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Regulation of nitrogen topdressing and paclobutrazol at different stages on spike differentiation of winter wheat

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Background. Optimal nitrogen application and plant growth regulator is essential for individual wheat productivity. This can help to improve yield level and ensure food security with limited resources in the Huang-Huai-Hai Plain of China (HPC). **Methods.** A 2-year field field experiments were conducted using a randomized block design with four treatments (TS–nitrogen topdressing at pseudostem erection stage; TPS–TS combined with paclobutrazol application; TJ–nitrogen topdressing at jointing stage; TPJ–TJ combined with paclobutrazol application) in 2011-2013. **Results.** We found that grain number per ear, thousand kernel weight and yield under TJ and TPJ were higher than those under TS and TPS. Grain number per ear, yield under TPJ and thousands kernel weight under TJ were significantly higher than those under TS and TPS. The floret number, significantly correlated with cytokinin content, was also significantly increased under TJ and TPJ at connectivum differentiation stage. The relative expression level of cytokinin oxidase gene (*TACKX2.2*) in response to cytokinin increasing was improved to regulate hormonal balance. **Conclusions**. Therefore, nitrogen topdressing at jointing stage had increased grain number per ear, thousand kernel weight, and grain yield of wheat. Paclobutrazol could delay spike differentiation and promote cytokinin accumulation that induced expression of *TACKX2.2*, maintaining hormonal balance and affecting wheat spike morphogenesis.

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Keywords topdressing stages, floret number, phytohormone, spike differentiation, *TACKX2.2*

INTRODUCTION

The North China Plain, one of the most important grain base, is experiencing prominent conflicts on limited natural resources and crop production. In order to pursue higher grain yield, excessive use of nitrogen fertilizer exists commonly in local wheat-maize planting system. This can also lead to large losses of nitrogen, resulting in serious environmental pollution (*Azam Shah et al. 2009; Wang et al. 2010*). Additionally, the planting area of winter wheat is shrinking for the sake of saving over-exploit underground water and improving ecological benefits (*Xu et al. 2005*). The yield pressure and resources cost such as farmland, water and fertilizer have become increasingly growing. Therefore, it is an important way to ensure China's food security by improving grain production capacity. Wang *et al*. (2015) referred that a key factor for increasing wheat yield was increasing the grain number of spike and seed weight, which was often influenced by nitrogen fertilizer in the high-yield wheat region of Hebei Province. Nitrogen fertilizer application cannot only promote allocation of assimilate, but also increase assimilation amount and contribution rate of post-anthesis photosynthate to grain (*Ma et al. 2008a*). There were three nitrogen absorption peaks of wheat at stage before winter, jointing-booting stage, and flowering-filling stage during the whole growth period. The transport of carbon assimilates to grain was more efficient with nitrogen topdressing at jointing stage than at flowering stage (*Zhai and Li, 2006*). But the nitrogen application should be delayed until a later booting stage under the super-high-yield conditions (*Kara and Uysal, 2009; Liu et al. 2019*). Additionally, the optimal nitrogen application time is often related to local water condition and wheat varieties. Under drought conditions or limited irrigation, nitrogen topdressing should be applied early, that would be more beneficial to transportation of carbon photosynthate to grain at jointing stage than at flowering stage (*Gevrek and Atasoy, 2012*). But, for spring wheat, it was more appropriate to topdressing nitrogen at flowering time (*Walsh et al. 2018*). Under irrigated conditions, the topdressing time should be delayed for enhancing photosynthesis and increasing wheat yield (*Chen et al. 2008*).

