An early auxin-responsive Aux/IAA gene from wheat (Triticum aestivum) is induced by epibrassinolide and differentially regulated by light and calcium

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Abstract

The plant hormone auxin plays a central role in regulating many aspects of plant growth and development. This largely occurs as a consequence of changes in gene expression. The Aux/IAA genes are best characterized among the early auxin-responsive genes, which encode short-lived transcriptional repressors. In most plants examined, including Arabidopsis, soybean, and rice, the Aux/IAA genes constitute a large gene family. By screening the available databases, at least 15 expressed sequence tags (ESTs) have been identified from wheat (Triticum aestivum), which exhibit high sequence identity with Aux/IAA homologues in other species. One of these Aux/IAA genes, TaIAA1, harbouring all the four conserved domains characteristic of the Aux/IAA proteins, has been characterized in detail. The expression of TaIAA1 is light-sensitive, tissue-specific, and is induced within 15–30 min of exogenous auxin application. Also, the TaIAA1 transcript levels increase in the presence of a divalent cation, Ca²⁺, and this effect is reversed by the calcium-chelating agent, EGTA. The TaIAA1 gene qualifies as the primary response gene because an increase in its transcript levels by auxin is unaffected by cycloheximide. In addition to auxin, the TaIAA1 gene is also induced by brassinosteroid, providing evidence that interplay between hormones is crucial for the regulation of plant growth and development.

Key words: Auxin, Aux/IAA, brassinosteroid, calcium, light regulation, wheat (Triticum aestivum).

Introduction

Plant growth and development is a carefully orchestrated event regulated by both environmental and endogenous signals. Phytohormones are a vital part of this developmental process and provide cues to regulate this process in a spatio-temporal manner. The importance of auxin for plant sustenance is both vital and readily apparent: auxin elicits a plethora of plant responses including embryogenesis, lateral root development, vascular differentiation, apical dominance, tropic responses, and flower development, along with cell division, elongation, and differentiation (Cleland, 1999; Quint and Gray, 2006). The cellular responses to auxin involve changes in gene regulation and stimulation of the transcription of numerous genes. The most well characterized auxin-responsive genes are represented by the members of the Aux/IAA (auxin/indoleacetic acid), GH3, and SAUR (small auxin up RNA) gene families (Guilfoyle, 1999; Jain et al., 2006a, b, c).

The Aux/IAA gene family is comprised of at least 29 members in Arabidopsis (Dharmasiri and Estelle, 2004) and of 31 members in the rice genome (Jain et al., 2006a). The Aux/IAA proteins harbour four conserved domains. Domain I has been assigned a repressor function (Tiwari et al., 2004), domain II is responsible for rapid degradation of the Aux/IAA proteins, while domains III and IV are responsible for homo- and heterodimerization among the
various members of the Aux/IAA and auxin response factor (ARF) proteins (Kim et al., 1997; Ouellet et al., 2001). The Aux/IAA genes were originally identified from soybean as mRNAs that are rapidly up-regulated in response to auxin (Ainley et al., 1988; Abel and Theologis, 1996). Within the promoters of these genes, cis-elements that confer responsiveness (referred to as auxin response elements or AuxREs) have been identified, and a family of trans-acting transcription factors (ARFs) that bind with specificity to AuxREs has been characterized (Hagen and Guilfoyle, 2002). Although ARFs, a plant-specific family of DNA-binding proteins, positively regulate the expression of Aux/IAA genes, the Aux/IAA proteins dimerize with ARFs to repress their activity. In fact, auxin initiates cascading events that lead to proteolysis of Aux/IAA proteins via the ubiquitin-ligase SCFTIR1 complex, thus allowing ARFs to dimerize and promote transcription of auxin-responsive genes (Gray et al., 2001; Kepinski and Leyser, 2004). The mechanism for auxin perception remained elusive for a long time, although recent studies demonstrated auxin action in a cell-free system (Dharmasiri et al., 2003; Thakur et al., 2005), pointing towards a soluble protein being the auxin receptor. In a pioneering work, very recently, TIR1 (an F-box protein) has been shown to be one of the auxin receptors mediating transcriptional responses to auxin (Dharmasiri et al., 2005a; Kepinski and Leyser, 2005). Evidence has also been provided whereby auxin may also interact with other F-box proteins (Dharmasiri et al., 2005b).

