Functional characterization of the rice root Germin-like protein gene-1 (*OsRGLP1*) promoter in *Nicotiana tabacum*

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Abstract

Germin (GER) and germin-like protein (GLP) genes play a very important role against various stresses. Promoter analysis provide significant insight into gene's function and regulation. Presently, upstream region (1228 bp) of the *OsRGLP1* gene was functionally characterized via heterologous expression. It was fused with the Glucuronidase (GUS) reporter gene and the expression cassette was used to transform *Nicotiana tabacum* using *Agrobacterium*-mediated transformation. Transgenic plants were examined via quantitative real-time PCR (qPCR) to analyze its role in wounding, salinity, drought, abscisic acid (ABA) and circadian rhythm. *OsRGLP1* was highly induced by ABA and drought by showing 28- and 25-fold changes in GUS mRNA level respectively as compared to wounding (fourfold change) and salinity (threefold change). However, no activity was observed in circadian rhythm. Histochemically, strong GUS activity was observed in leaf veins, midrib, epidermal hair, stomata guard cells, stem cortex, root hairs, xylem and phloem and at cellular level in cell wall, cytoplasm and its periphery. *OsRGLP1* promoter can be used to develop agronomically important transgenic plants in future food program.

Keywords Rice germin-like proteins · *OsRGLP1* · Germin · Germin-like protein · Promoter · GUS · Stress

Introduction

Germin (GER) was early identified in wheat's embryo as germination's specific marker (Thompson and Lane 1980) but later on recognized as oxalate oxidase (OXO), while proteins showing average similarity of 50% with it were considered "Germin-like proteins" (GLPs), characterized by two exons and cupin protein domain at its C-terminus (Finn et al. 2014). GERs and GLPs provide resistance against various biotic and abiotic stresses by employing oxalate oxidase (OXO), superoxide dismutase (SOD), ADP glucose pyrophosphatase/phosphodiesterase (AGPPase), polyphenol oxidase (PPO) and cysteine peptidase activities. Their potential role in various biotic, abiotic and mechanical stresses as well as in developmental, biochemical and physiological processes is well validated (Ilyas et al. 2016b), but still their function is not fully understood. However, more insight into their function can be obtained by analyzing important molecular components that control their spatiotemporal regulation.

Functional analysis of a promoter in homologous or heterologous system provides significant insight and indirect clues into its regulatory mechanism (Cartharius et al. 2005) by confirming the role of suspected *cis*-elements.

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Besides, it validates its potential ability to achieve tissue-specific expression and alongside solve the long-lasting issue of using viral origin (CaMV 35S) promoter in transgenic technology. Optimization of the expression cassette to direct predominant transgene expression in various parts of the plants relies on the availability of promoter with desired expression profiles (Peremarti et al. 2010). Various studies regarding the functional analysis of GERS and GLPs promoters with reported expression in various plant parts were elaborately discussed in review by Ilyas et al. (2016b). Previously, *BnGLP* promoter was used in the design of a complex construct to obtain seed's specific expression for enhancing the accumulation of Omega-3 long chain molecules in transgenic *Arabidopsis* seeds (Ruiz-López et al. 2012). Similarly, polyphenol oxidase (*PPO*) gene in an antisense direction was put under the control of wound-inducible promoter (*OsRLGP2*) to achieve wound-specific expression in browning-resistant potato (Mahmood et al. unpublished).

In rice, *OsRGLP2* promoter was characterized through in silico approaches by predicting numerous TFBSs for dehydration, light response and pathogenicity (Mahmood et al. 2007) and later functionally confirmed in tobacco (Mahmood et al. 2013) and potato (Munir et al. 2016). Previously, in our research group the 1228 bp upstream (5') promoter of *OsRGLP1* gene was isolated, cloned in *E. coli* and the sequence was submitted to NCBI (EU742684) (Yasmin et al. 2008). However, to gain further insight into its regulatory mechanism, the promoter was studied by analyzing its activity in transgenic tobacco. In the current study, *OsRGLP1* promoter was fused with GUS reporter gene and transformed into *N. tabacum* using *Agrobacterium*-mediated transformation. Its activity under wounding, salt, drought, ABA and light stresses were studied by analyzing GUS expression in various parts of the transgenic plant using RT-PCR.

