The Jasmonate Signaling Pathway Induction in Plants: A Response to Herbivore Chewing Insects

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Abstract

Both biotic and abiotic factors in the environment affect plants in differential ways. The abiotic factors primarily result in wounding in plants, whereas, the herbivore wounding (biotic) results in a response which cannot be mimicked by mechanical wounding. The herbivore insects constitute different feeding guilds which identify the feeding behavior of an insect toward a plant. Chewing insects transmit oral secretion and cause plant wounding by defoliation of leaves resulting in elicitation of defense responses in the plants. The main events in plants following a bite include electrical signaling and calcium ion fluxes, phosphorylation cascades, and release of system in, resulting in induction of the Jasmonate signaling pathway. Jasmonate signaling includes a Hexadecanoid pathway and an Octadecanoid pathway. The derivatives of the Jasmonate pathway, induced by the attack of a chewing insect (herbivore) reduce the rate of photosynthesis which eventually results in stunted growth among plants. Herbivores actually shift the plant metabolism by inhibiting the growth and inducing a defense mechanism.

Keywords: Jasmonate Signaling Pathway; Herbivore; Chewing Insects; plant immunity

Introduction

The co-occurrence of plant and insect interactions has occurred for more than 400 years. The association between these organisms has many beneficial activities including pollination while herbivore insects on the other hand are extremely harmful to plants and may result in death of the plant. In this predator-host relationship almost every plant is eaten by at least one species of insect. Insect herbivores are categorized into three groups based on their diets. The insects are considered monophagous when they are restricted often to a single plant genus, oligophagous when they feed on many plant species of different genera and polyphagous when they feed on plant species of different families. These relationships have given rise to the co-evolutionary theory that suggests insect feeding on plants is a fundamental factor for the increasing variety of species in both plants and herbivores [1]. As a host plant is attacked by an insect, it develops various defense mechanisms which include the production of defensive proteins, volatiles, secondary metabolites and trichomes [2-5]. In response to these defense strategies insects develop defensive mechanisms which include processes for detoxification of toxic compounds, avoidance mechanisms, sequestration of poison and alteration in gene expression [6-9].

Plants are very diverse in their shapes and sizes and range from a few millimeters, as seen in microscopic duckweeds (Lemnaceae), to over 100 meters in Californian redwood trees (Sequoia sempervirens). Insect herbivores that feed on more than one host belonging to different families of plants are termed as generalists (polyphagous) whereas specialists (monophagous and oligophagous) are insects which feed on one or few species belonging to the same family. The generalist insect can cope with a wide variety of defenses, but they face difficulties when feeding on evolved plants with unique defense mechanisms. The defense produced by specialist insect-plant interaction acts as stimulant for feeding and provides ovipositioning sites [10]. However, this view of feeding has been challenged as it concentrates only on extremes and leaves the situations between these extremes which include insects feeding on variety of plants in a continuum process [11].

Confined and Systemic Responses

The attack of insects on plants results in either a successful attack termed as compatible response or a resistance posed by plants against attack, termed as an incompatible response. Incompatible responses include both local and systemic responses. The localized response includes oxidative burst [12]. Plants show direct and indirect responses. The direct response includes direct action of the plant against herbivore attack, whereas, indirect includes production of secondary metabolites for attracting predatory insects against attacking herbivore insects.

Wounding results in the formation of prosystemin, made up of 200-amino acids and a precursor of systemin which is an 18-amino acid polypeptide. Systemin ultimately results in formation of linolenic acid which activates the octadecanoid signaling pathway [13]. JA and systemin acts as systemic signals which are transported to all parts of the plant by phloem [14].

Cross-Talk &Trade Off

There are three main signaling pathways: SA, JA and Ethylene. It is believed that SA-dependent induced systematic resistance (ISR) is caused against a pathogen attack, whereas, the JA-dependent pathway induces resistance against herbivore attack (IRH). Resistance induced by pathogens is also active against the herbivore attack and vice versa. This is termed as cross resistance of one group of enemy against the other group. For example, it was observed that pseudoplusia (soybean looper) causes defoliation of soybean, which induces resistance not only against soybean looper but also against two different fungal infections [15].

