Comparative Analysis of Wild and Domesticated Pigeon Pea using Functional Genomics: Towards Sustainable Food Production

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Introduction

The FAO stated that food productivity must increase by 70% to feed an additional 2.3 billion people. One approach towards achieving this goal is to significantly reduce crop loss to pests and pathogens (estimated at 40%) in a sustainable and cost effective way. One method is to enhance endogenous defence in crops against pest insects, without introducing genetic material from outside the available gene pool, via the identification of functional molecular markers for use in conventional breeding programmes.

Pigeon Pea (*Cajanus cajan*) are important legume crops, forming an important source of dietary protein, particularly in the Indian subcontinent, eastern Africa and Central America countries. Unfortunately these crops suffer an extensive loss due to heavy insect infestation (*Helicoverpa armigera)*. Wild relatives, have displayed high resistance to these pest. Such differences in the level of pest resistance conferred by these species have not yet studied at molecular level.

Aims

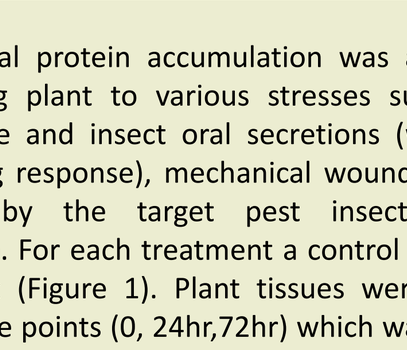
•To establish a molecular relationship between insects and crops by proteomic profiling with the help of available genomic information from Pigeon Pea.
•Understand how domesticated and wild varieties of pigeon pea respond to insect attack
•Identify functional molecular markers using proteomics

Methods and Materials

Differential protein accumulation was assessed upon subjecting plant to various stresses such as methyl jasmonate and insect oral secretions (which elicits a wounding response), mechanical wounding and insect feeding by the target pest insect (Helicoverpa armigera). For each treatment a control was applied to the plant (Figure 1). Plant tissues were collected at three time points (0, 24hr,72hr) which was immediately flash frozen and used for RNA isolation and protein extraction (Figure 1)

Protein accumulation following insect damage was analysed using a proteomics platform employing 2DE for protein separation, and Progensesis Same Spot software was for spot detection and quantification.

A comparative analysis of differentially expressed defensive genes was accomplished by using mass spectrometric techniques. Following this study, the expression of selected putative defense genes was then to be investigated for spatio-temporal responses using quantitative PCR.



Pigeon pea (*Cajanus cajan)*

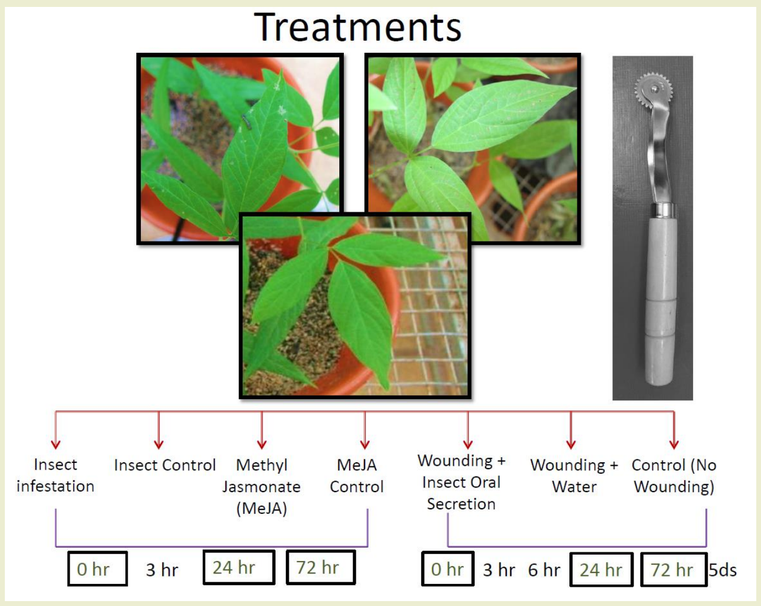


Figure 1: Treatments used to induce stress and controls. Time points in which plant tissues were collected are indicated by a bold box.

