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Effects of Phosphate Fertilizer Application on the Growth and Yield of Tartary Buckwheat under Low-Nitrogen Condition

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Abstract: This study aimed to clarify the effect of phosphorus fertilizer on the senescence and yield of Tartary buckwheat under low-nitrogen treatment. A two-year field experiment to investigate the characteristics was conducted on Tartary buckwheat (Qianku 5) under four phosphorus fertilizer application rates, 0(CK), 40(LP), 80(MP), and 120 kg·ha^{-1} (HP), in the absence of nitrogen treatment. Compared with CK, MP treatment increased the plant height, node number of main stem, branch number of main stem, root-morphology items, root activity, enzyme activity related to root nitrogen metabolism, leaf chlorophyll content, and antioxidant enzyme activity by an average of 27.82%, 36.00%, 31.76%, 70.63%, 103.16%, 45.63%, 19.42%, and 45.48%, respectively. MP treatment significantly decreased the malondialdehyde content by 23.54% compared with that of CK. Among all treatments, the HP treatment had the highest content. The grain number per plant, grain weight per plant, and yield under MP treatment were 1.54, 1.65, and 1.53 times those of CK, respectively. In summary, the appropriate phosphate fertilizer treatment (80 kg·ha^{-1}) can delay senescence, promote the growth, and increase the yield of Tartary buckwheat at low nitrogen levels. Such treatment is recommended for use in production to jointly achieve the high yield and high nitrogen conservation of Tartary buckwheat.

Keywords: Tartary buckwheat; phosphate fertilizer; low nitrogen levels; senescence; yield

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1. Introduction

Buckwheat belongs to *Fagopyrum* Mill of family Polygonaceae, and it is extensively distributed throughout the world. Buckwheat originated from China and has a long history of cultivation. It has gradually formed the northern buckwheat planting area represented by Inner Mongolia, Shaanxi, and Shanxi and the southern buckwheat planting area represented by Yunnan, Guizhou, and Sichuan [1]. Tartary buckwheat grains are rich in flavonoids, D-chiral inositol, and dietary fiber. It can lower blood pressure, blood lipid, and blood glucose. Tartary buckwheat also has unique health care value applications because of its anti-oxidation, anti-inflammation, and anti-tumor activities [2,3]. As an important staple food in China [1], it has great development value and market prospects.

Guizhou Province is one of the main areas producing Tartary buckwheat in China. Based on the traditional concept of low fertilizer tolerance of Tartary buckwheat, farmers often do not apply fertilizer when planting Tartary buckwheat for a long time, resulting in low yield. In recent years, farmers have realized the importance of fertilization in the yield of Tartary buckwheat, thereby emphasizing the application of chemical fertilizers, especially nitrogen fertilizer [4]. The yield of Tartary buckwheat improves to a certain extent compared with that when fertilizers are not used. The excessive application of nitrogen fertilizer causes low nitrogen-use efficiency and low economic benefits, as well as serious agricultural environmental problems [5,6]. An appropriate reduction of nitrogen fertilizer can increase the grain weight and final yield of Tartary buckwheat [4]. Jiang et al. (2022)

found that reducing the application of nitrogen fertilizer by 25% significantly reduces maize yield and improves the nitrogen-use efficiency [7]. Some scholars also believe that reducing the nitrogen application rate from 300 kg·ha^{-1} to approximately 225 kg·ha^{-1} can simultaneously increase the yield and nitrogen-use efficiency of wheat [8]. Therefore, the appropriate reduction of nitrogen fertilizer can jointly achieve fertilizer conservation and high yield.

As one of the three nutrient elements of plants, phosphorus fertilizer is in a special position. It is a component of macromolecular substances and various important compounds, and it participates in plant metabolism [9]. It is also one of the most important factors affecting crop yield [10]. Xue et al. (2020) found that the yield of maize increases with increased phosphate fertilizer application rate without nitrogen fertilizer, but it is lower than that under nitrogen fertilizer treatment [11]. Our previous studies on common buckwheat support the finding that when the application of nitrogen fertilizer is reduced, the yield of common buckwheat does not significantly differ from that of conventional nitrogen application by appropriately increasing the application of phosphorus fertilizer [12]. This condition may be associated with the low demand of common buckwheat for fertilizer. However, previous studies have observed this phenomenon only from the perspective of cultivation measures and have not analyzed the physiological mechanism of this phenomenon. In accordance with the result of Tartary buckwheat with lower fertilizer demand than common buckwheat [13], the current study hypothesized that a certain application of phosphorus fertilizer to replace nitrogen fertilizer may increase the yield of Tartary buckwheat by delaying senescence and promoting growth. However, relevant studies focusing on whether stable yield and nitrogen conservation of Tartary buckwheat can be jointly achieved by "regulating nitrogen with phosphorus" during production are lacking. In the present work, a large plantation area cultivar of Tartary buckwheat in Guizhou Province, named Qianku 5, was used as test material. It was treated with different phosphorus fertilizer applications, and the effects on the growth and yield of Tartary buckwheat in the absence of nitrogen treatment were analyzed. The results can serve as a theoretical basis to jointly achieve the high yield and high nitrogen conservation of Tartary buckwheat.

