

Short Communication

Interconnection between Wheat ABCC13 Transporter and Auxin-Related Genes during Lateral Root Development

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Abstract

TaABCC13 is member of wheat ABCC subclass of transporters. The RNAi mediated silencing of this transporter in wheat results in lowering of seed phytic acid level and other developmental defects. In addition to that, wheat ABCC13 was involved in cadmium detoxification as evident by the complementation assays in yeast. The appearance of early lateral roots in these transgenic seedlings speculated the possibility for studying the role of localized auxin-mediated effects. In the current study, firstly, the expression of auxin related genes was studied in the transgenic roots. Enhanced expression of genes pertaining to either auxin biosynthesis or its transport was observed in transgenic wheat seedling roots suggesting the direct effect of the hormone. Further, the early emergence of lateral roots in transgenic wheat seedlings was affected due to the presence of auxin-transport inhibitor suggesting the direct effect of hormones in root development. In conclusion, herein we provide the novel evidence for the auxin mediated regulation of lateral root emergence in *TaABCC13*:RNAi seedlings.

Key words: ABCC transporter; lateral roots; *Triticum aestivum*; auxin; transport inhibition

1. Introduction

Plasticity and adaptability against environmental stress are the most efficient abilities of plants. Adaptations with respect to soil related nutritional and other abiotic stresses majorly affect the root organization and architecture [1, 2]. In this regard, development of the lateral roots is the key determinant of agronomic performance by plants [3]. Studies with *Arabidopsis*, maize and wheat plants has emphasized on the phytohormones based regulation of lateral root formation. In particular, the plant response to environmental stresses like heavy metal and deprivation of nutrients are important factors [4-6]. At the anatomical level, contrasting development stages of lateral root origin was observed for *Arabidopsis* and cereal crops like maize and wheat [4]. Nonetheless, auxins have been considered as a common factor and determinant of root organization and lateral root origin among all plants.

Most of our understanding about role and mechanism of auxin action is comes from the study of *Arabidopsis* auxin signaling components [7-9]. At the molecular level, members from small gene families like *IAA*, *SAUR* (Small Auxin-Up RNA), *PIN* (PIN-FORMED) and *AUX/LAX* (*AUXIN1*/LIKE *AUX1*) are important markers for studying auxin responses [10]. Plant ABCC transporter has been extensively studied for their roles in wide range of cellular and physiological functions [11-12]. Previous reports have strongly supported the altered root phenotype in case of low phytic acid *Arabidopsis* and maize with mutations in genes of ABCC subfamily transporter [13-14].

Earlier, in an attempt to provide insight into this phenomenon of early lateral root emergence it was speculated that ABC transporters might control auxin dependent lateral root emergence [13,15]. Earlier, we validated the involvement of TaABCC13 in seed phytic acid accumulation and heavy metal responses in developing roots [6]. Interestingly, silencing of wheat transporter *ABCC13* resulted in enhanced emergence of short roots at the early stages of development. Herein, we

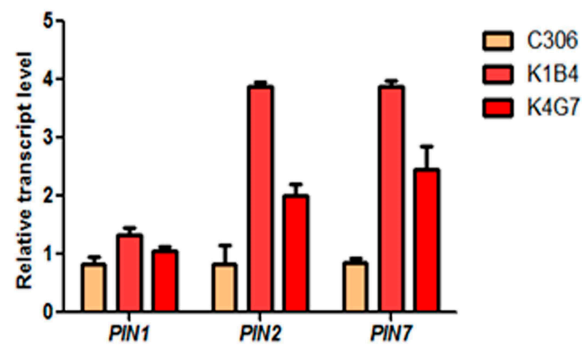
validated the involvement of auxin biosynthesis and transport related genes in the wheat transgenic showing enhanced lateral roots.

