Effects of Combined Salt and Drought Stress on Growth and Water Uptake in Wheat Seedlings: Postprint

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Abstract

To elucidate the effects of salt stress, drought, and combined salt-drought stress on wheat seedling growth and water absorption, thereby providing a theoretical basis for cultivation regulation under salt and drought stress, two wheat cultivars with different drought resistance (Yangmai 16 and drought-tolerant Luohan 7) were used as materials in a hydroponic experiment. Combined salt-drought stress was simulated using NaCl and PEG to investigate changes in wheat seedling growth, root morphology, photosynthetic characteristics, and water absorption characteristics. The results demonstrated that under salt, drought, and combined stress, the biomass, leaf area, total root length, root surface area, chlorophyll fluorescence, and net photosynthetic rate of wheat seedlings all decreased significantly; however, the reduction under combined stress was significantly less than that under single stress. Under combined salt-drought stress, root hydraulic conductivity and root bleeding sap intensity were significantly greater than under single stress, thereby enhancing leaf water potential and relative water content in wheat seedlings. The Na+/K+ ratio of wheat seedlings under salt stress was significantly higher than under combined stress, whereas the ABA content under combined stress was significantly lower than under single salt or drought stress. Therefore, combined salt-drought stress can maintain higher photosynthetic capacity by enhancing root water absorption and reducing ABA content in roots and leaves, which is an important mechanism for improved wheat adaptability under combined stress. Luohan 7 and Yangmai 16 showed essentially consistent responses to salt and combined salt-drought stress, but Luohan 7 exhibited marked tolerance under drought stress alone.

Full Text

Preamble

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Effects of Salt Combined with Drought Stress on Growth and Water Uptake of Wheat Seedlings

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Abstract

Salt and drought stress are two major limiting factors for wheat (*Triticum aestivum*) productivity. In northern and northwestern China, these stresses often occur simultaneously due to low rainfall and high evaporation during winter and spring, resulting in significant yield losses. Recent studies have indicated that certain crop species exhibit lower growth inhibition under combined salt and drought stress compared to individual salt or drought stress, yet the underlying adaptation mechanisms remain poorly understood. Drought-tolerant and susceptible cultivars may possess variable morphological and metabolic adaptation processes in response to salt and drought stress that differentially contribute to their stress adaptation capability.

This study investigated the combined effects of salt with drought stress (SD) on wheat seedling growth and water absorption characteristics to provide a theoretical basis for wheat cultivation management under salt and drought conditions. Two wheat cultivars with contrasting drought resistance—Yangmai 16 (drought-susceptible) and Luohan 7 (drought-tolerant)—were used in a hydroponic experiment to examine SD effects on root morphology, photosynthesis, and water absorption at the seedling stage. Sodium chloride (NaCl) and polyethylene glycol 6000 (PEG) were applied to nutrient solutions to simulate salt and drought stress, respectively. Leaf gas exchange, chlorophyll fluorescence parameters, leaf water potential, and root hydraulic conductivity were determined, and hormone concentrations were estimated using enzyme-linked immunosorbent assay.

The results showed that both salt and drought stress significantly affected plant growth and physiological activities in both cultivars. However, the combined SD effects on plant growth and dry matter production reduction were less severe than their individual effects. Root length, root surface area, and root volume in SD-treated plants were higher than those under single stress treatments, although still significantly lower than the control, indicating that SD had less negative impact on root growth than single stress. Similarly, chlorophyll content, chlorophyll fluorescence parameters (Fv/Fm, Fv /Fm), net photosynthetic rate, and stomatal conductance under SD treatment were all significantly higher than under single salt or drought stress, demonstrating that SD caused less damage to the photosynthetic apparatus.

Root hydraulic conductivity and xylem sap intensity under SD were significantly higher than under single stress, resulting in higher leaf water potential and relative water content compared to single stress treatments. The Na /K ratio in leaves and roots under SD treatment was significantly lower than under single salt stress, and abscisic acid (ABA) content in SD-treated plants was lower than in single stress-treated plants, though still significantly higher than the control. This indicated that SD could reduce root ABA formation compared to single stress. Overall, SD not only improved root water uptake capacity and leaf water status but also decreased Na and ABA content, thereby alleviating inhibition of leaf photosynthetic capacity.

Maintaining higher water absorption capacity and photosynthesis were the major contributors to wheat seedling adaptation to SD. Luohan 7 and Yangmai 16 responded similarly to salt and SD, whereas Luohan 7 showed more obvious tolerance to drought stress than Yangmai 16.

