Use of wild relatives to improve salt tolerance in wheat

Timothy D. Colmer1,2,*, Timothy J. Flowers2,3 and Rana Munns1,4

1 CRC for Plant-based Management of Dryland Salinity, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia
2 School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia
3 School of Life Sciences, University of Sussex, Falmer, Brighton, Sussex BN1 9QG, UK
4 CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

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Abstract

There is considerable variability in salt tolerance amongst members of the Triticeae, with the tribe even containing a number of halophytes. This is a review of what is known of the differences in salt tolerance of selected species in this tribe of grasses, and the potential to use wild species to improve salt tolerance in wheat. Most investigators have concentrated on differences in ion accumulation in leaves, describing a desirable phenotype with low leaf Na\(^+\) concentration and a high K\(^+\)/Na\(^+\) ratio. Little information is available on other traits (such as ‘tissue tolerance’ of accumulated Na\(^+\) and Cl\(^-\)) that might also contribute to salt tolerance. The sources of Na\(^+\) ‘exclusion’ amongst the various genomes that make up tetraploid (AABB) durum wheat (Triticum turgidum L. ssp. durum), hexaploid (AABBD) bread wheat (Triticum aestivum L. ssp. aestivum), and wild relatives (e.g. Aegilops spp., Thinopyrum spp., Elytrigia elongata syn. Lophopyrum elongatum, Hordeum spp.) are described. The halophytes display a capacity for Na\(^+\) ‘exclusion’, and in some cases Cl\(^-\) ‘exclusion’, even at relatively high salinity. Significantly, it is possible to hybridize several wild species in the Triticeae with durum and bread wheat. Progenitors have been used to make synthetic hexaploids. Halophytic relatives, such as tall wheatgrass spp., have been used to produce amphiploids, disomic chromosome addition and substitution lines, and recombinant lines in wheat. Examples of improved Na\(^+\) ‘exclusion’ and enhanced salt tolerance in various derivatives from these various hybridization programmes are given. As several sources of improved Na\(^+\) ‘exclusion’ are now known to reside on different chromosomes in various genomes of species in the Triticeae, further work to identify the underlying mechanisms and then to pyramid the controlling genes for the various traits, that could act additively or even synergistically, might enable substantial gains in salt tolerance to be achieved.

Key words: Aegilops, Agropyron, halophyte, Hordeum, K\(^+\)/Na\(^+\) selectivity, Lophopyrum, Na\(^+\) exclusion, potassium, salinity, sodium, stress tolerance, synthetic hexaploid, Thinopyrum, Triticum, Triticeae, tall wheatgrass, wild relatives, wide hybridization.

Introduction

Wheat (Triticum spp.) and rice are the world’s major cereal crops, with the annual production of wheat being over 627 million t in 2004 (http://faostat.fao.org). Wheat is grown under irrigated and rain-fed conditions: both types of agriculture are threatened by salinization (Ghassemi et al., 1995; Mujeeb-Kazi and Diaz de Leon, 2002). Data collected at CIMMYT suggest that 8–10% of the area planted to wheat in India, Pakistan, Iran, Egypt, Libya, and Mexico is affected by salinity (Mujeeb-Kazi and Diaz de Leon, 2002). In Western Australia, about half of the 13 475 farms, for most of which wheat is their major crop, are affected by salinity (ABS, 2005). If cropping is to continue on these salt-affected soils, substantial increases in the salt tolerance of crops are needed. Wild relatives, including some halophytes, might be sources of tolerance to improve wheat.

There is no precise definition of a halophyte. In ecological terms, halophytes are plants that are able to...
complete their life cycle in salt concentrations approaching those found in sea waters (around 500 mM NaCl). Aronson (1989) listed 1554 species of halophytic plants, of which 135 were grasses. Of these, the majority (76) were in the Chloridoideae, but there were 38 members in the Pooidae, with 13 species in the same tribe as wheat. Halophytic members of the Triticeae, as examples tall wheatgrass spp. (e.g. *Thinopyrum* spp.) and sea barleygrass (*Hordeum marinum*), are more salt-tolerant than wheat (see Fig. 1 in Colmer et al., 2005a). Importantly, many of the species within these genera can, using cytogenetic techniques, be hybridized with wheat.

The use of wild relatives in breeding programmes for abiotic stress tolerance is controversial because few salt-tolerant varieties (Farooq, 2004) have been released from previous attempts at employing this approach. Although wheat was the subject of early attempts to develop salt-tolerant hybrids by wide crossing with halophytes, new varieties have yet to reach farmers’ fields, some 25 years after the initial experiments were conducted (Flowers, 2004). The major limitation with some of these attempts has been the low yield-potential of the progeny. The introduction of deleterious characteristics from the wild parent has meant this has not been a popular approach for wheat breeders (summarized in Islam and Shepherd, 1991). Furthermore, interactions with other stresses, particularly waterlogging, have proved difficult to overcome (Hollington et al., 2002). By comparison, wild relatives have been useful as a source of disease resistance and other agronomically important traits for wheat (Feldman and Sears, 1981; Nevo and Beiles, 1989; Appels and Lagudah, 1990; Pienaar, 1990; Islam and Shepherd, 1991; Fedak, 1999). Such successes were helped by, for example, disease resistance being controlled by fewer genes than salinity tolerance, and by being easier to assess in breeding programmes.

In this review, knowledge on salt tolerance and associated Na+ ‘exclusion’ in species in the Triticeae is summarized, and the possibilities to improve salt tolerance of wheat using accessions of diploid or tetraploid progenitors and/or other wild species, including halophytic wild relatives, are discussed. Making ‘synthetic hexaploids’ might enable use of genetic variation for salt tolerance within the donor diploid species that was not captured during the evolution of durum or bread wheat. In addition, the halophytic wild relatives (Table 1) might also provide valuable sources of salt tolerance for new crops (Gorham and Wyn Jones, 1993; Mujeeb-Kazi et al., 1993; King et al., 1997b), and attempts to use these sources of tolerance are reviewed.

The Triticeae

Wheat and barley are members of a tribe of related species, the Triticeae [in the sub-family Pooidae, supertribe Triticeae, family Poaceae (syn. Gramineae)] (Watson and Dallwitz http://delta-intkey.com/grass/). The taxonomy of this group has been, and remains, contentious, being complicated by the multiple genomes present in some species and their past hybridizations. The nomenclature proposed by van Slageren (1994) for *Triticum* and *Aegilops* species has been adopted, and GRIN (http://www.ars-grin.gov/cgi-bin/npgs/html/taxeon.pl) has been used for other species in the Triticeae, and synonyms are provided in order to avoid ambiguity (Table 1). However, the uncertain taxonomy and the varying ploidy of the Triticeae complicate discussion of wild relatives of wheat and possible hybridizations.

The modern species commonly referred to as ‘wheat’ are bread and feed wheat (*Triticum aestivum* L. ssp. *aestivum*; henceforth abbreviated as *T. aestivum*) and durum (pasta or macaroni) wheat [*Triticum turgidum* L. ssp. *durum* (Desf.)]. *T. aestivum* is a hexaploid made up of the genomes A, B and D (AABBDD; 2n=42). *T. turgidum* ssp. *durum* is a tetraploid made up of A and B genomes (AABB; 2n=28). Tetraploid wheat probably originated as a natural hybrid between a diploid A genome species *T. urartu* (Khlestkina and Salina, 2001), and an unknown diploid B species; there is no diploid B species extant (McFadden and Sears, 1946). A member of the Sitopsis section (S genome) of the *Aegilops* genus is most probably the B genome donor species, and *Ae. speltoides* var. *speltoides* appears closest (Kerby and Kuspira, 1987). Hexaploid wheat is a natural hybrid between *Ae. tauschii* (DD; goat grass; syn. *Ae. squarrosa* and *T. tauschii*) (McFadden and Sears, 1946) and tetraploid (AABB) wheat. The possible sources of salt tolerance from diploid and tetraploid progenitors and wild relatives within the Triticeae are discussed below.

![Fig. 1.](attachment:image)

Fig. 1. Relative biomass after 35 d in 150 mM NaCl containing 15 mM CaCl₂ as compared to plants in 1 mM NaCl (n=9). Na⁺ concentrations were measured on the most recent fully expanded leaf after 15 d. The long arrow points to Kharchia (bread wheat), the short arrow to Modoc (durum wheat). The reason for the very low biomass in the three lowest genotypes was that about half the plants had died. Data recalculated from Schachtman et al. (1991).
Table 1. List of species that have been proposed as sources of salt tolerance in the Triticeae

Species names, synonyms, and genomes are given. For *Triticum* and *Aegilops* species, the nomenclature proposed by van Slageren (1994) has been adopted see http://www.k-state.edu/wgrc/Taxonomy/taxintro.html. Synonyms and common names come from GRIN (http://www.ars-grin.gov/cgi-bin/npgs/html/taxecon.pl).