The transcript levels of many auxin-responsive Aux/IAA genes increase in response to the protein synthesis inhibitor cycloheximide (CHX; Abel et al., 1995; Thakur et al., 2005), suggesting that some short-lived proteins repress Aux/IAA transcription. Because Aux/IAA proteins are extremely short lived in vivo (Gray et al., 2001), these proteins themselves may act as repressors of the auxin-mediated transcriptional responses. The Aux/IAA genes have been identified in dicots (soybean, pea, mungbean, Arabidopsis, tobacco, cucumber, tomato, and Populus), cereals (maize and rice), and pine tree (Abel et al., 1995; Fujii et al., 2000; Thakur et al., 2001; Hagen and Guilfoyle, 2002; Moyle et al., 2002; Goldfarb et al., 2003; Jain et al., 2006a). Despite intensive studies on the role of Aux/IAA proteins in the regulation of auxin-mediated gene expression, their function is not yet fully understood. Some Aux/IAA proteins are involved in light signalling (Liscum and Reed, 2002), and mutants defective in Aux/IAA proteins are insensitive to multiple phytohormones (Wilson et al., 1990; Leyser et al., 1996; Rogg et al., 2001). An Aux/IAA gene from tomato has been shown to respond to ethylene (Jones et al., 2002) and its mRNA accumulation during ripening coincides with their ethylene regulation in immature green fruits. brassinosteroids (BRs) also interact synergistically with auxin in hypocotyl elongation in several plant species (Sasse, 1999) and regulate changes in expression of Aux/IAA genes (Nakamura et al., 2006).

The Aux/IAA genes have not been found in bacterial, animal, or fungal genomes, and are therefore probably unique to plants.

Earlier studies on the isolation and characterization of an auxin-induced cDNA from rice (Oryza sativa), OsiIAA1 (Thakur et al., 2001, 2005), and identification of the Aux/IAA family in rice (Jain et al., 2006a), prompted the identification of its homologues in wheat as well. A database search revealed the existence of at least 15 cDNAs in wheat corresponding to homologues of Aux/IAA genes present in other plants. Simultaneously, an Aux/IAA cDNA (TaIAA1) was isolated by screening the wheat cDNA library using rice OsiIAA1 (Thakur et al., 2001) as a probe. This study provides evidence that this primary auxin-responsive gene encodes a nuclear-localized protein, whose levels are up-regulated by auxin and BR, and down-regulated by light. Calcium ions also stimulate TaIAA1 expression and probably mediate the action of auxin and/or BR in regulation of gene expression.

Materials and methods

Plant material and growth conditions

Wheat (T. aestivum) seeds were obtained from the Directorate of Wheat Research of the Indian Council Agricultural Research Institute, Karnal. Seeds were washed thoroughly with reverse osmosis (RO) water after disinfecting with 4% sodium hypochlorite for 30 min. Seedlings were grown on cotton saturated with RO water at 28 °C, either in the dark or in constant light provided by a bank of fluorescent tube lights (Philips TL 40 W/54, 6500 K) with a fluence rate of 70 μmol m⁻² s⁻¹, as per experimental requirements.

Coleoptile elongation assay

Wheat seedlings were grown in complete darkness for 72 h. The elongation zone (the middle segment) of 5 mm length of the etiolated coleoptile was excised. The segments were incubated in KPSC buffer (10 mM potassium phosphate, pH 6.0, 2% sucrose, 50 μM chloramphenicol) for 12 h, to deplete endogenous auxins; the buffer was replaced every 1 h (Thakur et al., 2001). The segments were then transferred to fresh KPSC buffer containing different concentrations of IAA, 2,4-dichlorophenoxyacetic acid (2,4-D) or epibrassinolide (EBL). Control segments were incubated in the KPSC buffer for the same duration. Similarly, the segments were first incubated in KPSC buffer for 14 h and then transferred to fresh KPSC buffer supplemented with 320 μM equivalent salt of the divalent cation, Ca²⁺, with or without 30 μM IAA/2,4-D. The calcium-chelating agent, EGTA, was used to demonstrate the specificity of the effect of Ca²⁺. The length of the coleoptile segments was recorded after the desired duration using a measuring scale. For every treatment, the length of at least 20 coleoptile segments was measured and the values plotted indicate the mean ± SE. The whole experiment was performed in a dark room under a green safe-light. Each experiment was performed at least twice and the data of only a representative experiment are presented.

