Journal für Kulturpflanzen, 61 (2). S. 53–61, 2009, ISSN 0027-7479 Verlag Eugen Ulmer KG, Stuttgart

Originalarbeit

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Breeding for resistance to insect-transmitted viruses in barley – an emerging challenge due to global warming

Züchtung auf Resistenz gegen insektenübertragene Viren bei der Gerste – eine zunehmende Herausforderung vor dem Hintergrund des Klimawandels

Abstract

Due to global warming longer periods of higher temperature in autumn and winter are expected which may result in an increasing importance of insect-transmitted viruses. Investigations carried out in Saxony-Anhalt from 1998 to 2008 on the incidence of *Barley yellow dwarf virus* (BYDV) and *Wheat dwarf virus* (WDV) revealed a clear relation between the number of infection days in autumn and the BYDV-attack in winter barley fields in the following spring. In additional experiments carried out in growth chambers under controlled conditions it turned out that 10°C is the minimum temperature for an efficient transmission of BYDV by *Rhopalosiphum padi*.

In order to enhance the level of resistance to BYDV, *Ryd2*, *Ryd3* and a QTL derived from the cultivar 'Post' located on chromosome 2HL were combined using DH-lines and molecular markers. Concerning symptom expression and virus extinction first results indicate a reduction in those lines combining especially *Ryd2* and *Ryd3*. Concerning WDV extensive screening programmes were conducted, but tolerance was only detected in cv. 'Post'. First results of genetic analysis using DH-lines give hint that this tolerance is inherited in a quantitative manner.

Key words: *Barley yellow dwarf virus* (BYDV), *Wheat dwarf virus* (WDV), aphids, leafhoppers, barley, resistance, tolerance, breeding, global warming

Zusammenfassung

Vor dem Hintergrund längerer Perioden mit höheren Temperaturen im Herbst und im Winter ist künftig von einer zunehmenden Bedeutung insektenübertragener Viren auszugehen, wie es die vorliegenden Untersuchungen zum Auftreten von *Barley yellow dwarf virus* und *Wheat dwarf virus* in Mitteldeutschland belegen. Dabei zeigte sich u. a. eine deutliche Beziehung zwischen der Anzahl Infektionstage im Herbst und dem BYDV-Auftreten in der Wintergerste im darauffolgenden Frühjahr. In Klimakammeruntersuchungen zum Einfluss der Temperatur auf die BYDV-Übertragung wurden 10°C als Temperaturgrenze ermittelt, bis zu der eine Virusübertragung durch *Rhopalosiphum padi* erfolgt.

Die ersten Ergebnisse zur Pyramidisierung von *Ryd2*, *Ryd3* und eines QTL aus der Sorte 'Post', der auf Chromosom 2H lokalisiert ist, mittels molekularer Marker und DH-Linien zeigen, dass Linien mit 3 bzw. 2 Resistenz-/Toleranzgenen eine deutliche geringere Symptomausprägung und einen verringerten Virustiter aufweisen. Dabei hat der QTL auf Chromosom 2H im Vergleich zu *Ryd3* und *Ryd2* einen deutlich geringeren Effekt.

Die genetische Basis der WDV-Toleranz ist sehr eng. Lediglich die Sorte 'Post' wies in den Infektionsversuchen im Freiland ein ausreichendes Toleranzniveau auf. Untersuchungen an Populationen doppelhaploider Linien lassen auf eine polygene Vererbung dieser Toleranz schließen.

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Accepted
July 2008

Stichwörter: Gerstengelbverzwergungsvirus (BYDV), Weizenverzwergungsvirus (WDV), Blattläuse, Zikaden, Resistenz, Toleranz, Züchtung, Klimaerwärmung

