European Journal of Plant Pathology **104:** 835–847, 1998.

Chromosomal location of genes for resistance to *Puccinia striiformis* in the wheat line TP1295 selected from the cross of Soissonais-Desprez with Lemhi

A. Calonnec[1,3] and R. Johnson[2,4]
[1] *Laboratoire de Pathologie Végétale, Institut National de la Recherche Agronomique, 78850 Thiverval-Grignon, France;* [2] *John Innes Centre, Colney lane, Norwich NR4 7UH, UK;* [3] *Present address: INRA Station de Pathologie Végétale, Domaine de la grande Ferrade, BP 81, 33883 Villenave d'Ornon cedex, France;* [4] *Present address: 16, Coppice Avenue, Great Shelford, Cambridge CB2 5AQ, UK*

Accepted 16 September 1998

Key words: monosomic analysis, yellow (stripe) rust, resistance gene, multidimentional analysis

Abstract

Crosses of a wheat line TP1295 with the cultivar Chinese Spring monosomic series were used to locate, on chromosome 1D, a major gene for resistance to isolate WYR 85-22 of race 6E0 of *Puccinia striiformis*. The gene is designated as *Yr25* and is probably present in several of the cultivars currently widely used for differentiating races of this pathogen. The expression of the gene was modified by the environment and by at least one minor gene which may be located on chromosome 6A. In F_2 and F_3 generations from a cross between TP1295 and euploid Chinese Spring, a wide range of variation in infection type (IT) was observed. This precluded the classification of the plants as either resistant or susceptible, so they were assigned to 6 classes and analyzed by factorial correspondence analysis and non-hierarchical classification. When all F_3 plants in a family were fully resistant, like TP1295 itself (IT ;), both *Yr25* and the modifying gene were assumed to be present and homozygous. In environments favourable to expression of the gene, families thought to carry *Yr25* alone had a distribution of ITs from fully resistant (IT ;) to intermediate (IT 2, rarely 3 or 3+). This F_3 analysis indicated that use of IT data alone, in the monosomic analysis, would not reveal the chromosomal location of the genes and that chromosome counting of numerous plants was necessary. As well as indicating the chromosomes carrying the genes for resistance to isolate WYR 85-22, the data showed that plants monosomic for chromosomes 5B and 5D were more resistant than the corresponding disomics, indicating that these chromosomes promote susceptibility and supporting other evidence of the effects of these chromosomes on yellow rust resistance.

Introduction

The wheat cultivar Soissonais-Desprez (Soissonais) (Mon désir × Hybride à Courte Paille) and the related cultivar Heines VII (H7) (Hybride à Courte Paille × Svalöv) were used as parents of many cultivars selected by the Plant Breeding Institute, Cambridge (PBI) (Lupton and Macer, 1962). H7 is also used as differential cultivar for determining virulence of races of *Puccinia striiformis*, the cause of yellow (stripe) rust (Johnson et al., 1972). A genetic analysis of F_3 families (from Minister × Heines VII and Minister × Soissonais) showed that both cultivars carried the resistance gene *Yr2*, and that other genes might modify its expression in H7 (Lupton and Macer, 1962). Labrum (1981) proposed that *Yr2* was located on chromosome 7B.

H7 and Soissonais were resistant (Infection types ;0 and $;2^+$ respectively) to a PBI isolate (WYR 85-23) of a race designated as 6E16 from Lebanon (Singh and Johnson, 1988). The resistance was not conferred by *Yr2* because the wheat cultivar Kalyansona, possessing *Yr2*, was susceptible to this isolate (IT 3^-4^-, on the Stakman and Levine scale, 1922). Singh and Johnson (1988) reported that a genetic analysis of F_2 populations from H7 × Kalyansona and H7 × Lee, using

WYR 85-23, suggested the presence of two genes in H7, one of which conferred only an intermediate level of resistance. Absence of segregation in the F_2 of H7 × Soissonais with the same race indicated that at least one of the genes in each cultivar might be identical or closely linked. Two lines with spring habit: TP981 and TP1295, were selected to separate this resistance from the gene *Yr2* (Singh and Johnson, 1988). TP981, was from the cross Lemhi × H7 and TP1295 from the backcross ((Lemhi × Soissonais) × Lemhi). Both lines were selected for resistance to WYR 85-23 and susceptibility to race 108E9 (WYR 81-20) which possesses avirulence for *Yr2*.

In addition to H7 four other differential cultivars, Strubes Dickkopf (SD, unidentified resistance genes), Heines Peko (HP) (*Yr2* and *Yr6*), Reichersberg 42 (*Yr7*) and Clement (*Yr9*) were resistant to WYR 85-23 and to another isolate WYR 85-22 identified as race 6E0 (Johnson, 1992) (Table 1). WYR 85-23 and WYR 85-22 possess virulence for *Yr2* and *Yr7*. In addition, WYR 85-22 (6E0) possesses virulence for *Yr6*, *Yr9* and *YrA*, and WYR 85-23 (6E16) for *Yr8*. A genetic analysis in F_3 showed that HP, SD and H7 possessed a resistance gene in common against WYR 85-22 (6E0), and this resistance was enhanced by at least one minor or modifier gene in each of the cultivars (Calonnec et al., 1997). The lack of segregation in F_2 of crosses between TP981 and SD and between TP1295 and HP strengthens the hypothesis that the major resistance gene present in the lines TP981 and TP1295 is also present in the differentials.

Lemhi, the other parent of TP981 and TP1295 is used to differentiate North American races of *P. striiformis* and possesses a gene for resistance to race CDL 21 (Chen and Line, 1992) which might be located on chromosome 1B (Chen et al., 1995a). Lemhi was used as a parent of both TP lines because of its susceptibility to all UK races; however, it was later found to have resistance to WYR 85-23 from outside the UK (Singh and Johnson, 1988). Lemhi also possessed resistance to WYR 85-22, expression of which was dependant on environmental conditions. Consequently part of the resistance selected in both TP lines may come from Lemhi.

TP981 and TP1295 are also resistant to other races that possess avirulence for SD, like 66E0 (isolate WYR 85-26) from Ecuador, 6E18 (isolate J8603) found on spring wheat in southern France, 82E16 (isolate IPO 90002) from Egypt and an Indian race 38A. Because several of the differential cultivars are resistant to the isolates WYR 85-22 and WYR 85-23 absent from western Europe, Johnson (1992) suggested that similar resistance might be present in many west European wheat cultivars.

