Microreview

Cell-wall invertases, key enzymes in the modulation of plant metabolism during defence responses

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SUMMARY

Most plant–pathogen interactions do not result in pathogenesis because of pre-formed defensive plant barriers or pathogen-triggered activation of effective plant immune responses. The mounting of defence reactions is accompanied by a profound modulation of plant metabolism. Common metabolic changes are the repression of photosynthesis, the increase in heterotrophic metabolism and the synthesis of secondary metabolites. This enhanced metabolic activity is accompanied by the reduced export of sucrose or enhanced import of hexoses at the site of infection, which is mediated by an induced activity of cell-wall invertase (Cw-Inv). Cw-Inv cleaves sucrose, the major transport sugar in plants, irreversibly yielding glucose and fructose, which can be taken up by plant cells via hexose transporters. These hexose sugars not only function in metabolism, but also act as signalling molecules. The picture of Cw-Inv regulation in plant–pathogen interactions has recently been broadened and is discussed in this review. An interesting emerging feature is the link between Cw-Inv and the circadian clock and new modes of Cw-Inv regulation at the post-translational level.

Keywords: cell-wall invertase, circadian clock, defence, metabolism, sugar signalling.

INTRODUCTION

Plants are exposed to a range of different pathogens, including fungi, bacteria, oomycetes and viruses. Plant pathogens have evolved different strategies to interact with their hosts. Generally, plant–microbe interactions can be classified as biotrophic, with the pathogen feeding from living tissue, or necrotrophic, with the pathogen feeding from dead host tissue, which has been actively killed by the pathogen. There is a wide range of trophic lifestyles of pathogens, e.g. hemibiotrophic pathogens show an initial biotrophic phase and a switch to a necrotrophic lifestyle later (Glazebrook, 2005). Plants are resistant to most micro-organisms they encounter as a result of pre-formed or activated defence

mechanisms. In basal resistance, pathogen-associated molecular patterns (PAMPs), such as fungal chitin or bacterial flagellin, are recognized by corresponding receptors, leading to the activation of signalling cascades, regulation of gene expression, production of reactive oxygen species and cell-wall modifications (Schwessinger and Ronald, 2012). Successful pathogens have evolved strategies to bypass plant defence reactions. Virulence is established by effector molecules which enable the pathogen to interfere with its recognition, or the downstream activation of plant defence. In a more specific line of defence, called effector-triggered immunity, the plant recognizes pathogen-derived effectors or effector activities, leading to the activation of strong defence reactions, including the hypersensitive cell death reaction (Jones and Dangl, 2006). Plant defence is accompanied by a profound and dynamic modulation of host metabolism. Common metabolic changes are the repression of photosynthesis, sink induction and the production of secondary metabolites (Berger *et al.*, 2007; Biemelt and Sonnewald, 2006). In this response, cell-wall invertase (Cw-Inv) plays a pivotal role in metabolic reprogramming and sugar sensing. Here, we summarize the role of Cw-Inv in defence reactions and extend the model to recent advances, such as a possible interconnection of Cw-Inv and the circadian clock and new modes of Cw-Inv regulation.

CW-INVS, KEY ENZYMES IN SOURCE–SINK TRANSITIONS

Plant tissues can be divided into two major categories: net exporters of sugars (carbon sources) and net importers of sugars (carbon sinks). Source tissues are typically photosynthetically active, CO₂-fixing mesophyll cells of mature leaves. However, there are different types of sink tissue that rely on an external supply of sugars, such as roots, flowers or fruits. The phloem transports sucrose, the main transport sugar in plants, from source to sink tissues. Phloem unloading can be mediated symplastically via plasmodesmata, or via sugar transporters. In the latter case, the extracellular sucrose is either imported into the sink cell via sucrose importers, or sucrose is cleaved irreversibly by Cw-Inv, yielding glucose and fructose, which are taken up by hexose transporters (Büttner and Sauer, 2000; Koch, 2004). In addition to Cw-Inv, sucrose cleavage is mediated by cytosolic sucrose synthase, which catalyses the

