Expression analysis of plant intracellular Ras-group related leucine-rich repeat proteins (PIRLs) in Arabidopsis thaliana

Md. Firose Hossain a, b, Mst Momtaz Sultana a, c, Ai Tanaka a, Amit Kumar Dutta a, d, Takushi Hachiya a, b, Tsuyoshi Nakagawa a, b, *

a Department of Molecular and Functional Genomics, Interdisciplinary Center for Science Research, Shimane University, Matsue, 690-8504, Japan
b Bioresource and Life Sciences, The United Graduate School of Agricultural Sciences, Tottori University, Tottori, 680-8550, Japan
c Department of Agricultural Extension (DAE), Ministry of Agriculture, Dhaka, 1215, Bangladesh
d Department of Microbiology, University of Rajshahi, Rajshahi, 6205, Bangladesh

ARTICLE INFO
Keywords:
Arabidopsis thaliana
Leucine-rich repeat
Promoter:GUS assay
Co-expression
Phylogenetic analysis
Pollen

ABSTRACT
Arabidopsis thaliana contains a family of nine genes known as plant intracellular Ras-group related leucine-rich repeat (LRR) proteins (PIRLs). These are structurally similar to animals and fungal LRR proteins and play important roles in developmental pathways. However, to date, no detailed tissue-specific expression analysis of these PIRLs has been performed. Therefore, in this study, we generated promoter:GUS transgenic plants for the nine A. thaliana PIRL genes and identified their expression patterns in seedlings and floral organs at different developmental stages. Most PIRL members showed expression in the root apical region and in the vascular tissue of primary and lateral roots. Shoot apex-specific expression was recorded for PIRL1 and PIRL8. Furthermore, PIRL1, PIRL3, PIRL5, PIRL6, and PIRL7 showed distinct expression patterns in flowers, especially in pollen and anthers. In addition, co-expression network analysis identified cases where PIRLs were co-expressed with other genes known to have specific functions related to growth and development. Taken together, the tissue-specific expression patterns of PIRL genes improve our understanding of the functions of this gene family in plant growth and development.

1. Introduction
Leucine-rich repeat (LRR) proteins contain a characteristic LRR domain consisting of 18–29 amino acid repeats rich in leucine residues. These proteins are widespread in plants, animals, and bacteria [1, 2]. In animals and lower eukaryotes, an intracellular LRR protein subfamily known as Ras-group LRR proteins with a variably long LRR domain are functionally involved in signal transduction [3–8]. Plants contain a small group of proteins that are structurally analogous to Ras-group LRRs, named plant intracellular Ras-group related LRR proteins (PIRLs) [4, 9].

The Arabidopsis thaliana genome has nine PIRL genes that are categorized into three subfamilies based on their evolutionary relationship [4]. To date, the functions of five PIRLs have been identified. PIRL6 has been found to be essential for male and female gametogenesis [10], while PIRL1 and PIRL9 redundantly work during the differentiation of microspores into pollen [11]. In addition, PIRL2 and PIRL3 have been found to be involved in pollen morphogenesis [12]. However, to elucidate the physiological functions of the remaining PIRL genes, it is requisite to know their precise expression patterns.

In this study, we used a promoter:GUS assay to examine in detail the expression patterns of PIRL genes in A. thaliana. We found that PIRL genes were expressed in the root, root hairs, shoot apex, leaves, anther, pollen, and pollen tube. We also performed a phylogenetic analysis of A. thaliana PIRL proteins with homologous PIRL proteins from other plants and in silico analyses examining the co-expression of PIRL genes. The results of these experiments should improve our understanding of the function of PIRL genes during plant growth and development.

2. Materials and methods
2.1. Phylogenetic analysis of PIRLs protein
The amino acid sequences of all nine A. thaliana PIRLs were retrieved...
from The Arabidopsis Information Resource (TAIR) database version 10 (https://www.arabidopsis.org/). A NCBI (https://www.ncbi.nlm.nih.gov/) BLASTP search was performed using the freeze-thaw method as per Weigel and Glazebrook [16].

2.2. Plasmid construction

The promoter entry clones pDONR201-ProPIRL1 to pDONR201-ProPIRL9 were prepared with the primers listed in Supplementary Table 2. The promoter:GUS binary constructs were prepared with the MyDomains-Image Creator (https://prosite.expasy.org/mydomains/).