The objective of this study was to determine the chromosomal location of the resistance of the TP1295 line to isolate WYR 85-22 of race 6E0 by monosomic analysis, in order to establish the identity of this resistance.

Table 1. Virulences (v) of European or non-European races of *Puccinia striiformis* on some wheat varieties with identified yellow rust resistance genes (*Yr*) or unnamed resistance genes (+)

Varieties	European race		Non-European race	
	virulent on *Yr2*, *Yr7*, *Yr9*	virulent on *Yr6*, *Yr9*	6E 0 WYR 85-22	6E 16 WYR 85-23
Lee (*Yr7*)[a]	v		v	v
Heines Kolben (*Yr2*, *Yr6*)[a]			v	
Strubes Dickkopf (+)[a]	v	v		
Clement (*Yr9*, +)[a]	v	v		
Reichersberg 42 (*Yr7*, +)[a]	v			
Heines Peko (*Yr2*, *Yr6*, +)[a]				
Heines VII (*Yr2*, +)[a]	v			
Kalyansona (*Yr2*)	v		v	v
Federation 4× Kavkaz (*Yr9*)	v	v	v	
Austerlitz (*Yr6*, +)		v		
Arcane (*Yr6*, +)		v	v	
TP981 (+)	v	v		
TP1295 (+)	v	v		
Soissonais (*Yr2*, +)	v			
Lemhi (+)	v	v		

[a] Differential variety.

Materials and methods

Wheat stocks

The Chinese Spring monosomic lines, provided by the Cereals Research Department, John Innes Centre, Norwich (UK) and euploid Chinese Spring were used as female parents in crosses with the line TP1295-L-2-9A-23-2.

F_1 monosomic plants were selected by mitotic chromosome counts on root tips from germinated seeds. For each cross, 2 to 5 F_1 monosomic plants were grown in a glasshouse and self-fertilized by bagging the ears. The seed production was rather poor especially for monosomics 1B, 2A, 2B, 2D, and 5A.

The following cultivars and populations were tested with isolate WYR 85-22 of race 6E0: BC1F6 plants from TP1295-L-2-9A-23-2-3 (later called TP1295), Soissonais-Desprez (Soissonais), Chinese Spring (CS), Lemhi, the 21 F_2 populations derived from monosomic hybrid of CS monosomics x TP1295 (later called monosomic F_2 populations) and F_2 populations from CS x TP1295 (later called euploid F_2 populations). Chromosomes were counted in mitotic cells of root tips in each monosomic F_2 populations. Because of the time necessary for preparation of the material for counting, tests were spread over time (Table 2) and some environmental variation occurred between tests.

To determine the number of resistance genes, all plants from the euploid F_2 population from test *d* (euploid F_2 *d*) were grown and selfed for F_3 progeny testing. Seventy-four families from F_2 plants in classes representative of the whole population (Chi-squared test for association, d.f $= 5$, $P = 0.99$) were analyzed. Each F_3 family comprised between 20 and 54 plants (average 43 plants).

Cytology

Seeds were placed at 24°C in Petri dishes on a filter paper moistened with tap water (24 h), followed by 2 days at 4°C to block cell division. Germination was then continued at 24°C (24 h or longer) until the primary and two lateral roots were 1 cm long. Two roots were cut and treated in freshly diluted α-bromonaphtalene for 5 h, fixed in glacial acetic acid overnight and stored in a mixture of Ethanol : Glacial acetic acid (70% : 30%), for up to three months. After the roots were taken, identified seedlings were grown for rust testing. For chromosome counts, roots were rinsed three times in tap water, hydrolysed in 1 N hydrochloric acid at 60°C for 11 min, stained with leuco-basic fuchsin for 30 min and the meristematic tips of roots were squashed in aceto-carmine on a microscope slide. The microscope set up was bright field, with 5× eyepiece and 100× objective with oil immersion.

Rust testing

Germinated seeds were planted in pressed peat pots, each with a single seedling to facilitate transplanting, and grown until GS 12 (Zadoks et al., 1974) (first leaf fully emerged, second leaf partly emerged). Plants were inoculated as described in Calonnec et al. (1997). Glasshouse temperatures ranged from 10°C night to maxima of 20°C in autumn or up to 35°C for short periods in the middle of the day in summer. Tests of F_2 populations were conducted at Norwich with the day-light supplemented by high intensity quartz lamps for 16 h, whereas F_3 families were tested in Grignon with the day-light supplemented by high pressure sodium vapour lamps for 16 h.

Plants were scored for IT about 15 days later, depending on rate of development. Reactions were scored on a scale ";" (non-sporulating fleck), 0 (chlorotic lesions without sporulation) and from 1 to 4 (decreasing chlorosis and increasing sporulation) according to a modified scale of Stakman and Levine (1922) with + and − used to show variation above or below the mean IT for the class. The scores were converted to a 6-class scale as described by Calonnec et al. (1997), with class 1 corresponding to full resistance (fleck ;) and class 6 to full susceptibility.

Mesothetic reactions with more than one IT on one leaf were frequent in some F_3 families. These reactions (IT $0/1^+$ to 0/4) were assigned to class 3 when the second leaf was resistant to intermediate and to class 5 for infection types $0/3^+$ and 0/4 when the second leaf was fully susceptible (IT 4).

Analyses

The ITs were classified into 6 classes, all of which were used for the analyses. Segregations of different F_2 populations into the 6 classes, were tested for homogeneity by Fisher's exact test (Conover, 1980). Each monosomic F_2 population was compared by chi-squared test for association, or Fisher's exact test (for expected counts less than 5), with one of the euploid

Table 2. Time-table for tests of Chinese Spring (CS), BC1F6 from TP1295-L2-9A-23-2-3 (called TP1295), Lemhi and Soissonais-Desprez (Soissonais), F_2 populations CS × TP1295 (called euploid F_2), F_3 families form CS × TP1295, and F_2 populations from the series of 21 CS monosomics × TP1295

Cultivar, F_2 population or F_3 families	Test	Date of sowing	Number of F_2 populations	Total number of seedlings tested
CS	*a*	from the 10th to 18th		13
TP1295		of June 1994		6
Lemhi				7
Soissonais				1
euploid F_2			1	96
1B[a]			5	33
2A			2	8
3B			3	62
4B			2	76
5B			3	84
6A			3	59
7B	*b*	from the 25th June to	2	72
4A		the 1st of July	2	74
5A			3	24
6B			3	75
7A			2	89
3A	*c*	from the 5th June to	2	80
2B		the 9th of July	3	12
1A			2	50
4D			1	63
3D			2	79
1D			2	72
Lemhi	*d*	from the 12th to		9
Soissonais		14th of July		8
euploid F_2			1	98
5D			2	70
6D			2	97
2D			3	22
7D			2	89
CS	*e*	from the 24th to		48
Lemhi		26th of October		19
euploid F_2			1	91
7D			1	31
1D			1	19
6D			1	30
CS	*f*	June 1995		12
Lemhi				41
Soissonais				68
TP1295				21
74 F_3 families, average number of seedlings per F3				43

[a] Female parent of the original cross: Chinese Spring monosomic for chromosome 1B.

