

Article

The Effects of Tillage and the Combined Application of Organic and Inorganic Fertilizers on the Antioxidant Enzyme Activity and Yield of Maize Leaves

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Abstract: The aim of this study was to explore the characteristics of the combined application of organic fertilizer and inorganic fertilizer using different tillage methods to delay the senescence of maize leaves. The yield and activities of GDH, CAT, APX, GR, and GSH enzymes in maize leaves were measured at different growth stages by using two tillage methods, three organic and inorganic combined applications (P_1 , P_2 , and P_3), and four control treatments. (1) During the growth period, the R + S and R treatments were P_1 treatments, with the highest enzyme activities noted for GDH, CAT, APX, GR, and GSH, which were 36.79–103.22% higher than those of CK. (2) The average yield of all R + S treatments was higher than that of R treatments, and the average yield of P_1 treatment was the highest under R + S, which was 13,663.79 kg hm^{-2} , which was 6.39%, 7.90%, and 14.67% higher than that of P_2 , P_3 , and CK, respectively, which was lower than that of R. The yield of P_1 treatment was 2.53% higher. (3) There was a significant positive correlation between APX activity, CAT activity, GR activity, GDH activity, GSH activity, grain number per ear, ear length, and 100-grain weight of maize leaves at the grain filling stage, and a significant negative correlation between bald tip length and yield. The treatment details had the strongest enzyme activity and the highest yield when using the rotary tillage + subsoiling (R + S) P_1 method, which was the most suitable tillage method and the best fertilizer ratio combination, which could be demonstrated and popularized in a large area in the dry farming area of spring maize in Shanxi Province.

Keywords: farming practices; fertilizer application; yield; antioxidant enzymes



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1. Introduction

Maize is one of the most important food crops in China and around the world, and it plays a vital role in food security, production, and the development of the national economy [1]. Since the advent of chemical fertilizers, fertilization has become the most important method of increasing crop yields [2–4]. Rational use of chemical fertilizers can improve soil fertility and replenish the nutrients needed by crops [4–6]. Organic fertilizers contain a large number of microorganisms and enzymes, which can provide sufficient nutrients for the soil [7]. Liu et al. [8] and Liu Gaojie [9] showed that organic fertilizer efficiency was low, and inorganic fertilizer effects were fast, which could promote the accumulation of dry matter in the early stages of crops. The combined application of organic and inorganic fertilizers has many positive effects, such as fertilizing the soil, preventing soil acidification and salinization, increasing yield, absorbing organic excreta, protecting the environment, and conserving resources [10–12]. Yadav et al. [11] and Ghosh et al. [13] The results showed that the combined application of organic and inorganic fertilizers had a significantly greater effect on crop yield than that of nitrogen, phosphorus, and potassium chemical fertilizer treatments. Li et al. [14] summarized a large number of

long-term positioning experiments, and the results showed that the combined application of organic and inorganic fertilizers was more conducive to the improvement of soil organic matter in dryland, and the yield of wheat was significantly higher than that when chemical fertilizer alone or organic fertilizer alone were used. Wei et al. [15] found that replacing 25% nitrogen fertilizer with organic fertilizer could ensure stable wheat yield, promote nitrogen uptake, and increase nitrogen use efficiency by 20%. Abbasi et al. [16] showed that the combined application of organic and inorganic fertilizers could improve soil fertility. Cai et al. [17] and Yu et al. [18] showed that the application of organic fertilizer to reduce the amount of chemical fertilizer used can increase the yield and fertilizer use efficiency of maize and rice and can also fertilize the soil and improve the activity of antioxidant enzymes. Therefore, the combined application of organic and inorganic fertilizers can not only make up for the shortcomings of insufficient fertilizer efficiency in the early stages of crop growth but also prolong the fertilizer efficiency in soil in the later stages, which can better maintain crop growth than inorganic fertilizer or organic fertilizer alone [19].

Rational tillage practices can improve soil physical properties and crop growth conditions, better meet crop demand, and increase crop yields [20,21]. Shanxi is a typical dry farming area in China, with severe interannual fluctuations in precipitation and uneven seasonal distribution; coupled with the use of conventional tillage methods during the fallow period, the bare surface accelerates water evaporation and dissipation, the soil water storage capacity is poor, and water deficit is the main limiting factor for crop yield increase [22–26]. Abiotic environmental stresses such as drought, high temperature, salinization, or ultraviolet radiation pose a serious threat to wheat growth and health and lead to wheat yield loss. Abiotic stress can induce membrane damage, protein denaturation, or reactive oxygen species (ROS) formation. Today, in addition to the fertilizers used in wheat cultivation, another group of compelling compounds is biostimulants, which have been found to alter plant physiological processes, optimize productivity, and improve plant physiology, especially when cultivated under stress [27]. However, although there are some studies that confirm the positive effects of biostimulants on plant productivity, their effects on plant metabolism and antioxidant status are poorly studied. However, it has also been suggested that organic biostimulants can mitigate the effects of abiotic stresses, including salinity, drought, and high and low temperatures, through the higher activity of antioxidant enzymes (catalase, peroxidase, and superoxide dismutase) and non-enzymatic antioxidants (glutathione, ascorbic acid, malondialdehyde, and hydrogen peroxide), but their mitigation effects associated with fungicide toxicity have not been extensively studied [28]. Deep plowing and subsoiling can break the hard bottom layer of the plow, increase porosity, facilitate root growth, promote the root utilization of nutrients in the soil, prolong the leaf functional period, and increase yield [29–32]. Meng et al. [33] showed that the activity of antioxidant enzymes in plant roots could be increased using different tillage methods. Therefore, increasing the water storage capacity and moisture conservation capacity of farmland and improving water use efficiency are the keys to the high yield and high efficiency of dryland maize. Compared with conventional tillage, subsoiling can improve soil structure and enhance soil permeability and the root morphology and root activity of maize, thereby increasing water and nutrient uptake, improving leaf photosynthetic performance, and prolonging the duration of green leaves, which is an important tillage measure for obtaining high yields [34,35]. The senescence process of maize leaves is a process of dysregulation of reactive oxygen species metabolism, which can induce the activity of a series of enzymes such as catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) to increase and scavenge the accumulation of reactive oxygen species. CAT is the most important reactive oxygen species scavenging system in plants. Wang et al. [36] concluded that maize leaves maintained higher CAT activity and soluble protein content in the late stage of grain filling, which reduced the degree of peroxidation of the leaf membrane and delayed leaf senescence. The application of organic fertilizer enhanced the antioxidant system of crop leaves [37] and scavenged the accumulation of reactive oxygen species, and the antioxidant defense pathways induced by different stresses

showed great differences [38,39]. Most of the current studies focus on the single effect of tillage or fertilization methods on antioxidant enzymes in crop roots or leaves but ignore the in-depth exploration of the combination of fertilizer and tillage methods. However, in actual agricultural production, the two are actually interrelated and mutually influential. In view of the above, this paper creatively and uniquely combines tillage practices with fertilizer combinations. Our goal was to gain insight into the changes in enzyme activity during the growth period of maize and their impact on yield. Using this comprehensive research method, we hope to provide a more scientific basis for corn cultivation in dryland areas and help to screen out more suitable farming methods and the most suitable fertilizer ratio. This will not only help to improve the yield and quality of maize but also have a positive impact on agricultural development in dryland areas, bring greater economic benefits to farmers, and contribute to the development of sustainable agriculture.

