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INTROGRESSION BREEDING – EFFECTS AND SIDE EFFECTS  
OF MARKER-BASED INTRODUCTION OF TWO NON-ADAPTED  
*QTL* FOR FUSARIUM HEAD BLIGHT RESISTANCE INTO ELITE WHEAT

MINI-REVIEW

ABSTRACT

Fusarium head blight resistance (FHB) can be achieved by using improved adapted varieties as crossing partners or by a marker-assisted introgression of mapped QTL from non-adapted sources. In this long-term study *Fhb1* on chromosome 3BS and *Qfhs.ifa-5A* located on chromosome 5A were introgressed into European elite spring and winter wheat to test effects on FHB resistance and side effects on agronomic performance and *F. graminearum* isolates and mixtures. The introgression of the QTL *Fhb1* and *Qfhs.ifa-5A* from the Sumai 3-descendant CM82036 could be performed in the shortest possible way by marker-assisted backcrossing. They were both validated in European elite wheat background. Effects on FHB resistance were, on average, only about half of the effect in the original mapping populations. In the best phenotypically and marker-selected BC<sub>0</sub> line of spring wheat FHB was reduced from 40 to 4.3% of disease symptoms by introgressing both QTL, in the best BC<sub>3</sub> line of winter wheat the reduction was 28 and 37% for the moderately resistant and highly susceptible recurrent parent, respectively. Introgression of both QTL simultaneously did not result in significantly higher FHB resistance than introgression of only one of both QTL. Small significant negative effects on grain yield were detected in the Anthus but not in the Opus BC<sub>3</sub>F<sub>2,5</sub> backcross population when both QTL were introgressed. Backcrossing with only *Qfhs.ifa-5A* did not reduce grain yield significantly. Differences in heading date, plant height and quality traits were in all cases small although often significant. Selection of lines with improved resistance level and similar high yield level like the recurrent parent was feasible. Stability of FHB resistance mediated by both QTL was stable across 22 *Fusarium* isolates from Europe and Canada and six binary mixtures. Competition effects between isolates in mixtures were obvious but could not be attributed to the resistance of the host. In conclu-

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sion, marker-based backcrossing is a feasible option for introgressing *Fhb1* or *Qfhs.ifa-5A* QTL into the high-yielding, quality-oriented European wheat gene pool.

Key words: FHB – QTL - resistance –wheat

## INTRODUCTION

Fusarium head blight (FHB) resistance is an important objective of most wheat breeding programs. The disease is caused by *Fusarium graminearum*, *F. culmorum* and some other *Fusarium* species. Winter wheat (*Triticum aestivum* L.) is a main crop in Germany grown on 3.33 million ha in 2010 (DESTATIS 2010). FHB resistance is quantitatively inherited with a considerable genetic variation among breeding materials (Mesterhazy 1995; Miedaner 1997). Highly resistant varieties reduce the mycotoxin levels significantly (Miller *et al.* 1985). To improve resistance levels and detect new sources of resistance tremendous efforts were made for identification, validation, and fine mapping of FHB resistance quantitative trait loci (QTL) in recent years. In a comprehensive meta-analysis Löffler *et al.* (2009) compared 101 out of 176 FHB published resistance QTL and found that most of the chromosomes of hexaploid wheat were associated with FHB resistance. The most important and widely used QTL is *Fhb1* on chromosome 3BS, which explained 20 to 40 % of the phenotypic variance in the mapping populations (Anderson *et al.* 2001; Bürstmayr *et al.* 2003; Zhou *et al.* 2002). A second important QTL is *Qfhs.ifa-5A*, which is located on chromosome 5A, and was detected in a cross between Remus and the Sumai3-derived CM-82036 (Bürstmayr *et al.* 2003). This QTL explained 23 % of the phenotypic variation in the original mapping population. Further major resistance QTL with comparably smaller effects are *Fhb2* and *Fhb3* that were fine mapped on chromosomes 6BS and 7AL, respectively (Cuthbert *et al.* 2007; Qi *et al.* 2008). *Fhb1* is used widely in North America, for example in the US cultivar Alsen (Gamotin *et al.* 2007; Mergoum *et al.* 2007). Sumai 3 and Frontana, however, are inferior for grain yield, lodging tolerance, and other disease resistances and therefore, not yet exploited in European wheat varieties. Because of the very high yield level of 7 and 8 t ha<sup>-1</sup> in Germany (DESTATIS 2010), European breeders are extremely cautious to use non-adapted germplasm and prefer resistance donors from their own programmes or European varieties. By rather intensive multi-step selection for FHB resistance accumulation of minor FHB resistance QTL in the European winter wheat pool has been achieved (Holzapfel *et al.* 2008) resulting in moderately resistant varieties. The overall objective of this long-term research was to exploit the feasibility of marker-assisted introgression of the non-adapted QTL *Fhb1* and *Qfhs.ifa-5A* from the Sumai 3-descendant CM82036 into European elite wheat populations. In particular, we aimed

