Chapter 9
Genetic Resistances

Leonard W. Panella and Enrico Biancardi

Abstract Soon after the first appearance of the disease, the presence of some traits of resistance to rhizomania was recognized in Italian varieties. In the mid-1980s, the breeding research led to the release of monogenic resistance, which reduced drastically the damage caused by beet necrotic yellow vein virus (BNYVV). At least two origins of the currently employed traits (Rz1 and Rz2) were identified in *Beta maritima* collected in the Po River Delta, Italy, and at Kalundborg Fjord, Denmark. Both traits are located on chromosome III and spaced far enough apart to be considered different loci. The crosses display an additive action, useful for increasing sugar yield even in the presence of Rz1 resistance-breaking strains of BNYVV. Some differences were detected in the mechanisms limiting the effects of the BNYVV, because the beets carrying the resistance Rz2 show reduced virus replication and more restricted cell-to-cell movement than Rz1. But the subject still is controversial. In the future, resistance to the vector, *P. betae*, could complement the effects of BNYVV resistances, if difficulties in the transfer of the trait would be overcome.

Keywords Sugar beet • Rhizomania • Genetic resistances • Rz1 • Rz2 • Genetic resources

Genetic resistance often has been recognized as the only viable mean for limiting soil-borne diseases. In sugar beet, it is only for beet necrotic yellow vein virus (BNYVV), i.e., rhizomania, that a set of fairly different and effective, single-gene resistances is currently available, while for other soil-borne diseases of sugar beet, multigenic traits with low heritability have been found. Varieties endowed with multiple resistances to different soil-borne diseases would be very useful, but so far nothing similar to the single-gene resistance to rhizomania exists (Harveson and Rush 2002).

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9.1 Sources of Resistance

The presence of varieties carrying some trait of resistance and a sufficient degree of genetic variability, originally were detected through differences within trials in fields naturally infected by the then unknown syndrome. The discovery of the disease outside Italy and the recognition of the unusually high yield losses captured the interest of research institutes, seed companies, growers' associations, and the beet sugar industry in Europe, Japan, and the USA. In relatively few years, thanks to international synergies and collaborations, the damage caused by the disease was almost completely eliminated, allowing the survival of the crop and the related industry. The probability of finding new sources of resistance, with the qualities demanded through the registration procedure, is quite low in commercial sugar beet germplasm. This primarily is due to the selection methods employed for breeding the currently used monogerm hybrid varieties and by their narrow genetic variability (Francis and Luterbacher 2003; Pavli et al. 2011; Litwiniec et al. 2015).

Seed companies prefer searching for new traits in germplasm adapted for sugar production, which has already been endowed with satisfactory levels of sugar yield, processing quality, and morphological traits. The search among other taxa of the genus *Beta* is more difficult and time consuming, but it should become more fruitful in the future. Hopefully, by means of molecular biology, the introgression of resistance genes into the cultivated germplasm will become easier than in the past (Francis and Luterbacher 2003). The transfer of monogenic and dominant genes of resistances into germplasm that already is regionally adapted is the easiest way (Meulemans et al. 2003).

9.2 Resistances to BNYVV

The first available type of rhizomania resistance with moderate effect on yield parameters was named "Alba type," because it was identified in varieties released by the Alba seed company, Padua, Italy (Biancardi et al. 2002) (Fig. 9.1). These genotypes surely were derived from cercospora leaf spot (CLS)-resistant lines, which, in turn, were obtained from crosses with *Beta maritima*, commonly named sea beet (Box 9.1). After 1970, Alba carried out mass selection on mother beets, cultivated in fields under 2-year rotations that gradually became severely infected by BNYVV, but which was not realized until later (Usai, personal communication). It is likely that for some years an efficient selection for rhizomania resistance was performed unconsciously in these fields. The association between Alba-type resistance and Munerati's germplasm is further confirmed because the seed company, which was founded in 1933, worked mainly with Rovigo materials, especially before the Second World War. Similar segregation patterns were found in some of Munerati's original families. Moreover, the multigerm variety Alba P was sold and utilized as a CLS-resistant variety. As is well known, the only currently available

Fig. 9.1 *Beta maritima* on the bank of Po di Levante River, "very near the Adriatic Coast." This photograph was found in 2015 on the original glass plate with only the year 1909 given. It most likely was taken in the June of 1909 by Munerati. The date and landscape correspond to the harvest of sea beet seed used for selection of resistance to cercospora leaf spot. Due to the similar lineage, it is likely that the depicted sea beet also has been the ancestor of the resistances to rhizomania employed worldwide in the last 30 years. (A print of this photo has been previously used by Biancardi et al. (2012) and was believed to date from 1951)

Box 9.1: A Fruitful Collaboration

In 1925, Coons was directed by the Agricultural Research Service (ARS) of the USDA to look for germplasm containing resistance to diseases (Coons 1936). He decided to look in the center of origin of the wild relatives of sugar beet, the North Atlantic Coast of Europe and within the European countries bordering the Mediterranean Sea. By the time Coons made his first trip to Europe in 1925, Munerati's work with resistance to cercospora leaf spot from *B. maritima* was well underway (Biancardi et al. 2012). But many of the commercial seed companies were reluctant to work with the germplasm because, as Coons commented on his first trip, "These plants, however, as seen by one of us in 1925, had not been freed from certain undesirable characteristics derived from the *B. maritima* parent-notably, the tendency to be multicrowned and to have sprangled roots, especially lateral roots emerging from the taproot at about a 90-degree angle" (Coons et al. 1955). However in 1935 when Coons returned again to Europe to collect germplasm, he commented, "Munerati had greatly improved his breeding stocks and furnished his American colleagues his family 'R 581' which, although not fully comparable with sugar beets in root or crown conformation, was externally resistant to Cercospora leaf spot and high in sucrose" (Coons et al. 1955).

(continued)

Box 9.1 (continued)

Seed of the Munerati's R 581 and other lines from his *Beta maritima* material such as "Mezzano" and "Cesena" were sent to USDA, university, and sugar beet industry researchers throughout the USA and incorporated into disease resistance germplasm. Despite some undesirable traits from the *Beta maritima* parents, as mentioned above, the USDA-ARS public plant breeders crossed it into many of their lines. It found its way into the programs at Salinas, California; Logan, Utah; Fort Collins, Colorado; East Lansing, Michigan; and Beltsville, Maryland (Panella and Lewellen 2005). Realizing that the collection of seed was dying in Beltsville, it was moved to Salinas, CA, where McFarlane worked to restore it, while incorporating disease resistance genes from *Beta maritima* (Panella and Lewellen 2007). It was also where a young scientist, Lewellen, began working with *Beta maritima* and, ultimately, the Rovigo Sugar Beet Research Station. Once Lewellen got his program established, he looked to the USDA-ARS gene bank, but also to Biancardi at Rovigo, for sources of resistance to important sugar beet disease. Until their retirement, Lewellen and Biancardi worked closely together on many projects. They, along with De Biaggi and Erichsen, were responsible for breeding the first varieties with resistance to rhizomania, with Rizor in Europe and the "Holly gene" in the USA. There were visits between the USA and Italy, as well as meetings at international congresses, but most importantly exchanges of breeding materials. Although they may be best known for their rhizomania research, they have worked together on cercospora leaf spot resistance, sugar beet cyst nematode resistance, and resistance to other diseases (Biancardi et al. 2012).

