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An assessment of the homoeology of six *Agropyron intermedium* chromosomes added to wheat

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Summary

Six wheat/*Agropyron intermedium* addition lines are described on the basis of their phenotype and biochemical markers. An assessment of homoeology of each addition chromosome is made. Chromosome morphology, plant phenotype, isozyme and protein studies are compared with similar data for other wheat/alien addition lines and other members of the *Triticeae*. These comparisons give consistent results and it is concluded that addition lines L1, L2, L3, L4, L5 and L7 carry *Agropyron* chromosomes of homoeologous groups 7, 3, 1, 4, 5 and 6 respectively. This agrees with previously published work with one exception: the L5 chromosome belongs to homoeologous group 5 and not group 2 as proposed by Figueiras *et al.* (1986).

1. Introduction

Agropyron species have an enormous potential for wheat improvement; useful genes include those for disease and pest resistance, salt, drought and cold tolerance (Cauderon, 1979). *Agropyron intermedium* is rich in genes conferring disease resistance. A hybrid between bread wheat (*Triticum aestivum* cv. 'Vilmorin 27') and hexaploid *A. intermedium* was produced as a first step in the transfer of genes to wheat (Cauderon, 1966). From this hybrid a partial amphiploid, TAF 46 ($2n = 56$), six disomic addition lines (L1, L2, L3, L4, L5 and L7) and two ditelosomic addition (L7d1 and L7d2) lines were produced in 'Vilmorin 27' by Cauderon. The disomic addition lines have all been intercrossed and are known to carry distinct *A. intermedium* chromosomes. However, the homoeology of the added chromosomes is unknown. The definitive way of assessing homoeology is to substitute alien chromosomes and demonstrate compensation for loss of a particular wheat chromosome. This is time consuming. A quicker approach is to compare the plant morphology of addition lines with those of tetrasomic lines of wheat; similar morphologies between an addition line and a homoeologous group of wheat tetrasomics can be indicative of homoeology (Miller & Reader, 1987). Likewise the presence of homoeoloci, on addition and wheat chromosomes, affecting a range of qualitative characters including pigmentation, isozymes and grain proteins can be used as additional evidence of homoeology (Hart, 1973; Payne *et al.* 1986; Miller & Reader, 1987).

This paper combines evidence from the study of chromosome and plant morphology and marker genes in determining the homoeology of the six *A. intermedium* chromosomes present in the 'Vilmorin 27' *A. intermedium* addition series.

2. Materials and Methods

The plant material used was produced by Y. Cauderon and consisted of *Triticum aestivum* cv. 'Vilmorin 27' ($2n = 6x = 42$, AABBDD), *Agropyron intermedium* (*Thinopyrum intermedium*) ($2n = 6x = 42$, $E_1E_1E_2E_2XX$ or $E_1E_1E_2E_2$NN, Cauderon 1958), the partial amphiploid between 'Vilmorin 27' and *A. intermedium*, TAF 46 ($2n = 8x = 56$, AABBDDXX), disomic addition lines L1, L2, L3, L4, L5 and L7 ($2n = 44$, 21 pairs of wheat and 1 pair of *Agropyron* chromosomes) and ditelosomic addition lines L7d1 and L7d2 ($2n = 44$, 21 pairs of wheat and one pair of *Agropyron* telocentric chromosomes).

Cytological studies at both mitosis and meiosis were carried out on material stained by the Feulgen technique. Plant morphology was studied on plants grown in a glasshouse. Isozymes were separated by flat-bed isoelectric focusing in polyacrylamide gels. Table 1 gives details of the enzymes and the procedures used. Endosperm proteins were fractionated as follows: high-molecular-weight glutenin subunits by

Table 1. *List of enzymes, enzyme symbols, gene symbols, tissue used, pH gradient of gel and assay reference*

