Chromosome aberrations in wheat nullisomic-tetrasomic and ditelosomic lines

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Summary Polymorphisms, deletions and translocations have been identified in some of the Chinese Spring nullisomic-tetrasomic and ditelosomic lines. In this paper, we present a comprehensive overview of the status of these materials.

Key Words Aberrations - Aneuploid stocks - Ditelosomic lines - Nullisomic-tetrasomic lines - Polymorphism

Introduction

The ability of hexaploid bread wheat, *Triticum aestivum* ($2n = 6x = 42$), to tolerate the addition or loss of chromosomes or chromosome segments was first exploited by the late Prof. Ernie Sears who constructed several series of aneuploid lines (Sears 1954; Sears 1966; Sears and Sears 1979). These materials, mostly in the variety 'Chinese Spring' (CS), have benefited both classical and molecular genetic studies in wheat. The development of the first molecular marker genetic maps was greatly facilitated by the use of nullisomic-tetrasomic (NT) and ditelosomic (Dt) lines. When RFLPs became available, the series of 42 NT lines, each lacking one chromosome pair in turn, compensated for by the presence of an additional pair of homoeologous chromosomes, presented an ideal tool for the localisation of the molecular markers to chromosomes without the need for polymorphism. The ditelosomic lines, each with 21 pairs of chromosomes but with one chromosome pair comprising only either the short or long arms, allowed determination of the chromosome arm location of probes and thus the positioning of centromeres on to the genetic maps.

Now, about half a century on, it is clear that the aneuploid stocks harbour a wealth of aberrations that can, and almost certainly have done, confound some experimental results arising from their use. The extensive use of aneuploid lines for mapping and tagging has led, indirectly, to their detailed characterisation. In this paper an attempt is made to bring together all cytological and molecular data available on polymorphisms and aberrations for the CS NT and Dt lines. This information should be of value to researchers who use these genetic stocks in future, and may help resolve difficulties in interpretation of results already obtained.

Materials and Methods

'Chinese Spring' NT and Dt lines, all originating from single sources, have been maintained independently in several wheat genetic resource centres and laboratories. Of the 42 NT lines (Table 1), N2A lines have very low fertility and N4B lines are sterile, and are therefore maintained as monosomic-tetrasomic lines. The N4DT4A line is available but also has very poor fertility due to the 4A tetrasomy. Thirty six ditelosomic lines are available (Table 1); Dt1AS, Dt1AL, Dt1BS, Dt1BL, Dt1DS, Dt1DL, Dt2AS, Dt2BL, Dt2DS, Dt2DL, Dt3AS, Dt3AL, Dt3BS, Dt3BL, Dt3DS, Dt3DL, Dt4AS, Dt4AL, Dt4BS, Dt4DS, Dt4DL, Dt5AL, Dt5BL, Dt5DL, Dt6AS, Dt6AL, Dt6BS, Dt6BL, Dt6DS, Dt6DL, Dt7AS, Dt7AL, Dt7BS, Dt7BL, Dt7DS and Dt7DL. Ditelosomic line Dt3BS frequently undergoes asynapsis during meiotic divisions and its progeny may therefore carry chromosomal deletions or translocations. Ditelosomic lines Dt2DL and Dt6BL have very low fertility are usually maintained and distributed as monotelodisomics (2DL'+2D' and 6BL'+6B'). The remaining six ditelosomics do not transmit through the male gametes and are sterile. These lines are maintained as dimonotelosomics (2AS'+2AL", 2BS"+2BL', 4BS'+4BL", 5AS"+5AL', 5BS"+5BL' and 5DS"+5DL') or monotelodisomics (2AL'+2A', 4BL'+4B', 5BS'+5B', 5DS'+5D').