2.3. Generation of transgenic A. thaliana

Transformation of Agrobacterium tumefaciens C58C1 (pMP90) was performed using the freeze-thaw method as per Weigel and Glazebrook [16]. Transformation of A. thaliana (Col-0 accession) was performed using the floral inoculation method [17]. Harvested T0 seeds were kept at 4 °C for 3 days, after which they were grown on Murashige and Skoog (MS) agar medium containing kanamycin (30 mg/L) and Cefotax (100 mg/L) (Chugai Pharmaceutical Co., Tokyo, Japan) at 22 °C under continuous light. Fifteen-day-old selected plants (T1) were transferred to Jiffy-7 plant pots (Jiffy Products International BV, Zwijndrecht, Belgium) and were grown at 22 °C under a 16-hr light/8-hr dark daily cycle.

2.4. Histochemical GUS staining

Plants were selected at different time points for histochemical staining, including 1-day-after-germination (DAG) seedlings, as well as plants at 4-DAG, 8-DAG, 16-DAG, and the flowers of 40-DAG plants. Staining was performed as described by Nakamura et al. [18] and plants were observed using a stereo microscope (SZX16, Olympus, Tokyo, Japan) equipped with a Nikon digital camera (DXM1200F) as well as an all-in-one microscope (BZ-710, Keyence, Osaka, Japan).

2.5. In silico expression and co-expression of PIRL genes

The expression value was obtained for each PIRL gene in several different tissues (cotyledon, hypocotyl, root, leaf, flower, sepal, petal, and pollen) using microarray data from the Arabidopsis eFP browser [19]. Expression values were then normalized using the log2 transformation. Finally, a heat map was generated by TBTools software [20]. Microarray data showing visual expression during different developmental stages was also retrieved from the Arabidopsis eFP browser [19]. The co-expression gene network of PIRLs and co-expressed genes were analyzed using the ATTED-II (https://atted.jp/) [21].

3. Results

3.1. Phylogenetic analysis shows a relationship between A. thaliana PIRLs and PIRLs found in other species

Phylogenetic analysis of amino acid sequences from A. thaliana (PIRL), Brassica rapa (BrPIRL), Capsella rubella (CrPIRL), Orzya sativa (OsPIRL), Hibiscus syriacus (HsPIRL), Zea mays (ZmPIRL), and Ricinus communis (RcPIRL) showed that, the PIRLs from A. thaliana showed some similarity to those from other plants. Our tree revealed that the phylogenetic relationships between and among species were clustered into six groups (Fig. 1A). In group I, PIRL4 and PIRL5 formed a cluster with CrPIRL4 and CrPIRL5, with bootstrap values of 93 and 87, respectively. The highest number of clusters was found in group III, where PIRL6 formed a cluster with BrPIRL6, and PIRL7 and PIRL8 formed a cluster with CrPIRL7 and CrPIRL8, respectively. Group IV contained PIRL2 and PIRL3, which formed a cluster with CrPIRL2 and CrPIRL3. Finally, PIRL1 and PIRL9 formed a cluster with BrPIRL1 and CrPIRL9, and these were grouped together into Group V. Next, we predicted the number of LRR in each A. thaliana PIRL (Fig. 1B and Supplementary Table 3). All PIRLs were found to contain the defining LRRs; PIRL4 and PIRL5 contained the highest number (11) of LRRs, while PIRL1, PIRL2, PIRL3, PIRL6, PIRL7, PIRL8, and PIRL9 all showed 10 LRRs (Fig. 1B and Supplementary Table 3).

