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# Molecular breeding for virus resistance in cereals

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**SUMMARY** – In Europe, soil borne viruses, i.e. Barley yellow mosaic virus (BaYMV) and Barley mild mosaic virus (BaMMV) on barley and Soil-borne cereal mosaic virus (SBCMV) on wheat as well as the aphid transmitted Barley yellow dwarf virus (BYDV) cause severe yield losses. Based on detailed genetic analyses different genes and QTL for resistance or tolerance to these viruses, respectively have been identified and molecular markers facilitating efficient marker based selection procedures, e.g. marker based backcrossing procedures and gene pyramiding, have been developed. In this paper the present state of the art concerning molecular breeding for resistance to these viruses is reviewed and future perspectives for gene isolation and breeding on the allelic level are briefly discussed.

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## Introduction

In Europe, soil borne viruses, i.e. Barley yellow mosaic virus (BaYMV) and Barley mild mosaic virus (BaMMV) both belonging to the genus *Bymovirus* and Soil-borne cereal mosaic virus (SBCMV) being a member of the Furoviruses, as well as the aphid transmitted Barley yellow dwarf virus (BYDV) cause severe yield losses in barley and wheat, respectively.

Concerning soil-borne viruses, which are transmitted by the plasmodiophorid *Polymyxa graminis* cultural practices as well as the application of chemicals as control measures are not effective, and concerning BYDV spraying of insecticides to prevent infection is not acceptable for ecological and economical reasons. Therefore, the only way to prevent high yield losses is breeding of cultivars resistant to soil-borne viruses or tolerant to BYDV, respectively.

## Barley yellow mosaic virus complex

In Europe, barley yellow mosaic virus disease is caused by two different strains of Barley yellow mosaic virus (BaYMV) and Barley mild mosaic virus (BaMMV) (Huth and Adams 1990, Hariri *et al.*, 2003, Kanyuka *et al.*, 2004a, Huth *et al.*, 2005). Screening programmes revealed genotypic differences concerning the reaction to the different members of the barley yellow mosaic virus complex (Ordon *et al.*, 1993) and genetic analyses resulted in the identification of different recessive resistance genes within the primary gene pool of barley (Götz and Friedt 1993, Ordon and Friedt 1993). Using molecular techniques these genes were mapped on chromosomes 1H, 3H, 4H, 5H, and 6H and easy to handle PCR based markers were developed (for overview cf. Ordon *et al.*, 2005a, Fig. 1). In addition to this, dominant resistance genes derived from *Hordeum bulbosum* have been mapped on chromosome 2H and 6H (Ruge *et al.*, 2003, 2006, Fig. 1).

Closely linked markers represent an efficient tool for barley breeding, since they facilitate the

selection of resistant plants without phenotypic analysis which concerning BaMYV/BaYMV-2 to a large extent relies on the climatic conditions during winter and spring time. In practise the availability of appropriate molecular markers allows doubled haploid populations (DHs) to be screened already *in vitro* and only those plantlets that carry the resistance encoding allele need to be transferred to the greenhouse.

Moreover, backcrossing procedures required to incorporate these resistance genes derived from low yielding exotic germplasms (Ordon and Friedt, 1994) into adapted high yielding cultivars can be considerably abridged by molecular markers (Ordon *et al.*, 2003). This holds especially true if genes only effective against BaYMV and BaYMV-2 have to be incorporated, because in contrast to BaMMV no efficient screening for these viruses can be conducted on the single plant level by mechanical inoculation in the greenhouse (Ordon *et al.*, 2005b). Besides this, these markers facilitate efficient pyramiding of resistance genes (Werner *et al.*, 2005, 2006). Pyramiding may become of special importance in the future as many of the recessive resistance genes known - except *rym11* - are not effective against all strains of the barley yellow mosaic virus complex (Kanyuka *et al.*, 2004a, Huth *et al.*, 2005). This approach will lead to an extended usability of these resistance genes in barley breeding, e.g. the combination of *rym5*, which at present is the sole basis of resistance to BaMMV, BaYMV and BaYMV-2 in European cultivars, with *rym9* being effective against BaMMV and BaMMV-SIL (Kanyuka *et al.*, 2004a) should result in resistance against all yellow mosaic inducing viruses known in Europe.

