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A new RAPD marker for *beet necrotic yellow vein virus* resistance gene in *Beta vulgaris*

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Abstract

RAPD markers linked to *beet necrotic yellow vein virus* (BNYVV) resistance genes were identified in two *Beta vulgaris* accessions Holly-1-4 and WB42 using bulked segregant analysis. The polymorphism revealed by the RAPD markers in the F_2 generations of WB42 was higher than that of Holly-1-4. The segregation distortion at marker loci was slightly lower in the *B. vulgaris* × *B. maritima* cross than in the *B. vulgaris* × *B. vulgaris* cross. For Holly-1-4, a RAPD marker was identified in a long distance from the resistance gene of Rz_1. However, a RAPD marker tightly linked with Rz_2 gene in repulsion phase was detected with an approximate distance of 0.036 rf. This marker was not generation specific and showed high repeatability. The distance between Rz_1 and Rz_2 genes was estimated as 0.464 rf. After the Rz_2Rz_2 and Rz_2rz_2 genotypes were identified using ELISA values and repulsion phase RAPD markers, comparison of their ELISA means revealed lack of the gene dosage effects. Nevertheless, under the field or severe infection conditions, the difference between ELISA mean values of the Rz_2Rz_2 and Rz_2rz_2 genotypes might be more than that observed in this study and the gene dosage effects of Rz_2 allele might be important.

Additional key words: bulked segregant analysis, gene dosage effect, rhizomania, tagging.

Introduction

Rhizomania disease is one of the most economically important diseases of sugar beet (Rush and Heidel 1995, Lennefors *et al*. 2000) and causes heavy losses of root yield and sugar content (Johansson 1985). The disease is caused by *beet necrotic yellow vein virus* (BNYVV, genus *Benyvirus*), which is transmitted by the soil-borne plasmodiophoride-like fungus, *Polymyxa betae* Keskin (Martelli 1997, Wisler and Duffus 2000). Breeding for resistance to BNYVV is the only possible mean to confront the disease (Johansson 1985, Lewellen *et al*. 1987, Putz *et al*. 1990, Wisler and Duffus 2000). *Beta vulgaris* L. subsp. *vulgaris* var. *altissima* Doll. (accesion Holly-1-4) and *Beta vulgaris* L. subsp. *maritima* Arcang (accesion WB42) are the most attractive sources of resistance to rhizomania (Lewellen *et al*. 1987, Scholten *et al*. 1996, 1997, 1999). Resistance in Holly-1-4 is controlled by a single dominant gene (Rz_1) (Lewellen *et al*. 1987, Scholten *et al*. 1996, 1997, Barzen *et al*. 1997, Redfearn and Asher 1997). Some investigations showed that resistance in WB42 is controlled by another dominant gene (Rz_2) (Lewellen 1995, Scholten *et al*. 1997, Francis *et al*. 1998, Francis 1999). But, the exact number of resistance genes remained unclear (Lewellen *et al*. 1987, Whitney 1989, Scholten *et al*. 1996, 1997,

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Abbreviations: BSA - bulked segregant analysis; RAPD - random amplified polymorphic DNA, rf - recombination fraction.
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1999). However, Amiri *et al.* (2003a, 2003b) reported that the resistance to rhizomania in WB42 is controlled by one dominant gene.

Use of molecular markers such as RAPD in combination with bulked segregant analysis (Lawson *et al*. 1998, Kumar *et al*. 2006), and recently cloning and analysis of differential expression fragments (Gu *et al*. 2008) are common methods to study of resistance genes. The molecular marker gives better discrimination of resistant from susceptible plants than the ELISA, because, there are no disease escaped plants in marker tests (Redfearn and Asher 1997, Francis *et al*. 1998, Francis and Redfearn 1998, Scholten and Lange 2000). The molecular markers linked to the resistance gene (Rz_1) in Holly-1-4 have been identified (Merdinoglu *et al*. 1995, Pelsy and Merdinoglu 1996, Scholten *et al*. 1997, Barzen *et al*. 1992, 1997, Giorio *et al*. 1997, Francis *et al*. 1998, Francis 1999). But, for WB42, the RAPD markers have only been identified at a relatively large distance from the resistance gene of Rz_2 (Scholten *et al*. 1997).

