Biochemical and Biophysical Research Communications 394 (2010) 178–183

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Functional role of rice germin-like protein1 in regulation of plant height and disease resistance

Joydeep Banerjee, Mrinal K. Maiti *

Department of Biotechnology and Adv. Lab. for Plant Genetic Engineering, Advanced Technology Development Center, Indian Institute of Technology, Kharagpur 721302, India

ARTICLE INFO

Article history:
Received 10 February 2010
Available online 24 February 2010

Keywords:
Germin-like protein
OsGLP1
Semi-dwarfing
siRNA
Superoxide dismutase

ABSTRACT

The functional role of rice (*Oryza sativa*) germin-like protein1 (OsGLP1) was elucidated through development of transgenic plants involving endogenous gene silencing in rice and heterologous gene expression in tobacco. Usually, the single copy OsGLP1 gene in rice plant was found to be expressed predominantly in green vegetative tissues. The transgenic rice lines showed significant reduction in endogenous OsGLP1 expression due to 26 nt siRNA-mediated gene silencing, displayed semi-dwarfism and were affected seriously by fungal diseases, compared to the untransformed plant. Structural homology modeling predicted a superoxide dismutase (SOD) domain in OsGLP1 protein which upon over-expression in transgenic tobacco plant clearly documented SOD activity. Our observations on the maintenance of cell dimension, cell wall-associated localization particularly in the sub-epidermal tissues and the SOD activity of OsGLP1 could explain its functional role in regulation of plant height and disease resistance in rice plant.

1. Introduction

Germin-like proteins (GLPs) constitute a diverse family of ubiquitous plant glycoproteins [1,2]. Most of the pre-proteins of GLPs contain the signal peptide sequence helping them to be exported from the cell and the mature GLPs possess at least one or two putative *N*-glycosylation sites at conserved positions and metal ion binding sites [1,3]. An important feature of the germins and GLP-related proteins is the presence of a motif derived from the cupin superfamily [2,4]. All proteins of the cupin superfamily contain a signature domain consisting of six β-strands within a conserved β-barrel structure. Comparing the amino acid sequences of different GLPs with the barley oxalate oxidase whose crystal structure has been determined, it has been observed that the cupin motifs of most of the GLPs contain three highly conserved histidines and one glutamate residue involved in binding a metal ion for active site [4,5].

The germins and GLPs have spatial and temporal expression in different plant species and are involved in diverse functions [6–10]. They may have single or combination of enzymatic activities, such as oxalate oxidase, superoxide dismutase (SOD) and ADP glucose pyrophosphatase/phosphodiesterase; act as structural protein or help in signal transduction by their receptor function [1,2]. Many GLPs have manganese-containing SOD activity [3,10]. A GLP from wheat leaf apoplast was found to having an inhibitory role to serine protease [11]. It has been proposed that GLPs function to protect the plants from the effects of oxidative stresses induced by abiotic or biotic factors [2,4].

Thus, considering various reports it is clear that GLPs have broad range of diversity in occurrence, expression and activity; and unique functions in specific plant system. Although, thorough investigations have not been carried out about the role of rice GLPs, in a recent study it has been reported that GLP family members function as a complex QTL conferring broad-spectrum disease resistance [12]. Moreover, an *in silico* study revealed the presence of several regulatory elements in a rice GLP promoter [13]. The objective of the present study was to clone and characterize the germin-like protein1 (OsGLP1) gene from an *indica* rice (*Oryza sativa*) cultivar; and to understand the functional role of OsGLP1 through development of transgenic plants for endogenous gene silencing in rice and heterologous gene expression in tobacco.

2. Materials and methods

2.1. Plant cultivars

The local cultivars of rice (*O. sativa* subsp. *indica*) and tobacco (*Nicotiana tabacum*) were used in the present study. All the untransformed and transgenic lines were grown in contained growth chamber and natural field condition for different analyzes.

2.2. Cloning of OsGLP1 gene from rice

The coding DNA sequence (CDS) of OsGLP1 gene was RT-PCR amplified from the leaf cDNA sample of *Badshahbhog* rice cultivar

* Corresponding author. Fax: +91 3222 278707 (Office).
E-mail addresses: mrinalkmaiti@gmail.com, maitimk@hijli.iitkgp.ernet.in (M.K. Maiti).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.02.142

by using two primers based on the available sequences in the GenBank (AF032975 and AB015593). The forward primer, 5′-TAC**GGATCC**ATGGCCAAGGCAGTGATGAT-3′ and the reverse primer, 5′-TAAT**AAGCTT**AACCGCTACCGCCGAGG-3′ were used, having an incorporated *Bam*HI (bold) and *Hin*dIII (bold) site, respectively. The PCR amplified product (~642 bp) was cloned into pUC18 vector and transformed into *Escherichia coli* DH10B (GIBCO BRL) cells [14]. The positive clones were screened by restriction digestion and confirmed by sequence analysis followed by bioinformatics study.