This collaboration continued when a new sugar beet researcher, Panella, joined the USDA-ARS sugar beet breeding program at Fort Collins, Colorado, in 1992. This was when rhizomania was being found in Colorado, and it was natural for him to begin to work with Lewellen and Biancardi, looking to *Beta maritima* for disease resistance genes to rhizomania and other diseases (Biancardi et al. 2012). Traveling to Italy to work with Biancardi on the *Beta maritima* book, Panella became acquainted with another young Italian sugar beet researcher, who had continued the sugar beet research from Rovigo at the University in Padua (Stevanato and Panella 2013). Together with Stevanato and Panella, ARS researchers, McGrath and Hanson (in East Lansing, Michigan), and Richardson (in Salinas, California), the longtime collaboration between USDA-ARS and the Italian sugar beet program born in Rovigo continues.

source of CLS resistance was the Rovigo germplasm (Skaracis and Biancardi 2000). Until 1980, Alba P was sold as multigerm variety and therefore potentially free to be used as a diploid pollinator by other seed companies. The first published use of mass selection on diseased soil began in 1966 at San Bonifacio and Albaredo d'Adige, Verona, Italy, and led to the identification of some diploid lines with the

required traits (Gentili and Poggi 1986). Through colchicine treatment, the Maribo seed company, Holeby, Denmark, obtained tetraploid pollinators and the triploid variety, "Ritmo," showing the features of quantitative resistance, both to rhizomania and cercospora leaf spot (Biancardi et al. 2002).

After the first observations on the spreading of the still unidentified syndrome (Donà dalle Rose 1954), the Sugar Beet Research Station at Rovigo went back to work on rhizomania resistance in 1976. Planting in April, in opportune soil temperature and moisture conditions, part of the institute's germplasm was evaluated in a naturally infected field at San Pietro in Casale, Bologna. In order to obtain uniform infection, a viruliferous *P. betae* inoculum was manually distributed after sowing and before a 26 mm rain (Ciafardini 1991). Individual selection was carried out in February on beets that had survived the winter, because their higher sugar content worked as a sort of antifreeze protection inside the roots. In some cases, it was possible to improve the selection based on the disease index (DI) (Table 11.1) or by means of Brix (°Bx) or refractometer degree of the root sap. The DI in Table 11.1 does not include symptoms on the leaves because they are not always correlated to the disease, because, among other things, they can be confused with nutrient deficiencies (Pavli 2010). The collection of brei samples necessary for more precise analyses was impossible due to the small size of the surviving roots. In these instances the use of the °Bx measurement was helpful in making selections. The storage and reproduction of the selected mother roots were critical, mainly because of the danger of the development of rot. Particular care and reduced water supply were adopted for the storage and for field transplanting of the roots for seed production.

Only one diploid, multigerm family, coded Ro 236, showed a relatively high percentage of survival. Root and leaf morphology were similar to the check variety, Alba P; the same was observed for the degree of CLS resistance (Biancardi et al. 2002). The rest of the entries, especially monogerm, O-Type, CMS lines, and tetraploid families, were almost completely destroyed. That the resistance was the multigenic form in the varieties Alba P and Ro 236 was suggested by the behavior of their F_2 progeny. This quantitative resistance was similar to that found in some lines of sea beet collected in the Po Delta of Italy (Figs. 2.3 and 9.1) (Biancardi and De Biaggi 1979; Lewellen and Biancardi 1990). Analyzing the improvements obtained in 3–4 cycles of backcross and recurrent selection on different genotypes carrying the Alba resistance, the heritability of the trait appeared relatively high (Biancardi, unpublished). But progress became more difficult in advanced cycles of selection. Histological analyses carried out on root tissue displayed a clear delay in the BNYVV diffusion through the xylem bundles (Giunchedi and Poggi-Pollini 1988). These observations suggested that the reduced symptoms and the better production under diseased condition were caused by an active reaction of the plant against the diffusion of pathogen (Lewellen and Biancardi 1990). This multigenic resistance probably was incorporated into the varieties Mezzano NP, Buszczynski CLR, GW 304, GW 359, GW 671, Monodoro, Dora, Ritmo (3×), Lena, Sanamono, and Bushel and in the varieties later released using the pollinators Ro 401 and Ro 412 (Biancardi et al. 2002) (Fig. 9.2). In field tests conducted in Germany in 1982 on rhizomania diseased fields, the varieties Dora and Lena produced 63 % and 85 % more white sugar, respectively, than the susceptible check (Bolz and Koch 1983).

