Title:

Use of ecologically-and evolutionary relevant transcriptomic data to infer functions of fungal pathogen gene orthologues important for limiting fungal stresses caused by interacting host plants and bacteria

Stefan Olsson[1,2*#], Osakina Aron[3], Hongchen Li[1], Quingfang Li[1], Bjoern Ost Hansen[1,4], Wei Tang[3], Zonghua Wang[1,3,5], Guodong Lu[1], Wenhui Zheng[1]

***# First and corresponding author**

[1]State Key Laboratory for Ecological Pest Control of Fujian and Taiwan Crops, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, China.

[2]Plant Immunity Center, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University, Fuzhou, China

[3]Fujian University Key Laboratory for Plant-Microbe Interaction, College of Life Science, Fujian Agriculture and Forestry University, Fuzhou, 350002, China.

[4]OmicsDriven, Østergade 76, DK-4340 Tølløse, Denmark

[5]Institute of Oceanography, Minjiang University, Fuzhou, China

Authors contributions divided per authors

SO Initial idea. Hypotheses generation from data and from literature, co-regulation analysis in relation to HPI indicative expression, overall responsible for driving the work forward and methods development. Main responsible for manuscript writing and the analysis. Coordination of manuscript writing and corrections.

OA Research concerning MoCpa1 and MoADE4, Manuscript correction

HL Research concerning *F. graminearum* NO production. Manuscript correction

QL Analysis of PARP/PARG in the secondary data. Manuscript correction

BOH Research concerning *F. graminearum* NO production and CK2 activity in *F. graminearum* and *M. oryzae* during different HPI normalized for growth. Download and preparation of secondary data. Manuscript correction.

WT Research concerning MoCpa1 and MoADE4. Manuscript correction.

ZW Research concerning *F. graminearum* NO production, CK2 activity in *F. graminearum* and *M. oryzae* during different HPI normalized for growth, MoCpa1 and MoADE4. Acquisition of financial support. Manuscript correction.

GL Research concerning *F. graminearum* NO production, Acquisition of financial support. Manuscript correction.

WZ Research concerning *F. graminearum* NO production. Manuscript correction.

Abstract: 278 words

Body without references abstract and title pages: 6348 words. Total 8222

21 Figs and 2 tables with 88 XY plots.

42 references

 doi:10.20944/preprints202012.0773.v1

ABSTRACT (278 words)

We identified key genes needed for maintenance and growth and homed in on genes where there could be a competition between maintenance requirements (stress) and growth requirements. Such processes are synthesis of arginine, synthesis of DNA-bases, nitric oxide synthesis needing arginine, autophagy, DNA synthesis and DNA repair. Using procedures previously developed for the use of sets of downloaded transcriptomic data to test hypotheses concerning at what time under the course of infection of plants genes are expressed for the two pathogens *Fusarium graminearum* and *Magnaporthe oryzae*, we constructed a simplified regulatory network for these genes for both organisms. Our analysis shows that the transcription effort (cost) to maintain the fungal cells (maintenance) are high before infection and in early infection. During the following biotrophic phase maintenance cost drops for later in the transition to the necrotrophic phase increase dramatically. Finally, in the necrotrophic phase, maintenance is lower again despite the high growth rate that can also cause stress. The expressions of all identified genes behaved almost similar for both fungi except the DNA repair genes PARP/PARG that was not responding or absent in the mainly clonal *M. oryzae* which might indicate this species is more subject to evolution by point mutations than *F. graminearum* where sexual reproduction is frequent. The potential consequences of these different roles for PARP/PARG in the development and the accelerated breakage of host species resistance in a Red Queen dynamics scenario is discussed. Our analysis demonstrates the possibility to use large transcriptome datasets and co-regulations between key genes to test hypotheses and discusses the advantages with this technique as complement to molecular techniques employing knockouts and over-expression of target genes to suggest gene roles.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

Introduction

Growth and maintenance are key concept both in cell biology and ecology. Maintenance is basically all the costs needed to maintain the integrity of the cell including repair of proteins and DNA [1]. Growth is simply the growing of biomass but more specifically as the growth of number of cells including all proteins needed for maintenance including copies of the genomes [2]. However, the maintenance concept has been questioned since it contains many different processes [3].

Wheat head blight caused by *F. graminearum* Schwabe (teleomorph stage: *Gibberella zeae* (Schwein.) Petch) leads to significant losses in grains like wheat and barley especially through the contamination of the seeds with mycotoxins [4]. *Magnaporthe oryzae* B.C. Couch (teleomorph *Pyricularia oryzae* Cavara) cause rice blast resulting in yield and economic losses world-wide [5]. Both fungi are studied by many researchers worldwide and are both considered model organisms [6,7]. One interesting difference between them is that *F. graminearum* often reproduces sexually [8] while *M. oryzae* is mainly clonal [9]. These pathogens are relatively closely related (Sordariomycetes) and in most cases gene expression patterns are mirrored in the two species [10]. Both pathogens infect as biotrophs and switches to necrotrophy at a later stage, hours post infection (HPI). At the plant surfaces they are exposed to environmental stresses possible biotrophic stresses from other organisms. They enter the plant and establishes biotrophic growth inside the plant and the plant defenses are low at this time. At about mid-time (HPI) the fungi are detected by the plant innate immune system that starts attacking the intruders with radical oxygen species (ROS). Then the pathogens switch to necrotrophy, killing the host cells and in the case of *F. graminearum* producing the toxic secondary metabolites deoxynivalenol (DON). At the end of the necrotrophic phase both fungi switch from biomass growth to conidia production emptying the vegetative mycelium of biomass to form conidia that can spread to other plants and infect them. In a previous study we found that the expression of the key autophagy gene ATG8 increases with HPI in both fungi and can be used as indicator for hours post infection (HPI) in the downloaded expression data from a large number of experiments [10]. In the same study we identified the His2b gene as an indicator of de novo DNA synthesis and growth since free histones not bound to DNA are cytotoxic [10,11]. In this study we define maintenance expression as gene expression effort to express a specific gene as the relative growth rate normalized expression of the particular gene, or in other words, gene expression per DNA synthesis.

We have previously studied conserved genes involved in maintenance and growth of fungi as ATG8 [12], DNA repair (PARP) [13,14], and recently we have worked with genes involved in synthesis of DNA bases [15], and arginine [16]. Arginine is also heavily used as the main source together with oxygen for production of nitric oxide (NO) that is a ROS produced in fungal innate immunity. It is triggered both during the transition between biotrophy to necrotrophy and when the fungus is exposed to bacterial MAMPs (microbial associated molecular patterns) [17]. Together these, through evolutions, very conserved genes fit into a conceptual model for how these genes are likely to be differentially expressed during different phases of the plant colonization by the pathogen. During growth dominated phases the purine synthesis genes are mainly used for making new DNA while arginine synthesis is mainly needed for making new proteins. ATG8 activity is also important for growth since fast growing causes need for recycling of misfolded proteins, protein aggregates and storage lipid droplets through autophagy [10,12,18] (**Fig. 1A**).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

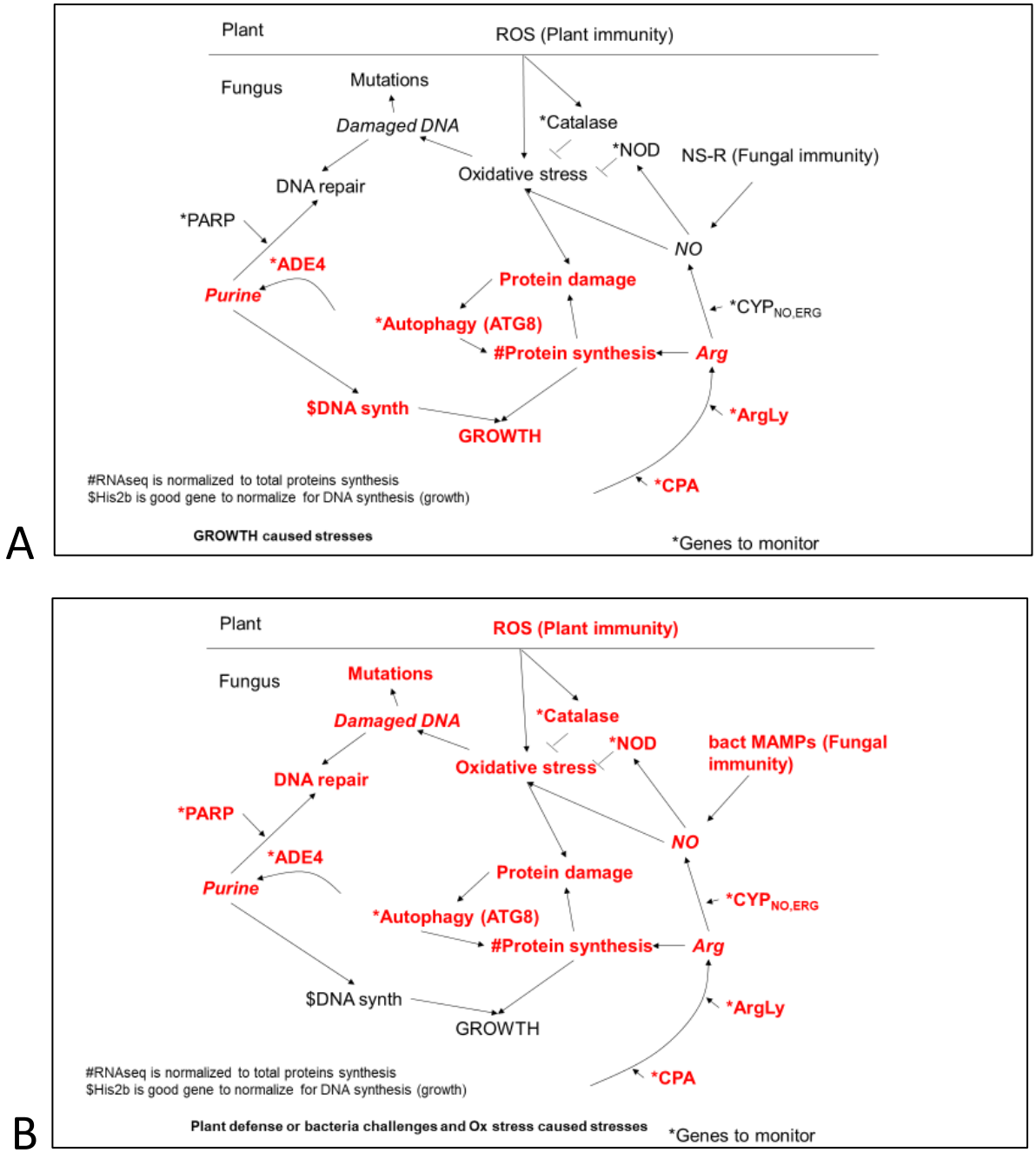


Figure 1. Transcriptional activity of identified genes **A**. Shows in red genes and processes dominating during formation of new biomass (growth). **B.** Shows in red genes and processes dominating during maintenance if expression is normalized for growth using the His2b gene [10] in plant pathogens highlights especially maintenance needed to counteract plant ROS defenses and ROS generated as NO by non-self-recognition fungal innate immunity. CPA=MoCPA1 [19] and FgCPA1 (this study), ArgLy=MoArgLy [20] and FgArgLy (this study), CYP(NO,ERG)=FgCYP(NO,ERG) [17] and MoCYP(NO,ERG) (this study), ATG8=MoATG8 [21] and FgATG8 [12], PARP=FgPrp (this study) and MoPrp (this study), ADE4=MoADE4 [15] and FgADE4 (this study), NOD=FgNOD1 and FgNOD2 [17] and MgNOD and (this study), finally catalases Catalase=FgCAT1-5 (this study) and MoCAT1&3 (this study). For more info information on these genes see **Table 2**.

