



Ento(o)mics: the intersection of ‘omic’ approaches to decipher plant defense against sap-sucking insect pests

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Plants are constantly challenged by insect pests that can dramatically decrease yields. Insects with piercing-sucking mouthparts, for example, aphids, whiteflies, and leaf hoppers, seemingly cause less physical damage to tissues, however, they feed on the plant’s sap by piercing plant tissue and extracting plant fluids, thereby transmitting several plant-pathogenic viruses as well. As a counter-defense, plants activate an array of dynamic defense machineries against insect pests including the rapid reprogramming of the host cell processes. For a holistic understanding of plant-sap-sucking insect interactions, there is a need to call for techniques with the capacity to concomitantly capture these dynamic changes. Recent progress with various ‘omic’ technologies possess this capacity. In this review, we will provide a concise summary of application of ‘omic’ technologies and their utilization in plant and sap-sucking insect interaction studies. Finally, we will provide a perspective on the integration of ‘omics’ data in uncovering novel plant defense mechanisms against sap-sucking insect pests.

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Introduction

Numerous biotic stressors constantly threaten crop productivity and global food security. Recently, it has been reported that biotic stressors can cause a global yield loss between 17–30% [1]. Because insects are highly diverse and abundant in nature, their emergence as a pest can have a devastating effect on crop production, if not dealt with in an efficient and timely manner. In response to insect attack, plants and their pests engage in complex-counter responses, including rapid reprogramming of the

transcriptome/proteome and changes in hormonal/metabolite composition as well as other signal transductions culminating in either plant resistance or an invasive triumph by the pest [2,3,4*].

Compared to other herbivorous insects, sap-sucking insects have a distinct feeding behavior. For example, aphids, one of the most damaging sap-sucking insect pests of plants, use their slender stylets to maneuver various plant tissues and to progress to the phloem tissue/sap to establish a suitable feeding site [3,4*]. Although considerable research progress has been made in the area of complex plant-sap-sucking insect interactions, the underlying mechanisms that contribute to plant resistance to these insect pests are not fully understood. As the dynamic interaction between plants and insects are hard to discern in its entirety with a single approach, several complementary ‘omic’ technologies are being increasingly utilized to investigate these complex interactions. Consequently, this will also provide a distinctive opportunity to integrate genomic and phenomic data to gain insight into the underlying basis of plant resistance to insect pests. Specifically, it is now possible to integrate various ‘omics’ data to answer biological questions [5]. In this opinion article, we present an overview of how these ‘omic’ technologies have impacted research in the field of plant–insect interactions. We limit our discussion to sap-sucking insects and direct readers to other excellent recent reviews [6,7] that predominantly focus on interactions between plants and chewing insects.

‘Omic’ studies on plant-sap-sucking insect interactions

(a) Transcriptomics

Plants activate defense mechanisms when being attacked by the insect pests, many of which involve transcriptional reprogramming [8]. Transcriptomic analysis on a cassava cultivar resistant to mealybug (*Phenacoccus manihoti*) revealed mixed regulation of genes encoding proteins mostly involved in secondary metabolic process, plant resistance, and signal transduction pathways [9]. Interestingly, genes encoding proteins such as 2-oxoglutarate, gibberellin oxidase and terpene synthase were found to be induced in the resistant cassava genotype after mealybug attack [9]. Several of these genes involved in the same pathways were also activated during sorghum (*Sorghum bicolor*) - sugarcane aphid (*Melanaphis sacchari*) interactions [10]. Similarly, feeding by *Tupiocoris notatus*, a

sap-sucking mirid bug, on tobacco (*Nicotiana attenuata*) engages detoxification strategy to rapidly remove plant defense metabolites that are dependent on the jasmonic acid (JA) signaling pathway [11]. The results suggest that a larger number of differentially expressed transcripts, predominantly transcripts involved in the detoxification of tobacco defense metabolites, were downregulated in mirid bugs when fed on JA-silenced plants. The most dramatic downregulation of these transcripts were noticed on RNAi-silenced *Allene Oxide Cyclase* (AOC) plants, which is deficient in all JA-inducible defenses, suggesting that AOC may function synergistically to mobilize tobacco's defense against mirid bugs [11]. Table 1 summarizes recent studies that compare monocot and dicot plant responses to sap-sucking insect infestation.

