Inheritance of Resistance to Red Clover Vein Mosaic Virus in Red Clover

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ABSTRACT

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Red clover (*Trifolium pratense*) clones, either resistant or susceptible to red clover vein mosaic virus, were intercrossed to study the mode of inheritance of resistance to this virus. Evaluation of parental, F_1, F_2, and backcross and other populations, indicated that the resistant reaction was controlled by a single dominant gene, *Rc*.

Additional key words: genetics of resistance.

Red clover vein mosaic virus (RCVMV) causes a characteristic chlorosis of leaf veins, veinlets, and tissue immediately adjacent to the veins in red clover (9); the disease decreases the yield of red clover (*Trifolium pratense* L.) by reducing the foliage growth, decreases persistence and increases the susceptibility to root rot organisms, particularly *Fusarium* spp. (1, 4, 10). In addition, red clover plants serve as reservoirs for RCVMV, which causes pea stunt (4, 5, 6).

The losses caused by RCVMV and the frequency of its occurrence in red clover in Wisconsin are variable. Surveys made during 1949-1952 showed that RCVMV was the most prevalent virus in red clover (6) and also in alsike clover and sweet clover (4). However, a later survey made by Stuteville and Hanson (13) indicated that RCVMV ranked third after bean yellow mosaic virus (BYMV) and pea streak virus.

Virus diseases of red clover can be controlled effectively by the use of resistant cultivars. Resistance to BYMV, pea common mosaic virus, and RCVMV has been reported in many breeding lines and commercial cultivars (6, 12). Partial resistance to BYMV recently has been incorporated into red clover cultivar Arlington (11). In the only reports on the mode of inheritance of resistance to virus diseases in red clover, Diachun and Henson (2, 3) found that the hypersensitive local lesion reaction to BYMV is controlled by a dominant gene.

This investigation was undertaken to determine the mode of inheritance of resistance to RCVMV in red clover. Such information would be of value in the development of multiple disease resistant germplasm.

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MATERIALS AND METHODS

To study the inheritance of resistance to RCVMV, resistant (R) and susceptible (S) red clover clones were crossed in all three combinations: resistant-by-resistant (R × R), resistant-by-susceptible (R × S), and susceptible-by-susceptible (S × S). Clones C1, C24, C72, and Pen 3 were the resistant parents and clones L12, C14, and Pen 4 were the susceptible parents. Clones Pen 3 and Pen 4 were selected from the cultivar Pennscott and C72 from breeding line C452, which was synthesized from a

TABLE 1. Segregation in F_1 or I_1 generations of red clover for reaction to red clover vein mosaic virus

Crosses[a]	Plants observed R (no.) : S (no.)
R × R	
C72 × Pen 3	102 : 28
C24 × Pen 3	97 : 23
C24 selfed (I_1)	21 : 5
R × S	
Pen 3 × L12	45 : 47
C24 × L12	41 : 37
C24 × C14	40 : 35
C1 × L12	102 : 0
S × S	
Pen 4 × L12	4 : 78
C14 × L12	0 : 70

[a]Abbreviations: R = resistant and S = susceptible. No reciprocal differences within a cross were observed, so data for a cross were pooled.

recurrent selection program for foliar disease resistance. Clones C1, C14, and C24 were selected by Stuteville and Hanson (12) from cultivars Dollard, Purdue, and Lakeland, respectively. Clone L12, a selection from Lakeland, was obtained from S. Diachun, University of Kentucky, Lexington. All of these clones are self-incompatible, but highly cross-compatible. The F_1 crosses and the subsequent backcrosses (BC-F_1) of F_1 plants to both parents were made in the greenhouse by controlled hand pollinations. The F_2 populations were obtained by intercrossing clones of five resistant F_1 plants by means of honeybees (*Apis millifera* L.) as the pollinating agent.

Selfed seeds from C24 were obtained by placing flower heads at 40 C for 48 hr followed by hand-aided self pollination using a toothpick to trip the floral mechanism (7).

