A Study of Adult Plant Resistance to Leaf Rust

Leaf rust of wheat caused by Puccinia recondita is currently the most important disease of wheat on a world-wide basis. The primary means of control has been through the use of resistant cultivars. Although resistance has often failed after a few years use, the adult plant resistance of a number of South American cultivars has been durable for many years. This resistance has been used in cultivars around the world. Frontana was a Brazilian cultivar that was widely used in developing with durable resistance to leaf rust. Genetics studies have shown Frontana has Lr13, 34 and T3, and its derivatives with Lr13 and 34 are resistant. Although there are a number of similar cultivars from Argentina, Brazil and Uruguay, it is unknown if the basis for their resistance is similar to Frontana. A number of cultivars are more resistant than Frontana in Brazil as measured by disease severity and area under the disease progress curve. Although they may have some or all of the resistance genes of Frontana, they must have at least one additional resistance gene.

To do a genetic study of resistance, it is desirable to have a susceptible parent into which to transfer the genes. A number of susceptible lines have been identified world-wide for this purpose. However, due to the unique soil and disease problems in Brazil, it is apparent that to make crosses and to make potential field tests possible, the susceptible cultivar chosen should be well adapted to Brazil. The chosen susceptible parent should be susceptible to at least the pathogen race chosen for the study, but it is desirable for it to be susceptible to most races if field studies are needed to eliminate possible interactions between resistance genes. A number of potential susceptible parents were selected (Table 1) and Coxilha 546, IAC13 and IAC160 seemed to be the most likely candidates. A preliminary test is required to make the final selection of a susceptible cultivar.

Although the resistance of any cultivar can be studied, the fewer genes present and the more effective the resistance, the easier the resistance is to study. Most resistance genes for which the inheritance has been studied are effective in the seedling stage. These genes are much easier to evaluate and require less expense and labor to study and therefore were selected for study. They have not always been the most effective resistances. In recent studies interactions have been shown between certain adult plant resistance and an ineffective seedling resistance gene. Therefore, the fewer resistance genes present, the simpler the study. Also, to be certain that the resistance observed is strictly due to an adult plant resistant gene, it is desirable to have a cultivar that is resistant in field tests, but susceptible to most pathogen races in the seedling stage. The resistant cultivar should be adapted to Brazilian conditions and similar enough in maturity to cross without too much difficulty to the

selected susceptible cultivar. Differences in growth habit can be accommodated by differences in planting dates, vernalization, and growing the parental cultivars at different temperatures or day lengths. Often these studies may require a season's experience before a cross can be made. Based on data collected in Brazil, Toropi is an excellent choice for study; it is susceptible to all Brazilian leaf rust races evaluated in the seedling stage and is resistant in the field. Toropi is more resistant than Frontana indicating the presence of a different gene than in Frontana. A preliminary test will be made to make the final choice of resistant cultivar to study (Table 2).

Pathogen race

The study can be done with any pathogen race that is virulent on the susceptible host and avirulent on the resistant host in the environment and at the host growth stage tested. However, some races will give a more distinct and/or constant infection type over a wider range of host growth stages. This difference in host response between pathogen races studied is due to whether the culture is homogygous or heterozygous avirulent and to the aggressiveness or adaptation of the race. Thus, results are often clearer and easier to obtain with certain races or cultures. A preliminary test will be conducted to select the culture to be used in the study (Table 3). The selected pure culture should be increased so that twice the anticipated inoculum required for all further tests is available and stored before testing of F_1 progeny is started.

Ideal pathogen race must be:

1) Virulent on susceptible parent at all growth stages.
2) Avirulent on resistant parent.
3) Characterized by the greatest possible contrast in response between the susceptible and resistant parents.
4) Characterized by a stable host response over the range of environmental and host growth stages to be studied.

Table 1. Brazilian wheat cultivars susceptible to leaf rust in the seedling and adult plant stages.