Table 1: Lists of available NT and Dt stocks and those analysed by John Innes Centre (JIC) and Cornell University (CU)

Stocks[1]	Comments	JIC	CU	Stocks[1]	Comments	JIC	CU
N1AT1B				N7BT7A		√	√
N1AT1D	Deletion in 7DL	√	√	N7BT7D			
N1BT1A		√		N7DT7A			√
N1BT1D			√	N7DT7B	Pilot, Thatcher and Hope in background	√	
N1DT1A			√				
N1DT1B		√		Dt1AS			√
N2AT2B	Very low fertility; Maintained as monosomic-tetrasomic line	√		Dt1AL		√	√
				Dt1BS	Deletion in 7AL	√	√
N2AT2D	Very low fertility; Maintained as monosomic-tetrasomic line			Dt1BL		√	√
				Dt1DS			√
N2BT2A				Dt1DL		√	√
N2BT2D		√	√	Dt2AS	Not pure CS background; Deletion in 3BS	√	√
N2DT2A		√	√				
N2DT2B				Dt2BL	Deletion in 4AL	√	√

N3AT3B				Dt2DS		√	√
N3AT3D		√	√	Dt2DL	Not pure CS background; Very low fertility		√
N3BT3A		√	√				
N3BT3D				Dt3AS		√	√
N3DT3A			√	Dt3AL			√
N3DT3B		√		Dt3BS	Undergoes asynapsis		√
N4AT4B				Dt3BL		√	√
N4AT4D		√	√	Dt3DS		√	√
N4BT4A	Sterile; Maintained as monosomic-tetrasomic line			Dt3DL		√	√
				Dt4AS			
N4BT4D	Sterile; Maintained as monosomic-tetrasomic line	√		Dt4AL	*T. durum/Ae. tauschii* in background	√	√
N4DT4A	Very low fertility			Dt4BS			√
N4DT4B		√	√	Dt4DS		√	√
N5AT5B		√	√	Dt4DL		√	√
N5AT5D				Dt5AL		√	√
N5BT5A	Carries a 2AS.5AL translocation			Dt5BL	Possible deletion in 2DS	√	√
				Dt5DL		√	√
N5BT5D	May carry translocations due to homoeologous recombination	√	√	Dt6AS		√	√
				Dt6AL	Not pure CS background; Pure CS line available from AJ Lukaszewski		√
N5DT5A		√					
N5DT5B			√	Dt6BS	Deletion in 2BS	√	√
N6AT6B				Dt6BL	Very low fertility		√
N6AT6D		√	√	Dt6DS		√	√
N6BT6A	Correct identification is N6BT6D	√		Dt6DL			√
				Dt7AS		√	√
N6BT6D			√	Dt7AL			√
N6DT6A				Dt7BS		√	√
N6DT6B	Not pure CS background	√	√	Dt7BL	Not pure CS background	√	√
N7AT7B				Dt7DS			√
N7AT7D		√	√				

[1] All stocks are maintained at and available from SM Reader at the John Innes Centre; A. Hang (P.O. Box 307, Aberdeen, ID 83210, USA); P. Gustafson at University of Missouri, Columbia (206 Curtis Hall, University of Missouri, Columbia, MI 65211, USA); and AJ Lukaszewski at University of California, Riverside, USA.

Results and Discussion

Polymorphisms between 'Chinese Spring' and the aneuploid lines

Not all Chinese Spring aneuploid lines were developed in a pure CS genetic background. It is known, for example, that the varieties 'Pilot', 'Thatcher' and 'Hope' are present in the background of N7DT7B (Sears 1954) and that varieties other than CS were involved in the generation of ditelosomic lines Dt2DL, Dt4AL, Dt6AL and Dt7DL and in the lines telocentric

for chromosomes 2BS, 4AS, 5BS and 5DS (Sears and Sears 1979; E.R. Sears, Pers. Comm.). The variety 'Gabo' was used in the production of the 5DS telocentric, and Dt4AL may have a synthetic hexaploid (*T. durum/T. squarrosa*) in its ancestry (E.R. Sears, Pers. Comm.). The original 5BS telocentric stock, produced by T.E. Miller, was extracted from 'Holdfast' and backcrossed into CS. A new 5BS monotelodisomic stock extracted from CS is available from S.M. Reader. The original Dt6AL line displayed both C-banding and RFLP patterns that were different from those of CS, and has been re-extracted in a pure CS background by A.J. Lukaszewski. The 4AS chromosome in the original Dt4AS-Mt4AL stock is acrocentric, and a true Dt4AS line of CS origin is available from A.J. Lukaszewski. RFLP studies also revealed a further three lines, N6DT6B (Fig.1), Dt2AS and Dt7BL, that did not have a pure CS background.

