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Nitrogen Fertilizer Modulates Plant Growth, Chlorophyll Pigments and Enzymatic Activities under Different Irrigation Regimes

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Abstract: Nitrogen fertilization and irrigation patterns have been extensively studied for common maize (*Zea mays* L.), but there is limited published work for Zhengda 619, especially in subtropical areas. Nitrogen (N) fertilizer and irrigation play an important role in crop growth and yield improvements. The study aimed to investigate the yield, growth, chlorophyll content, reactive oxygen species (ROS) and enzyme activities of hybrid maize (Zhengda 619) under greenhouse conditions. Individual plants grown in plastic pots were subjected to two irrigation types—low irrigation (LW; 60% field capacity) and high irrigation water (HW; 80% field capacity)—and five N rates. Our results demonstrate that the LW irrigation increased dry matter, kernel yield, leaf chlorophyll, total root length, root diameter, root volume, and root surface area, as well as soil enzymes and plant antioxidant enzymes, while it lowered malondialdehyde (MDA), proline, and ROS. Moreover, most of the above parameters increased with increasing N application rates up to N3 under LW irrigation due to the increased N availability to the plant and soil enzymes. It is concluded that increasing N rates could improve soil enzyme activities as well as plant antioxidant enzymes and decrease ROS, ultimately resulting in a higher kernel yield under LW irrigation.

Keywords: enzymatic activity; leaf chlorophyll; irrigation; nitrogen; maize

1. Introduction

Maize (*Zea mays* L.) is the third most important primary crop in China and is considered an important pillar of agricultural development [1]. Water and fertilizers, particularly nitrogen (N), are ideal for increasing productivity in regions with water scarcity or abundant rainfall. Nitrogen application increases root and shoot dry matter and kernel yield, as well as improving crop quality [2]. Sustainable intensification in modern agriculture requires an increased efficiency of resources while maintaining or increasing productivity and improving environmental quality [3]. Similarly, adopting suitable water management strategies, such as irrigation management, is critical for achieving high crop water use efficiency (WUE) and yield [4].

One of the most important research topics in agriculture is nitrogen regulation and irrigation management. Nitrogen is a key plant nutrient that promotes and inhibits plant growth [5]. High-yield crops like maize are regularly fertilized with a large amount of nitrogen fertilizer to achieve optimal yields. Agricultural yields are improved by increasing nutrients and water uptake with appropriate fertilization and irrigation techniques [6,7]. However, such strategies must be backed up with research-based knowledge that addresses key issues that could reduce yield [8]. Water stress has a negative impact on plant development, plant height, and leaf area [9]. Similarly, N deficiency restricts plant growth and decreases leaf area and biomass yield [10]. Water and nitrogen, particularly in terms of plant growth and crop production, are well known for their complex interaction. Maize yield decreases under limited water conditions with high N fertilizers [11]. In contrast, large amounts of N fertilizer are required when corn is cultivated in areas with no water stress [12].

Nitrogen fertilizers have been applied to maize immediately before planting and partially side-dressed during the V6 to V8 stages [13]. Delayed side-dressing could result in irreversible yield loss. Delaying N application until the V6 stage resulted in a near 12% loss in kernel yield, according to [14]. However, research was limited to normal maize hybrids. There is a growing interest in learning more about the effects of different irrigation and nitrogen applications during plant growth, when N is most important for maximum yield, especially in leafy and hybrid maize. During kernel filling, a number of annual cereals show genetic diversity in the degree and pace of leaf withering [13]. While it is critical to use the optimal amount of water and nitrogen, when the amount of fertilizer input exceeds the level of nutrient absorbed by crops, contamination of water resources occurs as a result of crop management [15]. As a result, scientific and public concern have finally increased, with an emphasis on water pollution caused by nitrogen from agricultural sources [16].

Inappropriate use of N and water may increase N nitrate losses through leachate, with negative environmental consequences [17]. Furthermore, the price of N fertilizer has risen rapidly during the last few decades [18]. As a result, in an irrigated agricultural system, it is critical to improve nitrogen management in order to maximize farm income and reduce environmental impact [19]. Irrigation water and N have been studied extensively in relation to maize production and WUE [20–22]. However, few studies have investigated the combined effect of water and nitrogen on maize biomass, kernel yield, and enzymatic activity in subtropical areas of China, particularly in high-precipitation areas. In addition, there are discrepancies in the results between the amounts of water used and the rates of nitrogen applied [23]. Maize for kernel yield requires different management approaches than silage maize. Compared to kernel corn, silage maize is harvested at an earlier stage of maturity, so it requires less water. To avoid decreasing the overall nutritional value of the maize, nitrogen management is essential during the early reproductive stages [24]. This research aimed to determine the combined effects of irrigation water and N on hybrid maize growth, biomass yield, kernel yield, WUE and enzymatic activity at different growth stages for maize cultivated in subtropical regions in controlled conditions.

2. Results

2.1. Kernel Yield and Yield Components

The yield and yield-related components from different N and irrigation treatments are shown in Table 1. We found that a high dose of N input with low irrigation water (LW) resulted in a higher yield. Our results show that kernel yield was significantly affected by irrigation rates ($p < 0.001$) and input N rate ($p < 0.001$), and the interactions between irrigation and N level ($p < 0.001$; Table 2) also affected kernel yield. There was no effect of irrigation treatment on the number of rows per ear, but N rate had a significant effect on the number of rows per ear, kernel per row, kernel per ear, ear length and ear diameter in both LW and HW irrigation. In contrast, N had no effect on ear diameter in HW irrigation, but the ear diameter was significantly increased in LW irrigation (Table 1). Regardless of

N fertilizer input, LW irrigation significantly increases crop yield and yield components compared to HW irrigation.

Table 1. Effect of different water and N treatments on yield and yield components of maize.

Irrigation	N-Fertilizer	Rows Number	Kernels Per Row	Kernels Per Ear	Ear Length (cm)	Ear Diameter (cm)	Kernels Yield (g Plant^{-1})
HW	N0	11.0 ± 0.6 b	10 ± 1.0 b	108 ± 13.6 c	7.5 ± 0.5 b	4.0 ± 0.2 a	32.2 ± 0.6 d
	N1	14 ± 0.5 a	13 ± 1.3 a	169 ± 19.3 b	8.4 ± 0.3 b	4.2 ± 0.1 a	47.1 ± 2.2 c
	N2	14 ± 0.5 a	13 ± 0.9 a	177 ± 3.1 b	8.6 ± 0.3 b	4.3 ± 0.1 a	38.5 ± 0.2 bc
	N3	14 ± 0.5 a	14 ± 0.5 a	199 ± 16.5 ab	8.8 ± 0.7 b	4.3 ± 0.1 a	39.4 ± 2.2 ab
	N4	15 ± 0.5 a	14 ± 0.8 a	220 ± 17.1 a	11.0 ± 0.7 a	4.3 ± 0.1 a	44.2 ± 1.3 a
LW	N0	12 ± 1.3 c	11 ± 1.5 b	119 ± 11.0 b	8.3 ± 1.0 b	4.1 ± 0.2 c	27.9 ± 1.7 d
	N1	13 ± 0.6 bc	15 ± 1.9 ab	197 ± 18.5 a	9.3 ± 0.9 b	4.2 ± 0.3 bc	37.6 ± 1.8 c
	N2	14 ± 1.0 abc	17 ± 1.6 a	222 ± 18.5 a	10.0 ± 0.5 b	4.3 ± 0.1 bc	57.2 ± 1.0 b
	N3	16 ± 0.8 a	18 ± 1.0 a	259 ± 22.7 a	12.3 ± 0.2 a	4.9 ± 0.1 a	70.0 ± 1.2 a
	N4	15 ± 1.0 ab	17 ± 1.1 a	234 ± 21.3 a	10.1 ± 0.7 ab	4.7 ± 0.1 ab	60.1 ± 0.9 b

Note: Means followed by different lowercase letters within each column indicate significant differences ($p < 0.05$) using the LSD test. The nitrogen treatments N0, N1, N2, N3 and N4 represent the application of nitrogen at the rate of 0, 200, 250, 300, and 350 kg ha^{-1}. HW and LW represent irrigation water at a rate of 80 and 60% of field capacity.

