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Molecular characterization of a set of wheat deletion stocks for use in chromosome bin mapping of ESTs

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Abstract The objective of this study was molecular characterization of a set of deletion stocks and other aneuploids for use in chromosome bin mapping of ESTs in wheat. Wheat aneuploid stocks including 21 nullisomic-tetrasomic (NT), 24 ditelosomic (Dt), and 101 deletion (del) lines were screened with 526 EST clones. A total of 1,951 loci were detected by 493 informative EST clones and tagged 150 of the 159 deletion intervals or chromosome bins. Previously described deletion lines del1AS-4, del6AL-2, del6BS-6, and del7DS-6 were found to have normal chromosome constitution. The short arm deletion in del3AS-3 may be translocated from an unknown chromosome as this stock is nullisomic for the 3AS arm. Thirty-five new deletions were detected in 26 lines. Most of the new deletions occurred in terminal regions of chromosomes and probably resulted from the loss of very small terminal fragments that were difficult to detect cytologically. Eleven chromosome aberrations were also detected in two NT and five Dt lines. Overall, the chromosome bin map provides a resolution of around 28 Mb for an anchor map of a basic set of seven chromosomes of the Triticeae. Any target gene can be allocated to a specific 28-Mb bin and associated ESTs, anchored to the other Triticeae/grass maps including rice and, therefore, amenable to molecular cloning by comparative and wheat-based positional cloning methods.

Keywords *Triticum aestivum* · Deletion lines · EST · Genomics

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Introduction

In the classical genetics literature, the terms deficiency or deletion have been used interchangeably to denote the loss of a chromosome segment, although Muller (1940) had suggested that the term deletion should be used for the loss of an internal segment and deficiency for the loss of a terminal segment. Chromosomes suffering loss are termed deficient or deletion chromosomes. The deficiency of an internal segment requires two breaks, followed by the loss of an acentric fragment and the rejoining of broken ends. Terminal segment deficiencies require one break, the loss of a distal segment, and healing of the broken end by the de novo addition of a telomere. Deficiencies were first reported in *Drosophila* and corn based on genetic and cytological tests (Bridges 1917; McClintock 1931; Mackensen 1935; see Burnham 1972 for an extensive review of the literature). Deficiencies were experimentally produced by cytogeneticists for physical mapping of genes on chromosomes by X-irradiation of pollen or sperm of wild-type males mated to recessive females. The chromosome constitution of rare F_1 individuals showing the recessive phenotype was analyzed to determine the nature (interstitial or terminal), size, and identity of the chromosome involved in the deficiency (McClintock 1931; Mackensen 1935; Khush and Rick 1968). McClintock (1941) used reverse tandem duplication to generate dicentric chromosomes that underwent break-fusion-bridge cycles to produce deficient chromosomes. In the vast majority of cases, only first-generation deficiency heterozygotes are available for analysis, as deficient chromosomes are not transmitted to the progeny. Smaller deficiencies may be transmitted through the female, and deficient chromosomes can be maintained as deficiency heterozygotes. However, mutants homozygous for a small deficiency involving a single gene or part of a gene can be isolated readily, and fast-neutron-produced deficiencies are poised to play a major role in plant functional genomics (Li et al. 2001).

In bread wheat, Endo (1988, 1990) discovered that certain *Aegilops* chromosomes readily induce chromo-

some deletions when introduced as monosomic additions into wheat. Chromosome breakage occurs in the gametophytes lacking the alien chromosome prior to the S phase of the first mitotic division (Nasuda et al. 1998). Most breaks involve the loss of terminal segments, and broken ends are healed by the additions of new telomeres (Werner et al. 1992a; Tsujimoto 1993; Friebe et al. 2001). Because the bread wheat genome is highly buffered due to polyploidy, deletion chromosomes can be isolated in homozygous condition. Some plants can be homozygous for up to four independent deletions. Endo and Gill (1996) reported on the isolation of 436 deletions, and these have been extensively used in molecular mapping of the wheat genome (Werner et al. 1992b; Kota et al. 1993; Gill et al. 1993, 1996a, b; Hohmann et al. 1994; Delaney et al. 1995a, b; Mickelson-Young et al. 1995; Faris et al. 2000; Sarma et al. 2000; Weng et al. 2000; Qi and Gill 2001). Deletion mapping uncovered highly recombinogenic gene-rich regions at the distal regions of wheat chromosomes. Gene density in these regions is comparable to that of rice (Gill et al. 1996a, b; Feuillet and Keller 1999). Based on map position or through genome-wide sequencing, gene-rich regions are candidates for cloning useful genes, most of which are specific to wheat and have made wheat the world's most important crop plant.

Recently, the United States and collaborating international wheat geneticists initiated a large project on the structure and function of the expressed portion of the wheat genome. A description of the project, progress reports, and other pertinent information can be found on the Internet at http://wheat.pw.usda.gov/NSF. The major goal of the project is to identify a wheat unigene set by EST (expressed sequence tags) analysis and map the unigene set into chromosome bins defined by deletion stocks. For chromosome bin mapping, we have selected a set of wheat aneuploids and deletion stocks. The description and molecular characterization of this set of stocks and chromosome bins are reported in this paper.

Materials and methods

Genetic stocks

All genetic stocks selected for EST mapping are listed in Tables 1, 2, and 3. The set includes 21 nullisomic-tetrasomic (NT) lines, 24 ditelosomic (Dt) lines (Sears 1954, 1966; Sears and Sears 1978), 74 single-deletion lines (one chromosome has a terminal deletion), 21 double-deletion lines (two different chromosomes have terminal deletions), and 6 triple-deletion lines (three different chromosomes have terminal deletions; Endo and Gill 1996). Most of the genetic stocks were developed in the genetic background of Chinese Spring wheat (CS), except for N7DT7B and Dt4AL which are in the background of varieties other than CS (Sears 1954, 1966; Sears and Sears 1978). RFLP analysis revealed polymorphism between N6DT6B, Dt2DL and Dt7BL and CS (Devos et al. 1999). All the genetic stocks are maintained at the Wheat Genetics Resource Center (WGRC), Department of Plant Pathology, Kansas State University, Manhattan, Kansas, United States of America.

Table 1 Nullisomic-tetrasomic(NT) and ditelosomic (Dt) lines of Chinese Spring used in the study (*TA* Triticeae accession number)

TA no.	Genetic stock
3258	N1AT1D
3260	N1BT1D
3262	N1DT1B
3263	M2AT2B
3266	N2BT2D
3267	N2DT2A
3270	N3AT3D
3272	N3BT3D
3274	N3DT3B
3278	N4AT4D
3276	M4BT4D
3279	N4DT4B
3063	N5AT5D
3065	N5BT5D
3067	N5DT5B
3152	N6AT6B
3154	N6BT6A
3157	N6DT6B
3281	N7AT7D
3284	N7BT7D
3286	N7DT7B
3113	Dt1BL
3087	Dt1DS
3131	Dt1DL
3103	Dt2AS
3114	Dt2BL
3123	Dt2DS
3124	Dt2DL
3116	Dt3BL
3193	Dt3DS
3192	Dt3DL
3086	Dt4AS
3117	Dt4AL
3106	Dt4BS
3125	Dt4DS
3126	Dt4DL
3107	Dt5AL
3118	Dt5BL
3127	Dt5DL
3108	Dt6AS
3120	Dt6BL
3128	Dt6DS
3129	Dt6DL
3122	Dt7BL
3130	Dt7DS

RFLP analysis

Procedures used for genomic DNA isolation, restriction endonuclease digestion, gel electrophoresis, and DNA gel blot hybridization were as described in Faris et al. (2000) with modifications (for detailed protocols, see project website). Twenty micrograms genomic DNA digested with *Eco*RI was electrophoresed in 0.8% agarose gel in 1× Tris-borate-EDTA (TBE). As a size marker, 150 ng lambda DNA digested with *Hin*dIII and *Bst*EII was used. Pre-hybridization was for 12–16 h at 65°C in 50 ml solution containing 6× SSPE (0.9 M NaCl, 0.6 M NaH_2PO_4), 5× Denhardt's solution [0.1% Ficoll, 1 mg/ml BSA, 1 mg/ml polyvinylpyrrolidone (PVP), 0.05 mg/ml salmon sperm DNA, and 0.5% sodium dodecyl sulfate (SDS)]. The pre-hybridization solution was replaced with 10 ml hybridization solution (6× SSPE, 5× Denhardt's solution, 0.05 mg/ml salmon sperm DNA, 0.5% SDS, and 20% dextran sulfate). The membranes were washed in 2× SSPE, 1× SSPE, and 0.5× SSPE plus 0.5% SDS for 30 min at 65°C each.

The Collaborations in Wheat Genomics Project funded by the United States National Science Foundation have contributed various cDNA libraries of *Triticum aestivum* and are identifying EST singletons (http://wheat.pw.usda.gov/NSF); 526 EST clones from this project were used for characterization of the deletion lines.