2. Materials and Methods

2.1. Overview of the Test Site

The test site is in the Dongyang Experimental Base of Shanxi Agricultural University (Shanxi Academy of Agricultural Sciences) (37°33'21" N, 112°40'2" E, 802 m above sea level). The region belongs to the warm temperate semi-humid continental monsoon climate, the annual average temperature is 9.7 °C, the annual average frost-free period is 158 days, rain and heat occur in the same season, and rainfall is mainly concentrated in July to September. The average annual temperature from May to September 2022 was 21.38 °C, the average annual temperature from May to September 2023 was 21.10 °C, the average annual rainfall from May to September 2022 was 385 mm, and the average annual rainfall from May to September 2023 was 222.9 mm. The soil of the experimental plot was yellow clay, and the soil organic matter content of the 0–20 cm tillage layer was 14.08 g kg⁻¹, the total nitrogen content was 0.96 g kg⁻¹, the alkali hydrolyzable nitrogen content was 67.1 mg kg⁻¹, the available phosphorus content was 11.0 mg kg⁻¹, the available potassium content was 110 mg kg⁻¹, and the pH value was 8.55.

2.2. Experimental Materials

(1) Seeds: Qiangsheng 388 corn seeds were selected, with compact plants, 317 cm high, 116 cm ears, 22 leaves, and a growth period of 129 days. The seedling leaf sheath was dark purple, the leaf margin was red, the filament was green, and they had medium resistance to large spot disease and high resistance to stem rot. The seeds were provided by Shanxi Qiangsheng Seed Industry Co., Ltd. (Taiyuan, China). (2) Fertilizer: organic fertilizer, net content of 40 kg, organic matter content ≥ 45.0%, and total nutrients (N/P/K) ≥ 5%, provided by Taiyuan Zhida Shun Compound Fertilizer High-tech Co., Ltd. (Taiyuan, China); inorganic fertilizer, urea, net content of 40 kg, total nitrogen content ≥ 46.0%, and particle size range 0.85 mm~2.80 mm ≥ 90%, provided by PetroChina Co., Ltd. (Beijing, China). Ethylenediamine phosphate, net content of 50 kg, total nutrients (N/P/K) ≥ 57%, and effective contents of N, P, and K of 15%, 42%, and 0, provided by Guizhou Kailin Group Co., Ltd. (Guiyang, China). (3) Kit: The glutamate dehydrogenase (GDH) kit, glutathione peroxidase (GSH) kit, glutathione reductase (GR) kit, catalase (CAT) kit, and ascorbate peroxidase (APX) kit used in this study were provided by Beijing Solaibao Technology Co., Ltd. (Beijing, China).

2.3. Experimental Design

As can be seen from Table 1, in this study a two-factor randomized block design was adopted, with the tillage method as the main area and two treatments: rotary tillage (R) and rotary tillage + subsoiling (R + S), in which the depth of rotary tillage reached 30 cm and the depth of subsoiling reached 50 cm. The combined application of organic and inorganic fertilizers and inorganic fertilizer was the sub-area, and there were 4 treatments: conventional fertilization CK and 3 kinds of organic and inorganic fertilizer combined application ratios. The application rate of inorganic fertilizer was 150 kg hm⁻² of urea, and

the application rate of ethylenediamine phosphate was 300 kg hm⁻². The planting density was 66,000 plants/hm², each plot was 15 m long and 4 m wide, and the area was 60 m², with the experiment repeated 3 times.

Table 1. Presentation of tillage practices and fertilizer application design.

Farming Methods	Fertilizer Treatment	Inorganic Fertilizers	Organic Fertilizer
R	CK	Urea 300 kg hm ⁻² , Pd 600 kg hm ⁻²	—
	P ₁	Urea 150 kg hm ⁻² , Pd 300 kg hm ⁻²	3000 kg hm ⁻²
	P ₂	Urea 150 kg hm ⁻² , Pd 300 kg hm ⁻²	6000 kg hm ⁻²
	P ₃	Urea 150 kg hm ⁻² , Pd 300 kg hm ⁻²	9000 kg hm ⁻²
R + S	CK	Urea 300 kg hm ⁻² , Pd 600 kg hm ⁻²	—
	P ₁	Urea 150 kg hm ⁻² , Pd 300 kg hm ⁻²	3000 kg hm ⁻²
	P ₂	Urea 150 kg hm ⁻² , Pd 300 kg hm ⁻²	6000 kg hm ⁻²
	P ₃	Urea 150 kg hm ⁻² , Pd 300 kg hm ⁻²	9000 kg hm ⁻²

Note: R: rotary tillage; R + S: rotary tillage + subsoiling; CK: conventional fertilization urea 300 kg hm⁻², phosphate diamine 600 kg hm⁻²; P₁: organic fertilizer 3000 kg hm⁻², inorganic fertilizer: 150 kg hm⁻², diamine phosphate 300 kg hm⁻²; P₂: organic fertilizer 6000 kg hm⁻², inorganic fertilizer: 150 kg hm⁻², diamine phosphate 300 kg hm⁻²; P₃: organic fertilizer 9000 kg hm⁻², inorganic fertilizer: 150 kg hm⁻², diamine phosphate 300 kg hm⁻². Pd: phosphate diamine.

2.4. Determined Items and Methods

2.4.1. Project

At the jointing stage, the big flare stage, the tasselling stage, the grouting stage, and the maturity stage, the upper functional completed leaves with normal color, the same height, and similar light were randomly selected from 9:00 a.m. to 11:30 a.m. on a sunny day, and the leaf veins were removed using scissors at both ends and the leaf veins were evenly removed in the middle part, scrubbed with alcohol, wrapped in tin foil placed in liquid nitrogen for preservation, and brought back to the laboratory.

2.4.2. Reference Method

The kit and reference were from Beijing Solaibao Technology Co., Ltd.; website: www.solarbio.com (accessed on 10 April 2023).

2.4.3. GDH Determination Method

(1) Weigh about 0.1 g of tissue and add 1 mL of extract for ice bath homogenization. Centrifuge at 8000 × g at 4 °C for 10 min; take the supernatant and put it on ice for testing.

(2) Take 1 mL of working solution and 0.05 mL of sample in a 1 mL quartz cuvette with an optical diameter of 1 cm, mix well, start timing while adding the sample, record the initial absorbance A₁ at 20 s and the absorbance A₂ at 5 min and 20 s at 340 nm wavelength, and calculate ΔA = A₁ - A₂.

