress (Fig. 3). However, this reduction might be mitigated after AMF inoculation. Therefore, AM seedlings displayed superior rubisco activity in all treatment, and the activity was greater than non-AM seedlings by 36.11% at 150 mM NaCl, and 23.21% at 300 mM NaCl, respectively.

DISCUSSION

Salinity has proved to be one of the foremost environmental elements restricting plant growth due to salt-excess effects (Evelin et al., 2009; Munns et al., 2006). In present study, maize plants were badly affected by salt and revealed morphological and physiological changes. When subjected to salt stress, maize plants showed obvious decrease in both leaf area and biomass, such reduction was possibly related to appearance of osmotic stress and increase on the efficiency for ion transfer (Farooq et al., 2009). Meanwhile, both higher leaf area and biomass were detected in AM plants (Ramos-Zapata et al., 2009; Sgrott et al., 2012), it can be explained that AMF might change biomass allocated to aboveground and belowground (Zhang et al., 2011). And the positive influence may also be a result of the enhancement of ability of nutritive equilibrium, absorbance, metabolism and utilization caused by mycorrhiza (Zandavalli et al., 2004). Thus, AMF inoculation could enhance the salt tolerance of maize and alleviate stress symptoms.

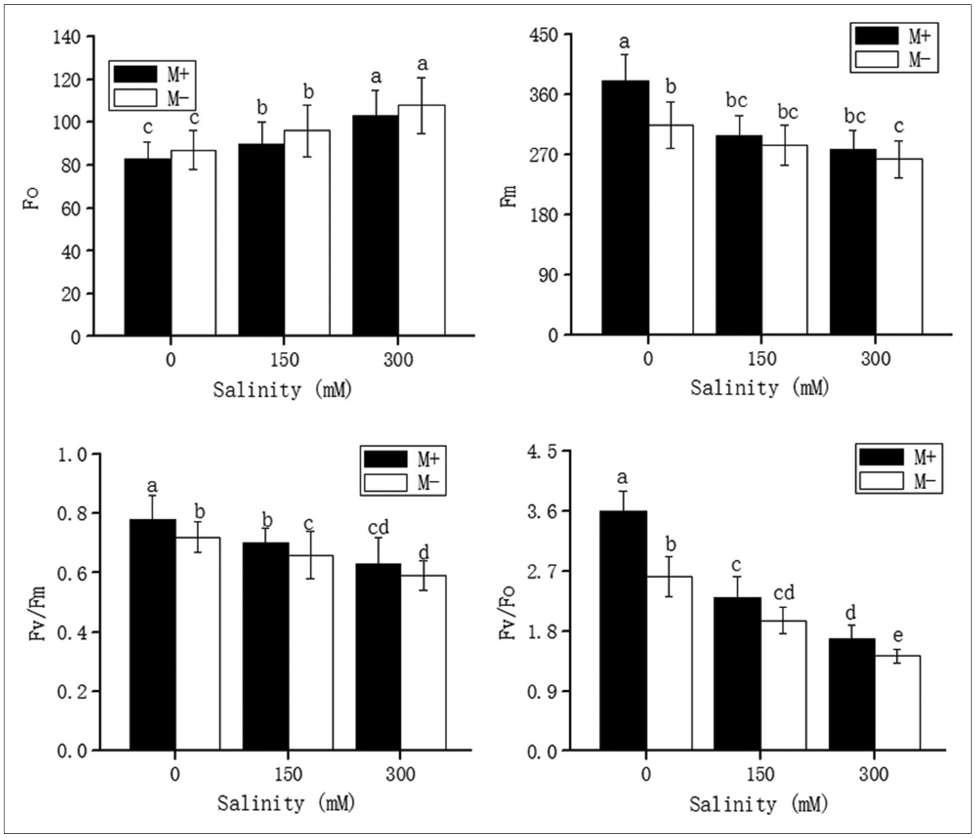

Fig 2. The effect of *Glomus. tortuosum* on Fo, Fm, Fv, Fv/Fm and Fv/Fo of maize plants under salt stress.

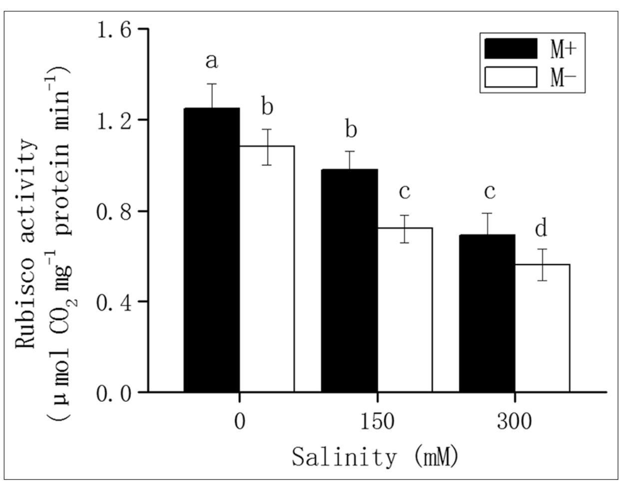

Fig 3. The effect of *Glomus. tortuosum* on rubisco activity of maize plants under salt stress.