Paclobutrazol (pp333), a plant growth regulator to inhibit synthesis of endogenous gibberellin, could be absorbed easily by root, stem, and leaf. The application of paclobutrazol could inhibit crop height, increase stress tolerance, promote tillering, and increase spike number and yield (*Hajihashemi et al. 2007; Gómez et al. 2011; Peng et al. 2014; Dwivedi et al. 2017*). Combing nitrogen topdressing with paclobutrazol could improve photosynthetic rate, increase grain weight per spike, and achieve further increase in wheat yield at jointing stage (*Yang et al. 2008*). Also, nitrogen topdressing at pseudostem erection stage with paclobutrazol application could increase dry matter accumulation, grain weight per spike, and grain yield (*Zhang et al. 2017*). On the one hand, appropriate amount of nitrogen and paclobutrazol could increase stem lodging resistance to guarantee high and stable yield (*Chen et al. 2011*). On the other hand, paclobutrazol regulated root morphological characteristics, maintained physiological function and root activities, increased grain yield by enhancing the levels of osmolytes, endogenous hormones contents, and antioxidant activities under disadvantage conditions (*Soumya et al. 2017; Kamran et al. 2018*). Additionally, nitrogen can also regulate endogenous hormone content and affect ear and flower development (*Ma et al. 2008a*). Spikelet number, regulated by hormones in spike and root, was closely related to nitrogen supply (*Chen et al. 2008; Zhang et al. 2009*). At early grain formation stage, zeatin riboside (ZR) content could effectively regulate panicle flower development and spikelet number (*Ma et al. 2008a*). Decreasing cytokinin and ABA contents in grain and root would be possible resulting in sterile spikelet (*Chen et al. 2008; Zhang et al. 2009*). Paclobutrazol application could influence hormone directly and modify flowering and development of plants (*Zhang et al. 2016*). During ear differentiation, cytokinins oxidation dehydrogenase (CKX), controlling endogenous cytokinins content, often negatively regulates flowering time and grain formation of crop (*Zalewski et al. 2010; Yeh et al. 2015; Ashikari et al. 2005; Bartrina et al. 2011*). For wheat, *TaCKX2.2* was involved in the formation of the spike grain number and yield (*Zhang et al. 2011*). Inhibiting *TaCKX2* expression by RNAi, the grain number per spike would be increased in bread wheat plants(*Li et al. 2018*). So far, it is still not clear when topdressing nitrogen combined paclobutrazol application is optimal for ear differentiation and individual wheat productivity; and how changing phytohormones and related gene expression in plant under different nitrogen topdressing treatments in the North China Plain. The objective of the present study was thus to evaluate the effect of topdressing nitrogen with paclobutrazol application on spike differentiation and to elucidate the possible mechanism of improving productivity of wheat. This study will help to provide guidance for optimizing irrigation and fertilizer and both combination measures in drought and semi-drought regions.

Materials and methods

Experimental site and soil

The field experiment was carried out from 2011 to 2013 on the super-high-yield testing farmland, Gaocheng, Shijiazhuang, China. The climate was classified as a subhumid continental monsoon. The mean annual rainfall is less than 520 mm, one-third of which would be allocated to wheat-growing season. Winter wheat-summer maize rotation is a typical planting system. The soil type was light-loamy Chao Soil. Soil properties are shown in Table 1.

Experimental design and crop management

The cultivar Shimai18 was provided by Shijiazhuang Academy of Agricultural Sciences. During the whole growth period, fertilizers were based applied with the rates of 112 kg ha^{-1} nitrogen, 60 kg ha^{-1} phosphorus, 87 kg ha^{-1} potassium and 9 kg ha^{-1} zinc. Another 112 kg ha^{-1} nitrogen topdressing was applied in next April. Fore-rotating corn straw crushed 2 times was returned to the field. Then rotary tillage operations were conducted 2 times with a 15 cm depth. Seeding rate was 150 kg ha^{-1}. Paclobutrazol was applied by a foliar spray of aqueous solution containing 10% wettable powder at effective concentration of 200 mg ha^{-1} and spraying amount of 675 kg ha^{-1}. Wheat was sown on October 7 in 2011 and October 9 in 2012. The equal row spacing was 0.15 m. Planting density was approximately 270◊10^4 plants ha^{-1}.

The experiment was a randomized block design with four experimental treatments including nitrogen topdressing at pseudostem erection stage (TS), TS with paclobutrazol application (TPS), nitrogen topdressing at jointing stage (TJ), TJ with paclobutrazol application (TPJ). Each treatment was replicated three times. Each plot area was 60 m^2.

Variable measurements

Young ears were sampled and then quickly-frozen at −80°C in the differentiation stages of stamen and pistils, early connectivum, late connectivum, and tetrad formation, separately. Partial young ears marked on the same flowering date would be sampled at 0, 3, 6, 9 and 12 days after flowering. From regreening stage, representative wheat plants were selected at 3 days intervals. The numbers of spikelets and florets were recorded in a constant state (on about 21st April) using anatomical lens (Stemi 2000-c, Germany).

Total ribonucleic acid (RNA) samples were extracted. RNA concentration and purity were detected by spectrophotometer (Nanodrop, USA); the RNA integrity was detected by agarose gel electrophoresis. And cDNA obtained by reverse transcription of RNA was used for subsequent fluorescence quantitative expression. Priming sequence: GGGAGAAGAAGCACTTTGGTC (*TaCKX2.2*-F); CCTGCAGTAAACTCAAACCATATC (TaCKX2.2-R). The optimal reference gene *GAPDH* was selected for mRNA transcription studies using quantitative real-time polymerase chain reaction (PCR).

Endogenous phytohormone content (IAA, auxin; ABA, abscisic acid; GA, gibberellin; cytokinin, CTK including isopentenyl adenosine-IPA and trans zein nucleoside-ZR) was measured by enzyme linked immunosorbent assay (ELISA). The test kit was provided by College of Agriculture and Biotechnology, China Agricultural University. Young ear samples were cut into

0.5 g pieces and were ground to a fine powder in liquid nitrogen with a pre-cooled mortar. The powder diluted with extracting solution was extracted 4 h at 4 ℃ in cold 80% methanol based on Oliver *et al*. (2007) protocol. The homogenate was then centrifuged at 3500 r min^{-1} for 20 min, and the supernatant was collected to be washed by 80% methanol in C-18 solid phase extraction column. Then, the filtered fluid of all the samples was pooled and dried in a vacuum chamber. Sample diluent was added to a final volume of 1 mL, which were diluted in tris-buffered saline and measured GA, CTK, ABA, IAA concentrations according to Jin *et al*. (2011).

