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Research article

# Stress inducible proteomic changes in *Capsicum annuum* leaves

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ABSTRACT

Herbivore attack induces defense responses in plants, activating several signaling cascades. As a result, molecules deterrent to the herbivores are produced and accumulated in plants. Expression of defense mechanism/traits requires reorganization of the plant metabolism, redirecting the resources otherwise meant for growth. In the present work, protein profile of *Capsicum annuum* leaves was examined after herbivore attack/induction. Majority of proteins identified as differentially accumulated, were having roles in redox metabolism and photosynthesis. For example, superoxide dismutase and NADP oxidoreductase were upregulated by 10- and 6-fold while carbonic anhydrase and fructose-1,6-bisphosphatase were downregulated by 9- and 4-fold, respectively. Also, superoxide dismutase, NADPH quinone oxidoreductase and NADP dependent isocitrate dehydrogenase transcripts showed a higher accumulation in induced leaf tissues at early time points. In general, proteins having role in defense and damage repair were upregulated while those involved in photosynthesis appeared downregulated. Thus metabolic reconfiguration to balance defense and tolerance was evident in the stress-induced leaves.

## 1. Introduction

To sustain and survive in nature, plants adopt strategies to combat varied biotic and abiotic stresses. Pathogenic microorganisms and herbivorous insects are primarily the main types of biotic stress encountered by plants. Various microbial/pathogen associated molecular patterns (MAMPs or PAMPs) or herbivore attack associated molecular patterns (HAMPs) enable the perception of the challenge posed by these attacks. HAMPs such as glucose oxidase, β-glucosidase, inceptin, fatty acid-amino acid conjugates (FACs), caeliferins and oviposition fluid are known to elicit plant defenses (Reymond and Farmer, 1998). Plant hormones namely, salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play significant role in regulating the signaling cascades during stress. SA is involved in plant defense against pathogens and induction of pathogenesis related (PR) genes. JA and ET pathways are fundamental to wounding and anti-herbivore defense in the plants. Also, it has been observed that a cross talk between these pathways exists, which actually governs the nature of plant response (Walling, 2000).

Plants have evolved a plethora of chemical strategies to deal with the phytopathogens and herbivores. Investigation of anti-herbivore defenses in plants has highlighted occurrence of several such molecules and signaling cascades, which ultimately restrict herbivore attack (Walling, 2000; Felton, 2005). Also systemic induction of defense traits indicates the existence of "complex regulatory networks". Mobile signaling molecules travel to the undamaged regions from the damaged parts of the plant to initiate defense reactions and herbivore attack alert signals in systemic tissues (Schilmiller and Howe, 2005). Plants sense the presence of herbivores and the changes in several signaling pathways are initiated, by means of $Ca^{2+}$ flux, membrane depolarization, kinase activation, etc. These pathways form sophisticated intertwined regulatory networks that orchestrate specific defense responses according to the attacking species of the herbivore. Redox associated or reactive oxygen species (ROS) mediated signaling has been found to play a major role in amplification of defense responses in plants (Mittler et al., 2004). Wounding/injury alone can activate multiple signal transduction pathways to initiate damage repair and prevent further damage. Wounding essentially accompanies herbivore attack, therefore, events like oxidative burst and release of oligosaccharides from damaged cell walls, take place in either of the conditions. However, plants perceive wounding and herbivore attack differently due to the presence of elicitors in insect oral secretion. Hence, despite similarities in plant responses to wounding and herbivore attack, they differ qualitatively as well as in magnitude (Walling, 2000). Treatments like wounding and

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*Abbreviations:* ET, Ethylene; JA, Jasmonic acid; OS, Oral secretion; 2-DE, Two-dimensional gel electrophoresis; ROS, Reactive oxygen species; Rubisco, Ribulose-1,5-bisphosphate carboxylase/oxygenase; SA, Salicylic acid.

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wounding followed by application of *Helicoverpa armigera* oral secretion (OS), led to the systemic induction of significant amounts of diverse protease inhibitors (PI) in *Capsicum annuum*. These PIs also show differential accumulation in response to the respective treatments, indicating differential perception of elicitors in the OS (Mishra et al., 2012).

The objective of the present study was to identify/characterize the proteomic changes in the induced *C. annuum* plants. *C. annuum* plants were subjected to two types of induction treatments namely, wounding followed by application of sterile water or *H. armigera* OS (to mimic insect infestation). Uninduced/control leaves were collected from unwounded healthy plants. The proteomic profiles of systemic leaves from the treated and untreated *C. annuum* plants, were analyzed by classical two-dimensional gel electrophoresis and mass spectrometric (nano-LC-$MS^E$) identification of proteins.We identified various proteins showing differential abundance, which were linked to different metabolic pathways/processes, in the induced leaf tissues. Selected proteins were further evaluated for their transcript accumulation by semi-quantitative PCR at successive time points after wounding to support the mass spectrometric identification results. Overall, proteins having role in defense and damage repair were upregulated, while those involved in photosynthesis were downregulated. Reconfiguration of metabolic processes, in the stress induced leaves, in order to balance defense and tolerance was apparent in this study.

