Germin like protein genes exhibit modular expression during salt and drought stress in elite rice cultivars

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Abstract

Background Germin-like proteins (GLPs) are ubiquitous plant proteins, which play significant role in plant responses against various abiotic stresses. However, the potential functions of GLPs in rice (Oryza sativa) against salt and drought stress are still unclear.

Methods and results In this study, transcriptional variation of eight OsGLP genes (OsGLP3-6, OsGLP4-1, OsGLP8-4, OsGLP8-7, OsGLP8-10, OsGLP8-11 and OsGLP8-12) was analyzed in leaves and roots of two economically important Indica rice cultivars, KS282 and Super Basmati, under salt and drought stress at early seedling stage. The relative expression analysis from qRT-PCR indicated the highest increase in expression of OsGLP3-6 in leaves and roots of both rice varieties with a significantly higher expression in KS282. Moreover, relative change in expression of OsGLP8-7, OsGLP8-10 and OsGLP8-11 under salt stress and OsGLP8-7 under drought stress was also commonly higher in leaves and roots of KS282 as compared to Super Basmati. Whereas, OsGLP3-7 and OsGLP8-12 after salt stress and OsGLP8-4 and OsGLP8-12 after drought stress were observed with higher relative expression in roots of Super Basmati than KS282. Importantly, the OsGLP3-6 and OsGLP4-1 from chromosome 3 and 4 respectively showed higher expression in leaves whereas most of the OsGLP genes from chromosome 8 exhibited higher expression in roots.

Conclusion Overall, as a result of this comparative analysis, OsGLP genes showed both general and specific expression profiles depending upon a specific rice variety, stress condition as well as tissue type. These results will increase our understanding of role of OsGLP genes in rice crop and provide useful information for the further in-depth research on their regulatory mechanisms in response to these stress conditions.

Keywords Oryza sativa · Germin-like proteins · Expression profiling · Abiotic stress

Introduction

Germin-like proteins (GLPs), member of cupin superfamily, have been found to be associated with various abiotic stresses in different plant species [1–6]. Previous studies have indicated that GLPs possess several enzymatic functions such as oxalate oxidase (OXO) and super oxide dismutase (SOD) and play a crucial role in cell wall reinforcement by cross linking of cell wall components under stress conditions [6–8]. Upon exposure to salt and drought stress in plants, GLP genes have been reported to exhibit stress and tissue-specific modulated expression providing evidence for their role in defense against these two stress conditions. Differential and tissue-specific expression of GLP genes has been reported in Arabidopsis and barley depending upon a particular abiotic stress type including osmotic and salt stress [9, 10]. Another study reported the accumulation of germin like protein under drought stress in leaves of Boea hygrometrica [11, 12]. Additionally, GLP genes have also been shown to exhibit cultivar-specific differences in their expression between stress tolerant and stress sensitive
cultivars. For example, transcriptional study of a spinach GLP (SoGLP) showed that the expression of SoGLP was relatively higher in salt resistant cultivar as compared to sensitive one [13]. Moreover, three GLPs depicted difference in their accumulation among the roots of tolerant and sensitive wheat varieties after exposure to drought stress [14].

Rice (Oryza sativa), a model monocot plant, is particularly affected by the salinity and drought stress which impede the growth and development of plants ultimately reducing the agricultural yield [15]. Both of these stress factors affect the developmental process of plants by osmotic shock and subsequent oxidative stress, however, salt stress has an added deleterious impact of ionic toxicity and nutrient imbalance [16]. Moreover, the effect of salt and drought stress in rice is greatly dependent on the growth period: early seedling stage of rice is potentially among the most sensitive stages for the imposition of both stress conditions [17–19]. Considering this aspect, the analysis of expression dynamics of stress responsive GLP genes in rice (OsGLP) at early seedling stage can be useful for screening the stress tolerance of different rice varieties.