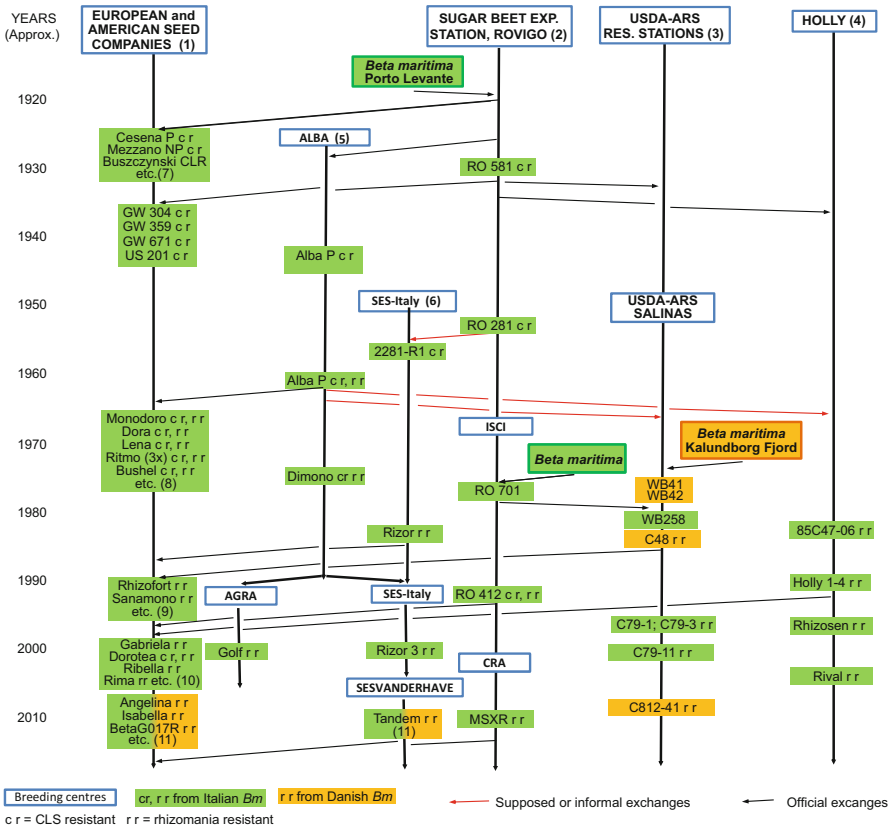

Fig. 9.2 Lineage of cercospora leaf spot and rhizomania resistances on the basis of published references or probable exchanges among the European and American research centers. Chronology in some cases is approximate due to graphic needs. It includes (**1**) Buszczynski, Synovie (PL); Centro Seme, Mezzano (I); Centro Produzione Seme, Cesena (I); KWS, Einbeck (D); Hilleshøg, Landskrona (S); Maribo, Holeby (DK); Lion Seeds, Maldon (UK); Great Western (now Western Sugar Coop); Denver (CO); American Crystal Sugar Company, Moorhead (MN); (**2**) Regia Stazione Sperimentale di Bieticoltura, Rovigo (I), named ISCI in 1968 and CRA in 2002; (**3**) USDA stations and other breeding activities at Beltsville (MD), Fort Collins (CO), Salt Lake City (UT), Salinas (CA), Riverside (CA), Waseca (MN), East Lansing (MI); (**4**) Holly Sugar, Colorado Springs (CO); (**5**) Alba Immobiliare, Ponte San Nicolò (I), joined with Agra; (**6**) SES-Italy, Massa Lombarda (I); (**7**) Commercial multigerm varieties endowed with CLS resistance; (**8**) monogerm varieties endowed with multigenic rhizomania resistance "Alba"; (**9**) varieties endowed with "Rizor" monogenic resistance similar to "Holly"; (**10**) varieties endowed with "Holly" monogenic resistance similar to "Rizor"; and (**11**) varieties with both monogenic resistances "Rz1" (Rizor and Holly) and "Rz2" (WB 42)

In 1979, the SES-Italy seed company, Massa Lombarda, Italy, began a research program aimed at discovering some source of resistance to rhizomania, possibly ready for a rapid development, and then releasing it quickly. It began with the field evaluation of the diploid germplasm belonging to the company (De Biaggi 1987).

Fig. 9.3 Field trial organized in diseased field where the first monogenic resistance to rhizomania has been detected. It is evident the quite normal green color of the leaves in the more resistant entry (highlighted plot) (Villa Serraglio, Italy, 1980)

Apart from the standard check varieties, the nursery included diploid pollinators and CMS lines with their maintainers (O-Type). The entries were sown in the spring of 1980 in rhizomania-infected fields located at San Martino in Argine, Bologna, and Villa Serraglio, Ravenna (Fig. 9.3). The fields revealed a severe and quite uniform infection, which allowed a satisfactory selection of the best individuals within the best entries. The beets, belonging to five multigerm families including 2,281, were selected on the basis of:

- Plant development and an intense green color of the leaves
- Regularly shaped roots, without internal or external symptoms of the disease
- Root weight

The remaining genotypes were almost totally destroyed by rhizomania. The 10–20 mother beets selected in each family, representing around 5 % of the original population, were overwintered (De Biaggi 1987).

In February 1981, the beets of each family were crossed with two F_1 male-sterile, high-yielding seed bearers (referred to as females in the text). In the year after, field tests of the ten hybrids were conducted at a site in France, where a more intense and uniform rhizomania infestation had been previously located. In July, the excellent performance of hybrids obtained with the pollinator 2,281 was confirmed by the French trials (Table 9.1). The remaining hybrids did not exceed the yield of Alba P and Domino (the hybrid monogerm version of Alba P) used as check.

Table 9.1 Field trials illustrating the performance of Rizor compared with other varieties

Locality (year) rhizomania	Variety (seed company)	Roots (t/ha)	Sugar content (%)	Sugar yield (t/ha)
Pithiviers – France (1982) severe	2281 Rizor (SES)	41.90	14.22	5.95
	Alba P (Alba)[a]	22.60	11.77	2.65
	Domino (Alba)[a]	20.90	10.08	2.32
	LSD (P = 0.05)	*9.60*	*2.01*	*2.33*
S. Martino – Italy (1983) moderate	Rizor (SES)	52.39	41.11	7.39
	Monodoro (Hilleshøg)[a]	37.69	14.94	5.63
	Ritmo (Maribo)[a]	14.11	13.46	5.53
	Monofort[b] (VDH)	22.90	13.43	3.07
	LSD (P = 0.05)	*14.30*	*0.96*	*1.49*

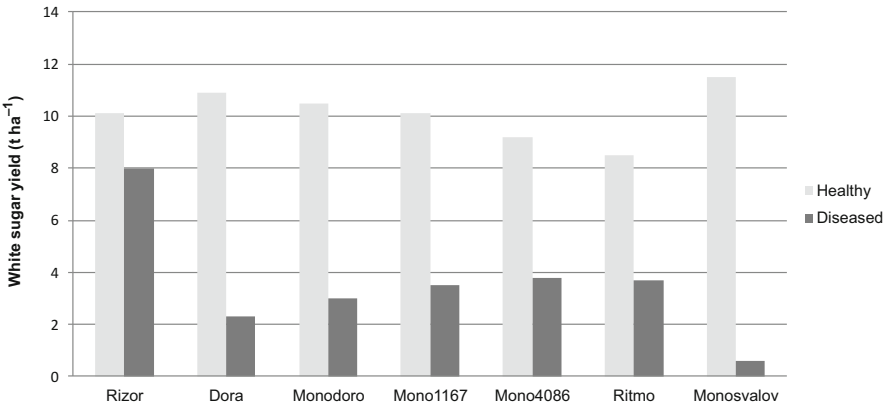

Fig. 9.4 Field trial conducted at Erstein, France, in diseased and healthy soils

The next year, the best hybrids were sown in France in two similar field trials, both conducted in infested and in rhizomania-free condition (Fig. 9.4). In addition, another two similar tests were sown in Italy (De Biaggi 1987). These trials confirmed that the resistance factor identified in the family 2281-R1 (used later in the hybrid "Rizor") offered a real possibility for significantly reducing the damage caused by rhizomania (De Biaggi 1987; De Biaggi et al. 2003). The virus concentration in the root tissues in susceptible check varieties and Rizor was 2,300 and 135 ng/g, respectively (De Biaggi 1987).

The French trials confirmed the higher level of resistance displayed by the monogenic factor carried by the first version of Rizor, when compared with the varieties of the "type Alba" (Bongiovanni 1984). However, the first available version of Rizor had some negative traits:

- A slight tendency toward bolting
- Unsatisfactory processing quality
- Sugar yield that was 10 % less than the susceptible varieties in rhizomania-free fields

But by using traditional breeding methods, Rizor was improved rapidly. In 1985, it was widely sown in France. At the same time, several experimental versions of Rizor were included in field trials in diseased districts all over the world. Until 1987, the breeding program for Rizor was carried out using normal family selection methods, integrated with artificial infection of individual plants followed by ELISA analyses. Later, the selected lines were multiplied using in vitro techniques (De Biaggi 1987; De Biaggi et al. 2003).

