Recent Advances in Breeding of Cereals for Resistance to Barley Yellow Dwarf Virus – a Review

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Abstract: The review focuses on recent progress in the breeding of small grain cereals (barley, wheat, oats) for resistance to the barley yellow dwarf virus (BYDV). First, the symptomatology of barley yellow dwarf (BYD) disease is briefly described and the genome of BYDV, its serotypes and mechanisms of its replication and translation in host plants are characterized. Great attention is paid to the description of resistance genes and sources of BYDV resistance that are currently used in some breeding programmes of barley, wheat and oats. In barley, the introduction of the *Ryd2* gene into high-yielding cultivars is still desirable. An example of recent success reached in a European programme aimed at a pyramiding of resistance genes is the registration of the Italian feeding barley cultivar Doria, carrying resistance genes *Ryd2*, *rym4* and *Rdg1*. The release of this cultivar resulted from the cooperation between EICR, Fiorenzuola d'Arda and CRI in Prague-Ruzyně in the field of virus resistance. Finally, some experiments employing transgenic techniques in the construction of resistant plants are mentioned. In conclusion, the advantages and disadvantages of classical breeding methods using crossing and transgenic techniques are compared and newly arising approaches are discussed.

Keywords: BYDV; biological characteristics; breeding; resistance genes; sources of resistance; barley; wheat; oats

Barley yellow dwarf (BYD) is an important disease of small grain cereals caused by a luteovirus (Barley yellow dwarf virus BYDV) which is worldwide spread and which significantly reduces the agronomical yield of cereals. The cause of the disease, i.e. the BYDV (the virus), was described for the first time more than 50 years ago (in 1951 by Oswald & Houston); however, efficient tools to eliminate the viral infection in plants are still missing. Adequate agronomical strategies such as the date of sowing or the control of the spreading of aphids by the use of pesticides can significantly contribute to the reduction of BYDV infection; however, the genetic resistance of cultivated cereals is the most efficient tool how to eliminate the economic losses caused by BYDV.

Genes which form the basis of natural resistance in some resistant grasses have been identified and introduced into cereal genomes via crossing – the most important of them is *Ryd2* (*Yd2*) gene, which comes from Ethiopian spring barley lines and which is widely used in breeding programmes in barley, and *Bdv2* gene, which originates from an intermediate wheatgrass (*Thinopyrum intermedium*) and which has been introduced into some wheat cultivars. Apart from these two genes, some other resistance genes have been used in breeding programmes in wheat and barley. Now, many breeders struggle to introduce these genes into agronomically valuable barley and wheat cultivars which exhibit high susceptibility to BYDV infection. To reach this aim, methods enabling the rapid and reliable detection of the presence of resistance genes in the plant material are crucial. Techniques of molecular biology have enabled us to detect the presence of a gene of interest on the basis of its unique sequence characteristics which can be used as molecular markers of the gene.

Now, molecular markers of the BYDV resistance genes are searched for. The most suitable markers are PCR markers since they enable us to detect the presence of a resistance gene in a very small amount of plant material.

Apart from the sources of natural resistance, modern techniques of transgenosis have been employed in the enhancement of cereal resistance to BYDV. However, the development of these techniques is just in progress now, and their practical use in cereal breeding is limited due to EU legislation.

In the 1990's, the knowledge of the BYD problem was summed up in the book by D'Arcy & Burnett (Barley Yellow Dwarf: 40 years of progress). The biology of the virus (i.e. its genome, serotypes, its transmission) was precisely described in a review by Miller & Rasochová (1997). In the present review, we would like to describe the structure of the BYDV genome and its transmission only briefly because we will focus on the sources of natural resistance against BYDV and on the techniques of detection of resistance genes which are used in marker-assisted selection.

It should also be noted that breeding of cereals for resistance to BYDV is usually associated with breeding for resistance to other viral diseases, especially diseases caused by the wheat dwarf virus (WDV) (Vacke 1961), by the soil-borne barley yellow mosaic virus complex BaYMV-1, BaYMV-2, BaMMV (Barley yellow mosaic virus type 1, 2 and Barley mild mosaic virus; Ikata & Kawai 1940; Huth & Lesemann 1978) and by a newly detected BaMMV-SIL strain (Hariri *et al.* 2003).