Feeding Guilds

Plants have the ability to recognize different feeding guilds; they can differentiate between herbivore and mechanical damage such as hail and wind. Two thirds of more than one million insects are leaf eating beetles (Coleoptera) or caterpillars (Lepidoptera) that feed on plants with the help of specialized mouthparts meant for chewing, snipping and tearing [16]. Leaf miners also termed as chewing insects feed on soft tissues of the leaf whereas other piercing-sucking herbivores, having a tube like mouthpart, feed on plants by a sucking action without causing external damage to the plants. Examples are spider, mites, aphids; whiteflies and other Hemiptera that have specified stylets for injecting in between intercellular spaces of cells or into phloem. In the case of root feeding herbivores, the majority are root chewers although some are root borers [17].

This review tries to cover the biochemical pathway induced for production of defense responses in plants against the attack of chewing insects.

Oral Secretions of Insects

In many experimental procedures mechanical wounding was used to mimic insect herbivore. These results suggested that in wounded plants up regulation of several genes and proteins was the same as observed by chewing herbivores [18]. However, it is now well established that herbivores induce a different set of physical and chemical pathways distinct from induction caused by mechanical wounding [19]. It was demonstrated by using a specimen of potato plant attacked by Manducasexta and evaluating transcriptomic changes induced by insect attack and mechanical wounding. A rapid accumulation of 3-hydroxy-3-methylglutaryl-coenzyme is ducates and wound-inducible protein inhibitor II (Pin-II) was seen with insect attack while a slower accumulation was monitored through mechanical wounding [20]. The oral secretion injected by insect herbivores upon feeding is the main reason for the changed transcriptomic analysis. The types of elicitors induced through oral secretion are listed and described below.

Volicitin

The union of plant and herbivore-induced precursor's results in production of fatty acid-amino acid conjugates (FACs). In Spodopteraexigua (beet armyworm) oral secretions, N-17-hydroxylinolenoyl-L-glutamine also termed as volicitin was the first acknowledged [21]. It was suggested that Volicitin selectively binds with the FAC receptor on the plasma membrane [22]. The action of volicitin seen in Zea mays (maize) was activation of the gene Ig-l for production of enzyme indole-3-glycerol phosphatase lyase (IGL) that catalyzed indole-3-glycerol for production of reactive free indoles [23]. However, it was seen that some plants which included Arabidopsis thaliana, *Gossypium hirsutum, P. lunatus* and *Vignaun guiculata* did not respond to exogenously treated FACs [24].

Inceptins

Inceptins are disulfide-bonded peptides formed in gut of *Spodoptera frugiperda* (fall armyworm). While feeding the fall army worm causes proteolytic fragmentation of chloroplastic ATP synthase γ-subunit present in leaves and the formation of inceptin [25]. There are no receptors yet known for inceptin.

Caeliferins

Caeliferins are disulfoxy fatty acids present in oral secretions of *Shistocerca Americana* (American bird grasshopper) and other grasshopper species [26]. Just like FACs, Caeliferins caused release of volatile terpenoids in maize seedlings. The exact function of these volatiles is not well known but recently the successful synthesis of Caeliferins have made it possible for further study of their function and the identification of plant receptors [27].

Bruchins and β-glucosidase

Bruchins are long-chain α,ω-diols, esterified at one or both oxygen atoms with 3-hydroxypropanoic acid. They are elicitors seen in Bruchuspisorum (pea weevil) and *Callosobruchus maculates* (cowpea weevil) [28]. β-glucosidase present in oral secretion of *P. brassicaelarvae* elicited release of volatile organic compounds (VOC's) resulting in the attraction of the parasitic was p, *Cotesia glomerata* [29].

In contrast to the explanation above there are also some elicitors in oral secretion which suppresses immune responses. One example is salivary glucose oxidase (GOX) secreted by Helicoverpa zea (corn earworm) [30].

Primary events during plant insect interaction

Most research on the topic of plant insect interactions is focused on the transcriptomic, proteomic and genomic changes induced by an insect attack. The early events taking place following the bite to the plants are poorly understood. Presently available knowledge related to these primary events is explained in this review.

