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Plant G-protein β subunits positively regulate drought tolerance by elevating detoxification of ROS

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ABSTRACT

Heterotrimeric guanine-nucleotide-binding proteins (G-proteins) consist of α, β and γ subunits and play important roles in response and tolerance to abiotic stresses in plants, but the function of the heterotrimeric G-protein β subunit in response to drought remains unclear. In the present study, the AGB1 mutants (*agb1-2-1* and *agb1-3-2*) were more sensitive to drought than the wild-type. The over-expression of mulberry (*Morus alba* L.) G-protein β subunit in transgenic tobacco (*Nicotiana tabacum* L.) significantly enhanced the plants' drought tolerance. The transgenic tobacco plants had higher proline contents and peroxidase activities, and lower malonaldehyde and hydrogen peroxide contents and superoxide free radical accumulations under drought conditions. Additionally, transcript levels of the tobacco antioxidative genes, *NtSOD* and *NtCAT*, increased in drought-stressed transgenic tobacco plants. Thus, the heterotrimeric G-protein β subunits positively regulate drought tolerance in plants.

1. Introduction

Plants live in adverse environmental conditions, including exposure to biotic and abiotic stresses, that are often unfavorable or stressful for growth and development [1]. Drought is a major environmental factor that limits agricultural productivity and crop yield [2]. Drought typically induces physiological and biochemical responses in plants, including stomatal closure, cell growth and photosynthesis repression, osmolyte accumulation, and reactive oxygen species (ROS) production [3].

Heterotrimeric guanine-nucleotide-binding protein (G-protein) signaling pathways represent an evolutionarily conserved extracellular signal transduction system, which consist of a G-protein complex (three subunits, Gα, Gβ and Gγ), G-protein-coupled receptors (GPCRs), and regulators of G-protein signaling proteins (RGSs). In animals, GPCRs catalyze nucleotide exchange (GTP for GDP) on the Gα subunit, causing GTP-bound Gα dissociated from the Gβγ dimer. Both the GTP-bound Gα and a Gβγ dimer regulate the activity of the downstream effectors. Gα hydrolyzes GTP and returns to the GDP-bound state, and then reforms the inactive heterotrimer with Gβγ. This hydrolysis is accelerated by RGS [4]. However, the plant Gα protein is self-activated without GPCRs, and the 7TM-RGS protein serves as the regulatory point of G-protein activation and keeps the complex in the inactive state [5]. Plant G-protein signaling pathways are engaged in a wide range of developmental and physiological processes, including seed germination, morphology, stomatal movements, stress responses, and the regulation of some key agronomical traits in crops [6–10].

Previous studies suggested that G-protein plays important roles in responses and tolerances to abiotic stresses, especially salt and drought stresses. Loss-of-function analyses of *Arabidopsis* mutants demonstrated that the loss of the Gβγ dimer (AGB1) confers hypersensitivity, while the loss of AtGPA1 confers hyposensitivity to salt stress [11]. AGB1 is involved in the regulation of Na^+ fluxes and translocation, and it positively regulates salt tolerance in *Arabidopsis* by changing the transcription of the genes related to proline biosynthesis, oxidative stress, ion homeostasis, and stress- and abscisic acid-responses [12,13]. The overexpression of rice (*Oryza sativa* L.) G-protein γ subunit (RGG1) enhances the tolerance of the

Abbreviations: CAT, Catalase; DAB, 3,3-diaminobenzidine; G-proteins, Heterotrimeric guanine-nucleotide-binding proteins; H_2O_2, hydrogen peroxide; MAPK, mitogen-activated protein kinase; *Ma-GY*62, *Morus atropurpurea cv.* Guiyou No.62; MDA, Malonaldehyde; PEG, polyethylene glycol; NBT, Nitro-blue tetrazolium; POD, peroxidase; qRT–PCR, quantitative reverse transcription–PCR; ROS, Reactive oxygen species; SOD, Superoxide dismutase; O^{2-}, superoxide free radicals.

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transgenic rice to salt stress by elevating the detoxification of ROS [14]. The function of the G-protein in response to drought has also been studied. The G-protein α subunit (RGA1) mutant, *d1*, exhibits a reduced sensitivity to drought stress compared with wild plants [15]. Transgenic tobacco (*Nicotiana tabacum* L.) overexpressing an apple (*Malus domestica* Borkh.) heterotrimeric G-protein α subunit (MdGPA1) was more sensitive to drought stress than the wild tobacco [16]. These results suggested that the G-protein α subunit plays a negative role in response to drought stress in plants. However, the role of the G-protein β subunit in tolerance to drought remains unknown. AGB1 negatively regulates drought tolerance by down-regulating AtMPK6-related pathways in *Arabidopsis* [17], while the rice G-protein β subunit (RGB1) is a positive regulator of drought adaption in rice plants [18]. To clarify this controversy, the role of the G-protein β subunit was investigated by loss-of-function analyses of *Arabidopsis* AGB1 mutants and the overexpression of the G-protein β subunit from a woody plant, mulberry (*Morus alba* L.), in tobacco. In this report, we show that the AGB1 mutants, *agb1-2-1* and *agb1-3-2*, reduced plant' tolerance to drought and that the overexpression of *MaGβ* enhanced the tolerance of the transgenic tobacco, which suggested that the G-protein β subunit is a positive factor in response to drought. In addition, the overexpression of *MaGβ* decreased malonaldehyde (MDA), hydrogen peroxide (H_2O_2) and superoxide free radical (O^{2-}) accumulation and increased the proline content, peroxidase (POD) activity and antioxidant-related gene expression levels. Our data provide new insight into the functions of plant G-protein β subunits in response to drought stress and also provide the basis for further characterizations of its physiological functions under abiotic stresses.

