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Sodium transport in the seedlings of two bread wheat (*Triticum aestivum* L.) genotypes showing contrasting salt stress tolerance

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Abstract

In many plant species, salt sensitivity is associated with the accumulation of sodium (Na^+) in photosynthetic tissues. Na^+ uptake to leaves involves a series of transport steps and for which only few candidates' genes have been so far characterized. In this study, we provide a physiological and molecular analysis of two Algerian bread wheat varieties (*Triticum aestivum* L.), Mahon-Demias (MD) a salt sensitive and Hidhab (HD) a salt tolerant varieties. The comparative analysis of Na^+ transport revealed two major differences between the two genotype i) a lower rate of transfer from the root to the shoot (xylem loading) in the salt tolerant genotype, and ii) A higher capacity of the leaf sheath in the tolerant genotype to extract and sequester Na^+ as it entered the leaf. In addition, an enhanced uptake of K^+ in leaves of Hidhab compared to Mahon-Demias resulting in a higher K^+/Na^+ ratio in leaf blades and hence improving cellular homeostasis in the tolerant variety. Moreover, correlation was observed between the expression patterns of the transcripts encoding the plasma membrane Na^+/H^+ antiporter (TaSOS1), two members of the HKT transporters family (HKT1;5 and HKT2;2) and the Na^+ fluxes from roots to leaves. All together, these results help to understand the differential salt stress tolerance between Hidhab and Mahon-Demias wheat varieties. More interestingly, our data may ultimately contribute to deciphering the physiological and molecular mechanisms of salt stress tolerance in bread wheat, and hence to assist breeders in selecting salt tolerant genotypes.

Keywords: salinity tolerance, bread wheat, leaf sheath, leaf blade, Na^+ transport.
Abbreviations: HD- Hidhab; HKT- high affinity K^+ transporters; MD- Mahon–Demias; RWC- relative water content; TaSOS1-wheat plasma membrane Na^+/H^+; antiporter TNHX1-wheat vacuolar Na^+/H^+ antiporter; TVP1- vacuolar H^+-pyrophosphatase pump; Ψw- water potential; QTL- quantitative trait loci

Introduction

Soil salinity is one of the major world wide environmental constraints affecting agricultural production in arid and semi-arid regions. Despite a significant progress achieved towards our understanding of a molecular mechanisms controlling plant's response to salinity, there have been a few, if any, cereal cultivars with improved salinity tolerance. Glycophytes such as bread wheat (*Triticum aestivum*) cope with salinity stress by excluding Na^+ from shoots (Munns and James, 2003; Colmer et al., 2005) and by tolerating high internal levels of Na^+, which is also referred to as tissue tolerance (Yeo and Flowers, 1983; Colmer et al., 2005; Munns et al., 2006; Tammam et al., 2008). When grown in saline environments, bread wheat is generally more salt tolerant than durum wheat, and this is likely to be largely due to its better Na^+ exclusion (Colmer et al., 2006). In contrast, barley is more salt tolerant than bread wheat or durum wheat despite having leaf blade Na^+ concentrations similar to those of durum wheat (Maas, 1986), which suggests greater levels of tissue tolerance to Na^+. Given the number of mechanisms that contribute to salt tolerance (Colmer et al., 2005), it is perhaps not surprising that salt tolerance is also a genetically complex trait. Genetic studies in rice (Gregorio and Senadhira, 1993) and wheat (Singh and Singh, 2000) have demonstrated that salt tolerance is governed by multiple genes with additive, dominance and reciprocal effects. Despite the complexity of salt tolerance, much of the recent work to improve the level of salt tolerance in wheat has focused on Na^+ exclusion in plant tissues as the most appropriate selection criteria. Genotypic differences in Na^+ exclusion (estimated by the Na^+ concentration in the leaf blade or whole shoot) can be demonstrated in wheat, but its relationship with salt tolerance is not consistent. Hexaploid bread wheat cultivars have slow rates of Na^+ transport to the shoot, and maintain a high K^+/Na^+ ratio in leaves. This enhanced K^+/Na^+ discrimination trait contributes to salt tolerance (Dvorak et al., 1994; Tammam et al., 2008). A locus for this trait, *kna*1 was mapped to the distal region of chromosome 4DL (Dubcovsky et al., 1996) of the bread wheat cultivar, whereas the tetraploid durum wheat lacks this trait. A homologue of the *kna*1 locus has not yet been found on either the A or B genomes of tetraploid wheat species. A new source of Na^+ exclusion was found in durum wheat, Line 149, which had a low Na^+ concentration and high K^+/Na^+ ratios in the leaf blade similar to bread wheat (Munns et al., 2000). Genetic studies indicated that two major loci *Nax*1 and *Nax*2 (Na^+ exclusion loci) controlled leaf blade Na^+

Table 1. List of the primers used for RT-PCR analysis of the wheat candidate genes.

