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Study on the Mechanism of Slow-Release Fertilizer and Nitrogen Fertilizer on the Senescence Characteristics of Quinoa Leaves

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Abstract: The objective of this study was to investigate how nitrogen and slow-release fertilizers affect the traits of leaf senescence and quinoa production in order to explore the optimal slow-release fertilizer and nitrogen fertilizer ratios suitable for quinoa production, as well as to provide theoretical references for the planting of quinoa fertilization methods and fertilizer amount. In this experiment, the main local strain Quinoa 77 was selected as the experimental material, and six treatments were set up: CK: no nitrogen fertilizer; T_1: 100% urea (N); T_2: 100% slow-release fertilizer (C); T_3: 5:5 (C_5N_5); T_4: 3:7 (C_3N_7); and T_5: 7:3 (C_7N_3). This was done in order to investigate how various treatments affect the activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD); malondialdehyde (MDA) content; and yield of quinoa leaves. The findings revealed the following: (1) As the reproductive period progressed, the activities of CAT, POD, and SOD in quinoa leaves treated differently showed a tendency to increase and subsequently decrease, and they reached the peak value at the early stage of filling. The activity of CAT, POD, and SOD in the T_3 treatment was the highest, and the average activities were 3148.74 $U{\cdot}g^{-1}$, 2197.84 $U{\cdot}g^{-1}$, and 118.51 $U{\cdot}g^{-1}$, respectively, which increased by 78.90%, 101.99%, and 108.14%, respectively, compared with CK. The content of MDA continued to increase with the progress of fertility. The average T_3 treatment was 36.41 $nmol{\cdot}g^{-1}$, which was 46.87% lower than that of CK. (2) Out of all the treatments, T_3 had the highest yield with an average of 3829.43 $kg{\cdot}hm^{-2}$, T_5 the second with an average of 3313.52 $kg{\cdot}hm^{-2}$, and T_4 the third with 2847.47 $kg{\cdot}hm^{-2}$, which increased yields by 96.18%, 69.75%, and 45.87%, respectively, compared with CK. (3) Yield was highly significantly and positively correlated with thousand kernel weight; number of grains per spike per plant; and the early filling stages of CAT, POD, and SOD sports, and it had a negative, extremely significant correlation with MDA content. Comprehensive analysis showed that slow-release fertilizer and nitrogen fertilizer can improve the antioxidant enzyme activity of quinoa leaves, inhibit MDA content, improve the physiological characteristics of quinoa, and delay the purpose of leaf senescence, with a better effect of yield and income, of which the T_3 treatment had the high-quality impact of increasing yields and was a more scientific and reasonable fertilization method.

Keywords: quinoa; slow-release fertilizer; nitrogen fertilizer; senescence characteristics; yield

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1. Introduction

Quinoa (*Chenopodium quinoa* Willd.), also known as South American quinoa, Indian wheat, etc., is an annual dicotyledonous plant of the subfamily quinoa. It is indigenous to South America's Andes Mountains and has been cultivated in the Andean area for thousands of years [1]. Quinoa has strong resistance to cold, salt, and drought, and it can grow normally on marginal soil. Quinoa grain is called a total nutritious food because of its excellent protein, extensive range of minerals, and wealth of vitamins. It has a variety of development and utilization values and has received extensive attention all over the world [2,3]. Therefore, increasing the yield of quinoa is of great significance to its development and utilization.

Nitrogen is an critical nutrient element for the increase and development of crops. The different types of nitrogen are organic nitrogen, ammonium nitrogen, and nitrate nitrogen. Plants absorb and use nitrate nitrogen and ammonium nitrogen [4,5]. Because the content of these two kinds of nitrogen in the soil is far from meeting the needs of normal plant growth [6], nitrogen fertilizer has become the most important factor restricting crop growth and development. The rational software of nitrogen fertilizer is very important for quinoa to obtain a high and stable yield.

Slow-release fertilizer is also known as controlled-release fertilizer, slow-acting fertilizer, and long-acting fertilizer. It refers to a fertilizer whose chemical composition is changed, or when the surface is coated with semi-permeable or impervious substances, and then the effective nutrients are released slowly in order to effectively regulate the release rate of nutrients [7]. Slow-release fertilizer acts by extending or controlling the nutrient release period so that the soil nutrient supply and crop nutrient demand are coordinated to realize long-lasting nitrogen supply management, improve the soil nutrient content, prolong the period of fertilizer efficacy, and enhance the utilization rate of fertilizer [8,9]. Slow- and controlled-release fertilizer are mainly based on nitrogen fertilizer, which can achieve simultaneous sowing of seed fertilizer. After a one-time base application, it can be gradually degraded by chemical and biological methods, and long-term nutrient release can be realized so that crop growth and nutrient release can be synchronized to meet the nutrient demand of the whole growth period of crops [10]. Applying slow-release fertilizer can not only reduce the number of fertilizations but also save labor costs and improve labor productivity. The study of Huang Hua et al. [11] showed that conventional fertilizer needs to be applied seven times to complete the growth of rice, while slow-release fertilizer only needs to be applied four times, which reduces the number of fertilization times and is conducive to saving labor costs. Slow- and controlled-release fertilizers can regulate the release rate of nutrients, reduce the loss of fertilizers caused by volatilization and leaching, improve the utilization rate of fertilizers, and increase crop yield [12]. Xie Peicai et al.'s [13] research showed that coated slow-release fertilizer could effectively improve the utilization rates of nitrogen, phosphorus, and potassium. Compared with common compound fertilizer, the utilization rates of nitrogen used on corn and wheat were increased by 5.04% and 9.14% on average, and the utilization rates of phosphorus were increased by 11.22% and 17.52% on average, respectively. The utilization rate of potassium was increased by 11.26% and 8.35%, respectively. And slow-release fertilizers meet the nitrogen needs of crops, making their growth stronger and improving their resistance to diseases and pests. Through the study of tomato growth, yield, and quality, Wu Liyan et al. [14] found that the two slow-release fertilizers were superior to traditional fertilizers and had fewer diseases and pests. Under the background of today's "low-carbon economy", the extensive use of slow- and controlled-release fertilizers can save fertilizer and medicine, playing an important role in the sustainable development of agriculture [7]. However, due to its high cost, it is difficult to obtain high returns in production, so in the current fertilizer industry, it is used in conjunction with urea to achieve the purpose of cost savings and efficiency. Urea combined with slow-release nitrogen fertilizer can effectively increase the content of soil total nitrogen and ammonium nitrogen, reduce the content of nitrate nitrogen, ensure soil fertility, and reduce the nutrient loss of sloping farmland runoff [15]. Wang Hui's [12] study found that compared with previous years of formula fertilization (only chemical fertilizer), the application of slow-release fertilizer has improved the fertilizer utilization rate, and the fertilizer reduction has increased the efficiency. Urea mixed slow-release nitrogen fertilizer treatment is mainly distributed in the 0–40 cm soil layer, which is not only conducive to crop absorption but also reduces the leaching loss of soil nitrogen to the deeper soil layer, as well as reducing the risk of deep soil environmental pollution [16]. Therefore, the research on the combined application of slow-release fertilizer and nitrogen fertilizer is of great significance to the protection of the ecological environment and the sustainable development of agriculture. In cultivation techniques, the impact of sluggish-release fertilizers on the yield of grain crops, which include rice, wheat, and maize, has been mentioned [17]. The application of slow-release fertilizer as base fertilizer in rice production can ensure the normal tillering of rice and the yield of rice under the condition of reducing the amount of chemical nitrogen fertilizer by 10% [18]. Gu Rui [19] found that the application of slow-release nitrogen fertilizer could not only improve soil fertility but also significantly improve the water and nitrogen use efficiency of wheat, and the quality of wheat was also improved. Xu Lili et al. [20] showed that the application of slow-release fertilizer could significantly improve the phenomenon of corn bald tip; increase ear length, ear thickness, ear number, ear weight, etc.; and increase yield. There are also corresponding studies on the combined application of slow-release fertilizer and nitrogen fertilizer. Yang Jinyu et al. [21] showed that the different ratios of slow-release nitrogen fertilizers and urea have certain differences in their effects on the growth status and production efficiency of crops and that slow-release nitrogen fertilizers and urea rationing can regulate the dynamics of wheat grouting and the composition of yields to achieve the purpose of increasing yields. Ji Jinghong et al. [22] showed that slow-release fertilizers and urea used in combination at a certain ratio could delay leaf aging, thus increasing spring corn yield and income. Zou Qifang et al. [23] found that the combined application of 100% urea +75% slow-release fertilizer increased the yield of winter wheat by 22.41% and 11.00%, respectively, compared with the treatment of 100% urea and 100% slow-release fertilizer. Lu Jinling et al. [24] found that under 50% polyurea formaldehyde slow-release fertilizer +50% urea treatment, wheat yield was significantly increased. In the study of flue-cured tobacco, Liu Fang et al. [25] found that the combined application of 50% base fertilizer +50% slow-release fertilizer could increase the yield, output value, and single-leaf weight of cured tobacco leaves to the maximum extent under the condition of constant nitrogen application. The combined application of slow-release fertilizer and nitrogen fertilizer has been studied in wheat, maize, and other crops, but it has not been found in quinoa.

