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Utilization of molecular markers in Czech wheat breeding programmes

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Abstract

Selection by molecular markers (MAS) might be an efficient breeding tool especially for programmes of strategic importance, where other test procedures are not effective, costly or difficult. The advantage of molecular markers can be also time, when breeders can receive information about the location of target genes in the plant or progeny. The Czech breeding company Selgen has utilized markers for a couple of decades. Determination of baking quality by protein markers is applied extensively and effectively especially in regard to the determination of low baking quality. Breeding for BYDV resistance was enhanced by the *Bdv2* marker. Fusarium head blight is widely studied and Sumai 3 based markers are already used but further studies are required. From our experiments it can be concluded that small and medium size breeding companies need a broad cooperation with universities and public research institutions to develop and utilize new markers.

Keywords

Baking quality, barley yellow dwarf virus, Fusarium head blight, selection, *Triticum aestivum*

Introduction

Practical breeding is different from academic research because new cultivars must combine many useful traits, not only one, two or three of them. The optimum numbers of plants and progenies for populations are growing with the selection of every new trait. Phenotype based selection for agronomically important traits is straightforward but it has several limitations. Environmental influence on symptom expression may result in inaccurate classification and for some traits phenotyping is costly and time consuming. Hence, selection by molecular markers, so called marker assisted selection (MAS), might be an efficient breeding tool especially for programmes of strategic importance, where other test procedures are not effective, costly or difficult. Some authors also stated that the number of crosses and tested progenies can be reduced (KNAPP 1998). In plant breeding the application of molecular markers can be carried out for several breeding steps: parental selection for crossing, backcrossing, three way crossing, segregating populations, plant (ear) progenies (F_3, F_4) and juridical protection of cultivars. The advantage of molecular markers is also time, when breeders can receive information about the location of target gene(s) in plant or progeny. For parental selection and crossing programmes we need the information before flowering, for F_2, F_3 and plant progenies before selection for harvest. The time limitation is important for consideration about numbers of populations planned for MAS. KOEBNER and SUMMERS (2003) reported three main advantages of MAS: (i) possibility to select on single plant bases, (ii) selection for traits under multigenetic control and (iii) detection of recessive genes in early generations and for backcross programmes.

Quality breeding and MAS

We would like to demonstrate our experience with markers in the wheat breeding programme of the plant breeding station Stupice. In wheat progress gene identification and marker development have been slow due to the hexaploid nature and large size of the wheat genome. In our wheat breeding programme attention to the improvement of protein composition is paid. Biochemical markers for baking quality, for frost resistance and disease resistance have been used. Electrophoretic analyses for glutenin and gliadin subunits are included on a broad scale since the last 30 years (ŠAŠEK et al. 1982, 1984). Annually 60-80 crosses of winter and spring wheat have been analyzed. Gliadin markers were partly also used for frost resistance selection. The correlation with our tests for frost resistance was high (r=0.44-0.61) (ŠAŠEK et al. 1982, 1984). Also widely used was protein marker *EP-D1b*, which predicts the presence of *Pch1*, the gene conferring resistance to eyespot. Unfortunately *Pch1* gene is negatively correlated with yield potential and our new breeding lines with eyespot resistance were not registered (HANIŠOVÁ et al. 1993).

Biochemical markers have been applied for parents and plant progenies in F_3 and F_4 (F_5) generations and for maintenance breeding. For classical electrophoresis from one grain we need at least 5 analyses per progeny. To increase the labour capacity the method of mixed samples was developed (KUBÁNEK et al. 1999). The mixed samples from plant progenies, rests after sowing (20-100 grains), are used for analyses. The codominant heritability enables to detect parental or recombinant gametes, homozygous or segregating progenies in HMW glutenin subunits. The effect of HMW glutenin subunits on the final baking quality and final quality group is not directly related. It is evident that

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Table 1: **Baking quality, Payne score (PAYNE and LAWRENCE 1983) and HMW glutenin subunits of registered varieties in Czech Republic**

