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Chromosomal location of resistance to Barley Yellow Mosaic Virus in German winter-barley identified by trisomic analysis

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Summary. In order to localize a gene for resistance to Barley Yellow Mosaic Virus (BaYMV) of German resistant varieties, cvs. 'Ogra' and 'Sonate' were crossed to a complete trisomic ($2n=2x+1=15$) set of 'Shin Ebisu 16'. Tests for resistance in F_2 strongly support the conclusion that the German gene for resistance to BaYMV is located on barley chromosome 3.

Key words: *Hordeum vulgare* – Barley Yellow Mosaic Virus (BaYMV) – Resistance to BaYMV – Trisomic analysis – Gene localization

Introduction

Barley yellow mosaic disease is caused by Barley Yellow Mosaic Virus (BaYMV), which is transmitted by a soilborne fungus, *Polymyxa graminis* (Toyama and Kusaba 1980). This virus disease was first discovered in Japan in 1940 (Inouye and Saito 1975). In the Federal Republic of Germany, the disease probably occurred only 20 years later and was first described by Huth and Lesemann (1978). The present area of distribution mainly covers the central and northern parts of the country, but recently BaYMV was also found in the south of West Germany (Huth 1988). Meanwhile the disease was also discovered in England (Hill and Evans 1980), France (Lapierre 1980), Belgium (Maroquin et al. 1982), The Netherlands (Langenberg and van der Wal 1986) and the German Democratic Republic (Proeseler et al. 1984).

In these countries, barley yellow mosaic became one of the most important cereal diseases. Because of its soil transmission, chemical measures against BaYMV are either inefficient or uneconomical. Up to now, the considerable yield losses caused by BaYMV could only be prevented by growing resistant cultivars.

Genetics of resistance to Barley Yellow Mosaic Virus

Resistance genes to BaYMV are present in a number of German and European winter barley cultivars as well as in numerous winter and spring barley stocks from different parts of the world (Takahashi et al. 1973; Friedt et al. 1985). Thus far, only a few stocks have been studied genetically. Immunity to BaYMV of German cultivars is probably due to one identical recessive gene, since their crosses to susceptible cultivars are susceptible and those to other German resistant cultivars are resistant in F_1; crosses among resistant cultivars do not segregate in F_2, where all plants are resistant, too (Friedt and Foroughi-Wehr 1986).

Another valuable source of resistance is the Chinese Spring barley 'Mokusekko 3'. Earlier genetic analyses carried out in Japan demonstrated that 'Mokusekko 3' carries a dominant resistance gene, designated as *Ym1* (Takahashi et al. 1973). In order to identify the chromosomal location of this gene, Takahashi et al. (1973) studied genetic relationships between *Ym1* and marker genes on six of the seven barley chromosomes. The results of their experiments indicated that the gene *Ym1* was inherited independently from the genes *n* for naked kernel on chromosome 1, *V* for two-rowed spike on chromosome 2, *B* for black kernel on chromosome 5, *o* for orange lemma base and node on chromosome 6 and *s* for short and hairy rachilla on chromosome 7. In contrast, evidence for linkage of *Ym1* to *K* (hooded lemma) was found in a cross with 'Colsess IV', in which excessive numbers of parental character combinations were observed. Therefore it was concluded that the gene *Ym1* of 'Mokusekko 3' was located on chromosome 4. However, no marker gene for chromosome 3 was used in this study.

Hybrid plants (F_1) from crosses of German cultivars to Asian resistant parents which carry the gene *Ym1*, like

