

# Induction of Specific Biochemical Pathways in Plants for Pest Management

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## ABSTRACT

The biochemical pathways encompassing induced resistance involve oxidizing enzymes such as PPO (Polyphenol Oxidase), PO (Peroxidase) and LOX (Lipoxygenase). Increased activities of these enzymes in plants serve as markers for visualizing induced resistance. Induced resistance, being an active defense mechanism, results from the expression of PR-proteins, low molecular weight compounds like phytoalexins and proteinase inhibitors. This paper highlights on the spatial induction, variability and persistence of induced defenses responses in *Lycopersicon esculentum* Mill. using lepidopterans, aphids and mechanical wounding as elicitors. The early signally events upon wounding by an insect resulting in the release of elicitors JA, Ethylene, Glycans and Absisic acid and the transmission of signals through unwounded sites is described. The practical utility of induced resistance is discussed based on the results of the systematic small scale laboratory essays.

## INTRODUCTION

Plants have developed and optimized a considerable diversity of defense mechanisms against adverse environmental conditions caused by either biotic or abiotic factors. These defenses are commonly divided into constitutive or passive and induced or active defenses. An understanding of induced resistance, a non-heritable qualitative and quantitative enhancement of a plant's defensive mechanisms against pest in response to external physical or chemical stimuli (Heinrichs, 1998, Dilawaria and Dhaliwal, 1993, Panda and Kush, 1995) is a key element in insect pest management programs. There is great scope for using chemical elicitors of plant resistance to protect plants against insect and pathogen attack. Work in the area of plant resistance to pathogens has advanced more rapidly than studies of plant resistance to herbivores and has resulted in the production and marketing of elicitors of salicylate-dependent plant resistance to pathogens (Lyon and Newton 1999, Tally *et al.*, 1999). However, the role of insects in induced resistance build up in plants is still not fully understood. Insect feeding has been reported to elicit local, as well as systemic responses in more than 100 plant species (Karban and Baldwin 1997). These responses might function either as direct resistance (physical or chemical traits that act directly against further attack or reduce herbivore performance) or as indirect resistance. The latter is based on the attraction of 'enemies of the plant's enemies' (Price *et al.*, 1980).

Induction of defense enzymes might be either a local or a systemic event (Edwards and Wratten, 1983). Occurrence of biochemical and physical changes confined at the site of injury represent localized defense response (Mauch-Mani *et al.*, 1998; Stout and Bostock, 1999; Norman *et al.*,

1999). Systemic induction is represented as molecular, chemical or morphological changes that occur in distant undamaged leaves (Zhang and Baldwin, 1997; Stratmann, 2003; Orians, 2005). Systemic induction can be uniform throughout the plant or vary between different plant parts (Jones *et al.*, 1993; Stout *et al.*, 1996b; Sanjayan, 2005). Induced responses can selectively affect the performance of herbivores and the behavior of natural enemies (Thaler, 1999a, 1999b; 2002; Agrawal, 2000; De Moraes *et al.*, 2001; van Poecke *et al.*, 2001).

Induced plant responses, especially different responses in different stages of development, will produce variability within individual plants (Baldwin and Karb, 1995, Zangerl and Rutledge 1996). Also, the responses of the plant may vary in relation to the species of insect attacking it. Against this background, the present study attempts to examine the response of the tomato plant, *Lycopersicon esculentum*, to different induction treatments *viz.*, 1) feeding by *Spodoptera litura*, *Aphis gossypii*, as well as chemical and mechanical injury, 2) the spatial mapping of chemical induction and 3) the degree and persistence of polyphenol oxidase enzyme activity against the exposure of tomato plants to *S. litura* larval feeding for 24 hours.

## MATERIALS AND METHODS

Tomato plants were grown in pots in a green house for about 30 days. They were subjected to damage on the third leaf from the cotyledon. Plants of similar age and size were left undamaged as a control. Four types of damage induction was tested namely 1) feeding by *S. litura* representing biting and chewing damage, 2) feeding by *A. gossypii* representing sucking type, 3) Mechanical damage and 4) Insecticidal soap treatment.