In transcriptomic experiments the expression of a gene is normalized against sum of the expression of all genes thus the expression of a specific gene reflects the need for that gene for both growth and maintenance. However, it is in addition possible to normalize for growth using the His2B expression as indicative for DNA-synthesis to focus on the relative gene expression needed for only

 doi:10.20944/preprints202012.0773.v1

cell maintenance [10]. Using a large number of transcriptomes from previous papers [10,17], we set out to test the hypothesis that that the stress weighted network (**Fig. 1B**) should be most active just before penetration and in the transition between biotrophy to necrotrophy when plant defenses are activated. During biotrophic growth and during later phases of necrotrophy the growth-related network (**Fig. 1A**) should be most active. For *F. graminearum* and *M. oryzae* we used 64 respective 47 transcriptomes sampled at different times post infection [10]. For *F. graminearum* we in addition also tested the activity of the genes of interest under non-growing conditions short time (<4h) after challenging with purified bacterial MAMPs in a dataset of 113 transcriptomes [17,22]. Exposure to bacterial MAMPs triggers NO production and should mainly be reflected as an increased response of the stress weighted network (**Fig. 1B**) with increasing expression of the cytochrome p450 gene (CYP(NO,ERG)) that is the gene mainly responsible for the intrinsic NO production with accompanying ROS stress [17].

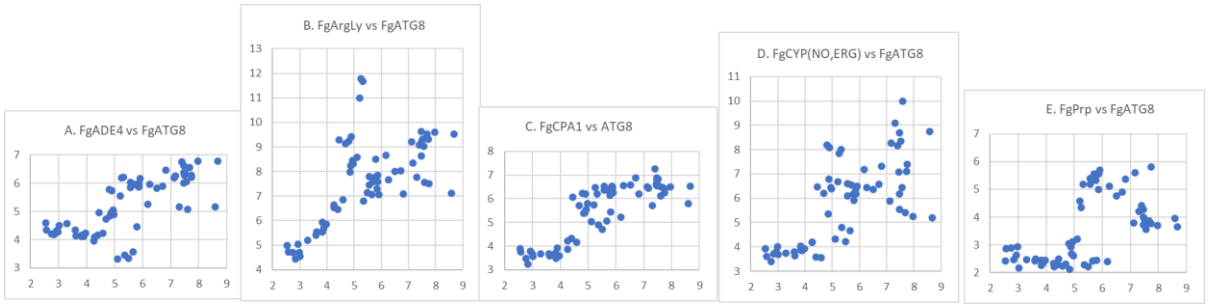
We find support for our hypothesis and in addition we find that the PARP gene necessary for DNA repair reacts very differently in *M. oryzae* and in *F. graminearum*. An orthologue for PARG necessary for de-PARylation of the PARP activity is also absent in *M. oryzae*. In our conclusion we suggest an interpretation that the found difference reflects the need for the mainly clonal *M. oryzae* [8,9] to generate mutational variation to overcome changes in host resistance without need for sexual recombination's. We also discuss the potential benefits transcriptomic analyses, like in this study, for suggesting relative importance of specific gene expressions and roles under relevant natural conditions.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

RESULTS AND DISCUSSION

Since the quality of the transcriptomic datasets we use, are better for *F. graminearum* [10] we first investigated the expression of the key genes (**Fig. 1**) in relation to the expression of FgATG8 an indicator of Hours Post Infection (HPI) in this dataset [10]. The switch from biotrophy-necrotrophy takes place around LOG2 ATG8 expression values of 4.5-6 as indicated by expression of the TRI4 gene involved in DON production is needed for counteracting plant defenses [10,17]. All 5 genes are particularly upregulated at the shift from biotroph to necrotrophy (**Fig. 2A.A-E**). In agreement with our hypothesis (**Fig. 1**) all 5 genes show the same pattern of growth normalized expression (**Fig. 2B.A-E**) indicating that all genes are relatively highly needed for maintenance due to stress at low HPIs (LowATG8 expression), therafter decrease in expression during biotrophy, then strongly increase during the biotrophy-necrotrophy transition for becoming low again during necrotrophy with a tendency to rise at very late necrotrophy. All figures from now on have equal LOG2 scales on both X-axis and Y-axis to facilitate visual comparisons of gene expressions. This is especially important when comparing different genes response patterns visually.

A



B

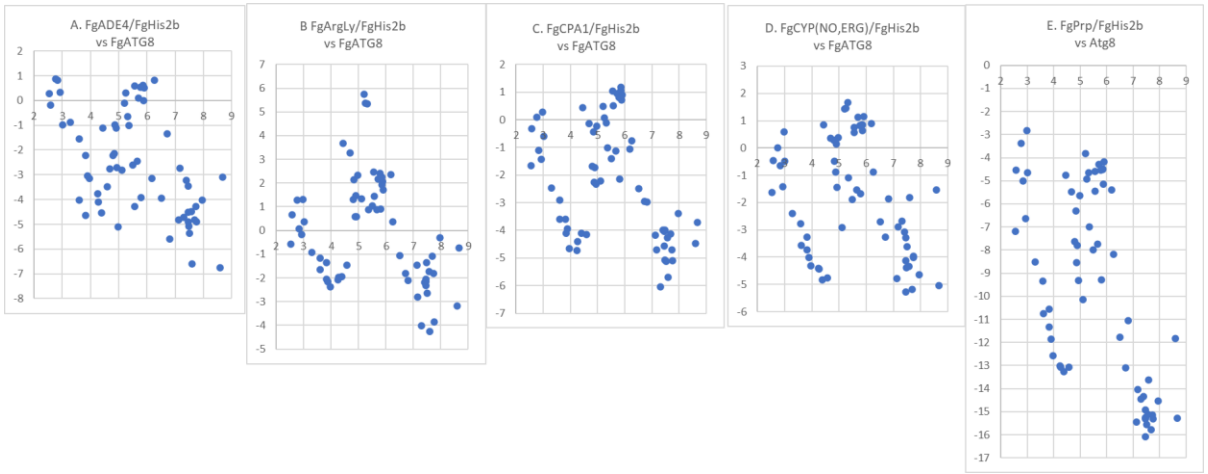
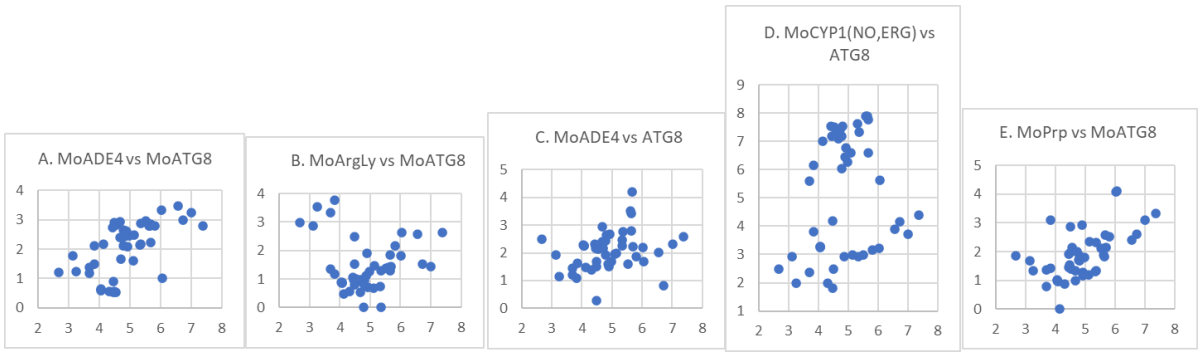


Figure 2. LOG2 expression of key genes in relation to LOG2 expression of the autophagy gene ATG8 that is increasingly expressed during plant infection. The transition between biotrophy to necrotrophy takes place at LOG2 ATG8 expression values 4.5-6. (**A.A-E**) Total expression (growth + maintenance related). (**B.A-E**) Maintenance related growth normalized expression as LOG2 of the ratio of respective gene expression divided by His2b expression. All plots are shown with equal scaling on both axes to facilitate comparisons.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

For *M. oryzae* a similar pattern as for *F. graminearum* but less pronounced was observed for all genes (**Fig. 3A.A-E**). Growth normalized FgPrp was strongly upregulated especially at the transition between biotrophy to necrotrophy while this was not found for MoPrp (**Fig. 3B.E**). This could indicate that there is either very little ROS stress at the transition between biotrophy to necrotrophy for *M. oryzae* or MoPrp is not functionally regulated in response to DNA damages due to oxidative stress or has lost both function and regulation.

A.



B

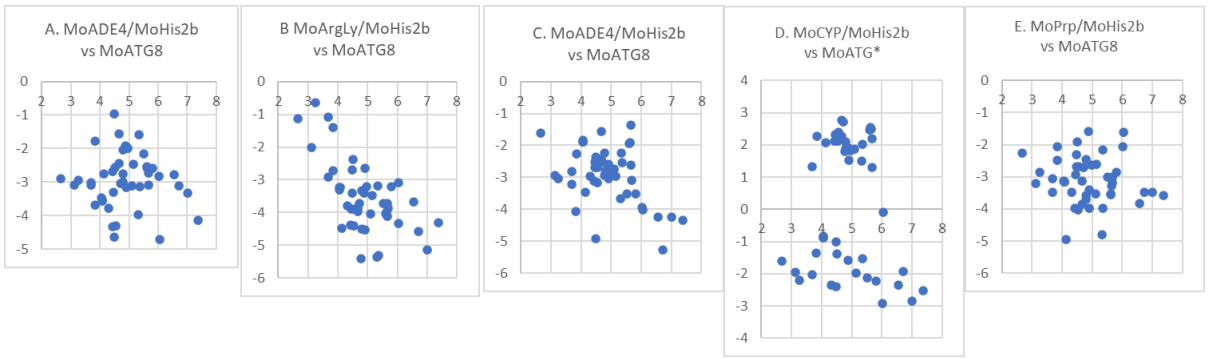


Figure 3. LOG2 expression of key genes in relation to LOG2 expression of the autophagy gene ATG8 that is increasingly expressed during plant infection. The transition between biotrophy to necrotrophy takes place at LOG2 ATG8 expression values 4.5-6. **A.A-E**. Total expression (growth + maintenance related. **B.A-E**. Maintenance related growth normalized expression as LOG2 of the ratio of respective gene expression divided by His2b expression. All plots are shown with equal scaling on both axes to facilitate comparisons.

Oxidative stresses during plant infection

Fusarium graminearum

To investigate if there is ROS stress, we decided to explore the relative expression for all catalase (CAT) orthologues in both fungi needed to counteract intrinsically and plant made H_2O_2 and all nitric oxide dismutase (NOD) orthologues needed to counteract intrinsically and plant made NO. We found 5 good candidate orthologues to yeast (*Saccharomyces cerevisiae*) CatA, genes, FgCAT1-5 (**Table 2**). All 5 are activated in the biotrophy to necrotrophy transition (**Fig. 4**). This is especially obvious when investigating the growth normalized expression where the expression profiles for all genes resembled the PARP orthologue (FgPrp) expression profile (**Fig. 2A.E and B.E**).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