Gene	N°. Access	Primer	Sequence
HKT1;5	DQ64633	HKT8_F3	5'-CTGTCGCTCTTCTGCGCCAT-3'
		HKT8_R3	5'-TTATACTATCCTCCATGCCT-3'
HKT2;2	DQ015706	HKT2_F3	5'-GATCCACTCAACTTCTCCAC-3'
		HKT2_R3	5'-TCATACTTTCCAGGATTTAC-3'
TNHX1	AY296910	B_3F	5'-TCGGAAAATTCCTCTACCTA-3'
		B_4R	5'-AGAACAACAATGATTGTGCT-3'
TVP1	AY296911	TVP_F	5'-GTCAGCAGAGCTGGTGTGAAG-3'
		TVP_R	5'-TCAGCTTGATGAGGATGTTGA-3'
TaSOS1	AY326952	KM_1F	5'-GCATCTTATTGGAAGGATTT-3'
		KM_2R	5'-CCTCTCAGGTGAGACTGCTA-3'
Actin	AB181991	Act_F	5'-GTGCCCATTTACGAAGGATA-3'
		Act_R	5'-GAAGACTCCATGCCGATCAT-3'

accumulation in Line 149 (Munns et al., 2003). Recently, a molecular marker linked to *Nax1* was identified and has facilitated the rapid transfer of this trait into commercial varieties of durum wheat (Lindsay et al., 2004). Physiological studies indicated that in a tolerant genotype, the control leaf blade Na^+ concentration is the result of the interaction between net xylem loading and leaf sheath sequestration (Davenport et al., 2005). Using near-isogenic lines, it was found that the major role of *Nax*1 in conferring salt tolerance was through a greater removal of Na^+ from the xylem in the roots and in the leaf sheath, thereby reducing Na^+ concentrations in the leaf blade (James et al., 2006). Under salinity, sodium gains entry into root cell cytosol through selective and non selective cation channels, or into the root xylem stream via an apoplastic pathway depending on the plant species. The HKT (High Affinity K^+ Transporters) transport systems are active at the plasma membrane and have been shown to function as Na^+/K^+ symporters and as Na^+-selective uniporters (Horie and Schroeder, 2004; Garciadeblas et al., 2003). Phylogenetic analyses of available HKT sequences revealed two major subfamilies named 1 and 2 (Platten et al., 2006). It was suggested that subfamily 1 (HKT1;x) includes HKT transporters permeable to Na^+ only, whereas subfamily 2 (HKT2;y) refers to transporters that are permeable to both Na^+ and K^+. HKT transporters appear to play an important role in the control of Na^+ transport in bread wheat (Laurie et al., 2002) and may also transport sodium and contribute to salt tolerance in durum wheat. The Na^+ compartmentation into vacuoles provides an efficient mechanism to avert the toxic effects of Na^+ in the cytosol. The transport of Na^+ into the vacuoles is mediated by cation/H^+ antiporters that are driven by the electrochemical gradient of protons generated by the vacuolar H^+-translocating enzymes, the H^+-ATPase and the H^+-PPase. These phosphatases generate the necessary proton gradient required for activity of Na^+/H^+ antiporters and homeostasis equilibrium (Chinnusamy et al., 2005). Vacuolar NHX (Na^+/H^+ exchange) transporters have been shown to play significant roles in endosomal pH regulation (Yamaguchi et al., 2001), cellular K^+ homeostasis and cell expansion (Apse et al., 2003), vesicular trafficking and protein targeting (Bowers et al., 2000; Sottosanto et al., 2004; Brett et al., 2005). A correlation between the expression of genes encoding NHX antiporters in salt-tolerant cultivars and their salt tolerance was shown in cotton (Wu et al., 2004). Similar results were observed in wheat (Saqip et al., 2005) suggesting that the higher expression of endogenous vacuolar Na^+/H^+ antiporters in roots and shoots of the salt-resistant wheat genotypes facilitated Na^+ exclusion from the cytosol, improving salt tolerance (Saqip et al., 2005). Previously, several reports provide the evidence that the overexpression of NHX genes improve salt tolerance of several plant species, indicating their role in vacuolar Na^+ sequestration (Brini et al., 2007; Yamaguchi and Blumwald, 2005; Tester and Davenport, 2003; Zhang and Blumwald, 2001; Apse et al., 1999). However, it was reported that NHX proteins act not only as Na^+/H^+ antiporters but possess also K^+/H^+ exchange activity with similar efficiency (Venema et al., 2002). Recently, Functional characterization of wheat Na^+/H^+ antiporter TNHX1 and vacuolar H^+-PPase pump TVP1 was reported by Brini et al., (2005). Transgenic *Arabidopsis* plants overexpressing the wheat vacuolar Na^+/H^+ antiporter TNHX1 and H^+-PPase TVP1 are more resistant to high concentrations of NaCl and to water deprivation than the wild-type plants (Brini et al., 2007). Sodium efflux from root cells prevents the accumulation of toxic levels of Na^+ in the cytosol and its transport to the shoot. Molecular genetic analysis of *Arabidopsis* sos (salt overly sensitive) mutants have led to the identification of a plasma membrane Na^+/H^+ antiporter, SOS1, which plays a crucial role in sodium exclusion from root epidermal cells under salinity. Understanding the molecular basis of salt-stress signalling and tolerance mechanisms in wheat becomes today mandatory for engineering and/or screening for local wheat genotypes more tolerant to salt stress. In this report, we performed physiological and molecular analysis on two Algerian bread wheat genotypes (*Triticum aestivum* L.), Mahon-Demias and Hidhab with contrasting tolerance to salinity. Our data provide the evidence for a functional correlation linking Na^+/ fluxes and the expression patterns of SOS and HKT-type transporters to salt stress tolerance in bread wheat.