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reversible cleavage of sucrose, yielding UDP-glucose and fructose (Koch, 2004). Symplastic phloem unloading is characteristic for tissues with a high demand for sugars, such as tubers. Cw-Invs are considered to be key enzymes in apoplastic phloem unloading and sink induction (Sturm and Tang, 1999). The functional coupling of hexose transporters and Cw-Inv is supported by a coordinated induction after cytokinin application, as shown in *Chenopodium rubrum* suspension cultures (Ehneß and Roitsch, 1997), and the co-expression of extracellular invertase and hexose transporters in the endosperm transfer cell layer of barley seeds (Weschke *et al.*, 2003). Cw-Invs are organized into small gene families with different isoenzymes characterized by tissue-, organelle- and development-specific expression patterns (Cho *et al.*, 2005; Sherson *et al.*, 2003). Specific functions have been shown for different isoenzymes, particularly for flower, fruit and pollen development, a fact which reflects the diversity of invertase functions in assimilate partitioning.

MODES OF CW-INV REGULATION

For a long time, the regulation of Cw-Inv activity appeared to take place mainly on the transcriptional level. Diverse stimuli, such as hormones, sugars, abiotic and biotic stress stimuli, regulate the transcript levels of Cw-Inv (Roitsch *et al.*, 2003; and references therein). However, recent studies have highlighted Cw-Inv regulation on the post-translational level. As a result of their glycan decoration, Cw-Invs are rather stable; hence, post-translational regulation is important to control their activity (Rausch and Greiner, 2004). Post-translational regulation of Cw-Inv activity is mediated by proteinaceous invertase inhibitors. These inhibitors target directly the active site of Cw-Inv in a pH-dependent manner and compete with sucrose for the same binding site. This suggests that Cw-Inv activity is regulated by the extracellular sucrose level and pH (Hothorn *et al.*, 2010). Bonfig *et al.* (2010) have shown that proteinaceous invertase inhibitors regulate Cw-Inv activity after pathogen challenge. They demonstrated a de-repression of invertase activity via the down-regulation of an invertase inhibitor, thus realizing a local sink induction at the site of the defence reaction, in the interaction of *Arabidopsis* with the hemibiotrophic bacterial pathogen *Pseudomonas syringae*. Moreover, reduction of Cw-Inv activity by the overexpression of an invertase inhibitor in roots was able to reduce clubroot gall development in *Arabidopsis* (Siemens *et al.*, 2011). An interesting novel mode of Cw-Inv regulation has been proposed by Le Roy *et al.* (2013). The authors analysed a tobacco Cw-Inv, which lacks sucrose-hydrolysing capacity because of the absence of certain essential amino acids responsible for stable substrate binding. *In vitro* experiments on pollen cell walls suggested that the inactive Cw-Inv Nin88 counteracts the binding of another, sucrolytically active, Cw-Inv (cwINV1) to pollen cell walls, thereby leading to higher activities of cwINV1, but also more efficient binding with the corresponding

Cw-Inv inhibitor. Defective Cw-Invs have been described in several plant species. Therefore, it would be interesting to determine whether such interactions between defective/active Cw-Invs play a role in the defence response. Further modes of Cw-Inv regulation are exon skipping (Bournay *et al.*, 1996) and differential transcript formation (Cheng *et al.*, 1999). The former study showed that the characteristic 9-bp mini-exon-II of Cw-Inv, which encodes three amino acids of the highly characteristic and conserved NDPNG/A-motif, was lost during cold stress. The latter study reported that the maize Cw-Inv gene *Incw1* encodes two different transcripts because of the different lengths of the 3'-untranslated region, whereas the 5'-untranslated region and the coding sequence remain unaffected. Interestingly, it was sufficient to add the metabolizable sugars, sucrose and glucose, to heterotrophic suspension cultures to increase the abundance of the smaller transcript, resulting in increased levels of INCW1 protein/activity. Non-metabolizable sugars increased the abundance of the larger transcript, but did not result in altered levels of INCW1 protein/activity. The authors speculated that the 3'-untranslated region of *Incw1* may contribute to different RNA processing/translation according to the presence/absence of the corresponding sugars.

