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Modification of the salinity response of wheat by the genome of *Elytrigia elongatum*

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Summary *Triticum aestivum* cv. Chinese Spring wheat, *Elytrigia elongatum* (tall wheatgrass), and the *Triticum-Elytrigia* amphiploid were grown in complete nutrient culture containing, in addition, 0, 40, 80 and120 m*M* NaCl. The 3 genotypes responded quite differently to increasing salinity; the Na concentration of wheat shoots increased in direct proportion to the increase in salinity of the external medium whereas the Elytrigia response was interpreted as showing high affinity for Na at low external Na (40 m*M*) but comparative exclusion of Na at high salinities (120 m*M*). In contrast, Na levels of the amphiploid were less than those of either wheat or Elytrigia under both low and high salinities. Thus the amphiploid behaved like wheat at 40 m*M* NaCl but more like Elytrigia at 120 m*M* NaCl because Na transport to the amphiploid shoot was restricted over the whole salinity range. The K concentration of the amphiploid shoot at high salinities was significantly greater than the K concentrations of either wheat or Elytrigia.

Introduction

The wheatgrass genus Elytrigia (= Agropyron) contains several species which possess considerable tolerance to salt[3,9,12,13]. A number of studies have shown that diploid *Elytrigia elongatum* ($2n = 2x = 14$) and the decaploid *Elytrigia pontica* ($2n = 10x = 70$) are highly tolerant species and are able to survive salt concentrations as high as $1\frac{1}{2}$ times seawater[3,12,13]. Significant intraspecific differences have also been reported[4,9,12,13].

However, the physiological responses of Elytrigia to salinity have been reported in only a few publications[5,8,11,15]. Two studies suggest that Na and Cl transport to the shoot at high salinities is restricted thereby maintaining high and favourable K/Na and K/Cl ratios in the shoot[8,11]. Maintenance of high K/Na is characteristic of many monocotyledonous halophytes[2]. Inter- and intraspecific differences in tolerance to salt within the genus Elytrigia have also been positively correlated to salt exclusion[8,13].

Elytrigia species have been used as a genetic source to transfer disease resistance to wheat[10]. Because of the high salt tolerance of Elytrigia species and their crossability to common wheat, the genus Elytrigia has been recognised as a valuable gene source to produce salt tolerant cultivars of wheat[12]. However, specific information is required on physiological mechanisms which confer salt tolerance upon monocotyledonous species such as Elytrigia and Triticum. This knowledge will assist in screening of cultivars for tolerance to salt and also in identifying those attributes which are responsible for the salt tolerance of Elytrigia and which ones can be transferred to wheat.

Preliminary results of a research programme designed to study chromosomal control of salt tolerance characters in a wheat-Elytrigia amphiploid are presented in this paper. Some of the physiological responses to salt stress of hexaploid Chinese Spring wheat ($2n = 6x = 42$),