The main reasons for leaf senescence in the late growth stage of plants are the reduction of antioxidant enzyme activity, the destruction of the inter-mobile reactive oxygen species era and scavenging mechanism, the immoderate accumulation of cellular reactive oxygen species, the deepening of the diploma of membrane lipid peroxidation, and a boom in the content material of MDA [26,27]. Senescence, as the very last stage in the natural development technique of plants, has an essential effect at the final yield of crops at some point of the duration of its incidence, so in-depth research at the regulatory mechanisms and influencing factors of crop senescence is of great significance in promoting crop yield enhancement. Predecessors' studies have shown that superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) are essential antioxidant-protecting enzymes in plants [28–30], and their contents are not only affected by genotypes [31–34] but also by sowing period, nitrogen application, irrigation, and other cultivation measures [35–39]. Duan Pengfei et al. [40] found that compared with normal fertilization with equal nutrients, a one-time basal application of controlled loss slow-release fertilizer enhanced the activities of antioxidant enzymes SOD and CAT, and reducedas well as reducing the content of MDA. The results of Wang, Y. et al. [41] showed that the activities of SOD, POD, and CAT in flag leaves of wheat after flowering were effectively maintained by 324 kg/hm^2 slow-release fertilizer, 324 kg/hm^2 compound fertilizer, and 15 kg/hm^2 water-retaining agent, and the content of MDA was decreased. Bai Yao et al. [42] found that when N300 kg/hm^2 was applied and slow-release fertilizer was sprayed, the enzyme activities of CAT, POD, and SOD were the highest, and the activity of malondialdehyde was the lowest. Yan Dongliang et al.'s [43] study on maize showed that the SOD and POD activities of ear position leaves could be significantly increased and the MDA content decreased when the nitrogen application level was 180 kg/hm^2 and the controlled release nitrogen to urea nitrogen ratio was 1:2. There are few reports on the effects of the combination of slow-release fertilizer and nitrogen fertilizer on the aging characteristics of crops, and there is no report on the study of quinoa, with the optimal ratio of slow-release fertilizer and nitrogen fertilizer on the aging characteristics and yield of quinoa remaining to be studied. Therefore, this study started from the ratio of slow-release fertilizer and nitrogen fertilizer to study the effects of fertilizer ratio on the aging characteristics and yield of quinoa leaves, analyzed the changes of antioxidant enzymes and MDA contents of quinoa leaves, and aimed to reveal the internal physiological mechanism of quinoa under different fertilizer ratios so as to screen out the optimal fertilizer application scheme suitable for quinoa growth. This was to provide a theoretical reference for the high-yield cultivation techniques of quinoa.

2. Materials and Methods

2.1. Overview of the Test Site

The experiment was carried out at the experimental demonstration base in Jingle County (38°3′ N, 119° E), Xinzhou City, Shanxi Province. The area is 1140~2421 m above sea level, with a temperate monsoon climate, four distinct seasons, warm and hot summers, large temperature difference between day and night, annual rainfall of 380~500 m, annual average temperature of 7.2 °C, over 2500 h of sunshine, and 120~135 days without frost experienced per year. The soil in this area is yellow loam, which is weakly alkaline and has a low organic matter content. In 2022, the soil layer of 0~20 cm of the test base contained 7.60 g/kg organic matter, 91.5 mg/kg total nitrogen, 128 mg/kg available potassium, and 20.23 mg/kg available phosphorus, as well as having an 8.14 pH. From 2022 to 2023, soil organic matter content was 7.87 g/kg, total nitrogen was 95.5 g/kg, available potassium was 132 mg/kg, and available phosphorus was 21.31 mg/kg, with a pH of 8.09.

2.2. Experimental Materials

The quinoa strain for testing was the local main variety, No. 77. Fertilizers for testing: nitrogen fertilizer was urea (N 46%) and slow-release fertilizer (abbreviated as C) ($N:P_2O_5:K_2O$ = 26:12:12). Chemical composition of slow-release fertilizer: the types of slow-release nutrients are nitrogen, with total nutrients ≥ 50%, slow-release nitrogen ≥ 8.0%, P_2O_5 ≥ 21%, and K_2O ≥ 21%. We used diammonium phosphate ($(NH_4)_2HPO_4$) and monoammonium phosphate ($NH_4H_2PO_4$) as phosphorus raw materials.

