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Nuclear and cytoplasmic gene control of resistance to loose smut (*Ustilago tritici* (Pers.) Rostr.) in wheat (*Triticum aestivum* L.)

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Summary. Using disomic chromosome substitution lines based on the susceptible wheat cultivar 'Chinese Spring', loose smut resistance of wheat cultivars 'Hope' and 'Thatcher' was shown to be conferred in each case by a single dominant major gene carried on chromosome *7A* ('Hope') or *7B* ('Thatcher'). Partial resistance was determined by genes on an additional eight 'Hope' or seven 'Thatcher' chromosomes, and similarities were evident between the partial resistance genotypes of 'Hope' and 'Thatcher'. 'Chinese Spring' exhibited a mean infection value of approximately 50%, indicating a significant level of partial resistance, which was found to be due, in part, to genes on the homoeologous chromosome arms $1A^S$, $1B^S$ and $1D^S$, and to cytoplasmic genes. Substitution of the 'Chinese Spring' nucleus into the cytoplasm of *Aegilops squarrosa, Ae. variabilis* or *Ae. mutica* resulted in increased susceptibility to *Ustilago tritici*. Several alloplasmic lines of the resistant wheat cultivars 'Selkirk' and 'Chris' exhibited race-specific susceptibility to *U. tritici*.

Key words: *Triticum* – *Ustilago tritici* – Chromosome substitution lines – Nuclear-cytoplasmic interactions – Disease resistance

Introduction

Ustilago tritici (Pers.) Rostr. is the fungus which causes the seed-borne disease termed wheat loose smut; plants grown from infected grains (following ovary infection) produce heads in which the entire floral tissue (with the exception of the rachis and awns) is replaced by a dark brown mass of ustilospores. The disease is found in all the wheat-growing countries of the world, being particularly prevalent in the more humid areas.

Relatively little has been published on the genetics or mechanisms of loose smut resistance in wheat. Conventional genetic studies on resistance to *U. tritici* involve progeny testing of inoculated plants in segregating populations. This strategy is feasible for analysis of complete resistance, which is due generally to one or two dominant genes (Tikhomirov 1983; Nielsen 1987), but is difficult to apply to partial resistance, although inoculation of reciprocal F_1 hybrids has revealed a maternal component of partial resistance to *U. tritici* in certain crosses (Gaskin and Schafer 1962). In this paper, we report on genetic analysis of complete and partial resistance to *U. tritici* using substitution (chromosomal and cytoplasmic) and ditelosomic wheat lines.

Materials and methods

Plant Material

The wheat (*Triticum aestivum*) genotypes used in this study were as follows: 'Chinese Spring' ('Hope') disomic chromosome substitution lines (seed provided by Dr. C.N. Law, Plant Breeding Institute, Cambridge, UK), 'Chinese Spring' ('Thatcher') disomic chromosome substitution lines (Prof. E. Sears, University of Missouri, USA), 'Chinese Spring' ditelosomics (Dr. C.N. Law), 'Chinese Spring' alloplasmic series (Prof. K. Tsunewaki, Kyoto University, Japan) and 'Chris' and 'Selkirk' alloplasmic series (Prof. S.S. Maan, North Dakota State University, USA).

Plants (from inoculated or non-inoculated seed) were grown in 12.5-cm plastic pots of soil-less compost, comprising three parts (by volume) Shamrock moss peat (Bord na Móna, Ireland) to one part washed sand; fertiliser (Bio P Base; Pan Britannic Industries, UK) was added at the rate of 1.5 kg m^{-3} compost. Plants were grown under glasshouse conditions, with natural daylight supplemented daily by 16-h illumination from 400 W

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high-pressure sodium plant irradiators. A minimum temperature of 18 °C was maintained throughout the year. Attacks of powdery mildew were controlled using sprays of Karathane (Rohm and Haas) because of the possible influence of systemic fungicides on *U. tritici,* and aphids were controlled by glasshouse fumigation with Bladafum (Bayer). Plants were routinely supported to prevent lodging.

Fungal material

Ustilago tritici races T1, T6 and T39 (Nielsen 1987) were used. Freshly-harvested mature spores were removed from the rachis of smutted heads and stored in tubes plugged with non-absorbent cotton wool in vacuo over desiccant at 4 °C. Spores stored in this way retained their viability (with germination rates greater than 70%) for at least 3 years. Unless stated otherwise, data refer to inoculation with race T6.