Isolation of TaIAA1 cDNA and sequencing

The dark-grown 4-d-old wheat seedling cDNA library was made using the cDNA synthesis kit, ZAP express™ and Gigapack III gold (Stratagene Cloning Systems, USA), according to the manufacturer’s
instructions (Kulshreshtha et al., 2005), and screened by using radiolabelled full-length OsilAA1 cDNA from rice as probe (Thakur et al., 2001). The hybridization was carried out at 58 °C in the buffer containing 6× SSC, 5× Denhardt’s solution, 0.5% SDS, and 100 µg ml⁻¹ denatured herring sperm DNA. After screening ~5×10⁶ recombinant plaques, 24 putative clones were selected and further purified through three rounds of successive screening. Single clone excision was done to obtain recombinant pBK-CMV phagemids according to the manufacturer’s instructions. T3 and T7 primers were used for sequencing, and the sequence from the 5’ end of one of the clones showed significant homology to Aux/IAA cDNAs. The clone was thus designated as TaIAA1. Complete sequencing was done using an automated ABI Prism 3700 DNA Analyzer (Applied Biosystems, USA), with the ABI Prism Big Dye Terminator V 2.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, USA), as per the manufacturer’s instructions.

**Completion of the 5’ untranslated region (UTR) by rapid amplification of 5’ cDNA ends (5’ RACE)**

First-strand cDNA was synthesized from total RNA (1 mg) isolated from leaf bases of 13-d-old light-grown wheat seedlings treated with 2,4-D (30 µM) for 1 d, using a gene-specific primer (GSP), 5’-GGAGATCTCCTCCTTACTCCTGCCCATC-3’ and Stratscript II reverse transcriptase (Gibco-BRL, USA). This was purified using a gel extraction kit (Qiagen) and a T3 adaptor, 5’-CCCTTTAGT-GAGGTTAATTTTCC-3’ (3AC), and was ligated to the 5’ end of the single-stranded cDNA by using T4 RNA ligase (Ambion Biochemicals, USA). The adaptor was modified at its 5’ end by phosphorylation and at its 3’ end by amino modification. The ligated product was again purified using a Qiagen column and then used as a template for PCR amplification with the T3 adaptor and GSP. The PCR product was cloned in pGEMT-easy vector and sequenced using T7 and SP6 primers.

**Northern analysis**

Total RNA was isolated from different tissues (Nagy and Schafer, 2002) and resolved on a 1.2% agarose gel containing 1.1% formaldehyde, at 120 V. After alkaline blotting to a Hybond-N⁺ membrane (Amersham), hybridization was carried out in 50% formamide, 5× SSC, 5× Denhardt’s solution, 0.1 M sodium phosphate buffer, pH 6.5, 10% dextran sulphate, and 250 µg ml⁻¹ of denatured herring sperm DNA at 42 °C, using [α-³²P]dATP-labelled full-length TaIAAI cDNA as a probe. The membrane was then subjected to three successive washes for 5, 15, and 15 min, with 2× SSC/0.5% SDS, 2× SSC/0.1% SDS, and 0.1× SSC/0.5% SDS, respectively, at room temperature. Autoradiography and X-ray film development were performed as described above, and ethidium bromide-stained rRNA served as a control to estimate the relative amounts of rRNA in each lane.

**Identification of Aux/IAA homologues in wheat (T. aestivum)**

To identify Aux/IAA homologues in wheat (T. aestivum), the National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/BLAST/nl/EST), and the Institute for Genomic Research (TIGR) database (http://www.tigrblast.tigr.org/euk-blast) resources were used. The amino acid sequences of all the rice Aux/IAA proteins were downloaded and used to search for their homologues in wheat using the TBLASTN program in the NCBI (nr and est) and TIGR databases, the redundant sequences were removed by the ClustalX program (version 1.83), and the full-length cDNA sequences and partial expressed sequence tags (ESTs) recovered. The search was limited to the identification of at least three domains in these ESTs to avoid retrieving the ARF sequences (Jain et al., 2006a).