Materials and methods

Plant material and vector construction

The tobacco variety *Nicotiana tabacum* (Sub-variety Samsun) was used as experimental material for *Agrobacterium*-mediated transformation. Full length *OsRGLP1*-GUS construct was prepared by fusing *OsRGLP1* promoter upstream (5' end) to the GUS reporter gene in p1391Z (pCAMBIA) vector which was electroporated into *Agrobacterium tumefaciens* i.e. strain EHA101. *Agrobacterium tumefaciens* (EHA101) harboring *OsRGLP1::GUS* construct was used for transformation.

Agrobacterium-mediated transformation and confirmation of integration

Leaf discs of the *N. tabacum* were agroinfiltrated (Curtis et al. 1995) and selection of the transformants was done using 50 mg/L Hygromycin [added to Murashige and Skoog (MS) media]. At first, integration of the transgene in putative transgenic *N. tabacum* was confirmed by Histochemical GUS staining (Jefferson 1989) and then with PCR. Total genomic DNA was isolated from leaves of the transgenic plants using CTAB (Cetyl tri-methyl ammonium bromide) method and PCR was carried out with *OsRGLP1* (F: 5' CCCGGGACCAACGAAAAG ATTGAA CA 3', R: 5' CCCGGGCATTGTCCATGGAGAGGAT 3') and Hygromycin-resistant gene (F: 5' GCTCCATAC AAGCCAACCAC 3', R: 5' CGAAAAGTTCGACAGCGT CTC 3') primers. Reaction mixture (25 µL) contained 1 µL (45 ng) of genomic DNA, 2.5 µL of PCR buffer (10×), 1 µL (50 pmol) each of the forward and reverse primer, 1.5 µL of MgCl₂ (25 mM), 1.5 µL of dNTPs (2 mM), 0.3 µL (1.5 U) of Taq DNA polymerase and 16.2 µL of nanopure water. The conditions for amplification consist of initial denaturation at 94 °C for 5 min; then 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min; and a final extension step at 72 °C for 20 min. Response of the *OsRGLP1* were tested in mature tobacco plant (T₀ generation) under various stresses by analyzing GUS expression using microscopy and qPCR.

Wound inducible activity

Transgenic tobacco was grown on MS media. Small pieces of the leaves, stem and root were excised from transformed plant and analyzed for GUS expression after 0, 0.5, 1, 2, 3, 4 and 5 h along with negative control. Similarly, small equal pieces of the leaves were excised from transformed plant at each experimental point and stored in liquid NO₂ for RNA isolation and qPCR analysis.

Salt stress

Transgenic tobacco was grown on MS media containing 0, 50, 150, 250 and 350 mM of NaCl. Samples were taken from each part (stem, root and leaves) at each treatment after 12, 24 and 48 h. Salt-inducible activity of the *OsRGLP1* promoter was studied by analyzing GUS expression in each part. For qPCR, 100 mg of leaf were taken from each stressed plant at each experimental point (12, 24, 48 h) and stored in liquid NO₂ for RNA isolation and qPCR analysis.

Drought assay

The assay was conducted according to the modified method of van der Weele et al. (2000). First, $\frac{1}{2}$ MS media were prepared and solidified (40 mL in each of six jars). The PEG-overlay solutions were prepared by dissolving 3, 6, 9, 12, 15 and 18 g of PEG-4000 (Applichem) in $\frac{1}{2}$ MS_(liq) (60 mL) media to make a final concentration of 0, 5%, 10%, 15%, 20%, 25% and 30%, respectively. They were poured over solid media to generate a final osmotic stress level of -0.3 , -0.6 , -0.9 , -1.2 , -1.5 and -1.8 megapascal (MPa). The jars were left for 24 h so that the PEG may properly infuse into the solid media. Next day, the liquid media were poured out and transgenic plants were grown in each jar at controlled condition (16/8 h light/dark cycles, 28 °C temperature and 50% humidity). GUS activity was monitored in leaves, stem and root after 24 h. Leaves were stored in liquid NO₂ and monitored for GUS mRNA level at each experimental point.

