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Determination of the transmission frequency of chromosome $4S^l$ of *Aegilops sharonensis* in a range of wheat genetic backgrounds

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Summary. The transmission of chromosome $4S^l$ from *Aegilops sharonensis* was observed in a range of wheat genetic backgrounds. Chromosome $4S^l$ was transmitted at a very high frequency (at least 97.8%) in all crosses. The genetic background appears to only have a small effect on transmission. The frequency of transmission of chromosome $4S^l$ was the same in each genetic background through both the male and female gametes.

Key words: Preferential transmission – *Triticum aestivum* – *Aegilops sharonensis*

Introduction

Bread wheat, *Triticum aestivum* ($2n=6x=42$) belongs to the tribe Triticeae, which also contains numerous wild and cultivated species with which it can be sexually hybridized. The genomes of some of these species are sufficiently diverged from those of hexaploid wheat, so that in hybrids that carry *Ph1*, the gene which prevents allosyndesis (Sears and Okamoto 1958; Riley and Chapman 1958), wheat and non-wheat (alien) chromosomes remain unpaired at meiosis. Thus, it is usually possible to add each pair from an alien genome to the A, B and D genomes of wheat to form a wheat/alien addition series.

The gametic transmission frequency of alien monosomes added to the euploid wheat complement is generally low. When such plants (somatic chromosome number $2n=43$) are allowed to self-fertilize, about 75% of the progeny are euploid ($2n=42$, no alien chromosomes), 25% are monosomic additions ($2n=43$, one alien chromosome) and only a very small proportion are disomic additions ($2n=44$, with two alien chromosomes) (Evans and Jenkins 1960; Islam et al. 1981; Miller 1982). In contrast, the frequency of 44 chromosome progeny produced upon self-fertilization of plants monosomic for *Aegilops sharonensis* chromosome $4S^l$ is close to 100% (Maan 1975; Miller 1982). Plants monosomic for chromosome $4S^l$ produce normal, viable gametes and inviable gametes which undergo chromosome fragmentation. The viable gametes are thought to contain chromosome $4S^l$, whilst the inviable gametes are thought to lack chromosome $4S^l$ (Finch et al. 1984).

If chromosome $4S^l$ is transmitted at a high frequency in a range of genetic backgrounds, it should be possible to exploit the phenomenon of preferential transmission to overcome instability in semidwarf wheat varieties (King et al. 1988) and for hybrid wheat production (King 1990). However, at present the transmission of chromosome $4S^l$ has only been studied in two genetic backgrounds, Chinese Spring (Miller et al. 1982) and Selkirk (Maan 1975). This study extends these observations by investigating the transmission of the $4S^l$ chromosome in a range of different genetic backgrounds and addresses the question of whether transmission frequency is the same through male and female gametes.

Materials and methods

The following genotypes were used: hexaploid wheat varieties April Bearded, Brigand, Cheyenne, Chinese Spring, Holdfast, Hope, Koga II, Little Joss, Lutescens 62; *Triticum sphaerococcum*; Chinese Spring ditelosomic lines for the short arms of chromosomes 4B* (DT4BS) and 4D (DT4DS) (Sears and Sears 1978) and the genetic stocks $4S^l$(4B) and $4S^l$(4D), in which

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* Chromosome 4B has recently been redesignated as chromosome 4A and vice versa (Miller and Koebner 1988). The new designations as agreed by the 7th International Wheat Genetics Symposium are used in this paper. Also, *β-Amy-1* and *Amp-2* nomenclature are used, as amended in McIntosh et al. (1990).

chromosome $4S^l$ is substituted for chromosomes 4B and 4D, respectively, in a Chinese Spring background (Miller et al. 1982).

$4S^l$(4B) and $4S^l$(4D) were crossed to the hexaploid wheats listed above to produce plants simultaneously monosomic for chromosomes $4S^l$ and 4B or 4D. The F_1 hybrids Cheyenne × $4S^l$(4B), Holdfast × $4S^l$(4B) and Koga II × $4S^l$(4B) were testcrossed separately as male and female to DT4BS, whilst the F_1 hybrid April Bearded × $4S^l$(4D) was testcrossed both as male and female to DT4DS. The F_1 hybrids *T. sphaerococcum* × $4S^l$(4B) and Hope × $4S^l$(4B) were testcrossed as male only to DT4BS, whilst the hybrids Little Joss × $4S^l$(4D) and Lutescens 62 × $4S^l$(4D) were testcrossed as male only to DT4DS.

