Response of Floret Development and Grain Setting to Nitrogen Fertilizer in Wheat Varieties with Different Spike Types: Postprint

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Date: 2017-11-08T00:00:00+00:00

Abstract

To explore the response of floret development and grain setting to nitrogen fertilizer in wheat varieties with different spike types, this experiment used the large-spike variety 'Zhoumai 16'and the multi-spike variety 'Yumai 49'as test materials, with different nitrogen application levels of 0 kg(N) · hm−2, 180 kg(N) · hm−2, and 360 kg(N) · hm−2, to observe and analyze the dynamic patterns of floret development and grain setting characteristics in the two spike-type wheat varieties. The results showed that with the increase of growing degree days (GDD), the dynamic change trends of floret development under different nitrogen levels were similar for the two varieties; floret differentiation conformed to a quadratic curve equation pattern, while degeneration and abortion conformed to a linear equation, with R2 reaching significant levels. The total number of floret differentiations in the large-spike variety 'Zhoumai 16' was significantly higher than that in the multi-spike variety 'Yumai 49', with extremely significant differences at 360 kg(N) · hm−2; the large-spike variety 'Zhoumai 16' showed that with increasing nitrogen application, the rates of floret differentiation and degeneration increased, which was conducive to the formation of fertile florets and increased grain number; ultimately, the grain number at 180 kg(N) · hm−2 was significantly higher than other treatments, with an average increase of 2.04 grains per spike compared to 360 kg(N) · hm−2. Although the multi-spike variety 'Yumai 49' had a relatively low total number of differentiated florets, it exhibited lower rates of floret degeneration and fertile floret abortion, showing high numbers of fertile florets and grain setting at the 180 kg(N) · hm−2 nitrogen level, though the differences were not significant compared to the high nitrogen treatment. This indicates that for increasing grain number per spike, 180 kg(N) · hm−2 was more suitable for both varieties. From the perspective of final yield and yield component analysis, 'Yumai 49' showed the same result as for grain

number per spike, whereas 'Zhoumai 16' could increase yield through increased spike number and grain number per spike under high nitrogen conditions.

Full Text

Response of Floret Development and Grain Formation in Different Spike-Type Wheat Cultivars to Nitrogen Application

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Abstract

To investigate the response of floret development and grain formation to nitrogen application in different spike-type wheat cultivars, we conducted a field experiment using the large-spike cultivar 'Zhoumai 16' and the multi-spike cultivar 'Yumai 49' under three nitrogen levels: 0 kg(N)·hm^{-2}, 180 kg(N)·hm^{-2}, and 360 kg(N)·hm^{-2}. The dynamic patterns of floret development and grain setting characteristics were observed and analyzed. The results showed that with increasing growing degree-days (GDD), both cultivars exhibited similar dynamic trends in floret development across nitrogen treatments. Floret differentiation followed a quadratic curve model, while degeneration and infertility followed linear equations, with R^2 values reaching significant levels. The large-spike cultivar 'Zhoumai 16' showed significantly higher total floret differentiation than the multi-spike cultivar 'Yumai 49', with extremely significant differences observed at 180 kg(N)·hm^{-2}. For 'Zhoumai 16', increased nitrogen application enhanced both floret differentiation and degeneration rates, which favored fertile floret formation and increased grain number. At 180 kg(N)·hm^{-2}, this cultivar achieved high grain number, significantly higher than other treatments, with an average increase of 2.04 grains per spike compared to 360 kg(N)·hm^{-2}. Although the multi-spike cultivar 'Yumai 49' had lower total floret differentiation, it exhibited lower rates of floret degeneration and fertile floret infertility, performing optimally at 180 kg(N)·hm^{-2}. Analysis of final yield and yield components revealed that 'Yumai 49' showed consistent results with grains per spike, while 'Zhoumai 16' could increase yield through enhanced spike number and grains per spike under high nitrogen conditions.

