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ORIGINAL ARTICLE

Cloning and characterization of auxin efflux carrier genes *EcPIN1a* and *EcPIN1b* from finger millet *Eleusine coracana* L.

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Abstract Auxin signaling events in plants play important role in developmental regulation as well as gravitropic responses and plays crucial role in the development of root, lateral root and root hairs. The gene that is known to be most important in the development of root, lateral root and root hairs is commonly known as auxin efflux carrier (*PIN*). Being commonly known as orphan plant, the genome sequence of *Eleusine coracana* is not known yet, and hence it was very difficult to conduct advanced research in root development in this plant. As *PIN* gene plays crucial role in root development, to have some advanced study we proposed to clone the *PIN* genes from *E. coracana*. We cloned two *PIN* genes in *E. coracana* and named them as *EcPIN1a* and *EcPIN1b*. The coding sequence (CDS) of *EcPIN1a* was 1779 bp and *EcPIN1b* was 1788 bp long that encodes for 593 and 596 amino acids, respectively. In-silico analysis shows the presence of transmembrane domain in EcPIN1a and EcPIN1b protein. Multiple sequence alignment of EcPIN1a and EcPIN1b protein shows the presence of several conserved motifs. Phylogenetic analysis of EcPIN1a and EcPIN1b grouped with the PIN gene of monocot plant *Oryza sativa*. This shows that *EcPIN* genes were monocot specific, and closely match with the PIN genes of *O. sativa*. The transcript analysis of *EcPIN1a* gene in leaf tissue shows gradual up-regulation from 7th to 28th days of developmental time period while the transcript level was found to be lower in root tissue. The transcript abundance of *EcPIN1b* was not detected. Gradual up-regulation of *EcPIN1a* gene in developmental stages signifies its important role in root development in *E. coracana*.

Keywords Auxin · Auxin influx carrier · Auxin efflux carrier · PIN · Transmembrane domain

Introduction

The sessile organism plant uses its roots to acquire water and nutrient molecules from soil as well as monitor the underground soil for a wide range of environmental conditions (Chapman et al. 2012; Craine and Dybzinski 2013; Barberon and Geldner 2014; Kong and Ma 2014). Moreover, the degree of root branching has lots of impact in the efficiency of the nutrient acquisition, water uptake and anchorage to the plant (López-Bucio et al. 2003; Hermans et al. 2006). Therefore, it is very important to understand the agronomic importance of root development. The cellular and molecular basis of root formation and its development has been studied extensively in model plants *Oryza sativa* and *Arabidopsis thaliana* (Coudert et al. 2010; Petricka et al. 2012; Mohanta et al. 2015; Singh et al. 2015). Lots of progress has been made to identify the important genes involved in root, lateral root and root hair formation in these plants (Bañoc et al. 2000; Casimiro et al. 2003; Péret et al. 2009). The model organism *Arabidopsis* possesses primary root that continuously branches to generate several lateral roots (Péret et al. 2009), whereas

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the monocot plant *O. sativa*, *Zea mays* and others predominantly contain adventitious roots (Lorbiecke 1999; Rebouillat et al. 2009; Liu et al. 2009). Availability of genomic data led to the identification of several genes that are common to lateral, adventitious and crown root development (Casimiro et al. 2003; Petricka et al. 2012). Availability of genomic data of crop plant *O. sativa* led to the development of several novel agronomic traits with superior root development and nutrient acquisition potential (Majumder et al. 1990; Wissuwa and Ae 2001; de Dorlodot et al. 2007; Shrawat et al. 2008). These agronomic traits are very useful for plants growing in hilly areas as well as in drought and dry soil to withstand adverse conditions. In hilly area, the roots provide proper anchorage to the plants while in drought and water starved area, roots help to absorb the underground soil moisture and help the plant to withstand the drought condition. Availability of genome sequence data in model plant like *O. sativa* led to decipher the potential role of different agronomic important traits. However, the genome sequence data of the orphan crop plant *Eleusine coracana* is not available yet. *Eleusine coracana* is an important monocot crop plant belonging to family Poaceae and cultivated throughout the world and used as a staple food crop in Africa and India as well. The grains of this plant contain important nutritional value as they are rich in vitamins (Shobana et al. 2013; Chandra et al. 2016; Gull and Ahmad 2016). Besides this, it is also very attractive source of dietary calcium content (Chandra et al. 2016). Therefore, the grains of *E. coracana* used to make dietary food and food product as well (Ramulu and Udayasekhara Rao 1997; Mangala et al. 1999; Mahadevamma and Tharanathan 2004). However, the crop yield of *E. coracana* is largely affected by soil texture, nutrient supplement and proper irrigation. In majority of cases, this plant is cultivated in dry and loamy conditions. Therefore, improvement of the rooting system in *E. coracana* will be very valuable to enhance the potential of crop yield.

The root development in plants is largely regulated by the phytohormone auxin (Vanneste and Friml 2009; Zazímalová et al. 2010; Mohanta and Mohanta 2013; Mohanta et al. 2014; Gururani et al. 2015). Several studies have demonstrated in model plants regarding root, and root hair formation. The phytohormone auxin is regulated by different auxin signaling genes including Aux/*IAA* (Indole-3-acetic acid) (Lewis et al. 2011; Carraro et al. 2012), *LAX* (auxin influx carrier) (Bainbridge et al. 2008; Swarup et al. 2008; Vandenbussche et al. 2010) and *PIN* (auxin efflux carrier) (Friml et al. 2003; Forestan and Varotto 2012). Previous studies demonstrated that auxin efflux carrier (*PIN*) protein conducts directional auxin flow and regulate root development and plant morphogenesis (Péret et al. 2013; Drdová et al. 2013; Band et al. 2014; Singh et al. 2015). Therefore, it was highly important to study the role of *PIN* genes in *E. coracana* to understand their potential role in root development. Hence, we cloned and characterized the auxin efflux carrier (*EcPIN*) genes from *E. coracana* and reported here.