Cultivar	Parentage ~~Percentage~~	Remarks
Coxilha S46	Girua/Purplestraw	
IAC 13 (Lorena)	Ciano 67/Iassi	early
IAC 160	IRN 303.70/IAS20	
RS 2 (Santa Maria)	S45/Kavkaz	
Tipton	Gal123/3/N10B/Tenmarq 112*Hadden/4/ CI13524/Asosan//Purdue 57148.3.11.7	Lr24
CEP 14 (Tapes)	Pel 72380/Arthur 71	Lr9
CEP 19		
CEP 21		Lr26
Londrina	IAS15/4/Yaqui 53/N10B//Yaqui 50/3/ Kentana 54B	
Peladinho		
CNT 7		Lr1, some Res. plts
CNT 9		Res. to B29
CNT 10		Res. to B29 & B30

Table 2. Brazilian cultivars thought to have adult plant resistance to leaf rust.

Cultivar	Pedigree	Remarks
Toropi CNT 8	Frontana/Quadernal/Petiblanco 8 IAS20/ND81	seedling res.
Jacui	Colotana 82451/Yaktana 54/Carazinho /3/Toropi	seedling res.
Maringa	Frontana/Kenya58//Ponta Grossa 1	may be like Frontana
BH1146 (Paulistinha)	Ponta Grossa 1//Fronteira/Mentana	may be like Frontana
BR 4	IAS20*3/Sinvalocho Gama	seedling, *Lr*2b
BR 14 res. Hadden	IAS63/Alondra's'//Gabato/Lagoa Vermelha	seedling res.

Table 3. Virulence/avirulence formulae of leaf rust races in Brazil available for testing.[1]

Race	Avirulence/Virulence
10	2a 3 3ka 9 10 16 17 18 21 23 24 26 11 30 Ald/1 2c 14a 14b 11 20
11	2a 3 3ka 9 10 16 17 18 21 24 26 11 30 Ald/1 2c 14a 14b 23 11 20
12	2a 3 3ka 9 16 17 18 21 23 24 26 11 30 Ald/1 2c 20 14a 14b 11 20 30
13	2a 3 9 16 17 18 21 23/1 2c 3ka 10 14a 14b
14	3 3ka 9 16 21 23 24 26 20 30 Ald/12a 2c 10 14a 14b 17 18 11 20
15	1 2a 3 3ka 9 10 16 17 18 21 23 24 26 Ald/2c 14a 14b 20
16	2a 2c 3 3ka 9 10 16 17 18 21 24 26 20 30 Ald/1 14a 14b 23 11 30
17	3 3ka 9 10 14a 16 21 23 24 26 11 Ald/1 2a 2c 14b 17 18
18	2a 2c 9 10 16 17 18 21 24 26 20 Ald/13 3ks 14a 14b 23 11 30
19	1 2a 3 3ka 9 14a 16 17 18 21 24 26 Ald/2c 10 14b 23 11 20
20	1 2a 3 3ka 9 10 16 17 18 21 23 26 11 30 Ald/2c 14a 14b 24 11 20 30
21	2a 2c 3 3ka 9 16 17 18 21 24 26 20 30 Ald/1 10 14a 14b 23 11 20 30
22	2a 3 3ka 9 16 17 18 21 24 26 11 30 Ald/1 2c 10 14a 14b 23 11 20 30
23	2a 2c 3 9 16 17 18 21 24 26 Ald/1 3ka 10 14a 14b 23
24	2a 3 3ka 9 10 16 17 18 21 23 24 26/1 2c 14a 14b Ald.
25	2a 2c 3 3ka 9 16 17 18 21 24 11 20 30/1 10 14a 14b 23 26 11 30 30 Ald
26	2a 3 3ka 9 16 17 18 21 24 11 20 30/1 2C 10 14a 14b 23 26 11 20 30 Ald
27	2a 2c 9 16 17 18 21 24 26 20 30 Ald/1 3 3ka 10 14a 14b 23 11 20 30
28	2a 3c 3ka 9 14b 16 17 18 21 24 26 20 30 Ald/1 3 10 14a 23 11
29	9 10 14b 16 21 23 26 20 Ald/12a 2c 3 3ka 14a 17 18 24 11 30
30	3 3ka 9 16 21 23 26 30 Ald/1 2a 2c 10 14a 14b 17 18 24 11 20 30
31	1 2a 2c 9 10 16 18 21 24 26 20 30 Ald/3 3ka 14a 14b 17 23 11 30
32	2a 2c 3 3ka 10 16 17 18 21 24 26 11 20 30 Ald/19 14a 14b 23 11 30
33	3ka 9 10 14a 18 21 23 24 26 11 30 Ald/12a 2c 3 14b 16 17 11 20 30
34	29 2c 3ka 9 14b 16 17 21 24 20/13 10 14a 18 23 26 11 30 Ald
35	2a 2c 3ka 9 10 14b 16 17 21 24 20 30/13 14a 18 23 26 11 30 Ald
36	2a 2c 9 14b 16 17 18 21 24 26/1 3 3ka 10 14a 14b 23 11
37	3 3ka 10 16 17 18 21 24 26/1 2a 2c 9 14a 14b 23