ABA stress

Transgenic plants were grown on MS media for 1 month at controlled conditions (mentioned in the above section). Fully mature plants at five leaflets stages were sprayed with 0, 50, 150, 250, 350 or 450 μ M of ABA solutions (10 mL) in a completely sterilized environment. Different parts (leaves shoot and roots) of the plant were obtained at each treatment and analyzed for GUS activity after 24 and 48 h. Leaves were stored in liquid NO₂ and GUS mRNA level was analyzed at each experimental point.

Circadian rhythm (dark/light assay)

The assay was conducted according to the modified method of Sassaki et al. (2014). Transgenic plants were grown on MS media at controlled condition for 30 days. At the end of light cycle, the plants were shifted into dark room for 48 h following their exposure to continuous light. Samples from leaves, stem and root were taken after 0, 1, 6 and 24 h post-exposure period and assayed for GUS expression. Three independent transgenic lines were used, and the experiment was randomized using two replicates.

Microscopy

Microscopic study of the leaves, stem and root in wounding, drought, salt, ABA and light stresses was conducted using LABOMED and OPTIKA (10 $\times$, 100 $\times$ and 200 $\times$) microscopes. Cross and longitudinal sections of each part was observed under high magnification to find

physiologically active region by analyzing GUS expression in each stress.

Real-time PCR

Activity of the *OsRGLP1* promoter was assessed in each stress by examining GUS transcript level using qPCR. Leaves taken from transformed tobacco at every experimental point in each stress, stored in liquid NO₂ were used for RNA extraction. Unstressed transgenic plants were used as a control and each experimental trial was repeated two times.

RNA extraction and cDNA synthesis: Leaves were grounded in pestle and mortar in the presence of liquid NO₂. Total RNA was extracted (100 mg of tissue) with RNA isolation kit (GeneJET plant RNA purification kit, ThermoScientific). RNA samples were purified from contaminated DNA after Turbo DNA-free DNase treatment. Quality and quantity was confirmed both by running on 1.5% agarose gel and using Nanodrop 2000. cDNA was synthesized using 2 μ l of purified RNA per 20 μ l of total reaction mixture which contained 2 μ l of reaction buffer (5 $\times$), 4 μ l of MgCl₂ (25 mM), 2 μ l of dNTP mix (10 mM), 0.5 μ l of RiboLock RNase inhibitor (20 U), 0.6 μ l of RevertAid Reverse Transcriptase (15 U) (Fermentas) and 1 μ l of oligo (dT)₁₈ primer (e-oligos) 25 pM (picomole) and 7.9 μ l of nuclease-free water. The mixture was incubated at 42 °C for 1 h followed by 70 °C for 5 min and finally at 5 °C for 10 min. The product was stored at -20 °C till further use.

Quantitative real-time PCR (qRT-PCR): qPCR was performed in 28 well reaction plate using LINEGENE system (BIOER technology). Total volume of the reaction mixture was 12 μ l which contained 2 μ l of cDNA, 1 μ l of primers (forward and reverse), 6 μ l of SYBR-Green Master mix and 3 μ l of H₂O_(DI). Reactions were carried out using a specific set of primers for a reporter (GUS [F: 5' CGGCAGAGA AGGTACTGGAA 3', R: 5' ATATCCAGCCATGCACAC TG 3']) and house-keeping genes (Actin [F: 5'GGAATC CACGAGACTACA 3', R: 5' TGAGGGAAGCCAAGATAG 3']). Condition comprised of pre-denaturation at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 1 min and extension at 72 °C for 40 s. The data were analysed in terms of *OsRGLP1* promoter activity regarding GUS gene expression. Relative quantification of the data was performed through 2 ^{$-\Delta\Delta C_t$} method using LineGeneK (Version 4) software. There was a selection of an internal control and calibrator for each treatment. The final data were retrieved in the form of excel sheet and presented in a graphical form.

