

EFFECT OF NITROGEN FERTILIZER APPLICATION RATE ON NITRATE REDUCTASE ACTIVITY IN MAIZE

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Abstract. In this paper, the effect of nitrogen application on nitrate reductase activity of the Sidan 19 maize (*Zea mays* L.) cultivar was studied. The Sidan 19 maize is a medium maturity maize single variety, which is a hybrid of 44/4 (Huangzao4 improved line) as the female parent and Mo17 as the male parent. It has high average hectare yield, good early-onset, easy to catch seedlings and other excellent properties, so it was selected as a model plant. In order to study the effect of nitrogen application on the activity of nitrate reductase in maize, the method of this paper is to prepare nitrate reductase, extract corn leaves, add trichloroacetic acid solution to stop the enzyme reaction, and use standard curve method to determine the content of maize nitrate reductase. The experimental results show that: (1) N application rate of nitrate reductase activity (NRA) was N300 > N200 > N100 > N0. (2) NRA activity of maize leaves was the lowest at the seedling stage, the highest at heading stage, and then decreased. Under 15% pec-6000 drought stress, 72 mmol/L salt stress and 72 mmol/L Saline-alkali stress, nitrate reductase activity of maize leaves and roots was the highest. (3) Under the same amount of nitrogen application, the nitrate reductase activity of maize roots increased, and the nitrate reductase (NR) activity of leaves was higher than that of roots under the same stress effect. (4) Under the same amount of nitrogen application, the nitrate reductase activity of maize leaves was the highest. At nitrogen levels of N₀, N_{0.1} and N_{0.5}, the activity of nitrate reductase enzymes in roots of each treatment from highest to lowest were sodium nitrate > urea > ammonium chloride. It showed that the activity of nitrate reductase increased with the increase of nitrogen application. The nitrate reductase activity of maize root was the highest at heading stage, and under low nitrogen condition.

Keywords: *single variety, model plant, trichloroacetic acid solution, standard curve method, heading stage*

Introduction

As the world's largest crop, maize is not only an important food crop, but also a feed and industrial raw material. It occupies an important position in the national economy (Zheng et al., 2018). Scientists have been working to increase their production and productivity in order to solve the food problem and the shortage of feed and industrial raw materials in China (Helmets et al., 2017). At present, with the increase of population, the expansion of animal husbandry and the shortage of energy, the total demand for crop production in the market are increasing day by day, and the demand for grain in China is increasing rigidly. At present, the number of cultivated lands in China is decreasing year by year, so improving the unit yield is one of the key measures to solve the food shortage (Russo et al., 2017). The increasing dependence of high-yielding varieties on chemical fertilizers, especially nitrogen fertilizers, has resulted in the increasing use of nitrogen fertilizers in China (Li et al., 2017). Excessive application of nitrogen fertilizer resulted in low efficiency of nitrogen fertilizer use in maize production in China, resulting in a large amount of nutrient loss to the environment and serious environmental problems (İlhan et al., 2018). How to improve the utilization rate of nitrogen fertilizer, reduce the loss of nitrogen fertilizer and the environmental

pollution caused by the application of nitrogen fertilizer in maize under the current difficult conditions is a difficult problem to be solved urgently in the maize planting industry (Juárezhernández et al., 2017). Breeding and screening nitrogen-efficient maize varieties is one of the main ways to solve the problems of food shortage, shortage of arable land and environmental pollution (Zhang et al., 2017). It is the premise and basis for breeding maize varieties with high nitrogen efficiency to study the nitrogen uptake regularity and identify and excavate candidate genes for high nitrogen efficiency (Iqbal et al., 2017). Nitrate reductase, as an important inducible enzyme in nitrogen metabolism of maize, is the first rate-limiting enzyme in the process of nitrogen uptake and utilization. Nitrate reductase plays a key biological role in the process of efficient nitrogen utilization in maize (Kang et al., 2017).

In the process of plant growth and development, many biological metabolic reactions, such as N metabolism, are involved. N is one of the essential elements in plants. N is an important factor in plant metabolic system related to yield. Nitrate, ammonia and amino acid are the three main forms of nitrogen uptake by plants, and nitrate is the most important form of nitrogen uptake. When external nitrogen enters plants, ammonia, amino acids and amides are formed in turn, and then they participate in the biosynthesis of other living substances for plant growth, development and metabolism (Li, 2017). Nitrate reductase plays an important role in plant nitrogen uptake and development. As a key enzyme, nitrate reductase plays an important role in plant nitrogen uptake and assimilation. Its activity can directly reflect the status of plant nitrogen uptake and utilization (Liu et al., 2018). The results showed that nitrate reductase activity was affected by environmental factors such as water, nitrate concentration, light, carbon dioxide, molybdenum and temperature, and exogenous hormone analogues such as cytokinin, abscisic acid, indole-3-acetic acid, gibberellin and ethephon could affect nitrate reductase activity (Sui et al., 2017). Nitrate reductase was found in all tissues of maize, but its activity was the highest in maize leaves. Nitrate reductase activity has an important impact on the utilization of nitrate nitrogen, is an important basis for crop selection of nitrogen fertilizer, and is also one of the breeding indicators for crop nitrogen efficient utilization (Castillogodina et al., 2018). The main content of this paper is to study the effect of nitrogen application rate on nitrate reductase activity of maize, so as to improve crop yield, avoid nitrogen loss, promote the development of agricultural technology and promote the progress of agricultural economy. Therefore, it is necessary to study the effect of nitrogen application rate on nitrate reductase activity of maize.

Materials and methods

This study was carried out in the experimental site of Sichuan Agricultural University in 2019. The GPS coordinates are N29° 58' 54.23" north latitude and E102° 59' 57.54" east longitude. The experimental variety is Sidan 19, and the sowing time is July. The method of sowing or strip sowing is adopted, the temperature is 20 °C, the water is sufficient, and the time is 5-7 days. The nutrient content of the soil is shown in *Table 1*.