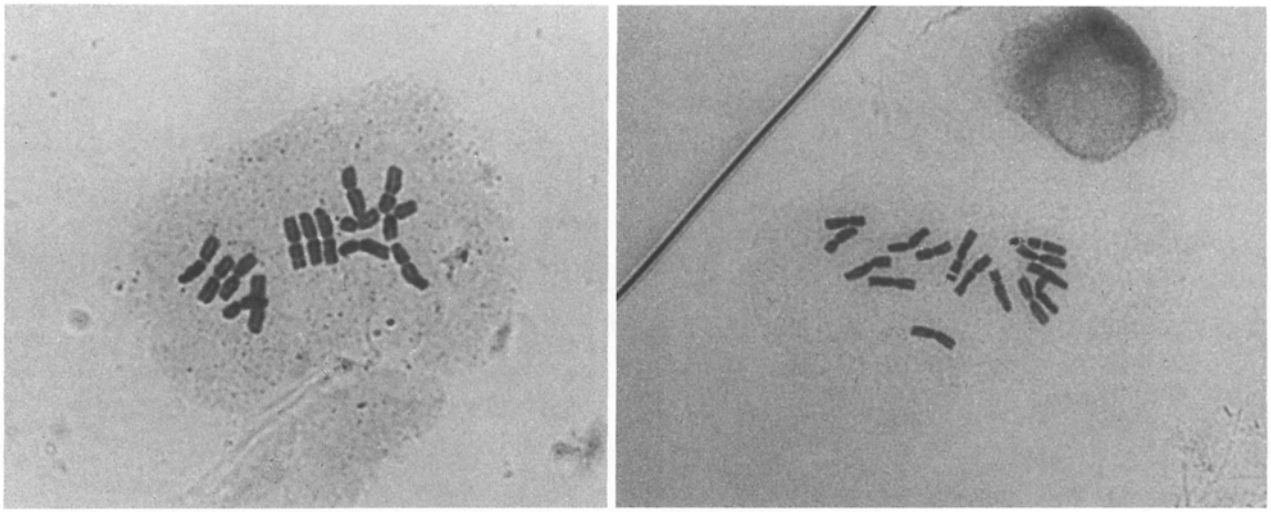

Fig. 1. Mitotic metaphase cells of disomic ($2n = 2x = 14$, left) and trisomic ($2n = 2x + 1 = 15$, right) barley (*Hordeum vulgare* L.)

'Mokusekko 3', are all BaYMV-resistant and the respective F_2-populations do not segregate susceptible individuals (Friedt and Foroughi-Wehr 1986; Friedt et al. 1987; Götz et al. 1988). It can be concluded, therefore, that the respective resistance genes are either allelic or very tightly linked.

In order to clarify the genetic basis of resistance or immunity to BaYMV, marker and trisomic analyses were carried out to localize the gene for resistance of German resistant cultivars. By means of marker analyses, it was verified that the German gene for resistance is inherited independently from the genes *n*, *V*, *B* and *o*. However, this marker analysis also indicates that the German resistance gene is inherited independently of the gene *K* for hooded lemma on chromosome 4 (Kaiser et al. 1988). Nevertheless, the definite genetic location of this resistance gene remained to be determined.

Materials and methods

Trisomic analysis was carried out with a complete trisomic set of the spring barley cv. 'Shin Ebisu No. 16', obtained from T. Tsuchiya, Fort Collins, USA. Its spring growth habit, short post-harvest seed dormancy and non-brittle rachis (unlike the series of primary trisomics of *Hordeum spontaneum*) make it very suitable for genetic studies (Tsuchiya 1967). The seven strains of primary trisomics were identified by means of morphological, cytological and genetical examination (Tsuchiya 1963, 1967). On the basis of their main morphological features, Tsuchiya (1967) designated the strains as 'Bush' (chromosome 1), 'Slender' (chromosome 2), 'Pale' (chromosome 3), 'Robust' (chromosome 4), 'Pseudonormal' (chromosome 5), 'Purple' (chromosome 6) and 'Semierect' (chromosome 7).

Tests for resistance to BaYMV were carried out in the greenhouse. Test plants were grown at 10°–15 °C until the four- to five-leave stage, which proved to be the most suitable stage for inoculation. For inoculum preparation, sap was extracted from young leaves of plants showing typical BaYMV symptoms, using a POLLÄHNE sappress. Plant sap was diluted (1:10) in a K_2HPO_4 buffer (0.1 M, pH 9.1) and carborundum (0.5 g/25 ml) was added; the temperature of inoculum was kept at +4 °C. Plants were inoculated with a spray gun (SATA Dekor/Z-UNIVERSAL) using an air compressor (8 bar). After inoculation, plants were watered briefly and kept in shadow for 24 h at 20 °C and subsequently for 4 weeks at natural light and 12 °C (Friedt 1983, 1984; Umbach 1987). By means of this mechanical inoculation technique, only BaYMV type M (BaYMV-M) is transmitted (Huth 1988).

One month after inoculation, all plants were examined serologically using ELISA (Casper and Meyer 1981). The antiserum for ELISA was kindly provided by W. Huth, Braunschweig, FRG.

Results

Trisomic plants for each barley chromosome were crossed as females to the German resistant cvs. 'Sonate' and 'Ogra'. In the F_1 generation, trisomic plants of each cross combination were identified by morphological and cytological (Fig. 1) examination (Tsuchiya 1963) and grown to maturity in the greenhouse.

To increase the ratio of trisomics, thin F_2 seeds were selected and sown. The F_2 plants were classified morphologically as disomics and trisomics two times, 1 day before and 1 month after inoculation.

