

Arbuscular mycorrhizal fungi-mediated resistance to salt spray in *Cinnamomum camphora* seedlings enhanced by leaf functional traits

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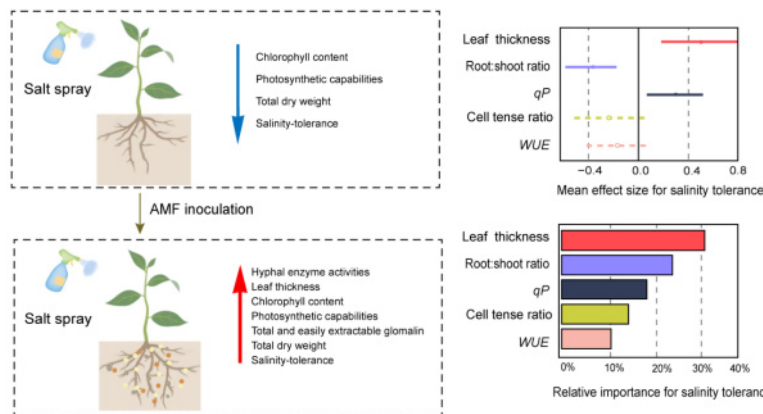
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ABSTRACT

- Salt spray is a natural disturbance in coastal area of Southern China.
- Arbuscular mycorrhizal fungi can mediate the detrimental effects of salt spray.
- Leaf thickness and photosynthetic ability are key parameters.
- Combined fungi may be beneficial for trees grown in coastal areas.

Salt spray is a natural disturbance in coastal region. Arbuscular mycorrhizal fungi (AMF) are recognized as bio-ameliorators of soil salinity in plants. However, the mechanism through which AMF protects *Cinnamomum camphora* against aerial salinity remains unclear. To address this knowledge gap, plants were subjected to four fungal regimes, namely sterilized fungal inoculum, *Glomus tortuosum*, *Funnelliformis mosseae*, or a combination of these two fungi, and exposed to three sprayed-salt regimes (0, 7, or 14 mg NaCl cm⁻² d⁻¹) in a greenhouse. Salt spray significantly decreased photosynthetic capabilities, total dry weight, and salinity tolerance of non-mycorrhizal plants. Mycorrhizal inoculation, particularly a combination of *G. tortuosum* and *F. mosseae*, evidently mitigated the detrimental effects induced by salt spray. Meanwhile, mycorrhiza-mediated protection depended on the intensity of sprayed salt and the identity of fungal taxa. Furthermore, the enhanced resistance of mycorrhizal *C. camphora* seedlings to aerial salinity was mainly owing to increased leaf thickness and photosynthetic capabilities. These findings imply that inoculation with combined fungi could be an optimal strategy for cultivating *C. camphora* plants in coastal regions. The results gained hold the potential to offer both theoretical and practical guidance for the managers of coastal ecosystems in soil restoration and conservation.

Keywords arbuscular mycorrhizal fungi, leaf thickness, photosynthetic capability, salinity-tolerance, salt spray



1 Introduction

Coastal areas comprise approximately 60% of the human population and host two-thirds of economically developed

cities in the world (Cai et al., 2008). Within these coastal habitats, salt spray (SP) is an important natural disturbance (Johnson et al., 2015; Luo, 2016; Du and Hesp, 2020; Toscano et al., 2020). SP originates from the bursting of bubbles in breaking waves, forming tiny salt droplets, which are then carried by wind, intercepted by coastal soil and plants, and subsequently redistributed in the soil after

precipitation (Hesp, 2002; Grythe et al., 2014). SP can cause water stress, reduce photosynthetic capabilities, disrupt enzyme systems, inhibit nutrient acquisition, and, in some cases, result in the death of plants (Kouali et al., 2017; Rajaniemi and Barrett, 2018; Du and Hesp, 2020; Itoh, 2021). Plant aerial parts, such as the leaves, are particularly susceptible as they are the primary route for salt to enter the plants (Tezara et al., 2003). These damages will retard aboveground growth and selectively inhibit the growth and survival of some salt-intolerant plants, thereby changing the vegetation zonation in coastal areas (Griffiths, 2006; Du and Hesp, 2020; Toscano et al., 2022). In China, especially in the southeastern region, SP has become a major concern in the socio-economic development of coastal areas (Cai et al., 2008; Luo, 2016). To mitigate the unfavorable effects of SP, researchers have explored various technologies involving structural and non-structural measures (Cai et al., 2008; Mastrocicco et al., 2013; Sintim and Flury, 2017), most of which are successful but beyond the economic means of developing nations (Cantrell and Linderman, 2000). Therefore, biotechnological, cost-effective, and environmentally friendly approaches enabling plants to withstand SP would be a good alternative in coastal areas.

Arbuscular mycorrhizal (AM) fungi (AMF) are one of the most important soil microorganisms building mutualistic symbiosis with the majority of terrestrial plants (Smith and Read, 2008; Brundrett and Tedersoo, 2018; Genre et al., 2020; Wang et al., 2022). AMF naturally grow in saline soils and can serve as bio-ameliorators for plants in exploring saline soils through some physiological processes including the protection of photosynthetic apparatus, which in turn enhances photosynthetic efficiency, stimulates nutrient uptake, and contributes to improved biomass accumulation (Chandrasekaran et al., 2014; Cotton, 2018; Evelin et al., 2019; Dastogeer et al., 2020). Glomalin and glomalin-related soil protein have also been mentioned as mechanisms employed by AMF to counter salinity stress owing to their correlations with soil aggregation (Rillig and Mummey, 2006; Holátko et al., 2020). Additionally, plants in coastal areas are often subjected to SP along with soil salinity, but the mechanisms underlying the tolerance to the different types of salinity appear independent (de Vos et al., 2010; Bian and Pan, 2018). Moreover, previous studies were mostly focused on soil salinity, and less attention was paid on SP tolerance in mycorrhizal plants, particularly in woody plants (Du and Hesp, 2020; Toscano et al., 2022). Consequently, the mycorrhizal efficiencies on the leaf trait and growth of trees under SP conditions remain unelucidated.

