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# Differentiation of strains of bean common mosaic virus

E. DRIJFHOUT[1], M. J. SILBERNAGEL[2] and D. W. BURKE[2]

[1] Research plant breeder, Institute for Horticultural Plant Breeding (IVT), Wageningen, the Netherlands
[2] Research Plant Pathologists, USDA, Agricultural Research Service, Irrigated Agriculture Research and Extension Center, Prosser, Washington, USA

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**Abstract**

Pathogenicity and symptom expression of seventeen described isolates of bean common mosaic virus (BCMV) and five previously unreported isolates were compared on many bean cultivars (*Phaseolus vulgaris* L.). From these cultivars, a standard set of differentials were assigned to nine groups with different disease reactions. The twenty-two virus isolates comprised seven strain (pathotype) groups, three of which were divided into two subgroups each. To promote international standardization in BCMV research, recommendations are given for test conditions and procedures, criteria for strain differentiation, and maintenance of differential cultivars and virus strains.

## Introduction and literature review

Bean common mosaic virus (BCMV) is a seed-borne pathogen of common bean (*Phaseolus vulgaris* L.) that is aphid-transmitted non-persistently. It is found in all parts of the world where beans are grown, and is economically important in many areas (Zaumeyer and Thomas, 1957; Bos, 1971a).

Grogan and Walker (1948) reported that at temperatures above 30 °C some resistant cultivars developed severe vascular necrosis in the leaves, stems, shoot tips and roots. This reaction was referred to as 'black root'. At normal (20–28 °C) growing temperatures, those cultivars were resistant to the type and NY15 strains, then prevalent in the United States.

Later, Ali (1950) showed that this resistance was governed by a dominant inhibitor gene *I*. This gene restricts virus multiplication at normal growing temperatures and permits development of 'black root' at high temperatures. Most garden bean cultivars developed for processing in the United States carry the *I* gene which protects against all known BCMV strains in the United States (Silbernagel, 1969).

Thomas and Fisher (1954) showed that the *I* gene could be detected in segregating breeding lines by local vein necrosis and systemic necrosis in inoculated plants at 32 °C. However, Zaumeyer and Goth (1964) found a strain (Florida) which did not induce a necrotic reaction at high temperatures, while Hubbeling (1963) reported other strains in the Netherlands which induced a severe systemic necrosis at lower (15–20 °C) temperatures in cultivars with gene *I*. Subsequently, European breeders of garden cultivars have not relied on the *I* gene for BCMV resistance as much as breeders have in the United States. Rather, European breeders have utilized more of the specific (vertical) genes (Drijfhout, 1978) that confer resistance to individual strains of the virus.

BCMV readily develops new strains by mutation, which infect previously resistant cultivars. Several isolates were studied in detail, and about twenty 'strains' were described. Those used in this study and their literature citations are shown in Table 1. For a detailed literature survey of these strains see Drijfhout (1978).

Table 1. Strains of bean common mosaic virus used in this study. For simplicity they are already arranged on the basis of their pathogenic relationship, detected during investigation (see Tables 3 and 4).

| Strain group | Isolate | Reference | Origin | Obtained from |
|---|---|---|---|---|
| I | *Type* | Richards & Burkholder (1943) | USA-Washington | Burke[1] |
| | Westlandia (NL1) | van der Want (1954) | Netherlands | Bos[2] |
| | Puerto Rico (PR9M) | Alconero et al. (1972, 1974) | Puerto Rico | Alconero[3] |
| | Iran | Kaiser (unreported) | Iran | Kaiser[4] |
| II | *NL7* | Drijfhout & Bos (1977) | Peru | Drijfhout[5] |
| | R220 | Burke (unreported) | USA-Washington | Burke |
| | S74 | Drijfhout (unreported) | Netherlands | Drijfhout |
| | ATCC-PV25 | Goth (unreported) | USA-New York | Goth[6] |
| III | *NL8* | Drijfhout & Bos (1977) | Netherlands | Drijfhout |
| IVa | *Florida* | Zaumeyer & Goth (1964) | USA-Florida | Zaumeyer[7] |
| IVb | *Idaho 123* | Dean & Wilson (1959) | USA-Idaho | Dean[8] |
| | Western | Skotland & Burke (1961) | USA-Washington | Burke |
| | Colana (NL6) | Hubbeling (1972) | Netherlands | Hubbeling[9] |
| | Bailif | Burke (unreported) | USA-Washington | Burke |
| Va | *NY15* (ATCC-PV28) | Richards & Burkholder (1943) | USA-New York | Zaumeyer |
| Vb | *RM* (NL2) | van der Want (1954) | Netherlands | Bos |
| | Imuna | Hubbeling (1963) | Netherlands | Drijfhout |
| VIa | *Michelite* (NL3) | Hubbeling (1963) | Netherlands | Drijfhout |
| VIb | *Jolanda* (NL5) | Hubbeling (1963) | Netherlands | Hubbeling |
| VII | *Great Northern* (NL4) | Hubbeling (1963) | Netherlands | Drijfhout |
| | Mexican | Silbernagel (1969) | Mexico | Silbernagel[10] |
| | Chile A5 | Alconero (unreported) | Chile | Alconero |