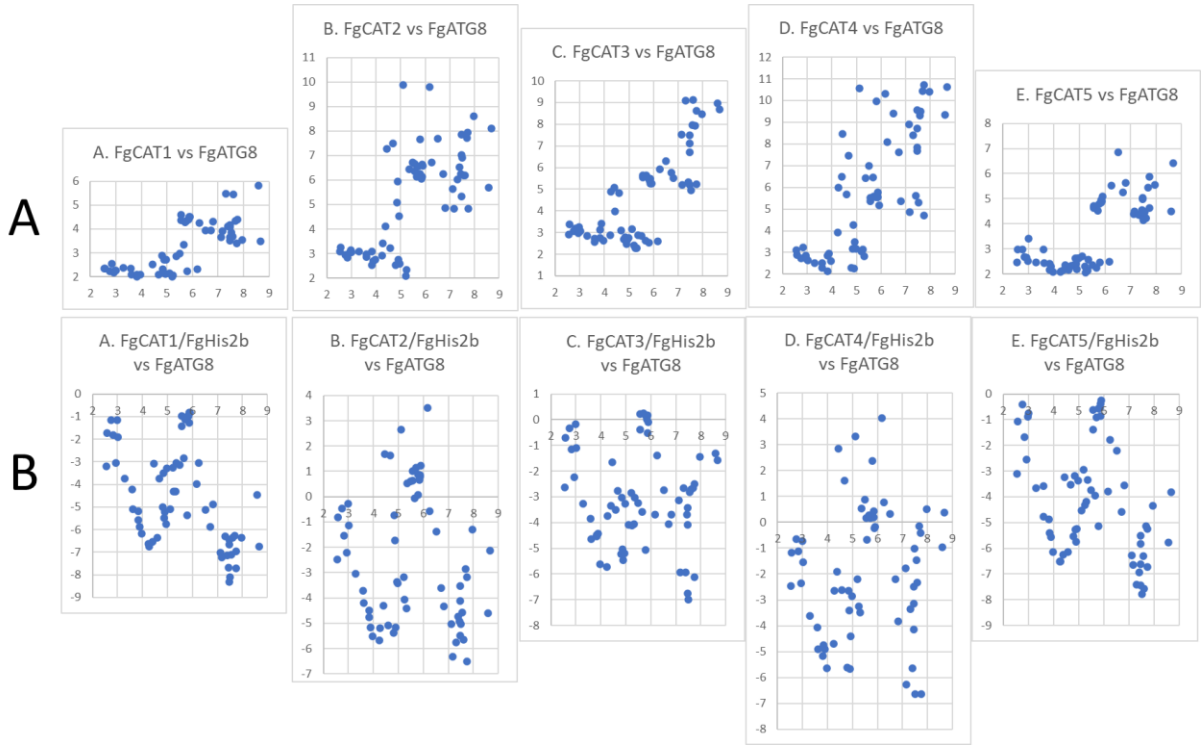


Figure 4 Catalase orthologues in *Fusarium graminearum*. **A.A-E.** Expression vs FgATG8 (HPI). **B.A-E.** Growth normalized expression using FgHis2b for normalization vs FgATG8 (HPI). All plots are shown with equal scaling on both axes to facilitate comparisons.

If the expression of these genes responds to oxidative stresses that also causes DNA damage, they should also be correlated with FgPrp expression and they are, especially when both gene expressions are growth normalized (**Fig. 5A-E**).

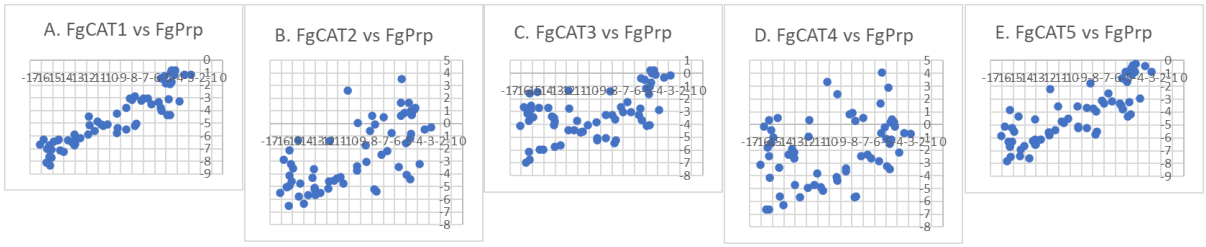


Figure 5.A-E. Catalase homologues vs FgPrp in *F. graminearum* both normalized for growth. There are strong correlations between the FgCATs and FgPrp as would be expected if all FgCATs and FgPrP help the fungus against H_2O_2. All plots are growth normalized and shown with equal scaling on both axes to facilitate comparisons.

Two catalase genes are annotated for *M. oryzae* (**Table 2**). These were also the only one we could find that have similarities to yeast CatA. Of these, only MoCAT1 seems to respond and be upregulated in the transition between biotrophy to necrotrophy (**Fig. 6**) and appear to stay high also growth normalized in the whole necrotrophic phase at high MoATG8 expression.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

Magnaporthe oryzae

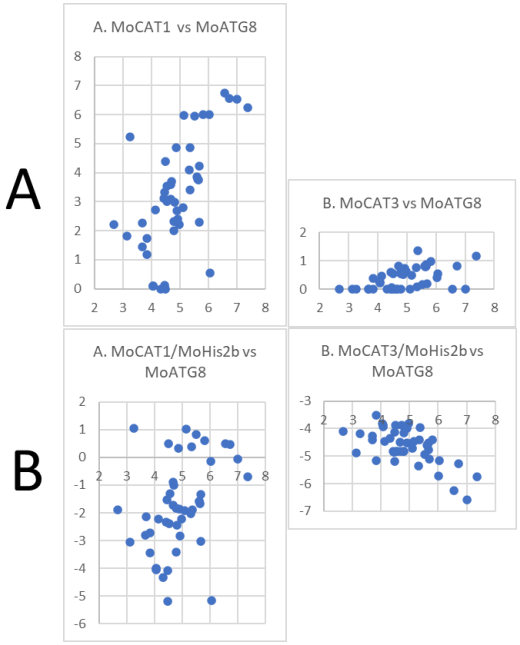


Figure 6. Catalase orthologues in versus MoATG8 (HPI). A.A-B normal expression. B.A-B normalized for growth rate (MoHis2B). All plots are shown with equal scaling on both axes to facilitate comparisons.

However, none of the two catalase orthologues are strongly positively correlated with the MoPrp gene (**Fig. 7.A-B**) as would be expected if any of the catalases helped the fungus defend against ROS. It appears like MoPrp is not activated although MoCat1 is activated indicating that MoPrp might not be upregulates due to oxidative DNA damages. This is a bit strange since the fungus apparently need to use catalase to withstand other damages being exposed to ROSs during necrotrophy (**Fig. 6.A and C**). Not activating or inhibiting the PARP gene can lead to increased mutations due to DNA damages [14,23].

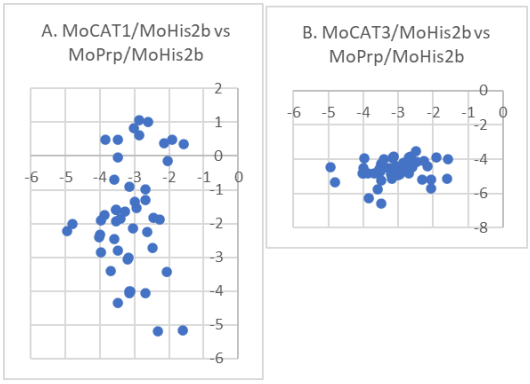


Figure 7A-B. Catalases in *M. oryzae* normalized for growth vs the DNA repair MoPrp normalized for growth. Both plots are shown with equal scaling on both axes to facilitate comparisons.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

Oxidative stresses due to NO

In *F. graminearum* there are two nitric oxide dismutase genes [17] FgNOD1 and FgNOD2. Both genes are sharply upregulated in the transition between biotrophy to necrotrophy (**Fig. 8A,B**) most likely because of plant generated NO instead of intrinsically generated NO that seem to dominate at low expression levels of ATG8 (HPI) . This pattern is even more pronounced when normalizing for growth (**Fig. 8C,D**). The likely response to plant generated NO in the transition is made even more likely if instead normalized for the main protein involved in intrinsic NO formation in the fungus since now it can be seen there is a balance between FgNODs and FgCYP(NO,ERG) before the biotrophy-necrotrophy transition (**Fig, 8E,F**).

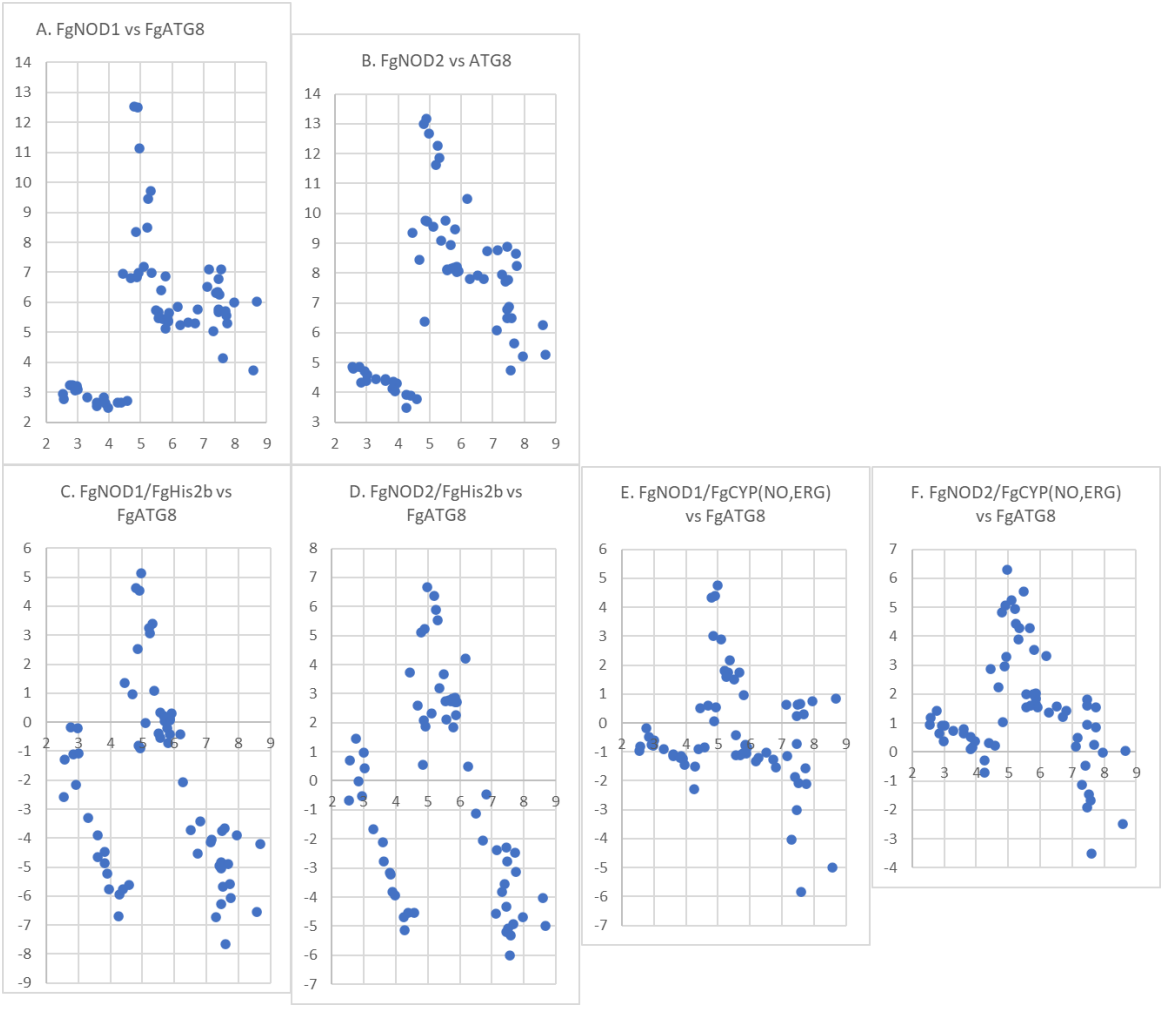


Figure 8. FgNOD1 and FgNOD2 in *F graminearum* vs HPI (FgATG8). A and B without growth normalization C and D with growth normalization using FgHis2b. E and F with normalization for the NO forming CYP using FgCYP(NO,ERG). All plots are shown with equal scaling on both axes to facilitate comparisons.

Thus plant NO stress most likely dominates inside the plant, while intrinsically produced NO probably dominates at low ATG8 levels (LOG2<4.5) (low HPIs before entering biotrophy). To test this idea, we plotted the ratio of the expression of the two NOD genes per FgCYP(NO,ERG) (FgNOD1 or 2/FgCYP(NO,ERG)) as indicator for intrinsically produced NO versus FgATG8 to see if intrinsically dominated NO is more likely at low FgATG8 levels (HPI), and it is (**Fig. 9A,B**). and also against intrinsic

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

NO generation indicated by FgCYP(NO,ERG) expression (**Fig. 9C, D**). This indicates that during plant infection after the biotrophy-necrotrophy transition intrinsically produced NO is negatively correlated with NO defenses pointing to that these defenses are most likelyagainst plant generated NO.