Materials and methods

Plant material and growth conditions

To grow *E. coracana*, first of all the compost soil were autoclaved and sterilized to remove any potential bacterial and fungal growth. Later, the seeds were surface sterilized by 1% (v/v) sodium hypochlorite for 5 min followed by washing with 70% (v/v) ethanol for one minute. To remove the residual ethanol, seeds were washed with sterilized double distilled water. Later the seeds were germinated in pot and allowed to grow in the greenhouse at 50–60% of relative humidity. The light intensity for growth of *E. coracana* was kept around 700 µmol m^{-2} S^{-1}. Later the plants were harvested at different developmental stages of 7th, 14th, 21st, and 28th days, respectively (at the interval of 1 week). The harvested plant samples (leaves and roots) were immediately transferred to the liquid nitrogen for further analysis. Three biological replicates of samples were harvested each time for the study.

Extraction of total RNA and cDNA synthesis

Total RNA was extracted from the root and leaf samples of *E. coracana* using Trizol method following manufacturer instructions. The isolated RNA was treated with DNase to remove the presence of any residual and contaminated DNA. The isolated RNA was used to synthesize cDNA for further use. RevertAid first strand synthesis kit was used to synthesize the first strand of cDNA. The reaction mixture was prepared by taking 2 µg of RNA sample followed by gentle heating and subsequent addition of oligo dT primers (500 ng/µl) and MLV reverse transcriptase (Promega, Madison, WI, USA). The resulted cDNA was diluted ten times with nuclease free water and kept at −20 °C for further use. A PCR reaction was run with *actin* gene to ensure that synthesis of cDNA was accomplished. The PCR reaction was checked in agarose gel electrophoresis. To confirm whether the DNase treatment was successful, another reaction was run with primers of *actin* gene in non-reverse transcribed total RNA. It was failed to amplify any product thus suggesting that the RNA and cDNA did not had any contaminated genomic DNA.

Primer design and cloning

Eleusine coracana is a monocot plant and the genome sequence of this orphan plant is not available yet. However, the genome sequence of monocot plant *O. sativa* is available and highly matches with the genome of *E. coracana*. Therefore, we used the *OsPIN1a* and *OsPIN1b* genes of *O. sativa* as orthologous gene to design the primers to clone the *EcPIN* genes in *E. coracana*. The forward and reverse primers of *EcPIN1a* were 5′-ATGATAACGGGGGC-3′ and 5′-CCCCAGCAGGATGTAGTACACC-3′, respectively, and forward and reverse primers of *EcPIN1b* were 5′-ATGATCACGGTGGT-3′ and 5′-GAGCCCCAGCAGTATGTAGTAG-3′, respectively. For qRT-PCR analysis, full length sequences of *EcPIN1a* and *EcPIN1b* were used to design the primers using Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/primer3/). The forward and reverse primers of *EcPIN1a* for qRT-PCR analysis were 5′-CATCGTCCTCGCGCTCCTCA-3′ and 5′-CCCATGACGAGCGTGTTGGG-3′, respectively, while the forward and reverse primers for *EcPIN1b* were 5′-GATGGTGCTGGCCATGCTCA-3′ and 5′-TGTTGGCGGCGGTGTCCGGG-3′, respectively. About 20 µl of PCR reaction mixture was prepared that contained 4 µl of high fidelity phusion buffer (5×), 0.5 µl of 10 mM dNTPs, 1 µl of 10 µM forward and reverse primer, 1 µl of cDNA template, 0.1 µl of phusion polymerase, 2 µl of DMSO solution, and 10.4 µl of nuclease free water. The thermal profile of the PCR reaction was as follows; initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, primer annealing at 58 °C for 30 s, polymerization at 72 °C for 2 min and final extension at 72 °C for 5 min. The resulted PCR products were separated by agarose gel electrophoresis and cloned into pGEMT vector. The cloned genes were subsequently sequenced to get their full length sequence.

In silico analysis of EcPIN genes

The resulted sequences of *E. coracana* were subjected to BLASTN search in *O. sativa* genome database and NCBI database. The BLAST result has shown similarity with PIN genes. The nucleotide sequences were translated to protein sequence using online server of Expasy bioinformatics portal (http://web.expasy.org/translate/) and Emboss Transeq of EMBL-EBI (https://www.ebi.ac.uk/Tools/st/emboss_transeq/). The resulted protein sequences were subjected to study the molecular masses and isoelectric point using protein calculator v3.4 (http://protcalc.sourceforge.net/). The presence of transmembrane domains in EcPIN proteins were studied using TMHMM server (http://www.cbs.dtu.dk/services/TMHMM/). The presence of different motifs in EcPIN protein was analyzed by MEME suit (http://meme-suite.org/) and motif scan software (http://myhits.isb-sib.ch/cgi-bin/motif_scan).

Multiple sequence alignment

To find out the sequence similarity with other PIN proteins, we conducted multiple sequence alignment of EcPIN proteins using Multalin server (http://multalin.toulouse.inra.fr/multalin/). Statistical parameters used to run the analysis were sequence input method, auto; alignment matrix, Blosum62-12-2; gap penalty, default; high consensus, 90% and low consensus was 50%.

