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# Mapping of the $K^+/Na^+$ discrimination locus *Kna1* in wheat

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**Abstract** In saline environments, bread wheat, *Triticum aestivum* L. (genomes AABBDD), accumulates less $Na^+$ and more $K^+$ in expanding and young leaves than durum wheat, *T. turgidum* L. (genomes AABB). Higher $K^+/Na^+$ ratios in leaves of bread wheat correlate with its higher salt tolerance. Chromosome 4D from bread wheat was shown in previous work to play an important role in the control of this trait and was recombined with chromosome 4B in the absence of the *Ph1* locus. A population of plants disomic for 4D/4B recombined chromosomes in the genetic background of *T. turgidum* was developed to investigate the genetic control of $K^+/Na^+$ discrimination by chromosome 4D. Evidence was obtained that the trait is controlled by a single locus, designated *Kna1*, in the long arm of chromosome 4D. In the present work, $K^+/Na^+$ discrimination was determined for additional families with 4D/4B chromosomes. The concentrations of $Na^+$ and $K^+/Na^+$ ratios in the youngest leaf blades clustered in two nonoverlapping classes, and all recombinant families could be unequivocally assigned to *Kna1* and *kna1* classes. The *Kna1* locus scored this way was mapped on a short region in the 4DL arm and was completely linked to *Xwg199*, *Xabc305*, *Xbcd402*, *Xpsr567*, and *Xpsr375*; it was also mapped as a quantitative trait. The results of the QTL analysis, based on the $K^+/Na^+$ ratios in the young leaves of greenhouse-grown plants and flag leaves of field-grown plants, agreed with the position of *Kna1* determined as a qualitative trait. Several aspects of gene introgression by manipulation of the *Ph1* locus are discussed.

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## Introduction

The species of the tribe Triticeae behave as partial $Na^+$ excluders when grown in saline substrates. The exclusion of $Na^+$ and compensatory accumulation of $K^+$ in the expanding leaf or the youngest leaf highly correlate with salt tolerance (Omielan et al. 1991; Colmer et al. 1995). Bread wheat, *Triticum aestivum* L. (2n=6x=42, genomes AABBDD), has a greater discriminating capacity between $Na^+$ and $K^+$ than durum wheat, *T. turgidum* (2n=4x=28, genomes AABB), under salt stress and is generally more salt-tolerant. Examination of $K^+/Na^+$ ratios in disomic substitution lines in which each of the seven D-genome chromosomes from bread wheat was individually substituted for homoeologous chromosomes of durum wheat cv 'Langdon' (Joppa and Williams 1988) showed that chromosome 4D accounts for 50–60% of the difference between bread wheat and durum wheat in this trait (Gorham et al. 1987; Dvořák and Gorham 1992).

To investigate the genetic basis of $K^+/Na^+$ discrimination conferred on durum wheat by bread wheat chromosome 4D, Dvořák and Gorham (1992) recombined chromosome 4D with durum wheat chromosome 4B by employing the *ph1c* mutant of durum wheat. The *ph1c* mutation facilitated the recombination between homoeologous chromosomes that is normally suppressed in durum wheat by the activity of the *Ph1* gene (Giorgi and Cuozzo 1980). The accumulation of $Na^+$ and $K^+$ was determined in young plants grown in 50 m*M* NaCl in the greenhouse (Dvořák and Gorham 1992). The families could be grouped in two clear-cut classes according to their $K^+/Na^+$ ratios, which were similar to those of the parents. This and cytogenetic evidence suggested that $K^+/Na^+$ discrimination is controlled by a single locus on the long arm of chromosome 4D, which was designated *Kna1*. Field experiments

showed that the *Kna1* families as a group had higher salt stress tolerance than those lacking *Kna1* (Dvořák et al. 1994).

To determine the position of *Kna1* on the linkage map, the population of the 4D/4B recombinant families was used to construct a genetic map using restriction fragment length polymorphism (RFLP). The enhanced $K^+/Na^+$ discrimination was placed on the map as a qualitative trait, on the basis of classification of greenhouse-grown plants to *Kna1* and *kna1* classes, and as a quantitative trait, on the basis of the actual ratios of accumulated $K^+$ to $Na^+$in the youngest leaves of the greenhouse-grown plants and the flag leaves of field-grown plants.