Variety	Baking quality (UKZUZ)	Payne score	Glu-A1	Glu-B1	Glu-D1	1BL/1RS
Vlasta	B	10	1	7+8	5+10	
Akteur	E	9	1	7+9	5+10	
Sulamit	E	8	0	17+18	5+10	
Bohemia	A	8	0	17+18	5+10	
Alana	A	8	0	7+8	5+10	
Samanta	B	8	0	7+8	5+10	
Sakura	C	8	0	7+8	5+10	
Cubus	A	7	0	7+9	5+10	
Dromos	C	7	1	7+9	2+12	
Ludwig	A/E	6	0	6+8	5+10	
Kerubino	A	5	0	7+9	2+12	
Rapsodia	C	4	0	17+18	2+12	+
Hedvika	B	4	0	6+8	2+12	
Etela	C	3	0	6+8	2+12	+

the presence of the 1BL/1RS translocation always predicts low baking quality (*Table 1*).

Disease resistance breeding and MAS

For MAS in wheat an increasing number of agronomically important genes have been tagged with linked microsatellite markers in recent years. Most of them are resistance genes. Some of the markers are not effective anymore since the resistance genes were overcomed, others, however, are already used in many programmes for a long time.

Selgen decided to apply MAS for traits which are difficult to evaluate with conventional methods, i.e. in resistance breeding against Fusarium head blight (FHB) and barley yellow dwarf virus (BYDV). Some resistance sources were used in these programmes for a long time, but with low effectiveness, because the screening in early generations was not possible by field test methods.

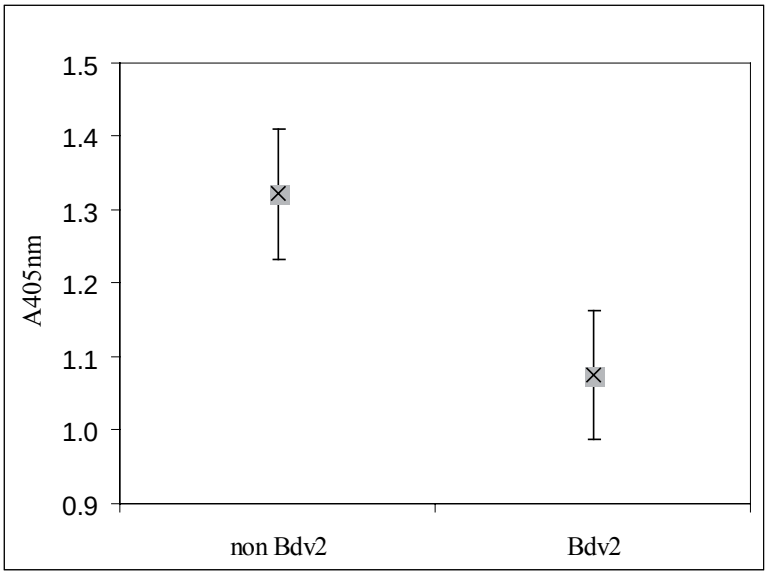

Figure 1: **Average absorbances in the infected plant leaves on the 11th day after inoculation with BYDV-PAV (ELISA, 405nm) and 95% Tukey HSD for groups of *Bdv2* and non-*Bdv2* spring wheat lines (VESKRNA et al. 2009)**

Resistance programme to virus diseases was started in 1992 in artificial infection tests in the Research Institute Praha-Ruzyně by Ing. J. Vacke. In these tests 15-20 breeding lines of winter and 10-15 lines of spring wheat were tested annually. Two winter wheat cultivars showed moderate resistance and two resistant lines, SG-S604-96 and SG-S26-98, were selected in spring wheat (BARTOŠ et al. 2002).

Resistance sources from the CIMMYT programme and translocated lines from *Thinopyrum intermedium* with known molecular markers for BYDV resistance were crossed in the wheat breeding programme of breeding station Stupice since 1997. Until now only the *Bdv2* marker was used for these crosses. The marker looked very promising due to a positive effect of the presence of *Bdv2* on absorbances in the infected plant leaves (*Figure 1*). In contrary, the effect of *Bdv2* on field evaluation after artificial inoculation with BYDV-PAV was very variable (*Figure 2*).

