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Enhanced Post-Drought Compensatory Growth and Water Utilization in Maize via Rhizosphere Soil Nitrification by Heterotrophic Ammonia-Oxidizing Bacteria

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Abstract: Heterotrophic ammonia-oxidizing bacteria (HAOB), crucial for soil nitrification, have unclear benefits for crop water use. This study explored the impact of a novel HAOB strain, S2_8_1, on maize drought resilience via pot culturing. The experiment included various treatments: control with sufficient water (CK), sufficient water + HAOB strain (WI), limited rewatering (DL), sufficient rewatering (DH), sufficient rewatering + HAOB strain (DHI), and limited rewatering + HAOB strain (DLI). The results revealed below-compensatory growth with DL compared to CK. Interestingly, the HAOB strain displayed survival resilience with a 96% increase in its copy numbers in the rhizosphere soils compared to CK during rewatering. The DLI treatment exhibited equal to compensatory growth, showing a remarkable 169% surge in the water use efficiency versus CK. This improvement was attributed to heightened rhizosphere soil nitrification by HAOB, enhancing the cytokinin production in roots and its transference to leaves, leading to a 25% higher leaf cytokinin concentration with DLI compared to CK during rewatering. Additionally, HAOB DHI prompted overcompensatory growth after sufficient rewatering, boosting nitrification and facilitating cytokinin root-to-leaf transport. However, its water use efficiency was 39% lower than DLI. The study highlights HAOB's importance in optimizing crop water use, particularly in scenarios of limited rewatering in cropland soils.

Keywords: heterotrophic ammonia-oxidizing bacteria; compensatory growth; post-drought growth; cytokinin; limited rewatering; maize

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1. Introduction

Water scarcity poses a pressing threat to maize (*Zea mays* L.) production in northern China, a region known for its vulnerability to droughts [1]. Enhancing maize water utilization efficiency is crucial in addressing this issue. Bacteria and other soil microorganisms play a significant role in enhancing water use efficiency [2,3]. Therefore, prioritizing research on manipulating soil bacteria to enhance maize water utilization is essential.

Various water-saving techniques, including regulated deficit and supplemental irrigations, as well as deficit irrigation, are extensively employed in agriculture [4–6]. These technologies are based on plant compensatory growth, where growth inhibited by drought stress is compensated for through accelerated growth upon rewatering. Compensatory growth can be categorized as undercompensation (biomass recovery below the lost levels), compensation (recovery equal to loss), and overcompensation (recovery surpassing lost biomass) [7]. Efficient water use during the rewatering period is the key to the water-saving mechanism of compensatory growth [8,9]. Both sufficient and limited water supplies can induce maize compensatory growth [10,11]. However, limited water supplies demonstrate better water-saving potential compared to abundant water supplies. Therefore, implementing limited rewatering strategies after drought conditions holds promise for water saving in maize production.

After drought, rewatering can trigger compensatory growth in crops, which was characterized by accelerated growth upon the reintroduction of water to the roots. Enhancing maize's compensatory growth has been observed with the application of nitrogen or organic fertilizer along with sufficient rewatering [12,13]. This phenomenon can be ascribed to the heightened availability of nitrate nitrogen (NO_3^-). Synthetic nitrogen or organic fertilizers typically raise the soil's NO_3^- levels through nitrification processes [14,15]. Research has observed that soil NO_3^- promotes cytokinin (CK) synthesis in maize roots, leading to CK transport to the leaves and stimulating maize growth during post-drought rewatering [16].

Another study found that rewatering with ample water induced the proliferation of the symbiotic bacterium *Ensifer sesbaniae* in maize rhizospheres [17]. This bacterium facilitates root-induced cytokinin delivery to leaves, promoting compensatory growth. The *Ensifer sesbaniae* belongs to heterotrophic ammonia-oxidizing bacteria, and is crucial for soil nitrification and NO_3^- release. The key point here is that soil nitrification primarily consists of two steps: first, ammonia-oxidizing bacteria and ammonia-oxidizing archaea convert ammonium nitrogen into nitrite nitrogen; then, nitrite-oxidizing bacteria oxidize nitrite nitrogen into NO_3^-. In soil, nitrite nitrogen is easily oxidized into NO_3^-. However, the oxidation of ammonium nitrogen into nitrite nitrogen is the rate-limiting step in soil nitrification. Ammonia-oxidizing bacteria are typically found in soil rich in organic nutrients, while ammonia-oxidizing archaea often appear in nutrient-poor soils. Soil moisture and nutrients are taken up by plant roots, which can secrete nutrients to provide a source of carbon and nitrogen for rhizospheric soil microorganisms. Therefore, ammonia-oxidizing bacteria typically inhabit the rhizospheric soil of plants and tend to be heterotrophic. In a recent study, a strain of heterotrophic ammonia-oxidizing bacteria (HAOB) was found to have a strong correlation with the transport of cell division hormones from the roots to the leaves [18]. However, limited research explores how soil microorganisms induce compensatory growth in maize through roots under limited rewatering after drought. Further studies are needed to investigate this aspect and deepen our understanding of the mechanisms involved.

The present study investigated the compensatory mechanism of maize seedlings, which are highly sensitive to drought stress and rewatering. Understanding these mechanisms is crucial for maize water-saving production, considering maize's significance as China's most productive grain crop. We hypothesized that heterotrophic ammonia-oxidizing bacteria (HAOB) would promote maize's compensatory growth during rewatering, especially with a limited water supply. To test this, we used quantitative fluorescence polymerase chain reaction (PCR) to measure the abundance of HAOB in the rhizosphere soil. We also measured the leaf cytokinin levels, photosynthesis, and soil nitrification rates and biomasses to study compensatory growth mechanisms following drought and limited rewatering.

