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Abstract

Drought and salt are major abiotic stresses that adversely affect crop productivity. Thus identification of factors that confer resistance to these stresses would pave a way to increasing agricultural productivity. When grown on soil in greenhouse longer than 5 weeks, transgenic Arabidopsis plants that over-express an ABC (ATP-binding cassette) transporter, AtABCG36/AtPDR8, produced higher shoot biomass and less chlorotic leaves than the wild type. We investigated whether the improved growth of AtABCG36-overexpressing plants was due to their improved resistance to abiotic stresses, and found that AtABCG36-over-expressing plants were more resistant to drought and salt stress and grew to higher shoot fresh weight than the wild type. In contrast, T-DNA insertional knockout lines were more sensitive to drought stress than the wild type. To understand the mechanism of enhanced salt and drought resistance of the AtABCG36-over-expressing plants, we measured sodium contents, and found that AtABCG36-over-expressing plants were lower in sodium content than the wild type. Our data suggest that AtABCG36 contributes to drought and salt resistance in Arabidopsis by a mechanism that includes reduction of sodium content in plants.
Overexpression of AtABCG36 improves drought and salt stress resistance in Arabidopsis

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Abstract
Drought and salt are major abiotic stresses that adversely affect crop productivity. Thus identification of factors that confer resistance to these stresses would pave a way to increasing agricultural productivity. When grown on soil in greenhouse longer than 5 weeks, transgenic Arabidopsis plants that over-express AtABCG36/AtPDR8 produced higher shoot biomass and less chlorotic leaves than the wild type. We investigated whether the improved growth of AtABCG36-overexpressing plants was due to their improved resistance to abiotic stresses, and found that AtABCG36-over-expressing plants were more resistant to drought and salt stress and grew to higher shoot fresh weight than the wild type. In contrast, T-DNA insertional knockout lines were more sensitive to drought stress than the wild type and were reduced in shoot fresh weight. To understand the mechanism of enhanced salt and drought resistance of the AtABCG36-over-expressing plants, we measured sodium contents, and found that AtABCG36-over-expressing plants were lower in sodium content than the wild type. Our data suggest that AtABCG36 contributes to drought and salt resistance in Arabidopsis by a mechanism that includes reduction of sodium content in plant.
**Abbreviations** - ABC, ATP-binding cassette; Fw, fresh weight; MS, Murashige and Skoog; PDR, pleiotropic drug resistance; PR, pathogenesis-related; Wt, Wild-type.

**Introduction**

Abiotic stresses, such as drought, salinity, low temperature, high light, heavy metals, adversely affect the plant growth and are important limiting factors in crop productivity (Raskin et al. 1997, Vinocur and Altman. 2005, Zhu et al. 2002). Extensive studies have been conducted to reveal the cellular processes elicited by environmental stresses and the mechanisms by which plants cope with these stresses (Denby and Gehring 2005). The abiotic stresses typically lead to the production of free radicals and reactive oxygen species (ROS) in plant cells, which in turn can act as a signal molecule and/or cause cellular damage and death (Moran et al. 1994, Schutzendubel A and Polle A 2002, Xiong et al. 2002). Transgenic approaches have been attempted to improve tolerance to abiotic stresses. A large number of genes, either involved in signaling and regulatory pathways, or encoding enzymes known to alleviate stress have been introduced to produce plants with increased stress resistance against salinity, oxidative stress, heavy metals or drought (Wang et al. 2002).

ATP-binding cassette (ABC) proteins are present in all living organisms and constitute a large gene family (Higgins 1992, Rea 2007) and most, if not all, of them are membrane proteins active in the transport of a broad range of substances (Jungwirth and Kuchler 2006, Linton et al. 1998, Martinoia et al. 2002, Rogers et al. 2001, Theodoulou 2000, Rea 2007). In *Arabidopsis*, approximately 130 ABC genes were reported and some of them were found to function in detoxification, pathogen resistance, plant growth and development (Garcia et al. 2004, Sanchez-Fernandez et al. 2001).

