SCIENTIFIC NOTE

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Herbivory induces differential gene expression in *Solanum lycopersicum* cultivars

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ABSTRACT

Solanum lycopersicum cultivars display differential response to biotic stress. Three agronomically important tomato cultivars viz. All Rounder, Lakshmi and Shaktiman were subjected to equal amount of *Spodoptera litura* Fab. damage at anthesis stage. Expression levels of four genes CORONATINE INSENSITIVE-1 (COI-1), Leucylaminopeptidases (LAP), Peptidyl propyl isomerase (PPI) and DNA binding protein (DBP) involved in Jasmonate signaling pathway, oxidative stress response and defense responses were determined by Semi-Quantitative RT-PCR. A significantly enhanced expression of COI-1 transcript (p=0.05) was observed in induced samples of all three cultivars as compared to their uninduced samples. In Cultivar Lakshmi, the expression of LAP transcript increased 2 fold in response to herbivory. PPI & DBP transcript levels were compromised and varied widely across cultivars. Within species variation was evident in case of COI-1 & LAP transcripts expression. Elucidating the differential expression levels of key targets provides us clues for developing insect tolerant cultivars.

Keywords: Semi-quantitative RT-PCR, *Spodoptera litura* Fab, Herbivory, CORONATINE INSENSITIVE-1, Leucylaminopeptidases, Peptidyl propyl isomerase.

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INTRODUCTION

Insect herbivory in crop plants is a serious problem accounting for approximately 25-40 % loss, world over annually (Maerere et al. 2010, Mark et al. 2012). Tomato, the second largest vegetable crop produced in world, undergoes a major loss annually by pest damage. Intense uses of chemical & biological pesticides on wide range of pest insects have not been able to control losses incurred in present agricultural scenario (Fenton et al. 2010).

Introduction of tolerant and resistant cultivars in agricultural practices is the best alternative to achieve pest control. Studying the molecular events elicited upon insect herbivory can help in understanding the mechanism of insect pest tolerance in tomato plants. The scientific literature has reports illustrating genome wide expression analysis upon herbivory in model plants like Arabidopsis and Nicotiana. Miersch et al. (2008) reported significant variation in response to pest attack within species, wherein 12-Hydroxy-jasmonyl accumulation is seen post wounding in two cultivars of tomato. The transcript analysis in 2 different species of Solanaceous taxa upon similar herbivore induction shows a species specific signaling cascade, downstream genes mediating herbivore resistance and a distinct transcriptional pattern (Schmidt et al. 2005). There is however very limited information on within species variation in responses to herbivory. In this report we present, a within species expression profiling of four key transcripts from the well described JA, SA and ethylene pathways that are modulated

differentially in few tomato cultivars. Four genes chosen for study are involved in oxidative stress, protein processing, gene expression modulation and Jasmonate signaling pathways upon herbivory in plants. CORONATINE INSENSITIVE-1 (COI-1), an F-box protein, involved in regulating Jasmonate mediated signaling pathways during herbivore damage (Howe et al. 2004) and promotion of glandular trichome-based defenses (Lei et al. 2004). DNA binding protein (DBP) is a transcription factor that binds to AT-hook motif is being evaluated for modulation in the expression levels (Aravind and David 1998). Leucyl aminopeptidase (LAP), an exopeptidase, catalyzes the release of N-terminal residues from proteins and peptide is chosen for this study. LAP and LAP-like proteins in tomato are induced locally and systemically on herbivory (Mahagamasekera and Team 2001, Yong-Qiang et al. 1999, VanDoorn et al. 2011). A Peptidyl propyl isomerase (PPI), involved in oxidative stress and protein processing during proteolytic events of a cell, they are considered as protein folding catalyst (Björn et al. 2009) is also used for evaluation of gene expression levels.

The expression levels of four transcripts were evaluated using semi-quantitative PCR which is a reliable tool being used in molecular studies recently (Jerry et al. 2006). The transcript levels of COI-1, LAP, PPI and DBP were analyzed in intact and herbivore, *S. litura* damaged leaves of *S. lycopersicum* cuv. All Rounder, Lakshmi and Shaktiman.