Materials and methods

Plant material, germination assay and stress conditions

The seeds of two bread wheat cultivars *Triticum aestivum* L Mahon- Demias (MD, salt sensitive) and *Triticum aestivum* L Hidhab (HD1220, salt tolerant) were supplied by the Agricultural Research Station of Sétif (ARSS- Algeria). Seeds of each line were sterilized in 0.5 % NaOCl for 15 min, then washed three times with sterile water and placed on Petri dishes with a single sheet of Whitman #1 filter paper for germination. The percentage of seed germination was

determined as the ratio of the number of seeds with radicals growing at least to 2 mm long, over the initial seed number soaked on wet Whitman paper. To test the response of the seeds to salt-stress, 30 seeds of the two wheat varieties were germinated on various concentrations of NaCl (0, 50, 100 and 200 mM) and incubated at 25°C in growth chamber under a 16 h light/8 h dark photoperiod and 60±10% relative humidity. Four days old seedlings were transferred to Eppendorf tubes floating on modified half-strength Hoagland's solution in containers (Epstein, 1972). When plants reach the third leaf stage, NaCl concentrations (0, 50, 100, and 200 mM) were applied progressively (salt treatment). All seedlings were grown in a glasshouse at 25±5°C, under photosynthetically active radiation of 280 µmol m^2s^{-1}, a 16 h photo-periods and 60±10% relative humidity. A first harvest was made at the beginning of salt treatment (initial harvest) and sequential harvests were made at different times (3, 7, 10 and 14 d) of exposure to salinity (final harvest). All the physiological tests were performed on leaves at the same developmental stage (leaf 1 or leaf 2).

Leaf surface determination

UTHSCSA image tool is a free image processing and analysis program. It can acquire, display, edit and analyze images (http://ddsdx.uthscsa.edu/dig/itdesc.html). Total leaf area of wheat seedlings in hydroponics system was calculated in centimetres square using image tool program. Photograph of plants were taken every 2 d.

Measurement of ions, relative water contents and water potential

Dry plant material was extracted in nitric acid (0.5% HNO3) at 70°C for at least 4 d. Na^+ and K^+ contents were estimated by HPLC. To measure the relative water content (RWC), leaves were excised and their fresh weight taken immediately. After floating on deionised water at +4°C overnight, their rehydrated weight was determined. Finally, they were dried in an oven at 70°C overnight and weighed. The RWC was calculated as (fresh weight–dry weight) / (rehydrated weight–dry weight). For Water potential measurement, the leaf discs (5 mm in diameter) from fully watered and salt-stressed plants were excised and loaded into the holder of a WESCOR C-52 chamber. After inserting the slide, the chamber was sealed, and leaf water potential (MPa) estimated with a vapour pressure psychrometer (WESCOR, PSWPROTM).

RNA extraction and RT-PCR assay

Total RNA from roots, leaf sheaths and leaf blades of 1-week-old plants treated with 100 mM NaCl for 3 d, were extracted using the RNeasy total RNA isolation kit (Qiagen). To remove contaminating DNA, RNAs (10µg) were treated with RNase-free DNase (Promega). DNase-treated RNA samples (0.5µg) were reverse-transcribed using M-MLV reverse transcriptase (Invitogen). The reverse transcription (RT) reactions were performed at 37°C for 1h using 2µM oligo-dT_{18}. Two µl of the first strand cDNAs were used as templates for PCR amplification with specific primers of candidate genes (Table 1). A wheat *Actin* gene fragment was used as an internal control. Samples were denatured for 5 min at 94°C and then run for 35 cycles of 30 sec at 94°C, 45 sec at 58°C and 2 min at 72°C with a final extension of 5 min at 72°C. The PCR products were separated by agarose gel electrophoresis.

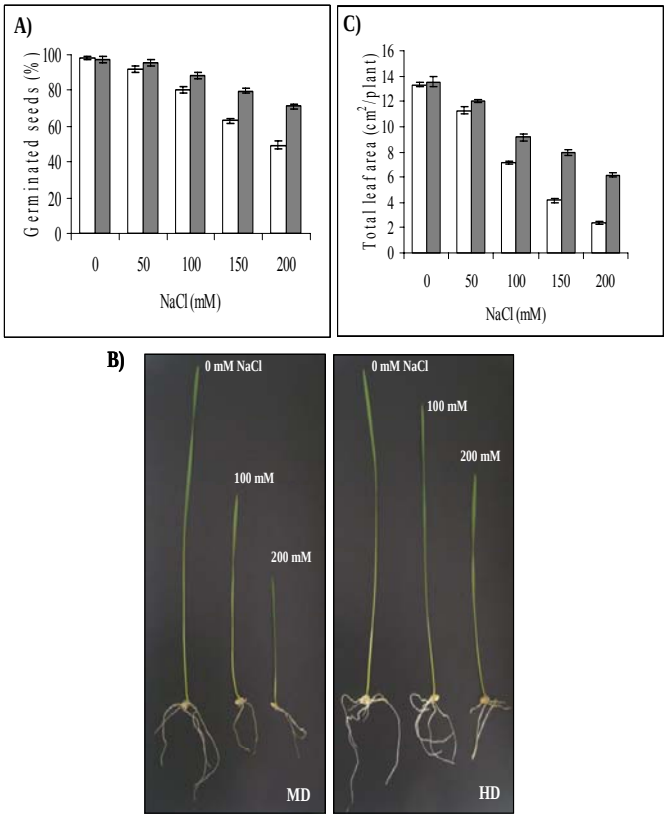

Fig 1. NaCl effect on the germination and growth of the two bread wheat HD and MD genotypes. (A), Comparison of seed germination rates of salt sensitive MD and salt tolerant HD. (B), Effect of salt stress on growth of HD and MD genotypes after 7 d of continuous treatment with 0, 100 or 200 mM NaCl. (C), Final total leaf area of seedling of MD (white bars) and HD (grey bars) after 14 d treatment. Values are means ± SD (n = 4).

