Molecular and Bioinformatic Characterization of the Rice ROOT UV-B SENSITIVE Gene Family

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Abstract

Background: ROOT UV-B SENSITIVE (RUS) genes exist in most eukaryotic organisms, and encode proteins that contain a DUF647 (domain of unknown function 647). Although the RUS genes are known to play essential roles in Arabidopsis seedling development, their precise functions are not well understood in other plants, including rice.

Findings: In this study, six OsRUS genes were cloned from rice root and leaf cDNA libraries. Our analysis showed that the sequence and open reading frame of cloned OsRUS3 cDNA differs from the predictions reported in the RAP-DB and RGAP databases. Public microarray, MPSS, and EST databases were used to analyze the expression profiles of the six OsRUS genes. Expression profiles for all OsRUS genes at different rice developmental stages were also analyzed by qRT-PCR. The signal peptide, GPI-anchor, transmembrane domain and subcellular localization of OsRUS proteins were predicted by various bioinformatics tools. Furthermore OsRUS1 was determined to be localized to the chloroplast by a protoplast experiment.

Conclusions: All the characterization of the OsRUS family generated from this study will provide a crucial foundation from which to further dissect how OsRUS genes function in rice development.

Keywords: DUF647, Expression profile, Oryza sativa, ROOT UV-B SENSITIVE, Subcellular localization

Findings

Identification and Cloning of OsRUS cDNA

RUS genes were first identified by Dr. He’s group in Arabidopsis (Tong et al. 2008; Leasure et al. 2009), and it was found that AtRUS1 and AtRUS2 play a role in very-low-fluence UVB response and VB6 homeostasis (Leasure et al. 2011). However, Dr. Estelle’s group discovered that the weak auxin response mutant wxr1 and wxr3 were caused by mutations in AtRUS2/WXR1 and AtRUS1/WXR3, respectively. Their results suggested a role for these two genes in the regulation of polar auxin transport (Ge et al. 2010; Yu et al. 2013). The inconsistencies between the results of these two research groups have not currently been resolved.

There are six AtRUS genes in the Arabidopsis genome, and they all contain a specific domain DUF647. There are six OsRUS genes annotated in the rice genome. OsRUS6 appears to have duplicated in the rice lineage to OsRUS6A and OsRUS6B, and there is no apparent ortholog for AtRUS4 (Leasure et al. 2009). The six OsRUSs are distributed on four rice chromosomes: OsRUS5 and OsRUS6A on chromosome 1; OsRUS1 and OsRUS2 on chromosome 4; OsRUS3 on chromosome 3; and OsRUS6B on chromosome 5 (Fig. 1a). The cDNA library of rice was reverse-transcripted from total RNAs extracted from young seedlings of Zhonghua 11 (Additional file 1: Materials and methods). The primers for cloning the six OsRUS cDNAs were designed to amplify their cDNAs (Additional file 2: Table S1). All six OsRUS cDNAs were amplified (Fig. 1b), which means that they are all functional genes. The PCR products of the six OsRUS cDNAs were cloned and sequenced. Surprisingly the sequence we obtained for the OsRUS3 cDNA (Additional file 3: Figure S1) was different from the sequences downloaded from the

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RGAP and RAP-DB databases (Fig. 2b, d and f). All of the other OsRUS cDNA sequences were consistent with both databases. The DUF647 domain and transmembrane domains of OsRUS3 were found in the RGAP database, the RAP-DB database and our cloned OsRUS3 (Fig. 2c, e and g). A 56aa cTP was found in the OsRUS3 from RGAP database, but was neither predicted in the OsRUS3 from RAP-DB database nor found in our cloned OsRUS3 (Fig. 2c, e and g). Whether the three types of OsRUS3 cDNA represent alternative splicing of LOC_Os03g11500, or only our cloned cDNA is real, needs further study.

Expression Profiles of OsRUS Genes During Vegetative and Reproductive Development

The expression profiles of genes are highly important for dissecting the functions of the genes (Fang et al. 2016). Here the expression profiles of the six OsRUS genes were data-mined from microarray, EST and MPSS publicly available databases and generated by qRT-PCR approach, respectively.

The expression profiles of OsRUSs during rice development were extracted from database RiceXPro (http://ricexpro.dna.affrc.go.jp/) (Sato et al. 2011) (Fig. 3). According to
this database, the expression level of OsRUS1 is much higher in roots and late embryos than in other organs. The expression levels of OsRUS2, OsRUS3, OsRUS6A and OsRUS6B during rice development are relatively high in all tissues examined, except for in leaf sheath at the reproductive stage and endosperm. The expression level of OsRUS5 in leaf is much higher than in other organs and stages. These results suggest that OsRUS2, OsRUS3, OsRUS6A and OsRUS6B function at similar development stages, while OsRUS1 and OsRUS5 function at different stages.