Fusarium head blight (FHB) is one of the most destructive diseases of wheat causing reductions in grain yield and quality. FHB resistance breeding via traditional methods is difficult because resistance is quantitative in nature and incomplete. In addition, the most resistant sources are not adapted, susceptible to other diseases and have poor combining ability (CHEN et al. 2003). Conventional testing of FHB resistance is also very costly. In the Selgen wheat breeding programme at Stupice FHB tests cost approx. 65 € per line. Markers have been proven to be a powerful tool for tagging genes associated with FHB resistance. For the use in breeding programmes, we developed STS markers for the 3BS QTL region, which was found to be more frequently transferred into recurrent backgrounds (CHEN et al. 2003). Crosses with sources for which molecular markers were described (e.g. Sumai 3, Ning 7840, CM 82036, Ernie, etc.) were prepared. We would like to control backcrossing programmes on plants and plant progenies by MAS. The effect of the presence of QTL on FHB infection in the F_2 population of the cross Sumai 3×Swedjet was tested. The presence of two markers 5A+3BS increased the ratio of plants with higher resistance, but still some percentage of

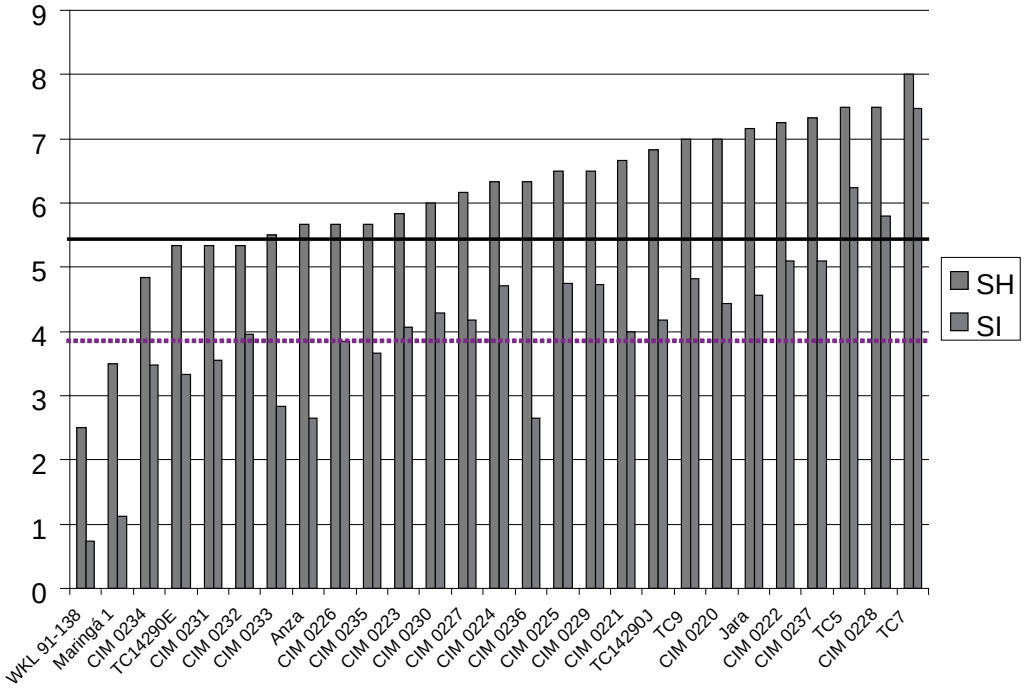

Figure 2: **Symptom evaluation (SH) and symptomatic index (SI) (0 resistant, 9 susceptible) of spring wheat (*Bdv2* positive) after artificial inoculation by BYDV-PAV (VESKRNA et al. 2009)**

susceptible ones were present (*Figure 3*). There is a number of plants with no verified marker for FHB resistance. After further selection of the best 10 lines in F_5 FHB occurence and DON content were evaluated. The group with the 3BS marker had lower DON content and lower FHB occurence (*Table 2*). Resistance of non-marker plants was lower, but the best lines were comparable to marker-positive ones. Many present varieties with good FHB resistance (e.g. Sakura, Simila, etc.) were selected by classical methods in the breeding station Stupice. Field tests demonstrated the broad variation of FHB resistance between different varieties and the low effect of fungicides (*Table 3*).