MATERIAL AND METHODS

Plant material and growing conditions. Twenty five day old plantlets of cultivar All Rounder, Lakshmi and Shaktiman germinated on coco peat based substrate were transferred to earthen pots (size: 26cm height, 26 cm diameter). Pot mix consist of red soil, sand and organic compost in 1:1:2 ratio, plants were grown in the green house [average temperature 28°C/20°C (day/night), RH (60-65%)] till anthesis stage. Third instar larvae of *S. litura* were starved for 12 hours before herbivore induction in experimental plants. Two larvae per plant were allowed on third leaf from top, to account a damage area of 5mm^2/per larva and were removed thereafter. Top three leaf samples were harvested post 4hrs of herbivory, frozen in liquid nitrogen and stored at -80°C until use.

RNA extraction and PCR conditions. Total RNA was isolated from 1g of leaf samples of all three tomato cultivars (uninduced and herbivory induced), using standard phenol extraction method (Cheng and Team 1998). RNA was quantified using Cary 50 UV-Vis spectrophotometer, (Varian Australia Pty Ltd, Mulgrave, Vic, Australia) at A260nm and qualitatively by A260/A280nm ratio (>1.8). The integrity of RNA was confirmed by electrophoresis on 1% agarose gel. cDNA was synthesized at 37°C for 30min, using 5 µg RNA in the presence of 4 U of M-MLV reverse transcriptase (USB corporation, Cleveland, Ohio, USA) after digesting with 2U DNase 1 (Invitrogen, Mexico, USA) following manufacturer's instructions. Gene specific primers (Table 1) designed for four target genes COI-1, DBP, LAP and PPI were used for PCR post cDNA synthesis. The cDNA was synthesized by priming with oligo dT primers. Plant Actin was optimized as reference transcript for cDNA synthesis and PCR amplification. RT-PCR was performed using 8 µl of cDNA template, 0.2 mM each of forward and reverse gene specific primer, 0.2 mM dNTPs, 1 U of FideliTaq DNA polymerase(USB corporation, Cleveland, Ohio, USA) and 1 x PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.6) in a final volume of 25 µl. PCR was carried out on a DNA Engine, Peltier Thermal Cycler (BIO-RAD, Rock way, NJ, US) using the following cycling conditions: initial denaturation at 94 °C for 3 min, followed by amplification cycles at 94 °C for 45 s, 50 °C for 45 s, and 72 °C for 1 min and then a final extension at 72 °C for 10 min. The PCR was done at two different cycle lengths for quantification of the transcripts. No enzyme (Reverse transcriptase) controls for each RNA sample were performed parallel to know genomic DNA contamination. PCR product were resolved using electrophoresis in 1.2% agarose gel stained with ethidium bromide (EtBr), documented under UV light on UVIpro platinum gel documentation system. All amplicons were sequenced to confirm the target gene specificity of designed primers (data not shown). Densitometric analysis of EtBr stained gel bands was performed on UVIBandMap image analysis software (Eurodyne Ltd, *Lindale,* Cumbria, UK).

Statistical analysis. Data of target genes band intensity value were normalized to reference (Actin) gene band intensity of similar cDNA samples. Mean and standard error of all samples were calculated after normalization to reference gene. A pairwise comparative Tukey's (HSD) test was performed using XLSTAT software (Addinsoft, Damrémont, Paris, France).

RESULTS AND DISCUSSION

Gene expression levels in uninduced state of plants shows variance across three cultivars. Performing pairwise comparative Tukey's (HSD) test on mean of uninduced expression shows that difference across was not significant. COI-1 and LAP expression levels are high in comparison with the other two transcripts in tomato cultivar.