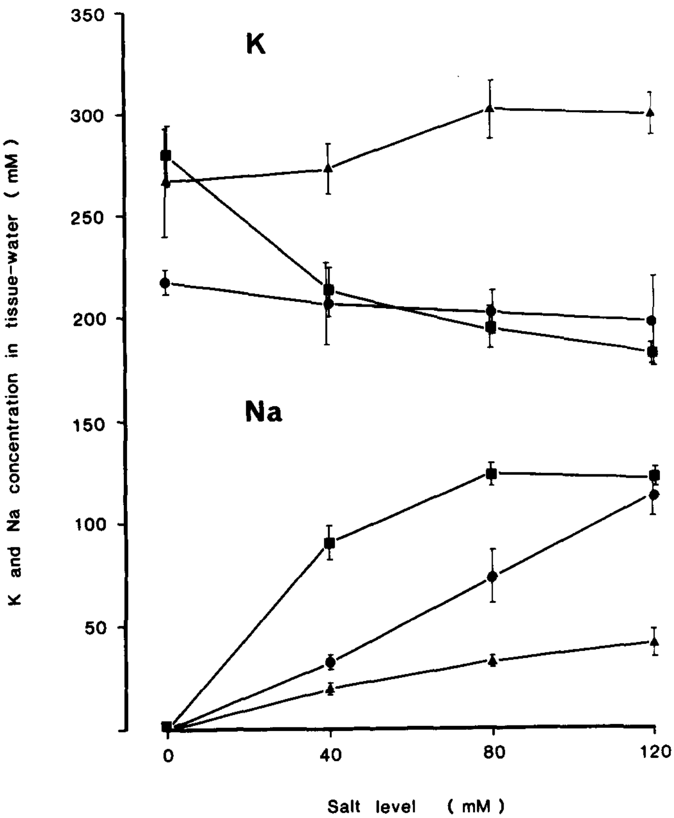

Fig. 1. Na and K concentrations in the shoot tissues of Chinese Spring wheat (•), Elytrigia (▪) and the wheat-Elytrigia amphiploid (▲). Vertical bars represent standard errors of four replicates.

Elytrigia elongatum (2 n = 2 x = 14) and the wheat – Elytrigia amphiploid (2 n = 8 x = 56) are described.

Methods

Seeds of *Elytrigia elongatum* and of the Chinese Spring wheat – *Elytrigia elongatum* amphiploid were obtained from J. Dvorak at the University of California, Davis. Seeds of Chinese Spring wheat were obtained from stocks currently held at the Waite Agricultural Research Institute.

Experiment 1

Wheat and wheat-Elytrigia amphiploid seeds were germinated on moist filter paper in petri dishes. Because of the slow growth of Elytrigia at the seedling stage these seeds were germinated in moist potting mix. Plants were transferred to full strength Hoagland's solution when the shoot fresh weight was approximately 0.5 g (10 plants per 10 l of solution) NaCl increments were added at the rate of 40 m*M* every 3 days to give final salt levels of 0, 40, 80 and 120 m*M*. The plants were grown at these salinities for 3 weeks in a glasshouse.

Table 1. The concentration of Na in the shoots of Chinese Spring wheat, Elytrigia and the wheat-Elytrigia hybrid grown in culture solution with a total [K + Na] content of 10 m*M* at 3 K/Na ratios

K/Na ratio of root medium	Tissue-Water Na (m*M*)*		
	Wheat	Wheat-Elytrigia	Elytrigia
9:1	1.8 ± 0.2	1.8 ± 0.1	4.8 ± 0.5
5:5	3.0 ± 0.1	3.3 ± 0.2	15.4 ± 0.8
1:9	4.8 ± 0.5	7.0 ± 0.5	24.6 ± 0.9

* Mean values with standard error of 4 replicates.

Experiment II

Seeds of Elytrigia, wheat and the wheat-Elytrigia amphiploid were germinated in moist potting mix. Seedlings were transferred to nutrient solution when the shoot fresh weight was between 0.2 g and 0.5 g (5 plants per 10 l of solution). The total [K + Na] concentration of the culture solution was 10 m*M* with K:Na ratios of 9:1, 5:5, 1:9. The plants were grown in the glasshouse to a final shoot fresh weight of 10–15 g during which the culture solution was changed 3 times.

Ion extraction and analyses

Shoots were rinsed briefly in distilled water, blotted dry, weighed, dried at 70°C and re-weighed. 0.1 to 0.2 g of dry material frozen in liquid N_2 was crushed to a fine powder with a glass rod. The plant material was extracted with 0.5 ml of 1 *N* HNO_3 at 95–100°C for 15 min. The sample, diluted with 5 ml of distilled water, was centrifuged and the supernatant decanted and stored. The pellet was extracted twice with a further 5 ml of water. The K and Na concentrations in the bulked extracts were determined by atomic absorption spectrophotometry.

