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Review paper

PLANT DEFENCE PROTEINS DURING APHID INFESTATION

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ABSTRACT: Parasitism by phloem-feeding insects, such as aphids and whiteflies, are widespread and often serious constraint on plant growth. Aphids successfully exploit a broad range of vascular plants. Despite the ubiquity of phloem feeding insects, in depth knowledge of plant defence and plant-microbe interactions is still lagging. This review summarizes the current knowledge about the phloem sap proteins that are involved in defence reactions with probable functions in wound and defence reactions.

Keywords: Aphid, phloem, Saliva, Sieve Element, p-protein

INTRODUCTION

Phytophages breach the integrity of plant tissues to recover nutrients from foliage, seeds, pollen, nectar, roots, or shoots. Herbivores cause extensive damage while phloem feeding insects such as aphids and whiteflies cause modest to barely perceptible damage. Phloem feeding insects challenges the plants by depleting photosynthates. They also introduce chemical and / or protein effectors that alter the plant defence signalling infestation symptoms and plant development (Kaloshian and Walling, 2005). the phloem – feeding insects cause heavy losses in agriculture and horticulture due to their broad host range, breeding strategies, and the emergence of the insecticide resistant strains. (Goggin, 2007).

With the tools of cell and molecular biology, genetics, genomics, electrophysiology, and biochemistry, investigators are providing novel insights into the complexity and dynamics of plant – herbivore interactions.

Whiteflies and aphids are members of the Hemipteran suborder Sternorrhyncha. Their life cycles, endo-symbiont populations, and feeding activities, are distinct (Baumann, 2005; Kaloshian and Walling, 2005). These insects have highly modified mouth parts (stylets) to navigate the cuticle, epidermis, mesophyll and establish feeding sites in phloem sieve elements (S.E).

Adult aphids and nymphs are mobile and utilize several feeding sites during their lifetime. In contrast, once the whitefly nymph establishes a feeding site on a minor vein of the phloem, nymphs feed at this site almost continuously for 21 to 30 days. The immobility of nymphs, longer lifecycle and prolonged nymph feeding are features that distinguish the whitefly – plant and aphid – plant interactions.

Aphid and whiteflies take advantage of their adept feeding strategies and avoid or deter many plant defences. These insects disguise themselves and deceive their hosts and natural enemies by using stylets to deliver salivary chemicals and / or proteins into the plant to influence wound healing, defence – signalling pathways, and volatile emissions. Similar deceptive strategies are routinely employed by phytopathogenic microbes to avoid recognition and combat plant defences (da Cunha *et al*., 2007).

Pathogens introduce effectors into plant cells manipulating many biochemical and cellular processes to enhance their invasion on host plants. This review will highlight the known class of defence – related phloem sap proteins.

Salivary glands and saliva composition of phloem feeding insects

The salivary glands of aphids are paired. The right and the left glands have two glandular units, a large principle gland and a smaller accessory gland. The salivary ducts of both glandular units join together on one side and then their common duct joins the one coming from the contra-lateral side. The principle gland is innervated and contains eight secretory cells, possibly secreting different components (Ponsen, 1972).

This gland seems to play a major role in the sheath saliva production. The accessory gland does not appear to be enervated, and its cells do not show much differentiation. Transmission studies of persistent / circulative plant viruses have shown that the accessory glands transfer the viruses from the haemolymph to the salivary canal in the stylets and into plants (Gray and Gildow, 2003). From this it has been inferred that the watery E1 saliva must come from the accessory glands since E1 salivation is responsible for inoculation of viruses (Prado and Tjallingii, 1994). It remains unclear whether the principle glands exclusively produce the sheath saliva. According to Peter Miles (1999), the protein content in aphid saliva revealed lot of contradictions, not only between but also within species. Possibly the variation is mainly due to the different diet compositions. It is believed that E1 salivation may suppress the wound responses. How the suppression works and in what stage the E1 saliva interferes with the wounding responses is still not clear.