With the recent aggravation of coastal erosion in the coastal areas of southern China, resistance to SP is a preferred trait in plants (Du and Hesp, 2020). *Cinnamomum camphora* (L.) Presl. (the camphor tree), one of the economically important evergreen broadleaf trees, is widely

distributed in southern China along the Yangtze River (Zhou et al., 2021) and is moderately adapted to soil salinity (Wang et al., 2016). Further, it is highly dependent on AMF, with root colonization up to 81.85% (Yan and Zhong, 2007). However, the protective effects of AMF on the plants under SP stress are less known (Xue et al., 2023). Given that AMF can enhance plant performance under saline conditions (Chandrasekaran et al., 2014; Evelin et al., 2019), we hypothesized that AMF inoculation may mitigate the adverse effects of SP as well on *C. camphora* plants. Furthermore, various AMF may modulate plant response to environmental stress differently, and the mycorrhizal efficiencies could depend on specific plant-AMF combinations and different saline conditions (Johnson et al., 1997; Evelin et al., 2019). Therefore, we also hypothesized that the resistance to SP, as conferred by AMF, might be influenced by the intensity of SP stress and the specific fungal isolates used. In light of these hypotheses, a greenhouse experiment was designed to assess the impact of various AMF on the growth, leaf functional traits, and glomalin concentrations of *C. camphora* seedlings exposed to simulated SP conditions. This study aimed to shed light on the mechanism underlying the alleviation of SP stress through mycorrhizal association and offer valuable insights for cultivating and managing *C. camphora* plants in coastal regions.

2 Materials and methods

2.1 Plant materials, soil, and fungal inocula preparation

In December 2020, seeds of *C. camphora* were collected in the research station of Zhejiang A&F University (30°14' N, 119°42' E), located near Hangzhou Bay in the East Sea of southeastern China (Wang et al., 2022). On February 22, 2021, the seeds were surface sterilized with 70% ethanol for 1 min, followed by 2.625% NaClO for 3 min and 70% ethanol for another 1 min, and washed with distilled water for 1 min (Liang et al., 2015). Subsequently, seeds were germinated in plastic trays filled with autoclaved sterilized sand (Wang et al., 2018). After 2 months, the germinated seedlings with similar size (approximately 4 cm in height with three leaves per plant) were transplanted in plastic pots (16.5 cm × 18 cm × 12 cm) with one seedling per pot. One week following transplantation, any seedlings that had died or were growing poorly were replaced with new ones (Liang et al., 2015). The substrates used in the experiment were obtained from the topsoil (0 – 10 cm) beneath a common understory at the research station. These substrates were thoroughly mixed and sterilized using gamma irradiation (25 kGy, 60 Co γ -rays) at Zhejiang Jiexiang Irradiation Technology Co., Ltd. These sterilization process was performed to eliminate indigenous soil biota before their

use in the experiment (Namara et al., 2003). Each pot was filled with 2 kg of sterilized soil substrate, as in a previous study (Wang et al., 2022). The soil properties were as follows: organic matter, 22.31 mg g⁻¹; available nitrogen, 0.083 mg g⁻¹; potassium (K⁺), 2.42 mg g⁻¹; sodium (Na⁺), 0.16 mg g⁻¹; calcium (Ca²⁺), 1.21 mg g⁻¹; magnesium (Mg²⁺), 1.62 mg g⁻¹; copper (Cu²⁺), 0.008 mg g⁻¹; zinc (Zn²⁺), 0.048 mg g⁻¹; iron (Fe²⁺), 14.77 mg g⁻¹; manganese (Mn²⁺), 0.297 mg g⁻¹; and pH, 5.83 (soil: water, 1:5).

Two mycorrhizal fungal species that were prevalent in stressful environments were selected as inocula: *Glomus tortuosum* N. C. Schenck & G. S. Smith (BGC NM05A) (Cabello, 2001) and *Funneliformis mosseae* (T. H. Nicolson & Gerd.) C. Walker & A. Schüßler (BGC XJ02) (Wang et al., 2018). Both were provided by the Bank of Glomeromycota in Beijing of China (BGC) and propagated individually with *Sorghum bicolor* for 5 months in pots filled with autoclaved fine sand as the substrate (Wang et al., 2022). Therefore, the AMF inocula comprised the spores, hyphae, sand, and colonized root fragments of host plants (Xia et al., 2021).

2.2 Experimental design

A 4 × 3 completely factorial design experiment was conducted involving four AMF inoculations (inoculated with sterilized fungal inoculum, *G. tortuosum*, *F. mosseae*, or a combination of these two AMF) and three SP regimes (0, 7, or 14 mg NaCl cm⁻² d⁻¹). This resulted in 12 treatment combinations, each with five replicates. During seedling transplantation, 40 g of a single fungal species (either *G. tortuosum* or *F. mosseae*) or 40 g of combined inoculum, which consisted of an equal ratio of the two AMF, was added at a depth of 10 cm below the substrate for each corresponding mycorrhizal plant (AM plant). The non-mycorrhizal plant (NM plant) received the same amount of sterilized combined-fungi inoculum, along with 40 mL of filtrate made from the combined-fungi inoculum to minimise the differences in other microbial communities (Wu et al., 2016).

To avoid the shock effects of SP on the development of AMF and fine roots, SP treatment was initiated on May 22 2021, nearly one month after transplantation. During the treatment, we set the regimes of SP as 7 and 14 mg NaCl cm⁻² d⁻¹, corresponding to low (LS) and high SP (HS) treatments, respectively. This setting was referenced from the reports that the salinity of seawater around the East Sea ranged from 10.32 mg NaCl cm⁻² d⁻¹ to 13.46 mg NaCl cm⁻² d⁻¹ (Xu and Kang, 2019), and the component of SP that was exposed to the plants grown in coastal areas was similar to that of seawater (de Vos et al., 2010). To closely simulate the actual situation in coastal areas while considering the pot size, each potted seedling was subjected to daily spraying with 100 mL of the corresponding NaCl solutions

between 16:00 and 18:00. SP was applied as a fine mist using a hand-held herbicide applicator. The control plants were treated similarly but with distilled water instead (Tezara et al., 2003). The pots were randomized once a week to avoid any possible impacts induced by environmental factors in the greenhouse. No additional fertilizer was added during the experiment, and plants were watered when needed. Additionally, all the seedlings were grown at a relative humidity of 70%, mean temperature of 29°C, and midday photosynthetic photon flux density of approximately 1200 μmol m⁻² s⁻¹. On November 22, 2021, the plants were harvested after more than 6 months of growth.

2.3 Determination of leaf photosynthetic traits, leaf chlorophyll fluorescence, and photosynthetic pigment

Before harvesting, photosynthetic traits (leaf net photosynthetic rate, P_n ; stomatal conductance, g_s ; intercellular CO₂ concentration, C_i ; transpiration rate, T_r) were measured between 9:00 and 11:00 am using a Li-6400 portable photosynthesis system (LI-COR, Inc., Lincoln, USA). The ambient CO₂ concentration, air flow rate, relative humidity of the leaf chamber, and leaf temperature were set as described by Wang et al. (2021). The instantaneous water use efficiency (WUE) was calculated using the equation P_n/T_r .

