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REVIEW: PART OF A SPECIAL ISSUE ON PLANT IMMUNITY

Should I fight or should I grow now? The role of cytokinins in plant growth and immunity and in the growth–defence trade-off

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• **Background** Perception and activation of plant immunity require a remarkable level of signalling plasticity and control. In *Arabidopsis* and other plant species, constitutive defence activation leads to resistance to a broad spectrum of biotrophic pathogens, but also frequently to stunted growth and reduced seed set. Plant hormones are important integrators of the physiological responses that influence the outcome of plant–pathogen interactions.
• **Scope** We review the mechanisms by which the plant hormone cytokinin regulates both plant growth and response to pathogens, and how cytokinins may connect these two processes, ultimately affecting the growth trade-offs observed in plant immunity.

Key words: Cytokinin, defence, plant immunity, pathogens, plant growth, plant development, growth–defence trade-offs, fitness costs.

INTRODUCTION

Upon pathogen perception, plants initiate the concerted activation of a complex suite of structural and physiological defence responses, a process that requires a remarkable degree of signalling plasticity and control. Insufficient or untimely immunity activation may fail to restrain the pathogen, resulting in the host succumbing to disease. Conversely, constitutive or excessive defence activation leads to resistance to a broad spectrum of pathogens, but also often to suppression of plant growth, a phenomenon described as the growth–defence trade-off.

The existence of growth–defence trade-offs associated with defence activation underscores the need of plants to maintain a delicate balance between growing and defending against pathogens. While the underlying mechanisms associated with growth suppression during increased states of immunity are not well understood, energy diversion to the production of defence proteins and metabolites, as well as alteration of developmental programmes that promote growth, have been proposed as possible means of growth suppression. Thus, in order to survive pathogen attack, as well as successfully grow and reproduce, plants must effectively integrate signals initiated upon defence activation with those responsible for growth and developmental programmes.

Several lines of evidence suggest the existence of plant fitness costs associated with mechanisms of defence activation against pathogens (Bergelson and Purrington, 1996). This is especially true in the case of resistance to biotrophic pathogens, which are pathogens that obtain their nutrients from living plant cells. For example, yield penalties associated with resistance controlled by recognition of biotrophic pathogen effectors by resistance (R) proteins [effector-triggered immunity (ETI)] have been reported (Bjornstad and Aastveit, 1990; Ortelli *et al*., 1996; Brown, 2002). In the model plant *Arabidopsis thaliana* (hereafter arabidopsis), effective association of *R* gene-mediated resistance and fitness costs has been demonstrated (Tian *et al*., 2003). Introgression of *RPM1*, a nucleotide binding leucine-rich repeat (NLR) *R* gene conferring resistance to the bacterium *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) expressing *avrRPM1* or *avrB*, into an *RPM1*$^{-}$ ecotype leads to fitness costs characterized by reduction of seed set, decreased number of siliques and lower dry biomass (Tian *et al*., 2003). Further, recognition by plant cells of certain pathogen-associated molecular patterns (PAMPs), such as the conserved flg22 epitope from the bacterial protein flagellin, leads not only to immunity activation [PAMP-triggered immunity (PTI)] but also compromised plant growth (Gomez-Gomez *et al*., 1999), indicating that fitness costs of resistance are not restricted to ETI activation.

Similarly to the effect of defence activation by pathogen perception, plants with mutations that lead to constitutive activation of defence responses are also frequently dwarf, often with reduced seed set, demonstrating the fitness costs of constitutive defence activation. For example, arabidopsis constitutive defence mutants *snc1* (*SUPPRESSOR OF NPR1 CONSTITUTIVE*), *cpr1* (*CONSTITUTIVE PATHOGENESIS-RELATED 1*) and *cpr5* (*CONSTITUTIVE PATHOGENESIS-RELATED 5*) are dwarf plants with reduced biomass, altered morphology and decreased seed yield compared with wild-type plants (Bowling *et al*., 1994, 1997; Li *et al*., 2001). The *snc1* plants contain a gain-of-function mutation in a gene encoding an NLR protein that leads to constitutive expression of *PATHOGENESIS-RELATED* (*PR*) genes and activated levels of defence responses, including accumulation of the plant defence hormone salicylic acid (SA). Likewise, *cpr1* and *cpr5* mutants also have elevated *PR* gene expression and SA levels, with *cpr5* exhibiting early senescence (Bowling *et al*., 1994, 1997).

Because activation of resistance to biotrophic pathogens is mostly dependent on SA signalling pathways (Glazebrook,

2005), and because SA-accumulating mutants tend to display decreased plant growth, high levels of SA have been suggested as one of the mechanisms by which the growth–defence trade-off may occur in plants. In agreement with this hypothesis, mutants with reduced SA content or signalling have been shown to display increased biomass in comparison with wild-type plants (Abreu and Munne-Bosch, 2009). How exactly SA mediates growth inhibition is still unknown.

The plant hormone cytokinin has long been associated with the regulation of plant growth and stress tolerance (Argueso *et al*., 2009). In recent years, cytokinins have been determined to play an important role in defence against biotrophic pathogens, which has led to the elucidation of hormonal crosstalk between SA and cytokinins in the orchestration of plant defence. In this review we highlight the cytokinin-regulated physiological and molecular processes associated with plant development and also with plant immunity, and point to a role for this class of plant hormones in the regulation of the growth–defence trade-offs in plants.

