DoGMP1 from *Dendrobium officinale* contributes to mannose content of water-soluble polysaccharides and plays a role in salt stress response

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GDP-mannose pyrophosphorylase (GMP) catalyzed the formation of GDP-mannose, which serves as a donor for the biosynthesis of mannose-containing polysaccharides. In this study, three GMP genes from *Dendrobium officinale* (i.e., DoGMPs) were cloned and analyzed. The putative 1000 bp upstream regulatory region of these DoGMPs was isolated and cis-elements were identified, which indicates their possible role in responses to abiotic stresses. The DoGMP1 protein was shown to be localized in the cytoplasm. To further study the function of the DoGMP1 gene, 35S:DoGMP1 transgenic *A. thaliana* plants with an enhanced expression level of DoGMP1 were generated. Transgenic plants were indistinguishable from wild-type (WT) plants in tissue culture or in soil. However, the mannose content of the extracted water-soluble polysaccharides increased 67%, 96% and 92% in transgenic lines #1, #2 and #3, respectively more than WT levels. Germination percentage of seeds from transgenic lines was higher than WT seeds and the growth of seedlings from transgenic lines was better than WT seedlings under salinity stress (150 mM NaCl). Our results provide genetic evidence for the involvement of GMP genes in the biosynthesis of mannose-containing polysaccharides and the mediation of GMP genes in the response to salt stress during seed germination and seedling growth.

GDP-mannose pyrophosphorylase (GMP, E.C. 2.7.7.13), also known as mannose-1-phosphate guanyltransferase, catalyzes the conversion of mannose-1-phosphate to GDP-mannose. A number of nucleotide sugars such as GDP-L-galactose, GDP-L-fucose and GDP-D-rhamnose are synthesized using GDP-mannose as the precursor\(^1,2\). Moreover, GDP-mannose is an important intermediate product related to a wide range of metabolic pathways in plants, such as N-glycosylation\(^3,4\) and the synthesis of ascorbic acid (AsA) and polysaccharides\(^1\). Insertion of the GMP gene into *Saccharomyces cerevisiae* restored the viability of alg1 N-glycosylation mutants\(^5\). GDP-mannose deficiency, which is caused by GMP deficiency, is responsible for N-glycosylation deficiency, and results in inhibited root growth in the presence of NH\(_4\)\(^+\)\(^6,7\).

In plants, the GMP gene is involved in AsA synthesis and has been shown to improve the tolerance of plants under abiotic stress. For example, in an ozone-sensitive *Arabidopsis thaliana* mutant (sozi/vct1/cyt1l) that contained only 30% of the wild-type (WT) AsA concentration, the VCT1 gene, which encodes a GMP, was shown to be responsible for AsA deficiency\(^8,9\). GDP from rice (*Oryza sativa* L.) improved salinity stress tolerance in tobacco (*Nicotiana tabacum* L.)\(^10\). A tobacco GMP is involved in tolerance to temperature stress\(^11,12\).

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Previous studies demonstrated that mannan synthase isolated from plant species such as pea (Pisum sativum L.), fenugreek (Trigonella foenum-graecum L.) and guar (Cyanopsis tetragonoloba L.), used GDP-mannose as a substrate to synthesize a mannan backbone in vitro. The cellulose synthase-like A (CSLA) family, which belongs to the cellulose synthase (CesA) superfamily of glycosyltransferase family 2 (GT2), encodes proteins responsible for mannan polysaccharides by using GDP-mannose as the substrate. Transformants of potato (Solanum tuberosum L.) with reduced GMP activity had 30–50% lower mannose content than WT plants.

Although the vast majority of the characterized GMPs from Arabidopsis thaliana, rice or other higher plant species have been analyzed, it has been proposed that the involvement of GMPs in AsA synthesis and stress tolerance is conserved. However, it is not possible to predict gene functions of possible GMPs based only on nucleotide or amino acid sequence similarities. For example, a probable GDP-mannose pyrophosphorylase (TAIR number: AT1G74910), predicted by amino acid sequence similarity, lacks GDP-mannose pyrophosphorylase activity.