One second instar larva of *S. litura* was confined to the third leaf (position LI) using the clip cage and allowed to feed for up to 24 h. Plants with a clip cage without larva served as the control. After 24 h, cages and insects were removed and the plants left for an additional 24 h. Similarly 45-50 *A. gossypii* (nymphs and adults) were confined to the same position and the same procedure was followed. For mechanical damage, two wounds were made perpendicular to the mid-vein of the terminal leaflet of the third leaf and for chemical damage the entire third leaf was immersed for 5-10 seconds in a 5% (v: v) soap solution. After two days, plants were transported to the laboratory and the leaflets from several positions on control and damaged plants were then excised at the petiole with a razor blade and assayed for Polyphenol oxidase (PPO), Lipoxygenase (LOX) and Peroxidase (POD). The activities of these proteins in leaflets from damaged plants were compared to activities in corresponding leaflets from control plants (Stout *et al.*, 1996a). In order to quantify the persistence of resistance induction, induced leaves were sampled at 1, 3, 5 and 7 days after feeding and PPO and POD analysis carried out. Leaflets from the four positions were sampled: damaged leaflets (terminal leaflet of the third leaf, designated position LI), undamaged leaflets from the damaged leaf, adjacent to the damaged leaflets (position A), leaflets from the leaf immediately below the damaged leaf (position L) and leaflets from the leaf above the damaged leaf (position U). This method is to assess induction at three spatial levels – leaflet-local induction (induction confined to the damaged leaflet), leaf-systemic induction and plant-systemic induction.

## RESULTS AND DISCUSSION

Plants have a generalized defensive response to wounding that can be divided into two phases- activation and induction. Activation represents the immediate response to cellular damage wherein cell integrity is lost and a variety of hydrolytic and oxidative enzymes are released from compartmentalization. This release results in the generation of chemical signals that trigger the systemic and/or local induction of defenses, and in the generation of chemically reactive products that lead to cell death through destruction of membranes and polymerization of cellular components. This polymerization is mediated by PPO, POD and LOX.

PPO and POD oxidizes phenolics to quinones which alkylates nucleophilic functional groups of proteins, peptides and amino acids (-SH, -NH<sub>2</sub>, -HN and OH). The amino acid becomes nutritionally inert and reduces the digestibility of the protein by tryptic and chymotryptic enzymes. Further nutritive value of the protein can be

lost via polymerization and subsequent denaturation and precipitation. POD is also capable of decarboxylating and deaminating free and bound amino acids to aldehydes. The aldehyde facilitates polymerization by forming Schiff's bases with -NH<sub>2</sub> of the protein molecules. POD can also initiate free radical formation on -SH and tyrosinyl functions of proteins which leads to polymerization of proteins. LOX converts polyunsaturated fatty acids (Linolenic and linoleic acid) into lipid hydroperoxides. The lipid hydroperoxides then form hydroperoxide radicals, epoxides and are degraded to form malondialdehyde. These products are strongly electrophilic and can 1) destroy amino acids by decarboxylative deamination 2) cause free radical mediated cross linking of proteins and 3) cause Schiff's base formation.

Table 1 indicate that the spatial pattern of protein induction varied with the damage type. Comparison of the grand means of the damage treatments through two factor ANOVA, indicate that an increased induction of PPO activity in *S. litura* (biting and chewing type), which was significantly different from the *A. gossypii* (sucking type), as well as the mechanical and chemical damages. At the spatial level, a higher induction in position D and A was found to be a general tendency for all treatments followed by positions U and L, the levels being primarily different significantly. Therefore PPO induction could be regarded as a plant systemic phenomenon. The induction of POD was maximum in position D for both *S. litura* and *A. gossypii* damage treatments. Maximum induction was in *S. litura* damaged plants followed by *A. gossypii* and mechanically damaged plants. Spatial difference in activity for POD as a whole for all the treatments was more or less the same with no significant difference for positions A, U and L indicating a localized induction.