Keywords: Wheat; Nitrogen level; Spike type; Floret development; Dynamic model; Grain setting characteristics

Introduction

Wheat (*Triticum aestivum* L.) is one of the major food crops in China. Under current conditions of limited arable land and water resources, stable increases in wheat yield are crucial for improving living standards and ensuring national food security [1]. Wheat yield improvement requires coordinated development among yield components, but insufficient grain number per spike represents a key factor limiting yield enhancement [2-3]. Research has indicated that the developmental process of young spikes is a critical factor affecting grain number per spike [4]. However, due to the long duration of floret differentiation, numerous differentiated florets, and substantial degeneration, floret development has become a bottleneck restricting increases in grain number per spike. Consequently, many studies have focused on the stage-specific characteristics of floret primordium differentiation and its regulatory factors [5-7]. Cui et al. [1] first divided the floret development process into ten stages based on the relationship between floret developmental progress and ecological conditions: floret primordium differentiation stage, stamen and pistil primordium differentiation stage, small concave stage, concave stage, large concave stage, stigma protrusion stage, stigma elongation stage, stigma feather protrusion stage, stigma feather elongation stage, and stigma feather formation stage.

Floret primordium differentiation in wheat is influenced by multiple factors, including temperature and light conditions, vegetative organ growth status, and regulatory measures. Among these, nitrogen is an essential nutrient element affecting wheat growth and development, and nitrogen fertilizer application significantly impacts floret differentiation number, reduces floret degeneration, and increases final grain number [8-10]. Wang et al. [11] demonstrated that nitrogen application rate consistently affects the development of middle florets within spikelets, total spikelet number, sterile spikelet number, and spike fertility rate. Due to the diverse cultivar types utilized in wheat production, final grain number per spike varies considerably among different spike-type cultivars [12]. Wang et al. [13] reported that different spike-type wheats show that larger spike types have higher floret differentiation rates and more florets differentiated per spike, resulting in the highest number of fertile florets and grains. While differences in grain setting characteristics among different spike-type wheats are small in superior floret positions, they are relatively large in inferior floret positions. Wheat floret development can be divided into three processes: floret differentiation, degeneration, and fertile floret infertility. However, few studies have examined the effects of nitrogen application on different stages of floret development across spike types. Therefore, this experiment was conducted to investigate the response of floret development and grain formation to nitrogen application in different spike-type cultivars, providing a reference basis for developing techniques to increase grain number per spike and stably improve yield using different spike-type wheat cultivars in production.

1.1 Experimental Materials and Design

The field experiment was conducted during the 2015–2016 growing season at the Science and Education Demonstration Park of Henan Agricultural University. The experimental soil was loam with organic matter content of 16.2 $g \cdot kg^{-1}$, total nitrogen of 1.00 $g \cdot kg^{-1}$, alkaline-hydrolyzable nitrogen of 119 $mg \cdot kg^{-1}$, available phosphorus of 19.1 $mg \cdot kg^{-1}$, and available potassium of 97 $mg \cdot kg^{-1}$ in the 0–20 cm soil layer. Before sowing, phosphorus fertilizer (calcium superphosphate) at 150 $kg \cdot hm^{-2}$ and potassium fertilizer (KCl) at 150 $kg \cdot hm^{-2}$ were applied. Nitrogen fertilizer (urea, containing 46% N) was applied 50% as basal fertilizer and 50% at the regreening stage.

The experiment used large-spike wheat cultivar 'Zhoumai 16' (V1) and multi-spike wheat cultivar 'Yumai 49' (V2) as test materials. Both cultivars have spindle-type spikes. 'Zhoumai 16' averages approximately 5.55 million spikes per hectare, with about 38 grains per spike and a thousand-grain weight of about 46 g, while 'Yumai 49' produces about 6.45 million spikes per hectare, with approximately 31.0 grains per spike and a thousand-grain weight of about 40.9 g [14].

A randomized block design was employed with three nitrogen levels: 0 $kg \cdot hm^{-2}$ (N1), 180 $kg \cdot hm^{-2}$ (N2), and 360 $kg \cdot hm^{-2}$ (N3), with four replications. Each plot measured 2.9 m × 7 m. Sowing occurred on October 8, with a basic seedling density of 2.25×10^6 plants per hectare.