[1] D. W. Burke, Prosser, Washington, USA.
[2] L. Bos, Wageningen, the Netherlands.
[3] R. Alconero, Mayaguez, Puerto Rico (via L. Bos).
[4] W. Kaiser, Washington, D.C., USA (via L. Bos).
[5] E. Drijfhout, Wageningen, the Netherlands.
[6] R. W. Goth, Beltsville, Maryland, USA.
[7] W. J. Zaumeyer, Beltsville, Maryland, USA.
[8] L. L. Dean, Twin Falls, Idaho (via A. W. Saettler, East Lansing, Michigan), USA.
[9] N. Hubbeling, Wageningen, the Netherlands.
[10] M. J. Silbernagel, Prosser, Washington, USA.

*Tabel 1. Stammen van het bonerolmozaïekvirus gebruikt in dit onderzoek. Voor het gemak zijn ze al gerangschikt op basis van hun pathogene verwantschap, vastgesteld tijdens het onderzoek (zie Tabellen 3 en 4).*

Some may have been described as new strains on insufficient grounds, considering the variation in number and type of differential cultivars, methods and environmental conditions employed. Such confusion prompted the International Working Group on Legume Viruses to suggest that a standard set of differential bean cultivars be selected out of those used by various authors and multiplied and distributed by a central agency (Bos, 1971b).

The purposes of the present study were (1) to develop a standard set of bean cultivars for differentiation of strains of BCMV, (2) to classify the previously reported strains and other isolates on those differentials, and (3) to recommend standardized BCMV strain identification procedures for international use.

## Materials and methods

*Terminology.* We have attempted to use terms consistent with the recommendations of the Federation of British Plant Pathologists (1973). We consider 'resistant' as the opposite of 'susceptible' and 'sensitive' as the opposite of 'tolerant'. The two couples of terms refer to basically different phenomena. The first couple indicates the difficulty or ease with which a plant becomes locally and systemically infected, while the second couple of terms indicates the ability or unability of a susceptible plant to produce symptoms and to become damaged as a result of systemic infection. An immune cultivar is incapable of being infected under any condition. A susceptible cultivar is systemically infectable and may be sensitive or tolerant, depending upon 1) the particular BCMV strain – cultivar genotype combination, 2) temperature, or 3) the assay procedure. Therefore precise delineation of the degree of susceptibility and sensitivity depends on standardization of these variables.

With the above limitations in mind we consider a cultivar *susceptible* if it becomes systemically infected and *resistant* if no systemic infection can be detected. A susceptible cultivar is *sensitive* if infection results in moderate to severe disease symptoms and considerable damage. It is *tolerant* if it harbours the virus systemically, but shows slight or no systemic symptoms and damage.

Our criteria for systemic infection of a cultivar are: the development of clear systemic symptoms, or demonstrated presence of virus in uninoculated leaves of plants without systemic symptoms or in plants with very mild or questionable systemic symptoms, by back-inoculation to a very sensitive cultivar like Dubbele Witte or Sutter Pink.

*Virus isolates.* All strains of BCMV described in the literature, of which material could be obtained, were used, as well as some previously unidentified isolates. The virus isolates are listed in Table 1.

Apparently the exact identity of the original type strain of BCMV is lost, since all reports from 1917 (Stewart & Reddick) to 1943 (Richards and Burkholder) presumed all strains to be pathogenically identical. Richards and Burkholder reported that the New York 15 strain was different from previously reported isolates because it caused mosaic in cultivars resistant to the original type strain. We therefore used Richards and Burkholder's report as the earliest description of the type and the NY15 strains. R. W. Goth supplied the American Type Culture Collection isolate PV25 as a representative of the type strain' However, after comparing the reactions of PV25 on a

number of cultivars with a type isolate obtained from D. W. Burke, the latter was chosen to represent the type strain because it produced more distinct symptoms than PV25. Moreover, PV25 fell into strain group II (Table 1), exhibiting a greater pathogenicity range than strain group I, which we feel represents the type group.

Phatak (1974) mentions a Rumanian strain of which the reactions in a range of differentials were compared with data found in a circular letter (Bos, 1971b). He concluded that the strain was closer to the Type strain than to the others mentioned in literature. As his publication reached us after the comparison of the strains had been started, the Rumanian strain was not included in our experiments.

All virus isolates were transmitted through seed of susceptible cultivars before their use in pathogenicity studies, to reduce chances of contamination by viruses that are not seed-borne.

