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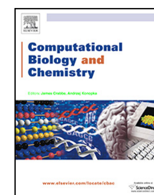
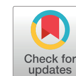


Research Article

Characterization of regulatory elements in *OsRGLP2* gene promoter from different rice accessions through sequencing and *in silico* evaluation



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ABSTRACT

Germins and germin-like proteins from cupin superfamily contribute resistance to heat denaturation, chemical degradation and against plant pathogens, further functions in plant growth and development. In this study, from three different *Oryza sativa* accessions KS-282 and Pak 7178 and Pak 7865, *Os*RGLP2 gene promoter region was amplified, sequenced and analyzed. Sequencing data was evaluated *via* different computational tools. The regulatory elements were predicted by Consite tool and mapping was done. Many transcription factors binding sites were discovered in *Os*RGLP2 gene promoter; among these factors, HFH-1 having a significant role in germination was picked for further investigation. To study the interaction between HFH-1 and corresponding regulatory factors, HADDOCK Webserver was used. Graphical models for the interactions of HFH-1 and related regulatory elements were studied by graphic molecular system PyMOL. Mapping of *cis*-acting regulatory elements in *Os*RGLP2 gene promoter from three rice accessions showed differences in their position and copy number. Important regulatory elements found in *Os*RGLP2 promoter region were TATA, CAAT Box, ARR1, GATA, AGAAA, CAAT and DNA-binding One Zinc Finger (Dof) factors, few of them contribute to the regulation of plant defensive system, light responses, developmental and growth activities. Furthermore, during DNA interaction studies, it was found that HFH-1 transcription factor participates in hydrogen bonds formation with thymine and adenine bases.

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1. Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop. According to the world rice production data 2015/2016 by United States Agriculture Department (USAD), the estimated rice production will be 469.32 million metric tons. According to Devos and Gale (1997) due to the complete sequenced genome, rice acts as a model crop plant and it provides basis to understand the fundamental traits, for example, yield, productivity, hybrid potency, stress tolerance and disease resistance. *Oryza sativa* genome is diploid (2n = 24), though tetraploid genome (4n = 48) is also in some *Oryza* genus. This genome is comparatively small amongst all the food crops; moreover, nearly a half of its genome comprise of repetitive sequences (Chang, 2003). Rice genome consists of twelve chromosomes, with wide-ranging genetic resources of cultivated and wild species (Nakagahra et al., 1997; Vaughan et al., 2013). In a report by Goff et al. (2002), the *Oryza sativa* genome represents a great diversity of genes and about 50,000 genes have been found.

Germins and germin-like proteins (GLPs) are the members of cupin superfamily revealing an important role in plant defensive system, against osmotic and homeostasis stress, (Braun et al., 1996) and also in oxidative stress resistance as a result of abiotic and biotic stimuli (Dunwell et al., 2008). Yasmin et al. (2015) recently have described germins and GLPs as a diverse family of proteins widespread in plants and these are associated with developmental processes. It has also been found that *Oryza sativa* genome harbours a large family of GLPs and, therefore, it acts as a model plant for the analysis of germins and GLPs to reveal their structural and functional properties. There are many reports for the occurrence and expression of GLPs in different plant tissues (Godfrey et al., 2007; Komatsu et al., 2010; Wang et al., 2013) and in response to various adverse environmental stresses (Yasmin et al., 2008; Banerjee and Maiti, 2010; Galván et al., 2011; Yang et al., 2013).

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GLPs reveal very diverse patterns of expression. Few of them are expressed at particular developmental points, such as initiation of flowering, fruit ripening and embryogenesis (Mathieu et al., 2006; El-Sharkawy et al., 2010). Several GLPs are stimulated under abiotic and biotic stress conditions, such as pathogens attack (Galván et al., 2011; Olvera-Guevara et al., 2012; Wang et al., 2013), herbivores (Lou and Baldwin, 2006) and osmotic stress (Wang et al., 2013). The barley *GER4c* gene promoter was discovered to be activated in response to pathogens (Himmelbach et al., 2010). In another study, Sassaki et al. (2014) carried out the expression and functional analysis of *Eg*GLP gene promoter from *Eucalyptus grandis* by *in silico* study, selecting an expressed sequence tag (EST) encoding a putative GLP having its expression in leaves, using the expression profile data and furthermore validated the promoter activity *via* reverse transcription PCR analysis. Moreover, two GLP gene promoters from two conifers (*Pc*GER1 from *Pinus caribaea* and *Lm*GER1 from hybrid larch) were investigated (Mathieu et al., 2003, 2006). The activity of *Pc*GER1 promoter was demonstrated during the cell growth (Mathieu et al., 2003), while in case of *Lm*GER1 promoter, reporter gene expression under the control of this promoter was studied in young embryo root caps and cotyledons (Mathieu et al., 2006).

