AgGMP encoding GDP-D-mannose pyrophosphorylase from celery enhanced the accumulation of ascorbic acid and resistance to drought stress in Arabidopsis

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ABSTRACT

Ascorbic acid (AsA) is an important nutrient in celery, the conversion of D-mannose-1-P to GDP-D-mannose catalyzed by GDP-D-mannose pyrophosphorylase (GMPase) represents the first committed step in the biosynthesis of AsA. To clarify the function of the AgGMP gene of celery, the AgGMP gene was cloned from celery cv. ‘Jinnan Shiqin’. It contains an open reading frame (ORF) with the length of 1,086 bp, encoding 361 amino acids. AgGMP protein was highly conserved among different plant species. Phylogenetic analysis demonstrated that the GMP proteins from celery and carrot belonged to the same branch. AgGMP protein was mainly composed of three α-helices and certain random coils. No signal peptide was found in the AgGMP protein. The subcellular localization indicated that the AgGMP protein was located in the cytoplasm. The relative expression levels of AgGMP in ‘Jinnan Shiqin’ were significantly up-regulated at 2 h and 4 h under drought stress treatments. AsA contents in transgenic Arabidopsis lines hosting AgGMP gene were higher than that in wild type plants, and the root lengths were also longer in the MS medium containing 300 mM mannitol. The present study provides useful evidence for the functional involvement of AgGMP in regulating AsA accumulation and response to drought stress in celery.

INTRODUCTION

GDP-D-mannose pyrophosphorylase (GMPase) is a rate-limiting enzyme in the L-galactose pathway, a key biosynthetic pathway for L-ascorbic acid (AsA) in plants. In this pathway, the initial step is the formation of GDP-D-mannose, which is catalyzed by GMPase (Wang et al., 2011). AsA is a water-soluble anti-oxygenic organic micromolecule widely found in plants. It’s essential for cardiovascular function, immune cell development,
connective tissue health, and iron utilization. As a key enzyme in AsA biosynthesis, GMPase in higher plants plays important roles in removing reactive oxygen species (ROS) generated by adverse environmental conditions (Xue et al., 2018), such as high temperature, low temperature (Li et al., 2018a; Liu et al., 2019; Huang et al., 2016), and salt stress (Zhang et al., 2012). In addition, the resistance of abiotic stress in plants could be improved through modulating GMP gene expression to increase the AsA content. Until now, GMP genes have been isolated from a number of higher plants, including Arabidopsis thaliana (Wang, Guo & Tang, 2011), Camellia sinensis (Xiao et al., 2015), Solanum esculentum (Wang et al., 2011), and Dendrobium huoshanense (He, Yu & Tei, 2017).

Plant resistance against abiotic stress was associated with the changes of antioxidant defense system, which consisted of enzymatic and non-enzymatic antioxidants (Garg, Varshney & Jain, 2014; Hasanuzzaman et al., 2017; Li et al., 2018a; Liu et al., 2021). AsA belongs to non-enzymatic antioxidants (Liu et al., 2019), drought stress affects AsA content by controlling the activities of enzymes involved in AsA metabolism (e.g. GMP, GalDH, APX, DHAR, MDHAR) (Huang et al., 2016; Liu et al., 2019). AsA can directly remove ROS (reactive oxygen species) produced by stress, and also indirectly remove H$_2$O$_2$ through the AsA-GSH cycle to protect tissues from harmful oxidative products and to keep certain enzymes in their required reduced forms (Liebler, Kling & Reed, 1986; Padh, 1990; Barth, Gouzd & Steele, 2009), as well as improve the ability of plants to resist abiotic stress.