Phylogenetic analysis

To construct the phylogenetic tree, PIN protein sequences of *O. sativa* and *A. thaliana* were downloaded from the rice genome annotation database and The Arabidopsis Information Resource portal, respectively. The EcPIN proteins along with the AtPINs and OsPINs were subjected to generate a clustal file using MUSCLE in EBI-EMBL (http://www.ebi.ac.uk/Tools/msa/muscle/) database. The resulted clustal file of PIN proteins was downloaded and converted to MEGA file format using MEGA6 software (Tamura et al. 2013). The MEGA file of the PIN protein was used to construct the phylogenetic tree. Following parameters were used to construct the phylogenetic tree; analysis, phylogeny reconstruction; statistical method, maximum likelihood; test of phylogeny, bootstrap method; number of bootstrap replicate, 1000, and gaps/missing data treatment, partial deletion.

Expression analysis by qRT-PCR

Transcript level of cloned *EcPIN* genes was studied using quantitative real-time PCR (qRT-PCR) using Mx3000P real-time PCR system (Stratagene, Santa Clara, CA, USA). The qRT-PCR was run using 25 µl reaction mixture that contained 12.5 µl of SYBR green/ROX master mix (Fermentas, USA), 1 µl of cDNA template, 1 µl of each forward and reverse primer, and 8.5 µl of nuclease free water. The *actin* gene of *E. coracana* was used as an internal control to normalize the gene of interest. Each sample was amplified in three biological replicates where each biological replicate contained three technical replicates as well. The thermal profile for qRT-PCR analysis was as follows: an initial step of denaturation at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and polymerization at 72 °C for 30 s. The expression levels of *EcPIN1a* and *EcPIN1b* were calculated using the $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak 2008).

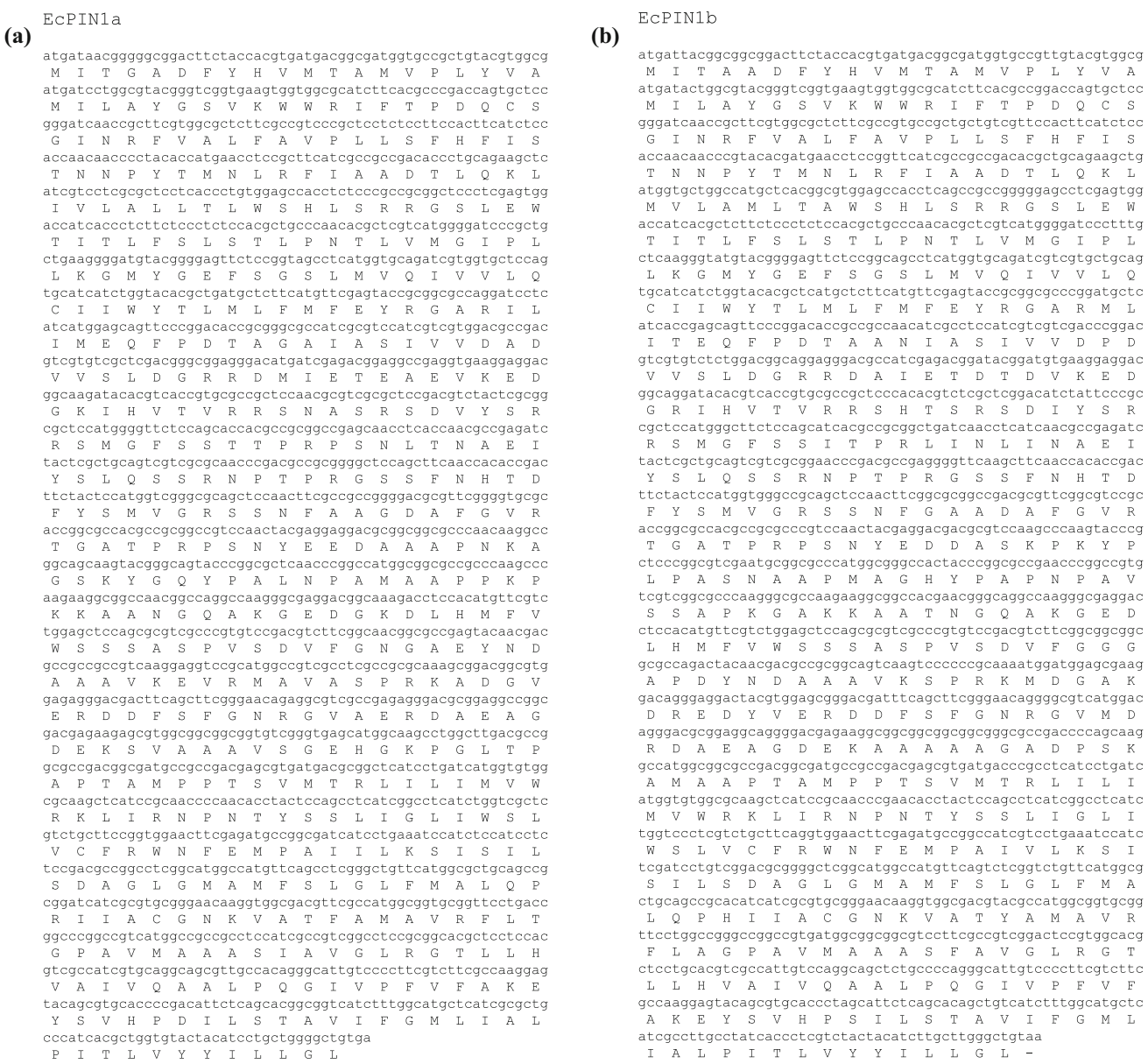

Fig. 1 **a** Cloned nucleotide sequence of *EcPIN1a*. It contains 1779 nucleotides that encode for 592 amino acids. **b** Cloned nucleotide sequence of EcPIN1b. It contains 1788 nucleotides that encodes for 595 amino acids

