

# Germin Like Protein Genes Exhibit Modular Expression During Salt and Drought Stress in Elite Rice Cultivars

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## Research Article

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## Abstract

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. In this study, transcriptional variation of 8 *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. 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.

## 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 8 *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 comprehensive analysis identified potential candidate *OsGLP* genes associated with salt and drought stress tolerance eventually providing functional basis for developing stress resistant rice cultivars. We identified common GLP genes expressed during both the stresses indicating a common 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 minutes then washed with autoclaved Milli-Q water (5 washes 1 min each), followed by 15 minutes shaking in 3.5% sodium hypochlorite and tween-20, another 5 minutes 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  $25^{\circ}\text{C} \pm 1$  with 16 hour photoperiod. After two weeks, seedlings were randomly divided into three groups for control, salinity and drought conditions (3 replicates in each group with 15 plants per replicate). Salt and drought stress treatments were applied to 14 days old seedlings for 24 hours. 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  $-80^{\circ}\text{C}$  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  $\mu\text{g}$  of total RNA using RevertAid First Strand cDNA Synthesis Kit (Thermo scientific, Lithuania) according to manufacturer's protocols and stored at  $-20^{\circ}\text{C}$ .

## Primer designing and gene expression profiling

To analyze the differential expression of 8 *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 8 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  $\mu\text{L}$  reaction mixture, containing 1  $\mu\text{L}$  sample cDNA, 12.5  $\mu\text{L}$  SYBR Green Master mixture, 2.5  $\mu\text{L}$  each specific primer and 6.5  $\mu\text{L}$  nuclease-free water. The program for qRT-PCR included 95  $^{\circ}\text{C}$  for 5 min and 40 cycles at 95  $^{\circ}\text{C}$  for 10 s and 60  $^{\circ}\text{C}$  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  $2^{-\Delta\Delta\text{CT}}$  and  $2^{-\Delta\text{CT}}$  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 8 *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 5 *OsGLP* genes (*OsGLP3-6*, *OsGLP4-1*, *OsGLP8-7*, *OsGLP8-10* and *OsGLP8-11*) in KS282 (Fig. 1a, 1c, 1e, 1f and 1g) and 2 *OsGLP* genes (*OsGLP3-6* and *OsGLP4-1*) in Super Basmati (Fig. 1a and 1c) 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 (Fig. 1a). Moreover, comprehensive 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). 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.

In KS282 leaves, compared to the untreated control, a significant up-regulation of 5 *OsGLP* genes (*OsGLP3-6*, *OsGLP4-1*, *OsGLP8-7*, *OsGLP8-10* and *OsGLP8-11*) under salt stress (Fig. 1a, 1c, 1e, 1f, 1g) and 4 *OsGLP* genes (*OsGLP3-6*, *OsGLP4-1*, *OsGLP8-7* and *OsGLP8-11*) under drought stress (Fig. 1a, 1c, 1e and 1g) was observed. However, relative expression of 4 *OsGLP* genes (*OsGLP3-6*, *OsGLP4-1*, *OsGLP8-10* and *OsGLP8-11*) under salt stress (Fig. 1a, 1c, 1f and 1g) and 3 *OsGLP* genes (*OsGLP3-6*, *OsGLP4-1* and *OsGLP8-11*) under drought stress (Fig. 1a, 1c and 1g) was up-regulated in leaf samples of Super Basmati. The fold change in expression of these up-regulated *OsGLP* genes in both varieties, except for *OsGLP4-1* and *OsGLP8-7* in Super Basmati, was significantly different in response to salt and drought stress. In this aspect, higher relative expression of *OsGLP3-6* and *OsGLP4-1* was observed under drought stress as compared to salt stress (Fig. 1a and 1c) whereas *OsGLP8-7*, *OsGLP8-10* and *OsGLP8-11* showed high level of expression in response to salt stress (Fig. 1e-1g) suggesting that these genes from chromosome number 8 might play a significant role in response to salt stress. 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 (Fig. 1b, 1d and 1h).

# **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 (Fig. 2b and Fig. 2c). Overall, relative change in expression of 5 *OsGLP* genes (*OsGLP3-6*, *OsGLP4-1*, *OsGLP8-7* *OsGLP8-10* and *OsGLP8-11*) under salt stress (Fig. 2b) and 3 *OsGLP* genes (*OsGLP3-6*, *OsGLP4-1* and *OsGLP8-7*) under drought stress (Fig. 2c) was significantly higher in KS282 than Super Basmati.