2. Results and Discussion

Early lateral roots in TaABCC13:RNAi seedling is auxin dependent

To test if auxin is involved during the early lateral root phenotype of *TaABCC13*:RNAi seedlings, mRNA expression for the subset of genes pertaining to either auxin transport or biosynthesis [16] (Table 1) was analyzed. In our experiment, the expression level of early auxin responsive genes was observed to be significantly high in transgenic roots (with lateral root phenotype) in comparison to roots from control seedlings. In particular, the expression of wheat *IAA1* and *IAA7* was higher in both RNAi lines while *IAA2* transcript was significantly high in K1B4-2-5. The genes encoding the auxin transporters *PIN2* and *PIN3* also expressed high in K1B4-2-5 as compared to control roots (Figure 1A and B). Although, PIN transporters have been extensively studied for their polar auxin transport activities in *Arabidopsis* [17], but limited studies were undertaken in cereal crops where it is supposed to be even more complex [18]. These observations are in agreement with previous report where treatment of wheat seedlings with exogenous auxin also resulted in enhanced expression of marker genes [16]. Thus, collectively we can hypothesized that increased mRNA transcript of *IAA* related genes in roots of wheat with *ABCC13* silenced lines represented a high localized auxin activity.

(A)



(B)

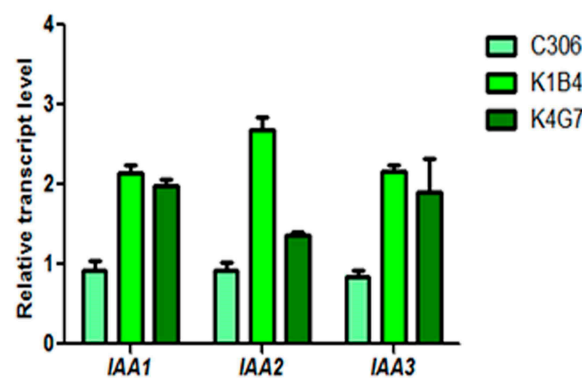


Figure 1

Effect of auxin transport inhibitor on lateral root development

To further confirm the involvement of auxin during the lateral root development, inhibitory studies were performed. Wheat seedlings were exposed to the inhibitor for polar auxin transport. One of the commonly used such inhibitor is NPA (*N*-1-Naphthylphthalamic acid). The application of auxin transporter inhibitor NPA resulted in apparent reversal of lateral root phenotype in

TaABCC13:RNAi lines (Figure 2). The short lateral roots visible in the transgenic wheat were not developed in presence of auxin inhibitor suggesting the possible involvement in structuring the root phenotype. Therefore, the suppression of polar auxin transport in presence of NPA resulted in reversal of lateral root phenotype. Our results confirmed the up-regulation of auxin marker response

genes in roots of RNAi seedlings with reduced *TaABCC13* transcript. Summarizing, through our observation one could reinforce the link between the root phenotype in *TaABCC13* silenced plants and importance of auxin homeostasis. Collectively, these observations provide the evidence that the enhanced localized auxin accumulation in root is due to defective *TaABCC13* mediated transportation mechanism.

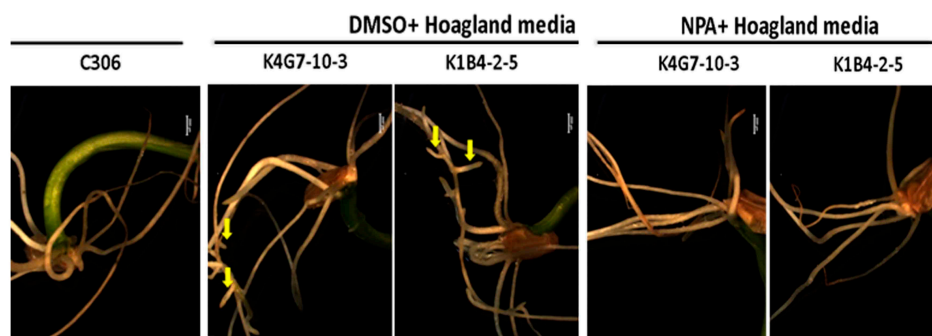


Figure 2

Plant roots may develop different phenotypes and anatomical features when exposed to metal stress. As a general tendency, higher plants commonly develop lateral roots in an attempt to block the radial transport of heavy metals [5,19]. Previously, we demonstrated that non-transgenic wheat roots also developed lateral roots under Cd stress reinforcing the conserved mechanism of sensitivity in plants but not in *TaABCC13*:RNAi seedlings [6]. The regulatory genes for suppression of auxin signaling in presence of cadmium stress are well known [20]. There is an altered auxin transport pattern has also been reported in case of mutation in *MIPS* gene [21]. The MIPS (myo-Inositol-1-phosphate synthase) protein is key enzyme of early phase of phytic acid biosynthesis and

thus there could be another possibility of feedback loop link between biosynthesis of higher inositol phosphates forms and root auxin homeostasis. This certainly needs to be explored and validated. Our previous report suggests reversal of early lateral root phenotype in presence of cadmium stress [6]. The regulatory components for this phenomenon are still unknown and needs to be explored in future. It could be interesting to investigate the molecular basis of lateral root (LR), cadmium (Cd) and auxin homeostasis as a combinatorial affect (Figure 3). The model presented, describes an interconnection between ABCC transporter pertaining to perturbation in either Cd uptake or development of LR as an effect of change in auxin distribution. In conclusion, the present observations coupled with previous evidence strongly suggested a possible interlinking role of plant ABCC transporters in determining lateral root formation under auxin or metal stress.

