Breeding for Fusarium head blight resistance in wheat update on the Fusarium research at IFA-Tulln

Hermann Bürstmayr^{1*}, Karin Huber, Abdallah Alimari, Barbara Steiner, Katharina Schießl, Maria Bürstmayr und Marc Lemmens

Abstract

During the past decade numerous studies have been published on molecular mapping of Fusarium head blight resistance in wheat: QTL for FHB resistance were found on all wheat chromosomes except chromosome 7D. Some OTL were found in several independent mapping studies indicating that such QTL are stable and therefore useful in breeding programs. We summarize and update current knowledge on the genetics of Fusarium head blight resistance in wheat resulting from published QTL mapping investigations and review and suggest FHB breeding strategies based on the available information and DNA markers. In addition we present current own results on the genetic analysis of novel Fusarium resistance QTL derived from Triticum macha a close relative of hexaploid bread wheat, as well as from T. dicoccum and T. dicoccoides which are relatives of tetraploid durum wheat.

Keywords: Fusarium, resistance, QTL, selection

Introduction

Resistance to Fusarium head blight is of ongoing interest to wheat breeders in many wheat growing regions worldwide, including most parts of Europe (TAYLOR 2004). Practical breeders in many countries have achieved considerable selection progress and farmers can nowadays choose moderately FHB resistant cultivars for wheat production. Despite that, it is still very challenging and resource demanding to develop winter wheat cultivars with an optimal combination of productivity, quality and disease resistance.

During the past years, several review articles have been published on Fusarium diseases of cereals covering different aspects. PARRY et al. (1995) reviewed the significance of the disease with an emphasis on phytopathological aspects.

Reviews of conventional breeding for FHB resistance were published by MIEDANER (1997) and MESTERHAZY et al. (1999). PLACINTA et al. (1999) documented the worldwide occurrence and significance of Fusarium mycotoxins. A comprehensive monograph edited by LEONARD and BUSHNELL (2003) reports in 18 book chapters a range of aspects on Fusarium diseases of small grain cereals, including the pathogen, the associated mycotoxins, resistance breeding and other control options as well as the social and economic impact of the disease. BAI and SHANER (2004) reviewed the management and resistance to FHB in wheat and barley including the knowledge on FHB resistance QTLs mainly from a North American perspective. HOLZ-APFEL et al. (2008) reported about QTL in winter wheat and neatly summarized own results together with published FHB resistance QTL. The most recent review on FHB resistance in wheat has been provided by BUERSTMAYR et al. (2008). The authors included results of 52 peer-reviewed studies reporting QTL for FHB resistance in wheat.

Of the 52 studies, 46 were done with hexaploid wheat, 4 with tetraploids and 2 with related species. Detailed lists including information on the mapping population, the phenotyping methods and the association of the detected FHB resistance QTL with other traits are illustrated in three tables and one figure. Currently, apart from a few exceptions not much is known on the actual function of FHB resistance genes.

No large effect FHB resistance gene (QTL) has been cloned to date from wheat itself.

Breeding for Fusarium head blight resistance

Basically two roads are available to improve FHB resistance: (1) classical breeding which relies on sexual recombination and selection of naturally occurring resistance alleles in the wheat gene pool; and (2) application of transgenic approaches, known as genetic transformation, in order to introduce novel genes from outside the wheat gene pool. Although a range of promising results on the use of transgenes for resistance improvement have been published already (see for instance LEONARD and BUSHNELL 2003) the following paragraphs in this article will exclusively cover classical plant breeding.

The basic preconditions for successful classical breeding are:

- 1) We need to find genetic variation for the trait of interest in the wheat gene pool(s).
- 2) We need to introduce the resistance trait into the regional breeding material.
- 3) We need selection tools that help us to find the genotypes possessing improved resistance.

1) Genetic variation for FHB resistance

Fortunately, large genetic variation for FHB resistance is available in the primary hexaploid wheat gene pool. Although no immune genotype has been reported to date, lines

¹ University of Natural Resources and Applied Life Sciences Vienna, Department IFA-Tulln, Konrad Lorenz Straße 20, A-3430 TULLN

^{*} Ansprechpartner: Dr. Hermann BÜRSTMAYR, hermann.buerstmayr@boku.ac.at

with remarkably high levels of FHB resistance have been found in different germplasm pools, like some wheats from Asia, Europe and Latin America (e.g. SNIJDERS 1990). On the other hand, often the best regionally adapted and highly productive cultivars are susceptible to FHB, mainly in cases where selection for FHB resistance has been neglected in the past. Only very limited variation for resistance to FHB has been reported in cultivated durum wheat (*Triticum durum*), therefore its relatives (e.g. *T. dicoccum, T. dicoccoides*) have been screened in order to find sources for useful resistance genes. Resistance in wild relatives has been studied and is existing and could be used in breeding in the future. However, it is usually a long and tedious process to introduce alien genes into productive cultivars. Even in wild relatives of wheat, so far no immune genotype has been found.