2.3. Test Methods

The experiment was a one-way randomized block design with six treatments, namely, (1) CK: control treatment without nitrogen fertilizer (CK); (2) T_1: 100% urea (N); (3) T_2: 100% slow-release fertilizer (C); the following mixing ratios for a single application of slow-release fertilizers with urea ratio for slow-release nitrogen fertilizers (C) and urea (N): (4) T_3: 5:5 (C_5N_5); (5) T_4: 3:7 (C_3N_7); (6) T_5: 7:3 (C_7N_3). The fertilizers were a one-time basal application. Each treatment was replicated three times with a plot area of 50 m^2 and a total of 18 plots. Protected rows and protected strips were set up around the perimeter. The seed was sown by hand in holes at a depth of two cm, with a spacing of 40 cm between flora and 40 cm between rows, at a density of 4682.34 plants/mu. Field management measures such as water and fertilizer, as well as pest and weed control, in the experimental field were carried out according to the local high-yield field. The fertilizer application rate is shown in Table 1.

Table 1. Experimental fertilizer rates.

Treatment	Application of N kg/hm^2	Fertilizer Proportioning Method	One-Time Basal Application kg/hm^2
CK	0	0	0
T_1	82.8	100% N	13.5 N
T_2	82.8	100% C	23.85 C
T_3	82.8	C:N = 5:5	12 C + 6.75 N
T_4	82.8	C:N = 3:7	7.2 C + 9.45 N
T_5	82.8	C:N = 7:3	16.65 C + 4.05 N

Note: CK: control treatment, no nitrogen fertilizer; T_1: apply 100% urea (N); T_2: apply 100% slow-release fertilizer (C); T_3: the ratio of slow-release fertilizer to nitrogen fertilizer was 5:5 (C_5N_5); T_4: the ratio of slow release fertilizer to nitrogen fertilizer was 3:7 (C_3N_7); T_5: the ratio of slow release fertilizer to nitrogen fertilizer was 7:3 (C_7N_3).

2.4. Measurement Indicators and Methods

2.4.1. Blade Sampling and Instrumentation

At the heading stage (HS), flowering stage (FS), initial grouting stage (IGS), Late grout stage (LGS), and mature stage (MS), 15~20 reversed four-leaf leaves were taken from the leaves at 9:30–11:30 a.m. and placed in ice pots on a sunny day, returned to the laboratory, and kept at −80 °C in an ultra-low temperature refrigerator. The activity of CAT, POD, and SOD and the content of MDA in the leaves were detected using the biochemical kits provided by Beijing Suolaibao Technology Co., Ltd. (Beijing, China). All were determined by ultraviolet spectrophotometry. Tissuelyser-96, a fully automatic sample grinder from Shanghai Jingxin Technology Co., Ltd. (Shanghai, China) was used for grinding. We weighed with the electronic balance HZT-A + 200 of Huazhi Electronic Technology Co., Ltd. (Putian, China). Centrifugation was carried out with an Eppendorf (Hamburg, Germany) centrifuge, Centrifuge 5810 R. The determination was performed with a UV-VIS spectrophotometer (UV9100D S/N: 1905UV1849) from Beijing Labtech Science and Technology Instrument Co., Ltd. (Beijing, China).

2.4.2. Determination of CAT Enzyme Activity

After the leaves of quinoa were mashed with liquid nitrogen, 0.1 g was weighed, added with 1 mL extract, homogenized in the ice bath, and centrifuged at 8000× *g* at 4 °C for 10 min; then, the supernatant was placed on ice to be tested. We took the 1 mL CAT detection solution in the 1 mL quartz colorimetric plate, then added the 35 uL sample, mixed it well for 5 s, and immediately determined the absorption value A1 of 240 nm and the absorption value A2 of 1 min at room temperature. We calculated the AA = A1 − A2.

CAT activity calculation:

Definition of unit: every g tissue catalyzes 1 umolH_2O_2 degradation per minute in the reaction system as an enzyme activity unit.

Calculation formula: CAT (U/g) mass) = [ΔA × V inverse total/(ε × d) × 10^6]/(W × V sample/V sample total)/T = 678 × ΔA/W

Note: V inverse total: total volume of the reaction system, 1.035×10^{-3} L; ε: molar absorption coefficient of H_2O_2, 43.6 L/mol/cm; d: light diameter of the cupola, 1 cm; V sample: add the sample volume, 0.035 mL; V sample total: add extraction liquid volume, 1 mL; T: reaction time, 1 min; W: sample quality, g; 10^6: unit conversion factor, 1 mol = 10^6 μmol.

2.4.3. Determination of SOD Enzyme Activity

After the leaves of quinoa were mashed with liquid nitrogen, 0.1 g was weighed, added with 1 mL extract, homogenized in the ice bath, and centrifuged at 8000× *g* at 4 °C for 10 min; then, the supernatant was placed on ice to be tested. We took the 90 uL sample supernatant; added the reagent according to the reagent adding table in the test kit instructions; mixed the reagent thoroughly; bathed it in 37 °C water for 30 min; placed it in a 1 mL glass cuvette to measure the absorbance at 560 nm; separately recorded it as A determination, A control, A1 blank, and A2 blank; and calculated ΔA determination = A determination − A control. ΔA blank = A1 blank − A2 blank.

SOD activity calculation:

Calculation of inhibition percentage: inhibition percentage = (ΔA blank − ΔA determination)/ΔA blank × 100%

Unit definition: When the inhibition percentage in the above xanthine oxidase coupling reaction system is 50%, the activity of the SOD enzyme in the reaction system is defined as an enzyme activity unit.

Calculation formula: SOD (U/g mass) = [inhibition percentage/(1 − inhibition percentage) × V inverse total]/(W × V sample/V sample total) × F = 11.11 × inhibition percentage/(1 − inhibition percentage)/W × F

Note: V inverse total: total volume of the reaction system, 1 mL; V sample: the volume of the sample added to the reaction system, 0.09 mL; V sample total: add extraction liquid volume, 1 mL; W: sample quality, g; F: sample dilution ratio.

2.4.4. Determination of POD Enzyme Activity

After the quinoa leaves were mashed with liquid nitrogen, 0.1 g was weighed; 1 mL of the extract was added; and the mixture was homogenized in the ice bath, centrifuged at 8000× *g* 4 °C for 10 min, and put on ice for testing. In the 1 mL glass colorimetric dish, the supernatant and reagent of 15 uL samples were added according to the sample determination table and immediately mixed and timed, and the absorbance values A1 and A2 after 30 s of 470 nm and Imin30 s were recorded. We calculated ΔA = A2 − A1.

POD activity calculation:

Unit definition: 0.01 per minute change in A470 per minute per g of tissue in the reaction is one unit of enzyme activity.

Calculation formula: POD (U/g mass) = ΔA × V inverse total/(W × V sample/V sample total)/0.01/T = 7133 × ΔA/W

Note: V inverse total: total volume of the reaction system, 1.07 mL; V sample: add the sample volume, 0.015 mL; V sample total: add extraction liquid volume, 1 mL; T: reaction time, 1 min; W: sample quality, g.