Keywords: wheat; salt combined with drought; photosynthesis; root hydraulic conductivity; water uptake

Introduction

Salt and drought have long been serious natural disasters limiting agricultural production. The frequency and intensity of salt and drought stress are increasing, directly threatening national long-term food security and social stability [1-4]. Research indicates that during drought, enhanced soil water evaporation leads to continuous surface salt accumulation, while plant root water uptake reduces soil moisture and increases salt concentration [5-6], resulting in combined salt-drought stress. Wheat is an important food crop in China, and seedling development directly affects yield and quality. The wheat seedling stage occurs during dry autumn and salt-returning spring seasons [7], making it practically significant to clarify the effects and physiological mechanisms of salt-drought combined stress on wheat seedling growth.

Both salt and drought cause osmotic stress, increase root water absorption resistance, and reduce photosynthetic rates. However, current research on combined salt-drought stress mainly focuses on osmotic adjustment substances and stress-resistance enzymes, with fewer studies on plant water absorption, transport, and photosynthetic capacity under combined stress [7-10]. Different crop species and treatment methods have led to varying conclusions [8-12]. Some studies suggest that salt-drought combined stress aggravates plant damage [8,10], while others indicate that plants can exhibit certain adaptability when facing dual stress [6-7,11-13], though explanations for this adaptability remain limited.

Building on previous research, this study used hydroponic experiments to simulate salt-drought combined stress with two wheat cultivars differing in drought resistance to analyze water absorption capacity, leaf photosynthesis, and seedling growth under combined stress, aiming to provide scientific evidence for the adaptive mechanisms of wheat seedlings to salt-drought combined stress and clarify the reasons for their adaptive performance.

1. Experimental Materials and Treatments

A hydroponic experiment was conducted in 2015 at the Pailou Experimental Station of Nanjing Agricultural University under a rain shelter. Two wheat cultivars with different drought resistance were used: drought-susceptible Yangmai 16 and drought-tolerant Luohan 7. Plump, uniform seeds were surface-sterilized with 10% H O for 10 minutes, then placed in a turnover box lined with two layers of sterilized filter paper for germination in the dark in a growth chamber at 20°C/8°C (day/night). When the second true leaf emerged, uniform, healthy seedlings were selected and transplanted to plastic containers (38 cm × 32 cm × 27 cm) containing 1/2 Hoagland nutrient solution. The experiment used a completely randomized design with three replicates per treatment.

Stress treatments were applied when the third leaf emerged: drought (D, 12.7% PEG, -0.25 MPa), salt (S, 50 mmol/L NaCl, -0.25 MPa), and salt-drought combined stress (SD, 12.7% PEG + 50 mmol/L NaCl, -0.45 MPa), with complete nutrient solution as control (CK). Solution water potential was measured using a water potential meter. An air pump maintained dissolved oxygen concentration at 6-8 mg/L. The pH was maintained at 5.5-6.0. Growth conditions were 15 h light/9 h dark with photosynthetically active radiation of 300 mol m ^{2} s ^{1}, and average temperature of 20°C/8°C (day/night).

2. Measurement Items and Methods

2.1 Plant Dry Weight and Root-Shoot Ratio

Plants were harvested and separated into shoots and roots, oven-dried at 75°C for 10 minutes, then dried to constant weight. Dry weights were measured and root-shoot ratio calculated.

2.2 Leaf Water Potential and Relative Water Content

Leaf water potential of the topmost fully expanded leaf was measured using a dew point water potential meter. Relative water content (RWC) was determined by the oven-drying method: RWC = (fresh weight - dry weight) / (saturated fresh weight - dry weight) × 100%.

2.3 Root Morphology

Total root length, surface area, and volume were determined using a WinRHIZO automatic root scanning analyzer. Fresh samples were snap-frozen in liquid nitrogen for physiological measurements.

2.4 Chlorophyll Content

Chlorophyll and carotenoid contents were determined spectrophotometrically after extraction in 95% ethanol (1:1 ratio) in darkness for 24 h [15].

2.5 Photosynthetic Physiological Parameters

Net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO concentration (Ci), and transpiration rate (Tr) were measured using a Li-6400 portable photosynthesis system with an open gas circuit and red-blue light source at 1000 mol m^{2} s^{1} photosynthetic photon flux density and 385 mol/L CO concentration. Five uniform topmost leaves were measured per treatment.

2.6 Chlorophyll Fluorescence Parameters

Chlorophyll fluorescence was measured using a PAM-2000 portable modulated fluorometer. After dark adaptation for 15 minutes, maximum photochemical efficiency (Fv/Fm) was measured under strong flash (3000 mol m^{2} s^{1}). Actual photochemical efficiency (Fv /Fm) was measured under actinic light.

