Running Head: Aquaporin's role in improving transpiration-use efficiency

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The Role of Tobacco Aquaporin 1 in Improving Water-Use Efficiency, Hydraulic Conductivity and Yield Production Under Salt Stress

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Tobacco (C3) plants increase their water-use efficiency (WUE) under abiotic stress and are suggested to show characteristics of C4 photosynthesis in stems, petioles and transmitting tract cells. The tobacco (*Nicotiana tabacum*) stress-induced aquaporin 1 (NtAQP1) functions as both water and CO₂ channel. In tobacco plants, overexpression of NtAQP1 increases leaf net photosynthesis (AN), mesophyll CO₂ conductance (gm) and stomatal conductance (gs), whereas its silencing reduces root hydraulic conductivity (Lp). Nevertheless, interaction between NtAQP1 leaf and root activities and its impact on plant WUE and productivity under normal and stress conditions have never been suggested. Thus, the aim of this study was to suggest a role for NtAQP1 in plant WUE, stress resistance and productivity. Expressing NtAQP1 in tomato (*Solanum lycopersicum*) plants (TOM-NtAQP1) resulted in higher gs, whole-plant transpiration and AN under all conditions tested. In contrast to controls, where, under salt stress, Lp decreased more than 3-fold, TOM-NtAQP1 plants, similar to maize (*Zea mays*) (C4) plants, did not reduce Lp dramatically (only by approx. 40 %). Reciprocal grafting provided novel evidence for NtAQP1’s role in preventing hydraulic failure and maintaining the whole-plant transpiration rate. Our results revealed independent, albeit closely related NtAQP1 activities in the roots and leaves. This dual activity, which increases the plant’s water use and AN under optimal and stress conditions, results in improved WUE. Consequently, it contributed to the plant's stress resistance in terms of yield production under all tested conditions, as demonstrated in both tomato and arabidopsis plants constitutively expressing NtAQP1. The putative involvement of NtAQP1 in tobacco's C4-like photosynthesis characteristics is discussed.
INTRODUCTION

Aquaporins (AQPs) are integral membrane proteins that increase the permeability of membranes to water, as well as to other small molecules such as CO₂, glycerol and boron (Uehlein et al., 2003; Kaldenhoff and Fischer, 2006). Of all the kingdoms, plants contain the largest AQP family, consisting of over 30 members (e.g. 35 in arabidopsis—Johanson et al., 2001 36 in maize—Chaumont et al., 2005 and 37 in tomato—Sade et al., 2009). AQPs were suggested to play a key role in plantwater balance and water-use efficiency (WUE) (Knepper, 1994; Tyerman et al., 2002; Aharon et al., 2003; Kaldenhoff and Fischer, 2006; Maurel, 2007).

Raising ambient CO₂ levels can potentially improve the growth rates of some C3 plants under optimal growth conditions (Kimball and Idso, 1983; Besford et al., 1990; Li et al., 2007), suggesting that membrane permeability to CO₂ is a growth-limiting factor in these plants (Loreto et al., 1992; Evans and von Caemmerer, 1996; Evans et al., 2000; Flexas et al., 2008). Impaired CO₂ conductance in the mesophyll following treatment with the non-specific AQP inhibitor HgCl₂ was one of the first pieces of evidence suggesting AQPs’ ability to conduct CO₂ (Terashima and Ono, 2002). The first direct evidence of this ability was reported for tobacco (*Nicotiana tabacum*) aquaporin 1 (NtAQP1) expressed in *Xenopus* oocytes (Uehlein et al., 2003). Those authors also induced NtAQP1 expression in tobacco plants and reported significant increases in photosynthetic rate, stomatal opening and leaf growth rate. A role for NtAQP1 in the photosynthetic mechanism has been recently reported in tobacco plants overexpressing NtAQP1, which showed a 20% increase in photosynthetic rate relative to controls, while *NtAQP1*-antisense plants showed a 13% decrease (Flexas et al., 2006). That study provided firm evidence (obtained by three independent research groups) for the role of NtAQP1 in increasing mesophyll CO₂ conductance (gₘ), net photosynthesis (Aₐ) and stomatal conductance (gₛ). However, no morphological, growth-rate or yield parameters were reported for any plants overexpressing the NtAQP1.

NtAQP1’s cellular localization has been reported in both the mesophyll plasma membrane (PM) and the chloroplast inner membrane (CIM) in mesophyll and guard cells (Uehlein et al., 2008). However, these two membranes showed opposite
permeability coefficients: while the water permeability of the CIM was threefold that of the PM, its CO₂ permeability was about five times lower than that of the PM. Moreover, while silenced plants showed a ca. 10-fold decrease in CIM CO₂ permeability, they did not show significantly decreased PM CO₂ permeability, and vice versa for water permeability (Uehlein et al., 2008). These results indicate a localization-function relationship in NtAQP1 cellular activity.

In addition to its role in controlling CO₂ and water permeability of mesophyll tissues and stomatal conductance (Uehlein et al., 2003; Flexas et al., 2006), NtAQP1 was originally suggested to control root hydraulic conductivity (Lₚ). Based on its high abundance in the roots, especially around the xylem vessels (Otto and Kaldenhoff, 2000; Siefritz et al., 2001), its impact on increasing the water permeability of Xenopus oocytes (Biela et al., 1999) and the decrease in root Lₚ of NtAQP1-silenced plants (Siefritz et al., 2002), NtAQP1 was suggested to play a role in controlling root Lₚ. This activity might be related to abiotic stress conditions since NtAQP1 is a stress-induced gene, it is regulated by an ABA-sensitive promoter (Siefritz et al., 2001) and it shows a significant increase in root transcript level during drought stress (Mahdieh et al., 2008). Moreover, NtAQP1-silenced plants reveal higher drought sensitivity than control plants (Siefritz et al., 2002).

Considerable progress has been made in the study of NtAQP1. Research on its water- and CO₂-transport and stress-resistance activities has progressed in parallel, but without much interaction. Furthermore, tobacco plants are known to significantly improve their WUE under drought conditions (Cakir and Cebi, 2009) and are suggested to have C4 photosynthesis characteristics in stems, petioles and transmitting tract cells (Jansen et al., 1992; Hibberd and Quick, 2002). Nevertheless, to the best of our knowledge, a specific role for AQPs in these mechanisms has never been suggested. Thus, the aim of this study was to address NtAQP1's role in the plant stress-resistance mechanisms by testing the hypothesis that NtAQP1 plays a key role in improving WUE and yield under abiotic stress.