Results and discussion

The *O. sativa PIN* genes available in public genome database were used to design the *EcPIN* family gene specific primers in *E. coracana*. The PCR reaction carried out with specific primers to clone two *EcPIN* genes resulted in amplification of two bands from *E. coracana*. The sequencing results followed by BLAST analysis of these PCR products showed similarity with PIN genes of other organism in NCBI, TAIR and rice genome annotation database. This confirms that the cloned genes belonged to *EcPIN* genes. The sequencing report revealed that *EcPIN1a* was 1779 bp and *PIN1b* was 1788 bp long, and was predicted to contain 592 and 595 amino acids, respectively (GenBank accession number for *PIN1a* and *PIN1b* are KY305428 and KY305429, respectively) (Fig. 1). Pairwise nucleotide sequence alignment between EcPIN1a and EcPIN1b genes show 84.5% similarity while pairwise sequence alignment of amino acid shows 87.9% similarity. The molecular mass of EcPIN1a and EcPIN1b was found to be 64.368 and 64.809 kDa, respectively, while the iso-electric point (pI) of EcPIN1a and EcPIN1b was 8.96 and 8.93, respectively. The charge at pH 7 of EcPIN1a and EcPIN1b was found to be 9.0 and 8.5, respectively. Motif analysis using MEME suit v4.11.2 shows the presence of at least 14 motifs in EcPIN1a and 12

Fig. 2 Multiple sequence alignment of EcPIN1a and EcPIN1b proteins with PIN proteins of model plant *Arabidopsis thaliana* and *Oryza sativa*. Alignment shows the presence of several conserved motifs in PIN proteins. Conserved motifs are *underlined* in the figure

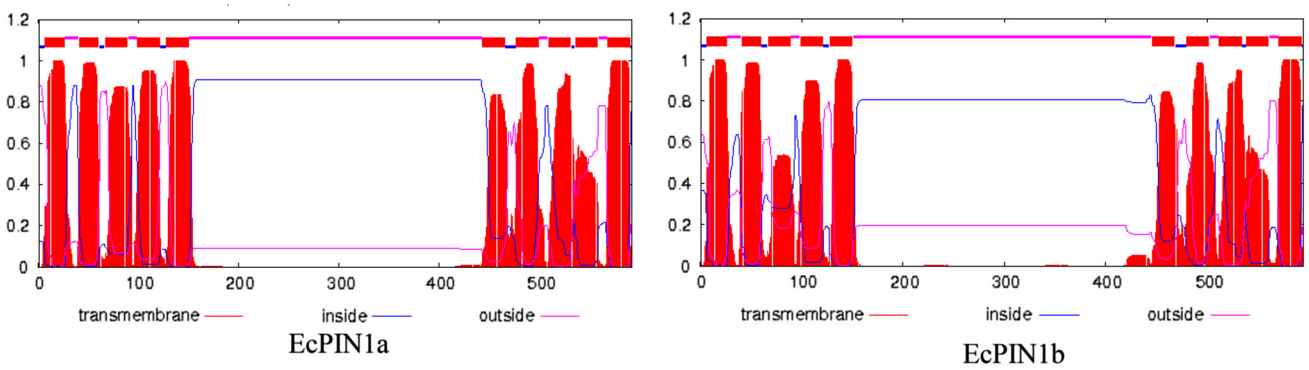

Fig. 3 Analysis for the presence of membrane bound transmembrane domain in EcPIN1a and EcPIN1b protein. Prediction shows the presence of membrane bound transmembrane helices in EcPIN1a and EcPIN1b. Prediction was conducted using online TMHMM server

motifs in EcPIN1b. Multiple sequence alignment with PIN proteins of *O. sativa* and *A. thaliana* shows several conserved motifs. Few of the conserved motifs of EcPIN1a and EcPIN1b along with PIN proteins of *O. sativa* and *A. thaliana* were F-x_2-D/E-Q-C, I-I-W, F-x_2-E, W-R-K-L-x_2-P-N-x-Y, G-x_2-W-x_{10}-L-P-x_5-S and V-A-I-x-Q-L-L-A-P-x_2-I-x_2-F-V-F-A-x-E-Y-x_2-H-x_4-T-x-V-I-x-G (Fig. 2). The transmembrane domain analysis revealed the presence of membrane spanning transmembrane domains in EcPIN1a and EcPIN1b (Fig. 3) protein. The EcPIN1a and EcPIN1b proteins were found to contain nine transmembrane helices. The crystal structure of PIN protein is not available yet. Hence, to know the probable structure of PIN protein, we undertake modeling approach to model EcPIN1a and EcPIN1b protein in SWISS model automatic modeling mode (Figs. 4, 5). From several models obtained during the modeling process, we selected the best one based on its lowest QMEAN *Z*-score (*Z*-score: −10.15). Further motif