Stylectomy (fast stylet amputation) during E2 waveforms were done to collect phloem sap exuding from severed stylets (Van Helden and Tjallingii 1994). However exudation from the phloem sap was seen to stop soon after stylectomy. Electron micrographs of the stylet stump in the plant showed the presence of coagulated lump of protein inside the food canal (Tjallingii and Hogen Esch, 1993). This suggests (i) that there is free protein in the sieve element; and (ii) when E2 salivation stops due to stylectomy, this protein will clog in the food canal. This shows that the E1 saliva does not prevent the release of the bound p-protein, but it may only prevent its coagulation in the sieve tube (Tjallingii, 2005).

Phloem wound responses on plant – insect interaction

The transported metabolites make S.Es an attractive target for insects that are specialised to feed exclusively on phloem sap, for example whiteflies or aphids. In contrast to herbivores, phloem – feeding insects established a sustained interaction with S.Es. They release saliva that inhibits plant stress responses and prevent closure of pierced S.Es by callose or polymerised proteins (Miles, 1999). This allows the insects to feed large amounts of phloem sap to obtain enough nutrients for their survival. Due to this feeding behaviour, phloem – sucking insects are directly exposed not only to the nutrients but to all components of the transport fluid, including proteins. Interestingly a high proportion of the phloem sap proteins so far identified is predicted to be involved in stress and defence reactions, although their exact physiological functions remain to be established.

Reactive Oxygen Species(ROS) , Calcium Phytohormones

Oxidative stress is one of the first general reactions to the injury caused by phloem – sucking insects penetrating the tissue. It is noticed that aphid salivary secretions can themselves alter oxidative conditions (Jiang and Miles, 1993; Walling, 2000). It was also proposed that the saliva and the injury caused by the aphid feeding induce not only a local but also a systemic production of reactive oxygen species (ROS) in the phloem (Moran *et al*., 2002; Zhu-Salzman *et al*., 2004; Divol *et al*., 2005). Several genes are up – regulated by aphid infestation at a whole plant level in *Arabidopsis thaliana* (Moran *et al*., 2002). Glutathione – S – transferases and the metal ion scavengers, metallothioneins, and the copper homeostasis factor can detoxify radicals (Marrs, 1996) and the expression of the corresponding gene was significantly up –regulated by aphid feeding in systematic phloem tissue (Divol *et al*., 2005).

Tissue damage is accompanied by an elevation in cystolic calcium. In un-disturbed S.Es, calcium is low and an increase upon wounding is thought to initiate a long distance signalling cascade (Fromm and Baur, 1994; Knoblauch *et al*., 2001; van Bel and Gaupels, 2004).

It was proposed that the elevation of phloem Ca^{+2} also participate in the regulation of phloem enzymes (Eschrich and Heyser, 1975). The occurrence of calcium – binding proteins in phloem sap was recognised early (Mc Euen *et al*., 1981) but quite a time elapsed before any of them could be identified. For example, enzymes and activity of calcium - activated protein kinases that act as major mediators in Ca^{+2} signalling that occurs in phloem sap. Annexins and calmodulins were also found in phloem samples from different species. There is evidence that calcium and calcium binding proteins are involved in the regulation of calmodulin gene expression and ROS generation (Harding *et al*., 1997). Calmodulin expression is seen to be increased by systemin, a peptide hormone found in S.E of Solanacea
(Narvaez- Vasquez *et al*., 1995).
Phloem sap contains enzymes involved in the synthesis of phytohormones, namely ethylene, jasmonic acid and salicylic acid. Plant transcriptional responses to pathogens and herbivores are determined in part by the coordinate regulation of salicylic acid, jasmonic acid and ethylene. Signalling pathways can have both synergistic and antagonistic interactions (Rojo *et al*., 2003). Exogenous application of jasmonates to cotton, wheat sorghum and tomato reduce aphid host preference, survival and fecundity (Omer *et al*., 2001; Bruce *et al*., 2003; Cooper *et al*., 2004; Zhu- Salzman *et al*., 2004; Cooper and Goggin, 2005). Results suggest that roles of salicylic acid and jasmonic acid in plant species, and between compatible and incompatible interactions. Further work is needed to explore the potential roles of other hormones, including auxin and gibberellins in plant responses to phloem feeding insects (Park *et al*., 2005).