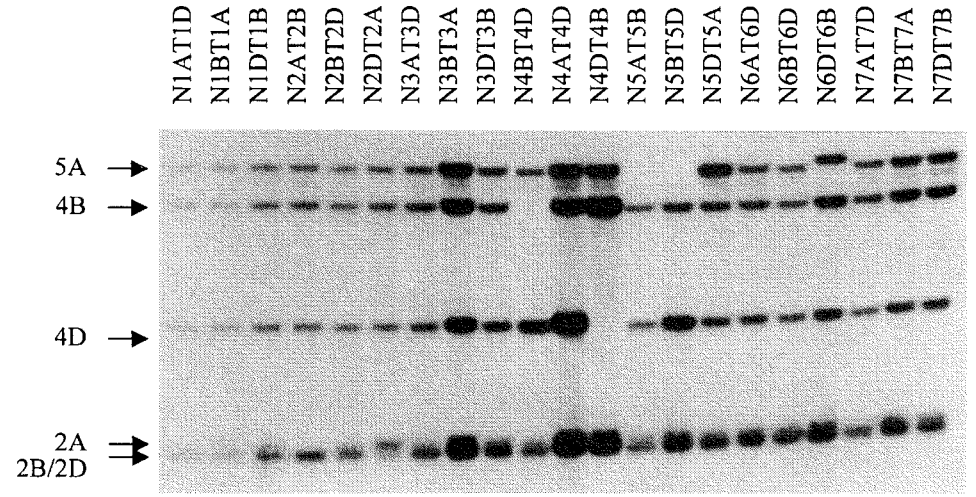

Figure 1 Autoradiograph showing hybridization of probe pcβC51 (Kreis et al. 1987) to 21 Chinese Spring nullisomic-tetrasomic lines. The probe sequence is located on chromosomes 5A, 4B and 4D and 2A, 2B and 2D, hence the absence of hybridizing fragments in lines N5AT5B, N4BT4D and N4DT4A, and the group 2 chromosomes. Line N5BT5D lacks the 5A fragment, but carries extra copies of the 4D fragment, indicating that homoeologous recombination has taken place between these two chromosomes. Lines N6DT6B displays a polymorphism of the 5A fragment.

Polymorphisms were found for most or all of the 5AS arm in the Dt5DL stock maintained at University of California, Davis. The same 5A polymorphism was observed in the deletion stocks 5DS-1 and 5DS-2 produced from a Dt5DL line at the Wheat Genetics Resource Center, Kansas State University (Endo and Gill 1996). The John Innes Centre (JIC) stock, obtained from the same source at an earlier date, appears to have a complete CS background.

Deletions

Terminal deletions were observed for chromosome arms 7DL in N1AT1D, 2DS in N5AT5B, 2BS in N7DT7B, 7AL in Dt1BS, 3BS in Dt2AS, 4AL in Dt2BL, 2DS in Dt5BL, 2BS in Dt6BS. The RFLP loci covered by the deletions in N1AT1D, Dt1BS, Dt2AS, Dt2BL, Dt5BL and Dt6BS are shown in Fig.2.