Table 2. Probability values (*p* values) of main and interaction effects for irrigation, nitrogen treatment and stage (V9, R1, R3, R6) for various maize parameters.

Parameters	Irrigation	Nitrogen	Stage	Irrigation × Nitrogen	Stage × Irrigation	Stage × Nitrogen	Stage × Irrigation × Nitrogen
Number of rows/ear	0.367	<0.001	—	0.442	—	—	—
Kernels per row	0.021	<0.001	—	0.775	—	—	—
Kernels per ear	0.005	<0.001	—	0.578	—	—	—
Ear length	0.029	0.002	—	0.045	—	—	—
Ear diameter	0.043	0.007	—	0.248	—	—	—
Kernel weight	<0.001	<0.001	—	<0.001	—	—	—
Shoot dry matter	0.009	<0.001	—	0.035	—	—	—
Root dry matter	0.002	<0.001	—	0.328	—	—	—
Plant height	<0.001	<0.001	<0.001	<0.001	0.006	<0.001	<0.001
Leaf area	0.035	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Malondialdehyde	<0.001	<0.001	<0.001	0.009	0.006	0.002	<0.001
Hydrogen peroxide	0.002	<0.001	<0.001	0.002	0.065	0.002	<0.001
Superoxide anion	<0.001	<0.001	<0.001	<0.001	0.043	<0.001	0.005
Proline	<0.001	<0.001	<0.001	0.004	0.018	<0.001	<0.001
Superoxide dismutase	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
Peroxidase	0.005	<0.001	<0.001	<0.001	0.040	<0.001	<0.001
Ascorbate peroxidase	<0.001	<0.001	<0.001	0.040	0.001	<0.001	<0.001
Chlorophyll a	<0.001	<0.001	<0.001	<0.001	0.024	<0.001	<0.001
Chlorophyll b	0.016	<0.001	<0.001	<0.001	0.019	0.002	0.017

2.2. Plant Dry Matter

Nitrogen fertilizer significantly increased the growth characteristics of maize under both LW and HW irrigations. As shown in Figure 1, the root and shoot dry matter of maize was significantly increased with increasing N rate in both LW and HW conditions ($p < 0.001$). LW irrigation had 29 and 17% higher root and shoot dry matter than HW irrigation, respectively ($p < 0.01$). Compared to N0, the root dry matter in N1, N2, N3, and N4 was 64, 73, 98, and 105% higher in LW, and 28, 43, 76, and 66% higher in HW irrigation, respectively. In contrast, the root dry matter in N3 and N4 treatments was statistically similar under both LW and HW irrigation.

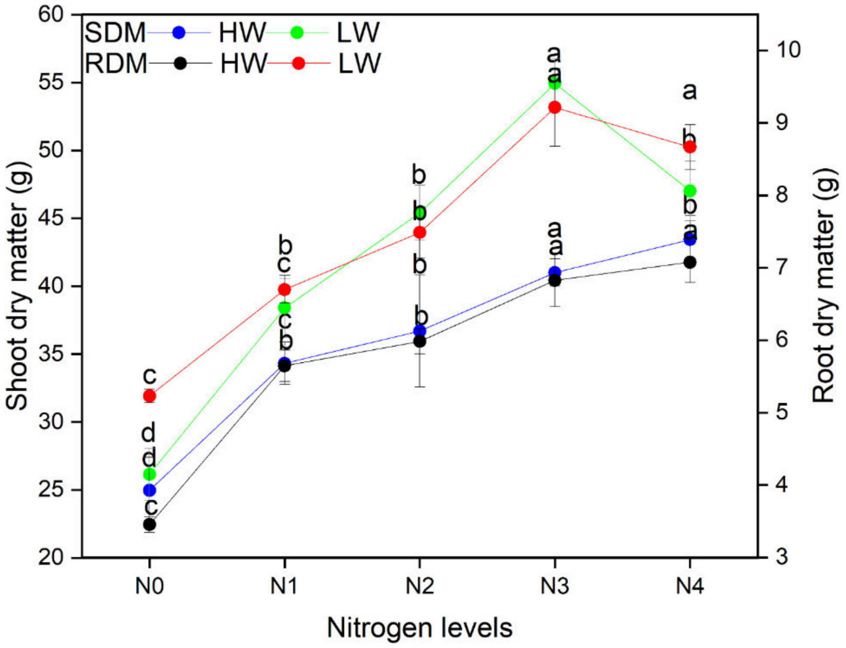


Figure 1. Effect of irrigation and nitrogen treatments on root and shoot dry matter. Means followed by different lowercase letters within each treatment indicate significant differences ($p < 0.05$). Bars represents standard errors. The nitrogen treatments N0, N1, N2, N3 and N4 represent the application of nitrogen at the rates of 0, 200, 250, 300 and 350 kg ha^{-1}. HW and LW represent irrigation water at the rate of 80 and 60% of field capacity. RDM and SDM are root and shoots dry matter, respectively.

Under both irrigation treatments, the accumulation of shoot dry matter in N-treated plants was significantly higher than in N0 plants. Averaged across N fertilizer applications, N3 markedly improved shoot dry matter by 88% compared to the N0 treatment (Figure 1). Similar to root dry matter, shoot dry matter was also significantly increased as the N level increased, but in the N2 and N4 treatments, the shoot dry matter was not statistically different. Our results show that the N rate had a better effect on shoot dry matter under LW compared to HW irrigation. The N3 treatment showed the most favorable influence on shoot dry matter, followed by the N4 and N2 treatments, indicating that N3 treatment significantly increased shoot dry matter by 110 and 64% compared to N0 in LW and HW irrigation, respectively.

2.3. Plant Height and Leaf Area

Irrigation, N fertilizer, stages and their interactions had significant effects on plant height and leaf area (Figure 2 and Table 2). The results show that plant height and leaf area were significantly increased with increased N rate ($p < 0.001$) under both irrigations treatments ($p < 0.01$) at different growth stages ($p < 0.001$). However, LW irrigation showed markedly higher plant height and leaf area than HW irrigation. LW irrigation showed 4.3 and 0.4% higher plant height and leaf area than HW irrigation, respectively. However, on average, the N4, N3, N2, and N1 treatments increased plant height by 10.7, 16.5, 9.5 and 5.7%, and leaf area by 24, 37.5, 13 and 6.5%, compared to N0. It is well understood that plant height and leaf area increase over time. Our findings reveal that the plant height at the R3 stage was statistically similar to the R6 stage, but significantly greater than at the V9 and R1 stages ($p < 0.001$). The maximum leaf area was obtained at R3 followed by R6, and the lowest leaf area was observed at the V9 growth stage.

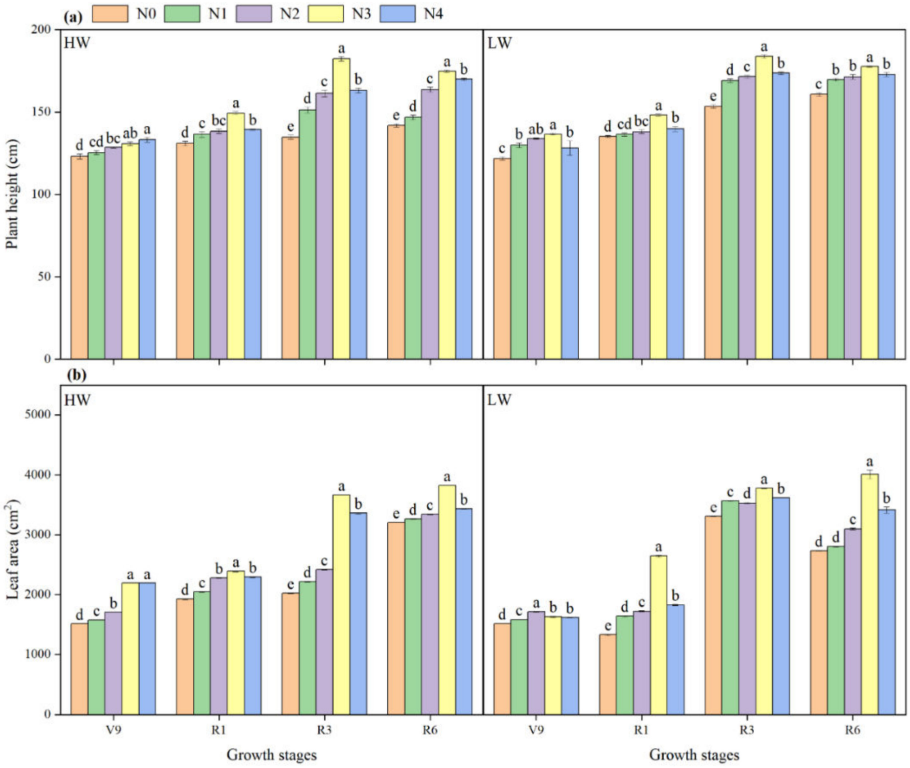


Figure 2. Effects of irrigation and nitrogen rates on plant height (**a**) and leaf area (**b**). HW and LW represent irrigation water at the rate of 80 and 60% of field capacity. Means followed by different lowercase letters within each treatment indicate significant differences ($p < 0.05$). Bars represents standard errors. The nitrogen treatments N0, N1, N2, N3 and N4 represent the application of nitrogen at the rates of 0, 200, 250, 300 and 350 kg ha^{-1}.

