Theor Appl Genet (1999) 99:740–746

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Inheritance of resistance to beet necrotic yellow vein virus in *Beta vulgaris* conferred by a second gene for resistance

Received: 29 October 1998 / Accepted: 12 March 1999

Abstract Rhizomania is a serious disease of sugar beet, caused by beet necrotic yellow vein virus (BNYVV). The disease can only be controlled by the use of resistant cultivars. The accession Holly contains a single dominant gene for resistance, called *Rz*. The identification of a locus for resistance that differs from *Rz* would provide possibilities to produce cultivars with multiple resistance to BNYVV. Inheritance of resistance to BNYVV was studied by screening progenies of crosses between resistant plants of the accessions *Beta vulgaris* subsp. *maritima* WB42 and *B. vulgaris* subsp. *vulgaris* Holly-1–4 or R104. Observed and expected segregation ratios were compared to elucidate whether the resistance genes in the three accessions are alleles or situated on different loci. STS markers, linked to the genes for resistance, were used to study the segregation in more detail. The results demonstrated that the genes for resistance to BNYVV in Holly-1-4 and WB42 are closely linked. The gene for resistance in R104 is at the same locus as in Holly-1-4, and also closely linked to the gene in WB42. As the Holly resistance gene has been named *Rz*, the name *Rz2* is proposed to refer to the resistance gene in WB42. Consequently, the gene *Rz* should be referred to as *Rz1*.

Key words Beet necrotic yellow vein virus · *Beta vulgaris* · Inheritance · Resistance genes · Rhizomania · STS markers

Introduction

Beet necrotic yellow vein virus (BNYVV) is the causal agent of rhizomania in sugar beet, *Beta vulgaris* L. (Tamada 1975). The virus is transmitted by the soilborne fungus *Polymyxa betae* Keskin, and severe infections of beets with BNYVV lead to significant decreases in yield and sugar content (Richard-Molard 1985). The most effective way to control the disease is the breeding and cultivation of rhizomania-resistant sugar beet cultivars (Schlösser 1988; Asher 1993).

Resistance to rhizomania occurs in *B. vulgaris* subsp. *vulgaris* accession Holly (Lewellen et al. 1987) and has been shown to be based on resistance to the virus rather than to the vector (Paul et al. 1993a). Inheritance of resistance was studied using progenies of crosses between Holly and susceptible plants (Lewellen et al. 1987; Scholten et al. 1996). The results of field experiments indicated that resistance in Holly is simply inherited and possibly conditioned by a single dominant gene, named *Rz* (Lewellen et al. 1987; Lewellen 1988). Results of segregation analyses using mixture models on virus concentration data obtained from individual plants tested in the greenhouse confirmed this hypothesis (Scholten et al. 1996). Due to the relative ease of introgressing single dominant resistance genes into breeding stocks, Holly has often been used as a source of resistance.

Resistance to BNYVV has also been found in *B. vulgaris* subsp. *maritima* (L.) Arcang. originating from Italy, France, England and Denmark, as for example, in WB42 (Fujisawa and Sugimoto 1979; Whitney 1986, 1989; Lewellen et al. 1987; Paul et al. 1993a). Field tests with progenies of crosses between resistant plants of accession WB42 and susceptible plants of subsp. *vulgaris* showed that resistance in WB42 is dominant, but the number of genes conferring resistance remained unclear (Lewellen et al. 1987; Whitney 1989). Segregation analyses, based on the results of greenhouse tests, supported the hypotheses that the resistance to BNYVV is based either on one (or more) dominant major gene(s) showing distorted segregation or on two complementary unlinked dominant genes, both of which required for resistance (Scholten et al. 1996).

