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REVIEW PAPER

Multidimensional approaches for studying plant defence against insects: from ecology to omics and synthetic biology

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Abstract

The biggest challenge for modern biology is to integrate multidisciplinary approaches towards understanding the organizational and functional complexity of biological systems at different hierarchies, starting from the subcellular molecular mechanisms (microscopic) to the functional interactions of ecological communities (macroscopic). The plant–insect interaction is a good model for this purpose with the availability of an enormous amount of information at the molecular and the ecosystem levels. Changing global climatic conditions are abruptly resetting plant–insect interactions. Integration of discretely located heterogeneous information from the ecosystem to genes and pathways will be an advantage to understand the complexity of plant–insect interactions. This review will present the recent developments in omics-based high-throughput experimental approaches, with particular emphasis on studying plant defence responses against insect attack. The review highlights the importance of using integrative systems approaches to study plant–insect interactions from the macroscopic to the microscopic level. We analyse the current efforts in generating, integrating and modelling multiomics data to understand plant–insect interaction at a systems level. As a future prospect, we highlight the growing interest in utilizing the synthetic biology platform for engineering insect-resistant plants.

Key words: Ecological genomics, plant defence, plant–insect interaction, omics, synthetic biology, systems biology.

Introduction

Plants are sessile organisms and continuously face different harsh environmental conditions as well as biotic invasions in their natural habitat. The interaction of biotic factors with plants can be both beneficial and deleterious, having a multitude of impacts on population dynamics, plant-insect co-evolution, and ecosystem nutrient cycling (Boyer, 1982; Stotz *et al.*, 1999; Lu *et al.*, 2014). Among all the biotic invaders, insects have been recognized to be the most significant herbivores (Crawley, 1989) considering the fact that almost half of the total 6 million insect species are herbivorous. Herbivorous insects have evolved a variety of feeding mechanisms to acquire nutrients from their host plants (Gripenberg *et al.*, 2010). In response to herbivory, plants have developed various morphological, biochemical, and molecular level defence survival strategies (Howe and Jander, 2008; War *et al.*, 2012).

Plants produce various toxins and defence proteins that target physiological processes in the insect (Mithofer and Boland, 2012). The biochemical and molecular mechanisms of defence against the herbivores are diverse, highly dynamic, and are mediated by both direct and indirect defences (Gatehouse, 2002; War *et al.*, 2012).

The partially known or hypothesized molecular mechanisms in higher plants in response to insect attack starts with the recognition of insect oral secretions, recognition of structural components of the insect mouth parts, and signals from injured plant cells (signal input), followed by signal transduction (calcium ion fluxes, phosphorylation cascades, and hormonal cross-talk), signal processing (reprogramming of the transcriptome, proteome, and metabolome), signal responses (production of defence compounds, proteins,

and secondary metabolites), and finally genotypic and/or phenotypic responses (adaptation, selection, and evolution) (De Vos *et al.*, 2005; Eulgem, 2005; Howe and Jander, 2008). Evidently, the plant defence response against insect attack is a multidimensional dynamic process and contains many levels of organizational and functional complexity, executed at different time scales ranging from fractions of seconds to millions of years (Bruce, 2014). Some responses of host plants to different insect herbivores are very general and provide protection against a variety of invaders, whereas others are more specific and target particular types of attackers (Stotz *et al.*, 2000; Kusnierczyk *et al.*, 2007; Ali and Agrawal, 2012; Stam *et al.*, 2014). Different plant species or in fact different ecotypes of the same species may react differently to the same insect species (Kusnierczyk *et al.*, 2007 2008; Moore *et al.*, 2014). Moreover, the response specificity and sensitivity towards diverse stress factors depend on the age and developmental stage of the plant in question. The intensity of responses may vary in time and space, being different in various organs of a plant. A rapidly changing global climate, and elevated CO_2 levels and temperature are altering the naturally co-evolving interactions between plants and insects (DeLucia *et al.*, 2012).

Like other organisms, plants are highly complex systems, composed of densely interconnected elements, arranged in a hierarchical manner from subatomic level to the whole plant and ecosystem level (Fig. 1). The emergent properties of any complex systems may not be understood well by studying the constituting elements separately (Spiertz *et al.*, 2007; Mazzocchi, 2008). Considering a plant as a complex system, spatial and/or temporal dynamics of parameters related to processes such as ecological interaction, photosynthesis, enzymatic reactions, and a broad class of fluxes could be associated with a greater capacity for system homeostasis and successive adaptation (Hammer *et al.*, 2004; Yuan *et al.*, 2008). Nowadays, plant biologists have been using high-throughput omics techniques (e.g. genomic, proteomic, transcriptomic, metabolomic, phenomic, interactomic, ionomic, etc.) extensively in their research on plant–insect interactions (Mochida and Shinozaki, 2011). Each of these omics approaches on its own can provide useful and novel information about plant–insect interactions, but data from several approaches may also be integrated together to facilitate the identification of genetic traits underlying a given phenotype. Utilizing available high-throughput multiomics data along with robust bioinformatics and data mining tools, scientists can now explore relevant correlations and construct mathematical or statistical models describing different biological processes related to plant–insect interaction (Fukushima *et al.*, 2009). Based on such predicted models from high-throughput omics data, new biological hypotheses can be generated, tested, rectified, and further be used for synthetic engineering of insect-resistant crop plants (Bowen *et al.*, 2008; Purnick and Weiss, 2009; Birkett and Pickett, 2014; O'Connor and Brutnell, 2014). Knowledge generated from such integrative plant–insect interaction studies can also be used for integrated pest management (Ahuja *et al.*, 2011)

and for developing plant-based insect repellents or eco-friendly green pesticides (Maia and Moore, 2011).

As described above, the field of plant–insect interaction is comprised of researchers with diversified research interests and they employ multidisciplinary approaches. This review aims at a wider community to summarize briefly contemporary research works conducted using the various omics techniques and integrated methods in the field of plant–insect interaction primarily focusing on the aspect of plant defence against insects. We will analyse the technological advancements, current limitations, and future prospects of the field such as multiomics data integration, integrative modelling, and synthetic biology-based approaches.