## **Expression profiling of *OsGLP* genes in roots**

A comparison of differential expression profiles of 8 *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 7 *OsGLP* genes (*OsGLP3-6*, *OsGLP3-7*, *OsGLP4-1*, *OsGLP8-4*, *OsGLP8-7*, *OsGLP8-10* and *OsGLP8-11*) in KS282 (Fig. 3a- 3g) and 5 *OsGLP* genes (*OsGLP3-6*, *OsGLP3-7*, *OsGLP4-1*, *OsGLP8-4* and *OsGLP8-12*) in Super Basmati (Fig. 3a-3d and 3h) 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 (Fig. 3a). Furthermore, 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).

Additionally, some *OsGLP* genes exhibited similar expression pattern in KS282 and Super Basmati depending on a particular stress type. In this aspect, the expression of 6 *OsGLP* genes (*OsGLP3-6*, *OsGLP3-7*, *OsGLP4-1*, *OsGLP8-7*, *OsGLP8-10* and *OsGLP8-11*) under salt stress (Fig. 3a, 3b, 3c, 3e, 3f and 3g) and 4 *OsGLP* genes (*OsGLP3-6*, *OsGLP4-1*, *OsGLP8-4* and *OsGLP8-11*) under drought stress (Fig. 3a, 3c, 3d and 3g) 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 (Fig. 3f). Importantly, 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. 3h).

## **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 4 *OsGLP* genes (*OsGLP3-6*, *OsGLP8-7*, *OsGLP8-10* and *OsGLP8-11*) under salt stress (Fig. 4b) and 2 *OsGLP* genes (*OsGLP3-6* and *OsGLP8-7*) under drought stress (Fig. 4c) was significantly higher in KS282 as compared to Super Basmati. Whereas, in comparison with KS282, 2 *OsGLP* genes (*OsGLP3-7* and *OsGLP8-12*) after salt stress (Fig. 4b) and 2 *OsGLP* genes (*OsGLP8-4* and *OsGLP8-12*) after drought stress (Fig. 4c) were observed with higher expression in Super Basmati.

## 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, 3 *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, *OsGLP 8-4* was most prominent with highest level of expression in roots followed by *OsGLP8-11* whereas *OsGLP3-6* and *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 sub-species (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* 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, 5 *OsGLP* genes in leaves and 7 *OsGLP* genes in roots of KS282 whereas 4 *OsGLP* genes in leaves and 8 *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.

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<sub>2</sub>O<sub>2</sub>. The accumulation of H<sub>2</sub>O<sub>2</sub> 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 upregulated 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.

## Declarations

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## Conflict of interest

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

## Availability of data and material

Not applicable

## Code availability

Not applicable

## Author contributions

Conceptualization: [Jazba Anum], [Tayyaba Yasmin]; Data Curation [Jazba Anum]; Methodology: [Charlotte O'Shea], [Jazba Anum], [Tayyaba Yasmin]; Formal analysis: [Jazba Anum], [M Zeeshan Hyder], [Sumaira Farrukh], [Saad Imran Malik]; Investigation: [Charlotte O'Shea], [Jazba Anum]; Validation: [M Zeeshan Hyder], [Tayyaba Yasmin]; Writing - original draft preparation: [Jazba Anum]; Writing - review and editing: [Sumaira Farrukh], [Saad Imran Malik], [Tayyaba Yasmin]; Funding acquisition: [Jazba Anum]; Resources: [Karen Skriver]; Project Administration: [Charlotte O'Shea], [Jazba Anum], [Karen Skriver]; Supervision: [Karen Skriver], [M Zeeshan Hyder], [Tayyaba Yasmin].

