# Analysis of the overexpression of vegetative cell wall protein *GP1* gene in rice against soda saline-alkali stress (#84908)



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# Analysis of the overexpression of vegetative cell wall protein *GP1* gene in rice against soda saline-alkali stress

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Plant growth and development are inhibited by the high levels of ions and pH of soda saline-alkali soil. However, plant cells can resist damage by regulating ion homeostasis, reactive oxygen species balance, and the action of some related enzymes. The cell wall serves as a crucial barrier against external stresses in plants. The full-length coding sequence of the vegetative cell wall protein gene *OsGP1* was cloned from Lj11 (*Oryza sativa longjing11*), and it contained 660 bp nucleotides, which encode 219 amino acids. Interacting protein network analysis results revealed possible interacting nodal proteins such as CESA1, TUBB8, and OsJ_01535, which are related to plant growth and cell wall synthesis. OsGP1 was localized in the cell membrane and cell wall. The plant height and fresh weight of rice overexpressing *OsGP1* were higher than those of wild-type (Lj11) under saline stress, showing the saline-alkali resistant growth of transgenic plants. The peroxidase and superoxide dismutase activities of transgenic rice under stress were significantly higher than those of Lj11, and that of malondialdehyde was lower than that of Lj11. Therefore, *OsGP1* overexpression improved saline-alkali stress tolerance in rice. OsGP1 may be involved in soda saline-alkali stress response pathway in rice and play a direct or indirect role in the mechanism of cell wall resistance to salinity stress.

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## Abstract

Plant growth and development are inhibited by the high levels of ions and pH of soda saline-alkali soil. However, plant cells can resist damage by regulating ion homeostasis, reactive oxygen species balance, and the action of some related enzymes. The cell wall serves as a crucial barrier against external stresses in plants. The full-length coding sequence of the vegetative cell wall protein gene *OsGP1* was cloned from Lj11 (*Oryza sativa longjing11*), and it contained 660 bp nucleotides, which encode 219 amino acids. Interacting protein network analysis results revealed possible interacting nodal proteins such as CESA1, TUBB8, and OsJ_01535, which are related to plant growth and cell wall synthesis. OsGP1 was localized in the cell membrane and cell wall. The plant height and fresh weight of rice overexpressing *OsGP1* were higher than those of wild-type (Lj11) under saline stress, showing the saline-alkali resistant growth of transgenic plants. The peroxidase and superoxide dismutase activities of transgenic rice under stress were significantly higher than those of Lj11, and that of malondialdehyde was lower than that of Lj11. Therefore, *OsGP1* overexpression improved saline-alkali stress tolerance in rice. OsGP1 may be involved in soda saline-alkali stress response pathway in rice and play a direct or indirect role in the mechanism of cell wall resistance to salinity stress.

## Introduction

The Songnen Plain of China is one of the three most concentrated saline-alkali lands in the world (*Li et al., 2003*), and it is an important reserve resource of cultivated land in China (*Jiang et al.,*

*2019*). The salt in the soil of Songnen Plain mainly exists in the form of alkaline salts ($NaHCO_3$ and $Na_2CO_3$), with alarmingly high salt and pH. Rice is one of the four major cereal crops (*Zhang et al., 2003*), and it is an extremely important food crop. The development of soda saline-alkali resistance in rice is an important research topic for improving the quality and efficiency of rice plantation and strengthening food security. Soda saline-alkali soil causes damage to plants mainly because of the high levels of $Na^+$, $CO_3^{2-}$, $HCO_3^-$, and high pH (*Wang J et al., 2022*). Salt stress inhibits rice seed germination, seedling growth, and reproductive development (*Li et al., 2003*), leading to a decrease in leaf area, stalk, stem diameter, root activity, nutrient synthesis, accumulation, and transport, and interferes with young spike differentiation in reducing its effective spike number (*Wang Y et al., 2022*). Salt-alkali stress also leads to various physiological and molecular changes and hinders plant growth by inhibiting photosynthesis, thereby reducing available resources and inhibiting cell division and expansion. Plants have evolved many physiological and biochemical reactions to cope with saline-alkali stress (*Pastori and Foyer, 2002*), mainly including ion-selective absorption, accumulation of osmotic adjustment substances, and scavenging of reactive oxygen species (ROS) (*Liang et al., 2018*). Salt stress leads to a large influx of $Na^+$ into plant cells to induce ion toxicity, resulting in an imbalance of intracellular ion homeostasis; plants can promote osmotic balance at the cellular level through the synthesis of soluble sugars, proline, and other substances. ROS accumulates because of salt stress (*Vaidyanathan et al., 2003*), and plants scavenge excess ROS by producing superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT) to avoid internal damage.

Plant cell walls surround cells and provide external protection and intercellular communication, ~~and~~ they are mainly composed of polysaccharides (cellulose, hemicelluloses, and pectins), polymers such as lignin, and a small amount of cell wall proteins (CWPs) (*Jamet and Dunand, 2020*). CWPs can be divided into nine functional classes according to *Jamet et al.* (*2008*); these classes include proteins acting on carbohydrates, oxidoreductases, proteases, proteins related to lipid metabolism, proteins possibly involved in signalling, proteins with predicted interaction domains, miscellaneous proteins, proteins of unknown function, and structural proteins (*Calderan-Rodrigues et al.,2019*). CWPs are major players in cell wall remodelling and signalling and play an important role in plant cell growth and development, morphogenesis, pathogen infection, and environmental response. FERONIA (FER), a plasma-membrane-localized receptor kinase from Arabidopsis, is necessary to sense the defects of the cell wall. Sensing of the salinity-induced wall defects is possibly a direct consequence of the physical interaction between the extracellular domain of FER and pectin. FER-dependent signalling elicits cell-specific calcium transients that maintain cell-wall integrity during salt stress (*Feng et al., 2018*). Leucine-rich repeat extensins (LRXs) are chimeric proteins in the cell wall. LRXs bind rapid alkalinization factor (RALF) peptide hormones that modify cell wall expansion and directly interact with the transmembrane receptor FER (*Herger et al., 2019*). RALF in turn interacts with FER. LRXs, RALFs, and FER function as a module to transduce cell wall signals to regulate plant growth and salt stress tolerance (*Zhao et al., 2018*).