In greenhouse tests, average virus concentrations in rootlets of resistant plants of WB42 were lower than in those of Holly (Scholten et al. 1996). Under high infec-

Communicated by G. Wenzel

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tion pressure, using viruliferous zoospore suspensions of *P. betae*, differences between the level of resistance in WB42 and Holly were even more clear (Paul et al. 1993b). Studies on the localisation and spread of BNYVV in rootlets, infected through zoospore suspensions, showed clear differences between WB42 and Holly (Scholten et al. 1994). These findings imply that the mechanism of resistance to BNYVV in Holly differs from that in WB42. This difference might have a genetical origin (Scholten et al. 1996). Combining genes conferring different mechanisms of resistance could provide higher levels of resistance with an improved durability.

Another accession with a single dominant gene for resistance to BNYVV is *B. vulgaris* subsp. *vulgaris* R104, with resistance originating from subsp. *maritima* (R.T. Lewellen personal communication). Linkage between sequence-tagged-site (STS) markers and the genes for resistance in Holly and R104 indicates the presence of identical or tightly linked loci in these two accessions (Scholten et al. 1997).

The aim of the investigation presented here was to elucidate the genetics of resistance of WB42 in relation to the genes of Holly and R104. Progenies of crosses between these accessions were used to determine segregation ratios. For progenies of crosses between Holly and WB42, the inheritance of STS markers linked to resistance of both accessions (Scholten et al. 1997) was also studied.

Materials and methods

Plant materials and crosses

Plant materials consisted of F_1 progenies, obtained by making pair crosses between resistant plants of *B. vulgaris* subsp. *maritima* WB42 and plants of the homozygously resistant sugar beet accessions Holly-1-4 (a selection of Holly) and R104. The WB42 plants originated from a bulk multiplication because selfing of inbred plants only resulted in small numbers of seed. Most plants of WB42 were homozygously or heterozygously resistant, but some susceptible plants also occurred. F_1 families were screened in the greenhouse for their level of resistance to BNYVV and appeared to be resistant. F_1 plants were selfed for the production of F_2 seed or crossed with susceptible male-sterile plants of *B. vulgaris* MS-2. The latter progenies will be referred to as BC (backcross) families.

Greenhouse test and genetical analysis

Plants were tested in the greenhouse as described by Paul et al. (1992). ELISA was applied to determine the concentration of BNYVV in the rootlets of individual plants. The $\log_{10}$ of the virus concentration was used for statistical analysis. Varying numbers of plants of the F_1, F_2, and BC families were tested. The parents and the susceptible cultivar 'Regina' were included in all experiments as a control.

Mixtures of normal distributions were fitted to the virus concentrations to estimate segregation ratios of resistant and susceptible plants in F_2 and BC families (Jansen 1993, 1994; Scholten et al. 1996). To assess major gene activity, the likelihood of the distribution obtained in the normal (non-mixture) model was compared with the likelihood of the distribution obtained in normal mixture models with two underlying components, each corresponding to an underlying genotype. If the number of plants with a high concentration of virus was small in comparison with the number of plants with a low concentration of virus, the mixture model could not be applied. In such situations, individual plants were called susceptible, if the virus concentration of such a plant was higher than the lowest virus concentration estimated for rootlets of 'Regina'.

Progenies of crosses between WB42 and Holly-1-4 or R104 were analysed under three main hypotheses: the resistance genes in the two accessions studied (1) are allelic, (2) are closely linked (approximately 20 cM) and (3) are unlinked. Each main hypothesis was split into two sub-hypotheses: the inheritance of resistance in WB42 is conferred (A) by one dominant major gene or (B) by two unlinked dominant major genes (Table 1). Because of the presence of homozygously and heterozygously resistant plants of WB42, the genotype of F_1 plants may differ (see Table 1). As will

Table 1 Expected segregation ratios in progenies of crosses between pairs of the accessions of WB42, Holly-1-4 and R104. The ratios are calculated on the basis of six hypotheses, considering one major gene for resistance to BNYVV in Holly-1-4 and R104 (R_1), and one or two major genes in WB42 (R_2 and R_3), together with three possibilities for allelism or linkage