In this study, we report on the cloning and characterization of three GMPs from D. officinale. We generated 35S:DoGMP1 A. thaliana transgenic lines, studied the relationship between DoGMP1 and mannose content of water-soluble polysaccharides, and assessed the tolerance of these lines to salinity stress. This work can provide insight into understanding the molecular mechanisms of polysaccharide biosynthesis in D. officinale. Furthermore, this work also has implications for the development of abiotic stress-tolerant crops to overcome environmental stress limitations and improve production efficiency in the face of a burgeoning world population.

Materials and Methods

Plant materials and growth conditions. Potted D. officinale plants used to clone genes were grown and maintained in a greenhouse (Guangzhou, Guangdong, China) under natural conditions. The stems of D. officinale (about one year old) were harvested, frozen rapidly in liquid nitrogen and kept at −80 °C until RNA extraction. A. thaliana (ecotype Columbia) was used as the WT in this study. WT and DoGMP1 overexpression lines were cultured in a growth chamber in a 16-h photoperiod (100 µmol m−2 s−1) at 22 °C. Plants were grown in pots (8 × 10 cm, diameter × height) filled with soil (topsoil and vermiculite; 1:2) and watered periodically with Hyponex fertilizer (N:P=6–10–5, diluted 1,000-fold; Hydroponic Chemicals Co., Ohio, USA).

Cloning GDP-pyrophosphorylase genes from D. officinale. According to the annotation of unigenes of an in-house transcriptome reference database of sequences, GDP-mannose pyrophosphorylase unigenes were identified and used to design primers. The total RNA of D. officinale was isolated by using Column Plant RNAout2.0 (Tiangz., Inc., Beijing, China) according to the manufacturer's protocol. Two µg of total RNA were reverse transcribed for the first-strand cDNA, which served as the template to generate 3′ and 5′ cDNA ends by using M-MLV reverse transcriptase (Promega, Madison, Wisconsin, USA). The SMARTer RACE cDNA Amplification Kit (Clontech Laboratories Inc., Mountain View, USA) was used to generate both 5′ and 3′ cDNA according to the manufacturer's protocol. PCR products were purified by a Gel Extraction Kit (Dongsheng, Biotech, Guangzhou, China), cloned into the pMD18-T vector (Takara Bio Inc., Dalian, China) and sequenced at the Beijing Genomics Institute (Shenzhen, Guangdong, China). Primer pairs for each gene designed to amplify 3′ and 5′ cDNA regions are listed in Supplementary Table 1.

Isolation and analysis the putative promoters of DoGMPs. To understand the regulatory mechanism of DoGMPs, the Genome Walking Kit (Takara Bio Inc.) was used to clone the putative promoters of DoGMPs according to the user's manual. Primers specific for each gene were designed by Primer Premier 5.0 (PREMIER Biosoft Palo Alto CA USA) and listed in Supplementary Table 2. The putative promoters were used to analyze the cis-regulatory elements by an on-line prediction soft (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

DoGMP1-YFP plasmid construction and localization analysis. The full-length coding sequences of the DoGMP1 gene (excluding the termination codon) were amplified with a pair of primers (DoGMP1YFPF/DoGMP1YFPR, listed in Supplementary Table 3) introduced as adaptor sequences at the 5′ and 3′ ends according to the pSAT6-EYFP-N1 vector sequences. The principles of adaptor sequence design followed In-Fusion® HD Cloning Kit (Clontech Laboratories, Inc.) instructions. The amplified product was inserted downstream of the 35S Cauliflower mosaic virus (CaMV) promoter in the unique Ncol site of the pSAT6-EYFP-N1 vector by using the In-Fusion® HD Cloning Kit according to the manufacturer's instructions. The DoGMP1-YFP recombinant plasmid was verified by DNA sequencing at the Beijing Genomics Institute. Transient transformation was performed with 10 µg of plasmid DNA transferred into mesophyll protoplasts from a 4–5 weeks-old A. thaliana plant by a polyethylene glycol (PEG)–mediated transfection system described by Yoo et al.21. Protoplasts were incubated for 20 h under standard light/dark conditions then yellow fluorescent protein (YFP) was localized via fluorescence microscopy. YFP fluorescence was visualized by a Zeiss LSM 510 confocal microscope (Zeiss, Jena, Germany).

