Effects of AM Fungi on Photosynthetic and Chlorophyll Fluorescence Characteristics of Elaeagnus angustifolia Seedlings under Salt Stress (Postprint)

Authors: Jia Tingting, Chang Wei, Fan Xiaoxu, Song Fuqiang

Date: 2018-03-08T00:00:00+00:00

Abstract

To investigate the effects of arbuscular mycorrhizal (AM) fungi on the photosynthetic and physiological characteristics of seedlings under salt stress, a pot experiment was conducted using Elaeagnus angustifolia seedlings inoculated with the AM fungus Glomus intraradices (GI) and non-inoculated controls (CK), which were subjected to NaCl treatments at concentrations of 0, 100, 200, and 300 mmol/L. Indices including net photosynthetic rate (Pn), gas exchange parameters (transpiration rate Tr, stomatal conductance Gs, intercellular CO concentration Ci), pigment contents (chlorophyll a, b, total chlorophyll, carotenoids), and chlorophyll fluorescence parameters (maximum photochemical efficiency Fv/Fm, photosystem II efficiency ΦPSII, photochemical quenching coefficient qP, non-photochemical quenching coefficient NPQ, apparent electron transport rate ETR, potential activity of PSII reaction centers Fv/Fo, heat dissipation rate HDR) were measured in leaves of E. angustifolia seedlings under different treatments. The results demonstrated that: (1) With increasing salt concentration, the changing trends of Pn, Tr, Gs, and Ci in leaves of E. angustifolia seedlings under GI and CK treatments were fundamentally consistent, all decreasing significantly. However, at identical salt concentrations, these indices in GI-inoculated leaves were significantly higher than those in the CK treatment group ($P < 0.05$). Moreover, compared with the salt-free control treatment, the magnitude of change in these parameters was significantly lower in the GI group than in the CK group. (2) For pigment content parameters in leaves of E. angustifolia seedlings, the changing trends with increasing salt concentration were fundamentally consistent between GI-inoculated and CK groups, with all parameters either decreasing or increasing. However, compared with the salt-free control treatment, the magnitude of change in the CK treatment group was significantly greater than that in the GI treatment. (3) With increasing salt concentration across treatments, Fv/Fm, ΦPSII, qP, ETR, and Fv/Fo in the GI-inoculated treatment exhibited a trend of initial increase followed by subsequent decrease, while NPQ and HDR showed a trend of initial decrease followed by subsequent increase. In contrast, the CK treatment group exhibited a significant decreasing trend for these values, whereas NPQ and HDR displayed trends of initial decrease followed by increase as well as gradual increase. Compared with the salt-free control treatment, the magnitude of change in the GI treatment group was significantly lower than that in the CK group. These findings further reveal that AM fungi play an important role in saline habitats by enhancing plant photosynthetic and chlorophyll fluorescence characteristics, and that salt stress intensity is also a factor influencing this functional role of AM fungi. The symbiosis between halophytes and AM fungi holds promising application prospects for saline-alkali soil remediation.

Full Text

Preamble

Acta Ecologica Sinica, Volume 38, No. 4, February 2018
DOI: 10.5846/stxb201701030011

Effects of Arbuscular Mycorrhizal Fungi on Photosynthetic and Chlorophyll Fluorescence Characteristics in *Elaeagnus angustifolia* Seedlings under Salt Stress

JIA Tingting, CHANG Wei, FAN Xiaoxu, SONG Fuqiang*
College of Life Sciences, Heilongjiang University, Harbin 150080, China

Abstract

To elucidate the effects of arbuscular mycorrhizal (AM) fungi on the photosynthetic physiology of seedlings under salt stress, a pot experiment was conducted with *Elaeagnus angustifolia* seedlings inoculated with *Glomus intraradices* (GI) and non-inoculated controls (CK) subjected to NaCl concentrations of 0, 100, 200, and 300 mmol/L. Gas exchange parameters (net photosynthetic rate Pn, transpiration rate Tr, stomatal conductance Gs, intercellular CO concentration Ci), chlorophyll fluorescence parameters (maximum fluorescence efficiency Fv/Fm, actual PSII efficiency ΦPSII, photochemical quenching coefficient qP, non-photochemical quenching coefficient NPQ, apparent electron transfer rate ETR, PSII reaction center potential activity Fv/Fo, heat dissipation rate HDR), and pigment contents (chlorophyll a, chlorophyll b, carotenoids) were measured in leaf tissues.

The primary findings were threefold. First, Pn, Tr, Gs, and Ci decreased significantly with increasing salt concentration in both CK and GI treatments. However, at the same salt concentration, these parameters were significantly higher in GI-treated seedlings compared to CK ($P < 0.05$), and the magnitude of decline was smaller in GI treatments. Second, chlorophyll and carotenoid contents showed consistent decreasing trends with increasing salt stress in both treatments, but the reduction amplitude was significantly less pronounced in GI treatments. Third, GI-treated seedlings exhibited an initial increase followed by decrease in Fv/Fm, ΦPSII, qP, ETR, and Fv/Fo with rising salt levels, whereas CK treatments showed consistent declines. NPQ and HDR displayed initial decreases followed by increases in GI treatments, while CK treatments showed gradual increases in NPQ and continuous decreases in HDR. Across all salt concentrations, the magnitude of change in these parameters was significantly lower in GI treatments compared to CK.