A) One major resistance gene in both accessions (R_1 and R_2)			B) One major resistance gene in one accession (R_1) and two unlinked major resistance genes in the other accession (R_2 and R_3)		
F_1	Expected segregation[a]		F_1	Expected segregation[a]	
	in F_2	in BC		in F_2	in BC
A1) R_1 and R_2 on the same locus			B1) R_1 and R_2 on the same locus		
R_1R_2	1 : 0	1 : 0	R_1R_2 R_3r_3	1 : 0	1 : 0
R_1r_2	0.75 : 0.25	0.50 : 0.50	R_1R_2 r_3r_3	1 : 0	1 : 0
			R_1r_2 R_3r_3	0.94 : 0.06	0.75 : 0.25
			R_1r_2 r_3r_3	0.75 : 0.25	0.50 : 0.50
A2) R_1 and R_2 on linked loci (20 cM)			B2) R_1 and R_2 on linked loci (20 cM)		
R_1r_1 R_2r_2	0.99 : 0.01	0.90 : 0.10	R_1r_1 R_2r_2 R_3r_3	1 : 0	0.95 : 0.05
R_1r_1 r_2r_2	0.75 : 0.25	0.50 : 0.50	R_1r_1 R_2r_2 r_3r_3	0.99 : 0.01	0.90 : 0.10
			R_1r_1 r_2r_2 R_3r_3	0.94 : 0.06	0.75 : 0.25
			R_1r_1 r_2r_2 r_3r_3	0.75 : 0.25	0.50 : 0.50
A3) R_1 and R_2 on unlinked loci			B3) R_1 and R_2 on unlinked loci		
R_1r_1 R_2r_2	0.94 : 0.06	0.75 : 0.25	R_1r_1 R_2r_2 R_3r_3	0.98 : 0.02	0.88 : 0.12
R_1r_1 r_2r_2	0.75 : 0.25	0.50 : 0.50	R_1r_1 R_2r_2 r_3r_3	0.94 : 0.06	0.75 : 0.25
			R_1r_1 r_2r_2 R_3r_3	0.94 : 0.06	0.75 : 0.25
			R_1r_1 r_2r_2 r_3r_3	0.75 : 0.25	0.50 : 0.50

[a] In case of distortion against genes or chromosomes of the subsp. *maritima* the percentage of resistant plants containing the resistance genes of R104 or WB42 will decrease

be explained later, the hypothesis of two unlinked complementary dominant genes in WB42, both required for resistance, could be left out. In the case of distortion of genes or chromosomes of the subsp. *maritima*, it is expected that a smaller percentage of resistant plants of the F_2 and BC families will contain the gene(s) for resistance of WB42 and R104. The χ^2 tests were carried out using a probability of 0.05.

Molecular analysis

Plant DNA was isolated following the procedure described by Van Der Beek et al. (1992) or using a modified procedure of Shure et al. (1983), in which about 1 g of ground leaf material is mixed with 1.5 ml 2× isolation buffer [0.6 M NaCl, 0.1 M TRIS, pH 7.5, 40 mM EDTA, 4% (w/v) Na-lauryl sarcosine, 1% (w/v) SDS], 1.5 ml 10 M urea and 150 µl phenol. After a thorough mixing with 3 ml phenol/chloroform, pH 8, the sample was centrifuged for 7 min at 3000 rpm. DNA was precipitated by adding isopropanol (0.7× the volume of the upper phase), washed with 70% ethanol and dissolved in TE (10 mM TRIS pH 8.0, 1 mM EDTA).

The development and application of STS markers has been described by Scholten et al. (1997). For the resistance gene of WB42 the random amplified polymorphic DNA (RAPD) marker OP-04 was converted into an STS marker, subsequently referred to as W, which previously was mapped at a distance of about 11 cM of a resistance gene. For Holly-1-4 the marker STS/OP-01, further referred to as H, was used. This marker was previously mapped at about 2 cM of *Rz*.