The pleiotropic drug resistance (PDR) subfamily ABC proteins are only found in fungi and plants. They were first characterized in the yeast *Saccharomyces cerevisiae*, in which nine PDR members were identified among thirty ABC proteins. Two of them, the plasma membrane localized PDR5 and SNQ2, mediate ATP-dependent efflux of hundreds of structurally and functionally unrelated compounds (Wolfger et al. 2001). The PDR-type ABC genes have been identified in many plant species as well. Plant PDR-type ABC proteins are involved in biotic and abiotic stress responses. A tobacco PDR, *NpABC1* has been shown to extrude sclarolide, an antifungal compound diterpene (Jasinski et al. 2001). A close homologue of *NpABC1* in the Arabidopsis, AtPDR12 has also a role in antifungal terpenoid secretion (Campbell et al. 2003), and ABA transport (Kang et al., in press). The transcription of *GmPDR12*, from soybean (*Glycine max*), was induced rapidly by salicylic acid (SA), a major plant hormone involved in systemic acquired resistance (Eichhorn et al. 2006). Probably the best characterized PDR is PDR8/ABCG36, which contributes to the pathogen resistance of
Arabidopsis thaliana (Kobae et al. 2006, Stein et al. 2006) by possibly facilitating the export of substances related to defense against pathogens across the plasma membrane. Very recently it was shown that PDR8 is required for accumulation of callose, which plays a central role in plant defense (Bednarek et al. 2009, Clay et al. 2009). Interestingly, in addition to contribution to biotic stress response, the two Arabidopsis PDRs, AtPDR8 and AtPDR12 have also been shown to confer heavy metal resistance. While AtPDR12 confers lead resistance (Lee et al. 2005), AtPDR8 has been shown to be important for cadmium resistance (Kim et al. 2007). Further hints that plant PDRs are involved in abiotic stress responses come from expression studies where it has been shown that transcripts of SpTUR2, the first characterized plant PDR protein, are increased by cold and salt stress and those of OsPDR9 strongly induced in response to osmotic, salt, and hypoxic stresses (Moons 2003, van den Brule et al. 2002). In addition, NtPDR3, a Nicotiana tabacum PDR3, was found to be induced in suspension cells under iron deficient condition (Duicos et al., 2005).

In order to characterize AtABCG36/PDR8 in more details, we performed further experiments and observed growth difference between wild type and AtABCG36 transgenic plants under water deficient condition. Here, we present many lines of evidence that suggest the involvement of AtABCG36 in drought and salt stress resistance of Arabidopsis.

Materials and methods

Plant growth and stress conditions

Seeds of wild type (ecotype Columbia-0) and AtABG36 transgenic plants were surface sterilized, placed in the dark at 4°C for 2 days, and then sown on MS (Murashige and Skoog, 1962) agar plates with 3% sucrose. For phenotype analysis in the 1/2 MS agar plate, seeds of wild-type and transgenic plants were germinated and grown on vertically-placed 1/2 MS agar plates with or without supplemented NaCl for 2 to 3 weeks with 16 h light (at 22°C, 40 µmol m⁻² s⁻¹)/8 h dark cycles (at 18°C). Plants were collected, weighed and their root lengths were measured.

To test drought resistance of AtABCG36 transgenic plants in soil, seeds of wild-type and transgenic plants were germinated and grown on vertically-placed 1/2 MS agar plates for 1 week, and plants of similar size and developmental stage were selected and transferred onto soil. To prevent pathogen infection, sterilized soil and pots were used, and the plants were kept under a controlled environment free of pathogens. They were grown for 2 weeks in a green house under 16 h light (at 22°C)/8 h dark (at 18°C) cycles with normal watering schedule. Then watering was withheld for 10 days, and the photographs were taken. Shoot parts of plants were harvested, rinsed twice briefly with
ice-cold water and then blot-dried for fresh weight measurement.

**Hydroponic cultures of Arabidopsis**

Seeds were germinated and grown on 1/2 MS agar plate for 10 days and then transferred to hydroponic solution (1.25 mM KNO$_3$, 0.025 mM KH$_2$PO$_4$, 0.5 mM MgSO$_4$, 0.5 mM Ca(NO$_3$)$_2$, 0.025 mM Fe-EDTA, 0.175 mM H$_3$BO$_3$, 0.055 mM MnCl$_2$, 0.005 mM ZnSO$_4$, 0.0625 mM NaMoO$_4$, 0.025 mM NaCl, 0.025 µM CoCl$_2$) and incubated for 1 week under 16 h light (at 22°C, 40 µmol m$^{-2}$ s$^{-1}$)/8 h dark (at 18°C) cycles. To subject the plants to drought stress, we opened the cap of the container completely and placed the container under low humidity condition (30% of humidity) for longer than 1 week.