2.4. Root Growth and Development

The number of roots, total root length, surface area, volume, and diameter were significantly affected by N treatment (Table 3; $p < 0.001$). Compared with N0, the total root length in N1, N2, N3, and N4 for LW was increased by 77, 141, 375, and 248%, while a 48, 80, 117, and 175% increase was observed for HW irrigation (Table 3). On average, the total root length in LW was 13% longer than that in HW irrigation. Similarly, averaged across irrigation, the N3 treatment increased total root length by 229% over N0. The maximum root surface area was observed for the N3 treatment in LW (881.3 cm^2) and the N4 treatment in HW irrigation (720.1 cm^2), which was significantly higher than for the N0 treatment, which resulted in the lowest root surface area (Table 3). The maximum root surface area was observed for LW compared to HW irrigation, but these results were not significant. Similarly, the root volume and average diameter were not significantly affected by irrigation treatment ($p > 0.05$), but they significantly increased with increasing N rate ($p < 0.001$; Table 3). Compared to N0, the root volume and average diameter were increased by 183 and 19.6%, respectively, in the N3 treatment, regardless of irrigation.

Table 3. Effects of irrigation and nitrogen application on number of roots, total root length, acreage diameter and surface area.

Irrigation	N-Fertilizer	Number of Roots	Total Root Length (cm)	Root Diameter (mm)	Root Surface Area (cm^2)	Root Volume (mm^3)
HW	N0	1829 e	856.69 e	0.8 b	162.3 d	5092.3 c
	N1	3014 d	1267.60 d	1.0 a	412.0 c	19,579.8 b
	N2	3584 c	1539.53 c	0.9 a	458.9 c	20,793.1 b
	N3	4222 b	1855.38 b	0.9 a	545.9 b	24,704.9 b
	N4	5183 a	2359.84 a	1.0 a	720.1 a	35,036.3 a
LW	N0	2323 c	663.45 e	0.7 c	160.0 c	20,197.8 c
	N1	2868 c	1172.20 d	1.1 a	398.4 b	21,697.8 c
	N2	3699 b	1599.53 c	0.9 b	472.4 b	21,571.4 c
	N3	6256 a	3148.81 a	0.9 b	881.3 a	47,005.9 a
	N4	4397 b	2306.67 b	1.1 a	818.6 a	37,606.5 b

Note: Means followed by different lowercase letters within each column indicate significant differences ($p < 0.05$) using LSD tests. The nitrogen treatments N0, N1, N2, N3 and N4 represent the application of nitrogen at the rates of 0, 200, 250, 300 and 350 kg ha^{-1}. HW and LW represent irrigation water at a rate of 80 and 60% of field capacity.

2.5. Chlorophyll a and b Contents

The application of N, irrigation treatment and their interaction significantly increased chlorophyll *a* and *b* content at different growth stages (Figure 3 and Table 2). The maximum content of chlorophyll *a* and *b* occurred at V9 and gradually decreased until R6. Under both HW and LW irrigation, the maximum chlorophyll *a* and *b* content was found at V9 with N3 treatment, and the minimum was found for N0. Our results show that chlorophyll *a* and *b* content increased with increasing N content up to the N3 treatment, but that higher N content (N4) decreased chlorophyll *a* and *b* contents. The application of N fertilization with LW irrigation resulted in higher chlorophyll *a* and *b* content compared with HW irrigation. The minimum chlorophyll *a* and *b* contents at all growth stages were obtained with HW in N0 treatment, and the maximum with LW in N3, followed by N2 treatment. The mean results for the four growth stages revealed that N1, N2, N3, and N4 significantly increased chlorophyll *a* content by 52, 71, 89, and 62%, respectively, and chlorophyll *b* content by 33, 66, 96, and 58%, respectively, compared to N0.

2.6. Malondialdehyde and Proline Content

The application of N and irrigation treatment significantly affected the MDA and proline content (Figure 4a,b). The application of N decreased the MDA content compared to the N0 treatment. The maximum MDA content was observed for N0 and the minimum was observed for N4 treatment in both HW and LW irrigations (Figure 4). This suggests that a higher N content has a negative impact on MDA content. For irrigation, the LW treatment decreased the MDA content by 29.72% compared to HW irrigation. The N0 treatment resulted in a higher MDA content compared to other N treatments. For fertilization, N4, N3, N2 and N1 significantly decreased the MDA content, by 51, 43, 26, and 17%, respectively. The results show that N content and irrigation have a significant effect on proline content under both irrigation types. The proline content decreased with increasing N content at LW and HW irrigations. However, on average, the high N content (N4) has a 57.7% lower proline content than that the N0 treatment (Figure 4b). The minimum proline content was observed at V9 under LW treatment, and the maximum was observed at R6 under HW treatment. The LW treatment significantly decreased the proline content by 19% compared to HW irrigation.

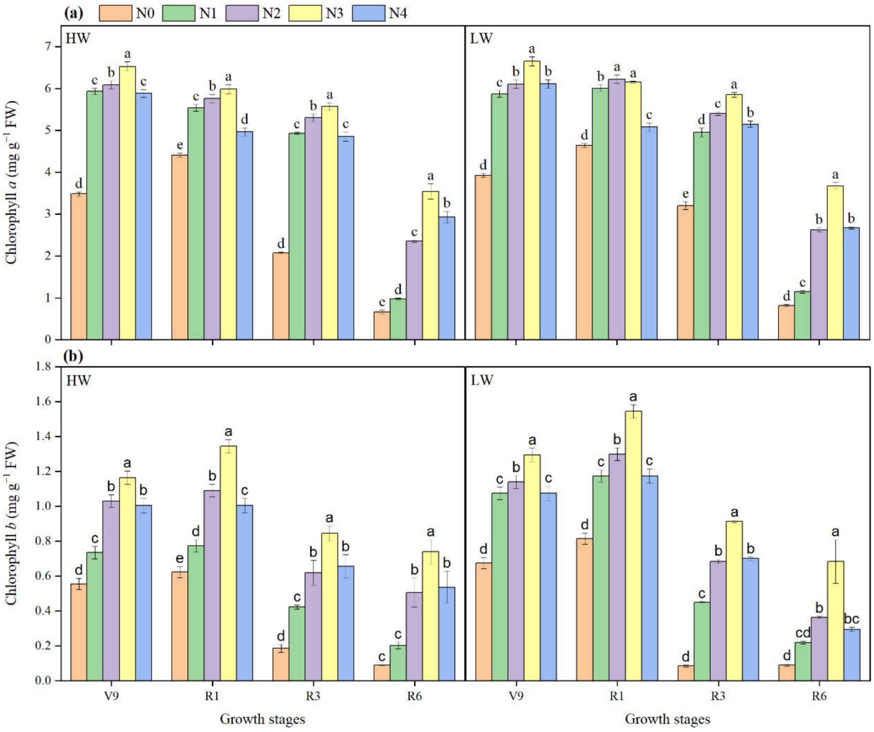


Figure 3. Effect of irrigation and nitrogen rates on chlorophyll *a* content (**a**) and chlorophyll *b* contents (**b**). HW and LW represent irrigation water at the rate of 80 and 60% of field capacity. Means followed by different lowercase letters within each treatment indicate significant differences ($p < 0.05$). Bars represent standard errors. The nitrogen treatments N0, N1, N2, N3 and N4 represent the application of nitrogen at the rates of 0, 200, 250, 300 and 350 kg ha^{-1}.

