EVALUATION AND INHERITANCE OF RESISTANCE IN FABA BEAN (*Vicia faba*) TO BEAN YELLOW MOSAIC VIRUS

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Inder Paul Singh Gadh

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FOREWORD

The format adopted for this thesis deviates from the conventional and has been approved by the Council of the Faculty of Graduate Studies at the University of Manitoba. Accordingly, materials, methods and results are presented in the form of three publications, the style of which complies with the requirements of the Canadian Journal of Plant Pathology. A general discussion follows and thesis terminates with bibliography and appendices.

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GENERAL ABSTRACT

Gadh, Inder Paul Singh. Ph.D., The University of Manitoba, May, 1982. Evaluation and Inheritance of Resistance in Faba Bean (Vicia faba) to Bean Yellow Mosaic Virus. Major Professor; Dr. C.C. Bernier.

Sixty-eight open-pollinated faba bean selections identified as resistant to bean yellow mosaic virus (BYMV) in preliminary field experiments were evaluated further against the 'mosaic' strain of BYMV in the field from 1978 to 1981. Most selections were variable for disease reaction. However, repeated testing and reselection resulted in a progressive increase in the number of symptomless plants in accessions 2N138-1, 2N23-3, 2N295-2 and 2N85. Within 2 years, several field and greenhouse selections from 2N138 and 2N23 were found to be uniformly immune and symptomless, respectively, in the greenhouse. Accession 2N138 was also immune to the 'necrotic' strain in the field and the greenhouse tests. Additional selections were heterogeneous with several plants resistant to both strains.

An immune and a highly resistant faba bean inbred line were crossed reciprocally with each of the two susceptible inbred lines. Results from testing F_1, F_2 and F_3 generations at 27° C indicated that the resistance of line 2N23-2GH-3-2 to the 'mosaic' strain and the immunity of line 2N138-1GH-3-5 to both virus strains were each controlled by two recessive genes. One of the two genes for susceptibility in line UMFB13-1GH-1-3 was masked in the greenhouse and expressed only in the field. Symptoms in many F_2 plants appeared 14 days after inoculation,

but the fact that symptoms did not appear until 21 or 28 days after inoculation in some other F_2 plants was attributed to the heterozygous condition of a dominant gene.

Temperature had little effect on the time required for virus or symptoms to appear in the host. Higher inoculum concentration or four or eight pea aphids (Acyrthosiphon pisum Harris) were required to infect plants of line 2N23-2GH-3-2 with the 'mosaic' strain. The immunity in line 2N138-1GH-3-5 was overcome in the greenhouse when plants were inoculated by exposing them to viruliferous aphids or when epidermal strips were removed from leaves before mechanical inoculation. However, inbred lines and field selections from 2N138 were immune when viruliferous aphids were used to inoculate the plots in the field.

GENERAL INTRODUCTION

Faba bean (Vicia faba L.) is an important legume crop in Western Canada and in many other parts of the world. It is primarily used as a protein source in the human diet and animal feed and occasionally as a green manure crop.

Several plant viruses, including bean yellow mosaic virus (BYMV), have been reported to infect field-grown faba beans and limit their production (Bos, 1970a; 1981). BYMV occurs commonly in the province of Manitoba and has been recognized as a potential threat to faba bean production (Bernier, 1975). Significant reductions in yield resulting from BYMV infection in faba bean have been reported (Heathcote and Gibbs, 1962; Izadpanah et al., 1969; Kaiser, 1973). Frowd and Bernier (1977) found that severe early BYMV infections could result in yield losses as high as 96.3%.

In the field, BYMV is readily transmitted by aphids in a non-persistent manner, has a wide range of hosts among legumes and non-legumes which include several perennial wild legumes such as clovers and vetches that are reservoirs of the virus (Bos, 1970a; Edwardson, 1974).

