Plant Growth Regul (2010) 62:93–99
DOI 10.1007/s10725-010-9494-5

SHORT COMMUNICATION

Solanum tuberosum Aux/IAA family: new members and characterization of StIAA1 interacting proteins

María Cecilia Terrile · Diego Fernando Fiol · Claudia Anahí Casalongué

Received: 14 March 2010 / Accepted: 9 June 2010 / Published online: 20 June 2010

Abstract Auxin/indole-3-acetic acid (Aux/IAA) proteins are transcriptional repressors that regulate auxin-mediated gene expression by interacting with members of the auxin response factor (ARF) family. We previously identified the first *Solanum tuberosum* Aux/IAA member, *StIAA1*, as a stress-responsive gene. In this report, we described that StIAA1 interacts with TIR1 auxin receptor suggesting a conserved participation in auxin signaling pathway. In addition, protein–protein interaction between StIAA1 and new members of *S. tuberosum* Aux/IAA (StIAA3 and StIAA4) and ARF (StARF1) families was demonstrated. Furthermore, thirteen other members of the *S. tuberosum* Aux/IAA family were identified by in silico analysis. This overall view of auxin signaling components in a Solanaceae contributes to enrich the understanding of this hormonal pathway in other plants phylogenetically distant from *A. thaliana*.

Keywords Auxin response factor (ARF) protein · Auxin/indole-3-acetic acid (Aux/IAA) protein · Auxin signaling pathway · *Solanum tuberosum*

Electronic supplementary material The online version of this article (doi:10.1007/s10725-010-9494-5) contains supplementary material, which is available to authorized users.

M. C. Terrile · D. F. Fiol · C. A. Casalongué (✉)
Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata, Funes 3250, CC 1245, 7600 Mar del Plata, Argentina
e-mail: casalong@mdp.edu.ar

C. A. Casalongué
Departamento de Biología, Universidad Nacional de Mar del Plata, Funes 3250, CC 1245, 7600 Mar del Plata, Argentina

Introduction

The plant hormone auxin, typified by indole-3-acetic acid (IAA), controls many aspects of plant development (Woodward and Bartel 2005). It exerts its effects by modulating gene expression through the action of two large families of transcription factors called Auxin/indole-3-acetic acid (Aux/IAA) and Auxin Response Factor (ARF). The *Arabidopsis thaliana* genome contains 29 and 23 different *Aux/IAA* and *ARF* members, respectively (Okushima et al. 2005; Overvoorde et al. 2005). *Aux/IAA* genes encode for proteins that contain four conserved domains, where domain I confers repressor activity and domains III and IV mediate ARF interaction. Domain II contains a 13-amino acid motif that is responsible for rapid degradation of Aux/IAA proteins (Worley et al. 2000; Ramos et al. 2001). ARF proteins bind directly to DNA and, depending on their type, either activate or inhibit transcription (Guilfoyle and Hagen 2007). Interaction between Aux/IAA and ARF proteins represses auxin-regulated gene expression. Auxin binding to the auxin receptor Transport Inhibitor Response 1 (TIR1) or its paralogs, Auxin Signaling F-box 1-3 (AFB1-3) promotes the recruitment and ubiquitin-dependent proteolysis of Aux/IAA proteins (Gray et al. 2001; Dharmasiri et al. 2005a, b; Kepinski and Leyser 2005; Tan et al. 2007). Consequently, Aux/IAA degradation leads to the activation of ARFs and subsequent auxin-responsive gene expression (Hagen and Guilfoyle 2002).

Although there exists an expanding comprehension of auxin signaling mechanism in *A. thaliana*, the actors that take part in other plant species have been less explored. The complete *Aux/IAA* gene family has also been characterized in *Oryza sativa* and *Populus trichocarpa* (Jain et al. 2006; Kalluri et al. 2007). Only two *Aux/IAA* members have been reported in *Solanum tuberosum* so far: *StIAA* and *StIAA2* (Zanetti et al. 2003; Kloosterman et al. 2006).

In this work, evidences on protein–protein interaction between StIAA1 (previously StIAA) and TIR1, as well as other potato Aux/IAA and ARF proteins are presented. In addition, a wide framework of Aux/IAA family in *S. tuberosum* is described.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana tir1-1 *GVG::TIR1-myc* transgenic line was described by Gray et al. (1999). Seedlings were grown on solid ATS medium (Wilson et al. 1990) in a growth chamber at 22–24°C under 100 μmol m^{-2} s^{-1} with 16:8 h light:dark cycles.