2. Materials and Methods

2.1. Experimental Design

2.1.1. Experimental Grouping and Treatments

The HAOB strain employed in this study is stored in the Chinese Center for Preservation of Typical Cultures (Wuhan University, Wuhan, China) with the deposit number "CCTCC NO: M2021374" and the GenBank accession number "ON667919". The strain, named S2_8_1, belongs to *Rhizobiaceae*, *Ensifer*, and was extracted and subjected to screening from the soil at Henan University of Science and Technology, where the study was conducted. The purified culture of S2_8_1 was as follows: 0.5 g CH3COONa, 0.03 g $MgSO_4 \cdot 7H_2O$, 0.25 g NaH_2PO_4, 0.01 g $MnSO_4 \cdot 4H_2O$, 5.0 g $CaCO_3$, 0.5 g $(NH_4)_2SO_4$, 0.75 g

KH_2PO_4, 1 L distilled water. When the culture was inoculated with S2_8_1, its number in the culture was about 2,160,900 cfu/L.

A potted maize experiment was conducted at Henan University of Science and Technology's experimental farm in Luoyang City, Henan Province, China (34°32′ N, 112°16′ E). The region experiences an annual average rainfall of 601 mm, a mean temperature of 14.2 °C, and approximately 2204.9 h of sunlight. "Zhengdan 958", known for its wide adoption in China and drought tolerance, was chosen as the maize variety. Starting on 5 June 2022, 15 maize seeds were sown in each of 300 plastic pots. These pots had a diameter of 21.5 cm and a height of 20.0 cm, containing approximately 5.8 kg of soil with approximately 24.7 g/kg organic carbon and 2.15 g/kg total nitrogen. Maize seedlings emerged within about 6 days. The experimental process is shown in Figure 1.

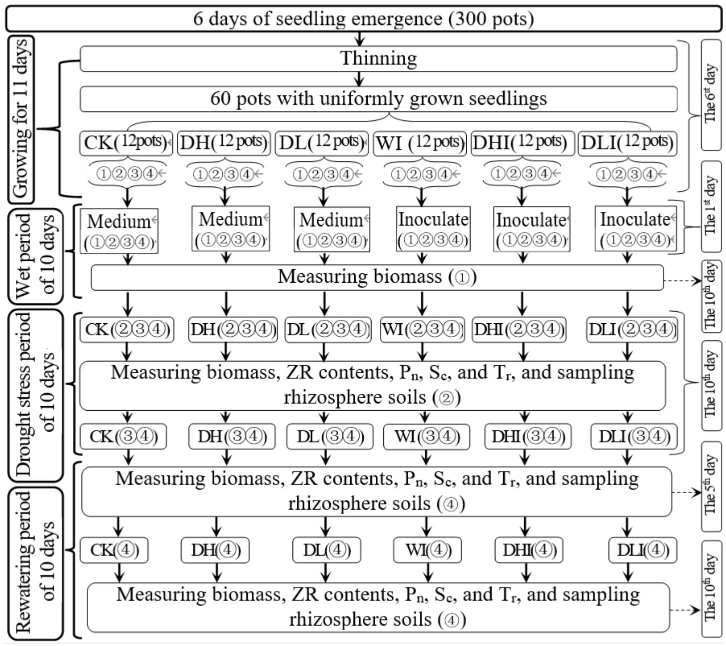

Figure 1. Schematic diagram for the experimental design. The abbreviations CK, DH, DL, WI, DHI, and DLI correspond to treatments of control with sufficient water supply, sufficient rewatering after drought, limited rewatering after drought, sufficient water supply with HAOB strain inoculation, sufficient rewatering after drought with HAOB strain inoculation, and limited rewatering after drought with HAOB strain inoculation. The numbers "①", "②", "③", and "④" represent the first, second, third, and fourth subgroups within each treatment, respectively. P_n represents the net photosynthetic rate, S_c represents stomatal conductance, and T_r represents the transpiration rate. The terms "Inoculate" and "Medium" refer to the addition of culture medium solution with and without HAOB stain, respectively.

On the 6th day after emergence, thinning was conducted, leaving five vigorous seedlings per pot. Subsequently, 72 pots with consistent growth were selected for the study, organized into six groups, each consisting of 12 pots. These six groups represented specific treatments: (1) control with sufficient water supply (CK), (2) sufficient water supply with HAOB strain inoculation (WI), (3) limited rewatering after drought (DL), (4) sufficient rewatering after drought (DH), (5) sufficient rewatering after drought with HAOB strain inoculation (DHI), (6) limited rewatering after drought with HAOB strain inoculation (DLI). Within each treatment group, the 12 containers were further divided into four subsets, each comprising three containers. These subsets served as replicates to improve measurement reliability.

2.1.2. Timeline of the Experiment

From the 12th to the 22nd day after emergence, there was a 10-day period of sufficient water supply for all treatments. At the beginning of this period, the S2_8_1 strain was introduced into the WI, DLI, and DHI treatments by adding 200 mL of its culture medium solution to the soil. The DH, DL, and CK treatments received 200 mL of culture medium solution without the strain. The first subgroup in each treatment had its maize biomass measured to assess plant water use efficiency.

