Effects of Nitrogen, Phosphorus, and Potassium Deficiency Stress on Agronomic Traits and Chlorophyll Fluorescence in Wheat

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Abstract. In order to investigate the effects of nitrogen (N), phosphorus (P), and potassium (K) deficiency stress on wheat growth and physiological characteristics, this study used wheat (Xinmai 296) as the experimental material. Four treatments were established: nitrogen-deficient treatment, phosphorus-deficient treatment, potassium-deficient treatment, and full fertilizer application treatment. The chlorophyll, plant height, leaf width and chlorophyll fluorescence parameters were systematically measured from the seedling stage to jointing stage. The results showed that the nitrogen deficiency significantly inhibited wheat chlorophyll synthesis, and its chlorophyll content, plant height, and leaf width were all lower than those of the other treatments. Among the chlorophyll fluorescence parameters, the F_v/F_m value was full fertilizer application treatment > potassium-deficient treatment > phosphorus-deficient treatment > nitrogen-deficient treatment, indicating that nitrogen deficiency had the most significant inhibitory effect on the activity of photosystem II (PSII). In summary, nitrogen deficiency had the most significant inhibitory effect on the photosynthetic system and agronomic traits of wheat, followed by potassium and phosphorus. This study provides a theoretical basis for optimizing wheat nutrient management strategies.

Keywords: Nitrogen, phosphorus and potassium; Chlorophyll Fluorescence; Wheat

1 Introduction

Wheat constitutes the principal food source for more than half of China's urban and rural population, ranking as the third-largest grain crop in the country and accounting for 20% of total grain production. Xinmai 296 wheat variety exhibits high yield, lodging resistance, disease resistance, and tolerance to dry and hot winds. Due to its excellent adaptability, comprehensive resistance, and substantial yield capacity, it has been extensively cultivated throughout the North China region.[1]

H. P. Singh et al. (eds.), *Proceedings of the 2025 International Conference on Chemical Engineering and Biological Science (CEBS 2025)*, Advances in Engineering Research 273,
https://doi.org/10.2991/978-94-6463-829-5_8

Photosynthesis underpins crop growth and quality. Nutrient uptake and utilization are closely linked to the photosynthetic system; imbalances in essential nutrients such as N, P, K, and microelement can impair photosynthesis. A scientifically sound supply of nitrogen, phosphorus, and potassium not only enhances crop yield but also improves crop quality and contributes to the sustainability of agricultural systems. However, nutrient imbalances in farmland soils often lead to single or multiple nutrient deficiency stresses in crops, resulting in reduced photosynthetic efficiency and impaired biomass accumulation.

Biomass indices serve as effective indicators for assessing plant growth characteristics and predicting crop yield. Upon absorption of light energy by chlorophyll molecules, excitation from the ground state to an excited state occurs. The return to the ground state involves energy dissipation through three primary pathways: photosynthetic activity within the reaction center, thermal dissipation, and fluorescence emission. These processes exhibit a competitive relationship. Indirect assessment of plant photosynthetic intensity is achievable through the measurement of leaf chlorophyll fluorescence variations. Chlorophyll fluorescence parameters provide a sensitive probe for evaluating the absorption, conversion, transfer, and distribution of light energy within plants. Under stress conditions, damage to the PSII reaction center can occur, leading to the inhibition of photosynthetic electron transport and PSII photosynthetic activity, thereby impeding photosynthesis and reducing photosynthetic rates.

Currently, a systematic investigation into the differential impacts of single nutrient deficiencies (N, P, and K) on the photosynthetic physiology of wheat is lacking. This study simulated different nutrient deficiency conditions to analyze the response characteristics of agronomic traits and chlorophyll fluorescence parameters of wheat, thereby providing a scientific basis for nutrient management and stress resistance regulation of wheat.

2 Materials and Methods

2.1 Site Description

The field experiment was conducted in Guan County, Shandong, China, which is located in a temperate monsoon region with a semi-arid continental climate. The average annual temperature in the county is 13.1°C, and the average annual precipitation over many years is 576.4mm. Data were collected in the middle and late March. At the time of measurement, the atmospheric temperature was approximately 24°C, the atmospheric humidity was about 40%, the light intensity was 70,000 to 80,000lux, and the wind speed was approximately 2.5m/s.

2.2 Experimental Design

The wheat variety Xinmai 296 as the experimental material. The experiment was set up with four treatments (Table 1), each with three replicates, and each replicate was $100m^2$. The application amounts and methods of various fertilizers are shown in Table 1.