In vitro GST pull-down assay and protein blot analysis

Ten day-old *tir1*-1 *GVG::TIR1-myc* seedlings were transferred into liquid ATS medium containing 30 μM dexamethazone for 24 h to induce TIR1-myc expression. Total protein was extracted by homogenizing seedlings in a buffer containing 50 mM Tris–HCl (pH 7.2), 100 mM NaCl, 0.1% Tween 20, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 10 μM MG132. Extracts were cleared of cell debris by centrifugation at 12,000*g* for 10 min. *StIAA1* coding sequence was cloned in-frame into *Sal*I/*Not*I sites of the glutathione S-transferase (GST) fusion vector pGEX-4T-3 and introduced into *Escherichia coli* strain BL21 (DE3) pLys. Stationary phase cells were diluted tenfold and grown at 37°C for 1 h before induction with 1.5 mM isopropyl-1-thio-β-D galactopyranoside (IPTG). Cells were collected after 4 h of growth, resuspended in PBS (25 mM sodium phosphate buffer pH 7.2, 150 mM NaCl) containing 0.5% Triton X-100, and lysed by sonication. GST-StIAA1 fusion protein was purified using glutathione-agarose beads (Molecular Probes, Oregon, USA). For GST pull-down reaction, 3–4 μg GST fusion protein was incubated with 800 μg crude *tir1*-1 *GVG::TIR1-myc* extract at 4°C for 3 h with gentle agitation. IAA was directly added to the pull-down reaction. After incubation glutathione-agarose beads were recovered by centrifugation and washed three times with 1 ml extraction buffer without protease inhibitors for 15 min. Beads were resuspended in sample buffer and proteins were resolved by 12% SDS–PAGE and analyzed by protein blot using anti-c-myc antibody (Sigma–Aldrich, St. Louis, MO, USA).

Yeast two-hybrid analyses

An oriented activation domain (AD)-tagged cDNA library was constructed from potato (*Solanum tuberosum* cv Desireé) stolons by using the HybriZAP kit with the pAD-GAL4 vector (Stratagene, La Jolla, CA, USA) and converted to a yeast plasmid library by in vivo excision (Chen et al. 2003). The Matchmaker two-hybrid system (Clontech Laboratories, Inc., Palo Alto, CA, USA) was used for yeast (*Saccharomyces cerevisiae*) two-hybrid screen. AH109 yeast strain transformation and plasmid rescue into DH5-α *E. coli* cells were carried out according to the manufacturer's instructions. StIAA1 coding sequence was cloned in-frame into pGBKT7 vector using *Sma*I/*Sal*I linker primers. This construct was used as a bait to screen the stolon two-hybrid library. Positive interactions were confirmed by cotransforming yeast cells with each purified pAD plasmids and pGBKT7:StIAA1. Untransformed yeasts were grown in yeast extract/dextrose/peptone (YPD) medium (Sigma–Aldrich) while selection of plasmids and/or interactions was performed on appropriate synthetic dropout (SD) media (Clontech). To select bait (BD) and prey (AD) plasmids, a non-selective medium lacking tryptophane and leucine (–Trp/–Leu) was used. Selective medium containing 40 mM 3-aminotrioazole (3-AT) and lacking tryptophane, leucine, histidine and adenine (–Trp/–Leu/–His/–adenine) allowed selection of bait-prey interaction.

For the semi-quantitative growth assay, one colony from a fresh plate was grown in non-selective medium. The OD_{600} of yeast culture was measured and adjusted to 2.0. Each sample was diluted 10, 100 and 1,000 times and 5 μl was plated on selective and non-selective media and incubated at 30°C for 6 days.

In silico identification and phylogenetic analysis

To identify new Aux/IAA family members in *S. tuberosum*, an exhaustive survey of EST databanks was performed. Search was performed utilizing sequences coding for conserved regions (domains I to IV) from every *A. thaliana* Aux/IAA protein as queries and using BLAST. Hits were aligned and full length ORFs were assembled from several ESTs in order to solve frequent sequencing errors. Sequences were confirmed, when available, with the data of partial *S. tuberosum* genome sequences at the Solanaceae Genomics Resource (http://solanaceae.plantbiology.msu.edu/).

ClustalX program was used to perform multiple alignments and for the construction of the unrooted phylogenetic tree based on the neighbor-joining method (Thompson et al. 1997). Bootstrap resampling (1,000 trials) was used to estimate the degree of confidence in the branching order.

Results and discussion

New partners of StIAA1 protein

In order to gain insight into StIAA1 function, protein–protein interaction assays were performed. For this purpose,

StIAA1 expressed as GST fusion protein and total protein extracts containing c-myc epitope-tagged AtTIR1 derivative were used in pull-down assays. Protein gel blot analysis using anti-c-myc antibodies revealed that TIR1 co-purified with GST-StIAA1 in the presence of IAA but was undetectable when IAA was omitted (Fig. 1a) supporting the idea that StIAA1 interacts with TIR1 protein in an auxin-dependent manner.

