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Mode of inheritance and genetic diversity of BaMMV resistance of exotic barley germplasms carrying genes different from *'ym4'**

F. Ordon and W. Friedt

Institute of Agronomy and Plant Breeding I, Justus-Liebig-University, Ludwigstrasse 23, D-6300 Gießen, FRG

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Summary. In order to obtain information about the mode of inheritance of BaMMV resistance in germplasms carrying genes different from the "German" gene *'ym4'*, F_1-tests for resistance as well as F_2-segregation analyses of crosses to susceptible German cultivars were carried out by mechanical inoculation in the greenhouse. In the F_1 the majority of plants of each combination tested reacted susceptible to BaMMV while in the F_2 a good fit to a segregation of 1r:3s or 7r:9s was observed. Therefore, the results of these tests revealed that the BaMMV resistance of all the varieties tested is inherited either by a single or by two recessive genes. By testing intercrosses of these resistant varieties segregation of BaMMV-susceptile plants was observed in the majority of combinations, revealing a high degree of genetic diversity in the barley gene pool.

Key words: *Hordeum vulgare L.* – Barley mild mosaic virus (BaMMV) – Resistance – Mode of inheritance – Genetic diversity

Introduction

The yellow mosaic-inducing viruses of barley – i.e., barley mild mosaic virus (BaMMV), barley yellow mosaic virus (BaYMV) and BaYMV-2 (Huth 1990; Huth and Adams 1990) – are responsible for one of the most important diseases of winter barley (*H. vulgare L.*) in Germany and other major European barley growing countries. High yield losses are frequently observed in susceptible winter barley crops (e.g., Friedt et al. 1990a). Such losses can only be prevented by growing resistant cultivars, because chemical measures are either inefficient or uneconomic due to the transmission of the viruses by the soilborne fungus *Polymyxa graminis* Led. (Toyama and Kusaba 1970). The resistance of German cultivars to BaMMV is determined by a recessive gene (Friedt et al. 1983) located on the long arm of chromosome 3 (Kaiser and Friedt 1989, 1992) and designated *'ym4'* by Friedt et al. (1990b). Genes *'Ym1'* from 'Mokusekko 3' (Takahashi et al. 1973), *'Ym2'* from 'Mihori Hadaka 3' (Takahashi et al. 1973), and *'ym3'* from the mutant 'Ea 52' (Ukai 1984), have also been described. But the BaMMV resistance genes of 'Mokusekko 3' and 'Mihori Hadaka 3' seem to be allelic to the German gene *'ym4'* (Götz 1991); however, *'ym3'* is not effective against BaMMV (Friedt et al. 1990a). Therefore, segregation analyses of crosses between foreign resistant germplasms and cultivars carrying *'ym4'* were initiated in order to broaden the genetic basis of resistance. In a first study, 19 varieties with genes different from *'ym4'* were described by Götz (1991). Nevertheless, it is still an open question whether genetic diversity in relation to BaMMV resistance does exist between varieties carrying genes different from *'ym4'*. Therefore, genetic analyses of crosses among these germplasms, as well as with susceptible cultivars, were carried out in order to obtain detailed information on the mode of inheritance of BaMMV resistance. Segregation analysis in the F_2 is still limited to BaMMV because, in contrast to BaYMV and BaYMV-2 (Huth 1990), this virus can be efficiently transmitted mechanically (Friedt 1983) and so can be handled in the greenhouse under controlled conditions.

