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Effects of Multiple Cropping with Green Manure on Yield and Agronomic Traits of Spring Wheat

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Abstract: A split-plot experiment was conducted to investigate the effects of green manure returning methods on wheat yield and phosphorus (P) accumulation. The main plots consisted of two fertilization levels for the subsequent wheat crop: no chemical fertilizer (N0) and chemical fertilizer application (N1, N 157.5 kg/ha + P_2O_5 78.75 kg/ha). The sub-plots comprised three green manure returning methods: no green manure (G0), green manure with root stubble retention (G1), and green manure with full incorporation (G2). Wheat yield, agronomic traits, and P content in various wheat organs were measured after harvest. The results showed that green manure returning significantly increased the yield and P accumulation of the subsequent wheat crop. Compared with chemical fertilizer alone (N1G0), the combination of green manure and chemical fertilizer significantly improved wheat yield. Specifically, the full incorporation treatment (N1G2) and root stubble retention treatment (N1G1) increased yield by 8.7% and 5.8% in 2024, and by 7.2% and 5.2% in 2025, respectively. The combined application of green manure and chemical fertilizer synergistically optimized agronomic traits and yield components, including grains per spike, thousand-grain weight, spike number, stem diameter, and plant height. Path analysis indicated that thousand-grain weight was the dominant factor affecting yield. Therefore, green manure full incorporation combined with chemical fertilizer is the optimal green manure-chemical fertilizer pattern for improving phosphorus availability in Qinghai region.

Keywords: Green Manure; Wheat Yield;Wheat Agronomic Traits

1 INTRODUCTION

Phosphorus is an essential macronutrient for crop growth and development, directly participating in key physiological processes such as energy metabolism, nucleic acid synthesis, and photosynthesis, and plays an irreplaceable role in yield formation [1-3]. In calcareous soils in northern China, phosphorus availability is generally low, and phosphate fertilizers applied to the soil are easily fixed into insoluble forms, with an immediate utilization rate of only 10%–20% [4-5]. Therefore, exploiting potential soil phosphorus sources and improving phosphorus use efficiency are important approaches for ensuring sustainable wheat production.As a clean and efficient organic fertilizer source, green manure has the dual function of improving soil fertility and promoting crop growth and development in agricultural production [6]. Green manure can fix atmospheric nitrogen through root symbiotic nitrogen fixation, while root exudates and decomposition after incorporation can mobilize insoluble phosphorus in the soil, thereby improving phosphorus availability [7-8]. Studies have shown that green manure return can significantly increase soil phosphatase activity, increase microbial biomass phosphorus content, and promote soil phosphorus transformation by regulating the structure of phosphate-solubilizing microbial communities [9-11]. However, current research on green manure return has mostly focused on changes in soil phosphorus pools and enzyme activity responses. Systematic studies on how green manure regulates soil phosphorus supply to subsequently control phosphorus uptake and accumulation in wheat and ultimately achieve yield increases remain insufficient.Based on a long-term positioning experiment, this study set up different green manure return methods and fertilization combinations to analyze the effects of multiple cropping green manure on yield components, grain yield, and plant phosphorus accumulation in spring wheat. The relationship between the yield-increasing effect of green manure return combined with chemical fertilizer and phosphorus uptake and accumulation was clarified, aiming to provide a theoretical basis for green and efficient wheat production in the agricultural areas of Qinghai.

2 MATERIALS AND METHODS

2.1 *EXPERIMENTAL SITE*

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This experiment was conducted at the experimental station of the Qinghai Academy of Agriculture and Forestry Sciences, Chenghbei District, Xining City, Qinghai Province (36° 43′ N, 101° 45′ E, altitude 2,300 m). The region features a continental plateau semi-arid climate, characterized by cool temperatures, an annual average temperature of 6 °C, annual evaporation of 1729.8 mm, annual precipitation of 367.5 mm (concentrated in summer), and an annual sunshine duration of 1939.7 h. The frost-free period is 220 days, suitable for crop growth. This long-term experiment was established in 2009. The current study was conducted from 2023 to 2024. The soil type is chestnut soil. Basic physicochemical properties of the 0–20 cm surface soil measured in 2009 are shown in Table 1. Precipitation and temperature changes during the wheat growing seasons of 2024 and 2025 are illustrated in Figure 1.