(3) Definition of units: consumption of 1 nmol of NADH per minute per g of tissue is defined as one unit of enzyme activity.

$$\text{GDH (U/g mass)} = [\Delta A \times V \text{ anti-total} \div (\epsilon \times d) \times 10^9] \div (V\text{-like} \times V\text{-like total}) \div T = 675 \times \Delta A \div W;$$

anti-total: the total amount of the reaction.

Total Vanti: total volume of reaction system, 1.05 × 10⁻³ L; ε: NADH molar extinction coefficient, 6.22 × 10³ L/mol/cm; d: cuvette aperture, 1 cm; V-sample: add sample volume,

0.05 mL; total V-sample: add the extract liquid volume, 1 mL; T: reaction time, 5 min; W: sample quality, g; 500: total number of bacteria or cells, 5 million.

2.4.4. GSH Determination Method

(1) Tissue: According to the ratio of tissue mass (g): extract volume (mL), the ratio is 1:5~10 (it is recommended to weigh 0.05 g tissue and add 1 mL of extract) for ice bath homogenization. Centrifuge at 5000 rpm at 4 °C for 10 min, take the supernatant, and put it on ice for testing (if the supernatant is not clear, it can be centrifuged for a longer time).

(2) Dilute the 80 µmol/mL standard solution into a 0.08 µmol/mL standard solution with diluent and prepare it for immediate use.

Mix well and let it stand at room temperature for 15 min. Measure the absorbance at 412 nm as soon as possible, denoted as A assay tube, A control tube, A standard tube, and A blank tube. Calculation:

$$\Delta A \text{ assay} = A \text{ control tube} - A \text{ assay tube}, \Delta A \text{ standard tube} = A - A \text{ blank tube}.$$

(3) Calculate according to the quality of the sample.

Definition of activity units: catalyzing 1 nmol of GSH oxidation per minute per g of sample in the reaction is defined as one enzyme activity unit.

$$\text{GPX (U/g mass)} = \Delta A \text{ determination} \div (\Delta A \text{ standard} \div C \text{ standard}) \times 1000 \times V \text{ enzymatic} \div \\ (V\text{-sample} \div V\text{-sample total} \times W) \div T = 200 \times \Delta A \text{ determination} \div \Delta A \text{ standard} \div W$$

Scale C: Concentration of standard mixture: 0.08 µmol/mL; V enzymatic: enzymatic reaction system volume, 1.25 mL; V-like: sample volume added to the enzymatic reaction, 0.1 mL; total V-sample: volume of extracted liquid, 1 mL; Cpr: supernatant protein concentration, mg/mL; T: reaction time, 5 min; number of cells: in 10,000; W: sample quality, g; 1000: conversion factor, 1 µmol = 1000 nmol.

2.4.5. GR Determination Method

(1) Tissue: According to the tissue mass (g): the volume of reagent (mL) is a 1:5~10 ratio (it is recommended to weigh about 0.1 g of tissue and add 1 mL of reagent one) for ice bath homogenization. Centrifuge at 10,000 rpm at 4 °C for 10 min and place the supernatant on ice for testing.

(2) After adding the reagent to the quartz cuvette, quickly blow it and mix it well, record the absorbance value of 340 nm at the 10th s, empty 1 (A measure 1), quickly place it in a 37 °C water bath or incubator for 3 min, take out the absorbance value A empty 2 (A measure 2) when quickly drying and measuring 3 min 10 s, and calculate $\Delta A \text{ blank tube} = A \text{ empty 1} - A \text{ empty 2}$ and $\Delta A \text{ measuring tube} = A \text{ measuring 1} - A \text{ measuring 2}$. Blank tubes only need to be measured 1–2 times.

(3) Definition of the active unit: At 37 °C and pH 8.0, each gram of sample catalyzes the oxidation of 15 mol NADPH per minute to one enzyme activity unit.

$$\text{GR enzyme activity (U/g mass)} = [(VA \text{ assay tube} - \Delta A - \Delta A \text{ blank tube}) \div (g \times d) \times V \text{ anti total} \times 106] \div \\ (V \text{ sample} \div V \text{ sample total} \times W) - T = 0.536 \times (\Delta A \text{ assay tube} - \Delta A \text{ blank tube}) = W$$

ϵ : NADPH molar extinction system.

2.4.6. CAT Determination Method

(1) Sample processing b. Tissue: According to the tissue quality (g): the extraction volume (mL), the ratio is 1:5–10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of extract) for ice bath homogenization. Centrifuge at 8000 × g at 4 °C for 10 min and then take the supernatant and set it on ice for testing.

(2) Put 1 mL CAT detection solution in a 1 mL quartz cuvette, add 35 mL of sample, mix well for 5 s, and immediately measure the initial absorbance value A_1 at 240 nm and the absorbance value A_2 after 1 min at room temperature. Calculate.

$$\Delta A = A_1 - A_2.$$

(3) Calculate according to the quality of the sample:

Definition of units: 1 μmol of H_2O_2 degradation per minute catalyzed by each g of tissue in the reaction system is defined as one unit of enzyme activity.

$$\text{CAT (U/g mass)} = [\Delta A \times V \text{ anti-total} \div (\epsilon \times d) \times 10^6] \div (W \times V\text{-sample} \div V\text{-sample total}) \div T = 678 \times \Delta A \div W$$

Total V: Total volume of reaction system, 1.035×10^{-3} L; ϵ : H_2O_2 molar absorbance coefficient, 43.6 L/mol/cm; D: cuvette aperture, 1 cm; V-sample: add the sample volume, 0.035 mL; total V-sample: add the extract liquid volume, 1 mL; T: reaction time, 1 min; W: sample quality, g; Cpr: supernatant protein concentration, mg/mL; 500: total number of cells or bacteria, 5 million; 10^6 : unit conversion factor, 1 mol = 10^6 μmol .

2.4.7. APX Determination Method

(1) Sample processing

According to the ratio of tissue mass (g): extract volume (mL), the ratio is 1:5~10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of extract) for ice bath homogenization. $13,000 \times g$, centrifuge at 4 °C for 20 min and then take the supernatant and put it on ice for testing.

(2) Add the above reagents to the quartz cuvette in turn, mix quickly, measure the absorbance value of 10 s and 130 s at 290 nm, record as A_1 and A_2 , and calculate ΔA measuring tube = A_1 measuring tube – A_2 measuring tube and ΔA blank tube = A_1 blank tube – A_2 blank tube. Blank tubes only need to be measured 1–2 times.

(3) APX vitality calculation

Definition of units: 1 μmol of AsA oxidized per minute per g of tissue is 1 enzymatic active unit.

$$\text{APX activity (U/g mass)} = (\Delta A \text{ assay tube} - \Delta A \text{ blank tube}) \div (\epsilon \times d) \times V \text{ anti-total} \times 10^6 \div (W \times V \text{ sample} \div V \text{ sample total}) \div T = 1.79 \times (\Delta A \text{ assay tube} - \Delta A \text{ blank tube}) \div W$$

ϵ : molar absorbance coefficient of AsA at 290 nm, 2.8×10^3 L/mol/cm; d: cuvette aperture, 1 cm; total V: total volume of the reaction system, 1000 L = 1×10^{-3} L; 10^6 : unit conversion factor, 1 mol = 1×10^6 μmol ; V-like: add to the reaction system.