After the period of sufficient water supply, drought stress and subsequent rewatering occurred from the 23rd to the 32nd day and from the 33rd to the 42nd day, respectively. During the drought stress period, subgroups 2, 3, and 4 of the DH, DL, DHI, and DLI treatments experienced drought stress, while the CK and WI treatments continued to receive sufficient water. In the rewatering period, the CK, WI, DH, and DHI treatments had adequate water supply, while the DL and DLI treatments had limited water supply. At the end of the drought period, assessments were made for maize biomass, zeatin riboside (ZR) content, net photosynthetic rate (P_n), stomatal conductance (S_c), and transpiration rate (T_r), both on the 5th and 10th days after rewatering. Rhizosphere soil samples were collected from subgroups 2, 3, and 4 of each treatment for further analysis.

2.1.3. Soil Moisture Control Methods

Research has established that soil moisture levels of 75 ± 5%, 60 ± 5%, and 45 ± 5% of field capacity indicate wetness, moderate drought, and severe drought, respectively [19]. Based on these findings, our study implemented three conditions: sufficient water supply, limited water supply, and drought stress, by maintaining soil moisture levels at 75 ± 5%, 60 ± 5%, and 45 ± 5% of field capacity. A preliminary experiment in this study showed that a 10-day period of drought stress significantly inhibited maize growth without causing substantial damage. To maintain the desired soil moisture levels, water was added when the soil moisture in the containers fell below 45%, 60%, or 75% of field capacity. Formula (1) was used to calculate soil water content [20].

$$\text{SWC} = \frac{B_t - B_d - B_e - B_p}{B_d \times \text{FWC}} \times 100\% \quad (1)$$

where SWC represents soil water content, while *Bt*, *Bd*, *Be*, and FWC, respectively, represent instantaneous total pot weight, net weight of dried soil, weight of empty pots, and approximated live plant weight. FWC signifies field capacity. *Bp* was measured using additional pots early.

2.2. Measurements and Data Processing

2.2.1. Biomass and Photosynthesis Indicators

Washing was employed to extract the roots from the soil. Fresh root, stem, and leaf samples underwent 72 h drying at 65 °C, yielding dry weights. Total biomass combined these dry weight components, while aboveground biomass only considered leaves and stems. Water use efficiency was calculated by dividing total biomass gain by the water utilized during drought stress and rewatering periods. Total biomass gain represented the increase throughout the entire period spanning from the initiation of drought stress to the end of rewatering. Water use referred to added water during drought stress and rewatering. The P_n, T_r, and S_c were assessed via the photosynthesis analyzer of the LI-6400 equipment from 10:30 a.m. to 12:30 p.m.

2.2.2. Soil Nitrification Rate and Zeatin Riboside Content

NH_4^+ and NO_3^- levels in rhizosphere soils were evaluated, respectively, using indophenol blue and phenol disulphonic acid colorimetric methods [21]. The net nitrification rate in the rhizosphere soil was determined by incubating soil samples undergoing a 7-day period at 25 °C, while preserving their original soil moisture levels. The daily net rhizosphere soil nitrification rate was computed by dividing NO_3^- increase resulting from incubation by 7 days.

After cutting the maize stem at its base, a precisely weighed section of absorbent cotton (1.0 g), encased in plastic film to limit moisture loss, was immediately placed over the cut to collect xylem sap across 12 h. The cotton weight gain was split by its density of 1 g/cm^3 to gauge the volume of the xylem sap. Subsequently, the cotton was compressed to extract sap for quantifying concentration of ZR in the xylem sap (C_{ZR}). The rate of ZR transport across the root-to-leaf pathway (R_{ZR}) during darkness was calculated as the C_{ZR} per hour. An enzyme-linked immunosorbent assay was used to measure the ZR concentrations in leaves and xylem sap [22]. Notably, ZR test kits utilized were sourced from the reputable Shanghai Enzyme-linked Biotechnology Co., Ltd. based in Shanghai, China.

2.2.3. Quantitative Real-Time PCR

The MO-BIO PowerSoil DNA Isolation Kit was employed to extract the total DNA from the soil genome, and a template was used for PCR amplification of the total DNA. The PCR amplification targeting the S2_8_1 gene utilized specific primers: F (5′-ATGTACTGCGCTC-AAATCCGA-3′), R (5′-ATGATGAAGGCAAAACCACGAT-3′), and probe P (5′-FAM-AC-AACGCAGAAGTCGCACGGAAG-BHQ1-3′). In a 25 µL reaction, 12.5 µL of 2× Premix Ex Taq (qPCR probe) solution, 0.5 µL of both forward and reverse primers (10 µM each), 0.5 µL of probe (at a concentration of 10 µM), 5 µL aliquot of the DNA template, 6 µL of double-distilled water were combined. Reaction conditions included initial denaturation under a temperature of 95 °C for a duration of 30 s, followed by denaturation by heating to 95 °C for 10 s, annealing at 60 °C for a duration of 45 s, and 45 cycles of amplification. Each soil sample underwent triplicate testing. Amplification was performed on the extracted total soil genome DNA, and the quantified Ct value obtained through fluorescence-based PCR was used within the equation of the standard curve for calculating the copy count of S2_8_1 (copies/g), reflecting bacterial quantity.