Results and discussion

Genetical analysis of resistance in crosses between WB42 and Holly-1-4

Segregation ratios of resistant and susceptible plants in BC and F_2 families resulting from crosses between WB42 and Holly-1-4 were obtained from four greenhouse experiments (Table 2). Average virus concentration of the resistant plants was slightly lower in Experiment 1 than in the other experiments. The control plants reacted as expected (data not shown). In all tests, the $\log_{10}$ virus concentration of the susceptible cultivar 'Regina' was well above 2.0 ng/ml. The $\log_{10}$ virus concentration of Holly-1-4 varied from 0.6 ng/ml, which is around the detection limit, to a maximum of 2.0 ng/ml. For WB42, 4 out of 80 plants were considered to be susceptible because they had a virus concentration comparable to that of 'Regina'. In the other WB42 plants the virus concentration was generally lower than in Holly-1-4. For the F_2(92.20) and BC(93.40) families the relative number of plants with high virus concentration was too low to fit mixtures of normal distributions to the data. Therefore, the segregation ratios in these families were based on the classification of plants as susceptible when the $\log_{10}$ virus concentration was higher than 2.0 ng/ml.

Table 2 Estimated mixture model parameters, based on $\log_{10}$ of the BNYVV concentration (in ng/ml) in rootlets of individual plants obtained after selfing F_1 plants of crosses between the resistant accessions WB42 and Holly-1-4, and plants obtained from crosses between F_1 plants and the susceptible accession MS-2

Plant materials[a]	*n*[b]	Observed segregation ratios R : S	95% confidence intervals[c]	Mean $\log_{10}$ virus concentration		SD
				Of resistant plants	Of susceptible plants	
Experiment 1						
F_2(92.29)	92	1 : 0		0.54		0.41
F_2(93.40)	32	1 : 0		0.74		0.30
F_2(93.41)	32	1 : 0		0.62		0.18
F_2(92.20)[d]	54	0.93 : 0.07		0.72	2.39	
F_2(93.42)	32	0.73 : 0.27	±0.14	0.67	1.80	0.34
F_2(93.43)	32	0.77 : 0.23	±0.12	1.27	2.00	0.28
Experiment 2						
BC(93.40)[d]	96	0.96 : 0.04		1.20	2.20	
BC(93.42)	96	0.54 : 0.46	±0.18	1.12	2.04	0.31
BC(93.43)	94	0.53 : 0.47	±0.16	1.53	2.37	0.20
Experiment 3						
F_2(92.20)	104	1 : 0		1.50		0.18
Experiment 4						
F_2(93.41)	60	1 : 0		1.37		0.21
F_2(92.20)	120	1 : 0		1.20		0.38
BC(93.40)[d]	96	0.90 : 0.10		1.37	2.20	

[a] ( ) = Identification number of the crosses. F_1 plants were selected among resistant F_1 families F_1(91.01), for the production of BC and F_2(93.42); F_1(91.10) for F_2(92.20) and BC and F_2(93.43); and F_1(91.37), for F_2(92.29), BC and F_2(93.40) and F_2(93.41)

[b] *n* = Number of plants

[c] The 95% confidence interval is based on the mean observed ratios ± 1.96 × standard error. If 0.75 fits within the 95% confidence interval for resistance the hypothesis is accepted that 75% of the plants can be resistant

[d] Due to the small number of susceptible plants the mixture models could not be applied. In that case SD varies for resistant and susceptible means

Studies by Scholten et al. (1996) resulted in the conclusion that resistance in WB42 may be conferred by one (or more) dominant major gene(s) or by two complementary unlinked dominant genes, both required for resistance. On the basis of the present results obtained for the F_2 families F_2(92.29), F_2(93.40) and F_2(93.41), in total 216 plants, the latter hypothesis could be rejected (χ^2 = 6.68 for the combined F_2 families). Additional evidence for the rejection of this hypothesis was derived from the results obtained from family BC(93.40), in which 7% of the plants appeared to be susceptible (expectation at least 25%, χ^2 = 32.11). The observed segregation ratios of the families BC and F_2(93.42) and BC and F_2(93.43) were of no use because they fit every possible hypothesis mentioned in Table 1.