These results demonstrate that AM fungi play an important role in improving photosynthetic and chlorophyll fluorescence characteristics under saline conditions, thereby promoting plant growth in salt-affected habitats. The study further reveals that salt stress intensity is a key factor influencing this symbiotic effect. Inoculation of halophytes with mycorrhizal symbionts shows potential application for saline-alkali land improvement.

Keywords: Arbuscular mycorrhizal fungi; salt stress; *Elaeagnus angustifolia*; chlorophyll content; chlorophyll fluorescence parameters

1. Materials and Methods

1.1 Experimental Materials

Elaeagnus angustifolia seeds were provided by Heilongjiang Jinxiu Dadi Bioengineering Co., Ltd. The AM fungus *Glomus intraradices* was propagated by the Restoration Ecology Laboratory of Heilongjiang University. The inoculum consisted of a rhizosphere mixture containing spores, mycelium, and mycorrhizal root fragments. The cultivation substrate was a mixture of forest soil, peat soil, and vermiculite (V:V:V = 6:2:2) sterilized at 120°C for 2 hours.

1.2 Experimental Design and Sampling

The experiment employed a two-factor design: AM fungal inoculation (GI vs. CK) and salt stress (0, 100, 200, and 300 mmol/L NaCl). The inoculation rate was 1% (g/g). For CK treatments, sterilized inoculum was applied. Surface-sterilized seeds were sown in pots (30 cm × 15 cm × 15 cm) containing the substrate. All pots were placed in a plastic greenhouse at Heilongjiang Botanical Garden for cultivation. Salt treatments were applied using distilled water solutions at the specified concentrations.

For physiological measurements, 10 uniform seedlings per treatment were randomly selected, and the 3rd to 5th functional leaves from the top were sampled between 9:00–11:00. For pigment analysis, leaf samples were immediately frozen in liquid nitrogen and stored at -80°C.

1.3 Measurement Methods

Mycorrhizal colonization was determined using the acid fuchsin staining method [19]. Gas exchange parameters (Pn, Tr, Gs, Ci) were measured using a Li-6400 portable photosynthesis system. Chlorophyll fluorescence was measured with a Li-6400-40 fluorometer after 30 minutes of dark adaptation, followed by light adaptation under 500 mol · m^{2} · s^{1} irradiance to determine Fv/Fm, ΦPSII, qP, NPQ, ETR, Fv/Fo, and HDR. Pigment contents were determined by ethanol extraction [20]. All measurements were performed in triplicate.

1.4 Data Analysis

Data were processed using Microsoft Excel 2003 and OriginPro 8.5. Two-way ANOVA and S-N-K tests were used to analyze significant differences among treatments. Change amplitude (A) was calculated following Wang et al. [21] as:
$A = (P - P)/P$
where P represents the parameter value under salt stress (100, 200, or 300 mmol/L) and P represents the control value (0 mmol/L). Positive A values indicate increases relative to control, while negative values indicate decreases. The absolute value of A reflects the magnitude of change.

2. Results

2.1 Mycorrhizal Colonization of *E. angustifolia* Roots

Acid fuchsin staining revealed extensive mycorrhizal structures in GI-treated roots, including abundant hyphae and vesicles with strong colonization intensity. Mycorrhizal infection rates exceeded a certain threshold across all salt concentrations, while no colonization was observed in CK treatments. These results confirm that *G. intraradices* forms effective symbiosis with *E. angustifolia* roots.

[Figure 1: see original paper] The hyphae and vesicles of AM fungi inside *E. angustifolia* roots

2.2 Effects on Net Photosynthetic Rate and Gas Exchange Parameters

Under salt stress, Pn, Tr, Gs, and Ci in both treatments decreased significantly with increasing NaCl concentration. However, at identical salt concentrations, GI-treated seedlings maintained significantly higher Pn, Tr, Gs, and Ci values compared to CK ($P < 0.05$). The decline patterns of Gs and Ci were consistent across treatments.

[Figure 2: see original paper] Effects of GI and CK treatments on net photosynthetic rate and gas exchange parameters in *E. angustifolia* leaves under salt stress

The amplitude of change analysis revealed that at 100 mmol/L NaCl, GI-treated seedlings showed reductions of 34%, 56%, 55%, and 26% in Pn, Tr, Gs, and Ci respectively, compared to non-salt controls, whereas CK seedlings exhibited greater reductions of 44%, 63%, 63%, and 34%. At 200 mmol/L, GI reductions were 50%, 71%, 76%, and 44%, while CK reductions reached 73%, 85%, 86%, and 66%. At 300 mmol/L, the differences in change amplitude between treatments were statistically significant ($P < 0.05$), with GI-treated seedlings showing significantly smaller declines.

2.3 Effects on Pigment Content

Pigment contents in both treatments decreased progressively with increasing salt concentration. However, chlorophyll a, chlorophyll b, and carotenoid contents were significantly higher in GI-treated leaves at all salt levels ($P < 0.05$). The chlorophyll a/b ratio in GI treatments remained lower than CK throughout the experiment.