A time-series transcriptomic analysis on sorghum - sugarcane aphid interaction identified several genes related to cell wall modification, photosynthesis and phytohormone biosynthesis [12[•]]. In addition, upregulation of transcripts related to several nucleotide-binding-site, leucine-rich repeat (NBS-LRR) and disease resistance genes have been identified in the sugarcane aphid-resistant sorghum plant [12[•]]. The upregulation of NBS-LRR genes in resistant sorghum plant is particularly intriguing, because *Mi-1.2* gene in tomato (*Solanum lycopersicum*) and the *Vat* gene in melon (*Cucumis melo*), both of which encode NBS-LRR type R proteins, confer resistance against various sap-sucking insect pests and the cotton-melon aphid, respectively [13–15]. This suggests that plants may have evolved a similar resistance mechanism to combat aphid attack. However, the downstream signaling pathways activated by the resistance genes upon aphid infestation may vary in different plant species. Similarly, cotton (*Gossypium hirsutum*) transcriptional response to whitefly (*Bemisia tabaci*) infestation, another sap-sucking insect, revealed several genes encoding protein kinases, transcription factors, metabolite synthesis, and phytohormone signaling [16]. Specifically, *GhMPK3* and *WRKY40* along with a copper transport protein were identified as key regulators of cotton defense against whitefly infestation [16]. Interestingly, *GhMPK3* knockdown led to susceptibility to whitefly feeding, mostly due to the downregulation of JA and ethylene (ET) pathways.

Chitin, a polymer composed of β -(1,4)-linked *N*-acetyl-D-glucosamine (GlcNac), has been reported in aphid exoskeleton and stylet [17]. Plants can sense chitin, a Microbe-Associated Molecular Pattern (MAMP), which can trigger immune responses to ward off chitin-containing organisms [18]. Recent transcriptomic study on soybeans (*Glycine max*) after soybean aphid (*Aphis glycines*) infestation identified several soybean genes that are involved in chitin regulation, indicating that chitin could function as one of Herbivore-Associated Molecular Patterns (HAMPs) that triggers soybean defense response [19^{••}]. Likewise, greenbug (*Schizaphis graminum*) feeding

on switchgrass (*Panicum virgatum*) plants revealed upregulation of switchgrass genes important for chitin recognition and degradation [20]. Collectively, these studies suggest plants can recognize conserved HAMPs, such as chitin, that could activate downstream plant defenses.

In addition to the insect-induced plant defenses, beneficial microbes can activate plant defenses. For example, tomato pretreatment with beneficial fungus (*Trichoderma harzianum*, T22) primed plant responses against aphid infestation by inducing changes in the transcriptome as well as metabolome signatures [21[•]]. Interestingly, the transcriptome data revealed that the defense priming was linked to the upregulation of several genes involved in terpenoid and carotenoid/apocarotenoid biosynthesis [21[•]], which are similar to volatile signals emitted by herbivore-infested plants that can activate indirect defenses [22]. The increased accumulation of these volatile signals in tomato plants pretreated with *Trichoderma* significantly attracted *Aphidius ervi* (parasitic wasp), which are the natural enemies of the attacking aphids [21[•],23]. In addition, *Trichoderma* pretreated tomato plants upregulated several defense-related transcription factors, thus suggesting that beneficial fungus can also activate direct defenses against aphid attack [21[•]].

(b) Metabolomics

Plants produce a wide range of metabolites that can be used for improving crop resistance to various stresses [24]. Progress in developing high-resolution instruments has made it possible for quantification of wide range of metabolites in plant samples using both targeted and non-targeted approaches. In maize (*Zea mays*), benzoxazinoids (BX) act as key defensive secondary metabolites against insect infestation [25]. One of the most abundant BX compounds found in the maize seedlings is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside (DIMBOA-Glc) [25,26]. DIMBOA-Glc and 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), a break-down product of DIMBOA-Glc, can contribute to enhanced resistance to corn leaf aphid (*Rhopalosiphum maidis*) by enhancing callose deposition [25,27], a defense strategy used by plants to unsettle aphid feeding and colonization. Similarly, maize plants that are resistant to bird cherry-oat aphids (*R. padi*), also displayed elevated levels of BX compared to susceptible maize plants [28]. Interestingly, 12-oxo-phytodienoic acid (OPDA), an intermediate in the JA biosynthesis pathway, provided heightened resistance to *R. maidis* by enhancing callose accumulation independent of the JA and BX pathways [29[•]]. In *Arabidopsis thaliana*, *ACTIN-DEPOLYMERIZING FACTOR3* (*ADF3*), which is involved in actin cytoskeleton reorganization, provided enhanced resistance to