Materials and methods

Nineteen varieties, carrying genes different from *'ym4'* (Götz 1991), as well as the variety 'Mokusekko 3', were crossed to the susceptible varieties 'Trixi', 'Igri', 'Magie', 'Alraune' and 'Copia'. F_1 plants of these crosses were inoculated mechanically in order

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Correspondence to: F. Ordon

Table 1. Results of crosses between resistant exotic germplasms and susceptible winter barley cvs 'Alraune', 'Copia', 'Igri', 'Magie' and 'Trixi'

Cross	F_1^a	F_2 segregation	$\chi^{2\,b}$	$\chi^{2\,b}$	Infect. rate (%)^c
		r : s	1r:3s	7r:9s	
10247 × Trixi	s	28 : 86	0.461	–	97
9048 × Alraune	s	22 : 93	2.113	–	100
Anson Barley × Trixi	s	22 : 97	2.692	–	100
Bulgarian 347 × Alraune	s	41 : 135	0.928	–	98
Chikurin Ibaraki 1 × Magie	s	26 : 94	0.711	–	100
Iwate Omugi 1 × Magie	s	36 : 76	0.007	–	90
Krasnodar 1920 × Alraune	s	28 : 92	3.279	–	92
Maksimirski 452 × Trixi	s	34 : 79	0.001	–	93
NIR 55 MN-28 × Copia	s	32 : 84	0.414	–	100
Ou 1 × Alraune	s	37 : 80	2.738	–	100
Rokkaku 1 × Magie	s	35 : 83	1.367	–	100
Russia 32 × Trixi	s	24 : 96	1.600	–	100
Russia 57 × Alraune	s	30 : 120	2.000	–	100
Taihoku A × Magie	s	21 : 93	2.632	–	100
Turkey 235 × Alraune	s	32 : 85	0.345	–	100
9043 × Alraune	s	42 : 68	4.777	3.105	95
Namji Milyang Native × Igri	s	92 : 77	37.076	1.733	90
Muju covered 2 × Copia	s	99 : 131	17.824	1.988	93
Mokusekko 3 × Magie	s	52 : 57	29.972	0.693	100
Zairai Rokkaku × Igri	s	41 : 70	8.435	2.094	100

r resistant; s, susceptible

[a] Infection rate for all crosses 100%

[b] χ^2-values arithmetically corrected for the rate of infection according to J. Jensen (cited in Kaiser 1989)

[c] Estimated by plants of the susceptible cv 'Gerbel' and only involving F_2 results

to obtain initial information about the mode of inheritance (dominant/recessive) of resistance and F_2 progeny were tested for the segregation ratio. For analyzing the genetic diversity of BaMMV resistance these exotic germplasms, as well as 'Mihori Hadaka 3', were intercrossed and tested in the F_2. The sample size necessary to distinguish between potential segregation ratios was estimated according to Seyffert (1962). Consequently, 120 plants were examined in crosses with susceptible varieties, corresponding to a probability level of 0.025 to 0.005. Agreement between observed segregation and theoretical expectation was tested by the chi-square test (χ^2) according to Mudra (1958) and χ^2-values were arithmetically corrected for the rate of infection if this rate (I) was $80\% < I < 100\%$ (Jensen, cited in Kaiser 1989). Samples with an infection rate of $I < 80\%$ were excluded. The rate of infection was estimated by using plants of the susceptible variety 'Gerbel' (ratio check plants: test plants = 1:3). In crosses between resistant varietes, χ^2 values were calculated on the basis of the mode of inheritance and the expected number of resistance genes, according to the results of crosses to susceptible varieties. In the latter type of crosses a shift of segregation ratio due to linkage in the repulsion phase can be excluded.

Plants were mechanically inoculated with BaMMV in the four- to five-leaf stage according to Umbach (1987, cited in Kaiser and Friedt 1989). For inoculum preparation, leaf material of BaMMV-infected barley cv 'Gerbel' was sampled, using young leaves with typical symptoms only. Plant sap was extracted by using a Pollähne sap-press. The sap was diluted (1:10) in K_2HPO_4 buffer (0.1 *M*; pH 9.1), and carborundum (mesh 300) was added (0.5 g/25 ml). Mechanical inoculation was carried out by a spraygun (Sata Dekor/Z-Universal) using 8-bar pressure (air compressor) and a nozzle set with an 0.5 mm diameter. The youngest and the second youngest leaf of each plant were sprayed from both sides with on average 2.5 ml of diluted sap. During sap preparation and mechanical infection the inoculum was kept at +4 °C. After inoculation the plants were briefly rinsed with tap water and kept in the shade for 24 h at +18 °C; subsequently plants were grown in the greenhouse under natural light conditions at approximately +12 °C. Plants were examined serologically 4 weeks after inoculation using Das-Elisa according to Clark and Adams (1977). Antisera were kindly provided by Dr. W. Huth, Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig. Optical density was estimated photometrically at a measurement wavelength of 405 nm and a reference wavelength of 620 nm (Easy Reader 400 ATX, SLT-Labinstruments, Crailsheim).