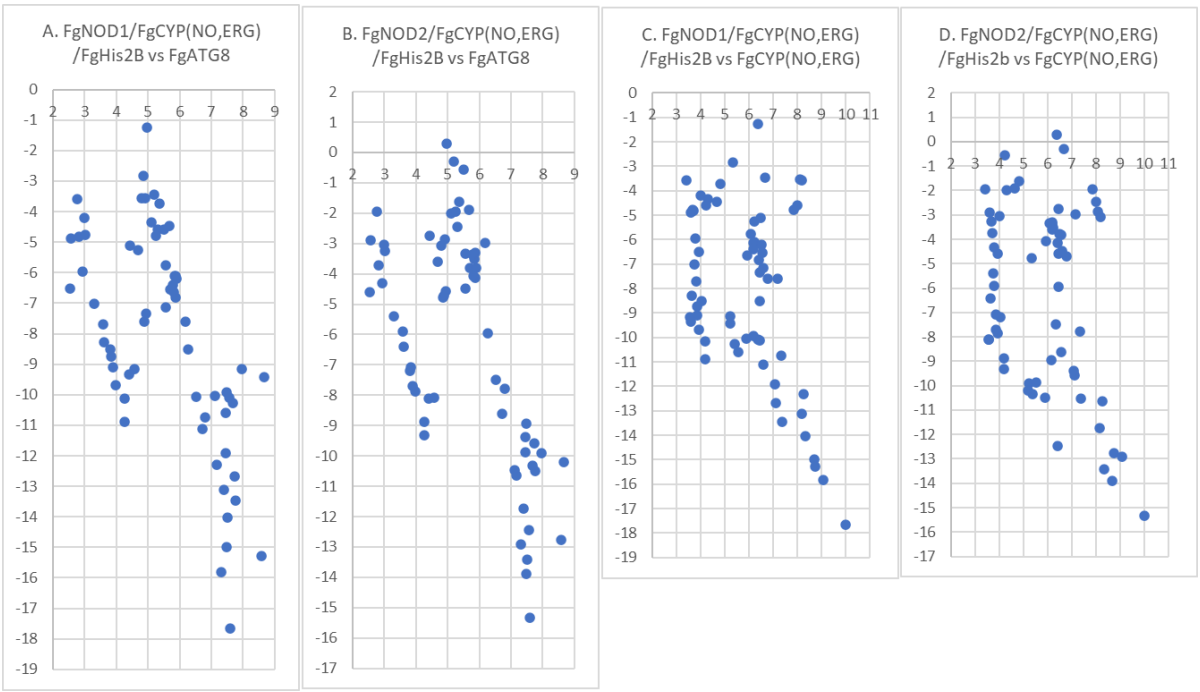


Figure 9. A-B shows NO stress (extrinsic) indicated as FgNODs/FgCYP(NO,ERG) is high at low HP and in the transition between biotrophy to necrotrophy but at high FgCYP(NO,ERG) expression (above 8-9) does not seem to be counteracted by NOD. C-D sshow the same ratios plotted against FgCYP(NO,ERG) and show that there is in principle an inverse relationship between intrinsic NO formation and defense against NO. All plots are shown with equal scaling on both axes to facilitate comparisons.

On the other hand, expression of both NODs that are indicative of needed ROS defenses due to NO are strongly correlated with PARP expression suggesting that more DNA repair is needed at the high NO levels mainly caused by the plant defenses (**Fig. 10A-B**).

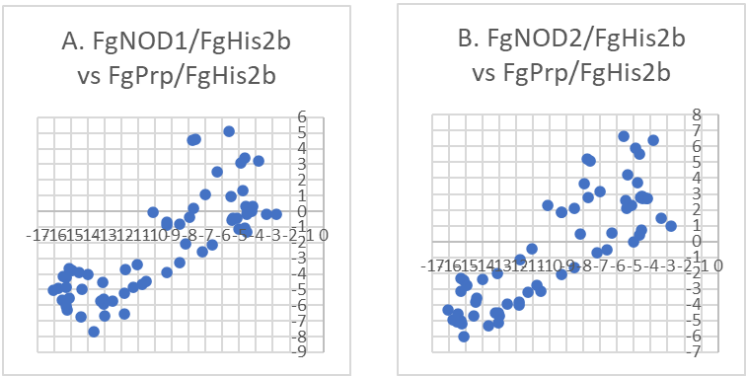


Figure 10A-B. The two FgNODs plotted versus FgPrp both normalized for growth. Strong correlation with is seen as would be expected if both helps the fungus against NO produced by itself and/or by the plant. Both plots are shown with equal scaling on both axes to facilitate comparisons.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

Magnaporthe oryzae

In *M. oryzae* there is only one good BLAST hit for a NOD orthologue, MoNOD (**Table 2**). For *M. oryzae* the pattern is less clear but MoNOD is as for *F. graminearum* sharply upregulated in the transition between biotrophy to necrotrophy (**Fig. 11A**). This is also most probably because of plant generated NO instead of intrinsically generated NO at low expression levels of MoATG8 (HPI) but might also be aided by intrinsically formed NO in the necrotrophic phase (at high MoATG8 levels). This pattern is even more pronounced when normalizing for growth (**Fig. 11 B**) where it can be seen that the MoNOD is sharply upregulated in the biotrophy-necrotrophy transition. In addition, the response to plant generated NO in the biotrophy-necrotrophy transition appear even more likely when we normalize for the main protein involved in intrinsic NO formation in the fungus since when growth adjusted MoNOD is high (**Fig, 11B**) at the same time MoNOD/MoCYP(NO,ERG) ratio is low (**Fig. 11C**). It is also clear that at late stages of infection, NO from the plant probably plays a large role since then this expression ratio increase (**Fig. 11C**).

To test the idea further that plant NO stress most likely dominates inside the plant while intrinsically produced NO probably dominates at low MoATG8 levels (low HPIs before entering biotrophy). We plotted the ratio of the expression of the NOD gene per MoCYP(NO,ERG) as indicator for intrinsically produced NO versus MoATG8 to see if intrinsically dominated NO is more likely at low ATG8 levels and it is (**Fig. 11D**) and against FgCYP(NO,ERG) (**Fig. 11E**) to confirm that it is negatively correlated at high levels. This indicates that during plant infection gene expression of the gene for intrinsically produced NO seem to be negatively correlated with the gene necessary for NO defenses (**Fig. 11D, E**) as for *F. graminearum* (**Fig. 9**).

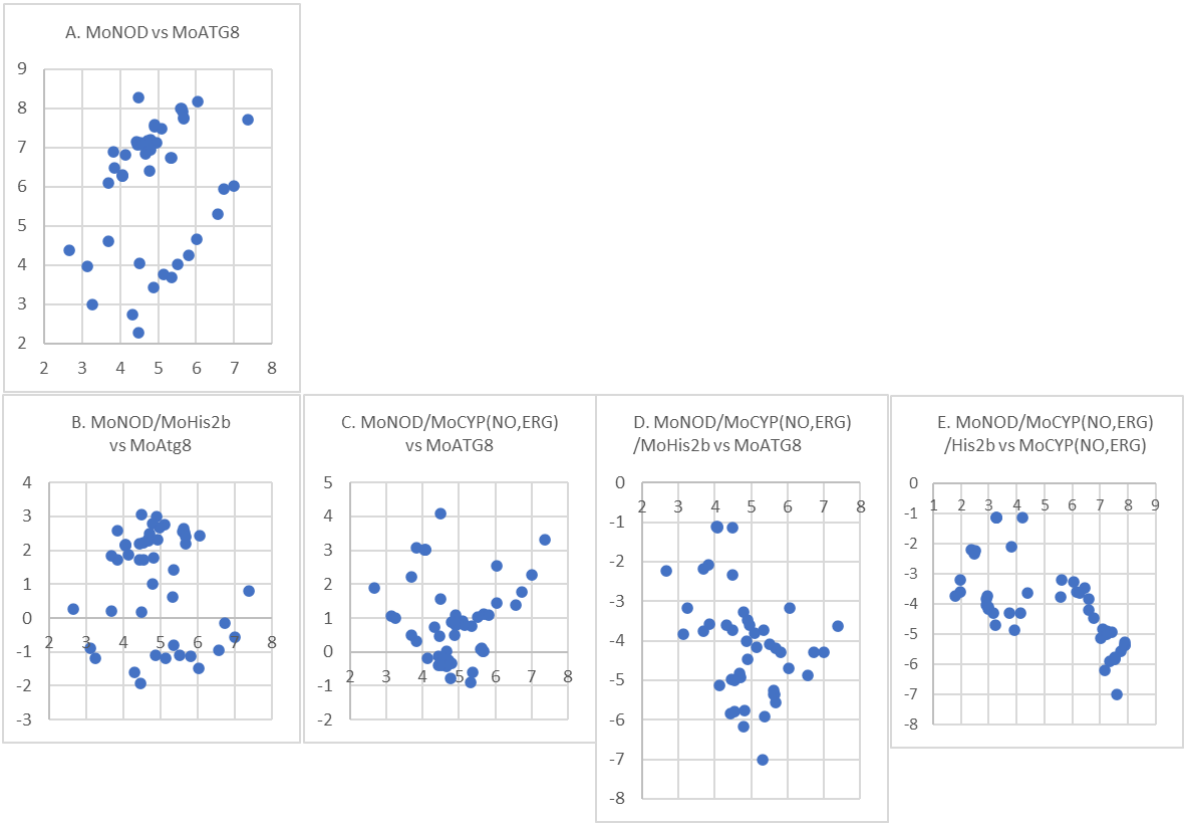


Figure 11. A. MoNOD versus MoATG8 (HPI). B. MoNOD growth normalized as MoNOD/MoHis2b versus MoATG8 (HPI). C. MoNOD MoCYP(NO,ERG) normalized versus MoATG8 (HPI). D. The ratio in C growth normalized versus MoATG8 (HPI). E. Same D but versus MoCYP(NO,ERG) showing basically an inverse relationship. All plots are shown with equal scaling on both axes to facilitate comparisons.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

Finally, since also NO might damage DNA and then DNA need to be repaired to remove point mutations by MoPrp. If MoPrp is active in repairing such damages we should see a positive correlation between MoPrp gene regulation and NOD gene regulation as we saw for the similar relation in *F. graminearum* (**Fig. 10A-B**) but no such pattern is clearly visible (**Fig. 12**), further supporting that in *M. oryzae* an increased DNA repair by MoPrp is not activated by the plant produced NO even if the fungus is stressed by plant produced NO.

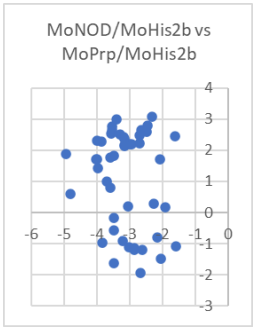


Figure 12. No strong correlation between MoNOD and MoPrp as would be expected if both were active to protect against NO as radical. It appears like MoPrp is not activated. Plot is shown with equal scaling on both axes to facilitate comparisons.

Since there is this difference between FgPrp and MoPrp we investigated if there can be differences in the "parylation toolbox" for the two fungi. Part of this signaling pathway is also poly(ADPribosyl) glycohydrolase (PARG) that is the de-PARylation counterpart to PARP, so we looked for PARG in the *F. graminearum* proteome. PARG enzymes have been described in *Fusarium oxysporum* (FoPARG) [24] and we found an orthologue in *F. graminearum* (Table 2). The *F. graminearum* protein FgPrg has high similarity and is identical to the FoPARG around the active site. There are many orthologues PARGs in fungi and PARGs appear to be well conserved within fungi. However, we could not find any orthologues in *Magnaporthe* sp. To test if the PARG orthologue seem to be active in *F. graminearum* we plotted FgPARG expression and the FgPrp in the *in planta* data together to see if they correlate and they are basically expressed in 1/1 ratio at all stages of infection (**Fig. 13A-B**).

