LINKAGE OF GENES FOR REACTION TO *PUCCINIA GRAMINIS* F. SP. *TRITICI* AND *P. RECONDITA* IN SELKIRK WHEAT AND RELATED CULTIVARS

By R. A. McIntosh* and N. H. Luig*

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Abstract

A previously unidentified gene, *Sr23*, for resistance to *P. graminis* in wheat cultivars Selkirk, Exchange, Warden, and possibly Etoile de Choisy is reported. *Sr23*, which is involved in a necrotic infection type, is closely linked ($\leqslant 0{\cdot}7\%$ recombination at $P = 0{\cdot}05$) in coupling with *Lr16* for resistance to *P. recondita* which is located in chromosome 4A.

Although no Australian cultures are virulent on seedlings with *Sr23*, the infection types varied with the particular cultures employed; some were stable whereas others were variable in different tests. In monogenic lines, *Sr23* appeared to be ineffective in conferring resistance to adult plants.

I. Introduction

Several instances of linked genes for resistance to different diseases of hexaploid wheat, *Triticum aestivum* L., have been reported. Genes *Sr15* for reaction to *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. (hereafter designated *P. graminis*), *Pm1* for reaction to *Erysiphe graminis* DC. f. sp. *tritici* Em. Marchal (*E. graminis*), and *Lr20* for reaction to *P. recondita* Rob. ex Desm. are very closely associated on the long arm of chromosome 7A (Watson and Luig 1966). The long arm of the homoeologous chromosome 7B carries genes *sr17*, *pm5*, and *Lr14a* respectively for resistance to the same three diseases, but in this instance the genes are not as closely linked (McIntosh *et al.* 1967). This suggests that if the two homoeologous group 7 gene series represent evolutionary divergence then genetic differences in chiasmata distribution must be involved.

Resistance to *P. graminis* transferred to hexaploid wheats, C.I. 12632 and C.I. 12633 (Allard and Shands 1954), and Timvera (Watson and Luig 1958) from *T. timopheevi* Zhuk. and controlled by gene *SrTt1* (McIntosh and Gyarfas 1971) is very closely associated in coupling with a gene (now designated *Pm6*) for resistance to *E. graminis* (Nyquist 1963; Jorgensen and Jensen 1972).

Resistance to *P. recondita* transferred from Rosen rye, *Secale cereale* L., by Driscoll and Jensen (1964) is, in the wheat background, closely associated with resistance to *E. graminis* derived from the same source (Driscoll and Jensen 1965). Unfortunately no genetic data are available to indicate the intensity of this linkage in the original rye chromosome.

* Department of Agricultural Botany, University of Sydney, Sydney, N.S.W. 2006.

This paper reports a further instance of close linkage of genes for reaction to different pathogens. Gene *Lr16* on chromosome 4A (Dyck and Kerber 1971) is shown to be closely linked with a previously undesignated gene for reaction to *P. graminis* in the wheat cultivar Selkirk. McIntosh (unpublished data) suggested the presence of an undesignated gene for seedling reaction to *P. graminis* in Selkirk from data collected in North America when this stock was infected with numerous cultures, including at least one with virulence on all the previously documented genes [viz. *Sr6*, *Sr7b*, *Sr9d* (formerly *Sr1*), and *sr17*] carried by this stock.

Further indications of the existence of this gene arose when similar infection types were noted with Selkirk, Exchange, and Warden wheats during a routine screening programme comparing certain newly acquired *P. graminis* cultures with standard cultures of the Sydney University collection. Since Selkirk had derived both *Lr16* and *Lr10* from Warden via Exchange the likelihood of linkage with one or other of these genes was hypothesized. Furthermore, there was no evidence suggesting that the resistance of Warden to *P. graminis* had been intentionally transferred to Exchange, and later to Selkirk.

