

## Effects of NaCl Stress on Biomass and Photosynthesis of Different *Ulmus pumila* Lines: Postprint

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**Date:** 2017-11-10T00:00:00+00:00

### Abstract

Employing three one-year-old *Ulmus pumila* varieties (Y65, Y1, Y34) as experimental materials, this study investigated the differential salt tolerance among the three elm varieties and the characterization of their photosynthetic parameters by measuring changes in biomass, chlorophyll content, and photosynthetic parameters under five NaCl treatment concentrations (0, 50, 85, 120, 155 mmol/L). The results demonstrated that, compared with the control, biomass and chlorophyll content of the elm varieties decreased progressively with intensifying NaCl stress, with high NaCl stress (> 85 mmol/L) significantly inhibiting biomass accumulation and disrupting chlorophyll synthesis. Under low NaCl stress (85 mmol/L), the photosynthetic mechanism of the three varieties was predominantly constrained by stomatal limitation; they enhanced water use efficiency (WUE) by increasing stomatal limitation values to reduce transpiration, thereby adapting to salt stress. Conversely, under high NaCl stress (> 85 mmol/L), non-stomatal limitation became predominant, reducing WUE to decrease root absorption of subsurface water and salt ions as a mechanism to sustain growth. Among the three elm varieties, the threshold NaCl stress concentration at which non-stomatal limitation became the primary factor causing Pn decline varied: the turning point was 85 mmol/L for Y65 and Y1, whereas it was 50 mmol/L for Y34. Conclusion: Among the three tested elm varieties, Y65 exhibited superior comprehensive salt tolerance and represents the preferred selection for elm cultivation in saline-alkali soils.

### Full Text

## Effects of NaCl Stress on Biomass and Photosynthetic Characteristics of Different *Ulmus pumila* L. Strains

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**Abstract:** Salt stress is one of the major environmental factors affecting plants, causing numerous metabolic changes such as alterations in chlorophyll content and composition and reductions in photosynthetic capacity and efficiency. Salt stress also inhibits biomass accumulation. Consequently, plant biomass, photosynthesis, and chlorophyll content are frequently used as indicators of salt stress injury. *Ulmus pumila* L. is a widely distributed, fast-growing hardwood timber species with excellent tolerance to drought, cold, salt, and wind. It represents the most important timber species in northern China, saline-alkali regions, and desert areas, where it is used for afforestation and shelterbelt establishment, making it a promising candidate for cultivation in saline soils.

In response to salt stress, plants have evolved diverse mechanisms to mitigate stress effects and enhance tolerance. This study examined one-year-old potted seedlings of three *Ulmus pumila* strains (Y65, Y1, and Y34) to investigate changes in chlorophyll content, photosynthetic characteristics, and biomass accumulation under different NaCl concentrations (0, 50, 85, 120, and 155 mmol/L). The results showed that under higher salt stress, chlorophyll content and biomass in all three strains were significantly inhibited, though Y65 showed no obvious biomass reduction at lower NaCl concentrations.

Under low salinity stress (NaCl = 85 mmol/L), net photosynthetic rate (Pn), stomatal conductance (Gs), and intercellular CO<sub>2</sub> concentration (Ci) decreased while stomatal limitation (Ls) increased, indicating stomatal limitation as the primary cause of photosynthetic reduction. Although no stomatal restriction occurred in the 120 and 155 mmol/L treatments, Pn decreased while Ci increased. Under low-salinity stress, increased Ls led to decreased transpiration rate (Tr) and increased water use efficiency (WUE), reducing leaf water loss and alleviating water supply-demand imbalance to improve salt tolerance. Under high-salinity stress (NaCl > 85 mmol/L), root absorption capacity decreased, and both Tr and WUE declined, causing more severe water imbalance.

Correlation analysis revealed that biomass was highly significantly positively correlated with total chlorophyll, chlorophyll a, Pn, and Gs, and significantly positively correlated with Tr. WUE was significantly positively correlated with Ls. We recommend Y65 as the preferred strain for afforestation in saline areas. Overall, Y65 demonstrated superior salt resistance compared to Y1 and Y34, providing a scientific basis for further research on salinity tolerance mechanisms in *Ulmus pumila*.