[1] 11 and 30 are often on both sides of the virulence formulae. This may be due to a genotype or environmental difference.

Environment

As the resistance to be studied is expressed in the field at Passo Fundo in September and October, then it is expected that this range of temperature, day length, light intensity, and soil fertility should permit the resistance to be expressed in the greenhouse or growth chamber. Depending on the host, pathogen and host growth stage selected to evaluate resistance, a change in temperature may make resistance easier to evaluate. The resistance genes Lr13 and Lr34 are present in Frontana and presumed to be present in many Brazilian cultivars. Lr13 is more effective in seedlings at 25 than at 20°C, while Lr34 is more effective in seedlings at 5 to 10°C than at 20°C.

It is desirable to use the best controlled environmental conditions possible. In the preliminary test and tests of F_1s, the use of a growth chamber is advised; however, due to the large number of F_2 plants to evaluate, the evaluation will probably be done in the greenhouse. F_3 tests (after the completion of the thesis) will be tested in the field, due to the space required.

Checks

It is critical in each test (preliminary, F_1, F_2, F_3 and test crosses) to have adequate checks derived from selected pure lines. Initial seed sources will be from CNP-TRIGO, about 100 grams per line. Checks in each test should include each susceptible parent line, each resistant parent line used and single gene lines for genes that have some likelihood to be present. For this study, Frontana, the basic adult plant resistance from Brazil (Lr13, 34, T_3), TcLr34, TcLr13, TcLrT_3 (single gene lines for the respective genes, TcLr34&T_3 and Thatcher, the background parent for the backcross lines, should be used as checks. If extra F_1 seeds exist, it would be useful as a check in both the F_2 and F_3 tests. It is desirable to have a row of each of the check lines planted in the field each wheat season so that any difference between the field and greenhouse resistance response can be observed. The rows in the field are for observation purposes only.

Time

A careful schedule must be developed so that planting, inoculation, evaluation and crossing can be done in the desired environment. The work schedule must take into account the availability of materials, equipment and space, as well as minimally interfere with classes, examinations and required reports. The preliminary experiment must start immediately and the entire time schedule should be determined immediately after

the preliminary tests.

Scoring

The existing diagrams (modified Cobb and James scales) for estimating disease severity (lesion number) and lesion size were probably marginal for use with wheat leaf rust. Therefore, a new diagram was constructed from which either James or Cobb scale severities can be determined. Lesion size and shape were adjusted for leaf rust, and host response (R, MR, MS, S) were diagramed as size 1, 2, 4, and 8, respectively, enabling quick assignment of both lesion numbers and size (Fig. 1).

Seed

A 100 grams of the purest seed source known are desirable to initiate this experiment. The particular plants to use will be selected in the preliminary experiment. However, even then that plant could be a heterozygote. If risk of heterzygous plants are great, and time permits, self plants before starting preliminary experiment. In this case, time is of the essence. To avoid later problems, seed of each selected plant in the preliminary should be maintained separately.