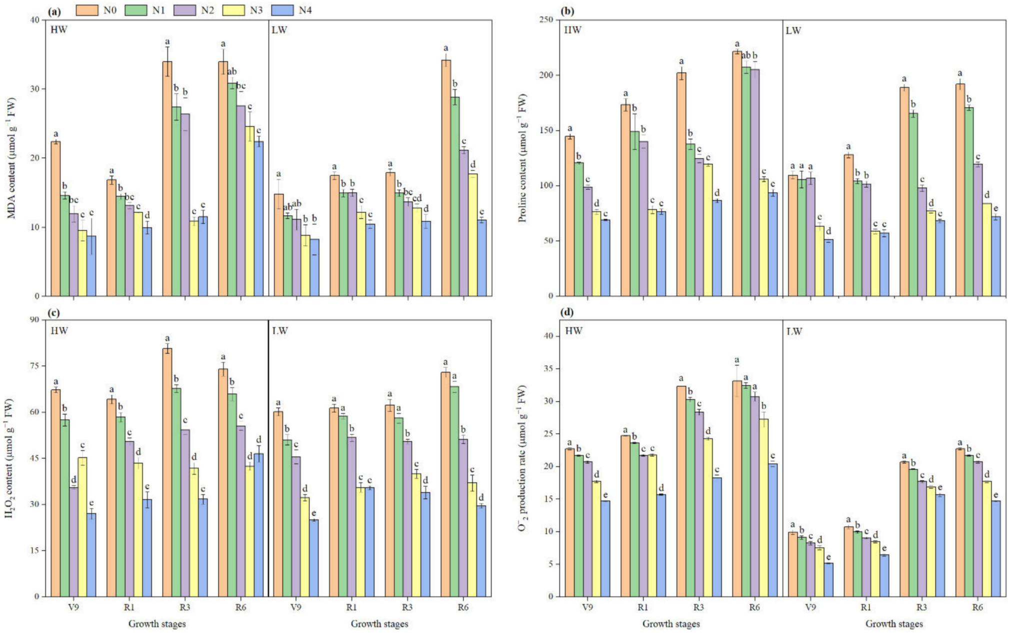


Figure 4. Effect of irrigation and nitrogen rates on malondialdehyde (MDA) content (**a**), proline content (**b**), H_2O_2 content (**c**), and O_2 content (**d**). HW and LW represent the irrigation water at the rate of 80 and 60% of field capacity. Means followed by different lowercase letters within each treatment indicate significant differences ($p < 0.05$). The bar represents the standard error. The nitrogen treatments N0, N1, N2, N3 and N4 represent the application of nitrogen at the rate of 0, 200, 250, 300 and 350 kg ha^{-1}.

2.7. Reactive Oxygen Species

Hydrogen peroxide and $O_2{}^{-}$ content decreased significantly with N rates in HW and LW irrigation. In the present study, both H_2O_2 and $O_2{}^{-}$ contents decreased with increasing N content, whereas the higher N content (N4) resulted in a lower ROS content compared to other N treatments (Figure 4c,d). Under both LW and HW, the minimum H_2O_2 and $O_2{}^{-}$ contents were observed for N4, while N0 resulted in a higher ROS content. The H_2O_2 content was higher at R3 and R6 in HW and LW irrigations, respectively. Likewise, a lower $O_2{}^{-}$ content was observed at V9 and gradually increased towards R6. On average, the LW irrigation resulted in an 8% lower H_2O_2 and a 43% lower $O_2{}^{-}$ content compared to the HW irrigation, indicating that N has a better response to ROS under HW irrigation. Similarly, compared to N0, the N1, N2, N3, and N4 treatments decreased the H_2O_2 content by 11, 27, 41 and 52% (Figure 4c), respectively, and the $O_2{}^{-}$ content by 5, 11, 19 and 37% (Figure 4d).

2.8. Antioxidant Enzymatic Activity

The N application, stages, and irrigation treatments significantly affected the antioxidant enzymatic activity (Figure 5). The results show that the antioxidant enzymatic activities increased with increasing N content under LW and HW irrigation. On the other hand, LW irrigation showed significantly higher SOD, POD, and APX activity than HW irrigation. The activities of SOD, POD, and APX increased from V9 to R3 and then decreased towards R6. Increasing N content significantly increased the activity of the antioxidant enzyme, but N4 treatment had lower activity than N3. In comparison to N0, the N1, N2, N3, and N4 treatments increased SOD activity by 32, 61, 86, and 65%, respectively, and HW decreased SOD activity by 5.7% compared to LW irrigation. The highest POD activity was observed for N3 treatment, which was 39 and 19% higher than the N0 and N4 treatments, respectively. Similarly, the APX activity was increased in the N3 treatment by 159 and 17% compared to the N0 and N4 treatments, respectively. The mean results based on four stages exhibited that the enzymatic activities (SOD, POD, and APX) were decreased in HW by 5.7, 4.8, and 12.2% compared to LW irrigation. The higher antioxidant enzymatic activities increase the yield by protecting the photosynthetic system of the maize crop.

2.9. Soil Enzyme Activity

As shown in Figure 6a–f, nitrogen fertilization significantly increased the activity of acid phosphatase, acid invertase, and urease, while the N4 treatment (350 kg N ha^{-1}) under LW irrigation resulted in lower enzyme activities than the N3 treatment (300 kg N ha^{-1}). Similarly, the activities of β-glucoside, catalase, and cellulase were significantly higher for the N3 treatment compared to control or other treatments of the group under the LW irrigation system. In addition, the N4 treatment under the HW irrigation system resulted in higher β-glucoside and urease activity, but β-glucoside and urease activity were not statistically different from N3 and N2 treatment, respectively. Under LW irrigation, cellulase activity was similar under N0, N1, and N2 treatments, but was significantly increased under N3 treatment.

2.10. Correlation Analyses

Leaf enzymes had a positive correlation with kernel per row, kernel per ear, root dry matter, shoot dry matter, plant height, kernels yield, leaf area, root diameter, root surface area, root volume, and total root length. However, MDA, proline H_2O_2 and $O_2{}^{-}$ had negative correlations with most of the above parameters. Moreover, a strong positive correlation was observed for chlorophyll *a* and *b* content with plant enzymes, dry matter, and yield. However, a slight positive correlation was observed with root diameter, ear diameter, total root length, root surface area, and volume. Additionally, MDA content had a lower negative correlation with almost all of the above parameters, but had a strong negative correlation with root diameter (Figure 7).

To assess the relationship between N fertilization and enzyme activities, Principal Component Analysis (PCA) was performed (Figure 8). Dim1 separated the plant ROS and antioxidant enzyme activities under both low and high irrigation. These findings reveal that Dim1 contributed 75 and 77.8% of the overall variation under low and high irrigation, respectively (Figure 8a,b). Furthermore, the plant enzyme activities were strongly correlated with N3 treatment, while ROS and proline were correlated to N0 treatment under both irrigation types. However, SOD and POD have a much greater contribution under LW compared to HW irrigation. Similarly, Dim1 showed 93.2 and 98.5% variation in soil enzymes activities under low and high irrigation regimes, respectively (Figure 8c,d). These results suggest that UR, AI, and CL were more correlated to N3 treatment and had a higher contribution compared to ACP and BGC activities under low irrigation (Figure 8c). However, under high irrigation regimes, CL, UR, and BGC were significantly correlated with N3 and N4 treatments, whereas CL and BGC had lower contributions compared to other soil enzymes (Figure 8d).