Under low and high temperature stresses, the relative expression of SlGMP, GMPase activity, contents of AsA and dehydroascorbic acid (DHA) were increased, and the content of malondialdehyde (MDA) was decreased in transgenic tomato plants (Wang et al., 2011). The overexpression of SlGMP also delayed the senescence of potato (Lin et al., 2011). OsGMP gene affected AsA synthesis and GMPase activity in rice (Oryza sativa), and overexpression of OsGMP reduced the inhibitory effect of NH$_4^+$ on root growth in A. thaliana (Li et al., 2010). The OsGMP gene also was reported to play a key role in the rice during its nutritional and reproductive stages under salt stress (Kempinski, Haffar & Carina, 2011; Qin et al., 2016a, 2016b). The GmGMP1 gene can be induced to express under high temperature, low temperature, drought and salt stresses. The overexpression of GmGMP1 gene in A. thaliana and soybean (Glycine Max) increases GMPase activity and AsA content, and enhances the ability for eliminating ROS (Xue et al., 2018). The tobacco (Nicotiana Tabacum) harboring PpGMP showed stronger resistance to salt or drought stresses by increasing the AsA content (Ai, Liao & Li, 2016). AtGMP positively regulates the synthesis of AsA. AsA content in transgenic lettuce (Lactuca sativa) overexpressing AtGMP gene increased to 2.5-fold of that in control (Wang, Guo & Tang, 2011).

Celery (Apium graveolens) growth is influenced by multiple environmental factors. In previous studies, it is reported that GMP gene participated in plant response to stress. We speculated that AgGMP may be involved in celery resisting abiotic stress through modulating the AsA accumulation. However, the characteristics and transcription regulation mechanism of AgGMP under drought stress in celery remain unclear. In this study, AgGMP, a gene encoding GDP-D-mannose pyrophosphorylase, was cloned from celery, and then its expression patterns were detected in celery under abiotic stress...
treatments. The transgenic *A. thaliana* plants overexpressed *AgGMP* were obtained to examine the AsA level and compared their root growth with wild-type under drought stress. This study further clarified the roles of *AgGMP* in abiotic stress, and provided a theoretical basis for stress response of celery.

**MATERIALS AND METHODS**

**Plant materials, growth conditions and stress treatments**
Celery cv. 'Jinnan Shiqin', *A. thaliana* ecotype Columbia (WT), and transgenic *A. thaliana* were grown in pots within a soil/vermiculite mixture in phytotron at the Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University (32°03′N, 118°84′E). The phytotron program was 25 °C/18 °C (day/night) for 16 h/8 h. The light intensity was 300 μmol m⁻² s⁻¹ at daytime, with relative humidity of 75%. Two-month-old (day after germinating) 'Jinnan Shiqin' plants were grouped and treated with 4 °C, 38 °C, 200 g·L⁻¹ PEG 6000 and 200 mM NaCl, respectively. The leaf blades of celery with the longest petiole were collected at 0, 1, 2, 4, 8, and 24 h after treatments. All the samples were frozen in liquid nitrogen immediately and then stored at −80 °C for RNA extraction. WT and AgGMP-OE lines were grown on Murashige and Skoog (MS) medium with or without 300 mM mannitol (control). Each experiment was performed with three biological replicates.

**Total RNA extraction, cDNA synthesis and AsA content determination**
Total RNA of celery and *A. thaliana* were extracted using the total RNA extraction Kit (Tiangen, Beijing, China) according to the manufacturer’s instructions. cDNA was obtained by Prime Script RT reagent Kit (TaKaRa, Dalian, China) based on the operation instruction. The content of AsA was determined using UPLC (ultra performance liquid chromatography) system according to the method described as previous study (*Liu et al., 2019*).

**Bioinformatics analysis**
The sequences of GMP proteins from other species were downloaded from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/). Primer Premier 6.0 was utilized to design primers. Nucleotide and encoded amino acid sequence of *AgGMP* gene was analyzed using BioXM software. MEGA7.0 software was used to construct the phylogenetic tree. The alignment of amino acid sequences of GMPs from celery and other plants were carried out by DNAMAN 8.0 software. SOPMA software (http://pbiol.ibcp.fr/) was used to predict the secondary structure of GMP protein. The protein tertiary structural model was established using CPH models 3.2 Server (http://www.cbs.dtu.dk/services/CPHmodels/). Signal peptide was predicted by Signal P software.

