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Full Length Research Paper

Isolation and characterization of an *Aux/IAA* gene (*LaIAA2*) from *Larix*

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The phytohormone auxin controls many aspects of plant development. Auxin/indole-3-acetic acid (*Aux/IAA*) transcriptional factors are key regulators of auxin responses in plants. To investigate the effects of auxin on gene expression during the rooting process of *Larix* cuttings, a subtractive cDNA library was constructed and 272 UniEST were obtained by using suppression subtractive hybridization (SSH). Based on a fragment of 272 UniEST, the full-length cDNA of *LaIAA2*, an *Aux /IAA* gene from *Larix* was isolated. Then, the response expression of *LaIAA2* to auxin was determined by treating with different sources and concentration of auxin and cycloheximide and the expression patterns of *LaIAA2* were examined in different tissues. The results show that *LaIAA2* appears to be the first response gene of auxin and *LaIAA2* gene was involved in the root development and auxin signaling. The express pattern of *LaIAA2* gene indicated that it might play a central role in root development, specially regulated lateral and adventitious root production.

Key words: Aux/IAA gene family, auxin, LaIAA2, Larix

INTRODUCTION

The plant hormone auxin, in particular indole-3-acetic acid (IAA), is a key regulator of virtually every aspect/process of plant growth and development, including apical dominance, phyllotaxy, lateral and adventitious root initiation, flower and fruit development, embryogenesis, cell elongation, cell division and vascular tissue differentiation (Muday, 2001; Jenik and Barton, 2005; Leyser, 2005). Auxin induces the transcription of several classes of genes as a rapid, primary response, in which one of the best characterised is the *Aux/IAA* family (Knox et al., 2003). Full-length cDNAs belonging to this large superfamily have been cloned from several species, includeing *Arabidopsis thaliana*, pea, *Pisum sativum L.*, soybean, *Glycine max (L.) Merrill*, mung bean, *Vigna radiata (L.) R. Wilcz*, tobacco, cucumber, loblolly pine and tomato (Goldfarb et al., 2003; Zhang et al., 2007).

The precise biochemical functions of *Aux/IAA* gene family members are not fully understood, but genetic and biochemical evidence suggests that they are transcriptional factors that act as either positive or negative regulators of other auxin-responsive genes (Abel et al., 1994). *Aux/IAAs* encode low abundance, nuclear proteins with extremely short half-lives, some as short as 5 min. Aux/IAAs proteins are characterised by a highly conserved four domain structure called Domains I, II, III and IV and contain functional nuclear localization signals (Abel and Theologis, 1995). Domain I is responsible for the repressing activity of proteins (Tiwari et al., 2004).

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Abbreviations: IAA, Indole-3-acetic acid; **IBA,** indole-butyric acid; **NAA**, -naphthalene acetic acid; **DAG,** days after germination; **AUX1,** auxin resistant 1; **Aux/IAA,** auxin/indole-3-Acetic Acid (Aux/IAA) transcriptional factors; **TIR1,** transport inhibitor response 1; **SCFTIR1,** Skp1-Cullin-F-box complex; **SSH,** suppression subtractive hybridization; **ARFs,** auxin-response factors; **ABA,** abscisic acid; **2, 4-D,** 2, 4-dichlorophenoxyacetic acid; **BA,** 6-benzylaminopurine; **GA,** gibberellic acid.

Domain II acts as a regulatory domain that confers instability to the Aux/IAA proteins (Worley et al., 2000; Ouellet et al., 2001; Ramos et al., 2001; Tiwari et al., 2001). Domain II interacts with an F-box protein, TIR1, which is a component of the SCF^{TIR1} ubiquitin ligase complex (Gray et al., 2001). Auxin increases this interaction in a dose-dependent manner, promoting the rapid degradation of Aux/IAA proteins through the ubiquitin-proteasome pathway (Gray et al., 2001; Zenser et al., 2001, 2003). Domains III and IV of Aux/IAA proteins serve as protein-protein interaction domains that promote both homodimerization with other *Aux/IAA* gene family members and heterodimerization with the auxin response factors (*ARFs*) (Ulmasov et al., 1997a, b; Ouellet et al., 2001). The Aux/IAA proteins regulate auxin-mediated gene expression by controlling the activity of ARFs by protein-protein interactions (Ulmasov et al., 1997b; Tiwari et al., 2003). In plant cells containing low levels of auxin, Aux/IAA-ARF heterodimers would be predicted to persist, resulting in inhibition of ARF-dependent transcription. By contrast, cells experiencing high levels of endogenous auxin would be expected to maintain little Aux/IAA protein, having ARFs dissociated from Aux/IAA proteins. This allows for expression changes in typically auxin-regulated genes.