Table 2 Single-deletion lines and fraction length (*FL*) values. All lines are homozygous unless otherwise indicated. Data were taken from Endo and Gill (1996)

TA no.	Deletion	FL value	Note
4510L3	1AS-3	0.86	
4510L1	1AS-1	0.47	
4511L1	1AL-1	0.17	
4511L3	1AL-3	0.61	
4512L9	1BS-9	0.84	
4512L10	1BS-10	0.50	
4513L6	1BL-6	0.32	
4513L1	1BL-1	0.47	
4514L5	1DS-5	0.70	
4514L1	1DS-1	0.59	
4515L4	1DL-4	0.18	
4515L2	1DL-2	0.41	Hemizygous
4515L5	1DL-5	0.70	Hemizygous
4516L5	2AS-5	0.78	
4517L1	2AL-1	0.85	
4518L3	2BS-3	0.75	
4518L1	2BS-1	0.53	
4520L5	2DS-5	0.47	
4520L1	2DS-1	0.33	
4521L3	2DL-3	0.49	
4521L9	2DL-9	0.76	
4522L3	3AS-3	0.71	
4522L4	3AS-4	0.45	
4522L2	3AS-2	0.23	
4523L3	3AL-3	0.42	
4523L5	3AL-5	0.78	
4524L1	3BS-1	0.33	
4525L2	3BL-2	0.22	
4525L10	3BL-10	0.50	
4525L7	3BL-7	0.63	
4527L3	3DL-3	0.81	
4528L1	4AS-1	0.20	
4529L12	4AL-12	0.43	
4529L5	4AL-5	0.66	
4530L1	4BS-1	0.81	1''[4BS-1] + t'[4BS]
4530L8	4BS-8	0.57	1''[4BS-8] +1' [4BL-11]
4530L4	4BS-4	0.37	1''[4BS-4] +1'[4B]
4531L1	4BL-1	0.86	
4532L3	4DS-3	0.67	
4532L1	4DS-1	0.53	
4533L13	4DL-13	0.56	
4534L3	5AS-3	0.75	
4535L12	5AL-12	0.35	Hemizygous
4535L10	5AL-10	0.57	
4535L17	5AL-17	0.78	
4535L23	5AL-23	0.87	
4536L6	5BS-6	0.81	
4536L5	5BS-5	0.71	
4536L8	5BS-8	0.56	
4537L6	5BL-6	0.29	Hemizygous
4537L1	5BL-1	0.55	Hemizygous
4537L14	5BL-14	0.75	
4537L9	5BL-9	0.76	
4537L16	5BL-16	0.79	
4538L2	5DS-2	0.78	
4538L5	5DS-5	0.67	
4538L1	5DS-1	0.63	
4539L1	5DL-1	0.60	
4539L5	5DL-5	0.76	
4541L2	6AL-2	0.89	
4542L2	6BS-2	1.05	
4542L6	6BS-6	1.02	
4542L5	6BS-5	0.76	
4543L5	6BL-5	0.40	
4543L6	6BL-6[a]	0.79	
4544L2	6DS-2	0.45	
4545L6	6DL-6	0.29	
4511L5	7AS-5	0.59	
4547L1	7AL-1	0.39	
4548L1	7BS-1	0.27	
4551L4	7DS-4	0.61	
4551L5	7DS-5	0.36	Hemizygous
4550L5	7DL-5	0.30	
4550L2	7DL-2	0.61	

[a] Del6BL-6 has an interstitial deletion in 6BL

Fraction length value and bin assignments

The fraction length (FL) value in a given deletion line identifies the breakpoint in the deleted chromosome and the length of the remaining chromosome arm from the centromere relative to the length of the complete arm. Calculation of FL value was described in detail by Endo and Gill (1996).

The bin assignment in this study indicates the physical location of each deletion interval in a chromosome according to the FL values. Figure 1 gives an example of bin assignment and localization of an EST clone to a chromosome bin. The deletion lines del3BL-7, del3BL-10, and del3BL-2 are missing 37%, 50%, and 78% of terminal segments of the 3BL arm, respectively. These deletions divide the long arm of chromosome 3B into four chromosome bins. The bin C-3BL2–0.22, in which C represents the centromere, and the last number is the FL value of del3BL-2, is located between the centromere and first deletion 3BL-2. Another bin, 3BL2–0.22–0.50, is located in an interval between del3BL-2 to del3BL-10. The two numbers in the bin are FL values of del3BL-2 and del3BL-10 respectively. In Fig. 1a, a 3BL fragment detected by EST clone KSU019BE445011 was missing in del3BL-2 and del3BL-10, as well as in N3BT3D, but was present in del3BL-7. Thus, the EST was mapped to the bin 3BL10–0.50–0.63 (Fig. 1c).

Results

The 101 deletion lines selected for this study divide the 21 chromosomes of wheat into 159 deletion intervals, i.e. 159 chromosome bins. An average of 7.6 bins is assigned to each chromosome, which allows the mapping of EST loci to any possible chromosome region. A total of 526 EST clones were hybridized to an array of nullisomic-tetrasomic, ditelosomic, and deletion lines; 493 of these clones were informative. A total of 2,413 restriction fragments were detected, and 1,951 loci were assigned to 150 of the 159 chromosome bins and tagged with molecular markers. Six hundred and forty-one loci were mapped to the A genome, 679 to the B genome, and 631 to the D genome. This degree of high-resolution mapping allows a detailed molecular characterization of these genetic stocks.

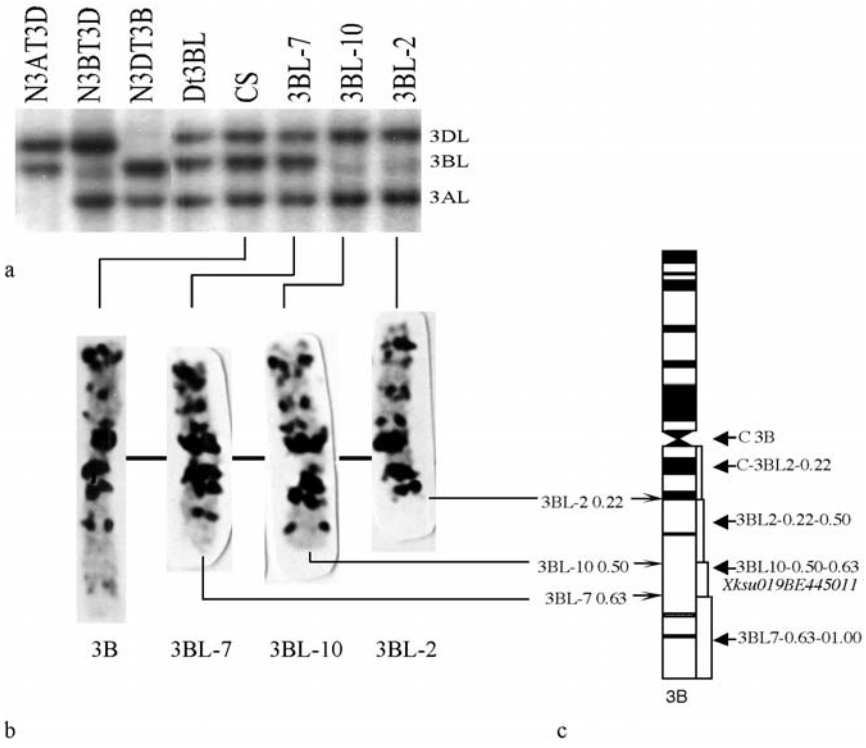


Fig. 1a An autoradiograph of Southern hybridization of EST KSU019BE445011 to genomic DNAs digested with *Eco*RI of nullisomic-tetrasomic (*NT*), ditelosomic (*Dt*), and deletion (*del*) lines of group 3, and Chinese Spring (*CS*) control. Three restriction fragments were assigned to the long arms of group-3 chromosomes. A 3BL fragment was missing in N3BT3D, del3BL-10, and del3BL-2, but present in del3BL-7. The EST was mapped to the bin 3BL10–0.50–0.63. **b** C-banded chromosomes of 3B, deleted 3BL-7, 3BL-10, and 3BL-2, which were provided by Dr. Endo. **c** The idiogram of the C-banded 3B chromosome indicates the breakpoints of deletion lines, bin assignments and one mapped EST. The C-banding pattern of 3B chromosome is taken from Gill et al. (1991)

Table 3 Double- and triple-deletion lines and fraction length (*FL*) values. All lines are homozygous unless otherwise indicated. Data were taken from Endo and Gill (1996)