In the present study, virus concentrations of individual plants of the F_2 generations of Holly-1-4 and WB42 accessions grown in the greenhouse were estimated using DAS-ELISA. Bulks of the most susceptible and resistant plants in the F_2 generations, were performed for identification of RAPD markers linked to rhizomania resistance genes in these accessions.

Materials and methods

Plant materials: This study was performed with the resistant sugar beet (*Beta vulgaris* L. subsp. *vulgaris* var. *altissima* Doll. accession Holly-1-4) and the resistant wild beet accession WB42 (*Beta vulgaris* L. subsp. *maritima* Arcang). Characteristics of both accessions were described by Scholten *et al*. (1996). Resistant F_1 families obtained after crossing of Holly-1-4 or WB42 accessions in pairs with plants of one susceptible sugar beet genotype 261 (which is a self-fertile genotype and segregates for nuclear male sterility A:aa) and one annual beet accession from Sugar Beet Seed Institute. Mapping F_2 generations were obtained by selfing resistant F_1 plants and were used to identify the RAPD marker(s) linked to the *Rz* resistance genes. All accessions were diploid with 2n=18.

Greenhouse tests and ELISA: A greenhouse test for screening sugar beet plants for resistance to BNYVV, described by Paul *et al*. (1992) and Amiri *et al*. (2003a), was used in the present study. Infested soil was collected from Shiraz, Iran, which contained the A type of BNYVV (Amiri *et al*. 2003a). *Beta vulgaris* cv. Regina (a susceptible cultivar from *Novartis Seeds AB*, Landskrona, Sweden) was used in all tests as negative or positive controls. Roots 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 *Nicotiana clevelandii* leaf (*Bioreba AG*, Reinach, Switzerland). Plants were considered susceptible if their samples showed an absorbtion value more than twice of the negative control (Amiri *et al*. 2003a).

DNA extraction and RAPD analysis: DNA was extracted from fresh or frozen leaves according to Van der Beek *et al*. (1992). Quality and quantity of genomic DNA were estimated using 0.8 % agarose gel electrophoresis and spectrophotometry (*Spectrolab-M350*, Newbury, UK), respectively. PCR reactions were performed in a total volume of 0.025 cm^3 containing 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.0025 cm^3 10× advanced biotechnology reaction buffer [750 mM Tris-HCL, pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.1 % *Tween 20*], 25 ng primer, 1 unit *Smartaq* polymerase (*Cinnagen*, Tehran, Iran) and 50 ng genomic DNA. DNA amplification was performed in a *Biometra T3* (*Whatman Company*, Göttingen, Germany) thermocycler. The PCR cycles were: 1 cycle of 5 min at 94 °C, followed by 40 cycles of 45 s at 94 °C, 45 s at 34.5 °C and 80 s at 72 °C, and finally 1 cycle of 10 min at 72 °C for final extension. The PCR products were separated by 1 or 1.2 % agarose gel electrophoresis with TAE buffer and stained with 1 μg cm^{-3} ethidium bromide solution.

RAPD markers linked to rhizomania resistance gene(s) were identified using bulked segregant analysis (Michelmore *et al*. 1991). Eight most susceptible and eight most resistant plants from the F_2 generations were used in each susceptible and resistant bulks based on their reactions. A total of 280 decamer primers (180 primers from *Advanced Biotechnology*, Surrey, UK, and 100 primers from *UBC kits*, Vancouver, Canada) were used to amplify DNA of the bulks. Primers detecting polymorphisms between the bulks were first tested on the individual plants of the each bulk and the parents. The RAPD markers showing linkage to resistance gene(s) based on individual plants of bulks were evaluated further on an additional number of individual plants from F_2 generation. The RAPD markers were named by the primer name which followed by size of the fragment, and c or r for coupling phase and repulsion phase markers, respectively. The sequence of RAPD primers which produced the nearest bands to the resistance genes is shown in Table 1.

Eventually, the selected repulsion phase RAPD marker was examined on a susceptible cultivar (Regina) and five resistant sugar beet cultivars: Dorothea

(a commercial hybrid from *Novartis Seeds AB*), 20506 O-type and 20507 male sterile lines, W114 and HM1990 (sugar beet diploid pollinators) of Sugar Beet Seed Institute.

Table 1. The sequence of primers which amplified the nearest bands to resistance genes.