*Bean cultivars.* Seed stocks of differential cultivars were maintained at IAREC, Prosser, Washington, United States, and at IVT, Wageningen, the Netherlands. The differential cultivars were increased in insectprotected greenhouses or screenhouses

Table 2. Bean host groups used for differentiation of BCMV strains. Per group the differentials are ordered to preference.

| Host group | Cultivar | Origin |
|---|---|---|
| A. With *ii* alleles of presumed inhibitor gene | | |
| 1 | Dubbele Witte | Netherlands |
| | Sutter Pink | USA |
| | Strinless Green Refugee | USA |
| 2 | Redlands Greenleaf C | Australia |
| | Puregold Wax | USA |
| | Imuna | Germany |
| 3 | Redlands Greenleaf B | Australia |
| | Great Northern UI 123 | USA |
| 4 | Sanilac | USA |
| | Red Mexican UI 34 | USA |
| | Michelite 62 | USA |
| 5 | Monroe | USA |
| | Great Northern UI 31 | USA |
| | Red Mexican UI 35 | USA |
| B. With *II* alleles of presumed inhibitor gene | | |
| 6 | Jubila | Germany |
| 7 | Topcrop | USA |
| | Improved Tendergreen 40031 | USA |
| 8 | Widusa | Netherlands |
| | Black Turtle Soup | Mexico |
| 9 | Amanda | Netherlands |

*Tabel 2. Groepsindeling van de toetsrassen voor stammendifferentiatie van BCMV. Per groep zijn de rassen gerangschikt naar voorkeur.*

to prevent contamination and then selected for health and trueness-to-type. Pure-line selections were made from all cultivars to insure uniform virus reactions. All available cultivars previously used for BCMV strain differentiation were tested for reaction to the isolates used in this study. The most useful cultivars are listed in Table 2 by country of origin. Cultivars were chosen as differentials on the basis of (1) kinds of systemic symptoms (mosaic vs necrosis, Fig. 1–4) induced by the different isolates and, (2) clearness and reproducibility of symptoms. Distinction was made between differential cultivars with presumed recessive alleles *i* of the inhibitor gene (Ali, 1950), which show mosaic in certain host-virus combinations but never systemic necrosis and cultivars carrying the dominant allele *I*, which show systemic necrosis but never mosaic.

Fig. 1. 'Dubbele Witte' inoculated with strain NL1; leaf rolling and mosaic.
Fig. 2. 'Redlands Greenleaf B' inoculated with strain NL5; green vein banding and flecking.

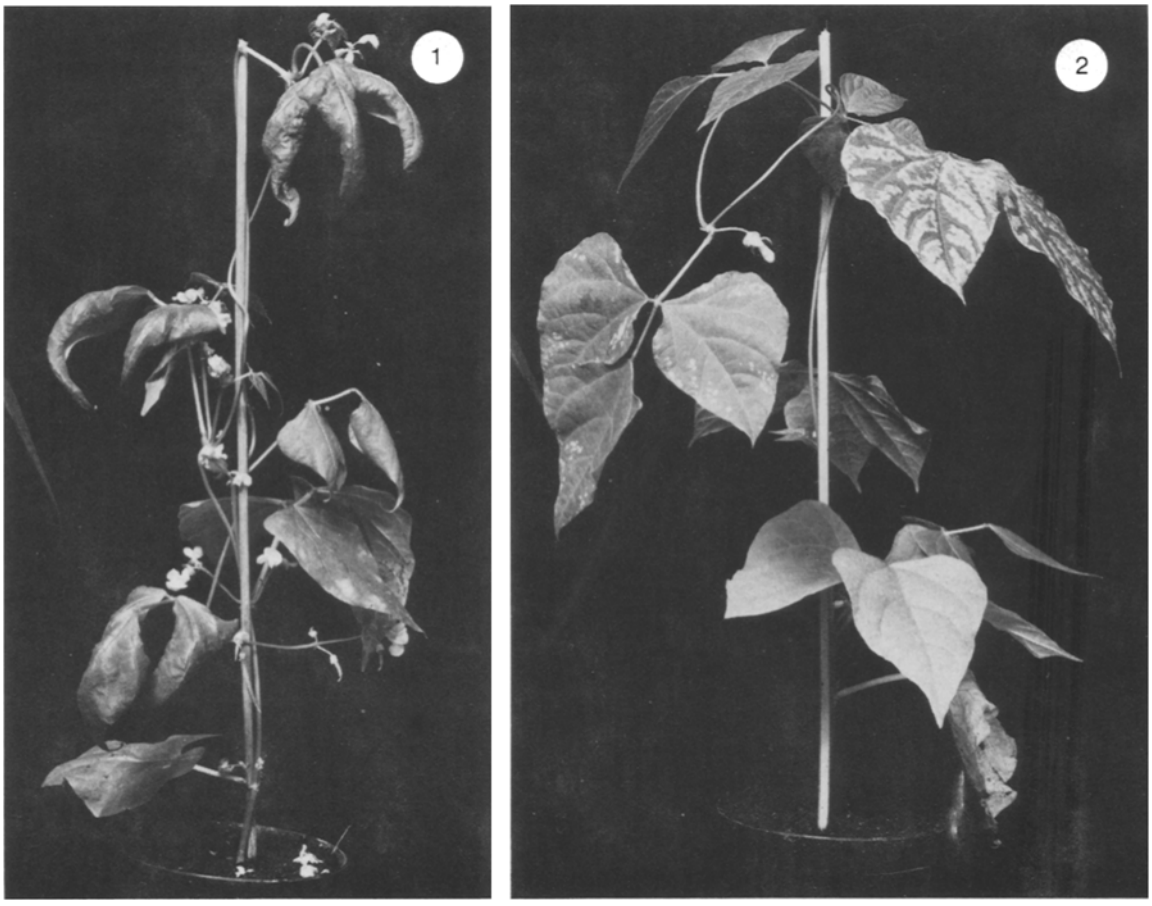
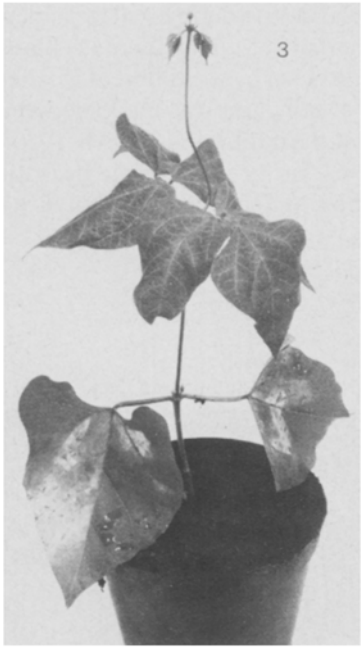