Plants in the four- to five-leave stage were inoculated mechanically by BaYMV-M. Transmission of BaYMV-M was usually complete, except in extremely heterogeneous populations, due to the weak growth habit of some trisomics. To control possible escape from infection, some check plants of the susceptible cv. 'Gerbel' were grown and infected together with each F_2 cross population. Populations where the control had more than 20% escapes were excluded, and those with less than 20% escapes were corrected arithmetically by ad-

justing the expected segregation ratio for the rate of escapes.

In all crosses with trisomics which do not carry the gene to be localized in triplicate, ordinary segregations of 3:1 are expected for the whole F_2 as well as for disomics and trisomics alone. The cross involving the extra chromosome on which the gene is located ("critical cross") is expected to have total segregation ratios in F_2 in the range of 8 : 1–17 : 1, depending on the transmission rate of the extra chromosome. For the disomic fraction only, a ratio of 8 : 1 is expected, whereas in the trisomic fraction, resistant plants are only expected in the rare cases of double reductions (Fig. 2).

Among F_2 disomics of crosses including cv. 'Ogra', the theoretically expected segregations were observed

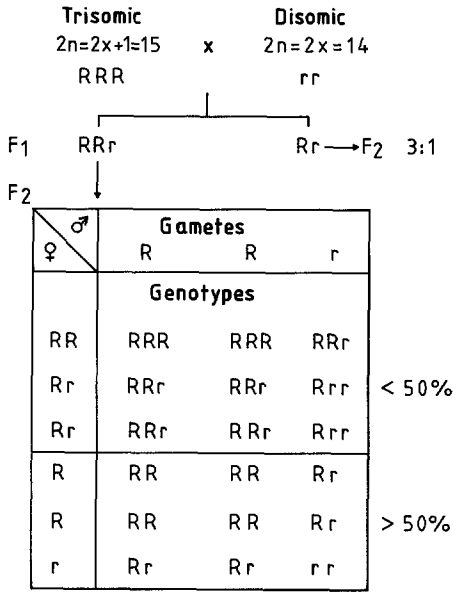

Fig. 2. Segregation for a recessive resistance gene (r) in the "critical cross" of a susceptible trisomic (RRR) to a resistant disomic (rr) line

(Table 1). In all seven F_2 populations, except the one including 'Pale' (chromosome 3) as a parent, good fit to the expected segregation, obtained by correcting the 3 : 1 ratio for escaped infections, was found; for the F_2 of 'Pale' a good fit to the corrected 8 : 1 segregation ratio was evident.

Similar results were obtained for the disomic fractions of F_2's including cv. 'Sonate' (Table 2). Data of all F_2 populations except the one of 'Pale' again show good fit to an expected segregation. On the contary, fit to the corrected 8 : 1 segregation was again obtained in disomic F_2's of 'Pale'.

Unexpected segregations with an excess of non-infected plants were found in some trisomic fractions, especially of the weakest trisomics. This finding could not be explained by the frequency of escapes as found in the controls. Because of the deleterious effects of the rough procedure of inoculation, the small and weak trisomic plants in the F_2 populations may have had higher escape rates than normal diploids. Furthermore, the rate of escape among the morphologically quite different trisomic types may vary. Therefore the trisomic data, which for completeness are given in Tables 3 and 4, do not contradict the conclusion that the resistance gene is located on chromosome 3.

Discussion and conclusions

A rather high frequency of trisomics was obtained in F_2 by selecting thin seeds. This should theoretically be an advantage; however, due to an apparently high frequency of escapes, the trisomic progenies could not be used for localization of the BaYMV-resistance gene.

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

Table 1. Segregation for reaction to BaYMV-M in F_2 disomics of crosses of 'Shin Ebisu 16' trisomics with the resistant cv. 'Ogra'

Trisomic type	Extra chrom.	Frequency of escape in control cv. 'Gerbel'	Frequency expected of non-infected	Number of disomic plants		Frequency of non-infected observed	χ^2 for deviation from exp. ratio	Probability for χ^2
				infect.	non-inf.			
Bush	1	0.08	0.31	113	55	0.33	0.24	0.70–0.50
Slender	2	0.11	0.33	126	60	0.32	0.08	0.80–0.70
Pale	3	0.11	0.33	69	17	0.20	7.04	< 0.01 *
Robust	4	0.04	0.28	109	39	0.26	0.20	0.70–0.50
Pseudonormal	5	0.00	0.25	125	46	0.27	0.33	0.70–0.50
Purple	6	0.00	0.25	65	20	0.24	0.10	0.80–0.70
Semierect	7	0.04	0.28	113	39	0.26	0.41	0.70–0.50