2.3. Preparation of constructs for OsGLP1 gene silencing and over-expression

The preparation of gene constructs has been described in Supplementary information.

2.4. Development of OsGLP1 down-regulated transgenic rice lines and OsGLP1 over-expressed transformed tobacco plant

The *Agrobacterium tumefaciens* strain EHA105 harboring the gene silencing construct was used to transform the rice calli following reported protocol [15]. For over-expression of OsGLP1 in tobacco, *A. tumefaciens* strain LBA4404 harboring the OsGLP1 over-expression construct was used for tobacco leaf disk transformation [16]. Putative transformants from both of the events were selected in hygromycin containing media. The selected plantlets were screened through PCR, confirmed by Southern hybridizations in T_0, T_1 and T_2 generation and analyzed by Northern hybridization and Western blotting.

2.5. Southern and Northern hybridization of untransformed rice plant

For Southern hybridization, 10 μg of undigested and different restriction digested DNA samples from untransformed rice were size-fractionated on 0.8% agarose gel with molecular weight marker. For Northern blotting, total RNA samples (30 μg) from root, stem, leaf, developing endosperm, husk and mature endosperm tissues were electrophoresed on 1.2% agarose-denaturing (formaldehyde) gel along with the RNA ladder marker. Transfer of nucleic acids onto nylon membrane and hybridizations with [α-32P] dCTP labeled OsGLP1 CDS-specific probe (642 bp) were performed following standard techniques [14].

2.6. siRNA detection in transgenic rice lines

Total RNA samples (30 μg) from the leaf of the untransformed and different transgenic lines were electrophoresed on 20% denaturing polyacrylamide gel. The presence of siRNA specific to OsGLP1 was detected following a reported procedure [17]. Three oligonucleotides, two OsGLP1 gene specific of 29 and 27 nt and another arbitrary sequence of 28 nt, were included in the same gel as markers. After electrophoresis, transfer onto nylon membrane and hybridization were performed as mentioned above.

2.7. Protein extraction and electrophoresis

Total protein from leaf tissues of the untransformed plant and different transgenic lines were isolated using extraction buffer [20 mM Tris–Cl (pH 8.0), 100 mM NaCl, 10 mM PMSF, 0.1% Triton × 100 with or without 2 mM β-mercaptoethanol] followed by centrifugation at 12,000×*g* for 10 min at 4 °C. After quantification by Bradford method, electrophoresis of the protein samples was performed under semi-native and denaturing 12% polyacrylamide gel.

2.8. Production of polyclonal antibody and Western blotting

The OsGLP1 gene was expressed in BL21 cells via pT7-7 vector by inducing with 0.5 mM IPTG at 25 °C. The induced protein sample was subjected to SDS-12% PAGE and the over-expressed OsGLP1 band was electro-eluted for production of polyclonal antibody in rabbit. After purification the serum was used in immunoblot. For Western blotting, total leaf protein samples were subjected to SDS-12% PAGE and transferred onto nitrocellulose membrane. The anti-OsGLP1 antibody was used as primary antibody and protein A-conjugated horseradish peroxidase was used as secondary antibody. The immunoblot was developed using the HPO color development kit (Bio-Rad).

2.9. Superoxide dismutase assay

In-gel SOD activity on semi-native polyacrylamide gel was performed following reported protocol [3], with minor modification in positive staining method. After illuminating the gel for 55 min, it was soaked in deionized water overnight.

2.10. Light microscopy and immunolocalization

Longitudinal stem sections of 0.1–0.2 mm thick and 0.5 cm long were collected from the immediate upper portion of the first node from 45 days old rice plants. Sections were immediately placed in 20% glycerol containing deionized water and finally observed under bright field with 40× magnifications by Leica MPS 60 microscope.

The cross-sections (0.1–0.2 mm thick) of stem were collected from the immediate upper portion of the first node from 21 days old rice plants. After fixing in PBS containing 4% formaldehyde, the sections were dechlorophyllized in 1:1 methanol and acetic acid followed by blocking in PBS containing 0.3% BSA. Following washing with 1× PBS, the samples were incubated with the anti-OsGLP1 as primary antibody and FITC (fluorescein isothiocyanate) conjugated anti-rabbit antibody as secondary antibody. After washing, the sections were observed under fluorescence microscope (Leica MPS 60). The control sections were incubated with pre-immune sera instead of the primary antibody keeping other conditions same.

2.11. Modeling of OsGLP1 using barley oxalate oxidase

The structural model of mature OsGLP1 protein, excluding the signal peptide sequence, was performed by first approach mode in Swiss-model (http://swissmodel.expasy.org/workspace/index.php?func=modelling_simple1). The model was analyzed and figure was generated using PyMOL.