Scarified seeds of all F_1, F_2, and backcross populations were germinated on moist filter paper in petri plates and then planted in sand. When the seedlings were 2 to 4 cm high, 50 seedlings were transplanted to a wooden flat (52 × 36 × 10 cm) or individually transplanted to 10-cm diameter pots containing a steamed mixture of soil, peat, vermiculite, and sand. Further propagation of individual plants was made by cuttings (12). Since RCVMV infection in red clover is systemic, cuttings of selected clones from each population were made prior to inoculation and these cuttings were maintained for future crosses.

Seeds were collected from each parent of a cross, and plants were grown and individually tested for virus reaction. Chi-square values were used for testing the goodness of fit and homogeneity.

Different isolates of RCVMV react differently with the specific genotypes of red clover (12). Therefore, a single RCVMV isolate, ATCC PV 110, was used in all the inoculations (8). It was maintained on red clover clone L12 and on pea (*Pisum sativum* L. 'Perfected Wales'). The inoculum was prepared by grinding RCVMV-infected pea or red clover leaves in water or in 0.05 M sodium borate buffer, pH 7.0, containing 0.02 M 2-mercaptoethanol (5 ml/g of leaves). The inoculum was rubbed on Carborundum-dusted leaflets. The first inoculation was made 4-6 mo after planting, and uninfected plants were inoculated subsequently twice at 1-mo intervals. Before inoculation, all but three to four

TABLE 2. Segregation for reaction to red clover vein mosaic virus in backcross and F_2 populations of red clover

Populations[a]	Plants observed R (no.)	:	S (no.)	Chi-square	*P*
F_1 from Pen 3 × L12		(Expect 3R:1S)			
$1RF_1$ × Pen 3	26		6	0.67	.25-.50
$2RF_1$ × Pen 3	34		8	0.80	.25-.50
$3RF_1$ × Pen 3	10		4	0.09	.75-.90
RF_2	30		6	1.33	.10-.25
		(Expect 1R:1S)			
$1RF_1$ × L12	20		16	0.44	.50-.75
$2RF_1$ × L12	22		17	0.64	.25-.50
$3RF_1$ × L12	13		9	0.73	.25-.50
$4SF_1$ × Pen 3	18		16	0.12	.50-.75
$5SF_1$ × Pen 3	16		12	0.57	.25-.50
		(Expect 0R:1S)			
SF_1 × L12	0		63	...	...
F_1 from C24 × L12		(Expect 3R:1S)			
$1RF_1$ × C24	13		3	0.33	.50-.75
$2RF_1$ × C24	29		7	0.58	.25-.50
$3RF_1$ × C24	17		7	0.22	.50-.75
RF_2	71		13	4.063	.01-.02
		(Expect 1R:1S)			
$1RF_1$ × L12	25		21	0.35	.50-.75
$2RF_1$ × L12	23		17	0.90	.25-.50
$4SF_1$ × C24	25		19	0.82	.25-.50
$5SF_1$ × C24	23		18	0.61	.25-.50
		(Expect 0R:1S)			
SF_1 × L12	0		32	...	...
SF_2	2		39	0.10	.75-.90
F_1 from C1 × L12		(Expect 1R:1S)			
$1RF_1$ × L12	36		30	0.55	.25-.50
$2RF_1$ × L12	14		13	0.04	.75-.90
$3RF_1$ × L12	28		22	0.72	.25-.50
		(Expect 3R:1S)			
$1RF_1$ × C24	45		9	2.00	.05-.10
$3RF_1$ × C24	23		5	0.76	.25-.50

[a]Abbreviations: RF_1 = resistant F_1 plant; SF_1 = susceptible F_1 plant; RF_2 = polycross from intermating RF_1 plants; and SF_2 = polycross from intermating SF_1 plants. Different F_1 plants of the same cross are indicated by different numbers.

leaves were removed. Plants of clone L12 were used as the susceptible control. Plants with symptoms of RCVMV infection were recorded and removed before the next inoculation. Six wk after the last inoculation, leaves from all plants without symptoms were indexed for the presence of RCVMV on pea or the local lesion host, *Chenopodium amaranticolor* Coste and Reyn. No symptomless RCVMV-infected plants were detected.