F_2 families used as a standard. Monosomic F_2 populations from tests *a* were compared to euploid F_2 *a*, monosomic F_2 populations from tests *d* compared to euploid F_2 *d*, and monosomic F_2 populations from test *e* compared to euploid F_2 *e*. There was no F_2 tested during tests *c* and *b*, therefore monosomic F_2 populations were compared to F_2 euploid *d* which had the distribution the most similar to the whole F_2 monosomics from tests *b* and *c*. Results of the rust tests were supplemented by chromosome counts in the F_2 plants to identify the critical crosses.

F_3 segregations from CS × TP1295 were recorded as the numbers of plants classified into each reaction class and were compared using a multidimensional analysis method described by Calonnec et al. (1997). Families with similar distributions were grouped in clusters by application of a factorial correspondence analysis (FCA) followed by a non-hierarchical classification (NHC) with *k*-means iterations. FCA and NHC were performed by classon2, an Splus function. CS, TP1295, Lemhi, Soissonais tested at the same time as F_3 families and the different euploid F_2 populations tested the year before were used as supplementary units, i.e. they were not used to calculate relative contribution to the axes for the F_3 family analyses but their coordinates were calculated to locate them on the FCA axis 1-2 configuration amongst family clusters determined by FCA-NHC analyses. They could then help the identification of some genotypes associated with clusters of F_3 families.

Results

Expression of resistance in the parents

Line TP1295 was fully resistant (class 1) in tests *a* and *f* (Table 3) whereas both its parents (Lemhi and Soissonais) gave variable reactions. Lemhi gave an intermediate IT in tests *a* and *d* and lower ITs in tests *e* and *f*. In test *f*, Soissonais was less resistant than Lemhi. CS varied in its response with plants ranging from class 4 to class 6.

Number of resistance genes and their expression in euploid crosses

The euploid F_2 populations gave a continuous range of response from class 1, as observed for TP1295, to the classes 4–6, as observed for CS. However, the distributions of the three euploid F_2 populations were significantly different over the 6 classes ($\chi^2 = 41.6$, d.f. $= 10$, $P < 0.01$). Euploid F_2 *d* differed from euploid F_2 *a* with fewer plants in classes 2 and 3 and more in higher susceptibility (classes 5–6) and euploid F_2 *e* showed the reverse with a shift towards greater resistance (Table 3). For euploid F_2 *a*, and euploid F_2 *d* the proportion of fully resistant plants (similar to TP1295) was less than 1/4 suggesting the segregation of more than one gene.

The first FCA and NHC analysis of F_3 families isolated two groups of 63 : 11 families, which did not fit the hypothesis of monogenic segregation (3 : 1, resistant-segregating : susceptible families) where more susceptible families would have been expected ($\chi^2 = 4.05$, d.f. $= 1, P = 0.044$). Further analyses isolated 5 groups of 14 : 11 : 38 : 4 : 7 families. Among the 14 most resistant families (cluster 1, Figure 1) 5 had all plants in class 1 and were coincident on Figure 1. A group of 11 families (cluster 2) was composed of segregating families. A group of 38 families (cluster 3) also segregated but had a higher proportion of plants in classes 2 and 3 (Figure 1). Two of these had no plants in classes 5 and 6 and the majority of plants in class 3 were mesothetic ITs. These two families were therefore considered to be resistant families (sub-cluster 1 – Figure 1). The groups of 4 (cluster 4) and 7 (cluster 5) families differed for the ratio of plants in classes 5 and 6. Cluster 1 plus sub-cluster 1 contained 16 families, which does not deviate significantly from 25% of the 74 F3 families, and hence, indicates the segregation of one major gene. However, the heterogeneity of cluster 1 and the low number (7) of susceptible families (18.5 susceptible families were expected for a hypothesis of one segregating gene) suggested segregation of at least one other resistance or modifier gene.

A genetic model for segregation of two resistance genes, *R1* and *R2* is therefore proposed (Table 4). The 5 families of cluster 1 (1/16th of F_3 families) having 100% of class 1 plants could be homozygous for both genes (*R1R1R2R2*). Two families of cluster 1 with 58% of plants in class 1 plus the two families from sub-cluster 1; these four fitted the expected 1/16 of the F_3 families and could be homozygous only for the stronger gene, hence giving a variable resistance response ranging from class 1 to class 4 (*R1R1r2r2*). Variability in resistance response could also be observed for plants having only one copy of the modifier gene (*R1R1R2r2*). Cluster 2, with 11 families, could fit the expected 1/4 of families segregating for *R1* but with *R2* homozygous,

Table 3. Distribution of plants for resistance to the race 6E0 of *P. striiformis* of monosomic F_2's, euploid F_2's, and controls Chinese Spring (CS), BC1F6 plants from TP1295-L-2-9A-23-2-3 (TP1295), Lemhi and Soissonais–Desprez (Soissonais)