THE ROLE OF CW-INVS IN PLANT-PATHOGEN INTERACTIONS

In addition to growth- and development-related functions, Cw-Invs play an important role during the course of plant-pathogen interactions. Numerous studies have shown an increase in Cw-Inv expression/activity on pathogen challenge in different plant species and corresponding compatible and incompatible pathogen interactions (Table 1). During the course of the incompatible interaction of photoautotrophic tobacco leaves challenged with *Phytophthora nicotianae*, a metabolic shift from photosynthesis/assimilatory metabolism to a non-assimilatory metabolism was observed. This was accompanied by an increase in Cw-Inv activity, reduced sucrose export and an increase in respiration at the infection site. Thereby, the reduced sucrose export from infected source leaves is probably a consequence of increased sucrose cleavage by Cw-Inv at the infection site (Scharte *et al.*, 2005). An induction of Cw-Inv is also observed in non-photosynthetic tissue, as described for the interaction of tomato roots with *Fusarium oxysporum*. Thereby, the induction of Cw-Inv proceeds more rapidly in incompatible plants relative to compatible plants. This induction of Cw-Inv may be crucial to the establishment of an enhanced flow of sucrose to the infection site to feed an increased metabolic activity when defence reactions are mounted (Benhamou *et al.*, 1991). The identification of monosaccharide transporters that are co-regulated with Cw-Inv by pathogen infection suggests a close relationship between apoplastic sucrose hydrolysis and hexose uptake during defence responses. Such a co-regulation was observed in the interaction of

Table 1 Examples of pathosystems characterized by the modulation of cell-wall invertase (Cw-Inv) expression/protein amount/activity during the course of infection.

Plant	Pathogen	Trophic style	Interaction *	Cw-Inv modulation†	Reference
Barley, leaf (WT/mlo/Mla12)	<i>Blumeria graminis</i> f.sp. <i>hordei</i>	Biotrophic	C, I	A-Ind	Swarbrick <i>et al.</i> (2006)
Wheat, leaf	<i>Blumeria graminis</i> f.sp. <i>tritici</i>	Biotrophic	C	T-Ind, A-Ind	Sutton <i>et al.</i> (2007)
<i>Arabidopsis</i> , leaf	<i>Albugo candida</i>	Biotrophic	C	T-Ind, A-Ind	Chou <i>et al.</i> (2000)
<i>Arabidopsis</i> , leaf	<i>Erysiphe cichoracearum</i>	Biotrophic	C	T-Ind, A-Ind	Fotopoulos <i>et al.</i> (2003)
<i>Arabidopsis</i> , leaf	<i>Pseudomonas syringae</i> pv. <i>tomato</i>	Hemibiotrophic	C	T-Ind, A-Ind	Bonfig <i>et al.</i> (2010)
<i>Arabidopsis</i> , root	<i>Plasmodiophora brassicae</i>	Biotrophic	C	T-Ind	Siemens <i>et al.</i> (2011)
<i>Arabidopsis</i> , root	<i>Heterodera schachtii</i>	Biotrophic	C	T-Rep/A-Rep	Cabello <i>et al.</i> (2014)
Tobacco, leaf	Potato virus Y		C	T-Ind/A-Ind	Herbers <i>et al.</i> (2000)
Tobacco, leaf	<i>Phytophthora nicotianae</i>	Hemibiotrophic	I	T-Ind/A-Ind	Scharte <i>et al.</i> (2005)
Tobacco, leaf	<i>Phytophthora nicotianae</i>	Hemibiotrophic	I	A-Ind	Essmann <i>et al.</i> (2008)
Tomato, leaf	<i>Xanthomonas campestris</i>	Hemibiotrophic	C	A-Ind	Kocal <i>et al.</i> (2008)
Tomato, leaf/fruit	<i>Botrytis cinerea</i>	Necrotrophic	C	A-Ind/T-Ind	Hyun <i>et al.</i> (2011)
Tomato, root	<i>Fusarium oxysporum</i> f.sp. <i>radicis-lycopersici</i>	Hemibiotrophic	C, I	P-Ind	Benhamou <i>et al.</i> (1991)
Tomato, root	<i>Glomus intraradices</i>	Symbiotic	S	T-Ind/A-Ind	Schaarschmidt <i>et al.</i> (2006)
Grapevine, leaf	<i>Erysiphe necator/Plasmopora viticola</i>	Biotrophic	C	T-Ind, A-Ind	Hayes <i>et al.</i> (2010)
<i>Vicia faba</i> , leaf	<i>Uromyces fabae</i>	Biotrophic	C	T-Ind (host) T-Ind/P-Ind (<i>U. fabae</i>)	Voegele <i>et al.</i> (2006)
Carrot, root/leaf	<i>Pectobacterium carotovorum</i>	Necrotrophic	C	T-Ind	Sturm and Chrispeels (1990)

*C, compatible interaction; I, incompatible interaction; S, symbiotic interaction.