1.2 Measurement Content and Methods

From the agricultural elongation stage (when basal internodes began elongating and 1.5–2.0 cm of internode was above ground) until 5 days after flowering when floret differentiation ended, samples were taken every 7 days. Five uniform wheat plants were sampled from each plot. Young spike developmental progress was observed under an EMZ-TA dissecting microscope, recording single-stem spikelet number, differentiated floret number (calculated at the peak differentiation period 28 days after elongation), fertile floret number (florets with stamens, pistils, ovary, stigma, and stigma feathers), and developmental progress at each stage, with final calculations converted to per-square-meter quantities. At maturity, 20 plants were sampled for yield component analysis, recording spikelet number, grains per spike, and thousand-grain weight, with 6 m^2 harvested for actual yield measurement.

Based on observations and yield data, the following parameters were calculated: differentiated floret number per square meter (21 days after elongation), fertile floret number per square meter (28 days after elongation), grain number per unit area (at maturity), floret setting rate (%), and fertile floret setting rate (%). Accumulated growing degree-days (GDD) were calculated from local meteorological data to measure accumulated heat for floret development.

1.3 Statistical Analysis

Microsoft Excel 2013 and SPSS 19.0 software were used for data plotting and statistical analysis.

Results

2.1 Effects of Nitrogen Application on Floret Differentiation, Degeneration, and Infertility in Different Spike-Type Wheat Cultivars

Analysis of [Figure 1: see original paper] revealed that under different nitrogen levels, both cultivars experienced floret development processes that transitioned from rapid differentiation to rapid degeneration and then slow infertility as accumulated growing degree-days after sowing increased. The differentiation stage conformed to a quadratic curve equation, while degeneration and infertility stages followed linear equations (), with R^2 values reaching significant levels. Under all three nitrogen levels, both spike-type cultivars conformed to the quadratic curve equation $y = -0.9708x^2 + 2839.9x - 2\times10^{6}$ ($R^2 = 0.8209$) for the differentiation stage and the linear equation $y = -484.51x + 781{,}833$ ($R^2 = 0.4838$) for the degeneration stage. The infertility stage showed minimal change in floret number, tending toward stability, indicating that accumulated growing degree-days after sowing significantly affected wheat floret differentiation under different nitrogen levels.

Further analysis showed that dynamic changes in floret differentiation, degeneration, and infertility differed between spike-type cultivars under various nitrogen levels. During the differentiation stage, both cultivars differentiated faster under N1 than under N2 and N3. Compared with the multi-spike cultivar V2, the large-spike cultivar V1 started floret differentiation later under N2 and N3 but entered the peak differentiation period earlier with a relatively longer peak duration, which favored the development of superior florets and laid the foundation for large spikes. During the fertile floret infertility stage, both cultivars showed essentially constant infertility rates under N2 and N3, with floret numbers declining slowly. These results demonstrate that the two spike-type wheat cultivars differed in sensitivity of floret differentiation to nitrogen application, and also indicated that large-spike cultivars possess the advantage of greater floret differentiation capacity.

2.2 Differences in Differentiated Floret Number, Fertile Floret Number, and Grain Number Among Spike-Type Cultivars Under Different Nitrogen Levels

[Figure 2: see original paper] shows significant differences in floret differentiation numbers between the two spike-type cultivars under different nitrogen levels. The large-spike cultivar V1 had higher floret differentiation numbers than the multi-spike cultivar V2 under all three nitrogen levels, with extremely significant differences observed at N3, where total floret number per square meter was

95,212 more than V2. Total floret number in the large-spike cultivar showed an increasing trend with nitrogen application, while the multi-spike cultivar showed an initial increase followed by a decrease. Fertile floret numbers in both cultivars increased with nitrogen application. Although the large-spike cultivar had significantly higher floret differentiation than the multi-spike cultivar at the same nitrogen level, the difference diminished after infertility. Final grain numbers per square meter for the large-spike cultivar under different nitrogen treatments were 13,294.23 (V1N1), 18,191.74 (V1N2), and 19,577.52 (V1N3), showing an increasing trend with nitrogen application. In contrast, V2N1, V2N2, and V2N3 produced 14,382.39, 19,263, and 17,897.34 grains per square meter, respectively, showing an initial increase followed by a decrease. These results indicate that increased nitrogen application benefits floret differentiation and grain number formation in large-spike wheat.