IF I DON'T FIGHT THERE WILL BE TROUBLE: CYTOKININS IN PLANT–PATHOGEN INTERACTIONS

Cytokinins are N^6-substituted adenine derivatives that were discovered based on their role in regulating cell division in plants. Since then these plant hormones have been shown to regulate several other aspects of plant development and physiology, as well as responses to the environment (Argueso *et al*., 2009). Cytokinins constitute a group of structurally similar compounds, which can be classified as isoprenoid or aromatic cytokinins depending on whether they have an isoprene-derived or an aromatic side chain at the N^6-terminus (Kudo *et al*., 2010). Isoprenoid cytokinins are considered the predominant type of cytokinin in plants and are synthesized through the transfer of an isopentenyl group to an ATP/ADP moiety, a reaction catalysed by isopentenyl transferase (IPT) enzymes (Kakimoto, 2001). The resulting isopentenyladenine (iP) ribosides can be converted to active free base forms by the lonely guy (LOG) enzymes (Kurakawa *et al*., 2007). Cytokinin content is also tightly regulated by cytokinin oxidase/dehydrogenases (CKXs), which catalyse degradation of cytokinins into either adenine or adenoside (Houba-Herin *et al*., 1999).

Cytokinin signal transduction in plant cells utilizes a two-component phosphorelay system, a signalling pathway commonly used by bacteria and fungi to perceive and respond to environmental signals (Hwang *et al*., 2012). Briefly, cytokinins are perceived by histidine kinases (HKs), which are mostly present on the endoplasmic reticulum membrane and act as cytokinin receptors. Binding of cytokinin to HKs leads to HK autophosphorylation and conformational changes. The cytokinin signal is then transduced from HKs to response regulators (RRs) through histidine-containing phosphotransfer proteins (HPs), which shuttle between the cytoplasm and nucleus (Hutchison *et al*., 2006; Punwani *et al*., 2010). Response regulators can be grouped into at least two classes, depending on the plant species. Type-B RRs contain DNA-binding domains and act as transcription factors, activating the transcription of primary cytokinin response genes, including type-A RRs (Argyros *et al*., 2008). Type-A RRs, on the other hand, lack any DNA-binding domains and function to inhibit cytokinin signalling, forming a negative feedback loop that regulates the cytokinin signalling pathway (To *et al*., 2004).

A role for cytokinins in plant–pathogen interactions has long been suggested, mostly from studies where exogenous application of molecules with cytokinin activity to plants resulted in altered levels of host resistance or susceptibility to pathogens. For example, plant cell cultures grown under high cytokinin concentrations showed increased expression of defence and stress genes (Schafer *et al*., 2000). Application of cytokinins to bean plants resulted in decreased susceptibility to white clover mosaic potexvirus, also accompanied by the induction of defence gene expression (Clarke *et al*., 2000). The results of exogenous application of cytokinins to plants raised the question of whether the reduction in pathogen growth originated from antimicrobial activities of biologically active cytokinins or from host-regulated processes that impeded pathogen growth.

Helping the plant defend: cytokinin-induced immunity

A definitive role for host cytokinins in plant immunity came from studies in arabidopsis. Exogenous application of high concentrations (10–100 μM) of the isoprenoid-derived synthetic cytokinin 6-benzylaminopurine (BA) to arabidopsis plants before pathogen inoculation led to decreased susceptibility to the biotrophic oomycete *Hyaloperonospora arabidopsidis* (*Hpa)* (Argueso *et al*., 2012). Similar results were obtained in arabidopsis treated with 1 μM of *trans*-zeatin, a natural isoprenoid biologically active cytokinin for which the cytokinin receptors have very high affinity, in response to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 (Choi *et al*., 2010). The same disease-protective effect of cytokinins could not be observed in *ahk2,3* plants, which harbour mutations in two of the three genes encoding cytokinin receptors (*ARABIDOPSIS HISTIDINE KINASE 2* and *3*), indicating that the action of cytokinins in this context is indeed due to cytokinin-regulated physiological processes (Choi *et al*., 2010; Argueso *et al*., 2012). An additional concern in elucidating a role of cytokinins in plant immunity was the fact that many of the experiments were performed with exogenous hormone applications to plants, therefore confounding the contributions of exogenous and endogenous levels of cytokinin to the process. This was conclusively addressed by the use of transgenic arabidopsis plants overexpressing *IPT* genes, in which the endogenous levels of cytokinin are increased up to 100-fold (Kakimoto, 2001). *IPT*-overexpressing arabidopsis plants showed a decrease in *Pst* growth, confirming that highly increased levels of cytokinins help deter pathogen growth, and that this can be achieved by either exogenous or endogenous cytokinins (Choi *et al*., 2010). The decreased pathogen growth observed in cytokinin-treated plants was accompanied by enhanced upregulation of defence gene expression and callose deposition, to levels far superior to those obtained with pathogen treatment alone (Choi *et al*., 2010; Argueso *et al*., 2012). It is important to note that treatment of plants with cytokinin alone, without pathogen challenge or elicitors, does not lead to high levels of defence activation. In this way, the action of cytokinin in plant immunity is similar to the action of chemicals known as

priming agents, which act to potentiate defence responses, but are only activated upon pathogen/elicitor perception (Conrath *et al*., 2015).

While the initial mechanistic studies on cytokinin action in plant immunity were primarily focused on arabidopsis, they were shortly followed by studies in other plant species. In tobacco, inducible expression of an *IPT* gene responsible for cytokinin biosynthesis, as well as exogenous application of cytokinins, substantially reduced disease progression of the biotrophic bacterial pathogen *Pseudomonas syringae* pv. *tabaci* (*P. s. tabaci*) (Grosskinsky *et al*., 2011). In rice, treatment of plants with high levels of cytokinins led to increased defence gene expression against the biotrophic rice blast fungus *Magnaporthe oryzae* (Jiang *et al*., 2013). Added to other reports from the literature where high levels of cytokinins in plants have been linked to resistance to viruses (Clarke *et al*., 2000; Pogany *et al*., 2004) and even nematodes (Shanks *et al*., 2016), these data point to a role for cytokinins in activating defence responses and contributing to physiological conditions that help contain invading biotrophic pathogens, a process we have now named cytokinin-induced immunity.