2.7 Root Hydraulic Conductivity and Xylem Sap

Root hydraulic conductivity (Lpr) was measured using a pressure chamber. Wheat seedlings were excised at the shoot base, and roots were placed in distilled water in the pressure chamber. Pressure was applied (0.2, 0.5, 1.0 MPa), and after sap flow stabilized, exudate was absorbed with cotton wool for 1 minute and weighed. The water flux per unit root weight per unit time per unit pressure was calculated as Lpr [16]. To determine aquaporin contribution, HgCl (50 mol/L) was added to block water channels, and Lpr was remeasured.

Xylem sap was collected by excising shoots and wrapping the cut surface with cotton wool covered with plastic film for 12 h (19:00-7:00). The cotton wool was weighed to determine bleeding intensity [15].

2.8 Hormone and Na /K Content

Abscisic acid (ABA) concentration was determined by enzyme-linked immunosorbent assay (ELISA) using kits provided by China Agricultural University. Na /K ratio was determined according to Wang et al. [14].

2.9 Data Analysis

Data were analyzed using Excel and SigmaPlot software. Duncan' s multiple range test (= 0.05) was used for significance testing.

3. Results

3.1 Effects of Salt-Drought Combined Stress on Wheat Seedling Growth

Both cultivars showed significantly reduced dry weight under salt, drought, and combined stress, with the greatest reduction under salt stress and the smallest under combined stress . Root-shoot ratio decreased significantly under salt stress but increased under drought and combined stress, with drought having a greater effect than combined stress. Leaf area decreased significantly under all three stresses, but combined stress had the smallest impact, indicating that seedlings maintained stronger leaf growth and biomass accumulation under combined stress. Cultivar comparison showed that Luohan 7 exhibited clear resistance to drought stress, with no significant differences from Yangmai 16 under salt or combined stress.

Effects of salt combined with drought stress on leaf area, plant dry weight and root-shoot ratio of wheat seedlings

3.2 Effects of Salt-Drought Combined Stress on Wheat Seedling Root Morphology

Total root length, surface area, and volume decreased significantly under all stress treatments, with salt stress having the greatest impact and combined stress the smallest , consistent with dry matter accumulation results. Under single salt or drought stress, seminal root changes were greater than primary root changes, while under combined stress, primary root changes were more pronounced. Studies on seminal and primary roots of both cultivars showed similar trends.

Effects of salt combined with drought stress on root length, root surface area and root volume of wheat seedlings

3.3 Effects of Salt-Drought Combined Stress on Leaf Water Potential and Relative Water Content

Leaf water potential and relative water content decreased significantly under all stresses in both cultivars. Under combined stress, Yangmai 16 showed significantly higher relative water content than under drought but lower than under salt stress, while its leaf water potential showed no significant difference between salt and drought but was significantly lower than under combined stress. In contrast, Luohan 7 showed significantly higher leaf water potential and relative water content under combined stress compared to single stresses. Under combined stress, Luohan 7 had significantly lower relative water content than under salt stress but not significantly different from drought, while its leaf water potential under salt stress was significantly lower than under drought and combined stress. This indicates Luohan 7 maintained higher water retention capacity under combined stress. As a drought-tolerant cultivar, Luohan 7 exhibited resistance to drought stress with higher water potential and water content [Figure 1: see original paper].

[Figure 1: see original paper] Effects of salt combined with drought stress on leaf water potential and relative water content of wheat seedlings

3.4 Effects of Salt-Drought Combined Stress on Chlorophyll Content

Chlorophyll (a+b) content decreased significantly while carotenoid content increased significantly under stress. Under combined stress, chlorophyll (a+b) and Chla/Chlb ratio were significantly greater than under single drought stress but significantly smaller than under salt stress. Luohan 7 showed significantly higher chlorophyll content under drought than under salt stress, with Chla/Chlb ratio significantly greater under drought. Under both stresses, differences in Chla/Chlb ratio were not significant. Luohan 7 demonstrated clear tolerance to drought stress [Figure 2: see original paper].