<table>
<thead>
<tr>
<th>Species</th>
<th>Genome</th>
<th>Alternative genome designation</th>
<th>Synonym</th>
<th>Common name</th>
<th>Examples of data on salt tolerance (italicised references primarily contain data on leaf ion concentrations)</th>
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<td><strong>Triticum species</strong></td>
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<td><em>Triticum aestivum</em> L. ssp. aestivum</td>
<td>AABBDD</td>
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<td>Bread wheat</td>
<td>Kingsbury and Epstein (1984)</td>
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<td>AA</td>
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<td>Tritium boeoticum; Tritium monococcum ssp. boeoticum</td>
<td>Wild einkorn</td>
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<td><em>Triticum timopheevii</em> (Zhuk.) Zhuk. ssp. armeniacum (Jakubz.) Slageren</td>
<td>GGAA</td>
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<td>Tritium araraticum; Tritium armeniacum</td>
<td>Einkorn</td>
<td>Datta et al. (1995)</td>
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<td>Tritium militinae; Tritium timopheevii</td>
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<td>Gorham (1990b)</td>
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<td>Tritium dicoccoides</td>
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<td>ABB</td>
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<td>Tritim durum</td>
<td>Durum wheat; Pasta wheat</td>
<td>Gorham et al. (1987); Munns and James (2003)</td>
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<td><strong>Aegilops species</strong></td>
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<td><em>Aegilops bicornis</em> (Forssk.) Jaub. &amp; Spach</td>
<td>SbSb</td>
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<td>Sitopsis bicornis; Triticum bicornum</td>
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<td>Farooq et al. (1989); Gorham (1990a)</td>
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<td>UUMM</td>
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<td>Aegilops macrochaeta; Aegilops lorentii; Tritium macrochaetum</td>
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<td>Tritium comosum</td>
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<td>Gorham (1990a)</td>
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<td><em>Aegilops cylindrica</em> Host</td>
<td>CCDD</td>
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<td>Tritium cylindricum</td>
<td>Jointed goat grass</td>
<td>Farooq et al. (1989); Gorham (1990a)</td>
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<td><em>Aegilops longisssima</em> Schweinf. &amp; Muschl.</td>
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<td>Sitopsis longissima; Triticum longissimum</td>
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<td>Gorham (1990a)</td>
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<td>Aegilops caudata</td>
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<td>C&quot;C&quot;M&quot;M&quot;</td>
<td>Aegilops geniculata; Triticum ovatum</td>
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<td>Gorham (1990a)</td>
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<td>C&quot;S&quot;</td>
<td>Tritium sharoneae</td>
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<td>Barb goat grass</td>
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<td>C&quot;C&quot;</td>
<td>Triticum umbellulatum</td>
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<td>C&quot;C&quot;S&quot;S&quot;</td>
<td>Aegilops peregrina var. peregrina</td>
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<td>J&lt;sub&gt;i&lt;/sub&gt;</td>
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<td>Tall wheatgrass</td>
<td>Aronson (1989); McGuire and Dvořák (1981)</td>
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<td>E&lt;sup&gt;e&lt;/sup&gt;E&lt;sup&gt;i&lt;/sup&gt;</td>
<td>J&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Agropyron bessarabicum; Elytrigia bessarabica</td>
<td>Tall wheatgrass</td>
<td>Gorham et al. (1985); Aronson (1989); Mujeeb-Kazi et al. (1993); Dewey (1960); McGuire and Dvořák (1981)</td>
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<td><em>Thinopyrum junceiforme</em> (Á. Löve &amp; D. Löve) Á. Löve</td>
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<td>Elymus cylindricus</td>
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<td>Criptesion jubatum</td>
<td>Foxtail barley</td>
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<td><em>Hordeum vulgare</em> L.</td>
<td>II</td>
<td></td>
<td>Criptesion jubatum; Hordeum caespitosum; Hordeum vulgare ssp. vulgare</td>
<td>Barley</td>
<td>Mano and Takeda (1998); Garthwaite et al. (1999)</td>
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<td><em>Hordeum marinum</em> Huds.</td>
<td>XX or XXXX</td>
<td></td>
<td>Hordeum marinimum; Criptesion marinum; ssp. marinum and ssp. gussoneanum</td>
<td>Sea barleygrass</td>
<td>Mano and Takeda (1998); Garthwaite et al. (1999)</td>
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Salt tolerance in durum and bread wheat

A number of characteristics, summarized by Colmer et al. (2005a), contribute to salt tolerance in wheat. Foremost among salt-specific traits is the ability to limit the concentration of Na$^+$ that enters the xylem. This trait is described here by the term Na$^+$ ‘exclusion’. Within a species, or even within a genus, the Na$^+$ concentration in the leaves can be used as an indicator of the relative ability to ‘exclude’ Na$^+$, particularly if well-defined tissues are sampled. The Na$^+$ concentration in a leaf, and its change with time, is determined by the concentration of Na$^+$ in the xylem, transpiration rate, growth rate, as well as the total volume of water already transpired during the life-span of the leaf; little Na$^+$ is remobilized in the phloem (Munns, 2005). In addition, genotypes can differ in tolerable Na$^+$ concentrations in leaves, presumably due to differences in the efficiency of compartmentation in leaf vacuoles and this trait has been characterized as ‘tissue tolerance’ (Yeoh and Flowers, 1983). Also of importance to salt tolerance in many grasses is an ability to accumulate Na$^+$ in the older leaves and the leaf sheaths; i.e. compartmentalization at the whole plant level (Yeoh et al., 1985; Boursier and Läuchli, 1989; Davenport et al., 2005). Maintaining K$^+$ uptake and transport to growing tissues is also crucial for salt tolerance (Greenway and Munns, 1980).

Variation in Na$^+$ ‘exclusion’ and K$^+$/Na$^+$ discrimination can be found amongst wheat genotypes, wheat progenitors, wild relatives (Gorham et al., 1987; Gorham, 1993), and in the halophytic species in the Triticeae (Gorham et al., 1985; Garthwaite et al., 2005). However, the ability to maintain low Na$^+$ concentrations in the xylem cannot simply be judged by the Na$^+$ concentration in leaves in all situations. For example, halophytes growing in high external NaCl concentrations must be very effective ‘excluders’ to prevent a massive build up of Na$^+$ in their shoots, but can have relatively high leaf Na$^+$ (and Cl$^-$) concentrations that contribute to balancing their water relations with that of the environment. Moreover, in the case of perennials with leaves having a longer life-span than for annuals, Na$^+$ will eventually accumulate to relatively high concentrations. Finally, although salt glands are an important component of salt tolerance in many halophytic grasses, particularly members of the Chloridoideae (Marcum, 1999), this is not a characteristic of species in the Triticeae.

The ability to maintain low Na$^+$ and high K$^+$ concentrations in leaves, is correlated with salt tolerance within cultivated wheat species (Francois et al., 1986; Gorham et al., 1987; Shah et al., 1987; Maas and Grieve, 1990; Dvořák et al., 1994; Munns and James, 2003; Poustiti and Siosemardeh, 2004), and in some wild species of the Triticeae (Schachtman et al., 1991; Garthwaite et al., 2005). There is little genetic variation in Cl$^-$ accumulation in leaves within cultivated wheat (Gorham et al., 1990a; Husain et al., 2004). However, considerable variation exists in some wild species in the Triticeae in capacity to restrict the rate of Cl$^-$ accumulation in leaves (Garthwaite et al., 2005).

Durum wheat has a higher rate of leaf Na$^+$ accumulation and a lower leaf K$^+$/Na$^+$ ratio (Gorham et al., 1987), than bread wheat. At 150 mM NaCl, bread wheat excludes 97–99% of the Na$^+$ from the transpiration stream (i.e. only 1–3% of the external Na$^+$ concentration enters as water is taken into the xylem); by contrast in durum wheat, 5–6% of the external Na$^+$ concentration enters the xylem (Munns, 2005). However, genetic variation for Na$^+$ accumulation exists in durum wheat (Munns et al., 2000). The relationship between leaf Na$^+$ concentration and salt tolerance, assessed as maintenance of shoot dry mass after 24 d at 150 μM NaCl, was examined for a range of durum wheat genotypes (Munns and James, 2003). The genotypes with the lowest leaf Na$^+$ concentrations suffered the least reduction in dry matter. The low-Na$^+$ genotypes had less leaf injury and a greater proportion of living to dead leaves (5% of total leaf area was dead) than the high-Na$^+$ genotypes (15% of total leaf area was dead).

In bread wheat, the D genome contains the Kna1 locus on the long arm of chromosome 4D (Dubcovsky et al., 1996), which contributes to lower rates of Na$^+$ accumulation and higher K$^+$/Na$^+$ ratio in leaves, compared with durum wheat (Table 2). However, low leaf Na$^+$ accumulation (and high K$^+$/Na$^+$ ratio), of similar magnitude to that of bread wheat, has been found in a durum landrace (Munns et al., 2000). The enhanced Na$^+$ ‘exclusion’ was shown to be controlled by two major genes (Munns et al., 2003). A molecular marker was found for one of them, and the locus (Nax1) mapped to the long arm of chromosome 2A (Lindsay et al., 2004). The enhanced Na$^+$ ‘exclusion’ in the landrace (Line 149), as compared with the Australian cultivar Tamaroi, results from lower rates of transport of Na$^+$ from roots to shoots and an enhanced capacity for the sheath to remove Na$^+$ from the xylem during its passage to

| Table 2. Cation concentrations in modern wheat and its progenitors (mM, measured in expressed leaf sap) in youngest fully emerged leaves of plants grown for 14 d at 50 mM NaCl (with nutrients and 2.5 mM CaCl$_2$). |
|---|---|---|---|---|
| Species | Genome | Number of accessions | Na$^+$ (mM) | K$^+$ (mM) | K$^+$/Na$^+$ |
| T. monococcum ssp. monococcum | AABB | 20 | 18 | 159 | 19 |
| T. urartu | AABB | 19 | 13 | 185 | 19 |
| T. monococcum ssp. aegilopoides | AABb | 24 | 13 | 176 | 23 |
| T. turgidum ssp. durum | AABB | 7 | 107 | 118 | 1 |
| T. turgidum ssp. dicoccoides | AABB | 5 | 100 | 122 | 1 |
| T. aestivum ssp. aestivum | AABBDD | 15 | 11 | 155 | 16 |
the leaf blade (Davenport et al., 2005). Thus, although the \textit{Nax1} and the \textit{Kn1} loci result in similar phenotypes (i.e. lower Na\textsuperscript{+} concentrations in the leaf blade, and higher K\textsuperscript{+}/Na\textsuperscript{+} ratio), the mechanisms producing this phenotype are different (R Munns, RA James, R Davenport, unpublished data). Many transport processes are likely to contribute to the amount of Na\textsuperscript{+} net uptake into roots, its entry into xylem, and ultimately the rate of arrival of Na\textsuperscript{+} in the leaves (Tester and Davenport, 2003), and whether any of the known cation transporter genes are found at the \textit{Nax1} locus in durum wheat or \textit{Kn1} locus in bread wheat remains to be determined.

\section*{Crosses between durum wheat and bread wheat}

To transfer genes for salt tolerance from durum wheat to bread wheat, an interspecific cross is made to produce an F\textsubscript{1} pentaploid (AABBBD; with homologous recombination between the corresponding chromosomes), which is then backcrossed to the bread wheat. The F\textsubscript{2} progeny is a mixture of tetraploid, pentaploid, and hexaploid seed. The pentaploid seed is morphologically different from the tetraploid and hexaploid, and can be eliminated visually. Cytological analysis or use of a D genome-specific marker such as the \textit{Dgas} probe (McNeil et al., 1994) can identify lines containing the D genome. The hexaploid seed is then tested for the presence of a DNA marker sequence that shows the durum donor chromatin has been recombined with its homologous chromosomes in the hexaploid wheat. To transfer genes on the A or B genome of hexaploid wheat to durum wheat, the reverse is done, and in this case the F\textsubscript{1} is backcrossed to the tetraploid parent, i.e. durum wheat. These methods have been successful in transferring genes for disease resistance from one species to the other (Fieldman and Sears, 1981), and are being used in an attempt to introduce the \textit{Nax1} gene for Na\textsuperscript{+} ‘exclusion’ found on the A genome of durum wheat (Lindsay et al., 2004) into hexaploid wheat that lacks this gene (R Munns, RA James, unpublished data).