Introduction

Drought and biotic stresses are two of the major challenges for barley production in Europe and Germany. Both are predicted to worsen over most of continental Europe in the climate change scenario foreseen by the Intergovernmental Panel on Climate Change (IPCC) in its last report (IPCC, 2007). Due to global warming it is expected that insect-transmitted viruses, such as the aphid-transmitted *Barley yellow dwarf virus* (BYDV) and *Cereal Yellow dwarf virus* (CYDV) belonging to the genera *Luteovirus* and *Polerovirus*, respectively as well as the leafhopper-transmitted *Wheat dwarf virus* (WDV) being a member of the genus *Mastrevirus* will become more important in the future, because longer and warmer periods in autumn will result in longer flight activities of the vectors leading to an increased risk of winter barley to get infected by these viruses. *Barley yellow dwarf virus* as the causal agent of dwarfing and leaf discoloration of barley was already detected in 1951 in California (OSWALD and HOUSTON, 1951) and is known today worldwide as a serious disease on barley (LISTER and RANIERI, 1995) causing yield losses e.g. up to 25% (PIKE, 1990). According to the transmission efficiency of aphid species 5 different strains were distinguished (ROCHOW, 1969, ROCHOW and MULLER, 1971, ZHANG et al., 1983), first. But today, these causal agents of barley yellow dwarf are classified as different viruses, i.e. *Barley yellow dwarf virus* and *Cereal yellow dwarf virus*, which are again sub-divided into different strains (MAYO and D'ARCY, 1999, FAUQUET et al., 2005). *Wheat dwarf virus* (WDV) which is transmitted persistently by the leafhopper *Psammotettix alienus* was first detected in Europe in the 1960 s in the former Czechoslovakia (VACKE, 1961) and was later on detected in different parts of Europe, e.g. Sweden (LINDSTEN et al., 1970), France (LAPIERRE et al., 1991), Germany (HUTH, 1994), Turkey (ILBAGI et al., 2005) and Finland (ERLUND, 2007). For WDV also different strains are known, i.e. a barley strain and a wheat strain (LINDSTEN and VACKE, 1991; COMMANDEUR and HUTH, 1991), which according to SCHUBERT et al. (2002, 2007) and KÖKLÜ et al. (2007) may be regarded as different viruses due to a different host range and sequence differences.

The only possibility to avoid yield losses caused by these viruses is combating the vectors by chemical sprayings. However, because of the high abundance of aphids and the high mobility of leafhoppers chemical measures are in general of limited effectiveness. In this respect growing of tolerant/resistant cultivars has to be considered as the most environmental sound and effective method to control both viruses. In case of barley yellow dwarf 3 genes conferring tolerance to BYDV/CYDV are known, i.e. *ryd1*, which was detected in the spring barley cultivar 'Rojo' (SUNESON, 1955), but was not used in barley breeding due to its low efficiency, *Ryd2*, which was detected in Ethiopian landraces and localized on the long arm of chromosome 3 near the centromere (SCHALLER et. al., 1963; COLLINS et al., 1996) and *Ryd3*, which was detected in the Ethiopian barley line L94. This gene was mapped to chromosome 6H and its effectiveness to BYDV-PAV infection is quite similar to that of *Ryd2* (NIKS et al., 2004). Furthermore, quantitative trait loci (QTL) for tolerance to BYDV have been mapped on different barley chromosomes (TOOJINDA et al., 2000; SCHEURER et al., 2001, 2003). Up to now, only *Ryd2* has been successfully used in breeding tolerant spring (e.g. ŠIP et al., 2006) and winter barley cultivars (DELOGU et al., 1995), e.g. cvs. 'Vixen' (PARRY and HABGOOD, 1986), 'Wysor' (STARLING et al., 1987), 'Venus' (BROWN et al., 1988) and 'Naturel'.

In contrast to BYDV up to now only small quantitative differences in the degree of attack by WDV are reported (VACKE and CIBULKA, 2000, 2001; BARTOS et al., 2002; LINDBLAD and WAERN, 2002; ŠIRLOVÁ et al., 2005).

Therefore, the aims of the present study are (i) to investigate the incidence of BYDV and WDV in the central part of Germany (Saxony-Anhalt) and to get information on the effect of temperature on virus transmission, (ii) to combine different genes and QTL for tolerance to BYDV by molecular markers and to get information whether pyramiding results in a higher level of tolerance, (iii) to identify sources of tolerance to WDV and get information on the mode of inheritance.

Material and Methods

Investigations on the incidence of BYDV and WDV and on the influence of temperature on virus transmission

The incidence of BYDV and WDV was monitored in about 10 to 15 winter barley fields in the period 1998 to 2008. In early spring 150 leaf samples per field (30 samples taken randomly at 5 different locations at each field) were analysed by double antibody sandwich – enzyme linked immunosorbent assay (DAS-ELISA) using polyclonal BYDV and WDV specific antibodies. DAS-ELISA was conducted according to CLARK and ADAMS (1977) and extinction at 405 nm was estimated on a microtitre plate reader (DYNATECH MR 5000).

Investigations on the influence of temperature on virus transmission were carried out using BYDV-PAV and *Rhopalosiphum padi* of a virus-free permanent aphid rearing in a growth chamber. An acquisition period of 4 days was followed by an inoculation period of 1, 2 or 4 days respectively at 10, 15, 20 or 25°C. 54 seedlings of the highly susceptible cv. 'Rubina' were inoculated with single aphids in each variant. After inoculation, the aphids were killed by insecticide spraying. Next, the plants were cultivated in a greenhouse at 20°C and 75% relative humidity. 6 weeks post inoculation the virus extinction of single plants was estimated by ELISA

as described above and the infection rate (%) in the different variants was calculated.