A standard curve was generated by amplifying primers designed specifically for S2_8_1. The resultant products underwent purification, followed by plasmid construction and transformation into bacterial cells of Escherichia coli. The *E. coli* harboring the plasmid with the target gene were cultured at 37 °C in shaking flasks. Plasmids were isolated utilizing the Axygen Plasmid Miniprep Kit (Axygen). Plasmid concentration was quantified using Qubit 3.0 (Life Biotech) from Shanghai, China, facilitating the computation of plasmid copy counts. Selected gradient dilutions of standard plasmids resulted in key points, e.g., 5.86 × 105, 5.86 × 104, etc. These points formed the basis for a linear standard curve relating Ct values and S2_8_1 concentration in maize rhizosphere soil. This technique establishes a robust foundation for quantitative fluorescence analysis, aiding in understanding S2_8_1 abundance.

Table 1 lists the abbreviations used in this study. Graphs display the mean values. Employing SPSS 23, a general linear model performed one-way ANOVA, followed by the Dunnett test ($\alpha = 0.05$).

Table 1. Symbol definition.

Symbol	Definition	Symbol	Definition
HAOB	Heterotrophic ammonia-oxidizing bacteria	S_c	Stomatal conductance
CK	Control with sufficient Water	T_r	Transpiration rate
WI	Sufficient water + HAOB strain	ZR	Zeatin riboside
DL	Limited rewatering	R_{ZR}	Delivery rate of ZR from roots to leaves
DH	Sufficient rewatering	C_{ZR}	ZR concentration in xylem sap
DHI	Sufficient rewatering + HAOB strain	B_t	Temporary weight of the whole pot
DLI	Limited rewatering + HAOB strain	B_d	Net weight of dried soil of pot
NO_3^-	Soil nitrate	B_e	Weight of the empty pot
NH_4^+	Soil ammonium	FWC	Field water capacity
P_n	Photosynthetic rate		

3. Results

3.1. Changes in Biomass

After rewatering on day 0, the CK and WI treatments displayed a notably higher aboveground biomass compared to the other treatments, which was also reflected in the total biomass (Figure 2), highlighting the drought stress's impact on maize growth. By days 5 and 10 post-rewatering, the WI, DLI, and DHI treatments showed significant improvements in the aboveground and total biomasses compared to the CK, DL, and DH treatments, showcasing the HAOB strain's positive effects on maize growth.

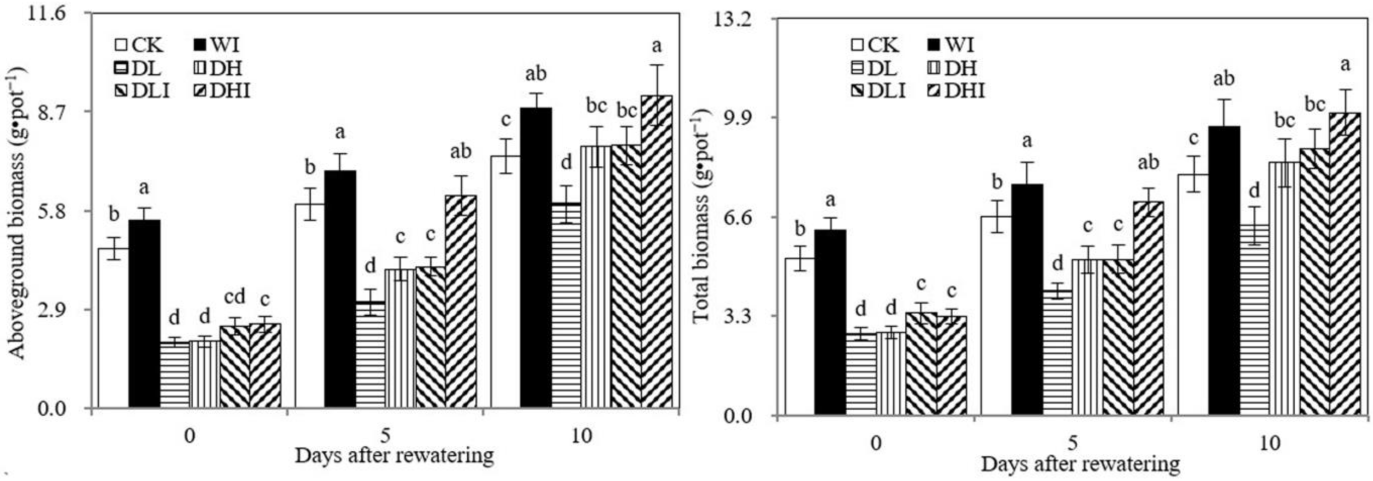

Figure 2. Biomass comparison among different treatments. The abbreviations CK, WI, DH, DL, DHI, and DLI correspond to treatments of control with sufficient water supply, sufficient water supply with HAOB strain inoculation, sufficient rewatering after drought, limited rewatering after drought, sufficient rewatering after drought with HAOB strain inoculation, and limited rewatering after drought with HAOB strain inoculation, respectively. The values represent the mean ± standard error (n = 3). Letters placed above the bars indicate significant differences between treatments at a significance level of $p \leq 0.05$.

On day 10 post-rewatering, the CK and DH treatments exhibited a similar total biomass, suggesting adequate rehydration promoted compensatory growth. Limited rewatering with HAOB strain inoculation resulted in compensatory growth, seen in DLI and CK, with a similar total biomass on the 10th day post-rewatering. Conversely, ample rewatering with HAOB strain inoculation triggered super-compensatory growth, increasing the DHI treatment's total biomass by 26% over CK on day 10 post-rewatering. In contrast, restrained rewatering without HAOB strain inoculation led to below-compensatory growth, reducing the DHI treatment's total biomass by 18% compared to CK on day 10 post-rehydration.

