

INHERITANCE OF RED CLOVER (*TRIFOLIUM PRATENSE* L.) RESISTANCE TO BEAN YELLOW MOSAIC VIRUS (BYMV)¹

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Summary. In the studies on the inheritance of red clover resistance to bean yellow mosaic virus (BYMV), the source of resistance were resistant genotypes of the strain Strugi R-104. Resistant genotypes were crossed with susceptible ones and the F_1 and F_2 generations were analysed.

On the basis of the obtained results it may be inferred that resistance to BYMV virus is determined by a single dominant gene, whereas the lack of phenotypic differences in reciprocal crosses is indicative of a nuclear inheritance to BYMV.

Virus diseases infecting red clover result in significant losses of green matter and decrease seed setting; they are also a source of infection for other cultivated papilionaceous plants. Losses estimated under experimental conditions were 23 - 56% in the USA (Kreitlow et al. 1957) and several dozens per cent in Europe (Kowalska 1973). Among viruses attacking red clover the dominant one is bean yellow mosaic virus (Hanson et al. 1961, Kowalska 1973).

Studies dealing with disease symptoms, degree of population infection and reaction to infection of various cultivars and lines of red clover showed the existence of resistance to bean yellow mosaic virus (Diachun et al. 1956, 1958, 1960; Błaszczak et al. 1971; Kowalska 1974; Fiedorow et al. 1977). The purpose of the present paper was to know the inheritance mechanism of resistance to bean yellow mosaic virus.

MATERIAL AND METHODS

As a result of many-year studies on resistance (Fiedorow et al. 1977) it was found that the strain Strugi R 104 displays a high resistance to bean yellow mosaic virus. The mentioned strain of red clover was selected in 1969 from the cv. Gloria. Twelve reciprocal crosses were made between genotypes resistant to BYMV virus

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originating from the strain Strugi 104 (designated R 104-1, R 104-2, R 104-3, R 104-4, R 104-5, R 104-6) and susceptible plants originating from the cultivars: Gloria (designated G-1, G-2), Hruszowska (designated H-1, H-2) and Skrzyszowicka (designated S-1, S-2). Flowers were manually castrated and crossed using bumble-bees (*Bombus hypnorum* L.):

At the stage of the leaf rosette, plants were clipped leaving 3 - 4 well-developed leaves, which were then mechanically inoculated with red clover isolate 48 of BYMV virus. The inoculum was prepared from pea plants of the cv. Flawanda infected with virus. The inoculum was diluted in the ratio 1:2 or 1:3 by phosphate buffer 0.5 M pH 7.6. In order to increase the inoculum effectiveness carborundum was used.

Inoculation was performed under glass-house conditions from July to October at the following five dates: July 19, July 24, August 14, August 30 and October 12. At the fourth inoculation date, darkness of plants was applied for 24 hours before inoculation. The number of diseased plants was ascertained on the basis of four observations on the occurrence of disease symptoms on red clover leaves.

The F_2 generation was obtained from reciprocal crosses of F_1 plants between: resistant and susceptible forms (R 104-4 \times H-2), susceptible and susceptible (R 104-4 \times G-1/6) and resistant and resistant (R 104-6/11 \times G-2/12). Crossing was made using bumble-bees *Bombus terrestris* L.

With the aim to reveal resistant and susceptible forms, F_2 hybrids were infected with six isolates of bean yellow mosaic virus. The inoculation was repeated twice and plants with mosaic symptoms were recognized susceptible to infection. The obtained data concerning the number of different phenotypic classes of resistant and susceptible plants were subjected to statistical analysis.

To estimate the chromosome number, samples for cytological analysis were taken from 12 parental plants and from 5 plants of each cross combination in F_1 generation (in combination 12 the samples were taken from four plants). The rootlets of the studied plants were fixed in Carnoy's solution and stored afterwards in 75% ethyl alcohol. The chromosome number was determined by the method of smear preparations from the root meristems. The rootlets were thermally macerated in 1 N HCl for 2 minutes and stained with aceto-carmin. The chromosome were counted at metaphase and mitotic anaphase.

RESULTS

It was observed that the first generation of hybrids (F_1) obtained from reciprocal crosses of resistant forms of the strain Strugi R 104 with susceptible genotypes of the cv. Gloria and Hruszowska (Table 1 cross combinations 1 - 6) segregated into resistant and susceptible plants in the phenotypic ratio of 5:1.