Statistical analysis

All the assays were repeated at least three times. The PROC GLM procedure was used to study the analysis of variance

(ANOVA) using SAS 9.4. The means within each treatment were compared using the least significant difference (LSD) test with a threshold probability of $P < 0.01$.

Results

Transformation and regeneration of transgenics

Agrobacterium-mediated transformation was used to transfer *OsRGLP1*-GUS fusion fragment into *N. tabacum* (Fig. 1). After co-cultivation, putative transformed leaf disks developed calli on the cut edges of the explant after 15–20 days on Hygromycin selection media. Adventitious shoots emerged after 3–5 weeks which were rooted on MS media and later transferred to soil and maintained in green house.

Molecular analysis

To verify the insertion of *OsRGLP1*-GUS fragment in putative transgenic plant; PCR was carried out with *OsRGLP1*- and Hygromycin resistant gene- specific primers (forward and reverse) resulting in the amplification of high-quality fragments of 1228 and 700 bp, respectively. A total of five independent transgenic lines (L1–L5) were identified through PCR among which a single line (L4) was further multiplied to obtain T_0 Plants. These T_0 plants were exposed to various stresses to analyze *OsRGLP1* regulated GUS expression through qPCR.

Wound inducibility

Morphologically, leaves, stem and root of the transformed plant showed no GUS activity at 0.5 h but maximum was observed after 4 and 5 h, mostly at the margins where the

cuts/mechanical injuries were produced. Stem and leaves exhibited a constant increase in GUS activity with time, while root exhibited uniform expression throughout its surface, especially at 3, 4 and 5 h, irrespective of the injuries. qPCR revealed no significant increase in GUS transcript level of leaves after 0.5 h, but almost twofold change was noted after 1 and 2 h which increased to fourfold after 4 and 5 h (Fig. 2a).

Salt assay

Visual observation revealed that GUS expression increased directly with NaCl concentration (mM) and time in all plants parts. Highest activity was observed in leaves as compared to shoot and root with prominent expression in the middle of the leaves, around the midrib or sometime on the whole surface. RT-PCR analysis revealed lowest activity at 50 mM, maximum at 250 which reduced at 350 mM as per concentration. However, as per time, no significant increase in GUS transcript level was observed after 12 h. But, highest expression was observed after 24 h (threefold change at 250 mM) which decreased at 48 h (Fig. 2b).

Drought stress

Visually, highest GUS activity was observed in root followed by leaves (tips and gradually spread throughout the leaf surface as the amount of PEG increased) and lowest in stem. Minimum activity was noted at 5% PEG while maximum at 30%. In qPCR analysis of leaves, lowest GUS expression was observed at 5% and highest at 30% by showing 2- and 25-fold change, respectively (Fig. 2c).

Fig. 1 Schematic diagram of the *OsRGLP1*-GUS construct showing GUS gene under the control of rice root Germin-like protein gene-1 (*OsRGLP1*) promoter. The construct was made using p1391Z vector (size 11.2 kb). Each segment is labelled and represented with a

distinct color. The maps were built with Illustrator for Biological Sequences (IBS 1.0) software. **a** Construct without insert (*OsRGLP1*), **b** construct with promoter inserted

