African Journal of Microbiology Research Vol. 5(6) pp. 714-719, 18 March, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.079
ISSN 1996-0808 ©2011 Academic Journals

*Full Length Research Paper*

# Effects of arbuscular mycorrhizal (AM) fungi *Glomus mosseae* on characteristics of leaf development of *Paeonia suffruticosa* under salt stress

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Accepted 28 March, 2011

**The effects of *Arbuscular mycorrhizal* (AM) fungi on the characteristics of leaf development of *Paeonia suffruticosa* at different levels of salt stress (0, 8, 16 and 24%) were studied. Potted 'Feng Dan' seedlings were inoculated with *Glomus mosseae*, and the non-inoculated was used as the control. The results showed that under salt stress leaf relative water content, sclerophyllous index of leaves and leaf succulence level of *P. suffruticosa* seedlings inoculated with *G. mosseae* were significantly higher than those of non-inoculated seedling, and specific leaf area was significantly lower than that of the control. Leaf water loss rate of the non-inoculated peony was faster, the percentage of water loss in the total amount of water each time point is the highest. These results suggest that *G. mosseae* may play an important role in the leaf traits and enhanced salt tolerance of tree peony seedlings.**

**Key words:** *Arbuscular mycorrhizal* fungi, characteristics of leaf development, *Paeonia suffruticosa*, salt stress.

## INTRODUCTION

Soil salinization seriously influences on plant growth and development. The change of plant morphology and growth characteristics is the important strategy that plants adapt to different environments, especially the leaf, sensitive to the environment, whose characters reflect the adaptation of plants to the environment and environmental effects on plants (Meziane et al., 1999). Succulent leaves, which can not only dilute the high salinity in cells but also storage adequate water reserves for cell physiological activities such as photosynthesis, is one of the ways that the plant adapt to saline environment (Wang et al., 2005). Zhang and Zhao (1996) believe that the succulent degree of plant leaves under salt stress can be seen as an important indicator of the level of salt tolerance of plants. Specific leaf area (SLA) can reflect the plant adaptations in different habitats (Poorter et al., 1999; Garnier et al., 2001), many studies found that plants with low SLA could better adapt to resource-poor and arid environment (Lambers et al., 1992); SLA are closely linked with measures of plant growth and survival, and close to other functional traits and plant leaves (such as plant photosynthesis, respiration, etc.) (Ackerly et al., 2002; Wei et al., 2009; Deng et al., 2010), but the the relationship of SLA to salt tolerance in plants is not yet see the report.

*Arbuscular mycorrhizal* fungi (AM) can improve the water absorption capacity of plants and increase water use efficiency and the salt tolerance by changing plant tissue structure (Davies et al., 1993). He (2007) found that relative water content of tomato leaves, leaf water potential and leaf water use efficiency and root hydraulic conductivity all reduced with salt concentration increased and prolonged salt stress, but inoculated tomato by AM fungi can reduce the degree of salt damage and water loss, and the higher salt concentrations the more obvious this effect was. AM fungi can significantly promote the absorption of mineral elements Peony (Chen, et al., 2010), mycorrhizal fungi can also improve the peony leaf osmotic adjustment (Guo et al., 2010), mycorrhizal fungi can also promote the growth and enhance salt tolerance of peony seedlings under salt stress. This study intended to determine water-retention capacity and morphology of peony leaves under salt stress, to explore morphological