## Materials and methods

### Plants

A disomic substitution line in which chromosome 4D of bread wheat was substituted for chromosome 4B of cv 'Langdon', henceforth DS4D(4B), was developed by Joppa and Williams (1988). The *T. turgidum* homozygous mutant *ph1c*, the source of chromosome 4B, was developed by Giorgi and Cuozzo (1980). The development of the families with 4D/4B recombinant chromosomes was described by Dvořák and Gorham (1992). Disomic plants for the 4D/4B chromosomes were produced by the self-pollination of double monosomic 4D/4B-4D $F_1$ plants and the selection of 4D/4B disomics by C-banding analysis or with the aid of molecular markers in the $F_2$ (Dvořák and Gorham 1992 and present data).

### RFLP mapping procedures

Of a total of 133 families produced, 2 were excluded because they were insufficiently characterized. Seventy-five families that were shown by studying chromosome pairing to have acquired an unrecombined chromosome were not subjected to RFLP analysis but were included in the data matrix in map construction; the remaining 56 families were used in the RFLP investigation. Two families were aneuploid and were excluded from the map construction, leaving a total of 129 families for map construction.

For the RFLP analysis, nuclear DNAs were isolated (Dvořák et al. 1988) from bulked leaves of a minimum of 7 randomly chosen $F_2$ plants per $F_2$ family. In some cases, a single $F_3$ or $F_4$ plant disomic for a recombined chromosome was used. Southern blotting and DNA hybridization were performed as described earlier (Dubcovsky et al. 1994). The RFLP map was constructed with the computer program MAPMAKER/EXP3.0 (Lander et al. 1987; Lincoln et al. 1992) using the Kosambi function (Kosambi 1943). $K^+/Na^+$ discrimination was also mapped using the actual $K^+$ and $Na^+$ concentrations in the greenhouse- (Dvořák and Gorham 1992) and field-grown plants (Dvořák et al. 1994) and the quantitative trait loci (QTL) option of the MAPMAKER program.

### Determination of $K^+/Na^+$ratio

The concentrations of $K^+$ and $Na^+$ and ratios of accumulated $K^+$ to $Na^+$ in the youngest leaves have been reported for most of the families by Dvorak and Gorham (1992). Seven additional families with 4D/4B chromosomes were investigated here. Two-week-old seedlings of the 7 families, 'Langdon', and DS4D(4B) were transferred in a completely randomized design to 100-l tanks in the greenhouse. The medium was an aerated 1/4 concentration Hoagland solution modified by the addition of 0.25 m*M* $Na_2SiO_3$ and 0.50 μ*M* FeEDTA; pH 5.5 (Huang et al. 1992). With regard to the inclusion of $Na_2SiO_3$, see Epstein (1994). After 1 week, the solution was renewed and adjusted to 50 m*M* NaCl; the pH was adjusted every 3 days, and the solution was changed once a week. The youngest fully expanded leaves were harvested after the plants had been in the salinized solution for 14 days; fresh weight and dry weight after 48 h at 60°C were recorded. Samples were ground and then shaken for 3 days in plastic vials with 15 ml of 0.5 *M* HCl for acid extraction of free cations. $K^+$ (1/400 dilution) and $Na^+$ (1/100 dilution) concentrations were determined in the presence of the LaCs reagent in an atomic absorption spectrophotometer (Perkin-Elmer).

**Table 1** Concentrations of $K^+$, $Na^+$, and $K^+/Na^+$ ratios (μmol $g^{-1}$ dry weight) in the youngest fully expanded leaves of $F_2$ plants in 7 families homozygous for 4D/4B recombinant chromosomes, 'Langdon', and DS4D(4B). The inferred *Kna1* and *kna1* genotypes are indicated by + and –, respectively

| Family | Number of plants | $K^+$ | $Na^+$ | $K^+/Na^+$ | *Kna1* |
|---|---|---|---|---|---|
| DS4D(4B) | 18 | 1431a[a] | 72a | 22.3a | + |
| Langdon | 18 | 1102c | 509d | 2.2b | – |
| 25 | 5 | 1407ab | 58a | 26.2a | + |
| 133 | 5 | 1392ab | 58a | 26.7a | + |
| 200 | 3 | 1499a | 42a | 38.7c | + |
| 67 | 1 | 1204abc | 572cd | 2.1b | – |
| 87 | 2 | 1221bc | 382bc | 3.4b | – |
| 168 | 5 | 1287b | 313b | 4.5b | – |
| 171 | 1 | 916b | 536cd | 1.7b | – |