Subsequently, the chlorophyll fluorescence parameters were determined with the same leaves used for the photosynthetic measurements using a PAM-2500 portable chlorophyll fluorometer (Walz, Effeltrich, Germany). Before determination, the leaves were adapted to dark for 30 min; the minimum (F_0) and maximum fluorescence (F_m) were measured via a saturating pulse of approximately 5000 μmol m⁻² s⁻¹. The leaves were then exposed to 800 μmol m⁻² s⁻¹ light. The data obtained were used to calculate the maximum quantum yield of PSII (F_v/F_m), photochemical quenching (qP), non-photochemical quenching (qN), and actual photosynthetic quantum ($Yield$) (Wang et al., 2019).

Finally, the total chlorophyll (Total Chl) of the corresponding leaves was extracted using 80% acetone, and the absorbance of the extracts was determined using a spectrophotometer (AA7000, Shimadzu, Japan) (Lichtenthaler, 1987).

2.4 Determination of leaf morphological parameters and plant biomass

During harvest, all the plants were carefully removed from the soil, rinsed with distilled water, and then separated into shoots and roots to determine their fresh biomass.

To examine the leaf anatomical structure, three healthy and mature leaves from each treatment combination were

selected and preserved in a formalin-aceto-alcohol (FAA) solution (Guo et al., 2008). The anatomical structures of all the leaves were processed with conventional paraffin sectioning method (Wu et al., 2022). Briefly, leaf specimens underwent desilicization using hydrogen fluoride, followed by dehydrated using a series of graded alcohol solutions. Subsequently, they were sliced into 7µm-thick sections using a rotary microtome (SYD-S3050, Shenyang, China). These sections were then stained with safranin and fast green and observed using a microscope (Leica DM2500, Heidelberg, Germany). The leaf, palisade tissue, and upper epidermis thicknesses were investigated using Qwin V3 software (Leica Microsystems Ltd., Mannheim, Baden-Württemberg, Germany). Additionally, the cell tenses ratio was calculated using the equation as the palisade tissue/leaf thickness.

Furthermore, three plants were chosen from each treatment group, and their roots were divided into four subsamples, three of which were left undisturbed to determine mycorrhizal colonization and fungal enzyme activities. The total dry weight of the remaining roots and shoots were recorded by oven-drying at 60°C for 72 h.

2.5 Determination of mycorrhizal colonization, fungal enzyme activities, and glomalin concentration

One of the selected undisturbed root subsamples was used to determine fungal colonization after clearing and trypan blue staining (Wang et al., 2018). These stained root subsamples were microscopically examined for mycorrhizal colonization using the gridline intersect method (Gosling et al., 2016). The other two root subsamples were used to measure the activities of fungal hyphal enzymes, namely succinate dehydrogenase (SDH) (Zhao et al., 1997) and alkaline phosphatase (ALP) (Tisserant and Gianinazzi, 1993; Wang et al., 2018).

The concentrations of total glomalin (T-GRSP) and easily extractable glomalin (EE-GRSP) were determined using the sodium citrate solution method previously described (Wright and Upadhyaya, 1996). Briefly, for EE-GRSP, 1 g of soil sample was incubated with 8 mL of 20 mM citrate solution (pH 7) and then autoclaved at 121°C for 30 min. T-GRSP was extracted from the remaining residue using 8 mL of 50 mM citrate solution (pH 8) and autoclaved at 121°C for 60 min. Bovine serum albumin served as the standard during this determination (Wu et al., 2015).

2.6 Data analysis

To quantify the mycorrhizal efficiencies, the mycorrhizal growth response (MGR) was calculated using the following equation (Johnson et al., 2015):

$$\text{MGR (\%)} = \log_e \left(\frac{DW_{AMF}}{\text{Avg}(DW_{non-AMF})} \right) \times 100$$

where DW_{AMF} and $\text{Avg}(DW_{non-AMF})$ represent the actual value of biomass for each AM plant and the average biomass of NM plants under identical saline levels, respectively.

Salinity tolerance (ST) of the plants was calculated using the following equation (Wang et al., 2018):

$$\text{ST (\%)} = \frac{DW_{ss} - \text{Avg}(DW_{ck})}{\text{Avg}(DW_{ck})} \times 100$$

where DW_{SS} and $\text{Avg}(DW_{ck})$ represent the dry weights of SP-stressed plants and the average dry weights of the controls, respectively.

The data were analyzed using a two-way analysis of variance to assess the effects of SP, AMF, and their interactions. Before conducting the analysis, all data were examined for equality of variance using Levene's tests to check, and for normality using the Shapiro-Wilk. In case where the interactive effects of SP and AMF were found to be significant ($p < 0.05$), Student-Newman-Keul (SNK) tests were employed to compare differences in plant traits among the various treatment combinations. Pearson's correlation analysis was conducted to examine pairwise trait relationships between all parameters of *C. camphora* plants across the 12 treatment combinations. Linear regression was performed to determine the relationships between MGR (or ST) and related parameters. The statistical analyses were carried out using R 4.0.2 (R Core Team, 2020).

3 Results

3.1 Mycorrhizal colonization and hyphal enzyme activity

In this study, no roots of NM plants were colonized by AMF; however, it successfully colonized the roots of AM plants with mycorrhizal colonization ranging from 28% to 58% (Fig. 1A). The presence of SP significantly promoted the root colonization of both *F. mosseae*-inoculated and the combined fungi-inoculated plants with exception of *G. tortuosum*-inoculated plants. Regardless of the plants grown in non-SP conditions, the mycorrhizal colonization of AM plants exposed to SP varied according to AMF isolates. SP also had notable impacts on the ALP activities of *F. mosseae*-inoculated and combined fungi-inoculated plants but not on that of *G. tortuosum*-inoculated plants (Fig. 1B). AMF significantly impacted the ALP activity of AM plants, with the highest value observed in combined fungi-inoculated plants grown under high-SP conditions. SP did not affect the SDH activity of AM plants (Fig. 1C). Irrespective of the plants grown under low-SP conditions, AMF had significant effects on the SDH activity of AM plants, and the mycorrhizal