Results

Germination and seedling growth

Under standard growth conditions, the overall seed germination rates of the cultivars Mahon-Demias (MD) and Hidhab (HD) were > 98% (Fig.1A). However, in the presence of increasing concentrations of NaCl, a gradual decrease in the germination rates was observed (Fig. 1A). Under high salinity (200 mM NaCl), the germination of the sensitive genotype (MD) was severely affected and did not exceed 48%, whereas in salt-tolerant HD genotype it was maintained to ≈ 70%. Moreover, these salt stress treatments affect seedling growth and result in both leaf and root length reductions which are greater in MD (Fig. 1B). Consequently, the estimated total leaf area was more dramatically reduced in MD than in HD (Fig. 1C).

Ion status

Following an exposure to 100 mM of NaCl, Na^+ concentrations were measured in individual leaves of both wheat genotypes. At the leaf sheaths (leaf 1 and leaf 2), sodium accumulates at similar rates in both genotypes after the first 3 d of salt treatment, but reaches later substantially higher levels in the HD variety especially at day 7 (Fig. 2A).

However, the leaf blades of MD accumulate Na^+ up to 250 mM, a concentration 5 times higher than the values registered in HD variety (Fig. 2B). By contrast, in the root of both genotypes, similar Na^+ concentrations were registered (Fig. 2C). Storage of Na^+ in the two wheat genotypes was investigated further by measuring the Na^+ content in the leaf sheaths and the leaf blades (leaves 1 and 2) after 7 d of exposure to increasing concentrations of NaCl. Both wheat varieties accumulated Na^+ at different levels in the leaf sheath, and HD accumulated a substantially higher Na^+ concentration than MD with no evidence of saturation of storage (Fig. 3). The two genotypes seem to have a contrasting capacity to store Na^+ in the leaf sheath and their leaf sheath cells may differ in their ability to extract Na^+ from the xylem stream. This possibility was supported by genotypic differences in the proportion of total leaf Na^+ content that was stored in the leaf sheath (Fig. 2C and 2F). HD sequestered up to 85% of total leaf Na^+ in the leaf sheath, and this capacity appeared to reach saturation since a slight decrease was observed at 200 mM NaCl (Fig. 3C and 3F). In contrast, salt sensitive MD stored only up to 65% of leaf Na^+ in the sheath, with little change in response to external NaCl levels (Fig. 3C and 3F). This result suggests that HD genotype possesses some additional mechanism for maintenance of low blade Na^+, involving efficient withdrawal of leaf Na^+ into the sheath.

To investigate whether the preferential accumulation of Na^+ in leaf sheaths of HD represents a general solute accumulation mechanism, we measured K^+ in the leaf blade and leaf sheath of both genotypes during 14 d growth in the presence of 100 mM NaCl. K^+ was accumulated to similar levels in the leaf sheath of each genotype, whereas in leaf blades, more K^+ accumulated in HD than MD (Fig. 4A and 4B) giving a higher K^+/Na^+ ratio in the salt tolerant genotype (Fig. 4C). In roots, K^+ content was higher in HD genotype compared to MD genotype (Fig. 4D). The root's ability to retain K^+ correlates with a higher salt tolerance in HD, compared to MD genotype.

Water potential and relative water content

Leaf water potentials (Ψw) of leaf blade and leaf sheath of unstressed seedlings were similar in HD and MD (~ -1.2 and -1.1 MPa, respectively) (Fig. 5). When challenged with salt stress (100 mM NaCl) for 7 d, the Ψw decreases at similar rates in the leaf blades of both cultivars (~ -2.5 MPa) (Fig. 5B). Whereas, in leaf sheath, we registered a lower Ψw in HD (~ -3.1 MPa) than in MD (~ -1.6 MPa) (Fig. 5A). The differences between Ψw values registered in leaf sheaths of both wheat varieties might be due to relatively higher rates of osmotic adjustment in HD. Osmotic adjustment may lower Ψw in the sheath, hence increasing the overall leaf:soil Ψw gradient which is the driving force for water uptake into the plant. This leads to greater water uptake at low soil solution Ψw which can occur due to salinization. MD and HD also responded to salinity with significant reduction in relative water content (RWC). After 7 d of salt stress (100 mM NaCl), RWC of HD was maintained at approximately 84% of unstressed plants, whereas the RWC of MD decreased to 58% of control values (Fig. 6A and 6B). After 14 d of stress, RWC in MD dropped to 20% and 31% of controls for the leaf blade and the leaf sheath, respectively. In contrast, losses of RWC by HD were much smaller and decreased to 48% of unstressed controls in the leaf blade and to 65% of controls in the leaf sheath. The maintenance of greater RWC in the leaves of HD is in agreement with the enhanced capacity of this variety for osmotic adjustment.