Calcium ion fluxes

In the active victim in plant-insect interactions, plants respond to attack by the formation of a diverse group of toxins and defensive proteins [31]. The elicitors were transmitted by injection of oral secretion resulting in initiation of signal transduction pathways including calcium ion fluxes, phosphorylation cascades and the Jasmonate pathway. All of these pathways play a key role in stimulating resistance to the extensive range of insects [32].

The elicitation of membrane potentials (Vm) and intracellular calcium influx was studied in Lima bean (Phaseolus lunatus) leaves attacked by Mediterranean climbing cutworm (Spodoptera littoralis). It was found that insect saliva contained an elicitor N-acyl Glns (volicitin, N-palmitoyl-Gln, N-linolenoyl-Gln) which caused a change in membrane potential first at the area of the bite gradually spreading along the length of leaf. N-acyl Gln doesn't show any change until combined with regurgitant of which the addition can enhance the effect as seen in transgenic aequorin-expressing soybean cells [33]. As the calcium enters the cell it activates the calcium binding proteins such as calmodulin, calcineurin B-like proteins and Ca^{2+}-binding protein kinases (CDPKs). This results in altered protein phosphorylation and gene expression [34]. In Medicago truncatula (barrel clover) the calcium concentration is decreased when ethephon, releasing source of ethylene, is added. The result is the decrease of Ca^{2+} ions demonstrating ethylene is the modulator of Ca^{2+} influx [35]. Little is known about other expected ion channels; therefore, the future studies could investigate the identification of ion channels and the expected downstream signaling cascade. In plant insect interactions calcium acts as a second messenger for triggering the signaling pathway, thus, has an important role in plants. The change in calcium influx can be monitored either by fluorescent probe or by bioluminescence-based aquorin technology. The aequorin technology is considered more preferable than Ca^{2+} sensitive fluorescent dyes for the analysis of calcium signaling in the cell. The protein aequorin contains three EF-hands meant for binding calcium. In the presence of oxygen the aequorin undergoes a chemical change and converts coelenterazine into excited Coelenteramide with the emission of carbon dioxide.
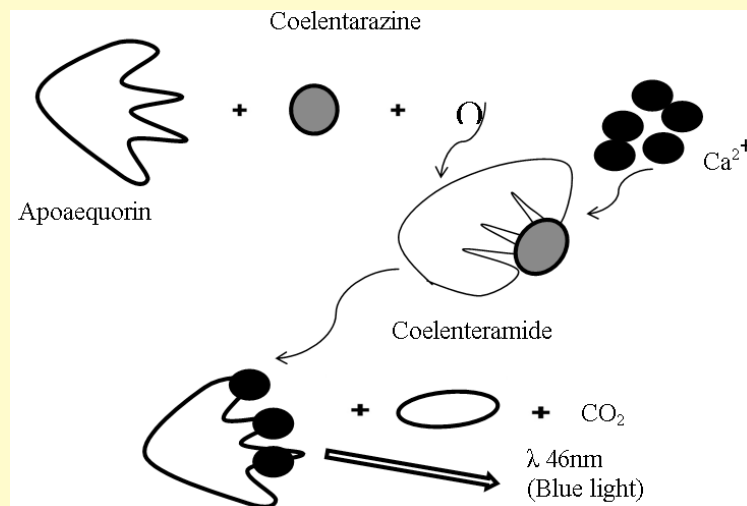
Figure 1: *Bioluminescence-based aequorin technology. This mechanism results in the emitting of light by the reaction of calcium with the apoaequorin.*

Insect Induced Jasmonate-Signaling Pathway

There are different intracellular wound signals induced by insect attacks such as JA, SA and ethylene. The JA pathway is regulated by the action of wounding and the attack of chewing insects [36]. The intracellular pathways such as systemic and Jasmonate signaling pathways are mainly discussed.

Systematic signaling means long term signaling, it includes the induction of several defense responses including systemin peptides produced by proteolytic action on large proteins [13]. Systemin is an 18-amino acid peptide, responsible for activation of the octade-canoid and hexadecanoid pathways which ultimately produce the Jasmonate hormone that functions in triggering genes and production of defense proteins. Prosystemin is polar containing many cleavage sites; the cleavage site for release of systemin is not specified, it could be a polar region or a conserved sequence motif. If cleavage occurs at the N-terminus it is between Leu-Ala and if it occurs at the C-terminus it is between Asp-Asn [31]. The location of prosystemin is inside parenchyma cells. After initiation of an insect attack, systemin is produced and released into the apoplast [37]. The systemin is then bound to its receptor (SR160). The binding induces many events such as membrane depolarization, increased cytosolic Ca^{2+} influx and activation of MAKP cascade which finally results in formation of JA.