3. Results

3.1. Predominant expression of single copy OsGLP1 gene in green vegetative tissues

A 642 bp CDS of OsGLP1 was cloned from an *indica* rice cultivar and sequence analysis revealed 100% nucleotide identity with the gene in rice genome of public database. The CDS of OsGLP1 was predicted (by ExPASy PortParam) to produce a polypeptide of 214 amino acids with a molecular weight of ~21.8 kDa. Similar to many other GLPs, the OsGLP1 possessed a signal peptide sequence of 24 amino acids (predicted by SignalP 3.0) and one putative *N*-glycosylation site (NTS) at 63 amino acid position (predicted by NetNGlyc 1.0 Server).

Southern hybridization of the rice genome using OsGLP1-specific probe revealed a single band signal with *Bam*HI, *Eco*RI and

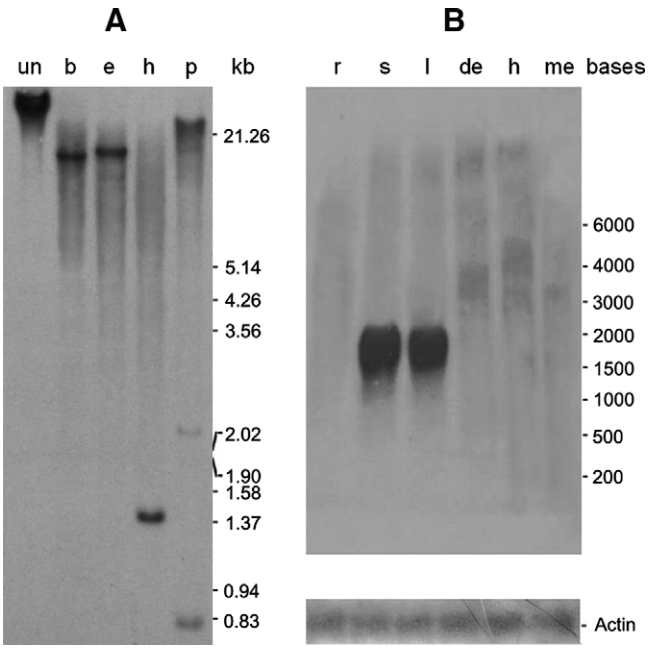

Fig. 1. Genomic organization and tissue specific expression of OsGLP1 in untransformed rice. (A) Southern blot of undigested (un), *Bam*HI (b), *Eco*RI (e), *Hin*dIII (h) and *Pst*I (p) digested genomic DNA samples of rice along with *Eco*RI + *Hin*dIII digested lambda DNA as marker. (B) Northern blot of RNA samples from root (r), stem (s), leaf (l), developing endosperm (de), husk (h) and mature endosperm (me) along with RNA ladder marker (Fermentas). Lower panel shows the loading control using rice actin probe.

*Hin*dIII digested genomic DNA samples, but in the *Pst*I digested sample two bands at ~0.82 kb and ~2.2 kb (Fig. 1A) were found due to one internal *Pst*I restriction site. The results indicated the presence of a unique sequence of OsGLP1 gene in this rice cultivar. Tissue specific expression pattern by Northern hybridization documented high level expression of OsGLP1-specific transcript of ~1900 bases in the stem and leaf tissues but no such expressions were detected in root, developing endosperm, husk and mature endosperm (Fig. 1B).

3.2. siRNA-mediated down-regulation of endogenous OsGLP1 gene in transgenic rice lines

To elucidate the role of OsGLP1 in rice plant it was down-regulated by hair-pin RNA-mediated gene silencing approach. The transgenic rice lines developed with the gene silencing construct (Fig. S1A) were confirmed by Southern hybridization in T_1 as well as in T_2 generation (data not shown). To verify further, they were examined for the presence of, if any, OsGLP1-specific siRNA which is the basis of post-transcriptional RNA interference strategy used in this study. The OsGLP1-specific siRNA band of ~26 nt was detected in the Northern blot of all the transgenic lines tested but not in the untransformed plant (Fig. 2A Blot). Additionally, the two OsGLP1 CDS-specific oligonucleotides (29 nt and 27 nt) used as markers in the same gel (Fig. 2A Gel) were found to be hybridized at their respective positions (Fig. 2A Blot). To check for any nonspecific hybridization, one arbitrary oligonucleotide (28 nt) was used in the same RNA gel (Fig. 2A Gel, lane 3) and found to produce no signal (Fig. 2A Blot, lane 3).