**Keywords:** NaCl stress; *Ulmus pumila* L.; photosynthetic characteristics; salt tolerance

## Introduction

Soil salinization represents a major natural adversity in plant growth. When salt ions in soil solution exceed threshold levels, they inhibit plant development and induce physiological and biochemical changes, including disruption of biological membrane function and structure, imbalance of cellular redox systems, and ion toxicity. Previous studies on salt tolerance in *Ziziphus jujuba* var. *spinosa*, ornamental groundcover bamboo, and different provenances of *Elaeagnus angustifolia* have demonstrated that as salt stress concentration increases, the inhibitory effect on biomass accumulation becomes more pronounced, chlorophyll content declines more significantly, and photosynthetic mechanisms shift from stomatal to non-stomatal limitation. Photosynthetic indices such as net photosynthetic rate and stomatal conductance decrease to varying degrees, with more pronounced changes in salt-sensitive varieties compared to relatively stable responses in salt-tolerant varieties.

Given that different plant species and even different strains within the same species exhibit substantial variation in salt tolerance, their performance in growth, physiological-biochemical indicators, and photosynthetic characteristics under salt stress differs considerably. Investigating plant salt tolerance is therefore crucial for screening salt-tolerant plants or varieties and for restoring and reconstructing saline-alkali soils. *Ulmus pumila* is China's most widely distributed and important fast-growing hardwood timber species, with excellent drought, cold, salt, and wind resistance, making it a premium afforestation tree species. Previous research has demonstrated that saline-alkali stress affects *Ulmus pumila* growth, free proline content, and soluble sugar changes. Evaluations based on proliferation rate and seedling height growth, photosynthetic and fluorescence parameters, and seedling damage have revealed that different *Ulmus pumila* strains exhibit strong, weak, or sensitive salt-alkali tolerance responses.

Based on these inter-strain differences in salt tolerance, this study selected three *Ulmus pumila* strains as experimental materials to investigate biomass accumulation, chlorophyll content, and photosynthetic parameters under different NaCl concentrations. The objectives were to preliminarily evaluate salt tolerance differences among strains, characterize photosynthetic parameter responses, and explore response patterns to salt stress, thereby providing a scientific basis for breeding salt-tolerant plants for saline-alkali soils.

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## 1. Study Site Overview

The experiment was conducted at the Forestry Experimental Station of Shandong Agricultural University, located in southern Tai'an City. The region experiences a temperate monsoon continental climate with an average temperature of 12.8°C, annual temperature range of -6.9 to 37°C, accumulated temperature 10°C of 4283.1°C, geographic coordinates of 117°11 E, 36°16 N, and a frost-free period of 186.6 days. The greenhouse was well-ventilated with temperature and

humidity conditions essentially consistent with natural outdoor conditions.

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## 2. Experimental Materials

The three tested *Ulmus pumila* strains (Y65, Y1, Y34) were provided as tissue culture seedlings by the Shandong Academy of Forestry Sciences. The explants were originally collected from Baiwa Forest Farm in Jinxiang County, Shandong Province—a tree improvement base designated by the former Ministry of Forestry and Shandong Provincial Forestry Department. These superior clones were selected from 69 *Ulmus pumila* germplasm resources based on high growth volume and strong pest resistance, then subjected to salt-tolerance tissue culture to obtain salt-resistant capabilities. Compared with common elm trees, these strains exhibit characteristics of rapid growth and good tree form.

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## 3. Experimental Design

In 2014, healthy, uniform-sized, pest-free seedlings of the three strains were transplanted into 20 cm diameter pots lined with non-woven fabric to prevent water and soil leakage. The cultivation substrate was prepared with a 2:1:1 dry mass ratio of fine sand to peat soil. Regular watering and fertilization with Hoagland nutrient solution ensured survival and growth. The experiment ran from June to October 2014.

Salt treatments consisted of five NaCl concentrations: 0 (control), 50, 85, 120, and 155 mmol/L. The soaking method was employed, where pots were immersed in salt solution for 6-10 hours, allowing absorption through bottom holes. To avoid salt shock effects, seedlings were gradually acclimated from low to high concentrations. Between soakings, pots were washed with tap water multiple times to eliminate salt accumulation. Except during salt treatments and washing, no additional watering or pruning was performed. All measurements commenced after treatment initiation.

