An Interactive Computer-Based System for Comparing Cultures of *Puccinia graminis* and Postulating *Sr* Genotypes in Wheat

A. P. Roelfs, F. D. Baker, and D. V. McVey

First and last authors, research plant pathologist, Cereal Rust Laboratory, Agricultural Research; second author, intergovernmental personnel act intern (operations research analyst), Statistical Applications Branch, Communications and Data Services; USDA, ARS, University of Minnesota, St. Paul 55108.

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ABSTRACT

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A computer-based system (IRIS) was developed to compare infection types produced by the interaction of cultures of *Puccinia graminis* on hosts with designated *Sr* resistance genes, and to postulate the rust resistance genotype of the host by the use of disease infection types. The user is guided by IRIS through Time Sharing Option command procedures to the appropriate program in a series of connected interactive Statistical Analysis System programs. IRIS is currently operational on the IBM 370/168 at the U.S. Department of Agriculture, Washington Computer Center in the South Building and can be accessed by remote terminal. IRIS sorts races and permits the user to compare infection types produced by all previously evaluated cultures on 45 "single" genes for resistance with newly evaluated cultures. Infection types of newly characterized cultures can be selectively added to the system, as well as infection types produced on newly characterized host resistance, *Sr* genes. The infection types produced by the 51 cultures most frequently used and the 46 characterized *Sr* genes were placed in a base file. The infection types in this file were used to postulate host resistance genotypes of host lines infected with some or all of the cultures.

Additional key words: wheat stem rust, race-specific resistance, gene postulation.

A large number of host lines can be evaluated with the same pathogen culture over a period of years (11), due to advances in handling and storage of cultures of wheat stem rust, *Puccinia graminis* Pers. f. sp. *tritici*. A stem rust culture can be characterized phenotypically for virulence by infecting host lines with "single genes" for resistance (1,2,4–6,8,12). Although more than 1,600 cultures had been evaluated on 42 host "single-gene" resistances by 1978 (11) it was not feasible to continue handling such large volumes of data without computer assistance (3). Postulated (4,8), hypothetical (5,6), or probable (2) host resistance genotypes are extremely useful, but the number of possibilities and the time required make postulation difficult in host lines with several genes for resistance. The potential value of postulation has been adequately demonstrated (1,2,4–6,8,12). Furthermore postulation can assist in obtaining genetic proof, where a line known to have the postulated resistance is crossed with the line postulated to have that gene (7). This is much simpler than the usual procedure of crossing the line of unknown resistance with a susceptible line and evaluating large numbers of F_2 and F_3 progenies.

The bases for postulation are cultures that vary from each other by a single virulence or avirulence gene. Thus, it is important to retain such cultures, but if cultures must be increased or temporarily stored pending a decision on their uniqueness, time and cost is incurred unnecessarily.

The objectives were to develop an interactive computer system that had the capacity to store infection-type data, to permit comparisons between previously characterized and newly characterized cultures, to postulate host genotypes from infection-type data, and to permit updating and retrieval of this information.

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MATERIALS AND METHODS

The data bank was built with 329 cultures retained from an earlier study (11). An initial data modification was to edit the infection types to a maximum of four characters to reduce time in entering data and space in printing outputs. This editing affected less than 5% of the data, and variation in environmental conditions and inoculum density between days and years probably rendered additional characterization of the infection meaningless.

Data were processed on the IBM 370/168 at the U.S. Department of Agriculture Washington Computer Center in the South Building. The computer was accessed from a remote terminal, via telephone lines. The programs were written in Statistical Analysis System (SAS) and are coordinated by Time Sharing Operations (TSO) command procedures.

The infection-types for the previously evaluated culture and host lines were entered into one of two data files (Fig. 1). Cultures frequently used in evaluating host resistance were placed in a file named BASE, and the remainder in a file named SECOND. The data were entered in a free format (not placed in designated columns) with the culture number, followed by the Cereal Rust Laboratory race designation (9,10), and then the infection type produced with each of the *Sr* genes (*Sr*5,6,7a,7b,8,9a,9b,9d, 9e,9f,9g,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27, 28, 29, 30, 31, 32, Tt-1, Tt-2, Tt-3, Tmp, McN, X, dp-2, LC, Kt'2', Gt, Wst-2, and Wld-1 in order). If an infection type was unavailable, a period was inserted. When these 329 cultures were compared to each other based on the infection-types produced by each culture on 46 host resistance genes, 39 cultures were judged to be near-duplicates and were deleted. The initial system then contained 50 cultures in BASE and 240 in SECOND data files.