The functions of Aux/IAA proteins in growth and development are determined by mutations in Arabidopsis or other plants. The gain-of-function mutations, including *shy2/iaa3* (Tian and Reed, 1999), *shy1/iaa6* (Kim et al., 1996; Reed, 2001), *axr2/iaa7* (Timpte et al., 1994; Nagpal et al., 2000), *bdl/iaa12* (Hamann et al., 1999, 2002), solitary root *(slr)/iaa14* (Fukaki et al., 2002), *axr3/iaa17* (Leyser et al., 1996; Rouse et al., 1998), *iaa18* (Reed, 2001), *msg2/iaa19* (Tatematsu et al., 2004) and *iaa28* (Rogg et al., 2001), have pleiotropic effects on plant growth. Some of the mutations suggest the potential roles of Aux/IAA proteins in root formation. For example, the *shy2-2/iaa3* mutant has reduced numbers of lateral roots (Tian and Reed, 1999) and the *iaa28-1* mutant forms fewer lateral roots (Rogg et al., 2001). In contrast, the *axr2-1/iaa7* mutant has increased numbers of lateral roots (Nagpal et al., 2000), while the *axr3-1/iaa17* mutant has increased numbers of adventitious roots (Leyser et al., 1996). These varied phenotypes in root formation might reflect the functional differences among the various Aux/IAA proteins and/or the transcriptional differences in *Aux/IAA* genes regarding organ/tissue specificity and responsiveness to auxin.

Auxin-stimulated adventitious root formation is required for vegetative propagation from stem cuttings; therefore, this process has an important application role in tree species transplantation. However, many tree species have stem cuttings that are recalcitrant to rooting, limiting the use of vegetative propagules. Root formation can be better understood by determining the effects of auxin on gene expression. Here, we report the isolation and characterization of a member of *Aux/IAA* genes in *Larix*, named *LaIAA2*, which is involved in adventitious root formation in *Larix* cuttings.

MATERIALS AND METHODS

Plant materials

Two clones of *Larix leptolepis*×*Larix olgensis*, which had a significant difference in rooting ability, were chosen as plant materials (clone 31-6 with high ability of rooting and clone 15-4 with low ability of rooting) for establishing the subtractive hybridization library. The stems (clone 31-6 and clone 15-4) were treated with 1×10^{-3} M 1-naphthal-eneacetic acid (NAA) for 30 min, callus and adventitious roots during the rooting process were used to isolate total RNA and to construct subtractive cDNA library.

Seeds of *Larix,* which were chilled at -20°C for at least 1 month, were dipped in 45°C water for 30 min, followed by a room temperature water soakage for 24 h. Then, seeds were sown between two wetness gauze and grown in a greenhouse. Seedings were harvested at 2 weeks and hypocotyl cuttings were prepared by severing the root at a point 2.5 cm below the cotyledons, but above the hypocotyl/root junction.

RNA extraction

Total RNA were extracted from stems, callus, adventitious roots using TRIzol_ Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's manual. Poly(A)$^+$ RNA was isolated from the total RNA with the polyATract mRNA isolation system kit (Promega, Madison, WI, USA) according to the manufacturer's instructions.

SSH

The subtractive library was constructed with ds cDNA of clone 31-6 (Tester) and 15-4 (Driver) based on BD PCR-select cDNA subtract kit (Clontech, mountain view, CA, USA) according to the protocols provided by the manufacturer. Positive clones were sequenced with an ABI 3730 at the Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). Nucleotide sequences and translated sequences were analyzed for homology in GenBank release 165.0 using the BLAST program (NCBI, Rockville Pike, Bethesda, MD, USA). *E*-values <0.001 from BLAST were considered to have significant similarities.

Reverse transcription and clone of *LaIAA2* gene

To a 0.2 ml thin-walled PCR tube on ice, add 2 µg of total RNA, 1 µl of O1igo-(dT) 18 primer and 1 µl of l0 mM dNTP. The mixture was denatured at 65°C for 10 min, followed by quick ice-quenching. To the stated mixture, add 4 µl of M-MLV buffer (Promega), 2 µl of RNase inhibitor and 2 µl of M-MLV reverse transcriptase (Promega). The mixture was mixed by gently pipetting and spins briefly to collect the content. The reverse transcription reaction was performed at 42°C for 1 h to synthesize the first strand cDNA.

A forward primer LaIAA2F1 (5´-ATGKAGAGGTTTGCCAAYGA-3´) based on *Aux/IAA* gene sequences of other plants and a reverse primer LaIAA2R1 (5´-CTAATTCTGGGACTTGGATT-3´) based on *Aux/IAA* gene sequence of *Larix* SSH library were designed for *LaIAA2* amplification. Total volume of PCR reaction system was 50 µl, including 5 µl of 10 × reaction buffers, 2.5 µl of template, 2.5 µl of 10 µM primers of LAIAA2RF1 and LAIAA2RR1, respectively, 1 µl of 10 mM dNTP, 2 µl of Taq polymerase (TIANGEN) and 33.5 µl of PCR-Grade water. Thermocycling was performed at 94°C × 30 s, 60°C × 30 s and 72°C × 1 min for 36 cycles. The PCR product (10