As a general rule, photosynthesis, as the central physiological process, is sensitive to stress circumstance. In our study, maize plants subjected to salinity stress showed to decrease dramatically in photosynthetic parameters such as Pn, E and gs, thereby resulting in the reduction on the photosynthetic capacity, such negative effects may be attributed to the variation occurred in osmotic potential and water potential of cellular tissue caused by salinity (Negrão et al., 2017). And it had adverse impact on the stomatal conductance (Chartzoulakis et al., 2002; Sudhir and Murthy, 2004), moreover, reduced gs can also affect photosynthetic CO_2 assimilation. Pn is referred as one great indicator of eco-physiological responses and sensitivity when exposed to environmental stress (Xu et al., 2008). The reduction on Pn is mainly due to be a result of the decline on related enzymes activities of carbon fixation and the damage of photosynthetic machinery in virtue of salinity (Sun et al., 2016). Consequently, salinity could bring about imbalanced gas exchange, and ultimately interfere with the photosynthetic apparatus. AMF was able to mitigate the harmful impacts of salinity on photosynthesis, in spite of salt stress conditions, hence AM plants still keep superior Pn, E and gs values. The improved gas exchange ability was determined by the rise of hormonal levels and the enhancement of water uptake ability in AM plants (Ruiz-Lozano et al., 2012), another reason is that AM could promote the interchange of carbohydrate, leading to improved photosynthetic capacity (Sun et al., 2016).

The main function of chlorophyll is to absorb light energy, and its content can directly affect the intensity of photosynthetic capacity (Takai et al., 2010). Maize plants exhibited a drastic reduction of photosynthetic pigment contents in the wake of the increase of salinity, it suggested that salt stress could enhance the activity of

Table 2: The effect of *Glomus. tortuosum* on Pn, E, Gs and Ci of maize plants under salt stress

Salinity (mM)	Inoculation	Pn	E	gs	Ci
0	M+	7.67±0.08a	6.80±0.07a	49.4±0.53a	136±1.58d
	M-	7.31±0.05ab	3.62±0.02d	46.3±0.0.49a	158±1.44d
150	M+	6.87±0.06b	5.47±0.06b	28.8±0.35b	229±2.30c
	M-	5.75±0.07c	3.75±0.04d	20.4±0.26b	280±3.01b
300	M+	4.59±0.05d	4.66±0.05c	12.1±0.17c	295±3.05b
	M-	3.06±0.03e	1.98±0.03e	6.48±0.08d	419±3.99a

Chl-degrading enzymes, meanwhile, the biosynthesis of the different chlorophyll fractions might also be restrained. Besides, chlorophyllase activity could be inhibited by the accumulated ions under salt stress conditions (Guo et al., 2014). Relatively higher photosynthetic pigment contents were detected in AM maize plants among all treatments. The results stemed from the fact that AMF could not only improve uptake ability of nutritive element, but also secrete substances to stimulate chloroplast development (Thanaa and Nawar, 1994). In addition, AM maize plants also revealed higher rubisco activity, approving a weaker limitation of photosynthesis due to salt stress than non-AM plants (Lima-Neto et al., 2014).

It has proved that Chl fluorescence parameters is of great use for the evaluation of photosynthetic capacity (Zhu et al., 2014). According to the research data analysis, found that salinity can to some extent reduce Fv/Fm, this may be due to non-photochemical energy dissipation and injury of PSII reaction centers, or the outcome caused by quenching of Fm. And inferior Fv/Fm suggested maize seedlings presented clear photoinhibition and decrease in efficiency of electron transportation (Briantais et al., 1998). Nevertheless, AMF could slow up PSII reaction center's damage from saline stress, so AM symbiosis might significantly reduce the decreasing amplitude of Fv/Fm. And it was able to alleviate injury from salinity and execute normal photosynthesis. To sum up, the adverse effect of saline stress on photosynthetic characteristics of maize seedlings could be relieved by means of inoculating AM fungi, which could help improve the salt tolerance of maize plants through enhancing the efficiency of light energy utilization (Pinior et al., 2005; Bagheri et al., 2011).

CONCLUSION

Our experiments suggested that AM colonization enhances plant growth, photosynthetic capacity and relevant photosynthetic enzyme activity under different salt stress condition in maize plants. It affirmed that AMF can mitigate the growth limitations and physiological damage owing to saline stress. From the results of our study the potential role of AM inoculation as an effective biological solution when confronted with salinity stress is firmly supported.

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Authors' contribution

H. X. wrote the article and corrected it. Y. L. and S. T. designed the study. H. X. and Y. L. conducted the experimental work.

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