F_2 population or cultivar[a]	Test	Reaction classes						Ph[b]	Pa[c]
		1	2	3	4	5	6		
1A	*c*	9	6	7	9	7	12	0.91	**0.0006**
2A(s)[e]	*a*	1	4	0	0	2	1	1	**0.02**
3A	*c*	19	7	3	7	8	36	0.14	0.31
4A	*b*	10	3	2	6	12	41	0.85	0.99
5A(s)[e]	*b*	8	2	4	2	1	7	0.88	**0.007**
6A	*a*	13	5	4	6	4	27	0.7	0.79
7A	*b*	23	6	2	12	11	35	0.11	0.11
1B(s)[e]	*a*	3	5	3	8	5	9	0.02	0.05
2B(s)[e]	*c*	6	0	1	2	1	2	0.02	**0.01**
3B	*a*	12	11	4	7	7	21	0.55	0.46
4B	*a*	17	10	5	12	10	22	0.13	0.09
5B	*a*	32	9	7	11	9	16	0.05	**0.0003**
6B	*b*	11	10	2	0	6	46	0.76	**0.032**
7B	*b*	9	6	1	11	11	34	0.77	0.31
1D	*c*	3	7	7	11	15	29	0.3	**0.006**
1D(s)[e]	*e*	7	8	0	2	0	2		0.24
2D(s)[e]	*d*	2	2	0	2	2	14	0.79	0.71
3D	*c*	17	7	1	3	10	41	0.16	0.56
4D	*c*	7	12	5	8	6	25		**0.007**
5D	*d*	9	6	2	14	12	27	0.06	0.05
6D	*d*	0	9	7	9	12	60	0.47	**0.0003**
6D	*e*	11	6	2	2	1	8		0.79
7D	*d*	4	11	4	12	15	43	0.29	**0.02**
7D	*e*	6	5	4	6	3	7		0.65
Total of mono. $F_2'S$[d]		180	127	62	135	150	514		0.29
Euploid F_2	*a*	15	14	5	10	5	47		
Euploid F_2	*d*	15	4	3	6	16	54	$\mathbf{9 \cdot 10^{-6}}$	
Euploid F_2	*e*	27	19	7	10	10	18		
CS	*a*	0	0	0	0	8	5		
	e	0	0	0	6	38	4		
	f	0	0	0	1	3	8		
TP1295	*a*	6	0	0	0	0	0		
	f	21	0	0	0	0	0		
Lemhi	*a*	0	4	3	0	0	0		
	d	0	0	2	7	0	0		
	e	11	4	4	0	0	0		
	f	36	5	0	0	0	0		
Soissonais	*a*	1	0	0	0	0	0		
	d	1	6	1	0	0	0		
	f	19	42	6	1	0	0		

[a] Female parent of the original cross: Chinese Spring monosomic for the chromosome indicated or Chinese Spring euploid (euploid F_2).
[b] Ph = Probability of homogeneity of the F_2 populations from a same cross on the 6 classes (Chi-squared test for association or Fisher's exact test for expected number less than 5).
[c] Pa = Probability of association of the monosomic F_{2s} and the euploid F_{2s}. Monosomics F_{2a}, F_{2d} and F_{2e} compared to euploid F_{2a}, F_{2d} and F_{2e} respectively. Monosomics F_{2b} and F_{2c} compared to euploid F_{2d}.
[d] Total of monosomic F_2's except 5B, 2A(s), 5A(s), 1B(s), 2B(s), 1D(s), 2D(s) and probability of association against the total of euploid F_2's.
[e] (s) = F2 populations of small size (less than 35 plants).

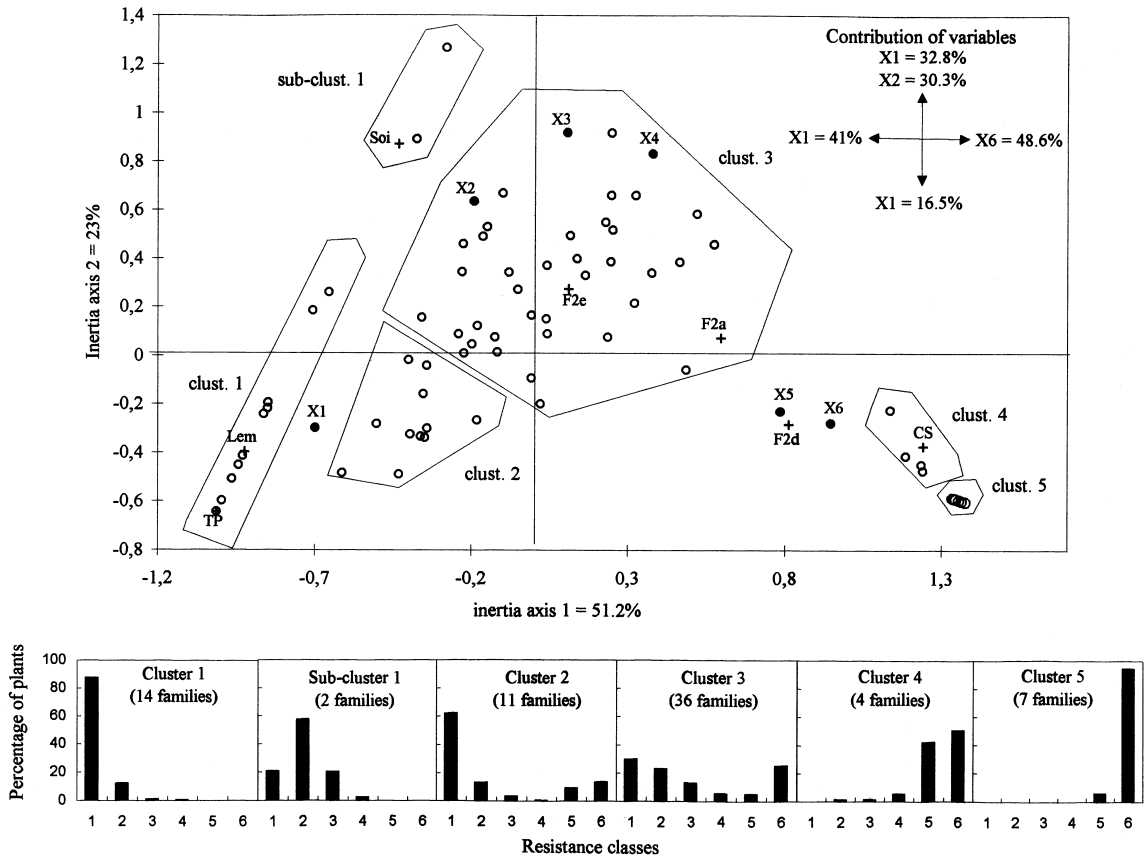

Figure 1. Distribution for resistance for each cluster of F_3 families determined by a non-hierarchical classification and position of each of the 74 F_3 families from Chinese Spring × TP1295, on the FCA axes 1–2 diagram (open circles). The supplementary individuals are TP1295 (TP, 21 plants), Lemhi (Lem, 41 plants), Soissonais-Desprez (Soi, 68 plants), F_2 from Chinese Spring euploid × TP1295 for tests *a*, *d* and *e* (F2a, F2d, F2e) and Chinese Spring (CS, 12 plants) (crosses). The six variables are denoted X1 to X6 (black points). Contribution of the most informative variables on the two axes 1 and 2 are indicated in percentage.

giving in the F3 genotypes *R1R1R2R2*, *R1r1R2R2* and *r1r1R2R2*. Cluster 3 could then be assumed to contain progenies from F_2 genotypes *R1r1R2r2*, *R1r1r2r2* and *r1r1R2R2*. Clusters 4 and 5 could show progenies from F_2 *r1r1R2r2* and *r1r1r2r2* respectively.