TABLE1 NUTRIENTS AND PH IN 0～20 CM TOPSOIL

SOC (g/kg)	TN (g/kg)	TP (g/kg)	TK (g/kg)	Available Nitrogen (mg/kg)	Available Phosphorus (mg/kg)	Available Potassium (mg/kg)	pH
9.81	1.09	2.14	26.25	77.00	10.72	167.00	8.31

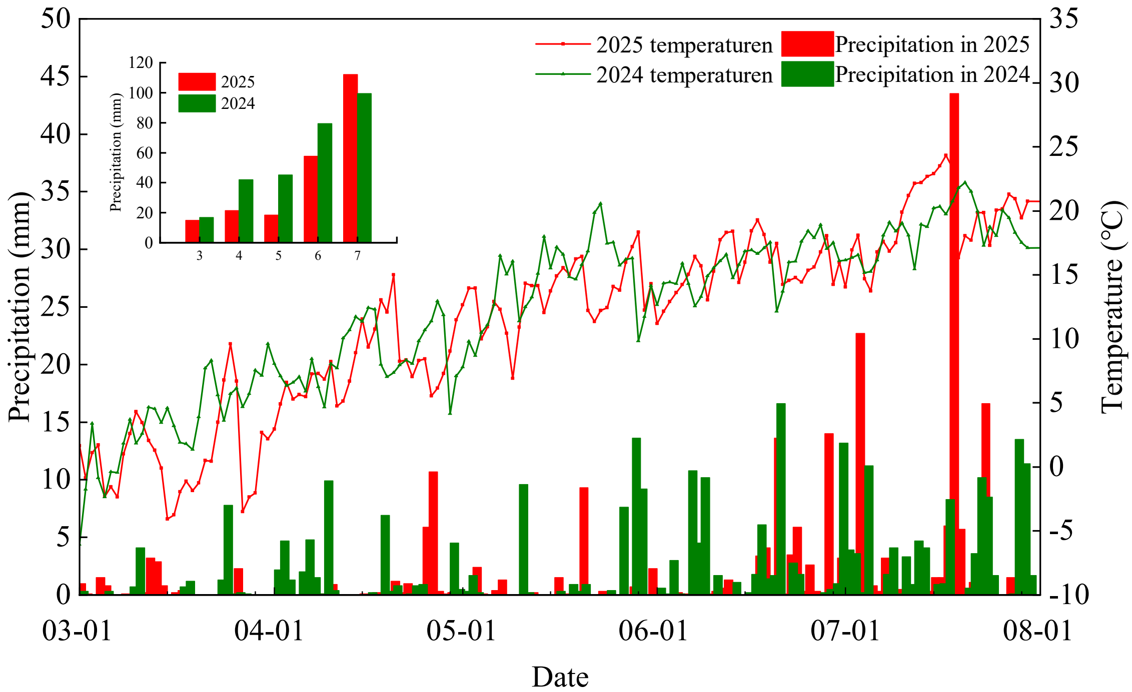

FIGURE 1 TEMPERATURE AND RAINFALL THROUGHOUT THE WHEAT GROWING SEASON IN THE EXPERIMENTAL REGION

2.2 *EXPERIMENTAL DESIGN*

A split-plot experimental design was adopted. The main plots consisted of two fertilization levels for the subsequent wheat crop (N): N0 (no chemical fertilizer) and N1 (chemical fertilizer: N 157.5 kg/hm² + P_2O_5 78.75 kg/hm²). The sub-plots comprised three green manure return methods (G): G0 (no green manure), G1 (green manure root stubble retention), and G2 (green manure full incorporation). The combination of these two factors resulted in a total of six treatments, each with three replicates. Each plot had an area of 20 m² (4 m × 5 m). The nitrogen fertilizer used was urea (46% N), of which 80% was applied as basal fertilizer incorporated into the plough layer during soil preparation before wheat sowing, and the remaining 20% was applied as topdressing at the four-leaf stage of wheat. The phosphorus fertilizer used was superphosphate (12% P_2O_5), applied as basal fertilizer together with nitrogen fertilizer before sowing. The above fertilization treatments were applied only to the subsequent wheat crop; no fertilizer was applied during the green manure growth period. The tested wheat (Triticum aestivum L.) cultivar was 'Qingchun 38', and the green manure

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crop was hairy vetch (Vicia villosa Roth.) cultivar 'Turkmen hairy vetch'.