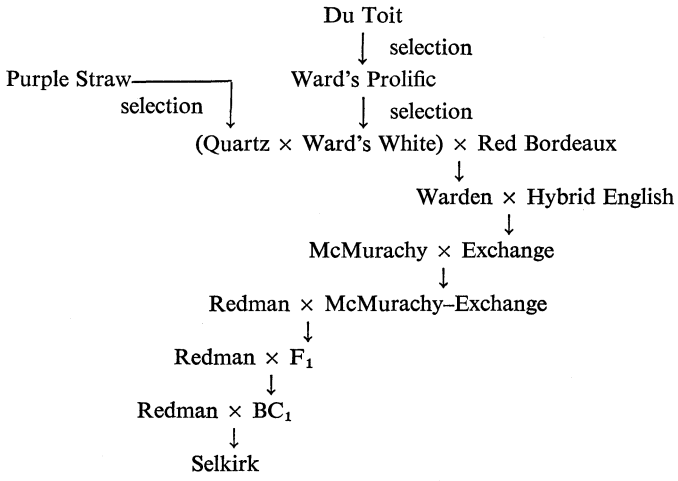
II. Materials and Methods

(a) *Wheat Cultivars and Lines*

Pathological tests were conducted with the Sydney University accessions Selkirk W2699, Exchange W1781, and Warden W217. A second accession of Exchange was provided by Dr. G. J. Green, Canada Department of Agriculture, Winnipeg, Manitoba. In addition, limited studies were made using Etoile de Choisy W3550, which possesses gene *Lr16* (Bartos *et al.* 1969) and an undesignated gene for reaction to *P. graminis* (Bartos *et al.* 1970).

TABLE 1

PART PEDIGREE OF SELKIRK WHEAT



Selkirk has been an important Canadian hard red spring cultivar, but in recent years was replaced primarily because of its widespread susceptibility to leaf rust. The segment of its pedigree pertaining to this paper is presented in Table 1. Basically it is a Redman (H-44) derivative which has,

in addition, gene *Sr6* for resistance to stem rust from McMurachy, and genes *Lr10* and *Lr16* for leaf rust resistance derived from Exchange.

Inheritance studies were based on F_3 lines from a cross between Selkirk and Chinese Spring. Seed of the Selkirk parent was provided by Dr. W. Q. Loegering, Department of Plant Pathology, University of Missouri, U.S.A.

(*b*) P. graminis *and* P. recondita *Cultures*

The main *P. graminis* culture used was 7316, which has the strain designation 126-Anz-1,6,7 on the system proposed by Watson and Luig (1963, 1966) and Luig and Watson (1970). In addition to the eight supplementary differentials described in these papers, one further addition has been made: "-9", denoting virulence on seedlings of a line designated TAF2, which is a common wheat stock carrying, in addition, a pair of chromosomes derived from *Agropyron intermedium* (Host) P.B.

Various additional *P. graminis* cultures used are listed in Table 2, or elsewhere in Section III.

All Australian *P. recondita* cultures maintained by this Department are avirulent on seedlings with *Lr16*, and all are virulent on seedlings carrying *Lr10*. Hence, in order to study *Lr16* in Selkirk the only prerequisite is to use cultures with virulence on seedlings possessing *Lr14a*, which Selkirk obtained from Redman. Two cultures of this type were used interchangeably:

67028 (strain designation 26-Anz-1,3—system of Watson and Luig 1961)

63312 (68-1,2,3,4).

"-3" in the strain designation denotes virulence on seedlings of Spica W2341 which carries *Lr14a*.

Cultivars and F_3 lines were inoculated at the 1–2 seedling leaf stage by spraying with suspensions of urediospores in Mobilsöl oil. After 16–20 hr in a room fitted with a misting device, inoculated seedlings were moved to greenhouse benches. Infection types were recorded after approximately 14 days for stem rust and 12 days for leaf rust.

III. Results

(*a*) *Characterization of Infection Types*

(i) P. graminis

Infection types recorded when Warden, Exchange, Selkirk, and Chinese Spring were inoculated with various test cultures are listed in Table 2. Those involving cultures 334, 7316, and 70386 were consistently lower than those involving other cultures, among which some variation has been noted over repeated tests. Indeed, in some instances with culture 70-L-5 on Warden, Exchange, and Selkirk, and with 59-51A on Warden, infection types have been recorded as 3+ rather than the 3 type necrotic reactions listed for the set of data in Table 2. The main characteristic of the reaction is necrosis which is usually more widespread on the upper leaf surface. Hence, with some cultures reactions of these stocks must be regarded as variable. Figure 1 illustrates comparative infection types involving cv. Exchange inoculated with three cultures including 334 and 70-L-5. In this test little necrosis was apparent with 70-L-5.

The range of infection types listed for Selkirk reflect interactions involving known genes. The only tests in which Selkirk can be compared directly with Warden involve cultures 7316, 70386, and 70-L-5. All other infection types listed for this stock can be attributed to *Sr6* or *sr17* or both, and in the case of 59-51A, to *Sr6*, *Sr9d*, and possibly other genes.

All tests with Exchange appeared comparable with Warden, excluding 59-51A. Apparently culture 59-51A possesses a gene(s) for avirulence different from the other listed cultures. A stock of Exchange supplied by Dr. G. J. Green produced slightly lower infection types than Exchange W1781 in comparative tests. For example, when

an infection type of 1++ was recorded for Exchange W1781, Green's stock was recorded as 1NN. Similarly, for 3CN the comparative result was 3−CN. The genetic nature of this small but consistent difference is unknown.