2.5. Yield

The middle 2 rows of each plot were taken to measure the yield, and the total number of plants and the actual number of harvested ears were recorded. At the same time, 20 fruit ears were randomly selected to be naturally air-dried, and when the moisture was less than 20%, the ear length (cm), bald tip length (cm), and grain number per spike were measured. The 100-grain weight (g) was determined after threshing and air drying, and the grain yield was calculated according to the grain moisture content of 14% by combining the seed test data with the field yield measurement data.

2.6. Data Processing and Analysis

Microsoft 2023 Excel v16.0.16924.20124 software was used for data collation, SPSS27.0 software was used for ANOVA analysis, a q-test was used to compare the significance of significant differences between different treatments ($p < 0.05$), and Origin2022 was used for plotting.

3. Results

3.1. Effect of Combined Fertilizer Application on Antioxidant Enzyme Activity in Maize Leaves Using Different Tillage Modes

3.1.1. CAT Activity

It can be seen from Figure 1 that with the advancement of the maize growth period, the CAT activity of combined fertilizer application using different tillage methods first increased and then decreased, reaching a peak at the grain filling stage and decreasing rapidly at the maturity stage. The CAT activity of P₁ treatment was at a high level, with an average of “742.13 U·g⁻¹”, which was significantly different from CK, P₂, and P₃. From the jointing stage to the tasselling stage, P₂ treatment was better than P₃, and P₃ treatment was better than P₂ at the filling stage–maturity stage. CK was the lowest.

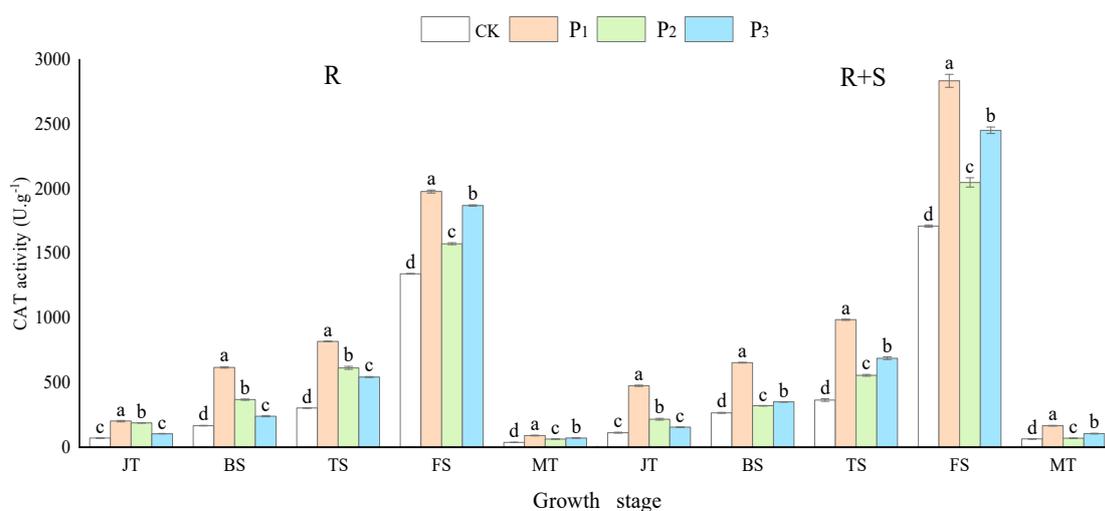


Figure 1. Effects of fertilizer application on CAT activity in maize leaves using different tillage methods. JT: jointing stage; BS: big trumpet stage; TS: tasselling stage; FS: filling stage; MT: maturity stage; R: rotary tillage; R + S: rotary tillage + subsoiling; CK: conventional fertilization urea 300 kg hm⁻², phosphate diamine 600 kg hm⁻²; P₁: organic fertilizer 3000 kg hm⁻², inorganic fertilizer: 150 kg hm⁻² diamine phosphate 300 kg hm⁻²; P₂: organic fertilizer 6000 kg hm⁻², inorganic fertilizer: 150 kg hm⁻², diamine phosphate 300 kg hm⁻²; P₃: organic fertilizer 9000 kg hm⁻², inorganic fertilizer: 150 kg hm⁻², diamine phosphate 300 kg hm⁻². a, b, c, d represent different levels of significance. a indicates a higher level of significance, such as 0.01 or 0.05, b may indicate a secondary level of significance, and c may indicate a lower level of significance.

Compared with R, all treatments of R + S were better than those of R treatment, and the CAT activity of R + S P₁ treatment was the strongest, ranking first, with an average of “1024.46 U·g⁻¹”, which was much higher than that of R + S treatment, which was 38.04% higher than that of RP₁ treatment. The CAT activity of all R + S treatments was the most active at the grain-filling stage. During the filling stage, the average enzyme activities of P₁, P₂, and P₃ reached “1024.46 U·g⁻¹”, “642.79 U·g⁻¹”, and “750.94 U·g⁻¹”, respectively, which were 103.22%, 27.51%, and 48.96% higher than that of CK.

3.1.2. GSH Activity

It can be seen from Figure 2 that with the advancement of the maize growth period, the activity of glutathione (GSH) in different tillage treatments with fertilizer increased and reached a peak at the maturity stage. Under R tillage, the average activity size of GSH was P₁ > P₂ > P₃ > CK, and the average value of GSH in P₁ treatment was “25.73 U·g⁻¹”, which was 24.78%, 25.07%, and 46.66% higher than that of P₂, P₃, and CK treatments.

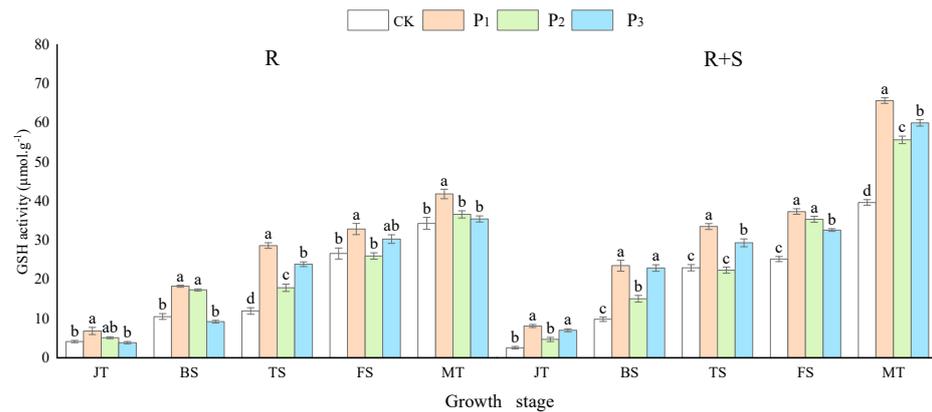


Figure 2. Effects of fertilizer application on GSH activity in maize leaves using different tillage methods. a, b, c, d represent different levels of significance. a indicates a higher level of significance, such as 0.01 or 0.05, b may indicate a secondary level of significance, and c may indicate a lower level of significance.

