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Inheritance of Resistance to Barley Yellow Mosaic Virus

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Barley yellow mosaic virus is widely distributed in Central and Western Europe. Considerable yield losses caused by this virus can only be prevented by cultivation of resistant cultivars (Huth, 1984).

Among barley varieties differential reaction to this disease can be observed. Resistance genes are present in a number of German and European winter barley cultivars as well as in numerous stocks from different parts of the world (Takahashi et al., 1973; Friedt et al., 1985). Immunity to BaYMV of German cultivars is probably due to one identical recessive gene, because their crosses are resistant in F_1 and do not segregate in F_2, where all plants are resistant, too (Friedt and Foroughi-Wehr, 1986).

Besides the German resistant cultivars, an important source of resistance is the Chinese spring barley 'Mokusekko 3'. In earlier genetic analyses carried out in Japan, it was shown, that 'Mokusekko 3' has one dominant resistance gene, called *Ym1* (Takahashi et al., 1973). Hybrid plants (F_1) from crosses of German cultivars to Asian resistant parents, which carry the gene *Ym1*, like 'Mokusekkor 3', are all resistant and the respective F_2-populations do not segregate susceptible individuals (Friedt and Foroughi-Wehr, 1986). Therefore it can be concluded, that the respective resistance genes are either allelic or very tightly linked.

In order to identify the chromosomal location of the resistance gene of 'Mokusekko 3', Takahashi et al. (1973) studied genetic relationships between resistance gene *Ym1* and several marker genes on the individual barley chromosomes. The results of their cross-experiments show, that the gene *Ym1* is inherited independently from the genes *n* for naked kernels on chromosome 1, *V* for two-rowed spikes on chromosome 2, *B* for black kernels on chromosome 5, *o* for orange lemma base and nodes on chromosome 6 and *s* for short and hairy rachilla on chromosome 7. No marker gene for chromosome 3 was used. On the contrary, evidence for linkage of *Ym1* to *K* (hooded lemma) was found in the cross with 'Colsess IV', in which excessive numbers of parental character combinations were observed. The observed frequencies of the four phenotypes did not fit to the calculated numbers for independent segregation (9 : 3 : 3 : 1), and it was

concluded therefore, that the gene *Ym1* of 'Mokusekko 3' was located on chromosome 4.

So far, only a few stocks have been studied genetically (e.g. Friedt and Foroughi-Wehr, 1986). In order to clarify the genetic basis of resistance or immunity, different cross-experiments have been initiated. Marker- and trisomic-analyses were carried out to localize the gene for resistance of German resistant cultivars like 'Birgit', 'Franka', 'Ogra' and 'Sonate'.

Materials and Methods

For marker-analyses, multiple as well as simple genetic markers were used. Multiple genetic markers 'Nigrinudum' and 'Colsess orange lemma', kindly provided by the Institute of Agricultural and Biological Sciences, Okayama University, Japan were studied. 'Nigrinudum' carriers alleles *n* for naked kernel (chr. 1), *V* for two-rowed spikes (chr. 2) and *B* for black kernel (chr. 5). 'Colsess orange lemma' has *K* for hooded lemma (chr. 4) and *o* for orange lemma base and nodes (chr. 6). For testing linkage to the remaining chromosomes, the single genetic markers *yst* (yellow stipe, chr. 3) and *mt2* (mottled leaves, chr. 7) obtained from Dr. Tsuchiya, Department of Agronomy, Colorado State University, Fort Collins, USA, were used.

Trisomic-analysis was started with a complete trisomic set of the cultivated spring barley variety 'Shin Ebisu 16' kindly provided by Dr. Tsuchiya, too.

Tests for resistance (immunity) to BaYMV are carried out in the greenhouse by mechanical inoculation. By means of this artificial inoculation technique only BaYMV type M is transmitted. Details of inoculum preparation, plant inoculation and maintenance of inoculated plants have already been described by Friedt (1983; 1984). This technique has been improved by Umbach (1987) by application of an efficient and highly reliable air-brush (spray-gun) inoculation.