Dynamic multiple herbivory studies are more important than static pairwise interaction studies

There has been a significant amount of research carried out to study the effect of pairwise interactions between one insect and one plant species at a time (Kessler and Baldwin, 2002; Schoonhoven *et al.*, 2005; Kusnierczyk *et al.*, 2007 2008). In a natural environment, plants are exposed to combinations of herbivores simultaneously, rather than in a pairwise pattern (Poelman *et al.*, 2008; Heidel-Fischer *et al.*, 2014). Host plant defence is influenced by a complex web of interactions with other insects and biotic or abiotic factors that co-exist in their respective environment (Ohgushi, 2005; Schenk *et al.*, 2012; Rasmussen *et al.*, 2013). Interaction effects of combinations of biotic or abiotic stresses are always severe to plants (Mittler, 2006; De Vos *et al.*, 2007; Heidel-Fischer *et al.*, 2014). Surprisingly, very few studies have been conducted to date to study the responses of plants to a combination of different herbivores, and these studies reported that the responses of plants to a combination attack were unique and could not be directly inferred from the response of plants to each individual attack (Pieterse and Dicke, 2007; Dicke *et al.*, 2009; Utsumi *et al.*, 2010). The cumulative effect of a putative defence response may be under- or overestimated when other interactions are not considered (Gols, 2014). Attack by different herbivores has dynamic and differential impacts on different levels of the biological organization, ranging from different trophic levels, visible phenotypic traits, to the molecular level (Heil, 2008; Mooney *et al.*, 2012). Also insect-associated organisms (e.g. parasites or endosymbionts) can affect plant–insect interactions, and the reader is referred to excellent recent reviews on this topic by Douglas (2013) and Zhu *et al.* (2014). In recent years, there has been a growing interest among researchers towards such multifactorial interaction studies using integrative approaches to explore plant–insect interactions (Baldwin *et al.*, 2001; Keurentjes *et al.*, 2011; Baldwin, 2012; Stam *et al.*, 2014). Such interests are giving rise to new disciplines such as ecological genomics, which is gaining popularity in studying herbivore-induced plant defence mechanisms (Zheng and Dicke, 2008; Bidart-Bouzat and Kliebenstein, 2011; Mathur *et al.*, 2013).
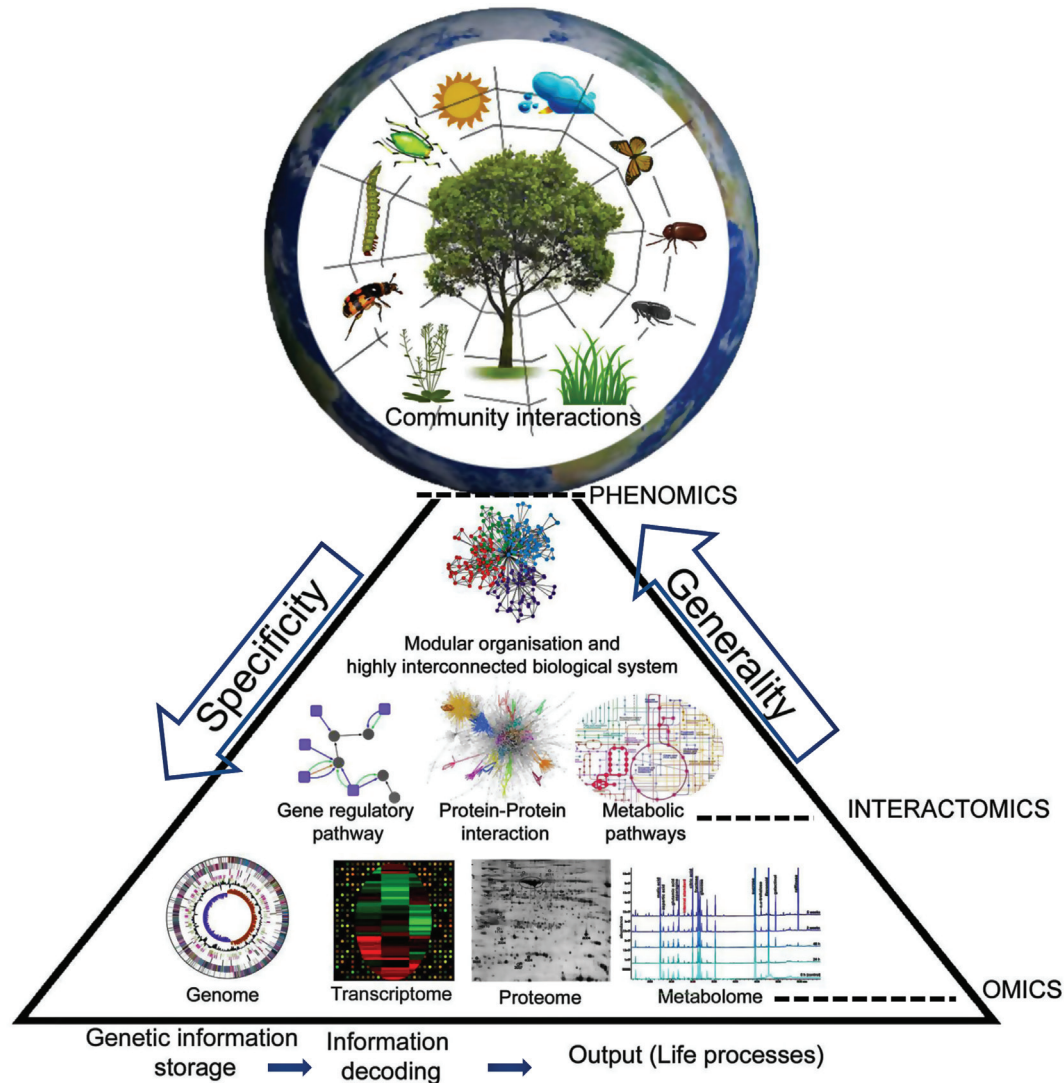
Fig. 1. Macroscopic to microscopic level complexity of plant–insect interaction. The highly interconnected hierarchical organization and functional complexity need a sophisticated integrated systems approach.