Feeding by *S. litura* resulted in the enhancement of induction of both POD and PPO. While the sucking pest, *A. gossypii* showed less induction when compared to *S. litura* damage but significant increase in induction was observed over the control plants. There are several mechanisms for this increase. Aphids may come into contact with inducible compounds via several routes, for example, mechanical damage induces PPO in tomato trichomes and phloem (Thipyapang *et al.*, 1997). Aphids walking on the surface break trichomes and may be entrapped by phenolics polymerized by PPO and POD (Duffey, 1986). The difference in induction for different pests *viz.*, *S. litura* and *A. gossypii* may be due to their feeding mechanisms and elicitor variability. Stout *et al.*, (1996a) worked out the spatial mapping for the induction of four foliar proteins against *Heliothis zea* and russet mite *Aeulops lycopersici* Masee and indicated that for *H. zea*, PI, PPO and POD were induced leaf systemically and

Table 1. Induction of PPO and POD activity in tomato against different damage treatments and leaf positions (LSD)\*

Damage/Location	PPO activity (OD increase/min/g sample)				Grand Mean
	A	U	D	L	
<i>S. litura</i>	0.6820 <sup>a</sup>	0.5720 <sup>b</sup>	0.6560 <sup>a</sup>	0.5780 <sup>b</sup>	0.6220 <sup>A</sup>
<i>A. gossypii</i>	0.5400 <sup>c</sup>	0.4860 <sup>c</sup>	0.5840 <sup>b</sup>	0.4760 <sup>c</sup>	0.5215 <sup>B</sup>
Soap solution	0.3400 <sup>de</sup>	0.3120 <sup>ef</sup>	0.4460 <sup>c</sup>	0.2920 <sup>f</sup>	0.3475 <sup>D</sup>
Mechanical damage	0.3740 <sup>d</sup>	0.3520 <sup>de</sup>	0.4800 <sup>c</sup>	0.3580 <sup>d</sup>	0.3910 <sup>C</sup>
Grand Mean	0.4840 <sup>B</sup>	0.4305 <sup>C</sup>	0.5415 <sup>A</sup>	0.4260 <sup>C</sup>	
Damage/Location	POD activity (OD increase/min/g sample)				Grand Mean
	A	U	D	L	
<i>S. litura</i>	0.7800 <sup>b</sup>	0.716 <sup>bcd</sup>	0.9760 <sup>a</sup>	0.7580 <sup>b</sup>	0.8075 <sup>A</sup>
<i>A. gossypii</i>	0.706 <sup>bcd</sup>	0.654 <sup>cd</sup>	0.7720 <sup>b</sup>	0.636 <sup>efg</sup>	0.6920 <sup>B</sup>
Soap solution	0.5200 <sup>i</sup>	0.5500 <sup>hi</sup>	0.5700 <sup>ghi</sup>	0.5140 <sup>i</sup>	0.5385 <sup>C</sup>
Mechanical damage	0.626 <sup>fgh</sup>	0.640 <sup>defg</sup>	0.7300 <sup>bc</sup>	0.632 <sup>efg</sup>	0.6570 <sup>B</sup>
Grand Mean	0.6580 <sup>B</sup>	0.6400 <sup>B</sup>	0.7620 <sup>A</sup>	0.6350 <sup>B</sup>	

Rows and columns followed by the same letter(s) are not significantly different at P<0.05. \* Values represent difference in OD (activity) between the experiment and control leaves

induction of PPO and PI was also plant systemic. Results for *S. litura* feeding damage in the present study corroborate with their observation. However the results for the insects with sucking type of mouthparts varied. Stout *et al* (1996b) showed that mite feeding brought about a plant systemic induction of PPO and POD in contrary to our results with *A. gossypii* damage (a sucking pest) which showed POD induction only up to leaf systemic level. Different pest damages in different stages of development will produce variability within individual plants (Baldwin and Karb, 1995, Zangerl and Rutledge, 1996). Sucking insects in particular cause only very local damage and seem to be recognized by the plants as 'pathogens' rather than as 'classical herbivores', thus eliciting ISR (Walling, 2000). Bostok *et al.*, (2001) reported strong induction of proteinase inhibitor and polyphenol oxidase for *Spodoptera exigua* and *Heliothis zea* feeding, but in contrast, aphid feeding damage (*Macrosiphum persicae* and *M. euphorbia*) induced PR proteins but did not elicit proteinase inhibitors (Fidantsef *et al.*, 1999, Stout *et al.*, 1999).

