Exploitation of chromosome 4S¹, from *Aegilops sharonensis*, for the production of stable 44-chromosome wheat lines

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A number of chromosomes from species related to wheat carry genes of potentially high agronomic value. However, wheat lines carrying an additional pair of such chromosomes have never been exploited commercially because they are unstable and the extra chromosomes are frequently lost during gametogenesis. In order to overcome this problem translocations involving the long arm of the *Aegilops sharonensis* chromosome 4S^I, which carries the gene(s) determining preferential transmission, and the long and short arms of chromosome 1U from *Aegilops umbellulata*, which carry respectively the genes *Glu-U1* and *Gli-U1*, which affect bread making quality, have been isolated. These translocations are stable and preferentially transmitted.

Keywords: Aegilops sharonensis, preferential transmission, protein quality, stable additions, Triticum aestivum.

Introduction

A number of chromosomes from species related to bread wheat (Triticum aestivum) have been shown to carry genes of potentially high agronomic value when introduced into wheat (e.g. Knott, 1968; Forster et al., 1988), but lines carrying an additional pair of such 'alien' chromosomes have never been exploited commercially. One reason for this is that the alien homologues in wheat alien addition lines sometimes fail to pair at meiosis (Riley, 1960), resulting in reversion to pure wheat. The phenomenon of preferential transmission exhibited by chromosome 4Si of Aegilops sharonensis, when present in a wheat background. ensures that all gametes lacking it are non-functional (Miller et al., 1982). Thus one way in which the cytological instability of disomic addition lines could be overcome would be to induce a translocation between an alien chromosome and the segment of the long arm of chromosome 4S¹, which carries the gene(s) for preferential transmission (King et al., 1991b). Lines disomic for such a novel chromosome (42 wheat chromosomes + two 4S'L/alien translocation chromosomes) would be expected to be stable 44-chromosome lines, as normal 21-chromosome wheat gametes (lacking the translocation) will be inviable due to the gametocidal action of the gene(s) for preferential transmission on 4Si. The simple way to induce the required

translocations is via centric breakage and fusion (Robertsonian translocations) which have been shown to occur between unrelated chromosomes (Driscoll & Sears, 1963; Davies *et al.*, 1985).

The current paper reports the production of three translocations between chromosome 4S¹ and chromosome 1U from Aegilops umbellulata. The latter chromosome carries genes encoding endosperm storage proteins of potential value in manipulating breadmaking quality: Glu-U1 on the long arm, encoding high-molecular-weight (HMW) subunits of glutenin, and Gli-U1 on the short arm, encoding gliadins.

Materials and methods

The genetic material consisted of the *T. aestivum* variety Chinese Spring (CS), and the Chinese Spring 4S¹ and 1U disomic addition lines. As the first step in the induction of 4S¹L/1U translocations, the 1U and 4S¹ additions were intercrossed, and progeny with 44 chromosomes were selected. When plants containing chromosome 4S¹ are used as the pollen parent in crosses to plants without 4S¹, chromosomal aberrations are frequently observed in the progeny (Endo, 1988). To avoid this problem, the CS 1U disomic addition line was used as the pollen parent and the CS 4S¹ disomic addition line as the female parent. The resultant double monosomic addition lines (42W+4S¹+1U)

were expected to form 21 bivalents and a 4S¹ and a 1U univalent at meiosis. As univalents occasionally misdivide and rejoin, some gametes would be expected to contain a 4S¹L/1U translocation chromosome. The double monosomic addition lines were subsequently used as the pollen parent in crosses with CS euploid. Although carrying out the cross in this direction would be expected to result in some of the progeny containing chromosomal aberrations, this was necessary because the presence of a monosomic chromosome 4S1 can result in a reduction in female fertility of up to 75 per cent (King, 1990). Although male fertility is also reduced, the problem is not severe due to the large pollen population. Identification of 4SIL/1U Robertthe $42W + 4S^{1} + 1U$ sonian translocations in ×CS F₁ individuals was facilitated by screening for the presence or absence of the following genetic markers: Amp-S'2 on 4S'S, encoding an aminopeptidase isozyme (King et al., 1991c); \(\beta\text{-}Amy\text{-}S'\text{1}\) on $4S^{1}L$, encoding certain β -amylase isozymes (King et al., 1991c); Gli-U1 on 1US, encoding gliadins (Brown et al., 1979); Glu-U1 on 1UL, encoding two HMW subunits of glutenin (Brown et al., 1979). All these nonwheat isozymes/proteins are electrophoretically distinct from their wheat equivalents. Since, as noted earlier, the gene(s) for preferential transmission is on $4S^{1}L$, $42W+4S^{1}+1U\times CS$ individuals carrying the desired translocation would be expected to possess β-Amy-S'1 on 4S'L and lack Amp-S'2 on 4S'S; they would also carry one or other of Glu-U1 on 1UL or Gli-U1 on 1US. Thus initial selection was carried out by screening for the absence of Amp-S'2. Individuals which lacked this marker were then screened for the remaining three markers.

