Chapter 5

Discussion

Defence genes are found in plants and their role is to elicit a response during interactions with possible pathogens and pests that attack the plant. To study these interactions it is necessary to identify some of the genes that are regulated during this interaction. Suppression subtractive hybridisation (SSH) is a highly effective method to identify expressed genes from biological interactions. In this study SSH was used to enrich for genes in three-week-old barley plants to study the effect of *F. graminearum* on young plants.

The majority of the isolated TDFs from this interaction were related to Rubisco and a light harvesting chlorophyll a/b protein precursor (SSH13) was also isolated. The occurrence of these proteins linked to photosynthesis may indicate that the 150:1 ratio of driver to tester used still allowed some background through. However, the ratio used was derived from an earlier study on plant-pathogen interaction in barley (Hein *et al*., 2004). It could therefore indicate that a change in carbohydrate metabolism occurs during this interaction. One such study of the interaction between wheat and *Diuraphis noxia* (Russian wheat aphid) showed that the aphid releases toxins that break down the chlorophyll content in wheat plants. This causes a response in the resistant plants and the expression of several defence genes. Eight fragments were isolated that were related to photosynthesis, where most of these fragments were up-regulated. This up-regulation could be a mechanism from the plant to help it recover or overcome these stress conditions (Botha *et al*., 2006).

Clone SSH10 was similar to a putative-selenium binding protein (SBP) found in *Oryza sativa* (Table 4.1) and was up-regulated at 24 hpi. It has been noted from literature that a response to *F. graminearum* is evident at 24 hpi in wheat (Pritsch *et al*., 2000). Furthermore, between 48 hpi and 72 hpi the fungus starts to successfully penetrate the host. In this time the plant responds by the expression of PR proteins such as PR-1 (antifungal), PR-2 (β-1,3-glucanase), PR-3 (chitinase), PR-4 (antifungal) and PR-5 (antifungal) (Pritsch *et al*., 2000). Therefore, the role that this fragment plays will in future be further investigated using quantitative RT-PCR, which is a more sensitive technique to study gene expression.

The role of SBP's has been suggested to accelerate the expression of PR proteins and phytoalexin accumulation (Sawada *et al*., 2004). This role was postulated to be secondary during defence responses. In the infection of rice with the rice blast fungus,

Magnaporthe grisea, SBP's were linked with a GTP-binding protein (Sawada *et al*., 2004) that leads to the activation of Ca^{2+} channels that plays a role in signal transduction, a mechanism the host uses to respond to pathogen infection (Gelli *et al*., 1997). The mRNA levels of the SBP homologue identified by Sawada *et al* (2004) was up-regulated at 12 hpi and highly up-regulated by 24 hpi during interaction with both a virulent and avirulent isolate of the rice blast fungus. This induction could lead to a signal transduction in the host and the expression of certain PR proteins.

Clone SSH11 was similar to a glutamine-dependent asparagine synthetase 1 found in *Hordeum vulgare* subsp. *vulgare* (Table 4.1). This TDF displayed low but significant regulation during the interaction between *F. graminearum* and Puma 15. There was an up-regulation after 24 hpi followed by down-regulation at 48 hpi. At 72 hpi there was an increase in the detectable levels in both the inoculated and control plants. Glutamine-dependent asparagines synthetase plays a role during leaf senescence by remobilising nitrogen. Nitrogen metabolism has several components e.g. certain amino acids play key roles (Lamb *et al*., 1989). Ammonia is incorporated in the amino acids glutamine and asparagine that are the nitrogen-carrying molecules in plants. These molecules (amino acids) are the building blocks for plant growth and development. Furthermore, nitrogen also helps the plant to recover from injuries or pathogen invasion (Snoeijers *et al*., 2000). Thus, a reduction in these amino acids could be expected during natural senescence and pathogenesis. An additional aspect could be that since necrotrophic fungi uses nitrogen as a source of nutrition that the plant will elicit a response to the reduction in nitrogen levels (Snoeijers et al., 2000).

Asparagine concentrations increase in barley when abiotic stress such as salt and water stress occurs (Gilbert *et al*., 1998). Two fragments (TaASN1 and TaASN2) related to barley asparagine synthetases were identified from wheat (Gilbert *et al* 1998). These fragments are involved in glutamine, ATP and aspartate binding. Since the fragments were extracted in seedling plants and SSH11 was also found in young seedling plants, a connection can be made between the two different hosts and their responses to stress conditions (Wang et al., 2005). However, in our study this fragment was up-regulated to very low detectable levels. This could be that the sensitivity of the Northern blot to detect regulation of this fragment is limited. This

fragment will be further analysed using RT-PCR to study the expression of this fragment during pathogen interaction.

Clones SSH12, SSH15 and SSH16 showed homology to different hypothetical proteins in different organisms (Table 4.1 and Table 4.2). There are no specific functions known for these hypothetical proteins and they could not be related to a specific defence response. Therefore, they were not immediately chosen for use in expression analysis. Their full length sequences will be obtained and the role that they play will be further investigated in a future study.

We know that the barley and *F. graminearum* interaction is not based on the guard hypothesis and there is no HR evident. This was reinforced by the results generated in this study where no HR related gene expression was detected. This might also explain why low levels of induction were detected with the Northern blots. Several different gene products in barley function in conjunction to combat the infection from the FHB causing pathogen. These levels of gene expression could be detected with SSH as it can detect very low levels of differential gene expression (Diatchenko *et al*., 1996) that is not necessarily detectable by Northern blot analysis.

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 previous *F. graminearum* infection studies (Pritsch *et al*., 2000). In this study it was found that the host will respond to infection between 24 hpi and 72 hpi. This response includes the expression of defence genes and other antifungal mechanisms (Pritsch *et al*., 2000). Although not all of the identified genes' functions are known, these analyses could bring insight into barley defence against a necrotrophic pathogen. Further elucidation of these genes and the mechanisms barley use against *F. graminearum* will complement what we have learned in wheat. This knowledge will better our understanding of how plants respond to fungal ingress and hopefully increase our capacity for developing new more tolerant cultivars.