2) Introduction of FHB resistance

into regional breeding material

This is the easiest part in the breeding process. If breeders rely on resistance sources available in the primary gene pool, crossing should be easy and straight forward, progeny should be fully fertile. In cases when 'alien genes' are used the development of translocation lines is needed, which needs long term research investments. Whether breeders should rely on so called 'exotic' resistance sources (e.g. Asian spring wheats) or on moderately effective 'native' resistance is subject to ongoing discussions. To my knowledge, so far in Europe no cultivar incorporating 'exotic' Asian spring wheat resistance has been released, but several cultivars have been released with a good level of resistance derived from the native winter wheat germplasm. Obviously, it was easier to select productive cultivars with moderate resistance within the adapted winter wheat gene pool. In case that 'exotic' resistance sources are introduced, breeding populations should be derived from backcrosses of the 'exotic' resistance source with regionally adapted lines instead of single crosses. Several cycles of crossing and selection may be needed in order to regain the desired productivity level.

3) Selection for improved resistance

Most breeders to date relied on phenotypic selection. Phenotypic selection is a very useful approach for selecting improved cultivars. Because Fusarium head blight occurs sporadically in nature in most wheat growing areas, breeders have to apply some tricks. The goal is to determine the level of genetically governed resistance on every line of the analysed population as precisely as possible. One of the main problems in testing for Fusarium resistance is reproducibility (DILL-MACKY 2003). The severity of FHB is a quantitative trait that is modulated by (1) genetic factors of the host (resistance factors in the plant) and of the pathogen (aggressiveness of the fungus) and (2) environmental influence on disease establishment and development leading to significant genotype-by-environment (GxE) interactions. Therefore, in most FHB resistance studies measures are taken to provoke Fusarium infections and apply uniform inoculum pressure over time (flowering period) and space (e.g. experimental field). FHB resistance is a complex trait and not one single, simple way of measuring FHB resistance is practiced. For a more detailed review on inoculation and evaluation methods see DILL-MACKY (2003). Selection is usually done in specific disease provocation nurseries. The question when in the breeding process FHB resistance screening should be started depends on the regional preferences and the relative importance of the trait. If FHB resistance is considered a key trait for a new cultivar in a certain area, selection should start as early as possible, for instance already in F3 head rows. If selection is practised over several subsequent generations, the selected population will shift significantly in its average resistance performance.

Although phenotypic selection is a very useful and successful approach, it is not simple and it is time consuming. Therefore, alternative selection procedures may be considered. Unfortunately, neither seedling tests nor *in-vitro* screening methods for FHB resistance have been established or validated to date.

However, in recent years numerous projects on molecular mapping of FHB resistance in wheat have been performed in many labs around the globe (see BUERSTMAYR et al. 2008) opening the way for molecular marker assisted selection. In this case selection is practised based on genetic fingerprints typical for resistance genes (QTL). In the ideal case perfect markers, which predict presence or absence of the desired allele at a resistance locus are preferable, but these are not available for most of the known FHB resistance OTL apart from Fhb1 (syn. Ofhs.ndsu-3BS) mapping to chromosome 3BS (LIU et al. 2008). In all other cases breeders have to rely on linked markers around the resistance locus. However, even linked markers, in most cases SSR (microsatellite) markers, which show a distinct haplotype for the resistance allele at the QTL, have been applied successfully in the selection process. The relative advantage of molecular marker assisted selection is that selection can be started very early in the selection process $(BC_1 \text{ or } F_2)$ and that desired QTL can be moved skilfully from exotic germplasm into well adapted lines in relatively short time. In several case studies marker assisted selection proofed to be efficient. For instance WILDE et al. (2007) showed that both phenotypic selection and marker based selection led to significant gain by selection, gain per unit time was larger in marker based selection. On the other hand, marker based selection can only utilize the mapped, large effect QTL and not quantitative minor QTL, which are usually missed in QTL mapping. The possibly best approach would be to skilfully combine marker selection with phenotypic selection: one could select in early generations of the breeding programs for presence of a few large effect QTL but keep the breeding populations large enough in order to allow further improvement by phenotypic selection in later generations.