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## 4. Measurement Methods

### 4.1 Biomass Determination

After all measurements, seedlings were harvested and brought to the laboratory. Soil was loosened with tap water, and plants were gently removed. Roots and shoots were washed with deionized water to remove sand and debris, then separated into aboveground and belowground portions. Samples were oven-dried at 105°C for 30 minutes, then at constant temperature to achieve constant weight for biomass determination.

#### 4.2 Photosynthetic Parameter Measurement

On clear, windless days between 9:00-11:00, healthy upper-middle leaves were selected from vigorous seedlings of each strain. A CIRAS-2 portable photosynthesis system (PP Systems, USA) with an open gas path and red-blue light source chamber was used to measure net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO<sub>2</sub> concentration (Ci). Measurement conditions included photosynthetically active radiation of 1000 mol·m<sup>-2</sup>·s<sup>-1</sup> and CO<sub>2</sub> concentration of 385 mol/mol. Water use efficiency (WUE = Pn/Tr) and stomatal limitation (Ls = 1 - Ci/Ca) were calculated, where Ca represents ambient CO<sub>2</sub> concentration. Each strain and treatment combination was measured with three replicates.

#### 4.3 Chlorophyll Content Determination

Concurrently with photosynthetic measurements, upper-middle leaves were collected, rapidly frozen in liquid nitrogen, and transported to the laboratory. Chlorophyll was extracted using the acetone method and quantified with a spectrophotometer to determine Chl(a+b), Chl a, and Chl b contents.

#### 4.4 Data Processing

Experimental data were processed using Excel and SPSS 19.0 statistical software. One-way ANOVA was used for significance testing, and correlation analysis was performed among indicators.

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### 5. Results and Analysis

#### 5.1 Effects of NaCl Stress on Biomass

Salt stress inhibited plant growth and development, significantly reducing biomass in all three *Ulmus pumila* strains, though the degree of reduction varied with concentration and strain. At 50 mmol/L NaCl, biomass decreased by 16.71%, 25.27%, and 27.45% for Y65, Y1, and Y34 respectively compared to controls. Between 50-85 mmol/L, biomass decline gradually plateaued. At 120-155 mmol/L, biomass decreased more dramatically, with reductions of 45.79%, 47.98%, and 47.07% at 155 mmol/L. Notably, Y65 maintained significantly greater biomass accumulation than Y1 and Y34 across all treatments, indicating superior salt tolerance.

#### 5.2 Effects of NaCl Stress on Chlorophyll Content

All three strains showed similar response patterns in chlorophyll content to salt stress. Chl(a+b) and Chl a contents decreased with increasing NaCl concentration. At 155 mmol/L, Chl(a+b) contents were 58.68%, 37.65%, and 48.96% of respective controls for Y65, Y1, and Y34. ANOVA revealed significant differences in Chl(a+b) and Chl a among treatments (P < 0.05). The Chl a/b ratio showed an initial increase followed by decrease, peaking at 120 mmol/L at 144.46%, 143.76%, and 144.20% of control values, then declining below control levels at 155 mmol/L. These changes were significant across concentration gradients (P < 0.05).

### 5.3 Effects of NaCl Stress on Photosynthetic Characteristics

#### *Net Photosynthetic Rate (Pn)*

At 50 mmol/L NaCl, Y65 showed a slight but significant increase in Pn (5.76% above control), while Y1 and Y34 exhibited non-significant reductions of 12.20%. At 85 mmol/L, all strains showed concentration-dependent Pn decreases, with reductions of 86.42%, 73.96%, and 88.98% at 155 mmol/L compared to respective controls ( $P < 0.05$ ).

#### *Transpiration Rate (Tr), Stomatal Conductance (Gs), Intercellular CO<sub>2</sub> (Ci), and Stomatal Limitation (Ls)*

Under low salinity (85 mmol/L), Tr and Gs decreased while Ls increased, consistent with stomatal limitation mechanisms. At 50 mmol/L, Y65's Gs increased significantly, promoting Tr and enhancing water and mineral transport, which improved Pn. At 85 mmol/L, Y65 and Y1 showed decreased Gs and Tr but maintained photosynthetic function through stomatal regulation. Under high salinity (>85 mmol/L), all strains exhibited decreased Gs, Tr, and Ls with increased Ci, indicating a shift to non-stomatal limitation. The transition point differed among strains: Y65 and Y1 shifted at >85 mmol/L, while Y34 shifted at >50 mmol/L.