The control of the disease lies in eradicating the primary sources of inoculum for the virus, in controlling insect vectors and producing cultivars with resistance to the virus. The first two measures may continue to play a significant role in an integrated disease control program. However, both measures involve a continuous and timely use of

chemicals and must be repeated year after year. Breeding for resistant plants provides the most efficient and effective method of virus control (Holmes, 1954). Diseases of faba beans have received very little attention in the past and limited attempts to find host resistance to BYMV have not been successful (Nitzany and Cohen, 1964; Kaiser et al., 1968; Fiederow, 1980). However, resistance to BYMV has been found and successfully used in peas (Yen and Fry, 1956; Johnson and Hagedorn, 1958; Schroeder and Provvidenti, 1962, 1971; Barton et al., 1964), in Phaseolus beans (Baggett, 1956; Baggett and Frazier, 1957; Provvidenti and Schroeder, 1973), in cowpeas (Reeder et al., 1972) and in soybeans (Provvidenti, 1975). In preliminary studies, some 360 faba bean accessions from different regions of the world were screened at the University of Manitoba and resistance to BYMV was identified in several segregating accessions (C. Bernier, unpublished data). In the present studies, the accessions previously identified as having some resistance were subjected to more rigorous evaluation and selection under different environmental conditions. The genetic basis of virus resistance was then examined in selected faba bean inbred lines.

The expression of symptoms in BYMV-infected plants is influenced by temperature and possibly by the quantity of virus introduced into plants. In the field, faba beans are repeatedly exposed to the virus depending on numbers and movement of the aphids. Therefore, the effects of two temperature regimes and varying inoculum densities on the reactions of selected inbred lines were also studied.

LITERATURE REVIEW

2.1 The Host

2.1.1 Faba Bean: A Brief History

Faba beans (*Vicia faba* L.) now grown in many regions of the world (Bond, 1976) are believed to have originated in the Near East (Cubero, 1974; Zohary and Hopf, 1973). The ancestry of *V. faba* is not known. *Vicia narbonensis* L. which was once considered to be the ancestor was found to be widely divergent cytogenetically (Zohary and Hopf, 1973). Similarly, *Vicia galilea* Plitm. et Zoh. has also been proposed to be the ancestor of *V. faba* because of its resemblance but Ladizinsky (1975) found that the albumin profiles of seed protein from *V. galilea*, *V. narbonensis* and *V. hyaeniscyamus* Mout. had little in common with the profile of *V. faba*. This, along with the fact that these species are cross incompatible with *V. faba* suggests that they are not the ancestors of faba beans (Conner, 1981).

Faba beans are thought to have been cultivated first in late Neolithic era (Zohary and Hopf, 1973). Their use has been largely confined to temperate zones and to the cool season in subtropical areas. The crop is grown extensively throughout the Middle East and China and also, to a lesser extent, in Southern Europe (Hawtin and Stewart, 1979) and Western North America (Evans and Slinkard, 1975). *Vicia faba* has been divided into three subspecies based on the seed size: *major*, *equina* and *minor* (Robinson, 1968; Evans and Slinkard, 1975). These subspecies include large, medium and small seed types, respectively. All these

subspecies are used for animal and human consumption (Cepeda, 1981). Small-seeded faba beans, however, have been found to be an excellent silage source and their ability to fix nitrogen in combination with suitable rhizobia makes them a good green manure crop. The small seed types are also preferred in Canada because seed can be manipulated with conventional cereal equipment and seed coats are lower than for large-seeded types (Crafts, 1979). Field trials have shown that the crop is well adapted to the moister growing areas of the Prairies (Evans et al., 1972). Current research is aimed at improving traits such as shattering resistance, earlier maturity and disease resistance (Furgal and Evans, 1976). Among other pathogens, bean yellow mosaic virus has been found to be quite prevalent and is regarded as a potentially serious pathogen in the province of Manitoba (Bernier, 1975; Frowd and Bernier, 1977).