TA no.	Primary deletion	FL value	Note	Secondary deletion	FL value	Note	Tertiary deletion	FL value	Note
4510L4	1AS-4	0.76		6BL-3	0.36				
4519L2	2BL-2	0.36		7AS-1	0.89				
4519L6	2BL-6	0.89		5AS-6	0.97	5AS-6+5A			
4524L9	3BS-9	0.57		1BS-4	0.52				
4528L4	4AS-4	0.63		6DL-10	0.80				
4529L13	4AL-13	0.59		7AL-21	0.74				
4529L4	4AL-4	0.80		6DL-1	0.47				
4531L5	4BL-5	0.71		6AL-4	0.55				
4533L9	4DL-9	0.31		1BS-18	0.50[b]				
4534L7	5AS-7	0.98		6AS-5	0.65				
4536L4	5BS-4	0.43		7AS-1	0.89				
4539L7	5DL-7	0.29	5DL-7+5D	5AS-1	0.40				
4539L9	5DL-9	0.74		4DL-12	0.71				
4540L1	6AS-1	0.35		1AS-5	0.20				
4542L3	6BS-3	1.05		6DL-12	0.68				
4544L6	6DS-6	0.99		3DS-7	0.30				
4544L4	6DS-4	0.79		1BS-19	0.31[b]				
4546L8	7AS-8[a]	0.45		7AL-17[a]	0.71				
4549L7	7BL-7	0.63		1DS-3	0.48				
4551L6	7DS-6	0.73		7BL-2	0.33				
4550L3	7DL-3	0.82		1BS-2	1.06				
4518L4	2BS-4[a]	0.84		2BL-4[a]	0.50		3DL-2	0.27	
4524L8	3BS-8	0.78		4AS-3	0.76		7BL-10	0.78	
4526L6	3DS-6	0.55		4AL-11	0.66		7AL-18	0.90	
4526L3	3DS-3	0.24		5AS-8	1.00		7AL-16	0.86	
4534L10	5AS-10	1.00		1BL-3	0.85		6DL-11	0.74	
4541L8	6AL-8	0.90		1BL-2	0.69		4DS-2	0.82	

[a] Two deletions in one chromosome
[b] The deletion breakpoint is located in the satellite of 1BS arm

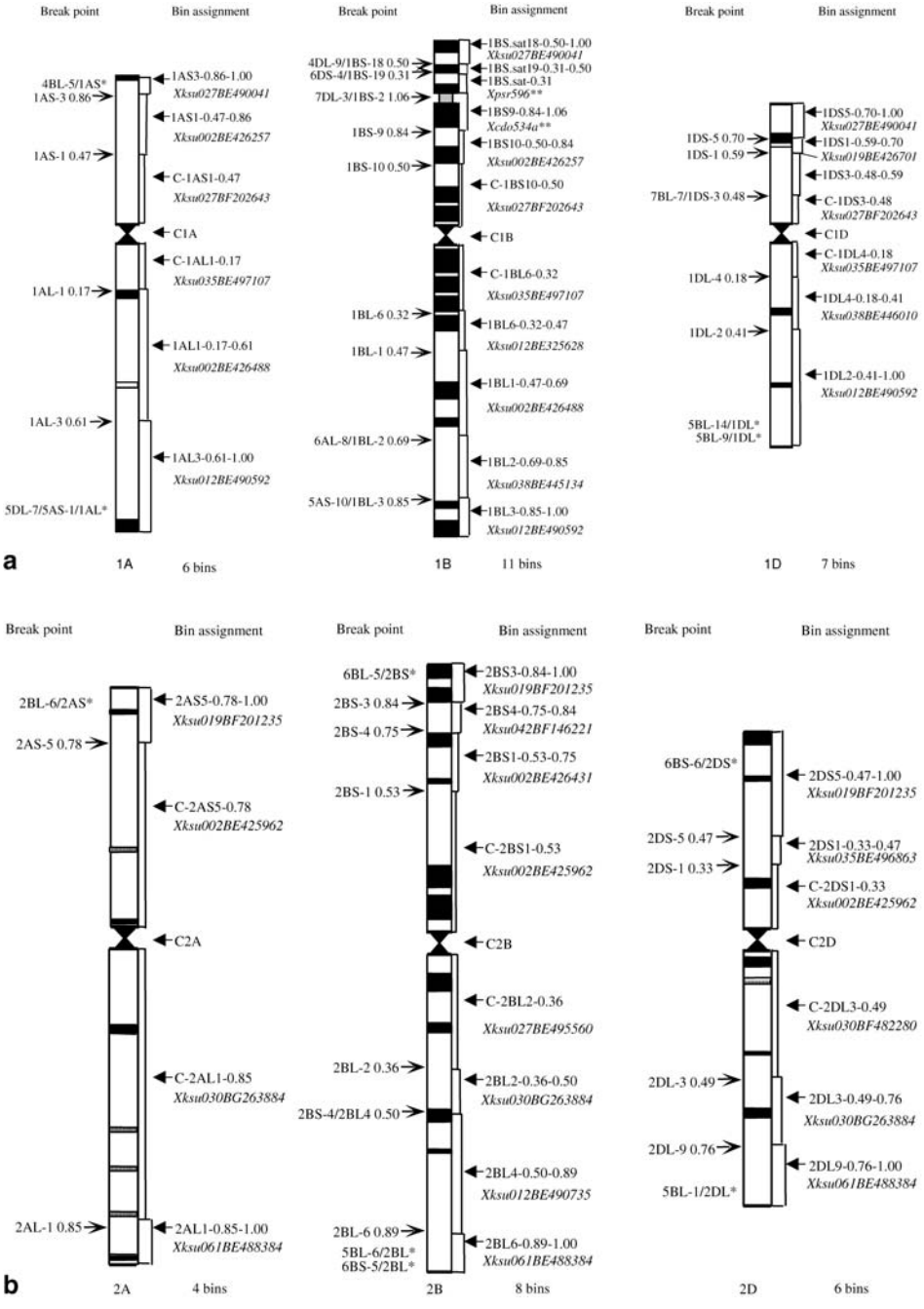


Fig. 2a–g The partitioning of the 21 wheat (*Triticum aestivum*, cv Chinese Spring) chromosomes into bins based on deletion breakpoints. The name of deletion lines and FL (fraction length) values of breakpoints are indicated on the *left*, and the bin assignments and diagnostic EST markers on the *right*. The C-banding patterns of chromosomes are taken from Gill et al. (1991). Wheat chromosome bin maps can be accessed at http://wheat.pw.usda.gov/NSF. *Single asterisk* new deletion detected in the present study, *double asterisk* RFLP markers selected from published reports (Gill et al. 1996b; Qi and Gill 2001)

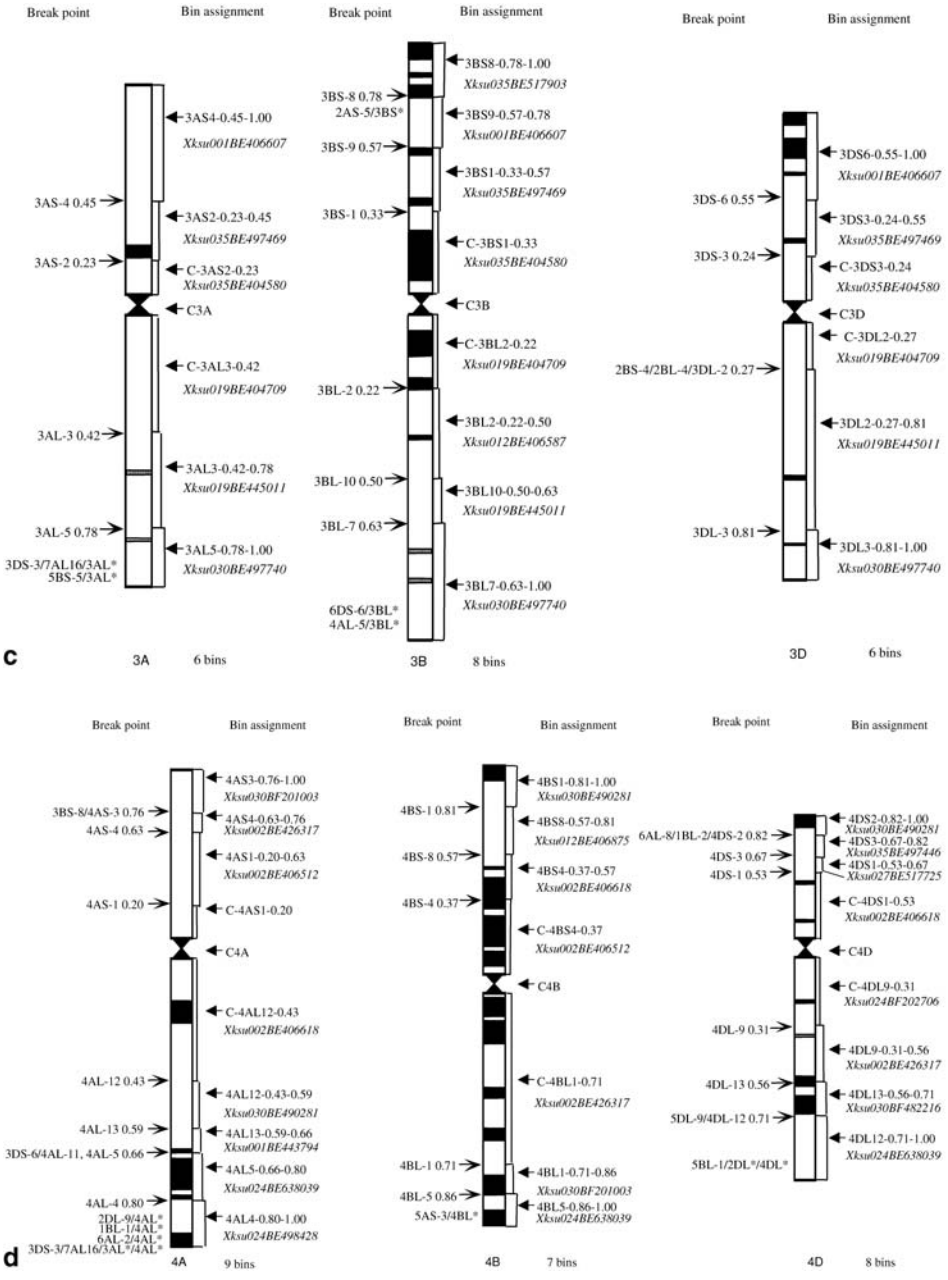
Molecular genetic characterization of previously described deletion lines