[a] Values in the column not sharing a common letter are significantly different at the 1% probability level

## Results

### $K^+/Na^+$ ratio

Langdon accumulated significantly less $K^+$ and more $Na^+$ than DS4D(4B), and had a lower $K^+/Na^+$ ratio than DS4D(4B) (Table 1). The recombinant families fell into two nonoverlapping classes in the accumulation of $Na^+$ and in the $K^+/Na^+$ ratio that were similar to those of 'Langdon' or DS4D(4B) (Table 1). The expression of *Kna1* primarily affected the exclusion of $Na^+$ rather than the preferential uptake of $K^+$. The tissue $K^+$ levels ranged from 1499 μmol $g^{-1}$ dry weight to a value of 916, for a high-to-low ratio of only 1.6 (Table 1). The corresponding values for $Na^+$ were 572 μmol $g^{-1}$ dry weight and 42, for a high-to-low ratio of 13.6. There was, however, a tendency for the families with low $Na^+$ concentrations and high $K^+/Na^+$ ratios to have higher $K^+$ concentrations than those with high $Na^+$ concentrations and low $K^+/Na^+$ ratios (Table 1). On the basis of $Na^+$ concentrations and $K^+/Na^+$ ratios, the 7 families were assigned to *Kna1* and *kna1* classes (Table 1).

### Mapping of *Kna1*

Twenty-nine RFLP loci were mapped in the 4D/4B population. Of 131 families, 2 were monosomic additions 4B

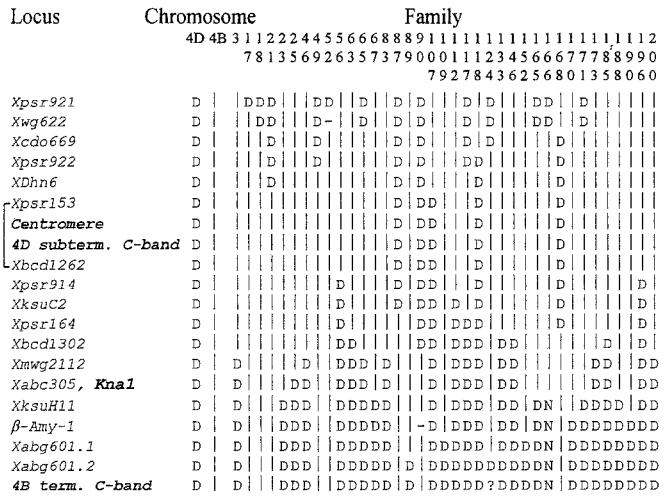


**Fig. 1** Graphical genotypes of the 4D/4B recombinant chromosomes. The numerical designations of families harboring the recombinant chromosomes are shown on the *horizontal axis*. For groups of completely linked loci, only a single, arbitrarily chosen locus is shown. The loci are designated as described in Fig. 2. Indicates 4B alleles, *D* 4D alleles, *N* null alleles, *?* atypical C-band, – alleles that were not scored. The null alleles in family 166 were caused by homoeologous crossing-over between 4BL and the translocated region of 5AL harboring the distal part of 4AL (Devos et al. 1995)