Hydroxyproline-rich glycoproteins (HRGPs) are a superfamily of CWPs. According to the 'Hyp contiguity hypothesis', contiguous and non-contiguous clustered Hyp residues are the sites attached by arabinoside and arabinogalactan polysaccharide, respectively (*Ma and Zhao, 2010*). HRGPs consists of three members: hyperglycosylated arabinogalactan proteins (AGPs), moderately glycosylated extensins (EXTs), and lightly glycosylated proline-rich proteins (PRPs), which function in diverse aspects of plant growth and development (*Showalter et al., 2010*). EXTs are involved in cell wall reinforcement in higher plants and in defence against pathogen attacks (*Castilleux et al., 2018, 2021*). Virus infection triggers several inducible basal defence responses. In *potato virus Y* ($PVY^{NTN}$)-infected potatoes, synthesis of EXTs is induced, whereas the synthesis of the catalytic subunit of cellulose synthase (CesA4) is reduced. The active trafficking of these proteins occurs as a step-in potato cell wall remodelling in response to $PVY^{NTN}$ infection (*Otulak-Kozieł et al., 2018*).

The remodelling and signal transduction functions of CWPs play an important role in abiotic stresses such as high temperature (*Pinski et al., 2021*), high salt (*Feng et al., 2018*; *Zhao et al., 2018*), and nutrient deficiency (*Wu et al., 2019*; *Ogden et al., 2018*) in plants. A total of 270 CWPs have been identified in *Oryza sativa* (*Calderan-Rodrigues et al., 2019*), including 69 AGPs (*Ma and Zhao, 2010*). However, the biological functions of rice vegetative cell wall proteins (GP1) involved in salt alkalinity resistance and related signal transduction and protease mechanisms have not been reported. In the current study, the *OsGP1* gene was cloned from rice leaves by reverse transcription PCR, and the functional site of OsGP1 protein was determined by subcellular localization. The genetic phenotypes of tolerance to soda saline-alkali stress in rice overexpressing *OsGP1* and wild-type were compared to clarify the role of OsGP1 under salinity stress. These results support the involvement of OsGP1 in the stress resistance mechanism of rice cell wall under soda saline-alkali stress.

## Materials & Methods

### Plant material

*O. sativa longjing11* (Lj11) seeds were donated by the research group of Qingyun Bu, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences.

### Soda Saline–Alkali Soil Eluent

The soda saline-alkali soil eluent (SAE) used for stress treatment were obtained as described by *Wang et al.* (*2018*). The 0-10 cm soil of heavy alkali patch was obtained from the Anda field experiment station (124°53'~125°55'E, 46°01'~47°01'N). The sample was dried, passed through a 5 mm×5 mm sieve, and mixed well. Approximately 4 l of water was poured into 2 l of saline-alkali soil. The mixture was stirred well and left for 12 h (stirring once every 4 h). The mixture was filtered using a filter paper to remove impurities, and experimental SAE was obtained. The different ratios of SAE required in the experiments are shown in Table 1.

### Gene cloning

According to the sequence of Nipponbare *OsGP1* gene (XM_015773690), specific primers (OsGP1F1: 5'-catggcgtcatcggcattgc-3'; OsGP1R2: 5'-gctccggcgaacgatcagac-3') were designed. The RNA of Lj11 was reverse-transcribed into cDNA as a template, and target DNA was

amplified by Blend-Taq DNA polymerase. The DNA was purified and inserted into the pMD18-T vector and then transformed into *Escherichia coli* JM109. The pMD18-T-*OsGP1* plasmid was identified by digestion with restriction endonuclease SalI/BamHI and sent for sequencing.

**Bioinformatics analysis**

The nucleotide sequence obtained by sequencing was used as the comparison benchmark, and the open reading frame (ORF) of the *OsGP1* gene was analysed based on NCBI (https://www.ncbi.nlm.nih.gov/). The physicochemical properties of the encoded protein were obtained using the ProtParam (https://web.expasy.org/protparam/). Conserved domains were analysed using the online website SMART (http://smart.embl-heidelberg.de) and InterPro (https://www.ebi.ac.uk/interpro/). Signal peptides were analysed using an online website (www.detaibio.com/tools/signal-peptide.html). The transmembrane structure of OsGP1 was predicted using TMHMM (https://services.healthtech.dtu.dk/service.php?TMHMM-2.0). The online tool (https://www.novopro.cn/tools/secondary-structure-prediction.html) was used to predict the protein's secondary structure. The tertiary structure was predicted using SWISS-MODEL (https://swissmodel.expasy.org/). The Locus ID (Os03g0852400) was found according to *OsGP1* (XM_015773690). *OsGP1* is located at the position of 35895654-35896833 (-strand) on chromosome 3. The sequence at 35896832-35898832 was taken as the *OsGP1* promoter, and the promoter elements in this sequence were analysed using Plant CARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and mapped using TBtools. The sequences with high homology were obtained by Blastp (NCBI) and aligned using Clustal Omega. The phylogenetic tree was constructed in MEGA6.0 by using the neighbour-joining (NJ) method (bootstrap value, 1,000). The subcellular location of OsGP1 was predicted by amino acid sequence (http://psort1.hgc.jp/form.html).

**Subcellular localization**

The recombinant plasmid pGWB5-*OsGP1* with OsGP1 fused with green fluorescent protein (GFP) was constructed using the Gateway method. The ORF of *OsGP1* was amplified using specific primers OsGP1-F, R (OsGP1-F:5'-cggagctagctctagaatggcgtcatcggcattgcc-3', OsGP1-R:5'-tgctcaccatggatccgacgagctcgaggcgggcg-3'). The samples were ligated using a Gateway™ LR Clonase™ Ⅱ Enzyme Mix (Thermofisher) and transformed into *E. coli* TOP10. Positive colonies were detected by PCR and sent for sequencing to obtain the pGWB5-*OsGP1* plasmid. The recombinant plasmids were transformed into onion cells by using a gene gun (Wealtec, GDS-80, Nevada, USA) and cultured in the dark at 22 ℃ for 16–24 h (*Zhao et al., 2013*). After culturing, the onion epidermal cells were torn to make pressed slices, and the results of the green fluorescence experiments of the onion epidermal cells were observed under a fluorescence microscope (ZEISS, Axio Imager 2, Gottingen, Germany).

**Rice transformation and identification**

According to the rice transgenic method of *Upadhyaya et al.* (*2000*) and *Toki et al.* (*2006*), Lj11 seeds were dehulled and sterilized, and the callus was induced on the medium added with 2,4-D. The pGWB5-*OsGP1* plasmid was electrotransformed into *Agrobacterium tumefaciens* EHA105 and used to infect rice callus. The infected rice callus was screened and differentiated in a

hygromycin medium to obtain transgenic T0 generation lines, and the integration of *OsGP1* was detected by PCR by using specific primers (OsGP1-F: 5'-cggagctagctctagaatggcgtcatcggcattgcc-3') and vector primers (B5Nos-R: 5'-atcggggaaattcgctagtg-3'). The T3 generation transgenic rice was obtained by twice germination selection of 50 mg/l hygromycin medium. The relative expression of *OsGP1* in T3 transgenic lines seedlings was detected by quantitative real-time PCR (qRT-PCR) in a fluorescent quantitative PCR instrument (Agilent, Mx3000p, Waldbronn, Germany). The seeds of overexpressed T3 generation lines were collected for subsequent experiments.