Results

In all crosses between resistant germplasms and susceptible German cultivars the majority of F_1 plants tested reacted susceptible to BaMMV. A few uninfected plants were detected in crosses with 'Chikurin Ibaraki 1' and 'Muju covered 2' only; in these plants mechanical inoculation had obviously failed (Table 1). Therefore, the results indicate an entirely recessive mode of inheritance of BaMMV resistance in all the varieties carrying genes different from '*ym4*', as well as in 'Mokusekko 3'.

In order to confirm the results of the F_1 tests and to obtain information about the number of genes involved in each variety, F_2 analyses for segregation were conducted with these crosses. For most of the crosses a good fit of the observed segregation ratio to the expectation of

Table 2. Results of intercrosses between different BaMMV-resistant parents

Cross	F_2 segregation	$\chi^{2\,a}$	P	Infect. rate (%)[b]
	r : s	(7r:9s)		
Iwate Omugi 1 × Mokusekko 3	118 : 0			90
Iwate Omugi 1 × Mihori Hadaka 3	30 : 0			90
§ × Muju covered 2	103 : 0			88
NIR 55 MN-28 × Iwate Omugi 1	117 : 0			100
Zairai Rokkaku × §	113 : 0			88
Zairai Rokkaku × Mihori Hadaka 3	89 : 0			93
10247 × Russia 57	55 : 63	0.127	0.80–0.50	92
Anson Barley × Russia 57	59 : 58	0.702	0.50–0.20	95
Bulgarian 347 × Russia 57	42 : 73	2.442	0.20–0.05	100
Iwate Omugi 1 × Russia 57	52 : 67	1.535	0.50–0.20	90
Krasnodar 1920 × Russia 57	53 : 67	0.008	0.95–0.80	100
Maksimirski 452 × Russia 57	53 : 66	0.030	0.95–0.80	100
Rokkaku 1 × Russia 57	60 : 59	0.0003	0.99–0.95	88
Russia 57 × Taihoku A	31 : 58	2.877	0.20–0.05	100
Anson Barley × Taihoku A	60 : 58	2.451	0.20–0.05	100
Taihoku A × Chikurin Ibaraki 1	50 : 68	0.091	0.80–0.50	100
Ou 1 × Taihoku A	64 : 55	1.176	0.50–0.20	91

§, Namji Milyang Native; r, resistant; s, susceptible; P, probability for χ^2

[a] χ^2 values arithmetically corrected for the rate of infection according to J. Jensen (cited in Kaiser 1989)

[b] Estimated by plants of the susceptible cv 'Gerbel'

1r:3s ($\chi^2 < 3.841$) was found indicating the presence of a single recessive gene (Table 1). In contrast to these results, an excess of resistant plants was observed in crosses with '9043', 'Namji Milyang Native', 'Muju covered 2', 'Mokusekko 3' and 'Zairai Rokkaku'. The observed segregation ratio estimated for these crosses does not fit an expected ratio of 1r:3s ($\chi^2 > 3.841$). However, a good fit of the observed segregation in these crosses is found with an expected ratio of 7r:9s ($\chi^2 < 3.841$). Such a ratio can be explained by the action of two independently inherited recessive genes in the resistant parents. As in the F_1 no indication of the presence of dominant genes (segregation 3r:1s) in any of the foreign resistant germplasms is found in the F_2. From these results of crosses to susceptible varieties it can be concluded, therefore, that the BaMMV resistance of all the exotic varieties tested is inherited either by a single or else by two recessive resistance genes.