Plant inoculation

Dry spores were suspended in distilled water (120×10^6 spores ml^{-1}) by repeated vortex-mixing, followed by filtering through muslin to remove debris. Wheat heads at the appropriate stage of development (yellow but non-dehisced anthers) were selected; less well-developed florets within a selected head were removed before inoculation. In the hybridisation studies, inoculation was delayed until 24 h after pollination. The tips of the lemma and palea were clipped prior to inoculation. Inoculation was carried out using a rubber bulb (containing the spore suspension) attached to a 21-gauge hypodermic needle; one drop (50 µl approximately) of spore suspension was deposited in each floret. At least eight heads of each genotype were inoculated. At maturity, seeds from inoculated heads were sown as ear rows, or bulked for use in embryo sectioning.

Embryo sectioning

Mature inoculated grains were soaked overnight at room temperature in 5% sodium hydroxide to aid isolation of the embryos. After rinsing, the embryos were fixed in 3:1 ethanol:acetic acid, then dehydrated in increasing concentrations of tertiary butanol (Johansen 1940), before being infiltrated and embedded in molten paraffin wax (Johansen 1940). Sections (10–12 µ thick) were cut on a rotary microtome and stained with safranin and thionin before being counter-stained in Fast Green and Gold Orange (Johansen 1940). Fungal mycelium stained purplish-red against a blue-green background of host tissue.

Statistical analysis

Analysis of data from substitution and ditelosomic lines involved analysis of variance, from a replicated randomized block design. Percentage infection data were subjected to arc sine transformation prior to analysis; similar results were obtained by analysis of transformed or non-transformed data, and non-transformed data are presented. Segregation data from hybridisation studies were analysed by the Chi-square test.

Results

Chromosomal location of genes conferring complete resistance

Artificial inoculation of wheat cultivars 'Hope', 'Thatcher' and 'Chinese Spring' revealed that, whereas the former two cultivars were resistant to infection by *U. tritici* race T6, 'Chinese Spring' exhibited approximately 50% infection (Table 1). Loose smut-resistance in 'Hope' and 'Thatcher' is controlled by independent genes (Nielsen 1987) and preliminary investigation of the mechanisms of resistance exhibited by these two cultivars supported this view. Examination of embryo sections (stained with safranin to highlight fungal tissue) from inoculated grains revealed that 'Chinese Spring' embryos contained abundant mycelium in the scutellum, embryonic axis, plumular bud, coleoptile and primary root, while 'Thatcher' embryos were completely free of mycelium (Table 2). On the other hand, mycelium was widespread throughout inoculated embryos of 'Hope' (resembling the situation in 'Chinese Spring'), although the levels of mycelial invasion in embryos of 'Hope' were less than those observed in inoculated 'Chinese Spring' embryos (Table 2). These findings show that wheat cultivar 'Thatcher' exhibited embryo resistance (Popp 1951; Gaskin and Schafer 1962; do Valle Ribeiro 1963) to *U. tritici* race T6, while cultivar 'Hope' exhibited embryo susceptibility but seedling resistance. Further examina-

Table 1. Percentage plant infection of 'Chinese Spring' ('Hope') and ('Thatcher') disomic chromosome substitution lines inoculated with *U. tritici* race T6

Chromosome	Percentage plant infection	
	'Hope'	'Thatcher'
1A	72.6[a]	80.5[a]
2A	51.4	49.6
3A	23.6[a]	48.5
4A	15.4[a]	9.1[a]
5A	57.8	56.7
6A	50.7	57.9
7A	0.0[a]	17.3[a]
1B	53.6	69.8[a]
2B	60.3	51.3
3B	57.0	57.4
4B	58.8	54.0
5B	60.2	73.9[a]
6B	55.9	50.3
7B	54.1	0.0[a]
1D	76.3[a]	61.1
2D	12.1[a]	55.8
3D	23.4[a]	57.1
4D	25.7[a]	63.3
5D	14.1[a]	22.1[a]
6D	61.2	8.0[a]
7D	50.8	59.1
Euploid	0.0[a]	0.0[a]
'Chinese Spring' euploid	55.3	
LSD ($P=0.01$)	11.4	

[a] Significantly different from Chinese Spring euploid ($P=0.01$)

Table 2. The presence and distribution of *Ustilago tritici* mycelium within embryos isolated from mature inoculated wheat grains