We constructed transgenic tomato (Solanum lycopersicum) plants (a well-characterized model crop plant) constitutively expressing NtAQP1 (TOM-NtAQP1) and compared their Aₘ, gₛ, CO₂ concentration in the substomatal cavity (Cᵢ), stomatal aperture, stomatal density, whole-plant transpiration, WUE, root Lₚ and yield production to control and reciprocally grafted plants under normal and salinity-stress conditions. Our results revealed independent, albeit closely related water and CO₂
activities induced by NtAQP1 in the roots and leaves, respectively. This dual activity, which increases the plant's water use and photosynthetic rate under optimal conditions and consequently improves its WUE, contributed to the plant's (tomato and Arabidopsis) yield production under all tested conditions.

RESULTS

NtAQP1 Increases Osmotic Water Permeability (Pf) of Tobacco Mesophyll Cells

In addition to its role as a CO₂ channel, NtAQP1 expression in Xenopus oocytes proved its activity as a water channel (Biela et al., 1999). It was also suggested to be involved in regulating the mesophyll osmotic water permeability coefficient (Pf) and root Lp, in particular under stress. However, NtAQP1's specific contribution to the water permeability of these tissues was never characterized in planta. Thus, our first objectives were to measure and characterize NtAQP1 water-channel activity in tobacco mesophyll cells and to estimate its role in regulating whole-plant hydraulic conductivity.

The impact of NtAQP1 on the Pf value of tobacco mesophyll protoplasts was measured by cell-swelling assay. Mesophyll protoplasts were isolated from tobacco (Uehlein et al., 2003) expressing NtAQP1 under a 35S tetracycline-inducible promoter. The induced cells had three times higher Pf values than the control non-induced cells (Fig. 1), indicating NtAQP1's activity as a functional water channel in mesophyll cells.

The Impact of NtAQP1 Water Conductivity and Photosynthesis in Tomato Plants Under Normal and Salt-Stress Conditions

To determine NtAQP1's impact on whole-plant water conductivity, Aₙ, and abiotic-stress resistance (which we defined as fruit yield under stress relative to controls), we introduced the NtAQP1 gene into tomato, producing TOM-NtAQP1.

Three different independent transgenic tomato lines expressing NtAQP1 under the constitutive promoter 35S were identified at the genomic DNA, mRNA and membrane protein levels (Supplemental Fig. S1).
The impact of NtAQP1 on leaf photosynthesis and stomatal conductivity was tested using a gas-exchange measuring system that enables monitoring CO₂ assimilation rate. As expected and similar to Uehlein et al. (2003) and Flexas et al. (2006), our TOM-NtAQP1 plants showed significantly higher A_N and g_s, while the C_i concentration remained the same relative to control plants under normal conditions (Table I and Supplemental Fig. S2). A higher g_s can result from changes in stomatal density and/or stomatal pore area. An anatomical characterization of the leaf epidermis of both TOM-NtAQP1 and control plants was performed to explore this issue. Imprints of the abaxial leaf surface revealed no significant change in stomatal density among plants, whereas a significantly larger stomatal pore area was measured in TOM-NtAQP1 plants under both normal and salt-stress conditions (Table I). The higher A_N was maintained under 100 mM NaCl, suggesting that NtAQP1's ability to transport CO₂ provides a substantial benefit in improving carbon's diffusive permeability under stress conditions. Calculating the instantaneous WUE (IWUE) revealed improvement in TOM-NtAQP1 plants under normal and salt-stress growth conditions compared to the control plants with the highest improvement seen in TOM-NtAQP1 plants under salt stress (Table I).

The rate of tension-induced root exudation from the de-topped root system of TOM-NtAQP1 plants differed from that of controls under normal irrigation. Moreover, salt application caused a considerable decrease in root exudation in control plants, while TOM-NtAQP1 plants retained their original rate of sap exudation (Supplemental Fig. S3). Root L_p, accounting for the total cross-sectional area of the xylem, in TOM-NtAQP1 plants did not differ from that in control plants under normal irrigation. However, when irrigated with water containing 50 mM NaCl, the TOM-NtAQP1 plants decreased their L_p only by about 40%, while L_p of control plants decreased more than 3-fold (Table I).

Both plant types yielded a low and stable sodium concentration in their xylem sap of around 2 mM Na⁺ during the 3 h prior to irrigation with 50 mM NaCl-containing water. Within a short time after irrigation with the salt-containing water, the sodium concentration rose dramatically in the xylem sap of both plants, with control plants reaching over 26 mM Na⁺, almost twice the Na⁺ concentration in the xylem sap of TOM-NtAQP1 plants. However, when the lower exudate volume collected from the control plants following the salt treatment relative to the TOM-NtAQP1 plants was taken into account, the amount of Na⁺ that was taken up by the
de-topped root systems (Na\(^+\) discharge) did not differ between the two treatments (Table I).

The higher \( g_s \) in TOM-NtAQPI compared to control plants was supported by the wider stomatal aperture measured; however under salinity stress, the \( g_s \) values in both plants were similar, while TOM-NtAQPI plants continued to show significantly wider stomatal aperture (Table I). This phenomenon suggested that the timing of the stomatal conductivity measurements might be important. We therefore conducted continuous daily monitoring of plant transpiration.

Using a multiple lysimeter system normalized for vapor-pressure deficit (VPD) and plant leaf area, we measured the daily transpiration rates in all TOM-NtAQPI and control plants simultaneously. Daily transpiration rate in the former was higher than in control plants under normal growth conditions (Fig. 2A), mainly during the hours of high water demand—1100 to 1500 h, resulting in significantly higher relative transpiration levels for the entire day (Fig. 2A insert). Total daily transpiration was defined by the area under the daily transpiration rate curve. The differences in transpiration between TOM-NtAQPI and control plants were significant during the salinity stress treatment (Fig. 2B,C), and during subsequent recovery from salinity stress (Fig. 2D).