2.3 Effects of Nitrogen Application on Rates of Floret Differentiation, Degeneration, and Infertility in Different Spike-Type Cultivars

As shown in the analysis, both cultivars had essentially equal initial differentiation rates, with about 800 differentiated florets $\cdot$ m^{-2} $\cdot$ GDD^{-1}. The V1N1 treatment had lower initial differentiation rates than other nitrogen levels, while V1N2 and V1N3 showed higher floret differentiation rates at all GDD values. During the degeneration stage, V1 had higher floret degeneration rates than V2 under all nitrogen levels, with the most significant difference observed at N3. The large-spike cultivar V1 showed increasing floret degeneration rates with increasing nitrogen levels, while the multi-spike cultivar V2 showed an initial increase followed by a decrease. During the fertile floret infertility stage, both cultivars showed the highest infertility rates at the highest nitrogen level, with V1N3 reaching 82.90 florets $\cdot$ m^{-2} $\cdot$ GDD^{-1} and V2N3 reaching 72.209 florets $\cdot$ m^{-2} $\cdot$ GDD^{-1}. These results demonstrate that within the experimental range, the large-spike cultivar had significantly higher floret differentiation than the multi-spike cultivar but also significantly higher degeneration rates, indicating that large-spike cultivars possess strong floret differentiation capacity alongside high degeneration rates. Conversely, multi-spike cultivars had lower floret differentiation but also lower degeneration rates and significantly lower fertile floret infertility rates. Both cultivars exhibited high infertility rates under the highest nitrogen application (N3), suggesting that increasing nitrogen application had limited effect on reducing fertile floret infertility under these experimental conditions.

2.4 Differences in Floret Setting Rate, Fertile Floret Setting Rate, and Spikelet Setting Rate Among Cultivars Under Different Nitrogen Levels

Analysis of [Figure 4: see original paper] revealed that the large-spike cultivar V1 showed decreasing trends in floret setting rate, fertile floret setting rate, and spikelet setting rate with increasing nitrogen levels. The multi-spike cultivar

V2 showed decreasing fertile floret setting rate and spikelet setting rate with increasing nitrogen, while its floret setting rate increased. V2 had significantly higher floret setting rates than V1 under all three nitrogen levels, with particularly significant differences at N3. However, V2 had lower fertile floret setting rates and spikelet setting rates than V1 at all nitrogen levels. These findings indicate that although large-spike wheat cultivars differentiate more florets, they also have greater degeneration, resulting in lower floret setting rates.

2.5 Effects of Nitrogen Application on Yield and Yield Components in Different Spike-Type Wheat Cultivars

Analysis of showed that differences in spike number between the two cultivars were significant under N1 but not significant under N2 and N3. Both cultivars exhibited the trend N1 > N2 > N3 for thousand-grain weight, with N1 being significantly higher than the other two nitrogen treatments. Grains per spike in both the large-spike cultivar V1 and multi-spike cultivar V2 showed an initial increase followed by a decrease with increasing nitrogen levels, though V1 had more grains per spike than V2 at all nitrogen levels. The yield of V1 was higher than V2 under N2 and N3, demonstrating the yield potential of large-spike cultivars. The yield trend matched that of grains per spike, with both spike-type cultivars achieving maximum yield at medium nitrogen application. These results indicate that under the experimental conditions, medium nitrogen application could ensure coordinated development of yield components and improve yield.