Table 1. Fertilization design for different treatments.

Treatments		Preseeding fertilization (kg/ 667m^2)			Additional fertilization (kg/ 667m^2)
		Urea	Calcium superphosphate	KCl	Urea
Nitrogen-deficient	ND	0	2	1.2	0
Phosphorus-deficient	PD	2.3	0	1.2	2.3
Potassium-deficient	KD	2.3	2	0	2.3
Full fertilizer application	FFA	2.3	2	1.2	2.3

2.3 Determination Indicators and Methods

The chlorophyll, plant height, leaf width and chlorophyll fluorescence parameters of 5 wheat plants were determined randomly in each plot. Chlorophyll was determined by SPAD-502 PLUS (Konica-Minolta). Chlorophyll fluorescence was measured by Yaxin-1162 chlorophyll fluorometer. One leaf per plant was selected for 20-minute dark adaptation, and its initial fluorescence (F_0), maximal fluorescence yield (F_m), and maximum photochemical efficiency (F_v/F_m) were determined under 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ saturation pluse.

2.4 Data Processing

SPSS 27.0 was used to conduct one-way ANOVA for different fertilization treatments ($P<0.05$), and least significant difference method (LSD) was used to compare the significance of mean differences among treatments. The results were expressed as "mean ± standard deviation" and origin 2021 was used for mapping.

3 Results and Analysis

3.1 Effects of Nitrogen, Phosphorus and Potassium Deficiency Stress on Wheat Agronomic Traits

Table 2 shows the effects of each treatment on wheat agronomic traits. Chlorophyll is the organelle of photosynthesis in plants, and the photosynthetic rate(Pn) increases with the increase of chlorophyll content in a certain range. The results in Table 2 showed that The chlorophyll content of PD and FFA treatments was much higher than that of ND and KD treatments. Compared with treatment FFA (SPAD, 56.18), the chlorophyll content in ND decreased by 11% ($P<0.05$), and KD decreased by 8.8% ($P<0.05$). Nitrogen is one of the important components of chlorophyll, and nitrogen deficiency will seriously affect the synthesis of chlorophyll. The results of this experiment also prove this theory.

There were also some differences in plant height and leaf width between nutrient deficiency treatments. Compared with FFA treatment (17.79cm), ND had the lowest plant height (16.16cm, 9.16% lower than FFA), but KD and PD had a slight increase in plant height(3.09% and 0.11%, respectively). Nitrogen deficiency had the most significant effect on wheat plant height. The increase of plant height under K deficiency treatment indicates that mild K deficiency may promote stem elongation[2].

There was no significant difference in leaf width under each treatment. Among them, the rate of treatment PD (9.82cm) is higher than that of FFA (9.46cm), and the leaf width of N deficiency treatment is the lowest (9.06cm).

The density of wheat under nutrient deficiency treatments were lower than that under FFA treatment ($33.25/m^{2}$). ND treatment decreased by 2.25%, PD decreased by 10.28% and KD decreased by 11.28% compared with FFA treatment. Table 2 shows that phosphorus and potassium deficiency have significant difference on wheat density, while nitrogen deficiency has little effect on wheat density.

Table 2. Effects of Nitrogen, Phosphorus and Potassium Deficiency Stress on Agronomic Traits of Wheat.

Treatment	SPAD	Plant height/cm	Blade width/cm	density/plant/m^2
ND	50.07±8.70b	16.16±0.96b	9.06±0.56a	32.50±7.14
PD	56.47±3.27a	17.81±0.58a	9.82±0.68a	29.83±6.85
KD	51.19±6.32ab	18.26±0.98a	9.1±0.63a	29.50±2.07
FFA	56.18±3.50a	17.79±1.48a	9.46±0.54a	33.25±6.18

Note: Different lowercase letters in the same column indicate significant differences between treatments($P<0.05$).