Continued work in arabidopsis and other plant species has shown that the decrease in pathogen growth seen in cytokinin-induced immunity is at least partially dependent on content and signalling of the plant hormone SA. Arabidopsis mutants in the gene encoding the SA biosynthetic enzyme isochorismate synthase 1 (ISC1) failed to show the same effect of suppression of biotrophic pathogen growth due to cytokinin treatment (Choi *et al*., 2010; Argueso *et al*., 2012; Naseem *et al*., 2012). A similar lack of cytokinin-induced immunity was also observed using *npr1* (*NON-EXPRESSOR OF PR-1*) plants, which contain a mutation in a known master regulator of SA signalling (Choi *et al*., 2010). The SA-dependence of cytokinin-induced immunity further supported the observed priming activity of cytokinins, in which cytokinin-treated plants show enhancement of SA-dependent gene expression upon pathogen perception (Choi *et al*., 2010; Argueso *et al*., 2012). Similarly, in rice, exogenous co-treatment of plants with the SA analogue benzothiadiazole *S*-methyl ester (BTH) and the cytokinin kinetin dramatically increased expression of the defence genes *OsPR1b* and *PBZ1*, while treatment with either hormone alone did not show a significant increase in defence gene expression, nor did co-treatment with SA plus several other hormones (Jiang *et al*., 2013). This potentiation of SA-dependent defence gene expression by cytokinin is determined by the major regulators of SA-dependent defence responses in rice (Wu *et al*., 2012), OsNPR1 and WRKY45 (Shimono *et al*., 2007; Sugano *et al*., 2010). Interestingly, the increased immunity state induced by cytokinin and SA treatment in rice does not translate into decreased *M.oryzae* growth. It is important to note that in arabidopsis and tobacco high levels of SA are produced in response to pathogen invasion, while in rice the basal levels of SA are already high and do not change significantly following pathogen invasion (Silverman *et al*., 1995). Therefore, the observed differences in disease outcome in cytokinin-induced immunity may reflect the differences in SA content and signalling between rice and dicotyledonous plant species.

Despite the recognizable role for SA in cytokinin-induced immunity described above, some evidence points to a role for cytokinins in the activation of defence responses in a manner that is independent of SA. Transgenic tobacco lines overexpressing the bacterial gene *nahG*, encoding an SA degradation enzyme, did not alter the protective effect of cytokinin against *P. s. tabaci* infection seen in wild-type plants (Grosskinsky *et al*., 2011). The positive effects of cytokinin on defence responses of wild-type tobacco plants were attributed to the production of the key phytoalexins scopoletin and capsidiol, which act to restrict pathogen growth. A time-course analysis showed that the production of these phytoalexins occurs early during infection in response to pretreatment with cytokinin, before SA accumulation (Grosskinsky *et al*., 2011). Therefore, the fact that cytokinin-induced immunity in this pathosystem seems to be independent of SA may be simply due to the timing of scopoletin and capsidiol accumulation, which occurs before SA-dependent defence responses are activated (Grosskinsky *et al*., 2011). Nevertheless, co-treatment of wild-type tobacco plants with cytokinin and *P. s. tabaci* increased SA levels and *PR-1a* expression significantly more during late infection stages than treatment with cytokinin or *P. s. tabaci* alone (Grosskinsky *et al*., 2011). Thus, while SA may not be essential for cytokinin-induced immunity in early defence responses such as scopoletin and capsidiol biosynthesis, it certainly contributes to the overall defence response. In agreement with this, a synergistic action of cytokinins and SA was found to regulate the production of another type of phytoalexin, known as diterpenoid phytoalexins, during defence responses of rice to *M. oryzae* (Ko *et al*., 2010; Akagi *et al*., 2014), and this response is dependent on both SA and the SA signalling regulator WRKY45 (Akagi *et al*., 2014).

The signalling mechanisms regulating immunity activation by cytokinins are slowly being revealed. Choi *et al*. (2010) demonstrated that ARR2 (arabidopsis response regulator 2), the a type-B positive regulator of the cytokinin signalling, directly interacts with the SA-responsive transcription factor TGA3 and master regulator of SA signalling NPR1, forming a transcriptional complex that activates expression of the SA-dependent defence marker *PR1*. On the other hand, type-A ARRs, which function as negative regulators of cytokinin signalling, also act to suppress SA-dependent defence gene expression, in a manner dependent on their phosphorylation status (Argueso *et al*., 2012). Stimulation of reactive oxygen species (ROS) has also been linked to cytokinin and is a likely mechanism by which defence responses can be modulated by this plant hormone. Transgenic tobacco plants overexpressing *IPT* genes showed an increase in activity of the antioxidant enzymes ascorbate peroxidase, glutathione S-transferase and catalase, accompanied by lower H_2O_2 content in infected leaves. This suggests that increases in cytokinin content in leaves provide more efficient ROS scavenging activity, inhibiting symptom development of necrotic lesions upon infection with tobacco necrosis virus (*TNV*) (Pogany *et al*., 2004), which is in agreement with the role of cytokinin in oxidative stress (Zavaleta-Mancera *et al*., 2007; Shi *et al*., 2014). In another study, overexpression in tobacco of *S*-adenosylhomocysteine hydrolase (SAHH), a key enzyme in transmethylation reactions, conferred resistance to infection by several host viruses, and transgenic tobacco plants overexpressing *SAHH* (Masuta *et al*., 1995). Through physiological analysis it was shown that resistance to viral infection was correlated with elevated levels of cytokinins (Masuta *et al*., 1995). Interestingly, a similar result was obtained with viral-

induced gene co-silencing of the three genes in the tomato genome that encode SAHHs (Li *et al*., 2015). The *SAHH*-silenced tomato plants showed increased activation of immunity and resistance to *Pst*, as well as growth alterations similar to *IPT*-overexpressing plants. Further, the transgenic plants also showed increased drought tolerance (Li *et al*., 2015), a phenotype associated with cytokinin accumulation. Together, these results point to a role for cytokinin-regulated ROS homeostasis as a factor in the defence activation observed during cytokinin-induced immunity.

