Identification of expressed genes involved in Fusarium head blight resistance of wheat

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Introduction

Fusarium head blight (FHB) is a devastating disease of wheat in many areas of the world. Molecular mapping projects led to the identification of two major FHB resistance QTL, Qfhs.ndsu-3BS (ANDERSON et al. 2001) and Qfhs.ifa-5A (BUERSTMAYR et al. 2003). The actual function of these resistance genes is still unknown. In this project we aim to identify expressed genes involved in the resistance reaction of wheat against FHB and contribute to the functional clarification of the resistance reaction.

Materials and Methods

Near isogenic lines (NILs) differing in the major FHB resistance QTL, Qfhs. ndsu-3BS and Qfhs.ifa-5A, were developed. At anthesis these NILs along with the parental lines were challenged by pipetting either *Fusarium graminearum* or water between the palea and lemma of the two basal florets of four spikelets per spike. 0h, 6h, 12h, 24h, 48h and 72h after inoculation treated spikelets were harvested, separated into the lemma, palea and the subtending section of rachis and the reproductive tissues and shock frozen in liquid nitrogen. As the first tissues to be colonized by the fungus are the inner surfaces of palea and lemma (KANG and BUCHENAUER 2000) we started to analyze these tissues.

Total RNA was extracted from 200 mg ground tissues of the wheat lemma, palea and the subtending section of the rachis. First and second strand cDNA were synthesized using standard procedures. To evaluate differential gene expression of the cDNA samples the cDNA-AFLP method is used. The separation of the PCR fragments is done on a LI-COR 4200 DNA dual-dye sequencing system.

Preliminary Results

Transcript derived fragments (TDFs) displayed by cDNA-AFLP ranged in size from 30-700 bp. Until now 96 and 50 AFLP primer combinations were applied to the parental lines and the NILs, respectively, resulting in 30 to 80 TDFs per primer combination. Although the majority of the bands revealed no change in intensity between the different wheat lines, treatments and time points, we detected genotype discrimative banding patterns, 272 transcripts specific for the parental lines, compared to 4 NIL specific TDFs. The low number of polymorphic transcripts between the NILs confirms the nearly identical character of these lines.

Altered expression patterns after Fusarium inoculation were observed for about 150 TDFs, corresponding to 3% of the analysed fragments. Most of these transcripts were up-regulated at 48 and 72 hours after inoculation and were unaffected by the analysed genotypes and their resistance level. 10 TDFs displayed differential expression after fungal attack depending on the genotype and the possession of the resistance QTL.

Interesting transcripts are isolated from polyacrylamid gels and reamplified. Isolating, cloning and sequencing of the TDFs is part of the current lab work.

References

ANDERSON, J.A., R.W. STACK, S. LIU, B.L. WALDRON, A.D. FJELD, C. COYNE, B. MORENO-SEVILLA, J. MITCHELL FETCH, Q.J. SONG, P.B. CREGAN, R.C. FROHBERG, 2001: Theor Appl Genet 102: 1164-1168.

BUERSTMAYR, H., B. STEINER, L. HARTL, M. GRIESSER, N. ANGERER, D. LENGAUER, T. MIEDANER, B. SCHNEIDER and M. LEMMENS, 2003: Theor Appl Genet 107, 503-508.

KANG, Z., H. BUCHENAUER, 2000: Mycol Res 104: 1083-1093.

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