Own results with 16 winter wheat backcross-two derived families differing in two major QTL from spring wheat indicated that on average over 6 experiments, presence of *Qfhs.ifa-5A* led to a reduction in FHB severity compared to the respective sister lines with no QTL of 16%, presence of *Fhb1* reduced disease severity by 29% and both QTL

combined by 35%. Yield tests with a subset of these sister lines gave no clear trend indicating that no yield penalty was associated with these QTL *per se*.

Special emphasis has been given in the past few years to the association of FHB resistance with plant height. Generally, a negative association between plant height and FHB susceptibility has been reported numerous times, i.e. short lines tend to be more susceptible than tall lines. It has been suggested that tall plants are possibly exposed to less FHB inoculum and a lower infection pressure compared to tall plants. Recently, several reports showed that the semi-dwarfing allele RhtD1b is strongly associated with increased FHB susceptibility, but not with plant height per se (DRAEGER et al. 2007, HOLZAPFEL et al. 2008). Whether or not the *RhtD1b* allele is causally involved in reduced resistance or linked to a susceptibility allele nearby needs further investigations. Possibly other known dwarfing genes are also associated with susceptibility, like RhtB1b and Rht8 (HANDA et al. 2008), but further research is needed to clarify this relation. Another interesting subject is the association between FHB resistance and wheat flowering, especially anther extrusion. There is substantial evidence that wheat lines with rapid and efficient anther extrusion exhibit lower FHB susceptibility (TAYLOR 2004, SKINNES et al. 2008).

Current ongoing research projects at the IFA-Tulln lab

After af few successful QTL mapping studies using spring wheat sources in the past (BUERSTMAYR et al. 2002, 2003, STEINER et al. 2004, LEMMENS et al. 2005), we focused recently on more distant resistance sources like hexaploid *Triticum macha* (Georgian spelt wheat) and tetraploid *T. dicoccum* (cultivated emmer) and *T. dicoccoides* (wild emmer). Especially the tetraploid resistance sources appear promising, because there is an urgent need to increase FHB resistance in durum wheat and introduction resistance from bread wheat in durum wheat was only partly successful so far.

QTL in *T. macha* were found on chromosomes 2A, 2B, 5A and 5B. Notably, the relatively largest QTL mapped at the Q-locus of chromosome 5A. In the *T. dicoccoides* accession 'Mt. Gerizim-52' from Israel QTL were found on chromosomes 3A and 6B. Two populations of BC1F5 derived lines from the *T. dicoccum* (cultivated emmer) 'line 161' crossed with either Helidur and Floradur were evaluated over three years. There was surprisingly little agreement in the QTL detected in these populations. While in the *T. dicoccum* x Floradur populaton the largest QTL mapped to chromosomes 3B and 6B in the *T. dicoccum* x Helidur population the largest effect was associated with the *RhtB1* locus and susceptibility was associated with the semi dwarf allele (*RhtB1b*). The detailed results from these ongoing mapping projects will be published in the coming year.

In addition, we intensively work on research projects to gain further insight into the genes and pathways that are involved on FHB resistance of wheat by applying functional genomics approaches. For further details see STEINER et al. (2008).

Acknowledgments

The presented work is based on several grant funded projects, primarily the Austrian Science Fund (FWF) projects 'AB-QTL mapping of Fusarium resistance' (P17310), and 'Identification and characterization of expressed genes involved in Fusarium head blight resistance of wheat' (P16724), and the Euro Trans Bio project 'Short Wheat', with support from the breeding company Saatzucht-Donau, Austria. The research work of IFA-Tulln is supported by the Government of Lower Austria.