**DNA isolation and real-time PCR analysis**

Total DNA was extracted using the RNase Plant mini kit (Qiagen, Germany) according to the manufacturer’s instructions, followed by DNase I treatment to remove any genomic DNA contamination. The quantitative real-time PCR analysis was performed as described earlier (Jain et al., 2006b). In brief, the cDNA samples synthesized from 3 µg of the total RNA using the High Capacity cDNA Archive kit (Applied Biosystems, USA) were used as template and mixed with 200 nM of each primer and SYBR Green PCR Master Mix (Applied Biosystems) for real-time PCR analysis, using the ABI Prism 7000 Sequence Detection System and Software (PE Applied Biosystems) according to the manufacturer’s instructions. Each pair of primers designed by using Primer Express 2.0 software (PE Applied Biosystems) was checked by the BLAST program in wheat sequences available in the TIGR database to ensure that the primers amplify a unique and desired cDNA segment. The primer sequences are listed in Supplementary Table 1 available at JXB online. The specificity of the reactions was verified by melting curve analysis. The relative mRNA levels for the TaIAAI and other Aux/IAA genes in RNA isolated from various tissue samples were quantified with respect to the internal standard, actin. At least two independent RNA isolations were used for cDNA synthesis, and each cDNA sample was subjected to real-time PCR analysis in triplicate.

**Results**

Cell elongation is one of the most rapid and well studied auxin-responsive phenomena that is generally accompanied by activation of a set of primary or early induced genes (e.g. Aux/IAA, GH3, and SAURs). These genes further influence the expression of secondary and/or late responsive genes governing the end product/phenotype controlled by auxins (Cleland, 1999). Although a number of early auxin-inducible genes have been sequenced and characterized in various dicots, OsilAA1 from rice was the first early auxin-inducible gene isolated and characterized from monocots, which may also have a probable role in cell elongation (Thakur et al., 2001, 2005). The work on the rice Aux/IAA family was extended further (Jain et al., 2006a), and the scope of the work was also enlarged to another important and rather more complex cereal crop, i.e. wheat.

**Spectrum of Aux/IAA genes in wheat**

In an attempt to identify Aux/IAA protein-coding genes in wheat, a TBLASTN search of cDNA clones or ESTs of wheat available at TIGR and NCBI (nr and est) was performed using 31 OsIAA (Jain et al., 2006a) proteins as query. In this search, 15 non-redundant clones (among a total of 64; including TaIAAI; DR740490) having high sequence similarity to OsIAA proteins could be identified. Their GenBank accession numbers, nucleotide length, and the conserved domains are given in Table 1 (see also Supplementary Fig. 1 available at JXB online). The absence of domain I in most of the sequences (10 out of 15) may be due to the incomplete cDNA/EST sequences available. Some of these genes were also examined for their auxin inducibility (described later). Simultaneously, one of the auxin-inducible cDNAs from wheat was isolated...
by screening a cDNA library made from 4-d-old etiolated wheat seedlings (Kulshreshtha et al., 2005), using rice OsIAA1 (Thakur et al., 2001) as a probe. The cDNA (accession no. AJ575098) thus isolated has been designated as TaIAA1 (T. aestivum IAA 1) and its characteristic features are described below.

Characterization of TaIAA1 cDNA

The TaIAA1 cDNA isolated originally by library screening was 1104 bp long with an open reading frame of 702 bp. The cDNA was further extended by 5′ RACE increasing its size to 1209 bp. A 181 bp 5′ UTR precedes the initiation codon, ATG. The 3′ UTR of 527 bp contains a poly(A) site and two potential polyadenylation signals at 59 bp and 201 bp upstream of the poly(A) site (see supplementary Fig. 2 available at JXB online). The cDNA encodes a protein of 234 amino acids with a predicted molecular mass of ~24.88 kDa. The predicted amino acid sequence of TaIAA1 showed significant identity (32–45%) with known Aux/IAA proteins. The TaIAA1 protein contains all the four domains (I–IV) that are highly conserved in Aux/IAA proteins. The TaIAA1 protein belongs to a single copy, the wheat genomic DNA was digested with different restriction enzymes and processed for Southern analysis at high stringency (50% formamide, 42 °C) using complete TaIAA1 cDNA as a radiolabelled probe. The autoradiogram shows that either two or three fragments prominently hybridized after restriction digestion with BamHI, PstI, EcoRI, and HindIII (see supplementary Fig. 5 available at JXB online). However, under low stringency conditions for hybridization, some more bands could be detected in the autoradiogram (data not presented). This indicates that TaIAA1 belongs to a multigene family, which is similar to the situation in most of the other plant species examined (Abel et al., 1995; Thakur et al., 2001; Moyle et al., 2002; Jain et al., 2006a).