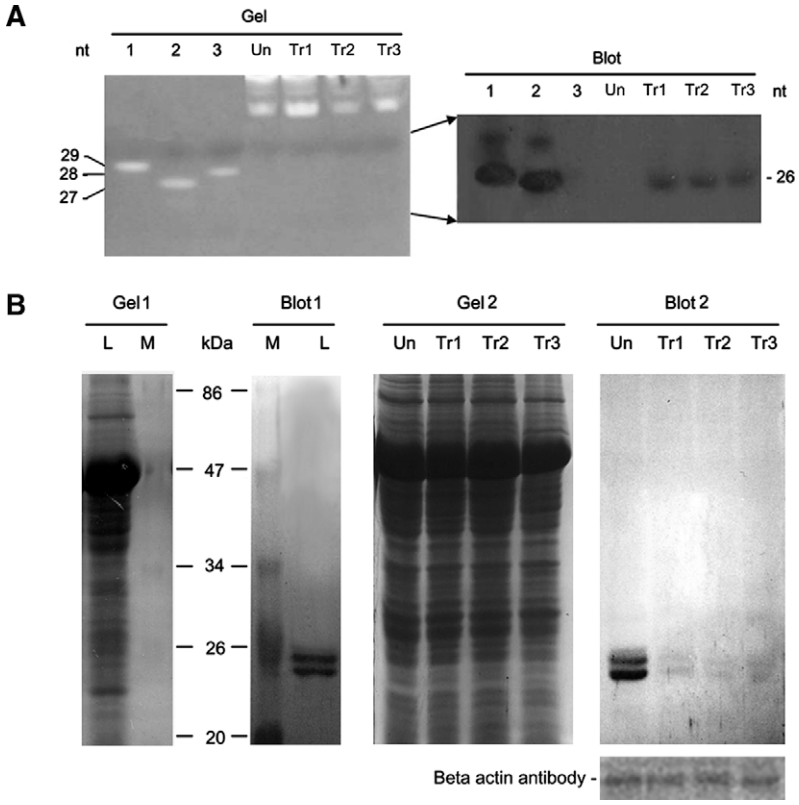

Fig. 2. siRNA-mediated down-regulation of OsGLP1 in transgenic rice lines. (A) The OsGLP1-specific siRNA detection by Northern hybridization (Blot) following gel electrophoresis and ethidium bromide staining (Gel). Lanes 1 and 2, two oligonucleotides (29 and 28 nt) specific to OsGLP1 gene; lane 3, an arbitrary oligonucleotide of 27 nt; lane Un and Tr1-3, RNA from untransformed and three transgenic lines, respectively. (B) Protein samples from leaf tissue were visualized by Coomassie blue staining of gel (Gel) and OsGLP1-specific polypeptides were detected by immunoblotting (Blot). Lane L, untransformed control (20 μg protein); lane M, protein molecular weight marker SM 0441 (Fermentas). Lane Un, Tr1, Tr2 and Tr3 represent leaf protein samples (40 μg) from untransformed plant, transgenic lines 1–3, respectively. Lower panel shows the loading control using anti-beta actin antibody as standard.

In Western blot of rice protein samples, two major bands of ~22 and ~24 kDa were detected in untransformed plant (Fig. 2B Blot1), whereas the expressions of these OsGLP1-specific proteins were found to be drastically reduced in the transgenic lines (Fig. 2B Blot2). Sequencing data revealed that the two immuno-detected bands were of OsGLP1 origin. Thus, the results together confirmed the down-regulation of the OsGLP1 protein by siRNA-mediated gene silencing in the transgenic lines.

3.3. Semi-dwarfism and disease susceptibility of the transgenic rice lines

Interestingly, all of these OsGLP1 down-regulated transgenic rice lines were found to display semi-dwarf phenotype in T_2 generation compared to the tall untransformed rice cultivar (Fig. 3A). When these plants were grown under irrigated condition in a region prone to infestations by the fungal diseases, e.g. sheath blight and blast; the transgenic lines were found to be more susceptible to both of these biotic stresses (Fig. 3B). The leaf sheath portions and leaves of all the transgenic lines became heavily infected by sheath blight and blast fungi, respectively, compared to very little infestation in the untransformed rice (Fig. 3B (a–h)). Upon maturity, the transgenic panicles contained predominantly chaffy-grains in contrast to mostly well-filled grains in untransformed panicle (Fig. 3B (i–l)).

3.4. Alteration of cell dimension in transgenic lines by cell wall associated OsGLP1

To understand the semi-dwarf phenotype of the transgenic plant at the cell anatomy level, longitudinal stem sections of the transgenic and control plants were analyzed under bright field microscope. The transgenic cells were found to be more shorter with a lower length:breadth ratio compared to the control (Fig. 3C). To examine the histological location of the OsGLP1 protein, it was immuno-detected *in situ* under fluorescence microscopy in the cross sections of rice stem. In the control plant, the OsGLP1 protein was present at higher level in the sub-epidermal cells but faintly in the vascular bundle (Fig. 3D). Moreover, this protein was predominantly localized in the cell wall region rather than being intracellular. However, the transgenic plant showed a lower level of OsGLP1 localized in the sub-epidermal cells along with the significant changes in cellular anatomy (Fig. 3D). The stem sections probed with pre-immunized serum lacking OsGLP1 antibodies gave no fluorescent signal under similar microscopic observation, as expected (data not shown).