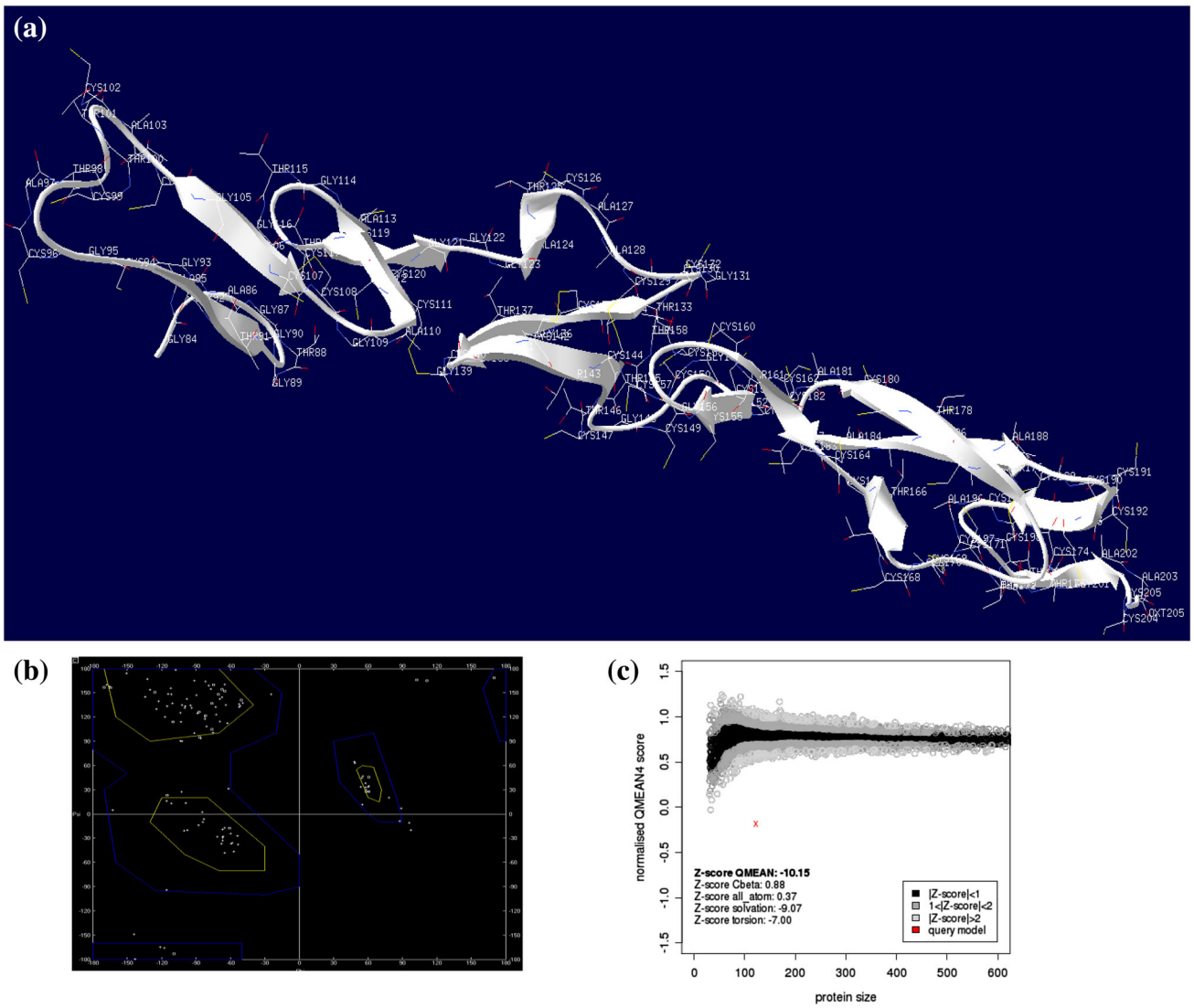

Fig. 4 In silico modeling of EcPIN1a protein. **a** Molecular model of EcPIN1a protein. **b** Ramachandran plot of EcPIN1a model. **c** It shows the model quality and QMEAN *Z*-score. The model quality along with distribution of amino acids in Ramachandran plot shows that the resulted model was the best model of EcPIN1a protein. Molecular modeling was conducted in SWISS-MODEL automatic mode

analysis shows the presence of one membrane transport and amidation site, three Asn glycosylation sites, one CAMP phospho site, five CK2 phospho sites, nine PKC phospho sites, and 12 myristoylation sites in EcPIN1a protein. Unlike EcPIN1a, EcPIN1b was also found to contain one membrane transport and amidation site, one Asn glycosylation site, one CAMP phospho site, four CK2 phospho sites, ten PKC phospho sites, 12 myristoylation and alanine rich sites (Table 1). The EcPIN1a protein contained three Asn glycosylation sites, whereas EcPIN1b protein contained only one Asn glycosylation site. Further, EcPIN1a protein contained five CK2 phospho sites while EcPIN1b protein contained only four CK2 phospho sites. The EcPIN1a protein contained only nine PKC phospho sites while EcPIN1b contained ten. Alanine rich region was not found in EcPIN1a while it was present in EcPIN1b protein. The phylogenetic analysis of EcPIN proteins with PIN proteins of *A. thaliana* and *O. sativa* shows closer relationship of EcPIN1a with OsPIN1d and OsPIN1a, while EcPIN1b shows close relationship with OsPIN1b (Fig. 6). The PIN proteins of *E. coracana* grouped with the PIN protein of monocot plant *O. sativa* while the PIN proteins of dicot plant *A. thaliana* grouped separately. This shows that EcPIN proteins are monocot specific and belonged to *E. coracana*.

Transcriptome analysis was performed to understand their transcript abundance at different developmental stages. The transcript level of *EcPIN1*a in leaf was found to be down-regulated at 7- and 14-day time periods while they were up-regulated at 21- and 28-day time periods (Fig. 7).