**Soda saline-alkaline stress tolerance analysis**

The tolerance of *OsGP1* transgenic rice to soda saline-alkali stress was tested by surface-sterilizing three independent homologous transgenic lines (T3-#2, #4, and #5) of the T3 generation and the non-transgenic control (Lj11) and culturing them in hoagland nutrient solution at 28 ℃ with 16 h light and 8 h dark photoperiod. Three-leaf stage seedlings were used as treatment materials. The compound salt mixed with sterilized water and SAE in different ratios was used as the stress treatment solution. The roots of each experimental line were soaked with different ratios of SAE, and the tolerance phenotypes and physiological indices were detected after 7 days, the determination method refers to *Chen and Zhang* (*2016*).

## Results

**Cloning of *OsGP1* gene**

The DNA was amplified by PCR with specific primers and electrophoresed to show a ~~target gene~~ of approximately 600 bp (Fig. 1A), purified and inserted into a pMD18-T vector, and transferred into *E. coli* JM109 to obtain a strain that contains recombinant plasmid pMD18-T-*OsGP1*. The plasmid DNA was extracted, digested with Sal Ⅰ/BamH Ⅰ, and detected by electrophoresis (Fig. 1B). Gel imaging results showed the DNA fragments consistent with ~~the target gene~~. The plasmid was then sent for sequencing. ~~*OsGP1* was obtained with an~~ identical nucleotide sequence comparison to the XM_015773690.

**Bioinformatics analysis of OsGP1**

The ORF of *OsGP1* contains 660 bp nucleotides and encodes 219 amino acids (Fig. 2A). The secondary and tertiary structures of OsGP1 protein were predicted using online tools (Fig. 2B, C). The results show that it has α-helix and β-fold, accounting for 36.07% and 5.48%, respectively, while the remaining structures are mainly irregularly coiled with a few extended chains. The conserved structural domain analysis of OsGP1 showed a proline-rich family protein region at sites 6-208, of which sites 178-197 are transmembrane helix region. According to the results of signal peptide prediction (Fig. 2D), OsGP1 has a site at the 26th amino acid site that can be recognized and digested by signal peptidase. Hence, a possible signal peptide structure was predicted between the 1st and 25th amino acid sites. The protein interactions of OsGP1 were predicted using STRING (Fig. 2E), and the results showed that it was co-expressed with proteins such as CESA1, TUBB8, and OsJ_28509. The analysis of the *OsGP1* promoter (Fig. 2F) revealed that it contains the cis-acting element ABRE involved in abscisic acid (ABA) response, LTR involved in low-temperature response, TCA-element involved in salicylic acid response,

and TGACG-motif involved in methyl jasmonate response. The sequences of 43 proteins were aligned from Blastp (NCBI) and a phylogenetic tree was constructed (Fig. 3A). Four sequences in the same branch with OsGP1 were selected for multiple sequence alignment (Fig. 3B), including that of *O. sativa* Indica group protein OsI_1436 had 100% similarity with the OsGP1 from *O. sativa* japonica group studied here. The similarity of OsGP1 to mucin-1-like from *Oryza brachyantha* was 87.88%, while its similarity to GUJ93 from *Zizania palustris* was 84.85%. The results of Clustal Omega were imported into Jalview, and the analysis showed that most of the conserved regions of the protein were from the 177th amino acid site to the end of the protein.

**Subcellular localization of OsGP1**

PSORT was used to predict the subcellular localization of OsGP1, and the results showed that the localization rates of OsGP1 protein were 46% in cytoplasmic membrane, 28% in the endoplasmic reticulum membrane, and 10% in the endoplasmic reticulum (Table 2). Fluorescence microscopy was used to observe the onion epidermal cells transformed with the gene gun. The green fluorescence of the OsGP1-GFP fusion protein was found to be expressed in the cell membrane and cell wall (Fig. 4), thus supporting the predicted localization at the cytoplasmic membrane.

**Identification of rice overexpressing *OsGP1***

*Agrobacterium tumefaciens* EHA105 containing pGWB5-*OsGP1* plasmid infected and transformed the rice callus (Fig. 5A) and differentiated on the selection medium containing hygromycin. The T0 generation plants were transplanted to pots and detected by PCR, and the results showed that *OsGP1* was integrated into the genome of the T0 generation lines (Fig. 5B). Transgenic rice seeds were harvested and screened for two generations under hygromycin to obtain T3 generation seeds. Total RNA was extracted from T3 generation lines and reverse-transcribed into cDNA as a template. qRT-PCR was performed to detect the expression of *OsGP1* under 35S promoter (Fig. 5C). The expression of #2 and #4 line was up to more than 10 times that of WT, and the expression of #5 line was approximately nine times that of WT. Therefore, overexpressing *OsGP1* T3 generation rice lines #2, #4, and #5 were selected for subsequent resistance analysis.

**Tolerance analysis of rice overexpressing OsGP1 to soda saline-alkali stress**

Three-leaf stage seedlings of T3 generation lines overexpressing *OsGP1* were treated with different concentrations of SAE, and phenotypes were observed after 7 days (Fig. 6A). With the increase in SAE content, injuries such as leaf wilting of seedlings gradually increased, and the transgenic lines were less damaged than WT. In the treatment groups with $H_2O$:SAE ratios of 4:1 and 3:1, the transgenic lines differed significantly from WT, and higher plant height and fresh weight were observed in rice overexpressing *OsGP1* than WT (Fig. 6B, C). The MDA content increased in all lines under saline-alkali stress, but the overexpression *OsGP1* lines had significantly less MDA than those of Lj11 (Fig. 6D). The antioxidant enzyme activities were detected, showing that the POD and SOD activities of overexpression lines were significantly higher than those of Lj11(Fig. 6E, F). Therefore, the overexpression of the *OsGP1* enhanced

saline-alkali stress resistance in rice, and OsGP1 protein functioned to protect against high salt ion injury at high pH.

