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ADDITIONAL HOSTS AND RESISTANCE SOURCES OF BEET NECROTIC YELLOW VEIN *FUROVIRUS*

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IZVLEČEK

DODATNI GOSTITELJI IN IZVORI ODPORNOSTI PROTI PESNEMU FUROVIRUSU NEKROTIČNEGA RUMENENJA ŽIL

Dodatno je bilo ugotovljenih devet novih lokalno občutljivih diagnostičnih gostiteljev (*Amaranthus mitchellii, A. quitensis, Acroglochin chenopoides, Chenopodium polyspermum, C. pumilio, C. sandwicheum, C. strictum* var. *strictum*, *C. suecicum, Rhagodia nutans*) pesnega furovirusa nekrotičnega rumenenja žil (BNYVV). *Amaranthus bouchonii*, nov trajni plevel na Madžarskem, je tudi reagiral s sistemičnimi mozaičnimi simptomi in ima lahko zelo pomembno vlogo v ekologiji virusa. *Axyris amaranthoides*, doslej v virologiji neznana rastlina, je nov lokalni in sistemični gostitelj BNYVV. *Nicotiana benthamiana*, najbolj znana indikatorska rastlina v virologiji, je reagirala s sistemičnim mozaikom, deformacijo listov in upočasnjeno rastjo in je zelo dober nov propagativni gostitelj za BNYVV.

Hipersenzitivna odpornost je bila izražena v različnih vrstah rodu *Beta* in v različnih izvorih (npr. *B. procumbens, B. vulgaris, B. webbiana*). Odpornost je bila najdena v šestih izvorih *Beta vulgaris* ssp. *maritima* in v dveh turških, enem poljskem in treh izvorih ameriških *Beta vulgaris*.

ABSTRACT

We have found nine new local susceptible diagnostic hosts (*Amaranthus mitchellii, A. quitensis, Acroglochin chenopoides, Chenopodium polyspermum, C. pumilio, C. sandwicheum, C. strictum* var. *strictum*, *C. suecicum, Rhagodia nutans*) of beet necrotic yellow vein *Furovirus* (BNYVV). *Amaranthus bouchonii*, a new, perennial weed plant in Hungary reacted also with systemic mosaic symptoms and may play a highly important role in the ecology of the virus. *Axyris amaranthoides*, a plant so far unknown in virology, is a new local and systemic host of BNYVV. *Nicotiana benthamiana*, the best known indicator plant in virology, reacted with systemic mosaic, leaf deformation and growth inhibition, and is a very good new propagative host for BNYVV.

Hypersensitive resistance was pointed out in different *Beta* species and accessions (e. g. *B. procumbens, B. vulgaris, B. webbiana*). We found resistance in six accessions of *Beta vulgaris* ssp. *maritima* and in two Turkish, one Polish and three American accessions of *Beta vulgaris*.

Introduction

Beet necrotic yellow vein *Furovirus* (BNYVV), the causal agent of rhizomania disease of sugar beet is very important. The economic loss caused by the virus may amount to 60-80% in beet root, 20-30% in sugar content and 70-80% in sugar output (Alghisi *et al.*, 1964; Horak and Schlösser, 1980; Winner, 1984; Giunchedi et al., 1987). The virus is a soilborne persistant virus transmitted by the *Polymyxa betae* fungus (family: Plasmodiophoraceae), a member of the *Furovirus* group (*fu*ngus-transmitted *ro*d shaped *virus*) (Brunt and Richards, 1989; Putz *et al.*, 1990; Richards and Tamada, 1992).

According to our knowledge BNYVV occurs in more than 25 countries (Table 1). The BNYVV has so far been isolated not only from sugar beet but also from spinach (*Spinacia oleracea*) and Swisschard (*Beta vulgaris* var. *cycla*) too (Russo *et al.*, 1981; Fujisawa *et al.*, 1982; Hess *et al.*, 1982).

The host range of BNYVV is relatively narrow. The importance of a some *Chenopodium* species (e. g. *C. amaranthicolor, C. quinoa*), of *Tetragonia expansa* (family: Aizoaceae) and *Gomphrena globosa* (family: Amaranthaceae) is to be emphasized (Tamada and Baba, 1973; Faccioli and Giunchedi, 1974). The virus tended to be restricted to the inoculated leaves of plants. In some plants (e. g. *Beta macrocarpa*, *B. vulgaris* and *Spinacia oleracea*) the virus often became systemic. Tamada and Baba (1973) failed to infect any of 84 species belonging to the following 15 families: Amaranthaceae, Apocynaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cruciferae, Cucurbitaceae, Gramineae, Leguminosae, Pedaliaceae, Plantaginaceae, Poligonaceae, Portulaceae, Scrophulariaceae, Solanaceae, although among the plants examined there were some test plants widely used in plant virology (e. g. *Cucumis sativus*, *Phaseolus vulgaris*, *Vigna sesquipedalis*, *Datura metel*, *D. stramonium*, *Nicotiana glutinosa*, *N. sylvestris*, *N. tabacum* cultivars, *Petunia hybrida* etc.).

