GENETIC INVESTIGATION OF RESISTANCE TO BEET NECROTIC YELLOW VEIN VIRUS (BNYVV) IN *B. VULGARIS* SUBSP. *MARITIMA*, ACCESSION WB42.

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ABSTRACT

Management of rhizomania includes planting in cool soils, proper irrigation and soil fumigation with methyl bromide. However, breeding for resistance to BNYVV is the most critical aspect of rhizomania control. In this study, the inheritance of resistance to *beet necrotic yellow vein virus* (BNYVV) in *B. vulgaris* subsp. *maritima*, wild beet (WB) accession WB42 was investigated. Crosses between resistant source and susceptible parents were carried out and F_1, F_2 and BC_1 populations were obtained . Another source of resistance , *B. vulgaris* subsp. *vulgaris*, accession Holly-1-4, was also crossed with WB42 and population of F_2 was obtained. Virus concentrations in WB42 and its F_1, F_2 and BC_1 populations were investigated by DAS-ELISA in greenhouse conditions. Observed ratios of susceptible and resistant plants in F_2 and BC_1 populations of WB42 were confirmed 3:1 and 1:1 ratios, respectively and adjusted χ^2 test were not statistically significant($P \leq 0.05$). This result was in agreement with hypothesis of one dominant major gene. Segregation of plants in F_2 populations obtained from crosses between Holly-1-4 and WB42 revealed that the resistant genes in Holly-1-4 and WB42 were nonallelic and linked loci with approximately distance of 35 cM.

INTRODUCTION

Rhizomania disease of sugar beet is one of the most economical important diseases of sugar beet (Rush and Heidel, 1995; Izadpanah et al., 1996) and causes heavy losses of root yield and sugar content (Johansson, 1985). Breeding for resistance to BNYVV depends on availability of resistance sources (Johansson, 1985; Lewellen et al., 1987; Wisler and Duffus, 2000).

Sources of resistance to rhizomania were found in USDA breeding program, lines developed at Colorado state for resistance to Rhizoctonia root rot, lines from Alba germplasm, and Holly sugar company source (Lewellen et al., 1987). Resistance in Holly is simply inherited by a single dominant gene (Rz_1)(Lewellen

et al., 1987; Scholten et al., 1996). Resistance to BNYVV has been obtained in several wild beet (WB) accessions *B. vulgaris* subsp. *maritima*, (for example, WB42) originally collected from France, UK, Denmark and Italy (Whitney, 1989; Lewellen, 1995). Resistance in WB42 is inherited by another dominant gene (Lewellen, 1995; Francis, 1999). But, the number of genes conferring resistance remained unclear (Lewellen et al., 1987; Whitney, 1989; Scholten et al., 1996). Scholten et al. (1999) studied the progenies of crosses between Holly-1-4 and WB42. They concluded that 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.

The objective of the present study was the more precise investigation of the number of genes in WB42. The F_2 populations of crosses between WB42 with Holly-1-4 were also studied to determine the approximate distance of these resistance genes.

MATERIALS AND METHODS

Plants of the resistant wild beet accession WB42 were crossed in pairs with susceptible sugar beet germplasm 261 and annual beet accession of Sugar Beet Seed Institute (SBSI). Segretating F_2 and BC1 populations for WB42 were obtained by selfing resistant F_1 plants and crossing resistant F_1 plants with susceptible sugar beet germplasm 261 and annual beet accession. The Holly-1-4 was also hand-crossed in pairs with WB42 to obtain F1 plants. Selfing of F_1 plants produced F_2 seed.

A greenhouse test described by Paul et al. (1992), was used in the present study to discriminate between resistant and susceptible plants. Rootlets of individual plants were analysed for the virus by standard double- antibody sandwich ELISA (DAS-ELISA) as described by Clark and Adams (1977) using a commercial polyclonal antiserum and BNYVV-infected *N. clevelandii* leaf (Bioreba AG, Switzerland).

Absorbance reading of ELISA values were transformed to natural logs. After analysis of variance, the means were transformed back to the original scale. Analysis of variance (Proc GLM) and comparison of means were performed by SAS. Yates adjusted chi-square test was used to compare observed and expected ratios (Steel and Torrie, 1980).

RESULTS

Two F1 progenies of WB42 (85-71 and A2-72, Table 1) segregated into plants with low virus concentrations as found in the resistant parent and high virus concentrations as found in the susceptible parents, while the rest of related F1 progenies were classified as resistant. The adjusted χ^2 for the test of 1:1 ratio in these two F1 progenies were 0.000 and 0.083, respectively, which were not statistically significant. Therefore, based on the one gene hypothesis, the genotype of male parents of 85-71 and A2-72 F_1 families (plant numbers of 71 and 72 from WB42) were determined as Rz_2rz_2 and in the same way the genotype of male parents of 93-76 , 99-80 , A1-111 and A2-112 F_1 families

(plant numbers of 76, 80, 111 and 112 of WB42) were determined as Rz_2Rz_2 (Table 1).

The hypothesis that resistance of WB42 is based on one dominant major gene was accepted for all F_2 and BC_1 populations. So that, we observed the ratios of 3:1 and 1:1 in F_2 and BC_1 populations, respectively and the adjusted χ^2 tests were not statistically significant (Table 2).