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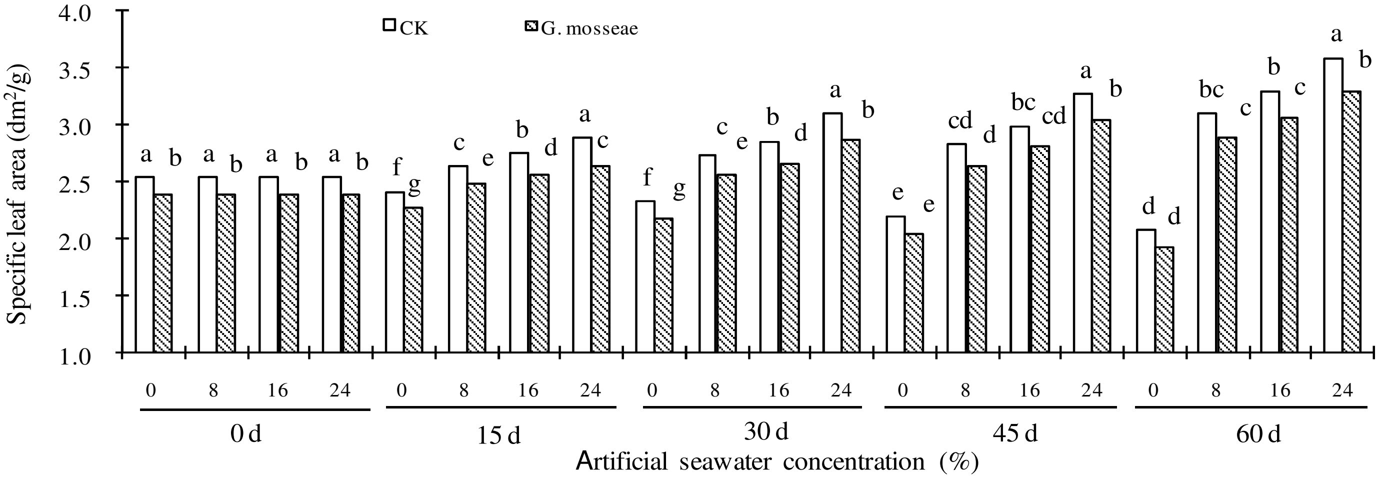

**Figure 1.** Effects of AM fungi *Glomus mosseae* on specific leaf area of *Paeonia suffruticosa* under salt stress. Values with different small letters meant significant difference at 0.05 Level.

responses of the peony leaves to salt stress after *Glomus mosseae* inoculation and to reveal the mechanism that AM fungi enhance the salt tolerance of Peony.

## MATERIALS AND METHODS

### Materials

Tested strains was *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe, used spores of mycorrhizal, mycorrhizal root segment and AM fungal mycelium as inoculum, provided by Institute of mycorrhizal Biotechnology of Qingdao Agricultural University.

The tested material is Peony 'Feng Dan' (Paeonia suffruticosa 'Fengdan') seeds taken from Heze Peony Garden. Perlite and peat soil were mixed in ratio of 1:3, screening and sterilizing (121ºC, 2 h) in the reserve for use, whose features are pH6.54, conductivity 700 μs·cm$^{-1}$, organic matter content 31.2%, available P 23.2 mg·kg$^{-1}$, available N 243.10 mg·kg$^{-1}$, available K 160.94 mg·kg$^{-1}$, soil salinity 0.03%.

Salt solution used artificial seawater (pH=7.8) (Epstein, 1972). Original solution (salt concentration 100%): 410.52mmol·L$^{-1}$NaCl, 9.93 mmol·L$^{-1}$KCl, 10.23 mmol·L$^{-1}$$CaCl_2$, 53.58 mmol·L$^{-1}$ $MgCl_2$, 28.25 mmol·L$^{-1}$$Na_2SO_4$, 2.34 mmol·L$^{-1}$$NaHCO_3$, 0.83 mmol·L$^{-1}$NaBr, 0.07 mmol·L$^{-1}$$SrCl_2$ and 0.44 mmol·L$^{-1}$ $H_3BO_3$. Obtaining the required concentrations of salt solution by diluting.

### Experimental design

The 'Feng Dan' seeds were treated by sand stratification and low temperature, select the seeds which have the same root length to sow, nursery container is nutrition pot (16 × 13 cm, sterilized by 0.5% potassium permanganate solution for 1 h). Inoculated about 5,000 inoculation potential units *G. mosseae* before sowing (Liu Runjin et al., 2007), control were inoculated the equal amount of sterilized inoculum and inoculum filtrate. Random order with 3 repeats.

Treated seedlings with salt stress when grow up to be clover and the plant height about 10 cm. The treatments contained 8, 16 and 24% artificial sea water with three repeats respectively. Irrigated one time with the designed salt water and keeping constant concentration of salts.

