eJBio

*Electronic Journal of Biology*, 2007, Vol. 3(1): 18-20

# Differential Gene Expression Patterns in the Basal Stems of Wheat at the Jointing Stage

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## Abstract

Improved differential display of mRNA was used to analyze alterations in gene expression during the internode elongation of basal stem of wheat plant. Three patterns of differential expression were observed: (I) bands observed in pre-elongation basal stem tissue, but not in elongated; (II) bands occurring in elongated but not in pre-elongation basal stem tissue; (III) bands vibrated during the phase. Three differentially expressed cDNA fragments (*WSR1-3*) had been cloned and sequenced. Sequence homology search in GenBank database showed that one (*WSR2*) of them has no homology hits; one transcripts (*WSR1*) had similarity to barley serine/threonine protein kinase; while the product of *WSR3* showed high similarity to Ty3/gypsy-type retrotransposon reverse transcriptase, which indicated diverse pathways may be involved in the internode elongation of wheat stem.

**Keywords:** Wheat; Internode elongation; Gene expression; Differential display.

## 1. Introduction

In general, an increase in wheat height can result in increased lodging possibility and severity [1,2]. The jointing stage is the key phase for the wheat height determination and certain genetic pathways involved in the molecular regulation of the rapid internode elongation of basal stems. Dwarf genes have been utilized extensively in wheat breeding to improve lodging resistance, and the introduction of dwarf cultivars was a major factor in the success of the "Green Revolution" in wheat [3]. The important dwarf genes that have been used in agriculture are mutations of genes in the gibberellin biosynthesis or response pathways [4], while little is know about the molecular mechanism of the wheat height regulation. The study of internode elongation in molecular level may be useful for improving wheat crop because many current cultivars are prone to stem lodging, which can lead to yield loss and difficulty harvesting.

In this paper, we report on the differential gene expression during the internode elongation of basal stem of wheat plant. Three differentially expressed cDNA fragments were cloned and sequenced, and possible roles in internode elongation were discussed.

## 2. Materials and Methods

### 2.1. Plant materials

Field grown identical plants of wheat variety Zhengmai 9023 were used. The pre-elongation basal stem tissue and 3 types of the elongated basal stems, of which the first basal internode are 0.2cm, 0.5cm and 1.0cm respectively, were dissected from wheat plants and immediately frozen in liquid nitrogen.

### 2.2 RNA extraction

Total RNA was prepared from each sample by using the RNeasy kit (Sangon, Shanghai, China) according to the manufacture's instruction. The RNA preparations were subjected to DNase digestion in the presence of recombinant ribonuclease inhibitor.

### 2.3 Reverse transcription

Equal amounts of 2μg RNA each were transcribed into cDNA in 20μl reactions containing 50mM Tris-HCl (pH8.3), 75 mM KCl, 3mM MgCl2, 10mM DTT, 50μM dNTPs, 200U MMLV reverse transcriptase (Promega, Madison, USA) and 50pmol either one base anchor oligonucleotides AAGCT11A, AAGCT11C, or AAGCT11G. Reverse transcription was performed for 60 min at 37℃ with a final denaturation step at 95℃ for 5 min.

### 2.4 PCR amplification of cDNA

The following primers were synthesized according to Von der Kammer's report [5].

3' end anchored primers are as follows:

HT11A: 5'-AAGCTTTTTTTTTTA-3'

HT11C: 5'-AAGCTTTTTTTTTTTC-3'

HT11G: 5'-AAGCTTTTTTTTTTG-3'

5' end oligonucleotide primers are as follows:

DD18: 5'-TGCCGAAGCTTTGGTCAC-3'

DD19: 5'-TGCCGAAGCTTTGGTCAG-3'

DD20: 5'-TGCCGAAGCTTTGGTCAT-3'

DD23: 5'-TGCCGAAGCTTGATTCCG-3'

DD32: 5'-TGCCGAAGCTTGGAGCTT-3'

DD60: 5'-TGCCGAAGCTTCGACTGT-3'

### 2.5 Electrophoresis

PCR products were separated on 0.4mm thick, 4% denaturing polyacrylamide sequencing gels in a temperature-regulated Bio-Rad Sequencing System (Bio-Rad, California, USA) at 50℃. Gels were silver-stained and photographed.

### 2.6 Cloning, sequencing and sequence analysis

Bands that showed differences were excised from the gel and reamplified using the following PCR conditions: 1 min at 94℃; 45sec at 94℃, 2 min at 60℃, 1 min at 72℃, followed by 40 cycles; One final step at 72℃ for 5 min was added to the last cycle. To ensure that there is no DNA contamination in RNA samples, a negative control was prepared without reverse transcription. The single specific band of PCR were ligated into pGEM-T easy vector (Promega, Madison, USA) and sequenced. A BLAST search was completed in NCBI (http://www.ncbi.nlm. nih.org).

## 3. Results and Discussion

### 3.1. Differential expression Patterns of wheat basal stems during the internode elongation

A total of 1529, 1507, 1475 cDNA fragments were amplified by using 18 primer combinations which include three one-base anchored primers and six 5' end oligonucleotide primers for the elongated basal stems (the first internode is about 0.2cm, 0.5cm and 1.0cm respectively), in which 331(21.7%), 340(22.6%) and 346(23.5%) were found to be differentially expressed as compared to those of the pre-elongation basal stem tissues. This indicated that significant alterations in gene expression occurred during the internode elongation of wheat stem.