Enzyme	Enzyme symbols	*Gene symbol*	Tissue used	pH gradient of gel	Reference for assay
α-Amylase-1	α-AMY-1	*α-Amy-1*	Endosperm	3·5–9·5	Gale *et al.* 1983
α-Amylase-2	α-AMY-2	*α-Amy-2*	Endosperm	4·0–6·5	Gale *et al.* 1983
Esterase	EST-5	*Est-5*	Endosperm	3·5–9·5	Ainsworth *et al.* 1984
Glucose-phosphate isomerase	GPI-1	*Gpi-1*	Endosperm	3·5–9·5	Chojecki & Gale 1982
Phosphoglucomutase	PGM-1	*Pgm-1*	Endosperm	4·0–6·5	Benito *et al.* 1983
Triosephosphate isomerase	TPI-2	*Tpi-2*	Young leaf	4·0–5·0	Pietro & Hart 1985
Trypsin inhibitor	TI-2	*Ti-2*	Endosperm	4·0–6·0	Koebner 1987

sodium dodecyl sulphate, polyacrylamide-gel electrophoresis (SDS–PAGE) as described by Payne *et al.* (1980); gliadins by acid; polyacrylamide-gel electrophoresis (APAGE) as described by Bushuk & Zillman (1978); and the group 5 non-storage proteins by isoelectric focusing in the first dimension and SDS-PAGE in the second as described by Jackson *et al.* (1980).

3. Results

The phenotypes of the lines studied in this paper are as follows:

TAF 46

The partial amphiploid contains 56 chromosomes which form 28 bivalents, or occasionally, 26 bivalents and a quadrivalent at meiosis. Of the 56 chromosomes four pairs have satellites, therefore in addition to the wheat chromosomes 1B and 6B the other two pairs must derive from *A. intermedium*. The morphology of the amphiploid is distinct, the seedlings have purple coleoptiles and the mature plants are tall with long, lax, tapering and slightly brittle ears. The leaves are glabrous.

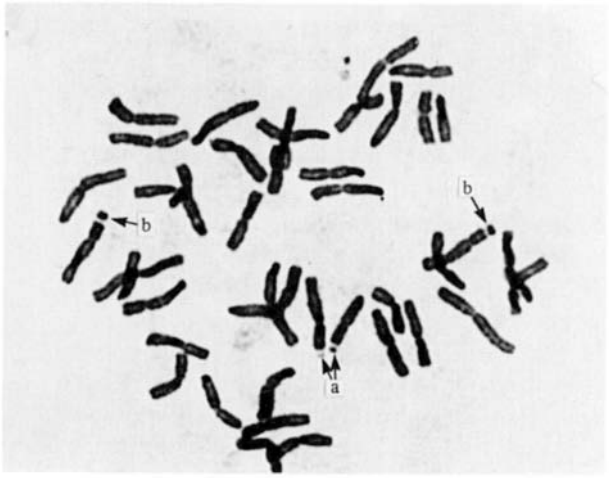

Fig. 1. Root tip chromosome spread of *A. intermedium*. Satellited chromosomes a and b are found in the addition lines L3 and L5 respectively.

L1

This addition line is shorter than 'Vilmorin 27'. The ears are very lax and tapering with supernumary florets. The coleoptile and straw are purple. L1 is resistant to black rust (Cauderon 1966) and carries *Agropyron* grain peroxidase (Cauderon *et al.* 1978) and acid phosphatase genes (Figueiras *et al.* 1986). An *Agropyron* α-AMY-2 isozyme is found in L1 (Fig. 2*a*).

L2

Morphologically L2 is similar to 'Vilmorin 27'. However, the ears are smaller with a slightly brittle rachis at maturity and the flag leaves are hairy. L2 expresses *Agropyron* isozymes for glutamic oxaloacetic transaminase (GOT-2) and malate dehydrogenase (MDH-2) (Figueiras *et al.* 1986) and carries resistance to brown rust (Cauderon 1966). Like TAF 46, L2 expresses two distinct grain esterase isozymes (Fig. 2*b*).

L3

Mitotic chromosome analysis showed L3 to carry the alien pair of chromosomes with the very small satellite, also seen in *A. intermedium* (Fig. 1). The nucleolus organiser region (NOR) is subterminal on the short arm of this alien chromosome. Plants of L3 are the shortest of all the addition lines and have many tillers with small ears. Electrophoretic analysis showed the presence of two *A. intermedium* grain GPI-1 isozyme bands plus a hybrid dimer (Fig. 2*c*, see also Figueiras *et al.* 1986). L3 also expresses *Agropyron* genes for HMW-glutenin (*Glu-1*) (Fig. 3*a*) and ω-gliadin (*Gli-1*) (Fig. 3*b*) (Cauderon *et al.* 1978).