2.4.5. Determination of MDA Content

After the quinoa leaves were mashed with liquid nitrogen, 0.1 g was weighed; 1 mL of the extract was added; and the mixture was homogenized in an ice bath and centrifuged at 8000× *g* 4 °C for 10 min. Then, the supernatant was taken and placed on ice for testing. We added the reagent according to the sample table, kept the mixture in a water bath at 100 °C for 60 min (cover tightly to prevent moisture loss), cooled it in an ice bath, placed it in a centrifuge at 10,000× *g*, room temperature, and centrifuged it for 10 min. We took the supernatant to the 1 mL glass colorimetric plate; determined the absorbance of each sample at 532 nm and 600 nm; and calculated them, respectively, ΔA532 = A532 determination − A532 blank, ΔA600 = A600 determination − A600 blank, ΔA = ΔA532 − ΔA600.

MDA content calculation:

MDA content (nmol/g mass) = [ΔA × V inverse total/(ε × d) × 10^9]/(W × V sample/V extraction) × F =32.258 × ΔA/W

Note: V inverse total: total volume of reaction system, 0.001 L; ε: molar absorption coefficient of MDA, 1.55 × 10^5 L/mol/cm; V sample: add sample volume, 0.2 mL; d: light diameter of the cupola, 1 cm; V extraction: add the extraction liquid volume, 1 mL; W: sample quality, g; 10^9: unit conversion factor, 1 mol = 10^9 nmol; F: dilution ratio.

2.4.6. Determination of Yield and Its Components

When the stems turned yellow and the leaves were 80% yellow, 10 representative plants with uniform growth were randomly selected from each plot to determine the main spike length (MSL), grain number per plant (GNPP), and effective branch number per plant (EBNPP). After drying, the seeds were threshed, dried cleaned, and weighed to decide the one thousand-grain weight (TGW) and yield.

2.5. Data Processing

Excel 2016 was used to calculate the mean and standard deviation and tabulate the experimental data; data were analyzed by ANOVA using DPS 7.05 software (significance level was taken as α = 0.05), and plots were made using Origin 2022.

3. Results and Analysis

3.1. Characterization of Quinoa Leaf Senescence

3.1.1. CAT Activity

As is visible from Figure 1, the CAT activity of quinoa leaves in different treatments showed a general trend of increasing and then decreasing with the process of fertility, reaching a peak value at the initial grouting stage. From the heading stage to the mature stage, the CAT activities of quinoa leaves showed as follows: $T_3 > T_5 > T_4 > T_1 > T_2 >$ CK. The average CAT activity of the T_3 treatment was 3148.74 U·g^{-1}, which was 78.90%, 24.93%, 45.69%, 16.21%, and 9.50% higher than that of CK, T_1, T_2, T_4, and T_5, respectively. The consequences confirmed that the combined application of sluggish-release fertilizer and nitrogen fertilizer could significantly increase the CAT activity of quinoa leaves. There were significant differences between T_3 treatment and other treatments at the flowering stage, initial grouting stage, and mature stage.

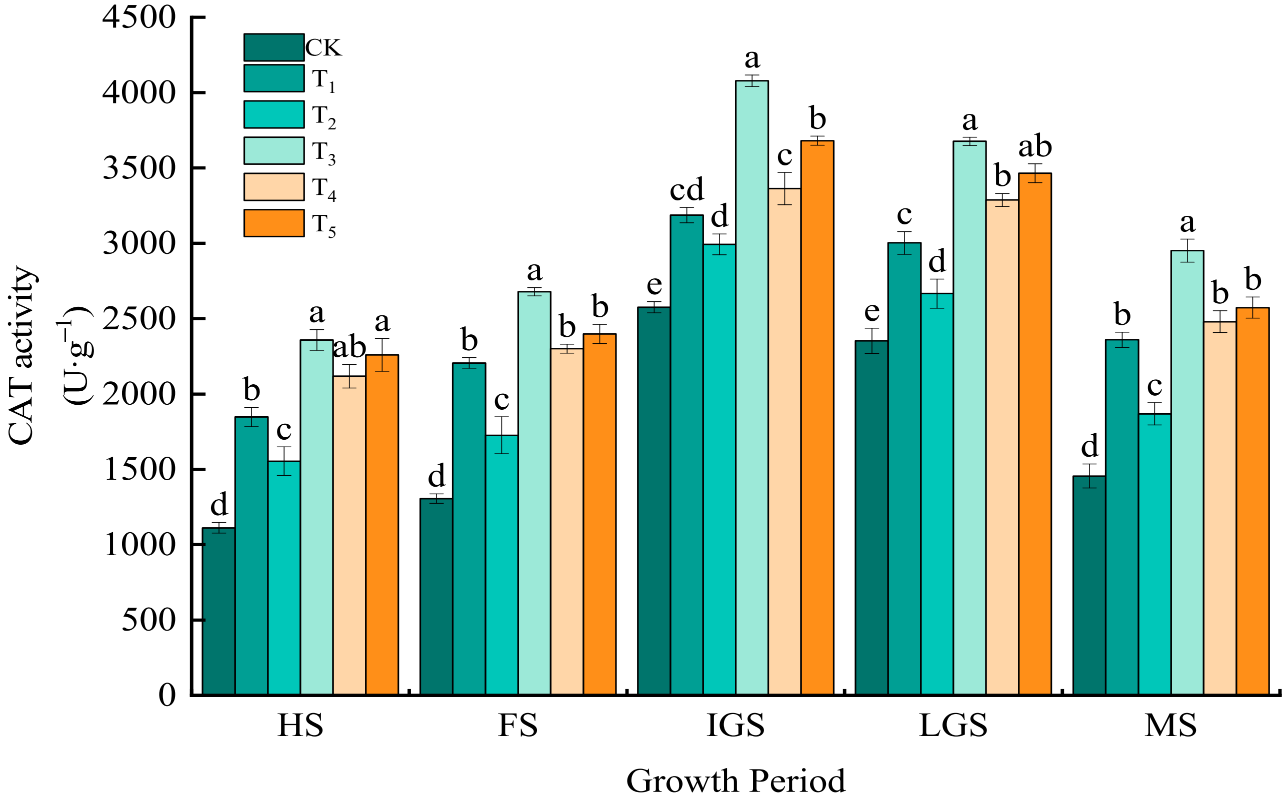

Figure 1. Changes of CAT enzyme activity in leaves underneath the mixed utility of slow-release fertilizer and nitrogen fertilizer. Note: Different lowercase letters indicate that there are significant differences among different treatments in the same period at 0.05 level.