Evaluation of F_1 Plants

This test is not essential, but I highly recommend it. 1) In the susceptible x resistant cross, F_1 plants can be used to determine if a cross was made; 2) show resistance is dominant, or with reciprocal crosses determine maternal inheritance. If several resistance genes are present, these tests for maternal inheritance may be inconclusive. In any case, F_1 test shows the heterozygous genotypes, phenotypic expression and allows a check of the chosen methodology from the preliminary experiment.

The number of F_1 plants required is determined by the number of F_2 required, which depends on the number of effective resistance genes segregating in the progeny (Table 4). Many cultivars have been found to have 3 to 4 effective genes.

Evaluation of F_2 Plants

This test is based on a single plant response. Note that plants cannot be replicated, however, depending on stage inoculated. A second inoculation of the same plant at a later date may be possible to eliminate possible escapes. In the case of crosses involving a seedling and an adult plant resistant or two seedling resistance genes, two races can be used. In general, if F_2 testing is done, it is the best procedure to save seed of each plants so, if necessary, a F_3 row can be used to confirm the F_2 genotype. The number of F_2 plants required per susceptible x

resistance cross is shown in Table 4. The easiest test for F_2 plants is the ratio of all other plants (not as susceptible as the susceptible parent) to the number of susceptible plants. The resistance to leaf and stem rust is generally dominant; however, recessive resistance will be recognized in the F_1 (susceptible) and the larger ratio of susceptible F_2 plants. Consult a genetics textbook for recessive genes and other gene interactions.

The F_2 tests should always include the parents if seed is available, the F_1 remnants. When evaluated where space or growth stage differences preclude a single test, then the checks must be included in each test. Difference between environments and inoculum density between tests may result in the need to express the results as a percent of the check, e.g., if in test one the susceptible check has a 80% severity and test line 40%, that has to be standardized with test two where the check was 100% and a test line was 40%, i.e. 50% and 40% severity, respectively.

Evaluation of F_3 Lines

The F_3 lines can be evaluated and from this the F_2 phenotypes determined. The advantages of this is that data is for a row instead of a plant (Table 4). The disadvantages are the space required. If several genes are involved, the F_3 tests exceed the space available in most greenhouse facilities. With field tests, the investigator loses control of the environment, and sometimes the pathogen may be a mixture of several phenotypes. F_3 tests also require the time of another generation. Thus, if time is limiting, F_2 testing is often done with verification by planting F_3 lines from specific F_2 plants. In this study, the F_3 testing should be done but not as a part of the thesis.

F_3 needs primarily to be scored as homozygous, susceptible, homozygous resistant, and segregating. Homozygous lines should be scored for lesion number (severity) and lesion size. This may help to identify the homozygous lines for 0, 1, 2 and --- genes for resistance. The only segregating lines one would be interested in are those segregating 3:1 which indicates a single heterozygous gene for resistance in the F_2 plant. The most resistant plants in the row should be selfed to obtain a line with a single gene for resistance, or return to resistant seed of that F_2 plant, which then must be selfed.

Check Crosses

As Lr13 and Lr34 are fairly likely to be present in this cultivar, it is desirable to make test crosses with TcLr13 and TcLr34. The object is to determine if these genes are present. A TcLr13 x resistant parent cross would result in a F_2 progeny

that would be homozygous resistant to a Lr13 avirulent culture. A maternal inherited gene in the resistant male parent would not be transferred to the progeny. If Lr13 is absent, then some susceptible plants would occur in the F_2 progeny. This test could be done in the field in Minnesota where the current leaf rust population is Lr13 avirulent and Lr34 virulent with a population size no greater than 64 plants. Checks included should be Thatcher, TcLr13, TcLr34, resistant parent, susceptible parent, and remnant F_1 seed.