Transcriptomic-based studies

One of the globally measurable effects of plant responses to herbivores are the changes in the levels of gene expression (Reymond *et al.*, 2000). Since the development of a first microarray chip detecting 45 transcripts of *Arabidopsis thaliana* (Schena *et al.*, 1995) up to full transcriptome microarrays and RNA sequencing (RNA-seq) (Gan *et al.*, 2011), the field of transcriptomics has gone through revolutionary changes and is currently considered as a major omics technique for studying plant responses to insect attack (Thompson and Goggin, 2006; Heidel-Fischer *et al.*, 2014). An oversimplified search in the GEO database (Edgar *et al.*, 2002) with the term 'herbivory' returned >300 gene expression data sets deposited since June 2014. These data sets were generated using diverse groups of plant species, for example *Saccharum* sp., *Oryza* sp., *Zea* sp., *Nicotiana* sp., *Solanum* sp., *Arabidopsis* sp., *Populus* sp., and *Picea* sp. A large number of transcriptomics experiments conducted to assess plant responses to different insects have made substantial contributions to our

understanding of the herbivore-induced defences of plants (Moran *et al.*, 2002; Reymond *et al.*, 2004; Barah *et al.*, 2013). Comparative transcriptomics can be used to identify common and attacker-specific gene expression patterns against multiple types of insect attack (Kusnierczyk *et al.*, 2007; Ali and Agrawal, 2012; Dubey *et al.*, 2013). However, the comparison of independent gene expression experiments and extraction of meaningful information from such comparisons is complicated and difficult in most cases, mainly due to the lack of common standards regarding plant cultivation, plant infestation, sampling stages, expression profile experiment platforms, and methods for evaluation of the resulting gene expression data (Finotello and Di Camillo, 2014; Kratz and Carninci, 2014). It needs robust and statistically powerful normalization and computational methods to tackle the issues of heterogeneity and experimental noise (Jorstad *et al.*, 2007; Klebanov *et al.*, 2007; Nuzzo, 2014).

A plant defence response is a highly dynamic process and the defence transcriptome is differentially regulated in time and space. From several microarray experiments conducted

to uncover transcriptional response patterns to different abiotic and biotic stresses, it is now understood that the transcriptional response initially is composed of a core set of genes responsive to multiple stresses, but becomes gradually more stress specific (De Vos *et al.*, 2005; Eulgem, 2005; Gulati *et al.*, 2013). The chronology and co-ordinated regulation of such a defence transcriptome can be deciphered by employing high-resolution temporal transcriptomic analysis as demonstrated by Windram *et al.* (2012), generating data from 24 time points using 2 h intervals over a 48 h period (Windram *et al.*, 2012). In addition, rapidly developing cost-effective next-generation sequencing (NGS)-based technologies may now be applied to carry out transcriptomic analysis (also known as RNA-seq) even in non-model plant and insect species (Strickler *et al.*, 2012). A few such examples of NGS-based methods for studying plant–insect interaction are given in the following subsections.

Comparative metatranscriptomics to study cross-kingdom cross-talk during plant–insect interactions

Invasion of plants by a combination of different insect species may trigger agonistic, antagonistic, or potentially unrelated defence responses (Gols, 2014), as also seen in other combined stress situations (Rasmussen *et al.*, 2013). Transcriptomic studies provided abundant information about cross-talk of different phytohormone pathways such as salicylic acid, jasmonate, and ethylene pathways, by interacting either positively or negatively for the fine-tuning of the production of a specific blend of the alarm signals responding to different types of attacks (Koornneef and Pieterse, 2008). Interestingly, there is also evidence of cross-kingdom cross-talk by hormones shared by both plants and their insect herbivores (Schultz and Appel, 2004). Understanding the co-evolution of plant–insect interactions can be enhanced by studying the commonalities, particularly common signals, rather than their differences. The comparative metatranscriptomic approach has been well utilized to study plant–microbe interactions and can further be used towards identifying potential shared components during plant–insect interactions by utilizing samples directly from the field and from controlled-environment conditions (Damon *et al.*, 2012; Knief, 2014). For example, a large number of pathogenic, symbiotic, and free-living organisms unrelated to the soybean genome were identified by deep sequencing of soybean small RNA libraries (Molina *et al.*, 2012). Comparative metatranscriptomic signatures of wood and paper feeding in the gut of the termite *Reticulitermes flavipes* were analysed to understand termite nutritional symbiosis and to identify potential genes as targets for termite control (Raychoudhury *et al.*, 2013).

NGS technologies: aiming for 5000 insect genomes and 1000 plant transcriptomes

NGS is a high-throughput technique to generate millions of sequences in parallel within a short span of time. Several NGS technology platforms have been developed during the last decade (Mardis, 2013). The rapid evolution and declining costs of such NGS technologies facilitate the large-scale analysis of various model and non-model organisms, including several plants and insects (Weber *et al.*, 2007; Egan *et al.*, 2012; Zhang and Yuan, 2013). The recently started 5000 insect genome (i5k) project (Robinson *et al.*, 2011) and the 1000 plant transcriptome (oneKP or 1KP) project (www.onekp.com/) may greatly contribute to the understanding of plant–insect co-evolution in coming days. The 'i5k' project takes the initiative to sequence the genomes of 5000 species of insects and other arthropods that comprise those known to be important to worldwide agriculture and food safety, medicine, and energy production; all species used as models in biology; the most abundant insects in world ecosystems; and representatives of insect relatives in every major branch of arthropod phylogeny in order to achieve an in-depth understanding of arthropod evolution. The '1KP' initiative is an international multidisciplinary consortium generating large-scale gene sequencing data for >1000 species of plants (Johnson *et al.*, 2012). Instead of generating ESTs (expressed sequence tags), the '1KP' project opts to reconstruct full-length transcripts by computational assembly of the produced shotgun libraries to generate 2 Gb of raw sequence per species. In due course, the generated data from these two ambitious projects may make the large-scale comparison of herbivore and plant genomes to reconstruct the co-evolutionary history possible.

Successful use of NGS technology to develop transcriptomic and genomic resources, including expressed genes and molecular markers for a non-model invasive aphid species *Aphis glycines*, was demonstrated by Bai *et al.* (2010). Studying the dynamic behaviour of food webs is essential for understanding the mechanistic processes behind community ecology and functional organization of the ecosystem. NGS-based approaches have been used for the identification of plant species consumed by herbivores through the characterization of DNA present in gut or faecal samples of the insects (Pompanon *et al.*, 2012). NGS-based DNA metabarcoding approaches allow the direct characterization of dozens of samples with several thousand sequences per PCR product, and has the potential to reveal the diversity of consumed plant species by insects simultaneously (Valentini *et al.*, 2009). Gase and Baldwin have demonstrated NGS to be a valuable tool to identify genetic loci for ecologically relevant traits to study transcriptomic changes in the non-model plant *Nicotiana attenuata* elicited by abiotic factors, as well as 34 different herbivore taxa and innumerable pathogens (Gase and Baldwin, 2012). Later they considered 'reverse genetic' approaches to produce genotypes that could be put out into the wild to determine the ecological importance of particular genes.

