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Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2)

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Abstract

Barley Yellow Mosaic Virus disease caused by different strains of BaYMV and BaMMV is a major threat to winter barley cultivation in Europe. Pyramiding of resistance genes may be considered as a promising strategy to avoid the selection of new virus strains and to create more durable resistances. However, this goal cannot be achieved by phenotypic selection due to the lack of differentiating virus strains. For pyramiding of resistance genes *rym4*, *rym5*, *rym9* and *rym11*, located on chromosomes 3H and 4H of barley two different strategies have been developed. These strategies are based on doubled haploid lines (DHs) and marker assisted selection procedures. On the one hand F_1 derived DH-plants of single crosses were screened by molecular markers for genotypes being homozygous recessive for both resistance genes. These genotypes were crossed to lines carrying one resistance gene in common and an additional third gene, leading to a DH-population of which 25% carry three resistance genes, 50% have two resistance genes and 25% possess a single resistance gene homozygous recessively. Alternatively, F_1 plants having one resistance gene in common were directly inter-crossed [e.g. (*rym4* × *rym9*) × (*rym4* × *rym11*)] and about 100 seeds were produced per combination. Within these complex cross progenies plants were identified by markers being homozygous at the common resistance locus and heterozygous at the others. From such plants, theoretically present at a frequency of 6.25%, DH-lines were produced, which were screened for the presence of genotypes carrying three or two recessive resistance genes in a homozygous state. Besides DH-plants carrying all possible two-gene combinations, 20 DH-plants out of 107 analysed carrying *rym4*, *rym9*, and *rym11* and 27 out of 187 tested carrying *rym5*, *rym9*, and *rym11* homozygously have been detected using the second strategy which is faster but needs co-dominant markers, because in contrast to the first strategy marker selection is carried out on heterozygous genotypes.

Introduction

As growing of resistant cultivars is the only way to combat soil-borne Barley Yellow Mosaic Virus disease, breeding barley for resistance to this disease is an ongoing program in Europe. In Germany the disease is caused by a complex of at least three viruses, i.e., *Barley Mild Mosaic Virus*

(BaMMV), *Barley Yellow Mosaic Virus* (BaYMV) and BaYMV-2 (Huth 1989; Huth and Adams 1990). Resistance to BaMMV and BaYMV in Europe has for a long time been exclusively based on the recessive resistance gene *rym4*, but during the last few years an increasing number of cultivars carrying *rym5* derived from the Chinese landrace 'Mokusekko 3'(Konishi et al. 1997; Graner et al. 1999), e.g. 'Tokyo' or 'Kamoto' (Friedt et al. 2000) have been released being also resistant to BaYMV-2. Besides these two genes, a number of different recessive resistance genes have been detected within the barley gene pool and were assigned to chromosomes (cf. Graner et al. 2000; Ruge et al. 2003; Werner et al. 2003; Le Gouis et al. 2004; Nissan-Azzous et al. 2005). These genes are well suited to broaden the genetic base of resistance in European barley breeding, which is an important task due to the risk of selection of new virus strains. Regarding the present situation in Japan and Europe this risk becomes obvious. In Japan seven strains of BaYMV and two of BaMMV have been described (Nomura et al. 1996) and in France variants of BaYMV and BaMMV have been reported, which are able to overcome several of the resistance genes known so far (Hariri et al. 2000; Hariri et al. 2003; Kanyuka et al. 2004). Besides the potential risk of a resistance breakdown, the durability of disease resistance is of special importance due to the economical importance of resistant winter barley cultivars. A potential strategy to create more durable and broad-spectrum resistances is the pyramiding of resistance genes (Liu et al. 2000), i.e. the combination of different resistance genes against the same pathogen in one breeding line or cultivar.

Pyramiding of resistance genes has been carried out in several cereal host-pathogen-systems already, e.g. *Hordeum vulgare* ssp. *vulgare* – *Rhynchosporium secalis* (Brown et al. 1996), *Triticum aestivum* L. – *Mayetiola destructor* (Dweikat et al. 1997), – *Puccinia recondita* f.sp. *tritici* (Kloppers and Pretorius 1997), – *Blumeria graminis* (Liu et al. 2000) and *Oryza sativa* L. (rice) – *Xanthomonas oryzae* pv. *oryzae* (Huang et al. 1997, Singh et al. 2001), and – *Pyricularia oryza* Cav. (Hittalmani et al. 2000). In most cases homozygous double and triple resistance gene combinations have been achieved, which led in most cases to a broader resistance spectrum. Studies on pyramiding virus resistances have been carried out in *Phaseolus vulgaris* combining several genes conferring resistance against *Bean Common Mosaic Virus* (BCMV, Kelly et al. 1995) as well as in *Capsicum anuum* concerning resistance against *Pepper Veinal Mottle Virus* (PVMV, Caranta et al. 1996). The latter identified a doubled haploid line completely resistant against all virus strains due to the complementary action of genes originating from the different parental lines.