Effects of different amounts of pure ammonia fertilizer on nitrate reductase activity in maize

Fixed the amount of phosphorus and potassium, four nitrogen fertilizer treatments were set up in the experiment:

- (1) N0: no nitrogen fertilizer
- (2) N100: 100 kg of pure nitrogen per hectare
- (3) N200: 200 kg of pure nitrogen per hectare
- (4) N300: 300 kg of pure nitrogen per hectare

The experiment was designed by random block design and repeated three times. The length of residential area is 5 m and the distance of 6 rows is 70 cm.

Table 1. Nutrient content of soil

Organic matter content	2.56%
Total nitrogen	0.134
Total phosphorus	0.064
Slowly available potassium	98
Hydrolyzable nitrogen	146.2
Available phosphorus	44.78
Available potassium	143.2
pH value	6.62

Effects of different proportion of nitrogen fertilizer applied by stages on nitrate reductase activity in maize

The total amount of chemical fertilizer application is fixed, which is pure N 370 mg/hm², pure P₂O₅ 150 kg/hm² and pure K₂O 150 kg/hm². Nitrogen fertilizer is applied in stages according to different proportions, as detailed in Table 2.

Table 2. Distribution ratio of nitrogen fertilizer in different growth periods

Handle	Base fertilizer	Topdressing at jointing stage	Topdressing at silking stage
N1	370	0	0
N2	296	74	0
N3	296	37	37
N4	222	148	0
N5	222	111	37
N6	222	74	74

Nitrate reductase activity in leaves and roots was measured at seedling stage, jointing stage, trumpet mouth stage, booting stage, silking stage, filling stage and maturity stage (Ma et al., 2017). The top fully unfolded leaves were selected before silking, and the ear leaves were selected after silking (Han et al., 2018), and the roots were selected to be measured at a distance of more than 1 cm from the root tip.

Effects of nitrogen fertilizer application rate on nitrate reductase activity in maize under different stress environment

Formulas of simulated solutions for different stress treatments are shown in Table 3. After 48 h of stress treatment, physiological indexes were measured (Wang et al., 2018).

Table 3. Formulas of solutions for different stress treatments

Stress treatment	Ratio of nitrogen fertilizer	Solution formulation	Solution J_e (MPa)	Solution pH
Drought stress	Application of pure nitrogen per hectare 100 kg	Hoaglangsolution + 15% PEC-6000 (Actual site conditions)	-0.363	6.8
		Hoaglangsolution + 15% PEC-6000 (Water culture experiment)		
Salt stress	Application of pure nitrogen per hectare 100 kg	Hoaglang solution + 72 mmol/L NaCl (Actual site conditions)	-0.363	6.7
		Hoaglang solution + 72 mmol/L NaCl (Water culture experiment)		
Saline-alkali stress	Application of pure nitrogen per hectare 100 kg	Hoaglangsolution + 72 mmol/L $NaHCO_3$ (Actual site conditions)	-0.363	8.9
		Hoaglangsolution + 72 mmol/L $NaHCO_3$ (Water culture experiment)		
Control (normal)	Application of pure nitrogen per hectare 100 kg	Hoaglangsolution (Hoaglangsolution + 72 mmol/L $NaHCO_3$)	-0.050	6.5
		Hoaglang solution (Water culture experiment)		

Effects of nitrogen fertilizer varieties and nitrogen fertilizer dosage on nitrate reductase activity in maize

The experimental settings are as shown in *Table 4*.

Table 4. Effects of nitrogen fertilizer varieties and nitrogen fertilizer dosage on nitrate reductase activity in maize

Nitrogen fertilizer varieties	Code name	Control level (g/kg)
Sodium nitrate	N ₀	0.00
	N _{0.1}	0.61
	N _{0.3}	1.82
	N _{0.5}	3.04
Ammonium chloride	N ₀	0.00
	N _{0.1}	0.38
	N _{0.3}	1.15
	N _{0.5}	1.91
Ammonium nitrate	N ₀	0.00
	N _{0.1}	0.28
	N _{0.3}	0.86
	N _{0.5}	1.43
Urea	N ₀	0.00
	N _{0.1}	0.22
	N _{0.3}	0.64
	N _{0.5}	1.07

Method of determination

(1) Nitrate reductase production (Hwang et al., 2018) could be induced by adding 50 mmol/L KNO_3 or $NaNO_3$ to the water of maize seedlings one day before sampling.

(2) 0.5 g of maize leaves were weighed (three parts were cut into about 1 cm segments) and put into three triangular bottles, one of which was used as control, and the other two were used for enzyme activity determination (Song et al., 2017).

(3) Reaction: First add 1 mL 30% trichloroacetic acid solution to the control triangle bottle, then add 9 ml 0.1 mol/L KNO_3 solution to each triangle bottle. After mixing, put it into the dryer immediately, and exhaust for 30 min (during several times, air is injected, then vacuum is pumped to make the blade sink completely into the bottom of the bottle, and then react in 25-degree darkness for half an hour, respectively (Ahamed et al., 2017). 1 m 130% trichloroacetic acid was added to the determination bottle (except the control bottle) to terminate the enzyme reaction.

(4) Colorimetric determination: Shake each bottle for 2 min, take 2 mL reaction solution, add 1 mL sulfonamide, shake well, add 1 mL vinylamine, and then color in 35 water baths for 15 min, and then colorimetric 540 nm.

(5) Blank solution: 2 mL distilled water + 1 mL sulfonamide, the same as the sample solution for 15 min.

