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Forum

Plant–Pathogen Maneuvering over Apoplastic Sugars

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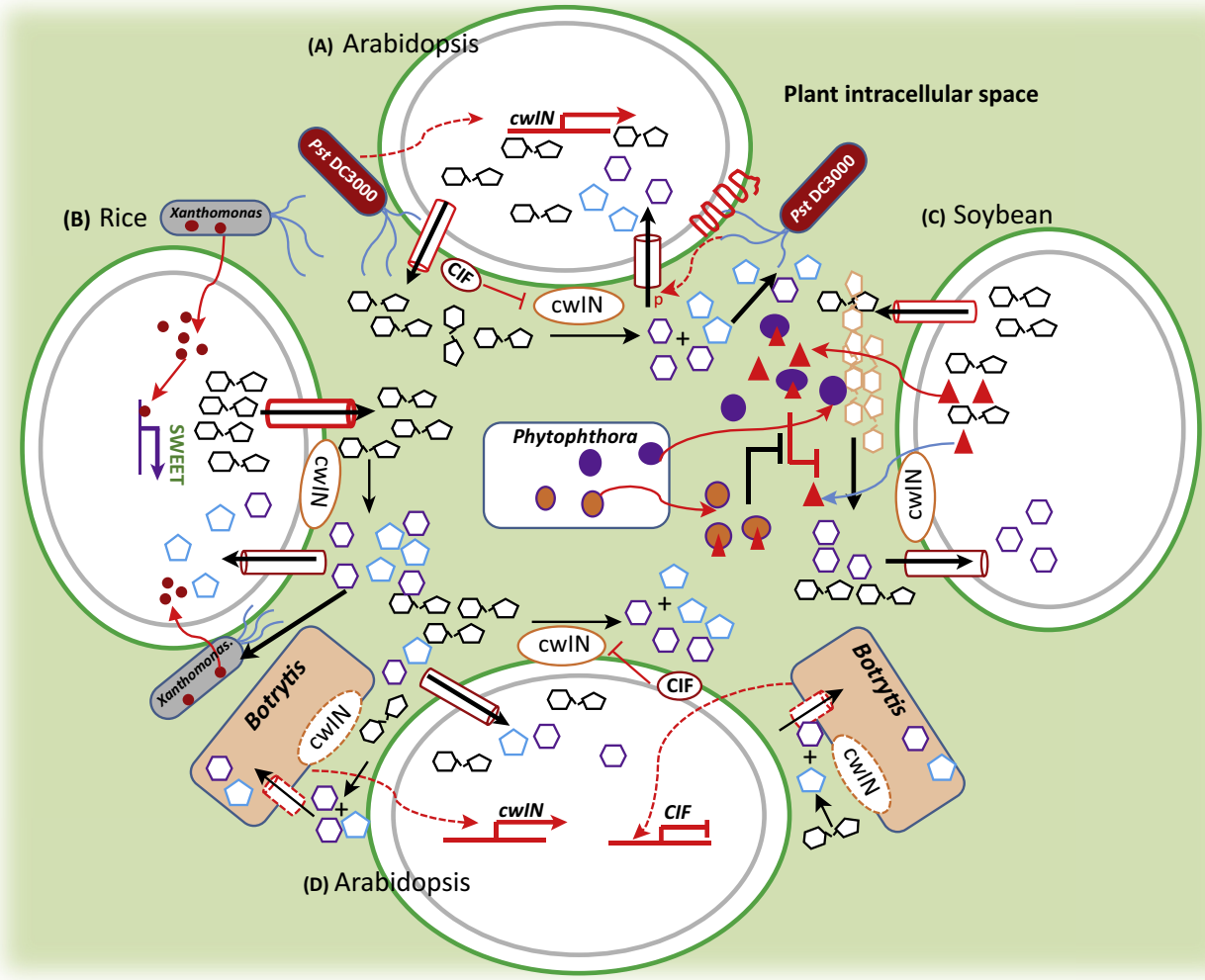
The nutrient-rich extracellular plant compartment, the apoplast, is an attractive niche for attacks by microbial pathogens. Here, we highlight recent trends in plant–pathogen competition for apoplastic sugars in the context of innate immune responses in various plant–pathogen interaction systems.