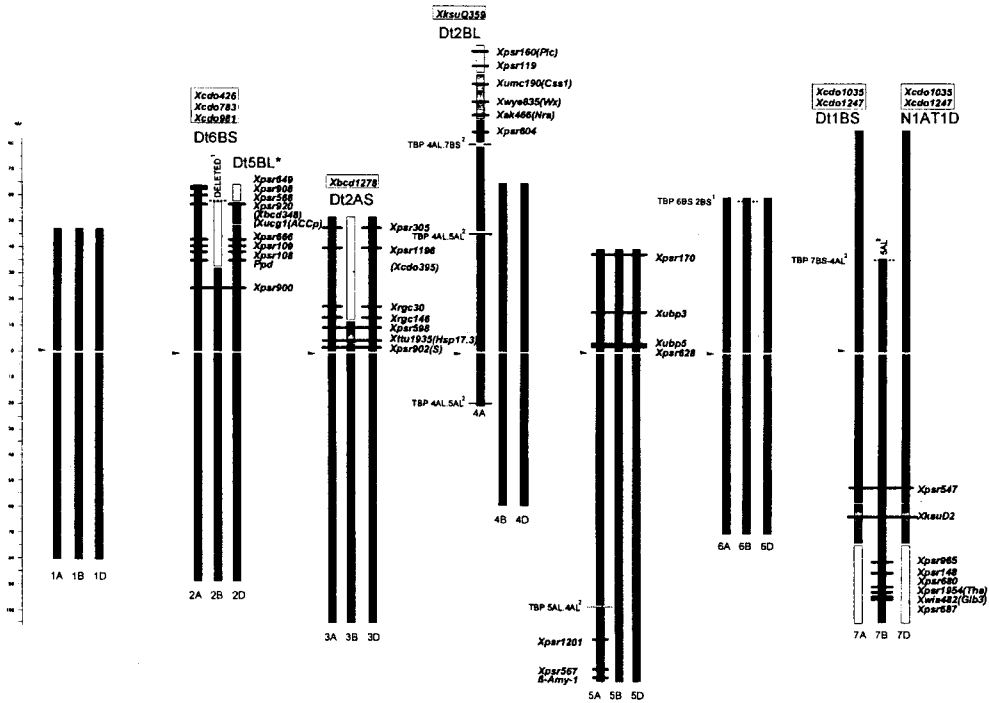

Figure 2 Chromosomal deletions observed in Chinese Spring nullisomic-tetrasomic and ditelosomic lines. The lines in which deletions have been observed are given at the top of the chromosomes. With the possible exception of the deletion in Dt5DL (indicated with *), all deletions are likely be present in E.R. Sears' original stocks. Open bars represent deletions. Loci that map within the hatched regions (indicated with '?') have not been tested against the set of nullisomic-tetrasomic lines. The most proximal marker on each chromosome maps outside the deletion. Probes that identify deletions but have not yet been genetically mapped are presented in boxes above the chromosomes. Detailed descriptions of the evolutionary translocations in wheat are given in Devos et al. (1993b)[1], Devos et al. (1995)[2], and Nelson et al. (1995)[2].

The 7AL deletion present in the Dt1BS stock was also found in a CS euploid line extracted at JIC from Sears' monosomic 1B line. Since the Dt1BS line is likely to be derived from the monosomic 1B line, we expect that the 7AL deletion was present in Sears' original monosomic 1B line. The 4AL deletion in Dt2BL can be observed cytologically and comprises approximately 8% of the arm. This aberration also exists in other telosomic 2BL lines, and was presumably present in Sears' original Dt2BL stock. The Dt6BS line with a background terminal deletion of chromosome arm 2BS is, in contrast to 'Chinese Spring', photoperiod sensitive (Islam-Faridi et al. 1996). This indicates that the deletion covers the *Ppd2* gene. With the exception of the 2DS deletion in Dt5BL, which may only be present in the JIC stock, all deletions presented in Fig.2 were observed in the stocks both at JIC and Cornell University (CU), and are likely to have taken place during the generation of these aneuploids. It is expected that they will be a universal feature of all lines derived from Sears' original N1AT1D, Dt1BS, Dt2AS, Dt2BL and Dt6BS stocks. The 2BS and 2DS deletions on the other hand, which comprise most of the short arms, in N7DT7B and N5AT5B, respectively, were present in the stocks maintained at CSIRO, Canberra, but not in the JIC and CU stocks.

Potential deletions have also been observed in N2BT2D, N2DT2A, Dt2BL, Dt3BS, Dt4AS, Dt4AL, Dt5DL and Dt6AL (Table 2). The absence of restriction fragments in these lines has, so far, been noted with only one probe and, thus, could be the result of polymorphism rather than deletions. Deletions in the Dt3BS line may be the consequence of asynapsis, and therefore different aberrations may be found in different stocks.

Table 2 : Potential deletions identified with one RFLP marker only

Stock	Chromosome arm carrying a potential aberration	Probe identifying the aberration[1]
N2BT2D	6BL	PSR908
N2DT2A	6DS	PSR10 (*Gli-2*)
Dt2BL	4AS	CDO1400
Dt3BS	1AS	ECD10
Dt4AS	2BS	UCG1
Dt4AL	2AL	BCD292
Dt5DL	1DL	BCD200
Dt6AL	2BS	BCD348

[1] Probe sources : BCD - Cornell University; PSR - John Innes Centre; ECD - Gallego et al. 1998; UCG1 - Gornicki et al. 1997

Wrongly identified stocks - Dt7DL and N6BT6A

All probes that hybridized to chromosome-arm 7AL and 7BL fragments failed to detect fragments on ditelosomic line Dt7DL. However, probes that detected loci on 7AS and 7BS hybridized to DNA isolated from the Dt7DL line. As homoeology between chromosome arms 7AS, 7BS and 7DS, and 7AL, 7BL and 7DL had previously been established (Riley and

Chapman 1964), the RFLP results clearly indicated that the line which had been identified as 7DL carried the short arm rather than the long arm of chromosome 7D. The misidentification of the Dt7DL stock had also been established using chromosome banding (Friebe et al. 1996) and chromosome pairing studies (S.M. Reader and T.E. Miller, unpublished). A correct Dt7DL stock is currently being extracted at JIC from the double ditelosomic stock.