Helping the pathogen grow: cytokinin-induced susceptibility

Adding to the complexity of the roles of cytokinins in plant–pathogen interactions is the fact that in some cases an increase in the levels of biologically active cytokinins in plants is associated with increased pathogen growth, a process that we have named cytokinin-induced susceptibility. Given its beneficial effect in pathogenic organisms, cytokinin-induced susceptibility is likely a pathogen-driven process, by which manipulation of *in planta* cytokinin signalling and/or content, or direct production of cytokinins by the pathogens themselves, culminates in host physiological responses that help the pathogen thrive.

Cytokinin-induced susceptibility is usually associated with low to moderate levels of cytokinin content in plants. In one of the first demonstrations of cytokinin-induced susceptibility, Argueso *et al*. (2012) showed that lower concentrations of cytokinins can actually help pathogen success. Exogenous application of low concentrations of the cytokinin BA (<1 μM) to arabidopsis led to increased growth of the oomycete *Hpa* on wild-type plants, in comparison with mock-treated controls (Argueso *et al*., 2012). Similarly, a moderate increase in the levels of biologically active cytokinins was associated with increased growth of powdery mildew on wheat leaves, rather than increased resistance (Babosha, 2009). How low to moderate concentrations of cytokinins may help pathogen success is unclear, but it likely involves improved physiological conditions for pathogen growth. For example, the fungal pathogen *M. oryzae* produces and secretes cytokinins. *Magnaporthe oryzae*-derived cytokinins are biologically active in the host plant, as shown by activation of the cytokinin responsive promoter of the type-A response regulator *OsRR6* (Jiang *et al*., 2013). Production of cytokinins by *M. oryzae* was shown to alter rice metabolism near sites of infection, leading to an increase in the levels of key sugars and amino acids, which may act to help support fungal growth, increasing plant susceptibility (Chanclud *et al*., 2016).

Another possible explanation for cytokinin-induced susceptibility is decreased defence activation. Consistent with this hypothesis, examination of the molecular mechanism by which the bacterial effector protein HopQ1 suppresses defence responses revealed that low levels of cytokinin signalling increase susceptibility of arabidopsis to *Pst* by decreasing defence responses (Hann *et al*., 2014). HopQ1 is an effector protein produced by *Pst* and secreted via the type III secretion system. Transgenic arabidopsis plants expressing *HopQ1* show suppression of PTI responses when exposed to flg22, including reduced accumulation of ROS and PTI-associated mitogen-activated protein kinase (MAPK) activation. The reduced PTI responses are attributed to the attenuated expression of the *FLS2* (FLAGELLIN-SENSITIVE 2) gene, encoding the receptor for flg22. This reduced expression of *FLS2* corresponds to increased levels of active cytokinins and increased expression of cytokinin-responsive genes. Interestingly, HopQ1-expressing plants had severe developmental defects, including reduced root growth and branching, anthocyanin build-up and loss of apical dominance, consistent with phenotypes observed in a cytokinin-signalling mutant. Further, exogenous application of a low concentration of *trans*-zeatin (100 nM) to wild-type arabidopsis plants can recapitulate the reduced levels of *FLS2* transcript and protein amounts seen in *HopQ1*-transgenic plants, along with suppression of both ROS accumulation and PTI-associated MAPK activation (Hann *et al*., 2014). The authors hypothesize that the bacterial effector HopQ1 acts similarly to the LOG enzymes in arabidopsis, converting inactive cytokinin nucleotides into moderate levels of cytokinin active forms. The moderate increases in active cytokinins attenuate *FLS2* expression, diminishing PTI responses and ultimately helping pathogen growth. The action of HopQ1 seems limited by the existing pool of the inactive forms, which fluctuates with the developmental stage and environmental conditions of the plant (Hann *et al*., 2014). As in other plant–pathogen interactions, HopQ1 is an example of effector-triggered susceptibility and of how pathogens can exploit hormone biosynthesis to facilitate colonization.

IF I DON'T GROW THERE WILL BE DOUBLE: REGULATION OF PLANT GROWTH BY CYTOKININS AND POTENTIAL AVENUES FOR REGULATION OF GROWTH–DEFENCE TRADE-OFFS

The main implication of a role of cytokinins in the growth–defence trade-off comes from the fact that this plant hormone regulates not only plant immunity, as described in the sections above, but also plant growth (Kieber and Schaller, 2014). The decreased cytokinin content observed in *ipt* mutant plants, as well as in transgenic plants overexpressing *CKX* genes, positively correlates with decreased shoot size and decreased shoot meristem activity (Werner *et al*., 2003; Miyawaki *et al*., 2006). Conversely, overexpression of *IPT* genes causes increases in cytokinin content, leading to larger embryos and often to increased shoot growth (Smigocki and Owens, 1988; Ma *et al*., 2002).

In the process of analysing the hormonal crosstalk between cytokinin and SA, SA was determined to also have an inhibitory effect on cytokinin signalling (Argueso *et al*., 2012). Plants lacking SA biosynthesis (*eds16* mutants) are more sensitive to root growth inhibition by cytokinin and express higher levels of cytokinin-regulated genes, indicating a negative regulatory effect of SA on cytokinin signalling in wild-type plants (Argueso *et al*., 2012). Therefore, it is possible to envision a scenario where plants that accumulate high levels of SA, due to mutations or immunity activation by pathogens, also have reduced cytokinin content and/or signalling, which can then be translated into reduced plant growth and the fitness costs seen in the growth–defence trade-off. In the next sections we discuss some of the cytokinin-regulated processes that may affect the

observed growth–defence trade-offs during immunity activation.

Cytokinins and the control of cell division

Plant growth depends on cell division and expansion. Cytokinins were first discovered as molecules with the ability to promote cell division in plant cells. Initial studies identified a substance present in autoclaved herring sperm DNA that could promote division of plant cells in culture as a purine derivative, today known as the cytokinin kinetin (Miller *et al*., 1955, 1956). Since then, several other cytokinin species have been identified, and while these molecules have now been found to play roles in many developmental processes and biotic/abiotic responses, promotion of cell proliferation continues to be the hallmark role of this class of plant hormones (Schaller *et al*., 2014).