3.2. PIRL expression in different vegetative tissues in A. thaliana

We subjected T2 or T3 plants from ProPIRL:GUS transgenic lines to histochemical GUS staining at different developmental stages. As shown in Fig. 2, seedlings (1-, 4-, 8-, and 16-DAG) exhibited different expression patterns in their vegetative organs. For PIRL1, GUS expression was observed at the junction between the root and hypocotyl at 4-DAG, at the primary root tip and in the differentiated region of the primary and secondary roots at 8-DAG, and in the shoot apex at 16-DAG (Fig. 2A). For PIRL2, we observed unique expression in root hair, and while lower expression was observed in the vascular tissues of the primary and secondary roots from 1- to 8-DAG, we also observed moderate expression in leaves and strong expression in stipules at 16-DAG (Fig. 2B). PIRL3, PIRL4, PIRL7, and PIRL9 showed two similar trends: for each of these PIRL genes, we observed GUS expression in root (1- to 8-DAG) but faint or no signal in aboveground organs at 16-DAG (Fig. 2C, D, G and I). For PIRL5, we observed expression in the root tip and in the vascular tissue of the first and secondary roots at 4- and 8-DAG, but again almost no expression in aboveground organs at 16-DAG (Fig. 2E). PIRL8 showed GUS expression in roots (8-DAG), the shoot apex region, and young leaves (Fig. 2H). We did not observe considerable expression of PIRL6 in any vegetative organs (Fig. 2F). All the results of GUS staining experiments are summarized in Supplementary Table 4.

3.3. PIRLs are expressed in various reproductive tissues in A. thaliana

We subjected the flowers of 40-DAG transgenic plants to GUS staining. For PIRL1, GUS was weakly expressed in petals and filaments, but was strongly expressed in anthers and pollen (Fig. 3A). Expression was also detected in the pollen tube (Fig. 3A). For PIRL2, expression was strong in flower buds but was lower in sepal, petals, filaments, and stigmas of open flowers (Fig. 3B). We observed distinct expression of PIRL3 in anthers and pollen, but not in the pollen tube (Fig. 3C). Next, for PIRL4, we did not find any expression in any floral organs whatsoever (Fig. 3D). Similarly, PIRL5 was detected very weakly and only in anthers (Fig. 3E). However, clear GUS expression was detected in both
Fig. 1. Phylogenetic tree of *A. thaliana* PIRLs including homologous PIRLs from other plants as well as the domain structure of the nine *A. thaliana* PIRLs. (A) An unrooted phylogenetic tree was created using the full-length protein sequences of the nine *A. thaliana* PIRLs and their homologs found in other plants. Group I, II, III, IV, V, and VI are denoted by blue, fuchsia, purple, lime, teal, and olive, respectively, while PIRLs from *A. thaliana* are indicated in red. The prefixes Br, Cr, Os, Hs, Zm, and Rc were used to designate *Brassica rapa*, *Capsella rubella*, *Oryza sativa*, *Hibiscus syriacus*, *Zea mays*, and *Ricinus communis*, respectively. BrPIRLs, CrPIRLs, HsPIRLs, ZmPIRLs, and RcPIRLs were numbered according to their registered names in the NCBI database. (B) Domain structure of the nine *A. thaliana* PIRLs. The following scheme was used to indicate domains: green squares represent disordered regions; orange horizontal pentagons indicate coiled coil regions; blue hexagons indicate LRR; gray horizontal pentagons indicate the GVYW motif.

Fig. 2. Expression patterns of PIRL genes in the vegetative tissues of *A. thaliana*. (A) ProPIRL1:GUS, (B) ProPIRL2:GUS, (C) ProPIRL3:GUS, (D) ProPIRL4:GUS, (E) ProPIRL5:GUS, (F) ProPIRL6:GUS, (G) ProPIRL7:GUS, (H) ProPIRL8:GUS, (I) ProPIRL9:GUS. GUS staining is shown for 1-DAG seedlings, 4-DAG seedlings, 8-DAG primary root tips (left), 8-DAG initiated lateral roots (center), 8-DAG differentiated roots (right), and 16-DAG aboveground organs of transgenic *A. thaliana*. Scale bars = 1 mm.
pollen and pollen tubes for both PIRL6 and PIRL7 (Fig. 3F and G). GUS activity was not observed in any floral organ for either PIRL8 or PIRL9 (Fig. 3H and I). All GUS staining results are summarized in Supplementary Table 4.