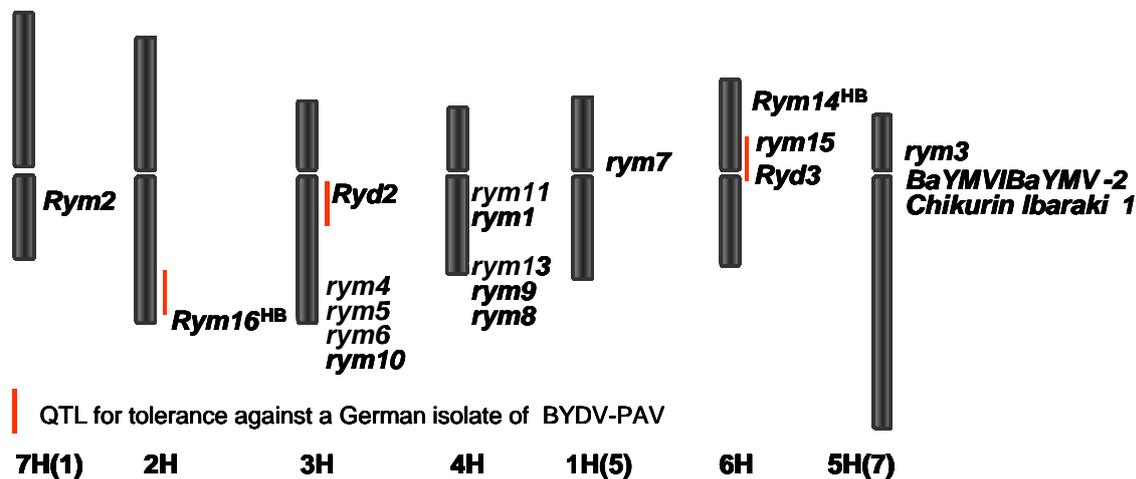


Fig. 1. Localisation of resistance genes against BaYMV/BaNMV (*rym*) and tolerance against BYDV-PAV (*Ryd*, for details cf. Ordon *et al.*, 2005a).

However, respective markers are based in general on polymorphisms around the locus of interest leading to the fact that recombination may lead to false selections. Recently, based on a high resolution map comprising about 7000 meioses (Pellio *et al.*, 2005) and BAC sequence analysis of about 440kb (Wicker *et al.*, 2005), the *Rym4/Rym5* locus located on chromosome 3H has been isolated and it turned out that this locus comprises the translation initiation factor 4e (*Hv-eIF4E*, Stein *et al.*, 2005). Specific SNPs in the nucleotide sequence of *Hv-eIF4E* confer resistance to the different strains of BaMMV and BaYMV (Stein *et al.*, 2005, Kanyuka *et al.*, 2005). Based on these SNPs efficient markers facilitating an allele specific selection have been developed and by analysing a broad barley germplasm collection different alleles of *Hv-eIF4E* have been detected which are actually analysed for their specific reaction to the different members of the barley yellow mosaic virus complex (Azhaguvel *et al.*, in prep.).

As the plant translation machinery besides *Hv-eIF4E* comprises different genes, which turned out to be involved in potyvirus resistance (Robaglia and Caranta 2006), these are valuable candidate genes for different loci encoding resistance to the barley yellow mosaic virus complex. Mapping of these candidate genes is in progress.

## Soil-borne cereal mosaic virus

Soil-borne cereal mosaic virus is becoming increasingly important in many European wheat growing areas (Koenig and Huth 2000, Yang *et al.*, 2001, Clover *et al.*, 2001). Resistance derived from the cultivar 'Cadenza' (*Sbm1*) being inherited in a monogenic manner and acting as a translocation resistance from the roots to the leaves (Kanyuka *et al.*, 2004b) has recently been located on chromosome 5DL (Bass *et al.*, 2006). Although not related by pedigree to 'Cadenza' it turned out by genetic analysis that the resistance of the French cultivars 'Tremie' and 'Claire' also follows a monogenic mode of inheritance (Perovic *et al.*, 2005) and by SSR- and bulked segregant analysis, resistance of these cultivars was also located on chromosome 5DL (Perovic *et al.*, in prep.). In order to saturate this locus with markers and to identify candidate genes, different strategies are followed. The resistance gene harbouring region of chromosome 5DL is syntenic to rice chromosome 3. Up to now 250 wheat ESTs were identified for this region located in deletion bin 5DL5 of which more than 80% showed the highest homology to rice chromosome 3 and now will be analysed in detail. Besides this, expression profiling using a 10K unigene array of barley has been conducted on parental genotypes and bulks of resistant and susceptible lines after natural SBCMV infection by *Polymyxa graminis* in growth chamber experiments. mRNA was extracted from roots, hypocotyl and leaves at the time when the virus was first detectable in these tissues in susceptible genotypes. Using this approach, about 80 genes differentially expressed in roots after SBCMV infection were identified, 11 in hypocotyls and four in leaves which are now subject to further investigations. Out of these, those differentially expressed between resistant and susceptible genotypes will be mapped in a first step leading to a structural and functional map of resistance to soil-borne viruses in wheat.