The water use efficiency showed similarities between the CK and WI treatments, while the DLI, DHI, DL, and DH treatments displayed significant increases of 169%, 96%, 94%, and 66% compared to CK, respectively (Figure 3). Limited rewatering with HAOB strain inoculation had the most pronounced effect on enhancing water use efficiency, followed by sufficient rewatering with HAOB strain inoculation and limited rewatering without HAOB strain inoculation. Sufficient rewatering with HAOB strain inoculation had the least pronounced effect on water use efficiency.

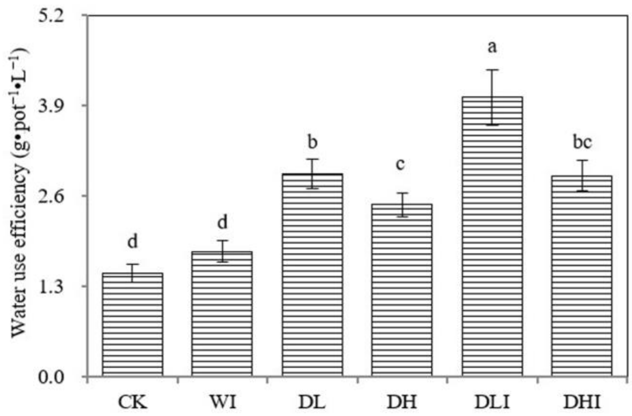

Figure 3. Water use efficiency across different treatments. The abbreviations CK, WI, DH, DL, DHI, and DLI correspond to treatments of control with sufficient water supply, sufficient water supply with HAOB strain inoculation, sufficient rewatering after drought, limited rewatering after drought, sufficient rewatering after drought with HAOB strain inoculation, and limited rewatering after drought with HAOB strain inoculation, respectively. The values represent the mean ± standard error (n = 3). Letters placed above the bars indicate significant differences between treatments at a significance level of $p \leq 0.05$.

3.2. Photosynthesis Analysis

Before rewatering, significantly higher P_n, T_r, and S_c readings were observed in the CK and WI treatments compared to others, indicating drought stress inhibited maize photosynthesis (Figure 4). Before rewatering and on days 5 and 10 post-rewatering, P_n, T_r, and S_c showed significantly higher values in the DHI treatment compared to DH, in the DLI treatment compared to DL, and in the WI treatment compared to CK, indicating that the HAOB strain boosted maize photosynthesis regardless of drought or rewatering. Adequate rewatering further enhanced photosynthesis, while limited rewatering hindered its recovery, as seen in the significant increases with DH compared to CK, and CK compared to DL on days 5 and 10 post-watering.

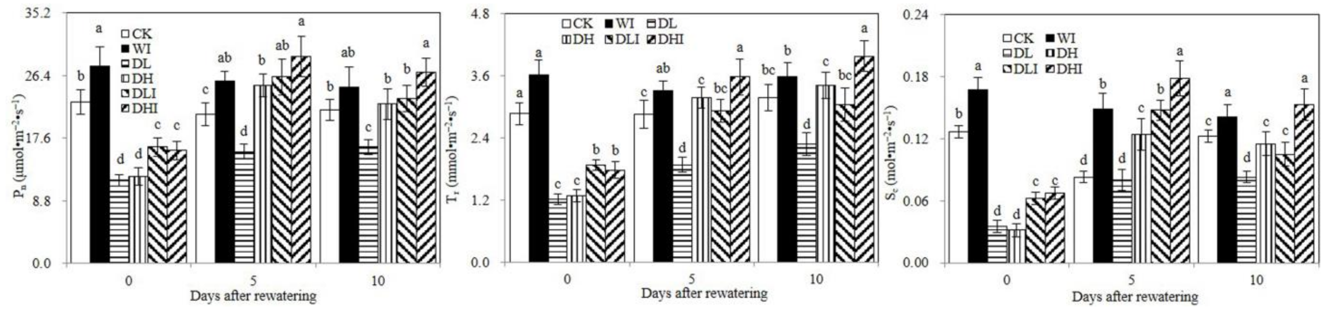

Figure 4. Photosynthesis rate (P_n), stomatal conductance (S_c), and transpiration rate (T_r) in different treatments. The abbreviations CK, WI, DH, DL, DHI, and DLI correspond to treatments of control with sufficient water supply, sufficient water supply with HAOB strain inoculation, sufficient rewatering after drought, limited rewatering after drought, sufficient rewatering after drought with HAOB strain inoculation, and limited rewatering after drought with HAOB strain inoculation, respectively. P_n represents the net photosynthetic rate, S_c represents stomatal conductance, and T_r represents the transpiration rate. The values represent the mean ± standard error (n = 3). Letters placed above the bars indicate significant differences between treatments at a significance level of $p \leq 0.05$.

On day 5 post-rewatering, DLI exhibited significant P_n increases compared to CK, indicating a more pronounced positive impact on photosynthesis with limited rehydration and HAOB strain inoculation. Moreover, significant P_n increases were observed in DHI compared to DLI, highlighting how sufficient rewatering under HAOB strain inoculation offered greater photosynthesis benefits than limited rewatering.