The TP1295 line and Lemhi were located in cluster 1 on the FCA 1-2 axes configuration, Soissonais was located close to sub-cluster 1, and CS in cluster 4. The location of the parents of TP1295 could lead to the hypothesis that Lemhi possessed the two genes whereas Soissonais possessed only one gene, but it does not indicate whether the TP1295 line received genes from both parents. The two batches of euploid F_2 tested in *e* and *a* were located in cluster 3 but euploid F_2 *d* was located between cluster 3 and 4 showing a higher proportion of susceptible plants. Lemhi was more susceptible during tests *a* and *d* (Table 3) and the position of euploid F_2 *d* on the FCA diagram suggested that a gene in Lemhi may have variable expression possibly dependant on environmental conditions. The position of CS in cluster 4 also indicates that gene(s) in CS could contribute to resistance.

Interpreting gene expression

On the basis on the proposed genetic model and the observed data, the resistance gene expression is analysed for different tests. 16 F_2 plants from test *d* generated resistant F_3 families, and were parents of cluster 1 and sub-cluster 1 families. They were assumed to be *R1R1*, irrespective of the condition of the genotype at the second locus, and were distributed from class 1 (11 plants) to class 5 (1 plant) (Table 4). In conditions of the test of F_3 families, similar to conditions

Table 4. Presumed genotypes and observed phenotypes for F_2 plants which are parents of the five clusters of F_3 families for the cross Chinese Spring × TP1295 line

F_2 plants							F_3 family				
Presumed genotype	Observed number of plants for each disease class[a]						Cluster	Number	Range of disease classes[b]	Frequency expected	Number expected
	1	2	3	4	5	6					
R1R1R2R2	5	0	0	0	0	0	1	5	1	1/16	4.62
R1R1R2r2	5	1	1	0	0	0		7	1–3	2/16	9.25
R1R1r2r2	1	1	1	0	1	0	1, sub-1	4	1–4	1/16	4.62
R1r1R2R2	0	0	0	2	6	3	2	11	1–6	2/16	9.25
R1r1R2r2	0	1	0	3	4	28	3	36	1–6	7/16	32.37
R1r1r2r2 *r1r1R2R2*											
r1r1R2r2	0	0	0	0	0	4	4	4	2–6	2/16	9.25
r1r1r2r2	0	0	0	0	0	7	5	7	5–6	1/16	4.62

[a] Under condition of test *d*.
[b] Under condition of test *e*.

of test *e*, plants with *R1R1* were distributed mainly in classes 1 and 2 and, more rarely, in classes 3 (3.4%) and 4 (0.4%) (cluster 1 and sub-cluster 1). The 47 F_2 plants that generated segregating F_3 families (Figure 1) were distributed from classes 2 (1 plant) to 6 (31 plants) (Table 4). Therefore, in the conditions of tests *a* to *d*, all F_2 plants in class 1 should have at least the genotype *R1R1*, but F_2 plants scored as class 1 in test *e* might also have included *R1r1*, since that test tended to be biased to higher resistance (Table 3). *R1r1R2R2* could comprise plants in classes 1–3 according to the distribution of plants in cluster 2 (1/4 *R1R1R2R2*, 1/2 *R1r1R2R2*, 1/2 *r1r1R2R2)*.

Expected ratios in monosomic analysis

Theoretically, in all monosomic F_2 and euploid F_2 populations, about 25% of plants should have genotype *R1R1* (Table 5). In the critical cross where CS was monosomic for the chromosome carrying *R1*, an additional 73% (approximately) of plants will be hemizygous for *R1* (i.e. *R1*-). In the other monosomic and euploid crosses, only a further 50% of plants will be *R1r1*. Therefore, assuming that all F_2 plants in class 1 in tests *a* to *d*, were *R1R1*, no excess of plants in class 1 can be expected in the critical cross compared to F_2 euploid populations. However, if the diagnosis is correct that plants heterozygous *R1r1* or hemizygous *R1*- gave intermediate to susceptible responses, a higher proportion of plants with intermediate responses could occur for the critical cross, with approximately 73% of hemizygous plants, compared with only 50% heterozygous plants in the non-critical crosses. The segregation of *R2r2* and *R2R2* would tend to shift the reactions towards resistance but the effect should be the same in both F_2's, so that the critical monosomic F_2 population should have a higher proportion of plants with intermediate ITs and fewer susceptible plants. Deviations towards a higher proportion of susceptible plants would be assumed to be due to chance, because these could not arrise from the segregation of chromosomes carrying resistance. Although no significant deviation for fully resistant plants (class 1) was expected for any chromosome, counting the chromosomes should allow the identification of those carrying the resistance genes. For the critical cross for *R1*, no nullisomic plant should occur in class 1 and no disomic plant (all *R1R1*) should be fully susceptible (class 6). Indeed, the majority of disomic plants (*R1R1*) should be fully resistant, whereas the distribution of disomic plants (*R1R1*, *R1r1*, *r1r1*) in the non-critical crosses should not be significantly different from the euploid F_2, covering all response classes.

The identification of the critical cross for the minor gene (*R2*) is more difficult: nullisomics could be from fully resistant to fully susceptible depending on the genotype at the *R1* locus and disomics *r1r1R2R2* could be fully susceptible. However, in the critical cross for the minor gene, disomic plants would have three genotypes, *R1R1R2R2* (1/4), *R1r1R2R2* (1/2), and *r1r1R2R2* (1/4) and therefore show, on average, a higher proportion of class 1 plants and a lower proportion of plants in class 6 than euploid F_2 populations.