Table 1: Occurrence of beet necrotic yellow vein *Furovirus* (BNYVV)

Country	Literature
Italy	Canova (1959, 1966), Faccioli and Giunchedi (1974)
Finland	Koch (1967)
Japan	Masuda *et al.* (1969, cit. Tamada and Baba, 1973), Tamada (1975)
Czech Repuclic	Dunning (1972), Novak and Lanzova (1984), Konecny (1994)
Poland	Dunning (1972), Koch (1982), Paczuski and Szyndel (1994)
Greece	Kouyeas (1973)
France	Putz and Vuittenez (1974), Putz et al. (1990)
Germany	Schäufele (1974, cit. Schäufele, 1983), Hamdorf et al. (1977), Koenig et al. (1984)
USA (California)	Falk and Duffus (1977), Al Musa and Mink (1981), Whitney (1984), Duffus et al. (1984)
Yugoslavia	Tošić et al. (1978), Šutić and Milovanović (1978)
Austria	Krexner (1981), Kurtz (1989)
Roumania	Codrescu et al. (1981)
Hungary	Virág (1982), Kobza et al. (1985), Horváth et al. (1989, 1990), Horváth and Virág (1990)
China	Gao et al. (1983), Jinliang et al. (1983)
Kyrgyzstan	Vlasov et al. (1983, cit. Vlasov, 1989), Valsov (1992)
Mongolia	Heling et al. (1983)
Switzerland	Häni and Bovey (1983)
Bulgaria	Jankulova et al. (1984)
Netherland	Heijbroek (1984, 1989)
USA (Texas)	Duffus et al. (1984), Duffus (1987), Duffus and Liu (1987)
Belgium	Van den Bossche et al. (1985)
Sweden	Lindsten (1986)
England	Asher (1987), Hill and Torrance (1989)
Spain	Raposo and Mateo-Sagasta (1988)
Russland	Kaverzneva et al. (1988)
Turkey	Schäufele (1989), Vardar and Erkan (1992)
Denmark[1]	Danielsen (1992)
Slovakia	Subikov (1993), Zatkov and Subikov (1993)

[1] During 1990-1991, the soil samples in Denmark were tested for the presence of beet necrotic yellow vein Furovirus (BNYVV) and its vector using a baiting plant method. Few BNYVV-like particles were found in a single soil sample using immunosor-bent electron microscopy (see Danielsen, 1992).

Resistance to the BNYVV can be found in cultivated *Beta vulgaris* and in the related wild species (Doney and Whitney, 1990). Among the accessions of *Beta vulgaris*, which were tested in the greenhouse, some interesting sources of resistance were found. In accession R39, the concentration of BNYVV was only 20 per cent of that of the virus susceptible cultivar Regina (Paul et al., 1991). In recent years some accessions of *Beta maritima* and *B. webbiana* showed high level of resistance or immunity to BNYVV (Whitney, 1989a; Horv th et al., 1989, 1990; Horv th and Vir g, 1990). The *Beta* germplasm collections are today very remarkable. According to Doney (personal information) a successful collection expedition for wild relatives (*Beta maritima*) of sugar beet was conducted along the Gulf of Lion (Ligurian Sea), along the entire Atlantic coast of France, The Channel Islands (Jersey and Guernsey), the major island of Denmark and along the Atlantic coast of Belgium. A total of 2992 individual plants in 19 populations in Denmark and 57 individual plant in 3 populations in Belgium were collected. Considering that the host range of the virus and the number of the resistant plants are relatively narrow, we carried out investigations in order to detect new hosts and resistant sources.

Materials and Methods

The virus used in this study was designated a Donna strain of BNYVV isolated in Hungary (BNYVV-DH) from diseased sugar beet (Horváth *et al.*, 1989, 1990). The leaf/root tissues were ground in 0.02 M phosphate buffer, pH 7.2, or in distilled water and inoculated onto Carborundumdusted leaves of indicator plants (e. g. *Chenopodium quinoa*, *Gomphrena globosa*, *Tetragonia expansa*). Indicator plants and experimental plants (see Table 3-5) were maintained in a glasshouse at 20-25°C. BNYVV was maintained in *Chenopodium quinoa* and *Gomphrena globosa* plants planted in sterile soil and was passed every 2-3 weeks.