The occurrence of the three resistant F_2 families also eliminated hypothesis A3 [Table 1, χ^2 = 9.96 for the combined F_2 families and χ^2 = 5.87 for F_2(92.29)]. Results obtained from family BC(93.40), which originated from the same F_1 family as the resistant F_2 families, also led to the rejection of hypothesis A3 (χ^2 = 32.11). In addition, the segregation data of family BC(93.40) led to rejection of three other hypotheses (Table 1): B3 (χ^2 = 4.03), A1 and B1. Thus, it was concluded that the resistance genes of WB42 and Holly are linked (hypotheses A2 and B2). Family F_2(92.20) was tested in three experiments (Table 2), and 4 out of 278 plants were classified as susceptible. This finding resulted in the rejection of hypothesis B2 (Table 1, genotype of F_1: R_1r_1 r_2r_2 R_3r_3, χ^2=9.92), and the acceptance of hypothesis A2 or B2 (genotype of F_1: R_1r_1 R_2r_2 or R_1r_1 R_2r_2 r_3r_3, χ^2 = 0.54). The possibility of R_1r_1 R_2r_2 R_3r_3 as the genotype of the F_1 parent was rejected as well.

Since BC(93.40) and F_2(92.20) are segregating, and their F_1 parents must have obtained at least one resistance gene derived from WB42 linked to R_1, these families are useful for estimating the distance between R_1 and R_2. Of the plants of family BC(93.40) 14 out of 192 were classified as susceptible. If the F_1 plant of BC(93.40) were genotypically R_1r_1 R_2r_2, the number of susceptible backcross plants represents half the number of the recombinant plants (= 0.5 × r), so that the distance between R_1 and R_2 can be estimated as 15 cM (r=2 × 14/192=0.15). With the χ^2 test the distance between R_1 and R_2 was determined to fall within an interval of 9–20 cM. Since the presence of an additional gene for resistance in WB42 could not be ruled out for BC(93.40), the genotype of the F_1 plant could also have been R_1r_1 R_2r_2 R_3r_3. The susceptible plants then represent 0.25 × r, leading to an estimated distance of about 29 cM between R_1 and R_2, which falls within an interval of 7–40 cM. For family F_2(92.20) 4 out of 278 plants were classified as susceptible. Since the hypothesis of an additional unlinked gene derived from WB42 had been rejected for this F_2 family, family F_2(92.20) originated from an F_1 plant with the genotype R_1r_1 R_2r_2. In that case the fraction of susceptible plants would have been 0.25 × r^2, meaning that the distance between R_1 and R_2 can be estimated to be 24 cM and falling within an interval of 17–38 cM.

In conclusion, an estimated distance of 17–20 cM between R_1 and R_2 explains all of the segregation ratios observed in these crosses. Thus, the resistance genes in WB42 and Holly-1-4 are closely linked, whereas the presence of an additional gene for resistance in WB42 could not be ruled out.

Genetical analysis of resistance in crosses between WB42 or Holly-1-4 and R104

Progenies of crosses between WB42 and R104 were tested in the greenhouse, and mixtures of normal distributions were fit to the virus concentrations to estimate the segregation ratios of resistant and susceptible plants in the BC families (Table 3). The level of infection was relatively high, and the lowest $\log_{10}$ virus concentration in rootlets of 'Regina' was 2.4 ng/ml. The $\log_{10}$ virus concentration in WB42 varied from 0.9 to 1.8 ng/ml, and all plants were considered to be resistant. The $\log_{10}$ virus concentration in the resistant accessions Holly-1-4 and R104 were slightly higher than in WB42 and varied from 1.0 to 2.1 ng/l. As expected, all F_1 families were completely resistant.