Plant height was measured at pseudostem erection, jointing, booting, and ripening stage, respectively. Yield and yield components were measured by aconventional method. The harvest area was 3 m^2 in each plot. All plants were harvested, threshed, and dried to weigh seed yield. 1000-kernel weight was determined by counting two 500-kernel samples and weighing them with the relative error was less than 5%. If not, the third 500-kernel sample would be weighted. The number of spikes was recorded by counting the samples in one-meter-length at mature stage. The number of kernels per spike was counted in a set of 20 plants per plot.

Statistical analysis

All data were run using analysis of variance (ANOVA) with three replicates according to Excel 2003, SPSS17.0 (SPSS Inc., Chicago, USA). The Duncan's new Multiple Range (DMR) test at 5% probability level was used to test the differences among the mean values. Significant differences were labelled based on DMR.

RESULTS

Plant height

Table 2 showed that plant height increased gradually with the growth and development of seedlings in all treatments. At pseudostem erection stage, there were no significant differences among all untreated plots. At jointing stage, plant height in TS increased rapidly and was significantly higher than that in TPS, TJ (2011-2012) and TPJ (2011-2012). No obvious changes were observed between TJ and TPJ. At booting stage, the plant height in TS was higher significantly than other treatments; and TPJ was significantly lower than others in 2012-2013. No significant differences were existed between TPS and TJ. At mature stage, plant height reached to a peak value under all treatments. Also, the same changing trend and arranged order of plant height were observed at booting stage. These findings suggested that paclobutrazol application could inhibit plant growth effectively at different stages.

Wheat yield and yield components

Data from both years showed that grains per spike, thousand grain weight, and yield of wheat in TJ and TPJ were higher than those in TS and TPS (Table 3). The yield was observed in a decreasing order as TPJ>TJ>TPS>TS, in which TPJ was significantly higher than that in TPS and TS. Whether or not spraying paclobutrazol, grain yield and yield components of wheat were not affected obviously comparing two treatments of nitrogen topdressing at the same time. The thousand grain weight has shown that TJ and TPJ were significantly higher than TS, and TPJ was significantly higher than TPS in 2011-2012; TJ was significantly higher than TS and TPS in 2012-2013. This result suggested that nitrogen topdressing at jointing stage was more beneficial to grain weight than at pseudostem erection stage. The grains per spike in TJ and TPJ were significantly higher than in TS and TPS, and that in TPJ was significantly higher than in TS and TPS in 2011-2012. This value of TJ was significantly higher than that of TS and TPS in 2012-2013. The results also showed that grains per spike could be increased by 1.81-3.81 under nitrogen topdressing with paclobutrazol application at jointing stage. There were no significant differences in spikes per hm^2 among four treatments.

Spike differentiation

The numbers of spikelet and floret of wheat in TPS were both less than those in other treatments, which suggested that nitrogen topdressing with paclobutrazol application at pseudostem erection stage had evident inhibitory effect on spikelet differentiation (Table 4). Spikelet of wheat in TS, the most one, was significantly higher than in TPS, but had no obvious differences with that in TJ and TPJ. Floret of wheat in TPJ, the most one in 2011-2012, was significantly higher than that in TPS and TS; and this value in TPS was significantly lower than that in other treatments in 2012-2013. The florets differentiated from stamen and pistil of wheat in TPJ was significantly higher than in TJ (2011-2012), TS, and TPS, respectively; this value in TJ was significantly higher than in TPS and TS (2012-2013). The floret number at connectivum differentiation stage in TPJ, the most one, was significantly higher than other treatments in 2011-2012 and in 2012-2013(TJ exception), respectively. These results illustrated that nitrogen topdressing at jointing stage with paclobutrazol application was more helpful in improving development of florets, inhibiting its degeneration in wheat, and increasing grains number per spike.