Table 1**Comparison of monocot versus dicot plant responses to sap-sucking insect infestation**

Defense metabolite/gene	Plant response		Reference
	Monocot	Dicot	
Absciscic acid (ABA)	Aphid feeding induced several genes involved in ABA signaling	Promoted aphid colonization	[10,67,68]
Actin-binding proteins	–	Provided enhanced resistance to aphids	[30]
Ascorbate peroxidase (Asp)	Aphid feeding induced accumulation of Asp	Aphid feeding induced accumulation of Asp	[69,70]
Benzoxazinoids (BX)/Glucosinolates (GS)	Provided enhanced resistance to aphids (BX)	Provided enhanced resistance to aphids (GS)	[25,27,71,72]
Chitin-related genes	Aphid feeding induced several genes involved in chitin recognition and degradation	Aphid feeding induced several genes involved in chitin regulation	[19 ^{••} ,20]
Ethylene (ET)	Contributed to enhanced resistance in maize against corn leaf aphids, whereas susceptibility in rice against brown planthoppers	Promoted susceptibility to aphids in Arabidopsis	[73–75]
Flavonoids	Provided enhanced resistance to aphids and brown planthoppers	Promoted enhanced resistance to aphids	[32,33,62]
Jasmonic acid (JA)	Aphid feeding induced several genes involved in JA pathway	Promoted enhanced resistance to aphids and whiteflies	[36,76,77]
NBS-LRR genes	Aphid feeding induced several genes that encode NBS-LRR type R protein	R genes provided heightened resistance to aphids and whiteflies	[13–15,20]
Non-protein amino acids	Aphid feeding enhanced accumulation of non-protein amino acids	Provided enhanced resistance to aphids	[78,79]
12-oxo-phytodienoic acid (OPDA)	Heightened resistance to aphids by enhancing callose accumulation	Provided enhanced resistance to aphids	[29 [•] ,80,81]
Pipecolic acid (Pip)	Aphid feeding induced accumulation of Pip	Aphid feeding induced accumulation of Pip	[20,62]
Salicylic acid (SA)	Aphid feeding induced genes involved in SA pathway	Aphid feeding induced genes involved in SA pathway and accumulation of SA	[62,76,77]
Terpenoids	Aphid feeding induced several genes involved in terpenoid biosynthesis pathway	Mealybug feeding induced several genes involved in terpenoid biosynthesis pathway	[9,76]
Trehalose	Aphid feeding induced accumulation of trehalose	Provided enhanced resistance to aphids	[20,82]

green peach aphids (*Myzus persicae*) [30], which feeds on a wide range of host plants. It was suggested that *ADF3* or *ADF3*-dependent mechanism controls the callose deposition in aphid-infested plants, thereby limiting the aphids to feed continuously on the host plant. It is clear that the plants respond to sap-sucking insect infestation by enhancing callose deposition as one of the defense mechanisms, however, based on the research reports there are multiple metabolites or routes involved in activating physical defenses upon insect attack.

Phenylpropanoid metabolites are involved in providing resistance to insect pests [31]. Long-term soybean aphid feeding on soybean plants accumulated several isoflavonoid compounds that may have an effect on aphid feeding or growth [19^{••}]. Interestingly, mass spectrometry imaging revealed that these isoflavones does not accumulate in the vascular tissues, rather these compounds were part of non-phloem defense against aphids, and accumulated likely in the mesophyll cells or

epidermis [19^{••}]. Similarly, corn leaf aphid fecundity was reduced on sorghum plants that accumulated 3-deoxyanthocyanidin flavonoids [32]. Along with spermidine, a polyamine compound, metabolic profiling revealed that the flavonoid quercetin contributed to rice (*Oryza sativa*) resistance to brown plant hopper (*Nilaparvata lugens*), a sap-sucking insect pest, infestation [33]. A recent untargeted metabolomics approach has identified pea aphid biotype-specific resistance on native and non-native host plants [34]. Among the major compounds identified, flavonoids were identified as one of the defense compounds that may be responsible for the differential performance of pea aphid biotypes on native and non-native host plants. These results suggest that phenylpropanoid-derived metabolites contribute to plant resistance to sap-sucking insect pests. However, it remains to be determined what specific metabolite(s) and downstream signaling pathways are required for the induction of effective resistance against plant sap-sucking insects.