To transfer a gene from the D genome of bread wheat into durum wheat is much harder than from the A or B genomes. Recombination between different genomes, i.e. homoeologous pairing, requires the use of the \textit{Ph1} mutation (Sears, 1977), which suppresses the normal inhibition of pairing between homoeologous chromosomes on the different genomes at meiosis. An important example is the transfer of the \textit{Kn1} locus of ‘K\textsuperscript{+}/Na\textsuperscript{+} discrimination’ from the D genome of hexaploid wheat into tetraploid wheat (Dvorák et al., 1994). Using the pairing mutant \textit{phlc} in the background of the durum cultivar Capelli, homoeologous recombination of the distal part of the long arm of chromosome 4D with chromosome 4B was obtained (Dvorák and Gorham, 1992). This has created novel tetraploid germplasm with enhanced K\textsuperscript{+}/Na\textsuperscript{+} discrimination; the leaf Na\textsuperscript{+} concentrations were not significantly different, whereas the K\textsuperscript{+}/Na\textsuperscript{+} ratios were higher than in the durum parent (Dvorák et al., 1994). When grown in the field on soils with intermediate and high salinity, about 5 and 10 dS m\textsuperscript{-1}, respectively, at harvest time, there was no significant difference in grain yield between +\textit{Kn1} and –\textit{Kn1} lines as a group (Dvorák et al., 1994), although there were promising yield differences between individual lines, which were borne out in a subsequent experiment in sand culture (Gorham et al., 1997). Concerned that genes being introgressed with the large chromosomal segment containing the \textit{Kn1} locus might cause a yield penalty, a second cycle of homoeologous recombination with the \textit{phlc} mutant was undertaken (Luo et al., 1996). This reduced the size of the segment from chromosome 4D in the durum chromosome 4B. However, no agronomically acceptable durum line with the introgression has been released.

\section*{Summary}

The ability to maintain low Na\textsuperscript{+} and high K\textsuperscript{+} concentrations in leaves is correlated with salt tolerance in durum and bread wheat. Bread wheat is, in general, a better Na\textsuperscript{+} ‘excluder’ than durum wheat, a trait controlled by the \textit{Kn1} locus on chromosome 4D (Dvorák et al., 1994). Attempts to introduce \textit{Kn1} into durum wheat have been hampered by the recombinant lines having a growth penalty under control conditions. However, considerable variation in capacity to ‘exclude’ Na\textsuperscript{+} has also been found in durum wheat, and a locus (\textit{Nax1}) mapped to chromosome 2A. This variation is being used in an attempt to develop durum wheat with improved salt tolerance (Lindsay et al., 2004).

\section*{Progenitors of durum wheat and bread wheat}

\subsection*{Diploid species (AA)}

\textit{T. urartu} (AA), the species that probably gave rise to the A genomes of durum and bread wheat, shows greater Na\textsuperscript{+} ‘exclusion’ and K\textsuperscript{+}/Na\textsuperscript{+} discrimination than durum wheat (AABB), as do the closely related A-genome species \textit{T. monococcum} ssp. \textit{monococcum} and \textit{T. monococcum} ssp. \textit{aegilopoidea} (syn. \textit{T. boeoticum}) (Gorham et al., 1991). The ‘exclusion’ of Na\textsuperscript{+} is accompanied by a higher accumulation of K\textsuperscript{+}, leading to much higher K\textsuperscript{+}/Na\textsuperscript{+} ratios in the leaves of diploid A genotypes, than in durum wheat (Table 2). The poorer Na\textsuperscript{+} ‘exclusion’ in durum wheat might be due to: (i) the actual donor of the A genome to durum wheat being inferior in this trait; not all diploid genotypes with the A genome ‘exclude’ Na\textsuperscript{+} to the same degree (Shah et al., 1987; R Munns, RA James, unpublished data); (ii) some negative interaction between the A and B genomes that reduces expression of this trait on the A genome, and/or (iii) the B genome bringing other gene(s) that act independently of the A genome to increase entry of Na\textsuperscript{+}, with a net result of increased Na\textsuperscript{+} transport to the leaves.

\textit{Triticum turgidum} ssp. \textit{dicoccoides}

\textit{T. turgidum} ssp. \textit{dicoccoides} (AABB; wild emmer wheat), the wild progenitor of cultivated tetraploid wheat, is
distributed over areas of the Middle East having dry and possibly saline soils (Nevo et al., 1993). Three accessions tested by Gorham et al. (1991) had poor Na⁺ ‘exclusion’ relative to T. aestivum and also appeared to lack its enhanced K⁺/Na⁺ discrimination trait, and were equal in this regard to T. turgidum ssp. durum (Table 2). However, populations of T. turgidum ssp. dicoccoides from Israel did have lower rates of Na⁺ uptake than the durum cultivar Langdon (Nevo et al., 1992), when tested by applying 22Na⁺ to 7-d-old seedlings for 2 d in 1 mM NaCl. In addition, some accessions were grown in 175 or 250 mM NaCl until maturity. The most tolerant accession (Gilboa) had average dry weights per plant at 175 mM NaCl that were 63% of the control (Nevo et al., 1993). This shows tolerance higher than in cultivated durum wheat, which is typically reduced to about 10% of control when grown to maturity at 150 mM NaCl (Husain et al., 2003). Gilboa did not have the lowest 22Na⁺ uptake (Nevo et al., 1992) in 1 mM NaCl, indicating that 22Na⁺ uptake from 1 mM NaCl is not a reliable indicator for salt tolerance.

_Aegilops tauschii_

_Ae. tauschii_ (DD; syn. _Ae. squarrosa, T. tauschii_) is the progenitor of the _D_ genome of bread wheat. Gorham and colleagues (Gorham et al., 1987, 1990b; Shah et al., 1987; Gorham, 1990b) tested several accessions of _Ae. tauschii_, and showed these had much lower Na⁺ concentrations and higher K⁺/Na⁺ ratios in leaves than did durum wheat, and were similar to bread wheat for these traits. Gorham and co-workers suggested that the _D_ genome imparted the Na⁺ ‘exclusion’ and enhanced K⁺/Na⁺ discrimination to bread wheat. For example, when grown in 50 mM NaCl for 14 d, the average Na⁺ concentration in sap expressed from the most recent fully-expanded leaf of _Ae. tauschii_ was 20 mM (12 accessions), synthetic hexaploid wheat was 17 mM (10 accessions), and durum wheat was 78 mM (4 accessions) (Gorham, 1990b). Cl⁻ concentrations in leaves were similar for all genotypes, the values were 111 mM for _Ae. tauschii_, 84 mM for synthetic hexaploids, and 100 mM for durum wheat (Gorham, 1990b).

There is variation in Na⁺ accumulation within _Ae. tauschii_. Testing of 415 accessions showed a 10-fold variation in Na⁺ concentration in the most recent fully-expanded leaf blade, from 200 to 2100 µmol g⁻¹ dry weight (about 40–400 mM Na⁺ in leaf sap, assuming a water content of 5 ml g⁻¹ dry weight), when grown at 150 mM NaCl (Schachtman et al., 1992). This spanned the range in Na⁺ concentrations in durum versus bread wheat, the salt-tolerant bread wheat Kharchia having a Na⁺ concentration of 300 µmol g⁻¹ dry weight and the salt-sensitive durum wheat Modoc being 1500 µmol g⁻¹ dry weight (Schachtman et al., 1991). K⁺ concentrations were inversely affected, so that the accessions with the lowest Na⁺ had the highest K⁺/Na⁺ ratios (Schachtman, 1991). This Na⁺ ‘exclusion’ was associated with salt tolerance, in terms of biomass production. The accessions with the lowest leaf Na⁺ accumulation generally had the least reduction in biomass accumulation when grown at 150 mM NaCl for 35 d (Schachtman et al., 1991; Fig. 1). This was not due to genotypic differences in growth rate or size (Schachtman et al., 1991) or due to the ability of some genotypes to ‘exclude’ Na⁺ from growing leaves (Schachtman and Munns, 1992). The more tolerant accessions had lower Na⁺ concentrations in older leaves, so that the reduced rate of Na⁺ accumulation prolonged leaf longevity and therefore CO₂ assimilation (Schachtman et al., 1991; Munns et al., 1995). Enhanced Na⁺ ‘exclusion’, however, does not always confer salt tolerance, as some individuals had low Na⁺ accumulation rates but were salt sensitive, as assessed by their low biomass accumulation (Schachtman et al., 1991; Schachtman and Munns, 1992), indicating other traits are also needed to confer tolerance. Unfortunately, no tolerant individuals were found that had high leaf Na⁺ concentrations, as these might then have provided a source of ‘tissue tolerance’. Nevertheless, as discussed below, the best sources of Na⁺ ‘exclusion’ in _Ae. tauschii_ might be used in breeding programmes by making synthetic hexaploids, to further improve this trait in bread wheat.

_Synthetic hexaploids: Aegilops tauschii×durum wheat_

The amount of genetic variation in the _D_ genome of bread wheat is restricted because during its evolution, between 8000 to 10 000 years ago, only a few genotypes of _Ae. tauschii_ were involved (Appels and Lagudah, 1990). Since the natural habitat of _Ae. tauschii_ includes dry and (moderately) saline areas (Schachtman et al., 1991), the production of synthetic hexaploids, to broaden the genetic variation contributed by the _D_ genome, could improve the salt tolerance of bread wheat (Schachtman et al., 1992; Mujeeb-Kazi and Diaz de Leon, 2002; Pritchard et al., 2002; Drecer et al., 2004). Synthetic wheat (AABBDD) is produced from _Ae. tauschii_ (DD) and durum wheat (AABB) by the process of crossing, embryo rescue, growth of haploid plantlets, and doubling of chromosomes by colchicine treatment (Limin and Fowler, 1982; Gill et al., 1988; Mujeeb-Kazi et al., 1996).