Occlusion of Sieve Elements

Plants have different mechanisms to plug affected sieve elements (S.Es) to avoid the loss of organic nutrients. This is regulated as a quick and straight forward response to the damage caused by the insects. Calcium is an important mediator for plugging S.Es and calcium antagonists such as EDTA have long been known to prevent sieve tube occlusion (King and Zeevaart, 1974).
Legumes contain unique crystalloid proteins, the so – called forisomes, that can undergo rapid and reversible conversations from the condensed resting state into a dispersed state in which they close S.Es both in-vivo (Furch, 2009) and in-vitro (Schwan, 2007; Knoblauch, 2005). Forisomes disperse above a threshold of about 50µ mol Ca^{+2}. In- case of S.E damage the required 1000 –fold increase in Ca^{+2} concentration is only attained in the proximity of the endoplasmic reticulum cisternae, where Ca^{+2} hotspots are created in the proximity of the forisome ends by (partly interactive) activation of local Ca^{+2} channels (Hafke, 2009).
In sword bean, *Canavalia gladiate* tailed forisomes and comparatively longer ones were observed. The size ranges between 20µm and 55µm in length. It is observed that there is 9- fold increase in volume of forisome on application of Ca^{+2}, thus the efficiency of S.E plugging is high. As a response to herbivore attack, phloem sap is squeezed from S.Es and accumulates at wounded sites. This phloem sap will prevent further herbivore attack and reduce the risk of infection of wounds with opportunistic pathogens like fungi (Christeller *et al*., 1998). The formation of phloem filaments by PP1 and PP2 as well as the closure constitute a potent physical barrier against further invasion.
In contrast, phloem sucking insects can locate and access S.Es avoiding the normal plant wound response. Components of aphid saliva injected immediately after phloem puncture inhibit the normal callose deposition and P- protein deposition gelation, therefore enables sap uptake without phloem sealing. After feeding sites are established, the stylets of phloem – piercing insects can stay in continous contact with the plant cells for hours to weeks (Walling, 2000). Recent study revealed that massive deposits of callose are caused by infection of phloem feeding aphids. Leaves that had been colonized by aphids but from which aphid had been removed showed extensive wound callose deposits, which persisted for up to 48 hrs after the removal of aphid colonies. This suggests that the damage caused by aphid feeding is a long term, transient event in non resistant plants (Botha and Matsiliza, 2004).
Protease inhibitors and lectins
Protease inhibitors are proteins which tightly bind proteolytic enzymes and thereby inhibit their activity.

Squash phloem exudates has shown to contain high amount of trypsin, chymotrypsin, serine, and aspartic protease inhibitors and cysteine protease inhibitors have been detected in Rape and Ricinus phloem sap.

In addition to phloem inhibitors the lectins, another group of defence proteins show a wide spread occurrence in phloem sap. Lectins are proteins that reversibly bind to specific mono- or oligosaccharides. Chitin – binding lectins from the Curcubitaceae are a small group of lectins that were first identified in curcubit phloem sap (Read and Northcote, 1983 b). Arabidopsis also contains homologous phloem expressed PP2 – like lectins. Many of these lectins are known to be toxic to both insects and vertebrates. Only a few of these lectins are known to be herbivore or wound induced (Chrispeels and Raikhel, 1991). Feeding experiments in insects showed its interference with chewing (Murdock *et al*., 1990) and sucking (Powell *et al*., 1993).

Other defence related proteins

Several components of the myrosinase system have been detected in phloem exudates from Brassica. The myrosinase system is able to produce cynates and nitriles (Bones and Rossiter, 1996) from glucosinolates that are transported inside the phloem (Chen, *et al*., 2001). These toxic hydrolysis products are induced by wounding, microorganisms, and insects that leads to deterrence of herbivorous and phloem feeding insects. Phloem sap contains additional proteins known to be induced by wounding (CSF-2, SN- 1) or insect feeding (SLW-1, SLW-3) but their mode of action is unknown. SLW proteins are specifically induced by whitefly feeding. SLW-1 (potentially produced in sieve elements) transcription is regulated by jasmonic acid and ethylene. SLW – 3 does not respond to any known wound signal indicating a probability of some new signalling pathway for activation (Walling, 2000).

Conclusion

Recent identification of numerous proteins in phloem sap of different plant species provides an insight to the potential functions of these polypeptides. A number of these proteins are functionally related to defence responses and therefore has an impact on plant – insect interactions. There is a worldwide interest in the molecular aspects as well as induced resistance to phloem feeders. There is still lack of knowledge in the molecular backgrounds of phloem insect interactions, composition and timing of salivary secretion which apparently is the key factor to different mechanism of resistance. This review will be useful to develop novel biotechnological strategies to enhance the resistance of crop plants against phloem feeding insects.