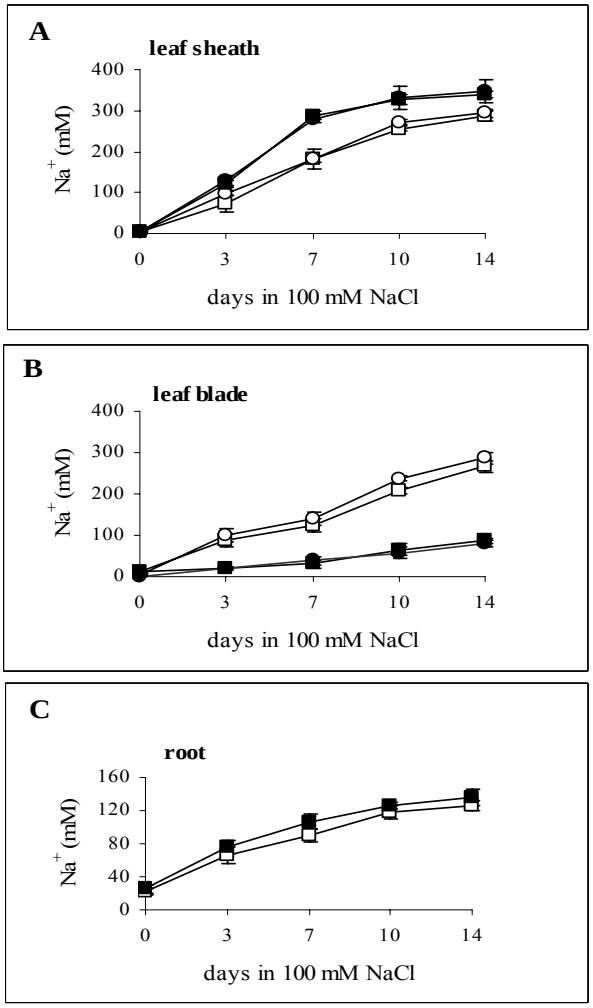

Fig 2. Increase in Na^+ concentrations in the leaf sheaths (A) and leaf blade (B) of leaf 1 (squares), and leaf 2 (circles), and roots (C), of salt tolerant HD (black symbols) and salt sensitive MD (white symbols) during 14 d exposure to 100 mM NaCl.

Expression analysis of candidate genes HKT1;5, HKT2;2, TNHX1, TVP1 and TaSOS1 in the two bread wheat varieties

Many genes were previously shown to play important roles in maintaining K^+ or Na^+ homeostasis in higher plants. We have studied the expression levels of five candidate genes involved in the control of uptake, transport and sequestration of Na^+ ions. RT-PCR analysis of two HKT encoding genes: HKT1;5 (previously named HKT8) and HKT2;2 (previously named HKT2) in cultivars MD and HD exposed to 100 mM NaCl showed a high expression levels of both genes in roots but neither in leaf sheaths nor in leaf blades (Fig. 7). Whereas HKT2;2 shows similar expression patterns in the roots of the two wheat genotypes, HKT1;5 transcripts seem to accumulate to higher levels in HD (Fig. 7). Regarding the vacuolar Na^+/H^+ antiporter TNHX-1, the transcript levels in the roots, sheaths and blades were greater in HD than in MD genotype (Fig. 7). More transcripts seem to accumulate in the roots and leaf sheaths compared to leaf blades in the two genotypes (Fig. 7). The expression level of the vacuolar H^+-Pyrophosphatase TVP1, was comparable to that observed with TNHX1. In fact, the roots and sheaths of the two genotypes accumulated more TVP1 transcript than the leaf

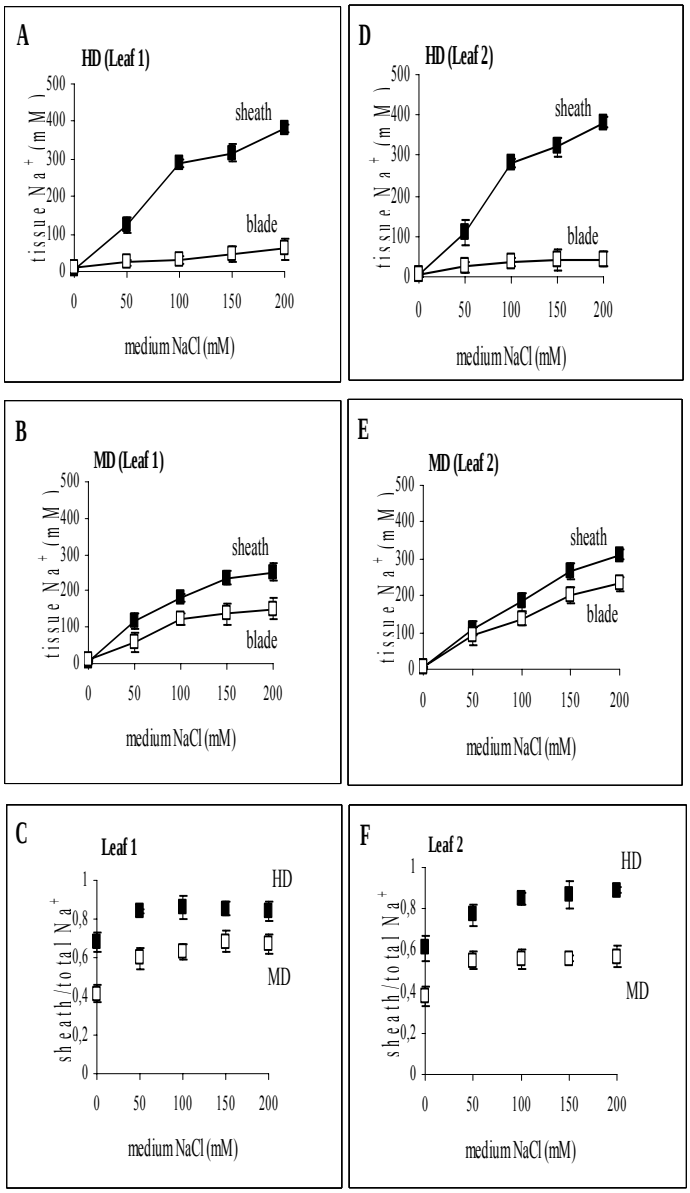

Fig 3. Na^+ content of leaves 1 (A-C) and 2 (D-F) after 7 d of exposure to increasing concentrations of NaCl. A, B, D, and E, Na^+ concentrations in the leaf blade (white symbols) and sheath (black symbols). C and F, Ratio of sheath Na^+ content to total leaf Na^+ (blade + sheath). Values are means ± SD (n = 5).

blade (Fig. 7). For TaSOS-1, a plasma membrane Na^+/H^+ antiporter, more transcripts accumulated in the roots and leaf sheaths of MD compared to HD (Fig. 7).