Plants are efficient energy reactors and produce organic compounds, mainly in the form of sugars (sucrose) by fixing atmospheric CO_2 in the presence of sunlight. However, there is a division of labor and only photosynthetically active green tissues (source) produce an excess of carbon assimilates. These assimilated sugars are translocated to growing tissues (sink) throughout the plant via intracellular and extracellular trafficking pathways. During growth and development, various sinks in the plants compete for carbohydrates [1,2]. Therefore, partitioning of the carbohydrates is tightly regulated to fulfill the metabolic needs of the individual sinks with respect to their ability (sink strength) to obtain assimilates in a competing system [1]. The extracellular space (apoplast) of plant tissue is a nutrient-rich niche and many microbial pathogens have evolved strategies to cause infection in this compartment. For their optimal multiplication, pathogens ensure the uptake of sugars and other required metabolites from the apoplastic compartment [1–3]. The presence of microbial pathogens with their own nutritional requirements inside the apoplast is an additional energy drain on the host cell [3–5]. The host plant perceives the apoplast-inhabiting pathogens as alien sinks and adopts ways and means to starve the pathogens in the apoplast. By releasing antagonistic compounds into the extracellular space, such as hydrolytic enzymes, proteinaceous inhibitors, and defense decoy and mimicry compounds, both the plant and pathogens maneuver over apoplastic sugar supplies and turn the extracellular space into a competitive arms race to acquire energy. Here, we highlight these recent trends in plant–pathogen competition for apoplastic sugars from the perspective of innate immune responses in various plant–pathogen interaction systems.

Sucrolytic Enzymes and Sugar Transporters: The Host Side of the Story

Plants deploy a plethora of mechanisms to regulate carbon fluxes in the apoplastic space. Sucrose is a key carbon source for many physiological processes, such as growth, development, and response to various stresses [1,2,5,6]. By virtue of their sucrolytic enzymes, such as cell wall-bound, cytoplasmic, and vacuolar invertases (cwINs, CINs, and VINs) and sucrose synthases (SUSs), plants catalyze the cleavage of sucrose into glucose and fructose [1,5,6]. Among these invertases, the function of cwINs is essential for the regulation of phloem unloading, a process that releases free hexoses into the apoplastic space [1,2,5]. These reducing sugars are taken up by the apoplast-adapted pathogens [4–7]. Plants stringently regulate the flux of apoplastic sugars via the activity of sugar transporter proteins (STPs) located on the plasma membrane [4–6]. Coordinated regulation of cwINs and STP proteins has been reported to shape the dynamics of various plant–pathogen interaction systems [3,6,7]. In terms of nutrient acquisition, it is difficult to generalize the nature of the interactions that occur in specific plant–pathogen interaction systems. However, accumulating evidence suggests that pathogen infection leads to the induction of the cwINs, which results in the release of free hexoses that provide nourishment to the apoplast-adopted pathogens (Figure 1). By contrast, the arabidopsis-mediated activation of Sugar Transporter Protein 13 (STP13) facilitates the influx of hexoses into the cytosol and, thus, keeps the pathogen deprived of metabolizable sugars, which affects both its growth and fitness [4,5,7].

A recent report by Yamada *et al.* [4] on the host-mediated deprivation of metabolizable sugars required for *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) growth demonstrates an elegant mechanism that connects basal immunity with carbohydrate metabolism in plants. Infection of *Pst* DC3000 increases the activity of cwIN and, thus, enhances the level of free hexoses in the apoplastic space (Figure 1). However, to keep the pathogen starved, the plant hexose sugar transporter *STP13* physically interacts with the innate immune receptor Flagellin Sensing 2 (FLS2). The co-receptor BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 (BAK1) phosphorylates the transporter to facilitate influx of hexose sugars from apoplast into the cytosol (Figure 1A [4]). This influx of hexose sugars culminates in retarded bacterial growth in the apoplast. Mutation of *STP13* enhances the susceptibility of arabidopsis (*Arabidopsis thaliana*) to pathogen infection, due to perturbed