(c) Proteomics

In the recent years, researchers have been increasingly using the proteomics approach to understand the plant resistance mechanisms to sap-sucking insect pests. Proteomic studies on rice-small/brown planthopper (*Laodelphax striatellus*) and pepper (*Capsicum annuum*)-whitefly interactions depicted a high degree of overlap on how plants respond to sap-sucking insect infestation [35,36,37^{*}]. These studies revealed that several protein networks involved in redox regulation, stress response, photosynthesis, carbon metabolism, protein metabolism, and lipid metabolism, were differentially upregulated in the respective resistant plants upon sap-sucking insect infestation [35,36,37^{*}]. However, the downstream defenses, as mentioned before, might function through different signaling routes. For example, salicylic acid pathway has been implicated in rice defense against small brown planthopper, whereas, pepper resistance to whitefly was mediated through the JA pathway [35,36]. These few examples indicate that the proteomics approach can be used to understand the plant resistance mechanisms to sap-sucking insect pests; however, the role of specific proteins involved in these interactions are yet to be determined.

(d) QTLomics

QTLomics include the integration of classical QTL mapping and identification/characterization of genes/metabolites/proteins that contribute to underlying quantitative traits. A recent study involving cabbage (*Brassica oleracea*) - cabbage whitefly (*Aleyrodes proletella*) interaction utilized QTLomics to understand the underlying resistance mechanisms in cabbage resistance to cabbage whitefly [38^{**}]. It was observed that the genes related to the phytohormone abscisic acid (ABA) signaling were congruent with whitefly induced ABA levels that contributed to age-dependent cabbage resistance to cabbage whitefly [38^{**}]. Indeed, significant QTL intervals identified on chromosome 2 and 9 for oviposition rate and adult whitefly survival, respectively, correlated with several genes involved in ABA signaling, thus suggesting that ABA could potentially play a significant role in plant defense against cabbage whitefly. Similarly, Genome-Wide Association Studies (GWAS) have been utilized for dissecting the complex traits in plants [39]. For example, GWAS on various soybean accessions identified significant associations between single nucleotide polymorphisms (SNPs) and insect resistance for potato leafhoppers (*Empoasca fabae*) and soybean aphids [40,41]. Interestingly, significant SNPs were found on chromosomes with known *Rag* (*Resistance against Aphis glycines*) genes that confer resistance to soybean aphids [41]. However, some SNPs were also significant on other

chromosomes where *Rag* genes have not yet been mapped, suggesting that other novel locus/genes for soybean aphid resistance are yet to be determined. Kloth *et al.* [42] recently demonstrated that feeding behavior of aphids using automated video tracking [43] in conjunction with GWA mapping identified the small heat shock-like *SIEVE ELEMENT-LINING CHAPERONE1 (SLI1-SLI1)* in Arabidopsis, which restricts aphid feeding from the phloem sap during heat stress. SLI1 is localized in sieve element margins and around mitochondria, thereby limiting phloem sap ingestion of aphids. However, SLI1 was not involved in occluding sieve tubes [42], indicating that the callose accumulation, a hallmark of sieve tube occlusion, is likely triggered by other plant defense mechanisms. Taken together, these studies suggest that combining QTL mapping with ‘omic’ technologies should hasten the discovery of candidate genes involved in plant resistance to insects. Table 2 summarizes the recent studies that have utilized the various ‘omic’ approaches to understand the mechanisms underlying plant-sap-sucking insect interactions.