[Figure 3: see original paper] Effects of GI and CK treatments on chlorophyll content in *E. angustifolia* seedlings under salt stress

Change amplitude analysis (Table 2) showed that at 100 mmol/L NaCl, GI-treated seedlings exhibited smaller reductions in chlorophyll a (2%) and total chlorophyll (1%) compared to CK (34% and 26% respectively), with differences reaching significant levels ($P < 0.05$). At 200 and 300 mmol/L, the divergence between treatments became more pronounced, with CK showing 56–61% reductions while GI showed only 9–18% reductions ($P < 0.01$).

2.4 Effects on Chlorophyll Fluorescence Parameters

Chlorophyll fluorescence parameters responded differently to salt stress between treatments. In GI-treated seedlings, Fv/Fm, ΦPSII, qP, ETR, and Fv/Fo initially increased slightly at low salt concentrations, peaking at 100 mmol/L before declining. In contrast, these parameters decreased consistently in CK treatments with increasing salt stress. NPQ and HDR showed initial decreases followed by increases in GI treatments, while HDR decreased gradually in CK treatments.

[Figure 4: see original paper] Effects of GI and CK treatments on chlorophyll fluorescence parameters in *E. angustifolia* leaves under salt stress

Change amplitude analysis (Table 3) demonstrated that GI-treated seedlings maintained significantly smaller changes in fluorescence parameters across all salt concentrations compared to CK ($P < 0.05$). For example, at 200 mmol/L NaCl, the reduction amplitude for ΦPSII was 28% in GI versus 56% in CK.

2.5 Two-Way ANOVA Analysis

Two-way ANOVA revealed that both salt level and mycorrhizal inoculation significantly affected all measured parameters ($P < 0.001$). Significant interactions between the two factors were detected for Pn, Ci, chlorophyll a, chlorophyll b, total chlorophyll content, and Fv/Fo ($P < 0.05$), indicating that the effect of AM fungi depended on salt concentration.

Two-way ANOVA for the effects and interactions of different treatments on *E. angustifolia* leaf parameters

3. Discussion

3.1 Photosynthetic Response of Mycorrhizal *E. angustifolia* to Salt Stress

Photosynthesis, the foundation of plant growth, is highly sensitive to environmental changes. Under salt stress, stomatal conductance decreases, limiting CO uptake and water loss. This study showed that Pn, Tr, Gs, and Ci declined significantly with increasing salinity, consistent with previous research [1]. However, GI-treated seedlings maintained significantly higher values and experienced smaller reduction amplitudes, demonstrating that AM fungi can enhance photosynthetic capacity and salt tolerance in *E. angustifolia.*

3.2 Chlorophyll Content Response of Mycorrhizal *E. angustifolia* to Salt Stress

Chlorophyll content reflects photosynthetic capacity and is crucial for light energy absorption, transfer, and conversion. Salt stress typically enhances chlorophyllase activity, accelerating chlorophyll degradation [24-26]. Our results align with these findings, but GI-treated seedlings showed significantly higher pigment contents and smaller reduction amplitudes. This suggests AM fungi can mitigate salt-induced chlorophyll degradation, particularly in young seedlings which are more salt-sensitive.

3.3 Chlorophyll Fluorescence Response of Mycorrhizal *E. angustifolia* to Salt Stress

Chlorophyll fluorescence analysis serves as an effective tool for diagnosing photosynthetic performance and plant stress tolerance [28]. AM fungi can alleviate damage to PSII reaction centers and enhance photochemical and non-photochemical quenching coefficients [32-33]. In this study, GI-treated seedlings showed initial increases in Fv/Fm, ΦPSII, qP, ETR, and Fv/Fo at moderate salt stress (100 mmol/L), suggesting enhanced photosynthetic efficiency. The subsequent decline at higher salinity indicates that the protective effect has limits. Nevertheless, the change amplitudes were consistently smaller in GI treatments, confirming that mycorrhizal symbiosis can protect the photosynthetic apparatus from salt damage.

The dual role of AM fungi—improving both chlorophyll content and fluorescence efficiency—helps maintain photosynthetic electron transport and reduces PSII damage under salt stress [34-35]. This synergistic effect significantly enhances net photosynthetic rate and overall salt tolerance.

4. Conclusion

Through pot experiments investigating the effects of *Glomus intraradices* inoculation on *Elaeagnus angustifolia* seedlings under 0, 100, 200, and 300 mmol/L NaCl stress, this study demonstrated that:

1. AM fungi significantly improved net photosynthetic rate, gas exchange parameters, chlorophyll content, and chlorophyll fluorescence characteristics under salt stress.
2. The magnitude of salt-induced changes was significantly smaller in mycorrhizal seedlings, indicating enhanced salt tolerance.
3. Salt concentration and mycorrhizal inoculation showed significant interactive effects on most photosynthetic parameters.

These findings reveal that AM fungi can alleviate salt stress damage by improving plant photosynthetic performance, suggesting potential applications for saline-alkali land improvement through halophyte-mycorrhizal symbiosis.

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