2. Materials and Methods

2.1. Plant Materials

The Tartary buckwheat variety Qianku 5, which has a large plantation area in Guizhou province, was used as the experimental material. Qianku 5 has excellent characteristics, such as high yield, disease and pest resistance, and lodging resistance. The recommended nutrient requirements for this variety were 135 kg·ha^{-1} nitrogen fertilizer, 70 kg·ha^{-1} phosphate fertilizer, and 5.0 kg·ha^{-1} potassium fertilizer. Its average yield in Guiyang, Guizhou Province was approximately 1230 kg·ha^{-1} [14].

2.2. Treatment

The experiment was performed in Xiaba's Cultivation Experimental Station of Guizhou Normal University, Guiyang City, Guizhou Province, China (1250 m, 106.95° E, 26.72° N) from 2021 to 2022. The soil in the test site is yellow loam. The soil used was yellow loam, and the nutrient contents of the shallow tillage layer (0–20 cm) of the test site are as follows: available nitrogen, 17.42 mg·kg^{-1}; available phosphorus, 40.01 mg·kg^{-1}; available potassium, 59.61 mg·kg^{-1}; and organic matter, 7.07 g·kg$^{-1.}$

The experiment was conducted using a single-factor randomized block design with three replicates. The area for each test plot had dimensions of 2 m × 5 m, and the plots were separated by a 25 cm-wide gap that was wrapped with agricultural film (polyethylene, 0.06 mm thick) to prevent water and fertilizer from mixing. In the absence of nitrogen fertilizer, the following four phosphate fertilizer (calcium superphosphate, containing 14% P_2O_5) treatments were established: 0 kg·ha^{-1} (CK), 40 kg·ha^{-1} (LP), 80 kg·ha^{-1} (MP), and 120 kg·ha^{-1} (HP). Potassium fertilizer (potassium chloride, containing 60% K_2O) was applied at the optimum local dosage of 5.0 kg·ha^{-1}. The two fertilizers were mixed and

applied once as base fertilizer, and no fertilizer was applied throughout the entire growth period. Seeds were sown in the plot on 21 March 2021 and 18 March 2022. The row spacing and seeding amounts were 0.33 m and 37.5 g·m^{-2}, and approximately 90–100 reserved plants were available for each square meter. Tartary buckwheat seeds were harvested on 25 June 2021 and 24 June 2022 (70% of the seeds matured). Artificial irrigation was performed according to the principle of "extreme drought and thoroughly irrigated", and other field-management and pest-control strategies were consistent with local high-yield cultivation.

2.3. Sampling

At the seedling, flowering, grain-filling, and maturity stages, 20 Tartary buckwheat plants with uniform growth were randomly excavated from each treatment plot. During excavation, the root system was excavated as completely as possible, and the roots were washed with running water. After filtering the water, the roots were cut off, from which five plants were used to determine the root-morphology index. The roots and leaves on 4–6 nodes (from top to bottom, main stem) of the 15 remaining plants were treated with liquid nitrogen for 30 s and then stored in a refrigerator at −80 °C. The leaves were used to determine chlorophyll and malondialdehyde (MDA) content and antioxidant enzyme activity, and the roots were used to determine nitrogen metabolism-related enzyme activity.

2.4. Determination

2.4.1. Agronomic Characters and Yield

According to the method of Zhang et al. (2021), the plant height, main stem node number, main stem branch number, grain number per plant, and grain weight per plant of Tartary buckwheat at the mature stage were determined [1]. In the middle of each treatment plot, the grains on all Tartary buckwheat plants within the 1 m^2 area (randomly selected, not sampled during the experimental process, excluding border plants) were used to determine the yield after air drying.

2.4.2. Root Morphology and Root Activity

As described by Zhang et al. (2021), a root-scanning analysis system (GXY-A, Zhejiang Tuopu Instrument Co., Ltd., Hangzhou, China) was used to determine the total root length, root surface area, and root volume [1]. Root activity was determined using the 2,3,5-triphenyl-tetrazolium chloride method [15].

2.4.3. Root Nitrogen Metabolism-Related Enzymes

The activities of glutamine synthetase (GS) and glutamate dehydrogenase (GDH) in roots were determined as described by Magalhaes and Huber (1991) [16]. Glutamate synthase (GOGAT) was determined according to the method of Singh and Srivastava (1986) [17].

2.4.4. Chlorophyll Content in Leaves

The contents of chlorophyll a, chlorophyll b, and carotenoids in leaves were determined by colorimetry [15].