Table 5. Expected ratios and number of plant chromosomes in case two independent genes segregate in F_2 of Chinese Spring (CS) × TP1295 (euploid F_2), CS monosomic for the chromosome carrying the major resistance gene × TP1295 (critical cross for R1), CS monosomic for the chromosome carrying the minor gene × TP1295 (critical cross for R2), CS monosomic for any other chromosome × TP1295 (non critical cross)

euploid F_2	genotypes	*R1R1R2R2*	*R1R1R2r2*	*R1R1r2r2*	*R1r1R2R2*	*R1r1R2r2*	*R1r1r2r2*	*r1r1R2R2*	*r1r1R2r2*	*r1r1r2r2*
	chromomes	42	42	42	42	42	42	42	42	42
	percentage[a]	6, 25	12, 5	6, 25	12, 5	25	12, 5	6, 25	12, 5	6, 25
critical cross	genotypes	*R1R1R2R2*	*R1R1R2r2*	*R1R1r2r2*	*R1 - R2R2*	*R1 - R2r2*	*R1 - r2r2*	*- - R2R2*	*- - R2r2*	*- - r2r2*
for *R1*	chromomes	42	42	42	41	41	41	40	40	40
	percentage[a]	6	12	6	18,25	36,5	18,25	0,75	1,5	0.75
critical cross	genotypes	*R1R1R2R2*	*R1R1R2-*	*R1R1- -*	*R1r1R2R2*	*R1r1R2 -*	*R1r1- -*	*r1r1R2R2*	*r1r1R2 -*	*r1r1- -*
for *R2*	chromomes	42	41	40	42	41	40	42	41	40
	percentage[a]	6	18,25	0,75	12	36,5	1,5	6	18,25	0,75
non critical cross	genotypes	*R1R1R2R2*	*R1R1R2r2*	*R1R1r2r2*	*R1r1R2R2*	*R1r1R2r2*	*R1r1r2r2*	*r1r1R2R2*	*r1r1R2r2*	*r1r1r2r2*
	chromomes	42 41 40	42 41 40	42 41 40	42 41 40	42 41 40	42 41 40	42 41 40	42 41 40	42 41 40
	percentage[a]	1.5 4.56 0.18	3 9.12 0.37	1.5 4.56 0.18	3 9.12 0.37	6 18.25 6	3 9.12 0.37	1.5 4.56 0.18	3 9.12 0.37	1.5 4.56 0.18

[a] A monosomic plant selfed produced on average 24% of disomic, 73% of monosomic and 3% of nullisomic plants (Morris and Sears, 1967).

Indeed, in euploid F_2 populations, each genotype *R1R1* (1/4), *R1r1* (1/2) and *r1r1* (1/4) would be combined with all possible combinations of segregation at the *R2* locus and not only with *R2R2* (Table 5).

Observed ratios in monosomic F_2 populations

Reactions to yellow rust. For monosomic F_2 populations 1B, 2A, 2B, 2D and 5A the deviation in rust reaction from the euploid F_2 distribution was not judged because of the small population size plus the fact that they were coming from different F1 plants (Table 3). Monosomic F_2 populations 1A, 1D, 6D, 7D deviated significantly from the euploid F_2population, with higher proportions of intermediate reaction classes 2–4, and monosomic 6B F_2 had a higher proportion of susceptible plants. Monosomic F_2 populations 1D, 6D and 7D (tests *c* and *d*) were retested (test *e*) and no deviations from the euploid occurred (Table 3). Monosomic 5B F_2 showed a significantly higher proportion of resistant plants (Table 6).

Chromosome counts

Small population. Among monosomic F_2 populations,1B, 2B, 2D and 5A, $2n = 42$ plants fully susceptible and/or a $2n = 40$ plant fully resistant were found, casting doubt on 1B, 2B, 2D and 5A as candidates on which R1 would be located. No conclusion could be reached for chromosome 2A because only two plants were counted.

Deviating populations. Among monosomic F_2 1A and 5B that deviated from the F_2 euploids, monosomics 1A did not have disomic plants in class 6 and chromosome 1A could potentially carry *R1*. However, a $2n = 40$ plant (probably nullisomic for chromosome 1A) was fully resistant casting doubt on 1A as candidate (Table 6). In order to understand the significant deviation for an excess of resistant plants for monosomic 5B F_2, chromosomes were counted in 35 plants from one F_2. Five disomic plants were found in class 6 and one $2n = 40$ (possibly nullisomic) plant in class 1 suggesting that chromosome 5B could not carry the major resistance gene. There was an abnormally high proportion (45%) of $2n = 42$ plants (Table 6), but the distribution for resistance of $2n = 42$ plants did not differ from the distribution of euploid F_2 *a* (Fisher's exact test, $P = 0.41$) suggesting that 5B is unlikely to carry the minor gene. The distribution of plants monosomic for 5B differed from the control with more resistant plants (Fisher's exact test, $P = 1.6 \cdot 10^{-4}$).

Populations with no deviation from the euploid F_2. Each F_2 populations 3A, 4A, 7A, 3B, 4B, 6B, 7B, 3D, 4D, 5D, 6D, 7D showed at least one $2n = 42$ plant that was fully susceptible. In the monosomic 1D F_2, all $2n = 42$ plants were fully resistant, all with $2n = 41$ were intermediate to susceptible and a probable nullisomic susceptible. The distributions for rust response of disomics and monosomics 1D plants differed from the F_2 euploid *e* (Fisher's exact test, $P = 0.04$ for $2n = 42$ plants and $P \leq 0.001$ for $2n = 41$ plants). This suggested that chromosome 1D carries resistance gene *R1*.

For chromosome 6A, $2n = 42$ plants ranged in response from fully resistant to intermediate (class 4, IT 3) with more class 1 plants than euploid F_2 *a* (Fisher's exact test, $P = 0.02$) and no fully susceptible $2n = 42$ plants. Chromosome 6A was therefore postulated to carry the gene *R2*. The response distribution of $2n = 41$ plants did not differ from that of the euploid F_2 *a* (Fisher's exact test, $P = 0.63$).

The monosomic 5D F_2 population did not deviate significantly from the euploid F_2 *d*. However, chromosome counts were obtained for 38 plants representative of the distribution of the whole population (Fisher's exact test, $P = 0.62$). The distribution for response of $2n = 42$ plants did not deviate from the euploid F_2 (Fisher's exact test, $P = 0.18$) but there was a significant deviation for more resistant plants among $2n = 41$ individuals (Fisher's exact test, $P = 6.6 \cdot 10^{-3}$).