3.4. In silico expression and co-expression analysis of PIRLs

We generated a heat map that used microarray datasets of PIRL genes sourced from the Arabidopsis eFP browser (Fig. 4A). This heat map analysis showed that PIRL6 was specifically expressed in pollen

![Supplementary Table 4.](image)

Fig. 3. Expression patterns of PIRL genes in the floral organs of *A. thaliana*. (A) ProPIRL1:GUS, (B) ProPIRL2:GUS, (C) ProPIRL3:GUS, (D) ProPIRL4:GUS, (E) ProPIRL5:GUS, (F) ProPIRL6:GUS, (G) ProPIRL7:GUS, (H) ProPIRL8:GUS, (I) ProPIRL9:GUS. GUS staining is shown for the inflorescence, flower, young anther, mature anther, style, stigma, and fruit tissue of 40-DAG transgenic *A. thaliana*. Scale bars = 1 mm.

Fig. 4. In silico gene expression and co-expression analyses of PIRLs. (A) Gene expression (Log2 transformed) heat map of *A. thaliana* PIRL genes at different developmental stages. PIRLs are shown on the right side, and tissues are indicated at the bottom. Red indicates a higher level of gene expression, while green indicates lower expression. (B) Co-expression networks were generated by the ATTED-II using PIRLs as query genes. Red, yellow, blue, sky-blue, and green circles indicate groups of genes involved in different metabolic pathways.
(Fig. 4A), that PIRL1 and PIRL3 were expressed strongly in pollen and moderately in vegetative organs (Fig. 4A), and that PIRL5 was expressed in pollen and roots (Fig. 4A). This analysis also showed that both PIRL4 and PIRL9 were expressed in a variety of organs, although PIRL4 was not expressed in pollen (Fig. 4A). Interestingly, weak expression in root tissue was observed for PIRL8 (Fig. 4A). Moreover, a microarray study using visual expression of PIRL genes during developmental stages revealed that PIRL1 and PIRL3 were significantly expressed in floral organs, specifically in mature pollen rather than vegetative organs (Supplementary Fig. S1). According to the microarray expression developmental map, PIRL4 and PIRL9 were expressed in a number of regions, while no expression was found for PIRL4 in pollen (Supplementary Fig. S1). PIRL5 and PIRL6 showed vigorous expression in mature pollen, whereas root-specific expression was also observed for PIRL4 and PIRL8, respectively. The microarray datasets contained no expression values for PIRL2 and PIRL7. Further, to identify the functional relationship between PIRLs and other genes, we generated a co-expression network using the ATTED-II (ath-m.c9-0 platform) (Fig. 4B). Of the nine PIRL genes, only PIRL1 and PIRL3 were found to co-express with each other. Furthermore, PIRL1 expression was correlated with the expression of VQ-motif containing protein 9 (VQ9, At1g78310), which is a class of transcription factor [22], and zinc finger nuclease 2 (ZFN2, AT2G39230) (Fig. 4B). We also found that PIRL3 co-expressed strongly with a member of the Type One Protein Phos- phatase family known as TOP8 (AT5G27840), as well as IBS5 (AT2G04550) and an F-box domain-containing protein (At3g17710) (Fig. 4B). Expression of PIRL4 was found to strongly correlate with expression of TMK1 (At1G66150) (Fig. 4B), while PIRL5 was weakly co-expressed with SK32 (AT4G00720) and CYP721A1 (AT1G75130) (Fig. 4B). PIRL6 was co-expressed with GLOX1 (AT1G67920) and PAB3 (AT1G22760) (Fig. 4B), two genes whose expressions are known to have been restricted to the anther and tapetum [23]. PIRL8 showed co-expression with IMMUNE ASSOCIATED NUCLEOTIDE BINDING 4 (IAN4, At1g33900) and a member of the Alpha Expansion gene family (EXPA17, AT4G01630) (Fig. 4B). The expression of EXPA17 is root-specific and it is vital for lateral root formation [24]. Finally, PIRL9 was found to co-express with UDP-DEPENDENT GLYCOSYL-TRANSFERASE 76B1 (UGT76B1, AT3G11340) and At4g02940 (Fig. 4B). Co-expression data was not available for PIRL2 and PIRL7.

