Field Crops Research 122 (2011) 165–172

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Review

Achievements and prospects in breeding for rhizomania resistance in sugar beet

Ourania I. Pavli[a], Piergiorgio Stevanato[b], Enrico Biancardi[c], George N. Skaracis[a,*]

[a] Department of Crop Science, Laboratory of Plant Breeding and Biometry, Agricultural University of Athens, Iera Odos 75, 11855, Greece
[b] Dipartimento di Biotecnologie Agrarie, Università degli Studi di Padova, Viale dell' Università 16, 35020 Legnaro, PD, Italy
[c] Centro di Ricerca per le Colture Industriali (CRA-CIN), Sede Distaccata di Rovigo, Viale Amendola 82, 45100 Rovigo, Italy

ARTICLE INFO

Article history:
Received 2 December 2010
Received in revised form 29 March 2011
Accepted 29 March 2011

Keywords:
Rhizomania resistance
Beet necrotic yellow vein virus
Sugar beet
Resistance breeding

ABSTRACT

Economic viability of a sugar beet crop largely depends on its successful protection against rhizomania, a most devastating disease that causes severe losses in root yield, sucrose content and quality. Rhizomania disease is caused by *Beet necrotic yellow vein virus* (BNYVV), a virus present in most sugar beet growing regions being vectored by the widely spread soil borne protoctist *Polymyxa betae* Keskin. The only practical means to control the disease is the use of genetically resistant varieties and, to date, such resistance is mainly based on a dominant gene (*Rz1*) that when present confers a sufficiently high level of protection against BNYVV. However, the emergence of virus strains capable of compromising the resistance employed in commercial varieties as well as a possible spread of more pathogenic isolates threatens crop's protection efficiency in the future. All these point to the necessity for exploiting new and more effective genetic sources of rhizomania resistance, both by classical and molecular breeding approaches, a practice that is being pursued by the relevant breeding firms. This article critically reviews the various issues related to the disease and its management and particularly to the ones pertaining to pathogen genetic diversity, types of genetic resistance currently employed, as well as to novel biotechnological approaches aiming at the development of better resisting cultivars.

Contents

1. The rhizomania disease

Historically, *Beet necrotic yellow vein virus*, the etiological agent of rhizomania disease (Tamada and Baba, 1973), is considered as one of the most important threats in worldwide sugar beet cultivation (Tamada, 1999; Lennefors et al., 2005). In the absence of efficient control measures, the disease causes severe economic losses due to a dramatic reduction in root yield, sugar content and purity (Tamada, 1999). The virus is the type species of the genus *Benyvirus* (Torrance and Mayo, 1997; Tamada, 1999) and is transmitted by the widely spread soilborne protoctist *Polymyxa betae* Keskin (Fujisawa and Sugimoto, 1976) which, due to its thick-walled resting spores, can survive in soil for years (Abe and Tamada, 1986). Rhizomania disease root symptoms mainly include a massive proliferation of secondary and tertiary roots that eventually become necrotic and give the root a bearded appearance, a profound constriction of the main taproot, a general plant stunting and a brown discoloration of the vascular stele (Richard-Molard,

* Corresponding author. Tel.: +30 2105294633; fax: +30 2105294622.
E-mail address: gskaracis@aua.gr (G.N. Skaracis).

0378-4290/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.fcr.2011.03.019

1985; Putz et al., 1990). Foliar symptoms are mostly manifested by a bright fluorescent yellowing which can be easily confused with nutrient deficiencies. The yellow vein appearance, that provides the name for the disease causal agent, is only rarely found and mostly confined to fields infected by a specific virus pathotype (Tamada, 1975). Diseased plants usually occur in patches, but can also be found scattered throughout the field. Disease responses at the physiology level include a reduced transpiration and CO_2 uptake, a reduced content of nitrogen, chlorophyll and carotenoid and an elevated amino nitrogen, sodium and potassium in the root sap (Steddom et al., 2003). For a detailed review on morphophysiological consequences of the disease the reader is referred to Rush (2003).

Disease diagnosis is usually performed by immunological tests such as DAS-ELISA, whereas pathotype differentiation was in the past mainly based on molecular techniques such as single-strand conformation polymorphism (SSCP) and restriction fragment length polymorphism (RFLP) analysis (Kruse et al., 1994; Koenig et al., 1995; Suarez et al., 1999). Since some years differentiation is mainly performed by partial or complete (re)sequencing (Koenig and Lennefors, 2000; Meunier et al., 2003; Schirmer et al., 2005).

2. Genetic features and diversity of BNYVV

BNYVV has a multipartite genome consisting of four genomic messenger-like RNAs, with some isolates also possessing a fifth RNA species, RNA 5. All genes required for basic house-keeping functions including replication, encapsidation and cellular translocation reside on RNAs 1 and 2, whereas the small RNA species RNA 3, 4 and the isolate-specific RNA 5 encode for genes involved in vector transmission and pathogenicity (Fig. 1) (Tamada, 1999) (for details see Tamada, 2002; McGrann et al., 2009). RNA 3-encoded p25 is a major determinant of disease expression in the sugar beet host but also acts as an avirulence factor in resistant sugar beet lines. The outcome of BNYVV-host-specific resistance interactions is mainly controlled by single amino acid changes in p25 (Acosta-Leal et al., 2008, 2010b; Chiba et al., 2008, 2011; Koenig et al., 2009; Pferdmenges et al., 2009).

BNYVV has been classified in three major pathotypes, referred to as A, B, and P (Koenig et al., 1995; Koenig and Lennefors, 2000). Type A is widespread in most European countries, the USA, China and Japan (Schirmer et al., 2005). Type B has a limited spread and is primarily found in Germany and France (Kruse et al., 1994), while it has been also incidentally reported in Sweden, China, Japan and Iran (Miyanishi et al., 1999; Lennefors et al., 2000; Sohi and Maleki, 2004; Koenig et al., 2008). BNYVV type P contains an additional genomic RNA (RNA 5) and is closely related to the A-type (Miyanishi et al., 1999; Schirmer et al., 2005). P-type was originally discovered in Pithiviers, France (Koenig et al., 1997) and was later also encountered in Kazakhstan (Koenig and Lennefors, 2000) and recently, in the UK (Ward et al., 2007) and Iran (Mehrvar et al., 2009). Other RNA 5-containing isolates have been reported in Japan, China (Tamada et al., 1989; Kiguchi et al., 1996; Miyanishi et al., 1999), the UK (Harju et al., 2002; Ward et al., 2007) and in Germany, where an Asian RNA 5-containing BNYVV isolate has been recently found to occur (Koenig et al., 2008). BNYVV isolates containing a fifth RNA species are generally considered as more aggressive than those containing RNAs 1-4 (Tamada et al., 1989, 1996; Heijbroek et al., 1999), presumably due to *in planta* transcription by the RNA 5-encoded p26 (Link et al., 2005).

Recent studies on the evolutionary history of BNYVV, based on the magnitude and complexity of sequence variation of four genes (RNA 2-*CP*, RNA 3-*p25*, RNA 4-*p31* and RNA 5-*p26* genes), revealed the existence of various reassortant isolates in China and Japan, as a result of mixed infections of different source isolates. It was thus suggested that the virus most probably originated in East Asia long before the beginning of sugar beet cultivation and that wild beet or related species might not have been the natural hosts of both BNYVV and *P. betae* (Chiba et al., 2011). The spread of BNYVV into the crop dates in recent years. Since the initial reports for the disease (Canova, 1959), the virus has colonized most sugar beet growing areas worldwide, yet generally showing a considerable genetic stability among virus populations separated in space and time (Koenig and Lennefors, 2000). BNYVV populations also present a relatively low incidence of reassortants or natural recombinants (Schirmer et al., 2005). Given its multipartite genome and the frequent occurrence of mixed infections with different BNYVV strains (Koenig et al., 1995), it has been assumed that natural selection poses constraints in an expected BNYVV diversification and conditions virus evolution by acting as a filter controlling the mutations that eventually become fixed (Acosta-Leal et al., 2008).