## 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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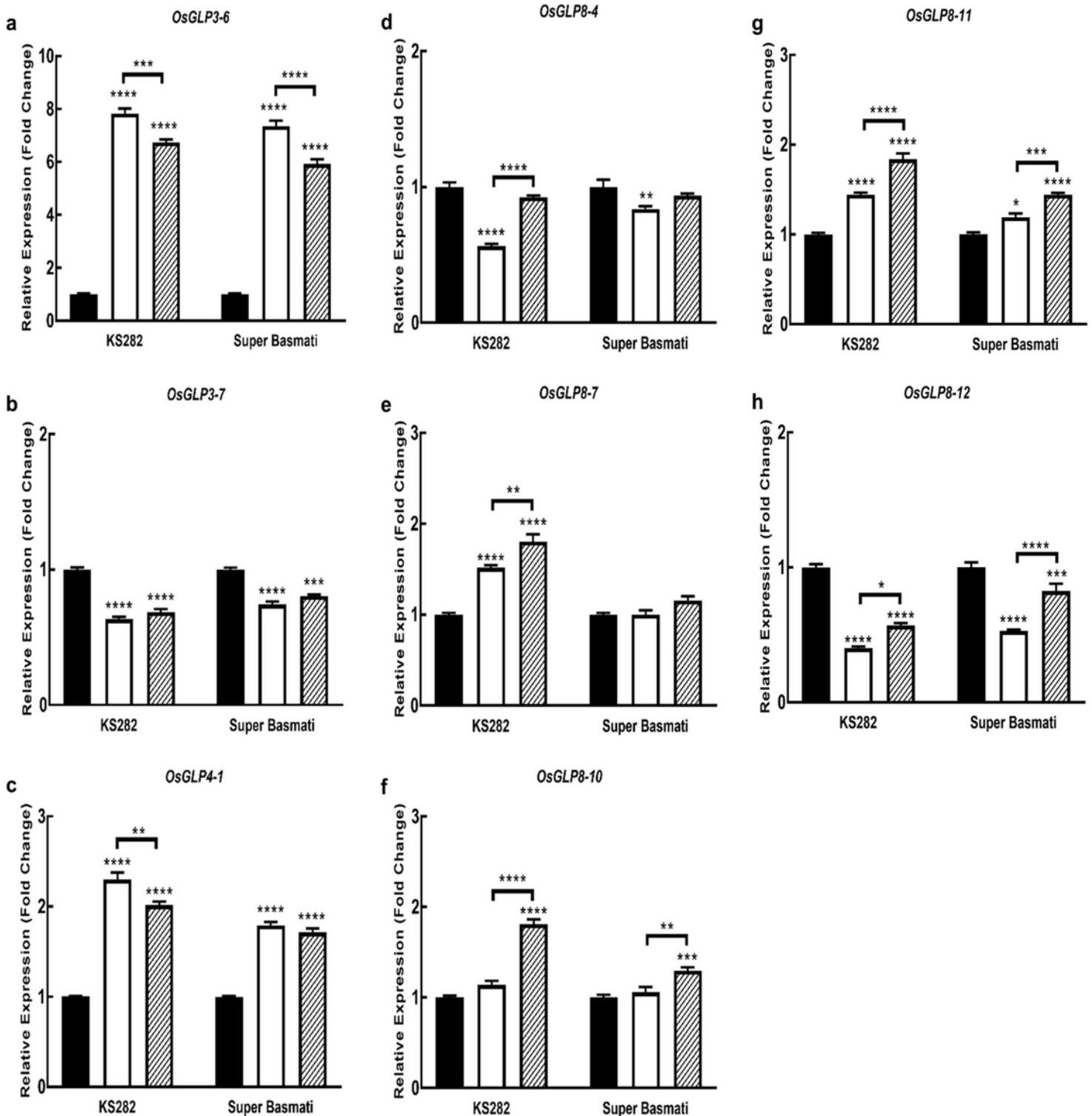
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