**Measurement of ion contents**

To measure ion contents of wild type and AtABCG36 transgenic plants, seeds were germinated and grown on 1/2 MS agar plate for 10 days and then transferred to hydroponic solution. After 1 week of incubation, NaCl was added to a final concentration of 50 mM to the solution. After incubation for a day, shoots and roots were harvested separately, rinsed twice briefly with ice-cold water, blot-dried and digested with 11 N HNO$_3$ at 200°C overnight. Digested samples were diluted with 0.1 N HNO$_3$ and ion contents were measured using ICP-ES (Inductively coupled plasma emission spectrometer).

**Results**

**AtABCG36 is involved in drought tolerance**

Wild-type (Wt) and AtABCG36 transgenic plants (Kim et al. 2007) grown for 4 weeks on MS agar plates or on soil did not differ in their size. However, after prolonged growth (more than five weeks), plants overexpressing ABCG36 had a taller shoot and more biomass than wild-type plants, while plants where ABCG36 was silenced were slightly smaller and produced less biomass (Fig. 1). In order to avoid that the variation in growth had an impact on our results, in the subsequent experiments we compared only plants which grew for less than five weeks.

To test whether AtABCG36 plays a role in drought resistance, we subjected the wild-type and AtABCG36 transgenic plants to drought stress. The plants were first grown on 1/2 MS agar plates for 1 week then transferred to soil where they grew for 2 weeks under normal conditions, followed by a period of 10 days where plants grew with or without watering. As shown in figure 2, no difference
was observed between wild type and AtABCG36 overexpressing plants under control conditions (Fig. 2A, left). In contrast, AtABCG36 overexpressing plants grew much better than the corresponding wild type when watering was withheld for 10 days (Fig. 2A, right). Quantitative analysis confirmed that the shoot fresh weights of AtABCG36 overexpressing plants and wild type plants were similar under control conditions but that under drought, AtABCG36 overexpressing plants exhibited a much higher fresh weight than wild-type plants (Fig. 2B). These results indicate that the increased expression of AtABCG36 is correlated with the enhanced drought tolerance of Arabidopsis plants.

Under the normal growth conditions, no difference between wild-type and AtABCG36 overexpressing plants was apparent during the entire life cycle of Arabidopsis, including flowering, seed formation, germination efficiency. Neither was there any difference in germination rate of the seeds of wild type and AtABCG36 plants grown under drought stress (data not shown). Therefore, we concluded that the overexpression of AtABCG36 resulted in improved drought resistance without any changes in developmental processes in Arabidopsis.

Because increased expression of AtABCG36 leads to enhanced drought tolerance, we were interested to test whether knock-out mutants of AtABCG36 are more drought sensitive than wild-type plants. We used two different alleles of T-DNA insertion mutants from the SALK Institute (SALK_000578; abcg36-1, SALK_142256; abcg36-2., Kim et al. 2007). Plants were grown as described above. The growth rates of wild type and knock-out plants were similar under control conditions (Fig. 3A, left). However, the two atabcg36 knock-out plants were much more sensitive to drought stress than wild-type plants (Fig. 3A, right), producing less than 50% of fresh weight compared to that of wild-type plants (Fig. 3B). These data indicate that loss of expression of AtABCG36 in Arabidopsis results in drought sensitivity. Altogether, these results strongly suggest that drought tolerance in Arabidopsis is linked to the expression level of AtABCG36.

To further characterize the AtABCG36-mediated drought tolerant phenotype, we cultivated wild-type and AtABCG36 transgenic plants in hydroponic solution. Seeds of wild type and AtABCG36 transgenic plants were germinated, grown on 1/2 MS agar plate for 1 week, transferred to containers with hydroponic culture solution, and grown for 1 week. Shoots were either exposed to low humidity condition (below 30% relative humidity) or saturated humidity. Under the highly humid condition, the growth of the wild type and AtABCG36 transgenic plants did not differ (data not shown). In contrast, under low humidity conditions, the growth of AtABCG36 overexpressing plants was more pronounced while that of knock-out plants was impaired compared to the growth of wild-type plants (Fig. 4).

