

1 **Enhancements of arbuscular mycorrhizal fungi on growth and**
2 **nitrogen acquisition of *Chrysanthemum morifolium* under salt stress**

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15 Running title: Effects of AM fungi and salt stress on *Chrysanthemum morifolium*

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Abstract:

The study aimed to investigate the effects of colonization with two arbuscular mycorrhizal (AM) fungi, *Funneliformis mosseae*, *Diversispora versiformis*, alone and in combination on the growth and nutrient acquisition of NaCl-stressed *Chrysanthemum morifolium* (Hangbaiju) plants in the greenhouse experiment. Mycorrhizal and non-mycorrhizal Hangbaiju plants were grown under different salinity levels imposed by 0, 50 and 200 mM NaCl for five months, following 6 weeks of non-saline pre-treatment. The results showed that root length, shoot and root dry weight, total dry weight, shoot and root N concentration were higher in mycorrhizal than in non-mycorrhizal plants under moderate saline conditions especially with *D. versiformis* colonization. As salinity increased, the mycorrhizal colonization, the mycorrhizal dependence (MD) decreased. Enhancement of tissue N acquisition is probably the main mechanism underlying salt tolerance in AM plants. It is suggested that the symbiotic associations between *D. versiformis* fungus and *C. morifolium* plants may be taken as a biotechnological practice in culture.

Key words: Arbuscular mycorrhiza; *Chrysanthemum morifolium*; N acquisition; Salt stress

1. Introduction

Salinity is a serious environmental problem, over 800 million hectares of the world's land surface is affected by excessive salt [1,2]. The saline soil in China are about 34.6 million hectares, mainly distributing in the northern China and the areas along the Changjiang River [3]. In addition to natural causes such as salty raining waters and weathering of native rocks, poor quality of irrigation water, land clearing, low precipitation, high temperature and over-exploitation available water resources have also aggravated increasing saline soil in many parts of the world [4-7]. It is expected that increasing salinization of arable land will have devastating global effects, resulting in 30% land loss within the next 25 years and up to 50% by the year 2050 [8-10]. High salinity causes both hyperionic and hyperosmotic stress, and can induce plants with decreased growth, lower nutrient acquisition, and even death at the end [5-7]. To deal with saline soils and minimize crop loss, many approaches are employed to combat salt stress, of which application of arbuscular mycorrhizal fungi (AMF) in saline soils are considered as bio-ameliorators in plants [7,10-12].

Arbuscular mycorrhizal fungi is a regular and universal component of the rhizosphere microflora, and nearly 70-90% of land plant species in all terrestrial ecosystems can become colonized by AMF [13-16]. AMF occur naturally in saline environments associated with native species including halophytes, hydrophytes and xerophytes [7,13,17]. Many studies have indicated that AMF can confer positive effects on plant growth and nutrient acquisition under salt stressed conditions [5,7,10,18-21]. Some investigations have suggested that enhancing nutrient acquisition, especially phosphorus, is the most important mechanism of salinity stress tolerance in AM plants [7,10], whereas others reported reduced acquisition of P by AMF inoculated plants grown under saline conditions [5,22,23]. In addition, not all AM fungi function equally well in improving plant growth in saline soils [4,7,10,11]. For example, some researchers reported that *Glomus fasciculatus* appeared to be the most efficient fungus in reducing the negative effects of salinity [10,22], while others suggested that *G. mosseae* was the better option to tackle salt stress [7,24]. These

inconsistency effects of AM fungi under salt stress could be resulted either from the salt tolerance of host species or the fungal species [7,22,25]. Furthermore, most studies on the interactions of mycorrhiza and salinity have been conducted with crop plants, few with medicinal plants [7,10,16].

Hangbaiju (*Chrysanthemum morifolium* (Ramat.) Tzvel), one cultivar of *Dendranthema* genus, is a perennial herbaceous medicinal plant, and the economical importance of it has been attributed especially to the world's largest producers and exporters of its flowers in China [26,27]. In addition to its unique taste as flower tea, Hangbaiju plants contain biologically active compounds such as phenolics, mainly flavonoids, which contribute to disease prevention and the enhancement of our general health status [28]. However, for medicinal plants, these secondary metabolites are usually produced and accumulated under stressful environment [29]. It is reported that this cultivar of Hangbaiju plants are of moderate salt tolerance [27], however, there has been no literature addressing how much benefit can be gained by the plants with AMF inoculation under saline conditions. The purpose of this study was to answer the following questions: (1) Can inoculation of the plants with AMF enhance growth and nutrient uptake under saline conditions? (2) Which fungal species is the better option for improving salt tolerance?

2. Materials and methods

2.1 Plant growth conditions

The experiment was conducted from April to October, 2016 in a greenhouse located in Hangzhou, Zhejiang province of Southeastern China (30°14' N, 119°42' E). During the experiment, 5h supplementary light per day were provided by 400 W high-pressure sodium lamps in the afternoon and evening. Through late February to April, 2016, *Chrysanthemum morifolium* seedlings were cultivated in plastic pots with the soils being sterilized by γ -irradiation (25kGy) [30]. On April 23, 2016, 60 similar-sized *C. morifolium* seedlings were transplanted into plastic pots (18cm

×11cm ×10cm) containing 1kg γ -irradiated medium (25kGy). The potting medium consisted of peat and local soil in a 1:2 (v/v) ratio. The medium had the following properties: 21.94 mg·g⁻¹ organic matter, total N 0.80mg·g⁻¹, Olson P 0.32 mg·g⁻¹, pH4.88 (water: soil= 5:1).