## Discussion

Rice is an important food crop in the world, and its yield is affected by soil salinity. Alkaline salt stress can inhibit the photosynthesis and growth of plants more than neutral salt stress. Under alkaline salt stress conditions, metal ions form a large number of precipitates, thus decreasing the availability of plant nutrients (*Guo et al., 2017*). Under the interaction of high pH and salt ions, alkaline salts are more restrictive than neutral salts in the germination of seeds and the growth of seedlings (*Wang et al., 2022*). When plants are subjected to salt stress, the plant first experiences several changes in the cell wall, including a reduction in cellulose content, disruption of pectin cross-linking, and accumulation of lignin, resulting in the inability of rice to grow normally (*Liu et al., 2022*). CWPs play an important role in plant defence against abiotic stresses. In this study, OsGP1 was found to be a proline-rich protein containing a signal peptide and a transmembrane structure. The Locus ID of OsGP1 (Os03g0852400) in RAP-DB (https://rapdb.dna.affrc.go.jp/transcript/?name=Os03t0852400-02) also labelled as OsAGP31, which was identified in a screen for AGPs in the rice genome. It belongs to the non-classical AGPs which contained an AGP-like region and other atypical regions, and was expressed in roots and panicles (*Ma and Zhao, 2010*). The prediction results of the OsGP1-interacting proteins showed that OsGP1 has co-expression with CESA1, TUBB8, OsJ_28509, and other proteins. Among these proteins, cellulose synthase gene 1 (CESA1) is required for the crystallization of β-1,4-glucan microfibrils, which is related to the main mechanism of cell wall formation (*Burn et al., 2002*). *OsCESA9*/*OsCESA9*$^{D387N}$ heterozygous plants enhance plant resistance to salt stress by deregulating the toxicity of ROS, scavenging ROS, and indirectly affecting related genes such as *OsCESA4* and *OsCESA7* (*Ye et al., 2021*). TUBB8 has the same protein sequence as the japonica rice β-tubulin protein OsTUB8. *OsTUB8* is mainly expressed in anthers and pollen and is an anther-specific microtubule protein that has a unique role in microtubule formation during anther and pollen development and pollen tube growth. Its expression is upregulated by gibberellin (GA3) and may be involved in GA-regulated anther and/or pollen development (*Yoshikawa et al., 2003*). In addition, the overexpression of *Arabidopsis thaliana TUB9* gene in rice increased the tolerance to salt stress (*Chun et al., 2021*). These proteins are involved in cellulose synthesis, plant growth and development, hormone signalling and regulation, and tubulin synthesis. Based on the prediction of *OsGP1* promoter, the promoter sequence has cis-acting elements related to hormone regulation (abscisic acid, salicylic acid, and methyl jasmonate) and low temperature response, and these acting elements are related to plant resistance to abiotic stress (*Wang et al., 2019*; *Chen et al.,1995*). Subcellular localization showed that the OsGP1 protein was localized to the cytoplasmic membrane, reflecting the specific expression characteristics of cell wall proteins. Saline-alkali-tolerant rice exhibited higher germination rate, root length, shoot length, fresh weight, and dry weight than sensitive rice under saline-alkali stress. Overexpressing *OsGP1* lines T3-#2, #4, and #5 and Lj11 were treated with different ratios of SAE for saline-alkali stress. The results showed that compared

with Lj11, the fresh weight and plant height of transgenic lines were higher, and the degree of leaf bleaching and yellowing was lower, indicating that the overexpression of *OsGP1* enhances plant resistance to external stresses. The MDA content can reflect the degree of membrane lipid peroxidation, which is an important parameter to reflect the antioxidant capacity of plants (*Gaweł et al., 2004*). SOD and POD are key enzymes for ROS scavenging, and their high intracellular activity usually alleviates the damage of ROS and restores ROS homeostasis in plant cells. The MDA content of overexpressing *OsGP1* lines is lower than that of Lj11, and the SOD and POD activities were significantly higher than those of Lj11 under stress, indicating that *OsGP1*-overexpressing plants have stronger resistance than wild-type plants under the high concentration of ion damage, and the overexpression of *OsGP1* improved saline-alkali stress tolerance in rice.

## Conclusions

In the current study, the vegetative cell wall protein gene *OsGP1* was cloned from *O. sativa longjing11* (Lj11). Bioinformatics analysis results indicate that OsGP1 may interact with proteins involved in plant growth and cell wall synthesis, and the *OsGP1* promoter sequence contains many functional elements that can respond to abiotic stress. Subcellular localization showed that OsGP1 may function in cell membrane and cell wall. The resistance experiments of overexpressing *OsGP1* rice lines and wild-type (Lj11) were carried out at different ratios of saline-alkali soil eluent, and the results show that the growth vigour of transgenic rice was better than that of Lj11. Moreover, the MDA content of overexpressing *OsGP1* lines was lower than that of Lj11, and the SOD and POD activities were significantly higher than those of Lj11 under stress. Therefore, the OsGP1 protein may be involved in the response to saline-alkali stress related pathways and participate in the cell wall resistance against soda saline-alkali stress.

## Acknowledgements

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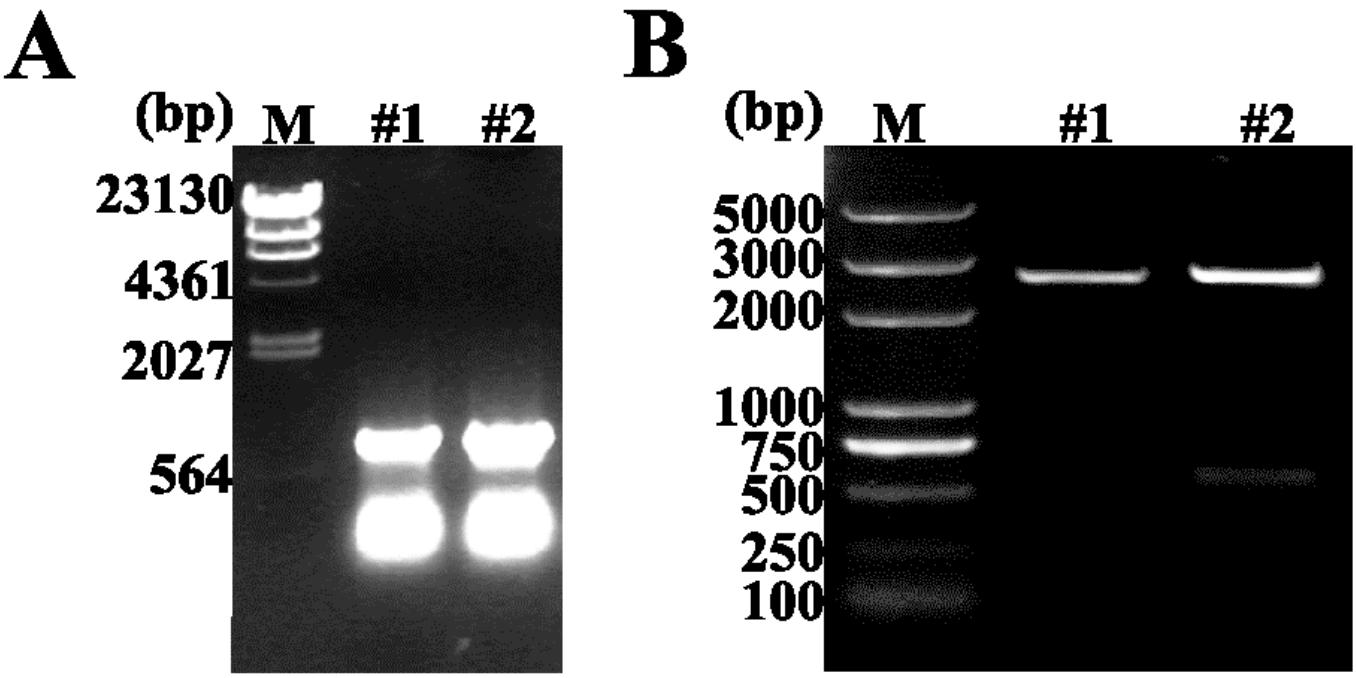
# Figure 1