A set of synthetic hexaploids was analysed by Shah et al. (1987) who concluded that the _D_ genome from _Ae. tauschii_ conferred enhanced Na⁺ ‘exclusion’. A second set was analysed by Gorham (1990b) and the importance of the _D_ genome for Na⁺ ‘exclusion’ confirmed; the Na⁺ concentration in the most recent fully-expanded leaf of the synthetic hexaploids was as low as that of _Ae. tauschii_ and bread wheat. In 50 mM NaCl, Na⁺ in the synthetic hexaploids averaged 17 ± 2 mM and in _Ae. tauschii_ the average was 20 ± 2 mM, both in contrast to 78 ± 11 mM in the durum wheat to which the _Ae. tauschii_ was hybridized (Gorham, 1990b). At higher external NaCl concentrations (150 and 200 mM), the differences between the synthetic hexaploids and durum wheat were less, but still statistically significant.
Synthetic hexaploids have also been produced at CIMMYT (Mujeeb-Kazi et al., 1996), where the trait for enhanced K⁺/Na⁺ discrimination was transferred from Ae. tauschii into the synthetic hexaploids, as shown by the much lower leaf Na⁺ concentrations and higher K⁺/Na⁺ ratios than in their likely durum parents when exposed to 50 mM NaCl (Mujeeb-Kazi et al., 1993; Mujeeb-Kazi and Diaz de Leon, 2002; Pritchard et al., 2002). For plants grown at 100 mM NaCl, there were positive relationships between shoot fresh weight and leaf K⁺/Na⁺ ratio within the durum parents (r=0.27), the CIMMYT set (r=0.37) and the elite set of synthetic hexaploids (r=0.44) (Pritchard et al., 2002).

In a subsequent screen of a large number of primary synthetic hexaploids from CIMMYT, considerable genetic variation in Na⁺ ‘exclusion’ ability was found (Dreccer et al., 2004). The leaf Na⁺ concentration in two synthetic hexaploids (300 μmol g⁻¹ dry weight) was significantly lower than in the lowest bread wheat cultivar, Janz (550 μmol g⁻¹ dry weight). These results indicate that primary synthetic wheat could be used as a source to increase the capacity for Na⁺ ‘exclusion’ of bread wheat. Evidence that the enhanced Na⁺ ‘exclusion’ in the Ae. tauschii donor of synthetic hexaploid wheat would increase its salt tolerance was shown by Schachtman et al. (1992). Hybridization of selections of Ae. tauschii with low Na⁺ accumulation with durum wheat (cv. Langdon) produced synthetic hexaploids that yielded, in the best case, 50% more grain than bread wheat (cv. Kharchia, regarded as being a salt-tolerant bread wheat) when grown at 150 mM NaCl (Schachtman et al., 1992; Mujeeb-Kazi and Diaz de Leon, 2002). The best synthetic hexaploid had a substantially lower leaf Na⁺ concentration than the durum parent (~250 versus ~1200 μmol g⁻¹ dry weight), but it was still above that in the bread wheat Kharchia (~150 μmol g⁻¹ dry weight), so the higher salt tolerance in some synthetic hexaploids presumably resulted from the improved Na⁺ exclusion, as well as other traits.

**Other synthetic hexaploids**

Synthetic hexaploids have also been made between durum wheat (AABB) and genotypes of T. monococcum ssp. monoccoccum, T. urartu, and T. monococcum ssp. aegilopoides (Limin and Fowler, 1982; Gill et al., 1988). These are denoted as AABBA*A*, the first AA coming from the durum parent and the second A*A* from the diploid A genome species used in the cross. The trait for low leaf Na⁺ concentration present in the A genome species, the donors of A*A*, was expressed in these synthetic hexaploids (Gorham, 1990b). When grown in 60 mM NaCl, the leaf sap Na⁺ concentrations in these hexaploids were much lower (109, 59, and 65 mM, respectively) than in durum wheat (201 mM). The better ‘exclusion’ of Na⁺ was accompanied by higher leaf sap K⁺ concentrations, so that K⁺/Na⁺ ratios were higher (1.9, 4.1, and 4.1, respectively) than in durum wheat (0.7), and in two cases approached that in bread wheat (6.4). Cl⁻ concentrations were not significantly different in the various genotypes, being about 150 mM (Gorham, 1990b). At higher salinity (200 mM), the leaf sap Na⁺ concentration increased 4-fold in bread wheat, but only 2-fold in the synthetic hexaploids (Gorham, 1990b), indicating that the mechanism for Na⁺ ‘exclusion’ on the A* genome was able to withstand a higher salinity than that on the D genome. However, salt tolerance of the AABBA*A* synthetic was not measured. Salt tolerance should be evaluated against that of bread wheat or the AABBD synthetic hexaploids, and even if these are more tolerant, use of AABBA*A* hexaploids might be restricted to animal fodder due to poor grain quality.

_T. timopheevii_ ssp. _timopheevii_ (GGAA) has also been hybridized with _Ae. tauschii_ (DD) to make the synthetic hexaploid (GGAADD) (Limin and Fowler, 1982; Gill et al., 1988). At 50 mM NaCl, _T. timopheevii_ ssp. _timopheevii_ had low leaf sap Na⁺ (19 mM) and high K⁺ concentrations and therefore high K⁺/Na⁺ ratio (11:1) (Gorham, 1990b). The G genome, like the B genome, does not exist today in a diploid species, the closest match to both these genomes being the S genome species, _Ae. speltoides_ var. _speltoides_ (Kerby and Kuspira, 1987). When exposed to 50 or 150 mM NaCl, leaf sap Na⁺ concentration and K⁺/Na⁺ ratio in the GGAADD synthetic hexaploid was generally similar to that of _Ae. tauschii_ (DD), although some hexaploid products had relatively high leaf Na⁺ concentrations (Gorham, 1990b). Na⁺ ‘exclusion’ in the _T. timopheevii_ ssp. _timopheevii_ accession used was not tested. There was no apparent advantage of the GGAADD hexaploid compared with Na⁺ ‘exclusion’ in bread wheat (AABDD) (Gorham, 1990b), so the GGAADD hexaploid does not seem a useful avenue to pursue.

**Possible limitations to combining genes via interspecific crosses**

There are likely to be difficulties in combining a number of traits using interspecific crosses, as the transfer of the desired gene can carry substantial ‘linkage drag’. With homologous recombinations, pairing occurs between the homologous chromosomes from the wild donor and the cultivated parent (e.g. between the A genome of _T. monococcum_ and of durum wheat). However, the frequency of pairing can be quite low and result in deleterious characters being transferred, along with the desired character, from the wild species into wheat (summarized by Islam and Shepherd, 1991). Chromosome segments (‘linkage blocks’) can be quite large, and can take many backcrosses to break up. Molecular markers can be used to monitor the size of the chromosome segment and reduce the linkage drag (Paterson et al., 1991), as reviewed for wheat by Fedak (1999).

In the case of introgressions from different genomes (e.g. homoeologous chromosomes of species in the Triticeae), a very large amount of alien genetic material can be transferred along with the desired gene, and render the plant unsuitable as a crop plant. Reducing the size of the
introduced chromosome segment requires ionizing radiation treatment, or suppression of the Ph gene (Islam and Shepherd, 1991; Sears, 1993). It may require a second cycle of homoeologous recombination with the phl mutant as undertaken by Luo et al. (1996) in transferring the Kna1 locus from chromosome 4D in bread wheat to 4B in durum wheat.

**Summary**

Significant variation for capacity to ‘exclude’ Na⁺ exists within the A genome of *Triticum*; evident both in some diploids (Gorham et al., 1991), but rarely expressed in the AABB tetraploid (Lindsay et al., 2004). The D genome contributes to lower rates of Na⁺ accumulation and higher K⁺/Na⁺ ratio in leaves, both in the diploid *Ae. tauschii* (DD) (Gorham et al., 1991) and also in hexaploid (AABBDD) wheat (Gorham et al., 1987). Considerable diversity in Na⁺ ‘exclusion’ is evident within *Ae. tauschii*, so use of low-Na⁺ lines of *Ae. tauschii* in the production of synthetic hexaploids is under investigation as a means to improve salt tolerance in bread wheat (Mujeeb-Kazi et al., 1996; Drecer et al., 2004), with the potential benefits of this approach documented (Schachman et al., 1992). The contributions to Na⁺ ‘exclusion’ of Kna1 on chromosome 4D and Naxl on chromosome 2A might, in the future, be pyramided, both into durum and bread wheat. However, there could be difficulties in combining a number of traits from genotypes with different genetic backgrounds, due to ‘linkage drag’ (Islam and Shepherd, 1991).

**Other Aegilops species (C, G, M, N, U, and S genomes)**

Gorham (1990a, b) evaluated leaf ion concentrations in a number of species of *Aegilops* when exposed to 50 mM NaCl. Although Cl⁻ did not differ significantly in any of the *Aegilops* species, there were differences in leaf Na⁺ and K⁺ concentrations. Amongst the diploid species, those with the M or U genome (*Ae. comosa* and *Ae. umbellulata*, respectively) had accessions with low Na⁺ concentrations, being in some accessions as low as those in *Ae. tauschii* (DD) (Gorham, 1990a). However, some genotypes of *Ae. umbellulata* (UU) had relatively high Na⁺ concentrations in leaves (Gorham, 1990a), indicating substantial variation within this species for capacity to ‘exclude’ Na⁺. In contrast to these two diploid species (MM or UU) with accessions with low Na⁺ concentrations, none of the diploid S genome species, or those with C or N genomes, had low Na⁺ concentrations. For example, leaf sap Na⁺ concentrations were 144±5 mM for six accessions of *Ae. sharonensis* (S⁴S⁴S⁴) grown in 50 mM NaCl, in contrast to 41±5 mM in *Ae. tauschii* (7 accessions) (Gorham et al., 1991).

Analysis of the results for tetraploids is more complex, with probable interactions between the genomes. For example, tetraploids containing the S genome together with U or D genomes all had high leaf Na⁺ concentrations (Gorham, 1990a), which most likely indicates that the S genome dominates over the U or D genomes (diploid S genotypes had high Na⁺, whereas U or D diploids both had low Na⁺). However, this result could also have been due to genetic variation for Na⁺ ‘exclusion’ in the U or D genome accessions that formed the tetraploids. By contrast, the strong Na⁺ ‘excluding’ ability conferred by the D genome was also seen in tetraploid species with C and D genomes, such as *Ae. cylindrica* (CCDD), even though the diploid C genome species *Ae. caudata* (syn. *Ae. markgrafii*) has high Na⁺ concentrations in leaves. At 50 mM NaCl, the Na⁺ concentration (mM) in expressed sap of young leaves of *Ae. cylindrica* (CCDD) was 6±1, whereas in *Ae. caudata* (CC) the concentration was 131±18 and in *Ae. tauschii* (DD) Na⁺ was 41±5 (Gorham, 1990a). So, not only was the Na⁺ ‘exclusion’ associated with the D genome expressed, but it appears to have been enhanced by the C genome. The lower leaf Na⁺ concentrations were also associated with higher K⁺ concentrations and thus enhanced K⁺/Na⁺ ratio. Several tetraploid UM species were also examined by Gorham (1990a) and differences in leaf Na⁺ concentrations reported. As examples, after 14 d at 75 mM NaCl, *Ae. ovata* (UUMM) had relatively low leaf sap Na⁺ concentrations (68 mM) and *Ae. biuncialis* (UUMM) had relatively high Na⁺ of 159 mM (Gorham, 1990a). However, only a few accessions were tested and the variance was high, suggesting also that wide genetic variation exists for the capacity to ‘exclude’ Na⁺ within these species.

