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RESEARCH ARTICLE

## Gene and protein expression profiling analysis of young spike development in large spike wheat germplasms



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CHEN Dan<sup>1,2\*</sup>, ZHANG Jin-peng<sup>1\*</sup>, LIU Wei-hua<sup>1</sup>, WU Xiao-yang<sup>1</sup>, YANG Xin-ming<sup>1</sup>, LI Xiu-quan<sup>1</sup>, LU Yu-qing<sup>1</sup>, LI Li-hui<sup>1</sup>

<sup>1</sup> National Key Facility for Crop Gene Resources and Genetic Improvement/Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R.China

<sup>2</sup> Biotechnology and Germplasm Resources Institute, Yunnan Academy of Agricultural Sciences/Key Laboratory of Southwestern Crop Gene Resources and Germplasm Innovation, Ministry of Agriculture/Scientific Observation for Rice Germplasm Resources of Yunnan, Ministry of Agriculture/Yunnan Provincial Key Laboratory of Agricultural Biotechnology, Kunming 650223, P.R.China

### Abstract

The wheat grain number per spike (GNPS) is a major yield-limiting factor in wheat-breeding programs. Germplasms with a high GNPS are therefore valuable for increasing wheat yield potential. To investigate the molecular characteristics of young spike development in large-spike wheat germplasms with high GNPS, we performed gene and protein expression profiling analysis with three high-GNPS wheat lines (Pubing 3228, Pubing 3504 and 4844-12) and one low-GNPS control variety (Fukuho). The phenotypic data for the spikes in two growth seasons showed that the GNPS of the three large-spike wheat lines were significantly higher than that of the Fukuho control line. The Affymetrix wheat chip and isobaric tags for relative and absolute quantitation-tandem mass spectrometry (iTRAQ-MS/MS) technology were employed for gene and protein expression profiling analyses of young spike development, respectively, at the floret primordia differentiation stage. A total of 598 differentially expressed transcripts (270 up-regulated and 328 down-regulated) and 280 proteins (122 up-regulated and 158 down-regulated) were identified in the three high-GNPS lines compared with the control line. We found that the expression of some floral development-related genes, including *Wknox1b*, the AP2 domain protein kinase and the transcription factor *HUA2*, were up-regulated in the high-GNPS lines. The expression of the *SHEPHERD* (*SHD*) gene was up-regulated at both the transcript and protein levels. Overall, these results suggest that multiple regulatory pathways, including the *CLAVATA* pathway and the meristem-maintaining KNOX protein pathway, take part in the development of the high-GNPS phenotype in our wheat germplasms.

**Keywords:** wheat, high grain number per spike, spike development, expression profiling

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CHEN Dan, Mobile: +86-13668714841, E-mail: [xiaoyezi09@163.com](mailto:xiaoyezi09@163.com);  
ZHANG Jin-peng, Mobile: +86-13269833221, E-mail: [zhangjinpeng@caas.cn](mailto:zhangjinpeng@caas.cn);  
Correspondance LI Li-hui, Tel: +86-10-62186670, Fax: +86-10-62189650, E-mail: [lilihui@caas.cn](mailto:lilihui@caas.cn)  
\* These authors contributed equally to this study.

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## 1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops throughout the world. Enhanced sustainable wheat production is therefore essential for meeting the increasing food demand caused by global population growth and climate change. Wheat yield increases depend on

three factors, namely the spike number per square (SNPS), grain number per spike (GNPS), and the thousand-kernel weight (TKW). In recent decades, increases in the SNPS and TKW have approached saturation, such that future wheat yield increases should focus on increased GNPS (Zhuang 2003).

Good genetic resources with high GNPS levels have significant applications for potential yield increases in crop breeding. Our previous studies have shown that 4844-12, Pubing 3504 and Pubing 3228, were derived from the same cross between wheat and its wild relative *Agropyron cristatum* (*A. cristatum*), represent three high-GNPS wheat lines (Li et al. 1998; Wu et al. 2006; Zhang et al. 2011). Cytogenetic analysis has demonstrated that the high-GNPS trait observed in the addition line 4844-12 is derived from the alien 6P chromosome of *A. cristatum* (Wu et al. 2006). Pubing 3228 and Pubing 3504 are both derived from the addition line 4844-12 and show high-GNPS, narrow-sense heritability along with wide environmental adaptability and genetic stability (Chen et al. 2012). Multiple important quantitative trait loci (QTLs) that control GNPS and yield-related traits in the Pubing 3228 line have been mapped to chromosomes 1A, 4A, 4B, 5A, and 6A (Wang et al. 2011). These germplasms with high GNPS values represent valuable materials for research on the molecular mechanisms of large-spike wheat formation.