for (1) validation of both QTL in European elite wheat background and, more recently, for the analysis of (2) FHB resistance effects in elite European spring and winter wheat, (3) possible side effects on grain yield and quality, (4) stability of FHB resistance concerning *Fusarium* isolates and binary mixtures.

All experiments incorporated disease resistance trials on two-row microplots after spray inoculation of the entries at full anthesis by the *F. culmorum* isolate FC46. As traits, FHB rating (0-100%) was visually rated several times on a whole-plot basis and deoxynivalenol (DON) content was measured by a commercially available immunotest (RIDASCREEN, r-biopharm, Darmstadt). Agronomic performance was evaluated on large drilled plots (about 5 m<sup>2</sup>) with usual agronomic measures. All trials were tested in two to three replicates at several environments (location-year combinations) across Germany.

#### MARKER-BASED INTROGRESSION OF THE QTL *FHB1* AND *QFHS.IFA-5A*

Introgression was started by incorporating three QTL, *Fhb1* and *Qfhs.ifa-5A* mapped in CM-82036, and *Qfhs.ifa-3A* mapped in Frontana, into the susceptible spring wheat varieties Nandu and Munk in 2000 by two to three closely linked DNA markers per QTL (Fig. 1).

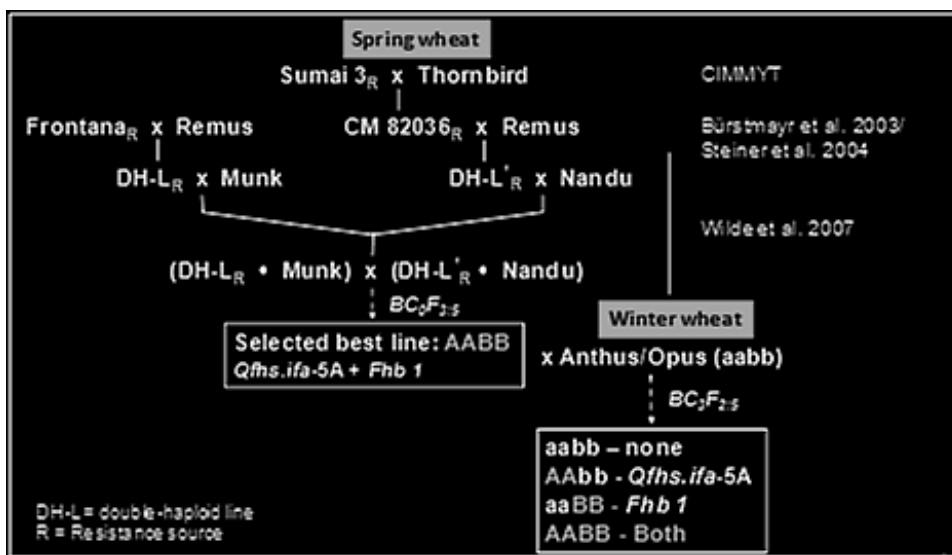


Fig. 1 Introgression of non-adapted QTL *Fhb1* and *Qfhs.ifa-5A* from CM-82036 and *Qfhs.ifa-3A* from Frontana into susceptible spring wheat and backcrossing of the best selected spring wheat line into the winter wheat varieties Anthus and Opus