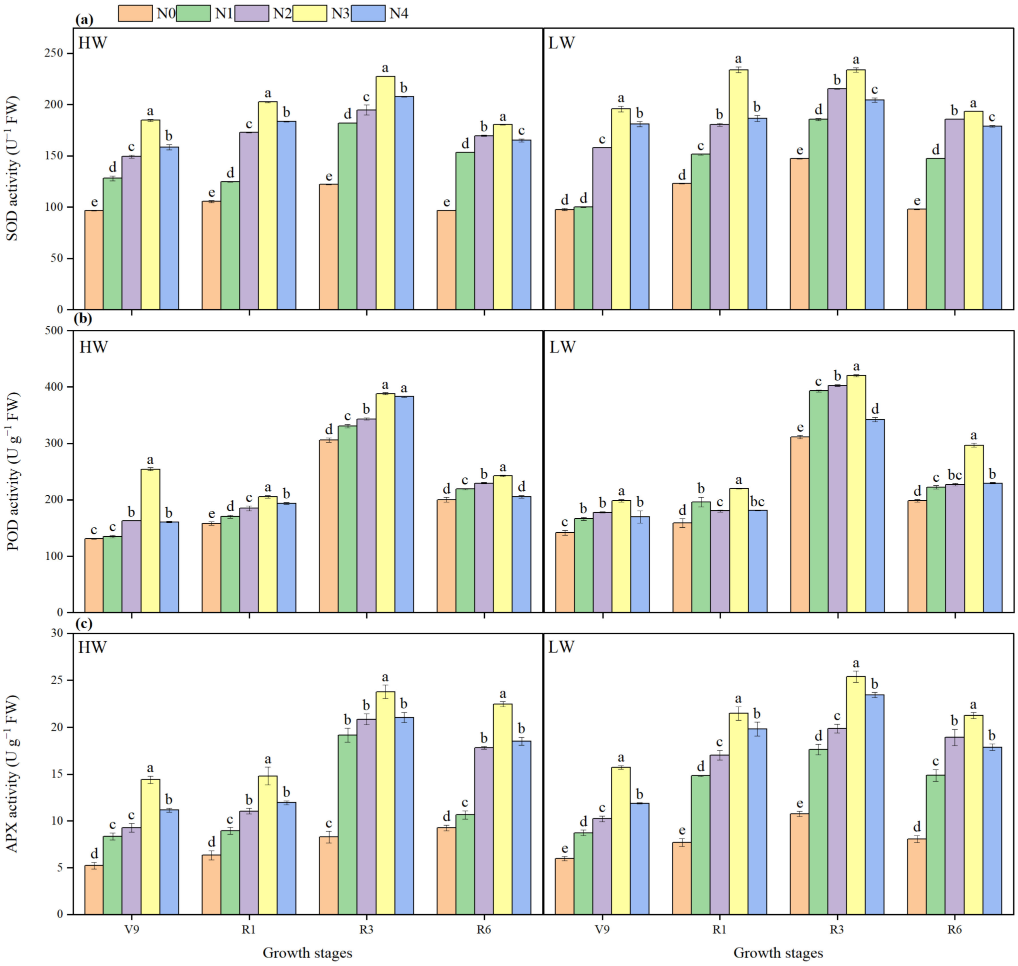


Figure 5. Effects of irrigation and nitrogen rates on SOD activity (**a**), POD activity (**b**), and APX activity (**c**). HW and LW represent irrigation water at the rate of 80 and 60% of field capacity. Means followed by different lowercase letters within each treatment indicate significant differences ($p < 0.05$). Bars represents standard errors. The nitrogen treatments N0, N1, N2, N3 and N4 represent the application of nitrogen at the rates of 0, 200, 250, 300 and 350 kg ha^{-1}.

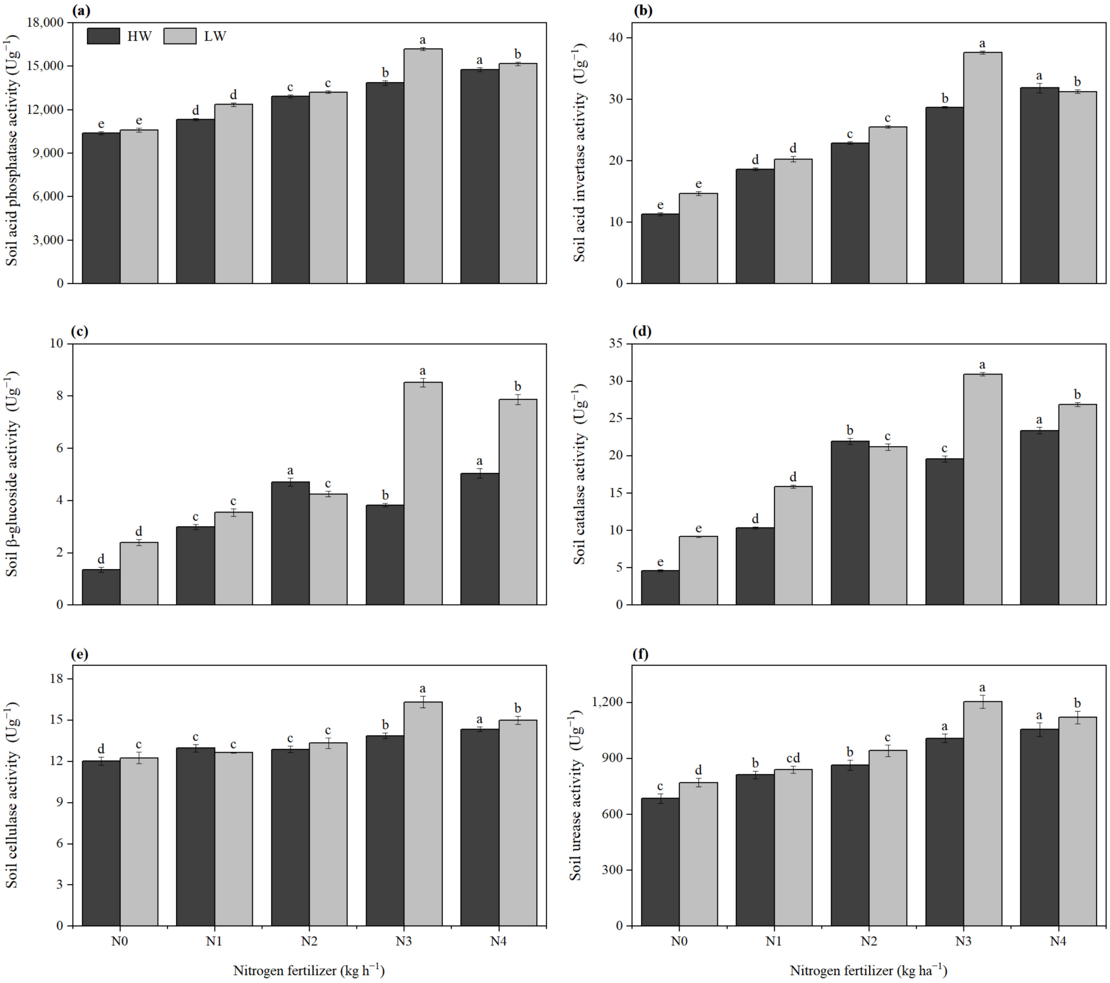
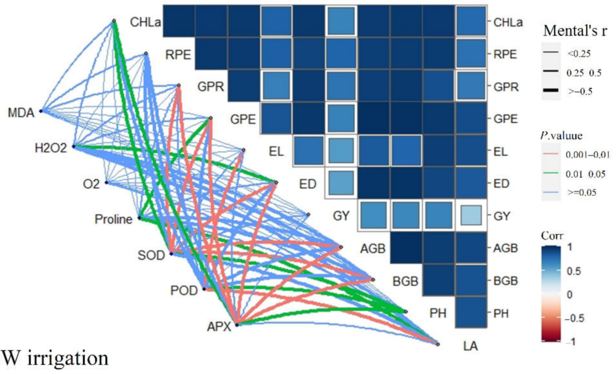
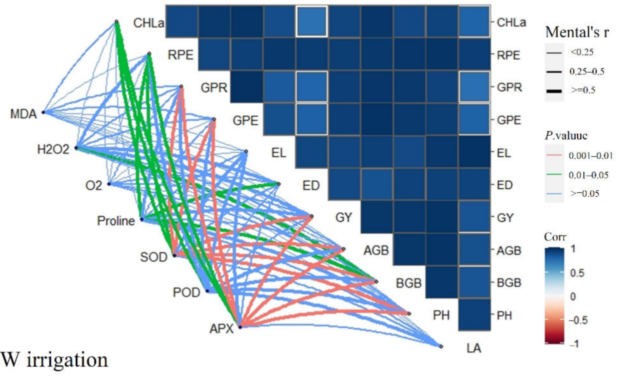


Figure 6. Effects of irrigation and nitrogen rates on soil acid phosphatase (**a**), soil acid invertase (**b**), β-glucosides (**c**), catalase (**d**), cellulose (**e**), and soil urease (**f**). HW and LW represent irrigation water at the rate of 80 and 60% of field capacity. Means followed by different lowercase letters within each treatment indicate significant differences ($p < 0.05$). Bars represent the standard errors. The nitrogen treatments N0, N1, N2, N3 and N4 represent the application of nitrogen at the rates of 0, 200, 250, 300 and 350 kg ha^{-1}.