Production of Jasmonate

As discussed earlier, systemic signaling results in the production of Jasmonate. The average level of Jasmonate is 10-40 ng in 1 gram of fresh leaves. In Arabidopsis thaliana, following insect attack, the maximum of a 40 fold increase occurred within 90 min. The decline in jasmonates was monitored after 9 hours and persisted at the same level for the next 24 hours [38]. The comparable inactive trans-conformation form of JA in non-invasive plants transforms to active cis-conformation upon wounding [39]. The JA spread through the whole plant including the wounded and unwounded regions. Jasmonate evoked the defense response by inducing the transcription of defense genes. Jasmonic acid (JA) belongs to a family of compounds that are collectively known as jasmonate or oxylipins, produced through the Jasmonate pathway discussed next.

Polyunsaturated fatty acid (PUFA) is released from the membranes of chloroplasts, thylakoids or cells. These PUFA usually get esterified within the membrane and are released by stimulation with lipases such as DAD1 (defective in anther dehiscence) and DGL (dongle) [40].

An experiment was designed to prove the activation of defense genes by the octadecanoid signaling pathway in the tomato plant [41]. A tomato mutant plant (JL5) also known as DEFl was used because normally it is deficient in the production of a defense response to Manducasexta attack. Incorporated intermediates related to the octadecanoid pathway stimulated the production of JA as an end product. Accordingly, it suggests that the octadecanoid pathway plays an important role in producing anti-herbivore agents.

Defense Responses Regulated By Jasmonate

During gene expression the (+)-7-epi-JA-L-Ile binds to coronatin-insensitive 1 (COI1) protein, it is the F-box subunit of the E3 ubiquitin ligase a type of Skip/Cullin/Fbox (SCF) protein [42]. F-box proteins are present in the kingdom of eukaryotes; they function in the direction of proteins for ubiquitin-mediated destruction. The association of F-box protein with cullin and Skp1 proteins results in construction of an E3 ubiquitin ligase known as the SCF complex. Thus, SCFCOI1 recognizes JA-Ile, and stimulates the binding of hormone with the JAZ motif (J) of Jasmonate Zim-domain protein (JAZ), resulting in ubquitination [43], and degradation by 26S proteasome. Following this the transcription factor, MYC2is released and together with the RNA polymerase 11 transcriptional machinery and the mediator complex (universal adaptor) gene expression is activated. In the absence of JA-IIe, the transcription factor (MYC2) remains bonded with the JAZ-protein, preventing the expression of JA- induced genes.

The JAZ proteins are also dependent on MYC2 and therefore only induced temporarily by Jasmonate [43]. The presence or absence of gibberllic acids (GAs) also effects JA-responsive gene expression. As seen in Arabidopsis thaliana the presence of JAs and absence of GA results in the binding of DELLA to the JAZ protein, releasing MYC2 for activation of JA related genes. On the other hand the presence of GAs results in degradation of DELLA protein which promotes the formation of JAZ-MYC complexes and thus represses JA responsive gene expression. Twelve genes have been seen in Nicotina attenuata, showing differential expression of JAZ with respect to location of shoots and roots [44].

Conclusion

Plants maintain balance between defense and growth according to changes applied by a biotic stress. Herbivore attack on Nicotiana attenuata results in induction of the Jasmonate pathway which reduces photosynthesis. The experiment compared wild type plants with mutant plants deficient in Jasmonate synthesis. The reduction of photosynthesis was increased in wild type plants compared to mutant type plants because in wild type plant the lipoxygenase signaling pathway affected the photosynthetic electron transport chain resulting in reduction of photosynthesis.

