

# Breeding for resistance to bymoviruses in Poaceae with special consideration for the barley yellow mosaic virus complex

W Friedt \*, F Ordon

*Institute of Crop Science and Plant Breeding, Justus-Liebig-University, Ludwigstr 23, D-35390 Giessen, Germany*

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**Summary** — Bymoviruses, *ie* BaYMV, BaMMV, WYMV, WSSMV, OMV and RNMV, cause serious damages to their host-plants, resulting in high yield losses in susceptible crops. Due to their transmission by the soil-borne fungus *Polymyxa graminis*, chemical measures against these diseases are neither efficient nor acceptable for economical and ecological reasons. Therefore, breeding resistant varieties is an important aim for plant breeding. Besides classical breeding methods completed by the production of DH-lines and marker-facilitated selection procedures, recombinant DNA-techniques may give way to new strategies in breeding for resistance to bymovirus in Poaceae in the near future.

**bymoviruses / Poaceae / resistance / barley yellow mosaic / breeding / recombinant DNA-techniques**

**Résumé** — Sélection pour la résistance aux bymovirus chez les poacées en considérant particulièrement le complexe des virus des mosaïques de l'orge. Les bymovirus : BaYMV, BaMMV, WYMV, WSSMV, OMV, RNMV causent de sérieux dégâts à leurs plantes hôtes, entraînant d'importantes pertes de rendement chez les cultures sensibles. À cause de leur transmission par le champignon du sol *Polymyxa graminis*, les traitements chimiques sont soit inefficaces, soit inacceptables pour des raisons économiques ou écologiques. Ainsi, la création des variétés résistantes est un objectif majeur de sélection. À côté des schémas classiques d'amélioration des plantes, complétés par les techniques de production de lignées haploïdes doublées par un marquage moléculaire, les méthodes de recombinaison de l'ADN pourront sans doute donner naissance dans un avenir proche à de nouvelles stratégies de sélection pour la résistance contre les bymovirus des poacées.

**bymovirus / poacées / résistance / mosaïque de l'orge / sélection / ADN recombinant**

## INTRODUCTION

The bymovirus-group founded by Usugi *et al* (1989) consists of viruses with a bipartite genome which are mostly serologically related and transmitted by the soil-borne fungus

*Polymyxa graminis*, *ie* barley yellow mosaic virus (BaYMV), barley mild mosaic virus (BaMMV), wheat yellow mosaic virus (WYMV), wheat spindle streak mosaic virus (WSSMV), oat mosaic virus (OMV), and rice necrosis mosaic virus (RNMV). Because WYMV and WSSMV show

\* Correspondence and reprints

very similar features they may be considered as strains of the same virus (Usugi and Saito, 1979; Usugi *et al*, 1989), while BaMMV and BaYMV are considered as distinct viruses (Huth and Adams, 1990). The host range of bymoviruses is quite limited, although BaMMV has been mechanically transmitted to triticale (*Triticosecale* Wittmack, Kegler *et al*, 1985), rye (Ordon *et al*, 1992) and *Triticum turgidum* (Proeseler, 1993). Despite of the limited host range, these viruses are infectious to crops of major agronomic importance, *ie* wheat, barley, oats and rice (table I). Depending on the climatic conditions they cause high grain yield losses in susceptible crops, *eg*, OMV in oats 66–78% (Catherall and Boulton, 1979) and up to 100% (Coffmann *et al*, 1962), WSSMV in wheat 3–87% (Miller *et al*, 1992; Cunfer *et al*, 1988) or BaYMV/BaMMV in barley 50–100% (Huth, 1984; Friedt *et al*, 1990). Due to the transmission by the soil-borne fungus *P graminis* chemical measures against the diseases are neither efficient nor acceptable for economical and ecological reasons. Therefore, breeding for resistance is the only way to prevent yield reductions in infested growing areas. Since RNMV is limited to Japan and India (Gosh, 1981), the following considerations on resistance breeding will focus on the viruses present in Europe with special emphasis on the type member of bymoviruses, barley yellow mosaic virus.