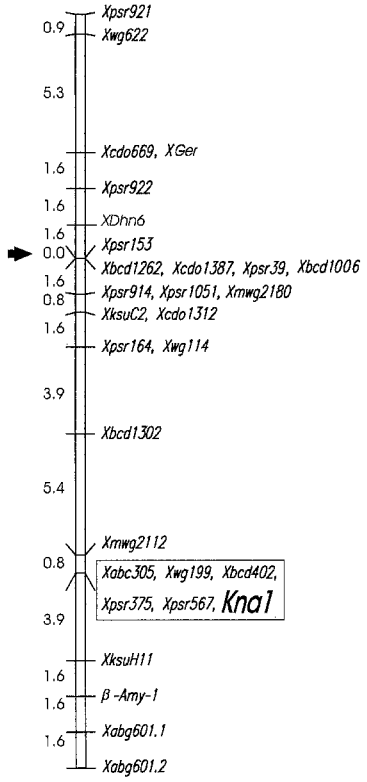


**Fig. 2** Linkage map produced from targeted homoeologous recombination between chromosome 4B of *T. turgidum* and 4D of *T. aestivum* in the absence of the *Ph1* locus. The distances are given in cM. The centromere is indicated by an *arrow*. Loci designated *psr* were mapped with clones described by Devos et al. (1995); those designated *wg*, *cdo*, and *bcd*, by Anderson et al. (1992); *ksu*, by Gill et al. (1991); *mwg*, by Graner et al. (1991); *bc* and *abg*, by Kleinhofs et al. (1993). *β-Amy-1* was mapped with a clone developed by Khursheed and Rogers (1988), *XDhn6* with a clone developed by Close et al. (1989), and *XGer* with a clone developed by W. J. Hurkman (GenBank Accession no. U01963). The markers completely linked to *Kna1* are *boxed*

(2n=29). These aneuploid families were eliminated from further analysis. Out of the remaining 129 euploid families (2n=28), 92 harbored either an unrecombined 4B (74 families) or 4D (18 families) chromosome. Of the 37 families with recombined chromosomes, 5 had a recombined chromosome with the 4D centromere and 32 had a recombined chromosome with the 4B centromere (Fig. 1). The genotype of each recombined chromosome is shown graphically in Fig. 1; those of the unrecombined chromosomes are not shown.

The 4D/4B homoeologous map, spanning the interval *Xpsr921* in the short arm to *Xabg601.2* in the long arm, was 33.8 cM long (Fig. 2). The most distal markers mapped on the 4D/4B map were the most distal markers currently mapped in the wheat and barley chromosomes of homoeologous group 4, except for the telomeres (Kleinhofs et al. 1993; Devos et al. 1995). Of 44 cross-over points mapped, 31 were singles and 12 were 4D/4B doubles. There was, additionally, one double cross-over that involved 4D/4B/5A (family 166 in Fig. 1).

To place *Kna1* in the 4D/4B linkage map, we used the assignments of individual recombinant families to *Kna1* or *kna1* classes reported earlier (Dvořák and Gorham 1992; Dvořák et al. 1994) or determined here (Table 1). Of 37 families with recombined chromosomes, 17 were *Kna1* and 20 were *kna1*. *Kna1* was mapped in the distal third of the long arm and was completely linked to markers *Xwg199*, *Xabc305*, *Xbcd402*, *Xpsr567*, and *Xpsr375* (Fig. 2). While the population of recombined chromosomes with the 4B centromere had cross-over points on either the proximal or distal sides of *Kna1*, those with the 4D centromere had cross-over points only on the proximal side of *Kna1*.

The *Kna1* family 25 (Fig. 1) showed a high $K^+/Na^+$ ratio (Table 1) and harbored a chromosome recombined between *Xmwg2112* and the group of markers completely linked to *Kna1*; this chromosome had the *Xmwg2112-4B* allele but the 4D alleles for the 5 linked markers. This result showed that *Kna1* cannot be more than 0.8 cM proximal to the group of completely linked markers including *Xwg199* and *Xbcd402* in the 4D/4B map. No distal RFLP marker was found to be tightly linked to the group of linked markers including *Kna1* on the 4D/4B map. Since no recombination was observed between *Kna1* and the 5 markers in a sample of 129 chromosomes, *Kna1* is expected to be no more than 2.3 cM from these markers with 95% probability on the 4D/4B map.

## Analysis of $K^+/Na^+$as a quantitative trait

The association between markers on the 4D/4B map and the $K^+/Na^+$ ratios in greenhouse-grown plants treated with