The transmission frequency of chromosome $4S^l$ was determined by using isoelectric focussing (IEF) to detect the presence or absence in each testcross individual of the isozymes β-amylase, encoded by *β-Amy-S*^{l}*1* (Ainsworth et al. 1987), and aminopeptidase (*Amp-S*^{l}*2*, Koebner and Martin 1989). *β-Amy-S*^{l}*1* is known to be located on the long arm of $4S^l$, as is the gene(s) for preferential transmission, whilst *Amp-S*^{l}*2* is located on its short arm (King 1990).

DT4BS and DT4DS were chosen as testcross parents because they lack the genes *β-Amy-B1* and *β-Amy-D1*, respectively. This deficiency allowed the presence or absence of 4B or 4D to be assessed in the testcross progenies by observation of their β-amylase phenotypes.

Isoelectric focussing of mature endosperm extracts was carried out following published procedures (β-amylase: Sharp et al. 1988; aminopeptidase: Koebner and Martin 1989). Aminopeptidase analysis necessitated the presence of the reducing agent dithiothreitol (10 m*M*) in the extraction solution.

Results

β-Amy-S^l*1* controls the production of two isozymes, both of which were visible in addition to the wheat *β-Amy-1* isozymes in the phenotypes of the following hybrids: Chinese Spring × $4S^l$(4B), Hope × $4S^l$(4B), *T. sphaerococcum* × $4S^l$(4B), Lutescens 62 × $4S^l$(4D) and Little Joss × $4S^l$(4D). However, in the hybrids Koga II × $4S^l$(4B), Holdfast × $4S^l$(4B), Cheyenne × $4S^l$(4B) and April Bearded × $4S^l$(4D), only the more anodically focussing isozyme could be detected, as each of these varieties carries 5A-encoded *β-Amy-A1* alleles (April Bearded, Cheyenne and Koga II: *A1b*; Holdfast: *A1d*, Ainsworth et al. 1983), which code for an isozyme cofocussing with the other *β-Amy-S*^{l}*1* product (Fig. 1A, B). It was not possible to detect *β-Amy-B1* (and hence the presence of chromosome 4B) in testcross populations involving Hope, as the β-amylase phenotype of this variety did not include an identifiable *β-Amy-B1* product. *Amp-S*^{l}*2* produces a single isozyme which was visualized in all of the testcross progenies (Fig. 1C), with the exception of those derived from the cross $4S^l$(4D) × April Bearded; the latter carries *Amp-B2b*, the product of which cofocusses with that of *Amp-S*^{l}*2*. The ($4S^l$(4D) × April Bearded) × DT4DS testcross progeny was therefore not screened for *Amp-S*^{l}*2*. The presence or absence of chromosomes $4S^l$, 4B and 4D was determined by inference from the relevant isozyme patterns.