Pairing analyses of test crosses carried out on the JIC N6BT6A stock, revealed that this line was, in fact, tetrasomic for chromosome 6D, and thus was a N6BT6D line. A correct N6BT6A line is currently being extracted at JIC.

Nulli-5B lines

Detailed analysis of the N5BT5D line revealed the absence of hybridizing fragments in chromosomes other than 5B. In some cases, this was coupled with the presence of fragments with double dose intensity on homoeologous chromosomes (Fig.1). This almost certainly reflects that fact that homoeologous recombination had taken place in the N5BT5D line, giving rise to deletions and duplications. Nulli-5B lines lack the pairing control gene, *Ph1*, which is located on chromosome arm 5BL. In theory, homoeologous recombination in nulli-5B lines can take place during each meiosis. Therefore, the chromosome aberrations can be expected to be different in independently maintained nulli-5B stocks and will continue to accumulate during further generations. Indeed, the current generation of the N5BT5D line maintained at JIC displays a deletion of a terminal segment of chromosome arm 5AS, possibly a small interstitial deletion on 1AL and evidence of recombination between homoeologous segments of chromosome arms 7AL and 7DL, and 5AL and 4DL, while an independently maintained N5BT5D stock at Cornell University carried a recombined 2AS-2DS.2DL chromosome (Anderson et al. 1992)(Table 3).

Table 3 : Aberrations identified in the N5BT5D stocks at JIC and CU

N5BT5D stock	Chromosome (arm) carrying a rearrangement	Probes identifying the rearrangement[1]
JIC	1AL	PSR12 (*Glu-1*)
	5AS	PSR170[2], UBP3[2], UBP5[2]
	5AS.5AL-4DL	PSR567[2], pcβC51[2,3]
	7AS.7AL-7DL	PSR680[2], PSR1954[2], pLW2.1[2,4], PSR687[2]
CU	2AS-2DS.2DL	BCD348[2], CDO418, CDO426, CDO783, CDO666, CDO981

[1] Probe sources : BCD, CDO - Cornell University; PSR - John Innes Centre; MGB - A. Blanco; pcβC51 - Kreis et al. 1987; pLW2.1 - Loi et al. 1988

[2] Map position of these probes are given in Fig.2.

[3] pcβC51 detects *β-Amy-1* loci

[4] pLW2.1 detect the *Xwia482(Glb3)* loci

Line N5BT5A was not examined using RFLP markers, but cytogenetic studies have indicated the presence of a centric 2AS.5AL translocation in the original N5BT5A stock, which is also present in the tetrasomic 5A stock. This line also produced embryoless grains at low frequently. A decrease in fertility has been observed over generations in both the N5BT5D and N5BT5A stocks at JIC, and new stocks are currently being constructed.

Breakpoints

Comparison of the deletion breakpoints in the aneuploid stocks and evolutionary translocation breakpoints in Triticeae chromosomes may indicate that certain chromosome regions are more prone to breakage than others. The distal part of the long arm of chromosome 7A is absent in line Dt1BS and the distal part of 7DL has been deleted in line N1AT1D. In both lines, the breakpoint may be flanked by the same RFLP markers. A translocation breakpoint in the same chromosomal region was also observed in rye (Devos et al. 1993a) and *Aegilops umbellulata* (Zhang et al. 1998), which may support a hypothesis that the ancestral 7L Triticeae chromosome arm carried a hot spot for chromosome breakage.

Conclusions

Polymorphisms, deletions and homoeologous recombination events characterise some of the wheat 'Chinese Spring' nullisomic-tetrasomic and ditelosomic lines. When employing these lines for genetic studies, it is important to be aware of the presence of these aberrations in the 'Chinese Spring' aneuploid materials to avoid misinterpretation of experimental results.

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