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Variation in pathogenicity and multiplication of beet necrotic yellow vein virus (BNYVV) in relation to the resistance of sugar-beet cultivars

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Abstract

Some partially resistant cultivars varied in their response to beet necrotic yellow vein virus (BNYVV), which could be due to the occurrence of different pathotypes. In the past three different types of BNYVV could be identified. Since in the field no consistent cultivar × virus source interaction could be detected, greenhouse trials were carried out under more standardised conditions, starting with a similar initial density of BNYVV. Cultivars with different degrees of resistance varied in their response to various types of beet necrotic yellow vein virus (BNYVV). The B type appeared to be less damaging than the A and P types. The virus content in the tap roots and the ratio of the virus content in tap roots to that in lateral roots were both higher in P type than in A or B type infections indicating that the P type moves more rapidly in the plants than the two other BNYVV types. The percentage of plants in which the virus reaches only a low concentration (less than 56 ng/ml of sap) is much lower in P type than in A or B type infections. Frequency distribution diagrams of individual plants showing different BNYVV levels reveal considerable differences between various cultivars.

Introduction

Rhizomania is the most important disease of sugar beet worldwide, caused by beet necrotic yellow vein virus (BNYVV), described by Tamada (1973). This furovirus is transmitted by zoospores of the soil-borne fungus *Polymyxa betae* (Keskin, 1964). Severe Rhizomania infections lead to reductions in root yield of 50% or more, whilst the sugar content and processing quality can be badly affected also. *Polymyxa betae* itself might cause a little damage at high temperatures, but as a pathogen it does not seem to be significant (Blunt et al., 1991).

Several plant breeding companies have developed cultivars partially resistant to BNYVV, originating mainly from the Rz1 gene (Holly-1-4) as described by Pelsy and Merdinoglu (1996) and in a later phase also Rz2 the wild beet accession WB42 from *B. vulgaris* subsp. *maritima* (Scholten et al., 1996). Some of these cultivars have shown a variable response in yield when grown in different countries. This could be caused by variation in pathogenicity due to differences in the RNA of BNYVV, as reported by Tamada et al. (1989). Koenig et al. (1997) found that RNA 1, 2, 3 and 4 are always present in naturally infected sugar-beet roots. The only difference in the RNA composition concerns the absence or presence of RNA 5. In Europe RNA 5 has been found only in the Pithiviers area (France). Apart from that, nucleotide sequences of some RNAs differ significantly (Koenig et al., 1995) and at least 3 types of BNYVV (A, B and P) can be identified.

Irrespective of the variation in RNA found in BNYVV a uniform serological response was observed in ELISA tests (Kuszala et al., 1986).

A joint research program within the Genetics and Breeding, and Pests and Diseases Study Groups of the International Institute of Sugar Beet Research (IIRB) was started in 1993 to detect whether the variation in the field reaction of resistant cultivars could be caused by the occurrence of pathotypes, or should be ascribed to external factors such as differences in climatic or soil conditions. BNYVV isolates from a number of fields

were analysed by reverse transcription polymerase chain reaction (RTPCR); in the same or neighbouring fields a standard set of partially resistant cultivars were incorporated in trials (Koenig et al., 1994). A total of 15 locations on contaminated and healthy fields in 7 countries were used. Soil samples were collected from diseased fields to determine the most probable number of infective units of BNYVV.

Initially, two clearly distinct major strain groups (A and B) of BNYVV could be discriminated in Europe (Kruse et al., 1994). The two types occurred in separate geographical regions and in borderline areas mixtures were found. Later, a third strain group P in the region around Pithiviers (France) was identified.

In trial fields no clear differences in the reaction of the standard set of partially resistant cultivars could be detected, probably because a large number of external factors, such as climatic conditions, soil type and inoculum density influenced the results (Paul et al., 1993). Therefore it was decided to set up experiments with the same set of cultivars in the greenhouse and climate cabinets under more standardised conditions and at the same initial inoculum level.

Since partially resistant cultivars differ widely, ranging from a very high level to nearly no resistance, combined with different degrees of tolerance, a more extensive series of these varieties was tested against one biotype (A) to check that any isolate × cultivar interaction that was detected was not due to this variation.