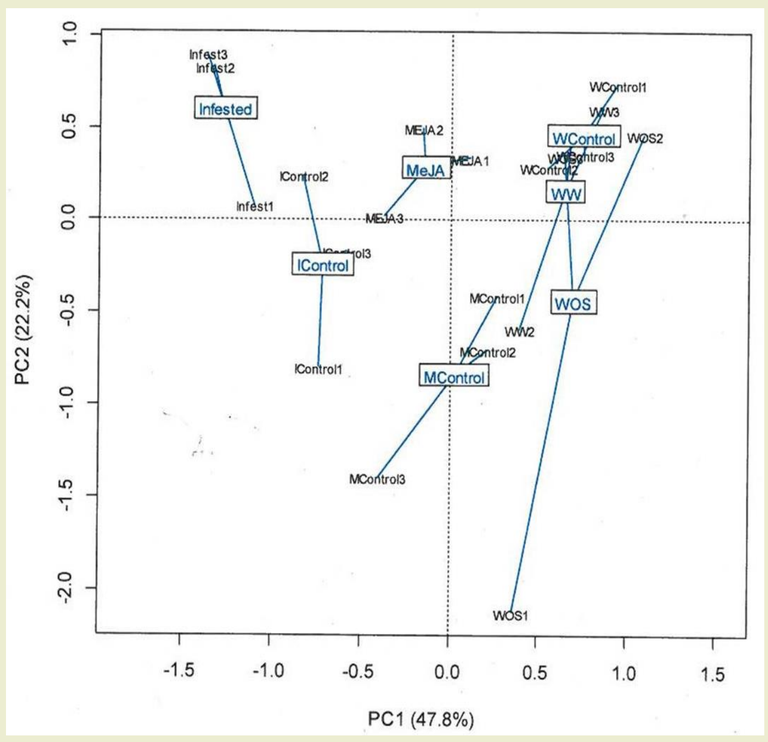
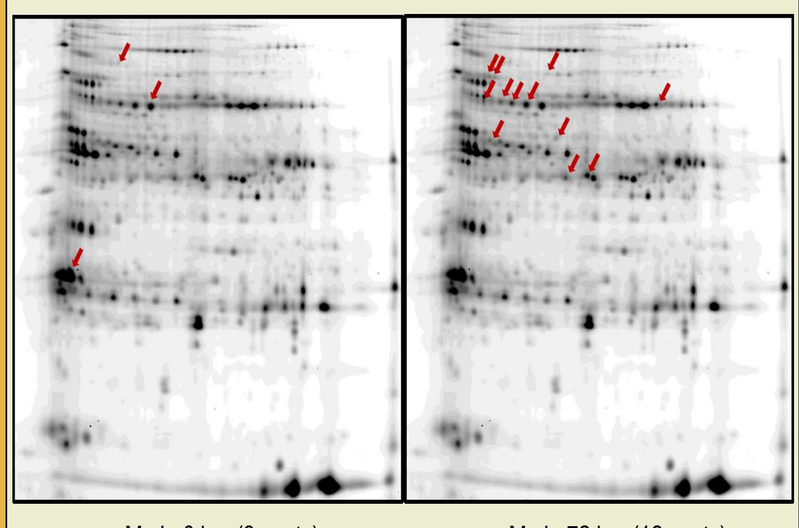
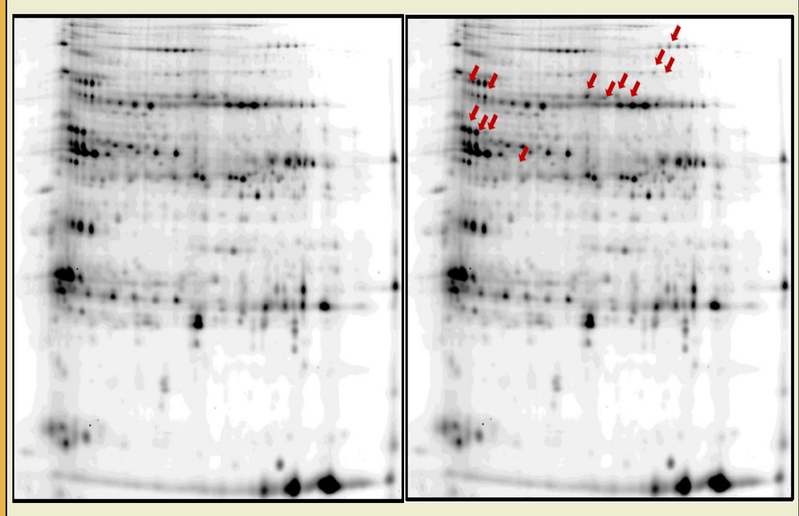


