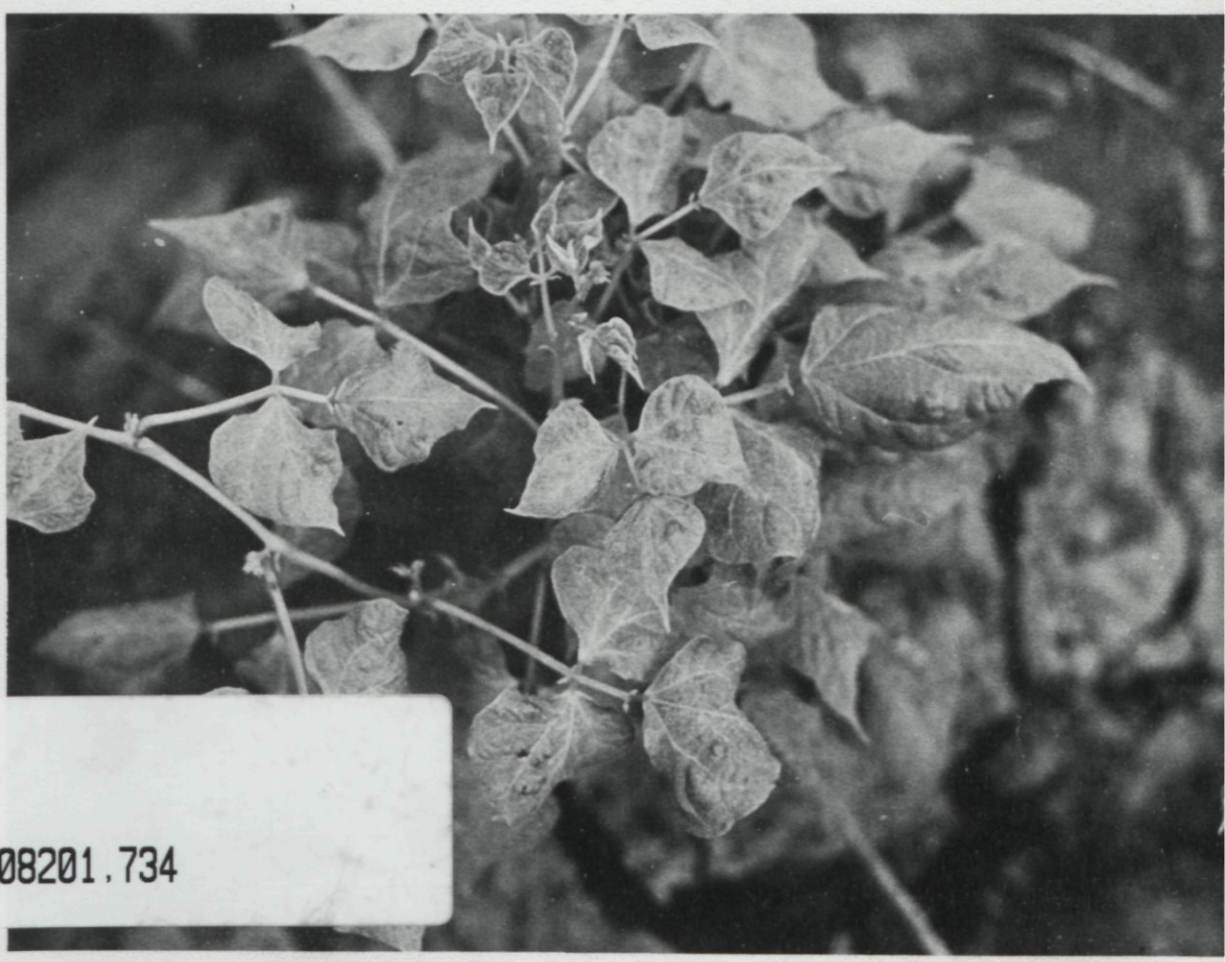
Genetic interaction between Phaseolus vulgaris and bean common mosaic virus with implications for strain identification and breeding for resistance

E. Drijfhout



Genetic interaction between Phaseolus vulgaris and bean common mosaic virus with implications for strain identification and breeding for resistance

Promotor: dr.ir. J. Sneep, hoogleraar in de plantenveredeling
Co-promotor: dr.ir. J.P.H. van der Want, hoogleraar in de virologie

E. Drijfhout

Genetic interaction between Phaseolus vulgaris and bean common mosaic virus with implications for strain identification and breeding for resistance

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op woensdag 20 september 1978
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

Centre for Agricultural Publishing and Documentation

Wageningen – 1978

Abstract

DRIJFHOUT, E. (1978) Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance. Agric. Res. Rep. (Versl. landbouwk. Onderz.) 872, ISBN 90 220 0671 9, (vii) + 98 p., 14 figs, 42 tables, 72 refs, 1 app., Eng. and Dutch summaries.
Also: Doctoral thesis, Wageningen.

Various strains of bean common mosaic virus (BCMV) occur in susceptible cultivars of bean. To compare these strains, a standard procedure for identification and a set of differential cultivars were established. The differentials are representatives of 11 resistance groups, determined by testing of about 450 bean cultivars with 8 to 10 strains. The virus strains and isolates were classified into 10 pathogenicity groups and subgroups, so that 10 strains were distinguished and the others considered as isolates of those strains.

Twelve differentials were intercrossed and their F_1 and F_2 tested with most of the strains for genetical analysis of resistance in bean. Seven genes were distinguished: a necrosis gene *I*, already known from the literature, 5 strain-specific resistance genes *bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2} and *bc-3*, and a strain-unspecific gene *bc-u*, complementary to the strain-specific ones. Genes *bc-1* and *bc-1*^{2} were allelic, as were *bc-2* and *bc-2*^{2}. The 5 loci segregated independently or nearly so. The 4 strain-specific genes *bc-1* to *bc-2*^{2} had a gene-for-gene relationship with 4 pathogenicity genes, likely to be present in the virus strains. Gene *bc-3* had not been overcome by a corresponding pathogenicity gene. Two bean genotypes were developed with resistance to all known strains. Some implications for resistance breeding are discussed.

Free descriptors: *Phaseolus vulgaris*, common bean, bean common mosaic virus, BCMV, breeding for resistance, genetics of resistance, host – virus relationship, pathogenic variation, screening for resistance, strain identification.

This thesis will also be published as Agricultural Research Reports 872.

Stellingen

1. De kosten en de benodigde tijdsduur voor het kweken en in het economisch verkeer brengen van een 'ideale' cultivar maken bezinning op nieuwe veredelingsmethoden noodzakelijk.

2. De internationale centra voor landbouwkundig onderzoek, onder toezicht van de Consultative Group on International Agricultural Research, kunnen de als doel gestelde opbrengstverhoging alleen bereiken, indien ze, samen met de nationale overheden en instellingen voor landbouwkundig onderzoek in de landen waarvoor zij hun activiteiten ontplooien, een systeem ontwerpen voor de vermeerdering, certificatie en distributie tegen lage prijs van zaaizaad en plantgoed van de cultivars waaraan zij kweekarbeid hebben verricht.

3. Identificatie van virusstammen kan sterk worden verbeterd door internationaal gebruik van een standaardserie differentiërende rassen, samengesteld na een vergelijkend onderzoek betreffende de identiteit en de genetische karakterisering van de in de diverse landen reeds gebruikte toetsrassen en virusstammen in hun waardplant – pathogeenrelatie.

Dit proefschrift.

4. Door de complementaire werking van het stam-aspecifieke resistentie-gen *bc-u* met één of meer van de stam-specifieke genen *bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2} en *bc-3* in de boon wordt de vermeerdering van het bonerolmozaïekvirus zodanig afgeremd dat systemische verspreiding in de plant onmogelijk wordt.

Dit proefschrift.

5. Het verschijnsel dat bepaalde stammen van het bonerolmozaïekvirus het in vele bonerassen aanwezige resistentiegevende necrose-gen *I* in meerdere of mindere mate hebben doorbroken, vertoont overeenkomst met de veelal monogeen bepaalde verschillen in resistentie van schimmels tegen systemische fungiciden.

Dit proefschrift.
J. Dekker, 1976. Annu. Rev. Phytopathol. 14: 405-428.
J.M. van Tuyl, 1977. Meded. Landbouwhogeschool Wageningen 77-2.

6. Tussen *Phaseolus vulgaris* L. en de schimmel *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi & Cav. bestaat een gen-om-gen relatie.

7. De vermeldingen in de literatuur dat de pronkboon (*Phaseolus coccineus* L.) vatbaar is voor het bonerolmozaïekvirus of voor de schimmel *Colletotrichum lindemuthianum*

- - -

(Sacc. & Magn.) Briosi & Cav. zijn onjuist en moeten berusten op aantasting van natuurlijke hybriden tussen *P. vulgaris* en *P. coccineus*.

G.C. Ainsworth, 1940. Ann. Appl. Biol. 27: 218-226.
R. Nelson, 1932. Agric. Exp. Stn. Mich., Tech. Bul. 118.
W.J. Zaumeyer & H.R. Thomas, 1957. USDA Tech. Bul. 868: 12.

8. In een waardplant – virussysteem kan zich een gen-om-gen relatie ontwikkelen, ook al is het aantal cistrons in het desbetreffende virusgenoom kleiner dan het aantal betrokken resistentie-genen in de plant.

9. De remstoffen in de zaden van pitvruchten zijn niet van belang voor de narijping van het embryo, maar spelen waarschijnlijk een rol bij de bescherming van het zaad tegen schadelijke bodemorganismen.

M. Kawase, 1958. J. Hortic. Assoc. Japan 27: 256-264.
O. Balboa-Zavala & F.G. Dennis, 1977. J. Am. Soc. Hortic. Sci. 102: 633-637.

10. De overheid, die al jaren de warmte-isolatie van particuliere woningen stimuleert, schiet te kort, indien zij zelf, als één van de grootste energieverbruikers, nalaat plannen te openbaren om de duizenden onder haar beheer vallende gebouwen te isoleren terwille van de energiebesparing.

11. In de wachtkamers van artsen staat het medisch beroepsgeheim op de tocht.

Stellingen behorend bij het proefschrift van ir. E. Drijfhout, getiteld 'Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance'. Wageningen, 20 september 1978.

aan de nagedachtenis van mijn ouders
aan mijn vrouw en kinderen

Woord van dank

Graag wil ik allen danken die mij bij het onderzoek en bij het voltooien van dit proefschrift behulpzaam waren.

Mijn promotoren prof. dr. ir. J. Sneep en prof. dr. ir. J.P.H. van der Want ben ik zeer erkentelijk voor de belangstelling die zij voor mijn werk toonden, voor de waardevolle kritische opmerkingen waarmee zij mijn manuscript voorzagen en voor de stimulerende discussies over het onderzoek. Mijn dank gaat tevens uit naar dr. ir. J.E. Parlevliet van het Instituut voor Plantenveredeling die in de discussies er toe bijdroeg dat mijn inzicht in de bestudeerde verhouding tussen waardplant en pathogeen zich verdiepte en naar dr. ir. L. Bos van het Instituut voor Plantenziektenkundig Onderzoek die waardevolle suggesties gaf voor de vormgeving van het manuscript en de virologische aspecten ervan bekritiseerde.

Mijn welgemeende dank gaat uit naar ir. C. Dorsman, directeur van het Instituut voor de Veredeling van Tuinbouwgewassen, die mij de gelegenheid bood het onderzoek te verrichten en het manuscript voorzag van kritische kanttekeningen.

Mijn collega's dr. ir. N.C. Hogenboom en dr. ir. L.D. Sparnaaij ben ik zeer erkentelijk voor de moeite die zij zich hebben getroost om het manuscript kritisch door te lezen, en voor hun waardevolle suggesties.

De heer A. van Steenbergen dank ik hartelijk voor de vakkundige en consciëntieuze wijze waarop hij technische assistentie verleende bij het onderzoek, de heren A.J. Cochius en C. Verkuijl voor de goede verzorging van de planten, de heer G. Berends voor het polijsten van de Engelse tekst, de dames van de typekamer voor het uitstekende typewerk en de fotografen van de BGD voor hun bijdrage. Mijn waardering gaat uit naar de heer J.C. Rigg voor de taalkundige en naar de heer J.A.M. van den Heuvel voor de technische redactie van het proefschrift en via hen naar het Pudoc voor de wijze waarop deze publikatie werd uitgegeven.

Tenslotte wil ik mijn vrouw en kinderen bedanken, omdat ze begrip toonden voor mijn geringe bijdrage aan het familieleven tijdens de voorbereiding van het proefschrift.

Curriculum vitae

Eelco Drijfhout, geboren op 18 februari 1930 te Harlingen, behaalde het einddiploma H.B.S.-B in 1947 aan de Rijks H.B.S. in zijn geboorteplaats. Van 1947 tot 1953 studeerde hij aan de Landbouwhogeschool te Wageningen, richting tuinbouw. Het doctoraal examen met als hoofdvak tuinbouwplantenteelt en als specialisaties fytopathologie, entomologie en bodemkunde werd op 26 juni 1953 behaald. Van 1953 tot 1961 was hij werkzaam bij de Rijkstuinbouwvoorlichtingsdienst, met achtereenvolgens de standplaatsen Leeuwarden en Barendrecht, en van 1961 tot 1963 bij de Directie Tuinbouw van het Ministerie van Landbouw in Den Haag. Sinds 1963 is hij verbonden aan het Instituut voor de Veredeling van Tuinbouwgewassen in Wageningen, waar hij als hoofd van de afdeling peulvruchtgewassen (Phaseolus, Pisum en Vicia) veredelingsonderzoek heeft verricht met betrekking tot o.a. soortkruisingen, resistenties tegen virussen, schimmels en insecten, en ontwikkeling van nieuwe planttypen. Het promotieonderzoek werd verricht als onderdeel van zijn onderzoekprogramma in het IVT. Momenteel verricht de auteur hoofdzakelijk resistentieveredelingsonderzoek in Phaseolus, voor een deel ten behoeve van Latijns-Amerika via een samenwerkingsovereenkomst met het Centro Internacional de Agricultura Tropical in Cali, Colombia.

Contents

1 General introduction

Dry seeds of common bean (*Phaseolus vulgaris* L.) are consumed all over the world. In developed countries fresh or processed pods are eaten as a vegetable, while dry seeds are a less popular food. In most developing countries, dry beans are an important protein component of the human diet. In 1974, the following areas (in 1000 ha) were planted with beans (FAO, 1975):

Asia	10 731	USA and Canada	895
Latin America	6 079	Europe	2 965
Africa	2 836	USSR	35
Australia	20	World	23 561

These figures indicate the great importance of the crop. The world total is slightly more than that of potatoes which in the same year was 22 million hectares (FAO, 1975).

The figures given are the totals for dry beans, green beans and green shelled beans. The FAO areas for dry beans also include other *Phaseolus* species. This means that in Asia the area of *P. vulgaris* (common bean) is considerably smaller than indicated above because of important cultures of *P. lunatus* (lima bean), *P. mungo* (black gram) and *P. radiatus* (green gram or mung bean) and in Africa and America somewhat smaller because of the culture of *P. lunatus*.

Bean common mosaic virus (BCMV) is also worldwide in distribution, mainly due to its seed transmission. The virus is easily spread by aphids in the non-persistent manner. In nature it seems to be confined to common bean, in which it may be extremely damaging since it may cause 'common mosaic', usually associated with leaf malformation, or severe vascular necrosis, which is generally known as 'black root disease', often accompanied by necrosis in pods and frequently resulting in plant death. The occurrence of symptoms, but also their nature and severity, depend on host cultivar, virus strain and environmental conditions. The economic damage consists of a severe reduction of the crop yield and of quality of the harvested product. Yield may be reduced between 50 and 80% (Kaiser et al., 1968; Leon & Calot, 1973).

To prevent virus spread by seed, the United States and Canada require the use of certified seed, guaranteeing infection rates of 0% for foundation seed and maximally 1% for certified seed (Association of Official Seed Certifying Agencies, 1971). However, no internationally accepted techniques for testing seed lots for virus infection are available so far and certification never warrants complete freedom from virus (Bos, 1976). Thus sources of infection, consisting of seedlings germinating from infected seed, cannot be completely avoided. Moreover, non-persistent transmission from such sources of infection in a newly sown crop or from nearby bean crops, even by probing aphids, cannot be precluded by insecticides.

In the early 1930s, plant pathologists and breeders became aware that selection and breeding for resistance to the virus is the only effective method of control. Since then plant breeders, especially in the United States and the Netherlands, have greatly contributed to control of the disease. At present, resistance to BCMV is one of the primary objectives in every breeding program in common bean. However, sometimes newly introduced resistant cultivars were attacked by new or overlooked strains of the virus. Moreover, bean cultivars are usually not of merely local importance. Several have world-wide distribution; they are sometimes multiplied in other parts of the world than the region where the crop is grown for consumption. Either way they may be infected by virus strains not occurring in the country where the new cultivars have been bred. It is therefore useful to study beforehand their reaction to strains occurring in other parts of the world.

This leads us to the phenomenon of the host-virus relationship and of the genetic variation of host and virus. Host variation permits the selection of genotypes resistant or tolerant to the virus genotypes (strains). However, the introduction of resistant host genotypes may, through natural selection, bring to the fore new strains of the virus attacking formerly resistant genotypes.

Until now, host genetics of resistance to BCMV is not well understood. The study of the genetics of plant viruses is still in its infancy, virus variants being described in terms of differences in host ranges and especially differences in main reactions of host cultivars. Some 18 strains of BCMV have been reported. But most identification of virus strains has been done under entirely different conditions, often with different test ranges or, when using the same cultivars, with different sub-lines. Direct comparison at one site of strains from different countries has been difficult because of quarantine regulations. To achieve some sort of standardization, the International Working Group on Legume Viruses is now multiplying seed of a limited number of selected homozygous differential bean cultivars for use in different countries.

The object of the present study is to investigate the genetics of the relationship between *Phaseolus vulgaris* and BCMV, in order to give breeding programs for BCMV-resistance a sounder basis. Host resistance and strain differentiation cannot be studied independently. Nothing definite can be said about strain differentiation without information on the resistance genes and without the availability of homozygous differential cultivars. Similarly, host genetics in relation to the virus cannot be properly studied without information on differentiation of the strains. Such investigations can assist plant breeders as well as plant virologists. Plant breeding and genetics and plant virology are two interdependent aspects of this study. However, emphasis will be given to the genetic aspect.

In a literature review (Chapter 2), the virus is briefly described, the host reaction tentatively analysed, and information given on the strains reported, leading to a better delimitation of the problems to be dealt with. After a description of materials and methods used (Chapter 3), the host reaction is further analysed by testing a large number of cultivars and other accessions with several strains of BCMV. A choice can then be made of a proper range of differential cultivars for comparison and identification of strains from the Netherlands and from other countries (Chapter 4). The inheritance of resistance

in host genotypes with recessive alleles of the necrosis gene is studied by testing progenies of crosses between differentials of this type with different virus strains (Chapter 5).

The resistance genotypes of the differentials with recessive alleles of the necrosis gene are then presented, while a gene-for-gene relationship is worked out between the genes for specific resistance of the host and the pathogenicity genes of the virus (Chapter 6). After determination of the resistance genotypes of the differentials with recessive alleles of the necrosis gene, those of the differentials with dominant necrosis gene are analysed (Chapter 7). Finally, some practical implications of the research are given for resistance breeding (Chapter 8).

Working in the area where plant breeding and plant pathology overlap, I use some terms from both sciences. In the literature some terms may have more than one meaning. For instance: resistance may mean 'resistance to the disease' or 'resistance to the pathogen', that is 'resistance to infection'. A plant not showing disease symptoms may be called resistant, despite the presence of a possible systemic infection in the plant. I use the term in the sense of complete resistance to systemic infection. Other terms, which might give rise to confusion, are defined where they are first used. Such terms are in accordance with Allard, 1960 (plant breeding), Riger et al., 1968 (genetics) and Federation of British Plant Pathologists, 1973 (plant pathology). Besides, some new terms will be introduced in this study. If a new term is used here for the first time, its definition will be given in italics.

Cultivar names often occur in the text. For readability they are not given between single quotation marks but are preceded by cultivar or cv. when first mentioned unless it is already evident from the context that we are dealing with a cultivar.

The term 'strain' is reserved here for virus strains and the term 'cultivar' (elsewhere sometimes called 'variety' or 'strain') for the host crop plant.

2 Review of the literature and aims of this study

2.1 Introduction

Unlike bacteria and fungi, viruses cannot grow on artificial cell-free media or be seen with the light microscope. This is why host reaction is still essential in identification of viruses or virus strains. Host reaction is also the basis for establishing differences in susceptibility of the host plant.

The following literature survey describes (1) the virus, (2) the host reaction, (3) the inheritance of resistance to the virus, and (4) the various strains of the virus described so far.

2.2 The virus

Bean common mosaic was first recognized in the United States as a virus disease by Stewart & Reddick (1917) and the incitant was called bean mosaic virus. Pierce (1934) gave a more detailed description of the biological properties of the virus and added the epithet 'common' to distinguish it from bean yellow mosaic virus. The disease had earlier been reported by Iwanowski (1899) in Russia. The virus is now known to occur in many countries, is evidently world-wide in distribution and probably coexistent with the host. The names common bean mosaic virus, bean mosaic virus, bean virus 1 and *Phaseolus* virus 1 have been used as synonyms (Martyn, 1968).

In nature, infection seems confined to *Phaseolus vulgaris*. Several aphid species can transmit the virus, even by probing. Uptake on infected plants and transmission to healthy ones is usually a matter of minutes or even seconds, and ability of aphids to transmit is rapidly lost, i.e. the virus is non-persistent. Artificially, the virus is readily transmissible within *Phaseolus vulgaris* by sap inoculation.

Reddick & Stewart (1919) were the first to prove that the virus is transmitted through seed and Reddick (1931), and Nelson & Down (1933) found that it was also transmitted to offspring from healthy bean plants through the pollen from infected plants. Seed transmission is irregular, depending on stage of growth at the time of infection, cultivar and virus strain. If infection occurs after flowering, the virus does not usually reach the seed (Nelson, 1932). Flower buds, which become infected just before or after fertilization, never produce seed infected with BCMV (Schippers, 1963).

Quantz (1961) mentioned as artificial hosts *P. atropurpureus*, *P. radiatus* (= *P. aureus*), *P. lunatus*, some *Vigna* spp., *Crotalaria spectabilis*, *Lupinus albus* and some other species of Leguminosae. These are all tropical or subtropical species, not occurring in Western Europe. Bos (1970) recovered the virus from inoculated leaves of *Chenopodium quinoa* and *Gomphrena globosa* and from uninoculated tip leaves of *Nicotiana clevelandii*. Drijfhout & Bos (1977) reisolated the virus from inoculated leaves of *Tetragonia expansa* with local lesions.

In expressed sap after heating for 10 minutes, most infectivity is usually lost around 60 °C but, depending on virus source, virus strain and environmental conditions, thermal inactivation may occur between 50 and 65 °C. The dilution end-point is usually between 10^{-3} and 10^{-4} and ageing in vitro at room temperature 1-4 days (Bos, 1971a). Information on the virus itself, gathered by Bos, is still scanty. In crude sap, the particles can now easily be seen with the electron microscope. They are flexuous filaments about 750 nm long and 15 nm wide. In particle morphology, mode of transmission and other biological and biophysical properties, BCMV resembles members of the potato virus Y group of viruses (potyvirus group) and is serologically related to several of them, especially to bean yellow mosaic virus (BYMV). The latter virus has a wider host range and is not seed-transmitted in *Phaseolus* beans.

2.3 Host reaction

The early literature mentions mainly mosaic, while leaf roll, growth reduction, vein chlorosis, yellow dots or a yellow mosaic may also be produced. Thus the epithets distinguishing bean common mosaic and bean yellow mosaic are more historical than descriptive and are slightly confusing.

Even severe vascular necrosis, stem discoloration and death may occur. This syndrome was described as a separate 'black root disease' (Jenkins, 1940) until Grogan & Walker (1948) discovered that it was also caused by BCMV. All vascular bundles of the plant may be affected (Jenkins, 1941) and the plant dies if infected while young. When infected later, parts of the plant may die and many of the pods, even on the apparently healthy parts, show black discolorations in the pod wall by vascular necrosis. Because of their appearance, such pods are unmarketable. Even low percentages are unacceptable in mechanical harvesting for processing because they have to be manually removed and lead to considerable economic loss.

According to Grogan & Walker (1948), systemic necrosis only appears in cultivars having a dominant type of resistance derived from cv. Corbett Refugee. They found that such plants were generally resistant to the Type and NY 15 strains but might show systemic necrosis if grafted on plants of cv. Stringless Green Refugee, which were inoculated with one of these strains, developing mosaic. Common mosaic was not found in plants with dominant resistance after inoculation with BCMV. Conversely, no systemic necrosis appeared in genotypes showing mosaic or possessing the resistance of the cvs Robust or Michelite. These cultivars proved resistant to the prevalent Type strain of the virus, but susceptible to NY 15 strain showing mosaic (Richards & Burkholder, 1943).

After inoculation, local lesions may be found before systemic necrosis. They arise as dark-brown pin-point lesions, mostly within a few days, enlarging to star-like local vein necroses by brown discoloration of the adjacent veinlets. The number of lesions is influenced by temperature. Quantz (1957) found with a temperature series of 15, 20, 25, 30 and 35 °C the highest numbers of lesions at the last two temperatures.

In more recent years, strains of BCMV were isolated that could easily induce local and systemic necrosis at 20 °C (Hubbeling, 1963, 1972). Local lesions are induced not only in plants with the dominant resistance from Corbett Refugee. Another type of local lesion is sometimes induced in plants susceptible to mosaic. Zaumeyer & Goth (1962b, 1963) mentioned small white necrotic ring lesions, 2-3 mm in diameter, on inoculated leaves of

some cultivars with some strains and brownish ring lesions, diameter 5-7 mm, on other genotypes with the same strains. The production of these types of local lesions was followed by systemic mosaic.

Hubbeling (1972) described the development of vein chlorosis followed by superficial necrosis in parenchyma tissues on the upper side of inoculated leaves ('local defence symptom'). This necrosis is limited to superficial browning near the veins, and is followed by dropping of the inoculated leaves. Sometimes the superficial browning appears in ring lesions.

Very small local lesions are found in cv. Monroe (Trujillo & Saettler, 1972, 1973; Saettler & Trujillo, 1972). They were described as circular dark-red spots of about 0.4 mm on the upper side of the leaves, arising about 4-5 days after inoculation. The lesions enlarge in the next 4-5 days to 0.8-1 mm. Number and clearness of lesions were high at 20 °C, good at 24 °C and poor at 16 and 28 °C. At the higher temperatures, more ring-shaped spots and superficial vein necrosis developed. Monroe is among the cultivars susceptible to some strains, showing mosaic, but resistant to others.

From this literature, it is clear that there are two types of local lesions. One type arises as pin-point lesions, mostly enlarging to star-like vein necrosis, sometimes followed by systemic necrosis but never by mosaic and only occurring in genotypes with the dominant resistance from Corbett Refugee. The second type may start as pin-point lesions but mostly as bigger spots, sometimes ring-shaped, first white or chlorotic, later brownish extending in a superficial necrosis above the veins. Sometimes this local reaction, only arising in genotypes not possessing the dominant resistance from Corbett Refugee, is followed by mosaic in higher leaves but never by systemic necrosis.

2.4 Genetics of resistance

The genetics of resistance to BCMV was initially studied in the United States. Pierce (1935) already pointed out that there are two types of resistance, of which one is inherited dominantly as in Corbett Refugee and the other recessively as in the cvs Robust and Great Northern UI 1.

Ali (1950) was the first to propose a genetic explanation for the segregations found in the F_2 of his crosses after inoculation with the 'Zaumeijer' strain of BCMV. Probably this strain was identical with the Type strain. Ali worked only with this strain. The F_2 of cvs Stringless Green Refugee x US 5 Refugee and Str. Green Refugee x Idaho Refugee segregated susceptible and resistant in the ratio 1:3.

Crosses between Str. Green Refugee and Robust gave an F_2 ratio of 3:1. Crosses between US 5 Refugee or Idaho Refugee and Robust segregated in the F_2 ratio 3:13. After grafting on a susceptible rootstock and inoculation of this stock, the F_2 ratio of mosaic, systemic necrosis and healthy was 3:9:4. The last two crosses between two resistant types gave evidence of two pairs of independently inherited genes.

Ali explained the results as follows: A dominant gene *A* is required for virus infection, rendering the tissues susceptible. Another dominant gene *I*, when present with gene *A*, inhibits symptom expression following rub-inoculation and conditions systemic necrosis, when there is a continuous supply of virus inoculum, after approach-graft inoculation. With *aa*, the plant becomes resistant to both mosaic and systemic necrosis. The genotypes

of the four cultivars are thus: Stringless Green Refugee *AAii* (susceptible); US 5 Refugee and Idaho Refugee *AAII* (resistant, systemic necrosis if grafted); Robust *aaii* (resistant, no systemic necrosis if grafted). The necrotic reaction after graft-inoculation is governed by gene *I* in the presence of *A*, most likely through a 'hypersensitivity' mechanism. Plants with the genotypes *aaI.*, *aaii* or *A.ii* do not react in this way. The first two genotypes remain healthy, while the third develops mosaic symptoms.

Andersen & Down (1954) analysed the F_2 of the crosses US 5 Refugee x Michelite (susceptible to resistant, 1:3) and Great Northern 31 x Michelite (3:1). They inoculated plants with the variant strain, later generally called NY 15 strain. They concluded that resistance in the former cross was governed by one dominant gene and in the latter by one recessive gene.

Petersen (1958) in Germany made crosses between the susceptible cultivars Bagnolais and Saxa, the 'hypersensitive' Topcrop and the resistant Great Northern UI 15. He tested with strain Voldagsen and distinguished 'hypersensitive' plants from resistant ones by grafting a virus containing leaf of an infected susceptible plant onto a plant not showing symptoms. The 'hypersensitive' plants showed systemic necrosis, and the resistant plants remained symptomless. F_2 ratios of 'susceptible', 'resistant' or 'hypersensitive' should be seen as relating to that strain:

Bagnolais x GN 15	3 susceptible:	1 resistant	
Topcrop x Bagnolais	1 susceptible:	3 hypersensitive	
Saxa x GN 15	15 susceptible:	1 resistant	
Topcrop x Saxa	1 susceptible:	3 hypersensitive	
Topcrop x GN 15	3 susceptible:	9 hypersensitive:	4 resistant

He explained these results as follows. The dominant, independently inherited genes A_v and S_v (v from Voldagsen) cause susceptibility. In the presence of the dominant gene A_v, the dominant gene I_v controls 'hypersensitivity'. Resistance is controlled by both recessive genes a_v and s_v, together with I_v or $i_v i_v$. On the basis of this hypothesis, Petersen arrived at the following genotypes: Saxa $A_v A_v S_v S_v i_v i_v$ (susceptible); Bagnolais $A_v A_v s_v s_v i_v i_v$ (susceptible); Topcrop $A_v A_v s_v s_v I_v I_v$ ('hypersensitive'); GN 15 $a_v a_v s_v s_v i_v i_v$ (resistant).

These results and their explanation are in agreement with the work of Ali. But Petersen showed that in some susceptible cultivars, like Saxa, two independent genes govern susceptibility in relation to a certain strain, instead of one gene as in Bagnolais. He used a letter code to identify genes governing reactions to a particular strain, suggesting that other genes might be involved in relation to other strains.

Since 1958, no important additonal data on the inheritance of resistance to BCMV have been reported. None of the earlier investigators studied the inheritance of resistance to different strains of the virus.

2.5 Virus strains

Since 1943, several strains of BCMV have been reported, mainly from the United States and the Netherlands. Richards & Burkholder (1943) mentioned that, as early as 1939, plants of Michelite were observed with symptoms of BCMV, as had Robust some years later. Both cultivars were immune to the virus and had remained so for years. They

used six isolates from bean plants grown in New York State for inoculation experiments. Robust and Michelite proved susceptible to two isolates but immune to the other four. This demonstrated the occurrence of a variant of BCMV . It was named New York 15 strain. Great Northern UI 15 was also susceptible to this strain, but resistance was found in the Great Northern cultivars GN 1 and GN 59. The original virus, to which Robust and Michelite were resistant, has since then been recorded as Type strain. Dean & Hungerford (1946) reported NY 15 strain in Idaho. Cultivar Red Mexican UI 34 was also susceptible to this strain, while GN 56, GN 81 and GN 123 besides GN 1 and GN 59 proved resistant.

Zaumeyer & Thomas (1947) mentioned a 'shiny or greasy pod strain'. In a later publication (Zaumeyer & Thomas, 1948), they concluded that the strain was not different from typical BCMV, as only cultivars susceptible to Type strain were attacked. Occasionally black root symptoms were found with this strain in some cultivars resistant to BCMV, like Idaho Refugee.

In Germany, Frandsen (1952) described strains Voldagsen and Marienau. Robust, Michelite and Red Mexican 34 were susceptible to strain Voldagsen. Michelite and RM 34 also produced circular local lesions in addition to mosaic. The strains differed from the NY 15 strain in not being pathogenic to GN 15. Cv. Wachs Rheinland was susceptible to strain Voldagsen and resistant to strain Marienau.

In the Netherlands, van der Want (1954) mentioned the isolation of strain W from a plant of cv. Westlandia. He also used a strain named RM (abbreviation for 'rolmozaïek'), isolated in 1948 from a plant of the dry bean cultivar Noordhollandse Bruine. Both strains gave symptoms in cvs Beka and Dubbele Witte differing in severity. Differential cultivars were not mentioned.

Quantz (1961) used isolates P487 and P1075. Dubbele Witte and Wachs Rheinland were susceptible to both isolates, while cvs GN 15, GN 31, GN 123, Pinto 111 and RM 34 were resistant. Cultivars to differentiate both isolates were not recorded.

Dean & Wilson (1959) reported a strain infecting GN 123 and GN 31. It had been discovered in Idaho in 1954, and was later recorded as Idaho or B strain. They stated that cultivars carrying dominant resistance to Type strain were either resistant or susceptible to the new strain. They considered cv. Improved Tendergreen (dominant resistance) as susceptible to Type strain and Idaho strain, but resistant to NY 15 strain.

Skotland & Burke (1961) described a virus in the west of the United States, bean western mosaic virus, infectious to GN 123. Michelite, Sanilac, Pinto 111, RM 34 and GN 31 proved to be resistant. Later, this virus was considered a strain of BCMV (Silbernagel, 1969), called Western strain.

Zaumeyer & Goth (1962a, 1964) reported Florida strain. The symptoms of Florida strain on susceptible cultivars were more severe than those caused by Type strain, NY 15 strain or Idaho strain. Stringless Green Refugee was susceptible to Florida strain but Pinto 111, Michelite, Sanilac, RM 34, GN 123 and GN 31 were resistant. Plants of cv. Topcrop did not show local necrosis when inoculated with Florida strain at 32 °C, as they did after inoculation with Type strain or NY 15 strain.

Hubbeling (1963) described the Imuna, Michelite and Great Northern strains from cvs Imuna, Michelite and GN 123, respectively. He compared these strains with strain W and differentiated among others on cvs Dubbele Witte, Imuna, Michelite, GN 123 and Widusa. While strain W only attacked Dubbele Witte, each of the strains Imuna and Michelite also gave symptoms both in Imuna and Michelite, but not in GN 123, which was susceptible

to Great Northern strain. Michelite strain differed from Imuna strain in giving local and systemic necrosis at 20 °C in Widusa and the other cultivars with dominant resistance. Thus a strain was found giving systemic necrosis at that moderate temperature.