The origin of the resistance shown by the variety Rizor (i.e., the multigerm, diploid family 2281-R1) was not entirely clear. But recently, through the analysis of molecular variance (AMOVA) and use of principal component analysis of SNP markers on the original genotypes (which had been stored at low temperature), it was established that the Rizor and Holly resistances are not discernable as separate genetic sources (Stevanato et al. 2015). With this evidence, it was recognized that the pollinator SES 2281-R1 almost certainly originated from the Ro 281 family or some similar germplasm, which had been bred in public and private research stations and then bought, likely from Holly Sugar, currently located at Sheridan WI, through normal germplasm exchanges.

The resistances, Rizor and Holly, were soon recognized as monogenic and dominant because the rhizomania-resistant F_1 hybrid varieties were produced from rhizomania-susceptible females. In some genetic backgrounds, the segregation of *Rz1* was disturbed by minor genes (De Biaggi 1987). At these times, the presence of susceptible (*rzrz*) plants in the hybrid was around 10 %. This percentage was not negligible, especially from the sugar production point of view, and was difficult to reduce. This task became easier with the availability and the use of molecular markers.

In the first Rizor releases, it was observed that the viruliferous zoospores of *P. betae* inoculated the rootlets of susceptible genotypes in the same way as in the Rizor pollinator and in the Rizor hybrid (Giunchedi et al. 1985). The zoospores only moved easily through the xylem tissues in the roots of susceptible genotypes, whereas the movement in the genotypes carrying the Rizor resistance appeared slightly reduced. At that time, it was not possible to establish the physiological mechanism, which reduced spreading of the BNYVV (De Biaggi et al. 1986; Giunchedi et al. 1987; Giunchedi and Poggi-Pollini 1988). Further research ascertained that in Rizor, the zoospores multiply normally in the rootlets, but their migration toward the taproot is reduced or delayed by the development of a sort of barrier

of suberized cells, which interferes with the diffusion of the viruliferous zoospore from the affected areas (Asher and Kerr 1996; Marciano et al. 1977; Poggi-Pollini and Giunchedi 1989). Moreover, it was observed that the reproduction of *P. betae* in the roots of Rizor seemed to be hindered because the zoosporangia rarely were visible. This should lead to a slower reproduction of the plasmodiophoromycete and, consequently, to a reduced soil inoculum level after harvest, when compared with the susceptible varieties (Merdinoglu et al. 1987) (Fig. 8.4). Accordingly, the resistant plant seemed to react actively against the diffusion and the reproduction of the BNYVV. It was unclear if the diffusion of the virus inside the root happened cell to cell or by means of the vascular bundles (Geyl et al. 1995). At least in the initial observations, the resistance to rhizomania did not seem to act through limited inoculation of the viruliferous zoospores of *P. betae*. Another quality of Rizor, at least in its first releases, was the suitable CLS resistance, similar to Ritmo, confirmed by the similar weight of leaves and crowns in the late harvests (Bongiovanni 1986).

In the summer of 1983, Erichsen observed very poor growth and a diffused yellowing of leaves in a variety trial at Tracy (California) conducted by the Holly Sugar. Only the beets of some three-way experimental hybrids, such as 85C47-06 (Table 9.2), which had been produced by crossing different pollinators with the same CMS female, were normal (Lewellen et al. 1987). After ELISA analyses, it became clear that the field was uniformly infested with rhizomania (Duffus et al. 1984; Duffus and Ruppel 1993). Notwithstanding the presence of at least 30 % susceptible beets in the F_1, the sugar yield of the Holly hybrid was better than some European resistant checks. In this case too, the resistance factor segregated partially according to the action of a single dominant gene, demonstrated by a chi-square test on the backcrosses. The next year, similar results were obtained at Salinas by Lewellen et al. (1987), and the single, dominant gene was named *Rz* (Lewellen 1988) (Table 9.2).

The O-Type and CMS pair of lines carrying the Holly resistance was sold in Europe in 1986, and the first variety endowed with the "Holly" trait was "Gabriela" (KWS, Einbeck, Germany) released in 1990. In the initial reproductions of hybrids bearing the Holly trait, the seed quality was fairly low. These problems were rapidly resolved because the allele *Rz* proved to be easily handled and the negative qualities (low germination ability, cold susceptibility, etc.) were not linked with the resistance (Wisler et al. 1999).

Attempts to trace the source of the Holly resistant gene have not been successful. It has been speculated that it was partially derived from sea beet, perhaps from the Italian CLS-resistant accessions incorporated around 1935 into the germplasm of the USDA-ARS stations and the Great Western Sugar Company and other American seed companies (Lewellen and Biancardi 1990) (Fig. 9.2).

The mechanism of resistance for the Holly gene, coded *Rz1* by Scholten et al. (1999), appeared to be related to a reduction in BNYVV replication. Wisler et al. (1999) observed that the allele *Rz1* was incompletely dominant with various degrees of penetrance, as later confirmed by Pelsy and Merdinoglu (1996). In F_2 segregating, heterozygous plants, *Rz1rz1*, Giorio et al. (1997) observed a 1:2:1 ratio, which was thought to be caused by some kind of codominance. The *Rz1* types did not perform with the same intensity in the backcrossed genotypes; resistance was

Table 9.2 Field trials organized at Salinas in 1985 and 1986

Locality (year) rhizomania	Variety (seed company)	Roots (t/ha)	Sugar content (%)	Sugar yield (t/ha)	Disease index[c]
Salinas CA, USA (1985) moderate	84C39 – 031[a] (Holly)	60.80	12.90	7.82	2.67
	Rizor (SES)	40.10	13.80	5.48	3.08
	Monodoro (Hilleshøg)	37.50	11.90	4.50	3.29
	Monohikari (Mitsui – Seedex)	25.50	12.20	3.20	3.38
	HH37[b] (Holly)	24.10	9.90	2.41	3.60
	USH 11[b] (USDA)	20.10	9.00	1.80	3.66
	LSD (P = 0.05)	*8.30*	*1.10*	*1.08*	*0.54*
Salinas CA, USA (1986) severe	85C47 – 06[a] (Holly)	39.80	14.60	5.88	2.98
	Rizor (SES)	25.80	14.30	3.72	3.58
	Monodoro (Hilleshøg)	19.90	13.30	2.68	4.38
	Monohikari (Mitsui – Seedex)	22.80	13.40	3.06	4.45
	HH37[b] (Holly)	19.90	12.40	2.46	4.54
	USH 11[b] (USDA)	15.40	11.60	1.79	4.45
	LSD (P = 0.05)	*5.30*	*1.00*	*0.77*	*0.40*

See also Table 11.1
From Biancardi et al. (2002), modified
[a]Monogenic-resistant varieties
[b]Susceptible check
[c]Disease index (0 = no symptoms, 9 = dead)

dependent on both their genetic background and the presence of modifying genes (Rush et al. 2006). The effect of the genetic background that accompanies the resistance gene cannot be disregarded because it may modify significantly the expression of the trait (Meulemans et al. 2003).

