# SALINE SOIL CONDITION DECREASES RHIZOMANIA INFECTION OF *BETA VULGARIS*

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## SUMMARY

Rhizomania, caused by the beet necrotic yellow vein virus (BNYVV), is a world-wide problem for sugar beet production. Although rhizomania was first detected in sugar beet in the northern Italian Po Valley, wild beet on the Adriatic coast near the Po estuary show no virus infection under natural conditions. Our aim was to examine whether this absence of virus infection is caused by endogenous wild beet resistance or by the saline soil conditions of coastal habitats. Greenhouse experiments were carried out with pot plants grown from coastal seed and with a BNYVV-susceptible sugar beet variety as control. The plants were grown in highly BNYVV infested soil. To simulate saline conditions, the pots were periodically irrigated with sea salt solutions of two different concentrations or with tap water as a control. The BNYVV levels in the plant were measured by ELISA. Without salt, a range of tolerances to BNYVV was observed in plants derived from different coastal populations. One population was highly virus resistant, two were more heterogeneous and three populations were highly susceptible. In the presence of salt the data showed a significant reduction of the number of infected plants. These results indicate that the absence of virus in wild beet populations is mainly due to the saline soil conditions in coastal habitats. This phenomenon will be important for the biosafety assessment of gene flow of BNYVV resistance genes from transgenic sugar beet to wild beet populations in coastal habitats.

## RIASSUNTO

**I SUOLI SALINI DIMINUISCONO LE INFEZIONI DI RIZOMANIA IN *BETA VULGARIS*.** La rizomania, causata dal virus dell'ingiallimento necrotico nervale della barbabietola (BNYVV), è ormai un problema di portata mondiale per la bieticoltura da zucchero. Benché la malattia sia stata originariamente segnalata nelle colture della valle del Po, le bietole selvatiche della costa adriatica nelle vicinanze della foce del fiume non sono apparentemente infette. Si è pertanto pensato di accertare se l'assenza d'infezione dipendesse da resistenza genetica intrinseca di queste popolazioni, ovvero dalle condizioni di salinità degli habitat costieri. Allo scopo, sono state condotte prove allevando in vaso in un terreno con un forte inoculo di BNYVV semenzali di bietola derivati da semi raccolti sulle coste. Come testimone è stata usata una varietà di bietola altamente suscettibile al virus. Per riprodurre le condizioni di salinità, i vasi sono stati periodicamente irrigati con soluzioni acquose di sali marini a due diverse concentrazioni, mentre i testimoni venivano irrigati con acqua di fonte. La concentrazione virale nelle piante è stata stimata mediante ELISA. I semenzali di diverse popolazioni costiere di bietole selvatiche irrigati con acqua dolce hanno mostrato un livello di tolleranza differenziale a BNYVV. Una popolazione è risultata altamente resistente, due hanno mostrato un comportamento intermedio, e tre si sono rivelate altamente suscettibili. L'irrigazione con acqua salata, invece, ha prodotto una notevole diminuzione del numero di piante infette. Questi risultati indicano che l'assenza di BNYVV nelle popolazioni spontanee di bietola degli habitat costieri è dovuta sostanzialmente più alle condizioni di salinità ivi presenti che a fattori di resistenza. Di ciò è importante tener conto negli accertamenti sulla diffusione naturale (gene flow) di geni di resistenza da piante transgeniche di barbabietola da zucchero alle popolazioni costiere di bietole selvatiche.

*Key words:* rhizomania, *Beta vulgaris*, saline soil condition, virus infection, transgenic BNYVV resistance, biosafety assessment.

## INTRODUCTION

Rhizomania caused by the beet necrotic yellow vein virus (BNYVV), has spread through the sugar beet fields of Europe, USA, Japan, and China (Cooper and Asher 1988; Asher, 1993). The virus is transmitted by

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the fungus *Polymyxa betae* Keskin (Tamada, 1975; Abe and Tamada, 1986). The disease leads to decreased sugar beet yields and a loss of up to 30% sugar content (Giunchedi *et al.*, 1987). Plant breeders are developing new resistant varieties (Mannerlöf *et al.*, 1996; Mechelke, 1997). And biosafety questions may arise due to potential ecological effects resulting from flow of resistance genes between cultivated and wild plant populations (Rissler and Mellon, 1996; Schmidt and Hankeln, 1996; Tomiuk *et al.*, 1996).