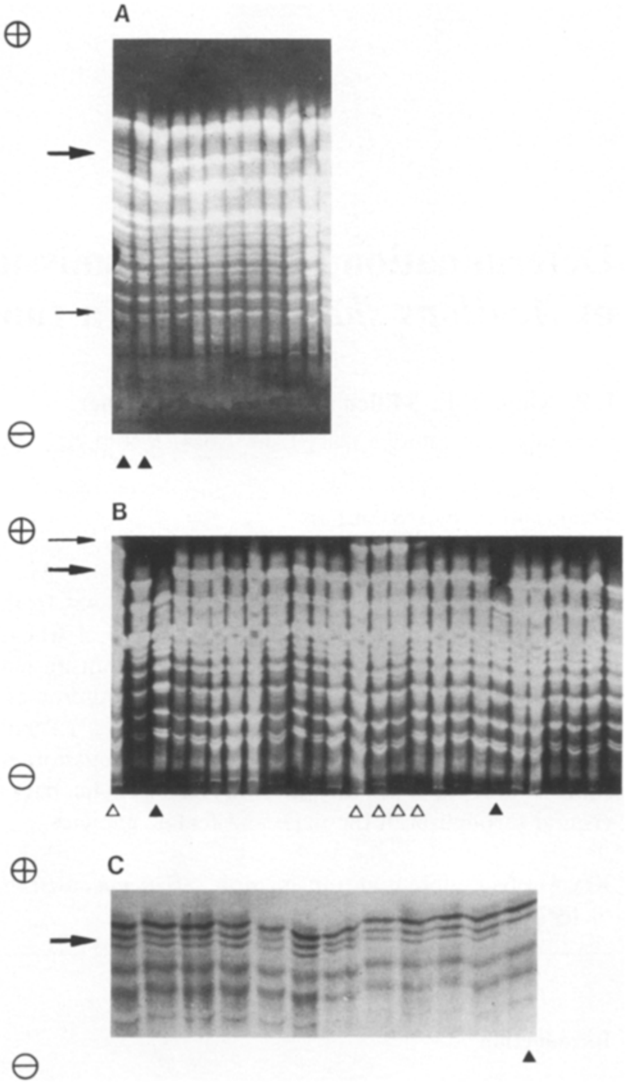

Fig. 1 A–C. Zymograms showing the presence or absence of *β-Amy-S*^{l}*1* and *β-Amy-B1* (**A**); *β-Amy-S*^{l}*1* and *β-Amy-D1* (**B**); *Amp-S*^{l}*2* (**C**). *Closed triangles* indicate tracks that $4S^l$-encoded isozymes, whilst *large arrows* indicate the positions of $4S^l$-encoded isozymes. **A** ($4S^l$(4B) × Koga II) × DT4BS. *Small arrow* indicates the position of the *β-Amy-B1* encoded isozyme. **B** [$4S^l$(4D × April Bearded)] × DT4DS. *Open triangles* indicate tracks that contain the isozyme encoded by *β-Amy-D1*, whilst the *small arrow* indicates its position on the gel. **C** [$4S^l$(4B) × *T. sphaerococcum*] × DT4BS

The transmission rates of *β-Amy-S*^{l}*1* and *Amp-S*^{l}*2* through the male and female gametes in a number of genetic backgrounds, shown in Table 1, were analysed by log linear contingency Chi-square analysis to test for homogeneity. The transmission frequency of *β-Amy-S*^{l}*1* and *Amp-S*^{l}*2* (and therefore, by inference, chromosome $4S^l$) appeared to be independent of whether the gamete was derived from the pollen or the egg, within each genetic background. Similar analysis showed that *β-Amy-S*^{l}*1* was not transmitted at the same frequency through the gametes of each of the five [4S^l(4B) × variety] genetic backgrounds ($\chi^2_{[4]}=14.57$, $P=0.006$, male and female data pooled), but was transmitted at the same frequency through the gametes of each of the [4S^l(4D) × variety] genetic backgrounds ($\chi^2_{[2]}=2.752$, $P=0.253$, male and female data pooled, (Table 2). The transmission of *Amp-S^{l}2* was independent of the genetic background [for the five 4S^l(4B) backgrounds, $\chi^2_{[4]}=5.69$, $P=0.224$; for the two 4S^l(4D) backgrounds, $\chi^2_{[1]}=2.704$, $P=0.100$]. The difference in the transmission frequency of *Amp-S^{l}2* and *β-Amy-S^{l}2* is presumably the result of misdivision of the complete 4S^l chromosome into its component telocentrics followed by the transmission of only one of these into a viable gamete containing chromosome 4S^l.

Although the majority of individuals in the testcross populations [variety × 4S^l(4B)] × DT4BS and [variety × 4S^l(4D)] × DT4DS possessed both *β-Amy-S^{l}1* and *Amp-S^{l}2*, a number of individuals had lost one or both of these loci (Table 2). Five of these lacked *Amp-S^{l}2* but retained *β-Amy-S^{l}1*, and contained two telocentric chromosomes, suggesting that the arm of chromosome 4S^l that carries *Amp-S^{l}2* was absent. Ten individuals lacked *β-Amy-S^{l}1* but retained *Amp-S^{l}2*. Of these, only three germinated and one died before it was possible to obtain root tips for cytological analysis. The second individual possessed 40 bibrachial and two telocentric chromosomes. One of the telosomes was 4BS, whilst the other was probably 4S^{l}S, a misdivision product of chromosome 4S^l. Twenty-two individuals derived from self-fertilization of this plant carried 40 bibrachial chromosomes and either one or two telocentric chromosomes. However, none of these plants possessed *Amp-S^{l}2*, indicating that there was strong selection against gametes carrying 4S^{l}S. The remaining selection carried 41 bibrachial and one telocentric chromosome and was male sterile. Six hybrid grains were obtained by cross-pollination with Chinese Spring. Analysis of these grains revealed the presence of *β-Amy-S^{l}1* throughout, despite the confirmed absence of the expression of this gene in the parental endosperm. Both the male sterility and the *β-amy-S^{l}1* expression are anomalous and this line is currently being studied in more detail. The seven individuals carrying *Amp-S^{l}2* but lacking *β-Amy-S^{l}1*, which failed to germinate, are assumed to have lost all, or a segment of, the long arm of chromosome 4S^l on which *β-Amy-S^{l}1* is located. None of the six individuals that lacked both *β-Amy-S^{l}1* and *Amp-S^{l}2* germinated. These embryos are assumed to have lost the complete 4S^l chromosome. A further two individuals of the cross [April Bearded × 4S^l(4D)] × DT4DS lacked *β-Amy-S^{l}1*. However, it was not possible to determine if the other arm was present, as these crosses could not be scored for the presence of *Amp-S^{l}2*. Neither of these grains germinated.