Discussion

By a combination of disease reactions and chromosome counts, a major gene (*R1*) for yellow rust resistance was located on chromosome 1D, on which no gene for resistance to this disease has previously been located. It is therefore suggested that *R1* is different from previously identified genes and the designation *Yr25* is proposed. The same or a closely linked gene might be present in the differential cultivars Heines VII, Heines Peko, Strubes Dickkopf, Clement and Reichersberg 42 (Calonnec et al., 1997). Plants homozygous for *Yr25* are expected to express resistance as IT 0; (zero fleck) to 1, but they sometimes gave higher ITs up to 2 and 3 or, rarely, even 3^+. The expression of *Yr25* was enhanced by at least one minor gene *R2*. Plants thought to possess

Table 6. Resistance reaction of plants and their chromosome number, for a sample of each F_2 population from the series of the 21 Chinese Spring monosomic lines crossed with the TP1295 line

Female parent[a]	nbr chr.	Test	Reaction classes 1	2	3	4	5	6
1A (*)	42	*c*	1				2	
	41		2	1				1
	40		**1**					
2A (s)	42	*a*					1	
	41		1					
	40							
3A	42	*c*	2	1				**2**
	41		2	2				8
	40							1
4A	42	*b*	1		1			**2**
	41		2			1		3
	40							
5A (s)	42	*b*	2					**1**
	41		1	1	2			4
	40							
6A	42	*a*	4	2		1		
	41		2	2	1	1	3	13
	40							
7A	42	*b*	2					**3**
	41		3	1				3
	40							
1B (s)	42	*a*						**2**
	41				4			1
	40							
2B (s)	42	*c*	1					**1**
	41		3					
	40		**1**					
3B	42	*a*	1	2			1	**1**
	41			2				7
	40		1	1				
4B	42	*a*	3	2		1		**3**
	41		4					
	40		**1**					
5B(*)	42	*a*	3	1	2	3	1	**5**
	41		9	6	2		2	
	40		**1**					
6B	42	*b*	1	1	1			**5**
	41		3	2			1	6
	40							
7B	42	*b*	4					**3**
	41		4	1				3
	40							
1D	42	*e*	7					
	41			8		2		1
	40							1
2D (s)	42	*d*						**2**
	41		1	2			1	4
	40							
3D	42	*c*	3					**3**
	41		2					2
	40							
4D	42	*c*		1				**2**
	41		1	2				2
	40							
5D	42	*d*	1			2		**2**
	41		7	3	2	7	6	7
	40						1	
6D(*)	42	*d*		4		1		**1**
	41			3		2	1	9
	40			1				
6D	42	*e*	1					
	41		2				1	2
	40							
7D (*)	42	*d*	2					**3**
	41		2	1				1
	40							
7D (*)	42	*e*	1					**2**
	41		3					1
	40							

[a] Female parent of the original cross: Chinese Spring monosomic for the chromosome indicated.
(*)Population deviating from the F_2 euploid.
(s) Population of small size.

both genes homozygously always produced IT 0;. The gene *R2* was tentatively located on chromosome 6A, although further data are required before a symbol can be allocated to it.

It is not clear from which parent, (Lemhi or Soissonais), TP1295 inherited these genes. If the major gene in TP1295 is the same as the allelic gene of H7, it is probably not inherited from Soissonais, because the F_3 from H7 × Soissonais segregated for resistance to isolate WYR 85-22 of race 6E0 (Calonnec, 1996). A gene for resistance to USA race CDL21 was identified in Lemhi and located on chromosome 1B (Chen et al., 1995a). This gene was different from *Yr10* and *Yr15* also located on chromosome 1B because Lemhi is susceptible to races avirulent for both these genes. In the present work, no gene was detected on chromosome 1B with race WYR 85-22, suggesting either that it possesses virulence for the gene, or that the gene described by Chen and Line (1995a) in Lemhi was not selected in the line TP1295. However, the gene in TP1295 on chromosome 1D might be a different gene derived from Lemhi.

Most yellow rust resistance genes are located on chromosomes of A and B genomes (McIntosh et al., 1995), except the two adult plant resistance genes *Yr16* on chromosome 2D (Worland and Law, 1986) and *Yr18* on 7D (Singh, 1992). Recently, resistance genes were proposed on chromosome 6D by monosomic analysis of the North American differential cultivars Fielder and Tyee and on chromosome 5D in the American cultivar Daws. However, these genes have unusual patterns. They are expressed at the seedling stage either as dominant genes that are not expressed when hemizygous (gene on 6D) or as recessive and expressed when hemizygous (gene on 5D) (Chen et al., 1995a,b), unfortunately, the localization data are not adequately supported by cytological analysis.

The first gene for yellow rust resistance located by monosomic analysis was reported on chromosome 6A of Cometa Klein, a cultivar from Argentina (Singh and Swaminathan, 1959). It was a recessive gene and acted as complementary to another resistance gene on chromosome 4B (earlier designated as 4A), for resistance to the Indian race H. TP1295 was not tested for resistance to the race H; however, Cometa Klein was also resistant to the Indian race 31 to which TP1295 is resistant (Nayar S.K., Indian Agricultural Research Institute, Simla, personal communication). Perhaps the gene in Cometa Klein for resistance to race H, is the same as the minor gene *R2* described in the present study and indicated to be on 6A for resistance to isolate WYR85-22 of race 6E0.

The euploid F_2 populations showed variable resistance depending on environmental conditions and gave a wide range of infection types consistent with the action of both a major and a minor gene that also displayed incomplete dominance. It was therefore necessary to compare the monosomic F_2 populations with the euploid populations rather than with a theoretical ratio (Knott, 1989). Classification of this variation into only two classes, viz. resistant and susceptible plants, as is usually done for monosomic analyses, would not have identified chromosomes carrying the resistance genes. For example, classification of the data into the two classes 1–4 and 5–6 fitted the hypothesis one dominant or partially dominant gene in euploid F_2 *e* but gave no significant deviation for monosomic 1D. Classification into the two classes 1 and 2–6 (class 1 being the resistance of TP1295) would lead to the conclusion of one recessive gene and no deviation would have been expected or observed for chromosome 1D. Therefore, if no chromosome counting was done and if any other cross deviated by chance, the location of the resistance gene could be misdiagnosed.

It was necessary to study F_3 families of the euploid crosses to understand the expression of the major gene and the presence of minor genes, and also to determine possible deviations that could be expected in the monosomic progenies. This showed that chromosome counts on significant numbers of plants is essential for identification of the chromosomes carrying the genes. Excesses of intermediate and susceptible responses observed for some monosomic populations was attributed to chance. Excesses of susceptible plants as deviations from theoretical ratios were found in monosomic analyses of cultivars such as Lemhi, Fielder, Clement and Tyee with various races but were not attributed to statistical chance (Chen et al., 1995a,b).