**NtAQPI's Role in Preventing Root-Shoot Hydraulic Failure and Improving Whole-Plant Stress Resistance**

Increasing \( g_s \) and transpiration on the one hand, while maintaining normal root \( L_p \) under osmotic stress conditions on the other, suggests dual independent roles for the NtAQPI channel in whole-plant hydraulic control. To estimate the relations between these activities of NtAQPI and the relative importance of each in the whole plant's response to stress, a reciprocal grafting experiment was conducted. Whole-plant transpiration rates and relative daily transpiration of all grafted plants were simultaneously measured (using the multiple lysimeter system and normalization of each plant to VPD and leaf area; see Materials and Methods). The grafting process did not affect the plants' behavior, as reflected by the fact that the control grafted plants, TOM-NtAQPI over TOM-NtAQPI (T/T) and control over control (C/C), maintained similar transpiration-rate patterns as their non-grafted counterparts (see Fig. 2), i.e. higher transpiration rate and relative daily transpiration of the transgenic plants under
both normal and salt treatments (Fig. 3A,B). Interestingly, the T/C grafted plants exhibited a considerable reduction, starting at midday, in the rate of the whole plant's daily course of transpiration. This midday "break" in transpiration rate in T/C plants (clearly seen under both normal and salt-stress conditions) might be explained by stomatal closure. Another explanation for the "break" might be a failure in hydraulic conductivity resulting from their higher gs and lower Lp (as was previously demonstrated in the non-grafted plants).

To estimate the relative impact of root hydraulic signals on shoot gs and AN under salt stress in the reciprocal grafted plants, both were measured in the grafted plants. These measurements were taken during two time intervals: morning (0800-1100 h) and noontime (1100-1400 h). During the morning period, no change in whole-plant transpiration rate (Fig. 3B) or in either gs or AN could be detected in the grafted plants (Fig. 3C,E). During the noon period, however, a "break" in transpiration rate was clearly seen in all but the T/T plants (Fig. 3B). While the reduction in whole-plant transpiration rate could be explained by stomatal closure for the C/C and C/T plants, this could not explain the high gs and AN values of T/C plants, which remained higher than controls and similar to T/T plants (Fig. 3D,F): both T/T and T/C plants maintained similar and significantly higher gs and AN values than C/C or C/T plants.

**TOM-NtAQP1 Plants Show Higher Yields than Controls Under Normal and Salt Treatments**

The fact that TOM-NtAQP1 plants showed significant increases in whole-plant transpiration and AN resulting in increased WUE suggests that NtAQP1 may contribute to plant vigor, biomass and yield parameters under both optimal and stress conditions. Thus, we conducted a greenhouse experiment to determine the impact of NtAQP1 on plant productivity under normal and salinity conditions.

Three independent transgenic TOM-NtAQP1 plants were grown in a controlled greenhouse under optimal or 100 mM NaCl conditions for an entire growing season. In each trial, the transgenic genotypes were compared to non-transformed plants as controls. In the salt-stress trial, all of the plants were continuously irrigated with water containing 100 mM NaCl. TOM-NtAQP1 plants did not appear to be more vigorous than control plants under either control or stress conditions.
irrigation; nevertheless, TOM-NtAQPI plants showed improved yield parameters, relative to controls, under both favorable and stressed growth conditions (Fig. 4). To rule out the possibility that NtAQPI’s impact is unique to tomato or to the Solanaceae, we conducted a complementary experiment with transgenic arabidopsis (*Arabidopsis thaliana*) plants expressing NtAQPI (Supplemental Fig. S4). These plants showed better tolerance to a salt stress of 100 mM NaCl than control plants, as reflected by their 125% higher dry biomass under salt stress and 81% higher dry biomass under control irrigation (Fig. 5).

**DISCUSSION**

NtAQPI is one of the few plant AQPs that has been thoroughly studied in the last decade. Early reports suggested that NtAQPI controls root \( L_p \), in particular under stress (Siefritz et al., 2002). Later studies suggested novel \( \text{CO}_2 \)-transport activity for this channel, launching fresh studies into its role in the mechanism governing plant photosynthesis (Uehlein et al., 2003; Flexas et al., 2006; Uehlein et al., 2008). However, despite the extensive research on this AQP, there have been no studies on the integrated impact of NtAQPI on whole-plant WUE in general and under stress. Moreover, the fact that NtAQPI overexpression triggers an increase in leaf \( g_s \), which leads to a higher rate of whole-plant transpiration, conflicts with the fact that this gene is naturally induced under stress. To expand our understanding of NtAQPI’s role in the whole plant's response to stress, we investigated its role in the water and \( \text{CO}_2 \) balance of tomato plants grown under optimal and abiotic stress conditions.

**NtAQPI’s Impact on Transpiration-Use Efficiency**

Although NtAQPI was shown to be an active water channel in *Xenopus* oocytes (Biela et al., 1999), its contribution to the water permeability of mesophyll cells was not obvious. Furthermore, the dual localization of NtAQPI in the CIM and PM (Uehlein et al., 2008) led us to measure NtAQPI water-transport activity *in planta*. Here we show that NtAQPI is indeed an active water channel in tobacco protoplasts, significantly increasing their \( P_t \) level relative to controls (Fig. 1). Thus we provide the first proof of its water-transport activity, in addition to its previously reported function as a \( \text{CO}_2 \) channel, in tobacco mesophyll cells. Interestingly,
NtAQP1 has a dual impact not only at the cellular level but also at the whole-plant level where its expression increases both transpiration and net photosynthetic fluxes. In addition, the fact that this channel is stress-induced (Siefritz et al., 2001; Mahdieh et al., 2008; Mahdieh and Mostajeran, 2009) suggests its unique involvement in the plant's mechanism of transpiration-use efficiency (biomass produced per unit of water transpired), particularly under stress. Reduced plant production under stress conditions can be explained and evaluated by the crop's transpiration-use efficiency ratio, which is linear and depends on crop characteristics (deWit, 1958; Sinclair et al., 1984; Kemanian et al., 2005).

As expected from previous reports (Uehlein et al., 2003; Flexas et al., 2006), TOM-NtAQP1 plants showed an increased response of AN to Ci under normal growth conditions. These plants retained this photosynthetic advantage under stress conditions (Supplemental Fig. S2). In addition, their leaf gs and whole-plant transpiration rate were significantly increased, due to wider stomatal aperture (Table I, Fig. 2), resulting in improved yield parameters in all treatments relative to controls (Fig. 4).

Under normal growth conditions, the above changes yielded a major improvement in productivity due to the high photosynthetic rate; however, this process was accompanied by a proportionally higher transpiration rate, thereby revealing only slightly higher IWUE (Table I). On the other hand, under stress conditions, the ratio between CO2 assimilation and transpiration increased, as reflected by the improved IWUE of TOM-NtAQP1 plants (Table I), which eventually led to higher biomass and/or yield (in comparison to controls) under stress, in both tomato and arabidopsis plants (Figs. 4 and 5).