Discussion

Salt tolerance reflects the ability of the plant to exclude Na^+ as well as the mechanisms associated with tolerance of the tissues to accumulate Na^+. These two components of salt tolerance are likely to operate independently and so salt tolerance will depend on their relative effects. Accordingly, salt tolerance of two Algerian bread wheat genotypes was evaluated in this study using a cluster of physiological and molecular parameters. Our data show that MD appeared to be more sensitive to salt than HD at the germination stage (Fig. 1A). High NaCl concentrations reduced plant growth,

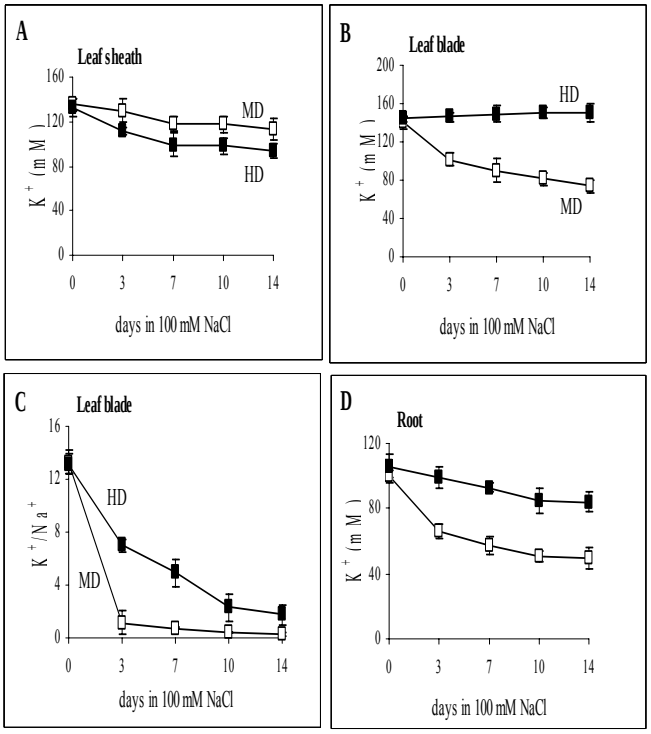

Fig 4. Effect of salt stress on the accumulation of K^+ ions in leaf sheaths (A), in leaf blades (B) and in roots (C); K^+/Na^+ ratio in leaf blade (D) in salt tolerant HD (black symbols) and salt sensitive MD (white symbols). Values are means ± SD (n = 5).

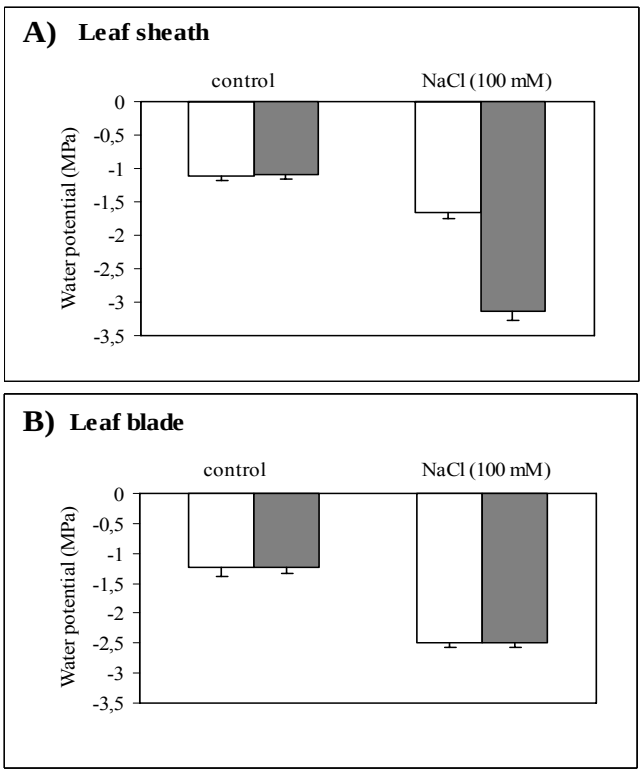

Fig 5. Water potential of salt sensitive MD and salt tolerant HD varieties exposed to salt stress (7 d in 100 mM NaCl) measured on leaf blades (A), and leaf sheath (B). Values for water potential of unstressed plants (control) are also presented. White bars: MD; grey bars: HD. Values are means ± SD (n = 4).