Several of the *Aegilops* species have also been examined for salt tolerance, assessed as survival when exposed to a mixture of salts at 30 dS m⁻¹, namely Na₂SO₄:CaCl₂:ZnCl₂:NaCl in a ratio of 10:5:1:4 by weight in Hoagland solution (Farooq et al., 1989). Many accessions of *Ae. tauschii* (DD), *Ae. cylindrica* (CCDD), and *Ae. ovata* (UUMM) survived, indicating a significant level of salt tolerance (although survival at high NaCl does not necessarily imply productivity; Rawson et al., 1988). As described in the next two paragraphs, *Ae. cylindrica* and *Ae. ovata* have been investigated as donors for salt tolerance in wheat breeding.

In the case of *Ae. cylindrica* (CCDD), about three-quarters of the plants from 11 accessions tested survived at 30 dS m⁻¹ for 7 weeks, and about 15% survived 40 dS m⁻¹ in treatments using the mixture of salts described above (Farooq et al., 1989). Hybrids were produced between one of the tolerant *Ae. cylindrica* accessions and bread wheat (Farooq et al., 1992a). The F₁ hybrids containing 35 chromosomes (ABDDCD) were backcrossed to the bread wheat parent and then selfed, and plants with 42 chromosomes screened for salinity tolerance in hydroponics (Farooq et al., 1992a) and in small field-plots, also irrigated with the hydroponics treatment solutions (Farooq et al., 1995). It is presumed that the aim was to introduce salt-tolerance genes
from *Ae. cylindrica* via direct crossing of the D genome of *Ae. cylindrica* with that of bread wheat, as C genome chromosomes would be eliminated. In addition, the D genome is more likely than the C genome to enhance Na⁺ ‘exclusion’ (discussed earlier in this review). RAPD markers were used to identify the presence of chromosomal introgressions from *Ae. cylindrica* into the wheat introgression lines containing 42 chromosomes (Farooq et al., 1994). In hydroponics at 15 dS m⁻¹, the best introgression line yielded 23% of the grain weight of its non-saline control, whereas the bread wheat did not produce grain (Farooq et al., 1992a). In the field plots irrigated to be moderately saline (EC=5–10 dS m⁻¹), one introgression line yielded 20% higher than the bread wheat (Farooq et al., 1995) and in soil with higher salinity (EC=20–34 dS m⁻¹), the best line had a 36% reduction in grain yield compared with a 54% reduction for a commercial bread wheat (Farooq, 2004). In a parallel programme by the same group, the F₁ hybrids containing 35 chromosomes (ABDCD) were screened for salt tolerance, the best F₁ hybrids were backcrossed to the bread wheat chromosomes (ABDCD) were screened for salt tolerance, and then selfed, and resulting plants with 42 and 44 chromosomes screened for salinity tolerance in hydroponics at 15 dS m⁻¹ (Farooq et al., 1992b). This second approach did not appear to enhance salt tolerance in the progeny above that obtained with the first approach described above, as the best line yielded only 16% of its non-saline control when grown at 15 dS m⁻¹ (Farooq et al., 1992b).

*Ae. ovata* (UUMM) has also been pursued by Farooq (2002) as a potential source for improvement of salt tolerance in wheat. The species contains genotypes that have a significant ability to ‘exclude’ Na⁺, at least when grown at 75 mM NaCl (Gorham, 1990a). Out of 21 accessions of *Ae. ovata* tested for salt tolerance, five survived at 30 dS m⁻¹ (mixture of salts); however, none survived 40 dS m⁻¹ (Farooq et al., 1989), so *Ae. ovata* appears to be less salt tolerant than *Ae. cylindrica* (see above). The more salt-tolerant accessions of *Ae. ovata* (UUMM) were hybridized with durum wheat (AABB), and the F₁ hybrids (UMAB) were crossed with hexaploid wheat (AABBDD) to produce progeny with 42–49 chromosomes. The lines containing 42 chromosomes (genome constitution unknown) were tested in saline fields ranging between 8–14 dS m⁻¹ (Farooq, 2004). The yield of these lines in non-saline soil was the same as that of several Pakistani wheat cultivars, but their yield in saline soil was reduced by only 22% compared with reductions of 65% for bread wheat. Genetic analysis of the derivative lines is needed to determine the amounts of chromosomal material introgressed from *Ae. ovata* into bread wheat.

**Summary**

In addition to the sources of Na⁺ ‘exclusion’ discussed above for the D genome (Kna¹) and A genome (Nax¹), variation for Na⁺ ‘exclusion’ has also been identified on other genomes (e.g. U and M) within the genus *Aegilops* (Gorham, 1990a, b), and some of these species have also been proposed as sources of salt tolerance for wheat breeding programmes (Farooq, 2004). However, recombination between the genomes of wheat and those of *Aegilops* species without A or D genomes, would require ionizing radiation treatment or use of the Ph¹ mutant (Sears, 1993).

**Tall wheatgrasses (E and J genomes)**

Amongst the halophytes within the Triticeae (listed in Table 1), tall wheatgrass species have received most attention as sources for improving salt tolerance in wheat. The diploid species, *Elytrigia elongata* (syn. *Lophopyrum elongatum*; EE, syn. JJJ° or EEJ; 2n=14) and *Thinopyrum bessarabicum* (JJ syn., EEEb; 2n=14) have been the focus of research in this area, as diploids are much more convenient for cytogenetic manipulations within wheat, than are polyploids (reviewed by Pienaar, 1990). *E. elongata* and *Th. bessarabicum* are closely related and are considered by some to represent two versions of the same basic genome (Wang and Hsiao, 1989). The programmes using diploid *E. elongata* or *Th. bessarabicum* as donors for salt tolerance into wheat are reviewed in some detail, as these are the most comprehensive examples of this approach using cytogenetic techniques and halophytic species in the Triticeae as donors for salt tolerance into wheat. In addition, several workers have used other (polyploid) wheatgrass species as potential sources of salt tolerance. The older work of this type will not be reviewed further (Wyn Jones and Gorham, 1986; Pienaar, 1990; Gorham, 1993, 1994; Mujeeb-Kazi et al., 1993), whereas several recent developments on introgressions from the decaploid *Thinopyrum ponticum* (EEEEEEEEEE), and the hexaploid *Thinopyrum junceum* (JJ JJJJJJ), will be discussed.

Two strategies have been proposed for use of wheat–tall wheatgrass hybrids in improvement of salt tolerance of wheat. The first is that wheat–tall wheatgrass amphiploids could be used as a new salt-tolerant cereal, *Trityprum* (name derived from *Triticum* spp. × *Thinopyrum* spp.) (King et al., 1997b). The resulting crop would not be expected to produce grain of bread or durum wheat quality, but it would be a feed wheat. The second strategy is that recombinant lines of wheat containing small segments of tall wheatgrass chromosomes might have improved salt tolerance without deleterious effects on yield or grain quality. Such an approach has enabled the use of tall wheatgrass as a source of disease resistance for wheat (Pienaar, 1990; Fedak, 1999).

**Elytrigia elongata**

*E. elongata* (EE; syn. *Lophopyrum elongatum*), the diploid tall wheatgrass, grows in salt marshes around the Mediterranean (Zhong and Dvorák, 1995) and survived exposure to 500 mM NaCl (McGuire and Dvorák, 1981). Physiological
data on salt tolerance in the diploid *E. elongata* do not appear to be available, whereas the physiological basis for tolerance in the decaploid tall wheatgrass *Thinopyrum ponticum* (EEEEEEE), a species used as a forage on saline lands (Dewey, 1960), has been studied and is summarized later in this review under the heading for that species.

A bread wheat–*E. elongata* amphiploid (2*n*=8x=56; genome AABBDDEE) has been produced, using *T. aestivum* cv. Chinese Spring and *E. elongata* as parents (Rommel and Jenkins, 1959). The *E. elongata* accession used came from Tunisia (Dvořáek and Knott, 1974). Unfortunately, this accession of *E. elongata* is no longer available and its salt tolerance has not been assessed, as the original objective of this programme was to study the evolutionary relationships between the genomes of *E. elongata* and bread wheat, as a basis for the further use of *E. elongata* as a donor to wheat of genes for disease resistance (Dvořáek and Knott, 1974). In addition to the bread wheat–*E. elongata* amphiploid, a complete set of disomic addition lines (Chinese Spring with one of each of the seven *E. elongata* chromosome pairs) was produced (Dvořáek and Knott, 1974; Dvořáek and Chen, 1984; Tuleen and Hart, 1988) and so were 20 of the 21 theoretically possible disomic chromosome substitution lines (Chinese Spring with chromosome pairs in each homoeologous group individually replaced by the respective *E. elongata* chromosome pairs) (Dvořáek, 1980; Tuleen and Hart, 1988). Numerous ditelosomic addition and substitution lines have also been produced (e.g. see the list in Zhong and Dvořáek, 1995). Although *E. elongata* is generally regarded as salt tolerant (see preceding paragraph), accessions differ in tolerance (Dewey, 1960; Shannon, 1978), so it is unlikely that the full potential for salt tolerance from *E. elongata* has been captured in this single amphiploid.