3.2 Effects of nitrogen, phosphorus and potassium deficiency stress on chlorophyll fluorescence parameters of wheat

3.2.1 F_0.

Figure 1 shows the comparison of F_0 parameters in wheat under different treatments. As shown in Fig.1, the value of F_0 parameters was in the order of ND > FFA > PD > KD, and F_0 of ND was significantly higher than that in the other three treatments. Compared with FFA treatment, F_0 in nitrogen deficiency treatment was increased by 69.3%. PD and KD treatments decreased by 13% and 18.49%, respectively. F_0 is the fluorescence yield when the PSII reaction center is completely open and all non-photochemical processes are at the minimum. The destruction or reversible inactivation of the PII photochemical reaction center can cause the increase of F_0, and the degree of change can be used to identify the different resistance tolerance of plants. F_0 in the N deficient treatment was significantly higher than that in FFA, indicating that the activity of PSII reaction center decreased, the electron transport efficiency decreased, and the initial fluorescence intensity increased due to N deficiency.

3.2.2 F_m.

Fig. 2 shows the comparison of F_m parameters in wheat under different treatments. As shown in Fig.2, the value of the F_m was in the order of FFA > ND > KD > PD. There was no significant difference of F_m between FFA and ND treatment, while there was a great difference in PD and KD treatments, among which the difference was greatest in phosphorus deficiency treatment. Compared with FFA treatment, F_m in ND, PD and KD treatments were decreased by 0.56%, 34.8% and 30.3%, respectively. F_m is the fluorescence output when the PSII reaction center is completely closed, which can reflect the electron transfer through PSII. The reduction of F_m can be used as a feature of photoinhibition, the larger the value, the more electrons transferred to PSII, and the final photosynthetically produced products are also increased. The results of Fig.2 showed that phosphorus and potassium deficiency had more obvious effects on the maximum fluorescence yield of wheat than nitrogen deficiency.

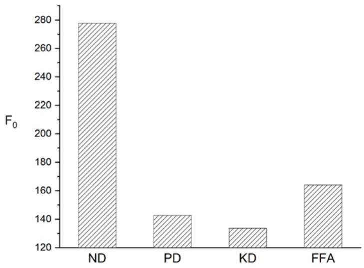

Fig. 1. Comparison of F_0 Parameters of Wheat under Different Treatments

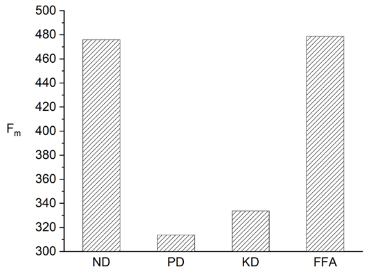

Fig. 2. Comparison of F_m Parameters of Wheat under Different Treatments

3.2.3 F_v/F_m.

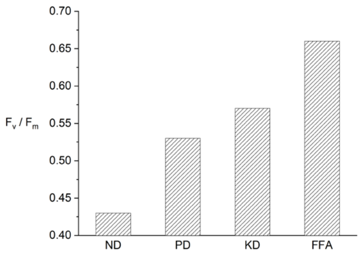

Fig. 3. Comparison of F_v/F_m Parameters of Wheat under different treatments

Fig. 3 shows the comparison of F_v/F_m parameters in wheat under different treatments. As shown in Fig.3, the value of the F_v/F_m parameter was in the order of FFA > KD > PD > ND. The value of F_v/F_m in N deficiency treatment, P deficiency treatment and K deficiency treatment decreased by 34.85%, 19.7% and 13.63%, respectively. The decrease of F_v/F_m in ND treatment was most obvious. F_v/F_m refers to the maximum photochemical efficiency when all reaction centers are open after dark adaptation, and the change of PSII maximum light energy conversion efficiency F_v/F_m is the most widely studied index of light inhibition, and it is an important parameter related to the dark adaptation state of plants, which can be used to detect light inhibition. The fluorescence parameter F_v/F_m of all the wheat treated with nutrient deficiency was lower than that of FFA treatment, indicating that due to the lack of elements, the activity of PSII was reduced, the photochemical quenching efficiency was reduced, the proportion of reaction center opening was reduced, PSII was seriously damaged, and the ability of electron transfer was relatively reduced. The results showed that the maximum photochemical efficiency of wheat was significantly inhibited under nitrogen deficiency, and the inhibition degree of phosphorus deficiency and potassium deficiency was less.

