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Serine-rich protein is a novel positive regulator for silicon accumulation in mangrove

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ABSTRACT

Silicon (Si) plays an important role in reducing plant susceptibility against a variety of different biotic and abiotic stresses; and also has an important regulatory role in soil to avoid heavy metal toxicity and providing suitable growing conditions for plants. A full-length cDNAs of 696 bp of *serine-rich protein* was cloned from mangrove plant (*Rhizophora apiculata*) by amplification of cDNA ends from an expressed sequence tag homologous to groundnut (*Arachis hypogaea*), submitted to NCBI (KF211374). This *serine-rich protein* gene encodes a deduced protein of 223 amino acids. The transcript titre of the *serine-rich protein* was found to be strongly enriched in roots compared with the leaves of two month old mangrove plants and expression level of this *serine-rich protein* was found to be strongly induced when the mangrove seedlings were exposed to SiO_2. Expression of the *serine-rich protein* transgenic was detected in transgenic *Arabidopsis thaliana*, where the amount of serine increased from 1.02 to 37.8 mg/g. The same trend was also seen in Si content in the roots (14.3%) and leaves (7.4%) of the transgenic *A. thaliana* compared to the wild-type plants under Si treatment. The biological results demonstrated that the accumulation of the serine amino acid in the vegetative tissues of the transgenic plants enhanced their ability to absorb and accumulate more Si in the roots and leaves and suggests that the *serine-rich protein* gene has potential for use in genetic engineering of different stress tolerance characteristics.

1. Introduction

One of the most plentiful elements found in the soil and the environment after oxygen is silicon (Si). Silicon, an ultra-trace element, the second highest abundant element (27.2%) present in the lithosphere, participates in the normal metabolism of plants and animals (Hossain et al., 2002; Likhoshway et al., 2008). While all plants contain Si in different tissues, the accumulation of Si varies between plant species, notably by up to 10% in dry weight (Epstein, 1994; Ma and Takahashi, 2002). Silicon is also bioactively used in semiconductors and associated with beneficial effects on the chemical and physical properties of various scientific and technical applications.

Many elements, especially essential trace elements, are important for biological processes and play an important role in the growth and development of uni- and multi-cellular organisms (Müller et al., 2011). Silica (SiO_2), for example, is commonly found in a crystalline state in nature and as granules in the cell walls of single-cell and multi-celled organisms, including protists, diatoms, sponges, molluscs and higher plants (Morse, 1999; Perry and Keeling-Tucker, 2000; Foo et al., 2004; Desclés et al., 2008). Silica-based materials are widely used in the manufacture of siloxane, in catalysis, chemical and biological separations, biomedical materials, food and drug technology, biotechnology devices and so on (Trewyn et al., 2007; Jin and Yuan, 2011; Patwardhan, 2011).

Biosilica studies prior to 2005 were mainly concerned with the characterization of Si uptake and the transporter system, though not at a molecular level, in diatoms and plants and the identification of silica-depositing proteins, propylamines, from diatoms and sponges. Recent molecular studies of biosilicifying organisms on a variety of biomineralizers, such as diatoms and sponges, have resulted in researchers successfully unveiling the mechanistic secrets of biosilica formation. Investigation of their in vitro properties may help identify the controls in the formation of silica structured in vivo. For instance silaffins, cingulins, silacidins and long-chain polyamines (LCPA) have been identified to better understand the mechanism of silica formation and discover the fundamental principles of biomineral morphogenesis in diatoms (Kröger et al., 2002; Poulsen et al., 2003; Scheffel et al., 2011).

Mangrove forests which cover vast areas in the tropical and subtropical zones have their own unique characteristics. The harsh

Abbreviations: SSH, suppression subtractive hybridization; Ser, serine; Glu, glutamic acid; SEM, scanning electron microscope; EDX, energy-dispersive X-ray spectroscopy; HPLC, High performance liquid chromatography.

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environmental conditions which mangrove plants have to endure having given them the important ability to survive during their development stages. Among others, mangrove plants are able to tolerate elevated salinity, high temperature and UV irradiance; and also survive under poor nutrient conditions and protracted periods of inundation (Dasgupta et al., 2012). Preliminary study reported that the *Rhizophora apiculata* is able to absorb and accumulate a large amount of Si from the soil Nurul Mayzaitul Azwa (Personal communication). This accumulated Si makes the roots of the *R. apiculata* stronger in comparison to the roots of other plants growing in tidal areas. This is because intercellular Si is able to decrease heavy metal toxicity (Williams and Vlamis, 1957), suppress deleterious effects of salinity stress (Gong et al., 2006; Savvas et al., 2009; Hashemi et al., 2010) and act as an obstacle to penetration of different harmful pathogens. Pre-messenger RNA splicing is an important process in all eukaryotes towards regulation of gene expression. Alternative splicing generates mature mRNAs leading to existing different proteins that vary in structures and functions through intron excision and exon ligation mechanisms (Cartegni et al., 2002; Maniatis and Tasic, 2002; Reddy, 2004; Isshiki et al., 2006). The arginine/serine-rich proteins are a conserved family protein structurally and functionally, related to non-snRNP proteins and consist of different roles in pre-messenger RNA splicing (Graveley, 2000; Sanford et al., 2003). The functions of serine/arginine rich proteins in mRNA metabolism are not limited to alternative and constitutive splicing. They are also playing important roles in mRNA stability, translation, and nuclear export (Isshiki et al., 2006). Alternative splicing regulation is an important mechanism in the physiology and development of higher plants. Although spliced transcripts for many plant genes have been considered but most of their functions are still unclear (Isshiki et al., 2006).