Concerning the Barley Yellow Mosaic Virus complex, gene pyramiding cannot be achieved by phenotypic selection due to the lack of differentiating virus strains. Therefore, the availability of markers facilitating an easy and reliable detection of respective alleles is an essential prerequisite. The present work focused on combining resistance genes *rym4*, *rym5*, *rym9* and *rym11* using different strategies. While *rym4* and *rym5* map to the same marker interval in the telomeric region of chromosome 3HL and are allelic regarding resistance to BaMMV (Graner et al. 1999), *rym9* is located in the telomeric and *rym11* in the centromeric region of chromosome 4HL (Bauer et al. 1997; Nissan-Azzouz et al. 2005). Combination of resistance genes was based on strategies relying on doubled haploid (DH) lines, as homozygous recessive genotypes are more frequent in DH-populations than in segregating F_2 populations. The aim of this study was on the one hand to combine resistance genes with different specificities in order to facilitate their use in barley breeding thereby broadening the genetic base of resistance and on the other hand to prove that marker based strategies of pyramiding are effective, opening new opportunities in breeding barley for resistance to the Barley Yellow Mosaic Virus complex.

Materials and methods

Plant material

German resistant cultivars (*rym4*), with the exception of cv. 'Tokyo' carrying *rym5*, as well as lines formerly used for mapping *rym5*, *rym9* and *rym11* (Bauer et al. 1997, Graner et al. 1999) were used for pyramiding (Table 1). The breeding lines, i.e. DHs (W548, W549, W550, W551, W575) and segmental recombinant inbred lines (1132, 1292, 1289) were derived from crosses of the respective resistance donor, i.e. W122/× (*rym*5), 'Bulgarian 347' (*rym9*),

Table 1. Resistant genotypes (cultivars/lines) used for pyramiding; their pedigree, resistance genes and linked molecular markers.

Resistance gene, chromosome	Cultivars/Lines	Number of lines	Cross*	Marker	References
rym4, 3HL	'Express'			BMac029	1
	'Krimhild'			OP-Z04H660	2
	'Labea'			OP-Z04A	3
	'Nixe'			OP-L14H910	4
rym5, 3HL	'W548'[a]	1	'**W122/35.2**' × 'Harmonika'	BMac029	1
	'W549'[a]	1	'**W122/35.2**' × 'Cosima'	OP-020H950	1
	'W550'[a]	1	'**W122/37.1**' × 'Alraune'	OP-C13H590	1
	'W551'[a]	2	'**W122/37.1**' × 'Posaune'	OP-Y14H660	1
	'W575'[a]	3	'**W122/54.2**' × 'Interbell'	OP-J06H1050	1
	'Tokyo'			OP-C14H950	1
rym9, 4HL	'1132'[b]	11	'**Bulgarian 347**' × 'Alraune'	HVM67	5
				STS-C04H910	5
				OP-C04H910	6
				OP-M04H610	6
rym11, 4HL	'1292'[b]	5	('**Russia 57**' × 'Alraune') × 'Alraune'	HVM03	7
				OP-G06H550	7
	'1289'[b]	2	'**Russia 57**' × 'Magie'	OP-F01H385	7
				OP-A04H450	7

*Resistance donor printed in bold; [a]DH-lines, [b]segmental inbred lines; [1]Graner et al. (1999), [2]Ordon et al. (1995), [3]Schiemann et al. (1997), [4]Weyen et al. 1996, [5]Werner et al. (2000), [6]Schiemann et al. (1998), [7] Bauer et al. (1997).

'Russia 57' (*rym11*) to susceptible German cultivars. For each of the four resistance genes closely linked PCR-based markers (RAPDs, STSs, SSRs) are available (Ordon et al. 1995; Bauer et al. 1997; Graner et al. 1999; Werner et al. 2000, Table 1).

Production of doubled haploid plants

Production of doubled haploid lines (DHs) from respective F_1-plants was carried out at the Resistenz Labor der Saatenunion GmbH, Hovedissen (Dr. Jens Weyen) and Pajbjergfonden, Odder, Denmark (Dr. Heidi Jaiser).