REFERENCES

1. Baumann P. (2005) Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annual Review of Microbiology* 59: 155–189.
2. Bones AM, Rossiter JT.(1996). The myrosinase–glucosinolate system, its organization and biochemistry. *Physiologia Plantarum* 97, 194–208.
3. Botha CEJ, Matsiliza B. (2004) Reduction in transport in wheat (Triticum aestivum) is caused by sustained phloem feeding by the Russian wheat aphid (Diuraphis noxia). *South African Journal of Botany* 70, 249–254.
4. Bruce TJ, Martin JL, Picket JA, Pye BJ, Smart LE,Wadhams LJ. (2003) Cis-Jasmone treatment induces resistance in wheat plants against the grain aphid, Sitobion avenae (Fabricius) (Homoptera: Aphididae). *Pest Management Science* 59, 1031–1036.
5. Chen S, Petersen BL, Olsen CE, Schulz A, Halkier BA. (2001) Long-distance phloem transport of glucosinolates in Arabidopsis. *Plant Physiology* 127, 194–201.
6. Chrispeels MJ, Raikhel NV. (1991) Lectins, lectin genes, and their role in plant defence. *The Plant Cell* 3, 1–9.

7. Christeller JT, Farley PC, Ramsay RJ, Sullivan PA, Laing WA. (1998) Purification, characterization and cloning of an aspartic proteinase inhibitor from squash phloem exudate. *European Journal of Biochemistry* 254, 160–167.
8. Cooper WR, Goggin FL. (2005) Effects of jasmonate-induced defences in tomato on the potato aphid, Macrosiphum euphorbiae. *Entomologia Experimentalis et Applicata* 115, 107–115.
9. Cooper WR, Jia L, Goggin FL.(2004) Acquired and R-genemediated resistance against the potato aphid in tomato. *Journal of Chemical Ecology* 30, 2527–2542.
10. da Cunha L, Sreerekha MV, Mackey D. (2007) Defense suppression by virulence effectors of bacterial phytopathogens. *Current Opinion in Plant Biology* 10: 349–357
11. Divol F, Vilaine F, Thibivilliers S, Amselem J, Palauqui J-C, Kusiak C, Dinant S. (2005) Systemic response to aphid infestation by Myzus persicae in the phloem of Apium graveolens. *Plant Molecular Biology* 57, 517–540.
12. Eschrich W, Heyser W. (1975) Biochemistry of phloem constituents.In: Zimmermann MH, Milburn JA, eds. Encyclopedia of Plant Physiology, Vol. 1. Berlin: Springer Verlag, 101–136.
13. Fromm J, Bauer T. (1994) Action-potentials in maize sieve tubes change phloem translocation. *Journal of Experimental Botany* 45, 463–469.
14. Furch, A.C.U. *et al*. (2009) Sieve element Ca2+ channels as relay stations between remote stimuli and sieve tube occlusion in Vicia faba. *Plant Cell* 21, 2118–2132.
15. Goggin FL (2007) Plant-aphid interactions: molecular and ecological perspectives. *Current Opinion in Plant Biology* 10: 399–408.
16. Gray S, Gildow FE. (2003) Luteovirus–aphid interaction. *Annual Review of Phytopathology* 41, 539–566.
17. Hafke, J.B. *et al*. (2009) Forisome dispersion in Vicia faba is triggered by Ca2+ hotspots created by concerted action of diverse Ca2+ channels in sieve elements. *Plant Signalling Behaviour* 4, 968–972.
18. Harding SA, Oh S-H, Roberts DM. 1997. Transgenic tobacco expressing a foreign calmodulin gene shows an enhanced production of active oxygen species. *EMBO Journal* 16, 1137–1144.
19. Jiang Y, Miles PW. (1993) Responses of a compatible lucerne variety to attack by spotted alfalfa aphid: changes in the redox balance in infested tissues. *Entomologia Experimentalis et Applicata* 67, 263–274.
20. Kaloshian I,Walling L.L. (2005) Hemipterans as plant pathogens. *Annual Review of Plant Biology* 43: 491–521.
21. King R, Zeevaart J. (1974) Enhancement of phloem exudation from cut petioles by chelating agents. *Plant Physiology* 53, 96–103.
22. Knoblauch M, Peters WS, Ehlers K, van Bel AJE. (2001) Reversible calcium-regulated stopcocks in legume sieve tubes. *The Plant Cell* 13, 1221–1230.
23. Knoblauch, M. *et al*. (2005) Corrigendum. *Nature Materials* 4, 353.
24. Marrs KA. (1996) The function and regulation of glutathione S-transferases in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 47, 127–158.
25. McEuen AR, Hart JW, Sabnis DD. (1981) Calcium-binding protein in sieve tube exudate. *Planta* 151, 531–534.
26. Miles PW. (1999) Aphid saliva. *Biological Reviews* 74, 41–85.
27. Moran PJ, Cheng Y, Cassell JL, Thompson GA. (2002) Gene expression profiling of Arabidopsis thaliana in compatible plant– aphid interactions. *Archives of Insect Biochemistry and Physiology* 51, 182–203.