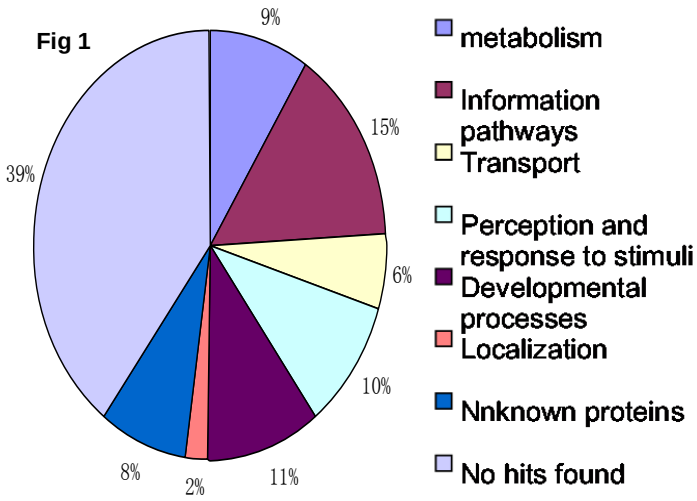

Figure 1. Distribution (as percentage) of predicted functions represented among the 272 genes.

μl) was separated by 1% agarose gel electrophoresis, staining with GoldViewTM nucleic acid stain.

The PCR amplified segment was inserted into pGEM-T-easy vector according to the manufacturer's instructions. Competent cells of *Escherichia coli* stain DH5α were prepared for transformation of the *LaIAA2*-T-easy vector recombinant. IPTG/X-gal blue-white screening was carried out under ampicillin selection (Sambrook et al., 1989). The white *E. coli* colonies were first confirmed by PCR and then, the positive clones were sequenced.

Sequence analyses

The sequence analyses of LaIAA2 cDNA were performed by DNA star (http://www.dnastar.com) and bioXM 2.2 (http://bioxm.go.nease.net). Other *AUX/IAA* gene sequences were retrieved at the National Center for Biotechnology Information (NCBI) at the web site (http://www.ncbi.nlm.nih.gov). Properties and structures of LaIAA2 protein were predicted by the software tools on scan prosite web site (http://au.expasy.org/prosite).

Real-time quantitative PCR expression analyses

The relative quantification was performed using the SYBR Premix Ex Taq^{TM} (Takara). The real-time quantitative PCR was carried out on the ABI 7500 system with the *LaIAA2* primers 5´-GCTCCTGGGCTTTCTATGTC-3´ and 5´-CCATCGTCTGTAGGCTTTGA-3´. The program for quantitative PCR was as follows: initial denaturation step of 95°C for 10 s, followed by 40 cycles of 95°C for 5 s, 60°C for 34 s. Specificity of the reaction was verified by both melting curve analysis and sequencing of amplification products. Real-time PCR experiment was performed in triplicate and normalized to 18S rRNA (with primer 5'-TTTCTGCCCTATCAACTTTC-3' and 5'-CGATGAGCCCAGTATTGTTA-3') by the ΔΔ *Ct* method.

Semi-quantitative PCR expression analyses

Total RNA were extracted from roots, stems, leaves, stems which were treated with 200 ppm IBA, callus and callus with adventitious root primordium. The cDNA synthesis was performed as described earlier. The cDNA pools from these tissues were then, amplified using the primers 5'-GCTCCTGGGCTTTCTATGTC-3' and 5'-CCATCGTCTGTAGGCTTTGA-3', respectively. These primers span the two introns and the amplified fragment was easily distinguished from genomic DNA contamination. The amplification reaction was as follows: initial denaturation step of 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s. A 218 bp fragment of the β-actin gene was amplified as a control using the primer set 5'-TGCTCCAGAAGAACACCCTA-3' and 5'-GGAACTGGTCTTGCCTGTCT-3'. The PCR products were separated on a 1.2% agarose gel.

Statistical analysis

Each experiment was independently performed at least three times. The data from real-time PCR were analyzed by one way analysis of variance (ANOVA) using SigmaStat 3.5 software (Systat Software, Inc., Richmond, CA). Differences between experimental groups were considered significant at $P < 0.05$.

RESULTS

Characterization of up-regulation genes for rooting of *Larix* cuttings

To reveal different expression of genes in cuttings of *Larix* which had significant difference in rooting ability, an SSH approach was used. The driver cDNA was synthesized from mRNA isolated from clone 15-4 of which rooting ability was low and the tester cDNA was produced from mRNA isolated from clone 31-6 of which rooting ability was high.

A large-scale screening of up-regulated genes in clone 31-6 was carried out by analysis of clones obtained from the SSH library. Out of 500 isolated ESTs, 272 were sequenced successfully and analyzed for their sequence.

Based on the documented functions of the genes from which these ESTs were derived, these ESTs were classified into 8 categories (Figure 1). In all the 272 UniESTs, 10 of them were related to the hormones. Furthermore, of these 10 UniESTs, 6 had relationship with auxin, 2 with gibberellin, 1 with ABA and 1 with jasmonic acid. In the 6 UniESTs related to auxin, 2 of them belonged to *AUX/IAA* family, 1 belonged to *NAC* gene family and another 3 belonged to the auxin response genes.