Wheat line	Total no. of embryos examined	Percentage embryo infection	Distribution of mycelium in infected embryos	Percentage plant infection in field
'Chinese Spring'	74	18.5	Plentiful in scutellum, plumular bud, primary root, coleoptile	17.7
'Hope'	91	15.4	Sparse in scutellum, plumular bud, primary root, coleoptile	0.0
'Thatcher'	88	0.0	–	0.0
'Chinese Spring' ('Hope' *7A*)	55	5.5	Sparse in scutellum, very sparse in plumular bud, none in primary root, coleoptile	0.0
'Chinese Spring' ('Thatcher' *7B*)	199	0.5	Very sparse in scutellum	0.0

tion of 'Hope' plants grown from inoculated seed revealed that, under glasshouse conditions, infected plants exhibited stunting and leaf-twisting symptoms characteristic of the so-called hypersensitive reaction (Király and Lelley 1957; Mantle 1961) often associated with the embryo-susceptibility seedling-resistance response to inoculation with *U. tritici;* this use of the term 'hypersensitive' pre dates the more widely accepted use of this term in resistance studies, but is retained here because of its continued use in loose smut resistance analyses.

To locate resistance genes from wheat cultivars 'Hope' and 'Thatcher' onto specific chromosome arms, the 21 disomic chromosome substitution lines of each cultivar in 'Chinese Spring' were inoculated with *U. tritici* race T6. Comparison of the infection levels exhibited by individual lines with that exhibited by 'Chinese Spring' euploid permitted the identification of chromosomes which carry loose smut resistance genes present in different allelic forms in the recipient ('Chinese Spring') and donor ('Hope' or 'Thatcher') cultivars. Studies of the 'Chinese Spring' ('Thatcher') chromosome substitution lines demonstrated that complete resistance to *U. tritici* race T6 was conferred by 'Thatcher' chromosome *7B* (Table 1). Inoculated embryos of the resistant 'Chinese Spring' ('Thatcher' *7B*) substitution resembled those of 'Thatcher' euploid in being completely free of mycelium (Table 2). Parallel studies with 'Chinese Spring' ('Hope') substitution lines revealed that the corresponding resistance major gene was carried on 'Hope' chromosome *7A* (Table 1). Seedlings of 'Chinese Spring' ('Hope' *7A*) grown from inoculated seeds exhibited the symptoms of 'hypersensitivity' similar to those shown by 'Hope' euploid, the mature plants producing healthy heads. Inoculated grains of the resistant substitution line 'Chinese Spring' ('Hope' *7A*) contained significant levels of *U. tritici* mycelium widely distributed within the embryo (cf. 'Hope' euploid), but the amounts of mycelium were appreciably less than those present in embryos of the resistant parent, 'Hope' (Table 2). The lower levels of embryo invasion in 'Chinese Spring' ('Hope' *7A*) than in either 'Chinese Spring' or 'Hope' suggest the presence of genes on other 'Hope' chromosomes which, relative to the corresponding 'Chinese Spring' alleles, promote mycelial invasion of the inoculated embryos.

Inoculation of F_1 hybrid embryos from crosses between 'Chinese Spring' and either 'Chinese Spring' ('Hope' *7A*) or 'Chinese Spring' ('Thatcher' *7B*) revealed that resistance was dominant in both cases (Table 3). To determine the number of major resistance genes carried on chromosomes 'Hope' *7A* or 'Thatcher' *7B*, the F_1 hybrid was backcrossed to the corresponding resistant substitution line; the offspring from this cross were self-pollinated and the embryos inoculated with *U. tritici* race T6. The resulting inoculated grains from each plant were sown as progeny rows, which were assessed for segregation for loose smut resistance/susceptibility; progeny rows containing one or more smutted heads were classified as susceptible. Chi-square analysis showed that the data from both resistant substitution lines approximated most closely to the 1:1 ratio expected from a single dominant major gene being responsible for the complete resistance conferred on 'Chinese Spring' by disomic substitution of either 'Hope' *7A* or 'Thatcher' *7B* (Table 3).

Chromosomal location of genes conferring partial resistance

Although 'Chinese Spring' is susceptible to infection by *U. tritici* race T6 and carries no known qualitative resistance gene against loose smut, inoculated seeds produce an average of only 50% infected plants. This may be due to flaws in the inoculation procedure, resulting in 'escapes', but the procedure used was carefully optimised (with respect to host stage of development, spore concentration, post-inoculation treatment, etc.) to yield maximum and reproducible infection levels in wheat cultivar 'Chinese Spring' (Dhitaphichit 1987); consistent levels of percentage infection in 'Chinese Spring' indicated the existence of significant levels of partial resistance to loose smut.