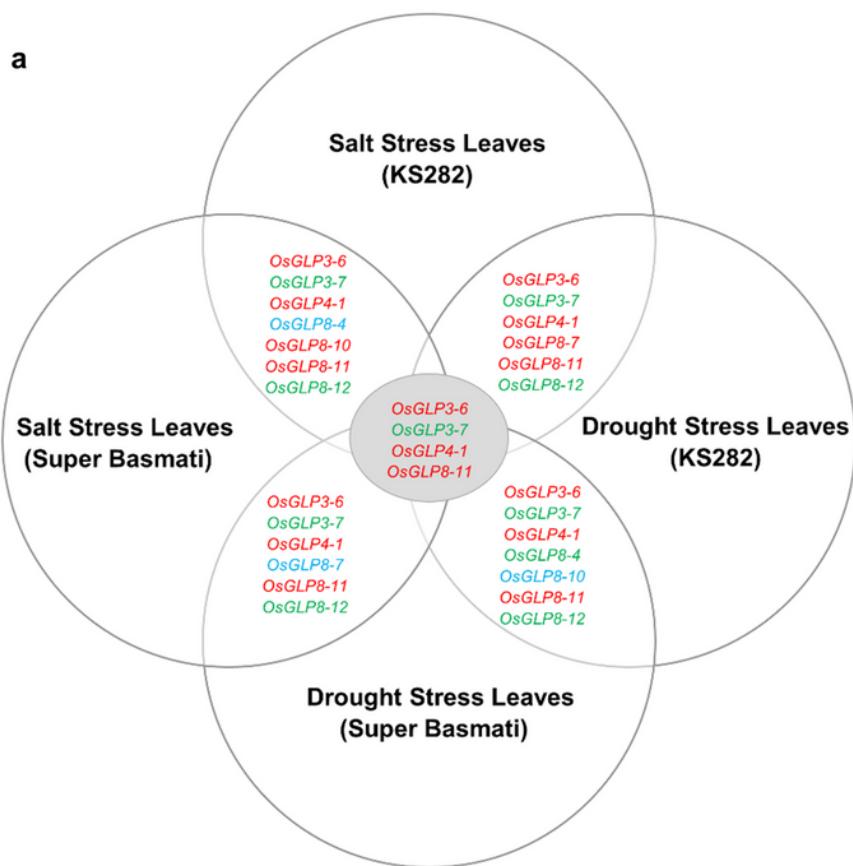
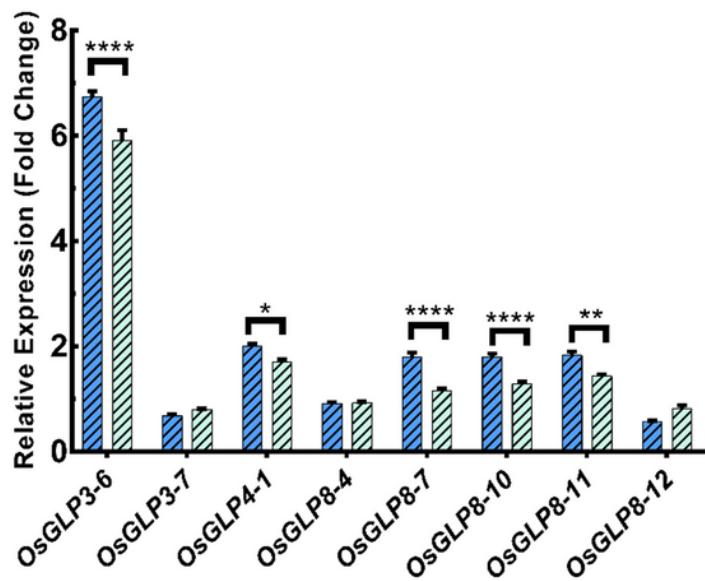
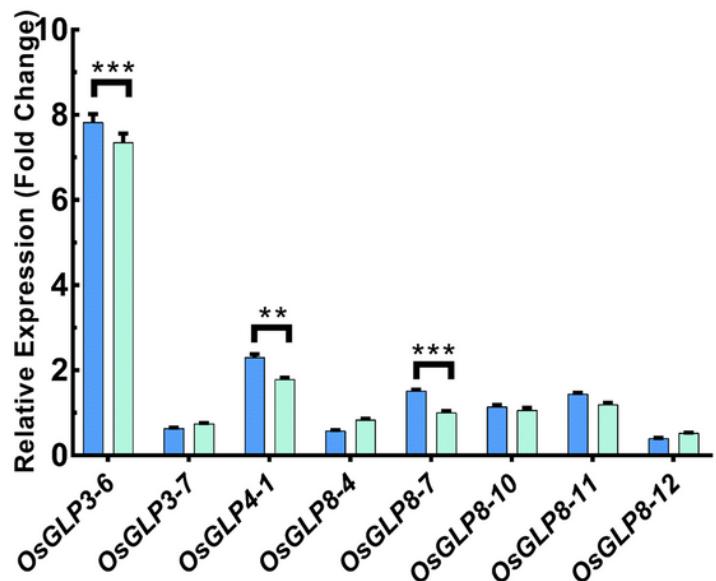
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## Figures