3.5. Presence of SOD structural domain in OsGLP1 and documentation of its SOD activity

To understand whether OsGLP1 possesses any domain for putative SOD activity, homology modeling of mature OsGLP1 protein,

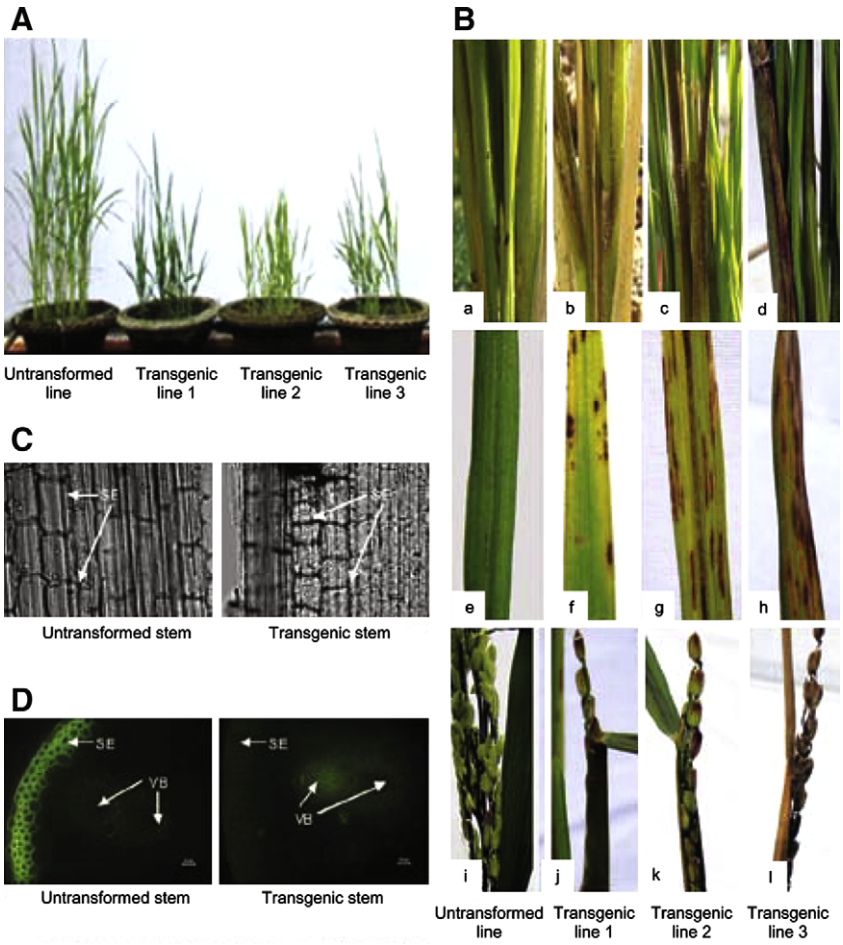

Fig. 3. Morphological differences between untransformed and transgenic rice lines with respect to plant height (A), disease infestation (B), cell dimension (C) and localization of OsGLP1 protein (D). (A) Semi-dwarf phenotype of transgenic lines. (B) Susceptibility of transgenic lines to fungal diseases. Sheath blight infestation in untransformed (a) and different transgenic lines (b–d). Blast infection in untransformed (e) and different transgenic leaves (f–h). Affected panicle in untransformed (i) and different transgenic lines (j–l). (C) Light microscopy of longitudinal stem sections of rice. (D) Immunolocalization using OsGLP1 polyclonal antisera and FITC labeled anti-rabbit antibody on transverse stem sections. In micrographs bars indicate 10 μm; SE, sub-epidermis; VB, vascular bundle.

excluding the signal peptide sequence was performed using barley oxalate oxidase as template (pdb 2ET1). The model of OsGLP1 predicted an active site composed of three histidines (His104, His106, His151) and one glutamate residue (Glu111) responsible for metal ion binding and SOD activity. The active site, analogous to barley oxalate oxidase, is protected within the jellyroll β-barrel structure comprised of 9 β-strands (Fig. 4A).

To examine the SOD function of OsGLP1, the total leaf protein sample from untransformed rice plant were subjected to in-gel SOD activity staining, where three major SOD-active bands were detected at ~47, 38 and 35 kDa regions (Fig. 4B SOD gel). Interestingly, the same protein sample immuno-detected the ~35 kDa band as OsGLP1-specific protein (Fig. 4B Immunoblot) indicating the OsGLP1 may possess SOD activity. For further confirmation, the full length OsGLP1 CDS was expressed transgenically in tobacco plant using an over-expression gene construct (Fig. S1B). It was observed that along with several endogenous bands, the transgenic tobacco possesses another distinct SOD-active band which is absent in untransformed tobacco plant (Fig. 4C). Immunoblot analysis confirmed that the additional SOD-active band in the transformed tobacco leaf sample was due to the heterologous expression of OsGLP1 (Fig. 4D lane 2). Moreover, under complete denaturing condition the transgenic tobacco plant showed two major OsGLP1-specific protein bands (Fig. 4D lane 1), similar to the situation in untransformed rice plant (Fig. 2B Blot1, lane L). It was noticed that the over-expressed OsGLP1-specific SOD-active band in the transformed tobacco was slightly smaller in size (~33 kDa) compared to that (~35 kDa) in rice. Taken together, these results undoubtedly documented that the OsGLP1 indeed possesses SOD activity.