Resistance monitoring

For monitoring of the resistance reaction, DH-lines derived from pyramiding were grown in a field at Giessen, Hesse (Germany), infested with BaMMV and BaYMV. Resistance was scored in 2001 by visual assessment and accordingly in 2002 for lines from pyramiding strategy II by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA, Clark and Adams 1976) using specific antisera against BaMMV and BaYMV (kindly provided by Dr. Frank Rabenstein, Federal Centre for Breeding Research on Cultivated Plants (BAZ), Aschersleben, Germany). Optical density was estimated photometrical at a measurement wavelength of 405 nm and a reference wavelength of 620 nm (Easy Reader 400 ATX, SLT-Lab Instruments, Crailsheim, Germany).

Molecular analysis

PCR for RAPD primers given in Table 1 was carried out in a reaction volume of 25 μl consisting of 25 ng genomic DNA, 0.4 mM dNTP; 6 mM $MgCl_2$, 0.3 μM primer and 1.5 U AmpliTaq DNA-polymerase Stoffel Fragment (Perkin Elmer, Norwalk, CT, USA) and its corresponding reaction buffer (cf. Ordon et al. 1995) using the following temperature profile: an initial denaturation step of 4 min at 94 °C was followed by 45 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C. The heating rate was fixed at 5 °C/min from annealing to extension and the polymerisation step (72 °C) was extended for 3 s/cycle at 72 °C. PCR products were separated by electrophoresis in 2% agarose gels and visualized by ethidium bromide staining and UV illumination (286 nm). SSRs HVM67 (*rym*9) and HVM03 (*rym11*) were amplified according to Liu et al. (1996) and Ramsay et al. (2000). Bmac029 (*rym4*, *rym5*) was amplified according to Graner et al. (1999). PCR-products were separated on an 8%

Long Ranger Gel solution (FMC Biozym, Hessisch Oldendorf, Germany) and detected fluorescence labelled on a LI-COR DNA Sequencer Genereadir 4200 (MWG Biotech AG, Ebersberg, Germany).

Results

In a first step, all cultivars and lines serving as parents for the pyramiding program were screened with the above mentioned markers (Table 1) in order to prove purity of seeds and to gain information about the suitability of respective markers for pyramiding, i.e. their polymorphism between parental lines. With the exception of OP-Y14H660 all markers turned out to be polymorphic in the combinations needed for pyramiding. Besides this, it was proven by these flanking markers that the lines used for pyramiding did not carry recombinations between marker loci and the respective resistance locus (data not shown).

In a next step single crosses between these lines were performed to combine respective genes, i.e., *rym4* × *rym9*, *rym4* × *rym11*, *rym5* × *rym9*, *rym5* × *rym11*, *rym9* × *rym11*. No crosses were carried out between carriers of *rym4* and *rym5* as they both map to the same marker interval (MWG838 – Bmac029) on chromosome 3HL and have been known to be allelic with respect to BaMMV (Graner et al. 1999). Concerning *rym9* and *rym11*, which are localised on the same chromosome arm (4HL), restricted recombination is expected, but as *rym11* is located in the centromeric region and *rym9* in the telomeric region of the long arm of chromosome 4H (Bauer et al. 1997) these genes were considered combinable.

Pyramiding strategy I

Strategy I is based on two single crosses each followed by the production of DH-lines. In Figure 1 the strategy for combining resistance genes *rym4*, *rym9* and *rym11* is presented. Starting from single crosses mentioned above F_1-plants were used for DH-production. By the use of respective PCR-based markers (Table 1) DH-plants carrying both genes homozygous recessively were identified. These genotypes, which theoretically occur at a frequency of 25% within a DH-population, were crossed again and the resulting F_1', being homozygous already at the resistance locus in common and heterozygous at the other two loci, was used for DH-production. Within these DH-populations genotypes carrying all three recessive genes homozygously were expected at a frequency of 25% and those carrying two resistance genes at a frequency of 50%.

The observed segregation within the doubled haploid populations of the first DH-production cycle based on marker results is shown in Table 2. In most cases the observed segregation fits to the theoretical expectation, being 25% for each of the four possible genotypes. DH-lines identified as homozygous-recessive at both loci were selected in the greenhouse directly and used for the next crossing step.