Materials and methods

Soil samples were collected from fields in which one of the distinct major strain groups A, B or P was determined by Koenig (pers. comm.). The A types originated from Nagele (Flevoland, the Netherlands) and Rovigo (Italy); the B types from Gross Gerau (Germany) and Elst (the Netherlands); the P type from Pithiviers (France). From these soil samples the most probable number (mpn) of infective units (i.u.) of BNYVV per 100 g soil were determined according to Tuitert (1990). Different levels of infestation were created by serial dilution of BNYVV infested soil from the above-mentioned origins. Soil samples were air-dried, ground, homogenised and diluted in steps of 10^1, 10^2 and 10^3 times with sterilised river sand. Two weeks old seedlings of the cultivar Univers were transplanted in 200 ml pots in a bioassay with 10 replicates per dilution in a climate cabinet at 22/15 °C with a photo period of 16 h. Water and once a week Steiner nutrient solution were added until after six weeks plants were harvested and roots analysed for the presence of BNYVV by double antibody sandwich ELISA, as described by Tuitert (1990). The data on BNYVV positive plants were analysed by a computer programme in Fortran, which calculates the mpn of infective units and confidence limits of the estimate for any number of dilution levels and any combination of replicates per dilution. Based on these values the soil samples were diluted to a mpn of 15 i.u. per 100 g soil, using river sand. Possible differences of nutrient supply in the dilutions were compensated for by adding nutrient solution (Steiner, 1968). Following dilution the soil samples were retested to check the accuracy of the method and gave values of 7–28 i.u. per 100 g of soil. All sources of BNYVV were tested separately in two series of experiments. To avoid the influence of cultivars always a series in not infected sand with nutrient solution was added. Root weights were calculated as percentage of not infested. After statistical analysis by means of GENSTAT using ANOVA and Student's *t*-test the results were arranged according to the biotypes of BNYVV and put together, because each biotype reacted similarly.

A standard set of cultivars was grown for 9 weeks in pots in a climate cabinet at temperatures of 23 °C (day) and 18 °C (night) and light of 30,000 lux for 16 h per day. The cultivars of Rizor, Gabriela and Stratos were tested, together with the very susceptible standard Accord. The experiment consisted of 5 replicates of 5 plants each. After harvesting the remaining soil was collected, mixed and used in a second test to determine the final i.u. values of BNYVV.

The set of cultivars was also grown in a medium consisting of river sand and nutrient solution (Steiner, 1968). After harvesting the roots, washing and superficially drying, root fresh weight was determined and sap was collected for determination of BNYVV by ELISA (Tuitert, 1990). By running a dilution series of purified BNYVV the actual virus content could be calculated (Alderlieste and Van Eeuwijk, 1992). Since the subsequent batches of purified virus reacted differently, two standards with high extinction values were used throughout.

All roots were estimated for brown discoloration caused by the infection and development of cystosori on a scale ranging from 1–10.

Variation in the degree of resistance of the cultivars against a single biotype was assessed by using the same technique with soil originating from Nagele (A type;

mpn = 59 i.u. BNYVV/100 g soil). In this test, modified after the method described by Paul et al. (1992), seedlings in the cotyledonous stage were transplanted and grown in a climate cabinet in blocks of 24 soil filled pipes in 4 replicates (96 plants). After growing for 6 weeks under the same conditions described above, the plants were harvested and, after washing and superficially drying, the fresh weight of the roots and their BNYVV content were determined. Results are reported from the cultivars Univers (susceptible standard), Rima, Roxane, Elisa, Ramona, Rialto, Patricia, FD 9414, H 4669, S 1569, Riant, Stratos and Saphir. The samples originated from seed lots of cultivars put forward for the official variety trials and selected to represent the wide range of resistant levels.

Results

The influence of BNYVV on root development

The weight of the tap roots after 9 weeks growth in soil infested with circa 15 i.u. per 100 g is given in Figure 1 as a percentage of the weight in a sterilized sand mixture.

Biotype B was the least pathogenic, particularly on the partially resistant cultivars Rizor and Gabriela which exceeded their weight under healthy conditions. The differences between the A and P types were minimal and, although there was a slight deviation in ranking order, this was not statistically significant.

The estimated discoloration of the lateral roots was equally high in Accord for all sources of BNYVV (A, B, and P type). Although partially resistant cultivars showed a less serious discoloration, no consistent differences between BNYVV types could be detected.

The replication and transport of BNYVV in the plant

The BNYVV contents of the lateral roots and tap roots were measured separately 9 weeks after transplanting the seedlings. BNYVV content in the tap root, expressed as percentage of the susceptible standard Accord (Figure 2a), showed considerably larger differences between A and P type than root weight (Figure 1) for the three cultivars.