To explore other putative targets of StIAA1, a yeast two hybrid strategy was adopted. GAL4 DNA-binding domain (BD) was fused to StIAA1 ORF (open reading frame) (BD-StIAA1). The fusion protein was correctly expressed in yeast and auto-activation of *HIS3* and *ADE2* reporter genes was not detected (data not shown). This construct was used as a bait to screen a potato cDNA library cloned as fusion to GAL4 DNA-activation domain (AD). More than 4 million independent potato cDNA clones were screened for their auxotrophy in selective media (–Trp/–Leu/–His/–adenine). After several rounds of screening, four different cDNA clones interacted stably with StIAA1. Figure 1b shows in vivo protein–protein interaction between StIAA1 and potato interacting partners. These four cDNAs were isolated and identified by single-pass sequencing. One of them matched to the same protein, StIAA1. The other three cDNA sequences corresponded to unidentified *S. tuberosum* genes. They were submitted to GenBank public database as GQ386947, GQ386948 and GQ386949 entries. GQ386947 and GQ386948 sequences matched with SGN-U269815 and SGN-U289713 unigenes, respectively, from Sol Genomics Database (http://sgn.cornell.edu) while GQ386949 matched with SGN-U293 936 and SGN-295715. Predicted GQ386947 and GQ386 948 amino acid sequences revealed high similarity with Aux/IAA proteins while GQ386949 showed homology with ARF proteins. GQ386947 showed 88% identity with *Nicotiana tabacum* IAA28 and its most homologue sequence from *A. thaliana* corresponded to IAA16 (78%). GQ386948 presented 68% identity with *Ricinus comunis* IAA27 and 52% with both *A. thaliana* IAA27 and IAA8. Based on their sequence homologies with other Aux/IAAs, GQ386947 and GQ386948 were classified as new potato Aux/IAA proteins and named StIAA3 and StIAA4, respectively. Functional characterization of StIAA3 and StIAA4 homologous, NtIAA28, AtIAA16, RcIAA27, AtIAA27 and AtIAA8 has not been described yet. However, AtIAA27 is known to interact with phytochrome A (PHYA) and the Tobacco mosaic virus (TMV) replicase (Choi et al. 1999; Soh et al. 1999; Padmanabhan et al. 2006). In addition, AtIAA8 and NtIAA28 have been classified as secondary auxin-responsive genes because of their delayed response to IAA and their dependence on de novo protein synthesis (Abel et al. 1995; Dargeviciute et al. 1998). Since StIAA1 is a late auxin-response gene (Zanetti et al. 2003), it is likely that the protein–protein interactions detected, StIAA1-StIAA3 and StIAA1-StIAA4, may occur in vivo and participate in the regulation of growth responses or environmental stimuli. Functional relevance of such interactions may be part of further studies.