2.2 AM fungal inoculum

Inoculums of *Funneliformis mosseae* (T. H. Nicolson & Gerd.) C. Walker & A. Schüßler (BGC HUN03B) (Fm) and *Diversispora versiformis* (P. Karst.) Oehl, G. A. Silva & Sieverd (BGC GD01C) (Dv) were sourced from the Glomales Germplasm Bank in China (Institute of Plant Nutrient and Resources, Beijing Municipal Academy of Agriculture and Forestry Science). The inoculums were multiplied in an open-pot with fine sand substrate. *Sorghum vulgare* L. was used as trap plant and cultured for 5 months in the greenhouse [20]. At harvest, *Sorghum* was cut at the ground level, and then the roots were chopped into small pieces and mixed with the substrate of the culture pots. Thus, inoculums consisted of sand, spores and mycelium of Fm or Dv and infected root fragments. The 100g (containing ~80 AM fungal propagules per 10 g soil for both fungi) of inoculum (Fm, Dv alone or the combination of Fm and Dv with each fungal species 50g, respectively) was placed in the growth medium before the seedlings were transplanted. Control treatments received no AMF inoculums but with 100ml of 100g combined inoculums filtrate that was sieved through a 25 μ m filter in an attempt to provide similar microbial populations (excluding AM fungi) in all treatments.

2.3 Experimental design

The experiment was designed with two factors: (1) four levels of mycorrhizal treatments: control (non-mycorrhiza, NM), inoculated with *F. mosseae* (Fm), inoculated with *D. versiformis* (Dv), and inoculated with combined inoculums of Fm and Dv (Fm+Dv); (2) three salinity levels of 0, 50 and 200 mM NaCl treatments. Thus,

there were 12 treatment combinations arranged in a randomized complete block design with 5 replicates. During the first 6 weeks, the plants were grown without addition of NaCl in order to obtain plants with functional mycorrhizas and avoid salt effects on AM establishment [18]. To avoid osmotic shock, NaCl was introduced gradually by successively adding 49ml of prescribed NaCl solution in each pot for 7 days, and then a total volume of 343 ml of the corresponding saline solution was added per pot in this experiment [20]. Thereafter, each pot was watered individually with deionized water when needed, and was supplied weekly with 10 ml of a nutrient solution based on Hoagland solution but with half of the normal N and P concentrations [31]. When leaching occurred, the leachate was collected and added to soil to maintain salinity treatments near target levels [32]. The experiment was terminated at October 23, 2016. Shoots and roots were harvested separately.

2.4 Measurements

At harvest, the plants were rinsed three times with deionized water, and then separated into shoots and roots to determine the initial biomass. Leaf images were obtained with a scanner (Epson V330, Japan), and then leaf area was measured with Image J (1.44p; National Institutes of Health, Bethesda, MD, USA). Subsequently, all plant shoots were dried at 70°C for 48 h to measure the dry masses. N concentration was determined with the elemental analyzer TruSpec CN (Leco, St. Joseph, MI, USA) according to the Australian ÖNORM L 1080 protocol; P concentration was measured by ammonium molybdate blue method [33].

Mycorrhizal colonization was detected by the following procedures: Roots from three randomly selected plants in each treatment were collected by gently washing out the soil under running tap water and rinsed three times in deionized water. A subsample of 0.5g fresh fine roots per plant was collected and cut into 1-cm-long pieces [18]. Then, the root segments was stained using the modification of Phillips and Hayman's method [34]. Stained roots were analyzed under a dissecting microscope for the total mycorrhizal colonization percentage, arbuscule colonization

percentage, and vesicle numbers per unit root length with the method described by Biermann and Linderman [35]. The remaining roots were washed free of soil and dried at 70°C for 48 h. N and P concentration were measured as described as above for shoots.

At the end of the experiment, the mycorrhizal dependency (MD) was calculated as follows [24]:

$$MD = \frac{\text{Dry weight of AM plants} - \text{mean value of Dry weight of non-AM plants}}{\text{Dry weight of AM plants}} \times 100$$

2.5 Statistical analysis

Two-way ANOVA (General Linear Model, SPSS 18.0, SPSS Inc., Chicago) was used to test the differences in response variables, with salt and AMF as fixed factors. Before analysis, the data on root length, total dry weight, shoot N concentration, shoot P concentration, root N concentration, N: P ratio of shoot biomass, and the numbers of vesicles were square root-, log 10-, log 10-, log 10-, square root-, log 10-, ln-transformed, respectively, based on the Levene's test for equality of variance and the Shapiro-Wilk test for normality, and the other data were analyzed without transformation. Also, LSD multiple range test was used to compare the differences in response variables between treatments at $P < 0.05$.

3. Results

3.1 Mycorrhizal colonization

180 In this experiment, none of the Hangbaiju plants from the non-inoculated
181 treatments were colonized by AM fungi. Plants inoculated with *F. mosseae* (Fm), *D.*
182 *versiformis* (Dv) and the combined inoculums had percentage of total colonization of
183 27.7%-56.9%, 34.6%-73% and 32.5%-60%, respectively, also, the extent of
184 mycorrhizal colonization were all significantly affected by salt and the interactive
185 effects of salt and AMF (Table 1). Except of the non-saline condition, there were no
186 significant differences in the percentage of total mycorrhizal colonization and
187 arbuscule colonization of Hangbaiju plants inoculated with different fungal species
188 (Table 1).

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190 **3.2 Plant growth**

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192 Except of root/shoot ratio, the growth variables of the plants were significantly
193 affected by salt; except of root length and root dry weight, the growth variables were
194 significantly affected by AMF; also, the interactive effects of salt and AMF were
195 significant on root length, root dry weight and total dry weight (Table 2). Compared
196 to the non-colonized plants, inoculated with fungal species had no positive effects on
197 the leaf area under saline or no-saline conditions (Figure 1A). In the absent of saline
198 treatment, inoculation with fungal species significantly decreased root length
199 compared to the non-colonized plants, while under saline conditions, inoculated with
200 fungal species had positive effects on root length, particularly at severe salinity (200
201 mM NaCl), and inoculated with Fm and Dv significantly increased the root length by
202 44% and 93%, respectively, but the combined inoculums did not induce such effect
203 (Figure 1B). In addition, the root length of the non-colonized plants was 40% lower at
204 200 mM NaCl compared to the non-saline treated plants, while in the AM-colonized
205 plants, this negative effect of NaCl supply was only about 13% lower by Fm and even
206 16% higher by Dv (Figure 1B).