Primer	Sequence	Primer	Sequence
AB1-4	5′GGACTGGAGT3′	AB6-15	5′AGTCGCCCTT3′
AB2-2	5′TGCCGGCTTC3′	AB9-3	5′AGCCAG GCTG3′
AB3-3	5′TCTCCGCTT G3′	UBC278	5′GACAACAGGA3′

Statistical analyses and mapping of the RAPD markers: The ELISA absorbance readings of most susceptible and resistant plants in each of the generations were compared using a completely random design with unequal replications. Normality of experimental errors was investigated by *Proc Univariate* and *Proc Capability* of *SAS* software (*SAS Institute*, Cary, NC, USA). Homogeneity of experimental errors was evaluated by Bartlett's test and correlation coefficients between mean and variance of treatments. Due to non-normality of data, heterogeneity of experimental errors, and correlation between mean and variance of treatments, the absorbance reading of the ELISA values were transformed to natural logs. After analysis of variance, the means were transformed back to the original scale. Yates adjusted χ^2-test (Steel and Torrie 1980) was used to compare observed and expected ratios in the F_2 generations. Yates has proposed this correction for continuity, to improve the approximation to the χ^2 distribution and thus be able to obtain a more exact probability value from the χ^2 table, applicable when the criterion has a single degree of freedom. This correction is intended to make the actual distribution of the criterion more nearly like the χ^2 distribution based on normal deviations (Steel and Torrie 1980). The distance between the markers and resistance gene(s) on the basis of recombination fraction (rf) was estimated by frequency of recombinant individuals (Paterson *et al*. 1991, Barzen *et al*. 1992, 1997) and linkage analysis was performed using the program *Mapmaker 3.0* (Lander *et al*. 1987). LOD score 3.0 with maximum distance of 40 cM were used as critical points for the analysis. The absorbance reading means for Rz_2Rz_2 and Rz_2rz_2 genotypes in the F_2 generation of WB42, which were identified by the repulsion phase RAPD markers, were compared using Student *t*-test and also completely random design with unequal replications. Analysis of variance (*Proc GLM*), comparison of means and *t*-test were performed by *SAS* software.

Results

Individual plants of two Holly-1-4 derived and one WB42 derived F_2 generations were classified as resistant and susceptible plants on the basis of DAS-ELISA values in the greenhouse test. Eight most resistant and eight most susceptible plants were selected according to virus concentrations in the roots for identification of the RAPD markers linked to the resistance gene(s).

DNAs of the most susceptible and the most resistant plants of each F_2 generation were mixed in equal ratio. Each set of the bulks were screened by 280 RAPD primers, which resulted in the amplification of more than 2700 DNA fragments per generation. Seven primers in two Holly-1-4 derived F_2 generations showed a polymorphic band between the bulks and the parents. In the WB42 derived F_2 generations, however, 25 primers amplified at least one RAPD marker either in a resistant or in a susceptible bulk and in one of the parents. The polymorphic primers for Holly-1-4 accession were tested in parents, F_1 and additional plants of F_2 generation. Only one RAPD marker [AB6-15-400c: 400 bp band in coupling (c) phase which was amplified by AB6-15 primer] was linked to the resistance gene (Rz_1) of Holly-1-4. Phenotype of this marker was determined in 80 plants (57 resistant and 23 susceptible) of the F_2

Table 2. Linkage analysis of Rz_2 locus and the RAPD markers, which identified by BSA technique, in the F_2 generation of WB42 by *Mapmaker 3.0* software (c and r refers to the coupling and repulsion phase RAPD markers, respectively; the markers with segregation distortion are underlined, because their adjusted χ^2 tests for the 3:1 ratio were significant at $P \leq 0.01$).

Linkage group	Marker or locus
1(chromosome 3)	AB2-2-1800r, AB3-3-710r, AB9-3-800r, AB1-4-680r AB1-4-710c, AB1-4-1250c, AB9-18-2300c, UBC278-1340c, Rz_2
2	AB9-3-560r, UBC238-850r, AM2-350c, AB3-3-580c
3	AB6-7-570r, AB2-16-570r, AB9-2-1700c
4	UBC238-450c, AB9-18-400c, AB2-16-1300c, AB5-9-1540c
Not linked	AM2-1650r, AM2950r, AB9-18-550r, AB2-16-350r, UBC278-680r AB3-4-1150r, AB9-3-1140c, AB6-7-1000c, AB5-9-840c, AB5-9-1130c, AB3-14-1200c, AB3-4-960c

Table 3. The recombination frequency of the markers in susceptible or resistant plants [%] and distances [rf] between Rz_2 locus and the RAPD markers on chromosome 3 (the markers were selected from Table 2; c and r refers to the coupling and repulsion phase RAPD markers, respectively).