From tests in growth chambers maintained at 16 C, 20 C, 24 C, and 28 C with a 12-hr photoperiod, we selected 24-28 C as the most suitable temperature for maximum RCVMV symptom development of inoculated red clover and pea. Thus, as far as possible, all plants were maintained and screened in a greenhouse maintained at approximately 24 C. In winter months, natural light was supplemented with incandescent and fluorescent lights.

RESULTS AND DISCUSSION

The results from the reciprocal crosses indicated no maternal inheritance, and, therefore, the data from each cross were pooled.

In the R × R crosses, C72 × Pen 3, C24 × Pen 3, and C24 × C24, the F_1 plants segregated three resistant to one susceptible (Table 1). In three of the four R × S crosses, Pen 3 × L12, C24 × L12, and C24 × C14, F_1 plants segregated in an approximate ratio of one resistant to one susceptible. In the fourth R × S cross, C1 × L12, F_1 plants were all resistant. When two susceptible parents (L12, C14, or Pen 4) were crossed, only four out of 152 F_1 plants were not susceptible. These results (Table 1) were indicative of the hypothesis that resistance to RCVMV in red clover was controlled by one gene, and that susceptibility was recessive to resistance and thus was expressed only in recessive homozygous conditions. To test this hypothesis, resistant and susceptible F_1 plants were backcrossed to the two types of parents, and also sib-mated to obtain the F_2 generation.

When the resistant F_1 (RF_1) plants, which were selected from the R × S crosses (Pen 3 × L12 and C24 × L12) were backcrossed to their resistant parent, each set of the backcrossed population segregated in a 3R:1S ratio. When the same RF_1 plants were backcrossed to the susceptible parent, or when susceptible F_1 (SF_1) plants were crossed by their resistant parents, the BC populations segregated in 1R:1S ratio. The progeny from the backcross of SF_1 plants by their susceptible parent were all susceptible. The polycross progenies (F_2) obtained from intermating selected RF_1 plants from the Pen 3 × L12 cross, segregated in a 3R:1S ratio. However, the similar polycross F_2 obtained from the C24 × L12 cross deviated significantly from the expected 3:1 ratio ($P = 0.01$ to 0.02).

The frequencies of resistant and susceptible plants of the F_1, BC, and F_2 plants (Table 2) are consistent with the view that the single dominant gene controls the resistance to isolate PV 110 of RCVMV in red clover. The susceptible parents are assumed to be homozygous for the recessive allele and three of the resistant parents (C24, C72, and Pen 3) apparently are heterozygous for this allele. Another resistant parent (C1) apparently is homozygous for the dominant allele. All the F_1 plants from C1 × L12 cross (R × S) were resistant and a segregation ratio of 1R:1S was obtained when these were backcrossed to susceptible L12 (Table 2). When these RF_1 plants, expected to be heterozygotes, were backcrossed to C24 (another heterozygote) the progeny segregated in a 3R:1S ratio.

Segregation of the selfed progeny of C24 in a ratio of 3R:1S further confirms the model. This would be expected for the self progeny of a heterozygous plant if resistance is controlled by a single dominant gene.

The Chi-square tests for goodness of fit ($P > 0.25$), within the individual crosses and pooled for the crosses within each group, were acceptable for a monogenic model. The gene for resistance to RCVMV isolate PV 110 in resistant red clover clone C24 is designated as *Rc*, since no other allele for the RCVMV reaction in red clover is known.

The putative genotype of parent clones C24 and Pen 3 would be *Rcrc* and of susceptible parent clones C14 and L12 would be *rcrc*.

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