The results of one study over-illustrating the pre-mRNA splicing effects of SR proteins on *Arabidopsis* indicate that serine/arginine genes are expressed in all plant parts, but regulation of splicing patterns depend on tissue-specific manner and developmental stage (Palusa et al., 2007). The SR proteins contain an arginine/serine-rich region (C-terminal) and one or two RNA binding domains (N-terminal), hence significantly contributing to the complexity of the proteome in higher eukaryotes. Multifunctional roles of SR proteins imprint their importance in gene expression regulation at various levels (Barta et al., 2010). The SR proteins affect the selection of splice site in a phosphorylation- and concentration-dependent fashion following contribution to the alternative pre-mRNA splicing (Duque, 2011).

Serine and proline-rich proteins might catalyze the localized depositing associated with silica at the attempted site regarding fungal penetration. The peptide-bound or polycationic-free oligo-N-methylpropylamines had been identified to cause the silica precipitation from orthosilicic acid soluble in a few moments. Subsequently it has been pointed out that proteins rich in proline have the ability to precipitate silica due to being strongly polycationic (Kauss et al., 2003).

Based on the finding regarding cell wall proteins, it has proposed that within diatoms, condensation regarding silicic acid to make covalently bonded inorganic/organic composites can be mediated by serine and threonine rich proteins (Harrison, 1996). Up to now the mechanism of bio-silica enhancement by creatures has been the main topics of vast investigation. At present, there are no reports available on the molecular mechanism of Si absorption in mangrove plants and thus the objective of this study was to understand the functional role of the *serine-rich protein* gene isolated from the roots of the *R. apiculata* and *Arabidopsis thaliana*.

2. Materials and methods

2.1. Plant materials

Seeds of mangrove plants (*R. apiculata*) were collected from Kuala Sepetang [04° 50.150″N, 100° 37.620″E] in Taiping, Perak, Malaysia. They were grown in hydroponic culture for two months and then treated with different concentrations of Si (0.5–1.5 mM SiO_2). *A. thaliana* seeds (col 24) were obtained from the Malaysian Palm Oil Board (MPOB) and cultured in special soil (peat-moss) in the transgenic plant green house.

2.2. RNA isolation and cDNA library construction

The roots of the mangrove plants (two months old seedlings) were washed and immediately frozen in liquid nitrogen and stored at −80 °C to facilitate the RNA extraction process. Total RNA was extracted from the Si treated plant's roots using modified CTAB method (Sahebi et al., 2013). PolyATracts® mRNA Isolation Systems Kit (Promega, USA) was used to purify Poly(A) mRNA from the total RNA. The SSH approach, based on the PCR-Select™ cDNA subtraction kit (Clontech Inc., CA, USA), was carried out to construct the cDNA library (Sahebi et al., 2014).

2.3. Bioinformatics analysis

The Blast2GO software (http://www.blast2go.org) v1.3.3 was performed to compute annotation of the mangrove EST datasets using the BLAST search at NCBI (http://www.ncbi.nlm.nih.gov/Blast/Genome/PlantBlast.shtml) (Altschul et al., 1997). The Wu-Blast from EBI (http://www.ebi.ac.uk/blast2) was used to determine the similarity of amino acid sequence (Altschul et al., 1990). Prediction of hydrophobicity and hydrophilicity of *serine-rich protein* gene was carried out online using TMHMM, MemBrain, and ProtScale in the toolkit of ExPASy (http://web.expasy.org/protscale/). Sub-cellular localization of *serine-rich protein* was investigated using Cell-PLoc, BaCelLo program and PSORT II Prediction. The secondary structure of protein was predicted using PsiPred program and http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html. The 3D structure of protein was obtained by PFAM program.

2.4. Cloning and sequencing of cDNA

A full-length cDNA sequence encoding protein rich in serine amino acid was obtained by searching the database, and then primers were designed according to the sequence. The PCR program was carried out using KAPA HiFi Hot Start. The PCR product was reclaimed and cloned into pDrive cloning vectors using PCR Cloning^{plus}Kit (Qiagen, Germany). The DNA sequence was determined based on the BLAST program in GenBank (Sahebi et al., 2014).

2.5. Semi-quantitative and Real-time RT-PCR

Reverse transcriptase RT-PCR and real-time qRT-PCR were performed to study the expressions and expression levels, respectively, of the candidate *serine-rich protein* gene in the roots of the mangrove plants in response to treatment with different concentrations of Si in comparison to the control (untreated) plants.

2.6. Construction of expression clone

Gateway® Technology with Clonase™II (Invitrogen, USA) was performed to construct the expression clone.

2.7. Construction of entry clone

The KAPA HiFi Hot Start DNA polymerase kit was used to generate DNA fragments (attB-PCR product) using the following primers:

Forward serin-anchor 5′ → 3′ GGGGACAAGTTTGTACAAAAAAGCAG GCTGTCATTCTGCCGAGTTCC.
Reverse serin-anchor 5′ → 3′ GGGGACCACTTTGTACAAGAAAGCTG GGTAATGCCCATTTATGTGACTTCG.