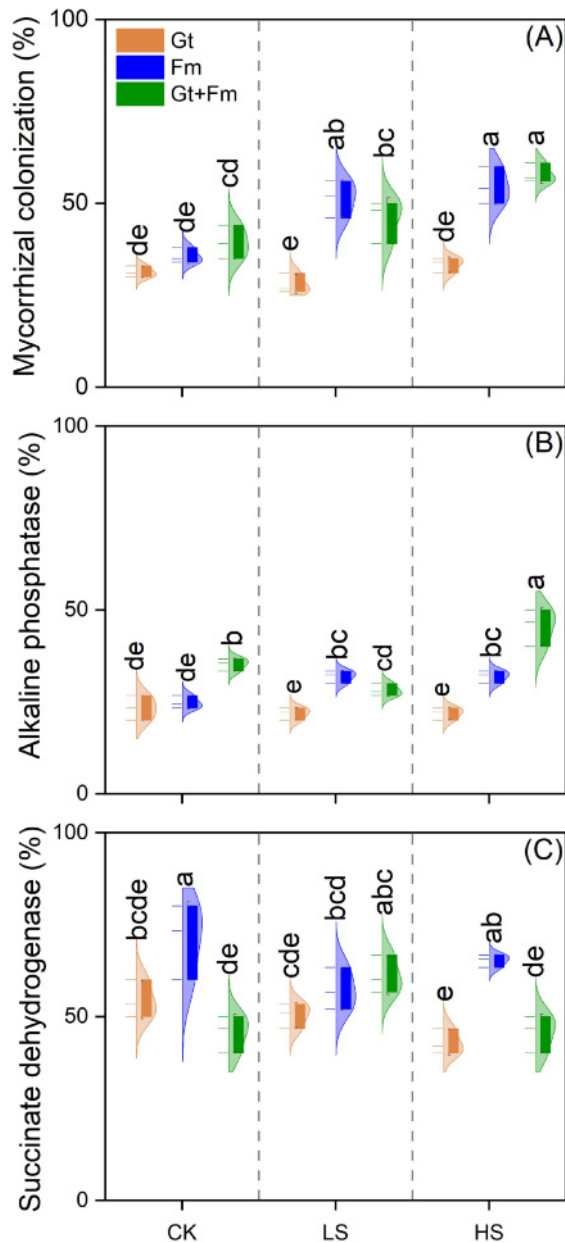


Fig. 1 Mycorrhizal colonization (A), alkaline phosphatase (B) and succinate dehydrogenase (C) of *Cinnamomum camphora* plants grown at non-salt spray (CK), low-salt spray (LS), and high-salt spray (HS) conditions. Gt, Fm, and Gt + Fm represent three mycorrhizal treatments: inoculation with *Glomus tortuosum*, *Funneliformis mosseae*, or their combination, respectively. Values are presented as the mean \pm SE ($n = 3$). Different lowercase letters indicate a significant difference according to SNK at $p < 0.05$.

efficiencies varied according to AMF isolates. Notably, there were significant interactions between SP and AMF in terms of mycorrhizal colonization and ALP and SDH activities (Table S1).

3.2 Leaf anatomical structure

The leaf thickness of NM plants exhibited an initial increase

followed by a decrease with increasing SP concentrations. Mycorrhizal inoculation, particularly with combined fungi, led to a significant 7.6% increase in leaf thickness compared to in NM plants under the harshest SP conditions (Fig. 2A). Both SP and AMF had variable effects on palisade tissue thickness (Fig. 2B). Neither SP nor AMF had an impact on the upper epidermis thickness of the plants (Fig. 2C). SP did not affect the cell tense ratio of plants regardless of AMF inoculation type (Fig. 2D). However, AMF significantly influenced the cell tense ratios of plants, with variations observed among different AMF isolates and SP levels. Notably, there were significant interactions between SP and AMF regarding the thicknesses of leaf, palisade tissue, and upper epidermis (Table S1).

3.3 Leaf photosynthetic traits and chlorophyll fluorescence

SP greatly reduced total *Chl* concentrations of plants (Fig. 3A). AMF significantly increased the total *Chl* concentrations of plants grown in non- and high-SP conditions, with the exception of the *G. tortuosum*-inoculated and combined-fungi inoculated plants grown under lower-SP conditions. SP decreased the P_n of AM plants (Fig. 3B). AMF significantly increased the P_n of plants grown in non-SP conditions; under low-SP conditions, *F. mosseae* conferred more benefits on P_n than did the other inocula, and under high-SP conditions, *F. mosseae* and combined-fungi performed the best with respect to P_n . SP significantly decreased the C_i of NM plants but did not affect those of AM plants (Fig. 3C). AMF did not significantly influence the C_i of plants at any SP level with the exception of combined-fungi inoculated plants grown under non-SP conditions. SP did not affect T_r of plants (Fig. 3D), whereas it had variable effects on the g_s of plants (Fig. 3E). AMF differently affected T_r and g_s with increasing SP levels, and under high-SP conditions, *F. mosseae* and combined-fungi significantly increased T_r by 107% and 122%, respectively, and increased g_s by 117% and 122%, respectively, compared with those in the corresponding NM plants grown under identical SP conditions. SP negatively affected the *WUE* of AM plants (Fig. 3F). AMF significantly enhanced the *WUE* of plants grown under non-SP conditions. Under low-SP conditions, only inoculation with *F. mosseae* promoted the *WUE* of plants. However, when the plants were grown under high-SP conditions, mycorrhizal inoculation did not have any effect on the *WUE* of plants. Significant interactions between SP and AMF were found in the photosynthetic traits (Table S1).

SP conferred no effects on *Fv/Fm* of NM plants, *F. mosseae*-inoculated plants, or combined fungi-inoculated plants but inhibited the *Fv/Fm* of *G. tortuosum*-inoculated plants (Fig. 4A). AMF did not affect the *Fv/Fm* of plants grown under non-SP conditions, and the mycorrhizal effects varied with SP levels. With increasing SP, the qP of