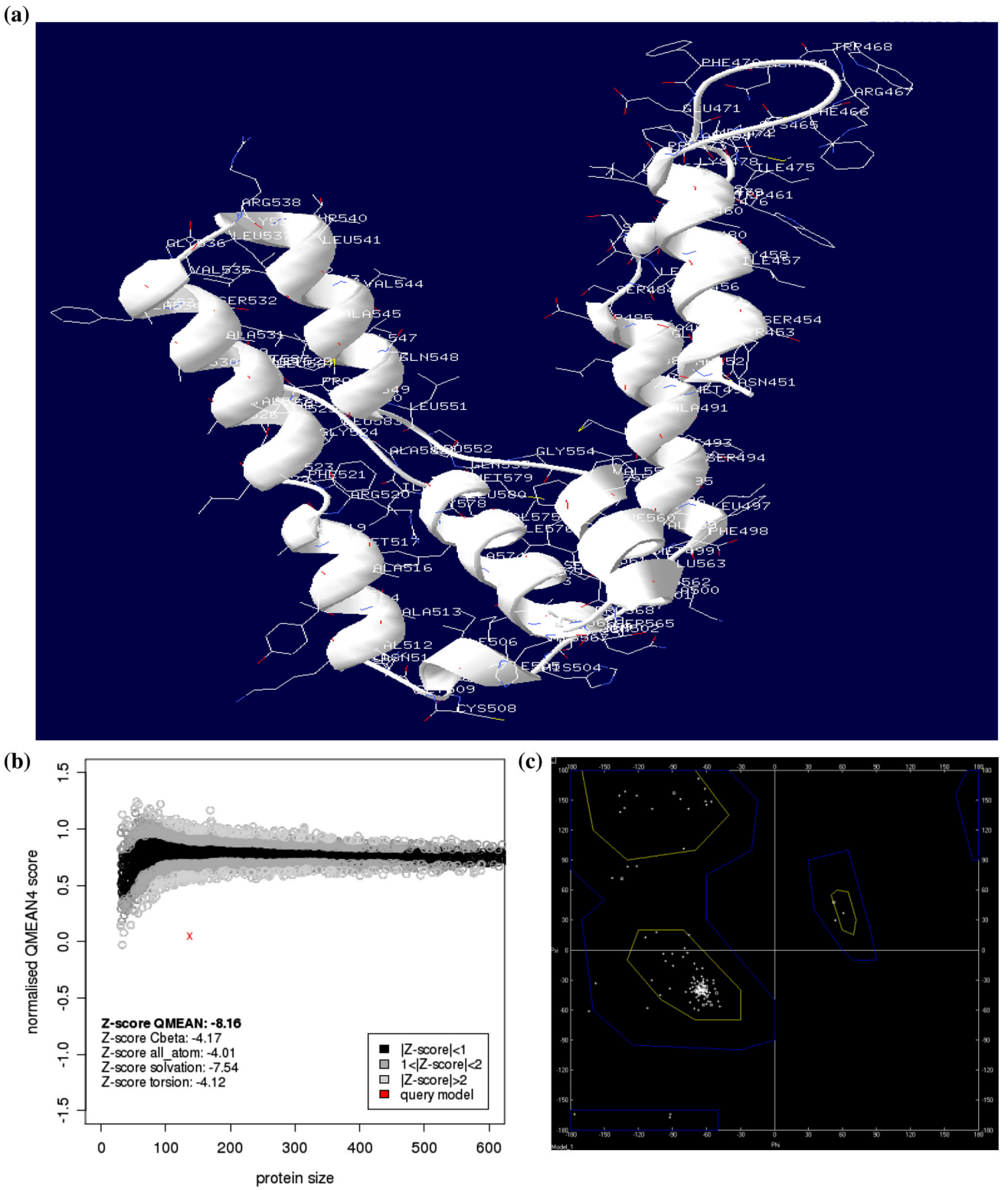

Fig. 5 In silico modeling of EcPIN1b protein. **a** Molecular model of EcPIN1b protein. **b** Ramachandran plot of EcPIN1b model. **c** It shows the model quality and QMEAN Z-score. The model quality along with distribution of amino acids in Ramachandran plot shows that the resulted model was the best model of EcPIN1b protein. Molecular modeling was conducted in SWISS-MODEL automatic mode

Table 1 In-silico prediction of possible functional domains of EcPIN1a and EcPIN1b protein

EcPIN1a		EcPIN1b	
Position	Domain	Position	Domain
9–587	Membrane transporter	9–590	Membrane transporter
185–188	Amidation	185–188	Amidation
211–214	Asn glycosylation	257–260	Asn glycosylation
234–237	Asn glycosylation		
257–260	Asn glycosylation		
94–97	CAMP phospho site	94–97	CAMP phospho site
3–6	CK2 phospho site	3–6	CK2 phospho site
193–196	CK2 phospho site	193–196	CK2 phospho site
213–216	CK2 phospho site	213–216	CK2 phospho site
236–239	CK2 phospho site	288–291	CK2 phospho site
288–291	CK2 phospho site		
27–29	PKC phospho site	27–29	PKC phospho site
93–95	PKC phospho site	93–95	PKC phospho site
206–208	PKC phospho site	206–208	PKC phospho site
219–221	PKC phospho site	212–214	PKC phospho site
229–231	PKC phospho site	219–221	PKC phospho site
245–247	PKC phospho site	229–231	PKC phospho site
250–252	PKC phospho site	245–247	PKC phospho site
284–286	PKC phospho site	250–252	PKC phospho site
373–375	PKC phospho site	284–286	PKC phospho site
170–175	Myristoylation	272–274	PKC phospho site
253–258	Myristoylation	253–258	Myristoylation
278–283	Myristoylation	272–277	Myristoylation
301–306	Myristoylation	278–283	Myristoylation
326–331	Myristoylation	326–331	Myristoylation
355–360	Myristoylation	334–339	Myristoylation
455–460	Myristoylation	415–420	Myristoylation
484–489	Myristoylation	458–463	Myristoylation
493–498	Myristoylation	487–492	Myristoylation
506–511	Myristoylation	496–501	Myristoylation
533–538	Myristoylation	509–514	Myristoylation
575–580	Myristoylation	536–541	Myristoylation
		578–583	Myristoylation
		403–427	Alanine rich