Cloning of *LaIAA2* gene

The open reading frame (ORF) of *LaIAA2* comprised 891 base pair, encoding a 296 amino acid protein (Figure 2). The LaIAA2 amino acid polypeptide was predicted with molecular weight (Mw) of 32.1 kD and isoelectric point (pI) of 5.94 using the software of DNAStar (http://www.dnastar.com). The derived protein comprised the four conserved domains characteristic of the *Aux/IAA* gene family, Domains I to IV and two nuclear localization signal (NLS) sequences. The predicted bipartite NLS comprised an invariant basic doublet KR in interdomain I/II and basic amino acids in Domain II. A second SV40-type NLS was located at Domain IV (Figure 2). Comparison of the LaIAA2 amino acid sequence against GeneBank database revealed a high degree of consensus over other Aux/IAA proteins. The highest score (83% identity) was found with the sequence of *Pinus pinaster* Aux/IAA protein (GeneBank, accession number CAC85936.1). The higher score (82% identity) was found with the sequence of *Pinus taeda* Aux/IAA protein (GeneBank, accession number AAP44405.1). Lower identities were detected with Aux/IAA amino acid sequences from other species: *Populus tremula x Populus tremuloides* Aux/IAA protein, Zinnia elegans Aux/IAA protein and *G. max* GH1 protein (GeneBank, accession number CAC84706.1, AAM12952.1 and AAB70005.1) (50, 50 and 48%). In all 29 Aux/IAA proteins of Arabidopsis, only AtIAA9 protein (GeneBank, accession number NP_569017.2) (46%) had higher degree of identity with LaIAA2 protein. These results show that LaIAA2 protein had higher degree of identity with gymnosperm Aux/IAA protein, instead of angiosperm Aux/IAA protein. Furthermore, the results indicate that there was large variation in LaIAA2 gene during evolution.

Furthermore, the predicted results of LaIAA2 protein secondary structure showed that a βsheet (183~188aa), two alpha helix α1 (200~219 aa) and α2 (232~239 aa) sequentially formed a βαα motif, which was thought as a DNA binding domain in prokaryotic and in Arabidopsis. Ten conserved protein kinase C (PKC) phosphorylation sites (T-56, S-66, S-96, T-128, S-161, T-166, T-205, S-211, T-221 and S-292), 3 casein kinase I (CKI)(S-28, S-79 and S-227) , 4 casein kinase II (CK2) phosphorylation sites (S-12, T-205, S-210 and S-211), 5 cAMP-dependent protein kinase (PKA)(S-44, S-66, S-135, T-166 and S-279) and 2 cdc2 protein kinase (Cdc2)(S-188 and S-204) were also present in LaIAA2 protein (Figure 2). The occurrence of these phosphorylation sites also implied that *LaIAA2* was a short-lived protein, similarly to Arabidopsis *Aux/IAA* members.

Deduced phylogeny of the LaIAA2, other gymnosperm and angiosperm members of the *Aux/IAA* gene family

An alignment of the amino acid sequence of LaIAA2 protein with other homologies was analyzed using DNAMAN software (Figure 3). Previous phylogenetic analyses of angiosperm Aux/IAA genes revealed four main classes in the family (Abel and Theologis, 1995; Dargenvicitiute et al., 1998; Liscum and Reed, 2002). However, the clustering pattern of the LaIAA2 amino acid sequences was different from that in angiosperm species. The *LaIAA2* and other gymnosperm *Aux/IAA* genes (*PtIAA1, PtIAA2*) were clustered in a single class (class I) and formed a discreet subgroup within that class.

The expression patterns of *LaIAA2* in *Larix*

The expression patterns of LaIAA2 in different tissues

Organ-specific expression patterns of *LaIAA2* in *Larix* were investigated with RT-PCR. The amplified product was a 0.2 kb fragment in length. The results show that *LaIAA2* was expressed at low levels in the leaf and root and yet at high levels in stem. The *LaIAA2* transcripts were detected in different phases of adventitious root formation in *Larix* cutting. Furthermore, after the cuttings were treated with 200 ppm IBA, the expression of *LaIAA2* increased obviously (Figure 4).

Timing of LaIAA2 expression

To monitor the kinetics of gene expression, we measured mRNA accumulation for up to 5 days with total RNA extracted from *Larix* hypocotyls treated with a control solution or 1.6 mM NAA for 10 min. The results show that expression of *LaIAA2* increased following NAA treatment. *LaIAA2* mRNA began to accumulate in abundance at 10 min after treatment and reached maximal levels at 1 day after treatment. The abundance of *LaIAA2* mRNA persisted above the basal levels for 3 days in NAA-treated hypocotyl cuttings (Figure 5).