Bibliography

1. Ehrlich PR and Raven PH. "Butterflies and plants: A study in coevolution". *Evolution* 18 (1964): 586-608.
2. Haruta M., *et al*. "AKunitz trypsin inhibitor gene family from trembling aspen (Populus tremuloides Michx.): cloning, functional expression, and induction by wounding and herbivory". *Plant Molecular Biology* 46.3 (2001): 347-359.
3. Birkett MA., *et al*. "New roles for cis-jasmone as an insect semiochemical and in plant defense". *Proceedings of the National Academy of the United States of America* 97.16 (2000): 9329-9334.
4. Kliebenstein DJ., *et al*. "Genetic control of natural variation in Arabidopsis glucosinolate accumulation". *Plant Physiology* 126.2 (2001): 811-825.
5. Fordyce JA and Agrawal AA. "The role of plant trichomes and caterpillar group size on growth and defense of the pipevine swallowtail Battus philenor". *Journal of Animal Ecology* 70.6 (2001): 997-1005.
6. Scott JG and Wen ZM. "Cytochromes P450 of insects: the tip of the iceberg". *Pest Management Science* 57.10 (2001): 958-967.
7. Ziegler J., *et al*. "Purification and characterization of allene oxide cyclase from dry corn seeds". *Plant Physiology* 114.2 (1997): 565-573.
8. Nishida R. "Sequestration of defensive substances from plants by Lepidoptera". *Annual Review of Entomology* 47 (2002): 57-92.

9.   Silva CP., *et al*. "Induction of digestive alpha-amylases in larvae of Zabrotes subfasciatus (Coleoptera: Bruchidae) in response to ingestion of common bean alpha-amylase inhibitor 1". *Journal of Insect Physiology* 47.11 (2001): 1283-1290.

10. Chew FS and Renwick JAA. "Host plant choice in Pieris butterflies". Carde RT and Bell WJ. Eds., Chapman and Hall: New York, NY, USA". *Chemical Ecology of Insects* II: 214-238.

11. Ali JG and Agrawal AA. "Specialist versus generalist insect herbivores plant defense". *Trends in Plant Science* 17.5 (2012): 293-302.

12. Lamb C and Dixon RA. "The oxidative burst in plant disease resistance". *Annual Review of Plant Physiology and Plant Molecular Biology* 48: 251-275.

13. Ryan CA and Pearce G. "Systemins: A functionally defined family of peptide signal that regulate defensive genes in solanaceae species". *Proceedings of the National Academy of the United States of America* 100.Suppl 2 (2003): 14577-14580.

14. Zhang Z-P and Baldwin IT. "Transport of (2-14C) jasmonic acid from leaves to roots mimics wound-induced changes in endogenous jasmonic acid pools in *Nicotiana sylvestris*". *Planta* 203.4 (1997): 436-441.

15. Padgett GB., *et al*. "Interactions among the soybean looper (Lepidoptera: Noctuidae), three corned alfalfa hopper (Homoptera: Membracidae), stem canker, and red crown rot in soyabean". *Journal of Entomological Science* 29 (1994): 110-119.

16. Schoonhoven LM., *et al*. "Insect Plant-biology: From Physiology to Evolution" Chapman and Hall: London, UK.

17. Houlihan DF. "The structure and behaviour of *Notiphilariparia* and *Erioptera squalida*, two root-piercing insects". *Journal of Zoology* 159: 249-267.

18. Duceppe MO., *et al*. "Wounding, insect chewing and phloem sap feeding differentially alter the leaf proteome of potato, Solanum tuberosum L". *Proteome Science* 10.1 (2012): 73.

19. Hilker M and Meiners T. "How do plants "notice" attack by herbivorous arthropods". *Biological Reviews of the Cambridge Philosophical Society* 85.2 (2010): 267-280.

20. Korth KL and Dixon RA. "Evidence for chewing insect-specific molecular events distinct from a general wound response in leaves". *Plant Physiology* 115.4 (1997): 1299-1305.

21. Turlings TCJ., *et al*. "Learning of Host Location Cues by Insect Parasitoids". *In Insect Learning*: *Ecological and Evolutionary Perspectives* Papaj DR and Lewis A. Eds., Chapman and Hall: New York, NY, USA, 51-78.

22. Truitt CL., *et al*. "A plasma membrane protein from Zea mays binds with the herbivore elicitor volicitin". *Plant Cell* 16.2 (2005): 523-532.