**Isolation of the *AgGMP* gene, overexpression vector construction and *A. thaliana* transformation**
The putative *AgGMP* gene sequence was retrieved from celery genome and transcriptome database (*Feng et al., 2018a; Li et al., 2020c*). The full lengths ORF (open reading frame) of
AgGMP was amplified with special primers (AgGMP-7736-F: 5′-TTTACAATTACCAGATCCATGAAGGCTCTTATTCTTGTA-GGA-3′; AgGMP-7736-R: 5′-ACCGATGATAACGAGGTCTCTCACATCAATCTCTGGCTCtCAAA-3′). The PCR product was cloned into the pCAMBIA1301 and then sequenced (Genscript, Nanjing, China). The recombinant plasmid (35S: AgGMP) was introduced into the Agrobacterium tumefaciens strain GV3101 via electroporation method. The floral-dip method was used for Agrobacterium-mediated transformation of A. thaliana (Zhang, Henriques & Lin, 2006). Transgenic A. thaliana were initially screened on MS medium containing hygromycin (40 mg/L), and then further confirmed by β-glucuronidase (GUS) assay, PCR amplification and sequencing.

**Subcellular localization**

The ORF of AgGMP without stop codon was amplified using specific primers (AgGMP-PA7-F: 5′-CACCATCACCATCACGCATGATGAAGGCTCTTATTCTTGTTGGA-3′ and AgGMP-PA7-R: 5′-CACATGATACGAACGAGCTCTCAGTCAAACATCTCTGGCTTCAA-3′). The amplification product was ligated into pA7 vector via Nco I site. The control vector (pA7-GFP) and recombinant vector (AgGMP-GFP) were bombarded into onion epidermal cells using the biolistic bombardment Biolistic PDS-1000 (Bio-Rad, Hercules, CA, USA). After 18 h of dark growth on MS solid medium, the GFP fluorescence of samples were observed using a laser confocal microscope LSM780 (Zeiss, Oberkochen, Germany).

**Real-time quantitative PCR analysis**

Real-time quantitative PCR (RT-qPCR) was conducted to detect the expression level of AgGMP. Premier 6.0 software was used to designed primers (AgGMP-qF: 5′-TGCTGGAACTTACCTGCTGAACC-3′, AgGMP-qR: 5′-TGCTGGAATCTACCTGCTGAACC-3′). The SYBR Premix Ex Taq (TaKaRa, Dalian, China) and Bio-Rad IQ5 real-time PCR System (Bio-Rad, Hercules, CA, USA) were used for RT-qPCR reaction. AgActin gene was used as internal standard (Li et al., 2016). Each reaction set three biological replicates. The relative expression data of AgGMP were analyzed using 2^−ΔΔCt method (Pfaffl, 2001).

**Statistical analysis**

All data in the text were obtained from the average of three biological repeats. Data significant difference was analyzed using SPSS 24.0 by one way ANOVA at a 0.05 level.

**RESULTS**

**Analysis of AgGMP sequence**

The full lengths cDNA sequence of AgGMP gene was obtained from ‘Jinnan Shiqin’ (GenBank No. OL757646), which contained an open reading frame (ORF) with the length of 1,086 bp, encoding 361 amino acids (Fig. 1A). The secondary structure was mainly composed of 30.77% α-helices, 29.09% extended strand, 9.07% β-turn and 37.67% random coils. Prediction results indicated that the protein tertiary structure of AgGMP protein was mainly composed of three α-helices and certain random coils (Fig. 1B). AgGMP
The protein has no signal peptide according to the prediction by Signal P software. The amino acid sequence of AgGMP were aligned with homologous sequences from *Daucus carota* (carrot, AQM57027.1), *Lycopersicon esculentum* (tomato, accession ID DQ449030), *Solanum tuberosum* (potato, NP_001275205.1), *Camellia sinensis* (tea plant, AGI78460.1), *Arabidopsis thaliana* (AT4G30570), *Oryza sativa* (rice, LOC4327472), *Malpighia glabra* (acerola, ABB53473.1), *Brassica rapa* subsp. *chinensis* (non-heading Chinese cabbage, AET14212.1), *Nicotiana tabacum* (tobacco, BAB62108.1) (Fig. 2). GMP proteins were highly conserved (90.80% of consistency) among different species. Phylogenetic analysis demonstrated that AgGMP protein had the closest evolutionary relationship with carrot (Fig. 3).

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**Figure 1** Bioinformatics analysis of AgGMP. (A) Nucleotide and encoded amino acid sequence of AgGMP gene. (B) The tertiary structural model of AgGMP protein.