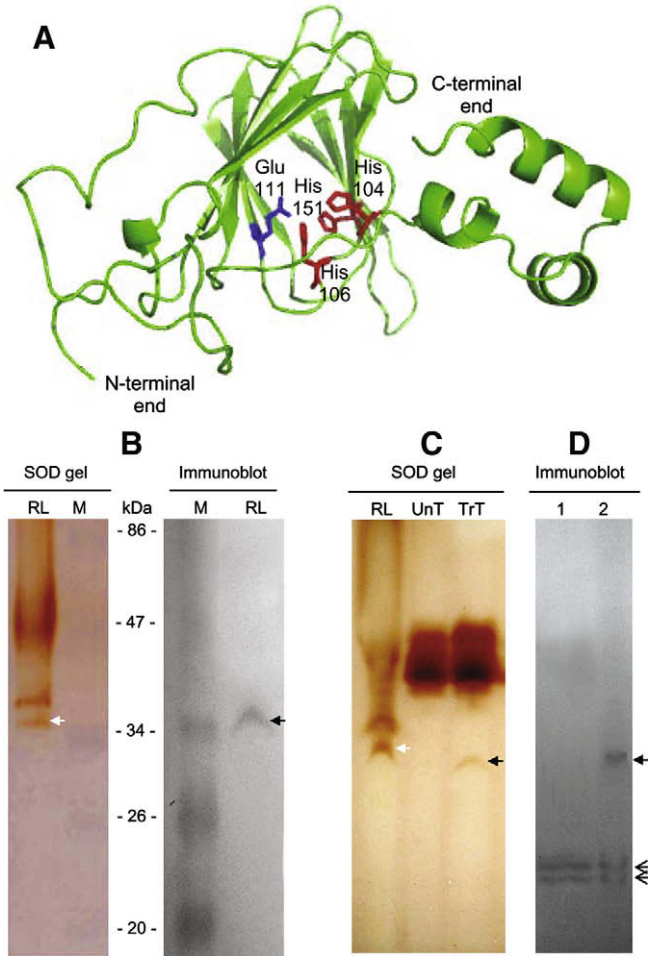

Fig. 4. Structure prediction of OsGLP1 and its SOD activity. (A) Modeling of OsGLP1 protein. The amino acids-His104, His151, His106 and Glu111 responsible for SOD domain are highlighted. (B) In-gel SOD activity and immunoblot of rice leaf protein (RL) under semi-native condition run along with Fermentas molecular weight marker (M). White and black arrows indicate OsGLP1-specific SOD-active and immuno-detected bands, respectively. (C) In-gel SOD activity of untransformed rice (RL), untransformed tobacco (UnT) and transgenic tobacco (TrT) leaf protein samples under semi-native condition. White and black arrows indicate OsGLP1-specific SOD-active bands in rice and tobacco samples, respectively. (D) Immunoblot detection of the heterologously expressed OsGLP1 protein in tobacco leaf sample under complete denaturing (lane 1) and semi-native (lane 2) conditions. Solid and hollow arrows indicate heterologous expression of OsGLP1 protein in dimeric and monomeric forms, respectively.

4. Discussion

Although, several studies have been reported regarding various functions of GLPs, a group of highly conserved proteins in different plant systems, no thorough investigation has been carried out in rice, the most important cereal crop of the world. In the present study, the OsGLP1 gene from an *indica* rice cultivar has been cloned and its physiological as well as enzymatic functions have been elucidated through development of transgenic plants both by endogenous gene silencing in rice and heterologous gene expression in tobacco.

The single copy OsGLP1 gene in untransformed rice is usually expressed abundantly in green vegetative tissues, i.e. in leaf and stem but this transcript was not detectable in root, developing endosperm, husk and mature endosperm (Fig. 1). This observation is someway analogous in barley, where HvGLP1 mRNA shows high and low level expressions in young and older leaves, respectively, but not in root tissues [6]. Although the CDS of OsGLP1 is 642 bp, the size of the transcript detected in this study is ~1900 bases, which is close to one EST sequence (1829 bases) of this gene reported in the databank (CT842946).