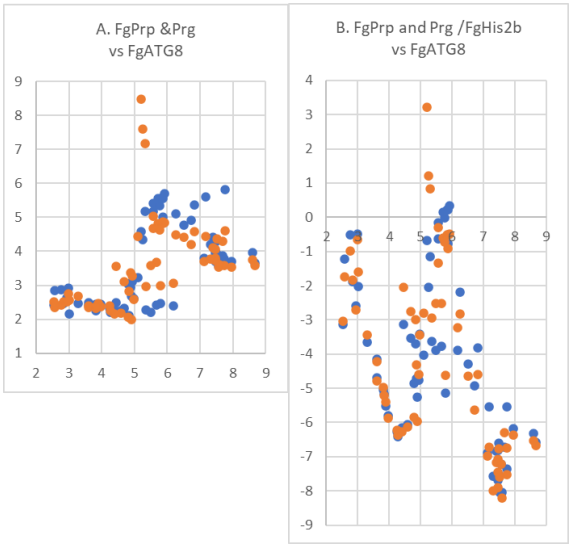


Figure 13 FgPrp putatively responsible for parylation and FgPrg putatively responsible for de-parylation versus FgATG8 (HPI). A. The two genes expression versus FgATG8 (HPI). B. Growth normalized. Blue dots=FgPrp and orange dots=FgPrg. Both plots are shown with equal scaling on both axes to facilitate comparisons.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

For *M. oryzae*, the lack of strong correlation of MoPrg with the ROS indicating genes (catalase and NOD) (**Fig. 7 and 12**) and the lack of a MoPrg all indicate that MoPrp is not active, or at least not in the same way in *M. oryzae* as in *F. graminearum* and most likely do not have the same role in repairing DNA-damage as the PARP/PARG system has in *F. oxysporum* [24] and appears to have in *F. greminearum*.

Non-growing *F graminearum* challenged 0,1,2,4 h with bacterial MAMPs triggering NO formation.

Since we have transcriptome data from 113 transcriptomes of non-growing *F. graminearum* mycelia short term exposed to bacterial MAMPs in water under non-growing conditions [17,22] we decided to also test if the maintenance specific network (**Fig1B**) is activated under these conditions. and if it responds to the intrinsic NO production caused by the FgCYP(NO,ERG) [17,22]. Under these non-growing conditions FgATG8 is not differentially regulated, instead we now use CYP(NO,ERG) expression as an indicator of increasing NO stress due to MAMPs challenges [17]. As can be expected the genes responsible for production of arginine, needed to produce NO, are both positively correlated with FgCYP(NO,ERG), as are purine synthesis FgCPA1 and FgPrp needed for DNA repair (**Fig. 14**). This gives support for the notion that intrinsic NO production indeed causes single nucleotide mutations that needs repair. Although the PARG gene FgPrg is upregulated it does not completely mirror the FgPrp gene as in the *in plant*a data. This can be due to the short time nature of the experiments (1-4h) since protein parylation by PARP is a very fast process [25] and quite fast again removed by PARG [26]. It could also be indicative of an increase in protein PARylation that is part of a fast reacting innate immune response [27].

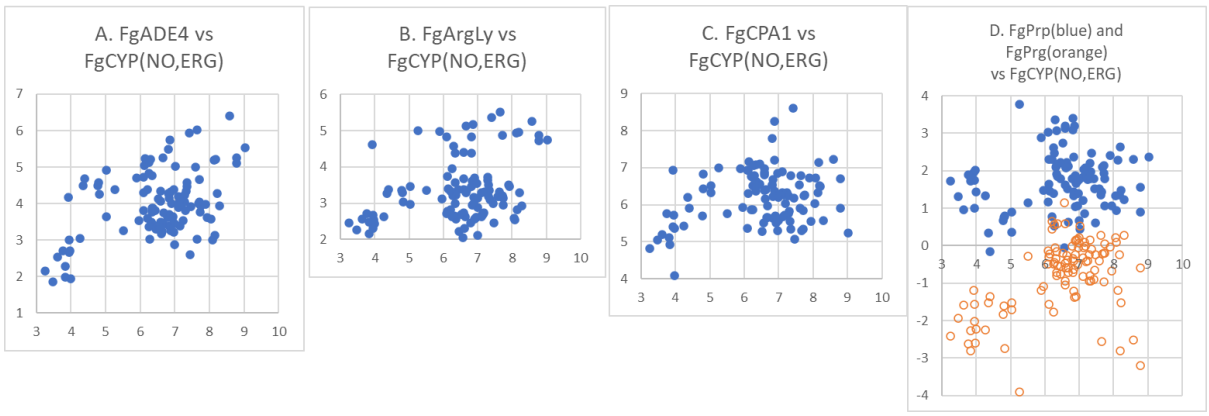


Figure 14A-D. Expression of genes versus FgCYP(NO,ERG) known to form NO when exposed to bacterial MAMPs. All plots are shown with equal scaling on both axes to facilitate comparisons.

ROS counteraction seems to be mainly detected by increased oxidative state through the transcriptional regulator sensor dependent on oxidative stress formed S bridges in the stress transcription factor YAP1 in yeast and its orthologue in *F. graminearum* FgAP1 [28]. Thus, the upregulation of the 5 catalases are most likely indicative of a general oxidative stress rather than a specific sensing of NO or other intrinsically generated ROSs (**Fig. 15**)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

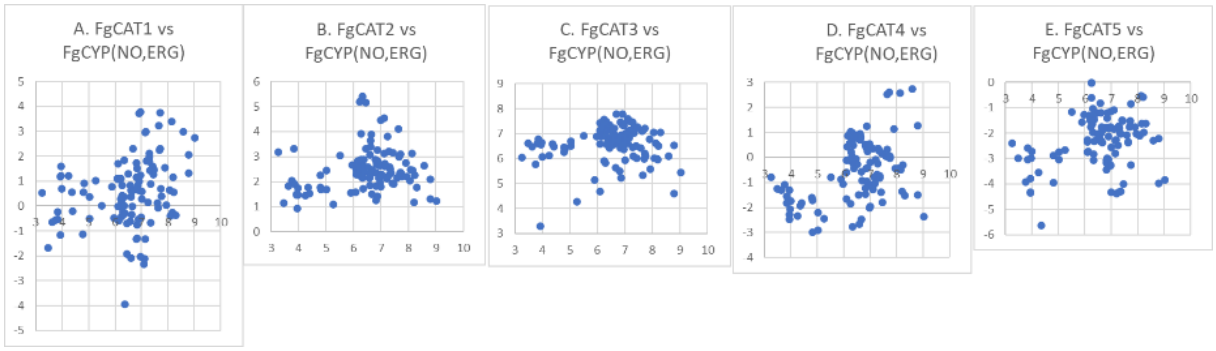


Figure 15. A-E Catalase gene expression seem to be a bit higher at higher NO gene expression. All plots are shown with equal scaling on both axes to facilitate comparisons.

The two NODs that are specifically necessary to regulate NO concentrations are both upregulated with increased expression of the NO generating FgCYP(NO,ERG) especially at the higher levels (**Fig. 16A-B**). NOD1 is localized in the cytoplasm and also in the nucleus while NOD2 is not in the nucleus [17]. The FgNOD1/CYP(NO,ERG) ratio can be expected to be negatively correlated with CYP(NO,ERG) to reach high levels of bacterial detection signaling when exposed to MAMPs. Thus it is expected that there is a very clear negative correlation between FgNOD1/CYP(NO,ERG) ratio and Fg(CYP(NO,ERG) and that is indeed the case (**Fig. 16 C**). There is also a negative correlation between FgNOD2/CYP(NO,ERG) but that seem to be less tightly regulated. FgNOD2 is located in the cytoplasm and in cytoplasmic puncta [17] and is less likely directly involved in directly affecting transcription factors activity. Finally, under these conditions the higher the level of NODs the lower the concentration of NO should be and consequently there should be less DNA damages needing repair by PARP. Thus, NODs and PARP genes are expected to be negatively correlated and they are (**Fig. 17**).

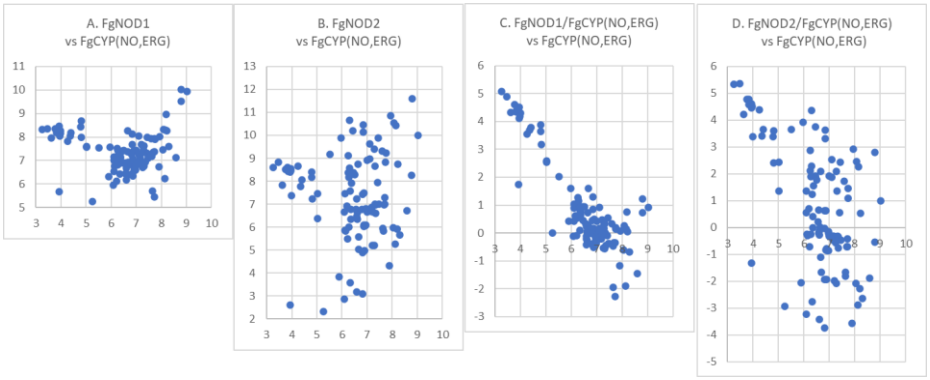


Figure 16. A-B the 2 FgNOD genes increase in activity with NO gene expression especially at the higher levels over 8. C-D FgNOD/FgCYP(NO,ERG) decreases with increasing FgCYP(NO,ERG) indicating increased NO signaling. FgNOD1 that is localized in the whole cytoplasm and also in the nucleus [17] nearly linearly related and probably the main counterpart to FgCYP(NO,ERG). All plots are shown with equal scaling on both axes to facilitate comparisons.

Since FgNOD1 is located in the nucleus and a part of the signaling system allowing NO to affect transcription factors the expression of FgNOD1 and Fg(CYP(NO,ERG) is not strongly correlated but roughly constant (+-Log2=1)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

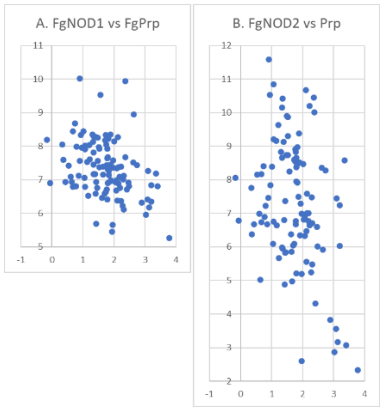


Figure 17. A-B the two FgNODs versus FgPrp. Negative correlations imply that when the FgNODs are high there is less need for FgPrp under these non-growing conditions when the fungus is challenged by bacterial MAMPs since the FgNODs keep NO concentration low.

Potential relative roles of autophagy and apoptosis during the infection phases (HPI) in both fungi.

