Screening for sodium exclusion in wheat and barley

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Abstract

Accessions and cultivars of the genus *Triticum (T. monococcum*, *T. urartu*, *T. tauschii*, *T. durum* and *T. aestivum)* and of *Hordeum (H. vulgare* and *H. spontaneum*), from different geographical locations, were screened for sodium exclusion. Species from both genera demonstrated a range of differences in Na^+ exclusion/accumulation in the shoot. The greatest polymorphism for Na^+ exclusion was found in *T. monococcum* accessions, where there was more than 50-fold difference between the lowest excluder and the highest accumulator, while *T. durum*, showed minimal variability in Na^+ exclusion, (less than 1.5-fold). These results are important for guiding a strategic choice of parental forms, and reflect the pressure of both natural and artificial selection, as well as on adaptation in both wild accessions and cultivated forms to high salinity environments.

Key Words

Na^+, K^+, accumulation, hydroponics.

Introduction

Salinity is one of the major factors limiting crop production in Australia and elsewhere in the world, with the area of land affected increasing constantly. The ability of plants to minimize Na^+ accumulation in their shoots generally results in improved salt tolerance. The use of plants from different saline environments and different genetic backgrounds provides a range of diversity and polymorphism for Na^+ exclusion. The use of supported hydroponic growth systems allows us to perform highly defined growth and sampling regimes which are cheap and high throughput, while flame photometry permits the rapid screening of cultivars, lines, new landraces and accessions of wheat, barley and close genetic relatives in the genera *Triticum*, and *Hordeum.* The main purpose of the work described here was to screen for individuals from *Triticum* and *Hordeum* species for the greatest ability to exclude Na^+ from the shoot, using a supported hydroponics system and flame photometry.

Methods

Plant material

Various accessions of both genus *Triticum* (*T. durum*, *T. monococcum*, *T. urartu* and *T. tauschii*) and *Hordeum* (*H. vulgare* and *H. spontaneum*) were supplied by the Australian Wheat Collection (Tamworth) and by the Vavilov Plant Research Institute (St.-Petersburg, Russia). Cultivars of bread wheat and barley were obtained from Dr. Tony Rathjen and Dr. Jason Eglinton, respectively, both from the University of Adelaide.

Supported hydroponic system

Seeds were germinated for 4 days on moist filter paper before being transferred to a supported hydroponic setup (Fig 1). Seedlings were transplanted into individual 280 mm long ? 40 mm diameter tubes filled with 3 mm diameter polycarbonate fragments, used as a soil substitute. Plants were supplied with a growth solution containing 5 mol m^{-3} KNO_3, 2 mol m^{-3} $Ca(NO_3)_2$, 2 mol m^{-3} MgSO4, 0.5 mol m^{-3} Na_2SiO_3, 0.2 mol m^{-3} NH_4NO_3, 0.1 mol m^{-3} KH_2PO_4, 0.05 mol m^{-3} NaFe(III)EDTA and micronutritients (50 mmol m^{-3} H_3BO_3, 10 mmol m^{-3} $ZnSO_4$, 5 mmol m^{-3} $MnCl_2$, 0.5 mmol m^{-3} $CuSO_4$ and 0.1 mmol m^{-3} Na_2MoO_4), pH = 6.5-7.0, in a 20 min pump/20 min drain cycle, for approximately 10 days, until the third leaf emerged. At third leaf emergence, NaCl was added twice daily in 25 mol m^{-3} steps until final concentrations of 50, 100 or 150 mol m^{-3} NaCl were achieved. Additional $CaCl_2$ was added to maintain the constant Ca^{2+} activity. After 10 days of Na^+ stress the third leaf was harvested, fresh, dry and tissue water weights recorded before the leaf was digested in 1% HNO_3 at 85^0C for 4 hrs. Concentrations of Na^+ and K^+ in the leaf were determined by flame photometry.

Figure 1. Hydroponic system for screening wheat and barley lines.

Results and discussion

Plants in both *Triticum* and *Hordeum* genera accumulated very different sodium concentrations. It was found that *T. monococcum* and *T. urartu* could not survive in highly saline conditions, with 100 mol m^{-3} Na^+ quickly leading to senescence. However, when accessions were grown from these lines at 50 mol m^{-3}, a wide range of Na^+ accumulation in the shoot could be observed, and accessions of *T. monococcum* showing more than 50-fold difference in Na^+ accumulation between the best salt excluder and the highest salt accumulator (Table 1, Fig 2). There was only a 10-fold difference in the accumulation of Na^+ in *T. urartu,* which showed a moderate range of Na^+ content in the shoot. *T. tauschii*, however, is very salt tolerant and was able to grow in 150 mol m^{-3} NaCl without showing any visual symptoms of the salinity stress. The *T. tauschii* accessions also showed a moderate range of sodium accumulation in the shoot. These data differ from that previously published, where little variability was reported in *T. monococcum* and *T. urartu* (Gorham et al. 1991; Colmer et al. 2006).