The involvement of NtAQP1 in the mechanism controlling gs, AN, Ci and gm has been previously reported (Uehlein et al., 2003; Flexas et al., 2006). Those studies showed increased gs in NtAQP1-overexpressing tobacco plants and decreased gs in NtAQP1-antisense plants. This impact on the guard cells might be related to a direct effect of NtAQP1 in transporting CO2 (Uehlein, 2003; Flexas et al., 2006) or water, as demonstrated in this study, although we cannot rule out other indirect effects; nevertheless, based on our grafting experiments, we suggest that the impact of NtAQP1 on gs and AN is independent of the conventional root-to-shoot signal.

**Root-to-Shoot Signaling and Its Impact on gs and AN**
Signals initiated under water stress (salinity or drought) are sensed by the roots, transported to the leaf and ultimately reduce stomatal conductance. These root-to-shoot signals may be either chemical or hydraulic (reviewed by Schachtman and Goodger, 2008). A hydraulic signal may form due to the sharp decrease in root $L_p$ in response to abiotic stress. Such a decrease has been reported as a general reaction in plants to many abiotic stresses (Steudle, 2000) and was recorded also in our control plants (more than 3-fold reduction in $L_p$) in response to 50 mM NaCl. In contrast, TOM-NtAQP1 plants reduced their $L_p$ by less than 40% under the same salt stress (Table I). A reduced root hydraulic signal might explain TOM-NtAQP1’s higher transpiration, $g_s$ and $A_N$ under stress conditions compared to stressed control plants. Yet, a TOM-NtAQP1 scion grafted on a control rootstock (T/C) and exposed to salt stress still exhibited higher $g_s$ and $A_N$, similar to T/T plants (Fig. 3C-F). This suggests that NtAQP1's activity in controlling $g_s$ and $A_N$ is dominant and nearly independent of root signals.

However, T/C plants showed a midday drop in transpiration rate under both normal and stressed conditions (Fig. 3A,B). This "drop" came just after these plants had reached their daily peak transpiration rate (assumed to be coupled with peak xylem tension). Replacing the control rootstock with TOM-NtAQP1 (T/T) revealed a mirror image of these results, i.e. a peak instead of a drop in midday transpiration rate, thereby indicating the roots’ involvement in this process. Moreover, since $g_s$ of T/C plants remained high during the midday drop in transpiration (Fig. 3C-F), we can conclude that this drop is not due to stomatal closure and that it might be due to hydraulic failure resulting from the combination of high $g_s$ and low $L_p$, leading to a decrease in plant water content.

Interestingly, maize (Zea mays, a C4 plant) exhibits a similar strategy of increasing root ZmPIPs expression level and $L_p$ in response to stress or ABA (Hose et al., 2000; Parent et al., 2009). Those authors suggested that ABA has long-lasting effects on plant hydraulic properties via ZmPIPs activity, which contribute to maintenance of favorable plant water status. Moreover, a recent study revealed that inhibiting maize root $L_p$ does not reduce plant transpiration under low VPD (Ehlert et al., 2009). A similar impact of ABA on tomato root $L_p$ (Thompson et al., 2007) and on tobacco root AQP (NtAQP1, NtPIP1;1, NtPIP2;1) transcript levels and an increase in $L_p$ (Mahdieh and Mostajeran, 2009) have been recently reported.
We hypothesize that TOM-NtAQP1's stress-resistance behavior is tightly related to NtAQP1 water-transport activity in the roots. Accordingly, NtAQP1 might act as the root's "emergency" hydraulic valve, i.e., release hydraulic tension by increasing root $L_p$ under higher transpiration rate or other stress, which in turn decrease xylem tension, thereby preventing hydraulic failure in the xylem system. Nevertheless, the fact that our model plants overexpressed NtAQP1 constitutively puts the physiological relevance of our findings to the in-vivo role of NtAQP1 in tobacco into question. In other words, have we really learned something about the role of NtAQP1, or are we just seeing what happens when we artificially increase membrane permeability?

The Endogenous Role of NtAQP1 in Improving Tobacco Plant WUE

To address the above question, we looked to related studies on the impact of other AQPs on the whole plant's water balance. Interestingly, constitutive expression of several AQPs has been reported to increase plant hydraulic conductivity, suggesting that increasing membrane permeability might be the dominant role of AQP in its impact on the whole plant. However, while overexpression of stress-induced AQPs resulted in improving the plant's stress resistance, expressing AQPs which are downregulated under abiotic stress had the opposite effect. For example the rice PIP1 AQP OsPIP1;3 is a stress-induced gene which increases its transcript level in both leaves and roots of the stress-resistant upland rice (Oryza sativa L. subsp. indica cv. Zhonghan 3) in response to stress (Lian et al., 2004, 2006). Overexpressing OsPIP1;3 in the stress-sensitive lowland rice (Oryza sativa L. subsp. japonica cv. Zhonghua 11) improved its water status during conditions of water deficit and revealed an increase in whole-plant transpiration and $L_p$ (Lian et al., 2004). In another study, constitutive expression of the tomato stress-induced aquaporin SlTIP2;2 (Sade et al., 2009) led to an increase in the transgenic plants' whole-plant transpiration and dramatically improved their stress resistance. In contrast, tobacco plants overexpressing AtPIP1b (which is downregulated under drought stress; Alexandersson et al., 2005) showed a significant increase in transpiration rate yet became more sensitive to water stress (Aharon et al., 2003). Unlike this latter study but similar to the previous ones, in the present study, plants overexpressing stress-induced AQPs presented superior stress resistance relative to their controls. In addition, the differential impacts of the
different AQPs on the plant-stress response (regardless of their constitutive expression profile), suggest that studying their involvement in the stress-resistance mechanism by overexpressing them in heterologous plants is a justifiable approach.

On the basis of our results, we suggest an explanation for the tobacco "C4-like" stress-resistance mechanism. It is possible that during evolution, tobacco plants developed "C4-like" characteristics of higher maximal CO₂ assimilation rates and Lₚ as stress-resistance mechanisms. Interestingly, tobacco plants have two other C₄-analogous behaviors: a) photosynthetic cells of stems and petioles that surround the xylem and phloem possess high activities of enzymes characteristic of C₄ photosynthesis, which allow the decarboxylation of 4-carbon organic acids from the vascular system and not from the stomata, similar to the bundle sheath cells of C₄ plants (Hibberd and Quick, 2002), and b) tobacco transmitting tract cells lack ribulose bisphosphate carboxylase (Rubisco). Photosynthesis in these cells is functionally coupled to phosphoenol pyruvate carboxylasemediated "dark reactions", which result in the formation of malate (Jansen et al., 1992). The fact that NtAQP1 is highly expressed in xylem-surrounding cells and in flowers (Biela et al., 1999; Otto and Kaldenhoff, 2000; Siefritz et al., 2001) gives additional support to this hypothesis.

