Red Clover Clones with Hypersensitive Reaction to an Isolate of Bean Yellow Mosaic Virus

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ABSTRACT

Clones of red clover resistant to bean yellow mosaic virus, cause of a major disease of red clover, have been selected as potentially useful breeding lines for development of red clover cultivars. The clones are first generation inbreds of a plant in which hypersensitive primary lesions result in localization of the virus. Resistance is controlled by a single dominant factor that is present in a homozygous condition in these clones.

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Additional key words: necrotic spots, local lesions, host resistance, progeny tests.

Red clover plants with symptoms of veinal chlorosis or mottling occur frequently in Kentucky and other states (1, 6). Bean yellow mosaic virus is a common cause of these symptoms in central Kentucky (1). Several variants of the virus have been recovered from naturally infected plants (3). Isolate 204-1 appears to be typical of the virus common in red clover in central Kentucky. In differential clones of red clover selected from the cultivar 'Kenland', isolate 204-1 induces vein chlorosis or mosaic in clone KyC36, primary necrotic lesions followed by lethal systemic necrosis in clone KyC71-8, and only hypersensitive localized necrotic lesions on inoculated leaves of clone KyC40-1 (2).

The purpose of this paper is to report development of inbreds of clone KyC40-1 that are homozygous for a dominant factor that controls the necrotic-spotting type of resistance to bean yellow mosaic virus isolate 204-1 which may serve as breeding lines for development of virus-resistant populations of red clover. An abstract has been published (5).

MATERIALS AND METHODS.— Most red clover plants are self-incompatible and normally set seed only after cross-pollination. Accordingly, it has been difficult or impossible to study the genetics of resistance in a simple straightforward manner; traditional F_1 and F_2 populations were relatively unavailable because of self-incompatibility. Recently, Kendall found that some seed set and matured on self-pollinated red clover plants previously considered to be self-incompatible, if flower heads were maintained at high temperature (35 to 40 C) while the rest of the plant was at lower temp (20 to 25 C) (7).

Flower heads of clone KyC40-1 were treated in this way and were self-pollinated by hand. A limited amount of seed set and matured. Seedlings from the seed were inoculated with isolate 204-1. Several plants that formed local necrotic spots were selected for progeny tests and cloned. They were selfed and cross-pollinated with the susceptible mottling clone KyC36 and with the parental clone KyC40-1. The seedlings were inoculated with isolate 204-1. The flow diagram below summarizes the procedure.

Inoculum of isolate 204-1 was prepared from young mottled leaves of 'Dwarf Gray Sugar' pea seedlings 7 to 12 days after inoculation with isolate 204-1. Leaves were crushed in a mortar with half their weight of 600-mesh Carborundum and 10 times their weight of a solution of either 0.01M Na_2SO_3 or 0.01M DIECA. Inoculum was rubbed onto clover leaflets or sprayed onto them with an airbrush. Plants that did not show symptoms were reinoculated at least three times with inoculum of isolate 204-1.

RESULTS.— Primary necrotic spots were formed on 20 of the 24 inbreds of clone KyC40-1 that were inoculated. The primary lesions on all 20 plants remained localized; none of the plants became systemically invaded. The other four plants became mottled.

Only a few viable seed developed from selfed plants (designated I_1) and from crosses between the I_1 plants and the parental clone KyC40-1, but seed developed in adequate numbers from the crosses with KyC36, so that progeny tests could be used to distinguish between I_1 plants that were homozygous for the hypersensitive resistance factor and those that were heterozygous. Results are shown in the flow diagram (below) and in Table 1.

All inoculated seedlings from crosses between the mottling clone KyC36 and three I_1 clones (accession numbers KyC40-$1I_1$8, KyC40-$1I_1$12 and KyC40-$1I_1$15) developed local necrotic spots. These results are interpreted to mean that these three I_1 inbreds carry the hypersensitivity factor in a homozygous condition.

About half of the plants from the crosses between clone

Flow Diagram

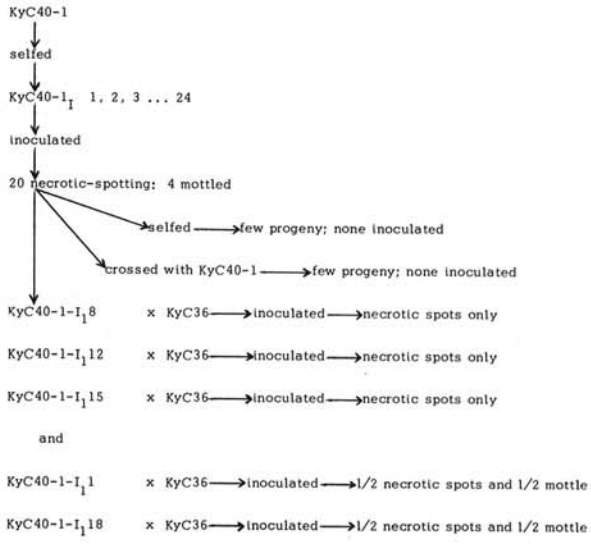

TABLE 1. Inheritance of hypersensitive resistance to bean yellow mosaic virus isolate 204-1 in F_1 generations of red clover clones

Population	Number of plants with		X^2 for local necrosis to mottle ratio
	Local necrosis	Mottle	
KyC40-1$I_1$8 × KyC36	130	0	.00 (1:0)
KyC40-1$I_1$12 × KyC36	56	0	.00 (1:0)
KyC40-1$I_1$15 × KyC36	106	0	.00 (1:0)
KyC40-1$I_1$1 × KyC36	10	8	.22 (1:1)
KyC40-1$I_1$18 × KyC36	22	23	.04 (1:1)

KyC36 and two I_1 clones (accession numbers KyC40-1$I_1$1 and KyC40-1$I_1$18) were necrotic-spotting, while the other half were mottled. This ratio indicates that these two clones are heterozygous for the resistance factor and are like the parental clone KyC40-1 in this regard.

DISCUSSION.— We have reported that a single dominant factor controls necrotic-spotting in clone KyC71-8 (4). The necrotic reaction in KyC71-8 is not localized but becomes systemic (2, 3); thus the necrotic reaction in KyC71-8 is different from the reaction in KyC40-1, in which necrosis results in localization of the virus. Despite the development of primary necrotic lesions, KyC71-8 is not resistant but, indeed, is very susceptible to isolate 204-1, whereas KyC40-1 is hypersensitive and for practical purposes is resistant. Hundreds of plants of each clone have been inoculated with isolate 204-1 over a period of about 15 years. In clone KyC71-8, systemic lethal necrosis almost invariably follows primary necrotic lesions. In clone KyC40-1, primary necrotic lesions develop on inoculated leaves, but infection is localized; typical systemic necrosis has not been observed in growth chambers or greenhouses. Plants of the clone have been under observation under field conditions for several years; typical systemic necrosis has not been seen. We have designated the necrotic factor in clone KyC71-8 as the N factor (4). At this time it is not known whether the localizing (resistant) factor in clone KyC40-1 and in its derivatives is at a locus separate and distinct from the non-localizing necrotic N factor present in clone KyC71-8, whether it is allelic with the N factor, whether KyC40-1 carries a localizing factor additive or complementary to the N factor, or whether some entirely different explanation is needed.

In addition to the three inbreds from clone KyC40-1, at least two other homozygous hypersensitive clones have been identified (accession numbers KyC101$I_1$7 and KyC1173T). Synthetic breeding lines of red clover with the property of localizing a common variant of bean yellow mosaic virus may be possible if some or all of these clones interpollinate.

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