2. Materials and methods

2.1. Plant material

Arabidopsis thaliana, including one wild-type ecotype [Columbia-0 (*col-0*)], two Gβ null mutants (*agb1-2-1* and a*gb1-3-2*; background *col-0*) were used as plant materials and were grown at 22 °C under a 16-h light/8-h dark photoperiod. Mulberry, *Morus atropurpurea* cv. *Guiyou* No.62 (*Ma-GY*62), was used for gene cloning. Tobacco (K326) was used for genetic transformation.

2.2. Drought-stress tolerance analysis of Arabidopsis

For the drought-stress tolerance analyses, 2- and 3-week-old *Arabidopsis* plants in soil had watering withheld. The growth phenotypes were observed and recorded.

2.3. Binary vector construction and transgenic plant generation

The full-length coding sequence of the mulberry G-protein β subunit (*MaGβ*) (KX099865) was obtained from *Ma-GY*62. Then, its full-length sequence was inserted between the *Sal*I and *Eco*RI sites of the pLGNL expression vector under the control of the CaMV35S promoter. The recombinant plasmid was transformed into *Agrobacterium tumefaciens* GV3101 and introduced into tobacco. The methods of transformation, GUS staining, genomic PCR and qRT-PCR were as described in our previous study [19]. The primers for these experiments are shown in Table S1.

2.4. Drought-stress tolerance analysis of transgenic tobacco

The transgenic lines and wild-type (WT) plants were grown on Murashige and Skoog (MS) medium until roots formed and were then transferred into soil in a climate chamber (24 °C, 16 h day/8 h night). After ~4 weeks, they were treated using drought conditions (30% PEG6000) until significant differences appeared between the transgenic lines and WT tobacco. When this occurred, the seedlings were collected, and the H_2O_2, proline and MDA contents, POD activity, and *NtSOD* and *NtCAT* expression levels were measured. The 7-*d*-old seedlings were incubated in 1/2 MS agar medium containing 300 mM mannitol for 7 d, and the root growth was recorded. For 3,3-diaminobenzidine (DAB) and nitro-blue tetrazolium (NBT) staining, 3-week-old tobacco seedlings were treated with 20% PEG for 24 h.

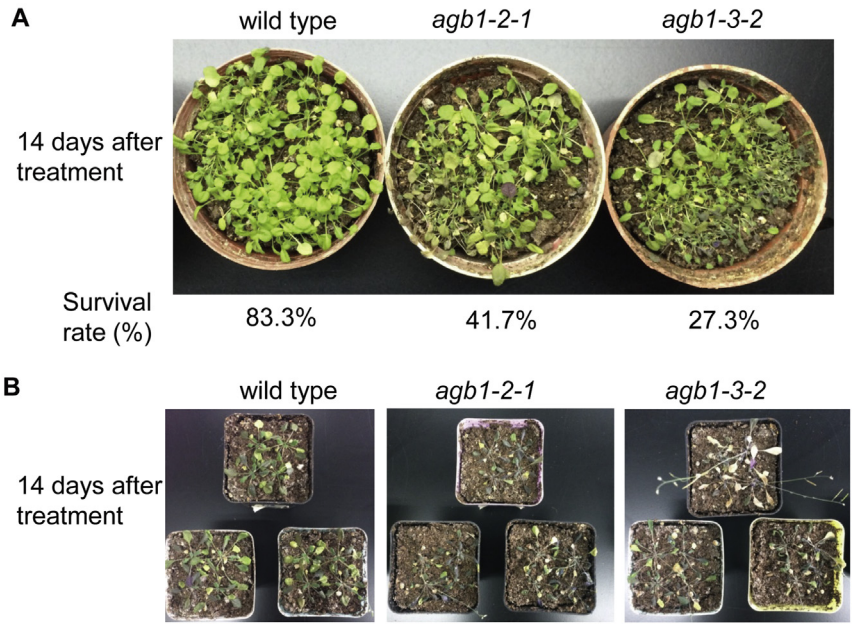

Fig. 1. Drought tolerance assay of WT (*Col-0*) and *agb1* mutants. (A) Two-week-old WT, *agb1-2-1* and *agb1-3-2* plants subjected to drought by withholding water. (B) Three-week-old WT, *agb1-2-1* and *agb1-3-2* plants subjected to drought by withholding water.

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