2.3 Sampling And Measurement

Chlorophyll Measurement: Chlorophyll content was measured using a SPAD-502Plus chlorophyll meter (Minolta, Japan). Measurements were taken every 15 days starting from the jointing stage, selecting intact green leaves and avoiding leaf veins.

Yield Measurement: After wheat harvest, each plot was harvested individually, threshed, weighed, and adjusted to a 14% moisture content to calculate yield.

Plant Sampling: 20 plants were randomly selected from each plot at seedling, flowering, and maturity stages. At flowering, wheat was divided into: leaf sheath + stem, leaves, and glume + rachis. At maturity, it was divided into: leaf sheath + stem, leaves, glume + rachis, and grain. Samples were deactivated at 105 °C for 30 min, oven-dried at 75 °C to constant weight, weighed, and ground to pass through a 100-mesh sieve for total P determination.

Plant Total Phosphorus: Measured via the H_2SO_4-H_2O_2 digestion-molybdenum blue colorimetric method using a T500 UV-Vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.). Phosphorus accumulation was the product of total phosphorus content and dry matter weight.

2.4 Data Analysis

Excel 2019 and Origin 2021 were used for data processing and visualization. SPSS 27.0 was used for two-way ANOVA and path analysis. Significant differences ($p < 0.05$) were compared using Duncan's new multiple range test.

3 Results And Analysis

3.1 Effect Of Multiple Cropping Green Manure On Spring Wheat SPAD Values

eaf SPAD value is an important indicator reflecting chlorophyll content and photosynthetic capacity of plants. As shown in Figure 2.2, under different fertilization treatments, the SPAD value of spring wheat showed a dynamic trend of first increasing and then decreasing throughout the growth period, with significant differences among treatments. The SPAD values of all treatments increased from the jointing stage to the full grain-filling stage, peaked at the full grain-filling stage, and then decreased sharply at the late maturity stage due to leaf senescence. Regarding the combined treatments, green manure combined with chemical fertilizer significantly increased leaf SPAD values at each growth stage, with the N1G2 treatment having the highest SPAD value. At most measurement stages, its SPAD value was significantly different from those of other treatments. The enhancing effect of green manure return on SPAD value was particularly evident under unfertilized conditions. Under N0 conditions, the SPAD values of G1 and G2 treatments were significantly higher than those of G0 treatment in the early and middle growth stages. This may be because green manure return releases nutrients through mineralization, effectively improving crop nutritional status. Under N1 conditions, the SPAD value of G2 treatment was higher than those of G1 and G0 treatments at all stages. At the full grain-filling stage, the SPAD value of N1G2 treatment was significantly higher than those of N1G1 and N1G0 treatments. This indicates that green manure incorporation may enhance the photosynthetic capacity of crops in the middle and late growth stages by improving the rhizosphere microenvironment and promoting nutrient uptake.

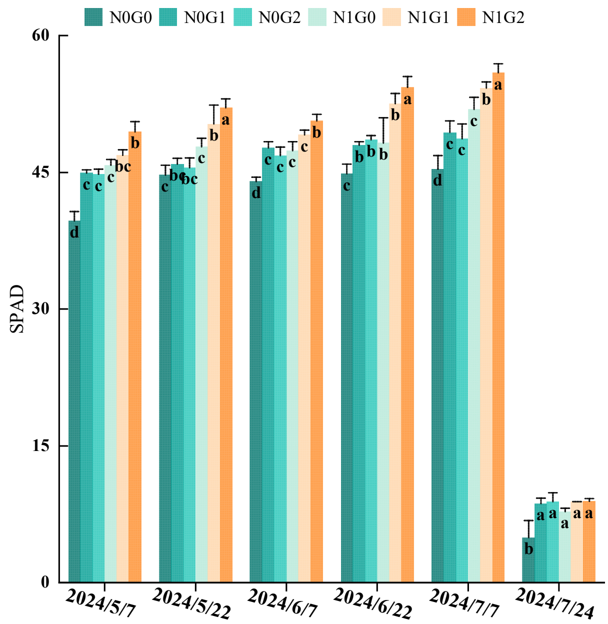

Figure 2. SPAD Values Of Spring Wheat Leaves At Different Growth Stages Under Different Treatments

Note：Bars marked without the same letters indicate significant difference at p<0.05. The same as below.