The ranking order for BNYVV content in tap roots remained identical (in decreasing order: Stratos–Rizor–Gabriela) for all three types. However, the differences in BNYVV content of the lateral roots between the susceptible and resistant cultivars were less than those of the tap roots and were often not significant (Figure 2b). Particularly the cultivar Stratos did

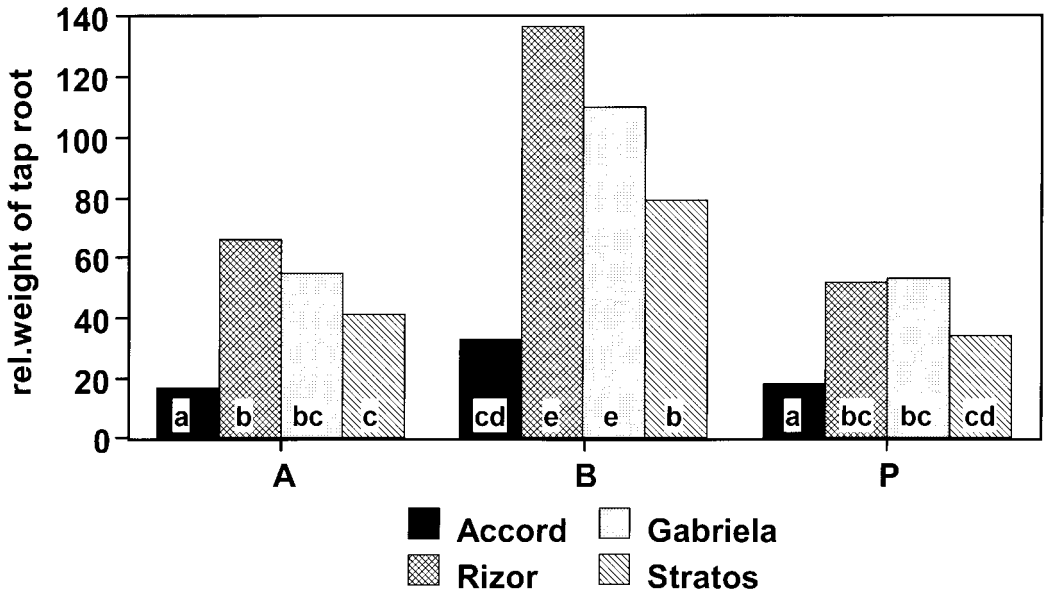

Figure 1. The fresh weight relative to uninfected of the tap roots (=100) of 4 cultivars after 9 weeks growth in the presence of 3 different types of BNYVV (A, B and P) in a climate cabinet; initial infection level of 15 infective units per 100 g of soil. Columns with the same letter do not differ significantly from each other at $p = 0.05$.