**CONCLUSION**

We suggest that the stress-induced AQP *NtAQP1* is involved in improving the stressed plant's transpiration-use efficiency by a combination of three independent but complementary means: the primary means involves increasing mesophyll CO₂ permeability, resulting in increased photosynthetic rate; the second involves increasing the stomatal aperture and the third involves maintaining a near constant root Lₚ, thus preventing hydraulic failure under higher xylem tensions. Obviously, this mechanism cannot function under severe stress. Nevertheless, as we show in this work, moderating the decrease of root Lₚ allows a higher transpiration rate and together with a higher CO₂ assimilation rate, the plant reveals significant improvement in productivity under normal and stress treatments.

**MATERIALS AND METHODS**

Construction of Transgenic Plants
The full-length cDNA of *NtAQP1* (a gift from Prof. Ralf Kaldenhoff) was digested from pCRII TOPO (Invitrogen, Carlsbad, CA) using *Bam*HI and *Xho*I restriction enzymes. *NtAQP1* was then cloned, using the same restriction enzymes, into binary plasmid pBIN203 (courtesy of Dr. Orit Edelbaum) under the regulation of the 35S constitutive promoter. M82 tomato (*Solanum lycopersicum*) lines were genetically transformed using disarmed *Agrobacterium tumefaciens* transformation methods (Barg et al., 1997). Arabidopsis plants were genetically transformed using the floral dip transformation method (Clough and Bent, 1998). Plants were assayed for the presence of *NtAQP1* by PCR (4 min initial denaturation at 94°C, followed by 33 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 1 min, and a final step at 72°C for 15 min) using the following primers: sense primer 35Sprom-FWD 5’- taccttcgcaagaccctcc-3’, and the reverse complementary primer NtAQP1-REV 5’-tgacctgctgtcgttttagat-3’, amplifying a 930-bp *NtAQP1* DNA fragment.

**mRNA Extraction and cDNA Construction**

Leaf tissue (100 mg) was taken from transgenic and control plants and total RNA was extracted using Tri-Reagent (Sigma-Aldrich, St. Louis, MO) according to the manufacturer’s protocol. To rule out the effect of any residual genomic DNA in the preparation, RNA was treated with TURBO DNA-free™ (Ambion, Foster City, CA) according to the manufacturer’s instructions. Total RNA (1 µg) was taken for RT-PCR using Reverse Transcriptase M-MuLV RevertAid™ according to the manufacturer’s protocol (Fermentas, Glen Burnie, MA). cDNA amplification was performed using the sense primer NtAQP1-RNA SENSE 5’-ccgggcaggtactatcc-3’, and the reverse complementary primer NtAQP1-REV 5’-tgacctgctgtcgttttagat-3’, amplifying an 830-bp *NtAQP1* cDNA fragment.

**Protein Extraction and Detection**

Leaf tissue (100 mg) was taken from transgenic and control plants and homogenized in three volumes of homogenization buffer (330 mM sucrose, 100 mM KCl, 1 mM EDTA, 50 mM Tris/0.05% MES pH 7.5, 5 mM DTT, 1 mM PMSF). The sample was then centrifuged twice for 15 min, once at 1000g (supernatant collected)
and then at 10,000g (supernatant collected). Finally the sample was centrifuged at 48,000g for 75 min to extract the microsomal phase. The pellet (microsomal fraction) was resuspended in membrane buffer (330 mM sucrose, 20 mM DTT, 50 mM Tris/0.05% MES pH 8.5).

Protein extracts were diluted in sample buffer (10% glycerol (v/v), 5% mercaptoethanol (v/v), 0.125 M Tris-HCl pH 6.8, 3% (w/v) SDS, 0.05% (w/v) bromophenol blue) and subjected to 10% SDS-PAGE. After electrophoresis, proteins were electroblotted onto a Hybond-C Extra membrane (Amersham Life Science, Buckinghamshire, UK) at 4°C for 2 h at 110 V, using transfer buffer (25 mM Tris HCl pH 8.3, 192 mM glycine) supplemented with 10% (v/v) methanol. The membranes were blocked for 1 h at 22 to 25°C with 2% (w/v) bovine serum albumin (BSA) in 10 mM Tris HCl pH 7.5, 150 mM NaCl containing 0.1% (w/v) Tween 20 (TBS-T). Briefly, membranes were incubated for 18 h at 4°C with primary antibody (1:5000 dilution, kind gift from Prof. Ralf Kaldenhoff). All subsequent steps were performed at 22 to 25°C. Following five washes of 10 min each in TBS-T, membranes were incubated for 1 h with horseradish peroxidase-linked secondary antibody. After intensive washes with TBS-T, immobilized conjugates were visualized by enhanced chemiluminescence (ECL, Amersham Life Science), followed by exposure to X-ray film.

**Plant Material and Growth Conditions**

The experiments were conducted on T$_2$-generation plants from three independent transgenic M-82 tomato (*Solanum lycopersicum*) lines overexpressing the tobacco PM aquaporin gene *NtAQP1*—TOM-NtAQP1, and non-transgenic plants as controls. The plants were transplanted to 3.9-L pots with ready mixed growing substrate and were grown for ca. 3 months (May to August) in a controlled greenhouse at 25°C and 50 to 60% relative humidity. The experimental design was completely randomized. Fertilization was added to the irrigation system automatically. Normal fertigation consisted of ca. 500 mL, three times a day. Salt treatment was applied by treating the plants with 1.5 L of 100 mM NaCl solution in the fertigation solution, given once a day.

The arabidopsis experiment consisted of two independent T$_2$ transgenic arabidopsis (*Arabidopsis thaliana*) lines overexpressing NtAQP1, and non-transgenic
plants as controls. All plants were grown in a controlled growth chamber at 22°C under short-day conditions (10 h of light) in 200-mL pots with commercial growing medium containing slow-release fertilizers. Plants were irrigated with tap water or 100 mM NaCl solution until shoot harvesting (45 days from transplanting).