Despite the relatively low general genetic diversity among BNYVV isolates, different degrees of selection pressure seem to operate, depending on the gene and geographic location. In this framework, the RNA 3-p25 was found to be subjected to the strongest selection pressure and, more importantly, it has been demonstrated that certain amino acid changes in p25 are associated with the emergence of BNYVV strains capable of compromising the commercially exploited partial resistance sources in the last decade (Schirmer et al., 2005; Acosta-Leal et al., 2008, 2010a; Chiba et al., 2008, 2011; Koenig et al., 2009). More specifically, it has been suggested that amino acids 67–70 of p25 are linked with symptom development in resistant cultivars (Schirmer et al., 2005), although other amino acid residues in p25 may also influence isolate virulence (Acosta-Leal and Rush, 2007; Liu and Lewellen, 2007; Chiba et al., 2008). However, conclusive evidence has been to date obtained only for positions 67 and 68. In this framework, it has been shown that the amino acid change A → V at position 67 is associated with increased virulence in cultivars endowed with the *Rz1* and/or *Rz2* resistance genes (see later section) (Acosta-Leal et al., 2008, 2010a,b; Koenig et al., 2009; Pferdmenges et al., 2009; Pferdmenges and Varrelmann, 2009). In addition, it has been reported that amino acid residue 68 plays a major role in pathogenicity (Acosta-Leal et al., 2008; Chiba et al., 2008) and furthermore, changes from F or Y to C, H, L, Q lead to an increased virulence and thus, to the manifestation of the *Rz1*-resistance breaking (RB) trait (Chiba et al., 2011). Although, based on these studies, the $V_{67}C_{68}$ motif of p25 is generally considered as responsible for RB, the occurrence of such isolates was not always found associated with more pronounced disease symptoms, higher virus accumulation in the roots and reduced performance of the varieties tested as a result of *Rz1*-RB (Liu and Lewellen, 2007; Pavli et al., in press).

The possible association between genetic diversity of the virus and resistance breaking ability has been investigated in a study of host effect, employing *Rz1*, *Rz2* and susceptible plants, on genetic diversification of BNYVV. Although no direct such association was evidenced, the study revealed a significant increase of virus diversity in proportion to the strength of host resistance and it was argued that the genetic structure of BNYVV populations is correlated with virulence and the magnitude of defence barriers to be defeated for disease occurrence (Acosta-Leal et al., 2010a).

3. Conventional and molecular breeding for rhizomania resistance

Rhizomania incidence and severity can be only very moderately reduced by preventive cultural practices such as rotation, avoidance of excessive soil moisture and early plantings. Consequently, the only substantial means to ensure a viable crop production in

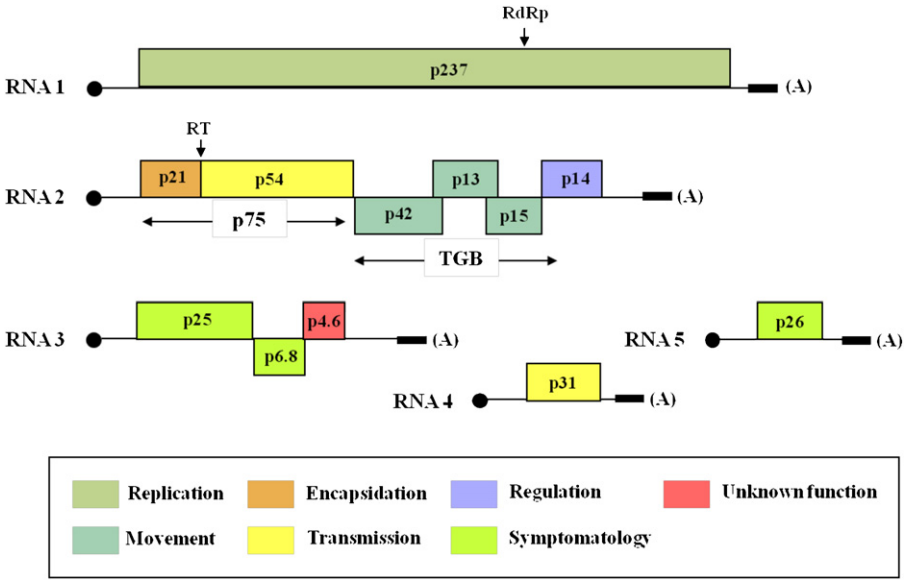

Fig. 1. Genome organization of *Beet necrotic yellow vein virus* and function of viral gene products. All RNAs are polyadenylated at the 3′ end and capped at the 5′ terminal. The boxes represent the open reading frames existing in the viral genome and colours indicate their corresponding functions. RdRp: RNA dependent RNA polymerase, TGB: triple gene block movement proteins, RT: read through protein. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

rhizomania incidence areas is the use of varieties specifically bred as resistant to the disease (Biancardi et al., 2002).

Following the initial evidence concerning the existence of genetic variability for rhizomania resistance (Bongiovanni and Lanzoni, 1964), systematic breeding efforts commenced in the late 60s using germplasm originating from the Italian multigerm variety "Alba P", a variety that had been originally bred for resistance to the cercospora leaf spot (*Cercospora beticola* Sacc.) disease (Biancardi et al., 2002). Selections, mainly based on symptom occurrence and severity along with accompanying yield reduction in terms of sucrose content, root yield and purity, resulted in the first commercial sugar beet cultivars that were widely used in infested fields and their resistance was of a quantitative nature (Lewellen and Biancardi, 1990). Further mass selection, effected in material of similar genetic background (Skaracis and Biancardi, 2000), supplemented with artificial infections and ELISA tests on a single plant basis, led to the development of the diploid monogerm hybrid variety "Rizor", characterized by a considerable higher level of resistance (De Biaggi, 1987). This variety was cultivated for a good number of years in heavily infested fields throughout Europe (Biancardi et al., 2002).

The production of resistant sugar beet hybrid varieties that have dominated the market over the last 15 years is based on a resistance source found in a commercial hybrid of Holly Hybrids in the USA. Following the first field observations in 1983, the results of only a few cycles of selection, based on individual plant performance, pointed to the high heritability of this "Holly source" (Lewellen et al., 1987; Lewellen, 1988). Later studies with segregating populations, confirmed that this resistance was simply inherited and conditioned by a single dominant gene first named *Rz* and later *Rz1* (Lewellen et al., 1987; Lewellen and Biancardi, 1990; Pelsy and Merdinoglu, 1996; Scholten et al., 1996). Although susceptible to infection by both *P. betae* and BNYVV, plants harbouring this gene typically present a low virus titer and significantly reduced disease symptoms. Due to its qualitative nature, the introgression of the *Rz1* gene has been extensively exploited in backcross breeding programs for the development of the majority of modern commercial sugar beet varieties (Biancardi et al., 2002). As to the relation between the "Holly" and the "Rizor" type resistances, they are most probably caused by the same gene (Barzen et al., 1997).