Full-size [DOI: 10.7717/peerj.12976/fig-1](https://doi.org/10.7717/peerj.12976/fig-1)

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**Expression profiles of AgGMP under abiotic stress in celery**

As shown in Fig. 4, the relative expression levels of AgGMP gene in ‘Jinnan Shiqin’ were up-regulated significantly under low temperature, high temperature, salt and drought treatments. The relative expression levels of AgGMP gene were obviously increased at 1 h after low temperature, high temperature and salt treatments, respectively (Figs. 4A–4C). In salt treatment, the relative expression levels of AgGMP gene were remarkably elevated at 8 h and 24 h than that at 0 h, which were 2.52 and 2.79-folds of that at 0 h, respectively. The highest expression of AgGMP gene were occurred at 2 h, 4 h, 1 h and 2 h of low temperature, high temperature, salt and drought treatments, which were 2.70, 2.43, 1.54, and 4.2-folds of the 0 h, respectively. Under drought stress, the expression profiles of
AgGMP exhibited an increase at 1 h, 2 h, 4 h, which were 1.11, 1.54 and 1.47, and then decreased at 8 h, 24 h, which were 0.82 and 0.85, respectively (Fig. 4D). The results indicated that AgGMP involved in the response to abiotic stress in celery.

**Subcellular localization analysis of AgGMP**

The vector AgGMP-GFP and pA7-GFP were transferred into the onion epidermis cell to detect the subcellular localization of AgGMP. The onion epidermal cell with AgGMP-GFP
and pA7-GFP displayed bright fluorescence throughout the entire cell, suggesting that the AgGMP protein was located in cytoplasm (Fig. 5A).

Identification of transgenic *A. thaliana*

To investigate the function of *AgGMP* gene, transgenic *A. thaliana* lines were generated via *Agrobacterium*-mediated transformation. The transgenic *A. thaliana* lines (AgGMP-1, AgGMP-2, and AgGMP-5) were screened on the MS medium containing hygromycin. The leaves of transgenic *A. thaliana* lines were immersed in X-gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid) and appeared blue (Fig. 5B). About 1,000 bp PCR products were observed only in the transgenic lines, AgGMP-1, AgGMP-2 and AgGMP-5, based on the PCR amplification (Fig. 5C). The results indicated that *AgGMP* was successfully transferred into *A. thaliana*, three OE lines (AgGMP-1, AgGMP-2 and AgGMP-5) harboring *AgGMP* gene were obtained.

Overexpression of *AgGMP* up-regulated the AsA content in *Arabidopsis*

There are no obvious differences observed in phenotype among three OE lines and WT plants (Fig. 6A). According to the AsA content determination, the transgenic *A. thaliana* lines contained higher AsA accumulation compared with WT (Figs. 6B and 6C). The AsA
The results indicated that overexpression of AgGMP up-regulated the AsA level in transgenic A. thaliana.

The change of seedling root lengths in A. thaliana under 300 mM mannitol treatment

The seeds of three A. thaliana OE lines and WT were germinated and grown on MS medium containing different mannitol concentrations. The root lengths of 7-day-old (day after germinating) A. thaliana plants were measured. On MS medium without mannitol, no significant difference in root lengths was observed between WT and transgenic A. thaliana lines hosting AgGMP gene. The root lengths of WT, AgGMP-1, AgGMP-2, and
AgGMP-5 lines, were 2.98, 2.55, 3.31 and 3.00 cm, respectively (Fig. 7A). Whereas, the roots lengths were inhibited in WT and three transgenic *A. thaliana* lines treated with 300 mM mannitol, which were 1.71, 2.08, 2.23, and 1.81 cm, respectively (Fig. 7B). On MS medium without mannitol, the root lengths of *A. thaliana* were longer, more lateral roots and root hairs, as well as better root growth. The root lengths of transgenic plants were longer than that of WT on MS medium containing 300 mM mannitol. In particular, the root lengths of AgGMP-2 were significantly increased compared with WT.