28. Murdock LL, Huesing JE, Nielsen SS, Pratt RC, Shade RE. (1990) Biological effects of plant lectins on the cowpea weevil. *Phytochemistry* 29, 85–89.
29. Narvaez-Vasquez J, Pearce G, Orozco-Cardenas ML, Franceschi VR, Ryan CA. (1995) Autoradiographic and biochemical evidence for the systemic translocation of systemin in tomato plants. *Planta* 195, 593–600.
30. Omer AD, Granett J, Karban R, Villa EM. (2001) Chemically induced resistance against multiple pests in cotton. *International Journal of Pest Management* 47, 49–54.
31. Park S-J, Huang Y, Ayoubi P. (2006) Identification of expression profiles of sorghum genes in response to greenbug phloem-feeding using cDNA subtraction and microarray analysis. *Planta* published online Nov. 15, 2005.
32. Ponsen MB. (1972) The site of potato leafroll virus multiplication in its vector, Myzus persicae. *Mededelingen van de Landbouwhoogeschool Wageningen* 16, 1–147.
33. Powell KS, Gatehouse AMR, Hilder VA, Gatehouse JA. 1993. Antimetabolic effects of plant lectins and plant and fungal enzymes on the nymphal stages of two important rice pests, Nilaparvata lugens and Nephotettix cinciteps. *Entomologia Experimentalis et Applicata* 66, 119–126.
34. Prado E, Tjallingii WF. 1994. Aphid activities during sieve element punctures. *Entomologia Experimentalis et Applicata* 72, 157–165.
35. Read SM, Northcote DH. 1983b. Subunit structure and interactions of the phloem proteins of Cucurbita maxima (pumpkin). *European Journal of Biochemistry* 134, 561–569.
36. Rojo E, Solano R, Sanchez-Serrano JJ. 2003. Interactions between signalling compounds involved in plant defence. *Journal of Plant Growth Regulation* 22, 82–98.
37. Schwan, S. *et al*. (2007) In vitro investigation of the geometric contraction behavior of chemo-mechanical P-protein aggregates [forisomes]. *Biophysical Chemistry* 125, 444–452.
38. Tjallingii WF, Hogen Esch Th. (1993) Fine structure of the stylet route in plant tissues by some aphids. *Physiological Entomology* 18, 317–328.
39. Tjallingii WF. (2006) Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of Experimental Botany*, Vol. 57, No. 4, 739–745.
40. van Bel AJE, Gaupels F. (2004) Pathogen-induced resistance and alarm signals in the phloem. *Molecular Plant Pathology* 5, 495–504.
41. Van Helden M, Tjallingii WF. (1994) Phloem sap collection from lettuce (Lactuca sativa): chemical comparison among methods. *Journal of Chemical Ecology* 20, 3190–3206.
42. Walling LL. (2000) The myriad plant responses to herbivores. *Journal of Plant Growth Regulation* 19, 195–216.
43. Zhu-Salzman K, Salzman RA, Ahn J-E, Koiwa H. (2004) Transcriptional regulation of sorghum defence determinants against a phloem-feeding aphid. *Plant Physiology* 134, 420–431.