Nitrate reductase activity in maize was determined by standard curve method (Johansson et al., 2017). The content of NO_2^- ($\mu g/mL$) was detected from the standard curve, and the activity of nitrate reductase in maize was expressed by the amount of NO_2^- produced per gram fresh weight per hour. The calculation method was as follows:

$$\text{Enzymatic activity} \quad [\mu g / (g \cdot h)] = \frac{(C_1 - C_0) \times V_t}{FW \times t \times V_s} \quad (\text{Eq.1})$$

In the formula, C_0 and C_1 are the amount of NO_2^- obtained from the standard curve ball at the beginning of the test and in the course of the test respectively; V_t is the total volume of the reaction liquid; V_s is the sampling volume at the time of determination; FW is the fresh weight; t is the reaction time.

Results

In order to further verify the effect of nitrogen application rate on nitrate reductase activity of corn, relevant test experiments need to be carried out. The research content of this experiment is as follows: effect of pure nitrogen fertilizer application rate on nitrate reductase activity in maize at different growth stages, effects of different proportion of nitrogen fertilizer applied by stages on nitrate reductase activity in maize, effects of nitrogen fertilizer application rate on nitrate reductase activity in maize under different stress environment and effects of nitrogen fertilizer varieties and nitrogen fertilizer dosage on nitrate reductase activity in maize. The experimental environment is shown in *Figure 1*.

Effect of pure nitrogen fertilizer application rate on nitrate reductase activity in maize at different growth stages

After analyzing the effect of nitrogen fertilizer application rate on nitrate reductase activity in maize at different growth stages, the results are shown in *Figure 2*.



Figure 1. Experimental environment diagram

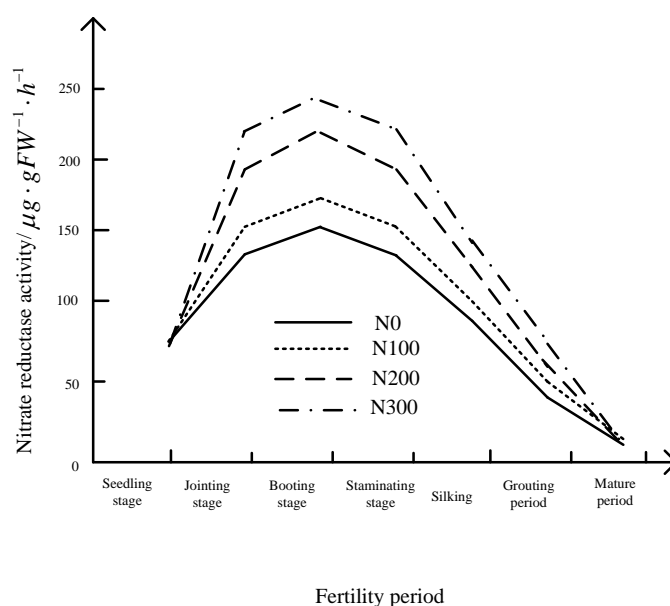


Figure 2. Effects of nitrogen fertilizer application rate on nitrate reductase activity in maize at different growth stages

The activity of nitrate reductase changed with the amount of nitrogen. The activity of nitrate reductase showed a single peak curve during the whole growth period. With the advancement of fertility process, it gradually increases and then decreases after reaching the peak. Nitrate reductase activity increased with the increase of nitrogen application (Yang et al., 2017). Nitrate reductase activity increased rapidly after jointing stage, reached its peak at booting stage, decreased gradually from booting stage to maturity stage, and decreased after heading stage.

After analyzing the effect of nitrogen application rate on nitrate reductase activity in ear leaves of maize during grain filling, the results are as shown in *Table 5*.

Table 5 shows that nitrate reductase activity in ear leaves of maize decreases gradually with the advance of grain filling, and increase with the increase of nitrogen nutrition level. From 7 to 49 days after silking, the activity of nitrate reductase was $N300 > N200 > N100 > N0$. With the increase of nitrogen fertilizer application rate, the nitrogen metabolism of maize maintained a high level at the later growth stage.

Table 5. Nitrate reductase activity in ear leaves of maize (silking in spite of spinning)

Handle	Number of days after spinning (d)						
	7	14	21	28	35	42	49
N0	71.31	50.91	38.41	22.41	16.91	14.21	13.11
N100	82.41	62.41	51.31	35.61	22.81	19.91	18.21
N200	100.91	85.81	60.61	42.61	27.31	22.81	19.91
N300	116.71	90.21	69.91	52.21	34.81	27.61	21.41

Effects of different proportion of nitrogen fertilizer applied by stages on nitrate reductase activity in maize

(1) Effect of different proportion of nitrogen fertilizer on nitrate reductase activity in maize leaves

The effects of different proportion of nitrogen fertilizer on nitrate reductase activity in maize leaves were analyzed. The results are shown in *Figure 3*.