In 1964, a strain was found by Silbernagel (1966, 1969) in a PI line of *Phaseolus vulgaris* (PI 197690S) from Mexico, which he indicated as Mexican strain (hereafter referred to as Mexico strain). That strain differed from those previously reported in being seed-transmitted through cv. Red Mexican 35 and by its inability to infect Improved Tendergreen. Symptoms induced by this strain on certain bean cultivars were as severe as those caused by the Florida strain. The necrosis induced by the Type and NY 15 strains on Topcrop, when subjected to 32 °C for 3 days, was also induced by the new strain.

Moreno et al. (1968) reported on a Costa Rican isolate. That isolate infected the cultivars that are also susceptible to Type strain, but, unlike Type strain, induced no local necrosis on Topcrop at 32 °C. There was no difference in pathogenicity spectrum (Section 2.6) of the Costa Rican isolate from Type strain and Florida strain. Stringless Green Refugee was susceptible to the Costa Rican isolate while Pinto 111, Michelite, Sanilac and Topcrop were resistant.

Gamez et al. (1970) reported Peru strain. They directly compared this with the Costa Rican isolate on test cultivars, and compared it with Type, NY 15, Florida, Idaho and Mexico strains on the basis of their bean varietal reactions as published. None of the used cultivars, resistant to Type strain, was attacked by Peru strain. But some cultivars susceptible to Type strain did not react to Peru strain or Costa Rica strain.

Hubbeling (1972) isolated another two strains in the Netherlands from the pods of Jolanda and Colana, both cultivars having dominant resistance. He added GN 31, RM 35 and Jubila to his 1963 list of differential cultivars. Jolanda strain, like Michelite strain, gave local and systemic necrosis at 20 °C in cultivars with dominant resistance. It differed from Michelite strain in giving rapid systemic necrosis at 20 °C in Jubila, in which Michelite strain induced local vein necrosis only. He noted that GN 31 was susceptible to Jolanda strain, but resistant to Michelite strain. Colana strain differed from Great Northern strain in its inability to infect GN 31 and giving systemic necrosis in Jubila, and from Jolanda and Michelite strains in not attacking Michelite and Sanilac.

Alconero et al. (1972) briefly described a strain in Puerto Rico, infectious to cultivars also susceptible to Type strain. Cv. Puregold Wax was also mentioned to be susceptible to this strain. In a later publication, Alconero & Meiners (1974) reported Puregold Wax as susceptible to Type and Puerto Rico strains. Neither publication clearly distinguished Puerto Rico strain from Type strain.

In the Netherlands, Drijfhout & Bos (1977) recorded two strains coded NL 7 and NL 8. Strain NL 7 was isolated in 1974 from an unknown Peruvian cultivar. It gave a typical greyish green mosaic in the differentials Dubbele Witte and Stringless Green Refugee. It attacked Imuna and Puregold Wax in contrast to strain W, while the other differentials with recessive *ii*, cvs Redlands Greenleaf B, GN 123, Michelite 62, Sanilac, GN 31 and RM 35 were resistant to that strain. There was no reaction in the differentials with dominant *I* gene: cvs Jubila, Topcrop, Improved Tendergreen 40031, Widusa, Black Turtle Soup and Amanda.

Strain NL 8 infected Sanilac, Michelite and RM 34 as well as Dubbele Witte and Stringless Green Refugee, both susceptible to all strains. It induced local necrosis at 20 °C in all differentials with dominant *I*, but systemic necrosis in Widusa and Black Turtle only.

Table 1. Literature review of bean varietal reactions after inoculation with the described virus strains. + = susceptible, systemic mosaic; +n = susceptible, systemic necrosis; ± = tolerant, systemic symptoms questionable or very weak, systemic virus spread recoverable by indexing; ±n = systemic necrosisis in some of the plants only; – = resistant, no systemic symptoms, virus spread not recovered by indexing; –? = literature data not clear. N.B. back-inoculations for indexing are mentioned by some of the authors only.

Differential cultivar	Virus strains																					
	Type	NY 15	Voldagsen	Marienau	Westlandia	RM	P 487	P 1075	Idaho or B	Western	Florida	Imuna	Michelite	Great Northern	Mexico	Costa Rica	Peru	Jolanda	Colana	Puerto Rico	NL 7	NL 8
Dubbele Witte	+	+			+	+	+	+	+	+	+	+	+	+	+			+	+		+	+
Str. Green Refugee	+	+			+				+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saxa			+	+			+	+														
Wachs Rheinland			+	–																		
Beka					+	+																
Puregold Wax	–, +	+			–				+	±	+, –	–	+	+	+			+	+	+	+	–
Imuna	–	±, –			–, ±				–	–	+, ±	+	±, –	+	±			+	+	±	+	–
Great Northern UI 123	–, ±	–	–		–		–	–	+	+	–	–	–	+	+			+	+	±	–	–
Great Northern UI 59	–	–					–	–			–											
Michelite	–	+	+	–?	–				–	–	–	+, ±	+	–, +	–	–	–	+	–	–	–	+
Sanilac	–	+, –			–				–	–	–	+, –	+	–, +	–	–	–	+	–	–	–	+
Red Mexican UI 34	–, ±	+	+	–?	–		–	–	±	–	–, ±, +	+, ±	+	±	–			+	–	±	–	+
Pinto UI 111	–, ±	+			–		–	–	±	–	–	±	+	–	±	–	–	+	–	±	–	+
Great Northern UI 15	–	+	–	–			–	–														
Robust	–	+	+	+																		
Great Northern UI 31	–	–	–	–	–		–	–	+	–	–	–	–	+	+		–	+	–		–	–
Red Mexican UI 35	–	–, ±			–				±	±	–, ±	±	±	+	+			±	±	±	–	–
Monroe	–	–									±											
Jubila	–	–			–				–	–	–	+n	+	±n	–			+n	+n		–	–
Topcrop	–, ±	–, ±	–	–	–		–	–	–	–	–, ±	±n	+n	–	–	–	–	+n	–	±	–	–
Improved Tendergreen	+, ±	–			–				±	±	±, +	–	+n	±	–			+n	–		–	–
Widusa					–							–	+n	–							–	+n

The crucial question is whether all these strains are indeed different and whether the reported varietal reactions are reproducible (Section 4.4). A review of the published varietal reactions of the strains described is given in Table 1.

2.6 Further delimitation of the problem

The literature on strains of BCMV clearly shows that there is much confusion and misunderstanding about their identification. Because there are no basic differences in symptom expression between strains of BCMV, a strain is mainly characterized by reaction of a range of differential cultivars, i.e. by its pathogenicity spectrum. Definition: *a pathogenicity spectrum is a sequence of positive or negative reactions that a virus strain induces in a standard range of differentials, indicating whether the pathogen can systemically infect each of them.* To standardize strain identification, an internationally accepted standard range of differentials is desirable. So far, test ranges used in different countries have differed, and there are not even clear agreements on the interpretation of terms like susceptibility and resistance, and on test methods and climatic conditions. Therefore some of the strains described may be identical.

From the literature it is also obvious that the genetics of resistance and susceptibility of common bean to BCMV is incompletely understood. There are clear differences in resistance spectra of some differentials. Definition: *a resistance spectrum is a sequence of positive or negative reactions of a cultivar after inoculation with a standard range of strains, indicating whether each of the strains can systemically infect it.* Of course, these differences are genetically controlled, and result from differences between the resistance genotypes of the differentials. Definition: *a resistance genotype is a combination of genes governing resistance, indicating the dominant or recessive alleles that are present.*

Resistance to the virus is controlled by at least two genes for resistance, indicated as *a/A* and *s/S* and one gene *I/i* for systemic necrosis. Whether a plant will show systemic mosaic or systemic necrosis depends on the combination of recessive and dominant alleles of these three genes, on the virus strain used and perhaps to some extent on the climatic conditions during the tests.

It is not known whether these genes are concerned with reaction to all strains or are specific for certain strains only, since the published studies were all with one strain. Other genes for resistance and necrosis might function for other strains. If more genes are involved than the three of which the independant inheritance was demonstrated, then the question arises whether these additional genes also act independently, or are linked or form multiple alleles.

Resistant cultivars have often been reported in literature, but they were all resistant to only some of the strains. In my preliminary trials, I found no cultivars with resistance to all strains, showing neither mosaic nor systemic necrosis.

Damage by systemic infection of cultivars with dominant *I* gene could be prevented by modifying their genotype in such a way that reaction remains local. One might also aim at breeding cultivars carrying $I^{t}I^{t+1}$, in which no systemic mosaic can be induced with any of

1. $I^{t}I^{t+1}$ replaces *ii* according to the new rules for gene symbols of beans (Comacho et al., 1977), which will be used from now on.

the known strains, and no systemic infection is detectable. To this end, a comprehensive study was made of the genes controlling resistance, in relation to all distinguishable virus strains. This study was intended to show whether resistance can be achieved to all existing strains.

The aims of this research were as follows:

a. To analyse the interaction between the resistance genes available in common bean and the pathogenicity genes present in the different strains of the virus and the inheritance of the resistance genes.

b. To obtain genotypes with dominant or recessive resistance to all strains of the virus. In practice, the breeding of these types was hampered by insufficient knowledge of the inheritance of the two types of resistance in relation to specialization of the virus.

To reach this aim, the following research program was completed:

1. Study of suitability of the test methods and their possible improvement. An attempt to standardize test methods and definitions of terms of plant reaction for identification of strains of BCMV.
2. Testing of a large number of cultivars and other accessions with the separate strains to determine resistance to each strain and the number of different resistance genotypes.
3. Comparison of all available strains of BCMV known until 1976 and of differential cultivars. Establishment of an international range of differential cultivars and final identification and classification of the virus strains.
4. Analysis of the inheritance of resistance by testing with each strain the F_1, F_2 and sometimes F_3 of the diallel crosses between the differential cultivars with recessive I^+ gene and by testing F_2 of some crosses with a mixture of strains.
5. Analysis of the different resistance genotypes with dominant resistancy by testing with some strains the F_1 and F_2 of crosses between the differentials with dominant I gene and a number of differentials carrying I^+I^+.

3 Materials and methods

3.1 Bean cultivars

Seeds of most cultivars came from stocks maintained at the Institute for Horticultural Plant Breeding (IVT). Most cultivars had been propagated at IVT several times from seed after receipt from an outside source.

The following persons or seed companies supplied seeds of the cultivars used as differentials: Dr M.J. Silbernagel (Prosser, Washington, United States) provided seeds of Black Turtle Soup, Great Northern UI 31 (GN 31), Great Northern UI 123 (GN 123), Improved Tendergreen 40031 (Impr. Tendergr.), Michelite 62 (Michelite), Monroe, Pinto UI 111 (Pinto 111), Pinto UI 114 (Pinto 114), Puregold Wax, Red Mexican UI 34 (RM 34), Red Mexican UI 35 (RM 35), Sanilac and Stringless Green Refugee (Str. Gr. Ref.). Miss Barbara Ballantine (Rydalmere, New South Wales, Australia) supplied Redlands Greenleaf B (RG-B) and Redlands Greenleaf C (RG-C). Jubila was received from the breeding firm Bruno Nebelung (Münster, West Germany), Imuna from Dippe (Herford, West Germany), Topcrop came from Rogers (Idaho Falls, Idaho, United States), Amanda from Nunhem (Haelen, the Netherlands), and Dubbele Witte (DW) from Pop Vriend (Andijk, the Netherlands). IVT provided Widusa and the breeding lines IVT 7214 and IVT 7233. IVT 7214 was selected after repeated testing with BCMV and BYMV for several generations from the American line PI 181954, supplied by Dr M.H. Dickson (Geneva, New York, United States). I selected IVT 7233 from a cross between GN 31 and Widusa.

3.2 Virus isolates and strains

Dr M.J. Silbernagel supplied Florida, Idaho, Mexico, New York 15, Type and Western strains. Dr. L. Bos (Wageningen, the Netherlands) supplied Puerto Rico strain, sent to the Netherlands by Dr R. Alconero (Mayaguez, Puerto Rico, United States). The Dutch Plant Protection Service gave permission to import the foreign strains. All strains described until 1975 were included in the tests except the German ones, of which material was no longer available, and Costa Rica and Peru strains, of which no material could be obtained.

The strains isolated in the Netherlands and originally from the Institute of Phytopathological Research (IPO), were maintained at IVT: Westlandia, Imuna, Michelite, Great Northern, Jolanda and Colana. For convenience, these strains were given NL numbers in order of description: NL 1, NL 2, NL 3, NL 4, NL 5 and NL 6, respectively. Two strains, NL 7 and NL 8, also maintained at IVT, were later added.

3.3 Handling the virus

3.3.1 Virus transmission

Freshly picked leaves of clearly diseased virus source plants, 4 to 6 weeks old, were ground in a mixer and the ground material squeezed through cheesecloth, after which the sap was diluted 1:10. Some carborundum powder (500 mesh) was then added and the primary leaves of the plants to be tested were rubbed with a small piece of foam plastic after dipping in the inoculum. The inoculated leaves were washed with tap-water. Best results were obtained by inoculating not yet fully expanded primary leaves nine to ten days after sowing when grown at 20 °C. All plants were inoculated a second time within a week, also on the primary leaves.

This was the usual method of virus transmission in our experiments. For virus transmission in the necrosis test and the infectivity test, see the descriptions of these tests (Sections 3.4.2 and 3.4.3).

3.3.2 Virus propagation and maintenance of strains

Propagation of the virus strains was in plants of Dubbele Witte. This cultivar is susceptible to all strains and very sensitive to mosaic, rapidly attaining a high content of virus. Leaves with distinct symptoms were picked for inoculum preparation about three weeks after inoculation. Four or five plants of each of the differentials Imuna, RG-B, Michelite, GN 31, Jubila and Widusa were always added to each propagation block of Dubbele Witte plants, to check strain purity. To keep the virus strains uncontaminated and to obtain a reliable differential reaction, some precautions were taken:

- Seed of the differential cultivars was produced in aphid-free greenhouses and suspected plants were removed to guarantee virus freedom.
- To maintain genetic purity (obtained by line selection) of the differential cultivars during seed multiplication, plants of deviating type were discarded.
- Batches of plants inoculated with different strains were isolated by distance or with plastic screens to prevent contamination by contact of plants.
- Hands and tools were disinfected with a mixture of trisodium phosphate and soft soap after working on plants infected with a particular strain and before proceeding to plants with another strain to avoid contamination.

The strains used for the genetical analysis viz. NL 1 to NL 8, NY 15 and Florida, were permanently maintained on the differential range Dubbele Witte, Imuna, RG-B, Michelite, GN 31, Jubila, Topcrop, Widusa and Amanda. Every eight weeks the strains were transferred to young plants of a new series for constant check of strain purity. If the differentials reacted characteristically, seeds were harvested from time to time from plants of Dubbele Witte, infected with one of the strains. About 30 to 80% of seeds were always infected. The strains were thus stored in dry seed for reisolation from seedlings if the stock of a given strain ran out or if strains were contaminated. This procedure proved essential as it was not always possible to reisolate a strain from a mixture.

Strain purification was sometimes possible by selective passage through a differential cultivar, but this could only be done if this differential was selectively susceptible to the strain wanted but resistant to the contaminant. The strain wanted was then passed twice

through this differential to eliminate traces of the contaminant. This procedure took more time than returning to infected seed and was only used if such seed was not available. Anyway, for many strains such a selective differential was not available.

If reactions were deviant, the isolate was identified by first determining the reaction of the range of differentials. If deviating from the pathogenicity spectra of the known strains, the isolate could be a new strain or a mixture of strains. The isolate was therefore back-inoculated twice from each susceptible differential onto new plants of the complete range of differentials. If the resulting pathogenicity spectra were identical, then the original isolate was concluded to be pure.

3.4 Plant testing

3.4.1 General test

Plants of cultivars or progenies of crosses were subjected to infection as follows. Seven days after sowing, the plants to be tested were transplanted to pots with a diameter of 12 cm, one plant per pot, and were grown in greenhouses at mean temperatures between 22 and 26 °C with a minimum day and night temperature of 20 °C and a maximum of 24 °C during winter or at cloudy days in summer, ranging up to 30 °C on sunny summer days (Fig. 1A, 1B). In the tests for comparison of strains and differentials, four plants of each differential were inoculated with each strain, and one plant was used as an uninoculated control (Fig. 2A, 2B). In the cultivar tests, five of six plants were inoculated with each strain used, one plant remaining uninoculated. When testing progenies of crosses, 10 F_1 plants were used including those of the reciprocal combination. From F_2, 160, 640 or 1280 plants were usually tested, including reciprocals, the number of plants depending on the expected segregation ratios. To each cultivar or progeny test, 10 plants of each of the differentials Dubbele Witte, Imuna, RG-B, Michelite, GN 31, Jubila and Widusa were added to check the strain purity. The first inoculation was about ten days after sowing and the second 3-5 days later. Reactions were recorded one, two, three and four weeks after the first inoculation.

If systemic necrosis was expected in a segregating progeny, the test plants were placed in a greenhouse at a constant temperature of 26 °C to favour this systemic reaction. After separating the plants with gene *I* (Chapters 4 and 5; Ali, 1950) from those with I^+I^+ (through the local necrosis reaction of the plants with dominant *I* or by means of the necrosis test, Section 3.4.2), the plants with I^+I^+ were moved to a greenhouse section at 20 °C. At that temperature, mosaic was mostly more pronounced than at 26 °C, where some masking occurred.

The plants with dominant *I*, not showing symptoms four weeks after the first inoculation or with local necrosis only, were considered resistant to the strain concerned. In those plants, one could not demonstrate systemic spread of virus. The plants developing systemic necrosis, whether or not preceded by local necrosis, were susceptible and sensitive to the strain concerned.

Cultivars showing systemic necrosis four weeks after inoculation in some of the plants only were also considered susceptible at the given temperature. The number of plants with systemic necrosis mostly varied in repeated tests and increased if tested at a higher temperature.

Plants with I^+I^+ were rated as follows. Plants showing clear symptoms of systemic mosaic were considered susceptible and sensitive to the strain concerned. Plants without symptoms three weeks after the first inoculation or with questionable or very weak systemic symptoms or with local discolorations only, were subjected to the infectivity test (Section 3.4.3). The plants in which systemic spread of virus could be detected were considered susceptible but tolerant to the strain used. Those reacting negatively in these tests were considered resistant.

During winter, when the light intensity was low, additional light was given with Philips high-pressure mercury vapour lamps (type HPL/N, 400 W), to promote growth. The intensity of the additonal light was about $10 W/m^2$.

3.4.2 Necrosis test

The necrosis test was used for genetic analysis to distinguish plants with gene *I* from those carrying I^+I^+ in a segregating progeny (Fig. 3A, 3B). This test is a modification of the 'dish test' of Quantz (1957) and was used as a supplementary test if the virus strain did not induce local necrosis in plants with dominant *I* gene (Section 4.3).

From each plant not showing symptoms after inoculation with the strain concerned, a leaflet was taken from the third or higher trifoliate leaf. This was then tagged, inoculated with strain NL 3 and placed in a box of asbestos cement. Each box had first been provided with a layer of cotton wool covered with a sheet of filter paper, both saturated with water. The boxes were covered with plastic to maintain 100% humidity, and placed in a greenhouse at a temperature, ensuring an inner box temperature between 27 and 30°C. If necessary, mercury lamps were placed above the boxes for additional light and maintenance of the temperature. After three to four days the leaves from plants with dominant *I* showed pin-point lesions and vein necrosis, whereas the leaves from plants with I^+I^+ showed no reaction or only superficial necrosis (local discoloration), clearly differing from vein necrosis.

3.4.3 Infectivity test

Plants not reacting with necrosis in the necrosis test (Section 3.4.2) and without distinct mosaic or without symptoms three weeks after inoculation were tested for infectivity by back-inoculation.

From these plants two or three leaflets each were taken from the third trifoliate leaf and stored overnight in small numbered plastic bags in the deep-freeze. Next day, each sample was thawed and some sap was expressed with thumb and forefinger (the hands covered with plastic gloves) on the carborundum-dusted primary leaves of two plants of Dubbele Witte sown ten days earlier. Then the sap was rubbed out with the forefinger. After each back-inoculation the gloved hands were carefully washed and disinfected with trisodium phosphate and soap. One, two and three weeks after inoculation the Dubbele Witte plants were examined for mosaic as usual (Fig. 4).

Fig. 1. Bean plants for testing of resistance to strains of BCMV. A. Young plants, just before inoculation. B. Inoculated older plants at final examination.

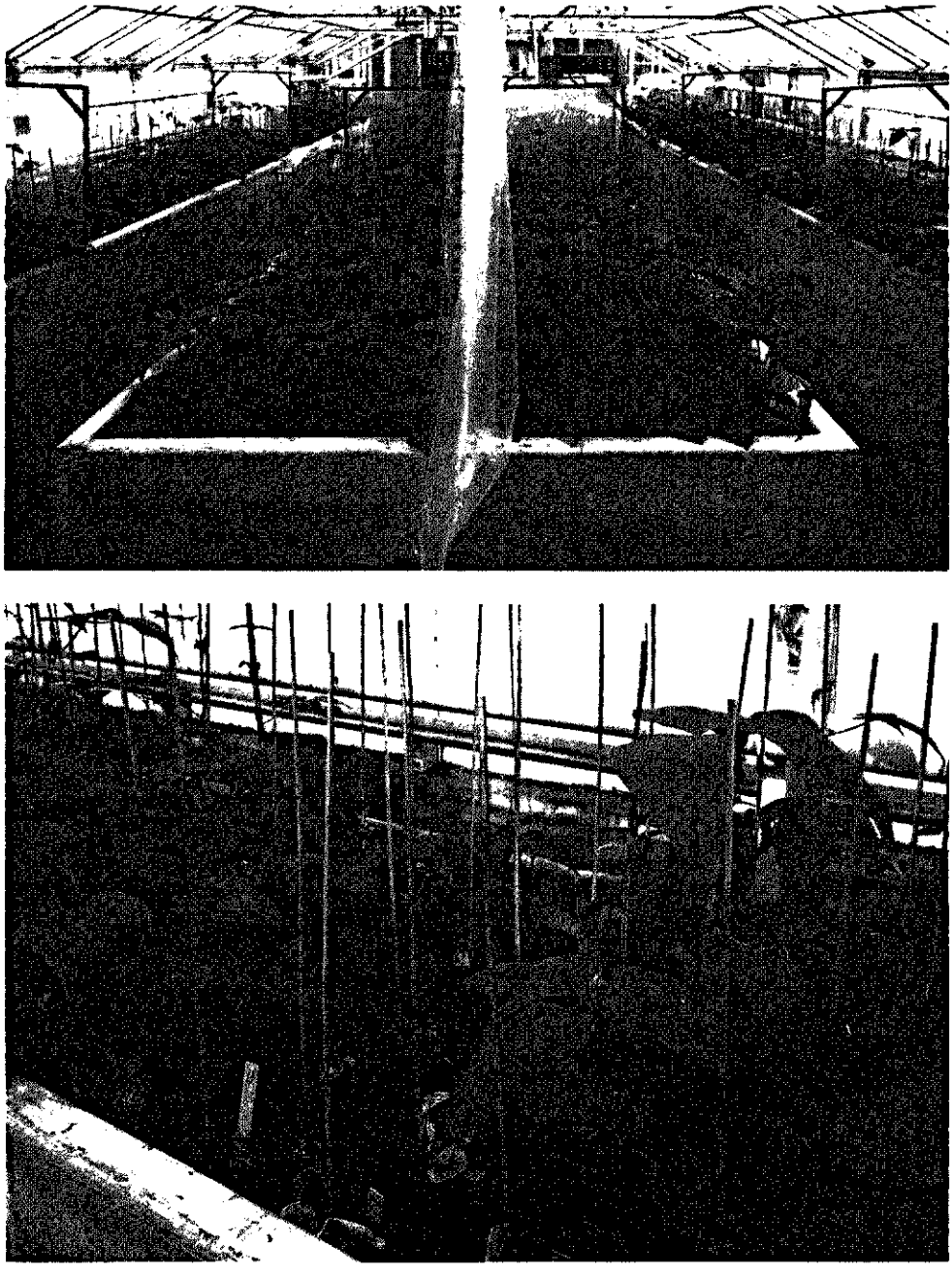


Fig. 2. Checking strains of BCMV for purity trough their reactions on a standard set of differential cultivars. A. General view. B. Detail of a block of plants inoculated with a necrosis-inducing strain, showing systemic necrosis in some differentials.

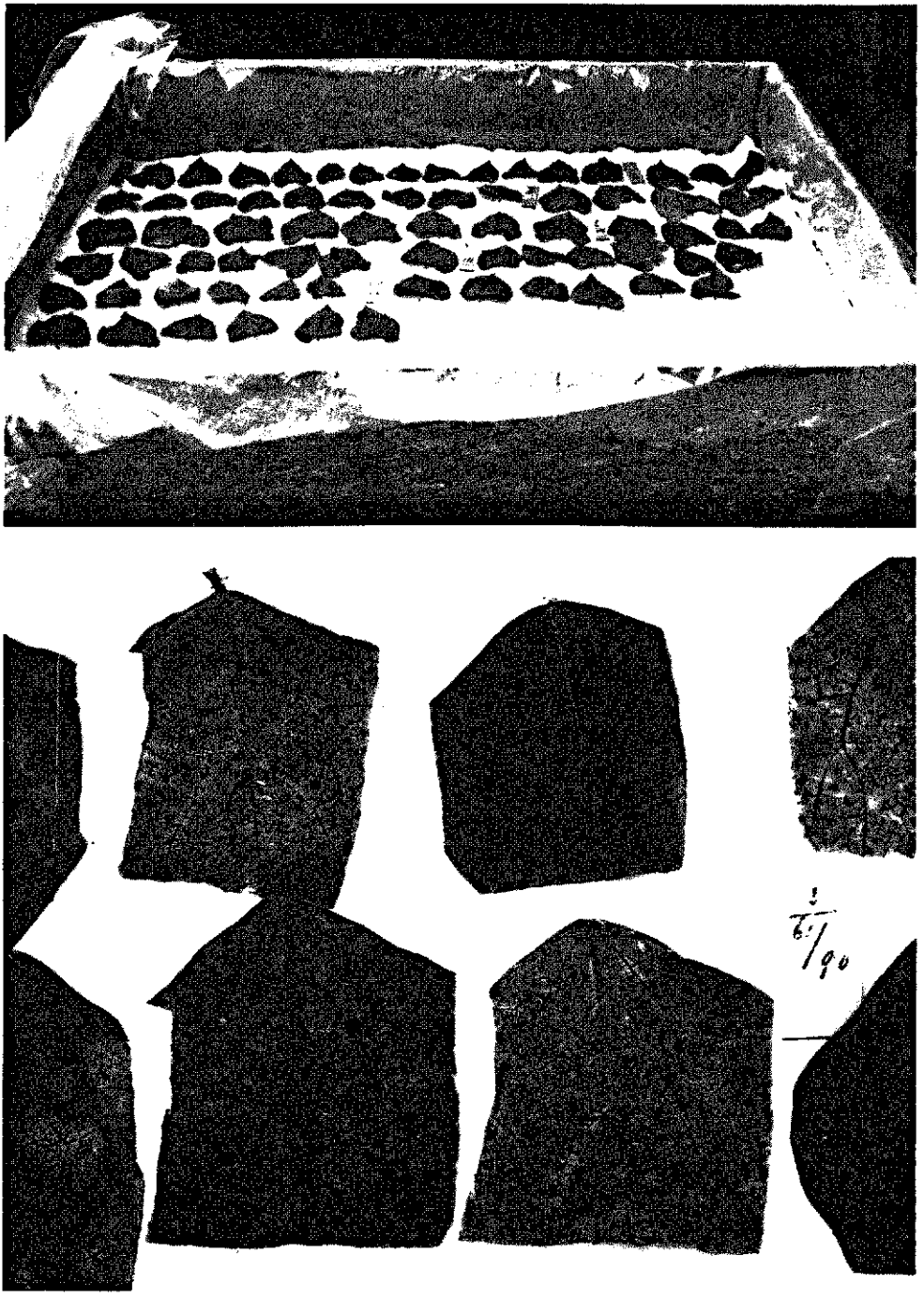
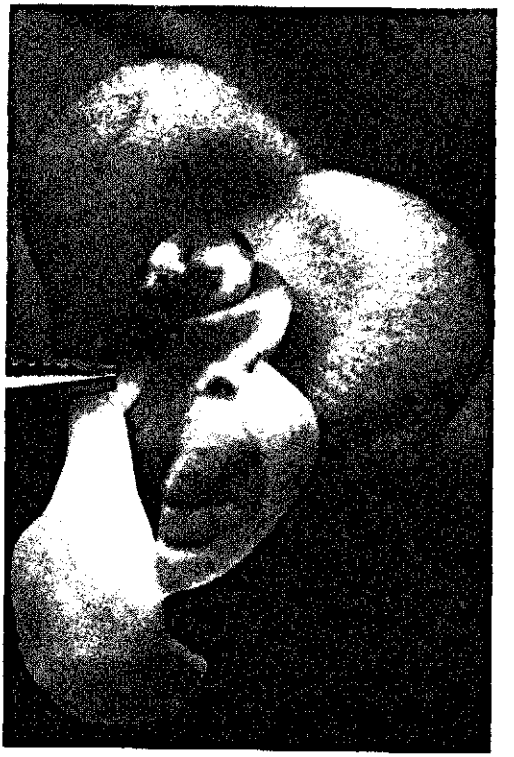


Fig. 3. Necrosis test with detached leaves. A. General view. B. Detail showing leaves with positive (vein necrosis) and negative reactions.

Fig. 4. Infectivity test with back-inoculations onto pairs of plants of Dubbele Witte. Some pairs are infected; other are healthy.

Fig. 5. Local discolorations on a primary leaf of GN 31, inoculated with strain NL 1.

A



B

C

Fig. 6. Crossing of beans A. Loosening and folding back the standard of a flower bud, about two days before anthesis. B. Forcing the stigma out of the twisted keel by pushing down one of the lateral wings of the flower bud. C. Hand-pollination by rubbing the pollen-covered stigma of a mature flower against the stigma of the flower bud to be pollinated.

Fig. 7. Leaf rolling in Dubbele Witte with strain NL 1.

Fig. 8. Vein chlorosis in Dubbele Witte with strain NL 2.

Fig. 9. A yellow mosaic in Redlands Greenleaf B with strain NL 5.

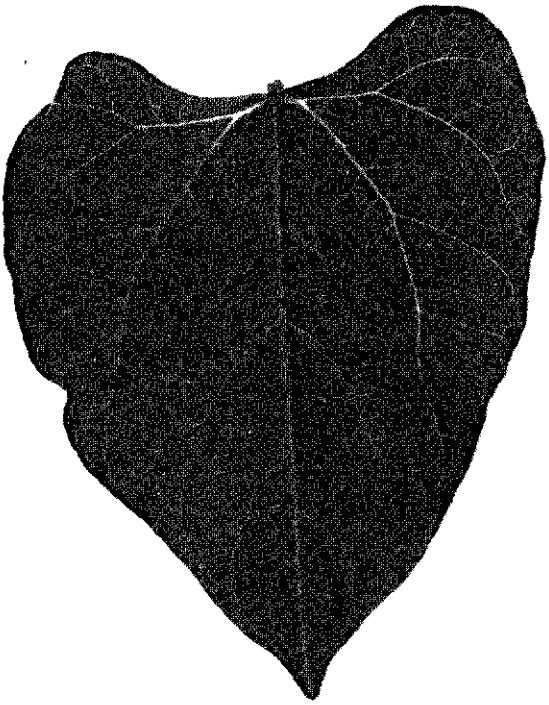


Fig. 10. Local necrosis (pin-point lesions) in a leaf of IVT 7233 with strain NL 3.

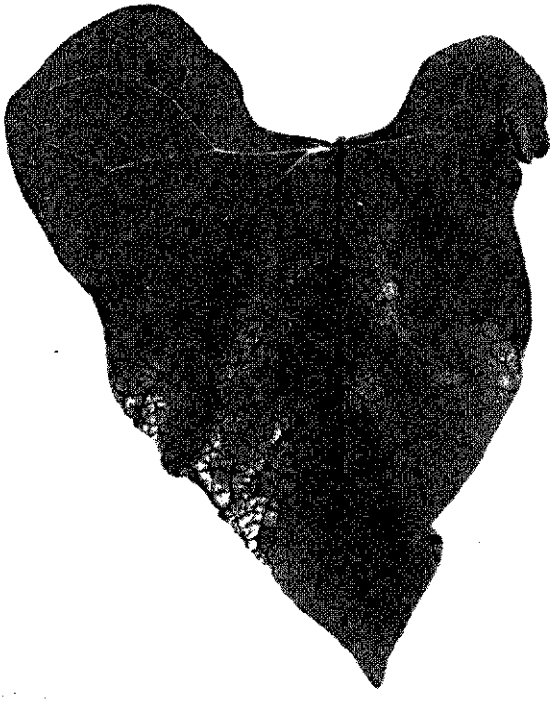


Fig. 11. Local vein necrosis in a leaf of Topcrop with strain NL 8.

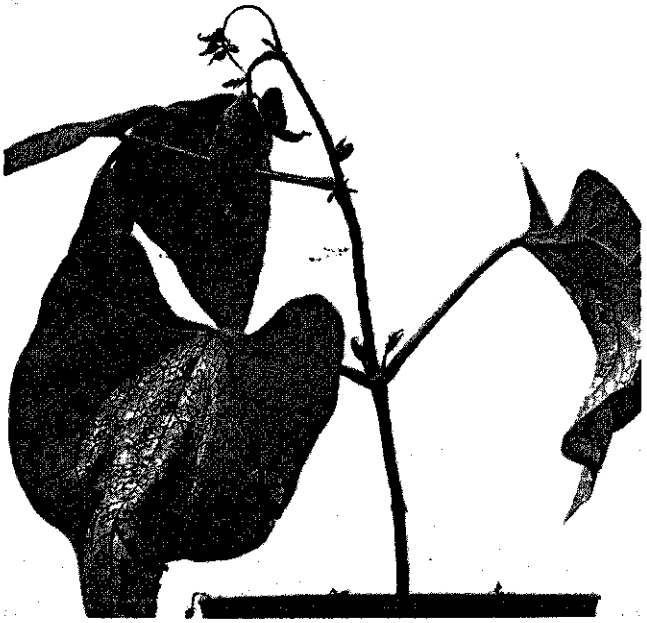


Fig. 12. Local and systemic necrosis in Widusa with strain NL 8.

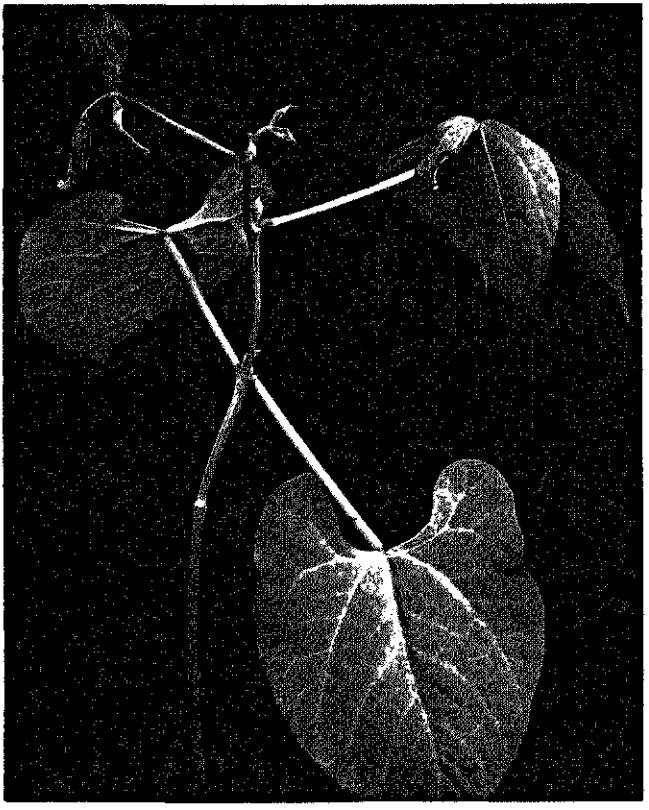


Fig. 13. Systemic necrosis but no local necrosis in Topcrop with strain NL 6.

