K^+/Na^+ discrimination in synthetic hexaploid wheat lines: Transfer of the trait for K^+/Na^+ discrimination from *Aegilops tauschii* into a *Triticum turgidum* background

D.J. Pritchard[1], P.A. Hollington[2,5], W.P. Davies[1], J. Gorham[2], J.L. Diaz de Leon[3] & A. Mujeeb-Kazi[4]

[1]Royal Agricultural College, Cirencester, GL7 6JS, UK

[2]Centre for Arid Zone Studies, University of Wales, Bangor LL57 2UW, Wales, UK

[3]Universidad Autonoma de Baja California Sur, Department of Agronomy, Apartado Postal 19-B, 23054 La Paz, B.C. S. Mexico

[4]CIMMYT (International Maize and Wheat Improvement Centre) Apartado Postal 6-641, 06600 Mexico DF, Mexico

[5]Corresponding author

Telephone: +44 1248 382285

Fax: +44 1248 364717

E-mail: p.a.hollington@bangor.ac.uk

Summary

Irrigation-induced salinity is a major constraint to crop production in many countries. K^+/Na^+ discrimination is a trait which enhances salinity tolerance in bread wheat compared to durum wheat, and is present in the wheat ancestor *Aegilops tauschii*. An experiment is described to assess K^+/Na^+ discrimination, and other traits, in a number of synthetic hexaploid wheat genotypes, produced by crossing *Ae. tauschii* with durum wheat. The durum parents of the synthetics were also used in the experiment, along with the CIMMYT test set of salt tolerant entries, and some synthetic hexaploid-based drought-tolerant germplasm. K^+/Na^+ ratios were lower in the durum parents than in the elite synthetics, confirming that the trait was present in the synthetics, and demonstrating its successful transfer from *Ae. tauschii* to the synthetic hexaploid wheat (2n=6x=42, AABBDD). The 14 best-performing synthetics had similar K^+/Na^+ ratios to the tolerant check S24. There were highly significant correlations between K^+/Na^+ discrimination and fresh weight within the durum parents, the elites, and the CIMMYT set. Backcrosses of some elite synthetics with the drought-susceptible hexaploid Opata failed to show the same level of discrimination as Opata.

The potential of two elite synthetics which were also tolerant to waterlogging, and one tolerant to multiple biotic stresses, for use in breeding programmes for stressed conditions was noted.

Keywords: K^+/Na^+ discrimination, wide hybridisation, salinity tolerance, synthetic hexaploid wheat, durum wheat, *Triticum turgidum*

Introduction

Salinity is a major constraint to crop production in irrigated agriculture in many parts of the world, and although bread wheat (*Triticum aestivum* L.) is commonly regarded as moderately tolerant to the stress durum wheat (*T. turgidum* L.) is more susceptible (e.g. Francois *et al.* 1986). One of the reasons for this is the absence in durum wheat of the trait for enhanced K^+/Na^+ discrimination, which is carried on the long arm of chromosome 4D in bread wheat (Shah *et al.* 1987) and is also present in D genome ancestors of wheat, for example the diploid *Aegilops tauschii.* (syn. *Ae. squarrosa; T. tauschii*) (Gorham, 1990a).

The D genome of *Ae. tauschii* is homologous to the D genome of bread wheat. For wheat improvement one route is therefore via bridge crosses that utilize synthetic hexaploids (SH). Synthetics are produced by crossing *T. turgidum* (2n=4x=28; AABB), with *Ae. tauschii* (2n=2x=14; D). *T. turgidum* is generally used as the female parent. Earlier work at CIMMYT has shown that there is less imbalance between chromosome numbers in the embryo and endosperm when higher ploidy species are used as the female parent (Mujeeb Kazi, 1995). Further, embryo regeneration is more efficient when durum wheats are the female parents in synthetic hexaploid production. The result of the cross is an F_1 hybrid with 21 chromosomes (ABD): these are doubled with colchicine to produce the 42 chromosome synthetic hexaploid (AABBDD) (Mujeeb Kazi *et al*, 1996). Approximately 800 synthetic hexaploids have been produced at the International Maize and Wheat Improvement Centre (CIMMYT), and an elite set of 95 was designated for further study in terms of morphology, growth, disease and abiotic stress resistances.