Proteomics

Developments in modern proteomic technologies have allowed for an efficient and simultaneous detection of a large number of proteins in plant samples (Vanderschuren *et al.*, 2013). High-throughput quantitative proteomics studies have gained substantive importance in plant research during the last few years to characterize proteomes and their differential

modulation during plant development, and biotic and abiotic stresses. A proteomic study was conducted to investigate physiological factors affecting feeding behaviour by larvae of the insect *Plutella xylostella* on herbivore-susceptible and herbivore-resistant *A. thaliana* recombinant inbred lines (RILs) (Collins *et al.*, 2010). The results revealed significant differences in the proteomes between the identified resistant and susceptible RILs. Proteomics studies showed that many proteins responsive to the insect attack were associated with the tricarboxylic acid (TCA) cycle and also involved in carbon metabolism, which concluded the alteration in carbon metabolism for defence during insect attack. High abundance of proteins such as ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) causes great difficulty for the whole-proteome characterization using shotgun plant proteomics. An improved proteomic method called Polyethyleneimine Assisted Rubisco Cleanup (PARC) was used to study the mechanisms of defence during plant–insect interactions (Y. Zhang *et al.*, 2013). A comparative proteomic analysis of the plant's response to wounding and herbivory was carried out, using potato plants (*Solanum tuberosum* L.) subjected to mechanical wounding, defoliation by the Colorado potato beetle *Leptinotarsa decemlineata*, or phloem sap feeding by the potato aphid (*Macrosiphum euphorbiae*). Nearly 500 leaf proteins were monitored by two-dimensional gel electrophoresis (2-DE) to illustrate the differential impacts of wounding and insect herbivory on defence- and photosynthesis-related components of the potato leaf proteome (Duceppe *et al.*, 2012). In another study, 2D-DIGE (difference gel electrophoresis) coupled with protein identification by MALDI-TOF-MS (matrix-assisted laser deionization-time of flight-mass spectrometry) was used to compare the proteome patterns during tritrophic interactions among avirulent and semi-virulent potato aphids and their bacterial endosymbionts on resistant and susceptible tomato lines (Francis *et al.*, 2010). This study concluded that there was a potential contribution of the symbionts to differential adaptation of aphids to host plant resistance. Differential protein expression in maize (*Zea mays*) in response to infestation with a chewing (*Spodoptera littoralis*) and a boring insect (*Busseola fusca*) attack was reported by George *et al.* (2011). Stability of plant defence proteins in the gut of insect herbivores was studied in tomato plants with a specialist (*Manduca sexta*) and generalist (*Trichoplusia ni*) using shotgun proteomics of midgut and faeces (frass) samples to identify hyperstable plant proteins that served important roles in defence (Chen *et al.*, 2007). Proteomic analysis was used in rice (*Oryza sativa* L.) mutants to identify differentially induced proteins during infestation by brown planthopper (*Nilaparvata lugens*) (Sangha *et al.*, 2013). The proteomic approach has also been used in large tree species such as Sitka spruce (*Picea sitchensis*) to study defence responses induced by mechanical wounding or feeding by white pine weevils (*Pissodes strobi*) (Lippert *et al.*, 2007).

Plant and insect secretomics

Insect oral secretions (Consales *et al.*, 2012) as well as secretions from the plant cell wall (Krause *et al.*, 2013) play a crucial role during plant–insect interactions. Proteomics has also been a major tool towards isolating, identifying, and exploring the biological significance of the plant secretome or the plant cell wall proteome during pathogen and insect attack (Isaacson and Rose, 2008; Agrawal *et al.*, 2010; Tanveer *et al.*, 2014). Similar proteomic approaches have also been used to perform comparative analyses of salivary secretion from different aphid species to reveal how aphids target plant processes and how the aphid and host plant interact (Vandermoten *et al.*, 2014).

Metabolomics

The evolution of the chemical defence system against herbivory in plants is connected to the plant-made chemical compounds that are not involved in the photosynthetic and central metabolic processes (Mithofer and Boland, 2012; Turley *et al.*, 2013). These compounds are known as secondary metabolites with no major involvement in the normal growth, development, or reproduction of the plant. Such compounds can act as signalling molecules (Zebelo and Maffei, 2012) or direct defence chemicals, and include alkaloids, terpenoids, cyanogenic glycosides, glucosinolates, and phenolics (Bennett and Wallsgrove, 1994). A dedicated field called 'chemical ecology' has evolved to study the chemicals involved in the interactions of living organisms, including the chemical defence system during plant–insect interaction (Feeny, 1992). While activating the defence response system mediated by the secondary metabolites against insect attack, plants have to compromise some of the central metabolism by allocating resources for this defence. Along with secondary metabolism, a plant's primary metabolism is also differentially affected during an insect or a pathogen attack (Barah *et al.*, 2013). Studying the differential regulation of primary or secondary metabolites during plant–insect interaction has been in practice for the last decades (Weckwerth and Kahl, 2013). We illustrate this with a few recent representative studies below. Metabolite profiling of *Senecio* hybrids exposed to the thrips *Frankliniella occidentalis* revealed that hybrids which were more resistant to the thrips accumulated higher amounts of some alkaloids and flavonoids than susceptible hybrids (Leiss *et al.*, 2009a). In chrysanthemum, the metabolome of cultivars that are resistant to the same thrips can be differentiated from that of susceptible cultivars by higher amounts of two phenylpropanoids (Leiss *et al.*, 2009b). In another study taking advantage of natural variation in insect resistance, metabolomics of *Barbarea vulgaris* plants from a population ranging from susceptible to resistant to the flea beetle *Phyllotreta nemorum* showed that the concentrations of two known saponins and two novel compounds likely to be saponins correlated positively with insect resistance (Kuzina *et al.*, 2009). In order to monitor herbivore-induced metabolite changes in local and systemic tissue, maize leaves, roots, root exudate, and vascular sap from stems were submitted to metabolite profiling after leaf infestation with *S. littoralis*. While 32 compounds were identified that showed changed concentration levels in leaves upon insect attack, no changes