3.4.4 F_3 test

As a third supplementary test, the F_3 progeny of F_2 plants showing no symptoms and not reacting in the infectivity test were also tested with the same strain as used for the F_2 plants to determine whether the healthy F_2 plants had escaped or had been really resistant.

For this test, seeds were harvested in a greenhouse from the F_2 plants reacting negatively in the infectivity test. Ten to twenty descendents of each F_2 plant were inoculated and examined as usual, after which the final segregation ratio of the F_2 generation of the cross could be determined.

3.5 Pollination technique

In *Phaseolus vulgaris*, self-pollination normally occurs. If a cross is to succeed, selfing must be prevented before the flower opens. The corolla of a *Phaseolus* flower consists of an erect standard, two lateral wings and a tubular keel. The keel, composed of two petals grown together and twisted, contains the ovary and the diadelphous stamens: one free and nine with the partially fused filaments forming a sheath around the ovary. The keel terminates spirally, and stamens and stigma are also twisted.

Our pollination technique to prevent selfing without emasculation was as follows: The standard of an unopened flower bud was lifted up with a pair of tweezers (Fig. 6A). Then the left wing was pressed downwards, forcing the stigma out of the keel, remaining in this position if the wing had been pressed down far enough (Fig. 6B). Then the stigma was rubbed with the desired pollen, using a stigma from an open flower of a male parent covered with pollen (Fig. 6C). Artificial pollination was done one or two days before anthesis and elongation of the standard. Until that time, selfing was impossible. When the stigma retracts into the keel after hand-pollination, self-pollination is not excluded but it is rare. With a skilled worker, the percentage of successful crosses is generally more than 95% if the climatic conditions are good (greenhouse temperature 20-26 °C, not too low relative humidity). The pollinations were twice as rapid as with emasculation. To detect possible selfing, plants with an easily visible recessive character were used as female parents wherever possible (e.g. absence of anthocyanin formation on stem and determinate growth are recessive).

4 Analysis of host reaction and identification of strains

4.1 Introduction

Host genetics underlying host reaction to the various strains of the virus cannot be properly understood without adequate knowledge of virus strain differentiation and vice versa. Both studies are interdependent. In the past, emphasis was on the description of strains of BCMV with very little information on host genetics in relation to resistance to the virus. So far, certain host groups differing in reaction to various apparently different strains had been distinguished empirically (Table 1, Chapter 2).

To elucidate host genetics, eight apparently different Dutch virus strains were chosen for tests to classify many bean cultivars by their resistance spectrum (Section 2.6). Each spectrum supposedly results from a specific resistance genotype (Section 2.6).

Through an International Working Group on Legume Viruses (Bos, 1971), direct liaison was established with Dr M.J. Silbernagel, Prosser, Washington, United States (Drijfhout et al., 1978) for the development of an internationally acceptable and standardized range of differential cultivars for identification of strains of BCMV. With this international range of differential cultivars, all strains of BCMV described in literature, of which material could be obtained, are here differentiated and classified. An attempt is made to analyse possible causes of contradictory results found in literature.

4.2 Cultivar testing

In eight trials about 450 cultivars and lines were tested with the strains NL 1 to NL 8 inclusive. On the basis of their resistance, these cultivars and lines could be classified into twelve groups (including two subgroups) as summarized in Table 2. The first six groups never reacted with local or systemic necrosis, but with mosaic to one or more strains. They thus bear the necrosis gene in recessive form (Ali, 1950). IVT 7214 of host group 7 reacted neither with mosaic, like cultivars with I^+I^+would do, nor with local or systemic necrosis, typical for cultivars with *II*. It carries I^+I^+as will be demonstrated in Chapter 5. Four other groups reacted to some strains with systemic necrosis and one with only local necrosis, but never with mosaic. They thus carry *II* as will be proved in Chapter 7. Host group 9 is divided into subgroups a and b. The differences in resistance spectra between the two subgroups seem to be rather quantitative than qualitative. The analysis of resistance genes in Chapter 7 will reveal whether Jubila and Topcrop as representatives of groups 9a and 9b carry different major genes for resistance.

No distinction is made in Table 2 between sensitivity and tolerance but only between susceptibility and resistance. With susceptibility, systemic spread of virus can be demonstrated outside the inoculated leaves; with resistance, it cannot.

Table 3 shows those cultivars and lines found in the different host groups, which I or

Table 2. Host groups as found by cultivar testing with eight strains. + = susceptible, systemic mosaic, or virus recoverable from uninoculated leaves by back-inoculation onto cv. Dubbele Witte; +n = susceptible, systemic necrosis; ±n = susceptible, showing systemic necrosis, or resistant, depending on temperature. Temperature range between days: 22-26 °C, mean day temperatures.

Host group	Number of cultivars or lines	Virus strain							
		NL 1	NL 2	NL 3	NI 4	NL 5	NL 6	NL 7	NL 8
Cultivars with recessive alleles of the necrosis gene									
1	251	+	+	+	+	+	+	+	+
2	53	–	+	+	+	+	+	+	–
3	19	–	–	+	+	+	+	–	–
4	12	–	+	+	–	+	–	–	+
5	11	–	+	+	–	+	–	–	–
6	4	–	–	–	+	–	–	–	–
7	1	–	–	–	–	–	–	–	–
Cultivars with dominant alleles of the necrosis gene									
8	34	–	–	+n	–	+n	±n	–	+n
9a	5	–	+n	+n	–	+n	+n	–	–
9b	40	–	±n	+n	–	+n	±n	–	–
10	2	–	–	–	–	+n	–	–	–
11	1	–	–	–	–	–	–	–	–

others have used as differentials. A list of all cultivars and other accessions tested is given as an appendix. Host groups 7 and 11 are represented by two of my breeding lines: IVT 7214, bearing I^+I^+ as will later appear and resistant to all strains used; and IVT 7233, carrying *II*, also resistant to all strains, showing local necrosis with NL 3, NL 5 and NL 8. Both lines were not only resistant to the eight strains used for cultivar testing, but also to all foreign strains used in studying the strain classification (Section 4.4). No commercial cultivar was found to be resistant to all strains of BCMV. By breeding these lines, one of the aims of this study was achieved: to obtain genotypes resistant to all known strains of the virus.

Representatives of host groups 1 to 7 inclusive (carrying I^+I^+) were inoculated with strains NL 1 to NL 8, and the plants were kept at constant temperatures of 17, 20, 23, 26 or 30 °C in regulated greenhouse sections of the IVT phytotron to determine whether the reactions of Table 2 were valid for a wider temperature range. Susceptible cultivars remained susceptible at all five temperatures and cultivars resistant at one temperature did not show systemic infection at the other temperatures. Temperature only influenced rapidity and severity of symptom development.

The same treatment was given to cultivars carrying *II* (Host groups 8, 9a, 9b, 10 and 11). However, some showed systemic necrosis at higher temperatures but none at lower temperatures or showed systemic necrosis in more plants at higher temperatures (Table 4). In these experiments, ten strains were used, representing all strain groups of Section 4.4. The ten strains could be arranged into three main groups: (1) strains NL 1, NL 4, NL 7, Florida and NY 15 that never induced systemic necrosis (Table 6; Section 4.4), they were omitted from Table 4; (2) strains NL 2 and NL 6 that induced systemic necrosis in

Table 3. Resistance of cultivars and lines, used in the literature or in this study as differentials, to eight strains of BCMV. See Table 2 for reaction symbols and temperatures.

Host group	Cultivar or line	Virus strain							
		NL 1	NL 2	NL 3	NL 4	NL 5	NL 6	NL 7	NL 8
Cultivars with recessive alleles (I'I') of the necrosis gene									
1	Beka Bountiful Common Red Mexican Dubbele Witte Prelude Red Kidney Saxa Stringless Green Refugee Wachs Rheinland	+	+	+	+	+	+	+	+
2	Imuna Puregold Wax Redlands Greenleaf C	–	+	+	+	+	+	+	–
3	Great Northern UI 1 Great Northern UI 59 Great Northern UI 123 Redlands Greenleaf B	–	–	+	+	+	+	–	–
4	Bo 19 Great Northern UI 15 Michelite 62 Pinto UI 78 Pinto UI 111 Red Mexican UI 34 Robust Sanilac	–	+	+	–	+	–	–	+
5	Pinto UI 114	–	+	+	–	+	–	–	–
6	Great Northern UI 16 Great Northern UI 31 Monroe Red Mexican UI 35	–	–	–	+	–	–	–	–
7	IVT 7214	–	–	–	–	–	–	–	–
Cultivars with dominant alleles (II) of the necrosis gene									
8	Black Turtle Soup Widusa	–	–	+n	–	+n	±n	–	+n
9a	Jubila	–	+n	+n	–	+n	+n	–	–
9b	Improved Tendergreen 40031 Processor Topcrop	–	±n	+n	–	+n	±n	–	–
10	Amanda	–	–	–	–	+n	–	–	–
11	IVT 7233	–	–	–	–	–	–	–	–

Table 4. Resistance of six differentials with dominant necrosis gene to five virus strains at five temperatures. Values present numbers of plants with systemic necrosis (s.n.) per plants inoculated. +n = some or all plants with severe s.n., cultivar susceptible at the given temperature(s); ± = plants with s.n. may or may not occur, depending on the temperature, the number of plants with s.n. mostly increasing with temperature, cultivar susceptible or resistant, conditioned by temperature; – = no plants with s.n., cultivar resistant at the recorded temperature(s).

Host group	Differential cultivar	Virus strain	Temperature (°C)					Systemic reaction at 20-26 °C	Systemic reaction at 30 °C
			17	20	23	26	30		
8	Black Turtle S.[1]	NL 2	0/5	0/5	0/5	0/5	4/5	–	+n
	Widusa		0/7	0/7	0/7	0/7	3/7	–	+n
9a	Jubila		4/7	7/7	7/7	7/7	7/7	+n	+n
9b	Impr. Tendergr.[2]		0/6	0/6	0/6	3/6	6/6	±n	+n
	Topcrop		0/7	0/7	0/7	2/7	7/7	±n	+n
10	Amanda		0/6	0/6	0/6	0/6	0/6	–	–
8	Black Turtle S.	NL 3	5/5	5/5	5/5	5/5	5/5	+n	+n
	Widusa		7/7	7/7	7/7	7/7	7/7	+n	+n
9a	Jubila		6/7	7/7	7/7	7/7	7/7	+n	+n
9b	Impr. Tendergr.		6/6	6/6	6/6	6/6	6/6	+n	+n
	Topcrop		7/7	7/7	7/7	7/7	7/7	+n	+n
10	Amanda		0/6	0/6	0/6	0/6	6/6	–	+n
8	Black Turtle S.	NL 5	5/5	5/5	5/5	5/5	5/5	+n	+n
	Widusa		7/7	7/7	7/7	7/7	7/7	+n	+n
9a	Jubila		7/7	7/7	7/7	7/7	7/7	+n	+n
9b	Impr. Tendergr.		6/6	6/6	6/6	6/6	6/6	+n	+n
	Topcrop		7/7	7/7	7/7	7/7	7/7	+n	+n
10	Amanda		0/6	4/6	6/6	6/6	6/6	+n	+n
8	Black Turtle S.	NL 6	0/5	0/5	0/5	2/5	5/5	±n	+n
	Widusa		0/7	0/7	0/7	1/7	6/7	±n	+n
9a	Jubila		7/7	7/7	7/7	7/7	7/7	+n	+n
9b	Impr. Tendergr.		0/6	0/6	2/6	4/6	6/6	±n	+n
	Topcrop		0/7	0/7	4/7	7/7	7/7	±n	+n
10	Amanda		0/6	0/6	0/6	0/6	2/6	–	+n
8	Black Turtle S.	NL 8	5/5	5/5	5/5	5/5	5/5	+n	+n
	Widusa		6/7	7/7	6/7	7/7	7/7	+n	+n
9a	Jubila		0/7	0/7	0/7	0/7	0/7	–	–
9b	Impr. Tendergr.		0/6	0/6	0/6	0/6	0/6	–	–
	Topcrop		0/7	0/7	0/7	0/7	0/7	–	–
10	Amanda		0/6	0/6	0/6	0/6	0/6	–	–

1. Black Turtle Soup.
2. Improved Tendergreen 40031.

cultivars of host groups 8 and 9b and NL 6 also in Amanda of group 10, depending on temperature; there was almost complete absence of local necrosis (local reactions not mentioned in Table 4); (3) strains NL 3, NL 5 and NL 8, inducing local and systemic necrosis at all temperatures in those genotypes that are susceptible to the strain concerned; these three strains caused clear local necrosis in all gentoypes with dominant *I*,

even the resistant ones, in contrast to all other strains, which at most induced a few scattered local vein necrosis. Thus, in some cultivars resistance to certain strains is dependent on temperature.

Distinction is made in Table 4 between systemic reaction within the range 20-26 ° and at 30 °C (constant temperatures). The range 20-26 °C is almost representative for conditions for bean cultivation in temperate regions and the subtropics (a constant temperature of 23 °C, for instance, is like an average of 23 °C for day and night). A mean temperature of 30 °C or more may occur in some parts of the tropics.

Breeding line IVT 7233, representing host group 7, is not mentioned in Table 4, as no systemic necrosis was found in it at any of the temperatures used.

4.3 Host reaction

According to data from the literature and data in Chapter 5, bean cultivars can be classified into two main groups: cultivars with recessive alleles I^+I^+ of the necrosis gene and those with dominant alleles (*II*). A cultivar with I^+I^+ may produce mosaic with some strains. Plants with *II* or II^+ may produce necrosis but never mosaic.

Plants with I^+I^+ may show no symptoms at all after inoculation, or only local discoloration (Fig. 5), or leaf rolling, vein chlorosis or systemic mosaic (Fig. 7-9), sometimes combined with local discolorations, according to virus strain and cultivar. The occasional superficial discolorations on the upper surface of the inoculated leaves of plants with I^+I^+ should not be confused with the distincter local necrosis of plants with dominant *I*. Local necrosis is clearly visible on both surfaces of the inoculated leaves. By contrast, local discolorations are clearly visible on the upper surface but hardly on the lower one, as they are limited to the tissue above the veins. 'Local discolorations', occurring in cultivars carrying I^+I^+, was used as a term to comprise several local symptoms like chlorotic lesions, flecks or rings, superficial browning above veins, brown rings, brownish diffuse discolorations, or small necrotic lesions or rings in Monroe. The 'local discolorations' were mostly easily distinguished from 'local necrosis' occurring in *II* cultivars, except the local lesions found in Monroe.

Plants without symptoms or with local symptoms only, without systemic infection as detected by back-inoculation (infection test, Section 3.4.3), were assigned as resistant to the strain concerned. Plants with systemic mosaic were classed as susceptible, as were plants with questionable or no systemic mosaic, in which systemic infection could be detected by back-inoculation.

Plants with *II* or II^+ never reacted with mosaic. If they reacted at all, their reaction was necrotic as pin-point lesions (Fig. 10) only, or such lesions rapidly extending into local vein necrosis (Fig. 11), or local vein necrosis followed by systemic necrosis (black root, Fig. 12), depending on virus strain, cultivar and sometimes temperature. No systemic spread of virus could be demonstrated by back-inoculation from plants with local necrosis only and seldom from plants with systemic necrosis. In some cultivars, systemic necrosis was induced with strains NL 2 and NL 6 practically without local necrosis (Fig. 13). *II* or I^+I^+ plants without symptoms at a given temperature or with only local necrosis were considered resistant to the strain concerned at that temperature and those with systemic necrosis susceptible to that strain.

Thus, in both the I^+I^+ and the *II* groups of cultivars, there was a type of resistance not

showing symptoms with certain strains. The two types were distinguishable by submitting a detached leaf of each plant to the necrosis test (Section 3.4.2). The leaf of a plant with II (or II^+) shows pin-point lesions, mostly followed by vein necrosis; the leaf of a plant with I^+I^+ does not.

Host reactions and their symptoms are summarized in Table 5. The symbols there were not used for strain differentiation but for genetic analysis in Chapters 5 and 7.

4.4 Grouping of the cultivars and strains

Part of this work was done in collaboration with Dr M.J. Silbernagel and Dr D.W. Burke Prosser, Washington, United States, who duplicated most of the experiments. For a tentative report see Drijfhout et al. (1978). Twelve cultivar groups were used as mentioned in Table 2. As differential cultivars preference was given to those mentioned in

Table 5. Plant reaction types and principal symptoms after inoculation with BCMV.

Alleles present of the necrosis gene	Reaction	Symptoms	Symbol
recessive (I^+I^+)	resistant	absent (negative in necrosis test[1] and infectivity test[2])	R^-
		local discolorations (negative in infectivity test)	Rd
	susceptible	absent (positive in infectivity test)	S
		local discolorations (positive in infectivity test)	
		mosaic, whether or not with local discolorations	
dominant ($I.$)	resistant	absent (positive in necrosis test)	R^+
		local pin-point necrosis	Rn
		local vein necrosis	
	susceptible	systemic necrosis, whether or not with local vein necrosis	Sn

1. A positive or negative reaction in the necrosis test means the development or absence of local necrosis in a detached leaf of a plant, inoculated with virus strain NL 3, indicating the presence or absence of (a) dominant allele(s) of gene I.
2. A positive or negative reaction in the infectivity test means whether virus can be detected outside the inoculated leaves or not.

literature. Table 6 contains data from several trials, at least eight for each strain mentioned.

4.4.1 Resistance groups of the cultivars

Host group 1 (Table 6) contains cultivars without resistance genes, susceptible to all strains. Dubbele Witte was used because of high sensitivity, showing severe symptoms with all strains. This cultivar is also a good indicator to detect the virus by back-inoculation from plants with questionable or no systemic symptoms.

Of the cultivars of host group 2, Imuna sometimes hardly reacted systemically to the NY 15 strain. Redlands Greenland C should not be confused with Redlands Greenleaf B of group 3.

Of the cultivars of host group 3, Redlands Greenleaf B gave more conspicuous symptoms with strains of group VI than Great Northern 123. Moreover RG-B is a bush type, whereas GN 123 has indeterminate growth. However GN 123 was maintained as it had often been mentioned as a differential in the literature.

All three cultivars of host group 4, to which Pinto UI 111 (also often mentioned in the literature) could be added, have the same resistance spectrum, although reports on their reactions sometimes conflict (Section 4.5).

Pinto 114 of host group 5 differs from Pinto 111 and the other differentials of group 4 in being resistant to NL 8.

The cultivars of host group 6 showed local discolorations with most strains. Monroe gave a local reaction consisting of small brown lesions with some strains. These were smaller and distincter than in other cultivars of the group and not easy to distinguish from the pin-point lesions arising with some strains in genotypes with dominant *I*.

Host group 7 comprises only IVT 7214, resistant to all strains.

The cultivars of group 8, the first group with *II*, were the only ones where systemic necrosis was induced by NL 8. Their systemic reaction with strains of IVb occurred only in a few plants in the temperature range 22-26 °C, but usually in all plants with the strains of group VI.

Jubila of host group 9a gave systemic necrosis with strains of groups IVb, Vb and VI. At first glance, host group 9b resembles group 9a in that systemic necrosis was induced by the strains of groups IVb, Vb and VI. However, the systemic reaction induced by strains of groups IVb and Vb was dependent on temperature, contrary to that in group 9a. After inoculation with NL 2, only a few plants reacted systemically in the range 22-26 °C.

Group 10 represented by Amanda was needed to distinguish NL 3 and NL 5. Finally, IVT 7233 of group 11 was the only genotype with *II* not showing any systemic necrosis with all strains available.

4.4.2 Pathogenicity groups of the virus strains

The virus strains were classed into seven groups. These groups could be distinguished by the reactions induced in host groups 1 to 6.

The reactions of the differentials of host groups 8 to 10 further supported the above strain differentiation. However, some strains with the same pathogenicity spectrum for

host groups 1 to 6, differed in their spectrum for host groups 8 to 10 carrying *II* (for the definition of the term pathogenicity spectrum see Section 2.6). Thus we subdivided strain groups IV, V and VI. Subgroups b contain virus strains more able to induce systemic necrosis than subgroups a. The strains of IVa and Va induced no systemic necrosis at all in the range 17-30 °C. The difference between NL 3 and NL 5 was based on the systemic reaction of Amanda induced by NL 5.

Hence, groups were created by reaction of $I^{+}I^{+}$ differentials and subgroups by reaction of *II* differentials.

Systemic necrosis (s.n.) induced by strain groups IVb and Vb was dependent on temperature. At a constant temperature of 30 °C, the strains of these two subgroups usually induced s.n. in all plants of differentials that showed s.n. in only some plants at the lower mean temperatures mentioned in Table 6. Strain NL 3 of subgroup VIa did not generally induce temperature-dependent reactions, but overcame the resistance of Amanda present at 17-26 °C, inducing s.n. in all plants at 30 °C.

As the representatives of host groups 7 and 11 did not react systemically to any known strain, they were of no use in distinguishing strains. However they might be useful for detecting new strains.

Finally, the resistance spectra of the host groups and the pathogenicity spectra of the strain groups are shown in Table 7, mentioning the representative differential cultivars and virus strains used in the analysis of inheritance in this study.

4.5 Meaning and usage of the terms resistant/susceptible and tolerant/sensitive

When mosaic was inconspicuous, but systemic infection was detected, the differential was recorded as tolerant to the strain concerned (+t). Such plants were susceptible but had little or no sensitivity; they tolerate systemic infection by the pathogen. So two pairs of terms are used in this study to describe the reaction of the plant: resistant/susceptible and tolerant/sensitive. They describe complete different phenomena and are thus likely to be controlled by different genes.

Resistance and susceptibility refer to the ability of the plant to act as a host to the pathogen. Both terms indicate the difficulty or ease with which a pathogen becomes established in the inoculated leaves (local infection), as well as the ease with which the pathogen multiplies and spreads throughout the plant (systemic infection). Plants with some resistance to local infection may escape systemic infection. From a pathological aspect, such plants still have to be considered (locally) susceptible. Complete resistance means insusceptibility, usually called immunity: infection even at the sites of inoculation is excluded. The less resistant the plant, the greater its susceptibility. For tests, a practical delimitation is needed between resistance and susceptibility. In plant breeding and in this study, the term resistance to a virus is used to indicate absence of systemic infection and inability of the plant to act as a practical host.

Plants noted here as resistant may have had local infection (in the inoculated leaves), but that was not determined by back-inoculation. The frequent occurrence of local reaction (discolorations, necrosis) suggest a high incidence of local infection (Drijfhout & Bos, 1977), but absence of local infection (immunity) may have occurred too. Since local infection was not tested, all plants without systemic infection were rated as resistant.

Table 6. Differentiation and grouping of the strains of BCMV. + = susceptible, sensitive, systemic mosaic; +t = susceptible, tolerant, systemic symptoms questionable or very weak, virus recovered from uninoculated leaves by back-inoculation onto Dubbele Witte; – = resistant, no systemic symptoms, virus not recovered from uninoculated leaves by back-inoculation; +n = susceptible, sensitive, usually all plants with systemic necrosis, not clearly dependent on temperature; ±n = susceptible or resistant, dependent on temperature, from none to all but mostly only a few plants with systemic necrosis, the number varying in repeated tests and increasing with temperature. Greenhouse mean temperature 22-26 °C, day and night fluctuation at most 20-24°C in winter and 20-30 °C in summer.

Resistance group of the host	Differential cultivar	Pathogenicity group of the virus														
		I			II	III	IVa	IVb			Va	Vb	VIa	VIb	VII	
		Westlandia NL 1	Type US 1	Puerto Rico PR 1	NL 7	NL 8	Florida US 5	Western US 4	Idaho or B US 3	Colana NL 6	NY 15 US 2	Imuna NL 2	Michelite- NL 3	Jolanda NL 5	Mexico US 6	Great North. NL 4
Cultivars with recessive alleles (I'I') of the necrosis gene																
1	Dubbele Witte	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Str. Gr. Ref.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Redl. Gr. C	–	–	–	+	–	+	+	+	+	+t	+	+t	+	+	+
	Puregold Wax	–	–	–	+	–	+	+	+	+	+t	+	+t	+	+	+
	Imuna	–	–	–	+t	–	+	+	+	+	+t	+	+t	+	+	+
3	Redl. Gr. B	–	–	–	–	–	+	+	+	+	–	–	+	+	+	+
	Gr. North. 123	–	–	–	–	–	+	+	+	+	–	–	+t	+t	+	+
4	Sanilac	–	–	–	–	+	–	–	–	–	+	+	+	+	–	–
	Michelite 62	–	–	–	–	+	–	–	–	–	+	+	+	+	–	–
	Red Mex. 34	–	–	–	–	+	–	–	–	–	+	+	+	+	–	–
5	Pinto 114	–	–	–	–	–	–	–	–	–	+	+	+	+	–	–
6	Monroe	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+
	Gr. North. 31	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+
	Red Mex. 35	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+
7	IVT 7214	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Cultivars with dominant alleles (II) of the necrosis gene

8	Widusa	–	–	–	–	+n	–	±n	±n	±n	–	–	+n	+n	–	–
	Bl. Turtle S.	–	–	–	–	+n	–	±n	±n	±n	–	–	+n	+n	–	–
9a	Jubiła	–	–	–	–	–	–	+n	+n	+n	–	±n	+n	+n	–	–
9b	Topcrop	–	–	–	–	–	–	±n	±n	±n	–	±n	+n	+n	–	–
	Imp. Tendergr.	–	–	–	–	–	–	±n	±n	±n	–	±n	+n	+n	–	–
10	Amanda	–	–	–	–	–	–	–	–	–	–	–	–	+n	–	–
11	IVT 7233	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 7. Resistance spectra (rows) of the differentials and pathogenicity spectra (columns) of the strains, used in the analysis of inheritance in this study. See Table 6 for reaction symbols and temperatures.

Resistance group of the host	Differential cultivar	Pathogenicity group and virus strain									
		I	II	III	IV		V		VI		VII
		NI 1	NL 7	NL 8	a US 5	b NL 6	a US 2	b NL 2	a NL 3	b NL 5	NL 4
Cultivars with recessive alleles (I^+I^+) of the necrosis gene											
1	Dubbele Witte	+	+	+	+		+		+		+
2	Imuna	–	+	–	+		+		+		+
3	Redl. Gr. B	–	–	–	+		–		+		+
4	Michelite	–	–	+	–		+		+		–
5	Pinto 114	–	–	–	–		+		+		–
6	Gr. North. 31	–	–	–	–		–		–		+
7	IVT 7214	–	–	–	–		–		–		–
Cultivars with dominant alleles (II) of the necrosis gene											
8	Widusa	–	–	+n	–	±n	–	–	+n	+n	–
9a	Jubila	–	–	–	–	+n	–	+n	+n	+n	–
9b	Topcrop	–	–	–	–	±n	–	±n	+n	+n	–
10	Amanda	–	–	–	–	–	–	–	–	+n	–
11	IVT 7233	–	–	–	–	–	–	–	–	–	–

Tolerance and sensitivity indicate the severity with which the plant becomes diseased, the inability or ability to react visibly and to produce symptoms, to become damaged by systemic infection. Complete tolerance means total absence of disease symptoms, thus insensitivity. The higher the sensitivity, the lower the tolerance. A plant with such a high tolerance that no symptoms can be observed is called a symptomless carrier, but it is still susceptible. Severity of symptoms may also depend on virus concentration. If so, a low susceptibility rather than tolerance would cause mild symptoms.

In some of my tables, the susceptible plants carrying I^+I^+ recessive are subdivided into tolerant and sensitive ones. Sharp delimitation between the categories does not exist. In practical tests, susceptible plants with very mild, questionable, delayed or no symptoms and little or no damage were classed as tolerant and plants with moderate to severe symptoms and medium to heavy damage as sensitive.

I had no reason to distinguish between tolerant and sensitive plants carrying *I* dominant. Systemic virus spread was detectable only in plants with systemic necrosis (s.n.), but not in plants that did not react. This last group was therefore not considered as tolerant. Even in plants with s.n., it is not always easy to recover virus by back-inoculation, probably because of a low virus concentration, due to a restraining influence of dominant *I* on virus multiplication.

The question arises how to denote the plants not reacting with s.n. in a test with a strain that gives a temperature-dependent reaction in *II* cultivars, when at a given tem-

perature only some of the plants of a cultivar – which can be considered as a pure line – react with s.n. Since systemic virus spread could not be demonstrated by back-inoculation in the plants which did not react systemically, these plants were denoted as resistant at the temperature used. They mostly reacted systemically within a few days, if placed at a higher temperature.

This variable reaction of plants of a pure line at a given temperature seems confusing. In such plants local multiplication of virus is limited and small differences in virus concentration in the inoculated leaves are probable, with small differences in temperature, light and physiological condition of the leaves. In plants with a slightly better aspect (temperature and light) or in slightly better condition (a more appropriate physiological age of the inoculated leaves), virus may earlier reach a local concentration sufficiently high for systemic spread and subsequent systemic reaction than in plants with a slightly less favourable aspect or physiological condition. Higher temperatures stimulate virus multiplication. Then, more plants react systemically until a temperature is reached at which all plants show s.n., irrespective of aspect or physiological condition.

So in both *II* or I^+I^+genotypes, systemic virus spread, as shown by systemic symptoms or by back-inoculation, has been chosen as delimitation between resistance and susceptibility.

Local reactions were not recorded since they confused rather than helped in strain differentiation. I disagree with Alconero & Meiners (1974) who superseded terms like resistant and tolerant by seven other symbols, covering different types of local and systemic reactions. I found the terms resistant and tolerant very useful for tests, as long as terms and methods were clearly described. For the plant breeder too the terms are necessary to classify plants or lines as susceptible, tolerant or resistant.

4.6 Pathogenicity spectra of the strains compared with literature data

The reports of Richards & Burkholder (1943) and of Dean & Hungerford (1946) about differential reaction to NY 15 strain are in agreement with the present results (Table 6). They used representatives of host groups 1, 3, 4 and 9b.

Dean & Wilson (1959) compared Idaho strain with Type and NY 15 strains on a series of differentials with representatives of host groups 1, 2, 3, 4, 6 and 9b. NY 15 strain induced reactions in agreement with the present results, but cultivars from all these host groups were susceptible to their Idaho strain, whereas in my experiments only groups 1, 2, 3 and 9b were susceptible. They worked carefully, using young GN 123 plants from infected seed as virus source for Idaho strain, preventing contamination with Type and NY 15 strains to which GN 123 is resistant. In this manner they also excluded contamination with BYMV, since this is not seed-borne. Nevertheless their Idaho strain might have been contaminated with the later described Mexico strain, to explain the mosaic in GN 16 and GN 31 (host group 6). There is no satisfactory explanation for the mosaic obtained in Pinto 72, Pinto 78 and Pinto 111 and in RM 3 and RM 34, all being cultivars of host group 4, susceptible to NY 15 strain but not to strains of group IV.

Another difference for Idaho strain between my results and those of Dean & Wilson is the reaction of Improved Tendergreen, one of the cultivars they used from host group 9b, together with Idaho Refugee and Idaho Bountiful, carrying dominant *I*. Although the two

last-mentioned cultivars were resistant to the three strains, Improved Tendergreen was noted as susceptible to Type and Idaho strains, developing mosaic, but being resistant to NY 15 strain. In numerous trials, I never found cultivars with dominant *I* developing mosaic symptoms as a result of systemic infection, detected by back-inoculation. Seed samples of Improved Tendergreen from different sources were examined and in one I found a few plants with mosaic, but they were off-plants, not carrying *II* as demonstrated in the necrosis test. Also the original cv. Tendergreen was examined and found susceptible to all strains, carrying I^+I^+, but developing only weak mosaic symptoms with Type and NY 15 strains. A possible explanation for the deviant reaction of Improved Tendergreen, as found by the above-mentioned authors, might be that the seed lot they used was not genetically pure for necrosis gene *I*, or it was an Improved Tendergreen still with recessive I^+I^+ from the old Tendergreen.

Zaumeyer & Goth (1964) concluded that bean cultivars resistant to Type, NY 15 or Idaho strain, were also resistant to the new Florida strain. This agrees with my results for Idaho strain, but for observation of the difference in pathogenicity spectrum from Type and NY 15 strains, the differential range was incomplete as no representatives of host group 2 were mentioned, while I found that GN 123 of host group 3 is susceptible to Florida strain, but resistant to Type and NY 15 strains. They differentiated Florida strain from Idaho strain on the data mentioned by Dean & Wilson (1959).

Hubbeling (1963) distinguished Imuna, Michelite, Great Northern, and Westlandia strains on cultivars of host groups 1, 2, 3, 4, 8 and 9b, while group 6, important for recognition of Great Northern strain, was lacking. He found Imuna to be tolerant to the Westlandia strain. In my trials, Imuna was resistant to that strain. The tolerance of Imuna might be due to slight contamination of Westlandia strain. Imuna and GN 123 were reported resistant to Michelite strain, but in my experiments they were tolerant. Michelite and Sanilac were called tolerant and resistant, respectively, to Imuna strain, but I found them both sensitive to that strain. A low virus concentration might be the reason why that author did not find a sensitive reaction. He recorded Michelite and Sanilac as susceptible and RM 34 as tolerant to the Great Northern strain, but in my experiments these cultivars were resistant. The different results of Hubbeling may have been due to contamination of Great Northern strain, probably by Imuna strain. With Michelite strain, Widusa would have shown systemic necrosis.

Silbernagel (1969) described Mexico strain and found Michelite to be susceptible. Later Drijfhout et al. (1978) mentioned Michelite as resistant to that strain. The discrepancy may be due to use by Silbernagel of a cultivar not genetically pure for resistance to that virus.

Hubbeling (1972) described two more strains, Colana and Jolanda strains, adding RM 35 and GN 31 (host group 6) and Jubila (group 9a) to this differential range. There are many differences between the cultivar reactions mentioned in that publication and those in my Table 6. He classed GN 123 as resistant and RM 35 as tolerant to Michelite strain, whereas I found tolerance and resistance, respectively. Hubbeling classed Pinto 111, RM 34 and RM 35 as tolerant to Imuna strain and Puregold Wax as resistant, but I found Pinto 111, RM 34 and Puregold Wax susceptible and RM 35 resistant. He classed Topcrop as resistant and RM 35 as tolerant to the Colana strain, but I found it susceptible (varying number of plants with systemic necrosis) and resistant, respectively (Table 6). I never

found mild mosaic symptoms in Improved Tendergreen as recorded by Hubbeling with some strains. He classed RM 34 as tolerant to Great Northern strain and Jubila as showing varying systemic necrosis with this strain, whereas I found both resistant (Table 6). The discrepances might indicate strain contamination, as do the notations 'susceptible' for GN 31 and 'tolerant' for RM 35 to Jolanda strain. My investigations showed that distinction between Michelite and Jolanda strain cannot be based on the reaction of Jubila, as this cultivar also showed systemic necrosis with Michelite strain at 20-26 °C, though more slowly than with Jolanda strain. Amanda, however, gave systemic necrosis with Jolanda strain, but local necrosis with Michelite strain in that temperature range. I therefore added that cultivar to the range of differentials.

Alconero & Meiners (1974) reported GN 123, Pinto 111, Puregold Wax, RM 34 and Topcrop to become systemically infected with Type and Puerto Rico strains. This contrasts with my results, but they used other environmental conditions and a different infectivity test (local lesion assay on Monroe), making comparison difficult. They did not describe how Puerto Rico strain differed from Type strain.