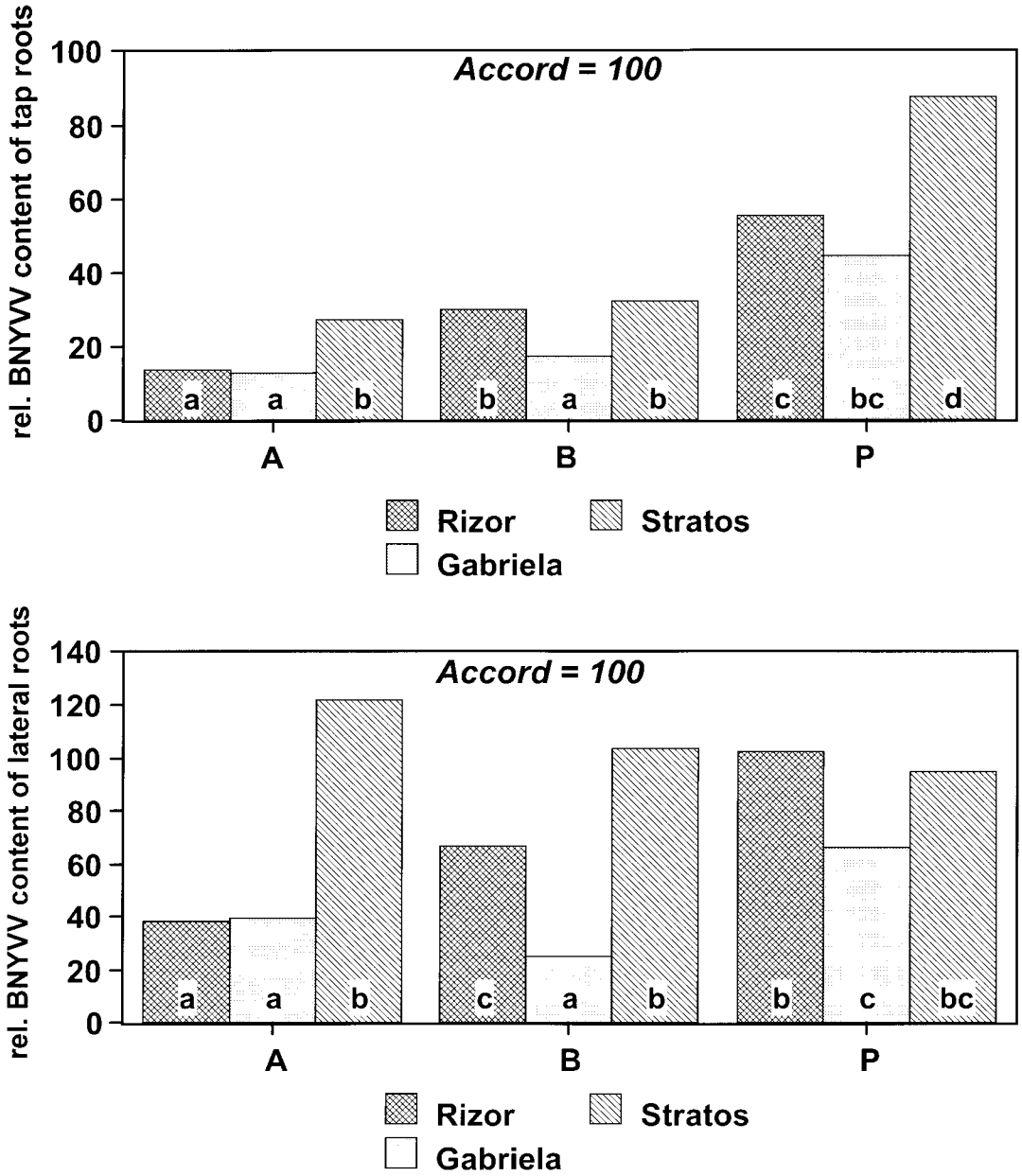

Figure 2. The relative BNYVV content of the tap root (a) and lateral roots (b) of three resistant cultivars compared with the susceptible Accord (=100) after 9 weeks growth in the presence of 3 different types of BNYVV (A, B and P). Tests in a climate cabinet at an initial infection level 15 infective units of BNYVV per 100 g of soil. Columns with the same letter do not differ significantly from each other at $p = 0.05$.

not differ from the susceptible standard and for type A even exceeded the BNYVV content of Accord. The actual contents of BNYVV for the P type were low in all four cultivars including Accord, since the majority of the lateral roots were destroyed.

The ratio of BNYVV content between tap root and lateral roots showed more clear differences (Figure 3), particularly in the cultivars Accord, Stratos and Gabriela. Rizor behaved differently showing a less pronounced ratio with type P.