The higher resistance of plants monosomic for chromosomes 5B and 5D of TP1295, compared with disomics in the same crosses or with the euploid F_2 (Table 6) could be the consequence of the presence of genes promoting susceptibility on these chromosomes in TP1295 and in CS. This corresponds with evidence of Pink et al. (1983) that long arms of chromosomes of homologous group 5 carried genes that increased susceptibility of CS to yellow rust. It has also been demonstrated that the long arms of chromosomes 5A and 5D of Cappelle-Desprez, Hybride de Bersée and

Hobbit sib all carry genes promoting susceptibility to yellow rust in the field (Law et al., 1973; Worland and Law, 1995). Results in the present paper demonstrate similar effects for these chromosomes at the seedling stage.

The results presented in this paper demonstrate the value of using a multivariate analysis method, combined with extensive cytological data to investigate the chromosomal location of genes for resistance to yellow rust by monosomic analysis, using aneuploids of the cultivar CS. This permitted the identification and chromosome location of a major resistance gene for resistance to yellow rust of wheat, *Yr25*, on chromosome 1D. The probable presence of this gene in several of the differential cultivars in current use is important for the diagnosis of virulence in *P. striiformis*.

Acknowledgments

AC thanks the Ministère de la Recherche et de l'Espace for a post-graduate grant and A. J. Worland for training in cytology. We are grateful for financial assistance from the British Council and INRA Alliance programme.

References

Calonnec A, Johnson R and de Vallavieille-Pope C (1997) Genetic analysis of resistance to *Puccinia striiformis* in the wheat differential cultivars Heines VII, Heines Peko and Strubes Dickkopf. Plant Pathology 46, 373–386

Calonnec A (1996) *Analyse génétique des variétés différentielles de blé utilisées dans la caractérisation des races de* Puccinia striiformis *agent de la rouille jaune*. France: Université Paris-sud, Ph.D. thesis

Chen X and Line RF (1992) Identification of Stripe rust resistance genes in wheat genotypes used to differentiate north American races of *Puccinia striiformis*. Phytopathology 82: 1428–1434

Chen X, Jones SS and Line RF (1995a) Chromosomal location of genes for stripe rust resistance inspring wheat cultivars Compair, Fielder, Lee and Lemhi and interactions of aneuploid wheats with races of *Puccinia striiformis*. Phytopathology 85: 375–381

Chen X, Line RF and Jones SS (1995b) Chromosomal location of genes for resistance to *Pucciniastriiformis* in winter wheat cultivars Heines VII, Clement, Moro, Tyee, Tres, and Daws. Phytopathology 85: 1362–1367

Conover WJ (1980) Practical Nonparametric Statistics, 2nd Edition. (p. 162), New York, John Wiley and Sons

Johnson R (1992) Reflections of a plant pathologist on breeding for disease resistance, with emphasis on yellow rust and eyespot of wheat. Plant Pathology 41: 239–254

Johnson R, Stubbs RW, Fuchs E and Chamberlain NH (1972) Nomenclature for physiologic races of *Puccinia striiformis* infecting wheat. Transactions of the British Mycological Society 58: 475–480

Knott DR (1989) The Wheat Rusts – Breeding for Resistance. (pp. 109–125), Springer-Verlag, Berlin

Labrum KE (1981) The location of *Yr2* and *Yr6*, genes conferring resistance to yellow rust. Proceedings of the Fifth European and Mediterranean Cereal Rusts Conference (1980), Bari and Rome, Italy, 41–45

Law CN, Gaines RC, Johnson R and Worland AJ (1979) The application of aneuploid techniques to a study of stripe rust resistance in wheat. Proceedings of the Fifth International Wheat Genetics Symposium (1978). (pp. 427–436), New Dehli, IARI

Lupton FG and Macer RCF (1962) Inheritance of resistance to yellow rust (*Puccinia glumarum* Erikss. and Henn.) in seven varieties of wheat. Transactions of the British Mycological Society 45: 21–45

McIntosh RA, Hart Gen and Gale MD (1995) Catalogue of gene symbols for wheat. Proceedings ofthe Eighth International Wheat Genetics Symposium (1993) Beijing, China, 1333–1451

Morris R and Sears ER (1967) The cytogenetics of wheat and its relatives. In: Heyne EG (ed.) Wheat and Wheat Improvement. (pp. 19–87), USA Madison: American Society for Agronomy

Pink DAC, Bennet FGA, Caten CE and Law CN (1983) Correlated effects of homoeologous group 5 chromosomes upon infection of wheat by yellow rust and powdery mildew. Zeitschrift für Pflanzenzüchtung 91: 278–294

Singh H and Johnson R (1988) Genetics of resistance to yellow rust in Heines VII, Soissonais and Kalyansona. Proceedings of the Seventh International Wheat Genetics Symposium (1987), TE Miller, RMD Koebner (eds.). (pp. 885–890), IPSR Cambridge, England

Singh MP and Swaminathan MS (1959) Monosomic analysis in bread wheat. III. Identification of chromosomes carrying genes for resistance to two races of yellow rust in Cometa Klein. Indian Journal of Genetics 19: 171–175

Singh RP (1992) Genetic association of leaf rust resistance gene Lr34 with adult plant resistance to stripe rust in bread wheat. Phytopathology 82: 835–838

Stakman EC and Levine MN (1922) The determination of biologic forms of*Puccinia graminis* on *Triticum* spp. Technical Bulletin of the Minnesota agricultural Experimental Station 8: 1–10

Worland AJ and Law CN (1986) Genetic analysis of chromosome 2D of wheat. I. The location of genes affecting height, day-length insensivity, hybrid dwarfism and yellow-rust resistance. Zeitschrift für Pflanzenzüchtung 96: 331–345

Worland AJ and Law CN (1995) Improving adult plant resistance to rusts and mildew in the wheat variety 'Hobbit sib'. Proceedings of the 8th International Wheat Genetics Symposium. Li ZS, Xin ZY (eds.). (pp. 907–913), China Agricultural Scientech Press, Beijing

Zadoks JC, Chang TT and Konzak GF (1974) A decimal code for the growth stages of cereals. Weed Research 14: 415–421