Phytohormone content

The variation of endogenous hormone levels in wheat spike were similar among all treatments during differentiation of the main stem spike (Fig.1). However, the content of endogenous hormones was different at various phases of spike differentiation. The changes in CTK, IAA, and GA content showed a single-peak curve with peaks of CTK and IAA appearing at stamen and pistil differentiated stage and with peak of GA appearing at floret primordia differentiation stage. The contents of CTK, IAA, GA in spike under different treatments exhibited a decreasing order of TS>TPS>TJ>TPJ during peak periods. This result showed that it was easier to promote cumulation of growth acceleration hormone under nitrogen topdressing at pseudostem erection stage than at jointing stage. IAA content of wheat spike with paclobutrazol application was obviously decreased comparing with that without paclobutrazol treatment at pseudostem erection stage. But this value was significantly higher under nitrogen topdressing with paclobutrazol application than that without paclobutrazol treatment at jointing stage. The differences between two paclobutrazol treatments were increasingly smaller during the panicle differentiation. ABA content in spike showed an increasing trend throughout the growth period. At floret primordium formation stage, this value showed a descending order as TJ>TPJ>TS>TPS. At booting stage, it reached the maximum value and showed a descending order as TS>TPS>TJ>TPJ. These results suggested that ABA content could be decreased under nitrogen topdressing with paclobutrazol application at jointing stage.

IPA and ZR content

Further research on CTK had been conducted after tetrad stage. Fig. 2 showed an "up-down" single-peak changing trend of IPA, ZR, (IPA+ZR) content in wheat grains under all treatments from tetrad stage to flowering stage. The peak values appeared mainly at 0–6 days after flowering. There were no significant differences in IPA content among all treatments at tetrad stage. IPA content, which was the highest in TPJ at 0, 3, 12, and 15 days after flowers, showed significantly higher in TJ and TPJ than in TS and TPS at 6 days after flowers. ZR contents in grains under TPS and TPJ were both significantly higher than that under TS and TJ at booting stage. And this value was higher in TPJ and TJ than in TS and TPS at 0, 12, 15 days after flowering. Additionally, the highest ZR content appeared in TPJ and TJ at 3 and 9 days after flowering, respectively. The IPA+ZR content, showing similar changing trend to ZR content, was higher in TJ and TPJ than in TS and TPS. This value in TPJ was significantly higher than others at 3 and 15 days after flowering.

TACKX2.2 **Expression**

TACKX2.2 expression trending was shown in Fig. 3 under different treatments during spike development. From stamen and pistil differentiation to tetrad differentiation stage, the expression level of *TACKX2.2* was very low in all treatments. Thereafter, it rose sharply and reached a peak on 6 days after flowering under TS and TPS. During 0–9 days after flowering, the *TACKX2.2* expression in spike under TJ and TPJ was higher than that under TS and TPS obviously. And there were the first peak appeared on 0 day and the second higher peak appeared on 6 days after flowering. On 9 days after flowering, *TACKX2.2* expression was substantially decreased in all treatments. Thereafter, it rose modestly under TJ and TPJ with a higher rate of increase than under

TS and TPS at 12 days after flowering. These results suggested that *TACKX2.2* expression could be induced by paclobutrazol at jointing stage and its expression in TPJ reached maximum value during the flowering stage.

Correlation Analysis

The floret number had a significant linear correlation with IPA and ZR content during 0-12 days after flowering (Table 5). At 0 day, 6 days, and 12 days after flowering, the florets numbers from stamen and gynoecium and from connectivum were all very significantly correlated to cytokinin (IPA+ZR) content. At 3 days, 9 days, and 15 days after flowering, there were a significant correlation between cytokinin (IPA+ZR) content and numbers of floret differentiated from stamen and gynoecium (15 days exception) and from connectivum. These results showed that both IPA and ZR could control and regulate floret number and development during full-blossom period.

DISCUSSION

It was reported that nitrogen topdressing at jointing stage could increase the number of grains per ear and wheat yield (*Li et al. 2010*). The application of paclobutrazol combined with nitrogen fertilizer can improve wheat yield mainly due to increase in nitrogen use efficiency and reduction in nitrogen loss (*Nouriyani et al. 2012b*). In this study, the number of grains per ear, thousand kernel weight, and yield of wheat in TPJ were significantly higher than those in TS and TPS treatment, which was in agreement with the report of Wu *et al*. (2014). These results showed that nitrogen topdressing combined with paclobutrazol at jointing stage promoted more photosynthate accumulation in grain and improved nitrogen use efficiency. Paclobutrazol application decreased plant height effectively from booting stage to maturity stage; and that would be further decreased when the application time was delayed from pseudostem erection stage to jointing stage. The inhibitory effect of paclobutrazol on overgrowth of wheat plants could avoid excessive consumption of nutrient substance. Therefore, it is an effective measure to increase wheat yield by combing nitrogen topdressing and paclobutrazol application at jointing stage. Although a single application of paclobutrazol had no significant effect on yield and yield components, the increment of topdressing nitrogen with paclobutrazol on yield indexes was higher than those without paclobutrazol application. It was reported that the best effect was achieved at the combination of 150 mg L^{-1} paclobutrazol and 160 kg ha^{-1} nitrogen, for which paclobutrazol increased the absorption and transportation of nitrogen in plant and significantly affected photosynthetic pigments to keep high duration of flag leaf area and increase the grain yield (*Nouriyani et al. 2012a*). Paclobutrazol could also reduce plant height and flag-leaf area of black rice, but increase sucrose and amylopectin content in the grain at a concentration of 50 ppm (*Dewi and Darussalam, 2018*). Previous report indicated that paclobutrazol was prone to regulation on physiological balance at early growth stage, not on final yield of winter wheat directly (*Yang et al. 2008*). Therefore, the regulatory effect of paclobutrazol on plant would be different duo to different developmental phase of plant and concentration variation from paclobutrazol or nitrogen or both.