Based on the one dominant major gene hypothesis, it was expected that the F_1 plants that obtained from crossing between Holly-1-4 and WB42 (plant number of 111 from WB42 as a male plant hand crossed with plant number of 110 from Holly-1-4 as a female plant and the resulting F_1 was selfed to produce F_2-HW-110G and F_2 – HW-110F populations , Table 2) had the genotypes of $Rz_1rz_1Rz_2rz_2$ (Rz_1rz_2/rz_1Rz_2) or Rz_1Rz_2. Because, the genotype of plant number of 110 from Holly-1-4 and plant number of 111 from WB42 (Table 2) were as Rz_1Rz_1 and Rz_2Rz_2, respectively. According to this expectation, the following genetical hypotheses were verified : The Rz_1 and Rz_2 are allelic and the Rz_1 and Rz_2 are nonallelic. If the Rz_1 and Rz_2 were allelic, then in F_2 populations the susceptible plants would not be observed. In F_2 – HW-110G and F_2– HW-110F populations, 6 and 2 plants were classified as susceptible, respectively. Therefore, 8 out of 257 plants (from combined F_2 populations) were susceptible. The adjusted χ^2 for test of 1:0 ratio was 56.468 and was significant ($P \leq 0.01$). Thus, it can be expected that the Rz_1 and Rz_2 are not allelic. Although the adjusted χ^2 for 15:1 ratio in F_2-HW-110G and F_2-HW- 110F were not significant (Table 2), this test for combined F_2 populations was statistically significant (χ^2_A=3.798, $P \leq 0.05$). This means that the distance between Rz_1 and Rz_2 could be lower than 50 cM. In F_2 populations, the fraction of susceptible plants would have been $0.25 \times r^2$. Thus, the distance between Rz_1 and Rz_2 was estimated as 35 cM. With adjusted χ^2 test the distance between Rz_1 and Rz_2 was estimated to fall within an interval of 22-45 cM.

CONCLUSIONS

Resistance to rhizomania in *B. vulgaris* subsp. *maritima*, accession WB42 is controlled by one dominant major gene as named by Rz_2. We did not observe any distortion of segregation, so that the hypothesis of one dominant major gene was accepted in all of the segregating populations of WB42 and in the progenies of crosses between Holly-1-4 with WB42. Perhaps, one reason for the lack of distorted segregation was due to larger number of plants (132 plants) that were tested in this study, whereas Scholten et al. (1996) had tested about 60 plants.

The WB42 gene is linked to another source of resistance to rhizomania, Holly gene (Rz_1). The approximate distance between Rz_1 and Rz_2 was estimated as 35 cM.

Table 1. DAS-ELISA absorbance reading means ($A_{405\,nm}$) of parents and F_1 populations in rootlets of individual plants (Duncan's multiple range test, separately)[a]

a Mean values followed by the same letter are not significantly different (P≤0.05)

b Number of plants

c Standard errors of means

Plant materials	Identification number of crosses	n[b]	Absorbance mean of resistant plants	n_R	Absorbance mean of susceptible plants	n_S	SE[c]
Parents			**Duncan grouping of parents**				
WB42		36	0.1906 b	32	0.3575 a	4	0.0138
261		26			0.3427 a		0.0167
Annual		17			0.3236 a		0.0241
Crosses with WB42			**Duncan grouping of F_1 populations**				
261×WB42	85-71	43	0.2120 b	22	0.3843 a	21	0.0182
261×WB42	93-76	31	0.1907 b				0.0100
261×WB42	99-80	26	0.1876 b				0.0092
Annual×WB42	A2-72	12	0.2255 b	7	0.4380 a	5	0.0362
Annual×WB42	A1-111	22	0.1955 b				0.0194
Annual×WB42	A2-112	17	0.1751 b				0.0273
Controls							
Regina root (infected)		47			0.5086		0.0200
BNYVV-infected *N. clevelandii* leaf		17			1.9806		0.0444
Regina root (Healthy)		54	0.1214				0.0080

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Table 2. Expected and observed ratios of R:S in segregating populations of WB42 and crosses between Holly-1-4 and WB42. The ratios were calculated on the basis of the one gene for each of resistance source (Rz_1 and Rz_2, respectively)

a n.s = non significant ($P \leq 0.05$)

b 111- WB42 plant (male parent) was hand- crossed with 110- Holly-1-4 plant (female parent) and F_1 was selfed to produce F_2-HW-110G and F_2 – HW-110 F populations.

c See text for more discription

Populations	N	Expected ratios of R:S	Observed ratios of R:S	Adjusted χ^{2} [a]	Accepted hypothesis
Populations of WB42					
F_2-93-76	170	3:1	0.694:0.306	2.541 [n.s]	One gene
F2-A2-111	167	3:1	0.725:0.275	0.449 [n.s]	One gene
BC_1-93-76	140	1:1	0.464:0.536	0.579 [n.s]	One gene
BC_1-85-71	59	1:1	0.627:0.373	3.322 [n.s]	One gene
Holly-1-4 with WB42 [b]					
F_2-HW-110G	171	15:1	0.965:0.035	1.750 [n.s]	Rz1 and Rz2 are nonallelic but linked loci [c]
F_2-HW-110F	86	15:1	0.977:0.023	1.640 [n.s]	

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