BYDV-resistance tests

For pyramiding of genes and QTL encoding BYDV-tolerance doubled haploid (DH-) lines of the crosses 'RIL K4-56' (*Ryd3*) x 'DH 21-136' (*Ryd2*, QTL of cv. 'Post' located on chromosome 2H, winter barley) and DH-lines of 'RIL K4-56' (*Ryd3*) x 'Coracle' (*Ryd2*, spring barley) were produced by microspore or anthere culture technique, respectively by KWS-Lochow GmbH and the Saaten-Union Resistenzlabor.

Phenotyping of DH-populations is carried out in four locations [Gudow (Nordsaat), Irlbach (Saatzucht Ackermann), Bernburg (Lochow-Petkus) and Quedlinburg (JKI)]. For this purpose 24 plants of 281 winter barley DH-lines and 188 spring barley DH-lines were artificially infected in the greenhouse in the one leaf stage using BYDV-PAV bearing aphids (10 aphids/plant) and simultaneously healthy control plants were grown. Plants of 'RIL K4-56' x 'DH 21-136' were transferred to the field at the four locations in October 2007 in two replications (2x12 plants per infected and control variant) and the same was done for the spring barley cross 'RIL K4-56' x 'Coracle' in March 2008.

The level of tolerance will be estimated using the methods described by SCHEURER et al. (2001). Up to now results on symptom expression (score 1 = plant without symptoms, score 9 = plant died) and ELISA values for selected DH-lines of the different genotypes (3 winter barley DH-lines and 6 spring barley DH-barley lines of each genotypic class with 10 to 12 plants per replication from three different locations) are available.

Genotyping

DNA was extracted using a modified CTAB method according to DOYLE and DOYLE (1990). DNA concentration was measured using the NanoDrop ND-1000 spectrophotometer (peqLab, Biotechnology GmbH, Erlangen) and adjusted to a final concentration of 30ng/µl for PCR.

For the detection of *Ryd2* the Capsmarker YlpPCRM was used according to FORD et al. (1998). Screening for the presence of *Ryd3* was conducted using the microsatellite marker HVM74 (NIKS et al., 2004) and the QTL on chromosome 2H derived from cv. 'Post' was analysed by the SSR HVCSG (SCHEURER et al., 2001). While YlpPCRM was detected on agarose gels the SSRs were detected by means of a capillary sequencer (Beckman Coulter CEQ™ 8000).

WDV-resistance tests

In the period 2002 to 2006 248 winter barley genotypes [accessions of the genebank of the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben (IPK) as well as breeding lines and cultivars] were evaluated for their reaction to WDV by artificial WDV-inoculation in the field using viruliferous leafhoppers of the species *Psammotettix alienus*. In the middle of September of each year, 12 seeds per accession were sown in two replications in an inoculated (I) and a non-inoculated (control, C) variant in the field. To increase the infection pressure, one WDV-infected barley plant was planted between each row of the I-variant shortly before inoculation. At the 1- to 2-leaf stage the plots of the I-variant were covered with a tunnel made of cotton and viruliferous leafhoppers were distributed in the tunnel in a density of approximately 1 leafhopper per plant. To keep the C-variant virusfree the plots were treated with an insecticide, regularly. After 4 weeks the cover was removed and the whole test was sprayed with an insecticide. Scoring of symptom expression was conducted at heading stage using a scale from score 1 = without symptoms to 9 = plant died. On the basis of these data the degree of attack (DA) was calculated as follows:

$$DA = \frac{\sum_{s=2}^{9} n^*(s-1)}{N^*8}$$

n = number of plants per scoring class
s = scoring class
N = number of plants with symptoms

DAS-ELISA was used to determine the virus titre of selected genotypes as described above. At harvest, plant height, number of ears per plant, kernel weight per plant and thousand kernel weight of both variants were determined. The level of tolerance was estimated as the results of the infected variant relative to the results of the control of the same genotype.

To get information on the genetics of the WDV-tolerance detected in cv. 'Post' in more detail, 2 independent populations of doubled haploid lines of the cross 'Post' x 'Vixen' comprising 86 (I) and 77 (II) lines were phenotypically analysed in gauze house tests in 2006 and 2007 as described above.

Statistical analysis

Statistics were conducted using the software package SAS 9.1. ANOVA was carried out using the GLM procedure. The significance of differences between means was tested by the Tukey-Test (α=0.05). The scores of symptom expression were compared by a bootstrap test (NEUHÄUSER and JÖCKEL, 2007) and significance was tested by contrast analyses with a t-test using the MULTTEST procedure.

The observed frequency distributions concerning tolerance to WDV were analysed for the fit to a Gaussian distribution by the Kolmogorov-Smirnov-Test.

On the basis of the existing genetic map developed by SCHEURER et al. (2001) for mapping QTL for BYDV-tolerance in the DH-population 'Post' x 'Vixen' (II), and the phenotypic data (relative values) scored for this population first preliminary QTL-analyses concerning WDV-tolerance were carried out (software MapQTL®5, Plant Research International B.V. and Kyazma B.V., Benelux and USA).