A third file named HOST (Fig. 1) was constructed to store infection-type data for cultures in BASE on host cultivars or lines with unknown genotypes for stem rust resistance. A fourth file, GENHOST, contains general information concerning the hosts in HOST file. The GENHOST file contains the host designation

(eight characters), the CI or PI numbers (Cereal Investigation [eg, C17783] or Plant Introduction [eg, 365882] numbers of the U.S. Department of Agriculture) and the cultivar name or line designation, not to exceed 25 characters, and up to six detected, postulated, *Sr* gene designations. These data were also entered in free format.

The 139 newly characterized cultures used to test IRIS represented the differences in virulence (races) on *Sr*5,6,7b, 8,9a,9b,9d,9e,10,11,15,17,Tt-1, and Tmp found during the 1978 and 1979 annual race surveys (11,12). One isolate of each virulence combination had been retained from each state in which it had been isolated.

Cultures were removed from ultralow-temperature storage (−45 to −50 C) and inoculated to a susceptible host that had been treated with maleic hydrazide to enhance spore production. Enough uredospores were collected after 14–19 days to inoculate 6–10 seedlings of 120 host differential lines. Plants were grown in vermiculite and fertilized 5 and 8 days after planting at a rate of 2.5 g (23-19-17, N-P-K) per 24 host lines. The 7-day-old plants were inoculated with a spore suspension in a lightweight mineral oil carrier, then placed in a dew chamber at 18 C overnight. The next morning the chamber was illuminated (10,000 lux) and the temperature was raised gradually to 30 C over a 4-hr period so the dew evaporated slowly. The plants were placed in a greenhouse at 18 C supplemented with 11,000 lux of fluorescent light. Infection types (11) were recorded 13 days after inoculation. High infection types (6) were recorded as an S.

A TSO-controlled interconnected system of interactive SAS programs was developed to compare races and postulate host genotypes. Copies of these programs are available from the Cereal Rust Laboratory. For convenience, this system has been named IRIS (Interactive Rust Identification System).

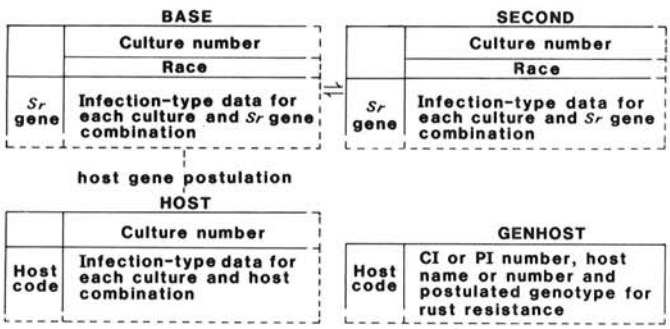

Fig. 1. Statistical Analysis System (SAS) data files for the computer-based Interactive Rust Identification System (IRIS). Newly characterized cultures are compared with those previously evaluated in BASE or SECOND files. Cultures can be added to either file or switched between files. Comparisons between data in BASE and HOST files permits postulation of genes for resistance, which can be added to GENHOST file.