The endogenous OsGLP1 gene expression in rice has been down-regulated by using siRNA-mediated gene silencing (Fig. 2). The OsGLP1 gene specific probe detected the siRNA band of ~26 nt in the transgenic lines tested but none in the control plant (Fig. 2A). Previously, it has been reported that during post-transcriptional gene silencing in plant system induced by virus and transgene, both shorter (~21 nt) and longer (~26 nt) classes of siRNAs were found to have a role in gene silencing [18]. However, in our transgenic rice lines siRNA of ~21 nt has not been found. It is possible that the size and functional diversity of siRNAs in plants is due to the multiplicity of DCL enzymes, which are Dicer-homologues [18]. A very recent study has predicted 41 members of GLP-related genes in rice genome and only 3 (loci Os08g35750, Os05g10830 and Os11g33110) are closely related to OsGLP1 (Os08g35760) belonging to GER2 subfamily based on barley HvGER proteins [12]. The possible off-target effect of siRNA-mediated gene silencing in this study has been analyzed by sequence alignment at nucleotide level showing insignificant homology among the OsGLP1-related genes (Fig. S2).

In the leaf protein sample of untransformed rice, the OsGLP1 is highly expressed and exhibits two major polypeptide bands of ~22 and 24 kDa under complete denaturing condition (Fig. 2B Blot1, lane L). The different sizes of polypeptide bands may perhaps be due to differential glycosylation of OsGLP1 protein as observed in other cases for PsGER1 and AlGLP [3,7]. In the transgenic rice lines, the OsGLP1 protein expression has been significantly decreased compared to the untransformed plant (Fig. 2B Blot2) and the minor differences in expression level among the transgenic lines may be due to the variable degree of gene silencing.

Our study reveals that the OsGLP1 down-regulated transgenic rice lines are semi-dwarf and significantly affected by two fungal diseases, i.e. sheath blight and blast in contrast to the tall and

relatively resistant untransformed plants (Fig. 3A and B). The cell morphology is also found to be different in transgenic lines (Fig. 3C) and this may have direct correlation to the semi-dwarf phenotype (Fig. 3A). The present study documents that the OsGLP1 is a cell wall associated protein in rice and is normally localized at higher level in the sub-epidermal cells but its abundance has been drastically minimized in the transgenic rice lines (Fig. 3D). This could be one of the reasons for susceptibility of the OsGLP1 down-regulated rice lines to fungal stresses, because the sub-epidermal cells constitute the major barrier tissues for natural pest and pathogen attack. Various earlier studies have shown that different GLPs are associated with the cell walls [2,8] and possess structural role in relation to cross linking of the cell wall after pathogen attack [19]. A very recent study in rice involving RNAi-mediated gene silencing by using a conserved region from 12 members of GLP gene family has identified a QTL that confers broad-spectrum disease resistance [12].

Analyzing the data of homology modeling with barley oxalate oxidase, a putative SOD domain was predicted in OsGLP1 (Fig. 4A). The expressions of OsGLP1 in native host (rice) and heterologous host (tobacco) clearly show its SOD enzyme activity (Fig. 4B–D). Comparing the immunoblot and the SOD assay of rice and tobacco protein samples under semi-native condition, it is inferred that the OsGLP1 possesses SOD activity in its dimeric form, i.e. ~35 kDa in rice and ~33 kDa in tobacco. This is in contrast to several studies that reported SOD-active GLPs are either tetrameric or higher molecular forms as described earlier [3,10]. Unlike the over-expressed AlGLP that shows higher molecular mass in transgenic tobacco plant [7], the heterologously expressed OsGLP1 displays slightly lower electrophoretic mobility, possibly because of differential glycosylation or proteolytic cleavage. Earlier literatures demonstrated that SOD activity has direct correlation with disease resistance [19,20].

5. Conclusion

To our knowledge this is the first report to demonstrate the detailed characterization of the OsGLP1 gene which, being present as a single copy in the rice genome, is expressed predominantly in green vegetative tissues. The down-regulation of endogenous OsGLP1 in an *indica* rice cultivar and over-expression in heterologous tobacco plant has established important physiological and biochemical role of this protein. The prominent function of OsGLP1 includes maintenance of cell dimension, association with cell wall and SOD enzyme activity attributing towards regulation of plant height and disease resistance.

Acknowledgments

The authors sincerely thank Prof. S. Dey, Prof. S. K. Sen and Dr. A. Basu for their suggestions during the research work. We also thank Dr. J. K. Jha for technical help during preparation of a genetic construct used in this study. We are thankful to Dr. Ashish Bhattacharjee and Dr. Belinda Willard of Lerner Research Institute, Cleveland Clinic, Ohio, USA for protein sequencing and to the members of rDNA Technology Lab., IIT-Kharagpur for assisting in certain experiments. Technical help from Mr. Sona Dogra and Mrs. Gayatri Aditya is gratefully acknowledged. Financial assistance in the form of research fellowship to J.B. from the CSIR, Government of India, and grant supports to this laboratory from the DBT and DST, Government of India are acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.02.142.