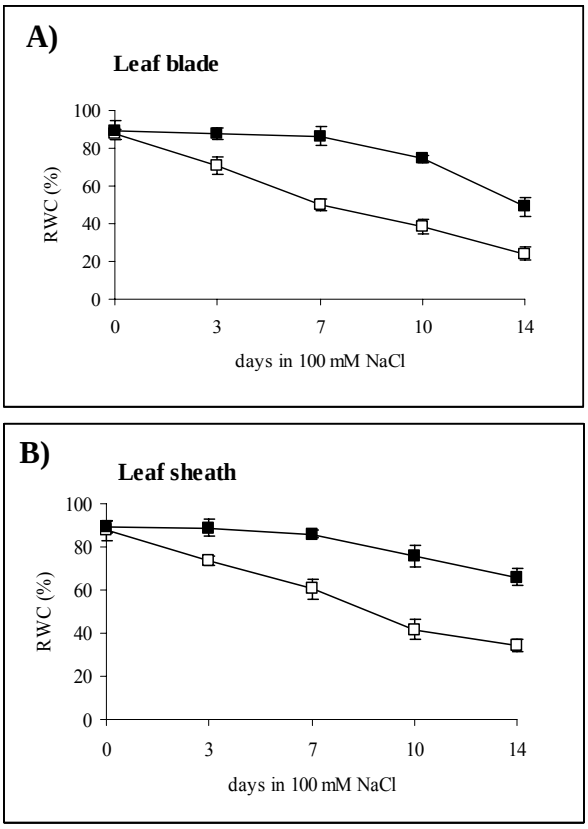

Fig 6. Relative water content (RWC) of leaf blades (A) and leaf sheathes (B) of salt sensitive MD (white symbols) and salt tolerant HD (black symbols) after growing in salt containing media (100 mM NaCl) for 0, 3, 7, 10 or 14 d. Black symbols. One leaf per plant was removed from each RWC analysis. Values are means ± SD (n = 4).

particularly leaf expansion, with more pronounced effect on the MD variety. The observed performance of HD might be due to a stronger osmotic adjustment, which is considered to be important for plant adaptation to salt stress since it contributes to the maintenance of turgor and cell volume. This notion is supported by a higher RWC in the leaf sheaths of tolerant HD in association with lower (more negative) leaf water potentials (Ψw) even though the sheaths contain less NaCl than the sensitive MD variety. One immediate response of plants to elevated salinity is a decrease in leaf expansion. This is more often associated with a loss in cell turgor pressure rather than a salt-specific toxic effect. In the salt-sensitive genotype, MD, salt was less effectively excluded from the transpiration stream as it entered the leaf blade, resulting in a greater accumulation. It is presumed that high levels of salt in leaf blades would enhance premature senescence of old leaves and inhibit photosynthetic performance of younger leaves (Colmer et al., 1995). While the two wheat genotypes studied here appeared to have similar Na^+ storage capacity in the roots, they showed different Na^+ accumulation rates in leaf blades. Na^+ accumulated more in the sheaths of the leaf 1 and 2 of HD compared to MD (Fig. 2). Sheath storage capacity may represent an advantage for HD by limiting the loading of Na^+ to the leaf blade thus, preserving its photosynthetic capacity. However, sheath retention of Na^+ itself would only delay the accumulation of Na^+ in leaf blades until a threshold was reached above which the Na^+ will reach leaf blades of both cultivars. It is possible that this trait of preferential sheath retention of Na^+ would interact with the low xylem loading. Similarly, differential sheath retention of Na^+ have been previously reported on two durum wheat varieties showing marked differences to salt and drought stress (Brini et al., 2009). Uptake of K^+ into leaf sheaths of salt treated plants showed no differences between the genotypes. This suggests that sheath sequestration of Na^+ could be Na^+ specific. However, an enhanced uptake of K^+ in leaves of HD compared to MD, resulted in a higher K^+/Na^+ ratio in leaf blades and this may benefit for cellular homeostasis. Salt tolerance is associated with low rates of transport of Na^+ to shoots with high selectivity for K^+ over Na^+ and therefore K^+/Na^+ ratio in young leaves is suggested as an important factor for metabolism and growth (Dvorak and Gorham, 1992; Husain et al., 2004; Poustini and Siosemardeh, 2004). K^+/Na^+ ratio is controlled by a QTL linked to *Kna*1 locus located at the distal region of chromosome 4DL of bread wheat (Gorham et al., 1987; Dubcovasky et al., 1996). Increasing evidence shows that, during root uptake, enhanced discrimination of K^+ over Na^+ is an important trait contributing to salt tolerance and, therefore, K^+/Na^+ ratio in plant tissues is a widely used parameter in distinguishing genotypes for their tolerance to NaCl toxicity in wheat and other cereal species (Gorham et al., 1990; Santa-Maria and Epstein, 2001; Munns and James, 2003). Reducing salt-induced K^+ efflux would allow its contribution towards osmoregulation, negating the need for a high investment into the production of organic solutes and allowing the critical maintenance of optimal cytosolic K^+/Na^+ ratio (Cuin et al., 2008). Salt tolerance of plants depend on HKT transporters, which mediate Na^+-specific transport or Na^+-K^+ transport and play a key role in regulation of Na^+ homeostasis (Rodriguez-Navarro and Rubio, 2006; Munns and Tester, 2008). Several genes belonging to the HKT family have been studied in wheat. TaHKT1 was the first HKT gene cloned from higher plants, showing expression in cortical cells (Schachtman and Schroeder, 1994). The down-regulation (by an antisense construct) of TaHKT2; 1 in wheat increased shoot fresh weight by 50 -100% in 200 mM NaCl under conditions of K^+ deficiency (Laurie et al., 2002). Following the down-regulation of TaHKT2;1, transgenic wheat had smaller Na^+-induced depolarisations in roots cortical cells and low $^{22}Na^+$ influx, indicating that TaHKT2;1 mediates Na^+ influx (Laurie et al., 2002). Further evidence using a root uptake system and a yeast transformation system also supported that TaHKT2;1 and HvHKT2;1 functioned as a Na^+ uniport (Haro et al., 2005). In durum wheat, the gene homologous to TmHKT7-A2 (from *Triticum monococum*) which is the best candidate for *Nax*1, could control Na^+ unloading from xylem in root and sheath of line 149 (salt tolerant) but not of Tamaroi (salt sensitive) (James et al., 2006). Upon a salt stress, the expression of the two HKT genes used in this study, were detected only in the roots of the two bread wheat genotypes. While HKT2;2 shows the same expression pattern in both varieties, a differential accumulation was observed for HKT1;5 transcripts which reach higher levels in the roots of HD. These findings suggest that both HKT genes might be involved in Na^+/K^+ transport through the plasma membrane of the root cortical cells with a more active role of HKT1;5 in the tolerant variety. The expression level of the wheat Na^+/H^+ antiporter gene (TNHX1) following salt stress was also investigated. The TNHX1 transcripts accumulate to higher amounts in roots and leaf sheaths of both MD and HD compared to leaf blades. The greater increase of TNHX1 expression in roots and sheaths treated with salt might be a response to more Na^+ accumulating in the corresponding vacuoles. Expression level of TVP1 seems to be similar to TNHX1 in the different tissues of the plant of the two