207 Root colonization by fungi species significantly enhanced biomass development,
208 especially under moderate salt stress (50 mM NaCl) (Figure 2). Under non-saline
209 conditions, compared to the non-inoculated plants, shoot dry weight of Fm, Dv and

the combined inoculums inoculated plants increased by 67%, 121% and 77%, respectively. In the presence of NaCl at 50 mM, inoculations with Fm, Dv and the combined inoculums increased shoot dry weight by 105%, 133% and 45%, respectively, in relative to the controls. As for the root dry weight, in the absence of NaCl, Fm, Dv and the combined inoculums increased it by 13%, 49% and 63%, respectively, compared to the controls. In the presence of NaCl at 50mM, inoculations with Fm and Dv increased the root dry weight by 38% and 9%, respectively in relative to the controls, but not with the combined inoculums. Clearly, the magnitude of the growth response to AMF was more effective in improving shoot development than root development under lower salt stress. Under non-saline conditions, inoculations with Fm, Dv and the combined inoculums increased the total dry weight by 49%, 97% and 73%, respectively, compared to the non-inoculated plants. In the treatments with 50 mM NaCl, the total dry weights of Fm, Dv and the combined inoculums inoculated plants were higher than the controls by 92%, 95% and 23%, respectively. Nevertheless, at any given NaCl level, there were no significant differences in root/shoot ratio irrespective of the colonizing fungi. Also, in the treatments with 200mM NaCl, shoot dry weight, root dry weight and the total dry weight of plants inoculated with or without AM fungi decreased more greatly compared to the non-saline conditions, and inoculated with AM fungi had no effects on them compared to the non-inoculated plants.

Furthermore, with the increase of salinity, the percentage of mycorrhizal dependence (MD) persisted stable under none or moderate salt stress (0 or 50 mM NaCl), but greatly decreased at severe salt stress (200 mM NaCl) (Figure 3). In addition, the plant growth dependence on mycorrhizal symbiosis was greatest when plants were colonized by Dv.

3.3 Plant nutrient

Salt, AMF and the interaction of salt and AMF had significant effects on the tissue N and P concentration, and also the N:P ratio of shoot biomass (Table 2). With

the increase of salinity, the shoot N and P concentration significantly increased while the root N and P concentration decreased; nevertheless, the effects of AM colonization on nutrient acquisition varied greatly in any given NaCl levels (Figure 4). Compared to the controls, inoculation with Fm increased shoot N concentration by 15%, whereas inoculation with Dv and the combined inoculums decreased it by 4% and 10%, respectively, under non-saline conditions. In the treatments with 50mM NaCl, the shoot N concentration of Fm and Dv plants decreased by 10% and 7%, respectively, while that of the combined inoculums inoculated plants increased by 9% in relative to non-inoculated plants. Compared to the non-inoculated plants, the shoot N concentration of Fm, Dv and the combined inoculums inoculated plants increased by 29%, 6% and 13%, respectively, under 200mM NaCl. Under non-saline conditions, inoculation with Fm significantly increased the shoot P concentration by 27%, while inoculation with Dv and the combined inoculums had no positive effects on it compared to the non-inoculated plants. Under saline condition, only inoculation with Dv had positive effects on shoot P concentration compared to non-inoculated plants, but not with Fm and the combined inoculums. In addition, inoculation with any of the fungal species had no positive effects on root N concentration compared to the controls under non-saline condition. In the treatments with 50mM NaCl, inoculation with Fm decreased root N concentration by 48%, whereas inoculations with Dv and the combined inoculums increased it by 17% and 13%, respectively, in relative to the non-inoculated plants. Nevertheless, in the treatments with 200mM NaCl, inoculation with Fm and the combined inoculums increased root N concentration by 20% and 77%, respectively, whereas inoculations with Dv decreased it by 20% in relative to the non-inoculated plants. At any given NaCl levels, inoculations with any of the fungal species significantly decreased the root P concentration compared to the controls.

In addition, with the increase of salinity, the N:P ratios of shoot biomass of plants inoculated with Fm and the combined inoculums significantly increased from 7 to 16, while the biomass N:P ratios of non-inoculated plants and Dv plants increased firstly and then decreased with the values under 10 (Figure 5).

4. Discussion

The longer root length, higher shoot and root dry weight, higher total dry weight, higher shoot and root N concentration of mycorrhizal plants under moderate NaCl stress conditions compared with non-mycorrhizal plants show that root colonization by fungi species, especially by *D. versiformis* (Dv), can alleviate the detrimental effects of NaCl stress. Also, the mycorrhizal dependence (MD) persisted at positive values under moderate salinity. These results showed that *C. morifolium* plants were highly dependent on AM colonization to reach the optimal growth under saline conditions, and the most active fungus was Dv. In addition, just as Feng et al. [18] pointed out the beneficial effects of AM fungi on plants were not specific process induced by salinity stress, which occurred not only during NaCl stress but also in non-stress conditions.

Plants growing in saline soil are subject to two primary physiological stresses [4,7]: Firstly, the toxic effects of specific ions such as sodium and chloride, which disrupt the structure of enzymes and other macromolecules, damage cell organelles, disrupt photosynthesis and respiration, inhibit protein synthesis and induce ion deficiencies. Secondly, plants exposed to the low osmotic potentials of saline solutions are at risk of “physiological drought”. In this experiment, salt stress induced plants with shorter root length, less biomass development, however, these deleterious effects of NaCl stress can be mediated by mycorrhizal colonization (Figures 1 and 2). These findings are consistent with previous reports for AM plants under salt stress [11,21,36-39]. Longer root length and more biomass accumulation of AM plants under salt stress are adaptive strategies for benefiting water and nutrient acquisition and then improving fitness under stressful environment[7]. It is suggested that improvement of plant phosphorus acquisition is the most important mechanism of salinity stress tolerance in AM plants [5,11,22,40]. However, some studies have shown that mycorrhizal plants grow better than non-mycorrhizal plants under salt stress even when mycorrhizal and non-mycorrhizal plants have a similar P status

300 [23,41]. In addition, Ruiz-Lozano et al. [41] argued that the mechanism underlying
301 AM plant growth improvement under saline conditions are based on physiological
302 process (increased carbon dioxide exchange rate, transpiration, stomatal conductance
303 and water use efficiency) rather than on nutrient uptake (N or P). Also, Feng et al. [18]
304 suggested that it is the higher soluble sugar accumulation in mycorrhizal plants in
305 relative to non-mycorrhizal plants not the P status that enhanced salt resistance.