Introgression resulted finally in superior spring wheat lines bearing three QTL individually and in all combinations (Miedaner *et al.* 2006, Wilde *et*

*al.* 2007). For further studies, the two FHB resistance QTL *Fhb1* and *Qfhs.ifa-5A* were introgressed from the best selected spring wheat line containing both resistance QTL homozygously into winter wheat (von der Ohe *et al.* 2010). The recipient parents were the moderately resistant variety Anthus and the susceptible variety Opus. Lines were marker selected with three flanking markers for *Fhb1* and two for *Qfhs.ifa-5A* after all backcross ( $BC_x$ ) generations to ensure that the QTL alleles were present. After the first two selfing steps the populations were again selected for the target QTL to be homozygous and assigned to the following four marker classes: *Qfhs.ifa-5A* (AAbb), *Fhb1* (aaBB), both QTL present (AABB) or neither of them (aabb) comprising 25 and 15 lines in the Anthus and Opus backcross populations, respectively. Selected  $BC_3F_2$  derived-bulks in generation  $BC_3F_{2:3}$  were propagated twice resulting in  $BC_3F_{2:5}$  and phenotypically tested in the field (von der Ohe *et al.* 2010).

#### VALIDATION IN EUROPEAN ELITE WHEAT BACKGROUND

The effects of two QTL of the Sumai 3-derivative CM 82036 and the QTL from Frontana could be validated in an independent European elite background. They were estimated, on average, as 10% reduced FHB rating for each of *Fhb1* and *Qfhs.ifa-5A* and as 5% reduction for *Qfhs.ifa-3A* (Miedaner *et al.* 2006). These values are considerably lower than those estimated in the original mapping populations (Bürstmayr *et al.* 2003, Steiner *et al.* 2004).

A crucial question for the breeder is, whether he achieves a higher selection gain for phenotypic *vs.* marker-based selection. We compared both procedures across one cycle of recurrent selection and tested both variants phenotypically thereafter. The mean realized response from selection per year was 3.2 *vs.* 4.4% for the phenotypic *vs.* marker variant, respectively (Wilde *et al.* 2007). They additionally reduced DON content from 24.9 mg kg<sup>-1</sup> in the unselected source population to 7.8 mg kg<sup>-1</sup> in the variant with two QTLs without any direct selection for DON. The best progeny had a similar resistance and DON content like the most resistant parent CM82036. *Fhb1* has also been validated in diverse North American backgrounds (Zhou *et al.* 2003, Anderson *et al.* 2007). The non-adapted donor-QTL alleles had a considerably higher effect than those from European winter wheat where the realized selection gain per year was 2.1 *vs.* 2.5% for the phenotypic *vs.* marker variant, respectively (Miedaner *et al.* 2009).

## FHB RESISTANCE EFFECTS IN ELITE EUROPEAN SPRING AND WINTER WHEAT

When comparing the best selected single lines containing both QTL (AABB) or neither QTL (aabb), disease symptoms decreased in highly susceptible backgrounds considerably (Table 1). The different effect of the resistance QTL on FHB resistance in spring and winter wheat could be explained by (1) varying proportion of the donor genome ( $BC_0$  vs.  $BC_3$ ), (2) different genetic background, i.e. genetically different recipient varieties and/or general genetic differences between spring and winter wheat, (3) additional minor QTL contained either in the donor or the recipient parent. In accordance with the findings of Salameh (2005), we confirmed that these QTL acted additively together independently of the background. This underlines the interest in combining major QTL to increase the resistance level (von der Ohe *et al.* 2010).

Wilde *et al.* (2007) suggested that the best way to use the full range of quantitative variation for resistance is to first apply marker-assisted selection (MAS) followed by phenotypic selection. This procedure allows the incorporation of minor QTL which have been undetected in QTL mapping studies.