TABLE 2

INFECTION TYPES RECORDED WHEN VARIOUS TEST CULTIVARS WERE INFECTED WITH TEST CULTURES OF *P. GRAMINIS*

Culture	Strain designation	Warden W217	Exchange W1781	Selkirk W2699	Chinese Spring W1806
334	126-6,7	1++NN	1++NN	;X=	3+
7316	126-1,6,7	1++NN	1++NN	1++NN	3+
70386	222-1,2,3,5,6	1++NN3CN	1++3CN	1++NN	3+
54129	21-0	3−CN3CN	3−N3CN	X2N	3+
69490	21-2	3CN	3−CN3CN	X	3+
71178	21-9	3CN	3−CN3CN	X	3+
69100	21-6,9	3−N	3−N	X	3+
71-L-1	17-0	3CN3+CN	3−CN	X−	3+
63640	34-5	3CN	3−N,3N	XX+3CN	3+
63688	34-2,7	3CN	3−CN3CN	X	3+
57096	34-4,7	3CN3+CN	3CN3N	X=X,X+	33+
72484	34-2,3,7	X3−CN	3−CN3CN	X	3+
70-L-5	222-1,2,3,4,5,6,7	3N	3N,33+	3N3+N	3+
71772	116-2,4,5	3CN	3−N3CN	2+N	3+
59-51A	59-5,7	3CN,3+CN	2=	O;	3+

Comparative infection types exhibited on Warden, Exchange W1781, Selkirk, and Chinese Spring inoculated with culture 7316 are illustrated in Figure 3.

The development of necrotic reactions followed a characteristic pattern. Whereas with many *P. graminis–Triticum* necrotic interactions visible necrosis occurs before pustules break through the leaf epidermis, in the interaction investigated in these studies pustule development was normal until the onset of necrosis, which occurred approximately 9–10 days after inoculation with culture 334 and 12–13 days with culture 70-L-5.

During the progress of these studies variability in infection types from one test to another suggested that temperatures and light intensity might be important. In order to study the effect of temperature, one member of paired pots of Exchange was inoculated with culture 68016 (21-2,3,4,5,7) and the other with 70-L-5. The pairs were held for increasing periods of time in a greenhouse at 15–20°C before removal to a considerably warmer house where previous tests had been conducted. Subsequent development of infection types could not be correlated with the period of controlled temperature treatment, but infection types with 68016 were consistently lower than those with 70-L-5. The variation obtained indicated that some environmental variable other than temperature might be important.

(ii) P. recondita

Following inoculation with cultures 67028 and 63312, Warden, Exchange, and Selkirk produced very similar infection types (Fig. 2), each of which is attributed to interaction involving *Lr16*.