Till now, 43 OsGLP genes have been reported in rice with prominent localization of their product in the extracellular region particularly in the cell wall. Although these OsGLP genes are found to be located on different rice chromosomes including chromosome 1, 2, 3, 4, 5, 8, 9, 11 and 12, the highest number of OsGLP genes was identified in chromosome 3 and 8 in the form of small clusters [9, 20]. The transcriptomic data from seedlings of japonica rice cultivar, Nipponbare revealed significant regulation of OsGLP4-1 and some OsGLP genes from chromosome 8 in response to salt and drought stress and reported their expression in cell wall with SOD activity [9]. In silico assessment in Japonica rice cultivar suggested that OsGLP genes from chromosome 3 and 8 are functionally more linked as compared to other OsGLPs by regulating in a combinatorial manner to deal with various abiotic stress conditions [21]. Although such studies indicated the salt and drought stress responsive nature of OsGLPs, however, not many reports are available on the comparative expression analysis of OsGLPs in multiple rice varieties under these stresses. For example, in shoot tissues of Japonica rice, the expression of a GLP gene (GLP4) was reported to be increased in tolerant variety but decreased in sensitive variety “M103” [22]. Taken together, scattered information on the functional characterization of OsGLPs mainly in Japonica sub-species of rice is available, but a comparison of differential regulation of OsGLP genes in two different Indica rice cultivars under salt and drought stress has never been performed. This lack of comparative studies is one of major obstacles in understanding the dynamic role and complex regulation of OsGLP genes underlying salinity and drought stress adaptation in rice cultivars.

We performed transcriptomic analysis to explore expressional variation of eight OsGLP genes (OsGLP3-6, OsGLP3-6, OsGLP4-1, OsGLP8-4, OsGLP8-7, OsGLP8-10, OsGLP8-11 and OsGLP8-12) from chromosome 3, 4 and 8 between two economically important Indica rice salt tolerant KS282 and sensitive Super Basmati cultivars under salt and drought stress. The main objectives of this study were to compare the differential expression pattern of OsGLP genes in leaves and roots of both cultivars under salt and drought stress, as well as to identify OsGLP genes showing specific and general response with respect to a particular rice cultivar and stress type at early seedling stage of rice. Our analysis identified potential candidate OsGLP genes associated with salt and drought stress tolerance eventually providing functional basis for developing stress resistant rice cultivars. We have found a few common OsGLP genes expressed during both the stresses indicating a communal regulatory mechanism in the identified genes.

Material and methods

Plant material, stress treatments and sample collection

Seeds of two rice cultivars KS282 and Super Basmati, classified as tolerant and sensitive specifically to salt stress respectively [23], were de husked and surface sterilized using 70% ethanol for 2 min then washed with autoclaved Milli-Q water (5 washes 1 min each), followed by 15 min shaking in 3.5% sodium hypochlorite and tween-20, another 5 min shaking in 3.5% sodium hypochlorite and then washed with autoclaved Milli-Q water (5 washes 2 min each).

For germination purpose, seeds were placed on Murashige and Skoog (MS) media in test tubes under aseptic conditions and then kept in a growth room at 25 °C ± 1 with 16 h photoperiod. After two weeks, seedlings were randomly divided into three groups for control, salinity and drought conditions (three replicates in each group with 15 plants per replicate). Salt and drought stress treatments were applied to 14 days old seedlings for 24 h. For salt stress, seedlings were subjected to 200 mM NaCl solution and drought stress was applied by placing seedlings on dried filter paper in aseptic conditions [9]. For each group (control, salinity and drought), leaves and roots from 45 plants were harvested in three biological replicates (15 plants for each replicate). Collected samples were frozen in liquid nitrogen and stored at – 80 °C for RNA extraction.

RNA isolation and cDNA synthesis

Total RNA was extracted from control and treated leaves and roots of KS282 and Super Basmati using RNeasy Plant Mini
Kit (Qiagen, Hilden, Germany) according to manufacturer’s instructions. RNA concentration and quality were analyzed using NanoDrop™ 1000 Spectrophotometer (Thermo Scientific) and gel electrophoresis (1% agarose gel). First strand cDNA was synthesized from 1 μg of total RNA using RevertAid First Strand cDNA Synthesis Kit (Thermo scientific, Lithuania) according to manufacturer’s protocols and stored at −20 °C.

**Primer designing and gene expression profiling**

To analyze the differential expression of eight *OsGLP* genes (*OsGLP3-6*, *OsGLP3-7*, *OsGLP4-1*, *OsGLP8-4*, *OsGLP8-7*, *OsGLP8-10*, *OsGLP8-11* and *OsGLP8-12*), qRT-PCR was conducted with cDNA from control and treated samples of leaves and roots of both varieties. For this purpose, all primers specific for selected eight rice *OsGLP* genes were designed using primer3 software (Table S1) and were subjected to BLAST to evaluate their specificity. Actin was selected as internal control in order to normalize the variance among samples.