*Fig. 1. 'Dubbele Witte' geïnoculeerd met stam NL1; bladrol en mozaïek.*
*Fig. 2. 'Redlands Greenleaf B' geïnoculeerd met stam NL5; groen nerfbandmozaïek en gevlektheid.*

Fig. 3. 'Dubbele Witte' inoculated with strain NL5; vein chlorosis and leaf malformation.
Fig. 4. 'Widusa' inoculated with strain NL3; local and systemic necrosis (black root).



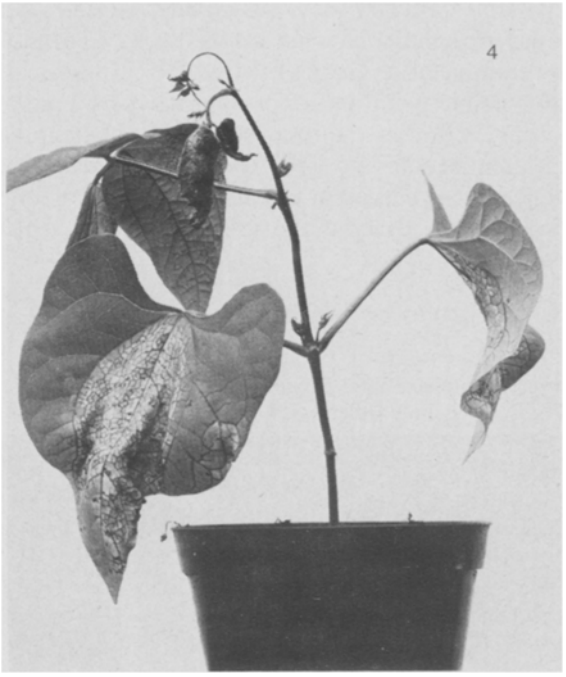




*Fig. 3. 'Dubbele Witte' geïnoculeerd met stam NL5; nerfchlorose en bladmisvorming.*
*Fig. 4. 'Widusa' geïnoculeerd met stam NL3; lokale en systemische necrose (zwarte-vaatziekte).*

*Virus propagation.* Blocks of 10 to 20 pots of 'Dubbele Witte' or 'Sutter Pink', very sensitive to systemic mosaic by all isolates of all strains, were used for virus propagation for inoculum production. A set of differentials with five plants of each cultivar was added to each virus propagation block to monitor virus strain purity.

The following precautions were taken to prevent contamination: (a) virus-free seeds were used for growing source plants and differentials; (b) blocks of plants inoculated with different strains were isolated by distance or with plastic screens; (c) hands and tools were disinfected between operations involving different virus strains; and (d) systemic insecticides were applied to plants weekly, even in apparently insect-free greenhouses and screenhouses.

*Inoculum preparation and inoculation.* Freshly picked leaves of clearly diseased two- to six-week old virus source plants were either triturated with a mortar and pestle in 0.01 M phosphate buffer pH = 7 (1:1, W/V), or ground for one minute in a blendor and the juice extracted through cheesecloth. The juice was diluted 1:10 (dilution end point usually $10^{-3}$ to $10^{-4}$). The primary leaves of the plants to be tested were dusted with 500-mesh carborundum powder and then rubbed lightly with a cotton-tipped applicator or a small piece of foam plastic dipped in the inoculum. Best results were obtained when 1/2- to 3/4-expanded primary leaves were inoculated, washed with tap water, and grown at 23–26 °C.