Table 1. Aux/IAA gene family in wheat

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<tr>
<th>Accession no.</th>
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<th>Domains present</th>
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<td>1107</td>
<td>I, II, III, IV</td>
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<tr>
<td>AL808316</td>
<td>524</td>
<td>II, III, IV</td>
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<td>1124</td>
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</tr>
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</table>

a GenBank accession number.

b Length of cDNA/EST in bp.

c Domains present in the predicted Aux/IAA proteins (some are partial clones).

The elongation response of three different segments of coleoptile towards auxins was also examined at the optimal concentration, i.e. 30 μM (Fig. 1B). The coleoptile was excised into 5 mm segments each at the base, middle, and the tip region, and the middle segment was observed to show the maximum response (~2.5-fold increase over the
control). The basal and tip segments also showed an increase in length, but not as great as that displayed by the middle segment (Fig. 1B).

To determine the changes in *TaIAA1* transcript abundance after depletion of endogenous auxin, the total RNA was isolated from etiolated segments floated in KPSC buffer at varying time intervals and subjected to northern analysis. As compared with the undepleted control, there was a slight decrease within 2 h in the steady-state transcript levels of *TaIAA1*, which gradually declined and became negligible after 12 h (Fig. 1C). For induction experiments, the endogenous auxin was depleted by floating the excised coleoptile segments in KPSC buffer for 14 h and then treated with 30 μM IAA for various times. The increase in transcript abundance was apparent within 15 min of incubation, increasing further with extended duration of IAA treatment (Fig. 1D). The expression of six other *Aux/IAA* genes of wheat was also examined by real-time PCR and all but one of these genes could be induced by auxin in etiolated coleoptile segments (Fig. 1E); maximum induction was recorded in clone CK20769.

**Fig. 1.** (A) Effect of various concentrations of IAA and 2,4-D on elongation growth of 3-d-old etiolated wheat coleoptiles after 14 h of endogenous auxin depletion in KPSC buffer. (B) Effect of IAA and 2,4-D on elongation of segments (basal, middle, and tip) of coleoptiles from 3-d-old dark-grown wheat seedlings. The measurements were taken after 20 h of auxin treatment and are expressed as the mean ±SE (C) The kinetics of decrease in *TaIAA1* transcript abundance during depletion of endogenous auxin in the excised coleoptile segments in KPSC buffer. (D) Northern blot hybridization showing the kinetics of increase in *TaIAA1* transcript abundance in excised coleoptile segments of 3-d-old etiolated wheat seedlings by 30 μM IAA, after 14 h of auxin depletion. As a control, RNA was isolated from 14 h auxin-depleted tissue. Each lane contains 20 μg of RNA, and ethidium bromide-stained rRNA represents the control in both (C) and (D). (E) The relative mRNA levels of five *Aux/IAA* cDNAs in control (14 h auxin depleted) and 2,4-D- (30 μM) (2 h) treated coleoptile segments of 3-d-old etiolated wheat seedlings. The error bars represent the mean ±SD of two biological replicates, each analysed with three technical replicates.

Auxin-induced *TaIAA1* expression is insensitive to CHX

CHX is known to induce the early auxin-responsive genes (Abel *et al.*, 1995; Thakur *et al.*, 2005). The steady-state transcript accumulation was monitored in excised etiolated wheat seedlings treated with 50 μM CHX for various times. The *TaIAA1* transcript levels increased within 30 min in the presence of exogenously supplied CHX, and a sustained
increase was registered for up to 5 h (Fig. 2A). The CHX treatment could not abolish the inductive effect of IAA and, in fact, their effects on the increase in TaIAA1 transcript accumulation were additive (Fig. 2B).

**Light-sensitive and tissue-specific expression of TaIAA1**

The changes in TaIAA1 transcript abundance were examined by northern analysis using the total RNA extracted from roots and shoots of both light-grown and etiolated wheat seedlings (Fig. 3). In etiolated wheat seedlings, the transcript level of TaIAA1 was most in the upper portion of shoots, followed by the lower portion, and least in the root. This expression was negligible in light-grown roots although moderately present in the shoot (Fig. 3A). In addition, the transcript was detectable in 1-month-old leaf base calli cultured on 2,4-D medium (Fig. 3B).