L4

Plants of L4 are similar in height to 'Vilmorin 27', but have a distinct phenotype. They are erect in habit with square parallel sided ears. Analysis confirmed the presence of an extra *A. intermedium* grain PGM-1 band (Fig. 2*d*, see also Figueiras *et al.* 1986). L4 also carries *Agropyron* genes *Adh-1* (Hsam & Zeller, 1982) and *β-Amy-1* (Cauderon *et al.* 1978).

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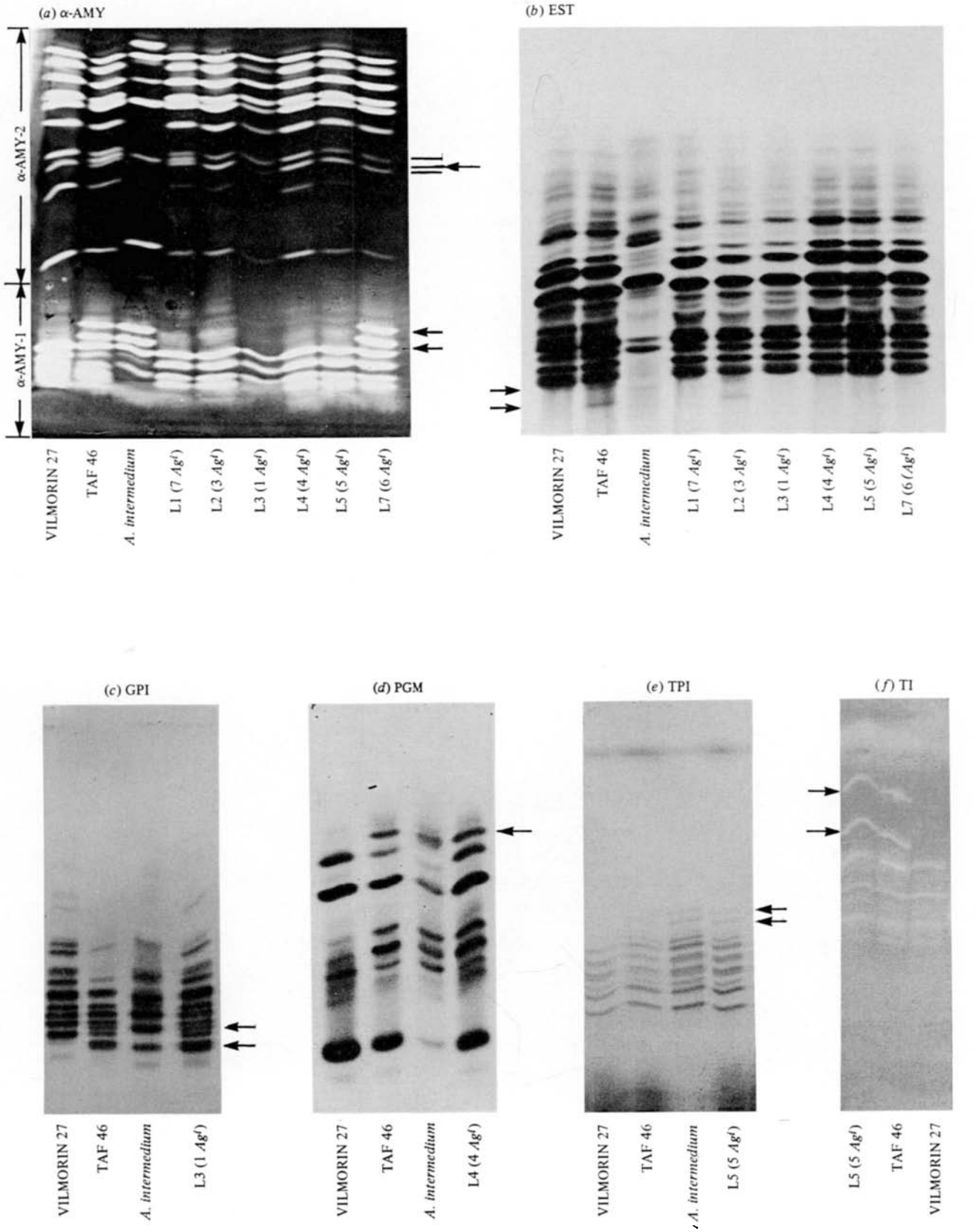

Fig. 2. Zymograms of α-AMY-1, α-AMY-2, EST-5, GPI-1, PGM-1, TPI-2 and TI-2. *Agropyron* bands present in the addition lines are arrowed.