2.4.5. Antioxidant Enzyme Activity and MDA Content

The activity of superoxide dismutase (SOD) was evaluated by its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT), as proposed by Li (2000) [15]. Measurements were made at 560 nm, and one unit of SOD was deemed to correspond with the amount of enzyme capable of inhibiting 50% of NBT photoreduction under the experimental conditions. Peroxidase (POD) activity was determined by guaiacol oxidation at 470 nm [15]. Catalase (CAT) activity was determined by potassium permanganate titration. About 2.5 g of sample was weighed and used to prepare a crude extract of the enzyme, H_2O_2 and H_2SO_4 were added, and the mixture was titrated with $KMnO_4$. Enzyme activity

was expressed by the milligrams of mg of H_2O_2 decomposed per gram of fresh sample within 1 min [15]. MDA content was determined using the thiobarbituric acid method [15]. About 1.0 g of sample was weighed, 2 mL of 10% trichloroacetic acid (TCA) was added, and grinding was performed until homogenization. After adding 8 mL of TCA for further grinding, the homogenate was centrifuged, 2 mL of the supernatant was collected, and 2 mL of 0.6% thiobarbiturate was added. After mixing, the mixture was reacted in a boiling water bath for 15 min and then centrifuged after rapid cooling. Absorbance was measured at 532, 600, and 450 nm.

2.5. Statistical Analysis

Microsoft Excel 2010 and SPSS 22.0 were used for data processing. One-way ANOVA was performed, and means were compared using the least significant difference at the 0.05 probability level. No significant difference existed between 2021 and 2022. Therefore, the data were presented as the average across the two study years, and the data of 2021 and 2022 were deposited as Supplementary Data.

3. Results

3.1. Effects of Phosphorus Fertilizer Application on Agronomic Traits and Yield under Low-Nitrogen Treatment

The plant height, number of main stem nodes, number of main stem branches, number of grains per plant, weight of grains per plant, and yield under MP treatment were remarkably higher than those under other treatments, and the HP treatment was the lowest (Table 1). Compared with CK, MP treatment increased the grain number per plant, grain weight per plant, and yield by 54.18%, 64.71%, and 53.24%, respectively.

Table 1. Effects of different phosphorus fertilizer application on the yield of Tartary buckwheat.

Treatment	Plant Height (cm)	Main Stem Node Number	Number of Main Stem Branches	Grain Number per Plant	Grain Weight per Plant (g)	Yield ($kg \cdot ha^{-1}$)
CK	97.4 ± 2.7 c	12.5 ± 1.4 b	8.5 ± 0.9 b	106.5 ± 5.9 c	1.7 ± 0.11 c	795.4 ± 19.7 c
LP	103.7 ± 3.2 b	14.0 ± 2.4 b	9.2 ± 0.8 b	135.2 ± 6.5 b	2.2 ± 0.18 b	961.7 ± 27.2 b
MP	124.5 ± 8.5 a	17.0 ± 1.6 a	11.2 ± 1.5 a	164.2 ± 9.2 a	2.8 ± 0.18 a	1218.9 ± 58.8 a
HP	96.0 ± 2.9 c	13.3 ± 1.5 b	6.8 ± 0.5 c	84.8 ± 5.0 d	1.5 ± 0.12 d	670.1 ± 19.9 d

Note: Data are presented as mean ± standard error of the mean. Small letter in the same column means significant difference at $p < 0.05$. CK: phosphorus fertilizer application was 0 $kg \cdot ha^{-1}$; LP: phosphorus fertilizer application was 40 $kg \cdot ha^{-1}$; MP: phosphorus fertilizer application was 80 $kg \cdot ha^{-1}$; HP: phosphorus fertilizer application was 120 $kg \cdot ha^{-1}$.

3.2. Effects of Phosphorus Fertilizer Application on Root Morphology and Root Activity under Low-Nitrogen Treatment

The total root length, root surface area, and root volume increased continuously with prolonged growth period and reached the maximum at maturity period (Table 2). Root activity initially increased and then decreased with prolonged growth period. Compared with CK, MP treatment increased the total root length, root surface area, root volume, and root activity by an average of 49.20%, 57.23%, 105.47%, and 103.16%, respectively.

3.3. Effects of Phosphorus Fertilizer Application on Enzymes Related to Root Nitrogen Metabolism under Low-Nitrogen Treatment

The GS, GOGAT, and GDH activities of roots initially increased and then decreased with prolonged growth period (Table 3). The activities of GS and GDH reached the maximum at the grain-filling stage, and the activity of GOGAT reached the maximum at the flowering stage. Comparison with the CK, MP treatment increased the GS, GOGAT, and GDH activities by an average of 43.22%, 64.68%, and 29.00%, respectively.

Table 2. Effects of different phosphorus fertilizer application on root morphology and root activity of Tartary buckwheat.