†A-Ind, activity induction; A-Rep, activity repression; P-Ind, protein amount induction; T-Ind, transcriptional induction; T-Rep, transcriptional repression.

Arabidopsis with *Erysiphe cichoracearum* (Fotopoulos *et al.*, 2003), wheat with powdery mildew (Sutton *et al.*, 2007) and grapevine with powdery and downy mildew (Hayes *et al.*, 2010). Elevated glucose levels serve as sugar signal and regulate gene expression, as outlined below. Voegele *et al.* (2001) characterized a hexose transporter, HXT1, from the biotrophic rust fungus *Uromyces fabae*, and showed a localization of HXT1 within the haustorial complex. Moreover, during the course of the *Vicia faba*–*U. fabae* interaction, an increase in pathogen invertase gene expression in the periphery of haustoria and of host Cw-Inv in infected leaves was observed (Voegele *et al.*, 2006). However, the role of the pathogen-derived Cw-Inv and hexose transporter is not yet clear; a possible functional link to support nutrient uptake of the pathogen is still under debate. Another possibility is that the *U. fabae* Cw-Inv conditions the host for source–sink transition (Voegele *et al.*, 2006).

RNA interference (RNAi)-mediated repression of Cw-Inv activity in tobacco resulted in an impaired defence response following *P. nicotianae* infection. During the course of infection, the accumulation of apoplastic sugars was reduced/delayed in RNAi plants, the expression of pathogen-related genes was reduced and the formation of hydrogen peroxide was weak (Essmann *et al.*, 2008). By contrast, overexpression of a rice Cw-Inv gene, *Grain Incomplete Filling 1 (GIF1)*, resulted in constitutive activation of defence-related genes, higher resistance to the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* and the fungal pathogen *Magnaporthe oryzae*, and higher levels of callose deposition during pathogen infection. Moreover, cell walls were thicker at the infection sites of *GIF1*-overexpressing lines relative to the wild-type (Sun *et al.*, 2014).

A detailed analysis of invertase and sucrose synthase gene expression/activities during the interaction of *Arabidopsis* and the cyst nematode *Heterodera schachtii* revealed that several sucrose synthase isogenes were induced and cytosolic, vacuolar and Cw-Inv genes were repressed in the infected roots. This resulted in lower cytosolic, vacuolar and Cw-Inv activities in syncytia of the infected root, but higher levels of all three classes of invertase activity in the systemic leaf of root-infected plants relative to non-infected controls. This indicates a local and systemic modulation of sucrose-cleaving enzymes following nematode infection (Cabello *et al.*, 2014). Cw-Inv is important in gall development during clubroot disease in the interaction of the biotrophic protist *Plasmodiophora brassicae* and *Arabidopsis*. This was demonstrated by the overexpression of invertase inhibitors in *Arabidopsis* roots, which resulted in reduced Cw-Inv activity and reduced development of clubroot symptoms (Siemens *et al.*, 2011). Transcriptional induction of a tomato Cw-Inv was also observed in a symbiotic plant–microbe interaction during the course of the mycorrhization of tomato roots (Schaarschmidt *et al.*, 2006).

Recently, a pepper Cw-Inv was shown to be a target of the *Xanthomonas campestris* effector XopB. Cw-Inv repression by XopB could interfere with sugar-mediated defence responses during infection (Sonnewald *et al.*, 2012). The fact that pathogens evolved effectors targeting the host Cw-Inv underlines the importance of Cw-Inv regulation in the defence response.