‘Omic’ approaches in identifying sap-sucking insect effectors

Insect effectors modulate plant defense pathways and/or mechanisms [3,4^{*}]. Although significant progress has been made in genome sequencing of various plant populations, sequencing of various sap-sucking insect pests and comparative genomics have been lagged until lately. Recent advances in genomics technologies are allowing us to increasingly understand the role of sap-sucking insect effectors and their role in modulating plant defenses [reviewed in Refs. 3,4^{*}]. Pea aphid salivary gland transcriptome identified and characterized the first aphid effector, C002, which is required for continuous feeding and colonization by aphids on host plants [44,45]. Subsequently, a functional genomics pipeline was developed to identify and characterize candidate aphid effectors [46–49]. These studies have identified a large repertoire of salivary proteins that are critical in modulating plant defenses. For example, a recent transcriptome analysis of the salivary glands of the grain aphid (*Sitobion avenae*) identified several putative secretory proteins, including calcium-binding proteins [50]. These calcium-binding proteins found in the aphid saliva can unplug the sieve tube occlusion [51], such as the removal of callose deposition as discussed before. Furthermore, genome sequences of several economically important sap-sucking insect pests are now available [52–60], which in combination with RNAi-mediated silencing or CRISPR-Cas9 genome editing tools can help unravel the molecular function of gene(s) that encode the putative salivary proteins. For example, genome-enabled predicted approach identified and characterized the potato aphid (*Macrosiphum euphorbiae*) salivary effector Me47, which is involved in modulating plant responses to aphid feeding [61]. Aphid fecundity was enhanced in plants expressing

Table 2**Omic approaches used to decipher plant defense against sap-sucking insect pests**

Approach	Host	Insect	Tissue	# of insects used for infestation	Duration of experiment	Reference
Transcriptome	Cassava (<i>Manihot esculenta</i>)	Mealybug (<i>Phenacoccus manihoti</i>)	Leaves	15 third stage instar mealybugs	24 and 72 HPI	[9]
Transcriptome	Sorghum (<i>Sorghum bicolor</i>)	Sugarcane aphid (<i>Melanaphis sacchari</i>)	Leaves	15 aphids	24 HPI	[10]
Transcriptome	Sorghum (<i>S. bicolor</i>)	Sugarcane aphid (<i>M. sacchari</i>)	Leaves	5 aphids	5, 10, and 15 DPI	[12*]
Transcriptome	Cotton (<i>Gossypium hirsutum</i>)	Whitefly (<i>Bemisia tabaci</i>)	Leaves	50 adults	0, 12, 24 and 48 HPI	[16]
Transcriptome, Metabolome	Soybean (<i>Glycine max</i>)	Soybean aphid (<i>Aphis glycines</i>)	Leaves	Thirty wingless, mixed age aphids	21 DPI	[19**]
Transcriptome	Switchgrass (<i>Panicum virgatum</i>)	Greenbugs (<i>Schizaphis graminum</i>)	Leaves	10 greenbugs	5, 10, and 15 DPI	[20]
Transcriptome, Metabolome	Tomato (<i>Solanum lycopersicum</i>)	Potato aphid (<i>Macrosiphum euphorbiae</i>) in the presence or absence of <i>Trichoderma harzianum</i> strain T22	Leaves	Five 1-day-old nymphs	48 HPI	[21*]
Transcriptome, Metabolome	Maize (<i>Zea mays</i>)	Bird cherry-oat aphid (<i>Rhopalosiphum padi</i>)	Leaves	50 adult aphids	0, 6 and 24 HPI (transcriptome) 0 and 48 HPI (metabolome)	[28]
Metabolome	Rice (<i>Oryza sativa</i>)	Brown planthopper (<i>Nilaparvata lugens</i>)	Seedlings	10 adults per seedling	0, 24, 48 and 96 HPI	[33]
Metabolome	Alfalfa (<i>Medicago sativa</i>)	Pea aphid (<i>Acyrtosiphon pisum</i>): clone L84 (Medicago race; MR), clone T3-8V1 (Trifolium race; TR), clone Colmar (Pisum race; PR)	Leaves	Five apterous female adults	48 HPI	[34]
	Red clover (<i>Trifolium pratense</i>) Pea (<i>Pisum sativum</i>) Broad bean (<i>Vicia faba</i>)					
Proteome	Rice (<i>O. sativa</i>)	Small brown planthopper (SBPH) (<i>Laodelphax striatellus</i>)	Outmost layers of leaf sheaths	15–20 SBPHs	0, 6, 12, 24, 36, 48, 72, and 96 HPI	[35]
Proteome	Pepper (<i>Capsicum annuum</i>)	Whitefly (<i>B. tabaci</i>)	Leaves	50 adults	48 HPI	[36]
Proteome	Rice (<i>O. sp.</i>)	Brown planthopper (<i>N. lugens</i>)	Stem	—	30 DPI	[37*]
QTL, Transcriptome, Metabolome	White cabbage (<i>Brassica oleracea</i> var. <i>capitata</i>)	Cabbage whitefly (<i>Aleyrodes proletella</i>)	Leaves	20 whitefly female adults of assorted ages	4 HPI	[38**]