3.1.2. POD Activity

As can be visible from Figure 2, the trend of changes in POD activity in quinoa leaves was generally the same for all treatments, growing first, and after this lowering, achieving the highest level at the initial grouting stage and rapidly reducing at the mature stage. During the whole growth period, the POD activity of quinoa leaves treated with different treatments was as follows: $T_3 > T_5 > T_4 > T_1 > T_2 >$ CK. T_3 had the best effect, with an average POD activity of 2197.84 U·g^{-1}. T_1, T_2, T_3, T_4, and T_5 increased by 44.10%, 19.51%, 101.99%, 55.58%, and 69.24%, respectively, compared with CK. From IGS to MS, the POD activity of T_3 treatment was still at a high level and reached a significant difference with other treatments.

3.1.3. SOD Activity

As can be seen from Figure 3, the direction of the SOD activity curve in quinoa leaves between treatments was basically similar to that of CAT and POD activity curves; with the advancement of the reproductive process, SOD showed a trend of increasing and then decreasing, reaching a peak at the IGS. From the whole reproductive period, the T_3 treatment SOD enzyme activity ranked first with an average of 118.51 U·g^{-1}, which was 108.14%, 43.92%, 72.67%, 31.92%, and 18.56% higher than CK, T_1, T_2, T_4, and T_5, respectively. Significant differences were reached between treatments at the IGS and LGS, except for T_1 and T_4.

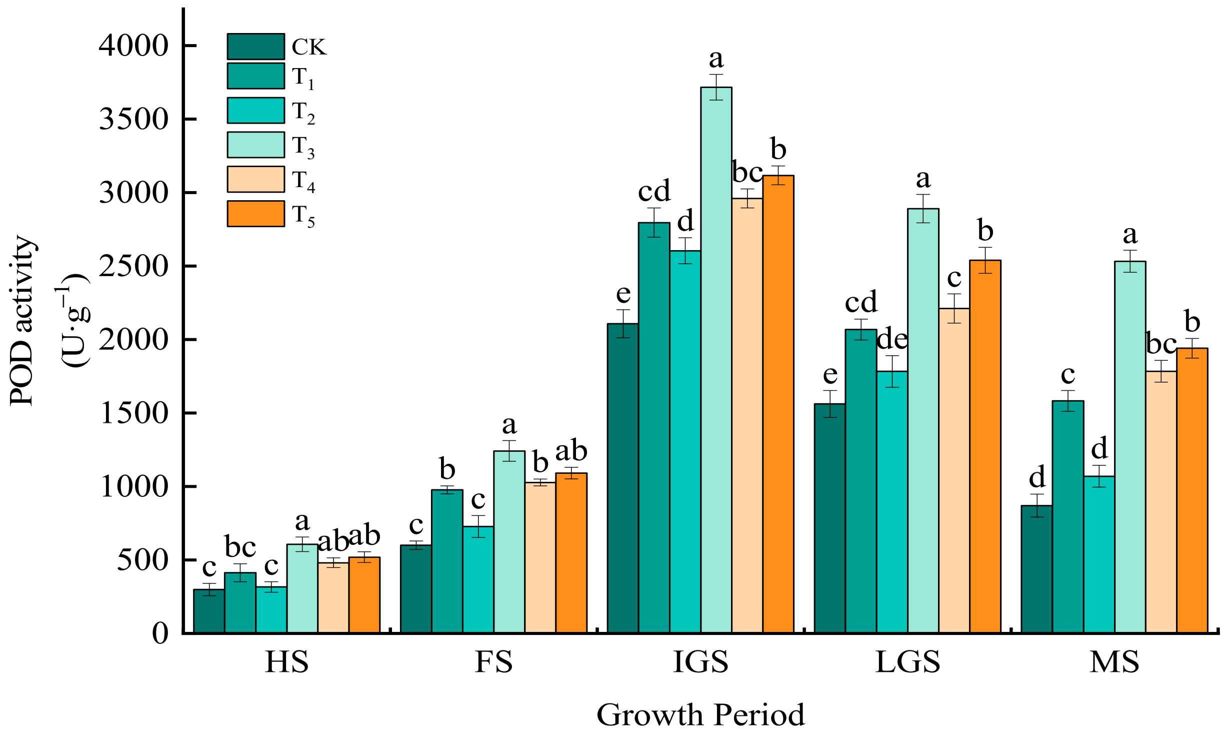

Figure 2. Changes of POD enzyme activity in leaves underneath the mixed utility of slow-release fertilizer and nitrogen fertilizer. Different lowercase letters indicate that there were significant differences among different treatments in the same period at the 0.05 level.

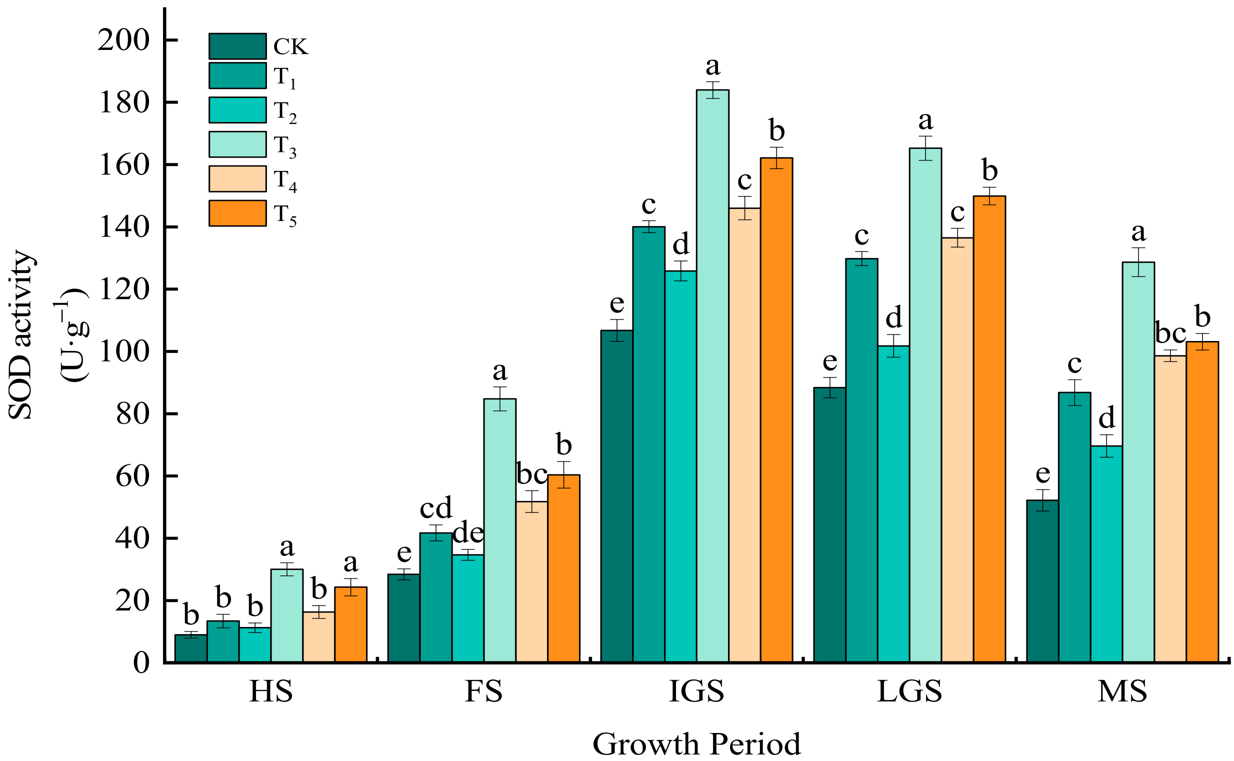

Figure 3. Changes of SOD enzyme activity in leaves underneath the mixed utility of slow-release fertilizer and nitrogen fertilizer. Different lowercase letters indicate that there were significant differences among different treatments in the same period at the 0.05 level.