Additional sources of rhizomania resistance have also been searched for in several collections of wild beet germplasm (reviewed in Scholten and Lange, 2000; Biancardi et al., 2002). As a result, resistance genes were found in the sea beet (*Beta vulgaris* ssp. *maritima*) accessions WB41 and WB42, both accessions collected in Denmark (Lewellen et al., 1987; Scholten et al., 1996, 1999). Resistance found in WB42 is controlled by a single dominant gene (*Rz2*), closely linked to the previously identified *Rz1* gene (Scholten et al., 1996; Amiri et al., 2003), and its effectiveness in crop protection occasionally exceeds that of the *Rz1* gene (Paul et al., 1993b; Scholten et al., 1996). Consequently, although in most cases *Rz1* sufficiently protects against the A and B types, when the disease is incited by highly virulent virus types, the employment of both the *Rz1* and the *Rz2* genes is already successfully realized (Liu and Lewellen, 2007). The major gene component conditioning resistance in WB41 was named *Rz3* and was mapped on chromosome III (Gidner et al., 2005). Based on a combined use of AFLP, SNP and RAPD markers, QTL analysis in a sugar beet mapping population has identified a novel resistance source (*Rz4*), which like *Rz1*, *Rz2* and *Rz3* is located on chromosome III (Grimmer et al., 2007). Given the continuous phenotypic variation (Lewellen et al., 1987; Whitney, 1989) and the distorted segregation patterns observed, it was not possible to clarify whether *Rz2* and *Rz4* were allelic or closely linked with either *Rz1* or *Rz3* (Scholten et al., 1996, 1997; Grimmer et al., 2007). Similarly, the *Rz2* and *Rz3* genes could also be different alleles at the same locus (Grimmer et al., 2007). Recently, a novel resistance gene originating from WB 258 accession, designated as *Rz5*, was mapped at the same location as *Rz1* and *Rz4*, indicating that these genes might represent different alleles (Grimmer et al., 2008). In addition, the work of Lein et al. (2007), who mapped four resistance gene analogues (RGAs) as molecular markers, further provided evidence that the five major genes for rhizomania resistance reside at two distinct, proximal loci, with the first locus being represented by the allelic series *Rz1*, *Rz4* and *Rz5* and the second by that of *Rz2* and *Rz3*.

Apart from the above well studied and mapped sources of resistance, individuals with varying degrees of resistance to the virus have also been identified in many other accessions of *B. vulgaris* ssp. *maritima* such as R04, WB 151, WB 169, WB 258 (Lewellen, 1995)

as well as in *Beta corolliflora*, *Beta intermedia*, *Beta macrorhiza* and *Beta lomatogona* (Paul et al., 1993a; Luterbacher et al., 2005).

In addition to searching for new genes against BNYVV, improving disease resistance has also been pursued by means of exploiting probable resistance to the transmitting vector, *P. betae*, itself. To this end, resistance to the vector has in the past been found in the Procumbentes (Paul et al., 1992; Barr et al., 1995) and Corollinae (Paul et al., 1993a) sections, in hybrids of *B. vulgaris* with wild *Beta* species as well as in different monosomic addition lines of *Beta procumbens* in *B. vulgaris* (Paul et al., 1992). However, in contrast to the possibilities for gene transfer offered by the sexual compatibility with the sea beet (*B. vulgaris* ssp. *maritima*), the use of genes from species in these sections is severely hampered due to their poor hybridization with the cultivated beet. Recently, a two-gene system (*Pb1*|*Pb2*) conferring resistance against *P. betae* has been identified and mapped (Asher et al., 2009). The resistance to the vector is simply inherited and acts additively to the *Rz1* resistance against BNYVV, while it also confers comparable to the *Rz1* protection in individuals lacking this gene. The combined use of genes controlling resistance against the pathogen and the vector could lead to a more durable resistance to be exploited by the breeding programs (Asher et al., 2009).

4. Transgenic strategies towards rhizomania resistance

In addition to conventional breeding methodologies, including marker-assisted backcross breeding, that led to all rhizomania resistant sugar beet varieties currently in commercial use, various genetic engineering approaches have also been studied for the purpose of further improving disease resistance. These approaches include the pathogen-derived resistance (PDR), relying on the transgenic expression of a key gene from the pathogen, the resistance based on non-viral genes and the RNA silencing-mediated resistance, the most successful variant of PDR.

Genetic transformation of sugar beet, a crop species whose recalcitrance is generally recognized, is characterized by a very low efficiency owing to the poor competence of its cells to both transformation and regeneration procedures (Wozniak, 1999; Skaracis, 2005). Several efforts for the development of efficient transformation protocols have focused on the optimization of various relevant factors, such as explant type, gene transfer technique, selection system, tissue culture conditions and type/concentration of hormones used. Despite the progress achieved, reproducibility of transformation protocols among different laboratories tends to be poor and transformation frequencies are still much lower than those of other crop species (Joersbo, 2007). To circumvent the problems arising from sugar beet's recalcitrance in general and specifically in evaluating resistance to rhizomania through a stable transformation, a protocol for *Agrobacterium rhizogenes*-mediated production of transgenic hairy roots has been developed (Pavli and Skaracis, 2010). This protocol provides an attractive platform for the study of transgene expression in genetically engineered roots, prior to the tedious and low in efficiency processes of transformation and plant regeneration. Therefore, the approach may assist at improving resistance to root pathogens, whereas it could also be employed as a molecular breeding tool aiming at other important traits i.e. nitrogen and water use efficiency.

4.1. Pathogen-derived resistance

The pathogen-derived resistance (PDR) concept, as proposed by Sanford and Johnston (1985), gave rise to a series of research initiatives for the development of transgenic virus resistance over the years. Following the initial demonstration that the expression of a viral coat protein (CP) confers a varying level of resistance to the pathogen (Powell-Abel et al., 1986; Beachy et al., 1990), it was later evidenced that the PDR-approach could be efficiently extended to a wide range of plant viruses, including the rhizomania-causing BNYVV.

In the framework of obtaining resistance against BNYVV, Kallerhoff et al. (1990) showed that protoplasts of *CP*-transformed sugar beet suspension cells, though amenable to infection, presented lower virus multiplication rates in comparison to protoplasts of non-transformed cells. Further, Ehlers et al. (1991) described a protocol for the generation of CP-expressing hairy roots obtained through *A. rhizogenes*-mediated transformation. CP-based resistance at the plant level however, obtained via *Agrobacterium tumefaciens*-mediated transformation, was first reported by Mannerlöf et al. (1996) using two constructs carrying the coding region of the *CP*. Progenies obtained after two cycles of selfing, were challenged with BNYVV and evaluated for resistance. Although accumulation of the viral protein could not be detected, expression of the *CP* gene was found correlated with reduced virus titers both in greenhouse and field trial experiments. Presently, such a discrepancy between translatable levels and reduced virus accumulation has been explained on the basis of additional mechanisms such as RNA-mediated interferences (RNAi) (for a review see Prins et al., 2008).

Another PDR approach involves the employment of either functional or truncated versions of virus movement proteins (MP) as a means to interfere with virus cell-to-cell movement. In this line, the "triple gene block" (TPG) movement proteins, found among several genera, have served as potential targets for interfering with virus movement (Beck et al., 1994). Towards this direction, the finding that over-production of BNYVV-p15 relative to p13 results in inhibition of TGB-based cell-to-cell movement (Bleykasten-Grosshans et al., 1997), provides evidence that the transgenic expression of p15 in sufficient amounts may be employed for generating resistance to BNYVV.

4.2. RNA silencing-mediated resistance

RNA silencing has become the focus of interest in the broad field of molecular biology since the early 1990s with the then unexplained observation that transgene introgression into the plant genome triggered a co-suppression phenomenon, evidenced by the silencing of both the transgene and homologous endogenous counterparts (Napoli et al., 1990; van der Krol et al., 1990). Its recently unravelled mechanism of innate sequence-specific RNA degradation, also referred to as post-transcriptional gene silencing (PTGS), RNA silencing or RNAi, is nowadays considered as a highly promising biotechnological approach for building up plant virus resistance (Baulcombe, 1999; Ding and Voinnet, 2007).

