Euphytica **90:** 293–299, 1996.

Reduced titer of BNYVV in transgenic sugar beets expressing the BNYVV coat protein

Marie Mannerlöf, Britt-Louise Lennerfors & Paul Tenning*
Hilleshög AB, Box 302 S-261 23 Landskrona, Sweden; (author for correspondence)*

Received 24 October 1995; accepted 31 January 1996

Key words: Beet necrotic yellow vein virus, *Beta vulgaris*, coat protein, field trial, rhizomania, sugarbeet

Summary

Rhizomania is a disease of sugar beet caused by the furovirus beet necrotic yellow vein virus (BNYVV). Coat protein mediated resistance has been reported for a number of viral diseases. This approach to virus resistance was therefore attempted for control of rhizomania. Two constructs of the coat protein gene of BNYVV were introduced into sugar beet by *Agrobacterium*-mediated transformation. The mRNA level was estimated to be 0.01% of the poly A^+ RNA. Expression of the coat protein gene was under the detection limit of our western blotting protocol i.e. below 0.01 μg /50 μg (0.02% of the total soluble protein). One transformation event per construct was tested in a greenhouse assay and in rhizomania infested soil in a field trial. In the greenhouse assay, transgenic plants showed a strong reduction of virus multiplication when compared to non-transgenic plants. This result was confirmed in the field trial, where a significant difference in virus multiplication was shown between plants with and without the coat protein gene.

Introduction

Rhizomania has become a major threat to sugar beet (*Beta vulgaris* L.) crops in most growing areas of the world (Bouzoubaa 1986, Duffus 1987, Tamada 1973). Symptoms of the disease are massive lateral proliferation of rootlets and a discoloration of the vascular tissue. This results in a severe stunting of the root with a consequent reduction in sugar yield. In 1992 it was estimated that 10% of the sugar beet acreage in western Europe was affected by the disease (E. Johansson, personal communication). Rhizomania is caused by the furovirus, beet necrotic yellow vein virus (BNYVV) (Tamada 1975). Furoviruses are a defined taxonomic group of fungal-transmitted rod-shaped single-stranded viruses with divided, typically bipartite genomes (Brunt 1986, Brunt 1989). BNYVV is transmitted by the soil-borne fungus, *Polymyxa betae* (Abe 1986, Fujisawa 1977), member of the Plasmodiophoraceae. BNYVV is a positive sense, single-stranded RNA virus. The virus consists of four RNA components, the coat protein gene is located at the 5' end of RNA 2 (Bouzoubaa 1986). Except for fumigation of the soil by methyl bromide, no chemical control is available for eliminating the transmitting fungus. Thus, if the disease invades a field, the only option open to the sugar beet farmer is the use of genetically resistant cultivars. Resistant cultivars means the plant do not develop any symptoms or suffer from the BNYVV disease. At present, only cultivars that are tolerant,i.e. plants are developing mild symptoms when infected with BNYVV are available on the market. Rhizomania has therefore been the focus of attention for biotechnologists.

Resistance to viral diseases through the introduction of viral DNA sequences is a well studied phenomenon (Beachy 1990). Coat protein-mediated resistance has been shown for a wide range of virus diseases (Hanley-Bowden 1990). Thus far, no report has addressed the coat protein-mediated resistance to a furovirus based on results from mature plants. There has been one report by Kallerhoff et al. (1990) on suspension cells of sugar beet that contain the coat protein gene of BNYVV introduced by *Agrobacterium*-mediated transformation. Protoplasts were isolated from transgenic suspension cells and infected with

BNYVV. By immunological methods it was shown that transgenic protoplasts were less effective than non-transgenic protoplasts in supporting viral multiplication. Ehlers et al. (1991) cloned and expressed the coat protein gene of BNYVV in sugar beet hairy root cultures. However, multiplication of the virus in these cells was not studied.