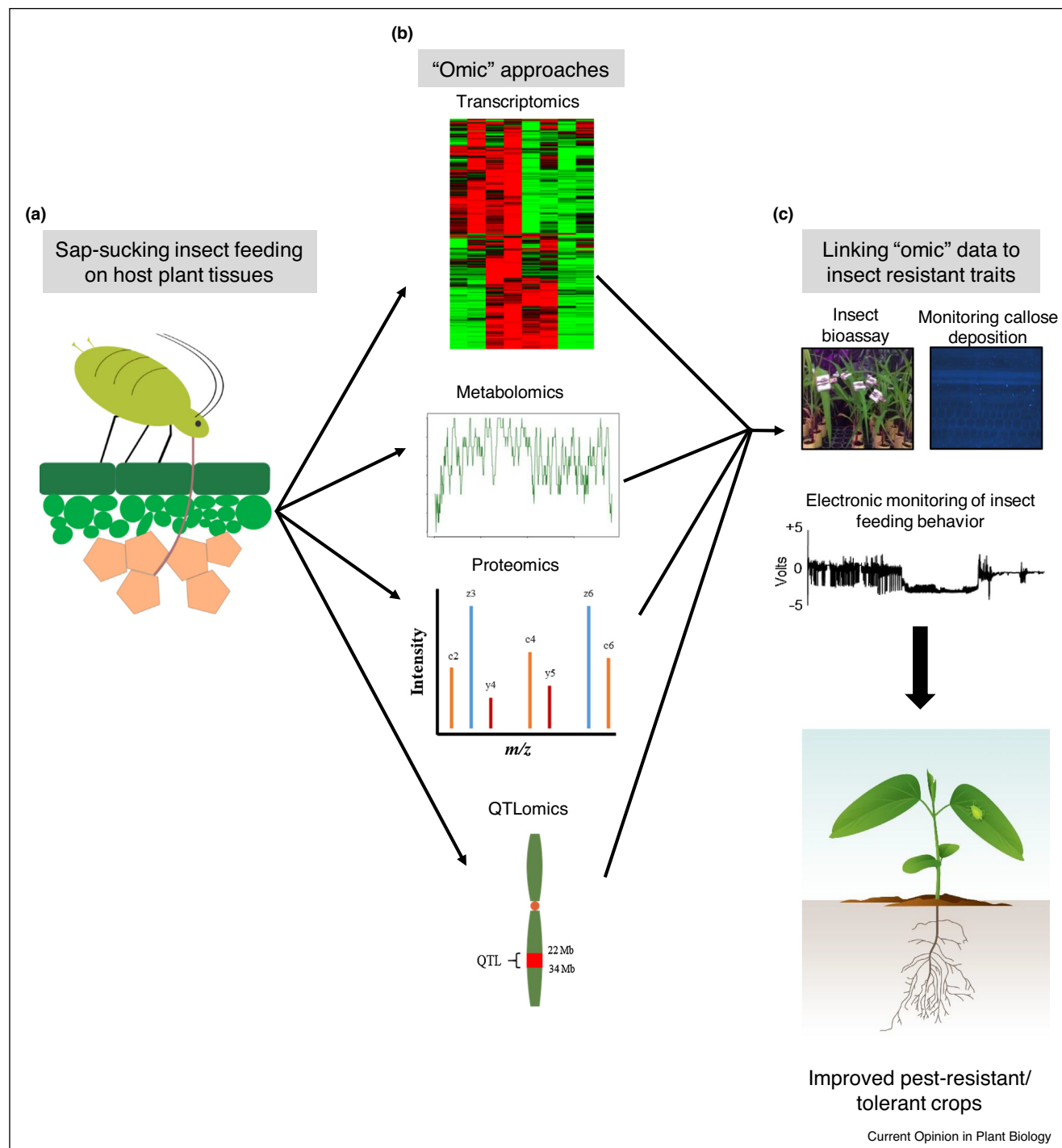
HPI - Hours post infestation; DPI - Days post infestation.