References

[1] F. Bernier, A. Berna, Germins and germin-like proteins: plant do-all proteins. But what do they do exactly? Plant Physiol. Biochem. 39 (2001) 545–554.
[2] J.M. Dunwell, J.G. Gibbings, T. Mahmood, S.M.S. Naqvi, Germin and germin-like proteins: evolution, structure, and function, Crit. Rev. Plant Sci. 27 (2008) 342–375.
[3] S. Gucciardo, J.P. Wisniewski, N.J. Brewin, S. Bornemann, A germin-like protein with superoxide dismutase activity in pea nodules with high protein sequence identity to a putative rhicadhesin receptor, J. Exp. Bot. 58 (2007) 1161–1171.
[4] S. Khuri, F.T. Bakker, J.M. Dunwell, Phylogeny, function, and evolution of the cupins, a structurally conserved, functionally diverse superfamily of proteins, Mol. Biol. Evol. 18 (2001) 593–605.
[5] E.J. Woo, J.M. Dunwell, P.W. Goodenough, A.C. Marvier, R.W. Pickersgill, Germin is a manganese containing homohexamer with oxalate oxidase and superoxide dismutase activities, Nat. Struct. Biol. 7 (2000) 1036–1040.
[6] L. Vallelian-Bindschedler, E. Mösinger, J.P. Métraux, P. Schweizer, Structure, expression and localization of a germinlike protein in barley (*Hordeum vulgare* L.) that is insolubilized in stressed leaves, Plant Mol. Biol. 37 (1998) 297–308.
[7] T. Tabuchi, T. Kumon, T. Azuma, T. Nanmori, T. Yasuda, The expression of a germin-like protein with superoxide dismutase activity in the halophyte *Atriplex lentiformis* is differentially regulated by wounding and abscisic acid, Physiol. Plant 118 (2003) 523–531.
[8] C. Heintzen, R. Fischer, S. Melzer, K. Kappeler, K. Apel, D. Staiger, Circadian oscillations of a transcript encoding a germin-like protein that is associated with cell walls in young leaves of the long-day plant *Sinapis alba* L, Plant Physiol. 106 (1994) 905–915.
[9] B. Kukavica, Z. Vucinić, M. Vuletić, Superoxide dismutase, peroxidase, and germin-like protein activity in plasma membranes and apoplast of maize roots, Protoplasma 226 (2005) 191–197.
[10] C. Carter, R.W. Thornburg, Tobacco nectarin I. Purification and characterization as a germin-like, manganese superoxide dismutase implicated in the defense of floral reproductive tissues, J. Biol. Chem. 275 (2000) 36726–36733.
[11] C.I. Segarra, C.A. Casalongué, M.L. Pinedo, V.P. Ronchi, R.D. Conde, A germin-like protein of wheat leaf apoplast inhibits serine proteases, J. Exp. Bot. 54 (2003) 1335–1341.
[12] P.M. Manosalva, R.M. Davidson, B. Liu, X. Zhu, S.H. Hulbert, H. Leung, J.E. Leach, A germin-like protein gene family functions as a complex quantitative trait locus conferring broad-spectrum disease resistance in rice, Plant Physiol. 149 (2009) 286–296.
[13] T. Yasmin, T. Mahmood, M.Z. Hyder, S. Akbar, S.M.S. Naqvi, Cloning, sequencing and *in silico* analysis of germin-like protein gene 1 promoter from *oryza sativa* L. ssp. *indica*, Pak. J. Bot. 40 (2008) 1627–1634.
[14] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, second ed., Cold Spring Harbor Laboratory Press, New York, 1989.
[15] K.K. Kumar, S. Maruthasalam, M. Loganathan, D. Sudhakar, P. Balasubramanian, An improved *Agrobacterium*-mediated transformation protocol for recalcitrant elite *indica* rice cultivars, Plant Mol. Biol. Rep. 23 (2005) 67–73.
[16] R.B. Horsch, J.E. Fry, N.L. Hoffman, D. Eichholtz, S.G. Rogers, R.T. Fraley, A simple and general method for transferring genes into plants, Science 227 (1985) 1229–1231.
[17] J.K. Kundu, P. Briard, J.M. Hily, M. Ravelonandro, R. Scorza, Role of the 25–26 nt siRNA in the resistance of transgenic *Prunus domestica* graft inoculated with plum pox virus, Virus Genes 36 (2008) 215–220.
[18] M. Matzke, W. Aufsatz, T. Kanno, L. Daxinger, I. Papp, M.F. Mette, A.J. Matzke, Genetic analysis of RNA-mediated transcriptional gene silencing, Biochim. Biophys. Acta 1677 (2004) 129–141.
[19] A.B. Christensen, H. Thordal-Christensen, G. Zimmermann, T. Gjetting, M.F. Lyngkjaer, R. Dudler, P. Schweizer, The germinlike protein GLP4 exhibits superoxide dismutase activity and is an important component of quantitative resistance in wheat and barley, Mol. Plant Microbe Interact. 17 (2004) 109–117.
[20] G. Zimmermann, H. Bäumlein, H.P. Mock, A. Himmelbach, P. Schweizer, The multigene family encoding germin-like proteins of barley, regulation and function in basal host resistance, Plant Physiol. 142 (2006) 181–192.