### Test method

Leaves were cut from the leaf base to measure leaf area 0, 15, 30, 45 and 60 d after salt stress, respectively. The leaves were immersed in distilled water, soaked in water to the saturation under the dark for 12 h after weighed fresh mass, wiped away excess moisture of leaf surface, weighed saturated fresh weight, and dried to constant weight at 75℃, weighed the dry weight.

Leaf relative water content (RWC) determined by weighing method (Li, 2000):

RWC% = [(Fresh mass - Dry mass) / (Saturated fresh weight - Dry mass)]×100.

Specific leaf area (dm$^2$/g) = Leaf area / Leaf dry weight;

Sclerophyll index (g/dm$^2$) = Leaf dry weight / Leaf area (Wei, 2005).

Succulence = Fresh mass / Dry mass (Sun, 2000).

Five leaves were randomly taken from seedling 45 days after salt stress, and put in room to let the natural water loss, weighed the fresh weight at regular intervals until constant, weighing accuracy is 1 / 1000. The ratio of Leaf water loss to total water at each time point represents the water-retention capacity.

### Data analysis

Analyzed the data with software EXCEL2003 and SPSS13.0.

## RESULTS AND ANALYSIS

### Effects of AM fungi on specific leaf area of Peony under salt stress

Leaf area of the Peony inoculated *G. mosseae* was significantly lower than that of control before salt stress

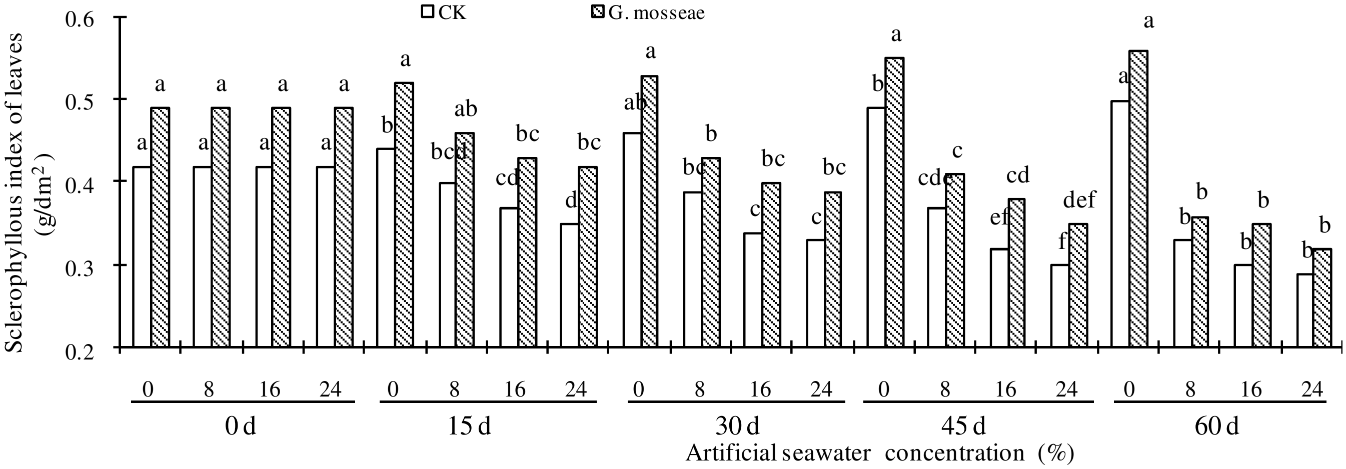

**Figure 2.** Effects of AM fungi *Glomus mosseae* on sclerophyllous Index of leaves in *Paeonia suffruticosa* under salt stress.