The observed segregation ratios of resistant and susceptible plants of the three BC families of crosses between WB42 and R104, tested in Experiment 5, fit the ratio 0.5 : 0.5. This result indicates that only the resistance gene of R104 could have been present in the F_1 plants used to produce the BC families. The same was true for the observed segregation ratios of BC families BC(96.82) and BC(96.94), tested in Experiment 6. However, in BC(96.106) (Experiment 6), most plants were resistant and contained $\log_{10}$ virus concentrations between 1.1 and 1.9 ng/ml. Three plants were identified with $\log_{10}$ virus concentrations as high as those in 'Regina'. The presence of these 3 plants indicates that the genes for resistance in WB42 and R104 are not alleles at the same locus, but that R_1 and R_2 are linked, and that the F_1(WB42 × R104) parent plant can be described as being R_1r_1 R_2r_2.

Although the number of plants tested is small, the number of susceptible plants was used to estimate the distance between the genes from WB42 and R104, which appeared to be 19 cM and falling within an interval of 13–44 cM. This is about the same as the estimated distance between the loci in WB42 and Holly-1-4. This finding indicates that the loci in R104 and Holly-1-4 are identical, which was already considered to be plausible on the basis of results of studies with STS markers (Scholten et al. 1997). The conclusion of identical loci was confirmed by the results of Experiment 7 (χ^2 = 0.00)

Genetical analysis of resistance using the STS markers W and H

In addition to the above results concerning the inheritance of resistance in WB42, the inheritance of the STS

Table 3 Estimated mixture model parameters based on $\log_{10}$ of BNYVV concentration (in ng/ml) in rootlets of individual plants obtained from crosses between the susceptible accession MS-2 and the F_1 of a cross between the resistant accessions WB42 and R104 (Experiments 5 and 6), or the resistant accessions Holly-1-4 and R104 (Experiment 7)

Plant materials[a]	*n*[b]	Observed segregation ratios R : S	95% confidence intervals[c]	Mean $\log_{10}$ virus concentration		SD
				Of resistant plants	Of susceptible plants	
Experiment 5						
BC(96.44)	104	0.60 : 0.40	0.12	1.73	2.44	0.18
BC(96.50)	104	0.44 : 0.56	0.14	1.77	2.56	0.14
BC(96.74)	104	0.46 : 0.54	0.14	1.70	2.51	0.17
Experiment 6						
BC(96.82)	32	0.44 : 0.56	0.24	1.69	2.22	0.16
BC(96.94)	32	0.41 : 0.59	0.24	1.60	2.31	0.19
BC(96.106)[d]	32	0.81 : 0.09		1.48	2.13	
Experiment 7						
BC(96.40)	103	1 : 0		1.40		0.21
BC (96.59)	104	1 : 0		1.49		0.27
BC(96.74)	104	1 : 0		1.53		0.23

[a] ( ) = Identification number of the crosses. F_1 plants were selected among the resistant F_1 families F_1(95.190), for the production of BC(96.44) and BC(96.82); F_1(95.191) for BC(96.50) and BC(96.94); F_1(95.192) for BC(96.74) and BC(96.106); F_1(95.177) for BC(96.40); and F_1(95.179) for BC(96.59) and BC(96.74)
[b] *n* = Number of plants
[c] The 95% confidence interval is based on the mean observed ratios ± 1.96 × standard error. If 0.75 fits within the 95% confidence interval for resistance the hypothesis is accepted that 75% of the plants can be resistant
[d] Due to the small number of susceptible plants the mixture models could not be applied. In that case SD varies for resistant and susceptible means

Table 4 The presence of markers W (linked to resistance in WB42) and H (linked to resistance in Holly-1-4) in resistant plants of resistant and segregating BC and F_2 families of crosses between WB42 and Holly-1-4. The hypothesis was tested, explaining that resistance in both Holly-1-4 (R_1) and in WB42 (R_2) is conferred by two separate major dominant genes which are closely linked