Table 1. Primers used in RT and Semi-Quantitative PCR analysis

Gene group	Gene Code	Gene Bank Accession No	RT-PCR product size(bp)	Primer Sequence (FP = forward primer, RP = reverse primer)
Proteolytic enzymes	COI-1	NM_001247535.1	400	FP: 5′-ATGGAGGAACGGAACT-3′ RP: 5′-GCAGGAACGAGAAATAG-3′
Amino peptidases	LAP	U50152.1	342	FP: 5′-GCAATGGCAAGGAGAC-3′ RP: 5′-CATAAGTGGACGCAATC-3′
Important isomerase	PPI	AK320378.1	500	FP: 5′-TCAAGAAGGGGAGTGTG-3′ RP: 5′-TGGGATCTTTGGTGGAG-3′
DNA binding proteins	DBP	NM_001247725.1	400	FP: 5′-AACAAGCAAAGGCACGA-3′ RP: 5′-GACATTCTCTCACATAAAC -3′
Cytoskeleton proteins	ACT	JQ435884.1	500	FP: 5′GTATTGTGTTGGACTCTGGTGATGGTGT-3′ RP: 5′-GTATTGTGTTGGACTCTGGTGATGGTGT-3′

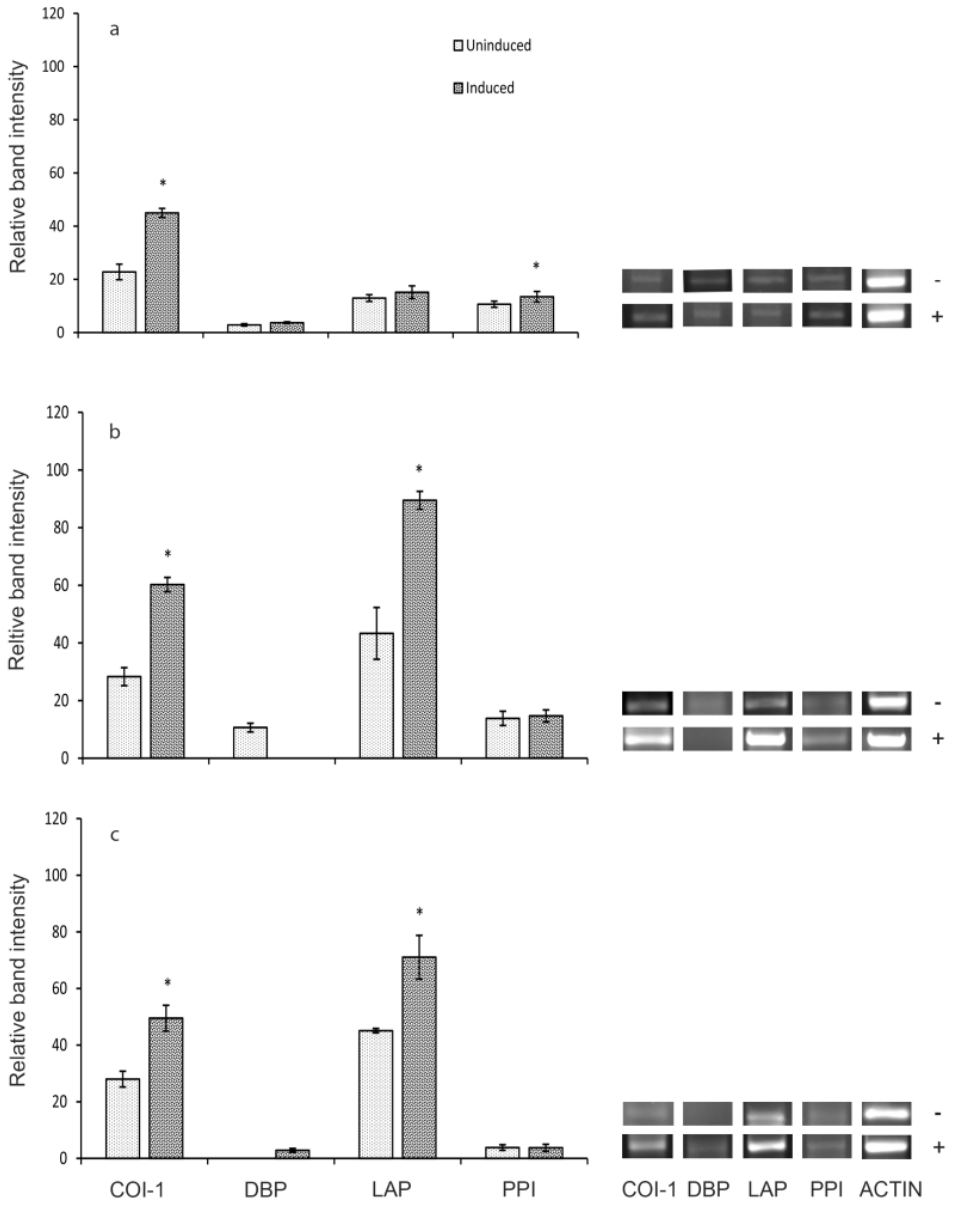

Figure 1. Expression levels of 4 transcripts COI-1, LAP, PPI & DBP in herbivore induced leaf sample (**+**) and uninduced leaf sample (-) across 3 tomato cultivars All Rounder (a), Lakshmi (b), Shaktiman(c). Each value is the mean of 4 independent replicates ± SE,* indicates significance at $p< 0.05$ based on Tukey's test. Band intensity captured at 29th cycles of PCR.

COI-1 expression levels were significant (p 0.006, 0.004, 0.004) across all the three tomato cultivars with herbivore damage (Fig1). Though the levels of expression in uninduced sample across 3 cultivars were similar, their induced expression levels vary distinctly. COI-1 expression was high in cultivar Lakshmi as compared to other two cultivars. A significant (p 0.001, p 0.002) LAP expression was observed in cultivar Lakshmi and Shaktiman, of which cultivar Lakshmi shows predominant 2 fold increased expression (Fig1b). Though slight increase in expression levels of LAP was observed in herbivore damaged All- Rounder cultivar it was statistically not significant (p 0.130). PPI transcripts were detected in all three tomato cultivars without a significant value and low expression in uninduced and induced samples (Fig1). DBP shows an inconsistent expression in all three tomato cultivars and is not detected in control samples of varieties Lakshmi and Shaktiman. We observe a clear varietal difference in the levels of expression of these select genes (Fig-1). It is inconclusive with respect to DBP as these are master controllers and possess a range of functions.