Because recent fingerprinting analyses confirmed that the resistances of Rizor and Rz1 are almost identical, it is very probable that the Holly 1-4 line carrying the *Rz1* gene and the resistant family, SES 2281, were derived from the same common parent (Stevanato et al. 2015). The differences between the Holly line and the SES family evidently are due to diverse genetic backgrounds (De Biaggi, unpublished). It appeared possible to accumulate additive traits, which increase the effects of *Rz1*. The supposed modifying genes seemed to be the similar to those working in the Rizor resistance. Additionally, both alleles showed incomplete dominance, which means higher production in the homozygous state than in the heterozygous (Meulemans et al. 2003). In resistant genotypes, the virus was localized in the epidermis, cortex parenchyma, endodermis, and interstitial parenchyma but rarely in the vascular tissues (Scholten et al. 1994).

The combination of multiple types of resistance may be advantageous to provide higher levels of protection against BNYVV (Lewellen and Biancardi 1990). The effect of the Alba multigenic resistance on sugar yield in diseased fields is less than the monogenic or near monogenic sources like Rizor and Holly, and the hybrids between Alba and Rizor did not perform better than the parents. The expected het-

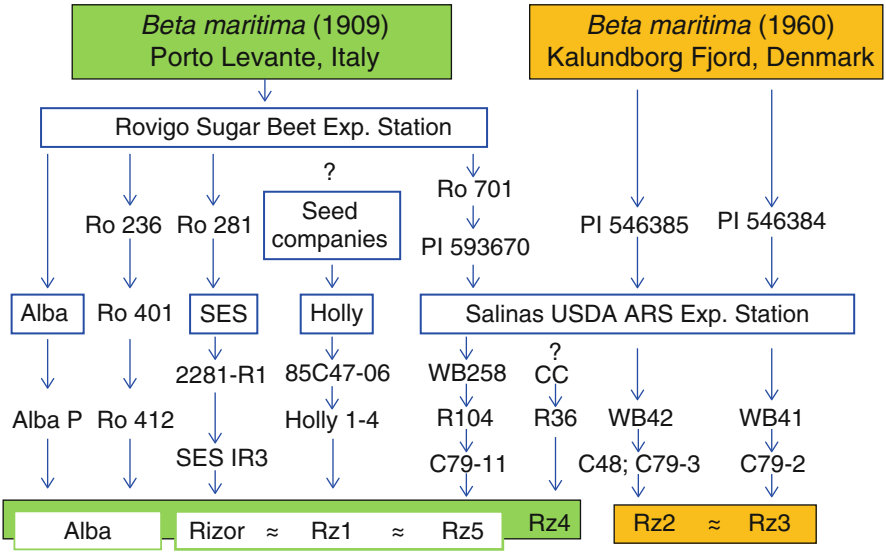

Fig. 9.5 Sources, year of sampling (between parentheses), and partial derivation of the currently employed resistances to rhizomania (Alba, Rizor, Holly, Rz2) or those resistances still under evaluation (Rz3, Rz4, and Rz5). *CC* Composite cross, ≈ similar Breeding centres

erosis effect also was not evident in hybrids between Rizor and Holly sources. Therefore, their equivalence began to be hypothesized. According to Barzen et al. (1992), the resistances of Rizor and Holly likely are due to the same major gene with incomplete dominance, located in chromosome III and interacting both with minor (or modifying) genes and the presence of different genetic backgrounds (Meulemans et al. 2003). Scholten et al. (1999) analyzed segregation in F_2 and backcross generations of a cross between the Salinas line R104 and Holly 1-4 (Rz1) and placed both resistance loci in the identical position on chromosome III. Because the line R104 is derived from the *B. maritima* accession Ro 701, collected in the Po Delta in 1978 (Biancardi et al. 2002), the common lineage of Rizor and Holly sources is confirmed (Stevanato et al. 2015) (Fig. 9.5).

The variety Rizor became available for farmers in 1985 (De Biaggi 1987), that is, 5 years before the marketing of the Holly Rz1 resistance, which was sold to the European seed companies in the form of a CMS and its corresponding O-Type. According to Dürr et al. (2000), some negative traits of the first Rz1 and the derived European varieties were evident, especially during emergence, mainly caused by the limited nitrogen uptake of the seedlings (Rush et al. 2006).

Notwithstanding the intense screening carried out in public and private breeding centers, no other traits of resistance were found in the cultivated sugar beet germ-

plasm. Consequently, attention turned to wild beets and in particular to *B. maritima* (van Geyt et al. 1990). The transfer of the monogenic resistance trait from *B. maritima* to sugar beet genotypes is relatively easy and usually is performed by means of backcrosses or recurrent selection, aided by the use of molecular markers, which have improved greatly the rate of success (Geyl et al. 1995). The accessions collected in different parts of the world and stored in gene banks were carefully checked. The wild germplasms of USDA-ARS collection were analyzed in the field and greenhouse at the USDA-ARS Research Station in Salinas, California. Differing degrees of rhizomania resistance were found in 17 entries (Whitney 1989). After crossing with susceptible beets, the segregating generations suggested the monogenic and dominant nature of the resistance, which appeared quite simply inherited. The most promising accessions were WB 41 and WB 42 (PI 546385), corresponding to *B. maritima* populations collected in 1960 at Kalundborg Fjord, Denmark, by Lund (Amiri et al. 2003; Doney and Whitney 1990; Lewellen 1991, 1997). In some accessions, including WB 42, high levels of resistance to rhizomania, CLS, root maggot, and *Erwinia carotovora* were detected (Doney and Whitney 1990). Using recurrent selection, the resistance trait of WB 42 was transferred into the high-yielding pollinator C37 (Lewellen et al. 1985) also carrying resistance to curly top, erwinia root rot, beet western yellows virus (BWYV), and beet yellows virus (BYW). The resulting line, C79-3 (Lewellen 1997), endowed with WB 42 trait, displayed a higher level of resistance to rhizomania than Rz1 (Scholten et al. 1999). The gene, coded *Rz2* by Scholten et al. (1999), was localized on chromosome III at a genetic distance of 20–35 cM from *Rz1*. The Rz2 resistance seems to be based on a single, dominant, major gene displaying distorted segregation as observed both in Rizor and Rz1 sources. It was predicted that, because the genes carrying the resistance Rz1 and Rz2 were at different loci, they would provide some heterotic effects after crossing (Amiri et al. 2003).

In accession WB 41 collected very close to the WB 42 site, another resistant gene was discovered (Figs. 9.2 and 9.5). The gene was named *Rz3* and the locus conferring resistance was localized on the same chromosome III, very near (5 cM) to *Rz*2. It is believed that the WB 41 and WB 42 resistances are induced by the same gene, perhaps belonging to the same allelic series and interacting with different modifying factors (Grimmer et al. 2007). In relation to WB 42, Scholten et al. (1997) put forward the hypotheses of either one or two major genes with distinct segregation or of two complementary major genes both necessary for expression of the resistance. The genomic region of 800 kb including *Rz2* is currently being analyzed in search of new candidate genes for resistance (Capistrano et al. 2014).