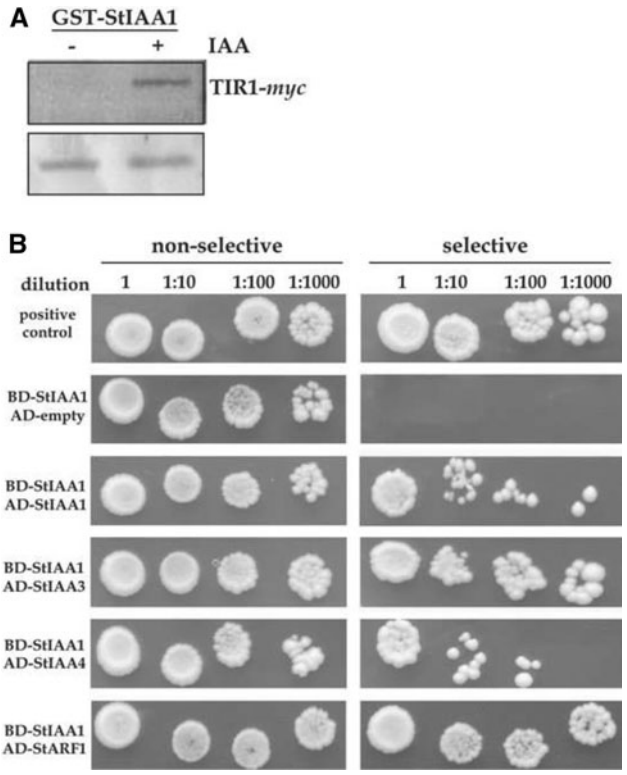

Fig. 1 StIAA1 protein–protein interactions. **a** In vitro StIAA1-TIR1 protein–protein interaction. Protein extracts prepared from *GVG::TIR1-myc* seedlings were incubated with purified GST-StIAA1 in the presence of 50 μM IAA. After several washes, bounded proteins were electrophoresed in a 12% SDS–PAGE and immunoblotted with anti-c-myc antibody. The lower panel shows control protein loading. **b** In vivo StIAA1 protein–protein interaction. The plasmid pGBKT7-StIAA1 was used as a bait to screen a potato cDNA library constructed in a pAD-GAL4-2.1 vector. AH109 yeast cells containing pGBKT7-StIAA1 plasmid were grown on liquid medium lacking tryptophan (–Trp) and were transformed with the cDNA library. Transformants were plated on non-selective (–Trp/–Leu) or selective media (–Trp/–Leu/–His, containing 20 mM 3-AT) in serial dilutions for semi-quantitative growth assay. Positive control corresponds to a well-characterized protein–protein interaction, AtγCA2/AtγCAL2 (Perales et al. 2004). As negative control, yeast cells harbouring the pGaD424 were used (BD-StIAA1/AD-empty)

Finally, the fourth interacting protein, coded by GQ386949 gene was named *StARF1* based on its high similarity with ARF2 from *Cucumis sativus* and *Gossypium arboretum*. GQ386949 sequence includes a partial *StARF1* gene (coding for 268 aminoacids in contrast with over 1,100 aminoacids contained in both ARF2 from *C. sativus* and *G. arboretum*). In order to obtain a more complete StARF1 sequence, *S. tuberosum* ESTs identical to GQ386949 were searched in EST databases using basic local alignment

Table 1 *Solanum tuberosum Aux/IAA* family members

Gene	Length (aa)	*AtIAA* homologs (% identity)	GenBank ID	Reference
StIAA1	349	*AtIAA8* (62) *AtIAA9* (61) *AtIAA27* (56)	AY098938	Zanetti et al. (2003)
StIAA2	213	*AtIAA7* (71) *AtIAA14* (73) *AtIAA17* (70) *AtIAA16* (70)	EF053504	Kloosterman et al. (2006)
StIAA3	249	*AtIAA16* (78) *AtIAA7* (65) *AtIAA14* (65) *AtIAA17* (62)	GQ386947 ESTs: CK278440, CV470264, DV624641, BQ114777, BQ114861, CK270862	This paper
StIAA4	295	*AtIAA8* (52) *AtIAA9* (45) *AtIAA27* (52)	GQ386948 ESTs: DV626685, BM113677, G887295, BE471882	This paper
StIAA5	182	*AtIAA1* (62) *AtIAA2* (61) *AtIAA3* (62) *AtIAA4* (63)	ESTs: BG597838, BM407632, BF153569, EG015968	Predicted in silico
StIAA6	176	*AtIAA5* (58) *AtIAA6* (58) *AtIAA19* (57)	ESTs: CV505892, CV499480, CV499493, CN215626, DR037782, DN589141	Predicted in silico
StIAA7	155	*AtIAA1* (65) *AtIAA2* (65) *AtIAA3* (65) *AtIAA4* (65)	ESTs: CV499207, DR037782, DN589141, CN215626	Predicted in silico
StIAA8	277	*AtIAA8* (53) *AtIAA9* (51) *AtIAA27* (62)	ESTs: DV625819, DV624641, DN939464, CV470264	Predicted in silico
StIAA9	236	*AtIAA7* (72) *AtIAA14* (75) *AtIAA17* (70) *AtIAA16* (69)	ESTs: CN514914, CK268524, CN514887, CX700132, CK268523, CK863890	Predicted in silico
StIAA10	216	*AtIAA7* (66) *AtIAA14* (69) *AtIAA17* (66) *AtIAA16* (71)	ESTs: CX162197, BQ505047, CV501053, BG592836, FG550880, BI175834	Predicted in silico
StIAA11	283	*AtIAA28* (55) *AtIAA18* (50) *AtIAA26* (50)	ESTs: CK249849, CK249850	Predicted in silico
StIAA12	282	*AtIAA12* (55) *AtIAA13* (57)	EST: CV503304	Predicted in silico
StIAA13	213	*AtIAA6* (43) *AtIAA19* (43)	SGN-U272708	Predicted in silico
StIAA14	220*	*AtIAA10* (40) *AtIAA11* (52)	ESTs: CN213675, BG594573, BG096268	Predicted in silico
StIAA15	222	*AtIAA29* (33) *AtIAA10* (27) *AtIAA11* (29)	EST: BQ120731	Predicted in silico
StIAA16	195	*AtIAA1* (69) *AtIAA2* (66) *AtIAA3* (68) *AtIAA4* (69)	ESTs: AM907737, AM908966, CX700174, DN848977, CK851465, BQ119535	Predicted in silico
StIAA17	190	*AtIAA1* (67) *AtIAA2* (65) *AtIAA3* (65) *AtIAA4* (65)	ESTs: BG592184, BG095837, BQ117045	Predicted in silico