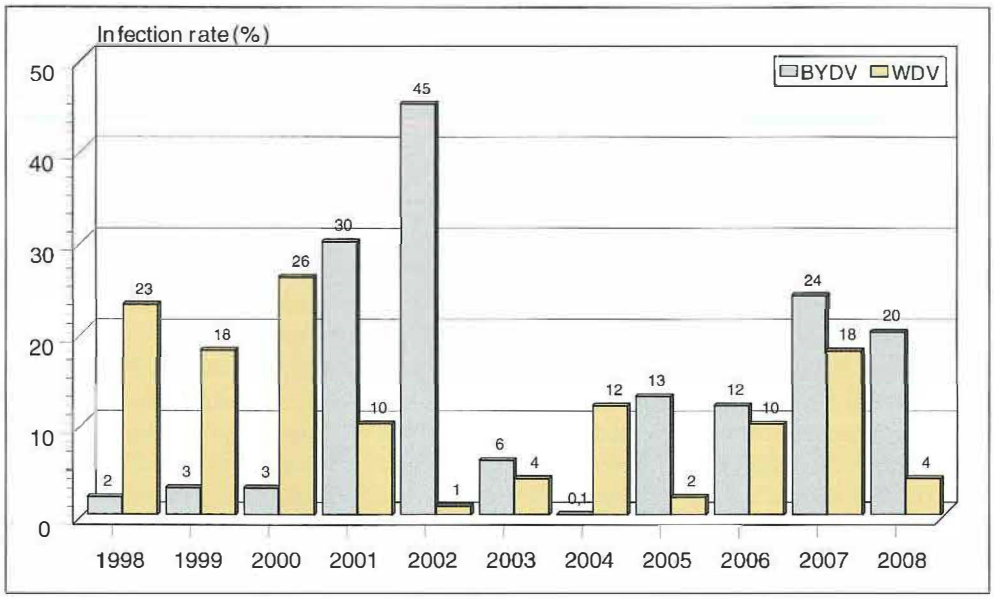


Fig. 1. Incidence of BYDV and WDV in winter barley in Saxony-Anhalt during 1998-2008.

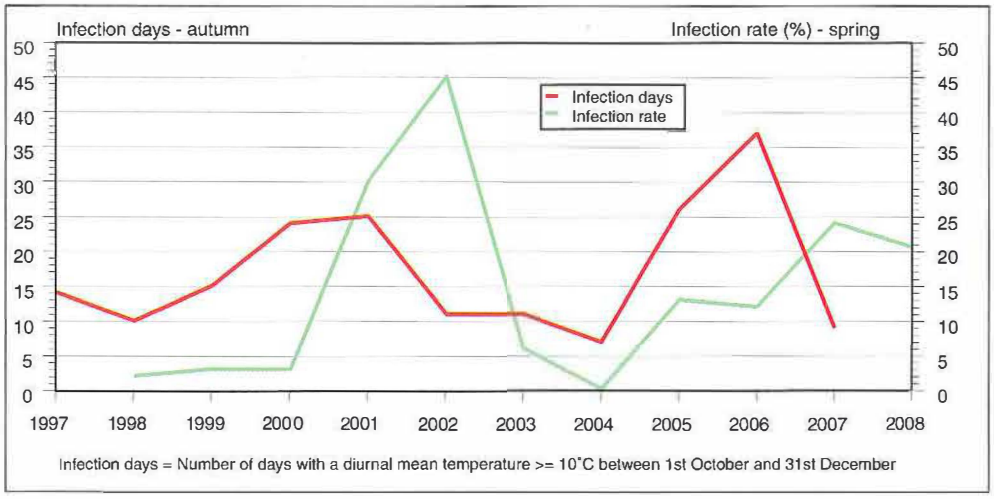


Fig. 2. Relation between BYDV-infection rate of winter barley in spring and temperature in autumn (1997-2008).