I also recommend a TcLr34 x resistant cross to check for the presence of Lr34. This F_2 progeny could be evaluated in Obregon, Mexico where the leaf rust population is virulent to Lr13 and avirulent to Lr34. Some population size and checks are required in this test as in the Lr13 test.

Table 3 Expected F_2 and F_3 genetic ratios for the effective number of resistance genes assuming dominance, and independence which normally have been observed for resistance to wheat leaf rust. Genotypes will be the same if the assumption is untrue; however, phenotypes will be altered. Consult a genetic textbook.

1 gene for resistance F_2 (16 plants required)		F_3 rows
1	RR	homozygous resistant
2	Rr	segregates
1	rr	homozygous susceptible

2 genes for resistance F_2 (64 plants required)		F3 rows
1	RR TT*	homogygous resistant
2	RR Tt	homozygous R, single gene line for R
1	RR tt	segregates 15 all other:1 sus.
2	Rr TT	segregates 3 all other:1 sus.
4	Rr Tt**	homozygous T, single gene line for T
2	Rr tt	segregates 3 all other:1 sus.
1	rr TT	homozygous susceptible
2	rr Tt	
1	rr tt***	

3 gene ratio F_2 (256 plants required)			F3 rows
1	RR TT WW		homozygous resistant
2	RR TT Ww		homogygous
1	RR TT ww		
2	RR Tt WW		
4	RR Tt Ww		
2	RR Tt ww		
1	RR tt WW		homozygous
2	RR tt Ww		single gene line for R
1	RR tt ww	homozygous	
1	Rr TT WW		
4	Rr TT Ww		
2	Rr TT ww		
4	Rr Tt WW		
8	Rr Tt Ww**		Segregates 63 all other:1
4	Rr Tt ww		Segregates 15 all other:1 susceptible
2	Rr tt WW		
4	Rr tt Ww		Segregates 15 all other:1 susceptible
2	Rr tt ww		Segregates 3 all other:1 susceptible
1	rr TT WW		homozygous
2	rr TT Ww		single gene line for T
1	rr TT ww	homozygous	

Table 3 (continued)

2	rr Tt WW		
4	rr Tt Ww	—	Segregates 15 all other:1 susceptible
2	rr Tt ww		Segregates 3 all other:1 susceptible
1	rr tt WW	homozygous	single gene line for W
2	rr tt Ww		Segregates 3 all other:1 susceptible
1	rr tt ww***		homozygous susceptible

4 genes segregating

F_2 (1024 plants required)			F_3 rows
1	QQ RR TT WW*		
2	QQ RR TT Ww		homozygous
1	QQ RR TT ww		homozygous
2	QQ RR Tt WW		
4	QQ RR Tt Ww		
2	QQ RR Tt ww		
1	QQ RR tt WW		homozygous
2	QQ RR tt Ww		
1	QQ RR tt ww		homozygous
2	QQ Rr TT WW		
4	QQ Rr TT Ww		
2	QQ Rr TT ww		
4	QQ Rr Tt WW		
8	QQ Rr Tt Ww		
4	QQ Rr Tt ww		
2	QQ Rr tt WW		
4	QQ Rr tt Ww		
2	QQ Rr tt ww		
1	QQ rr TT WW		homozygous
2	QQ rr TT Ww		
1	QQ rr TT ww		homozygous
2	QQ rr Tt WW		
4	QQ rr Tt Ww		
2	QQ rr Tt ww		
1	QQ rr tt WW		homozygous
2	Qq rr tt Ww		
1	QQ rr tt ww	homozygous	single gene line for Q
2	Qq RR TT WW		
4	Qq RR TT Ww		
2	Qq RR TT ww		
4	Qq RR Tt WW		
8	Qq RR Tt Ww		
4	Qq RR Tt ww		
2	Qq RR tt WW		
4	Qq RR tt Ww		
2	Qq RR tt ww		
4	Qq Rr TT WW		
8	Qq Rr TT Ww		
4	Qq Rr TT WW		

Table 3 (continued)