3.1.4. MDA Content

Figure 4 showed that the MDA content of quinoa leaves in different treatments increased continuously with the change of plant senescence and reached the maximum at the mature stage. During the whole growth period, T_3 treatment was the best, followed by T_5 treatment, and CK treatment had the worst inhibitory effect on MDA content in quinoa leaves. The average MDA content of T_3 treatment was 36.41 nmol·g^{-1}, which decreased by 46.87%, 21.55%, 33.94%, 26.26%, and 13.93%, respectively, compared with CK, T_1, T_2, T_4, and T_5, indicating that the T_3 treatment had the best inhibitory effect on MDA, which could delay crop senescence and increase the yield of quinoa.

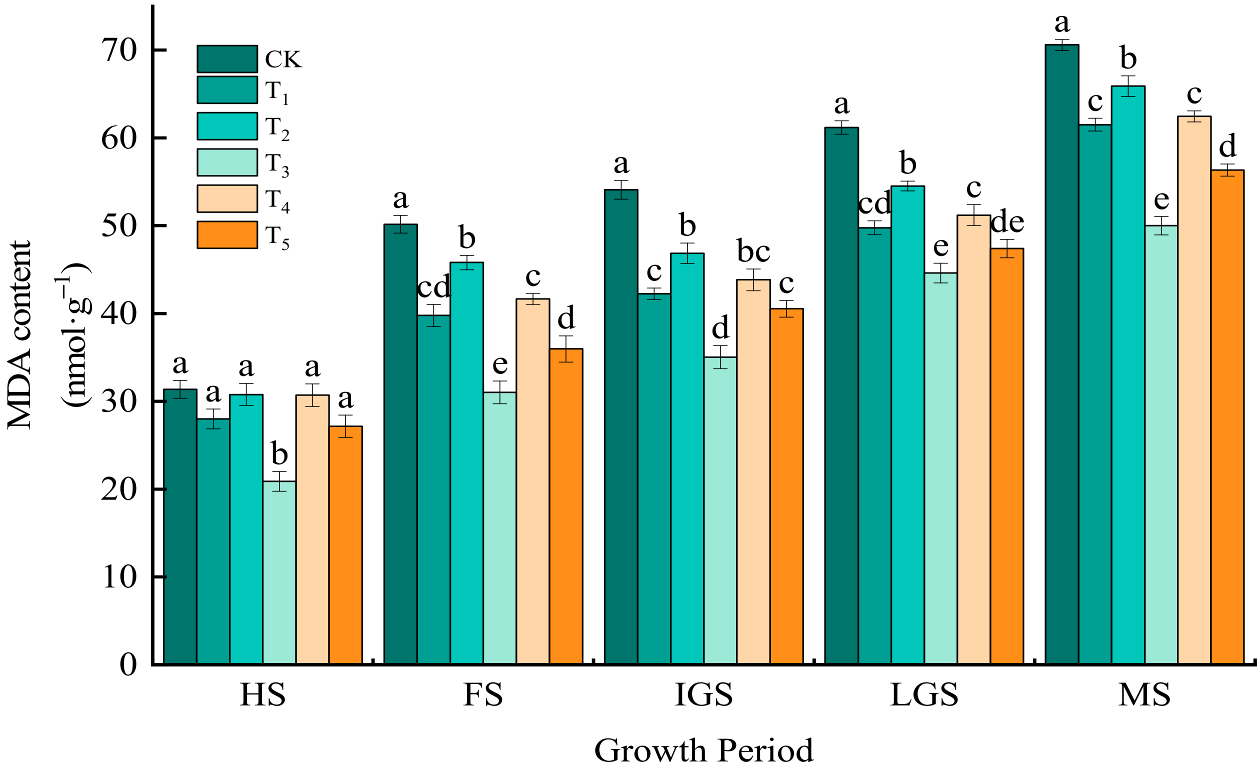

Figure 4. Changes of MDA content in leaves under the blended software of slow-release fertilizer and nitrogen fertilizer. Different lowercase letters indicate that there were significant differences among different treatments in the same period at the 0.05 level.

3.2. Analysis of Yield and Its Components

As shown in Table 2, compared with no fertilization, fertilization treatment can significantly increase quinoa yield. Among them, T_3 had the highest yield, with an average of 3829.43 kg·hm^{-2}; T_5 had the second, with an average of 3313.52 kg·hm^{-2}; and CK had the lowest yield, with an average of 1952.05 kg·hm^{-2}. Compared with CK, T_1, T_2, T_3, T_4, and T_5 increased production by 38.93%, 36.71%, 96.18%, 45.87%, and 69.75%, respectively.

Analyzing the yield components, the main spike period of quinoa within the T3 treatment was once drastically better than the opposite redress, 66.60 cm, which was 4.72%, 11.37%, 12.12%, 7.07%, and 6.05% higher than that of CK, T_1, T_2, T_4, and T_5, respectively. The maximum number of grains per plant appeared in the T_3 treatment, which increased by 72.60%, 43.91%, 48.98%, 46.61%, and 16.63% compared with CK, T_1, T_2, T_4, and T_5, respectively. The effective branch number per quinoa plant ranged from 18.40 to 25.20, with the T_3 treatment showing the highest number, which increased by 23.53%, 16.67%, 36.96%, 32.63%, and 27.27% compared with CK, T_1, T_2, T_4, and T_5, respectively. The 1000-grain weight varied significantly among treatments and was the highest in T_3. Compared with CK, T_1~T_5 1000-grain weight increased by 15.90%, 13.74%, 23.41%, 14.76%, and 18.07%, respectively.

Table 2. Yield and its components in quinoa under different fertilization treatments.