WDV is a dsDNA geminivirus spread by the leafhopper *Psammotettix alienus* and has geminate capsids with icosahedral symmetry. Two strains of WDV which are specific to wheat and barley infections were distinguished (Lindsten & Vacke 1991). It was found out by Collin *et al.* (1996) that the two non-structural proteins encoded by the WDV genome and transcribed into –ssRNA, which are involved in viral replication, encode retinoblastoma-binding proteins, thus altering the course of the cell cycle in infected cells. It is the only known example of a plant virus which encodes proteins by the retinoblastoma-binding motif; before this paper was published, these proteins were known only from animal viruses. Sources of natural resistance to WDV are not known; therefore, transgenic techniques are an important alternative way of constructing resistant lines. Some experiments (e.g. Laufs *et al.* 1990; Matzeit *et al.* 1991) were carried out that were aimed at the development of vectors derived from +ssRNA encoded by WDV in order to construct efficient vectors for the transformation of some monocotyledonous plants (*Triticum monococcum*, *Zea mays*, *Oryza sativa*, *Lolium multiflorum*, etc.). Later, Xie *et al.* (1999) characterised two members of wheat GRAB proteins which interact with the specific C-terminal domain of replicase A protein of WDV and the researchers reported that the expression of GRAB proteins inhibited WDV DNA replication in cultured wheat cells. However, little information on the progress in the enhancement of WDV resistance in wheat and barley is still available in public databases.

Barley yellow mosaic viruses are ssRNA viruses with a helical capsid and are transmitted by the fungus *Polymyxa graminis*. The resistance to BaYMV in the primary gene pool of barley is determined by the presence of *rym*(*ym*) genes in the homozygous recessive state which can be quite easily accessible via the construction of doubled haploid (DH) lines from F1 microspore or anther cultures. Until now, *rym* genes named *rym1* to *rym13* and *rym15* have been described in the genome of resistant cultivars of *Hordeum vulgare* (Le Gouis *et al.* 2004; Ordon *et al.* 2004). Apart from these recessive resistance genes, two dominant resistance genes, $Rym14^{Hb}$ and $Rym16^{Hb}$, have been introgressed into cultivated barley from *Hordeum bulbosum* (Ruge *et al.* 2003; Ruge-Wehling *et al.* 2006). A comprehensive survey of progress in breeding of barley resistant to BaYMV was given by Ordon *et al.* (2004). In wheat breeding programmes, some species of the genus *Thinopyrum* are widely used as sources of combined resistance to BYDV and to various rusts (see e.g. Larkin *et al.* 1995; Ayala-Navarrette *et al.* 2007; etc.).

Disease symptoms and basic virological and biological characteristics of viruses causing BYD

Barley yellow dwarf (BYD) is a world-wide spread disease of many cultivated cereals, especially of wheat, barley, triticale and oats, which causes substantial economic losses to farmers. The main symptoms of the disease are as follows: stunting of plants, reduction in plant growth (dwarf appearance), yellowing (wheat, barley) or reddening (oats) of leaf blades along the vascular bundles,

especially of leaf tips. These colour changes are associated with the reduction of chlorophyll amount and photosynthesis, which leads to a substantial reduction in grain yield. The virus may also cause the phloem degradation and collapse of sieve elements. Moreover, the unripe grains of ill plants are more susceptible to various fungi (fungal infections) when compared with the healthy ones (D'Arcy 1995).

Originally, it was thought that the symptoms characteristic of BYD were caused by farming in wet and cool soils until the isolation of Barley yellow dwarf virus (BYDV) by Oswald and Houston in 1951. BYDV is a positive single-stranded RNA (+ss RNA) luteovirus which exists at least in six different serotypes (strains) that can multiply in the plants. The serotypes are divided into two sub-groups: subgroup I comprising BYDV-PAV, BYDV-MAV (the most frequently found serotypes in small grain cereals) and BYDV-SGV while subgroup II, often characterised as a separate virus species, containing cereal yellow dwarf virus (CYDV) includes CYDV-RGV, CYDV-RPV and CYDV-RMV. Recently, another BYDV serotype named BYDV-ORV (oat red-leaf virus) was identified by Robertson and French (2007) in isolates of diseased oat plants from Alaska. Until now, around 100 monocotyledonous species have been characterised as susceptible to either BYDV or CYDV. Of these species, economically important small grain cereals, especially wheat, barley, triticale and oats, are most notable. The virus is spread exclusively by some species of aphids: when the aphid accepts some virions of BYDV during the sucking of the phloem sap of an infected plant, it becomes a vector of BYDV and spreads the virus to other healthy plants via the sucking of the phloem sap.