Pyramiding strategy II

The second pyramiding strategy for the genes *rym4*, *rym9* and *rym11* is presented in Figure 2. In contrast to strategy I no DHs have been produced from F_1-plants, but respective F_1-plants being heterozygous at two resistance loci each and having one locus in common were inter-crossed directly. Within the resulting segregating population the genotype being homozygous recessive at the resistance locus in common and heterozygous at the other two loci, is theoretically present at a frequency of 6.25%. Respective genotypes have been identified by marker assisted selection (Table 1) and used for DH-line production, leading to an offspring of 25% having three resistance genes and 50% having two genes fixed homozygous-recessively.

To carry out this strategy, four F_1 plants of each single cross combination were grown and the following crosses were carried out: (*rym4* × *rym9*) × (*rym4* × *rym11*); (*rym9* × *rym4*) × (*rym9* × *rym11*); (*rym11* × *rym4*) × (*rym11* × *rym9*); (*rym5* × *rym9*) × (*rym5* × *rym11*); (*rym9* × *rym5*) × (*rym9* × *rym11*); (*rym11* × *rym5*) × (*rym11* × *rym9*). At least 100 seeds were produced per combination in order to ensure the selection of the desired genotypes at a sufficient probability. The co-dominant SSR-markers Bmac029, HVM67 and HVM03 were used for selection in the segregating F_1'-population, as they facilitate differentiation between homozygous and heterozygous genotypes (Figure 2). In a

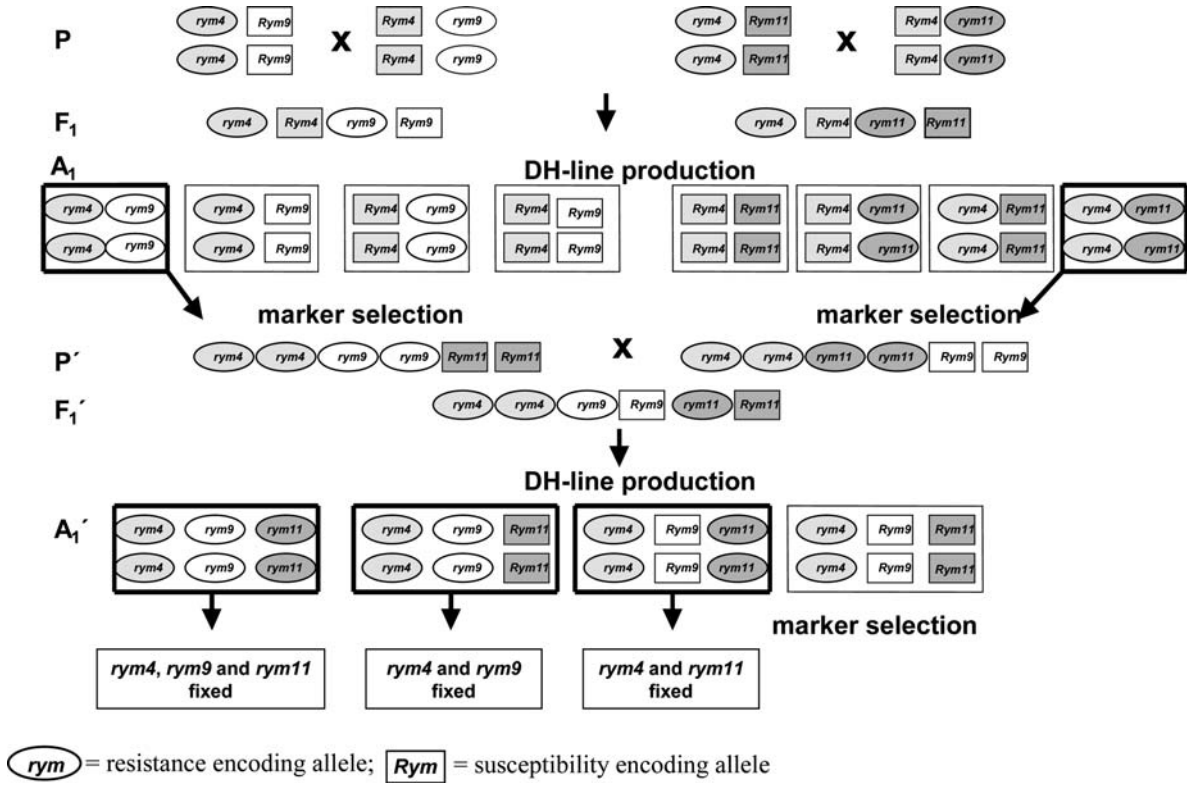

Figure 1. Scheme of pyramiding resistance genes *rym4*, *rym9* and *rym11* by two haploidy steps (strategy I).

Table 2. Strategy I: Expected and observed segregation ratios within the DH-populations derived from the first cycle of DH-production.