Treatment	Main Spike Length/cm	Grain Number per Plant	Effective Branch Number per Plant	1000-Grain Weight/g	Yield (kg/hm^2)
CK	63.60 ± 0.66 b	7068.43 ± 19.95 d	20.4 ± 0.95 bc	3.93 ± 0.06 d	1952.05 ± 80.07 e
T_1	59.80 ± 0.37 c	8477.39 ± 42.67 c	21.60 ± 0.75 b	4.56 ± 0.04 bc	2711.89 ± 66.81 cd
T_2	59.40 ± 0.88 c	8189.05 ± 260.29 c	18.40 ± 0.96 c	4.47 ± 0.03 c	2668.55 ± 97.79 d
T_3	66.60 ± 1.47 a	12199.96 ± 28.28 a	25.20 ± 1.16 a	4.85 ± 0.07 a	3829.43 ± 30.07 a
T_4	62.20 ± 0.86 b	8321.45 ± 75.90 c	19.00 ± 0.71 c	4.51 ± 0.01 c	2847.47 ± 82.65 c
T_5	62.80 ± 0.31 b	10460.30 ± 32.27 b	19.8 ± 0.57 bc	4.64 ± 0.06 b	3313.52 ± 40.57 b

Note: The data in the table represent the mean ± standard deviation. Different lowercase letters after the data indicate the difference between different treatments at the 0.05 level.

3.3. Analysis of the Correlation between Leaf Physiological Indexes, Spike Biological Characters, and Yield of Quinoa at the IGS

From Figure 5, it was found that yield was significantly positively correlated with 1000-grain weight, grain number per plant, CAT, POD, and SOD activity at the IGS, and the correlation coefficients were 0.94, 0.96, 0.98, 0.97, and 0.97, respectively, being negatively correlated with MDA content, with a correlation coefficient of −0.93, indicating that the yield of quinoa was closely related to the biological characters of spike and the internal physiological mechanisms of the plant. The 1000-grain weight was notably positively correlated with the number of grains per plant; activity at IGS connected to CAT, POD, and SOD, having a negative correlation with MDA content. The effective branching number was once definitely correlated with GNPP, MSL, POD, and SOD activity at the IGS, with correlation coefficients of 0.64, 0.52, 0.57, and 0.56, respectively. There originally existed an exceptionally strong positive correlation between the GNPP and the IGS activities of CAT, POD, and SOD, with correlation coefficients of 0.94, 0.93, and 0.95, respectively, and being highly negatively correlated with the MDA content, with a correlation coefficient of −0.88. At the IGS, there was a highly significant negative connection with MDA content and a negative correlation with CAT, POD, and SOD activity, indicating that the increase in the protective enzyme activity could inhibit the MDA content.

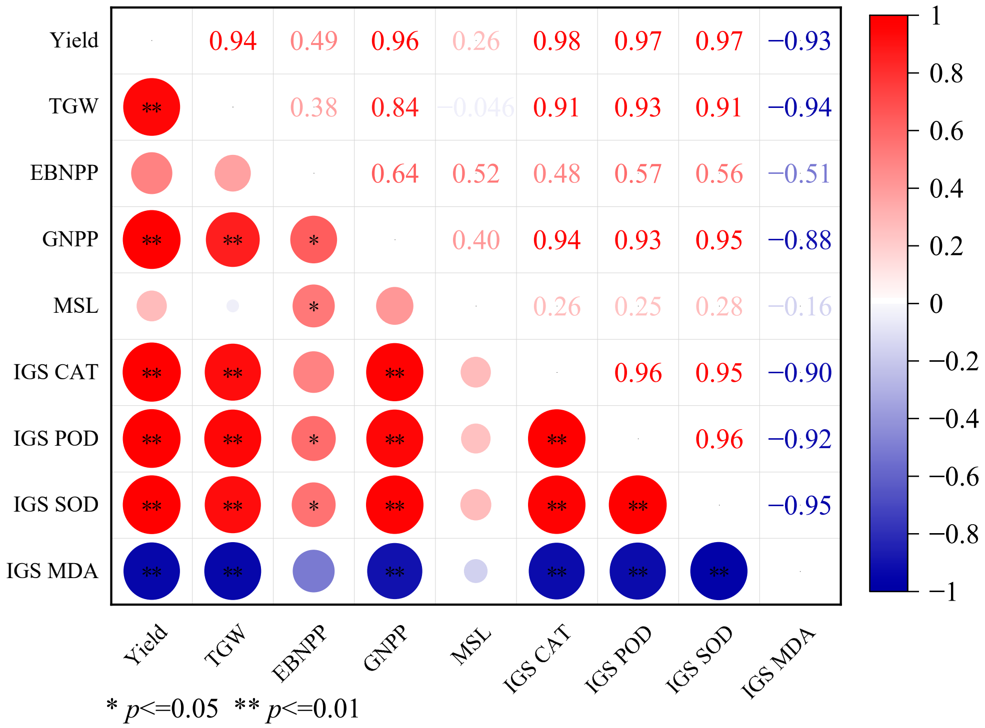

Figure 5. Relationship between yield and its components of quinoa and leaf enzyme activity at the early filling stage.

4. Discussion

4.1. Mechanistic Study of Anti-Aging Properties

The middle and upper leaves of the quinoa plant are the source of nutrition supply for the growth and development of the quinoa grain. At the flowering stage, the plant changes from vegetative growth and reproductive growth to the reproductive growth stage, and the vegetative organs such as stems and leaves essentially stop growing and enter the senescence stage [44]. Yang Shushen believes that the dynamic equilibrium between the production and scavenging of superoxide free radicals and reactive oxygen species in plants is broken during the senescence process, and the concentration of free radicals and reactive oxygen species exceeds the injury "threshold", which accelerates the senescence [45]. CAT is one of the key enzymes in the reactive oxygen species protection machine, especially clearing H_2O_2. SOD is the first line of protection to get rid of reactive oxygen species, being able to prevent the oxidation of superoxide free radicals to the biofilm system. The POD enzyme can effectively remove excessive oxygen free radicals from the cell and cooperate with SOD [46,47]. Consequently, these three enzymes work together to keep the balance of reactive oxygen species in the plant, protect cells, delay leaf senescence, lengthen the time of photosynthesis, and accumulate organic matter, which are all conducive to the increase in quinoa yield.