HW irrigation



LW irrigation

Figure 7. Correlation analysis of the yield, enzymes, chlorophyll, and ROS related parameters. CHLa, chlorophyll a; CHLb, chlorophyll b; GY, kernel yield; AGB, shoot dry matter, BGB; root dry matter; PH, plant height; LA, leaf area; MDA, malondialdehyde; H_2O_2, hydrogen peroxide; $O_2{}^{-}$, superoxide anion; SOD, superoxide dismutase; POD, peroxidase; and APX, ascorbate peroxidase.

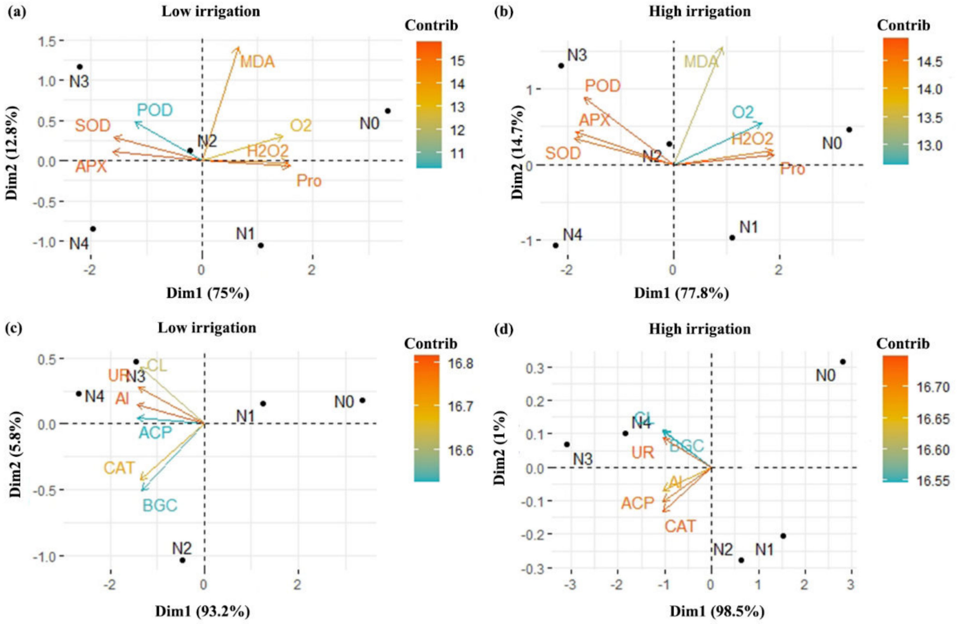


Figure 8. Principal component analysis (PCA) indicates that the largest separation among antioxidant enzymes is the spatial distribution of irrigation water (Dim1) and the second largest source of variation is nitrogen fertilizer (Dim2). Plant enzymes activities in low irrigation (**a**), plant enzymes activities in high irrigation (**b**), soil enzymes activities in low irrigation (**c**), and soil enzymes activities in low irrigation (**d**).

3. Discussion

Nitrogen (N) and water are the major governing factors in agricultural systems worldwide [15]. Nitrogen fertilizer is one of the key elements influencing crop growth. It is severely used in crop production due to its significant crop yield and the demands of the current population [25]. High water and fertilizer inputs are commonly seen to achieve high yields [26]. However, excessive fertilization and poorly planned irrigation systems are common for farmers, and these methods are substantial in causing environmental problems related to soil [27]. The application of N significantly increased the maize kernel yield, as well as root and shoot dry matter under both LW and HW irrigation. The higher yield and yield components under LW irrigation with N application are attributed to the higher chlorophyll content and an enhanced antioxidant enzymatic activity defense system. The higher N content of the LW irrigation system protected the photosynthetic system and enzymatic activity, resulting in higher yields. For better plant adaptation to stress conditions, it is important to maintain a high antioxidant enzymatic activity system [28]. Previous literature reported that N application increased the yield of different crops by reducing the loading rate and enhancing photosynthetic performance [16,29,30]. Ahmad et al. [31] reported that N application increased maize kernel yield with a medium plant population density by reducing leaf senescence at the bottom. In the present study, N3 under LW resulted in a higher kernel and dry matter yield, but N4 had a higher kernel yield under the HW irrigation system, which is not statistically different from the N3 treatment. These findings are consistent with previous findings [32,33], which show that high irrigation water causes oxidative membrane damage in maize crops. The ideal application rate reported is intended to maximize crop yield while conserving resources and the environment [34].

Root morphology is quite flexible; it can readily respond to the available mineral nutrients in the soil [35]. We found that the N3 treatment resulted in a higher root dry weight, implying that the association between the N fertilizer rates and root systems is not linear and positive. In fact, N input may be detrimental to root development and growth. In the current study, N fertilizer application promoted root growth in both the HW and LW irrigation systems and increased the proportion of roots in LW on average compared to HW, indicating that N application has a significant impact on root growth development under well irrigation systems. Root and shoot dry matter and proportion in the HW and LW irrigation systems were negatively affected by the N0 treatment, suggesting that an absence of N will be harmful for early plant growth is described as premature growth. Not only nutrient absorption and root morphology are inextricably linked, but crop development, yield, and crop growth are all also closely connected to the spatial distribution of root systems [36]. The root system is distributed efficiently in N4 under HW irrigation, with a higher root length density, diameter, surface area, and volume, compared to N3 under LW irrigation, resulting in larger and deeper infiltration scales. Nitrogen rate-induced increases in kernel yield are also aided by gentler root senescence in the N3 treatment. These results are supported by [37], which demonstrated the ideal root system distribution in N225 treatment with a greater total root length and surface area, which is the main contributor to the N-induced increase in kernel yield. According to previous studies, the root environment's relative stability is effective in promoting the root system's buffer capacity in detrimental soil environments, as well as resulting in a high kernel yield and utilization efficiency [37,38]. Our results for LW irrigation exhibit that disproportionate N (N4) adversely affects root growth as compared to N3, while in HW irrigation, N4 has better root growth compared to the other N treatments. The observed decrease in crop yield can be attributed to a variety of factors. Excessive N application may cause slight reductions in crop yield due to adverse effects on root development during the early stages of plant growth or different aging mechanisms leading to a relative nitrogen shortage during the reproductive stage [37]. Root thinning and longitudinal expansion are induced by N deficiency, which promotes root development in the soil, whereas high N inhibits root vertical expansion [37,39]. From the perspective of root morphology and development, our findings describe the role of excessive N and its deficiency on maize yield under two different irrigation regimes.

Reduced chlorophyll content and leaf area per plant are closely associated with leaf senescence [31,40], and the degradation of chlorophyll reduces photosynthetic efficiency [41]. For a high-density plant population, it is critical to reduce maize's accelerated leaf senescence and protect the photosynthetic apparatus [31]. Our results show that the chlorophyll *a* and *b* contents were significantly higher at V9, and the lowest value was observed at R6 under both HW and LW irrigation. The maximum chlorophyll *a* and *b* contents were observed for N3 treatment at all stages under LW irrigation compared to other N treatments and the control. Recently published studies have reported that the decrease in chlorophyll content in the later stages is due to an increase in leaf senescence in older plants [31,42]. The application of N with LW irrigation increased leaf area per plant and chlorophyll *a* and *b* content, suggesting that N had a crucial effect on enhancing the leaf area, plant growth and photosynthetic efficiency. The leaves stopped growing at the silking stage and became senescent as the plant grew older. The loss of leaf greenness due to chlorophyll loss, a result of chloroplast degradation, was the first symptom of senescence [43,44]. The primary element that maintains leaf photosynthesis is the leaf chlorophyll content [45], with leaves providing up to 50–80 percent of the photosynthesis required by kernels [43]. Lower dry matter and kernel yield in HW irrigation are primarily caused by a reduced chlorophyll content and enzymatic activities. This may be due to drought damage to the chloroplast structure [46], and a disorder of the N metabolism and down-regulation of the enzymatic activities [47,48]. All these adverse changes reduced plant photosynthesis and induced a decrease in dry matter accumulation, which ultimately decreased kernel yield [43,49].