Fig. 2 Relative expression of GUS gene under the control of *Oryza sativa* root Germin-like protein gene-1 promoter (*OsRGLP1*) in transgenic *Nicotiana tabacum* var. Samsun using qPCR in response to various stresses. **a** Leaves of the transgenic tobacco were pinched with forceps and its mRNA was quantified after 1/2, 1, 2, 3, 4 and 5 h post-wounding period using qPCR. **b** For salt stress, transgenic plants were grown on Murashige and Skoog (MS) media supplied with 50, 150, 250 and 350 mM of NaCl. Leaves were taken after 12, 24 and 48 h and analyzed for GUS mRNA level using qPCR. **c** For drought stress, transgenic plants were grown on MS media supplied with 5,

10, 15, 20, 25 and 30% polyethylene glycol (PEG-4000). Leaves were analyzed for GUS mRNA level after 24 h using qPCR. **d** For abscisic acid (ABA) stress, transgenic plants were sprayed with 50, 150, 250, 350 and 450 μ M of ABA solutions. Leaves were analyzed for GUS mRNA level after 24 and 48 h using qPCR. In all these stresses, untreated transgenic plants were used as a control and it has given a value of “1”. The data are the means $\pm$ SE of three replicates ($n=3$). The bars on graphs within each treatment indicate significant differences at $P<0.01$

Abscisic acid (ABA) stress

GUS expression was observed in root, shoot and relatively higher in leaves which increased with ABA level and time by showing lowest activity at 5 μ M of ABA while highest at 450 μ M after 24 h. RT-PCR data of leaves showed significant GUS activity at 450 μ M after both 24 and 48 h by showing 5.57- and 28-fold changes, respectively (Fig. 2d).

Circadian rhythm (light/dark assay)

In the present study, dark/light assay did not induce *OsRGLP1* promoter activity by not showing any prominent GUS expression at 0, 1, 6, 24 and 24 h post-treatment period.

Microscopy

Microscopically, some parts of the plants were found highly active in all stresses, while others showed variable expression. In leaves, veins, interveinal area stomata guard's cells, midrib and its central part, epidermis,

epidermal hair (tips), root hair showed maximum GUS activity in all stresses. In wounding, the activity was mostly wound-specific also found in petiole, petiole stem junction. In stem, highest activity was observed in epidermis (except in drought stress), staminal hairs, hydathodes, xylem and phloem. However, cortex showed the highest activity in wounding and drought, weak in salt but not in ABA. Similarly, no activity was noted in endodermis, pericycles and pith cells in all stresses except in ABA, where pith cells showed weak activity. Root showed uniform expression in all stresses with maximum expression in epidermis, xylem, phloem, root hair (tip) while cortex showed maximum activity in case of drought, reduced in wounding and salt but not in ABA. Similarly, pith showed highest activity in wounding and drought but reduced in salt and ABA stresses. Cytologically, leaf showed highest expression in cytoplasm while stem cells showed highest activity in cell wall, with granular cytoplasmic expression in case of salt stress. However, root cells showed enhanced cytoplasmic expression in wounding (mostly in cell wall, cytoplasm and its periphery) and drought but reduced in salt and ABA stresses (Fig. 3).

Fig. 3 Histochemical detection of GUS activity in various parts of the transgenic *Nicotiana tabacum*. The plants parts were stained in GUS buffer overnight at 37 °C. Cross and longitudinal sections (CS and LS) of each part was observed under high magnification (10, 100 and 200×) to find physiologically active region by analyzing GUS expression in various tissues using LABOMED and OPTIKA microscopes. **a** GUS expression at the margins of the excised leaf piece, **b** GUS expression at the edges of cut stem, **c** LS of the root which showed

uniform expression particularly at the central cylinder, **d** GUS expression at the surface of the leaf where veins and central midrib showed prominent expression, **e** CS of stem where xylem and phloem showed prominent expression, **f** GUS expression in epidermis and cortex of stem in which at cellular level prominent expression was observed in cell wall, **g** GUS expression in root hair, **h** GUS expression in stomata. These microscopic parts with enhanced GUS expression were found active in all stresses