[Figure 2: see original paper] Effects of salt combined with drought stress on chlorophyll and carotenoid content in leaves of wheat seedlings

3.5 Effects of Salt-Drought Combined Stress on Photosynthetic Characteristics

Net photosynthetic rate decreased significantly under all stresses, with salt stress having the greatest impact and combined stress the smallest. Under drought, Luohan 7 maintained significantly higher photosynthetic rate than Yangmai 16. Under combined stress, wheat seedlings maintained higher Gs and Tr to sustain greater transpiration pull. Luohan 7 showed significantly higher Gs and Tr under combined stress than under single salt or drought stress. Under salt stress, Gs was greater than under drought while Ci was lower, indicating that salt stress mainly reduced photosynthetic rate through non-stomatal factors. Both cultivars showed consistent trends in Fv/Fm, which decreased significantly under stress, with salt stress having the greatest impact and combined stress the smallest. Fv /Fm also decreased significantly under stress, showing the same trend as Fv/Fm. Both parameters were significantly higher under drought than under salt stress but significantly lower than under combined stress, indicating that salt stress damaged the photosynthetic apparatus and reduced photosynthetic capacity. Combined stress caused less damage to the photosynthetic apparatus, resulting in smaller reductions in photosynthetic capacity [FIGURE:3-4].

[Figure 3: see original paper] Effects of salt combined with drought stress on photosynthetic parameters in leaves of wheat seedlings

[Figure 4: see original paper] Effects of salt combined with drought stress on chlorophyll fluorescence parameters in leaves of wheat seedlings

3.6 Effects of Salt-Drought Combined Stress on Root Hydraulic Conductivity and Bleeding Intensity

Root hydraulic conductivity (measured without HgCl) was significantly higher under combined stress than under single stress. HgCl is a specific aquaporin inhibitor that reacts with amino acid residues at water channel proteins, causing physical blockage [16-17]. After HgCl addition, root hydraulic conductivity under combined stress was significantly smaller than under single stress, indicating that combined stress seedlings expressed more aquaporins to resist stress. Under combined stress, wheat seedlings showed significantly higher bleeding intensity than under drought (not significantly different from salt stress), demonstrating that salt and combined stress enabled roots to maintain higher water absorption capacity [Figure 5: see original paper].

[Figure 5: see original paper] Effects of salt combined with drought stress on root hydraulic conductance and root bleeding intensity of wheat seedlings

3.7 Effects of Salt-Drought Combined Stress on Na /K Ratio and ABA Content

Under combined stress, both cultivars showed significantly lower Na /K ratios in roots and leaves compared to single salt stress, indicating that combined stress reduced Na accumulation and alleviated ion toxicity. ABA content was significantly lower under combined stress than under single stresses, though still higher than control. This suggests that combined stress reduced root ABA formation compared to single stress, promoting stomatal opening and increasing transpiration pull, which enhanced root and shoot growth for stress resistance [Figure 6: see original paper].

[Figure 6: see original paper] Effects of salt combined with drought stress on ABA content and Na /K ratio in leaves and roots of wheat seedlings

4. Discussion

Plants exhibit certain adaptability when facing combined salt and drought stress [6-7,11]. Previous research has focused on osmotic adjustment substances and stress-resistance enzymes, with less attention to water absorption, transport, and photosynthetic capacity under combined stress [7-10]. This study investigated water absorption and photosynthetic capacity to explain the adaptive mechanisms.

Osmotic stress reduces root bleeding intensity, causing stomatal closure and decreased transpiration pull, making water uptake difficult [18-19]. Compared to single drought stress, combined stress increased root bleeding intensity and hydraulic conductivity, elevating leaf water potential and relative water content, which ensured relatively efficient photosynthesis. Efficient photosynthesis increased stomatal conductance and transpiration rate, enhancing transpiration pull. Osmotic stress induces large ABA accumulation, causing stomatal closure and photosynthetic rate reduction [18-20]. Excessive Na accumulation causes osmotic stress and ion toxicity, disrupting Na /K balance and damaging photosynthetic apparatus, leading to decreased photosynthetic rates [21-23].

Under combined stress, wheat seedlings maintained higher water absorption capacity and plant water content compared to drought stress. They also reduced Na /K ratio to alleviate ion toxicity and maintained lower ABA content, ensuring relatively efficient photosynthetic rates. This demonstrates that combined stress enhances adaptability through improved water relations and reduced ion toxicity, consistent with previous findings [6-7,11].

5. Conclusion

Consistent with previous studies [6-7,11], salt-drought combined stress enhanced wheat seedling root water absorption capacity and leaf water content compared to single drought stress, alleviating inhibition of leaf photosynthetic capacity and ensuring plant growth, thereby exhibiting certain adaptability. Maintaining high water absorption and reducing Na /K ratio are important mechanisms for wheat adaptation to combined salt-drought stress.

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Note: Figure translations are in progress. See original paper for figures.