Construction of DoGMP1 overexpression vector and Arabidopsis thaliana transformation. The full-length coding sequences of the DoGMP1 gene (excluding the termination codon) were amplified and cloned into the pCAMBIA1302 vector at the Ncol site, driven by the 35S CaMV promoter. After verification by full sequencing at the Beijing Genomics Institute, the recombinant vector was transformed into Agrobacterium tumefaciens EHA105 by using the freeze-thaw method then used for A. thaliana transformation. Transgenic plants were generated by a floral dip transformation method. The primer pairs designed for construction of 35S:DoGMP1 vector are listed in Supplementary Table 3.

Western blot assay. Seven-day-old A. thaliana seedlings (0.5 g), grown on half-strength Murashige and Skoog medium containing 2% sucrose and 0.8% agar (pH 5.7) (basal medium, BM), were harvested and immediately ground in liquid nitrogen with a mortar and pestle. Cells were lysed in 700 µL extraction buffer (50 mM...
Tris-HCl at pH 7.4, 150 mM NaCl, 2 mM MgCl₂, 1 mM dithiothreitol (DTT), 20% glycerol, 1% Nonidet P-40) containing a protease inhibitor cocktail (Cat. No. 04693132001, Roche, Basel, Switzerland), then centrifuged at 4 °C and 14,000 g for 20 min. The supernatants containing total proteins were fractionated by SDS-PAGE and analyzed on Western blots. Immunoprecipitated recombinant DoGMP1-GFP fusion proteins were visualized on Western blots with an anti-GFP antibody (product code ab290, Abcam, Cambridge, U.K.) and goat anti-rabbit IgG-HRP (catalog number sc-2301, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA).

**Measurement of and mannose content of water-soluble polysaccharides.** The above-ground parts (leaves, flowers and stems) from two-month-old *A. thaliana* plants in the reproductive stage were used to determine water-soluble polysaccharide content. Samples were powdered by a DFT-50 pulverizer (Xinno Instrument Equipment Inc., Shanghai, China) after drying in an oven at 85 °C for 6 h. The supernatants containing total proteins were fractionated by SDS-PAGE and analyzed on Western blots. Immunoprecipitated recombinant DoGMP1-GFP fusion proteins were visualized on Western blots with an anti-GFP antibody (product code ab290, Abcam, Cambridge, U.K.) and goat anti-rabbit IgG-HRP (catalog number sc-2301, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA).

**Germination assays under salinity stress.** Seeds of all genotypes used in germination assays were grown simultaneously, and harvested and stored under similar conditions. Three transgenic lines of T5 homozygote plants and WT plants were used to determine water-soluble polysaccharide content. Samples were powdered by a DFT-50 pulverizer (Xinno Instrument Equipment Inc., Shanghai, China) after drying in an oven at 85 °C for 6 h. The supernatants containing total proteins were fractionated by SDS-PAGE and analyzed on Western blots. Immunoprecipitated recombinant DoGMP1-GFP fusion proteins were visualized on Western blots with an anti-GFP antibody (product code ab290, Abcam, Cambridge, U.K.) and goat anti-rabbit IgG-HRP (catalog number sc-2301, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA).

**Salinity stress treatment for Arabidopsis thaliana seedlings.** Sterilized seeds were germinated on BM at 22 °C under a 16-h photoperiod (100 μmol m⁻² s⁻¹) after stratification for 2 days at 4 °C in the dark.
Five-day-old seedlings were transferred to fresh BM supplemented with 150 mM NaCl and cultured at 22 °C under a 16-h photoperiod. Root length was measured and photographs were taken after 7 days. Twelve plants were used in each experiment, and all experiments were repeated three times.

Hydrogen peroxide staining. A. thaliana seedlings (12-d old) that were grown on BM were transferred to fresh BM supplemented with 150 mM NaCl, or not, and then cultured at 22 °C under a 16-h photoperiod. Seedlings transferred to BM served as the control. Seedlings were harvested after 48 h and stained with 3,3′-diaminobenzidine (DAB) (D5637, Sigma-Aldrich)25. Eight plants of each treatment were used in each analysis, and all experiments were repeated three times.