Item	Treatment	Period			
		Seedling Stage	**Flowering Stage**	**Filling Stage**	**Mature Stage**
Root length (cm)	CK	41.54 ± 2.49 bc	72.76 ± 3.25 c	121.70 ± 4.91 b	171.85 ± 6.91 b
	LP	47.52 ± 2.71 b	118.31 ± 3.86 b	164.35 ± 7.24 a	228.50 ± 12.05 a
	MP	55.88 ± 3.53 a	142.81 ± 7.18 a	175.28 ± 8.23 a	234.54 ± 8.94 a
	HP	38.92 ± 2.90 c	55.88 ± 2.73 d	87.56 ± 6.00 c	130.88 ± 5.46 c
Surface area (cm^2)	CK	8.88 ± 0.28 b	13.95 ± 2.55 b	21.94 ± 1.87 b	37.74 ± 2.32 c
	LP	9.73 ± 0.59 ab	19.76 ± 2.00 a	33.29 ± 2.24 a	50.84 ± 2.57 b
	MP	9.97 ± 0.34 a	21.71 ± 2.77 a	37.59 ± 1.90 a	60.46 ± 2.10 a
	HP	7.10 ± 0.41 c	9.92 ± 0.81 c	17.16 ± 1.87 c	29.81 ± 1.57 d
Volume (cm^3)	CK	0.27 ± 0.03 bc	0.54 ± 0.03 c	0.72 ± 0.07 b	1.76 ± 0.15 b
	LP	0.30 ± 0.03 b	0.78 ± 0.06 b	1.32 ± 0.12 a	3.74 ± 0.28 a
	MP	0.38 ± 0.05 a	0.90 ± 0.05 a	1.36 ± 0.08 a	4.12 ± 0.13 a
	HP	0.22 ± 0.03 c	0.35 ± 0.04 d	0.77 ± 0.11 b	1.44 ± 0.20 b
Root activity ($\mu g \cdot g^{-1} \cdot h^{-1}$)	CK	27.34 ± 1.33 b	55.67 ± 3.51 c	79.09 ± 3.76 c	28.76 ± 1.90 c
	LP	51.11 ± 2.56 a	90.23 ± 4.95 b	92.68 ± 5.31 b	38.03 ± 2.31 b
	MP	58.10 ± 3.76 a	122.79 ± 7.69 a	148.88 ± 8.29 a	57.98 ± 3.47 a
	HP	21.32 ± 1.69 b	49.88 ± 1.84 c	53.30 ± 3.45 d	15.85 ± 1.29 d

Note: Data are presented as mean ± standard error of the mean. Small letter in the same column means significant difference at $p < 0.05$.CK: phosphorus fertilizer application was 0 $kg \cdot ha^{-1}$; LP: phosphorus fertilizer application was 40 $kg \cdot ha^{-1}$; MP: phosphorus fertilizer application was 80 $kg \cdot ha^{-1}$; HP: phosphorus fertilizer application was 120 $kg \cdot ha^{-1}$.

Table 3. Effects of different phosphorus fertilizer application on root nitrogen metabolism related enzymes of Tartary buckwheat.

Item	Treatment	Period			
		Seedling Stage	**Flowering Stage**	**Filling Stage**	**Mature Stage**
Synthetase (GS, $U \cdot g^{-1} \cdot h^{-1}$)	CK	1.67 ± 0.10 b	2.02 ± 0.18 b	2.14 ± 0.16 c	1.62 ± 0.16 bc
	LP	1.89 ± 0.15 b	2.14 ± 0.24 b	2.43 ± 0.28 b	1.78 ± 0.16 b
	MP	2.22 ± 0.19 a	2.54 ± 0.28 a	3.77 ± 0.21 a	2.14 ± 0.19 a
	HP	1.28 ± 0.12 c	1.71 ± 0.16 c	1.92 ± 0.18 c	1.50 ± 0.19 c
Glutamate synthase (GOGAT, $U \cdot g^{-1} \cdot h^{-1}$)	CK	6.28 ± 0.24 b	18.27 ± 1.51 b	9.79 ± 0.79 c	7.02 ± 0.57 b
	LP	7.75 ± 0.37 b	19.62 ± 1.76 b	12.00 ± 1.77 b	8.65 ± 0.66 b
	MP	9.41 ± 0.73 a	28.61 ± 2.60 a	19.93 ± 1.86 a	10.16 ± 1.06 a
	HP	3.93 ± 0.42 c	12.37 ± 1.46 c	5.91 ± 0.56 d	3.88 ± 0.36 c
Glutamate dehydrogenase (GDH, $U \cdot g^{-1} \cdot h^{-1}$)	CK	4.98 ± 0.35 c	5.93 ± 0.31 c	10.14 ± 1.13 b	1.02 ± 0.09 c
	LP	5.66 ± 0.23 b	6.53 ± 0.25 b	11.13 ± 0.92 a	1.27 ± 0.10 b
	MP	6.54 ± 0.41 a	8.25 ± 0.34 a	11.80 ± 0.88 a	1.88 ± 0.15 a
	HP	4.52 ± 0.23 d	5.10 ± 0.22 d	8.81 ± 0.53 c	0.88 ± 0.07 d

Note: Data are presented as mean ± standard error of the mean. Small letter in the same column means significant difference at $p < 0.05$.CK: phosphorus fertilizer application was 0 $kg \cdot ha^{-1}$; LP: phosphorus fertilizer application was 40 $kg \cdot ha^{-1}$; MP: phosphorus fertilizer application was 80 $kg \cdot ha^{-1}$; HP: phosphorus fertilizer application was 120 $kg \cdot ha^{-1}$.