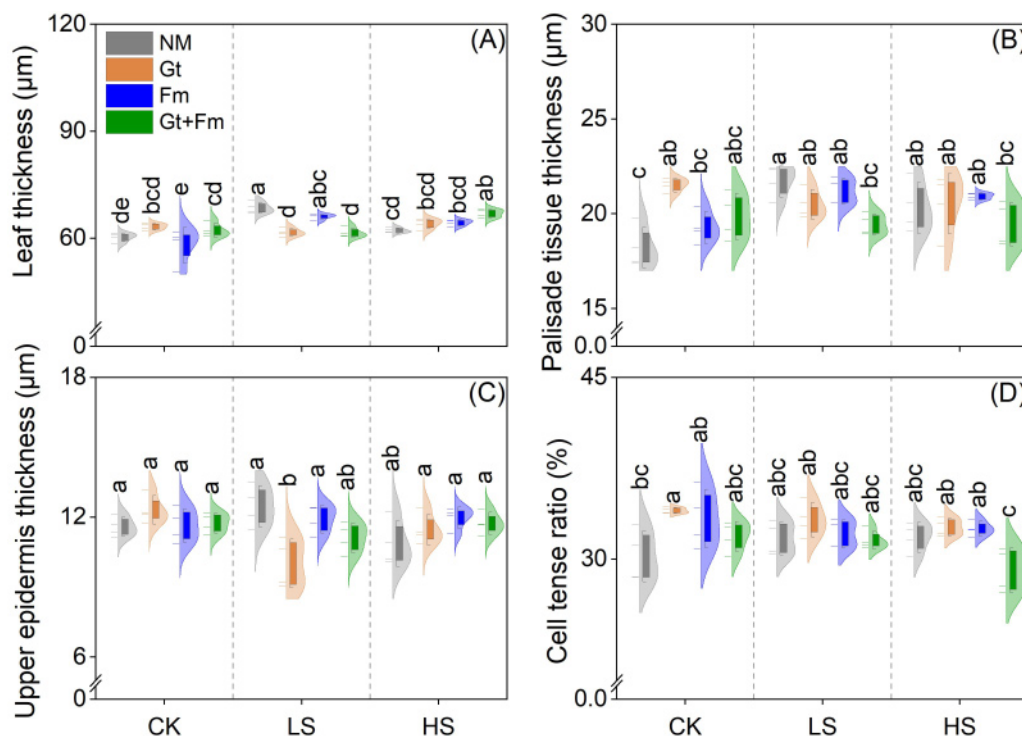


Fig. 2 Effect of arbuscular mycorrhizal fungi on leaf thickness (A), palisade tissue thickness (B), upper epidermis thickness (C), cell turgor ratio (D) of *Cinnamomum camphora* plants grown at non-salt spray (CK), low-salt spray (LS), and high-salt spray (HS) conditions. NM, Gt, Fm and Gt + Fm represent the four mycorrhizal treatments: inoculation with sterilized mycorrhizal fungi, *Glomus tortuosum*, *Funneliformis mosseae*, or their combination, respectively. Values are expressed as the mean \pm SE ($n = 4$). Different lower-case letters indicate a significant difference according to SNK at $p < 0.05$.

G. tortuosum-inoculated plants and combined fungi-inoculated plants increased (Fig. 4B). Mycorrhizal efficiencies on qP varied according to AMF isolates. SP did not affect the qN of NM plants or combined fungi-inoculated plants (Fig. 4C). By contrast, it promoted qN of *G. tortuosum*- and *F. mosseae*-inoculated plants. AMF did not affect the qN of plants grown under the harshest SP, and only the qN values of combined fungi-inoculated plants were significantly higher than those of their counterparts grown under both non- and low-SP conditions. SP decreased the *Yield* of plants (Fig. 4D). AMF had no benefits on the *Yield* of plants at all SP levels. Significant interactions between SP and AMF were observed in terms of Fv/Fm , qP , and qN (Table S1).

3.4 Total and easily extractable glomalin

The values of EE-GRSP and T-GRSP significantly increased with increasing SP levels, except for *G. tortuosum*-inoculated plants (Figs. S1A and S1B). AMF inoculation had a substantial positive impact on both EE-GRSP and T-GRSP across all SP levels, with the highest values observed in combined fungi-inoculated plants grown under the harshest SP conditions. Significant interactions between SP and AMF were found in terms of EE-GRSP and T-GRSP (Table S1).

3.5 Biomass accumulation and allocation

SP significantly decreased the total dry weights of NM plants (Fig. 5A). AMF inoculation greatly improved the total dry weights of plants grown under non-SP conditions; under low-SP condition, only *F. mosseae* inoculation conferred benefits on total dry weight; under high-SP condition, the benefits were posed by both *F. mosseae* and combined fungi. SP did not affect the root:shoot ratio of NM plants but had variable effects on those of AM plants (Fig. 5B). AMF had variable effects on the root:shoot ratio. Significant interactions between SP and AMF were found in terms of total dry weight and root:shoot ratio (Table S1).

3.6 Mycorrhizal efficiency, salinity tolerance, and their relationships with other plant variables

SP significantly decreased the ST of NM plants (Fig. S2A). Under low SP, regardless of *F. mosseae*, AMF inoculation significantly decreased the ST of plants; under high-SP conditions, only inoculation with combined fungi significantly promoted the ST of plants. With the increase of SP, MGR first decreased and then increased (Fig. S2B). AMF had variable effects on MGR, and the mycorrhizal effects varied within fungal species. Under non- and low-SP conditions, the highest value of MGR was observed in

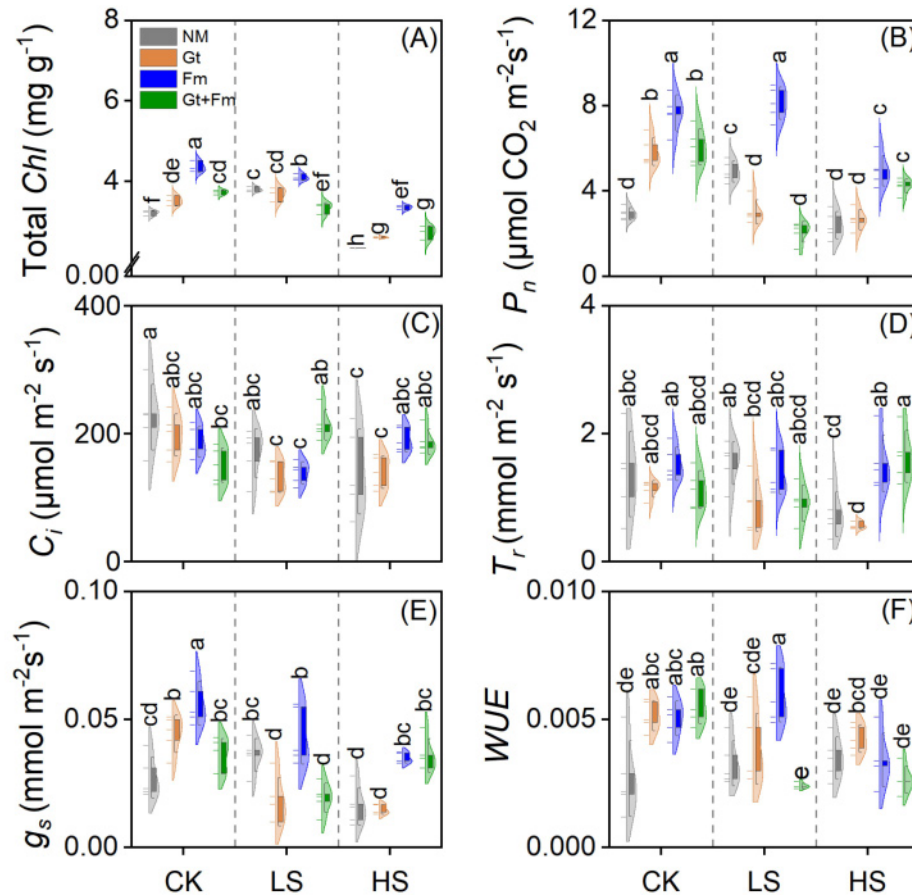


Fig. 3 Effect of arbuscular mycorrhizal fungi on total *Chl* (A), net photosynthetic rate (P_n) (B), intercellular CO_2 concentration (C_i) (C), transpiration rate (T_r) (D), stomatal conductance (g_s) (E), instantaneous water use efficiency (WUE) (F) of *Cinnamomum camphora* plants grown at non-salt spray (CK), low-salt spray (LS), and high-salt spray (HS) conditions. NM, Gt, Fm and Gt + Fm represent the four mycorrhizal treatments: inoculation with sterilized mycorrhizal fungi, *Glomus tortuosum*, *Funneliformis mosseae*, or their combination, respectively. Values are expressed as the mean \pm SE ($n = 3$). Different lower-case letters indicate a significant difference according to SNK at $p < 0.05$.