An F_2 analysis of the genetic diversity of BaMMV resistance within these varieties was also carried out. Segregation of BaMMV-susceptible plants in the F_2 derived from intercrosses of resistant varieties is an indication of the presence of different resistant genes, while completely resistant F_2 progeny is indicative of allelic genes. Out of 59 crosses tested only six combinations did not segregate susceptible plants (Table 2). Therefore, the BaMMV resistance genes involved in these crosses can be assumed to be allelic at least for one gene. In the remaining 53 crosses segregation of BaMMV-susceptible plants was observed. The results of some of these crosses are presented, as an example, in Table 2. In all of them a good fit to a segregation of 7r:9s was observed which indicates the presence of a different recessive gene in each variety. Furthermore, a good fit to a segregation ratio of 37r:27s, expected in the presence of three recessive genes, was found (data not shown) in crosses between varieties with a single recessive gene and those which had shown a digenic-recessive mode of inheritance in crosses to susceptible cultivars; e.g., 'Mokusekko 3' × 'Taihoku A' ($\chi^2_{37:27} = 0.049$), 'Zairai Rokkaku' × 'Russia 57' ($\chi^2_{37:27} = 0.837$).

The results of all the crosses tested are summarized in Table 3. In most of the combinations a good fit between observed and expected segregation was found, which is in agreement with the number and type of genes involved as estimated in crosses to susceptible varieties. However, an excess of resistant plants was found in some combinations (for details see Ordon 1992). Nevertheless, the occurrence of susceptible plants in the F_2 of crosses between resistant varieties is an indication of the genetic diversity of the parents. In summary, the results of F_2 analyses lead to the conclusion that a high degree of genetic diversity in relation to BaMMV resistance is present among those varieties which carry genes different from '*ym4*'.

Discussion

The results of the studies on the mode of inheritance revealed that BaMMV resistance of all the varieties tested is inherited recessively. In contrast to these results, the resistance of 'Mokusekko 3' and 'Mihori Hadaka 3' to Japanese BaYMV was earlier claimed to be inherited by a dominant gene (Takahashi et al. 1973). In studies with BaMMV, a digenic-recessive mode of inheritance for

Table 3. Summary of the results obtained in F_2 analyses of crosses between exotic varietes carrying genes different from '*ym4*'

Variety	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. 10247	–	–	+	▤	+	–	▤	–	▤	▤	–	–	–	▤	–	–	▤	–	–	–
2. 9043		+	–	–	▤	–	–	–	–	▤	–	–	–	–	–	–	–	–	–	–
3. 9048			–	–	▤	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4. Anson Barley				▤	–	–	+	–	–	–	+	+	–	–	+	–	▤	▤	+	–
5. Bulgarian 347					▤	–	–	–	–	▤	–	–	–	–	–	–	▤	–	–	–
6. Chikurin Ibaraki 1						–	+	–	–	–	–	+	–	+	+	–	–	▤	+	+
7. Iwate Omugi 1							–	–	A	A	–	–	A	–	–	–	▤	–	–	–
8. Krasnodar 1920								–	–	–	–	–	–	–	–	+	▤	–	▤	–
9. Maksimirski 452									–	▤	–	–	–	–	–	–	▤	–	–	–
10. Mihori Hadaka 3										–	+	–	–	–	+	–	–	–	▤	A
11. Mokusekko 3											▤	–	–	▤	–	▤	–	▤	▤	▤
12. Muju covered 2												A	–	▤	–	–	–	–	–	–
13. Namji Milyang Native													–	–	–	–	+	–	–	A
14. NIR 55 MN-28														–	–	–	–	–	–	–
15. Ou 1															–	–	–	▤	–	–
16. Rokkaku 1																–	▤	–	–	–
17. Russia 32																	+	–	–	–
18. Russia 57																		▤	▤	▤
19. Taihoku A																			–	–
20. Turkey 235																				–
21. Zairai Rokkaku																				