The qRT-PCR was performed on a CFX96 Real-Time PCR System (Bio-Rad) with QuantiFast SYBR Green PCR kit (Qiagen, Germany) in a 25 μL reaction mixture, containing 1 μL sample cDNA, 12.5 μL SYBR Green Master mixture, 2.5 μL each specific primer and 6.5 μL nuclease-free water. The program for qRT-PCR included 95 °C for 5 min and 40 cycles at 95 °C for 10 s and 60 °C for 30 s. Three independent experiments were performed in duplicate for all real-time PCR reactions and relative quantification of gene expression for each target gene was calculated using 2^−ΔΔCT and 2^−ΔCT method [24, 25].

**Statistical analysis**

The statistical analysis of data was performed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California USA). The statistical significance was determined by two-way ANOVA test and p < 0.05 was considered as a significant difference.

**Results**

**Expression profiling of OsGLP genes in leaves**

To examine the effect of salt and drought stress on the transcriptomic abundance of *OsGLP* genes in leaves of two rice varieties (KS282 and Super Basmati), the relative expression pattern of eight *OsGLP* genes (*OsGLP3-6*, *OsGLP3-7*, *OsGLP4-1*, *OsGLP8-4*, *OsGLP8-7*, *OsGLP8-10*, *OsGLP8-11* and *OsGLP8-12*) was determined by RT-qPCR. Taking the two-way interaction of abiotic stress treatments and rice varieties into account, statistical analysis identified five *OsGLP* genes (*OsGLP3-6, OsGLP4-1, OsGLP8-7, OsGLP8-10* and *OsGLP8-11*) in KS282 and two *OsGLP* genes (*OsGLP3-6 and OsGLP4-1*) in Super Basmati that were differentially expressed by nearly 2-folds or more than 2-folds in response to at least one of the stress treatments. Among these *OsGLP* genes, *OsGLP3-6* was observed with highest up-regulation under salt and drought stress in both rice varieties. Apart from this, some of the *OsGLP* genes exhibited significantly different relative expression in response to both stress treatments however, the relative expression of some *OsGLP* genes was same under both stresses depending on a particular rice cultivar (Fig. 1).

In KS282 leaves, compared to the untreated control, up-regulation of five *OsGLP* genes (*OsGLP3-6, OsGLP4-1, OsGLP8-7, OsGLP8-10* and *OsGLP8-11*) under salt stress and four *OsGLP* genes (*OsGLP3-6, OsGLP4-1, OsGLP8-7* and *OsGLP8-11*) under drought stress was observed. However, relative expression of four *OsGLP* genes (*OsGLP3-6, OsGLP4-1, OsGLP8-10* and *OsGLP8-11*) under salt stress and three *OsGLP* genes (*OsGLP3-6, OsGLP4-1* and *OsGLP8-11*) under drought stress was up-regulated in leaf.
samples of Super Basmati. Further, OsGLP3-7, OsGLP8-4 and OsGLP8-12 showed similar expression patterns by being down-regulated in both varieties in response to at least one of the stress treatments. Expression based hierarchal clustering showed that OsGLP3-6 was clustered in a separate group while all other OsGLP genes were clustered in one group, indicating their differential regulation (Fig. 1). Additionally, overlap analysis demonstrated that, in leaves of both rice varieties, changes in the expression pattern of OsGLP genes under salt and drought stress are both general and specific to a particular variety as well as to a particular stress (Fig. 2a).

Comparison of differential expression of OsGLP genes in leaves of KS282 and Super Basmati under salt and drought stress

Among the up-regulated OsGLP genes in leaves under salt and drought stress, relative expression of OsGLP3-6, which showed highest up-regulation in both rice varieties, was significantly higher in KS282 in comparison with Super Basmati. Overall, relative change in expression of five OsGLP genes (OsGLP3-6, OsGLP4-1, OsGLP8-7 OsGLP8-10 and OsGLP8-11) under salt stress and three OsGLP genes (OsGLP3-6, OsGLP4-1 and OsGLP8-7) under drought stress was significantly higher in KS282 than Super Basmati (Fig. 2b, c).