Test of corrected 8:1 ratio for Pale (chromosome 3): $\chi^2 = 0.064$, $P = 0.90–0.80$
* highly significant

Table 2. Segregation for reaction to BaYMV-M in F_2 disomics of crosses of 'Shin Ebisu 16' trisomics with the resistant cv. 'Sonate'

Trisomic type	Extra chrom.	Frequency of escape in control cv. 'Gerbel'	Frequency expected of non-infected	Number of disomic plants		Frequency of non-infected observed	χ^2 for deviation from exp. ratio	Probability for χ^2
				infect.	non-inf.			
Bush	1	0.02	0.27	55	27	0.33	1.74	0.20–0.10
Slender	2	0.07	0.30	91	42	0.32	0.11	0.80–0.70
Pale	3	0.10	0.33	110	20	0.15	17.36	small *
Robust	4	0.00	0.25	123	40	0.25	0.02	0.90–0.80
Pseudonormal	5	0.00	0.25	139	49	0.26	0.11	0.80–0.70
Purple	6	0.04	0.28	85	36	0.30	0.18	0.70–0.50
Semierect	7	0.00	0.25	78	19	0.20	1.52	0.30–0.20

Test of corrected 8:1 ratio for Pale (chromosome 3): $\chi^2 = 1.731$, $P = 0.20–0.10$
* highly significant

Table 3. Segregation for reaction to BaYMV-M in F_2 trisomics of crosses of 'Shin Ebisu 16' trisomics with the resistant cv. 'Ogra'

Trisomic type	Extra chrom.	Infect. rate (%)	Number of plants			χ^2 for 3:1[a] ratio	χ^2 for 9:0[a] ratio
			infect.	non-inf.	Total		
Bush	1	92	118	58	176	0.31	148.6*
Slender	2	89	24	71	95	73.67*	396.5*
Pale	3	89	42	34	76	4.52*	87.7*
Robust	4	96	98	43	141	0.44	260.1*
Pseudonormal	5	100	71	58	129	27.41*	336,310.1*
Purple	6	100	77	32	109	1.10	102,345.4*
Semierect	7	96	71	53	124	13.37*	480.2*

[a] Corrected for the rate of escapes
* significant at $P < 0.05$

Table 4. Segregation for reaction to BaYMV-M in F_2 trisomics of crosses of 'Shin Ebisu 16' trisomics with the resistant cv. 'Sonate'

Trisomic type	Extra chrom.	Infect. rate (%)	Number of plants			χ^2 for 3:1[a] ratio	χ^2 for 9:0[a] ratio
			infect.	non-inf.	Total		
Bush	1	98	92	35	127	0.07	430.9*
Slender	2	93	56	81	137	54.13*	571.1*
Pale	3	90	65	28	93	0.24	41.8*
Robust	4	100	80	38	118	3.27	144,336.3*
Pseudonormal	5	100	71	28	99	0.57	78,351.9*
Purple	6	96	74	45	119	5.69*	350.8*
Semierect	7	100	44	30	74	9.53*	89,952.2*

[a] Corrected for the rate of escapes
* significant at $P < 0.05$

This result is in contraction to earlier results of Takahashi et al. 1973, who reported the gene *Ym1* derived from 'Mokusekko 3' to be linked to the gene *K* on chromosome 4. This gene *Ym1* was shown to be allelic to the "German gene" of 'Franka' and other cultivars (Friedt and Foroughi-Wehr 1986).

However, our above conclusion is supported by recent results of Konishi and Matsuura (1987). They found that the Chinese landrace 'Mokusekko 3' and some resistant cultivars derived from it, as well as the progeny of their crosses to other cultivars, always carry the esterase isozyme pattern of 'Mokusekko 3'. These results indicate that a resistance gene of 'Mokusekko 3' would be linked to an esterase isozyme gene block at the terminal end of the long arm of chromosome 3. The authors claimed this gene as an additional resistance gene to the gene *Ym1*.

Our own results, together with the results of Takahashi et al. (1973) and Konishi and Matsuura (1987), can therefore be interpreted as follows: either the "German

gene" for resistance is allelic or closely linked to the dominant gene *Ym1* of 'Mokusekko 3', which then must also be located on chromosome 3, or the "German gene" is allelic to the postulated second, minor gene of 'Mokusekko 3' on chromosome 3. Further analysis will be necessary to clarify this open question.

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