# The Genetic Basis of Breeding Winter Barley for Resistance to Barley Yellow Mosaic Virus

W. Friedt
Biologische Bundesanstalt für Land- und Forstwirtschaft,
Institut für Resistenzgenetik

## Introduction

In recent years, winter barley crop in central and northern Europe has frequently been damaged by the soil-borne barley yellow mosaic virus (BaYMV) (HILL and Evans 1980, HUTH 1981). Increasing winter barley growing areas are infested with this new European disease, which has recently for the first time been recorded in southern Germany, too (HUTH pers. comm.). The virus is only multiplied in the barley plant when temperatures are below 15°C. Therefore, there is no doubt, that the rapid spread of the barley yellow mosaic disease was initiated by the conside-

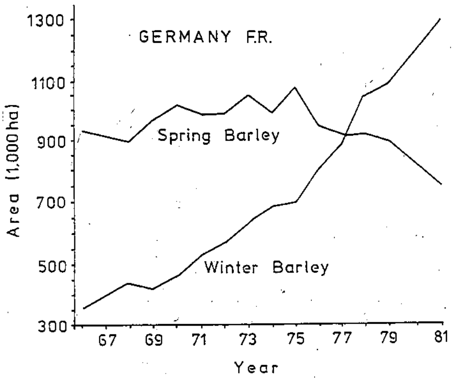


Fig. 1. Cultivation area of spring and winter barley in the Federal Republic of Germany, 1966-1981

rable extension of winter barley cultivation in the last years (Fig. 1).

As a consequence of BaYMV-infection, most of the winter barley cultivars suffer considerable yield losses, which are further modified by the climatic conditions (e.g. temperature) in early spring. On the average, the grain yield of susceptible varieties can be reduced by 70% or more as compared to a resistant cultivar like 'Birgit' (Fig. 2). Since the virus cannot be controlled by chemicals rentably, yield losses can only be prevented by growing resistant cultivars. However, the presently recommended resistant varieties are not fully satisfactory in yield at non-virus places. Therefore, it is one of the major breeding goals in winter barley to combine resistance to BaYMV with superior grain quality and yield. For that purpose it would be very helpful to know the genetic basis of BaYMV-resistance. Diverse sources of resistance should be identified and used for practical breeding. Besides conventional schemes, haploidy techniques are applied simultaneously to accelerate our breeding programme.

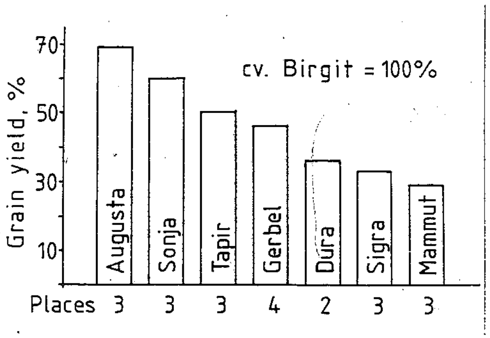


Fig. 2. Average grain yield of BaYMV-susceptible cultivars at 2 to 4 places as related to the resistant cv. 'Birgit' (=100%) (drawn from HUTH 1984)

## Materials and methods

The following winter barley ( *Hordeum vulgare* L.) cultivars and their hybrids were used:

(a) BaYMV-resistant, six-row cvs.: 'Barbo','Birgit', 'Franka','Ogra','Dea'(BGRC 4881),'Roschützer'(BGRC 4919),'Esaw'(BGRC 4970),'Wigo'(BGRC 10603) (cf. HUTH 1982), 'Mokusekko 3'(TAKAHASHI et al. 1973);

(b) BaYMV-resistant, two-row cvs.: 'Sonate','Resistant Ym No. 1' (MURAMATSU 1976);

(c) BaYMV-susceptible, six-row cvs.: 'Arma','Bosquet', 'Corona','Freya','Gerbel','Hasso','Largo','Leuta', 'Illia', LP 61444,'Mammut','Marko','Robur','Thibaut';

(d) BaYMV-susceptible, two-row cvs.: 'Alpha','Igri', and LP 8.34218.

Tests for resistance to BaYMV were carried out in a BaYMV-infested field at Sunstedt near Braunschweig and in the laboratory by mechanical inoculation (FRIEDT 1983). For inoculum preparation, leaves of plants with clear BaYMV-symptoms, i.e. yellow streaks running along the veines, were homogenized, and the sap was diluted in 0.1M $K_2HPO_4$ buffer solution (1:2 w:w). Carborundum (300 mesh) was added as an abrasive and test plants were inoculated in the 4-5 leaves stage by using a sponge. The plants were kept for one day at about 20°C and were subsequently grown at temperatures below 12°C in a greenhouse or growth cabinet. First BaYMV-symptoms became visible after about 3 weeks and were usually present in all susceptible plants 4 weeks after inoculation, provided that the inoculation fluid was prepared and handled carefully (FRIEDT and FOROUGHI-WEHR 1984).