Expression profiling of OsGLP genes in roots

A comparison of differential expression profiles of eight OsGLP genes (OsGLP3-6, OsGLP3-7, OsGLP4-1, OsGLP8-4, OsGLP8-7, OsGLP8-10, OsGLP8-11 and OsGLP8-12) was conducted in root samples of KS282 and Super Basmati under salt and drought stress. Statistical analysis revealed that the expression of seven OsGLP genes (OsGLP3-6, OsGLP3-7, OsGLP4-1, OsGLP8-4, OsGLP8-7, OsGLP8-10 and OsGLP8-11) in KS282 and five OsGLP genes (OsGLP3-6, OsGLP3-7, OsGLP4-1, OsGLP8-4 and OsGLP8-12) in Super Basmati was increased by nearly 2-folds or more than 2-folds after exposure to at least one of the stress conditions. As in leaves, OsGLP3-6 was observed with highest increase in its expression under salt and drought stress in roots of both rice varieties. Moreover, some OsGLP genes exhibited similar expression pattern in KS282 and Super Basmati depending on a particular stress type. In this aspect, the expression of six OsGLP genes (OsGLP3-6, OsGLP3-7, OsGLP4-1, OsGLP8-7, OsGLP8-10 and OsGLP8-11) under salt stress and four OsGLP genes (OsGLP3-6, OsGLP4-1, OsGLP8-4 and OsGLP8-11) under drought stress was commonly up-regulated in both rice varieties indicating that salt stress positively induced the expression of more OsGLP genes in comparison to drought stress. Further, a significant decrease in expression of OsGLP8-10 was also observed in both cultivars after exposure to drought stress. However, expression of OsGLP8-12 was significantly up-regulated in Super Basmati after exposure to both stress treatments while its expression in KS282 was decreased after drought stress and nearly unchanged after salt stress (Fig. 3).

As a result of expression based hierarchal clustering in root samples, OsGLP genes were clustered in two groups. Similar to leaves, OsGLP3-6 was observed to make a separate group as compared to other OsGLP genes (OsGLP3-7, OsGLP4-1, OsGLP8-4, OsGLP8-7, OsGLP8-10, OsGLP8-11 and OsGLP8-12) (Fig. 3). In addition to this, comparison of regulation pattern of OsGLP genes by overlap analysis suggested that, while not all OsGLP genes, some OsGLP genes were differentially regulated in both rice cultivars after exposure to different stress conditions (Fig. 4a).

Comparison of differential expression of OsGLP genes in roots of KS282 and Super Basmati under salt and drought stress

Although most of the OsGLP genes showed up-regulation in roots of both rice varieties in response to either salt or drought stress, the relative change in the expression of several OsGLP genes was significantly higher in one variety than the other. For example, the expression of four OsGLP genes (OsGLP3-6, OsGLP8-7, OsGLP8-10 and OsGLP8-11) under salt stress and two OsGLP genes (OsGLP3-6 and OsGLP8-7) under drought stress was significantly higher in KS282 as compared to Super Basmati. Whereas, in comparison with KS282, two OsGLP genes (OsGLP3-7 and OsGLP8-12) after salt stress and two OsGLP genes (OsGLP8-4 and OsGLP8-12) after drought stress were observed with higher expression in Super Basmati (Fig. 4b, c).

Comparative analysis of tissue specific expression pattern of OsGLP genes under control and stress conditions

To get insight into the tissue specific role of OsGLP genes, the expression level of OsGLP genes was analyzed in leaves and roots of both varieties under control and stress (salt and drought) conditions. In general, results indicated same expression pattern of OsGLP genes in both rice varieties, however, the expression level of several OsGLP genes was different in leaves and roots (Fig. 5). For example, in both varieties, three OsGLP genes from chromosome number 8 including OsGLP8-4, OsGLP8-7 and OsGLP8-11 were observed with higher expression in roots in comparison with leaves. On the contrary, OsGLP3-6 and OsGLP4-1 were expressed at higher level in leaves as compared to roots. It is noteworthy that OsGLP8-12 which exhibited almost
same expression pattern in both tissues was observed with higher expression level in Super Basmati roots as compared to leaves under salt stress. Among all OsGLP genes, OsGLP8-4 was most prominent with highest level of expression in roots followed by OsGLP8-11 whereas OsGLP3-6 and