2.1.2 Faba Bean Uniformity

Studies by Toynbee-Clark (1974) and Poulsen (1975) have indicated that V. faba is subject to cross-fertilization ranging from 19-79%. This large disparity found in outcrossing in different lines has been attributed to a number of factors such as the bee activity, flower colour and flowering time (Poulsen, 1975; De Vries, 1978). Plant density may also have an effect on the degree of cross-fertilization, although the evidence is conflicting (Holden and Bond, 1960). Poulsen (1975) found that earlier formed flowers tend to outcross more than later ones. However, the late flowers ensure higher seed production even under low bee activity. Some type of self-incompatibility controlled by a number of deleterious recessive genes that favour cross-fertilization has been proposed by Rowlands (1960). There is little evidence to support this theory, although

Holden and Bond (1960) have shown that after several generations of inbreeding the fertility declines even when there is selection for self-fertility. Poulsen (1975), on the contrary, suggests that fertility declines for the first few generations of selfing and later levels off as genes controlling autofertility become fixed. Nevertheless, the evidence that flowers do require tripping to produce pods (Rowlands, 1960; Toynbee-Clark, 1974) supports the fact that cross-pollination is favoured. To limit the amount of cross-pollination for inbreeding selections, an understanding of isolation distances is required. Pope and Bond (1975) found that an isolation distance of 134 m reduced cross-fertilization to 1-2%, while low levels of contamination could still occur at distances of 1.1 km or more.

2.1.3 Faba Bean as a Host to BYMV and Other Viruses

Presently, 16 different viruses have been reported from field-grown faba beans in various parts of the world (Bos, 1981). In addition, *V. faba* L. has been used as a test plant in research and found to be susceptible to a number of other viruses. The faba bean crop is thus potentially vulnerable to virus diseases. In fact, in the early 1920's, with a rapidly increasing interest in mosaic diseases (a name at that time equivalent to virus diseases), mosaic-diseased faba bean crops were among the first to attract the attention of virologists as in the U.S.A. (Dickson, 1921; Elliot, 1921), the Netherlands (Vandermeulen, 1927), Germany (Boning, 1927), Bermuda (Ogilvie, 1928) and Japan (Fukushi, 1930). Soon thereafter, mosaic diseases of faba bean were reported in many other countries (Quantz, 1953). It was later observed, however, that viruses not only cause mosaic, but various other symptoms as well. Stubbs

(1947) in Australia, described a virus that caused a destructive vascular wilt of broad beans. The virus now known as broad bean wilt virus is reported to be widespread in various parts of the world as a very damaging pathogen of several crops, including non-legumes and even woody ornamentals (Taylor and Stubbs, 1972). Gradually, other viruses such as alfalfa mosaic virus and cucumber mosaic virus were shown to cause diseases of faba beans (Bos, 1981). Within bean yellow mosaic virus, special strains have been detected that cause severe necrosis in faba beans (Bos *et al.*, 1974; Frowd and Bernier, 1977).

Knowledge of mechanisms by which viruses are transmitted facilitated host range studies which in turn helped to differentiate viruses and strains of a virus. It also provided an insight into knowing ways of natural spread and ecology of diseases concerned (Bos, 1981). Thus, Quantz (1950, 1953) was the first to detect seed transmission of a special mosaic virus of broad bean which he called Echtes Ackerbohnenmosaik virus (broad bean mosaic virus). Up to 15% of the seeds from diseased plants were found to carry the virus. Six of the 16 viruses reported in *Vicia faba* are now known to be transmitted through seeds obtained from naturally infected faba beans. Bos (1981) has also described some of the characteristics of the pertinent viruses that have so far been isolated from naturally infected faba beans. The information summarized by Bos (1981) was obtained from the studies of Inouve (1969) who reviewed reports on viruses detected on legumes including *Vicia faba* in Japan, from Cockbain (1980) and Sjodin (1980) who provided information on the incidence and importance of viruses infecting faba beans in England and Sweden, respectively, from Fiedorow (1980) in Poland and Schmidt *et al.* (1977a) in East Germany.