It has been shown in transgenic plants that the coat protein gene of one virus may protect plants from closely- and also distantly-related viruses (Anderson 1989, Stark 1989). Beet soil-borne virus (BSBV), another furovirus that is closely related to BNYVV (Hutchinson 1992), is found in most soils in western europe. It is a rod-shaped virus and vectored by *P. betae* but has no serological relationship to BNYVV. The ability to protect beet cells against BSBV by expression of the coat protein gene of BNYVV has not been tested. BSBV causes no obvious symptoms on sugar beet, but some researchers have reported reduced seedling growth in greenhouse studies (Kaufmann 1993).

In the present study, the coat protein gene of BNYVV was evaluated for its efficacy in providing resistance to rhizomania.

Materials and methods

Plant material and gene constructs

Two diploid transgenic sugar beet plants were obtained from Plant Genetic Systems N.V., Ghent, Belgium (PGS). Both lines containing one copy of the T-DNA insert, Commission de Genie Biomoleculaire. (B/F/92.02.01)

The sugar beet material used for the transformation experiments was from an population that has been selfed two times (S2). The plants were transformed with two different constructs pGSBNYC2 and pGSBNYC3, by *Agrobacterium*-mediated transformation (D'Halluin 1992).

The coding sequence of the BNYVV coat protein gene was isolated as a 721 basepair cDNA.

The two plasmid constructs, pGSBNYC2 and pGSBNYC3 (see Figure 1), contains the coat protein gene (cp), isolated as a 721 basepair cDNA. The cp gene was driven by the cauliflower mosaic virus promoter (35S) and followed by a 3' nopalin synthase (nos) terminator. The constructs also contain the *bar* gene (De Block 1987) that encodes an acetyl transferase which confers resistance against the herbicide BASTA® (phosphinotricin). The constructs also contain the *nptII* gene (Bevan 1983) that encodes a neomycin phospotransferase which confers resistance to several aminoglycoside antibiotics, such as neomycin, Kanamycin and G418. The *bar* gene and the *nptII* gene were driven by TR promoter originating from *A.tumefaciens*. The bar gene was followed by a 3' gene 7 from *A.tumefaciens* (g7) terminator and the *nptII* gene was followed by a 3' octopine synthase (ocs) terminator. The difference between the constructs resided in the length of their untranslated coat protein leader sequences. pGSBNYC2 had a 57-basepair untranslated leader and pGSBNYC3 a 145-basepair untranslated leader. One transformation event was obtained per construct. Original transformants (S2) were selfed and seeds (S3) were germinated. Plants segregating for the introduced genes were identified by the BASTA® paint assay (see below). The transgenic plants (S3) expressing the *bar* gene were again selfed. These plants, representing the S4 generation, were then used for further study. Thus, the tested material was a segregating S4 population with a mixture of homozygous and heterozygous plants with respect to the introduced genes. The plants segregating for the introduced genes were used as controls in greenhouse and in fieldtrial.

The sugar beet cultivars Hilma and Rhizor were used as susceptible and tolerant controls, respectively. Hilma as a susceptible control, developing severe symptoms. Rhizor as a tolerant control, developing mild symptoms when infected with BNYVV. To monitor expression levels tobacco was transformed according to Horsch et al. (1986).

DNA isolation and PCR

Isolation of root DNA was carried out as described (Tai 1990) with minor modifications, a 2 x extraction buffer was used and after precipitation the DNA was further purified with CTAB (Murray 1983). PCR was performed in a 25 μl volume (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 3 mM $MgCl_2$, 0.01% gelatin, 200 μM of each dNTP, 2.5 units *Taq* polymerase (Boehringer Mannheim) and 1.25 μM of each primer). A control amplification was done with internal primers directed to *nad5* (5'TAGCCCGACCGTAGTGATGTTAA 3' and 5'ATCACCGAACCTGCACTCAGGAA 3' (Ecke 1990). Specific primers were made to complement the coat protein gene of BNYVV (5'CGTGTTTCGGACGTCGTGAGTGT 3' and 5'GGTGGACTGGTTCTACCTTGACA 3') (Bouzoubaa 1986).