Closest *A. thaliana* homologs are shown. GenBank ID (if available) or corresponding ESTs are indicated for each gene. SGN is indicated if no EST data is available. Asterisk indicates a partial sequence

search tool (BLAST). Several other partial sequences coding for StARF1 were also identified (Online Resource 1A). The assembly of identified ESTs extended StARF1 sequence to over 2,200 base pairs, including an ORF coding for 593 aminoacids. Then, translation of extended StARF1 was aligned with most similar ARFs (Online Resource 1B). The C-terminal region of ARF proteins contains the well-conserved domains III–IV which allows heterodimerization with Aux/IAA proteins. Moreover, StARF1 contains these conserved domains that most likely allowed protein–protein interaction with StIAA1. Hitherto, only one *S. tuberosum* ARF (ARF6) has been characterized (Faivre-Rampant et al. 2004).

Collectively, our data indicated that StIAA1 is able to form both homo and heterodimers with members of Aux/IAA and ARF families as previously reported for different members of *A. thaliana* Aux/IAA proteins (Kim et al. 1997; Ulmasov et al. 1999). Taking together all these findings, we suggest that StIAA1 is a functional member of the potato Aux/IAA family. To our knowledge, this is the first report of the physical interaction between a member of Aux/IAA family and TIR1 auxin receptor in a species other than *A. thaliana*. Future studies will allow a better understanding on the precise role of these protein–protein interactions and dimerization preferences between different auxin signaling components.

In silico identification of new *StIAAs*

In an attempt to acquire a more complete framework of *Aux/IAA* family members in *S. tuberosum*, an exhaustive survey of EST databanks was performed. Thirteen new