In Canada, Bernier (1975) first noticed that viral infections could potentially lead to severe yield losses in faba beans. Frowd and Bernier (1977) later isolated a virus characterized as BYMV and reported two strains from the field-grown faba beans of Manitoba. They also observed that pea aphid (Acyrthosiphon pisum Harris) was the chief vector for the virus spread in the fields. In other parts of the world, other aphid species are also known to transmit the virus.

Multiple infections with other viruses and completely different pathogens are now known to complicate symptoms and detection of pathogenic viruses in V. faba. In complex infections, interactions between viruses may range from antagonism to synergism. Viruses that are normally latent in plants may greatly aggravate the effect of other viruses (Bos, 1981). Symptoms of virus diseases may easily be mistaken for symptoms of diseases caused by other pathogens. Certain fungi may only attack plants already virus-infected or may be more pathogenic in virus-infected plants. Leaf roll-diseased faba bean plants are more susceptible to Botrytis fabae (Tinsley, 1959) and root rot inducing fungi (Kaiser, 1973).

2.2 The Virus

Bean yellow mosaic was first recognized by Pierce (1934) in a bean plant of Red Vallentine variety grown in the fields of the University of Wisconsin, Madison. The causal agent was called yellow bean mosaic virus or bean virus 2 by Pierce, but was later more often referred as bean yellow mosaic virus (BYMV) or more technically BYMV */* : */* : E/E : S/Ap. The virus is now known to occur in many countries and is evidently worldwide in distribution (Bos, 1970a, 1981). The names, pea mosaic virus (Doolittle and Jones, 1925), bean virus 2 (Pierce, 1934), Phaseolus virus 2 (Smith, 1937) and gladiolus mosaic virus (Smith and Brierley,

1944) have been used as some of the synonyms for the virus (Bos, 1970a).

The virus has flexuous particles about 750 nm long, is readily transmissible by sap inoculation and by aphids in a non-persistent manner and causes diseases in economically important plant species such as peas, french beans, faba beans, clovers, gladiolus and in several other species. It can also infect a number of wild legumes and non-legumes such as Liliiflorae (Bos, 1970a). The literature on host range is further reviewed in subsection 2.2.3.

The stability of the virus depends greatly on virus source, test plant and conditions. The expressed sap loses most of its infectivity after heating 10 min at 60° C and depending on the virus source and strain, the thermal inactivation temperature is between 50 and 70° C. The dilution end point is usually between 10^{-3} and 10^{-4} and aging *in vitro* at room temperature is 1-7 days (Bos, 1970a).

2.2.1 Transmission

More than 20 aphid species have been reported to transmit the virus (Kennedy *et al.*, 1962). The important aphid species include *Acyrthosiphon pisum*, *Macrosiphum euphorbiae*, *Myzus persicae* and *Aphis fabae*. *A. pisum* biotypes have been found to differ greatly in transmission efficiency (Sohi and Swenson, 1964). The loss of transmissibility by some isolates has not been found to be related to repeated sap transmissions (Swenson *et al.*, 1964; Kamm, 1969). Transmission of virus by aphid species in the field may depend upon the geographical area and the virus strains involved. In Manitoba, the pea aphids which is the principal aphid species colonizing *Vicia faba* has been found to be a vector of BYMV (Frowd and Bernier, 1977).

Although seed infection is uncommon, the virus has been reported to

be occasionally seed-borne in faba bean by Zschau (1961), Evans (1973) and Kaiser (1973). Seedlings from such infected plants, therefore, have the potential to be primary foci of disease outbreaks. The findings of some other workers (Grogan and Walker, 1948; Zaumeyer and Fisher, 1953; Frowd and Bernier, 1977; Drijfhout, 1978), however, do not agree that the virus is seed-borne. Out of 20 broad bean lines from nine countries, Kaiser (1973) detected 0.1-0.9% BYMV infection in seeds of 12 lines, while Cockbain et al. (1976) did not see a single case of seed-borne infection in 30 crops of spring-sown faba beans grown between 1970 and 1976 in England. Only two infected in about 60 samples (0.2%) of seeds grown in a glasshouse were detected.