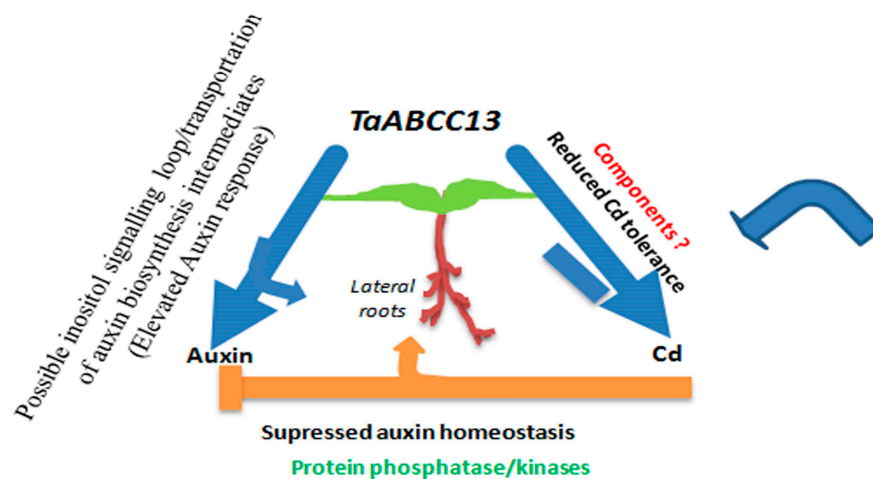


Figure 3

3. Materials and Methods

3.1. Plant material and growth conditions

T₄ seedlings from the previously confirmed wheat transgenic lines (K1B4 and K1G7) were used for the current study [6]. After overnight stratification the seeds were imbibed for two days and subjected to germination. All seedling treatment experiments were carried out in plant growth chamber with photoperiod of 12 hours (400 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The relative humidity was 70% and temperature conditions 25 °C, 18 °C for day and night respectively.

3.2. Root phenotypes and auxin transport inhibition assay

For root phenotypes sterilized T₄ *TaABCC13*:RNAi germinating seedlings from selected lines, along with control were cultured in Hoagland's media in hydroponic system. To validate the role of auxin in root morphology, auxin transportation inhibitor assay performed using *N*-1-Naphthylphthalamic acid (NPA; 20 μM supplemented in Hoagland media). *TaABCC13*:RNAi seedlings after 4 days of germination were exposed to NPA dissolved in DMSO (20 μM) in Hoagland hydroponic system, while control seedlings were incubated in Hoagland media supplemented with DMSO only. The root phenotypes after NPA treatment were observed after 10 days of germination under light microscope. RNA samples were collected at this stage of experiment for further molecular studies.

3.3. RNA extraction and cDNA preparations

Total RNA was isolated from roots of wheat seedlings during this course of the study. Total RNA extraction from seed was performed manually using TRIzol® reagent (Ambion®, Invitrogen™, USA). The isolated RNA samples were treated with TURBO DNase™ (Ambion, Life Technologies, NY, USA) to remove any genomic DNA contamination. Two micrograms of total RNA was used to synthesize the cDNA. The expression analysis was performed with the primers those were designed

and are mentioned in Table 1. Quantitative real time PCR reactions were performed from the above mentioned cDNA of root samples.

3.4. Quantitative real time PCR analysis of genes involved in auxin biosynthesis and transport

For expression analysis of candidate genes investigated during this study, an optimized Quantitative real time-polymerase chain reaction (qRT- PCR) was performed while following instructions and guidelines published by peer groups [23]. The threshold cycles (C_t) of each target genes were normalized in each qRT-PCR experiment case with C_t of the internal controls, 18s rRNA or wheat *ADP-ribosylation factor* (ARF) genes. Normalization and quantification of relative changes in gene expression or fold changes were calculated using $2^{-(\Delta\Delta C_t)}$ [24]. The specificity of amplified PCR products was verified by melt-curve analysis. The amplicons generated during the reaction were also sequenced to confirm the genes.