Each BYDV strain is preferentially spread by quite a narrow range of aphid species: BYDV-PAV is spread by the oat bird-cherry aphid (*Rhopalosiphum padi*) and by the English grain aphid (*Macrosiphum* (*Sitobion*) *avenae*), erratically by the greenbug (*Schizaphis graminum*). BYDV-MAV is transmitted prevalently by the English grain aphid (*Macrosiphum* (*Sitobion*) *avenae*) and rarely by the oat bird-cherry aphid (*Rhopalosiphum padi*), the corn leaf aphid (*Rhopalosiphum maidis*) and the greenbug (*Schizaphis graminum*). BYDV-SGV is borne regularly by the greenbug (*Schizaphis graminum*) and rarely by the English grain aphid (*Macrosiphum* (*Sitobion*) *avenae*), the oat bird-cherry aphid (*Rhopalosiphum padi*) and the corn leaf aphid (*Rhopalosiphum maidis*). CYDV-RMV is spread by the corn leaf aphid (*Rhopalosiphum maidis*), but infrequently also by the oat bird-cherry aphid (*Rhopalosiphum padi*), the English grain aphid (*Macrosiphum* (*Sitobion*) *avenae*) and the greenbug (*Schizaphis graminum*). CYDV-RPV is transmitted preferentially by the oat bird-cherry aphid (*Rhopalosiphum padi*) and erratically by the greenbug (*Schizaphis graminum*), the corn leaf aphid (*Rhopalosiphum maidis*) and the English grain aphid (*Macrosiphum* (*Sitobion*) *avenae*).

Both BYDV and CYDV are +ss RNA luteoviruses of the total size of the genome 5.67 kbp (Miller *et al.* 1987). They form icosahedral virions of 25 to 30 nm in diameter, consisting of the RNA and of 180 subunits of 22 kDa coat protein (Miller & Rasochová 1997). The sequence of the total viral genome of the BYDV-PAV serotype was published for the first time by Miller *et al.* (1988).

The 5' half of viral RNA of both BYDV and CYDV encodes two overlapping viral polymerase genes (ORF1 and 2). ORF1 is expressed by itself (in its most abundant form) or fused with ORF2. In contrast, ORF2 is not expressed by itself, but only in a fused form with ORF1. The fused translation of ORF1 and 2 is provided by a mechanism of -1 ribosomal frameshifting. This means that during the elongation of ORF1 translation, a fraction of translating ribosomes slips back one base before the initial codon and starts translating a new ORF. This shift then allows the ribosomes to bypass the stop codon of ORF1. Apart from these two genes, the 5' half of the CYDV genome contains an extra ORF (ORF 0).

The 3' half is conserved among all *Luteoviridae* (it is also called '*Luteoviridae* block') and it consists of ORF3, ORF4 which is situated within ORF3, and ORF5. ORF3 encodes a coat protein. ORF5 is expressed in some cases together with ORF3 by a ribosomal readthrough mechanism (i.e. the translation does not stop at the 3´terminus of ORF3, but it continues till the end of ORF5). Therefore, the virus can produce two types of coat protein. The alternative type of coat protein synthesized from both ORF3 and ORF5 by the ribosomal readthrough mechanism plays an important role in the transmission of BYDV virions by aphid vectors. ORF4 encodes a movement protein which is involved in the widening of plasmodesmata during the transport of virions in the phloem across the plant. Except these ORFs, BYDV contains an extra ORF6 which is located at the 3' terminus.