A cytological analysis of the parental forms showed that R 104-1, R 104-2 and R 104-3 plants had 15 chromosomes, whereas the remaining parental forms, i.e. R 104-4, R 104-5, R 104-6, G-1, G-2, H-1, H-2, S-1, S-2, had 14 chromosomes each. An analysis of F_1 hybrids also showed the occurrence of trisomic ($2x+1=15$ chromosomes) and diploid ($2x=14$ chromosomes) plants (Table 1).

Table 1. Segregation in F_1 generation obtained from crossing BYMV-resistant and -susceptible forms and their cytological characterization

No.	Cross combinations	Proposed genotypes of parental plants	Number of plants	Segregation into resistant and susceptible forms		Theoretical segregation ratio	Chi^2 calc.	Number of chromosomes in F_1
				obtained o:p	expected o:p			
1	R 104-1 o × p G-1	$Re Re re \times re re$ $re re \times Re Re re$	24	20:4	20:4	5:1	0.000	$2x+1; 2x$
2	G-1 p × o R 104-1		13	11:2	10.8:2.2	5:1	0.040	$2x+1; 2x$
3	R 104-2 o × p G-2		52	42:10	43.3:8.7	5:1	0.442	$2x+1; 2x$
4	G-2 p × o R 104-2		22	20:2	18.3:3.7	5:1	0.912	$2x+1; 2x$
5	R 104-3 o × p H-1		18	12:6	15:3	5:1	3.600	$2x+1; 2x$
6	H-1 p × o R 104-3		32	29:3	26.7:5.4	5:1	1.230	$2x+1; 2x$
7	R 104-4 o × p H-2	$Re Re \times re re$ $re re \times Re Re$	27	27:0	1:0	—	—	$2x$
8	H-2 p × o R 104-4		25	25:0	1:0	—	—	$2x$
9	R 104-5 o × p S-1		33	33:0	1:0	—	—	$2x$
10	S-1 p × o R 104-5		65	65:0	1:0	—	—	$2x$
11	R 104-6 o × p S-2		9	9:0	1:0	—	—	$2x$
12	S-2 p × o R 104-6		5	5:0	1:0	—	—	$2x$

$\chi^2_{1;0.05} = 3.84$. x — doubled chromosome number, o — plants resistant to BYMV virus, p — plants susceptible to BYMV virus, Re — gene determining resistance to bean yellow mosaic virus.

No plants infected with bean yellow mosaic virus were observed in the F_1 generations of those cross combinations (Table 1, 7 - 12), which beside the strain Strugi R 104 involved infection-susceptible genotypes of the cv. Gloria and Skrzyszowicka. Resistance in F_1 dominated over susceptibility, no phenotypic differences being found in reciprocal crosses.

The second hybrid generation (F_2) derived from resistant nonsegregating F_1 hybrids was characterized by segregation into resistant and susceptible forms in the ratio 3:1. In the case of crossing F_1 plants — resistant × resistant and susceptible × susceptible — no segregation into resistant and susceptible forms was observed (Table 2).

Table 2. Segregation in F_2 hybrid generation obtained from crossing BYMV-resistant and -susceptible forms

No.	Cross combination	Proposed F_2 genotypes	Plant number	Segregation into resistant and susceptible forms		Theoretical segregation ratio	Chi^2 calc.
				obtained	expected		
1	R 104-4 o × p H-2	$Re Re, 2 Re re,$ $re re$	171	145:28	142.5; 38.5	3:1	0.011
2	H-2 p × o R 104-4		34	29:5	28.5:5.7	3:1	0.095
3	R 104-4 (1 p × p G-1) 6	$re re$ $re re$	28	0:28	0:1	—	—
4	G-1/6 p × p R 104-4		36	0:36	0:1	—	—
5	R 104-6 (11 o × o S-2) 12	$Re Re$	165	165:0	1:0	—	—
6	S-2 (12 o × o R 104-6) 11		154	154:0	1:0	—	—

$\chi^2_{1;0.05} = 3.84$. o — plants resistant to BYMV virus, p — plants susceptible to BYMV virus, Re — gene determining resistance to bean yellow mosaic virus.

The agreement of the obtained segregation ratios with hypothetical ones in F_1 and F_2 generations was proved by the Chi^2 -test.

