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Evaluation of disease resistance in wheat supplemented with *Pm3b*

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Abstract

The introduction of genes into the wheat genome by genetic modification (GM) technology may change phenotypic traits in the wheat other than the introduced one. The present experiments aim at testing the efficacy of an additional *Pm3b* gene against powdery mildew, caused by *Blumeria graminis* f.sp. *tritici* into the spring wheat variety 'Bobwhite' and examines alterations in the resistance reaction against yellow rust and *Fusarium* head blight. The experiments have been realised with four independently transformed 'Bobwhite' lines and their corresponding sister-lines. The results obtained show clearly that the additional resistance gene reduces the disease severity significantly in all transformed lines but not in the sister-lines. The stability of the resistance depends on the stability of the expression of the transgene as shown elsewhere. One transgenic line shows enhanced resistance against yellow rust and two transgenic lines are slightly more susceptible towards *Fusarium* head blight infections. In the four transgenic events studied here, two lines showed changed resistance reactions against yellow rust or FHB. Therefore, side effects of the genetic modifications seem to be quite frequent. These results are important for breeding of GM wheat varieties or the testing of such varieties.

Keywords

Genetic engineering, *Fusarium* head blight, GMO, powdery mildew, transgenic wheat, unintended effects, yellow rust

Introduction

Genetic modification (GM) technology offers the possibility to introduce traits into cultivated plants which are difficult, time consuming or even impossible to transfer by other means. By this, plants can be obtained that show improved resistance to biotic and abiotic stresses, enhanced quality criteria or produce new or higher contents of secondary metabolites (SLATER et al. 2008, GUPTA 2011). Genetically modified plants bear therefore very interesting agronomic and economic potentials. However, because of environmental and health safety apprehensions as well as social and economic considerations, the use of genetically modified plants is highly controversial (DAVISON 2010). Agronomic tests of GM plants often focus on the verification of the improvement of the target trait assuming equivalence in substance. Only limited information is available on non-target effects in genetically modified crop plants under field cropping conditions (SAINT PIERRE et al. 2012). From a physiological point of view, the introduction of a foreign gene into an existing genetic background can have different types of consequences. In the first step of transformation, the insertion of the foreign gene into the genome is highly fortuitous and can hardly been directed by the operator (HANSEN and WRIGHT 1999). This fortuitous insertion may disrupt genes, provoke mutations and alter expression of other genes (BREGITZER et al. 1998). During the regeneration process of the modified cell to plants, different cultivation steps usually on agar media have to be passed (BRUNNER et al. 2011). The occurrence of physiological and genetically rearrangements such as somaclonal variations during regeneration are well documented (BAIRU et al. 2011). Finally, the expression of the new gene itself can be modified by pre-existing genes and it can modify the expression of pre-existing genes itself; plants may show pleiotropic effects (RAVEL et al. 2009).

The present work examines the influence of a transgenically introduced specific powdery mildew resistance gene in the spring wheat variety 'Bobwhite' on the reaction against the target disease and two other common fungal diseases of wheat. The tests are realised in field trials with artificial infections. The resistance reactions of the transgenic lines are compared with those of the original variety 'Bobwhite', isogenic sisterlines and recent commercial, non-transgenic, spring wheat varieties.

Material and Methods

Plant material

Experiments were performed with the spring wheat genotypes described in *Table 1*. Transgenic *Pm3b* lines are based on the CIMMYT variety 'Bobwhite S28'. Control consisted in the original variety 'Bobwhite S28', non-transgenic sister-lines and four recently released Swiss varieties. For each resistance test, particularly resistant or susceptible varieties were planted.

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Experimental design

The experiments were conducted at the research centre Pully of Agroscope Changins-Wädenswil, in 2009. Separate trials have been planted to test the resistance against powdery mildew, yellow rust and *Fusarium* head blight. The resistance tests were separated by two lines of the spring triticale 'Trado' (Agroscope/DSP). The varieties were seeded by hand in pockets consisting of 40 seeds each respecting 30 cm distance between the pockets in each direction. The replicates of each disease resistance test were separated by one line (infection line) of a particularly susceptible variety (*Table 1*). No fungicide treatment was applied. The presence of the frit fly (*Oscinella frit*) was monitored until heading (BBCH stage 40). When more than 1 egg·m^{-2} was counted, chemical control using Karate Zeon (Syngenta Agro AG, Dielsdorf, Switzerland; 75 l in 300 l water·ha^{-1}) was applied.