Yield Parameters

Total number and weight of fruits from the transgenic TOM-NtAQP1 and control plants were measured for each plant under normal and salt-stress (100 mM NaCl) conditions. Average fruit weight was calculated by dividing the total weight of the fruits by their number. The fresh weight of the aboveground shoots was measured. Harvest index was calculated by dividing total weight of fruits per plant (fruit number x individual fruit weight) by fresh weight per plant.

Gas-Exchange Measurements

The response of $A_N$ to $C_i$ was recorded in three independent $T_2$ transgenic TOM-NtAQP1 and control plants inside a controlled greenhouse on fully expanded leaves, under all tested irrigation conditions, using a Li-6400 portable gas-exchange system (Li-Cor Inc., Lincoln, NE). All measurements were conducted between 0800 and 1400 h.

Photosynthesis was induced in saturating light (1200 μmol m$^{-2}$ s$^{-1}$) with 370 μmol mol$^{-1}$ CO$_2$ surrounding the leaf ($C_a$). The amount of blue light was set to 15% PFD (photosynthetically active photon flux density) to optimize stomatal aperture. The leaf-to-air VPD was kept at around 1 to 2.5 kPa during all measurements. Leaf temperature for all measurements was ca. 26°C (ambient temperature). Once a steady state was reached, a CO$_2$-response curve was measured and finally, the $A_N$-$C_i$ curve was plotted.

Osmotic Water Permeability Coefficient ($P_f$) Measurements

Protoplasts were isolated from tobacco (Nicotiana tabacum) leaf mesophyll (Uehlein et al., 2003) and subjected to 10 mg L$^{-1}$ tetracycline for ca. 1 h to induce NtAQP1 expression. $P_f$ was measured from the initial (videotaped) rate of volume
increase in a single protoplast in response to hypotonic solution. The $P_f$ was
determined by a numerical approach (off-line curve-fitting procedure using several
algorithms), which has been proven to yield accurate $P_f$ values over a large range of
water permeability values. The analyses were performed with the “$P_f$Fit
program” incorporating these equations, as described in detail in Moshelion et al. (2002) and
Volkov et al. (2007).

Measurements of Whole-Plant Transpiration Rate

The calculation of whole-plant transpiration rate was based on the plant’s rate
of weight loss. The examined plants were planted in 3.9-L pots. Each pot was placed
on a temperature-compensated load cell with digital output. To monitor the temporary
variation in water demand in the greenhouse, we used a vertical wet wick, made of
0.14-$m^2$ cotton fibers, that was partially submerged in a 1-L water tank. The wick
system was located on a load cell. Evaporation from the growth-medium surface was
prevented by covering the pot surface with aluminum foil. Each pot was immersed in
a non-transparent plastic container (13 x 21.5 x 31.5 cm H, W, L) through a hole in its
upper cover. The container was sealed to prevent evaporation. The load cell output
was monitored every 10 s and the average readings over 3 min were logged in a data
logger for further analysis. The whole-plant transpiration rate was calculated by a
numerical derivative of the load cell output after a data-smoothing process. The
plants’ daily transpiration rate was normalized to their total leaf area (measure by Li-
Cor area meter, model Li-3100) and the neighboring submerged wick (i.e. mean daily
evaporation was averaged for a given treatment, over all plants; wick daily amount =
100%).

The plants were fertigated once a day by adding a commercial fertilizer
solution to the container. The salinity stress consisted of a solution of 100 mM NaCl
to which the normal dosage of nutrients was added. The salinity treatment was applied
for 3 consecutive days. Normal irrigation was resumed at the end of the stress
treatment to examine the plants' recovery patterns.

Stomatal Aperture and Density
Abaxial leaf stomata were imprinted on glass as detailed in Geisler and Sack (2002). All samples were collected at around 1100 h (at peak transpiration). Counting and photographing were performed under a bright-field inverted microscope (Zeiss 1M7100; Zeiss, Jena, Germany) mounted with a Hitachi (Hitachi, Japan) HV-D30 CCD camera. Stomatal images were later analyzed to determine aperture using the ImageJ software (http://rsb.info.nih.gov/ij/) area-measurement tool. A microscopic ruler (Olympus, Tokyo, Japan) was used for the size calibration.

**Grafting Experiment**

Seedlings from three independent T<sub>2</sub> transgenic TOM-NtAQP1 plants (2 weeks after germination) were used as scions on control rootstocks and vice versa. As controls, we used both TOM-NtAQP1 plants and control plants grafted on themselves. Two weeks after grafting, the plants were transplanted to 3.9-L pots and grown in a semi-controlled greenhouse, cooled by a compact evaporative air-cooling unit that allows lowering the temperature of the air passing through the evaporative pad (ca. 30°C). Plants (8 weeks old) were exposed to a series of measurements including whole-plant transpiration rate and gas exchange (leaf temperature was ca. 30°C, ambient temperature) as described above.

**Root Conductivity (L<sub>p</sub>)**

Plants from two independent T<sub>2</sub> transgenic TOM-NtAQP1 plants and control plants were used. On the night before the experiment, the main stem was cut with a razor 5 cm aboveground and the stump was sleeved with a silicone tube with an air-tight seal. The plants were then irrigated with fresh nutrient solution till drainage. The next morning (0800 h), the plants were irrigated again and a vacuum pump (RK 400, Today's Instruments Co., Taiwan) was connected to the sleeve via a custom-made liquid trap (for details, contact R. Seligmann) and vacuum was adjusted to a suction of 80 kPa. The first 15 min of exuded sap was discarded and thereafter the sap was collected every 30 min. After 3 h, excess salt solution was added (50 mM NaCl) and sap collection was continued for another 3 h. Sap fractions were weighed and plant discharge was calculated. Samples were then stored in the cold (5°C) for later mineral element analysis. Stem cross sections (250 µm) were taken using a vibratom...
(VT1000S, Leica, http://www.leica.com) and photographed under a bright-field inverted microscope (Olympus-IX8 Cell-R) mounted with an Orca-AG CCD camera (Hamamatsu, Hamamatsu, Japan). Image analysis of the sections was used to determine the area of the xylem elements using the ImageJ software area-measurement tool. A microscopic ruler (Olympus) was used for the size calibration. Sap discharge was normalized to flux (average velocity) based on conductive area. Hydraulic conductivity (Lp) was calculated using the general flow equation and accounted for both hydrostatic (P) and osmotic (π) pressure gradients (Joly, 1989). The Osmotic component included only sodium concentrations as the osmotic component calculated for the other cations in the sap was relatively small, and therefore it was neglected. The reflection coefficient (σ) of the entire root system was assumed to be 0.5 (based on Steudle, 2001).