Table 1. Na^+ concentration in the third leaf of accessions of *Triticum* and *Hordeum* species. Plants were grown for approximately 10 days in standard growth conditions, until the third leaf emerged. Thereafter they are supplied for the next 10 days with the NaCl concentrations shown in the table below. Values for individual accessions are the means of 4 replicates ? standard error.

Species	Number of accessions	NaCl application (mol m^{-3})	Overall average $[Na^+]$ (mol m^{-3})	Range of average $[Na^+]$ (mol m^{-3}) in individual accessions	
				Minimum	Maximum
		Triticum species:			
T.?monococcum	43	50	56 ? 25	6 ? 2	510 ? 345

T. urartu	22	50	28 ? 6	9 ? 3	82 ? 10
T. tauschii	17	150	212 ? 91	61 ? 14	595 ? 121
T. durum	37	100	214 ? 21	79 ? 2	285 ? 87
		Hordeum species:			
H. vulgare	105	150	207 ? 8	139 ? 7	285 ? 16
H. spontaneum	40	100	120 ? 21	51 ? 15	221 ? 41

The tetraploid species, *T. durum*, in contrast, showed minimal variability in Na^+ exclusion, with less than 1.5-fold difference between most accessions, with the exception of two Na^+ excluding lines that had a shoot sodium concentration approximately half that of the majority of lines (Table 1, Fig. 3). A similar result with *T. durum* has previously been published (Munns and James 2003), where a single line had significantly less Na^+ accumulation than other cultivars/accessions. Wild type barley accessions, *H. spontaneum*, contained a relatively high range of Na^+ accumulation in the shoot (approximately 4.5-fold), while cultivated barley accessions, *H. vulgare*, showed only a 2-fold variation (Table 1).

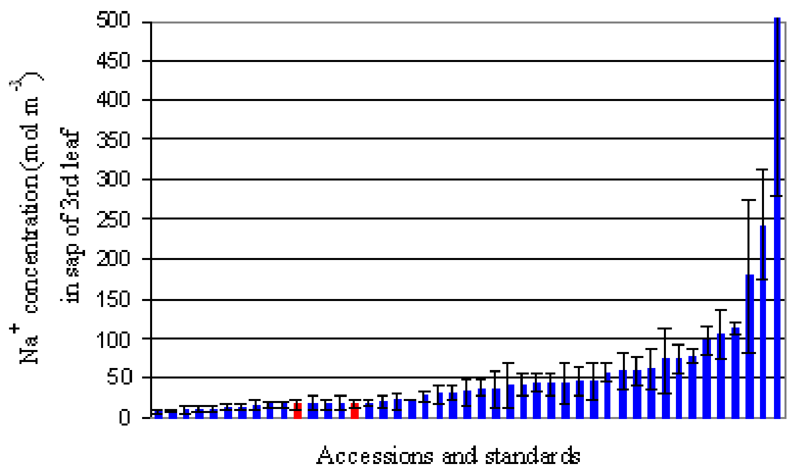

Figure 2. Na^+ concentration in the third leaf of 43 accessions of *T. monococcum* (in blue) in comparison with two bread wheat cultivars, Kharchia and Krichauff, as standards (in red). Plants were grown for approximately 10 days in standard growth conditions, until the third leaf emerged, with growth for the next 10 days in the presence of 50 mol m^{-3} NaCl. Values for individual accessions are means of 4 replicates ? standard error.

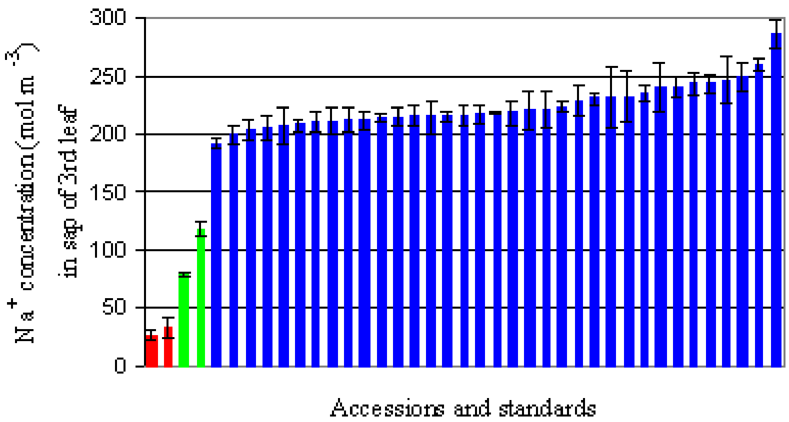

Figure 3. Na^+ **concentration in the third leaf of 37 accessions of *T. durum* (in blue) in comparison with two bread wheat cultivars, Krichauff and Kharchia (in red). Plants were grown for approximately 10 days in standard growth conditions, until the third leaf emerged, with growth for the next 10 days in the presence of 100 mol** m^{-3} **NaCl. Two *T. durum* accessions (in green) were identified as the best sodium excluders. Values for individual accessions are means of 4 replicates ? standard error.**

Similar trends could be observed with K^+ accumulation (Table 2), which correlates negatively with Na^+ concentration. *T. monococcum* and *T. urartu* accumulated the highest concentrations of K^+, while cultivated durum wheat and barley were the poorest K^+ accumulators. This observation is reflected in the $[K^+]$ / $[Na^+]$ ratio of the different species (Table 2).