Plant materials[a]	*n*[b]	Presence of markers in resistant plants				Expected presence of markers in resistant plants				Hypothesis accepted (χ^2)[c]	
		HW	-W	H-	–	HW	-W	H-	–		
Only H present in F_1. Hypothesis of possible F_1 genotype: $R_1r_1\ r_2r_2$											
F_2(93.43)	22			22	0			21.99	0.01	Yes	(0.01)
BC(93.43)	41			39	2			40.18	0.82	Yes	(1.73)
Only H present in F_1. Hypothesis of possible F_1genotype: $R_1r_1\ R_2r_2$ or $R_1r_1\ r_2r_2\ R_3r_3$											
F_2(92.20)	68			47	21			51.00	17.00	Yes	(1.25)
H and W present in F_1. Hypothesis of possible F_1 genotype: $R_1r_1\ R_2r_2$, markers H and W linked (5 cM)											
F_2(93.40)	26	9	7	10	0	13.00	6.50	6.50	0.00	Yes	(3.15)
BC(93.40)	75	2	33	38	2	1.88	35.62	35.62	1.88	Yes	(0.37)
F_2(93.41)	29	14	8	7	0	14.50	7.25	7.25	0.00	Yes	(0.10)

[a] ( ) = Identification number of the crosses
[b] *n* = Number of resistant plants tested
[c] If the χ^2 is larger than 7.81 (for four classes of segregation, *df*=3) or 3.84 (for two classes of segregation, *df*=1) the probablity of the expected segregation ratio is smaller than 0.05

markers W and H, linked to resistance genes from WB42 and Holly-1-4, respectively, was analysed (Table 4). The segregating families BC and F_2(93.42) could not be used since both markers were neither amplified in resistant plants of these families nor in the original F_1 plant.

The segregation ratios of BC and F_2(93.43) (Table 2) indicated that in the F_1 parent only one resistance gene was present. Since the Holly-1-4 parent was homozygously resistant, this resistance gene must have been the Holly gene. The WB42 parent must have been heterozygously resistant, and the results regarding these families do not contribute to the segregation analysis. This was supported by the results of the STS markers, which demonstrated that only the marker linked to resistance in

Holly-1-4 was amplified in the F_1 parent and the segregating families (Table 4).

The segregation ratio in F_2(92.20) indicated that resistance in this family was conferred by two resistance genes, one originating from WB42 and the other from Holly-1-4. Therefore, amplification of both STS markers was expected. However, only marker H was amplified in the F_1 parent and in some plants of the F_2 family. The absence of marker W must have been the result of a recombination event between W and the R_2 gene. Thus, the F1 plant can only genotypically be described as R_1r_1 R_2r_2, where R_1 and R_2 are closely linked.

In the non-segregating families F_2(93.40) and F_2(93.41) and the segregating family BC(93.40), both STS markers were amplified (Table 4). If, in BC(93.40) the markers H and W would have been unlinked, equal segregation ratios of 25% would be expected for each of the groups HW, -W, H- and –. This hypothesis was firmly rejected (χ^2 = 60.57). The segregation ratio of the markers in the BC family then was used to estimate the distance between the two linked markers. Out of 75 resistant plants, 4 recombinant plants were identified, indicating that the estimated distance between W and H is about 5 cM, and fits within an interval of 2–17 cM. An estimation of 5 cM also fits to both F_2(93.40) (χ^2 = 3.15) and F_2(93.41) (χ^2 = 0.10) (Table 4). The result of this finding leads to the conclusion that W and H are closely linked.