3.4. Effects of Phosphorus Fertilizer Application on Chlorophyll Content under Low-Nitrogen Treatment

The contents of chlorophyll a, chlorophyll b, and carotenoids in leaves initially increased and then decreased with prolonged growth period and reached the maximum at the flowering stage (Figure 1). Compared with CK, MP treatment increased the content of chlorophyll a, chlorophyll b, and carotenoids by an average of 15.24%, 22.54%, and 20.48%, respectively.

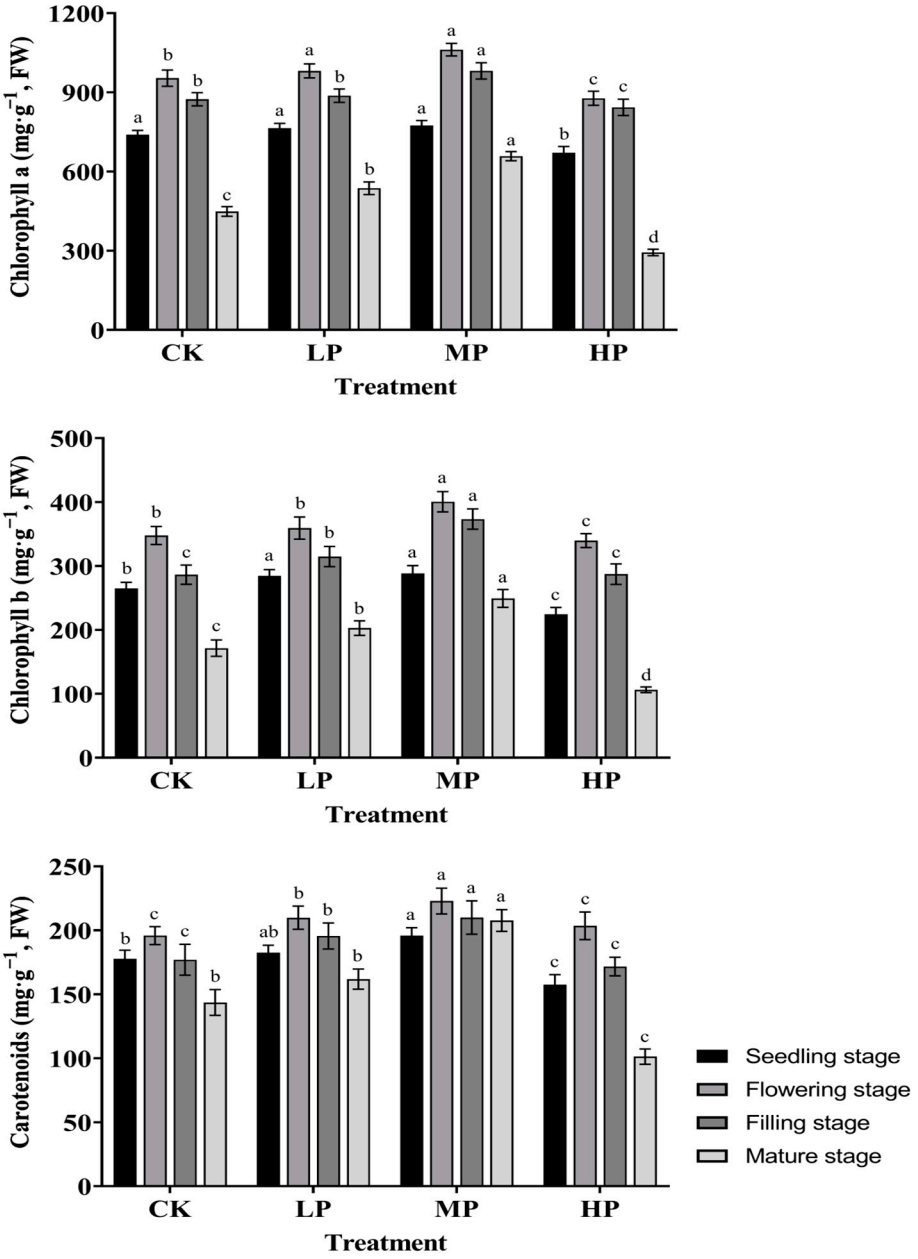


Figure 1. Effects of different phosphorus fertilizer application on chlorophyll content of Tartary buckwheat. Note: Data are presented as mean ± standard error of the mean. Small letter in the same column means significant difference at $p < 0.05$. CK: phosphorus fertilizer application was 0 kg·ha^{-1}; LP: phosphorus fertilizer application was 40 kg·ha^{-1}; MP: phosphorus fertilizer application was 80 kg·ha^{-1}; HP: phosphorus fertilizer application was 120 kg·ha^{-1}. FW: fresh weight.