When comparing the patterns of differentially expressed cDNAs between elongated and pre-elongation basal stems, it was found that both qualitative and quantitative difference can be observed. Since quantitative differences could not be accurately examined, we only analyzed the qualitative differences which include three categories, that is, bands observed in pre-elongation basal stem tissue, but not in elongated (BBnE, Fig1.A); bands occurring in elongated but not in pre-elongation basal stem tissue (BEnB, Fig1.B) ; bands vibrated during the phase (BV, Fig1.C1-3).

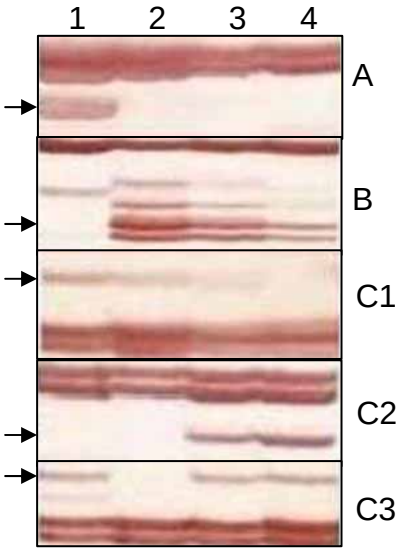


Fig1 Differential expression Patterns in wheat basal stems during the internode elongation. 1:The pre-elongation basal stem tissue; 2-4: the elongated basal stems, of which the first basal internode are 0.2cm，0.5cm and 1.0cm respectively; A, bands observed in pre-elongation basal stem tissue, but not in elongated ones (BBnE); B, bands occurring in elongated but not in pre- elongation basal stem tissue (BEnB); C1-3, bands vibrated during the phase (BV).

### 3.2 Cloning and sequencing of differentially expressed cDNAs

Three differentially expressed cDNA fragments (*WSR1-3*), which belong to BBnE, BBnE and BEnB expression patterns respectively, were cloned and sequenced. TBLASTX search of sequenced cDNAs in GenBank showed that *WSR2* has no homology hits, *WSR1* can encode a serine/threonine kinase - like protein; while *WSR3* can encode a polypeptide highly similar to the Ty3/gypsy-type retrotransposon reverse transcriptase (Table 1).

Protein phosphorylation is the most common mechanism of protein functional regulation known to date. In eukaryotes, serine/threonine protein kinases is a large family of functional enzymes that transfer the terminal phosphate from ATP to a specific Ser or Thr residue on protein substrates [6]. It has been proved that the barley (*Hordeum vulgare* L.) stem rust (*Puccinia graminis f. sp. tritici*) resistance gene Rpg1 is a serine/threonine protein kinase with two tandem kinase domains [7]. In this study, the differentially expressed gene *WSR1* can encode a serine/threonine kinase-like protein; further analysis of *WSR1* revealed it can encode a polypeptide with an kinase catalytic domain. It seems that *WSR1* might participate the regulation of multiple cellular

responses during the internode elongation of wheat stems and the functional analysis of *WSR1* is still ongoing.

Retrotransposons are ubiquitous in plants genome and play a major role in plant gene and genome evolution [8], it can generate mutations by inserting near or within genes, and these elements may provide regulatory sequences for gene expression and alter the expression of adjacent genes [9, 10]. These studies indicated that the retrotransposon activity changes might cause alterations in the expression patterns of other genes. In this study, the change of *WSR3* expression profile may contribute to modifications in the expression of other genes during the internode elongation of wheat plant.

These differentially expressed genes in the basal stems indicated diverse pathways may be involved in the internode elongation of wheat basal stems at the jointing stage.

Table 1. Expression patterns and BLAST search of differentially expressed cDNAs

| Name | Expression pattern based on DDRT | Best homology | The accession numbers of the homologous sequences | E-value |
|---|---|---|---|---|
| *WSR1* | BBnE | barley serine/threonine kinase | DQ469714 | 5e-14 |
| *WSR2* | BBnE | None | ----- | ------ |
| *WSR3* | BEnB | Zea mays Ty3/gypsy-type retrotransposon reverse transcriptase | AF030633 | 1e-45 |

## 4. Conclusion

The jointing stage is the key phase for plant height determination, lodging control and even high yield formation, and the rapid elongation of the wheat stems must be regulated by certain genetic pathways. The results from these experiments demonstrate that more than 1000 cDNAs were found to be differentially expressed (qualitatively or quantitatively) during the internode elongation. Analysis of three cloned cDNAs showed that diverse pathways may be involved in the internode elongation. In general, many genes participate the regulation of internode elongating and the cloned cDNAs *WSR1* and *WSR3*, which coding a kinase-like and retrotransposon reverse transcriptase-like protein respectively, will provide a new starting point to understand the regulatory mechanism of internode elongation of wheat.

## Acknowledgements

We are grateful to Yongwei Zhang and Jing Han for helpful assistance in experiments. This work was supported by the Innovation Foundation for Natural Science and Technology of Henan Agricultural University, China (No. 2006-CX-022).

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