While durum wheat is not yet a major crop of irrigated wheat-producing areas such as the Indo-Gangetic basin in India and Pakistan, there is increasing interest in its use as a higher value crop for such situations, and there is therefore interest in increasing its salinity tolerance. It is also subject to salinity stress in many parts of the Middle East and the Mediterranean basin where it is a major cereal. The objective of the work described here was to characterise the elite set for K^+/Na^+ discrimination, in an attempt to identify those lines with potential use in breeding programmes to increase first the salinity tolerance in bread wheat, and subsequently use cytogenetic protocols to transfer the D genome diversity into durum cultivars.

Materials and Methods

Experiments were carried out to assess K^+/Na^+ discrimination in a range of material obtained from CIMMYT, including a population of elite synthetic hexaploid genotypes. A total of 140 accessions were used: 97 elite synthetic hexaploids, 35 of the tetraploid parental lines, and 13 hexaploid genotypes from the CIMMYT salinity test set (Diaz de Leon *et al.* 1999), but with Pasban 90 and PDW 34 substituted by Ciano 79 and the highly tolerant genotype S24, the progeny of a cross of the two tolerant wheats LU26S from Pakistan and Kharchia 65 from India (Ashraf and O'Leary, 1996). Opata is a CIMMYT wheat with known drought susceptibility. Backcrosses were made at CIMMYT of this genotype with some of the drought-tolerant elite synthetic hexaploids (Trethowan *et al*, 2000), and 5 tolerant progeny, together with Opata, were tested to see whether they were also positive for salt tolerance.

The trial was conducted at the University of Wales, Bangor, field station at Pen-y-Ffridd, in a glasshouse with heating and supplementary lighting. There were 4 replications of a randomised block design, grown in plug trays over John Innes No. 1 compost irrigated with saline nutrient solution. As the objective of the work was to study differences in K^+/Na^+ discrimination, no non-saline controls were used, and salinity was gradually raised after germination to a final level of 100 mol m^{-3} NaCl, with $CaCl_2$ added to maintain an Na:Ca ratio of 20:1. The plants grew rapidly. They were grown for 21 days after attaining the final salinity level, a total of 35 days growth in saline conditions, and were harvested when most had 6 leaves. Assessments were made of above ground fresh weight, height and number of leaves, and between 2 and 4 fully expanded leaves per accession were removed and frozen. Sap was extracted using the methods of Gorham *et al.* (1984) and analysed for Na^+ and K^+ using a flame photometer.

Results and Discussion

Results will be discussed with particular reference to the performance of the elite synthetic material. The durum parents were of significantly lower fresh weight than the CIMMYT test set and the elite synthetics (which were similar to each other), but heavier than the backcrosses. The durums were shorter than the CIMMYT test set, and the elite synthetics. The elite synthetics were taller than the synthetic hexaploid/Opata backcross lines (data not shown).

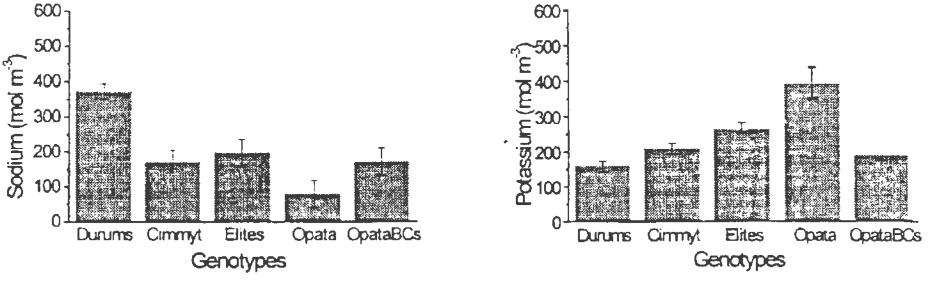

Figure1. Shoot sodium and potassium concentrations of wheat genotypes grown at 100 mol m^{-3} NaCl. Data are means +/- standard errors for the various classes.