2.2.2 Symptoms

Symptoms caused by BYMV vary greatly with type and variety of plant and the virus strains involved. The earlier literature mentions that symptoms like yellow mosaic (Pierce, 1934), necrotic local lesions (Zaumeyer and Fisher, 1953), stem or top necrosis (McWhorter and Boyle, 1946) and pod-distortion (Grogan and Walker, 1948) may be produced in susceptible bean varieties. In Vicia faba the virus is reported to cause a mild mosaic with irregular dark green islands with a slight malformation of young leaves and stunting of plants. Characteristically, the virus produces crystalline inclusion bodies in infected plant cells (McWhorter, 1941). Later, Zaumeyer and Goth (1963) reported isolation of a red clover necrosis virus isolate that produced brown primary necrotic lesions followed by systemic leaf necrosis, stem streaking and death of plants. More recently, BYMV isolates were also obtained that produced local and systemic necrotic lesions followed by tip and/or vascular necrosis and rapid collapse of susceptible faba bean plants (Frowd and

Bernier, 1977).

On pea plants (Pisum sativum), Doolittle and Jones (1925) reported a disease that characteristically resulted in a bright yellow mosaic and considerable distortion of plants. The virus then named pea mosaic virus was later identified as a strain of bean yellow mosaic virus (Bos et al., 1974).

On white sweet clover (Melilotus alba), symptoms of BYMV first appear as small light yellow spots on the leaves. The spots then usually enlarge and coalesce with others producing small light green blotches interspaced with dark green areas followed by slight dwarfing and ruffling of leaves in severe cases of infection (Smith, 1972). Symptoms produced on other legume and non-legume plants are further discussed under host range studies.

2.2.3 Host Range

Since the first description of BYMV by Pierce in 1934 (Bos, 1970a), many isolates with widely varying host ranges have been reported (Kovachevsky, 1968; McCord and Gudauskas, 1968; Bos, 1970b; Bos et al., 1974; Edwardson, 1974; Jones and Diachun, 1977; Hampton et al., 1978). In a comprehensive study on host ranges, Kovachevsky (1968) found 77 leguminous and three non-leguminous species (opium poppy, Circium arvense and Chenopodium album) as natural hosts of BYMV in Bulgaria, 45 of them being new hosts. Of the tested legume species, only Lotus corniculatus and L. conjugatus were found immune to the virus, while certain Trifolium species and Melilotus species were found to serve as its winter hosts. Bos (1970a) gave a summary of host range and symptomatology of BYMV isolates that infect not only leguminous species but certain non-leguminous species as well. He divided hosts into three categories as follows:

Propagation species which serve as host to propagate virus and maintain the cultures are: Pisum sativum, Vicia faba, Phaseolus vulgaris and Nicotiana clevelendii.

Diagnostic species which provide an aid in diagnosis of virus by presence of inclusion bodies of typical morphology and localization are: Phaseolus vulgaris, Pisum sativum, Vicia faba, Chenopodium amaranticolor, C. quinoa, Gomphrena globosa, Nicotiana tabacum, Spinacia oleracea and Tetragonia expansa.

Assay species that have been used to measure the relative titres of virus are C. amaranticolor, C. quinoa and for some BYMV strains, Crotaloria spectabilis, Phaseolus aureus and some Kenland red clover clones.