Results

The Na concentration on a tissue water basis in the shoot of Elytrigia was significantly greater than the Na concentration of the rooting medium at both 40 and 80 m*M* (Fig. 1). However, a further increment (40 m*M*) in NaCl did not increase the Na concentration of the shoot. In contrast, the Na concentration in Chinese Spring wheat increased in direct proportion to the increase in salinity of the external medium. The Na response of the amphiploid was different to that of either parent. The Na concentration of the amphiploid was lower than that of wheat at all salinities. At 120 m*M* NaCl the Na tissue concentration was 40 m*M* compared to 120 m*M* in wheat and Elytrigia.

The K response of the amphiploid also was different to that of either wheat or Elytrigia (Fig. 1). While the K concentration in the amphiploid may have increased at high salt concentrations, the K concentration decreased in Elytrigia and changed little in wheat.

Na accumulation by Elytrigia from a solution of low concentration of K + Na (10 m*M*) was high at all 3 K/Na ratios compared to that of wheat and the amphiploid (Table 1). Under these growth conditions the Na response of the amphiploid was more like that of wheat.

Discussion

The changes in the K and Na concentrations of *Elytrigia elongatum* grown in NaCl culture solution were characteristic of many of the monocotyledonous halophytes[2] which tend to restrict Na accumulation in the shoot to maintain a K/Na ratio frequently greater than unity. Dicotyledonous halophytes, however, are generally characterised by a high affinity for Na, even at very low salinities, with K/Na ratios frequently less than unity. At 100 m*M* the K/Na ratio in the leaf tissue of the dicotyledonous halophyte *Atriplex spongiosa*[15] was less than 0.25 while Elytrigia grown in 120 m*M* NaCl had a K/Na ratio greater than 2.

Nevertheless, the affinity of Elytrigia for Na was greater than that of the glycophyte, wheat, at low salinities (Fig. 1 and Table 1). However, the high affinity towards Na shown by Elytrigia was not evident at 120 m*M* NaCl and indeed the data suggested that further Na accumulation in the shoot was restricted above 80 m*M* NaCl.

While the Na concentration in the shoot tissue of wheat increased in direct proportion to the change in external salinity, the addition of the Elytrigia genome to the wheat genomes in the amphiploid resulted in a lower Na level in the shoot. The decrease in Na content was particularly significant at the higher salinities suggesting that the Elytrigia genome may have modified Na transport processes in the root. For a more definitive interpretation of these results, further study of Na localization and fluxes in the roots of these three genotypes is warranted.

The wheat-Elytrigia amphiploid contains three genomes derived from wheat and one from Elytrigia. Nevertheless, the gene or genes which restrict Na accumulation in the shoot of Elytrigia at high salinities appear to be epistatic in their effect in the amphiploid phenotype over wheat characters for accumulation of Na. However, at low salinities, the low affinity shown by wheat for Na appeared to be epistatic over the high affinity response towards Na shown by Elytrigia.

Thus the phenotype of the amphiploid appears to express characters of both wheat and Elytrigia. At low salinities Na is excluded from the shoot of the amphiploid like wheat, while at high salinities the amphiploid responds more like Elytrigia where Na transport to the shoot is restricted.

Na exclusion from the shoot is an important salt resistance mechanism of many glycophytes including cereals. Although little data exists for wheat genotypes, the salt tolerance of barley cultivars has been positively correlated to Na and Cl exclusion from the shoot[7,14]. Shannon[13] from a study of 32 accessions of *Elytrigia elongatum* also concluded that the most tolerant varieties were those with lower Na and Cl levels in the leaves. Thus it appears that a salt exclusion mechanism that confers salt resistance upon both wheat and tall wheatgrass genotypes may be transferred by the Elytrigia genome to wheat and be subsequently expressed in that phenotype. Cl exclusion from the leaf and stem tissues of salt tolerant soybean cultivars has been shown to be regulated by a single dominant gene[1]. Similarly, restriction of Na transport to the shoot of Elytrigia may also be controlled by a single gene or a few genes which can be used to modify the salinity response of common wheat. The results presented in this paper show that *Elytrigia elongatum* strains represent a valuable gene source for the manipulation of physiological processes in the wheat plant to increase resistance to salt stress.

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