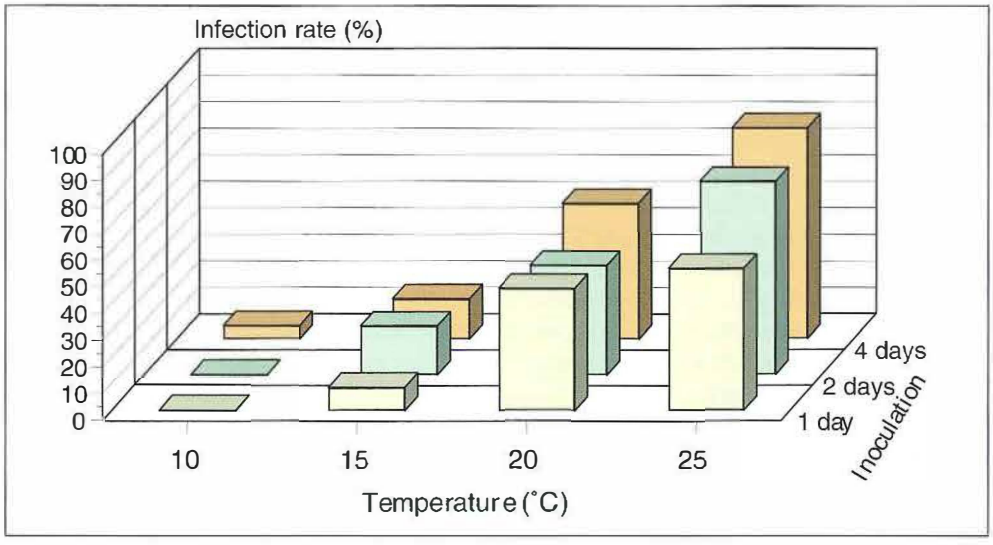
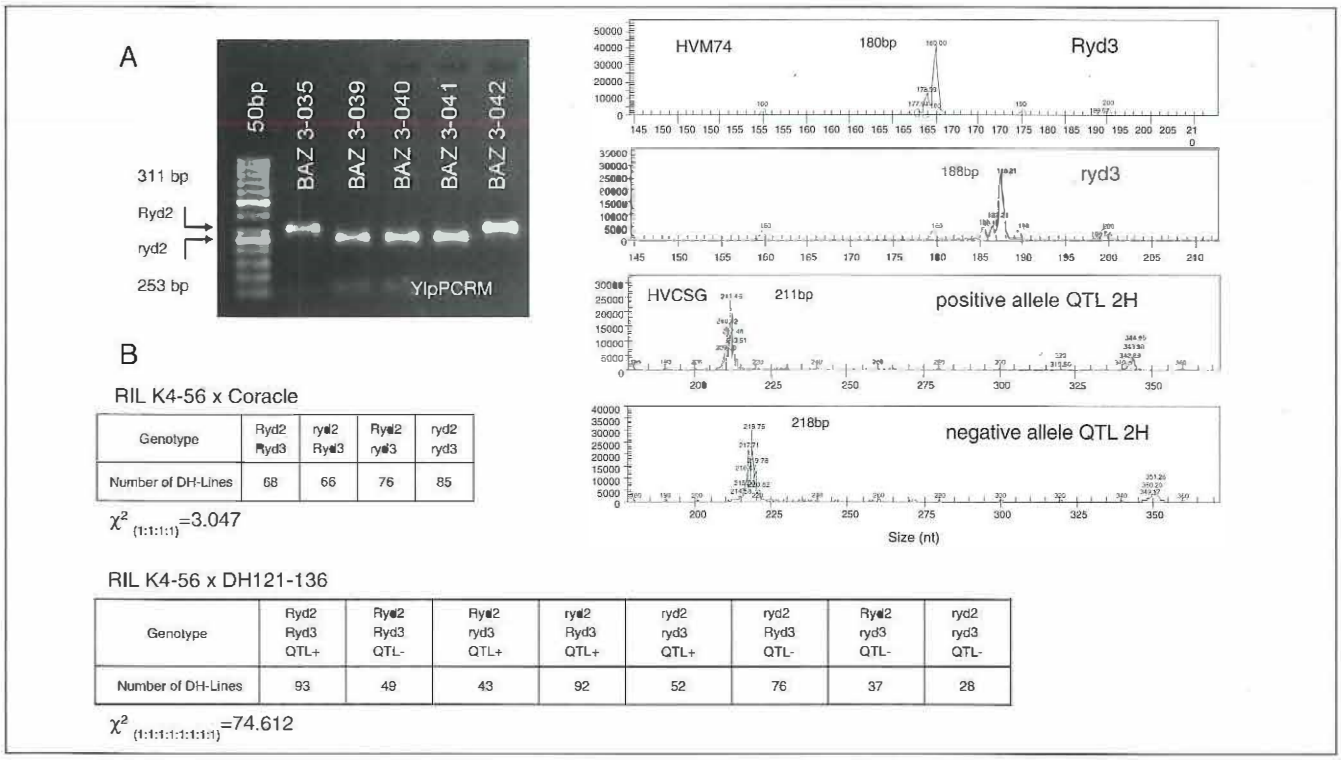


Fig. 3. Infection rate (%) of cv. 'Rubina' in relation to temperature and time of inoculation.