**DISCUSSION**

Celery is one of important leafy vegetables with rich nutrients (*Kooti & Daraei, 2017; Li et al., 2018b*), such as anthocyanin (*Feng et al., 2018b, 2021*), apigenin (*Tan et al., 2017; Yan et al., 2019; Wang et al., 2021*), carotenoids (*Li et al., 2019; Yin et al., 2020; Ding et al., 2021*), AsA (*Liu et al., 2022*), and dietary fiber (*Duan et al., 2020*). AsA is not only one of important nutrients, but also a key mediator that triggers plant response to various abiotic stress (*Barth, Gouzd & Steele, 2009*). AsA accumulation is affected by abiotic stress, such as salt (*Zhang et al., 2012*), low temperature, high temperature (*Wang et al., 2011*), drought stress (*Padh, 1990*), light (*Li et al., 2020a*), and a variety of nutrient elements (*Li et al.,
which is speculated to accomplished by increasing the activity of related enzymes involved in the AsA biosynthesis pathway in plants.

In plants, there are four pathways for AsA biosynthesis, including L-galactose pathway, myo-inositol pathway, L-gulose pathway, and D-galacturonate pathway. The GMP gene, encoding GMPase, plays an essential role in the L-galactose pathway. GMPase catalyzes the initial steps of AsA biosynthesis to form GDP-D-mannose, the precursor of AsA. Thus, GMP gene could affect AsA content by controlling the activities of GMPase. Our present result indicated that AgGMP involved in the response to abiotic stress in celery. The identified function of AgGMP is similar to that of soybean GmGMP1, GMP genes expression were both significantly induced by heat, cold and salt stresses. Under abiotic stress treatments, the expression level of GmGMP1 peak at 1 h and then decreased (Xue et al., 2018). Under drought stress, the expression levels of the AgAPX1 and AgGMP of celery were also similar, which significantly higher than that of the control at 2 h, and peaked at 4 h followed by a decrease (Liu et al., 2019).

It is acknowledged that drought is an important factor affecting agricultural production, improving plant drought resistance also is an ongoing hot topic. Considering evidences have suggested the relationship between AsA and stress resistance (Wang et al., 2011; Zhang et al., 2012). One of the consequences of abiotic stress is that it triggers an oxidative burst due to formation of reactive oxygen species. AsA could directly remove ROS produced by stress, and also indirectly remove H$_2$O$_2$ through the AsA-GSH cycle to protect tissues from harmful oxidative products, as well as keep certain enzymes in their required reduced forms. GMPase is a key rate limiting enzymes in AsA biosynthesis. Declined expression of GMP gene, encoded GMPase, usually was followed by a decreased resistance to drought stress in plants. Overexpression of the GMP gene of soybean enhanced the plant drought resistance in transgenic plants (Xue et al., 2018). In this study, we found that the relative expression levels of AgGMP gene were up-regulated significantly under low temperature, high temperature, salt and drought treatments, which reached the peak at 2 h, 4 h, 1 h, and 2 h, respectively. The transcript of AgGMP in celery were induced by drought stress, the expression of AgGMP in celery was significantly up-regulated of AgGMP under drought stress at 2 h and reach peak at 4 h and then declined.

To further investigate the function of AgGMP gene in response to drought stress, the AsA content and root lengths of three A. thaliana OE lines and WT were measured. Compare with the WT, overexpression of AgGMP up-regulated the AsA content in three transgenic A. thaliana lines, the root lengths were also longer when subjected to 300 mM mannitol. It is possible that overexpression of AgGMP can improve the activity of GMPase that involved in AsA biosynthesis pathway in A. thaliana, and increase the AsA accumulation to neutralize part of the stress effects. AsA could remove ROS and H$_2$O$_2$ produced by drought stress, protect tissues from harmful oxidative products and keep certain enzymes in their required reduced forms (Wang et al., 2011; Zhang et al., 2012). The three transgenic A. thaliana lines showed reduced root damage caused by mannitol and presented greater tolerance to drought stress. We speculated that AgGMP was participated in the process of celery resisting drought stress and overexpression of AgGMP
can induce an increased resistance to drought stress in transgenic plants by enhancing the accumulation of AsA.

**CONCLUSION**

GMPase was a rate-limiting enzyme in the L-galactose pathway, a key biosynthetic pathway for L-ascorbic acid (AsA) in plants. Here, the gene AgGMP encoding the AgGMPase was identified and characterized. The AgGMP gene contained an ORF of 1,086 bp, encoding 361 amino acids. Sequence alignment suggested that AgGMP protein was highly conserved among different plant species. The transcript of AgGMP was induced by abiotic stress in celery, the heterologous overexpression of AgGMP in *A. thaliana* proved the role of AgGMP in regulating AsA accumulation and modifying drought stress resistance. These findings suggested that AgGMP acted a regulator in AsA accumulation and response to abiotic stress in celery. In the future, we hope to achieve its homologous over-expression through transgenic technology and knock it out using the CRISPR/Cas9 method in celery.