Gene combination	ΣDH-lines	Number of expected DHs in each class (25%, segregation 1:1:1:1)	observed segregation (A : B : C : D)[a]	$\chi^2_{(1:1:1:1)}$ (χ^2_{Tab} = 7.814; α = 0.05)
rym4 × *rym9*	261	65.25	58 : 62 : 83 : 58	6.60
rym4 × *rym11*	18	4.50	4 : 4 : 1 : 9	7.33
rym5 × *rym9*	104	26.0	20 : 36 : 22 : 26	5.85
rym5 × *rym11*	382	95.50	73 : 99 : 87 : 123	14.10
rym9 × *rym11*	117	29.25	21 : 32 : 31 : 33	3.17

[a]A = *gene1* and *gene2* homozygous recessive, B = *gene1* homozygous recessive, *gene2* homozygous dominant, C = *gene2* homozygous recessive, *gene1* homozygous dominant, D = *gene1* and *gene2* homozygous dominant.

first step, genotypes being homozygous recessive at the resistance locus in common were identified using the respective SSR-markers. In a next step these genotypes were screened for being heterozygous at the other resistance loci. Out of 100 F_1'-plants, 6.25 plants of the respective genotype are theoretically expected. Due to the fact that within four of the crosses less than the expected plants were identified, also those genotypes have been selected being heterozygous at all three resistance loci (Table 3).

In a next step altogether 62 (29 + 33) selected genotypes (cf. Table 3) were used for DH-production and within the resulting DH-populations the 'pyramided' genotypes carrying three or two resistance genes, respectively were identified using the above-mentioned markers. The DH-lines were pooled to form populations based on combinations with the genes *rym5*, *rym9*, *rym11* (187 lines) and *rym4*, *rym9*, *rym11* (107 lines) in order to get a sample size sufficient for statistical analysis. Within these two populations a further separation