Salt tolerance in the wheat–*E. elongata* amphiploid was evaluated in a series of glasshouse experiments and also in field plots. Dvořáek and Ross (1986) reported that exposure to 250 mM NaCl caused a large proportion of amphiploid and Chinese Spring plants to die (only 17–23% of plants survived); however, when exposed to marine salts at a total EC similar to that of 250 mM NaCl, survival of the amphiploid was 68% (with grain production at 10% of the level in the non-saline control), whereas only 13% of the Chinese Spring plants survived and none of these produced grain (Dvořáek and Ross, 1986). In a subsequent experiment, plants were grown at 100 mM NaCl from day 5 onwards; final dry weight of the amphiploid (at 19.5 g) was twice that of Chinese Spring, and grain yield of the amphiploid (2.72 g per plant) was 39% higher than that of Chinese Spring (Dvořáek et al., 1988). At 250 mM, dry weight of the amphiploid was only 1.70 g and yield was only 0.17 g; but both were 28-times the respective values in Chinese Spring. In field experiments conducted by Omielan et al. (1991), small plots were irrigated with water of different salinities to obtain different soil salinities (characterized by the electrical conductivity of a saturated soil paste, ECs), which were of ‘low’ (1.1–1.2 dS m⁻¹), ‘intermediate’ (8.0–9.7 dS m⁻¹) or ‘high’ (13.9–15.6 dS m⁻¹) salinity (1990 season). Grain yield declined for Chinese Spring to 70% (intermediate saline) and then 8% (high saline) of control values; whereas for the amphiploid yield was not affected by intermediate salinity and was still 46% of the control at high salinity. A similar result occurred for the 1989 season. Grain yield of the amphiploid in control conditions was equal to, or slightly higher than, Chinese Spring, so absolute yields of the amphiploid under salinity were much higher than for Chinese Spring. Thus, the wheat–*E. elongata* amphiploid is more salt tolerant than Chinese Spring, but productivity is still severely diminished by soil salinity (ECs) less than one-third of the EC of seawater.

Salt tolerance in the wheat–*E. elongata* amphiploid could be, at least partly, due to its greater Na⁺ ‘exclusion’ and enhanced levels of K⁺, as measured in flag leaves by Omielan et al. (1991). Earlier experiments by Schachtman et al. (1989) in hydroponics with 250 mM NaCl showed that the amphiploid had a superior capacity to ‘exclude’ Na⁺ (and Cl⁻) from its shoots and to maintain higher leaf K⁺ concentration, compared with Chinese Spring. The enhanced capacity for Na⁺ ‘exclusion’ by the amphiploid, as compared with Chinese Spring, was also evident at low (40 mM) to moderate (80 mM) levels of NaCl (Storey et al., 1985). Analyses of solutes in different-aged leaf blades of both genotypes showed ~5-fold lower Na⁺ concentration, better maintenance of K⁺, and enhanced levels of glycinebetaine, in the youngest leaf blades of the amphiploid, when grown at 200 mM NaCl (Colmer et al., 1995). Na⁺ concentrations in the older leaves were also lower in the amphiploid than in Chinese Spring, although the magnitude of the differences became progressively less in the older leaves (Colmer et al., 1995). Upon exposure to NaCl, the amphiploid absorbed and transported less Na⁺ to the youngest leaf than Chinese Spring (Santa-Maria and Epstein, 2001). In contrast to all these studies, Deal et al. (1999) found that Na⁺ concentrations in the youngest fully-expanded leaf blades of Chinese Spring and the amphiploid did not differ after 14 d exposure to 100 or 250 mM NaCl. Finally, differences in gene expression between the amphiploid and wheat, following suddenly-imposed salinity (250 mM NaCl), have been recorded (Galvez et al., 1993; Shen et al., 2001), but the significance of these differences for salt tolerance requires elucidation.

Salt tolerance in *E. elongata*, as expressed in wheat, has been further dissected using the sets of disomic addition and substitution lines of *E. elongata* chromosomes in Chinese Spring. Dvořáek et al. (1988) recommended that disomic substitution lines are more useful than disomic addition lines for assessing stress tolerance, because of the confounding effects of aneuploidy in the addition lines.
However, the complexity of results observed by Zhong and Dvorák (1995), led to their recommendation that a full picture is only obtained by using both the disomic addition and substitution lines. A complex system, in which several of the *E. elongata* chromosomes enhance salt tolerance in an additive manner, but also with some epistasis between chromosomes, was revealed. Nevertheless, chromosome 3E was identified in a number of experiments to have a major dominant effect on salt tolerance (i.e. growth), and on regulation of leaf Na⁺ concentrations (Dvorák et al., 1988; Omielan et al., 1991; Gorham, 1994; Zhong and Dvorák, 1995).

The influence of individual chromosome pairs from *E. elongata* on salt tolerance in Chinese Spring was best demonstrated in the field experiments by Omielan et al. (1991). The substitution lines containing chromosome 3E showed superior ‘exclusion’ of Na⁺ and better maintenance of K⁺ in flag leaves, and higher dry mass and grain yields, when compared with Chinese Spring (Table 3). Omielan et al. (1991), therefore, suggested that introgression of loci from *E. elongata* chromosome 3E has the potential to increase salt tolerance in wheat, particularly since the increased salt tolerance did not cause reduced performance in non-saline conditions. Omielan et al. (1991) indicated that recombinant wheat lines (recombination between chromosomes 3E and 3A) had already been produced by Dvorák and colleagues, but whether or not any of these lines show superior salt tolerance has not been reported.

**Thinopyrum bessarabicum**

*Th. bessarabicum* (JJ) was described by Gorham et al. (1985) as ‘a slow-growing, perennial wheatgrass native to coastal regions of the Black and Mediterranean Seas (Tsvelev, 1976)’. The growth response of *Th. bessarabicum* to NaCl salinity, as well as the physiological basis of salt tolerance, have been studied in detail (Gorham et al., 1985, 1986a, b). *Th. bessarabicum* survives prolonged exposure to 350 mM NaCl, although growth reductions are large even at 250 mM (Gorham et al., 1985). After 66 d at 250 mM NaCl, a salinity that usually causes the death of bread wheat, shoot and root fresh masses of *Th. bessarabicum* were 40% and 50% of values in non-saline controls, although in the final week of this treatment, RGR was only 18% of that in non-saline conditions (Gorham et al., 1985). So, although *Th. bessarabicum* is much more salt tolerant than bread wheat, productivity is still greatly diminished when salinity reaches half that in seawater. Salt tolerance in *Th. bessarabicum* is associated with a capacity to: (i) regulate leaf Na⁺ and Cl⁻ concentrations to those required for ‘osmotic adjustment’, (ii) maintain leaf K⁺ concentrations when exposed to salinity, and (iii) synthesize glycinebetaine.

A wheat (cv. Chinese Spring)–*Th. bessarabicum* amphiploid (2n=8x=56; genome AABBDDJJ) has been produced (Forster and Miller, 1985). Growth and yield components of the amphiploid were compared with those in wheat (cv. Chinese Spring) and *Th. bessarabicum*, in nutrient solution (Gorham et al., 1986b). Growth data were not presented, but summarized by Gorham et al. (1986b) as: ‘the amphiploid grew much faster than *Th. bessarabicum*, so that early vegetative growth resembled that of Chinese Spring’. Fertility in the amphiploid was poor; grain number per head was only 18% of that in Chinese Spring, so that although individual grains were 50% heavier, grain yield per plant was only 28–33% of that in Chinese Spring (Gorham et al., 1986b). In addition, the amphiploid was genetically unstable, with about 10% of progeny not breeding true when selfed (Forster et al., 1987).

The effect of 150 mM NaCl on yield of the amphiploid was evaluated in two experiments reported by Gorham et al. (1986b), and in one experiment by Forster et al. (1987). In the first experiment by Gorham et al. (1986b),

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Flag leaf Na⁺ concentration (µmol g⁻¹ dry weight)</th>
<th>Grain yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Saline</td>
</tr>
<tr>
<td><em>Triticum aestivum</em></td>
<td>9</td>
<td>522</td>
</tr>
<tr>
<td>Wheat–<em>E. elongata</em> amphiploid</td>
<td>8</td>
<td>96</td>
</tr>
<tr>
<td>Substitution lines 1E(1A), 1E(1B), 1E(1D)</td>
<td>5–11</td>
<td>354–533</td>
</tr>
<tr>
<td>Substitution lines 2E(2A), 2E(2B), 2E(2D)</td>
<td>5–8</td>
<td>381–532</td>
</tr>
<tr>
<td>Substitution lines 3E(3A), 3E(3B), 3E(3D)</td>
<td>5–8</td>
<td>107–195</td>
</tr>
<tr>
<td>Substitution lines 4E(4A), 4E(4B), 4E(4D)</td>
<td>9–25</td>
<td>303–645</td>
</tr>
<tr>
<td>Substitution line 5E(5D)</td>
<td>7</td>
<td>420</td>
</tr>
<tr>
<td>Substitution lines 6E(6A), 6E(6B), 6E(6D)</td>
<td>5–7</td>
<td>372–492</td>
</tr>
<tr>
<td>Substitution lines 7E(7A), 7E(7B), 7E(7D)</td>
<td>6–12</td>
<td>373–471</td>
</tr>
</tbody>
</table>
plants were not vernalized, causing Chinese Spring to flower later than the amphiploid; whereas in their second experiment (Gorham et al., 1986b) and also in the work of Forster et al. (1987) plants were vernalized to synchronize flowering times. At 150 mM NaCl (without vernalization), grain yield for Chinese Spring was reduced to only 21% of that in control plants (tillering and thus numbers of ears, seeds per ear, and mean seed weight were all reduced by salinity), whereas for the amphiploid yield was still at 89%. However, because fertility of the amphiploid was poor, grain weights per plant at 150 mM NaCl were probably not statistically different (4.3±1.4 g for the amphiploid; 3.5±1.1 g for Chinese Spring). In the second experiment reported by Gorham et al. (1986b) (with vernalization), 150 mM NaCl reduced yields by 31–32% in both genotypes, so that the amphiploid only yielded 0.9 g and Chinese Spring 2.9 g. At 250 mM NaCl, Chinese Spring died but the amphiploid yielded 18% of the grain produced in non-saline conditions (Gorham et al., 1986b). However, Forster et al. (1987) reported that at 250 mM NaCl, the amphiploid yielded 59% of the non-saline control [although non-saline controls in the experiment by Forster et al. (1987) only yielded 25% of those in Gorham et al. (1986b)]. Unfortunately, Forster et al. (1987) did not compare their findings with the results in Gorham et al. (1986b). Physiological data are not available for the plants grown by Forster et al. (1987), preventing further interpretations [the data on leaf ion concentrations in Forster et al. (1987) are the same as those presented earlier by Gorham et al. (1986b)].