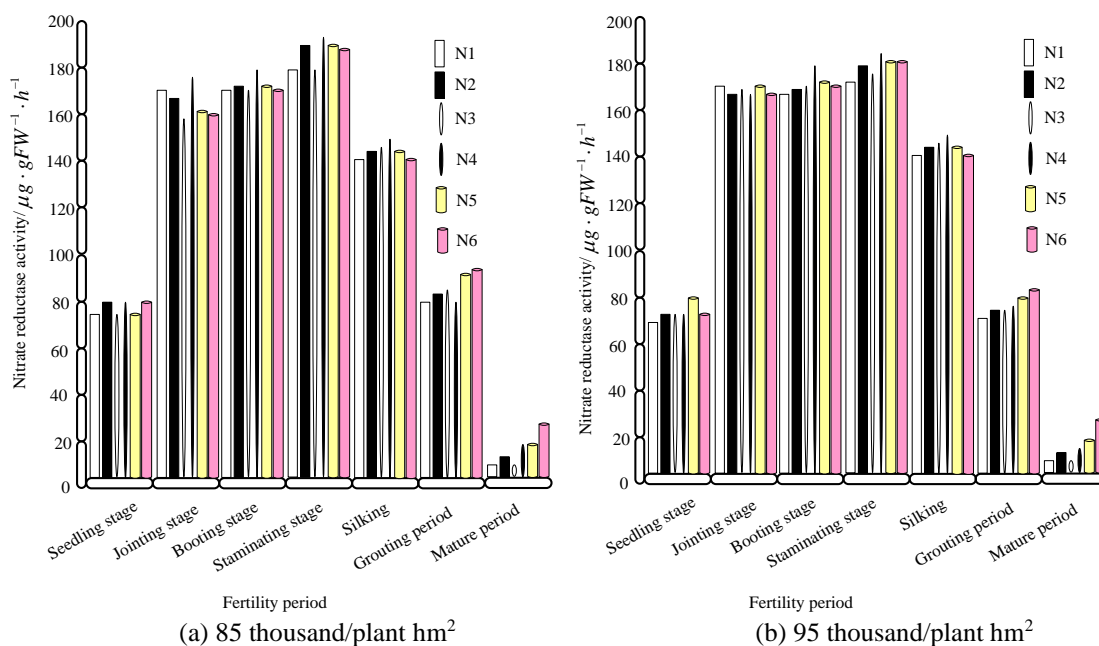


Figure 3. Effects of different proportion of nitrogen fertilizer applied in stages on nitrate reductase activity in maize leaves

Figure 3 shows that nitrate reductase activity is lower at seedling stage and reaches the maximum at male-drawing stage, then it shows a downward trend, and changes in a single peak curve during the whole growth period. Because the male-drawing stage is the key period for the morphological formation of maize reproductive organs, carbon and nitrogen metabolism is the most vigorous, and nitrate reductase activity is the highest in the male-drawing stage, so a large number of nitrates can be assimilated into leaves to meet the needs of crop growth and development.

Nitrate reductase activity changed with the application ratio of nitrogen fertilizer at different growth stages. Nitrate reductase activity of maize leaves treated with N6

maintained higher activity of nitrate reductase than that of other treatments in the process of decreasing from silking stage (Chelladurai, 2017). Nitrogen metabolism in maize is an integral part of photosynthesis, which determines the accumulation of dry matter and has an important impact on yield.

By comparing the changes of nitrate reductase activity in maize leaves under two planting densities, it can be seen that the nitrate reductase activity of maize leaves under 85,000 thousand/plant hm^2 density and 95,000 thousand/plant hm^2 density had little difference at seedling and jointing stages, and the nitrate reductase activity under 85,000 thousand/plant hm^2 density began to be higher than 95,000 thousand/plant hm^2 from booting stage, indicating that the activity of nitrate reductase in the leaves of high-density planted maize was lower.

(2) The effect of different proportion of nitrogen fertilizer applied by stages on nitrate reductase activity in maize roots

After analyzing the effect of different proportion of nitrogen fertilizer on nitrate reductase activity in maize roots by stages, the results are shown in *Figure 4*.

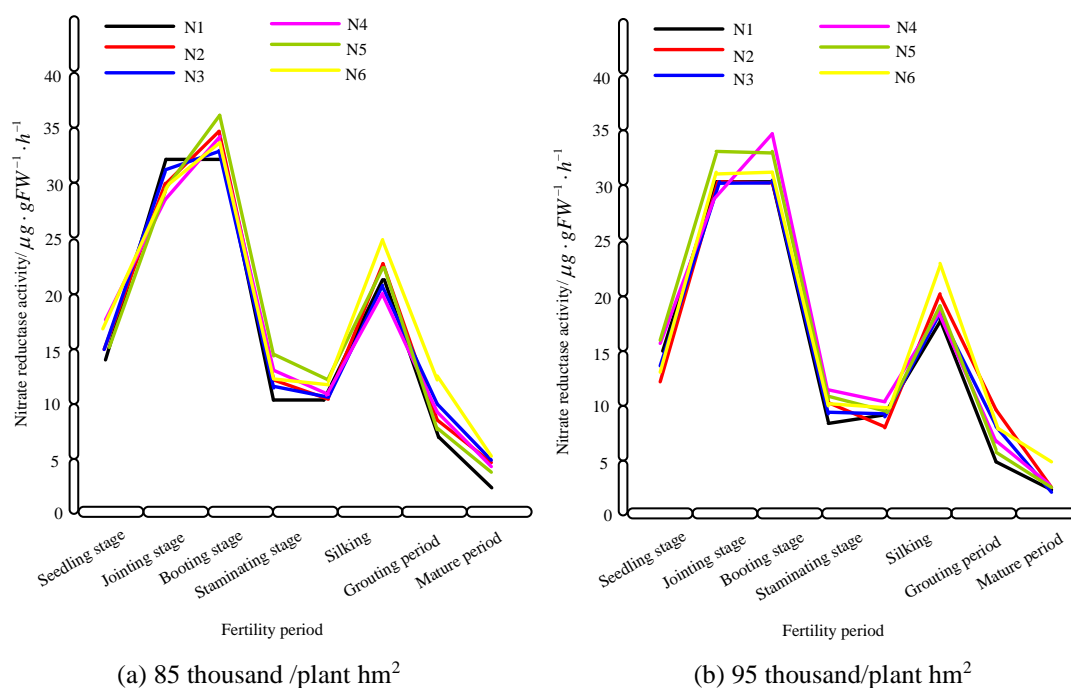


Figure 4. Effects of different proportion of nitrogen fertilizer on nitrate reductase activity in maize roots

From *Figure 4*, it can be seen that the nitrate reductase activity in roots changes in a bimodal curve with the development of the growth process. It is highest at booting stage, then decreases sharply, and begins to rise after silking stage, and then decreases after the second peak at grain filling stage.