Hybrid plants from crosses of BaYMV-resistant and susceptible parents were tested for their BaYMV-reaction. They were either multiplied for subsequent genetic analyses and pedigree selection, or were used as anther donor plants. For that purpose, plantlets were vernalized for 6 weeks at 3-4°C and 10 h light (4,000 lux) and subsequently grown in a growth cabinet at 10-12°C and 14 h light (18-20,000 lux). The plants were subsequently cultivated under natural conditions in a greenhouse and anthers were collected in the uninucleate stage of microspores. The anther culture procedure has already been described by FOROUGHI-WEHR et al. (1976) and FOROUGHI-WEHR and FRIEDT (1984). Androgenetic green plants ($A_1$-generation) were vernalized in vitro at 5°C (10 h light, approx. 3,000 lux) for 6 weeks. These plants were subsequently potted, tested for their BaYMV-reaction and grown to maturity.

Table 1

BaYMV-reactions of crosses of resistant parents to the susceptible cv. 'Igri'

| Hybrid<br>Igri x | $F_1$ | $F_2$ | Total | Suscep. | Resist. | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|
| x Birgit(♀) | Susc. | Obs. | 125 | 89 | 36 | 0.77 | 0.3-0.5 |
| | | Exp. | | 93.75 | 31.25 | | |
| x Franka(♂) | Susc. | Obs. | 129 | 95 | 34 | 0.12 | 0.7-0.8 |
| | | Exp. | | 96.75 | 32.25 | | |
| x Ogra (♀) | Susc. | Obs. | 124 | 90 | 34 | 0.39 | 0.5-0.7 |
| | | Exp. | | 93 | 31 | | |
| x Sonate(♂) | Susc. | Obs. | 128 | 92 | 36 | 0.67 | 0.3-0.5 |
| | | Exp. | | 96 | 32 | | |

Expected values are based on a single recessive gene for BaYMV-resistance.

## Results

All the tested cultivars and cross progenies showed corresponding, resistant or susceptible, reactions to BaYMV in field- and laboratory-tests. Hybrid plants ($F_1$) from crosses of resistant, German cultivars to susceptible varieties were uniformly susceptible to BaYMV (Tab. 1). The respective $F_2$-populations segregated into about 75% susceptible and 25% resistant plants (Tab. 1). On the contrary, hybrid plants derived from crosses of the resistant cultivar 'Mokusekko 3' (TAKAHASHI et al. 1973) were all resistant to BaYMV and the corresponding $F_2$-population contained 25% susceptible and 75% resistant plants (Tab. 2).

Table 2

BaYMV-reactions of crosses segregating for gene Ym 1

| Hybrid | $F_1$ | $F_2$ | Total | Suscep. | Resist. | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|
| Mokusekko x Igri | Res. | Obs. | 69 | 15 | 54 | 0.39 | >0.5 |
| | | Exp.(1:3) | | 17.25 | 51.75 | | |
| Igri x Resist.Yml | Res. | Obs. | 94 | 13 | 81 | 6.25 | <0.05 |
| | | Exp.(1:3) | | 23.5 | 70.5 | | |

Donors of gene Ym 1 described by TAKAHASHI et al. (1973) are underlined

'Mokusekko 3' contains the dominant gene Ym 1, which is also present in the two-row line 'Resistant Ym No.1' described by MURAMATSU (1976). However, $F_2$-populations derived from crosses of this line to susceptible cultivars showed an excess of resistant plants (Tab. 2). Hybrid plants from crosses of different resistant parents were all resistant and the derived $F_2$-populations did not segregate susceptible individuals (Tab. 3).

Table 3

BaYMV-reactions in crosses of two resistant parents

| Hybrid | $F_1$ | $F_2$ Total | Suscep. | Resistant |
|---|---|---|---|---|
| Barbo x Franka | res. | 31 | 0 | 31 |
| Franka x Birgit | res. | 74 | 0 | 74 |
| Birgit x Franka | res. | 44 | 0 | 44 |
| Mokusekko x Res.Ym-l | res. | 99 | 0 | 99 |
| Birgit x Res.Ym-l | res. | 300 | 0 | 300 |
| Franka x Res.Ym-l | res. | 69 | 0 | 69 |
| Sonate x Res.Ym-l | res. | 70 | 0 | 70 |
| Dea x Res.Ym-l | res. | 100 | 0 | 100 |
| Wigo x Res.Ym-l | res. | 93 | 0 | 93 |

'Res.Ym-l'='Resistant Ym No.l'(MURAMATSU 1976)

Several hybrid plants of various crosses involving cv. 'Franka' were used as anther donors. The results of anther culture are summarized in Table 4. From 17 crosses of 'Franka' to susceptible cultivars, a total of 445 androgenetic lines (A-lines) were regenerated. From a total of 261 tested lines, 159 (61%) proved to be resistant to BaYMV (FOROUGHI-WEHR and FRIEDT 1984). Out of 2 crosses of 'Franka' to resistant parents, 51 A-lines were derived which all proved to be resistant. Three of the former crosses included the two-row parents 'Alpha', LP 8.34218, and 'Igri'. From these 3 hybrids, a total of 282 A-lines were regenerated. When 132 of these were tested for their reactions to BaYMV, 79 (60%) were resistant and 30 of these, i.e. 23% of the total, were two-rowed (Tab. 4).

