

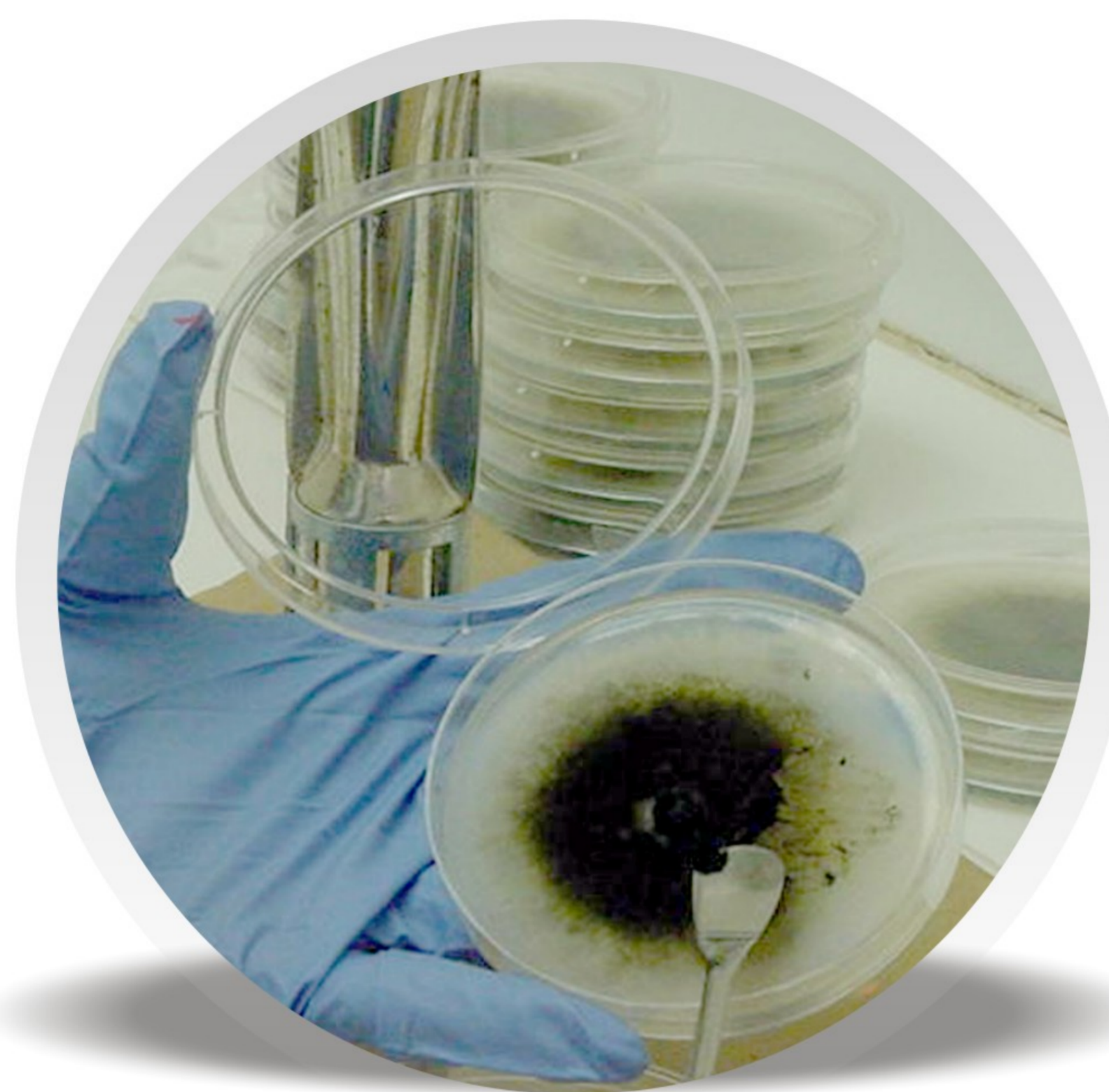
# Transcriptomic Analysis of the *Alternaria alternata* response to 2-Propenyl-Isothiocyanate