The virus is not enveloped (i.e. the virions of BYDV contain no membranaceous structures, no lipids), viral RNA is only protected by the two types of coat protein which form the viral capsomere. The translation of viral RNAs takes place on cellular ribosomes and is mediated by a cap-independent mechanism which is common for many other viruses (e.g. Tobacco necrosis virus). For the cap-independent mechanism of translation, a specific 3' UTR, located between ORF5 and ORF6 on the viral RNA, is crucial. Translation of ORF1, which is situated within ORF0 in subgroup II luteoviruses, and ORF4, which is situated within ORF3, is done by the mechanism of leaky scanning (i.e. avoiding the first AUG codon, which is situated in a poor context for the initiation of translation, and starting translation at the second AUG codon, which is in a better context).

Apart from the genomic +ss RNA, additional 322-nt RNA which is called satellite RNA has been identified in greenhouse isolates of an Australian CYDV-RPV strain. This satellite RNA replicates by a rolling circle mechanism and exhibits a self-cleave activity (i.e. it functions as a ribozyme = RNA with an enzymatic activity). It forms a hammerhead tertiary structure which has been described as one of the structures of ribozymes (after Miller & Rasochová 1997).

Types of resistance to BYDV

When the resistance of cereals to BYDV is discussed, two different forms of resistance are usually distinguished:

(1) virus resistance – i.e. a low virus titre in infected plants;

(2) field resistance – i.e. reduced symptoms of BYDV infection independent of the virus titre. This type of resistance is also called tolerance to BYDV, i.e. the plant can contain substantial amounts of BYDV virions, but it can cope with them (i.e. it can eliminate the symptoms of BYDV infection and reduce yield losses). The virus titre (the accumulation of BYDV virions) is determined usually by ELISA tests using a specific anti-viral antibody raised against the viral coat protein.

Breeding of cereals with enhanced resistance to BYDV

Currently, two main ways of breeding cereals for enhanced resistance to BYDV are used:

(1) Classical breeding programmes which are aimed to increase the resistance of susceptible cultivated lines through transfer of a single gene or more genes of resistance from resistant relative species via crossing;

(2) The programmes which are aimed to increase the resistance of susceptible cultivars via transgenosis (transgenes derived usually from the viral genome, associated with the use of molecular mechanisms of inactivation of gene expression, e.g. posttranscriptional gene silencing PTGS, etc.).

(1) Classical breeding programmes

The natural sources of BYDV tolerance in barley, wheat and oats as well as in selected wild *Poaceae* species, characterised till the mid-1990's, are reviewed in Burnett *et al.* (1995) and advances in the breeding of wheat and barley for resistance to BYDV in Chrpová *et al.* (2006).

Barley

In barley, natural resistance to BYDV was described for the first time by Suneson (1955) in cultivar Rojo. The author found a single gene whose recessive allele named *ryd1*(*yd1*) was responsible for the resistance. However, the *ryd1* allele exhibited only a low efficiency in a wide range of cultivated barleys, thus it has been used in barley breeding programmes only rarely. In contrast to *ryd1*, the second resistance gene described, *Ryd2* (*Yd2*), was introduced into many barley cultivars and until now it has remained the main resistance gene used in barley breeding programmes. The *Ryd2* gene was described for the first time in 1959 by Rasmusson and Schaller in Ethiopian spring barley lines. The barley cultivars carrying the *Ryd2* gene exhibit tolerance to the two most spread BYDV serotypes, BYDV-PAV and BYDV-MAV, but the gene may be ineffective in inducing resistance to CYDV-RPV (Niks *et al.* 2004). In resistant barley lines, this gene is located on 3HL close to the centromere (Collins *et al.* 1996). It was shown by Holloway and Heath (1992) that a gene tightly linked to *Ryd2* in resistant genotypes encodes a subunit of vacuolar proton-translocating ATPase (Miller & Rasochová 1997). The *Ryd2* gene was then transferred to chromosome 3H of American spring barley cultivar Atlas 68 via crossing (Schaller & Chim 1969). Several authors (e.g. Chalhoub *et al.* 1995) suggested the