Figure1 shows the sodium and potassium contents of the various classes. In terms of leaf Na^+ content, there were significantly higher concentrations in the durum wheats, while the elites had a similar level to the CIMMYT test set (Figure 1). Potassium was lowest in the durum parents and the Opata backcrosses, and highest in Opata. The elite synthetics showed much better K^+/Na^+ discrimination than their durum parents (Figure 2), and had a similar level to the CIMMYT test set. Overall, the highest K^+/Na^+ levels were in Opata, with its backcross derivatives being similar to the CIMMYT test set.

It is clear from these results that the trait for K^+/Na^+ discrimination had been successfully transferred from *Ae. tauschii* into the synthetics, and in no case was the K^+/Na^+ discrimination of the synthetic lines lower than in the durum parent. This confirms the work of Gorham (1990a), who concluded after examination of amphiploid hybrids that the trait was dominant in crosses of *Ae. tauschii* with species in which the trait was absent, including *T. turgidum*. He also (1990b) found low leaf Na^+ and high leaf K^+ concentrations in synthetic hexaploids produced by crossing tetraploid *Triticum* species with *Ae. tauschii*, confirming the dominance of the trait in synthetic hexaploid wheats.

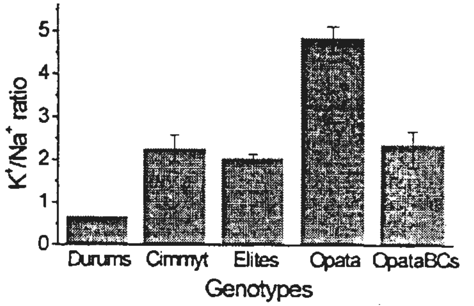

Figure 2. Shoot K^+/Na^+ ratios of wheat genotypes grown at 100 mol m^{-3} NaCl. Data are means +/- standard errors for the various classes.

There were highly significant correlations on an overall basis (all genotype means) between K^+/Na^+ discrimination and fresh weight (Table 1), indicating that at this early growth stage K^+/Na^+ discrimination was reflected in terms of salinity tolerance. Within the durum parents, the CIMMYT set and the elites there were also significant or highly significant correlations. However, the correlation of K^+/Na^+ with fresh weight in the Opata lines was just non-significant at the 0.05 level.

Table 1: Correlation coefficients (r) between K^+/Na^+ ratio and shoot fresh weight of wheat genotypes. Individual plant basis.

	Overall	Durums	CIMMYTs	Elite synthetics	Opatas
r	0.433	0.271	0.373	0.443	0.455
P	<0.001	0.001	0.009	<0.001	0.050

There was great variability among the elite synthetics for K^+/Na^+ discrimination (Figure 3), with genotype mean values ranging from almost 5 to less than 0.5, with a mean of 2.04 and standard deviation of 1.79. This value compares well with the mean of the CIMMYT set of 2.26. However, there was no significant correlation between the K^+/Na^+ ratio in the synthetics and that of their respective durum parents (r = 0.038, p = 0.715), neither was there any association of particular *Aegilops* parents with good K^+/Na^+ discrimination in the synthetics(r=0.011, p=0.915).

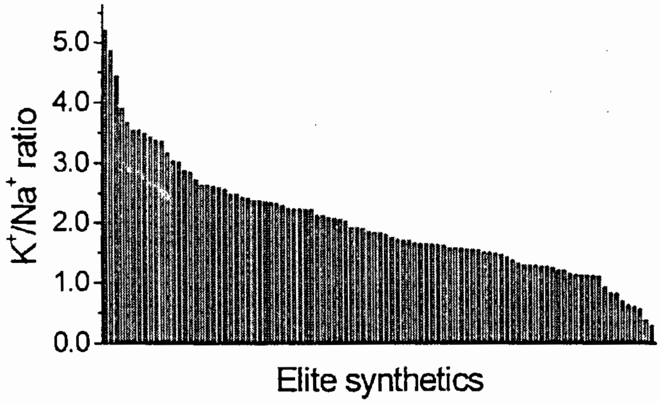

Figure 3. K^+/Na^+ ratios of elite synthetic hexaploid wheat genotypes, grown at 100 mol m^{-3} NaCl for 35 days, showing the range of variation within the population. Genotypes are ranked from highest to lowest: space does not permit individual identification.