	AB2-2 1800r	AB3-3 710r	AB9-3 800r	AB1-4 680r	UBC287 1340c	AB9-18 2300c	AB1-4 1250c	AB1-4 710c
Susceptible	5.8	13.5	19.2	23.1	47.2	56.5	61.5	55.8
Resistant	-	-	-	-	15.8	22.2	9.5	21.6
Distance	0.036	0.114	0.159	0.19	0.311	≥0.50	≥0.50	≥0.50

Table 4. DAS-ELISA absorbance reading means (A_{405}) of Rz_2Rz_2 and Rz_2rz_2 genotypes in the greenhouse, which were identified by the four repulsion phase RAPD markers in the F_2 generation of WB42. Means followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$; n_1 - number of Rz_2Rz_2 genotypes, n_2 - number of Rz_2rz_2 genotypes, n.s and ∗ - not significant and significant at 0.05 probability level determined by Student *t*-test, respectively.

Marker	Rz_2Rz_2	n_1	Rz_2rz_2	n_2	*t*-test
AB2-2-1800r	0.13149 abc	37	0.15017 a	77	1.5100 n.s
AB3-3-710r	0.10736 bc	25	0.14231 ab	46	2.3878 ∗
AB9-3-800r	0.10386 c	25	0.13937 ab	49	2.6102 ∗
AB1-4-680r	0.10888 bc	25	0.13752 ab	49	2.0774 ∗

generation. This fragment was not amplified in 12 resistant plants, whereas 8 susceptible plants showed the marker. On the basis of these data, the distance between the marker and Rz_1 was estimated to be 0.25 rf (20/80 = 0.25) by the frequency of recombinant individuals (Paterson *et al*. 1991, Barzen *et al*. 1992, 1997). Linkage analysis using *Mapmaker 3.0* software estimated the distance between the marker and the resistance gene as

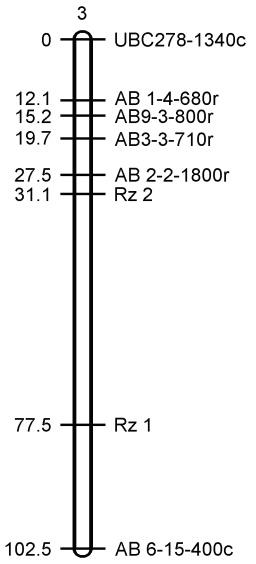

Fig. 1. Short map showing the position of the RAPD markers linked to the resistance loci in segregating generations of Holly-1-4 and WB42 (chromosome 3). The distances were estimated by *Mapmaker 3.0* software and frequency of recombinant individuals, with the exception of the repulsion phase RAPD markers for which the distances were estimated by the latter method. See text for more description.

35.5 cM. AB6-15-400c marker was also evaluated in WB42, 261, F_1, eight most resistant and eight most susceptible plants of the F_2 generation from 261 × WB42 cross. The marker was not shown in any of the 19 DNA samples. Thus, the right order of the marker and the resistance genes of WB42 and Holly-1-4 was found to be Rz_2, Rz_1 and AB6-15-400c marker. Segregation distortion test for AB6-15-400c marker fitted the data into a 3:1 ratio in F_2 generation ($\chi^2_A = 2.817$, $n = 80$, df = 1), indicating no segregation distortion for the marker.