Nitrate reductase activity in roots varied with the proportion of nitrogen fertilizer applied in different growth stages. Nitrate reductase activity was the highest in N4 treatment at booting stage.

Before booting stage, the nitrate reductase activity in maize roots had little difference between the two densities (Sarkar et al., 2017). From booting stage, the nitrate reductase

activity of 85,000 thousand/plant hm^2 densities were higher than 95,000 thousand plant/ hm^2 .

Effects of nitrogen fertilizer application rate on nitrate reductase activity in maize under different stress environment

Nitrate Reductase (NR) is an important regulator and rate-limiting enzyme in plant nitrogen metabolism. NR plays an important role in plant growth and development, yield formation and protein content. NR activity is induced by substrates and is influenced by many other environmental factors. The effects of nitrogen application rate on nitrate reductase activity in maize under different stress environments, as shown in Table 6.

Table 6. Effects of nitrogen fertilizer application rate on nitrate reductase activity in maize under different stress environments

Stress treatment	NR activity/ $\mu\text{g} \cdot \text{gFW}^{-1} \cdot \text{h}^{-1}$		Activity ratio
	Root system	Blade	
Contrast	36.52 ± 3.10	106.22 ± 6.77	65.6
15% PEC-6000	37.70 ± 2.00	$34.00 \pm 4.99^{**}$	-9.8
72 mmol/L NaCl	$61.96 \pm 5.24^{**}$	$38.93 \pm 2.24^{**}$	-59.2
72 mmol/L	$142.58 \pm 8.40^*$	$43.2 \pm 3.00^{**}$	-230.0

* Activity ratio (%) = (leaf NR activity - root NR activity), leaf NR activity 100; ** t test reached a very significant level

Table 6 shows that the nitrate reductase activity of maize roots increases under different stress environments with a certain amount of nitrogen fertilizer. In 72 mmol/L saline-alkali stress environment, the increase of NR activity was 69.7% compared with the control, and the t test also reached a very significant level (Ramezani et al., 2017). In 15% PEC-6000 drought stress environment, the increase of NR activity was the smallest, which was 3.2% higher than the control, and t test was not significant. For comparison, we also measured the NR activity of maize seedling leaves under the same stress conditions as the root. As shown in Table 6, NR activity in leaves was higher than that in roots, reaching $106.22 \mu\text{g} \cdot \text{gFW}^{-1} \cdot \text{h}^{-1}$, which was 2.9 times higher than that in roots. However, NR activity in leaves decreased significantly under different stress environments, and reached a significant level by t-test analysis compared with the control values. The NR activity ratios of roots treated with the same treatment were -9.8% under drought stress, -59.2% under salt stress and -230.0% under salt stress, respectively. The results showed that leaf NR activity stress was greater than root NR activity stress under the same stress.

Effects of nitrogen fertilizer varieties and nitrogen fertilizer dosage on nitrate reductase activity in maize

(1) The effect of different nitrogen fertilizer varieties and dosage on nitrate reductase activity in maize leaves

Figure 5 is a comparative study of nitrate reductase activity in maize leaves under different nitrogen fertilizer varieties and nitrogen levels. The results showed that the

variety and amount of nitrogen fertilizer had significant effects on the activity of nitrate reductase in maize leaves.

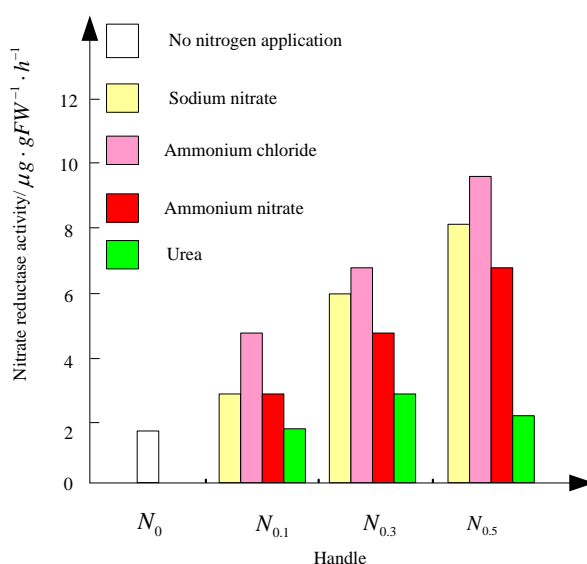


Figure 5. Effects of different nitrogen fertilizer varieties and amounts on nitrate reductase activity in maize leaves

Nitrogen fertilizer application could significantly increase the activity of nitrate reductase in maize leaves. The results showed that the nitrate reductase activity of maize leaves treated with sodium nitrate, ammonium chloride, ammonium nitrate and urea was higher than that of maize leaves without nitrogen application. The nitrate reductase activity of maize Treated with sodium nitrate increased significantly from $N_{0.1}$ to $N_{0.5}$, and increased 59.5%, 200.5% and 299.5% under three levels of nitrogen application compared with N_0 , respectively. When the nitrogen supply level increased from N_0 to $N_{0.5}$, the nitric acid in leaves of maize Treated with ammonium chloride increased. The activity of nitrate reductase increased significantly with the increase of nitrogen application level. There was no significant difference in the activity of nitrate reductase between the treatments with ammonium nitrate application amount of N_0 , $N_{0.1}$ and $N_{0.5}$, but the activity of nitrate reductase in leaves increased significantly with the further increase of nitrogen application amount of $N_{0.5}$. The activity of nitrate reductase in urea treatment was significantly higher than that in $N_{0.3}$ treatment. Compared with other nitrogen supply levels, the activity of nitrate reductase was 107.1%, 87.1% and 30.8% higher than that of N_0 , $N_{0.1}$ and $N_{0.5}$, respectively. There was no significant difference in the activity of nitrate reductase between $N_{0.5}$ and $N_{0.1}$ levels.