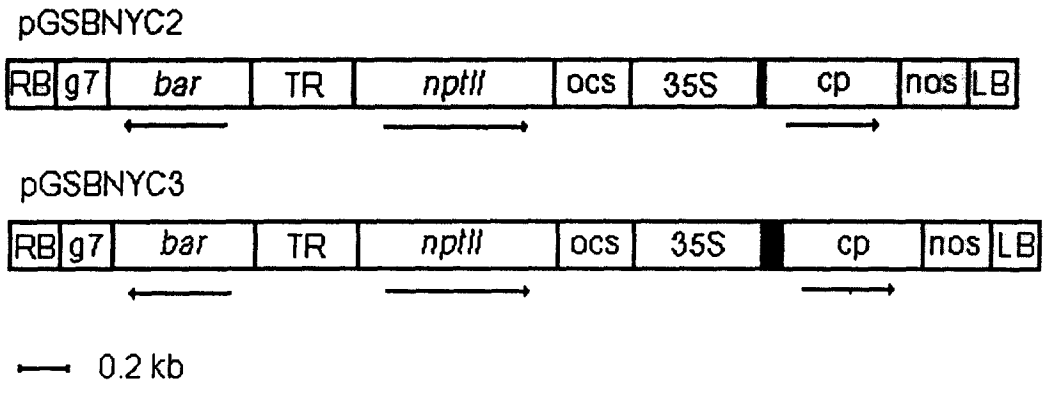

Figure 1. A schematic representation of the T-DNA in the plasmids pGSBNYC2 and pGSBNYC3. The T-DNA of pGSBNYC3 is identical to pGSBNYC2 except that the leader sequence in this latter construct is shorter.

Basta paint assay

Plants with and without the introduced DNA were discriminated with a BASTA® paint assay. A filter paper containing 100 μl of a 10 mg/ml solution of phosphinothricin was attached to a leaf with a clip. Fourteen days after the application, the filter paper was removed and the leaf area under the filter paper was inspected. Plants with the *bar* gene showed no sign of wilting, whereas plants segregating for the *bar* gene showed a wilted area under the filter.

Greenhouse test

Plants transformed with the gene construct pGSBNYC3 were screened for tolerance/resistance against BNYVV in a greenhouse assay. Twenty-one plants from the S4 generation were screened. The greenhouse test was essentially performed as described by Buercy and Buettner (1985). Ten days after sowing the sugar beet plants were transplanted into a BNYVV infested soil/sand mixture.

Twenty-one days after transplantation the roots were harvested individually, washed and an extract made from each root for detection of virus content in ELISA.

Field trial

Five plots per transgenic line were grown in a randomized fashion. One plot consisted of 3 rows, each row was 5 meters long. Planting density was 5 plants per meter, i.e. in total 75 plants per plot. Seeds were drilled in early May and roots were harvested in late October. The roots were harvested individually, washed and an extract made from each root for detection of virus content in ELISA. Normal agricultural practice was followed during the growth period. The field trial was performed in BNYVV-infected soil in Pithivers, France in 1992. A permit for the trial was obtained from Commission de Genie Biomoleculaire.

ELISA and western blot

Polyclonal antibodies against BNYVV were obtained from Boehringer Mannheim (cat #814 296, Mannheim, Germany). Polyclonal antibodies against BSBV were obtained from Professor Klas Lindsten (Swedish University of Agricultural Sciences, Uppsala, Sweden). The ELISA protocol recommended by the manufacturer was followed. For detection of coat protein by western blot, 500 mg of leaf material was homogenised and diluted in 5 x Laemmli loading buffer (Laemmli 1970). Protein was determined by Lowry et al. (1951). Fifty μg of soluble protein was separated by SDS-PAGE, electroblotted to a nitrocellulose membrane (0.45 μm) and immunodetected by an amplified alkaline phosphatase assay kit (cat #1706412 Bio-Rad Laboratories, Richmond, CA, USA).