Table 4

Results of anther culture of 'Franka'-hybrids ($F_1$) §)

| Hybrid type 'Franka' x | No.of comb. | Number of androgentic lines Total | Tested for BaYMV-reaction Total | Res.(%) | 2row (%) |
|---|---|---|---|---|---|
| x 6row-suscep. | 14 | 163 | 129 | 80 (62) | - - |
| x 2row-suscep. | 3 | 282 | 132 | 79 (60) | 30 (23) |
| x 6row-resist. | 2 | 51 | 31 | 31(100) | - - |
| Grand total | 19 | 496 | 292 | 190 (65) | |

§) rearranged from FOROUGHI-WEHR and FRIEDT (1984)

## Discussion

The BaYMV-reactions in crosses of German, resistant varieties to susceptible cultivars demonstrate, that resistance is recessive to susceptibility in these cases. The observed segregation in $F_2$ further demonstrates that the resistance of German modern varieties is most likely inherited by a single major gene (Tab. 1 and 2), which also seems to be present in a large number of recently developed breeding lines. Although the results do not completely rule out the possibility, that additional minor resistance genes are effective in cultivars like 'Franka', it is quite evident that the genetic basis of BaYMV-resistance in our native, adapted varieties is very limited.

Besides that, numerous resistant stocks have been identified among materials stored at the Germplasm Center of Okayama University, Japan (TAKAHASHI et al. 1973) and at the German Genebank, Institut für Pflanzenbau und Pflanzenzüchtung, FAL, Braunschweig (HUTH 1982 and unpubl. data). Most of the resistant samples originate in East Asia. One of them is the Chinese spring barley 'Mokusekko 3', which carries a dominant resistance gene Ym 1 on chromosome 4 (TAKAHASHI et al. 1973). This six-rowed barley has long and very weak straw and can therefore not directly be used for commercial breeding purposes. However, the gene Ym 1 was transferred to modern, two-rowed barley types like 'Resistant Ym No.1' described by MURAMATSU (1976). When this line was crossed to German resistant cultivars, no segregation of susceptible plants was observed in the $F_2$-generation (Tab. 3). Since these native varieties possess recessive resistance genes, a segregation into 3 susceptible and 13 resistant $F_2$-plants would be expected. The fact, that no one susceptible individual occurred among 572 plants (Tab. 3) indicates therefore, that 'Resist. Ym No.1' carries an additional gene for BaYMV-resistance which is allelic to the major gene of 'Franka', 'Birgit','Sonate', and 'Dea'.
New materials from Japan, like 'Resist. Ym No.1' are distinguished by stiff straw, but unfortunately they are usually spring types. On the other hand, the resistant materials of the German Genebank described by HUTH (1982) usually show winter type, but most of these stocks are characterized by negative, "wild" traits, particularly susceptibility to lodging. Consequently, the resistances of all these non-adapted samples have to be transferred into adapted genetic background, in order to provide the commercial breeders with useful cross parents.

For the above reasons, the practical breeding for BaYMV-resistance is at present solely based on our released varieties as a source of resistance. It is therefore advisable to introduce additional germplasm in our breeding programmes to broaden the genetic basis of BaYMV-resistance.

The incorporation of new genes is most rapidly brought about by applying haploidy techniques. Via anther culture, more than 500 androgenetic lines were recovered from crosses to the resistant cv.'Franka'. Of 292 A-lines tested, 65% proved to be resistant to BaYMV (Tab. 4). From three crosses of 'Franka' to susceptible, two-row cultivars, 23% two-row, resistant lines were obtained. This frequency is close to the expected 25% (6row-susceptible : 6row-resistant : 2row-susceptible : 2row-resistant = 25% : 25% : 25% : 25%). The comparable theoretical frequency of homozygous resistant-2row genotypes in a classical $F_2$-population is 1 : 16 (i.e. 6.25%). Therewith it is demonstrated that a (recessive) character like BaYMV-resistance can be efficiently and rapidly combined with other favourable characters via haploidy techniques.

Summary

The soil-borne barley yellow mosaic virus (BaYMV) causes increasing damage in German and European winter barley crop. Such yield losses can only be prevented by growing resistant cultivars, since the virus cannot be controlled by chemicals rentably.
The presently recommended resistant German varieties are not fully satisfactory in yield at non-virus locations. They are therefore used as cross parents to study the

genetic basis of resistance and to breed new resistant lines. The results demonstrate that the resistance of all modern German breeding materials is most probably identically controlled by a single recessive gene. However, there are a great number of resistant stocks available in different germplasm centers. These materials should be introduced into adapted winter barley to broaden its genetic basis of BaYMV-resistance.

The anther culture technique has been applied for developing new resistant lines, besides the conventional pedigree selection. Within one year, about 500 androgenetic lines were regenerated from 19 'Franka'-crosses. About 300 doubled haploid lines proved to be resistant to BaYMV. They are now field-tested for other agronomic characters.

## Literature


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Address of author: Dr. W. Friedt, Institut für Resistenzgenetik der Biologischen Bundesanstalt, Grünbach, 8059 Bockhorn, FRG