Light and phytohormones profoundly influence plant growth and development. There are some compelling results that suggest that light, besides its effect on plant growth and development, can regulate Aux/IAA expression at the molecular level (Colon-Carmona et al., 2000; Thakur et al., 2001, 2005). To determine whether TaIAA1 is up- or down-regulated by light, the kinetics of accumulation of the TaIAA1 transcript were examined in seedlings exposed to white light for various times. There was a distinct decrease in TaIAA1 transcript levels within 3–4 h in both 3- and 5-d-old etiolated wheat seedlings exposed to light (Fig. 3C, D).

**Calcium-induced changes in TaIAA1 expression and elongation growth**

Calcium, a second messenger in many hormone-regulated responses, plays a key role in various cellular and physiological processes of higher plants (Harper, 2001). In the present study, the effect of calcium was examined on TaIAA1 gene expression and elongation growth of coleoptile segments. The middle segments of 3-d-old etiolated coleoptiles of wheat were first incubated in KPSC buffer for 14 h and then transferred to fresh KPSC buffer supplemented with 30 μM 2,4-D and/or 320 μM CaCl2 for 20 h in the dark. There was increased elongation in the presence of both auxin and calcium. The chelating agent, EGTA, virtually arrested the elongation growth of cut segments in the presence of auxin alone or even when incubated together with calcium (Fig. 4A). The etiolated wheat coleoptile segments showed a more conspicuous curving response when both 2,4-D and CaCl2 were supplied simultaneously (Funke and Edelmann, 2000). Northern studies also revealed essentially a similar picture with respect to changes in TaIAA1 transcript levels on treatment with Ca2+ and/or auxin. The TaIAA1 transcript abundance in the excised etiolated coleoptiles treated with 320 μM CaCl2 increased with or without auxin, which could be checked by EGTA application (Fig. 4B).

**Induction of TaIAA1 transcripts by BR**

Plants exhibit different BR sensitivities, depending on endogenous or exogenous factors, such as organ type, environment, and growth stage (Nakamura et al., 2006). To determine the optimal effective concentration of EBL,
real-time PCR was performed with total RNA isolated from etiolated shoots and roots of the seedlings treated with varying concentrations of EBL. This analysis revealed that concentrations which were inhibitory to shoots (200 nM) were promoting expression of \textit{Aux/IAA} transcript in roots, and concentrations which were inhibitory to roots were promoting expression of \textit{Aux/IAA} transcript in shoots (100 nM and 1 lM) (Fig. 5A, B). Northern analysis revealed increased \textit{TaIAA1} transcript accumulation in etiolated shoots in the presence of EBL, although it was less effective as compared with auxin (at least at 100 nM) (Fig. 6A). The expression was undetectable in roots of dark-grown seedlings (Fig. 6B). The kinetic studies revealed that the pattern of \textit{TaIAA1} transcript accumulation was essentially similar to auxin; the increase in transcript abundance was visible within 1 h and, thereafter, a gradual increase was observed up to 16 h (Fig. 6C, D). Subsequently, the etiolated shoots were depleted of endogenous auxin in KPSC buffer for 14 h and then incubated with BR (10 nM and 100 nM) alone and/or with auxin (30 lM) for 2 h. The level of \textit{TaIAA1} transcript increased much more in the combined presence of both auxin and BR (Fig. 6E).

Discussion

\textit{Aux/IAA} gene family in wheat

The \textit{Aux/IAA} genes are present as a multigene family in nearly all plants examined, including soybean (Ainley \textit{et al.}, 1988), pea (Oeller \textit{et al.}, 1993), mungbean (Yamamoto \textit{et al.}, 1992), tobacco (Dargeviciute \textit{et al.}, 1998), tomato (Nebenfuhr \textit{et al.}, 2000), \textit{Arabidopsis}}
(Liscum and Reed, 2002), *Populus* (Moyle et al., 2002), and loblolly pine (Goldfarb et al., 2003). Very recently, the rice genome has been shown to contain 31 Aux/IAA genes (Jain et al., 2006a). Southern analysis indicated that Aux/IAA genes may be represented as a multigene family in the wheat genome, as has been shown in the case of rice (Thakur et al., 2001; Jain et al., 2006a). The overall in silico analysis (present study) also revealed that there are at least 15 ESTs (or cDNAs) of auxin-inducible Aux/IAA genes present, although the expected number in the hexaploid genome of wheat may be much more, bearing in mind the fact that rice has >30 members of this family (Jain et al., 2006a). For the present, the *TaIAA1* cDNA has been isolated and characterized in detail from the hexaploid wheat (*T. aestivum*).