The *EcPIN1a* was down-regulated 10.63-fold at 7 days followed by 1.88-fold down-regulation while it was up-regulated 1.95-fold at 21 days and 5.98-fold at 28-day time period. The transcript abundance gradually increased with increasing time period in leaf tissue. However, the transcript abundance of *ECPIN1a* in root was found to be down-regulated at all the four time points. At 7-day time period it was found to be down-regulated 61.23-fold while it was down-regulated 5.08-fold in the 14 days. Although it was down-regulated in both the time periods, transcript level was comparatively up-regulated at 14 days compared to the 7-day time period. *The EcPIN1b* was down-regulated at 21- and 28-day time periods as well. The transcript abundance of *EcPIN1b* was detected very minutely in both root and leaf tissue.

Development of plant organs and its morphogenesis largely depends upon the dynamic relationship between the regulation of gene expression and fine tuning of physico-chemical processes. The phytohormone auxin plays a major role in vegetative (Ljung et al. 2001; Reinhardt et al. 2003), reproductive (Sundberg and Østergaard 2009; Jain and Khurana 2009) and root development (Mohanta and Mohanta 2013; Singh et al. 2015) in plants. Auxin was found to play important role in root hair formation (Lee and

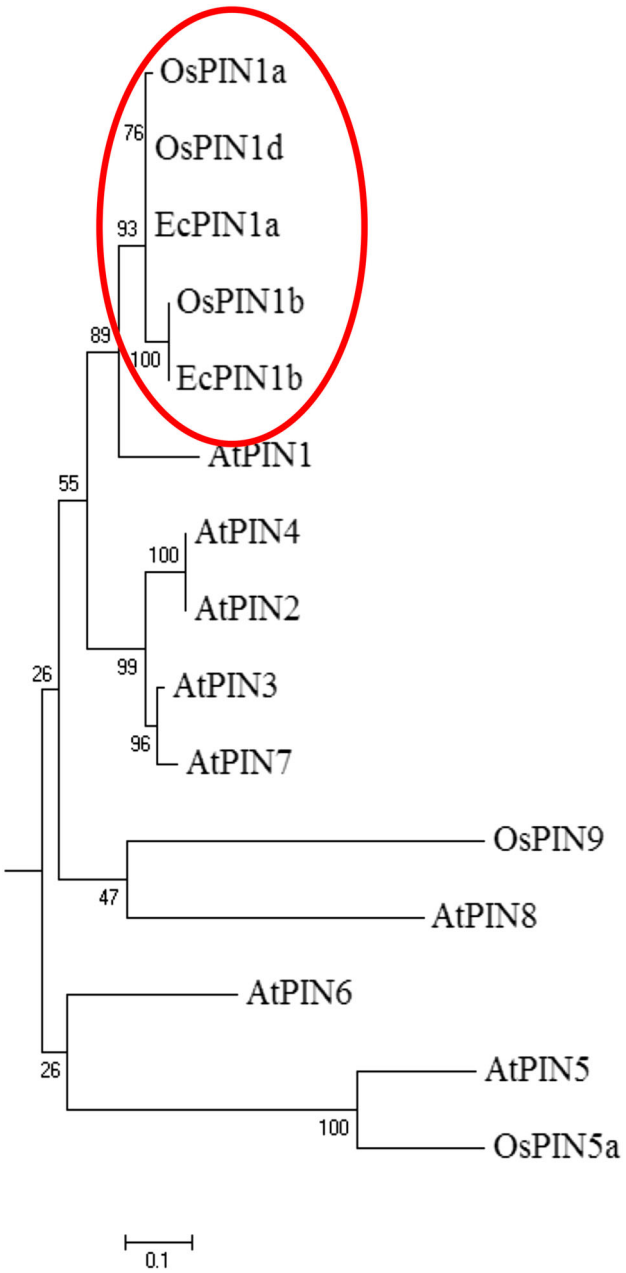

Fig. 6 Phylogenetic relationship of EcPIN1a and EcPIN1b with PIN proteins of model plants *A. thaliana* and *O. sativa*. Phylogenetic result shows close relationship of EcPIN1a with OsPIN1a and OsPIN1d and EcPIN1b with OsPIN1b. This show the EcPIN proteins were closer to monocot plant *O. sativa*. Phylogenetic tree was constructed using MEGA6 software with 1000 bootstrap replicates