306 Our study shows that the enhancement of nutrient uptake with AMF inoculation
307 mainly appeared in the tissue N concentration, not the P concentration (Figure 4). It
308 has been reported that the application of AMF may improve nitrogen assimilation by
309 host plants [10]. For example, Giri and Mukerji [42] recorded higher accumulation of
310 N in the shoots of mycorrhizal *Sesbania grandiflora* and *S. aegyptiaca* than
311 nonmycorrhizal control plants. The increased nutrient uptake observed may be
312 explained by the fact that the hyphae of AMF often penetrate some 7cm or more into
313 the soil beyond the rhizosphere, where they can absorb water and nutrients under
314 different conditions of osmotic potential than at the root surface [11,43]. Improved N
315 uptake may help to reduce the toxic effects of Na⁺ ions by regulating its uptake and
316 indirectly help to maintain chlorophyll content of the plant [9,10]. Also, the biomass
317 N:P ratio in this experiment can provide another support for the enhancement of N
318 uptake underlying the mechanism of AM plants to tackle saline stress. Biomass N:P
319 ratio has been mainly used to assess whether N or P is more limiting for biomass
320 production, usually, N:P ratios < 10 and > 20 correspond to N- and P-limited biomass
321 production [44]. As salinity increased, the biomass N:P ratios of non-inoculated plants
322 and Dv plants persisted under 10, indicating that it is the N-limited biomass
323 production. Whereas, for plants inoculated with Fm and the combined inoculums, it
324 increased from 7 to 16, indicating that it is still the N-limited biomass production and
325 there is transition between N- and P-limited biomass productions.

326 Furthermore, AM fungi differ in their ability to enhance growth and nutrient
327 uptake even though there were small differences among the fungal species in terms of
328 their ability to colonize the roots (Table. 1; Figure 4). This finding is in agreement
329 with the previous studies [24,43]. Specific mechanisms conferring functional

330 differences between AM fungi could be expected from changes in fungal
331 characteristic such as length of external mycelium, hyphae distribution, and /or
332 nutrients translocation [24]. Actually, the positive effects of AM fungi on the growth
333 and nutrient acquisition of *C. morifolium* plants under saline condition can be reduced
334 in the severe salt stress, which is because that soil salinity may also influence the
335 growth and activity of AM fungi [4]. With the increasing sodium chloride levels, the
336 mycorrhizal colonization varied from the highest percentage of colonization 73% for
337 Dv at no added NaCl to approximately 35% at 200mM NaCl (Table 1). The reduced
338 colonization with salt application is consistent with the observations of others
339 [22,32,45].

340 The above results show that AM fungi differ in their ability to enhance growth
341 and nutrient uptake of *C. morifolium* plants under saline conditions, of which Dv
342 fungus is the most active and effective one compared to others. Actually, there is
343 specific compatibility relationship existing among symbionts, which underscore the
344 importance of host-endophyte selection to maximize growth and nutrition of the
345 plants. AM symbiotic efficiency attributed to plant is dependent on plant species and
346 AM fungal species, and the selection of the most suitable AM fungus for a specific
347 plant is of practical interest for improving the effectiveness under particular
348 environmental conditions [24].

349

350 5. Conclusions

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352 This study addressed that colonization with fungi species improved the growth
353 and N uptake of *C. morifolium* plants under moderate saline conditions. Nevertheless,
354 AM fungi differ in their effects on *C. morifolium* plants under saline conditions, of
355 which *D. versiformis* (Dv) is the most active fungus. These observations consolidate
356 evidence for the potential of Dv to protect *C. morifolium* plants against moderate salt
357 stress and may pave the way for the exploitation of the symbiosis as a
358 biotechnological practice in culture.

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References

1. Munns, R. Genes and salt tolerance: Bring them together. *New Phytol.* **2005**, *167*, 645-663.
2. Rengasamy, P. World salinization with emphasis on australia. *J. Exp. Bot.* **2006**, *57*, 1017-1023.
3. Wu, X.; Ni, J.W.; Zhang, H.X.; Liu, T.; Zhang, L. Effects of salt stress on osmotic adjustment substances in three species of *Nitraria*. *J. Northeast Forest. Univ.* **2012**, *40*, 44-47, 69 (in Chinese).
4. Juniper, S.; Abbott, L. Vesicular-arbuscular mycorrhizas and soil salinity. *Mycorrhiza* **1993**, *4*, 45-57.
5. Al-Karaki, G.N. Growth of mycorrhizal tomato and mineral acquisition under salt stress. *Mycorrhiza* **2000**, *10*, 51-54.
6. Mahajan, S.; Tuteja, N. Cold, salinity and drought stresses: An overview. *Arch. Biochem. Biophys.* **2005**, *444*, 139-158.
7. Evelin, H.; Kapoor, R.; Giri, B. Arbuscular mycorrhizal fungi in alleviation of salt stress: A review. *Ann. Bot.* **2009**, *104*, 1263-1280.
8. Szabolcs, I. Soil sand salinisation. In *Handbook of plant and crop stress*; Pessarakali, M., Ed.; Marcel Dekker: New York, United States, 1994; pp. 3-11.
9. Garg, N.; Chandel, S. The effects of salinity on nitrogen fixation and trehalose metabolism in mycorrhizal *Cajanus cajan* (L.) Mill sp. plants. *J. Plant Growth Regul.* **2011**, *30*, 490-503.
10. Chandrasekaran, M.; Boughattas, S.; Hu, S.J.; Oh, S.H.; Sa, T.M. A meta-analysis of arbuscular mycorrhizal effects on plants grown under salt stress. *Mycorrhiza* **2014**, *24*, 611-625.
11. Ojala, J.C.; Jarrell, W.M.; Menge, J.A.; Johnson, E.L.V. Influence of mycorrhizal fungi on the mineral nutrient and yield of onion in saline soil. *Agron. J.* **1983**, *75*, 255-259.
12. Dodd, I.C.; Pérez-Alfocea, F. Microbial amelioration of crop salinity stress. *J. Exp. Bot.* **2012**, *63*, 3415-3428.
13. Khan, A.G. The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes, and of endogone spores in adjacent soils. *J. Gen. Microbiol.* **1974**, *81*, 7-14.
14. Smith, S.E.; Read, D.J. *Mycorrhizal symbiosis*. Academic press: New York, United States, 2008.