3.3. Changes in Soil Nitrification Rate and Soil Concentrations of NO_3^- and NH_4^+

Compared to the DL and DH treatments, the rhizosphere soil nitrification rates significantly increased with CK and WI on day 0 post-rehydration (Figure 5), indicating water scarcity suppresses this. However, on the 5th and 10th days post-rewatering, the DH treatment showed significant increases in rhizosphere soil nitrification rates compared to CK, suggesting sufficient rewatering promotes it. Regardless of HAOB strain inoculation, sufficient rewatering led to higher rhizosphere soil nitrification rates than limited rewatering, as observed in the significantly higher rates with DHI compared to the DLI treatment, as well as with DH compared to DL treatment on days 5 and 10 post-rehydration.

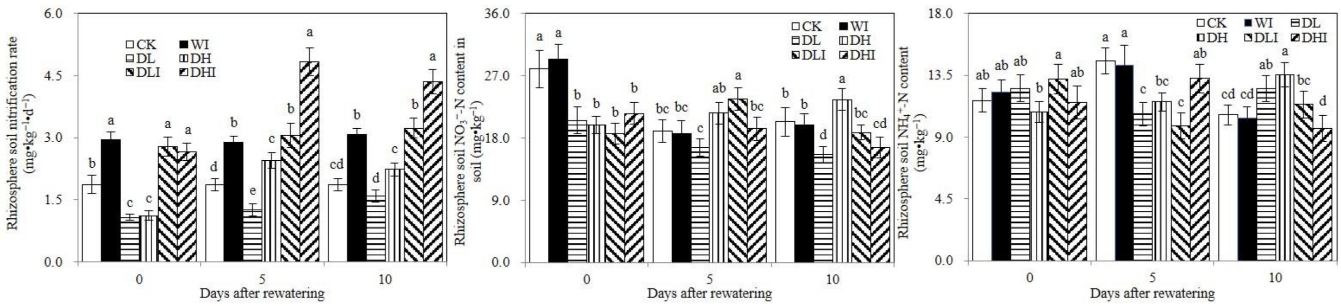

Figure 5. Soil nitrification rate, soil ammonium, and nitrate nitrogen contents in different treatments. The abbreviations CK, WI, DH, DL, DHI, and DLI correspond to treatments of control with sufficient water supply, sufficient water supply with HAOB strain inoculation, sufficient rewatering after drought, limited rewatering after drought, sufficient rewatering after drought with HAOB strain inoculation, and limited rewatering after drought with HAOB strain inoculation, respectively. The values represent the mean ± standard error ($n = 3$). Letters placed above the bars indicate significant differences between treatments at a significance level of $p \leq 0.05$.

On days 5 and 10 post-rewatering, limited rewatering with HAOB strain inoculation (DLI treatment) exhibited significant increases in the rhizosphere soil nitrification rates compared to CK, indicating a stronger positive effect on soil nitrification than sufficient water supply. However, NO_3^- and NH_4^+ concentrations within the rhizosphere soil among treatments during the rewatering period displayed irregular results, suggesting that neither the HAOB strain nor rewatering increased their concentrations.

3.4. Changes of Zeatin Riboside

At the beginning of rewatering, the CK treatment showed a notable increase in the leaf ZR content and C_{ZR} compared to the DH and DL treatments. Similarly, the WI treatment exhibited a higher ZR content and C_{ZR} compared to the DHI and DLI treatments (Figure 6). These observations suggest that drought stress negatively affects the cytokinin content in both leaves and xylem sap, as ZR is considered one of the principal forms of cytokinins.

The ZR content in leaves, as well as R_{ZR} and C_{ZR}, all significantly increased with DHI compared to DH, with DLI compared to DL, and with WI compared to CK on day 5 or 10 after rewatering. This indicates that the HAOB strain contributes to elevated cytokinin levels in leaves and xylem sap, as well as enhanced cytokinin transportation from roots to leaves. Similarly, rewatering was also found to be beneficial, as shown by significant increases in DH and DL compared to CK, and in DHI and DLI compared to WI on day 5 or 10 after rewatering.

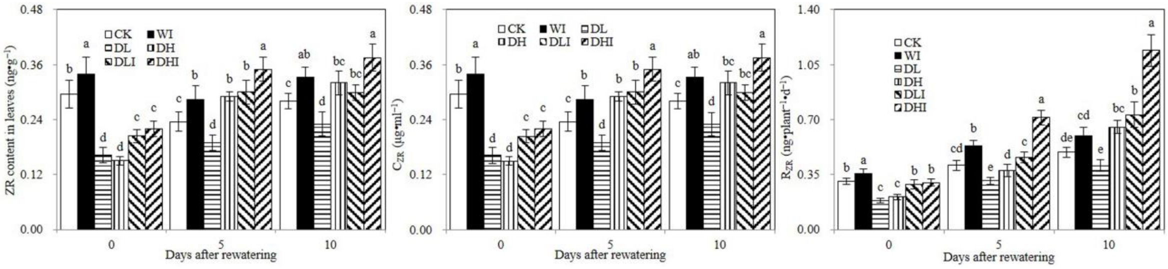

Figure 6. Leaf ZR content, C_{ZR}, and R_{ZR} in different treatments. The abbreviations CK, WI, DH, DL, DHI, and DLI correspond to treatments of control with sufficient water supply, sufficient water supply with HAOB strain inoculation, sufficient rewatering after drought, limited rewatering after drought, sufficient rewatering after drought with HAOB strain inoculation, and limited rewatering after drought with HAOB strain inoculation, respectively. ZR stands for zeatin riboside, C_{ZR} represents the concentration of ZR in xylem sap, and RZR indicates the delivery rate of ZR from roots to leaves under darkness. The values represent the mean ± standard error (n = 3). Letters placed above the bars indicate significant differences between treatments at a significance level of $p \leq 0.05$.