Physiological traits associated with salt tolerance were evaluated in the wheat–Th. bessarabicum amphiploid and its parents, by analyses of the third-youngest leaf for a range of ions, metabolites, and sap osmotic potential (Gorham et al., 1986b). At 150 mM NaCl, Th. bessarabicum showed a superior capacity to ‘exclude’ Na⁺ from its leaves (concentrations were 40% lower) and to accumulate glycinebetaine (5-fold higher), as compared with Chinese Spring. On the other hand, leaf K⁺ concentrations suffered larger reductions and Cl⁻ tended to be higher in Th. bessarabicum, than in Chinese Spring. The superior capacity for Na⁺ ‘exclusion’ in Th. bessarabicum was displayed by the amphiploid (~50% lower leaf Na⁺ concentration than in Chinese Spring); however, glycinebetaine levels were not enhanced in the amphiploid. Glycinebetaine was enhanced, however, in backcross derivatives obtained from another wheat–Th. bessarabicum hybrid (stated in Gorham et al., 1986b).

Th. bessarabicum has also been hybridized with nine cultivars of durum wheat: eight durum wheat–Th. bessarabicum amphiploids were evaluated for fertility, and then two were also evaluated for salt tolerance (King et al., 1997b). Fertility of the durum wheat–Th. bessarabicum amphiploids ranged between 29–51%, in conditions of self-pollination; encouragingly, all these were higher than the fertility of the Chinese Spring-based amphiploid at 18%. However, although meiosis in the amphiploids was ‘generally regular’, cases of failure in pairing of chromosomes were observed for all genotypes. King et al. (1997b) discussed this fertility problem in comparison with a similar problem encountered during development of triticale (reviewed by Muntzing, 1979), and suggested that the approach used to improve fertility in triticale might also be used to solve this problem in the development of durum wheat–Th. bessarabicum amphiploids as a new crop, ‘tritipyrum’.

Two of the durum-based amphiploids (Langdon–Th. bessarabicum and Neodur–Th. bessarabicum) were evaluated for salt tolerance, in hydroponics (King et al., 1997b). In non-saline conditions, both amphiploids produced a greater number of spikes per plant than their respective durum parents, so that grain numbers per plant were higher in the amphiploids, even though these had lower fertility. The Langdon-based amphiploid produced 77% more grains than the Neodur-based amphiploid. Unfortunately, data on mean grain weights and/or on grain yields per plant were not presented. At 150 mM NaCl, both durum wheat cultivars produced no grain, whereas the amphiploids produced 25% (Langdon-based) and 75% (Neodur-based) of their grain numbers under non-saline conditions. At 200 mM NaCl, the respective values for grain numbers produced per amphiploid plant were 20% and 38% of those by non-saline controls. At 250 mM NaCl, both amphiploids only produced 8–9% of the number of grains for plants in non-saline conditions.

Only two addition lines (namely additions 2J and 5J), of the seven possible disomic chromosome addition lines for Th. bessarabicum into bread wheat, were available at the time experiments were conducted by Forster and colleagues. Growth and yield in saline hydroponics of these two addition lines was compared with that of Chinese Spring, and also with lines tetrasomic for homoeologous group 2 and 5 from Chinese Spring (to assess for possible gene-dosage effects, as compared with specific effects of the added Th. bessarabicum chromosomes) (Forster et al., 1988). Lines with group 2 chromosomes were evaluated at 150 mM and lines with group 5 chromosomes at 200 mM. Disomic addition line 2J, and also lines with an additional set of group 2 chromosomes from wheat, all grew worse and yielded less than Chinese Spring at 150 mM NaCl. By contrast, at 200 mM NaCl Chinese Spring and all three tetrasomic group 5 lines died, whereas the 5J disomic addition line grew similarly to the wheat–Th. bessarabicum amphiploid and yielded at least some grain, although only 24% of that produced by the amphiploid (g per plant). Unfortunately, no data were presented for non-saline controls and the physiological basis for the apparent salt tolerance in disomic addition line 5J, or apparent sensitivity in line 2J, were not evaluated. Nevertheless, it was concluded by Forster et al. (1988) that chromosome 5J
must contain a major dominant gene (or genes) for salt tolerance.

A subsequent study showed that the 5J disomic addition line was superior to Chinese Spring, in 'exclusion' of Na\(^+\) from both 'mature' and 'newly-developed' leaves (Mahmood and Quarrie, 1993). When grown at 200 mM NaCl for 15 d, Na\(^+\) concentrations in the 5J disomic addition line were only 8% in old leaves, and 46% in newly-developed leaves, of those in Chinese Spring. Emptically, however, Na\(^+\) concentrations in the more salt-sensitive 2J disomic addition line (Forster et al., 1988) were also considerably lower than in Chinese Spring; being only 31% in old leaves, and 77% in newly-developed leaves, thus raising doubts regarding the exact physiological basis for salt tolerance (and sensitivity) in these two disomic addition lines. Furthermore, when exposed to 200 mM NaCl in a different study, only 63% of the 5J disomic addition line plants survived, whereas all Chinese Spring plants survived during that same experiment (Koebner et al., 1996).

Despite much uncertainty regarding the traits contributing to salt tolerance in the 5J disomic addition line (discussed above) and its poor grain yield under salinity when compared with the amphiploid (Forster et al., 1988; indicating that other Th. bessarabicum chromosome(s) also contribute to salt tolerance in the amphiploid), work to make wheat recombinant lines focused on chromosome 5J (summarized in King et al., 1997a). However, further progress in making wheat recombinant lines with salt tolerance from 5J has not been reported by this group.

As far as is known, the bread wheat–E. elongata and bread wheat–Th. bessarabicum amphiploids have not been compared directly in the same experiment. Neither can absolute comparisons be made between the two amphiploids from data in Dvóřák and Ross (1986) and Gorham et al. (1986b), since grain yields of Chinese Spring in these experiments were, respectively, 2.5 and 17.1 g per plant. Fertility in the wheat–E. elongata amphiploid is 83% of that in Chinese Spring (Dvóřák and Sosulski, 1974), this being much higher than the 18% fertility in the wheat–Th. bessarabicum amphiploid (Gorham et al., 1986b). The two amphiploids differ in time to flowering; the E. elongata-based amphiploid flowers 2 weeks later than Chinese Spring (Dvóřák and Sosulski, 1974), whereas the Th. bessarabicum-based amphiploid flowers 2 weeks earlier (Gorham et al., 1986b) (in both cases plants were not vernalized). Furthermore, although E. elongata and Th. bessarabicum are closely related, and are considered by some to represent two versions of the same basic genome (Wang and Hsiao, 1989), different chromosomes in the two species were identified as playing major roles in determining Na\(^+\) ‘exclusion’, i.e. 3E and 5J; although the enhanced Na\(^+\) ‘exclusion’ in wheat resulting from 5J appears to be much less than that from 3E. Forster (1994) suggested the apparent disparity between the effects of chromosomes 3E and 5J on Na\(^+\) ‘exclusion’ might be due to chromosome translocation differences between the genomes of E. elongata and Th. bessarabicum; at least two translocations that differ between the genomes of these two species were identified (Wang and Hsiao, 1989). A definitive explanation will only be possible when the loci, and genes, involved in the enhanced Na\(^+\) ‘exclusion’ in both species are identified. The possible effects of other chromosomes from Th. bessarabicum, especially 3J, when in wheat should also be evaluated. Five of the seven disomic chromosome addition lines for Th. bessarabicum in wheat are now available, but unfortunately addition line 3J is still to be isolated (William and Mujeeb-Kazi, 1995; Zhang et al., 2002). So, although Mujeeb-Kazi and Diaz de Leon (2002) published a preliminary analysis of K\(^+\)/Na\(^+\) in leaves of seven disomic chromosome addition lines for Th. bessarabicum into bread wheat, lines 3J and 6J presumably were not valid. As Mujeeb-Kazi and Diaz de Leon (2002) regarded their own experiment as ‘preliminary’, and since units for leaf Na\(^+\) and K\(^+\) were not given, additional physiological evaluations of the five available disomic addition lines are needed to improve knowledge on salt tolerance in Th. bessarabicum, as expressed in bread wheat.

**Thinopyrum ponticum**

*Th. ponticum* (EEEEEEEEEEE) is a perennial grass with salt tolerance, as evidenced by its use as forage on salt-affected lands. Several accessions survived 750 mM NaCl (McGuire and Dvóřák, 1981) and some also maintained reasonable growth at an EC\(_e\) of 13.9 dS m\(^{-1}\) (Dewey, 1960). Salt tolerance in decaploid tall wheatgrass was associated with a capacity to restrict the rate of accumulation of Na\(^+\) and Cl\(^-\) in shoots (Greenway and Rogers, 1963; Shannon, 1978; Weinberg and Shannon, 1988), and also with the accumulation of glycinebetaine in leaf tissues (Weinberg and Shannon, 1988).

Somatic hybridization techniques were used by Xia et al. (2003) to transfer *Th. ponticum* chromosomes, or chromosomal fragments, into bread wheat. Fertile plants were regenerated from asymmetric somatic hybrids produced by fusing protoplasts of *Th. ponticum* irradiated by UV with protoplasts from *T. aestivum*. A number of introgression lines were shown to contain *Th. ponticum* chromatin (Xia et al., 2003). Salt tolerance was tested for the parents, and selected F\(_2\) generation introgression lines, in hydroponic experiments (Chen et al., 2004). The NaCl concentrations were stepped up over 9 d, and although plants were only exposed to the final NaCl concentrations for a further 5 d, growth of two introgression lines was much less inhibited by NaCl (up to 250 mM) than for bread wheat. At 250 mM NaCl, dry mass of wheat plants was only 52% of the non-saline control, whereas it was 80% and 85% of the control in the two introgression lines. However, leaf Na\(^+\) concentration was similar in the bread wheat parent and the
Thinopyrum junceum

Th. junceum L. (A. Löve) is a hexaploid (J1J2J3J2EE) composed of the genomes of Th. elongatum and Th. bessarabicum (Charpentier, 1992), sometimes expressed as E1E2E3E4E5E6 (Wang et al., 2003b). Data on salt tolerance do not appear to be available for Th. junceum; although Gorham (1994) evaluated K+/Na+ ratios in several wheatgrass species, including Th. junceum, data were not reported for individual species. Gorham et al. (1986a), however, presented data on solute concentrations in sap expressed from ‘mature’ leaves after 4 months at 200 mM NaCl; Na+ was 146 mM, Cl− was 248 mM, and K+/Na+ was only 1.0.