## SIDE EFFECTS ON GRAIN YIELD AND BAKING QUALITY

Using effective QTL from non-adapted sources in commercial wheat breeding requires to estimate their potential side effects on other agronomic traits due to linkage drag or background effects. With each of 25 and 15 lines, the four QTL classes were tested in the Opus and Anthus  $BC_3F_{2.5}$  population, respectively, across five locations and two years (von der Ohe 2010). FHB resistance was significantly ( $P < 0.05$ ) improved between classes carrying either one or two QTL. One of the two non-adapted QTL already sufficed for the maximal effect. In the class containing both QTL grain yield was significantly negatively affected by 1.6 % in the Anthus but not in the Opus  $BC_3F_{2.5}$  population. Entries with only one of either QTL showed no significantly different grain yield compared to the class without QTL. Due to the significant phenotypic variation for FHB resistance and grain yield within each class selection of lines with improved resistance level and similar high yield level than the recurrent parent would be feasible. For other agronomic and quality traits, differences were small in all cases and negligible but often significant. Quite similar results were obtained by Salameh (2005), who is stressing the effect of the recurrent parent on the final FHB resistance level. Interestingly, both studies came to the conclusion that highly susceptible European varieties can be significantly improved by introgression of these QTL, but they never reached the level of already moderately resistant varieties (Table 1).

Table 1  
**Mean FHB rating (%) of the best selected lines containing either *Fhb1* (aaBB), *Qfhs-ifa.5A* (AAbb), both QTL (AABB) or none of them (aabb). The spring wheat lines were tested across nine I isolate mixtures at four environments and the winter wheat lines were inoculated with one isolate across ten environments.**

Entry	Spring wheat	Winter wheat	
		Opus background	Anthus background
AABB	4.3	26.9	14.2
aaBB	9.5	36.0	16.5
AAbb	9.2	30.7	15.6
aabb	40.0	42.9	19.6

#### STABILITY OF FHB RESISTANCE CONCERNING *FUSARIUM* ISOLATES AND MIXTURES

To test stability of FHB resistance two routes were followed. Firstly, 21 isolates of *Fusarium graminearum* from Canada and Germany were inoculated on the spring wheat line with both FHB QTL (AABB) and a moderately susceptible variety (Taifun). Although Taifun significantly ( $P < 0.01$ ) differentiated the isolates varying from 10 to 22% FHB rating, the same isolates on line AABB ranged from 2 to 4% only with no significant difference ( $P > 0.1$ ). Similarly DON content ranged from 1.3 to 26.5 mg  $\times$  kg<sup>-1</sup> for Taifun and 0.4 to 4.8 mg  $\times$  kg<sup>-1</sup> for AABB (von der Ohe 2010). In a second attempt, seven binary mixtures of a total of six *F. graminearum* isolates were inoculated on four spring wheat lines containing either *Fhb1* (aaBB) or *Qfhs.ifa-5A* (AAbb), both (AABB) or neither of them (aabb, von der Ohe and Miedaner 2010). Re-isolates were sampled from plots inoculated with the binary mixtures to identify the percentage of each mixing partner by simple sequence repeat (SSR) markers and to detect competition effects. Resistant host lines showed a high stability to all isolates inoculated individually and to the mixtures as well. Host by mixture interactions were detected, but no change in rank order occurred. Significant ( $P < 0.05$ ) deviations from the inoculated 1:1 proportions occurred in 34 of 49 cases illustrating that competition effects appeared in most instances. These effects, however, could not be attributed to the level of host resistance, type and amount of mycotoxins and aggressiveness of isolates tested individually, but depended mainly on environmental effects.

In conclusion, both resistance QTL are effective and stable in elite spring and winter wheat backgrounds. For improvement of FHB resistance both QTL are valuable, but *Qfhs.ifa-5A* would suffice for European breeding programs. Resistant spring wheat lines were less affected by the tested *Fusarium* isolates and

mixtures and, therefore, confirmed a high stability of these *QTL*. Directed selection of highly aggressive isolates due to the resistance *QTL* seems to be unlikely on the short term.

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