Costa Rica and Peru strains described by Moreno et al. (1968) and Gamez et al. (1970) could not be obtained for direct comparison. Moreno et al. (1968) claimed the first one as a new strain, but did not demonstrate a difference in pathogenicity spectrum between 'Costa Rica strain' and Type and Florida strains. Costa Rica strain cannot be identified with certainty on the basis of that paper because of its incomplete range of differentials.

Gamez et al. (1970) reported Peru strain, which they compared with Costa Rica strain on test cultivars. None of the cultivars they used, that were resistant to Type strain, was attacked by Peru strain. Some cultivars susceptible to Type strain gave no reaction with Peru strain or with Costa Rica strain. Likewise some cultivars susceptible to Costa Rica strain gave no reaction with Peru strain. Cultivars susceptible to Type strain are, in my opinion, susceptible to all other strains. A low concentration of virus in inoculum may explain why cultivars susceptible to Type strain did not show mosaic symptoms. Costa Rica and Peru strains need further investigation.

4.7 Conclusions

There are several discrepancies between data from the literature and my data in Table 6, as are between some published data from different sources. Experience from my tests suggests the following possible causes of these discrepancies: (1) the unknowing but frequent use of an incomplete range of differentials; (2) the use of different ranges of differentials; (3) the use of a line of a cultivar with a deviant resistance spectrum and unreliable nomenclature of such lines; (4) genetic impurity of differential cultivars; (5) the use of seed samples of differentials already partially infected during field production of seed; (6) contamination of the virus strains used; (7) a low concentration of virus in inoculum; (8) different understandings of the terms 'resistant', 'tolerant' and 'susceptible'; (9) different ways of determining these properties, e.g. checking or not by back-inoculation; (10) tests at different temperatures; (11) differences in age of the test plants at inoculation.

Some measures have now been worked out by Drijfhout et al. (1978) for proper differentiation of strains, further to earlier recommendations by Bos (1971b).

The virus strains now known can be arranged into ten groups and subgroups each with a different pathogenicity spectrum. Strains with other pathogenicity spectra can be expected, likewise host groups with other resistance spectra than mentioned in Table 7 may be discovered, although testing of about 450 cultivars has not revealed more than the twelve resistance groups mentioned. Table 6 shows that some strains are identical, not only strains from different countries, but sometimes also from the same country. The latter is true for Idaho and Western strains, both from the United States, and for RM and Imuna strains both from the Netherlands (Drijfhout & Bos, 1977).

If strains are identical, we may consider them as isolates of the same strain, so Westlandia (NL 1) and Puerto Rico strains are merely isolates of the older Type strain, Western and Colana strains are isolates of Idaho strain, and Mexico strain is an isolate of Great Northern (NL 4) strain. With the present range of differentials, we can discern 10 strains instead of some 20 as described in literature.

There is no internationally accepted uniform system for nomenclature of virus strains. In the past, some strains were named after cities, others after states, countries or regions, again others after symptoms, and yet other strains after cultivars from which they were isolated. This last practice is often confusing, especially when the cultivars are also used for differentiation. I suggest to denote a virus strain, isolated and described in a given country, by the international two letter country code (ISO-S 3166, 1974), followed by a number in sequence of description of the strains of that virus in the country concerned. For example: RM strain was the second strain described in the Netherlands. It is now coded NL 2, or, if we also wish to indicate the virus, BCMV-NL 2.

This nomenclature has also been applied to races of *Bremia lactuacae* (Tjallingi & Rodenburg, 1967) and was proposed for international use for that fungus (Blok, 1973). Designation of a strain by country should be considered as preliminary. After international comparison of races or strains and a genetic study of the relation between genes for resistance and pathogenicity of host and pathogen, a gene code can be added to the designation of each genetically distinct race or strain, for the pathogenicity genes present. Such a proposal for international nomenclature was suggested for races of *Phytophthora infestans* (Black et al., 1953), of *Cladosporium fulvum* (Day, 1956) and for strains of tobacco mosaic virus (TMV) (Pelham, 1972). Gene codes for strains of BCMV will be discussed in Chapter 6.

5 Inheritance of resistance of plants with recessive alleles of the necrosis gene

5.1 Introduction

The results of the strain differentiation and classification (Table 7) show that twelve resistance spectra can be distinguished, one for each host group. Seven spectra belong to differential cultivars with recessive alleles of necrosis gene *I* (I^+I^+ differentials) and five to differentials with dominant alleles of *I* (*II* differentials). We may expect that different genes for resistance underlie the different resistance spectra. Likely more genes are involved than the resistance genes *a* and *s* and the necrosis gene *I* (Petersen, 1958), as three genes maximally result in 2^3 different homozygous genotypes, which is less than the twelve found.

The genes for resistance present in the I^+I^+ differentials or at least some of them may also be expected in the *II* differentials, to which gene *I* can be added. Corbett Refugee, probably a mutant, was the first cultivar with *II* and was selected from Stringless Green Refugee, which carries I^+I^+ and does not show any resistance to BCMV (Pierce & Walker, 1933). All other cultivars with *II* derived their *I* alleles ultimately from Corbett Refugee. I first analyse the resistance genes of the I^+I^+ differentials and then, with the knowledge thus obtained, try to determine these genes in the *II* differentials (Chapter 7). The resistance genes are provisionally named after the cultivars in which they are discerned. More definitive gene symbols are given in Chapter 6.

The F_1 and F_2 of diallel crosses between I^+I^+ differentials were tested for resistance with several strains. The F_2 of the cross Michelite 62 x Great Northern UI 31 was inoculated with mixtures of strains, to see if by recombination a genotype could be selected with resistance to all strains, as was found in IVT 7214. This could be expected if the supplementary resistance of both cultivars (Table 7) were governed by different genes that were not allelic or completely linked. No mutual influence was observed in preliminary trials on the action of the components of the strain mixture, compared with their action in single-strain inoculation.

5.2 Crosses between differentials with recessive alleles of the necrosis gene

Diallel crosses were made between the I^+I^+ differentials Dubbele Witte, Imuna, Redlands Greenleaf B, Michelite 62, Great Northern UI 31 and IVT 7214, representing host groups 1, 2, 3, 4, 6 and 7, respectively, of Table 7. Until then Pinto UI 114 of host group 5 had shown the same resistance spectrum as Michelite 62 and therefore was considered to belong to host group 4. However, Pinto 114 and Michelite 62 could recently be distinguished with the later detected strain NL 8.

To obtain some information about the inheritance of the resistance of Pinto 114, it was crossed with Dubbele Witte and Great Northern 31. The incomplete representation of Pinto 114 in the diallel crosses did not hinder analysis of its resistance genes.

Some crosses were made with other differentials of the same host group: Puregold Wax x Michelite, Imuna x Monroe, RG-B x Monroe and Michelite x Monroe, to compare the F_1 test results with those of Imuna x Michelite, Imuna x GN 31, RG-B x GN 31 and Michelite x GN 31, respectively.

5.3 Testing of F_1 generation

The F_1 of the 17 crosses between the I^+I^+ differentials was tested with all ten strains of Table 7. Table 8 shows the results of tests with seven of them. The results of the tests with the strains US 5, US 2 and NL 3 (Table 7), which were exactly the same as those with NL 6 and NL 2 and NL 5, respectively, are omitted.

The symbols + and – are not used in Table 8 to indicate susceptibility and resistance as till now, but the letters S and R from the symbols shown in Table 5.

Besides the tests listed in Table 8, F_1 Puregold Wax x Michelite was tested with NL 1, Imuna x Monroe with NL 1 and NL 8, RG-B x Monroe with NL 1, NL 7 and NL 8 and Michelite x Monroe with NL 1, NL 7 and NL 6. As the F_1 of these crosses gave the same reaction as the F_1 of the crosses they were compared with, their results are not shown in Table 8.

Generally 20 F_1 plants were used for testing per strain: 10 plants of the cross shown in Table 8 and 10 of the reciprocal one. Some tests were repeated several times, especially if few or no mosaic symptoms were detected. Then the plants were indexed for systemic infection through back-inoculation onto Dubbele Witte.

From Table 8 the following is concluded:

1. Resistance to BCMV is recessive.
2. Local discoloration, induced by some strains in differentials of host group 6, is inherited recessively.
3. The F_1 of two cultivars, each resistant to a given strain, is sometimes also resistant to that strain (Imuna x RG-B, Imuna x GN 31, RG-B x GN 31, Michelite x GN 31, Michelite x IVT 7214, Pinto 114 x GN 31 and GN 31 x IVT 7214), but in other cases susceptible (Imuna x Michelite, RG-B x Michelite, GN 31 x IVT 7214, Imuna x IVT 7214 and RG-B x IVT 7214), although sometimes difficult to detect if tested with NL 1 as in the last two crosses.

Imuna, RG-B and GN 31 must have either a gene in common or allelic resistance genes. The same applies to Michelite, GN 31 and IVT 7214, and to Pinto 114 and GN 31. Each of the couples Imuna and Michelite, Imuna and IVT 7214, RG-B and Michelite, RG-B and IVT 7214 and GN 31 and IVT 7214 must have recessive resistance genes at different loci.

4. F_1 GN 31 x IVT 7214 is resistant to some strains (NL 1, NL 7 and NL 6), to which both parents are also resistant, but susceptible to other strains (NL 8, NL 2, NL 5), that cannot infect the parents. These cultivars must have a resistance gene in common or two allelic genes as well as genes at different loci. This means that either both or one of these two differentials must have at least two genes for resistance.

Table 8. Testing F_1 of crosses in both directions between *I**/*I** differentials with strains of all pathogenicity groups. S = Susceptible, systemic mosaic, or systemic infection detectable by back-inoculation. (S) = Susceptible, systemic infection detected by back-inoculation in some experiments only. R = Resistant, no symptoms, no systemic infection detectable. Rd = Resistant, local discoloration, no systemic infection detectable.

Cross P_1		P_2	Pathogenicity group and strain																				
			I Nl 1			II NL 7			III NL 8			IV NL 6			V NL 2			VI NL 5			VII NL 4		
			P_1	P_2	F_1	P_1	P_2	F_1	P_1	P_2	F_1	P_1	P_2	F_1	P_1	P_2	F_1	P_1	P_2	F_1	P_1	P_2	F_1
1 DW	x	Imuna	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
2 DW	x	RG-B	S	R	S	S	R	S	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S
3 DW	x	Michelite	S	R	S	S	R	S	S	S	S	S	R	S	S	S	S	S	S	S	S	R	S
4 DW	x	Pinto 114	S	R	S	S	R	S	S	R	S	S	R	S	S	S	S	S	S	S	S	R	S
5 DW	x	GN 31	S	Rd	S	S	Rd	S	S	R	S	S	Rd	S	S	Rd	S	S	R	S	S	S	S
6 DW	x	IVT 214	S	R	S	S	R	S	S	R	S	S	R	S	S	R	S	S	R	S	S	R	S
7 Imuna	x	RG-B	R	R	R	S	R	S	R	R	R	S	S	S	S	R	S	S	S	S	S	S	S
8 Imuna	x	Michelite	R	R	S	S	R	S	R	S	S	S	R	S	S	S	S	S	S	S	S	R	S
9 Imuna	x	GN 31	R	Rd	R	S	Rd	S	R	R	R	S	Rd	S	S	Rd	S	S	R	S	S	S	S
10 Imuna	x	IVT 7214	R	R	(S)	S	R	S	R	R	S	S	R	S	S	R	S	S	R	S	S	R	S
11 RG-B	x	Michelite	R	R	S	R	R	S	R	S	S	S	R	S	R	S	S	S	S	S	S	R	S
12 RG-B	x	GN 31	R	Rd	R	R	Rd	R	R	R	R	S	Rd	S	R	Rd	R	S	R	S	S	S	S
13 RG-B	x	IVT 7214	R	R	(S)	R	R	S	R	R	S	S	R	S	R	R	S	S	R	S	S	R	S
14 Michelite	x	GN 31	R	Rd	R	R	Rd	R	S	R	S	R	Rd	R	S	Rd	S	S	R	S	R	S	S
15 Michelite	x	IVT 7214	R	R	R	R	R	R	S	R	S	R	R	R	S	R	S	S	R	S	R	R	R
16 Pinto 114	x	GN 31	R	Rd	R	R	Rd	R	R	Rd	R	R	Rd	R	S	Rd	S	S	Rd	S	R	S	S
17 GN 31	x	IVT 7214	Rd	R	R	Rd	R	R	R	R	S	Rd	R	R	Rd	R	S	R	R	S	S	R	S

5.4 Testing of F_2 generation

To test the F_2 of the seventeen crosses, a strain of each group or subgroup was used. Only a few tests were done with the strains US 5, US 2 and NL 3 of the subgroups 'a' (Table 7) to check whether the segregation ratios found with these strains were the same as those obtained with their 'parallel' strains of the subgroups 'b'.

Not every F_2 progeny was tested with all remaining seven strains, as I expected in general sufficient information from testing the F_2 progenies of three or four different crosses per differential with one strain instead of the F_2 progenies of all five or six crosses available. An exception was Pinto 114, which was crossed with only two other I^+I^+ differentials, so that the F_2 progenies of no more than two crosses with this cultivar could be tested with one strain. Testing of progenies with strains to which one or both parents are resistant gives more information than testing with a strain to which both parents are susceptible. Therefore strains NL 5 and NL 4 were used less often than strains NL 1, NL 7, NL 8, NL 6 and NL 2, as is shown in Table 9, presenting the scheme of the F_2 tests.

The number of tests (not including the repetitions) of the F_2 of the different crosses with each differential is shown in Table 10. The numbers were reasonably spread over the strains, except over US 5, US 2 and NL 3 as already mentioned. The number of tests of the F_2 of crosses with Pinto 114 was considerably lower because of the small number of crosses with that cultivar, while the number of tests of the F_2 of crosses with IVT 7214 was somewhat lower on account of shortage of F_2 seed of the crosses with Imuna and GN 31.

The first part of an F_2 test was done as described in Section 3.4.1 (General test). The plants without mosaic were indexed for infection by back-inoculation onto Dubbele Witte (Infectivity test, Section 3.4.3). Sometimes seeds were harvested from the not systemically infected plants, after which the F_3 progenies were tested with the same strain (F_3 test, Section 3.4.4) to determine whether the F_2 plant had been resistant or had escaped infection. This F_3 test was only done if there were no more than seventy F_2 plants to be tested, otherwise it would be too laborious. Thereafter the final segregation ratios could be determined. The results of the virus tests and their statistical evaluation are presented in the following section.

In Tables 12-18 the number of plants in each test is given minus the number of cripple plants observed. In all F_2 populations from crosses involving Imuna or RG-B, cripple plants occurred, of which only a part could be recognized and removed before inoculation. The degree of crippling varied considerably from yellowing and wilting of young seedlings with puckered primary leaves, the plants being smaller than normal, to leaf crumpling of plants of about normal size. The difference between leafroll and mosaic symptoms of BCMV-infected normal plants and the crumpling and puckering of cripple plants was mostly sufficiently clear, but for a few plants classification into normal and cripple plants was rather difficult.

No cripple plants were found in the F_1 generation, while in the F_2 mostly about 20% of the plants were cripples, pointing to two genes with epistatic interaction (13:3 ratio). Because of the large variation in cripple expression, minor genes may also be involved.

In some tests as many inoculated cripple plants as possible were indexed for virus infection by back-inoculation onto Dubbele Witte (infectivity test), to determine the

*Table 9. Scheme of tests of F_2 progenies of the crosses between I*I* differentials.*

F_2 progenies tested			Pathogenicity group and strain										Number of strains used per F_2 progeny
			I NL 1	II NL 7	III NL 8	IVa US 5	IVb NL 6	Va US 2	Vb NL 2	VIa NL 3	VIb NL 5	VII NL 4	
1 DW	x	Imuna	x[1]	x	x				x		x	x	6
2 DW	x	RG-B	x	x	x	x	x	x	x		x	x	7 + 2
3 DW	x	Michelite	x	x	x		x		x	x	x	x	7 +1
4 DW	x	Pinto 114	x	x	x				x		x	x	6
5 DW	x	GN 31	x	x	x		x		x	x	x	x	7 + 1
6 DW	x	IVT 7214	x	x			x				x	x	5
7 Imuna	x	RG-B	x	x	x		x		x		x	x	7
8 Imuna	x	Michelite	x	x	x	x	x				x	x	6 + 1
9 Imuna	x	GN 31	x	x	x		x	x	x				5 + 1
10 Imuna	x	IVT 7214	x		x		x					x	4
11 RG-B	x	Michelite	x	x	x		x	x	x				5 + 1
12 RG-B	x	GN 31	x	x	x	x	x		x		x	x	7 + 1
13 RG-B	x	IVT 7214	x	x	x		x		x			x	6
14 Michelite	x	GN 31	x	x	x		x		x	x	x	x	7 + 1
15 Michelite	x	IVT 7214	x	x			x		x		x	x	6
16 Pinto 114	x	GN 31		x	x		x		x				4
17 GN 31	x	IVT 7214			x		x		x		x		4
Total no. of F_2 progenies tested			15	15	15	3	15	3	14	3	12	13	99+9

1. x = F_2 progeny of cross mentioned in row tested with strain noted in column.

Table 10. Number of tests per strain of F_2 progenies of different crosses with each I^+I^+ differential.

Cross	Virus strain										
	NL 1	NL 7	NL 8	US 5	NL 6	US 2	NL 2	NL 3	NL 5	NL 4	Total
Dubbele Witte x ...[1]....	6	6	5	1	4	1	5	2	6	6	42
Imuna x	5	4	5	1	4	1	3	0	3	4	30
Redl. Gr. B x	5	5	5	2	5	2	5	0	3	4	36
Michelite x	5	5	4	1	5	1	4	2	4	4	35
Pinto 114 x	1	2	2	0	1	0	2	0	1	1	10
Gr. North 31 x	4	5	6	1	5	1	6	2	4	3	37
IVT 7214 x	4	3	3	0	5	0	4	0	3	4	26

1. The names of the other parents of the crosses tested with each strain can be found in Table 9.

ratio between susceptible and resistant cripple plants. The impression was gained that in cripples this ratio was the same as in normal plants of the F_2 of the same cross. But in all cases a number of the cripple plants was no longer available for infectivity test or F_3 test because of premature death or low fertility. So cripples were as much as possible excluded from the determination of the segregation ratios susceptible to resistant plants in the F_2.

5.4.1 Results of tests with individual strains

The segregation ratios of all F_2 tests are presented in Table 11. The results per cross are shown in Tables 12-28, which give the number of plants tested with each strain, the numbers of susceptible and resistant plants, the appropriate segregation ratios according to graphs drawn on binomial probability paper (Ferguson, 1956), and the Probability(P)-values of the chi-square tests of these ratios.

Let us first consider crosses 1-6 (Table 11), where Dubbele Witte is one of the parents. Dubbele Witte is susceptible to all strains studied, so we may assume that no genes for resistance are present. NL 1, the 'type' strain of BCMV, can infect only cultivars of host group 1, which have no resistance genes. So in testing F_2 of crosses with NL 1, all resistance genes present contribute to the observed segregation, except in allelism or complete linkage. The ratios of the tests of F_2 of crosses 1-6 with NL 1 therefore well reflect the number of resistance genes present in the second parent.

A 15:1 ratio was found in F_2 of crosses 1, 2 and 3, suggesting two complementary recessive genes for resistance. Resistance occurs only if both recessive alleles of both genes are present.

In testing F_2 of crosses 4, 5 and 6 (Table 11) with NL 1 a 57:7 ratio was found, indicating three genes for resistance. This ratio can be explained by assuming that one gene is complementary to the other two.

Apparently the differentials Imuna, RG-B and Michelite each have at least two complementary recessive genes for resistance and Pinto 114, GN 31 and IVT 7214 each at least three recessive genes, one of them being complementary to the others. In testing F_2

Table 11. Suggested ratios susceptible to resistant (S:R) in testing F_2 of crosses between I^+I^+ differentials with strains of all pathogenicity groups. n.t. = not tested.

Cross			Pathogenicity group and strain						
			I NL 1 S:R	II NL 7 S:R	III NL 8 S:R	IV NL 6; US 5 S:R	V NL 2;US 2 S:R	VI NL 5;NL 3 S:R	VII NL 4 S:R
1 DW	x	Imuna	15:1	1:0	15:1	n.t.	1:0	1:0	1:0
2 DW	x	RG-B	15:1	15:1;15:1	15:1	1:0;1:0	15:1;15:1	1:0	1:0
3 DW	x	Michelite	15:1	15:1	1:0	15:1	1:0	1:0;1:0	15:1;15:1
4 DW	x	Pinto 114	57:7	15:1	15:1	n.t.	1:0	1:0	15:1
5 DW	x	GN 31	57:7	>57:7<15:1;57:7[1]	>57:7<15:1[2]	15:1	57:7;57:7	15:1;15:1	1:0
6 DW	x	IVT 7214	57:7	57:7	n.t.	57:7	n.t.	15:1	57:7
7 Imuna	x	RG-B	0:1	3:1	0:1	1:0;1:0	3:1	1:0	1:0
8 Imuna	x	Michelite	9:7	3:1	3:1	3:1;3:1	n.t.	1:0	3:1
9 Imuna	x	GN 31	0:1	>9:7<3:1	0:1	3:1	9:7;9:7	n.t.	n.t.
10 Imuna	x	IVT 7214	19:45;19:45	n.t.	9:7	>9:7<3:1;9:7	n.t.	n.t.	9:7
11 RG-B	x	Michelite	>9:7<3:1	9:7	3:1;3:1	3:1	3:1;3:1	n.t.	n.t.
12 RG-B	x	GN 31	0:1	0:1;0:1	0:1	3:1;3:1	0:1	3:1	1:0
13 RG-B	x	IVT 7214	19:45;19:45	27:37;27:37	9:7;9:7	>9:7<3:1;9:7	9:7	n.t.	9:7
14 Michelite	x	GN 31	0:1;0:1	0:1	27:37;9:7	0:1	9:7	3:1;3:1	3:1;3:1
15 Michelite	x	IVT 7214	0:1	0:1	n.t.	0:1	3:1	3:1	0:1
16 Pinto 114	x	GN 31	n.t.	0:1	0:1	0:1	9:7	n.t.	n.t.
17 GN 31	x	IVT 7214	n.t.	n.t.	27:37	0:1	9:7;27:37	9:7	n.t.

1. Two tests with different results.
2. Suggested ratio lies between the two theoretic ratios mentioned.

of crosses 7-17, no ratios were found that would suggest complementary gene action. The results could be explained by a recessive gene, complementary to the other recessive resistance genes present, in all I^+I^+ differentials except Dubbele Witte, so that its action is seen only in crosses with that differential.

Turning back to F_2 of cross 1 (Table 12), a 15:1 ratio was found in the tests with strains NL 1 and NL 8, to which Imuna is resistant. All plants were susceptible in the tests with the other strains. I consider one of the two resistance genes of Imuna as a strain-specific resistance gene, which I provisionally denote as 'Imuna' gene.

F_2 of cross 2 (Table 13) gave a 15:1 ratio in tests with NL 1, NL 7, NL 8 and NL 2 (or

Table 12. Testing F_2 of cross 1, Dubbele Witte x Imuna. Number of plants is number of inoculated normal plants; cripple plants were excluded. Suggesting ratio is according to segregation graphs of theoretically possible ratios drawn on binomial probability paper. The column for ratio 2 (necessary in following tables) is for a second suggested ratio if the observed segregation lies within the significance areas of two theoretical ratios (suggested ratios = theoretical ratios) or it gives the second theoretical ratio if the observed segregation lies between the significance areas of two theoretical ratios (suggested ratio ≠ theoretical ratios). At P 0.05, the deviation of the obtained results from the theoretical ratio is significant (*) and at P 0.01 highly significant (**).

Virus strain	Number of plants	Segregation		Suggested ratio(s) S:R		P-value χ^2 test	
		S	R	1	2	1	2
NL 1	268	250	18	15:1		0.75	
NL 7	154	154	0	1:0		1.00	
NL 8	311	287	24	15:1		0.29	
NL 2	304	304	0	1:0		1.00	
NL 5	306	306	0	1:0		1.00	
NL 4	121	121	0	1:0		1.00	

Table 13. Testing F_2 of cross 2, Dubbele Witte x Redlands Greenleaf B. Details as in Table 12.

Virus strain	Number of plants	Segregation		Suggested ratio S:R		P-value χ^2 test	
		S	R	1	2	1	2
NL 1	726	687	39	15:1		0.33	
NL 7	294	268	26	15:1	57:7	0.07	0.25
NL 7	495	456	39	15:1		0.13	
NL 8	1002	930	72	15:1		0.22	
NL 6	142	142	0	1:0		1.00	
US 5	303	303	0	1:0		1.00	
NL 2	508	471	37	15:1		0.36	
US 2	296	270	26	15:1	57:7	0.07	0.24
NL 5	112	112	0	1:0		1.00	
NL 4	114	114	0	1:0		1.00	

US 2), the strains to which RG-B is resistant. No resistant plants were found in the test with NL 6. The same result could be expected if F_2 of cross 2 were screened with NL 5 or NL 4. In RG-B too, I assume two complementary recessive genes for resistance. One at least must be different from that in Imuna, because RG-B is resistant to more strains than Imuna. I consider one of the resistance gens of RG-B as a strain-specific resistance gene, different from the 'Imuna' gene, which I provisionally denote as 'RG-B' gene.

In F_2 of cross 3 (Table 14), a 15:1 ratio was found in tests with NL 1, NL 7, NL 6 and NL 4, to which Michelite is resistant. Screening this F_2 with NL 8, NL 2 and NL 5 (or NL 3) gave no resistant plants since Michelite is susceptible to these strains. These results can be explained by assuming that Michelite has two complementary recessive resistance genes, at least one being different from those of Imuna and RG-B, because the resistance was overcome by another range of strains than the resistance of Imuna and RG-B. One of these genes is denoted 'Michelite' gene and is considered as a strain-specific resistance gene.

In testing F_2 of cross 4 (Table 15) with NL 1, a 57:7 ratio was found, suggesting three recessive resistance genes, of which one is complementary to the others. Testing with NL 7 resulted in a 15:1 ratio, so one of the resistance genes of Pinto 114 was not effective against that strain. The tests on F_2 of crosses 1, 2, 3, 5 and 6 with NL 7 in comparison with the tests with NL 1 (Table 11) show that only the genes of Imuna were ineffective

Table 14. Testing F_2 of cross 3, Dubbele Witte x Michelite 62.

Virus strain	Number of plants	Segregation		Suggested ratio S:R	*P*-value χ^2 test
		S	R		
NL 1	321	298	23	15:1	0.50
NL 7	290	272	18	15:1	0.98
NL 8	161	161	0	1:0	1.00
NL 6	323	304	19	15:1	0.79
NL 2	160	160	0	1:0	1.00
NL 5	322	322	0	1:0	1.00
NL 3	160	160	0	1:0	1.00
NL 4	356	337	19	15:1	0.48
NL 4	326	300	26	15:1	0.20

Table 15. Testing F_2 of cross 4, Dubbele Witte x Pinto UI 114.

Virus strain	Number of plants	Segregation		Suggested ratio S:R	*P*-value χ^2 test
		S	R		
NL 1	1299	1163	136	57:7	0.59
NL 7	330	306	24	15:1	0.44
NL 8	348	323	25	15:1	0.47
NL 2	180	180	0	1:0	1.00
NL 5	168	168	0	1:0	1.00
NL 4	642	608	34	15:1	0.32

against NL 7. Thus it is most likely that the strain-specific resistance gene of Pinto 114, ineffective in F_2 of cross 4 to NL 7, is the 'Imuna' gene. The second strain-specific resistance gene of Pinto 114 was ineffective against the strains NL 8, NL 2 and NL 5. It was ineffective against NL 8, since a 15:1 ratio was found with that strain, suggesting that one of the genes of Pinto 114 was ineffective against NL 8. As already shown, that gene cannot be the 'Imuna' gene, also present in Pinto 114. It was ineffective against the strains NL 2 and NL 5 because no resistant plants were found in testing F_2 with these strains. Considering the results of the tests of F_2 of crosses 2, 3 and 5 with strains NL 8, NL 2 and NL 5 (Table 11), only the 'Michelite' gene, present in cross 3, was ineffective against all three strains, as might be one of the genes of IVT 7214 in cross 6. Therefore, I assume that the second strain-specific resistance gene in Pinto 114 is the 'Michelite' gene. The effectiveness of that gene against NL 4, as shown in testing F_2 of cross 4, supports this supposition.

In F_2 of cross 5 (Table 16) a 57:7 ratio was found with NL 1, suggesting three independently inherited recessive genes in GN 31, as in Pinto 114, of which one is complementary to the others. The independent inheritance was not always observed: one of the tests with NL 7 and that with NL 8 gave segregations between two ratios, as in the screening of cross 9 with NL 7. Of the thirteen tests in Table 11, in which GN 31 was the only resistant parent and the segregation suggested either two recessive resistance genes or two genes and a complementary gene, ten tests suggest independent inheritance and three some linkage. Thus some linkage between two resistance genes of GN 31 might exist. GN 31 is only susceptible to strain NL 4. In the screening of cross 5 with that strain, no resistant plants were found. One of the genes of GN 31 is ineffective against NL 4 but governs resistance to NL 5 (15:1 ratio of cross 5 with NL 5). As the resistance genes in Imuna, Rg-B, Michelite and Pinto 114 are all ineffective against NL 5, one of the genes in GN 31 must be different from the genes in these differentials. It is named the 'GN 31' gene. The other strain-specific resistance gene of GN 31 is effective against NL 7, NL 8 and NL 2. Of the genes of Imuna, RG-B, Michelite and Pinto 114, only the 'RG-B' gene is effective against these three strains, so the second resistance gene of GN 31 could be the 'RG-B' gene.

Table 16. Testing F_2 of cross 5, Dubbele Witte x Great Northern UI 31.

Virus strain	Number of plants	Segregation		Suggested ratio S:R		*P*-value χ^2 test	
		S	R	1	2	1	2
NL 1	1310	1153	157	57:7		0.23	
NL 7	1323	1201	122	>57:7	<15:1	0.05	<0.01**
NL 7	377	327	50	57:7		0.15	
NL 8	1267	1158	109	>57:7	<15:1	<0.01**	<0.01**
NL 6	324	308	16	15:1		0.33	
NL 2	162	148	14	57:7	15:1	0.35	0.21
NL 2	700	609	91	57:7		0.09	
NL 5	338	322	16	15:1		0.25	
NL 3	328	310	18	15:1		0.57	
NL 4	185	185	0	1:0		1.00	

In screening cross 6 (Table 17) with NL 1, NL 7, NL 6 or NL 4 a 57:7 ratio was found, suggesting three recessive resistance genes, one complementary to the others. At least one of the resistance genes of IVT 7214 differs from those of Imuna, RG-B, Michelite or GN 31, because IVT 7214 is resistant to all strains. It is provisionally designated as '7214' gene. The other strain-specific resistance gene is ineffective to NL 5 (15:1 ratio), but confers resistance to NL 7, NL 6 and NL 4 (57:7). This could be the 'Michelite' gene (Table 14, cross 3). Testing F_2 of cross 6 with NL 8 or NL 2 would then give a 15:1 ratio. There is little evidence of linkage between the two strain-specific resistance genes of IVT 7214. In F_2 of cross 6, no linkage was found. In F_2 of cross 10 tested with NL 6, a segregation between 9:7 and 3:1 was observed, but in a retest a true 9:7 ratio was found. The same observation was made in testing cross 13 with NL 6. In eight of the ten tests of Table 11, in which IVT 7214 was the only resistant parent and the segregation gave evidence of two recessive resistance genes or of three genes, one complementary to the others, a true 9:7 or 57:7 ratio was found and in two tests a segregation between 9:7 and 3:1. If there is any linkage between the two strain-specific resistance genes of IVT 7214, it must be a weak one.

No susceptible plants were found in tests of F_2 of cross 7 (Table 18) with NL 1 or NL 8, to which both parents are resistant. Imuna and RG-B each have a strain-specific resistance gene, different from one another as concluded earlier. Thus either these genes

Table 17. Testing F_2 of cross 6, Dubbele Witte x IVT 7214.

Virus strain	Number of plants	Segregation		Suggested ratio S:R		*P*-value χ^2 test	
		S	R	1	2	1	2
NL 1	603	549	54	57:7		0.12	
NL 7	600	546	54	57:7		0.13	
NL 6	330	291	39	57:7		0.61	
NL 5	303	276	27	15:1	57:7	0.06	0.26
NL 4	289	249	40	57:7		0.11	

Table 18. Testing F_2 of cross 7, Imuna x Redlands Greenleaf B.

Virus strain	Number of plants	Segregation		Suggested ratio S:R	*P*-value χ^2 test
		S	R		
NL 1	167	0	167	0:1	1.00
NL 7	164	113	51	3:1	0.07
NL 8	146	0	146	0:1	1.00
NL 6	159	159	0	1:0	1.00
NL 6	157	157	0	1:0	1.00
NL 2	154	118	36	3:1	0.64
NL 5	159	159	0	1:0	1.00
NL 4	167	167	0	1:0	1.00

are allelic or RG-B carries the 'RG-B' gene and the 'Imuna' gene, and the two genes are strongly linked.

A 9:7 ratio was observed in testing F_2 of cross 8 (Table 19) with NL 1, suggesting independent inheritance of the 'Imuna' and 'Michelite' genes.

The 'Imuna' gene and one of the genes of GN 31, the 'RG-B' gene or the 'GN 31' gene, are allelic or GN 31 also has an 'Imuna' gene, strongly linked with one of the two other strain-specific genes, since no susceptible plants were found in the tests of F_2 of cross 9 (Table 20) with NL 1 and NL 8. According to the results of cross 7, this is the 'RG-B' gene of GN 31. Both genes of this cultivar cannot be allelic or strongly linked with the 'Imuna' gene, in view of the previous conclusion that the genes of GN 31 are inherited independently or may at most be weakly linked.

In two tests of F_2 of cross 10 (Table 21) with NL 1, the number of susceptible plants was too low to fit a 27:37 ratio. In one test the segregation fits a 81:175 ratio, but the number of susceptible plants in the other test was even too low for that ratio. That result does not agree with results reported so far, according to which three independent or weakly linked genes, the 'Imuna' gene of Imuna and the 'Michelite' and '7214' genes of IVT 7214, contribute to a segregation in this test with NL 1, which should result in a 27:37 ratio, or in one between 27:37 and 9:7, but not in an 81:175 ratio, which would suggest four genes. It was rather difficult to prove the susceptibility of the F_1 of cross 10 to NL 1. Systemic infection as detected by back-inoculation could only be demonstrated in a few of the tests.

Table 19. Testing F_2 of cross 8, Imuna x Michelite 62.

Virus strain	Number of plants	Segregation		Suggested ratio S:R	*P*-value χ^2 test
		S	R		
NL 1	522	305	217	9:7	0.32
NL 7	145	112	33	3:1	0.53
NL 8	158	121	37	3:1	0.65
NL 6	124	98	26	3:1	0.30
US 5	140	109	31	3:1	0.44
NL 5	125	125	0	1:0	1.00
NL 4	281	223	58	3:1	0.09

Table 20. Testing F_2 of cross 9, Imuna x Great Northern UI 31.

Virus strain	Number of plants	Segregation		Suggested ratio S:R		*P*-value χ^2 test	
		S	R	1	2	1	2
NL 1	123	0	123	0:1		1.00	
NL 7	145	95	50	>9:7	<3:1	0.02*	<0.01**
NL 8	130	0	130	0:1		1.00	
NL 6	259	200	59	3:1		0.39	
NL 2	1783	1039	744	9:7		0.09	
US 2	242	122	120	9:7		0.07	

Table 21. Testing F_2 of cross 10, Imuna x IVT 7214.