#### *Water Use Efficiency (WUE)*

WUE showed an initial increase followed by decrease across treatments. At 50 mmol/L, Y65 and Y1 WUE increased to 119.95% and 257.61% of controls respectively, significantly reducing leaf water loss and alleviating water imbalance. Y34 reached maximum WUE at 85 mmol/L (193.57% of control). Under high salinity (>85 mmol/L), all strains showed decreased WUE, with Y65 maintaining relatively higher efficiency.

### 5.4 Correlation and Membership Function Analysis

Correlation analysis revealed biomass was highly significantly positively correlated with total chlorophyll ( $r = 0.771$ ), Chl a ( $r = 0.711$ ), Pn ( $r = 0.970$ ), and Gs ( $r = 0.647$ ), and significantly correlated with Tr ( $r = 0.548$ ). WUE was significantly positively correlated with Ls ( $r = 0.661$ ). Comprehensive evaluation using membership functions ranked salt tolerance as: Y65 > Y1 > Y34.

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## 6. Discussion and Conclusion

Excess soil salt causes osmotic stress and disrupts nutrient ion balance, affecting osmotic adjustment, lipid metabolism, and ultimately biomass accumulation. Biomass represents a comprehensive indicator of plant salt stress response and a direct measure of salt tolerance, providing reliable criteria for evaluation. In this study, all three *Ulmus pumila* strains exhibited biomass reduction under salt stress, with Y65 maintaining significantly greater accumulation than Y1 and Y34, demonstrating superior salt tolerance. As salt concentration increased, the other two strains showed progressively greater biomass reductions.

Chlorophyll is essential for photosynthesis, playing critical roles in light absorption and conversion. Correlation analysis showed Chl(a+b), Chl a, and Chl b were highly significantly positively correlated with Pn and biomass ( $P < 0.01$ ). Under salt stress, enhanced chlorophyllase activity promotes chlorophyll degradation, reducing photosynthetic rate and affecting growth and yield. The Chl a/b ratio reflects the proportion of thylakoid membranes in different stacking states within chloroplasts; smaller fluctuations under stress indicate stronger adaptability. In this study, Chl a/b values fluctuated within the normal range of 2.0-5.0 under low salinity (85 mmol/L), suggesting an adaptive mechanism to reduce light energy capture and avoid salt damage. At 120 mmol/L, increased Chl a/b may limit light-harvesting pigment proteins, consistent with findings in winter wheat under salt stress. Y65 showed the smallest Chl(a+b) reduction, indicating minimal damage to its chlorophyll system and superior salt tolerance.

Photosynthetic efficiency reduction results from two main factors: stomatal limitation due to partial stomatal closure, and non-stomatal limitation from decreased mesophyll cell photosynthetic activity. The primary diagnostic criterion is the direction of  $C_i$  change. This study revealed different photosynthetic response mechanisms among strains: under low salinity (85 mmol/L), stomatal limitation dominated as Pn and  $C_i$  changed concordantly. Plants regulated Gs to control Tr and  $C_i$ , reducing water loss while maintaining Pn. Under high salinity (>85 mmol/L), decreased Gs, reduced Tr, increased  $C_i$ , and decreased Ls indicated that non-stomatal factors became the main limitation, as excessive salt ions accumulated in cells, reducing water potential, damaging chloroplast structure, and degrading photosynthetic pigments.

Water use efficiency ( $WUE = Pn/Tr$ ) reflects the integrated response of photosynthetic and transpiratory characteristics. According to stomatal optimization theory, stomata operate to maximize carbon gain while minimizing water loss. Under low salinity, increased WUE in Y65 and Y1 represented an adaptive response, significantly reducing transpiration while maintaining photosynthesis. Under high salinity, decreased WUE in all strains indicated reduced water use efficiency and weaker stress tolerance. Y65 and Y1 could reduce root absorption of water and salt ions to maintain growth, while Y34's lower water use efficiency under high salt suggested poorer salt tolerance.

Through comprehensive membership function analysis of nine indicators under salt stress, this study objectively reflected the salt tolerance of tested strains. The ranking  $Y65 > Y1 > Y34$  indicates Y65's superior comprehensive adaptability to saline-alkali soils, making it the preferred choice for afforestation in such areas.

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