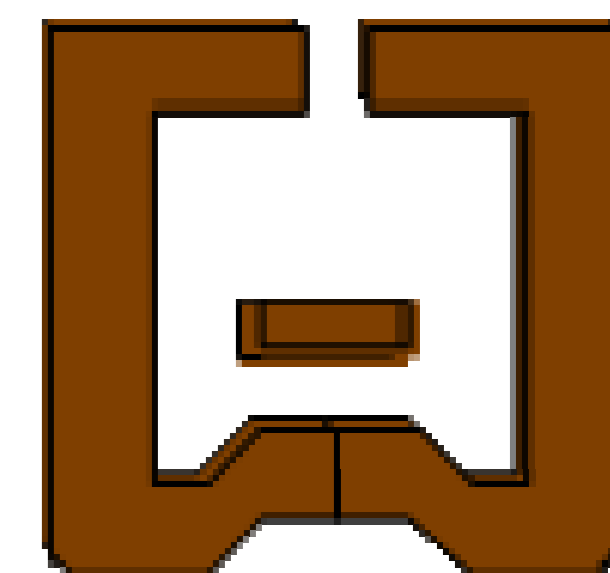


Differentially expressed cDNAs in *Alternaria alternata* tolerant to 2-propenyl-isothiocyanate



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Introduction

The development of resistant pests to synthetic fungicides is a serious problem in agriculture. It highlights the importance of environmentally friendly fungicide compounds like isothiocyanates (ITCs). Although ITCs are potent fungicides against different fungal species under *in vitro*, *in vivo* and *in solum* conditions¹, it was found that *A. alternata* can tolerate lethal concentrations of 2-propenyl-isothiocyanate (2-pITC) after a chronic exposition. The possibility of fungi developing resistance against ITCs, makes important to understand the molecular basis of this phenomenon, e.g. what genes are expressed in response to this natural compound and what proteins are involved in the fungal defence mechanism.

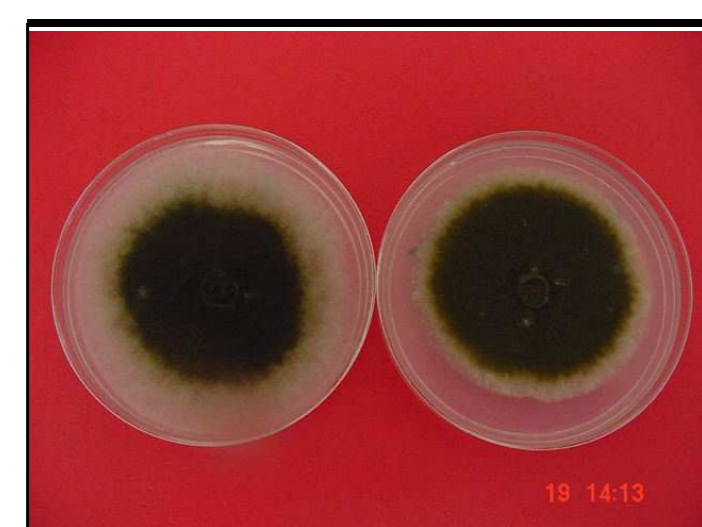
Objective

Isolate and identify the differentially expressed cDNAs in *A. alternata* tolerant to 2p-ITC

Materials and Methods



1. Inoculation on PDA (treated and control fungus)



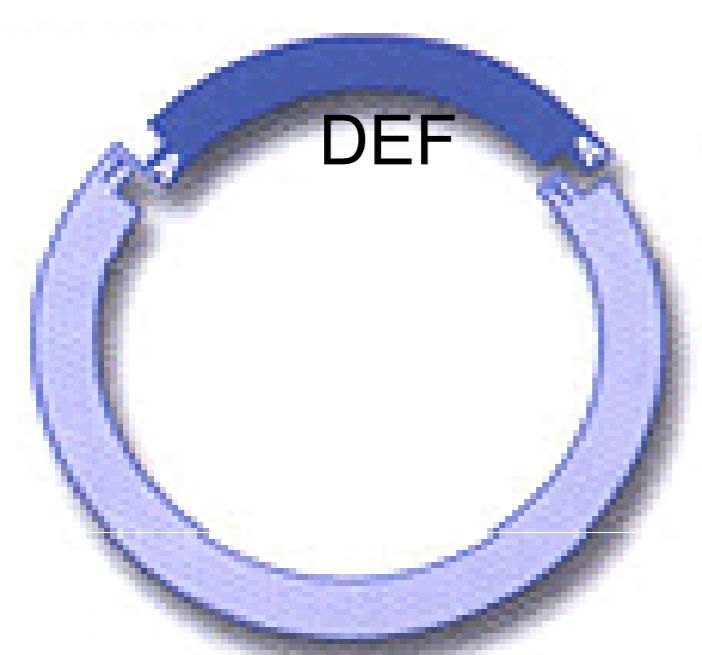
2. Treatment with 9.7 μ M 2p-ITC (treated fungus)