Table 1. Transmission of *β-Amy-S^{l}1* and *Amp-S^{l}2* through the male and female gametes in various backgrounds

Background	Gamete	*β-Amy-S^{l}1*		*Amp-S^{l}2*	
		+	−	+	−
[4S^l(4B) × Cheyenne] × DT4BS	male	95	4	96	3
	female	86	4	89	1
[4S^l(4B) × Koga II] × DT4BS	male	91	6	93	4
	female	17	0	17	0
[4S^l(4B) × Holdfast] × DT4BS	male	27	0	27	0
	female	20	0	20	0
[4S^l(4D) × April Bearded] × DT4DS	male	46	2		
	female	24	0		

+ = presence of *β-Amy-S^{l}1* or *Amp-S^{l}2*
− = absence of *β-Amy-S^{l}1* or *Amp-S^{l}2*

The frequency of transmission of 4B from the double monosomics 4S^l(4B) × Cheyenne, Koga II × 4S^l(4B), Holdfast × 4S^l(4B) and of 4D in April Bearded × 4S^l(4D) (Table 3) was independent of whether the

Table 2. Overall transmission of *β-Amy-S^{l}1* and *Amp-S^{l}2* (male and female data pooled)

β-Amy-S^{l}1 *Amp-S^{l}2*	+			−			Totals
	+		−	+		−	
[4S^l(4B) × *T. sphaerococcum*] × DT4BS	95		1	0		0	96
[4S^l(4B) × Cheyenne] × DT4BS	177		4	8		0	189
[4S^l(4B) × Holdfast] × DT4BS	46		0	0		0	47
[4S^l(4B) × Koga II] × DT4BS	108		0	2		4	114
[4S^l(4B) × Hope] × DT4BS	56		0	0		0	56
[4S^l(4D) × Little Joss] × DT4DS	28		0	0		0	28
[4S^l(4D) × Lutescens 62] × DT4DS	28		0	0		2	30
[4S^l(4D) × April Bearded] × DT4DS[a]		70			2		72
Totals	539	70	5	10	2	6	632

[a] [4S^l(4D) × April Bearded] × DT4DS testcross progeny were only screened for *β-Amy-S^l 1*

+ = presence of *β-Amy-S^{l}1* and/or *Amp-S^{l}2*

− = absence of *β-Amy-S^{l}1* and/or *Amp-S^{l}2*

Table 3. Transmission of *β-Amy-B1* or *β-Amy-D1* through the male and female gametes of four genotypes derived from crosses involving either $4S^l(4D)$ or $4S^l(4D)$

β-Amy-B1	Gamete	+	−
[$4S^l(4B)$ × Cheyenne] × DT4BS	male	28	71
	female	25	65
[$4S^l(4B)$ × Koga II] × DT4BS	male	17	80
	female	3	14
[$4S^l(4B)$ × Holdfast] × DT4BS	male	3	24
	female	2	18
β-Amy-D1			
[$4S^l(4D)$ × April bearded] × DT4DS	male	8	40
	female	5	19

+ = presence of *β-Amy-B1* or *β-Amy-D1*
− = absence of *β-Amy-B1* or *β-Amy-D1*

Table 4. Transmission of *β-Amy-B1* and *β-Amy-D1* in a range of genotypes

β-Amy-B1	+	−
[$4S^l(4B)$ × *T. sphaerococcum*] × DT4BS	23	73
[$4S^l(4B)$ × Cheyenne] × DT4BS	53	136
[$4S^l(4B)$ × Koga II] × DT4BS	20	94
[$4S^l(4B)$ × Holdfast] × DT4BS	5	42
β-Amy-D1		
[$4S^l(4D)$ × Little Joss] × DT4DS	10	18
[$4S^l(4D)$ × April Bearded] × DT4DS	13	59
[$4S^l(4D)$ × Lutescens 62] × DT4DS	6	24