With the perspective of engineering resistance against rhizomania disease, Andika et al. (2005) produced transgenic plants of *Nicotiana benthamiana* expressing the CP (21 kDa) or the CP-RTD ORF (54 kDa) of BNYVV. Upon challenge inoculation, only the RTD-transformed plants showed various levels of resistance to BNYVV: (a) highly resistant plants, (b) plants with delayed symptom appearance and eventual recovery, and (c) susceptible plants. Analyses of transgene mRNA and transgene-derived siRNA accumulated prior and post infection, revealed that enhanced resistance was based on transgene-induced RNA silencing, whereas the recovery phenotype was triggered by virus-induced silencing. In addition, based on results of mRNA degradation and siRNA accumulation in leaves and roots of silenced plants, it was suggested that RNA silencing-mediated resistance is less effective in roots than in leaves. It is worth noting however, that the transgenes employed in this study were transcribed as ssRNA, which is generally regarded as a weak silencing inducer, therefore leading to a reduced activity of transgene-induced RNA silencing.

In sugar beet, RNA silencing-based resistance against BNYVV was explored by Lennefors et al. (2006) who transgenically expressed a replicase-derived dsRNA molecule and, following inoculation using the transmitting vector, assessed resistance on the basis of virus titers. Transgenic plants presented equal or higher levels of resistance, both under greenhouse and field conditions, as compared to conventionally bred resistant plants. Further, Pavli et al. (2010a) developed three different hairpin constructs, carrying parts of a highly conserved region from the BNYVV replicase gene and evaluated their transgenic expression in sugar beet hairy roots, by means of an *A. rhizogenes* shortcut approach. Upon BNYVV inoculation, the composite seedlings showed a significant delay in symptom development as compared to the wild type ones. At the same time, the transgenic root system of these seedlings was virus-free or presented marginally positive values while the non-transformed aerial parts of the same plants proved infected. These findings support the conclusion that the expression of BNYVV replicase-derived dsRNA leads to resistant hairy roots, presumably as a result of an RNA silencing mechanism.

4.3. Resistance based on non-viral gene products

As an alternative to the widely explored PDR approach, other strategies involving the transgenic expression of non-viral sequences such as antibodies against a conserved domain in a key viral protein/enzyme have been further elaborated to achieve virus resistance. In sugar beet, a plantibody approach was employed by Fecker et al. (1997) who explored the potential of *in vitro* expressing single chain antibody fragments (scFv), specific to the viral coat protein or to the RNA 3-encoded p25 protein, in conferring protection against BNYVV. To this purpose, scFv-carrying constructs were used to transform *N. benthamiana* plants which were subsequently challenged by means of mechanical inoculation and through the use of the transmitting agent *P. betae*. Although confined in the endoplasmic reticulum, the CP-specific scFvs resulted in the inhibition of early infection and the development of milder symptoms at later stages of infection. This transgenic approach however, has not been further elaborated in recent years for BNYVV.

Another non-viral gene approach for achieving resistance against BNYVV, pertained to the expression of the harpin Z_{Psph} protein from *Pseudomonas syringae* pv. *phaseolicola*, in a canonical and a plant-secreted form (SP/HrpZ$_{Psph}$), in transgenic *N. benthamiana* plants (Pavli et al., 2010b, 2011). Although the protein could be readily detected at similar levels in plants expressing either HrpZ$_{Psph}$ or SP/HrpZ$_{Psph}$, transgenic plants showed significant differences in terms of the number of plants that became infected, the timing of infection and the disease symptoms displayed. Plants expressing HrpZ$_{Psph}$, presented similar phenotypic features with the non-transgenic plants regarding resistance to BNYVV. In contrast, plants expressing SP/HrpZ$_{Psph}$ were highly resistant to BNYVV, as evidenced by a complete absence of symptoms or a significantly delayed symptom development as well as a considerable reduction of virus multiplication, resulting in plants that were either virus-free or contained very low virus titer. These findings support the conclusion that the resistant phenotype is correlated with the extracellular secretion of harpin. In addition, the SP/HrpZ$_{Psph}$ protein has been expressed in sugar beet roots, through *A. rhizogenes*-mediated root transformation, as a means to evaluate the SP/HrpZ-based resistance in the sugar beet host (Pavli et al., 2011). Transgenic hairy roots showed high-level resistance to BNYVV, manifested by absence of disease symptoms as well as no or very low content of virus titer, whereas the non-transgenic parts of the same plants had both symptom development and virus content comparable to those of the control seedlings. Although the molecular mechanisms underlying the expression of the observed resistance were not in this study adequately elucidated, these data strengthen the proposal that harpins could add a novel tool towards generating a broad-spectrum resistance in plants (Shao et al., 2008). It is of interest that, apart from conferring enhanced rhizomania resistance, the expression of SP/HrpZ$_{Psph}$ in transgenic plants also resulted in a significantly increased plant growth rate and a higher final biomass. The latter, could prove important if sugar beet were to be considered as raw material for bioethanol and related products.

5. Trends and future prospects

Although breeding efforts have so far been met with considerable success, resistant cultivars may still suffer losses compared to their potential sugar yield under disease-free conditions. At the same time, the observed changes in field and molecular BNYVV epidemiology, as manifested by the recent emergence of RB mutants for the *Rz*-mediated resistances, most probably reflect an ensuing new endemic disease development that would require major adjustments in mainstream breeding programs if they were to keep providing a durable crop protection through the use of appropriate cultivars. Although further molecular and biological studies are needed – mainly on the basis of disease inducing capacity – the emergence of deviating strains with RB properties poses an obvious necessity to search for additional and more effective genetic sources of rhizomania resistance. Such a strategy, apart from effectively ensuring the economic viability of the crop, could also lead to a loss of pathogen fitness so as to eventually prevent the endemic spread of highly pathogenic BNYVV variants. Additional elements for an increased level of resistance to BNYVV may be sought for in the previously identified and/or developed germplasm originating from the cultivated beet forms as well as the wild beet relatives and especially the sea beet (*B. vulgaris* ssp. *maritima*) accessions where several genes have proved efficient in conferring resistance to the disease (Lewellen et al., 1987; Scholten et al., 1996, 1999; Liu and Lewellen, 2007). The further development of suitable molecular markers for the various sources/genes identified, as has been already achieved for *Rz1* (Barzen et al., 1997; Lein et al., 2007), *Rz2* (Scholten et al., 1999), *Rz3* (Gidner et al., 2005) and *Rz4* (Grimmer et al., 2007), strengthens the possibilities of gene pyramiding that could eventually provide a more durable resistance, specifically if it is combined with the finding of probable efficient sources against the vector, *P. betae* (Asher et al., 2009). Also, the use of such markers will significantly facilitate the isolation of homozygous *Rz*-based parents that will allow for a complete desirable heterozygosity in commercial hybrids. Such practice will increase the frequency of plants endowed with the resistance gene, which at present is only around 80% in the cultivars used (Biancardi et al., 2010; De Biaggi et al., 2011).

Obtaining durable rhizomania resistance through pyramiding though, still faces limitations due to the relative few existing different resistance genes. Possibilities will increase as new genes, mainly from the sea beet or other cross-compatible wild relatives become available. To this end, TILLING and EcoTILLING approaches represent valuable tools in generating and/or identifying beneficial mutants in chemically mutagenized populations or natural accessions respectively (Till et al., 2007). Apart from the recognition of such genes however, the method can be further exploited for the identification of polymorphism associated with host specificity in terms of the virus and/or the transmitting vector. In this manner, induced mutations might presumably create variation that results in silencing of viral/vector receptors or other host components which are essential for the establishment of a compatible interaction leading to disease.