3.2 Effect On Subsequent Wheat Yield

Based on the interaction effect, both green manure utilization method (G) and fertilization level (N), as well as their interaction (G × N), had highly significant effects on grain yield. Both green manure utilization method and fertilization level promoted wheat grain yield, with N1G2 and N1G1 showing good promoting effects and differing significantly from other treatments. Under the same fertilization level, grain yield in G2 and G1 treatments was higher than that in G0 treatment. At the N0 level, compared with G0, grain yield significantly increased by 83.0% and 84.3% in G1 and G2, respectively. At the N1 level, compared with G0, grain yield significantly increased by 5.8% and 8.7% in G1 and G2, respectively. Compared with N0G0, grain yield in N1G2 and N1G1 increased by 231.4% and 222.4%, respectively, all reaching significant differences (see Figure 3). Regardless of whether fertilizer was applied or not, grain yield increased with green manure return compared with G0, reaching the maximum under G2 treatment. At the N0 level, compared

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with G0, grain yield significantly increased by 78.2% and 76.7% in G1 and G2, respectively. At the N1 level, compared with G0, grain yield significantly increased by 5.2% and 7.2% in G1 and G2, respectively. Compared with N0G0, grain yield in N1G2 and N1G1 increased by 229.7% and 223.4%, respectively, all reaching significant differences. Data from two years indicate that, regardless of fertilizer application, grain yield increased with green manure return compared with G0, reaching the maximum under G2 treatment.