We examined various *Beta* species for resistance to BNYVV, with special regard to the species *B. maritima, B. procumbens, B. vulgaris* and *B. webbiana*. Seeds of the plants were sown in glasshouse, in plastic pots of 10 cm diameter containing sterile soil. The seed-coats in species belonging to the Procumbentes section were removed before sowing. The young beet plants were artificially inoculated with the DH isolate of BNYVV. After the inoculation the plants were symptomatologically checked every 7-10 days, then on the 30th day the leaves and on the 60th day the rootlets of the infected plants were examined by DAS-ELISA technique and biological test on *Chenopodium quinoa* and *Gomphrena globosa* indicator plants.

Results and Discussion

The results of new host plants are contained in Table 2. We have found 13 new hosts in three families (Amaranthaceae, Chenopodiaceae,

Table 2: New experimental hosts of beet necrotic yellow vein *Furovirus* (BNYVV)

Hosts	Symptoms[1] local/systemic
AMARANTHACEAE	
Amaranthus bouchonii	BNl/Mo
A. mitchellii	Chl, Y, Led/-
A. quitensis	Chl, Y, Led/-
CHENOPODIACEAE	
Axyris amaranthoides	Chl/La
Acroglochin chenopoides	Chl-Nl/-
Chenopodium polyspermum	Chl-Nl/-
C. pumilio	Chl-Nl/-
C. sandwicheum	Chl-Nl/-
C. strictum var. strictum	Chl-Nl/-
C. suecicum	Chl-Nl/-
Rhagodia nutans	Chl-Nl/-
Spinacia turkestanica	Chl-Nl/Mo, Gr
SOLANACEAE	
Nicotiana benthamiana	-/Mo, Ldef, Gr

[1] Symptoms: B, brown; Chl, chlorotic lesions; Gr, growth reduction; La, latent or symptomless; Ldef, leaf deformation; Led, leaf drop; Mo, mosaic; Nl, necrotic lesions; Y, yellow spots; -, no reaction.

Solanaceae). *Amaranthus mitchellii* and *A. quitensis* responded with local chlorotic yellow symptoms to the vi-rus; later the inoculated leaves came off. *Amaranthus bouchonii*, a new, very important weed plant in Hungary, responded with brown, necrotic local lesions. Owing to its systemic susceptibility the latter plant may play a highly important role in the ecology of BNYVV. The plants belonging to the family Chenopodiaceae equally showed local susceptibility to the virus. The chloroticnecrotic lesions appeared 6-8 days after the inoculation. Particularly expressed symptoms were shown by *Chenopodium suecicum* which can be used well to detect the virus.

The newly found virus susceptible *Chenopodium* species enable a wider laboratory examination of the virus. We should like to call special attention to *Axyris amaranthoides*, a plant so far unknown in virology, that we found locally and systemically susceptible to the virus; it may be highly important in studies on the ecology and other biological characteristics of BNYVV. The systemically latent (symptomless) plant can be regarded as definitely dangerous from a virological point of view. Or again, very remarkable is the *Spinacia turkestanica*, which responses with chloroticnecrotic local lesions and systemic mosaic to the virus. Among the plants examined *Nicotiana benthamiana* is very important. The plant we inoculated with the DH isolate of BNYVV responded with systemic mosaic, leaf deformation and growth inhibition. The conclusion drawn from the number of local lesions shown on the *Chenopodium quinoa* plants inoculated with the virus obtained from the infected plants is that the *Nicotiana benthamiana* is a very good propagative host for BNYVV. It is known to be susceptible to more than 200 viruses (Horv th, 1993).

In the course of experiments we have found different *Beta* species and accessions responded with necrotic local lesions to infection by BNYVV (Table 3). Owing to their hypersensitive resistance, these *Beta* species and accessions can deserve attention in breeding work. From the top leaves and rootlets of inoculated plants the virus could not be back-isolated nor detected even by DAS-ELISA method.

In various accessions of *Beta vulgaris* ssp. *maritima* we have found 111 resistant plants to BNYVV (Table 4). The resistance of *Beta vulgaris* ssp. *maritima* to BNYVV is very important. In the course of examinations of 97 inoculated plants of the Hungarian specific hybrid DN 9017 *Beta maritima* (Magassy, personal information) 27 plants were found to be resistant. The virus could not be detected in the roots and leaves of the plants either by DAS-ELISA technique or with back-inoculation test. According to a work (Whitney, 1989a) published at the same time with our earlier experiment results (see Horv th et al., 1989, 1990) *Beta maritima* accessions from Denmark, England, France - susceptible to the *Polymyxa* vector - proved resistant to the virus. Results of American and Hungarian investigations call attention to the above sources of virus resistance. Considering, that *Beta maritima* is rather readily crossed with *B. vulgaris*, and the virus resistance shows dominant inheritance in the