*Plant testing.* All tests were done in Wageningen and Prosser. The test plants were grown in, or transplanted to, 8 or 12 cm diameter pots, with one plant per pot. They were kept at a mean temperature between 23 and 26 °C (minimum day or night temperature 20 °C, and occasional maximum 30 °C). Additional light was given during winter for 14–16 h a day with cool white fluorescent or high pressure mercury lamps of 400 W (about 100 W/m$^2$).

Four plants of each differential cultivar were inoculated with each strain, and one plant was left uninoculated as a control. The first inoculation was made about ten days after sowing at both locations. At IVT a second inoculation was made 3–5 days later, while at Prosser the second inoculation of symptomless plants was made 12–14 days later. Reactions were recorded one, two, three, and four weeks after the first inoculation. For back-inoculations, from each plant to be assayed three leaflets were taken from the topmost trifoliate leaf and either assayed immediately or after storage and freezing in a small plastic bag overnight. The inoculum was prepared as described above with mortar and pestle or some drips were squeezed by thumb and forefinger out of the defrosted leaflets onto the carborundum-dusted primary leaves of two plants of 'Dubbele Witte' or 'Sutter Pink'. The gloved hands were washed after each back-inoculation.

*Verification of strain purity.* If a virus isolate produced in a standard set of differential cultivars a range of reactions, different from those of known strains, the isolate was considered to be new or a mixture of strains. The isolate was then back-inoculated twice from each susceptible cultivar to a fresh set of the differential cultivars. If all resulting reaction ranges were identical, then the original isolate was considered pure. Deviant reaction ranges produced by passage through one or more sets of the differentials was taken as evidence of strain mixtures. Single-lesion serial transfer and/or passage through selective hosts was sometimes required to obtain pure-culture isolates.

*Maintenance of strains.* Pure isolates were maintained in dry seeds from symptom-expressing plants of 'Dubbele Witte', 'Sutter Pink', or other susceptible cultivars. Usually, 20–80 % of such seeds were infected and pure isolates could be reisolated from such seedlings when needed.

*Criteria for selection of differential hosts and for strain classification.* A cultivar was placed in one of the differential host groups 1 to 5 (Table 2), depending upon which of the virus isolates induced mosaic, leaf malformation, stunting or symptomless infection of that cultivar. Because systemic necrosis could not be induced at high temperatures in any cultivar in these groups, they apparently carried the recessive *i* and not the dominant *I* gene.

New tip growth of plants without or with very mild or questionable systemic symptoms three to four weeks after the first inoculation was assayed for systemic infection by back-inoculation to sensitive plants. Those differential cultivars requiring back-inoculation to prove systemic infection were considered tolerant to the virus strain used. Those cultivars whose assay hosts reacted negatively were considered resistant. Plants recorded as resistant were not considered immune because usually the virus could be recovered from the inoculated leaves for some time before senescen-

ce. Nevertheless, this is a higher level of resistance than is found in cultivars classified as tolerant and we consider this as a critical distinction for plant breeders to note.

The cultivars with dominant alleles of gene *I* that did not have systemic symptoms or expressed local necrosis only were rated as resistant to the inoculum strain. Systemic virus spread in these symptomless plants was very unusual. Severe systemic vascular necrosis (N) usually resulting in tip kill on most plants, indicated a cultivar to be sensitive (N) to an inoculum strain.

Cultivars showing systemic necrosis in only a few of the plants, four weeks after inoculation, were considered variably sensitive (V) to the strain used at a particular temperature. The number of plants with systemic necrosis varied from test to test and usually increased with higher temperatures.

Most experiments were repeated several times both in Wageningen and Prosser, using the same virus isolates and seed lots of the differentials.

## Results

*Differential cultivar groups.* Those cultivars inoculated with the 22 isolates listed in Table 1 that expressed the clearest and most consistent reactions to BCMV strains were chosen as differentials. Nine differential host groups were recognized (Table 2) by their distinctive range of reactions to the 22 isolates. Cultivars showing an identical (or nearly identical) range of reactions were placed in the same host group. To identify a strain of BCMV, a minimum of one cultivar from each of the nine groups of differentials was needed. However, for precise strain identification at least two cultivars were used, where available, in each host group. The cultivars in each host group are listed in order of our preference for use as differentials (Table 2).

*Strain classification.* The 22 virus isolates (Table 1) were classified in seven virus strain groups (Tables 1, 3 and 4) and six subgroups (Tables 5 and 6) on the basis of the disease reactions they produced on the nine differential host groups.