Of the 74 single-deletion lines, del3AS-3, which has a distal terminal deletion (FL 0.71), is actually nullisomic for the entire 3AS arm. All RFLP fragments assigned to the group-3 short arms were missing in del3AS-3. The short arm of 3AS-3 could be translocated from an unknown chromosome. Lines del6AL-2 and del6BS–6 have normal 6A and 6B chromosomes, respectively. Of 22 EST clones mapping to the distal region of 6AL, none were missing in del6AL-2. Of the 9 EST clones mapping to the distal region of 6BS, none were missing in del6BS-6. N- and C-banding analysis also confirmed the presence of normal chromosomes 6A and 6B in these two lines. An interstitial deletion was confirmed in del6BL-6 as was mentioned in Endo and Gill (1996).

Among the 21 double-deletion lines (Table 3) analyzed, the primary deletion 1AS-4 has a complete 1AS arm. Sixteen EST clones were mapped to the distal 1AS arm; all were present in del1AS-4. A complete 7DS is present in the primary deletion 7DS-6. None of the group-7 EST clones, some mapping to the distal 11% of the arm, detected missing fragments in del7DS-6.

Seventeen secondary deletions in 21 double-deletion lines previously described by Endo and Gill (1996) were

Fig. 2a–g (continued)



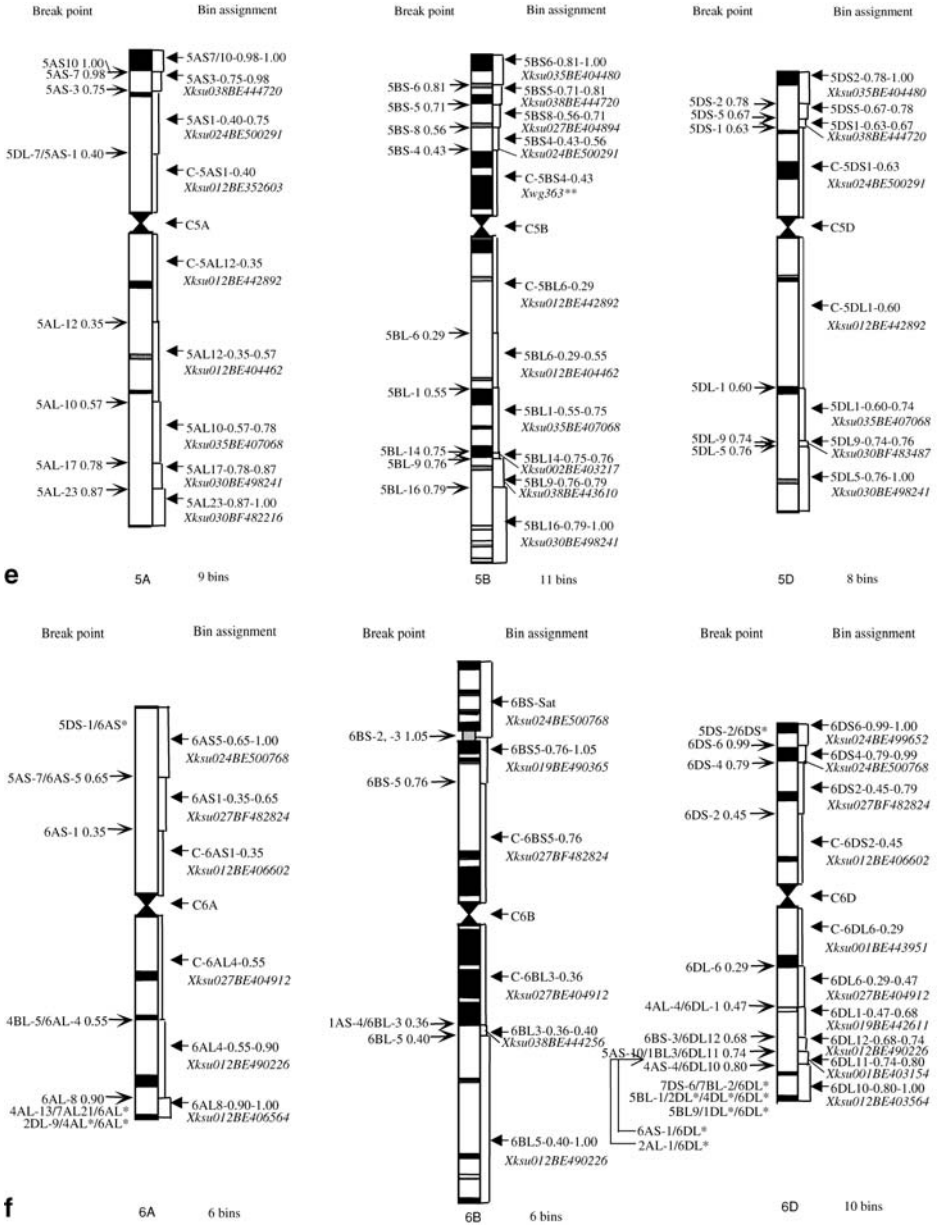
confirmed in this study. The secondary deletion 5AS-6 (FL 0.97) was not detected because no EST clone used in this study mapped distal to del5AS-6. Three secondary deletions, del1BS-4, del1AS-5 and del3DS-7 are reported as proximal deletions, but clones mapping to distal regions of the corresponding chromosome arms did not detect specific fragments missing in these deletion lines. These deletions are identified incorrectly.

In the six triple-deletion lines, all primary, secondary and tertiary deletions were confirmed except for del5AS-8. Similar to the secondary deletion del5AS-6, del5AS-8 (FL 1.00) was not detected because it either is the smallest deletion or has a normal 5AS arm.

We found two cases of discrepancy between cytological and mapping data. For del4BL-5 (FL 0.71) and del4BL-1 (FL 0.86), our mapping data suggest the revision of the FL values of these two lines. Most ESTs mapped to the distal region of 4BL detected the 4BL fragments missing in both lines. However, three ESTs, KSU019BE497134, KSU030BF201003, and KSU063BE443004, mapped in our lab., and six ESTs mapped in other labs. of the NSF project, detected the 4BL specific fragments missing in del4BL-1, but present in del4BL-5 (Fig. 2; http://wheat.pw.usda.gov/NSF). This result suggests that the deleted fragment in del4BL-1 is larger than that in del4BL-5, thus, the FL values are changed accordingly: del4BL-1 FL 0.71 and del4BL–5 FL 0.86 (Fig. 2).

A similar case was also found in two deletion lines del2BS-3 (FL 0.75) and del2BS-4 (FL 0.84).

Fig. 2a–g (continued)



Three ESTs, KSU042BF146221, UNL054BG274305, and UMC085BG275030, detected the specific 2BS fragments missing in 2BS4, but present in 2BS3 (Fig. 2) indicating that the deleted fragment in del2BS-4 is larger than that in del2BS-3. The FL values are changed accordingly: del2BS4 FL 0.75, and 2BS3 FL0.84 (Fig. 2).

The 4BS homozygous deletion lines are male sterile, because the gene controlling male fertility is located in the distal region of the 4B short arm (Sears and Sears 1978; Endo et al. 1991). These lines are normally maintained as segregating populations by crossing with either normal CS or Dt4BS. Six lines, del1DL-2, del1DL-5, del5AL-12, del5BL-1, del5BL-6, and del7DS-5, are hemizygous deletions. Del5DL-7 has an extra 5D chromosome. Pre-selection of these lines by either cytogenetic or molecular marker analyses is necessary. In the present study, we failed to select any plants with a deleted chromosome of 1DL-5. All chromosome 1D-specific fragments for both arms are missing in del1DL-5, indicating that this line is nullisomic for 1D. Plants with del5DL-7 but lacking 5D were not found in the progenies analyzed.

All available deletion lines identified in the study are presented in Fig. 2. A total of 150 diagnostic ESTs selected from 493 ESTs mapped were assigned to each chromosome bin. The images and data of all mapped ESTs in the present study are available at http://wheat.pw.usda.gov/NSF.