**ABBREVIATIONS**

| Abbreviation | Definition |
|--------------|------------|
| AsA          | L-ascorbic acid |
| DHA          | Dehydroascorbic acid |
| GFP          | Green fluorescent protein |
| GMPase       | GDP-D-mannose pyrophosphorylase |
| GUS          | β-glucuronidase |
| MDA          | Malondialdehyde |
| MS medium    | Murashige and Skoog medium |
| ORF          | Open reading frame |
| PCR          | Polymerase Chain Reaction |
| ROS          | Reactive oxygen species |
| RT-qPCR      | Real-time quantitative PCR |
| UPLC         | Ultra Performance Liquid Chromatography |
| X-gluc       | 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid |

**ADDITIONAL INFORMATION AND DECLARATIONS**

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Competing Interests
The authors declare that they have no competing interests.

Author Contributions
• Yan-Hua Liu conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
• Hao Wang performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
• Jie-Xia Liu performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
• Sheng Shu analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
• Guo-Fei Tan analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
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• Ao-Qi Duan performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
• Hui Liu performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
• Ai-Sheng Xiong conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:
The raw data are available in the Supplemental File.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.12976#supplemental-information.

REFERENCES
Ai T, Liao X, Li R. 2016. GDP-D-mannose pyrophosphorylase from Pogonatherum paniceum enhances salinity and drought tolerance of transgenic tobacco. Zeitschrift Fur Naturforschung Section C 71(7–8):243–252 DOI 10.1515/znc-2015-0145.

Barth C, Gouzd ZA, Steele HP. 2009. A mutation in GDP-mannose pyrophosphorylase causes conditional hypersensitivity to ammonium, resulting in Arabidopsis root growth inhibition, altered ammonium metabolism, and hormone homeostasis. Journal of Experimental Botany 61(2):379–394 DOI 10.1093/jxb/erp310.
Ding X, Jia LL, Xing GM, Tao JP, Sun S, Tan GF, Li S, Liu JX, Duan AQ, Wang H, Xiong AS. 2021. The accumulation of lutein and β-carotene and transcript profiling of genes related to carotenoids biosynthesis in yellow celery. *Molecular Biotechnology* 63(7):638–649 DOI 10.1007/s12033-021-00332-9.

Duan AQ, Tao JP, Jia LL, Tan GF, Liu JX, Li T, Chen LZ, Su XJ, Feng K, Xu ZS, Xiong AS. 2020. AgNAC1, a celery transcription factor, related to regulation on lignin biosynthesis and salt tolerance. *Genomics* 112(6):5254–5264 DOI 10.1016/j.ygeno.2020.09.049.

Feng K, Hou XL, Li MY, Jiang Q, Xu ZS, Liu JX, Xiong AS. 2018a. CeleryDB: a genomic database for celery. *Database* 2018:bay070 DOI 10.1093/database/bay070.2018.

Feng K, Liu JX, Duan AQ, Li T, Yang QQ, Xu ZS, Xiong AS. 2018b. AgMYB2 transcription factor is involved in the regulation of anthocyanin biosynthesis in purple celery (*Apium graveolens* L.). *Planta* 248(5):1249–1261 DOI 10.1007/s00425-018-2977-8.

Feng K, Xing GM, Liu JX, Wang H, Tan GF, Wang GL, Xu ZS, Xiong AS. 2021. AgMYB1, an R2R3-MYB factor, plays a role in anthocyanin production and enhancement of antioxidant capacity in celery. *Vegetable Research* 1(1):2–12 DOI 10.48130/VR-2021-0002.

Garg R, Varshney RK, Jain M. 2014. Molecular genetics and genomics of abiotic stress responses. *Front Plant* 5:398 DOI 10.3389/fpls.2014.00398.