After the discoveries of Rz2 and Rz3, other presumed new sources of resistance were isolated by means of QTL analyses in accession R36 derived from composite crosses obtained at Salinas, and in WB 258 (formerly coded Ro 701, R104, and PI 546426), derived from *B. maritima* harvested in July 1978 in the Po Delta by De Biaggi and Biancardi (Biancardi et al. 2002; Lewellen 1991), likely near the same site where Munerati collected the seed in 1908 (Fig. 9.1). These hypothesized new resistances, termed Rz4 by Gidner et al. (2005) and Rz5 by Grimmer et al. (2008), respectively, showed evidence of distorted segregation and mapped very near to

Rz1, likely representing another case of an allelic series (Grimmer et al. 2007). All the listed resistances were released in 1997, included in the Salinas germplasm series C79-1 to C79-11, developed after backcrossing with the common recurrent parent C37 (Lewellen et al. 1985; Lewellen 1995a, 1997) (Figs. 9.2 and 9.5). Two populations developed from a combination of all of the germplasms in this series will be released as FC1740 and FC1741 (Panella, personal communication). As regards further types of resistances, Pelsy and Merdinoglu (1996) mentioned some Turkish sugar beet breeding lines and *B. maritima* ecotypes, but further developments of these materials are not known.

Another approach has been applied to search for new traits of resistance (Doney and Whitney 1990). The individually selected *B. maritima* mother roots of different populations were pooled and open pollinated. In the derived heterogeneous population, chance, but potentially useful, combinations of major and minor genes of rhizomania resistance can be found. From similar composite crosses, some new sources of rhizomania resistance were obtained by Lewellen (1995b) and Lewellen and Whitney (1993). Using the approach described above, one of the germplasm developed led to the creation and release of C79-8, where the Rz4 resistance was discovered (Gidner et al. 2005). With the same system, the lines C39R and C47R were identified, which bore quantitative traits of resistance, allowing the same level of production under rhizomania conditions as did Rizor or Rz1 (Lewellen 1995c). Both lines reduced the disease symptoms, but not the virus concentration in the roots (Rush et al. 2006). Potentially useful traits of resistance were located in *Beta corolliflora*, *Beta intermedia*, and *Beta lomatogona* (Paul et al. 1993).

9.3 Multiple Resistances

The level of resistance of Rizor ≈ Holly (Rz1) improves in crosses with *Rz2* (De Temmerman et al. 2009; Meulemans et al. 2003). The differences among the above-mentioned monogenic resistances, as well as the heterosis, are evident only in the case of Rz1 x Rz2 crosses (Amiri et al. 2003). First in 2002, some commercial varieties carrying the double resistance were introduced in the USA and in France, where they displayed better sugar yield both in the presence of the BNYVV-P strain and in other resistance-breaking BNYVV strains (De Temmerman et al. 2009; Rush et al. 2006; Smith et al. 2010). To the best of our knowledge, these results indicate that the accessions of *B. maritima* collected in Italy and more recently in Denmark are the only sources of resistances to rhizomania commercially deployed today (Pavli et al. 2011) (Figs. 9.2 and 9.5).

Heijbroek et al. (1999) tried to find an interaction between varieties carrying different sources of resistance and the three pathotypes of BNYVV (A, B, and P), known at that time. Because no significant differences were detected in field trials, the experiments were continued in the glasshouse, using substrate with the same concentration of the diverse BNYVV pathotypes. Pathotype B was less damaging for all the measured parameters, while pathotype P confirmed its already known

high level of virulence, likely due to it moving more rapidly inside the roots than either the A or B type (Heijbroek et al. 1999). Significant differences also were evident among the response of resistant varieties, but not among the pathotypes.

Resistance-breaking strains of BNYVV appeared around the year 2000 on varieties with the Rz1 resistance. The evolution of these strains after about 30 years of continuous employment of Rz1 almost was expected. The same happens for every disease, when the crop is protected for a long time by the same chemical or a single-gene resistance (Van Der Plank 1975). In the Imperial Valley of California, similar loss of resistance by Rz1 was observed during the 2002 campaign, evidently due to mutations in the virus. The new strain was coded IV-BNYVV. Satisfactory degrees of resistance were identified in the C79-9 germplasm released by Lewellen (1997), coming from the *B. maritima* accession coded WB 151 (PI 546397), collected in Denmark together with WB 41 and WB 42. Additional resistance-breaking strains observed in other parts of the USA (Minnesota, North Dakota) are similar but not identical (Acosta-Leal et al. 2009, 2010; Liu and Lewellen 2007). It is yet to be ascertained whether the resistance-breaking episodes occur independently or, simply, are due to contamination, i.e., through movement of infected soil (Bornemann et al. 2015). The first option appears more likely and indicates that the local genotype x environment interactions can modify the frequencies and the molecular background of the virus mutations. This means that similar strains may appear everywhere after a given period of virus multiplication, if the local conditions favor the disease agents.

The genomic composition of *P. betae* displays variability, dependent on geographical adaptation as well. Therefore, the vector could have a role in the resistance-breaking occurrences (Pferdmenges 2007). According to the same author, the resistance-breaking episodes were unconnected with the *P. betae* concentration in soil (Pferdmenges and Varrelmann 2009). Soon after, proof of these occurrences in soils infected by BNYVV type A was discovered in varieties endowed with Rz2 and also in the more recent varieties with the double-resistant Rz1 + Rz2 (Hleibieh et al. 2007; Scholten and Lange 2000).

9.4 Resistance to *Polymyxa betae*

Beet necrotic yellow vein virus (BNYVV), the causal agent of rhizomania, is vectored by the plasmodiophorid, *P. betae* (reviewed by Rush 2003). *Polymyxa betae* is ubiquitous in every beet-growing country and can carry several more or less harmful soilborne viral diseases (Lennefors et al. 2008; Rush 2003). BNYVV is not always present inside the cystosori, e.g., only 45 % of the cystosori tested in Californian soil samples were infected (Gerik and Duffus 1988). Normally, the plasmodia alone seem asymptomatic for the crop (Desoignies et al. 2014; Hleibieh et al. 2007; Scholten and Lange 2000) or cause minimal damage (Rush et al. 2006). But it has been reported in greenhouse tests that a viruliferous *P. betae* induced a significant depression in emergence and seedling growth (Liu and Lewellen 2008;

Wisler et al. 2003). Similar damage in field conditions was described by Davarani et al. (2013) but only in warm soil, which also was observed also by Blunt et al. (1991). The opinions are quite controversial, likely because the behavior of the plasmodiophorid depends not only on genotype x environment interactions but also on the behavior of host plant (Pferdmenges 2007). According to Abe and Tamada (1986), isolates of *P. betae* coming from plants other than sugar beet are unable to transmit the BNYVV.