Results

Expression of the coat protein gene

Northern blot analysis on leaf material from transgenic sugar beet revealed a mRNA transcript of expected size. Transcription was estimated to 0.01% of the

polyA$^+$ RNA. No difference in transcription level could be seen between the two transformation events. In our assay system, the detection limit for a western blotting analysis of purified virus is 0.01 μg / 50 μg (0.02% of the soluble protein). No expression of the coat protein gene could be detected in transgenic sugar beet by western blot.In transgenic tobacco both constructs expressed the coat protein gene to 0.1% of the soluble protein (data not shown).This was estimated with an average of 13 stably-transformed tobacco plants.

Greenhouse test

Extracts from sugar beet plants that have been grown in BNYVV infested soil were used to estimate the virus content by ELISA and to isolate DNA for PCR. Plants with the coat protein gene showed a reduced level of virus content when compared to sugar beets that had segregated for the gene (i.e. cp-, Figure 2). All plants of the variety Hilma, the susceptible control, showed a high virus content. The rhizomania-tolerant variety, Rhizor, showed a more complex pattern. Some plants were low in virus content while others were high. This is also seen with Rhizor grown in commercial sugarbeet fields (E. Johansson, personal communication). No clear difference in virus content could be seen between the two transgenic lines (not shown).

Field trial

Seeds from the same cross that were evaluated in the greenhouse test was used for the field trial in infested soil in France. Fifteen hundred transgenic plants were planted in the field. Hilma and Rhizor were used as susceptible and tolerant controls, respectively.

A positive correlation was found between the presence of the coat protein gene (PCR) and a low content of BNYVV, not as clear as for the greenhouse trial (Figure 3). Again the tolerant control, Rhizor, showed great variation in virus content between plants. However the soil in the field was heavily infested with BNYVV and there could have been a heterogenous infection-pressure in the field. All plants were severely infected with beet mosaic virus. The plants were highly inbred and showed poor vigour, thus, they may have been compromised to attack by a second pathogen. No obvious correlation was found between the content of BSBV and the presence of the coat protein gene (data not shown).

Table 1. Statistical analysis of the ELISA values from field trials on the S4 population. The difference between the constructs pGSBNYC2 and pGSBNYC3 is the length of the leader sequence preceeding the coat protein gene. Cp+ indicate plants with the coat protein gene and cp- indicates plants segregating for the coat protein gene. N values indicate the size of the test sample. P values indicate the significance of the difference between plants containing the cp and plants segregating for the cp obtained after an analysis of variance.

Line	Mean OD ± SD	N	P
pGSBNYC2 cp$^+$	0.32 ± 0.07	60	0.0119
pGSBNYC2 cp$^-$	0.61 ± 0.09	18	
pGSBNYC3 cp$^+$	0.21 ± 0.04	58	0.0004
pGSBNYC3 cp$^-$	0.61 ± 0.11	22	

Statistical analysis of the field trial results

A statistical analysis was performed on the ELISA and PCR data from the S4 material evaluated in the fieldtrial. This analysis of variance presents the mean ELISA values from a total of 158 S4 sugar beet plants with and without the coat protein gene (Table 1). The results show significantly higher ELISA values for plants without the coat protein gene, irrespective of the length of the leader. No clear difference could be found in the degree of tolerance between the two constructs, based on ELISA values.

No correlation could be found after a statistical analysis between the presence of the BNYVV coat protein gene and the level of BSBV in the transgenic plants (data not shown).

Discussion

Sugar beet was transformed with *A. tumefaciens* carrying a coat protein gene from BNYVV. Two gene constructs were used that differed in the length of the leader sequence. These transgenic plants were assayed in rhizomania infected soil in a greenhouse assay and in a field trial. Both experiments show that virus multiplication was reduced in the plants carrying the coat protein gene. No clear difference was recorded between the two gene constructs. One transformation event was tested per construct.