In *Metarhizium robertsii* (evolutionary not far from *F. graminearum*) the expression of MrBI-1 has been identified linked to apoptosis [29]. Apoptosis is needed to completely empty hyphae (autolysis) for use in other hyphae (reallocation of resources) or for production of conidia [13]. There is only one orthologue to this gene in *F. graminearum* so we name it FgBI1 (**Table 2.**). First, we look at *F. graminearum* growth as measured by FgHis2b expression versus HPI as measured by FgATG8 expression (**Fig. 18A**). In principle growth increases as expected during the course of infection although with different rates. However, FgATG8/FgHis2b expression that is indicative of the use of internal stored resources is high early, then drops during biotrophy, increases in the transition to necrotrophy and then drops again going into necrotrophy for increasing again during last conidiation phases of necrotrophy (**18 B**) while the mirror image FgHis2b/FgATG8 (**18 C**) is consequently indicative of use of external resources, in this case plant biomass.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

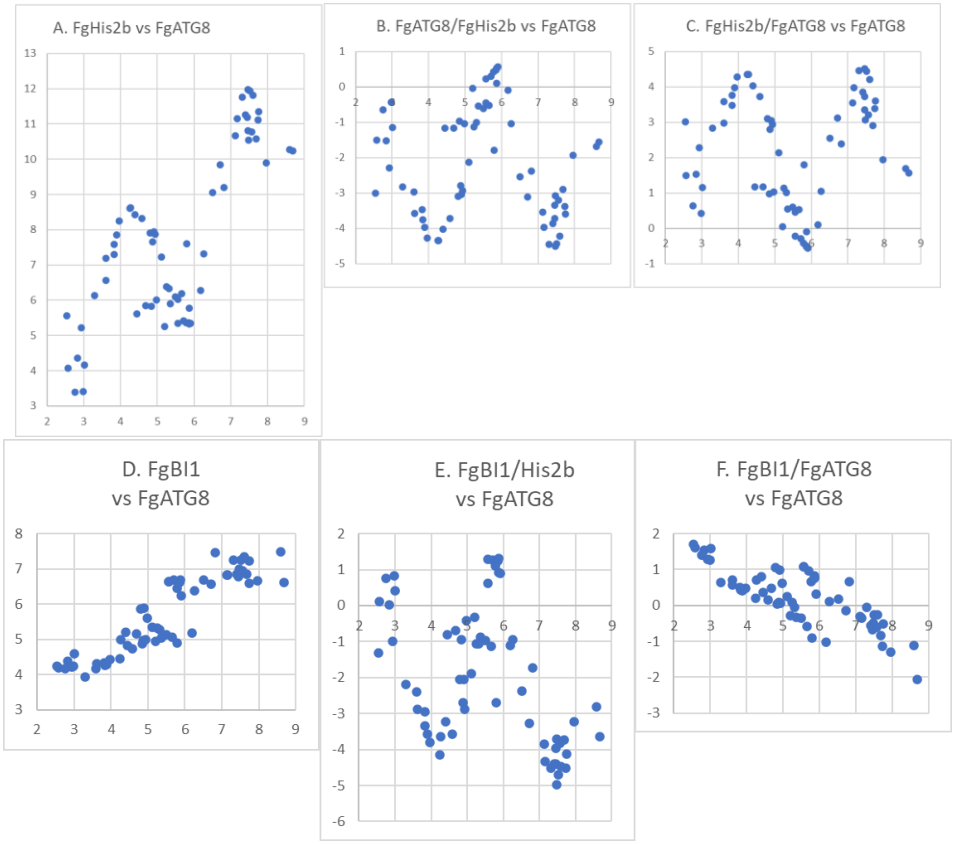


Figure 18. Relationship between His2b (DNA synthesis and growth) and FgATG8 (use of stored resources and repair) versus FgATG8 (HPI). A. With increasing HPI (FgATG8) the fungus increases in growth (FgHis2b). B. Growth corrected MoATG8 expression versus Mo TG8 to indicate when internal resources are used. C. The mirror image of B shows when plant resources are used. D Showing that the apoptosis indicated by FgBI1 increases with FgATG8 (HPI). E. Growth corrected FgBI1 indicate apoptosis is highest before plant entry and in the Biotrophy necrotrophy transitions. F. Apoptosis in relation to autophagy decrease with FgATG8 (HPI). All plots are shown with equal scaling on both axes to facilitate comparisons.

The expression of FgBI1 increases with FgATG8 (HPI) as expected (**Fig. 18D**) since reallocation of resources from non-productive hyphae to productive hyphae are keys in mycelium development [30,31]. Consequently, growth normalized FgBI1 expression vs HPI (ATG8) also looks like the ATG8 curve (**Fig. 18D**). Apoptosis seems to play a relatively slightly decreasing role compared to autophagy with increasing HPI (ATG8) since the ratio FgBI1/FgATG8 seems to decrease with HPI (ATG8) (**Fig. 18E**).

From **Figure 18** and previous figures we can suggest these phases for the infection of wheat by *F. graminearum*

1. Starts growing using mainly fungal internally stored resources. The arrival pre-penetration stage (<3 LOG2FgATG8-expression).
2. Enters plant and grows considerably without much stress from the plant defenses. The biotrophic stage (3-4.5 LOG2FgATG8-expression)
3. Plant discover the fungus and mounts defenses (ROS stress). Biotrophic growth resources might also have gone scarce causing internal (ROS) stresses similar to when running out of nutrients. The biotrophy-necrotrophy transition stage (4.5-6.5 LOG2FgATG8-expression)
4. Plant cells are killed or inhibited by DON. Growth resumes and reach a higher rate. The necrotrophic stage. (LOG2FgATG8-expression 6.5-8).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

5. Plant cells are consumed and new growth stops and fungus uses autophagy and probably also a fair bit of apoptosis to evacuate vegetative mycelium and form spores [13]. The “emigration”-conidiation stage. LOG2FgATG8-expression >8).

The situation for *M. oryzae* is similar but also different. Growth rate measured as His2b does not increase substantially until in the transition to necrotrophy and in the necrotrophic phase (**Fig. 19 A**). At the switch to necrotrophy and in the necrotrophic phase the relative importance of autophagy to growth is higher indicating stress and/or reallocation of nutrients through autophagy (**Fig. 19 A-C**). On the other hand, apoptotic emptying of hyphae (autolysis) seems to be much more important at an earlier phase in *M. oryzae* than in *F. graminearum.* This is indicative of considerable both growth and sporulation triggered at the transition from biotrophy to necrotrophy as also apoptosis MoBI1 expression increases and stays high at the same level of MoATG8 expression (HPI) non growth normalized and ATG8 normalized. But for this fungus also, there seem to be a slight decrease before a final spurt in apoptosis (aiding conidiation) at the very high levels of MoATG8 expression (HPI) (**Fig. 19 D-F**).

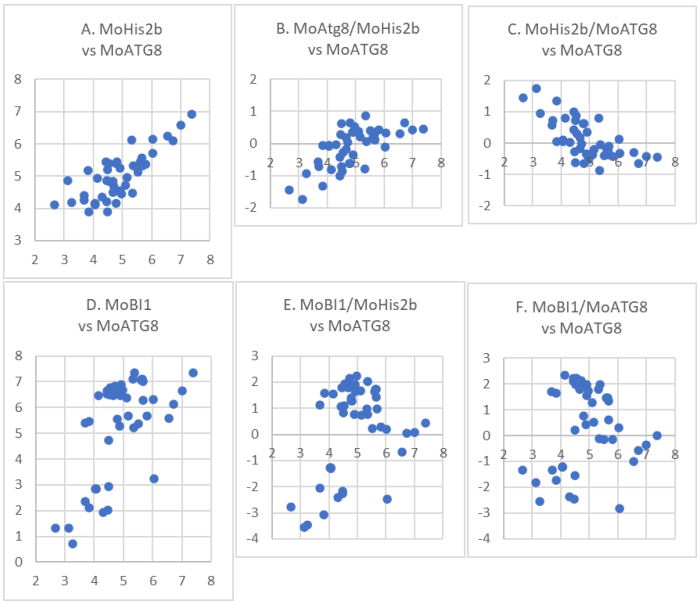


Figure 19 Relationship between His2b (DNA synthesis and growth) and MoATG8 (use of stored resources and repair) versus MoATG8 (HPI). A. With increasing HPI (MoATG8) the fungus increases in growth (MoHis2b). B. Growth corrected MoATG8 expression versus MoATG8 to indicate when internal resources are used. C. The mirror image of B shows when plant resources are used. D Showing that the apoptosis indicated by MoBI1 increases with MoATG8 (HPI). E. Growth corrected MoBI1 indicate apoptosis is highest in the Biotrophy necrotrophy transitions. F. Apoptosis in relation to autophagy is high at intermediate MoATG8 expression(HPI). All plots are shown with equal scaling on both axes to facilitate comparisons.

From **Figure 19** we can suggest these phases for the infection of rice by *M. oryzae*

1. No detectable extra stress in pre-penetration stage or this stage is short so not in the data.
2. Starts using plant resources biotrophically for slow growth/maintenance (<4 LOG2MoATG8-expression).
3. Plant discover Mo and mounts defenses (ROS stress) and/or biotrophic growth resources might have become limited. Killing of the plant and the resources released from the plant speeds up growth but also stresses imposed by the plant increases. Plant ROS stresses induce melanization of fungal cell walls [32]. Fungus starts emptying mycelium to form conidia (4-6 LOG2MoATG8-expression).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

4. Plant cells are killed and resources become scarce autophagy and apoptosis are used to empty mycelium and fill spores. This spore filling probably by mainly by apoptosis starts already early in the transition from biotrophy to necrotrophy (>6 LOG2MoATG8-expression).

Compared to *F. graminearum*. The data indicate *M. oryzae* does not have an extended necrotrophic phase with buildup of vegetative mycelium but starts forming conidia and emptying the mycelium already at the start of the necrotrophic phase. It also appears like apoptosis could play a more important role in this process in *M. oryzae* than in *F. graminearum*. This difference could be the reason for the relatively small lesions seen for *M. oryzae* on rice leaves compared to whole wheat grains and whole wheat heads or seedlings infected by *F. graminearum*. The available resources for the functional mycelium unit (FMU)[30,31] that is the fungal individual seems to be much smaller in *M. oryzae,* and that would be reflected in a short to non-existent "happy" non-stressed growth in the necrotrophic stage.

Conclusion

Testing the hypothesis

Our analysis gives support for the hypothesis presented in the introduction and discussed in relation to the results (above). We now summarize this in a conceptual model in **Figure 20**.

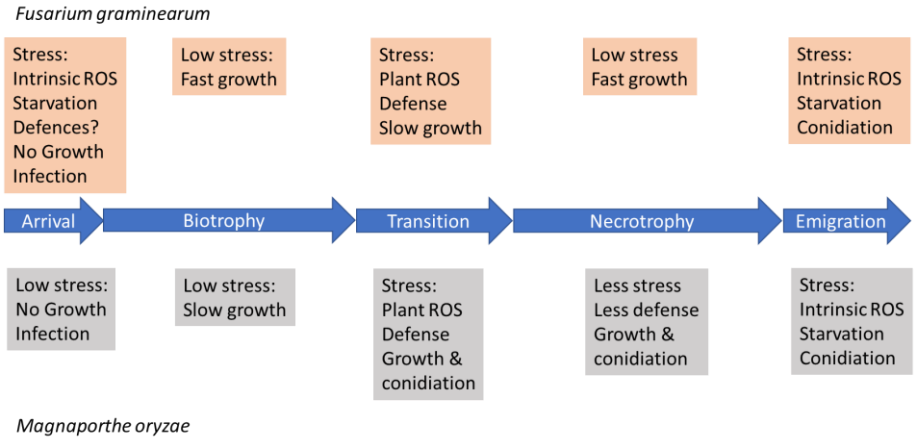


Figure 20. Conceptual model for the progression of the plant interaction in relation to the genes monitored from "arrival" to "departure" for the two plant pathogens, both having a biotrophic stage and a necrotrophic stage and a transition stage in between. The model highlights the found similarities and differences.