Figure 2: Principle component analysis (PCA) of each treatment at 0h (1),24h (2) and 72h (3). Infested (insect feeding), MeJa (methyl Jasmonate), Wos: (Wounding oral secretion), WW (wounding + water), Wcontrol (wounding control)



MeJa 0 hrs (3 spots) MeJa 72 hrs (12 spots)



Insect infested 0 hrs (0 spots) Insect infested 72hr (12 spots)

Figure 3: Two dimensional gel electrophoresis results for MeJa (methyl jasmonate) and insect infected treatment at 0 and 72 hours. Red arrows indicate the upregulated protein spots which were picked for furhter MALDI analysis.

Results

Following 2DE and statistical analysis it was revealed that after 72 hours of treatment there was large number of proteins being upregulated in insect infested and Methyl Jasmonate treatments but not with other treatments. The 2DE gel results are shown in figure 3, which highlights the upregulated proteins spots that were picked for further MALDI analysis. These protein spots were picked due to a statistically significant increase in expression following the treatment. Protein identification results from MALDI are shown in Table 1 below.

Protein ID	Function	Treatment
Beta Tubulin	For the formation of microtubules	Insect (72 hr)
UDP Glucose phosphorylase	formation of glucose-1-P and UDP from UDP-glucose and Pi	Insect (72hr)
S- adenosyl methionine synthase	ATP + L-methionine + H2O phosphate + diphosphate + S-adenosyl-L-methionine	MeJA (72 h)
RuBisCO Activase	Rubisco activase is one of a new type of chaperone, which in this case functions to promote and maintain the catalytic activity of Rubisco.	Insect (0hr/ 72hr)
ATPase subunit A	Membrane-bound complex that combine ATP synthesis and/or hydrolysis with the transport of protons across a membrane.	MeJA (0hr)
NADP dependent Malic enzyme	NADP-dependent enzyme that generates NADPH for fatty acid biosynthesis.	Insect (0hr)
RuBisCO	Catalyzes the carboxylation of ribulose-1,5-bisphosphate (also known as RuBP).	Insect (0hr/72hr) MeJa (72 hr)
ATP synthase	Synthesis of ATP	Insect (0 hr/72 hr)
Ferritin-1	The iron-storage protein, which is localized in the plastids in plants, plays roles during development and under stress conditions	Insect (0hr/72hr)
Chlorophyll A-b binding protein	Use in Light Harvesting Complex	MeJA (0hr)

Table 1: The results obtained from MALDI analysis of upregulated proteins. The protein classification, function of the protein and the type of treatment in which the protein spot was obtained from is detailed within the table. Proteins that are involved in the stress response are highlighted in light red

Conclusions

- After 72 hours of treatment there was large number of proteins being upregulated in insect infested and Methyl Jasmonate treatment but not with other treatments.
- The differential expression of defence related genes that play a vital role against chewing insects are identified in Table 1.
- It was evident that many photosynthetic proteins had been up-regulated upon insect infestation (Table 1) which clearly demonstrates that plant perceives the danger and starts making more food for its own survival though photosynthesis.
- Proteins involved in the stress response were significantly upregulated (Table 1), indicating that the plant has activated the expression of genes involved in herbivore deterrence, wound healing, and other defence-related processes.

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