F. mosseae-inoculated plants; under high-SP conditions, the highest value was found in combined fungi-inoculated plants.

The data analysis showed that MGR was positively correlated with ALP, SDH, T_r , WUE , and upper epidermis thickness but negatively correlated with total *Chl* (Figs. 6A and S3). Thereafter, we built the following model to quantify their relationships ($R^2 = 0.7178$, $p < 0.001$):

$$\text{MGR} = 0.7592X_1 + 0.7448X_2 + 0.3797X_3 + 0.2862X_4 + 0.2526X_5 - 0.3255X_6$$

X_1 – X_6 represent the values of ALP activity, SDH activity, T_r , WUE , upper epidermis thickness, and Total *Chl*. The coefficients in the model represent the correlation indexes between the parameters and MGR.

Meanwhile, ALP activity, SDH activity, T_r , Total *Chl*, WUE , and upper epidermis thickness were responsible for 27.6%, 27.1%, 13.8%, 11.8%, 10.4%, and 9.2% of the variance in MGR, respectively, which accounted for 99.9% of the contribution to MGR (Fig. 6B).

Furthermore, ST was positively correlated with leaf

thickness and qP but negatively correlated with root:shoot ratio, cell tense ratio and WUE (Figs. 6C and S3). Additionally, we constructed a model to quantify their relationships as follows ($R^2 = 0.6765$, $p < 0.001$):

$$\text{ST} = -0.2273X_1 - 0.379X_2 - 0.169X_3 + 0.489X_4 + 0.292X_5$$

where X_1 – X_5 represent the cell tense ratio, root:shoot ratio, WUE , leaf thickness, and qP , respectively. The coefficients in the model represent the correlation indexes between the parameters and ST.

Moreover, leaf thickness, root:shoot ratio, qP , cell tense ratio, and WUE were responsible for 31.4%, 24.4%, 18.8%, 14.6%, and 10.9% of the variance in ST, respectively, accounting for the whole contribution to ST (Fig. 6D).

4 Discussion

In this study, SP had negative impacts on the total chlorophyll content, photosynthetic capability, and total dry weight of NM plants, as well as their abilities to withstand aerial

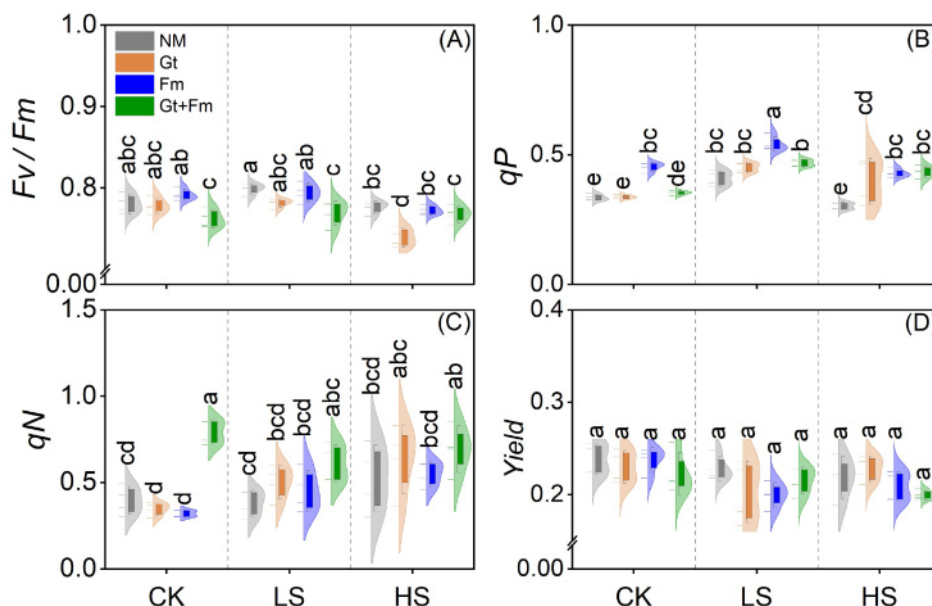


Fig. 4 Effect of arbuscular mycorrhizal fungi on maximum photosynthetic quantum (Fv/Fm) (A), photochemical quenching (qP) (B), non-photochemical quenching (qN) (C), actual photosynthetic quantum (Yield) (D) of *Cinnamomum camphora* plants grown at non-salt spray (CK), low-salt spray (LS), and high-salt spray (HS) conditions. NM, Gt, Fm and Gt + Fm represent the four mycorrhizal treatments: inoculation with sterilized mycorrhizal fungi, *Glomus tortuosum*, *Funneliformis mosseae*, or their combination, respectively. Values are expressed as the mean \pm SE ($n = 3$). Different lower-case letters indicate a significant difference according to SNK at $p < 0.05$.

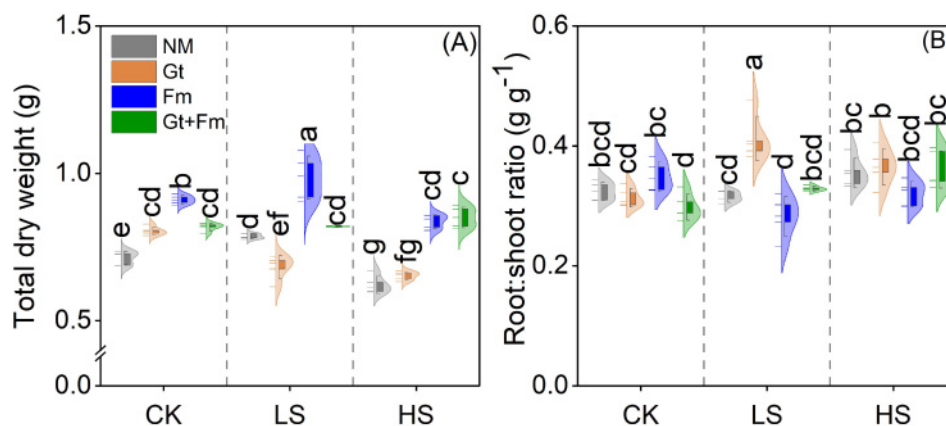


Fig. 5 Effect of arbuscular mycorrhizal fungi on total dry weight (A) and root:shoot ratio (B) of *Cinnamomum camphora* plants grown at non-salt spray (CK), low-salt spray (LS), and high-salt spray (HS) conditions. NM, Gt, Fm and Gt + Fm represent the four mycorrhizal treatments: inoculation with sterilized mycorrhizal fungi, *Glomus tortuosum*, *Funneliformis mosseae*, or their combination, respectively. Values are expressed as the mean \pm SE ($n = 5$). Different lower-case letters indicate a significant difference according to SNK at $p < 0.05$.