L5

This addition carries the pair of *A. intermedium* chromosomes with the larger satellite (see Fig. 1). The NOR is located in a submedian position on the short arm of this alien chromosome and not subterminally as on the L3 addition chromosome. Phenotypic differences from 'Vilmorin 27' include a marked

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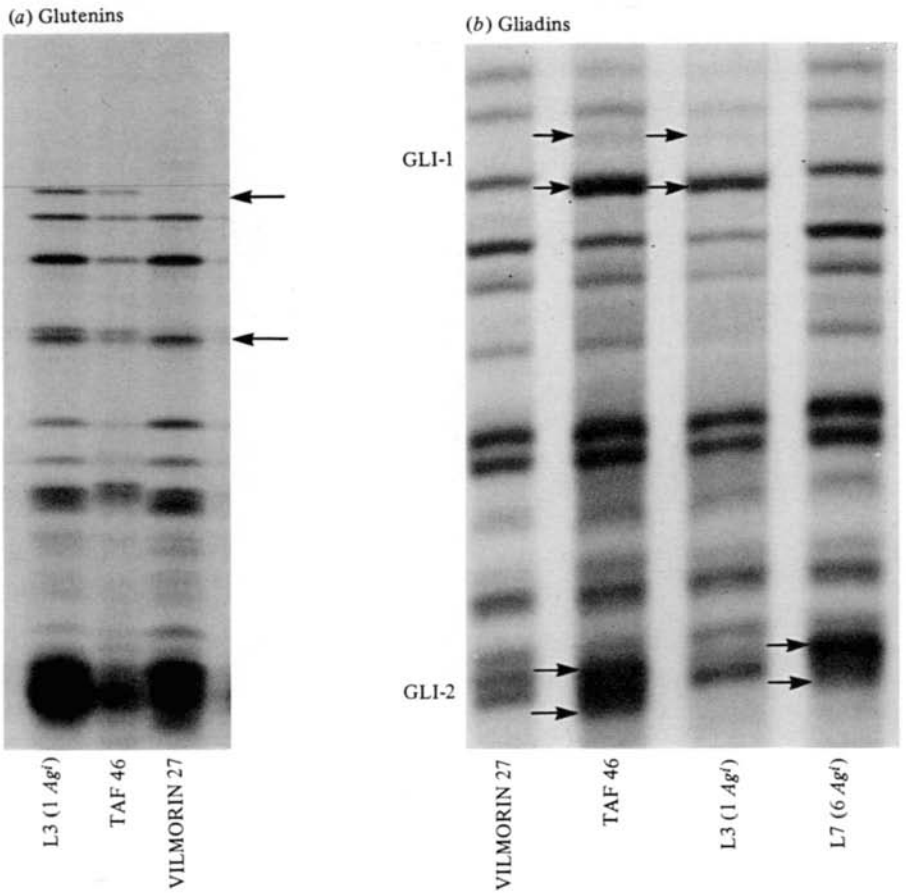

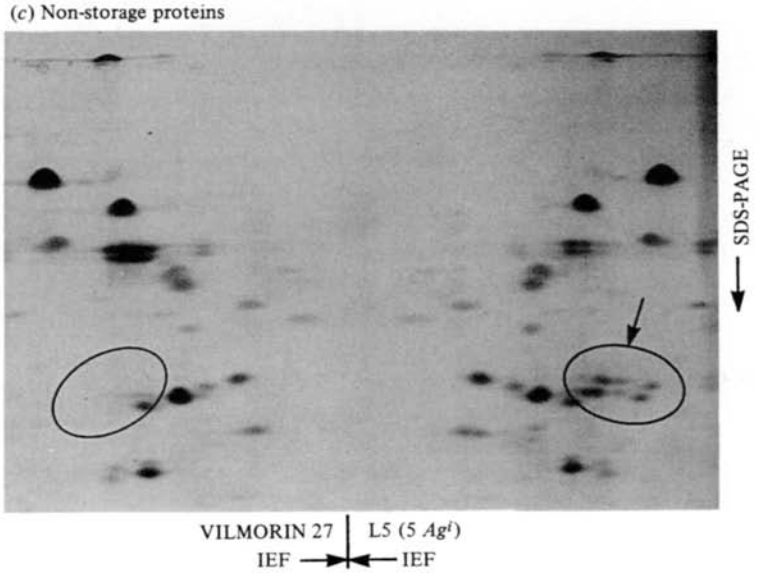

Fig. 3. Fractionation of HMW glutenin subunits, gliadins and group 5 non-storage endosperm proteins. *Agropyron* proteins are indicated.

susceptibility to powdery mildew, short straw, laterally flattened and tapered ears with elongated glumes. The *Agropyron* isozymes TPI-2 and TI-2 are expressed in L5 (Figs. 2*e*,*f*). This line also contained a group of endosperm proteins (Fig. 3*c*), shown to be associated with group 5 homoeologues in wheat (Payne *et al.* 1985).