Table 1: **Origin and description of the wheat lines and varieties tested in this work**

Name	Description	Origin/Breeder
Pm3b-1tg	Transgenic line with *Pm3b*	Univ Zürich, B. Keller
Pm3b-1sl	Non transgenic sister-line of Pm1tg	Univ Zürich, B. Keller
Pm3b-2tg	Transgenic line with *Pm3b*	Univ Zürich, B. Keller
Pm3b-2sl	Non transgenic sister-line of Pm2tg	Univ Zürich, B. Keller
Pm3b-3tg	Transgenic line with *Pm3b*	Univ Zürich, B. Keller
Pm3b-3sl	Non transgenic sister-line of Pm3tg	Univ Zürich, B. Keller
Pm3b-4tg	Transgenic line with *Pm3b*	Univ Zürich, B. Keller
Pm3b-4sl	Non transgenic sister-line of Pm4tg	Univ Zürich, B. Keller
Bobwhite	Original variety of the *Pm3b* transgenic lines	CIMMYT, Mexico
Frisal	Commercial variety	Agroscope/DSP
Toronit	Commercial variety	Agroscope/DSP
Fiorina	Commercial variety	Agroscope/DSP
Casana	Commercial variety	Agroscope/DSP
Rubli	Commercial variety	Agroscope/DSP
Oï--	Experimental line; comparison for powdery mildew	Agroscope
OïS	Experimental line; comparison for powdery mildew	Agroscope
Eridano	Commercial variety; comparison for yellow rust	SPS Bologna, Italy
Aletsch	Commercial variety; comparison for yellow rust	Agroscope/DSP
Nadro	Commercial variety; comparison for FHB	Agroscope/DSP
Sonalika	Commercial variety; comparison for FHB	CIMMYT, Mexico

Artificial infections

Isolates of all pathogens have been collected on the Swiss territory and represent the virulences present (MASCHER et al. 2010, 2012a). Powdery mildew isolates (*Blumeria graminis* fsp. *tritici*) were maintained in planta and multiplied in the greenhouse on the particularly sensible genotypes 'Kanzler' (Saatzucht Engelen) and 'Oï$^{2-}$' under cellophane bags, to keep isolates isolated. Infected plants were planted in-between the infection lines of the powdery mildew test. Yellow rust isolates (*Puccinia striiformis*) were conserved freeze-dried at 3°C and multiplied on the susceptible varieties 'Coker' and 'Eridano' (SPS Bologna). Plants presenting a big amount of spores of the pathogen were planted into the infection lines of the powdery mildew tests. Additionally, spores suspended in mineral oil were spray-inoculated. Infections in the *Fusarium* head blight resistance tests were realised with isolates of *Fusarium culmorum*. Isolates were mass-produced on humidified oat kernels in Erlenmeyer flasks. Wheat genotypes were infected 3 times at flowering at a concentration of 10^6 spores·ml^{-1}.

Symptom scoring

In the powdery mildew and yellow rust tests, severity was scored according to the infected surface of leaf on a 1 to 9 scala (1: no infection; 9:100% infection) following a logistic progression scheme (*Table 2*). For *Fusarium* head blight, data on the disease incidence, i.e. frequency of infection, were collected. Here, number of infected ears on a sample of 30 spikes per replicate was counted. According to the onset and the duration of the infection, symptoms were scored in average five times.

Statistical analysis

The experiment was set up as a complete randomized block with four replicates.

Table 2: **Scoring scheme for the estimation of disease severity**

Score	Surface with visible symptoms (%)
1	0
2	2.5
3	12.5
4	25
5	50
6	75
7	87.5
8	97.5
9	100

Each block was one replicate and harboured 18 wheat varieties. The disease severity and incidence scorings were integrated with the duration of observation (in days post infection) result in the calculation of the area under the disease progress curve (AUDPC). Subsequent statistical analyses were done on the relative AUDPC, (AUDPC value divided by days of observation).

Statistical analyses consisted of testing the significance of differences between all varieties using ANOVA (Analysis of Variance). Single differences between individual varieties were tested with Tukey's HSD post-hoc test. All tests were retained significant at $P<0.02$. Statistical calculations were done with SigmaPlot 11.0 (Systat Software Inc., Chicago, USA).

Results

Powdery mildew

The results of the powdery mildew test are displayed in *Figure 1*. The four transgenic lines bearing the resistance gene *Pm3b* showed an elevated level of resistance in comparison to the original 'Bobwhite' line and their corresponding sister-lines. The commercial cultivar 'Toronit' that contains the *Pm3b*-gene (O. Moullet, pers. commun.) was also highly resistant.