in the abundance of secondary metabolites were detected in roots (Marti *et al.*, 2013). In another whole-plant metabolomics study, profiling of undamaged leaf, apex, stem, and root tissue from tomato after infestation with one of two insect herbivores (*M. sexta* or *Helicoverpa zea*) revealed quantitative and qualitative differences in metabolite changes among tissues and between insects, although the biological implications of these differences were not further tested (Steinbrenner *et al.*, 2011). Jansen *et al.* (2009) monitored metabolic changes not just in plant tissues but also in the insect in the *Brassica oleracea–Pieris rapae* interaction, identifying phenylpropanoids that were induced in plant tissue and present in the insect upon feeding (Jansen *et al.*, 2009). Hence, metabolomics analyses can provide valuable information about plant defence to insects on its own (e.g. by identifying new interesting compounds, by looking at local and systemic changes) but may also be combined (as described later) with other omics approaches in an attempt to link phenotype and genotype (Macel *et al.*, 2010). Particular attention in metabolite profiling of plant–insect interactions has been given to the analysis of volatile organic compounds (VOCs), whether constitutively emitted compounds or herbivore-induced plant volatiles (HIPVs), and the characterization of their roles in direct and indirect defence against herbivores and plant to plant signalling (Dicke and Baldwin, 2010; Holopainen and Gershenzon, 2010; Holopainen and Blande, 2013). Possible intraspecific variation of metabolite composition (Keurentjes *et al.*, 2006), the effect of growth conditions on HIPVs (Gouinguene and Turlings, 2002), and the difficulty in analysing plant volatiles under natural conditions (Kallenbach *et al.*, 2014) are adding to the complexity when studying a given plant–insect interaction.

Recent developments in high-throughput metabolite profiling methods and advanced combinatorial protocols such as liquid chromatography–mass spectrometry (LC-MS), gas chromatography–mass spectrometry (GC-MS), ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS), Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), nuclear magnetic resonance (NMR), and flow-injection electrospray ionization mass spectrometry (FI-ESI-MS) have, however, accelerated the field of plant metabolomics (Weckwerth and Kahl, 2013; Foito, 2014). However, analysing the enormous diversity of plant metabolites generated using these methods is computationally challenging (Allwood *et al.*, 2008; Ernst *et al.*, 2014). The role of bioinformatics is very crucial in analysing and integrating highly complex biological data.

Integrative omics studies and role of bioinformatics

The highly complex hierarchical organization of plant systems and the importance of an integrative systems approach to study plant–insect interaction has been mentioned at the beginning of this review. In a recent integrative study to analyse herbivore-induced changes in plants, combinations of genome-wide gene expression, defence-related pathways,

and secondary metabolite profiles were monitored. The results showed that plant responses were not influenced by the degree of specialization of insect herbivores, but were more strongly regulated by their different feeding modes (Bidart-Bouzat and Kliebenstein, 2011). Integrated NGS-based transcriptomics and metabolomics studies have been used in non-model large trees to decipher differences in the resistance of pedunculate oak to the herbivore *Tortrix viridan* (Kersten *et al.*, 2013). The study also found that the resistant oak type seemed to prefer a strategy of constitutive defence responses, in contrast to more induced defence responses of the susceptible oaks triggered by feeding. Thompson and Goggin (2006) have reviewed integrative approaches used by the combination of transcriptomics and functional genomics to study plant defence induction by phloem-feeding insects. Combined analysis of the transcriptome and metabolome in tobacco plants during the attack by two insect pests *Spodoptera litura* and *Helicoverpa armigera* suggested that transcription factor MYB12-expressing plants showed changes in gene expression profiles and metabolites, and were more resistant to these two insects (Misra *et al.*, 2010). To obtain a comprehensive picture of the role of roots in defence–tolerance trade-offs during shoot herbivory, Gulati *et al.* (2014) have used multivariate time-series data to evaluate leaf herbivory-elicited transcriptional and metabolic dynamics in the roots of *N. attenuata*. Combination of proteomics and transcriptome sequencing was able to identify active plant cell wall-degrading enzymes in a leaf beetle *Phaedon cochleariae*, which helped the highly successful herbivores to overcome the defence mechanism of their host plants (Kirsch *et al.*, 2012). Parallel proteomic and metabolomic analyses were used to investigate larval haemolymph associated with diapause in the insect cotton bollworm, *H. armigera* (Q. Zhang *et al.*, 2013). Defence responses during citrus greening disease or HLB (Citrus Huanglongbing) in citrus plants were analysed by comparative iTRAQ proteome and transcriptome techniques (Fan *et al.*, 2011). Combined transcriptomic and proteomic analysis of a compatible tomato–aphid interaction revealed a predominant salicylic acid-dependent plant response to the *Macrosyphum euphorbiae* aphid (Coppola *et al.*, 2013). The molecular changes occurring in *N. attenuata* when attacked by the tobacco hornworm *M. sexta* were extensively studied at the transcriptome, proteome, and metabolome level by Baldwin's lab (Hui *et al.*, 2003; Giri *et al.*, 2006; Gaquerel *et al.*, 2009).

With the developments in high-throughput phenotyping methods and multidimensional genomic information, genome-wide association mapping is rapidly becoming an important approach to explore the genetic architecture of complex traits in plants for studying plant resistance to insect herbivores (Atwell *et al.*, 2010; Kloth *et al.*, 2012). Chan *et al.* (2011) combined genome-wide association mapping and transcriptional networks to identify novel genes controlling glucosinolates in *A. thaliana* (Chan *et al.*, 2011). Enzymatic and regulatory genes related to the plant defence metabolism were identified through quantitative trait locus (QTL) mapping in *A. thaliana* (Brotman *et al.*, 2011).

Bioinformatics tools for multiomics data integration

Increasingly available high-throughput multiomics data and public repositories are opening up the possibilities for comparative *in silico* analysis to understand plant–insect interaction in multiple non-model insects and plant species (Fukushima *et al.*, 2009). The biggest challenges are involved in integrating the heterogeneous omics information to produce biologically meaningful hypotheses (Keurentjes *et al.*, 2011). Few user-friendly computational platforms are developed for the integration of multiomics data. Babelomics is an example of a computational platform for the integrative analysis of transcriptomic, proteomic, and genomic data with advanced functional profiling (Medina *et al.*, 2010). TraitCapture is a platform for genomic and environment modelling of plant phenomic data (Brown *et al.*, 2014). Plant volatile analysis needs intensive bioinformatics expertise for detecting signal from noise in increasingly complex profiles (van Dam and Poppy, 2008). PlantMetabolomics. org (PM) is a user-friendly platform for exploring, visualizing, and downloading plant metabolomics data (Bais *et al.*, 2010). PLAZA is an online resource for plant genomics that has been developed to integrate and distribute comparative genomics data for both computational and experimental plant biologists (Van Bel *et al.*, 2012). VirtualPlant integrates genomic data and provides visualization and analysis tools for rapid and efficient exploration of genomic data (Katari *et al.*, 2010). Gulati *et al.* (2013) have described an integrative statistical method to explore herbivory-specific responses in plants through the construction of interactive motifs by combining an extended self-organizing maps-(SOM) based dimensionality reduction method with bootstrap-based non-parametric analysis of variance (ANOVA) models.