15. Bonfante, P.; Genre, A. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. *Nat. commun.* **2010**, *1*, 1-11.
16. Fester, T.; Sawers, R. Progress and challenges in agricultural applications of arbuscular mycorrhizal fungi. *Crit. Rev. Plant Sci.* **2011**, *30*, 459-470.
17. Wilde, P.; Manal, A.; Stodden, M.; Sieverding, E.; Hilderbrandt, U.; Bothe, H. Biodiversity of arbuscular mycorrhizal fungi in roots and soils of two salt marshes. *Environ. Microbiol.* **2009**, *11*, 1548-1561.
18. Feng, G.; Zhang, F.S.; Li, X.L.; Tian, C.Y.; Tang, C.; Rengel, Z. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza* **2002**, *12*, 185-190.
19. Hajiboland, R.; Aliasgharzadeh, N.; Laieghi, S.F.; Poschenreider, C. Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. *Plant Soil* **2010**, *331*, 313-327.
20. Evelin, H.; Giri, B.; Kapoor, R. Contribution of glomus intraradices inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed *Trigonella foenum-graecum*. *Mycorrhiza* **2012**, *22*, 203-217.
21. Balliu, A.; Sallaku, G.; Rewald, B. AMF inoculation enhances growth and improves the nutrient uptake rates of transplanted, salt-stressed tomato seedlings. *Sustainability* **2015**, *7*, 15967-15981.
22. Hirrel, M.C.; Gerdemann, J.W. Improved growth of onion and bell pepper in saline soils by two vesicular-arbuscular mycorrhizal fungi. *Soil Sci. Soc. Am. J.* **1980**, *44*, 654-655.
23. Poss, J.A.; Pond, E.; Menge, J. Effect of salinity on mycorrhizal onion and tomato in soil with and without additional phosphate. *Plant Soil* **1985**, *88*, 307-309.
24. Porras-Soriano, A.; Soriano-Martin, M.L.; Porras-Piedra, A.; Azcon, R. Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *J. Plant Physiol.* **2009**, *166*, 1350-1359.
25. Wilson, G.W.T.; Hartnett, D.C. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. *Am. J. Bot.* **1998**, *85*, 1732-1738.
26. Zheng, C.Z. *Flora of Zhejiang*. Zhejiang Science & Technology Press: Hangzhou, China, 1993; p. 271.
27. Zhang, P.; Wang, K.C.; Zhu, G.M.; Zhao, X.M.; Zhao, J.; Guo, Q.H.; Cheng, M.C.; Zhou, X.S. Evaluation of salt tolerance of 7 *chrysanthemum* germ plasm. *Jiangsu Agric. Sci.* **2015**, *43*, 234-238.
28. Guan, Z.Y.; Chen, S.M.; Chen, F.D.; YIN, D.M.; Liu, Z.L.; Tang, J.; Yang, F. Salt tolerance screening of 32 taxa from *chrysanthemum* and its relative genera. *Sci. Agr. Sin.* **2010**, *43*, 4063-4071.
29. Huang, L.Q.; Guo, L.P. Secondary metabolites accumulating and geoherb formation under environmental stress. *Chin. J. Chin. Mater. Med.* **2007**, *32*, 277-280 (in Chinese).
30. McNamara, N.P.; Black, H.I.J.; Beresford, N.A.; Parekh, N.R. Effects of acute gamma irradiation on chemical, physical and biological properties of soils. *Appl. Soil Ecol.* **2003**, *24*, 117-132.
31. Veiga, R.S.L.; Faccio, A.; Genre, A.; Pieterse, C.M.J.; Bonfante, P.; van der Heijden, M.G.A.

- Arbuscular mycorrhizal fungi reduce growth and infect roots of the non-host plant *Arabidopsis thaliana*. *Plant Cell Environ.* **2013**, *36*, 1926-1937.
32. Al-Karaki, G.N.; Hammad, R. Mycorrhizal influence on fruit yield and mineral content of tomato grown under salt stress. *J. Plant Nutr.* **2001**, *24*, 1311-1323.
33. Allen, S.E. *Chemical analysis of ecological materials*. 2nd ed.; Blackwell Scientific Publications: London, United Kingdom, 1989.
34. Phillips, J.; Hayman, D. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.* **1970**, *55*, 158-161.
35. Biermann, B.; Linderman, R.G. Quantifying vesicular-arbuscular mycorrhizas: A proposed method towards standardization. *New Phytol.* **1981**, *87*, 63-67.
36. Colla, G.; Roupael, Y.; Cardarelli, M.; Tullio, M.; Rivera, C.M.; Rea, E. Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. *Biol. Fertil. Soils* **2008**, *44*, 501-509.
37. Cantrell, I.C.; Linderman, R.G. Preinoculation of lettuce and onion with vesicular-arbuscular mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant Soil* **2001**, *233*, 269-281.
38. Giri, B.; Kapoor, R.; Mukerji, K.G. Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass and mineral nutrient of *Acacia auriculiformis*. *Biol. Fert. Soils* **2003**, *38*, 170-175.
39. Turkmen, O.; Sensoy, S.; Demir, S.; Erdinc, C. Effects of two different AMF species on growth and nutrient content of pepper seedlings grown under moderate salt stress. *Afr. J. Biotechnol.* **2008**, *7*, 392-396.
40. Bolan, N.S. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant Soil* **1991**, *134*, 189-207.
41. Ruiz-Lozano, J.M.; Azcón, R.; Gomez, M. Alleviation of salt stress by arbuscular mycorrhizal *glomus* species in *Lactuca sativa* plants. *Physiol. Plantarum* **1996**, *98*, 767-772.
42. Giri, B.; Mukerji, K.G. Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions: Evidence for reduced sodium and improved magnesium uptake. *Mycorrhiza* **2004**, *14*, 307-312.
43. Ruiz-Lozano, J.M.; Azcón, R. Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *glomus* sp. from saline soils and *glomus deserticola* under salinity. *Mycorrhiza* **2000**, *10*, 137-143.
44. Güsewel, S. N:P ratios in terrestrial plants: Variation and functional significance. *New Phytol.* **2004**, *164*, 243-266.
45. Duke, E.R.; Johnson, C.R.; Koch, K.E. Accumulation of phosphorus, dry matter and betaine during NaCl stress of splitroot citrus seedlings colonized with vesicular-arbuscular mycorrhizal fungi on zero, one or two halves. *New Phytol.* **1986**, *104*, 583-590.