There is a complex temporal and spatial array of signalling events in wounded plants. The earliest known events detected in wounded leaves include ion fluxes across the plasma membrane, changes in cytoplasmic calcium concentration, the generation of active oxygen species and changes in protein phosphorylation patterns. These early events occur in the first few minutes following damage, and are probably not directly responsible for inducing defense gene expression. Instead defense gene

expression is mediated primarily through the synthesis and action of Jasmonic acid. Other hormones with roles in regulating wound gene expression are ethylene and abscisic acid (ABA). The synthesis of JA and ethylene is well characterized, with many of the genes encoding their biosynthetic enzymes being up-regulated within 30-40 minutes of wounding, leading to peaks in hormones synthesis in wounded leaves at 1-2hours. Other elicitors of wound responses have also been identified, The most important include cell wall glycans, such as oligogalacturonides (OGAs) and systemin. These elicitors of wound response may either be primary signals released upon cellular damage, or may function to amplify the response in the wounded leaf. In addition, they may also perform a key role in systemic signaling. Proposed mechanisms for the transmission of signals to unwounded sites include electrical activity, the active transport of elicitors in the phloem and the passive transport of elicitors via hydraulic mass flow in the xylem. Wound inflicted by insect herbivory also results in signaling beyond the plant itself to mediate an indirect form of defense. Plants under attack from herbivores produce characteristic blends of volatiles that serve to attract predators and parasitoids of those herbivores.

An important criteria for practical usage of induced resistance techniques is to evaluate the persistence of induction in the plant. The PPO activity was persistent in tomato plants at levels significantly different from the controls even on the seventh day after a single feeding induction schedule. The degree of resistance was highest

with *S. litura* damage treatment. Very meager information is available on the persistence and magnitude of PPO induction in tomato due to herbivory. Many researchers reported that the external spray of JA mimics the herbivore damage in the induction of plant defense phenolics. Thaler *et al.*, (2001) reported increased activities of PI and PPO in JA (1.5mM=0.315mg of JA/plant) induced plants and was maximum at 13 days after spray. But for the herbivore damage (*S. litura* and *A. gossypii*) difference between control and induced plants, the PPO induction was maximum during 3<sup>rd</sup> day after damage treatment and then significant reduction in induction was observed, but still there was an increase in induction when compared to control. We may not yet fully understand the persistence of induced responses to herbivory. It is obviously important to determine the pattern of persistence for different types and combinations of herbivory.

A central signalling molecule in induced responses against herbivores is Jasmonic Acid (JA). In response to wounding or insect feeding, linolenic acid is released from membrane lipids and then converted enzymatically into JA. JA in turn causes the transcriptional activation of genes encoding proteinase inhibitors (PIs) and of enzymes involved in the production of volatile compounds or of secondary compounds such as nicotine, numerous phenolics and other defence related compounds.

Oligosaccharides and oligogalacturonides released from damaged cell walls bring about the general wound response and also some specific elicitors such as systemin. Systemin is an 18-amino acid polypeptide released upon wounding from a 200- amino acid precursor-pro-systemin, and leads to the release of linolenic acid. This activates the octadecanoid signalling cascade. Both JA and Systemin can be transported in the phloem and this may act as systemic signals. Besides systemin, cellulysin, a mixture of several cell wall-degrading enzymes can also induce JA responsive volatile.

It is therefore evident that the diversity of feeding types among insect pests plays a central role in the plant's response, which is frequently altered by insect-specific elicitors, giving plants to optimize their defenses. Both the plant variety and herbivore species affect the composition of induced volatiles, and it is becoming clear that both the predators and parasitoids are able to differentiate between various blends of herbivore induced volatiles to an amazing degree. It is to be noted that under the natural environmental conditions plants are exposed to a wide array of predators, either simultaneously or periodically. With each predator attack the plant shows characteristic biochemical induction. With numerous biochemical pathways underway, there is a lot of interactions between the molecules and their response to the predator.

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