Virus strain	Number of plants	Segregation		Suggested ratio S:R		*P*-value χ^2 test	
		S	R	1	2	1	2
NL 1	494	153	341	19:45	81:175	0.53	0.75
NL 1	313	81	232	19:45	81:175	0.14	0.03*
NL 8	525	281	244	9:7		0.22	
NL 6	377	255	122	> 9:7	< 3:1	<0.01**	<0.01**
NL 6	248	148	100	9:7		0.31	
NL 4	299	156	143	9:7		0.16	

I suppose that the F_1 of this cross with three heterozygous recessive genes at different loci, each of them conferring resistance to strain NL 1 if in homozygous condition, would also give some resistance to this strain because of incomplete recessiveness of the genes and their cumulative effect. The first number in the ratio 27:37 stands for plants in which no homozygous recessive gene is present. Of these 27 plants, 8 are heterozygous for all three genes. If this last genotype confers a certain resistance to NL 1, the segregation will not fit 27:37 but 19:45 or between the two ratios. However, the ratios 81:175 and 19:45 are so close that if a segregation fits a 19:45 ratio, it mostly also fits a 81:175 ratio. But to explain the segregation, a 19:45 ratio is more appropriate than 81:175, because no other results (e.g. from F_2 DW x Imuna or F_2 DW x IVT 7214 tested with NL 1) confirm the presence of a second strain-specific resistance gene in Imuna or a third in IVT 7214. Because of the many healthy plants, no F_3 test was done. In testing F_2 of cross 10 with NL 8, a 9:7 segregation was found, suggesting no linkage between the 'Imuna' gene and the '7214' gene.

In the test of F_2 of cross 11 (Table 22) with NL 1, the segregation did not fit a 9:7 ratio but was between 9:7 and 3:1. This does not point to a linkage between the 'RG-B' and 'Michelite' genes, because in that case a segregation between 9:7 and 1:1 would be expected. The ratio 9:7 found in a test of cross 11 with NL 7 also suggests an inde-

Table 22. Testing F_2 of cross 11, Redlands Greenleaf B x Michelite 62.

Virus strain	Number of plants	Segregation		Suggested ratio S:R		*P*-value χ^2 test	
		S	R	1	2	1	2
NL 1	409	276	133	>9:7	<3:1	<0.01**	<0.01**
NL 7	126	73	53	9:7		0.70	
NL 8	152	116	36	3:1		0.71	
NL 8	2092	1540	552	3:1		0.14	
NL 6	138	111	27	3:1		0.14	
NL 2	113	89	24	3:1		0.36	
US 2	145	102	43	3:1		0.20	

pendent inheritance of both genes. The slightly excessive number of susceptible plants may have been caused by a slight contamination of strain NL 1.

No susceptible plants were found in tests of F_2 of cross 12 (Table 23) with NL 1, NL 7, NL 8 and NL 2, to which both parents are resistant, suggesting that GN 31 has also an 'RG-B' gene. The parents have a gene in common governing resistance to those strains. Strains NL 6 and NL 5 infect RG-B, against which the 'RG-B' gene of GN 31 is also ineffective, resulting in a 3:1 ratio based on the resistance of the 'GN 31' gene.

A 19:45 ratio was found in F_2 of cross 13 (Table 24) when tested with NL 1 and a 27:37 ratio when tested with NL 7, both as a result of the independent inheritance of the 'RG-B' gene and the two strain-specific resistance genes of IVT 7214. The explanation for the deviating ratio 19:45 obtained with NL 1 is the same as for this ratio in F_2 Imuna x IVT 7214 tested with NL 1. A 9:7 ratio was observed in tests with NL 8 and NL 2, which overcame the 'Michelite' gene of IVT 7214. The same ratio was found in tests with NL 6 and NL 4, to which RG-B is susceptible. Statistically the segregation of F_2 of cross 13 tested with NL 4 did not fit a 9:7 ratio, but it was much closer to this ratio than to 3:1 or

Table 23. Testing F_2 of cross 12, Redlands Greenleaf B x Great Northern UI 31.

Virus strain	Number of plants	Segregation		Suggested ratio S:R	*P*-value χ^2 test
		S	R		
NL 1	129	0	129	0:1	1.00
NL 7	113	0	113	0:1	1.00
NL 7	91	0	91	0:1	1.00
NL 8	149	0	149	0:1	1.00
NL 6	233	182	51	3:1	0.27
US 5	130	93	37	3:1	0.36
NL 2	162	0	162	0:1	1.00
NL 5	215	165	50	3:1	0.56
NL 4	155	155	0	1:0	1.00

Table 24. Testing F_2 of cross 13, Redlands Greenleaf B x IVT 7214.

Virus strain	Number of plants	Segregation		Suggested ratio S:R		*P*-value χ^2 test	
		S	R	1	2	1	2
NL 1	482	131	351	19:45	81:175	0.23	0.04*
NL 1	375	100	275	19:45	81:175	0.20	0.04*
NL 7	419	184	235	27:37		0.47	
NL 7	380	166	214	27:37		0.56	
NL 8	554	286	268	9:7		0.03*	
NL 8	471	282	189	9:7		0.11	
NL 6	554	394	160	> 9:7	< 3:1	<0.01**	0.04*
NL 6	374	197	177	9:7		0.16	
NL 2	437	255	182	9:7		0.38	
NL 4	2204	1174	1030	9:7		<0.01**	

27:37. As there was no seed available for retesting, the 9:7 ratio is used in Table 11. In one test with NL 6, a segregation between 9:7 and 3:1 was also observed.

F_2 of cross 14 (Table 25) gave no susceptible plants in the tests with NL 1, NL 7 and NL 6, to which both parents are resistant. As three different recessive genes are involved, two of them must be allelic. GN 31 carries the 'RG-B' and 'GN 31' genes. The 'Michelite' and 'RG-B' genes are not allelic, so the 'Michelite' and 'GN 31' genes must form multiple alleles. The 9:7 ratio in the tests with NL 8 and NL 2 results from independant inheritance of the 'RG-B' and 'GN 31' genes of GN 31, because the 'Michelite' gene is ineffective against these strains. The 27:37 ratio observed in another test with NL 8 was probably due to a low virus concentration of the inoculum. A 3:1 ratio was found with NL 5, because the 'Michelite' gene was overcome by that strain as was the 'RG-B' gene of GN 31; a 3:1 ratio was obtained with NL 4 because GN 31 is susceptible to that strain.

In the tests of F_2 of cross 15 (Table 26) with NL 1, NL 7, NL 6 and NL 4, to which both parents are resistant, no susceptible plants were found. Presumably both parents have the 'Michelite' gene, preventing segregation of susceptible recombinants.

In the F_2 of cross 16 (Table 27), two pairs of allelic genes are probably involved: the

Table 25. Testing F_2 of cross 14, Michelite 62 x Great Northern UI 31.

Virus strain	Number of plants	Segregation		Suggested ratio S:R	*P*-value χ^2 test
		S	R		
NL 1	305	0	305	0:1	1.00
NL 1	577	0	577	0:1	1.00
NL 7	160	0	160	0:1	1.00
NL 8	641	245	396	27:37	0.04*
NL 8	487	290	197	9:7	0.14
NL 6	164	0	164	0:1	1.00
NL 2	334	209	125	9:7	0.02*
NL 5	337	254	83	3:1	0.88
NL 3	325	258	67	3:1	0.07
NL 3	168	136	32	3:1	0.08
NL 4	331	240	91	3:1	0.30
NL 4	162	122	40	3:1	0.93

Table 26. Testing F_2 of cross 15, Michelite 62 x IVT 7214.

Virus strain	Number of plants	Segregation		Suggested ratio S:R	*P*-value χ^2 test
		S	R		
NL 1	168	0	168	0:1	1.00
NL 7	638	0	638	0:1	1.00
NL 6	640	0	640	0:1	1.00
NL 2	250	176	74	3:1	0.09
NL 5	246	182	64	3:1	0.71
NL 4	644	0	644	0:1	1.00

Table 27. Testing F_2 of cross 16, Pinto UI 114 x Great Northern UI 31.

Virus strain	Number of plants	Segregation S	Segregation R	Suggested ratio S:R	*P*-value χ^2 test
NL 7	332	0	332	0:1	1.00
NL 8	174	0	174	0:1	1.00
NL 6	330	0	330	0:1	1.00
NL 2	350	184	166	9:7	0.17

'Imuna' gene of Pinto 114 with the 'RG-B' gene of GN 31 and the 'Michelite' gene of Pinto 114 with the 'GN 31' gene of GN 31. Hence no susceptible plants were found in tests with NL 7, NL 8 and NL 6, to which both parents are resistant. Screening with NL 2, to which Pinto 114 is susceptible, resulted in a 9:7 ratio based on the two strain-specific resistance genes of GN 31.

In testing F_2 of cross 17 (Table 28) with NL 8 or NL 2 a 27:37 ratio was found, which can be explained by the action of the two strain-specific resistance genes of GN 31 and the '7214' gene. The 'Michelite' gene of IVT 7214 was overcome by these strains. In another test of F_2 of cross 17 with NL 2, a 9:7 ratio was found, probably through contamination of NL 2. Also a 9:7 ratio was observed in a test of F_2 of cross 17 with NL 5. The 'RG-B' gene of GN 31 and the 'Michelite' gene of 7214 are ineffective against this strain. Some susceptible plants were found in the test of F_2 of cross 17 with NL 6, but too few to fit any predictable ratio. According to the conclusion that the 'GN 31' gene of GN 31 and the 'Michelite' gene of 7214 are allelic, no susceptible plants were expected. An incidental strain contamination could explain the small number of infected plants. No seed was left to repeat the test.

Identical results were obtained from testing F_2 of a cross with two strains from subgroups a and b (Table 7) of the same group: F_2 of crosses 2, 8 and 12 with US 5 (Florida) and NL 6 (Tables 13, 19 and 23), F_2 of crosses 2, 9 and 11 with US 2 (NY 15) and NL 2 (Tables 13, 20 and 22) and F_2 of crosses 3, 5 and 14 with NL 3 and NL 5 (Tables 14, 16 and 25). No different ratio was found in the F_2 of the same cross.

Table 28. Testing F_2 of cross 17, Great Northern UI 31 x IVT 7214.

Virus strain	Number of plants	Segregation S	Segregation R	Suggested ratio S:R	*P*-value χ^2 test
NL 8	644	251	393	27:37	0.10
NL 6	614	76	538		
NL 2	644	373	271	9:7	0.39
NL 2	332	149	183	27:37	0.32
NL 5	648	380	268	9:7	0.22

5.4.2 Results of testing F_2 Michelite x GN 31 with strain mixtures

Table 7 shows that a cross between Michelite and GN 31 allows selection of genotypes in the F_2 that combine the resistance of both cultivars, conferring resistance to all strains mentioned. This is only possible if the resistance genes of both differentials are present at different loci, i.e. if there are no allelic genes. By combining the resistances of both differentials in homozygous condition at different loci in one genotype, a resistance might be obtained comparable with that of IVT 7214, though probably based on other genes.

F_2 of cross 14, Michelite x GN 31 and its reciprocal cross were screened with the strain mixtures NL 2 + NL 4, NL 3 + NL 4 and NL 2 + NL 3 + NL 4. Strains NL 2 and NL 3 infect Michelite but not GN 31, and NL 4 attacks GN 31 but not Michelite. Presumably each of these strains cannot infect a genotype in which the homozygous recessive resistance genes of Michelite and GN 31 are combined. This assumption is justified because in a preliminary trial, in which the F_2 of cross 8, Imuna x Michelite, was tested with strain mixture NL 7 + NL 8, a 15:1 ratio was found, being the sum of the results with the individual strains (3:1 ratio; Table 19). Apparently the plants of the F_2 progeny, susceptible to one of the strains of the mixture, were infected as if they had been inoculated with that particular strain. The plants, however, in which the recessive resistance genes from both parents were homozygously combined, were resistant as they would have been after inoculation with the separate strains. Thus no strain interaction could be observed when a mixture of strains was used.

The results of the tests are summarized in Table 29. Of the 9550 plants tested, none was resistant. These results strongly support the conclusion that the 'Michelite' and 'GN 31' genes are allelic, because in testing almost 10 000 plants no plant was found that combined both resistance genes in a homozygous recessive way at separate loci, conferring resistance to these strains in a mixture.

Table 29. Testing F_2 Michelite x GN 31 with strain mixtures.

Strain mixture	Number of plants	Segregation		Ratio S:R	*P*-value χ^2 test
		S	R		
NL 2 + 4	330	330	0	1:0	1.00
NL 3 + 4	329	329	0	1:0	1.00
NL 3 + 4	320	320	0	1:0	1.00
NL 3 + 4	2047	2047	0	1:0	1.00
NL 3 + 4	1996	1996	0	1:0	1.00
NL 2 + 3 + 4	642	642	0	1:0	1.00
NL 2 + 3 + 4	623	623	0	1:0	1.00
NL 2 + 3 + 4	660	660	0	1:0	1.00
NL 2 + 3 + 4	571	571	0	1:0	1.00
NL 2 + 3 + 4	2032	2032	0	1:0	1.00
All tests	9550	9550	0	1:0	1.00

5.5 Discussion and conclusions

The test results show that Imuna, RG-B and Michelite each have at least two recessive complementary genes governing resistance and Pinto 114, GN 31 and IVT 7214 each at least three recessive genes, one of them being complementary to the others. One of the genes of Imuna, RG-B and Michelite and two of Pinto 114, GN 31 and IVT 7214 were designated as strain-specific, because resistance depends on the strain used. The strain-specific genes of Imuna, RG-B and Michelite and one of these genes of GN 31 and IVT 7214 proved to be different, whereas the two strain-specific genes of Pinto 114 and the second ones of GN 31 and IVT 7214 were recognized as occurring also in Imuna, RG-B and Michelite. So five strain-specific resistance genes were distinguished, which were provisionally denoted as 'Imuna', 'RG-B', 'Michelite', 'GN 31' and '7214' gene.

I assume that the second gene in Imuna, RG-B and Michelite and the third one in Pinto 114, GN 31 and IVT 7214, having a complementary action to the other genes present, are the same gene in all differentials investigated. Then this gene must not be strain-specific because it is only effective together with at least one strain-specific gene effective to the strain used. The effect of the strain-unspecific gene does not depend on the strain used but on the combination of the strain-specific gene(s) present and the attacking strain(s).

The tests of F_2 cross 7, Imuna x RG-B, show that the 'Imuna' gene and the 'RG-B' gene are allelic, or that RG-B carries both the 'RG-B' gene and the 'Imuna' gene and that the two genes are strongly linked, since no susceptible plants were found in tests with NL 1 or NL 8. Choice between the two explanations would require a virus strain that attacks RG-B but not Imuna. Such a strain would prove that the 'Imuna' gene is not present in RG-B, otherwise both differentials would have been susceptible. As long as no definite proof can be given, the first explanation, being the simplest and requiring the smallest number of genes, will be used for the construction of the genotype formulae in Chapter 6.

The same conclusion can be drawn for GN 31. If the 'RG-B' gene is allelic with the 'Imuna' gene, then GN 31 has the 'GN 31' and the 'RG-B' gene only. If not allelic, then also an 'Imuna' gene is present in that differential. Proof would require a virus strain attacking GN 31 but not Imuna. Tentatively I take the explanation of allelism as the most acceptable and consider GN 31 as having two strain-specific genes, 'RG-B' and 'GN 31'.

The situation with the 'Michelite' and 'GN 31' genes is different. These genes are allelic. Here the other explanation of also a 'Michelite' gene in GN 31 with strong linkage is not possible because strain NL 4 attacks GN 31 but not Michelite, while the latter has only one strain-specific gene. Thus GN 31 cannot carry the 'Michelite' gene, otherwise Michelite would also be susceptible to NL 4. A strong linkage between the 'Michelite' and 'GN 31' genes is also unlikely because nearly 10 000 plants were tested with strain mixtures and no resistant plant, i.e. no double-recessive recombinant was found. For the 'Michelite' and 'GN 31' genes, I can only conclude that the two genes are allelic.

It has not become clear why in testing F_2 of cross 13 a 19:45 ratio was found with NL 1 and a 27:37 ratio with NL 7, both as a result of the independent inheritance of the

'RG-B' gene and the two strain-specific resistance genes of IVT 7214. I explained the first ratio by assuming that the plants carrying all three genes in heterozygous condition exhibit a certain resistance to NL 1, because of incomplete recessiveness of these genes and their cumulative effect. This resistance was not found in plants having only two of these three genes in heterozygous condition, as in F_1 and F_2 of crosses 6, 8 and 11, so the cumulation to three heterozygous genes must give the effect. The question arises why this resistance was found only in testing with NL 1 and not with NL 7. Neither could a significant lower number of susceptible plants be found when testing F_2 of cross 17 with NL 8. It seems that the resistance of plants with three heterozygous strain-specific resistance genes at different loci only occurs in tests with NL 1. This resistance of triple heterozygous plants was found in F_1 and F_2 of crosses with IVT 7214. Perhaps the '7214' gene must be one of these genes to obtain this resistance. NL 1 differs from all other strains in apparently having no host-specific pathogenicity genes. It cannot attack a differential carrying any strain-specific resistance gene. It might be that this feature is responsible for the inability of NL 1 to overcome three recessive strain-specific resistance genes in heterozygous condition.

In all tests on F_2 of cross 14, Michelite x GN 31, either with NL 3 or NL 5, to which Michelite is susceptible but GN 31 resistant, or with NL 4, to which Michelite is resistant but GN 31 susceptible, a segregation ratio 3:1 was obtained. The results of other tests of this F_2 population show that the 'Michelite' gene and the 'GN 31' gene must be allelic. This 3:1 ratio indicates that a plant, carrying the recessive and allelic 'Michelite' and 'GN 31' genes, is susceptible to a strain of BCMV if one of the genes is ineffective against that strain. In other words: a recessive gene, ineffective against a certain strain and present with a recessive allelic gene that is effective against that strain, behaves like a dominant allele of the effective gene, making the latter ineffective. Resistance only exists if both recessive alleles of a strain-specific gene, effective against the attacking BCMV strain(s), are present, or if two such genes occur as alleles, in either case together with the recessive alleles of the strain-unspecific gene.

The results obtained will now be compared with the few published data. Ali (1950) tested F_2 Stringless Green Refugee x Robust with the 'Zaumeyer' strain, probably identical with Type strain or NL 1 (Zaumeyer & Thomas, 1948) and obtained a segregation fitting a 3:1 ratio. Stringless Green Refugee belongs to host group 1 and Robust to group 4 (Table 3). The results of this test are comparable with those of F_2 of cross 3, in which a 15:1 ratio was obtained. The resistance genotypes of Dubbele Witte and Stringless Green Refugee are apparently different. The latter probably has either only a strain-unspecific gene or only a strain-specific gene. Str. Green Refugee is in both cases susceptible to all strains but genetically different from Dubbele Witte. This may be the reason why Stringless Green Refugee has a lower sensitivity than Dubbele Witte, making the latter more suitable for back-inoculations. Apparently not all cultivars of host group 1 have the same resistance genotype, but a further subdivision of this group is difficult because the differences in reaction to virus infection are only quantitative and the difference in resistance genotype can only be recognized by testing F_2 of crosses with these cultivars.

Andersen & Down (1954) analysed F_2 Great Northern 31 x Michelite after inoculation with the 'variant' strain, also known as NY 15 strain, and obtained a 3:1 ratio. This is

not in line with my comparable test of F_2 of cross 14 with NL 2, in which a 9:7 ratio was obtained. The cause of this difference is difficult to establish, as Andersen & Down published only an abstract.

Petersen (1958) mentioned the following results after testing with strain Voldagsen: F_2 Saxa x Great Northern 15, 15:1; F_2 Bagnolais x GN 15, 3:1. The identity of strain Voldagsen and the resistance genotype of Bagnolais being not fully known, one cannot say which strain and cross we have to compare with. However, 15:1 and 3:1 ratios were found in my tests of the F_2 of different crosses and with several strains. Evidently, Saxa has no resistance genes, like Dubbele Witte, making a 15:1 ratio possible. My results are not contrary to those of Petersen.

Finally the conclusions concerning the genes governing resistance in the 'non-necrosis' differentials are summarized as follows:

1. Resistance is governed by recessive genes. One of these is strain-unspecific and complementary to a series of strain-specific genes. Resistance only occurs if the strain-unspecific gene is present together with at least one strain-specific gene for resistance that is effective to the virus strain involved. The strain-unspecific gene is present in recessive condition in all 'non-necrosis' differentials used in these crosses except in Dubbele Witte.
2. The differentials Imuna, RG-B, Michelite, GN 31 and IVT 7214 each have a different strain-specific gene for resistance besides the strain-unspecific gene.
3. Pinto 114, GN 31 and IVT 7214 each have two strain-specific genes for resistance besides the strain-unspecific gene. The 'Michelite' and the 'Imuna' gene are present in Pinto 114, GN 31 has the 'RG-B' gene as second strain-specific resistance gene and IVT 7214 the 'Michelite' gene.
4. The 'Imuna' gene and the 'RG-B' gene are either allelic or strongly linked. If the latter, RG-B and GN 31 also have the 'Imuna' gene. The 'Michelite' and 'GN 31' genes are allelic. If the 'Imuna' and 'RG-B' genes are also allelic, the five strain-specific resistance genes are situated at three loci.
5. The strain-specific genes at the three loci are inherited independently or there may be a weak linkage between them or between some of them. The inheritance of the strain-unspecific gene is independent of the strain-specific genes.

6 Genotypes for resistance and pathogenicity

6.1 Introduction

In Chapter 5, six recessive genes of bean were distinguished, one strain-unspecific, recessively present in all differentials except Dubbele Witte, and five strain-specific genes of which four govern resistance to different ranges of virus strains, while the fifth confers resistance to all known strains. The strain-unspecific gene is necessary for complete action of the strain-specific genes. The latter were temporarily designated by the name of the differential in which they showed their specific action.

In this Chapter, the genes are given symbols, and the genotypes of the differentials are presented in accordance with inheritance of resistance of the differentials to different strains, as demonstrated in the F_1 reaction and in segregation ratios of F_2.

The interactions between differentials and virus strains are explained by a gene-for-gene relationship between strain-specific genes for resistance of the differentials and pathogenicity genes of the virus strains. Extending this to a theoretically more complete gene-for-gene system allows prediction of further differential host-genotypes, still to be found or to be made by crossing, and not yet discovered virus genotypes (strains).

Finally suggestions are made about evolution of strains of BCMV, and selection of lacking differentials is outlined.

6.2 Genotype formulae: a gene-for-gene relationship

Petersen (1958) used the gene symbols *a* and *s* for the two genes governing resistance of Great Northern UI 15 to his strain Voldagsen. Dominant alleles of the two genes were present in Saxa, like Dubbele Witte susceptible to all strains. As these symbols were also used for other genes of bean (Bean Improvement Cooperative, Bean Germ Plasm Committee, 1965), they are not descriptive. Moreover, I found more genes involved in resistance to BCMV. Therefore, I propose the following six new gene symbols, replacing *a* and *s*:

bc-u (strain-)unspecific resistance gene, necessary for complete action of the following strain-specific genes;
bc-1 'Imuna' gene;
bc-1^2 'RG-B' gene;
bc-2 'Michelite' gene;
bc-2^2 'GN 31' gene;
bc-3 'IVT 7214' gene.

These symbols are in accordance with Comacho et al. (1977). The letters '*bc*' refer to bean common mosaic virus, to which the genes confer resistance. The abbreviation agrees with the earlier used gene symbol *By* for a gene conferring resistance to bean yellow mosaic virus (Schroeder & Provvidenti, 1968). The letter combination '*bc*' is proposed for

all six genes, because they all concern resistance to the same virus. The suffix '*-u*' denotes the (strain-) unspecific gene, while the suffixes '*-1*', '*-2*' and '*-3*' designate the three different loci for the strain-specific genes. The superscript '2' of *bc-1*^{2} and *bc-2*^{2} indicates that these genes are allelic with *bc-1* and *bc-2*, respectively. Permission to use these gene symbols was granted by the Bean Germ Plasm Committee (Dr D.H. Wallace, Chairman, Cornell University Department of Plant Breeding and Biometry, Ithaca, N.Y., United States, 1977-08-02, letter).

The resistance genotypes of the I^tI^t differentials are as noted in Table 30. Four conveniance, only one allele of each pair is mentioned. The dominant alleles of the four loci are designated by adding a superscript '+' to the symbols *bc-u*, *bc-1*, *bc-2* and *bc-3*. For completeness, recessive allele I^t of the necrosis gene is also added.

According to the theoretical model of Person (1959) for a gene-for-gene relationship between host and parasite, NL 1, the virus strain only able to attack the differential without genes for resistance, has no genes for pathogenicity corresponding with resistance genes of the host and is genotypically designated P0. Strain NL 7 overcame gene *bc-1* and carries pathogenicity gene P1. NL 4 overcame genes *bc-1*, *bc-1*^{2} and *bc-2*^{2}, and can be genotypically designated as P1.1^2.2^2 etc.

In accordance with this gene-for-gene model, the resistance of a differential to a given strain is determined by the presence of a resistance gene not overcome by a pathogenicity gene of the virus strain. The pathogen can only attack the host if it has pathogenicity genes corresponding with all genes for specific resistance of the host. Thus a resistance gene in the host confers resistance to all strains lacking the corresponding pathogenicity gene.

Elaborating this for the seven strains (one of each pathogenicity group), one strain has no pathogenicity gene at all, two strains could carry one gene, two strains two genes, and two strains three pathogenicity genes each (Table 30). With this system of four pathogenecity genes corresponding with four resistance genes, of which some are allelic, and a fifth resistance gene (*bc-3*) sofar without a corresponding P3 gene, the results of this study can be explained. The pathogenicity genes P1, P1^2 and P2 or P1, P1^2 and P2^2 are apparently not allelic, but allelism of P2 and P2^2 is not excluded.

6.3 Expected genotypes for resistance and pathogenicity

Not all differentials with the possible combinations between *bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2} and *bc-3* have been found, nor have the virus strains carrying all possible combinations between the supposed pathogenicity genes P1, P1^2, P2 and P2^2. We know that the allelic resistance genes *bc-1* and *bc-1*^{2}, and *bc-2* and *bc-2*^{2} cannot be combined in one genotype homozygous for these strain-specific genes. Thus the number of possible homozygous combinations will not be 2^5, as would be expected if all five genes had different loci, but 18 (Table 31).

Only 7 of 18 differential resistance genotypes have been found. Of the latter, 9 carry gene *bc-3* of IVT 7214. Existing combinations with *bc-3* other than IVT 7214 are unlikely, because IVT 7214 is not a commercial cultivar and this genotype may not have been used for breeding purposes. Of the 9 remaining resistance genotypes, 6 were recognized. This does not mean that the others do not exist. Two resistance genotypes cannot be recognized because of absence of corresponding virus strains.

Table 30. Genotypes for resistance (host) and pathogenicity (virus) with a gene-for-gene relationship between the strain-specific genes for resistance and the supposed pathogenicity genes. One allele of each pair of resistance genes is mentioned. Gene symbols are explained in the text (page 53). + = positive relationship resulting in systemic infection.

Differential cultivar	Resistance genes	Strain group, virus strain and pathogenicity gene code						
		I NL 1 P0	II NL 7 P1	III NL 8 P2	IV NL 6 or US 5 P1.1[3]	V NL 2 or US 2 P1.2	VI NL 5 or NL 3 P1.1[3].2	VII NL 4 P1.1[3].2[3]
DW	*bc-u*$^+$*bc-1*$^+$*bc-2*$^+$*bc-3*$^+$*1*$^+$	+	+	+	+	+	+	+
Imuna	*bc-u bc-1 bc-2*$^+$*bc-3*$^+$*1*$^+$	–	+	–	+	+	+	+
RG-B	*bc-u bc-1*^{3} *bc-2*$^+$*bc-3*$^+$*1*$^+$	–	–	–	+	–	+	+
Michelite	*bc-u bc-1*$^+$*bc-2 bc-3*$^+$*1*$^+$	–	–	+	–	+	+	–
Pinto 114	*bc-u bc-1 bc-2 bc-3*$^+$*1*$^+$	–	–	–	–	+	+	–
GN 31	*bc-u bc-1*^{3}*bc-2*^{3}*bc-3*$^+$*1*$^+$	–	–	–	–	–	–	+
IVT 7214	*bc-u bc-1*$^+$*bc-2 bc-3 1*$^+$	–	–	–	–	–	–	–

Table 31. Genotypes for resistance (host) and pathogenicity (virus), possible with the strain-specific resistance genes found and the supposed pathogenicity genes. The four pathogenicity genes are supposed to be present at different loci.

Possible differentials	Theoretical combinations of strain-specific genes[1]	Observed differentials	Possible virus strains with their pathogenicity genes and observed strains															Theoretical number of attacking strains
			V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	
				P1	P1²	P2	P2²	P1 P1²	P1 P2	P1 P2²	P1² P2	P1² P2²	P2 P2²	P1 P1² P2	P1 P1² P2²	P1 P2 P2²	P1² P2 P2²	P1 P1² P2 P2²	
			NL 1	NL 7		NL 8		NL 6 US 5	NL 2 US 2					NL 5 NL 3	NL 4				
H1		DW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16
H2	*bc-1*	Imuna	–	+	–	–	–	+	+	+	–	–	–	+	+	+	–	+	8
H3	*bc-1²*	RG-B	–	–	+	–	–	+	–	–	+	+	–	+	+	–	+	+	8
H4	*bc-2*	Michelite	–	–	–	+	–	–	+	–	+	–	+	+	–	+	+	+	8
H5	*bc-2²*		–	–	–	–	+	–	–	+	–	+	+	–	+	+	+	+	8
H6	*bc-3*		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
H7	*bc-1 bc-2*	Pinto 114	–	–	–	–	–	–	+	–	–	–	–	+	–	+	–	+	4
H8	*bc-1 bc-2²*		–	–	–	–	–	–	–	+	–	–	–	–	+	+	–	+	4
H9	*bc-1 bc-3*		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
H10	*bc-1² bc-2*	'X'	–	–	–	–	–	–	–	–	+	–	–	+	–	–	+	+	4
H11	*bc-1² bc-2²*	GN 31	–	–	–	–	–	–	–	–	–	+	–	–	+	–	+	+	4
H12	*bc-1² bc-3*		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
H13	*bc-2 bc-3*	IVT 7214	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
H14	*bc-2² bc-3*		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
H15	*bc-1 bc-2 bc-3*		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
H16	*bc-1 bc-2² bc-3*		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
H17	*bc-1² bc-2 bc-3*		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
H18	*bc-1² bc-2² bc-3*		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0

1. As long as *bc-1* and *bc-1²*, and *bc-2* and *bc-2²* are allelic.

Genotypes *bc-2*^{2} (H5) and *bc-1 bc-2*^{2} (H8) cannot be distinguished from *bc-1*^{2} *bc-2*^{2} (H11, GN 31) with strain P1.1^2.2^2 (NL 4). For that purpose, the theoretical strains P2^2 (V5) and P1.2^2 (V8) or P1^2.2^2 (V10) would be required. Thus, host group 6 (Tables 6 and 7) may comprise cultivars with *bc-2*^{2} or with *bc-1 bc-2*^{2} besides cultivars with genotype *bc-1*^{2} *bc-2*^{2} like GN 31. As long as the strains P2^2 and P1.2^2 or P1^2.2^2 have not been found, the recognition in host group 6 of genotypes different from *bc-1*^{2} *bc-2*^{2} is only possible by making test crosses and by screening their F_1 with appropriate virus strains.

Thus F_1 Imuna x Monroe was tested with NL 1 and NL 8, as was done with F_1 Imuna x GN 31. The F_1 of both crosses was resistant to these strains. Then Monroe, like Imuna, has a resistance gene at locus 1, either *bc-1* or *bc-1*^{2}, as well as *bc-2*^{2} at locus 2. F_1 RG-B x Monroe was tested with NL 1, NL 7 and NL 8, and also proved to be resistant, like F_1 RG-B x GN 31, while the F_1 of both crosses was susceptible to NL 6. These results suggest the presence of *bc-1*^{2} in Monroe, as in GN 31. Test crosses were only made with Monroe, not with other cultivars of host group 6. So there may be cultivars in this group with genotypes different from those of GN 31 and Monroe.

Only resistance genotype *bc-1*^{2} *bc-2* (H10) is left that could have been recognized. The resistance spectrum of this genotype differs from *bc-2* of Michelite (Table 30) in negative reactions (resistance) to NL 8 and NL 2. Only positive reactions can be expected with NL 3 and NL 5. I selected this genotype from the F_2 RG-B x Michelite (*bc-1*^{2}*/bc-1*^{2} x *bc-2/bc-2*) where the following segregation could be expected: 9 *bc-1*$^+$*/. bc-2*$^+$*/.* + 3 *bc-1*^{2}*/bc-1*^{2} *bc-2*$^+$*/.* + 3 *bc-1*$^+$*/. bc-2/bc-2* + 1 *bc-1*^{2}*/bc-1*^{2} *bc-2/bc-2*. In testing the F_2 of this cross with a mixture of strains NL 2 + NL 6 (P1.2 + P1.1^2), only the last mentioned host genotype is resistant (15S:1R). In this way, plants of the resistance genotype *bc-1*^{2}*/bc-1*^{2} *bc-2/bc-2* could be selected, that were resistant to NL 2 (P1.2) or NL 8 (P2), but susceptible to NL 3 or NL 5 (P1.1^2.2). A pure line of one of these plants provided the lacking differential H10, designated 'X'.

Resistance group 1 (Tables 6 and 7) is represented by DW, which has no genes for resistance. This group comprises cultivars susceptible to all known strains. However, other resistance genotypes besides that of DW may be found in this group. Cultivars without strain-specific genes for resistance but with strain-unspecific gene *bc-u* are susceptible to all strains and also belong to host group 1. Probably they are phenotypically not distinct from cultivars like DW in their severe symptom development with all strains. Cultivars with one or even more strain-specific genes for resistance but without the strain-unspecific gene are also susceptible to all strains and thus belong to resistance group 1. They will show only weak mosaic symptoms if infected with a strain, against which they have an effective gene.

I do not expect a large number of such genotypes in host group 1, because during selection for resistance to BCMV in segregating progenies they will have proved to be susceptible and will have been removed. However, some may have resulted from breeding programs in which no selection for BCMV resistance was practised. Among the large number of cultivars susceptible to all eight Dutch strains (Appendix), some were only

2. Assuming *bc-u* to be present in all existing and future differentials with *I*$^+$*I*$^+$ except DW, it is omitted for readability, unless presentation is wanted for a better understanding.

slightly susceptible to NL 1. These cultivars could have *bc-1* without *bc-u*. Proof could be obtained by making test crosses with Imuna and Michelite and testing the F_2 of the test crosses with NL 1.

The number of possible combinations between the four pathogenicity genes is more difficult to determine. P1, $P1^2$ and P2, and P1, $P1^2$ and $P2^2$ must be present at different loci, but there are no data about P2 and $P2^2$. Assuming that all pathogenicity genes are present at separate loci, 2^4 genotypes for pathogenicity would be possible (Table 31). A theoretical P3, corresponding with resistance gene *bc-3*, would lead to 2^5 genotypes for pathogenicity. However, if P2 and $P2^2$ should be allelic, only 12 combinations between the four known pathogenicity genes were possible.

Of the 16 possible virus strains listed in Table 31 (or 12 strains if P2 and $P2^2$ are allelic), only 7 have been found. I could not have found 4 of the strains because of missing resistance genotypes: V5 (indistinguishable from V1 because of lacking H5), V8 (indistinguishable from V2 because of lacking H5 and H8), V11 (indistinguishable from V4 because of lacking H5) and V14 (indistinguishable from V7 because of lacking H5 and H8). It may still be possible to find 5 strains, V3, V9, V10, V15 and V16, with the known differentials including H10. The isolation of V10 will make it possible to select resistance genotypes H5 and H8.