New deletions in lines with known deletions

In the known deletion stocks 66 new deletions were found, of which 31 putative deletions were detected with

Fig. 2a–g (continued)

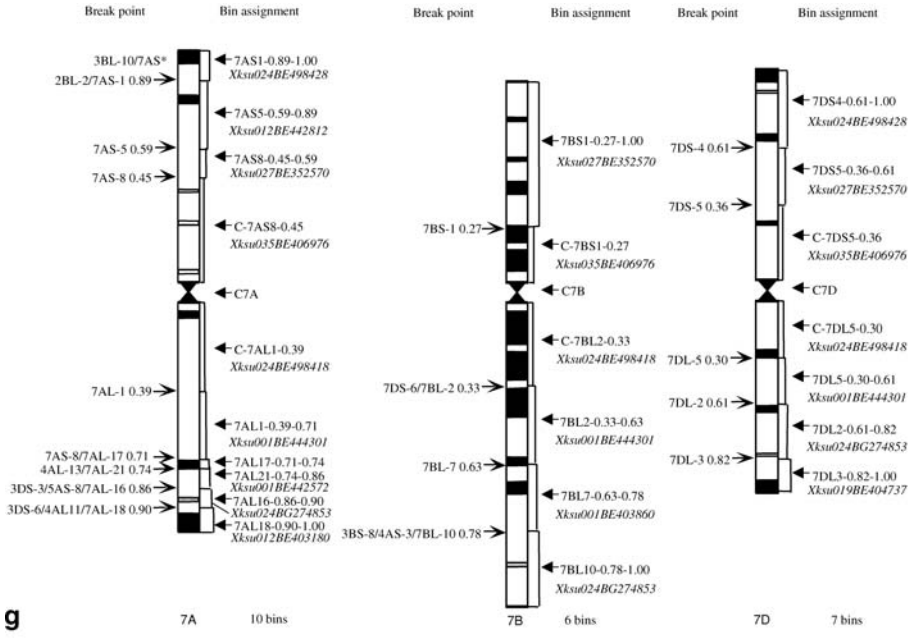


Table 4 Putative new deletions in known deletion lines detected by only one EST clone

Stock	Putative new deletion	EST clone detecting new deletion	Ratio[a]	Location (bin) of EST clone
TA4539L7 5DL-7	1AS	KSU012BE403153	1/10	1AS1–0.47–0.86
TA4542L6 6BS-6	1AS	KSU030BE606926	1/16	1AS3–0.86–1.00
TA4534L3 5AS-3	1AL	KSU027BE590822	1/16	1AL3–0.61–1.00
TA4519L2 2BL-2	1BL	KSU001BE443720	1/12	1BL2–0.69–0.83
TA4541L2 6AL-2	1BL	KSU030BE637473	1/13	1BL1–0.47–0.69
TA4524L8 3BS-8	1DS	KSU027BE517941	1/19	1DS5–0.70–1.00
TA4537L6 5BL-6	1DL	KSU019BE490584	1/44	1DL2–0.41–1.00
TA4537L6 5BL-6	2AS	KSU001BE443747	1/16	2AS5–0.78–1.00
TA4537L1 5BL-1	2AS	KSU001BE443747	1/16	2AS5–0.78–1.00
TA4522L4 3AS-4	2AL	KSU001BE444596	1/5	2AL1–0.85–1.00
TA4522L2 3AS-2	2AL	KSU001BE444596	1/5	2AL1–0.85–1.00
TA4548L1 7BS-1	2BS	KSU001BE443747	1/10	2BS3–0.84–1.00
TA4551L4 7DS-4	2BS	KSU012BE443439	1/10	2BS3–0.84–1.00
TA4512L9 1BS-9	2BL	KSU001BE444596	1/9	2BL6–0.89–1.00
TA4537L6 5BL-6	2DS	KSU024BF484829	1/26	2DS5–0.47–1.00
TA4519L6 2BL-6	3BS	KSU001BE444576	1/14	3BS8–0.78–1.00
TA4510L3 1AS-3	3BL	KSU024BE499348	1/7	3BL2–0.22–0.50
TA4541L2 6AL-2	3BL	KSU024BE499348	1/10	3BL2–0.22–0.50
TA4537L14 5BL-14	5AL	KSU024BE499927	1/12	5AL23–0.87–1.00
TA4510L4 1AS-4	5DS	KSU012BE490728	1/4	5DS2–0.78–1.00
TA4537L6 5BL-6	5DS	KSU012BE490728	1/4	5DS2–0.78–1.00
TA4541L2 6AL-2	6AS	KSU019BE494189	1/12	6AS5–0.61–1.00
TA4510L4 1AS-4	6BS	KSU019BE494189	1/7	6BS–sat
TA4512L9 1BS-9	6DS	KSU012BE442721	1/3	6DS6–0.99–1.00
TA4535L12 5AL-12	6DS	KSU012BE442721	1/3	6DS6–0.99–1.00
TA4535L12 5AL-12	6DL	KSU012BE403950	1/9	6DL10–0.80–1.00
TA4510L4 1AS-4	7AL	KSU001BE444301	1/11	7AL1–0.39–0.71
TA4514L1 1DS-1	7BL	KSU027BE517627	1/12	7BL10–0.78–100
TA4530L8 4BS-8	7BL	KSU019BE404737	1/12	7BL10–0.78–1.00
TA4537L1 5BL-1	7BL	KSU019BE404737	1/12	7BL10–0.78–1.00
TA4537L9 5BL-9	7DL	KSU012BE404744	1/6	7DL3–0.82–1.00

[a] Ratio = number of the EST clones detecting new deletion/number of the EST clones mapped to specific bin

only one EST clone (Table 4). The absence of restriction fragments expected in these lines may result from either polymorphism or very small deletions and needs to be confirmed with additional markers. New deletions that were detected with at least two EST clones were found in 26 deletion lines (Table 5). Of these, 21 lines had one, and 3 lines had two new deletions. One new deletion and one translocation chromosome were identified in

Table 5 New deletions in known deletion lines detected by EST clones

Stock	New deletion	EST clone detecting new deletion	Ratio[a]	Location (bin) of EST clone
TA4531L5 4BL-5	1AS	KSU001BE399288 KSU002BE426715 KSU012BE406450 KSU012BE406438 KSU012BE442682 KSU024BE500104 KSU030BE606926 KSU035BE405834	8/16	1AS3–0.86–1.00
TA4539L7 5DL-7	1AL	KSU038BE446672 KSU019BE495150	2/14	1AL3–0.61–1.00
TA4537L14 5BL-14	1DL	KSU012BE490592 KSU019BE490584 KSU027BE590822 KSU038BE445134 KSU038BE446672	5/44	1DL2–0.41–1.00
TA4537L9 5BL-9	1DL	KSU012BE490592 KSU019BE490584 KSU027BE590822 KSU038BE445134	4/44	1DL2–0.41–1.00
TA4519L6 2BL-6	2AS	KSU001BE443747 KSU001BE444297	2/16	2AS5–0.78–1.00
TA4537L1 5BL-1	2AL	KSU001BE442858 KSU002BE403217 KSU012BE403556 KSU038BE444851	4/37	C–2AL1–85
TA4543L5 6BL-5	2BS	KSU001BE443747 KSU012BE490444 KSU012BE443439 KSU019BF201235 KSU027BE517877 KSU035BE496951	6/10	2BS3–0.84–1.00
TA4537L6 5BL-6	2BL	KSU001BE444596 KSU001BE444178 KSU035BE497600	3/9	2BL6–0.89–1.00
TA4542L5 6BS-5	2BL	KSU001BE444596 KSU001BE444178	2/9	2BL6–0.89–1.00
TA4542L6 6BS-6	2DS	KSU002BE425962 KSU002BE426158 KSU019BE443112 KSU030BE636802 KSU035BE403404 KSU035BE406923 KSU038BE444638 KSU038BE446243	8/11	C–2DS1–0.33
TA4542L6 6BS-6	2DS	KSU035BE496863 KSU035BE496900 KSU035BE497171	3/6	2DS1–0.33–0.47
TA4542L6 6BS-6	2DS	KSU002BE426431 KSU012BE406474 KSU012BE490444 KSU012BE406808 KSU012BE404385 KSU019BE518306 KSU019BE201235 KSU024BE499478 KSU024BE498252 KSU024BE499648 KSU024BF484829 KSU027BE517877 KSU035BE497622 KSU035BE496951	14/26	2DS5–0.47–1.00
TA4537L1 5BL-1	2DL	KSU001BE444596 KSU012BE404385 KSU038BE444894	3/16	2DL9–0.76–1.00

Table 5 (continued)