The long leader construct has an intact hairpin structure (Meulewater 1989) in contrast to the short leader construct. The importance of viral leader sequences for gene expression is well known (Gallie

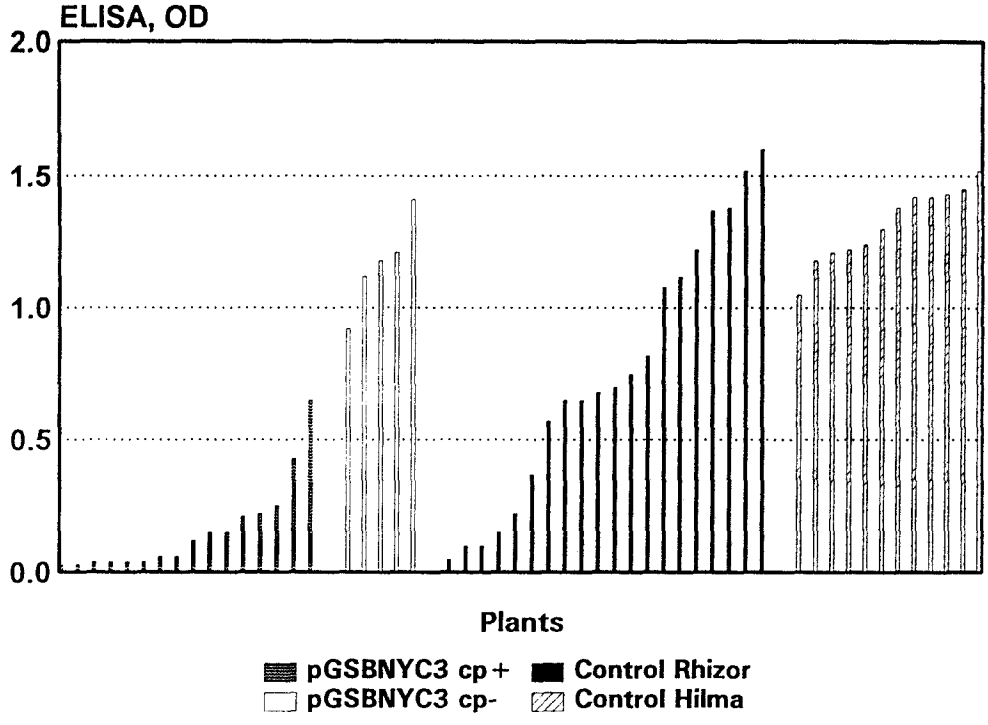

Figure 2. Results from the greenhouse trial. Each bar represents one plant. The transgenic plants (S4 generation) were transformed with pGSBNYC3. Cp+ and indicate plants with the coat protein and cp- indicates plants segregating for the introduced coat protein. Hilma is a sensitive and Rhizor is a tolerant cultivar.