The expression profiles of the OsRUS genes were also extracted from the NCBI EST database (http://www.ncbi.nlm.nih.gov/nucest) (Additional file 4: Table S2). The expression of all six OsRUS genes can be detected in callus and rice leaf, but the expression level of OsRUS1, OsRUS3 and OsRUS5 is much lower than that of OsRUS2, OsRUS6A and OsRUS6B. OsRUS6B is not only the sole gene expressed in all of the tissues examined, but also the only OsRUS gene expressed in root and SAM, and its expression in SAM is much higher than in other tissues.

According to the information generated from the MPSS database, all six OsRUS genes express in callus, all OsRUS genes except for OsRUS2 express in 14d young rice leaves, and all OsRUS genes except for OsRUS1 express in NOS (Ovary and mature stigma) and NIP (90 days - Immature panicle). The expression of OsRUS1 was only detected in 14d young rice leaves and callus. OsRUS3 expresses in almost all development stages except for NGS (3 days - Germinating seed). OsRUS6A and OsRUS6B are highly expressed in all development stages examined. Salt induces the expression of OsRUS1 in 14d young rice roots and leaves. Cold greatly up-regulates the expression of OsRUS6A in 14d young rice leaves. Salt, drought and cold down-regulate the expression of OsRUS6B in 14d young rice roots, but highly up-regulate the expression of OsRUS6B in 14d young rice leaves (Additional file 5: Table S3).

In this paper, qRT-PCR approach was used to verify the expression profiles of the six OsRUSs at different rice development stages (Additional file 1: Materials and methods). By using the primers designed for qRT-PCR of six OsRUSs (Additional file 6: Table S4), the expression profiles of six OsRUSs at different development stages were generated by qRT-PCR (Fig. 4). From the qRT-PCR results, we observed that the six OsRUS genes were expressed in all tissues and stages examined. The expression levels of the six OsRUS genes in leaves were
higher than in other tissues at all stages. Generally speaking, the expression levels of the six OsRUS genes were lower than the house-keeping gene OsACTIN1, except for OsRUS6A and OsRUS6B at seedling and flowering stages.

When the expression profiles of OsRUS genes from above three databases and our qRT-PCR experiment were analyzed together, it was found that some results were consistent, while some were not. For example, all six OsRUS genes were found to be expressed in all tissues examined in the RiceXPro database and our qRT-PCR experiments. However, only OsRUS6A and OsRUS6B were found to be expressed in all tissues in the MPSS database, and only OsRUS6B was found to be expressed in all tissues in the EST database. The expression level of OsRUS1 was relatively low in the three databases and the qRT-PCR results. OsRUS1 expression was only detected in the MPSS database in NYL (14 days Young leaves) and NCA (35 days Callus), and in the EST database it was detected only in callus, leaf, panicle and stem. In the EST database only expression of OsRUS6B was detected in roots, while in the MPSS database OsRUS2, OsRUS3, OsRUS6A and OsRUS6B were detected in roots. The reasons for this inconsistency are typically complicated, and may be due to cultivar, environment, tissue stage and/or method sensitivity (Ma et al. 2011).

**Subcellular Localization of OsRUS Proteins**

The post-translational modifications of a protein are highly important for its function (Guerra et al. 2015). Here the signal peptides (SPs) and GPI-anchor modification signals of the six OsRUSs were predicted by SignalP 4.0 (http://www.cbs.dtu.dk/services/SignalP/) and BigPi (http://mendel.imp.ac.at/gpi/plant_server.html), respectively. None of the OsRUSs was found to have an N-terminal secretion signal (SPs) or a GPI-anchor, indicating that these proteins neither target to the endoplasmic reticulum nor localize to the plasma membrane.

Transmembrane proteins often play important roles in signal transduction or metabolite transport across membranes. Transmembrane domains of OsRUS proteins were predicted using web-based transmembrane domain prediction programs (Additional file 7: Table S5). OsRUS1, OsRUS2, OsRUS3 and OsRUS5 have at least one transmembrane domain predicted by TopPred, TMpred, TMHMM, HMMPred and SACS HMMPred tools. OsRUS6A and OsRUS6B have one or three transmembrane domains predicted by TopPred, TMpred, HMMPred and SACS HMMPred, but no transmembrane domain predicted by TMHMM. According to the above predictions, OsRUS proteins are likely to be transmembrane proteins.

Determining the subcellular localization of a protein is important for understanding its function. There are many
reliable bioinformatics tools available to predict protein subcellular localization. Here the subcellular localizations of OsRUSs were predicted by TargetP, Plant-mPloc, Yloc, ESLpred2, TargetLoc and MultiLoc2 (Table 1), respectively. OsRUS1 and OsRUS5 were predicted to localize to the chloroplast by all six programs used. Although the subcellular localizations of the other OsRUS proteins predicted by the above six programs were not consistent, the chloroplast was the primary predicted subcellular localization: OsRUS2.1 (2/6); OsRUS2.2 (4/6); OsRUS3 (2/6); OsRUS6A (4/6); OsRUS6B.1 (3/6); and OsRUS6B.2 (3/6). The mitochondrion was the second predicted localization for some OsRUS proteins: OsRUS3 (2/6); OsRUS6B.1 (3/6); and OsRUS6B.2 (3/6).