Yellow rust

The results of the yellow rust tests are displayed in *Figure 2*. Infection levels of the susceptible control variety 'Eridano'

displayed the elevated infection pressure in the resistance test. 'Bobwhite' and the derived transgenic and sister lines showed a high infection level. Exception made for the transgenic line *Pm3b*-2tg showing a low infection level, comparable with the commercial varieties which are regarded as resistant.

Fusarium head blight

The incidence of the *Fusarium* head blight infections are in *Figure 3*. In comparison to the susceptible control varieties 'Sonalika', the infection levels are rather low. 'Bowhite' shows an intermediate resistance, that is statistically not different with the other 'Bobwhite' derived transgenic and sister-lines. When comparing transgenic lines and their sister-lines, the transgenic lines *Pm3b*-1tg and *Pm3b*-2tg show a statistically significantly higher disease incidence than their sister-lines.

Discussion

In this study, we compared the disease resistance of four transformation events of 'Bobwhite' with the *Pm3b* gene (BRUNNER et al. 2011). For each event, we used the transgenic line and its sister-line. The sister-line is the non-transgenic isoline obtained after segregation in the T1 generation. The sister-line has passed therefore the same transformation and regeneration process as the transgenic line without hosting the transgene. This is therefore a perfect tool to study eventual phenotypically modifications due to the biotechnological processes, as for instance somaclonal variations.

The powdery mildew resistance test shows clearly that the supplementary *Pm3b* resistance gene reduced strongly the disease severity in comparison to the original variety

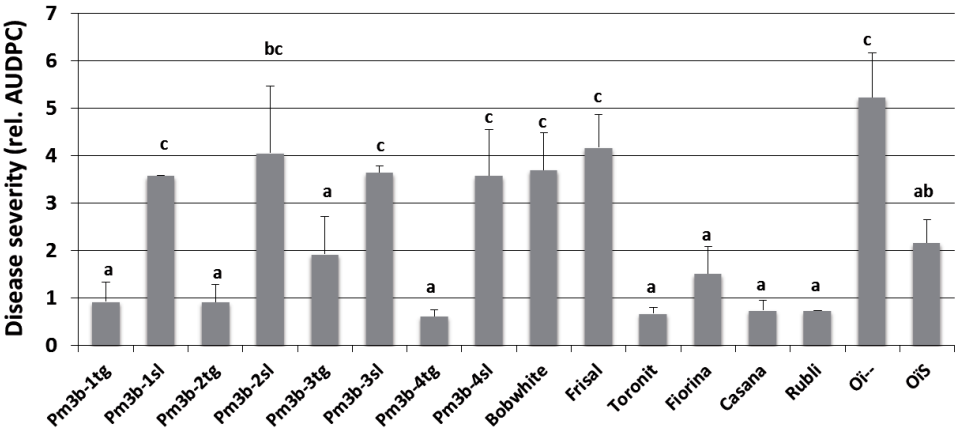

Figure 1: **Disease severities of the wheat genotypes tested in the resistance test with powdery mildew.** Bars designate the standard deviation; different letters indicate statistically significant differences at $P<0.02$.

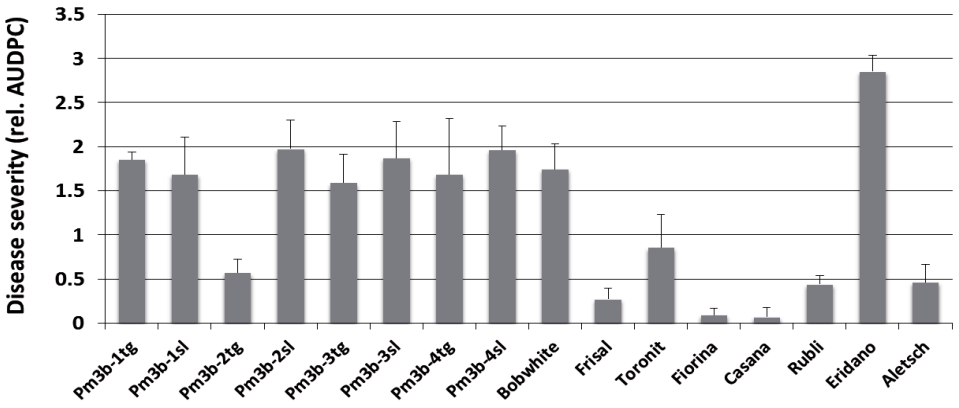

Figure 2: **Disease severities of the wheat genotypes tested in the resistance test with yellow rust.** Bars designate the standard deviation; different letters indicate statistically significant differences at $P<0.02$.