4. Discussion

LRR proteins are found in many plant species, where they perform important biological functions linked to growth and development. For instance, many proteins from the LRR-receptor-like kinases (RLKs) and related-receptor-like proteins (RPLPs) families play crucial roles in plant growth and development and in defense-related pathways [25-27]. In A. thaliana, nine PIRLs have been identified, some of which have been previously examined for functional roles in developmental signaling. In the present study, we used promoter:GUS assays and in silico approaches to analyze the amino acid sequences and detailed expression patterns of nine PIRLs found in A. thaliana. Phylogenetic analysis revealed the relationships of the A. thaliana PIRL proteins with other plants PIRLs and resolved all sequences into six groups (Fig. 1A). Each A. thaliana PIRL was found to be close in sequence to one of the PIRLs found in C. rubella (CrPIRL) and others from B. rapa (BrPIRL). A. thaliana PIRL1 and PIRL9 belong to Group V, a group that also contains PIRLs from C. rubella and B. rapa. A. thaliana PIRL4 and PIRL5 belong to a clade that includes PIRL4 and PIRL5 from both C. rubella and B. rapa, designated here as Group I. This group also contained PIRL4 and PIRL5 from O. sativa, Z. mays, H. syriacus, and R. communis. PIRL6, PIRL7, and PIRL8 from A. thaliana, C. rubella, and B. rapa all belong to Group III, a group that also includes PIRL1 from O. sativa and Z. mays. PIRL2 and PIRL3 from A. thaliana, C. rubella, and B. rapa belong to the same clade, designated here as Group IV. Interestingly, Groups IV and V did not include PIRLs found in O. sativa and Z. mays. Group VI contains PIRLs only from rice and maize. Focusing on A. thaliana and rice, there is no OsIRL corre- sponding to PIRL1, PIRL9, PIRL2 and PIRL3, and there is no PIRL corresponding to OsIRL2 and OsIRL3. PIRL1 and PIRL9 have been reported to be essential for pollen development [11]. Although the function of OsIRL2 and OsIRL3 is not yet known, they may be redundantly involved in pollen development like PIRL1 and PIRL9 because they are most similar to PIRL1 and PIRL9 among OsIRLs, and their expression was shown in stamens [9]. OsIRL2 and OsIRL3 may have diverged and ac- quired additional functions in rice. For example, expression of OsIRL2 and OsIRL3 is also shown in endosperm [9]. Since the osir3 mutant exhibited a normal phenotype, analysis of osg2/osir3 double mutant will be a clue to clarify their function. Analysis of the domain organi- zations by Uniprot showed that the number of LRRs present in A. thaliana PIRLs is 10 or 11 (Fig. 1B and Supplementary Table 3). The number of LRRs found in PIRL1, PIRL2, PIRL3, PIRL6, PIRL7, PIRL8, and PIRL9 was one more than reported by Forsthoefel et al. [4]. This dif- ference may be due to differences in the length of the first LRR between the previous prediction and the Uniprot prediction used in this study. Our expression experiments revealed that PIRL1, PIRL3, and PIRL6 were preferentially expressed in pollen (Fig. 4A). Microarray developmental map, RNA-seq [19,28] and promoter:GUS assays (Fig. 3 and Supplemen- tary Fig. S1) also indicated strong expression of PIRL1, PIRL3, and PIRL6 in pollen. These results are consistent with reports that have demonstrated that PIRL1 and PIRL9 are vital for the differentiation of microspores into pollen [11]. In addition, recent studies have also shown that pirl2pirl3 double mutant generated a wide range of abnormal pollen morphologies [12]. Moreover, our promoter:GUS assay of PIRL6 showed expression also in the ovule (Fig. 3F), a finding that is consistent with a previous result that showed that PIRL6 was essential for both male and female gametogenesis processes in A. thaliana [10]. Forsthoefel et al. [10] reported abundant functional transcript in flowers and a small amount of non-functional transcript by alternative