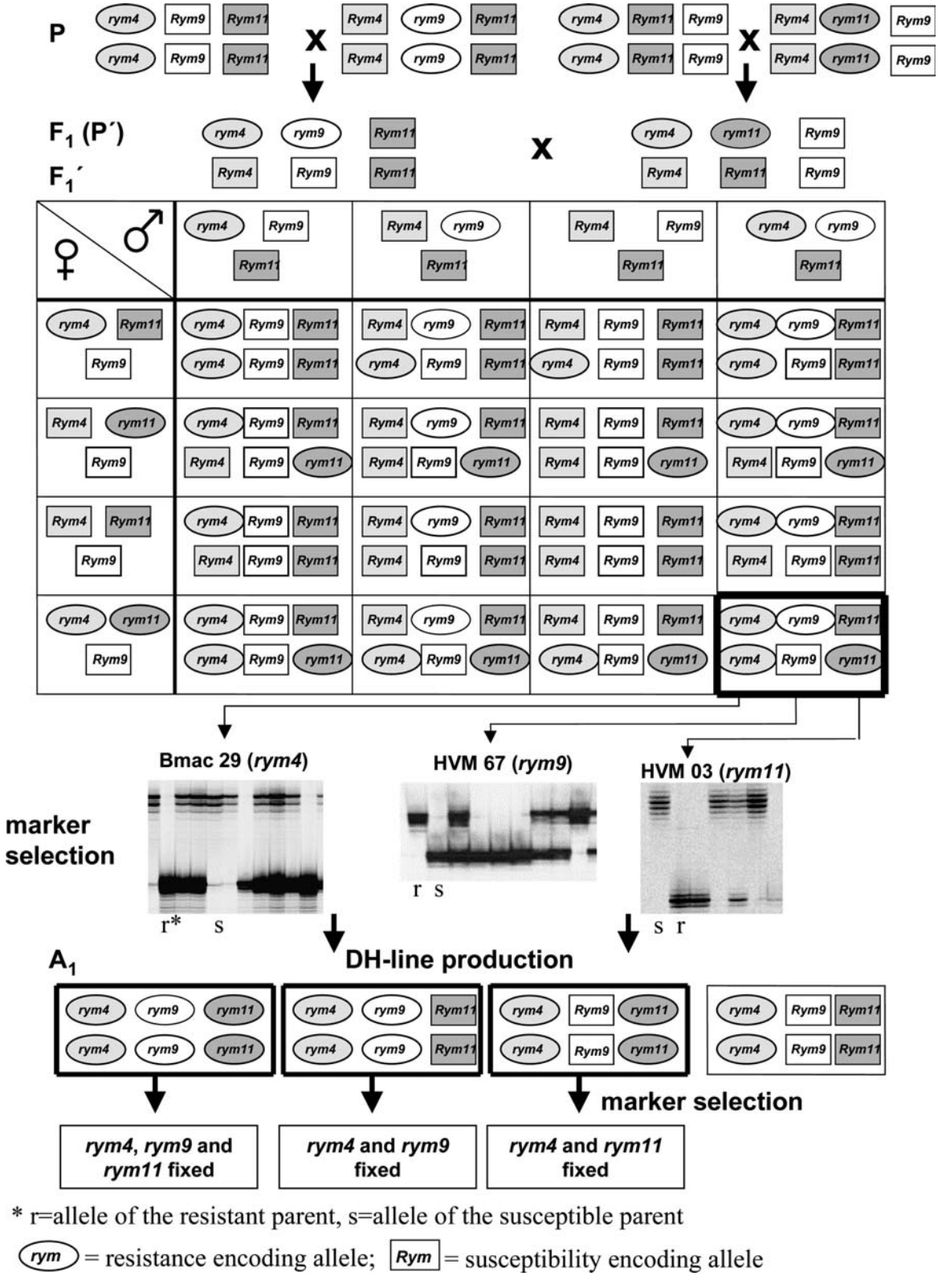

Figure 2. Scheme of pyramiding resistance genes *rym4*, *rym9* and *rym11* by one haploidy step (strategy II).

Table 3. Strategy II: Number of detected genotypes in F′$_1$ suitable for pyramiding resistance genes by one DH-step.

Cross combination	1 gene homozygous, 2 genes heterozygous		3 genes heterozygous	
	Number of genotypes*	$\chi^2_{(1:15)}$ (χ^2_{Tab} = 3.841; α = 0.05)	Number of genotypes*	$\chi^2_{(2:14)}$ (χ^2_{Tab} = 3.841; α = 0.05)
(*rym4* × *rym9*) × (*rym4* × *rym11*)	1	4.704	5	5.143
(*rym9* × *rym4*) × (*rym9* × *rym11*)	11	3.850	–	–
(*rym11* × *rym4*) × (*rym11* × *rym9*)	3	1.803	14	0.206
(*rym5* × *rym9*) × (*rym5* × *rym11*)	3	1.803	6	3.863
(*rym9* × *rym5*) × (*rym9* × *rym11*)	3	1.803	8	1.851
(*rym11* × *rym5*) × (*rym11* × *rym9*)	8	0.523	–	–
	29		33	

*Out of 100 F′-plants tested.

into sub-populations derived from genotypes being homozygous-recessive at one locus and heterozygous at the other two loci (92 lines of population '*rym5* × *rym9* × *rym11*'; 62 lines of population '*rym4* × *rym9* × *rym11*'), and those derived from plants being heterozygous at all loci, i.e. 95 for those employing *rym5* and 45 lines for *rym4*, was carried out in order to be able to calculate expected frequencies for χ^2-analyses. Results of these analyses are shown in Table 4. Segregation analysis for each combination is based on one of the four sub-populations (Table 4) or a combination of sub-populations, depending on the gene combination analysed. In this respect it has to be noted that the combination *rym9* × *rym11* can derive from both populations while susceptible lines can derive only from F$_1$′-plants being heterozygous at three loci. The χ^2-results demonstrate that in most cases the number of observed DH-lines fits to the number of theoretically expected DH-lines, except for the number of lines carrying the combination *rym4* × *rym9*.

Table 4. Strategy II: Segregation within the DH-populations derived from different F′$_1$ genotypes concerning genes *rym4*, *rym5*, *rym9* and *rym11*.