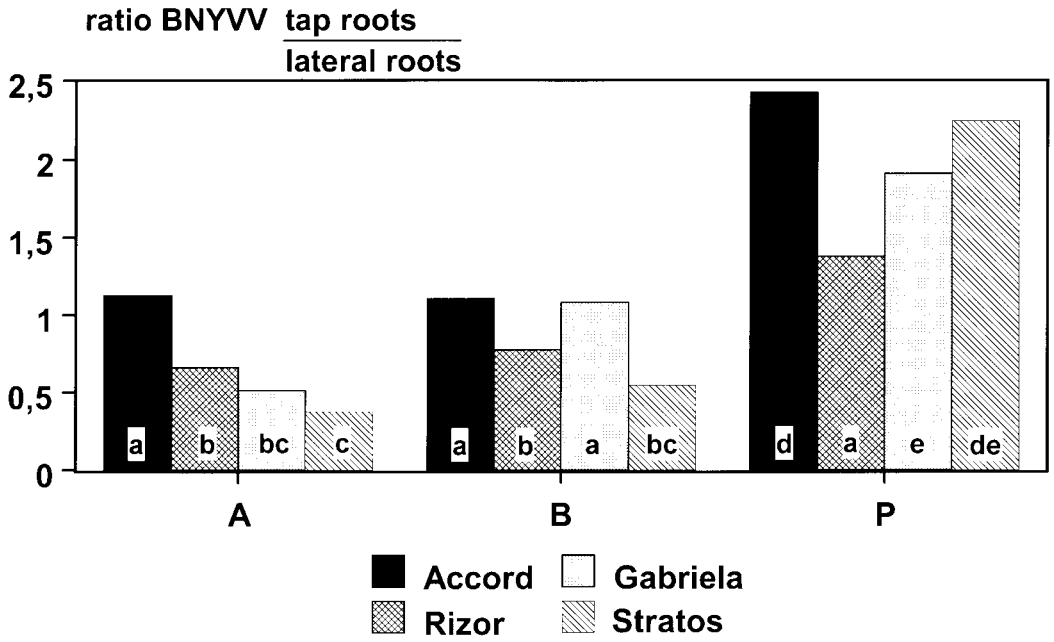

Figure 3. The ratio of BNYVV content tap root/lateral roots of four cultivars after 9 weeks growth in the presence of 3 different types of BNYVV (A, B and P) in a climate cabinet; initial infection level of 15 mpn per 100 g of soil. Columns with the same letter do not differ significantly from each other at $p = 0.05$.

In all experiments the percentage of resistant plants (BNYVV content less than 56 ng/ml of sap) was also recorded and is shown in Figure 4. Here the susceptible standard Accord and sometimes also the partially resistant cultivar Stratos (biotype P) had few resistant plants whereas Gabriela showed the largest number. For all cultivars the percentage of resistant plants found with biotype P was considerably less than with A and B.

The multiplication rate (Pf/Pi) *of BNYVV in soil*

In the same greenhouse test the mpn of i.u. before and after 9 weeks of growth was determined and from the results the multiplication rate (Pf/Pi) was calculated. The results summarized in Table 1 indicate distinct differences between the partially resistant cultivars and susceptible standard Accord. Following growth of the partially resistant cultivars Rizor and Gabriela no increase in types A and B of BNYVV in the soil could be detected. The susceptible standard Accord increased all three biotypes considerably. The P type multiplied in all partially resistant cultivars, although less than in Accord.

Differences in partial resistance against BNYVV type A

Four series of trials were conducted, each consisting of 5 resistant cultivars and a common susceptible standard (Univers) arranged in 4 blocks of 24 soil-filled pipes containing 5 g i.u. of BNYVV type A per 100 g of soil. The average BNYVV contents of the roots after 6 weeks of growth were determined. The BNYVV content of the susceptible standard Univers was similar in all trials (Figure 5) and did not differ significantly from two possible resistant varieties, Stratos and Saphir.

All other cultivars differed significantly ($P < 0.01$) from this group which is shown in decreasing order of virus content. Older cultivars such as Rima and Roxane and new selections from these showed the lowest BNYVV content. In the newer, highly productive cultivars such as Elisa, Patricia and Rialto the BNYVV content is significantly higher. In addition to the average BNYVV content the segregation of resistance within a resistant cultivar was examined by producing frequency distribution diagrams of individual plants showing different BNYVV levels (Figure 6). A fairly normal distribution pattern was observed