Results and Discussion

Incidence of insect-transmitted viruses and relation to temperature

The aphid-transmitted *Barley yellow dwarf virus* and the leafhopper-transmitted *Wheat dwarf virus* were detected in Saxony-Anhalt each year but in different frequencies. Since its first detection in Germany in 1990 (Vacke, pers. commun.) WDV has gained evident importance in winter barley (Fig. 1). In the period 1998 to 2000, and in 2004, WDV was the predominant insect-transmitted virus in winter barley in Central Germany, whereas BYDV was epidemic



Genotype	Ryd2 Ryd3	ryd2 Ryd3	Ryd2 ryd3	ryd2 ryd3
Number of DH-Lines	68	66	76	85

$\chi^2_{(1:1:1:1)}$=3.047

Genotype	Ryd2 Ryd3 QTL+	Ryd2 Ryd3 QTL-	Ryd2 ryd3 QTL+	ryd2 Ryd3 QTL+	ryd2 ryd3 QTL+	ryd2 Ryd3 QTL-	Ryd2 ryd3 QTL-	ryd2 ryd3 QTL-
Number of DH-Lines	93	49	43	92	52	76	37	28

$\chi^2_{(1:1:1:1:1:1:1:1)}$=74.612

Fig. 4. Detection of *Ryd2*, *Ryd3* as well as of the QTL of cv. 'Post' located on chromosome 2H by molecular markers (A) and observed segregation ratios (B).

Fig. 5. Reaction of genotypic different DH-lines of the population 'RIL K4-56' x 'DH 21-136' after BYDV-PAV inoculation in the field 2008.

with infection rates of 30% and 45%, respectively in 2001 and 2002. In 2006 a quite similar incidence of WDV and BYDV, i.e. 18% for WDV and 24% for BYDV was observed.

As shown in Fig. 2 there is an obvious relation between the number of days with >= 10°C mean temperature (infection days) in autumn (from the 1st of October to the first day with temperatures lower than -5°C) and the incidence of BYDV in the following spring. As can be seen in Fig. 3 the temperature limit to which BYDV-PAV is efficiently transmitted by *Rhopalosiphum padi* is at about 10°C as estimated in growth chamber experiments.

Pyramiding of BYDV-tolerance loci

Genotyping of the DH-lines was carried out by using known molecular markers. Fig. 4 shows the fragment pattern of respective markers and the number of genotypes observed for each genotypic class by analysing 470 winter barley lines of the combination 'RIL K4-56' x 'DH 21-136' and of 295 spring barley lines of the combination 'RIL K4-56' x 'Coracle'. In the spring barley combination a good fit to the expected segregation of 1:1:1:1 was observed (χ^2=3.047), while in the winter barley population a significant deviation from the expected 1:1:1:1:1:1:1:1 was detected (χ^2 =74.612).

Tab. 1. Symptom expression and virus titre (DAS-ELISA, extinction at 405 nm) of selected DH-lines representing different genotypic classes

Genotype	Mean score of symptom expression	Mean of virus extinction
A) RIL K4-56 x DH 21-136 (Means of Gudow, Irlbach and Quedlinburg)		
Ryd2/Ryd3/QTL+[1)]	2.14a[2)]	0.30e
Ryd2/Ryd3/QTL-	2.07a	0.38e
Ryd2/ryd3/QTL+	2.56a	1.37bc
ryd2/Ryd3/QTL+	2.52a	1.11d
ryd2/ryd3/QTL+	4.10c	1.56a
ryd2/Ryd3/QTL-	2.15a	1.26c
Ryd2/ryd3/QTL-	3.05b	1.50ab
ryd2/ryd3/QTL-	5.97d	1.22cd
B) RIL K4-56 x Coracle (Means of Bernburg, Irlbach and Quedlinburg)		
Ryd2/Ryd3	2.35a	0.74c
Ryd2/ryd3	3.13c	1.42a
ryd2/Ryd3	2.86b	1.32b
ryd2/ryd3	6.40d	1.26b

[1)] Capital letters and + represent alleles positively contributing to BYDV-tolerance.
[2)] Means with the same letter are not significantly different.