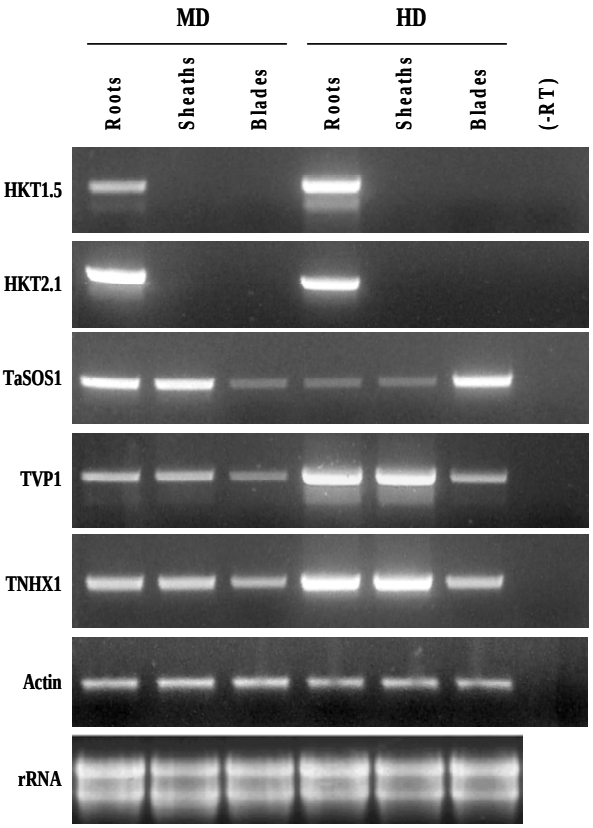

Fig 7. RT-PCR analysis of the expression levels of HKT1;5, HKT2;2, TNHX1, TVP1 and TaSOS1, in roots, sheaths and blades of salt sensitive MD and salt tolerant HD genotypes using specific gene primers. The expected size of the cDNA HKT1;5 is 600pb, HKT2;2 is 450 pb, TNHX1 is 640 pb, TVP1 is 550 pb and TaSOS1 is 680 pb. (-RT): without Reverse Transcriptase; A 380 pb Actin fragment was amplified by RT-PCR as internal control. The Ribosomal RNA (rRNA) samples stained by ethidium bromide are also indicated.

genotypes, MD and HD (Fig. 7). Thus, the Na^+/H^+ antiporter acts in concert with the vacuolar H^+-PPase and ATPase to sequester cations in the vacuole and prevacuolar compartments. The similar expression patterns of TNHX1 and TVP1 observed in MD and HD, suggest that vacuolar compartmentation acts with comparable efficiency in both genotypes. High salinity induction of V-PPase gene expression in roots has been reported for AVP1, HVP1, HVP10 and TsVP (Fukuda et al., 2004; Gao et al., 2006). Vacuolar compartmentation of excess Na^+ would provide a cheap osmoticum for osmoregulation under saline conditions, an osmolarity that could be matched by cytosolic retention of K^+. Indeed, Overexpression of the *Arabidopsis* tonoplast $Na^+(K^+)/H^+$ antiporter AtNHX1 (that would increase Na^+ influx into vacuole) improved Na^+ tolerance without increasing Na^+ content of transgenic wheat plants (Xue et al., 2004). Transcript accumulation of the plasma membrane Na^+/H^+ antiporter, TaSOS1, was lower in roots and leaf sheaths of HD than in MD; whereas, in leaf blades, the expression of TaSOS1 in HD was slightly greater than in MD (Fig. 7). These expression patterns suggest that beside an efficient Na^+ retention in the sheath, the HD variety may avoid Na^+ accumulation in leaf blades by activating sodium efflux through a higher expression of SOS1 in this compartment. Similar results were previously confirmed by Brini et al., (2009). In fact, a correlation was obtained between the expression pattern of TaSOS1 in the roots and sheaths of both durum wheat varieties and the Na^+ fluxes from roots to leaves. However, other recent findings have reported no apparent correlation between leaf Na^+ content and wheat salt tolerance (Genc et al., 2007). Thus, it appears that excluding Na^+ is not itself always sufficient to increase plant salt tolerance and other physiological traits should also be considered.

Conclusion

Tolerance to high saline concentrations in bread wheat seems to be related to an ability to avoid accumulation of toxic levels of Na^+ an enhanced capacity for osmotic adjustment and, or to maintain adequate levels of K^+, especially in the leaf blade. This information will be helpful in selecting more adapted wheat varieties for future breeding programmes.

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