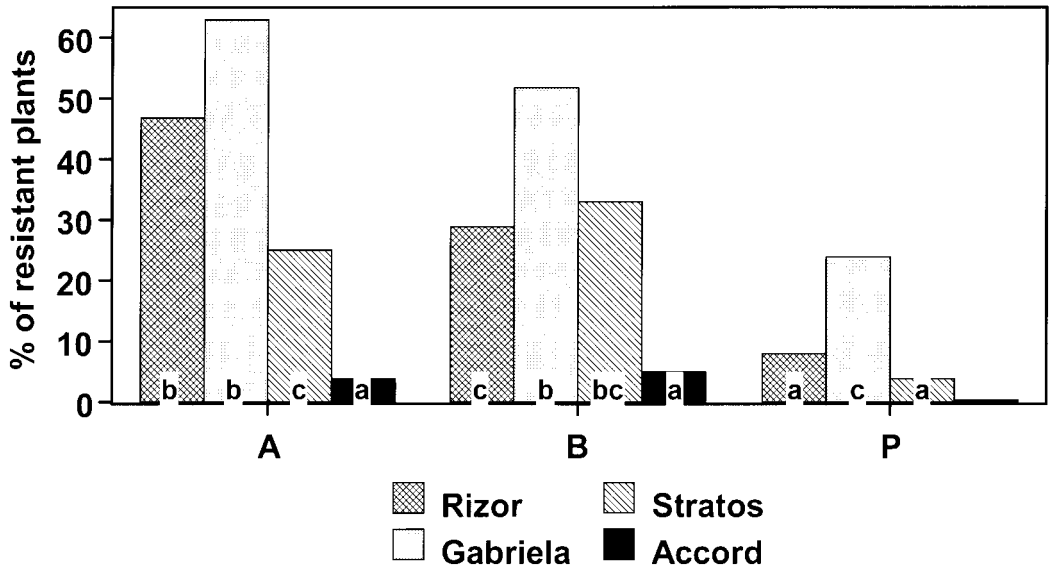

Figure 4. The percentage of BNYVV resistant plants of four cultivars after 9 weeks growth in the presence of 3 different types of BNYVV (A, B and P) in a climate cabinet; initial infection level of 15 infective units per 100 g of soil. Columns with the same letter do not differ significantly from each other at $p = 0.05$.

Table 1. The multiplication rates of BNYVV (Pf/Pi) following growth of the different cultivars for 9 weeks

mpn g/100 g of soil	Type of BNYVV		
	A	B	P
Pi	8	27	7
Pf/Pi per cultivar:			
Accord	78 a[1]	>185 b	87 a
Rizor	1 c	0.6 c	3 c
Gabriela	1 c	0.3 c	3 c
Stratos	3 c	2 c	17 d

[1]Figures with the same letter are not significantly different at $p = 0.05$.

amongst populations of cultivar Univers without plants in the most resistant classes.

Rima showed the highest percentage of plants in the most resistant category (less than 56 ng BNYVV) and no plants exceeding the level of 1085 ng/ml of sap. In the newer resistant cultivars Elisa and Patricia a high percentage of plants occurred in the class with less than 56 ng, but also in the 56–165 ng category. At the same time in these cultivars more highly susceptible plants could be detected.

Both features were reflected in the mean BNYVV level and the percentage of resistant plants measured after 6 weeks. Depending on the nature of the resistance, in some cultivars a few highly susceptible plants occurred whereas others were characterized by a relatively high percentage of moderately susceptible plants. On the contrary the frequency diagram of Stratos did not deviate significantly from Univers, as Saphir even showed an increased percentage of plants with BNYVV more than 2693 ng/ml.

Discussion and conclusions

Variation in pathogenicity of BNYVV

The relative weight of infected tap roots measured after 9 weeks as compared to their weight under healthy conditions (Figure 1) showed a serious reduction in response to both the A and P type of BNYVV for all cultivars. Surprisingly this also occurred in the partially resistant cultivars. This could have been caused by the infection with *Polymyxa betae* and BNYVV in the early stages of development, causing extensive root necrosis. However, the B type caused less reduction and, in the partially resistant cultivars, the weight of tap roots exceeded that of the healthy controls. This may be explained by the less vigorous growth of plants in sand with a nutrient solution.