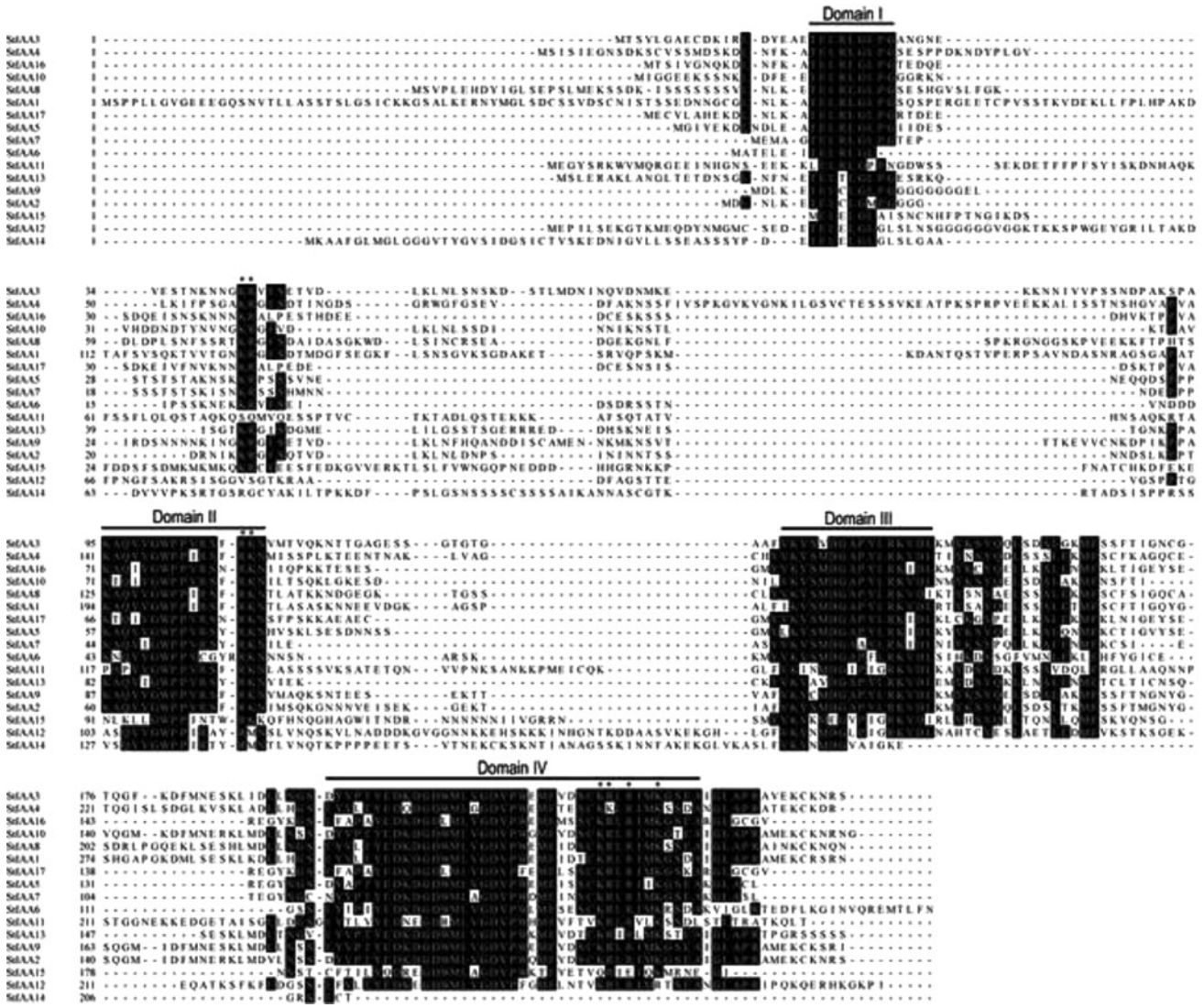

Fig. 2 Alignment of deduced amino acid sequences of *S. tuberosum* Aux/IAA proteins. For the alignment analysis, the computer program ClustalX was used. Conserved domains I to IV are indicated. *Asterisks* show the conserved NLS

S. tuberosum Aux/IAA sequences (named *StIAA5* to *StIAA17*) were identified (Table 1). The percentage of identity between each *S. tuberosum* Aux/IAA sequences is shown in Online Resource 2. A pair-wise analysis of protein sequences indicated that the overall identities range from 24 to 81%. However, the amino acid identity within the conserved domains reached over than 90%. To examine in more detail the domain organization of StIAA proteins, a multiple sequence alignment of the Aux/IAA proteins was done using ClustalX program (Fig. 2). Conserved domains I to IV are present in all 17 StIAA protein sequences. Key residues for the bipartite nuclear localization signal (NLS) and SV40-type NLS located in domain III are also conserved among all analyzed sequences.

Phylogenetic analysis of *S. tuberosum* and *A. thaliana* Aux/IAA members showed that all identified and predicted proteins belong to previously described subfamilies (Fig. 3). Nine *S. tuberosum* Aux/IAA proteins clustered into subfamily I, which is the most populated and divergent group: StIAA5, 7, 16 and 17 clustered with AtIAA1-4; StIAA6 clustered with AtIAA5, 6, 15 and 19; StIAA11 clustered with AtIAA18, 26 and 28; StIAA12 and 14 clustered with AtIAA10-13; StIAA15 clustered with AtIAA29. On the other hand, within the subfamily I no StIAA members were identified for the cluster given by AtIAA20, 30 and 31 (not included in Fig. 3). StIAA2, 3, 9 and 10 clustered with AtIAA7, 14, 16 and 17 into subfamily II. StIAA8 clustered with AtIAA27 in subfamily III while StIAA1 clustered with AtIAA8 and 9 into subfamily IV. Subfamilies III and IV did not diverge early in our results, but grouped together; even StIAA4 clustered within this group. Finally, StIAA13 did not cluster with any