A total of eight 'Hope' and seven 'Thatcher' chromosomes were shown to affect the level of partial resistance when substituted into 'Chinese Spring' (Table 1). Of these, substitution of several group 1 chromosomes from either donor parent and 'Thatcher' chromosome *5B* into 'Chinese Spring' resulted in increased loose smut susceptibility (Table 1), while the other chromosomes (including the homoeologous chromosomes 'Hope' *3A* and *3D*, 'Hope' *4A* and *4D* and 'Thatcher' *4A*) increased the loose smut resistance of 'Chinese Spring' (Table 1). To further analyse the effect of the group 1 chromosomes on the loose smut susceptibility of 'Chinese Spring', the available 'Chinese Spring' group 1 ditelosomics (which lack both copies of a specific chromosome arm) were inoculated with *U. tritici* race T6. Loss of the homoeologous short arms of chromosomes *1A*, *1B* and *1D* resulted in increased levels of infection following inoculation (Table 4), demonstrating that each of these arms of 'Chinese Spring' carries one or more genes conferring quantitative loose smut resistance, the corresponding alleles on 'Hope' *1A* and *1D* and on 'Thatcher' *1B* and *1D* being less active than the 'Chinese Spring' alleles.

Although only two principal classes of response are exhibited by plants from inoculated seed (resistant, producing healthy heads; and susceptible, producing smutted heads), an intermediate class, in which partially smutted heads are produced, occurs at low frequency. In this case, a portion (usually, but not always the upper part) of the head is unaffected, and may produce normal grains. The reason for this phenomenon is not known, though do Valle Ribeiro (1963) suggested that it occurred because of inhibited mycelial growth within the developing plant rather than as the result of reduced mycelial invasion of the embryo. High frequencies of partial smutting appear to be a genetically determined response to infection, involving a specific host-race interaction (do Valle Ribeiro 1963); of the infected heads produced by the loose smut differential wheat line PI 298554 × CI 7795 (Nielsen 1987) following inoculation with *U. tritici* race T6, 85% were partially smutted (Dhitaphichit 1987). The mean frequency of partial smutting in 'Chinese Spring' euploid was 1.8%, and similar levels were exhibited by most of the disomic chromosome substitution lines investigated. Substitution of two 'Hope' group 5 chromosomes, *5A* and *5B*, into 'Chinese Spring', however, resulted in partial-smutting levels of 15.8% and 16.7%, respectively, in the substitution lines. It appears that genes on these two homoeologous 'Hope' chromosomes affected the host/pathogen interaction, but this was not translated into the more conventional classes of partial resistance, the two substitution lines exhibiting values of percentage plant infection similar to that of 'Chinese Spring' euploid (Table 1).

Comparing individual substitution lines with the recipient cultivar euploid only identifies those chromo-

Table 3. Genetic analyses of the major loose smut resistance genes from wheat cultivars 'Hope' and 'Thatcher'

Genotype[a]	Resistant: Susceptible	χ^2	*P*
CS × CS (H7*A*)	108: 0[b]	–	–
CS × CS (Th7*B*)	131: 0[b]	–	–
{[CS × CS (H7*A*)] × CS (H7*A*)}[2]	74:71[c]	0.06[d]	0.5–0.9
{[CS × CS (Th7*B*)] × CS (Th7*B*)}[2]	69:76[c]	0.34[d]	0.5–0.9

[a] CS – 'Chinese Spring'; CS (H7*A*) – disomic 'Hope' *7A* chromosome substitution line of 'Chinese Spring'; CS (Th7*B*) – disomic 'Thatcher' *7B* chromosome substitution line of 'Chinese Spring'

[b] Response of individual F_1 plants to artificial inoculation

[c] Response of progeny rows; a row containing one or more smutted plants was classified as susceptible

[d] Assuming a 1:1 segregation ratio

Table 4. Percentage plant infection of ditelosomic lines of wheat cv 'Chinese Spring' inoculated with *U. tritici* race T6