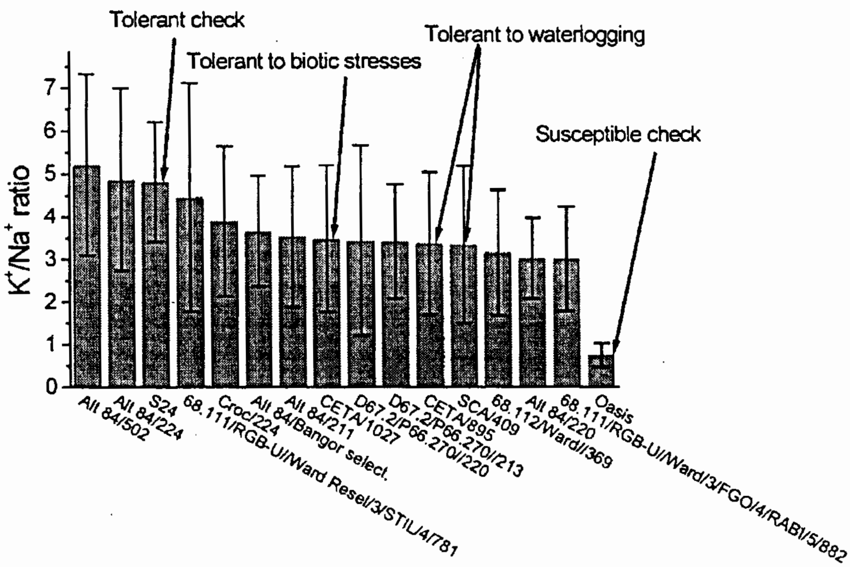

Figure 4. K^+/Na^+ ratios of the 14 best-performing synthetic hexaploids, together with tolerant (S24) and susceptible (Oasis) check varieties from the CIMMYT test set. The number after the oblique identifies the *Ae. tauschii* accession. Vertical bars represent standard errors.

Figure 4 shows the best performing elite synthetic lines, in terms of K^+/Na^+ ratio, together with the tolerant check S24 and the susceptible check Oasis. Although standard errors were very high, all the synthetics had similar K^+/Na^+ ratios to S24, and ratios were significantly higher than in Oasis. Of the elite lines CETA/*Ae. tauschii* 895 and SCA/*Ae. tauschii* 409 are also tolerant to waterlogging (Villareal *et al.* 2001), although the latter is susceptible to leaf and stripe rust. CETA/*Ae. tauschii* 1027 has multiple biotic stress resistance (Mujeeb-Kazi *et al.* 1999). There would therefore appear to be considerable potential for this germplasm in saline, and saline-waterlogged situations. Unfortunately, the elite synthetic genotypes are not yet fit agronomically, being difficult to thresh, and so require further backcrossing with well-adapted cultivars of regional specificity.

Munns *et al.* (2000) found low Na^+ accumulation and high K^+/Na^+ discrimination, of similar magnitude to bread wheat, in several selections of durum wheat, and suggested that these had the potential to improve salt tolerance in durum breeding programmes. However, from our analysis it does not appear that the durum cultivars which were the parents of the elite synthetic lines had K^+/Na^+ levels that could contribute to durum wheat improvement.

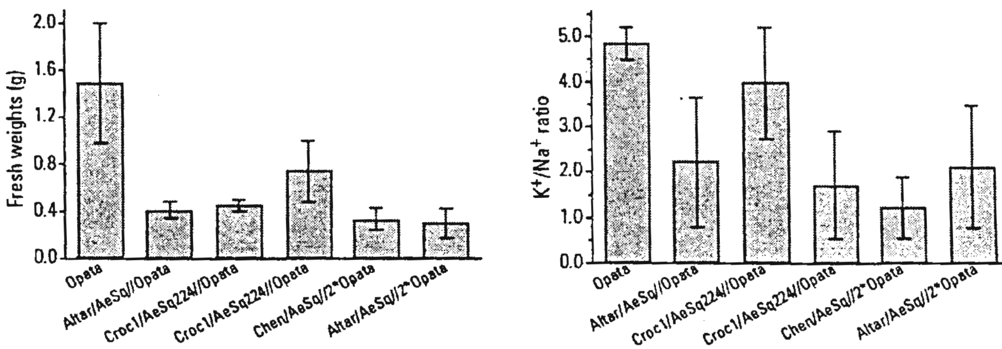

Figure 5. Fresh weights and K^+/Na^+ ratios of Opata and backcrosses of Opata with selected elite synthetic hexaploid wheats.