Compared to limited rehydration, sufficient rewatering led to an increased leaf cytokinin and xylem sap cytokinin content, as well as enhanced cytokinin transport along the root-to-leaf pathway, regardless of HAOB strain inoculation. Notably, the ZR content in leaves, along with the R_{ZR} and C_{ZR}, increased with DHI vs. DLI, and DH vs. DL on day 5 or 10 post-rehydration. On days 5 and 10 post-rewatering, the DLI treatment significantly boosted the ZR content in leaves and the R_{ZR} compared to CK, indicating a strong positive impact on the leaf cytokinin levels and their transport from roots to leaves.

3.5. Copy Number Analysis

The quantity of S2_8_1 copies in the rhizosphere soil significantly increased with WI compared to CK, with DLI compared to DL, and with DHI compared to DH on days 0, 5, and 10 after rewatering (Figure 7). These findings indicate that the HAOB strain's inoculation contributed to the augmentation of its population in the rhizosphere soils. Moreover, both sufficient and limited rewatering had comparable effects on the S2_8_1 copy numbers. Specifically, on day 0 after rewatering, there was a 51% and 44% higher quantity of S2_8_1 copies with WI relative to DHI and DLI, respectively. However, on days 5 and 10 following rewatering, similar quantities of S2_8_1 copies were observed among the WI, DLI, and DHI treatments.

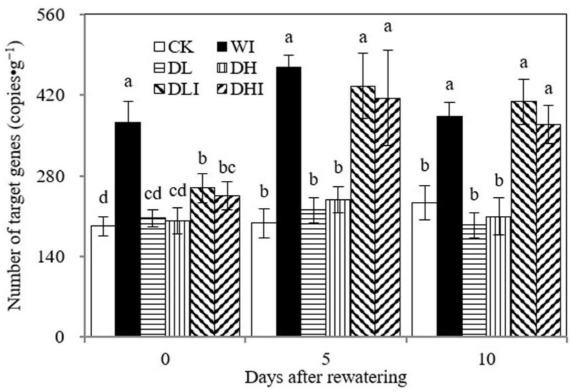

Figure 7. S2_8_1 number of rhizosphere soil in different treatments. The abbreviations CK, WI, DH, DL, DHI, and DLI correspond to treatments of control with sufficient water supply, sufficient water supply with HAOB strain inoculation, sufficient rewatering after drought, limited rewatering after drought, sufficient rewatering after drought with HAOB strain inoculation, and limited rewatering after drought with HAOB strain inoculation, respectively. The values represent the mean ± standard error (n = 3). Letters placed above the bars indicate significant differences between treatments at a significance level of $p \leq 0.05$.

4. Discussion

4.1. Limited Rewatering Inhibiting Compensatory Growth

In this study, during adequate rewatering, the rhizosphere soil's nitrification rate in the DH treatment was 1.3 times higher than in the CK (control) treatment. This led to an increased release of NO_3^- into the soil, subsequently enhancing root cytokinin synthesis. The root tip's apical meristem, crucial for cytokinin synthesis and nutrient absorption, witnessed heightened cytokinin production in response to NO_3^- stimulation [23,24]. Studies have demonstrated that plant root cytokinins can travel to aboveground parts via xylem sap [17,25], further supporting enhanced cytokinin transport to leaves under both dark and sunlight conditions with DH versus CK. Specifically, the DH group exhibited a 14% higher R_{ZR} (root-to-leaf cytokinin transport speed in the absence of light) compared to the CK group. Under sunlight, the DH group displayed a 24% higher C_{ZR} (cytokinin concentration in xylem sap) than the CK group, despite similar T_r (transpiration rate) values. The outcome of multiplying the T_r by C_{ZR} reflects cytokinin transport along the root-to-leaf pathway under sunlight, as transpiration propels sap flow toward the roots. Consequently, during adequate rewatering, the DH group exhibited an 18% higher leaf cytokinin content than the CK group, resulting in an approximate 12% enhancement in the leaf P_n (photosynthetic rate) compared to the CK group. Furthermore, the DH group corrected the initial 30% total biomass gap versus the CK group, achieving equal to compensatory growth.

Post-drought sufficient rewatering boosted maize rhizosphere soil nitrification, as previously shown [21], shedding light on the increased rate with DH during rewatering. Comparable NO_3^- levels in the DH and CK rhizosphere soil during rewatering resulted from significant NO_3^- utilization in both treatments during rapid growth. Cytokinins have an established role in enhancing photosynthesis, cell division, elongation, and organic matter accumulation [26–28], explaining the decrease in the leaf cell division hormone levels with DL during rehydration.

In the cases of limited rewatering (DL treatment), the water scarcity impeded nitrification, causing a 23% and 39% decline in the rhizosphere soil nitrification rates compared to the CK and DH treatments during limited rewatering. This hindered root cytokinin synthesis and root-to-leaf cytokinin transport, as evidenced by over 20% lower R_{ZR}, C_{ZR}, and T_r values with DL compared to CK and DH. The water scarcity with DL may also directly suppress root cytokinin synthesis, as suggested by drought impact research [29]. Consequently, the DL group exhibited an 18% and 31% decrease in the leaf cytokinin levels compared to the CK and DH group during rewatering, resulting in around a 25% lower P_n and 27% lower total biomass than the CK group, and a 33% lower P_n and 21% lower total biomass than the DH group. This led to the below-compensatory growth with DL.