Evaluation of WB42, 261, F_1 and additional plants of F_2 generation using 25 polymorphic primers for Rz_2 revealed 33 informative RAPD markers for rhizomania, which were amplified using 14 primers. At first, the informative markers were assigned to different linkage groups using *Mapmaker 3.0*. The markers were classified into four linkage groups and one unlinked markers group (Table 2). Approximately, 21 % of the markers (7 out of 33 markers) showed segregation distortion ($P \leq 0.01$). Group I (or chromosome 3) contained four RAPD markers in coupling phase, four RAPD markers in repulsion phase and Rz_2 locus. Distances of Rz_2 locus and the markers in group I were estimated by the frequency of recombinant individuals (Paterson *et al*. 1991, Barzen *et al*. 1992, 1997) (Table 3). The resistant plants without the marker and the susceptible plants having the markers were considered as the recombinant individuals in coupling phase. The number of recombinant plants in repulsion phase was taken as twice of the recombinant susceptible individuals. Distances estimated by *Mapmaker 3.0* for c-markers were in agreement with those obtained by the frequency of recombinant individuals. Therefore, the distance of UBC278-1340c marker was 0.32 rf and the distances of the other coupling

phase markers were more than 0.50 rf (no linkage). For repulsion phase (r) markers, however, only the order of the markers was similar to the results of the frequency of recombinant individuals. The distance between AB2-2-1800r and Rz_2 was about 37.4 cM and the distance of the other r-markers were more than 50 cM.

The polymorphic markers for Rz_2 were examined in Holly-1-4, 261, annual beet, F_1 generations and F_2 individual plants derived from the annual × Holly-1-4 and 261 × Holly-1-4 crosses. The results revealed no linkage between these markers and the resistance gene in Holly-1-4. Therefore, these markers were not located between Rz_1 and Rz_2. The distance of the nearest marker from Rz_2 was 0.036 rf (Table 3). Regarding the linkage of the resistance loci in WB42 and Holly-1-4 (Scholten *et al*. 1999, Amiri *et al*. 2003a, 2003b), the approximate distance of Rz_2 and Rz_1 were estimated to be 0.464 rf. On this basis, the RAPD markers linked to the resistance gene(s) in WB42 and Holly-1-4 were used to construct a short map around the resistance loci (Fig. 1). Eventually, AB2-2-1800r marker was tested on a susceptible (cv. Regina) and five resistant genotypes. This marker was observed in all of the tested plants.

The four nearest RAPD markers to Rz_2 were in repulsion phase (Table 3). Thus, using these markers and DAS-ELISA absorbance reading, it was possible to distinguish Rz_2rz_2 from Rz_2Rz_2 genotypes (Table 4). Absorbance reading means of Rz_2rz_2 and Rz_2Rz_2 genotypes were statistically different only for AB9-3-800r marker as revealed by Duncan's multiple range test, but not for the other markers. Nonetheless, absorbance reading means of these genotypes were only statistically different for AB2-2-1800r marker as shown by *t*-test. The classification precision of the plants obtained based on AB2-2-1800r marker was more than the three other markers, since the distance of this marker from Rz_2 gene was smaller than the others. The results indicated no gene dosage effects *i.e*. complete dominance under the conditions of the study. The ratio of Rz_2rz_2 plants : Rz_2Rz_2 plants identified by the four r-markers was found to be 2:1 as revealed by adjusted χ^2-test. The adjusted χ^2 values for the AB2-2-1800r, AB3-3-710r, AB9-3-800r and AB1-4-680r markers were 0.007, 0.041, 0.000 and 0.000, respectively, which were not statistically significant. Therefore, the observed ratios corresponded to the expected 2:1 ratio.

Discussion

Scholten *et al*. (1996) reported segregation distortion for rhizomania resistance genes of *maritima* in crosses between WB42 and susceptible plants of the subsp. *vulgaris*. However, this was not observed in our study, implying that the hypothesis of one dominant major gene is acceptable in the segregating generation of WB42. On the other hand, the segregation distortion for RAPD markers in the F_2 generation of WB42 (*vulgaris* × *maritima* cross) was about 21 %, *i.e.*, slightly lower than that of the *vulgaris* × *vulgaris* cross as reported by Uphoff and Wricke (1995). This may be explained by the selective elimination of male gametes as a result of pollen sterility or incompatibility (Wagner *et al*. 1992, Scholten *et al*. 1996, Subudhi and Huang 2002). Zygotic selection could be another explanation due to differences in fitness between the zygotes (Wagner *et al*. 1992) and selective elimination of female gametes (Subudhi and Huang 2002). It seems that the genetic background of subsp. *maritima* does not influence the amount of the segregation distortion in *vulgari* × *maritima* cross. Our results, therefore, were different from those of Scholten *et al*. (1996). One possible reason for the lack of segregation distortion for resistance to rhizomania in the present study was the larger number of plants tested as compared with Scholten *et al*. (1996).