Hasanuzzaman M, Nahar K, Hossain MS, Mahmud JA, Rahman A, Inafuku M, Oku H, Fujita M. 2017. Coordinated actions of glyoxalase and antioxidant defense systems in conferring abiotic stress tolerance in plants. *International Journal of Molecular Sciences* 18(1):200 DOI 10.3390/ijms18010200.

He C, Yu Z, Tei XR. 2017. DoGMP1 from Dendrobium officinale contributes to mannose content of water-soluble polysaccharides and plays a role in salt stress response. *Scientific Reports* 7(1):41010 DOI 10.1038/srep41010.

Huang W, Wang GL, Li H, Wang F, Xu ZS, Xiong AS. 2016. Transcriptional profiling of genes involved in ascorbic acid biosynthesis, recycling, and degradation during three leaf developmental stages in celery. *Molecular Genetics and Genomics* 291(6):2131–2143 DOI 10.1007/s00438-016-1247-3.

Kempinski CF, Haffar R, Carina C. 2011. Toward the mechanism of NH₄⁺ sensitivity mediated by *Arabidopsis* GDP-mannose pyrophosphorylase. *Plant Cell and Environment* 34(5):847–858 DOI 10.1111/j.1365-3040.2010.02162.x.

Kooti W, Daraei N. 2017. A review of the antioxidant activity of celery (*Apium graveolens* L.). *Journal of Evidence-Based Complementary & Alternative Medicine* 22(4):1029–1034 DOI 10.1177/2156587217717415.

Li MY, Feng K, Hou XL, Jiang Q, Xu ZS, Wang GL, Liu JX, Wang F, Xiong AS. 2020c. The genome sequence of celery (*Apium graveolens* L.), an important leaf vegetable crop rich in apigenin in the Apiaceae family. *Horticulture Research* 7(1):9 DOI 10.1038/s41438-019-0235-2.

Li MY, Hou XL, Wang F, Tan GF, Xu ZS, Xiong AS. 2018b. Advances in the research of celery, an important Apiaceae vegetable crop. *Critical Reviews in Biotechnology* 38(2):172–183 DOI 10.1080/07388551.

Li Q, Li BH, Kronzucker HJ, Shi WM. 2010. Root growth inhibition by NH₄⁺ in *Arabidopsis* is mediated by the root tip and is linked to NH₄⁺ efflux and GMPase activity. *Plant, Cell & Environment* 33(9):1529–1542 DOI 10.1111/j.1365-3040.2010.02162.x.

Li H, Liu H, Wang Y, Teng RM, Liu J, Lin S, Zhuang J. 2020b. Cytosolic ascorbate peroxidase 1 modulates ascorbic acid metabolism through cooperating with nitrogen regulatory protein P-II in tea plant under nitrogen deficiency stress. *Genomics* 112(5):3497–3503 DOI 10.1016/j.ygeno.2020.06.025.
Li H, Liu JX, Wang Y, Zhuang J. 2020a. The ascorbate peroxidase 1 regulates ascorbic acid metabolism in fresh-cut leaves of tea plant during postharvest storage under light/dark conditions. Plant Science **296**:110500 DOI 10.1016/j.plantsci.2020.110500.

Li H, Liu ZW, Wu ZJ, Wang YX, Teng RM, Zhuang J. 2018a. Differentially expressed protein and gene analysis revealed the effects of temperature on changes in ascorbic acid metabolism in harvested tea leaves. *Horticulture Research* **5**(1):65 DOI 10.1038/s41438-018-0070-x.

Li JW, Ma J, Feng K, Xu ZS, Xiong AS. 2019. Transcriptome profiling of β-carotene biosynthesis genes and β-carotene accumulation in leaf blades and petioles of celery cv Jinnanshiqin. *Acta Biochimica et Biophysica Sinica* **51**(1):116–119 DOI 10.1093/abbs/gmy141.

Li MY, Wang F, Jiang Q, Wang GL, Tian C, Xiong AS. 2016. Validation and comparison of reference genes for qPCR normalization of celery (*Apium graveolens*) at different development stages. *Frontiers in Plant Science* **17**(7):313 DOI 10.3389/fpls.2016.00313.

Liebler DC, Kling DS, Reed DJ. 1986. Antioxidant protection of phospholipid bilayers by alpha-tocopherol Control of alpha-tocopherol status and lipid peroxidation by ascorbic acid and glutathione. *Journal of Biological Chemistry* **261**(26):12114–12119 DOI 10.1016/S0021-9258(18)67210-2.