Nitrogen fertilizer varieties also had a great influence on nitrate reductase activity. According to *Figure 5*, the difference of nitrate reductase activity in maize leaves treated with low-nitrogen $N_{0.1}$ was as follows: ammonium chloride > sodium nitrate > sodium nitrate > urea due to different varieties of nitrogen fertilizer. That is to say, when ammonium chloride was used as nitrogen source, the nitrate reductase activity of maize leaves was the highest, reaching $4.72 \mu\text{g} \cdot \text{gFW}^{-1} \cdot \text{h}^{-1}$, and the value was 2.4 times of the control at this time. When urea was used as nitrogen source, the nitrate

reductase activity of maize leaves was the lowest, only $1.86 \mu\text{g} \cdot \text{gFW}^{-1} \cdot \text{h}^{-1}$, which was 1.1 times of the control. The nitrate reductase activity of maize leaves treated with medium nitrogen $N_{0.3}$ and high nitrogen $N_{0.5}$ were the highest with ammonium chloride and sodium nitrate as nitrogen sources. There was no significant difference in nitrate reductase activity between the two nitrogen fertilizer varieties, and the nitrate reductase activity of maize leaves treated with four nitrogen fertilizer varieties was the same under the same nitrogen application level.

It can be seen that the nitrate reductase activity of leaves treated with nitrogen fertilizer increased to varying degrees, regardless of which nitrogen fertilizer variety was used as nitrogen source.

(2) Effects of different nitrogen fertilizer varieties and amounts on nitrate reductase activity in maize roots.

The effects of different nitrogen fertilizer varieties and amounts on nitrate reductase activity in maize roots are shown in *Figure 6*.

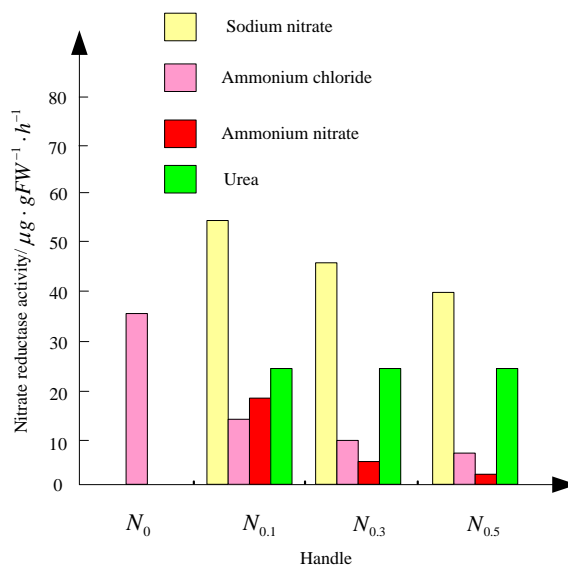


Figure 6. Effects of different nitrogen fertilizer varieties and amounts on nitrate reductase activity in maize roots

The results of *Figure 6* showed that the activity of nitrate reductase decreased with the increase of nitrogen fertilizer application. The nitrate reductase activity of roots treated with sodium nitrate increased in varying degrees compared with the control, while the nitrate reductase activity of roots treated with other three fertilizers was lower than that of the control. (1) Nitrate reductase activity was most affected by nitrogen application, which was significantly decreased under three levels of fertilization: $N_{0.1}$, $N_{0.3}$ and $N_{0.5}$; (2) Nitrate reductase activity in urea treatment was least sensitive to the amount of nitrogen application, and there was no significant difference among the four nitrogen treatments.

(3) The nitrate reductase activity of ammonium chloride and ammonium nitrate treatments was not significantly different under three levels of nitrogen application. The nitrate reductase activity of ammonium nitrate treatments of $N_{0.3}$ and $N_{0.5}$ were significantly lower than that of $N_{0.1}$.

The difference of nitrate reductase activity in roots caused by different nitrogen fertilizers was as follows: at the same level of nitrogen application, the nitrate reductase activity in roots of different treatments was sodium nitrate > urea > ammonium chloride, ammonium nitrate, ammonium chloride and ammonium nitrate, but there was no significant difference between the nitrate and urea, and between the other two nitrogen fertilizer varieties.

According to the results of nitrate reductase activity in maize Leaves under different nitrogen fertilizer varieties and different amounts of nitrogen fertilizer, the nitrate reductase activity in leaves and roots of maize treated with urea as nitrogen source did not change much compared with the other three nitrogen sources, while the nitrate reductase activity in leaves treated with other three nitrogen sources were all in the same condition. The nitrate reductase activity of roots was the highest under high nitrogen and low nitrogen. Nitrate reductase activity in leaves and roots showed opposite trends in four fertilizers when nitrogen application increased.

Discussion

Combining with the research contents in this paper, the technical principles of improving the utilization rate of nitrogen fertilizer are discussed. The utilization rate of nitrogen fertilizer in China's agricultural production is low and its loss is great, and the effect of increasing production is far from being fully exerted. The potential of reducing the loss of nitrogen fertilizer, improving its utilization rate and increasing production effect is also great. According to the experimental analysis, under the condition of increasing application of pure nitrogen fertilizer, the nitrate reductase activity of maize increased gradually at the start-up stage and before, and decreased gradually at the beginning of endurance stage; different application proportion of nitrogen in each period will have an impact on the nitrate reductase activity of maize; under different stress environment, a certain amount of nitrogen is applied Under the same stress, the NR activity of leaves was higher than that of roots; the effect of nitrogen fertilizer on NR activity of leaves was the greatest. Following is a brief description of the relevant technical principles:

(1) Avoiding excessive accumulation of mineral nitrogen in soil as far as possible

Exchangeable ammonium and nitrate nitrogen in soil are not only available nitrogen that crops can absorb directly, but also the common source of various nitrogen loss processes. It is undoubtedly necessary to have a proper amount of available nitrogen in soil, but excessive nitrogen will increase the loss of nitrogen. Therefore, it is one of the important principles for effective application of nitrogen fertilizer to avoid its excessive presence in soil.