Charpentier (1992) produced several disomic addition lines and partial amphiploids from a hybridization between bread wheat (cv. Chinese Spring) and Th. junceum, which were subsequently tested for salt tolerance by Wang et al. (2003b). Three partial amphidiploids and one addition line containing chromosome 5 from Th. junceum (AJDAj5) were ranked as salt tolerant. Wang et al. (2003a, b) produced recombinant lines of wheat containing segments of chromosome 5J. Following screening for ‘salinity tolerance’, although the approach used was far from ideal (discussed below), two recombinant lines (W4909 and W4910) were identified as having tolerance higher than Chinese Spring. However, based on the data in Wang et al. (2003b), tolerance in these lines is not certain, since: (i) the salinity treatments imposed were predominately CaCl2, (ii) absolute values were not presented (even for controls), (iii) it is unclear how shoot dry weight can be reduced by 78–84% at 22 dS m−1, yet grain weight was not reduced at all, and (iv) performance of the lines under field conditions at La Paz, Mexico, was reported as being ‘close to that in Kharchia 65’; showing that introduction of the alien genes did not result in substantial gains in salt tolerance above that already present in bread wheat (albeit a cultivar regarded as the most tolerant bread wheat). A future publication providing details of salt tolerance in the lines was foreshadowed in Wang et al. (2003b), and hopefully will clarify the levels of tolerance and physiological mechanisms in the lines, although this has not been published to date.

Notwithstanding the above concerns, an interesting observation from this work was that the Phb line used in the cytogenetic work might also possess higher salt tolerance than standard Chinese Spring wheat (Wang et al., 2003a, b). In order to induce meiotic pairing between wheat and Thinopyrum chromosomes, the Phb gene in wheat must be suppressed or removed (Sears, 1977; Chen et al., 1994). Wang et al. (2003b) used a Chinese Spring line having the Phb allele from Ae. speltoides var. speltoides (SS) (Chen et al., 1994). Wang et al. (2003b) reported that the Phb line was as salt tolerant as AJDAj5, and suggested that Ae. speltoides might have been the source of this salt tolerance. However, Gorham et al. (1991) found S genome species to have relatively poor Na+ ‘exclusion’, and Farooq et al. (1989) also found these to show poor survival, so this putative source of salt tolerance needs to be confirmed. If this source of tolerance is confirmed, Ae. speltoides var. speltoides might be used to generate synthetic hexaploids with improved salt tolerance, as this species is regarded as the most closely related to the donor of the B genome of durum wheat and bread wheat.

Summary

Wide-hybridization of tall wheatgrass species with wheat appears promising as an avenue to improve salt tolerance. Most convincing was the much greater yield under saline field conditions (namely 13.9–15.6 dS m−1) of a wheat–E. elongata amphiploid relative to Chinese Spring and also to a ‘tolerant’ check cultivar (Omielan et al., 1991). Halophytic wild relatives might also be sources of new genes for enhanced Na+ ‘exclusion’ (Omielan et al., 1991; Colmer et al., 1995), and with the possibility that these sources of Na+ ‘exclusion’ might continue to be effective even at high external NaCl concentrations. Enhanced Na+ ‘exclusion’ from E. elongata was attributed to chromosome 3E (Omielan et al., 1991) and that from Th. bessarabicum to 5J (Mahmood and Quarrie, 1993). The gene(s), and therefore mechanism(s) involved, in the enhanced Na+ ‘exclusion’ by these tall wheatgrasses might differ from the Kna1 locus in bread wheat on chromosome 4D (Dubcovsky et al., 1996) and Nax1 in durum wheat on chromosome 2A (Lindsay et al., 2004). The improved Na+ ‘exclusion’ could be introduced into bread wheat by making recombinant lines, although whether this leads to substantial improvements in salt tolerance awaits evaluation. Moreover, Th. ponticum introgressions into bread wheat appear to have resulted in improved salt tolerance (Chen et al., 2004). Finally, to make use of amphiploids as a new salt-tolerant feed wheat, the most tolerant accessions of wild relatives should be used to make amphiploids with a range of modern high-yielding, locally-adapted varieties for the target environments (as recommended for Th. bessarabicum by Gorham et al., 1986b). In many cases, issues of low
productivity and fertility, and stability, of the amphiploids would also need to be overcome (Islam and Shepherd, 1991); although at least in the case of the wheat–*E. elongata* amphiploid, productivity in field plots even under non-saline conditions was equal to that of the wheat parent (Omielan et al., 1991).

**Hordeum species (I, H and X genomes)**

Several *Hordeum*×bread wheat hybrids and cytogenetic stocks have been reported (Islam and Shepherd, 1990). As examples, wheat–*H. vulgare* and wheat–*H. chilense* disomic chromosome addition lines have been produced, and these have been studied for aspects related to salt tolerance. Barley (*H. vulgare* ssp. *vulgare*; genome II) is regarded as being more salt tolerant than bread wheat, but still has much lower tolerance than the wheatgrass species discussed in the preceding section (Colmer et al., 2005a). Barley disomic chromosome addition lines in bread wheat (Betzes barley added to Chinese Spring) were produced by Islam et al. (1981). Analyses of Na⁺ and K⁺ concentrations in leaves of these addition lines when exposed to 60 mM NaCl (Gorham et al., 1990a) revealed that there are genes on individual barley chromosomes that could enhance the ability of bread wheat to maintain low Na⁺ concentrations in leaves. In particular, addition of the chromosome pair 1I (designated 7H in its homeologous relationship with wheat chromosomes) resulted in significantly lower leaf sap Na⁺ concentration (being 17 mM) and higher K⁺ concentration (192 mM) than in Chinese Spring (Na⁺, 63 mM; K⁺, 152 mM). Addition of chromosome pair 6I (6H) also resulted in significantly lower leaf Na⁺ concentration (22 mM) than in Chinese Spring (63 mM). These effects of chromosomes from *H. vulgare* to enhance Na⁺ ‘exclusion’ in bread wheat are very interesting, as *H. vulgare* has much higher leaf Na⁺ concentrations than bread wheat (142 versus 63 mM), indicating a positive interaction between the individual *H. vulgare* chromosomes and the bread wheat genome to enhance Na⁺ ‘exclusion’.

Growth and grain yield of the wheat–*H. vulgare* addition lines were assessed for plants exposed to 175 and 200 mM NaCl, with treatments applied to 1-week-old seedlings by adding 25 mM NaCl in daily increments (Forster et al., 1990). Comparisons between these genotypes for salt tolerance were complicated by differences in biomass and development (e.g. flowering times) in non-saline controls. At 175 mM NaCl, fresh mass of Chinese Spring wheat was 33% of the control, whereas that of Betzes barley was 25%. Addition lines 1I (7H) and 6I (6H) had relatively low leaf Na⁺ concentrations when grown at 60 mM NaCl (Gorham et al., 1990a), but when exposed to 175 mM NaCl these two lines suffered reductions in fresh mass equal to that of Betzes, whereas addition line 2I (2H) was only reduced to 49% of the control. Forster et al. (1990) did not measure leaf Na⁺ concentrations, so it is not possible to make further comparisons with the results of Gorham et al. (1990a). At 200 mM NaCl both parents and all addition lines, except 3I (3H), had fresh mass values that were only 21–24% of the non-saline controls.

Hybrids of *H. chilense* (*H³H⁷*)xwheat were produced as part of a programme to develop a cereal with improved resistance to diseases (Martin et al., 1999). *H. chilense* is not, however, regarded as being particularly salt tolerant (von Bothmer et al., 1995), but the wheat–*H. chilense* disomic chromosome addition lines have been examined for growth responses when exposed to 175 and 200 mM NaCl (Forster et al., 1990). At 175 mM NaCl, the best addition line was 4Hch (42% of control) and the worst was 6Hch (14% of control); *H. chilense* itself was not tested. Neither leaf Na⁺ concentrations nor K⁺/Na⁺ ratios were reported in this study.

*H. marinum* (XX) is tolerant of salinity (Mano and Takeda, 1998; Garthwaite et al., 2005), and can be hybridized with wheat (Jiang and Liu, 1987). *H. marinum* displays a capacity to control concentrations of Na⁺ (and also Cl⁻) in leaves, even when exposed to very high (namely 450 mM) concentrations of NaCl in the root-zone (Garthwaite et al., 2005). Recently, a *H. marinum*–bread wheat amphiploid was produced by AKMR Islam (Colmer et al., 2005b). In addition to salt tolerance, *H. marinum* also showed better root aeration and higher waterlogging tolerance, as compared with several other species in the Triticeae, including wheat (McDonald et al., 2001; Garthwaite et al., 2003). Salt and waterlogging tolerance in the amphiploid was reported to be intermediate to that of its parents (Colmer et al., 2005b).

**Summary**

*H. marinum* is tolerant of both salinity and waterlogging, so *H. marinum*–wheat amphiploids could help address the challenging issue of the severe adverse interaction between waterlogging and salinity on cereal production. As land affected by dryland salinity is often also prone to waterlogging, these combined stresses have been suggested as a reason why wheat cultivars bred for salt tolerance have had little success in farmers’ fields (Hollington et al., 2002; Barrett-Lennard, 2003).

**Conclusions**

Several sources of enhanced Na⁺ ‘exclusion’, and higher salt tolerance (as compared with durum and bread wheat), have been identified within the Triticeae, both within close and more distant relatives. Although introgression of traits from closer relatives is easier, it is the more distant relatives, such as the halophytes (e.g. tall wheatgrass and sea barleygrass), that might ultimately provide most opportunity for substantial gains in salt tolerance. In contrast to bread wheat, the halophytes display a capacity for good Na⁺ ‘exclusion’, and in some cases Cl⁻ ‘exclusion’, even at relatively high
salinity. That many of these species can be hybridized with common wheat has been demonstrated, with some notable examples of improved Na⁺ ‘exclusion’ and enhanced salt tolerance in the progeny, as compared with the wheat parent. Even so, yields were still severely reduced by a salinity of about one-third of that in seawater. As several sources of improved Na⁺ ‘exclusion’ for species in the Triticeae are now known to reside on different chromosomes, further work to identify the underlying mechanisms, and then to pyramid the controlling genes for traits that could act additively, or even synergistically, might achieve substantial gains in tolerance (cf. Yeo and Flowers, 1986). This assertion is supported by several examples of positive interactions between genomes on the capacity of plants to ‘exclude’ Na⁺ from leaves. Moreover, traits in addition to Na⁺ ‘exclusion’, for example ‘tissue tolerance’, should also be evaluated in the Triticeae, as salt tolerance requires the coordinated action of a number of traits. Not withstanding this positive outlook, challenges are to be expected in any attempt to combine a number of traits from genotypes, especially as the genetic distance increases between the donor parents, and this might limit the possible gains in tolerance. Improvements in knowledge and techniques in genetics, including molecular and transgenic approaches, together with identification of the physiological mechanisms and genes involved, should enhance our capacity ultimately to breed crops with improved salt tolerance.

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