Isoelectric focusing separations of the isozymes were carried out according to Sharp *et al.* (1988) for β -amylase and Koebner & Martin (1989) for aminopeptidase. Glutenins and gliadins were fractionated using 10 per cent SDS-PAGE under reducing conditions as described by Payne *et al.* (1981), except that 2-mercaptoethanol was replaced by dithiothreitol (50 mm) as the reducing agent in the sample buffer.

Somatic chromosome counts were obtained from root-tip preparations and meiotic configurations from pollen mother cells, both stained by the standard Feulgen procedure.

Results

Two thousand and thirty-nine individual grains were produced from the $42W+4S^1+1U\times CS$ cross; of these, 1839 individuals possessed Amp-S'2 and were therefore discarded. Eighty-nine of the remaining 200 individuals lacked β -Amy-S'1 as well as Amp-S'2, and

presumably lacked chromosome 4S¹, these individuals were not studied further. Ninety-six of the Amp- $S^{\prime}2^{-}$. β-Amy-S'1+ selections carried neither Glu-U1 nor Gli-U1 and hence, presumably, lacked chromosome 1U entirely. Mitotic observations of the 96 seedlings revealed the presence of 42 bibrachial chromosomes and a single telosome, probably 4S¹L. Of the remaining 15 selections 12 had both Glu-U1 and Gli-U1 and carried 43 bibrachial chromosomes and one telosome, indicating the likely presence of a complete 1U chromosome and a 4S1 telosome. Two of the remaining three individuals (306 and 348), possessed Glu-U1 but not Gli-U1, while the other (304) had Gli-U1 but not Glu-U1. All three plants contained 43 bibrachial chromosomes and formed 21 bivalents and a univalent at metaphase I of meiosis. The univalent chromosomes possessed a single distinctive telomere (Fig. 1a and b). In contrast, $4S^{\bar{i}}$ univalent chromosomes possess two distinctive telomeres (King et al., 1991a). Thus the presence of only one distinctive telomere on the univalent chromosome, in each of the three plants, is consistent with the presence of a translocation involving 4S1. The biochemical phenotypes, the absence of telosomes, plus the meiotic configuration, indicated that plants 306 and 348 contained a 4SIL/1UL translocation, while plant 304 contained a 4SIL/1US translocation. The short arm of chromosome 1U possesses a nucleolus organizer region (NOR) which suppresses the activity of the four wheat NORs (Martini et al., 1982). Thus in Chinese Spring 1U addition lines, only the 1U NORs are visible. Mitotic preparations of plant 304 revealed that only one NOR chromosome was usually observed. At metaphase I of meiosis the NOR was located on the univalent chromosome, confirming the presence of a 4S'L/1US translocation in this plant (Fig. 1b). Four NOR chromosomes were regularly seen in mitotic and meiotic preparations of plants 306 and 348. At metaphase I of meiosis, the univalent in these plants did not appear to have a NOR (Fig. 1a).

In order to determine whether the translocations were preferentially transmitted, plants 306, 348 and 304 were test-crossed as pollen parents to Chinese Spring and the progeny screened with the biochemical markers. The majority of individuals in the three test-cross populations had both β -Amy-S'1 and Glu-U1 (84.6 per cent of the 306 × CS test-cross progeny and 87.5 per cent of the 348 × CS test-cross progeny), or Gli-U1 (93.8 per cent of the 304 × CS test-cross progeny), consistent with the preferential transmission of the 4S'L/1U chromosome (Table 1). Note that plant 348 was relatively male sterile and only eight 348 × CS progeny were obtained.