NAA dose response of LaIAA2 expression

To analyze the effect of NAA concentration on the

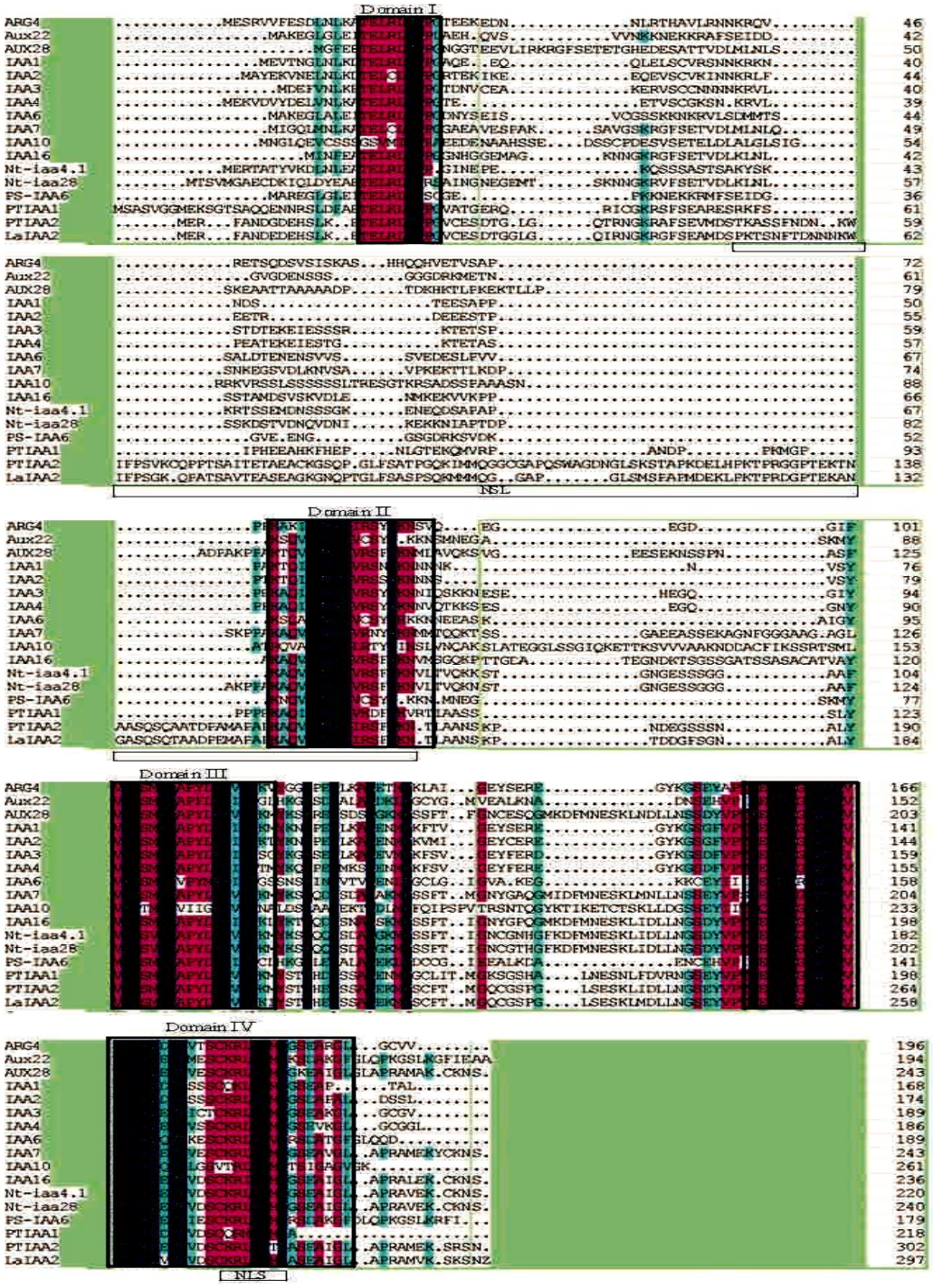

Figure 2. Sequence alignment of LaIAA2 and other Aux/IAA genes based on their amino acid sequences. Alignment was limited to full-length sequence and was analyzed using DNAMAN.

expression of *LaIAA2*, we performed a dose-response experiment using solutions with NAA concentrations ranging from 0 to 1×10^{-3} M. The highest levels of *LaIAA2* mRNA resulted from 1×10^{-3} M NAA treatment. This result was different from Arabidopsis seedling, in which 1×10^{-3} M IAA was supra-optimal for IAA mRNA abundance (Abel et al., 1995). However, the two experiments were different, whole seedlings were treated with IAA for 2 h in Arabidopsis experiment, whereas hypocotyl cuttings were treated with NAA for only 10 min in our experiment (Figure