Fig. 2 a Venn diagram demonstrating the expression pattern of OsGLP genes regulated by drought and salt stress in leaves of KS282 and Super Basmati. Expression profiles of OsGLP genes (in leaves) regulated commonly regarding particular stress type (drought and salt) and particular rice variety (KS282 and Super Basmati). Red, Up-regulation; Green, Down-regulation; Blue, No change in expression b Salt stress induced comparative expression of OsGLP genes in leaves of KS282 and Super Basmati. Comparison of relative expression (Fold Change) of OsGLPs (OsGLP3-6, OsGLP3-7, OsGLP4-1, OsGLP8-4, OsGLP8-7, OsGLP8-10, OsGLP8-11, and OsGLP8-12) was performed between KS282 leaves and Super Basmati leaves under salt stress. Values indicate mean ± Standard Error Mean (SEM). Statistical analysis was performed with two-way ANOVA (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001) c Drought stress induced comparative expression of OsGLP genes in leaves of KS282 and Super Basmati. Comparison of Relative expression (Fold Change) of OsGLPs (OsGLP3-6, OsGLP3-7, OsGLP4-1, OsGLP8-4, OsGLP8-7, OsGLP8-10, OsGLP8-11, and OsGLP8-12) was performed between KS282 leaves and Super Basmati leaves under drought stress. Values indicate mean ± Standard Error Mean (SEM). Statistical analysis was performed with two-way ANOVA (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). (Color figure online)
OsGLP4-1 were observed with highest levels in leaves of both rice varieties.

Discussion

Considering the functional characterization and expression analysis of GLP genes in rice, previous studies have mainly focused on Oryza sativa Japonica as compared to other subspecies (Indica and Javanica) of rice crop [9, 20, 21]. Moreover, not many studies have compared the genomic responses of OsGLP genes across tolerant and sensitive rice varieties under abiotic stresses mainly including salt and drought stress. The current study was designed to gain insight into the transcriptomic abundance of OsGLP genes in two different but economically important Indica rice cultivars (KS282 and Super Basmati) under salt and drought stress. The leaves and root specific expression dynamics of OsGLP genes was determined in both cultivars under control and stress conditions at early seedling stage.

The transcriptomic profiles indicated that several OsGLP genes were differentially expressed in leaves and roots following same expression pattern in both varieties under control condition. Expression level of OsGLP3-6 and OsGLP4-1 was higher in leaves as compared to roots whereas the expression of OsGLP8-4, OsGLP8-7 and OsGLP8-11 was higher in roots as compared to leaves, indicating their tissue specific roles. Conversely, OsGLP3-7, OsGLP8-10 and OsGLP8-12 were observed with nearly same expression in both tissues. Interestingly, in microarray data analysis of OsGLP genes in young leaves and roots of a Japonica cultivar, the expression pattern of some OsGLP genes (OsGLP3-6, OsGLP3-7, OsGLP8-4, OsGLP8-10, OsGLP8-11 and OsGLP8-12) was consistent with our results of both Indica varieties. However, in contrast to our findings, OsGLP4-1, OsGLP8-7 and OsGLP8-11 expressed at same level in roots and young leaves of Japonica cultivar [9]. It can therefore be suggested that OsGLP genes have diversified expression pattern in leaves and roots of distinct rice sub-species. It is worthy to mention that several OsGLP genes showed variation in their tissue specific expression pattern after salt and drought stress treatment. For example, OsGLP8-12 showing nearly same level of expression in leaves and roots was observed with higher expression level in roots of Super Basmati as compared to leaves under both stress treatments.

Although most of the selected OsGLP genes showed modulated expression in both leaves and roots in response to salt and drought stress, the overall analysis demonstrated that more OsGLP genes were upregulated in roots as compared to leaves in both varieties under a particular stress treatment. In accordance with this observation, five OsGLP genes in leaves and seven OsGLP genes in roots of KS282 whereas four OsGLP genes in leaves and eight OsGLP genes in roots of Super Basmati were upregulated in response to at least one of the stress types. According to various studies, under abiotic stresses including salt and drought stress, the level of plant hormone ABA increases which initiates the signal transduction and eventually leads to cellular stress responses [26]. ABA has been observed to modulate the expression of several stress induced GLP genes suggesting its importance in regulation of GLP genes under salt and drought stress. In a recent study, ABA increased the transcript level of CpGLP1 in Craterostigma plantagineum under drought stress [11]. According to another study, the expression of most of the peanut GLPs was induced in response to ABA [27]. Similarly, a rice GLP (OsGLP2-1) also showed increased expression in response to ABA [28]. One of the possible explanations for the increased expression of OsGLP genes in roots under salt and drought stress could be the role of GLP genes in specific tissue development. It has been demonstrated that two Arabidopsis thaliana germin like proteins (plasmodesmata germin like proteins 1 and 2) play significant role in controlling root development by exhibiting predominant abundance in root tissue [6, 29]. Also, the accumulation of ABA in roots under abiotic stress (specifically salt and drought stress) has been reported.
to elongate the roots and induce the expression of stress responsive genes [30]. Thus, the presence of ABA in roots might induce the expression of certain GLPs to promote the root elongation to make them adaptive for source utilization and to cope with salt and drought stress.