Six test-cross progeny (three from $304 \times CS$ and three from $306 \times CS$) lacked the relevant storage pro-

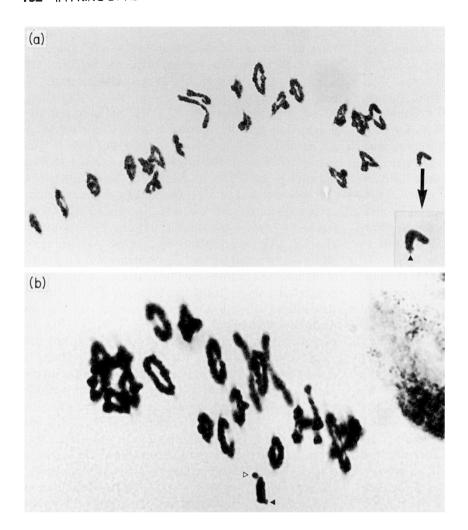


Fig. 1 Two photomicrographs; one from plant 306 (a) and the other from plant 304 (b). Each shows 21 bivalents and a univalent chromosome [the univalent in (b) is enlarged in an insert]. Each univalent has a distinctive telomere (\triangle). The univalent from plant 304 also possesses a NOR (\triangle).

Table 1 The number of individuals from the three test-cross progenies with or without β -Amy-S'1, Glu-U1 and Gli-U1

	β -Amy-S $^{\prime}I^{+}$	β -Amy-S $^{l}I^{+}$	β -Amy-S $^{l}I^{+}$	β -Amy-S $^{\prime}l^{-}$
Test cross	Glu-U1 - Gli-U1+	Glu-U1+ Gli-U1-	Glu-U1 ⁻ Gli-U1 ⁻	Gli-U1 Gli-Ul
304 × CS	45		3	_
$306 \times CS$	_	22	3	1
$348 \times CS$	_	7	_	1

tein (Gli-UI and Glu-UI, respectively) but had β -Amy-S'I. Of these one plant from each test-cross progeny possessed a dicentric chromosome. The biochemical phenotype of these individuals could be explained by a translocation which involved the $4S^1/1U$ translocation chromosome and a wheat chromosome, such that β -Amy-S'I was included in the dicentric chromosome, while the 1U segment was lost as an acentric fragment. Two plants from each test-cross progeny possessed 42 bibrachial chromosomes and one telosome. The loss of

either Glu-U1 or Gli-U1 in these plants was probably the result of misdivision of $4S^lL/1U$ followed by loss of the 1U telosome. A further two plants, one from test-cross progeny $306 \times CS$ and the other from test-cross progeny $348 \times CS$, lacked both β -Amy- S^l1 and Glu-U1 and had 42 bibrachial chromosomes. It appears that the $4S^lL/1UL$ translocation chromosomes were not transmitted to these two individuals. A number of individuals from each test-cross progeny, which possessed the relevant $4S^l$ and 1U markers, contained

Table 2 Chromosome number of plants produced upon self-pollination of plants monosomic for the three 4SⁱL/1U translocations

	Chromosome number of selfed progeny		
Parents	44	45	
304	17	_	
306	21	2	
348	22		
Totals	60	2	

chromosomal aberrations, deletions and dicentric chromosomes.

To reaffirm that the three translocations were preferentially transmitted, 43-chromosome monosomic addition individuals (42W+4S¹/1U) from each of the three progenies were selected and those forming 21 bivalents and a single univalent at metaphase I of meiosis were allowed to self-fertilize. With only two exceptions, the progeny produced from these plants contained 44 bibrachial chromosomes, demonstrating that the translocations are preferentially transmitted (Table 2). The two exceptions, derived from the selffertilization of plants monosomic for the 306 translocation, contained 45 chromosomes, indicating trisomy for an, as yet unidentified, chromosome. In contrast to the three test-cross progenies CS × 306, $CS \times 348$, and $CS \times 304$, none of the progeny derived from self-fertilization of the 42W+4SL/1U individuals contained chromosome aberrations.

Discussion

The transmission frequency of alien monosomes added to the euploid wheat complement is normally low. When these plants are allowed to self pollinate, about 75 per cent of the progeny are euploid (2n = 42, noalien chromosome), about 25 per cent are monosomic additions (2n = 43, including one alien chromosome),and only a small proportion are disomic additions (2n = 44, including a pair of alien chromosomes)(Evans & Jenkins, 1960; Islam et al., 1981; Miller, 1982). However, for the three translocated chromosomes produced in the current study, all but two of the 62 progeny derived from self-fertilization of the 42W+4S¹L/1U individuals possessed 44 chromosomes and none contained less than 44 chromosomes. Furthermore, the majority of progeny produced from each of the three crosses with the euploid CS possessed β -Amy-S'1 and the relevant 1U marker. It is therefore concluded that the three 4SIL/1U translocations are preferentially transmitted.