The presence or absence of systemic infection (usually inducing mosaic) in host groups 1 to 5 was the primary basis for virus strain differentiation (Tables 3 and 4). Table 3 shows the reactions to 22 virus isolates by two cultivars each of differential host groups 1 to 5. Reactions are recorded as sensitive (S) tolerant (T) or resistant (R). Table 4 presents a simplified overview of the detailed data in Table 3, to help clarify the basic differences in host-group by strain-group interactions. This view suggests that many strains are not yet discovered or reported, since there are many possible combinations of reactions not shown in Table 4.

*Strain subgroups.* The necrotic reactions of cultivar groups 6 to 9 (presumably containing dominant gene *I*) were considered secondary classification criteria and were used to distinguish subgroups (a and b) within a strain group (Tables 5 and 6). Virus strains in subgroups b induced systemic necrosis more readily than those in subgroups a. For instance, Florida strain (IVa), unlike the strains of IVb, did not induce systemic necrosis at any temperature. The difference between strains NL3 (VIa) and NL5 (VIb) was in the systemic necrosis produced on the cultivar Amanda (Tables 5 and 6). Table 5 lists the systemic necrotic reactions produced at a mean greenhouse temperature between 23 and 26 °C (range 20–30 °C) on cultivars of differential groups 6 to 9.

Table 3. Systemic reactions of cultivars from host groups 1 to 5, inoculated with 22 isolates of bean common mosaic virus, arranged according to strain groups of the virus[1].

| Cultivar[2] | Host group | BCMV strain group and isolates | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | | | | II | | | | III | IV | | | | | V | | | VI | | VII | | |
| | | Type | NL1 | PR9M | Iran | NL7 | R220 | S74 | PV25 | NL8 | Florida | Idaho | Western | NL6 | Bailif | NY15 | NL2 | Imuna | NL3 | NL5 | NL4 | Mexican | Chile |
| Dubbele Witte | 1 | S[3] | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| Sutter Pink | | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| RGL-C | 2 | R | R | R | R | S | T | S | T | R | S | S | S | S | S | T | S | S | T | S | S | T | S |
| Puregold W. | | R | R | R | R | S | T | S | T | R | S | S | S | S | S | T | S | S | T | S | S | S | S |
| RGL-B | 3 | R | R | R | R | R | R | R | R | R | S | S | S | S | S | R | R | R | S | S | S | S | S |
| GN-123 | | R | R | R | R | R | R | R | R | R | S | S | S | S | S | R | R | R | T | T | S | S | S |
| Sanilac | 4 | R | R | R | R | R | R | R | R | S | R | R | R | R | R | S | S | S | S | S | R | R | R |
| RM-34 | | R | R | R | R | R | R | R | R | S | R | R | R | R | R | S | S | S | S | S | R | R | R |
| Monroe | 5 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | S | S | S |
| GN-31 | | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | S | S | S |

[1] Greenhouse tests, 16 h daylight, mean day temperature 23–26 °C (range 20–30 °C).
[2] Cultivars of host groups 1 to 5 presumed to carry recessive alleles of inhibitor gene *I*.
[3] S = Sensitive: moderate to severe systemic mosaic, virus recoverable by assay from tip growth. T = Tolerant: systemic symptoms may be mild, atypical, delayed or absent. Virus recoverable by assay from tip growth. R = Resistant: no systemic symptoms. Virus not recoverable by assay from tip growth.

*Tabel 3. Systemische reacties van rassen uit toetsrasgroepen 1 tot 5, geïnoculeerd met 22 isolaten van het bonerolmozaïekvirus, gerangschikt naar stammengroepen van het virus.*

Table 4. Summary of the interactions between host groups and strain groups from Table 3.

| Host group | BCMV strain group | | | | | | |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII |
| 1 | + | + | + | + | + | + | + |
| 2 | − | + | − | + | + | + | + |
| 3 | − | − | − | + | − | + | + |
| 4 | − | − | + | − | + | + | − |
| 5 | − | − | − | − | − | − | + |

− = Resistant; no systemic symptoms, virus not detectable by assay from new tip growth.
+ = Sensitive or tolerant; moderate to severe systemic mosaic, or systemic symptoms mild, absent, atypical or delayed, but virus detectable by assay from tip growth.

*Tabel 4. Samenvatting van de interacties tussen toetsrassengroepen en stammengroepen uit Tabel 3.*

These cultivars are susceptible to some strains but have resistance at certain temperatures to other strains of BCMV because of the dominant inhibitor gene *I*. However, because the major gene *I*, which confers both a type of horizontal resistance plus necrotic sensitivity, is genetically different from the genes for specific resistance, which condition reactions observed in host groups 1 to 5, we used the symbols I (from inhibitor gene) to indicate resistance, N to indicate sensitivity showing systemic necrosis, and V (for variable) if only a few of the test plants developed systemic necrosis. Table 6 presents a summary of Table 5 showing the major virus strain-differential host reactions.

Local reactions (necrotic and chlorotic) occurred with several virus-host combinations, but these symptoms were not sufficiently reliable for use in strain differentiation.

