Effects of Saline-Alkali Stress on Bacterial and Fungal Community Structure and Abundance in the Rhizosphere Soil of Cucumber Grafted Seedlings: Postprint

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Abstract

To understand the effects of saline-alkali stress on the rhizosphere soil bacterial and fungal community structures of cucumber grafted seedlings, this study employed two saline-alkali tolerant rootstocks, 'Huazhen 108' (T1) and 'Shenli Tiemuzhen' (T2), and two saline-alkali sensitive rootstocks, 'Huitailang' (S1) and 'Jingxinzhen 6' (S2), as experimental materials, with self-rooted seedlings serving as the control (CK). A treatment solution of mixed salts (molar ratio NaHCO3:Na2SO4:NaCl:Na2CO3 = 4:2:2:0.15) at a concentration of 100 mmol L 1 and pH 9.0 was applied for 20, 30, and 40 days (30, 40, and 50 days after transplanting). Using PCR-DGGE technology, the effects of saline-alkali stress on the structure and abundance of rhizosphere soil microbial communities in cucumber seedlings grafted onto different rootstocks were investigated. The results demonstrated that the fungal DGGE profile band numbers in the rhizosphere soil of saline-alkali tolerant rootstock varieties T1 and T2 were significantly higher than those of the saline-alkali sensitive variety S2 and the self-rooted seedling control CK. Furthermore, the Shannon-Wiener index and evenness index of soil bacteria for the saline-alkali tolerant variety T2 were significantly higher than those of the saline-alkali sensitive varieties S1 and S2, as well as the self-rooted seedling control CK. The bacterial 16S rDNA gene copy number of the saline-alkali tolerant variety T1 at 50 days after transplanting was significantly higher than that of the saline-alkali sensitive varieties and self-rooted seedlings. At 40 days after transplanting, the fungal ITS gene copy number of the saline-alkali tolerant rootstock T2 was significantly higher than that of the saline-alkali sensitive varieties and cucumber self-rooted seedlings. At 50 days after transplanting, the fungal ITS gene copy number of saline-alkali tolerant rootstocks was significantly higher than that of saline-alkali sensitive varieties, but showed no significant difference from self-rooted seedlings. Differences existed in the composition and abundance of rhizosphere soil microbial communities among cucumber seedlings grafted onto rootstocks with different saline-alkali tolerance. The above study indicates that with increasing saline-alkali stress duration, grafted seedlings of rootstocks with different saline-alkali tolerance exhibited substantial differences in rhizosphere soil microbial community abundance and structural diversity, indirectly altering the soil microecological environment and causing changes in soil microbial quantity and richness. Saline-alkali tolerant rootstock varieties may enhance their own saline-alkali tolerance characteristics by improving the soil microenvironment.

Full Text

Effects of Saline-Alkali Stress on Structure and Abundance of Bacterial and Fungal Communities in Rhizosphere Soil of Grafted Cucumber Seedlings

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Abstract: This study investigated the effects of saline-alkali stress on microbial community structure and abundance in the rhizosphere soil of grafted cucumber seedlings using two saline-alkali tolerant rootstocks ('Huazhen108' [T1] and 'Shenlitiemuzhen' [T2]) and two saline-alkali sensitive rootstocks ('Huitailang' [S1] and 'Jingxinzhenliuhao' [S2]), with self-rooted seedlings serving as controls. A mixed salt solution ($NaHCO_3$:Na_2SO_4:NaCl:Na_2CO_3 = 4:2:2:0.15 molar ratio) at 100 $mmol \cdot L^{-1}$ concentration and pH 9.0 was applied 10 days after transplanting. Soil samples were collected at 30, 40, and 50 days after transplanting (corresponding to 20, 30, and 40 days of saline-alkali stress), and PCR-DGGE was used to analyze rhizosphere microbial communities. The results showed that the number of fungal DGGE bands for tolerant rootstocks T1 and T2 was significantly higher than that of sensitive rootstock S2 and self-rooted controls. The Shannon-Wiener index and evenness index for bacteria in T2 rhizosphere soil were significantly higher than those of sensitive rootstocks (S1, S2) and controls. At 50 days after transplanting, the copy number of bacterial 16S rDNA genes in T1 was significantly higher than in sensitive rootstocks and self-rooted seedlings. At 40 days after transplanting, the copy number of fungal ITS genes in T2 was significantly higher than in sensitive rootstocks and controls, and at 50 days, tolerant rootstocks showed significantly higher fungal ITS gene copy numbers than sensitive rootstocks, though not significantly different from controls. These findings demonstrate that different rootstock varieties significantly affect rhizosphere microbial community composition and abundance under saline-alkali stress, with differences becoming more pronounced over time. Tolerant rootstocks may enhance their saline-alkali resistance by improving soil microenvironment conditions.