None of the synthetic hexaploid/Opata backcrosses with superior agronomic types showed K^+/Na^+ discrimination to the same level as Opata, and neither were their fresh weights as high (Figure 5). Hence a rapid output to fit agricultural goals was not realized through these 5 elite drought tolerant lines.

Acknowledgements

We are grateful to Dr. Mohammed Ashraf for the contribution of seed of S24. Dr Hollington's contribution to this work was funded by DG XII of the European Commission under the INCO-DC Programme, contract number ERBIC 18CT 980305. We thank Mr. Julian Bridges for technical assistance.

References:

Ashraf, M. and O'Leary, J.W. (1996) Responses of some newly developed salt-tolerant genotypes of spring wheat to salt stress: 1. Yield components and ion distribution. J Agron Crop Sci **176**: 91-101

Diaz de Leon, J.L., Escoppinichi, R., Zavala, R. and Mujeeb Kazi, A. (2000) A sea-water based salinity testing protocol and the performance of a tester set of accumulated wheat germplasm. Ann Wheat Newsl **46:** (HTML Edition) Items from Mexico.

Francois, L.E., Maas, E.V., Donovan, T.J. and Youngs, V.L. (1986) Effect of salinity on grain yield and quality, vegetative growth and germination of semi-dwarf and durum wheat. Agron J **78**: 1053-1058

Gorham, J. (1990a) Salt tolerance in the Triticeae: K^+/Na^+ discrimination in *Aegilops* species. J Exp Bot **41**: 615-621

Gorham, J. (1990b) Salt tolerance in the Triticeae: K^+/Na^+ discrimination in synthetic hexaploid wheats. J Exp Bot **41**: 623-627

Gorham, J., Wyn Jones, R.G. and McDonnel, E. (1984) Pinitol and other solutes in salt-stressed *Sesbania aculeata.* Z Pflanzenphysiol **114**: 173-178

Gorham, J., Hardy, C., Wyn Jones, R.G., Joppa, L. and Law, C. (1987) Chromosomal location of a K^+/Na^+ discrimination character in the D genome of wheat. Theor Appl Genet **74**: 584-588

Munns, R., Hare, R.A., James, R.A. and Rebetzke, G.J. (2000) Genetic variation for improving the salt tolerance of durum wheat. Aust J Agric Res **51**: 69-74

Mujeeb Kazi, A (1995) Interspecific crosses: hybrid production and utilization. Chapter 3 in: A.M. Kazi and G.P. Hettel (eds):Utilizing Wild Grass Biodiversity in Wheat Improvement: Fifteen Years of Wide Cross Research at CIMMYT. CIMMYT Research Report No. 2, ISSN 0188-2465.

Mujeeb-Kazi, A., Rosas, V. and Roldan, S. (1996) Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* auct. non L.) in synthetic hexaploid wheats (*T. turgidum* L. s. lat. x *T. tauschii*; 2n=6x=42, AABBDD) and its potential utilization for wheat improvement. Genet Res Crop Evol **43**: 129-134

Mujeeb-Kazi, A., Fuentes-Davila, G., Gilchrist, L.I., Velazquez, C. and Delgado, R. (1999) D-genome based synthetic hexaploids with multiple biotic stress resistances. Ann Wheat Newsl **45**:105-106.

Shah, S., Gorham, J., Forster, B. and Wyn Jones, R.G. (1987) Salt tolerance in the Triticeae: the contribution of the D genome to cation selectivity in hexaploid wheat. J Exp Bot **38**: 254-269.

Trethowan, R., Van Ginkel, M. and Mujeeb-Kazi, A. (2000) Performance of advanced bread wheat x synthetic hexaploid derivatives under reduced irrigation. Ann Wheat Newsl **46**:87-88.

Villareal, R.L., Sayre, K., Banuelos, O. and Mujeeb-Kazi, A. (2001) Registration of four synthetic hexaploid wheat (*Triticum turgidum/Aegilops tauschii*) germplasm lines tolerant to waterlogging. Crop Sci **41**:274.

Received 30 July, 2001, accepted 6 March, 2002