474 **Table 1** Colonization of *Chrysanthemum morifolium* by arbuscular mycorrhizal fungi
475 under salt stress.

Treatments	Total colonization(%)	Arbuscule (%)	Numbers of vesicles (no./mm root length)				
0mM NaCl+Fm	35.8 ± 9.2 cd	5.3 ± 1.2 ab	1.9 ± 1.0 bc				
0mM NaCl+Dv	73.0 ± 5.2 a	12.0 ± 4.9 a	3.1 ± 1.4 ab				
0mM NaCl+Fm+Dv	60.0 ± 9.6 ab	5.0 ± 2.1 ab	1.6 ± 0.5 bc				
50mM NaCl+Fm	56.9 ± 7.0 abc	6.7 ± 3.2 ab	1.1 ± 0.4 bc				
50mM NaCl+Dv	45.0 ± 9.2 bcd	2.2 ± 1.5 b	5.3 ± 0.9 a				
50mM NaCl+Fm+Dv	47.4 ± 8.5 bcd	4.8 ± 2.7 ab	1.7 ± 0.2 bc				
200mM NaCl+Fm	27.7 ± 4.0 d	3.6 ± 1.2 b	1.5 ± 0.8 bc				
200mM NaCl+Dv	34.6 ± 4.6 cd	7.9 ± 1.5 ab	2.8 ± 1.8 abc				
200mM NaCl+Fm+Dv	32.5 ± 7.5 d	1.0 ± 0.9 b	0 c				
Levels of significance							
	<i>F</i>	Sig	<i>F</i>	Sig	<i>F</i>	Sig	<i>df</i>
Salt	3.956	*	0.534	ns	0.068	ns	2
AMF	1.927	ns	0.569	ns	1.773	ns	2
Salt × AMF	3.904	*	1.660	ns	2.539	ns	4
Error							14

476 Data in the table are expressed as mean ± SE. Values in columns followed by the same letter do not differ
477 significantly at $P < 0.05$ by LSD multiple range test. Fm, Dv and Fm+Dv represent inoculation with *Funneliformis*
478 *mosseae*, *Diversispora versiformis* and the combination of *F. mosseae* and *D. versiforme*, respectively. *ns* not
479 significant; * $P < 0.05$.

480 **Table 2** Effects of salt, arbuscular mycorrhizal fungi (AMF) and their interactions
481 on the variables of *Chrysanthemum morifolium* plants.

Variable	Salt	AMF	Salt × AMF
Leaf area	7.203** (2,30)	6.016** (3,30)	1.069 ^{ns} (6,30)
Root length	15.810*** (2,30)	0.512 ^{ns} (3,30)	4.703** (6,30)
Shoot dry weight	8.101** (2,30)	4.821** (3,30)	1.671 ^{ns} (6,30)
Root dry weight	8.044** (2,30)	1.578 ^{ns} (3,30)	2.698* (6,30)
Total dry weight	16.787*** (2,30)	2.931* (3,30)	3.812** (6,30)
Root/shoot ratio	0.745 ^{ns} (2,30)	4.810** (3,30)	0.977 ^{ns} (6,30)
MD	7.177** (2,35)	0.335 ^{ns} (2,35)	0.429 ^{ns} (4,35)
Shoot N concentration	3333.173*** (2,24)	129.896*** (3,24)	197.711*** (6,24)
Shoot P concentration	647.177*** (2,24)	490.471*** (3,24)	181.258*** (6,24)
Root N concentration	394.410*** (2,24)	333.397*** (3,24)	534.881*** (6,24)
Root P concentration	808.550*** (2,24)	261.131*** (3,24)	26.549*** (6,24)
N:P ratio of shoot biomass	1574.122*** (2,24)	326.351*** (3,24)	170.134*** (6,24)

482 Data in the table are expressed as *F*-values and followed by *df* values in parentheses. MD: mycorrhizal dependence.

483 * $P \leq 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, $P > 0.05$.

484

485 **Figure Captions**

486 **Fig. 1** Effects of arbuscular mycorrhizal fungi on leaf area (A) and root length (B) of
487 *Chrysanthemum morifolium* plants under 0, 50 and 200mM NaCl. NM, Fm, Dv and
488 Fm+Dv represent inoculation with non-mycorrhizal, *Funneliformis mosseae*,
489 *Diversispora versiformis* and the combined inoculums, respectively. Values represent
490 mean \pm SE. Values followed by the same letter do not differ significantly at $P < 0.05$
491 by LSD multiple range test.

492 **Fig. 2** Effects of arbuscular mycorrhizal fungi on shoot dry weight (A), root dry
493 weight (B), total dry weight (C) and root/shoot ratio (D) of *C. morifolium* plants under
494 0, 50 and 200mM NaCl. Symbols as in Fig.1.

495 **Fig. 3** The mycorrhizal dependence of *C. morifolium* plants under 0, 50 and 200mM
496 NaCl. Symbols as in Fig.1.

497 **Fig. 4** Effects of arbuscular mycorrhizal fungi on shoot N content (A), shoot P content
498 (B), root N content (C) and root P content (D) of *C. morifolium* plants under 0, 50 and
499 200mM NaCl. Symbols as in Fig.1.

500 **Fig. 5** Effects of arbuscular mycorrhizal fungi on N:P ratio of *Chrysanthemum*
501 *morifolium* plants under 0, 50 and 200mM NaCl. Symbols as in Fig.1.