Keywords: saline-alkali stress; grafted cucumber; saline-alkali resistant rootstock; soil microbial community

Introduction

China' s saline-alkali soils cover approximately 34.87 million hectares, with 7.6 million hectares of cultivated land affected by varying degrees of salinization—about one-quarter of the total arable land area. In Heilongjiang Province alone, saline-alkali land spans 1.16 million hectares, representing 3% of the province' s total land area [1-2]. The expanding area and increasing severity of soil salinization are significantly impacting protected vegetable production and quality, thereby hindering the development of China' s protected vegetable industry [3]. Cucumber (*Cucumis sativus* L.) is a major crop in protected vegetable cultivation, but its root system has poor regenerative capacity and weak salt tolerance. Mitigating soil salinity damage has become an urgent challenge in cucumber production.

Grafting has been shown to substantially improve cucumber salt tolerance [4], offering a practical, cost-effective, and safe solution. Grafting cultivation of cucurbit and solanaceous vegetables represents an effective measure to overcome soil salinization [5]. Soil environmental changes affect microbial community composition, population size, and distribution [6]. Saline-alkali stress reduces microbial activity and carbon/nitrogen content, decreasing organic matter decomposition [7] while also affecting plant growth, photosynthesis, and nutrient uptake and transport [8]. As a major abiotic stress factor, soil salinization significantly impacts crop development and yield [9].

Soil microorganisms play crucial roles in organic matter decomposition and nutrient mineralization, serving as an active pool of plant nutrients and representing an important indicator of soil quality [10]. Research indicates that soil microbial populations decrease with increasing salinity, with bacterial and actinomycete numbers showing significant negative correlations with total soil salt content, while bacterial numbers show extremely significant correlations with soil organic matter content [11-14]. Different cultivars of the same plant species produce varying root exudate compositions, altering rhizosphere microbial community structure and consequently affecting soil nutrient formation, accumulation, and plant stress resistance [15]. Grafting with different rootstock species can enhance bacterial diversity in watermelon [*Citrullus lanatus* (Thunb.) Matsum. et Nakai] rhizosphere soil, thereby improving resistance [16]. Similarly, grafting can alter microbial population structure and increase beneficial bacteria in eggplant (*Solanum melongena* L.) rhizosphere, enhancing disease resistance [17].

Previous research on vegetable grafting has primarily focused on disease resistance [18], waterlogging tolerance, and low temperature tolerance, with fewer studies examining salt resistance [19], particularly regarding complex saline-alkali tolerance mechanisms of rootstocks. Moreover, the relationship between grafting with different rootstock varieties of the same species and its effects on rhizosphere microbial community structure and abundance remains poorly understood. This study aims to clarify the relationship between saline-alkali stress and soil microorganisms in protected vegetables by simulating saline-alkali soil composition typical of Heilongjiang region. Using PCR-DGGE technology, we investigated how saline stress affects soil microbial community structure and abundance in cucumbers grafted onto rootstocks with different saline-alkali tolerance levels, providing theoretical insights into the mechanisms of grafted cucumber tolerance and offering reliable scientific basis for saline-land improvement.

1. Materials and Methods

1.1 Experimental Materials The experiment was conducted from February to November 2013 at the Energy-Saving Solar Greenhouse of the Facility Engineering Center and the Vegetable Physiology and Ecology Laboratory of Northeast Agricultural University.