![Venn diagram](image)

**Fig. 4** a Venn diagram demonstrating the expression pattern of OsGLP genes regulated by drought and salt stress in roots of KS282 and Super Basmati. Expression profiles of OsGLP genes (in roots) regulated commonly regarding particular stress type (drought and salt) and particular rice variety (KS282 and Super Basmati). Red, Up-regulation; Green, Down-regulation; Blue, No change in expression. b Salt stress induced comparative expression of OsGLP genes in roots of KS282 and Super Basmati. Comparison of relative expression (Fold Change) of OsGLPs (OsGLP3-6, OsGLP3-7, OsGLP3-8, OsGLP4-1, OsGLP6-7, OsGLP8-10, OsGLP8-11, and OsGLP8-12) was performed between KS282 roots and Super Basmati roots under salt stress. Values indicate mean ± Standard Error Mean (SEM). Statistical analysis was performed with two-way ANOVA (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001) c Drought stress induced comparative expression of OsGLP genes in roots of KS282 and Super Basmati. Comparison of relative expression (Fold Change) of OsGLPs (OsGLP3-6, OsGLP3-7, OsGLP4-1, OsGLP8-4, OsGLP8-7, OsGLP8-10, OsGLP8-11, and OsGLP8-12) was performed between KS282 roots and Super Basmati roots under drought stress. Values indicate mean ± Standard Error Mean (SEM). Statistical analysis was performed with two-way ANOVA (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). (Color figure online)
Most of the GLP genes have been reported to possess SOD activity which constitutes the defense against redox imbalance created by salt and drought stress by catalyzing the dismutation of superoxide radicals to H$_2$O$_2$. The accumulation of H$_2$O$_2$ eventually leads to increased tolerance against stress conditions by reinforcement of cell wall components [11, 31, 32]. Our results for transcriptional response of OsGLP genes under salt and drought stress indicated that fold change in the expression of various OsGLP genes is associated with a particular stress and tissue type, suggesting the distinct regulation of their expression. For example OsGLP8-4 showed increase in its expression only under drought stress in roots of both varieties whereas OsGLP4-1 showed same expression pattern under salt and drought stress in leaves and roots of both varieties by being up-regulated in response to both stresses. In contrast to our findings, OsGLP8-4 and OsGLP4-1 were found to be down-regulated under both salt and drought stress in a study done on seedlings of Japonica cultivar “Nipponbare” [9]. We also demonstrated that, especially in roots, the expression of most of the OsGLP genes including OsGLP3-7, OsGLP4-1, OsGLP8-7, OsGLP8-10 and OsGLP8-11 was significantly higher under salt stress in comparison with drought stress. Previously, GLP genes have been shown to exhibit up-regulation in barley roots under salt stress [33]. Also, the expressional study of GLP genes from peanut depicted increased expression in roots after salt stress [27]. In another proteomic analysis in wheat leaves, GLP was up-regulated at second and third day of salt stress [34]. The mechanisms reported for plant responses to salt and drought stress have high similarity indicating that both stresses must be perceived as reduced water potential, however, salt stress has an extra component of ionic stress [30]. This ionic aspect might involve a different signaling pathway for activation of OsGLP genes in response to salt stress. It can be inferred from current and previous studies that OsGLP genes might have both general and specific regulatory mechanisms and roles depending upon a specific stress as well as tissue type.