3.5. Effects of Phosphorus Fertilizer Application on Antioxidant Enzyme Activity and MDA Content under Low-Nitrogen Treatment

The activities of SOD, POD, and CAT in leaves initially increased and then decreased with prolonged growth period (Table 4). The content of MDA increased continuously with prolonged growth period. Compared with CK, MP treatment increased the activities of SOD, POD, and CAT by an average of 18.40%, 73.93%, and 44.11%, respectively. MP treatment significantly decreased the MDA content by 23.54% compared with that of CK.

Table 4. Effects of different phosphorus fertilizer treatments on antioxidant enzyme activity and MDA content of Tartary buckwheat.

Item	Treatment	Period			
		Seedling Stage	Flowering Stage	Filling Stage	Mature Stage
Superoxide dismutase (SOD, U·g^{-1}·h^{-1})	CK	99.4 ± 3.7 c	250.8 ± 8.4 c	232.1 ± 11.5 b	195.4 ± 6.7 b
	LP	128.8 ± 5.5 b	259.7 ± 9.5 b	253.2 ± 12.9 a	208.3 ± 8.5 b
	MP	160.5 ± 8.4 a	275.1 ± 10.8 a	260.9 ± 11.2 a	224.3 ± 10.8 a
	HP	78.0 ± 3.4 d	230.8 ± 9.5 d	209.1 ± 11.6 c	174.2 ± 8.8 c
Peroxidase (POD, U·g^{-1}·h^{-1})	CK	415.6 ± 9.9 c	619.6 ± 16.2 b	1092.0 ± 25.5 c	884.5 ± 17.3 c
	LP	489.6 ± 15.8 b	639.8 ± 15.7 b	1553.7 ± 29.9 b	1339.0 ± 22.0 b
	MP	614.8 ± 15.3 a	758.6 ± 16.8 a	2289.6 ± 29.3 a	1575.4 ± 27.4 a
	HP	367.3 ± 10.7 d	543.7 ± 13.5 c	858.1 ± 31.0 d	659.4 ± 18.1 d
Catalase (CAT, U·g^{-1}·h^{-1})	CK	1390.2 ± 35.7 c	2683.4 ± 46.3 c	4473.4 ± 54.7 c	2773.4 ± 54.0 c
	LP	1520.3 ± 40.5 b	3573.4 ± 39.6 b	5986.8 ± 58.7 b	3846.7 ± 54.3 b
	MP	2033.7 ± 39.5 a	3766.8 ± 42.5 a	6466.8 ± 45.9 a	4046.7 ± 51.0 a
	HP	1106.9 ± 41.3 d	1906.7 ± 52.1 d	2906.7 ± 60.0 d	1280.0 ± 32.0 d
Malondialdehyde (MDA, μmol·g^{-1})	CK	2.23 ± 0.14 b	3.08 ± 0.14 b	6.35 ± 0.13 b	6.52 ± 0.15 b
	LP	1.82 ± 0.13 c	2.43 ± 0.14 c	5.86 ± 0.20 bc	6.25 ± 0.19 b
	MP	0.94 ± 0.11 d	1.96 ± 0.11 d	5.32 ± 0.14 c	5.68 ± 0.19 c
	HP	2.65 ± 0.12 a	3.78 ± 0.13 a	6.73 ± 0.15 a	8.06 ± 0.26 a

Note: Data are presented as mean ± standard error of the mean. Small letter in the same column means significant difference at $p < 0.05$.CK: phosphorus fertilizer application was 0 kg·ha^{-1}; LP: phosphorus fertilizer application was 40 kg·ha^{-1}; MP: phosphorus fertilizer application was 80 kg·ha^{-1}; HP: phosphorus fertilizer application was 120 kg·ha^{-1}.

3.6. Correlations of Items and Yield

A significant positive correlation existed between yield and root activity and synthetase (Table 5). A significant positive correlation existed between yield and plant height, main stem node number, root surface area, glutamate dehydrogenase activity, chlorophyll content, and SOD and POD activities.

Table 5. Correlations of agronomic character, root morphology items, root activity, enzyme activity, leaf chlorophyll content, and yield.

Correlations with	Grain Number per Plant	Grain Weight per Plant	Yield
Plant height	0.922	0.962 *	0.959 *
Main stem node number	0.862	0.924	0.905
Number of main stem branches	0.982 *	0.967 *	0.984 *
Root length	0.953 *	0.91	0.913
Root surface area	0.998 **	0.986 *	0.986 *
Root volume	0.957 *	0.947	0.932
Root activity	0.990 **	0.985 *	0.996 **
Synthetase	0.977 *	0.992 **	0.995 **
Glutamate synthase	0.968 *	0.924	0.943
Glutamate dehydrogenase	0.964 *	0.987 *	0.988 *
Chlorophyll a	0.986 *	0.957 *	0.973 *
Chlorophyll b	0.980 *	0.947	0.966 *
Carotenoids	0.984 *	0.965 *	0.983 *
Superoxide dismutase	0.987 *	0.957 *	0.972 *
Peroxidase	0.992 **	0.977 *	0.975 *
Catalase	0.936	0.878	0.893
Malondialdehyde	−0.917	−0.857	−0.889

Note: *, ** indicate the significant difference at $p < 0.05$ and $p < 0.01$ level, respectively.