Ditelosomic	Percentage infection	Ditelosomic	Percentage infection	Ditelosomic	Percentage infection
Ditelo-$1A^L$	61.2[a]	Ditelo-$1B^L$	65.7[a]	Ditelo-$1D^L$	64.7[a]
-$1A^S$	47.9	-$1B^S$	50.8	-$2D^L$	44.5
-$2A^S$	49.3	-$2B^L$	43.7	-$2D^S$	59.1
-$3A^\alpha$	42.4	-$3B^L$	52.2	-$3D^\alpha$	50.8
-$3A^\beta$	38.3	-$3B^S$	53.3	-$3D^\beta$	47.4
-$4A^\alpha$	68.7[a]	-$4B^L$	65.9[a]	-$4D^L$	58.2
-$5A^L$	60.8[a]	-$5B^L$	45.9	-$4D^S$	45.0
-$6A^\alpha$	40.2	-$6B^L$	45.1	-$5D^L$	67.4[a]
-$6A^\beta$	49.5	-$6B^S$	48.0	-$6D^\alpha$	53.7
-$7A^L$	48.7	-$7B^L$	55.6	-$6D^\beta$	55.8
-$7A^S$	52.1	-$7B^S$	53.1	-$7D^S$	59.3
'Chinese Spring' euploid	43.9	'Chinese 'Spring' euploid	48.9	'Chinese Spring' euploid	50.8
LSD ($P=0.05$)	11.6	LSD ($P=0.05$)	8.9	LSD ($P=0.05$)	12.7

[a] Significantly different from 'Chinese Spring' euploid ($P=0.05$)

somes which carry resistance genes present in different allelic forms in the donor and recipient cultivars. It was considered likely, then, that 'Chinese Spring' chromosomes other than those of group 1 carried genes which contributed to the quantitative resistance of this cultivar to loose smut. To assess this possibility, the available 'Chinese Spring' ditelosomics were inoculated with *U. tritici* race T6. Removal of several homoeologous arms of both group 4 and 5 chromosomes resulted in increased levels of loose smut infection (relative to 'Chinese Spring' euploid) (Table 4), indicating the presence on these arms of genes conferring quantitative resistance to *U. tritici*. Interestingly, group 4 and 5 chromosomes had been cited as responsible for quantitative resistance in wheat cultivars 'Hope' and 'Thatcher' (Table 1).

Cytoplasmic control of loose smut resistance

An additional potential source of quantitative resistance lies among the cytoplasmic genomes; earlier studies had demonstrated a maternal influence on the expression of loose smut resistance in both wheat (Gaskin and Schafer 1962) and barley (Skoropad and Johnson 1952), although no attempts had been made to distinguish between true cytoplasmic inheritance (due to mitochondrial or chloroplast genes) and maternal pre-determination (due, e.g., to the presence of maternally derived nuclear-encoded 'determinative' molecules in the ovary). To assess the effect of the former phenomenon on loose smut susceptibility/resistance, alloplasmic series of wheat lines (a common nuclear genotype substituted into different but related cytoplasm types) were inoculated, and the percentage infection levels were compared with that exhibited by the control (euplasmic) line. Of the seven alloplasmic lines of 'Chinese Spring' investigated, three exhibited infection levels significantly different from that of euplasmic 'Chinese Spring' (Table 5); there was no link between plasma type and effect on loose smut susceptibility. Substitution of the 'Chinese Spring' nucleus into the cytoplasm of either *Aegilops squarrosa* or *Ae. variabilis* reduced the quantitative resistance of 'Chinese Spring' (Table 5).

On the other hand, all plants of (*Ae. mutica*)-'Chinese Spring' grown from inoculated seed produced unsmutted heads (Table 5); closer examination of the data, however, showed that the effect of *Ae. mutica* cytoplasm on 'Chinese Spring' response to loose smut infection was to increase susceptibility to a lethal degree, rather than to confer resistance. Inoculated plants of the *Ae. mutica* alloplasmic line exhibited low seed set, producing only 15 seeds from seven heads, as opposed to 116 from seven control (uninoculated) heads of this line. Of these, only 3 (20%) of the inoculated seeds germinated, compared with 104 (90%) of the uninoculated seeds. The percentage reductions in seed set and germination of the other alloplasmic lines and euplasmic 'Chinese Spring' following inoculation were much smaller, and approximated the average losses exhibited by partially resistant wheat cultivars following loose smut inoculation using the procedure employed here (Dhitaphichit 1987). The detrimental effect of loose smut inoculation on seed set and germination had been reported previously for both wheat and barley (Moore 1936; Shands and Schaller 1946; Wells and Platt 1949; Doling 1968). Wells and Platt (1949) reported that highly susceptible barley cultivars exhibited particularly high percentage reductions in the production of viable seed following inoculation with the loose smut fungus. Similarly, the *Ae. mutica* data can be interpreted as the response of a highly susceptible host to high levels of infection; infected ovaries either failed to develop (resulting in low seed set) or produced inviable seeds (resulting in low seed germination). Of the 110 florets inoculated, a plausible model would be that 3 escaped infection, probably as a result of experimental error, and the resulting seeds germinated, the 3 plants producing unsmutted heads.