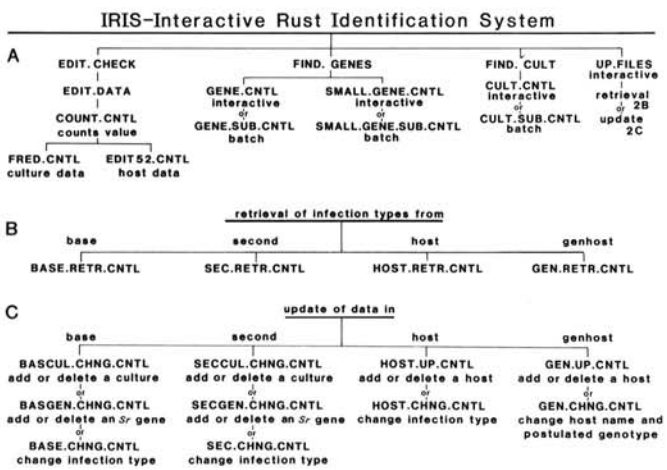

Fig. 2. The interconnected programs in the computer-based Interactive Rust Identification System (IRIS): **A,** EDIT.CHECK provides a series of programs that counts entries and notifies the user of shortages or excess of input data, FIND.GENES is a series of programs for postulating the presence of host genes for resistance, FIND.CULT compares newly characterized races with those in the system and the UP.FILES section for data updating and retrieval; **B,** the retrieval section; and **C,** the updating section of UP.FILES.

RESULTS AND DISCUSSION

Update and retrieval of data. To update any file in the data base the data must be placed in temporary files with specific names (Fig. 1A, B, and C). Three actions are possible: add (A), delete (D), or switch (S). Each action code is preceded by an asterisk to indicate a different host or culture, and each action code calls a different SAS program to complete the indicated action. New data for the system are normally entered with an action code. A series of programs in EDIT.DATA (Fig. 2A) determines the proper action to take, calculates the numbers of infection types and verifies that they are present for each culture, and signals the operator of errors. To add a new host (*Sr*) gene to BASE or SECOND data files, complete infection-type data are required with periods to indicate missing data. To add *Sr*33 to BASE or SECOND, a temporary file called UNKH.UP.DATA is created before entering IRIS; the action code (A) and gene (_33) are followed in order by infection types for each culture in the data file. Note that each numeric gene must be preceded by a _ for identification for IRIS. To add a culture a temporary file called UNKC.DATA is created with the action code (A), the culture number, and race designation followed in order by the infection types produced with each host line in BASE or SECOND data files. After the creation of the temporary files, the user enters IRIS and responds to the programmed options.

To delete a host line or culture from BASE or SECOND data files, the action code is (D) followed by the host line designation (eg, _33) or culture number (eg, 78470246A). Infection-type data are not required. It is also possible to switch cultures between SECOND and BASE data files. The action code(s) for switching culture(s) is followed by the culture number; infection-type data are not required.

To add (A) a host to HOST data, a temporary file designated UNKH.UP.DATA is created. The action code (A) is followed by host code and the infection types in order that were produced when the host was infected with the cultures in the BASE data file. To delete a host, (D) is substituted for (A) and infection types are not required.

To update data in the GENHOST file, a temporary file called GEN.UP.DATA is created. The format is (A) host code, C plus CI number or the PI number, host cultivar name or line number, and up to six postulated *Sr* genes for stem rust resistance.

These temporary files should be created before entry into IRIS; however, IRIS will prompt the user for the necessary files. Specific infection types are changed in IRIS by identifying the resistance gene or host code and culture number, and providing the new value. In the GENHOST file, only the host code is necessary for identification changes in host name or postulated genotype.

Retrieval of information from IRIS is an easy procedure through a series of four programs in UP.FILES (Fig. 2A and B). By following instructions provided by IRIS the appropriate program and file are accessed. IRIS creates a temporary file that "queries" the appropriate data base for the information. For BASE, SECOND, and HOST files the user provides IRIS with the culture number and *Sr* gene or host code combination for which infection-type data are desired. To recover information from the GENHOST file only the host code is required.

Comparison of cultures. One option, FIND.CULT (Fig. 2A) compares the race code of each newly entered culture with those in BASE and in SECOND. If an identical race is found, a printout is generated that lists the infection type for each *Sr* gene of the newly entered culture(s) and of the BASE or SECOND culture matched; eg, Table 1, MBCT and QFBS. In instances where the newly

entered culture does not match any previous culture, the infection type of only the newly entered culture is printed (eg, Table 1, GBCQ). The investigator must then decide to add the culture(s) to the BASE or SECOND data file or to delete the culture(s) from the system. This decision is based on anticipated use of the culture and depends mainly on differences in infection types among cultures. FIND.CULT offers the investigator two options to obtain the same goal, the interactive CULT.CNTL, or the slower and cheaper noninteractive CULT.SUB.CNTL. The slower option normally executes in a few hours but never longer than overnight.