Beets are wind pollinated, and due to the large potential spread over many kilometres it is not possible to control pollen dispersal (Bartsch and Pohl-Orf, 1996). The Po Valley is the most important breeding district in Europe and flowering cultivated and wild beets are only a few kilometres apart. Additionally, from this area BNYVV was first reported (Faccioli and Giunchedi, 1974). In this area, commercial cultivar seeds of *Beta vulgaris* ssp. *vulgaris* are produced on 4500 ha (KWS, personal communication). Each ha containing approximately 50,000 flowering bolters. There is a strong influence of the resulting $2.25 \times 10^8$ flowering sugar beet on an estimated number of $4 \times 10^4$ flowering wild beet in an area of 4000 $km^2$ in north-eastern Italy (Bartsch and Schmidt, 1997). Thus in the case of genetically modified rhizomania-resistant plants, there is a potential risk that this trait will spread into wild beet populations. Increased fitness of such hybrid plants after unintentional gene flow via resistance gene containing pollen, would influence the genetic diversity of these populations. However, in 1994, Bartsch *et al.* (1996) did not observe any virus infection in a representative number of coastal populations of wild beet, suggesting that an increase in wild beet fitness is unlikely at these sites but not necessarily at all sites (Whitney, 1989).

The aim of this study was to examine whether this absence of virus infestation is caused by endogenous wild beet resistance or by the saline soil conditions in natural coastal habitats. Assessing the effects of the introgression of transgenic virus resistance into wild beet populations is of great importance for ecological biosafety research of transgenic sugar beet.

## MATERIALS AND METHODS

**Plants.** Seeds from 6 representative wild beet populations on the Adriatic coast (Table 1) were collected in 1995 and sown in 1996 for greenhouse studies. A rhizomania susceptible sugar beet variety 'Edda' was used as control. Between 28 and 30 plants per population were used.

**Table 1.** Origin of *B. vulgaris* ssp. *maritima* from a south-western to north-eastern transect of the northern Adriatic coast.

| Population | Origin | Habitat |
|---|---|---|
| A | Bocassette | Po delta at Maestra |
| B | Rosolina Mare | Island of Albarella |
| C | Chioggia | Leisure boat harbour |
| D | Porto di Malamocco | Island of Pelestrina |
| E | Fusina | Lagoon of Venice |
| F | Grado | Lagoon of Grado |

**Growth conditions.** Plants were grown at 15-30°C (night/day). After 12-16 days growth in virus-free soil, seedlings were pricked out into single 250 ml pots containing naturally infested BNYVV-soil (Büttner and Mangold, 1997). The infested soil was prepared by mixing field soil of a known BNYVV-sugar beet field near Mainz (Germany) with peat culture substrate 1:1. Salt irrigation treatments were used to simulate saline conditions according to Ellenberg (1992). The plants were irrigated twice a week with sea salt solutions (0.5 % or 1.0 % salt) or with tap water . To supply the soil with sufficient moisture, plants were additionally watered with tap water whenever necessary. The electric conductivity of the soil in each pot was measured to evaluate the resulting soil salinity at the end of the experiment. Conductivity values were transformed to salinity using a calibration curve (Table 2).

**Table 2.** Effect of irrigation treatment on conductivity (± SE) and calculated salinity in soil at the end of the experiment.

| Irrigation treatment | Conductivity [mS $cm^{-1}$] | Salinity of soil [% wt] |
|---|---|---|
| water | 2.5 (± 0.3) | 0.1 |
| 0.5 % salt | 9.7 (± 0.2) | 0.5 |
| 1.0 % salt | 17.1 (± 0.5) | 1.2 |

**ELISA.** After 95-106 days of incubation (Whitney, 1989; Geyl *et al.*, 1995) the root material was sampled (3 replicates per plant), and tested for BNYVV infection by ELISA (Alderlieste and Van Eeuwijk, 1992; coat protein test from LOEWE, Ingelheim).

The ELISA extinction values ($E_{405}$) were classified in three infection categories:

| extinction | infection |
|---|---|
| 0.00-0.29 | none |
| 0.30-0.90 | low |
| > 0.90 | high |

**Statistics.** Statistical analyses were done with SIGMA-STAT. The extinction values were examined for significant differences in relation to the genotype of test plant and salt treatment in a TWO-WAY analysis with the Tukey-test ($P < 0.05$).

## RESULTS

**BNYVV susceptibility depending on population genotype.** For plants irrigated only with tap water, the six wild beet populations tested showed a wide range of resistance to rhizomania (Fig. 1). There was a significant virus infection rate at least in some plants of all populations tested (Table 3). One population (A) was highly resistant, only 4 of 32 plants falling within the low infection category by the virus. Most plants of populations B and F were not infected or only slightly, but a few were highly infected, indicating polymorphism for BNYVV-resistance/susceptibility in these populations. Population C, D and E were classified as highly susceptible (Table 3). The cultivar 'Edda' was significantly more highly infected than all wild beet populations, whereas population A always showed the highest resistance.