Table 5. Percentage plant infection of euplasmic and alloplasmic lines of wheat cultivar 'Chinese Spring' inoculated with *U. tritici* race T6

Cytoplasmic parent	Plasma type[1]	Percentage plant infection
Triticum aestivum cv 'Chinese Spring'	B	56.0
T. boeoticum	A	51.9
Aegilops bicornis	S^b	62.1
Ae. squarrosa	D	76.4[2]
Ae. variabilis	S^v	84.1[2]
Ae. mutica	Mt	0.0[2]
Ae. ovata	M^o	59.4
LSD ($P=0.05$)		11.3

[1] After Tsunewaki (1988)
[2] Significantly different from euplasmic 'Chinese Spring' ($P=0.05$)

The influence of cytoplasm on the expression of major genes conferring complete resistance to *U. tritici* was then investigated, using alloplasmic series of wheat cultivars 'Selkirk' (which carries the same resistance gene as wheat cultivar 'Hope') (Nielsen 1969) and 'Chris'. Plants of each alloplasmic line were inoculated with races T1, T6 and T39 (Nielsen 1987), against which both euplasmic 'Chris' and 'Selkirk' had been shown to be resistant, although the two parent cultivars contained different major resistance genes. In certain cases, cytoplasmic substitution resulted in low levels of infection of both 'Selkirk' and 'Chris' (often expressed as partially smutted heads), but only when race T6 was used (Table 6). Inoculation of euplasmic 'Selkirk' with spores from infected alloplasmic lines failed to cause infection, proving that infection of the alloplasmic lines was due to increased host sensitivity,

Table 6. Percentage plant infection of alloplasmic lines of wheat cultivars 'Selkirk' and 'Chris' inoculated with *U. tritici* race T6

Cytoplasmic parent	Plasma type[a]	Percentage plant infection	
		'Selkirk'	'Chris'
Ae. juvenalis	D^2	0	0
Ae. cylindrica	D	3.3	0
Ae. variabilis	S^v	0	0
Ae. squarrosa	D	0	2.5
Ae. uniaristata	M^u	1.7	0
Ae. ventricosa	D	0	0
Haynaldia villosa	V	1.7	2.5
T. macha (PI140191)	B	1.7	–[b]
T. macha (PI190923)	B	0	–[b]
T. turgidum	B	0	–[b]
T. aestivum	B	0	0

[a] After Tsunewaki (1988)
[b] Corresponding 'Chris' alloplasmic lines not available

rather than to the presence of a novel 'Selkirk'-virulent race in the inoculum. In a series of studies, more than 2,000 seeds were obtained following inoculation of euplasmic 'Selkirk' with *U. tritici* race T6, but no infected plants were produced. Again, no trend was evident between plasma type and loose smut susceptibility (Table 6), although substitution of either 'Chris' or 'Selkirk' nucleus into the cytoplasm of *Haynaldia villosa* (the donor parent most distantly related to *T. aestivum*) resulted in disease susceptibility (Table 6). Variation in cytoplasmic influence on *Ustilago* resistance was evident even between the two accessions of *T. macha*, only one (*T. macha*)-'Selkirk' alloplasmic line exhibiting resistance to the loose smut fungus.

Discussion

Disomic inter-varietal chromosome substitution lines based on 'Chinese Spring' have proved invaluable in the genetic analysis of resistance to a range of important wheat pathogens, including *Septoria nodorum* (Kleijer et al. 1980), *Puccinia striiformis* (Pink 1982) and *Erysiphe graminis tritici* (Johnson 1976). Technical difficulties associated with conventional genetic analysis of loose smut resistance (due to the long disease cycle and to problems in identifying the resistance genotype of individual plants) mean that substitution lines are particularly appropriate to the analysis of this character.