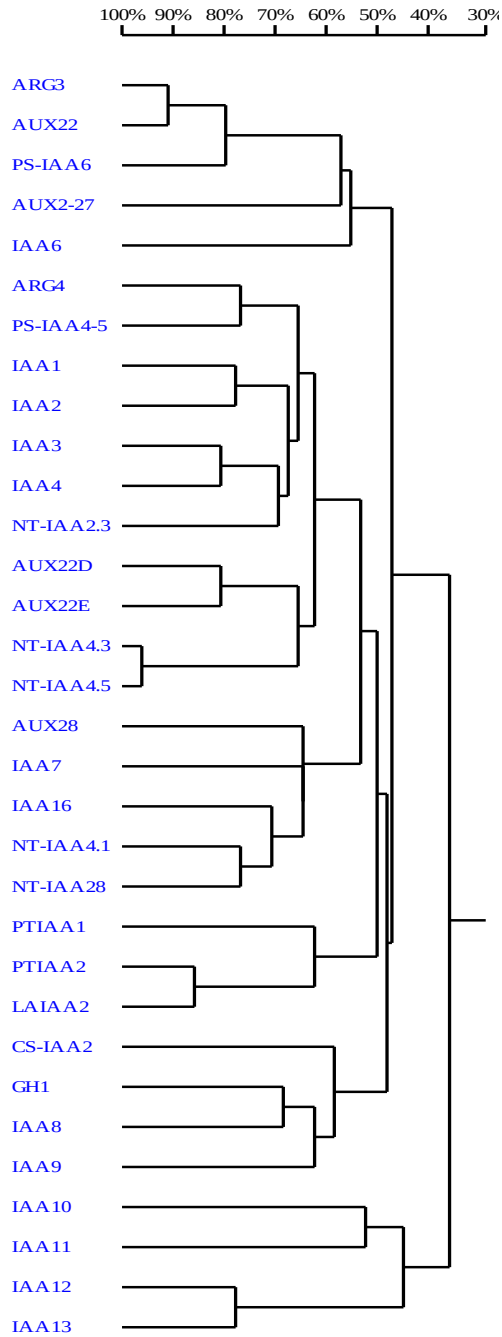

Figure 3. The phylogenetic tree of *LaIAA2.* The phylogenetic tree of 31 Aux/IAA proteins from other plants and 1 from Larix. The tree was constructed based on the amino acid alignment with selected sequences found in GenBank/EMBL bank.

LaIAA2 expression in response to auxins from different sources

To determine the changes of *LaIAA2* mRNA abundance after being treated with a variety of naturally occurring or synthetic auxins, we carried out real-time quantitative PCR to analyze hypocotyl cuttings treated with NAA (1.6 mM), indole-3-acetic acid (IAA)(8 mM), indole-3-butyric acid (IBA)(3.2 mM), 2, 4-dichlorophenoxyacetic acid (2, 4-D)(0.4 mM) and 9.5% ethanol. The results show that the mRNA levels of *LaIAA2* increased after treated by all auxins. *LaIAA2* was most strongly induced by NAA, followed by IAA and the lowest level of induction for *LaIAA2* was observed in the hypocotyls treated with 2, 4-D and IBA. *LaIAA2* levels did not change after treatment of hypocotyl cuttings with tryptophan (Figure 7).

LaIAA2 expression in response to different hormones and cycloheximide

To determine the changes of *LaIAA2* mRNA abundance after being treated with different hormones and cycloheximide, hypocotyls cuttings were treated by 6-benzylaminopurine (BA)(1.6 mM), abscisic acid (ABA)(3.2 mM), gibberellic acid (GA)(1.6 mM) or cycloheximide (CHX)(with or without NAA (1.6 mM))(0.08 mM), then real-time quantitative PCR was performed.

In the same way, mRNA abundance of *LaIAA2* following NAA treatment increased. Treatment with ABA did not increase mRNA levels of substantially greatly when compared with the controls for *LaIAA2*. The BA and GA treatment caused a slight increase in mRNA abundance in *LaIAA2*. Expression of LaIAA2 was unaffected by CHX (Figure 8).

DISCUSSION

Previous researches of rooting ability of cuttings in conifer and other plants revealed that rooting ability of cuttings were affected by auxin and its family members (Greenwood and Weir, 1994; Syros et al., 2004; Wiesman et al., 1989). The ability of auxins to stimulate adventitious root formation is well documented (Hartmann and Kester, 1983; Sircar Chatterjee, 1973). Exogenously supplied auxins, especially IAA and IBA, could improve rooting quantity of cuttings. At the same time, rooting success varied markedly with genotype and 5-fold differences among full-sib families had been observed (Foster, 1990); whereas, its poor understanding of the molecular basis for auxin and genotype in rooting ability is desirable. In molecular level, does the research about the genes which related to rooting have big significance?. It can explore the rooting mechanism about *Larix* cutting and it may also improve the rooting efficiency. In our research, by the SSH method, we successfully constructed the cDNA library between two *Larix* clones which have difference rooting ability (Feng, 2010). The result shows that some of early auxin response genes (*AUX/IAA* family) that is found in the cDNA library were important for us to understand the function of auxin in rooting of *Larix* cuttings. So based

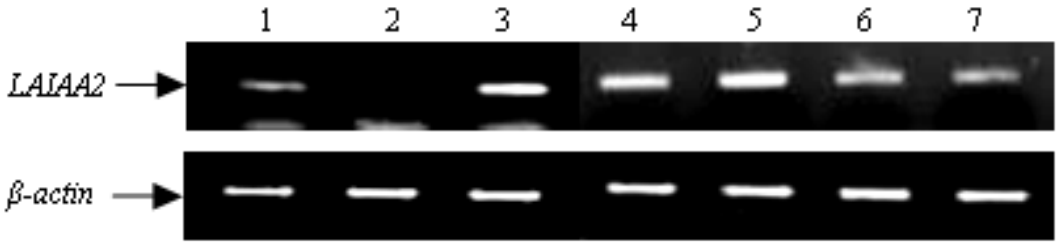

Figure 4. The expression of *LaIAA2* in root, stem, leaf and rooting of *Larix* cuttings.