The control of cell growth and proliferation by cytokinins is directly tied to cell cycle regulation. The levels of cytokinins are known to change during cell cycle progression in cultured plant cells (Redig *et al*., 1996; Hartig and Beck, 2005). In addition, cytokinin treatment induces the expression of *CYCLIN D3* genes in arabidopsis (Riou-Khamlichi *et al*., 1999), which are conserved regulators of the gap transitions during cell cycle progression in plants and animals. Cyclin proteins activate cyclin-dependent kinases (CDKs), whose ultimate function is the activation of the EF2 protein complex, which regulates G_1/S and G_2/M gap transitions. Further connecting cytokinins and cell cycle control, the overexpression of *CYCLIN D3* genes in plants can bypass the requirement for cytokinin in culture media for shoot regeneration (Riou-Khamlichi *et al*., 1999). Moreover, treatment of tobacco BY-2 cells with a cytokinin biosynthesis inhibitor demonstrated that cytokinin biosynthesis is indispensable for the G_2/M transition (Laureys *et al*., 1998), and a delay in the G_2/M transition is observed in root cells of cytokinin receptor (*ahk*) multiple mutants. Interestingly, perturbations of the cell cycle can lead to activation of plant immunity (Bao *et al*., 2013). Recently, two cell cycle CDK proteins, SIAMESE (SIM) and SIM-RELATED 1 (SMR1), were found to be essential for cell cycle control and proper activation of programmed cell death during ETI (Wang *et al*., 2014; Hamdoun *et al*., 2016). The activation of SIM and SMR1 is also dependent on the *CPR5* gene, encoding a nuclear envelope protein, and whose mutations show constitutive activation of defence and growth defects. It would be interesting to know whether the activation of these CDK proteins is dependent on the transcriptional regulation of *CYCLIN D3* by cytokinins. It is noteworthy that cytokinins have also been implicated in the control of HR-like programmed cell death, through mechanisms that may involve cell cycle control (Suda *et al*., 2009; Novak *et al*., 2013).

Cytokinins and the control of meristem function

Given their important role in cell cycle control and regulation of cell division, it is not surprising that cytokinins have a direct role in the regulation of meristem function. Cytokinins have long been known to promote, in conjunction with auxin, the induction of organogenesis, with a predominant role for cytokinins in shoot initiation (Kieber and Schaller, 2014). In the shoot apical meristem (SAM), plants with decreased cytokinin content due to disruption of cytokinin biosynthesis or signalling have smaller SAMs (Miyawaki *et al*., 2006; Kurakawa *et al*., 2007; Kuroha *et al*., 2009) and show reduced growth rate (Higuchi *et al*., 2004; Nishimura *et al*., 2004; Miyawaki *et al*., 2006; Kurakawa *et al*., 2007). On the other hand, disruption of *CKX* genes or mutations in the type-A RRs, which increased levels of cytokinin content and signalling respectively, led to an enlarged SAM (To *et al*., 2004; Leibfried *et al*., 2005; Bartrina *et al*., 2011). Transgenic arabidopsis plants expressing *CKX* genes specifically in young shoot tissue have a lower cytokinin content and a dramatic decrease in the size of the leaves and number of leaf epidermal cells (Werner *et al*., 2003; Holst *et al*., 2011). The reduction of cytokinin content in the SAM also compromises the ability of the plant to form new leaf primordia and flowers (Holst *et al*., 2011), which is consistent with the increased number of inflorescences and seed yield seen in *ckx* mutant plants (Bartrina *et al*., 2011).

Maintenance and differentiation of SAM cells is under the spatial–temporal transcriptional control of the meristem-defining transcription factor WUSCHEL (WUS), the signalling peptide CLAVATA3 (CLV3) and its cognate receptor CLAVATA1 (CLV1) (Fletcher *et al*., 1999; Brand *et al*., 2000). The link between meristem size and cytokinins comes from data showing that cytokinin upregulated *WUS* expression (Lindsay *et al*., 2006). In turn, WUS upregulation induces *CLV3* expression, which then binds to its receptor, CLV1 (Mayer *et al*., 1998; Schoof *et al*., 2000). Binding of CLV3 to CLV1 then represses *WUS*, forming a negative feedback loop that regulates meristem organization. In addition, upregulation of WUS was shown to downregulate expression of type-A ARRs, including ARR7 and ARR15, relieving inhibition of cytokinin signalling in the SAM (Leibfried *et al*., 2005) and forming a second feedback loop that amplifies cytokinin signalling in the SAM. Because SA was determined to negatively regulate cytokinin signalling (Argueso *et al*., 2012), it is tempting to speculate a function for SA in the inhibition of cytokinin signalling in the SAM, leading to altered shoot development and growth trade-offs during defence.

In the root apical meristem (RAM) auxin regulates cell division while cytokinin regulates cell differentiation, and a balance between these two growth hormones is critical for proper RAM maintenance. Exogenous application of cytokinins leads to a reduced cell division zone in the RAM, culminating in reduced root growth and root branching, while cytokinin receptor mutants have the opposite phenotype, indicating a role for cytokinins in root growth inhibition (Bertell and Eliasson, 1992; Riefler *et al*., 2006). The control by cytokinins of RAM function is achieved by inhibition of auxin signalling and transport. Cytokinin signalling activates type-B ARRs, which promotes transcription of the *SHY2*/*IAA3* (SHORT HYPOCOTYL 2/INDOLE-3-ACETIC ACID INDUCIBLE 3) gene (Dello Ioio *et al*., 2008). The SHY2 protein, in turn, inhibits auxin signalling by forming heterodimers with auxin response factors (ARFs), which are responsible for the expression of auxin response genes. SHY2/ARF dimerization limits the number of cell divisions in the RAM before differentiation occurs, leading to decreased root growth (Dello Ioio *et al*., 2008). SHY2 also downregulates expression of *PIN* genes, responsible for auxin