502

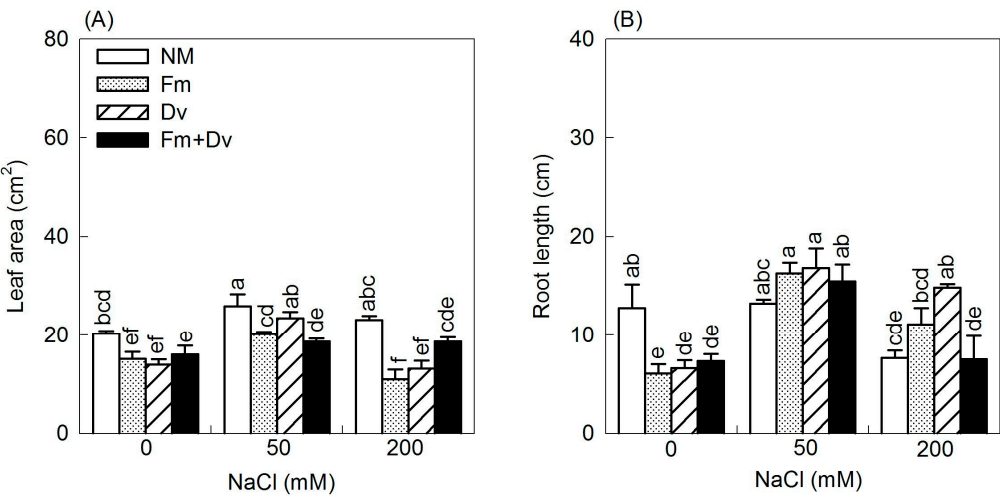


Figure 1

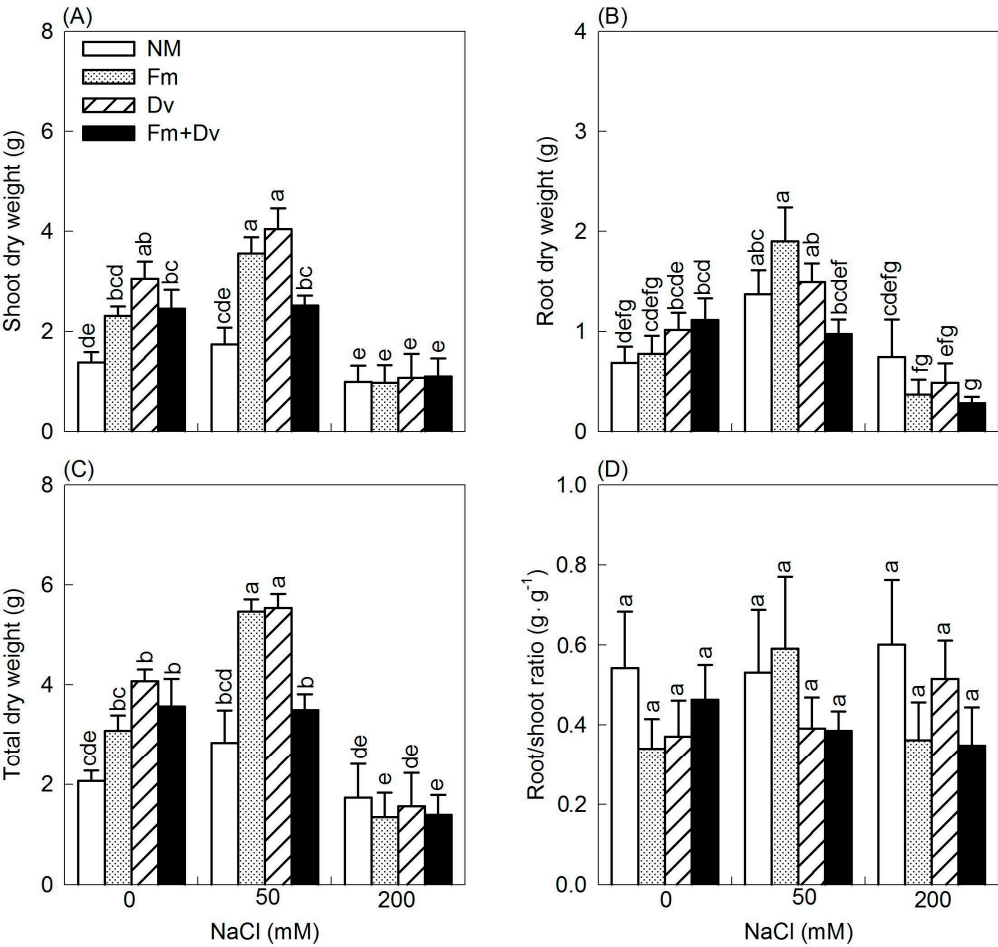


Figure 2

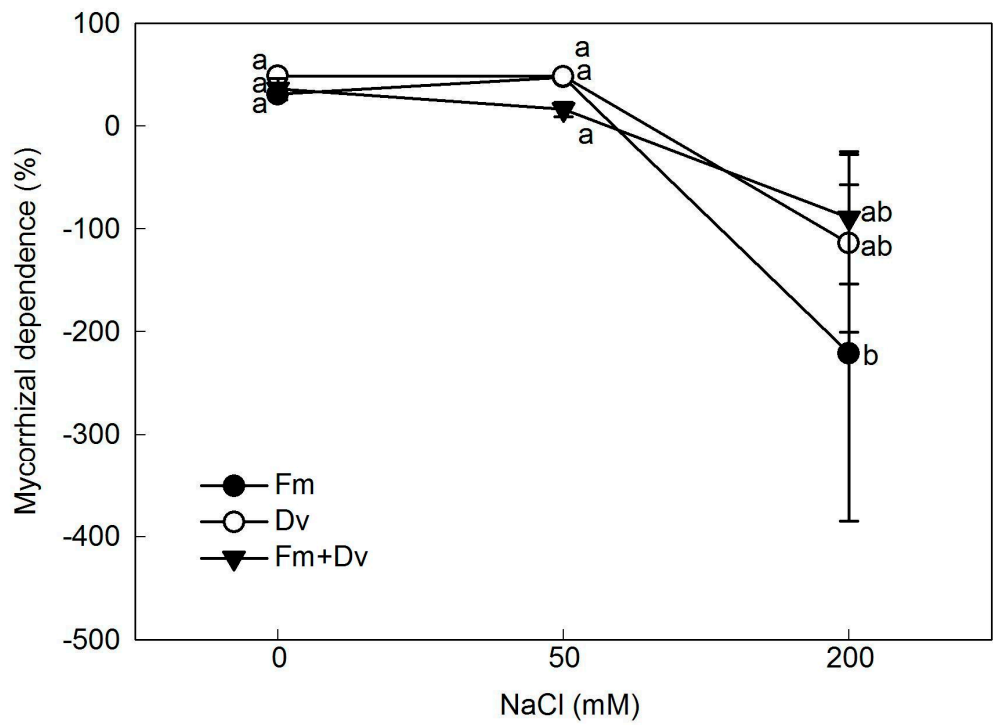