+ = presence of *β-Amy-B1* or *β-Amy-D1*
− = absence of *β-Amy-B1* or *β-Amy-D1*

gamete was derived from the pollen or the egg. The overall transmission of chromosome 4B (Table 4) appeared to be affected by the genetic background ($\chi^2_{[3]} = 9.44$, $P = 0.024$, transmission = 22.7%), while the transmission of chromosome 4D was not (for 4D $\chi^2_{[2]} = 3.48$, $P = 0.176$, transmission = 22.6%). The transmission frequency of chromosome 4B in gametes that had lost one or the other arm of chromosome $4S^l$ was 46.7%, i.e. higher than the overall transmission frequency of chromosome 4B. This may be a result of a selective advantage of $21 + t$ chromosome gametes over those with $20 + t$ (which possess a telocentric $4S^l$ chromosome, but lack chromosome 4B). The six individuals that had lost the complete $4S^l$ chromosome all possessed the relevant wheat chromosome (either chromosome 4B or 4D). This, however, is not surprising as gametes lacking chromosome $4S^l$ and 4B (or 4D) would be aneuhaploid and would thus be at a selective disadvantage over euhaploid gametes.

Discussion

The statistical analysis indicated that the transmission of *β-Amy-S^l1*, located on $4S^l$L, and by inference the genes for preferential transmission, also located on $4S^l$L (King 1990), may be affected by genetic background. However, none of the genetic backgrounds contained a gene(s) that substantially suppressed the action of the gene(s) for preferential transmission. This is in contrast to the situation found with the preferentially transmitted chromosome 3C of *Ae. triuncialis* where a gene, designated *Igc1*, which suppresses the transmission of chromosome 3C, is present in certain Japanese varieties (Tsujimoto and Tsunewaki 1985). No distinction between the frequency of transmission of chromosome $4S^l$ through the male or female gametes was observed. Again, this is in contrast to chromosome 3C, which is transmitted at a higher frequency through the female gametes in some genetic backgrounds (Endo 1979). Out of the total population of 632 testcross individuals, 614 (97.2%) had the *β-Amy-S^l1* marker and, by inference, the gene(s) for preferential transmission. Of the 18 plants that lacked *β-Amy-S^l1*, only two germinated. Excluding the anomalous individual mentioned above, the overall transmission of the $4S^l$L arm to viable progeny was 99.8% (630/631).

A possible reason for the failure of 16 grains to germinate and give rise to viable plants could be due to the chromosome fragmentation property of chromosome $4S^l$. Plants monosomic for chromosome $4S^l$ produce two types of gametes. At first pollen grain mitosis, one type of gamete behaves normally, but the other shows a high frequency of chromosome breakage. Finch et al. 1984) postulated that the normal gametes contain chromosome $4S^l$, while the abnormal ones lack chromosome $4S^l$, where the absence of $4S^l$ results in chromosome fragmentation, ultimately leading to the failure of these gametes to contribute to the next generation. However, some gametes that lack chromosome $4S^l$ might occasionally undergo only minor chromosome fragmentation, and these may survive to fuse with an egg cell. The resulting embryo would therefore possess chromosomal aberrations that might be sufficiently serious to prevent germination. A similar situation is thought to occur in certain lines carrying a single *Ae. triuncialis* or *Ae. cylindrica* cuckoo chromosome, where in some genetic backgrounds gametes lacking 3C are thought to undergo severe chromosome damage and die, while in other backgrounds, chromosome damage is reduced, so that gametes without 3C survive and give rise to viable progeny with chromosome aberrations (Endo 1988; Tsujimoto and Noda 1988). The situation observed with chromosome 3C, however, differs from that observed with chromosome $4S^l$, as in some genetic backgrounds many of the progeny derived from gametes that have lost 3C are nonetheless viable. Therefore, $4S^l$ possibly causes a higher level of chromosome fragmentation than 3C, so that gamete survival rate is lower in the former case. It should be noted that the five testcross individuals that lacked *Amp-S^{l}2* but had retained *β-Amy-S^{l}1* were all viable. This suggests that the long arm of chromosome 4S^l [and possibly the gene(s)] that conditions preferential transmission also conditions protection against chromosome fragmentation.

No distinction between the transmission frequency of chromosomes 4B and 4D was observed through the male and female gametes. Both 4B and 4D were transmitted at a frequency of approximately 23% although the frequency of transmission of 4B appeared to be effected by the genetic background, while the transmission of chromosome 4D was not. The overall frequency of transmission is similar to the random transmission of a univalent through the female gametes, i.e. 25% (Sears 1954). Thus, in the presence of a complete 4S^l chromosome, there appears to be no selection for or against either male or female gametes without chromosome 4B (or 4D).

The work in this paper has demonstrated that chromosome 4S^l is transmitted at a high frequency in a range of varieties through both the male and female gametes. It should therefore be possible to utilize chromosome 4S^l to ensure the preferential transmission of agronomically useful genes.

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