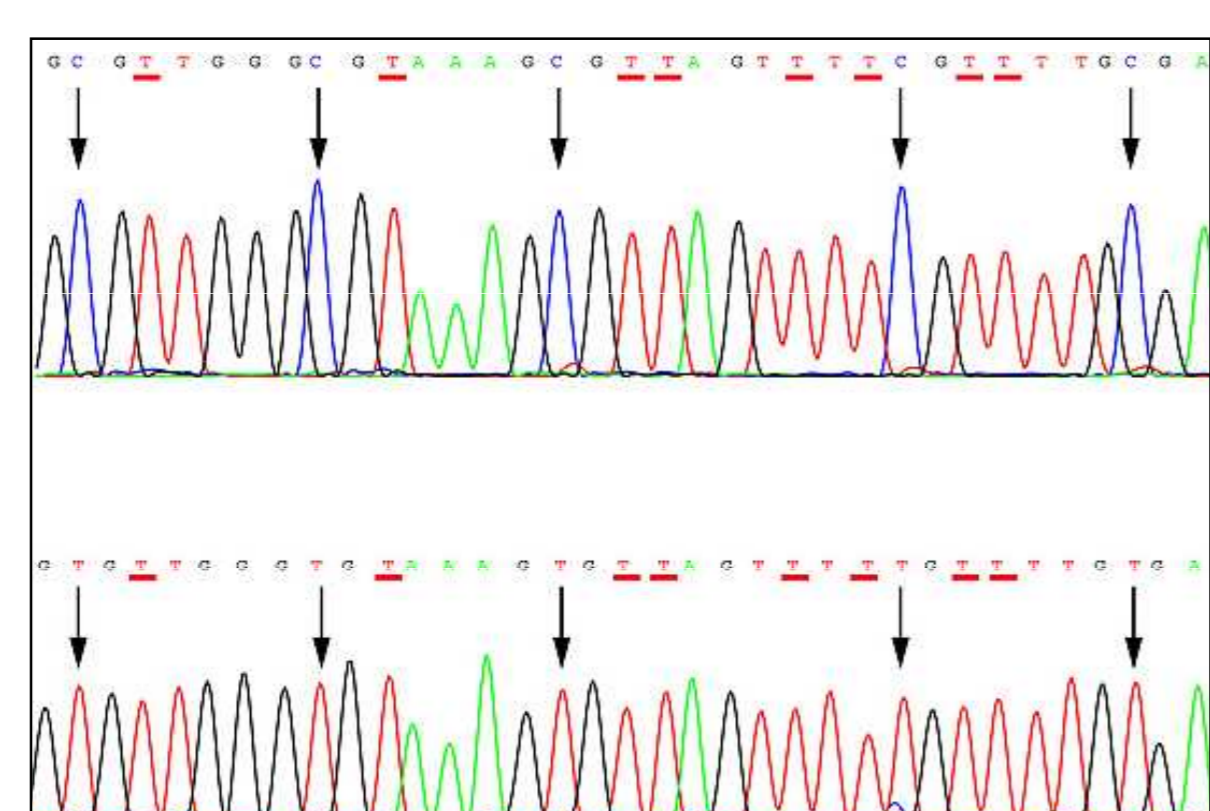
3. Growth (10 days at 28°C)

4. RNA extraction and cDNAs synthesis (Smart cDNA synthesis kit, Clontech)

5. Suppressive subtractive hybridization; obtention of differentially expressed fragments (DEF) PCR select cDNA subtraction kit, Clontech



6. Cloning of DEF into p-GEM vector



7. Clone sequencing

8. Bioinformatic analysis: CAP3 Sequence Assembler Program²; GenBank database using Blastn and Blastx algorithms³

Results

A total of 106 recombinant clones were obtained, with inserts (DEF) size varying from 250 to 750 bp (Fig1). After sequencing and assembly, 55 non redundant DNA fragments were found. The Blast results analysis are summarized in Figures 2 and 3.