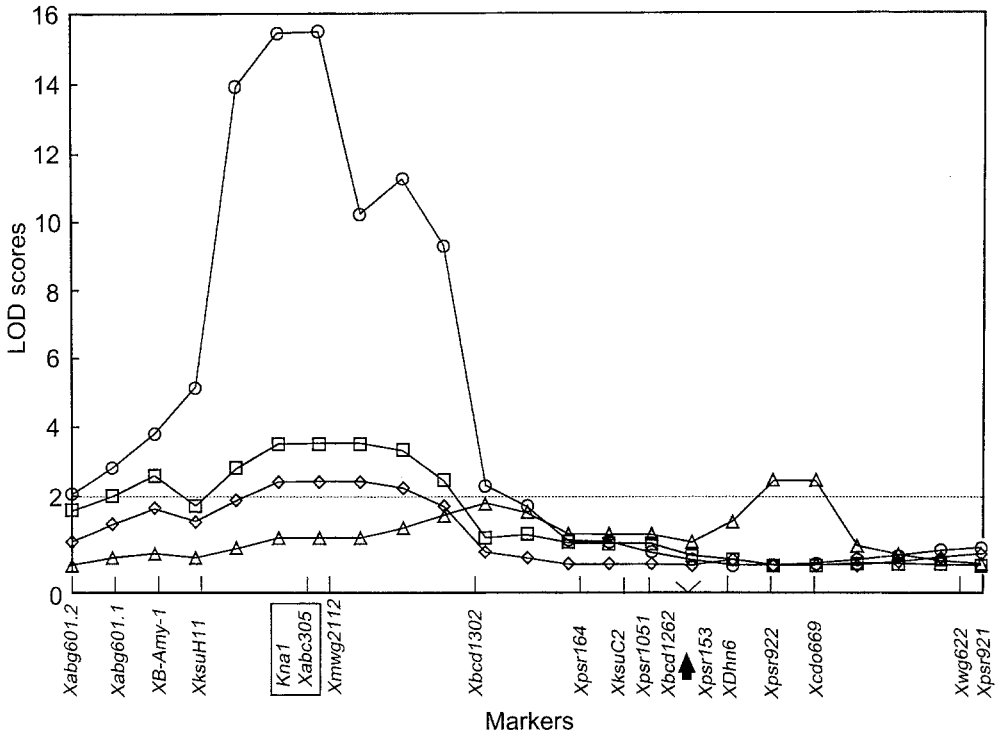


**Fig. 3** Log-likelihood values (LOD) for the association of markers in the 4B/4D map with the $K^+/Na^+$ ratio in the youngest leaf of young, greenhouse-grown plants treated with 50 m*M* NaCl (○), adult, field-grown control plants (△), and field-grown plants exposed to intermediate- (□) and high-salt stress (◇). The position of the centromere is indicated by an *arrow* and the distances are in cM

50 m*M* NaCl (Table 1 and Dvořák and Gorham 1992) and field-grown plants under control conditions (low salt) and intermediate- and high-salt conditions (Dvořák et al. 1994) was investigated with the MAPMAKER-QTL computer program. The association between the $K^+/Na^+$ ratio in the greenhouse plants and the markers peaked in a region of the map similar to where *Kna1* was mapped as a qualitative trait (Fig. 3). The peak in the interval *Kna1–Xmwg2112* showed a high LOD score. The LOD scores were lower for the field-grown plants grown under intermediate and high levels of salinity. The LOD score peaks were shifted proximally for the field-grown plants, because family 25, the only one with a cross-over between *Kna1* and *Xmwg2112*, was included in the greenhouse data but not in the field data. No peak was observed at the *Kna1* locus but there was a peak with LOD>2 centered over *Xpsr922* in the short arm under low salinity (control).

## Discussion

A genetic map constructed from recombination between 4D and 4B in the absence of the *Ph1* locus was 33.8 cM long, which is about one-third of the length of a consensus genetic map of chromosomes 4B and 4D in *T. aestivum* (Dvořák et al. 1995). In spite of this overall reduction in the length of the 4D/4B map, recombination was detected in all intervals, except for only a few short ones, in which recombination occurred between the 4D or 4B homologues. Detailed comparisons of the 4D/4B map with the 4B-4D consensus map showed that homoeologous recombination between 4D and 4B was disproportionately lower in the short arm than in the long arm and that in the long arm, the frequencies were disproportionately lower in the proximal region than in the distal region (Dvořák et al. 1995). Potential reasons for these observations are discussed in Dvořák et al. (1995). Keeping these quantitative differences in mind, the present results suggest that recombination between wheat and alien chromosomes in the absence of *Ph1* may be expected in any chromosome segment where recombination is observed between homologous chromosomes, provided that there are no structural differences between the chromosomes.