salinity. However, mycorrhizal inoculation partially alleviated the unfavorable effects induced by SP on *C. camphora* plants, confirming our first hypothesis that AMF can mitigate the detrimental effects conferred by SP. Moreover, the mycorrhizal efficiencies differed within fungal isolates, with the combination of *G. tortuosum* and *F. mosseae* showing the greatest improvement in salinity resistance. These benefits were more pronounced under high-SP conditions, supporting our second hypothesis that mycorrhizal efficiency depended on the intensity of SP and the AMF isolates used. Most importantly, our results suggest that the leaf thickness

and qP contributes greatly to the SP-resistance of *C. camphora* plants with mycorrhizal symbiosis.

4.1 Mycorrhizal efficiencies on plant growth

SP noticeably reduced the biomass of *C. camphora* plants without mycorrhizal inoculation (Fig. 5), consistent with the results of some previous reports (Benes et al., 1996; Kekere, 2014; Toscano et al., 2022) but contrary to those of a few others (Griffiths and Orians, 2003; de Vos et al., 2010). This controversy can be owing to the differences in the sensitivities

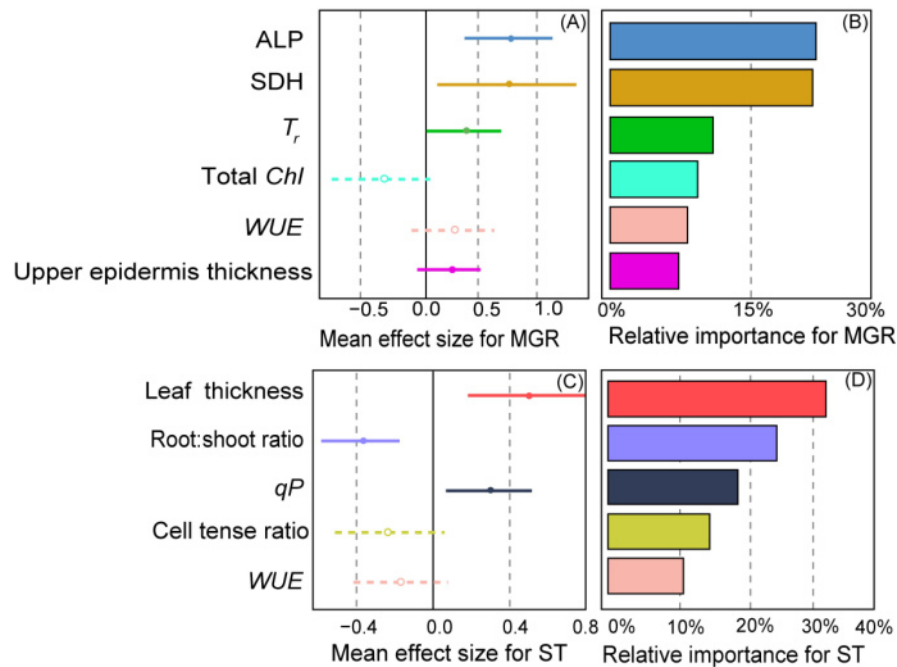


Fig. 6 Mean effect sizes of observed variables on mycorrhizal growth response (MGR) (A) and salinity tolerance (ST) (C) and their relative contributions to MGR (B) and ST (D) in *Cinnamomum camphora* plants. Solid lines with filled circles demonstrate significant effects ($p < 0.05$), and the dotted lines with hollowed circles represent non-significant effects ($p > 0.05$).

of plant species and intensities of SP (Kekere, 2014). Furthermore, SP-induced ionic effects on salt-specific response of biomass can be responsible for the various influences of airborne salinity, which has been suggested in the biphasic model (Munns, 1993). Similarly, soil salinity could also pose unfavorable effects on plant growth (Munns and Tester, 2008; van Zelm et al., 2020; Zhao et al., 2020; Hu and Schmidhalter, 2023). Mycorrhizal inoculation, to some extent, evidently enhanced plant growth under high-SP conditions (Fig. 5A), indicating that AMF can mitigate the detrimental effects of SP on plant growth. This aligns with the suggestion made by Maze and Whalley (1992), who proposed that the reduction in plant growth under SP stress primarily stems from nutrient absorption inhibition. Remarkably, when subjected to salinity, most AM plants can acquire more nutrients than NM plants, resulting in increased biomass in AM plants (Chandrasekaran et al., 2014). However, the present data showed that the enhancement of plant biomass was ascribed to higher mycorrhizal colonization, hyphal enzyme activities (ALP and SDH), and P_n (Fig. S3). Plants are often more sensitive to SP than to soilborne salinity, which may be because SP can simultaneously influence the plant aerial parts and soil salinity (Benes et al., 1996). Therefore, compared to their responses to soilborne salinity, plants may employ different strategies against aerial salinity. This difference has been observed as SP conferred no effects on biomass allocation between the shoot and root (Fig. 5B), whereas soil salinity often induces more biomass allocated to plant root than shoot (Chandrasekaran et al.,

2014; Evelin et al., 2019). Another potential explanation for growth enhancement is that mycorrhizal colonization protects the host plant from disease by improving immune system of the plant (Romero et al., 2023).

4.2 Mycorrhizal efficiencies on leaf traits

Leaves, as the main pathway for aerial salinity to enter the plants, play important roles in resistance to SP (Tezara et al., 2003; de Vos et al., 2010). Notably, the responses found in the leaf tissues of plants suffering from SP are indicators of plant growth in coastal areas (Du and Hesp, 2020). In this experiment, as the intensity of SP increased, leaf thickness initially increased under low-SP conditions and then decreased under high-SP conditions (Fig. 2A). This pattern suggests that plants may exhibit adaptability to low-SP conditions. It is worth noting that salt-tolerant plants often respond to aerial salinity by developing thicker leaves, a phenomenon known as leaf hypertrophy or cell enlargement. This response can lead to increased leaf thickness, as observed in *Scaevola sericea* seedlings, particularly on their windward-facing leaves, when grown in coastal areas (Alpha et al., 1996). This leaf thickness response probably reduced chloride toxicity and improved osmotically active solutes in leaves, resulting in a dilution effect in leaves (Alpha et al., 1996; Albaladejo et al., 2017).