Useful repositories

Universal sharing of available data and knowledge is a key focus of modern biology. Independent databases containing genetic information for different plants and insects are being developed (Clement and Quisenberry, 1998). A search for instances of genome projects using the term 'Viridiplantae' in NCBI (http://www.ncbi.nlm.nih.gov/genome/?term=Viridiplantae) returns 139 records of plant genome projects and their related information. A similar search using the term 'Insecta' (http://www.ncbi.nlm.nih.gov/genome/?term=insecta) returns 112 matches for various insect genome projects and their related information (as of October 2014). Whole-genome sequence data have become the primary set of resources for designing microarrays, tilling arrays, or molecular markers, and also an important reference for integrating other omics-derived information with genome sequences. Several comparative genomics projects among green plants such as Phytozome (Goodstein *et al.*, 2012) (http://www.phytozome.net/) and Gramene (Monaco *et al.*, 2014) (http://www.gramene.org/) have proven to be successful to grasp the biological properties of each plant species and to accelerate gene discovery and the functional analyses

of biologically important genes in plants. A few insect-related repositories are presented in Table 1.

Despite the availability of separate resources for plants and insects, combined repositories containing information about plant–herbivore interactions are rare. The 'Interaction Web Database' currently contains published data sets on species interactions from several communities in different parts of the world. It currently contains data for a variety of interaction types, including plant–pollinator, plant–frugivore, plant–herbivore, plant–ant mutualist, and predator–prey interactions (https://www.nceas.ucsb.edu/interactionweb/resources.html). The 'BRC–Database of Insects and their Food Plants' contains 47 000 interactions for ~9300 invertebrate taxa (insects and mites) and their host plants (http://www.brc.ac.uk/dbif/homepage.aspx). HOSTS is a database of the world's lepidopteran host plants which contains 180 000 records comprising taxonomically 'cleaned' host plant data for ~22 000 Lepidoptera species drawn from ~1600 published and manuscript sources (Robinson *et al.*, 2010).

From ecological studies to genes, metabolites, and pathways

Ecologists have a long-lasting interest in studying population dynamics models of plant–insect interaction (Varley *et al.*, 1974; Schowalter, 2006). Ecologists are mainly interested in issues related to insect pest control and conservation of endangered species, and study the effects of various types of disturbances (introduction of natural enemies, vegetation diversity, and pesticides) on insect populations etc. (Tonhasca and Byrne, 1994; Adams *et al.*, 2005; Bezemer *et al.*, 2014). Such studies mainly use field-generated ecological and behavioural data to be analysed with diverse mathematical modelling approaches such as multivariate statistical techniques, ordinary least squares techniques (Marsolan and Rudd, 1976; Adams *et al.*, 2005), etc. With the availability of omics data, interest has been growing in merging molecular and ecological approaches together to generate a systems-level understanding of plant–insect interaction from the community to the gene level (Baldwin *et al.*, 2001; Stam *et al.*, 2014). The increased use of meta-analysis in plant ecology over the last two decades (Gomez-Aparicio and Lortie, 2014) has proven the usefulness of integrated approaches to study contemporary topics in different areas of plant ecology from population level to single genes and interacting pathways (Ernst *et al.*, 2014). Considering the consequences of a changing global climate on plant–insect interactions (DeLucia *et al.*, 2012), integrated field experiments and corresponding meta-analyses have been carried out to examine the effects of elevated carbon dioxide (CO_2) levels on plant–herbivore interactions (Stiling and Cornelissen, 2007). The results from this study showed that elevated CO_2 significantly decreased herbivore abundance, increased relative consumption rates, development time, and total consumption, and significantly decreased relative growth rate, conversion efficiency, and pupal weight. Elevated CO_2 alters the entire defence chemistry and signalling mechanism of plants to affect the ecological

Table 1. *A list of insect-related databases*

Database	Description	URL
DBM-DB (Tang *et al.*, 2014)	The diamondback moth genome database	http://iae.fafu.edu.cn/DBM/
BmTEdb (Xu *et al.*, 2013)	A collective database of transposable elements in the silkworm genome	http://gene.cqu.edu.cn/BmTEdb/
KONAGAbase (Jouraku *et al.*, 2013)	A genomic and transcriptomic database for the diamondback moth, *Plutella xylostella*	http://dbm.dna.affrc.go.jp/px/
Insect Innate Immunity Database (Brucker *et al.*, 2012)	An annotation tool for identifying immune genes in insect genomes	http://bordensteinlab.vanderbilt.edu/IIID/test_immunity.php
ELM Pave Database (Buchel *et al.*, 2012)	EST database for identifying leaf beetle egg-induced defence genes	http://www.agcol.arizona.edu/pave/elm/
Hymenoptera Genome Database (Munoz-Torres *et al.*, 2011)	Integrated community resources for insect species of the order Hymenoptera	http://hymenopteragenome.org/
KAIKObase (Shimomura *et al.*, 2009)	An integrated silkworm genome database and data mining tool	http://sgp.dna.affrc.go.jp/KAIKObase/
Fourmidable (Wurm *et al.*, 2009)	A database for ant genomics	http://antgenomes.org/
Dung beetle database |(Khanyile *et al.*, 2008)	Comparison with other invertebrate transcriptomes	http://flylab.wits.ac.za/EI/est2uni/home.php
WildSilkbase (Arunkumar *et al.*, 2008)	An EST database of wild silkmoths	http://www.cdfd.org.in/wildsilkbase/home.php
AphidBase (Gauthier *et al.*, 2007)	A database for aphid genomic resources	http://w3.rennes.inra.fr/AphidBase/
DroSpeGe (Gilbert, 2007)	Rapid access database for new *Drosophila* species genomes	http://insects.eugenes.org/DroSpeGe/
InSatDb (Archak *et al.*, 2007)	A microsatellite database of fully sequenced insect genomes	http://cdfd.org.in/INSATDB/home.php
SPODOBASE (Negre *et al.*, 2006)	An EST database for the lepidopteran crop pest *Spodoptera*	http://bioweb.ensam.inra.fr/spodobase/
LocustDB (Ma *et al.*, 2006)	A relational database for the transcriptome and biology of the migratory locust (*Locusta migratoria*).	http://locustdb.genomics.org.cn/
cuticleDB (Magkrioti *et al.*, 2004)	A relational database of Arthropod cuticular proteins	http://bioinformatics.biol.uoa.gr/cuticleDB/

and evolutionary responses of plants to insect attack (Zavala *et al.*, 2013).