Senescence increases the MDA content in leaves, which can be harmful to plant growth [50]. Our results show that MDA was lower at V9 and increased gradually with leaf senescence. The N-treated plants resulted in a lower MDA content in both HW and LW irrigation compared to N0. Averaged across irrigation, a lower MDA content was obtained in all growth stages under N4, and the maximum was found under N0 treatment. In addition, MDA accumulation has been found to be increased during the leaf senescence process in a variety of crops [41]. The MDA content is commonly used to assess lipid peroxidation. The MDA content was higher in the HW treatment than in the LW treatment in this study (Figure 4a), implying that the leaf cell membranes were damaged as a result of the high irrigation. Furthermore, MDA content was lower in N4 under HW than at other nitrogen application rates, implying that increasing nitrogen fertilizer supply rates (up to 350 kg N ha^{-1}) could reduce lipid peroxidation in maize leaves caused by high irrigation. Tian et al. [33] reported that the greatest reduction in lipid peroxidation in leaves was observed with N4, implying that a high nitrogen fertilizer supply can aid in the recovery of lipid peroxidation in leaves following high irrigation.

Under both HW and LW irrigation, proline accumulation was highest in N0-treated plants at all growth stages compared to other N-treated plants, while the lowest value was observed in N4 (Figure 4b). Plants treated with N have a reduced MDA content. This might be due to the accumulation of nitrogen-containing compounds (i.e., proline), which play an adaptive role by helping to stabilize sub-cellular structures, scavenge free radicals, and buffer cellular redox potential in stressful situations [51]. Previous studies have reported that proline metabolism has a significant impact on cellular redox potential, which could be important for stress tolerance signaling. Additionally, proline reduces equivalent N and carbon dioxide as an osmolyte and antioxidant as well as a source of energy [52].

In this study, we found that N application decreased the H_2O_2 and O_2^- contents in both HW and LW irrigation, indicating that N may be beneficial in maintaining aquaporin activity by reducing H_2O_2 accumulation. The maximum H_2O_2 and O_2^- contents were observed at R6 in N0 under HW irrigation, but the minimum was observed at V9 in N4 under LW irrigation, suggesting that high irrigation water may have caused damage to the leaf cell membranes. H_2O_2 is a ROS that is produced by cellular metabolism and is a measure of a plant's ability to scavenge ROS under stress. Previous research has largely demonstrated that the accumulation of H_2O_2 can lead to a significant reduction in aquaporin activity [53,54]. The key enzymes in the ROS scavenging system are SOD and POD, where SOD catalyzes the disproportionation of O_2^-, while POD metabolizes H_2O_2 [33]. For plants to adapt to hypoxic stress, it is critical to maintain high antioxidant enzyme activity [28]. It has been shown that increasing nitrogen supply rates can reduce H_2O_2 and $O^-{}_2$ accumulation in maize leaves, reducing high-water-stress-induced oxidative membrane damage [33]. Waterlogging causes an increase in ROS production in plants, which affects cell membrane stability through lipid peroxidation [33]. Furthermore, the MDA content was lower in N3 and N4 under HW than at other nitrogen application rates, implying that increasing nitrogen fertilizer supply rates could reduce lipid peroxidation in maize leaves caused by high irrigation.

Antioxidant enzyme activity regulation is an innate plant response to prevent oxidative stress caused by a variety of external biotic and abiotic stress factors [55,56]. In the present study, a significant treatment effect on the antioxidant defenses in maize leaves was detected. Results show that in HW irrigation, there were lower SOD, POD, and APX activities at each N treatment compared to LW (Figure 5). The maximum SOD, POD, and APX contents were observed in N3 under LW irrigation at R3, whereas the minimum activities were observed in N0 and N4 under HW. The optimum higher N3 and LW irrigation increased the enzymatic activity contact with improved plant growth, root development, and yield, according to the correlations between the parameters investigated (Figure 7). According to previously published findings, waterlogging damages the membrane of maize [55]. Furthermore, increasing N fertilizer application in winter rape can improve plant enzyme activities in leaves [28]. Similarly, [57] reported that antioxidant enzyme activities (SOD and POD) were increased at higher N rates. Ahmad et al. [31] reported that N application significantly increased antioxidant enzyme activities and reduced leaf senescence in maize leaves up to the optimum level of N, and that a further increase in N had an adverse effect on antioxidant enzyme activities. The application of N has been shown to have a significant positive effect on SOD and POD synthesis [58]. Furthermore, under high irrigation water stress, appropriate nitrogen fertilizer application can help induce the expression of related antioxidant enzyme genes, resulting in increased antioxidant enzyme activity [59]. These findings suggest that increasing nitrogen supply rates can reduce H_2O_2 and O_2^- accumulation in maize leaves, reducing waterlogging-induced oxidative membrane damage.

Soil enzymes are commonly utilized as markers of soil quality because of their relationship to soil biology, their accessibility, and their quick reaction to alterations in soil management [60]. Excessive amounts of nitrogen can cause the buildup of harmful compounds such as ammonia, which harms plants and hamstrings microbial growth, as well as lower soil pH, which inhibits enzyme activity [61,62]. Similarly, a recent study reported that high doses of mineral nitrogen fertilizer can cause a considerable decrease in enzyme activity [62]. Compared to a control, the application of medium and high doses of N into the soil significantly increased urease activity by 42.9 and 23.6%, respectively [62]. In the current study, LW irrigation, together with appropriate N fertilizer (300 kg N ha^{-1}) input, significantly increased soil nutrient availability and provided a suitable environment for soil microorganisms, which resulted in higher enzyme activities (Figure 6; [60]). Under LW irrigation, all enzymatic activities were enhanced by N3 treatment, although N3 is statistically similar to N4 under HW irrigation, which is consistent with Zhou et al. [60] and Pathan et al. [63]. Acid phosphatase, acid invertase, β-glucoside, catalase, cellulase, and urease enzymatic activity were markedly affected by irrigation and nitrogen application, and all treatments showed great variability in their enzyme activities. This is mostly because of the usage of urea fertilizer, which supplies a substrate for the urease reaction [64]. Interestingly, the activity of cellulase showed slight variations with irrigation, and this may be because the substrate-binding portion of the enzyme developed resistance to fertilizer anions [5].

4. Materials and Methods

4.1. Experimental Site

A pot experiment was carried out at Guangxi University in the subtropical region of China (22°50′, 108°17′), in a greenhouse with a controlled environment and nutrition systems. The area is characterized as a warm and temperate region with a mean air temperature of 21.7 °C and mean annual rainfall of 1298 mm. According to Chinese Soil Taxonomy, the texture of soil was clay loam, with a pH of 5.6, a field capacity of 44%, soil bulk density of 1.40 g cm^{-3}, soil organic matter of 20 g kg^{-1}, and available nitrogen, phosphorus, and potassium of 127.0 mg kg^{-1}, 40.0 mg kg^{-1}, and 126 mg kg^{-1}, respectively. A pot was filled with a mixture of soil collected from the greenhouse, which has not been in use for the last six years.