Trends in Plant Science

Figure 1. Apoplastic Maneuvering over Sugar Molecules Exemplified by Various Plant–Pathogen Interaction Systems. The plant intracellular space (apoplast) among cells of a tissue (leaf) is a nutrient-rich niche [only carbohydrates (glucose: violet; hexose, fructose: blue pentagonal) and orange-colored long-chain carbon compounds are shown] infected by apoplast-adopted pathogens [*Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) and *Botrytis cinerea, Xanthomonas oryzae*, and *Phytophthora sojae*] in their respective host plants (arabidopsis, rice, and soybean). Moreover, pathogen-secreted effectors [Ps xyloglucan-specific endoglucanase (PsXEG1): violet-filled circles] with sugar-splitting enzyme activity and paralogous decoy proteins (PsXLP1: orange-filled circles) with no enzymatic activity have differential binding affinities for the plant secreted inhibitor, GmGIP1 (red triangles), a protein that attenuates XEG1 activity. (A) *Pst* DC3000 induces the activity of cell wall invertases (cwINs) in arabidopsis, which hydrolyze sucrose to glucose and fructose. The initiation of innate immunity via the interaction of bacterial flagellin with Flagellin Sensing 2 (FLS2: red spiral on the plasma membrane) activates Sugar Transporter 13 (STP13: cylindrical shape with an inward arrow) via phosphorylation. This causes the influx of hexose sugars from the apoplast towards the cytoplasm. (B) *Xanthomonas* bacteria use transcription factor-like effectors (TALEs; red circles) to activate the expression of sugar transporters genes (SWEET: cylindrical shapes with outward arrows), leading to the accumulation of sucrose in the apoplast. (C) In soybean, *Phytophthora sojae* secretes the sugar-splitting enzyme XEG1 as an effector, and this intervened with by the host surveillance receptor GmGIP1, resulting in the decreased accumulation of reducing sugars (glucose is shown here). The pathogen further secretes a decoy protein (XLP1) to engage the inhibitor more stringently and, hence, enable the XEG1 effector to further increase the level of reducing sugars by engaging the host-driven inhibitors. (D) Infection with *Botrytis* (in arabidopsis) induces the activity of invertases and, hence, increases the concentration of hexose sugars in the apoplast. Analogous to plant invertases and hexose transporters, *Botrytis* also harbors sucrolytic enzymes and hexose transporters to acquire metabolizable sugars from the apoplast. Plant invertase inhibitors (CIF) are instrumental to the regulation of the activity of cwINs and have been shown to be repressed during pathogen infection.

hexose availability [4]. More intriguingly, this perturbation also results in aberrant activation of the pathogen type III secretion system (T3SS) and, thus, discourages the delivery of bacterial effectors into the plant host. Whether the reduction in pathogen growth is directly related to nutrient limitation has yet to be clarified. Thus, the interaction of carbohydrate metabolism with plant defenses and the regulatory effects of host sugar metabolism on pathogen

virulence and pathogenicity mechanisms are also worth considering.

Plant cell wall-bound invertases have a pivotal role in maintaining metabolizable sugars in the apoplast, which has a relevance to the immune response of plants to pathogen infections [5,6]. Generally, invertases are transcriptionally controlled, but they are also regulated at the post-translational level by low-molecular-weight invertase inhibitor (AtCIF1 and AtCIF2) proteins (Figure 1A,D) [2,5,6]. These inhibitors control the activity of cwINs by binding directly to them. For instance, the isoform AtCIF1 binds to AtCWI in the apoplast and their genes are co-expressed in a spatiotemporal manner [2]. Interestingly, the loss-of-function mutant of *AtCIF1* (*cif1-1*) showed a drastic increase in AtCWI activity and boosted hexose levels [2]. Therefore, CIFs thought to be are instrumental in modulating apoplastic hexose levels. However, to date, the implications of these inhibitors in shaping the dynamics of apoplast-adapted pathogens are still not fully understood. Two more reports suggest that infection with *Pst* DC3000 and/or the necrotrophic fungal pathogen *Botrytis cinerea* in arabidopsis strongly regulate the expression level of *AtCIF1* and repress the level of *AtCIF2* genes [5,6]. It is likely that both host and pathogen manipulate the inhibitory potential of these small proteinaceous inhibitors of sugar-splitting enzymes in their favor.