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L7

Plants of L7 are the tallest of all the addition lines, being similar to TAF 46 in height. Like the amphiploid the leaves are glabrous. The ears are small with rounded glumes and rounded grains. Biochemical analyses showed the presence of Agropyron α-AMY-1 (Fig. 2*a*), and gliadin bands (GLI-2) (Fig. 3*b*). L7 is resistant to stripe rust (Cauderon & Rhind 1976) and carries *A. intermedium* esterase genes (Figueiras *et al.* 1986). The ditelosomic addition line L7d1 also expresses *Agropyron Gli-2* genes (Fig. 3*b*), but not *Agropyron α-Amy-1*, whereas line L7d2 expresses *Agropyron α-Amy-1*, but not *Agropyron Gli-2*.

4. Discussion

These descriptions of the cytological, morphological and biochemical features of the six addition lines provide a good indication of the homoeology between the *A. intermedium* and *Triticum aestivum* chromosomes.

Satellited chromosomes of many species of the *Triticeae* belong to homoelogous groups 1, 5 and 6 (Miller *et al.* 1983). The satellited addition chromosomes of lines L3 and L5 therefore probably belong to two of these three groups. The plant morphology of L3 suggests homoeology of the addition chromosome to group 1. This conclusion is supported by the presence of *A. intermedium* homoeoalleles for *Gli-1, Glu-1* and *Gpi-1*, all of which are normally associated with group 1 chromosomes (McIntosh, 1985). Plant morphology of L5 is that expected of a group 5 addition (Miller & Reader, 1987). Moreover, the novel bands for TPI-2, TI-2 and non-storage endosperm proteins found in this line are all determined by genes with homoeoalleles located on group 5 chromosomes. Figueiras *et al.* (1986) proposed that L5 belongs to homoeologous group 2 on the evidence of increased staining intensity of leaf peroxidase bands, explained as being due to overlapping wheat and *A. intermedium* isozymes, and decreased intensity of a 'Vilmorin 27' alkaline phosphatase band. Such evidence can be inconclusive since band intensity is often affected by differences in protein concentration between samples. The possibility of L5 being a Robertsonian centric fusion product between chromosomes of group 2 and 5 of *A. intermedium* is also unlikely since group 5 markers are found on both chromosome arms: the satellite is located on the short arm, and *Tpi-2* (Pietro & Hart, 1985), *Ti-2* (Koebner, 1987) and non-storage protein genes (Payne *et al.* 1985, Payne *et al.* 1986 and Forsyth unpublished) have all been mapped to the long arm of wheat group 5 homoeologues. Moreover, L5 shows none of the typical morphological effects of additional group 2 chromosomes (Miller 1984).

Line L1 has a plant morphology consistent with additions of homoeologous group 7. It has the purple culms and coleoptiles normally associated with the short arms of the group 7 chromosomes and produces an α-AMY-2 isozyme similar to those of the *α-Amy-2* series found on the long arms of wheat chromosomes of this homoeologous group. Figueiras *et al.* (1986) found L1 to express *Agropyron* leaf acid phosphatase isozymes. The genes responsible for this are usually associated with the group 4 chromosomes, with the exception of rye, *Secale cereale*, where *Acph-R1* is located on the chromosome designated 7R, a result of a translocation involving chromosomes 4R and 7R relative to the wheat chromosomes. Such a translocation is ruled out in *A. intermedium* as L1 has a definite marker associated with group 7 on each chromosome arm. The acid phosphatase found by Figueiras *et al.* (1986) probably forms part of another gene series.

L2 has several characteristics associated with the addition of alien chromosomes of homoeologous group 3. The brittle rachis character is typically carried on group 3 chromosomes and the presence of *Agropyron* EST-5 (Fig. 2*c*), GOT-2 and MDH-2 isozymes (Figueiras *et al.* 1986) all support the homoeology of the *Agropyron* chromosome with this group.