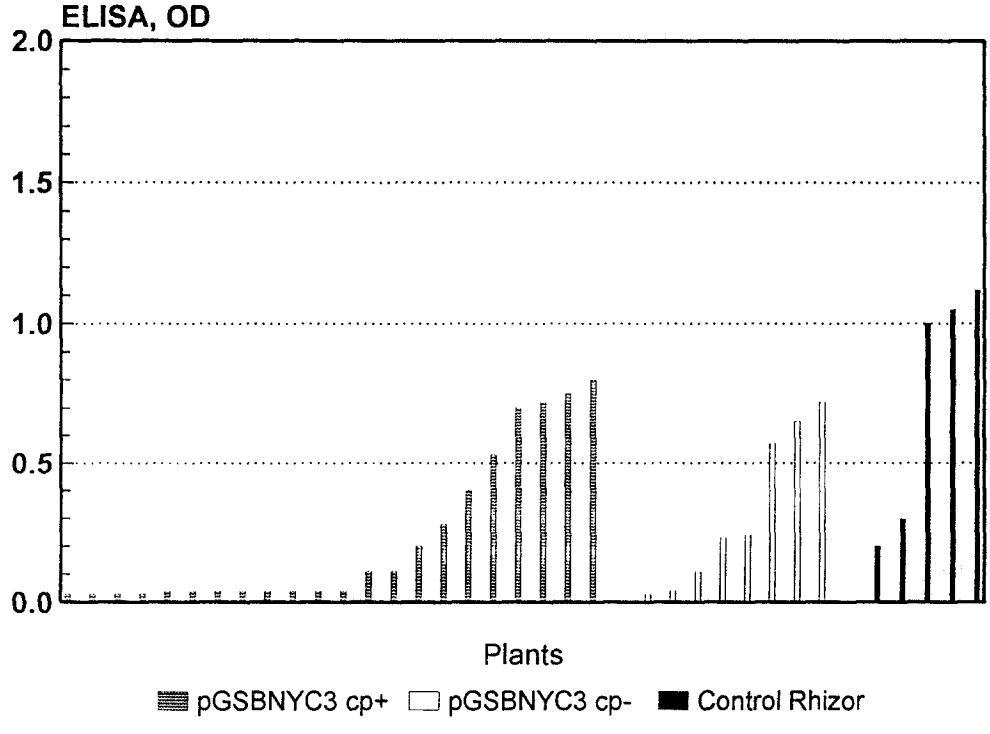

Figure 3. Representative part of the field trial. Each bar represents one plant. pGSBNYC3 cp+ indicates plants with the coat protein gene, pGSBNYC3 cp- indicates plants segregating for the introduced coat protein. Rhizor is a tolerant cultivar used as a control.

1987), and it might be expected that the long leader construct permits a higher expression level compared to the short leader and, thus, a higher degree of resistance. In this study only one transformation event per construct was used. This is not enough to make an evaluation of the importance of the leader sequence of the coat protein gene. In the greenhouse trial a clear correlation was found between the presence of the coat protein gene and low virus content. This was not as obvious in the field trial. The reason for this discrepancy is unclear, however, a number of factors may be involved. The material was a mixture of homozygous and heterozygous plants, with respect to the cp gene. A gene dosage effect in resistance could be envisaged depending on whether the plants had one or two copies of the coat protein gene. Kaniewski et al. (1990) reported on variability in the degree of expression of a potato virus X coat protein gene in several transformed plants due to altered genetic background. If this was also the case in the cp+ sugar beet material, the result could be a variation in resistance.

Kaufmann et al. (1993) showed a negative impact of BSBV on the growth of sugar beet. Stark and Beachy (1989) and Anderson et al. (1989) showed that transgenic plants expressing one coat protein gene were protected from infection by related viruses of the potyvirus group. An estimate of the virus content in the transgenic plants was therefore done with the highly related BSBV (Hutchinson 1992). No correlation could be shown between presence of the coat protein gene and the amount of this related furovirus. This report is the first published field and greenhouse data on the efficacy of the BNYVV coat protein gene to protect against rhizomania. One suggested mechanism for coat protein mediated resistance is recoating of the partly uncoated virus particle (Quillet 1989). The recoated virus might then be impaired in transport to the next cell, since the viral encoded movement protein is not produced in the infected cells. If BNYVV is transmitted from cell to cell as an intact virus particle (Meshi 1987), the movement protein might be needed to enlarge the periplasmic channel (Lindbo 1992b).

An assay of resistance to rhizomania is therefore more accurately assessed with fully grown plants than with protoplasts. Such a discrepancy between resistance found in fully grown plants and protoplasts was described in transgenic tobacco carrying the coat-protein gene of tobacco etch virus (Lindbo 1992a). Studies have been done with coat protein genes in a number of crop/virus combinations (Beachy 1990). Two types of resistance mechanisms can be distinguished: protein mediated (Kawchuk 1991, van der Vlugt 1993) and RNA mediated (Gielen 1991, Lindbo 1992b). Kaniewski et al. (1990) demonstrated resistance to potato virus X and Y in transgenic tobacco carrying the coat-protein gene. This resistance could not be correlated to the expression of the introduced gene.par

In our study it was not possible to detect any coat protein in a western blotting assay. However, it cannot be ruled out that a low, undetectable level of the coat protein expression was determining the recorded resistance.

Acknowledgement

We thank Kathleen D'Halluin and Frank Meulewater (PGS) for providing transgenic sugar beet lines.

References

Abe, H., & T.Tamada, 1986. Association of beet necrotic yellow vein virus with isolates of *Polymyxa betae* Keskin. Ann. Phytopathol. Soc. Jpn. 52: 235–247.