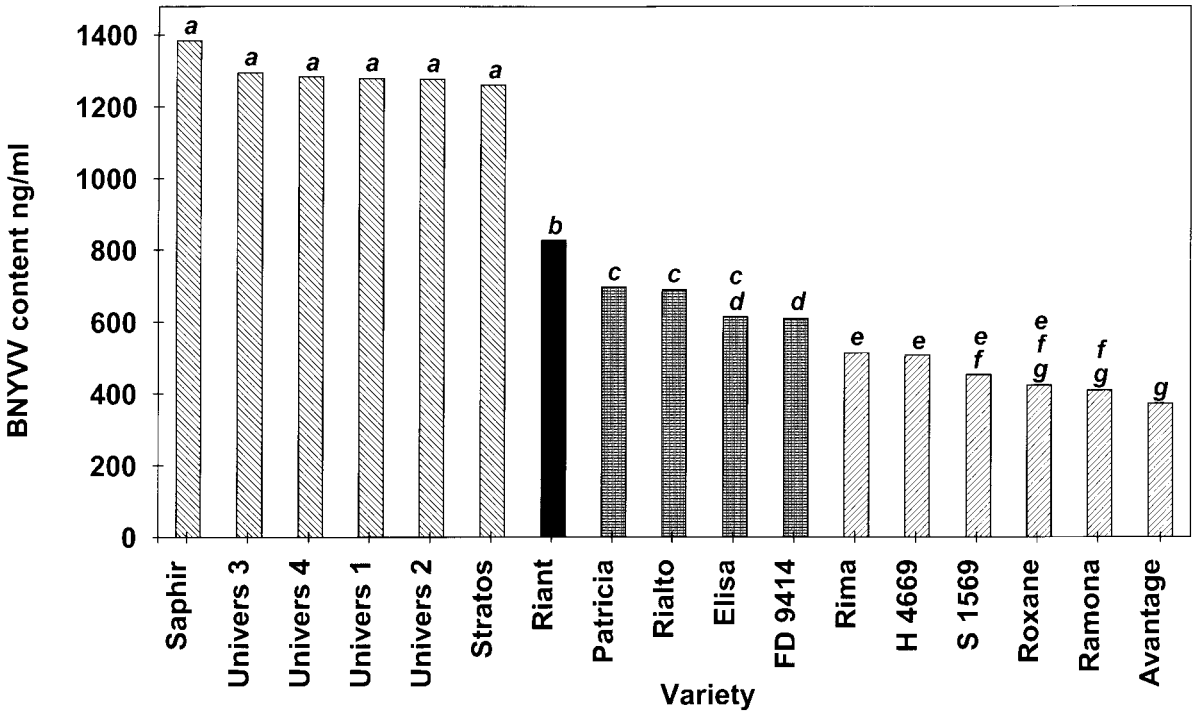

Figure 5. The mean BNYVV content of resistant cultivars in 4 trials as compared to the susceptible standard Univers, after 6 weeks growth in a climate cabinet in the presence of type A of BNYVV (source Nagele); initial inoculum level of 59 infective units per 100 g of soil. Columns with the same letter do not differ significantly from each other at $p = 0.05$.

Multiplication of BNYVV in plant and soil

The relative BNYVV content of type A and B in the tap roots was low in all partially resistant cultivars and no significant interaction between cultivar and these two types of virus could be demonstrated. In the cultivar Stratos the BNYVV level of type P was nearly as high as in the susceptible standard, which was in accordance with the low root weight. In the lateral roots of the cultivar Stratos (Figure 2b) the content of BNYVV was not significantly deviating from the susceptible standard (Accord), indicating less resistance and probably some tolerance when comparing tap root weights (Figure 1). Very little differences could be detected for type P (Figure 2b); here the lateral roots were largely destroyed, which resulted in low contents of BNYVV. Based on these observations the cultivars Rizor and Gabriela seem to have partial resistance, inhibiting BNYVV replication and transport. The ratio between virus contents in tap roots and lateral roots is a measure of the efficiency of transport of BNYVV in the root system. In all cultivars the ratio was increased when infected with the P type as compared to the A and B types, suggesting that the P type is more pathogenic and also more rapidly transported through the vascular bundles. This is supported by the observation that only plants infected with the P type showed necrotic yellow veins on the leaves, the symptom which is correlated with systemic movement of BNYVV. The most striking feature of the P type is the presence of RNA 5, which does not occur in the other two types (Koenig et al., 1997). Some of these BNYVV RNA 5 encoded proteins share a stretch of six amino acids with RNA 3, being involved in pathogenicity.

The multiplication rates in the soil (Pf/Pi) depend largely on the quantity of already killed lateral roots, since the roots still alive were taken out for measurements. The susceptible cultivar Accord showed high Pf/Pi values (Table 1) particularly with regard to the B type, which could not be measured within the dilution series of 10^4.

Within the set of partially resistant cultivars this difference could not be detected. It is also remarkable that Pf/Pi rates in type P are not higher than of type A or B, but this could possibly be caused by the fact that after 9 weeks the increased quantity of BNYVV in the tap