Many efforts have been made to understand the genetic and molecular mechanisms underlying spike development in cereals. Some key genes and proteins that take part in spike development have been identified (Chuck et al. 2002; Komatsu et al. 2003). Phytohormones also play a crucial role in meristem determination and differentiation (Barazesh and McSteen 2008). Moreover, the *CLAVATA* (*CLV*) pathway plays a critical role in the differentiation of stem cells by restricting the expression of *WUSCHEL* (*WUS*); the *CLV*/*WUS* pathway is also involved in CTK-regulated signaling circuits and cellular processes in *Arabidopsis* (Brand et al. 2000; Leibfried et al. 2005; Sablowski 2009; Yadav et al. 2011). In monocot plants, the key genetic and molecular switches have been reviewed in two model grass species,

namely rice and maize; such switches include meristem identity, meristem size and maintenance, the initiation and outgrowth of axillary meristems, and organogenesis (Zhang and Zheng 2014).

However, the molecular mechanisms of high-GNPS development in wheat remain unclear. The purpose of this study was to analyze the molecular mechanisms of spike development in our high-GNPS lines. We attempted to identify interesting genes or proteins that were involved in the formation of the high-GNPS phenotype by analyzing differentially expressed genes at both the RNA and protein levels during the spike differentiation stage, thereby providing a molecular basis for cloning candidate genes and for future breeding applications.

## 2. Results

### 2.1. Investigation of spike morphological features in the high-GNPS germplasm

Morphological observation of the young spikes showed that each developmental stage was retarded in the high-GNPS lines compared with the control Fukuho cultivar (Appendix A). The differentiation stage for Fukuho occurred on March 22th, whereas that of the three high GNPS lines occurred 5–7 d later. However, the length of the floret primordia differentiation stage in the young spikes did not significantly differ between the three high-GNPS lines and the Fukuho control. Investigation of the spike traits at the mature stage indicated that the three high-GNPS lines showed significant differences ( $P < 0.01$ ) compared to the Fukuho cultivar in GNPS, spikelet number per spike (SPN), kernel number per spikelet (KNS), and spike length (SL) (Table 1, Fig. 1-D and E). In two growing seasons, the average GNPS in the three high-GNPS lines ranged from 76.51 to 117.36, whereas that of Fukuho was 59.23. In the high-GNPS lines, the average SPN ranged from 22.31 to 25.29, and the average KNS ranged from 5.92 to 7.19. The SPN and KNS values for the Fukuho cultivar were 18.68 and 4.35, respectively. The spike feature data suggested that both SPN and KNS

**Table 1** Investigation of mature spike traits in three high-GNPS wheat lines<sup>1)</sup>

Material	Chromosome composition <sup>2)</sup>	GNPS		SPN		KNS		SL	
		2007–2008	2008–2009	2007–2008	2008–2009	2007–2008	2008–2009	2007–2008	2008–2009
Pubing 3504	42W	118.90 A	116.88 A	24.80 A	25.44 A	7.00 A	7.25 A	13.95 A	11.48 A
Pubing 3228	42W	88.10 B	105.83 B	24.20 A	23.43 B	5.30 B	6.83 B	12.28 B	10.31 B
4844-12	42W+2P	92.30 B	71.07 C	23.00 B	22.07 C	6.30 A	5.79 C	11.28 C	8.47 D
Fukuho	42W	59.70 C	59.07 D	19.20 C	18.50 D	4.10 C	4.43 D	10.19 D	9.56 C

<sup>1)</sup> GNPS, grain number per spike; SPN, spikelet number per spike; KNS, kernel number per spikelet; SL, spike length.

<sup>2)</sup> W and P denote wheat and *Agropyron cristatum* chromosomes, respectively.

Values followed by the same letters were not significantly different at  $P < 0.01$  (Duncan's multiple range test,  $n=30$ ). The data are means.

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