One month after inoculation all plants were examinated serologically by ELISA-test (Casper and Meyer, 1981). The antiserum for this test was kindly provided by Dr. Huth, Biologische Bundesanstalt, Braunschweig, Germany F. R.

Results and Discussion

To identify the chromosomal location of the resistance gene of German varieties, cvs. 'Birgit' and 'Franka' were crossed to multiple genetic marker stocks like 'Colsess orange lemma' and 'Nigrinudum', as described above. The F_1-generation was susceptible to BaYMV, as expected. Segregation in F_2-generation (Table 1) indicates, that the German resistance gene is inherited independently of gene *n* (naked kernels) on chromosome 1, *V* (two-rowed spike) on chromosome 2, *B* (black lemma and pericarp) on chromosome 5, and *o* (orange lemma base and nodes) on chromosome 6.

Table 1

Linkage analysis of "German" BaYMV-M resistance by the use of genetic markers (Kaiser 1988)

Cross: Marker x	Marker$_1$ X	x	suscept. X	x	resist. X	x	Total	X_2	P
	Chrom.1$_2$								
Franka x	N	n	299	121	118	44	582	6.07	.20—.10
Birgit x	N	n	305	95	94	40	534	2.02	.70—.50
	Chrom.2$_3$								
Franka x	V	v	312	108	126	36	582	3.35	.50—.30
Birgit x	V	v	279	120	90	44	534	9.87	.025—.01*
	Chrom.4$_4$								
Birgit x	K	k	318	110	88	46	562	6.45	.10—.05
Franka x	K	k	190	72	68	29	359	2.96	.50—.30
x Franka	K	k	263	98	91	34	486	1.35	.80—.70
	Chrom.5$_5$								
Franka x	B	b	305	115	120	42	582	3.80	.30—.20
Birgit x	B	b	312	88	102	32	534	2.01	.70—.50
	Chrom.6$_6$								
Birgit x	O	o	313	115	98	36	562	1.45	.70—.50
Franka x	O	o	190	72	67	30	359	3.58	.50—.30
x Franka	O	o	258	103	99	26	486	3.72	.30—.20

[1] 'Colsess-orange lemma' or 'Nigrinudum' [2] N = hulled, n = naked [3] V = two-rowed, v = six-rowed [4] K = Kapuze (hood), k = awned [5] B = black, b = yellow lemma and pericarp [6] O = yellow, o = orange lemma.

The marker gene *V* shows deviation from the expected independent segregation in the cross-combination with 'Birgit' (Table 1). This difference to the comparable cross with 'Franka' can be explained by difficulties in the classification of marker characters, because of the occurrence of various intermedium-types of spikes. In this cross-combination, *V* shows linkage to the genes *n* and *B*, too, which would not be expected, because the genes *n*, *V* and *B* are definitive located on different chromosomes. Segregation of *V* and *v* did not fit to the expected 3 : 1 ratio.

The marker-analyses in F_2-generation (Table 1) indicates also, that the German resistance gene is inherited independently of gene *K* for hooded lemma on chromosome 4. This result is in contradiction to earlier results, because the gene *Ym1* was said to be linked with the gene *K* on chromosome 4 (Takahashi et al., 1973) and the "German gene" has proved to be allelic to the gene *Ym1* (Friedt and Foroughi-Wehr, 1986). Therefore, it might be possible, that the gene *Ym1* is not located on chromosome 4. Our results could alternatively be explained by the presence of an additional, recessive gene in 'Mokusekko 3', which then should be allelic to the gene in the German cultivars (Friedt et al., 1987).

Results of linkage between the marker genes *yst* (yellow stripe, chromosome 3) and *mt2* (mottled leaves, chromosome 7) with the "German gene" for resistance are not available yet.