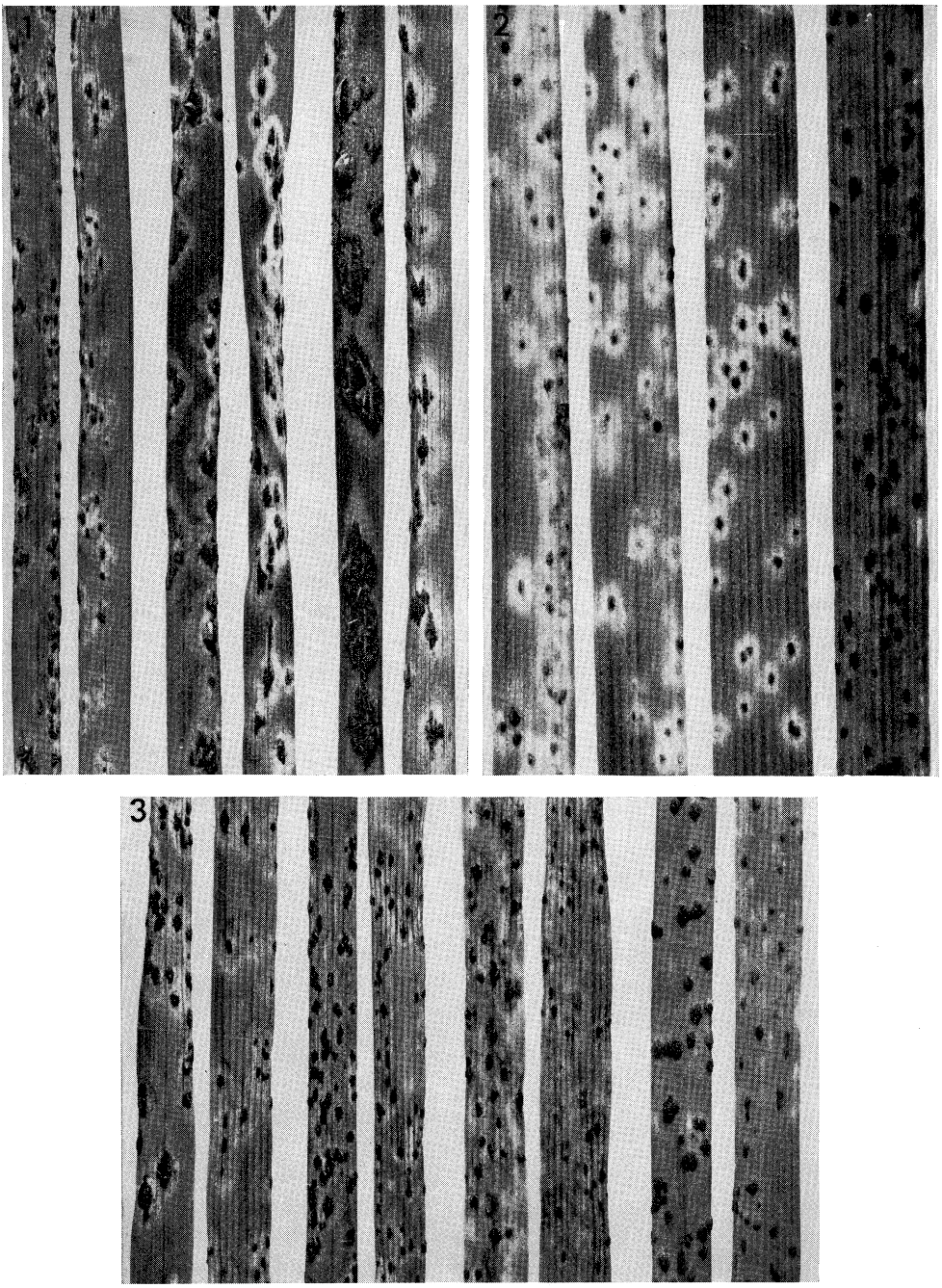


Fig. 1.—Left to right: lower and upper leaf surfaces showing infection types on wheat cultivar Exchange inoculated with *P. graminis* cultures 334, 68016 (21-2,3,4,5,7), and 70-L-5 respectively.

Fig. 2.—Left to right: infection types with *P. recondita* culture 63312 on cultivars Warden, Exchange, Selkirk (resistant), and Chinese Spring (susceptible) respectively.

Fig. 3.—Left to right: upper and lower leaf surfaces showing infection types on cultivars Warden, Exchange, Selkirk (resistant), and Chinese Spring (susceptible) respectively, inoculated with *P. graminis* culture 7316.

(*b*) *Genetic Studies*

A total of 207 F_3 lines from the cross Selkirk × Chinese Spring were inoculated with *P. graminis* culture 7316 and *P. recondita* cultures 67028 or 63312 or both. The following array of phenotypic classes was established:

51 homozygous resistant to both *P. graminis* and *P. recondita*,

105 segregated to both,

51 homozygous susceptible to both.

Apparently the same, or two closely linked, genes conferred resistance to both pathogens ($\chi^2_{1:2:1} = 0{\cdot}044; P > 0{\cdot}9$). With segregating lines both types of resistance appeared to be partially dominant.

For a population of 207 lines tested by the above procedures, the maximum recombination frequency (p) which can be applied to the observed results at the 5% level of probability can be calculated (Hanson 1959) from the expression:

$$(2p-\tfrac{3}{2}p^2) = 1-(0{\cdot}05)^{1/207}$$

$$[1-(2p-\tfrac{3}{2}p^2)]^{207} = 0{\cdot}05$$

$$p = 0{\cdot}007$$

Of the 207 F_3 lines, 54 were inoculated with *P. graminis* culture 334 (126-6,7). In this test segregation occurred for *Sr6* as well as for the newly identified gene. Infection types involving *Sr6* were epistatic, but in lines segregating for *Sr6*, or in which *Sr6* was absent, segregation with respect to the new factor was identical to that obtained with culture 7316.

In a further study, duplicate sowings of 30 lines were inoculated with *P. graminis* cultures 7316 and 70386. Again, complete agreement in segregation behaviour indicated that the same resistance gene was involved.

(*c*) *Field Reaction of Warden and Exchange*

During 1972, observations of field reactions of Warden and Exchange, where the predominant *P. graminis* strains were 21-1,2,5 and 34-2,4,5, indicated that the necrotic gene identified in seedlings has little effect on field reaction. Both stocks were susceptible. Although direct isolations and seedling tests were not conducted from these adult plants, it is unlikely that these strains differed from the standard cultures which are avirulent on seedlings of Warden and Exchange.