presence of multiple alleles at the *Ryd2* locus. It has been found out that the presence of *Ryd2*gene does not negatively influence important agronomic traits such as yield, kernel weight, time of heading, etc. in cultivated barley. However, the level of tolerance induced by this gene is also influenced by the genetic background of the cultivar, the isolate of the virus and the growth conditions. Besides Atlas 68, a six-rowed spring barley, the *Ryd2* gene has been introduced into other cultivars, e.g. into the two-rowed winter barley cultivar Vixen, which was derived from the cross (Coracle × Igri) × Igri (Coracle was the donor of *Ryd2* gene) (Parry & Habgood 1986). To detect the presence of *Ryd2* gene in the lines derived from a crossing with a cultivar which possesses *Ryd2* gene (e.g. Atlas 68), molecular markers which co-segregate with the *Ryd2* gene and which are easily detectable by PCR have been searched for. Two PCR markers associated with the presence of the *Ryd2* gene have been described until now – an SSR marker (CAPS marker) Ylp (Ford *et al.* 1998) and a co-dominant AFLP marker YLM (Paltridge *et al.* 1998). While Ylp represents polymorphism in a gene tightly linked to the *Ryd2* gene, YLM is not so tightly linked to *Ryd2* – the genetic distance between *Ryd2* and YLM is ca 0.7 cM (Paltridge *et al.* 1998). The authors have hypothesized that YLM may be useful in screening programmes working with large numbers of cultivars. In our set of DH lines obtained from a crossing between Atlas 68 (a resistant spring barley cultivar possessing *Ryd2* gene) and Igri (a susceptible winter barley cultivar lacking *Ryd2* gene), we used two PCR markers – an AFLP marker YLM and a microsatellite marker Ylp and it was confirmed that only Ylp marker can be recommended for the detection of the presence of *Ryd2* gene in the segregants (Ovesná *et al.* 2000). In addition, Šíp *et al.* (2006) screened the DH lines between Atlas 68 × Igri for the content of BYDV-PAV virions 9 days after the virus inoculation having used a specific quantitative ELISA technique and found out that the resistance (i.e. low virus titre in roots of seedling plants) correlated well with the presence of *Ryd2* gene in the genome of selected DH lines. However, the same authors (Šíp *et al.* 2004) previously showed on a selected set of Atlas 68 × Igri DH lines that the resistance to BYDV mediated by *Ryd2* gene was quite strongly dependent on ambient environmental conditions.

Another example presents the resistance gene *Ryd3*(*Yd3*), which is also of Ethiopian origin and which was introduced by Niks *et al.* (2004) into some barley lines derived from the Ethiopian barley line L94 × Vada cross. The resistant lines carried the *Ryd3* gene on chromosome 6H and showed highly reduced levels of BYDV virions, thus exhibiting real viral resistance rather than only tolerance to BYDV. The authors also showed that the *Ryd3* gene co-segregates with several PCR-detectable molecular markers – SSR markers HVM22, HVM14, HVM65, HVM74, Bmac0018 and Bmac0009 – which can be used for the detection of the presence of *Ryd3* gene during the breeding process.

Apart from *Ryd2* and *Ryd3* genes, other sources of natural resistance to BYDV exist in barley. Some examples of tolerant cultivars that are not derived from Ethiopian barley are winter barley Perry and Sigra (Ovesná *et al.* 2000) or winter barley Post (Burnett & Mezzalama 1990; Habekuss *et al.* 2000). In spring barley cultivars grown in the Czech Republic, genes non-allelic with *Ryd2* were detected by genetic analyses and the Ylp marker in moderately resistant cultivars Malvaz, Atribut and Madras (Ovesná *et al.* 2000).

The existence of other BYDV-tolerant cultivars that do not possess any of the known resistance genes has led to the formulation of a hypothesis of the joint effect of a larger number of minor genes. Scheurer *et al.* (2001) identified several QTLs for PAV-BYDV resistance on 2HL, 3HL (at a region containing the *Ryd2* gene), 4H and 7H in a set of doubled haploid (DH) lines derived from the Post × Vixen cross. Analogously, Toojinda *et al.* (2000) identified QTLs for resistance to BYDV-PAV and -MAV serotypes on 1H, 4H and 7H in a set of 94 DH lines from the Shyri × Galena cross.