Inheritance of resistance in WB42

In previous studies the number of genes conferring resistance in WB42 remained unclear (Whitney 1989, Scholten et al. 1996). Results of the present study indicate that resistance to BNYVV in WB42 is conferred by at least one dominant major gene. As the observed segregation ratios of F_2 and BC families including WB42 did not fit the expected ratios for a single resistance gene, the presence of a second resistance gene in some F_1 plants of WB42 could not be ruled out. For other F_1 plants, the presence of an additional resistance gene from WB42 could neither be confirmed nor excluded. Distortion of segregation of *maritima* chromosomes or genes was not observed in the so-called BC families obtained from crosses between WB42 and Holly-1-4 or R104. Also, the number of plants in which the STS marker of Holly was amplified, compared to the number of plants in which the marker of WB42 was amplified, did not differ significantly in the BC and F_2 families analysed. The difference in segregation in crosses between WB42 and Holly, compared to crosses between WB42 and susceptible plants of the subsp. *vulgaris* (Scholten et al. 1996), is not understood. Maybe one of the ancestors of Holly also belonged to subsp. *maritima*, resulting in a decreased distortion in crosses between WB42 and Holly compared to other plants belonging to subsp. *vulgaris*.

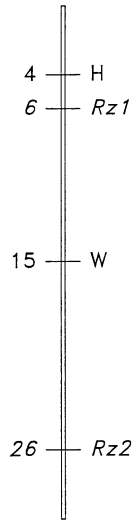

Fig. 1 Short-range map showing the position of the resistance genes of Holly-1-4 (*Rz1*) and WB42 (*Rz2*) and the markers *H* and *W*, originally identified as linked to these genes for resistance

Concluding remarks

Lewellen (1988) proposed using the name *Rz* to refer to resistance against BNYVV from Holly. For the resistance gene in WB42 the name *Rz2* is now proposed to emphasise the identification of a new gene in section *Beta* that confers resistance to BNYVV. Consequently, the gene *Rz* should be referred to as *Rz1*. In a previous study the markers H and W were mapped at 2 cM and 11 cM from the resistance genes in Holly-1-4 and WB42, respectively (Scholten et al. 1997). The present study demonstrated that a distance of about 20 cM between *Rz1* and *Rz2* is most likely, as this distance explains all the segregation ratios obtained in progenies of crosses between WB42 and Holly or R104. The markers H and W are linked with a distance of 2–17 cM. This information was used to construct a short-range map containing these four loci using the computer programme DRAWMAP (Van Ooijen 1994) (Fig. 1). The hypothetical distance of 20 cM between *Rz1* and *Rz2* fits very well with a distance of 11 cM between H and W.

Combining both genes in one cultivar will not be an easy task because of their tight linkage. Molecular markers flanking the resistance genes at short distances are needed to select plants which contain both resistance genes. Several markers linked to the Holly gene have already been identified (Barzen et al. 1992, 1997, Pelsy et al. 1995, Pelsy and Merdonoglu 1996, Scholten et al. 1995, 1997) and are now in use commercially. Such markers, as well as linkage maps, also have great potential for studying the phenomenon of possible distorted segregation in more detail. Another option in breeding sugar beet hybrids is the development of maternal and paternal families containing, *Rz1* and *Rz2*, respectively, in a homozygous state. The genes could then be combined after crossing the two families to produce a hybrid. Alternatively, the resistance genes of Holly and WB42 may be isolated and cloned, followed by transformation of plant material. Combining resistance from Holly and WB42 may not only result in a higher level of resistance

but also provide a more durable resistance against rhizomania.

Acknowledgements The authors thank Dr. Robert T. Lewellen for kindly sending seed of Holly, R104 and WB42. Dr. Rob W. Goldbach and Dr. Dick Peters (WAU Department of Virology) and Dr. (J.) Coosje Hoogendoorn (CPRO-DLO) are acknowledged for critically reading the manuscript. This work was supported by a PhD grant of the Dutch Ministry of Agriculture, Nature Management and Fisheries, and by the following breeding companies: Fa. A. Dieckmann-Heimburg Saatzucht Sülbeck, Germany; Maison Florimond Desprez Veuve & Fils, France; Hellenic Sugar Industry SA, Greece; KWS Kleinwanzlebener Saatzucht AG, Germany; Novartis Seeds AB, Sweden and Societa Produttori Sementi S.p.A., Italy.

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