

## Summary

A variety of micro-organisms are constantly exploiting plants. Barley is no exception and various pathogens attack it. This interaction between the plant and the pathogen can be divided into two groups. A compatible interaction occurs when the plant shows susceptibility to the pathogen. An incompatible interaction occurs when the plant shows resistance to the pathogen. This incompatible interaction is usually associated with necrotic lesions at the infected area. The aim of this project was to study the interaction between *Hordeum vulgare* (barley) and *Fusarium graminearum* (causal agent of head blight / scab). Further objectives were to look at the expression of defence genes in the cultivar Puma 15 when infected with *F. graminearum*. Juvenile plants were used in this study and infected at the third leaf stage. Suppression subtractive hybridisation (SSH) was performed on inoculated and uninoculated plants to enrich for differentially transcribed genes. The resulting transcript derived fragments (TDF) were cloned and sequenced. Several stress related (biotic or abiotic) genes were identified that included putative selenium-binding proteins (SSH10), glutamine-dependent asparagine synthetase 1 (SSH11) and hypothetical proteins. Upon inspection of the homology at nucleotide level of one of the hypothetical proteins (SSH16), it became evident that it was from the 26S ribosomal gene. A selection of the identified genes was further evaluated to the role that they play during this specific interaction by Northern blot analyses. Northern blot analysis of the putative selenium binding homologue (SSH10) showed low levels of induced gene expression in the inoculated plants as compared to the control at 24 hpi. For the asparagine synthetase homologue (SSH11) there was a significant up-regulation in the inoculated sample at 24 hpi. From the results obtained in this study it was found that there are certain defence related, stress related, and unknown genes that are expressed during infection of the barley cultivar Puma15 with *F. graminearum*. A correlation could be made between the results presented here and literature on *F. graminearum* infection studies. When *F. graminearum* infects the host it will respond to that infection between 24 hpi and 72 hpi. This response includes the expression of defence genes and other antifungal mechanisms. The results from this study complements previous studies and will in future aid in the understanding of barley responses after infection by necrotrophic pathogens.

## Opsomming

Plante word op 'n gereelde basis deur mikro-organismes aangeval. Gars is geen uitsondering nie en word deur verskillende patogene aangeval. Die interaksies tussen die plant en die patoogeen kan in twee groepe verdeel word naamlik 'n nie-vatbare interaksie, waar die plant weerstand bied teen die patoogeen en 'n vatbare interaksie waar die plant nie-weerstandbiedend is teen die patoogeen. Nie-vatbare interaksies bestaan gewoonlik uit nekrotiese letsels op die besmette area. Die doel van hierdie studie was om te kyk na die interaksie tussen *Hordeum vulgare* (gars) en *Fusarium graminearum* (aarskroei). Verdere doelwitte was om te kyk na die uitdrukking van weerstandsgene in die kultivar Puma 15 wanneer dit geïnfekteer word met *F. graminearum*. Jong plante was gebruik en is geïnfekteer tydens die drie-blaar stadium. Aftrekking-onderdrukkings-hibridisasie ("Suppression subtractive hybridisation", SSH) was gebruik op die ingeënte en oningeënte plante vir die verryking van differensieël uitgedrukte gene (TDF's). Die TDF's was gekloneer en gebruik vir die bepaling van hulle basispaarvolgordes. Vele stres verwante gene (bioties en abioties) was geïdentifiseer naamlik waarskynlike selenium-bindingsproteïene (SSH10), glutamien-afhanklike asparagien sintetase 1 (SSH11) en vele hipotetiese proteïene. Die homologie van een van hierdie waarskynlike proteïene (SSH16) op nukleotiedvlak het gedui dat dit verwant is aan 26S ribosomale geen. Die resultate van die TDF's is verder gebruik vir die ondersoek na hul spesifieke funksie in die patoogeen interaksie. Dit is gedoen deur gebruik te maak van Northern klad analyses. Die Northern klad van SSH10 het lae vlakke getoon van geïnduseerde geen uitdrukking in die ingeënte sowel as die oningeënte plante na 24 hpi. SSH11 het 'n merkwaardige induksie getoon in die ingeënte plant na 24 hpi. Die resultate toon duidelik daarop dat daar wel sekere stres-; weerstands- en ander onbekende gene uitgedruk word gedurende die interaksie tussen kultivar Puma 15 en *F. graminearum*. 'n Belangrike vergelyking kan gemaak word tussen hierdie studie en vorige literatuur. Dit toon dat wanneer *F. graminearum* die plant infekteer die plant 'n reaksie toon tussen 24 hpi en 72 hpi. Hierdie reaksie sluit in die uitdrukking van weerstandsgene en ander meganismes teen swamme. Hierdie studie lewer verdere insig op die reaksie wat gars toon nadat dit met 'n nekrotrofe patoogeen geïnfekteer word.