Anderson, E.J., D.M. Stark, R.S. Nelson, P.A. Powel, T.E. Tumer & R.N.Beachy, 1989. Transgenic plants that express the coat protein genes of tobacco mosaic virus or alfalfa mosaic virus interfere with disease development of some non-related viruses. Phytopathol 79: 1284–1290.

Beachy, R.N., S. Loesch-Fries & T.E. Tumer,1990. Coat protein-mediated resistance against virus infection. Annu. Rev. Phytopathol 28: 451–474 (1990).

Bevan, M.W., R.B. Flavell & M.D. Chilton, 1983. A chimaeric antibiotic resistance gene as a selectable marker for plant cell transformation. Nature 304: 184–187.

Brunt, A.A. & K.E. Richards, 1989. Biology and molecular biology of furoviruses. Adv. Virus Res. 36:1–32.

Brunt, A.A. & E. Shikata, 1986. Fungus transmitted and similar labile rod-shaped viruses. Pages 305- 335 in: The Plant Viruses. Vol. 2, The Rod-Shaped Plant Viruses. M.H.V. Van Regenmortel and Heinz Fraenkel-Conrat, eds Plenum Publishing, New York.

Bouzoubaa, S., V. Ziegler, D. Beck, H. Guilley, K. Richards & G. Jonard G, 1986. Nucleotide sequence of beet necrotic yellow vein virus RNA-2. J Gen Virol 67: 1689–1700.

Buercy, K. & G. Buettner, 1985. Ansaetze zur Selection rhizomaniatoleranter Zuckerrueben waehrend der Jugendentwicklung. I. Virustiter. Zuckerindustrie 110: 997–1000.

De Block, M., J. Botterman, M. Vanderwiele, J. Docks, C. Thoen, V. Gosselé, R. Movva, C. Thompson, M. van Montagu & J. Leemans, 1987. Engineering herbicide resistance in plants by expression of a detoxifying gene. EMBO J 6: 2513- 2518.

Duffus, J.E. & H.Y. Liu, 1987. First report of rhizomania of sugar beet from Texas. Plant Dis. 71:557.

D'Halluin, K., M. Bossut, B. Mazur, J. Leemans & J. Botterman, 1992. Transformation of sugar beet (*Beta vulgaris* L.) and evaluation of herbicide resistance in transgenic plants. Bio/technology 10: 309–314.

Ecke, W., U. Schmitz & G. Michaelis, 1990. The mitochondrial *nad5* gene of sugar beet (*Beta vulgaris*) encoding a subunit of the respiratory NADH dehydrogenase. Curr Genet 18: 133–139.

Ehlers, U., U. Commandeur, R. Frank, J. Landsmann, R. Koenig & W. Burgermeister, 1991. Cloning of the coat protein gene from Beet Necrotic Yellow Vein Virus and it's expression in sugar beet hairy roots. Theor Appl Genet 81: 777–782.

Fujisawa, I. & T. Suzimoto, 1977. Transmission of beet necrotic yellow vein virus by *Polymyxa betae*. Ann Phytopathol Soc Japan 43: 583–586.

Gallie, D.R., D.E. Sleat, J.W. Watts, P.C. Turner & T.M.A. Wilson, 1987. A comparison of eucaryotic vir 5'-leader sequences as enhancers of mRNA expression in vivo. Nucl Acids Res 15: 8693–8711.

Gielen, J.J.L., P. de Haan, A.J. Kool, D. Peters, M.Q.J.M van Grinsven & R.W. Goldbach, 1991. Engineered resistance to tomato spotted wilt virus, a negative strand RNA virus. Bio/technology 9: 1363–1367.

Hanley-Bowdon, L. & C. Hemenway, 1990. Expression of plant viral genes in transgenic plants. In Genetic Engineering with Plant Viruses, Michael T, Wilson A, Davies JW, (eds), pp. 251–275. CRC Press, Boca Raton.