L4 has the erect habit and ear morphology of group 4 addition lines. Homoeology of this *Agropyron* chromosome to group 4 is reinforced by the presence of *Agropyron* group 4 marker genes for both chromosome arms; *Pgm-1* and *Adh-1* are located on the opposite arm to *β-Amy-1* in wheat (Benito *et al.* 1984; Hart, 1973; Ainsworth *et al.* 1983).

The L7 line has a plant morphology consistent with group 6 additions and carries *A. intermedium Gli-2* and *α-Amy-1* genes. The *α-Amy-1* series of genes are located on group 6 chromosomes of all the *Triticeae* genomes studied (Ainsworth *et al.* 1987). The absence and presence respectively of *Agropyron α-Amy-1* and *Gli-2* genes in the lines L7d1 and L7d2 indicate that they contain, respectively, the short and the long arm only of the group 6 *Agropyron* chromosome.

A. intermedium has been assigned the genome symbols $E_1E_1E_2E_2XX$ (Dewey, 1984), where *X* denotes an unknown genome. The partial amphiploid TAF 46 does not possess all three genomes of *A. intermedium*. Autosyndetic pairing between the E_1 and E_2 genomes was observed in the original hybrid and subsequent backcrosses (the F1 had up to 27 bivalents, Cauderon, 1966), and this should have favoured the retention of the E genome chromosomes. It is however possible that the *X* genome (N in Cauderon, 1958) has been preferentially retained, or that a mixed genome derived from E_1, E_2 and *X* has been isolated. Since the alien genome of TAF 46 cannot be identified with certainty, gene symbols for the *A. intermedium* genes of TAF 46 should not infer the E or the *X* genomes. We suggest, therefore, that the alien genes carry the genome designation Ag^i (thus, for example, *α-Amy-*Ag^i*1* and *α-Amy-*Ag^i*2*). A list of these new gene symbols is

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given in Table 2. Since the homoeology of the addition chromosomes is now established we propose that both the chromosome and the addition line be named accordingly, i.e. $1Ag^t$ $3Ag^t$ etc. (see Table 2).

There was no evidence of a Robertsonian translocation in any of the addition lines. This was surprising as translocations between alien chromosomes often occur in the development of disomic addition lines. The lack of such translocations could be the result of the autosyndetic pairing observed in the early hybrids.

5. Conclusions

The partial amphiploid, TAF 46, used in this study contains an *A. intermedium* genome of unknown origin consisting of a pair of chromosomes from six of the seven homoeologous groups together with an unknown pair. The *Agropyron* chromosomes present in the six addition lines have been assigned to homoeologous groups. The seventh chromosome which is currently being isolated is expected to be a member of homoeologous group 2.

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Table 2. *A list of the 'Vilmorin 27'*/A. intermedium *addition lines classified by homoeology to wheat, and their associated marker genes*

Addition line (previous name)	Gene symbols (synonyms)	Reference
$1Ag^t$(L3)	*Gli-Ag^{t}1*	Cauderon *et al.* 1978
	Glu-Ag^{t}1	
	Gpi-Ag^{t}1 (Gpi-X1)	Figueiras *et al.* 1986
	Nor-Ag^{t}1	
$3Ag^t$ (L2)	*Est-Ag^{t}5*	
$4Ag^t$ (L4)	*Adh-Ag^{t}1 (Adh-E^{t}1)*	Hsam and Zeller, 1982
	β-Amy-Ag^{t}1 (β-Amy-E^{t}1)	Cauderon *et al.* 1978
	Pgm-Ag^{t}1 (Pgm-X1)	Figueiras *et al.* 1986
$5Ag^t$ (L5)	*Nor-Ag^{t}3*	
	Ti-Ag^{t}2	
	Tpi-Ag^{t}2	
$6Ag^t$ (L7)	*α-Amy-Ag^{t}1*	
	Gli-Ag^{t}2	
$7Ag^t$ (L1)	*α-Amy-Ag^{t}2*	
	Per-Ag^{t}3	Cauderon *et al.* 1978

Other genes mapped by Figueiras *et al.* (1986) include an acid phosphatase on $7Ag^t$ and $4Ag^t$, glutamic oxaloacetic acid transaminase and malate dehydrogenase on $3Ag^t$, peroxidases $7Ag^t$ and $1Ag^t$ and esterase on $6Ag^t$.

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