Under R + S tillage, the GSH activity of P₁ treatment was the strongest, ranking first, with an average value of “33.68 U·g⁻¹”, which was much higher than that of P₁ in R, with it being 30.92% higher. There was no significant difference between P₁ and P₃ at the jointing stage, the big flare stage, and the grouting stage, but there were significant differences with CK and P₂. During the whole growth period, the average activity of GSH was as follows: P₁ > P₃ > P₂ > CK.

3.1.3. APX Activity

It can be seen from Figure 3 that with the advancement of the maize growth period, the APX activity of combined fertilizer application using different tillage methods decreased, and the maturity stage decreased to the minimum. Under the R tillage method, the P₁ treatment was significantly different from the other treatments. The average value of APX was “0.129 U·g⁻¹”, which was 75.19%, 32.42%, and 33.01% higher than that of CK, P₂, and P₃, respectively. P₂ > P₃ at the big horn stage–maturity stage, and P₃ > P₂ at the jointing stage–filling stage, with the lowest APX activity noted in the CK treatment.

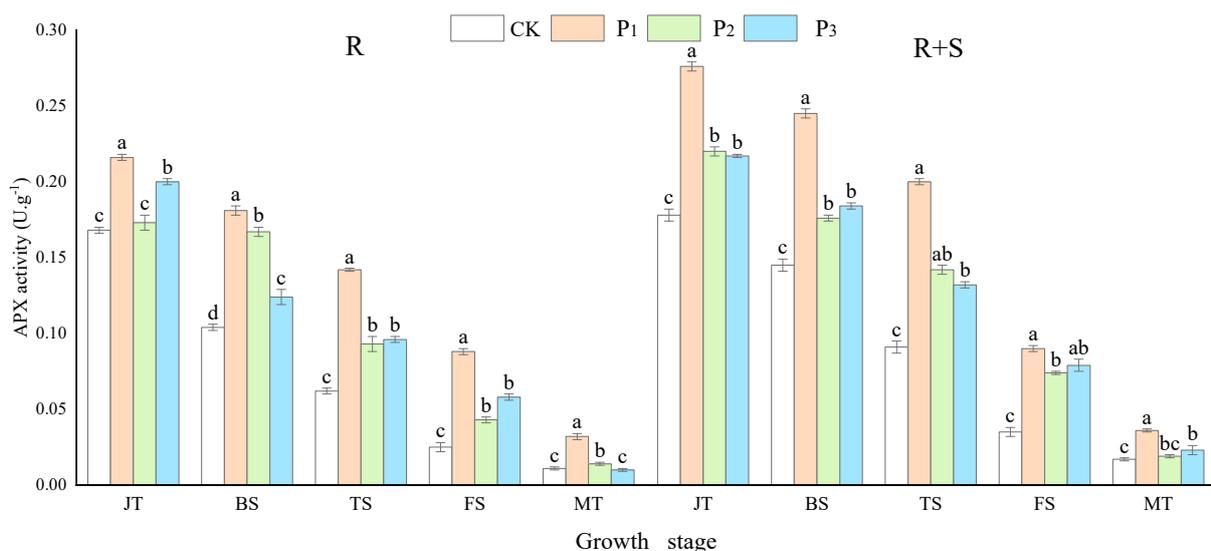


Figure 3. Effects of fertilizer application on APX activity in maize leaves using different tillage methods. a, b, c, d represent different levels of significance. a indicates a higher level of significance, such as 0.01 or 0.05, b may indicate a secondary level of significance, and c may indicate a lower level of significance.

Compared with R tillage, the R + S P₁ treatment had the strongest APX activity, with an average value of “0.169 U·g⁻¹”, which was much higher than that of R treatment, with it being 30.46% higher. At the jointing stage, the APX activities of P₁, P₂, and P₃ treatments were “0.276 U·g⁻¹”, “0.220 U·g⁻¹”, and “0.217 U·g⁻¹”, respectively, which were 54.72%, 23.57%, and 21.70% higher than those of CK.

3.1.4. GDH Activity

It can be seen from Figure 4 that with the advancement of the maize growth period, the change trend of GDH activity in maize leaves subjected to different fertilizer combinations using different tillage methods is basically the same, showing a trend of first increasing and then decreasing, and the activity reaches a peak at the grain filling stage and then begins to decline rapidly. Under R tillage, the GDH activity of P₁ treatment was at a high level, with an average of “93.02 U·g⁻¹”, which was significantly different from CK, P₂, and P₃, and the activity of P₁ > P₂ > P₃ > CK at the grain filling stage was 28.72%, 12.60%, and 7.85% higher than that of CK, respectively.

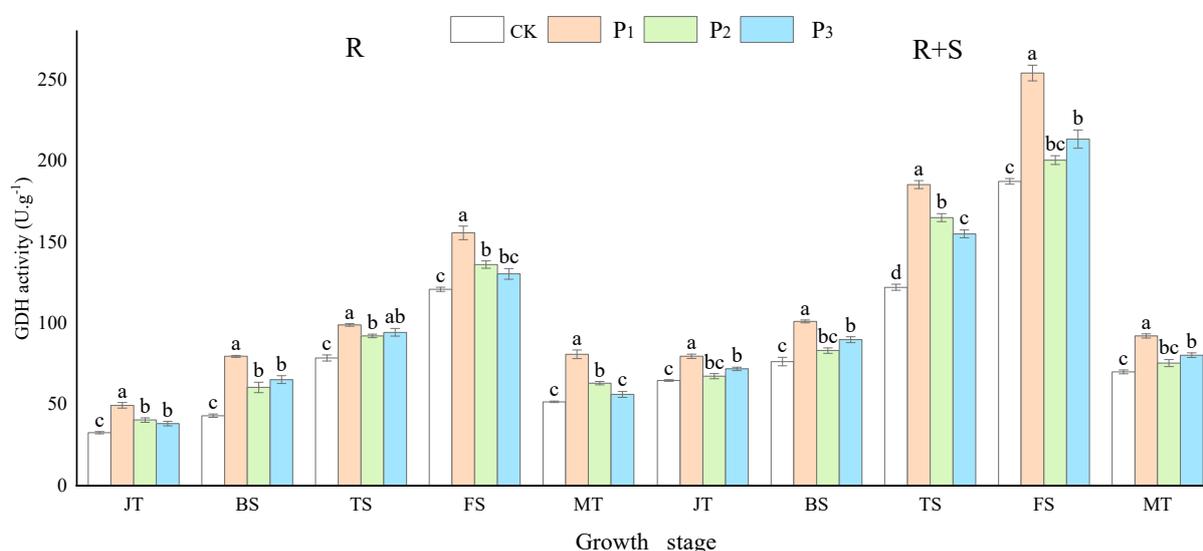


Figure 4. Effects of fertilizer application on GDH activity in maize leaves using different tillage methods. a, b, c, d represent different levels of significance. a indicates a higher level of significance, such as 0.01 or 0.05, b may indicate a secondary level of significance, and c may indicate a lower level of significance.