Semi-quantitative RT-PCR. One hundred sterilized seeds of transgenic lines #1 and #3, as well as WT were germinated on BM at 22 °C under a 16-h photoperiod (100 μmol m⁻² s⁻¹). Total RNA was extracted from seven-day-old A. thaliana seedlings using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. Two μg of each RNA sample were reverse transcribed for the first-strand cDNA using M-MLV reverse transcriptase (Promega, Madison, WI, USA) according to the manufacturer’s instructions after treatment with RNase-free DNase (Takara Bio Inc.) to remove any residual genomic DNA. The DreamTaq™ Green PCR Master Mix Kit (Takara Bio Inc.) was used for amplification. The following thermocycling conditions were applied: initial denaturation at 94 °C for 1 min; 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min; final extension at 72 °C for 10 min in a LabCycler Standard Plus PCR system (SENSOQUEST, Hannover, Germany). The amplified products were separated on a 1.5% agarose gel stained with ethidium bromide (EtBr) and photographed in a Bio Sens SC 710 system. The gene-specific primers for DoGMP1, which was used in semi-quantitative RT-PCR, and the A. thaliana ubiquitin 10 gene (AtUBQ10, TAIR accession number: AT4G05320.2), which was used as the control, are listed in Supplementary Table 4. AtUBQ10 was used as the internal control based on the advice of Zhao et al.26.

Quantitative real-time PCR (qRT-PCR) analysis. The cDNAs described above also used for qRT-PCR analysis. The gene-specific primer pairs were designed for qRT-PCR by online Primerquest software (listed in...
Supplementary Table 4). qRT-PCR was performed using the SYBR Premix Ex Taq™ Kit (Takara Bio Inc.) in an ABI 7500 Real-time system (ABI, CA, USA). Amplification conditions were 95 °C for 2 min, followed by 40 cycles of amplification (95 °C for 15 s, 60 °C for 1 min) and plate reading after each cycle. *AtUBQ10* served as the control based on the recommendation of Zhao et al. The gene-specific primers used for qRT-PCR are listed in Supplementary Table 4.

**Statistical analyses.** All data were analyzed using SigmaPlot12.3 software (Systat Software Inc., San Jose, California, USA) using one-way analysis of variance (ANOVA) followed by Dunnett's test. *P < 0.05* was considered to be statistically significant.
Mannose-1-phosphate binding site of GMP27. To examine the relationship between the three DoGMP proteins, a nucleotidyl transferase domain, a metal binding site (D, DxG or D, QxK) and the search for conserved domains, GMP proteins were shown to contain four conserved motifs: the pyrophosphatase of these primary sequences indicated that these proteins had a similar catalytic function. In a further analysis, confirming that DoGMP1 protein is indeed a cytoplasmic protein (Fig. 2).

MEGA 428. These were divided into two groups: GMPA and GMPB. DoGMP1 and DoGMP3 formed part of the GMPB group while DoGMP2 made up the GMPA group (Fig. 1).

Analysis of stress-related cis-regulatory elements in the putative promoters of DoGMPs. Studies have demonstrated that GMP family members are involved in tolerance to abiotic stresses. To understand the possible stress-related cis-regulatory elements in the promoter regions of DoGMPs, about 1200, 1000 and 1800 bp of the translation start site of DoGMP1, DoGMP2 and DoGMP3 were obtained namely, respectively. 1 kb of the promoter sequences of each gene was used to predict the stress-related cis-regulatory elements (Supplementary Text 1–3). A low-temperature responsiveness element and defense and stress responsiveness elements were found both in DoGMP1 and in DoGMP2 (Fig. 3). The HSE element, which is involved in heat stress responsiveness, was found both in DoGMP1 and in DoGMP3 (Fig. 3). In addition, the three DoGMPs contained several plant hormone responsiveness elements such as an ethylene responsive element, a salicylic acid (SA) inducible element, a methyl jasmonate (MeJA) inducible element and an abscisic acid (ABA) responsive element (Fig. 3).