The panicle primordium differentiation stage is a critical phase inreproductive organ construction and the formation of final yield of wheat. Nitrogen could increase floret survival rate and grain number in wheat (*Ferrante et al. 2010*). And paclobutrazol could promote fertile florets differentiation and coordinate the vegetative and reproductive growth (*Hampton et al. 2006*). Further observation showed that the differentiation of young panicle could be obviously inhibited and the floret number was decreased under nitrogen topdressing with paclobutrazol at pseudostem erection stage comparing with at jointing stage. That may explain why grain number per ear and yield of wheat with nitrogen topdressing at pseudostem erection stage was lower than at jointing stage. It is known that the growth of young ears and development of spikelet was closely related to hormone content (*Zhang et al. 2009*). A recent study reported that paclobutrazol elevated endogenous auxin and abscisic acid levels, suppressed gibberellins (GA4) and trans-zeatin concentrations of plant (*Opio et al. 2020*). However, another report showed that paclobutrazol significantly decreased the content of GA3 and IAA, and increased ABA contents in leaves of wheat (*Aly et al. 2011*). This study indicated that paclobutrazol application took a decreasing effect on the number of spikelet and floret at pseudostem erection stage, but took an increasing effect on floret number at jointing stage according to two-years data. The application of paclobutrazol inhibited not only GA content during spike differentiation but also CTK, IAA, and ABA content before tetrad stage. Maybe this is possible due to dynamic phytohormone levels and an inconstant inhibitory effect of paclobutrazol on spike differentiation. The mutual antagonism and interactions between those hormones varied in different growth stages of crops (*Wu et al. 2019*). Additionally, IAA, CTK, and GA contents in spike were lower or not different under nitrogen topdressing with or without paclobutrazol at jointing stage comparing with that at pseudostem erection stage. This result suggested that the differences in number of spikelets and florets among all treatments were not determined mainly by the phytohormone levels at this stage. At tetrad stage, ABA content in spikelet under TPS was higher than that under TPJ, suggesting that other promoting hormone would take possible regulatory effect on panicle and floret development after tetrad stage.

Cytokinin, which was degraded by cytokinin oxidizes dehydrogenase to IPA and ZR, has played an important role in early spikelet development and panicle differentiation (Ma *et al*. 2008b; Jameson *et al*. 2015). From tetrad stage to anthesis stage, cytokinin content in spikelet was further researched to reveal the possible regulatory mechanism. At tetrad stage, ZR content in spikelet under nitrogen topdressing with paclobutrazol treatment was significantly higher than that without paclobutrazol treatment, which help to increase yield sink capacity (*Dewi and Darussalam, 2018*). From this stage onwards, the contents of ZR and IPA and the expression level of *TACKX2.2* were increased obviously with nitrogen topdressing during 0–6 days after flowering; and the increment was higher at jointing stage than at pseudostem erection stage. It may be suggested that cytokinin oxidation dehydrogenase would perform stronger degradation to maintain hormone balance when accumulation of cytokinin in ear exceeded demand, which was often resulted from the rapid development of wheat ears with nitrogen topdressing during reproductive growth (*Werner et al. 2006; Zhang et al. 2011*). Also, panicle development was faster under nitrogen topdressing at jointing stage, when *TACKX2.2* expression was higher than that at standing stage. Meantime, paclobutrazol application would increase *TACKX2.2* expression to inhibit cytokinin content. Our study revealed that the contents of IPA and ZR in TPJ, ZR and (IPA+ZR) in TPS at 9days and 3 days after flowering, respectively, were suppressed by paclobutrazol, which was partly consistent with Opio *et al*. (2020). However, the contents of ZR and ZR + IPA in TPJ were elevated significantly by paclobutrazol at 3 days after flowering. These results can be interpreted that paclobutrazol induced early flowering by increasing ABA and cytokinins contents in buds, which would regulate increases in leaf water potential and carbon-nitrogen ratio of mango (*Upreti et al. 2013*). This study also showed that cytokinin (IPA+ZR) content was significantly correlated with the florets number at stamen and gynoecium differentiation and at connectivum differentiation stage. It was established that paclobutrazol had regulated floret differentiation and development indirectly by regulating cytokinin content and influenced the final yield. There were also lots of environmental factors, such as water status, paclobutrazol concentrations, growth stage, etc, should be involved in the process of understanding the regulatory process. Additionally, the differences of gene expression in spike among treatments was not only influenced by cytokinin content but also by multiple hormone interactions and other hormone levels changing at crucial stage. Further research still required to elucidating the main molecular mechanism by which assimilate of grain increased by nitrogen and paclobutrazol treatment