Compared with the R tillage method, the GDH activity of R + S P₁ treatment was the strongest, with an average of “142.60 U·g⁻¹”, which was much higher than that of R P₁ treatment, with it being 53.31% higher. The GDH activities of P₁, P₂, and P₃ treatments were “254.25 U·g⁻¹”, “200.58 U·g⁻¹”, and “213.58 U·g⁻¹”, respectively, which were 35.60%, 6.98%, and 13.91% higher than those of CK.

3.1.5. GR Activity

It can be seen from Figure 5 that with the gradual advancement of the maize growth period, the variation trend of GR activity in the maize leaves was about the same as that of the APX curve using different tillage modes, and the value reached the lowest value at the maturity stage. The GR activity of R P₁ was always at a high level, with an average value of “0.135 U·g⁻¹”, which was 114.24%, 55.01%, and 37.88% higher than that of CK, P₂, and P₃, respectively, and there were significant differences between them and the other treatments. The P₂ and P₃ treatments rose and fell from the jointing stage to the grouting stage, while the CK treatment was at the lowest level.

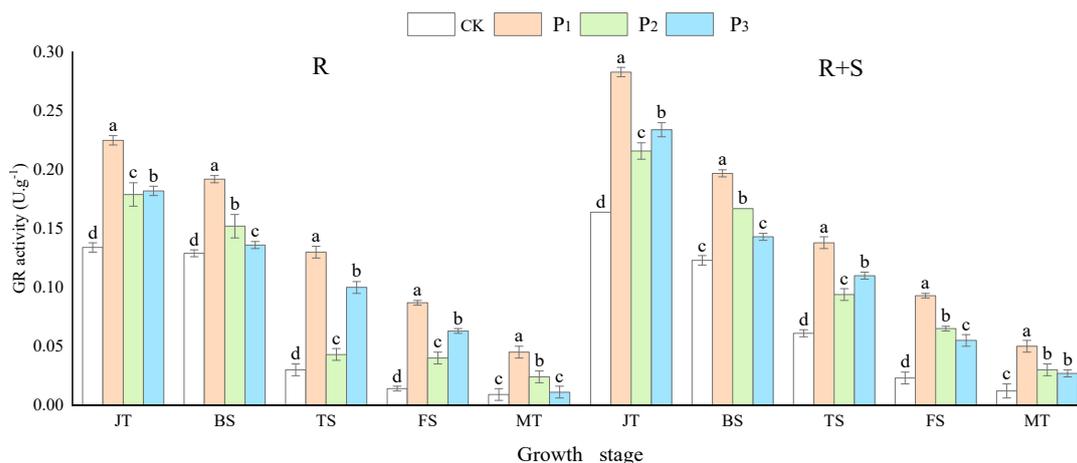


Figure 5. Effects of fertilizer application on GR activity in maize leaves using different tillage methods. a, b, c, d represent different levels of significance. a indicates a higher level of significance, such as 0.01 or 0.05, b may indicate a secondary level of significance, and c may indicate a lower level of significance.

In comparison, R + S P₁ treatment had the strongest GR activity, ranking first, with an average of “0.152 U·g⁻¹”, which was much higher than that of R + S P₁ treatment, which was 12.18% higher. The GR activities of P₁, P₂, and P₃ treatments at the jointing stage were 0.283 U·g⁻¹, 0.216 U·g⁻¹, and 0.234 U·g⁻¹, respectively, which were 72.46%, 31.92%, and 42.72% higher than those of CK.

3.2. Influence of Output and Production Components

It can be seen from Table 2 that there was no significant difference in yield between R and R + S in 2022, while R + S tillage was slightly higher than R, and the yield increase effect was not obvious, and in 2023, the yield of all R + S tillage treatments was significantly higher than that of R treatment.

Table 2. Effects of different fertilizer combinations on maize yield and constituent factors using different tillage methods.

Year	Tillage Method	Treatment	Bare Top Length (cm)	Ear Length (cm)	Ear Grain Number	100-Grain Weight (g)	Yield (kg hm ⁻²)
2022	R	CK	2.20 ± 0.23 a	18.47 ± 0.61 c	616.64 ± 10.59 bc	45.51 ± 1.28 c	11,726.43 ± 140.46 d
		P ₁	0.52 ± 0.18 c	21.05 ± 0.41 a	660.17 ± 11.77 a	51.72 ± 1.49 a	13,499.80 ± 123.92 a
		P ₂	1.09 ± 0.24 b	20.68 ± 0.48 a	630.61 ± 12.30 bc	52.45 ± 0.35 a	12,686.24 ± 194.48 b
		P ₃	0.83 ± 0.06 c	19.43 ± 0.51 b	642.19 ± 15.48 ab	49.36 ± 0.60 b	12,453.76 ± 125.09 c
	R + S	CK	1.67 ± 0.11 a	17.97 ± 0.53 c	629.70 ± 9.02 c	40.35 ± 1.03 c	12,046.75 ± 205.50 d
		P ₁	0.56 ± 0.02 c	25.65 ± 1.02 a	673.43 ± 11.12 a	51.19 ± 1.76 a	13,898.44 ± 113.57 a
		P ₂	0.93 ± 0.03 b	22.44 ± 0.64 b	650.31 ± 10.95 b	45.83 ± 1.66 b	12,979.22 ± 166.41 b
		P ₃	0.72 ± 0.20 b	19.63 ± 0.44 b	655.32 ± 13.53 b	45.45 ± 4.62 a	12,800.95 ± 122.29 c
2023	R	CK	2.24 ± 0.25 a	18.85 ± 0.76 a	583.66 ± 12.46 b	30.78 ± 1.50 d	11,319.90 ± 208.12 d
		P ₁	0.73 ± 0.30 d	19.36 ± 1.08 a	608.55 ± 12.35 a	40.47 ± 0.87 a	13,161.75 ± 134.34 a
		P ₂	1.29 ± 0.15 c	18.70 ± 0.83 a	600.23 ± 9.71 ab	37.72 ± 1.37 b	12,456.75 ± 244.12 b
		P ₃	1.72 ± 0.36 b	18.42 ± 0.39 a	586.84 ± 11.29 ab	33.12 ± 0.71 c	12,227.55 ± 187.22 c
	R + S	CK	1.71 ± 0.38 a	18.78 ± 0.46 b	584.78 ± 10.78 b	37.35 ± 0.05 d	11,791.90 ± 192.87 d
		P ₁	0.56 ± 0.34 c	24.26 ± 0.65 a	609.84 ± 12.08 a	49.83 ± 1.47 a	13,436.70 ± 207.97 a
		P ₂	0.90 ± 0.09 b	19.21 ± 0.32 b	587.58 ± 11.71 b	42.45 ± 0.54 b	12,716.30 ± 200.91 b
		P ₃	0.83 ± 0.15 b	19.59 ± 0.41 b	600.61 ± 16.54 ab	40.29 ± 0.59 c	12,536.34 ± 167.96 c