Plant materials: Cucumber rootstocks included saline-alkali tolerant varieties 'Huazhen108'(T1) and 'Shenlitiemuzhen'(T2), and saline-alkali sensitive varieties 'Huitailang' (S1) and 'Jingxinzhenliuhao' (S2) [20]. The scion was 'Jinchun 9' cucumber from Tianjin Kernel Cucumber Research Institute, with self-rooted 'Jinchun 9' seedlings serving as controls (CK).

Soil: Collected from a greenhouse at the Facility Engineering Center with long-term continuous cucurbit cropping. Basic physicochemical properties were: organic matter 21.7 $g \cdot kg^{-1}$, total nitrogen 0.125%, alkali-hydrolyzable nitrogen 54.6 $mg \cdot kg^{-1}$, available phosphorus 36.94 $mg \cdot kg^{-1}$, available potassium 177.9 $mg \cdot kg^{-1}$, pH 7.37, and EC 593 $\mu S \cdot cm^{-1}$.

1.2 Experimental Design The main salt components in Heilongjiang saline-alkali soils are NaCl, Na_2SO_4, Na_2CO_3, and $NaHCO_3$, with HCO_3^- accounting for about half of total anions and a $(CO_3^{2-}+HCO_3^-)/(SO_4^{2-}+Cl^-)$ mass ratio of 1-2, averaging pH 8.72 [13]. This experiment used a mixed salt solution at 100 $mmol \cdot L^{-1}$ concentration ($NaHCO_3$:Na_2SO_4:NaCl:Na_2CO_3 = 4:2:2:0.15 molar ratio). Five treatments were established: four grafted combinations (T1, T2, S1, S2) and self-rooted controls (CK), with 12 pots per replicate and three replicates arranged randomly with guard rows.

Cucumber scions were conventionally propagated and grafted using the insertion method. After grafting, when scions reached the three-leaf-one-heart stage, seedlings were transplanted into plastic pots (180 mm × 230 mm) containing 3 kg soil. Fertilizer (diammonium phosphate and potassium sulfate at 1:1 ratio) was applied at 8 g per pot. Ten days after transplanting, 1 L of saline-alkali solution was added to each pot to achieve a final soil salt concentration of 3.08 $g \cdot kg^{-1}$ ($NaHCO_3$ 1.37 $g \cdot kg^{-1}$, Na_2SO_4 1.16 $g \cdot kg^{-1}$, NaCl 0.48 $g \cdot kg^{-1}$, Na_2CO_3

0.065 $g \cdot kg^{-1}$) without leaching (pots had no drainage holes). Standard management practices followed thereafter.

Soil samples were collected at 30, 40, and 50 days after transplanting (corresponding to 20, 30, and 40 days of saline-alkali stress). Three cucumber plants were randomly selected from each replicate, and rhizosphere soil was collected using the root-shaking method, passed through an 80-mesh sieve, and stored at -80 °C for total DNA extraction.

1.3 Measurement Methods **Soil DNA extraction:** Total microbial DNA was extracted from rhizosphere soil using the Power Soil® DNA Isolation Kit (MO BIO Laboratories, CA, USA). Bacterial 16S rDNA was amplified using universal primers GC-338f and 518r [21]. Fungal ITS regions were amplified via nested PCR using universal primers ITS1-F, ITS4, GC-ITS1-F, and ITS2 [22].

Denaturing gradient gel electrophoresis (DGGE): 8% polyacrylamide gels were prepared with denaturant concentration increasing from top to bottom. Bacterial denaturant ranged from 40% to 75% (100% denaturant = 7 $mol \cdot L^{-1}$ urea and 40% deionized formamide). Electrophoresis was performed using a D-code System (Bio-Rad Lab, LA, USA) at 75 V and 60 °C for 12 h. Gels were stained with GelRed (Biotium, USA) at 1:3000 (v/v) for 25 min and photographed using a UVP labwork 4.6 imaging system.

Real-time quantitative PCR: Analyzed using the IQ5 real-time PCR system (Bio-Rad Lab, LA, USA).

1.4 Data Analysis Raw data were processed using Microsoft Excel (Office 2010). Statistical analysis was performed using SAS 8.1 (ANOVA procedure) and SPSS 16.0 (cluster analysis). DGGE fingerprint analysis was digitized and standardized using Bio-Rad Quantity One 4.5 software, and principal component analysis was conducted using Canoco for Windows 4.5 software [23].