Marker-analysis has one main problem. If the marker gene and the gene in question are on the same chromosome, so that 50% recombination can occur, the data will show independent inheritance. Therefore, trisomic-analyses were carried out with a complete trisomic set of 'Shin Ebisu 16'. Trisomic plants of each barley chromosome were crossed as females to the German resistant cvs. 'Sonate' and 'Ogra'. In F_1-generation trisomic plants of each cross combination were identified by morphological and cytological examinations (Tsuchiya, 1963) and grown to maturity.

In F_1-generation the plants were morphological classified as disomics and trisomics two times (one day before and one month after infection); each of the F_2-populations has been heterogeneous because of the presence of disomic and trisomic plants. Heterogeneity was also evident between F_1-populations because of the different morphology of each trisomic itself.

In the 4- to 5-leaves stage the plants were infected mechanically with BaYMV-M. Transmission was not always complete, because of the heterogeneous populations and the weak growth habit of some trisomic types. Because of previous classification, it was possible to evaluate the segregation for reaction to BaYMV-M for trisomics and disomics separately. F_2-populations with less than 80% infection-rate in the control plants (cv. 'Gerbel') were excluded and populations with less than 100% infection were corrected arithmetically for the rate of escapes.

In the trisomic fractions, especially of the weakest trisomics 'Slender', 'Pale' and 'Semi-erect', unexpected segregations with an excess of resistant plants were found. This finding may be explained by deleterious effects of the severe inoculation in the weak trisomic plants. But for the disomic fractions, which were more uniform and vigorous, clear results were obtained. Among disomics of crosses with cv. 'Ogra' the theoretically expected segregations have been observed (Table 2). In all seven F_2-populations, except the one including 'Pale' as a parent, a good fit to the uncritical segregation (3 : 1) was found, whereas the F_2 derived from crosses to 'Pale' (trisomic for chromosome 3) a good fit to the critical segregation of 8 : 1 for the disomics was evident.

In the disomic fractions of F_2's of crosses with cv. 'Sonate', identical results were obtained (Table 3). Data of all F_2'-populations except the one of 'Pale' again indicate good fits to an expected uncritical segregation. Fit to the critical segregation (8 : 1) in disomic F_2's of 'Pale' was obtained, too.

Table 2

Segregation for reaction to BaYMV-M in F_2 disomics of crosses of 'Shin Ebisu 16' trisomics with the resistant cv. 'Ogra' (Kaiser, 1988)

Trisomic-type	Extra chrom.	Infect. rate (%)	Suscep. (n)	Resist. (n)	Total (n)	X_2 for 3 : 1 ratio	P
Bush	1	92	113	55	168	0.237	0.70—0.50
Slender	2	89	126	60	186	0.083	0.80—0.70
Pale	3	89	69	17	86	7.044	<0.01*
Robust	4	96	109	39	148	0.199	0.70—0.50
Pseudonormal	5	100	125	46	171	0.329	0.70—0.50
Purple	6	100	65	20	85	0.098	0.80—0.70
Semierect	7	96	113	39	152	0.414	0.70—0.50

n = number of plants examinated.
Test of 8 : 1 ratio for Pale (Chr. 3): $X^2 = 0.0649$; P = 0.90—0.80.

Table 3

Segregation for reaction to BaYMV-M in F_2 disomics of crosses of 'Shin Ebisu 16' trisomics with the resistant cv. 'Sonate' (Kaiser, 1988)

Trisomic-type	Extra chrom.	Infect. rate (%)	Suscep. (n)	Resist. (n)	Total (n)	X_2 for 3 : 1 ratio	P
Bush	1	98	55	27	82	1.739	0.20—0.10
Slender	2	93	91	42	133	0.111	0.80—0.70
Pale	3	90	110	20	130	17.359	small *
Robust	4	100	123	40	163	0.018	0.90—0.80
Pseudonormal	5	100	139	49	188	0.114	0.80—0.70
Purple	6	96	85	36	121	0.184	0.70—0.50
Semierect	7	100	78	19	97	1.516	0.30—0.20

n = number of plants examinated.
Test of 8 : 1 ratio for Pale (Chr.3): $X^2 = 1.7310$; P = 0.20—0.10.