Based on the subcellular localization, non-GPI-anchor modification, and transmembrane predictions, we postulated that OsRUS proteins highly possible localize to the chloroplast membrane.

In order to evaluate the above subcellular predictions for OsRUS proteins, a protoplast transient-expression approach was used to detect the subcellular localization of OsRUS1 (Additional file 1: Materials and methods). OsRUS1 was predicted to contain a 35aa cTP and be localized to the chloroplast. There is enough information present in the cTP for chloroplast protein sorting (Lee et al. 2008). A transient expression vector of OsRUS1(1-160aa)::GFP was constructed and transformed into rice leaf sheath protoplasts. OsRUS1(1-160aa)::GFP was clearly observed to be localized to the chloroplast membrane (Fig. 5b). To our best knowledge this is the first time that the localization of a RUS protein has been experimentally confirmed to be localized to the chloroplast membrane (Tong et al. 2008; Leasure et al. 2009; Ge et al. 2010; Yu et al. 2013).

**Conclusions**

There are six OsRUS genes in the rice genome, distributed on four chromosomes. The cDNA sequences of five OsRUS genes are the same as the predictions of the RGAP and RAP-DB databases, while the cDNA sequence of OsRUS3 is not. Whether or not this new OsRUS3 cDNA represents a newly-identified alternative splicing variant has not been resolved. All six OsRUS proteins contain a specific DUF647 domain. The six OsRUS genes are expressed in tissues throughout rice development, and they all express more highly in leaves than in other organs. Some OsRUS genes have similar expression profiles during rice development. By using available bioinformatics tools, OsRUS proteins are predicted to lack both signal peptides

| Table 1 | Subcellular localizations of OsRUSs predicted by bioinformatics tools |
|---------|-------------------------------------------------|
| **Genes** | **TargetP** | **Plant-mPloc** | **Yloc** | **ESLpred2** | **TargetLoc** | **MultiLoc2** |
| OsRUS1 | Chloroplast | Chloroplast | Chloroplast | Chloroplast | Chloroplast | Chloroplast |
| OsRUS2.1 | Other | Chloroplast | Cytoplasm | Chloroplast | Other | Cytoplasm |
| OsRUS2.2 | Chloroplast | Chloroplast | Chloroplast | Chloroplast | Chloroplast | Cytoplasm |
| OsRUS3 | Mitochondrion | Cell membrane | Chloroplast | Chloroplast | Mitochondrion | Secretary pathway |
| OsRUS5 | Chloroplast | Chloroplast | Chloroplast | Chloroplast | Chloroplast | Chloroplast |
| OsRUS6A | Other | Chloroplast | Chloroplast | Chloroplast | Other | Chloroplast |
| OsRUS6B.1 | Mitochondrion | Chloroplast | Chloroplast | Chloroplast | Mitochondrion | Mitochondrion |
| OsRUS6B.2 | Mitochondrion | Chloroplast | Chloroplast | Chloroplast | Mitochondrion | Mitochondrion |

Fig. 5 Subcellular localization of OsRUS1 in rice sheath protoplasts. a, GFP control. b, OsRUS1(1-160aa)::GFP. Individual and merged images of GFP and chlorophyll autofluorescence (Chl), and brightfield (Bright) images of protoplasts are shown. Scale bars = 5 μm
and GPI-anchors, contain transmembrane domains, and be mainly localized to the chloroplast. Combining these predictions together, we postulate that most OsRUS proteins, if not all, localize to the chloroplast membrane. This postulation is supported by the OsRUS1 subcellular localization experiment using a rice protoplast transient-expression approach. All of the work in this paper will support the further dissection of the functions of OsRUS proteins during rice development.

Additional files

**Additional file 1:** Materials and methods. (DOCX 27 kb)

**Additional file 2:** Table S1. Primers for cloning of 6 OsRUS cDNAs. (DOCX 16 kb)

**Additional file 3:** Figure S1. The sequence of cloned OsRUS3 cDNA. (DOCX 15 kb)

**Additional file 4:** Table S2. The expression profiles of OsRUS genes from NCBI EST database. (DOCX 14 kb)

**Additional file 5:** Table S3. The expression profiles of OsRUS genes from the MPSS database. (DOCX 15 kb)

**Additional file 6:** Table S4. Primers for qRT-PCR of OsRUS genes. (DOCX 15 kb)

**Additional file 7:** Table S5. Transmembrane domains of OsRUSs predicted by bioinformatics tools. (DOCX 14 kb)

**Abbreviations**

cTP: Chloroplast transient peptide; DUf647: Domain of unknown function 647; EST: Expressed sequence tag; GFP: Green fluorescent protein; GPI-cTP: Chloroplast transient peptide; DUf647: Domain of unknown function

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**Authors’ Contributions**

NY and YL performed the experiments. NY and XH performed the bioinformatics analysis. XP and XH designed the experiments and bioinformatics analysis. NY and XH wrote the manuscript. All authors read and approved the final manuscript.

**Competing Interests**

The authors declare that they have no competing interests.

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