Finally, pathogen-derived extracellular invertases and sugar metabolism add to the complexity of metabolic regulation in plant–pathogen interactions. An increase in host and pathogen invertase gene expression was observed in the *V. faba*–*U. fabae*

Fig. 1 Function and regulation of cell-wall invertase (Cw-Inv) in plant–pathogen interactions (exemplified for fungal/bacterial leaf pathogens). Cw-Inv cleaves sucrose (Suc), the major transport sugar, yielding glucose (Glc) and fructose (Frc). Pathogen challenge leads to an induction of Cw-Inv expression or de-repression of Cw-Inv activity, mediated by the down-regulation of proteinaceous Cw-Inv inhibitors, resulting in enhanced sucrose cleavage. After uptake, hexoses are further metabolized to support respiration or the synthesis of secondary metabolites, notably the formation of callose or phenolic compounds. In addition, sucrose and hexoses act as signalling molecules. Therefore, Cw-Inv locates at an integration point of hormonal, sugar, defence and diurnal/circadian regulation. The latter is a newly emerging aspect of Cw-Inv regulation and could be crucial to the coordination of diurnal growth patterns and defence reactions. Moreover, Cw-Inv is a target of pathogen effectors, underlining the importance of Cw-Inv in the defence response. Black boxes represent sugar transporters. Dotted arrows depict metabolic reactions, broken arrows sugar transport and full arrows signalling interactions. PAMP, pathogen-associated molecular pattern; ROS, reactive oxygen species.

interaction (Voegelé *et al.*, 2006). Moreover, a fungal invertase plays a role in the grapevine–*Botrytis cinerea* (Ruiz and Ruffner, 2002) and sunflower–*Sclerotinia sclerotiorum* (Jobic *et al.*, 2007) interactions. A model illustrating a possible function and regulation of Cw-Inv in plant–pathogen interactions is shown in Fig. 1.

THE ROLE OF CW-INV IN THE INTEGRATION OF SUGAR AND DEFENCE SIGNALLING

Soluble sugars, mostly hexoses and sucrose, not only provide energy and carbon resources, but also act as signalling molecules (Jang and Sheen, 1997; Koch, 1996; Rolland *et al.*, 2006; Wind *et al.*, 2010). The role of hexokinase in plant sugar signalling is well established (Jang and Sheen, 1997; Xiao *et al.*, 2000). In addition to hexokinase, further sugar-sensing pathways have been described: glucose sensing at the plasma membrane via a G-protein-coupled pathway (Grigston *et al.*, 2008), sugar sensing via Snf-related protein kinases (Baena-González *et al.*, 2007) and the possibility of hexose sensing in the secretory pathway (Herbers *et al.*, 1996). Sucrose signalling is poorly understood; yet, a recent study describes a sucrose-induced receptor kinase in *Arabidopsis* (Wu *et al.*, 2013). There is evidence for sucrose acting as a signalling molecule in plant defence (Gómez-Ariza *et al.*, 2007). Sugar-mediated transcriptional regulation of Cw-Inv is widely observed and, in most reports, Cw-Inv expression is induced by sugars.

Glucose and sucrose increase the level of a Cw-Inv of pea (Zhang *et al.*, 1997), *Chenopodium rubrum* (Roitsch *et al.*, 1995) and tomato (Proels and Roitsch, 2009). Sugar-induced expression of Cw-Inv is of particular interest, as this mechanism allows for the maintenance or amplification of the initial induction of invertase via feed-forward regulation (Roitsch *et al.*, 2000). Moreover, sucrose cleavage by Cw-Inv alters the ratio of sucrose to hexoses in the apoplast, and can therefore actively modulate sugar signalling. Sugar partitioning can be altered by SWEET-type sugar transporters, which are targeted by bacterial effectors (Chen *et al.*, 2010). SWEETs may not only be relevant to support pathogen growth, but may also interfere with sugar signalling. Increasing glucose levels result in a repression of photosynthetic gene expression and an induced expression of pathogenesis-related (PR) genes (Berger *et al.*, 2007; Biemelt and Sonnewald, 2006). The down-regulation of photosynthesis gene expression, most probably via hexose signalling, is a central feature following pathogen attack (Bischof *et al.*, 2011; Bonfig *et al.*, 2006; Chou *et al.*, 2000; Swarbrick *et al.*, 2006). The impaired photosynthetic activity in infected tissue amplifies metabolic source–sink transitions. A number of defence-related genes have been found to be induced by soluble sugars (Gómez-Ariza *et al.*, 2007; Herbers *et al.*, 1996). Overexpression of a yeast invertase in the vacuole or apoplast of tobacco results in the constitutive induction of defence-related genes, elevated callose content and peroxidase

activities (Herbers *et al.*, 1996). Infection of tobacco plants with *Potato virus Y* leads to the inhibition of sugar export, induction of defence responses and a higher resistance towards viral attack (Herbers *et al.*, 2000). Based on these findings, Herbers *et al.* (2000) proposed a model of sugar-enhanced plant defence.