8	Qq Rr Tt WW		
32	Qq Rr Tt Ww	segregates 256 all others:1 susceptible	
8	Qq Rr Tt ww	segregates 63 all others:1 susceptible	
4	Qq Rr tt WW		
8	Qq Rr tt Ww		
4	Qq Rr tt ww	segregates 15 all other:1 susceptible	
2	Qq rr TT ww		
4	Qq rr TT Ww		
2	Qq rr TT ww		
4	Qq rr Tt WW		
8	Qq rr Tt Ww		
4	Qq rr Tt ww	segregates 15 all other:1 susceptible	
2	Qq rr tt WW		
4	Qq rr Tt Ww		
2	Qq rr tt ww	segregates 3 all other:1 susceptible#	
1	qq RR TT WW	homozygous	
2	qq RR TT Ww		
1	qq RR TT ww	homozygous	
2	qq RR Tt WW		
4	qq RR Tt Ww		
2	qq RR Tt ww		
1	qq RR tt WW	homozygous	
2	qq RR tt Ww		
1	qq RR tt ww	homozygous	single gene line for R
2	qq Rr TT WW		
4	qq Rr TT Ww		
2	qq Rr TT ww		
4	qq Rr Tt WW		
8	qq Rr Tt Ww		
4	qq Rr Tt ww		
2	qq Rr tt WW		
4	qq Rr tt Ww		
2	qq Rr tt ww	segregates 3 all other:1 susceptible#	
1	qq rr TT WW	homozygous	
2	qq rr TT Ww		
1	qq rr TT ww	homozygous	single gene line
2	qq rr Tt WW		
4	qq rr Tt Ww		
2	qq rr Tt ww	segregates 3 all other:1 susceptible#	
1	qq rr tt WW	homozygous	single gene line
2	qq rr tt Ww	segregates 3 all other:1 susceptible#	
1	qq rr tt ww***	homozygous	like susceptible parent

* like resistant parent

** like F_1

*** like susceptible parent

to obtain single gene lines, identify lines where all genes are effective with similar host response. It may be necessary to select the lines that segregate 3:1 and then self to get homozygous, and then intercross to show which lines are alike, no segregation or different F_2 segregates for susceptibility.

Preliminary test

Goal: To make the final decision on:

1) susceptible parental cultivar
2) resistant parental cultivar
3) pathogen race to use
4) environment to use
5) host growth stage at which to evaluate resistance
6) inoculation density to use

Because of the available background information in this case, most of these items are really being verified.

Procedure: Plant 10 to 20 seeds of each host and check in small pots for each race, environment and inoculum density to be evaluated (in many cases environment and inoculum density are known to not be important). When parents differ in maturity more than a few days, it will be necessary to plant every 7 days for a month or more, depending on the number of days' difference in flowering, to permit crossing. Note: Some wheats will require vernalization before planting for this test. Normally for vernalization, seeds can be placed in vermiculite or moistened sand or placed on moistened filter paper in a petri dish for 24 hours at 20° C before the cold exposure and then exposed to 15-45 days of 2-4°C. The cold exposure at 2-4°C for 15-45 days (depending on cultivar) can be done in the dark. In later planting dates of the preliminary test, it may be possible to eliminate cultivars, races, inoculum densities, growth stages or environments if these decisions have been made by planting time. It is always necessary to assure that the cultivars used are of the chosen phenotype by testing for resistance or susceptibility.

Three to five races should be selected to evaluate in the preliminary test. Experience has shown that some races more effectively distinguish the resistance than others. This may be due to the difference between the homozygous avirulent and heterozygous avirulent cultures. Resistance to some cultures are more effective over a wider environmental or host growth stage range.

For adult plant resistance, 3 to 4 growth stages are initially evaluated, the first being the seedling leaf and the last being the flag leaf. I like to inoculate the flag leaf as soon as completely emerged, so I can determine the host response before making crosses. If the resistance is adequately differentiated in an early growth stage, late stages may not need tested. Evaluation is normally done at the earliest stage where the resistance is clearly identifiable.