(2) The countermeasures for the main loss ways of nitrogen fertilizer

Due to the different environmental conditions of different soils and crops, the loss of nitrogen through different mechanisms and pathways and their proportion in total loss of nitrogen fertilizer are quite different, so the technology to reduce the loss of nitrogen fertilizer should also be different. However, there are some internal links between different ways of loss. Therefore, when taking one of the loss mechanisms and ways as

the control object to formulate technical countermeasures, we should take into account its possible impact on other loss mechanisms and ways.

A. Reducing ammonia volatilization

Ammonia volatilization rate is a function of partial pressure of ammonia in surface water and wind speed above surface water. The former is also a function of $[NH_3 + NH_4^+] - N$, pH and temperature of surface water. Therefore, reducing $[NH_3 + NH_4^+] - N$ and pH in surface water after fertilization is the key technology to reduce ammonia volatilization. In addition, ammonia volatilization can be reduced by inhibiting the loss of ammonia from surface water of maize fields to the atmosphere.

For dry farming, these principles are basically applicable, but because there is no water layer in dry farming soil, the key to reducing ammonia volatilization loss is to try to apply ammonia fertilizer in a certain depth of soil layer to reduce the partial pressure of ammonia on the soil surface.

B. Inhibition of nitrification

Nitrification of ammonium not only produces a small amount of N_2O , but also forms nitrate nitrogen which is easy to pass through denitrification (also produces part of N_2O) and leaching loss. Therefore, for ammonium nitrogen fertilizer and ammonia fertilizer that can be formed, delaying or maintaining nitrification is one of the important ways to reduce losses, improve utilization rate and reduce environmental impact.

(3) Improving the ability of crops to absorb mineral nitrogen

There is a competition between the uptake of mineral nitrogen and the loss of nitrogen in crops. Therefore, eliminating all kinds of limiting factors affecting crop growth, such as lack of phosphorus and potassium, drought and flood, to improve the ability of crops to absorb mineral nitrogen, will help to improve the utilization rate of nitrogen fertilizer and reduce losses. In addition, in the period of vigorous crop growth, nitrogen fertilizer applied will be absorbed rapidly because of the strong absorption ability of root system, which is beneficial to reduce its loss; moreover, because of the high vegetation coverage on the ground, it is also helpful to reduce the wind speed across the soil (dry farming) or water surface, thus reducing ammonia volatilization.

In the process of this experiment, the experimental environment is in line with the actual measurement conditions, and the experimental data is accurate and reliable. Therefore, we can draw relevant conclusions through the experimental results, which has scientific guidance and statistical significance. The results showed that the nitrate reductase activity could be significantly increased by increasing nitrogen content. The amount of nitrogen should be controlled at the early stage of crop growth, and the top dressing should be focused at the vigorous stage of crop growth.

Conclusions

In order to deeply analyze the effect of nitrogen fertilizer application rate on nitrate reductase activity of maize, the effects of different application rates of pure ammonia fertilizer on nitrate reductase activity of maize, the effects of different proportions of nitrogen fertilizer applied in stages on nitrate reductase activity of maize, the effects of

nitrogen fertilizer application rate on nitrate reductase activity of maize in different stress environments, and nitrogen fertilizer products were studied. The effects of seed and nitrogen fertilizer on nitrate reductase activity in maize were analyzed in four aspects. The results showed that:

Conclusion 1: Nitrate reductase activity tends to increase with the increase of nitrogen application. Nitrate reductase activity increases rapidly after jointing stage, peaks at booting stage, decreases gradually from booting stage to maturity stage, and decreases after heading stage.

Conclusion 2: Because the male-drawing stage is the key period for the morphological formation of maize reproductive organs, carbon and nitrogen metabolism is the most vigorous, and nitrate reductase activity is the highest in the male-drawing stage; nitrate reductase activity in roots varies with the proportion of nitrogen fertilizer applied in different growth stages. Nitrate reductase activity was the highest in N4 treatment at booting stage.

Conclusion 3: Nitrate reductase activity in maize roots increased under different stress environments and with a certain amount of nitrogen fertilizer.

Conclusion 4: Nitrate reductase activity of maize leaves was higher than that of maize leaves without nitrogen application. When ammonium chloride was used as nitrogen source, the nitrate reductase activity of maize leaves was the highest. When nitrogen source was urea, the nitrate activity of leaves and roots increased with the increase of nitrogen application. Compared with the other three nitrogen sources, the activity of nitrate reductase in maize leaves was the highest under high nitrogen conditions, and the activity of nitrate reductase in roots was the highest under low nitrogen conditions.

To study the effect of nitrogen application on nitrate reductase activity of maize is helpful to improve the quality and yield of maize, promote the economic development of our country, and improve the international status of our country. In order to further understand the effect of nitrogen application rate on nitrate reductase activity of maize, we can study the environmental temperature, soil moisture and other factors, so as to better realize the research of this part.