Table 2. K^+ **concentration and** K^+ / Na^+ **ratio in third leaf of accessions of *Triticum* and *Hordeum* species. Plants were grown for approximately 10 days in standard growth conditions, until the third leaf emerged, with growth for the next 10 days in the presence of NaCl. Values for individual accessions are means of 4 replicates ? standard error.**

Species	Number of accessions	NaCl application (mol m^{-3})	Overall average $[K^+]$ (mol m^{-3})	Range of average $[K^+]$ (mol m^{-3}) in individual accessions		Ratio $[K^+]/[Na^+]$
				Minimum	Maximum	
			Triticum species:			
T.?monococcum	43	50	231 ? 19	192 ? 29	344 ? 45	12.3 ? 5.7
T. urartu	22	50	248 ? 18	186 ? 10	335 ? 13	11.4 ? 3.1
T. tauschii	17	150	213 ? 29	121 ? 27	288 ? 35	1.6 ? 0.6

T. durum	37	100	116 ? 12	89 ? 7	185 ? 10	0.6 ? 0.2
			Hordeum species:			
H. vulgare	105	150	130 ? 4	88 ? 3	195 ? 10	0.6 ? 0.1
H. spontaneum	40	100	209 ? 13	173 ? 17	274 ? 13	1.9 ? 0.4

Several popular bread wheat cultivars have also been screened for sodium exclusion (Table 3). Pugsley, Cascade, Kharchia and Krichauff were found to be the best excluders, while Drysdale, Bobwhite and Waylkatchem demonstrated the highest sodium accumulation. This is also reflected in potassium accumulation and $[K^+]$ / $[Na^+]$ ratio.

Table 3. Comparison of the accumulation of Na^+ and K^+ in the third leaf of popular domestic and foreign bread wheat cultivars. Plants were grown for approximately 10 days in standard growth conditions, until the third leaf emerged, with growth for the next 10 days in the presence of 100 mol m^{-3} NaCl. Values for individual accessions are means of 4 replicates ? standard error.

N	Name	$[Na^+]$ (mol m^{-3})	$[K^+]$ (mol m^{-3})	Ratio K^+ / Na^+
1	Pugsley	14.2 ? 1.3	252 ? 7	17.7 ? 1.0
2	Cascade	14.4 ? 2.5	282 ? 11	19.6 ? 1.8
3	Kharchia	14.5 ? 1.2	261 ? 9	18.0 ? 0.9
4	Krichauff	14.6 ? 2.1	274 ? 8	18.8 ? 1.8
5	Cranbrook	15.2 ? 3.0	268 ? 5	17.6 ? 1.0
6	Chinese Spring	15.4 ? 1.2	237 ? 5	15.4 ? 0.5
7	Excalibur	15.4 ? 2.4	259 ? 6	16.8 ? 1.4
8	Halberd	15.9 ? 2.4	274 ? 6	17.2 ? 1.5
9	Yitpi	16.6 ? 1.7	257 ? 8	15.5 ? 1.4
10	Kukri	16.7 ? 1.3	239 ? 20	14.3 ? 0.8

11	Kite	16.9 ? 3.2	244 ? 9	14.4 ? 1.7
12	Opata	17.4 ? 1.1	242 ? 11	13.9 ? 0.8
13	Pitic	22.6 ? 2.2	277 ? 5	12.3 ? 0.5
14	Waylkatchem	25.3 ? 3.0	263 ? 5	10.4 ? 1.2
15	Bobwhite	25.6 ? 2.7	231 ? 7	9.9 ? 0.7
16	Drysdale	27.4 ? 2.7	282 ? 12	10.3 ? 2.3
	Average	18.0 ? 2.1	259 ? 8	15.1 ? 1.2

Conclusion

These results are important for guiding a strategic choice of accessions for use as parental lines to cross in beneficial traits for salt tolerance. The variation observed in all lines is believed to reflect natural and artificial selection, as well as the adaptation of wild accessions and cultivated forms to high saline environments. The low variability in polyploid wheat is also likely to reflect the reduced genetic variability arising from the bottleneck occurring at the time of polyploidisation that lead to durum and bread wheat. Several accessions from each species studied have been identified as the best Na^+ excluders. These are now being used in genetic studies and breeding programmes with the aim of transferring high levels of Na^+ exclusion, and potential salinity tolerance, to hybrids of both wheat and barley. The hybridization program will involve crossing within and between different species for both crop improvement and to produce segregating populations for mapping.

References

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