Mannose content of water-soluble polysaccharides increased in 35S:DoGMP1 transgenic lines. The vtc-1 (known as cyt1) mutant of A. thaliana is hypersensitive to abiotic stresses such as ozone stress, sulfur dioxide, ultraviolet B irradiation and salt stress. This indicates that AtCYT1 plays an important role in improving the abiotic stress tolerance of plants. Comparison of the GMP protein sequences in A. thaliana and...
D. officinale revealed that DoGMP1 was most similar to AtCYT1 and may thus play a similar role. Therefore, to analyze the functional role of the DoGMP1 gene, transgenic A. thaliana plants that constitutively overexpressed the DoGMP1 gene driven by the CaMV 35S promoter (35S:DoGMP1-GFP) were generated (Fig. 4A). Semi-quantitative RT-PCR and qRT-PCR were used to determine DoGMP1 gene expression in transgenic plants. The DoGMP1 gene could not be detected in WT plants but was detected in all three transgenic lines (Fig. 4B,C). Western blot analyses confirmed the expression of DoGMP1-GFP fusion proteins of correct size in all of the transgenic lines (Fig. 4D). No obvious phenotypic changes were observed among WT and transgenic plants when cultured in soil (Fig. 4E).

GDP-mannose, the product of the GMP enzyme, is the mannose donor for the synthesis of mannose-containing polysaccharides. To gain insight into the mannose content of these extracted polysaccharides, mannose content was determined by HPLC, which showed that mannose content in the transgenic lines increased significantly more than in the WT plant (Fig. 5). Over-expression of the DoGMP1 gene resulted in an obvious increase in the content of mannose in A. thaliana plants, suggesting that the DoGMP1 gene is involved in the biosynthesis of water-soluble polysaccharides, which are mannose-containing polysaccharides. Previous studies demonstrated that the backbone of mannan synthase, which is encoded by CSLA family members, is made by using GDP-mannose as the donor15,35. The CSLA family belongs to the CesA superfamily and includes nine CSLA members in A. thaliana: AtCSLA1, AtCSLA2, AtCSLA3, AtCSLA7, AtCSLA9, AtCSLA10, AtCSLA11, AtCSLA14 and AtCSLA1536. Since the mannose content increased significantly when the DoGMP1 gene was overexpressed...
in *A. thaliana*, the relative expression level of these nine AtCSLA genes were analyzed. *AtCSLA1*, *AtCSLA7*, *AtCSLA10*, *AtCSLA11*, *AtCSLA14* and *AtCSLA15* were significantly down-regulated in all 35S:DoGMP1 transgenic lines and WT. *p* < 0.05, ** indicates *p* < 0.01 between WT and transgenic lines by ANOVA/Dunnet's test. Each data bar represents the mean ± SD of 100 seeds. Bar = 0.5 mm. WT, wild-type; three 35S:DoGMP1 transgenic lines: line #1, line #2 and line #3.

**Germination of 35S:DoGMP1 transgenic lines improved under salinity stress.** Salinity, a critical factor influencing seed germination, reduces germination rate and delays seed emergence. Soluble sugars or polysaccharides may play important roles in the mechanisms of salt defense. To investigate the effect of salinity stress on the germination of seeds from WT and transgenic lines, seeds were germinated on BM containing 150 mM NaCl (or not) to explore germination ability under salt stress. In the control group, seeds from all three transgenic lines germinated later than WT seeds one day after stratification, although seeds from WT and transgenic lines showed no obvious differences in the following days when germination was scored (Fig. 6A). In contrast, a significant difference in germination was observed between 35S:DoGMP1 lines and WT when MS medium was supplemented with 150 mM NaCl. The three 35S:DoGMP1 transgenic lines exhibited a higher germination percentage than WT seeds on all scoring days (Fig. 7B). In particular, two days after stratification on 150 mM NaCl there was a significant difference in the germination percentage of 35S:DoGMP1 lines #1, #2 and #3 (65.7%, 72.0%, and 69.0%, respectively) compared with 26.0% for WT seeds (Fig. 7B). The seedlings of both WT and the three transgenic lines showed a similar phenotype when germinated on half-strength MS medium without NaCl (Fig. 7C). However, roots of transgenic lines were longer than the roots of WT seedlings and seedling
growth was more vigorous in transgenic lines than WT seedlings when seeds were germinated under salinity stress at 7 days after stratification (Fig. 7C). This implies that DoGMP1 plays a role as a positive regulator in seed germination of *A. thaliana* under salt stress.