transport, and this relocation of auxin stimulates cell differentiation (Dello Ioio *et al*., 2008). In contrast to shoots, the control of plant immunity in roots has been largely understudied. Recent work has demonstrated that FLS2 is under different transcriptional control in roots and shoots (Beck *et al*., 2014). Given the importance of cytokinin for root development and the recent discovery of transcriptional regulation of FLS2 through altered cytokinin levels (Hann *et al*., 2014), it is possible to suggest that cytokinins may be involved in the control of root immunity, conceivably by regulation of the *FLS2* transcript levels in root cells. While most examples of growth–defence trade-offs have been focused on shoots and inflorescences, reduced root growth or altered root architecture has severe consequences for shoot growth, including inflorescence growth and seed set. It is interesting to note that cytokinins are synthesized in the roots and transported to the shoot, where they exert control of different physiological processes (Kudo *et al*., 2010). Therefore, changes in development that result in reduced root growth may lead to diminished overall cytokinin content in the plants, and likely to the shoot growth defects that are typical of reduced cytokinin levels.

Cytokinins and the control of the source–sink relationships

Since the work of Mothes and Engelbrecht (1961), which showed that application of cytokinins can redirect the localization of plant assimilates in leaves of fava beans, cytokinins have been considered to have a fundamental function in the regulation of source–sink relationships. Today cytokinins are known to regulate the metabolism and transport of amino acids and carbohydrates important for plant growth, as well as several macronutrients, including nitrogen, phosphorus, sulphur and iron (Argueso *et al*., 2009).

One of the main ways by which cytokinins regulate the establishment of source and sink tissue is through the mediation of carbohydrate availability and transport (Roitsch and Ehness, 2000). Overexpression in tobacco of *CKX* genes, which encode enzymes that degrade cytokinin, led to a dramatic decrease in soluble sugar content in sink tissues accompanied by reduced shoot growth and seed set; however, no significant changes were observed in source tissues (Werner *et al*., 2008). These results suggest that cytokinin may be responsible for the availability of soluble sugars in sink tissues, such as young leaves, developing roots, fruits and seeds, positively affecting plant growth and yield.

A potential mechanism by which cytokinins may affect sugar availability in sink tissues involves invertases, which hydrolyse phloem-transported sucrose into hexose sugars. While plant cells possess different types of enzymes with invertase activity that differ in subcellular localization, cell wall invertase activity during phloem unloading constitutes an essential part of the establishment of metabolic sinks (Tauzin and Giardina, 2014). During attack by biotrophic pathogens, infected tissues such as leaves are known to transition from source to sink, and this transition is accompanied by increased cell wall invertase activity and increased expression of genes encoding sucrose transporters (Fotopoulos *et al*., 2003; Hayes *et al*., 2010). In some cases, as in the case of gall-forming pathogens, increased cell wall invertase activity helps pathogen growth, possibly by increasing the supply of sugars to pathogens (Siemens *et al*., 2011). In other instances, increased cell wall invertase activity is linked to increased resistance; silencing of a cell wall invertase in tobacco led to plants that displayed reduced defence responses and that were more susceptible to the oomycete *Phytophthora nicotianae*, indicating that availability of carbohydrates is essential to support defence reactions to invading pathogens (Essmann *et al*., 2008). Similarly, downregulation of proteinaceous invertase inhibitors during the defence reaction of arabidopsis to *Pst* culminates in elevated cell wall invertase activity that is needed for defence (Bonfig *et al*., 2010). It is interesting to note that exogenous application of cytokinin and overexpression of cytokinin biosynthetic *IPT* genes results in increased expression of genes encoding cell wall invertases (Gan and Amasino, 1995; Ehness and Roitsch, 1997; Kim *et al*., 2006), while plants with reduced cytokinin content have decreased cell wall invertase activity and plant growth (Werner *et al*., 2008). Thus, cytokinins are considered positive regulators of cell wall invertase activity, and are important for plant growth, defence and pathogen success.

Another possible role for cytokinin in source-sink relationships and plant growth is through the regulation of sugar transporters, which transport sucrose between photosynthetically active cells and phloem for further transport to sink tissues. During cytokinin-induced susceptibility, pathogens are able to manipulate cytokinin signalling and/or content to create physiological conditions that help their growth. In this scenario, sucrose transporters could also be targeted by pathogens to increase nutrient availability at sites of infection. A well-known example is the formation of green islands, which occurs in cereals infected with some rust and powdery mildew fungi. Green islands are regions of photosynthetically active leaf tissue at sites of infection surrounded by tissue undergoing senescence (Walters *et al*., 2008). Cytokinin content was demonstrated to be higher in green islands than in the surrounding senescing tissue (Lopez-Carbonell *et al*., 1998), where the effect of cytokinins on cell wall invertase activity is thought to prevent senescence and maintain the metabolically active tissue to support biotrophy and pathogen growth (Lara *et al*., 2004). Similarly, transcriptional activation of the *SWEET* class of rice sucrose transporters by *Xanthomonas oryzae* effectors has been implicated in the susceptibility of rice to this pathogen, possibly as a way to increase sucrose transport into the apoplast for pathogen feeding (Chen *et al*., 2010). Whether sucrose transporter expression and activity are dependent on cytokinins is unknown, but evidence exists supporting this hypothesis (Lee and Huang, 2013). Given the role of cytokinins in carbohydrate allocation during plant growth, and manipulation of these processes by invading pathogens, it could be hypothesized that competition among host and pathogen for cytokinin-regulated metabolic sinks is a potential avenue for the growth–defence trade-off to occur.