The differences between *F. graminearum* and *M. oryzae* in PARP/PARG gene activation and possible consequences for their respective co-evolution with their hosts

The only one of the genes identified to be important in the network (**Fig. 1**) that did not follow the expected pattern for both fungi was PARP needed for DNA repair of point mutations [23]. The PARP gene FgPrp is activated *in planta* in *F. graminearum* but that does not happen for the orthologue MoPrp in *M. oryzae* (**Figs. 2A.E, 2B.E, 3A.E, 3B.E, 5A-E, 7A-B, 10, 12**). In addition *M.oryzae* completely lack an orthologue to the FgPrg necessary for de-PARylation. *M. oryzae* is mainly clonal [9] while *F. graminearum* is commonly sexually reproducing [8]. Having a non-functioning or non-reacting PARP/PARG system should during infection exposure to plant ROS defenses lead to increased mutation rate as have been shown for a *F. oxysporum* PARG mutant [24]. *F. graminearum*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

and *M. oryzae*, although relatively closely related differ in their way of generating offspring genetic variation. *F. graminearum* uses sexual reproduction and the fungus is also homothallic and self-fertile having both necessary mating type loci making it possible to combine positive mutations in haploid nuclei [7,8,33]. As mainly a clonal fungus [9] *M. oryzae* is dependent on mutations within the same nuclei and/or epigenetic changes to overcome resistance development in the host. In conclusion, host ROS caused mutations due to DNA-damages needs to be corrected in *F. graminearum,* but for *M. oryzae,* although immediately lowering infection success for the fungus, DNA damages will speed up mutation rate [24] and shorten number of generations necessary to break plant resistance. In a pathogen with frequent sexual recombination, especially if this is necessary for infection or triggered during infection, the sexual recombination can generate collections of beneficial mutations enough in offspring strains to overcome plant resistance development. In a clonal pathogen, if PARP/PARG is not activated or lost, the host defenses will in itself generate a stress that will increase mutation rate of the pathogen. Both and especially the last creates a Red Queen dynamics for the host pathogen relationship [34] since increased resistance in host creates increases stress in pathogen that directly creates increased variation in the fungus. This results in the fungus being able to break host resistance creating more disease in the host. More resistant hosts survives better when the pathogen is present, and increased resistance in host……..and so on and so on (**Fig.21**). It could be expected that such an automatic host resistance caused increased mutation rate as indicated for *M. oryzae* is more beneficial to the fungus if fungal host range is narrow and infection cycles are short with ample production of infectious conidia.

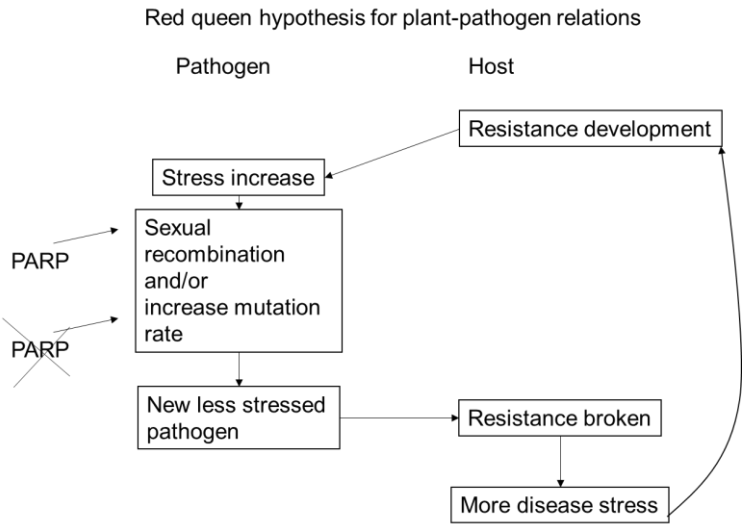


Figure 21. Stress caused by the host causes sexual recombination or increased mutation rate of the fugal pathogens. A non-functioning PARP (PARP/PARG) can potentially increase mutation rate to the benefit of a clonally reproducing pathogen.

Eco-physiological ranges of conditions and gene expressions – the advantage of inferring gene functions from large sets of transcriptomic data

Growth media are generally far too rich and outside eco-physiological ranges for the microorganism of study [31,35]. Thus, under such conditions genes are likely to show gene regulation mainly as a response to these artificial conditions and also additional gene functions not seen within normal ranges [36]. Complete deletion of a gene not only removes that gene function from an early developmental stage (for example spore germination) and can sometime be considered lethal even if its role is not crucial at later stages. This can also cause artifacts by formation of protein complexes

[37]. For most genes that are expressed at different levels during growth in the natural environment a complete deletion creates a physiological situation far outside what the organism can theoretically meet in nature and is prone to cause artifacts of transcriptional adaptation and genetic compensation [38]. Artificial overexpression can cause similar problems by inducing expression levels far outside natural ranges. This is especially a problem if the gene product is cytotoxic if produced in surplus, like for histones [11]. Analyzing correlations between gene expression of genes belonging to physiologically relevant connected processes in transcriptome datasets from natural conditions (not normal lab media) like in the present study can potentially overcome some of these limitations, and we think should be interrogated more generally by researchers. This insight also calls for development of not just tools for conditionally up or downregulation of genes, but also for graded regulations as well as *in-vitro* studies under environmentally more relevant nutrient availabilities and composition than often routinely used. Most problematic is perhaps that there are no fungi growing in isolation in nature. They are always surrounded by their own microflora of bacteria [39] and other microorganisms . This is also why transcriptomic studies of pathogens during natural infection of plants can be better trusted for inferring gene functions than “normal lab media”. For the organism we write about here we especially lack a large number of transcriptomic studies for *F. graminearum* on debris at different ages since incorporation into soil on debris is part of its life cycle. We also lack transcriptomic studies from the rhizosphere of seedlings at different times since a root tip passed since coming close to an inoculum might trigger fungal rhizosphere activities [40,41]. Such studies will be tricky since the amount of RNA will be very small but is today possible [42]. For *M. oryzae* it is obvious in our downloaded transcriptomic data [10] that the amount of RNA has not been large so similar techniques for using small amount of RNA might be used to solve these problems in future research.

Author Contributions

Conceptualization, Stefan Olsson; Data curation, Stefan Olsson and Bjoern Oest Hansen; Formal analysis, Stefan Olsson; Funding acquisition, Zonghua Wang and Guodong Lu; Investigation, Stefan Olsson and Quingfang Li; Methodology, Stefan Olsson and Bjoern Oest Hansen; Resources, Stefan Olsson, Osakina Aron, Hongchen Li and Wenhui Zheng; Supervision, Stefan Olsson, Wei Tang and Zonghua Wang; Validation, Stefan Olsson; Visualization, Stefan Olsson; Writing – original draft, Stefan Olsson; Writing – review & editing, Stefan Olsson, Osakina Aron, Bjoern Oest Hansen, Zonghua Wang, Guodong Lu and Wenhui Zheng.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

Material and methods

All procedures are described in the Results and Discussion section and mainly comprises plotting of expression data against expression data. All data used for this paper are secondary data and has been described in previous papers and are publicly available (**Table 1**).

Table 1. supplemental material available at public websites.

RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
M. oryzae transcriptomic data matrix covering a range of experiments of plant infection	[10]	DOI: 10.6084/m9.figshare.7068857
F. graminearum transcriptomic data matrix covering a range of experiments of plant infection	[10]	DOI: 10.6084/m9.figshare.7068860
F. graminearum transcriptomic data matrix covering a range of experiments of stationary F. graminearum mycelia in water exposed short times (1,2,4h) to purified bacterial MAMPs	[17] Supplementary data	DOI: https://doi.org/10.1101/2020.07.12.191361

Table 2. Genes analyzed in this paper and how these genes were identified

From published papers or annotated at NCBI		
Fg or Mo genes	Abbr. /ID	**SOURCE**
Fg CYtochrome P450 $CYP_{(NO,ERG)}$	$FgCYP_{(NO,ERG)}$ FGSG_01000	[17]
Fg Nitric Oxide Dioxygenase1	FgNOD1 FGSG_00765	[17]
Fg Nitric Oxide Dioxygenase2	FgNOD2 FGSG_04458	[17]
Fg ATG8	FgATG8 FGSG_10740	[12]
Mo ATG8	MoATG8 MGG_01062	[21]
Fg Histone 2b	FgHis2b FGSG_ 11626	[10]
Mo Histone 2b	MoHis2b MGG_03578	[10]
Mo carbamoyl phosphate synthetase small subunit	MoCPA1 MGG_01743	[16]
Mo Arg Lyase (ARG4)	MoArgLy MGG_17278	[20]
Mo Amido phosphoribosyl transferase	MGG_04618 MoADE4	[15]
Mo Catalase1	MoCAT1 MGG_10061	XP_003717445.1

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 December 2020 doi:10.20944/preprints202012.0773.v1

Mo Catalase1	MoCAT3 MGG_06442	XP_003717126.1

Through BLAST comparison with proteins using NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) *Fusarium graminearum* genes				
Organism (Code)	**Annotated gene**	**Orthologue/ID**	**E-value (%ID)**	**Coverage %**
M. oryzae	MoCPA1 See above	FgCPA1 FGSG_09554	0.0 (79.82)	94
M. oryzae	MoArgLy See above	FgArgLy FGSG_03694	0.0 (70.11)	97
M. oryzae	MoADE4 See above	FGSG_05278 FgADE4	0.0 (65.60)	84
Metarhizium robertsii	MrBI-1 XP_007826493.1|	FgBI1 FGSG_09422	0.0 (88.41)	100
Aspergillus nidulans	PrpA AAQ23182.1	FgPrp FGSG_05924	2e-175 (50.83)	78
Saccharomyces cerevisiae	CatA DAA12096.1	FgCAT1 FGSG_02881	9e-134 (43.27)	91
S. cerevisiae	CatA DAA12096.1	FgCAT2 FGSG_05695	1e-125 (42.31)	95
S. cerevisiae	CatA DAA12096.1	FgCAT3 FGSG_06596	2e-105 (40.00)	86
S. cerevisiae	CatA DAA12096.1	FgCAT4 FGSG_06554	6e-85 (41.97)	67
S. cerevisiae	CatA DAA12096.1	FgCAT5 FGSG_06733	1e-83 (39.34)	69
Fusarium oxysporum	FoPARG1 XP_018241514.1	FgPrg FGSG_09290	0.0 (70.34)	91
***Magnaporthe oryzae* gene (mostly identified through comparisons with *F. graminearum*)**				
F. graminearum	FgCYP(NO,ERG) See above	MoCYP1(NO,ERG) MGG_04432	0.0 (72.11)	99
F. graminearum	FgCYP(NO,ERG) See above	MoCYP2(NO,ERG) MGG_04628	0.0 (55.78)	94
F. graminearum	FgNOD1 and 2 See above	MoNOD MGG_00198	2e-116 (42.73)	90
Metarhizium robertsii	MrBI-1 XP_007826493.1|	MoBI-4* MGG_00198	5e-145 (74.47)	100
Aspergillus fumigatus	PrpA AAQ23182.1	MoPrp MGG_08613	0.0 (48.24)	87