**AtABCG36 overexpression confers salt resistance**
Drought stress has commonality with osmotic and salt stress since under all three stress conditions the plant suffers water deficit. Therefore we tested whether AtABCG36 contributes to salt resistance in *Arabidopsis*. When AtABCG36 overexpressing plants were germinated and grown on 1/2 MS agar plates, in the presence or absence of the NaCl concentrations indicated for 2 to 3 weeks, a substantial difference in growth could be observed. In the presence of 60 and 80 mM NaCl, AtABCG36 overexpressing plants grew better than the wild-type plants (Fig. 5A, middle and bottom), while in the control 1/2 MS plate, no growth difference was observed (Fig. 5A, top). Quantification of this effect revealed that the shoot biomass was increased to 1.5 to 1.7 fold, while the increase in root length was negligible at 60 mM NaCl and low (1.2 fold), but statistically significant at 80 mM (Fig. 5B).

We also tested whether *abcg36* knock-out mutants are more sensitive to salt stress compared to wild-type plants. These plants were germinated and grown on 1/2 MS agar plates in the presence or absence of 120 mM NaCl and grown for 17 days. The *abcg36* knock-out mutants grew similarly to the wild-type plants on 1/2 MS control plates (Fig. 6A, top). However, in the presence of 120 mM NaCl, growth of the *abcg36* knock-out mutants was more impaired than that of the wild-type plants (Fig. 6A, bottom). Quantitative analyses confirmed that the *abcg36* knock-out mutants had decreased resistance to salt. When grown in NaCl-containing medium, the shoot fresh weight and the root length of the *abcg36* knock-out mutants were significantly lower than those of the wild-type plants (Fig. 6B). To obtain clues about the function of AtABCG36 in conferring drought and salt tolerance, we measured ion contents of wild type and AtABCG36 transgenic plants. Seeds of wild type and AtABCG36 transgenic plants were germinated and grown on 1/2 MS agar plates for 2 weeks and subsequently cultivated in hydroponic solution for 1 week followed by a treatment with 50 mM NaCl for 24 hrs. This low NaCl concentration was used to avoid introducing too much difference in growth between wild-type and transgenic plants, thus to avoid secondary effects resulting from the reduced growth, or a distortion of the data during normalization with fresh weight. Sodium contents in shoots and roots of the AtABCG36 overexpressing plants were lower than those in wild type Arabidopsis (Fig. 7A and 7B, top panels). This effect was slightly more pronounced in roots. In contrast, sodium contents of roots and shoots in knock-out plants were only slightly, but not significantly higher compared to wild-type plants (Fig. 7A and 7B, bottom panels). The concentrations of other ions (Ca, K, Mg, P) were not significantly different between wild-type and AtABCG36 overexpressing (Fig. S1, Supplementary material) or *atabcg36* knock-out (Fig. S2, Supplementary material) mutants in both shoot and root. These results suggest that increased expression of AtABCG36 affect sodium contents in shoots and roots, thereby altering the Na$^+$ resistance of the plant (Fig. 5). Although all of these results suggest that AtABCG36 is involved in salt and drought resistance, the expression of AtABCG36 itself was not
induced by NaCl or PEG treatment (Fig. S3. Supplementary material).

Discussion

In this study, we showed many lines of evidence that support the role of an Arabidopsis PDR-type ABC transporter, AtABCG36, as a drought resistance factor. First, AtABCG36 overexpressing plants are resistant to drought stress, and atabcg36 T-DNA knock-out mutants are sensitive to drought (Figures 2, 3, and 4). Second, AtABCG36 overexpressing plants grow much better in sodium-containing 1/2 MS agar plates than wild type plants (Fig. 5). Third, sodium contents of AtABCG36 overexpressing plants are lower than those of wild type plants in both shoots and roots (Fig. 7). These data suggest that AtABCG36 contributes to drought and salt resistance of Arabidopsis by reducing sodium contents, at least in part.