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## INTRODUCTION

The ability of fungi developing resistance to either synthetic or natural fungicides highlights the need to elucidate the molecular basis of these phenomena. This knowledge will allow developing environmentally friendly strategies for fungi control. We had studied fungal tolerance to natural compounds using the model *Alternaria alternata* and 2-propenyl-isothiocyanate (2pITC). To isolate genes induced by 2pITC, a forward suppressive subtractive hybridization was carried out using cDNA from *A. alternata* ITC treated as “tester” and cDNA from the untreated fungus as “driver”. The fungal response appears focused in a “non degradative” ITC remotion from the cell, the structural maintenance and growth of hypha, and an oxidative stress response. The fungal resistance phenotype to 2pITC apparently could involve calcium ion and 2p-ITC efflux by an ABC transporter.



## MATERIALS AND METHODS

1. Obtention of differentially expressed fragments in *A. alternata* 2p(ITC) treated and cloning (Suppressive Subtractive hybridization; p-GEM T Easy).

2. Clone sequencing, Assembly and Analysis (VecScreen, CAP3 Assembler, BLAST).

3. Gene Ontology Annotation (Blast2GO v.2).

4. Gene Expression Evaluation (qReal time RT-PCR) and analysis (Rest software).

## OBJECTIVE

Identify genes induced by 2-pITC in *Alternaria alternata* and evaluate the regulation of expression by real time RT-PCR.

## RESULTS

Suppressive subtractive hybridization protocol identified a total of 245 recombinant clones with inserts varying from 250 to 887 bp with an average length of 482. After sequencing and assembly 124 no redundant DNA fragments were found (40 contigs and 84 singletons). The Blast results analysis are summarized in Figure 1.

The Blast2GO annotation provided = 1GO terms for 68 sequences (54%), retrieving a total of 233 annotations for the total set. The percent of sequences assigned to each GO category are shown in Figure 2. Distribution of Gene Ontology Terms is showed in Figure 3.

Out of six genes evaluated following the 2p(ITC) by qReal Time RT-PCR, four showed a statistically significant up-regulation (Table 1).