Based on this information, Bos et al. (1974) selected a list of hosts that could be used to differentiate isolates of bean mosaic, pea yellow mosaic and pea necrosis strains of BYMV, pea necrosis virus and pea seed-borne mosaic virus. The differentials used were Phaseolus vulgaris vars. Double White Princess and Great Northern 123; Pisum sativum vars. Koroza and Juwel; Vicia faba var. Compacta, Chenopodium amaranticolor and C. quinoa. The choice of host species or varieties used as differentials, however, could vary from one person to another depending upon geographical area and the virus isolates being studied. Hence, the set of differentials used by Thomas and Zaumeyer (1953), El-Attar et al. (1971), Jones and Diachun (1977), Frencel and Pospieszny (1977) and Allam et al. (1979) differed not only from that of Bos et al. (1974) but also from each other. For lack of standard identification criteria, therefore, similar or identical strains could be reported by different workers and given different names. To solve this problem and to promote international

communication and cooperation among plant pathologists working with legume viruses, a group called 'The International Working Group on Legume Viruses' was established in 1962 (Hampton et al., 1978). The group comprised eight investigators located at different places in the world and carried out a comprehensive test to study the reaction of 23 selected plant hosts against 38 legume viruses in an effort to standardize host range and reaction for type cultures of important legume viruses of northern temperate zones on uniform plants. Seedlots of plant hosts were gathered at one location, subdivided into eight equal quantities for distribution to the eight investigators. Thus, each investigator tested documented isolates of viruses on plant host germplasm identical to that employed by other investigators. An earlier working group published a revised list of host plants proven useful for definitive work with legume viruses (Vanderveken, 1976). Based on 1700 symptomatological and host range data, the group organized by Hampton et al. developed a key that could be used to identify and distinguish 38 viruses. Although the information for structuring the key was quite comprehensive, some limitations were recognized (Hampton et al., 1978). A major limitation was that with the exception of five viruses: alfalfa mosaic virus, BYMV, red clover vein mosaic virus, white clover mosaic virus and pea stunt virus, only one isolate of the other 33 viruses was used while different strains are known to induce different symptoms on the same set of hosts. For this reason, the authors believe that final identification of virus and strains should be confirmed by serology and, where possible, by other properties of the virus particles. In the host range analyses, it was found that BYMV could infect as many as 11 of the 23 hosts tested. The symptoms included local chlorotic spots to necrotic spots, vein clearing and severe mosaic coupled in some hosts with severe stunting and necrosis of stem tissue.

2.2.4 Strains of BYMV

Since 1934, several strains of BYMV have been reported from different areas of the world. The work by Doolittle and Jones (1925), Pierce (1935) and Murphy and Pierce (1937) led to the belief that (common) pea mosaic virus (PMV) that also causes mosaic in broad beans (Boning, 1927; Merkel, 1929), mottling in red clover (Zaumeyer and Wade, 1935) and 'sore shin' in yellow lupin (Chamberlain, 1935) is a distinct entity from BYMV. One of the main distinctions between BYMV and PMV was that the later virus was not able to infect beans (Phaseolus vulgaris). However, it was occasionally reported that PMV could infect beans (Zaumeyer and Wade, 1935; Johnson and Jones, 1937), although it was much less pathogenic to bean than normal BYMV (Schroeder and Provvidenti, 1966; Taylor and Smith, 1968).

Serologically, Goodchild (1956b) and Taylor and Smith (1968) could not distinguish between BYMV and typical isolates of PMV. Genetic studies done by Barton et al.(1964) showed that resistance in pea, especially Perfection type varieties, to isolate of BYMV (BYMV-B25) and PMV (F210) was conditioned by the same genotype. These findings, therefore, support the argument that PMV is only a strain of BYMV.

Grogan and Walker (1948) reported a strain that could infect Idaho Refugee bean and other varieties resistant to the type strain (Pierce, 1934). The pods on infected plants are severely warted, misshapen and disfigured. This strain differed from the type strain and other strains in the sense that it does not infect broad beans, scybeans, tobacco and sweet clovers (Thomas and Zaumeyer, 1953).

In Oregon (USA), McWhorter and Boyle (1946) discovered a black root x-disease strain of BYMV which caused a variable symptom in Phaseolus