Conclusion

In this paper, it could be concluded that nitrogen topdressing at jointing stage significantly increased floret number, kernels number per spike, and yield. And this result was partly related to cytokinin (especial ZR) content increasing after tetrad stage. Application of paclobutrazol could delay spike development to some extent and promote cytokinin content elevation, along with *TACKX2.2* relative expression increasing to keep hormonal equilibrium and regulate spikelet and floret number. It is crucial to focus future research on the deep mechanism of interaction between paclobutrazol and phytohormone. Therefore, our study shows still an effective measure to promote wheat productivity by nitrogen topdressing with paclobutrazol at jointing stage.

ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Dongxiao Li conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Shaojing Mo analyzed the data, authored or reviewed drafts of the paper.
- Ruiting Cheng and Hongguang Wang analyzed the data, authored or reviewed drafts of the paper, and approved the final draft
- Ruiqi Li conceived and designed the experiments, authored or reviewed drafts of the paper, funding, and approved the final draft.

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Table 1(on next page)

Soil conditions of the experimental field

Each data indicates the nutritious elements contents in different soil layers.

Table 1. Soil conditions of the experimental field.

Year	Soil layers (cm)	Organic Matter ($g \cdot kg^{-1}$)	Total N ($g \cdot kg^{-1}$)	Available N ($mg \cdot kg^{-1}$)	Available P ($mg \cdot kg^{-1}$)	Available K ($mg \cdot kg^{-1}$)
2011-2012	0-20	13.2	1.1	148.8	33.0	128.6
	20-40	5.6	0.6	60.5	7.3	50.0
2012-2013	0-20	20.3	0.9	124.5	21.4	133.3
	20-40	9.6	0.4	42.0	7.5	52.6

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Table 2(on next page)

Plant height of wheat under different treatments

Each data point indicates the average plant height of 5 repeats plants at different treatments.

Table 2. Plant height of wheat under different treatments.

Year	Treatment	Plant height (cm)			
		Pseudostem erection stage	Jointing stage	Booting stage	Maturity stage
2011-2012	TS	17.8a	29.7a	60.3a	69.2a
	TPS	17.5a	28.1b	58.6b	67.6b
	TJ	19.0a	27.6c	57.1bc	66.4b
	TPJ	18.3a	27.6c	56.3c	64.1c
2012-2013	TS	19.1a	27.1a	54.4a	68.7a
	TPS	18.7a	23.6b	51.3b	67.1b
	TJ	19.0a	26.0a	50.7b	66.0b
	TPJ	19.4a	26.0a	48.1c	61.7c

Note: Different letters in each column indicate significant differences among four treatments, assessed by ANOVA ($P \leqslant 0.05$). TS, nitrogen topdressing at erecting stage; TPS, TS combined with paclobutrazol application; TJ, nitrogen topdressing at jointing stage; TPJ, TJ combined with paclobutrazol application.

Table 3(on next page)

Wheat yield and yield components under different treatments

Each data point indicates the wheat yield and yield components in three repeats testing plots with different treatments.

Table 3. Wheat yield and yield components under different treatments.

Year	Treatments	Spikes ($\times10^4\cdot ha^{-1}$)	Grains Per spike	Thousand grain weight (g)	Yield ($t\cdot ha^{-1}$)
2011-2012	TS	796.7a	33.6b	39.2c	8.8b
	TPS	752.3a	34.4b	39.4bc	8.9b
	TJ	798.9a	36.2a	40.6ab	9.4ab
	TPJ	807.8a	36.9a	41.6a	9.7a
2012-2013	TS	829.4a	33.3bc	28.0b	7.5b
	TPS	834.9a	32.9c	27.8b	7.6b
	TJ	806.0a	35.8ab	31.7a	7.8ab
	TPJ	823.4a	36.7a	30.5ab	8.1a

Note: Different letters in each column indicate significant differences among four treatments, assessed by ANOVA ($P \leqslant 0.05$). TS, nitrogen topdressing at erecting stage; TPS, TS combined with paclobutrazol application; TJ, nitrogen topdressing at jointing stage; TPJ, TJ combined with paclobutrazol application.

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Table 4 (on next page)

Spike differentiation of wheat under different treatments

Each data point indicates the average performance of 5 repeats in spike differentiation indexes under different treatments.

