Reactive oxygen and antioxidants modulate the interaction between *Myzus persicae* (Sulzer) and plant hosts

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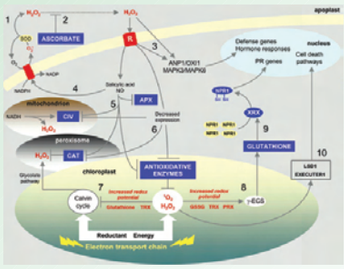


Introduction

In recent years, evidence for reactive oxygen signalling and/or defence in plant-aphid interactions has been accumulating. Numerous microarray studies have demonstrated upregulation of oxidative stress response genes[1-3], direct measurements revealed increased activities of reactive oxygen detoxification[4] and generating enzymes[5], and direct[5] measurements have suggested accumulation of reactive oxygen species (ROS). A scheme outlining ROS generation and detoxification and the link between ROS and plant signalling and defence pathways is outlined in figure 1[6]. Here we present preliminary findings of an investigation into the role of ROS in the interaction between the aphid *Myzus persicae* and its key UK host the potato (*Solanum tuberosum*).

Figure 1 Oxidant and antioxidant signalling in cell death and acclimation

Methods

M. persicae clones collected in Scotland (genotype G[7]) were reared in transparent Perspex cages on 10-12 week old *S. tuberosum* cv. Desiree plants at 18°C under a 16:8h light:dark regime. For infestation experiments, 60 adult aphids were confined to the abaxial leaf surface using a 2.5 cm diameter clip cage.

Glutathione[8] and ascorbic acid[9] were extracted and quantified as previously described. Peroxidase activity was estimated spectrophotometrically using guaiacol as substrate.

Total RNA was extracted using the Plant RNeasy kit (Quiagen) according to the manufacturers instructions and following DNase treatment, cDNA was generated using Ready-to-Go You Prime First Strand Beads (GE Healthcare) according the manufacturers instructions. Relative gene expression was estimated using SYBR Green (Applied Biosystems) with EF-1 as a control.

Results

Aphid infestation perturbs plant redox homeostasis

Two days following aphid infestation the intracellular redox status was significantly shifted towards the reduced as evidenced by enhanced concentrations of reduced ascorbate and a reduction in the oxidised to reduced glutathione ratio (Fig. 2).

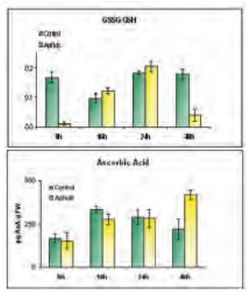


Figure 2 Impact of aphid infestation on cellular redox status of potato leaves

Expression levels of genes encoding ROS removing enzymes were also altered with strong downregulation of Fe-superoxide dismutase (SOD) from the earliest time point following infestation. On the contrary Cu/Zn-SOD was initially marginally upregulated before returning to the expression level found in uninfested plants after 48 h (Fig. 3).

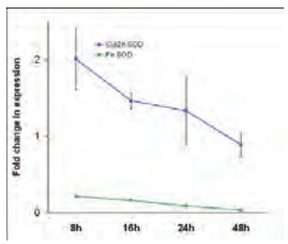


Figure 3 Impact of aphid infestation on superoxide dismutase gene expression in potato leaves

Aphid infestation results in activation of both JA- and SA- mediated defence pathways

Gene expression studies of JAZ-1, a jasmonate responsive gene and PR-1, a salicylate responsive gene, revealed that both were upregulated following aphid infestation although with different amplitude and kinetics (Fig. 4). JAZ-1 was upregulated approximately 8-fold 8h after infestation and expression continued to rise up to 16h. PR-1 expression was slightly elevated at 8h and continued to rise throughout the experiment to achieve a maximal 700-fold expression 48h following infestation.

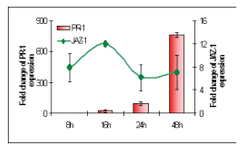
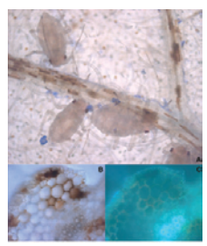


Figure 4 Impact of aphid infestation on SA/JA responsive gene expression in potato leaves

Aphid feeding causes a distinct vein banding pattern in potato leaves



48h following aphid infestation a distinct dark brown deposit was observed along the major veins. Fluorescence microscopy suggested the presence of phenolic compounds primarily accumulating in the apoplast supporting the hypothesis that the deposit consisted of melanins (Fig. 5).

Figure 5 Induction of vein banding in potato leaves following aphid infestation for 48h A) Bright field image of abaxial leaf surface following ethanol decolourisation (5X) B) Bright field image of leaf section (20X) C) Fluorescent image of section shown in B.

Peroxidase activity was also observed to increase over the course of infestation, rising to 1.25 times higher than that in control plants after 48h (Fig. 6).

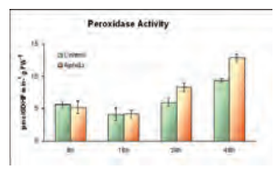


Figure 6 Impact of aphid infestation on peroxidase activity in potato leaves

Conclusions and Future Work

- *M. persicae* infestation significantly alters the cellular redox balance in potato leaves.
- Both jasmonate and salicylate responsive pathways are upregulated in potato leaves following *M. persicae* infestion – possible link with cellular redox status.
- The accumulation of melanins suggests oxidative processes are directly involved in aphid defence.
- Global analysis of gene expression following aphid infestation will be undertaken using microarray technology.
- Redox sensitive dyes and fluorescent protein fusions will be used to analyse the spatiotemporal kinetics of ROS production and downstream signalling.

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