Recent advances in our understanding on host–virus molecular interactions, include the unravelled antiviral pathways of RNA silencing and the more versatile identification of novel resistance

sources due to common sequence features with previously identified *R* genes. In view of such advances and the possibilities offered by marker assisted breeding approaches as well as the exploitation of modern 'omic' technologies, the problems with plant virus diseases can be faced in the near future in a considerably better perspective (Maule et al., 2007). In particular, bioinformatics will increasingly allow breeders to capitalize on the vast genomic information – both at the structural and functional levels – as this becomes available for a good number of major crops, including sugar beet of which a large part of its genome has already been sequenced. Additionally, such availability would allow for the identification of DNA segments that exert a function analogous to vector elements and therefore their subsequent exploitation for generating intragenic vector systems (Conner et al., 2007; Schouten and Jacobsen, 2008). Such an approach could prove extremely valuable in incorporating resistance genes to a particular genotype(s) by a much more efficient backcrossing devoid of linkage drag problems, thus requiring considerably less time (Conner et al., 2007; Rommens et al., 2007). The possibility of acquiring resistance from the repertoire of the crops' gene pool however, is delimited by two factors: (i) the scarcity of natural genetic sources of resistance to plant viruses in general and BNYVV in particular and (ii) the known high plasticity of viral genomes (Roossinck, 1997) that negatively affects durability of resistance (Garcia-Arenal and McDonald, 2003). As a consequence, the interest is justifiably concentrated to the more "classical" transgenic approaches.

There is no doubt that, transgenic approaches are capable to substantially complement conventional breeding methodology in diminishing or even eliminating the problem of sugar beet rhizomania disease, a problem ever maintaining its significance given the repeatedly occurring virus variants that seem to breakdown resistance. The commercial use of such resistant sugar beet transgenic varieties as they become available however, not only will depend on an official approval of the specific events – single or stacked – involved, but also on the compliance with specific rules concerning the co-existence of genetically modified (GM), conventional and organic crops as well as the economic viability of the relevant measures to be implicated. Overall concerns have already shifted research and practice of GM technology towards relaxing possible risks as exemplified by the adoption of antibiotic marker-free selection systems. "Intragenesis", earlier described, as well as "cisgenesis" are further steps towards the same direction. While a dramatic reversal of the current attitude is not likely to occur in the near future, it is reasonable to expect that GM cultivars might eventually find their way in the EU. Considering such a probable development, and in view of no significant difficulties encountered in achieving an economically sound co-existence in sugar beet (JRC, 2006), the future perspective of deploying rhizomania resistant transgenic sugar beet varieties should be expected as a promising strategy in complementing and enriching the breeder' arsenal.

Among the various GM approaches available, the antiviral pathways of the naturally occurring mechanism of RNA silencing are anticipated to assume a most central role in engineering virus resistance as well as resistance to biotic and abiotic stresses in plants. Such an expectation is based on the facts that RNA silencing: (i) has the potential to accomplish a high level resistance or even immunity, (ii) offers the possibility to build multiple resistance to a range of, even distantly related, viruses, (iii) due to its mode of function, exclusively acting on a sequence specific manner, it eliminates the risks of food safety in terms of possible allergenicity as well as environmental safety; issues associated with constitutive expression of viral gene segments are relatively relaxed due to the decreased risk of recombination, synergism and transcapsidation (Fuchs and Gonsalves, 2007). In terms of achieving durable rhizomania resistance, an interesting approach could rely on the employment of RNA silencing to simultaneously target multiple genes that assume central roles in virus multiplication in the sugar beet host and disease establishment. By analogy to the previously demonstrated efficiency of building multiple virus resistance (Bucher et al., 2006), this "co-targeting" approach would be expected to, apart from providing a high level resistance or immunity against the virus, prevent or considerably delay a counteracting virus adaptation leading to fitness gain and possibility for eventual breaking of resistance. Although such a multigene-based resistance seems quite promising, its possible approval under current regulatory framework appears particularly difficult. Under field conditions further, with plants being exposed to a plethora of viral pathogens, effectiveness and durability of the RNA silencing-mediated resistance could be threatened upon infection by a heterologous virus that encodes silencing-suppressor proteins (Brumin et al., 2009). Such probable reversal of plant immunity to susceptibility due to suppression of the consistently activated resistance response highlights the need for the design of new strategies for engineering RNA silencing-based resistance capable of counteracting these suppression phenomena.

6. Conclusions

There is no doubt that breeding ingenuity has resulted in a very successful control of sugar beet rhizomania disease throughout the world, thus ensuring crop's viability and profitability. At the same time, evolutionary changes in the pathogen continuously pose new challenges and require the intensive exploitation of all novelties as they become available. In the short and maybe in the midterm, classical/molecular breeding techniques are expected to exert a most important role. Molecular markers will accelerate new gene introduction into elite material and allow for the pyramiding of such genes towards a desirable durable resistance. At a later stage, marker-assisted breeding will be complemented and/or replaced by sequence-based breeding that relies on whole genome profiling. In the mid to long term, when problems associated with the approval and acceptance of GM technology are relaxed, relevant varieties have the possibility to also significantly contribute to a better and stable crop protection against the disease.

Acknowledgements

The authors wish to thank Prof. N.J. Panoloulos and Dr. Ir. M. Prins for fruitful discussions and critical review of the manuscript.

References

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

Acosta-Leal, R., Bryan, B.K., Rush, C.M., 2010a. Host effect on the genetic diversification of *Beet necrotic yellow vein virus* single-plant populations. Phytopathology 100, 1204–1212.

Acosta-Leal, R., Bryan, B.K., Smith, J.T., Rush, C.M., 2010b. Breakdown of host resistance by independent evolutionary lineages of *Beet necrotic yellow vein virus* involves a parallel C/U mutation in its *p25* gene. Phytopathology 100, 127–133.

Acosta-Leal, R., Fawley, M.W., Rush, C.M., 2008. Changes in the intraisolate genetic structure of *Beet necrotic yellow vein virus* populations associated with plant resistance breakdown. Virology 376, 60–68.

Acosta-Leal, R., Rush, C.M., 2007. Mutations associated with resistance-breaking isolates of Beet necrotic yellow vein virus and their allelic discrimination using TaqMan technology. Phytopathology 97, 325–330.

Amiri, R., Moghaddam, M., Mesbah, M., Sadeghian, S.Y., Ghannadha, M.R., Izadpanah, K., 2003. The inheritance of resistance to *Beet necrotic yellow vein virus* (BNYVV) in *B. vulgaris* subsp. *maritima*, accession WB42: statistical comparisons with Holly-1-4. Euphytica 132, 363–373.

Andika, I.B., Kondo, H., Tamada, T., 2005. Evidence that RNA silencing-mediated resistance to *Beet necrotic yellow vein virus* is less effective in roots than in leaves. MPMI 18, 194–204.

Asher, M.J.C., Grimmer, M.K., Mutasa-Goettgens, E.S., 2009. Selection and characterization of resistance to *Polymyxa betae*, vector of *Beet necrotic yellow vein virus*, derived from wild sea beet. Plant Pathol. 58, 250–260.

Barr, K.J., Asher, M.J.C., Lewis, B.G., 1995. Resistance to *Polymyxa betae* in wild *Beta* species. Plant Pathol. 44, 301–307.