DISCUSSION

The obtained and statistically proved phenotypic ratio of segregations 5:1 into resistant and susceptible forms suggested that plants of the Strugi R 104 strain (R 104-1, R 104-2, R 104-3) characterized by resistance to bean yellow mosaic virus were trisomic forms ($2x+1$). Cytological studies showed the existence of genotypes of the strain Strugi R 104 with 15 and 14 chromosomes. A trisomic plant with the genetic composition *Re Re re* crossed with a homozygote with *re re*, in the case of dominance in six theoretical zygotes, gives in its progeny five zygotes with predominance of a dominant trait and one with the phenotype of a recessive trait. The occurrence of trisomics and disomics in the studied populations of parental plants and F_1 generation explains phenotypic segregation into BYMV — resistant and — susceptible forms in the ratio of 5:1.

The lack of segregation in the F_1 generation in cross combinations of resistant genotypes of the strain Strugi R 104 (R 104-4, R 104-5, R 104-6) with susceptible genotypes originating from the cv. Gloria and Skrzyszowicka provides evidence to infer that alleles determining the studied alternations occur in the parental forms in homozygous systems. In the F_1 generation, resistance dominated over susceptibility and the lack of phenotypic differences in reciprocal crosses indicates that resistance is determined by nuclear genes.

Segregation in the ratio 3:1 observed in F_2 hybrid generation obtained from crossing resistant and susceptible plants and the lack of segregation in progenies obtained as a result of crossing resistant with resistant, as well as susceptible with susceptible plants, support the accepted mode of genetic determination of resistance to bean yellow mosaic virus (BYMV).

A similar mode of inheritance was found while studying genetic determination of red clover resistance to red clover vein mosaic virus (RCVMV) infection (Khan et al. 1978). An analysis of the parents and first and second hybrid generations, as well as backcrosses, showed that this trait is controlled by a single dominant gene *Rc*.

As a result of many-year studies on resistance the new variety of red clover, Arlington, with a moderate resistance to bean yellow mosaic virus cultivated in the north-eastern part of the United States, as well as clones of the cv. Kenstar displaying complete resistance to bean yellow mosaic virus expressed in oversusceptibility (Diachun et al. 1965), have been obtained in the USA.

Our studies made it possible to obtain plant material, which is a source of resistance adapted to soil-climatic conditions of Poland, and to obtain strains, which were subjected to breeding examination.

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DZIEDZICZENIE ODPORNOŚCI KONICYZNY CZERWONEJ
(*TRIFOLIUM PRATENSE* L.) NA WIRUS ŻÓLTEJ MOZAIKI FASOLI (BYMV)

Streszczenie

W badaniach nad dziedziczeniem odporności konicyzny czerwonej na wirus żółtej mozaiki fasoli (BYMV), źródłem odporności były genotypy odporne rodu Strugi R 104. Krzyżowano genotypy odporne z podatnymi i analizowano pokolenie F_1 i F_2 .

Na podstawie otrzymanych wyników można wnioskować, że odporność na wirus BYMV jest uwarunkowana pojedynczym genem dominującym, a brak różnic fenotypowych w krzyżówkach wzajemno-przemiennych wskazuje na jądrowe dziedziczenie odporności na BYMV.

НАСЛЕДОВАНИЕ УСТОЙЧИВОСТИ КРАСНОГО КЛЕВЕРА
(*TRIFOLIUM PRATENSE* L.) К ВИРУСУ ЖЁЛТОЙ МОЗАИКИ
ФАСОЛИ (BYMV)

Резюме

В исследованиях наследования устойчивости красного клевера к вирусу жёлтой мозаики фасоли (BYMV) источником устойчивости были устойчивые генотипы линии Strugi R-104. Скрещивались податливые генотипы с устойчивыми и анализировались поколения F_1 и F_2 . В первом гибридном поколении (F_1) комбинации скрещивания 1 - 6 сегрегировались в отношении 5 : 1, а комбинации 7 - 12 сегрегировались в отношении 3 : 1 в поколении F_2 . Цитологический анализ обнаружил существование трисомических систем $2x + 1 = 15$ хромосом, а также диплоидных с $2x = 14$ хомосом. На основании этих результатов можно сделать вывод, что устойчивость к вирусу BYMV обуславливается одним доминирующим геном, а отсутствие фенотипических различий в взаимно-переменных скрещиваниях указывает на ядерное наследование устойчивости к BYMV.