Abscisic acid carrier gene and transgenic plant expressing same

1. Executive Summary

Abscisic acid (ABA) is a ubiquitous phytohormone involved in many developmental processes and stress responses of plants. We identified that (AtPDR12)/ABCG40 is a plasma membrane ABA uptake transporter. ABA uptake is important for rapid responses to environmental stress such as drought stress. The transgenic Arabidopsis overexpressing ABCG40 increased yield under drought stress.

We also found that Arabidopsis thaliana Pleiotropic drug resistance transporter PDR12 (AtPDR12)/ABCG40 is an ABC transporter that contributes to Pb(II) resistance in Arabidopsis. The ABCG40 overexpressed transgenic arabidopsis showed resistance against heavy metals and salts as compared to wild-type plants.

2. Detailed Description of technology

Key Technology Highlights

Mechanism of action

- Drought stress resistance

Abscisic acid (ABA) is a ubiquitous phytohormone involved in many developmental processes and stress responses of plants. We identified that the ATP-binding cassette (ABC) transporter Arabidopsis thaliana Pleiotropic drug resistance transporter PDR12 (AtPDR12)/ABCG40 is a plasma membrane ABA uptake transporter. Uptake of ABA into yeast and BY2 cells expressing AtABCG40 was increased, whereas ABA uptake into protoplasts of atabcg40 plants was decreased compared with control cells. In response to exogenous ABA, the up-regulation of ABA responsive genes was strongly delayed in AtABCG40 plants, indicating that ABCG40 is necessary for timely responses to ABA. Stomata of loss-of-function AtABCG40 mutants closed more slowly in response to ABA, resulting in reduced drought tolerance. Our results integrate ABA-dependent signaling and transport processes and open another avenue for the engineering of drought-tolerant plants.

- Pb(II) resistance

Arabidopsis (Arabidopsis thaliana) contains about 130 ATP-binding cassette (ABC) proteins, which are likely to contribute to the transport of diverse materials, including toxic substances. However, the substrates of ABC transporters remain unknown in most cases. We tested which ABC transporter is involved in detoxification of lead [Pb(II)]. Among the many tested, we found that the message level of only AtPDR12 increased in both shoots and roots of Pb(II)-treated Arabidopsis, suggesting that it may be involved in the detoxification of Pb(II). AtPDR12-knockout plants (atpdr12) were used to further test this possibility. In Pb(II)-containing medium, atpdr12
plants grew less well and had higher Pb contents than those of wild-type plants. In contrast, AtPDR12-overexpressing Arabidopsis plants were more resistant to Pb(II) and had lower Pb contents than wild-type plants. The mutant phenotypes and their Pb contents, as well as the localization of the GFP:AtPDR12 fusion protein at the plasma membrane, suggest that AtPDR12 functions as a pump to exclude Pb(II) and/or Pb(II)-containing toxic compounds from the cytoplasm. Inhibition of glutathione synthesis by addition of buthionine sulfoximine to the growth medium exacerbated the Pb(II)-sensitive phenotype of AtPDR12 plants, consistent with a glutathione-dependent detoxification mechanism operating in parallel with an AtPDR12-dependent mechanism. Thus, we propose that AtPDR12 is an ABC transporter that contributes to Pb(II) resistance in Arabidopsis.

**Proven efficacy**

- AtABCG40-overexpressing Arabidopsis plants are more resistant to Pb(II)

![Fig. 1](image1.png)

**Fig. 1** Enhanced Pb(II) resistance of AtABCG40-overexpressing Arabidopsis. A, Growth in control medium. B, Growth in medium containing 0.75 mM Pb(NO$_3$)$_2$. T3 homozygous lines of AtABCG40-overexpressing plants and wild-type plants were grown vertically on one-half MS agar media with 1.5% Suc with or without 0.75 mM Pb(NO$_3$)$_2$. Photographs were taken 2 weeks after sowing. P12-1, -2, and -3, Three independent lines of AtABCG40-overexpressing T3 homozygous plants.

- Pb(II) resistance conferred by AtABCG40 is independent of glutathione

![Fig. 2](image2.png)

**Fig. 2** Glutathione-independent Pb(II)-resistance mechanism of AtABCG40 (atpdr12). A, Growth of AtABCG40 mutants in control medium. B, Growth in 1 mM BSO-containing medium. C, Growth in medium containing 0.75 mM Pb(NO$_3$)$_2$. D, Growth in both 1 mM BSO and 0.75 mM Pb(NO$_3$)$_2$-containing medium. AtPDR12-overexpressing (P12-1), knockout (atpdr12-1), and wild-type (WT) plants were grown vertically on one-half MS agar media without or with Pb(NO$_3$)$_2$ or BSO. Photographs were taken 3 weeks after sowing.
AtABCG40 knock-out mutants are impaired in stress tolerance

Fig. 3 Stomata of ABG40 knock-out mutants(abcg40-1, abcg40-2) are less sensitive to ABA. (A) Two-week-old soil-grown plants (24°C; 16-h light and 8-h dark conditions) were exposed to drought stress by withholding water for 8 days. (B) Delayed elevation of leaf temperature after ABA treatment of atabcg40 plants compared with wild type. Leaf temperature was monitored using an Infrared Thermal Imaging Camera (FLIR systems; P25) after the addition of ABA into the hydroponic culture medium to a final concentration of 1 µM. Results representative of three experiments with similar results are shown. (C) Increase in leaf temperature (Δ Temperature) after ABA treatment of plants is shown. The leaf temperature was quantified from three independent experiments, including the one shown in B. Data represent change in temperatures of the whole-leaf area of the plants. ΔTemperature = (Temperature at indicated time point) - (Temperature at 4 min). The initial temperatures were similar in WT and atabcg40-1. Data are mean ±SEM (*P < 0.05; **P < 0.01 compared with WT under the same treatment conditions by Student’s t test).

ABCG40 protects yield under drought stress

Fig. 4 Transgenic Arabidopsis overexpressing ABCG40 increased yield under drought stress when plants were subjected to drought stress by withholding water for 14 days.

Current Progress
- The transgenic arabidopsis overexpressing ABCG40 showed that functions related to tolerance for abiotic stress against a heavy metal and salt as compared to wild-type plants and would be useful as one of suitable trait for various plants.

Patent Information
- Patent number: WO10/058114
- Patent title: Abscisic acid carrier gene and transgenic plant expressing
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