splicing in leaf and root. These and our promoter:GUS results indicated transcriptional and post-transcriptional regulation of PIRL6. Next, our co-expression analysis showed that the expression of PIRL1 and PIRL3 are correlated with each other and that PIRL1 expression is also associated with the expression of VQ9 and ZFN2 (Fig. 4B). The essential role of VQ proteins in vegetative plant growth, differentiation, and seed development has been previously described [22]. The relative expression of VQ9 has also been reported in roots by Hu et al. [29], a finding that is consistent with the observed expression of PIRL1 in roots. Taken together, these findings suggest a contribution of PIRL1 to root development in addition to its role in pollen formation. PIRL1 and PIRL9 are closely related gene pairs, and pirl1pirl9 double knockout plants (pirl1/-,-, pirl9 -/-) were not established due to the developmental defect of pirl1pirl9 microspore (pirl1 -,- pirl9 -) into viable pollen [11]. Analysis of double mutants using weaker alleles may reveal their function in the development of vegetative organs, including roots. Next, we also identified TOPP8 and IBS5 as genes that were co-expressed with PIRL3. TOPP8 is also known as ATUNIS2 (AUN2) and plays a vital role in pollen germination and pollen tube growth [30]. Pollen- and root-specific expression of AUN2 has also been reported by Franck et al. [30], who used a microarray analysis that showed a pattern of expression resembling that of PIRL3, further sug- gesting their functional similarity. We also found that PIRL6 expression was strongly correlated with the expression of genes coding for glyoxyl-oxidase-related protein (GLOX1) and PAB3. GLOX1 was re- ported to be important for pollen development; Phan et al. [25] found GLOX1 promoter-driven GUS activity in mature and released pollen and buds as well as strong GUS activity in mature and released pollen grains. Belostotsky [31] also described tapetum and pollen-specific expression of PAB3 after performing promoter:GUS assays in A. thaliana. These results support the possibility that the function of PIRL6 in pollen development is related to GLOX1 and PAB3. Although no expression values were available in the database for PIRL7, our promoter:GUS assay revealed clear expression of PIRL7 both in pollen and in the pollen tube (Fig. 3G), suggesting that PIRL7 plays a role in pollen development. The
promoter:GUS assay of PIRL2 showed unambiguous staining of filaments (Fig. 3B). Therefore, the requirement of PIRL2 for pollen development [12] may be exerted in the filament. We obtained root-specific promoter:GUS expression for PIRL4 (Fig. 2D). As shown in Fig. 4, co-expression analysis revealed that PIRL4 was co-expressed with LRR-RLK TMK1 [32], which has been found to be expressed in roots by RT-PCR and promoter:GUS assays [33]. Dai et al. [33] also reported that the root lengths of tmk1; tmk4 double mutants were approximately one-third of that of the wild type. The biological functions of PIRL4 remain unknown; however, co-expression of PIRL4 and TMK1 in roots suggests that PIRL4 may be involved in the growth and development of roots. Furthermore, we also observed promoter:GUS expression of PIRL5, PIRL8, and PIRL9 in the root apical region, as well as in the primary and secondary roots (Fig. 2E, H, and I). According to the heat map and microarray developmental map, PIRL5, PIRL8, and PIRL9 are also known to be expressed in roots (Fig. 4A and Supplementary Fig. S1). In addition, we found that PIRL8 showed co-expression with EXP17; a previous study by Lee and Kim [24], who used the promoter:GUS of EXP17 and recorded its expression in lateral root and lateral root primordia. Therefore, they suggested that EXP17 may be required to promote lateral root formation during auxin response. Together, these findings signify that PIRL8 may have root-specific functions.