In most croplands, the soil water content undergoes alternating wetting and drying cycles, with water scarcity causing decreases that are typically replenished by rainfall or irrigation. Ensuring an ample water supply for croplands is rare, often occurring briefly after significant rainfall or irrigation events. More commonly, croplands face limited water supply due to moderate or even minor rainfalls or irrigation. According to this study, limited water supply can easily lead to below-compensatory growth in crops, which is detrimental to their yields. Therefore, understanding how to achieve healthy crop growth under water shortage conditions is of utmost importance.

4.2. Limited Rewatering with HAOB Increasing Compensatory Growth

This was supported by a substantial 1.6-fold increase in the total biomass under limited rewatering with the HAOB strain (DLI), compared to a 1.3-fold increase without the HAOB strain (DL). Consequently, the DLI group achieved a similar biomass level to the CK group after rewatering, while the DL group still lagged by 21% compared to the CK group. This led to a state of equal-compensatory growth in the DLI group but below-compensatory growth in the DL group. Therefore, the incorporation of HAOB into the soil under conditions of limited rewatering after drought demonstrates a valuable potential for converting ineffective water, whether from rainfall or irrigation, into a more efficient water source in agricultural fields. This approach facilitates the enhanced utilization of scarce rainfall and moderate irrigation water, effectively mitigating the adverse effects of water scarcity on crop growth.

Specifically, within the DLI treatment, the HAOB strain exhibited survival resilience during post-drought rewatering, evident in the roughly 35% and 96% increases in the S2_8_1 copy numbers in the rhizosphere soils compared to the CK group during both drought stress and rewatering, respectively. This led to over 1.3 times higher rates of nitrification within the rhizosphere soil in the DLI group than the CK group during rewatering, primarily due to the HAOB-mediated enhancement of soil nitrification. Similar to the effects of the DH treatment on compensatory growth mentioned earlier, heightened nitrification in the DLI treatment triggered increased cytokinin production in roots and its subsequent transportation to leaves, resulting in elevated leaf cytokinin levels and improved photosynthesis. This intricate mechanism facilitated maize growth under limited rewatering conditions, culminating in a state of balanced compensatory growth within the DLI group.

Generally, bacteria and other microorganisms exhibit greater drought resistance than plants [30]. That is why the copy numbers of S2_8_1 were comparable in both the low-water supply (DLI) and high-water supply (DHI) treatments following drought stress. However, the inoculation of the HAOB strain significantly enhanced the rhizosphere soil nitrification by 1.5-fold in the DHI group compared to the DLI group during rewatering. This contrast might be attributed to the limited water availability with DLI, which hampers nitrification. The increased rhizosphere soil nitrification facilitated the transport of root cytokinins to leaves, resulting in higher cytokinin levels and photosynthetic rates (P_n) in the DHI group compared to the DLI group. This condition favored biomass accumulation, leading to an approximate 26% increase in the total biomass in the DHI group during rewatering, thus explaining the overcompensatory growth with DHI compared to the equal to compensatory growth with DLI.

4.3. Achieving Efficient Water Use and Promoting Healthy Crop Growth

Despite the DL group's 96% increase in water use efficiency versus the CK group, it resulted in below-compensatory growth, negatively impacting the maize. In contrast, the DLI and DH groups showed equal to compensatory growth, while the DHI group displayed overcompensatory growth, yielding minimal negative impacts on the maize amidst total drought stress and rewatering. This phenomenon indicates the positive role of soil bacteria in regulating maize compensatory growth. Simultaneously, they significantly improved the water use efficiency: DLI by 152%, DH by 69%, and DHI by 94% versus CK. This enhancement was attributed to root-derived cytokinin, amplifying growth with similar or reduced water supplies during rewatering. Among all the treatments, DLI stood out for its superior water-saving impact and notably more robust growth tendencies, emphasizing its significance in the study. HAOB holds the potential to achieve water conservation while promoting agricultural productivity. Further research is warranted to explore how to apply it in practical settings and strive to solve the problem of water shortage in agricultural production.

Despite a research report about pseudomonas bacteria in rapeseed, showing soil bacteria promote the compensatory growth of limited rewatering post-drought [31], this research uncovered the mechanisms through which soil bacteria regulate this compensatory growth, achieving water conservation and promoting healthy crop growth. Furthermore, in the natural environment, there are many soil microorganisms similar to HAOB that are yet to be discovered and studied to validate the mechanism we have revealed. As such, rewatering has practical implications for efficient maize water use. Based on our study, field experiments should be conducted to apply these findings practically.

5. Conclusions

The inoculation of the HAOB strain resulted in its colonization in the rhizosphere soil. Under the limited rewatering condition, the strain notably bolstered soil nitrification, fostering the production of cytokinins within roots and promoting its subsequent transport to the foliage. This, in turn, heightened the leaf cytokinin content and photosynthesis. Consequently, when treating with HAOB, maize plants subjected to limited rewatering displayed remarkable compensatory growth and efficient water usage, surpassing their counterparts with merely sufficient water supply. Conversely, sufficient rewatering with HAOB led to overcompensatory growth, yet lower water use efficiency. Therefore, limited rewatering with HAOB strain inoculation demonstrated superior water-saving effects with a minimal negative impact on maize growth.

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