## BREEDING

### *Via sexual recombination*

A prerequisite for resistance breeding is the identification of resistant sources and a detailed knowledge of the genetics of resistance. Concerning OMV extensive screening programmes were carried out identifying resistant varieties and lines in *Avena sativa*, *A byzantina*, the diploid species *A strigosa* (Graham *et al*, 1969; Catherall and Valentine, 1987) as well as in *A sterilis*, the hexaploid progenitor of cultivated oats (Uhr and Murphy, 1992). However, in contrast to the yellow mosaic inducing viruses of barley, resistance to OMV is not complete, *ie* even highly resistant plants may be infected but display only slight symptoms and do not suffer from the disease like susceptible plants. Genetic analysis revealed that resistance/tolerance to OMV is quantitatively inherited (table I) and the degree of heritability was estimated to be in the range of 0.59–0.88 (*ie* 59–88%; Byrd *et al*, 1971; Uhr and Murphy, 1992).

In contrast to oats and OMV, resistance of wheat to WSSMV is complete and several sources of resistance were identified in *T aestivum* (Wiese *et al*, 1974; Jackson *et al*, 1976;

**Table I.** Members of the bymovirus group, their hosts, occurrence, sources of resistance, genetics of resistance and status of breeding.

<i>Virus</i>	<i>Crop species (Country)</i>	<i>Occurrence</i>	<i>Sources of resistance</i>	<i>Genetics of resistance</i>	<i>Breeding</i>
BaYMV BaMMV	Barley	France, UK, Germany, Belgium, The Netherlands, Italy, Ukraine, Japan, China	<i>H vulgare</i> <i>H spontaneum</i> <i>H bulbosum</i>	Simply inherited dominant and recessive genes	Resistant cultivars available
OMV	Oats	UK, Japan, New Zealand, USA	<i>A sativa</i> <i>A byzantina</i> <i>A strigosa</i> <i>A sterilis</i>	Quantitative, H = 0.7–0.8	Resistant cultivars available
WSSMV WYMV	Wheat	Canada, USA, France, Germany	<i>T aestivum</i> <i>T durum</i> <i>T monococcum</i> <i>T tauschii</i>	Two loci, additive dominant	Resistant cultivars available
RNMV <sup>a</sup>	Rice	Japan, India	<i>O sativa</i> spp <i>Japonica</i> , <i>Indica</i>		

<sup>a</sup> Of minor importance today due to raising of seedlings in sterile soil before transplantation.

Haufler and Fulbright, 1986) and *T durum* (Rubies-Autonell and Vallega, 1990). In the USA 2 varieties were recently released carrying resistance genes derived from *T tauschii* (Cox *et al*, 1994). Resistance of *T aestivum* was reported to be inherited by a single dominant gene (Wiese *et al*, 1974) or in an additive dominant manner (table I) with 3 alleles at the A-locus and 2 alleles at the B-locus, respectively (van Koeveering *et al*, 1987).

The most extensive information on resistance and genetics of resistance is available for the yellow mosaic inducing viruses of barley. Extensive screening programmes carried out in Japan (*eg*, Kawada, 1991) and Germany (*eg*, Friedt *et al*, 1990; Huth, 1991; Ordon *et al*, 1993) revealed that resistance is present in many varieties and can also be found in the ancestor of cultivated barley, *Hordeum spontaneum* (Erdogan *et al*, 1994). In genetic analyses carried out on the resistance of barley against BaMMV it turned out that different resistance genes are present within the gene pool of barley (Götz and Friedt, 1993; Ordon and Friedt, 1993; table I). These genes may even act in a specific way to the different virus of the barley yellow mosaic virus complex (Ordon *et al*, 1992b). Furthermore, genetic analysis revealed that resistance to the German isolate of BaMMV is inherited recessively in any case (Ordon and Friedt, 1993). However, since all these resistance genes encode for complete (qualitative) resistance the widespread use of only one or very few of these genes may be the reason for the selection of new virus-strains. In Japan 6 strains of BaYMV and 2 of BaMMV have already been described (Kashiwazaki *et al*, 1990b). In Europe the extensive use of the resistance gene *ym4* may be the major reason for the selection of the new strain, BaYMV-2 (Huth, 1991).

Since resistance to bymoviruses is only 1 breeding goal among many others, genes for virus resistance have to be combined with numerous other genes necessary for excellent agronomic performance. This 'combination breeding' usually requires several breeding steps. The major hosts of bymoviruses, *ie* barley, oats, rice and wheat, are self-pollinating species ('inbreeders'). Cultivars of such species are usually inbred lines and the breeding methods are often summarized as 'line breeding' methods. Specific breeding methods are, for example, the pedigree method, the bulk-population breeding method, and modifications of them.