Electropherogram of *OsGP1* DNA

A, Electropherogram of PCR amplification product ~~of *OsGP1*~~. M, Marker, λDNA digested with HindⅢ; #1 and #2, PCR amplification products of *OsGP1*. B, ~~Identification of~~ pMD18-T-*OsGP1* plasmid digested with SalI/BamHI. M: Marker, DL5000; #1, pMD18-T-*OsGP1* plasmid; #2, pMD18-T-*OsGP1* plasmid digested with SalI/BamHI.



# Figure 2

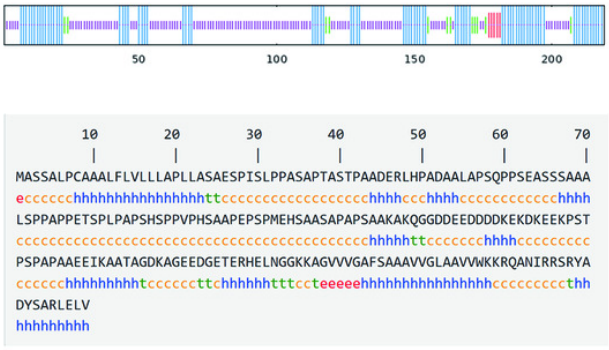
The bioinformatics analysis of OsGP1

A, Nucleotide sequence and deduced amino acid sequence of *OsGP1* coding region. B, Secondary structure prediction. C, Tertiary structure prediction. D, Prediction of the signal peptide. E, Protein interaction relationship. F, Analysis of *OsGP1* promoter.

A

```
1    atg gcg tca tcg gca ttg ccc tgc gcc gcc gcg ctc ttc ctc gtc ctc ctc ctc gcg ccg ctg ctc gcc tcc gcc gag tcg ccc atc tcg   90
1     M   A   S   S   A   L   P   C   A   A   A   L   F   L   V   L   L   L   A   P   L   L   A   S   A   E   S   P   I   S   30

91   ctg ccg cct gcg tcc gcg ccc acc gcc tcc acc ccg gct gca gac gag cgc ctc cac ccc gcc gac gcc gcc ctc gct ccg tcg cag ccg  180
31    L   P   P   A   S   A   P   T   A   S   T   P   A   A   D   E   R   L   H   P   A   D   A   A   L   A   P   S   Q   P   60

181  cct tcc gag gcc tcc tcc tcc gcc gcc gcg ctc tcc cct ccc gcg cct cct gag acc tcc cct ctc ccc gcg ccc tcc cac tcg ccc ccc  270
61    P   S   E   A   S   S   S   A   A   A   L   S   P   P   A   P   P   E   T   S   P   L   P   A   P   S   H   S   P   P   90

271  gtc ccg cat tcc gcg gca ccc gag ccg tcg ccc atg gag cat tcc gcc gcg tcc gcg ccg gcc ccc tcc gcc gcc aag gcc aag cag ggc  360
91    V   P   H   S   A   A   P   E   P   S   P   M   E   H   S   A   A   S   A   P   A   P   S   A   A   K   A   K   Q   G   120

361  ggc gac gac gag gag gac gac gac gat aag gag aaa gac aag gag gag aag ccg tca aca ccg tcg cct gcc ccc gcc gcc gag gag ata  450
121   G   D   D   E   E   D   D   D   D   K   E   K   D   K   E   E   K   P   S   T   P   S   P   A   P   A   A   E   E   I   150

451  aag gcc gcc acc gcg ggc gac aag gcg ggg gag gag gac ggg gag acg gag agg cac gag ttg aac ggc ggc aag aag gcc ggc gtc gtg  540
151   K   A   A   T   A   G   D   K   A   G   E   E   D   G   E   T   E   R   H   E   L   N   G   G   K   K   A   G   V   V   180

541  gtc ggc gcc ttc tcg gcc gcc gcg gtg gtg ggt cta gcc gcc gtc gtg tgg aag aag cgg cag gcc aac atc cgg cgg tcc agg tac gcc  630
181   V   G   A   F   S   A   A   A   V   V   G   L   A   A   V   V   W   K   K   R   Q   A   N   I   R   R   S   R   Y   A   210

631  gac tac tcc gcc cgc ctc gag ctc gtc tga                                                                              660
211   D   Y   S   A   R   L   E   L   V   -                                                                              219
```

B

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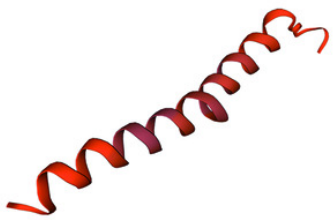

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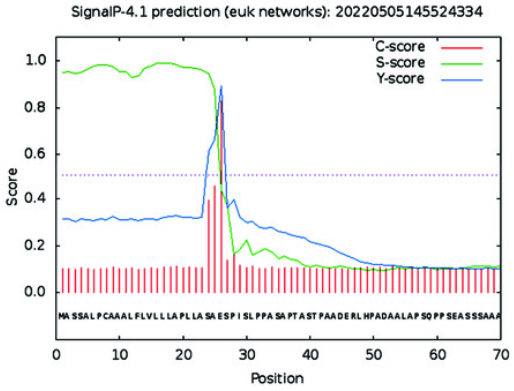


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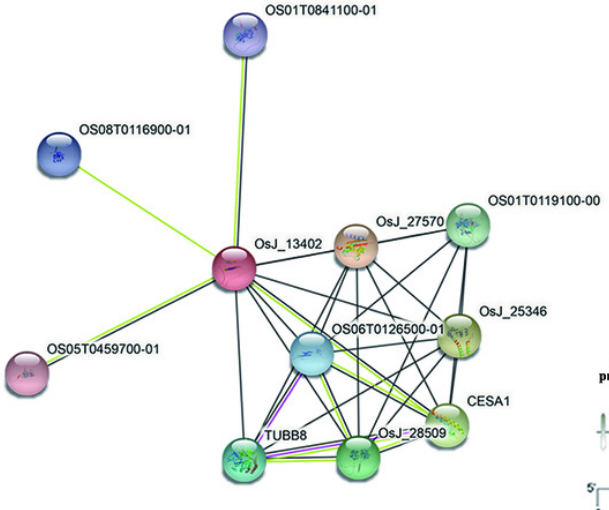
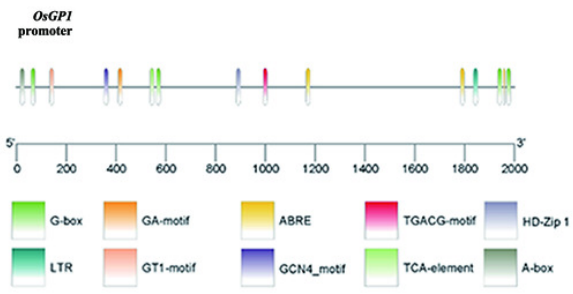