SO YOU'VE GOT TO LET ME KNOW: SHOULD I FIGHT OR SHOULD I GROW? REGULATION OF GROWTH–DEFENCE TRADE-OFFS BY CYTOKININS

In immunity against pathogens, modes of action in the cytokinin regulation of defence responses are dependent on the

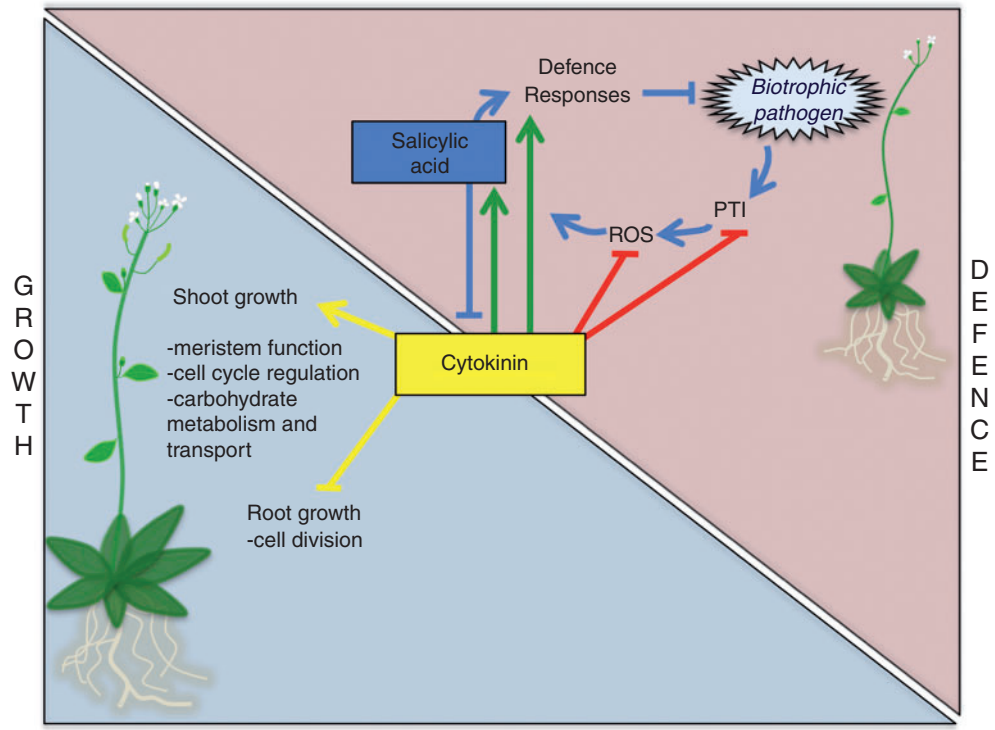

FIG. 1. Schematic representation of the role of cytokinins in plant growth and defence against biotrophic pathogens, and in the growth–defence trade-off. Under normal growing conditions, cytokinin promotes shoot growth while inhibiting root growth (yellow arrows). Infection by a biotrophic pathogen stimulates pattern-triggered immunity (PTI) activation, oxidative stress (ROS) and salicylic acid biosynthesis, culminating in salicylic acid-dependent defence responses that suppress biotrophic pathogen growth (blue arrows). Cytokinins can enhance defence activation by salicylic acid-dependent and -independent processes (cytokinin-induced immunity; green arrows). Cytokinins can also help pathogen growth, by mechanisms that include suppression of PTI and ROS (cytokinin-induced susceptibility; red arrows). Increased salicylic acid content/signalling inhibits cytokinin-regulated processes, potentially causing inhibition of plant growth, a likely mechanism by which the growth–defence trade-off may occur. Arrows indicate positive interaction; blunt ends indicate negative interaction (inhibition).

cytokinin concentration and the stage of infection. Moderate levels of cytokinins can help the biotrophic pathogen thrive by creating favourable physiological conditions (Argueso *et al*., 2012; Hann *et al*., 2014), while higher concentrations of cytokinin activate plant immunity primarily through SA-dependent processes (Choi *et al*., 2010; Argueso *et al*., 2012).

There are multiple areas of growth and development regulated by cytokinin that are likely processes during which the growth–defence trade-off could occur (Fig. 1). Defence gene expression, production of ROS and phytoalexin biosynthesis are processes that are partly mediated by cytokinins and also known to have a major role in the success of pathogen infection and disease progression. As cytokinins appear to both positively and negatively regulate sucrose transport and differentially regulate the expression of various hexose transporters, definition of sink tissues by cytokinins affords plants the ability to prioritize carbohydrate transport and metabolism to young growing tissues during optimal environmental conditions, while allowing for adjustment of sink tissue identity upon pathogen perception, leading to downregulation of growth. While metabolic reprogramming and competing resource allocation may account for growth suppression during immunity activation, several pieces of evidence indicate that such a scenario may not represent a complete picture of growth–defence trade-offs. For example, activation of PTI by the PAMP chitin does not lead to growth suppression (Wan *et al*., 2008; Petutschnig *et al*., 2010), which is in stark contrast to the effect of another PAMP, flg22 (Gomez-Gomez *et al*., 1999). Further, metabolic shifts under nitrogen- and carbon-limiting conditions have not been found to correlate with defence activation (Kleessen *et al*., 2014). Finally, decreased plant growth does not necessarily translate into increased immunity, as seen by the phenotype of cytokinin receptor mutants, which display reduced shoot growth but not increased defence activation. It is therefore more probable that some combination of energy diversion and altered regulation of developmental control pathways is responsible for the growth fitness costs of defence activation. In this regard, the roles of cytokinins in both energy partitioning and the control of cell division and meristem function indicate that this class of plant hormones is likely to play an important part in the regulation of growth–defence trade-offs. This existence of several physiological mechanisms that could account for growth–defence trade-offs stimulates one to hypothesize that growth suppression can be decoupled from defence activation. Studies that examine the fitness cost of defence have found that ROS produced during pathogen invasion are partly responsible for growth suppression independently of defence activation (Zhu *et al*., 2013).