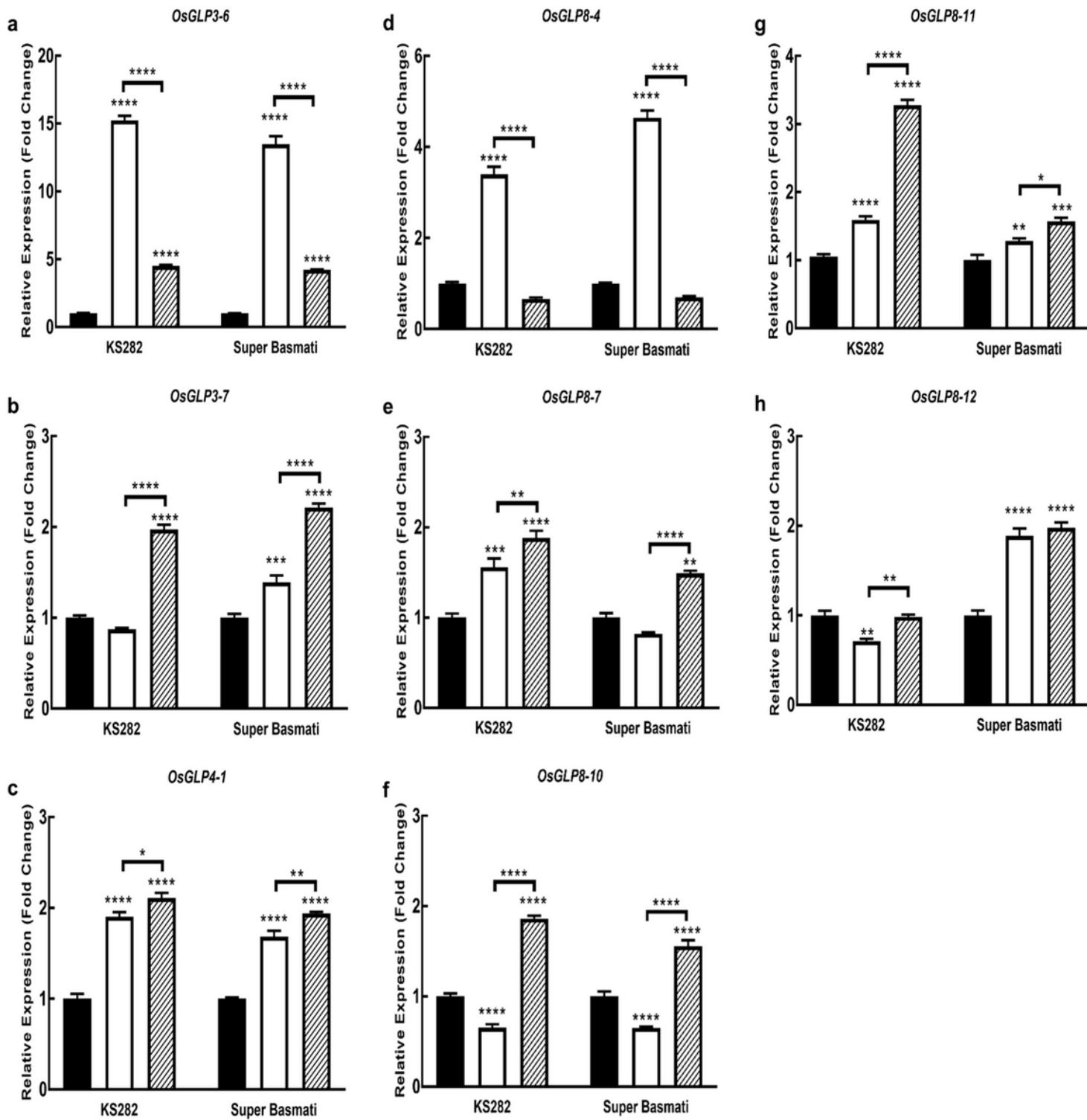


**Figure 1**

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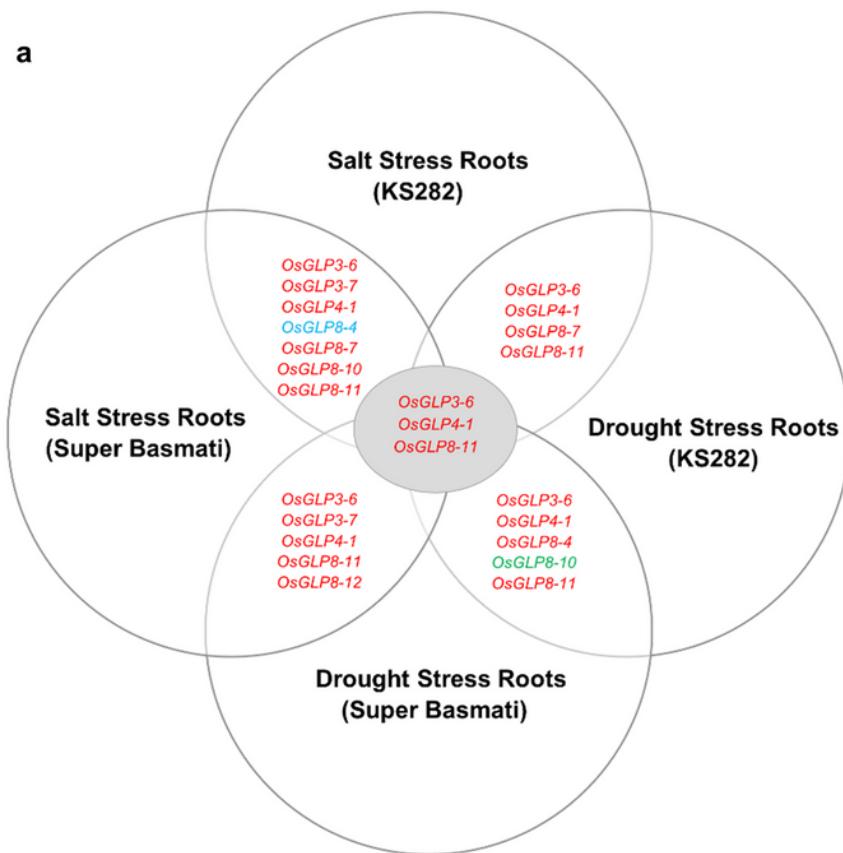