Plants carrying gene combinations or single genes homozygous recessively	Number of expected DH-lines carrying respective genes homozygous recessively (expected ratio in %); [total number of DH-plants]			Number of observed DH-lines	χ^2(χ^2_{Tab} = 3.841; α = 0.05)
	Genetic constitution of F′$_1$: 1 gene homozygous recessive, 2 genes heterozygous	Genetic constitution of F′$_1$: 3 genes heterozygous	Σ		
rym5 × *rym9* × *rym11*	23 (25%); [92]	11.875 (12.5%); [95]	34.875	27	2.19
rym4 × *rym9* × *rym11*	15.5 (25%); [62]	5.625 (12.5%);[45]	21.125	20	0.07
rym5 × *rym9*	15.3 (16,66%); [92]	11.875 (12.5%); [95]	27.175	31	0.63
rym5 × *rym11*	15.3 (16,66%); [92]	11.875 (12.5%); [95]	27.175	21	1.64
rym4 × *rym9*	10.3 (16,66%); [62]	5.625(12.5%); [45]	15.925	26	7.49
rym4 × *rym11*	10.3 (16,66%); [62]	5.625(12.5%); [45]	15.925	17	0.09
rym9 × *rym11*	25.6 (16.66% = 15.33 + 10.33)*; [154 = 92+62]	17.5 (12.5% = 11.875 + 5.625)*; [140 = 95 + 45]	43.1	42	0.03
rym5	7.7 (8.33%); [92]	11.875 (12.5%); [95]	19.575	17	0.38
rym4	5.2 (8.33%); [62]	5.625 (12.5%); [45]	10.825	16	2.75
rym9	12.9 (8.33% = 7.7 + 5.2)*; [154 = 92 + 62]	17.5 (12.5% = 11.875 + 5.625)*; [140 = 95 + 45]	30.4	33	0.25
rym11	12.9 (8.33% = 7.7 + 5.2)*; [154 = 92 + 62]	17.5 (12.5% = 11.875 + 5.625)*; [140 = 95 + 45]	30.4	28	0.21
Susceptible (=homozygous-dominant at all loci)		17.5 (12.5% = 11.875 + 5.625)*; [140 = 95 + 45]	17.5	16	0.15
				294	

*Genotype derived from both sub – populations, i.e. (*rym4, rym9, rym11*), (*rym5, rym9, rym11*).

In total 20 DH-plants out of 107 analysed carrying *rym4*, *rym9* and *rym11* and 27 out of 187 tested carrying *rym5*, *rym9* and *rym11* homozygous recessively, have been detected. Besides this, DH-lines carrying all possible two-gene-combinations were achieved by this strategy.

Evaluation of BaMMV/BaYMV-resistance

Resistance assessment of DH-lines in a BaMMV-/BaYMV-infested field showed that in nearly all cases data gained by phenotypic selection correspond to the results predicted based on the data of molecular analyses. For example the DH-progenies of the cross *rym4* × *rym9* showed the following patterns of resistance: (i) lines being resistant to BaMMV and BaYMV in the field turned out to be carriers of *rym4* or carriers of *rym4* and *rym9* by molecular analysis, (ii) susceptible lines showed the fragment pattern indicative for the dominant alleles or turned out to be carriers of *rym9* which is not effective against BaYMV. Very rarely exceptions, e.g. a line being resistant in the field but only carrying *rym9* by marker analysis, were observed.

Discussion

Pyramiding of resistance genes

The basic idea of pyramiding respectively combining different resistance genes in one line or variety (Nelson 1978) aims at the creation of longer-lasting or durable resistance. In the present study the resistance genes *rym4*, *rym5*, *rym9* and *rym11* have been combined in winter barley breeding lines. For this purpose, two different strategies have been applied in parallel, both being based on marker assisted selection (MAS) and the production of DH-lines. The aim of this approach was to prove the efficiency of these strategies and to compare both strategies regarding their potential application in practical barley breeding. The four resistance genes of the present study differ in their mode of action, i.e. not each gene confers resistance against all viruses or virus strains. Whereas genotypes carrying *rym5* are resistant to BaMMV, BaYMV and BaYMV-2, *rym4* confers no resistance against BaYMV-2 and *rym9* is effective against BaMMV, only (Bauer et al. 1997). The *Rym11* locus which at the beginning of these studies had been reported to provide BaMMV-resistance only (Bauer et al. 1997, Ordon et al. 1999) was recently shown to be also effective against BaYMV and BaYMV-2 (Nissan-Azzouz et al. 2005).

By combining the different genes in one line a broader resistance spectrum is created in some cases. This may be of special importance in the future as it turned out recently that against the *rym5* resistance breaking strain of BaMMV detected in France (BaMMV-Sil; Hariri et al. 2003) *rym9* is still effective (Kanyuka et al. 2004). Therefore, complete resistance to BaYMV, BaYMV-2, BaMMV and BaMMV-Sil should have been achieved by the procedure mentioned above. However, *rym11* seems to be effective against all these viruses (Kanyuka et al. 2004). Although at the moment the entire use of *rym11* will result in complete resistance combining of resistance genes can extend the utility of resistance genes being overcome by single strains as preliminary results obtained in field tests in France give hint that lines carrying *rym5* and *rym9* are resistant to all types of BaMMV and BaYMV-2 known so far in Europe (H. Jaiser pers. comm). In this respect it has to be mentioned that recently additional genes effective against BaYMV/BaYMV-2 or BaMMV only have been mapped (Werner et al. 2003; Le Gouis et al. 2004) which are suitable for broadening the genetic basis for pyramiding. This may lead to more durable resistance, provided that the different combined genes control different resistance mechanisms (Fraser 1990).