(*d*) *Etoile de Choisy W3550*

Seedlings of this stock inoculated with *P. graminis* culture 7316 produced infection type 1−NN which was distinctly lower than those obtained with the same culture on the cultivars listed in Table 2. Because of the characteristic necrosis it is suggested that Etoile de Choisy possesses the same gene as that identified in Warden, Exchange, and Selkirk. However, the distinctly lower infection type suggests the presence of an additional gene.

Infection types produced with *P. recondita* culture 67028 were very similar to those produced on Warden and Exchange controls inoculated with the same culture, indicating the presence of the same resistance gene, *Lr16*.

IV. Discussion

A previously undescribed gene for seedling resistance to *P. graminis*, very closely linked with *Lr16* in chromosome 4A (Dyck and Kerber 1971), has been identified and described. Because no previously identified gene for *P. graminis* resistance has been placed on this wheat chromosome, a new designation, *Sr23*, has been allocated.

Although low infection types associated with *Sr23* are more stable with some cultures than with others, no virulent cultures have been identified in this country. At least two, and possibly three, groups of cultures are indicated, viz. very low, intermediate, and variable high intermediate. Watson and Luig (1968) described progressive increases in virulence in cultures of *P. graminis* produced experimentally in the laboratory, and obtained from race surveys. These involved stepwise increases in characteristic infection types for genes *Sr6* and *Sr11*. Furthermore, considerable data are accumulating within this laboratory to suggest that such stepwise changes are common and that demonstration is possible following synthesis of appropriate stocks for use in suitably controlled environments. This situation appears to present itself with *Sr23*. The extremes in infection type are represented by cultures 334 and 7316 on the one hand, and 70-L-5 and possibly 59-51A on the other. The former are pre-1954 strains and are distinct from later types (Watson and Luig 1966). Culture 70-L-5 arose from culture 68016 (21-2,3,4,5,7,) following recurrent EMS*-treatment which produced mutations to virulence with respect to host genes *Sr5*, *Sr6*, and *Sr8*. A new strain designation 34-1,2,3,4,5,6,7 was allocated; however, in subsequent tests an apparent concurrent change in infection type on Acme from "3" to "2−" was noted necessitating an alteration to 222-1,2,3,4,5,6,7. Since the parent culture 68016 conditions a stable 3CN infection type on Warden and Exchange, the unstable and higher infection type of 70-L-5 must be attributable to a further mutation(s). Culture 59-51A is of United States origin and its possible similarity with 70-L-5 and with North American cultures with respect to virulence on *Sr23* requires further investigation.

The finding of close, or complete, linkage of *Sr23* and *Lr16* in Selkirk is supported by the facts that its progenitors, Exchange and Warden, both carry the combination, and that *Sr23* was transferred without conscious selection from Warden to Exchange, and thence to Selkirk, during transfer of *Lr16*. Only one other cultivar, Etoile de Choisy, has been reported to carry *Lr16* and the present results suggest that this stock also carries *Sr23* as well as at least one other gene, possibly that identified by Bartos *et al.* (1970). Unfortunately, these authors did not list the infection types produced on Etoile de Choisy, so no direct comparison with present results is possible.

Warden and Etoile de Choisy are the only presumably unrelated wheats carrying *Lr16*. An inspection of the pedigree of Warden (Table 1) suggests that *Lr16* may be derived from Red Bordeaux which, like Etoile de Choisy, is presumably of French origin. Unfortunately, no information is available regarding Red Bordeaux, but the Australian parental cultivars, Quartz and Ward's White, are not documented as having leaf rust resistance.

Since Warden and Exchange are susceptible in the adult plant stage with strains presumed to be avirulent on seedlings, it is apparent that *Sr23* will be of no use as a monogenic source of resistance in wheat breeding. Apart from the very limited

* Ethyl methanesulphonate.

observations of the first author, there is no evidence that *Sr23* has been recognized in North American studies involving Selkirk, further suggesting that it may be of scientific interest only.

As shown by Green (1971) *Sr6* in Selkirk is by no means solely responsible for the long-term resistance shown by this cultivar in North America. Race survey data indicate that frequently one or other of genes *Sr7b*, *Sr9d*, and *sr17*, rather than *Sr6*, may confer resistance. In addition the adult plant resistance attributed to *Sr2* is probably of utmost importance (Green 1971). In such a background of five resistance genes, the sixth, *Sr23*, could well display interactions not yet recognized.

V. Acknowledgments

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