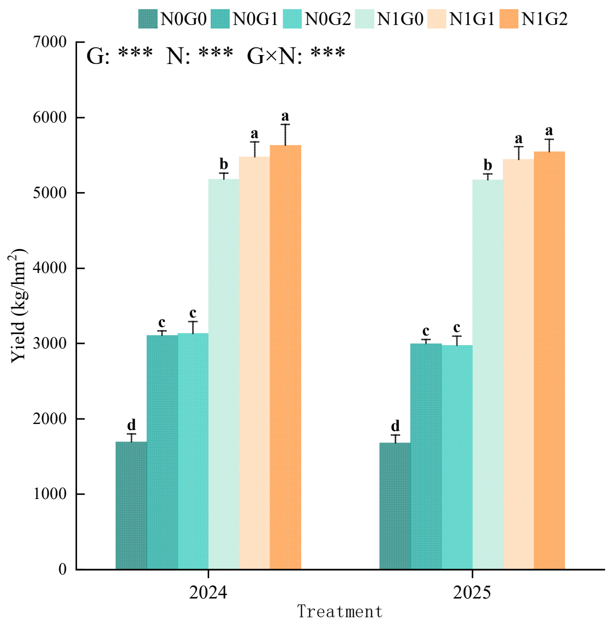

FIGURE 3 SPRING WHEAT YIELD UNDER DIFFERENT TREATMENTS IN 2024 AND 2025

3.3 *EFFECT ON AGRONOMIC TRAITS*

As shown in Tables 2.2 and 2.3, both fertilization level (N) and green manure return method (G) had significant effects on yield components and agronomic traits of spring wheat in 2024 and 2025, with consistent trends across the two years. Fertilization level (N) had highly significant effects on grains per spike, thousand-grain weight, spike number, stem diameter, and plant height, indicating that chemical fertilizer plays a stable role in promoting the growth and yield formation of spring wheat. Green manure return method (G) also reached significant or highly significant levels for most indicators, demonstrating its impact on plant traits in both years. Under fertilized conditions, the grains per spike, thousand-grain weight, spike number, stem diameter, and plant height of all treatments in 2024 and 2025 were significantly higher than those in the N0 treatment, with increases ranging from 37.9% to 83.7% for grains per spike, 13.6% to 16.7% for thousand-grain weight, 13.5% to 24.3% for stem diameter, and 25.7% to 30.6% for plant height. This indicates that fertilization increases yield by synergistically improving agronomic traits and yield components. Green manure return showed positive effects under both fertilized and unfertilized conditions. Under no chemical fertilizer conditions, compared with G0, the G1 treatment increased grains per spike, thousand-grain weight, spike number, stem diameter, and plant height by 27.9%–33.7%, 6.6%–7.2%, 9.2%–9.9%, 8.1%–10.8%, and 11.2%–12.0%, respectively, over the two years; the G2 treatment increased these five indicators by 34.3%–42.1%, 7.2%–8.2%, 12.8%–13.9%, 8.1%–10.8%, and 12.2%–13.9%, respectively. The improvement was better under G2. Under fertilized conditions, green manure return further promoted wheat growth, but the magnitude of increase was smaller than that under unfertilized conditions. The G1 treatment increased grains per spike, thousand-grain weight, spike number, and stem diameter by 3.3%–5.6%, 1.2%–1.5%, 6.2%–7.2%, and 2.4%–4.8%, respectively, over the two years, with little change in plant height; the G2 treatment increased the five indicators by 12.3%–14.8%, 2.4%–2.7%, 10.5%–14.0%, 7.1%–9.5%, and 2.5%–3.9%, respectively. This indicates that green manure return provides greater improvement under unfertilized conditions, demonstrating a significant nutrient compensation effect.

Stem diameter and plant height are important agronomic traits reflecting crop growth vigor. The stem diameter and plant height of N1G2 were significantly higher than those of other treatments, increasing by over 24% and 30%, respectively, compared with the N0G0 treatment. This indicates that this treatment provides the best morphological structure and physiological basis for high yield.

Fertilization is the foundation for improving crop traits, while green manure return, especially under unfertilized (N0) conditions, greatly alleviates nutrient limitations and promotes crop growth. Under sufficient fertility conditions (N1), green manure return mainly plays a synergistic role. The combination of green manure incorporation and chemical fertilizer application (N1G2) optimizes yield components such as spike number, grains per spike, and thousand-grain weight, making it the most effective cultivation model for achieving high yield.

TABLE 2 YIELD COMPONENTS AND AGRONOMIC TRAITS OF SPRING WHEAT UNDER DIFFERENT TREATMENTS IN 2024

Fertilizer level	Treatment	Grains per spike	1000-grain weight (g)	Spike number (10^4/hm^2)	Stem diameter (mm)	Plant height (cm)
N0	G0	19.0±3.5d	36.0±1.3c	472.6±15.6d	3.7±0.4c	73.9±5.5c
	G1	25.4±2.1c	38.6±0.3b	519.5±27.8cd	4.0±0.2bc	82.8±1.6b

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	G2	27.0±4.3bc	38.6±0.4b	538.4±27.4c	4.0±0.2bc	84.2±3.5b
N1	G0	30.4±3.1abc	40.9±0.5a	552.3±19bc	4.2±0.1b	93.7±1.7a
	G1	32.1±2.5ab	41.5±0.6a	591.9±19.8ab	4.3±0.1ab	92.9±3.6a
	G2		42.0±0.3a	629.9±46.4a	4.6±0.2a	96.5±3.5a
F-statistic						
Fertilizer level（N）		38.562***	38.147***	38.029	19.385***	74.011***
Utilization pattern（G）		6.900**	13.4281***	10.036	4.495*	5.354*
N×G		0.998	3.248	0.178	0.824	3.253

TABLE 3 YIELD COMPONENTS AND AGRONOMIC TRAITS OF SPRING WHEAT UNDER DIFFERENT TREATMENTS IN 2025

Fertilizer level	Treatment	Grains per spike	1000-grain weight（g）	Spike number (10^4/hm^2)	Stem diameter（mm）	Plant height（cm）
N0	G0	20.4±2.6e	36.5±1c	476.3±19.4d	3.7±0.3d	74.3±3.0c
	G1	26.1±1.5d	38.9±0.8b	528.5±27.2c	4.1±0.2c	83.5±0.6b
	G2	28.7±2.7cd	39±0.6b	545.4±32.7c	4.1±0.2c	84.5±2.6b
N1	G0	31.2±2.1bc	41.2±0.7a	561.4±10.9bc	4.2±0.1bc	94.1±2a
	G1	32.7±1.9ab	42±0.7a	608.2±20.4ab	4.4±0.1b	93.3±2.8a
	G2	35.5±0.8a	42.4±0.6a	623.3±41.9a	4.7±0.1a	97.1±2.4a
F-statistic						
Fertilizer level（N）		70.005***	113.539***	39.451**	45.023***	158.664***
Utilization pattern（G）		14.477***	11.512**	9.374**	14.256***	12.018***
N×G		2.003	1.963	0.029	1.419	7.106**