Signalling pathways enable plant cells to sense the changes in their environment, to integrate external or internal signals, and to respond to them by changing transcriptional activity, metabolism, or other regulatory measures (Mulligan *et al.*, 1997; Hetherington and Bardwell, 2011). Plants recognize herbivorous insects by complex signalling networks (Bonaventure, 2014). Dynamic modelling and simulation of signal transduction pathways is an important topic in systems biology (Klipp and Liebermeister, 2006). Such modelling approaches can be used to understand response specificity in plant–insect interactions and to identify new signalling molecules necessary for fine-tuning plant defence signalling (Beckers and Spoel, 2006; Erb *et al.*, 2009). Similarly, differential gene regulatory networks (Krouk *et al.*, 2013) and metabolic flux networks (Zebelo and Maffei, 2012) can be used for generating global pictures of plant defence mechanisms during insect attack. Such systems biology-based integrative approaches may be helpful in minimizing the gap between community- and gene-level knowledge of plant–insect interactions by correlating genotype to phenotype (Benfey and Mitchell-Olds, 2008).

A comprehensive understanding of molecular processes alone is not sufficient to test the paradigm of ecological fitness of a plant species, because fitness can also be influenced by external processes (Baldwin, 2001). The interest in linking field-based community studies to genes, pathways, and fitness traits has been growing significantly (Pierik *et al.*, 2014; Stam *et al.*, 2014). That the plant genotype/phenotype affects the severity of herbivory and even herbivore community composition has been shown in many plant–insect interaction studies (for a review, see Stam *et al.*, 2014), although the underlying traits for this effect have not always been identified. Poelman *et al.* (2009) observed, for example, that in *Brassica oleracea* cultivars herbivore abundance and species richness were negatively correlated with the concentration of glucoiberin, a compound belonging to the glucosinolate family of secondary metabolites (Poelman *et al.*, 2009). In *Boechera stricta*, a QTL contributing to the resistance to the generalist cabbage

looper (*Trichoplusia ni*) was also found to affect the glucosinolate profile, consisting in this case of the ratio between glucosinolates derived from branched-chain amino acids and those derived from methionine (Schranz *et al.*, 2009). The underlying genetic trait was later identified as variations in cytochrome P450s leading to novel enzyme function in the biosynthesis of valine- and isoleucine-derived glucosinolates (Prasad *et al.*, 2012). In maize, a QTL for natural variation in resistance to the maize leaf aphid (*Rhopalosiphum maidis*) was associated with an *O*-methyltransferase activity involved in the biosynthesis of the plant defence-related secondary metabolites benzoxazinoids. Maize lines showing higher susceptibility to the aphid contained increased levels of HDMBOA-Glc, by methylation of DIMBOA-Glc, and reduced callose deposition (Meihls *et al.*, 2013). Fine *et al.* (2013) have studied insect herbivore-mediated chemical innovation, and the evolution of habitat specialization in two native ecotypes of Amazonian trees *Protium subserratum* (Burseraceae) (Fine *et al.*, 2013). In a study to explore how natural enemies drive geographic variation in plant defences, Zust *et al.* (2012) have used 39 years of field data on *A. thaliana*, coupled to aphid abundance, to demonstrate that geographic patterns in a polymorphic defence locus (GS-ELONG) were strongly correlated with changes in the relative abundance of two specialist aphids (Zust *et al.*, 2012). Using a multigeneration selection experiment, they later demonstrated the differential selection by the two aphids on the polymorphic defence locus.

Electrical penetration graph (EPG) for studying behaviour to physiological aspects of plant–insect interaction

After discussing many of the modern high-throughput technologies, let us go back to the electrophysiological monitoring of plant–insect interactions using the electrical penetration graph (EPG). The history of EPG monitoring of insect feeding goes back to 1964, when such a system was first developed by McLean and Kinsey, for the pea aphid, *Acyrtosiphum pisum* (McLean and Kinsey, 1964). Electronic monitoring of feeding behaviour of piercing–sucking insects involves passing an electronic signal through an insect, which has been attached to a thin gold wire. The insect creates variable impedance when it salivates or ingests, which increases the current flow. These variations in voltage produced by salivation and ingestion are amplified, rectified, and then recorded via a strip chart recorder. Since then, this approach has been widely used and the techniques evolved tremendously (Backus, 1994). A recent significant development is reported by Backus and Bennett (2009) with a new universal EPG design having flexible input resistors to produce three simultaneous, time-synchronized, output signals from a single insect, via AC and DC signal processing circuitry. This system can be used for any piercing–sucking hemipteran. An EPG was, for example, used to investigate any differential effect of the insecticide pymetrozine on the feeding behaviours of four major rice sap-sucking insect species to find that their feeding behaviour was disturbed by pymetrozine and exhibited similar patterns of sharp decline in activity in the phloem tissue (He *et al.*, 2011). Salvador-Recatala *et al.* (2014) have applied EPG as a novel approach to plant electrophysiology that allows cell-specific, robust, real-time monitoring of early electrophysiological responses in plant cells to damage, and is potentially applicable to a broad range of plant–herbivore interactions.

High-throughput phenotyping is the next big challenge

With the advancement of genomics, transcriptomics, proteomics, and metabolomics technology, high-throughput and high-resolution phenomics tools have been evolving rapidly for the measurement of phenotypic traits of organisms in response to genetic mutation and external factors (Furbank and Tester, 2011; Fiorani and Schurr, 2013). Phenomics is a highly interdisciplinary field which needs co-ordinated expertise of non-invasive imaging, spectroscopy, image analysis, robotics, and high-performance computing (Finkel, 2009). The Australian plant phenomics facility (http://www.plant-phenomics.org.au/), a new $40 million venture that is the first national lab of its kind in the world, opened its high-resolution plant phenomics centre in Canberra. High-throughput plant phenomics has been considered as being the next big technology in studying the ecology of insect–plant communities (Snoeren *et al.*, 2007; Houle *et al.*, 2010). Significant work is in progress to develop technologies towards computerized video tracking, movement analysis, and behaviour recognition in insects (Hanan *et al.*, 2002; Noldus *et al.*, 2002). High-throughput phenotyping methods have been developed to identify plants with an increased resistance against aphids in large plant collections (Chen *et al.*, 2012). Field-based phenomics for plant genetics research is another challenge (White *et al.*, 2012). Large-scale data acquisition, transformation, and data analysis for high-throughput plant phenotyping experiments create new challenges in bioinformatics (Eberius and Lima-Guerra, 2009).