4. Conclusions

This study provides the evidence for the link between the wheat ABCC transporters and genes involved in the biosynthesis or transport of hormones like auxins. As evident from this and previous study we conclude that ABCC transporter is involved in the distribution of auxins in wheat seedling roots, thereby causing the lateral root phenotype.

Disclosure of potential conflict of Interest

No potential conflict of interest was disclosed.

Acknowledgements and Funding

The authors would like to thank the Executive Director of NABI for facilities and support. Authors also thank Dr. Ram Yadav, IISER-Mohali for scientific discussion. Part of this research was funded by the Department of Biotechnology (DBT), Government of India (BT/PR5989/AGII/106/867/2012) to AKP and NABI core grant. KKB acknowledge the Department of Biotechnology (DBT), Government of India for doctoral research fellowships.

Author Contributions: Kaushal K Bhati and Ajay K Pandey designed the research experiments. KKB and Anil Kumar performed the experiments. KKB and AKP wrote the manuscript. All the authors checked and approved the final version.

Abbreviations

IAA:	indole-3-acetic acid
ABCC:	ATP-binding cassette protein type C
ARF:	ADP-ribosylation factor
NPA:	<i>N</i> -1-Naphthylphthalamidic acid
PIN:	PIN-formed
qRT-PCR:	quantitative real time polymerase chain reaction
DMSO:	dimethyl sulfoxide

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Legend for Figures

Figure 1. The auxin mediated regulation of early lateral root emergence in *TaABCC13*:RNAi seedlings; (A and B) Relative expression level of wheat auxin related markers genes (*PIN1*, *PIN2*, *PIN7*, *IAA1*, *IAA2* and *IAA3*) in *TaABCC13*:RNAi lines with respect to control plants. Ct values were normalized with wheat internal control gene *TaARF* as mentioned previously [6]. The cDNA was prepared from 2 µg DNA free RNA and qRT-PCR analysis was performed using SYBR green based assay. Each bar indicates the mean of three biological replicates (3 technical replicates).

Figure 2. The assay for polar auxin transporter inhibition and reversal of lateral root phenotype of *TaABCC13*:RNAi lines. These auxin inhibition assays were carried out in hydroponic growth system with half strength Hoagland media. The seedling from RNAi (K1B4-2-5 and K4G7-10-3) and C306 (control) lines were exposed to 20 µM NPA (*N*-1-Naphthylphthalamidic acid in DMSO) for 10 days after germination while experimental controls were incubated with DMSO only under appropriate growth conditions mentioned in materials and methods.

Figure 3. Proposed complex circuit controlled by *TaABCC13* for the development of early lateral root (LR) by controlling the distribution of auxin. This also raises the possibility for the interaction of auxin and heavy metals like Cadmium (Cd).

Table 1. List of auxin responsive genes and primer sequences used.

Gene (accession)	Primer sequences
<i>TaIAA1</i> (AJ575098.1)	F: TGACAACAGTAGAATAAAATCACCAAGTAG R: GGTGGACCAGAAGCAATAATCTATC
<i>TaIAA2</i> (CK213604.1)	F: GTGGGCAGCGACCGTCTTAGTTCTTA R: CTGGATGCTGGTTGGTGACGTCCCAT
<i>TaIAA3</i> (CK170519.1)	F: CCACTGGAAATGGAAGCTACCGACAG R: ATCGGAGGCGGTGGCCTCGAAGG
<i>TaPIN1</i> (DR735521.1)	F: ATCAACCGCTTCGTCGCGCTCTTC R: CGTGGAGAGGGAGAAGAGCGTGAT
<i>TaPIN2</i> (JZ883406.1)	F: TACGTGGCCATGTTTCATGGCGTAC R: CAGGAAGCGGTAGTCCATGGCGTA
<i>TaPIN3</i> (CK208792.1)	F: CGCTTCATCGCCGCCGACAC R: TTGAGCAGCGGGATGCCCCATGAC
<i>TaARF1</i> (AB050957.1)	F: TGATAGGGAACGTGTTGTTGAGGC R: AGCCAGTCAAGACCCTCGTACAAC