Wheat

In bread wheat cultivars, resistance to BYDV was increased by the introduction of *Bdv1* and *Bdv2* genes. *Bdv1* gene probably originates from the Brazilian wheat cultivar Frontana and was introduced by Singh *et al.* (1993) into the North American bread wheat cv. Anza. The *Bdv1* gene was later located by Singh (1993) on 7DS in the linkage with genes *Lr34* and *Yr18* conferring adult plant resistance to leaf and stripe rusts. *Bdv2* gene comes from intermediate wheatgrass (*Thinopyrum intermedium*), a hexaploid species containing two copies of E genome and one copy of *Ti* genome. *Bdv2* gene is located in

the terminal part of chromosome 7E in *T. intermedium*. *T. intermedium* was crossed with wheat and as a result, an amphidiploid was produced between *T. intermedium* and wheat carrying a translocation of the terminal (distal) part of 7DL (the original part of 7DL of wheat genome was substituted by the terminal (distal) part of 7EL from *T. intermedium* genome carrying the resistance gene *Bdv2*). This amphidiploid was then back-crossed with wheat and as a result, wheat lines carrying the translocation of 7EL (which contains the *Bdv2* gene) were obtained. Ayala *et al.* (2001) described a microsatellite marker *Xgwm37* which can be used to detect the presence of *Ti* translocation (i.e. the presence of *Bdv2* gene) in wheat. Zhang *et al.* (2004) developed two sequence-characterized amplified region (SCAR) markers, *SC-gp1* and *SC-D04*, in order to detect the presence of *Bdv2* gene in wheat breeding programmes more easily than by conventional phenotypic screening. Analogously to barley, several QTLs for BYDV-PAV resistance were already identified in wheat – e.g. 22 QTLs in Opata × Synthetic population and 7 QTLs in Frontana × INIA66 population – one of them coincided with the position of the *Bdv1* gene on 7DS (Ayala *et al.* 2002).

However, the results of field tests in CRI in Prague did not show a high resistance level in the examined *Bdv1* and *Bdv2* gene carriers after the infection with PAV strain of BYDV and, therefore, it is highly desirable to use also some other BYDV resistant spring wheat lines with not yet identified resistance genes in both spring and winter wheat breeding programmes (Chrpová *et al.* 2006).

Oats

Additionally, several QTLs for BYDV resistance were already identified in oats by Jin *et al.* (1998) and by Barbosa-Neto *et al.* (2000). However, no single gene (major gene) conferring BYDV resistance in oats has been characterised yet, although two to four genes were proposed to confer the resistance of oats to BYDV by McKenzie *et al.* (1985).

Good sources of BYDV resistance have been found in wild relatives of cultivated oats, especially in the hexaploid species *Avena sterilis* and *A. fatua* and in the diploid *A. strigosa* cv. Saia; however, their use in the breeding of cultivated oats is still limited (see e.g. Ladizinsky 2000).

Breeding of tolerant oat cultivars has a long tradition at the Illionis Agricultural Experiment Station of the University of Illinois, Urbana-Champaign, U.S.A. In recent years, some tolerant, high-yielding cultivars of oats have been released by this institution, e.g. Rodeo, Blaze and Chaps in 1999 (Kolb *et al.* 1999a, b, c), Spurs (Kolb & Smith 2006) and others (Kolb *et al.* 2006a, b). Especially the lines IL 85-6467 and IL 86-4189 showed high resistance to BYDV in field infection tests (Vacke *et al.* 1996) and could be recommended for the use also in European oat breeding programmes.

(2) Breeding programmes using transgenosis

In recent years, breeding programmes for resistance to BYDV which use modern techniques of transgenosis have been developing in the United States. These new techniques that are used especially in the breeding of barley and oats use transgenes originating from the viral genome. McGrath *et al.* (1997) transformed oats with a modified gene which encodes the viral coat protein and observed the increased resistance of transformants to BYDV.

Koev *et al.* (1998) transformed oats with 5' half of BYDV-PAV genome which encodes polymerase genes and found the enhanced resistance to BYDV-PAV and BYDV-MAV in the transformants. The molecular mechanism of resistance is unknown; however, the authors proposed that in the transformants which express the 5' half of BYDV-PAV genome the duplexes between 5' half of the viral genome and 3' half of the -ss RNAs of the BYDV are formed during viral propagation. The duplexes are then recognized and split into small fragments of 25–30 nt by cellular enzymes.