AtABCG36 is mainly expressed in epidermal cells (Kim et al. 2007). Epidermal cells constitute the main barrier between the soil and the plants, and transporters that recognize and export toxic compounds at the epidermis can play a critical role in plant survival. AtABCG36 may contribute to exclusion of sodium from the epidermal cells, since overexpression of AtABCG36 decreases sodium contents. This decrease in sodium contents may be the direct cause of the observed drought resistance of AtABCG36 overexpressing plants. It is known that sodium has a toxic effect on many enzymes if present at high concentrations. In addition, high salt conditions in the environment cause low water availability, making it difficult for the plant to acquire water and nutrients. Therefore, salt stress results in a water deficient condition in the plant, which resembles a physiological drought. Conversely, drought decreases water contents in the cell and thereby increases the salt concentration. Therefore, high salt, drought, and osmotic stresses, which commonly result in dehydration of the cell and osmotic imbalance (Blumwald et al. 2000, Mahajan et al. 2005), can lead to the same defense mechanism involving AtABCG36.

In a previous report, we suggested a role for AtPDR8/AtABCG36 as an efflux pump of Cd$^{2+}$ or Cd-conjugates at the plasma membrane of Arabidopsis cells (Kim et al. 2007). In addition, two other laboratories provided evidence for the role of the protein in pathogen resistance and thus suggested that it may transport defense-related substances (Kobae et al. 2006, Stein et al. 2006). Many other PDR-type ABC transporters have also been suggested to have a role in pathogen resistance (Campbell et al. 2003, Jasinski et al. 2001, Sasabe et al. 2002, Stukkens et al. 2005, van den Brüle et al. 2002) and transcript levels of some Arabidopsis PDR-type ABC transporters are regulated by various biotic and abiotic stresses (Crouzet et al. 2006, Moons 2003, Smart and Fleming 1996, van den Brüle and Smart 2002). There are reports which suggest close correlation between heavy metal and other...
abiotic stress resistance. ScYCF1, an ABC transporter of *Saccharomyces cerevisiae* that contributes to cadmium resistance in yeast (Ghosh et al. 1999, Li et al. 1997) and in Arabidopsis (Song et al. 2003), has also been shown to confer salt tolerance in *Arabidopsis* (Koh et al. 2006). A Na⁺/H⁺ exchanger, an important factor for Na⁺ tolerance (Nass et al. 1997), is activated by heavy metals such as cadmium and zinc (Koutsogiannaki et al. 2006). Cadmium responsive genes are also up-regulated by abiotic stresses, e.g., dehydration, chilling, high salt, and heat (Minglin et al. 2005). Further studies on the mechanisms of diverse abiotic stress resistance may reveal how a transporter such as AtABCG36 contributes to both heavy metal and other stress tolerance.

In the present work, we showed that AtABCG36 confers drought and salt resistance, too. Interestingly, some genes induced transcriptionally by osmotic stress also participate in plant defense responses to wounding and pathogen attack. They include peroxidase, PR-1, PR-10, and osmotin (PR-5) genes (Ingram et al. 1996, Zhu et al. 1995). Pretreatment of plants with SA, an important regulator of systemic acquired resistance and inducer of many pathogenesis-related (PR) genes (Sticher et al. 1997), induces antioxidant enzymes and leads to increased salinity, drought, or chilling tolerance (Jacob et al. 2005, Kang and Saltveit 2002, Shakirova et al. 2003).

An interesting question to be resolved is whether AtABCG36 indeed transports compounds as different as cadmium, sodium, and organic solutes for pathogen defense. In this case, the transporter may be similar in its broad substrate specificity to the yeast PDR transporters PDR5 and SNQ2 which have been shown to transport hundreds of unrelated compounds (Wolfger et al. 2001). An alternative explanation is that AtABCG36 acts as a modulator as well as a transporter. Some ABC transporters function as regulator of other transporters and channels (Campbell et al. 2003, Matsuo et al. 2003), and their substrate specificity is modulated by different effectors. In case of AtMRP1 and AtPGP1, both of which interact with the TWD1 immunophilin, such interactions and modulations of transport activity have been demonstrated (Geisler et al. 2003, 2004). Finally, AtABCG36 may transport some potent chemicals such as plant hormones or second messengers which have broad-range effects covering pathogen resistance/heavy metal resistance/drought resistance. Our results on water stress resistance are in line with these possibilities. During the water deficit conditions, sugars (such as trehalose and sorbitol), sugar alcohols (such as mannitol), amino acids (such as proline), and amines (such as glycine betaine) are accumulated in plants and function as osmolytes, antioxidants or scavengers that help plants avoid and/or tolerate stresses. AtABCG36 may also transport such molecules that confer water stress resistance, or hormones and/or second messengers generated during water stress. In fact, salt stress, water stress, and drought stress are commonly related with oxidative stress, and thus AtABCG36 may contribute to resistance to these stresses by reducing oxidative stress. This possibility deserves further studies.
Previously, we showed that AtABCG36 transcript is induced by cadmium, lead or copper to confer heavy metal resistance to plant (Kim et al. 2007). However, salt or drought stresses did not induce the expression of AtABCG36 (Fig. S3. Supplementary material). It is possible that AtABCG36 activity is regulated by phosphorylation or other post-transcriptional modification to confer salt or drought resistance. Indeed, AtABCG36 has many potential phosphorylation sites and is a heavily phosphorylated protein (Alejandro and Martinoia, unpublished result).