## Barley yellow dwarf virus

In contrast to BaMMV/BaYMV or SBCMV no complete or translocation resistance to the aphid transmitted Barley yellow dwarf virus (BYDV) is known in barley. Genes conferring tolerance, i.e. *ryd1* derived from the cultivar 'Rojo' (Suneson 1955) and *Ryd2* previously identified in Ethiopian landraces (Rasmusson and Schaller, 1959), have been detected soon after the first discovery of the disease. In contrast to *Ryd2*, *ryd1* was only rarely used in barley breeding due to its low efficiency. The detection and exploitation of tolerance to BYDV is hampered by the fact that for an efficient selection, cumbersome inoculation procedures using viruliferous aphids are needed. Therefore, attempts were carried out to develop molecular markers. In this respect *Ryd2* has been located on chromosome 3HL (Collins *et al.*, 1996, Paltridge *et al.*, 1998, Ford *et al.*, 1998) and several QTL for tolerance against BYDV-MAV and -PAV have been mapped on chromosomes 7H, 4H and 1H (Toojinda *et al.*, 2000). Using a German isolate of BYDV-PAV a continuous variation regarding the relative yield after BYDV infection was observed and a QTL for this trait has been located in the vicinity of *Ryd2* (Scheurer *et al.*, 2001, Friedt *et al.*, 2003, Fig. 1). Besides this, an additional QTL for the relative yield after BYDV-infection derived from the cultivar 'Post' was detected on chromosome 2HL (Scheurer *et al.*, 2001). These two QTL together explain about 50% of the phenotypic variance of the relative yield after BYDV infection. Besides this, recently a new locus called *Ryd3* also derived from an Ethiopian landrace and explaining about 75% of the phenotypic variance regarding tolerance to BYDV was identified and located on chromosome 6H (Niks *et al.*, 2004). Further efforts now aim at pyramiding these different QTL by the combined use of molecular markers and DH-techniques in order to enhance the level of tolerance. Besides this, using parental lines and DH-lines carrying positive and negative alleles at respective QTL, cDNA-AFLPs are carried out in order to identify and map genes involved in the pathosystem barley – BYDV.

In wheat, resistance to BYDV has been detected in several *Thinopyrum* species. Resistance harboring chromosome segments from *Th. intermedium* have been transferred to wheat (Banks *et al.*, 1995) and a marker specific for this resistance effective against Mexican isolates of BYDV-PAV has been developed (Ayala *et al.*, 2001). In own experiments using artificial inoculation with a German BYDV-PAV isolate and genotyping by the marker developed by Ayala *et al.* (2001) it was shown, that these introgressions also considerably reduce both the infection rate and the virus titre of infected plants with respect to German isolates of BYDV-PAV (Habekuß *et al.*, in prep).

## Conclusions

Molecular markers already facilitate efficient breeding for virus resistance/tolerance in barley and

wheat. In the last few years several resistance genes against viruses have been isolated in different plant species. Knowledge on these genes representing candidate genes for virus resistance in cereals as well as expression profiling techniques and knowledge on synteny between the sequenced rice genome and barley and wheat will lead to an enhanced isolation of virus resistance genes in cereals and a deeper understanding of respective pathosystems in the future. The isolation of genes involved in resistance and/or tolerance will transfer breeding for virus resistance in cereals to the next level facilitating the identification of novel alleles and their directed use in molecular breeding strategies in order to enhance virus resistance.

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