Wang *et al.* (2000) transformed barley with hpRNA of the 3' half of the viral genome. As a consequence of the transformation, low virus titre and enhanced resistance were found in the transformants. The authors proposed that the double-stranded RNA which is formed by the stem of hpRNA expressed in the transformants activated cellular enzymes which recognize and cleave not only dsRNA but also ssRNA of the same sequence (i.e. the 3´ half of the viral genome). Thus, the transformants exhibited the low virus titre and enhanced resistance to BYDV.

Conclusions and future prospects

In recent years, significant progress has been made in the breeding of cereals for higher resist-

ance to BYDV. Genes that confer the resistance to the virus have been introduced into a relatively wide range of barley and wheat cultivars by crossing. The use of resistance genes in breeding programmes has enabled us to eliminate the use of pesticides in the control of the spreading of aphid populations. Moreover, the development of PCR markers has simplified the detection of resistance genes in plant material during the breeding process, which is crucial for the success of many breeding programmes aimed at the enhancement of resistance to BYDV.

Additionally, the analysis of the viral RNA and the boom of transgenic techniques have led to the production of transgenic plants conferring reduced levels of BYDV virions in the phloem sap. As the main reason of this fact, the inhibition of viral reproduction and formation of new virions has been supposed.

However, many problems associated with both the classical breeding approach and the transgenic approach remain still unresolved. First, the relatively low efficiency of the use of resistance genes (e.g. low efficiency in reduction of BYDV levels in slowly growing cultivars) and their negative effects on some important agronomical characteristics (e.g. reduction of grain yield) have been observed in some cultivars. Moreover, it becomes evident that the efficiency of resistance genes depends on many other factors, presumably on the genetic background of the cultivar where the resistance gene has been introduced, on the serotype of the virus (e.g. it has been reported for *Ryd2* that it is effective only against BYDV-PAV and BYDV-MAV serotypes, but not against the CYDV-RPV serotype), and additionally, on the ambient growth conditions. In Europe, the *Ryd2* gene was incorporated only into a very limited number of barley cultivars (Delogu *et al*. 1995) although the examination of different vegetative, grain yield and malting quality characters separately for *Ryd2* and non-*Ryd2* lines did not show any evidence of the adverse effect of the *Ryd2* gene on any character (Šíp *et al*. 2004). Recently, significant success in the breeding of *Ryd2*-carrying barley was achieved by Experimental Institute for Cereal Research, Section of Fiorenzuola d'Arda, Italy, where the *Ryd2*-carrying feeding, two-rowed barley cultivar Doria (Fior 7898) was released in 2006 (detailed characteristics of this cultivar are available in the documentation of Ministero delle Politiche Agricole e Forestali, 29 November 2006). The cultivar was derived from a series of crosses between *Ryd2*-carrying lines, tested for BYDV resistance in CRI in Prague-Ruzyně (Vacke & Šíp, unpublished), and the frost-tolerant winter barley Nure, within a programme aimed at pyramiding resistance genes. Besides *Ryd2* gene, two other important resistance genes were introduced into this cultivar, namely *rym4* gene, effective against BaYMV1 and BaMMV, and *Rdg1* gene, conferring "Vada" resistance to *Pyrenophora graminea*.

As discussed for the classical breeding programmes using resistance genes introgressed into cultivated cereals from natural sources of resistance, transgenic methods that have been applied to breeding for resistant genotypes have also many specific problems. First, there is a question of potential harmful effects on the physiological and agronomical characteristics of cultivars with transgene introgression that need to be tested. The second major question is the stability of the transgene in the plant genome (i.e. the possibility of its removal by natural plant mechanisms, e.g. by restriction endonucleases). Third, the activity of the transgene in the individual plant tissues should also be taken into account. It is well known from various transgenic studies that the transgene can be present in the genome, but its products cannot be detected in the plant material. The cause of this result can be very different, but presumably, the mechanisms of transcriptional and post-transcriptional gene silencing (TGS and PTGS) should be considered as the major cause.

Acknowledgements. Supported by the Ministry of Agriculture of the Czech Republic, Projects No. 1G57060 and QG50073. The authors thank Prof. N. Pecchioni from the University of Modena, Italy, for providing the registration data of the variety Doria.

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Received for publication January 29, 2008
Accepted after corrections March 3, 2008

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