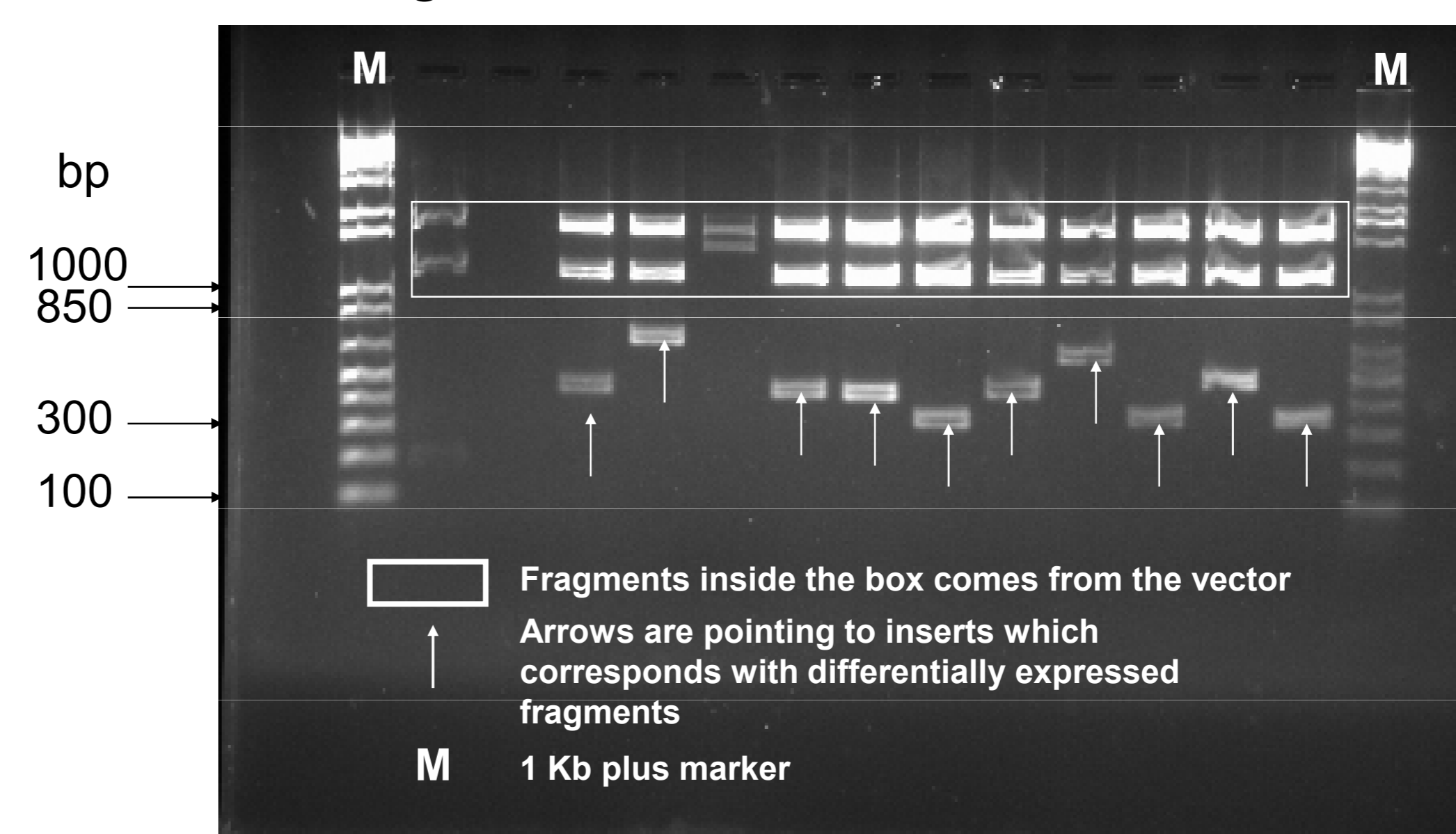


Fig. 1. Electrophoresis gel of DEF obtained from the recombinant clones by digestion