Table 6. Summary of the interactions between host groups and strain groups from Table 5.

| Host group | BCMV strain group | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IVa | IVb | Va | Vb | VIa | VIb | VII |
| 6 | − | − | − | − | + | − | + | + | + | − |
| 7 | − | − | − | − | + | − | + | + | + | − |
| 8 | − | − | + | − | + | − | − | + | + | − |
| 9 | − | − | − | − | − | − | − | − | + | − |

− = Resistant at the temperatures mentioned in Table 5. No systemic symptoms. Virus not recoverable by assay from plant tips.
+ = Necrotic tip kill of some to all plants in the used temperature range. Virus usually not recoverable by assay from tips of symptomless plants (sensitive or variably sensitive).

*Tabel 6. Samenvatting van de interacties tussen toetsrasgroepen en stammengroepen uit Tabel 5.*

Table 5. Systemic reactions of cultivars from host groups 6 to 9, inoculated with 22 isolates of bean common mosaic virus, arranged according to strain groups of the virus[1].

| Cultivar[2] | Host group | BCMV strain group and isolates | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | | | | II | | | | III | IVa | IVb | | | | Va | Vb | | VIa | VIb | VII | | |
| | | Type | NL1 | PR9M | Iran | NL7 | R220 | S74 | PV25 | NL8 | Florida | Idaho | Western | NL6 | Bailif | NY15 | NL2 | Imuna | NL3 | NL5 | NL4 | Mexican | Chile |
| Jubila | 6 | I[3] | I | I | I | I | I | I | I | I | I | N | N | N | N | I | N | N | N | N | I | I | I |
| Topcrop | 7 | I | I | I | I | I | I | I | I | I | I | V | V | V | V | I | V | V | N | N | I | I | I |
| Imp. Tgn | | I | I | I | I | I | I | I | I | I | I | V | V | V | V | I | V | V | N | N | I | I | I |
| Widusa | 8 | I | I | I | I | I | I | I | I | N | I | V | V | V | V | I | I | I | N | N | I | I | I |
| Blk Tur. | | I | I | I | I | I | I | I | I | N | I | V | V | V | V | I | I | I | N | N | I | I | I |
| Amanda | 9 | I | I | I | I | I | I | I | I | I | I | I | I | I | I | I | I | I | I | N | I | I | I |

[1] Greenhouse tests, 16 h daylight, mean day temperature 23–26 °C (range 20–30 °C).
[2] Cultivars of host groups 6 to 9 presumed to carry dominant alleles of inhibitor gene *I*.
[3] I = Disease expression inhibited by *I* gene: resistant at the above temperatures. No systemic symptoms. Virus not recoverable by assay from plant tips. V = Variably sensitive: some plants may show systemic necrosis at the temperatures mentioned. Virus usually not recoverable by assay from tips of symptomless plants. N = Necrotic tip kill of most or all plants: sensitive.

*Tabel 5. Systemische reacties van rassen uit toetsrasgroepen 6 tot 9, geïnoculeerd met 22 isolaten van het bonerolmozaïekvirus, gerangschikt naar stammengroepen van het virus.*

## Discussion

A comparison of our data with literature reports discloses several discrepancies. Some of the possible causes are: (1) different interpretations of the terms resistant, tolerant, sensitive, susceptible and immune; (2) ommission of, or insufficiently sensitive back-inoculation (assay) procedures, as well as use of inoculated leaves instead of new growth for assay tests; (3) use of different temperature ranges; (4) use of an incomplete set of differential cultivars; (5) use of other cultivars or isolates; (6) use of virus-contaminated differentials; (7) use of sources of a cultivar that may vary genetically in disease reaction; (8) isolate contamination by other viruses or by other isolates of BCMV; and (9) misidentification of another virus as BCMV.

According to present virological standards, many reports of strains of BCMV did not clearly establish that the author(s) were indeed working with BCMV. Conclusive proof should include such lines of evidence as symptoms, seed transmission, thermal inactivation, dilution end point, longevity in vitro, serology and particle photomicrographs and measurements (Bos, 1971a). Most early workers used only seed transmission and symptoms induced in bean plants as identification criteria.

The direct comparisons of virus strains on differentials at Wageningen and Prosser, using the same virus isolates and the same seed lots of the bean cultivars, have enabled us to classify the seventeen described strains available and the five unreported isolates into ten pathotype groups. In fact, within these groups the isolates cannot be distinguished, hence they are identical and isolates of one strain. Then the oldest name, printed in italics in Table 1, has priority.

Back-inoculation onto a very sensitive cultivar, preferably 'Dubbele Witte' or 'Sutter Pink', is essential in cases of no or doubtful systemic mosaic symptoms. The sometimes used local lesion assay to 'Monroe' (Trujillo and Saettler, 1972) gives excellent results with some strains, but, from our experience, with other strains (NL3, NL5, NL8, NL4) an insufficient reaction.