Postulation of host *Sr* genotypes. Postulation requires that an infection type be obtained with each culture, or a subset of cultures, in the BASE data file. These infection types are entered into a temporary data file called UNKH.DATA, either in a free format with the host code followed by the infection-type data for each culture in BASE or for the subset of BASE used. The infection-type data must be entered in the same order that the cultures used appear in BASE data; missing data are indicated by periods. The UNKH.DATA file is edited by a series of programs in EDIT.CHECK (Fig. 2A) and, if necessary, corrections are made before the information is added by IRIS to the HOST file. Four options are available for host-gene postulation depending on whether the results are required immediately or overnight, or whether all or nearly all of the cultures in the BASE data file or only a small subset are being used. The programs GENE.CNTL and SMALL.GENE.CNTL provide interactive analysis for the entire set or for a subset of the cultures in the BASE file, respectively. GENE.SUB.CNTL and SMALL.GENE.SUB.CNTL provide the same functions noninteractively at a reduced cost.

Only the first character of each infection type was used in postulation of host genotype. A comparison was made between the infection-type data for each *Sr* gene with cultures in BASE data and the line under postulation. To make this comparison possible and still use the familiar infection-type data, each infection type (11) was assigned priority order ascending from 0; (fleck) 1 2 3 X, Y, or Z and S. The order of infection types 0 through 3 and S corresponds to the descending order of epistasis. The exact ranking for X, Y, and Z is unknown, but is probably between 2 and S depending on the host, pathogen, and environmental interactions. If the initial character of the infection type produced by the host-culture combination being evaluated is greater than the infection type of the *Sr* gene and the same culture (in BASE file), then that *Sr* gene is eliminated from the list of genes that the computer postulates to be present. If missing data occur in either BASE or HOST data file, no comparison is made for that host-culture combination; however, this does not result in eliminating an *Sr* gene from the postulated genotype.

This system requires that infection types in the BASE data file have the most frequent character of the infection type first, not in ascending order as sometimes has been done. For example, if the observed infection type if 2− with a few flecks, then it must be recorded as 2−; not ;2−. To prevent elimination of *Sr* genes that were present from the postulated resistance genotype, the infection types in the BASE file had to be adjusted to their normal upper limit. This was especially a problem when the *Sr* gene used in establishing BASE was a diploid or tetraploid wheat and the line being postulated was a hexaploid. For example the data base was built with *Sr*21 in the cultivar Einkorn, a monococcum with a low infection type of ;1=, while the hexaploid derivative resulted in an infection type of 12− when infected with the same culture. The *Sr*27 gene often resulted in an infection type, about equally divided between 0 and flecks. However, in the BASE file it was necessary to set the infection type at ;0 to avoid an incorrect postulation.

Usually the outlined procedure eliminates many of the known *Sr* genes from the postulated genotype (Table 2). Next, the investigator must compare the entire infection-type data (four characters) with data of the *Sr* lines having the resistance genes postulated to be present. Often additional confirmation of the postulated genotype is provided, but occasionally probably host genes are eliminated from the postulated genotype. For example, infection-type S; with a host line and race 15-TNM and 0 with race 151-QSH would be accepted as evidence of the presence of *Sr*Tt-1, however, infection types of S and ;1+C, with these two races, respectively, would probably eliminate *Sr*Tt-1 from further consideration. If the genotype for the parental lines of the host under consideration is known, *Sr* genes absent from the parental lines are normally considered absent, and those present in parental lines and postulated to be present in the test line are assumed to be present. Caution must be used with pedigrees, however, as several cases were found where *Sr* genes were present in the progeny but did not occur in the parents as shown in the pedigree. Some *Sr* genes are difficult to separate by differences in infection type with cultures occurring in BASE data file. In these cases, it may be necessary to use a culture from the SECOND data file, or to retest the host line with selected cultures at another temperature (ie, *Sr*6,

TABLE 1. A listing of infection types when making a comparison of wheat stem rust races by the computer-based Interactive Rust Identification System (IRIS). IRIS indicates newly characterized culture(s) (0) and pairs them with previously characterized races (1) in either a BASE or SECOND data file. If a similar race is not found, the culture is listed separately