## 2. Results and discussion

Biotic stress entails restrain on normal plant growth and development. Plants try to evade the damage by activating defenses, which in turn requires metabolic reorganization (Fig. 1A) (Walling, 2000). Induction treatments to *C. annuum* leaves, showed differential accumulation of proteins with respect to uninduced leaves. These proteins were involved in varied functions including cellular processes, metabolism and stress response (Fig. 1B and Table 1). The detailed information of the identified proteins and the fold differences in abundance of proteins under different induction treatments is listed in Table 1. Twenty two protein spots showing differential protein accumulation were identified. The proteins were identified with sequence coverage ranging from 12.3% to 65.5% and the number of matching peptides ranged from 9 to 101. In the present study, both the induction treatments showed similar trends of protein accumulation. The upregulated proteins were NADPH quinone oxidoreductase (spot 1, 2, 10, 22); glycolate oxidase (spot 12); pectinesterase (spot 13); chloroplast ferredoxin NADP oxidoreductase (spot 14); superoxide dismutase Cu/Zn (spot 15); 23 kDa polypeptide of photosystem II (spot no. 16), while chloroplast sedoheptulose-1,7-bisphosphatase (spot 4, 5, 6, 11), fructose-1,6-bisphosphatase (spot 3) and carbonic anhydrase (spot 7, 19, 20) showed lower fold accumulation as compared to uninduced (Fig. 2) leaves. Superoxide dismutase showed the highest upregulation (10-fold) in either of the induced tissues while, carbonic anhydrase was downregulated by 9-fold in wounded leaves treated with *H. armigera* OS. Spots showing marked visual difference in protein accumulation are represented as magnified images in Supplementary Fig. 1. To validate the accumulation patterns of proteins, the transcript accumulation was examined for few candidate genes, at 3, 6, 9 and 12 h after induction. Since the proteomic responses in both types of inductions were similar, only wound induced tissue was used for gene expression analysis. Except for carbonic anhydrase, the transcript accumulation of selected genes was in accordance with the proteomic data.

Glycolate oxidase (GOX) converts glycolate into glyoxylate, during photorespiration, producing $H_2O_2$ as a by-product. Upregulation of GOX and consequent generation of $H_2O_2$ is also associated with the hypersensitive response in plants. $H_2O_2$ was also found to induce SA signaling cascades and regulate JA and ET pathways as well (Rojas et al., 2012). Redox signaling associated with photorespiration aids in maintaining cellular homeostasis (Foyer et al., 2009). $H_2O_2$, which is a small diffusible molecule, has been widely accepted as a signaling molecule when present in low concentrations. At high concentrations it induces programmed cell death leading to hypersensitive response. It also plays a role in regulating the cascades involving physiological processes like non-host resistance, phytoalexin production, strengthening of cell wall, senescence, photosynthesis, and the cell cycle. Plant growth and defense responses are also regulated by redox signaling cascades. Transcription factors like CDPKs, WRKY, DREB, bZIP, HSF and MYB have been found to be associated with $H_2O_2$ signaling/burst during biotic or abiotic stress (Petrov and Breusegem, 2012). Apart from signaling, the accumulated $H_2O_2$ also plays a direct defensive role against herbivores and pathogens, which in turn might subject the plant to oxidative stress (Ruuhola and Yang, 2006).

NADPH quinone oxidoreductase (NQOR) was upregulated by 2.3 fold (spot no. 9) in case of leaves treated with wounding followed by OS than that in leaves treated with wounding. NQOR plays a role in detoxification of plant quinones (Sparla et al., 1996). Lipid peroxides generated in plants during biotic stress can be degraded to form toxic aldehydes like α,β-unsaturated aldehydes. P1-ζ-crystallin, an NADPH quinone oxidoreductase in *Arabidopsis thaliana* was found to be induced under oxidative stress to detoxify the

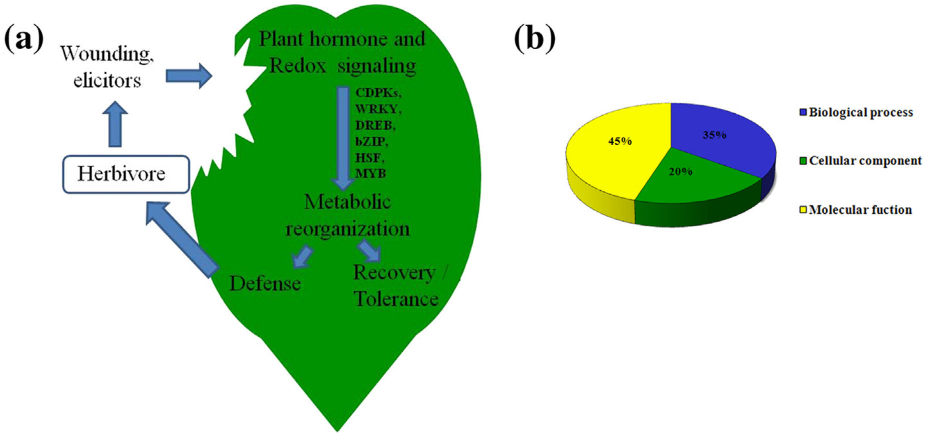

**Fig. 1.** Illustration of (A) Schematic mechanisms of plant and pest interaction. Damage by herbivore elicits a defense response in the damaged tissue, which requires re-allocation of resources and metabolic reorganization. (B) Classification of proteins identified in the present study on the basis of gene ontology.