POSSIBLE ROLES OF CW-INV S AS TARGETS AND MODULATORS OF CIRCADIAN REGULATION

Plants must anticipate light/dark cycles for optimal photosynthetic performance. To this end, they possess a sophisticated molecular machinery, the circadian clock, representing an internal timing mechanism consisting of autoregulatory interlocked transcriptional feedback loops (Carré and Veflingstad, 2013). The circadian clock can anticipate the 24-h periodicity of the day length and regulate physiological responses, such as plant growth and development. Circadian regulation interplays with other stimuli, such as hormones and sugars. Interestingly, among the sugar signals, it is mostly sucrose which affects the circadian clock (Dalchau *et al.*, 2011). Moreover, Haydon *et al.* (2013) have shown that endogenous sugars derived from photosynthesis can entrain the circadian clock. An overview of the interconnection of light and sugar signals, the clock and immune responses has been provided by Roden and Ingle (2009) and Bolouri Moghaddam and Van den Ende (2013). Here, we briefly state the role of the circadian clock in regulating defence responses and discuss a possible connection of the circadian clock and Cw-Inv. *Arabidopsis* plants are least susceptible to *Pseudomonas syringae* pv. *tomato* DC3000 infection in the subjective morning, and PAMP receptors and PAMP-triggered callose deposition is higher at this time of the day. In arrhythmic plants, no such temporal differences were observed (Bhardwaj *et al.*, 2011). Wang *et al.* (2011) demonstrated that CCA1 controls resistance (*R*)-gene-mediated resistance in *Arabidopsis* against downy mildew. Thereby, the circadian control of *R* genes allows for the anticipation of infection when pathogen challenge is highest—in this pathosystem, as a result of a diurnal pattern of spore dispersion. Leaf sucrose levels and invertase expression have been shown to follow a diurnal or circadian expression profile (Bläsing *et al.*, 2005; Ruts *et al.*, 2012). A tomato Cw-Inv, *Lin6*, has been reported to follow a diurnal cycle in photoautotrophic cell cultures. Furthermore, in a heterologous expression system, it has been shown that CCA1 and LHY, transcription factors acting in the core of the *Arabidopsis* circadian clock, activate the *Lin6* promoter (Proels and Roitsch, 2009). With regard to the central role of Cw-Invs in the defence response, a circadian/diurnal regulation of Cw-Inv could be crucial to coordinate source–sink transitions during diurnal growth patterns and defence reactions. Thereby, Cw-Inv-mediated changes in the sucrose to hexose level might modulate sugar signalling.

CONCLUDING REMARKS

Cw-Invs play a central role in the carbohydrate supply of sink tissues and the regulation of source–sink transitions. This places Cw-Inv in the centre of metabolic regulations during the course of plant–pathogen interactions, which are characterized by a profound reorganization of metabolism to meet an increased metabolic activity. Cw-Invs are regulated by sugar-, hormone- and stress-related signals and do not only serve as metabolic enzymes, but can generate sugar-based signals themselves via the modulation of sucrose/hexose ratios. Hence, several signals converge at the site of Cw-Inv regulation. Cw-Inv-generated sugar signals, in turn, can modulate the gene expression of pathogen-related or metabolic genes. Therefore, Cw-Inv acts at an integration point of hormonal, sugar, defence and diurnal/circadian regulation. The latter is a newly emerging aspect of Cw-Inv regulation and could be crucial to the coordination of diurnal growth patterns and defence reactions. Epidermal cells, which often come into contact with pathogens first and mount the initial layer of defence (Fig. 1), are characterized, in most plants, by an absence of chloroplasts, and thus depend on sucrose import from neighbouring mesophyll cells. Hence, there should be communication and carbon flux between epidermal and mesophyll cells to allow for an appropriate defence response. In most studies on plant–pathogen interactions, a dissection of epidermal and mesophyll cells has not been addressed. For a comprehensive understanding of metabolic reprogramming in defence reactions, it would be interesting to dissect epidermal and adjacent mesophyll cells and analyse underlying signalling events after pathogen attack.

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