Intra-arm double cross-overs are desirable in interspecific gene introgression by homoeologous recombination since they minimize the amount of the introgressed alien genetic material. All double cross-overs in the population of the 4D/4B chromosomes were in opposite arms; there were no double cross-overs within an arm. The structure of the chromosomes generated by homoeologous recombination between wheat chromosomes 4B and 4D dramatically contrasts with the structure of rice chromosomes found in backcross progeny of hybrids between *Oryza sativa* L. and *O. officinalis* Wall. (genomes AA) to *O. sativa* (genomes CC) (Jena et al. 1992). A number of short intercalated *O. officinalis* segments were found in the *O. sativa* chromosomes, indicating that frequent double cross-overs occurred between the chromosomes of the two species. Chromosome pairing in *Oryza* hybrids shows that *O. sa-*

*tiva* chromosomes are more differentiated from those of *O. officinalis* (Katayama 1965) than the wheat chromosomes 4B and 4D are differentiated from each other. The absence of double cross-overs between the 4B and 4D homoeologues but their unexpected presence between the *Oryza* homoeologues provides a strong support for the possibility, suggested by Jena et al. (1992), that some unconventional recombination mechanism operated in the rice hybrids.

The absence of a double cross-over within an arm in a sizable population of recombined chromosomes as investigated here illustrates the impracticality of searching for intercalated alien segments produced by homoeologous double cross-overs. More practical alternatives are either two sequential rounds of homoeologous recombination or the use of homologous recombination between two recombined chromosomes with single cross-overs on each side of the targeted locus (Sears 1981).

On the basis of the C-banding of *Kna1* and *kna1* chromosomes, Dvořák and Gorham (1992) concluded that *Kna1* is distal to the subterminal C-band in the long arm of metaphase chromosome 4D. RFLP mapping substantiated this and indicated that *Kna1* must be near the terminus of the metaphase chromosome and that this region of 4D shows a high frequency of homologous recombination. The subterminal C-band of chromosome 4D cosegregated with the group of 4 centromeric markers (*Xbcd1262*, *Xcdo1387*, *Xpsr39*, and *Xbcd1006*) and was completely linked to the centromere in the 4D/4B map (Fig. 1). While it is not known which of the centromeric markers is proximal and which is distal to the C-band, all other mapped markers commencing with *Xpsr914* must be distal to the C-band. On a map of *T. monococcum* chromosome 4A, *Xpsr914* was 6.6 cM from the centromere (Devos et al. 1995). In a consensus map of *T. aestivum* chromosomes 4B and 4D, *Xpsr914* was 13 cM from the centromere and 44 cM from the distal marker *β-Amy-1* (Dvořák et al. 1995). While the region terminus–subterminal C-band is only 25% of the metaphase chromosome arm length, it comprises a minimum of 77% of the length of the linkage map of the 4DL arm. Clearly, chromosome 4D shows the same distortion of the linkage map relative to the metaphase chromosome map as chromosomes of the B genome, as was shown first for chromosome 6B (Dvorak and Chen 1984) and recently for all B-genome chromosomes (Lukaszewski and Curtis 1993). A corollary of these findings is that *Kna1* is in a high recombination region of the 4DL arm. This information is important for designing *Kna1* cloning strategies.

In a previous analysis, the arm involved in each cross-over was inferred by C-banding analysis (Dvořák and Gorham 1992). With the banding technique employed, parental chromosome 4B shows a large terminal C-band in the long arm, whereas chromosome 4D shows no terminal C-band. The C-banding and RFLP analyses were in agreement with respect to the arm location of cross-over points for 34 4D/4B chromosomes, but disagreed with respect to the chromosomes in families 17, 91, 124, and 152, all with the 4B centromere. Dvořák and Gorham (1992) reported that the chromosomes did not have the 4B terminal C-band in families 17 and 91 and concluded that the cross-overs had to be in the long arms of these chromosome. They also reported that the chromosomes had the 4B terminal C-band in families 124 and 152 and concluded that the cross-overs had to be in the short arms of these chromosomes. However, the RFLP analysis showed that a cross-over was in the short arm of chromosome 17, no cross-over was in chromosome 91, a double cross-over was in chromosome 124, and a single cross-over was in the long arm of chromosome 152 (Fig. 1). Reinvestigation of C-banding showed that, consistent with the RFLP analysis, a normally sized terminal C-band was in the long arm of chromosome 17 and, consequently, that the chromosome had an unrecombined 4BL arm. No terminal C-band was in chromosome 152. Hence, the chromosome acquired a single cross-over in the long arm. The reinvestigation of C-banding showed that the remaining 2 chromosomes, 91 and 124, both had a terminal C-band of a reduced size. Detailed cytogenetic work is needed to clarify the origin of these C-bands.