the potato aphid salivary protein Me47 in tobacco (*N. benthamiana*) and tomato, whereas Me47 expression in *Arabidopsis* reduced aphid fecundity [61]. This suggests that the impact of Me47 is dependent on the host plant. Recombinant Me47 has glutathione *S*-transferase activity [61], however, it is not clear how glutathione *S*-transferase activity relates to modifying plant responses to aphid infestation.

Conclusions and future directions

To meet the future agricultural challenges using sustainable technologies, it is imperative that holistic approaches based on detailed understanding of plant defense mechanisms be used to develop crops that can naturally resist herbivore attack. Although significant progress has been made in understanding the plant responses to sap-sucking insect pests, linking resistance responses and phenotypes

Figure 1



Ento(o)mics to understand plant defense against sap-sucking insect pests. **(a)** Illustration depicting sap-sucking insect (e.g. aphid) feeding on the host plant. Aphids and other sap-sucking insects utilize their slender stylets present in their mouthparts to penetrate the plant tissue to consume plant sap. **(b)** Various 'omic' approaches can be utilized to investigate the underlying mechanisms that contribute to plant resistance to sap-sucking insect pests. **(c)** Linking/integrating the 'omic' data to physiological functions and phenotypes in plants is critical to fully understand plant resistance mechanisms against insects. For example, linking 'omic' data to phenotypic traits that contribute to insect resistance, such as monitoring insect performance on host plants (e.g. insect bioassay), monitoring plant's physiological defenses (e.g. callose deposition), electrical monitoring of insect feeding behavior [66] to investigate the details of plant resistance to sap-sucking insects, and so on, will aid us to develop an improved insect resistant/tolerant crop. Images taken by Suresh Varsani and illustration of aphid feeding on plants (in panel (c)) by Nick Sloff.

to genes and metabolic/genetic pathways in plants in response to insect attack is one of the bottlenecks in identifying and characterizing resistant traits. In order to fully understand the complexity of plant-insect interactions, we need to foster interdisciplinary research teams to attain these goals (Figure 1). Consequently, it is critical that several disciplines such as entomology, plant biology, molecular biology, biochemistry, systems biology, statistics, and informatics, should work in tandem to develop novel crop protection strategies. For example, mass spectrometry imaging (MSI) based on matrix-assisted laser desorption ionization (MALDI) can be used to image a wide range of internal leaf metabolites during plant-sap-sucking insect interactions [62]. Klein *et al.* [62] demonstrated the capability to visualize the salicylic acid and isoflavone based resistance during soybean-soybean aphid interaction. MSI-MALDI approach and/or other similar MSI methods can be used as a complement and a powerful tool to identify novel plant metabolites and also monitor how plant metabolites are being altered during plant-sap-sucking insect interactions. Another approach, for example, high-throughput phenotyping [43,63,64,65], which has not been discussed in this review, will also help to determine the crop resistance and physiological responses to insect infestation. Linking genes and pathways to physiological functions and phenotypes in plants is not a trivial task, however, utilizing these transdisciplinary approaches will help us to better understand the complex signaling networks and phenotypic traits at all levels of plant-sap-sucking insect interactions.

Conflict of interest statement

Nothing declared.

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- of outstanding interest

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In this study, a combination of transcriptomic analysis, insect bioassays and electronic monitoring of aphid feeding behavior were utilized to understand the sorghum responses to aphid feeding.

Using mass spectrometry imaging authors show that there are compounds present in the non-phloem tissues that could modulate soybean defenses against soybean aphids.

Authors show that exogenous application of *Trichoderma harzianum* strain T22 on tomato provided enhanced resistance to aphids by inducing both direct and indirect defenses.

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