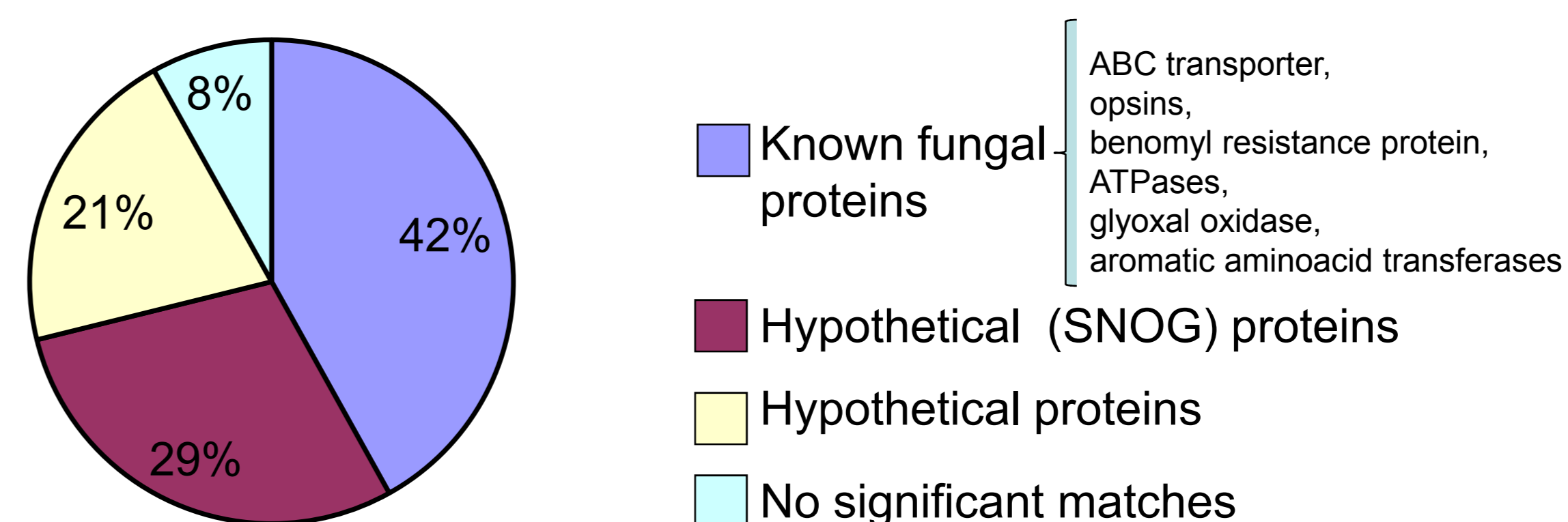


Fig 2. Blast results from non redundant GenBank CDS database

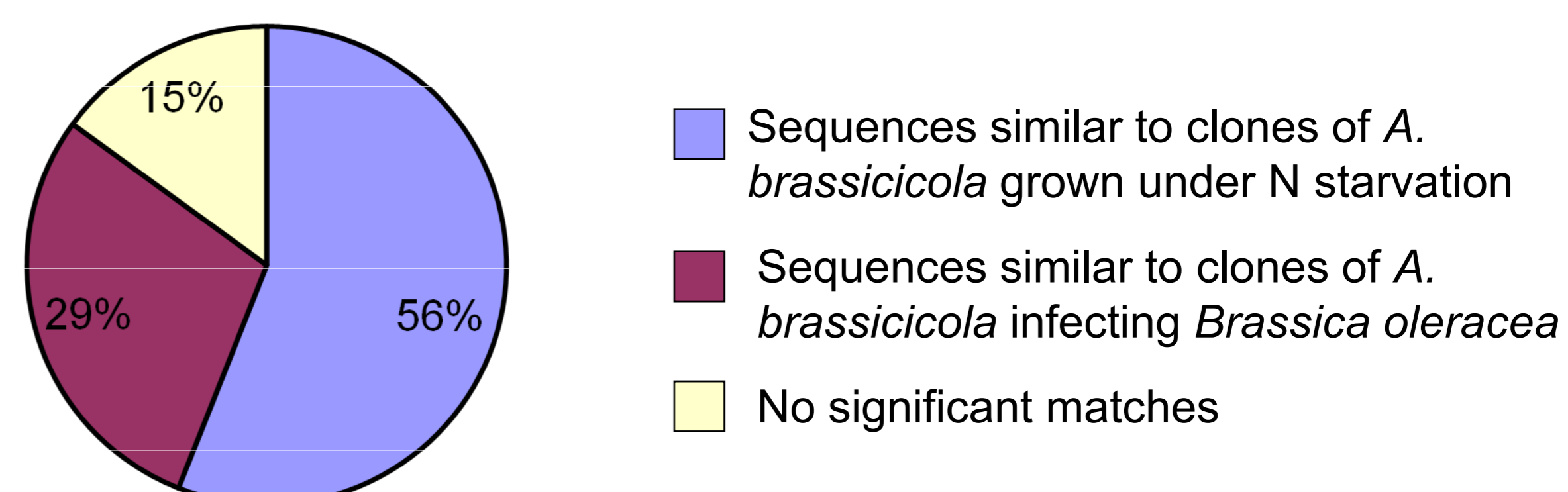


Fig 3. Results of Blast analysis in *Alternaria* ESTs GenBank database

Conclusions

The differentially expressed genes in *A. alternata* 2p-ITC tolerant suggest the induction of:

- "Non degradative" ITC remotion from the cell (membrane integral proteins e.g. ABC transporters, opsins and ATPases)
- Structural maintenance and growth of hypha (e.g. aromatic aminoacid aminotransferase, glyoxal oxidase)
- Oxidative stress response (SNOGs proteins)

Acknowledgements

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References

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