Off-type and weak plants should be discarded throughout the

experiment. Plants may have to be transplanted one or two times in order to ensure good disease response and seed production. However, to keep the space and work involved moving pots for inoculation to a minimum, it is necessary to use small pots when the plants are small and plant number high.

Crosses

The primary crosses are between the susceptible and resistant cultivars. Normally, the susceptible plant is used as the female. This allows the determination in the F_1 of seeds not resulting from a cross between cultivars as nearly all resistances in wheat have been dominant to leaf and stem rust. Also, any female inherited adaptation factors will be present in the progeny. If there is any chance of maternal inheritance, then the reciprocal cross should be made.

Crosses can be made and studied between two resistant cultivars, but it is not generally recommended as it increases F_2 and F_3 population sizes needed and increases the possibilities of interactions of resistance genes and allelism.

Crosses are also desirable between the resistant parent and line(s) thought to have an effective resistance gene that may also be present in the resistant parent. F_2 plants from these crosses should not segregate for resistance if the gene is present and susceptible plants should occur if the gene is absent. Note: if the resistant line has several effective genes for resistance, many F_2 plants or F_3 lines will need to be evaluated but any susceptible plants indicate the resistance is different than that of the test line.

If maternal inheritance is expected, evaluate the resistance (female) by susceptible (male) cross and determine the number of genes segregating in F_2 plants or F_3 lines. Then the analysis of reciprocal cross (must have same resistant plant for a parent) should have 1 less gene segregating if maternal inheritance occurred or the same number of resistance genes if maternal inheritance was not involved. By testing in this order, the number of progeny of the second population that must be evaluated can be estimated. In cases of maternal inheritance, 1 gene less will segregate than in the resistant x susceptible cross.

Number of F_1 seed required for each cross depends on resistant genes that may be segregating in the F_2. A general rule of thumb is as follows:

Number of resistance genes thought to be segregating	Number of required F_1 Seeds	F_2 Plants	F_3 Lines
1	16	16	16
2	64	64	64
3	256	256	256
4	1024	1024	1024
5	4096	4096	4096
6	16384	16384	16384

Crossing method

Several crossing methods are available but the transfer of 1 or 2 anthers per floret to the emasculated female ensures the male plant can be maintained for seed production. Crosses can also be made by bagging the emasculated female and male spikes together (remove awns and tips of glumes, lemma and palea from both parents to improve chances of successful pollination). Be sure to maintain seed of individual parental plants as checks.

Each cross between a susceptible and a resistance cultivar should involve more than one (three are often used) resistant plants. As long as the susceptible plant is susceptible, it is less important to use a specific number of plants. Plants of self pollinated crops are homozygous, but the cultivar is sometimes heterogenous. Thus, the following are the systems recommended:

susceptible plant 1 x resistant plant 1
susceptible plant 2 x resistant plant 2
susceptible plant 2 x resistant plant 3

If maternal inheritance may be involved, then:

susceptible plant 1 x resistant plant 1
susceptible plant 2 x resistant plant 2
resistant plant 1 x susceptible plant 3
resistant plant 2 x susceptible plant 4

Testing for presence of a specific gene:

Single gene line for resistance x resistant parent

As no maternal inherited resistance gene is currently known in wheat, reciprocal crossing need not be made in test crosses. If the resistance cultivar has 1, 2, 3 or 4 effective resistance

genes, then population sizes of F_2 plants or F_3 lines derived from F_2 plants would be 16, 64, 258 and 1024, respectively. Thus, susceptible plants would be rare if many resistant genes were present, giving a false impression that the gene tested for was present.

To make and evaluate all the lines necessary to determine all the genes involved and their chromosome location, it currently takes 5 to 10 years; however, to determine the number of effective genes from an F_2 population and to determine from test cross the presence or absence of a suspected gene is in the realm of a Ph.D. thesis. Parental cultivars, lines and pathogen cultures should be maintained.

Roelfs' Wheat Leaf Rust Severity Scale