If the translocations are to be exploited they must be stable, i.e. they must not revert at high frequency into their component telosomes. Misdivision of the translocation was detected only four times in the three test-cross progenies, and never in individuals derived from self-fertilization of the monosomic additions (42W+4S¹L/1U). These data demonstrate that the three translocations are sufficiently stable for use in plant breeding programmes.

If the unrelated alien monosomes in a double monosomic addition act independently their frequencies of fission and transmission should be predictable from those observed in the two relevant single monosomic additions. The frequencies of occurrence in the progeny of the newly formed telosomes 4S1L, 1UL and 1US, from their respective complete monosomes, have been observed to be 0.89 (King et al., 1991b), 6.0 and 5.1 per cent (Koebner & Shepherd, 1987), and one therefore would expect to obtain individuals simultaneously carrying the two telosomes 4S¹L and 1UL, or 4S¹L and 1US at frequencies of 0.05 and 0.04 per cent. respectively, equivalent to 1.0 (4SIL and 1UL) and 0.8 (4S¹L and 1US) in a population of 2039. Although individuals carrying a 4S¹L and a 1U telosome were not observed, three 4S¹L/1U translocations were isolated which seems to indicate that the products of misdivision of chromosomes 4S¹L and 1U are very likely to fuse if they occur in the same cell as suggested by Sears

Several individuals from each test-cross progeny contained chromosomal aberrations such as deletions and dicentric chromosomes. In contrast the progeny derived from self-fertilization of plants monosomic for the translocations did not. The significant difference here could be that in the former case only the pollen contained 4S¹L, and hence chromosome aberrations were expected (Endo, 1988), while in the latter case both male and female viable gametes should have contained 4S¹L.

The three translocations described in this work demonstrate the potential of utilizing the long arm of chromosome 4S1 for the production of stable addition lines. However, the successful application of these translocations to breeding programmes will depend on their effect on agronomic characters. Lines which carry the translocations may be inferior to euploid wheat due to either the presence of deleterious genes on the alien chromatin (Knott, 1968; 1980), or increased gene dosage. In the first case, attempts could be made to mutate the deleterious genes, while in the second case, selection for null alleles might alleviate the problem. In some circumstances, the agronomic advantages conferred by the additional chromosomes may be so significant that some reduction in yield is acceptable. For example, chromosome 5E^b from *Thinopyrum bessarabicum* confers tolerance to relatively high salt concentrations (Forster *et al.*, 1988). If a 4S^IL/5E^b translocation can be induced, 44-chromosome plants carrying it may be able to grow in areas where the salt concentration is too high for normal 42-chromosome wheat varieties. In this situation a reduction in yield observed under normal salt concentration, as a result of the alien translocation chromosome, would be acceptable.

Returning to the current study, the translocations involving 1UL carry Glu-U1 encoding two HMW subunits of glutenin, which, by the construction of the stably inherited addition chromosomes described here. have been added to the wheat genome. There is some evidence that adding extra doses of particular HMW subunits, or increasing the number of different subunits carried by a wheat, leads to increases in gluten strength (Rogers, et al., 1990; 1991), which is an important factor in bread-making. There is also evidence that the particular subunits encoded by Glu-U1 in the accession of Ae. umbellulata, used here, increase gluten strength in wheat when a chromosome segment carrying Glu-U1 is substituted for the segment carrying the Glu-A1 locus on chromosome 1A. Thus, there is good reason to believe that the lines produced here would show increases in gluten strength. The effect of the other translocation line, carrying Gli-U1 added to the wheat genome, appears less predictable; in a CS background, gluten strength is enhanced when a chromosome segment carrying this locus replaces the segment carrying Gli-A1 on 1A, but is reduced when it replaces the corresponding segments carrying Gli-B1 on 1B or Gli-D1 on 1D (Islam-Faridi, 1988; Harris, 1983; Rogers et al., 1987). Additionally, there are possible yield advantages associated with segments carrying Gli-U1 (Islam-Faridi, 1988, Harris, 1983). Further work is therefore required to assess the agronomic potential of lines carrying the three 4S1L/1U translocations.

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