5. Conclusion

In this study, we investigated the detailed expression patterns of nine A. thaliana PIRL genes at different developmental stages. Promoter:GUS assay showed that most PIRLS were expressed in roots and in the vasculature of the primary and lateral roots. In addition, we observed that some PIRLS were strongly expressed in floral organs, suggesting their possible biological function to regulate the growth and development of these organs. Moreover, co-expression network analysis indicated that functionally similar PIRLS are expressed simultaneously. Therefore, these results will be helpful for future tissue-specific expression analyses of other LRR proteins and may provide useful information to improve our understanding of their biological function.

Author contribution statement

MFH, AT, AKD and TN conceived the project and designed the experiments. Promoter:GUS assay of PIRL2 was expressed in roots and in the vasculature of the primary and lateral roots. In addition, we observed that some PIRLS were strongly expressed in floral organs, suggesting their possible biological function to regulate the growth and development of these organs. Moreover, co-expression network analysis indicated that functionally similar PIRLS are expressed simultaneously. Therefore, these results will be helpful for future tissue-specific expression analyses of other LRR proteins and may provide useful information to improve our understanding of their biological function.

Declaration of competing interest

The authors declare that this study was conducted in the absence of any commercial relationships that could lead to any potential conflicts of interest.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by a KAKENHI Grant from the Japan Society for the Promotion of Science (JSPS) [Grant-in-Aid for Scientific Research (C) No. JP15K07109 to TN]. The authors would like to thank Enago (www.enago.jp) for the English language review.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2022.101241.

References

[1] A.V. Kajava, Structural diversity of leucine-rich repeat proteins, J. Mol. Biol. 277 (1998) 519–527.
[2] B. Koke, J. Deisenhofer, The leucine-rich repeat: a versatile binding motif, Trends Biochem. Sci. 19 (1994) 415–421.
[3] C. Claudianos, H.D. Campbell, The novel flightless-I gene brings together two gene families, actin-binding proteins and leucine-rich repeat proteins involved in Ras signal transduction, Mol. Biol. Evol. 12 (1995) 405–414.
[4] N.R. Forsthoefel, K. Cutler, M.D. Port, T. Yamamoto, D.M. Vernon, PIRLs: a novel class of plant intracellular leucine-rich repeat proteins, Plant Cell Physiol. 46 (2005) 913–922.
[5] K.W. Jeong, Y.-H. Lee, M.R. Stallcup, Recruitment of the SWI/SNF chromatin remodeling complex to steroid hormone-regulated promoters by nuclear receptor coactivator flightless-I, J. Biol. Chem. 284 (2009) 29298–29309.
[6] Y.-H. Lee, H.D. Campbell, M.R. Stallcup, Developmentally essential protein flightless I is a nuclear receptor coactivator with actin binding activity, Mol. Cell Biol. 24 (2004) 2103–2117.
[7] W. Li, M. Han, K.-L. Guan, The leucine-rich repeat protein SUR-8 enhances MAP kinase activation and forms a complex with Ras and Raf, Genes Dev. 14 (2000) 895–900.
[8] D.S. Sieburt, Q. Sun, M. Han, SUR-8, a conserved leucine-rich protein with leucine-rich repeat domains, positively regulates Ras-mediated signaling in C. elegans, Cell 94 (1998) 119–130.
[9] C. You, X. Dai, X. Li, L. Wang, G. Chen, J. Xiao, C. Wu, Molecular characterization, pattern and functional analysis of the OsRGL gene family encoding intracellular Ras-group-related LRR proteins in rice, Plant Mol. Biol. 74 (2010) 617–629.
[10] N.R. Forsthoefel, K.A. Klag, S.R. McNichol, C.R. Vernon, W.W. Wood, D.M. Vernon, Arabidopsis PIRL6 is essential for male and female gametogenesis and is regulated by alternative splicing, Plant Physiol. 178 (2018) 1154–1169.
[11] N.R. Forsthoefel, T.P. Dao, D.M. Vernon, PIRL1 and PIRL9, encoding members of a novel plant-specific family of leucine-rich repeat proteins, are essential for differentiation of microspores into pollen, Plants 232 (2010) 1110–1114.