23. Frey M., *et al*. "An herbivore elicitor activates the gene for indole emission in maize". *Proceedings of the National Academy of the United States of America* 97.26 (2000): 14801-14806.

24. Schmelz EA., *et al*. "Phytohormone-based activity mapping of insect herbivore-produced elicitors". *Proceedings of the National Academy of the United States of America* 106.2 (2009): 653-657.

25. Schmelz EA., *et al*. "Fragments of ATP synthase mediate plant perception of insect attack". *Proceedings of the National Academy of the United States of America* 103.23 (2006): 8894-8899.

26. Alborn HT., *et al*. "Disulfoxy fatty acids from the american bird grasshopper Shistocerca americana, elicitors of plant volatiles". *Proceedings of the National Academy of the United States of America* 104.32 (32): 12976-12981.

27. O'Doherty I., *et al*. "Synthesis of caeliferins, elicitors of plant immune responses: Accessing lipophilic natural products via cross metathesis". *Organic Letters* 13.21 (2011): 5900-5903.

28. Doss RP., *et al*. "Bruchins: Insect-derived plant regulators that stimulate neoplasm formation". *Proceedings of the National Academy of the United States of America* 97.11 (2000): 6218-6223.

29. Mattiacci L., *et al*. "beta-Glucosidase-An elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps". *Proceedings of the National Academy of the United States of America* 92.6 (1995): 2036-2040.

30. Bos JI., *et al*. "A functional genomics approach identifies candidate effectors from the aphid species Myzus persicae (green peach aphid)". *PLoS Genetics* 6.11 (2010): e1001216.

31. Furstenberg-Hagg J., *et al*. "Plant defense against insect herbivores". *International Journal of Molecular Sciences* 14.5 (2013): 10242-10297.

32. Wu J and Baldwin IT. "New Insights into plant responses to the attack from insect herbivores". *Annual Review of Genetics* 44 (2010): 1-24.

33. Maffei M., *et al*. "Effects of Feeding Spodoptera littoralis on lima bean leaves. I. Membrane potentials, intracellular calcium variations, oral secretions, and regurgitate components". *Plant Physiology* 134.4 (2004): 1752-1762.

34. Howe GA and Jander G. "Plant immunity to insect herbivores". *Annual Review of Plant Biology* 59: 41-66.

35. Arimura G., *et al*. "Herbivore-induced terpenoid emission in Medicago truncatula: concerted action of jasmonate, ethylene and calcium signaling". *Planta* 227.2 (2008): 453-464.

36. Arimura G.I., *et al*. "Recent advances in plant early signaling in response to herbivory". *International Journal of Molecular Sciences* 12.6 (2011): 3723-3739.

37. Schilmiller AL and Howe GA. "Systemic signaling in the wound response". *Current Opinion in Plant Biology* 8.4 (2005): 369-377.

38. Glauser G., *et al*. "Spatial and temporal dynamics of jasmonate synthesis and accumulation in Arabidopsis in response to wounding". *The Journal of Biological Chemistry* 283.24 (2008): 16400-16407.

39. Schulze B., *et al*. "Profiling of structurally labile oxylipins in plants by in situ derivatization with pentafluorobenzyl hydroxylamine". *Analytical Biochemistry* 348.2 (2006): 269-283.

40. Dave A and Graham IA. "Oxylipin signaling: A distinct role for the jasmonic acid precursor cis-12-oxo-phytodienoic acid". *Frontiers in Plant Science* 3: 42.

41. Howe GA., *et al*. "An octadecanoid pathway mutant (JL5) of tomato 1s compromised in signaling for defense against insect attack". *The Plant Cell* 8.11 (1996): 2067-2077.

42. Thines B., *et al*. "JAZ repressor proteins are targets of the scfcoi1 complex during jasmonate signaling". *Nature* 448.7154 (2007): 661-662.

43. Chini A., *et al*. "The JAZ family of repressors is the missing link in jasmonate signaling". *Nature* 448.7154 (2007): 666-672.

44. Oh Y., *et al*. "Najazh regulates a subset of defense responses against herbivores and spontaneous leaf necrosis in Nicotiana attenuate plants". *Plant Physiology* 159.2 (2012): 769-778.