Finally, the contribution of other plant hormones in conjunction with cytokinin in the regulation of the growth–defence trade-off also has to be considered (Belkhadir *et al*., 2014; Huot

TABLE 1. *Summary of research studying the effects of cytokinin on plant–pathogen interactions*

Pathogen	Host plant	Cytokinin alteration	Effect observed	Reference
Bacteria				
Pseudomonas syringae pv. *tomato* DC3000	*Arabidopsis thaliana*	Exogenous *trans*-zeatin (1 μM) and endogenous increase (*IPT*/*CKX* overexpression)	CII	Choi *et al*. (2010)
Pseudomonas syringae pv. *tabaci*	*Nicotiana tobacum*	Exogenous kinetin (1–18 μM) and endogenous increase (upregulated *IPT*)	CII	Grosskinsky *et al*. (2011)
Pseudomonas syringae pv. *tomato* DC3000	*Arabidopsis thaliana*	Exogenous kinetin (10 μM)	CII	Naseem *et al*. (2012)
Pseudomonas syringae pv. *tomato* DC3000	*Arabidopsis thaliana*	Exogenous *trans*-zeatin (0·001–1 μM)	CIS	Hann *et al*. (2014)
Rhodococcus fascians	*Arabidopsis thaliana*	Pathogen-secreted isopentenyladenine, *trans*-zeatin, *cis*-zeatin	CIS	Pertry *et al*. (2009)
Oomycete				
Hyaloporenospora arabidopsidis	*Arabidopsis thaliana*	Exogenous benzyladenine (0·01–100 μM)	CIS and CII	Argueso *et al*. (2012)
Fungi				
Erysiphe graminis f. sp. *tritici*	*Triticum aestivum*	Exogenous *trans*-zeatin (0·25–9 μM)	CIS and CII	Babosha (2009)
Magnaporthe oryzae	*Oryza sativa* subsp. *japonica*	Exogenous kinetin or isopentenyladenine (1–100 μM) plus SA analogue	CII	Akagi *et al*. (2014), Jiang *et al*. (2013)
Magnaporthe oryzae	*Oryza sativa* subsp. *japonica*	Pathogen-secreted *cis*-zeatin nucleotide, isopentenyladenine, *cis*-zeatin riboside, isopentenyladenosine	CIS	Chanclud *et al*. (2016)
Pyrenopeziza brassicae	*Brassica napus*	Pathogen-secreted zeatin riboside, isopentenyl adenosine	CIS	Ashby (2000)
Viruses				
Cucumber mosaic virus, tobacco mosaic virus, potato virus X, potato virus Y	*Nicotiana tobacum*	Endogenous upregulation (*SAHH* overexpression)	CII	Masuta *et al*. (1995)
Tobacco necrosis virus	*Nicotiana tobacum*	Endogenous increase (*IPT* overexpression)	CII	Pogany *et al*. (2004)
White clover mosaic potexvirus	*Phaseolus vulgaris*	Exogenous dihydrozeatin (0·0025 μM)	CII	Clarke *et al*. (2000)
Tobacco mosaic virus	*Nicotiana tobacum*	Endogenous increase (*RGPT1* overexpression)	CII	Sano *et al*. (1994)
Plasmodiophoromycetes				
Plasmodiophora brassicae	*Arabidopsis thaliana*	Endogenous decrease (*CKX* overexpression)	CIS	Siemens *et al*. (2011)
Nematodes				
Heterodera schachtii	*Arabidopsis thaliana*	Cytokinin-hyper/hyposensitive signalling mutants	CII and CIS	Shanks *et al*. (2016)

CII, cytokinin-induced immunity; CIS, cytokinin-induced susceptibility.

et al., 2014; Lozano-Duran and Zipfel, 2015). For example, auxin and cytokinin signalling pathways interact in multiple ways to modulate key aspects of growth and development (Schaller *et al*., 2015). Salicylic acid represses expression of auxin signalling genes, resulting in inhibition of pathogen growth (Wang *et al*., 2007). Furthermore, *Pst* DC3000 stimulates auxin production upon infection in arabidopsis, presumably to facilitate colonization (Naseem *et al*., 2012). Auxins and cytokinins appear to act antagonistically in the mediation of immune responses, with auxin promoting pathogen growth while cytokinin inhibits growth. Co-treatment of plants with both hormones shows decreased pathogen growth relative to treatment with auxin alone (Naseem *et al*., 2012). This illustrates the well-known fact that it is the interactions between multiple hormone signalling pathways that regulate defence responses, and unravelling the complexities of the growth–defence trade-off requires accounting for hormonal signalling crosstalk.

Understanding of the role of cytokinin in the growth–defence trade-off requires more research that should define cytokinin actions in the responses of plants to pathogens, using different pathosystems, as well as examination during various stages of infection. Considerations that should be accounted for include cytokinin origin (host or pathogen), as well as types and concentrations of cytokinins, as differences in biological activity and effective concentrations can lead to states of increased immunity or susceptibility (Table 1). The matter of tissue specificity of growth–defence trade-offs should also be further explored, and in this regard the opposing regulation of shoot and root growth by cytokinins indicates that this class of plant hormones may play an important role. For example, plants overexpressing genes encoding *CKX* enzymes involved in cytokinin degradation display decreased shoot growth but increased root growth; however, whether this also translates into mutually decreased

susceptibility to shoot pathogens and increased susceptibility to root pathogens is not known.

In a breeding programme for new varieties of crops, disease resistance is only one of the several factors taken into account when deciding whether a cultivar may be of commercial significance, and any fitness costs associated with traits that increase resistance to pathogens must be weighed against other traits of agronomical importance, especially crop yield (Brown, 2002). Therefore, understanding of the mechanisms that regulate the growth–defence trade-off in plants is an important step for the production of advanced crop varieties that are both high-yielding and resistant to biotrophic pathogens. Possible applications exist for the breeding and engineering of crop species with enhanced, broad-spectrum, durable disease resistance to biotrophic pathogens with reduced yield penalties by uncoupling defence activation from growth reduction through manipulation of cytokinin levels and signalling.

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