**Sap Ion Concentration**

ICP analysis (ARCOS-SOP, SPECTRO Analytical Instruments GmbH, Germany) was used to determine sodium (Na+) sap concentration and Na+-uptake rate was quantified by multiplying Na+ concentration by sap discharge.

**Statistical Analysis**

Statistically significant differences between plants with altered NtAQP1 expression and their respective controls were analyzed using Student’s t-test for comparison of the means. In all cases, the level of significance was set at \( P < 0.05 \). \( P \)-value was computed using Fisher's Exact Test statistic. All of the results, unless otherwise specified, are presented as mean ± SE. Root Lp and sap ion concentration results were grouped into pre- and post-salt treatment and analyzed for significance using EMS followed by Student’s t-test: with \( \alpha = 0.05 \) (JMP, SAS Institute Inc., Cary, NC).

**Accession Numbers**

Sequence data from this article can be found in GenBank data libraries under the following accession number: *Nicotiana tabacum aquaporin 1* (AJ001416).
Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Screening of tomato plants that regenerated following co-cultivation of explants with *Agrobacterium*.

Supplemental Figure S2. The response of net photosynthesis (\(A_N\)) to substomatal CO\(_2\) concentration (\(C_i\)) in control and TOM-NtAQP1 transgenic plants.

Supplemental Figure S3. Root-system sap exudate discharge, measured from de-topped plants under vacuum, before and after application of 50 mM NaCl.

Supplemental Figure S4. Screening of arabidopsis plants following flower co-cultivation with *Agrobacterium*.

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Figure legends:

Figure 1. NtAQP1 expression increases osmotic water permeability coefficient
(Pf) of tobacco mesophyll membrane.
Columns represent the mean Pf(±SE) of tobacco mesophyll protoplasts expressing
NtAQP1 (white bar; n = 15) and control protoplasts (black bar; n = 9). *Significant
difference between the means (t test, P < 0.05).

Figure 2. Whole-plant daily transpiration rate and relative transpiration of
TOM-NtAQP1 and control plants grown under normal irrigation and salt stress
in a controlled greenhouse.
(A) Mean daily transpiration rate (±SE; normalized to leaf area) of TOM-NtAQP1
(gray line, n = 3) and control plants (black line, n = 3) under normal irrigation regime.
(B) and (C) Mean daily transpiration rate (±SE; normalized to leaf area) in plants after
1 and 3 days, respectively of 100 mM NaCl salinity stress, and (D) their first day of
recovery (receiving normal irrigation again), \( (n_{\text{NtAQP1}} = 3; n_{\text{control}} = 3) \). All inserts represent mean daily relative transpiration (%), normalized to VPD and leaf area, of the plants presented in the graphs. Data is given as mean ± SE for each 10th sampling point. When not seen, SE is smaller than the symbol. *Significant difference between means (\( t \) test, \( P < 0.05 \)).

Figure 3. Grafted plants: daily transpiration rate, \( g_s \), and \( A_N \) under normal and 100 mM NaCl irrigation.

(A) Whole-plant mean daily transpiration rate normalized to leaf area of all reciprocal grafted TOM-NtAQP1 and control plants measured simultaneously under normal irrigation and (B) after 3 days of 100 mM NaCl irrigation \( (T/T n = 3, C/C n = 3, C/T n = 8, T/C n = 6) \). SE is given for each 10th sampling point; when not seen, the SE bar is smaller than the line. Green square represents morning hours, 0800-1100 h, and light-red square represents noon hours, 1100-1400 h. At these hours and under the same salt conditions, parallel measurements of reciprocal grafted scions were conducted for stomata conductance \( (g_s) \) and leaf net photosynthesis \( (A_N) \), during the morning hours (green square) and during the noon hours (light-red square; C and E, D and F, respectively). Bars represent mean ± SE of grafted plants (white for TOM-NtAQP1 part and black for control part; \( T/T n = 3 \) for morning and noon; \( C/C n = 4 \) for morning and \( n = 11 \) for noon; \( C/T n = 6 \) for morning and \( n = 5 \) for noon; \( T/C n = 5 \) for morning and \( n = 6 \) for noon). Different letters indicate significant difference (\( t \) test, \( P < 0.05 \)). All measurements were performed in a semi-controlled greenhouse (see Materials and Methods); gas-exchange measurements were conducted with a portable apparatuses (Li-Cor 6400) on young, fully expanded leaves.

Figure 4. Yield parameters of TOM-NtAQP1 (white bars) and control plants (black bars).

Plants grown for 3 months in a controlled greenhouse under normal \( (n_{\text{NtAQP1}} = 8, n_{\text{control}} = 3) \) and 100 mM NaCl \( (n_{\text{NtAQP1}} = 5, n_{\text{control}} = 5) \) irrigation conditions. Columns represent mean (± SE) of (A) individual fruit weight, (B) fruit number, (C) shoot fresh weight and (D) harvest index. Different letters indicate significant difference (\( t \) test, \( P < 0.05 \)).
Figure 5. NtAQPI impact on arabidopsis plant dry weight under normal and 100 mM NaCl irrigation