Interestingly, no leaf necrosis was found in our study, probably because the plant was grown in a greenhouse, a protective environment different from the natural field.

Consequently, the leaves had few stomata to reduce the entry points for SP (Kekere, 2014). Mycorrhizal inoculation, especially of the combined fungi, evidently improved the leaf thickness compared to that of the NM plants at high SP, demonstrating that fungal colonization can improve plant adaptation at high SP.

Growth could also be inhibited by salinity damage to photosynthetic machinery, resulting in the inhibition of photosynthetic capabilities (Zhu, 2001). In this study, SP significantly decreased the chlorophyll concentrations of leaves (Fig. 3A), which may be due to K^+ deficiency and the toxicities of Na^+ or Cl^- from sprayed salt (Debez et al., 2004; Kouali et al., 2017). Although low SP had negligible effects on P_n (Fig. 3B), AMF differently increased chlorophyll content and P_n at all SP levels, which is consistent with the results of a previous study that photosynthetic capabilities are often higher in mycorrhizal plants than in their counterparts (Augé et al., 2016). Meanwhile, in our study, the change in P_n was synchronised with g_s but not with C_i after mycorrhizal inoculation. This suggests that the photosynthetic rate was co-limited by both stomatal and non-stomatal factors (Wang et al., 2019). Thus, enhancing chlorophyll contents and photosynthetic efficiency with mycorrhizal inoculation can promote biomass increment.

As for the chlorophyll fluorescence, in this experiment, the Fv/Fm and qP values of NM plants were unaffected by SP (Figs. 4A and 4B), demonstrating the insensitivity of the PSII system to this stress. Similar responses have been reported in *Lycium nodosum* (Tezara et al., 2003). Notably, qP , the reoxidation of the primary acceptor (the QA pool), was associated with the electron transporting activities of PSII (Scheuermann et al., 1991). AMF species differently improved qP at all SP levels, indicating that photorespiration was not suppressed under SP stress.

4.3 Underlying mechanisms for plant resistance to salt spray

For plants grown in salinity-stressed environments, three interconnected strategies are important to achieve salt tolerance: ① prevent or alleviate the salinity damage; ② re-build ionic homeostasis under the new, stressful environment; and ③ resume plant growth (Zhu, 2001). The present data somehow corroborated the implementation of these strategies. Mycorrhizal inoculation, especially with the combined fungi, significantly improved plant growth and salinity tolerance under high-SP conditions, primarily through the enhancement of leaf traits involving leaf thickness and photosynthetic capabilities. However, other researchers argued that plant resistance to salt spray is primarily attributed to improved nutrient acquisition (Maze and Whalley, 1992; Xue et al., 2023) or evoking biochemical changes (Toscano et al., 2022).

Several mechanisms may be responsible for the

mycorrhizal-mediated alleviation of salinity involving the increase of nutrient absorption, maintenance of ionic homeostasis, and stimulation of biochemical, physiologic, molecular, and ultra-structural changes (Chandrasekaran et al., 2014; Evelin et al., 2019). Given that the salinity caused by sprayed salt differs from soil salinity, the underlying mechanisms for plant resistance to salinity may also be disputed (Goldstein et al., 1996; Du and Hesp, 2020). Moreover, although mycorrhizal inoculation greatly improved T-GRSP and EE-GRSP (Fig. S1), the two dominant factors regulating soil aggregate stability (Qiao et al., 2016) were unrelated to salinity-tolerance. Therefore, we argue that further research involving more plant parameters is needed to completely understand how mycorrhizal symbiosis alleviates the detrimental effects induced by sprayed salt and optimize physiologic processes involved in salinity resistance (Azcón-Aguilar et al., 2009).

Furthermore, our results demonstrated that mycorrhizal efficiencies on *C. camphora* seedlings were primarily observed under high-SP conditions, aligning with the stress-gradient model that suggests that the strength of positive effects from the partners is enhanced by the magnitude of the stressful environment (Bertness and Callaway, 1994). Meanwhile, in this experiment, the combination of *F. mosseae* and *G. tortuosus* imparted better salinity tolerance than did a single fungus, possibly owing to functional complementarity among fungal species (Koide, 2000; Jansa et al., 2008).

Nevertheless, extrapolating the current findings to plants grown in the field must be exercised cautiously because of the following possible explanations: ① the NaCl solution applied in this experiment mimicked the impact of sprayed salt-induced by seawater; however, other ions in seawater can alter the nature of injury induced by this sprayed salt (Maun, 2009); ② heavy damages to plants are recorded in the greenhouse within a short period, which may not be the case in the coastal areas, where plants are exposed to continuous and long-term SP (Du and Hesp, 2020); ③ in the field, the seedlings are protected by adult or taller plants, and the population structure may also influence the response to SP (Griffiths and Orians, 2003); and ④ in the field, some biotic and abiotic factors usually co-occur with SP, which can alter the impacts of aerial salt on plant growth (Cheplick and White, 2002).

5 Conclusion

Plants in coastal areas are often exposed to SP, with the aerial parts of plants being more vulnerable than the underground parts. Our study revealed that mycorrhizal inoculation, especially with a combination of *F. mosseae* and *G. tortuosus*, conferred more benefits in alleviating the

detrimental effects of SP on plant growth and salinity resistance than that by either of the mycorrhizal fungus alone. These improvements in salinity resistance achieved from mycorrhizal inoculation were primarily attributed to changes in leaf functional traits. Furthermore, these mycorrhizal efficiencies depended on the intensity of SP and the specific fungal taxa used. It is worth noting that *C. camphora* seedlings were exposed to sprayed salt in the greenhouse, which was different from those grown in the field. Thus, further research under contrasting aerial salinity conditions and involving a broader range of fungal species is necessary to confirm that the plant leaf traits are linked to mycorrhizal efficiencies in coastal environments.

Author contributions

Y.H. Wang proposed and organized the experiment, wrote and reviewed the manuscript. X.P. Wang, N.L. Zhang, Y. Li, L.J. Dong, A.P. Wu, Q.Q. Wu, H. Liu, and M.S. Zhao interpreted the results and reviewed the final manuscript. X. Li and D. Zheng did the laboratory work, analyzed the data and prepared the figures. All the authors contributed to the final draft of the manuscript.

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Declaration of competing interest

The authors declare no competing interests.

Electronic supplementary material

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