Methods complementing the genetic resistance or alternative mechanisms for limiting the effects of BNYVV have been investigated. A primary target should be delaying or hindering the multiplication of *P. betae* by means of genetic mechanisms to prevent or reduce either the entry of the vector into the rootlets or the multiplication of the virus (Mesbah et al. 1997). In fact, the plasmodiophorid, as was shown by Lubicz et al. (2007), can assume the function of host, because the virus reproduces inside it as well. Several approaches to find resistance to *P. betae* have been tried without concrete results. No useful source of resistance to the plasmodiophorid has been discovered in screening sugar beet genotypes; on the contrary, a higher number of cystosori were found in BNYVV-resistant varieties than in the susceptible ones (Paul et al. 1993). In some entries belonging to the sections *Procumbentes* (now genus *Patellifolia*) and *Corollinae*, it has been observed that the zoospores penetrated the root of the resistant wild beets normally, but further diffusion was rarely observed (Barr et al. 1995) or further development of cystosori (Paul et al. 1993). Through monosomic addition lines, it was demonstrated that the genes inducing this sort of behavior are located on chromosomes IV and VIII of the host plant. Notwithstanding the dominance of the monogenic resistance (Barr et al. 1995; Paul et al. 1993), attempts to transfer the traits into cultivated genotypes were unsuccessful, mainly due to the cross-incompatibility among sugar beet and the species included in sections *Procumbentes* and *Corollinae* (e.g., *Patellaris*) (Box 1.5). The same attempts were undertaken using genotypes of *B. maritima* (Doney and Whitney 1990). Here, the traits of resistance to *P. betae* were "surprisingly common" and appeared to be quantitatively inherited (Asher et al. 2009; Asher and Barr 1990; Luterbacher et al. 2004, 2005). The resistance loci to the vector were located on chromosomes IV and IX and were termed *Pb1* and *Pb2*, respectively. In backcrosses with sugar beet germplasm, by screening with recombinant antibody, it was possible to transfer the resistance traits. BNYVV alone moves very slowly inside the root, being transferred more easily cell to cell by *P. betae* (Prillwitz and Schlösser 1993). Therefore, the degree of aggressiveness of the plasmodiophorid seems to have effects in spreading the virus inside the beet and in the related damage (Gerik and Duffus 1988). The same authors found significant differences of behavior in US isolates of *P. betae*. The multiple resistances, both to virus and to vector, could be a powerful means for further reduction of the damages caused by rhizomania due to the very different but complementary mechanisms.

9.5 Resistant Varieties

Owing to:

- The very limited or ineffective effects of crop rotation and other agronomic measures after soil infection
- The present and future difficulty of finding effective means of chemical control
- The encouraging, but still scarcely working, biological control systems for reducing the spread and the damage of rhizomania

The only affordable and relatively inexpensive means of management is given by genetic resistance (Pavli et al. 2011). The statement is justified by the availability of several already employed, effective, different types of resistance (Hull 1994). Other new traits are possible candidates to replace the currently used resistance sources in case of a possible reduction in efficacy. Using molecular biology-assisted techniques, the wild beet genomes (including almost all the species of the genera *Beta* and *Patellifolia*) could become further sources of suitable traits, stimulating future development of sustainable agriculture (Martin and Sauerborn 2013). Additionally, nothing is more environmental friendly than genetic resistance.

Another promising area of research is represented by the transgenic resistances (see. Chap. 10). Some transgenic varieties have shown quite normal sugar production under very diseased field conditions (Lennefors 2006). These effective resistances, near to immunity, are ready to be released and may be an important mean to reduce the effects of rhizomania, also under the condition of very aggressive virulence (Hleibieh et al. 2007; Mannerlöf et al. 1996; Pavli et al. 2011).

The rhizomania resistance traits in *B. maritima* are very unlikely to have originated by natural selection in presence of the disease factors. Bartsch and Brand (1998) did not find the presence of either *P. betae* or BNYVV in soils and roots of *B. maritima* at six sites along the coast of the North Adriatic Sea, including the Po River Delta, where the Rz1 resistance is found. This was mostly due to the lack of *P. betae*, which is decreased in the soil by the high salt content where *B. maritima* normally grows. The same lack of rhizomania and vector likely occurs in the Danish soils, where the sea beets that coded WB 41 and WB 42 were collected (Driessen 2003). Therefore, if rhizomania resistance in *B. maritima* has originated and developed by natural selection in diseased soils, as hypothesized above, this must have happened elsewhere or in some other manner.

Sugar yield of the first versions of the monogenic-resistant varieties was about 10 % lower than the susceptible ones in healthy soils (Whitney 1989). But this weakness gradually has been overcome. Today, the sugar yield of resistant and susceptible varieties is almost the same in rhizomania-free conditions. These varieties don't display significant yield variation either in absence or presence of rhizomania, excluding the cases of severe infections (Graf 1984) and resistance-breaking BNYVV (Fig. 9.5).

In conclusion, the dynamics of the host-pathogen relationship in rhizomania depend on numerous factors and reciprocal interactions, which still are quite

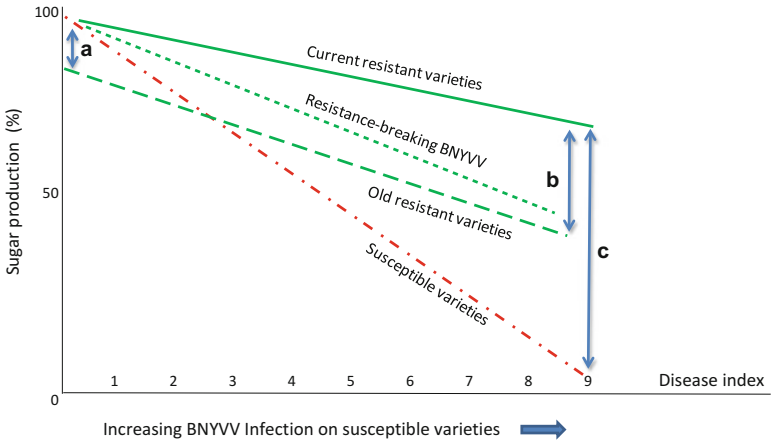

Fig. 9.6 Sugar yield varied with increasing infection. After release, resistant varieties were yielding less than the susceptible ones in healthy soil (*a*). Over time, this gap has been completely erased. The resistant varieties (old or new) are not immune to rhizomania and yield decreases with increasing infection but not nearly as much as yield decreases in susceptible varieties. The breeding progresses in the last 30 years and the effects of rhizomania resistances on sugar yield are represented by the *double arrows* (*b*) and (*c*), respectively

unknown or not predictable due to their extreme variability. The future development of disease management will require, as in the past, a continuing increase in multidisciplinary research projects (Fig. 9.6).