4.2. Experimental Design and Management

A 2 × 5 factorial experiment was carried out in a completely randomized design with four replications, with a total of 40 pots, in a controlled-environment greenhouse in Guangxi, China (Figure 1). The experimental treatments were two irrigation levels, i.e., low irrigation water (LW; 60%) and high irrigation water (HW; 80%), field capacity, and five nitrogen rates, i.e., control (N0), 200 kg N ha^{-1} (N1), 250 kg N ha^{-1} (N2), 300 kg N ha^{-1} (N3), and 350 kg N ha^{-1} (N4). On 28 September 2020, five uniformly sized hybrid maize seeds of the Zhengda 619 variety, which is the most commonly grown variety in the subtropical areas of China, were planted per pot (with a length of 32.5 cm and a height of 29.0 cm). The seeds were obtained from CP seed industry, Yunnan Zhengda Seed Co. Ltd., China. The selected seeds permission was granted from the respective authority. The base fertilizers (P and K) and 1/2 of N were thoroughly mixed with soil before sowing, and the remaining 1/2 of N was applied as a top dressing at the nine-leaf stage (V9). The phosphorus (P) and potash (K) fertilizers were used in accordance with local fertilization standards, at 100 kg P ha^{-1}, and 100 kg K ha^{-1}, to ensure that all experimental treatments had equal P and K concentrations. The fertilizer types used in our experiment were urea (46% N), phosphorus pentoxide P_2O_5 (18% P), and potassium oxide K_2O (60% K).

Maize crops were trimmed to four plants per pot at the three-leaf stage to facilitate better adaptability to the pot environment. Throughout the growth stage, plants were watered with tap water to maintain soil moisture at 60 and 80% of the field's water holding capacity. Micro-tensiometers were used to measure the temperature of the soil in each pot (Nanjing Institute of Soil Science, Chinese Academy of Sciences).

4.3. Sampling and Measurements

4.3.1. Determination of Yield and Growth Attributes

At four growth stages (V9, R1, R3, and R6), data on several physiological aspects of maize crops were collected. The plants, ear width, and ear length were measured at physiological maturity. The number of rows per ear and the number of seeds per row were manually counted after harvesting at full maturity. Following threshing, yield characteristics such as kernel yield and kernel per ear were recorded. Each plant's components were then divided into kernels, leaves, stalks, and roots. Following that, the plant dry matter was sun-dried for three days before being oven-dried at 70 °C for 72 h to determine plant dry matter.

For the measurement of root length, diameter and surface area, root samples were taken after harvesting, washed, scanned, and analyzed by root image analysing software. In each treatment, four plants were selected, and the leaf area was calculated using the following formula.

$$LA\left(\mathrm{cm}^2\right) = L \times W \times factor\ (0.75)$$

where LA represent the leaf area (cm^2), L is the length (cm), W is the width (cm), and 0.75 is the constant-coefficient factor for the maize leaf area.

4.3.2. Determination of Antioxidant Enzyme Activity

Four plants from each replicate were selected at four growth stages (V9, R1, R3 and R6) for antioxidant enzyme activity, and leaf samples were put in liquid N_2 for 1 min before being stored at 80 °C for biochemical and physiological analyses. The activity of superoxide dismutase (SOD) was measured with the nitro blue tetrazolium (NBT) illumination technique [65]. The 50% decrease in absorbance at 560 nm was used to represent one unit of SOD activity and was expressed as U g^{-1} fresh weight (FW). Peroxidase (POD) activity was analyzed according to MacAdam et al. [66]. The reaction mixture included a phosphate buffer (50 mM), guaiacol (16 mM), and 0.2 mL of enzyme extract, followed by H_2O_2 (10 mM). The absorbance at 470 nm was measured until 5 readings were taken at 30 s intervals. The activity of ascorbate peroxidase (APX) was determined by Nakano and Asada [67]. The reaction mixture included a 50 mM phosphate buffer, 0.1 mM EDTA, 0.5 mM AsA, and 1.0 mM H_2O_2, along with 0.2 mL crude enzyme extract. The following formula was used to calculate the change in absorbance of the mixture at 290 nm.

$$APX\ activity\left(U\ \mathrm{mg}^{-1}\right) = \frac{\Delta A_{290} \times Vt}{2.8 \times M \times V \times t}$$

4.3.3. Chlorophyll Content

With slight modifications, the chlorophyll content was determined using the method described in [68]. To avoid light from altering the results, the leaf samples from each treatment were chopped up and immersed in a graduated tube with 80% acetone. When chlorophyll had been extracted, the supernatant was then removed and placed in a new tube, and the absorbance was recorded at wavelengths of 663, 645, and 470 nm to measure the content of chlorophyll *a* and *b*, and 80% acetone was utilized as a blank control.

4.3.4. Determination of ROS, MDA and Proline Content

The superoxide anion ($O_2{}^{-}$) content was determined by following the method of [69]. Briefly, a fresh sample of the leaf (500 mg) was homogenized with a 65 mM potassium phosphate buffer (pH 7.8), and centrifuged for 10 min at 8000× *g* at 4 °C. Next, 2 mL of supernatant was mixed with 0.5 mL of potassium phosphate-buffer (PBS; 65 mM, pH 7.8), and 0.1 mL of hydroxylamine hydrochloride solution (10 M) was mixed together and kept at 25 °C for 1 h. One mL of amino benzene sulfonic acid solution (58 M) and 1 mL of an α-naphthylamine solution (7 M) was added, and incubated at 25 °C for 20 min. The pigments were then extracted with 1 mL of chloroform. The mixture was centrifuged for 10 min at 10,000× *g* at 4 °C. The absorbance at 532 nm was determined by collecting the upper pink supernatant, while the H_2O_2 content was assayed according to Ohto et al. [70].

Malondialdehyde, a product of lipid peroxidation, was measured in plant leaves using the method described by Weisany et al. [71]. Trichloroacetic acid (TCA; 0.1% w/v) was used to extract 200 mg of fresh leaf, and the extract was centrifuged at 12,000× *g* for 5 min at 4 °C. Subsequently, 20% of TCA was added to the solution and thoroughly mixed with 0.5% of 4 mL thiobarbituric acid (TBA) and incubated for half an hour in a hot water bath at 90 °C. The entire extract was kept on ice. The absorbance values were measured at 532 nm, and the nonspecific absorption at 600 nm was subtracted from the absorbance data.

The ninhydrin procedure, described by Bates et al. [72], was used to determine the free proline content. A fresh leaf sample (500 mg) was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and centrifuged at 10,000× *g* for 15 min. Following the filtration of the homogenized solution, 2 mL of the filtered solution was transferred to test tubes and treated with acid ninhydrin (2 mL) and glacial acetic acid (2 mL). The tubes were kept warm at 80 °C for 1 h. To stop the process, the tubes were placed in an ice bath. The liquid was aggressively stirred for a few seconds with a mixer after adding 4 mL of toluene. After separating the toluene chromophore from the aqueous phase, the absorbance at 520 nm was measured.

4.3.5. Soil Enzyme Activity Analysis

The activities of soil acid phosphatase (BC0145), acid invertase (BC3075), β-glucosides (BC0165), catalase (BC0105), cellulose (BC0155), and soil urease (BC0125) were determined using the soil enzyme kit from Solarbio Science & Technology Co. (Beijing, China). The methods of determination are described in detail in the manual.

4.4. Statistical Analysis

A mixed-model analysis of variance (ANOVA) was used to calculate the effects of N rates and irrigation on yields, enzymatic activity, and kernel quality during the four growth seasons. The analysis of variance (ANOVA) was performed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). The least significant difference (LSD) test was used to separate means and interactions. Statistical significance was evaluated at $p \leq 0.05$.

5. Conclusions

Low irrigation water (LW) resulted in higher plant height, leaf area, root and shoot dry matter, SOD, POD, APX in leaves, kernels per row, kernels per ear, root length, root volume, root diameter and kernel yield of hybrid maize plants. Related to yield, antioxidant enzymes, soil enzymes, and root parameters also improved with increased N application rates up to 300 kg N ha^{-1} under LW irrigation. However, MDA, proline, H_2O_2 and O_2^- contents in leaves showed the opposite response to N application rates. Similarly, SOD, POD, and APX activities were associated with a relatively higher N content in the soil. Thus, increasing application rate of N (up to 300 kg N ha^{-1}) improved leaf-physiological characteristics and consequently produced a considerable maize yield under LW irrigation. This knowledge can be applied to need-based N applications, reducing potential N loss and non-point pollution. More research is needed to determine the impact of N fertilizer rates and irrigation on N uptake, kernel quality, and the leachate of N to groundwater.

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Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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