From comparative analysis of transcriptomes, we found that KS282 and Super Basmati demonstrated different molecular responses to both stress conditions by exhibiting significantly different expression of several OsGLP genes. This finding was supported by the observation that fold change in expression of some OsGLP genes was significantly higher in KS282 under salt stress (OsGLP3-6, OsGLP4-1, OsGLP8-7, OsGLP8-10 and OsGLP8-11 in leaves and OsGLP3-6, OsGLP8-7, OsGLP8-10 and OsGLP8-11 roots) and drought stress (OsGLP3-6, OsGLP4-1 and OsGLP8-7 in leaves and OsGLP3-6 and OsGLP8-7 in roots) as compared to Super Basmati. However, some OsGLP genes presented higher relative expression in Super Basmati compared to KS282 in response to salt stress (OsGLP3-7 and OsGLP8-12 in roots) and drought stress (OsGLP8-4 and OsGLP8-12 in roots). The differential expression of OsGLP genes from largest cluster of chromosome 8, OsGLP4-1 from chromosome 4 and that of OsGLP3-6 from chromosome 3 has been
documented previously under salt and drought stress [9] but current study indicates that modulation in expression of these genes is dependent upon a particular rice cultivar. It is worthy to mention that in a transcriptomic study conducted in spinach, the relative expression of a GLP gene was found to be higher in salt tolerant variety in comparison with sensitive one [13]. In this context, findings from present study might have implications in providing information regarding candidate OsGLP genes associated with salt and drought stress tolerance, functional basis for developing stress resistant rice cultivars.

Regulation of gene expression is a significant molecular response controlled by utilization of several TFs which modulate the expression of stress responsive genes by binding with their promoters [35]. The presence of appropriate TFbs in the promoter of a gene directs the expression level of that gene under a particular stress condition [36]. Earlier, promoters of OsGLP genes have been analyzed for the presence of abiotic stress associated TFbs mainly including bHLH, bZIP, MYB and AP2/ERF [9, 20]. Among these TFs, MYB and AP2/ERF members have been reported for their association with salt and drought stress management in rice. AP2/ERFbs were found with the highest occurrence in the promoter region of OsGLP genes. Among our selected OsGLP genes, OsGLP3-6 which showed significant upregulation in leaves and roots of both rice varieties under salt and drought stress was found with highest number of MYB TFbs in its promoter region followed by OsGLP8-11. As a whole, promoter region of OsGLP8-11 depicted the highest number of TFbs in its promoter for above mentioned four TFs whereas OsGLP8-12 contained least amount of TFbs [20]. Interestingly, in current expression analysis, OsGLP8-12 was the only gene which exhibited a significant decrease or no change in its expression in both leaves and roots of KS282 under both stress conditions. In addition to this, most of the OsGLP genes (OsGLP3-6, OsGLP4-1, OsGLP8-7 and OsGLP8-11) with significantly higher modulated expression in KS282 as compared to Super Basmati were found to have more number of binding site for these TFs. This suggests that detailed analysis of salt and drought stress related TFbs in promoter regions of OsGLP genes with modulated expression in both rice varieties might help to further understand the regulatory mechanisms of OsGLP genes under stress conditions.

Conclusion

The present study compared the differential regulation of OsGLP genes in leaves and roots of two rice varieties under salt and drought stress. The results indicated that roots exhibited a greater number of up-regulated OsGLP genes, and the relative expression of most of the up-regulated OsGLP genes was higher in KS282 in comparison with Super Basmati. Importantly, among various OsGLP genes with modulated expression, OsGLP3-6 was the most prominent with highest increase in its expression under salt and drought condition in leaves and roots of both rice varieties with a significantly higher expression in KS282 than Super Basmati. Overall analysis suggested that OsGLP genes might have both general and specific regulatory mechanisms and roles depending upon a specific rice variety, stress condition as well as tissue type. Although the mechanism of differential regulation of OsGLP genes is still largely unexplored; our work, provided the information regarding the potential candidate OsGLP genes associated with salt and drought stress tolerance for the development of stress resistant rice cultivars. Moreover, it sheds light on the involvement of GLPs in common regulatory cross talks during salt and drought stresses in plants.

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**Author contributions** Conceptualization: [JA], [TY]; Data Curation: [JA]; Methodology: [CO], [JA], [TY]; Formal analysis: [JA], [MZH], [SF], [SIM]; Investigation: [CO], [JA]; Validation: [MZH], [TY]; Writing—original draft preparation: [JA]; Writing—review and editing: [SF], [SIM], [TY]; Funding acquisition: [JA]; Resources: [KS]; Project Administration: [CO], [JA], [KS]; Supervision: [KS], [MZH], [TY].

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**Data availability** Not applicable.

**Code availability** Not applicable.

**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest relevant to the content of this article.

**Consent to participate** A consent has been taken from all participants of the study.

**Consent to publish** All the participants have agreed on the publication of the results.
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