Breeders need to modify breeding methodology continuously. Breeders should increase collaboration with molecular genetics, plant pathologists and other research workers. MAS is a type of indirect selection and breeders would like to use it more and more in the future. The extent of their application in breeding process depends on: (i) sources with desired resistance or other useful traits, (ii) number of markers, (iii) number of entries and (iv) working capacities. Costs of analyses and esspecially reliability of markers in breeding programmes will play more and more important role.

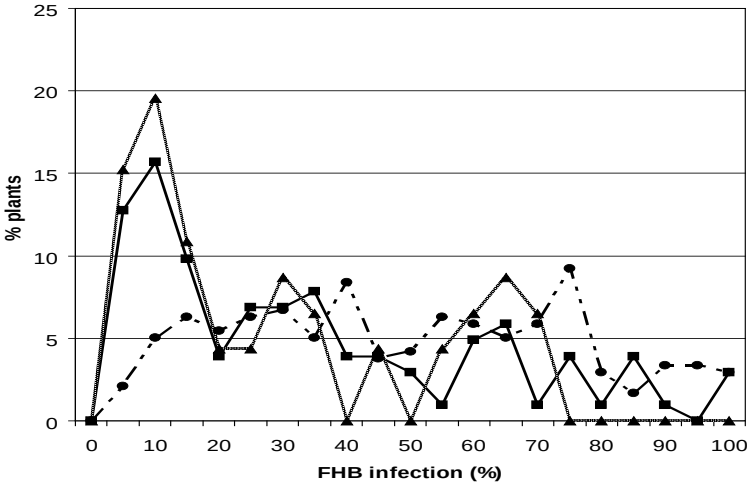

Figure 3: **Effect of the presence of FHB resistance QTLs (▲ 5A+3BS, ■ 3BS, ● none) on infection level in the F_2 population of Sumai 3×Swedjet**

Table 2: **Effect of the presence of the 3BS FHB marker on FHB infection occurence and DON content in the best 10 F_5 breeding lines of Sumai 3×Swedjet**

	FHB (%)			DON (ppm)		
Genotype/Marker	average	min	max	average	min	max
Sumai 3×Swedjet F_5						
533 3BS	6,8	7	10	12,2	5,0	21,3
none	11,6	5	20	17,5	9,3	23,7
Swedjet	50,0			35,0		
Sumai 3	5,0			14,0		

Table 3: **Variety group (R, medium tolerant; M, medium susceptible; S, susceptible) means of inoculated plots (I) and plots treated with fungicide (IF) for DON content, FHB disease severity (1, no symptoms visible) and relative yield reduction (% to uninfected control) in 2007-2009 experiments at two locations**

	DON (ppm)		FHB (1-9)		Relative yield (%)	
Variety group	IF	I	IF	I	IF	I
R	0,52	0,92	0,8	1,4	100	97
M	1,62	2,60	2,0	2,6	98	92
S	3,67	7,82	3,3	4,5	101	87

Molecular markers are now available for many traits. Their high costs still restrict their use in middle sized companies like Selgen. Therefore, close cooperations with research institutes like Research Institute of Crop Production in Prague-Ruzyně for the development of gene maps, molecular markers, primers, methods, training and consultations of specialists working in breeding, pre-breeding and development of new sources, including transgenic sources is important.

MAS will remain laboratory-based breeding, an indirect method, which must be confirmed by conventional tests. It is necessary to test breeding lines in interactions with climates, environments, under different biotic and abiotic stresses and to test end-use quality. KOEBNER and SUMMERS (2003) mentioned that MAS should remain servant of the field breeder and not its master. Wheat breeding will continue to be driven primarily by selection in breeder's plots rather than by detection in laboratory plates.

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