Discussion

Functional analysis

Wound inducibility

Wound inducibility is prevalent among GERs and GLPs particularly in GER1 subfamily due to their critical role in pathogenicity (Manosalva et al. 2009; Davidson et al. 2009). Previously, various GERs and GLPs genes showed wound inducibility (Ilyas et al. 2016b) with very few studies related to their promoters. *OsRGLP2* promoter showed such property in tobacco (Mahmood et al. 2013) and potato (Munir et al. 2016) and the same pattern was observed for *OsRGLP1* which may be due to the presence of wounding response elements. Multiple W-box were found at −555, −561, −568 positions in *OsRGLP1* which play an important role in wounding (Yasmin et al. 2008) as previously shown in PAMPs trigger immunity of GER4c promoters (Himmelbach et al. 2010). GUS activity was noted after 30 min which is higher than the response time of *OsRGLP2* (10 min) (Mahmood et al. 2013) but lower than *AIGLP*

and *TaOXO* (1 h) (Tabuchi et al. 2003), *G-OXO* (2 h) (Le Deunff et al. 2004) and *CaGLP1* (6 h) (Park et al. 2004). Such property showed the potential ability of *OsRGLP1* promoter in deriving gene expression during protection against pest and pathogen attacks. Wounding up-regulate the secondary metabolic pathways involved in the activation of defense responses during which MYB elements regulate the expression of flavonoid genes (Cheong et al. 2002). It is an agreement to TFBSs analysis, in which MYB was the most frequent element (Ilyas et al. 2016a). Histochemically, its induction pattern is comparable to *OsRGLP2*, *GERB* and *GERF* promoters (Federico et al. 2006; Mahmood et al. 2013). Leaf vein, midribs and stomata guard cells were most active showing its role in resource allocation and gaseous exchange during wounding (Sasaki et al. 2014; Yang et al. 2013). In root, xylem and phloem were most active, which beside its role in transportation also act as a strategic checkpoint for resistance against pests and pathogens (Turgeon and Wolf 2009). At cellular level, uniform GUS expression in the cytoplasm of leaf cells suggest its role in various developmental processes. However, in stem and root cell wall specific expression suggested its role in plant defense,

which is similar to *Ger4a* to *Ger4h* (Zimmermann et al. 2006), *AhGLPs* (Wang et al. 2013) and *CaGLP* (Park et al. 2004) expression, signifying that strengthening cell wall is the prominent resistant mechanism against biotic and biotic stresses.

Salt assay

Induction of *OsRGLP1* (particularly in leaves and roots) is comparable to *OsRGLP2* (Mahmood et al. 2013) representing similarity in their *cis*-elements. *OsRGLP1* was highly induced by 250 mM of NaCl after 24 h showing threefold change followed by reduction at 350 mM (1.25-fold change) which is comparable to the expression of *SoGLP* showing 2.25-fold change at 150 mM (Bai et al. 2014) but contradictory to the study of Tabuchi et al. (2003) and Park et al. (2004) where no response was observed against salinity. *GmGER9* and *AhGLPs* (*AhGLP2* and *AhGLP3*) showed maximum expression at 150 and 50 mM but reduced at 350 and 100 mM after 12 and 24 h, respectively (Lu et al. 2011; Wang et al. 2013). *OsRGLP1* showed decreased activity at 12 and 48 h which is similar to the results of *GhMPK6a* promoter which increased at 48 and decreased at 72 h in salinity (Li et al. 2013). Similarly, *CsPPO* promoter showed no induction at 8, maximum at 48 but reduced at 72 h in wounding. Such variation in promoter activity may be contributed to the fact that *OsRGLP1* is time and concentration dependent leading to the expression of GUS gene under a certain time and concentration. Such properties are largely governed by specific *cis*-regulatory elements that control its activity among which YTBP (19 copies) is the most important by helping in the binding of transcriptional machinery. *OsRGLP1* also contained numerous copies of MYBS (10), MYBL (13), NAC (10) and DREB (2) elements which regulate promoter activity during salt stress (Ilyas et al. unpublished). Histochemically, *OsRGLP1* was highly active in various parts that control transportation (vein, midrib, xylem, phloem) and the inflow of molecules [stomata, epidermal hair (tips, pits), root hair] to plant during salinity. It may be due the strong role of vascular bundle in transport of water and food, during which epidermal and root hair control the inflow of water and other molecules (Turgeon and Wolf 2009). At cellular level, stem showed low and granular activity as compared to leaves and root's uniform expression, which may seem organelle-specific expression of *OsRGLP1* as previously noted for *AtGLP4* (Golgi bodies) (Yin et al. 2009).