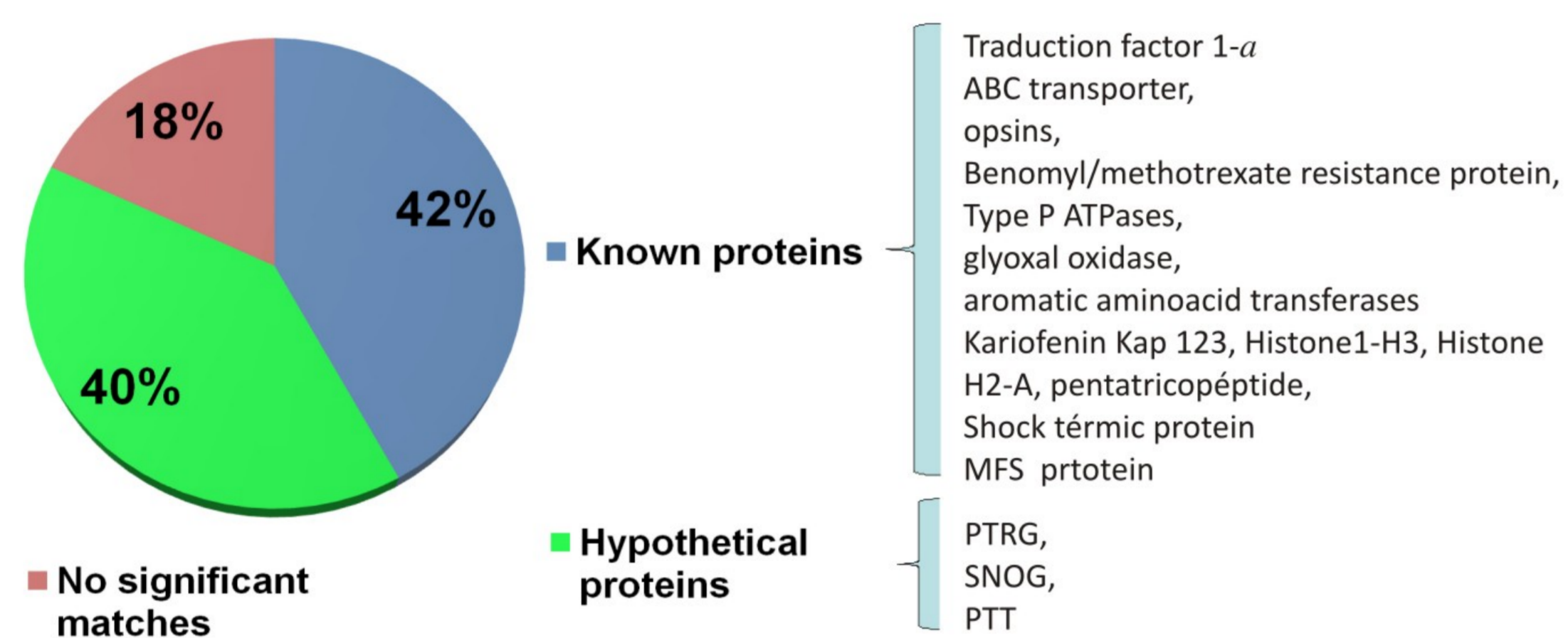


Fig 1. Blastx results from non redundant GenBank CDS database

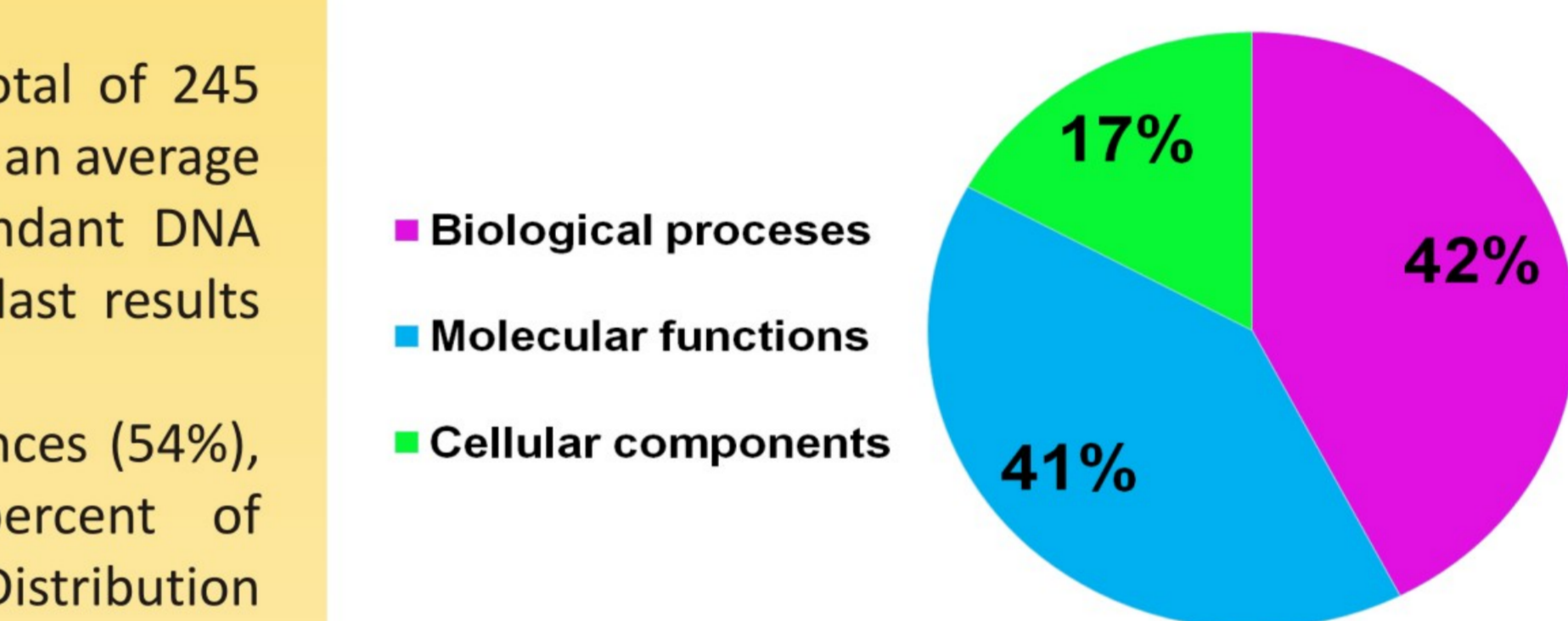


Fig. 2. Percent of sequences assigned to each GO category.