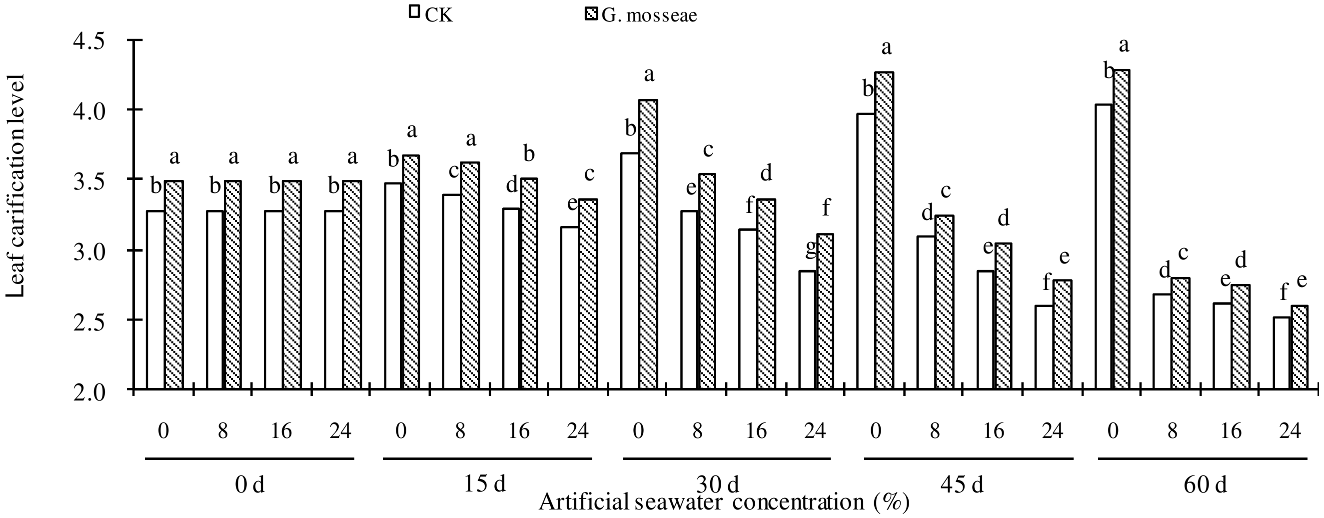

**Figure 3.** Effects of AM fungi *Glomus mosseae* on leaf carification level of *Paeonia suffruticosa* under salt stress.

(P<0.05) (Figure 1). With salt concentration increasing and prolonged stress, Peony leaf area showed an increasing trend. During the whole course, specific leaf area of the Peony inoculated *G. mosseae* were all significantly lower than CK (P<0.05); 60 days after salt stress at concentrations of 8, 16 and 24%, specific leaf area of the Peony inoculated *G. mosseae* were 6.4, 6.9 and 7.8% lower than CK respectively.

## Effects of AM fungi on sclerophyll index of Peony under salt stress

Sclerophyll index of peony inoculated *G. mosseae* was significantly higher than that of CK (Figure 2). Without salt stress, the sclerophyll index of peony showed a increasing trend, but with salt concentration increasing and prolonged stress, sclerophyll index of peony showed a decreasing trend. 60 days after salt stress, Sclerophyll index of peony inoculated *G. mosseae* had no significant difference with CK at all salt concentrations.

## Effects of AM fungi on leaf succulence of Peony under salt stress

Leaf succulence of Peony inoculated *G. mosseae* were significantly higher than that of CK (P<0.05) (Figure 3). Without salt stress, the leaf succulence of peony showed a increasing trend, but with salt concentration increasing and prolonged stress, leaf succulence of peony showed a decreasing trend. With salt concentration increasing and