To analyze the growth of transgenic seedlings under salinity stress, five-day-old seedlings of 35S:DoGMP1 transgenic *A. thaliana* versus WT plants were grown on BM containing 150 mM NaCl (or not) to further characterize the response of the over-expressing transgenic plants to abiotic stress at the seedling stage. When the seedlings of WT and transgenic lines were grown under control conditions, no obvious differences were observed in root length (Fig. 8A,C). However, in the presence of 150 mM NaCl, the roots of WT plants were markedly shorter than those of transgenic lines (Fig. 8B,C). The root length of WT seedlings was about 0.75 cm, while that of transgenic lines #1, #2 and #3 was 1.39, 1.33, and 1.24 cm, respectively. The salinity stress test showed that transgenic seedlings could grow better than WT seedlings under salinity stress. These results suggest that DoGMP1 is able to provide *A. thaliana* seedlings with tolerance to salinity stress.

To investigate whether the better growth of transgenic lines relative to WT was associated with accelerated ROS accumulation, DAB staining was carried out in WT and transgenic lines after control and salt stress treatment. No coloration was observed on the leaves when the seedlings of WT and transgenic lines were cultured in...
BM while the roots were slightly stained (Fig. 9A). In contrast, the leaves and roots from both WT and transgenic lines under salt stress were strongly stained relative to the control (Fig. 9B). The WT seedlings contained a higher level of H₂O₂ in leaves and root tips than seedlings of transgenic lines when cultured in BM supplemented with 150 mM NaCl (Fig. 9B).

Discussion

GMP catalyzes the reaction from mannose-1-phosphate to GDP-mannose and uses cofactor Mg²⁺ as a key switch for effective and continuous enzyme production⁴²,⁴³. Two types of GMP genes, namely GDP-mannose pyrophosphorylase A (GMPA) and GDP-mannose pyrophosphorylase B (GMPB), were found in fungus (Saccharomyces cerevisiae)⁴⁴,⁴⁵, pig (Sus scrofa)⁴⁶, humans (Homo sapiens)²⁹ and a higher plant, rice¹⁰. The protein sequences of GMPA and GMPB are very similar, but those of GMPA members are generally longer than those of GMPB members. Three DoGMP genes cloned from D. officinale showed high similarity with GMP sequences of other plant species (Supplementary Fig. 1). DoGMP1 and DoGMP3 belong to the GMPB group while DoGMP2 is a member of the GMPA group (Fig. 1). These three DoGMP proteins are likely to have the same catalytic function similar to other GMP proteins. DoGMP1 was localized in the cytoplasm, as was observed in other species such as Leishmania parasites, Homo sapiens and O. sativa¹⁰,⁴⁷,⁴⁸.

Studies have demonstrated that GDP-mannose is the active nucleotide sugar that provides mannose for the biosynthesis of mannan polysaccharides by mannosyltransferase⁴⁹. Transgenic potato plants whose GMP was inhibited by an antisense construct, showed 30–50% less mannose content than WT level⁴. In the present study, the DoGMP1 gene contributed to the mannose content of water-soluble polysaccharide, consistent with previous studies. This indicates that the DoGMP1 gene plays an important role in mannose-containing polysaccharide synthesis in D. officinale. Feedback regulation is very common in plant metabolism to maintain a balance. For example, carbon metabolites have feedback regulation during photosynthesis⁵⁰ while amino acids play a role in the feedback regulation of amino acid biosynthetic pathways⁵¹. Six genes (AtCSLA1, AtCSLA7, AtCSLA10, AtCSLA11, AtCSLA14 and AtCSLA15) related to mannose-containing polysaccharide synthesis were down-regulated in all of transgenic A. thaliana lines (Fig. 6). The AtCSLA genes were down-regulated significantly when BM was supplemented with 10 mM exogenous mannose (Supplementary Fig. 2). This suggests that high mannose-containing polysaccharide content has a negative feedback regulation of upstream genes.