In this study, the multiple linear regression equation of x1 (thousand-grain weight), x2 (grains per spike), x3 (spike number) with y (yield) is: y = -17170.4 + 448.7x1 + 61.5x2 + 2.8x3. The standardized regression equation is: y = 0.657x1 + 0.226x2 + 0.104x3, with R^2 = 0.9034. This indicates that the three independent variables—thousand-grain weight, grains per spike, and spike number—together explain 90.34% of the variation in wheat yield. The model shows a good fit and adequately reflects the relationship between yield and its components. Path analysis reveals that thousand-grain weight has the largest direct effect and is the most dominant factor affecting yield; grains per spike and spike number mainly exert indirect effects by increasing thousand-grain weight.

TABLE 4 PATH ANALYSIS OF SIMPLE CORRELATION COEFFICIENTS FOR WHEAT YIELD

Independent variable	Simple correlation coefficient with y	Path coefficient (direct effect)	Indirect path coefficient (indirect effect)			
			x1	x2	x3	total
x1	0.940	0.657	—	0.196	0.086	0.282
x2	0.881	0.226	0.570	—	0.213	0.655
x3	0.834	0.104	0.546	0.184	—	0.730

4 DISCUSSION

Reasonable agronomic measures promote crop growth and development by regulating soil water, nutrients, air, and heat, ultimately increasing yield. Green manure can perform biological nitrogen fixation during its growth period and, after being returned to the field, can supply nitrogen and phosphorus nutrients throughout the entire growth period of wheat, improve soil physicochemical properties and the microbial environment, enhance crop photosynthetic efficiency, and finally achieve yield increase [12-13]. This study shows that the combination of green manure incorporation and chemical fertilizer application has the best effect on increasing wheat yield, while significantly optimizing yield components and agronomic traits such as spike number, grains per spike, thousand-grain weight, stem diameter, and plant height. This is consistent with the findings of Xiao Yutao et al.[14], who reported that under the condition of reducing chemical fertilizer by 30%, multiple cropping and incorporating green manure combined with chemical fertilizer significantly increased spring wheat yield in Qinghai. The main reasons may be as follows: First, the decomposition and nutrient release of green manure after incorporation are slow, providing a continuous supply of nutrients to the crop, while chemical fertilizers supply nutrients rapidly. The combined application of green manure and chemical fertilizer effectively compensates for the shortcomings of both, thereby improving crop nutrient uptake and promoting wheat yield increase [15]. Second, crop yield is closely related to dry matter accumulation. Green manure incorporation promotes soil nitrogen cycling, increases soil nitrogen nutrient content [14], enhances crop growth and development, increases photosynthetic area and photosynthetic product accumulation, and promotes dry matter accumulation, thereby increasing crop yield [16]. Third, the incorporation of hairy vetch (Vicia villosa) carbon and nitrogen promotes soil microbial proliferation and the release of organic matter nutrients [17], increases soil available nutrient content, promotes wheat growth and development, and thus increases wheat yield. Therefore, the combination of green manure incorporation and chemical fertilizer application can provide sufficient nutrients for wheat growth and development, thereby achieving yield increase.

5 CONCLUSION

The combined application of green manure incorporation and chemical fertilizer significantly increases the chlorophyll content of spring wheat leaves, delays leaf senescence, and maintains photosynthetic capacity in the middle and late growth stages. This model achieves a balanced improvement of the three yield components—spike number, grains per spike, and thousand-grain weight—through synergistic optimization, making it the best cultivation practice for achieving high yield. Additionally, this treatment improves agronomic traits such as plant height and stem diameter, enhancing lodging resistance and biomass accumulation.

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