Strong similarities were observed between 'Chinese Spring', 'Hope' and 'Thatcher' with respect to the chromosomes involved in determining response to loose smut inoculation, and several homoeologous series of resistance genes were noted, e.g. on group 1, 3, 4 and 5 chromosomes. The presence of both major resistance genes on group 7 chromosomes raises the possibility of common ancestry of the two genes, although the responses to *U. tritici* race T6 which they confer are quite different; embryo resistance in 'Thatcher', embryo susceptibility but seedling resistance in 'Hope'. Interestingly, Gaskin and Schafer (1962) reported that resistance against an unclassified *U. tritici* race exhibited by a 'Hope' derivative operated at the embryo level. In his study of loose smut wheat differentials inoculated with 41 races of *U. tritici*, Nielsen (1987) reported that 11 differentials could exhibit either embryo resistance or seedling resistance (exhibited as 'hypersensitivity'), depending on the pathogen race.

Studies using chromosome substitution lines identified a large number of chromosomes which conferred partial resistance to loose smut. The large number of genes involved in determining the interaction between wheat and *U. tritici* relative to, say *Septoria nodorum* (Kleijer et al. 1980) probably reflects the long period of contact (covering the entire host life cycle) between host and pathogen. The effect of individual chromosomes on quantitative resistance to loose smut probably overstates the levels of background resistance in the donor cultivars. Several of the cited chromosomes are homoeologues, and it is likely that the effects of such genes will not be strictly additive. Nevertheless, the data suggest that partial resistance (conferred by nuclear and cytoplasmic genes) represents a significant barrier to loose smut infection in wheat.

A maternal influence on loose smut resistance had previously been reported from studies of F_1 hybrids from reciprocal wheat crosses (Gaskin and Schafer 1962). Maternal pre-determination would be expected to influence the early stages of infection, involving the newly fertilised ovary but, until this study, there was no firm evidence that cytoplasmic (mitochondrial and/or chloroplast) host genes affected the host-pathogen interaction. Cytoplasmic substitution has been shown to detrimentally affect complete resistance of wheat to *Puccinia recondita* (Washington and Maan 1974), and partial resistance to *Puccinia striiformis, Pseudocercosporella herpotrichoides* (Worland et al. 1987) and *Septoria nodorum* (Keane 1989), and the data presented here suggest that nuclear-cytoplasmic interactions can affect expression of both partial and complete resistance to *U. tritici*, cytoplasmic substitution resulting in increased susceptibility.

Apart from increasing the information available on the genetic control of loose smut resistance, these studies may have wider applications. As a result of these investigations, near-isogenic loose smut susceptible ('Chinese Spring') and resistant lines ('Chinese Spring' ['Hope' *7A*] and 'Chinese Spring' ['Thatcher' *7B*]) have been identified. The availability of near-isogenic wheat lines has already proved invaluable in the investigation of resistance mechanisms operating against pathogens of wheat (Daly 1972; Johnson 1976), most notably stem rust

(Loegering and Harmon 1969; Daly 1972). Very little is known of the molecular bases of resistance to wheat loose smut; proposed mechanisms have included grain phenolics (Gupta et al. 1979), high peroxidase levels (Saini et al. 1985) and low levels of unidentified infection-stimulatory factors on gynoecia (Michalikova 1970). The availability of near-isogenic resistant/susceptible host lines and virulent/avirulent pathogen races would facilitate these studies. Further inter-varietal chromosome substitution lines involving the loose smut resistant cultivars 'Timstein', 'Cappelle-Desprez' and 'Bersée' are also available, while Nielsen (1987) is developing near-isogenic lines carrying resistance genes from 'Little Club' and 'Carma' by backcrossing to the wheat cultivar 'Reward'. Detailed studies of these lines would permit analysis of the mechanisms of action of the corresponding major resistance genes against *U. tritici*.

The localisation of major loose smut resistance genes on specific wheat chromosomes opens up the possibility of using closely linked marker genes to facilitate the identification of loose smut resistant lines in wheat breeding programmes. To be effective, such a marker gene should be dominant, easily identified at the seedling level and tightly linked to the target resistance gene (Shands 1946). Preliminary linkage studies have shown that the 'Thatcher' resistance gene and the gene for purple culm (*Pc*) in wheat cultivar 'Hope' (Law 1967) are situated 11.9 centimorgans apart on chromosome *7B*. Although the *Pc* gene conforms to the former two requirements of a marker gene, it is not sufficiently closely linked to the resistance gene.

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