2. Results

2.1.1 Differences in Soil Bacterial Community Structure At 30 days after transplanting (20 days stress), DGGE profiles showed no obvious differences in rhizosphere bacterial communities among rootstock treatments [Figure 1: see original paper]. Principal component analysis (PCA) revealed that PC1 and PC2 accounted for 26.5% and 14.5% of total variance, respectively. Self-rooted seedlings (CK) were distinctly separated from grafted treatments, indicating different bacterial community structures. However, tolerant and sensitive rootstock treatments were relatively uniformly distributed, suggesting similar bacterial community composition at this early stage.

At 40 days after transplanting (30 days stress), DGGE profiles remained similar across treatments [Figure 2: see original paper]. PCA showed that tolerant rootstock treatments (T1, T2) were separated from other treatments, while sensitive rootstocks clustered together but were distinct from self-rooted seedlings, indicating emerging differences in bacterial community structure.

At 50 days after transplanting (40 days stress), DGGE patterns were still visually similar [Figure 3: see original paper]. PCA revealed that tolerant rootstocks T1 and T2 occupied the same quadrant, while sensitive rootstocks were separated from self-rooted seedlings, confirming substantial differences in bacterial community structure among treatments.

As shown in Table 1 , at 30 days after transplanting, the number of bacterial DGGE bands in self-rooted controls was significantly higher than in tolerant rootstock T2 ($P<0.05$), though Shannon-Wiener and evenness indices showed no differences. At 40 days, no differences in band numbers were observed, but tolerant rootstock T1 showed significantly higher Shannon-Wiener and evenness indices than sensitive rootstock S1 ($P<0.05$). At 50 days, self-rooted controls had significantly more bands than sensitive rootstock S1, while tolerant rootstock T2 exhibited significantly higher Shannon-Wiener and evenness indices than sensitive rootstock S1 ($P<0.05$).

2.1.2 Differences in Soil Fungal Community Structure At 30 days after transplanting (20 days stress), fungal DGGE profiles showed no obvious differences among treatments [Figure 4: see original paper]. PCA indicated that tolerant rootstocks T1 and T2 clustered closely together and were separated from self-rooted seedlings (CK) and sensitive rootstocks (S1, S2), suggesting distinct fungal community structures in tolerant rootstocks compared to other treatments.

At 40 days after transplanting (30 days stress), fungal DGGE profiles remained visually similar [Figure 5: see original paper]. PCA showed that tolerant rootstocks T1 and T2 occupied the same quadrant, while sensitive rootstocks (S1, S2) clustered in another quadrant, and self-rooted seedlings (CK) were distinctly separated, indicating significant differences between grafted and self-rooted seedlings. The tight clustering of replicate points for each treatment demonstrated good reproducibility.

At 50 days after transplanting (40 days stress), fungal DGGE patterns showed no obvious visual differences [Figure 6: see original paper]. PCA revealed that tolerant rootstocks T1 and T2 clustered closely, while sensitive rootstock S2 was nearby and S1 was more distant, indicating some variation between sensitive varieties. All grafted treatments were distinctly separated from self-rooted seedlings (CK), confirming substantial differences in fungal community structure.

At 30 and 40 days after transplanting, no significant differences were observed in band numbers, Shannon-Wiener indices, or evenness indices among treatments ($P<0.05$). However, at 50 days, tolerant rootstocks (T1, T2) showed significantly higher band numbers than sensitive rootstock S2 and self-rooted controls ($P<0.05$). Additionally, tolerant rootstock T2 exhibited significantly higher Shannon-Wiener and evenness indices than sensitive rootstocks (S1, S2) and controls ($P<0.05$) .

2.2 Differences in Microbial Community Abundance Fungal ITS gene copy numbers increased with cucumber growth [Figure 7: see original paper]. At 30 days after transplanting (20 days stress), sensitive rootstock S1 showed significantly lower fungal ITS gene copy numbers than other treatments and controls. At 40 days (30 days stress), tolerant rootstock T2 had significantly higher fungal ITS gene copy numbers than sensitive rootstocks (S1, S2) and self-rooted controls. At 50 days (40 days stress), tolerant rootstocks (T1, T2) showed significantly higher fungal ITS gene copy numbers than sensitive rootstocks, though not significantly different from controls. Throughout the 30-50 day period, the salt-sensitive S1 treatment consistently exhibited lower fungal ITS gene copy numbers than other treatments and controls.