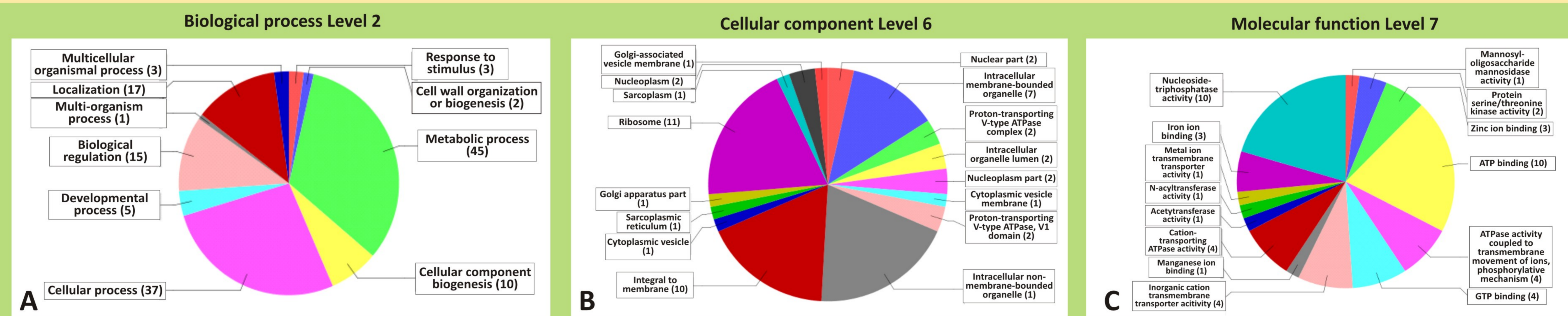


Figure 3. Distribution of Gene Ontology Terms (Level 2) for transcripts from *Alternaria alternata* treated with 2p(ITC). The numbers in graph categories show the frequency for each GO term in the unigenes set. (A) Biological process (Level 2). (B) Molecular function (Level 7). (C) Cellular components (Level 6).

Gene name	Fold change RQ ( $2^{\Delta\Delta Ct}$ ) $\pm$ SD			
	30 min	1h	2h	12h
Opsin	<b>1.39</b> $\pm$ 0.05	0.64 $\pm$ 0.03	0.13 $\pm$ 0.12	0.62 $\pm$ 0.00
Ca <sup>++</sup> ATPase	<b>2.67</b> $\pm$ 0.4	<b>6.24</b> $\pm$ 0.15	<b>3.92</b> $\pm$ 0.23	<b>1.50</b> $\pm$ 0.19
5MH4-PteGlu	0.22 $\pm$ 0.28	0.25 $\pm$ 0.20	0.76 $\pm$ 0.13	0.61 $\pm$ 0.04
EFhand protein	1.48 $\pm$ 0.20	<b>2.29</b> $\pm$ 0.28	<b>1.92</b> $\pm$ 0.26	0.42 $\pm$ 0.13
SNOG protein	1.54 $\pm$ 1.0	0.81 $\pm$ 0.90	1.12 $\pm$ 0.93	1.87 $\pm$ 0.61
ABC transporter	<b>95.8</b> $\pm$ 0.00	<b>25.6</b> $\pm$ 0.04	<b>3.85</b> $\pm$ 0.04	1.38 $\pm$ 0.03

Table 1. Time course expression in six genes in *A. alternata* 2p(ITC) treated. Method: comparative CT; normalization with the CT values from the  $\beta$ -tubuline housekeeping gene. Results are the mean of three repetitions indicating the standard deviation. Shown in bold are the data statistically significant ( $p > 0.05$ ).

## CONCLUSIONS

The fungal response to the toxic effects of 2p(ITC) appears to involve the isothiocyanate efflux mediated by an ABC transporter and the participation of calcium activating ATPases and calmodulin.

## LITERATURE CITED

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