*Annotated as MoBI-4 at NCBI, XP_003720584.1

References

1. Lipson, D.A. The Complex Relationship between Microbial Growth Rate and Yield and Its Implications for Ecosystem Processes. *Front. Microbiol.* **2015**, *6*, doi:10.3389/fmicb.2015.00615.
2. Dörter, I.; Momany, M. Fungal Cell Cycle: A Unicellular versus Multicellular Comparison. *Microbiol Spectr.* **2016**, *4*, 1–20, doi:10.1128/microbiolspec.FUNK-0025-2016.
3. van Bodegom, P. Microbial Maintenance: A Critical Review on Its Quantification. *Microb. Ecol.* **2007**, *53*, 513–523, doi:10.1007/s00248-006-9049-5.
4. Ponts, N. Mycotoxins Are a Component of *Fusarium Graminearum* Stress-Response System. *Front. Microbiol.* **2015**, *6*, doi:10.3389/fmicb.2015.01234.
5. Dean, R.; Van Kan, J.A.L.; Pretorius, Z.A.; Hammond-Kosack, K.E.; Di Pietro, A.; Spanu, P.D.; Rudd, J.J.; Dickman, M.; Kahmann, R.; Ellis, J.; et al. The Top 10 Fungal Pathogens in Molecular Plant Pathology. *Mol. Plant Pathol.* **2012**, *13*, 414–430, doi:10.1111/j.1364-3703.2011.00783.x.
6. Ebbole, D.J. Magnaporthe as a Model for Understanding Host-Pathogen Interactions. *Annu. Rev. Phytopathol.* **2007**, *45*, 437–456.
7. Cavinder, B.; Sikhakolli, U.; Fellows, K.M.; Trail, F. Sexual Development and Ascospore Discharge in *Fusarium Graminearum*. *J. Vis. Exp.* **2012**, doi:10.3791/3895.
8. Kelly, A.C.; Ward, T.J. Population Genomics of *Fusarium Graminearum* Reveals Signatures of Divergent Evolution within a Major Cereal Pathogen. *PLOS ONE* **2018**, *13*, e0194616, doi:10.1371/journal.pone.0194616.
9. Zhong, Z.; Chen, M.; Lin, L.; Han, Y.; Bao, J.; Tang, W.; Lin, L.; Lin, Y.; Somai, R.; Lu, L.; et al. Population Genomic Analysis of the Rice Blast Fungus Reveals Specific Events Associated with Expansion of Three Main Clades. *ISME J.* **2018**, *12*, 1867–1878, doi:10.1038/s41396-018-0100-6.
10. Zhang, L.; Zhang, D.; Liu, D.; Li, Y.; Li, H.; Xie, Y.; Wang, Z.; Hansen, B.O.; Olsson, S. Conserved Eukaryotic Kinase CK2 Chaperone Intrinsically Disordered Protein Interactions. *Appl. Environ. Microbiol.* **2020**, *86*, 12.
11. Singh, R.K.; Liang, D.; Gajjalaiahvari, U.R.; Kabbaj, M.-H.M.; Paik, J.; Gunjan, A. Excess Histone Levels Mediate Cytotoxicity via Multiple Mechanisms. *Cell Cycle* **2010**, *9*, 4236–4244, doi:10.4161/cc.9.20.13636.
12. Josefsen, L.; Droce, A.; Sondergaard, T.E.; Sørensen, J.L.; Bormann, J.; Schäfer, W.; Giese, H.; Olsson, S. Autophagy Provides Nutrients for Nonassimilating Fungal Structures and Is Necessary for Plant Colonization but Not for Infection in the Necrotrophic Plant Pathogen *Fusarium Graminearum*. *Autophagy* **2012**, *8*, 326–337, doi:10.4161/auto.18705.
13. Thrane, C.; Kaufmann, U.; Stummann, B.M.; Olsson, S. Activation of Caspase-like Activity and Poly (ADP-Ribose) Polymerase Degradation during Sporulation in *Aspergillus Nidulans*. *Fungal Genet. Biol.* **2004**, *41*, 361–368, doi:10.1016/j.fgb.2003.11.003.
14. Kothe, G.O.; Kitamura, M.; Masutani, M.; Selker, E.U.; Inoue, H. PARP Is Involved in Replicative Aging in *Neurospora Crassa*. *Fungal Genet. Biol.* **2010**, *47*, 297–309, doi:10.1016/j.fgb.2009.12.012.
15. Aron, O.; Wang, M.; Guo, J.; Otieno, J.F.; Zuriegat, Q.; Lu, S.; Li, M.; Wang, Z.; Tang, W. *De Novo* Purine Nucleotide Biosynthesis Mediated by Amidophosphoribosyl Transferase Is Required for Conidiation and Essential for the Successful Host Colonization of *Magnaporthe Oryzae* 2020a.
16. Aron, O.; Wang, M.; Mabeche, A.W.; Wajjiha, B.; Yang, S.; You, H.; Wang, Z.; Tang, W. MoCpa1-Mediated Arginine Biosynthesis Is Crucial for Fungal Growth, Conidiation, and Plant Infection of *Magnaporthe Oryzae*. *bioRxiv* **2020**, doi:10.1101/2020.12.27.424512.
17. Zheng, W.; Li, H.; Ipcho, S.; Fang, W.; Hennessey, R.; Hansen, B.O.; Lu, G.; Wang, Z.; Newman, M.-A.; Olsson, S. Evolutionary Conserved Nitric Oxide Synthesis Proteins Responding to Bacterial MAMPs Are Located to the Endoplasmic Reticulum and Are Also Involved in

Secondary Metabolite Synthesis and Sterol Production. *bioRxiv* **2020**, http://biorxiv.org/lookup/doi/10.1101/2020.07.12.191361, doi:10.1101/2020.07.12.191361.

18. Wong, E.; Cuervo, A.M. Integration of Clearance Mechanisms: The Proteasome and Autophagy. *Cold Spring Harb. Perspect. Biol.* **2010**, *2*, 1–19, doi:10.1101/cshperspect.a006734.
19. Aron, O.; Wang, M.; Mabeche, A.W.; Wajjiha, B.; Yang, S.; You, H.; Wang, Z.; Tang, W. MoCpa1-Mediated Arginine Biosynthesis Is Crucial for Fungal Growth, Conidiation, and Plant Infection of *Magnaporthe Oryzae* 2020b.
20. Zhang, Y.; Shi, H.; Liang, S.; Ning, G.; Xu, N.; Lu, J.; Liu, X.; Lin, F. MoARG1, MoARG5,6 and MoARG7 Involved in Arginine Biosynthesis Are Essential for Growth, Conidiogenesis, Sexual Reproduction, and Pathogenicity in *Magnaporthe Oryzae*. *Microbiol. Res.* **2015**, *180*, 11–22, doi:10.1016/j.micres.2015.07.002.
21. Veneault-Fourrey, C.; Barooha, M.; Egan, M.; Wakeley, G.; Talbot, N.J. Autophagic Fungal Cell Death Is Necessary for Infection by the Rice Blast Fungus. *Science* **2006**, *312*, 580–583, doi:10.1126/science.1124550.
22. Ipcho, S.; Sundelin, T.; Erbs, G.; Kistler, H.C.; Newman, M.-A.; Olsson, S. Fungal Innate Immunity Induced by Bacterial Microbe-Associated Molecular Patterns (MAMPs). *G3 GenesGenomesGenetics* **2016**, *6*, 1585–1595, doi:10.1534/g3.116.027987.
23. Javle, M.; Curtin, N.J. The Role of PARP in DNA Repair and Its Therapeutic Exploitation. *Br. J. Cancer* **2011**, *105*, 1114–1122, doi:10.1038/bjc.2011.382.
24. Araiza-Cervantes, C.A.; Meza-Carmen, V.; Martinez-Cadena, G.; Roncero, M.I.G.; Reyna-Lopez, G.E.; Franco, B. Biochemical and Genetic Analysis of a Unique Poly(ADP-Ribosyl) Glycohydrolase (PARG) of the Pathogenic Fungus *Fusarium Oxysporum* f. Sp. *Lycopersici*. *Antonie Van Leeuwenhoek* **2017**, 11, doi:10.1007/s10482-017-0951-2.
25. Krüger, A.; Bürkle, A.; Hauser, K.; Mangerich, A. Real-Time Monitoring of PARP1-Dependent PARylation by ATR-FTIR Spectroscopy. *Nat. Commun.* **2020**, *11*, 2174, doi:10.1038/s41467-020-15858-w.
26. O'Sullivan, J.; Tedim Ferreira, M.; Gagné, J.-P.; Sharma, A.K.; Hendzel, M.J.; Masson, J.-Y.; Poirier, G.G. Emerging Roles of Eraser Enzymes in the Dynamic Control of Protein ADP-Ribosylation. *Nat. Commun.* **2019**, *10*, 1182, doi:10.1038/s41467-019-08859-x.
27. Lasola, J.; Hodgson, A.; Sun, X.; Wan, F. The PARP1/ARTD1-Mediated Poly-ADP-Ribosylation and DNA Damage Repair in B Cell Diversification. *Antibodies* **2014**, *3*, 37–55, doi:10.3390/antib3010037.
28. Montibus, M.; Ducos, C.; Bonnin-Verdal, M.-N.; Bormann, J.; Ponts, N.; Richard-Forget, F.; Barreau, C. The BZIP Transcription Factor Fgap1 Mediates Oxidative Stress Response and Trichothecene Biosynthesis But Not Virulence in *Fusarium Graminearum*. *PLOS ONE* **2013**, *8*, 12.
29. Chen, Y.; Duan, Z.; Chen, P.; Shang, Y.; Wang, C. The Bax Inhibitor MrBI-1 Regulates Heat Tolerance, Apoptotic-like Cell Death and Virulence in *Metarhizium Robertsii*. *Sci. Rep.* **2015**, *5*, 10625, doi:10.1038/srep10625.
30. Olsson, S. Nutrient translocation and electrical signalling in mycelia. In *The Fungal Colony (Eds. N.A.R Gow, G.D. Robson and G.M. Gadd)*; Gow, N.A.R., Robson, G.D., Gadd, G.M., Eds.; Cambridge University Press: Cambridge, United Kingdom, 1999; pp. 25–48 ISBN 978-0-511-54969-4.
31. Olsson, S. Colonial growth of fungi,. In *Biology of the Fungal Cell*; Howard, Gow, N.A.R., Eds.; The Mycota; Springer Berlin Heidelberg, 2001; pp. 126–141.
32. Crowe, J.D.; Olsson, S. Induction of Laccase Activity in *Rhizoctonia Solani* by Antagonistic *Pseudomonas Fluorescens* Strains and a Range of Chemical Treatments. *Appl. Environ. Microbiol.* **2001**, *67*, 2088–2094, doi:10.1128/AEM.67.5.2088-2094.2001.
33. Mirzaghaderi, G.; Hörandl, E. The Evolution of Meiotic Sex and Its Alternatives. *Proc. R. Soc. B Biol. Sci.* **2016**, *283*, 20161221, doi:10.1098/rspb.2016.1221.

34. Brockhurst, M.A.; Chapman, T.; King, K.C.; Mank, J.E.; Paterson, S.; Hurst, G.D.D. Running with the Red Queen: The Role of Biotic Conflicts in Evolution. *Proc. R. Soc. B Biol. Sci.* **2014**, *281*, 20141382, doi:10.1098/rspb.2014.1382.
35. Zengler, K. Central Role of the Cell in Microbial Ecology. *Microbiol. Mol. Biol. Rev.* **2009**, *73*, 712–729, doi:10.1128/MMBR.00027-09.
36. Pérez-Llano, Y.; Rodríguez-Pupo, E.C.; Druzhinina, I.S.; Chenthamara, K.; Cai, F.; Gunde-Cimerman, N.; Zalar, P.; Gostinčar, C.; Kostanjšek, R.; Folch-Mallol, J.L.; et al. Stress Reshapes the Physiological Response of Halophile Fungi to Salinity. *Cells* **2020**, *9*, 525, doi:10.3390/cells9030525.
37. Hölzel, M.; Rohrmoser, M.; Orban, M.; Hömig, C.; Harasim, T.; Malamoussi, A.; Gruber-Eber, A.; Heissmeyer, V.; Bornkamm, G.; Eick, D. Rapid Conditional Knock-down–Knock-in System for Mammalian Cells. *Nucleic Acids Res.* **2007**, *35*, e17–e17, doi:10.1093/nar/gkl1055.
38. Zimmer, A.M.; Pan, Y.K.; Chandrapalan, T.; Kwong, R.W.M.; Perry, S.F. Loss-of-Function Approaches in Comparative Physiology: Is There a Future for Knockdown Experiments in the Era of Genome Editing? *J. Exp. Biol.* **2019**, *222*, jeb175737, doi:10.1242/jeb.175737.
39. Deveau, A.; Bonito, G.; Uehling, J.; Paoletti, M.; Becker, M.; Bindschedler, S.; Hacquard, S.; Hervé, V.; Labbé, J.; Lastovetsky, O.A.; et al. Bacterial–Fungal Interactions: Ecology, Mechanisms and Challenges. *FEMS Microbiol. Rev.* **2018**, *42*, 335–352, doi:10.1093/femsre/fuy008.
40. Olsson, S.; Nordbring-Hertz, B. Microsclerotial Germination of *Verticillium Dahliae* as Affected by Rape Rhizosphere. *FEMS Microbiol. Ecol.* **1985**, *31*, 293–299.
41. Olsson, S.; Bååth, E.; Söderström, B. Growth of *Verticillium Dahliae* Kleb. Hyphae and of Bacteria along the Roots of Rape (*Brassica Napus* L.) Seedlings. *Can J Microbiol* **1987**, *33*, 916–919.
42. Kanter, I.; Kalisky, T. Single Cell Transcriptomics: Methods and Applications. *Front. Oncol.* **2015**, *5*, doi:10.3389/fonc.2015.00053.