In a recent study, Munir et al. (2016) have reported the pathogen inducible property of this *Os*RGLP2 gene promoter in transgenic potato in response to two fungal pathogens that were *Fusarium solani* (Mart.) Sacc. and *Alternaria solani* Sorauer. The results were validated *via* quantitative real time PCR analysis and GUS histochemical data. According to earlier investigation by Yasmin et al. (2008), in rice, GLPs have an important role in plant defensive system. Furthermore, the sequencing data of the *Os*RGLP1 promoter has shown the presence of numerous regulatory elements that vary in density ranging from 2 to 9 in sequenced region and are found to be associated with defensive approach and gibberellin signaling pathway. Similarly, in another investigation, Mahmood et al. (2013) analyzed the activity of the *Os*RGLP2 gene promoter and demonstrated its wound-inducible property in transgenic tobacco. The main objective of the present work was the *in silico* characterization of the regulatory elements and putative transcription factor binding sites in the OsRGLP2 gene promoter by using computational tools. Furthermore, the interactions between the transcription factors and related regulatory elements were analysed. From different transcription factors discovered in the *Os*RGLP2 gene promoter, HFH-1 factor with a significant role in germination was picked for the evaluation of DNA-protein interactions *via* computational analysis.

2. Materials and methods

2.1. Plant material

Seeds of three different *Oryza sativa* accessions were obtained from National Agriculture Research Centre (NARC) Pakistan. Three accessions KS-282, Pak 7865 and Pak 7178 were selected. After seed surface sterilization, these were cultured on MS basal media under aseptic conditions.

2.2. Primer designing and PCR amplification

For the amplification of *Os*RGLP2 gene promoter region from selected rice accessions, a particular set of primers was designed from available sequences in GenBank of NCBI (National Center of Biotechnology Information) with the help of primer 3 (version 0.4.0) and the sequence of primers is mentioned below;

Forward Primer: 5′ CTGGTCTACTTGGCATTGT 3′

Reverse Primer: 5′ TTCTCTGCTGAATTATTTGCT 3′

Genomic DNA was isolated from the leaf tissues by Cetyl Trimethyl Ammonium Bromide (CTAB) protocol described by Richards (1997). Extracted DNA was used for the amplification of *Os*RGLP2 gene promoter. To amplify the *Os*RGLP2 gene promoter region, 25 μl PCR reaction mixture was prepared with 16.2 μl of nanopure water, 2.5 μl of 10× PCR buffer, 1.5 μl of 25 mM $MgCl_2$, 1.5 μl of 0.5 mM dNTPs, 1 μl of each 25 pmol forward and reverse primer, and 0.3 μl of *Taq* DNA polymerase and the reaction was run; the conditions were pre-denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 54.3, 55 and 56 °C for 30 s and extension at 72 °C for 1 min. Final extension cycle was set at 72 °C for 20 min.

2.3. Sequencing and sequence analysis

Amplified PCR products were purified using GeneJet PCR purification kit (Thermo Scientific) before getting these products sequenced from Macrogen (Seoul, Korea). Various bioinformatics tools, sequence searches and databases were used for the analysis of sequencing data. Basic local alignment search tool (BLAST) was used to detect resemblance between sequences. Nucleotide Blast (BLASTn) was applied to find the sequences similarity with previously submitted sequences in sequence databases (http://blast.ncbi.nlm.nih.gov).

A bioinformatics tool ConSite (http:/www.phylofoot.org/consite) was used for the detection of regulatory factors in *Os*RGLP2 gene promoter region in the studied rice accessions. Another software DOG 2.0 tool (www.mybiosoftware.com/protein-sequence-analysis) was used for the mapping of *cis*-acting regulatory elements. Furthermore, Protein data bank (PDB) accessible at (http://www.rcsb.org/pdb/) was used for the structural modeling of biomolecules. Protein residues were assembled by using online software DISPLAR (http://pipe.scs.fsu.edu/displar.html). DNA-protein modeling and docking was done *via* HADDOCK Webserver (http://haddock.chem.uu.nl/) while DNA-protein interactions were studied and visualized using the PyMOL Molecular Graphic System (http://www.pymol.org/). High-quality data for transcription factor HFH-1 (Accession number, 1KQ8) was selected from PDB based on the structure type, resolution and amino acid length for further evaluation. Protein-DNA docking was explored *via* HADDOCK software. The structure and active residues of particular *cis*-acting elements of *Os*RGLP2 gene promoter corresponding to HFH-1 were obtained using HADDOCK Webserver from studied rice accessions.

3. Results

3.1. Amplification and sequencing of OsRGLP2 gene promoter

The amplified products of *Os*RGLP2 gene promoter from all rice accessions were used for the sequencing. The sequenced data obtained for *Os*RGLP2 gene promoter region from Pak 7865, KS-282 and Pak 7178 showed 95, 93 and 87% similarity, respectively, with the *Os*RGLP2 promoter sequence from *Oryza sativa* var. japonica (Accession No. AP005531.3). Final sequences were submitted to Genbank for the allotment of accession number. The accession numbers obtained are KJ958556, KJ958557 and KJ958558 for Pak 7865, KS-282 and Pak 7178, respectively.

3.2. Prediction of regulatory elements

For all studied rice accessions, the regulatory elements in *Os*RGLP2 promoter region were predicted by Consite bioinformatics tool. The putative regulatory elements that were found on *Os*RGLP2 gene promoters from studied rice accessions are as follows; TATA and CAAT Box, ARR1, GATA, AGAAA, CAAT and DNA-binding One Zinc Finger (Dof) factors. These regulatory elements are known to be involved in various functional activities in plants including growth,

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