Lin LL, Shi QH, Wang HS, Qin AQ, Yu XC. 2011. Over-expression of tomato GDP-mannose pyrophosphorylase (GMPase) in potato increases ascorbate content and delays plant senescence. *Agricultural Sciences in China* **10**(4):534–543 DOI 10.1016/S1671-2927(11)60034-5.

Liu JX, Feng K, Duan AQ, Li H, Yang QQ, Xu ZS, Xiong AS. 2019. Isolation, purification and characterization of an ascorbate peroxidase from celery and overexpression of the AgAPX1 gene enhanced ascorbate content and drought tolerance in *A. thaliana*. *BMC Plant Biology* **19**(1):488 DOI 10.1186/s12870-019-2095-1.

Liu JX, Wu B, Feng K, Li MY, Duan AQ, Shen D, Yin L, Xu ZS, Xiong AS. 2021. A celery transcriptional repressor AgERF8 negatively modulates abscisic acid and salt tolerance. *Molecular Genetics and Genomics* **296**(1):179–192 DOI 10.1007/s00438-020-01738-x.

Wang WP, Guo XB, Tang KX. 2011. Studies on transformation of *A. thaliana* AtGMP gene into lettuce. *Journal of Shanghai Jiaotong University (Agricultural Sciences)* **43**:49 DOI 10.3969/J.ISSN.1671-9964.2011.02.008.

Wang H, Liu JX, Feng K, Li T, Duan AQ, Liu YH, Liu H, Xiong AS. 2021. AgMYB12, a novel R2R3-MYB transcription factor, regulates apigenin biosynthesis by interacting with the AgFNS gene in celery. *Plant Cell Reports* **41**(1):139–151 DOI 10.1007/s00299-021-02792-4.
Wang HS, Yu C, Zhu ZJ, Yu XC. 2011. Overexpression in tobacco of a tomato GMPase gene improves tolerance to both low and high temperature stress by enhancing antioxidation capacity. *Plant Cell Reports* 30(6):1029–1040 DOI 10.1007/s00299-011-1009-y.

Xiao Y, Zhou TS, Li J, Zhang JX, Yu YB. 2015. Cloning and expression analysis of GDP-D-mannose pyrophosphorylase cDNA from *Camellia sinensis*. *Journal of Tea Science* 35(1):55–63 DOI 10.13305/j.cnki.jts.2015.01.010.

Xue CC, Xu JY, Wang C, Guo N, Hou JF, Xue D, Zhao JM, Xing H. 2018. Molecular cloning and functional characterization of a soybean GmGMP1 gene reveals its involvement in ascorbic acid biosynthesis and multiple abiotic stress tolerance in transgenic plants. *Journal of Integrative Agriculture* 17(3):539–553 DOI 10.1016/S2095-3119(17)61727-1.

Yan J, Yu L, He L, Zhu L, Xu S, Wan Y, Wang H, Wang Y, Zhu W. 2019. Comparative transcriptome analysis of celery leaf blades identified an R2R3-MYB transcription factor that regulates apigenin metabolism. *Journal of Agricultural and Food Chemistry* 67(18):5265–5277 DOI 10.1021/acs.jafc.9b01052.

Yin L, Liu JX, Tao JP, Xing GM, Tan GF, Li S, Duan AQ, Ding X, Xu ZS, Xiong AS. 2020. The gene encoding lycopene epsilon cyclase of celery enhanced lutein and β-carotene contents and confers increased salt tolerance in *Arabidopsis*. *Plant Physiology and Biochemistry* 157:339–347 DOI 10.1016/j.plaphy.2020.10.036.

Zhang X, Henriques R, Lin SS. 2006. *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* using the floral dip method. *Nature Protocols* 1(2):641 DOI 10.1038/nprot.2006.9.

Zhang Z, Wang J, Zhang R, Huang R. 2012. The ethylene response factor AtERF98 enhances tolerance to salt through the transcriptional activation of ascorbic acid synthesis in *Arabidopsis*. *Plant Journal for Cell and Molecular Biology* 71(2):273–287 DOI 10.1111/j.1365-313X.2012.04996.x.