Discussion and Conclusion

In wheat production in the southern Huang-Huai region of China, spike number reaches approximately 6 million per hectare and thousand-grain weight generally exceeds 40 g, but grains per spike average only about 30 [15], making low grain number a primary factor limiting wheat yield improvement. Research suggests that increasing grains per spike depends mainly on increasing spikelet and floret numbers [15]. However, in the Huang-Huai wheat region, the double-ridge stage (wintering stage) is relatively long, typically resulting in numerous differentiated spikelets, with each spike capable of differentiating 150–180 floret primordia. Nevertheless, most florets (70%) cease development during the degeneration stage, with only a small portion developing into fertile florets [16]. Our previous research also noted that fertile florets are the backbone florets for grain formation, but they still experience infertility rates exceeding 30% during grain development [1]. Therefore, reducing fertile floret infertility is essential for improving grain setting rate and increasing grain number per spike. González et al. [17] reported that floret survival plays a decisive role in wheat grain number formation and that this process may be regulated by resource availability. The period from floret differentiation to fertile floret infertility coincides with rapid vegetative growth, and floret setting characteristics are jointly influenced by environmental conditions and genotype [18]. Thus, appropriate growth conditions and nutrient supply are beneficial for reducing floret degeneration and fertile floret infertility to increase grain number per spike.

Li et al. [19] studied the relationship between floret development in winter and spring cultivars and growing degree-days after sowing, noting that rising curves and rapid-slow linear models could accurately and completely reflect the dynamic processes of normal young spike development, floret differentiation, degeneration, and infertility. However, regulation of these three processes remains unclear. Our results demonstrate the dynamic patterns of floret differentiation, degeneration, and fertile floret infertility in large-spike and multi-spike cultivars under different nitrogen levels, and reveal differential responses to nitrogen among spike types. Increased nitrogen application enhanced floret differentiation in large-spike cultivars but also increased floret degeneration rates, thus having limited effect on improving floret setting rate. For multi-spike cultivars, floret differentiation number was less sensitive to nitrogen increase, but nitrogen application reduced floret degeneration rates. Although large-spike wheat cultivars had higher floret degeneration rates than multi-spike cultivars, their greater total differentiated spikelet and floret numbers resulted in relatively higher fertile floret and spikelet setting, which represents an important reason for their higher grain number per spike. Both cultivars showed high fertile floret infertility rates under high nitrogen conditions, indicating that fertile floret development into grains is determined not only by genetic characteristics but also by crop growth conditions. This suggests that appropriate nitrogen application rates could be used to reduce floret differentiation and fertile floret infertility rates in wheat production.

Numerous studies have investigated optimal nitrogen application rates for wheat, with traditional production practices determining rates based primarily on yield targets. Research by Hao et al. and Li et al. [20-21] indicated that under high-yield conditions in Henan, nitrogen levels of 180–270 $kg \cdot hm^{-2}$ were favorable for wheat biomass production, accumulation, translocation, and yield formation. Wu [22] analyzed soil testing and formulated fertilization data from Henan Province, concluding that optimal nitrogen application rates for wheat in most regions ranged from 150–200 $kg \cdot hm^{-2}$. Our results indicate that 180 $kg \cdot hm^{-2}$ could meet the floret number requirements for grain formation in both spike-type cultivars. Although large-spike cultivars showed increasing floret number per square meter with increasing nitrogen, both cultivars achieved optimal final grain number at 180 $kg \cdot hm^{-2}$. Analysis of final yield and yield components showed that 'Yumai 49' exhibited results consistent with grains per spike, while 'Zhoumai 16' could increase yield through enhanced spike number and grains per spike under high nitrogen conditions. Sinclair and Jamieson [23] studied the relationship between nitrogen content in spikes at flowering and grain number, concluding that nitrogen accumulation in wheat spikes at flowering was highly correlated with grain number. This suggests that appropriately delaying nitrogen topdressing to meet nutrient requirements during the fertile floret infertility stage could be feasible for reducing fertile floret infertility and increasing grain number in both spike-type cultivars, warranting consideration in future research.

The physiological mechanisms underlying the response of floret development and grain formation to nitrogen application in different spike-type cultivars require further investigation.

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Note: Figure translations are in progress. See original paper for figures.