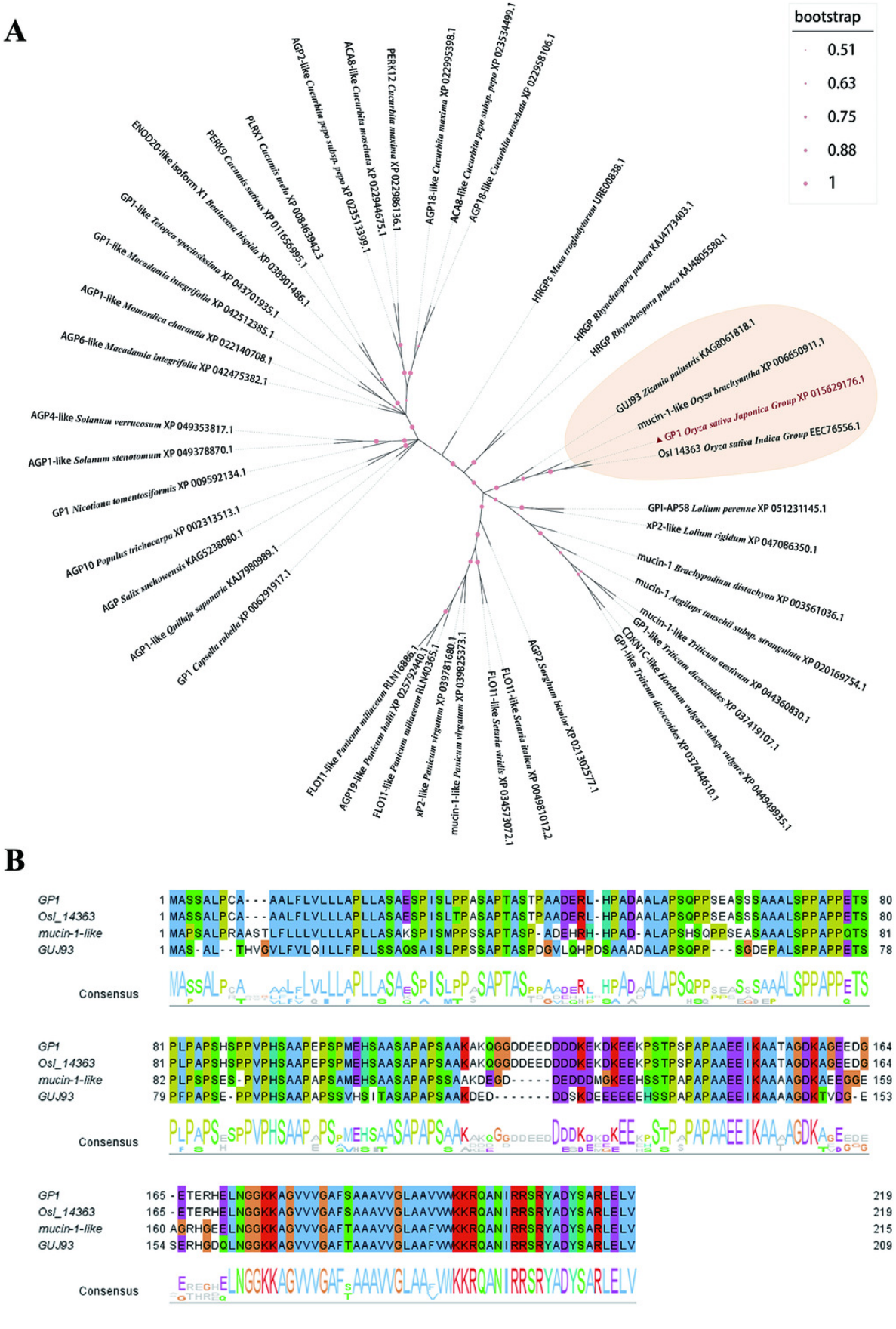

F



# Figure 3

Phylogenetic tree and multiple sequence alignment of OsGP1 protein

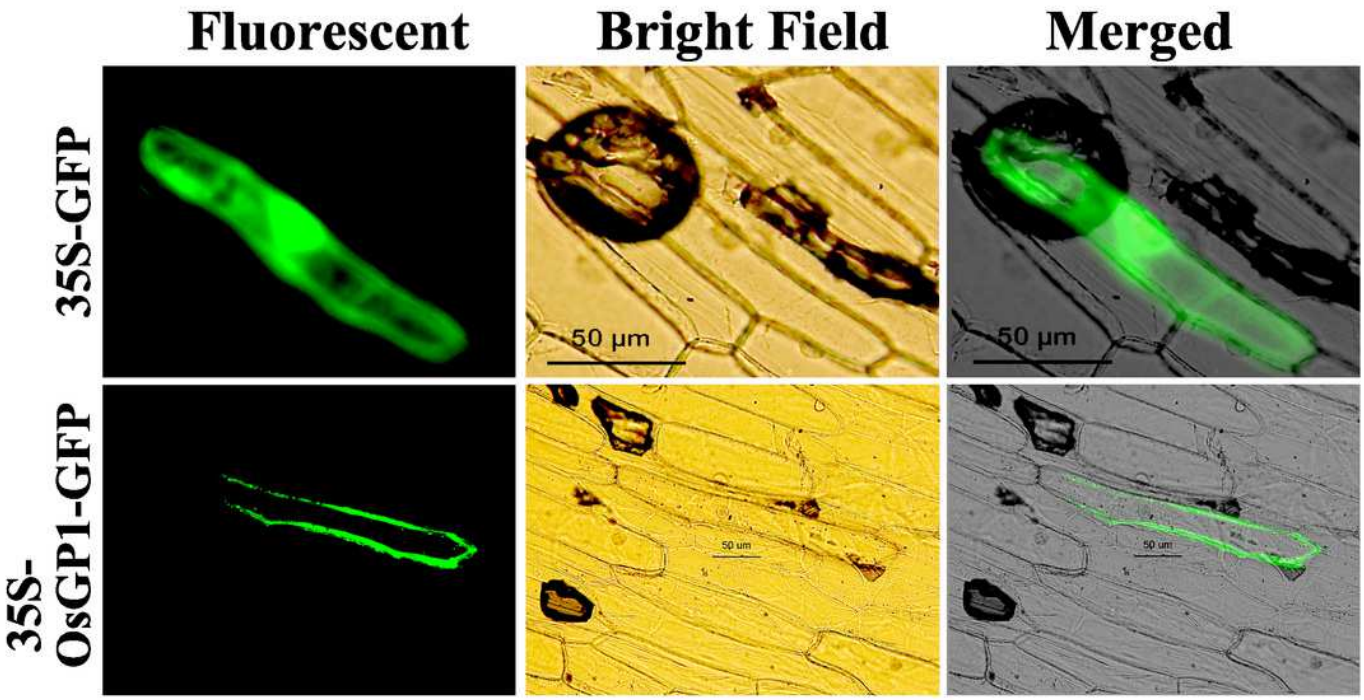
A, Phylogenetic tree. B, Multiple sequence alignment of four sequences in the same branch with OsGP1.


