



Plant–microbe interactions: tipping the balance

This virtual issue of the *Journal of Experimental Botany* for the XVIII International Congress on Molecular Plant Microbe Interactions in Glasgow (2019) focuses on research that tackles the consequences of plant–microbe interactions, for both plant–mutualist and plant–pathogen scenarios. When organisms interact, there can be direct and indirect effects on both plant- and microbe-directed processes. The extent to which the balance is tipped towards susceptibility, resistance, or mutualism depends on the degree of change for these plant and microbe processes. Moving from the study of key molecules and mechanisms underlying the balance, to the study of niche dynamics, it looks at the insights that could enable engineering of more robust crops in the future.

Facilitating host–microbe interactions: making a connection

We know that recognition of microbes relies on a litany of specialized extracellular and intracellular receptors such as pattern recognition receptors (PRRs) and resistance ‘R’ genes. However, despite our extensive understanding, new discoveries are continually being made. For example, by studying the well-known PRR, FLS2, Jelenska *et al.* (2017) found that FLS2 is internalized and acts as a transporter that enables mobilization of its ligand, flg22, to distal organs. This is a radical finding, suggesting that the ligand–receptor complex is being transported over a long distance as part of a communication mechanism. Analogous examples have been found in animal models [such as the intercellular transport of sonic hedgehog (reviewed in Simon *et al.*, 2016)], suggesting a conserved mechanism could exist. Similarly, multiple examples of nucleotide binding–leucine–rich repeat (NB-LRR) activation during plant–microbe interactions are known. An additional layer of complexity was demonstrated in work by Meteignier *et al.* (2016) where it was found that activation of NB-LRR signalling during viral infection induces translational repression of viral transcripts and the formation of RNA processing bodies. This only occurs in cells in which a defence response has been activated and which also contain viral RNAs being translationally inhibited.

As well as molecular interactions, microbes require physical connections to their plant host cells. In the case of interaction between the blight pathogen and rice, this interaction has been found to occur after initial recognition, facilitating

the delivery of effectors, even before the pathogen becomes virulent (Li *et al.*, 2019). Physical connections must be formed using cellular–derived material, and, by utilizing the pathosystem of barley–powdery mildew, Kwaaitaal *et al.* (2017) found that the extra–haustorial membrane (EHM) is not simply dependent on conventional secretion from the endoplasmic reticulum (ER). This suggests that it is generated from an unconventional secretory pathway, raising the possibility that the pathogen is remodelling the host cell to gain benefit, possibly via effectors that control plant vesicle trafficking.

Taking advantage of the situation: tipping the balance

It is well known that pathogen effectors are targeted to multiple locations within the cell. Studying both the location and activity of effectors could help us understand how they regulate host processes and tip the balance towards disease (Wang *et al.*, 2019). Combinatorial activity of effectors could enable stronger effects on plant cells. It has been found that co-expression of PiRXLR effectors that target different immune pathways has an additive enhancement; those targeting the same pathway do not (Wang *et al.*, 2019). Cell to cell communication is not, however, a simple one-way process; plant molecules can regulate microbial functions. These molecules are often secreted from plant cells, but it has also been found that they can be released in packages. For example, extracellular vesicles (EVs) from sunflower apoplastic fluid can be taken up by fungi such as *Sclerotinia* (Regente *et al.*, 2017). In this case, the EVs were found to contain proteins including cell wall–remodelling enzymes and defence proteins that affect fungal viability.

Complexity of recognition: it’s complicated

Plant–microbe interactions are not simply differentiated by whether the microbes are friend or foe—the molecular and phenotypic outcome depends on which microbial strain is present. Liang *et al.* (2019) demonstrate that *Lotus* can exhibit duality in how it is colonized, with some rhizobial species entering transcellularly via infection threads and some intercellularly via ‘peg’-like structures. Once microbes enter, the level of colonization and host physiological changes can

vary, for example in the well-known pathway of autoregulation of nodulation (AON) that modulates rhizobial colonization of legumes. In addition to strain-specific differences, legumes vary in how they regulate AON. In *Lotus*, the central controller *TML* is involved in CLE peptide-regulated AON (Tsikou *et al.*, 2018) but in *Medicago* two *TML* genes exist (Gautrat *et al.*, 2019). *MtTML1* and *MtTML2* seem to be functionally redundant, but are expressed slightly differently (Gautrat *et al.*, 2019). Regulation of plant-microbe interactions occurs with a high degree of spatial resolution, as has been found for many biotic and abiotic encounters (Walker *et al.*, 2017; Rich *et al.*, 2018, Preprint). This is also the case at the molecular level where modulation of spatio-temporal patterns and gene expression levels can fine-tune the production of reactive oxygen species (ROS), a central element of the plant immune response. *RbohD* and *RbohF* were found to be exquisitely regulated during *Arabidopsis* responses to both the hemibiotrophic bacterial pathogen *Pseudomonas syringae* pv. tomato and the necrotrophic fungus *Plectosphaerella cucumerina*, as well as upon treatment with microbe-associated molecular patterns (MAMPs) (Morales *et al.*, 2016).