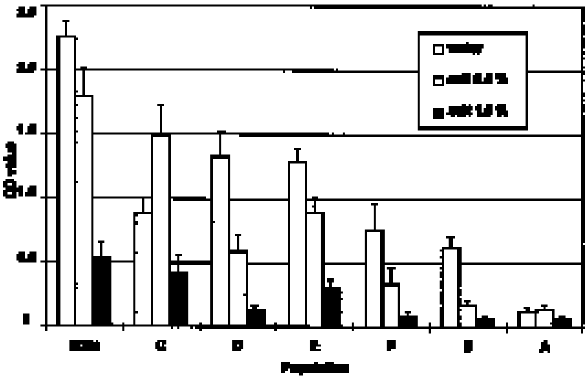

**Fig. 1**. BNYVV content of *B. vulgaris* populations under different irrigation treatments. The plants were grown in highly virus infested soil. Optical density represent the BNYVVcoat protein content. Origins of the wild beet populations A-F are given in Table 1. Bars represent ± SE.

**BNYVV susceptibility in relation to salt treatment.** As shown in Table 3, the number of rhizomania-infected plants significantly decreased with increasing salt treatment, and significant differences in virus infestation between the populations were observed. The effect was independent of plant genotype, with the exception

**Table. 3**. Number and status of BNYVV exposed *B. vulgaris* plants depending on salt irrigation treatment (water, 0.5% or 1.0%).

| Population | Treatment | No. infection | Low infection | High infection | Total |
|---|---|---|---|---|---|
| | water | 28 | 4 | 0 | 32 |
| A | 0.5 | 26 | 6 | 0 | 32 |
| | 1.0 | 30 | 2 | 0 | 32 |
| | water | 9 | 14 | 7 | 30 |
| B | 0.5 | 23 | 6 | 0 | 29 |
| | 1.0 | 31 | 0 | 0 | 31 |
| | water | 9 | 8 | 15 | 32 |
| C | 0.5 | 9 | 6 | 17 | 32 |
| | 1.0 | 21 | 6 | 5 | 32 |
| | water | 5 | 10 | 17 | 32 |
| D | 0.5 | 16 | 7 | 8 | 32 |
| | 1.0 | 28 | 3 | 1 | 32 |
| | water | 2 | 4 | 25 | 31 |
| E | 0.5 | 9 | 8 | 15 | 32 |
| | 1.0 | 21 | 9 | 2 | 32 |
| | water | 19 | 3 | 8 | 30 |
| F | 0.5 | 24 | 4 | 3 | 31 |
| | 1.0 | 29 | 1 | 0 | 30 |
| | water | 0 | 1 | 29 | 30 |
| Edda | 0.5 | 7 | 2 | 23 | 32 |
| | 1.0 | 13 | 10 | 5 | 28 |

of population A, which had very low virus titres even at the control water treatment. The surprising increase of virus infection in population C under low salt irrigation (Fig. 1), was not statistically significant.

## DISCUSSION

This study demonstrates a high degree of genetic polymorphism in the resistance of *B. vulgaris* to rhizomania. Endogenous resistance of the wild form of *B. maritima* has been reported (Geyl *et al.*, 1995; Mechelke, 1997). Whitney (1989) classified 27 % of the populations examined from Denmark, U.K., France and Italy as resistant to BNYVV, and the United States Department of Agriculture Database (1997) reports a rate of 17% resistance in their beet germplasm.

The data showed a significant reduction of infection rates in the presence of salt, and indicate that absence of the virus in wild beet populations (Bartsch *et al.*, 1996) is mainly due to the saline soil conditions in coastal habitats. The effect has been attribute to the strong negative influence of high nutrient (salt) supply on virus infection rates (Koch, 1982). Bürcky and Büttner (1985) reported delayed outbreak of virus infection in plants grown with powdered complete fertiliser. It is still uncertain whether soil salinity operates directly on BNYVV multiplication or causes inactivation of vector zoospores or, influences endogenous plant factors such as root physiology and morphology.

Not much is known about the biosafety aspects and potential ecological impact of transgenic plants expressing viral proteins. The spreading of virus resistance genes to non-target plant populations may bring several disadvantages as well as some advantages in ecological fitness. Virus resistance is likely to be ecologically relevant in wild populations if the disease is widespread and has significant effects on plant fitness. Effects might therefore only be observable in natural beet habitats with high levels of BNYVV and susceptible *B. vulgaris* genotypes. Under these circumstances, introgression of transgenic resistance genes would surely alter the natural genetic constitution of wild beet populations. Because saline soil conditions decrease the BNYVV infection in beet, dramatic effects are unlikely in wild beet populations in Adriatic coastal habitats.

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