The number of the polymorphic bands between the bulks in the F_2 generation of WB42 was higher than that in the F_2 generations of Holly-1-4. This may be related to the higher level of polymorphism in subsp. *maritima* than subsp. *vulgaris* (Lewellen *et al*. 1987, Doney and Whitney 1990, Desplanque *et al*. 1999, Biancardi *et al*. 2002).

Hackett and Broadfoot (2003) stated that the segregation of the markers with a ratio expected for a generation type is one of the ideal conditions of molecular markers data for linkage mapping. However, less attention has been paid to the effects of distorted segregation ratios on map order and map length (Hackett and Broadfoot 2003). The study of segregation distortion will have practical implications in breeding effort, since the genes closer to the genes with distorted segregation on the chromosome will be less heritable hence limiting the recombination of characters (Subudhi and Huang 2002). Thus, it is expected that the segregation distortion is confounded with the physical linkage between two markers or genes, because the linkage maps are constructed on the basis of the Mendelian expected ratios in the generation being considered (Liu 1998). Out of 33 markers in the F_2 generation of WB42, only 7 markers showed distorted segregation. However, the skewed markers did not affect the results of the study. Our objective was to identify the RAPD markers on the chromosome 3. None of the markers on this chromosome showed distorted segregation (linkage group I, Table 2). This was in agreement with the reports of lethal genes on the sugar beet chromosomes. Lethal genes were only reported on chromosomes 1, 2 and 5 in sugar beet (Schondelmaier and Jung 1997, Francis and Asher 2000).

Nevertheless, we constructed the final map without skewed markers by *Mapmaker 3.0*. Clearly, there was no change in linkage groups. On the other hand, we used two methods to estimate the marker distances. The distances of the c-markers, which were obtained by *Mapmaker 3.0* (cM) were slightly higher than those of the frequency of recombinant individuals (rf). Mapping was performed using Haldane's mapping function in which there is supposedly no interference (Lander *et al*. 1987, Liu 1998). In this condition, some of the parental gametes would be regarded as recombinant gametes due to the presence of double crossing-over. Thus, the estimation of the distances would slightly be more than that obtained from the frequency of recombinant individuals since the latter method does not consider double crossing-over. Moreover, as the distances of the markers increase, the difference of the distances estimated by the two methods will also increase since more crossing-over will occur in longer distances. In the present study the difference of the estimated distances by the two methods was high for r-markers.

Mapmaker 3.0 uses maximum likelihood function for the estimation of the distances (Lander *et al*. 1987). In the F_2 generation, recombination calculation using dominant markers such as RAPD (Narain 1990) in the r-markers is only impressed from the frequency of the fourth phenotypic group (susceptible plants in this study which did not have the r-marker). Thus, the estimation of the recombination frequency by maximum likelihood function would be biased and the amount of the bias will increase with decreasing of the frequency of the fourth phenotypic group (Liu 1998). Therefore, it can be concluded that the estimations obtained by the software were biased. In this regard, we only estimated the distances between the r-markers and Rz_2 locus by the frequency of the recombinant individuals (Fig. 1).

Although, Scholten *et al*. (1999) obtained three distance maxima of 20, 40, and 38 cM between Rz_1 and Rz_2 from two different generations (20 and 40 cM from a backcross generation and 38 cM from an F_2 generation). In some generations not included in the results and conclusions of the study, Rz_1 and Rz_2 genes were unlinked. Amiri *et al*. (2003a) estimated a distance maximum of 45 cM for the interval of Rz_1 and Rz_2 genes in an F_2 generation. In the study of Scholten *et al*. (1999), the average number of tested plants in two related generations was about 235, whereas Amiri *et al*. (2003a) used 257 plants. In the present study, we estimated the distance of Rz_1 and Rz_2 genes by RAPD markers independently from the greenhouse data used by Amiri *et al*. (2003a). The distance between WB42 and Holly-1-4 STS markers and the resistance genes (Rz_1 and Rz_2) was already obtained in 60 plants (30 resistant and 30 susceptible plants) (Scholten *et al*. 1997), whereas the distance between Rz_2 and AB2-2-1800r marker in the current study was estimated using 166 plants. Thus, we tested more plants than the previous studies (Scholten *et al*. 1997, 1999).