Horsch, R.B., J. Fry, N.L. Hoffman, M. Wallroth, D. Eickholtz, S.G. Rogers & R.T. Fraley, 1986. A simple and general method for transferring genes into plants. Science 227: 1229–1231.

Hutchinson, P.J., C.M. Henry & R.H.A. Coutts, 1992. A comparison, using dsRNA analysis, between beet soil borne virus and some other tubular viruses isolated from sugar beet. J Gen Virol 73: 1317- 1320.

Kallerhoff, J., P. Pascual, S. Bouzoubaa, S. Ben Tahar & J. Perret, 1990. Beet necrotic yellow vein virus coat protein-mediated protection in sugar beet (*Beta vulgaris* L.) protoplasts. Plant Cell Rep 9: 224–228.

Kaniewski, W., C. Lawson, B. Sammons, L. Haley, J. Hart, X. Delannay & N. Tumer, 1990. Field resistance of transgenic Russet burbank potato to effects of infection by potato virus X and potato virus Y. Bio/technology 8: 750–754 (1990).

Kaufmann A., R. Koenig & H. Rohloff, 1993. Influence of beet soilborne virus on mechanically inoculated sugar beet. Plant Pathol 42: 413–417.

Kawchuk, L.M., R.R Martin & J. McPherson, 1991. Sense and antisense RNA-mediated resistance to potato leafroll virus in Russet Burbank potato plants. Mol Plant-Microbe Interac 4: 247–253.

Keskin, B., 1964. *Polymyxa betae* n.sp., ein Parasit in den Warzeln von *Beta vulgaris* Tournefort, besonders während der Jugendentwicklung der Zuckerube. Arch Mikrobiol 49: 348–374.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685.

Lindbo, J.A. & W.G. Dougherty, 1992a. Untranslatable transcripts of the tobacco etch virus coat protein gene seguence can interfere with tobacco etch virus replication in transgenic plants and protoplasts. Virology 189: 725–733.

Lindbo, J.A. & W.G. Dougherty, 1992b. Pathogen- derived resistance to a potyvirus: Immune and resistant phenotypes in transgenic tobacco expressing altered forms of a potyvirus coat protein nucleotide sequence. Mol Plant-Microbe Interac 5: 144–153.

Lowry, O.H., N.J. Rosebrough, A.L. Farr & R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol; Chem. 193: 265–275.

Meshi, T., Y. Watanabe, T. Saito, A. Sugimoto, T. Maede & Y. Okada, 1987. Function of the 30 kd protein of tobacco mosaic virus involvement in Cell-to-cell movement. EMBO J 6: 557–256.

Meulewaeter, F., P. Soetert & J. van Emmelo, 1989. Structural analysis of the coat protein gene in different BNYVV isolates. Med. Fac. Landbouw, Rijksuniv. Gent. 54/2b: 465–468.

Murray, H.G. & W.F. Thomson, 1983. Rapid isolation of high molecular weight DNA. Nucl Acids Res 8: 4321–4325.

Quillet, L., H. Guilley, G. Jonard & K. Richards, 1989. In vitro synthesis of biologically active beet necrotic yellow vein virus RNA. Virology 172: 293–301.

Stark, D.M & R.N. Beachy, 1989. Protection against potyvirus infection in transgenic plants: Evidence for broad spectrum resistance. Bio/technology 7: 1257–1262.

Tai, T.H. & S.D. Tanksley, 1990. A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. Plant Mol Biol Rep 8: 297–303.

Tamada, T., 1975. Beet necrotic yellow vein virus. Commonwealth Mycological Institute/Association of Applied Biologists descriptions of Plant Viruses 144: 5.

Tamada, T. & T. Baba, 1973. Beet necrotic yellow vein virus from rhizomania-affected sugar beet in Japan. Ann Phytopathol Soc of Japan 39: 325–332.

van der Vlugt, R.A.A. & R.W. Goldbach, 1993. Tobacco plants transformed with the Potato Virus Y coat protein gene are protected against different PVY isolates and against aphid-mediated infection. Transgenic Res 2: 109–114.