Bacterial 16S rDNA gene copy numbers increased gradually with cucumber growth [Figure 8: see original paper]. At 30 days after transplanting (20 days stress), self-rooted seedlings showed significantly higher bacterial 16S rDNA gene copy numbers than tolerant rootstocks (T1, T2) ($P<0.05$). At 40 days (30 days stress), self-rooted seedlings and tolerant rootstocks (T1, T2) had significantly higher bacterial 16S rDNA gene copy numbers than sensitive rootstock S1 ($P<0.05$). At 50 days (40 days stress), tolerant rootstock T1 exhibited significantly higher bacterial abundance than all other treatments ($P<0.05$).

3. Discussion and Conclusion

Soil microorganisms serve as sensitive indicators of soil environmental conditions, with community structure and diversity reflecting soil quality [24]. Rhizosphere microbial populations and ratios influence plant growth and yield, soil health, and the incidence and severity of soil-borne diseases [25]. Soil bacteria, as major components of the soil microbial community, outnumber other soil organisms in both quantity and diversity and can decompose various organic substances. Research indicates that saline-alkali stress disrupts soil microbial community structure and reduces bacterial content [26]. Fungi, common soil microorganisms, decompose cellulose, starch, gums, lignin, and readily degradable proteins and carbohydrates, playing important roles in humus formation and soil aggregate stabilization.

Many common bands appeared in bacterial and fungal DGGE profiles across different rootstock treatments and self-rooted seedlings, indicating that these microorganisms are stable in soil and unaffected by grafting, sampling time, or saline-alkali treatment, consistent with Bao’ s findings [27]. Principal component analysis of DGGE data showed that PCA distances between rootstock treatments with different saline-alkali tolerance increased with stress duration, indicating diverging microbial community structures, which aligns with Zhao’ s research [16].

Microbial diversity influences soil ecosystem structure, function, and processes, representing an important component for maintaining soil productivity [28]. Common indices in microbial community studies include evenness, richness, and diversity indices [29]. This study found that Shannon-Wiener and evenness indices for both fungi and bacteria in tolerant rootstock rhizosphere soil were significantly higher than in sensitive rootstocks and self-rooted controls, demonstrating rootstock-dependent changes in microbial community structure, consistent with Wu et al. [15]. Previous studies showed that under salt stress, salt-tolerant cucumber varieties had higher fungal ITS and bacterial 16S rDNA gene copy numbers than salt-sensitive varieties, reaching significance at 30 and 50 days after transplanting [3]. In this study, fungal ITS gene copy numbers increased with cucumber growth under saline-alkali stress. At 50 days, tolerant rootstocks T1 and T2 showed significantly higher fungal ITS gene copy numbers, and T1 exhibited significantly higher bacterial 16S rDNA gene copy numbers, demonstrating significant cultivar differences. This suggests that saline-alkali stress may inhibit organic matter decomposition and humus formation, thereby altering fungal and bacterial populations. These differences likely arise from variations in root exudate composition and quantity among rootstock varieties with different saline-alkali tolerance levels, similar to Ling et al.' s conclusions [30].

In conclusion, as saline-alkali stress duration increased, differences in rhizosphere microbial community structure among rootstock treatments became more pronounced. This may result from saline-alkali components affecting soil physicochemical properties and root growth, with different degrees of growth inhibition among plants, while changes in beneficial root exudates differentially impacted microbial growth and reproduction [31-32]. Consequently, significant differences emerged in microbial community structure among treatments. Saline-alkali tolerant rootstocks may enhance their tolerance by improving soil microbial quantity and richness. The combination of real-time PCR and PCR-DGGE methods in this study revealed consistent trends in microbial abundance and DGGE band numbers, demonstrating that these complementary approaches can effectively characterize soil microbial dynamics, providing a theoretical basis for high-quality cucumber production in saline-alkali soils.

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