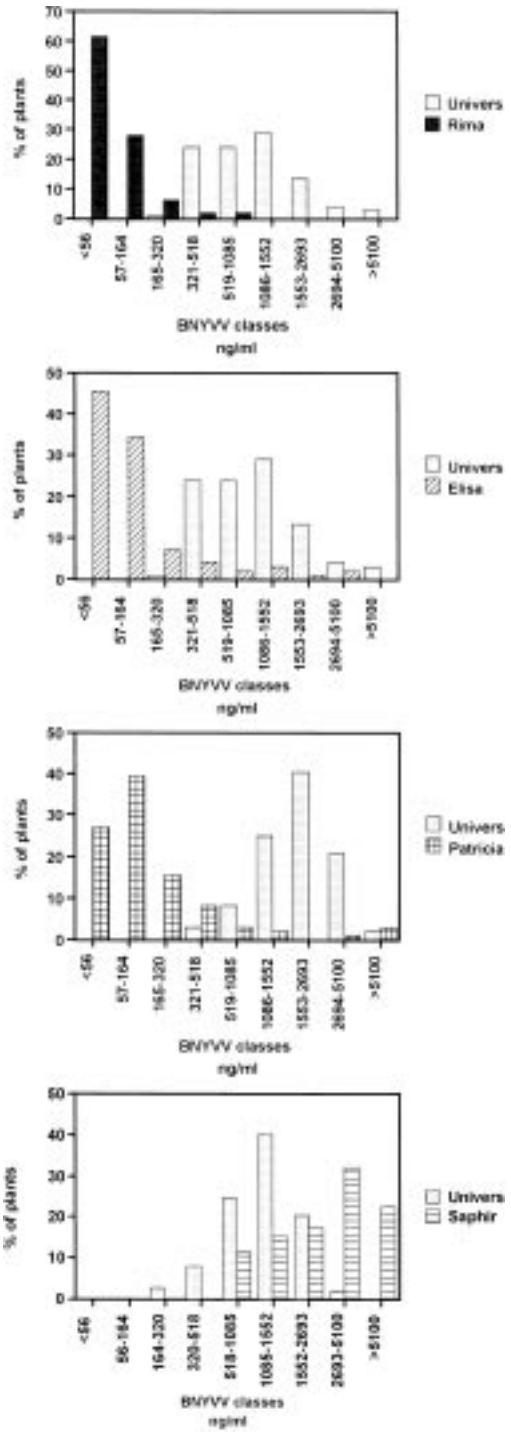

Figure 6. Frequency distribution diagrams of BNYVV levels in the partially resistant cultivar Rima, Elisa and Patricia and the tolerant cultivar Saphir as compared to the susceptible standard Univers.

root was removed completely at harvest before determining Pf by mpn of infective units.

For the first time Pf/Pi rates for BNYVV on host crops were recorded under controlled conditions. No conclusions on multiplication rates in field situations can be drawn from this single trial, particularly because under wet conditions clusters of cystosori can fall apart disturbing the estimation of infective units in soil.

Differences in partial resistance against BNYVV type A

In the tests in climate cabinets a range of different partially resistant cultivars were compared under identical conditions against a single isolate of BNYVV type A. The cultivars Stratos and Saphir did not show any reduced BNYVV content in the roots as compared to the susceptible standard in these tests. Nevertheless, they yield quite well in BNYVV infested fields, if the level of infestation is not too high. This suggests that these cultivars exhibit a degree of tolerance in the field.

As compared to the BNYVV content measured in the pathogenicity trials the cultivar Stratos was less resistant in this test. This may be explained by the fact that seed for the pathogenicity trials was obtained in 1990 and stored to be sure of an identical genotype, whereas seed for the single isolate tests was obtained in 1996. During this time the cultivar Stratos has changed, having been selected for improved yield. Similar changes were observed in other partially resistant cultivars, although this would be difficult to prove in field trials since infection levels vary.

BNYVV content varied considerably in the remaining partially resistant cultivars. The older cultivars Rima and Roxane contained less BNYVV than the new highly productive cultivars such as Elisa, Patricia and Ballerina. Some additional tolerance has probably also been selected in these.

Although to a lesser extent, differences in BNYVV content between partially resistant cultivars that were not negatively correlated with yield, were also reported by Bürcky and Büttner (1991). Apart from the mean level of BNYVV content in a cultivar the frequency of occurrence of susceptible plants will also be important. These are the plants that will be noticed in the field, because they often show the pale yellow discoloration of the leaves, which is symptomatic of rhizomania.

In general, good quantification of the level of resistance is obtained from the mean BNYVV content and

the percentage of resistant plants, with virus content below 56 ng/ml sap. The latter criterion is rather arbitrary and not as informative as a frequency distribution diagram for each cultivar, but in combination with the mean BNYVV content it is very useful for comparing a series of cultivars quickly.

Acknowledgements

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