As BaMMV-resistance is inherited by a single recessive gene, selection may be carried out as

early as in the F<sub>2</sub> generation efficiently. In contrast, selection for more complex inherited WSSMV-resistance and especially OMV-resistance will be more efficient in later generations. Donors of resistance, *eg*, primitive forms or landraces, are in general of inferior agronomic performance; this is at least the case for resistance to the yellow mosaic inducing viruses of barley (Ordon and Friedt, 1994). Therefore, backcrossing programmes have to be applied in order to combine virus resistance with sufficient agronomic performance.

The breeding methods mentioned above are time consuming and it must be taken into account that uniformly infested fields or highly efficient mechanical inoculation methods must be available for an effective selection of single F<sub>2</sub>-plants. Therefore, the introgression of resistance genes into adapted varieties may be abridged and selection in early generations becomes much easier by the use of doubled haploid (DH) lines (Foroughi-Wehr and Wenzel, 1990). Especially in breeding for quantitatively inherited type of resistance, like that of oats against OMV, DH-lines would be highly useful for an efficient recurrent selection procedure in order to accumulate resistance genes to this virus in breeding populations. Furthermore, the use of RFLP and RPAD markers offers the opportunity of an efficient marker-based selection for virus resistance today. The BaYMV/BaMMV-resistance gene *ym4* has been shown to be closely linked to an RFLP marker (Graner and Bauer, 1993) and to an RAPD marker (Ordon *et al*, 1995), respectively. Both facilitate marker-based selection avoiding the need of uniformly infested fields or mechanical inoculation procedures. Furthermore, the identification of RAPD or RFLP markers for different resistance genes offers the opportunity to combine these genes in 1 variety efficiently, *ie* the 'pyramiding' of resistance genes.

### **By recombinant DNA-techniques**

The conventional breeding methods mentioned above – including the use of DH-lines and marker-assisted selection – are the most commonly used methods in practical plant breeding today. However, recombinant DNA-techniques may offer new strategies in breeding for resistance to bymoviruses in the future. Detailed RFLP-maps of barley (*eg*, Graner *et al*, 1994), wheat (Liu and Tsunewaki, 1991) and rice (Tanksley *et al*, 1992) are available. Starting from these maps, corre-

sponding resistance genes may be isolated by map-based cloning strategies. The effectiveness of map-based cloning in isolating resistance genes has already been proven in *Arabidopsis thaliana* (Bent *et al*, 1994) and tomato (Martin *et al*, 1993). Different resistance genes against BaMMV were already integrated into the RFLP-map of barley and, notably, the *ym4* locus has been well characterized by RFLP (Graner and Bauer, 1993), RAPD (Ordon *et al*, 1995), and isozyme markers (Le Gouis *et al*, 1995) so that map-based cloning strategies may be applied to this locus in the near future. However, regeneration of protoplasts and genetic transformation of Poaceae are still problematic, although evident progress has been made recently. Nevertheless, besides a deeper knowledge of resistance, the direct transfer of isolated resistance genes into adapted cultivars would avoid the segregation of negative agronomic traits, which otherwise always takes place in breeding by sexual recombination.

The strategies mentioned are based on resistance genes derived from plant species. However, resistance to bymoviruses may also be engineered by using the corresponding genetic information of the viruses. The RNA-sequences of German and Japanese isolates of BaYMV and BaMMV – at least of the sequences encoding for the coat protein – are known (Kashiwazaki *et al*, 1989, 1990a, 1991, 1992; Davidson *et al*, 1991; Peerenboom *et al*, 1992; Bendiek *et al*, 1993; Schlichter *et al*, 1993; Foulds *et al*, 1993). Similar information is available for WSSMV (Sohn *et al*, 1994). Therefore, coat protein mediated resistance or resistance derived from anti-sense constructs – as already known from many dicot species (Steinbiss, 1993) – may be available in the future, too. However, the effectiveness of this strategy also requires efficient transformation and regeneration systems for Poaceae.

Traditional breeding schemes will definitely be supplemented by genetic transformation systems in the future. However, it must always be taken into account that most of the strategies in breeding for resistance to bymoviruses are dealing with simply inherited qualitative resistances, which may be easily overcome by new virus strains. An exception is OMV, where the quantitative resistance of cv 'Fulghum' has been working for more than 40 years (Uhr and Murphy, 1992). Therefore, despite all attempts and success already made, the race between resistance of Poaceae and virulence of bymoviruses is still on.

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