The consequences of the present study confirmed that CAT, POD, and SOD enzyme activities of quinoa leave from different treatments showed an increasing and then decreasing trend throughout the whole growth period, which is consistent with the trend reported by Xu Junfei et al. [48]. The activities of these three enzymes reached the peak at the early filling stage, which may be on account of the excessive accumulation of reactive oxygen species (ROS) in the duration from flowering to early filling, which induced the enhancement of the activities of the above protective enzymes and then improved the scavenging ability of reactive oxygen free radicals, effectively controlling membrane peroxidation, maximizing cell stability, and delaying the senescence process. However, after the filling stage, due to the mass transfer of nutrients and the production of reactive oxygen species, the activities of CAT, POD, and SOD began to decrease slowly, which led to the senescence of leaves. Zhu Kunlun et al. [49] believed that optimization of integrated agronomic measures such as rational nitrogen application could improve the activities of SOD, CAT, and POD, which was conducive to the synergistic improvement of yield and nitrogen efficiency. Yu Haodong et al. [50] found that compared with ordinary urea, controlled release fertilizer with a controlled-release time of 60 to 70 days and a nitrogen control ratio of 30% could significantly improve SOD, POD, and CAT enzyme activities in leaves at the later growth stage and delay the senescence of functional leaves. Cheng L's study in millet showed that applying slow-release fertilizer and topdressing nitrogen fertilizer significantly increased the activity of SOD and POD in leaves [51]. Wang J's study found that compared with the traditional single fertilization method, the combination of slow-release nitrogen fertilizer and ordinary nitrogen fertilizer could increase the activities of SOD, CAT, and POD in maize leaves, and the optimum ratio of ordinary nitrogen fertilizer/slow-release fertilizer was 1:2 [52]. However, the optimal fertilizer ratio in this study was nitrogen fertilizer/slow-release fertilizer = 5:5, which may be because different crops have different nitrogen absorption ranges, so the optimal fertilizer ratio is different. The results of this analysis showed that T_3 treatment significantly increased the activities of CAT, POD, and SOD in quinoa leaves at the later growth stage, followed by T_5 and T_4 treatments. The enzyme activities of slow-release fertilizer combined with nitrogen were drastically higher than those of single and control treatments, possibly because formula fertilization provided a balanced supply of nutrients at different growth stages of quinoa.

MDA content material is an important index to measure the degree of membrane lipid peroxidation in plants under stress conditions. Chen Yeting, Li Guiping, and Zhan Xiumei et al. [53–55] showed that with the development of maize leaves in the late growth period, the degree of senescence gradually deepened, the diploma of membrane lipid peroxidation intensified, and the content of malondialdehyde in maize leaves also increased. This study showed that MDA content continued to increase during the whole growth period of quinoa, which was much like the consequences of the work of Zhang Zhenbo et al. [56]. Dou K L's research showed that basal application of slow-release fertilizer and topdressing of urea could reduce the content of MDA in maize leaves in the later growth stage [57]. In this experiment, T_3 treatment had the finest inhibitory effect on MDA content, followed by T_5, and CK had the worst effect, which indicated that T_3 treatment could significantly reduce MDA content, lessen the diploma of membrane lipid peroxidation, delay the early senescence of quinoa, and keep the leaves with high vitality. This study further confirmed that a reasonable combination of slow-release fertilizer and nitrogen fertilizer could maintain high antioxidant enzyme activity, inhibit MDA content, delay leaf senescence, promote grain weight increase, and increase quinoa yield in the late growth period.

4.2. Yield

Nitrogen is the main index to measure the high yield of crops. Urea is favored by farmers because of its high N content, but the unreasonable fertilization method will cause fertilizer waste and reduce the yield [58,59]. Slow-release fertilizer can provide continuous nutrient demand for crops in the middle and later stages by controlling the release rate of fertilizer. Consequently, the mixed application of sluggish-release fertilizer and urea can efficiently condition the supply of nutrients, meet the fertilizer demand in the growth manner of quinoa, and facilitate the formation of yield. Zhang Pingzhen et al. [60] confirmed that the grain yield expanded with the boom of fertilizer application rate in a certain range but reduced beyond this scope. Chen Fu et al. [61] showed that overapplication of nitrogen fertilizer caused overgrowth of quinoa, poor lodging resistance, and reduced yield, while no fertilization or too little fertilization made the growth and development of quinoa slow, agronomic characters worse, and yield decrease. In a study of rice and wheat, Yu Z et al. [62] found that the mixed treatment of conventional urea and 70% slow-release urea had the highest rice yield. The mixed treatment of conventional urea and 30% slow-release urea had the highest wheat yield, which was 25.6% and 29.4% higher than that of 100% conventional urea, respectively. Zhi-Guo L et al. [63] found that the mixed application of ordinary urea and controlled release urea could increase the yield per unit area of rice by 0.51~0.92 t/hm^2 compared with the single application of controlled release urea, and the treatment with controlled release urea/ordinary urea = 7:3 had the highest yield. Gu Xiaobo et al. [64] found that under the condition of 180 kg/hm^2 nitrogen application, when the ratio of slow-release nitrogen fertilizer and urea application was 1:1, the green high yield and high efficiency of winter wheat could be achieved, and the yield was 7458 kg/hm^2. In the study of winter wheat, Li L et al. [65] found that under the same nitrogen fertilizer application, 1000-grain weight, grain number, and grain yield of 80% controlled release urea +20% conventional urea and 60% controlled release urea +40% conventional urea treatments were significantly increased, and grain yield was increased by 7.42~13.12%. The consequences showed that compared with CK, fertilization should considerably increase the yield, grain number per plant, and 1000-grain weight of quinoa, indicating that the yield-increasing effect of different fertilization treatments on quinoa was caused by the increase in 1000-grain quality and grain number per plant. T_3 treatment had the perfect effect on increasing the yield of quinoa, with the highest yield of 3829.43 $kg\cdot hm^{-2}$, followed by T_5 and T_4, which increased by 96.18%, 69.75%, and 45.84% compared with CK, respectively, indicating that fertilizer application had a better impact on increasing yield than proper quinoa, and too little or too much fertilizer would lead to a decrease in quinoa yield, which was much like the results of Zhao Zhiwei et al. [66]. In this experiment, T_3 treatment was found to be the optimal ratio of sluggish-release fertilizer and nitrogen fertilizer. Correlation analysis confirmed that quinoa yield was markedly positively correlated with 1000-grain weight; grain number per plant; and CAT, POD, and SOD activity on the early filling stage, and negatively correlated with MDA content. CAT was once extensively undoubtedly correlated with POD and SOD, whilst negatively correlated with MDA content, indicating that the yield of quinoa was closely related to the increase in protecting enzyme activity and the decrease in MDA content, each treatment through growing the activity of antioxidant enzymes and inhibiting MDA content material in quinoa leaves, wherein the treatments delayed the senescence process of leaves, extended the functional period of quinoa leaves, and accordingly executed the effect of increasing yield.

5. Conclusions

Comprehensive analysis showed that among different treatments, the responses of leaf senescence characteristics and yield of quinoa to the combined application of slow-release fertilizer and nitrogen fertilizer were the same. The effect of T_3 treatment was the optimal, followed by T_5 and T_4 treatment, and CK treatment was the worst. When the ratio of slow-release fertilizer and nitrogen fertilizer was 5:5, the activities of CAT, POD, and SOD in quinoa leaves were significantly increased, and the MDA content was decreased. Under the T_3 treatment, the yield, main panicle length, number of grains per spike, and 1000-grain weight reached the maximum. The results of correlation analysis showed that the yield of quinoa was significantly positively correlated with 1000-grain weight; the number of grains per plant; and the activities of CAT, POD, and SOD at the early filling stage, and negatively correlated with MDA content. Based on these findings, it can be concluded that T_3 treatment is the most suitable fertilization ratio for quinoa planting, which can delay leaf senescence, provide abundant assimilation products for grains, and ensure a high and stable yield of quinoa, which is worth popularizing.

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