In conclusion, our results show that AtABCG36 is important for drought and salt stress resistance in Arabidopsis, either directly or indirectly by reducing sodium contents in plants. For application, it might be useful to either search for crop varieties with constitutively high expression of the AtABCG36 homologue or to produce AtABCG36-overexpressing plants to develop crops with enhanced tolerance to abiotic stresses such as drought, salt, cadmium, and pathogen. Leafy vegetable crops equipped with such enhanced tolerance would be highly valuable.

**Acknowledgements**

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**Supplementary material**

The following supplementary material may be found in the online version of this article:

**Figure S1.** Ion content of wild-type and AtABCG36 overexpressing plants exposed to salt stress in hydroponic solution.

**Figure S2.** Ion content of wild-type and AtABCG36 knock-out plants exposed to salt stress in hydroponic solution.

**Figure S3.** The expression of AtABCG36 analyzed by quantitative real-time PCR. AtABCG36 transcript levels were quantified in whole plant of 2-week-old Arabidopsis treated with 1/2 MS solution (control), 30% PEG, 150 mM NaCl or 300 mM mannitol for the indicated times. β-tubulin was used as an expression control. The relative expression values were normalized by the expression value of control condition. Plants were grown as described in Experimental procedures. Data represent means of two biological replicates and two technical replicates.
Figure legends

Fig. 1. Growth of AtABCG36 transgenic plants under normal green house condition. (A) AtABCG36 transgenic and wild type plants grown on soil for 6 weeks under the regime of 16 h light at 22°C and 8 h dark at 18°C cycles in 50% humidity. (B) Shoot fresh weight of wild-type and AtABCG36 transgenic plants shown in (A). Note that ABCG36-1 overexpressing plants (ABCG36-1) grew much better than wild type (Wt), which in turn, grew better than ABCG36-1 RNAi plants (ABCG36i-2). The number of plants was the same for the three pots. In (B), significant differences from Wt as determined by Student’s t-test are indicated (*P < 0.05). Bars = S.E.

Fig. 2. Enhanced drought resistance in AtABCG36 overexpressing plants. (A) Growth of wild-type and AtABCG36 overexpressing plants on soil under either normal watering condition (left) or after drought treatment (right). For drought treatment, water was withheld from 3-week-old plants for 10 days. (B) Shoot fresh weight (Fw) of wild type and AtABCG36 overexpressing plants shown in (A). In (B), significant differences from Wt as determined by Student’s t-test are indicated (*P < 0.01). Bars = S.E.

Fig. 3. Decreased drought resistance in atabcg36 knock-out mutants. (A) Growth of wild-type and atabcg36 knock-out mutants on soil under either normal watering condition (left) or after drought treatment (right). Experimental procedures were the same as described in figure 2. (B) Shoot fresh weight of wild type and atabcg36 mutants shown in (A). In (B), significant differences from Wt as determined by Student’s t-test are indicated (*P < 0.01). Bars = S.E.

Fig. 4. AtABCG36 transgenic plants grown in hydroponic solution and exposed to dry air. (A) and (B) photographs of representative wild-type (Wt), AtABCG36 overexpressing (ABCG36-1, -2, -3) and knock-out (abcg36-1, -2) plants. Seeds were germinated and grown on 1/2 MS agar plate for 10 days and then transferred to hydroponic solution for 10 days. To expose the plants to drought stress, the cap
of the container with hydroponic solution was open and the container was placed under low humidity condition. (C) and (D) Shoot fresh weights (Fw) of wild-type and AtABCG36 transgenic plants shown in (A) and (B), respectively. In (C) and (D), significant differences from Wt as determined by Student’s t-test are indicated (*P < 0.05). Bars = S.E.