The amplification of AB2-2-1800r marker in cv. Regina indicated that this marker was not generation-specific and could be used in all generations. It was known that 20506 O-type line, 20507 male-sterile line, W114 and HM1990 diploid pollinators contained dominant homozygote genotypes for resistance to rhizomania. Therefore, the presence of the related marker in these genotypes indicated that Rz_2 locus did not contribute to the resistance of those lines. However, resistance to rhizomania in Dorothea was probably in heterozygote condition. Thus, it is concluded that the Rz_2 locus neither contributed to the resistance of this line nor it was in heterozygote state (Rz_2rz_2).

No gene dosage effect, *i.e*., complete dominance was observed under the conditions of this study, which is in agreement with the previous studies (Scholten *et al*. 1996, Amiri *et al*. 2003a). However, in this study the difference between Rz_2Rz_2 and Rz_2rz_2 genotypes from the view of ELISA values was higher than that reported by Amiri *et al*. (2003a) who used WB42 and the respective F_1 families (the conditions of both studies were identical). However, we can not rule out the lack of gene dosage effects for Rz_2 allele, because of the higher differences of ELISA values of the genotypes in the field trial as compared to the greenhouse tests (Amiri *et al*. 2003a), and the lower ELISA values of the root samples in this study compared with the results of Scholten *et al*. (1996, 1999) and Paul *et al*. (1992). Thus, it is likely to observe the gene dosage effects, *i.e*., incomplete dominance of the Rz_2 allele in the field or under severe infection conditions. On the other hand, the confirmation of the 2:1 ratio between Rz_2rz_2 and Rz_2Rz_2 plants while being in agreement with the hypothesis of one dominant major gene in WB42 proved the reliability of the r-markers.

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The crucial effect of soil environment on plant growth is generally well recognized but our understanding of all contributing parts is still not satisfactory. Soil compartment is of very complex nature, with plethora of highly variable physico-chemical processes and biotic interactions. Exact study of root responses to separate abiotic and biotic components of the soil environment is extremely difficult and reliable data are, therefore, rather scarce. The main aim of the reviewed book is to provide a comprehensive overview of recent advances in this problematic.

The first three chapters are devoted to root exudates. Main types of organic compounds released by plant roots are listed, together with their biological activity and possible roles in mineral nutrient acquisition or protection against pathogens. Quite useful is description of different techniques used for collection of root exudates in solution culture and in soil systems. Mechanisms of root exudation are clearly explained at cellular level. Dependence of root exudation rate on physiological status of a plant and on soil conditions (*e.g.*, nutrient deficiency, strong acidity, aluminium toxicity) is also treated.

The following block of four chapters is focused on transformations of mineral nutrients in the rhizosphere and their uptake by roots. Special attention is devoted to nitrogen and phosphorus dynamics, and to extracellular enzymatic activity. New methodical approaches to the study of soil transformation processes are discussed. Functions of special iron chelating agents (siderophores) secreted by microorganisms and grass roots in iron-limiting growth conditions are treated in detail. Very interesting are also the two chapters newly added to this second edition, discussing the mechanisms by which soil nutrient availability may modify root morphology and architecture, and also detail description of membrane processes in roots relevant to nutrient acquisition.

Direct interactions of plant roots with nodulating bacteria, mycorrhizal and pathogenic fungi are treated in three separate chapters. Much attention is devoted to the recent considerable advances in our understanding resulting from extensive application of molecular methods. This has deeply modified our view on signalling mechanisms of interacting roots and fungi or bacteria, their genetic background and evolution. Practical applications of such research in biocontrol of crop plants could be undoubtedly possible and very helpful.

The following topics are of more synthetic character, presenting a general overview of chemical communication in the rhizosphere, approaches to the mathematical modelling of soil processes, carbon flow in soil systems, and microbial population dynamics. The last contribution is devoted to mechanisms of natural gene transfer among soil microorganisms.

All chapters of this nicely printed and bound book are arranged in a similar form of concise review articles with many figures and abundant list of references. The book will be undoubtedly of continuous interest to plant scientists, soil biologists and practising plant ecologists as an invaluable source of basic information. It might be certainly useful to students and teachers of biological disciplines as an advanced textbook.

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