Note: R: rotary tillage; R + S: rotary tillage + subsoiling; CK: conventional fertilization urea 300 kg hm⁻², phosphate diamine 600 kg hm⁻²; P₁: organic fertilizer 3000 kg hm⁻², inorganic fertilizer: 150 kg hm⁻², diamine phosphate 300 kg hm⁻²; P₂: organic fertilizer 6000 kg hm⁻², inorganic fertilizer: 150 kg hm⁻², diamine phosphate 300 kg hm⁻²; P₃: organic fertilizer 9000 kg hm⁻², inorganic fertilizer: 150 kg hm⁻², diamine phosphate 300 kg hm⁻². The values with different lowercase letters in the same column vary significantly at the 5% probability level.

(1) The average yield of P1 was 13,330.78 kg hm⁻², which ranked first, which was 15.69%, 6.04%, and 8.02% higher than that of CK, P₂, and P₃, the weight of 100 grains was 51.46 g, which was 19.27%, 3.50%, and 11.29% higher than that of CK, P₂, and P₃, and the length of bald tip length was significantly shorted than that of CK, P₂, and P₃. The average spike Bare top length and ear grain number were significantly better than those of each treatment.

(2) The trends of all treatments under R + S tillage were consistent with those under R + S tillage, and P₁ was also the best. The average yield of P₁ was 13,667.57 kg hm⁻², which was 14.67%, 6.38%, and 7.89% higher than that of CK, P₂, and P₃, and the weight of 100 grains was 60.51 g, which was 23.87%, 11.77%, and 10.96% higher than that of CK, P₂, and P₃. Under the different tillage methods, the average yield of R + S P₁ was the best, which was 2.49% higher than that of R P₁ treatment, 56.25% lower than that of R P₁, and 24.62% higher bare top length than R P₁, and the ear grain number per spike increased by 1.15% compared to R P₁.

3.3. Correlation Analysis

As can be seen from Figure 6, yield was significantly positively correlated with ear grain number, bare top length, and 100-grain weight, with correlation coefficients of 0.58, 0.87, and 0.72, respectively, and negatively correlated with bare top length, with a correlation coefficient of “-0.77”, while bare top length was negatively correlated with ear grain number and ear length, with correlation coefficients of “-0.57” and “-0.60”, respectively, and negatively correlated with 100-grain weight. The correlation coefficient was “-0.50”, the 100-grain weight was significantly correlated with ear length, the correlation coefficient was 0.69, and the ear length was significantly positively correlated with the 100-grain weight, with a correlation coefficient of 0.69.

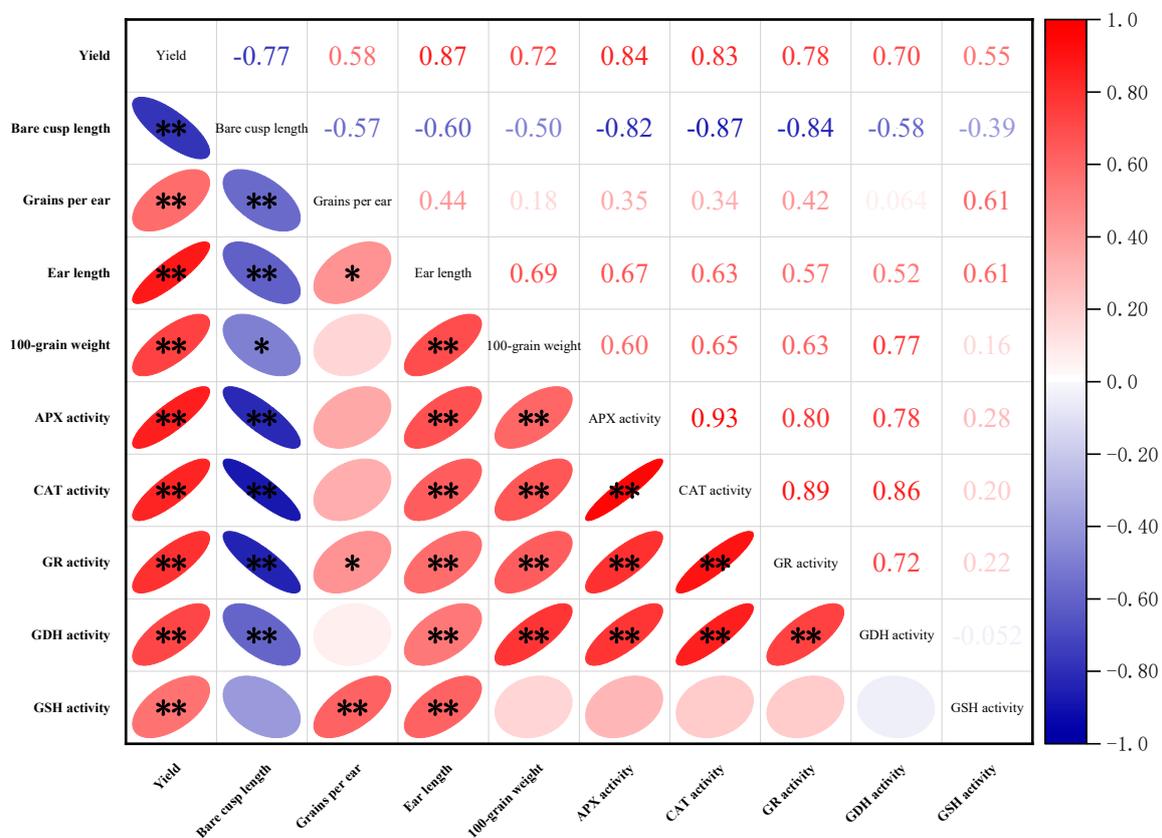


Figure 6. Correlation analysis of yield, yield components, and antioxidant characteristics. Note: * represents significant difference ($p < 0.05$); ** represents a significant difference ($p < 0.01$).

Yield was significantly positively correlated with APX activity, CAT activity, GR activity, GDH activity, and GSH activity, with correlation coefficients of 0.84, 0.83, 0.78, 0.70, and 0.55, respectively, APX was significantly positively correlated with CAT activity, GR activity, and GDH activity, with correlation coefficients of 0.93, 0.80, and 0.78, respectively, CAT activity was significantly positively correlated with GR activity and GDH activity, with correlation coefficients of 0.89 and 0.86, and GR activity was significantly positively correlated with GDH activity, with a correlation coefficient of 0.72.