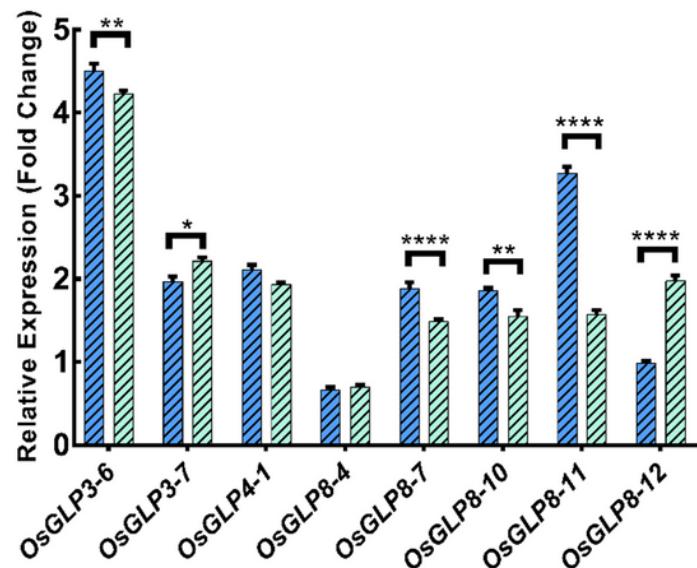
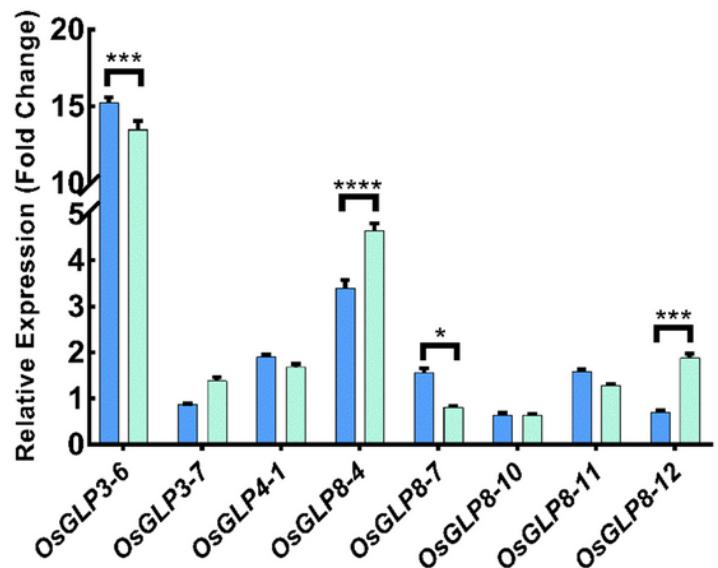
**a****b****c****Figure 2**