4. Discussion

4.1. Phosphate Fertilizer Treatment Delayed the Senescence of Tartary Buckwheat under Low-Nitrogen Treatment

MDA is a product of membrane lipid peroxidation. Excessive MDA content can cause plant metabolic disorders, loss of normal regulatory functions in cells, and even toxic effects on plant cells, leading to plant senescence and death [18]. SOD, POD, and CAT, as important protective enzymes in plants, can scavenge intracellular reactive oxygen species, inhibit MDA production, and alleviate plant premature senescence [19–21]. Senescence reduces the activity of antioxidant enzymes such as SOD in plant leaves. Consequently, the dynamic balance between the production and scavenging of reactive oxygen species in the body is broken, resulting in a large accumulation of reactive oxygen species, membrane damage or destruction, and increased MDA content. Nitrogen is closely correlated with crop growth [22]. Nitrogen deficiency causes the premature senescence of leaves, thereby accelerating the maturation of whole plants and seriously reducing crop yield [23]. The results of the present experiment showed that MP treatment had the highest protective-enzyme activities (SOD, POD, and CAT) and the lowest MDA content. It is suggested that the appropriate phosphorus fertilizer application may have delayed the senescence of Tartary buckwheat caused by nitrogen deficiency, which may explain why the MP treatment had the maximum chlorophyll content (Figure 1). However, the senescence was aggravated with increased phosphorus fertilizer application. This phenomenon may have occurred because the application of high phosphorus caused a nutritional imbalance between phosphate and nitrogen, resulting in accelerated senescence of Tartary buckwheat grown in nitrogen-deficient plots.

4.2. Phosphate Fertilizer Treatment Promoted the Root Growth of Tartary Buckwheat under Low-Nitrogen Treatment

Root attributes are closely correlated with the growth and development of above-ground parts and yield formation [24,25]. Fertilizer conditions in soil can significantly affect root growth and development. Sufficient soil nutrients can improve the nutrient competitiveness of roots in the soil ecological environment. Inappropriate soil nutrients affect the root growth of roots, resulting in a decreased number of roots and decreased competitiveness of roots to nutrients [1,26]. The amount of nitrogen fertilizer is closely correlated with the growth of crop roots, and insufficient nitrogen supply inhibits root growth [27]. In the current study, compared with the CK treatment, the LP and MP treatments promoted the increase in root length, root surface area, root volume, and root activity. The increase in amplitude of MP treatment was greater than that of LP treatment. This finding indicated that the appropriate phosphorus fertilizer application can alleviate the inhibition of root growth of Tartary buckwheat caused by nitrogen deficiency. This phenomenon occurred possibly because the application of appropriate phosphorus led to a nutritional balance between phosphate and nitrogen. Compared with the CK treatment, HP treatment reduced the root length, root surface area, root volume, and root activity. The reason may be that the application of high phosphorus accelerated the senescence of Tartary buckwheat, thereby weakening the photosynthetic capacity above ground and the ability of synthetic assimilates. Thus, few assimilates were transported into the roots, and the growth of Tartary buckwheat roots was inhibited [20]. Meanwhile, a specific threshold value of phosphorus fertilizer application rate can reportedly alleviate nitrogen-deficient stress. If this value were exceeded, the growth of Tartary buckwheat roots would be inhibited.GS, GOGAT, and GDH are enzymes related to nitrogen metabolism, and their activities can measure the level of nitrogen metabolism in crops [28]. The application of appropriate phosphate fertilizer significantly increases the activity of nitrogen metabolism-related enzymes and promotes nitrogen metabolism, whereas excessive phosphate fertilizer inhibits the activity of nitrogen metabolism-related enzymes [29]. In the present study, the activities of GS, GOGAT, and GDH in the roots of Tartary buckwheat initially increased and then decreased with increased phosphorus fertilizer when nitrogen fertilizer was not applied, which was

consistent with the above results [29]. This finding indicates that appropriate phosphorus fertilizer application can alleviate the damage inflicted by nitrogen deficiency to a certain extent. It may be correlated with the increase in nitrogen metabolism-related enzyme activities in the roots of Tartary buckwheat by appropriate phosphate fertilizer treatment, thereby improving the efficiency of ammonium nitrogen assimilation. However, the activity of nitrogen metabolism-related enzymes was inhibited by high phosphorus fertilizer application. This phenomenon may have been due to the application of high phosphorus causing a nutritional imbalance between excessive phosphate and low nitrogen.