4. Discussion

4.1. Effects of the Combined Application of Organic and Inorganic Fertilizers on Maize Yield Using Different Tillage Modes

The results of this experiment showed that the P₁ treatment had the best yield, 100-grain weight, spike length, grain number per spike, and bald tip length, and the R + S tillage method was better than the R tillage method, which is consistent with the research results of Zhou et al. [40] and Shen Xiaolin et al. [41]. The combination of organic and inorganic application can effectively promote the transformation of nutrients, improve organic matter and soil basic nutrients, improve soil structure, enhance the ability of soil fertilizer to supply nutrients, and promote an increase in 100-grain quality and yield. The combined application of organic fertilizer and inorganic fertilizers increased the content of soil organic matter and nitrogen, phosphorus, and potassium to promote plant absorption, thereby affecting the rapid jointing of plants at the jointing stage and the increase in maize grain quality at grain filling stage, thereby improving yield, which is consistent with the research results of Jin et al. [42] and Wang et al. [43]. Organic fertilizer can improve soil physicochemical properties, increase water infiltration, inhibit evaporation, and increase soil effective water content [44,45] and crop water use efficiency [46,47]. The combination of organic and nitrogen fertilizers can also enhance the water absorption and utilization of winter wheat [48]. Jin et al. [49] showed that the application of organic fertilizer could increase soil water content and increase soil storage capacity, and Su et al. [50] found that it had a significant effect on improving crop yield and soil water use efficiency. Good tillage practices and organic fertilizer application can improve soil water and fertilizer status, which can help in yield formation. Compared with rotary tillage, subsoiling can break the bottom layer of the plow, promote the development and rooting of crops, ensure the supply of water and fertilizer in the late growth stage, and increase the number of grains per ear and the weight of 100 grains [51]. Subsoiling can also improve soil porosity and water retention, increase soil microbial activity, improve maize growth and development conditions, and increase wheat yield [52,53]. Cao et al. [54] also showed that subsoiling significantly improved yield indexes such as spike length, grain number per spike, and 100-grain weight compared with continuous tillage treatment, and the dominant factor influencing yield was the coordination between spike length and grain number per spike, and the subsoiling effect was better.

4.2. Effects of the Combined Application of Organic and Inorganic Fertilizers on Antioxidant Enzymes in Maize Leaves Using Different Tillage Modes

Reactive oxygen species in plants usually maintain dynamic equilibrium, and under external adversity, the equilibrium is broken and the accumulation of reactive oxygen species increases. As signaling molecules, reactive oxygen species (ROS) excite and induce enhanced activity of a variety of antioxidant enzymes through signaling, which is a protective system formed by plants to scavenge excess ROS and mitigate oxidative stress damage [55–58]. The biological free radical hypothesis proposed by Fridovich [59] has attracted extensive attention in the study of plant stress resistance and aging mechanisms. The leaf senescence process is related to the imbalance of reactive oxygen species metabolism, and CAT and APX are the most important reactive oxygen species-scavenging systems in plants. Jiao et al. [60] found that nitrogen fertilizer could increase GDH activity. Lü et al. [61] found that CAT decomposes H₂O₂ and protects cells from oxidative damage, and plants increase their synthesis under oxidative stress. Sun et al. [62] found that APX

can scavenge hydrogen peroxide and other reactive oxygen species, and in the early stage of leaf development, APX uses AsA as an electron donor to remove H_2O_2 in cells, which can prevent the toxicity caused by reactive oxygen species to cells. Reduced glutathione (GSH) and glutathione reductase (GR) could alleviate the effects of drought and nutrient deficit on reactive oxygen species metabolism in maize leaves. In harsh environments, Shao et al. [63] and Islam et al. [64] found that the activity of the intrinsic protective enzyme system in leaves was induced to increase.

This study showed that the combined application of organic fertilizer and inorganic fertilizers with P1 had a great effect on antioxidant enzyme activity. Compared with R, subsoiling can break the bottom layer of the plow, promote the development and rooting of crop roots, increase the water and fertilizer use efficiency of roots in the soil, and improve the antioxidant capacity of maize leaves, which is consistent with the results of Wang et al. [29]. For the improvement of enzyme activity, the combined application of organic and inorganic fertilizers was more obvious than that of tillage. Regarding the combination of organic fertilizer and inorganic fertilizer: (1) GDH activity and CAT activity showed a trend of increasing first and then decreasing, and GDH could maintain plant carbon and nitrogen balance and improve plant tolerance to drought and alkali stress at the grain filling stage. The CAT enzyme catalyzes the decomposition of hydrogen peroxide, enhances maize antioxidant defense, reduces the damage caused by reactive oxygen species to cells, delays leaf senescence and promotes grain filling, increases the number of corn ears and 100 grains, and thus improves maize yield, which is consistent with the research results of Cao et al. [32]. (2) The activity of APX and GR showed a gradual downward trend, and the reason for the highest enzyme activity at the jointing stage was that in the early stage of leaf development, APX used AsA as an electron donor to remove H_2O_2 in cells, which could prevent the toxicity of reactive oxygen species to cells. GR converts hydrogen peroxide into oxygen and water, which is also detoxifying. At the same time, GR is able to maintain glutathione levels, thereby protecting plants from oxidative damage, which is consistent with the study results of Sun et al. [62]. (3) GSH activity showed a gradual upward trend, which is consistent with the results of previous studies, and the gradual increase in GSH activity was due to the increase in AsA electron content, and the activity of GSH was also accompanied by an increase and reacted with hydrogen peroxide, which was affected by drought after the filling stage, and a large amount of reactive oxygen species would be produced, which would promote the enhancement of GSH's activity, scavenge reactive oxygen species, and improve plant stress resistance, which is consistent with the study results of Sun et al. [62].

5. Conclusions

Using different farming methods, when the combination of organic and inorganic fertilizers is applied, will bring a series of surprising effects. This treatment significantly increased the activity of CAT (catalase), GDH (glutamate dehydrogenase), GR (glutathione reductase), GSH (glutathione), and APX (ascorbate peroxidase) activity in corn leaves. These enhancements are significant, as they can effectively delay the senescence process of corn leaves, keep the leaves green for longer, and reduce the bald growth of corn. At the same time, they can also promote the growth of corn ears, increase ear length, ear grain number, and 100-grain quality, and finally achieve corn yield.

In this study, R + S P₁ treatment performed particularly well, significantly increasing maize yields and having a positive effect on the activity of maize physiological internal enzymes, further delaying leaf senescence. Therefore, R + S P₁ treatment can be considered the best tillage practice and the most suitable fertilization ratio. We should vigorously promote this treatment method so that it can be demonstrated and applied in a large area, so as to provide a solid theoretical basis for high and stable corn yield in the dryland farming area of Shanxi. Only in this way can we better guarantee the yield and quality of corn and make greater contributions to the development of agriculture.

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Data Availability Statement: Data are contained within the article.

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