Stock	New deletion	EST clone detecting new deletion	Ratio[a]	Location (bin) of EST clone
TA4526L3 3DS-3	3AL	KSU012BE442818 KSU012BE442875 KSU024BE499186 KSU027BF482769 KSU030BE497740 KSU030BE499696 KSU030BE485004	7/20	3AL5–0.78–1.00
TA4536L5 5BS-5	3AL	KSU012BE442818 KSU012BE442875 KSU024BE499186 KSU027BF482769 KSU030BE497740 KSU030BE499696 KSU030BF485004	7/20	3AL5–0.78–1.00
TA4516L5 2AS-5	3BS	KSU035BE403373 KSU035BE403471	2/6	3BS9–0.57–0.78
TA4516L5 2AS-5	3BS	KSU002BE425222 KSU019BE422466 KSU035BE517903 KSU038BE446244	4/4	3BS8–0.78–1.00
TA4529L5 4AL-5	3BL	KSU019BE494450 KSU019BE442624	2/20	3BL7–0.63–1.00
TA4544L6 6DS-6	3BL	KSU012BE442875 KSU024BE499303 KSU024BE405348	3/20	3BL7–0.63–1.00
TA4521L9 2DL-9	4AL	KSU001BE443412 KSU001BE444470 KSU001BE442648 KSU012BE406897 KSU024BE498428 KSU024BE499049 KSU030BE490186 KSU035BE497624 KSU038BE443120	9/14	4AL4–0.80–1.00
TA4513L1 1BL-1	4AL	KSU001BE443412 KSU001BE444470 KSU001BE442648 KSU012BE406897 KSU024BE498428 KSU024BE499049 KSU030BE490186 KSU038BE443120	8/14	4AL4–0.80–1.00
TA4541L2 6AL-2	4AL	KSU001BE443412 KSU001BE444470 KSU001BE442648 KSU012BE406897 KSU024BE499049 KSU030BE490186 KSU038BE443120	7/14	4AL4–0.80–1.00
TA4526L3 3DS-3	4AL	KSU001BE444470 KSU030BE490186	2/14	4AL4–0.80–1.00
TA4534L3 5AS-3	4BL	KSU001BE444473 KSU027BE590745	2/24	4BL5–0.86–1.00
TA4537L1 5BL-1	4DL	KSU001BE444473 KSU001BE444421 KSU024BE499664 KSU024BE498038 KSU035BE406989 KSU035BE404665 KSU035BE403378	7/16	4DL13–0.56–0.71
TA4537L1 5BL-1	4DL	KSU024BE499546 KSU024BE499685	2/8	4DL12–0.71–1.00
TA4537L6 5BL-6	T5DS-5AS·5AL-5DL[b]	KSU001BE406368 KSU024BE500291 KSU024BE498878 KSU035BE403618 KSU035BE496951	5/8	C–5DS1–0.63

Table 5 (continued)

Stock	New deletion	EST clone detecting new deletion	Ratio[a]	Location (bin) of EST clone
TA4537L6 5BL-6	T5DS-5AS·5AL-5DL	KSU002BE406545 KSU012BE403556 KSU012BE403761 KSU012BE442892 KSU035BE406996	5/23	C–5DL1–0.60
TA4538L1 5DS-1	6AS	KSU024BE500768 KSU027BE495217	2/8	6AS5–0.61–1.00
TA4529L13 4AL-13	6AL	KSU001BE444363 KSU012BE403950 KSU012BE403564 KSU019BE498785 KSU035BE406840 KSU035BE497099 KSU038BE443643 KSU038BE442605	8/22	6AL8–0.90–1.00
TA4521L9 2DL-9	6AL	KSU001BE444363 KSU012BE403950 KSU012BE403564 KSU019BE498785 KSU035BE406840 KSU035BE497099 KSU038BE443643	7/22	6AL8–0.90–1.00
TA4538L2 5DS-2	6DS	KSU012BE442721 KSU027BE590879	2/3	6DS6–0.99–1.00
TA4540L1 6AS-1	6DL	KSU001BE403154 KSU002BE425207 KSU024BE498092	3/8	6DL11–0.74–0.80
TA4540L1 6AS-1	6DL	KSU001BE405507 KSU012BE403950 KSU012BE403564 KSU019BE498785 KSU024BE500840 KSU035BE404596 KSU038BE489323 KSU038BE443643 KSU038BE444279	9/9	6DL10–0.80–1.00
TA4517L1 2AL-1	6DL	KSU002BE425207	1/8	6DL11–0.74–0.80
TA4517L1 2AL-1	6DL	KSU001BE405507 KSU012BE403950 KSU012BE403564 KSU019BE498785 KSU024BE500840 KSU035BE404596 KSU038BE489323 KSU038BE443643 KSU038BE444279	9/9	6DL10–0.80–1.00
TA4551L6 7DS-6	6DL	KSU001BE405507 KSU012BE403950 KSU012BE403564 KSU024BE500840 KSU035BE404596 KSU038BE443643 KSU038BE444279	7/9	6DL10–0.80–1.00
TA4537L1 5BL-1	6DL	KSU001BE405507 KSU012BE403950 KSU012BE403564 KSU024BE500840 KSU038BE443643	5/9	6DL10–0.80–1.00
TA4537L9 5BL-9	6DL	KSU001BE405507 KSU012BE403950 KSU012BE403564	3/9	6DL10–0.80–1.00
TA4525L10 3BL-10	7AS	KSU012BE406897 KSU019BE422466 KSU024BE499049	3/16	7AS1–0.89–1.00

[a] Ratio = number of the EST clones detecting new deletion/number of the EST clones mapped to the specific bin
[b] Translocation chromosome

del5BL-6. Two new interstitial and three new distal deletions were observed in del5BL-1 (Table 5). Twenty chromosome arms are involved in the new deletions, including 1AS, 1AL, 1DL, 2AS, 2AL, 2BS, 2BL, 2DS, 2DL, 3AL, 3BS, 3BL, 4AL, 4BL, 4DL, 6AS, 6AL, 6DS, 6DL, and 7AS. Most of the new deletions were detected by EST clones mapping in distal chromosome regions. Not all clones mapping to a bin could detect each new deletion, however, indicating that the new deletions were different from known deletions (Table 5). Most of these deletions may have resulted from the loss of very small terminal fragments that are difficult to detect by cytological analysis. All new deletions in known deletion lines are described below. Most of these with terminal deletion are indicated with an asterisk in Fig. 2.

Homoeologous group 1

Four new deletions were detected in group-1 chromosomes and provide welcome breakpoints for previously poorly marked chromosome arms 1AL and 1DL.

Chromosome 1A

New terminal deletions were observed for 1AS in del4BL-5. Half of ESTs (8/16) mapped to the distal region of 1AS detected the 1AS fragments absent in this line. The del5DL-7 has a new, very small, distal deletion for 1AL. Two of the 14 ESTs mapping in bin 1AL3–0.61–1.00 detected this deletion.

Chromosome 1D

Two new terminal deletions for 1DL with different breakpoints are present in lines del5BL-14 and del5BL-9. The EST KSU038BE446672 can differentiate between these two lines. The 1DL fragment of KSU038BE446672 is absent in del5BL-14, but present in del5BL-9 (Table 5).

Homoeologous group 2

Nine new deletions, six terminal and three interstitial, were detected for group-2 chromosomes.

Chromosome 2A

Del2BL-6 has a new terminal deletion for 2AS. This deletion is assumed to have resulted from the loss of a very small piece from the terminal fragment of 2AS because only 2 of the 14 ESTs mapped to the distal region of 2AS detected the 2AS-specific fragments missing in this line (Table 5). An interstitial deletion for 2AL was found in del5BL-1.

Chromosome 2B

A new terminal deletion for 2BS is present in del6BL-5. New terminal deletions for 2BL were observed in both del5BL-6 and del6BS-5. The EST KSU035BE497600 can distinguish the different breakpoints of new deletions in these two lines (Table 5).

Chromosome 2D

Two interstitial deletions and one terminal deletion for 2DS are present in del6BS-6. EST clones mapping to three chromosome bins of 2DS detected the 2DS fragments absent in this line (Table 5). Because not all 2DS fragments are missing in del6BS-6, this line has a complex 2DS arm. A new terminal deletion for 2DL is present in del5BL-1.

Homoeologous group 3

Four new terminal deletions were detected in group-3 chromosomes.

Chromosome 3A

The deletion lines del3DS-3 and del5BS-5 have a new terminal deletion for 3AL. We infer that the same new deletion is present in both lines because identical sets of 3AL EST fragments were missing in both lines.

Chromosome 3B

A new deletion for 3BS is present in del2AS-5 with a breakpoint between del3BS-9 and del3BS-8. All ESTs mapped to the distal region of 3BS and two of six ESTs mapped to chromosome bin 3BS9–0.57–0.78 detected the 3BS fragments missing in del2AS-5. Two lines of del4AL-5 and del6DS-6 have new deletions for 3BL. However, the new deletions in both lines were detected by different ESTs, which mapped to the distal region of 3BL (Table 5). One of these lines may possibly have a small interstitial deletion in the distal 37% of 3BL, which does not overlap with the new terminal deletion in 3BL in the other line.

Homoeologous group 4

Six terminal and one interstitial deletions were detected for group-4 chromosomes. The distal 20% of 4AL appears to be a hotspot for breakage as four new breakpoints were detected for this region and three of the breakpoints were differentiated by one marker each only (see Table 5).