4 Discussion

Nitrogen, phosphorus and potassium are the core elements of plant metabolic network, and their deficiency stress interferes with the photosynthetic efficiency and growth of wheat through multiple mechanisms. Nitrogen is directly or indirectly involved in photosynthesis, such as the formation of chlorophyll molecules, the formation of ribulose 1, 5-diphosphate carboxylase/oxygenase (Rubisco), and the formation of various enzyme proteins. Nitrogen plays an important role in plant growth and development. Under nitrogen deficiency treatment, wheat chlorophyll synthesis is inhibited, the activity of PSII reaction center is decreased, the photosynthetic organs of plants absorb, transform and dissipate light energy and capture the primary quinone acceptor (QA) energy less, and the photosynthetic efficiency is reduced[3]. It can be seen from Table 1 that different treatments for nutrient deficiency were carried out during wheat fertilization. In the absence of a certain nutrient, crops can only use the soil's natural nutrient reserves. The results of this study showed that nitrogen deficiency had the most significant inhibitory effect on the photosynthetic system of wheat. In the case of nitrogen deficiency, chlorophyll content decreased significantly by 11% and F_v/F_m value decreased by 34.85%. This indicates that nitrogen loss directly destroys the structural stability of PSII reaction center, so increasing nitrogen application can reduce the damage caused by excessive excitation energy to the photosynthetic apparatus to a certain extent, and keep the maximum photochemical efficiency of PSII at a high level.

The chlorophyll content of PD treatment was slightly higher than that of FFA treatment, which may be due to the dynamic regulation of P on carbon metabolism. Phosphorus is a key component of ATP, NADPH and phospholipids. Under phosphorus deficiency stress, plants enhance C4 metabolic pathway to maintain chloroplast CO_2 concentration by up-regulating phosphoenolpyruvate carboxylase (PEPC) activity, thus temporarily delaying chlorophyll degradation[4]. The results of this study showed that F_0, F_m and F_v/F_m values of wheat decreased significantly under the condition of P deficiency, and some studies were consistent with the results of this experiment. It indicated that the photochemical efficiency of PII reaction center under the condition of P deficiency was greatly affected by phosphorus. Studies have also shown that there is a significant correlation between phosphorus deficiency and photosynthetic fluorescence characteristics during the growth and development of plants, and phosphorus deficiency will lead to the reduction of Fm, ΦPSII, Fv/Fm and electron transfer rate (ETR), thus affecting the photosynthetic rate.

As a necessary mineral element for plant growth and development, potassium plays an important role in maintaining cell osmotic pressure, regulating stomatal movement, guaranteeing enzyme activity, increasing photosynthesis, promoting the transport of sugar in the body and increasing yield[5]. Under potassium deficiency treatment, wheat seedling showed thinness and short stature, but the plant height of the potassium deficiency group increased by 3.09%, which may be related to the fact that wheat was at the seedling stage. Wang's experiment suggested that potassium deficiency did not significantly inhibit plant height during the vegetative growth stage, which may be related to the preferential distribution of potassium by plants to new organs[2]. Potassium deficiency resulted in a significant decrease in density of 11.28%, which may indicate that the tillering ability of wheat was weakened under potassium deficiency. In this study, the F_v/F_m value in the potassium-deficient treatment was reduced by 13.63%, which was consistent with the findings of Huang et al. in potassium-deficient walnuts[6]. The lack of potassium resulted in the decrease of the content of Rubisco and the activated enzyme of Rubisco, and the CO_2 fixation was lower than the maximum capacity of Rubisco, which restricted the increase of net photosynthetic rate and biological yield.

In addition, the density of the three nutrient deficiency treatments were lower than that of FFA, suggesting that nutrient stress may regulate the population structure by changing the source-sink relationship. Under nitrogen deficiency conditions, plants preferentially allocate resources to roots to enhance nutrient uptake, resulting in a decrease in aboveground biomass[7], while phosphorus and potassium deficiency may restrict the development of tillers by reducing the output efficiency of photosynthetic products.

5 Conclusion

This study systematically revealed the differential effects of N, P and K deficiency stress on wheat agronomic traits and photosynthetic physiology. Nitrogen deficiency had the most significant inhibitory effect on wheat, followed by potassium deficiency, and phosphorus deficiency showed a complex effect. In addition, all nutrient deficiency treatments resulted in a decrease in population density. In conclusion, nitrogen supply plays a key role in wheat high-yield cultivation, followed by potassium and phosphorus. The results of this study provide theoretical basis for optimizing fertilization strategies and stress regulation, and emphasize the importance of precise nutrient management for maintaining photosynthetic efficiency and population structure stability.

Acknowledgement

This research were funded by y Central Guiding Local Science and Technology Development Special Project (YDZX2023013); Educational innovation subject in Shandong Agriculture and Engineering University (22XJKTY08, 23XJSZZ01, 24XJSZZ02).

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