Salinity stress has negative effects on seed germination and plant growth, and osmotic stress decreases water potential, consequently restricting water uptake by dry seeds and roots⁵²,⁵³. Water uptake during seed germination can be divided into three phases: Phase I, the initial absorption of water (imbibition), is primarily a physical...
process; Phase II, a plateau with stable water content; Phase III, water uptake by protrusion of the radicle\(^2\)\(^3\)\(^4\)\(^5\). At the imbibition stage, proteins and polysaccharides are involved in the absorption of water by the dry seed\(^6\). As salinity stress builds up, water potential declines, limiting the germination of seeds by negative water potentials. Low molecular weight sugars such as monosaccharides and oligosaccharides serve as an important compatible solute to assist plants in dealing with osmotic stress caused by salinity stress\(^7\). Water-soluble polysaccharides may serve as a hydrophilic substance and help dry seeds to initially absorb water. The content of low molecular weight sugars, which were extracted by hot 80% ethanol, in the mature seeds of 35S:DoGMP1 transgenic lines #1–3 was 54.3, 59.9 and 58.6 mg·g\(^{-1}\) DW, respectively, while that of WT was 42.3 mg·g\(^{-1}\) DW (Supplementary Fig. 3A). The content of water-soluble polysaccharides in the mature seeds of the three transgenic lines was also significantly higher (35.6, 40.4 and 37.7 mg·g\(^{-1}\) DW, respectively) than that of WT plants (31.6 mg·g\(^{-1}\) DW, Supplementary Fig. 3B). The mature seeds of 35S:DoGMP1 transgenic lines had a higher content of low molecular weight sugars and water-soluble polysaccharides than WT plants. This may help transgenic seeds absorb water from a saline environment at the imbibition phase. Thus, the germination of seeds of transgenic lines was higher than that of WT seeds.

Salinity stress not only intervenes in seed germination, but also influences seedling establishment. The decrease in water potential caused by salinity stress results in reduced cell growth, root growth and shoot growth\(^2\). The root growth of WT plants was distinctly inhibited by salinity stress (Figs 7C and 8). Salt stress reduces the ability of plants to take water up from the soil\(^8\)\(^9\). To deal with water stress, plants accumulate compatible solutes such as proline and sugars to reduce the cell water potential and absorb water from soil\(^10\). The roots of transgenic plants contained higher levels of low molecular weight sugars and water-soluble polysaccharides than WT plants (Supplementary Fig. 4), which can be used as compatible solutes to reduce cell water potential allowing water to be absorbed from a saline environment. Furthermore, excessive radical oxygen species (ROS) are produced when plants undergo various environmental stresses such as drought, chilling, metal toxicity, UV-B radiation and salinity\(^11\). GMPs are involved in the synthesis of AsA, which acts as a non-enzymatic antioxidant playing important roles in plants’ responses to stresses, and serves to eliminate excess ROS in plant cells\(^12\)\(^13\)\(^14\). The AsA content increased significantly in the three transgenic lines (5.31, 5.37 and 6.71 \(\mu\)mol·g\(^{-1}\) FW, respectively; Supplementary Fig. 4) compared to the WT plant (3.70 \(\mu\)mol·g\(^{-1}\) FW). This is likely to have increased ROS scavenging. In DoGMP1-overexpressing plants, \(\text{H}_2\text{O}_2\), one form of ROS, was found at low levels than in WT plants under salt stress, as revealed by DAB staining (Fig. 9). These results suggest that DoGMP1 may enhance salt stress tolerance through the up-regulation of water-soluble sugars and AsA, thereby reducing ROS. In contrast, WT plants are unable to mediate the equilibrium of ROS production and scavenging, making the uptake of water from a saline medium very difficult, and subsequently suffering from oxidative damage and water stress, eventually developing a stressed phenotype.

In conclusion, three DoGMP genes were cloned from an important traditional Chinese herb, \(D. officinale\), which contains abundant mannose-containing polysaccharides. The DoGMP1 protein located in the cytoplasm catalyzed the synthesis of GDP-mannose. These results provide evidence for the involvement of the DoGMP1 gene in the biosynthesis of mannose-containing polysaccharides, paving the way for studies on the biosynthesis of bioactive polysaccharides in \(D. officinale\). 35S:DoGMP1 transgenic plants showed higher germination and grew better than WT plants under salinity stress. This indicates that the GMP gene can be utilized as a candidate gene for improving abiotic stress tolerance in plants.

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**Author Contributions**
J.D. conceived the experiments and experimental design. J.L. designed the draft manuscript. C.H. analyzed the data. Z.Y. conducted mannose content analysis and DAB staining. X.W. cloned the *DoGMP3* gene. K.W., J.Z., J.T. and X.Z. conducted tissue culture and cultivated plants. C.H., JATdS, X.L., S.Z. and G.M. collectively interpreted the results and wrote all drafts of the manuscript. All 13 authors approved the final draft for submission and take full public responsibility for the content of the manuscript.

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