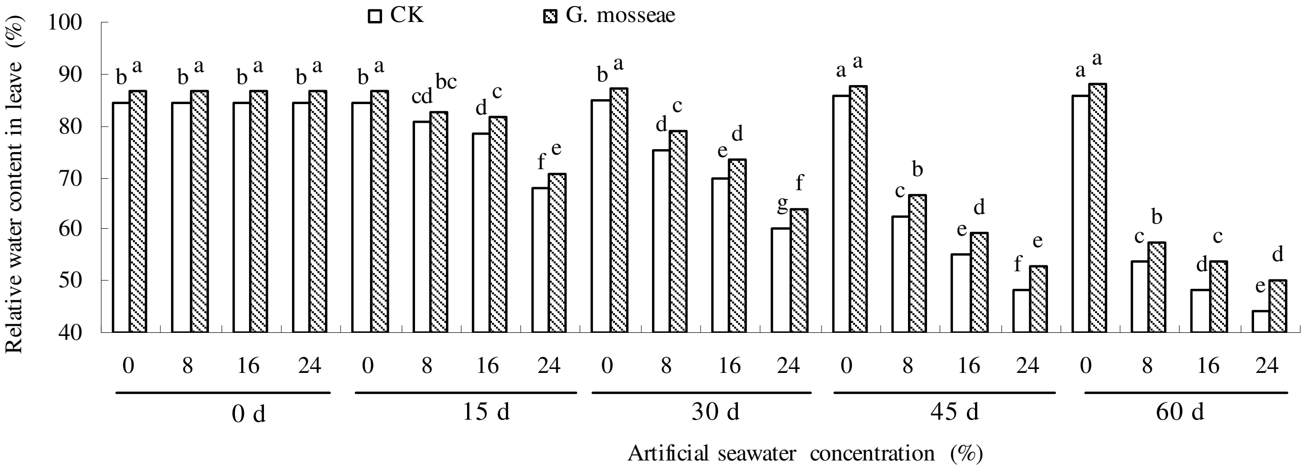

**Figure 4.** Effects of *Glomus mosseae* on leaf relative water content of *Paeonia suffruticosa* under salt stress.

prolonged stress, the leaf succulence of peony significantly decreased.

## Effects of AM fungi on leaf relative water content of Peony under salt stress

Leaf relative water content of Peony inoculated *G. mosseae* were significantly higher than that of CK when without salt stress ($P<0.05$). With salt concentration increasing and prolonged stress, leaf relative water content of peony also showed a decreasing trend, but leaf relative water content of Peony inoculated *G. mosseae* was significantly higher than that of control ($P<0.05$) (Figure 4). 60 days after salt stress, inoculated plants and non-inoculated plants at low-salt (8%), the relative water content in leaves were 34.5 and 37.5% lower than that without salt stress; high-salt treatment 42.9 and 48.5% lower respectively. *G. mosseae* inoculation slow down water loss due to salt stress, which was more obvious at higher salt concentrations, so after *G. mosseae* inoculation the peony has a stronger ability to maintain the plant water balance.

## Effects of AM fungi on leaf water-retention capacity of peony under salt stress

Leaf water loss of peony inoculated with *G. mosseae* was significantly lower than CK (Figure 5). At all salt concentrations, Peony leaf non-inoculated have rapid water loss rate, especially at 24% salt concentration water loss of uninoculated plant is the highest. At 16% salt concentration, the CK lose 80% water in 8 h, but after inoculation with *G. mosseae* the time prolong to 24 h. Peony seedlings inoculated with *G. mosseae* had lower water loss at all time points, time to reach constant mass was 84-108 h, but CK was 48-84 h.

## DISCUSSION AND CONCLUSION

Specific leaf area, sclerophyll index and the succulence degree of leaf can characterize the capacity of plants to maintain water balance and water storage, so they are important indicators to reflect morphological characteristics of leaf structure (Dahlman, 1993). You et al. (2009) believed that the more drought-tolerant plants, the more higher sclerophyll index and Succulence of leaf. Castro et al. (1997) found that the plant leaf area increased with the environment gradient drought. In this study, sclerophyll index and the succulence degree of leaf of peony seedling inoculated *G. mosseae* was significantly higher and specific leaf area was significantly lower than the control; with the increase of stress time and salt concentration, sclerophyll index and the succulence degree of leaf of peony seedling became smaller and specific leaf area increase, which showed that *G. mosseae* inoculation significantly enhanced the ability to adapt to salt stress of the peony.

Colla et al. (2008) proved that the inoculation of AM fungi under salt stress can increase leaf relative water content of the squash (*Cucurbita pepo*). In this paper, the peony leaf inoculated with AM fungi *G. mosseae* had significantly higher relative water content than the control, and the higher the concentration, the more obvious, which showed that *G. mosseae* inoculation can enhance the salt tolerance of peony. He (2007) reached the same result on tomato seedlings by inoculating AM fungi.