Application of molecular markers for the pyramiding of resistance genes

The availability of molecular markers closely linked to the respective resistance genes is an essential prerequisite for an efficient gene combination (Brown et al. 1996, Sanchez et al. 2000). It is often extremely difficult or even impossible to reliably combine and detect several resistance genes in one genotype by phenotypic selection, solely (Brown et al. 1996; Dweikat et al. 1997). Main reasons for this are dominance, epistactic effects and/or masking of the effect of one gene by another (Huang et al. 1997, Hittalmani et al. 2000, Singh et al. 2001). Concerning the Barley Yellow Mosaic Virus complex a phenotypic identification of geno-

types carrying one or more resistance genes is impossible, due to a lack of differentiating virus strains. However, if molecular markers linked to the resistance genes are available respective resistance encoding alleles can be easily identified (Paterson et al. 1991, Huang et al. 1997). For the resistance genes *rym4*, *rym5*, *rym9* and *rym11* used in this study, RAPD- and SSR-markers were available or have been developed in parallel, e.g. markers STS-C04H910 and HVM67, closely linked to *rym9* (Werner et al. 2000). However, results of resistance assessment emphasize the importance of a close linkage between respective markers and genes as in some rare cases recombination was observed. The risk of selecting recombinants may be minimized by using flanking markers which however will result in doubling of the molecular analyses needed. Due to their co-dominant mode of inheritance, SSR-markers facilitate the detection of heterozygous genotypes, which cannot be achieved by dominant RAPDs. In the case of *rym11* the two closely linked RAPD-markers OP-A04H450 and OP-G06H550 are available (Bauer et al. 1997). In combination they can be applied like a co-dominant marker, as OP-A04H450 generates an additional fragment linked to the resistance encoding allele, and OP-G06H550 generates an additional fragment on susceptible lines. Admittedly, this requires additional expenditures and labour, as two PCR-analyses per genotype are needed. In strategy I (cf. Figure 1) selection is only carried out on DH-lines, thus RAPD-markers are as informative as SSR-markers. If MAS is carried out within a segregating progeny (cf. Strategy II, Figure 2), application of co-dominant markers is clearly favoured and advantageous as was also shown by Huang et al. (1997). By the use of co-dominant markers, the authors were able to select rice plants in F_2, carrying two or three genes conferring resistance against *Xanthomonas oryzae* pv. *oryzae* homozygously without any further resistance tests in subsequent generations.

Comparison of both pyramiding strategies

Pyramiding strategies in the present study were based on DH-lines since homozygous recessive genotypes are much more frequent than in F_2-populations. Besides this, the application of DHs in such a breeding strategy results in a substantial time gain, as within one generation complete homozygosity is achieved and respective genotypes can be efficiently selected in this generation, already. The application of the haploid-technique has proven its efficiency already in backcrossing-programmes for combining BaYMV-resistance with agronomical characters (Foroughi-Wehr and Wenzel 1990).

Due to the fact that the DH-line-production itself is time consuming and costly, strategy II, which includes one haploid step only, is more time and cost efficient than strategy I. Regarding the number of molecular analyses to be carried out there are no differences between strategy I and II as in both cases molecular analyses have to be conducted twice. However, strategy I facilitates the application of dominant marker systems, since they are as informative as co-dominant markers due to the absence of heterozygous genotypes.

Especially regarding strategy I, high automation of the molecular analysis is required, as e.g. described by Schiemann and Backes (2000) and Pellio et al. (2005). DNA-extraction and PCR-analyses for selecting the respective genotypes have to be finished prior to anthesis. In conclusion, strategy II appears to be the method of choice for application in practical barley breeding, especially as the costs for DH-line production are lower and the strategy itself is faster. However, the efficiency, usefulness and manageability in terms of pyramiding resistance genes against the Barley Yellow Mosaic Virus complex could be demonstrated for both strategies. But, with respect to using such strategies in practical barley breeding, it has to be mentioned that after each crossing the procedure of pyramiding has to be repeated as respective genes are segregating in the progeny. Nevertheless, these strategies are used by some companies in breeding for resistance to the Barley Yellow Mosaic Virus complex, already (e.g. Tuvesson et al. 2004).

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