6.4 Possible evolution of strains of BCMV

Table 31 shows that 7 of the 16 BCMV strains, of which the genotypes form possible combinations between the supposed pathogenicity genes, have been found.

The strains are likely to evolve as indicated in Figure 14. Development along the line P1, $P1.1^2$ has made most progress. The development shown in Figure 14 seems to support a theory of step-by-step evolution to genotypes involving a larger number of genes. If P2 and $P2^2$ are not allelic, a strain may be found in future, in which the genes P1, $P1^2$, P2 and $P2^2$ are combined, attacking all cultivars with alleles I^+I^+ except the line IVT 7214. This strain might be a temperature-independent necrosis-inducing strain (Section 4.2), as

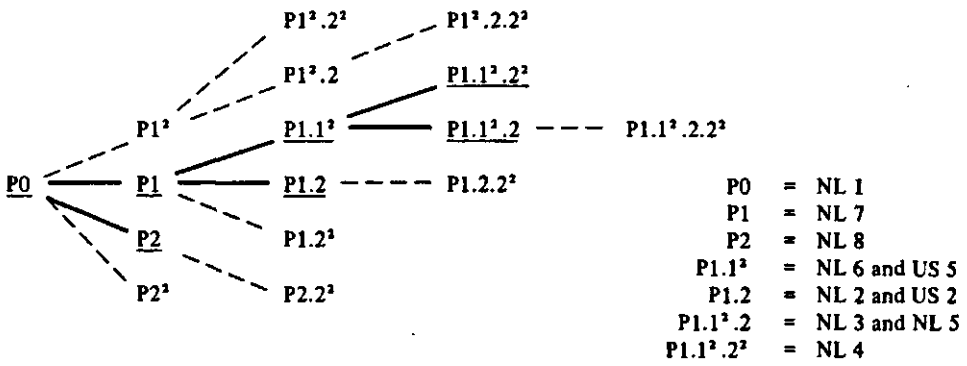


Fig. 14. Possible evolution of observed and expected strains of BCMV; the underlined strains have been found.

the genes P1, $P1^2$ and P2 are also combined in NL 3 and NL 5. Then differential IVT 7233 (shown in Section 7.2.5 to have the genotype *bc-u* $bc\text{-}1^2$ $bc\text{-}2^2$ *I*), showing only local pin-point lesions with the now known temperature-independent necrosis-inducing strains, would develop systemic necrosis, because gene $bc\text{-}2^2$ will then have been overcome by that strain.

6.5 Possible breeding of lacking differentials

It is theoretically possible to select the lacking differentials H5, H6, H8, H9, H12 and H14 to H18 (Table 31) from F_2 populations of crosses between differentials after testing the F_2 with an appropriate virus strain, test-crossing the resistant plants and testing the F_1 of the test crosses. The breeding method will now be outlined. It could be used for further research. Breeding of the missing resistance genotypes and their use for differentiation of virus isolates will considerably increase the chance of isolating the virus strains not yet found.

H5 ($bc\text{-}2^2$) could be bred by crossing DW ($bc\text{-}u^+$ $bc\text{-}1^+$ $bc\text{-}2^+$) and GN 31 (*bc-u* $bc\text{-}1^2$ $bc\text{-}2^2$) and testing the F_2 with a virus strain against which $bc\text{-}2^2$ is effective but not $bc\text{-}1^2$ (NL 3, NL 5 or NL 6). The F_2 segregates 60:4 for susceptible to resistant. The resistant plants with $bc\text{-}2^2/bc\text{-}2^2$ are 1 $bc\text{-}1^+/bc\text{-}1^+$ $bc\text{-}2^2/bc\text{-}2^2$ + 2 $bc\text{-}1^+/bc\text{-}1^2$ $bc\text{-}2^2/bc\text{-}2^2$ + 1 $bc\text{-}1^2/bc\text{-}1^2$ $bc\text{-}2^2/bc\text{-}2^2$. These plants are test-crossed with RG-B ($bc\text{-}1^2$ $bc\text{-}2^+$), resulting in the following F_1 genotypes: (a) $bc\text{-}1^+/bc\text{-}1^2$ $bc\text{-}2^+/bc\text{-}2^2$; (b) $bc\text{-}1^+/bc\text{-}1^2$ $bc\text{-}2^+/bc\text{-}2^2$ + $bc\text{-}1^2/bc\text{-}1^2$ $bc\text{-}2^+/bc\text{-}2^2$ and (c) $bc\text{-}1^2/bc\text{-}1^2$ $bc\text{-}2^+/bc\text{-}2^2$. In testing (a), (b) and (c) with a strain that has not overcome $bc\text{-}1^2$, (a) is susceptible, (b) segregates 1S:1R and (c) is resistant. The wanted H5 ($bc\text{-}1^+/bc\text{-}1^+$ $bc\text{-}2^2/bc\text{-}2^2$) is detected by test cross (a).

Similarly, differential H6 (*bc-3*) could be bred by crossing DW and IVT 7214 (*bc-2* *bc-3*), testing the F_2 with a strain against which *bc-3* is effective but *bc-2* is not, test-crossing the resistant plants with Michelite (*bc-2*) and testing that F_1 with a strain to which *bc-2* gives resistance.

Having obtained H5 and H6, one could select H8 in the F_2 of the cross between H5 and Imuna, H9 in F_2 of cross H6 x Imuna, H12 in F_2 of cross H6 x RG-B and H14 in F_2 of cross H6 x H5. The F_2 plants, resistant to the strain that has not overcome the resistance gene of the first parent are test-crossed with the second parent to detect the wanted genotype.

Breeding of differentials H15 to H18 is more complicated. For breeding of H15 (*bc-1bc-2 bc-3*), H6 (*bc-3*) is crossed with Pinto 114 (*bc-1 bc-2*) and the F_2 tested with a strain to which *bc-3* gives resistance but *bc-1* and *bc-2* do not. The resistant F_2 plants are test-crossed with the second parent. The resulting F_1 is separately tested with NL 7, to which *bc-2* gives resistance but *bc-1* does not, and with NL 8, to which *bc-1* gives resistance but *bc-2* not. The F_1 progeny resistant to both strains indicates the wanted genotype. It should be present in the orginal F_2 in the proportion 1:63, and the ratio susceptible to resistant in that generation should be 48:16. Of every 64 F_2 plants, 16 have to be test-crossed and their F_1 tested with two strains. Of these 16 F_1 progenies, 1 indicates the wanted genotype. Differential H17 can be bred similarly.

The breeding of H16 and H18 would be somewhat different. To obtain H16 ($bc\text{-}1$ $bc\text{-}2^2$ *bc-3*), crosses are made between H6 (*bc-3*) and H8 ($bc\text{-}1$ $bc\text{-}2^2$). The F_2 is tested

with NL 4 to select the plants with *bc-3/bc-3* (ratio 48:16 for susceptible to resistant) and the resistant plants are test-crossed with H5 (*bc-2²*), to detect the plants with *bc-2²/bc-2²*, as tested with NL 7. The F_2 plants thus selected for homozygosity of *bc-2²* and *bc-3* are then selected for double recessive *bc-1* by crossing the F_1 testcross plants resistant to NL 7 with Imuna (*bc-1*) and testing these F_1 plants with NL 1. The resistant plants indicate the F_2 plants of the original cross that were homozygous recessive for all three genes. Differential H18 would be bred similarly.

6.6 Discussion

Most gene-for-gene relationships described relate to fungi. A large number of genes is involved in some of them. A classic example is that between resistance genes of flax and pathogenicity genes of flax rust, *Melampsora lini* (Flor, 1956). Twenty-five dominant genes for resistance were reported to be present in flax cultivars at five loci. Examples of host-virus combinations with a suggested gene-for-gene relationship are rare.

Cockerham (1955) showed that strains of potato virus X (PVX) could be classed into four groups, which were differentiated with four potato cultivars involving two dominant genes for resistance. Another example of such a host-virus relationship was found between tomato and tobacco mosaic virus (TMV) (Pelham, 1972). Three genes for resistance were distinguished, *Tm-1*, *Tm-2* and *Tm-2²*, the last two being allelic, and four strains were identified, genotypically denoted 0, 1, 2 and 1.2. Rast (1975) mentioned strain 2^a(= 2^2) which was said to have overcome gene *Tm-2²*. The specific resistance genes against PVX and TMV are dominant, unlike those for BCMV. However, the specific resistance genes of pepper (*Capsicum annuum* L.) against potato virus Y are recessive and seem to be allelic, while dominant alleles, conditioning systemic necrosis, are present at another locus (Pochard, 1977).

Oligogenic specific resistance is usually determined by single dominant genes. Also in fungi there are examples of specific resistance determined by single recessive genes, like some resistance genes of various rusts in wheat, powdery mildew in peas and victoria blight in oats (Day, 1974). Resistance to BCMV in bean can now be added to this series.

The gene-for-gene system allows prediction of the strains of the pathogen that are to be expected on the basis of the known host genotypes for resistance, and reversely, the possible resistance genotypes based on the different strains isolated. Since the publication of the *Solanum-Phytophtora* system (Black et al., 1953), all races and cultivars predicted on that gene-for-gene basis were indeed found (Person, 1959). The general validity of that concept leads to the expectation of the existence, development or creation of the resistance genotypes and virus strains mentioned in Table 31 but not yet found.

7 Inheritance of resistance of plants with dominant necrosis gene

7.1 Introduction and crosses

After determining the resistance genes in differentials with I^+I^+, those in differentials with *II* were analysed. The same genes, or at least some of them, could be expected besides gene *I*, as the *II* differentials descend from I^+I^+ cultivars.

Each of the differentials Widusa, Jubila, Topcrop, Amanda and IVT 7233, representing resistance groups 8, 9a, 9b, 10 and 11, respectively, was crossed with Imuna, Michelite and GN 31, and the F_2 tested with appropriate strains. The resistance genes of the I^+I^+ parent and the pathogenicity genes of the virus strain being known, the resistance genes of the *II* parent could be determined from the segregation results of F_2.

It was not necessary to cross with representatives of all resistance groups with I^+I^+. The crosses with Imuna, Michelite and GN 31 gave sufficient information. These differentials carry genes *bc-1*, *bc-2* and *bc-1*^{2} plus *bc-2*^{2}, respectively, allowing homozygosity for each of the genes in F_2, if also present in the *II* parent. These genes can be identified through the seggregation ratios between susceptible and resistant I^+I^+ plants if tested with virus strains against which they are effective. The absence of *bc-3* or any other gene, not overcome by the known strains, was determined by testing the crosses involving GN 31 with NL 4 and those involving Michelite with NL 5. If no resistant plants with I^+I^+ segregated, only genes *bc-1*, *bc-1*^{2}, *bc-2* or *bc-2*^{2} could be present.

In analysis of resistance genes in *II* differentials, the segregation ratio in F_2 for susceptible and resistant plants carrying I^+I^+ ($S_{I^+I^+}$ and $R_{I^+I^+}$ plants) is important as well as the ratio between susceptible and resistant plants with *I*. ($S_{I.}$ and $R_{I.}$ plants). The first ratio is found with each strain, the second only if a 'necrosis-inducing' strain is used. Preferably a temperature-independent strain should be used, to avoid absence of systemic necrosis in plants that are genetically capable of that reaction in the host-strain combination concerned. Strain NL 5 was used to determine ratio $S_{I.}:R_{I.}$. As this strain overcame *bc-1*, *bc-1*^{2} and *bc-2*, the information from this ratio about strain-specific genes is limited.

Tests of F_1 are not presented. Genes for specific resistance are hypostatic to necrosis gene *I*, which prevents mosaic. Systemic necrosis may occur through presence of dominant allele *bc-u*$^+$, in spite of the homozygous condition of a specific resistance gene effective against the strain used. Absence of systemic necrosis, however, is not conclusive for homozygous presence of an effective gene for specific resistance and simultaneous absence of an allele *bc-u*$^+$, as systemic necrosis sometimes not only depends on temperature but also on genetic background (Sections 7.2.1-7.2.5). All this makes F_1 tests of crosses, of which *II* cultivars are one of the parents, of little value for the identification of genes for specific resistance.

Segregation ratios between plants with *I*. and I^+I^+ are also not presented. Published data

(Ali, 1950; Petersen, 1958) are sufficiently conclusive about the inheritance of gene *I*. Although in some of my tests the segregation between *I*. and I^+I^+ plants did not quite fit a 3:1 ratio, the total results gave no reason to doubt the existence of one gene *I*, acting against all strains.

7.2 Testing of F_2 generation

Strains NL 1, NL 2, NL 4 and NL 5 were used for testing the F_2 of the fifteen crosses and sometimes NL 3 and NL 6 for additional data. The crosses with GN 31 and those with Michelite were tested with NL 5 ($P1.1^2.2$) and NL 4 ($P1.1^2.2^2$) to detect *bc-2*^{2}, *bc-2* and *bc-u*. In testing the two crosses with NL 5, ratio S_I.:R_I. could also give information about the presence of *bc-u*. The crosses with Michelite were tested with NL 2 (P1.2) to detect the presence of *bc-1*^{2}, effective against that strain. Finally the crosses with Imuna were tested with NL 1 (P0) to detect the presence of *bc-1*, giving resistance to that strain.

In all tests with NL 4, NL 2 or NL 1, the plants without mosaic or obvious local discoloration or systemic necrosis (the last reaction being possible with NL 2) were subjected to the necrosis test to determine whether the resistant plants carried *I*. or I^+I^+. Then the I^+I^+ plants were subjected to the infectivity test for detection of (symptomless) systemic infection. The I^+I^+ plants not systemically infected were usually subjected to the F_3 test to determine whether plants had escaped infection.

Cripple plants were observed in F_2 of the crosses of Jubila and Topcrop with Michelite and GN 31, and removed as early as possible from the test to avoid confusion with virus infected plants. There was no indication for a linkage between genes for crippling and those for resistance or between genes for crippling and the necrosis gene.

7.2.1 Results of testing F_2 of crosses with Widusa

The results of the tests are shown in Table 32. The segregations obtained were used to determine the suggested S:R ($S_{I^+I^+}$:$R_{I^+I^+}$) and, if tested with NL 5, also ratio Sn:Rn (S_I.:R_I.).

The F_2 of cross 19, Widusa x Michelite, was tested with NL 5 ($P1.1^2.2$). All I^+I^+ plants were susceptible, indicating that Widusa does not have any strain-specific gene different from *bc-1*, *bc-1*^{2} or *bc-2*, and consequently does not have *bc-2*^{2}. The test of F_2 of cross 20, Widusa x GN 31, with NL 4 ($P1.1^2.2^2$) gave the same result: all I^+I^+ plants were susceptible. That result shows that Widusa does not have *bc-2*. Testing F_2 of cross 19 with NL 2 (P1.2), also revealed no resistant I^+I^+ plants, suggesting that Widusa does not have *bc-1*^{2}. The test of the same F_2 with NL 4 and of F_2 of cross 20 with NL 5 resulted in a 15:1 ratio for S and R, indicating the absence of strain-unspecific gene *bc-u* in Widusa. The only specific resistance gene that might still be present was *bc-1*. This was investigated in testing F_2 of cross 18, Widusa x Imuna, wit NL 1 (P0), resulting in a 15:1 ratio for S and R. indicating that Widusa does not have *bc-1*, otherwise a 3:1 ratio would be expected. Analysis of the resistance genes of Widusa is summarized in Table 33.

From this table it is concluded that Widusa has neither a strain-specific nor the strain-unspecific resistance gene. Some segregations suggest not only a 15:1 but also a

Table 32. Testing F_2 of Widusa crosses with some virus strains. Number of plants is number of inoculated normal plants; cripple plants were excluded. See Table 5 for the meaning of S, R, Sn, Rn and R⁺. Suggested ratios are according to segregation graphs of theoretically possible ratios, drawn on binomial probability paper. Ratio b is a second suggested ratio if the observed segregation lies within the significance areas of two theoretical ratios (suggested ratios = theoretical ratios) or it is the second theoretical ratio if the observed segregation lies between the significance areas of two theoretical ratios (suggested ratio ≠ theoretical ratios). Ratio 1 is the segregation ratio S_{I+I+} to R_{I+I+} and ratio 2 is S_{I_-} to R_{I_-}. Ratio 2 is only applicable in tests with NL 5 or NL 3. At-P <0.05, the deviation of the obtained results from the theoretical ratio is significant (*) and at P<0.01 highly significant (**).

Cross		Virus str.	No of pl.	Segregation S	R	Sn	Rn	R⁺	Suggested ratios 1 S:R a	b	2 Sn:Rn a	b	P of χ^2 test per ratio 1 a	b	2 a	b
18 Widusa x	Imuna	NL 1	317	61	7			249	15:1	57:7			0.17	0.86		
19 Widusa x	Michelite	NL 1	636	151	18			467	15:1	57:7			0.02*	0.90		
		NL 1	647	149	8			490	15:1				0.55			
		NL 2	335	92	0			243	1:0				1.00			
		NL 4	643	179	18			446	15:1	57:7			0.09	0.42		
		NL 4	648	159	10			479	15:1				0.86			
		NL 5	631	148	0	483	0		1:0		1:0		1.00		1.00	
		NL 5	324	79	0	245	0		1:0		1:0		1.00		1.00	
		NL 6	647	148	10	22		467	15:1				0.97			
20 Widusa x	GN 31	NL 1	648	123	12			513	57:7	15:1			0.45	0.20		
		NL 2	652	119	13			520	57:7	15:1			0.69	0.09		
		NL 3	642	118	9	439	76		15:1		>3:1<15:1		0.70		< 0.01**	< 0.01**
		NL 4	330	73	0			257	1:0				1.00			
		NL 5	320	63	4	241	12		15:1	57:7	15:1		0.92	0.19	0.32	
		NL 6	648	138	12	19		479	15:1	57:7			0.38	0.25		

Table 33. Analysis of the resistance genes of Widusa.

F_2 of cross	Strain	Ratio S:R	Conclusion
19 Widusa x Michelite	NL 5 (P1.1^3.2)	1:0	Widusa has not *bc-2*^{3}
20 Widusa x GN 31	NL 4 (P1.1^3.2^2)	1:0	Widusa has not *bc-2*
19 Widusa x Michelite	NL 2 (P1.2)	1:0	Widusa has not *bc-1*^{2}
	NL 4	15:1	Widusa has not *bc-u*
20 Widusa x GN 31	NL 5	15:1	
18 Widusa x Imuna	NL 1 (P0)	15:1	Widusa has not *bc-1*

57:7 ratio (Table 32). However the second ratio is not applicable in any of the tests, if studied in the sequence of the listed results in Table 33.

In testing F_2 of cross 20 with NL 5, a 15:1 ratio was obtained for the segregation of S and R plants, carrying I^+I^+, as well as for that of Sn and Rn plants with *I* (Table 32). This ratio proves that genotypes *bc-u*$^+$*/.(bc-1*^{2}*/bc-1*^{2}*)bc-2*^{2}*/bc-2*^{2} *I/.* (with and without *bc-1*^{2}*/bc-1*^{2}) can have complete expression of allele *bc-u*$^+$, with all plants showing systemic necrosis.

The results obtained allow designation of the genotype of Widusa as follows, mentioning one allele of each pair:

bc-u$^+$ *bc-1*$^+$ *bc-2*$^+$ *bc-3*$^+$ *I*

7.2.2 Results of testing F_2 of crosses with Jubila

The results are shown in Table 34. Using the same determination scheme and arguments as for identification of the genes of Widusa, the conclusions can be listed as in Table 35.

Testing F_2 of cross 21 with NL 1 resulted in 17 susceptible and 77 resistant I^+I^+plants (Table 34). If Jubila does not carry *bc-1*, unlike Imuna, a 15:1 ratio would be expected, and if present, a 3:1 ratio, based on segregation for *bc-u* (3 *bc-u*$^+$*/. bc-1/bc-1* + 1*bc-u/bc-u bc-1/bc-1*). The latter possibility is more likely.

The most probable explanation for the shortage of susceptible plants seems to me an inhibiting influence of the combined genetic background of Jubila and Imuna on the expression of allele *bc-u*$^+$. It cannot be the influence of Imuna alone, because in the test of F_2 of cross 1, DW x Imuna (Table 12, Section 5.4.1), a true 15:1 ratio was found. Also the influence of Jubila alone is unlikely, because the test of F_2 of cross 22 with NL 1 yielded a 57:7 ratio for S and R, indicating that complete expression of *bc-u*$^+$ is possible in the combined genetic background of Jubila and Michelite.

No Rn plants were expected in testing F_2 of cross 22 with NL 5, because no strain-specific genes effective to NL 5 occur in that cross. The six plants with only local necrosis (Rn, Table 34) may have escaped systemic infection. A 15:1 ratio was expected for Sn and Rn plants in the test of F_2 of cross 23 with NL 5, based on segregation for genes *bc-u* and *bc-2*^{2}, assuming that plants *bc-u*$^+$*/. bc-2*^{2}*/bc-2*^{2} *I/.* would show systemic necrosis. However, the data suggest a 3:1 ratio, indicating that allele *bc-u*$^+$ had no expression in the combined genetic background of GN 31 and Jubila.

These results allow designation of the genotype of Jubila as:

bc-u$^+$ *bc-1 bc-2*$^+$ *bc-3*$^+$ *I*

Table 34. Testing F_2 of Jubila crosses with some virus strains. Details as in Table 32.

Cross			Virus str.	No. of pl.	Segregation					Suggested ratios				P of χ^2 test per ratio			
					S	R	Sn	Rn	R+	1 S:R		2 Sn:Rn		1		2	
										a	b	a	b	a	b	a	b
21 Jubila	x	Imuna	NL 1	472	17	77			378	> 0:1	< 3:1			<0.01**	<0.01**		
22 Jubila	x	Michelite	NL 1	580	126	18			436	57:7	219:37			0.55	0.50		
			NL 2	593	151	0	159		283	1:0				1.00			
			NL 4	297	59	4			234	15:1				0.97			
			NL 4	591	149	11			431	15:1				0.74			
			NL 5	627	167	0	454	6		1:0		(<)1:0		1.00		<0.01**	
			NL 6	611	154	17	265		175	15:1	57:7			0.05	0.68		
23 Jubila	x	GN 31	NL 2	468	86	11	22		349	57:7	15:1			0.90	0.04*		
			NL 4	265	45	0			220	1:0				1.00			
			NL 5	656	98	6	419	133		15:1		3:1		0.84		0<2	
			NL 6	579	143	16	137		283	15:1	57:7			0.05	0.72		

Table 35. Analysis of the resistance genes of Jubila.

F_2 of cross	Strain	Ratio S:R	Conclusion
22 Jubila x Michelite	NL 5 (P1.1^2.2)	1:0	Jubila has not *bc-*2^2
23 Jubila x GN 31	NL 4 (P1.1^2.2^2)	1:0	Jubila has not *bc-2*
22 Jubila x Michelite	NL 2 (P1.2)	1:0	Jubila has not *bc-*1^2
	NL 4	15:1	Jubila has not *bc-u*
23 Jubila x GN 31	NL 5	15:1	
21 Jubila x Imuna	NL 1 (P0)	>0:1<3:1	Jubila has *bc-1*

7.2.3 Results of testing F_2 of crosses with Topcrop

The results are noted in Table 36. The conclusions of the genetic analysis are as in Table 37.

This table shows that Topcrop has only gene *bc-1*, like Jubila. Allele *bc-*u^+ had a better expression in F_2 of cross 24 than in F_2 of comparable cross 21 with Jubila as one of the parents: a true 3:1 ratio was attained in one of the tests.

The numbers of Sn and Rn plants (Table 36) suggest a ratio intermediate between the theoretic 3:1 and 15:1, indicating that allele *bc-*u^+ also did not have a complete expression in the combined genetic background of cross 26, Topcrop x GN 31.

The following resistance genotype can be inferred for Topcrop:

bc-u^+ *bc-1 bc-*2^+ *bc-*3^+ *I*

7.2.4 Results of testing F_2 of crosses with Amanda

The results are shown in Table 38, and the conclusions about the determination of the resistance genes are given in Table 39.

The analysis in Table 39 indicates the presence of *bc-*1^2 in Amanda. As I assume allelism between *bc-*1^2 and *bc-1*, the presence of the latter is excluded. Ratio 57:7, sometimes found together with 15:1, is not applicable.

The expected ratio 3:1 for S and R was not attained in cross 27, probably because of incomplete expression of allele *bc-*u^+ in the combined genetic background of Amanda and Imuna. These results are comparable with those of F_2 of cross 21, Jubila x Imuna. The segregations of S and R plants in the tests of F_2 of cross 27 with NL 1 fit a 7:9 ratio. I have no explanation for this ratio and consider the consistency with it as a coincidence.

The expected ratio 15:1 for Sn and Rn was also not attained (cross 29), but the segregation results suggest a 3:1 ratio. I would explain this shortage of susceptible plants in the same way as the deviant S:R ratio.

The resistance genotype of Amanda can be presented as:

bc-u^+ *bc-*1^2 *bc-*2^+ *bc-*3^+ *I*

7.2.5 Results of testing F_2 of crosses with IVT 7233

The results are presented in Table 40, and the conclusions on the resistance genes are as in Table 41.

The presence of *bc-u* and *be-*2^2 is obvious. The latter came from GN 31, a parent of

Table 36. Testing F_2 of Topcrop crosses with some virus strains. Details as in Table. 32.

Cross	Virus str.	No of pl.	Segregation S	R	Sn	Rn	R*	Suggested ratios 1 S:R a	b	2 Sn:Rn a	b	P of χ^2 test per ratio 1 a	b	2 a	b
24 Topcrop x Imuna	NL 1	477	91	20			366	3:1				0.09			
	NL 1	679	81	45			553	> 0:1	< 3:1			<0.01**	<0.01**		
25 Topcrop x Michelite	NL 1	593	136	11			446	15:1	57:7			0.54	0.18		
	NL 1	585	129	10			446	15:1	57:7			0.65	0.16		
	NL 2	572	127	0	11		434	1:0				1.00			
	NL 4	576	128	6			442	15:1				0.40			
	NL 5	152	35	0	117	0		1:0		1:0		1.00		1.00	
26 Topcrop x GN 31	NL 2	560	69	9	11		471	57:7	15:1			0.86	0.05		
	NL 4	554	102	0			452	1:0				1.00			
	NL 5	611	102	4	458	47		15:1		>3:1<15:1		0.29		<0.01**	<0.01**

Table 37. Analysis of the resistance genes of Topcrop.

F_2 of cross	Strain	Ratio S:R	Conclusion
25 Topcrop x Michelite	NL 5 (P1.1^2.2)	1:0	Topcrop has not *bc-*2^2
26 Topcrop x GN 31	NL 4 (P1.1^2.2^2)	1:0	Topcrop has not *bc-2*
25 Topcrop x Michelite	NL 2 (P1.2)	1:0	Topcrop has not *bc-*1^2
	NL 4	15:1 }	Topcrop has not *bc-u*
26 Topcrop x GN 31	NL 5	15:1 }	
24 Topcrop x Imuna	NL 1 (P0)	3:1 }	Topcrop has *bc-1*
	NL 1	>0:1<3:1 }	

IVT 7233. These two genes together give resistance to all necrosis-inducing strains and in IVT 7233 allow only development of local pin-point lesions with these strains. Whether *bc-*1^2 is also present is less obvious. Ratio 3:1 in F_2 of cross 31 with NL 2 suggests the absence of that gene. If so, a 9:7 ratio can be expected in the test of F_2 of cross 30 with NL 1, whereas no susceptible plants can be expected in the presence of *bc-*1^2 (ratio 0:1). However, the ratio obtained lies between the two theoretical ones. This deviant ratio could not be caused by incomplete expression of one of the strain-specific genes, because this was never found in any other test. Allele *bc-*u^t, having an incomplete expression in several other crosses, is not present in this cross. The explanation must be that the IVT 7233 plants, used for crossing with Imuna, had different genotypes: some plants with, others without gene *bc-*1^2. This was not detected during the selection of IVT 7233, because all selected F_2 plants and their progenies gave the same local pin-point lesions after inoculation with necrosis-incucing virus strains.

Assuming that two genotypes of IVT 7233 were used for the crosses with Imuna, it can be calculated that the two genotypes with and without *bc-*1^2 were present in F_2 of cross 30 in the ratio 1:1. This also means that in crossing IVT 7233 with Imuna, about the same number of plants of IVT 7233 was used with *bc-*1^2 as without that gene. Of the 155 plants of F_2 of cross 30 tested with NL 1 in two tests, 43 plants were susceptible. The theoretical number of susceptible plants, assuming both genotypes to be present in equal numbers, would be 43.6, so that the suggested explanation is likely to be the correct one.

However, the test of F_2 of cross 31 with NL 2 gave a 3:1 ratio, suggesting the absence of *bc-*1^2 in the presence of *bc-*2^2. But with two genotypes, with and without *bc-*1^2 in the ratio 1:1, the expected segregation ratio is not 3:1 but (3:1) + (9:7)/2 or 10.5:5.5. Then, 55 susceptible and 29 resistant plants could be expected from the 84 I^+I^+plants found in the test of F_2 of cross 31 with NL 2, while 61 susceptible and 23 resistant plants were found. A χ^2 test shows that the deviation of the actual segregation from the expected one is not significant ($P_{10.5:5.5}$ is 0.18).

The conclusion from testing F_2 of cross 30 with NL 1 was that *bc-*1^2 or *bc-1* was present in some plants of IVT 7233. However, presence of the latter gene was not possible since it was not present in GN 31 and Widusa, the parents of IVT 7233.

A 3:1 ratio was found for Sn and Rn plants in the test of F_2 of cross 31 with NL 5. This ratio results from segregation for gene *bc-*2^2 and confirms the conclusion that *bc-u* is present. In absence of the latter, a 15:1 ratio would be expected.

Table 38. *Testing F_2 of Amanda crosses with some virus strains. Details as in Table 32.*

Cross	Virus str.	No of pl.	Segregation					Suggested ratios				P of χ^2 test per ratio			
			S	R	Sn	Rn	R*	1 S:R		2 Sn:Rn		1		2	
								a	b	a	b	a	b	a	b
27 Amanda x Imuna	NL 1	275	28	38			209	> 0:1	< 3:1			<0.01**	<0.01**		
	NL 1	562	47	66			449	> 0:1	< 3:1			<0.01**	<0.01**		
	NL 1	629	55	75			499	> 0:1	< 3:1			<0.01**	<0.01**		
28 Amanda x Michelite	NL 1	629	130	21			477	57:7	219:37			0.24	0.85		
	NL 2	663	155	8			500	15:1				0.48			
	NL 2	643	159	8			476	15:1				0.44			
	NL 3	162	48	0	114	0		1:0		1:0		1.00		1.00	
	NL 4	632	151	14			467	15:1	57:7			0.24	0.31		
	NL 4	640	135	13			492	15:1	57:7			0.20	0.40		
	NL 5	598	144	0	454	0		1:0		1:0		1.00		1.00	
29 Amanda x GN 31	NL 2	647	112	33			502	3:1				0.53			
	NL 3	640	97	19	166	357		> 3:1	< 15:1	>0:1	<3:1	0.03*	<0.01**	<0.01**	<0.01**
	NL 4	342	67	0			275	1:0				1.00			
	NL 4	648	142	0			506	1:0				1.00			
	NL 5	641	119	11	391	120		15:1	57:7	3:1		0.30	0.37	0.43	

Table 39. Analysis of the resistance genes of Amanda.

F_2 of cross	Strain	Ratio S:R	Conclusion
28 Amanda x Michelite	NL 5 (P1.1^3.2)	1:0	Amanda has not *bc-*2^2
29 Amanda x GN 31	NL 4 (P1.1^3.2^2)	1:0	Amanda has not *bc-2*
28 Amanda x Michelite	NL 2 (P1.2)	15:1	Amanda has *bc-*1^2
	NL 4	15:1 }	Amanda has not *bc-u*
29 Amanda x GN 31	NL 5	15:1 }	
27 Amanda x Imuna	NL 1 (P0)	>0:1<3:1	Amanda has *bc-*1^2 or *bc-1*

This analysis of the resistance genes present in IVT 7233 prompted reselection in the differential for uniform presence of gene *bc-*1^2.

From these results I found the following genotypes for IVT 7233:

bc-u bc-1^2 *bc-*2^2 *bc-*3^+ *I* and *bc-u bc-*1^+ *bc-*2^2 *bc-*3^+ *I*

The first one will be considered as IVT 7233 and the second one as a new differential.

7.3 Observed and theoretical genotypes for resistance with dominant necrosis gene

Jubila and Topcrop have the same major gene for resistance and Widusa does not have any of those genes. This conclusion has consequences for the classification of the resistance groups (Table 7, Section 4.4.2). Thus, Widusa is comparable with DW, differing only in *II* alleles, and was rightly mentioned as representative of the first resistance group with *II*: group 8. Jubila and Topcrop belong to the same group, in contrast with an earlier proposal in Chapter 4 to place them in subgroups 9a and 9b. Their difference in reaction on inoculation with necrosis-inducing strains must be quantitative rather than qualitative. Both cultivars are now classed in group 9.

There is no obvious influence of the type of virus strain on the expression of allele *bc-*u^+ in I^+I^+genotypes. In *II* genotypes, however, *bc-*u^+ was expressed only with NL 5 and to some extent with NL 3 (Table 4 in Section 4.2; Tables 32, 34, 36, 38 and 40). The reactions of the *II* differentials in Table 4 only depended on the presence or absence of a strain-specific gene effective against the strain used, independent of the presence of allele *bc-*u^+, as can be concluded from the absence of reaction of Jubila, Topcrop and Amanda with NL 8 and of Amanda with NL 2. But a positive reaction might be expected with NL 5 in a differential carrying *bc-*u^+ and *bc-*2^2, effective against that strain.

The observed and theoretical *II* differentials and their observed and expected systemic reactions with the necrosis-inducing strains are shown in Table 42. The observed reactions are those of Table 4, found at 30 °C. The theoretical differentials are supposed to bear also *bc-u*. The combinations of strain-specific genes in the *II* differentials are the same as for I^+I^+ differentials (Table 31), but fewer genotypes have been identified and positive interactions are limited to the known necrosis-inducing strains. The strain-unspecific gene, not participating in the proposed gene-for-gene relation, is omitted from Table 42.

Table 40. Testing F_2 of IVT 7233 crosses with some virus strains. Details as in Table32.

Cross	Virus strain	Number of plants	Segregation					Suggested ratios			P of χ^2 test per ratio		
			S	R	Sn	Rn	R+	1 S:R		2 Sn:Rn	1		2
								a	b		a	b	
30 IVT 7233 x Imuna	NL 1	254	15	35			204	>0:1	< 9:7		<0.01**	<0.01**	
	NL 1	596	28	77			491	>0:1	< 9:7		<0.01**	<0.01**	
31 IVT 7233 x Michelite	NL 1	314	0	71			243	0:1			1.00		
	NL 2	324	61	23			240	3:1			0.61		
	NL 4	319	73	18			228	3:1			0.25		
	NL 5	157	37	7	87	26		3:1	57:7	3:1	0.16	0.29	0.62
32 IVT 7233 x GN 31	NL 2	324	0	91			233	0:1			1.00		
	NL 4	317	80	0			237	1:0			1.00		
	NL 5	159	0	44	0	111		0:1		0:1	1.00		1.00

Table 41. Analysis of the resistance genes of IVT 7233.

F_2 of cross	Strain	Ratio S:R	Conclusion
32 IVT 7233 x GN 31	NL 4 (P1.1^2.2^2)	1:0	IVT 7233 has not *bc-2*
31 IVT 7233 x Michelite	NL 5 (P1.1^2.2)	3:1	IVT 7233 has *bc-2*^{2}
	NL 2 (P1.2)	3:1	IVT 7233 has *bc-2*^{2} or *bc-1*^{2}
	NL 4	3:1 }	IVT 7233 has *bc-u*
32 IVT 7233 x GN 31	NL 5	0:1 }	
30 IVT 7233 x Imuna	NL 1 (P0)	>0:1<9:7	Part of IVT 7233 has *bc-1*^{2} or *bc-1*

Table 42. Observed and theoretical differentials, carrying *II* and combinations of the strain-specific genes, and their observed or expected positive reactions with the necrosis-inducing strains found, resulting in systemic necrosis. Temperature 30 °C.