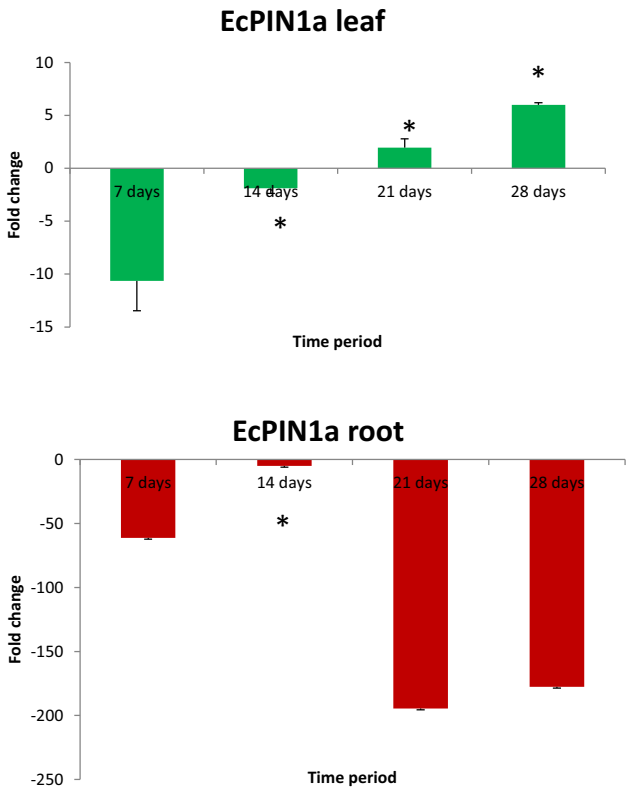

Fig. 7 Transcript analysis of *EcPIN1a* gene. **a** The transcript profile of *EcPIN1a* gene was found to be down-regulated at 7- and 14-day (1st and 2nd week) time periods while it was up-regulated at 21- and 28-day (3rd and 4th week) time periods. The transcript level was gradually up-regulated by increase in time period. **b** The transcript profile of *EcPIN1a* in root tissue shows down-regulated in all four time periods. At the 14-day time period, it was comparatively up-regulated compared to 7-day time period. Transcript profile of *EcPIN1b* gene was not detected during this study. Metric bars indicated the standard error (SE). *Asterisk* in the graph indicates statistically significant differences: * $p < 0.05$. Statistical analysis was done using unpaired *t* test

Cho 2006; Band et al. 2014), root gravitropism (Luschnig et al. 1998; Friml et al. 2002b), phyllotactic patterning (Vernoux et al. 2000; Reinhardt et al. 2000; Furutani et al. 2004) and leaf vein formation (Rolland-Lagan and Prusinkiewicz 2005; Wenzel et al. 2007; Scarpella et al. 2010). It is synthesized in the localized aerial parts of the plant and mediates uneven distribution throughout the plant and maintains homeostasis with the help of auxin efflux carrier (PIN) proteins that leads to proper growth and development of plants (Mohanta et al. 2015; Singh et al. 2015). The regulated and directional polar auxin transport within different plant tissues is unique to auxin and it has not been found in any other signaling molecules. Therefore, cloning of *EcPIN1a* and *EcPIN1b* gene was uncalled for and reported in the manuscript. Previous studies reported that PIN protein contains membrane spanning transmembrane domain which help to flux the auxin molecules in directional manner (Friml and Palme 2002; Petrásek and Friml 2009). Our analysis using TMHMM software shows that EcPIN1a and EcPIN1b proteins contain transmembrane domains which closely match with the previous studies (Chen et al. 1998; Friml et al. 2002a). Both EcPIN1a and EcPIN1b contain ten transmembrane helices. Chen et al. (1998) also reported the presence of same number of transmembrane helices in *A. thaliana* (Chen et al. 1998).

Arabidopsis thaliana AGRAVITROPIC 1 (*AGR1*) encodes for auxin efflux carrier and mutation in *AGR1* conferred increased root-growth sensitivity auxin and decreased sensitivity to ethylene, thus causing retention of exogenously added auxin in root tip cells (Chen et al. 1998). Chen et al. (1998) used positional cloning method to know whether *AGR1* encodes putative transmembrane domain and its homologies shares with bacterial transporter protein. Upon expression in *Saccharomyces cerevisiae*, *AGR1* promoted an increase in efflux of radiolabeled IAA from the cells and confers increased resistant to toxic fluoro-IAA (Chen et al. 1998). Li et al. (2012) cloned and characterized auxin efflux carrier *MiPIN1* in *Mangifera indica* associated with adventitious root formation in cotyledon segment (Li et al. 2012). Chawla and DeMason (2004) cloned *PsPIN1* gene, a putative auxin efflux carrier from *Pisum sativum* (Chawla and DeMason 2004). The *PsPIN1* gene was found to be ubiquitously expressed throughout the plant, more specifically in growing tissues. The phylogenetic analysis of EcPIN1a and EcPIN1b with PIN proteins of monocot and dicot plant grouped them in monocot lineage, suggesting their monocot specific origin in plant kingdom.

Conclusion

Cloning of auxin efflux carrier gene *EcPIN1a* and *EcPIN1b* were done in *E. coracana* and their expression analysis revealed gradual up-regulation of *PIN1a* genes from 7th to 28 days of developmental stages. Although *EcPIN1a* was down regulated at 7th and 14th days, they were up-regulated at 21 and 28-day time periods. Auxin efflux carrier genes play significant roles in plant growth and development in model plant including *A. thaliana* and *O. sativa*. Our study will help to understand the role of *EcPIN1a* and *EcPIN1b* gene in root and vegetative development in *E. coracana* plant.

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Compliance with ethical standards

Conflict of interest Author declares that there is no competing interest towards the publication of this manuscript.

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