Table 6 Putative chromosome aberrations in NT, and Dt lines detected by only one EST clone

Stock	Aberrations	EST clone detecting new deletion	Ratio[b]	Location (bin) of EST clone
TA3263 M2AT2B[a]	Deletion in 2BL	KSU001BE444596	1/9	2BL6–0.89–1.00
TA3267 N2DT2A	Deletion in 6DS	KSU012BE443439	1/3	6DS4–0.79–0.99
TA3065 N5BT5D	Deletion in 1AL	KSU030BF200980	1/14	1AL5–0.66–0.80
TA3065 N5BT5D	Deletion in 1DL	KSU027BE495028	1/44	1DL2–0.41–1.00
TA3065 N5BT5D	Deletion in 2DS	KSU024BF484829	1/26	2DS5–0.47–1.00
TA3124 Dt2DL	Deletion in 5BL	KSU002BE403217	1/2	5BL14–0.75–0.76
TA3124 Dt2DL	Deletion in 5DS	KSU012BE490728	1/4	5DS2–0.78–1.00
TA3117 Dt4AL	Deletion in 2BS	KSU001BE443747	1/10	2BS3–0.84–1.00
TA3117 Dt4AL	Deletion in 3AS	KSU001BE398929	1/14	3AS4–0.45–1.00
TA3117 Dt4AL	Deletion in 4AL	KSU001BE443794	1/8	4AL13–0.59–0.66
TA3117 Dt4AL	Deletion in 4AL	KSU012BE426715	1/14	4AL4–0.80–1.00
TA3117 Dt4AL	Deletion in 7AL	KSU012BE490790	1/3	7AL18–0.86–0.90
TA3117 Dt4AL	Deletion in 7DL	KSU012BE403180	1/6	7DL3–0.82–1.00
TA3127 Dt5DL	Deletion in 1DS	KSU019BE426701	1/2	1DS1–0.59–0.70
TA3127 Dt5DL	Deletion in 1DS	KSU030BG313350	1/18	1DS5–0.70–1.00
TA3127 Dt5DL	Deletion in 3AL	KSU019BE445011	1/21	3AL3–0.42–0.78
TA3127 Dt5DL	Deletion in 7AS	KSU024BE498428	1/16	7AS1–0.89–1.00
TA3122 Dt7BL	Deletion in 1DL	KSU001BE443703	1/44	1DL2–0.41–1.00

[a] N2AT2B plant was selected from the progeny of M2AT2B
[b] Ratio = number of the EST clones detecting new deletion/number of the EST clones mapped to the specific bin

Chromosome 4A

The new terminal deletions for 4AL were observed in four lines, del2DL-9, del1BL-1, del6AL2, and del3DS-3. The breakpoints of the new deletions in these lines are different. The new deletions for 4AL in del2DL-9 and del1BL-1 were differentiated by KSU035BE497624 and in del1BL-1 and del6AL-2 by KSU024BE498428. The new 4AL deletion in del3DS-3 is the smallest one in four lines (Table 5).

Chromosome 4B

A new, very small, terminal deletion for 4BL is present in del5AS-3. Two out of 24 ESTs mapping to the distal region of 4BL detected specific 4BL fragments absent in del5AS-3.

Chromosome 4D

Del5BL-1 has one new interstitial and one new terminal deletion for 4DL. Seven out of 16 ESTs mapped to 4DL13–0.56–0.71 and 2 out of 8 ESTs mapped to the distal region of 4DL detected the 4DL fragments missing in del5BL-1.

Homoeologous group 5

No new deletion but one homoeologous recombinant event was observed for group-5 chromosomes.

Chromosome 5D

A translocation chromosome 5DS-5AS·5AL-5DL is present in del5BL-6. ESTs mapped to the centromere regions of both arms of chromosome 5D detected specific 5D fragments absent in del5BL-6. The 5A fragments detected by these ESTs are more intense in del5BL-6, indicating that the increased signal of 5A results from the extra 5A fragments present in this translocation chromosome (http://wheat.pw.usda.gov/NSF).

Homoeologous group 6

Nine new breakpoints were observed for group-6 chromosomes. The distal region of the 6DL arm appears to be a hotspot region for chromosome breakage.

Chromosome 6A

Del5DS-1 has a new terminal deletion for 6AS and deletion lines del4Al-13 and 2DL-9 have new deletions for 6AL. The specific 6AL fragment of the EST KSU038BE442605 is absent in del4AL-13 but present in del2DL-1 indicating different breakpoints for the new 6AL deletions (Table 5).

Chromosome 6D

A new terminal deletion for 6DS is present in del5DS-2. New terminal deletions for 6DL were observed in the five lines del6AS-1, del2AL-1, del7DS-6, del5BL-1, and del5BL-9. The breakpoints of the new deletions for 6DL in del6AS-1 and del2AL-1 are located in the bin 6DL11–0.74–0.80 and were distinguished by the ESTs KSU001BE403154 and KSU024BE498092, which detected the 6DL fragments absent in del6AS-1 but present in del2AL-1. The new 6DL deletions in del7DS-6,

Table 7 Chromosome aberrations in NT, and Dt lines detected by EST clones

Stock	Aberrations	EST clone detecting new deletion	Ratio[a]	Location (bin) of EST clone
TA3258 N1AT1D	Deletion in 7DL	KSU012BE404744 KSU019BE404737 KSU038BE446244	3/6	7DL3–0.82–1.00
TA3065 N5BT5D	Deletion in 2AL	KSU001BE442858 KSU002BE403217	2/37	C-2AL1–0.85
TA3065 N5BT5D	Deletion in 7AS	KSU012BE442812 KSU024BE499049-3	2/11	7AS5–0.59–0.89
TA3065 N5BT5D	Deletion in 7AS	KSU001BE443794 KSU001BE442648 KSU002BE403518 KSU012BE489999 KSU012BE406897 KSU019BE422466 KSU024BE498428 KSU024BE499049-1 KSU030BE490186 KSU035BE497624	10/16	7AS1–0.89–1.00
TA3103 Dt2AS	Deletion in 3BS	KSU035BE403373 KSU035BE403471	2/6	3BS9–0.57–0.78
TA3103 Dt2AS	Deletion in 3BS	KSU002BE425222 KSU019BE422466 KSU035BE517903 KSU038BE446244	4/4	3BS8–0.78–1.00
TA3114 Dt2BL	Deletion in 4AL	KSU012BE489999 KSU012BE490790 KSU012BE443444 KSU012BE406897 KSU024BE498428 KSU024BE499049 KSU030BE490186 KSU035BE497624 KSU038BE443120	9/14	4AL4–0.80–1.00
TA3086 Dt4AS	Deletion in 2BS	KSU012BE490444 KSU012BE443439 KSU019BE201235 KSU027BE517877	4/10	2BS3–0.84–1.00
TA3117 Dt4AL	Deletion in 4AL	KSU002BE403518 KSU012BE442812	2/11	4AL5–0.66–0.80
TA3117 Dt4AL	Deletion in 7DS	KSU012BE490728 KSU024BE498428	2/25	7DS4–0.61–1.00
TA3120 Dt6BL	Deletion in 3DS	KSU002BE425222 KSU019BE422466	2/9	3DS6–0.55–1.00

[a] Ratio = number of the EST clones detecting new deletion/number of the EST clones mapped to specific bin

del5BL-1, and del5BL-9 are in the distal region of the 6DL arm; all have different breakpoints (Fig. 2, Table 5).

Homoeologous group 7

Only one terminal deletion was observed for 7AS.

Chromosome 7A

Del3BL-10 has the new terminal deletion for 7AS. Three of the 16 ESTs that mapped to the distal region of 7AS detected specific 7AS fragments missing in del3BL-10, indicating this new deletion has lost a very small piece of the 7AS fragment (Table 5).

Chromosome aberrations in NT and Dt lines

Putative chromosome aberrations detected in NT and Dt lines are listed in Table 6. The deletions detected by one EST clone only were observed in N2AT2B, N2DT2A, N5BT5D, Dt2DL, Dt4AL, Dt5DL, and Dt7BL. More clones are needed to further confirm these results.

Terminal deletions were detected for 7DL in N1AT1D, 3BS in Dt2AS, 4AL in Dt2BL, 2BS in Dt4AS, and 3DS in Dt6BL (Table 7).

N5BT5D has an interstitial and a terminal deletion for 7AS (Fig. 3, Table 7) and an interstitial deletion for 2AL. Additionally, putative terminal deletions for 1AL, 1DL, and 2DS were also observed in N5BT5D, which were detected with only one clone (Table 6).