**Fig. 5.** Enhanced salt resistance of AtABCG36 overexpressing plants. (A) Growth of wild-type (Wt) and AtABCG36 overexpressing plants (ABCG36-1, 2, 3) on NaCl-containing 1/2 MS plates. Seeds were germinated and grown for 2-3 weeks on agar plates containing 1/2 MS medium with or without supplemented NaCl. (B) Shoot fresh weights (FW) and root lengths of AtABCG36 overexpressing plants shown in (A). In (B), significant differences from Wt as determined by Student’s t-test are indicated (*P < 0.05). Bars = S.E.

**Fig. 6.** Decreased salt resistance of abcg36 knock-out mutants. (A) Growth of wild-type (Wt) and abcg36 knock-out mutant (abcg36-1) on NaCl-containing 1/2 MS plates. Seeds were germinated and grown for 2-3 weeks on agar plates containing 1/2 MS medium with or without supplemented NaCl. (B) Shoot fresh weights (Fw) and root lengths of abcg36 knock-out mutant shown in (A). In (B), significant differences from Wt as determined by Student’s t-test are indicated (*P < 0.05). Bars = S.E.

**Fig. 7.** Sodium content of wild-type and AtABCG36 transgenic plants grown in hydroponic solution. Shoot (A) and root (B) sodium content of wild-type, AtABCG36 overexpressing (top), and knock-out (bottom) plants. Seeds were germinated and grown on 1/2 MS agar plate for 10 days and then transferred to hydroponic solution. After 1 week of incubation, NaCl was added to the solution to a final concentration of 50 mM. After 24 h, shoot and root were collected separately and ion contents were measured using ICP-ES (Inductively coupled plasma emission spectrometer). The values are averages ± S.E. of three different experiments with 4 replicates each. In (B), significant differences from Wt as determined by Student’s t-test are indicated (*P < 0.05). Bars = S.E.

**Figure S1.** Ion content of wild-type and AtABCG36 overexpressing plants exposed to salt stress in hydroponic solution. Experimental procedures were the same as described in figure 7.

**Figure S2.** Ion content of wild-type and AtABCG36 knock-out plants exposed to salt stress in hydroponic solution. Experimental procedures were the same as described in figure 7.
**Figure S3.** The expression of AtABCG36 analyzed by quantitative real-time PCR. AtABCG36 transcript levels were quantified in whole plant of 2-week-old Arabidopsis treated with 1/2 MS solution (control), 30% PEG, 150 mM NaCl or 300 mM mannitol for the indicated times. β-tubulin was used as an expression control. The relative expression values were normalized by the expression value of control condition. Plants were grown as described in Experimental procedures. Data represent means of two biological replicates and two technical replicates.
Figure 1.

(A) ABCG36-1

Wt

ABCG36i-2

(B) Shoot Fw (mg)

<table>
<thead>
<tr>
<th></th>
<th>Wt</th>
<th>ABCG36-1</th>
<th>ABCG36i-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot Fw</td>
<td>800 ± 50</td>
<td>1600 ± 100</td>
<td>400 ± 10</td>
</tr>
</tbody>
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* indicates statistical significance.
Figure 2.

(A) Watering

No watering

(B) Shoot Fw (mg)

Watering

No watering
Figure 3.

(A) Watering

(B) No watering

Shoot Fw (mg)

Watering

No watering

Wt  

abcg36-1  abcg36-2

abcg36-1  abcg36-2
Figure 4.
Figure 5.

(A) Wt 1 2 3

control
NaCl 60 mM
NaCl 80 mM

(B) Wt
ABCG36-1
ABCG36-2
ABCG36-3

Shoot Fw (mg) / 3 plants

control NaCl 60 mM NaCl 80 mM

Root length (cm)

control NaCl 60 mM NaCl 80 mM

(*)
Figure 6.

(A) Wt  abcg36-1

control  NaCl 120 mM

NaCl 120 mM

(B)

Shoot Fw (mg) / 3 plants

control  NaCl 120 mM

Root lenght (cm)

control  NaCl 120 mM

*
Figure 7.