	Race[a] MBCT		Race QFBS		Race GBCQ
Sr gene	78470346A	59001900	78210215B	511951A	78BB01330
_5	S	S	;	0;	;
_6	;	;	;	0;	;
_7A	S	S	23C	23CN	S
_7B	S	S	2	2	2
_8	2⁻	2⁻	S	S	2⁻
_9A	2⁻	2⁻	S	S	2⁻
_9B	2	2	2⁻	2	2
_9D	;1	1	S	S	S
_9E	;	;1	;1	;	;
_9F	S	S	S	S	S
_9G	S	S	S	S	S
_10	S	S	;1N	;1N	S
_11	;	;	;	0;	;
_12		X⁼	X⁺	S	;
_13	2	2	2	2	2⁺
_14	;	21CN	;1	2⁼C	2
_15	S	S	S	S	S
_16	S	S	S	S	S
_17	S	S	S	S	X⁻
_18	S	S	S	S	S
_19	S	S	;1CN	S	S
_20	S	S	11⁺C	S	S
_21	1	2⁻	S	X	1
_22	1	2⁼	;1	;	;1⁻
_23	23C	23C	S	23CN	S
_24	2	2⁻	2	2	2
_25	2	2⁻	2	2	2
_26	2⁼	2⁼	;2⁼	;2⁼	;1
_27	;	;	0;	0;	;
_28	S	S	0;	S	S
_29	2	2⁻	2	2⁻	1
_30	2	2⁻	2⁼	2⁻	2⁼
_31					
_32					
Tt-1	0	0;	0;	0	0
Tt-2	;	0;	;	0;	;
Tt-3	2	1	S	0;	;
Tmp	S	S	2⁼	2⁼	2⁼
McN	S	S	S	S	S
X	S	S	1N	21C	S
dp-2	2	2⁻	S	S	2
LC	S	S	S	S	S
Kt'2'	S	S	S	S	S
Gt	2	2	2	2	2
Wst-2	S	S	2	2	S
Wld-1	2⁼	2⁼	2⁼	2⁼	2⁼
IRIS	BASE	BASE	SECOND	SECOND	BASE
pairs	0	1	0	1	0

[a] Figures in vertical columns in the boxheading are culture numbers.

TABLE 2. Comparisons between the postulation by the computer-based Interactive Rust Identification System (IRIS) for *Sr* genes in wheat lines selected for resistance to *Puccinia graminis*

Cultivar	IRIS postulation for *Sr* gene(s)[a]	*Sr* genotype for seedling resistance from the literature
Baart	Perfect LC	LC
Maruccos 9623	New gene	gene not previously detected
Thatcher	5,9f,9g,12,15,16,18,19,20, 21,23,McN,LC,Kt'2'	5,9g,12,16
Selkirk	6,7b,9d,9f,9g,12,15,16,17, 18,19,20,21,23,McN,LC	6,7b,9d,17,23
Agent	7a,7b,8,9a,9b,9f,9g,12,13, 15,16,18,21,22,24,25,26,30, Tmp,LC,Kt'2',Gt,Wst-2,Wld-1	24,Ag-1,Ag-3,Ag-4[b]

[a]IRIS using available data found evidence of: Perfect (no additional *Sr* genes); New gene (*Sr* gene not included in BASE.DATA). *Sr* gene(s) postulated are those not eliminated by IRIS search of available information.

[b]Ag-1,Ag-3, and Ag-4 may be identical to one of the numbered *Sr* genes.

13, or 15) (11). To prove the postulation of the resistance genotype it is necessary to cross the host line under study with a line having each of the postulated genes. If the gene is present there should be no susceptible plants in the F_2 progeny when evaluated with the appropriate culture; ie, one avirulent on that *Sr* gene but virulent, or avirulent with a higher infection type, on the other postulated genes. If such a culture is unavailable, the progeny line would need to be crossed with a susceptible line and postulation and crossing done with F_2 or F_3 lines. Undescribed *Sr* genes are often detected; ie, those that result in infection type not produced by any of the described *Sr* genes singly or in combination. These also must be evaluated by crossing them with a susceptible host. It has been the practice to include the "single-gene" lines in the test as checks when postulating host genotypes; thus when a genotype can be postulated within 24 hr it is possible to compare the infection types of the line under study with the infection types on the "single-gene" lines with the postulated genes.

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