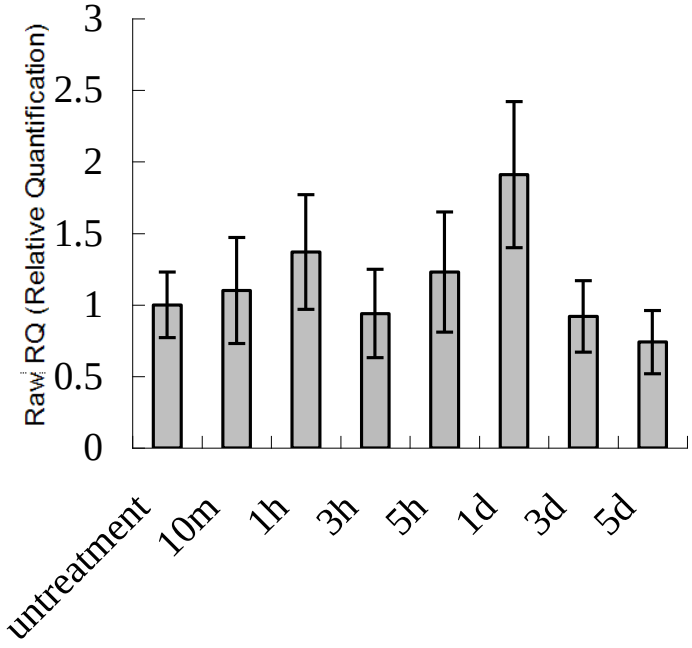

Figure 5. NAA dose response of *LaIAA2.*

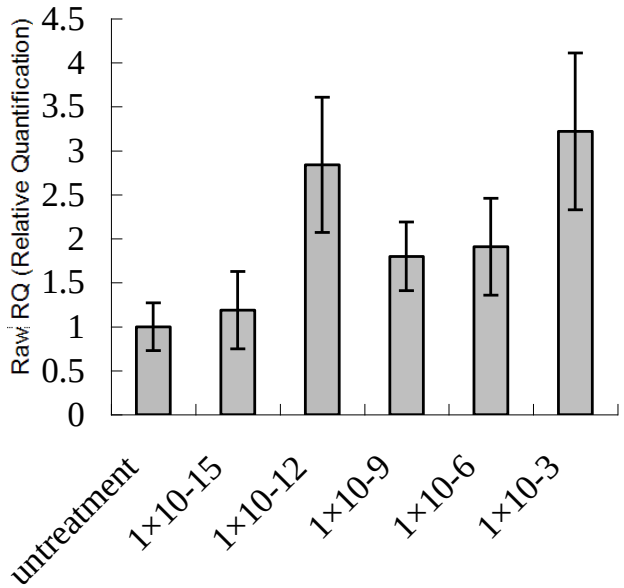

Figure 6. The hormone response of *LaI*

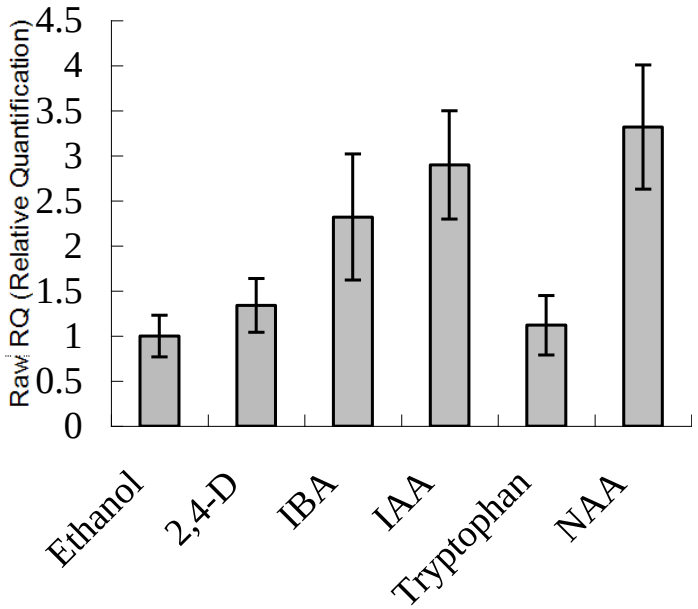

Figure 7. The auxin response of *LaIAA2.*

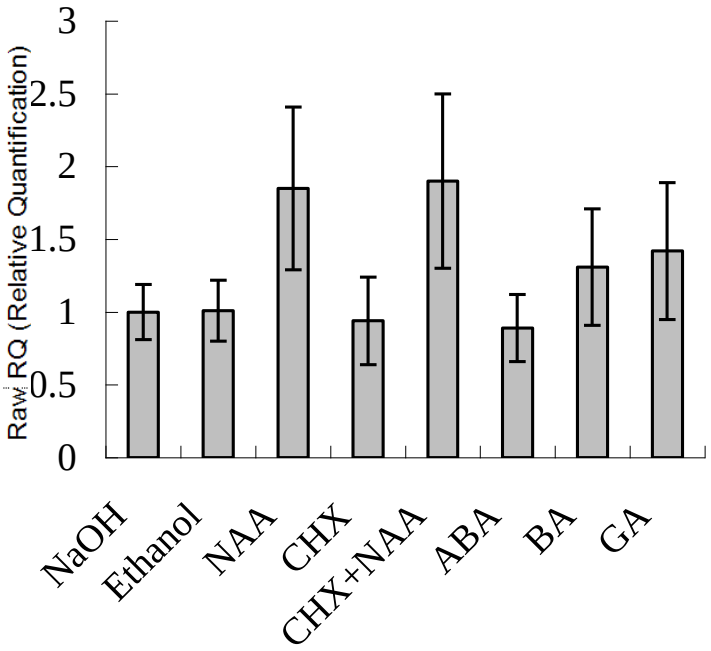

Figure 8. The expression of *LaIAA2* since NAA treatment.

on the segment of *AUX/IAA* family in cDNA library, we isolated and characterized an *Aux/IAA* gene from *Larix*, named *LaIAA2*. *LaIAA2* gene is identified as a member of the *Aux/IAA* family based on its amino acid sequence similarities to Aux/IAA proteins and its rapid and relatively specific induction by auxin. The deduced amino acid sequence of *LaIAA2* gene has all four domains of a canonical Aux/IAA protein. Compared with other Aux/IAA proteins, however, they are highly conserved for the characteristic domains I-IV. This property suggests that *LaIAA2* gene might have similar regulatory mechanisms to its homologous proteins in auxin-regulated events. In contrast, less conservation is observed between the *LaIAA2* amino acid sequence and its homologous proteins within the interdomain regions. These divergent domains might be the trace of evolutions and they might

mediate interactions with other proteins and confer a specific function on different members of the *Aux/IAA* family in different plants. Based on its fast induction kinetics and the non-inhibiton of its induction by cycloheximide treatment, *LaIAA2* appears to be the first response gene to auxin in *Larix*.

Auxins inhibit root elongation, increase lateral root production and induce adventitious roots. Abundant lateral and adventitious root is found in few mutants that overproduce auxin. Conversely, long primary roots, few lateral roots are found in mutants that were deficient in auxin responses (Zhang et al., 2007). As a big auxin response gene family, *Aux/IAA* genes are also expressed differentially in different plant tissues, organs and development stages (Abel et al., 1995). In *Arabidopsis*, gain-of-function mutations cause either enhanced (Leyser et al., 1996) or reduced (Rogg et al., 2001) root formation. In tomato, the *Sl-IAA3* promoter droves GUS expression predominantly in root cap