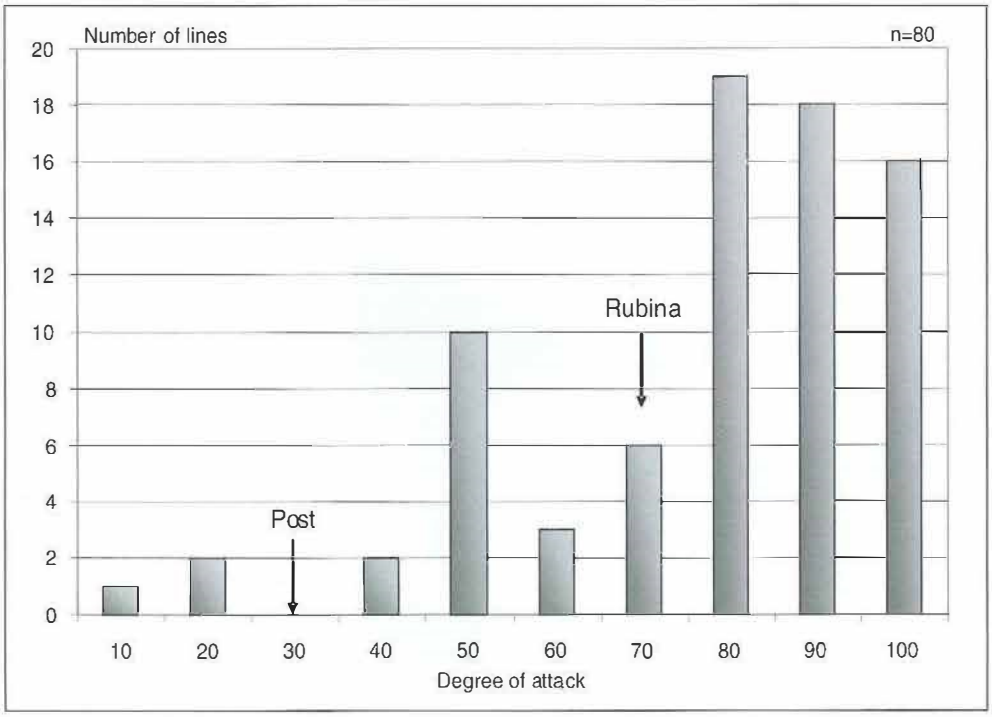


Fig. 6. Degree of WDV-attack of winter barley lines in comparison to cvs. 'Rubina' and 'Post' in the field 2006.

In the field tests 2007/2008 a clear phenotypic differentiation between DH-lines carrying no or two tolerance encoding alleles concerning symptom expression (Fig. 5) was detected. This holds true also for the virus concentration. As can be seen in Tab. 1 (A) especially those DH-lines combining *Ryd2* and *Ryd3* showed a significantly lower virus titre in contrast to DH-lines carrying only *Ryd2* or *Ryd3*. In contrast to this, the effect of the QTL on chromosome 2H on the virus concentration seems to be rather small. The same results concerning *Ryd2* and *Ryd3* was detected in the spring barley cross (Tab. 1, B). These first results which have to be replicated give hint that a combination of *Ryd2* and *Ryd3* leads to a significant reduction of the virus titre.

Screening of WDV-resistance

Out of the 248 barley accessions tested, only cv. 'Post' which is also tolerant to BYDV and 3 breeding lines having this accession in their pedigree revealed a higher level of tolerance to WDV (Fig. 6). However, no reduction in

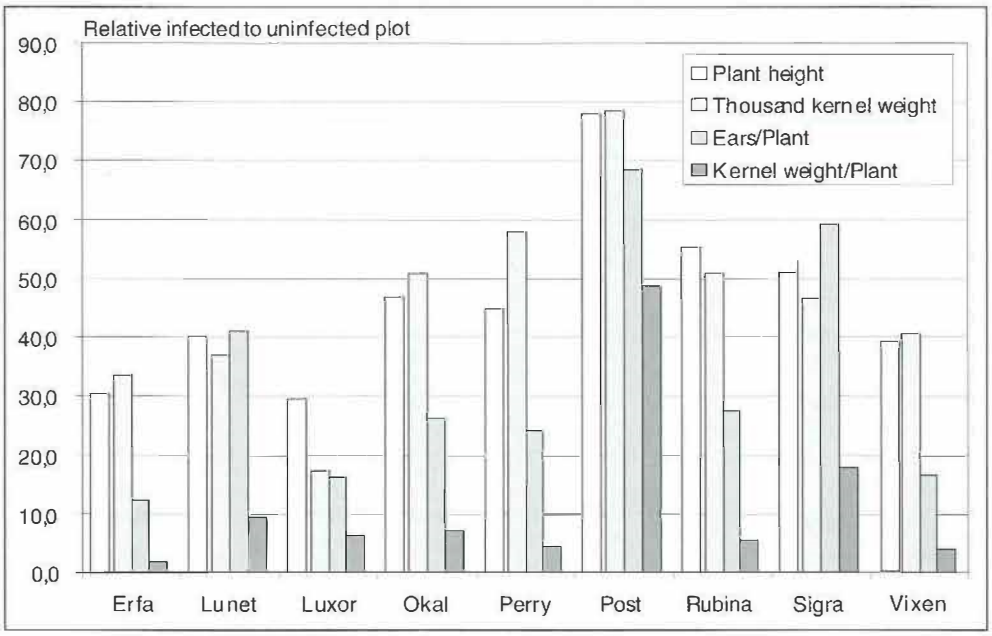


Fig. 7. Relative performance of barley genotypes concerning plant height, thousand kernel weight, ears/plant and kernel weight/plant after WDV-infection in the field 2006.