The molecular mass of Aux/IAA proteins in general ranges from 19 kDa to 36 kDa (Abel and Theologis, 1995; Guilfoyle et al., 1998). The *TaIAA1*-encoded protein also falls in this range, with a calculated molecular mass of 24.88 kDa. The *TaIAA1*-encoded polypeptide shares four conserved domains, I, II, III, and IV, and seven invariant residues in the intervening region. Overall sequence identity between the conserved domains is highly variable (36–87%). The amino acid alignment of *TaIAA1* with known Aux/IAA proteins shows conserved regions of basic amino acids. Domain III, along with five invariant hydrophobic residues, forms the $\beta\alpha$ structure which shows similarity to the $\beta\alpha$ DNA-binding domain of prokaryotic repressor Arc and MetJ (Pabo and Saver, 1992; Gray et al., 2001). This putative prokaryotic $\beta\alpha$
DNA-binding motif is required for protein dimerization (Kim and Harter, 1997) and protein–protein interaction. The Aux/IAA proteins are short-lived nuclear proteins, and domain II is critical for rapid degradation via the SCF^TIR1 complex. In a very recent study in Arabidopsis, it has been shown that regions outside of Aux/IAA domain II could independently regulate the proteolysis of specific Aux/IAA family members (Dreher et al., 2006). Domain III and IV are dimerization domains that are conserved not only among the Aux/IAA proteins but also among most ARFs (Kim and Harter, 1997; Ulmasov et al., 1999).

The TaIAA1 protein, like other Aux/IAA proteins (Abel et al., 1995; Jain et al., 2006b), has a Matx2-like putative NLS sequence and an SV40-type NLS sequence. Both these sequences were found to be functional in targeting the GUS–protein fusion to the nucleus in onion epidermal cells. The Aux/IAA proteins are known to interact with ARFs (known transcription factors) to function as repressors of auxin-induced gene(s) (Ulmasov et al., 1999).

The TaIAA1 is a primary auxin-responsive gene

Besides a fast induction phenomenon, early auxin-responsive genes are characterized by increased mRNA accumulation even when de novo protein synthesis is blocked. The mechanism for such induction is supposed to involve both stabilization of mRNAs and derepression of transcription (Koshiba et al., 1995). Earlier studies employing nuclear run-on transcription assays and metabolic inhibitors also indicate that many Aux/IAAs are transcriptionally regulated (Guilfoyle, 1999). In the present study, although CHX itself up-regulated TaIAA1 transcript accumulation, the addition of IAA further augmented the response. This CHX insensitivity towards auxin action indicates that TaIAA1 is a primary auxin-responsive gene and that its expression is up-regulated by auxin independently of de novo protein synthesis.

Tissue-specific and auxin-induced changes in TaIAA1 expression and coleoptile elongation growth

Auxin-mediated cell elongation is one of the fastest known hormonal responses (with a lag period of 15–25 min) (Abel et al., 1995). Exogenously supplied auxin affects the elongation of excised coleoptiles, and this is supported by the present study. The TaIAA1 transcripts declined on auxin depletion and this is consistent with most of the auxin-inducible genes that have a short half-life. An increase in elongation of the middle segment of the excised coleoptiles occurred with the application of 30 μM 2,4-D or IAA. The ability of exogenous auxin to promote cell elongation in excised stem and hypocotyl has been studied extensively previously (Hagen et al., 1984). Most Aux/IAA genes respond to exogenous auxin within the first 30 min of treatment, similar to the SAUR genes, the fastest responding genes known to be induced by auxin (Gee et al., 1991). In the case of TaIAA1 too, transcripts started accumulating as early as 15–30 min after auxin application. The TaIAA1 transcripts were present in shoots of 5-d-old light-grown wheat seedlings but were almost undetectable in roots, whereas its expression in the dark could be detected in roots, although it was still lower than in shoots. Essentially a similar tissue-specific profile was reported for rice OsiIAA1 (Thakur et al., 2001).