A
bootstrap
0.51
0.63
0.75
0.88
1
AGP2-like Cucurbita pepo subsp. pepo XP 023513399.1
ACA8-like Cucurbita moschata XP 022944675.1
PERK12 Cucurbita maxima XP 022986136.1
AGP18-like Cucurbita maxima XP 022995398.1
ACA8-like Cucurbita pepo subsp. pepo XP 023534499.1
AGP18-like Cucurbita moschata XP 022958106.1
PLRK1 Cucumis melo XP 008463942.3
PERK9 Cucumis sativus XP 011656995.1
ENOD20-like isoform X1 Benincasa hispida XP 038901486.1
GP1-like Telopea speciosissima XP 043701935.1
GP1-like Macadamia integrifolia XP 042512385.1
AGP1-like Momordica charantia XP 022140708.1
AGP6-like Macadamia integrifolia XP 042475382.1
AGP4-like Solanum verrucosum XP 049353817.1
AGP1-like Solanum stenotomum XP 049378870.1
GP1 Nicotiana tomentosiformis XP 009592134.1
AGP10 Populus trichocarpa XP 002313513.1
AGP Salix suchowensis KAG5238080.1
AGP1-like Quillaja saponaria KAJ7980989.1
GP1 Capsella rubella XP 006291917.1
FLO11-like Panicum miliaceum RLN16886.1
AGP19-like Panicum hallii XP 025792440.1
FLO11-like Panicum miliaceum RLN40365.1
xP2-like Panicum virgatum XP 039781680.1
mucin-1-like Panicum virgatum XP 039825373.1
FLO11-like Setaria viridis XP 034573072.1
FLO11-like Setaria italica XP 004981012.2
AGP2 Sorghum bicolor XP 021302577.1
GP1-like Triticum dicoccoides XP 037446610.1
CDKN1C-like Hordeum vulgare subsp. vulgare XP 044949935.1
GP1-like Triticum dicoccoides XP 037419107.1
mucin-1-like Triticum aestivum XP 044360830.1
mucin-1 Aegilops tauschii subsp. strangulata XP 020169754.1
mucin-1 Brachypodium distachyon XP 003561036.1
xP2-like Lolium rigidum XP 047086350.1
GPI-AP58 Lolium perenne XP 051231145.1
HRGP Rhynchospora pubera KAJ4805580.1
HRGP Rhynchospora pubera KAJ4773403.1
HRGP5 Musa troglodytarum URE00838.1
GUJ93 Zizania palustris KAG8061818.1
mucin-1-like Oryza brachyantha XP 006650911.1
GP1 Oryza sativa Japonica Group XP 015629176.1
Osl 14363 Oryza sativa Indica Group EEC76556.1
B
GP1
Osl_14363
mucin-1-like
GUJ93
Consensus

# Figure 4

Subcellular localization of OsGP1-GFP fusion protein in onion epidermal cells

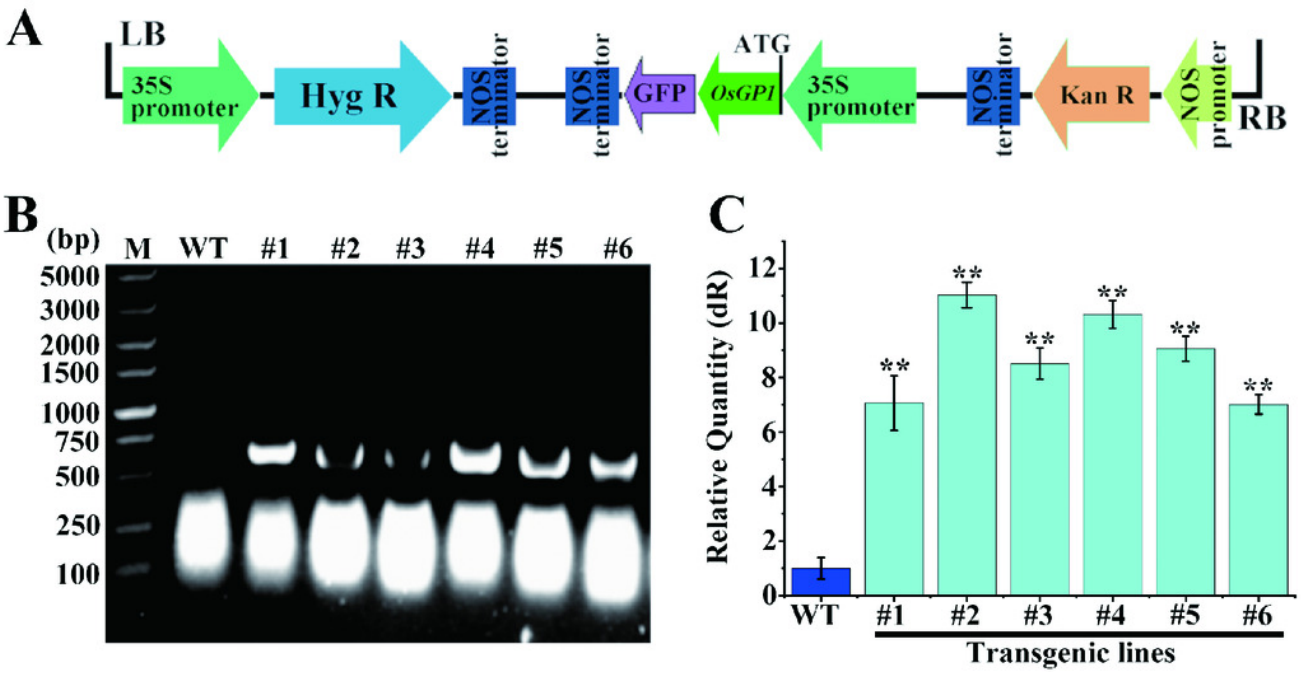
GFP and OsGP1-GFP driven by 35S promoter under green fluorescence, bright field, and merged views. Bar, 50 μm.