Conclusions

As mentioned above, German resistant cultivars carry an identical, recessive resistance gene. From the data presented above, it can be concluded, that the gene for resistance to BaYMV-M of these cultivars is located on barley chromosome 3.

This conclusion is supported by recently published results of Konishi and Matsuura (1987). They found, that the Chinese landrace 'Mokusekko 3' and some

resistant cultivars derived from 'Mokusekko 3', always carry the same esterase isozyme pattern (of 'Mokusekko 3') after hybridizations to other cultivars. These results indicate, that the minor resistance gene in 'Mokusekko 3' would be linked to an esterase isozyme gene block at the terminal end of the long arm of chromosome 3.

Our own results, together with the results of Takahashi et al. (1973) and Konishi et al. (1987), can therefore be interpreted as follows: either the German gene for resistance is allelic to a minor gene of 'Mokusekko 3' on chromosome 3, or the "German gene" is allelic to the dominant gene *Ym1* of 'Mokusekko 3', which then could also be located on chromosome 3. Further analyses will be necessary to clarify this open question.

Literature

Casper, R. and S. Meyer (1981): Die Anwendung des ELISA-Verfahrens zum Nachweis pflanzenpathogener Viren. Nachrichtenbl. Deut. Pflanzenschutzd., 33 (2), 49–54.

Friedt, W. (1983): Mechanical transmission of soil-borne Barley Yellow Mosaic Virus. Phytopath. Z. 106, 16–22.

Friedt, W. (1984): The genetic basis of breeding winter barley for resistance to barley yellow mosaic virus. In: Breeding for Disease Resistance and Oat Breeding. Proc. Eucarpia Cer. Sect. Meet., Freising. Vortr. Pflanzenzüchtg. 6, 145–155.

Friedt, W. and B. Foroughi-Wehr (1986): Herkunft, Eigenschaften und züchterische Erschließung von Resistenzquellen gegen Barley Yellow Mosaic Virus. Vortr. Pflanzenzüchtg. 10, 82–93.

Friedt, W., Huth; H. Mielke and S. Züchner (1985): Resistenzträger gegen Barley Yellow Mosaic Virus. Nachrichtenbl. Deut. Pflanzenschutzd. 37, 129–135.

Friedt, W., R. Kaiser; R. Bötz; H. Umbach and B. Foroughi-Wehr (1987): Genetic basis of breeding for resistance to barley yellow mosaic virus. Meeting of the Assoc. of Applied Biologists, St. Andrews, Scotland.

Huth, W. (1984): Die Gelbmosaikvirose der Gerste in der Bundesrepublik Deutschland — Beobachtungen seit 1978. Nachrichtenbl. Deut. Pflanzenschutzd. 36 (4), p. 49–55.

Kaiser, R. (1988): Untersuchungen zur Genetik der BaYMV-Resistenz bei einheimischen Wintergerste-Sorten. Ph. D. Thesis, Univ. Gießen (in prep.).

Konishi, T. and S. Matsuura (1987): Variation of esterase isozyme genotypes in a pedigree of Japanese two-rowed barley. Japan. J. Breed. 37, 412–420 (jap. with engl. summary).

Takahashi, R., J. Hayashi; T. Inouye; I. Moriya and C. Hirao (1973): Studies on resistance to yellow mosaic disease in barley. I. Tests for varietal reactions and genetic analysis of resistance to the disease. Ber. Ohara Inst. 16, 1–17.

Tsuchiya, T. (1963): Chromosome aberrations and their use in genetics and breeding in barley — trisomics and aneuploids. Barley Genetics I, Proc. 1st. Intern. Barley Genet. Symp., 116–150.

Umbach, H. (1987): Entwicklung einer Sprühmethode zur mechanischen Inokulation von Gerstenpflanzen mit Barley Yellow Mosaic Virus. Dipl. Thesis, Univ. Gießen.