An interstitial deletion for 4AL is present in Dt4AL. Two ESTs mapped to bin 4AL5–0.66–0.80 detected re-

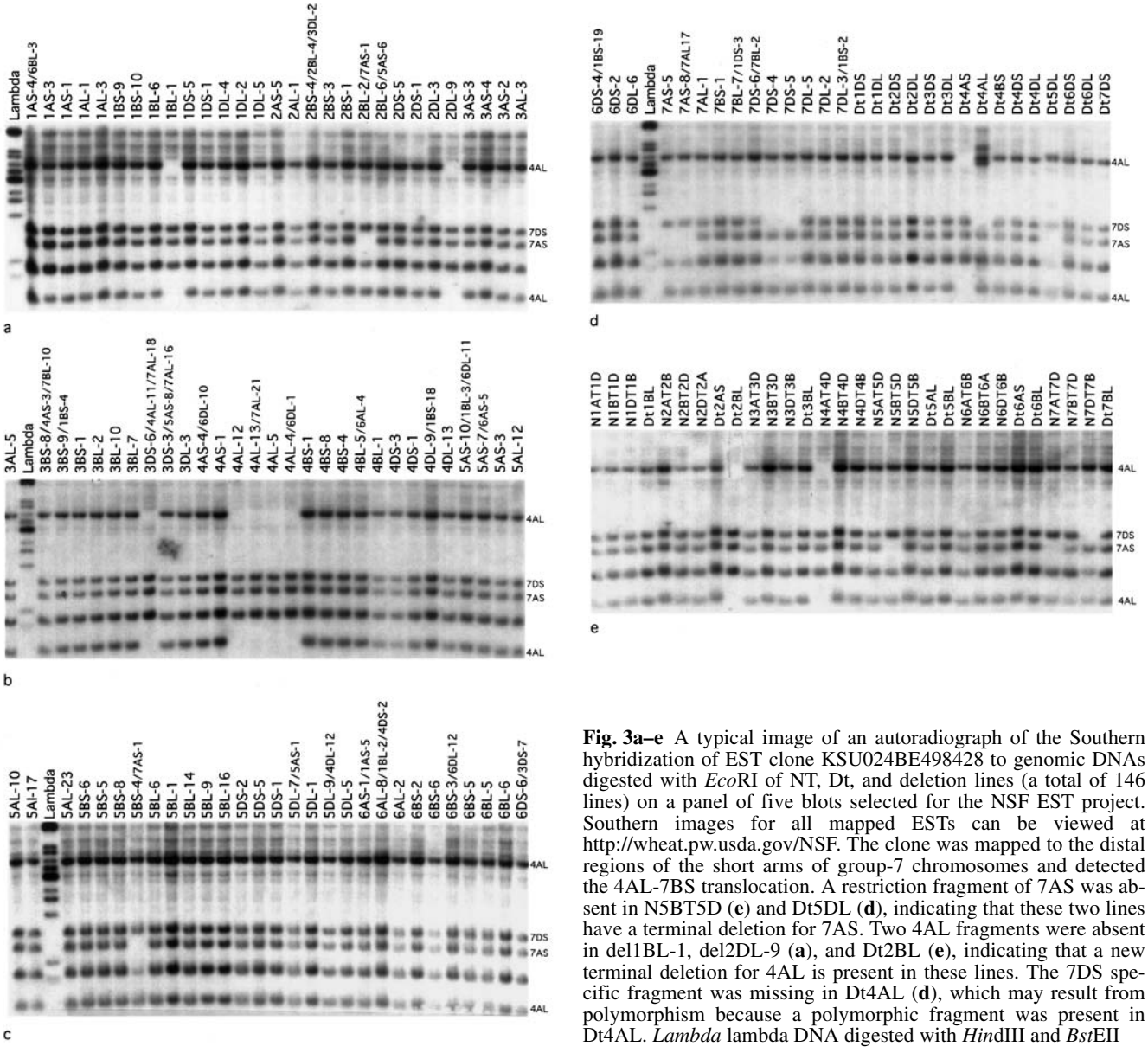


Fig. 3a–e A typical image of an autoradiograph of the Southern hybridization of EST clone KSU024BE498428 to genomic DNAs digested with *Eco*RI of NT, Dt, and deletion lines (a total of 146 lines) on a panel of five blots selected for the NSF EST project. Southern images for all mapped ESTs can be viewed at http://wheat.pw.usda.gov/NSF. The clone was mapped to the distal regions of the short arms of group-7 chromosomes and detected the 4AL-7BS translocation. A restriction fragment of 7AS was absent in N5BT5D (**e**) and Dt5DL (**d**), indicating that these two lines have a terminal deletion for 7AS. Two 4AL fragments were absent in del1BL-1, del2DL-9 (**a**), and Dt2BL (**e**), indicating that a new terminal deletion for 4AL is present in these lines. The 7DS specific fragment was missing in Dt4AL (**d**), which may result from polymorphism because a polymorphic fragment was present in Dt4AL. *Lambda* lambda DNA digested with *Hin*dIII and *Bst*EII

striction fragments absent in Dt4AL. The 7DS fragments absent in Dt4AL were detected by two ESTs, one of which may result from polymorphism (Fig. 3).

Discussion

The aneuploids of bread wheat, including nullisomic-tetrasomic and ditelosomic lines (Sears 1954, 1966; Sears and Sears 1978), have been extensively used to assign genes and molecular markers to individual chromosomes and chromosome arms. Chromosome aberrations detected in these genetic stocks were summarized by Devos et al. (1999). Terminal deletions in N1AT1D, Dt2AS, Dt2BL, and Dt4AS discovered in our study are in agreement with previous results. Additionally, we found that Dt4AL has interstitial and terminal deletions in the 4AL arm. N5BT5D maintained at WGRC has interstitial and terminal deletions in the 7AS arm, a small interstitial deletion in 2AL, and putative terminal deletions in 1AL, 1DL, and 2DS. Different changes in chromosome structure were observed in the N5BT5D stocks maintained in different laboratories (Devos et al. 1999), which resulted from the effect of the *Ph1* gene, a pairing control gene located on the long arm of chromosome 5B. Homoeologous recombination occurs at meiosis in lines nullisomic for 5B, which lack the *Ph1* gene. Similarly, deletion lines of del5BL-1 and del5BL-6 missing the *Ph1* gene have more complex chromosome aberrations resulting from homoeologous recombination. The translocation chromosome 5DS-5AS·5AL-5DL present in del5BL-6 is strong evidence that homoeologous recombination occurred in this line.

With the discovery and application of gametocidal chromosomes (genes; reviewed in Endo 1990), Endo and Gill (1996) isolated 436 different terminal deletions covering all 21 chromosomes of wheat opening a new era in localizing genes to specific chromosome segments. The cytogenetic analysis is not sensitive enough to detect some smaller deletions. In the last decade, more than 1,000 molecular markers (RFLPs and microsatellites) from genetic maps have been assigned to the deletion-based physical maps of each homoeologous group in wheat (Werner et al. 1992a, b; Kota et al. 1993; Gill et al. 1993, 1996a, b; Hohmann et al. 1994; Delaney et al. 1995a, b; Mickelson-Young et al. 1995; Roder et al. 1998; Faris et al. 2000; Weng et al. 2000; Qi and Gill 2001). Putative new deletions involving chromosomes of other groups were not detected because only markers corresponding to individual homoeologous group deletions were used for mapping. In the present study, the 526 EST clones were hybridized to all NT, Dt, and selected lines with known deletions and thereby provided the opportunity for detection of new deletions. We found 35 new deletions involving 20 chromosome arms in 26 known deletion lines. These new deletions resulted from either the effect of the gametocidal gene during production of the deletion lines or spontaneous chromosome breakage. From the present mapping data, we found an identical new deletion for 3BS in del2AS-5 and Dt2AS, which was detected by the same ESTs (Tables 5, 7). This result indicates those independent deletions for 3BS in del2AS-5 and Dt2AS might have occurred spontaneously. New terminal deletions for 4AL are present in four deletion lines, del1BL-1, del2DL-9, del3DS-3, and del6AL-2 and ditelosomic line 2BL. All occur in the distal 20% of the 4AL arm with different breakpoints, confirming this region of 4AL as a hotspot for chromosome breakage (Endo and Gill 1996). Similarly, 20% of the distal region of 6DL is another hotspot for chromosome breakage.

Chromosome deletions induced by gametocidal genes are normally stable, because broken chromosome ends are healed by the addition of telomere repeats (Werner et al. 1992a, b; Tsujimoto 1993; Friebe et al. 2001). Of 122 homozygous deletions selected, changes in chromosome structure were detected in the progenies of five lines, del1AL-3, del2BL-6, del3AS-2, del5BL-16, and del6BS-2, and these lines were re-isolated from the original stocks. Three secondary deletions that were presumed to be homozygous for del1BS-4, del1AS-5, and del3DS-7 were not confirmed as correct. Deleted chromosomes for 1AS, 6AL, 6BS, and 7DS were not detected in del1AS-4, del6AL-2, del6BS-6, and del7DS-6, respectively. Line del3AS-3 had a pair of chromosome 3AS-3 (G. Linc, personal communication). Our study indicated that 3AS is missing in del3AS-3, and the remainder of the short arm may have resulted from a translocation from an unknown chromosome.

Detailed characterization of deletion stocks will further enhance the use of deletion stocks in wheat genome analysis. Based on known deletions, we had estimated that they divide the wheat genome into 159 chromosome bins; 150 bins have been tagged with molecular markers. The diagnostic markers will be useful for the identification of specific deletion stocks. The new terminal deletions provide 30 new breakpoints and divide the wheat genome into 189 chromosome bins. The total genome size of bread wheat is 16×10^9 for 21 chromosomes or $16/3\times10^9$ for a basic set of 7 chromosomes. Because gene synteny and colinearity is conserved between A, B and D genome chromosomes, 189 chromosome bins can be used to order the ESTs for the basic set of 7 chromosomes and the calculated size of each chromosome bin is 28 Mb. Thus, any EST or target gene can be mapped at a resolution of 28 Mb. As the wheat genome is partitioned into gene-rich and gene-poor regions, many gene-rich regions of the genome will be mapped at a resolution of 28 Mb. The ESTs mapped to gene-rich chromosome bins will provide starting material for molecular cloning of useful genes in wheat by selective sequencing of gene-rich chromosome bins, and by comparative mapping in silico to the sequenced genome of rice.

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