# Figure 5

Identification of *OsGP1* transgenic rice lines

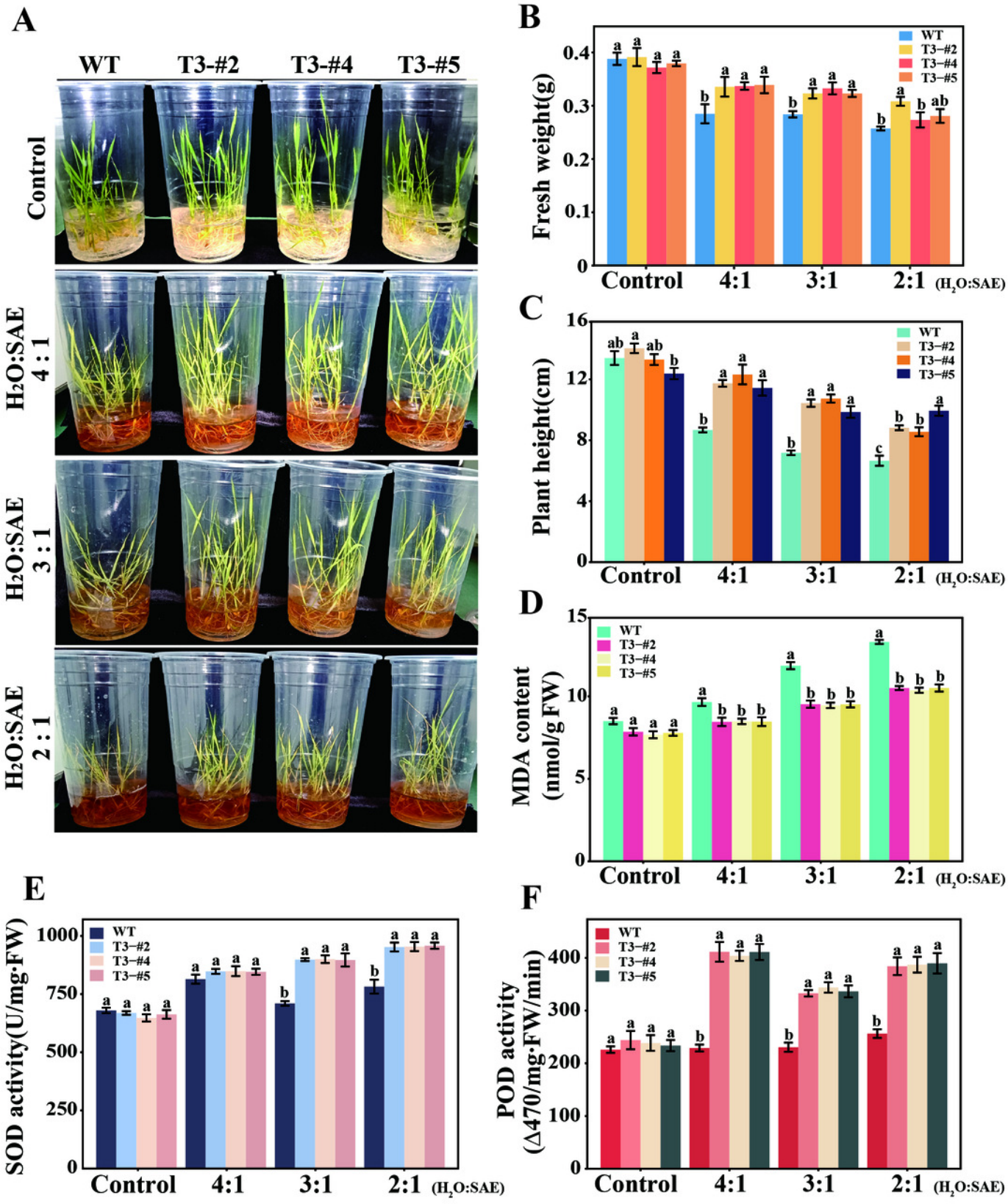
A, Schematic diagram of the T-DNA insertion site in pGWB5-*OsGP1* plasmid. B, Identification of T0 generation transgenic rice. M, Marker, DS5000. C, Expression detection of *OsGP1* in T3 generation transgenic rice. WT, Wild-type line, Lj11; #1-#6, *OsGP1* transgenic lines. Asterisks indicate significant mean differences between *OsGP1* overexpression lines and Lj11 (**$P < 0.01$). Data show the mean ± SD of three replicates.



# Figure 6

Tolerance analysis of rice overexpressing *OsGP1* to soda saline-alkali stress

A, Phenotypes of three-leaf stage overexpression lines T3-#2, #4, #5 and Lj11 seedlings under different ratios of SAE for 7 days, and water as control. B, Fresh weight of 5 seedlings. C, Plant height. D, MDA content. E, SOD activity. F, POD activity. Data show the mean ± SD of three replicates. Statistical differences are labelled with different letters using Duncan test ($P < 0.05$, one-way ANOVA).


A
WT
T3-#2
T3-#4
T3-#5
Control
H2O:SAE 4 : 1
H2O:SAE 3 : 1
H2O:SAE 2 : 1
B
Fresh weight(g)
C
Plant height(cm)
D
MDA content (nmol/g FW)
E
SOD activity(U/mg·FW)
F
POD activity (Δ470/mg·FW/min)
Control
4:1
3:1
2:1 (H2O:SAE)

## Table 1(on next page)

Configuration and characteristics of different ratios of soda saline-alkali soil eluent

Independent triplicate measurements were averaged and the standard deviation (SD) was calculated.

| Leachate | Configuration method | EC (μS·cm-1) | pH |
|---|---|---|---|
| SAE (Stock solution) | 100ml SAE | 10290±36 | 9.65±0.08 |
| $H_2O$:SAE=2:1 | 66ml $H_2O$ + 33ml SAE | 3903±38 | 9.21±0.06 |
| $H_2O$:SAE=3:1 | 75ml $H_2O$ + 25ml SAE | 2927±35 | 9.08±0.05 |
| $H_2O$:SAE=4:1 | 80ml $H_2O$ + 20ml SAE | 2530±26 | 8.95±0.06 |
| Control ($H_2O$) | 100ml $H_2O$ | 82.6±0.1 | 6.98±0.04 |

1

## Table 2(on next page)

Subcellular localization prediction of OsGP1

| location | probability |
|---|---|
| plasma membrane | 0.460 |
| endoplasmic reticulum membrane | 0.280 |
| endoplasmic reticulum | 0.100 |
| outside | 0.100 |

1