▤, unlinked genes; A, allelic genes; +, excess of resistant plants observed in F_2; –, not tested

'Mokusekko 3' and a trigenic-recessive inheritance for 'Mihori Hadaka 3' was reported by Götz (1991). Most of the results presented in the present paper (Tables 1, 3) fit well with these findings and recent Japanese studies also provide evidence for a recessive mode of inheritance in 'Mokusekko 3' (Kawada 1991). Because of the segregation ratio observed in a cross to the resistant German variety 'Diana', Götz (1991) assumed a dominant gene in the variety 'Zairai Rokkaku'. In crosses to the susceptible variety 'Igri' (Table 1), as well as in crosses to resistant varieties like 'Russia 57' (Table 3), we failed to obtain any evidence for a dominant gene in 'Zairai Rokkaku'. These findings highlight the necessity to study crosses to susceptible varieties in order to gain detailed information about the mode of inheritance and the number of genes involved, because in intercrosses of resistant varieties a shift of segregation ratio due to linkage in the repulsion phase may occur. Thus, in crosses between resistant varieties carrying a single recessive gene, segregation can be shifted from 7r:9s to a maximum of 1r:1s by linkage in the repulsion phase. As presented in Table 3 an excess of resistant plants, which cannot be due to linkage, was observed in some combinations. These deviating ratios are probably due to specific gene interactions which cannot as yet be explained. An excess of resistant plants in the F_2 of some crosses was also observed by Götz (1991).

Apart from the question of segregation ratio and the mode of inheritance the genetic analysis revealed a high degree of genetic diversity concerning BaMMV resistance so that different genes are now available for broadening the genetic basis of barley. Unfortunately, the question of whether the results obtained for BaMMV are also valid for BaYMV and BaYMV-2 cannot be answered at present. Screening programmes carried out to identify

BaYMV-2-resistant germplasms revealed different types of resistance to the yellow mosaic-inducing viruses (Ordon 1992) in some of the varieties used in the present study. For example e.g., 'Anson Barley', 'Bulgarian 347', 'Krasnodar 1920', 'Maksimirski 452' and 'Turkey 235' are resistant to BaMMV only, while others are resistant to BaMMV and BaYMV but susceptible to BaYMV-2. Nevertheless, some are also resistant to all of the yellow mosaic-inducing viruses (Götz 1991). Because genetic diversity concerning BaMMV was also detected in varieties resistant to BaYMV-2, different BaYMV-2 genes may be introduced simultaneously by using germplasms like 'Russia 57' and 'Zairai Rokkaku', which show genetic diversity and complete resistance with respect to BaMMV, BaYMV and BaYMV-2. However, it is still not known whether resistance to the different viruses is determined by one gene effective against all of the viruses, or by different specific genes each acting against one virus. Even, so by incorporating different BaMMV resistance genes into adapted varieties a selection of different BaMMV strains, as already reported from Japan (Kashiwazaki et al. 1990), may be avoided. Selection mechanisms due to the extensive use of the resistance gene '*ym4*' in German barley breeding programmes have also to be taken into account in the case of BaYMV-2 (Huth 1991).

The incorporation of foreign resistance genes will be a time-consuming process, because the exotic varieties are of inferior agronomic performance (Ordon 1992). They are low yielding and generally susceptible to lodging, winter-killing and fungal pathogens, especially *Rhynchosporium secalis*. Therefore, long-lasting backcross programmes are needed to combine the new resistance genes with good agronomic performance. To shorten this process haploid techniques are a very useful tool (Foroughi-Wehr and Wenzel 1990). The latter may be combined with marker-based selection procedures because, at least for the '*ym4*' gene, an RLFP marker has already been identified (Graner and Bauer 1992). On a longer term one can anticipate that such molecular markers will also be identified for genes different from '*ym4*'.

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