Drought stress

Comparatively highest GUS expression in roots and leaves may be due to the root- and leaves-specific behaviour of *OsRGLP1* during drought. In leaves, maximum activity

was observed at 25 and 30% PEG solutions by showing 15- and 25-fold changes respectively in GUS transcript level after 24 h, which is higher than the value of *SoGLP* which showed threefold change at 10% PEG after 6 h (Bai et al. 2014) but contradictory to the results of *AtGLPs* (Bray 2004) and *AlGLP* (Tabuchi et al. 2003), where no expression was reported. Such observation confirmed the effectiveness of NAC, MYB and DREB *cis*-elements in *OsRGLP1* that are important against drought stress. In root and stem, its expression is like *OsRGLP2* which can be justified by their similar origin through duplication, but in leaves it showed clear expression in comparison to diffused expression of *OsRGLP2*, which may be due to variations in their regulatory elements. Histochemically, the highest activity of *OsRGLP1* in veins, stomata guard's cells, xylem, phloem epidermal and root hair confirmed their significant role in drought tolerance, suggesting that it control and regulate the inflow of various molecules during drought stress.

ABA stress

A number of studies reported GLPs responses to various signalling molecules (Ilyas et al. 2016b), but few dealt with ABA. Increased GUS expression with increase in ABA and time, suggested *OsRGLP1*'s role in drought and salinity because ABA transduce signals under these stresses (Thaler and Bostock 2004). GUS expression was observed in root, shoot and relatively higher in leaves, which is similar to *OsRGLP2* (Mahmood et al. 2013). Relative quantification analysis, showed that ABA serves as enhancer of *OsRGLP1* activity. The data revealed significant promoter activity at two experimental points (24 and 48 h) which clearly indicate the presence of certain ABA responsive elements in *OsRGLP1*. Previously, no such comprehensive study has been conducted regarding GLP promoter induction under ABA stress, except the study of Tabuchi et al. (2003), where no significant increase in the transcript level of *AlGLP* was observed after 24 h by exposing transgenic tobacco to 50 μ M of ABA solution. Histochemically, strong GUS activity in leaves indicate *OsRGLP1* role in material transport and gaseous exchange under ABA stress. Plants synthesize ABA during stress, which initiates defense mechanisms and regulate stomata to control pathogens (Lim et al. 2014). Strong expression in the vascular bundle (xylem), staminal hairs and pith of the stem and central cylinder, root tip, root's hair and epidermis of root suggested its role in resource allocation and in controlling of the inflow of various molecules in plant under ABA stress. At cellular level, leaves showed cytoplasmic specific expression, similar to *OsRGLP2*, but stem showed cell wall localized expression (Wang et al. 2013). Generally, in wound-inducible promoters certain upstream *cis*-elements form an intricate regulatory system by acting in response to numerous defensive signaling

molecule. The present results clearly indicate the presence of such elements in *OsRGLP1* promoter.

Conclusion

Promoters with desired expression profile are critical to crop biotechnology application. The study revealed that *OsRGLP1* is a useful promoter capable of deriving gene expression under wounding, salt, drought and ABA stresses which make it a valuable tool to direct predominant transgene expression in various parts of the plants. The study will pave the way for production of agronomically important transgenic crops against various stresses.

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Author contributions MI and TM conducted experiments while SR, WA and SMSN helped in manuscript writing.

Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest in the publication.

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