If it is assumed that 4D does not pair with 4B in the *Ph1ph1* background, a cross-over would have to be assigned to the short arm in a chromosome when a chromosome has the 4B centromere, the 4B telomeric C-band in the long arm, and pairs with chromosome 4D. In families 68 and 151, the putative 4D/4B chromosome showed these attributes; they paired with chromosome 4D in 11% and 3% of the cells, respectively (Dvořák and Gorham 1992). The RFLP analysis showed that neither of these chromosomes were recombined in the *Xpsr921–Xabg601.2* interval. The observed pairing can be explained by a cross-over between *Xpsr921* and the terminus of the short arm that resulted in a transfer of a short terminal segment of 4D onto chromosome 4B. It is equally likely, however, that the observed pairing of chromosomes 68 and 151 with 4D could be a residual pairing between 4D and 4B in the *Ph1ph1* background. The frequency of this residual pairing was estimated to be 0.9% of the pollen mother cells (Dvořák et al. 1995).

Classification of the families into *Kna1* or *kna1* classes on the basis of $K^+/Na^+$ discrimination in the greenhouse-grown plants was used to determinate the position of *Kna1* on the map. *Kna1* was mapped 14.1 cM from the centromere in the long arm and was found to be completely linked to 5 RFLP markers on the 4D/4B map. That *Kna1* behaves as a single locus and is in the long arm agrees with the previous conclusion of Dvořák and Gorham (1992).

On a consensus map of the 4B and 4D chromosomes, marker *Xpsr375*, which is completely linked to *Kna1*, was 47 cM from the centromere and 10 cM from the distal marker *β-Amy-1* (Dvořák et al. 1995). Since it is not known whether *Kna1* is between *Xmwg2112* and the 5 markers, among them, or between them and *β-Amy-1*, it is best to assume that the position of *Kna1* in 4D is delimited by *Xmwg2112* on the proximal side and *β-Amy-1* on the distal side. The 2 markers were simultaneously mapped in *T. monococcum* where they were 21.1 cM apart (Devos et al. 1995).

The determination of the genotype at the *Knal* locus and the cross-over position in each recombined chromosome will facilitate further manipulation of *Knal* in durum wheat, such as the removal of the distal part of the 4D segment. This is important practically since genes on 4D have been shown to be detrimental to yield in durum wheat (Dvořák et al. 1994). Because of a strong segregation distortion operating against 4D in the durum genetic background (Dvořák et al. 1995), only 5 recombinant chromosomes with the 4D centromere were found among 37 4D/4B chromosomes obtained. Since none of them had a cross-over distal to the *Knal* locus (Fig. 1), the method of generating a chromosome with an intercalated alien segment by homologous recombination between two *Knal* chromosomes, one with the 4B centromere and a cross-over proximal to *Knal* and the other with the 4D centromere and a cross-over distal to *Knal*, could not be used. As an alternative, an intercalated segment can be generated by a second round of homoeologous recombination induced by the *ph1c* mutation.

QTL analysis placed the $K^+/Na^+$ discrimination in a region of the chromosome similar to that where *Knal* was mapped as a qualitative trait. Because of a large difference between the $K^+/Na^+$ ratios in the leaves of young greenhouse-grown, salt-stressed *Knal* and *knal* genotypes, the QTL analysis of greenhouse data was clear-cut. No other factor influencing $K^+/Na^+$ discrimination in the 4B/4D chromosome pair, except for the *Knal* locus, was detected. A similar analysis using the field data was only marginally significant, although it was based on a trial of 26 families with three replications at each salinity level (Dvořák et al. 1994). The association found here between the $K^+/Na^+$ ratios and the chromosome region involving the *Knal* locus was weaker, possibly because the division between the *Knal* and *knal* genotypes in the field was less clear-cut and the fact that ion concentrations were analyzed in the flag leaves of adult plants. This analysis also did not reveal any other chromosome region, except for *Knal*, influencing the $K^+/Na^+$ ratio in this chromosome pair. In the control plants (low salinity), the *Knal* locus had no effect on the $K^+/Na^+$ ratio, but a minor effect was associated with the *Xpsr922* and *Xcdo669* loci. This effect was associated with the 4D alleles. Since only 2 of the 26 families had this 4D region this result must be considered with caution. The QTL analysis of the field data showed that it will be difficult to select unequivocally *Knal* genotypes in segregating populations in the field and that the use of linked markers, such as the 5 RFLP markers identified here, is essential for the deployment of *Knal* in breeding programs.

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