[12] N.R. Forsthoefel, K.A. Klag, B.P. Simelis, B. Reiter, L. Brougham, D.M. Vernon, The Arabidopsis plant intracellular Ras-group LRR (PIRL) family and the value of reverse genetic analysis for identifying genes that function in gametophyte development, Plants 2 (2013) 507–520.
[13] N. Saitou, M. Nei, The neighbor-joining method: a new method for constructing phylogenetic trees, Mol. Biol. Evol. 4 (1987) 406–425, https://doi.org/10.1093/oxfordjournals.molev.a004054.
[14] S. Kumar, G. Stecher, K. Tamura, MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, Mol. Biol. Evol. 33 (2016) 1870–1874.
[15] M.M. Sultana, T. Ichiya, A.K. Dutta, K. Nishimura, T. Suzuki, A. Tanaka, T. Nakagawa, Expression analysis of genes encoding maize-like domain (MLD)- and leucine-rich repeat (LRR)-containing proteins in Arabidopsis thaliana, Biosci. Biotechnol. Biochem. 84 (2020) 154–158.
[16] D. Weigel, J. Glazebrook, Arabidopsis: a Laboratory Manual, CSSH Press, 2002.
[17] M. Narusaka, T. Shirashii, M. Iwabuchi, Y. Narusaka, The floral inoculating protocol: a simplified Arabidopsis thaliana transformation method modified from floral dipping, Plant Biotechnol. 27 (2010) 349–351.
[18] N. Sutamara, T. Suzuki, M. Kawamukai, T. Nakagawa, Expression analysis of Arabidopsis thaliana small secreted protein genes, Biosci. Biotechnol. Biochem. 76 (2012) 436–446.
[19] D. Winter, B. Vinegar, H. Nahal, R. Ammar, G.V. Wilson, N.J. Provart, An ‘Electronic Fluorescent Pictograph’ browser for exploring and analyzing large-scale biological data sets, PLoS One 2 (2007) e718.
[20] C. Chen, H. Chen, Y. Zhang, H.R. Thomas, M.H. Frank, Y. He, R. Xin, TBtools: an integrative toolkit developed for interactive analyses of big biological data, Mol. Plant 13 (2020) 1194–1202.
[21] T. Obayashi, Y. Aoki, S. Takada, Y. Kagaya, K. Kinoshita, ATTED-II in 2018: a plant gene expression database based on investigation of the statistical property of the mutual rank index, Plant Cell Physiol. 59 (2018) e3–e3.
[22] C. Liu, H. Liu, C. Zhou, M.P. Timko, Genome-wide identification of the VQ protein gene family of Tobacco (Nicotiana tabacum L.) and analysis of its expression in response to phytohormones and abiotic and biotic stresses, Genes 11 (2020) 284–293.
[23] B. Ho. Phan, S. Iacuone, S.F. Li, W.R. Parish, The MYBB80 transcription factor is required for pollen development and the regulation of tapetal programmed cell death in Arabidopsis thaliana, Plant Cell 23 (2011) 2209–2224.
[24] H.W. Lee, J. Kim, EXPAN3,17 up-regulated by LBD18/ASL20 promotes lateral root formation during the auxin response, Plant Physiol. 54 (2013) 1600–1611.
[25] S. Jeong, A.E. Trotchonad, S.E. Clark, The Arabidopsis CLAVATA2 gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase, Plant Cell 11 (1999) 1925–1933.
[26] K. Lease, E. Ingham, J.C. Walker, Challenges in understanding RLK function, Curr. Opin. Plant Biol. 1 (1998) 388–392.
[27] S. S. Shi, A.B. Blecker, Plant receptor-like kinase family: diversity, function, and signaling, Sci. STKE 2001 (2001) re22–re22.
[28] A.E. Loraine, S. McCormick, A. Estrada, K. Patel, P. Qin, RNA-seq of Arabidopsis pollen uncovers novel transcription and alternative splicing, Plant Physiol. 162 (2013) 1092–1109.
[29] Y. Hu, L. Chen, H. Wang, L. Zhang, F. Wang, D. Yu, A rabidopsis transcription factor WRKY 8 functions antagonistically with its interacting partner VQ 9 to modulate salinity stress tolerance, Plant J. 74 (2013) 730–745.
[30] C.M. Franck, J. Westermann, S. Bürssner, R. Lentz, D.S. Litoviev, A. Boisson-Dernier, The protein phosphatases ATUNIS1 and ATUNIS2 regulate cell wall integrity in tip-growing cells, Plant Cell 30 (2018) 1906–1923.

[31] D.A. Belostotsky, Unexpected complexity of poly (A)-binding protein gene families in flowering plants: three conserved lineages that are at least 200 million years old and possible auto-and cross-regulation, Genetics 163 (2003) 311–319.

[32] C. Chang, G.E. Schaller, S.E. Patterson, S.F. Kwok, E.M. Meyerowitz, A.B. Bleecker, The TMK1 gene from Arabidopsis codes for a protein with structural and biochemical characteristics of a receptor protein kinase, Plant Cell 4 (1992) 1263–1271.

[33] N. Dai, W. Wang, S.E. Patterson, A.B. Bleecker, The TMK subfamily of receptor-like kinases in Arabidopsis display an essential role in growth and a reduced sensitivity to auxin, PLoS One 8 (2013), e60990.