Resistance group	Theoretical combinations of strain-specific genes[1]	Observed differentials	Pathogenicity group, virus strain and supposed pathogenicity genes			
			III NL 8 P2	IVb NL 6 P1.1^2	Vb NL 2 P1.2	VIa, VIb NL 3, NL 5 P1.1^2.2
8		Widusa	+	+	+	+
9	*bc-1*	Jubila, Topcrop	–	+	+	+
10	*bc-1*^{2}	Amanda	–	+	–	+
	bc-2		+	–	+	+
(11)[2]	*bc-2*^{2}		–	–	–	–
	bc-3		–	–	–	–
	bc-1 bc-2		–	–	+	+
	bc-1 bc-2^2		–	–	–	–
	bc-1 bc-3		–	–	–	–
	bc-1^2 *bc-2*		–	–	–	+
11	*bc-1*^{2} *bc-2*^{2}	IVT 7233	–	–	–	–
	bc-1^2 *bc-3*		–	–	–	–
	bc-2 bc-3		–	–	–	–
	bc-2^2 *bc-3*		–	–	–	–
	bc-1 bc-2 bc-3		–	–	–	–
	bc-1 bc-2^2 *bc-3*		–	–	–	–
	bc-1^2 *bc-2 bc-3*		–	–	–	–
	bc-1^2 *bc-2*^{2} *bc-3*		–	–	–	–

1. As long as *bc-1* and *bc-1*^{2}, and *bc-2* and *bc-2*^{2} are allelic.
2. Obtained from a genotype mixture of the origenal IVT 7233.

7.4 Discussion

The results presented in this Chapter show that four of the five differentials with *II* carry also the dominant *bc-u*$^+$ alleles. This is in contrast with the *I*$^+$*I*$^+$differentials, which all have gene *bc-u*, except Dubbele Witte, which also carries no genes for specific resistance. How could this difference between the two main groups of bean cultivars carrying

I^+I^+ and *II*, respectively, have developed? The explanation may be that I^+I^+ plants with *bc-u*$^+$ were removed during breeding of resistant cultivars, because these plants were susceptible to all strains and thus easily detected. During breeding of new *II* cultivars, however, the prevalent strains, especially outside the Netherlands, could usually not induce a systemic necrosis reaction or only a temperature-dependent one, which was not likely at the prevailing field temperatures. The plants were resistant at those temperatures and did not show symptoms. Plants with and without *bc-u* could not be distinguished then. Also if strains inducing systemic necrosis were available during selection, it was not easy to distinguish plants with and without *bc-u*, because this had only been possible if an effective strain-specific gene was also present, so that the reaction of plants with *bc-u* would remain local and without that gene would be systemic.

However, the preceding sections show that expression of *bc-u*$^+$, if combined with an effective strain-specific gene, also depends on genetic background and sometimes on virus strain. Complete expression, resulting in systemic necrosis, occurred in only few combinations, limiting again the distinction between plants with and without *bc-u*. Furthermore, several crosses to obtain new cultivars with *II* will have been made between parents that both lack *bc-u*, making selection of genotypes with that gene impossible.

The phenomenon of incomplete expression of allele *bc-u*$^+$ needs further investigation. It seems to be due to interaction between virus strain, genetic background and sometimes temperature, and is more frequent in *II* than in I^+I^+ plants of the same cross. From the F_2 test results of several crosses I concluded that in some the combined genetic background of the two parents might prevent complete expression of *bc-u*$^+$. Complementary minor genes of the two parents probably inhibit the virus production allowed by allele *bc-u*$^+$.

This was illustrated in the F_2 tests of the crosses with Jubila and Topcrop. F_2 Jubila x Imuna tested with NL 1 (Table 34) gave a lower expression of *bc-u*$^+$ in the I^+I^+ plants (ratio S:R) than F_2 Topcrop x Imuna (Table 36). The same applies for the *II* plants (ratio Sn:Rn) in the crosses of those differentials with GN 31 tested with NL 5, but the expression of *bc-u*$^+$ was lower in the *II* plants than in the I^+I^+ plants. Thus, the combined genetic backgrounds of Topcrop with Imuna or with GN 31 are more favourable than those of Jubila with the same cultivars, and the expression of *bc-u*$^+$ is better in plants with I^+I^+ than with *II*. Comparing the tests of F_2 Jubila x Imuna and Topcrop x Imuna with Jubila x Michelite and Topcrop x Michelite, all tested with NL 1, complete expression of *bc-u*$^+$ occurred in the I^+I^+ plants of the crosses with Michelite, in contrast with a low expression in the Imuna crosses. Michelite seems to supply a better genetic background than Imuna. However, complete expression was obtained in testing F_2 Widusa x Imuna with NL 1 (Table 32), indicating that the genetic background of Imuna is unfavourable in combination with Jubila or Topcrop, but not with Widusa.

The reduced expression of *bc-u*$^+$ in *II* plants compared with I^+I^+ plants can be explained by a lower virus concentration in the former plants. It is very likely that *II* plants with systemic necrosis generally have a lower virus concentration than I^+I^+ plants with mosaic, assuming absence of an effective strain-specific gene in both categories of plants. Gene *I* is considered to inhibit the virus production ('inhibitor' gene, Ali, 1950). The low virus concentration in *II* plants was demonstrated by the fact that it was usually impossible to reisolate the virus from plants with systemic necrosis. The absence of seed transmission in *II* plants in contrast with I^+I^+ plants, might also be caused by too low a virus concentra-

tion. It is also likely that in *II* as well as in I^+I^+ plants the possibility of virus production is smaller if an effective strain-specific gene is present but not the strain-unspecific gene. My hypothesis is that gene *bc-u* confers on an effective strain-specific gene the capacity to be completely effective, that is, to prevent virus in the inoculated leaves from reaching such a concentration that systemic spread is possible. If, instead of *bc-u*, dominant *bc-u*$^+$ is present together with an effective strain-specific gene in homozygous condition, then the latter gives incomplete resistance and cannot prevent systemic production and spread of virus. Though virus concentration is likely to be low, it is sufficient to induce moderate to weak mosaic in I^+I^+ plants or to detect the virus by back-inoculation if the genetic background is not too unfavourable. In *II* plants with the same combination of *bc-u*$^+$ and an effective strain-specific gene, however, the virus production is so low that any inhibiting action of the genetic background largely or totally prevents systemic infection.

I have no conclusive proof for my supposition that a plant combining *bc-u*$^+$ and an effective strain-specific gene is less susceptible than a plant not carrying the latter. There is no sharp phenotypic distinction between plants with such a difference in genotype. Mosaic in I^+I^+ plants may vary from severe and rapid (arising in about one week) through very mild and slow (appearing after three weeks or more) to complete absence of the symptom, infection being only detectable by back-inoculation. Systemic necrosis may vary in the same degree, from very quickly killing the main stem within one week to the appearance of starlike vein-necrotic dots ascending slowly from leaf to leaf without killing the plant. It is impossible to distinguish two separate phenotypes in the expression range of the two symptoms. Proof could be obtained for I^+I^+ plants by determining virus titres in plants of isogenic lines, not carrying the strain-unspecific gene, and differing only by the presence or absence of an effective strain-specific gene.

8 Some implications for breeding

One of the tasks of a plant breeder is the search for resistance genes against new races or strains of a pathogen that threatens a crop. He has to incorporate these genes into the cultivars, to prevent damage and to allow the production of a healthy crop with a high yield. This holds especially for virus diseases, where chemical control is impossible and other agronomic measures are usually insufficient to prevent damage. To fulfil his task the plant breeder has to know which strains of the virus are present. He investigates which resistances exist against these strains and which of them are preferred for the defence of his crop. Especially this part of the plant breeder's activities has been the subject of the preceding chapters, applied to common bean and BCMV.

Bean cultivars can be divided into two main groups with and without the dominant alleles *II* of the necrosis gene. This gene hampers virus production, prevents mosaic but allows systemic necrosis if the plant is susceptible to the particular virus strain. The breeding of *II* cultivars reduces damage by the virus. Only the necrosis-inducing strains can cause systemic necrosis in these cultivars: the temperature-independent ones at all growing temperatures and the temperature-dependent strains only at high growing temperatures. All other virus strains do not induce systemic necrosis. In countries where temperature-independent necrosis-inducing strains are not prevalent, as in the United States, breeding of cultivars with *II* has been a solution to the BCMV problem, at least temporarily. However, temperature-independent necrosis-inducing strains may also arise in such countries or may be introduced through seed since the virus is seed-borne in I^+I^+ but not in *II* plants. Spread of these virus strains by seed is thus only possible through susceptible I^+I^+ cultivars. Sowing imported I^+I^+ cultivars first in aphid-free greenhouses to harvest virus-free seed from plants without virus symptoms considerably limits the risk of introducing new strains of BCMV.

Also in countries like the Netherlands, where temperature-independent necrosis-inducing strains seem common, the predominant use of *II* cultivars limits damage by the virus. Such cultivars are in general infected more incidentally than those with I^+I^+, especially when large areas of one cultivar are grown, mainly because there is no plant to plant infection by aphids within the crop. Virus transfer through aphids from plants with necrosis has never been observed and plants infected through seed are not found in *II* cultivars. Aphids can only introduce the virus from nearby crops of I^+I^+ cultivars.

In fact, BCMV infection is almost completely prevented by using exclusively *II* cultivars. Transmission through beans is then impossible, so that virus maintenance and spread are strongly impeded, particularly because *Phaseolus vulgaris* is the almost exclusive natural host of the virus. World-wide use of only *II* cultivars would practically eradicate the virus. To achieve a rapid decline in BCMV damage, the world capacity to breed beans has to be enlarged considerably. At present however, too many I^+I^+ cultivars are maintained in cultivation, because they often tolerate prevalent strains, or are only susceptible to strains not prevalent in the bean-growing area, so that damage by the virus remains

limited. Moreover these cultivars often have valuable characteristics for yield, consumption quality and plant type or are resistant to other diseases. The grower is then willing to neglect the disadvantage of susceptibility to BCMV. But keeping I^+I^+ cultivars in cultivation preserves the virus and its sources of infection. Simultaneous production on nearby fields of I^+I^+ and *II* cultivars may easily lead to systemic necrosis in the latter. With backcross breeding, I^+I^+ cultivars can be transformed into those with *II*, while retaining their valuable cultural characteristics.

However, as long as I^+I^+ cultivars are grown alongside those with *II*, the simple exchange of I^+I^+ alleles with *II* is insufficient. The chance of such *II* plants contracting systemic necrosis is too high. Additional incorporation is needed of strain-specific genes, such as *bc-2*^{2}, not overcome by the necrosis-inducing strains and present in GN 31 and other cultivars of resistance group 6. For this purpose, IVT 7233 (*bc-1*^{2} *bc-2*^{2} *I*) could also be used as source of resistance. The desired plants in segregating progenies are easily recognized by pin-point lesions if inoculated with one of the temperature-independent necrosis-inducing strains.

Simultaneous mutation of more than one pathogenicity gene, which would result in break-down of more than one corresponding resistance gene, seldom occurs. Hence one should incorporate at least two genes for resistance not yet overcome by the pathogen, to give the cultivar a better chance of lasting resistance than if only one effective gene were present. Therefore it is advisable to introduce also strain-specific gene *bc-3* of IVT 7214 (*bc-2 bc-3* I^+), effective against all known strains. Based on present knowledge, the best combination of resistance genes seems *bc-u bc-1*^{2} *bc-2*^{2} *bc-3 I*, in which resistance genes from IVT 7233 and IVT 7214 are combined.

However, with more genes involved, breeding becomes more complicated, requiring more time and skill. For simultaneous incorporation, for instance, of *bc-2*^{2} *bc-3 I*, the resistant F_2 plants have to be test-crossed with GN 31 and the F_1 testcross progenies have to be screened with a strain mixture (NL 3, NL 4, NL 5), to indicate those F_2 plants in which all three genes are homozygously present.

Breeding for resistance to BCMV in common bean is essential for several developing countries, especially in Latin America and Africa, where beans are a major source of protein in human diet. To satisfy the demand for food by the increasing population, a quick rise in average yield is necessary. Incorporation of resistance to diseases (including BCMV) and pests into the prevalent cultivars of those countries would substantially improve yield. Good results in limiting BCMV damage can be expected by incorporation into *II* cultivars of *bc-2*^{2} and *bc-3* and into I^+I^+ cultivars additionally *I*. The complicated and time-consuming incorporation of two or three resistance genes into several cultivars could be made the responsibility of different plant breeding institutes, even on different continents, so that more resistant cultivars would become available more rapidly. If the necessary virus strains for selection for resistance be available in another place than where the resistant cultivars are needed, it would be desirable to concentrate the basic breeding program for this resistance at the place where the strains occur, thus avoiding questionable introduction of foreign virus strains for selection. Such an agreement has been concluded between CIAT (Centro Internacional de Agricultura Tropical) in Colombia and IVT in the Netherlands on breeding for BCMV resistance in the latter institute for Latin America.

Summary

1. Introduction. Common bean (*Phaseolus vulgaris* L.) is grown and consumed all over the world. The total crop area is about the same as that of potatoes. Bean common mosaic virus (BCMV) is also world-wide in distribution. Its main natural host is *Phaseolus vulgaris*. The virus is one of the major disease problems in common bean, especially in developing countries where dry beans are an important source of protein.

2. Literature and aims of this study. The literature on BCMV shows much confusion about the identification of its strains, of which some twenty have been tentatively described. However, researchers often used different differentially reacting cultivars, worked under different conditions, used different test methods and different criteria for the plant reactions.

Resistance of common bean to the virus has been claimed to be controlled by two genes for resistance (*s* and *a*) and one gene (*I*) for necrosis, but previous research had been done with single strains only. Other genes might be involved, since there are several cultivars that are resistant, however, to only some of the strains. The problems of strain identification and resistance to the virus could not be studied separately, both being interdependent aspects of a dynamic host – pathogen system.

The aims of this research were (a) to analyse the interaction between resistance genes in bean and pathogenicity genes in the virus, and the inheritance of resistance; (b) to obtain bean genotypes resistant to all strains. Therefore (1) test methods for identification of virus strains were standardized; (2) a large number of cultivars was tested with several strains to determine resistance to each strain and number of resistance genotypes; (3) all available strains of BCMV were compared and an efficient set of differentials was established, and the virus strains were finally identified and classified; (4) the genetics of resistance were analysed and those of pathogenicity postulated through a gene-for-gene relationship.

3. Materials and methods. Fifteen of the twenty-two strains described in the literature were compared, the other ones were no longer available. The about 450 cultivars tested came from 36 countries, distributed over West and East Europe, North and Latin America, Africa, Asia and Australia, and are supposed to be a good representation of the world genetic diversity of common bean (Appendix).

The best methods for inoculum preparation and inoculation, virus propagation and maintenance of strains, determination of purity and purification of strains, as well as a general virus test and an infectivity test are described after being determined experimentally. They are proposed as standard methods for identification of BCMV strains, to promote international comparability of results. A necrosis test and an F_3 test are described to separate resistant plants of different genotype and to detect escapes from infection in segregating F_2 populations.

4. Analysis of host reaction and identification of strains. About 450 cultivars and lines were tested with the strains NL 1 to NL 8, known to represent well BCMV variation. On the basis of their resistance or susceptibility to each of these strains, the cultivars could be classified into eleven resistance groups. Cultivars of groups 1 to 6 never reacted with systemic necrosis, but with mosaic to one or more strains. They carry recessive alleles I^+I^+ of the necrosis gene. IVT 7214 of resistance group 7 reacted neither with mosaic, nor with local or systemic necrosis. It also has I^+I^+ as is demonstrated in Chapter 5. Cultivars of resistance groups 8 to 10 reacted to some strains with systemic necrosis, but never with mosaic, and IVT 7233 of group 11 only with local necrosis. They carry the dominant alleles *II* as is proved in Chapter 7. The most suitable cultivars were chosen for a set of differentials with representatives of each resistance group.

Resistance groups 7 and 11 are represented by breeding lines IVT 7214 with I^+I^+, and IVT 7233 with *II*, respectively. Both lines are resistant to all strains. By selecting these lines, one of the aims of this study was achieved: to obtain genotypes resistant to all known strains of the virus.

The I^+I^+ differentials, being susceptible or resistant to certain of the strains remained so at all growing temperatures. However, among the *II* cultivars, some showed plants with systemic necrosis at higher temperatures than normally used for strain identification but none at normal or lower temperatures, or showed systemic necrosis in more plants at higher temperatures.

The strains could be arranged in three main groups: 1. Strains that never induce systemic necrosis; 2. Strains that induce systemic necrosis in cultivars of some *II* resistance groups, according to temperature (temperature-dependent necrosis-inducing strains); 3. Strains inducing local and systemic necrosis at all temperatures in *II* genotypes susceptible to the strain concerned (temperature-independent necrosis-inducing strains).

The virus strains were finally classed into seven pathogenicity groups, three of which were divided into two subgroups each, according to the reactions they induced in each differential of the standard set. Thus, the actual number of strains to be distinguished was reduced to ten.

It is proposed to officially denote the virus strains by the international two letter country code followed by a chronological number. After international comparison of strains and a study of the genetics of resistance and pathogenicity, also a genetic code is given for each genetically distinct strain.

5. Inheritance of resistance of plants with recessive alleles of the necrosis gene. Diallel crosses were made between the I^+I^+ differentials Dubbele Witte, Imuna, Redlands Greenleaf B, Michelite 62, Pinto 114 (incomplete diallels), Great Northern 31 and IVT 7214, representing resistance groups 1 to 7. The F_1 of the 17 crosses was tested with a strain of each pathogenicity group and the F_2 with most of them.

The results lead to the following conclusions about the resistance genes in the I^+I^+ differentials:

1. Resistance is governed by recessive genes. One of these is strain-unspecific and complementary to a series of strain-specific genes. Resistance occurs if the strain-unspecific gene is present together with at least one strain-specific resistance gene effective to the particular virus strain. The strain-unspecific gene is present in recessive condition in all I^+I^+ differentials used in these crosses except Dubbele Witte.

2. The differentials Imuna, RG-B, Michelite, GN 31 and IVT 7214 each have a different strain-specific gene for resistance besides the strain-unspecific gene.
3. Pinto 114, GN 31 and IVT 7214 each have two strain-specific genes for resistance besides the strain-unspecific gene. Pinto 114 has the Michelite and Imuna genes, GN 31 has the RG-B gene as second strain-specific gene, and IVT 7214 the Michelite gene.
4. The Imuna and RG-B genes are either allelic or strongly linked. If the latter, RG-B and GN 31 also have the Imuna gene. The Michelite and GN 31 genes are allelic. If the Imuna and RG-B genes are also allelic, the five strain-specific resistance genes are situated at three loci. The strain-specific genes at these three loci are inherited independently or weakly linked.
5. Inheritance of the strain-unspecific gene is independent of the strain-specific genes.

6. Genotypes for resistance and pathogenicity. Petersen (1958) used symbols *a* and *s* for two genes governing resistance of GN 15 to strain Voldagsen. As these symbols were also used for other genes of bean, they are not descriptive for BCMV. The following six new gene symbols are proposed instead: *bc-u* for (strain-)unspecific resistance gene, *bc-1* for Imuna gene, *bc-1*^{2} for RG-B gene, *bc-2* for Michelite gene, *bc-2*^{2} for GN 31 gene and *bc-3* for IVT 7214 gene.

Pathogenicity genes P1, P1^2, P2 and P2^2, corresponding with four resistance genes of which some are allelic, are postulated for BCMV. The results of this study can be explained with this system of four resistance and pathogenicity genes, a fifth resistance gene (*bc-3*) so far without a corresponding pathogenicity gene, a strain-unspecific gene and a necrosis gene. Most pathogenicity genes are apparently not allelic, but allelism of P2 and P2^2 is not excluded. The gene-for-gene system presented in Table 30 is the most extensive one that has been investigated so far in a host – virus relationship.

A complete gene-for-gene model for resistance and pathogenicity was worked out (Table 31). Only seven of the eighteen theoretically possible differentials with combinations between the five resistance genes have been found, and one was selected from a cross between RG-B and Michelite. Of the remaining ten, eight carry gene *bc-3*. Combinations with *bc-3* other than in IVT 7214 are unlikely to be found. The two remaining resistance genotypes were not detectable because of the absence of appropriate virus strains for differentiation.

Assuming all four pathogenicity genes to be present at separate loci, sixteen genotypes for pathogenicity are possible, or twelve if P2 and P2^2 were allelic. Of the sixteen or twelve theoretically possible virus strains, seven have been found. Four could not have been recognized because of missing resistance genotypes for differentiation. The known differentials would allow the detection of five more strains. The strains are likely to evolve as indicated in Figure 14. This suggests a step-by-step evolution into genotypes with one more gene for pathogenicity.

Selection of the ten missing differentials is theoretically possible from F_2 populations of crosses between differentials, by testing the F_2 with an appropriate virus strain, test-crossing the resistant plants and testing the F_1 of the test crosses to determine the desired genotype.

7. Inheritance of resistance of plants with dominant necrosis gene. Each of the differentials Widusa, Jubila, Topcrop, Amanda and IVT 7233, representing resistance groups 8,

9a, 9b, 10 and 11, was crossed with Imuna, Michelite and GN 31, and the F_2 tested with appropriate strains. Since the resistance genes of the I^+I^+ parent and the pathogenicity genes of the virus strain were known, the resistance genes of the *II* parent could be determined from the segregation of F_2. Strains NL 1, NL 2, NL 4 and NL 5 were used to test the F_2 of the fifteen crosses and sometimes NL 3 and NL 6 for additional data.

The test results of the crosses with Widusa showed that this cultivar has neither a strain-specific nor the strain-unspecific resistance gene. Jubila and Topcrop have only *bc-1*, and Amanda carries *bc-1*^{2}. In IVT 7233, *bc-u*, *bc-1*^{2} and *bc-2*^{2} are present, but *bc-1*^{2} is lacking in some plants. Jubila and Topcrop are of the same resistance group. Hence, difference in reaction to inoculation with necrosis-inducing strains is in degree rather than in type.

The observed and theoretical combinations of strain-specific genes in *II* differentials are shown in Table 42. Four of the five examined differentials with *II* carry the dominant *bc-u*$^+$ alleles, in contrast with the I^+I^+ differentials, which all have gene *bc-u* except Dubbele Witte. This is explained by the resistance of cultivars with *II* to strains not inducing necrosis. These strains were common in the areas where cultivars were being bred for *II* resistance, so that genotypes with and without *bc-u* could not be distinguished. A second cause might be the incomplete expression of allele *bc-u*$^+$, expecially in *II* genotypes.

8. Some inplications for breeding. Breeding and use of *II* cultivars reduces crop loss by the virus. Disease incidence in an *II* crop is mostly lower than in one with I^+I^+ because (1) the virus is not seedborne in *II* plants; (2) aphids can only introduce the virus from a nearby I^+I^+ crop; (3) virus transmission by aphids from *II* plants with systemic necrosis has never been observed, so further spread within an *II* crop does not occur. Exclusive cultivation of *II* cultivars would completely prevent BCMV infection since *Phaseolus vulgaris* is practically the only natural host of the virus.

As long as I^+I^+ cultivars are grown alongside those with *II*, protection by *II* is insufficient. Additional strain-specific genes not overcome by the necrosis-inducing strains have to be incorporated. Preferably two such genes should be used, to have a better chance of lasting resistance. Present knowledge shows the combination of resistance genes *bc-u bc-1*^{2} *bc-2*^{2} *bc-3 I*, in which genes from IVT 7233 and IVT 7214 are combined, to be the best. Genes *bc-2*^{2} and *bc-3* are both effective against all necrosis-inducing strains and *bc-3* against all other strains too.

Samenvatting

Genetische interactie tussen Phaseolus vulgaris en bonerolmozaïekvirus, met gevolgtrekkingen voor stammenidentificatie en resistentieveredeling.

1. Inleiding. Bonen (*Phaseolus vulgaris* L.) worden over de gehele wereld geteeld en gegeten. Het wereldareaal is ongeveer gelijk aan dat van aardappelen. Het bonerolmozaïekvirus (BCMV) heeft eveneens een wereldwijde verspreiding, met *Phaseolus vulgaris* als nagenoeg enige natuurlijke waardplant. Het virus vormt één van de voornaamste ziekteproblemen in de boon, vooral in ontwikkelingslanden, waar droge bonen een belangrijke bron van eiwitten zijn in het voedselpakket.

2. Literatuur en doeleinden van deze studie. Uit de literatuur over de stammen van BCMV, waarvan er ongeveer twintig beschreven zijn, blijkt verwarring en misverstand betreffende de identificatie ervan. Onderzoekers gebruikten vaak verschillende series toetsrassen, werkten onder verschillende omstandigheden, gebruikten verschillende toetsmethoden en verschillende criteria ter beoordeling van de reacties van de planten. Een overzicht van de beschreven stammen is gegeven in Tabel 1.

Volgens de literatuur berust de resistentie van de boon tegen het virus op twee resistentie-genen *s* en *a* en een necrose-gen *I*, maar desbetreffend onderzoek werd steeds uitgevoerd met slechts één virusstam. Aangezien er verscheidene resistente rassen zijn, die elk echter slechts resistentie hebben tegen bepaalde stammen, lijkt het waarschijnlijk dat er meer genen bij betrokken zijn. Uit de literatuur werd duidelijk dat stammenidentificatie en resistentie tegen het virus niet afzonderlijk kunnen worden bestudeerd, daar het onderling afhankelijke aspecten zijn van een dynamisch systeem tussen waardplant en pathogeen.

De doeleinden van dit onderzoek waren: (a) analyse van de overerving van de resistentie en van de interactie tussen de resistentie-genen in de boon en de pathogeniteitsgenen in het virus; (b) het verkrijgen van genotypen die resistentie geven tegen alle stammen. Daartoe werden (1) methoden gestandaardiseerd voor het identificeren van en toetsen met virusstammen, (2) vele rassen getoetst met verscheidene stammen om de resistentiespectra en het aantal resistentiegroepen te bepalen, (3) alle beschikbare stammen van BCMV vergeleken en een efficiënte serie toetsrassen samengesteld, en (4) de overerving van de resistentie geanalyseerd en die van de pathogeniteit gepostuleerd via een gen-om-gen relatie.

3. Materialen en methoden. Vijftien van de 22 in de literatuur beschreven stammen werden vergeleken; de andere waren niet meer beschikbaar. De ongeveer 450 getoetste rassen kwamen uit 36 landen, verdeeld over West- en Oost-Europa, Noord- en Latijns-Amerika, Afrika, Azië en Australië, zodat kon worden aangenomen dat ze een goede afspiegeling vormden van de in de wereld aanwezige genetische variabiliteit van de boon.

De beste methoden voor inoculumbereiding en inoculatie, virusvermeerdering en in-

standhouding van stammen, zuiverheidscontrole en scheiding van stammen, alsmede een algemene virustoets en een infectietoets worden beschreven na experimenteel te zijn vastgesteld. Voorgesteld wordt om ze te gebruiken als standaardmethoden voor de identificatie van BCMV-stammen, ter bevordering van de internationale vergelijkbaarheid van de resultaten. Een necrosetoets en een F_3-toets worden beschreven waarmee in een splitsende F_2-populatie onderscheid kan worden gemaakt tussen resistente planten van twee verschillende genotypen en waarmee kan worden nagegaan of de planten zonder symptomen resistent zijn of slechts ontsnapten aan infectie.

4. Analyse van de waardplantreactie en identificatie van de stammen. Ongeveer 450 rassen en lijnen werden getoetst met de stammen NL 1 tot NL 8, waarvan was vastgesteld dat ze de stammenvariatie in BCMV goed vertegenwoordigen. Op basis van hun resistentiespectrum konden de rassen en lijnen worden ingedeeld in elf waardplant- of resistentiegroepen (Tabellen 2 en 3 en Appendix). Rassen van de groepen 1 tot 6 reageerden nooit met systemische necrose, maar op één of meer van de stammen met mozaïek. Ze hebben de recessieve allelen I^+I^+ van het necrose-gen. IVT 7214 van resistentiegroep 7 reageerde noch met mozaïek, noch met lokale of systemische necrose en heeft ook I^+I^+, zoals wordt aangetoond in Hoofdstuk 5. Rassen van de resistentiegroepen 8 tot 10 reageerden op sommige stammen met systemische necrose, maar nooit met mozaïek, en IVT 7233 van groep 11 alleen met lokale necrose. Ze hebben de dominante allelen *II*, zoals wordt bewezen in Hoofdstuk 7. De meest geschikte rassen werden gebruikt voor het samenstellen van een serie toetsrassen met vertegenwoordigers van iedere resistentiegroep.

De resistentiegroepen 7 en 11 worden vertegenwoordigd door de lijnen IVT 7214 met I^+I^+ en IVT 7233 met *II*. Beide zijn resistent tegen alle stammen. Met de selectie van deze lijnen was één van de doeleinden van dit onderzoek bereikt, namelijk de ontwikkeling van genotypen die resistentie geven tegen alle bekende stammen van het virus.

De I^+I^+-toetsrassen behielden hun resistentiespectrum bij alle groeitemperaturen. Sommige *II*-rassen echter, die bij normale toetstemperatuur of bij lagere temperaturen zonder necrose bleven, kregen bij hogere temperaturen in een aantal planten systemische necrose, andere kregen bij hogere temperaturen systemische necrose in méér planten (Tabel 4). De reactietypen van de plant en de belangrijkste symptomen zijn samengevat in Tabel 5.

De stammen konden worden gerangschikt in drie hoofdgroepen: 1. Stammen die nooit systemische necrose induceren; 2. Stammen die systemische necrose induceren in rassen van bepaalde *II*-resistentiegroepen, afhankelijk van de temperatuur (temperatuurafhankelijke necrose-inducerende stammen); 3. Stammen die lokale en systemische necrose induceren in alle *II*-rassen die vatbaar zijn voor de desbetreffende stam, onafhankelijk van de groeitemperatuur (temperatuuronafhankelijke necrose-inducerende stammen).

De stammen werden ingedeeld in zeven pathogeniteitsgroepen, waarvan er drie ieder in twee subgroepen werden onderverdeeld, overeenkomstig de reacties die ze induceerden in elk van de standaardserie toetsrassen. Aldus werd het aantal werkelijk onderscheidbare stammen teruggebracht tot tien (Tabellen 6 en 7).

Voorgesteld wordt om de virusstammen voortaan aan te duiden met de internationale landencode van twee letters, gevolgd door een chronologisch nummer. Na een internationale stammenvergelijking en bestudering van de overerving van resistentie en pathogeniteit wordt aan de landencode van iedere genetisch te onderscheiden stam een gencode toegevoegd. Stammen met dezelfde gencode, hoewel de landencode verschillend kan zijn,

hebben hetzelfde resistentiespectrum op de standaardserie toetsrassen en zijn identiek wat hun pathogeniteitsgenen betreft.

5. Overerving van de resistentie van planten met recessieve allelen van het necrose-gen. Diallele kruisingen werden gemaakt tussen de I^+I^+-toetsrassen Dubbele Witte, Imuna, Redlands Greenleaf B, Michelite, Pinto 114, Great Northern 31 en IVT 7214, als vertegenwoordigers van de resistentiegroepen 1 tot 7. De F_1 werd getoetst met een stam van elke pathogeniteitsgroep en de F_2 met de meeste van deze stammen (Tabellen 8, 9 en 10). De reacties van de F_1 zijn weergegeven in Tabel 8, de uitsplitsingsverhoudingen van alle F_2-toetsingen in Tabel 11, terwijl de resultaten per kruising zijn vermeld in de Tabellen 12 tot 28.

De volgende conclusies konden worden getrokken betreffende de resistentie-genen in de I^+I^+-toetsrassen.

1. Resistentie berust op een aantal recessieve genen. Eén ervan is stam-aspecifiek en complementair met elk van een serie stamspecifieke genen. Er is resistentie als het stam-aspecifieke gen aanwezig is samen met ten minste één stamspecifiek resistentie-gen dat effectief is tegen de betrokken stam. Het stam-aspecifieke gen is aanwezig in alle in deze kruisingen gebruikte I^+I^+-toetsrassen, behalve in Dubbele Witte.
2. De toetsrassen Imuna, RG-B, Michelite, GN 31 en IVT 7214 hebben behalve het stam-aspecifieke gen ieder een verschillend stamspecifiek resistentie-gen, voorlopig genoemd naar het ras waarin het werd aangetroffen.
3. Pinto 114, GN 31 en IVT 7214 hebben ieder twee stamspecifieke resistentie-genen naast het stam-aspecifieke gen. Pinto 114 heeft het Michelite-gen en het Imuna-gen, GN 31 heeft het RG-B-gen als tweede stamspecifieke gen, en IVT 7214 het Michelite-gen.
4. De Imuna- en RG-B-genen zijn allel of sterk gekoppeld. Als het laatste het geval is, hebben RG-B en GN 31 ook het Imuna-gen. De Michelite- en GN 31-genen zijn allel. In toetsingen van ongeveer 10 000 planten van de F_2 Michelite x GN 31 werd geen enkele dubbelrecessieve recombinant gevonden (Tabel 29). Als de Imuna- en RG-B-genen ook allel zijn, zijn de vijf stamspecifieke resistentie-genen aanwezig op drie loci. De op deze loci aanwezige genen erven onafhankelijk van elkaar over of zijn zwak gekoppeld.
5. De overerving van het stam-aspecifieke gen is onafhankelijk van de stamspecifieke genen.

6. Genotypen voor resistentie en pathogeniteit. Petersen (1958) gebruikte de symbolen *a* en *s* voor twee genen van GN 15, die resistentie gaven tegen stam Voldagsen. Deze symbolen werden echter ook gebruikt voor andere genen van de boon en werden niet geautoriseerd voor resistentie tegen BCMV. De volgende zes gensymbolen, die *a* en *s* vervangen, worden daarom nu voorgesteld: *bc-u* voor het stam-aspecifieke resistentiegen, *bc-1* voor het Imuna-gen, *bc-1*^{2} voor het RG-B-gen, *bc-2* voor het Michelite-gen, *bc-2*^{2} voor het GN 31-gen en *bc-3* voor het IVT 7214-gen. De resistentiegenotypen van de toetsrassen met I^+I^+ zijn vermeld in Tabel 30.

In overeenstemming met het gen-om-gen model (Person, 1959) heeft een stam die de resistentie-genen van één of meer toetsrassen heeft doorbroken, pathogeniteitsgenen die corresponderen met deze resistentie-genen. Aldus kunnen de pathogeniteitsgenen P1, P1^2, P2 en P2^2 worden verondersteld aanwezig te zijn in BCMV, verdeeld over de stammen zoals weergegeven in Tabel 30. Met dit systeem van vier pathogeniteitsgenen, die cor-

responderen met vier resistentie-genen waarvan sommige allel zijn, een vijfde resistentiegen (*bc-3*), tot dusver zonder een overeenkomend pathogeniteitsgen, een stam-aspecifiek resistentie-gen en een necrose-gen, kunnen de resultaten van dit onderzoek worden verklaard. De meeste pathogeniteitsgenen zijn blijkbaar niet allel, maar allelie van P2 en $P2^2$ is niet uitgesloten. De gen-om-gen relatie, weergegeven in Tabel 30, is de meest uitgebreide die tot nu toe is beschreven bij een waardplant – virusrelatie.