and developing lateral roots (Chaabouni et al., 2009). In this study, we characterized that *LaIAA2* gene was expressed differentially in various organs or development stages. The results indicate that transcriptional levels of *LaIAA2* gene were regulated by some development-related elements. Semi-quantitative RT-PCR showed that *LaIAA2* was highly expressed in all phases of adventitious root formation in *Larix* cutting. Especially, the expression of *LaIAA2* gene increased obviously after the cuttings were treated with 200 ppm IBA. All of these results indicate that *LaIAA2* gene was regulated by auxin and might play a central role in root development. Then, a series of experiments to test *LaIAA2* gene expression patterns were done. In the test of timing of *LaIAA2* expression, *LaIAA2* mRNA reached maximal levels at 1 day after treatment; this differed from *Aux/IAA* genes in tobacco seedlings, which declined to near basal levels within 24 h of NAA treatment (Dargeviciute et al., 1998). The persistence of elevated mRNA levels of the *LaIAA2* could be the result of the continued presence of NAA in its active form in *Larix* cuttings. In the test of NAA dose response of *LaIAA2* expression, the highest levels of *LaIAA2* mRNA resulted from 1×10^{-3} M NAA treatment. This result is same with physiological experiment that the quantity of roots of *Larix* cuttings treated with 1×10^{-3} M NAA was most (Wang et al., 2006). In the test of *LaIAA2* expression in response to auxins from different sources or different hormones, *LaIAA2* was most strongly induced by NAA. These four experiments indicate that *LaIAA2* gene was induced by exogenous auxin treatment of *Larix* cuttings, prior to the morphological changes that led to adventitious root formation and at auxin concentrations that cause root formation. Lastly, the alignment showed that the amino acid sequence of LaIAA2 protein was more similar with *PTIAA1* and *PTIAA2* genes. Both *PTIAA1* and *PTIAA2* genes are possibly enhanced adventitious root formation (Goldfarb et al., 2003). All of these results indicate that *LaIAA2* gene is involved in adventitious root formation and enhanced root formation.

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