9.6 Mechanisms of Resistance

Some analytical techniques, such as immunogold-silver labeling, electron microscopy etc., have been used to detect the location of the BNYVV inside root tissues, with the aim of explaining the mechanisms, which allow the resistant beets to limit the damage caused by rhizomania. Because BNYVV can multiply both inside the *P. betae* and in the root cell (Geyl et al. 1995), it is not easy to establish the stage(s) of the viral pathogenesis as influenced by the genetic host resistance. According to Fraser (1990), the reaction of resistant beets against vectored viruses similar to BNYVV works mainly by limiting:

- The transmission of the virus
- Its multiplication inside the root
- Its movement inside the root
- Its pathogenicity

The major portion of research papers reported no difference between the BNYVV concentration in the rootlets of resistant and susceptible beets under similar inoculum condition. This means that the resistance does not reduce the entry of virulifer-

ous cystosori. But in the case of Rz2, a lower concentration of BNYVV was detected (Scholten et al. 1994). The inhibition of BNYVV multiplication seems to behave in a way that influences the reduction of the damage. In fact, the epidermal cell of the resistant rootlets infected by cystosori contains more BNYVV than in susceptible genotypes (Giunchedi and Poggi-Pollini 1988).

The reduced mobility of the virus has been recognized as the major effect of genetic resistance. The short-distance movement happens cell to cell and has been detected by means of virus antigen, which located virus in cells neighboring the cell containing the viruliferous cystosori (Hull 1989). The possibility of long-distance movement, i.e., through the vascular bundles, is still controversial (Scholten et al. 1994). In some cases the BNYVV was detected neither in the bundles of resistant nor of susceptible beets. In other cases, the xylem vessels of susceptible genotypes were infected by the virus, as seen by the inoculum concentration (Scholten et al. 1994). In resistant beets, the vascular bundles appeared smaller than in the susceptible ones, likely limiting in this way the movement of the virus. It was hypothesized that there was development of suberin barriers in the cell, which hinders the movement of the virus from lateral rootlets to the taproot (Poggi-Pollini and Giunchedi 1989). The ability of the BNYVV to spread in the roots depends also on the beet's age. In fact, if the infection happens after the seedling stage, the virus spreads in the rootlets, but not in the taproot, also in susceptible genotypes (Hull 1989), thus explaining the minor damage of the disease if the crop is sown early. Similar differences were detected when comparing the currently deployed resistances, which display quite diverse mechanisms in limiting the effects of the BNYVV. For example, the beets carrying the resistance Rz2 show minor virus replication and more restricted cell-to-cell movement than Rz1. In order to explain this behavior, Scholten et al. (1994) hypothesized the presence of different genetic systems or mechanisms of action in Rz1 and Rz2, which was later demonstrated.

9.7 Germplasm Conservation in the Service of Plant Breeding

Over the past 60 years, we slowly have come to realize that the crop wild relatives of sugar beet, especially sea beet, have become a crucial genetic resource in the breeding of sugar beet and other cultivated beet crops. During this time, we have seen a tremendous increase in our knowledge of the life history of this critical resource (Biancardi et al. 2012). But it is only in the last 30 years that we have acknowledged that the wild germplasm was vanishing (Doney et al. 1995; Pignone 1989) and that without this resource, we might not have the genetic means to improve the sugar beet crop (De Bock 1986; Doney and Whitney 1990; Doney 1993; Lewellen and Skoyen 1991; van Geyt et al. 1990). We have begun to understand that an effective conservation strategy must be grounded on a thorough understanding of the taxonomy, genetic diversity, and distribution of the crop wild relatives (Frese 2010).

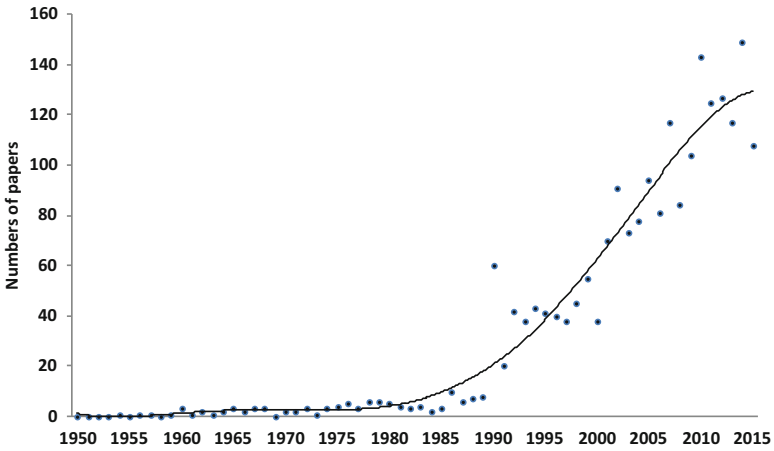

Fig. 9.7 Number of journal articles regarding rhizomania published per year. Until 1963, the papers were written only in Italian. An article in German regarding *Polymyxa betae* was edited in 1964, though not yet linked with rhizomania. To the best of our knowledge, the first papers on the disease in German or in English were published in 1967 and 1971 respectively (Summarized from different sources)

Some of our sources of resistance to rhizomania are the result of plant collections by the pioneers in this field. Coons of the USDA-ARS was a major supporter of using the wild *Beta* species, especially sea beet as a source of genetic diversity to improve cultivated beet in the USA. He made collection trips in 1925 and 1935 (Coons 1936) and again in 1951 and 1971 (Coons 1975), long before anyone in the USA was aware of rhizomania, yet some of the accessions he collected provided the genes for resistance to rhizomania. Similarly, Munerati may have transferred rhizomania resistance unintentionally from sea beet to cultivated beets as he worked on resistance to Cercospora *Cercospora* leaf spot.

Although there was a reluctance to use sea beet germplasm because of some of the undesirable traits, by the mid-1980s, commercial breeding programs had begun to reconsider (Frese et al. 2001). In Europe, Bosemark (1989) created the framework for plant breeders to introgress effectively the germplasm of crop wild relatives into elite breeding populations. This activity was mirrored in North America by the development of the Sugar Beet Crop Advisory Committee to work with the curator of the USDA-ARS *Beta* collection to provide evaluation data for plant breeders interested in crop improvement, especially for improved disease resistance (Doney 1998; Janick 1989; Panella and Lewellen 2007). Frese (1990) used many of these ideas to develop a strategy to enhance the genetic foundation of the sugar beet gene pool. Together these researchers founded the World *Beta* Network under the IBPGR to improve international collaboration among researchers and gene bank curators of *Beta* germplasm collections worldwide (Bosemark 1989). Today this effort of conserving genetic resources is a collaborative effort of the international community.

We know that species evolution is arrested in an ex situ collection, which provides only a snapshot of part of the existing genetic diversity at that place in time and space. There can be no additional adaptation to the changing environment, only adaptation to the changing gene bank seed reproduction process. Consequently, we have seen increasing awareness in conservation of beet wild relatives in situ and sea beet in particular (Biancardi et al. 2012; Frese 2010; Frese and Germeier 2009; Jarvis et al. 2015; Van Dijk 1998). It is up to all of us, researchers, plant breeders, and beet processors, to preserve these resources for those who will need them in the future (Fig. 9.7).

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