Een volledig gen-om-gen model voor de resistentie- en pathogeniteitsgenen is uitgewerkt in Tabel 31. Zeven van de achttien theoretisch mogelijke toetsrassen met verschillende combinaties van de vijf resistentiegenen zijn gevonden en één werd geselecteerd uit een kruising tussen RG-B en Michelite. Van de resterende tien combinaties zijn er acht met het gen *bc-3*. Het bestaan van deze combinaties met *bc-3*, die anders zijn dan die in IVT 7214, is niet waarschijnlijk. De twee dan nog resterende resistentiegenotypen konden niet gevonden worden, omdat de geschikte virusstammen voor herkenning ontbraken.

Zestien genotypen voor pathogeniteit zijn mogelijk, als wordt aangenomen dat alle vier pathogeniteitsgenen aanwezig zijn op verschillende loci (Tabel 31), of twaalf, indien P2 en $P2^2$ allel zijn. Zeven van de zestien of twaalf mogelijke virusstammen zijn gevonden. Vier konden niet gevonden worden wegens ontbrekende resistentiegenotypen, nodig voor differentiatie. Met de nu bekende toetsrassen kunnen nog vijf stammen worden gevonden, of drie, indien P2 en $P2^2$ allel zijn. De stammen evolueren blijkbaar, zoals aangegeven in Figuur 14. Dit lijkt een ontwikkeling waarbij door mutatie steeds één nieuw gen wordt toegevoegd aan de bestaande genen voor pathogeniteit.

Het is theoretisch mogelijk om de tien ontbrekende toetsrassen te selecteren uit F_2-populaties van kruisingen tussen toetsrassen. Hiervoor moet de F_2 worden getoetst met een voor iedere kruising geschikte virusstam, moeten toetskruisingen worden gemaakt met de resistente F_2-planten en de F_1 daarvan worden getoetst met passende virusstammen om de gewenste genotypen te bepalen.

7. Overerving van de resistentie van planten met dominant necrose-gen. Ieder van de toetsrassen Widusa, Jubila, Topcrop, Amanda en IVT 7233, die de resistentiegroepen 8, 9a, 9b, 10 en 11 vertegenwoordigen, werd gekruist met Imuna, Michelite en GN 31, waarna de F_2 werd getoetst met verschillende stammen. Daar de resistentie-genen van de ouder met I^+I^+ bekend waren, evenals de pathogeniteitsgenen van de virusstammen, konden de resistentiegenen van de ouder met *II* worden afgeleid uit de splitsingsverhoudingen van de F_2. De stammen NL 1, NL 2, NL 4 en NL 5 werden gebruikt om de F_2 te toetsen, terwijl NL 3 en NL 6 soms werden gebruikt voor aanvullende gegevens.

Uit de toetsingsresultaten van de kruisingen met Widusa (Tabellen 32 en 33) is geconcludeerd dat dit ras noch een stamspecifiek, noch het stam-aspecifieke gen bezit. Jubila (Tabellen 34 en 35) heeft alleen *bc-1*, evenals Topcrop (Tabellen 36 en 37). Amanda (Tabellen 38 en 39) heeft $bc\text{-}1^2$. In IVT 7233 (Tabellen 40 en 41) zijn *bc-u*, $bc\text{-}1^2$ en $bc\text{-}2^2$ aanwezig, maar $bc\text{-}1^2$ ontbreekt in sommige planten. Jubila en Topcrop behoren tot dezelfde resistentiegroep. Hun verschil in reactie na inoculatie met necrose-inducerende stammen is kwantitatief en niet kwalitatief.

De gevonden en theoretische combinaties van stamspecifieke genen in toetsrassen met *II* zijn vermeld in Tabel 42. Vier van de vijf onderzochte toetsrassen met *II* hebben de dominante $bc\text{-}u^+$-allelen, in tegenstelling tot de I^+I^+-toetsrassen, die allemaal gen *bc-u* bezitten, behalve Dubbele Witte. Dit wordt verklaard door de resistentie van rassen met *II*

tegen de stammen die geen necrose induceren. Deze stammen kwamen overwegend voor in de gebieden waar de resistentieveredeling van bonen door inkruisen van *II* plaats vond, zodat genotypen zonder en met *bc-u* niet van elkaar konden worden onderscheiden. Een tweede oorzaak zou de onvolledige expressie van allel *bc-u*$^+$ kunnen zijn, vooral voorkomend in genotypen met *II*.

8. Enige gevolgtrekkingen voor de veredeling. Gebruik van *II*-rassen vermindert het door het virus veroozaakte oogstverlies. De virusaantasting is in een *II*-gewas meestal lager dan in één met *I*$^+$*I*$^+$, omdat (1) het virus in *II*-planten niet met zaad overgaat, (2) bladluizen het virus alleen maar kunnen introduceren vanuit een nabijgelegen *I*$^+$*I*$^+$-gewas, (3) virusoverdracht door bladluizen vanuit *II*-planten met systemische necrose nooit is waargenomen, zodat verdere verspreiding binnen een *II*-gewas niet plaats vindt. Het uitsluitend gebruik van rassen met *II* zou infectie door BCMV volledig voorkomen, omdat *Phaseolus vulgaris* nagenoeg de enige natuurlijke waardplant van het virus is.

Zolang *I*$^+$*I*$^+$-rassen worden geteeld naast die met *II*, is de bescherming door *II* onvoldoende. Het is noodzakelijk om eveneens stamspecifieke genen in te kruisen, die niet doorbroken zijn door de necrose-inducerende stammen. Bij voorkeur zouden twee van zulke genen moeten worden gebruikt, om een grotere kans te hebben op een duurzame resistentie. Gebaseerd op de huidige kennis lijkt de beste combinatie van resistentie-genen *bc-u bc-1*^{2} *bc-2*^{2} *bc-3 I* te zijn, waarin resistentie-genen uit IVT 7233 en IVT 7214 zijn gecombineerd. De genen *bc-2*^{2} en *bc-3* zijn beide effectief tegen alle necrose-inducerende stammen en het laatste gen bovendien tegen alle andere stammen.

Acknowledgements

This study was carried out at the Institute for Horticultural Plant Breeding (IVT), Wageningen, the Netherlands.

I am much indebted to Ir. C. Dorsman, director of the IVT, and to the board of the institute for the opportunity to perform this study.

My sincere thanks are due to Prof. Dr. J. Sneep and Prof. Dr. J.P.H. van der Want for their stimulating interest and discussions and for their suggestions in the preparation of the manuscript, and to Dr. J.E. Parlevliet for discussing the host – virus system.

Thanks are extended to my friends and colleagues Dr. L. Bos, for discussing the virus aspects, and to Dr. N.C. Hogenboom and Dr. L.J.D. Sparnaay, for criticizing the genetical and breeding aspects.

Mr. A. van Steenbergen is gratefully acknowledged for technical assistance, and Mr. G. Berends for polishing the English text.

References

Alconero, R., J.P. Meiners & A. Santiago, 1972. A new strain of common bean mosaic in Puerto Rico. Phytopathology 62: 667 (Abstr.).

Alconero, R. & J.P. Meiners,1974. The effect of environment on the response of bean cultivars to infection by strains of bean common mosaic virus. Phytopathology 64: 679-682.

Ali, M.A., 1950. Genetics of resistance to the common bean mosaic virus (bean virus 1) in the bean (*Phaseolus vulgaris* L.). Phytopathology 40: 69-79.

Allard, R.W., 1960. Principles of plant breeding. First edition, John Wiley & Sons, New York, London.

Andersen, A.L. & E.E. Down, 1954. Inheritance of resistance to the variant strain of the common bean mosaic virus. Phytopathology 44: 481 (Abstr.).

Association of Official Seed Certifying Agencies, 1971. Bean, pea and cowpea certification standards. Association of Official Seed Certifying Agencies (AOSCA) certification handbook, Publication 23, Supplement 1974: 21.

Bean Improvement Cooperative, Bean Germplasm Committee, 1965. List of genes of *Phaseolus vulgaris* L. Bean Improvement Cooperative Report 8: 4-20.

Black, W., C. Mastenbroek, W.R. Mills & L.C. Peterson, 1953. A proposal for an international nomenclature of races of *Phytophtora infestans* and of genes controlling immunity in *Solanum demissum* derivates. Euphytica 2: 173-179.

Blok, I., 1973. First circular letter Working Group International *Bremia* Project. IPO, Wageningen.

Bos, L., 1970. The identification of three new viruses isolated from *Wisteria* and *Pisum* in the Netherlands, and the problem of variation within the potato virus Y group. Netherlands Journal of Plant Pathology 76: 8-46.

Bos, L., 1971a. Bean common mosaic virus. Commonwealth Mycological Institute/Association of Applied Biologists. Description of Plant Viruses 73.

Bos, L., 1971b. Circular letter to members of the International Working Group on Legume Viruses involved in research on BCMV. IPO, Wageningen, May 14, 1971.

Bos, L., 1976. Problems and prospects in plant virus identification. European and Mediterranean Plant Protection Organisation (EPPO) Bulletin 6: 63-90.

Cockerham, G., 1955. Strains of potato virus X. Proceedings of the second conference on potato virus diseases, Lisse – Wageningen, 25-29 June 1954. In: Publicatie Laboratorium voor Bloembollenonderzoek, Lisse, no. 4: 89-92.

Comacho, L.H., S. Dana, L.H. Hudson & D.H. Wallace, 1977. Report of the Bean Germplasm Committee. Gene symbol nomenclature. Bean Improvement Cooperative Report 20: 1-3.

Day, P.R., 1956. Race names of *Cladosporium fulvum*. Tomato Genetics Cooperative Report 3: 7-8.

Day, P.R., 1974. Genetics of host – parasite interaction. W.H. Freeman & Co., San Francisco.

Dean, L.L. & C.W. Hungerford, 1946. A new bean mosaic in Idaho. Phytopathology 36: 324-326.

Dean, L.L. & V.E. Wilson, 1959. A new strain of common bean mosaic in Idaho. Plant Disease Reporter 43: 1108-1110.

Drijfhout, E. & L. Bos, 1977. The identification of two new strains of bean common mosaic virus. Netherlands Journal of Plant Pathology 83: 13-25.

Drijfhout, E., M.J. Silbernagel & D.W. Burke, 1978. Differentiation of strains of bean common mosaic virus. Netherlands Journal of Plant Pathology 84: 13-26.

F.A.O., 1975. Production Yearbook 1974. Food and Agriculture Organisation, Rome.

Federation of British Plant Pathologists, Terminology Subcommittee, 1973. Guide to the use of terms in plant pathology. Commonwealth Mycological Institue (C.A.B.), Kew, Phytopathological Papers 17.

Ferguson, J.H.A., 1956. Some applications of binomial probability paper in genetic analysis. Euphytica 5: 329-338.

Flor, H.H., 1956. The complementary genic systems in flax and flax rust. Advances in Genetics 8: 29-54.

Frandsen, N.O., 1952. Untersuchungen zur Virusresistenzzüchtung bei *Phaseolus vulgaris* L. 1. Phytopathologische Untersuchungen. Zeitschrift für Pflanzenzüchtung 31: 381-420.

Gamez, R., A. Osores & E. Echandi, 1970. Una raza nueva del virus del mosaico común del frijol. Turrialba 20: 397-400.

Grogan, R.G. & J.C. Walker, 1948. The relation of common mosaic to black root of bean. Journal of Agricultural Research (Washington, D.C.) 77: 315-331.

Hubbeling, N., 1963. Complicaties bij de toetsing van bonerassen op resistentie tegen *Phaseolus* virus 1 ten gevolge van het voorkomen van afwijkende virusstammen. Mededelingen van de Landbouwhogeschool en de Opzoekingsstations van de Staat te Gent 28: 1025-1033.

Hubbeling, N., 1972. Resistance in beans to strains of bean common mosaic virus. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent 37: 458-466.

I.S.O.-3166, 1974. Codes for the representation of names of countries. International Organisation for Standardization, Geneva.

Iwanowski, D., 1899. Über die Mosaikkrankheit der Tabakspflanze. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abt. II, 5(8): 250-254.

Jenkins, W.A., 1940. A new virus disease of snap beans. Journal of Agricultural Research (Washington, D.C.) 60: 279-288.

Jenkins, W.A., 1941. A histological study of snap bean tissues affected with black root. Journal of Agricultural Research (Washington, D.C.) 62: 683-690.

Kaiser, W.J., D. Danesh, M. Okhovat & H. Mossahebi, 1968. Diseases of pulse crops (edible legumes) in Iran. Plant Disease Reporter 52: 687-691.

Leon, C. & L. Calot, 1973. Efecto del bean mosaic virus (BMV) sobre el rendimiento en dos variedades comerciales de poroto (*Phaseolus vulgaris*). Anales de la Sociedad Cientifica Argentina 196: 21-27.

Martyn, E.B., 1968. Plant virus names, an annotated list of names and synonyms of plant viruses and diseases. Commonwealth Mycological Institute, Kew, Surry, Phytopathological Papers 9.

Martyn, E.B., 1971. Plant virus names, an annotated list of names and synonyms of plant viruses and diseases. Commonwealth Mycological Institute, Kew, Surry, Phytopathological Papers 9, Supplement 1.

Moreno, R., R. Gamez & L.C. Gonzales, 1968. El virus del mosaico común del frijol (*Phaseolus vulgaris* L.) en Costa Rica. Turrialba 18: 257-263.

Nelson, R., 1932. Investigations in the mosaic disease of bean. Michigan Agricultural Experiment Station, Technical Bulletin 118.

Nelson, R., & E.E. Down, 1933. Influence of pollen and ovule infection in seed transmission of bean mosaic. Phytopathology 23: 25.

Pelham, J., 1972. Strain – genotype interaction of tobacco mosaic virus in tomato. Annals of Applied Biology 71: 219-228.

Person, C., 1959. Gene-for-gene relationships in host: parasite systems. Canadian Journal of Botany 37: 1101-1130.

Petersen, H.J., 1958. Beiträge zur Genetik von *Phaseolus vulgaris* L. auf Infektion mit *Phaseolus* Virus 1 Stamm Voldagsen. Zeitschrift für Pflanzenzüchtung 39: 187-224.

Pierce, W.H., 1934. Viroses of the bean. Phytopathology 24: 87-115.

Pierce, W.H., 1935. The inheritance of resistance to common bean mosaic in field and garden beans. Phytopathology 25: 875-883.

Pierce, W.H. & J.C. Walker, 1933. The development of mosaic resistant Refugee beans. Canner 77: 7-9.

Pochard, E., 1977. Etude de la résistance aux souches européennes de virus Y de la pomme de terre (PVY) chez le piment. Compte-rendu du 3e Congrès Eucarpia sur la génétique et la sélection du piment (Avignon-Montfavet), p. 109-118.

Quantz, L., 1957. Ein Schalentest zum Schnellnachweis des gewöhnlichen Bohnenmosaikvirus (*Phaseolus* Virus 1). Nachrichtenblatt für den Deutschen Pflanzenschutzdienst, (Stuttgart) 9: 71-74.

Quantz, L., 1961. Untersuchungen über das gewöhnliche Bohnenmosaikvirus und das Sojamosaikvirus. Phytopathologische Zeitschrift 43: 79-101.
Rast, A.T.B., 1975. Variability of tobacco mosaic virus in relation to control of tomato mosaic in glasshouse tomato crops by resistance breeding and cross protection. Agricultural Research Reports 834, Pudoc, Wageningen.
Reddick D., 1931. La transmission du virus de la mosaique du haricot par le pollen. Rapport du Deuxième Congrès international de Pathologie comparée (Paris, 1931) 1: 363-366.
Reddick, D. & V.B. Stewart, 1919. Transmission of the virus of bean mosaic in seed and observations on thermal death point of seed and virus. Phytopathology 9: 445-450.
Richards, B.L. & W.H. Burkholder, 1943. A new mosaic disease of beans. Phytopathology 33: 1215-1216.
Rieger, R., A. Michaelis & M.M. Green, 1968. A glossary of genetics and cytogenetics. Third edition, Springer-Verlag, Berlin, Heidelberg, New York.
Saettler, A.W. & G.E. Trujillo, 1972. Monroe bean as a local lesion host for bean common mosaic virus. Phytopathology 62: 489-490.
Schippers, B., 1963. Transmission of bean common mosaic virus by seed of *Phaseolus vulgaris* L. cv. Beka. Acta Botanica Neerlandica 12: 433-497.
Schroeder, W.T. & R. Provvidenti, 1968. Resistance of bean (*Phaseobus vulgaris*) to the PV2 strain of bean yellow mosaic virus conditioned by the single dominant gene By. Phytopathology 58: 1710.
Silbernagel, M.J., 1966. Mexican common bean mosaic virus, a new seedborne mosaic of beans. Bean Improvement Cooperative Report 9: 36.
Silbernagel, M.J., 1969. Mexican strain of bean common mosaic virus. Phytopathology 59: 1809-1812.
Skotland, C.B. & D.W. Burke, 1961. A seedborne bean virus of wide host range. Phytopathology 51: 565-568.
Stewart, V.B. & D. Reddick, 1917. Bean mosaic. Phytopathology 7: 61.
Tjallingii, F. & C.M. Rodenburg, 1967. Onderzoek van slarassen op vatbaarheid voor drie fysio's van valse meeldauw (*Bremia lactucae*). Zaadbelangen 21: 104-105.
Trujillo, G.E. & A.W. Saettler, 1972. Local lesion assay of bean common mosaic virus (BCMV) on 'Monroe' bean. Plant Disease Reporter 56: 714-718.
Trujillo, G.E. & A.W. Saettler, 1973. Algunos aspectos relativos a la produccion de lesiones locales en caraota (*Phaseolus vulgaris* L.) por el virus mosaico común de la caraota (BCMV). Agronomía Tropical (Maracay, Venezuela) 23: 379-391.
Want, J.P.H. van der, 1954. Onderzoekingen over virusziekten van de boon (*Phaseolus vulgaris* L.). Mededelingen Instituut voor Plantenziektenkundig Onderzoek 85.
Zaumeyer, W.J. & R.W. Goth, 1962a. A new strain of common bean mosaic virus. Phytopathology 52: 758 (Abstr.).
Zaumeyer, W.J. & R.W. Goth, 1962b. New types of primary necrotic lesions produced by common bean mosaic virus. Phytopathology 52: 1222 (Abstr.).
Zaumeyer, W.J. & R.W. Goth, 1963. Two new types of local lesions produced on beans by the common bean mosaic virus. Phytopathology 53: 490-491.
Zaumeyer, W.J. & R.W. Goth, 1964. A new severe symptom-inducing strain of common bean mosaic virus. Phytopathology 54: 1378-1385.
Zaumeyer, W.J. & H.R. Thomas, 1947. Greasy pod, a new virus disease of beans. Phytopathology 37: 25 (Abstr.).
Zaumeyer, W.J. & H.R. Thomas, 1948. Shiny pod (greasy pod) virus and its identity with black root virus. Phytopathology 38: 29 (Abstr.).

Appendix

List of cultivars tested with several strains of BCMV to determine their resistance group.

Cultivar	Country of origin	Resistance group
Alabama 1	United States	8
Alace Ayse	Turkey	1
Alpha Bonte	Netherlands	1
Amanda	Netherlands	10
Amateur	Netherlands	8
Aranyesö	Hungary	2
Arian	France	9
Aries	France	8
Arla	Sweden	1
Asta	Poland	2
Aurigena	Netherlands	2
Aurora	Switzerland	1
Banat 1	Romania	2
Banat 3	Romania	3
Bataaf	Netherlands	2
Bayerische Alpen	Fed. Rep. Germany	1
Beals	United States	1
Beda	United States	1
Běloruské Polni	Poland	1
Benbe Ayse	Turkey	1
Benishibari	Japan	1
Bequ	United States	1
Bere	United States	1
Berna	Netherlands	1
Beurré Aiguillette	France	2
Beurré Crayon	France	1
Beurré Gloire d'Allanville	France	1
Beurré Perfection	France	1
Beurré sans Rival	France	2
Biala Wyborowa	Poland	5
Bigbend	United States	3
Bismarck	Fed. Rep. Germany	1
Black Mexican	United States	1
Black Turtle Soup	United States	8
Blanc Nain d'Amélioré	France	1
Blanco 137	Guatemala	8 (mixture)[1]
Bland	Australia	8
Blanda	Netherlands	1
Bo 19	Fed. Rep. Germany	4
Bo 22	Fed. Rep. Germany	1
Bomba	Poland	1
Bomba Belaja	Czechoslovakia	1
Boterkoningin	Belgium	10
Bounteous	United States	5
Bountiful	United States	1
Brasil 343 Mulatinho	Brazil	8
Bronowicka	Czechoslovakia	1

Cultivar	Country of origin	Resistance group
Brown Beauty	Australia	1
Bulharsko 2638	Poland	1
Burnley Conquest	Australia	9
Bush Blue Lake	United States	9
Buter Boranya	Yugoslavia	1
Butterzart	Fed. Rep. Germany	2
Cabanais Hâtif	France	8
Cabanais Tardif	France	9
California Small White	United States	1
Canellini	Italy	1
Canieu	France	9
Canning King	United States	9
Caralto	Netherlands	1
Cardinal zöldhüvelnú szalhamentes	Czechoslovakia	1
Carlos Favorite	Denmark	2
Carmencita	Denmark	1
Ceka	Netherlands	1
Centrum	Netherlands	1
Charlottetown	United States	1
Cherokee	United States	1
Chocolade Bruine z.dr.	Netherlands	1
Choctaw	United States	9
Citroengele	Netherlands	3
Coco Blanche	France	9
Coco Rose d'Eyragues	France	1
Colana	Netherlands	9
Columbia Pinto	United States	3
Common Red Mexican	United States	1
Commodore	United States	1
Complet	France	1
Comtesse de Chambord	France	1
Conserva	Netherlands	1
Cornell 49-242	United States	8
Crussol	France	1
Cuilapa 72	Guatemala	8
Declivus Romulus	German Dem. Rep.	1
Delicata z.dr.	Netherlands	1
Delikat	Sweden	1
Dickfleischige o.f.	Fed. Rep. Germany	1
Dixie Belle	United States	9
Domaci Rose	Yugoslavia	5
Domina	Fed. Rep. Germany	1
Drabant 50	Sweden	2
Dromois (1e)	France	1
Dubbele Witte z.dr.	Netherlands	1
Dublette	Fed. Rep. Germany	1
Du Bua	France	9
Early Giant	United Kingdom	2
Early Marrow Pea	United States	4

Cultivar	Country of origin	Resistance group
Early Warwick	United Kingdom	1
Early Wax	United States	1
Eastern Butterwax	United States	1
Eastern Horticultural	United States	1
Ecuador 299	Ecuador	1
Emerson 15	United States	8
Empereur de Russie	France	3
Enfant de Mont Calme	Switzerland	1
Erfurter Speck m.F.	Fed. Rep. Germany	2
Evolutie	Netherlands	9
Exponent	Netherlands	1
Extender	United States	9
Famos	Fed. Rep. Germany	3
Fana	Poland	1
Favorit	Fed. Rep. Germany	1
Feine von Montreux	Switzerland	1
Feltham Prolific	United Kingdom	1
Festivo	Netherlands	1
Fijne Trosprinses m.dr.	Netherlands	1
Finalto	Netherlands	1
Fin de Linas	France	1
Fin des Fins	France	1
Fin de Bagnols	France	1
Fiskeby	Sweden	1
Flageolet à Feuille d'Ortie	France	1
Flageolet Blanc à Longue Cosse	France	3
Flageolet Chevrier	France	1
Flageolet du Vitry Blanc	France	1
Flageolet Roi de Vert	France	1
Flageolet très Hâtif d'Etamps	France	1
Flight	United States	9
Friesenfreund	Fed. Rep. Germany	1
Full Measure	United Kingdom	1
Gan	Netherlands	9
Geca	Netherlands	8
Giant Stringless Greenpod	United Kingdom	1
Gill's Reliable	United States	1
Gloire d'Aubagne	France	1
Gloire de Deuil	France	1
Gloire de Saumur	France	1
Glückauf	Fed. Rep. Germany	1
Golden May	Netherlands	1
Golden Pod Wax	United States	2
Golden Wax	United States	1
Grandessa	Fed. Rep. Germany	1
Great Northern Nebraska 1	United States	3
Great Northern 1140	United States	3
Great Northern Tara	United States	1
Great Northern UI 1	United States	3
Great Northern UI 15	United States	4

Cultivar	Country of origin	Resistance group
Great Northern UI 16	United States	6
Great Northern UI 31	United States	6
Great Northern UI 59	United States	3
Great Northern UI 61	United States	2
Great Northern UI 123	United States	3
Greencrop	United States	1
Greenstar	Netherlands	2
Gustoza	Netherlands	2
Hanacka belosemenna	Czechoslovakia	1
Harvester	United States	9
Hawkesbury Wonder	Australia	1
Heinrichova obrivoskovka	Czechoslovakia	5
Herold	Fed. Rep. Germany	1
Hinrichs Riesen bunt m.F.	Fed. Rep. Germany	1
Hinrichs Riesen bunt o.F.	Fed. Rep. Germany	1
Hinrichs Riesen weiszgrundig m.F.	Fed. Rep. Germany	1
Hinrichs Riesen weiszgrundig o.F.	Fed. Rep. Germany	1
Horoz	Turkey	1
Hundert für Eine	Switzerland	1
Hylowská Bilá	Czechoslovakia	2
Hylowská hnědá žlutá	Czechoslovakia	3
ICA Bunsi	Colombia	8
ICA Duva	Colombia	2
ICA Guali	Colombia	2
ICA Tui	Colombia	8
Ideal	Switzerland	2
Ima	Poland	2
Improved Tendergreen	United States	9
Imuna	Fed. Rep. Germany	2
L'Inépuisable	France	1
IVT 7214	Netherlands	7
IVT 7233	Netherlands	11
Jaguar	Fed. Rep. Germany	9
Jamapa	Mexico	8
Jaune du Canada	France	1
Jaune de la Chine	France	1
Jolanda	Netherlands	9
Jubila	Fed. Rep. Germany	9
Jutta	German Dem. Rep.	1
Kaboon	Netherlands	1
Kabumbu	Kenia	1
Kairyo Chunaga	Japan	1
Kairyo Otebo	Japan	1
Kidney Wax Stringless	United States	1
Kievit Koekoek	Netherlands	1
Kintoki	Japan	1
Kitahara Beninaga	Japan	1
Kleine Weisse	Switzerland	1

Cultivar	Country of origin	Resistance group
Koda	Czechoslovakia	1
Koda	Poland	3
Konservova Voskovska	Czechoslovakia	1
Kora	Czechoslovakia	2
Koralle	Fed. Rep. Germany	9
Kozienicka	Poland	1
Krakovska	Poland	1
Krombek	Netherlands	1
Kuizura	Japan	1
Kustovaja	Czechoslovakia	1
Landreth's Stringless	United Kingdom	1
Lastovici	Czechoslovakia	5
Lednicka Pravda	Czechoslovakia	8
Leeton	Australia	8
Limelight	United Kingdom	1
Lintorpa Attraktion	Fed. Rep. Germany	2
Lintorpa Frühe	Fed. Rep. Germany	1
Lit	Netherlands	9
Litago	Norway	1
Londoner Markt m.F.	Fed. Rep. Germany	1
Londynska Trzni	Czechoslovakia	1
Longimuna	Fed. Rep. Germany	2
Lorex	Fed. Rep. Germany	1
Lotus	Netherlands	1
Marafax	United States	1
Marché de Saumur	France	1
Marocain	France	1
Master	Denmark	9
Masterpiece	United Kingdom	1
Medford	United States	3
Medra	German Dem. Rep.	1
Meridional	France	9
Meteor	Fed. Rep. Germany	1
Métis	France	5
Métis du Sultan Hâtif	France	1
Métis extra pour Maraîchers	France	1
Metorex	Fed. Rep. Germany	9
Mexican	Mexico	1
Mexican 142	Kenia	1
Michigan	United States	9
Michelite	United States	4
Michelite 62	United States	4
Mignon	Netherlands	1
Ministry	Iran	9
Mironovskaja 14	Russia	1
Mogul	Sweden	2
Monroe	United States	6
Mont d'Or	France	1
Moravia	Czechoslovakia	2
Multima	Fed. Rep. Germany	1

Cultivar	Country of origin	Resistance group
Mwezi Moja	Kenia	1
Myrto	France	9
Negus	Poland	9
Nep 2	Costa Rica	8
New Abundance	Canada	8
Nimbus	Sweden	1
Nobila	German Dem. Rep.	1
Noir et blanc	France	1
Nouvel Ermitage	France	3
Nova	Fed. Rep. Germany	1
Nyampuny	Kenia	1
Oblongus	Poland	1
Olivka Zelenaja	Czechoslovakia	1
Olsok	Sweden	1
Olomouckà Zelenoluskà	Czechoslovakia	2
Omonuri	Kenia	1
Orionbiale	Poland	1
Otebo	Japan	1
Pallas	Fed. Rep. Germany	1
Parathan Viasz	Czechoslovakia	1
Pasuljica	Yugoslavia	1
Perreux (du)	France	1
Peru 257	Peru	9
Petit Potager	France	1
Pfalzgräfin	Fed. Rep. Germany	9
Phaseolus aborigineus	Fed. Rep. Germany	1
Phaseolus tuberosus	Fed. Rep. Germany	1
Phenix Claudia	France	1
Phönix Zuckerbrech o.F.	Switzerland	1
Pink Eye Bean	United States	1
Pinto	United States	5
Pinto UI 78	United States	4
Pinto UI 111	United States	4
Pinto UI 114	United States	5
Podrige de Courtry Blanc	France	1
Podrípska	Czechoslovakia	1
Polskaya	Poland	4
Porrillo Sintetico	El Salvador	8
Porynska Voskovka Pravá	Czechoslovakia	2
Précose Nain	France	1
Précose de Saumur	France	2
Prédome nain	France	1
Predule	Netherlands	1
Premier	United Kingdom	2
Prince (the)	United Kingdom	1
Prinzessa	Fed. Rep. Germany	1
Probatine	Netherlands	1
Probator	Netherlands	1
Processor	United States	9

Cultivar	Country of origin	Resistance group
Proxa	Hungary	1
Puebla 152	Mexico	1
Puebla 304	Mexico	1
Puregold Wax	United States	2
Recent	Netherlands	1
Rekord	Denmark	2
Red Kidney	United States	1
Redlands Autumncrop	Australia	8 (mixture)
Redlands Greenleaf B	Australia	3
Redlands Greenleaf C	Australia	2
Redlands Pioneer	Australia	2
Red Mexican	United States	4
Red Mexican UI 34	United States	4
Red Mexican UI 35	United States	6
Red Mexican UI 36	United States	2
Red Mexican UI 37	United States	3
Reflex	Denmark	1
Régalfin	France	3
Regina	Fed. Rep. Germany	1
Regula	Fed. Rep. Germany	1
Regulex	Fed. Rep. Germany	9
Remus	German Dem. Rep.	2
Richgreen	United States	9
Robust	United States	4
Roem van Holland	Netherlands	1
Rofin	Netherlands	9
Rognons à la Coque	France	1
Rojo 70	El Salvador	1
Rondelle	Netherlands	9
Royal Red	United States	9
Rustproof Golden Wax	United States	1
Sabre nain	France	1
Sacharnaja Gribovskaja	Czechoslovakia	1
Saconel	France	9
Sadaf	Iran	1 (mixture)
Salia	Fed. Rep. Germany	9
San Andres 1	Venezuela	9
Sans Rival	France	1
Sansy	Fed. Rep. Germany	9
Sartre Nain	France	1
Saxa	Fed. Rep. Germany	1
Saxa 70	Poland	2
Seafarer	United States	8
Seaway	United States	8
Selecta	German Dem. Rep.	1
Shad	Iran	1
Simplo	Netherlands	1
Sirokostručnája	Czechoslovakia	1
Sitan Beli	Yugoslavia	5
Slavia	Yugoslavia	2

Cultivar	Country of origin	Resistance group
Slovenska Perlicka	Czechoslovakia	1
Small White UI 74	United States	4
Soisson Blanc Hâtif	France	1
Soisson Gros Pied à Parchemin	France	1
Spirit	Netherlands	9
Stella	Sweden	1
Stolka	Poland	1
Stringless Green Refugee	United States	1
Stringless Red Valentine	United States	1
Succulent	France	1
Suisse Blanc Hâtif	France	1
Suisse Blanc Lingot	France	1
Suisse Rouge	France	5
Sulphur	United States	1
Sultan	Czechoslovakia	1
Superlative	United Kingdom	1
Super Phénix à Grain Blanc	France	1
Surecrop Black Wax	United States	1
S-182-N	Costa Rica	8
Taisho Kintoki	Japan	1
Taisho Shirokintoki	Japan	1
Tendergreen	United States	1
Tendergreen No 32304	United States	9
Tenderlong 15	United States	1
Titan	Chile	9
Topcrop	United States	9
Topmost	United States	8
Toscanelli	Italy	8
Transvaalse Bonte z.dr.	Netherlands	1
Transvaalse Bonte m.dr.	Netherlands	1
Triomphe de Farcy	France	2
Tristan	Fed. Rep. Germany	9
Triumf Sacharnyj	Czechoslovakia	1
Troketta	Fed. Rep. Germany	9
Trujillo 4	Venezuela	1
Tsunetomi Nagauzura	Japan	1
Turon	Australia	1
Turrialba 1	Costa Rica	8
Turrialba 4	Costa Rica	8
Unima	Fed. Rep. Germany	1
Unrivalled Wax	United States	1
Venus	Netherlands	1
Verbeterde Perfect z.dr.	Netherlands	1
Victoire (la)	France	2
Viouret Ermitage	France	1
Volgers	Netherlands	1
Voskovka Rýnska	Czechoslovakia	2

Cultivar	Country of origin	Resistance group
Wachs Aurora	Fed. Rep. Germany	1
Wachs Beste von Allen	Fed. Rep. Germany	1
Wachs Express	Sweden	2
Wachs Füllhorn m.f.	Fed. Rep. Germany	2
Wachs Gärtnerstolz	Fed. Rep. Germany	1
Wachs Gemma o.F.	Fed. Rep. Germany	2
Wachs Goldene Ernte	Fed. Rep. Germany	3
Wachs Goldvital	Fed. Rep. Germany	1
Wachs Mont d'Or	France	1
Wachs Protecta	Fed. Rep. Germany	1
Wachs Resista o.F.	Fed. Rep. Germany	2
Wachs Rheinland m.F.	Fed. Rep. Germany	1
Wachs Saxagold	Fed. Rep. Germany	2
Wachs Triumph	Sweden	2
Wachs Tschermaks o.F.	Fed. Rep. Germany	1
Wachs Unerschöpfliche	German Dem. Rep.	1
Wachs Wunder o.F.	Fed. Rep. Germany	1
Wädenswil o.F.	Switzerland	1
Walcherse Witte	Netherlands	1
Walo	Fed. Rep. Germany	2
Wardwell Wax .	United States	1
Watex	Fed. Rep. Germany	1
White from Bontoc	United States	1
White Marrowfat	United States	1
Wild Goose	United States	1
Widuco	Netherlands	8
Widusa	Netherlands	8
Wiejska	Poland	1
Wintergreen	South Africa	9
Witte Reuzen	Netherlands	1
Wyla	Fed. Rep. Germany	2
Yanco	Australia	8
Yes Ayse	Turkey	1
Zeltaja Gora	Czechoslovakia	1
Zenevská Tržni	Czechoslovakia	1
Zlatni Voscovac	Yugoslavia	1
Zlatno Zrno	Yugoslavia	2
Zlaty Klnenot	Czechoslovakia	1
Zlaty Roh	Czechoslovakia	2
Zlotka	Czechoslovakia	1
Zluta Charbinska	Czechoslovakia	1
Zutotrban	Yugoslavia	5
Zwarte Belgische	Belgium	1

1. Mixture of resistance genotypes, most of the plants belonging to the resistance group mentioned.