F_1 generation (resistant x susceptible), the importance of *B. maritima* in breeding for virus resistance is invaluable. The importance of the

Table 3: *Beta* species reacted with hypersentsitive reaction (necrotic local lesions) to beet necrotic yellow vein *Furovirus* (BNYVV)

Beta species
$1_{B.}$ *procumbens* (Donor Nr. 884465; Collection Nr. W29b)
$1_{B.}$ *vulgaris* ssp. *vulgaris* convar. *cicla* var. *cicla*
$1_{B.}$ *vulgaris* ssp. *vulgaris* convar. *cicla* var. *flavescens* f. rhodopleuta
$1_{B.}$ *vulgaris* ssp. *vulgaris* convar. *cicla* var. *flavescens* f. leucaplero
$1_{B.}$ *vulgaris* ssp. *vulgaris* convar. *vulgaris* var. *altissima*
$1_{B.}$ *vulgaris* spp. *vulgaris*
$1_{B.}$ *vulgaris* ssp. *vulgaris* convar. *vulgaris* var. *vulgaris*
$1_{B.}$ *vulgaris* var. *altissima*
$2_{B.}$ *vulgaris,* P.I. 173844
$2_{B.}$ *vulgaris,* P.I. 163182
$2_{B.}$ *vulgaris,* P.I. 181717
$2_{B.}$ *vulgaris,* P.I. 105335
$2_{B.}$ *vulgaris,* P.I. 164805
$3_{B.}$ *vulgaris* ssp. *adanensis*, BGRC 36500
$3_{B.}$ *vulgaris* ssp. *cicla*, BGRC 49749
$3_{B.}$ *vulgaris* ssp. *maritima*, BGRC 49834
$3_{B.}$ *vulgaris* ssp. *maritima*, BGRC 49845
$3_{B.}$ *vulgaris* ssp. *maritima*, BGRC 49847
$3_{B.}$ *vulgaris* ssp. *maritima*, BGRC 54778
$3_{B.}$ *vulgaris* ssp. *maritima*, BGRC 54779
$1_{B.}$ *webbiana* (Donor Nr. 884455)

[1] Centre for Genetic Resources, Wageningen, The Nether-lands.

[2] National Gene Bank Laboratorium, Beltsville, Maryland, USA.

[3] Gene Bank of FAL, Braunschweig, Germany.

resistance of *Beta maritima* to BNYVV is increased by the fact that - as pointed out by Whitney (1989b) in another work - in some accessions of the species there is resistance to the powdery mildew

fungus (*Erysiphe polygoni*) of beet and to the bacterium *Erwinia carotovora* spp. *betavasculorum* too. Among the fungus- and bacterium

Table 4: Resistant accessions of *Beta vulgaris* ssp. maritima plants to beet necrotic yellow vein *Furovirus* (BNYVV)

*P*lant *I*ntroduction or donor number[1]	Number of plants resistant/invesigated
P.I. 546396	27/31
P.I. 546413	10/11
P.I. 546419	30/42
P.I. 546425	7/36
P.I. 868838	18/18
Ames 4218	19/35

[1] North Central Regional Plant Introduction Station, USDA, Iowa State University, Ames, Iowa 50011 and Centre for Genetic Resources, Wageningen, The Netherlands.

resistant accessions the above author found some resistant to BNYVV as well. According to Doney (personal information) the North Atlantic accessions of *Beta maritima* are resistant to the beet mild yellowing *Luteovirus* (BMYV) too. The possibility of simultaneous selection of fungus-, bacterium- and virus resistant plants may become highly important in the work of breeding.

Table 5: Resistant accessions of *Beta vulgaris* to beet necrotic yellow vein *Furovirus*

*P*lant *I*ntroduction or donor number [1]	Origin
P.I. 169023	Turkey
P.I. 169030	Turkey
P.I. 266100	Poland
Ames 2661	USA - Utah
Ames 3049	USA - California
Ames 3051	USA - California

[1] National Gene Bank Laboratorium, Beltsville, Maryland, USA.

In the course of examining further *Beta vulgaris* accessions we found six accessions resistant to BNYVV (Table 5). In these plants the virus could not be detected either by DAS-ELISA technique or with

back-inoculation test. According to Paul (1990) in various accessions of *Beta vulgaris* a reduced level of BNYVV was found, but immunity to the virus could not be pointed out. Lewellen and Biancardi (1990) reported on both quantitative inherited and monogenic resistance in *Beta vulgaris*.

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