(A) Arabidopsis plants (45 days old) constitutively expressing NtAQPI (upper panel) and control plants (lower panel) grown in a controlled growth room under normal irrigation regime (left panel) and under 100 mM NaCl irrigation regime (right panel) for 33 days. (B) Mean (± SE) of shoot dry weight of arabidopsis plants overexpressing NtAQPI and control plants under normal (n_{NtAQPI} = 11; n_{control} = 8) and 100 mM NaCl (n_{NtAQPI} = 12; n_{control} = 6) irrigation conditions. Different letters indicate significant difference (t test, P < 0.05).
Normal irrigation | Salt irrigation
---|---
\( A_N \) (\( \mu \)mol CO\(_2\) m\(^{-2}\) s\(^{-1}\)) | Tom-NtAQP1 (n) | Control plants (n) | Tom-NtAQP1 (n) | Control plants (n)
24 ± 1.3 (20)* | 17 ± 1.5 (10) | 17 ± 0.8 (13)* | 15 ± 0.8 (9) |
g\(_s\) (mol H\(_2\)O m\(^{-2}\) s\(^{-1}\)) | 0.43 ± 0.04 (20)* | 0.31 ± 0.03 (10)* | 0.16 ± 0.02 (13)* | 0.16 ± 0.01 (9) |
\( C_i \) (\( \mu \)mol CO\(_2\) mol\(^{-1}\)) | 260 ± 8 (20)* | 248 ± 6 (10)* | 151 ± 15 (13)* | 158 ± 10 (9) |
T\(_r\) (mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\)) | 5 ± 0.4 (20)* | 4 ± 0.3 (10)* | 2.4 ± 0.3 (13)* | 2.5 ± 0.3 (9) |
stomatal density (0.01 mm\(^2\)) | 22 ± 1 (18)* | 24 ± 3 (12)* | 19 ± 1 (18)* | 21 ± 2 (12)* |
stomatal pore area (\( \mu \)m\(^2\)) | 52.9 ± 1.1 (445)* | 29.2 ± 2.0 (110) | 25 ± 1.1 (200)* | 16.1 ± 1.5 (74) |
IWUE (\( \mu \)mol CO\(_2\) mmol\(^{-1}\) H\(_2\)O) | 4.9 ± 0.3 (20)* | 4.3 ± 0.2 (10)* | 8.2 ± 1 (13)* | 6.3 ± 0.4 (9) |
L\(_p\) (mm s\(^{-1}\) MPa\(^{-1}\)) | 229 ± 68 (6)* | 225 ± 65 (6)* | 141 ± 53 (6)* | 66 ± 15 (6)* |
Na discharge (picomole s\(^{-1}\)) | 6 ± 2 (4)* | 5 ± 2 (4)* | 38 ± 2 (4)* | 35 ± 2 (4)* |

Table I. Photosynthetic characteristics (as determined at 1200 \( \mu \)mol m\(^{-2}\) s\(^{-1}\), ~25°C and 370 \( \mu \)mol mol\(^{-1}\) CO\(_2\)) and root hydraulic characteristics of TOM-NtAQP1 and control plants treated with normal irrigation and 100 mM NaCl in a controlled greenhouse.

Data are given as mean ± SE (n, number of replicates, indicated in table). Different letters in a row indicate significant difference (t test, P < 0.05).

*For the root hydraulic measurements (L\(_p\), Na discharge) plants were treated with 50 mM NaCl.
Figure 1. NtAQP1 expression increases the tobacco mesophyll membrane osmotic water permeability coefficient ($P_f$).

Columns represent the mean $P_f$ (±SE) of tobacco mesophyll protoplasts expressing NtAQP1 (white bar; $n=15$) and control protoplasts (black bar; $n=9$). * denotes significant difference between means ($t$ test, $p<0.05$).
Figure 2. Whole plant daily transpiration rate and relative transpiration of TOM-NtAQP1 (grey line and white bar) vs. control plants (black line and black bar) grown under normal irrigation and 100mM NaCl in a controlled greenhouse. (A) Mean daily transpiration rate (±SE; normalized to leaf area) of TOM-NtAQP1 (grey line n=3) and control plants (black line n=3) under normal irrigation regime. (B) and (C) Mean daily transpiration rate (±SE; normalized to leaf area) of the 1st and 3rd days of plants subjected to 100mM NaCl salinity stress, respectively and (D) their first day of recovery (receiving normal irrigation again), (n_{NtAQP1}=3; n_{control}=3). All insertions represent normalized to VPD and leaf area, mean daily relative transpiration (%) of same plants presented in the graphs. Data is given as mean ± SE for each 10th sampling point. When not seen, SE is smaller than the symbol, * denotes significant difference between means (t test p<0.05).
Figure 3. Grafted plants daily transpiration rate, $g_s$ and $A_N$ under normal and 100mM NaCl irrigation.

(A) Whole plant mean daily transpiration rate normalized to leaf area of all reciprocal grafted TOM-NtAQP1 and control plants measured simultaneously under normal irrigation and (B) 3 days of 100mM NaCl irrigation ($T/T \ n=3$, $C/C \ n=3$, $C/T \ n=8$, $T/C \ n=6$). SE is given for each 10th sampling point, when not seen the SE bar in smaller than the line. Green square represent the morning hours, 08:00-11:00, and the light-red square represent the noon hours, 11:00-14:00. At these hours and under the same salt conditions, a parallel measurement of reciprocal grafted scions were conducted for stomata conductance ($g_s$) and leaf net photosynthesis ($A_N$), during the morning hours (green square) and during noon hours (light-red square ; $C$ and $E$, $D$ and $F$, respectively). Bars represents the mean $\pm$ SE of grafted plants (white for TOM-NtAQP1 part and black for control part; $T/T \ n=3$ for morning and noon; $C/C \ n=4$ for morning and $n=11$ for noon; $C/T \ n=6$ for morning and $n=5$ for noon; $T/C \ n=5$ for morning and $n=6$ for noon). - a,b,c,d different letters indicates significant difference ($t$ test $P<0.05$).

All measurements were performed in a semi-controlled greenhouse (see material and methods), gas exchange measurements was conducted using portable apparatuses (Li-cor 6400) on young, fully expanded leaves.
Figure 4. Yield parameters of TOM-NtAQP1 (white bars) and control plants (black bars).
Plants grown for 3 months in a controlled greenhouse under normal ($n_{NtAQP1}=8, n_{control}=3$) and 100 mM NaCl ($n_{NtAQP1}=5, n_{control}=5$) irrigation condition. Columns represent the mean ($\pm$ SE) of (A) Individual fruit weight, (B) fruit number, (C) Shoot fresh weight and (D) harvest index. a,b,c,d - different letters indicates significant difference ($t$ test $P<0.05$).
Figure 5. NtAQ1P1 impact on Arabidopsis plant dry weight under normal and 100mM NaCl irrigations.

(A) 45 days old arabidopsis plants constitutively expressing NtAQ1P1 (upper panel) and control plants (lower panel) grown in a controlled growth room under normal irrigation regime (left panel) and under 100mM NaCl irrigation regime for 33 days (right panel). (B) Mean (± SE) of shoot dry weight of arabidopsis plants over-expression NtAQ1P1 and control plants under normal ($n_{NtAQ1P1}=11$, $n_{control}=8$) and 100mM NaCl ($n_{NtAQ1P1}=12$, $n_{control}=6$) irrigation condition. a,b,c,d - different letters indicates significant difference ($t$ test $P<0.05$).