1 **Table 4. Spike differentiation of wheat under different treatments.**

Year	Treatments	Differentiation Spikelet number	Differentiation Floret number	Floret number differentiated from stamen and gynoecium	Floret number differentiated from connectivum
2011-2012	TS	20.8a	140.8b	118.4b	52.2c
	TPS	19.2b	138.2b	102.2c	56.8b
	TJ	20.8a	145.4ab	115.4b	59.0b
	TPJ	20.4ab	150.2a	123.6a	62.6a
2012-2013	TS	19.4a	148.8a	99.4b	67.0b
	TPS	18.4b	141.8b	96.4b	66.2b
	TJ	18.8ab	150.4a	112.6 a	87.6a
	TPJ	19.0ab	149.6a	113.8a	85.8a

2 Note: Different letters in each column indicate significant differences among four treatments, assessed by
3 ANOVA ($P \leqslant 0.05$). TS, nitrogen topdressing at erecting stage; TPS, TS combined with paclobutrazol
4 application; TJ, nitrogen topdressing at jointing stage; TPJ, TJ combined with paclobutrazol application.

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Table 5(on next page)

The correlation between (IPA+ZR) content and floret number

Each data point indicates the correlation coefficient of 12 runs between (IPA+ZR) content and floret number differentiated from connectivum, stamens and pistils.

Table 5. The correlation between (IPA+ZR) content and floret number. **P < 0.01 and *P<0.05.

content of (IPA+ZR)	florets differentiated from connectivum	florets differentiated from stamen and gynoecium
0 day after flowering	0.825**	0.855**
3 days after flowering	0.685*	0.63*
6 days after flowering	0.766**	0.725**
9 days after flowering	0.600*	0.683*
12 days after flowering	0.913**	0.875**
15 days after flowering	0.695*	0.549

Notes. *, ** significant at the 0.05 and 0.01 probability levels, respectively, ns, no significant. Each data point represents the average of the measured data.

Figure 1(on next page)

Dynamic of endogenous hormone levels in ears during spike differentiation

E Each data point indicates the average performance of 5 repeats in phytohormone contents with different stages.

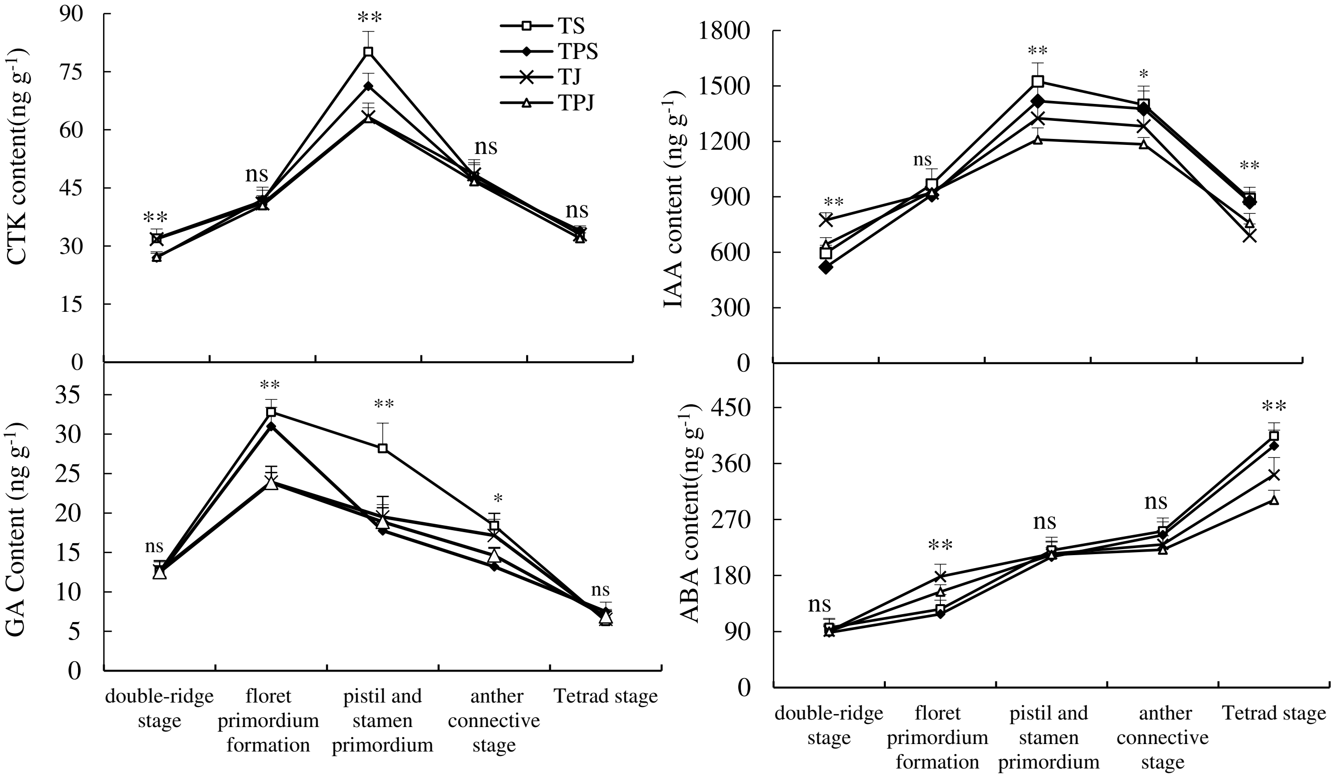

Figure 1 Dynamic of endogenous hormone levels in ears during spike differentiation. *, ** significant at the 0.05 and 0.01 probability levels, respectively, ns, no significant. Each data point represents the average of the measured data.