Transgenic and synthetic biology technologies for engineering insect-resistant plants

Despite the ongoing controversies regarding genetically modified (GM) crops, genetic engineering technologies have been widely accepted and successfully implemented to develop various insect-resistant crops in agriculture by introducing foreign genes into crop genomes (Gatehouse, 2008). For example, cotton resistant to lepidopteran larvae (caterpillars) and maize resistant to both lepidopteran and coleopteran larvae (rootworms) are now extensively being used worldwide and contributing to reduce pesticide usages, increased crop yield, and hence to increase crop production and bring down agriculturalp costs (Hellmich and Hellmich, 2012; Krishna and Qaim, 2012). Growing resistance of insects

against such GM crops has been raising some concerns. A case of field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* (*Bt*) toxins in transgenic maize was reported by Gassmann *et al.* (2014). Despite the scientific consensus that the genetically engineered crops on the market are safe, concurrent concerns over issues related to its risk assessment and biosafety have to be handled carefully (Gilbert, 2013; Ronald, 2014). Two recently published independent studies claimed that GM *Bt* crops had no negative effects on two beneficial insect predators or on a beneficial, entomopathogenic nematode (Gautam *et al.*, 2014; Tian *et al.*, 2014). As an alternative to introducing foreign genes into the plant genome, enhancing the existing plant defensive proteins has also shown significant promise (Morton *et al.*, 2000; Campbell *et al.*, 2011). However, all these approaches mostly rely on introducing or manipulating one or a few genes, proteins, or a small component of some highly interconnected biological pathways. Considering the functional and organizational complexity of the plant system, a more holistic and predictive approach can be useful to tackle such issues. Holistic approaches for molecular characterization using omics platforms can be used for the safety assessment of GM crops. Houshyani *et al.* (2014) have shown that a meta data analysis approach by measuring the transcriptome distance to the untransformed wild-type was a useful screening method to obtain insight in the pleiotropic effects of genetic modification in *A. thaliana* engineered for indirect insect defence (Houshyani *et al.*, 2014). Even though concerns related to GM technology is a very important topic, we limit our analysis here considering the scope of this article.

A next-generation approach to develop insect-resistant plants can be achieved by integrating diverse knowledge from ecology to genes and pathways together, to design sustainable plant systems. Synthetic biology is such a promising field (Small and Puchta, 2014). The underlying concept of synthetic biology is the rational design of a biological organism, through characterizing many biological components to generate a library of modules that can be assembled within an organism to give predictable and sustainable outcomes for enhanced performance of beneficial traits (Silver *et al.*, 2014). Significant progress has been achieved in microbial systems (Gibson *et al.*, 2010), but synthetic design of complex multicellular organisms such as plants poses great challenges. Despite such challenges, plant biologists have been putting great effort into developing new technologies for artificial design of plant systems (Puchta and Fauser, 2014). During the 2010 International Genetically Engineered Machines (iGEM) competition, a framework for engineering the model plant *A. thaliana* with standardized, BioBrick-compatible vectors and parts was introduced to encourage synthetic biologists to use plants as a genetic chassis (Boyle *et al.*, 2012). GoldenBraid 2.0 (GB2.0) is a platform that provides a framework to exchange both information and physical DNA elements among bioengineers to help implement plant synthetic biology projects (Sarrion-Perdigones *et al.*, 2013). Targeted genome engineering (or genome editing) using zinc finger nucleases (ZFNs) (Shukla *et al.*, 2009) and TAL effector nucleases (TALENs) (Schornack *et al.*, 2013) has emerged

as an alternative to classical plant breeding and transgenic (GMO) methods to improve crop plants. The newly developed CRISPR/Cas9 system for targeted gene knockout or editing has been shown to be more efficient in plants for genome editing (Feng *et al.*, 2013; Jiang *et al.*, 2014).

Engineering secondary metabolism of plant defensive compounds by increasing the flux in respective pathways can be of tremendous importance in plants for developing future insect-resistant crop varieties (Dudareva *et al.*, 2013; Jirschitzka *et al.*, 2013). Kristensen *et al.* (2005) have reported metabolic engineering of dhurrin, a cyanogenic glycoside in transgenic *A. thaliana* plants with minor effects on the whole metabolome and transcriptome. Another study has demonstrated that plant volatile emissions can be manipulated to enhance the effectiveness of biological control agents, by which to develop the way for novel and ecologically sound strategies to fight a variety of insect pests (Degenhardt *et al.*, 2009). Metabolic engineering of raffinose family oligosaccharides in the phloem of *A. thaliana* revealed alterations in carbon partitioning and enhanced resistance to green peach aphid (*Myzus persicae*) feeding (Cao *et al.*, 2013).

Concluding remarks

The study of plant–insect interactions has manifold aspects spanning from community ecology to molecular-level interactions. This topic attracts researchers from diverse disciplines such as plant biology, entomology, crop biology, mathematics, molecular biology, chemistry, and synthetic biology, which makes it an interesting interdisciplinary field. Ecological data in plant–insect interactions have been available for a long period. The advancements in modern high-throughput molecular biology technologies have produced large amounts of genome-scale data for both plants and insects. However, there is still a significant gap between the community-level and molecular-level understanding of plant–insect interaction. Integrative systems biology-based approaches can be useful to minimize such gaps to generate a better understanding from the community to the genes and pathways level. The combination of high-throughput profiling techniques, bioinformatics tools, and the data from ecological studies will provide ways by which to achieve a comprehensive systems-level understanding of various aspects related to plant defence responses during insect attack at different hierarchical levels. Such knowledge or biological hypotheses can be used to translate this understanding to develop better crop varieties using transgenic tools or rapidly developing synthetic biology techniques. However, proper ethical and safety guidelines have to be strictly followed during synthetic or transgenic projects.

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