Figure 3

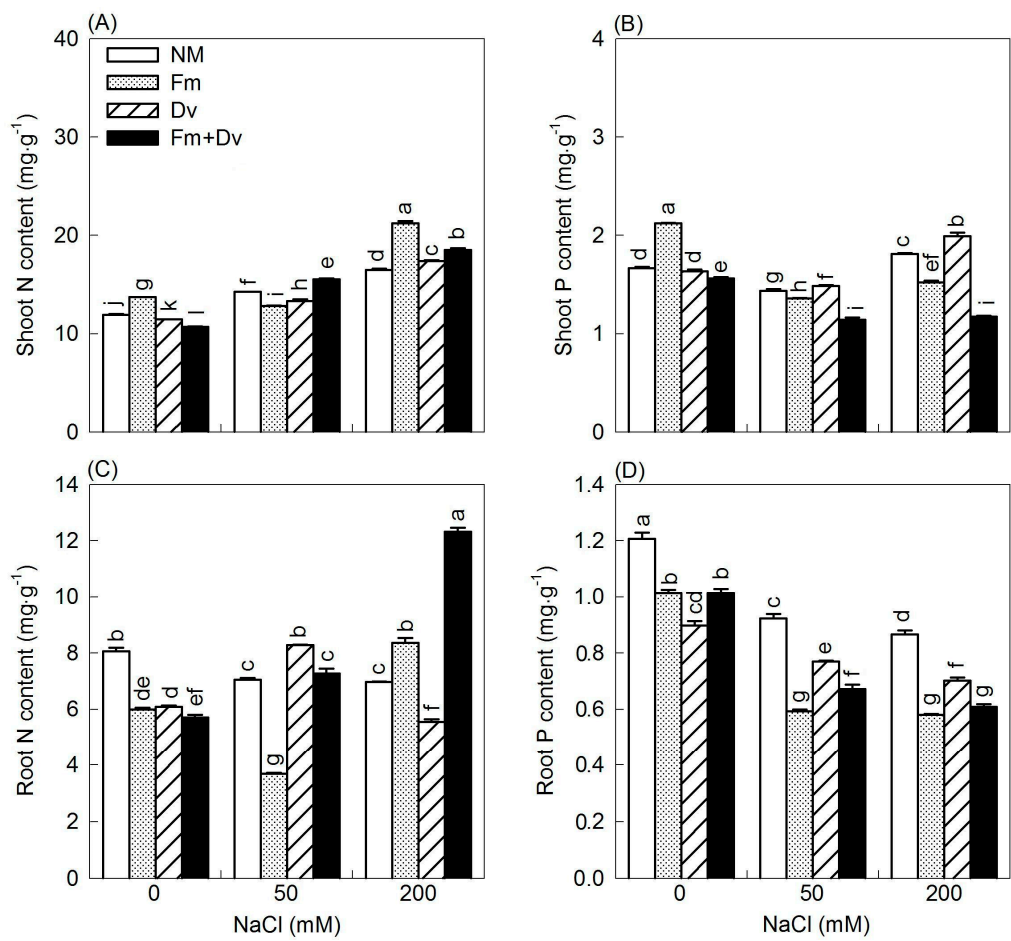


Figure 4

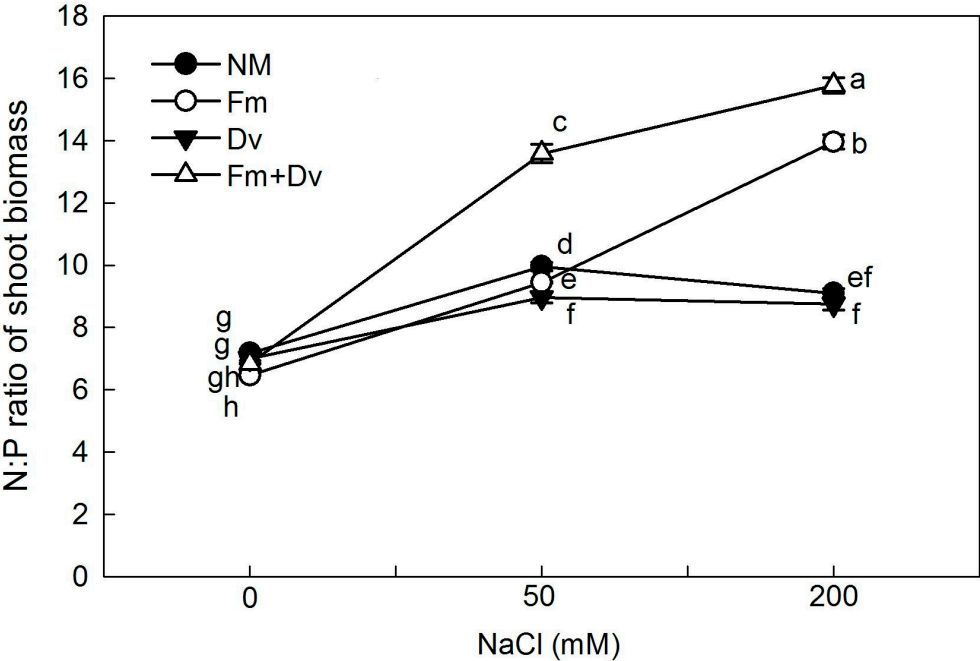


Figure 5