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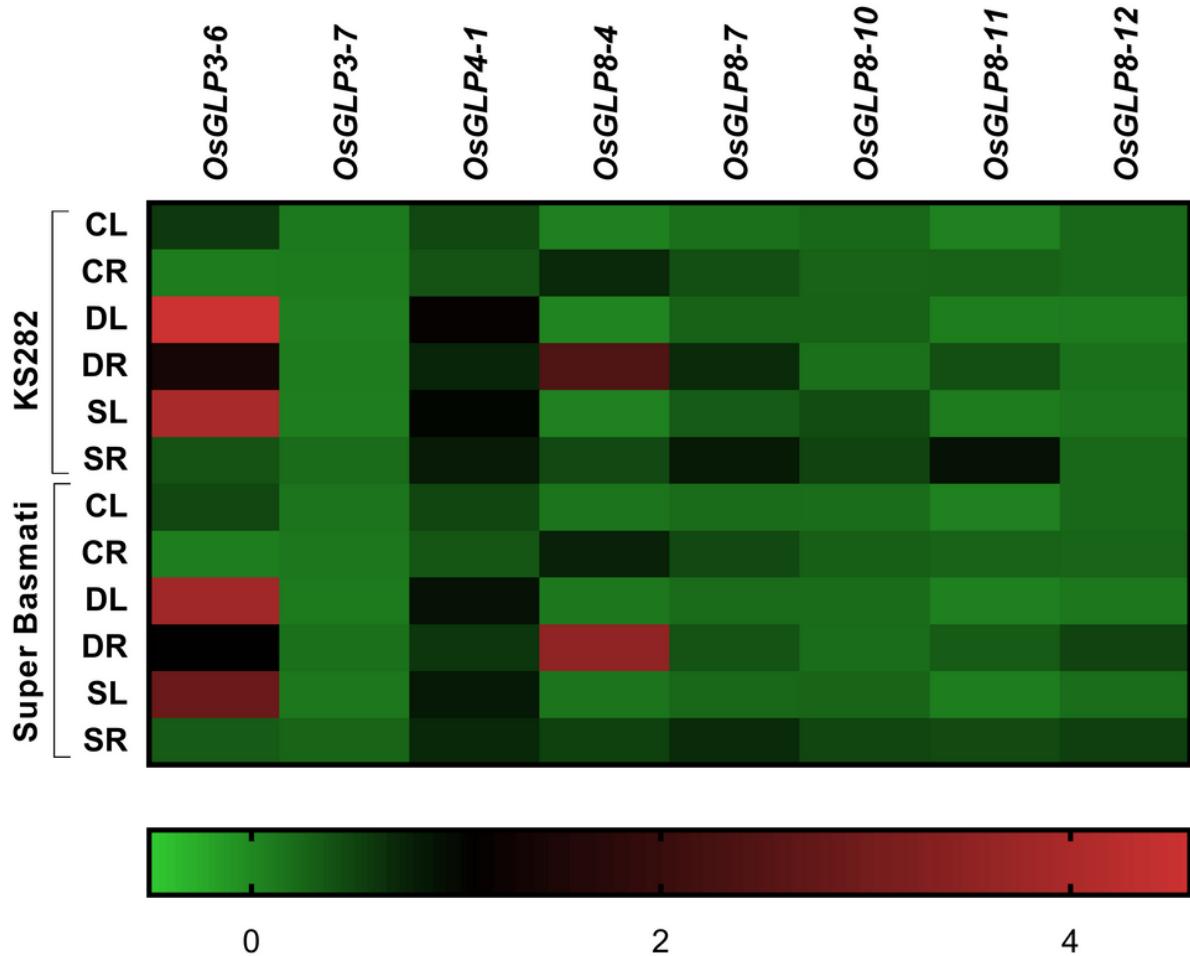


**Figure 3**

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**a****b****c****Figure 4**

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**Figure 5**

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