The Pathogen Perspective of the Apoplastic Conflict

We still do not know with certainty whether plant apoplast-adapted pathogens merely rely on host sucrolytic enzymes and the manipulation of host sugar uptake systems or harbor their own sugar hydrolases and hexose uptake machinery. The model apoplastic pathogen *Pst* DC3000 is expected to harbor sucrolytic enzymes to manifest resilience when the availability of metabolizable sugar is constrained by the arabidopsis STP13 proteins upon pathogen infection [4]. Whether *Pst* DC3000 bacteria have invertase-analogous enzymes is still unclear. However, they do contain levansucrases, which are extracellular glycoside hydrolases, and use sucrose as a substrate to synthesize oligosaccharides [8]. However, their exact role in the plant–pathogen interaction is far from understood. More compelling evidence of a pathogen-based long-chain sugar-splitting enzyme that results in metabolizable sugars has been published. A recent paper reported that *Phytophthora sojae* secretes xyloglucan-specific endoglucanase (PsXEG1: effector) into the apoplast of soybean [*Glycine max* (L.) Merr.], which converts xyloglucan to reducing sugars, such as various monosaccharides [7]. To limit the hydrolytic activity of the pathogen-secreted PsXEG1, the host also releases a glucanase inhibitor (GmGIP1) into the apoplast that can bind to PsXEG1, thereby reducing the production of hexose sugars. As a counterattack, the pathogen adds a paralogous 'decoy molecule' (PsXLP1) to the apoplast that is identical to the effector PsXEG1, but lacks the endoglucanase enzyme catalytic domain and has a higher binding affinity for GmGIP1. The interaction between the decoy PsXLP1 and GmGIP1 prevents the interaction with PsXEG1 and, thus, more free sugar molecules are available for the pathogen to use to grow (Figure 1C). Another recent study showed that *B. cinerea* has its own functional sucrolytic machinery that facilitates the breakdown of sucrose into hexoses, which is independent of the host cell wall invertases (Figure 1D) [5].

Whether plant pathogens harbor their own sugar uptake system or have the ability to exploit host-based sugar efflux transporters to release sugars from the cytosol into the apoplast has yet to be determined. However, *Xanthomonas* bacteria deliver transcription factor-like effectors (TALEs) into the host, where they activate the expression of SWEET family sugar transporter genes by binding to their promoters [3]. This results in the efflux of sugar from the cytoplasm into the apoplast, thus promoting bacterial nutrition (Figure 1B). Moreover, *Botrytis* also has a multigene hexose uptake system that facilitates the influx of hexose sugar from the plant apoplast into the pathogen [5]. Therefore, apoplastic pathogens are likely to have evolved sophisticated mechanisms to ensure that their nutritional needs are met despite the fact that the plant host triggers immune responses (discussed in the previous section) that directly or indirectly target that nutrient acquisition.

Concluding Remarks and Future Perspectives

Both plant and pathogens have the potential to deploy sucrolytic enzymes and sugar transporters to customise carbon fluxes in their favor. So far, the host-devised strategies for securing its carbon reserves in the apoplast have attained more attention than those on the side of the pathogen. However, recent data have shown that pathogens have tools and tricks that facilitate the acquisition of nutrients from the host apoplast. In this regard, the importance of levansucrases [8] and pathogen-based invertases merit further investigation. Likewise, a quest for the identification of plant-like STPs in pathogens should be the focus of future research. Moreover, the apoplast is not exclusively enriched in carbon compounds: it also contains other nutrients, such as nitrogenous compounds, that are also important for pathogen growth. Thus, future work to reveal mechanisms for other metabolites is also warranted.

Recent reports on apoplast-adapted pathogens and their interactions with the host plant highlight new avenues in plant–pathogen interaction research. The host strives to keep the pathogen starved by transporting sugars away from the apoplast into the cytoplasm via transporters [4]. This action, combined with the secretion of the pathogen (*P. sojae*) xyloglucanase (effector) and its decoy by the host, which prevents access of the plant inhibitor to the sugar-splitting enzymes [7], nicely support plant innate immunity responses at

the metabolic and physiological levels. Another recent report, which investigated the soaking of plant leaf (water abundance) tissue upon infection with *Pst* DC3000, substantiates the fact that an aqueous apoplastic environment could facilitate the flow of nutrients to bacteria and accelerate the spread of the pathogen [9]. This pathogen-mediated water soaking of plant tissue has a direct impact on the availability and solubility of sugar molecules that the pathogen acquires from the apoplast.

In summary, these new data point to the apoplast as the site of a critical battle between the host and invading pathogens, with both trying to leverage an advantage.

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