4.3. Phosphate Fertilizer Treatment Increased the Yield of Tartary Buckwheat under Low Nitrogen Treatment

Phosphorus amount is closely correlated with crop growth and development [30]. Phosphorus application can significantly increase crop grain weight and yield, but when the amount of phosphorus applied exceeds a certain threshold, the number of grains per plant, grain weight, and the yield decrease [31]. The rational application of nitrogen fertilizer can effectively increase crop yield [32]. Studies on Tartary buckwheat have shown that appropriate nitrogen fertilizer treatment can significantly increase the yield, and that decreasing or increasing the amount of nitrogen fertilizer significantly reduces the yield [33]. Herein, appropriate phosphorus fertilizer application increased the yield under nitrogen-deficient treatment. Combined with the correlation results, we considered that the appropriate phosphorus fertilizer application may have promoted the increase in nitrogen metabolism-related enzyme activity in the roots. It also promoted root growth and increased the absorption of rhizosphere soil nutrients, thereby increasing the aboveground biomass and final yield. Compared with CK, HP treatment decreased the grain number per plant, grain weight per plant, and yield by 20.38%, 11.77% and 15.75%, respectively. This phenomenon occurred possibly because when the phosphorus application rate exceeded the required amount of Tartary buckwheat, the nutrient metabolism became excessive, the respiratory intensity accelerated, the carbohydrate was consumed, and the amount of carbohydrate transported into the sink organ was reduced [31]. The result was a decreased number of grains per plant and grain weight per plant, ultimately affecting the yield of Tartar buckwheat. It may also be correlated with the application of high phosphorus causing a nutritional imbalance between phosphate and nitrogen.

Notably, in our experiment, the yield of Qianku5 was 1218.9 kg·ha^{-1} when the amount of phosphorus fertilizer was 80 kg·ha^{-1} without nitrogen fertilizer treatment, and this value did not significantly differ from the average yield of 1230 kg·ha^{-1} of this variety in Guizhou area, which may be due to the low demand of Tartary buckwheat for fertilizer. Compared with the local conventional 135 kg·ha^{-1} nitrogen fertilizer and 70 kg·ha^{-1} phosphate fertilizer [34], this experiment increased only the amount of phosphate fertilizer by 10 kg·ha^{-1} when no nitrogen fertilizer was used. Namely, 135 kg·ha^{-1} of nitrogen fertilizer was saved, and 10 kg·ha^{-1} of phosphorus fertilizer was increased. The price of nitrogen and phosphate fertilizer in Guizhou was USD 2·kg^{-1}, meaning that a total of USD 250·ha^{-1} was saved. The production cost was greatly reduced, but the yield did not considerably differ from the local average. This finding indicates that the stable yield and nitrogen saving of Tartary buckwheat can be achieved by "adjusting nitrogen with phosphorus" during production.

5. Conclusions

Appropriate phosphorus fertilizer application can increase the nitrogen metabolism-related enzyme activity in roots, promote root growth, increase the absorption of rhizosphere soil nutrients, and delay senescence, thereby increasing the aboveground biomass and final yield of Tartary buckwheat grown in nitrogen-deficient plots. A specific threshold (80 kg·ha^{-1}) for phosphate fertilizer application can alleviate nitrogen-deficient stress. After exceeding this threshold, the inhibition of nitrogen-deficient stress on the growth of Tartary buckwheat was intensified by causing a nitrogen and phosphorus imbalance. For Tartary

buckwheat with low demand for fertilizer, the stable yield and nitrogen conservation of Tartary buckwheat can be jointly achieved by "adjusting nitrogen with phosphorus" during production. Notably, each index was analyzed and discussed only from the physiological point of view. The relationship between the phosphorus fertilizer application and the phosphorus content in Tartary buckwheat plant tissues will be discussed in the future. The mechanism of the effect of phosphorus fertilizer on the growth of Tartary buckwheat under nitrogen-deficient stress will also be analyzed from the molecular point of view.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13071886/s1, Table S1: Effects of different phosphorus fertilizer application on the yield of Tartary buckwheat of 2021; Table S2: Effects of different phosphorus fertilizer application on root morphology and root activity of Tartary buckwheat of 2021; Table S3: Effects of different phosphorus fertilizer application on root nitrogen metabolism-related enzymes of Tartary buckwheat of 2021; Table S4: Effects of different phosphorus fertilizer application on chlorophyll content of Tartary buckwheat of 2021; Table S5: Effects of different phosphorus fertilizer treatments on antioxidant enzyme activity and MDA content of Tartary buckwheat of 2021. Table S6: Effects of different phosphorus fertilizer application on the yield of Tartary buckwheat of 2022; Table S7: Effects of different phosphorus fertilizer application on root morphology and root activity of Tartary buckwheat of 2022; Table S8: Effects of different phosphorus fertilizer application on root nitrogen metabolism-related enzymes of Tartary buckwheat of 2022; Table S9: Effects of different phosphorus fertilizer application on chlorophyll content of Tartary buckwheat of 2022; Table S10: Effects of different phosphorus fertilizer treatments on antioxidant enzyme activity and MDA content of Tartary buckwheat of 2022.

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