Figure 2

IPA and ZR content of spikes under all treatments at different stages

Each data point indicates the average performance of 5 repeats in spike with different treatments during all flowering stage.

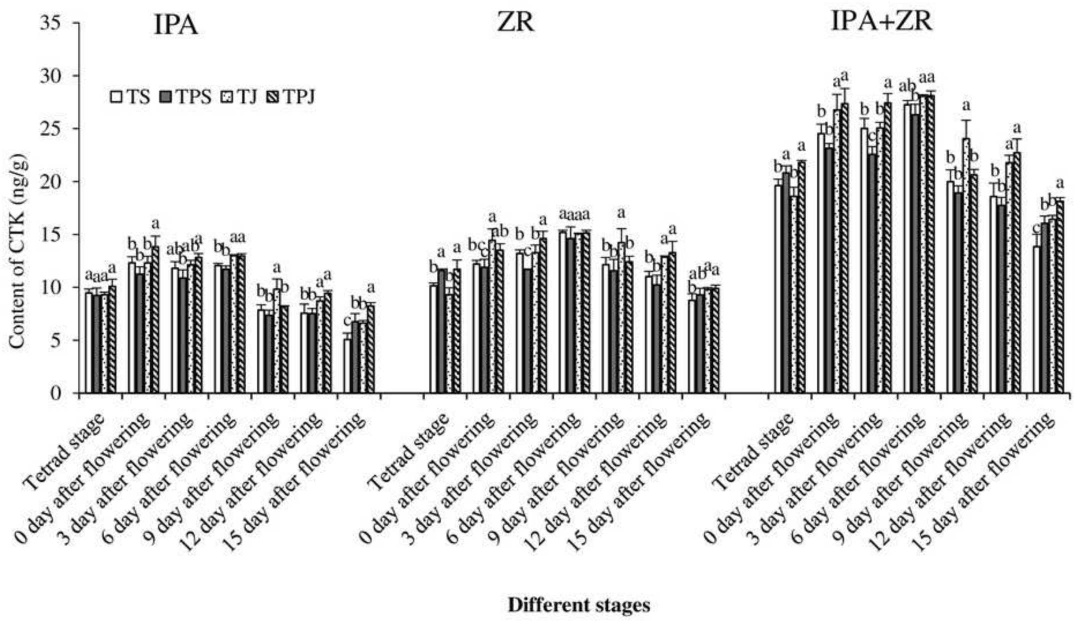

Fig.2. IPA and ZR content of spikes under all treatments at different stages. Different letters indicate significant differences among four treatments, assessed by ANOVA (P ≤ 0.05).

Figure 3

The changing trend of relative expression of TACKX2.2 at different stages

Each data point indicates the average performance of 3 repeats samples after randomly choosing with all treatments at different spike differentiational stages

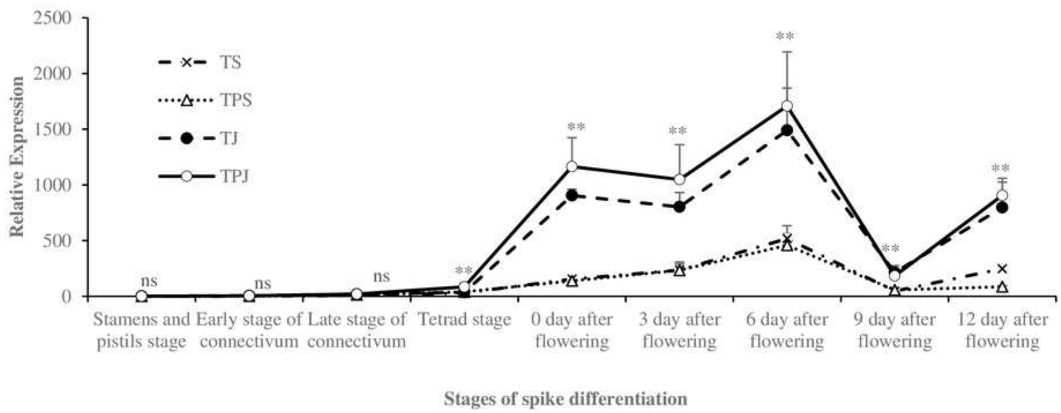

Figure 3 The changing trend of relative expression of *TACKX2.2* at different stages. *, ** significant at the 0.05 and 0.01 probability levels, respectively, ns, no significant. Each data point represents the average of the measured data.