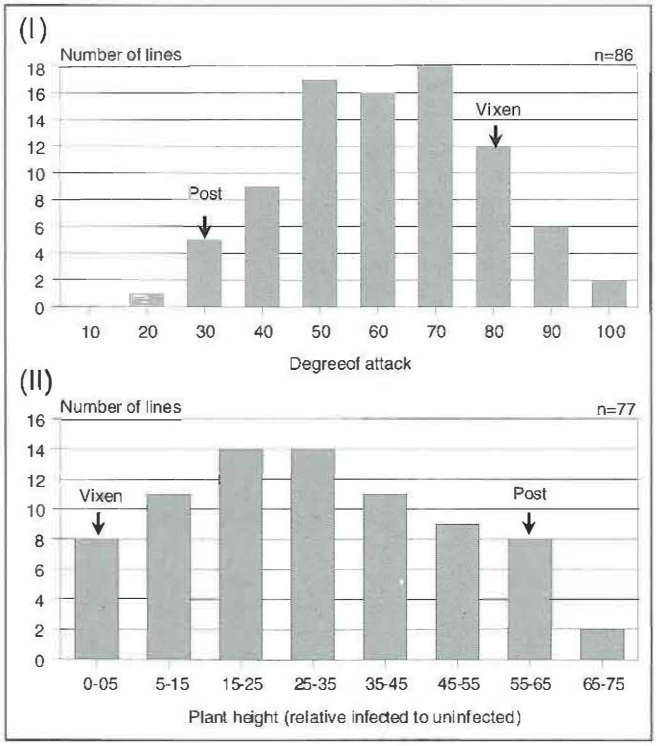


Fig. 8. Reaction of DH-population (I) and (II) of the combination 'Post' x 'Vixen' to WDV-infection in comparison to their parents in gauze house tests in 2006 (I) and 2007 (II).

virus concentration was detected in these genotypes (data not shown).

Concerning all the characters investigated, cv. 'Post' showed the highest relative values, i.e. the smallest reduction of these traits after WDV-infection. For example the kernel weight per plant was approx. 50% in cv. 'Post', but less than 10% in the other cultivars (Fig. 7).

In 2006 the DH-population (I) of the cross 'Post' x 'Vixen' was analysed for the reaction to WDV-inoculation in a gauze house. Concerning the frequency distributions a good fit to a Gaussian distribution was detected for the characters investigated, e.g. for the degree of attack ($P>0.15$) (Fig. 8, upper part). These observations indicate a polygenic inheritance of the WDV tolerance detected in cv. 'Post'. In the test of the second DH-population of this combination (II) in 2007 the high level of tolerance of cv. 'Post' was confirmed and again cv. 'Vixen' turned out to be highly susceptible (Fig. 8, lower part). Concerning the relative plant height after WDV infection also a good fit to a Gaussian distribution was observed ($P>0.15$). On the basis of these first phenotypic data of the 77 DH-lines and the available genetic map for this population (SCHEURER et al., 2001) up to now one QTL for the relative plant height after WDV infection could be detected on chromosome 4H (LOD 4.76) explaining about 26% of the phenotypic variance. However, in order to get reliable results which are a prerequisite for QTL-analyses the phenotypic characterisation of the DH-population (II) will be repeated during the next years. Respective QTL-flanking markers will offer the opportunity to simultaneously incorporate tolerance to BYDV and WDV derived from cv. 'Post' into adapted high yielding cultivars.

These first preliminary results give hint that due to global warming not only insects themselves will become more important pathogens in cereals and insects from southern regions may migrate northward e.g. the Russian wheat aphid (*Diuraphis noxia*) (CHIMELEWSKI et al., 2007), but that with rising temperatures also insect transmitted viruses will gain evident importance. In this respect it has to be taken in mind additionally, that aphids may survive the winter in an anholocyclic manner in the future causing permanent virus infections.

With respect to BYDV, molecular markers are available facilitating efficient marker based selection and marker based backcrossing procedures (ORDON et al., 2003) as well as pyramiding strategies (WERNER et al., 2005, 2007). These markers will be developed for WDV in the future. Therefore, in the future a simultaneous incorporation of tolerance to BYDV and WDV into adapted cultivars by

markers may be facilitated, avoiding the need of artificial inoculations using virus bearing aphids or leafhoppers which can not be integrated into applied breeding schemes. These tolerances to insect-transmitted viruses may be marker based combined with additional resistances to viral and fungal diseases as molecular markers are available for many resistance genes and QTL in barley (FRIEDT and ORDON, 2007). Applying such molecular breeding strategies in barley will considerably rise the level of tolerance to BYDV and WDV being a prerequisite for an environmental sound and consumer protecting barley production in case of rising average temperature in the future.

Acknowledgment

We thank the Federal Ministry of Education and Research, the Federal Ministry for Food, Agriculture and Consumer Protection and the Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V. for financial support of parts of these studies (BMBF 03i0607A, BLE-28-1-41.002-06).

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