Light-mediated down-regulation of TaIAA1

Light and auxins are known to interact with each other in various stimulus–response processes in plants. A direct interaction between light and auxin has been demonstrated by phosphorylation of Aux/IAA proteins (SHY2/IAA3, AXr3/IAA17, and AXR2/IAA7) by phytochrome A (Colon-Carmona et al., 2000). In the present study, the TaIAA1 expression in wheat seems to be downregulated by light, as its transcripts do not accumulate in light-grown tissues, leaf blades, and coleoptiles, while the transcript is abundant in the dark, in both root and shoot. This was further corroborated by northern analysis of the RNA isolated from 3- or 5-d-old coleoptile samples harvested at various time points upon transfer from the dark to white light. The down-regulation of TaIAA1 transcript accumulation in 3/5-d-old etiolated seedlings became visible as early as 3–4 h following irradiation with white light. The OsiIAA1 transcript levels are also affected by white light in a similar manner in rice (Thakur et al., 2001). In a subsequent study, the role of red, far-red, and blue light has also been demonstrated in triggering the down-regulation of OsiIAA1 expression (Thakur et al., 2005).

Brassinosteroid-mediated responses

While both auxin and BR promote elongation, their induction kinetics are quite different, with auxin generally showing a short lag time of 10–15 min (Sasse, 1999; Bao et al., 2004) and BR showing a gradual and continuous increase (Nakamura et al., 2006). This difference in kinetics is also seen at the level of gene expression in the case of TaIAA1 (present study) and also in Arabidopsis, where auxin induces members of the IAA, SAUR, and GH3 gene families generally much more rapidly than BR (Goda et al., 2002; Müssig et al., 2002; Nakamura et al., 2006). Earlier studies also reported that in a BR-deficient det2 mutant of Arabidopsis, Aux/IAA levels were lower than in the wild type, even though the endogenous auxin levels per gram of fresh weight were higher in the det2 mutant than in the wild type (Nakamura et al., 2003). The interactions of the BR and auxin signalling pathways in the best characterized auxin-insensitive mutants iaa7/axr2 and iaa17/axr3 have revealed that these genes are regulated by the Aux/IAA proteins in response to both auxin and BR (Nakamura et al., 2006). Whether auxin and BRs interact to regulate expression
of TaIAA1 remains to be worked out, but exogenous IAA induced accumulation of TaIAA1 transcripts quickly and transiently, whereas exogenous BR induced it gradually and in a sustained manner.

**Calcium-regulated changes in TaIAA1 expression and coleoptile elongation**

Plant cells are reported to contain all the elements essential for a calcium-based messenger system that couple the external stimuli to various physiological responses (Pleith, 2005). There is increasing evidence that auxin action is also mediated by an intracellular change of calcium levels and that calcium acts as a second messenger during auxin-mediated cellular responses (Yang and Poovaiah, 2000).

The present study too substantiates the role of calcium in auxin-mediated cell elongation. The presence of calcium, along with exogenously supplemented 2,4-D, induced curving of the excised coleoptiles (data not presented), suggesting a differential accumulation/distribution of auxin in the presence of calcium. Northern analysis revealed that the transcript levels of TaIAA1 increased when the endogenous auxin-depleted explants were incubated with calcium alone or in combination with 2,4-D, suggesting the positive influence of calcium on the relative abundance of the TaIAA1 transcripts. EGTA, when supplied in the presence of auxin and/or calcium, caused a decrease in the TaIAA1 transcript levels, which was concomitant with its inhibitory influence on coleoptile elongation in the dark. These data provide evidence for a role for calcium in auxin-inducible gene expression in wheat, but whether this effect is exerted at the transcriptional or post-transcriptional level, remains to be established. In an earlier study with a calmodulin (CaM) antagonist, it was suggested that CaM mediates regulation of ZmSAUR1 not at the transcriptional level but rather at the post-transcriptional level (Yang and Poovaiah, 2000).

The curving of coleoptile segments in the presence of calcium is an interesting observation because such a response is usually quite apparent when hypocotyl segments or internodes are treated with a BR (Sasse, 1999). Moreover, recent studies have also shown that some of the Aux/IAA genes are indeed induced by BR (Nakamura et al., 2003, 2006) and BR action is mediated by calcium (Du and Poovaiah, 2005). It is thus imperative to find out if Ca$^{2+}$ is involved in auxin or BR signalling, or in both, for coleoptile elongation and curving, and induction of Aux/IAA genes.

Although the precise role of TaIAA1 remains to be elucidated, there is correlative evidence for its probable role in cell elongation in seedling coleoptiles. It is intended to try, in the near future, to validate TaIAA1 functionally by modulating its expression in transgenics. There are probably chances that the Aux/IAA family will be much larger in this hexaploid wheat but its complexity will be unravelled only as the wheat sequencing project advances and high throughput genome data become available for computational analysis.

**Supplementary data**

Supplementary data can be found at JXB online.

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