For BCMV strain identification, a daily mean temperature within 23–26 °C is preferred, with the daily maximum not exceeding 30 °C. At higher mean temperatures, qualitatively different reactions in differentials were sometimes observed. Alconero and Meiners (1974) also found different reactions at a constant temperature of 35 °C as compared with constant temperatures of 24 °C, or 20 °C at night and 30 °C at day. The genetic relationship between *Phaseolus vulgaris* and bean common mosaic virus and its strains will be further dealt with in detail by Drijfhout (1978).

## Proposed standardized procedure

Once it is established that a disease is caused by an isolate of BCMV, the test procedures and differential cultivars we propose can be used to determine if the isolate is a pure culture (or mixture), and whether it is similar to or different from previously reported strains. Internationally comparable results can best be obtained in the future if authors use the same procedures, conditions, differential cultivars and virus isolates used in the present study. To this end, the present authors propose to take responsibility for seed and/or strain distribution to researchers concerned with identification of strains of BCMV. Small seed samples will be sent on request for further propagation by the receiver in his greenhouse.

Seed samples of the differentials will also be deposited in the National Seed Storage Laboratory at Fort Collins, Colorado, USA, where they will be available for future research. The virus isolates (in seed) will be deposited in the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA, where they will remain available for virologists.

**Samenvatting**

*Differentiatie van de stammen van het bonerolmozaïekvirus*

Zeventien beschreven stammen van het bonerolmozaïekvirus en vijf niet geïdentificeerde isolaten (Tabel 1) werden bestudeerd op een uitgebreide reeks van toetsrassen. De meeste van deze toetsrassen waren in de literatuur als zodanig vermeld, maar door de desbetreffende onderzoekers waren vaak verschillende series toetsrassen gebruikt, hetgeen de onderlinge vergelijking van de stammen bemoeilijkte.

De bedoeling van dit onderzoek was: vergelijking en indeling van de virusstammen, samenstelling van een standaard-toetsrassenserie en het ontwerpen en beschrijven van een standaard-werkwijze voor de stammenidentificatie van dit virus. De proeven werden zowel in Wageningen als in Prosser, Washington, USA, uitgevoerd met dezelfde virusisolaten en dezelfde zaadmonsters van de toetsrassen.

De toetsrassen konden op grond van hun differentiële reacties na inoculatie met de virusstammen worden ingedeeld in negen groepen. De rassen binnen een groep hebben hetzelfde 'resistentiespectrum' t.o.v. een standaardserie virusstammen. Uit elke groep werden op grond van hun geschiktheid (duidelijkheid en reproduceerbaarheid van de symptomen) één of meer vertegenwoordigers gekozen, waaruit een standaardserie van toetsrassen werd samengesteld (Tabel 2).

De 22 stammen en isolaten werden op grond van hun 'pathogeniteitsspectrum' t.o.v. de standaardserie van toetsrassen ingedeeld in tien groepen en subgroepen (Tabel 1). De stammen en isolaten binnen een groep of subgroep hebben eenzelfde 'pathogeniteitsspectrum' (Tabellen 4 en 6) en worden op grond daarvan als identiek beschouwd. De differentiële reacties tussen de rassen van de standaardserie en de virusstammen en -isolaten zijn vermeld in de Tabellen 3 en 5. Voorgesteld wordt om de naam van de eerstbeschreven stam van iedere groep te handhaven en de andere stammen in een groep of subgroep op te vatten als isolaten daarvan.

De toetsmethodiek wordt uitvoerig beschreven om standaardisatie van de stammenidentificatie te bevorderen. Ter verklaring van de in de literatuur gevonden tegenstrijdigheden in de differentiële reactie van de toetsrassen wordt een negental mogelijke oorzaken genoemd, o.a. het gebruik van planten van toetsrassen die reeds vanuit zaad met een onbekende stam waren besmet en het gebruik van onzuivere virusstammen (mengisolaten).

De auteurs stellen zich verantwoordelijk voor het distribueren (op aanvraag) van kleine zaadhoeveelheden van de toetsrassen en, op beperkte schaal, van in zaad aanwezige zuivere virusstammen aan onderzoekers die betrokken zijn bij de identificatie van de stammen van dit virus. Bovendien zal zaad van de standaardserie van toetsrassen worden gedeponeerd in het National Seed Storage Laboratory te Fort Collins, Colorado, USA, terwijl de virusstammen (in zaad) in bewaring worden gegeven bij de American Type Culture Collection te Rockville, Maryland, USA, waar ze beschikbaar zullen blijven voor verder onderzoek.

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## Addresses


E. Drijfhout: Instituut voor de Veredeling van Tuinbouwgewassen (IVT), P.O. Box 16, Wageningen, the Netherlands.

M. J. Silbernagel and D. W. Burke: Irrigated Agriculture Research and Extension Center (IAREC), Prosser, Washington 99350, USA.