Barzen, E., Rainer, S., Fuchs, E., Borchardt, D.C., Salamini, F., 1997. Development of coupling-repulsion-phase SCAR markers diagnostic for the sugar beet Rr1 allele conferring resistance to rhizomania. Mol. Breed. 3, 231–238.
Baulcombe, D., 1999. Viruses and gene silencing in plants. Arch. Virol. Suppl. 15, 189–201.
Beachy, R.N., Loesch-Fries, S., Tumer, T.E., 1990. Coat protein-mediated resistance against virus infection. Ann. Rev. Phytopathol. 28, 451–474.
Beck, D.L., Van Dolleerd, C.J., Lough, T.J., Balmori, E., Voot, D.M., Andersen, M.T., O'Brien, I.E., Forster, R.L.S., 1994. Disruption of virus movement confers broad spectrum resistance against systemic infection by plant viruses with a triple gene block. Proc. Natl. Acad. Sci. U. S. A. 91, 10310–10314.
Biancardi, E., Lewellen, R.T., DeBiaggi, M., Erichsen, A.W., Stevanato, P., 2002. The origin of rhizomania resistance in sugar beet. Euphytica 127, 383–397.
Biancardi, E., McGrath, J.M., Panella, L.W., Lewellen, R.T., Stevanato, P., 2010. Sugar beet. In: Bradshow, J. (Ed.), Handbook of Plant Breeding (vol. 4), Tuber and Root Crops. Springer, New York, pp. 173–219.
Bleykasten-Grosshans, C., Guilley, H., Bouzoubaa, S., Richards, K.E., Jonard, G., 1997. Independent expression of the first two triple gene block proteins of *beet necrotic yellow vein virus* complements virus defective in the corresponding gene but expression of the third protein inhibits viral cell-to-cell movement. MPMI 10, 240–246.
Bongiovanni, G.C., Lanzoni, L., 1964. La rizomania della bietola. Prog. Agric. 2, 209–220.
Brumin, M., Stukalov, S., Haviv, S., Muruganantham, M., Moskovitz, Y., Batuman, O., Fenigstein, A., Mawassi, M., 2009. Post-transcriptional gene silencing and virus resistance in *Nicotiana benthamiana* expressing a *Grapevine virus A* minireplicon. Transgenic Res. 18, 331–345.
Bucher, E.C., Lohuis, D., van Poppel, P.M.J.A., Geerts-Dimitriadou, C., Goldbach, R., Prins, M., 2006. Multiple virus resistance at a high frequency using a single transgene construct. J. Gen. Virol. 87, 3697–3701.
Canova, A., 1959. Appunti di patologie della barbabietola. Inform. Fitopatol. 9, 390–396.
Chiba, S., Kondo, H., Miyanishi, M., Andika, I.B., Han, C., Tamada, T., 2011. The evolutionary history of *Beet necrotic yellow vein virus* deduced from genetic variation, geographical origin and spread, and the breaking of host resistance. MPMI 24 (2), 207–218.
Chiba, S., Miyanishi, M., Andica, I.B., Kondo, H., Tamada, T., 2008. Identification of amino acids of the beet necrotic yellow vein virus p25 protein required for induction of the resistance response in leaves of *Beta vulgaris* plants. J. Gen. Virol. 89, 1314–1323.
Conner, A.J., Barrell, P.J., Baldwin, S.J., Lokerse, A.S., Cooper, P.A., Erasmuson, A.K., Nap, J.-P., Jacobs, J.M.E., 2007. Intragenic vectors for gene transfer without foreign DNA. Euphytica 154, 341–353.
De Biaggi, M., 1987. Méthodes de sélection, un cas concret. In: Proceedings of the 50th Congress of the I.I.R.B ,. International Institute for Beet Research, Brussels, Belgium, pp. 157–163.
De Biaggi, M., Stevanato, P., Trebbi, D., Saccomani, M., Biancardi, E., 2011. Sugar beet resistance to rhizomania: state of the art and perspectives. Sugar Tech 12 (3–4), 238–242.
Ding, S., Voinnet, O., 2007. Antiviral immunity directed by small RNAs. Cell 130, 413–426.
Ehlers, U., Commandeur, U., Frank, R., Landsmann, J., Koenig, R., Burgermeister, W., 1991. Cloning of the coat protein gene from *beet necrotic yellow vein virus* and its expression in sugar beet hairy roots. Theor. Appl. Genet. 81, 777–782.
Fecker, L.F., Koenig, R., Obermeier, C., 1997. *Nicotiana benthamiana* plants expressing *beet necrotic yellow vein virus* (BNYVV) coat protein-specific scFv are partially protected against the establishment of the virus in the early stages of infection and its pathogenic effects in the late stages of infection. Arch. Virol. 142, 1857–1863.
Fuchs, M., Gonsalves, D., 2007. Safety of virus-resistant transgenic plants two decades after their introduction: lessons from realistic field risk assessment studies. Annu. Rev. Phytopathol. 45, 173–202.
Fujisawa, I., Sugimoto, T., 1976. Transmission of *Beet necrotic yellow vein virus* by *Polymyxa betae*. Ann. Phytopathol. Soc. Jpn. 43, 583–586.
Garcia-Arenal, F., McDonald, B.A., 2003. An analysis of the durability of resistance to plant viruses. Phytopathology 93 (8), 941–952.
Gidner, S., Lennefors, B.-L., Nilsson, N.-O., Bensefelt, J., Johansson, E., Gyllenspetz, U., Kraft, T., 2005. QTL mapping of BNYVV resistance from the WB41 source in sugar beet. Genome 48, 279–285.
Grimmer, M.K., Kraft, T., Francis, S.A., Asher, M.J.C., 2008. QTL mapping of BNYVV resistance from the WB258 source in sugar beet. Plant Breed. 127, 650–652.
Grimmer, M.K., Trybush, S., Hanley, S., Francis, S.A., Karp, A., Asher, M.J.C., 2007. An anchored linkage map for sugar beet based on AFLP, SNP and RAPD markers and QTL mapping of a new source of resistance to Beet necrotic yellow vein virus. Theor. Appl. Genet. 114, 1151–1160.
Harju, V.A., Mumford, R.A., Bockley, A., Boonham, N., Clover, G.R.G., Weekes, R., Henry, C.M., 2002. Occurrence in the United Kingdom of *beet necrotic yellow vein virus* isolates which contain RNA 5. Plant Pathol. 51, 811.
Heijbroek, W., Musters, P.M.S., Schoone, A.H.L., 1999. Variation in pathogenicity and multiplication of *Beet necrotic yellow vein virus* in relation to the resistance of sugar beet cultivars. Eur. J. Plant Pathol. 105, 397–405.
Joersbo, M., 2007. Sugar beet. In: Pua, E.C., Davey, M.R. (Eds.), Biotechnology in Agriculture and Forestry, Transgenic Crops IV. Springer-Verlag, Berlin Heidelberg, pp. 355–379.
J.R.C. Technical Report EUR 22102 EN, 2006. New case studies on the coexistence of GM and non-GM crops in European agriculture. JRC Technical Report Series.
Kallerhoff, J., Pascual, P., Bouzoubaa, S., Tahar, S.B., Perret, J., 1990. *Beet necrotic yellow vein virus* coat protein-mediated protection in sugar beet (*Beta vulgaris* L.) protoplasts. Plant Cell Rep. 9, 224–228.
Kiguchi, T., Saito, M., Tamada, T., 1996. Nucleotide sequence analysis of RNA-5 of five isolates of *beet necrotic yellow vein virus* and the identity of a deletion mutant. J. Gen. Virol. 77, 575–580.
Koenig, R., Haeberle, A.M., Commandeur, U., 1997. Detection and characterization of a distinct type of *beet necrotic yellow vein virus* RNA 5 in a sugar beet growing area in Europe. Arch. Virol. 142, 1499–1504.
Koenig, R., Kastirr, U., Holtschulte, B., Deml, G., Varrelmann, M., 2008. Distribution of various types and p25 subtypes of *Beet necrotic yellow vein virus* in Germany and other European countries. Arch. Virol. 153, 2139–2144.
Koenig, R., Lennefors, B.-L., 2000. Molecular analysis of European A, B and P type sources of beet necrotic yellow vein virus and detection of the rare P type in Kazakhstan. Arch. Virol. 145, 1561–1570.
Koenig, R., Loss, S., Specht, J., Varrelmann, M., Luddecke, P., Deml, G., 2009. A single U/C nucleotide substitution changing alanine to valine in the beet necrotic yellow vein virus p25 protein promotes increased virus accumulation in roots of mechanically inoculated, partially resistant sugar beet seedlings. J. Gen. Virol. 90, 759–763.
Koenig, R., Luddecke, P., Haeberle, A.M., 1995. Detection of *beet necrotic yellow vein virus* strains, variants and mixed infections by examining single-strand conformation polymorphisms of immunocapture RT-PCR products. J. Gen. Virol. 76, 2051–2055.
Kruse, M., Koenig, R., Hoffmann, A., Kaufmann, A., Commandeur, U., Solovyev, A.G., Savenkov, I., Burgermeister, W., 1994. Restriction fragment length polymorphism analysis of reverse transcription-PCR products reveals the existence of two major strain groups of *beet necrotic yellow vein virus*. J. Gen. Virol. 75, 1835–1842.
Lein, J.C., Asbach, K., Tian, Y.Y., Schulte, D., Li, C.Y., Koch, G., Jung, C., Cai, D.G., 2007. Resistance gene analogues are clustered on chromosome 3 of sugar beet and cosegregate with QTL for rhizomania resistance. Genome 50, 61–71.
Lennefors, B.-L., Lindsten, K., Koenig, R., 2000. First record of A and B type beet necrotic yellow vein virus in sugar beets in Sweden. Eur. J. Plant Pathol. 106, 199–201.
Lennefors, B.-L., Savenkov, E.I., Bensefelt, J., Wremerth-Weich, E., van Roggen, P., Tuvesson, S., Valkonen, J.P.T., Gielen, J., 2006. dsRNA-mediated resistance to *Beet Necrotic Yellow Vein Virus* infections in sugar beet (*Beta vulgaris* L. ssp. *vulgaris*). Mol. Breed. 18, 313–325.
Lennefors, B.-L., Savenkov, E.I., Mukasa, S.B., Valkonen, J.P.T., 2005. Sequence divergence of four soil-borne sugarbeet-infecting viruses. Virus Genes 31, 57–64.
Lewellen, R.T., 1995. Performance of near-isolines of sugar beet with resistance to rhizomania from different sources. In: Proceedings of the 58th IIRB Congress ,. International Institute for Beet Research, Brussels, Belgium, pp. 83–92.
Lewellen, R.T., 1988. Selection for resistance to rhizomania in sugar beet. In: Proceedings of the 5th International Congress Plant Pathology , Kyoto, Japan, p. 455.
Lewellen, R.T., Biancardi, E., 1990. Breeding and performance of rhizomania resistant sugar beet. In: Proceedings of the 53rd Congress of the IIRB ,. International Institute for Beet Research, Brussels, Belgium, pp. 79–87.
Lewellen, R.T., Skoyen, I.O., Erichsen, A.W., 1987. Breeding sugarbeet for resistance to rhizomania: evaluation of host-plant reactions and selection for and inheritance of resistance. In: Proceedings of the 50th Congress of the IIRB ,. International Institute for Beet Research, Brussels, Belgium, pp. 139–156.
Link, D., Schmidlin, L., Schirmer, A., Klein, E., Erhardt, M., Geldreich, A., Lemaire, O., Gilmer, D., 2005. Functional characterization of the *Beet necrotic yellow vein virus* RNA-5-encoded p26 protein: evidence for structural pathogenicity determinants. J. Gen. Virol. 86, 2115–2125.
Liu, H.Y., Lewellen, R.T., 2007. Distribution and molecular characterisation of resistance breaking isolates of *Beet necrotic yellow vein virus* in the United States. Plant Dis. 91, 847–851.
Luterbacher, M.C., Asher, M.J.C., Beyer, W., Mandolino, G., Scholten, O.E., Frese, L., Biancardi, E., Stevanato, P., Mechelke, W., Slyvchenko, O., 2005. Sources of resistance to diseases of sugar beet in related *Beta* germplasm. II. Soil-borne diseases. Euphytica 141, 49–63.
Mannerlöf, M., Lennefors, B.-L., Tenning, P., 1996. Reduced titer of BNYVV in transgenic sugar beets expressing the BNYVV coat protein. Euphytica 90, 293–296.
Maule, A.J., Caranta, C., Boulton, M.I., 2007. Sources of natural resistance to plant viruses: status and prospects. Mol. Plant Pathol. 8, 223–231.
McGrann, G.R.D., Grimmer, M.K., Mutasa-Gottgens, E.S., Stevens, M., 2009. Progress towards the understanding and control of sugar beet rhizomania disease. Mol. Plant Pathol. 10, 129–141.
Mehrvar, M., Valizadeh, J., Koenig, R., Bragard, C.G., 2009. Iranian beet necrotic yellow vein virus (BNYVV): pronounced diversity of the p25 coding region in A-type BNYVV and identification of P-type BNYVV lacking a fifth RNA species. Arch. Virol. 154, 501–506.
Meunier, A., Schmit, J.F., Bragard, C., 2003. Sequence analysis of Belgian BNYVV and development of a simultaneous detection of soil-borne sugar beet viruses by RT-PCR. In: Rush, C.M., Merz, U. (Eds.), Proceedings of the 5th Symposium of the International Working Group on Plant Viruses with Fungal Vectors. American Society of Sugar Beet Technologists, Zürich, Switzerland, pp. 9–12.
Miyanishi, M., Kusume, T., Saito, M., Tamada, T., 1999. Evidence for three groups of sequence variants of *beet necrotic yellow vein virus* RNA 5. Arch. Virol. 144, 879–892.