Water-retention capacity of plant leaves are usually used to indicate the ability of anti-dehydration of leaf tissue. The more water loss per unit time the worse water holding capacity, and vice versa, the stronger the water retention (Li, 1991). Qi et al. (2004) found that AM fungal

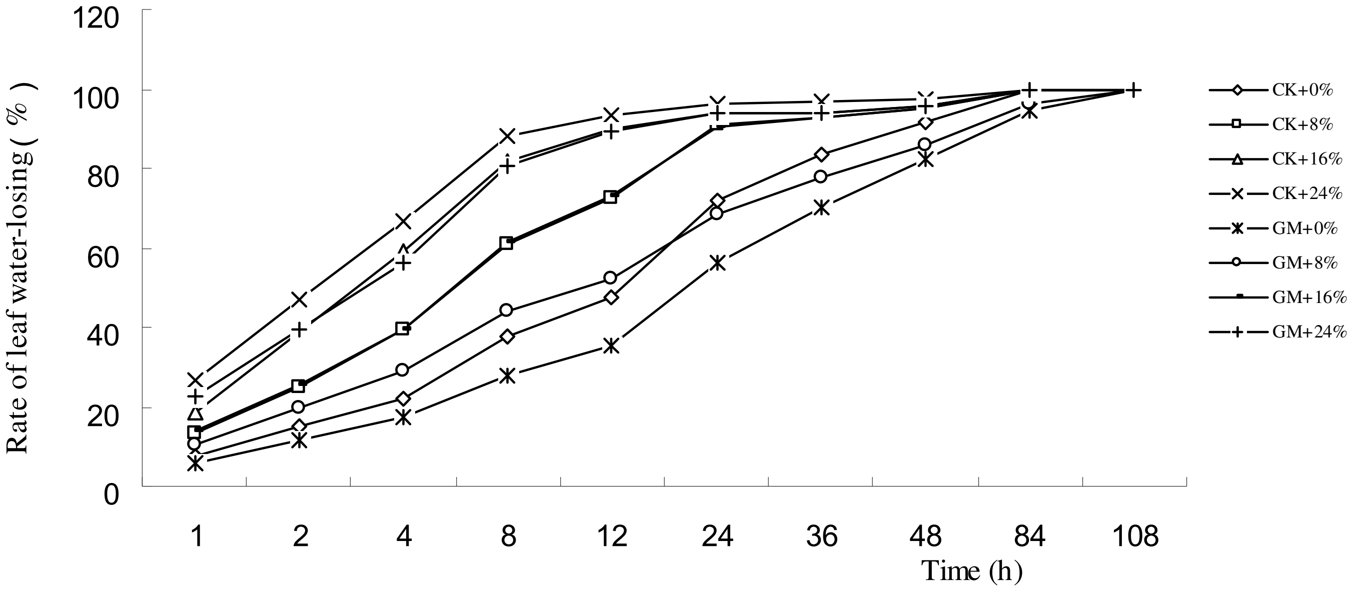

**Figure 5.** Effects of AM fungi *Glomus mosseae* on leaf water-holding ability of *Paeonia suffruticosa* under salt stress.

inoculation significantly increased the leaf water retention capacity of jujube seedlings. You et al. (2009) found that paliurus had high water-retention capacity and strong salt tolerance. Liu (2010) proved that AM fungi *G. mosseae* inoculation can improve the water holding capacity of seedlings of Licorice (*Glycyrrhiza inflata Bat*). This study showed that early in the leaf under salt stress, leaves inoculated with *G. mosseae* loss of water of peony was significantly lower than non-inoculated plants, which proved that *G. mosseae* inoculation can increase the salt tolerance of peony.

## ACKNOWLEDGEMENT

This research was supported by the program of Science and Technology Bureau of Qingdao City (No. 08-1-3-20-jch and 09-1-3-57-jch).

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