A semi quantitative estimation of transcript levels in the infested leaves reveals conserved downstream events as observed in Solanaceous plants. An up-regulation in COI-1 transcripts points to an induced Jasmonate defense signaling on herbivory, across cultivars. Differential expression levels of COI-1 observed across cultivars is probably due to varied Jasmonate signaling pathway in plant, making it highly complex to understand defense mechanism based on single plant model. DBP that are known for transient up regulation on touch related stimuli (Yasuaki et al. 2009) could be substantiated by this study, as we observe an up regulation 4 hours post herbivore attack. The DBP expression levels in uninduced Lakshmi cultivar as compared to induced sample are inconclusive. Further replications and downstream studies will be required to understand this trend. Increase in LAP transcript, indicates an elimination of mis-folded proteins which usually accumulate in a plant under stress. A LAP expression needs > 90min to mount a systemic defense in *Solanum nigrum* plants (VanDoorn et al. 2011) and we observe a similar phenomenon in *S. lycopersicum*. Among the cultivars, Lakshmi and Shaktiman were prominent in expression of LAP transcripts which group them separate from other cultivars of same species. COI-1 and LAP expression could be used as marker for identification of tomato plants under herbivore attack. PPI are involved in flower transition of plants (Yu et al. 2009), their low expression across cultivars is because sampling for analysis was done at the flowering stage. In our previous study (Raghava et al. 2010) we reported the variance of volatile organic compounds across tomato cultivars in the event of insect attack. Current study reveals that variance in the volatile organic compounds profile is a function of characteristic genetic response as seen with these transcript analyses.

CONCLUSION

In nutshell, our results reveal a highly varietal specific response of key genetic targets in the event of herbivory. These targets carry the blueprint of information that distinguishes between insect tolerant and susceptible cultivars. Further studies involving more varieties, induction time points and targets can help us in identifying key hot spots for developing insect tolerant tomato plants.

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