Napoli, C., Lemieux, C., Jorgensen, R., 1990. Introduction of a chimeric chalcone synthase gene into *Petunia* results in reversible co-suppression of homologous genes in *trans*. Plant Cell 2, 279–289.

Paul, H., Henken, B., De Bock, Th.S.M., Lange, W., 1992. Resistance to *Polymyxa betae* in *Beta* species of the section *Procumbentes*, in hybrids with *B. vulgaris* and in monosomic chromosome additions of *B. procumbens* in *B. vulgaris*. Plant Breed. 109, 265–273.

Paul, H., Henken, B., Scholten, O.E., De Bock, Th.S.M., Lange, W., 1993a. Variation in the level of infection with *Polymyxa betae* and its effect on infection with beet necrotic yellow vein virus in beet accessions of the sections Beta and Corollinae. In: Proceedings of the 2nd Symposium of the International Working Group on Plant Viruses with Fungal Vectors , Montreal, Canada, pp. 133–136.

Paul, H.B., Henken, B., Scholten, O.E., Lange, W., 1993b. Use of zoospores of *Polymyxa betae* in screening beet seedlings for resistance to beet necrotic yellow vein virus. Neth. J. Plant Pathol. 99 (3), 151–160.

Pavli, O.I., Kelaidi, G.I., Tampakaki, A.P., Skaracis, G.N., 2011. The hrpZ gene of *Pseudomonas syringae* pv. *phaseolicola* enhances resistance to rhizomania disease in transgenic *Nicotiana benthamiana* and sugar beet. PLoS One 6 (3), e17306, doi:10.1371/journal.pone.0017306.

Pavli, O.I., Panopoulos, N.J., Goldbach, R., Skaracis, G.N., 2010a. BNYVV-derived dsRNA confers resistance to rhizomania disease of sugar beet as evidenced by a novel transgenic hairy root approach. Transgenic Res. 19, 915–922.

Pavli, O.I., Tampakaki, A., Skaracis, G.N., 2010b. Expression of $HarpinZ_{Psph}$ in transgenic N. benthamiana results in BNYVV-induced tissue necrosis and enhanced rhizomania resistance. In: Proceedings of the 5th EPSO Conference , Olos, Finland, p. 65.

Pavli, O.I., Skaracis, G.N., 2010. Fast and efficient genetic transformation of sugar beet by *Agrobacterium rhizogenes*. Protoc. Netw. Nat. Protoc., doi:10.1038/nprot.2010.98.

Pavli, O.I., Prins, M., Goldbach, R., Skaracis, G.N. Efficiency of *Rz1*-based rhizomania resistance and molecular studies on BNYVV isolates from sugar beet cultivation in Greece. Eur. J. Plant Pathol., in press.