References

- BAI, G.H. and G. SHANER, 2004: Management and resistance in wheat and barley to Fusarium head blight. Ann. Rev. Phytopathol. 42, 135-161.
- BUERSTMAYR, H., M. LEMMENS, L. HARTL, L. DOLDI, B. STEI-NER, M. STIERSCHNEIDER and P. RUCKENBAUER, 2002: Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). Theor. Appl. Genet. 104, 84-91.
- BUERSTMAYR, H., B. STEINER, L. HARTL, M. GRIESSER, N. AN-GERER, D. LENGAUER, T. MIEDANER, B. SCHNEIDER and M. LEMMENS, 2003: Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor. Appl. Genet. 107, 503-508.
- BUERSTMAYR, H., T. BAN and J.A. ANDERSON, 2009: QTL mapping and marker assisted selection for Fusarium head blight resistance in wheat - a review. Plant Breeding, 128, 1-26.
- DILL-MACKY, R., 2003: Inoculation methods and evaluation of Fusarium head blight resistance in wheat. In: K. J. Leonard, and W. R. Bushnell (eds.) Fusarium Head Blight of Wheat and Barley, 184-210. The American Phytopathological Society, St. Paul.
- DRAEGER, R., N. GOSMAN, A. STEED, E. CHANDLER, M. THOM-SETT, SRINIVASACHARY, J. SCHONDELMAIER, H. BUERST-MAYR, M. LEMMENS, M. SCHMOLKE, A. MESTERHAZY and P. NICHOLSON, 2007: Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. Theor. Appl. Genet. 115, 617-625.
- HANDA, H., N. NAMIKI, D.H. XU and T. BAN, 2008: Dissecting of the FHB resistance QTL on the short arm of wheat chromosome 2D using a comparative genomic approach: from QTL to candidate gene. Mol. Breed. 22, 71-84.
- HOLZAPFEL, J., H.H. VOSS, T. MIEDANER, V. KORZUN, J. HÄ-BERLE, G. SCHWEIZER, V. MOHLER, G. ZIMMERMANN and L. HARTL, 2008: Inheritance of resistance to Fusarium head blight in three European winter wheat populations. Theor. Appl. Genet. 117, 1119-1128.
- LEONARD, K.J. and W.R. BUSHNELL, 2003: Fusarium head blight of wheat and barley American Phytopathological Society (APS Press), St. Paul, USA.
- LEMMENS, M., U. SCHOLZ, F. BERTHILLER, C. DALL'ASTA, A. KOUTNIK, R. SCHUHMACHER, G. ADAM, H. BUERSTMAYR, A. MESTERHAZY, R. KRSKA and P. RUCKENBAUER, 2005: The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for Fusarium head blight resistance in wheat. Mol. Plant-Microbe Interactions 18, 1318-1324.
- LIU, S., M.O. PUMPHREY, B.S. GILL, H.N. TRICK, J.X. ZHANG, J. DOLEZEL, B. CHALOUB and J.A. ANDERSON, 2008: Towards positional cloning of *Fhb1*, a major QTL for Fusarium head blight resistance in wheat. Cereal Res. Comm. 36, Suppl. B, 195-201.

- MESTERHAZY, A., T. BARTOK, C.G. MIROCHA and R. KOMOROC-ZY, 1999: Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. Plant Breeding 118, 97-110.
- MIEDANER, T., 1997: Breeding wheat and rye for resistance to Fusarium diseases. Plant Breeding 116, 201-220.
- PARRY, D.W., P. JENKINSON and L. MCLEOD, 1995: Fusarium ear blight (scab) in small grain cereals - a review. Plant Pathol. 44, 207-238.
- PLACINTA, C.M., J.P.F. D'MELLO and A.M.C. MACDONALD, 1999: A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. Animal Feed Sci. Technol. 78, 21-37.
- VAN EGMOND, H.P., 2004: Natural toxins: risks, regulations and the analytical situation in Europe. Anal. Bioanal. Chem. 378, 1152-1160.
- SNIJDERS, C.H.A., 1990: Genetic variation for resistance to Fusarium head blight in bread wheat. Euphytica 50: 171-179.
- SKINNES H., Y. TARKEGNE, J.A. DIESETH and A. BJORNSTAD, 2008: Associations between antehr extrusion and Fusarium head blight in European wheat. Cerea Res. Comm 36, Suppl. B, 223-231.

- STEINER, B., M. LEMMENS, M. GRIESSER, U. SCHOLZ, J. SCHON-DELMAIER and H. BUERSTMAYR, 2004: Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theor. Appl. Genet. 109, 215-224.
- STEINER B., H. KURZ, M. LEMMENS and H. BUERSTMAYR, 2009: Differential gene expression of related wheat lines with contrasting levels of head blight resistance after *Fusarium graminearum* inoculation. Theor. Appl. Genet. 118, 753-764.
- TAYLOR, M., 2004: Incorporation of Fusarium head blight resistance into European winter wheat breeding programs. In: Canty, S.M., Boring, T., Wardwell, J. and Ward, R.W (Eds.), Proceedings of the 2nd International Symposium on Fusarium Head Blight; incorporating the 8th European Fusarium Seminar, 11-15 December, Orlando, FL, USA. Michigan State University, East Lansing, MI., 195-196.
- WILDE, F., V. KORZUN, E. EBMEYER, H.H. GEIGER and T. MIEDA-NER, 2007: Comparison of phenotypic and marker-based selection for Fusarium head blight resistance and DON content in spring wheat. Mol. Breed. 19, 357-370.