Crosstalk of immunity and plant homeostasis: nothing is for free

As the plant-microbe interaction progresses to resistance, susceptibility, or mutualism, changes come with either a net cost or benefit that can tip the balance in one direction or the other. Some of these immune responses can directly change homeostasis. For example, production of ROS at the plasma membrane (PM) has been found to increase membrane order in response to a wide range of elicitors (Sandor *et al.*, 2016). This seems to be part of a general stimulation of defence response, with alterations of lateral membrane fluidity found in more specific cases (Sandor *et al.*, 2016). Autophagy is a general process controlling abiotic and biotic stress responses but is also key for maintaining cellular homeostasis and, when plant-pathogen interactions regulate autophagy, the balance between resistance and susceptibility becomes complicated, as reviewed in Leary *et al.* (2018). Even more fundamental to the cell are transcriptional and translational mechanisms. Meteignier *et al.* (2017) used RNaseq and TRAPseq to identify effector responses at the transcriptome and translome levels in *Arabidopsis* plants. They identified genes that have uncoupled transcription and translation during plant-pathogen interactions, specifically during the effector-triggered immunity (ETI) response (Meteignier *et al.*, 2017).

Crosstalk is found in plant-directed processes but also in microbe-directed processes. The phenotypic outcome depends on whether a particular balance is reached and held, or tipped in one direction or the other. The ubiquitin-proteasome system (UPS) has been found to be key for this balance since it brings together stress responses mediated by the phytohormones salicylic acid (SA) and jasmonic acid (JA), to control growth and immunity (reviewed in Adams and Spoel, 2018). Similarly, histone acetylation and deacetylation have been identified as key regulators of immunity (Ramirez-Prado *et al.*, 2018). Recently, Liu *et al.* (2019) found that the wheat WD40-repeat protein TaHOS15 functions with the wheat histone deacetylase

TaHDA6 to fine-tune the defence response to *Blumeria graminis* f.sp. *tritici*, via controlling the expression of defence-related genes. TaHOS15 is also known to regulate plant acclimation to cold stress in *Arabidopsis* (Zhu *et al.*, 2008), highlighting the degree of crosstalk complexity in multistress responses.

Crosstalk is also found to impact plant processes indirectly. For example, at a structural level, cell wall organization is affected during plant defence responses (reviewed in Castilleux *et al.*, 2018), and hormone pathways are frequently the site of indirect crosstalk action. Multiple developmental pathways are affected during plant symbiosis and it has been found that different symbiotic nodule types (indeterminate and determinate) produce auxin maxima in different mechanistic ways as part of the regulation of microbial housing (reviewed in Kohlen *et al.*, 2018). Plant-derived auxin can antagonize plant defences, virulence gene expression, and stress responses. Microbe-produced auxins have been found to regulate plant cellular processes both indirectly and directly (reviewed in Kunkel and Harper, 2018).

It has long been known that different hormone pathways can act antagonistically during plant growth versus immunity responses, as a mechanism of crosstalk. Some organisms can directly modulate these pathways; for example, it has been found that infestation of rice by the phloem-feeding brownhopper results in suppression of the brassinosteroid (BR) pathway while simultaneously activating the SA and JA pathways (Pan *et al.*, 2018). Brownhopper susceptibility is altered in BR signalling mutants and increased following treatment with high levels of BR that promote JA synthesis and signalling and reduce SA levels (Pan *et al.*, 2018). Crosstalk also occurs during mutualism; for example, strigolactones are key regulators of plant arbuscular mycorrhizal fungi (AMF) interactions and they are part of crosstalk with auxin, abscisic acid (ABA), and gibberellic acid (GA) pathways (reviewed in Lanfranco *et al.*, 2018). Strigolactones can also influence the AMF directly, resulting in changes in fungal gene expression and also microbial secretion of small proteins that could act as host non-specific effector proteins to modulate the outcome of AMF interactions (reviewed in Lanfranco *et al.*, 2018).

Environmental input: we are not alone

The balance of disease versus resistance can be further tipped depending on environmental factors, something of clear importance as we look to develop plants for a future of climate change. Identifying target genes that could be modulated in order to mitigate enhanced climate effects could be key for this. Tomato resistance to *Frankliniella occidentalis* (thrips) is modulated depending on UV levels, and Escobar-Bravo *et al.* (2019) find that this modulation is probably associated with the activation of JA- and SA-associated signalling. Interestingly this seems to be an indirect effect, again highlighting the high degree of crosstalk between abiotic and biotic responses. The mitogen-activated protein kinase gene *MPK4* has found to be CO₂ responsive and to affect plant responses to aphids by regulating both stomatal aperture and JA-dependent resistance (Guo *et al.*, 2017). This is certainly a checkpoint that could be manipulated, alongside the use of classical approaches to enhance resilience of crops (Piquerez *et al.*, 2014).

Finally, we must look both underground and overground for regulators. Plant–microbe interactions are mediated by other microbes in the environment, in both the rhizosphere and phyllosphere. Understanding root exudation to potentially encourage particular communities to develop, and investigating the bacterial genes that affect colonization are promising directions. We can exploit new advances in the methods to study plant–microbe interactions across chemical, molecular, metagenomic, and exometabolomic scales, as reviewed in [Jacoby and Kopriva \(2019\)](#).

Resistance, susceptibility, or mutualism—the outcome is finely balanced and comes with costs to plant and microbe. The latest research in plant–microbe interactions must take this into account, but could also exploit it to develop high-yielding and resistant new crop varieties. The key information that will enable us to realize food security comes from study of plant–microbe interactions at a fundamental level and its implementation through traditional breeding and plant engineering.

Keywords: Food security, hormones, immunity, plant defence, plant–microbe, plant–mutualist, resistance, symbiosis, transcriptional regulation.

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