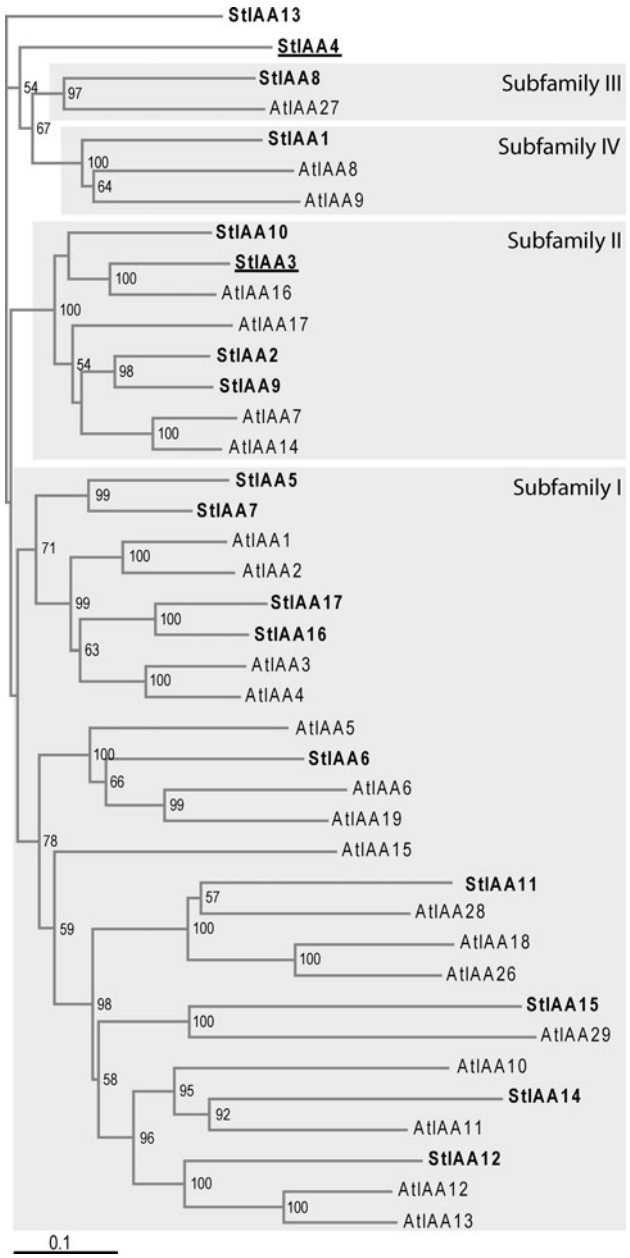

Fig. 3 Phylogenetic analysis of predicted Aux/IAA protein sequences. An unrooted phylogenetic tree was generated using the neighbour-joining program ClustalX package. Aux/IAA proteins experimentally identified in this work are *underlined*. *Numbers* at each node indicate the percentage of trees representing the particular node out of 1,000 bootstrap replicates (only values > 50 are shown)

A. thaliana Aux/IAA protein and it could not be included in any subfamily.

Concluding remarks

The complexity of auxin regulatory activity may be, at least in part due to the large size of *Aux/IAA* and *ARF* gene families, as well as to the variation in dimerization affinities, expression patterns, and auxin-mediated transcriptional and post-transcriptional regulation. Functional characterization of Aux/IAA and ARF proteins from different plant species is a critical step to go forward in the understanding of how these genes translate into a diverse suite of auxin-mediated functions even in plants with specific features. Although there are common traits between Aux/IAA and ARF proteins from *S. tuberosum* and *A. thaliana*, auxin dependent gene regulation is still unexplored in most dicotiledoneous plants. *StIAA1* is up-regulated by biotic and abiotic stresses in potato tubers (Zanetti et al. 2003). Its closest *S. lycopersicum* gene, *SlIAA9* is involved in fruit development and leaf morphogenesis (Wang et al. 2005). On the other hand, *StIAA2* is involved in petiole hyponasty and shoot morphogenesis (Kloosterman et al. 2006). In agreement with Kazan and Manners (2009), auxin regulation is intimately linked to plant growth and adaptative response against environmental stress. However, further studies are necessary to fully understand how auxin signaling components act and interact with endogenous partners.

Acknowledgments We thank Dr. Mark Estelle (UCSD, USA) for providing us the *tir1*-1 *GVG::TIR1-myc* seeds, Dr. Salomé Pratt for the gift of the potato cDNA library and Dr. Eugenia Zanetti for her technical assistance with yeast two-hybrid system. This work was supported by grants from National Research Council (CONICET), Universidad Nacional de Mar del Plata and ANPCyT (Argentina). MCT, DFF and CAC are members of CONICET.

References

Abel S, Nguyen MD, Theologis A (1995) The PS-IAA4/5-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana*. J Mol Biol 251:533–549

Chen H, Rosin FM, Prat S, Hannapel DJ (2003) Interacting transcription factors from the three-amino acid loop extension superclass regulate tuber formation. Plant Physiol 132:1391–1404

Choi MS, Yi H, Lee J, Kwon YK, Soh MC, Shin B, Luka Z, Hahn TR, Song PS (1999) Phytochrome signalling is mediated through nucleoside diphosphate kinase 2. Nature 401:610–613

Dargeviciute A, Roux C, Decreux A, Sitbon F, Perrot-Rechenmann C (1998) Molecular cloning and expression of the early auxin-responsive Aux/IAA gene family in *Nicotiana tabacum*. Plant Cell Physiol 39:993–1002

Dharmasiri N, Dharmasiri S, Estelle M (2005a) The F-box protein TIR1 is an auxin receptor. Nature 435:441–445

Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, Hobbie L, Ehrismann JS, Jurgens G, Estelle M (2005b) Plant development is regulated by a family of auxin receptor F-box proteins. Dev Cell 9:109–119

Faivre-Rampant O, Cardle L, Marshall D, Viola R, Taylor MA (2004) Changes in gene expression during meristem activation processes in *Solanum tuberosum* with a focus on the regulation of an auxin response factor gene. J Exp Bot 55:613–622

Gray WM, del Pozo JC, Walker L, Hobbie L, Risseeuw E, Banks T, Crosby WL, Yang M, Ma H, Estelle M (1999) Identification of

an SCF ubiquitin-ligase complex required for auxin response in *Arabidopsis thaliana*. Genes Dev 13:1678–1691

Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001) Auxin regulates SCF(TIR1)-dependent degradation of Aux/IAA proteins. Nature 414:271–276

Guilfoyle TJ, Hagen G (2007) Auxin response factors. Curr Opin Plant Biol 10:453–460

Hagen G, Guilfoyle T (2002) Auxin-responsive gene expression: genes, promoters and regulatory factors. Plant Mol Biol 49:373–385

Jain M, Kaur N, Garg R, Thakur JK, Tyagi AK, Khurana JP (2006) Structure and expression analysis of early auxin-responsive Aux/IAA gene family in rice (*Oryza sativa*). Funct Integr Genomics 6:47–59

Kalluri UC, Difazio SP, Brunner AM, Tuskan GA (2007) Genome-wide analysis of Aux/IAA and ARF gene families in *Populus trichocarpa*. BMC Plant Biol 7:59

Kazan K, Manners JM (2009) Linking development to defense: auxin in plant-pathogen interactions. Trends Plant Sci 14:373–382

Kepinski S, Leyser O (2005) The Arabidopsis F-box protein TIR1 is an auxin receptor. Nature 435:446–451

Kim J, Harter K, Theologis A (1997) Protein-protein interactions among the Aux/IAA proteins. Proc Natl Acad Sci USA 94: 11786–11791

Kloosterman B, Visser RGF, Bachem CWB (2006) Isolation and characterization of a novel potato Auxin/Indole-3-Acetic Acid family member (StIAA2) that is involved in petiole hyponasty and shoot morphogenesis. Plant Physiol Biochem 44:766–775

Okushima Y, Overvoorde PJ, Arima K, Alonso JM, Chan A, Chang C, Ecker JR, Hughes B, Lui A, Nguyen D, Onodera C, Quach H, Smith A, Yu G, Theologis A (2005) Functional genomic analysis of the AUXIN RESPONSE FACTOR gene family members in *Arabidopsis thaliana*: unique and overlapping functions of ARF7 and ARF19. Plant Cell 17:444–463

Overvoorde PJ, Okushima Y, Alonso J, Chan A, Chang C, Ecker J, Hughes B, Liu A, Onodera C, Quach H, Smith A, Yu G, Theologis A (2005) Functional genomic analysis of the AUXIN/INDOLE-3-ACETIC ACID gene family members in *Arabidopsis thaliana*. Plant Cell 17:3282–3300

Padmanabhan MS, Shiferaw H, Culver JN (2006) The tobacco mosaic virus replicase protein disrupts the localization and function of interacting Aux/IAA proteins. Mol Plant Microbe Interact 19:864–873

Perales M, Parisi G, Fornasari MS, Colaneri A, Villarreal F, González-Schain N, Echave J, Gómez-Casati D, Braun HP, Araya A, Zabaleta E (2004) Gamma carbonic anhydrase like complex interact with plant mitochondrial complex I. Plant Mol Biol 56:947–957

Ramos JA, Zenser N, Leyser O, Callis J (2001) Rapid degradation of auxin/indoleacetic acid proteins requires conserved amino acids of domain II and is proteasome dependent. Plant Cell 13:2349–2360

Soh MC, Hong SH, Kim BC, Vizir I, Park DH, Choi G, Hong MY, Chung YY, Furuya M, Nam HG (1999) Regulation of both light- and auxin-mediated development by the *Arabidopsis IAA3/SHY2* gene. J Plant Biol 42:239–246

Tan X, Calderon-Villalobos LI, Sharon M, Zheng C, Robinson CV, Estelle M, Zheng N (2007) Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature 446:640–645

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Ulmasov T, Hagen G, Guilfoyle TJ (1999) Dimerization and DNA binding of auxin response factors. Plant J 19:309–319

Wang H, Jones B, Li Z, Frasse P, Delalande C, Regad F, Chaabouni S, Latche A, Pech JC, Bouzayen M (2005) The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. Plant Cell 17:2676–2692

Wilson AK, Pickett FB, Turner JC, Estelle M (1990) A dominant mutation in Arabidopsis confers resistance to auxin, ethylene and abscisic acid. Mol Gen Genet 222:377–383

Woodward AW, Bartel B (2005) Auxin: regulation, action, and interaction. Ann Bot (Lond) 95:707–735

Worley C, Zenser N, Ramos JA, Rouse D, Leyser HM, Theologis A, Callis J (2000) Degradation of Aux/IAA proteins is essential for normal auxin signalling. Plant J 21:553–562

Zanetti ME, Terrile MC, Godoy AV, San Segundo B, Casalongue CA (2003) Molecular cloning and characterization of a potato cDNA encoding a stress regulated Aux/IAA protein. Plant Physiol Biochem 41:755–760