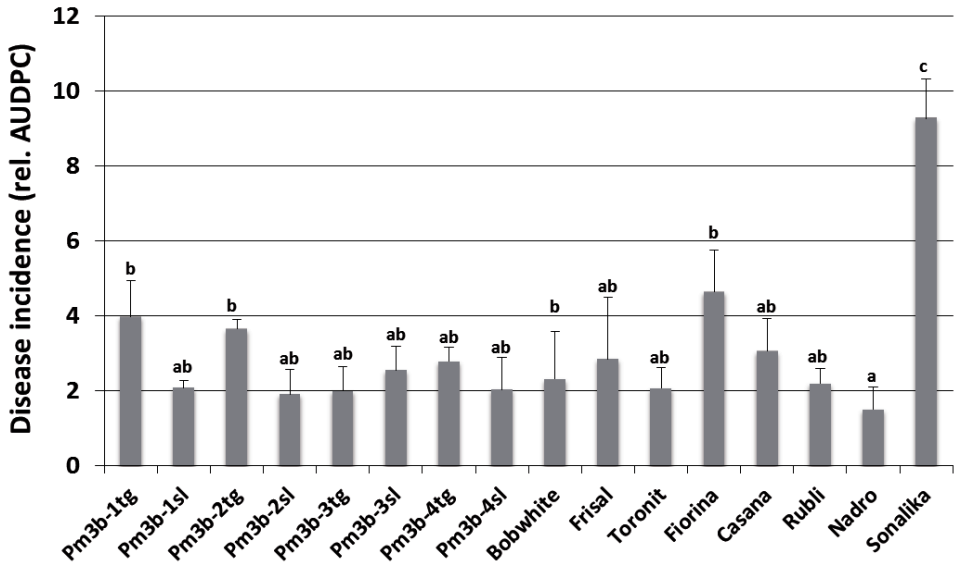

Figure 3: **Disease incidences of the wheat genotypes tested in the resistance test with *Fusarium* head blight.** Bars designate the standard deviation; different letters indicate statistically significant differences at $P<0.02$.

'Bobwhite' and the sister-lines. This has been proven in several other independent experiments (BRUNNER et al. 2011, ALVAREZ-ALFAGEME et al. 2011). The resistance reaction of the transgenic line *Pm3b*-3tg shows huge standard deviation (*Figure 1*). Previous expression studies have given evidence that the transgene is not expressed equally among all the individual plants of this genotype. In certain individuals, the gene is silenced (BRUNNER et al. 2011).

The transgenic line *Pm3b*-2tg shows enhanced resistance against yellow rust. BRUNNER et al. (2011) demonstrate that this line overexpresses the *Pm3b* gene. It is conceivable that an overexpressed resistance gene leads to a resistance reaction against another pathogen. This hypothesis applies exclusively to yellow rust since there was no alteration in the resistance to brown rust, *Septoria* leaf and ear diseases or smuts (results not shown). The finding of an interaction of a specific powdery mildew reaction with yellow rust might be an interesting starting point for complementary studies.

In the FHB study, the presence of the *Pm3b* gene seemed to enhance susceptibility to *Fusarium* infection of the spikelets. It is well known, that resistance against FHB is governed by a myriad of genes (BUERSTMAYR et al. 2009). It is conceivable that the presence of supplementary genes might interfere directly with minor resistance genes or housekeeping genes.

Obviously, transgenesis conferred not only novel traits to the variety 'Bobwhite', but induced also changes in its resistance reaction against other diseases in field trials. This is an important finding that has to be considered in eventual future variety trails with transgenic wheat. For breeding, it is probably necessary to test a large number of transformed lines to be able to select those that do not show non-desired side effects induced by genetic modifications.

Modern wheat breeding is based on pedigree selection (FOSSATI and BRABANT 2003). The progress made with genomic breeding is continuously included in the selection processes (MOULLET et al. 2008). The information obtained in the present work contributes to the understanding of the way resistance genes act. Genes such as *Pm3b* are routinely used in breeding programmes and it is of utmost importance to make the use of these resistance genes more durable. Testing of transgenic wheat lines must include not only the common yield, quality and resistance tests but must consider eventual side effects and other phenological variations.

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