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REFERENCES

- [1] Ahamed, A. J., Loganathan, K., Ananthakrishnan, S., Ahmed, J., Ashraf, M. A. (2017): Evaluation of graphical and multivariate statistical methods for classification and evaluation of groundwater in Alathur Block, Perambalur District, India. – *Applied Ecology and Environmental Research* 15(3): 105-116.
- [2] Berber, M. R., Hafez, I. H. (2018): Synthesis of a new nitrate-fertilizer form with a controlled release behavior via an incorporation technique into a clay material. – *Bulletin of Environmental Contamination and Toxicology* 101(6): 751-757.
- [3] Castillogodina, R. G., Foroughbakhchpournavab, R., Benavidesmendoza, A. (2018): Effect of selenium on elemental concentration and antioxidant enzymatic activity of tomato plants. – *Journal of Agricultural Science* 18(1): 233-244.

- [4] Chelladurai, G. (2017): Influence of diets on growth and biochemical parameters of *Babylonia spirata*. – *Geology, Ecology, and Landscapes* 1(3): 162-166.
- [5] Han, T., Zhan, W., Gan, M. (2018): Phosphorylation of glutaminase by PKC ϵ is essential for its enzymatic activity and critically contributes to tumorigenesis. – *Cell Research* 28(6): 655-669.
- [6] Helmers, M. J., Zhou, X., Baker, J. L. (2017): Nitrogen loss on tile-drained Mollisols as affected by nitrogen application rate under continuous corn and corn-soybean rotation systems. – *Canadian Journal of Soil Science* 92(3): 493-499.
- [7] Hwang, K. S., Yang, J. Y., Lee, J. (2018): A novel anti-melanogenic agent, KDZ-001, inhibits tyrosinase enzymatic activity. – *Journal of Dermatological Science* 89(2): 165-171.
- [8] İlhan, Ö., Okyay, G., Ulaş, A. (2018): Coating of nonwovens with potassium nitrate containing carboxymethyl cellulose for efficient water and fertilizer management. – *Cellulose* 25(2): 1527-1538.
- [9] Iqbal, S., Thierfelder, C., Khan, H. Z. (2017): Maximizing maize quality, productivity and profitability through a combined use of compost and nitrogen fertilizer in a semi-arid environment in Pakistan. – *Nutrient Cycling in Agroecosystems* 107(2): 197-213.
- [10] Johansson, M., Lundh, Å., De, V. R. (2017): Composition and enzymatic activity in bulk milk from dairy farms with conventional or robotic milking systems. – *Journal of Dairy Research* 84(02): 154-158.
- [11] Juárezhernández, E. O., Casadosvázquez, L. E., Bideshi, D. K. (2017): Role of the C-terminal and chitin insertion domains on enzymatic activity of endochitinase ChiA74 of *Bacillus thuringiensis*. – *International Journal of Biological Macromolecules* 102(2017): 52-59.
- [12] Kang, J., Zhang, Y., Zhang, Z. Y. (2017): Preparation and performance of nano array composite electrodes for lithium battery. – *Chinese Journal of Power Sources* (12): 1701-1702.
- [13] Li, D., Huang, Z. X., Lu, J. C. (2017): Research on the concept and mechanism of military information system based on cloud computing architecture. – *Journal of China Academy of Electronics and Information Technology* 12(4): 365-370.
- [14] Li, R. T. (2017): Design and application of electrical automation system for intelligent building. – *Automation & Instrumentation* (5): 164-166.
- [15] Liu, R., Siththanandan, V., Yang, Y. (2018): The enzymatic activity and cellular localization of drosophila myosin 7a is regulated by a novel binding protein. – *Biophysical Journal* 114(3): 210a-211a.
- [16] Ma, Y. Y., Zhou, C., Li, Q. M. (2017): Simulation research on optimization of intrusion node information in network. – *Computer Simulation* 34(8): 315-318.
- [17] Ramezani, M. R., Bavani, A. R. M., Jafari, M., Binesh, A. (2017): Evaluating gridded BIOME-BGC for simulating LAI at Kasilian watershed-Iran. – *Geology, Ecology, and Landscapes* 1(4): 225-231.
- [18] Russo, T. A., Tully, K., Palm, C. (2017): Leaching losses from Kenyan maize cropland receiving different rates of nitrogen fertilizer. – *Nutrient Cycling in Agroecosystems* 108(2): 195-209.
- [19] Sarkar, M. I. U., Islam, M. N., Jahan, A., Islam, A., Biswas, J. C. (2017): Rice straw as a source of potassium for wetland rice cultivation. – *Geology, Ecology, and Landscapes* 1(3): 184-189.
- [20] Song, M. S., Rossi, J. J. (2017): Molecular mechanisms of Dicer: endonuclease and enzymatic activity. – *Biochemical Journal* 474(10): 1603-1618.
- [21] Sui, C. H., Xu, Y. W., Yan, G. L. (2017): Preparation of a 76-Mer selenium-containing peptide with GPx and SOD double enzyme activity and its cell-penetrating effects. – *Journal of Jilin University (Science Edition)* 55(01): 168-174.

- [22] Wang, H., Li, P., Yu, D. (2018): Unraveling the enzymatic activity of oxygenated carbon nanotubes and their application in the treatment of bacterial infections. – *Nano Letters* 18(6): 3344-3351.
- [23] Yang, X. P., Luo, N., Zong, Y. Y., Jia, Z. H., Liao, X. J. (2017): Quantum dots extraction coupled with high-performance liquid chromatography for the determination of polycyclic aromatic hydrocarbons in water. – *Applied Ecology and Environmental Research* 15(3): 171-186.
- [24] Zhang, R., An, J. C., Hao, L. C. (2017): Influence of transformer magnetic saturation on low-voltage frequency converter driving high-voltage submersible motor system. – *Journal of Power Supply* 15(4): 162-167.
- [25] Zheng, J., Mmari, W. N., Nishigaki, T. (2018): Nitrogen availability to maize as affected by fertilizer application and soil type in the Tanzanian highlands. – *Nutrient Cycling in Agroecosystems* 112(2): 1-17.