Pelsy, F., Merdinoglu, D., 1996. Identification and mapping of random amplified polymorphic DNA markers linked to a rhizomania resistance gene in sugar beet (*Beta vulgaris* L.) by bulked segregant analysis. Plant Breed. 115, 371–377.

Pferdmenges, F., Korf, H., Varrelmann, M., 2009. Identification of rhizomania-infected soil in Europe able to overcome *Rz1* resistance in sugar beet and comparison with other resistance-breaking soils from different geographic origins. Eur. J. Plant Pathol. 124, 31–43.

Pferdmenges, F., Varrelmann, M., 2009. Breaking of *Beet necrotic yellow vein virus* resistance in sugar beet is independent of virus and vector inoculum densities. Eur. J. Plant Pathol. 124, 231–245.

Powell-Abel, P., Nelson, R.S., De, B., Hoffmann, N., Rogen, S.G., Fraley, R.T., Beachy, R.N., 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232, 738–743.

Prins, M., Laimer, M., Noris, E., Schubert, J., Wassenegger, M., Tepfer, M., 2008. Strategies for antiviral resistance in transgenic plants. Mol. Plant Pathol. 9 (1), 73–83.

Putz, C., Merdinoglu, D., Lemaire, O., Stocky, G., Valentin, P., Wiedemann, S., 1990. *Beet necrotic yellow vein virus*, causal agent of sugar beet rhizomania. Disease profile. Rev. Plant Pathol. 69, 247–253.

Richard-Molard, M.S., 1985. Rhizomania: a world-wide danger to sugar beet. Span 28, 92–94.

Rommens, C.M., Haring, M.A., Swords, K., Davies, H.V., Belknap, W.R., 2007. The intragenic approach as a new extension to traditional plant breeding. Trends Plant Sci. 12 (9), 1360–1385.

Roossinck, M.J., 1997. Mechanisms of plant virus evolution. Annu. Rev. Phytopathol. 35, 191–209.

Rush, C.M., 2003. Ecology and epidemiology of Benyviruses and plasmodiophorid vectors. Annu. Rev. Phytopathol. 41, 567–592.

Sanford, J.C., Johnston, S.A., 1985. The concept of pathogen derived resistance. J. Theor. Biol. 113, 395–405.

Schirmer, A., Link, D., Cognat, V., Moury, B., Beuve, M., Meunier, A., Bragard, C., Gilmer, D., Lemaire, O., 2005. Phylogenetic analysis of isolates of *Beet necrotic yellow vein virus* collected worldwide. J. Gen. Virol. 86, 2897–2911.

Scholten, O.E., De Bock, T.S.M., Klein-Lankhorst, R.M., Lange, W., 1999. Inheritance of resistance to beet necrotic yellow vein virus in *Beta vulgaris*, conferred by a second gene for resistance. Theor. Appl. Genet. 99, 740–746.

Scholten, O.E., Jansen, R.C., Keizer, L.C.P., De Bock, T.S.M., Lange, W., 1996. Major genes for resistance to beet necrotic yellow vein virus (BNYVV) in *Beta vulgaris*. Euphytica 91, 331–339.

Scholten, O.E., Klein-Lankhorst, R.M., Esselink, D.G., de Boch, T.S.M., Lange, W., 1997. Identification and mapping of random amplified polymorphic DNA (RAPD) markers linked to resistance against *beet necrotic yellow vein virus* (BNYVV) in *Beta* accessions. Theor. Appl. Genet. 94, 123–130.

Scholten, O.E., Lange, W., 2000. Breeding for resistance to rhizomania in sugar beet: a review. Euphytica 112, 219–231.

Schouten, H.J., Jacobsen, E., 2008. Cisgenesis and intragenesis, sisters in innovative plant breeding. Trends Plant Sci. 13, 260–261.

Shao, M., Wang, J., Dean, R.A., Lin, Y., Gao, X., Hu, S., 2008. Expression of a harpin-encoding gene in rice confers durable nonspecific resistance to *Magnaporthe grisea*. Plant Biotechnol. J. 6, 73–81.

Skaracis, G.N., 2005. Molecular biology and biotechnology, genetic engineering. In: Biancardi, E., Campbell, L.G., Skaracis, G.N., De Biaggi, M. (Eds.), Genetics and Breeding of Sugar Beet. Science Publishers, Inc., Enfield, NH, USA, pp. 255–268.

Skaracis, G.N., Biancardi, E., 2000. Breeding for cercospora resistance in sugar beet. Cercospora beticola Sacc. Biology, Agronomic Influence and Control Measures in Sugar Beet. Advances in Sugar Beet Research, vol. 2. IIRB, Bruxelles, Belgium, pp. 177–195.

Sohi, H., Maleki, M., 2004. Evidence for presence of types A and B of beet necrotic yellow vein virus (BNYVV) in Iran. Virus Genes 29, 353–358.

Steddom, K., Heidel, G., Jones, D., Rush, C.M., 2003. Remote detection of rhizomania in sugar beets. Phytopathology 93, 720–726.

Suarez, M.B., Grondona, I., Garcia-Benavides, P., Monte, E., Garcia-Acha, I., 1999. Characterization of *beet necrotic yellow vein virus* furovirus from Spanish sugar beets. J. Int. Microbiol. 2, 87–92.

Tamada, T., 2002. Beet necrotic yellow vein virus. CMI/AAB Description of Plant Viruses, vol. 391. Association of Applied Biologists, Wellesbourne, UK.

Tamada, T., 1999. Benyviruses. In: Webster, R.G., Granoff, A. (Eds.), Encyclopedia of Virology. , 2nd edn. Academic Press, London, UK, pp. 154–160.

Tamada, T., 1975. Beet necrotic yellow vein virus. In: CMI/AAB Descriptions of Plant Viruses. Association of Applied Biologists, Wellesbourne, UK, p. 144.

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

Tamada, T., Kusume, T., Uchino, H., Kiguchi, T., Saito, M., 1996. Evidence that *beet necrotic yellow vein virus* RNA-5 is involved in symptom development of sugar beet roots. In: Sherwood, J.L., Rush, C.M. (Eds.), Proceedings of the 3rd Symposium of the International Working Group on Plant Viruses with Fungal Vectors. American Society of Sugar Beet Technologists, Dundee, UK, pp. 49–52.

Tamada, T., Shirako, Y., Abe, H., Saito, M., Kiguchi, T., Harada, T., 1989. Production and pathogenicity of isolates of *beet necrotic yellow vein virus* with different numbers of RNA components. J. Gen. Virol. 70, 3399–3409.

Till, B.J., Comai, L., Henikoff, S., 2007. Tilling and Ecotilling for crop improvement. In: Varshney, R.K., Tuberosa, R. (Eds.), Genomics-Assisted Crop Improvement, Vol. 1: Genomics Approaches and Platforms. Springer, pp. 333–349.

Torrance, L., Mayo, M.A., 1997. Proposed re-classification of *Furoviruses*. Arch. Virol. 142, 435–439.

van der Krol, A.R., Mur, L.A., Beld, M., Mol, J.N., Stuitje, A.R., 1990. Flavonoid genes in petunia: addition of a limited number of gene copies may lead to a suppression of gene expression. Plant Cell 2, 291–299.

Ward, L., Koenig, R., Budge, G., Garrido, C., McGrath, C., Stubbley, H., Boonham, N., 2007. Occurrence of two different types of RNA-5-containing *Beet necrotic yellow vein virus* in the UK. Arch. Virol. 152 (1), 59–73.

Whitney, E.D., 1989. Identification, distribution, and testing for resistance to rhizomania in *Beta maritima*. Plant Dis. 73, 287–290.

Wozniak, C.A., 1999. Transgenic sugar beet: progress and development. Appl. Plant Biotechnol. 10, 301–324.